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PARASITES OF FRANCISCANA (*PONTOPORIA BLAINVILLEI*) FROM SÃO PAULO AND PARANÁ STATES, BRAZIL

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Abstract – The franciscana, *Pontoporia blainvillei*, is a threatened small cetacean of the western South Atlantic Ocean and is commonly caught incidentally in fishing nets along its distribution. Detailed studies regarding parasite loads of *P. blainvillei* have been recommended as a tool to evaluated ecological segregation and stock structure. We analyzed metazoan parasites from stomach, intestines and lungs of 41 franciscanas from three close geographical areas: Group 1, Guarujá and Praia Grande (São Paulo State), n=12 (24°00'S, 46°17'W); Group 2, Cananéia (São Paulo State), n=17 (25°00'S, 47°56'W); Group 3, Paraná State, n=12 (25°30'S, 48°23'W). The sample consists mainly of immature specimens, less than 3 years old. No parasites were found in the lungs analyzed. The acanthocephalan (*Polymorphus* sp) found in two stomachs were considered occasional. The trematode *Hadwenius pontoporiae* (Digenea: Campulidae) was the only parasite found in the intestines with prevalence of 100% for Group 1, 82.35% for Group 2 and 58.33% for Group 3. The mean intensity of *H. pontoporiae* for Groups 1, 2 and 3 was 39.5, 25.05 and 13.5 parasites per host, respectively. In this study there was a significant difference between the number of parasites among the three groups (Kruskal-Wallis test, p=0.012). Groups 2 and 3 are geographically close, and exhibited non-significant differences in the total number of parasites found (Mann-Whitney test, p=0.063). Groups 1 and 3, that are further apart geographically, differed significantly in the total number of parasites found (Mann-Whitney test, p=0.003). Through the analysis of data from the areas where *H. pontoporiae* was found [i.e. Areas 1, 2, 3 (this study), Rio Grande do Sul State and Argentina], it could be noticed that the prevalence and mean intensity increase as latitude increases. *H. pontoporiae* may continue to be useful as a biological tag for identifying franciscana's ecological stocks, together with information from genetic, morphometric and other biological studies. However, it is essential that standardized methodology be used in the collection of the parasite data.

Resumo – A toninha, *Pontoporia blainvillei,* é um cetáceo ameaçado do Atlântico Sul Ocidental. Captura acidentais ao longo de sua distribuição são freqüentes. O uso de informações a respeito dos parasitas de *P. blainvillei* é recomendado para avaliar segregações ecológicas e diferenciar estoques populacionais. Estômagos, pulmões e intestinos de 41 animais foram analisados buscando parasitos metazoários. Os animais são provenientes de 3 áreas geográficas próximas: Grupo 1, Guarujá e Praia Grande (Estado de São Paulo), n=12 (24°00'S, 46°17'W); Grupo 2, Cananéia (Estado de São Paulo), n=17 (25°00'S, 47°56'W) e Grupo 3, Estado do Paraná, n=12 (25°30'S, 48°23'W). A amostra consiste basicamente de animais imaturos, entre 0 e 3 anos de idade. Nenhum parasito foi encontrado nos pulmões analisados. O acantocéfalo *Polymorphus* sp, encontrado em dois estômagos foi considerado ocasional. O trematóide *Hadwenius pontoporiae* (Digenea: Campulidae) foi o único parasito encontrado nos intestinos com prevalência de 100% para o Grupo 1, 82,35% para o Grupo 2 e 58,33% para o Grupo 3. A intensidade média de *H. pontoporiae* nos Grupos 1, 2 e 3 foi de 39,5; 25,05 e 13,5 parasitos por hospedeiro, respectivamente. Houve uma diferença significativa entre o número de parasitas dos três grupos (Kruskal-Wallis test, p=0,012). Os grupos 2 e 3 são geograficamente mais próximos e não apresentaram diferença significativa (Mann-Whitney test, p=0,063), enquanto os grupos 1 e 3, mais distantes, exibiram uma diferença significativa no número total de parasitas encontrados (Mann-Whitney test, p=0,003). Através da análise das áreas onde *H. pontoporiae* foi encontrado: áreas 1, 2, 3 (este estudo), Estado do Rio Grande do Sul e Argentina, verificou-se uma tendência de aumento da prevalência e intensidade média de acordo com o aumento da latitude. *H. pontoporiae* pode continuar sendo usado como marcador biológico para toninhas, com metodologia padronizada, juntamente com outras características, sempre considerando-se todos os fatores que podem afetar os resultados.

Keywords: *Pontoporia blainvillei*, parasites, stock identification, western South Atlantic, Brazil.

Introduction

The franciscana, *Pontoporia blainvillei*, is considered one of the most threatened small cetacean in the western South Atlantic Ocean (Secchi *et al*., in press a) and is the most common cetacean incidentally caught in fishing nets along the coast of São Paulo and Paraná states, Brazil (e.g. Rosas, 2000; Bertozzi and Zerbini, 2002; Santos *et al*., 2002). Studies on the stock structure and abundance of franciscana in its restrict home range are urgently needed to evaluate the impact of this incidental mortality on local populations (Secchi *et al*., 2001; Secchi *et al*., in press b).

Parasites have been useful as an additional source of information on various aspects of host biology, including stock identity. Comparisons of infection levels of one or more species of parasites among host groups within a given geographic area can aid in the identification of populations that are segregated, potentially comprising different ecological stocks (e.g. Szidat, 1964; MacKenzie, 1987; Dailey and Vogelbein, 1991; Moser, 1991; Aznar *et al.*, 1995; Balbuena *et al*., 1995; Aznar *et al*., 1997a, Walker, 2001). The use of such information as a tool to examine the stock structure of the franciscana has already been recommended (Reeves and Leatherwood, 1994; IBAMA, 1997).

Previous studies of the parasite fauna of franciscana along the coast of southern Brazil (Rio Grande do Sul State), Uruguay and Argentina suggest the occurrence of two segregated populations (Aznar *et al*., 1995; Andrade, 1996).

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However, few parasite data are available for franciscanas from the coastal waters of Santa Catarina up to Espírito Santo states (29°05'S -18°25'S), and the stock structure of the species along this latitudinal gradient is not completely known.

The aim of this work is to present a preliminary description of the parasite community of specimens of *P. blainvillei* from Paraná and São Paulo states. The results can be useful in stock differentiation for conservation purposes.

Material and Methods

We analyzed metazoan parasites from the stomach, intestines and lungs of 16 female and 25 male franciscanas. Due to the small sample sizes for some areas, specimens were grouped according to their sampling location (Figure 1). Group 1 (n=12) includes animals from Guarujá (n=3) and Praia Grande (n=9) (ca. 24°00'S, 46°17'W), on the central coast of São Paulo State. Group 2 includes franciscanas from Cananéia (n=17) (ca. 25°00'S, 47°56'W), southern São Paulo State. Specimens from Baía de Paranaguá (n = 12) (ca. 25°30'S, 48°23'W), northern Paraná State, were included in Group 3.

Intestines of one franciscana from Ubatuba and one from Itanhaém, São Paulo State, were also sampled.

The total length and gender of each specimen were recorded. Lungs were opened with scissors inside a plastic tray and all the contents were washed in a sieve (mesh =150µm) to collect parasites. All macroscopic parasites seen during the analysis of food remains in the stomachs were collected. Small and large intestines were identified macroscopically according to Yamasaki *et al*. (1975). Based on the methodology used by Andrade (1996), the small intestine was divided into five equal-sized sections and only the first third of each section was sampled. The entire large intestine was examined. The intestinal contents were washed in a sieve (mesh = 150µm) and examined on a stereoscopic microscope (10x) to collect the parasites.

All parasites were fixed and stained according to Dailey (1978) and Gardiner (2000). Species identification was based mainly on Price (1932), Raga *et al*., (1994), Fernandez *et al*., (1995) and Yamaguti (1963).

Prevalence equal to or greater than 50% was used as a criterion to identify the parasite community. Pearson's correlation coefficient was applied to examine the correlation between infection levels (number of parasites per host) and total body length. Kruskal-Wallis and Mann-Whitney tests were used to compare mean intensity between the groups. A Mann-Whitney test was also applied to compare the mean intensity between males and females. Linear regression was used to examine trends in mean intensity along the latitudinal gradient, based on mean

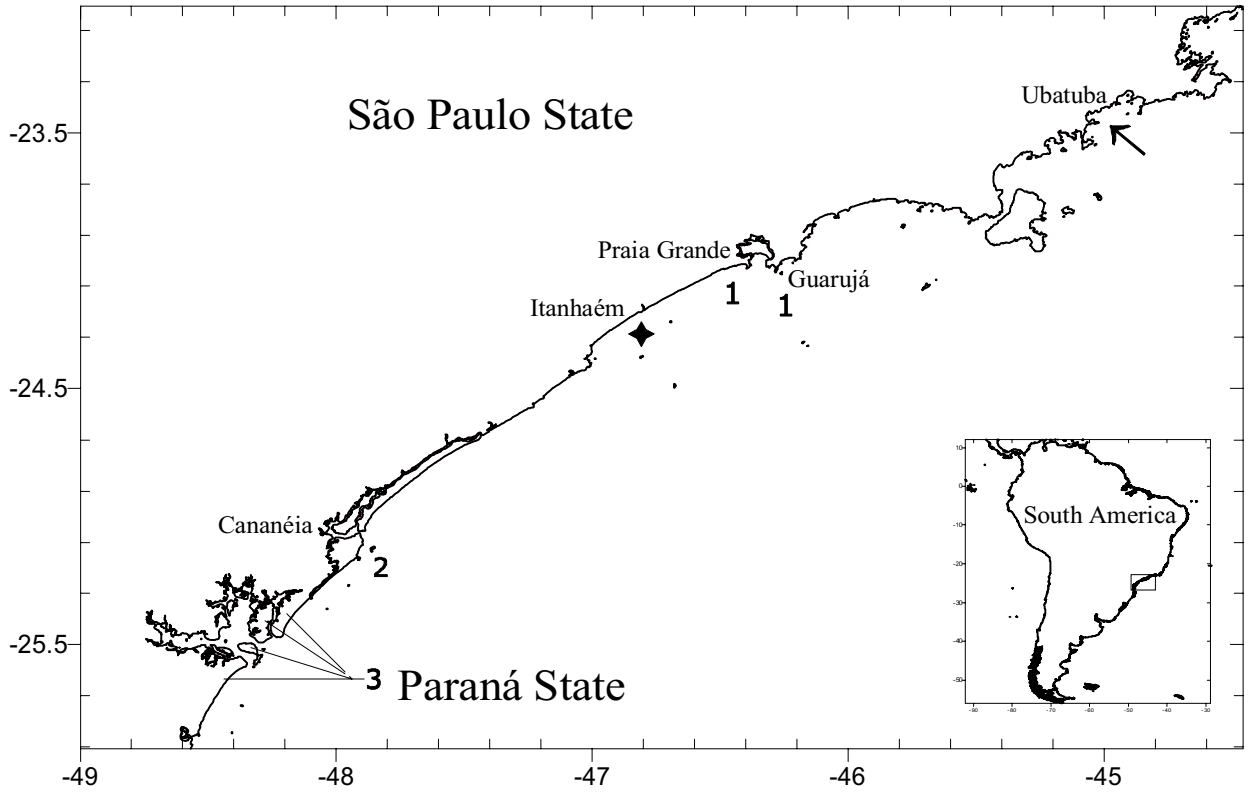

Figure 1. Map of São Paulo and Paraná states, showing the main sampling areas for franciscanas – (Area 1) Group 1: Guarujá and Praia Grande, São Paulo State (n=12); (Area 2) Group 2: Cananéia, São Paulo State (n=17); (Area 3) Group 3: Paraná State (n=12). (←) 1 animal from Ubatuba, São Paulo State; (F) 1 animal from Itanhaém, São Paulo State.

intensity and prevalence data from other regions, in addition to the data presented in this study.

Most franciscanas had their age determined by Rosas (2000). A Kruskal-Wallis test was applied to verify if total body length of specimens from the three groups differed. A significance level of 0.05 was used in all tests, unless stated otherwise.

Results and Discussion

Specimens

Osteological (Pinedo, 1991) and genetic studies (Secchi *et al*., 1998) suggest the existence of at least two distinct populations of *P. blainvillei*: a smaller northern geographic form (latitudes between 22º and 27ºS) and a larger southern form (latitudes between 32º and 38ºS). Considering the geographic location of the areas sampled, and the lack of a significant difference in the total length between animals from the three groups (Kruskal-Wallis test, p=0.388), we believe that all animals examined belong to the northern population.

Rosas (2000) analysed gonads and determined the ages of the same franciscanas examined in this paper. This author estimated that sexual maturity of franciscanas from Groups 2 and 3 is attained when males and females are 112-116cm and 122-126cm long, respectively (Figure 2), and that the age of attainment of sexual maturity was between 4 and 5 years for both sexes. The age of the animals from these groups ranged from 0 to 16 years, with the majority consisting of immature specimens less than 3 years old. Age data are not available for animals from Group 1; however, based on their mean total length (111.13 ± 16.40cm for females and 107.25 ± 8.07cm for males), it is suspected that most are immature specimens under 4 or 5 years. However, these results should be interpreted with caution because individuals from adjacent geographic locations may show different growth patterns.

Parasite Analysis

- Lungs

The lungs of franciscanas from Group 1 have not yet been analyzed. The lungs of the animals from Groups 2 and 3 were not infected by helminths; this is consistent with the fact that no parasites have ever been reported in lungs of franciscanas. At the coast of Paraná State (Group 3), other sympatric cetacean species, *Sotalia guianensis* (Cetacea: Delphinidae), is often found with high pulmonary infections by the nematode *Halocercus brasiliensis* (Marigo *et al*., 2000), which could be justified by the close host-specificity of this parasite (Dougherty, 1943).

- Stomachs

Stomachs from Group 1 are yet to be analyzed. Only two specimens of the acanthocephalan *Polymorphus* sp. were found in two stomach contents of two individuals from Group 2. Since only one parasite was found in each host, those parasites can be considered rare or even occasional because of their very low prevalence (2/29= 6.89%) and intensity of infection.

Andrade (1996) refers to *Anisakis typica, Polymorphus cetaceum* and *Corynosoma australe* in the stomachs of franciscanas from Rio Grande do Sul State. The present results are almost similar to, and may confirm, those results obtained by Santos *et al.* (1996), where no parasites were found in the stomachs of 42 animals from Rio de Janeiro State. In contrast, the sympatric *S. guianensis* from Rio de Janeiro and Paraná states harbors *Anisakis* sp. in the stomach (Santos *et al*., 1996; Marigo *et al*., 2001).

Overall, the occurrence of a particular parasite in any geographic location depends upon the presence of a suitable host, suitable intermediate host(s), and complex biological factors which impart a strict interdependency on the organisms comprising the host-parasite complex (Dailey and Vogelbein, 1991). In addition, a host's parasite

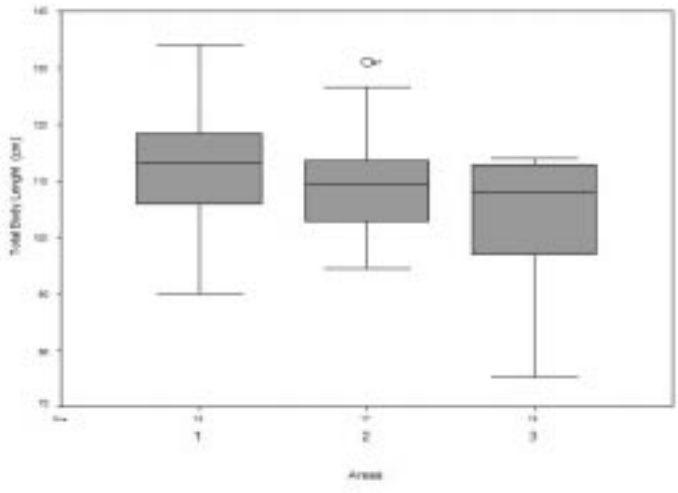

Figure 2. Dispersion of the total body length data (in cm) within the various sampling areas. The total number of samples (N) for each area is also shown on the x-axis. (Area 1) Guarujá and Praia Grande, São Paulo State; (Area 2) Cananéia, São Paulo State; (Area 3) Paraná State.

community can also be viewed as a characteristic of the specific host. Many parasite species are legacies of extinct host ancestors, so similarities between related species of hosts are usually not a result of convergence or recent acquisitions related to host ecology, but probably because they share a recent common ancestor (Poulin, 1996). *P. blainvillei* and *S. guianensis* are not phylogenetic closely related, which may explain in part why their parasite community is different, even though they occur in the same habitat. Aznar *et al.* (1994) mention that Pontoporiidae dolphins have adapted secondarily to freshwater habitats at least three times, and that franciscanas may have had one or several freshwater ancestors, which would involve remarkable habitat shifts for its helminth parasites. Evolutionary restrictions may be responsible for the relatively low richness and diversity of helminths found in the franciscana.

The lack of stomach parasites in specimens from this study, in comparison with specimens from southern Brazil, may be a reflection of differences in the franciscana's food habits and/or the absence of suitable intermediate hosts at lower latitudes.

- Intestines

In all groups, using a prevalence equal to or greater than 50% as a criterion to identify the parasite component community, franciscanas also showed a low diversity in the intestinal parasitic fauna, which was represented only by one species, the trematode *Hadwenius pontoporiae* (Digenea: Campulidae). The prevalence of this parasite was 100% for Group 1, 82.35% for Group 2 and 58.33% for Group 3 (Table 1).

In a previous study, franciscana dolphins from Rio Grande do Sul State (Andrade, 1996) presented a more diverse parasite community, harboring in the intestines three parasite species: *H. pontoporiae, Corynosoma australe,* and *Bolbosoma turbinella.* In Uruguay, no parasites have been found in their intestines (e.g. Brownell, 1975; Kagei *et al,* 1976; Praderi, 1984). Franciscanas from Argentina had *H. pontoporiae* and *Pholeter gastrophilus* (Aznar *et al.,* 1994) (Table 2).

Hadwenius pontoporiae does not seem to have a constant distribution in the intestine, occurring only in the initial portion of this organ. This pattern of distribution has been observed in franciscanas from Buenos Aires Province, Argentina (Aznar *et al.*, 1997b), and from Rio Grande do Sul (Andrade, 1996), Paraná and south of São Paulo states (Marigo *et al.*, 1999).

The mean intensity of *H. pontoporiae* for Groups 1, 2 and 3 was 39.5, 25.05 and 13.5 parasites per host, respectively (Table 1). One animal from Group 1 presented a total of 125 *H. pontoporiae*, and another individual from Group 2 harbored 84 trematodes. The only animal from Ubatuba (a 106cm long male), northern coast of São Paulo State,

Table 1. Comparison of identified gastrointestinal helminth species in specimens of *Pontoporia blainvillei* from São Paulo (Areas 1, 2 - SP), Paraná (Area 3 - PR) and Rio Grande do Sul (RS) states, Brazil, and from Uruguay and Argentina*.

Helminths	Site	Brazil				Uruguay	Argentina	References
		Area 1 SP	Area 2 SP	Area 3 PR	RS			
Anisakis typica	S	-	-	-	▒	▒	-	1,2,3
Anisakis simplex	S	-	-	-	-	-	▒	4
Contracaecum sp	S	-	-	-	-	▒	▒	4,5,6
Procamallanus sp	S	-	-	-	-	▒	-	1,3
Hadwenius pontoporiae	S, I	▒	▒	▒	▒		▒	2,4,7,8
Polymorphus (P.) cetaceum	S	-	-	-	▒	▒	▒	1,2,3,4
Corynosoma australe	S, I	-	-	-	▒	-	-	2
Bolbosoma turbinella	S	-	-	-	▒	-	-	2
Pholeter gastrophilus	S	-	-	-	-	-	▒	4

*Adapted from Andrade, 1996.
(▒) presence, (-) absence, (S) stomach, (I) intestine

References:

1 - Kagei *et al.,* 1976 (n=1)
2 - Andrade, 1996 (n=53)
3 - Praderi, 1984 (n=157)
4 - Aznar *et al.,* 1994 (n=46)
5 - Dailey and Brownell, 1972 (n=?)
6 - Brownell, 1975 (n=191)
7 - Marigo *et al.,* 1999 (n=19)
8 - Present study (n=41)

Table 2. Mean intensity (mean number of parasites per specimen) and prevalence (percentage of specimens infected) of the trematode *Hadwenius pontoporiae* from franciscanas from São Paulo (Areas 1, 2 - SP), Paraná (Area 3 - PR) and Rio Grande do Sul (RS) states, Brazil, and from Argentina.

Hadwenius pontoporiae	BRAZIL				Argentina
	Area 1 SP	Area 2 SP	Area 3 PR	RS	
Mean intensity	39,5	25,05	13,5	166,3	397
Prevalence	100%	82,35%	58,33%	97,70%	100%
References	1	1,2	1,2	3	4

References:

1 - Present study (n=41)
2 - Marigo *et al.,* 1999 (n=19)
3 - Andrade, 1996 (n=53)
4 - Aznar *et al.,* 1994 (n=46)

harbored only 6 *H. pontoporiae*. The single animal (a 120cm long male) from Itanhaém, south of Area 1, harbored 375 parasites, the highest infection level in this study. Sampling wild animals is often opportunistic, so that samples are rarely suitably age-stratified or diverse enough to detect heterogeneity in host-parasite associations (Gulland, 1997).

No significant correlation between total body length and infection levels was observed (Pearson's correlation coefficient = 0.51, $p > 0.01$). Franciscanas from Rio Grande do Sul State showed a negative correlation between mean intensity and total body length and age of the host, suggesting that the immune response increases with age (Andrade, 1996).

No significant quantitative differences in parasite infection levels were observed between sexes (males and females from the three areas together, p=0.239 and p=0.721, respectively). However, recognition of a pattern of sex differences in infections has two potential problems: 1) ensuring a sample large enough to detect a sex difference, if one is present, and 2) distinguishing between differential exposure and differential susceptibility. Sex differences in parasite prevalence or intensity are usually attributed to one of two causes: ecological or physiological, nearly always hormonal in origin (Zuk and McKean, 1996).

Another issue that was not resolved was a possible seasonal variation of infection levels due to the small sample sizes, and also that specimens of franciscana from each group were obtained at different time periods. According to Andrade (1996), seasonal variations, when present, may result not only in changes in the presence of intermediate stages and water temperature, but may also suggest host movements to other areas during a certain period of time.

In this study there was a significant difference between the number of parasites among the three groups (Kruskal-Wallis test, p=0.012). Groups 2 and 3 are geographically close, and exhibited non-significant differences in the total number of parasites found (Mann-Whitney test, p=0.063). Groups 1 and 3, which are further apart geographically, showed a significant difference in the total number of parasites (Mann-Whitney test, p=0.003).

The low infestation levels in specimens from the three groups suggest differences between franciscanas from those groups and franciscanas from Rio de Janeiro State that had no parasites (Santos *et al.*, 1996), as well as from those from Rio Grande do Sul State that harbored *H. pontoporiae* in all five segments of the small intestine with total mean intensity of 166.3 parasites per host (Andrade, 1996). These differences corroborate with a putative stock subdivision proposed by Secchi *et al.*, (in press b), where franciscanas from Paraná and São Paulo states belong to the same stock whilst franciscanas from Rio Grande do Sul and northern Rio de Janeiro are part of other two stocks. Marigo *et al.* (1999), in an analysis of 19 animals from Areas 2 and 3, found a significant quantitative difference (χ^2 test, $p<0.001$) in the prevalence of *H. pontoporiae* between the intestines of franciscanas from these areas (prevalence = 68.4%) and those of franciscanas from Rio Grande do Sul (prevalence = 97.7%; Andrade *et al.*, 1997). This corroborates the hypothesis of different stocks of franciscanas in the two above-mentioned locations, which was already suggested by Pinedo (1991) and Secchi *et al.* (1998), based on osteological and genetic characteristics, respectively.

A regression analysis of mean intensity as a function of the areas where *H. pontoporiae* was found [Areas 1, 2, 3 (this study), Rio Grande do Sul State, and Argentina] shows a tendency of increase in the response according to the geographic area (Figure 3). And despite the slight decrease in prevalence for Area 3, there is a linear tendency of increase in prevalence with distance as latitudes increase (Figure 4). Those latitudinal trends may reflect differences in the aquatic environment that can affect the parasite cycles, including pollution and water temperature. Aquatic pollution may act on free-living parasite stages, as well on the intermediate or definite host population (Möller, 1987). Some surveys of warm-water fishes show relatively smaller numbers of digenean species than in cold-water fishes, but numbers of fish examined were small or species of only one fish family were examined (Rohde and Heap, 1998). Delyamure (1955) made the first attempt to perform a global zoogeographic analysis on helminths infecting marine

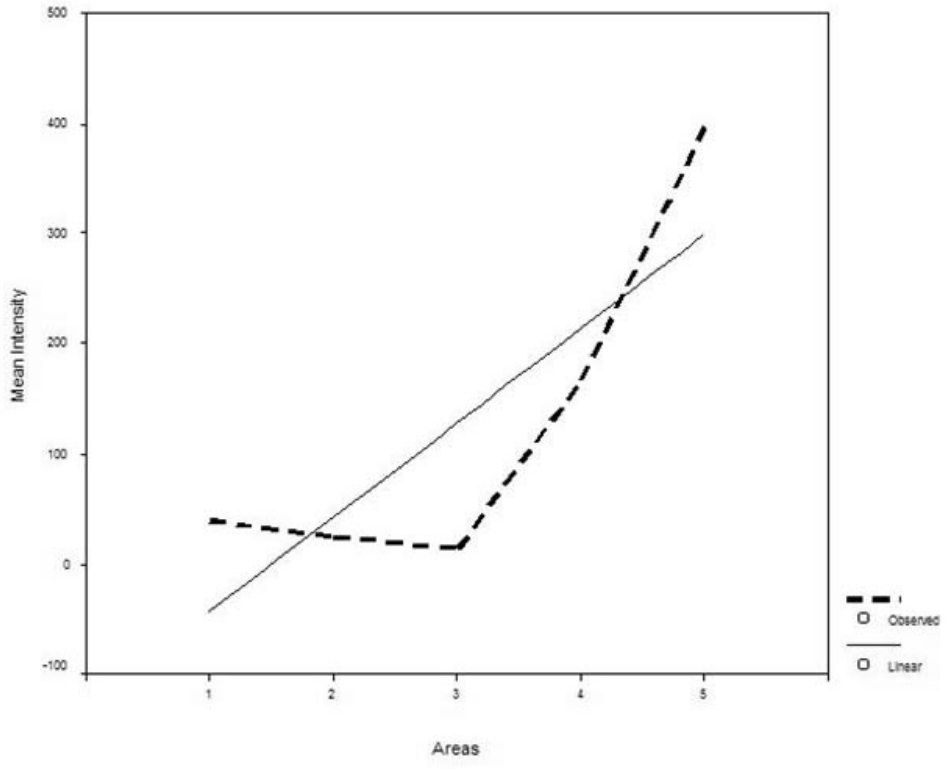

Figure 3. Regression line of mean intensity (mean number of *H. pontoporiae* per host) from the various sampling areas. (Area 1) Guarujá and Praia Grande, São Paulo State; (Area 2) Cananéia, São Paulo State; (Area 3) Paraná State; (Area 4) Rio Grande do Sul State; (Area 5) Argentina.

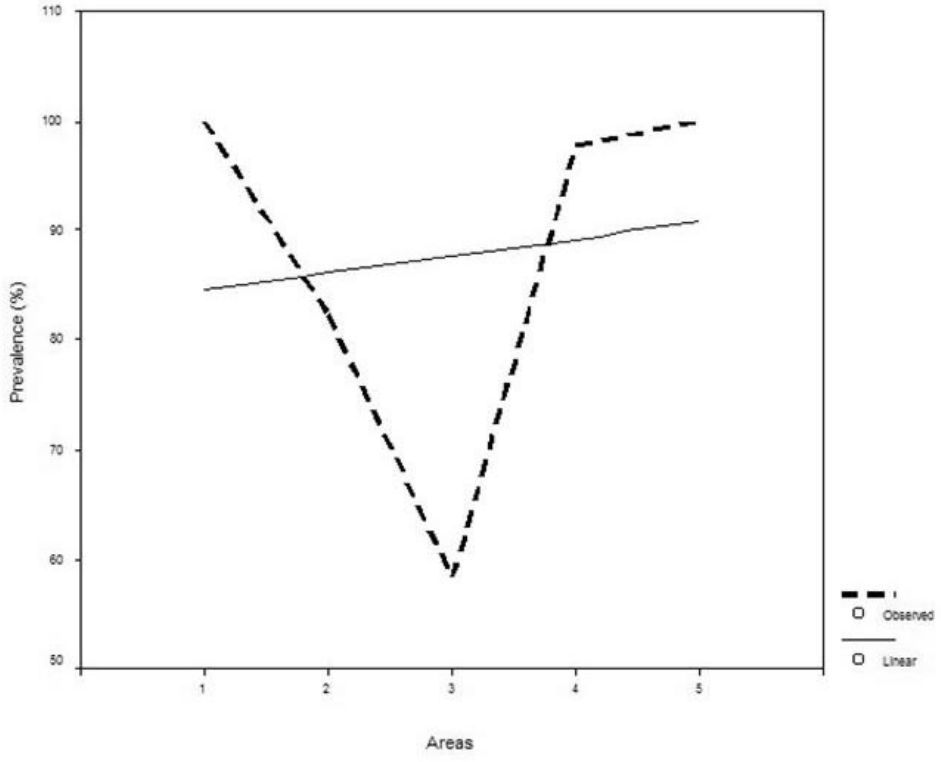

Figure 4. Regression line of the prevalence of *H. pontoporiae* from the various sampling areas. (Area 1) Guarujá and Praia Grande, São Paulo State; (Area 2) Cananéia, São Paulo State; (Area 3) Paraná State; (Area 4) Rio Grande do Sul State; (Area 5) Argentina.

mammals and found that helminth diversity is high in temperate waters, sharply dropping towards polar areas.

H. pontoporiae may continue to be useful as a biological tag for identifying franciscana's ecological stocks, together with information from genetic, morphometric and other biological studies. However, it is essential that standardized methodology be used in the collection of the parasite data.

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