

UNIVERSIDADE DE LISBOA
FACULDADE DE CIÊNCIAS
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Homing in *Lipophrys pholis*.
An evolutionary perspective.

Joana Andreia Guerreiro Fernandes Martins

DISSERTAÇÃO

MESTRADO EM BIOLOGIA EVOLUTIVA E DO DESENVOLVIMENTO

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Resumo

Lipophrys pholis é uma das espécies de blenídeos mais comuns da zona costeira Portuguesa. Residente em poças da zona intertidal, esta espécie apresenta um comportamento exploratório bastante desenvolvido durante o período de preia-mar, onde pode estar implícita a sua capacidade para memorizar características do território. Alguns autores sugerem que esta espécie de peixes não apresenta uma distribuição aleatória. Ao invés, selecionam ativamente determinadas poças de maré disponíveis na sua área vital. Durante a época de reprodução, que decorre de Outubro a Maio, os machos de *L. pholis* nidificam geralmente dentro de fendas e buracos, os quais podem ficar emersos por longos períodos de tempo durante o período baixa-mar. Em alternativa estes machos também podem nidificar sob pedras, onde em ambos os casos, as fêmeas, que não fornecem quaisquer cuidados parentais, depositam os ovos. Encontrar e escolher o ninho certo é crucial para os machos terem sucesso reprodutor pois são estes que prestam os cuidados parentais. Em alguns estudos é mencionado que a agregação de ninhos e a proximidade entre machos é um fator importante para a atração das fêmeas.

Assim, o conhecimento prévio das características específicas de uma determinada área, ou seja, a localização de potenciais refúgios, locais de nidificação, bem como o conhecimento de rotas de fuga preferenciais, pode desempenhar um papel importante na sobrevivência destes peixes, os quais, estão sujeitos a elevada pressão predatória. A memorização da posição dos refúgios e dos ninhos bem como a direção das rotas de fuga preferenciais têm como referencial pistas visuais conspícuas do habitat. Estas pistas visuais conspícuas “landmarks” são integradas a nível cerebral num referencial de “landmarks”, formando mapas espaciais da área familiar que os peixes usam para uma constante atualização da sua localização. As pistas do terreno podem ser proximais, i.e. localizadas dentro das poças habitadas pelos peixes, ou distais, i.e. localizadas fora das poças. Experiências de orientação mostraram que os indivíduos desta espécie também conseguem determinar a direção da área de residência a partir de locais não familiares localizados a várias dezenas de quilômetros do local de captura.

Com este trabalho pretende-se, através da análise de registos de presenças de *L. pholis* ao longo de três anos sucessivos, testar a capacidade de *homing* e mais concretamente testar a fidelidade dos machos aos locais de nidificação e aos ninhos em particular. O comportamento de *homing* é definido no seu sentido restrito como o regresso de um animal a um local espacialmente definido onde possui um abrigo. Um dos benefícios do *homing* é que assegura que o animal volta

a uma área familiar, conferindo-lhe assim uma vantagem adaptativa (ver acima), apoiando a ideia de que a familiaridade é importante para a sobrevivência dos indivíduos. A maioria dos estudos sobre *homing*, em peixes intertidais rochosos, indica que embora os peixes regressem muitas vezes a um local específico, geralmente não estão exclusivamente vinculados a esse local. Assim, um peixe pode utilizar uma rede de locais ou abrigos, mais ou menos desenvolvida, que visita regularmente, sem estar permanentemente ligado a nenhum deles.

Em *L. pholis*, e considerando que se trata de uma espécie aparentemente não-migratória, questiona-se qual será a funcionalidade de um sistema de navegação tão desenvolvido? Uma vez que se trata de uma população aberta a viver no seu habitat natural, e como não dispomos de meios que nos permitam registar os movimentos diários dos indivíduos, neste estudo utilizamos como uma aproximação de *homing* a capacidade que os machos adultos têm para retornar aos mesmos locais de nidificação após uma ausência mais ou menos prolongada durante o período não reprodutor. Assim, desde 2011 temos vindo sistematicamente a marcar e registar a presença de animais adultos de uma população de *L. pholis* estabelecida no Cabo Raso, Cascais, Portugal.

No total, 211 animais adultos foram capturados e marcados individualmente com recurso a marcação electrónica por meio de cirurgia, libertados e seguidos periodicamente. Cento e quinze indivíduos foram recapturados considerando um intervalo mínimo de tempo nunca inferior a quinze dias após a data da sua marcação. A inspeção da área de estudo foi realizada duas vezes por mês durante os dias de maior amplitude de maré (i.e. no período de lua nova e de lua cheia). Durante as inspeções procurou-se ativamente registar a presença dos animais marcados. Indivíduos novos (i.e. adultos não marcados) e encontrados durante as inspeções, dentro da área de estudo, foram sendo sucessivamente marcados. A presença, posição relativa (coordenadas do local) e medidas corporais foram registadas para todos os peixes capturados. Para identificar os abrigos durante estes três anos, várias marcações metálicas foram inseridas na rocha com a ajuda de um berbequim. Ninhos espacialmente próximos uns dos outros foram agregados em secções. Foram ainda calculadas as distâncias entre as diversas secções de ninhos assim como as distâncias particulares entre os diversos ninhos.

Estando descrito que esta espécie apresenta uma maior atividade exploratória durante a preia-mar do período diurno, assumiu-se que, se os peixes marcados, que apresentam posteriormente registo de recaptura, não foram amostrados é porque se encontram noutra local fora da área de amostragem. A análise dos registos de recaptura mostrou que os machos de *L.*

pholis, em geral, passam significativamente mais tempo na área amostrada durante a época de reprodução do que fora da época de reprodução. O mesmo não se verifica nas fêmeas com uma presença homogénea dentro e fora da época de reprodução. Interessante é que 54% dos machos regressaram ao mesmo ninho em épocas de reprodução distintas e esse número aumenta para 83% se considerarmos o comportamento de fidelidade ao sector. Importante de mencionar é o facto de que as maiores distâncias entre recapturas observadas correspondem a indivíduos que foram capturados e marcados primeiramente em poças e posteriormente reencontrados em ninhos.

O retorno dos machos ano após ano às mesmas áreas de nidificação, suporta a hipótese de que os peixes adquirem e armazenam vasta informação do meio envolvente utilizando-a posteriormente aquando do retorno em épocas de reprodução distintas. As observações resultantes deste trabalho indicam que os machos de *L. pholis* têm um comportamento de *homing*, mas mais importante é que representam um caso extremo deste comportamento, particularizando a sua fidelidade à área local até ao nível do ninho (ou seja, eles apresentam um comportamento de *nest fidelity*). Uma vez que nos é impossível seguir estes indivíduos devido a limitações técnicas (não existem dispositivos de GPS para estes animais), permanece em aberto a distância coberta nestas hipotéticas migrações verticais sazonais.

Em conjunto, esses resultados suportam a ideia de que os mecanismos de navegação são característica geral dos vertebrados e que poderão ter evoluído a partir dum ancestral comum e manter-se nas diferentes classes: Peixes (e.g. salmões e enguias), anfíbios (e.g. salamandras), aves (e.g. pombo-correio), mamíferos (e.g. elefantes e morcegos) e répteis (e.g. tartarugas marinhas). Curiosamente, um artigo recentemente publicado em gobídeos, onde foram testadas as capacidades de memorização e aprendizagem, sugere que o comportamento de orientação/navegação está dependente da história de vida das espécies em questão. Numa arena artificial onde foram simulados os ciclos de maré, as espécies que habitam o intertidal rochoso, e contrariamente às espécies que habitam em areia aprendem rapidamente a memorizar a posição de poças o que lhes permitem evitar a sobre-exposição ao sol aquando do recuo da maré. Os autores sugerem que a ausência de necessidade das espécies que habitam em areia em procurar locais de abrigo contribuiu claramente para este resultado. Assim, dependendo do ambiente onde os animais estão inseridos e através de seleção natural os comportamentos de orientação/navegação poderão ser otimizados ou reduzidos.

Palavras-chave:

Lipohrys pholis; fidelidade à área de reprodução; 'homing'; comportamento; evolução; navegação; orientação; vertebrados

Abstract

Lipophrys pholis is one of the most common species of blennies that inhabits the Portuguese rocky shores. Usually residing in intertidal rocky pools, they perform exploratory behavior during high tide, which may elicit their ability to memorize terrain characteristics. For a non-migrant species, it has excellent homing abilities. Despite the ability to memorize the position of refuges based on conspicuous visual cues, displacement experiments showed that they can determine the homeward direction even at unfamiliar places tens of kilometers away from their home range; they can perform true navigation. But, why does a non-migrant species need such complex navigational skills? To answer this question we have started tagging adult animals from a population of *L. pholis* at Cabo Raso, Cascais, Portugal in 2011. Adults were captured, tag individually with a microchip introduced into the abdominal cavity and subsequently released in the place where they were previously captured. During field inspections, performed twice a month (i.e. during days of new and full moon), we actively searched for the presence of tagged animals as well as for new animals to be tagged. The presence, relative position (spatial coordinates) and body measures were recorded for all fish. The findings showed that many males return to the same area and to particular nests (i.e. Nest-site fidelity) in successive breeding seasons supporting the hypothesis that fish acquire large information of the surroundings, which they may use when returning to a previous breeding area. Therefore, these findings suggest that navigational mechanisms are widespread in vertebrates and may have evolved from a common ancestor to the different classes. A recent publication on gobies suggested that depending on the habitat where species live, natural selection have evolved to maximize or reduce the use of the navigational skills.

Key Words: *Lipophrys pholis*; Nest-site fidelity; Homing; Behavior; Evolution; Navigation; Orientation; Vertebrates

Introduction

Homing behavior, generally defined as “*the ability of an animal to return to a spatially restricted location that it previously occupied following displacement to an unfamiliar site,*” has been documented in a wide range of vertebrate species and it is expected to significantly improve fitness (Williams, 1957; Gerking, 1959; Papi, 1992; Bélichon *et al.*, 1996; White and Brown, 2013). One benefit related with homing behavior is that it ensures that the animal returns to a familiar area, where the location of food sources and predators may be known. Thus, supporting the idea that site familiarity is important to survival (Braithwaite and Girvan, 2003). However, for many species, homing poses extraordinary bioenergetics and navigational challenges (Reese, 1989a; Dittman and Quinn, 1996; Bingman and Cheng, 2005; Lohmann *et al.*, 2008; White and Brown, 2013).

Homing is advantageous when the costs are overshadowed by the benefits, which may vary with sex and/or age (juveniles, immatures or adults) of the individuals (Gibson, 1968; Bélichon *et al.*, 1996; Börger *et al.*, 2008). Homing to familiar nesting areas (i.e. breeding site fidelity), supported with local knowledge of resources (e.g. sheltering or nesting sites and feeding) (Béslisle, 2005) or associated with stable social interactions (e.g. established dominance hierarchies and territorial boundaries) (Hoover, 2003), may increase fitness, by reducing the predation rate of nests and by enhancing the survival of adults, and reduce risks associated with nesting in new habitats (Burt, 1943; Bergerud and Gratson, 1988). Moreover, breeding success, defined as hatching a large number of descendants, can increase the probability of breeders return in subsequent years to the same area (Greenwood and Harvey, 1982). Site fidelity behavior has vital consequences for ecological processes, such the distribution and abundance of organisms or community structure (Fagan *et al.*, 2007; Wang and Grimm, 2007), habitat selection (Hutto, 1985), predator-prey dynamics (Lewis and Murray, 1993) and infection spread (Kenkre *et al.*, 2007).

Habitat selection is a process involving a series of innate and learned behavioral decisions made by an animal about what habitat is suitable for its use (Hutto, 1985). Classical studies of habitat selection by deer mice (*Peromyscus maniculatus*) revealed that heredity and experience play a role in that decision (Wecker, 1964). Others factors driving habitat selection are: 1) forage quality and quantity; 2) cover availability; and 3) resting or denning sites; and each of these may

vary seasonally. However, if animals remain in their natal territories or return there to reproduction it represents a case of natal philopatry and may affect patterns of gene flow and fitness components like survival and reproduction. Increased inbreeding results as a price paid for some other advantages (see above) and may result in speciation of those populations (Reese, 1989a). Alternatively, philopatry may result from processes of dispersal or migration, and with reaching sexual maturity, individuals start returning to the same location to breed, year after year; (breeding-site philopatry).

Dispersal processes involve the movement of organisms from their location of birth to other locations at which they will reproduce and it can be divided into two components: 1) natal dispersal of young prior to first breeding; or 2) breeding dispersal of breeding adults (Greenwood and Harvey, 1982). Dispersal processes are relatively undirected in contrast to migration, which is the cyclical movement between specific regions (e.g. migratory birds and fish) and its evolution may be affected by both, individual selection and group selection. Importantly, animal movement is intrinsically dependent of the animals' ability to orient and navigate (Greenwood and Harvey, 1982; Bergerud, 1988).

Griffin (1952, 1955) characterized the navigational process according to their complexity and it allowed a first classification of the navigation behavior: **Type I**, it is the simplest possible navigational strategy, based on visual landmarks within the familiar territory and the use of random exploration when released at unfamiliar territory. This strategy is commonly called "piloting"; **Type II**, it is more complex than type I and it is defined as the process by which animals are able to move into a certain direction even when crossing unfamiliar territory; it require the use of one external referential (i.e. compass); **Type III**, it is the most complex navigational strategy, it requires sophisticated mechanisms and it makes use of at least two external referential (i.e. a map and a compass), it is called "true navigation" (Fig. 1). After Griffin's classification a number of studies have monitored the homing ability of various animals, particularly birds (e.g. barn swallow, pigeons, little blue penguins), fishes (e.g. salmonids, codfish, eels) and sea turtles (e.g. green turtles and loggerhead turtles) (Matthews, 1951; Able, 1980; Brannon, 1982; Groot, 1982; Nastase, 1982; Reese, 1989a, 1989b; Pledger and Bullen, 1998; Robichaud and Rose, 2001; King and Withler, 2005; Westenberg *et al.*, 2007; Lohmann *et al.*, 2008).

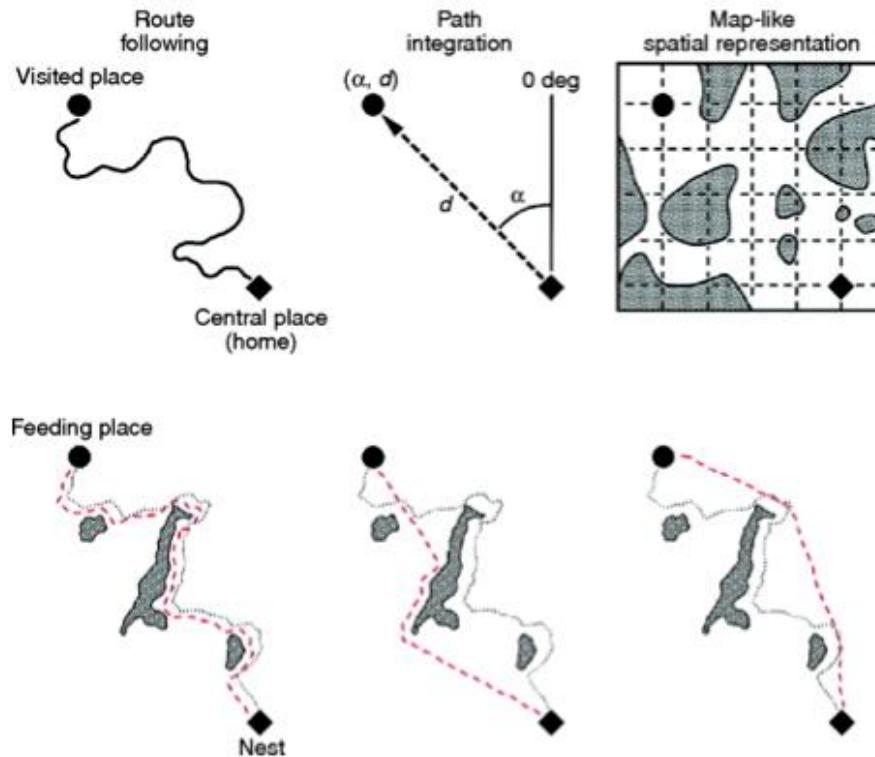


Fig. 1 – The three different, though not mutually exclusive, modes of navigation presented here. (Left) Route following; (middle) path integration; and (right) map-like spatial representation. Labelling is given for left column: central place, e.g. home or nest, is indicated as a black diamond; visited site, e.g. feeder, as a filled circle. In the bottom diagrams, outbound paths in search of food are indicated as black dotted lines, homebound paths after a successful visit to a food source are shown as red dashed lines. In route following, the outbound path is retraced when returning home from the visit to a distant goal. In path integration, the homebound vector is incrementally updated by monitoring angles steered (α) and distances covered (d) during outbound travel; the home vector is played out when returning to the nest, and the straight path indicated by the home vector may be interrupted (with the vector still being continuously updated) when obstacles need to be got round (indicated by hatched areas). When using a map-like spatial representation, the animals are informed about the spatial layout of their surroundings, including obstacles; when homing, they should select the shortest (or otherwise preferred, e.g. sheltered) route back home. These different homing performances can be used to distinguish between navigation strategies, in particular, when designing appropriate homing experiments (Figure adapted from Wolf H., 2011 *Journal of Experimental Biology*, **214**, 1629-1641).

The navigational strategy used by animals is then shaped by physiological constraints (e.g. age and sex) of the individual (Almada *et al.*, 1992; Jorge *et al.*, 2006, 2011a) and ecological demands (e.g. mountains or oceans) of the route (Bell, 2005; Marques *et al.*, 2009). Also, in the marine environment, teleost fish face similar problems, with salmonids facing similar challenges when returning to their natal rivers to spawn (Nastase, 1982; Dittman and Quinn, 1996). Ocean currents represent a key environmental factor that greatly affects the movement and behavior of marine animals (Chapman *et al.*, 2011). For instance, currents may influence the distribution and movements of pelagic animals in a variety of ways: 1) by determining the distribution of food

resources (Olson *et al.*, 1994; Bost *et al.*, 2009); or 2) by physically influencing their displacement (Girard *et al.*, 2006; Cotté *et al.*, 2007). Therefore, homing behavior and/or site fidelity are relatively common in marine systems and assume particular importance in environments with fluctuating conditions. The intertidal zone is one of those examples where conditions change over space and time and may have consequences for population processes such as recruitment and survival (Marnane, 2000). Moreover, fidelity to spawning places may result in reproductive isolation of those populations (see above “natal philopatry”).

Among oviparous fish, parental care may be provided to eggs and/or offspring by one or both parents, with male parental care being the most common, through a wide range of mating behaviors. These include establishing a nest, defending, cleaning and providing appropriate conditions for the fertilized eggs to hatch and releasing the larvae from the nest (Blumer, 1979; Almada *et al.*, 1990b; King and Withler, 2005).

For intertidal fish, like *L. pholis*, survival and reproductive fitness can be dependent of the individual’s ability to return to its home, rock pool or nest after high tide feeding excursions (Bélichon *et al.*, 1996; Ros *et al.*, 2006). The males of this specie establish nests in crevices or holes that become immersed during the low tide. They defend a breeding territory centered on the nest, and almost never leave the area during the breeding season. When disturbed they show a large number of agonistic behaviors and produce a characteristic sound (Almada, 1990b). On the other hand, females must travel longer distances visiting several nests and spawning with males (Costa *et al.*, 2011). Therefore, male blennies are hypothetically more site-attached than females (Almada and Santos, 1995; Costa *et al.*, 2011). Interestingly, the motivation for homing is present only in the adult phase (Jorge *et al.*, 2011b) but it appears to be independent of the sex and/or familiarity with the captured site (Ros *et al.*, 2006; Jorge *et al.*, 2011b; Thyssen *et al.*, 2014).

At the morphological level, *L. pholis* are benthic fish with a long and laterally elongated body (Faria *et al.*, 1996). They also have mechanisms of resistance to transport, no swim bladder and large well developed pectoral fins that allow locomotion associated with the substrate (Faria *et al.*, 1996). Moreover, they have great mobility of the head and have well developed eyes (Almada *et al.*, 1990a). Their small size and high maneuvering capabilities allows them to occupy small cavities and crevices that provide protection against predators and/or accidental displacement due to water turbulence (Faria *et al.*, 1996). The mucus associated with the absence of scales acts as a lubricant to facilitate movements in confined spaces and confer resistant to

drying (Faria *et al.*, 1996). *L. pholis* have a life span up to ten years (Almada *et al.*, 1990a; Faria *et al.*, 1996), with a reproductive period that occurs during the cold-water period, from October to May (Almada *et al.*, 1990a; Faria *et al.*, 1996; Ferreira *et al.*, 2012).

Overall, rocky-intertidal fishes have restricted movements and good homing abilities (Williams, 1957; Gibson, 1969; Green, 1999). *L. pholis* appears to be capable of learning complex spatial relationships of familiar landmarks forming mental representations of the local environment (i.e. familiar map). In addition to their singular characteristics (see above) and abundance (i.e. it is one of the species that is a consistent component of the Portuguese littoral fish fauna), it offers an outstanding biological model to test navigational hypotheses and ultimately provide answers. In this work is described for the first time the degree of homing/site fidelity behavior in *L. pholis*. Site fidelity can be examined in two ways: either by measuring the long term persistence of individuals at home sites or by examining the drive of individuals to return to home sites after being displaced (Hartney, 1996). Here, site fidelity/homing was examined by recording the presence of tagged fish in the study area over three consecutive years. It is suggested that *L. pholis* are partial residents but not migrants. Therefore, it is important to clarify whether it is the onset of the reproductive behavior that is conditioning the development of the navigational system.

Using an individually tagged population of adult *L. pholis*, it is the aim of the present study to understand their annual movements at the study area and how it conditioning the development of navigational strategies. Considering that *L. pholis* are mentioned as residents (see above) it is first hypothesized that adult individuals should spent equal time at the study area throughout life (i.e. between breeding and non-breeding seasons). Accordingly, the re-sight frequencies (f) of adult individuals should be equal in the breeding and non-breeding seasons. Alternatively, if this hypothesis is rejected either by the entire population or even by a particular sub-group (e.g. males or females) of the population, this means that the population or the sub-group of the population is asymmetrically distributed on time at the study area. For example, individuals can just use the area for breeding or resting and feeding. Evidences that individuals appear in successive seasons, leads to the second hypothesis, that is, the distribution of individuals (d) in two distinct seasons, at the study area, is independent of its previous experience (here, d is given by the distance in meters between two re-sights in distinct seasons). Interestingly, the rejection of the second hypothesis may suggest that individuals

should return to specific sectors of the area (e.g. site fidelity) or even to a particular point in the area (e.g. nest fidelity). However, because homing is costly (see above), all individual may not return in the next season. Therefore, it will be interesting to observe whether the body condition of homing and non-homing individuals (K_b) is equal (third hypothesis).

Methods

Subjects and Study Area

At Cabo Raso, Portugal (38°42'N, 9°29'W) we monitored a 4000 m² portion of an intertidal rocky-shore area of a vast rocky platform (Fig. 2A). The rocky platform is characterized by the presence of a large number of “appropriate” shelters (i.e. wholes or crevices that fish may use as refuge or nest) suggesting that shelters should not be regarded as a limiting resource. Holes and crevices in vertical walls were often occupied by individuals from different fish species, such as *Lipophrys trigloides* (Valenciennes, 1836) or *Coryphoblennius galerita* (Linnaeus, 1758). Invertebrates, mostly crabs (*Carcinus maenas* and *Eriphia verrucosa*), algae and sediments were also observed inside those shelters throughout the year, inferring important ecological transitions in shelters and surrounding areas.

From April of 2011 to May of 2014, a set of more than two hundred shelters and four pools were inspected twice a month during full-moon and new-moon periods. Seventy shelters that contained *L. pholis* eggs were classified as nests. The shelters/nests were identified with metallic marks that were attached to the rock with the help of an electric drill and grouped into sectors according to their position and proximity to other nests (see appendix I and Fig. 2B). Distances between sectors and between nests were measured, with a flexible ruler, to the nearest centimeter. Sectors length varied from 3 and 19 meters with the mean sector length of 10 meters (Table 1). The shortest distance between nearby sectors varied between 5m and 38m and the mean distance between nearby sectors was 20 m.

A group of four persons actively searched for the presence of adult *L. pholis*. Individuals that were previously tagged as well as individuals that were captured as new and tagged for the first time were registered for their presence, relative position, presence or absence of eggs in the shelter, and body measures (total body length and weight). In total, 211 adult shannies (i.e. total body length \geq 70mm), of both sexes were captured and marked.

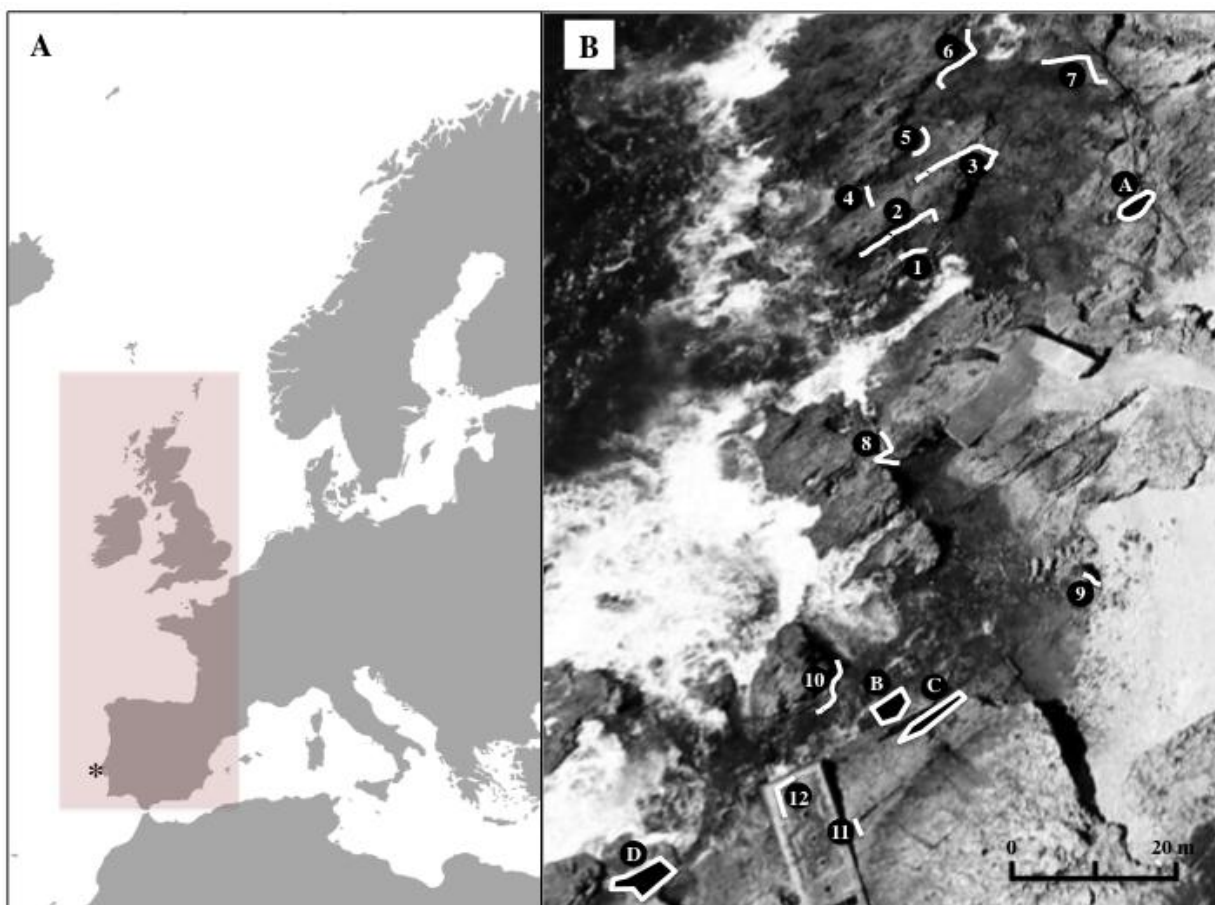


Fig. 2 – Study area in the context of the species distribution range (A). Asterisk represents the geographical location of the sheltered rocky platform at Cabo Raso, Portugal, (38°42'N, 9°29'W). The maroon area represents the highest rate of occurrence of *L. pholis* in the Eastern Atlantic (information taken from www.fishbase.org). Details about the location and spatial distribution of the nest sectors (B). Letters from A to D represent the intertidal pools (white polygons). Numbers from 1 to 12 represent the sectors of shelters and nests (white lines).

Table 1 - Sectors lengths and pools areas. Total number of occupied nests by sector. For relative position of sectors and pools in the study area see Fig. 2B.

Sector	Number of nests	Perimeter of the sector	Pool	Area of the pool
1	6	6,28m	A	14,3 m ²
2	14	17,80m		
3	12	19,20m		
4	2	4,1m	B	12,5 m ²
5	3	9,43 m		
6	8	17,0m	C	12,5 m ²
7	3	15,3m		
8	2	10,9m		
9	2	3,0m	D	18,0 m ²
10	2	9,81m		
11	14	2,10m		
12	2	6,33m		

Experimental procedure

Individuals were captured by carefully removing them from their holes by means of a blunt hook or from their tide pools by means of hand nets. Captured fish were anaesthetized with MS-222 (tricaine methanesulphonate, Sigma) and went through surgery for tagging; an electronic tag (IA-100A, TROVAN) was introduced into the abdominal cavity of each single fish. An antiseptic solution containing 9-12% of povidone-iodine was used for minimize the risk of infection. Fish were sutured with absorbable suture line and were placed in an aerated tank until complete recovery. Body weight, total and standard length and sex were determined while fish were anaesthetized (see appendix I). The entire procedure took approximately 10 min and was extremely safe since no casualties due to manipulation were reported in 211 surgeries.

Electronic tags were identified with a microtransponder reader (LID-560 Pocket Reader, TROVAN) adapted to a flexible cable (length: 1m; diameter: 1cm) holding the probe at the extremity (see appendix II). This adaptation to the microtransponder reader allowed the identification of tagged fish without the need to remove them from the shelter/nest. Therefore, this adaptation minimizes the re-sight impact on tagged fish once they did not need to be removed from the shelter/nest.

Statistical analysis

In most cases, data did not follow a normal distribution failing normality tests or other parametric assumptions (see appendix III). Although parametric analysis is often more powerful than non-parametric alternatives if sample size is small or parametric assumptions are not completely met the inverse is true (Siegel and Castellan, 1988). Therefore, Non-parametric tests were implemented whenever necessary.

Firstly, it was hypothesized that individuals spent equal time throughout live at the study area. Therefore, the null hypothesis to be tested is: frequencies of re-sight (f) at the breeding and the non-breeding seasons is equal [$H_0: f(\text{Breeding}) = f(\text{Non-breeding})$; $H_1: f(\text{Breeding}) \neq f(\text{Non-breeding})$]. The presence of males ($n=64$) and females ($n=13$) in the breeding and non-breeding seasons in the study area were compared with the Wilcoxon matched pair test. Since individuals were tagged over a large period of time and because tagging times were discrepant

among individuals, re-sights rates were calculated for each fish having in account the date of the first capture. Median times of presence in breeding and non-breeding seasons for males and females were also calculated. Moreover, the time spent by males in incubation was calculated based on records of presence/absence of eggs.

Secondly, it was hypothesized that individuals were random distributed between seasons. Homing, nest-site fidelity was tested using the distance of re-sight of single individuals in two distinct breeding seasons (n=29). To avoid potential biases, each individual only contributed once with a pair of re-sights to the pull. The pair of re-sights was randomly selected. The distances of re-sights were then grouped as following: Sector fidelity, individuals that returned to the same nest [0] or to a place less than 5 meters distant from the previous re-sight]0-5]; Possible sector fidelity, individual that returned to a place that distance 5-15 meters from the previous re-sight]5-10] and]10-15]; Sector change, individual that returned to a place that distance more than 15 meters from the previous re-sight]15-20] and]20-∞[. Classes were defined based on the average perimeter of sectors (see Methods – Subjects and Study Area). Therefore the null hypothesis to be tested here is: the place of re-sight in a given season is independent from the place of re-sight in the next season, that is given by the distance between the two re-sights [H0: [0] =]0-5] =]5-10] =]10-15] =]15-20] =]20-∞[; H1: At least one class is different from the others]. A uniform distribution of data among the various classes was analyzed using the χ^2 test. Comparisons between groups (i.e. Sector fidelity, Possible sector fidelity and Sector change) were performed using the Fisher exact test.

Thirdly, it was hypothesized that homing is influenced by the individual body condition.

The total body length (TL) and weight (W) were used to calculate the condition factor (Kb), which is independent of length and constant for equally nourished fishes (Le Cren, 1951).

$$Kb = \text{weight} \times 100 / (\text{length})^b$$

Where b is a constant. The constant b is determined by calculating the regression line of the logarithmic length weight relationship for the whole population (see appendix III). Therefore, the null hypothesis to be tested is: the Kb of homing and non homing individuals is equal [H0: Kb (Homing) = Kb (Non-homing); H1: Kb (Homing) \neq Kb (Non-homing)]. A t-test was used to search for differences between homing and non-homing males in terms of mean and a F-test to search for differences in terms of variance of Kb values.

Statistical analyses were performed with Statistica v.12 (Statsoft Inc., Tulsa, USA).

Results

During the three years survey 211 fish were captured and tagged: 161 males and 50 females. From those 113 were at least re-sighted once giving a re-sight rate of 60% for males (N=95) and 36% for females (N=18). Re-sight rates within the study area during the breeding and non-breeding seasons showed significant differences for males ($Z= 2.85$, $p<0.01$; Fig. 3A). No significant differences were found for females ($Z=1.29$, $p>0.05$; Fig. 3B). Accordingly, the first null hypothesis [$H_0: f(\text{Breeding}) = f(\text{Non-breeding})$] was rejected for males but not for females. Many males were absent from nests/shelters and tide pools during the non-breeding season (Fig. 3A), suggesting that they move elsewhere. Moreover, during the breeding season, males spent 17 days in average at the study area and 21 days with egg incubation (Fig. 3A and C).

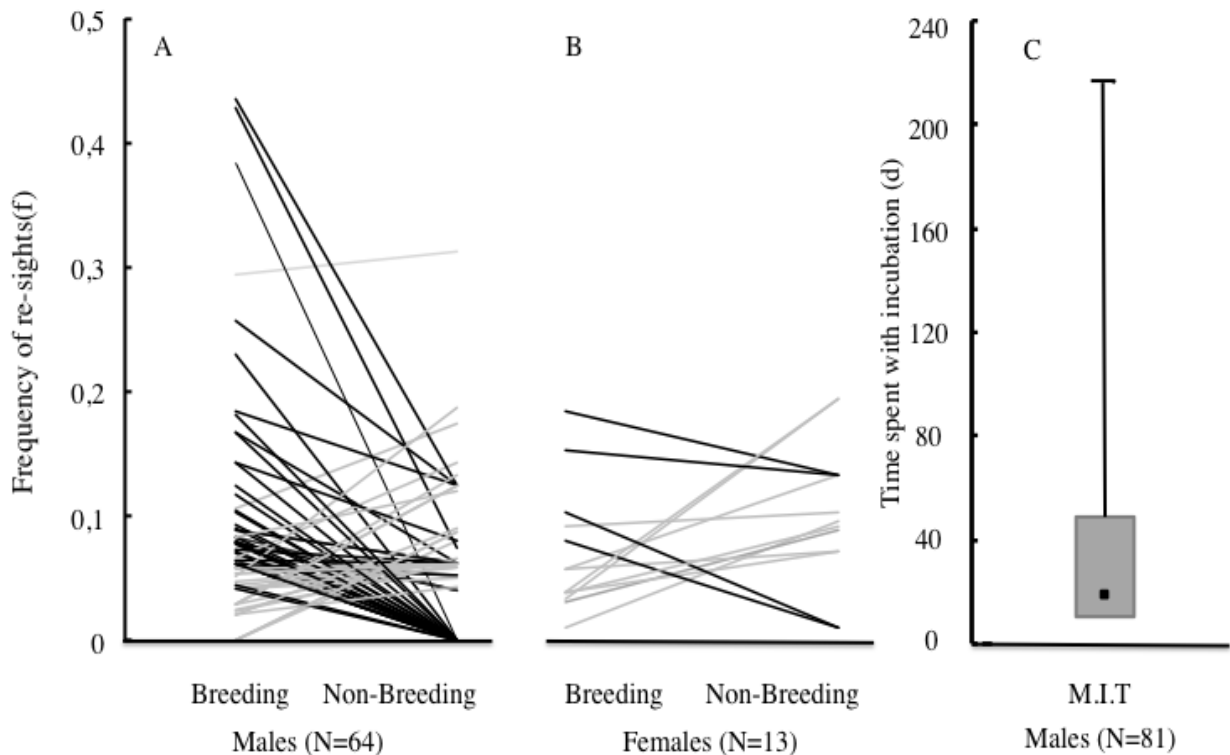


Fig. 3 – Distribution of re-sights by the breeding and non-breeding seasons (**A**) males re-sight frequency; (**B**) females re-sight frequency; Lines represent single individuals. Black lines have a positive slope; grey lines have a negative slope. (**C**) MIT - Male incubation time. Black symbol represents the median value (21 days); box represents the quartiles and; whiskers the maxim and minim values.

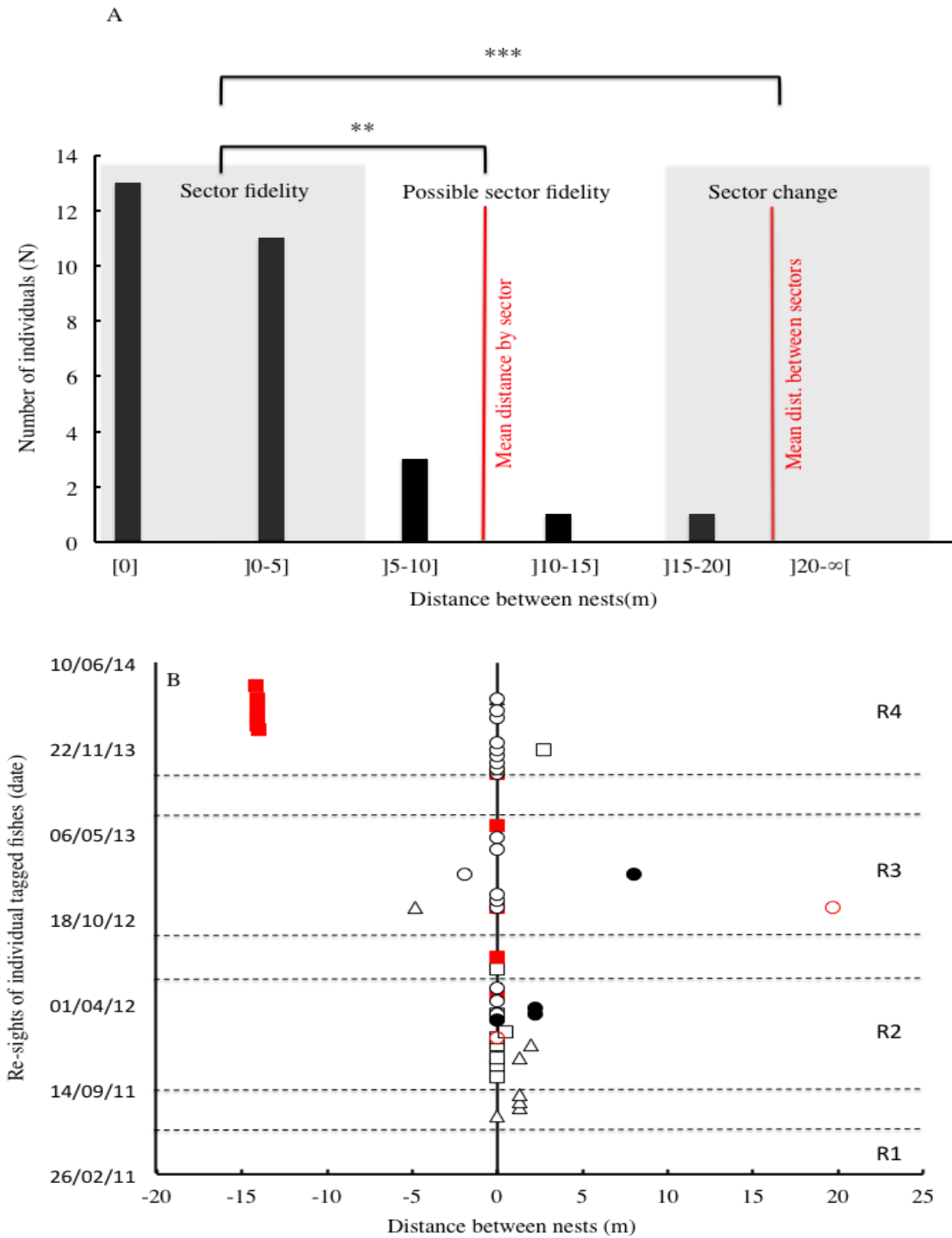


Fig. 4 – Homing in male *L. pholis*. **(A)** Distribution of males re-sighted in successive breeding seasons. Classes of distances (in meters) were aggregated as following: $[0; 5]$ sector fidelity; $]5; 15]$ possible sector fidelity and $]15; \infty[$ sector change. Differences were tested using χ^2 test. Statistical significance is given as follows: $**p < 0.01$; $***p < 0.001$. **(B)** Profile of re-sighting for six representative males that showed sector fidelity (White symbols with black outline - sector fidelity; black symbols and red symbols- possible sector fidelity; white symbols with red outline- sector change). Date is set by the format day / month / year. The banded areas with R's define re-sightings in the breeding season.

Because many males were not present in the study area outside the breeding season (Fig. 3A), it is important to observe whether in successive breeding seasons males return (home) to the same sectors of nests or to the same nest (Fig. 4). Findings show that the distribution of males from one breeding season to the next is not randomly distributed ($\chi^2_{(5)} = 22.4$ $p < 0.001$). Therefore, the second null hypothesis [$H_0: [0] =]0-5] =]5-10] =]10-15] =]15-20] =]20-\infty[$] was rejected by the subpopulation of adult males. Breeding males that arrived to the area in successive breeding seasons had in consideration their previous location (i.e. “sector fidelity” is different from “possible sector fidelity” or “Sector change” Fisher exact test, $p < 0.01$ and $p < 0.001$ respectively, see Fig. 4A). A detailed analysis showed that 45% of the males returned to the same nest (Fig. 4A and see black open circles in Fig. 4B). This number increased to 83% if we consider a 5 meters radius area around the nest of the previous breeding season (Fig. 4A and see black open symbols in Fig. 4B). The remaining 17% of the cases in which the distance calculated may suggest a change of sectors (Fig. 4A), field observation confirm that there is no real change of sectors. Moreover, field records showed that the two individuals that changed sectors might be on the move to their previous nests once they were re-sighted in pools followed by re-sights nearby their previous nests (Fig. 4A and see red symbols in Fig. 4B).

Homing is generally associated with body condition. For this population the regression equation resultant from the logarithmic length weight relationship was:

$$\text{Log } W = -1.8 + 2.9 * \text{Log } TL$$

Where b is equal to 2.9 (Fig. 5A). The comparison of the condition factor K_b between homing and non homing males showed that there was no differences in terms of means ($t = 0.03$, $p > 0.05$; Fig. 5B), but there is differences in terms of variances with homing males presenting much lower variance around the mean value than non-homing males ($F = 1.77$, $p < 0.05$; Fig. 5B). Interestingly, the third null hypothesis [$H_0: K_b (\text{Homing}) = K_b (\text{Non-Homing})$] was not rejected although differences in the variance but not in the mean may suggest that K_b is playing a role in this process.

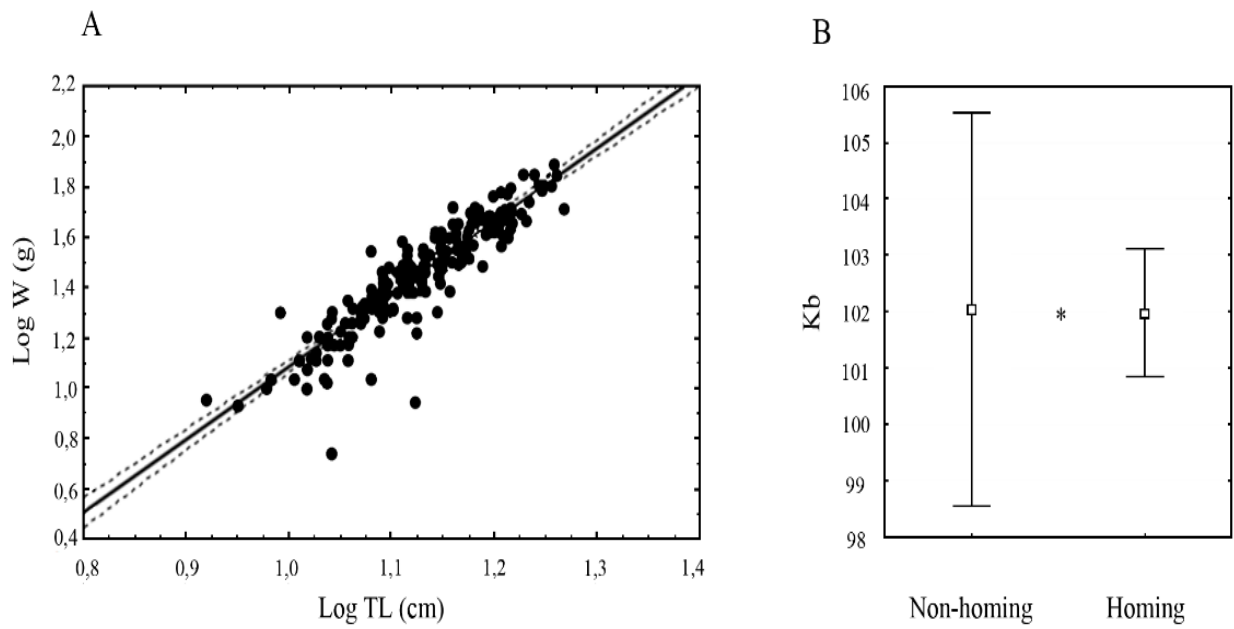


Fig. 5 – Condition factor K_b . Regression line from the logarithmic length (Log TL) weight (Log W) relationship (A). Data is available in Appendix III. Mean (squares) body condition factor (K_b) and the 95% confidence interval of the mean (whiskers) for non-homing and homing males (B). Significant variance is given by the F test as following: * $p < 0.05$.

Discussion

Our results indicated, as in many other animals (Thorrol *et al.*, 2001), homing behavior is an important component of the annual cycle of *L. pholis*. Because it was shown that males of this intertidal fish left the study area during the non-breeding season and returned to the same sector of nests, and in many cases returning to the same nests year after year (Fig. 3A, 4A and B). This behavior represents a striking example of nest-site fidelity. Interestingly, the time spent by males in the breeding area is similar to that spent on incubation (Fig. 3C), suggesting that breeding males do not spend much time searching for appropriated nests and generally prefer to make use of previous known sites. Also relevant is the fact that homing males, in general, had very low variance for the body condition factor (Fig. 5B), suggesting that this is a key feature influencing the return of males in successive breeding seasons.

The return of fish, year after year, to the same breeding sectors, after a more-or-less prolonged absence during the non-breeding season, is very interesting as this represents a first record of this behavior in *L. pholis*. Site fidelity supported by a homing mechanism has obvious implications at the intraspecific level with increased competition associated with territoriality and mating, and vulnerability to density-dependent effects. Consequently, site fidelity may limit mating opportunities, increase the strength of localized competitive interactions (e.g., due to localized depletion of resources), and/or increase vulnerability of individuals to habitat degradation (e.g., Wall and Herler, 2008). At the interspecific level site fidelity as several advantages allowing knowledge on other species that inhabits the same holes and crevices (see Introduction). For example, *L. pholis* released low agonistic responses against *Coryphoblennius galerita*, being frequently ignored (Almada *et al.*, 1990b), but strongly attack crabs (*Carcinus maenas*) with vigorous biting until drive them out from the shelters (Almada *et al.*, 1990b). Thus, philopatric individuals may benefit by breeding with others having similar co-adapted genes well adapted to local conditions (Shields, 1982). The fact that this species present a higher number of agonistic behaviors than sister species corroborates this idea (Francisco *et al.*, 2011).

Long-term data series on re-sighted individuals are the best way to learn about philopatric and migratory behaviors (e.g., Tuttle 1976; Dwyer, 1984). In many species a balance is maintained between the proportions of individuals dispersing and the proportion remaining

philopatric such that inbreeding and resource competition are minimized (Gompper *et al.*, 1998). Findings showed that some males return in successive years while others don't, suggesting that inbreeding, could be maintained low by this process. Indeed, Francisco *et al.* (2006) showed that there is no significant population genetic structure along the Portuguese coast in *L. pholis*. Alternatively, gene flow might be maintained through larval dispersal processes. After hatching, the highly developed larvae may be dispersed along the coastal area with the help of permanent southward surface circulation characteristic of the breeding season time in Portugal. Consequently, biological and behavioral characteristics of larvae may play a role in all this process allowing gene flow and preventing population divergence.

L. pholis have a long breeding season (see Introduction) although, males only spent 21 days on average with incubation per breeding event (Fig. 3C), which raises a number of questions. Is reproduction so costly that males are only able to have one single reproductive event per breeding season? Or, alternatively, do males undergo several reproductive events, but in areas so far apart that this has not yet been observed? Interestingly, the incubation time largely exceeds the larvae development cycle of 15 days (Faria *et al.*, 2001), suggesting that males maximize time incubating more than one single oviposition. Field observations corroborate this idea with several reports of nests with eggs in three distinct stages of the development: egg and embryo with distinct yellowish; egg with white/silvery coloration; hatched egg (see appendix III – Fig.4). On the other hand, empirical data also suggests that a good body-condition factor is a key to males return to the home territory in successive breeding seasons, as too-slim or too-fat males do not generally return. Altogether, these findings highlight that one aspect counting for the short time period spent with incubation is the higher costs it may represent.

Because the time spent by males in the breeding season (17 days) is shorter than the incubation time (Fig. 3B and 3C; and see above), it suggests that many males visit the area without reproducing (short visits). The reasons for that may be several and may be dependent of the physiological state of the animal (Vehrencamp 1979, 1983; West-Eberhard, 1984; Emlen, 1991). Accordingly, males that were not in good shape may opt by leaving the area without reproducing in that breeding season (Fig. 5B), or in contrast they may opt to have a poor reproductive event with consequences that, in the worse scenario, it will cause the individual's death. In all this process shelter/nest is an important resource that individuals have to balance

when taking a decision. In *Salaria pavo* keeping the right nest is crucial for the individual reproductive success (Gonçalves *et al.*, 2003).

Habitat selection is therefore crucial in the life cycle of animals, because it can affect most components of fitness. It has been proposed that some animals cue on the reproductive success of conspecifics to select breeding habitats (Danchin *et al.*, 1998). The fish are one of the groups of the animal kingdom with more reproductive plasticity. Have multiple reproductive phenotypes, since the gonochorism (Gonçalves *et al.*, 1996), through imitation of morphological and behavioral female characters by some male and ending in hermaphroditism with sex changing once in a lifetime (Sunobe and Nakazono, 1993), or even with multiple sex changes throughout life (Sunobe, 1993; Oliveira *et al.*, 1999). This plasticity allows a specific population to adjust their reproductive response in terms of the environment in which it operates.

Colony size is also important in the performance of breeding (Salomon *et al.*, 2008). In some cases the aggregation of nests can also be an advantage in appealing to females, even suggesting a better reproductive display (Faria *et al.*, 2000; Faria and Almada, 1999, 2001; Amorim, 2011). However, breeding in colonies strongly enhance sexual selection and drives species evolution towards speciation (Møller *et al.*, 1998). Although there are costs associated with the increased size of the colony, such high rates of transmission of parasites, higher competition for food and other limiting resources like nests. Factors like breeding density, habitat and nest characteristics and sex have also related to nest-site fidelity (West-Eberhard, 1984; Switzer, 1993; Brockman, 1997; Dubois *et al.*, 1998; Pyle *et al.*, 2001; Beheler *et al.*, 2003). Low food availability may increase competition and inhibit reproduction by some individuals (Clutton-Brock *et al.*, 2001; De Luca and Ginsberg, 2001).

Nest-site fidelity is considered to be an adaptative strategy in numerous groups (Vergara *et al.*, 2006). In birds, also it can result in higher survival rates for adults, and contributing to the maintenance of the social status, however over successive breeding seasons it might increase ectoparasitism (Sonerud, 1987, 1988; Vergara *et al.*, 2006). Nevertheless, the absence of males during the non-breeding season to return in the next breeding season clearly solves this problem (Fig. 2A). In birds, migrant populations have shown to have lower rates of ectoparasites when compared to sedentary populations (Pyle *et al.*, 2001; Vergara *et al.*, 2006). Also affecting nest-site fidelity is the breeding success. Failing in breeding, producing fewer or no offspring, tend to be the most likely reason to change nest-sites in the next breeding season. Within population first

breeders tend to be more affected by this problem than old more experienced breeders (Pyle *et al.*, 2001). Changes of nest are more frequent in young individuals and usually followed by mate changes (Schulz, 1998; Naves *et al.*, 2006).

The Pacific salmon is able to precisely locate the same tributaries and streams of their natal river, to spawn after years feeding offshore (Dittman and Quinn, 1996). Our findings suggest some similarities between the behavior patterns of salmon and *L. pholis*, since each of these species has a very precise homing mechanism and a very good spatial memory. We have reports of males failing to return in the following breeding season, to return 2 years later, to the same nests. This, clearly supports the idea that fishes acquire and memorize large information of the surroundings which, they can recall in successive breeding seasons when they want to pinpoint a specific nest (Braithwaite and Girvan, 2003; Burt de Perera and Guilford, 2008; White and Brown, 2014). The re-sightings of fish invariably found less than ten meters away from the site of the first capture (Fig. 4B), suggests that homing males have a very precise navigational mechanism (Jorge *et al.*, 2011b). Our findings corroborate previous studies, indicating that: 1) *L. pholis* has excellent navigational abilities, being able to accurately determine homeward direction, even in unfamiliar places; 2) *L. pholis* is able to acquire and memorize a large amount of information on the surrounding features, needed for pinpointing specific locations in the home area.

Although we have an area 20 meters long (Fig. 4B), the largest distances between re-sightings found in this work (i.e. 20m) correspond to individuals that were first captured in pools and then found in nests (see red symbols in Fig. 4B). Probably, in those re-sights, the individuals were moving into the area to nest, while in subsequent re-sight they were found in the chose nest. Interestingly, this suggests that fish do not reach the area randomly but instead search for appropriated corridors that lead them to the chose nests. This supports the common idea of high resilience of these fish to a specific spawning area (Faria and Almada, 1999, 2001).

The hypothesis that homing males might have several reproductive events in a given breeding season is, nevertheless, very interesting. If this were the case, then it could be expected that such behavior would enhance the development of a navigational system. It is suggested that while some fish have the ability to use both visual (Burt de Perera and Guilford, 2008) and olfactory marks (White and Brown, 2013; Burt de Perera and Guilford, 2008), the absence of adaptive benefits from its use, simply make them not use these qualities. Fish living in the

intertidal zone have a highly accurate awareness of their geographic location and therefore, an animal's behavior and cognition is shaped by the habitat in which they live (Burt de Perera and Guilford, 2008). For example, sand species of gobies show almost no flexibility to develop spatial learning abilities (White and Brown, 2013). Others can use panoply of environmental cues to learn the position of the refuge (Gibson, 1968; Dodd *et al.*, 2000; Jorge *et al.*, 2011a; White and Brown, 2013).

Altogether, these findings support the idea that navigational mechanisms are widespread in vertebrates and could have either evolved repeatedly in different lineages or it was a legacy of a common ancestor and maintained in various lineages across the different classes (Rodda and Phillips, 1992): from fish (Brannon, 1982; Groot, 1982; Reese, 1989a; Walker *et al.*, 1997) to amphibians (Phillips *et al.*, 1995; Diego-Rasilla *et al.*, 2005), to reptiles (Lohmann *et al.*, 2008), to birds (Nastase, 1982; Jorge *et al.*, 2009; Pledger and Bullen, 1998), to mammals (Holland *et al.*, 2006).

Future perspectives

One of the central questions of this work was to observe whether males, absent from the study area in the non-breeding season, return to the same sectors of nests, or to the same nest in successive breeding seasons, and how these life history traits affect the development of the navigational system. Interestingly, the findings showed that males spent a short time period with incubation leaving the question whether males invest in several reproductive events or alternatively the costs of each reproductive event are so high that they only invest in a single reproductive event per breeding season.

An organism's environment may be thought as everything other than itself that may affect its development, survival, or reproduction. Interactions with environmental factors also affect the spatial distribution of organisms, and therefore their population structure. Understanding the process of colonization of new habitat patches is critical to clarify the proximate mechanisms involved in the distribution of a species and particularly in the formation of breeding aggregations. Colonial breeding is a form of social mating behavior, in which individuals aggregate within densely distributed breeding territories that contain no resources other than nesting sites. It is a widespread phenomenon in animals, but still lacks a consensus on how coloniality has evolved. The oldest explanation for coloniality is that high density reduces nest predation as an answer to dilution effects, group vigilance and communal defense (Götmark & Andersson, 1984; Campagna *et al.*, 1992). Conversely, many examples suggested that predation risk increase with nest density and might result from colonial nests are more conspicuous to predators than solitary nests. (e.g. Tinbergen *et al.*, 1967; Birkhead and Hudson, 1977; Burger, 1987; Götmark and Andersson, 1984; Bellinato & Bogliani, 1995; Brown, 1999). Contributing to conspicuity of colonial nests are the higher rates of acoustic, visual and olfactory cues emanating from them, leading to higher attack rates in colonies (Götmark and Andersson, 1984; Varela, 2007; Chapman *et al.*, 2011). Therefore, for future work is important to observe the function of the agglomeration of nests, and how they impact the courtship display and the reproductive success. The effect of individual's behavior on their fitness often depends on what other members of the population are doing. In this instance, the fitness associated with a particular trait is frequency-dependent, it depends on the traits of at least some other members of the population and the optimization of this theory uses the concept of an evolutionarily stable strategy. We need

to test whether breeding failure or productivity in the previous breeding season is affecting nest-site fidelity. If breeding failure does not affect nest-site fidelity, we corroborate that with high rates of recaptures, as obtained in this work.

Another interesting question to answer is whether larvae are able to imprint on features from the natal area and return as adults to the same places. Using microsatellites and paternity tests we intend to test whether future breeding males in the area are descendants from males that presently breed there. Thus may be able to confirm if inbreeding is minimized and the proportion of individuals dispersing and remaining philopatric is balanced.

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APPENDIX I

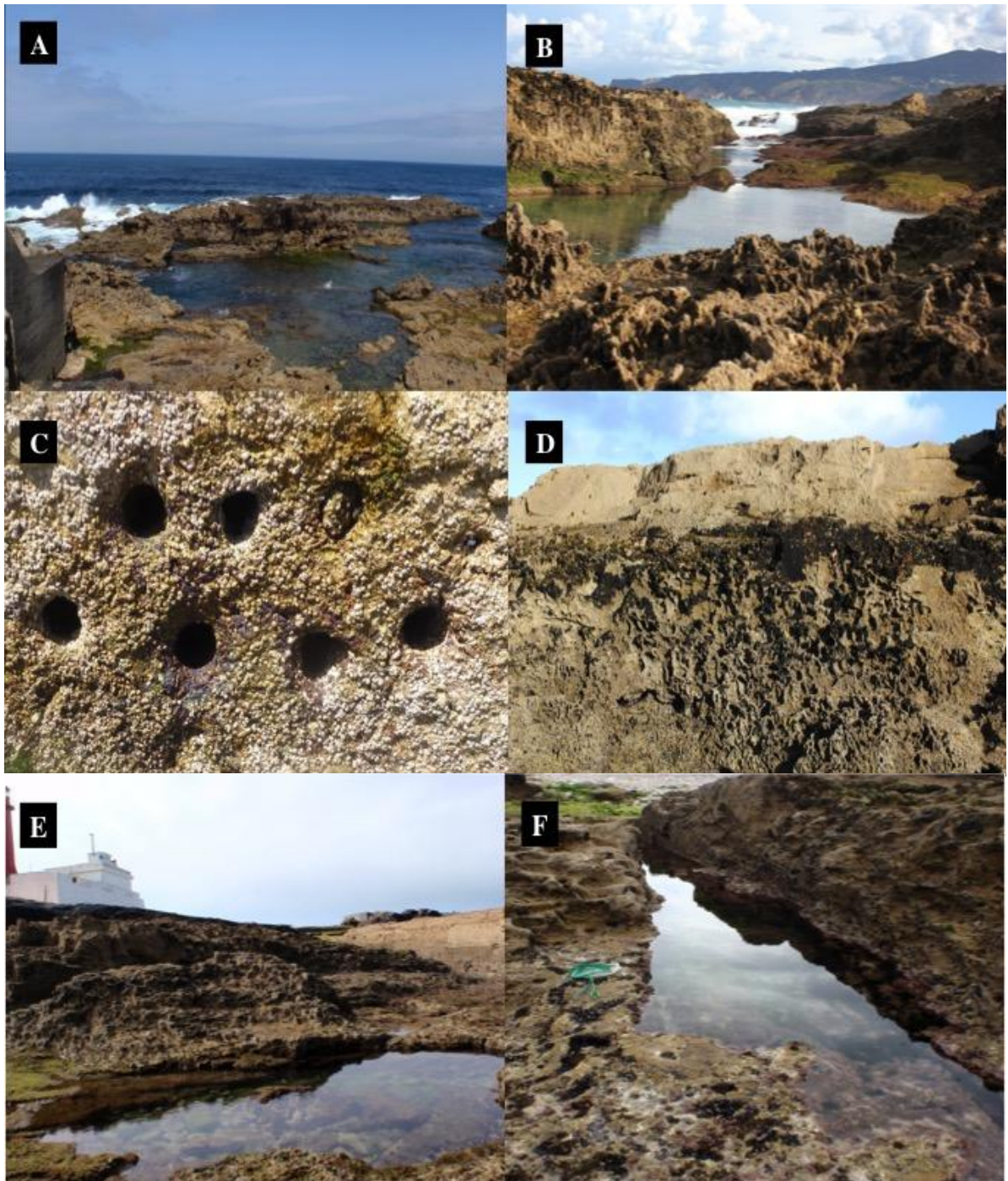


Fig. 1 – Overview of sectores. (A) Enlarged view of sectors between 1 and 6; (B) Enlarged view of sectors 6 and 7; (C) Structuring of the nests in sector 11; (D) Wall with shelters in sector 2; (E) Pool B (F) Pool C.



Fig. 2 – Tagging procedures: Sighting (A); Capture (B); Record conditions of development of eggs (C); anaesthetization, surgery (D), recovery (F) and release (G). Determining total and standard length while fish were anaesthetized (E); Fish went through surgery to introduce an electronic tag (IA-100A, TROVAN) (D); Fish placed in an aerated tank until complete recovery (F).

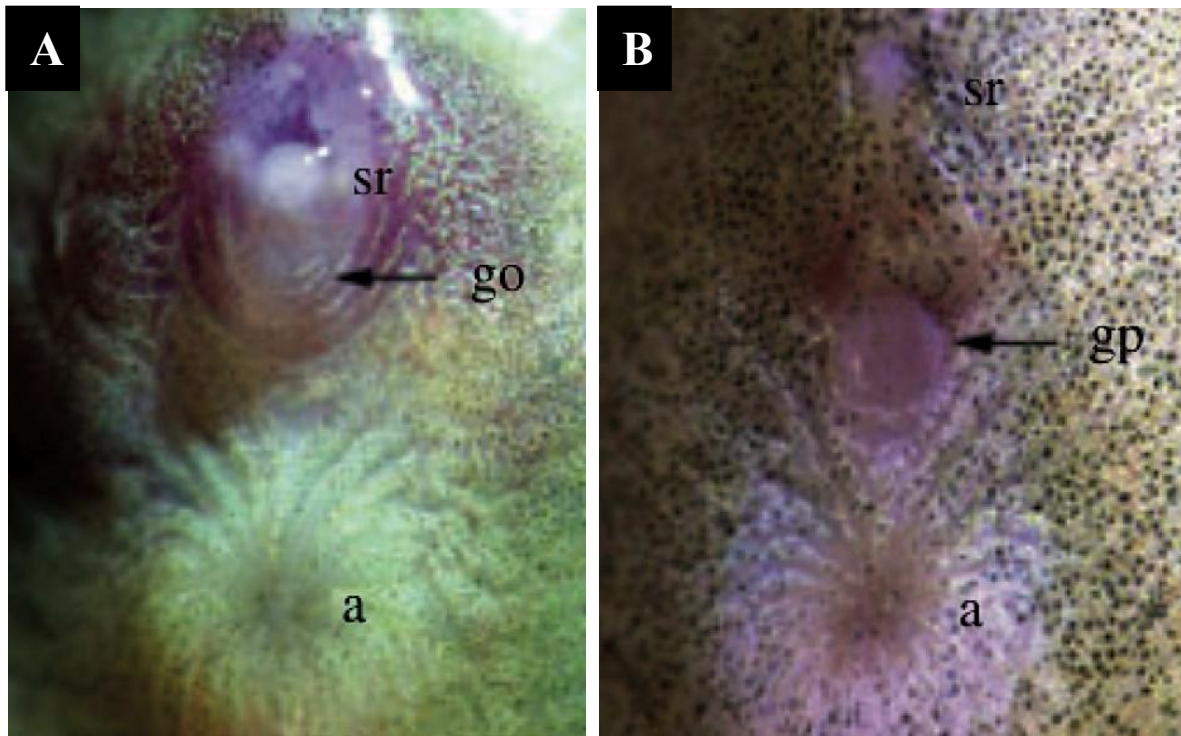


Fig. 3 - The external morphology of *L. pholis*: the male's genital papilla was located about midway from the anus to the first soft anal ray and was characterized by a globular protuberance (A). The female's genital papilla, however, was located closer to the first soft ray, which was largely embedded in dermal tissue, with only the tip of the ray remaining visible (B). In both views of the external morphology of the ano-genital area (a) anus; (sr) tip of first soft anal ray and (gp) genital opening. (Adapted from Ferreira et al., 2010).

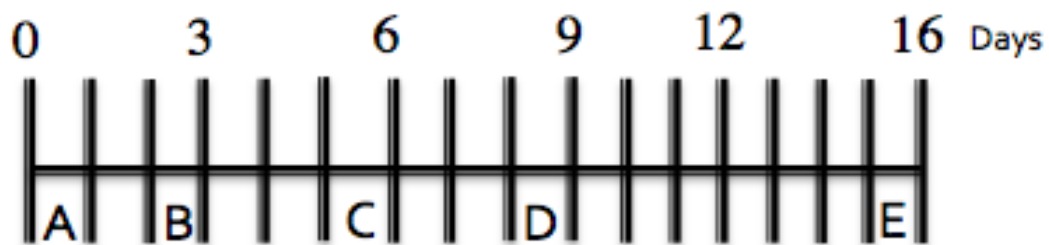


Fig.4 - Events of embryonic development of *Lipophrys pholis*: Day 1- Blastocist (A); Day 2-Embryo recognizable (B); Day 5- Embryo almost reaching the margin of the yolk (C); Day 8- Embryo longer than egg major axis (D); Day 16- Hatching (E); Above, the numbers indicated the total number of days that the eggs take to hatch. In field eggs are in three distinct stages of the development: egg and embryo with distinct yellowish (B); Egg with white/silvery coloration (D); Hatched egg (E) (Adapted from Faria et al., 2000).

APPENDIX II

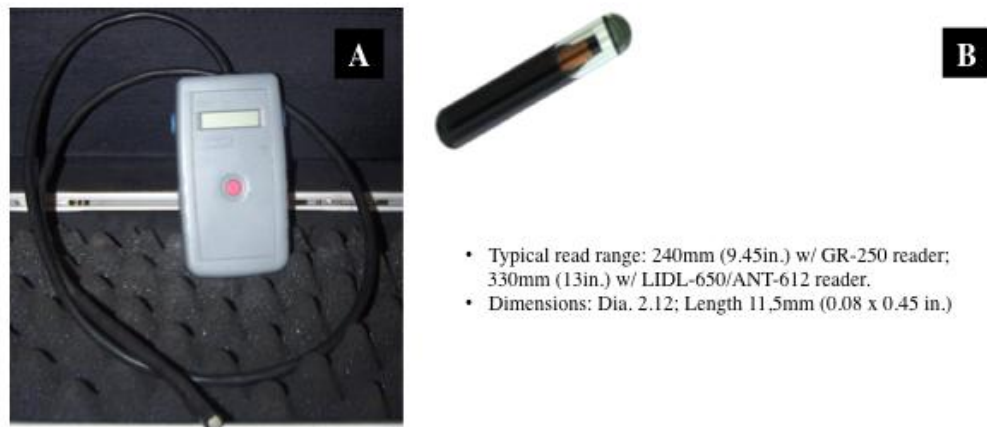


Fig. 1 – Microtransponder reader (LID-560 Pocket Reader, TROVAN) adapted to a flexible cable (1cm of diameter)(A). Electronic tags (ID100-A T.CRISTAL ISO FDXA)(B)

APPENDIX III

Statistical analysis – Tests of Normality

The Tests of Normality tables shows the Kolmogorov-Smirnov test with Lilliefors correction and adjustment yet another test developed especially for normal law, the Shapiro-Wilk test. For samples of size greater than or equal to 30 advises the Kolmogorov-Smirnov test with Lilliefors correction; for samples of smaller size is more suitable to the Shapiro-Wilk test. The Kolmogorov-Smirnov easily accepts the H0 hypothesis that the Kolmogorov-Smirnov test with Lilliefors correction.

Table 1 – *Test of Normality table for distances between sectors of the study area.*

	Shapiro-Wilk		
	Statistic	df	Sig.
Distances (meters) between sectors	0,646	29	0,000001

Table 2 – *Test of Normality table for weights from both sexes of L. pholis.*

	Kolmogorov-Smirnov ^a		
	Statistic	df	Sig.
Weights (grams) from both sexes	0,100	119	N.S.

Table 3 – *Test of Normality table with total lengths (cm) from females and males of L. pholis.*

	Kolmogorov-Smirnov ^a		
	Statistic	df	Sig.
Total lengths (centimeters) from both sexes	0,052	119	N.S.

^a Lilliefors Significance Correction

Table 4 – Test of Normality table with total lengths (cm) and weights for males of *L. pholis* with homing and non-homing.

Shapiro-Wilk			
	Statistic	df	Sig.
Weight (g) in homing and non-homing males	0,959	144	0,000001
Total lengths (cm) in homing and non-homing males	0,985	144	N.S.

Table 5 – Table with the Total lengths (cm), weights (g), sex discrimination (N=190) and definition of individuals who return two successive breeding seasons (N=26). Totalizing 127 marks in males that may or may not be recaptured but are not sighted two successive breeding seasons.

Individual	Sex	TL (Total length)	W (weight)	Successive breeding seasons
074D_1A67	M	16	49	
074D_1B3E	F	16	50	
074D_1C16	M	14	42	
074D_210A	M	13,2	30	
074D_217A	M	12,8	27	
074D_2438	M	15,4	47	
074D_24C3	M	14,5	40	
074D_24C5	M	13,7	34	
074D_271A	F	12,9	31	
074D_27B6	M	11,9	22	
074D_28ED	F	12,2	17	
074D_2989	M	12,9	29	
074D_2C08	M	16,3	60	
074D_3811	M	14	30	
074D_3B8E	M	14	35	
074D_3CF8	M	13	28	
074D_3F20	F	14	38	
074D_436F	M	16,1	37	
074D_442C	M	13	36	
074D_44E3	M	16,1	61	
074D_4B5A	M	12,3	21	
074D_4E35	M	11,5	21	
074D_505A	F	13,2	30	
Individual	Sex	TL	W	Successive
074D_505E	M	18,2	71	
074D_5279	M	18,1	77	
074D_5495	M	12,5	26	
074D_5496	M	12,7	29	
074D_58DE	M	17,3	71	
074D_5A42	M	16,4	63	
074D_5C72	M	16,3	51	
074D_5F42	M	13	34	
074D_60AB	F	13,8	40	
074D_6217	F	12,3	19	
074D_6585	M	15,3	52	
074D_68E4	M	12,9	38,5	
074D_6993	M	15,5	47	
074D_6AD9	M	11,3	18	
074D_6ADC	M	12,9	29	
074D_6ADE	M	14,7	36	
074D_6ADF	M	12,2	24	
074D_6E16	M	13,1	26	
074D_7095	M	13,5	30	
074D_7D2F	M	15,8	47	
074D_7E21	M	14,4	32	
074D_7F8E	M	12	25	
06DE_39F1	F	11	5,5	
06DE_94CD	M	16,1	50,5	

06DE_9884	F	15	50	
06DE_A8F4	M	16,4	44	
06DE_C810	M	11,7	21	X
06DE_AA8A	M	18	64	
06DE_EFF3	M	13	28,2	
06DF_0DFC	F	10,9	10,5	
06DF_0EC7	F	12	21	
06DF_0E61	M	13,8	31,4	X
06DF_0F2E	M	9,8	20	
06DF_0F31	M	11,7	18	
06DF_0F32	M	10,9	13	
06DF_0F9A	M	13,9	20	
06DF_100B	F	14,1	32	
06DF_1071	M	16,2	47	
06DF_1149	?	12,4	23	
06DF_114D	M	11,4	18	
06DF_11B5	M	13,5	26	X
06DF_11B9	M	16,3	60	X
06DF_1222	M	12	23	
06DF_1227	M	17	47	
06DF_1291	M	15,8	59	
06DF_13D0	M	13,4	30	X
06DF_1369	M	10,6	13	
06DF_143E	M	11,4	18	
06DF_143F	M	11,2	15	
06DF_14B	F	10,8	11	
06DF_14B1	F	11,8	21,5	
06DF_1530	M	11,4	13	
06DF_162D	F	12,5	24	
06DF_1634	M	13,5	27	
06DF_172E	F	11,5	21	
06DF_1732	M	16,1	41,5	
06DF_17AE	F	14,9	40	
06DF_17B3	M	10,9	15	
06DF_1831	M	16,2	52	
06DF_18B4	M	14,7	33,5	
06DF_18B6	M	14,9	36	
06DF_1AC7	M	17,7	64,5	X
06DF_1AC9	M	17,5	67	X
06DF_1B53	M	14	39	
06DF_1BD9	F	15	45	
06DF_1BDB	F	11,8	19	
06DF_1C5C	F	11,7	19,5	

06DF_1D6B	M	13,9	28	
06DF_1D6D	M	9,5	10	X
06DF_1D6E	M	15,5	48	
06DF_1DFD	M	13	19	X
06DF_1E7F	M	13,1	27,5	
06DF_1F04	F	13,3	16,5	
06DF_2010	F	13,5	36	
06DF_2073	M	17,4	64,5	
06DF_2097	F	13,2	24	
06DF_211A	M	12	21	
06DF_211E	M	14,5	44	X
06DF_222B	F	11,4	16	
06DF_2334	M	12,8	27,5	X
06DF_2335	M	10,2	13	
06DF_2442	F	14	35	
06DF_2449	M	15,6	45	
06DF_244D	M	12,8	26	X
06DF_24C9	F	13,6	31,5	
06DF_24D1	M	13,3	19	
06DF_26ED	M	13,6	32,7	
06DF_26F0	F	11	19	
06DF_26F1	M	16	46	
06DF_266D	M	12,3	31	X
06DF_2663	M	12,4	23,1	X
06DF_2773	M	18,5	52	
06DF_2778	M	15,5	41	
06DF_27FC	M	15	33	
06DF_27FE	M	16,1	45,5	
06DF_2887	M	14,6	45	
06DF_2911	M	15,4	30,5	
06DF_299C	M	16,4	53	
06DF_2A20	M	14,1	39	
06DF_2A23	M	12,7	28	X
06DF_2A27	M	14,2	35	
06DF_2A2D	F	11	19	
06DF_2AB5	M	14,1	31	
06DF_2ABB	M	11,4	22,5	
06DF_2B37	M	12,7	21	X
06DF_2B44	M	14,2	32,5	
06DF_2C57	M	13,5	31	X
06DF_2C58	M	13,8	42	
06DF_2C61	M	10,1	11	
06DF_2CB3	M	12,4	21	

06DF_2D73	M	17,1	59	X
06DF_2D76	M	17,1	56	
06DF_2DFC	M	12,9	26	
06DF_2E02	M	13,9	30	X
06DF_2E88	M	14,5	36	X
06DF_2E8C	F	14,6	36	
06DF_2FA0	M	16	45,5	
06DF_3026	F	12,5	20	
06DF_302A	M	15,7	45	
06DF_302D	M	13,9	31	
06DF_313B	M	14	26	
06DF_313D	M	15,6	41	
06DF_31C1	F	11,4	15	
06DF_324A	M	15,8	42	
06DF_32D4	F	11,1	15	
06DF_3356	M	11	15	
06DF_3357	F	13,6	24,5	
06DF_3359	M	13,1	26	
06DF_33DB	M	11,5	18	
06DF_33DE	M	16,5	45	X
06DF_33E0	M	14	28	X
06DF_34E8	M	12,2	22,5	
06DF_34EC	F	12,6	21	
06DF_356B	M	12	21,5	X
06DF_35F3	F	12,2	21	
06DF_35F6	M	14,9	41	X
06DF_3644	F	14,6	31	
06DF_366F	M	15,3	50	
06DF_3676	F	14,9	37	
06DF_36F0	M	12,6	20,5	

06DF_36F3	F	10,6	14	
06DF_36F8	F	14	31,5	
06DF_3773	F	12,3	29	
06DF_37F6	F	10,9	15,5	
06DF_3872	M	14,3	24,5	
06DF_38EF	F	12	21	
06DF_38F0	M	13,1	31	
06DF_38F7	M	13,6	29	
06DF_3970	M	12,9	25	
06DF_39E7	M	11,5	16	
06DF_3A63	M	10,4	16	
06DF_3AD9	M	11,8	20,5	
06DF_3ADD	M	13,4	27,1	
06DF_3ADE	M	16,5	50	
06DF_3ADF	M	16,4	43,66	
06DF_3B57	M	15,2	45,5	
06DF_3B58	M	13,4	24,5	X
06DF_3BD0	M	13	29,5	
06DF_3C43	F	10,9	16	
06DF_3C44	F	14,3	40	
06DF_3CB3	M	14,4	45	X
06DF_3D26	F	12,2	20	
06DF_3D28	M	10,5	13	
06DF_3E04	M	12	11	
06DF_3E75	M	14,1	30	
06DF_5DFF	M	10,4	10	
0713_5DAF	M	11,2	17	