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Survey of the Metazoan Ectoparasites of the European Flounder *Platichthys flesus* (Linnaeus, 1758) along the North-Central Portuguese Coast

Francisca I. Cavaleiro and Maria J. Santos*, Universidade do Porto, Faculdade de Ciências, Departamento de Zoologia-Antropologia, Praça Gomes Teixeira, 4099-002 Porto, Portugal, and CIMAR Laboratório Associado/CIIMAR, Centro Interdisciplinar de Investigação Marinha e Ambiental, Rua dos Bragas, 289, 4050-123 Porto, Portugal; *To whom correspondence should be addressed. *e-mail: mjsantos@fc.up.pt*

ABSTRACT: A survey was undertaken to identify metazoan ectoparasite species on the European flounder, *Platichthys flesus* (Linnaeus, 1758), in 4 different locations off the north-central Portuguese coast. Parasites of 7 different taxa were found: *Caligus diaphanus*, *Caligus* sp., and *Lepeophtheirus pectoralis* (Copepoda: Caligidae); *Acanthochondria cornuta* (Copepoda: Chondracanthidae); *Holobomolochus confusus* (Copepoda: Bomolochidae); *Nerocila orbignyi* (Isopoda: Cymothoidae); and praniza larvae (Isopoda: Gnathiidae). *Lernaeocera branchialis*, a common European flounder parasite in the North and Baltic Seas, was not observed among the surveyed fish. *Caligus diaphanus*, *Caligus* sp., and *Nerocila orbignyi* are new host records. The high prevalence and intensity values recorded for *L. pectoralis* and *A. cornuta* suggest that both parasite species are common to the European flounder along the north-central Portuguese coast. In contrast, infection levels with respect to the other parasite taxa were, in most cases, comparatively lower, thereby indicating that they only occur occasionally among flounders in the surveyed area.

The European flounder *Platichthys flesus* (Linnaeus, 1758) (Teleostei: Pleuronectidae) is a catadromous flatfish species that spends much of its life cycle in estuarine and brackish aquatic environments, going to the open sea to spawn in early spring. Its geographic distribution extends along the Atlantic coast, from the White Sea in the north, to northern Africa in the south, including also the Mediterranean and the Black seas (Lucas and Baras, 2001). It is an important species to the Portuguese fisheries, occurring along the entire coast of Portugal (Sobral and Gomes, 1997).

Several metazoan ectoparasite species have already been recorded on

TABLE I. Metazoan ectoparasitic species recorded for the European flounder *Platichthys flesus* (Linnaeus, 1758) in different studies of the literature and respective prevalence values (range).

Group: Family Species	Geographic location	Prevalence (%)	Reference
Monogenea: Gyrodactylidae			
Gyrodactylus unicopula Glukhova, 1955	Baltic Sea	0.4–2.0	Chibani and Rokicki, 2004; Chibani et al., 2005
	North Sea	*	MacKenzie and Gibson, 1970
Gyrodactylus flesi Malmberg, 1957	Baltic Sea	0.1–0.5	Chibani and Rokicki, 2004; Chibani et al., 2005
Gyrodactylus sp.	North Sea	1.1	Schmidt, 2003
Copepoda: Caligidae			
Caligus curtus Müller, 1785	Norwegian Sea	*	Lile et al., 1994
Caligus elongatus von Nordmann, 1832	North Sea	3.3–28	Boxshall, 1974; Schmidt, 2003
Lepeophtheirus pectoralis (Müller, 1777)	North Sea	78.4–96	Boxshall, 1974; Schmidt, 2003
	Ythan Estuary	*	MacKenzie and Gibson, 1970
	Thames River	0.5–13.3	El-Darsh and Whitfield, 1999
	Norwegian Sea	*	Lile et al., 1994
	Atlantic Ocean	52.5–79.4	Marques et al., 2006
Lepeophtheirus europaensis (Zeddam, Berrebi, Renaud, Raibaut, and Gabrion, 1988)	Mediterranean Sea	*	Zeddam et al., 1988
Copepoda: Pennellidae			
Lernaeocera branchialis (L.)	Baltic Sea	4–88	Køie, 1999
	North Sea	67–92.6	Boxshall, 1974; Schmidt, 2003
	Ythan Estuary	*	MacKenzie and Gibson, 1970
	Thames River	8.9	El-Darsh and Whitfield, 1999
	Norwegian Sea	*	Lile et al., 1994
Copepoda: Chondracanthidae			
Acanthochondria cornuta (Müller, 1776)	North Sea	50–63.7	Boxshall, 1974; Schmidt, 2003
	Ythan Estuary	*	MacKenzie and Gibson, 1970
	Atlantic Ocean	10.5–76.3	Kabata, 1959; Marques et al., 2006
	Norwegian Sea	*	Lile et al., 1994
Acanthochondria soleae (Krøyer, 1838)	Atlantic Ocean	*	Kabata, 1959
Acanthochondria limandae (Krøyer, 1863)	Atlantic Ocean	*	Kabata, 1959
Copepoda: Bomolochidae			
Holobomolochus confusus (Stock, 1959)	Baltic Sea	32	Køie, 1999
	North Sea	4.7	Schmidt, 2003
Isopoda: Gnathiidae			
Gnathia sp.	Atlantic Ocean	1.3	Marques et al., 2006

* Present.

the European flounder, *P. flesus* (L.), and reported in different studies of the literature (see Table I). However, for south European waters, only a single record indicating a flounder's infection by a new species, *Lepeophtheirus europaensis*, in the Mediterranean Sea (Zeddam et al., 1988), and a survey reporting flounder's infection by 3 different ectoparasite species in the south-central Portuguese coast (Marques et al., 2006), are known. Indeed, as far as we are aware, no parasitological survey has yet been conducted for flounders off the northern Portuguese coast, the geographic area where the economic income from flounder fishing is most important. Moreover, according to Lile et al. (1994), fish parasite communities often vary considerably in composition over short to moderate distances. Therefore, the main aim of the present study was to characterize the flounder's metazoan ectoparasite assemblage along the north-central Portuguese coast from different sampling locations.

On 2 and 8 September 2005, 120 flounders from 4 locations off the north-central Portuguese coast, i.e., Viana do Castelo (VC) (41°40′N, 8°50′W), Matosinhos (M) (41°10′N, 8°42′W), Aveiro (A) (40°38′N, 8°45′W), and Figueira da Foz (FF) (40°8′N, 8°52′W) (Fig. 1), were collected for examination of metazoan ectoparasites. In each location, 30 fish were collected by random sampling from the nets of local fishing boats. All the fish were kept frozen at −20 C until they could be examined. Each specimen was weighed (mean ± SD [minimum–maximum] = 279.2 ± 172.8 [160.7–1,090.4] g [VC]; 314.5 ± 217.6 [139.4–1,124.0] g [M]; 267.4 ± 122.0 [113.6–613.8] g [A]; 409.9 ± 207.3 [158.4–836.2] g [FF]), measured (27.6 ± 3.7 [24.2–42.8] cm [VC]; 28.7 ± 4.9 [23.5–42.7] cm [M]; 27.5 ± 4.3 [19.8–38.6] cm [A]; 30.9 ± 5.0 [23.6–41.6] cm [FF]), and sexed (20 males and 10 females [VC]; 10 males and 20 females [M]; 9 males and 21 females [A]; 11 males and 19 females [FF]). The body skin, eyes, fins, branchial chambers (subopercular surfaces, walls, gill arches, and pseudobranchiae), and nasal and buccal cavities were examined for metazoan ectoparasites using a stereomicroscope. Collected specimens were cleaned and then fixed in 70% alcohol. Later, copepods were cleared in 90% lactic acid (Humes and Gooding, 1964). Parasites were identified according to Naylor (1972) and Bruce (1987) for Isopoda, and to Kabata (1979, 1992) for Copepoda. It was not possible to identify the gnathiid pranizae at the species level because the identification keys require adult male specimens that were not found in our survey. Nevertheless, all the female larvae presented the same morphological type, which is, presumably, an indication of a single species.

After evaluating the sites of parasite infection on the host's body surface, the following ecological parameters were determined according to Bush et al. (1997) for each of the 4 sampled locations: prevalence (number of infected fish/percentage of infected fish [95% confidence

TABLE II. Metazoan ectoparasitic taxa recorded on flounders from the 4 sampled locations off the north-central Portuguese coast, their sites of infection, infection parameters (number of infected fish/prevalence [95% confidence interval]%, mean intensity ± SD [range]), and first-order jackknife estimator of species richness (estimated richness ± SD [N = 30 fish for all sampled locations]).

Parasite group			Sampled location			
Family	Taxa	Host site*	Viana do Castelo	Matosinhos	Aveiro	Figueira da Foz
Copepoda						
Caligidae	*Caligus diaphanus*	B	—	—	1/3 (0–17) (1)	—
	Caligus sp.	B; F	—	5/17 (6–35) (1)	—	—
	Lepeophtheirus pectoralis	B; F	6/20 (8–39) 7.2 ± 7.2 (1–19)	30/100 (88–100) 14.1 ± 9.9 (3–53)	29/97 (83–100) 7.6 ± 6.9 (1–34)	28/93 (78–99) 9.5 ± 10.0 (1–50)
Chondracanthidae	*Acanthochondria cornuta*	B; F; SOS; GA; P	5/17 (6–35) 22.0 ± 12.8 (5–41)	30/100 (88–100) 47.6 ± 22.6 (8–96)	29/97 (83–100) 34.4 ± 24.2 (2–110)	30/100 (88–100) 38.1 ± 25.9 (4–104)
Bomolochidae	*Holobomolochus confusus*	NC	—	1/3 (0–17) (1)	—	—
Isopoda						
Cymothoidae	*Nerocila orbignyi*	F; GA	—	3/10 (2–27) (1)	—	—
Gnathiidae	Praniza larvae	B; F; BC; GA	20/67 (47–83) 1.7 ± 0.9 (1–4)	—	1/3 (0–17) (3)	—
	Estimated richness S_{JK}		3 ± 0.0	6 ± 1.0	6 ± 1.3	2 ± 0.0

* B, body; BC, buccal cavity; F, fins; GA, gill arches; NC, nasal cavities; P, pseudobranchiae; SOS, subopercular surfaces.

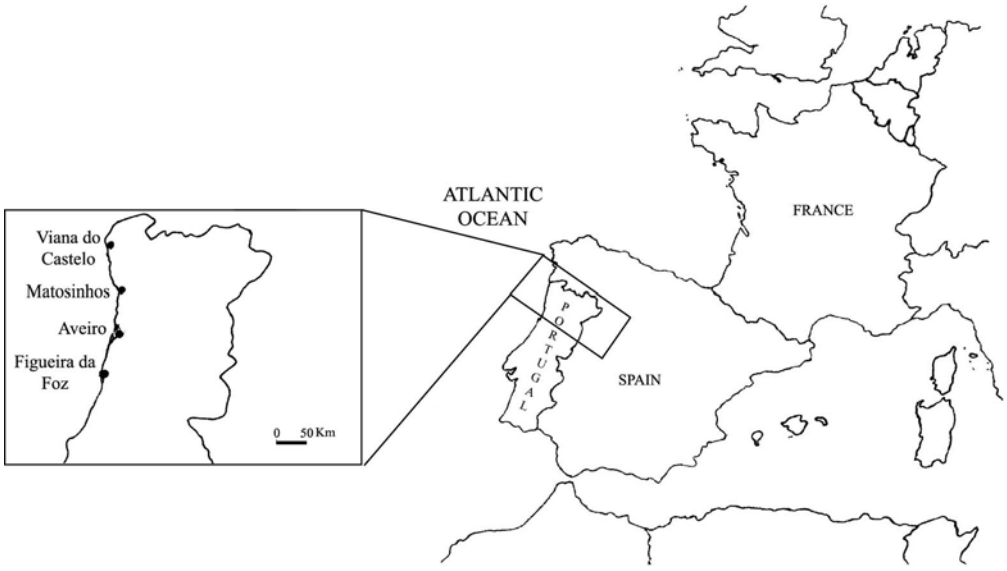

FIGURE 1. Geographic location of the 4 sampled areas (VC, Viana do Castelo; M, Matosinhos; A, Aveiro; and FF, Figueira da Foz) along the north-central Portuguese coast.

interval]) and mean intensity ± SD (range). Besides that, the first-order jackknife estimator of species richness (S_{JK}) rounded to the nearest integer and respective standard deviation values were evaluated using EstimateS software (Colwell, 2005).

Parasites of 7 different taxa were identified on the flounders examined: *Caligus diaphanus* von Nordmann, 1832, *Caligus* sp., 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 larvae (Isopoda: Gnathiidae) (Table II). Infected host specimens were quite common, varying from 21 fish (70 [51–85]%) off Viana do Castelo to 30 fish (100 [88–100]%) off Matosinhos, Aveiro, and Figueira da Foz. Multiple infections were more frequent off Matosinhos, with all the infected host specimens (30 fish/100 [88–100]%) harboring more than 1 parasite species, followed by Aveiro and Figueira da Foz (28 fish/93 [78–99]%), and Viana do Castelo (7 fish/23 [10–42]%). Copepod specimens were found on 7 (23 [10–42]%) fish off Viana do Castelo and all (30/100 [88–100]%) fish off Matosinhos, Aveiro, and Figueira da Foz. Isopods were found on 20 (67 [47–83]%), 3 (10 [2–27]%), and 1 (3 [0–17]%) fish off Viana do Castelo, Matosinhos, and Aveiro, respectively. In contrast to what was previously described for the northern Europe flounder populations, and similar to what was observed in the south-central Portuguese coast, neither *Lernaeocera branchialis* (L.) nor any monogenean species was found during our study.

The infection of the European flounder *P. flesus* off the north-central Portuguese coast by ectoparasitic metazoans seems to be quite common, judging by the total number of infected fish found in our study. Furthermore, copepods were the most frequent parasites, whereas the isopods occurred only on rare occasions. With the exception of *C. diaphanus*, *Caligus* sp., and *N. orbignyi*, which, as far as we know, are new host records, all the other species have already been recorded on flounders from the Atlantic Ocean, and from the North, Norwegian, and Baltic seas.

The number of parasitic species recorded varied across locations, ranging between 2 and 5. However, while in VC and FF the observed and estimated richness values coincided, in M and A they did not, thereby indicating that the true species richness for the latter locations is higher than the one observed in our survey. The minimum value documented for the observed species richness was recorded for *Lepeophtheirus pectoralis* and *A. cornuta*, 2 species common to all the sampled locations. In fact, prevalence and intensity values recorded for these 2 species suggest that they are probably common parasites of flounders throughout the north-central Portuguese coast. Both copepods were dominant off Matosinhos, Aveiro, and Figueira da Foz, whereas off Viana do Castelo the highest prevalence value was recorded for gnathiid pranizae. In the North Sea, *Lepeophtheirus pectoralis* and *A. cornuta* also appear to be common parasites of the European flounder (Boxshall, 1974; Schmidt, 2003). All other identified parasites, i.e., *C. diaphanus*, *Caligus* sp., *H. confusus*, and *N. orbignyi*, exhibited comparatively lower prevalence and total intensity values, indicating that they are probably not common in flounders from the studied area. For the latter 4 species, differences in host age may help to explain their diverse occurrence on the fish samples. Moreover, all were absent from FF, the sampling location where older fish, i.e., fish possessing higher mean total weight and length values, were collected. The absence of *Lernaeocera branchialis*, a parasite that can constitute a severe pest with significant economic impact (Kabata, 1979), is noteworthy, since this species is a common parasite on flounders from the North (Schmidt, 2003) and Baltic Seas (Køie, 1999). This result is probably related to the absence of the main definitive host species (gadoid fishes) from the area under study (Kabata, 1979; Svetovidov, 1986).

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Early Migration of *Sarcocystis neurona* in Ponies Fed Sporocysts

E. Elitsur, A. E. Marsh, S. M. Reed*, J. P. Dubey†, M. J. Oglesbee‡, J. E. Murphy*, and W. J. A. Saville§, Department of Veterinary Preventive Medicine, College of Veterinary Medicine, The Ohio State University, Columbus, Ohio 43210-1092; *Department of Veterinary Clinical Sciences, College of Veterinary Medicine, The Ohio State University, Columbus, Ohio 43210-1092; †United States Department of Agriculture, Agricultural Research Service, Animal and Natural Resources Institute, Animal Parasitic Diseases Laboratory, Beltsville, Maryland 20705-2350; ‡Department of Veterinary Biosciences, College of Veterinary Medicine, The Ohio State University, Columbus, Ohio 43210-1092; §To whom correspondence should be addressed. *e-mail: saville.4@osu.edu*

ABSTRACT: *Sarcocystis neurona* is the most important cause of equine protozoal myeloencephalitis (EPM), a neurologic disease of the horse. In the present work, the kinetics of *S. neurona* invasion is determined in the equine model. Six ponies were orally inoculated with 250×10^6 *S. neurona* sporocysts via nasogastric intubation and killed on days 1, 2, 3, 5, 7, and 9 postinoculation (PI). At necropsy, tissue samples were examined for *S. neurona* infection. The parasite was isolated from the mesenteric lymph nodes at 1, 2, and 7 days PI; the liver at 2, 5, and 7 days PI; and the lungs at 5, 7, and 9 days PI by bioassays in interferon gamma gene knock out mice (KO) and from cell culture. Microscopic lesions consistent with an EPM infection were observed in brain and spinal cord of ponies killed 7 and 9 days PI. Results suggest that *S. neurona* disseminates quickly in tissue of naive ponies.

Equine protozoal myeloencephalitis (EPM) is a serious neurologic disease and *Sarcocystis neurona* is the most important cause (Dubey et al., 1991). *Sarcocystis neurona* has a 2-host life cycle, including a meat-eating definitive host, the opossums *Didelphis virginiana* and *Didelphis albiventris* (Dubey, Lindsay, Kerber et al., 2001; Dubey, Lindsay, Saville et al., 2001). There is a wide range of intermediate hosts, including the raccoon (Dubey, Saville et al., 2001), armadillo (Cheadle, Tanhauser et al., 2001), skunk (Cheadle, Yowell et al., 2001), sea otter (Dubey et al., 2002), and the domestic cat (Dubey and Hamir, 2000; Dubey et al., 2000; Turay et al., 2002). The horse is considered an aberrant intermediate host (Dubey, Lindsay, Saville et al., 2001). Schizonts and merozoites are the only stages known in the horse, and they are found only in the central nervous system (CNS) following an uncharacterized migratory route. Attempts to demonstrate *S. neurona* in tissues of horses fed sporocysts have been unsuccessful despite the fact that horses developed neurological signs (Fenger et al., 1997; Lindsay et al., 2000; Cutler et al., 2001; Saville et al., 2001; Sofaly et al., 2002). In the present article, we have attempted to follow the migration of *S. neurona* in tissues of ponies by orally inoculating them with large numbers of sporocysts and examining at shorter postchallenge intervals.

Eight seronegative ponies (Table I) were randomly assigned to treatment (n = 6) or control (n = 2) groups and housed in separate stalls. Neurologic examinations were conducted before the initiation of the project and daily there after, including the date of termination. The examinations were performed by a coauthor (S.M.R.). Physical examinations were also performed daily. On day 0, cerebral spinal fluid (CSF) and blood samples were collected from each horse, and treatment ponies were inoculated with sporocysts via nasogastric intubation with 250×10^6 sporocysts (25 ml) and 120 ml doses of phosphate buffered saline (PBS) to ensure complete dosing. The sporocysts were of the raccoon isolate SN 37-R and had been obtained from the intestines of the laboratory-raised opossums fed tissues of experimentally infected raccoons as described (Sofaly et al., 2002; Stanek et al., 2002).

Control ponies were given saline solution (25 ml) and 120 ml doses of PBS via the nasogastric tube. Disposable gloves and plastic boots were used upon entrance into the control ponies' stalls to avoid cross-contamination and were immediately discarded afterwards. An empty stall was maintained between the control ponies and treatment ponies as well. Blood for serology was collected daily (days 1–9) and for buffy coat culture on terminal dates. Treatment ponies were randomly assigned to serial killing on days 1, 2, 3, 5, 7, and 9 PI, and the control ponies were killed on days 3 and 9 PI. Ponies were humanely killed with an overdose of Euthasol euthanasia solution (Delmarva Laboratories, Midlothian, Virginia), and CSF was collected via the atlanto-occipital space at postmortem.

Necropsy was performed on all ponies. At necropsy, samples of lung, liver, mesenteric lymph nodes, and mesenteric artery were removed aseptically for *S. neurona* isolation. Additional tissue samples were fixed in 10% buffered formalin for routine microscopic examination, including the heart, lung, diaphragm, liver, spleen, adrenal gland, kidney, tongue, mesenteric lymph node, mesenteric artery, cecum, sciatic