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Potential environmental and host gender influences on prevalence of *Haemogregarina platessae* (Adeleorina: Haemogregarinidae) and suspected *Haemohormidium terraenovae* (incertae sedis) in Brazilian flounder from the Patos Lagoon Estuary, Southern Brazil

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Abstract. Flounder, *Paralichthys orbignyanus* (Valenciennes), were captured in polluted and non-polluted sites within the Patos Lagoon Estuary, southern Brazil, over four seasons. Blood films showed a high prevalence of infection with a haemogregarine, or mixed parasitaemias of this and an organism resembling *Haemohormidium terraenovae* So, 1972. Haemogregarine gamont stages conformed to existing descriptions of *Desseria platessae* (Lebailly, 1904) Siddall, 1995 from flatfishes, but intraerythrocytic division of meronts was observed, leading to the recommendation for nomenclatural correction, placing the haemogregarine in the genus *Haemogregarina* (sensu lato) Danilewsky, 1885. Statistical analyses suggested that although sample sizes were small, infections with meront stages, immature and mature gamonts were all influenced by site, and possibly therefore, by pollution. Season also appeared to determine likelihood of infection with meronts and immature gamonts, but not mature gamonts, while adult fish gender apparently affected infection with immature and mature gamonts, but not meronts. The *H. terraenovae*-like organism exhibited unusual extracellular forms and did not match closely with the type description of *H. terraenovae*; precise identification was therefore difficult. Data analyses suggested that parasitism by this organism was influenced by site and fish gender, since females and males from non-polluted water were infected, but only females from the polluted site. Season was also important and significantly more adult fish of both sexes were infected with this parasite in the Brazilian summer and autumn, compared with winter and spring. Finally, these appeared to be the first observations of *Haemogregarina platessae*, and possibly *H. terraenovae*, from the southern hemisphere.

Haemogregarines (Apicomplexa, Adeleorina, Haemogregarinidae) are broadly elongate apicomplexans occurring in the red and white blood cells of a variety of vertebrates and widely distributed among fishes, especially in marine environments (Davies and Johnston 2000). Although most fish haemogregarine life cycles are unknown, fishes are likely intermediate hosts, and leeches or gnathiid isopods probable definitive hosts (see Molnár 2006). Siddall (1995) partially revised the taxonomy of fish haemogregarines and created the genus *Desseria* Siddall, 1995 for those lacking intraerythrocytic merogony, retaining those with division in circulating erythrocytes in the genus *Cyrilia* Lainson, 1981, or in *Haemogregarina* (sensu lato) Danilewsky, 1885.

Members of the genus *Haemohormidium* Henry, 1910 are also fairly common intraerythrocytic parasites of marine fishes (Henry 1910, 1913a). They are often irregularly round in shape, exhibit peripheral staining with Romanowsky dyes, and show evidence of intraerythrocytic division (Henry 1913b, Laird and Bullock 1969, So 1972, Davies 1980, Barta 1991). Khan (1980, 1984) demonstrated transmission of *Haemohormidium beckeri* So, 1972 and similar infections between fishes by haematophagous leeches, believing the blood parasites to be piroplasms, but controversy has remained surrounding the taxonomy of the genus *Haemohormidium* and the equally enigmatic genus *Haematractidium* Henry, 1910 (see Henry 1910, 1913c, Johnston 1975, MacLean 1980, Bodammer and MacLean, 1985, Davies 1995). This uncertainty was summarised by Siddall et al. (1994) who concluded that *Haemohormidium* and *Haematractidium* are synonymous genera, with the former taking priority; moreover, the identity of these parasites was not established, but they were not considered apicomplexan. Finally, Davies et al. (2003) suggested affinities between these organisms and microsporidians, but their classification remains unclear (see Molnár 2006).

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Recently, as part of a study of biomarkers of exposure and effects of contaminants on Brazilian flounder, Amado et al. (2006) reported adult *Paralichthys orbignyanus* (Valenciennes) from a polluted area of the Patos Lagoon estuary, southern Brazil, showing likely damage to their DNA repair systems and seasonal oxidative stress, in comparison with flounder from a non-polluted locality in the same estuary. As an extension to the previous study, the current research reports on three intriguing aspects of blood samples taken from a similar-sized population of Brazilian flounder, captured at polluted and non-polluted sites in the same (Patos Lagoon) estuary. Firstly, the blood films apparently contain a haemogregarine and a species of *Haemohormidium*, both formerly recorded only from the northern hemisphere. Secondly, the distribution of the two parasites among sites, seasons and mature fish when analysed statistically, shows interesting trends, with these factors potentially influencing parasitism by the various development stages of the two haematozoans. Thirdly, the parasites have features not noted previously, and in the case of the haemogregarine, this finding alters its taxonomic status.

MATERIALS AND METHODS

Brazilian flounder, *Paralichthys orbignyanus* (Paralichthyidae, Pleuronectiformes), were captured by bottom trawl in the Patos Lagoon Estuary, southern Brazil (see Amado et al. 2006). Sampling (n = 50) was undertaken in spring (November and December) of 2002, and summer (February and March), autumn (April and May) and winter (July and September) of 2003, and water surface temperature was recorded at each catch. Flounders were captured from two sites, Coroa do Boi (CB) which was considered polluted and Saco do Justino (SJ), a relatively non-polluted area (see Amado et al. 2006). Following capture, blood was taken immediately from branchial vessels by heparinized syringes and stored on ice. Flounders were then killed by spinal section and kept on ice for transport to the laboratory. Here, the fish were sexed if possible, measured (cm, total length TL), weighed (g), and blood samples were prepared as described elsewhere (Amado et al. 2006). Stained blood smears were then examined with a Nikon Eclipse 80i microscope. Images were captured by digital camera (Nikon DS-5M) and measured with a Nikon NIS 2.10 image analysis system, calibrated to a stage micrometer. Finally, the influence of pollution, season and fish gender on parasitism by the different parasite stages was analysed by loglinear regression analysis with hierarchical backward elimination. This yielded likelihood ratios, preferred to chi-square for small samples (Quinn and Keough 2004), indicating the statistical significance of the various combinations of influences on prevalence of infection. To increase the power of the tests, the data for autumn and winter, as well as for spring and summer, were combined, and immature fish were omitted from the analysis owing to the small numbers sampled.

RESULTS

Fifty flounders (28 females, 14 males and 8 immature individuals) were examined. Of these, 23 fish originated from the polluted site, CB (12 females, 5 males, 6 immature individuals) and 27 from the non-polluted area, SJ (16 females, 9 males, 2 immature fish) (Table 1). In spring of 2002, 4 females, 4 males and 1 juvenile flounder were examined, while the remaining 41 fish were studied over the summer, autumn and winter of 2003 (see Table 1). Water surface temperatures (minimum and maximum) for each season were: spring 23–26°C; summer 23–27°C; autumn 18–21°C; and winter 13–14°C. Two taxonomically distinct parasites were observed within peripheral blood films from fishes captured at both sites, one indistinguishable from the haemogregarine, *Desseria platessae* (Lebailly, 1904) Siddall, 1995 and the other similar to *Haemohormidium terraenovae* So, 1972 (see Figs. 1–15). The distribution of parasite stages among the flounder is recorded in Table 1.

Infection with *Desseria platessae*

Prevalence of infection between sites

Haemogregarines, identified as *Desseria platessae* (Lebailly, 1904) Siddall, 1995 (but, see Remarks and a recommended nomenclatural correction to *Haemogregarina platessae* in the Discussion), were found in 96% (48/50) of *P. orbignyanus* (Table 1). Infected fish included 27 females (19.8–44 cm TL, 85–1,015 g), 13 males (18.5–38 cm TL, 70–555 g) and 8 immature individuals (14.5–23 cm TL, 35–110 g). Infected fish were obtained from both CB and SJ (Table 1); thus only 2 individuals, 1 male (23 cm TL, 95 g) caught at CB in winter, and 1 female (26 cm TL, 165 g) captured at SJ in the same season, were apparently uninfected. Parasites were all intraerythrocytic, occurring typically in 1/1,500 to 1/3,500 mature erythrocytes, depending on the smear examined.

Parasite stages in relation to infection prevalence, host gender, and site of capture

Mature gamonts were observed most frequently (33/50, 66% of fish), while immature gamonts (21/50, 42% of fish) and meronts (16/50, 32% of fish) were less common (Table 1). Meront stages that were undergoing, or had undergone recent nuclear and/or cytoplasmic division (4/50, 8% of fish), were found least often (Table 1). For adult fish, loglinear analysis confirmed that there was a statistically significant interaction between the parasite stage and prevalence of infection (likelihood ratio = 22.69, $P<0.001$).

Meronts were found in immature fish (4/8, 50%), but only at CB, and only in summer and autumn. They were also seen in 16/28 (57%) of adult females from both CB and SJ in all seasons, and 6/14 (42%) of adult males, but in winter from both sites, and from SJ in spring and summer (Table 1). Loglinear analysis indicated that the presence of the meront stage was influenced by a combination of season and site (likelihood ratio = 5.189, P = 0.023); a greater proportion of adult fish were parasitized by meronts in spring/summer than in autumn/winter, but only from the non-polluted site (7/8, or 87%, versus 7/17, or 41%, respectively). Proportions of adults infected with meronts from the polluted site at the two times of the year did not differ significantly.

Division stages occurred in 1/8 (13%) immature individuals, 2/28 (7%) of female fish and 1/14 (7%) of infected males; immature fish and females were caught at CB in spring and summer, and the male at SJ in autumn (Table 1). Too few fish were infected with this stage for statistical analysis to be conducted on the influences of season, fish gender and site on infection.

Half of immature individuals (4/8, 50%) had immature gamonts, including 3 fish caught at CB in summer, and 1 captured at CB in autumn. Immature gamonts in adult females (11/28, 39% of females) were located in spring and autumn at CB and in all seasons at SJ, while adult males (6/14, 43% of males) had these stages in spring at CB (3 individuals) and in summer, autumn and winter (1 individual in each season) at SJ (Table 1). For adult fish, loglinear analysis confirmed statistically this dependence of infection on site, gender and season (likelihood ratio = 4.28, P = 0.039).

Mature gamonts were found in 6/8 (75%) of immature fish over three seasons at CB (spring, summer, autumn) and in autumn at SJ. In adult fish they occurred in all seasons at CB and SJ (17/28, 61% of females; 10/14, 71% of males), although 2 females captured in spring at SJ lacked this stage, and no males were caught in summer and autumn at CB. Statistical analysis revealed no seasonal influence, but a combination of site and adult fish gender (loglinear analysis: likelihood ratio = 5.58, P = 0.018) appeared to affect prevalence of infection; adult females were infected in polluted (9/12, or 75%) and non-polluted (8/16, or 50%) waters, but only 2/5 (40%) of males in polluted water compared with 8/9 (89%) in non-polluted waters.

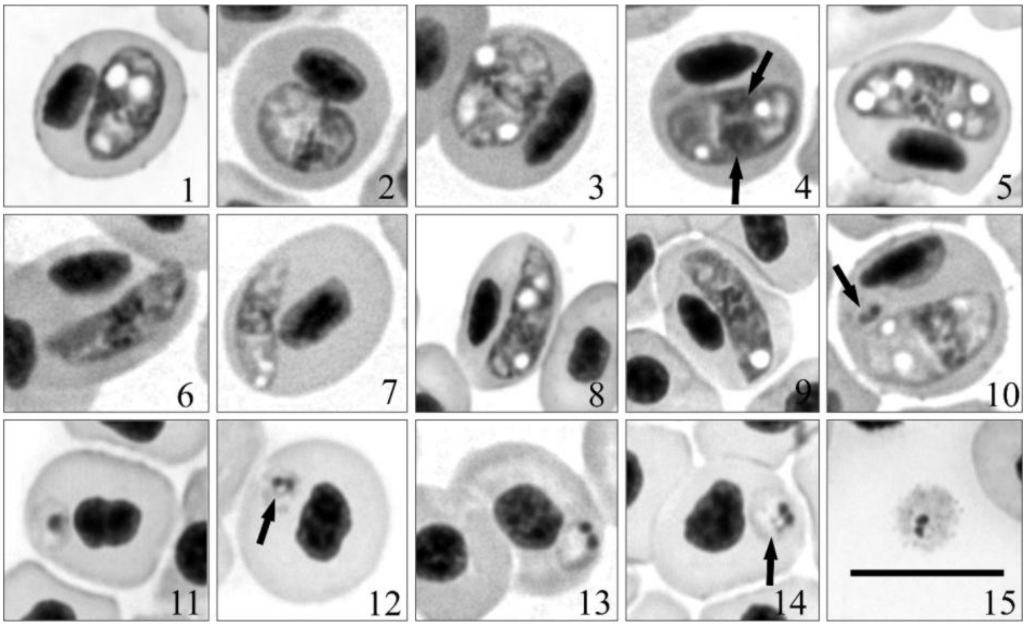
Thus, although sampled host numbers were low, statistical analyses raised the possibility that pollution within the site CB may have reduced the likelihood of parasitism of fish by haemogregarines. However, this influence also appeared dependent on season and host gender. Meront stages did not show the increased prevalence in spring/summer at CB that they did from the non-polluted site SJ, and a greater proportion of adult male fish from non-polluted water was infected by mature gamont stages of the parasite than males from polluted water.

Description of the parasite stages

Meronts: broad stages with rounded ends, oval, occasionally slightly kidney-shaped, sometimes one pole slightly broader than other (Figs. 1–3, 10), 8.2 ± 1.0 µm (n = 25) long by 5.1 ± 0.6 µm (n = 25) wide; large nucleus with loosely arranged chromatin, deep-purple stained, 2.5 ± 0.4 µm (n = 25) long by 5.0 ± 0.6 µm (n =

Table 1. Sites and seasons of capture, host flounder characteristics (imm = immature individuals, ♀ females, ♂ males; total length, TL in cm; weight, Wt in g), haemogregarine stages detected (M = meronts, D = division stages, Ig = immature gamonts, Mg = mature gamonts) and *Haemohormidium*-like organism (Hm).

Coroa do Boi	Host	Host	Host	Haemogregarine stages				Hm	Saco do Justino	Host	Host	Host	Haemogregarine stages				Hm
(polluted)	sex	TL	Wt	M	D	Ig	Mg		(non-polluted)	sex	TL	Wt	M	D	Ig	Mg	
Spring 2002	1imm 2♀ 3♂	14.5 32.5, 44 18.5–31	35 380, 1015 70–340	0/1 1/2 0/3	0/1 1/2 0/3	0/1 2/2 3/3	1/1 1/2 1/3	0/1 1/2 0/3	Spring 2002	0imm 2♀ 1♂	– 25, 38 22.9	– 155, 545 145	– 2/2 1/1	– 0/2 0/1	– 1/2 0/1	– 0/2 1/1	– 0/2 0/1
Summer 2003	3imm 3♀ 0♂	15.5–18.3 19.8–29.5 –	40–70 85–245 –	2/3 2/3 –	1/3 1/3 –	3/3 0/3 –	2/3 2/3 –	1/3 1/3 –	Summer 2003	0imm 2♀ 3♂	– 28, 32.5 26.5–33	– 240, 380 185–375	– 2/2 2/3	– 0/2 0/3	– 1/2 1/3	– 1/2 2/3	– 1/2 2/3
Autumn 2003	2imm 4♀ 0♂	20.5, 23 30.5–34 –	85, 110 255–390 –	2/2 2/4 –	0/2 0/4 –	1/2 2/4 –	1/2 3/4 –	1/2 3/4 –	Autumn 2003	2imm 7♀ 3♂	21, 21.5 22–35.8 22.7–35	85, 95 110–540 120–400	0/2 2/7 0/3	0/2 0/7 1/3	0/2 3/7 1/3	2/2 5/7 3/3	0/2 3/7 3/3
Winter 2003	0imm 3♀ 2♂	– 24–32.5 23, 29	– 120–320 95, 225	– 2/3 1/2	– 0/3 0/2	– 0/3 0/2	– 3/3 1/2	– 0/3 0/2	Winter 2003	0imm 5♀ 2♂	– 26–44 34, 38	– 165–885 380, 555	– 3/5 2/2	– 0/5 0/2	– 2/5 1/2	– 2/5 2/2	– 1/5 0/2
Total	23			12/23	3/23	11/23	15/23	7/23	Total	27			14/27	1/27	10/27	18/27	10/27



Figs. 1–15. Giemsa-stained blood films from *Paralichthys orbignyanus* with *Haemogregarina platessae* (Figs. 1–10) and the *Haemohormidium terraenovae*-like organism (Figs. 10–15). **Figs. 1–3.** Intraerythrocytic meront stages of the haemogregarine. Note the vacuolated cytoplasm of most parasites and displacement of host erythrocyte nuclei. **Figs. 4, 5.** Meronts in division showing lateral nuclei (arrows, Fig. 4). **Fig. 6.** Closely apposed, immature, individuals, likely resulting from binary fission. **Fig. 7.** Immature gamont. **Figs. 8, 9.** Mature gamonts. **Fig. 10.** Host erythrocyte with a haemogregarine meront and the *Haemohormidium*-like organism (arrow). **Fig. 11.** *Haemohormidium*-like organism with a single nucleus. **Figs. 12–14.** Same organism with a loop-like structure running between paired nuclei (arrow, Fig. 12), or with rod-like extensions from paired nuclei (arrow, Fig. 14). **Fig. 15.** Extracellular stage. Scale bar = 10 µm.

25) wide, sometimes nearer to one pole of meront; cytoplasm of meronts generally deep-blue stained, a few pink granules, prominent vacuoles up to 1.8 µm ± 0.3 µm (n = 25) in diameter (Figs. 1, 3, 5).

Division of meronts: by longitudinal binary fission (stage D in Table 1), two nuclei located laterally within elongate parasite body (Figs. 4, 5); two immature haemogregarines (pregamonts?) in close apposition within erythrocyte cytoplasm, likely outcome of division (Fig. 6).

Immature gamonts: about same length as meronts, 7.7 ± 1.1 µm (n = 25) long, but more slender, 2.3 ± 0.6 µm (n = 25) wide (Fig. 7); nuclei normally centrally placed, 2.1 ± 0.4 µm by 2.4 ± 0.4 µm (n = 25), slightly oval, deep-pink stained, chromatin more condensed than in the meront; cytoplasm deep-blue stained, a few pink granules, one or two small vacuoles.

Mature gamonts: elongate, curved, one pole (anterior?) more rounded, other extremity bluntly pointed (Figs. 8, 9); longest stages, 9.4 ± 0.9 µm (n = 25), intermediate in width between immature gamonts and meronts, 3.1 ± 0.4 µm (n = 25); nuclei centrally located, or nearer to one pole, especially to rounded end, deep-purple stained, similar in chromatin structure to those of immature gamonts, 3.3 ± 0.3 µm (n = 25) long by 2.9 ± 0.5 µm (n = 25) wide; cytoplasm deep-blue stained with pink granules, large vacuoles, 1.4 ± 0.2 µm (n = 25) in diameter, both anterior and posterior to the nucleus; no stained anterior caps observed.

Displacement and compression of host erythrocyte nuclei by meronts, dividing meronts and mature gamonts, evident in blood films (Figs. 1–6, 8–10); infected erythrocytes with meronts wider, 12.3 ± 1.7 µm by 10.6 ± 1.1 µm (n = 25), than uninfected counterparts, 12.6 ± 1.3 µm long by 8.1 ± 0.9 µm (n = 25) wide.

Remarks

Haemogregarina platessae was first described by Lebailly (1904) in European plaice, *Pleuronectes platessa* L. (syn. *Platessa vulgaris*), captured at Luc-sur-Mer, France. The haemogregarine was curved, notable for its vacuolated appearance, and measured 9 by 2 µm; smaller forms lacked vacuolation. Nuclear length was about a third of the total length of the haemogregarine, and nuclear width approximately the same as the parasite body. Both intracellular and extracellular haemogregarines were observed. In the same publication, Lebailly (1904) recorded two further new species from Luc-sur-Mer, namely *Haemogregarina flesi* from the flounder *Platichthys flesus* (L.), and *Haemogregarina laternae* from a flatfish that he identified as “*Platophrys laterna*”, possibly *Arnoglossus laterna* (Walbaum). Both of these latter haemogregarines, he reported, resembled *H. platessae.* Moreover, Lebailly

Table 2. Hosts and location records for *Haemogregarina platessae*.

Host fishes by orders and families	Common names for host orders, families and fishes	Location	Authors
Pleuronectiformes	Flatfishes		
1. Achiridae	American soles		
Achirus lineatus (L.)	Lined sole	Mississippi Sound and adjacent waters, USA	Becker and Overstreet 1979
Trinectes maculatus (Bloch et Schneider)	Hogchoker	St Augustine, Florida, USA;	Saunders 1955*;
		Patuxent River Estuary, Maryland, USA;	Laird and Morgan 1973;
		Mississippi Sound and adjacent waters, USA	Becker and Overstreet 1979
2. Bothidae	Lefteye flounders		
"*Platophrys laterna*", possibly *Arnoglossus laterna* (Walbaum)	Scaldfish	Luc-sur-Mer, France	Lebailly 1904
3. Cynoglossidae	Tonguefishes		
Cynoglossus senegalensis (Kaup)	Senegalese tonguesole	Cross River Estuary, Nigeria	Obiekezie 1986
Symphurus plagiusa (L.)	Blackcheek tonguefish	Mississippi Sound and adjacent waters, USA	Becker and Overstreet 1979
4. Paralichthyidae	Largetooth flounders		
Hippoglossina oblonga (Mitchill) (syn. *Paralichthys oblongus*)	American fourspot flounder	Cape Hatteras, North Carolina to Gulf of Maine, USA	Khan and Newman 1981
Paralichthys dentatus (L.)	Summer flounder	Woods Hole, Massachusetts, USA	Laird and Bullock 1969
Paralichthys lethostigma Jordan et Gilbert	Southern flounder	Mississippi Sound and adjacent waters, USA	Becker and Overstreet 1979
5. Pleuronectidae	Righteye flounders		
Glyptocephalus cynoglossus (L.)	Witch	Grand Banks, Newfoundland, Canada	So 1972
Microstomus kitt (Walbaum) (syn. *Pleuronectes microcephalus*)	Lemon sole	Shetland, UK;	Henry 1913a;
		Plymouth, UK	Noble 1957
Platichthys flesus (L.) (syn. *Pleuronectes flesus*)	Flounder	Luc-sur-Mer, France;	Lebailly 1904;
		Millport, UK	Robertson 1906
Pleuronectes platessa L. (syns. *Flesus vulgaris*, *Platessa vulgaris*)	European plaice	Luc-sur-Mer, France;	Lebailly 1904;
		Millport, UK;	Robertson 1906;
		Port Erin Bay, UK;	Henry 1910; 1913a;
		Plymouth, UK	Henry 1913d
Pseudopleuronectes americanus (Walbaum)	Winter flounder	Eastern Canada (location not specified);	Fantham et al. 1942;
		St. Andrews, New Brunswick, Canada	Laird and Bullock 1969
Reinhardtius hippoglossoides (Walbaum)	Greenland halibut	Ungava Bay, Quebec, Canada	Khan 1986
6. Scophthalmidae	Turbots		
Scophthalmus aquosus (Mitchill)	Windowpane	Grand Banks, Newfoundland, Canada	So 1972
Scophthalmus rhombus (L.) (syn. *Bothus rhombus*)	Brill	Luc-sur-Mer, France	Lebailly 1905
Lophiiformes	Anglerfishes		
1. Lophiidae	Goosefishes		
Lophius americanus Valenciennes**	American angler	Casco Bay, Gulf of Maine, USA;	Bridges et al. 1975;
		Cape Hatteras, North Carolina to Gulf of Maine, USA	Khan and Newman 1982

*Saunders (1955) originally named this haemogregarine *Haemogregarina achiri*, but Laird and Morgan (1973) considered it *Haemogregarina platessae*; **a possible host for *H. platessae*.

(1905) noted a fourth new species, *Haemogregarina bothi*, from the brill, *Scophthalmus rhombus* (L.) (syn. *Bothus rhombus*); this last haemogregarine exhibited characteristics of the other three species, so that *H. flesi*, *H. laternae* and *H. bothi* were all likely synonyms of *H. platessae* (see Becker and Overstreet 1979). Later, Robertson (1906), Henry (1910, 1913a, d) and Noble (1957) all reported *H. platessae* from UK waters. Thus, the haemogregarine appeared to be common among the flatfishes of northern Europe (see Table 2).

Subsequent reports of *H. platessae* came from the western North Atlantic and from West Africa. Laird and Morgan (1973) summarized the information available at the time, concluding that haemogregarines in their study of hogchoker, *Trinectes maculatus* (Bloch et Schneider) and those reported from a variety of North American flatfishes by Fantham et al. (1942), Saunders (1955), Laird and Bullock (1969), and So (1972) were likely all *H. platessae* (see Table 2). Later, Becker and Overstreet (1979) considered that flatfishes collected in the Gulf of Mexico all contained haemogregarines resembling *H. platessae* (Table 2), while Obiekezie (1985) described the haemogregarine in Nigerian flatfish, the only report of *H. platessae* from Africa (Table 2). Finally, Siddall (1995), in a partial systematic revision of the haemogregarines of fishes, placed this haemogregarine in the genus *Desseria* (as *D. platessae*), presumably because it lacked erythrocytic merogony.

The haemogregarine found in the current study is indistinguishable from published descriptions of *D. platessae* in its general size, appearance and occurrence within the erythrocytes of a flatfish (*P. orbignyanus*). The parasite is therefore identified as *D. platessae*, and its distribution has thus been extended to South America. Moreover, a new observation in this study over four seasons, and in different ages of fish, is that the parasite undergoes intraerythrocytic division. This development removes it from the genus *Desseria* (see Discussion).

Infection with cf. *Haemohormidium terraenovae*

This organism, identified as probable *Haemohormidium terraenovae* So, 1972 was found in 34% (17/50) of *P. orbignyanus* and all infected fish had haemogregarine stages (Table 1). Infected immature fish (2/8, 25%) (16.5, 20.5 cm TL, 45, 85 g) were found only at CB, and in summer and autumn. Infected adult females (10/28, 36%) (19.8–44 cm TL, 85–1,015 g) were captured at both CB and SJ in spring, summer and autumn, but only once was infection observed in winter (at SJ, in a female of 27 TL, 305 g). Males (5/14, 36%) (22.7–35 cm TL, 120–400 g) were also parasitized and these were located only at SJ, in summer and autumn (Table 1).

Significantly more adult fish, female and male, were infected with the *H. terraenovae*-like parasite in the Brazilian summer and autumn (13/22) compared with the winter and spring (2/20) (likelihood ratio = 15.07, $P = 0.002$). Loglinear analysis supported the observation that the numbers of adult fish parasitized by this organism were influenced by the interaction of collection site and fish gender (likelihood ratio = 4.971, $P = 0.026$); both female and male fish from non-polluted water (SJ) were infected (5/16 and 5/9, respectively), whereas only female fish (5/12) from polluted water (CB) were parasitized. Thus, pollution may have reduced likelihood of infection, especially in male fish, as suggested by the apparent relationship between site and infection by the *H. terraenovae*-like infection.

The infection was intraerythrocytic or occasionally extracellular, but parasitaemias were generally low (<1 in 100,000 erythrocytes infected), only twice attaining 1/500 erythrocytes, and once, 1/200 erythrocytes. Cells of the white cell series were not affected. Furthermore, the parasite was found mostly singly in mature erythrocytes and once formed a mixed infection with the haemogregarine within a single host cell (Fig. 10).

Intracellular parasites were intracytoplasmic, small, rounded or oval, 3.3 ± 0.6 µm long by 2.9 ± 0.1 µm wide ($n = 25$), with pinkish-blue stained, uniform, cytoplasm (Figs. 10–15). Parasite nuclei, were small, oval, or slightly elongate and red-purple stained, measuring 0.6 ± 0.1 µm wide by 1.0 ± 0.1 µm long ($n = 25$). Forms with a single nucleus were detected (Fig. 11), but the majority were binucleate, sometimes with a faint loop-like structure running between closely apposed paired nuclei (Fig. 12); infrequently, two indistinct rod-like extensions from the paired nuclei were evident, suggesting the formation of tetranucleate individuals (Fig. 14). Host cells were apparently unaffected by the presence of any stage of this parasite.

Unusual extracellular forms were seen on three occasions and a finely irregular outline was evident, giving them a 'powder puff' appearance (Fig. 15); these parasites were ~4.8 µm long by 4.5 µm wide ($n = 3$), and thus apparently larger than any intracellular stage. They were also binucleate (Fig. 15), with nuclei measuring 1.0 µm long by 0.7 µm wide ($n = 6$).

Remarks

So (1972) named *Haemohormidium terraenovae* from a number of fishes from the Grand Banks, Newfoundland, including the American sand lance *Ammodytes americanus* De Kay, haddock *Melanogrammus aeglefinus* (L.), white hake *Urophycis tenuis* (Mitchill), witch *Glyptocephalus cynoglossus* (L.), Atlantic halibut *Hippoglossus hippoglossus* (L.) and yellowtail flounder *Limanda ferruginea* (Storer) (Table 3). So (1972) also noted that Laird and Bullock (1969) had recorded probably the same parasite from the sea raven *Hemitripterus americanus* (Gmelin), as well as *H. hippoglossus* from St Andrews, New Brunswick (Table 3). The parasites illustrated by Laird and Bullock (1969) and So (1972) resembled *Haemohormidium cotti* Henry, 1910, (see Henry 1910, 1913b) in morphology and size.

Subsequently, Khan and Newman (1982), Khan et al. (1982, 1991) and Khan (1984, 1986) all reported *H. terraenovae* in largely pleuronectiform fishes from various marine sites in eastern Canada and the eastern USA (Table 3). Furthermore, Khan et al. (1980) suggested that *H. terraenovae* could represent a composite of species.

Table 3. Hosts and location records for *Haemohormidium terraenovae*.

Host fishes by orders and families	Common names for host orders, families and fishes	Location	Authors
Gadiformes	Cods		
1. Gadidae	Cods and haddocks		
Boreogadus saida (Lepechin)	Polar cod	Ungava Bay, Quebec, Canada; Davis Straight and Baffin Bay, Canada	Khan 1986; Khan et al. 1991
Gadus morhua L.	Atlantic cod	Davis Straight and Baffin Bay, Canada	Khan et al. 1991
Gaidropsaurus ensis (Reinhardt)	Threadfin rockling	Davis Straight and Baffin Bay, Canada	Khan et al. 1991
Melanogrammus aeglefinus (L.)	Haddock	Grand Banks, Newfoundland, Canada	So 1972
2. Macrouridae	Grenadiers		
Macrourus berglax Lacépède	Roughhead grenadier	Davis Straight and Baffin Bay, Canada	Khan et al. 1991
3. Merlucciidae	Merluccid hakes		
Merluccius bilinearis (Mitchill)	Silver hake	Cape Hatteras, North Carolina to Gulf of Maine, USA	Khan and Newman 1982
4. Moridae	Morid cods		
Antimora rostrata (Günther)	Blue hake	Davis Straight and Baffin Bay, Canada	Khan et al. 1991
5. Phycidae	Phycid hakes		
Urophycis chuss (Walbaum)	Red hake	Cape Hatteras, North Carolina to Gulf of Maine, USA	Khan and Newman 1982
Urophycis tenuis (Mitchill)	White hake	Grand Banks, Newfoundland, Canada	So 1972
Perciformes	Perch-like fishes		
1. Ammodytidae	Sand lances		
Ammodytes americanus De Kay	American sand lance	Grand Banks, Newfoundland, Canada	So 1972
2. Anarhichadidae	Wolffishes		
Anarhichas lupus L.	Wolf-fish	Davis Strait and Baffin Bay, Canada	Khan et al. 1991
Anarhichas minor Olafsen	Spotted wolfish	Davis Strait and Baffin Bay, Canada	Khan et al. 1991
3. Sciaenidae	Drums or croakers		
Leiostomus xanthurus Lacépède	Spot croaker	Cape Hatteras, North Carolina to Gulf of Maine, USA	Khan and Newman 1982
Pleuronectiformes	Flatfishes		
1. Pleuronectidae	Righteye flounders		
Glyptocephalus cynoglossus (L.)	Witch	Grand Banks, Newfoundland, Canada	So 1972
Hippoglossoides platessoides (Fabricius)	American plaice	St. Andrews, New Brunswick, Canada; Ungava Bay, Northern and Southern Labrador, Canada; Grand Banks, Newfoundland, Canada; Davis Strait and Baffin Bay, Canada; St. Lawrence River Estuary, Matane, Quebec, Canada	Laird and Bullock 1969*; Khan 1986; So 1972; Khan et al. 1991; Siddall et al. 1994

(continued)

Table 3. Continued.

Host fishes by orders and families	Common names for host orders, families and fishes	Location	Authors
Hippoglossus hippoglossus (L.)	Atlantic halibut	Davis Strait and Baffin Bay, Canada	Khan et al. 1991
Limanda ferruginea (Storer)	Yellowtail flounder	Grand Banks, Newfoundland, Canada	So 1972
Pseudopleuronectes americanus (Walbaum)	Winter flounder	Experimental transfer to this host	Khan 1984**
Reinhardtius hippoglossoides (Walbaum)	Greenland halibut	Ungava Bay, Northern and Southern Labrador, Canada; Davis Strait, south and west coasts of Newfoundland, Canada; Davis Strait and Baffin Bay, Canada	Khan 1986; Khan et al. 1982; Khan et al. 1991
Scorpaeniformes	Scorpionfishes and flatheads		
1. Cyclopteridae	Lumpfishes		
Eumicrotremus spinosus (Fabricius)	Atlantic spiny lumpsucker	Ungava Bay, Quebec, Canada	Khan 1986
2. Hemitripteridae	Sea ravens, sailfin sculpins	St. Andrews, New Brunswick, Canada;	Laird and Bullock 1969*;
Hemitripterus americanus (Gmelin)	Sea raven	Cape Hatteras, North Carolina to Gulf of Maine, USA	Khan and Newman 1982
3. Psychrolutidae	Fatheads		
Cottunculus microps Collett	Polar sculpin	Davis Straight and Baffin Bay, Canada	Khan et al. 1991
4. Sebastidae	Rockfishes, rockcods and thorny heads		
Sebastes marinus (L.)	Redfish	Davis Strait and Baffin Bay, Canada	Khan et al. 1991

*Considered by So (1972) to be records of *H. terraenovae*; **Khan (1984) reported transfer of *H. terraenovae* from American plaice to winter flounder.

Most recently, Siddall et al. (1994) also examined *H. terraenovae* from *H. hippoglossus*, although the organisms in their micrographs, showing mono-, bi-, tetra- and octonucleate stages in erythrocytes, did not closely match So's (1972) original drawings. Since the parasite found in the current study comes from a flatfish, it has a loop-like structure between some nuclei as in So's (1972) illustrations, and it resembles Siddall et al.'s (1994) light micrographs of *H. terraenovae*, it is logical to identify it provisionally as this organism. Its likeness to *Haematractidium scombri* Henry, 1910 is also noted (see Henry 1910, 1913c, Johnston 1975, MacLean 1980, Davies 1995). However, extracellular forms, like those seen in our material, were not recorded by any authors who previously reported *H. terraenovae*.

DISCUSSION

Few protozoan blood parasites of marine fishes are suspected to exist in both the northern and southern hemispheres. Among haemogregarines, examples may include *Desseria mugili* (Carini, 1932) Siddall, 1995 and *Haemogregarina* (sensu lato) *bigemina* (Laveran et Mesnil, 1901) Siddall, 1995, the latter apparently being distributed among several fish families and many fish species (see Eiras et al. 1995, Davies and Smit 2001, Davies et al. 2004). Since it is impossible to distinguish the Brazilian haemogregarine from *Desseria platessae* morphologically or morphometrically and it occurs in a flatfish, the known distribution of this haemogregarine now appears to cover the northeast, northwest, and southwest Atlantic, and includes a broad range of predominantly flatfish hosts (Table 2).

Immature stages and suspected meronts of *D. platessae* (as *H. platessae*) may have been observed previously (Saunders 1955, Laird and Morgan 1973, Obiekezie 1985), but binucleate meronts and paired, immature, haemogregarines likely resulting from division, as seen in the current study, have not been recorded. Since members of the genus *Desseria*, by definition, lack erythrocytic merogony (Siddall 1995), it is recommended that *D. platessae* is placed in the repository genus *Haemogregarina* (sensu lato) (see Siddall 1995), at least, until its life cycle can be established fully. The following nomenclatural correction is therefore recommended: *Haemogregarina* (sensu lato) *platessae* (Lebailly, 1904) [syns. *Haemogregarina platessae* Lebailly, 1904; *Desseria platessae* (Lebailly, 1904) Siddall, 1995] in the erythrocytes of *Pleuronectes platessa* L. and other flatfishes.

Haemohormidium terraenovae also seems to have a broad distribution from Canada, along the eastern seaboard of the USA (see Table 3), and perhaps to South America, if the parasite from Brazil is this species. Like the haemogregarine, *H. terraenovae* is not host-specific within flatfishes, but it also parasitizes other hosts, such as a broad range of cods, hakes, scorpionfishes and flatheads (Table 3).

Although numbers of host fish studied were low, it was interesting to note that prevalence of *H. platessae* was high, especially among female fish from polluted (Coroa do Boi, CB) and non-polluted (Saco do Justino, SJ) regions of the Patos Lagoon estuary, in the Brazilian summer, autumn and winter. Conversely, Laird and Morgan (1973) reported absence of infection in hogchokers caught in winter in North America. In the current study, the influence of season on parasitism with the haemogregarine in adult fish was only observed with the meront at SJ, with fewer fish having this stage in the Brazilian autumn/winter. Furthermore, there was an indication that pollution reduced the likelihood of infected fish carrying meronts and mature gamonts of *H. platessae*, with male fish being even less likely than females to sustain mature gamonts in polluted water. Similar observations were made for the *H. terraenovae*-like organism, although individual parasite stages were not analysed.

Since meronts and immature gamonts of *H. platessae* were found in immature fishes captured from the polluted site (CB) in summer and autumn, this suggests that the haemogregarine is acquired by young fishes in this environment, and that its hosts, including its vector, are to some extent tolerant of the pollution. On the other hand, the *H. terraenovae*-like organism was detected in fish from CB and SJ, but only once during the Brazilian winter (July–September, water surface temperature, 13–14°C). This contrasts with Siddall et al. (1995), who reported that American plaice held in captivity over winter in Canada showed increased intensity of infection with *H. terraenovae* between December and March. Dual transmission by marine leeches of *Haemohormidium* and trypanosomes, and of haemogregarines and trypanosomes, has been reported (Khan 1984, Hayes et al. 2006), and leeches and haemogregarines of freshwater turtles may tolerate urban contamination (Brites and Rantin 2004). Perhaps simultaneous transmission of *H. platessae* and the *H. terraenovae*-like organism occurs in both polluted and non-polluted regions of the Patos Lagoon Estuary, although if the two infections have a common leech vector, differences in prevalence and perhaps seasonality of the two infections are difficult to explain.

Much has been written about the usefulness of parasites as indicators of environmental health (see reviews of Khan and Thulin 1991, Williams and MacKenzie 2003, Sures 2004, Marcogliese 2005). Of the two sites fished in the present study, CB is rich in domestic effluent, with raised ammonium, phosphate, copper and zinc, and significant petroleum hydrocarbon levels; conversely, SJ has no known contaminant inputs (see Amado et al. 2006). Although the current research involves only 50 fish, a similar sized population to that in the original biomarker study (see Amado et al. 2006), some interesting trends are indicated, namely differences in blood parasite loads between CB and SJ. Since *P. orbignyanus* occurs in estuaries and along the coast of Argentina and Uruguay, as well as in Brazil (see Amado et al. 2006), a broader study involving more sites and host fish might better indicate the potential of the two blood parasites as biological tags and pollution exposure indicators.

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