

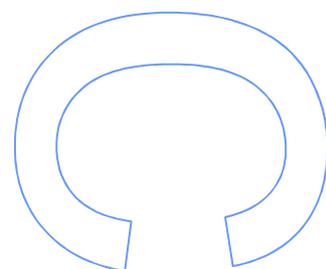
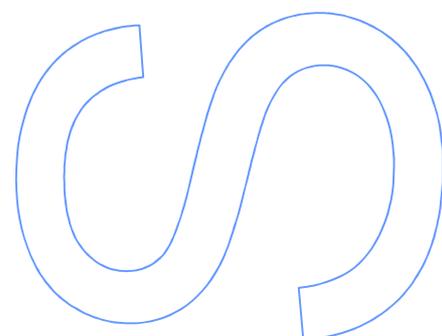
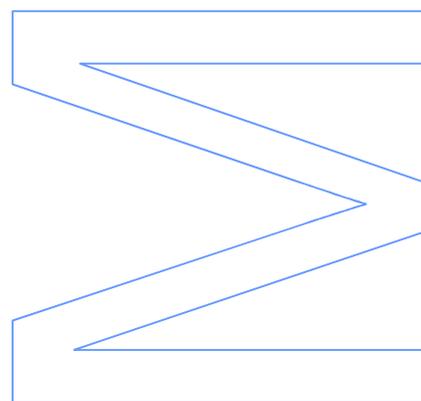
Ectoparasites of Atlantic mackerels (*Scomber colias* and *S. scombrus*): biological tags and macro and microhabitats dynamics

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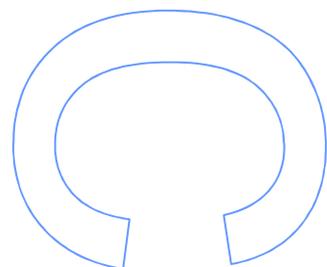
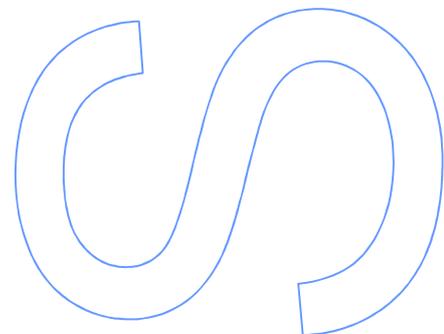
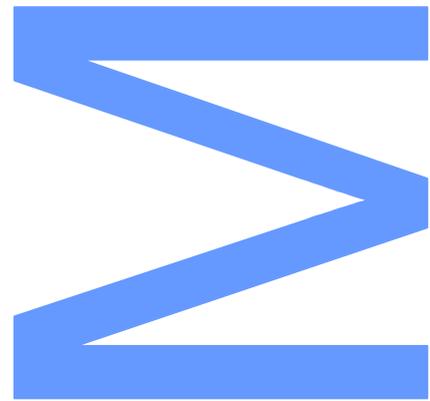
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Abstract

Atlantic chub mackerel, *Scomber colias* Gmelin, 1789, and Atlantic mackerel, *Scomber scombrus* L., 1758, (Teleostei: Scombridae) are two fish species inhabiting the Atlantic Ocean coastal waters, very important to the Portuguese and EU fisheries. The species identification and differentiation when entering the commercial circuit present some difficulties due to their similar morphology and partial habitat overlap. Despite not being present in the identification keys for mackerels, the empirical knowledge of fishermen refers the eye size of *S. colias* and *S. scombrus* as a good characteristic to differentiate both species, as the former presents a larger eye than the later. Furthermore, parasites of these species can be used as biological tags to differentiate both hosts. This study aims to verify the usefulness of these two solutions to easily solve the said misidentification problem. For this, fish obtained from commercial catches from northern Portuguese waters were analyzed. It was verified that the eyes of *S. colias* were significantly larger ($\approx 1.66\times$) than those from *S. scombrus* of the same size. Additionally, four ectoparasitic species were selected as good biological tags to distinguish between these two mackerels: *Pseudokuhnia minor* was recorded only for *S. colias* (97.5% prevalence and mean abundance of 23.95 worms/fish); *Caligus pelamydis* was recorded only for *S. scombrus* (69.0% prevalence and mean abundance of 3.12 copepods/fish); and *Grubea cochlear* and *Kuhnia scombri* were recorded for both fish, but presented significantly higher prevalence and abundance for *S. scombrus* (28.6% and 66.7% prevalence and mean abundance of 0.4 and 2.0 worms/fish, respectively), than for *S. colias* (2.5% and 17.5% prevalence and mean abundance of 0.03 and 0.5 worms/fish, respectively). Taking into account these results, the eye size and the macro-ectoparasites of *S. colias* and *S. scombrus* can be useful to successfully identify and differentiate both Atlantic mackerel species.

Parasites, especially ectoparasites, are affected by the environment that their hosts occupy. Also, they show a specific distribution among their hosts, living in a well-defined and restricted microhabitat. Apart from the above mentioned goals, this study also aims to describe the *S. scombrus*' ectoparasite fauna dynamics at the macro and microhabitat levels. Fish from Matosinhos and Figueira da Foz (two different Portuguese coastal regions) were examined for macro-ectoparasites. Differences in parasites' infection levels from fish of both regions were found, indicating different preferences in macrohabitat conditions. Regarding the microhabitat of the ectoparasites reported, it could be seen that every species have a very specific distribution within the host. No interspecific interactions were found among the

component species, which supports the idea that the driving forces of community structure of parasites are not the interspecific competitions, but the reinforcement of reproductive barriers and thus, enhancement of the chance to mate.

Keywords: *S. colias*; *S. scombrus*; ectoparasites; fish discrimination; biological tags; macrohabitat and microhabitat distribution; reproductive barriers.

Resumo

A cavala, *Scomber colias* Gmelin, 1789, e a sarda, *Scomber scombrus* L., 1758, (Teleostei: Scombridae) são duas espécies de peixe que habitam as águas costeiras do Oceano Atlântico, constituindo uma importante fonte de rendimento no setor pesqueiro português e europeu. A identificação e diferenciação das duas espécies, aquando da sua entrada no circuito comercial, apresentam algumas dificuldades devido à sua similaridade morfológica e à sobreposição parcial do seu habitat. Apesar de não estar presente nas chaves de identificação de scombrídeos, o conhecimento empírico dos pescadores indica que o tamanho do olho de *S. colias* e *S. scombrus* é uma boa característica para diferenciar as duas espécies, já que a primeira espécie apresenta um olho maior que a segunda. Para além disso, os parasitas destas espécies podem ser usados como marcadores biológicos para distinguir os dois peixes. Este estudo tem como objetivo verificar a utilidade destas duas soluções para facilmente resolver o já mencionado problema de identificação. Para isso, foi analisado peixe proveniente de capturas comerciais de Matosinhos. Foi verificado que os olhos de *S. colias* são significativamente maiores ($\approx 1.66\times$) que os de *S. scombrus* do mesmo tamanho. Adicionalmente, quatro espécies ectoparasitas foram selecionadas como sendo bons marcadores biológicos na distinção das duas espécies: *Pseudokuhnia minor* foi registada apenas na cavala (97.5% de prevalência e abundância média de 23.95 vermes/peixe); *Caligus pelamydis* foi registado apenas na sarda (69.0% de prevalência e abundância média de 3.12 copépodes/peixe); *Grubea cochlear* e *Kuhnia scombri* foram registadas em ambos os peixes, mas apresentaram prevalência e abundância significativamente maiores para a sarda, *S. scombrus* (28.6 e 66.7% de prevalência e abundância média de 0.4 e 2.0 vermes/peixe, respetivamente) do que para a cavala, *S. colias* (2.5 e 17.5% de prevalência e abundância média de 0.03 e 0.5 vermes/peixe, respetivamente). Tendo em conta estes resultados, o tamanho do olho e os macro-ectoparasitas de *S. colias* e *S. scombrus* podem ser úteis para identificar e distinguir com sucesso as duas espécies.

Os parasitas, especialmente os ectoparasitas, são afetados pelo ambiente que os seus hospedeiros ocupam. Estes apresentam também uma distribuição específica dentro dos hospedeiros, vivendo num microhabitat restrito e bem definido. Para além dos objetivos já mencionados, este estudo tem como propósito descrever a dinâmica da fauna ectoparasítica de *S. scombrus* ao nível do macro e microhabitats. Peixe proveniente de Matosinhos e da Figueira da Foz foi sujeito a um exame parasitológico. Foram encontradas diferenças significativas nos níveis de infeção de várias espécies

parasitas, o que pode indicar diferentes preferências em relação às condições do macrohabitat. No que diz respeito ao microhabitat dos parasitas reportados, pode ser visto que todas as espécies têm uma distribuição específica no hospedeiro. Não foram encontradas relações interespecíficas dentro das espécies componentes, o que suporta a ideia de que a estrutura da comunidade parasítica não se deve à competição interespecífica, mas sim ao reforço das barreiras reprodutivas e, conseqüentemente, ao aumento das hipóteses de acasalamento e reprodução.

Palavras-chave: *S. colias*; *S. scombrus*; ectoparasitas; distinção de espécies; marcadores biológicos; distribuição no macro e microhabitats; barreiras reprodutivas.

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Abbreviations

C – *Scomber colias*

ED – Eye diameter

D.F. – Degrees of freedom

DFA – Discriminant function analysis

g – Gills

i – Integument

K-W – Kruskal-Wallis test

M-W – Mann-Whitney's U-test

o – Opercula

pb – Pseudobranches

PD – Pupil diameter

poc – Preopercular channels

S – *Scomber scombrus*

s.d. – Standard deviation

χ^2 – Chi-square test value

Chapter 1

General Introduction

1. General Introduction

“Few people realize that there are far more kinds of parasitic than nonparasitic organisms in the world.”

Roberts and Janovy, 2009

Parasitism is a symbiotic relationship between two organisms in which one, the parasite, depends on the other, the host, harming and taking some benefit from it (Rohde, 2005). Technically, this definition of parasite includes many bacteria, viruses and fungi, but traditionally, these groups are the object of study of Microbiology. In this work, the strict definition of parasite, that include only protistan and metazoan parasites will be considered. Parasites can be classified according to its location in the host: if the organisms live on the host's surface, they are called ectoparasites; if they live internally in the host, they are endoparasites (Roberts and Janovy, 2009). As can be seen, parasites cannot be studied without knowing the hosts in which they live. This study will focus only on ectoparasites of two host species from the genus *Scomber*: *Scomber colias* and *Scomber scombrus*.

1.1. Genus *Scomber*

Presently, the genus *Scomber* Linnaeus, 1758 comprises only 4 valid species; however, more than 124 species were described inside this taxon (Froese and Pauly, 2012). The later were mainly new descriptions (done between the XVIII and XIX centuries), whose names either declined - and are now synonymies of *Scomber colias* Gmelin, 1789 (Atlantic chub mackerel), *S. japonicus* Houttuyn, 1782 (chub mackerel), *S. australasicus* Cuvier, 1832 (blue mackerel) or *S. scombrus* Linnaeus, 1758 (Atlantic mackerel) - or were moved to a different genus inside the families Scombridae or Carangidae. Recently, the species *S. japonicus*, traditionally recognized as a cosmopolitan species, was split in 2. Moreover, the fish living in Indo-Pacific waters kept the name *S. japonicus*, whereas those inhabiting Atlantic Ocean waters and related seas were assigned as *S. colias*. The latter species, already named and described in 1789, has been re-erected in the XXI century, in the sequence of recent molecular studies (Scoles et al., 1998; Infante et al., 2007; Catanese et al., 2010).

Fish from the genus *Scomber* present an elongate rounded body covered with small scales. Front and hind margins of the eyes are covered by an adipose eyelid (Collette, 1986). The most used fishing gears to catch mackerels are the purse seines and the pelagic pair trawls (Collette and Nauen, 1983; Cochrane, 2002). They are marketed fresh, frozen, canned, smoked and salted (Collette and Nauen, 1983).

1.1.1. *Scomber colias*

Atlantic chub mackerel, *S. colias* (Figure 1), is a coastal pelagic fish species, occurring at depths of 250-300 meters. It shows schooling and migratory behaviors (Collette and Nauen, 1983; Collette, 1986). The diet of adults ranges from copepods and other invertebrates to small pelagic fishes and squids. Moreover, this species plays a key role in the food web of the Atlantic Ocean ecosystems, being an essential element of the diet of larger pelagic fishes and mammals (Zardoya et al., 2004). Concerning the *S. colias* morphological distinctive characteristics, it shows 9 or 10 spines in the first dorsal fin, the space between the first and the second dorsal fin is approximately equal to the length of the first dorsal fin, the swimbladder is present and, finally, the belly is clearly marked with spotting (Collette and Nauen, 1983; Collette, 1986).

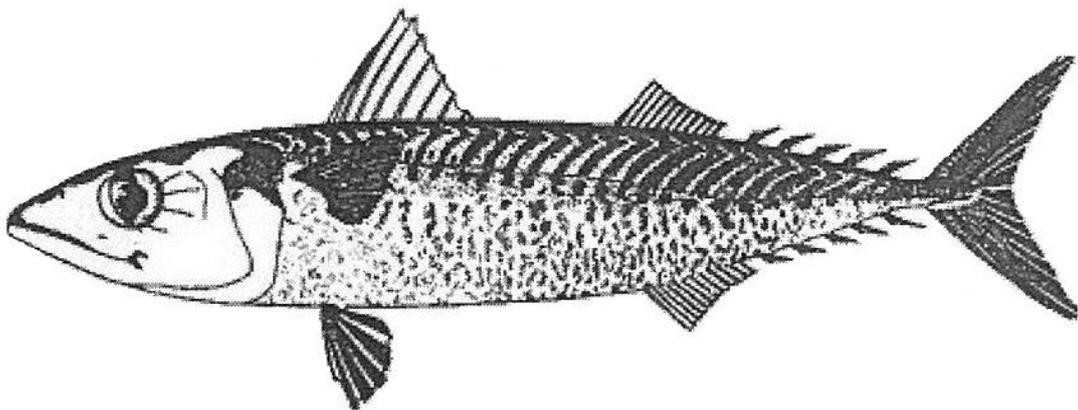


Figure 1. Atlantic chub mackerel, *Scomber colias*. (Adapted from Collette, 1986).

The distribution of *S. colias* can be seen on Figure 2. This species occupies the warm eastern and western coastal areas of the Atlantic Ocean and the adjacent seas (Scoles et al., 1998; Velasco et al., 2011). Atlantic chub mackerel is an important species for fisheries in Portugal, being the second most fished species in this country, with a total of 30,457 tons in 2011 (DGRM, 2012).

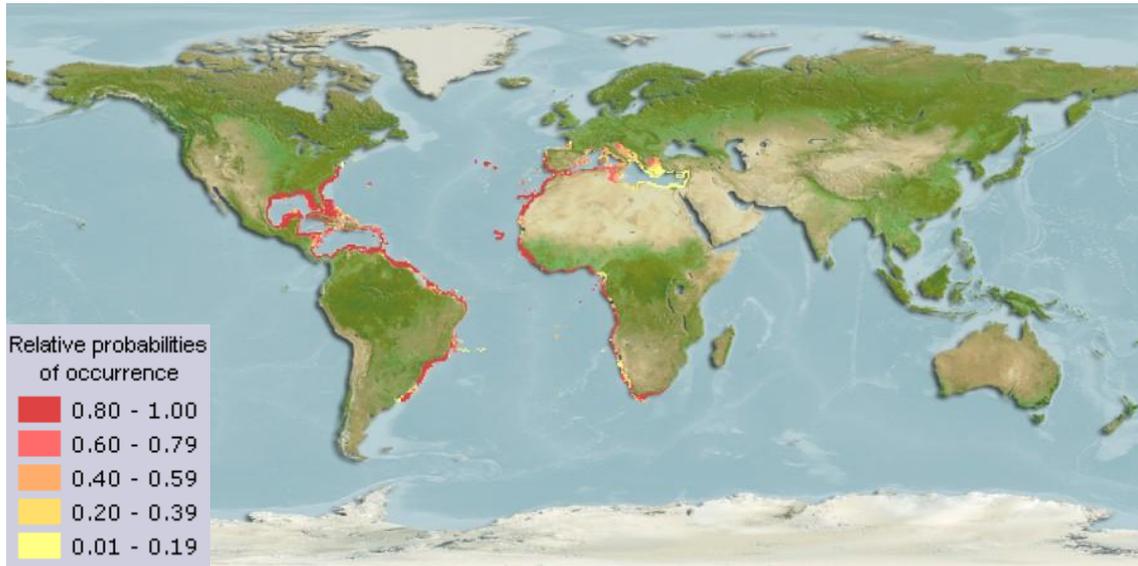


Figure 2. Distribution map for *Scomber colias* showing the relative probability of occurrence for each region. (Adapted from Aquamaps, 2012a).

1.1.2. *Scomber scombrus*

Just like *S. colias*, Atlantic mackerel, *S. scombrus*, is a coastal pelagic fish species, but occurring in lower depths, ranging from 200 to 250 meters (Collette and Nauen, 1983) (Figure 3). Fish from this species also show schooling and migratory behaviors (Collette, 1986). Regarding the diet of *S. scombrus*, they feed mainly on pelagic crustaceans, but also crab larvae, amphipods and young fishes (Collette, 1986; Zardoya et al., 2004). The characteristics that differentiate this *Scomber* species are the number of spines in the first dorsal fin (11 to 13), the space between the first dorsal fin and the second dorsal fin clearly greater than the length of the former, the swimbladder is absent and the unmarked belly (Collette and Nauen, 1983; Collette, 1986).

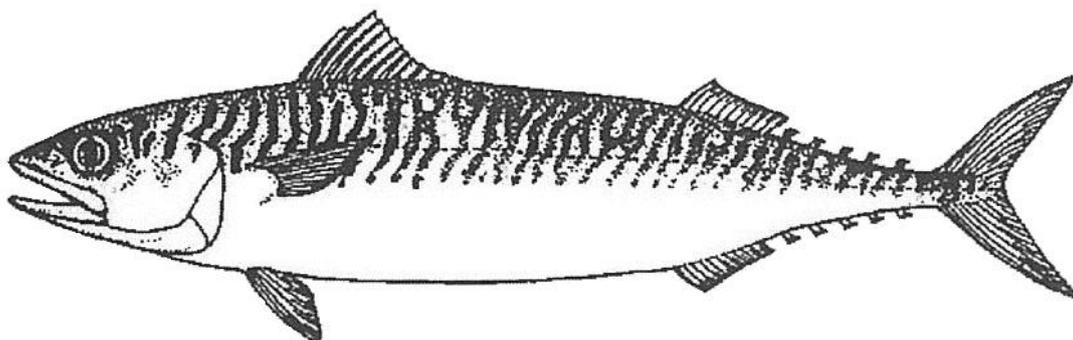


Figure 3. Atlantic mackerel, *Scomber scombrus*. (Adapted from Collette, 1986).

Figure 4 shows the distribution of *S. scombrus*. It can be seen that it has a northern distribution, compared with *S. colias*. Some overlap of the two species exists in the Mediterranean Sea, Southwest of Europe and North of Africa (Scoles et al., 1998; Velasco et al., 2011). In 2011, 959 tons of *S. colias* were fished by Portuguese fisheries (DGRM, 2012).

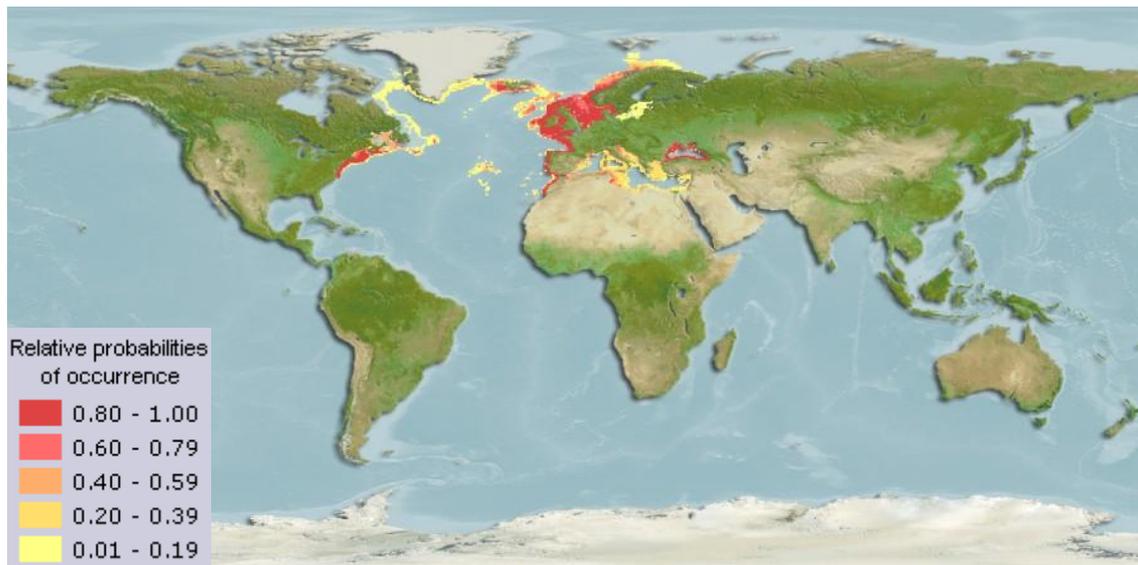


Figure 4. Distribution map for *Scomber scombrus* showing the relative probability of occurrence for each region. (Adapted from Aquamaps, 2012b).

1.1.3. Misidentification problem

Despite the small morphological differences between *S. colias* and *S. scombrus*, these two species can be easily misidentified and mistakenly assigned as the other species, at fishery landings. Matsui (1967) and Scoles et al. (1998) showed that *S. colias* presents phenotypic variations, among them, the existence of individuals with an unmarked belly (which is an important characteristic for the distinction of mackerels). Moreover, due to the non-selective fish gear used to catch mackerels, they are caught together (Cochrane, 2002). For instance, Cissé and Belghyti (2004) reported that, in Morocco fishery landings, mackerels are only identified as *Scomber*, without identification to the species level.

In terms of fisheries management and in setting fish quotas among the members of the European Union, the correct identification of the species is important (Begg et al., 1999; Abaunza et al., 2008; Gordo et al., 2009). In order to achieve this, alternative methods must be employed. One of them is used empirically by the

fisherman and consists in the observation of the fishes' eye size: the eyes of *S. colias* are bigger than the ones of *S. scombrus*. This method is also applied, with the same purpose, by Erguden et al. (2009). Although the usefulness of this method to identify these two mackerels, it is not used in the available identification keys (Collette and Nauen, 1983; Collette, 1986). Another alternative method is the use of parasites to distinguish species.

1.2. Parasites as biological tags

Parasites have been successfully used as tools to understand several aspects of their hosts' biology, namely, identify and discriminate fish stocks (Timi, 2007; Santos et al, 2009), study population structures (Pozdnyakov and Vasilenko, 1994; Larsen et al., 1997) and clarify taxonomic relations (Luque and Poulin, 2008; Oliva et al., 2008). The use of parasites with these purposes is based on the fact that the host can only become infected with a particular parasite species within the endemic area of that parasite (MacKenzie and Abaunza, 1998). The endemic area can be defined as the geographical region in which conditions are suitable for the transmission of the parasite and the completion of its life cycle (MacKenzie and Abaunza, 1998; Rohde, 2005). Thus, when a fish, infected by a parasite, is found outside the endemic area of the said parasite, it can be inferred that the fish was, at some time, in the endemic area (MacKenzie and Abaunza, 1998; Rohde, 2005). Taking this into account, and given the fact that *S. colias* and *S. scombrus* occupy different areas, it is likely that parasites of these species can be useful in distinguishing them.

Not all parasites can be useful as biological tags. In order to be a good one, there are certain criteria that should be met: (a) the parasite should have significantly different levels of infection in different parts of the study area. Prevalence, abundance and infection data, as defined by Bush et al. (1997), should be used (MacKenzie and Abaunza, 1998); (b) the parasite should have a long life span in the target host, at least long enough to cover the time scale of the investigation (Williams et al., 1992); (c) parasites with direct single-host life cycles are easier to use as tags, but those with two or more hosts can be useful, since there is information on biotic and abiotic factors influencing parasites transmission (MacKenzie and Abaunza, 1998; Rohde, 2005); (d) the parasite should have constant levels of infection from year to year (Rohde, 2005); (e) the parasite should be easily detected and identified, and the examination of the host should involve the minimum of dissection (MacKenzie and Abaunza, 1998; Rohde,

2005); (f) the parasite should not cause high pathogenicity in the host nor changes in host behavior (Williams et al., 1992, MacKenzie and Abaunza, 1998).

1.3. Ectoparasites

As abovementioned, ectoparasites are the ones that live on the host's surface. Typically, and among the metazoan species, ectoparasites can be found among Monogenea and Crustacea (Roberts and Janovy, 2009). Monogeneans, jointly with the copepods, are the most diverse group of metazoan ectoparasites of marine fishes (Whittington, 2005). Within this group of parasites, many species can be found with great ecological and economic importance. For instance, monogeneans and copepods are known to cause damage to fish in aquaculture or even mass mortalities in the sea (Sepulveda et al., 2004; Rohde, 2005; Costello, 2006).

Monogeneans, also known as ectoparasitic flukes, are small (0.03 mm to 20.00 mm) hermaphroditic flatworms and mainly external parasites of vertebrates, particularly fish (Roberts and Janovy, 2009) (Figure 5). Most species have a high degree of host specificity, i.e., they infect only a single host species or few related ones (Hayward, 2005). Morphologically, monogeneans present in the anterior region of the body an organ called prohaptor, which is constituted by various adhesive and feeding organs (Roberts and Janovy, 2009). In the posterior part of the body, monogeneans have an attachment organ called ophishaptor. This organ consists of various attachment structures, which show an enormous variety of forms and sizes and are important in species identification (Hayward, 2005; Whittington, 2005).

There are two subclasses within the Monogenea: Monopisthocotylea and Polyopisthocotylea. The main differences between these two groups are in the ophishaptor: the attachment organ of the former are constituted by small or large hooks (hamuli) which can be in a haptoral sucker whilst the ophishaptor of polyopisthocotyleans possesses clamps (Hayward, 2005; Whittington, 2005; Roberts and Janovy, 2009). Other differences can be found in diet (Monopisthocotylea feed on epithelial cells whilst Polyopisthocotylea feed on host's blood) and on the movement (most mobile behavior on monopisthocotyleans). The life cycle of most monogeneans is direct and with a single host and the larval stage is called oncomiracidium (Rohde, 2005).

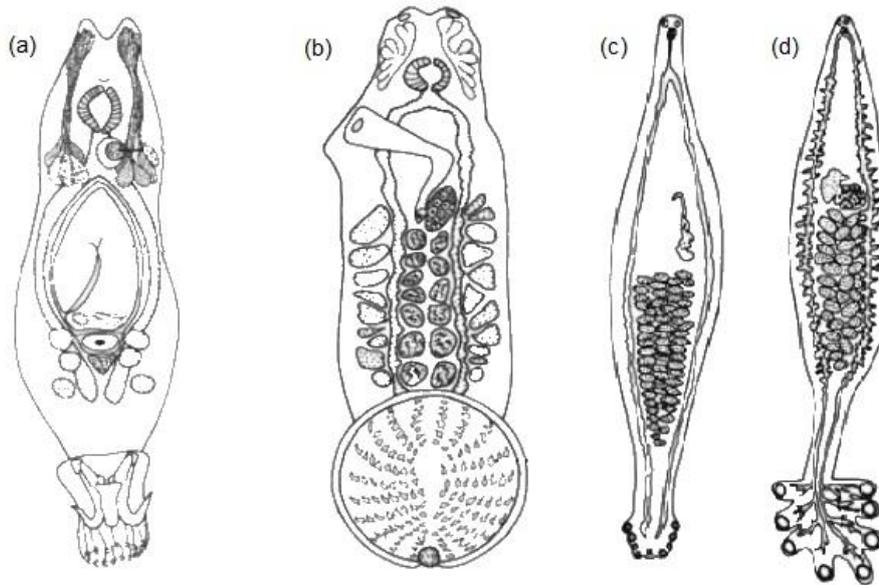


Figure 5. Marine Monopisthocotylea and Polyopisthocotylea Monogenea diversity. (a) Gyrodactylidae; (b) Acanthocotylidae; (c) Mazocraeidae; (d) Dicliphoridae. (Adapted from Rohde, 2005).

Another common and diverse group of ectoparasites is the Copepoda. Copepods present an amazing variety of evolutionary adaptations which enables infection of and survival on the hosts (Boxshall, 2005; Roberts and Janovy, 2009). Like other crustaceans, they show a segment armored exoskeleton with a set of appendages (Kabata, 1979; Boxshall, 2005). As mentioned before, parasitic copepods evolved in order to adapt to the parasitic life. Some of the adaptations are the reduction in locomotor appendages, the development of adaptations for adhesion, increase in size, change in body proportions (disproportionate growth of genital or reproductive regions) and loss of external evidence of segmentation (Roberts and Janovy, 2009).

There are many different groups within Copepoda, namely, Caligidae, Ergasilidae, Lernaepodidae, Chondracanthidae, among others (Figure 6). The Caligidae family is the one with higher number of species among the fish parasitic copepods and one of the most well studied because of its economic importance for aquacultures (Boxshall, 2005). Caligids show some adaptations for prehension, tend to be larger than most free-living groups, and have some dorsoventral flattening to promote adhesion to host's surface. Some tend to be more sedentary, being mostly confined to the branchial chambers of fish (Roberts and Janovy, 2009).

In most cases, copepods' life cycles are direct, involving only a single host and comprises three phases: naupliar, copepodid and adult (Boxshall, 2005).

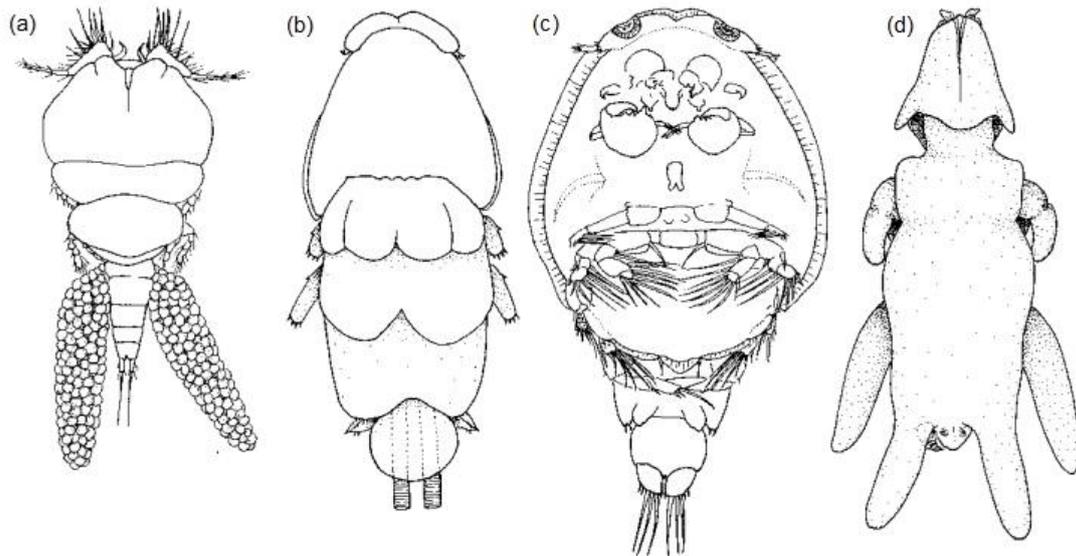


Figure 6. Morphological diversity of copepod families parasitic on fish. (a) Bomolochidae; (b) Pandaridae; (c) Caligidae; (d) Chondracanthidae female with male attached. (Adapted from Boxshall, 2005).

1.3.1. Ectoparasite fauna of *Scomber colias* and *Scomber scombrus*

Throughout the years, many studies on parasite and, more specifically, on ectoparasite fauna of *S. colias* and *S. scombrus* have been made. For instance, some monogeneans are common to both host species, namely, *Grubea cochlear* Diesing, 1858, *Kuhnia scombri* (Kuhn, 1829) and *Kuhnia sprostonae* Price, 1961 (Rego and Santos, 1983; Rego et al., 1985; Rohde and Watson, 1985b; Rohde, 1986, 1989; Romuk-Wodoracki, 1988; Somdal and Schram, 1992; Cremonte and Sardella, 1997; Alves et al., 2003; Shukhgalter, 2004; Costa et al., 2007, 2011). Other monogeneans were only found parasitizing *S. colias*: *Kuhnia scombercolias* Nasir and Fuentes Zambrano, 1983 and *Pseudokuhnia minor* (Goto, 1984) (Rohde and Watson, 1985a; Rohde, 1989; Alves et al., 2003; Shukhgalter, 2004; Costa et al., 2007, 2011). Moreover, some Copepoda species are frequent parasites of *S. colias* and *S. scombrus*: *Caligus pelamydis* Kroyer, 1863 and *Clavellisa scombri* (Kurtz, 1877) (Cressey and Cressey, 1980; Somdal and Schram, 1992; Alves et al., 2003; Öktener and Trilles, 2009).

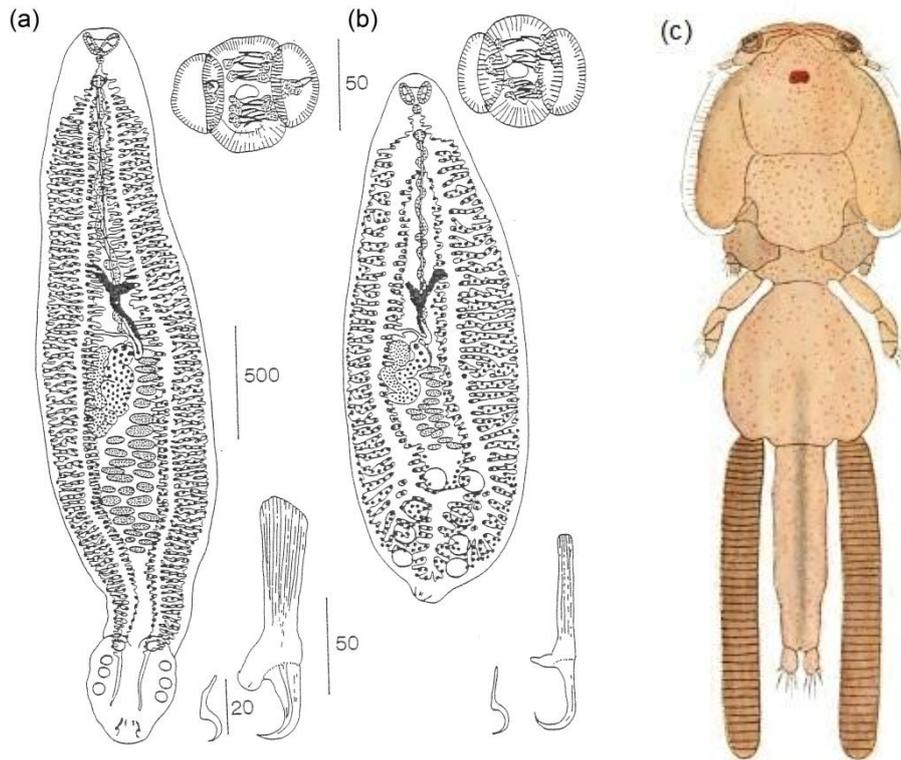


Figure 7. Some ectoparasites of *S. colias* and *S. scombrus*. (a) *Kuhnia scombri* and (b) *Kuhnia sprostonae* (Monogenea); (c) Female individual of *Caligus pelamydis* (Copepoda). Full body of Monogenea is shown, as well as hamuli and copulatory organ. Scales in μm . (Adapted from Rohde and Watson, 1985b and Scott and Scott, 1913).

1.4. Ecology of parasites

Ecology is the science that deals with interactions of organisms and their environment. One important concept in ecology is the ecological niche, which can be defined, according to Hutchinson (1957) as a multi-dimensional hypervolume determined by environmental variables, biotic and abiotic, where a species can exist. The number of these dimensions is almost infinite, but a few are sufficient to characterize the niche of a parasite species, namely, macrohabitat, microhabitat, host specificity, geographical range, sex and age of host, season and food (Rohde, 1993; Rohde and Rohde, 2005). Two of the most important dimensions for parasites are the macro- and microhabitat, which are detailed below.

1.4.1. Macrohabitat

The macrohabitat of a parasite is the part of the host habitat in which the parasite can also be found, i.e., the niche components which also represent the habitat of its host(s) (Rohde, 1984). Three scenarios are possible: (a) the macrohabitat of the parasite species is narrower than that of its host (the parasite only occur in some part of the area occupied by its host); (b) the parasite macrohabitat corresponds to the one of its host; (c) the parasite infects several hosts with different macrohabitats, having a wider one (Rohde, 1993). The distribution of a marine parasite species in the macrohabitat is dependent on some physical and chemical factors, such as temperature, water salinity, depth causing different light and pressure conditions and diet of hosts (Rohde, 1993, 2005; Tinsley and Jackson, 2002; Rohde and Rohde, 2005; Costello, 2006).

As expected, the preferences of parasites will lead to different distributions and some geographical gradients can be noticed. For instance, there is a latitudinal gradient of marine parasites, where the species richness increases from high latitudes to the tropics (Rohde, 2005). However, this gradient is much more marked in ectoparasites than in endoparasites, because the former are much more affected by the macrohabitat than the latter (Rohde, 2005) (Figure 8). This type of gradient is due to differences in sea water temperature, according to Rohde (1993) and Rohde et al. (1995). Longitudinal and depth gradients are not well studied, but Rohde (2005) refers that the parasite species diversity seems to decrease with depth.

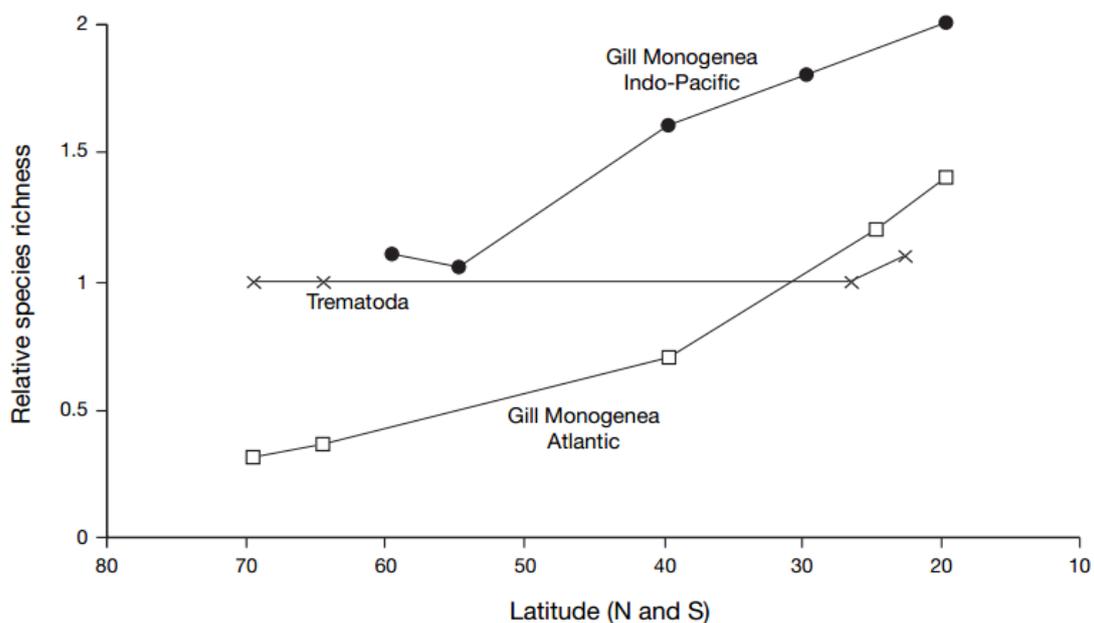


Figure 8. Relative species diversity (number of parasite species per host species) of digeneans (endoparasites) and monogeneans (ectoparasites) of marine teleosts at different latitudes. (Adapted from Rohde, 2005).

1.4.2. Microhabitat

Parasites cannot be found infecting all tissues of the host. Instead, they show preferences for specific tissues or organs. The place where a species can be found on the host is called the microhabitat (Rohde, 1984, 2005; Euzet and Combes, 1998). There are several arguments trying to explain the choice of parasites for a certain microhabitat. One is the avoidance of interspecific competition and is based on the idea that niches are saturated with species. This fact means that parasite species would compete for space and food. To avoid this competition, parasites restrict their niche (Holmes, 1973; Ramasamy et al., 1985). However, this argument is refuted by Rohde, who concluded, studying fish parasites, that several hundreds of niches are available for parasites to occupy (Rohde, 1979, 1980, 1994). Instead of the idea of avoidance of interspecific competition, Rohde (1993, 1994, 2005) proposes that niche restriction in parasites is due to reinforcement of reproductive barriers and facilitation of mating. For instance, evidence to support the reinforcement argument can be found in Rohde, 1980, Rohde and Hobbs (1986) and Lo and Morand (2000). In these works, it was verified that congeneric species show less microhabitat overlap than non-congeners and, when congeners have overlapping microhabitats, they differ significantly in size or in the copulatory organs' shape. Then, in order to prevent interspecific hybridization, congener species choose different microhabitats. Frequencies and intensities of infection of parasites are usually low and then, they would hardly have a chance to establish contact with mating partners if their microhabitats were not restricted and well defined (Rohde, 1993). Rohde (1980), Geets et al. (1997), Simková et al., (2000) and Dippenaar et al., (2009), for example, show evidence that individuals of the same species are aggregated in the same microhabitat, concluding that this distribution promotes and facilitates mating and reproduction.

A large part of the studies on habitat selection are made with gill parasites. In fact, data on gill ectoparasites can be examined quantitatively in a short period of time, fish are easily available in large numbers, the marine habitat is less variable than the terrestrial and species rich communities of ectoparasites can be found (Koskivaara et al, 1991; Rohde 1993, 1994). Additionally, gills can be divided in several ways (transversely, longitudinally, vertically and laterally), which can define precisely the microhabitat where the parasites occur (Rohde, 1993).

1.5. Aims

This study aims to increase knowledge about the ectoparasite fauna of *S. colias* and *S. scombrus* from the northern Portuguese coastal waters, presenting qualitative and quantitative data on their parasites.

In addition, given the misidentification problems of the two host species, this work will evaluate new ways to distinguish the species, namely, using the eye size and their parasites as biological tags.

At last, a characterization of the macro and microhabitats of *S. scombrus* ectoparasites will be performed.

Chapter 2

The two morphologically similar Atlantic mackerels *Scomber colias* and *S. scombrus* can be easily differentiated by eye size and/or ectoparasites

The two morphologically similar Atlantic mackerels *Scomber colias* and *S. scombrus* can be easily differentiated by eye size and/or ectoparasites

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Running headline: **Atlantic mackerels differ in eye or parasites**

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Abstract

Atlantic chub mackerel, *Scomber colias* Gmelin, 1789, and Atlantic mackerel, *Scomber scombrus* L., 1758, (Teleostei: Scombridae) are 2 fish species inhabiting the Atlantic Ocean, very important to the Portuguese and European fisheries. Their similar morphology and habitat partial overlap make them difficult to differentiate when entering the commercial circuit. In this study, the usefulness of eye size and macro-ectoparasites whilst species diagnostic feature and biological markers, respectively, was investigated in fish obtained from commercial catches from waters of the northern Portuguese coast. The eyes of *S. colias* ($n = 22$) were significantly larger ($\approx 1.66\times$) than those of *S. scombrus* ($n = 22$), for fish of a similar size class. For fish of different sizes, the macro-ectoparasites could be used as biological markers of both species. Moreover, among the 8 taxa reported in both Atlantic mackerels - *Pseudokuhnia minor*, *Grubea cochlear* and *Kuhnia scombri* (Monogenea) and 1 Gnathiidae (Isopoda) (new host record) were recorded for *S. colias* ($n = 40$); and *K. scombri*, *Kuhnia sprostonae* and *G. cochlear* (Monogenea), *Caligus pelamydis* and *Clavellisa scombri* (Copepoda) and 1 Aegidae (Isopoda) (new host record) for *S. scombrus* ($n = 42$) - 4 ectoparasites were selected as good biological markers to distinguish between them. *P. minor* was recorded only for *S. colias* (97.5% prevalence and mean abundance of 23.95 worms/fish); *C. pelamydis* was recorded only for *S. scombrus* (69.0% prevalence and mean abundance of 3.12 copepods/fish); and *G. cochlear* and *K. scombri* were recorded for both fish, but presented significantly higher prevalence and abundance for *S. scombrus* (28.6% and 66.7% prevalence, and mean abundance of 0.4 and 2.0 worms/fish, respectively), than for *S. colias* (2.5% and 17.5% prevalence, and mean abundance of 0.03 and 0.5 worms/fish, respectively). The eye size in conjunction with the ecology of macro-ectoparasites data, or the later alone, can be useful for further Atlantic mackerel species differentiation.

Keywords: *S. colias*; *S. scombrus*; eye size; macro-ectoparasites; fish discrimination; biological tags

Introduction

Presently, the genus *Scomber* L., 1758 (Teleostei: Scombridae) comprises only 4 valid species: *Scomber australasicus* Cuvier, 1832 (blue mackerel), *Scomber colias* Gmelin, 1789 (Atlantic chub mackerel), *Scomber japonicus* Houttuyn, 1782 (chub mackerel) and *Scomber scombrus* L., 1758 (Atlantic mackerel) (Froese & Pauly, 2011). *S. colias* and *S. scombrus* are 2 fish species inhabiting the Atlantic Ocean, with importance to both Portuguese and European fisheries (Scoles *et al.*, 1998; Zardoya *et al.*, 2004; Martins, 2007; DGPA, 2011). The recognition of *S. colias* in the Atlantic as a separate species from *S. japonicus* was recently achieved based on mtDNA analysis (Scoles *et al.*, 1998). Its geographic distribution shows however, some overlapping with that of *S. scombrus*, that has a more restricted and northern distribution (Zardoya *et al.*, 2004). Both Atlantic mackerel species live at similar depths, ranging from 250 to 300 m for *S. colias*, and from 200 to 250 m for *S. scombrus* (Collette, 1986). They are caught with pelagic pair trawls and purse seines, which are non-selective devices. Therefore, in a single catch, diverse fishes - like sardines, anchovies or different mackerels - can be captured together (Cochrane, 2002).

The morphology of both Atlantic mackerels is very similar, so that they can be easily misidentified at the landing auction. In accordance to the morphological descriptions of Collette & Nauen (1983) and the species identification key of Collette (1986), a number of characteristics, including, the number of spines in the first dorsal fin and the belly pigmentation, are needed to carefully observe to efficiently differentiate between them. Although the size of the eyes was not considered in that key, the empirical knowledge of the fishermen suggests that this feature can be useful in specimens' identification to the species level. In Turkish waters, *S. scombrus* is already distinguished from *S. colias* on the basis of its smaller eye diameter (Erguden *et al.*, 2009).

Parasites have been widely and successfully used as tools to understand several aspects of host fish biology, to distinguish fish populations and even to clarify systematic relations of close related hosts (Williams *et al.*, 1992; MacKenzie, 2002; MacKenzie & Abaunza, 2005; Luque & Poulin, 2008), being likely that they might, as well, be useful in distinguishing between similar species of fish, namely, *S. colias* and *S. scombrus*. To date, a number of macro-ectoparasites were reported in both Atlantic mackerel species collected from different biogeographic regions (Table I). In this study, macro-ectoparasites were considered in order to tentatively distinguish the 2 fish species. According to MacKenzie & Abaunza (1998), the optimal parasite as a biological marker should have significant levels of infection on hosts, persist for a long period of time, have only one host, a constant level of infection, be easily detectable

and identifiable and not cause high pathogenicity in the host. Macro-ectoparasites were used here because they fulfill most of these features, if not all. Moreover, their survey do not compromise the fish flesh integrity, and can be easily implemented without rendering the surveyed fish unmarketable.

Table 1. Ectoparasites (Monogenea and Crustacea) recorded in *Scomber colias* and *S. scombrus*, off the Atlantic Ocean and adjacent seas.

Parasite taxa	<i>S. colias</i> - C <i>S. scombrus</i> - S	Prevalence (%)	Locality (number of analyzed fish)	References
Monogenea				
<i>Grubea cochlear</i> Diesing, 1858	C	3.3	Madeira (151)	Costa <i>et al.</i> , 2007
	C	18; 6; -	Brazil (100); (50); (-)	Alves <i>et al.</i> , 2003; Rego & Santos, 1983; Rohde, 1986
	C	10.3; 1.1; 3;5.3	Azores – Irving Bank (29); Morocco (182); Western Sahara (232); Mauritania (76)	Shukhgalter, 2004
	S	2.5	Portugal mainland (80)	Rego <i>et al.</i> , 1985
	S	-	Mediterranean Sea, Atlantic Ocean (-)	Rohde, 1986
<i>Kuhnia arabica</i> Mamaev & Parukhin, 1986	C	6.7; 10.3	Azores (Az) – Hyeres Bank (15); Az – Irving Bank (29)	Shukhgalter, 2004
<i>Kuhnia scombercolias</i> Nasir & Fuentes Zambrano, 1983	C	39.1; 13.3;	Madeira (151); (30);	Costa <i>et al.</i> , 2007
	C	1.5	Canary Islands (68)	Costa <i>et al.</i> , 2011
	C	46; -	Brazil (100); (-)	Alves <i>et al.</i> , 2003; Rohde, 1989
<i>Kuhnia scombri</i> (Kuhn, 1829)	C	43.7; 43.3	Madeira (151); (30)	Costa <i>et al.</i> , 2007
	C	1.5	Canary Islands (68)	Costa <i>et al.</i> , 2011
	C	11; 44; -	Brazil (100); (50); (-)	Alves <i>et al.</i> , 2003; Rego & Santos, 1983; Rohde & Watson, 1985b
	C	1.1; 1.1	ArgentinaSea: Mar del Plata (90); El Rincón (83).	Cremonete & Sardella, 1997
	C	-	Brazil; Mediterranean; South Africa (Cape Town); Argentina	Rohde & Watson, 1985b
	C	24.1; 15.2; 19.2; 4.3; 10.5	Azores (Az) – Irving Bank (29); Az – Great Meteor Bank (46); Morocco (182); Western Sahara (232); Mauritania (76)	Shukhgalter, 2004
	S	37.5	Portugal mainland (80)	Rego <i>et al.</i> , 1985
	S	-	North America, North Sea, Bay of Biscay; Mediterranean; Guernsey	Rohde & Watson, 1985b
	S	23.3; 60; 75; 11.9	Northern North Sea (60); North Sea (70), Skagerrak (148); Ireland (Celtic Sea) (176)	Somdal & Schram, 1992
	S	45	North Caroline, Cape Hatteras (80)	Romuk-Wodoracki, 1988
	<i>Kuhnia sprostonae</i> Price, 1961	C	0.7	Madeira (151)
C		-	Brazil	Rohde & Watson,

	S	-	Madeira (-)	1985b Rohde, 1989
	S	-	North Sea	Rohde & Watson, 1985b
	S	10; 6.8; 11.9	North Sea (70), Skagerrak (148); Ireland (Celtic Sea) (176)	Somdal & Schram, 1992
<i>Pseudokuhnia minor</i> (Goto, 1984)	C	40; 17.2; 17.4; 78;57.3; 94.7	Azores (Az) – Hyeres Bank (15); Az – Irving Bank (29); Az – Great Meteor Bank (46); Marocco (182); Western Sahara (232); Mauritania (76)	Shukhgalter, 2004
	C	98.7; 86.7	Madeira (151); (30)	Costa <i>et al.</i> , 2007
	C	54.4	Canary Islands (68)	Costa <i>et al.</i> , 2011
	C	-	South Africa; Atlantic Spain; Mediterranean Sea	Rohde & Watson, 1985a
Copepoda				
<i>Kabatazus paradoxus</i> (van Beneden, 1851) [= <i>Advena paradoxa</i> (van Beneden, 1851)]	S	0; 0.6	North Caroline, Cape Hatteras (80); (500)	Romuk-Wodoracki, 1988
	S	0.6	Ireland (Celtic Sea) (176)	Somdal & Schram, 1992
<i>Caligus elongatus</i> von Nordmann, 1832	S	1.4; 0.6	North Sea (70); Ireland (Celtic Sea) (176)	Somdal & Schram, 1992
<i>Caligus mutabilis</i> Wilson, 1905	C	-	Gulf of Mexico (Campeche)	Cressey & Cressey, 1980
<i>Caligus pelamydis</i> Kroyer, 1863	C	-	Gulf of Mexico, Florida, Gulf of Guinea	Cressey & Cressey, 1980
	C	2	Brazil (100)	Alves <i>et al.</i> , 2003
	S	-	France	Cressey & Cressey, 1980
	S	5; 17.1; 0.7; 34.7	Northern North Sea (60); North Sea (70); Skagerrak (148); Ireland (Celtic Sea) (176)	Somdal & Schram, 1992
<i>Clavellisa scombri</i> (Kurtz, 1877)	C	-	Gulf of Mexico, Sierra Leoa, Liberia; Brazil	Cressey & Cressey, 1980
	C	2	Brazil (100)	Alves <i>et al.</i> , 2003
	S	-	Mediterranean (Trieste)	Cressey & Cressey, 1980
	S	6	Turkey (26)	Öktener & Trilles, 2009
	S	5; 2.9; 3.4; 0.6	Northern North Sea (60); North Sea (70), Skagerrak (148); Ireland (Celtic Sea) (176)	Somdal & Schram, 1992
<i>Peniculus</i> sp. von Nordmann, 1832	S	0.6	Ireland (Celtic Sea) (176)	Somdal & Schram, 1992
<i>Pumiliopes capitulatus</i> Cressey & Boyle, 1973	C	-	Gulf of Guinea, Mauritanea	Cressey & Cressey, 1980

The aims of the present work were 4-fold: (i) to confirm the misidentification problem of the 2 fish species in the beginning of their commercial circuit; (ii) to evaluate if the size of the eye can be used to discriminate between the 2 fish species; (iii) to present a summary report of the macro-ectoparasites found in *S. colias* and *S. scombrus* of the northern Portuguese coast; and (iv) to evaluate if these parasites are good biological markers or tools to discriminate between the 2 fish species.

Materials and Methods

Host identification and morphological characterization

While doing a parasitological survey of *S. colias* and *S. scombrus* caught at the northern Portuguese coast (nearby waters off Matosinhos, 41°10'N, 8°42'W), it was noticed that, for most of the study samples, the fish identification at the selling place did not correspond to the identification made at the laboratory, which was carried out using the diagnostic key of Collette (1986), in most of the samples. To be sure about the existence of a real species identification problem at the landing place, 2 large samples of each fish species were analyzed. The first one was collected in June 2010, consisting of 70 fish of a same size class, and identified as "*S. scombrus*" at the fishing auction. The fish were measured (mean \pm standard deviation - s.d. - [range]) [32.7 \pm 3.0 (21.8 - 38.6) cm] and identified in accordance with the main criteria in the above mentioned key - i.e., the number of spines in the first dorsal fin (9-10 / 11-13), the aspect of the integument belly (marked / unmarked) - and also, the eye size (large / small) (for *S. colias* and *S. scombrus*, respectively). The second sample was collected in February 2012, consisting of a total of 41 fish, also of the same size class, and identified as "*S. colias*" at the fishing auction. These fish were also measured [31.8 \pm 1.5 (28.4 - 35.5) cm] and identified in accordance with the 3 above mentioned criteria.

In order to test if the size of the eye could indeed be used to discriminate between both mackerels, a subsample of 22 fish from each species was considered. The total body length was assessed for both subsamples and the normal distribution of the data was evaluated using the one-sample Kolmogorov-Smirnov's test ($0.74 < Z < 0.91$; $0.374 < P < 0.653$). Owing to the normal distribution of the data, the Levene's test was run to analyze the equality of variances. As these did not vary significantly among the 2 fish species ($F = 2.38$, $P = 0.130$), the corresponding statistics of the Student's *t*-test, which compared between the mean values for both species, were considered. Two metric dimensions were recorded from the eye, that is, the eye diameter (ED) and the pupil diameter (PD). As the total body length differed significantly between the 2 samples of the 2 fish species, it was necessary to normalize these 2 measurements. So, 2 new parameters were created: "ED ratio" and "PD ratio", obtained by the division of ED and PD by the fish total body length, respectively. The normal distribution of ED ratio and PD ratio was also evaluated using the one-sample Kolmogorov-Smirnov's test (ED ratio: $0.65 < Z < 0.85$, $0.471 < P < 0.786$; PD: $0.50 < Z < 0.55$, $0.927 < P < 0.966$).

Owing to the normal distribution of the 2 variables, the Levene's test was run (ED ratio: $F = 2.03$, $P = 0.161$; PD ratio: $F = 0.13$, $P = 0.726$) and the variables compared, for both species, using the Student's *t*-test.

Parasitological survey

A total of 40 *S. colias* and 42 *S. scombrus* were captured at northern Portuguese waters (nearby waters off Matosinhos, 41°10'N, 8°42'W) and surveyed for macro-ectoparasites. The samplings were conducted throughout the year for both *S. colias* and *S. scombrus* and as follows: from October to December 2009 ($n = 19$ and 21, respectively) and from January to June 2010 ($n = 21$ for both species). At the laboratory, the fish total body length was measured: 32.6 ± 3.6 (25.4 – 39.2) cm for *S. colias*, and 31.9 ± 2.3 (21.8 – 36.4) cm for *S. scombrus*. The occurrence of macro-ectoparasites was evaluated under a stereo-dissecting microscope (30x magnification). The body regions scanned for parasites included the integument, eyes, nasal and buccal cavities, fins, pre-opercular channels, opercula and gills. All parasites were collected, cleaned and fixed in 70% ethanol for posterior identification. Taxonomic identifications of parasites were carried out following the descriptions and keys of Rohde and Watson (1985a, b) and Rohde (1986, 1989) for Monogenea, Kabata (1979, 1992) for Copepoda, Naylor (1972) for pranzia larvae of Gnathiidae (Isopoda), and Brusca and Iverson (1985) for Aegidae (Isopoda). Parasitological parameters, such as presence-absence data, prevalence (in %) and abundance of infection (mean, standard deviation – s.d.- , and range) were calculated for each parasite species and recorded for each of the host (Bush *et al.*, 1997). They were compared between Atlantic mackerels, for parasite species common to both fish species and component (at least in one of the host) [prevalence $\geq 10\%$, Bush *et al.* (1990)]. Comparison between the presence-absence data was conducted using the χ^2 -test; that between the abundance of parasites used the Mann-Whitney's *U*-test, owing to the non-normal distribution of the abundance data (one sample Kolmogorov-Smirnov's test, $Z > 1.75$, $P < 0.004$). A discriminant function analysis (DFA) was run on the abundance data (method: independents entered together) to evaluate whether the 2 species of mackerel could be separated on the basis of their component parasite fauna. Four groups of fish, defined on the basis of the total body length - lower than the mean value (small fish); greater than the mean value (large fish) -, were considered for analysis. They were as follows: *S. colias* small (group 1, $n=20$); *S. colias* large (group 2, $n=20$); *S. scombrus* small (group 3, $n=22$); and *S. scombrus* large (group 4, $n=20$).

Data were compiled and analyzed using the Microsoft Office Excel 2007 and the statistical program package SPSS for Windows, version 17.0. The results were considered as significant when $P < 0.05$.

Results

Host identification and morphological characterization

In the first sample of 70 fish examined in June 2010, nominated “Atlantic mackerel (*S. scombrus*)”, 13 fish were identified as *S. colias* and 57 as *S. scombrus*, which meant that around 1 in each 5 were misidentified at the auction. All specimens of *S. colias* presented 8 ($n = 1$), 9 ($n = 11$) or 10 ($n = 1$) spines in the first dorsal fin, marked belly with spotting or wavy broken lines and large eyes. On the other hand, the specimens of *S. scombrus* presented 11 ($n = 30$), 12 ($n = 22$) or 13 ($n = 5$) spines in the first dorsal fin, unmarked belly and small eyes. Pooled together, the specimens of *S. colias* ($n = 13$) measured 29.6 ± 3.6 (25.4 - 38.6) cm, whereas those of *S. scombrus* ($n = 57$) measured 33.5 ± 2.4 (21.8 - 37.2) cm. In the second sample (February 2012), 41 fish nominated “Atlantic chub mackerel (*S. colias*)”, the identification showed that all were *S. colias*. Thus, in this case, no mistakes occurred in the identification at the auction. Those fish hold 8 ($n = 2$), 9 ($n = 35$) or 10 ($n = 4$) spines in the first dorsal fin. In some, the belly was marked with spotting or wavy broken lines ($n = 29$), while in some others the marking was faded ($n = 10$) or even absent ($n = 2$). All of them presented large eyes. All fish morphologically studied were of the same size class at the auction, which allowed the comparison between samples, for further analysis.

With respect to the 2 subsamples of 22 fish used to test the usefulness of the eye size in the discrimination between species, we checked and compared their total lengths. The total length of the fish was of 32.2 ± 1.4 (29.4 - 35.5) cm for *S. colias* and 34.6 ± 1.8 (30.0 - 37.2) cm for *S. scombrus*, varying significantly between the 2 hosts (Student's t -test: $t = 5.01$, D.F. = 42, $P = 0.000$). For the eye, *S. colias* presented an ED ratio of 0.056 ± 0.003 (0.050 - 0.065) and a PD ratio of 0.031 ± 0.002 (0.028 - 0.036), whereas *S. scombrus* presented an ED ratio of 0.034 ± 0.002 (0.030 - 0.040) and a PD ratio of 0.022 ± 0.002 (0.017 - 0.026). Comparison between both eye measures for *S. colias* and *S. scombrus* showed the existence of significant differences for both of them (see Fig. 1): ED ratio (Student's t -test: $t = 23.99$, D.F. = 42, $P = 0.000$) and PD ratio (Student's t -test: $t = 11.27$, D.F. = 42, $P = 0.000$).

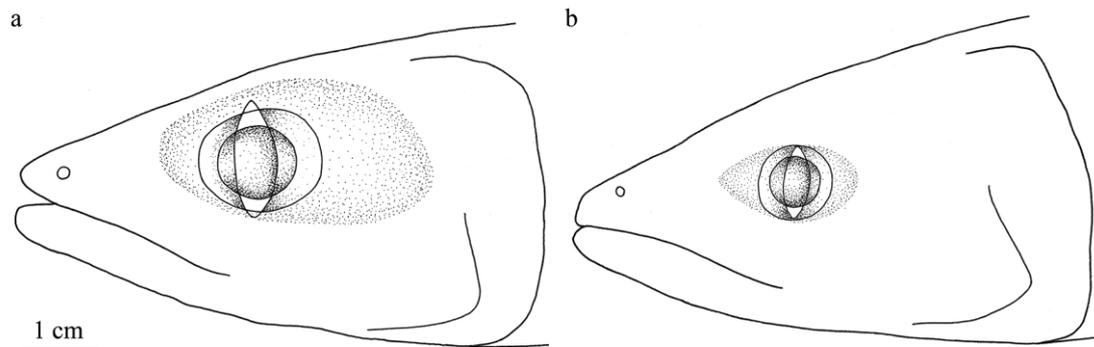


Figure 1. Schematic representation of the heads of *Scomber colias* (a) and *S. scombrus* (b), depicting the different sizes of the eye and pupil.

Parasitological survey results

The parasitological survey results, for both Atlantic mackerels of the northern Portuguese coast, are shown in Table II. In total, 8 macro-ectoparasite species - that is, 4 Monogenea and 4 Crustacea (2 Copepoda and 2 Isopoda) - were isolated from the 2 species of fish. From *S. colias*, 4 parasite species were detected, including 3 monogeneans (Mazocraeidae), as *Grubea cochlear* Diesing, 1858, *Kuhnia scombri* (Kuhn, 1829), and *Pseudokuhnia minor* (Goto, 1984), and a praniza larvae (Isopoda: Gnathiidae), being the latter a new host record. From *S. scombrus*, 6 parasite species were found, including *G. cochlear*, *K. scombri*, *Kuhnia sprostonae* Price, 1961 (Monogenea: Mazocraeidae), *Caligus pelamydis* Kroyer, 1863 (Copepoda: Caligidae), *Clavellisa scombri* (Kurz, 1877) (Copepoda: Lernaeopodidae) and Aegidae (Isopoda) being the latter a new host record. The 2 mackerels shared 2 parasite species, i.e., *G. cochlear* and *K. scombri* (Monogenea), and each have exclusivity for the remaining recorded parasitic species. Most of those parasites were collected from a single site location in the host, i.e., the fish gills; however, *C. pelamydis* was also found in the opercula (inner surface), whereas Aegidae was found on the integument exclusively.

The comparison of the infection levels recorded for the component parasite species shared by the 2 mackerels showed the existence of statistically significant differences. The distribution patterns of those parasite species are shown in Fig. 2. In *S. colias*, *P. minor* was the species recording the highest prevalence value (97.5%), whereas in *S. scombrus* the prevalence was higher for *C. pelamydis* (69.0%). As already mentioned, both parasite species were host exclusive, the first recording a

mean abundance of 23.95 worms/fish and the second a mean abundance of 3.12 copepods/fish. The other component species were the monogeneans *G. cochlear* and *K. scombri*, both of which recorded significantly higher infection values for *S. scombrus* (χ^2 -test: $\chi^2 = 10.4$, $p = 0.001$ for *G. cochlear*; $\chi^2 = 20.2$, $p = 0.000$ for *K. scombri*).

Table 2. Site of infection, prevalence (% and number of infected fish) and abundance (mean \pm standard deviation, range) of parasites in *Scomber colias* ($n = 40$) and *S. scombrus* ($n = 42$), off the north Portuguese coast. Abbreviations: g – gills; i – integument; o – opercula.

Parasites taxa	Infection site	Prevalence % (number of infected fish)		Abundance mean \pm SD (range)	
		<i>S. colias</i> ($n = 40$)	<i>S. scombrus</i> ($n = 42$)	<i>S. colias</i> ($n = 40$)	<i>S. scombrus</i> ($n = 42$)
Monogenea					
<i>Grubea cochlear</i>	g	2.5 (1)	28.6 (12)	0.03 \pm 0.16 (0-1)	0.38 \pm 0.66 (0-2)
<i>Kuhnia scombri</i>	g	17.5 (7)	66.7 (28)	0.53 \pm 1.71 (0-9)	2.00 \pm 2.58 (0-10)
<i>Kuhnia sprostonae</i>	g	0 (0)	2.4 (1)	0	0.10 \pm 0.62 (0-4)
<i>Pseudokuhnia minor</i>	g	97.5 (39)	0 (0)	23.95 \pm 22.34 (0-95)	0
Copepoda					
<i>Caligus pelamydis</i>	g, o	0 (0)	69.0 (29)	0	3.12 \pm 4.33 (0-20)
<i>Clavellisa scombri</i>	g	0 (0)	4.8 (2)	0	0.05 \pm 0.22 (0-1)
Isopoda					
Gnathiidae	g	7.5 (3)	0 (0)	0.35 \pm 1.76 (0-11)	0
Aegidae	i	0 (0)	2.4 (1)	0	0.02 \pm 0.15 (0-1)

Moreover, the prevalence values recorded were of 28.6% and 66.7%, respectively, for the latter fish host, and of 2.5% and 17.5%, respectively, for *S. colias*. Concerning the mean abundance, a similar pattern was found for both parasite species, with 0.38 and 2.00 worms/host for *S. scombrus*, and 0.03 and 0.53 worms/host for *S. colias*. Moreover, the comparison of the abundances of *G. cochlear* and *K. scombri* between the 2 mackerels also showed the existence of significant differences (Mann-Whitney's *U*-test: $U = 619.0$, $P = 0.001$, for *G. cochlear*; $U = 417.0$, $P = 0.000$, for *K. scombri*). As concerns the DFA conducted on the abundance data for the component parasite species, it was found that it accounted for 90.0% of the variance found, however only discriminant function 1 was statistically significant (axis 1: Wilks' Lambda = 0.358, $\chi^2 = 78.993$, D.F. = 12, $P = 0.000$; axis 2: Wilks' Lambda = 0.862, $\chi^2 = 11.465$, D.F. = 6, $P =$

0.075), Individuals of *S. scombrus* accumulated more expressively in its positive end, whereas those of *S. colias* were mostly located in the negative end. As a consequence, the positioning of the centroids for the 2 species along discriminant function 1 indicates a clear segregation between them, as concerns the component parasite fauna. It is worse to note that, variability within a species was more expressive between small and large *S. colias* than between small and large *S. scombrus* (Fig. 3).

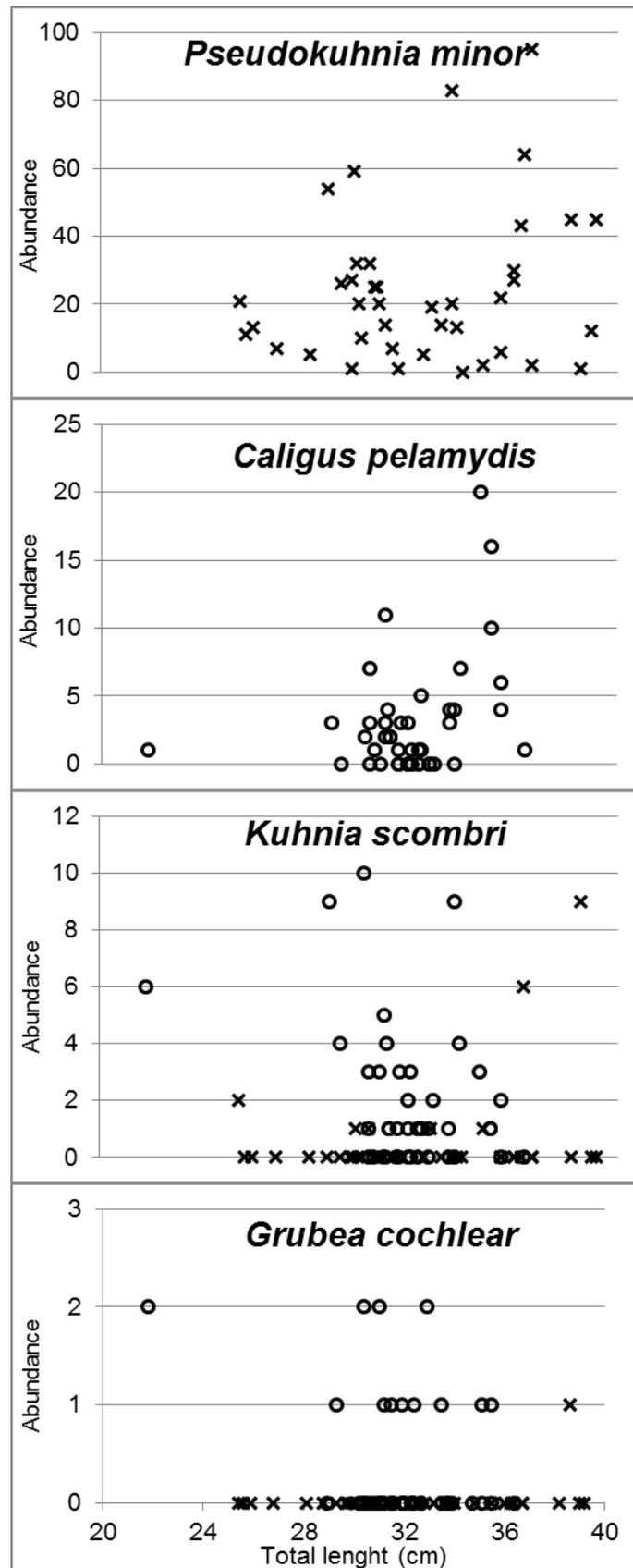


Figure 2. Distributions of abundances of the parasites selected as good biological markers - *Pseudokuhnia minor*, *Caligus pelamydis*, *Kuhnia scombri* and *Grubea cochlear* - of *S. colias* and *S. scombrus* in the northwest coast of Portugal, in accordance to the fish length. (x - *S. colias*; O - *S. scombrus*).

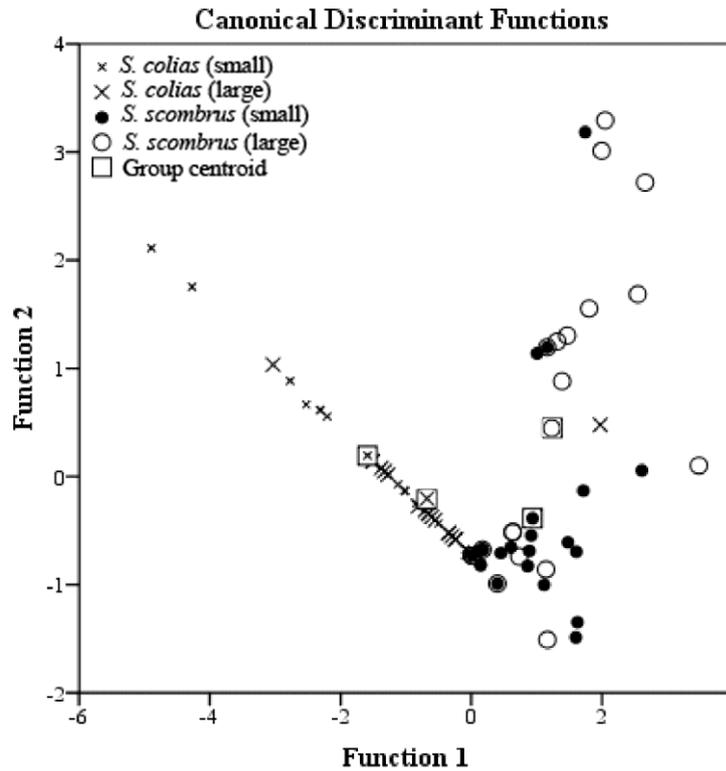


Figure 3. Discriminant function analysis run on the abundance data recorded for the component parasite species (*Pseudokuhnia minor*, *Caligus pelamydis*, *Kuhnia scombri* and *Grubea cochlear*) - projection of the specimens of *Scomber colias* and *S. scombrus* on discriminant functions 1 and 2.

Discussion

The morphological identification of Atlantic mackerels, *S. colias* and *S. scombrus*, to the species level appears to be problematic at landing places of the northern Portuguese coast, with some of the fish inaccurately assigned to a given mackerel species. This problem was previously pointed out for other geographical localities, like Morocco, where, at landing, both species are simply assigned as mackerels, and not identified at all to the species level (Cissé & Belghyti, 2004). In the present study, it was not aimed to quantify the number of fish misidentified, but rather show that species identification can be problematic and suggest additional morphological features to be included in the diagnostic key of Collette (1986). The correct identification of a species is important in terms of management that is, in setting of the fish quota among the members of the European Union (Abaunza *et al.*, 2008; Gordo *et al.*, 2009). Accordingly, all possible efforts should be made to guarantee the accurate fish identification at the species level. With respect to the 2 species of Atlantic mackerel considered in this study, their similar morphology, habitat partial overlap and utilization

of the same fishing device to catch them, make it difficult to have a proper and quick differentiation of the species at the landing place. Following the observations made in this study, misidentifications seemed to be particularly common for *S. scombrus*. A possible cause of this situation would also be the different price that *S. scombrus* can get on the market, which is 2 to 4 times higher than that for *S. colias*. Moreover, according to the official national statistical data of 2010 (DGPA, 2011), at the Portuguese auction they can cost, in average, 1.01€ and 0.25€, respectively. This will lead that the identification problem is biased to the same side, and that most of the time, the number of *S. scombrus* is reported by excess. This procedure may question the data reported in another Portuguese official document (MADRP-DGPA, 2007). In this latter, *S. scombrus* is classified as overexploited, but with great reproductive ability. Although *S. colias* is very important to Portuguese fisheries, being the second most fished species in Portugal (21,996 tons in 2010, DGPA, 2011), it has a much lower value as fresh fish. Indeed, this species is mostly sold canned, where it achieves a better price, 5 to 10 times higher than when it is sold fresh, as could be noticed in a quick survey in the internet for canned mackerel at the more important supermarkets in Portugal. The recorded metric data suggested that the size of the eyes is a good species' diagnostic feature, once the ED ratio of *S. colias* was 1.66 times higher than the one of *S. scombrus*. As an example, a 30 cm specimen of *S. colias* presents a 1.66 cm ED, whilst a specimen of *S. scombrus* with the same body size presents a 1.00 cm ED. Also, the PD seems to constitute a good diagnostic feature, with 1.45 times higher in *S. colias* than in *S. scombrus*. The 2 metric dimensions can be easily differentiated only when comparing between fish from the same size class. Nevertheless, if the sample consists of fish of very different sizes, this can be less accurate and even problematic, so that, other features must be used. For instance, parasites might be useful, since they were evenly distributed among fish of different sizes. The high species richness reported in the literature for both species of mackerel might deal with their large geographic distributions, which includes both sides of the Atlantic and the Mediterranean (Collette & Nauen, 1983). Moreover, fish species with larger geographic distributions are expected to have more parasite species, because they have more possibilities of coming into contact with parasites present in different habitats (Rohde, 1984). Besides this, other factors might as well determine high species richness recorded in the literature for the 2 mackerels: the host distributions overlap promotes the transfer of parasite species among host species; and the broad geographic distributions of the host species probably overlap with the distributions of other host species, promoting the horizontal transmission of parasites. Another important point for the transmission of parasites from fish to fish is fish host density (see Poulin & Morand, 2004; Poulin, 2007). This was observed for *S. colias*, which having a wider geographic

distribution than *S. scombrus*, was so far reported infected in the literature with a higher number of ectoparasites (10 versus 8). In this study, 8 species were recorded for the 2 fish (considered together), with *S. colias* holding only 4 species and *S. scombrus* 6. *S. colias* recorded a lower species richness than *S. scombrus*, a difference that might be related with the fact that, the habitat of the 2 mackerels is not the same, with *S. colias* living at greater depths, and thus, in a darker environment. Moreover, the light intensity has been recognized as an important environmental factor influencing the host finding behavior of caligid copepods, which are positively phototactic (Rae, 2002). Other than this, the 2 fish are under the influence of different water temperatures and pressures, what might also help to justify the result found (Rohde, 1984).

From the parasites reported in this work, 4 species might constitute good biological markers of the studied species of mackerel; 2 of them for their specificity in the host - i.e., *P. minor* for *S. colias* and *C. pelamydis* for *S. scombrus* - and the other 2 for their different levels of infection (prevalence and abundance) - i.e., *K. scombri* or *G. cochlear*, with higher infection levels in *S. scombrus*. Indeed, the results of the DFA reinforce the fact that the 2 species of mackerel can be distinguished on the basis of their component parasite fauna. However, for each species we found more homogeneity inside the *S. scombrus* fish (smaller and bigger specimens) than in *S. colias*, what could be related to the difference of the range of distribution that they have access and is expressed in their parasite fauna.

There seems to be a problem in the identification of *S. scombrus* at northern Portuguese fish harbors, and it is likely that misidentifications also occur in markets of other countries; for instance in Morocco both Atlantic mackerels are not differentiated at all. The results of the fish morphological analysis found in this study suggest another feature (i.e., the size of the eye) that should be added to the others currently used to distinguish between both species of Atlantic mackerel. However, the eye size is only easily used if the fish belong to a same size class. If this is not the case, other methods, like the use of parasites, will probably obviate the problem. Four macro-ectoparasites species are likely to constitute good biological markers for *S. colias* and *S. scombrus*, either by their specificity (*P. minor* on *S. colias* and *C. pelamydis* on *S. scombrus*), or by different infection levels (*K. scombri* and *G. cochlear* are more prone of *S. scombrus* than of *S. colias*), and easily separate both Atlantic mackerels.

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Chapter 3

Ectoparasites of Atlantic mackerel, *Scomber scombrus*: macro and microhabitat distribution

Ectoparasites of Atlantic mackerel, *Scomber scombrus*: macro and microhabitat distribution

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Abstract

Atlantic mackerel, *Scomber scombrus*, is a common fish species in Portuguese and Northern Europe coastal waters. Its parasite fauna dynamics are poorly studied. It is known that parasites are affected by the environment where their hosts live. Also, they have a specific distribution among their hosts, occupying a well-defined microhabitat. The present work aims to describe the ectoparasite fauna distribution at the macro and microhabitat levels. For that, fish from two different Portuguese regions, Matosinhos ($n = 40$) and Figueira da Foz ($n = 39$) were examined for macro ectoparasites. *S. scombrus* off Matosinhos presented 5 different species: *Grubea cochlear* and *Kuhnia scombri* (Monogenea), *Nematobothrium scombri* (Digenea), *Caligus pelamydis* (Copepoda) and an Isopoda. In Figueira da Foz, 6 species were collected: *Kuhnia sprostonae* in addition to the two above mentioned Monogenea, *N. scombri*, *C. pelamydis* and *Clavellisa scombri* (Copepoda). The main differences between infection values of fish from the two localities were found in *G. cochlear* (higher infection rates in Matosinhos) and *C. pelamydis* plus *K. sprostonae* (higher values in Figueira da Foz). Regarding the microhabitat of the ectoparasites reported, it could be seen that every species have a very specific distribution within the host. *G. cochlear* and *K. scombri* have a preference for the inner medial areas of gills; *K. sprostonae* can only be found in the pseudobranches; *C. pelamydis* prefer the internal wall of opercula. No interspecific interactions were found among the component species. The results support the idea that the driving forces of community structure of parasites are not the interspecific competitions, but the reinforcement of reproductive barriers and thus, enhancement of the chance to mate.

Keywords: *Scomber scombrus*; macro-ectoparasites; macrohabitat and microhabitat distribution; reproductive barriers.

Introduction

Atlantic mackerel, *Scomber scombrus* Linnaeus, 1758 (Scombridae) is a fish species occurring all over the Portuguese coast and an economically important species due to large fishing activities (Scoles et al., 1998; Martins, 2007). Studies on parasites of *S. scombrus* inhabiting its south distribution area are still scarce. Indeed, there is only one study concerning the helminthofauna of this fish species off the Portuguese coast (Rego et al., 1985). This study reports two monogenetic species parasitizing the gills of *S. scombrus*: *Kuhnia scombri* (Kuhn, 1829) and *Grubea cochlear* Diesing, 1858.

According to Rohde (1984), the environment of parasites, unlike the free-living organisms, comprises two components: the macrohabitat and the microhabitat. The macrohabitat of a parasite species can be defined as all the niche components which represent the habitat of the host (Rohde, 1993). Some physical and chemical factors, such as temperature, water salinity, depth causing different light and pressure conditions and diet of hosts, affect the macrohabitat of a parasite (Rohde, 1993; Tinsley and Jackson, 2002; Rohde, 2005; Costello, 2006).

The microhabitat of a parasite is the host itself, more specifically, the site within the host where the parasite can be found (Rohde, 1984). This microhabitat is not the same for all the parasitic species and each species show a preference for a specific site in the host (Euzet and Combes, 1998; Rohde, 2005). There are several hypotheses trying to explain the factors that lead a parasite species to a specific site or niche: intra and interspecific competition, reinforcement of reproductive barriers, a suitable substrate for attachment and feeding and enhancement of the chance to mate (Rohde, 1979; Rohde, 1993; Rohde, 1994; Ramasamy et al., 1985; Koskivaara et al., 1992; Lo and Morand, 2001; Scott-Holland et al., 2006; Cavaleiro and Santos, 2011). Usually, this kind of studies is focused on the fish gills, where species rich communities of ectoparasites can be found (Koskivaara et al., 1991).

Concerning the parasite species of *S. scombrus*, there is a work by Rohde (1980) which addresses the issue of parasite's microhabitat. However, this study only considers 3 parasitic species: *Caligus pelamydis* Kroyer, 1863, *K. scombri* and *Kuhnia* sp. Accordingly to Rego et al. (1985) and Castro et al. (personal communication), there is one more parasitic species occurring in the gills of *S. scombrus* – *G. cochlear* - which can imply new interactions between species and changes in the parasite's niches.

First, this study aims to make the current characterization of Atlantic mackerel's ectoparasitic fauna at the macrohabitat level, in two localities off the Portuguese coast (Matosinhos and Figueira da Foz), in the summer season, 2011. Second, and at the

microhabitat level, it will focus on the distribution pattern of the ectoparasites, considering the addition of a new species in the microhabitat, and will evaluate the possible interactions among the gills' parasites.

Material and Methods

Characterization of parasites macrohabitat

A total of 40 adult specimens of Atlantic mackerel, *Scomber scombrus* Linnaeus, 1758 (Teleostei: Scombridae) were sampled in July 2011, off Matosinhos (Northern Portuguese coast, 41°11' N, 8°41' W). Additionally, a total of 39 fish from the same species were caught at a Southern location in the Portuguese coast, off Figueira da Foz (40° 8' N, 8°51' W), in September 2011. Fish identification was confirmed using the identification key of Collette (1986). All fish were measured, weighed and sexed. Their total length and weight [mean \pm s.d. (range)] were 35.4 \pm 3.1 (28.5 – 42.0) cm, and 441.9 \pm 137.2 (206.2 – 756.6) g, with 32 females and 8 immature for Matosinhos fishes, and 35.4 \pm 1.26 (37.8 – 33.1) cm, and 406.4 \pm 47.1 (329.5 – 490.6) g, with 11 females and 28 immature for Figueira da Foz fishes.

The fish were surveyed for macro-ectoparasites. The body regions scanned included the integument, eyes, nasal and buccal cavities, fins, pre-opercular channels, opercula and gills. A stereo-dissecting microscope (30 \times magnification) was used to search for parasites. All of them were collected, cleaned and fixed in 70% ethanol for posterior identification. Taxonomic identifications of parasites were carried out following the descriptions and keys of Rohde and Watson (1985a, b) and Rohde (1986, 1989) for Monogenea, Baylis (1938) and Pozdnyakov and Gibson (2001) for Digenea, Kabata (1979, 1992) for Copepoda and Brusca and Iverson (1985) for Isopoda. Parasitological parameters, such as presence-absence data, prevalence (in %) and abundance of infection (mean, standard deviation and range) were calculated, accordingly to Bush et al. (1997), for each parasite species and recorded for fish samples from both localities (Matosinhos and Figueira da Foz). Then, comparisons between the studied localities were conducted: parasite presence-absence data was compared using the χ^2 -test; for abundance values of parasites, the Mann-Whitney's U-test (M-W) was used. Non-parametric tests were preferred over parametric ones owing to the non-normal distribution of the abundance data (one sample Kolmogorov-Smirnov's test, $Z > 1.45$, $p < 0.029$ in Matosinhos and $Z > 0.69$, $p < 0.733$ in Figueira da Foz). The significance level was set at $p < 0.05$ for all statistic tests.

Characterization of parasites microhabitat

In order to characterize the microhabitats of parasites infecting *S. scombrus*, the fish belonging to the above mentioned two samples were used (n = 79). Both branchial chambers were examined for macro-ectoparasites. In each chamber, 52 possible infection sites were considered for analysis: (1) inner surface of the operculum; (2) the pre-opercular channel; (3) the internal wall of the chamber; (4-51) the 4 holobranches; (52) the pseudobranch. Concerning the holobranches, they were successively numbered from I to IV, being the outermost assigned as the number I and the innermost the number IV. Additionally, as shown in Figure 1, each holobranch was divided into 3 segments (p-proximal, m-medial, d-distal), 2 gill areas (i-inner, o-outer) and 2 hemibranches (A-anterior, P-posterior).

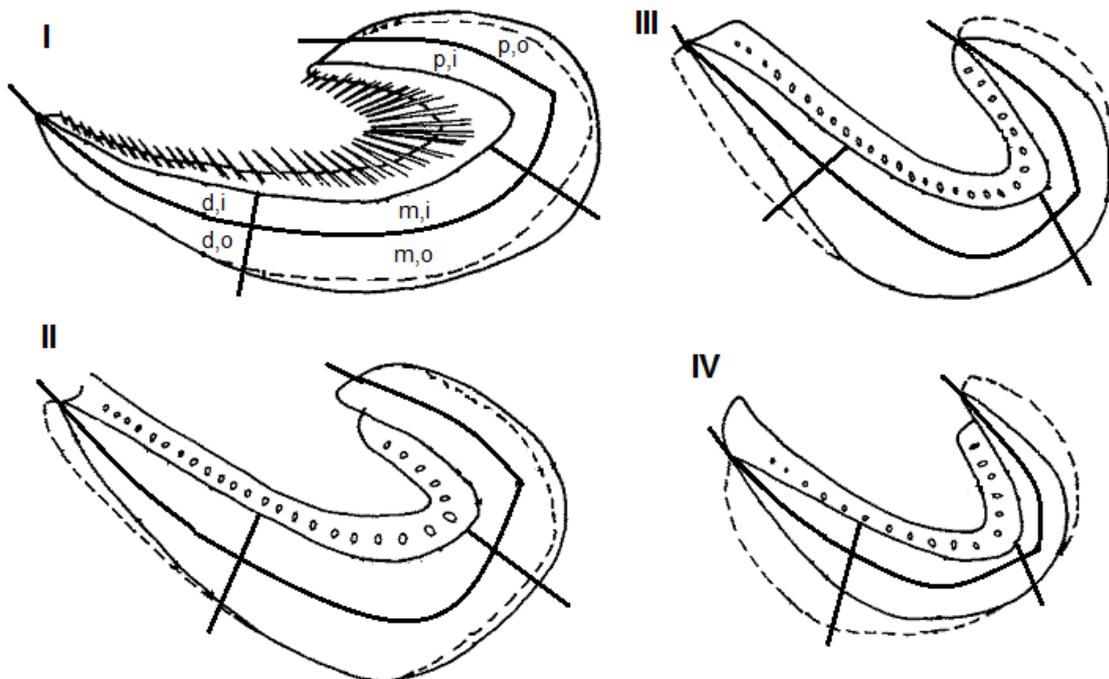


Figure 1. Delimitation of parasites' spatial distribution on the fish gills. Each holobranch was divided in 3 segments (p-proximal, m-medial, d-distal), 2 gill areas (i-inner, o-outer) and 2 hemibranches (anterior, posterior). Only the anterior hemibranch is shown. (Adapted from Rohde, 1984).

In order to determine the microhabitats of the parasites, comparisons between the presence-absence and abundance data of parasites in each possible infection site were conducted. The Cochran test was used to compare parasite presence-absence data between the 4 holobranches, whilst the Friedman was used with abundance data. These same tests were used with proximal, medial and distal segments' comparison. Posterior

multiple comparison tests were conducted. Concerning the gill areas (inner and outer) and the hemibranchs (anterior and posterior), presence-absence data was compared using the McNemar test and abundance data was compared with the Wilcoxon Signed Ranks test. Since the differences on presence-absence and abundance values between the left and the right branchial chambers were not significant (McNemar test: $p > 0.09$; Wilcoxon Signed Ranks test: $Z < -0.92$, $p > 0.14$), data on the both sides were pooled together.

Intra and interspecific relationships were assessed. The existence of significant relationships between the mean abundance of each parasite species and the correspondent number of infection sites was evaluated by the Spearman's rank correlation test. This test was also conducted in order to determine possible relationships (positive or negative) between pairs of species. However, only component species, with prevalence over 10% (Bush et al., 1990) were considered.

Results

Characterization of parasites macrohabitat

The parasitological parameters, for both sampling localities (Matosinhos and Figueira da Foz), are shown in Tables 1 and 2. In total, 7 macro-ectoparasite species - that is, 3 Monogenea, 1 Digenea and 3 Crustacea (2 Copepoda and 1 Isopoda) - were isolated from the 2 regions. From Matosinhos, 5 parasite species were detected, including, 2 monogeneans, as *G. cochlear* and *K. scombri*, 1 digenea [*Nematobothrium scombri* (Taschenberg, 1879)], 1 copepoda (*C. pelamydis*) and an isopoda specimen. From Figueira da Foz, 6 parasite species were found, including *G. cochlear*, *K. scombri*, *Kuhnia sprostonae* Price, 1961 (Monogenea: Mazocraeidae), *N. scombri* (Digenea; Didymozoidae), *C. pelamydis* (Copepoda: Caligidae) and *Clavellisa scombri* (Kurz, 1877) (Copepoda: Lernaepodidae). 3 parasite species were exclusive from each locality (Isopoda from Matosinhos and *Kuhnia sprostonae* and *Clavellisa scombri* from Figueira da Foz).

The comparison of the infection levels recorded for the parasite species shared by the 2 mackerels showed the existence of statistically significant differences. In fact, both *K. sprostonae* and *C. pelamydis* showed higher prevalence and abundance in fish from Figueira da Foz, than from Matosinhos. But on the contrary, *G. cochlear* presented higher infection levels in Matosinhos.

Table 1. *S. scombrus* ectoparasites prevalence data and infection site from Matosinhos (n=40) and Figueira da Foz (n=39). Occurrence was compared with Chi-square test for component species, at least in one of the samples. g = gills; o = opercula; pb = pseudoranches; poc = preopercular channels; w = branchial chamber internal wall; χ^2 = Chi-square test value; p = probability value; * = significant value.

Species	Infection site	Prevalence % (n of infected fish)		χ^2 (p)
		Matosinhos N=40	Figueira da Foz N=39	
Monogenea				
<i>Grubea cochlear</i>	g	45.0 (18)	18.0 (7)	6.7 (0.01)*
<i>Kuhnia scombri</i>	g	72.5 (29)	76.9 (30)	0.2 (0.65)
<i>Kuhnia sprostonae</i>	pb	0 (0)	12.8 (5)	5.5 (0.02)*
Digenea				
<i>Nematobothrium scombri</i>	poc	5.0 (2)	2.6 (1)	-
Copepoda				
<i>Caligus pelamydis</i>	g,o,w	70.0 (28)	100 (39)	13.8 (0.00)*
<i>Clavellisa scombri</i>	g	0 (0)	2.6 (1)	-
Isopoda	g	2.5 (1)	0 (0)	-

Table 2. *S. scombrus* ectoparasites abundance data from Matosinhos (n=40) and Figueira da Foz (n=39). Abundance was compared with Mann-Whitney's *U*-test for component species, at least in one of the samples. s.d. = standard deviation; M-W = Mann-Whitney's *U*-test value; p = probability value; * = significant value.

Species	Abundance: mean \pm s.d. (range)		M-W (p)
	Matosinhos N=40	Figueira da Foz N=39	
Monogenea			
<i>Grubea cochlear</i>	1.1 \pm 2.14 (0-12)	0.3 \pm 0.73 (0-3)	569.0 (0.01)*
<i>Kuhnia scombri</i>	3.5 \pm 4.52 (0-18)	3.0 \pm 3.23 (0-16)	767.0 (0.90)
<i>Kuhnia sprostonae</i>	0	0.3 \pm 0.98 (0-5)	680.0 (0.02)*
Digenea			
<i>Nematobothrium scombri</i>	0.1 \pm 0.22 (0-1)	0.0 \pm 0.16 (0-1)	-
Copepoda			
<i>Caligus pelamydis</i>	3.7 \pm 5.14 (0-23)	17.0 \pm 10.56 (3-48)	135.5 (0.00)*
<i>Clavellisa scombri</i>	0	0.0 \pm 0.16 (0-1)	-
Isopoda	0.0 \pm 0.16 (0-1)	0	-

Characterization of parasites microhabitat

All parasites were found in the branchial chamber. Within this space, parasites were attached in five main regions: gills, opercula internal wall, internal wall of the branchial chamber, pseudobranches and preopercular channels (Figure 2).

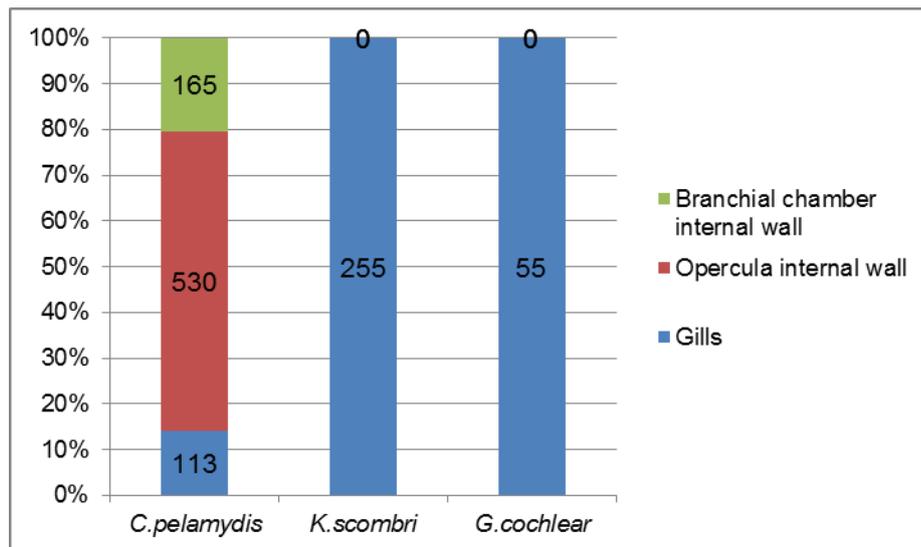


Figure 2. Infection sites of component ectoparasite species of *S. scombrus*. Within the bars, it is indicated the total number of parasite specimens in each site found for the 79 sampled fish.

The distribution of parasites, among the above considered gills regions, is shown on Figure 3. *G. cochlear* presented preference for the medial segment (Cochran test: $Q = 3.15$, $p = 0.00$; Friedman test: $\chi^2 = 4.13$, $p = 0.00$) and the inner area of the gills, only on abundance (Wilcoxon Signed Ranks test: $Z = -3.10$, $p = 0.00$), independently of the holobranches considered. *K. scombri* was the only parasite species showing preference for specific holobranches (I and II) (Cochran test: $Q = 98.33$, $p = 0.00$; Friedman test: $\chi^2 = 102.75$, $p = 0.00$). Additionally, this species is mostly found in the inner (McNemar test: $p = 0.00$, Wilcoxon Signed Ranks test: $Z = -6.47$, $p = 0.00$) and medial (Cochran test: $Q = 95.28$, $p = 0.00$; Friedman test: $\chi^2 = 105.31$, $p = 0.00$) areas of the gills. *K. sprostonae* occurs only on the pseudobranches. At last, *C. pelamydis* have three attachment sites (opercula, gills and internal branchial chamber wall). The preferred site of this species is the opercula internal wall (Cochran test: $Q = 9.89$, $p = 0.01$; Friedman test: $\chi^2 = 59.47$, $p = 0.00$). Even so, it also showed preference for certain regions in the gills, more specifically, the medial segment (Cochran test: $Q = 11.54$, $p = 0.00$; Friedman test: $\chi^2 = 11.40$, $p = 0.00$).

and the external area (McNemar test: $p = 0.00$, Wilcoxon Signed Ranks test: $Z = -4.44$, $p = 0.00$).

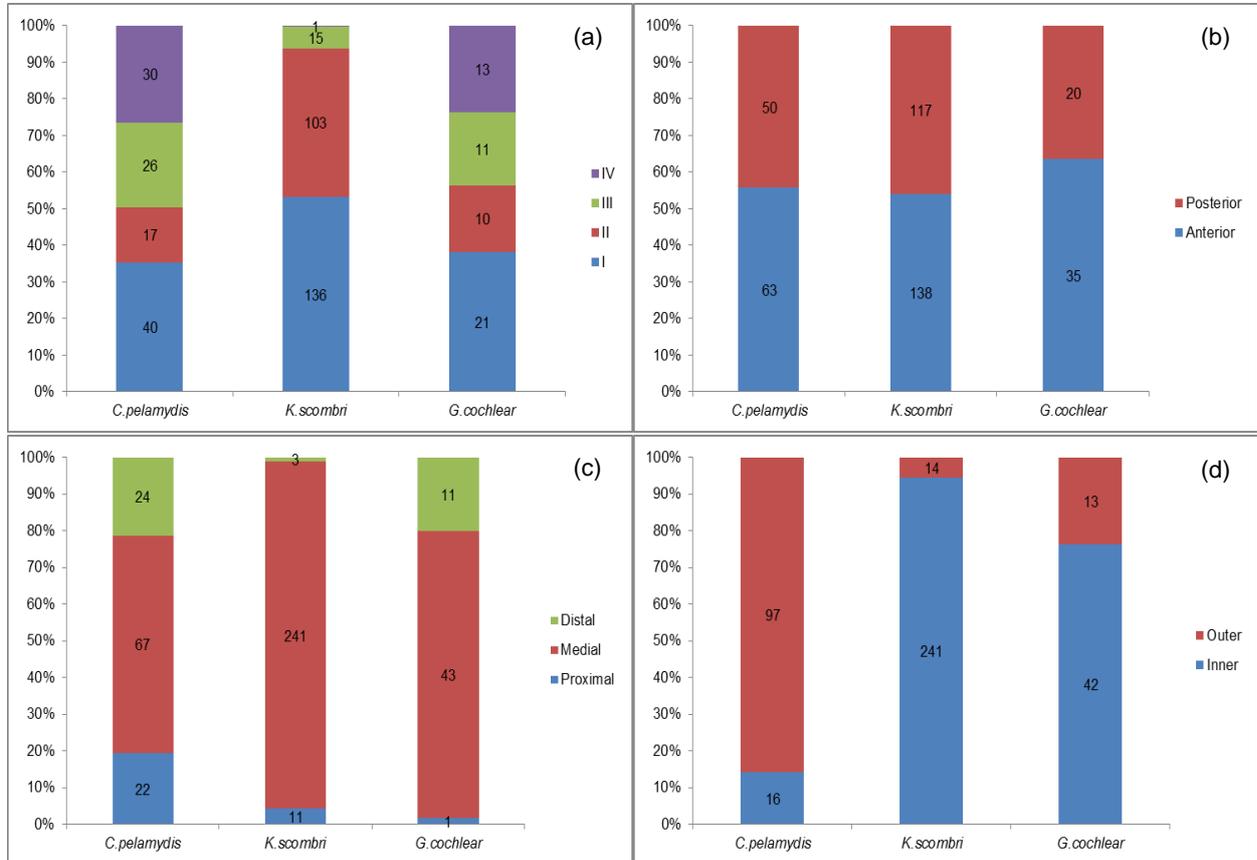


Figure 3. Distributions of parasites among the considered infection sites of the gills (a) in different gill arches (I to IV); (b) in the anterior or posterior hemibranches; (c) in each gill at the distal, medial or proximal site; (d) in each gill at the outer or inner site. Within the bars, it is indicated the total number of parasite specimens in each site found for the 79 sampled fish. Only component parasite species were considered.

The recorded data support the existence of a positive correlation between the parasite abundance and the number of infection sites in the host. Regarding the interspecific relationships of the component ectoparasitic species, there were no significant correlations between pairs of species: *C. pelamydis*/*K. scombri* ($\rho = 0.01$, $p = 0.93$); *C. pelamydis*/*G. cochlear* ($\rho = -0.09$, $p = 0.41$); *K. scombri*/*G. cochlear* ($\rho = 0.05$, $p = 0.62$).

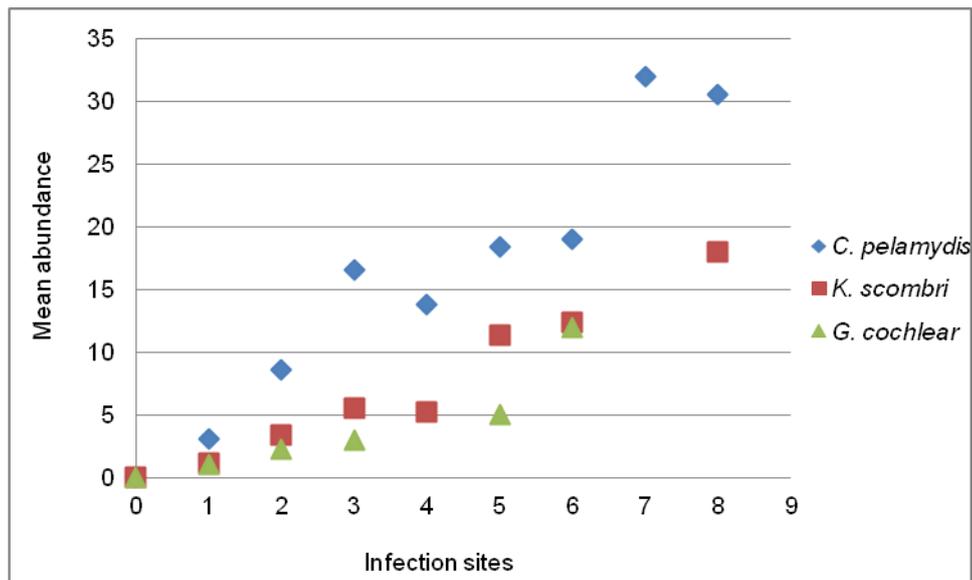


Figure 4. Relationship between the mean abundance of *G. cochlear*, *K. scombri* and *C. pelamydis*, and the number of infection sites on the host's body.

Discussion

There are some studies concerning the ectoparasite fauna of *S. scombrus* (Cressey and Cressey, 1980; Rego *et al.*, 1985; Rohde and Watson, 1985b; Romuk-Wodoracki, 1988; Rohde, 1986, 1989; Somdal and Schram, 1992; Oktener and Trilles, 2009). However, there is only one made in the last 20 years (Oktener and Trilles, 2009), so an update on this subject is needed. In this study, none of the species reported as parasites of *S. scombrus* are new host records. Comparing the present data with the work of Rego *et al.* (1985) - the previous study made in Portugal mainland with fish from lower latitude than the ones from this study - there are two species shared by both works: *G. cochlear* and *K. scombri*. In terms of prevalence values, the ones reported by Rego *et al.* (1985) are much lower – 2.5% against 45.0% (Matosinhos) and 18.0% (Figueira da Foz) for *G. cochlear* and 37.5% against 72.5% (Matosinhos) and 76.9% (Figueira da Foz) for *K. scombri*. In addition, Castro *et al.* (Personal communication) report some data on ectoparasites of *S. scombrus* from Matosinhos. Comparing the results, no evident differences on occurrence and abundance data can be seen between species present in both studies. Matching the results with the other studies, it can be seen that the prevalence values for *G. cochlear* in the present work are the highest. The same can be verified for *K. scombri*, with an exception on the work of Somdal and Schram (1992), where they found a prevalence of 75% in an area near the North Sea. Regarding the Copepoda species, the situation is similar: the only available

quantitative data indicates prevalence values ranging from 0.7% to 34.7% (Somdal and Schram, 1992). The values from Matosinhos (70.0%) and Figueira da Foz (100%) are much higher. This can be due to lower latitude of Portugal, where the sea water temperatures are higher. It is known that copepods are sensitive to water temperature, with higher rates of infection related to higher temperatures (Costello, 2006).

In terms of macrohabitat of the *S. scombrus* ectoparasite fauna, some conclusions can be made. According to the National Oceanographic Data Center (<http://www.nodc.noaa.gov>), sea water temperature tends to increase as the latitude low. Moreover, as mentioned above, the rate of infection by copepods increases with higher temperatures. These facts agree with the observations made on fish from Matosinhos (higher latitude) and Figueira da Foz (lower latitude), the latter reporting higher prevalence and abundance values. Additionally, the aforementioned comparison between this study and the one of Somdal and Schram (1992) supports also this statement. In the case of Monogenea, some authors refer that the rate of development of these organisms is inversely proportional to sea water temperature (Whittington et al., 1999; Tinsley and Jackson (2002). The present results on *G. cochlear* second this idea, being the highest values of infection presented by fish from Matosinhos. Moreover, the comparison between the prevalence values of *K. scombri* from Matosinhos and Figueira da Foz (approximately 75%) with the ones from Rego et al. (1985) (37.5%), is consistent with what has been stated above.

At the microhabitat level, each parasite species showed a preferential distribution within the branchial chamber. It is known that there are differences in water volumes passing over the 4 holobranches, which can affect the opportunities of parasites to attach and live (Llewellyn, 1956). Geets et al. (1997) also refers that the inner areas of the gills are calmer with slower water flow. It is in these areas that the monogeneans *G. cochlear* and *K. scombri* prefer to attach. Moreover, polyopisthocotyleans feed on host's blood (Rohde, 2005), which is available in a large quantity in the fish gills. On the other hand, the Copepoda *C. pelamydis* prefers the opercular internal walls of the host. This species feed on host mucus and tissues, which can explain its location. Also, *C. pelamydis*, when infecting the holobranches, is mostly found in the outer areas, where there is a higher quantity of mucus.

There are two main arguments concerning the niche restriction of parasites. The first one refers the interspecific competition as the main factor determining community structure of parasites (Holmes, 1973; Ramasamy et al., 1985). Oppositely, Rohde (1979, 1994) mentions that interspecific competition plays a minor role in niche restriction, being the reinforcement of reproductive barriers and the enhancement of the chances to mate the real driving forces of community structure. The results of this study seem to support the last theory. Firstly, from the 52 possible infection sites considered, only a few were infected by

parasites in each host – the higher number of infected sites reported per species is 8 – which represents many vacant niches available. Secondly, no significant interactions were verified between the component ectoparasite species. The reinforcement of reproductive barriers seems to play an important role in the niche restriction of *K. sprostonae*. This species can only be found in the pseudobranches, while his congeneric species – *K. scombri* – is present in the gills. This fact can be due to the similarity of their copulatory organs, so they are apart from each other in order to avoid interspecific mating (Rohde, 1994). Moreover, Rohde and Hobbs (1986) report that, among ectoparasites of marine fishes, congener species show less overlap than non-congeners, which is in line with what was observed. Also, *G. cochlear* and *K. scombri* (non-congeners) showed some microhabitat overlap. In addition to the reinforcement of reproductive barriers, more restrict niches lead to an increase of intraspecific contact, which can facilitate mating of the individuals (Rohde, 1994).

Comparing the microhabitat distribution of *S. scombrus* ectoparasites reported by Rohde (1980) with the distributions of this study, it can be seen that there is one more species occupying the gills' environment – *G. cochlear*. Nevertheless the addition of a new species, the microhabitat distribution of the other parasites remains unaffected. Again, this fact supports the idea that interspecific competition is not important in defining the community structure of parasites.

Chapter 4

Final remarks

General discussion

Fish parasitology has been strongly motivated by economic reasons. Parasites are known to cause diseases and mass mortalities on fish from aquacultures and, in order to control and eradicate parasites, hundreds of studies on parasite fauna, immunological responses or histopathology of farmed fish were made in last decades (Pike and Wadsworth, Sakai, Scholz, 1999; Jones, Kent et al., 2001; Costello, 2006). More recently, increasing attention has been paid to the ecology of parasites and their relationships with hosts and ecosystems. For instance, topics like transmission of parasites to hosts, specificity of parasites to hosts and microhabitats and structure of marine parasite communities have been study thoroughly because, despite their relative small size and biomass compared with their hosts, parasites play an important role in ecosystems' structure (Loreau et al., Rohde, 2005).

The present work identified the ectoparasite fauna of *S. colias* and *S. scombrus* caught off northern Portuguese coastal waters. It was mostly composed by monogeneans (*G. cochlear*, *K. scombri*, *K. sprostonae* and *P. minor*) and copepods (*C. pelamydis* and *C. scombri*). Also, most of the parasite species are new geographical records for both hosts, due to the scarce number of published parasitological surveys for *S. colias* and *S. scombrus* from these waters.

Additionally, this study tried to describe the macrohabitat preferences of *S. scombrus*' ectoparasites and which factors determine these preferences. According to the National Oceanographic Data Center (<http://www.nodc.noaa.gov>), sea water temperature tends to increase with lower latitudes. Regarding monogeneans, higher values of occurrence and abundance of *G. cochlear* infecting fish from Matosinhos (45.0%) (higher latitude) than from Figueira da Foz (18.0%) (lower latitude) supports the idea of some authors who refer the sea water temperature as the main factor affecting the growth of these organisms, being the rate of development inversely proportional to sea water temperature (Whittington et al., 1999; Tinsley and Jackson 2002). Also, the prevalence value of *G. cochlear* is higher than the one reported by Rego et al. (1985) (2.5%), which was obtained from fish of a more southerly location. Moreover, the comparison between the prevalence values of *K. scombri* from Matosinhos and Figueira da Foz (approximately 75%) with the ones from Rego et al. (1985) (37.5%), is consistent with what has been stated above. Concerning copepods, it is known that they are sensitive to water temperature, with higher rates of infection related to higher temperatures (Costello, 2006). A gradient can be seen on infection values of *C. pelamydis*, with higher ones at the lowest latitude (Figueira da Foz: prevalence = 100%; mean abundance: 17.0 worms/fish), decreasing in Matosinhos

(prevalence = 70.0%; mean abundance: 3.7 worms/fish) and lowest values are found in the work of Somdal and Schram (1992), with fish from higher latitude (prevalence values varying between 0.7 and 34.7). These results second the argument of Costello (2006).

This work also focused on the subject of microhabitats of *S. scombrus*' ectoparasite species. Each one showed preference for a specific and restricted microhabitat on the host. Blood feeders such as *G. cochlear* and *K. scombri* preferred to attach in the inner areas of host gills. In addition to the blood supply, these areas present a slower water flow, facilitating their attachment (Geets et al., 1997). On the other hand, *C. pelamydis*, which feeds on host's tissues and mucus, preferred the opercular internal walls to live. Also, within the gills, it is mostly found in the outer areas, where the amount of mucus is higher. Apart from these reasons to site selection of parasites, there are other arguments trying to explain microhabitat selection and niche restriction of parasites, namely interspecific competition, reinforcement of reproductive barriers and facilitation of mating (Holmes, 1973; Rohde, 1979, 1980; 1993; 1994; Ramasamy et al., 1985; Lo and Morand, 2000). This study supports the last two arguments for many reasons: only a few of the 52 infection sites considered were infected by parasites in each host; inexistence of significant interactions between the component ectoparasite species; congeneric species (*K. scombri* and *K. sprostonae*) were always found segregated from each other in the branchial chamber; non congeners species (*G. cochlear* and *K. scombri*) shared part of their habitat; and restricted niches may lead to an increase of chances to mate. Finally, when comparing the distribution of ectoparasites in this study with the one of Rohde (1980), it could be seen that the addition of a new species (*G. cochlear*) did not affect the distribution of the remaining parasites.

Overfishing is one of the issues that most concerns scientist and national authorities due to its effects on coastal ecosystems (Jackson et al., 2011). The correct identification of fish species and stocks is extremely important for fisheries management and setting of fish quota among nations (Begg et al., 1999; Abaunza et al., 2008; Gordo et al., 2009). Owing to misidentification problems, capture values of *S. colias* and *S. scombrus* from fisheries may be wrong and biased towards one species. Trying to find solutions, this study presented two alternative methods to correctly identify the Atlantic species of mackerel: the measurement of the eye size and utilization of ectoparasites as biological tags. The first one seems to constitute a good method to distinguish both species, as the eye of *S. colias* is more than 1.5x larger than the one of *S. scombrus*. However, this method can only be easily implemented on fish of the same size and so, the second method shall be used. Indeed, four parasite species seemed to constitute good biological tags to easily distinguish *S. colias* and *S. scombrus*, two by their host specificity (*P. minor* for *S. colias* and *C.*

pelamydis for *S. scombrus*) and two by their different levels of infection (*K. scombri* and *G. cochlear* are more prone of *S. scombrus* than of *S. colias*).

Conclusions

At the end of this work, some ideas must be highlighted. Regarding the misidentification problem of *S. colias* and *S. scombrus*, two new methods for more easily distinguish both species should be considered: the comparison of the eye size and the use of ectoparasites as biological tags.

The macrohabitat of parasites is mostly influenced by temperature, which controls infection levels of ectoparasites on hosts. Monogenea seems to prefer colder waters, whereas the opposite happens with Copepoda. Microhabitat and niche selection of ectoparasites of *S. scombrus* is due to feeding habits, reinforcement of reproductive barriers and facilitation of chances to mate.

Chapter 5

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