Seasonality of metazoan ectoparasites in marine European flounder *Platichthys flesus* (Teleostei: Pleuronectidae)

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SUMMARY

Seasonal occurrence of metazoan ectoparasites is described for the first time in marine European flounder, *Platichthys flesus* (L.). The parasitofauna, in this study monitored during 1 year, was found to be similar to that previously recorded for flounder. Moreover, specimens of *Caligus* sp. Müller, 1785 and *Lepeophtheirus pectoralis* (Copepoda: Caligidae), *Acanthochondria cornuta* (Copepoda: Chondracanthidae), *Holobomolochus confusus* (Copepoda: Bomolochidae) and *Nerocila orbignyi* (Isopoda: Cymothoidae), and also, a praniza larva (Isopoda: Gnathiidae), were isolated. From these, *L. pectoralis* and *A. cornuta* were the dominant parasites in all samples of flounder, while *Caligus* sp., *H. confusus*, *N. orbignyi* and the gnathiid praniza seemed to infect the flounder only occasionally. As far as the seasonality of infections is concerned, it differed considerably from that described for estuarine environments. Indeed, both prevalence and abundance of *L. pectoralis* and *A. cornuta* reached significant peaks in the summer, whereas the literature identifies the autumn as the season of maximum infection on estuarine flounder. Thus, the former period seems more favourable for the occurrence of epizooties of *L. pectoralis* and *A. cornuta* in flounder culturing systems running on seawater and operated in the studied or similar environments.

Key words: seasonal occurrence, epizooties, metazoan ectoparasites, *Platichthys flesus*.

INTRODUCTION

The incidence of many pathogens varies conspicuously from season to season (e.g. Altizer *et al.* 2006). In particular, for marine fish parasites, seasonality patterns were recorded in many species, although they seem to be absent in some others (Rohde, 1982). Seasonal occurrence may suggest periods during which epizootic outbreaks are likely to be favoured, guiding the identification of optimal moments for prophylactic measures in fish-culturing systems. So, this knowledge is extremely important in preventing economic losses for the fishermen and fish farm owners.

The European flounder, *Platichthys flesus* (Linnaeus, 1758) (Teleostei: Pleuronectidae), is a flatfish species of high commercial value in several European countries, where it represents a considerable portion of the total flatfish catches. Furthermore, it constitutes an important source of income for the emerging aquaculture industry of many such countries.

The infection of the European flounder by different metazoan ectoparasitic taxa has been characterized through several studies devoted to different geographical locations. These extend from the Norwegian Sea, in the north, to the far south as the Mediterranean Sea. In a recent study, the authors provided a review of the pertinent literature on the subject (Cavaleiro and Santos, 2007). Some of the identified parasites may inflict severe pathological lesions to the host fish, which justifies a detailed study of their dynamics. Moreover, *Lepeophtheirus pectoralis* (Müller, 1777) (Copepoda: Caligidae), one of the most reported parasites of marine origin, has been associated with the occurrence of skin laceration, erosion and haemorrhaging, and fin erosion and destruction (Scott, 1901; Mann, 1970; Boxshall, 1977). In intensive culturing systems, some of these lesions may become fairly extensive, since the fish are kept close together, a situation that favours opportunistic behaviour of parasites. Therefore, investing in prophylactic measures is mandatory for minimizing economic losses.

Although previous studies have already reported seasonal occurrences of European flounder's endoparasites, e.g., *Cucullanellus minutus* (Rudolphi, 1802) and *Cucullanus heterochrous* (Rudolphi, 1802) (Nematoda: Cucullanidae) (Sulgostowska *et al.* 1987), as far as we are aware, no information is

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available on the seasonal occurrence of marine European flounder's ectoparasite infections. Indeed, all reported studies on flounder's ectoparasites either concern estuarine environments (van den Broek, 1979; Schmidt *et al.* 2003) or the Baltic Sea (Chibani and Rokicki, 2004), which is known as the world's largest brackish water sea area (Leppäkoski *et al.* 2002).

In this study, the seasonal occurrence of metazoan ectoparasites in marine European flounder is investigated for the first time. This provides a more complete knowledge of the flounder's metazoan ectoparasite richness and dynamics in the studied geographical area, i.e., the northern Portuguese coast.

MATERIALS AND METHODS

Fish sampling and parasitological examination

The present study was intended to investigate the seasonal occurrence of metazoan ectoparasites in marine European flounder.

During 1 year, i.e., from Summer 2005 to Spring 2006, fish caught via demersal bottom trawling in marine coastal waters off the northern Portuguese town of Matosinhos (41°10'N, 8°42'W) were collected seasonally at the city harbour. The sampling times could not be uniformly distributed along the year, as they depended on the availability of flounder at the harbour. Actually, they were set as follows: Summer 2005 – 2 September; Autumn 2005 – 17 November; Winter 2006 – 2 March; Spring 2006 – 23 May. The specimens (N=30 per season) were randomly collected, and then placed, individually, in plastic bags. They were brought to the laboratory, located in Porto's University campus, and kept frozen at –20 °C until being examined for parasites.

During the survey, they were first measured for total body length, sexed and the weight of their gonads recorded. Following the Hutchinson's definition of ecological niche (Hutchinson, 1957), several locations on the flounder's body surface i.e. skin, fins, eyes and branchial, nasal and buccal cavities, were examined for metazoan ectoparasites under a stereoscopic microscope. The isolated parasite specimens were cleaned in 35‰ saline solution and fixed in 70% ethanol. Later, copepods were cleared for about 1 h in 90% lactic acid (Humes and Gooding, 1964).

Identification of parasites to the species level followed the identification keys in Kabata (1979, 1992) for Copepoda, and Naylor (1972) and Bruce (1987) for Isopoda. Species identification of female gnathiid pranzia was not possible because the keys found in the technical literature are exclusively based on the male's morphology. The stage of development was identified in accordance with Boxshall (1974a) and Heegaard (1947) for specimens of *L. pectoralis* and *Acanthochondria cornuta*, respectively. Sea water

temperature values were obtained from the Physical Oceanography Distributed Active Archive Center (PO.DAAC) at the NASA Jet Propulsion Laboratory (NASA Jet Propulsion Laboratory, 2009), while the light intensity figures were acquired via the Portuguese Meteorology Institute website (Instituto de Meteorologia – IP Portugal, 2009). According to the data presented in an European Environmental Agency's report (European Environmental Agency, 2008), salinity levels were assumed constant along the year, with values close to 35‰.

Data analysis

As far as the fish characteristics are concerned, we started to compare fish lengths among sampling seasons. For that, the Kruskal-Wallis test was used, since the data did not follow a normal distribution, as proved by the Kolmogorov-Smirnov test ($0.419 \leq Z \leq 1.266$; $P \geq 0.081$). The seasonal variations of females and males gonads weights were used to determine the flounder's spawning period and, thus, the migration timings between estuarine environments and the open sea, since no such information was available in the literature for fish of the Portuguese coast. Box-plots were used to depict the distribution of body length and gonads weight in the samples.

With respect to infection parameters, prevalence (%) and abundance were assessed in accordance to Bush *et al.* (1997) and used to express occurrence of parasites in the samples. Total abundance is the sum of all abundance values recorded for the 30 fish in each sampling season. Values corresponding to the total abundance of identified stages of development were also determined. Dependence of parasite abundance on fish body size i.e. total body length, and sex was evaluated using the Spearman's correlation test and the Mann-Whitney's *U* test, respectively. Besides this, the infection levels were analysed with respect to their seasonal variation during the sampled period. Tests of statistical difference were only performed for component parasitic species (Prevalence > 10%) (Bush *et al.* 1990), in all samples using the χ^2 -test (comparison between all samples) and the Fisher's exact test (pairwise sample comparisons) for prevalence, and the Kruskal-Wallis test (comparison between all samples) and the Mann-Whitney's *U* test (pairwise sample comparisons) for abundance. Evaluation of seasonal trends in parasite abundance was also conducted exclusively for the component species. It followed an *r*-squared for cubic fit regression analysis, the results of which served also to reveal the occurrence of infections at the infrapopulation level. Aggregation of component species among flounders was evaluated using the variance to mean abundance ratio (Poulin, 2007). All analyses, as well as the box-plots and the regression plots, were performed using version 16.0 of SPSS

Fig. 1. Distributions of fish body size data (total length) concerning the 4 samples (Summer 2005, Autumn 2005, Winter 2006 and Spring 2006) of European flounder *Platichthys flesus* (Linnaeus, 1758) caught off Matosinhos, northern Portuguese coast.

for Windows (SPSS Inc., Chicago, IL, USA). The results for the statistical tests were considered significant for $P \leq 0.05$.

RESULTS

Fish body length varied between 20.1 and 42.7 cm (mean $\pm$ s.d. = 28.22 ± 4.42 cm) and is depicted in the box-plot chart of Fig. 1. In each box-plot, the range identifies the values encompassing 95% of the recorded data, while the outliers and the extremes are located outside that range. No significant differences were found among sampling seasons when the analysis was made with respect to that fish characteristic (Kruskal-Wallis test: $\chi^2 = 0.269$; D.F. = 3; $P = 0.966$). The sex distribution in the 4 samples of flounder was as follows: 20 ♀♀, 10 ♂♂ (Summer 2005); 17 ♀♀, 13 ♂♂ (Autumn 2005); 11 ♀♀, 19 ♂♂ (Winter 2006); and 21 ♀♀, 9 ♂♂ (Spring 2006). Seasonal variations on gonads weight were found similar for females and males, although they were more pronounced in the case of females (Fig. 2). Moreover, weight values remained fairly constant throughout the year, except in Winter 2006, the season when considerably higher values were recorded.

The recorded environmental data, i.e. sea surface temperature and light intensity, are presented in Fig. 3. This shows that both of these parameters varied along the sampled period, being considerably more expressive in late Summer 2005 and Spring 2006.

Parasites from 6 metazoan ectoparasitic taxa were identified. They included exclusively the following crustaceans: *Caligus* sp. Müller, 1785 and *Lepeophtheirus pectoralis* (Müller, 1777) (Copepoda: Caligidae); *Acanthochondria cornuta* (Müller, 1776) (Copepoda: Chondracanthidae); *Holobomolochus confusus* (Stock, 1959) (Copepoda: Bomolochidae); *Nerocila orbignyi* (Guérin-Méneville, 1832)

(Isopoda: Cymothoidae); and praniza larva (Isopoda: Gnathiidae).

L. pectoralis and *A. cornuta* were component parasitic species in all seasonal samples of flounder. The former occurred mainly on the fish's body skin and beneath the pectoral and pelvic fins, while the latter was mostly observed inside the gill cavities. Overall prevalence and abundance (mean $\pm$ s.d.) levels were as follows: 80% and 8.8 ± 10.1 parasites per host for *L. pectoralis*; and 86.7% and 21.6 ± 23.0 parasites per host for *A. cornuta*. Association between abundance of component species and fish body size/sex was never found, either when samples were considered separately, or when all fish were pooled together in the same analysis (Spearman's correlation test for *L. pectoralis*: $-0.180 \leq r_s \leq 0.186$, $P \geq 0.326$ and for *A. cornuta*: $0.000 \leq r_s \leq 0.306$, $P \geq 0.100$; Mann-Whitney's *U* test for *L. pectoralis*: $66.0 \leq U \leq 1728$, $P \geq 0.071$ and for *A. cornuta*: $77.0 \leq U \leq 1618$, $P \geq 0.428$).

Concerning prevalence, the sample of Summer 2005 recorded the maximum possible level i.e. 100%, for both *L. pectoralis* and *A. cornuta* (Fig. 4A). The lowest levels were recorded for Autumn 2005: *L. pectoralis*: 43% and *A. cornuta*: 67%. Levels regarding the 4 seasons of sampling were found to present significant differences (χ^2 -test for *L. pectoralis*: $\chi^2 = 36.250$, D.F. = 3, $P = 0.000$ and for *A. cornuta*: $\chi^2 = 14.460$, D.F. = 3, $P = 0.002$). Consequently, pairwise comparisons were performed using the Fisher's exact test (Table 1). Such analyses revealed that the pair (Summer 2005 – Autumn 2005) was unique, as it presented significant differences in prevalence for both *L. pectoralis* and *A. cornuta*. Prevalence of *Caligus* sp. was more expressive in Summer 2005 (17%) and Spring 2006 (20%), while that of *N. orbignyi* showed a single peak in Summer 2005 (10%). Depending on the sampling season, prevalence values for all other identified taxa i.e. *H. confusus* and gnathiid pranizae were of 3% or

Fig. 2. Seasonal variations on females and males gonads weight concerning the 4 samples (Summer 2005, Autumn 2005, Winter 2006 and Spring 2006) of European flounder *Platichthys flesus* (Linnaeus, 1758) caught off Matosinhos, northern Portuguese coast.

Fig. 3. Monthly variations (from July 2005 till June 2006) in sea surface temperature and in light intensity levels off Matosinhos, northern Portuguese coast.

0%. For both *L. pectoralis* and *A. cornuta*, prevalence values recorded for adults were always more expressive than those regarding the other stages of development, i.e. pre-adults and juveniles, reaching their maximum values in Summer 2005 (Fig. 4B). Furthermore, as far as the adult life-cycle stage is concerned, seasonal variations in prevalence were found to be more noticeable for *L. pectoralis*. This was also verified for pre-adults and juveniles.

As shown in Fig. 5A, the seasonal variations of total abundance were found to be similar to those recorded for prevalence. The highest values were recorded in Summer 2005 for both *L. pectoralis* – 423 parasites – and *A. cornuta* – 1 429 parasites, while the lowest were found in Autumn 2005 – *L. pectoralis* – 34 parasites – and *A. cornuta* – 197 parasites. Because of the non-normality of abundance data in some of the samples, as revealed by the

Fig. 4. Seasonal prevalence levels (Summer 2005, Autumn 2005, Winter 2006 and Spring 2006) concerning: (A) the metazoan ectoparasitic taxa found infecting the European flounder *Platichthys flesus* (Linnaeus, 1758) off Matosinhos, northern Portuguese coast; (B) the life-cycle stages of the identified component parasitic species, i.e., *L. pectoralis* and *A. cornuta*.

Table 1. Pairwise comparisons of prevalences (results of the Fisher's exact test) concerning the 4 seasonal samples (Summer 2005, Autumn 2005, Winter 2006 and Spring 2006) of European flounder *Platichthys flesus* (Linnaeus, 1758) caught off Matosinhos, northern Portuguese coast (* indicates significant results)

	Autumn 2005	Winter 2006	Spring 2006
<i>L. pectoralis</i>			
Summer 2005	$P=0.000^*$	$P=0.052$	$P=0.492$
Autumn 2005		$P=0.003^*$	$P=0.000^*$
Winter 2006			$P=0.424$
<i>A. cornuta</i>			
Summer 2005	$P=0.001^*$	$P=0.112$	$P=0.237$
Autumn 2005		$P=0.125$	$P=0.057$
Winter 2006			$P=1.000$

Kolmogorov-Smirnov test (*L. pectoralis*: $0.897 \leq Z \leq 1.930$, $0.001 \leq P \leq 0.398$; *A. cornuta*: $0.611 \leq Z \leq 1.572$, $0.014 \leq P \leq 0.849$), the Kruskal-Wallis test

was used to compare abundance of component parasitic species among sampling seasons. Furthermore, similar to what was described for prevalence, it was found that the recorded abundance levels presented significant differences among the 4 sampling seasons (Kruskal-Wallis test for *L. pectoralis*: $\chi^2=54.473$, D.F.=3, $P=0.000$ and for *A. cornuta*: $\chi^2=54.586$, D.F.=3, $P=0.000$). The results for the pairwise comparisons (using a Mann-Whitney's *U* test) are presented in Table 2. In this case, only the pair (Winter 2006 – Spring 2006) did not present any significant difference in abundance levels. Adults of *L. pectoralis* and *A. cornuta* were always found to be dominant over the other life-cycle stages i.e. pre-adults and juveniles, reaching maximum levels in Spring 2006 for *L. pectoralis* and Summer 2005 for *A. cornuta* (Fig. 5B). In addition, in both cases, the total abundance was found to vary notoriously throughout the year. Occurrence of pre-adults and juveniles was more expressive and variable in the case of *L. pectoralis*. Seasonal trends of variation in abundance levels (component species only) are presented in the regression plots of Fig. 6A and

Fig. 5. Seasonal total abundance levels (Summer 2005, Autumn 2005, Winter 2006 and Spring 2006) concerning: (A) the metazoan ectoparasitic taxa found infecting the European flounder *Platichthys flesus* (Linnaeus, 1758) off Matosinhos, northern Portuguese coast; (B) the life-cycle stages of the identified component parasitic species, i.e., *L. pectoralis* and *A. cornuta*.

B. Such plots show evident and synchronized trends for *L. pectoralis* and *A. cornuta*, with pronounced occurrences in Summer 2005. Moreover, they show clear evidence that, in most cases, the occurrence of both of these species was fairly expressive at the infrapopulation level. When present, parasitic taxa other than *L. pectoralis* and *A. cornuta* were always found to record rather low total abundance values (*Caligus* sp. – 5 specimens in Summer 2005 and 6 specimens in Spring 2006; *N. orbignyi* – 3 specimens in Summer 2005; *H. confusus* – 1 specimen in Summer 2005; gnathiid praniza – 1 specimen in Autumn 2005).

Although the mean abundance varied between sampling seasons, the variance was always greater than the mean. In fact, the variance to mean abundance ratio ranged from 6.9 to 10.9 for *L. pectoralis* and from 10.8 to 20.8 for *A. cornuta* (Fig. 7). This suggests that the populations of those 2 species were aggregated among flounders. The smallest aggregation levels were recorded in Summer 2005 and

Autumn 2005 for *L. pectoralis* and in Summer 2005 for *A. cornuta*.

DISCUSSION

As far as richness is concerned, all 6 identified taxa of metazoan ectoparasites had already been recorded in previous surveys (see Cavaleiro and Santos, 2007). However, Chibani and Rokicki (2004) found a very distinct parasitofauna infecting the European flounder of the Gulf of Gdańsk, Baltic Sea. In that study, monogeneans i.e., *Gyrodactylus unicopula* (Glukhova, 1955) and *G. flesi* (Malmberg, 1957) (Monogenea: Gyrodactylidae), were recorded, contrary to parasitic crustaceans. Such a situation might reflect the different salinity conditions in the 2 environments. In fact, the Baltic Sea constitutes a very unique aquatic environment, as it is a semi-enclosed basin of about 415 000 km² (European Science Foundation, 2007) separated from the North Sea and the north-eastern Atlantic Ocean by both

Fig. 6. Seasonal abundance trends of *L. pectoralis* (A) and *A. cornuta* (B) concerning the 4 samples (Summer 2005, Autumn 2005, Winter 2006 and Spring 2006) of European flounder *Platichthys flesus* (Linnaeus, 1758) caught off Matosinhos, northern Portuguese coast.

Table 2. Pairwise comparisons of abundances (results of the Mann-Whitney's *U* test) concerning the 4 seasonal samples (Summer 2005, Autumn 2005, Winter 2006 and Spring 2006) of European flounder *Platichthys flesus* (Linnaeus, 1758) caught off Matosinhos, northern Portuguese coast (* indicates significant results)

	Autumn 2005	Winter 2006	Spring 2006
<i>L. pectoralis</i>			
Summer 2005	<i>U</i> = 20 000 <i>P</i> = 0.000*	<i>U</i> = 232 000 <i>P</i> = 0.001*	<i>U</i> = 339 500 <i>P</i> = 0.102
Autumn 2005		<i>U</i> = 147 500 <i>P</i> = 0.000*	<i>U</i> = 72 000 <i>P</i> = 0.000*
Winter 2006			<i>U</i> = 332 500 <i>P</i> = 0.082
<i>A. cornuta</i>			
Summer 2005	<i>U</i> = 38 000 <i>P</i> = 0.000*	<i>U</i> = 83 500 <i>P</i> = 0.000*	<i>U</i> = 142 500 <i>P</i> = 0.000*
Autumn 2005		<i>U</i> = 309 000 <i>P</i> = 0.036*	<i>U</i> = 187 500 <i>P</i> = 0.000*
Winter 2006			<i>U</i> = 324 000 <i>P</i> = 0.062

geographical and ecological (e.g. coldness and low salinity) barriers (Leppäkoski *et al.* 2002). Its area-averaged sea-surface temperature may vary from 5 to 10 °C (European Science Foundation, 2007). In this study, the temperature values of the Atlantic were always higher than these. Thus, since temperature is generally accepted as the most important abiotic factor influencing the occurrence of parasites, including Copepoda (Kabata, 1981), the

differences found for this environmental parameter may help to justify the variations recorded for parasitofauna. Additionally, salinity is also recognized as an important environmental factor limiting the distribution of many marine organisms in coastal waters (Gunter, 1961; Schmidt *et al.* 2003). Thus, since many ectoparasites (e.g. copepods) are known as stenohaline (e.g. Kabata, 1979; Knudsen and Sundnes, 1998), being only capable of surviving in rather narrow ranges of high salt concentrations, it is probable that salinity also influenced the differences recorded in the parasitofauna. To support this hypothesis, it should be mentioned that, while studying stenohalinity of *L. pectoralis* and *A. cornuta*, Möller (1978) reported a lower capacity of survival in reduced salinity levels for both species.

According to the infection levels herein reported, we may conclude that the infection of the European flounder by *L. pectoralis* and *A. cornuta* is probably quite frequent in the northern Portuguese coast. Conversely, the low infection levels of *Caligus* sp., *H. confusus*, *N. orbignyi* and gnathiid praniza indicate that these species are probably rare in European flounder. Nevertheless, it is noteworthy that the infection by gnathiid praniza may be underestimated. In fact, when praniza larva infects teleost fishes, it remains attached just for a few hours, a period of time in which it feeds on the host's blood. Besides this, many species were found to leave their hosts just after their capture, while others only feed at night (Lester, 2005). Concerning *N. orbignyi*, the species has been frequently reported in fish of the north-west African shelf (Rokicki, 1997). Still regarding the Portuguese coast, reports on infection of fish other

Fig. 7. Seasonal variations on the variance to mean abundance ratio of *L. pectoralis* and *A. cornuta* concerning the 4 samples (Summer 2005, Autumn 2005, Winter 2006, Spring 2006) of European flounder *Platichthys flesus* (Linnaeus, 1758) caught off Matosinhos, northern Portuguese coast.

than European flounder (Cavaleiro and Santos, 2007) and Lusitanian toadfish *Halobatrachus didactylus* (Bloch and Schneider, 1801) (Marques *et al.* 2005) were not found in the literature. This may suggest that the parasite might not be so common in this geographical area, preferring warmer waters.

As far as the seasonality is concerned, we should first analyse the results of the statistical tests performed on fish sex and body size data, as these variables are sometimes concurrent inducers of prevalence and abundance variations beyond the seasonality itself. Indeed, one of the reasons generally accepted for explaining the increase in infection levels is that larger fish come into contact with bigger volumes of water, having larger binding surfaces, and more nutrients available. Besides this, it is sometimes observed that one of the sexes is more prone to infection than the other (Rohde, 1984). In this study, not only the fish presented no significant differences in body size among samples, as no relation was found between the recorded abundance levels and the fish body size or sex. Therefore, the differences found between the infection levels recorded along the sampling period should be attributed to seasonality. Indeed, both *L. pectoralis* and *A. cornuta* were previously suggested to have annual occurrences in European flounder (van den Broek, 1979) and in closely-related flatfish species i.e. the plaice *Pleuronectes platessa* (Linnaeus, 1758) (Boxshall, 1974b).

In this research, all the 6 identified parasitic taxa have been investigated for seasonal occurrence in marine European flounder. Nevertheless, only *L. pectoralis* and *A. cornuta* presented seasonality, as these were the only ones to show expressive occurrences in flounder along the sampled period. Moreover, in both of these species, infections reached significant maxima in prevalence and abundance in the summer season, showing concurrent behaviour throughout the sampled period. Such a situation might have been influenced by their monoxenous

life cycles and direct modes of transmission. Besides this, the temperature and the light intensity levels may also have influenced the recorded trend, since they showed a similar evolution during the sampling period, with highest levels being recorded in late spring and summer. Moreover, as far as the light intensity is concerned, at least the copepodids of *L. pectoralis* seem to be positively phototactic (Boxshall, 1976). Furthermore, durable changes in light intensity levels might be expected to directly impact the parasite's host-finding success, as they might influence its swimming performance. Thus, this may help to justify the increase of infection levels verified in the summer season.

It should be noted that previous studies in the literature already reported increases in infection levels of ectoparasitic crustaceans, copepods included, in the summer (Boxshall, 1974b; Hogans and Trudeau, 1989; Rokicki and Strömberg, 1991; Schram *et al.* 1998; Hakalahti and Valtonen, 2003; Costello, 2006). Indeed, such increases were also noticed for some other ectoparasites and hosts, like *Trichodina* sp. (Oligohymenophorea: Trichodiniidae), *Diplectanum* sp. (Monogenea: Diplectanidae) and *Caligus* spp. (Copepoda: Caligidae), found infecting the sea bass, *Dicentrarchus labrax* (Linnaeus, 1758), off the northern Portugal (Santos, 1998).

The notorious decrease of infection levels recorded for autumn may also be considered as an indication that flounder may have recently migrated from lower salinity areas. Further, in the winter season, flounders probably entered the spawning period, as suggested by the notorious increase verified in both females and males gonads weight. So, the gradual increase of infection levels, recorded for winter and following seasons, may also indicate that the fish are now in a higher salinity environment, favourable for the life-cycle and survival of many parasitic copepods.

In estuarine environments, both *L. pectoralis* and *A. cornuta* were shown to exhibit a clear seasonal

pattern. Indeed, in the course of a study on the parasites infecting the European flounder at 5 different locations in the German Bight, North Sea, with sampling campaigns performed only in the spring and autumn, Schmidt *et al.* (2003) reported a marked seasonality for *L. pectoralis*. Moreover, the latter species was found to present significantly higher infection levels i.e. prevalence and intensity, in the autumn. This seasonal trend was comparatively less clear for *A. cornuta*, as it was only recorded in one of the sampling localities. In another study devoted to the temporal dynamics of the copepod infections in the European flounder of the Medway Estuary, North Sea, the trends of variation recorded for *L. pectoralis* and *A. cornuta* also differed markedly from the ones now reported (van den Broek, 1979). In that study, the prevalence of both parasite species reached 100% between October and December (i.e. in autumn), while the minimum was recorded on April (i.e. in spring) for *L. pectoralis* (4.2%), and between February and June (i.e. in winter and spring) for *A. cornuta* (21.4%). Mean intensity was also found to exhibit seasonal variation, with maximum levels occurring in December (i.e. in autumn) for both *L. pectoralis* (≈ 9 parasites/fish) and *A. cornuta* (21 parasites/fish), and minimums occurring on April (i.e. in spring) for *L. pectoralis* (≈ 1 parasite/fish) and throughout the whole year for *A. cornuta*.

In summary, the ectoparasitofauna of European flounder in estuarine and marine environments is similar, the component species exhibiting seasonal variations in both environments. However, there is a clear difference in the seasonal pattern identified in the literature for estuarine flounder and the one now reported for the examined marine specimens.

One factor that may help to justify this difference is the disparate values of solar influx typical of the North Sea and the northern Portuguese coast. Indeed, such environmental parameter has been recognized as an important factor influencing the strong seasonality observed for parasites in northernmost regions of the Earth (Hemmingsen *et al.* 1995). In addition, the daily variations in salinity levels that are more pronounced in winter than in summer, and which are typically found in estuarine environments, may have also contributed to some extent, to the differences herein reported.

Besides the identification of ectoparasite species and the seasonality of their occurrence, this work further contributes to the characterization of the parasitofauna of marine European flounders by reporting the evolution of life-cycle stages and aggregation levels during the year.

Starting with the infection levels due to the various life-cycle stages, the recorded data indicate that the reproduction cycle of *L. pectoralis* and *A. cornuta* runs throughout the year. However, the infection levels due to pre-adults and adults, in summer, suggest that this season is more favourable for the

occurrence of epizootic outbreaks of these 2 parasite species.

It was also in the summer that these component parasitic species presented a lower aggregation level. This situation might be explained by different factors acting together, including the host's higher susceptibility to infection, the greater exposure times to parasites or even the host's greater aggregation (Bandilla *et al.* 2005; Poulin, 2007). The latter is particularly relevant in culturing systems, where fish are usually kept at high density.

In conclusion, the results reported in this work, and the correspondent discussion, provide clear evidence that summer is the period when epizooties of *L. pectoralis* and *A. cornuta* will be likely to be favoured in marine flounder. Therefore, it seems that, if prophylactic measures are considered as a possible means to avoid epizootic outbreaks caused by ectoparasites, these should preferably take place during the spring and summer if applied to flounder raised in marine water farms.

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