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GASTROINTESTINAL HELMINTHS OF THE DUSKY DOLPHIN, *LAGENORHYNCHUS OBSCURUS* (GRAY, 1828), OFF PATAGONIA, IN THE SOUTHWESTERN ATLANTIC

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ABSTRACT

The stomachs and intestines of 23 dusky dolphins incidentally caught in a trawl fishery off Patagonia were surveyed for helminths. All the dolphins were parasitized, with a total of 3,936 helminth individuals. Only five species occurred, of which three were common (prevalence ≥10%): *Anisakis simplex*, *Braunina cordiformis* and *Hadwenius* sp. *A. simplex* was present in all the dolphins and showed the highest abundance and mean intensity (104.9 individuals/infected host). *B. cordiformis* was next most common (87%), and *Hadwenius* sp. third (52.2%). *A. simplex* was found mostly in the stomach (94.8%), *B. cordiformis* in the duodenal ampulla (51.4%), and *Hadwenius* sp. in the stomach (64.5%). The rare species *Corynosoma australe* (immature specimens) and *Pholeter gastrophilus* occurred in the stomach. *A. simplex* and *B. cordiformis* showed a clumped distribution along the intestine. Brillouin's diversity and evenness indices for the intestinal helminth community were 0.329 and 0.393, respectively. The diversity values and the number of parasite species were within the range for other small cetaceans.

Key words: *Anisakis simplex*, *Braunina cordiformis*, *Hadwenius* sp., *Corynosoma australe*, *Pholeter gastrophilus*, helminths, parasites, *Lagenorhynchus obscurus*, Cetacea, southwestern Atlantic Ocean.

The dusky dolphin, *Lagenorhynchus obscurus* (Gray, 1828), is one of the most common small cetaceans off the Patagonian coast (SW Atlantic). The species is subject to by-catches in a shrimp trawl fishery, which operates between 42° and 47°S and from the coastline to 150 nm offshore (Crespo *et al.* 1997, Dans *et al.* 1997*a*). In the last five years several studies of the biology and ecology of the species have been carried out, including density estimations (Schiavini *et al.* 1999), reproductive biology (Dans *et al.* 1997*b*), and diet composition (Koen Alonso *et al.* 1998). However, several aspects of the life history and stock identity still must be elucidated in order to model the dynamics of the populations. This includes natural mortality factors and the identification of potential stocks which may be affected differentially by the fishery. The latter is particularly important with respect to populations in Chile and Peru.

The helminths of marine mammals can provide data on the population ecology of their hosts, such as natural mortality factors (Perrin and Powers 1980; Raga *et al.* 1997), stock identity (Dailey and Vogelbein 1991, Balbuena *et al.* 1995), distribution and diet (Dailey and Otto 1982) and social behavior and spatial segregation (Balbuena and Raga 1994, Aznar *et al.* 1995). Under the assumption that different parasitic faunas would indicate that the host populations are isolated or nearly so, parasites have been used as biological indicators of stocks of fishes (Mackenzie 1983, Williams *et al.* 1992), small odontocetes (Dailey and Otto 1982, Walker *et al.* 1984, Walker 1990, Aznar *et al.* 1995), and baleen whales (Dailey and Vogelbein 1991).

The parasites of the dusky dolphin have been previously studied only off Chile and Peru in the southeastern Pacific (Van Waerebeek 1992, Van Waerebeek *et al.* 1993), although some preliminary data from New Zealand are also available (Cipriano 1992). For the southwestern Atlantic, the previously available information is scanty. This study also provides baseline data for future comparison with populations in other geographical areas (New Zealand, South Africa, Chile, and Peru).

Materials and Methods

The sample consisted of 23 dusky dolphins incidentally caught in the trawl fishery off Patagonia between 1990 and 1995. The animals were frozen on

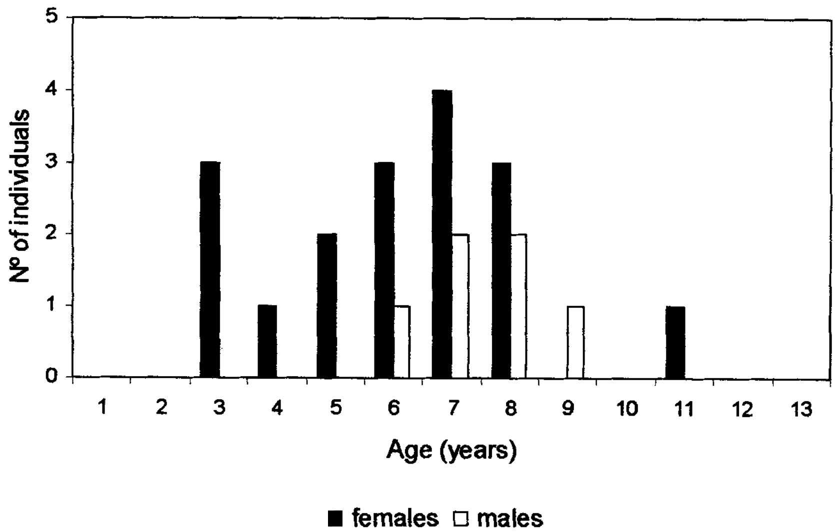

Figure 1. Age structure of dusky dolphins (Lagenorhynchus obscurus) from Patagonia analyzed in this study.

board and dissected in the laboratory. The contents of stomachs and intestines were extracted and sorted using a series of sieves. Helminths were removed, washed in saline solution, fixed in 70% ethanol or 5% formalin, and then preserved in 70% ethanol. Helminth species were determined by using standardized methodology and available systematic keys (Baylis 1932, Price 1932, Yamaguti 1951, Dailey and Brownell 1972, Zdzitowiecki 1991). The specimens collected from the stomach and intestines (including the duodenal ampulla) were collected and counted separately. The intestines were divided into 30 sections of equal length to describe more precisely the linear distribution of each helminth species.

The terms "prevalence" (number of hosts infected with a particular parasite species divided by the number of hosts examined for that parasite), "mean intensity" (average number of individuals of a particular parasite species per host infected with that species), and "mean abundance" (total number of individuals of a particular parasite species divided by the total number of hosts examined, both infected and uninfected) conform with Bush *et al.* (1997). Parasite species were categorized into "component" (prevalence $\geq$10%) and "rare" (<10%) (Bush *et al.* 1990). To compare with other delphinid species, Brillouin's diversity and evenness indices (based on natural logarithms) were calculated for both gastrointestinal and intestinal infracommunities (Magurran 1988; Krebs 1989, 1992).

Prevalence and intensity of parasite species may be a factor of host age. It is important to insure that the age structures of host samples are comparable for meaningful comparisons between cetacean populations (Walker 1990). Figure 1 shows the age distribution of the sample of dolphins, as determined by examination of tooth layers.

Site selection was analyzed for each helminth species on an infrapopulation (individual dolphin) basis. The relative abundance (*RA*) of each species in the stomach, duodenal ampulla, and the 30 intestinal sections of each infrapopulation were calculated as:

$$RA_i(x) = n_i(x)/n_i \times 100$$

where $n_i(x)$ is the total number of individuals of the helminth species i located in site x, and n_i is the total number of individuals of the helminth species i in the individual dolphin.

The spatial arrangement of the helminths in the intestine was expressed by the following indices of dispersion (sample size or number of quadrats corresponds to total number of sections): (1) variance-to-mean ratio I (Krebs 1989, 1992); significance assessed with a χ^2 statistical test and (2) Standardized Morisita's index I_p (Krebs 1989, 1992). The advantage of this index is that it varies between -1 and $+1$, and values above 0.5 correspond to clumped distributions with a confidence interval of 95%.

A negative binomial distribution was fitted to the observed frequency distribution along the intestine, by estimating the k parameter with a maximum likelihood method (Krebs 1992). To test whether the distribution model describes the observed counts, a chi-square goodness-of-fit test or a U-goodness-of-fit test was used. The U test is adequate for small sample sizes; it compares the observed and expected variances of the negative binomial distribution. The expected value of U is 0, and the negative binomial distribution is rejected when U exceeds two standard errors of U (Krebs 1989).

Results

A total of 3,936 helminth individuals belonging to five species were collected. They were one nematode (*Anisakis simplex*), one acanthocephalan (*Corynosoma australe*), and three digenetic trematodes: *Braunina cordiformis* (Brauninidae), *Pholeter gastrophilus* (Troglotrematidae), and *Hadwenius* sp. (Campulidae). The prevalence, mean intensity, and abundance of the species are shown in Table 1.

Three species were classified as component (*A. simplex*, *B. cordiformis*, and *Hadwenius* sp.), while the remaining two were rare (*P. gastrophilus* and *C. australe*). *A. simplex* and *B. cordiformis* showed prevalences higher than 85%. *A. simplex* also showed the highest mean intensity and abundance. *B. cordiformis* and *Hadwenius* sp. showed lower and similar mean intensities and ranges of intensity (Table 1).

All the dolphins were parasitized, more than half of them (57%) harboring three of the five parasite species. Only one dolphin harbored four species and none harbored all five species. *Anisakis simplex* seemed to dominate the infracommunities, accounting for 61% (SD = 29.07) of the total gastrointestinal helminth individuals, while the brauninid and campulid accounted for 28% (SD = 24.28) and 11% (SD = 17.89), respectively. Both gastrointestinal and intestinal infracommunities had low helminth diversity (mean Brillouin's in-

Table 1. Prevalence, intensity of infection, and abundance of helminth species found in 23 dusky dolphins off Patagonia in southwestern Atlantic.

Helminth species	Hosts n	Prevalence %	Intensity range	Intensity $\bar{X} \pm SD$	Abundance $\bar{X} \pm SD$	Abundance N
Nematoda						
Anisakidae						
Anisakis simplex	23	100	11–353	104.9 ± 97.2	104.9 ± 97.2	2,412
Trematoda						
Brauninidae						
Braunina cordiformis	20	86.96	3–178	50.5 ± 45.5	43.9 ± 45.5	1,010
Campulidae						
Hadwenius sp.	12	52.17	1–171	42.6 ± 51.0	22.2 ± 42.1	511
Troglotrematidae						
Pholeter gastrophilus	1	4.35	1	1	0 ± 0.2	1
Acanthocephala						
Polymorphidae						
Corynosoma australe	1	4.35	2	2	0.1 ± 0.4	2

n: number of hosts.
N: number of helminth individuals.

Table 2. Values of Brillouin's diversity index (*H*), eveness (*E*), number of helminth species (*S*), and total abundance of helminths (*N*) from intestinal and gastrointestinal infracommunities of dusky dolphins off Patagonia in southwestern Atlantic.

	H	*E*	*S*	*N*
Intestinal				
$\bar{X}$	0.329	0.393	1.826	39.348
SD	0.297	0.388	0.650	36.026
Gastrointestinal				
$\bar{X}$	0.574	0.374	2.522	171.087
SD	0.335	0.413	0.846	128.612

dices 0.57 and 0.33, respectively), and low species richness (mean number of species: 2.5 and 1.8, respectively) (Table 2).

Most of the *A. simplex* specimens (86.45%, SD = 23.84) were located in the stomach; the remainder occurred along the intestine and were third-stage larvae. *B. cordiformis* occurred mostly in the duodenal ampulla (RA_A = 53.29%, SD = 34.02) but also in the stomach and the intestine. *Hadwenius* sp. was found mostly in the stomach (RA_S = 58.84%, SD = 48.86) but also in the intestine. The rare *C. australe* and *P. gastrophilus* were found only in the stomach.

All the species found in the intestine showed clumped distributions. A negative binomial distribution model fit the observed counts well both for *A. simplex* (k =0.169, χ^2 = 0.784, 2 df, $P < 0.05$) and *B. cordiformis* (U being 0.5 SE) (Table 3). According to the values of the dispersion indices, *Hadwenius* sp. also showed an aggregated pattern (Table 3), but the small sample size did not allow a fit of a negative binomial distribution model to the data. *B.*

Table 3. Indices of dispersion, *k*-parameter, and goodness of fit tests of helminth spatial arrangement in the intestines of dusky dolphins off Patagonia (I: variance to mean ratio; I_p: Standardized Morisita's index).

Helminth species	*I*	I_p	Negative binomial distribution
A. simplex	I = 1.975 χ^2 = 886.84 449 df p<0.05	0.505	k = 0.169 χ^2 = 0.7835 2 df p<0.05
B. cordiformis	I = 7.078 χ^2 = 3121.53 441 df p<0.05	0.666	k = 0.035 U = −0.263 SE(U) = 0.554
Hadwenius sp.	I = 5.709 χ^2 = 2563.5 449 df p<0.05	0.600	k = 0.028 U = 0.12 SE(U) = 0.061

cordiformis was located only in the first section (duodenum). In a plot of relative abundance on intestinal section, *A. simplex* did not show a clear distributional pattern but seemed to be located mostly in the first and last sections, while *Hadwenius* sp. was located in the anterior sections.

Discussion

The gastrointestinal helminth communities of dusky dolphins off Patagonia seem to be depauperate, with low species richness. The communities are dominated by *A. simplex* (which also dominates numerically) and *B. cordiformis*.

Most of the individuals of *A. simplex* (86.45 %) were found in the stomach, which is the habitat for the maturation process of this group (Smith and Wootten 1978). The remaining were found clumped along the intestine and were larval stages, which could be related to a digestion and elimination pattern. The presence of *A. simplex* in the intestine could be due to unsuccessful worms coming from the stomach. This may account for the apparent concentration of individuals in the anterior sections (nematodes that possibly died recently) and the last section (accumulation with fecal materials).

Anisakids are a very generalist nematode group, and *A. simplex* has been described from a wide range of intermediate or paratenic hosts (crustaceans, cephalopods, and fishes) and definitive hosts (pinnipeds and mainly cetaceans) (Smith and Wootten 1978). The most frequent prey species of the dusky dolphin in Argentinean waters include the southern anchovy (*Engraulis anchoita*), Argentine squid (*Illex argentinus*), Patagonian squid (*Loligo gahi*) and common hake (*Merluccius hubbsi*) (Koen Alonso *et al.* 1998). Larval stages of *Anisakis* sp. have been reported in all these prey species (Szidat 1955, Nigmatullin 1989, Nigmatullin and Shukhgalter 1990, Sardella *et al.* 1990), and therefore it is plausible to assume that the dolphins are frequently exposed to *Anisakis* infections, accounting for the high levels of prevalence and intensity.

The presence of acanthocephalans in the intestines of many cetaceans and pinnipeds is well documented (Raga 1992, 1994), but only three acanthocephalan species (*Corynosoma hamanni*, *Polymorphus arctocephali*, and *P. cetaceum*) are known to have colonized the stomachs of marine mammals (Zdzitowiecki 1984, Smales 1986, Aznar *et al.* 1994). In the Argentine dusky dolphins, only two immature individuals of *C. australe* were found in the stomach of one animal. This finding may suggest that the individuals were ingested with prey, probably the southern anchovy, a species reported as an intermediate host of *C. australe* in the area.[1] Furthermore, the southern anchovy is a common prey for dusky dolphins in Patagonian waters (Koen Alonso *et al.* 1998). The rarity and immature condition of the worms suggest that this dolphin is an unsuitable definitive host for this acanthocephalan species.

The digenean *P. gastrophilus* was previously recorded from the stomach and

[1] Personal communication from J. Timi and N. Sardella, Laboratorio de Parasitología. Departmeto de Biología, F.C.E.yN., Universidad Nacional de Mar del Plata, Funes 3350 (7600) Mar del Plata, Buenos Aires, Argentina, Spetember 1998.

intestine of many odontocete cetaceans, including dusky dolphins from Peruvian waters (southeastern Pacific; Van Waerebeek 1992). This parasite seems to have a wide range of distribution among small odontocetes, while the life-cycle and intermediate hosts remain unknown (Raga 1994).

B. cordiformis was mainly found fixed to the duodenal ampulla and also showed a clumped pattern. This pattern was also observed in the anterior sections of the intestine. The family Brauninidae is specific to delphinids (Raga 1994). It has been recorded in 40% of dusky dolphins from Peru (Van Waerebeek 1992, Van Waerebeek *et al.* 1993) and recently from Commerson's dolphins (*Cephalorhynchus commersonii*) in Argentine waters (author's unpublished data).

The intestine has been described as the main habitat for the species of the genus *Hadwenius* (Fernández 1996). Our observations agree with this general pattern. Moreover, the intestinal distribution, mostly clumped in the anterior sections of the intestine, is consistent with data for *H. pontoporiae*, the intestinal distribution of which has been well documented by Aznar *et al.* (1997). However, a significant number of individuals (around 60%) was found in the stomachs. This result is not congruent with recent data for *Hadwenius* spp. and particularly that of *H. pontoporiae.* The latter followed a distribution suggesting an ontogenetic migration through the anterior intestinal sections (see Aznar *et al.* 1997). The observed pattern in the dusky dolphin would agree with this evidence only if the hypothetical anteriad migration included the stomach. Campulids of the genus *Hadwenius* have been described from several odontocete species belonging to Pontoporiidae, Monodontidae, Phocoenidae, and Delphinidae, and seem to have a cosmopolitan distribution (Fernández 1996).

The diversity values for gastrointestinal and intestinal infracommunities obtained for the dusky dolphins were compared with those for a neritically feeding coastal species, the franciscana (*Pontoporia blainvillei*) (Aznar *et al.* 1994), and a pelagic species, the long-finned pilot whale (*Globicephala melas*) (Balbuena and Raga 1993) (Fig. 2). Diversity values for intestinal communities for the pilot whale were 0.04, 0.09, and 0.14 for three different age groups (the second value, corresponding to individuals of 11–20 yr, was chosen for this comparison, since the dusky dolphins in this study were mainly young mature animals). These helminth communities are all depauperate, with low diversity and species richness. The pilot whale showed the lowest diversity values, while the dusky dolphin showed the highest (Fig. 2). Species richness was similar in the three cetacean species, but the franciscana showed higher mean total abundance of helminths.

The ecological processes explaining these findings may relate to host vagility, range of prey species, or trophic specialization in intermediate hosts. Pilot whales may be feeding on a less parasitized pelagic community (Balbuena and Raga 1993) and have a locally specialized diet with few prey items per individual (Desportes and Mouritsen 1993). On the other hand, franciscanas seem to be sedentary, feeding on a neritic community richer in highly parasitized intermediate hosts, thus leading to higher diversity and total abundance of helminths (Aznar *et al.* 1994). Although dusky dolphins feed on only a few

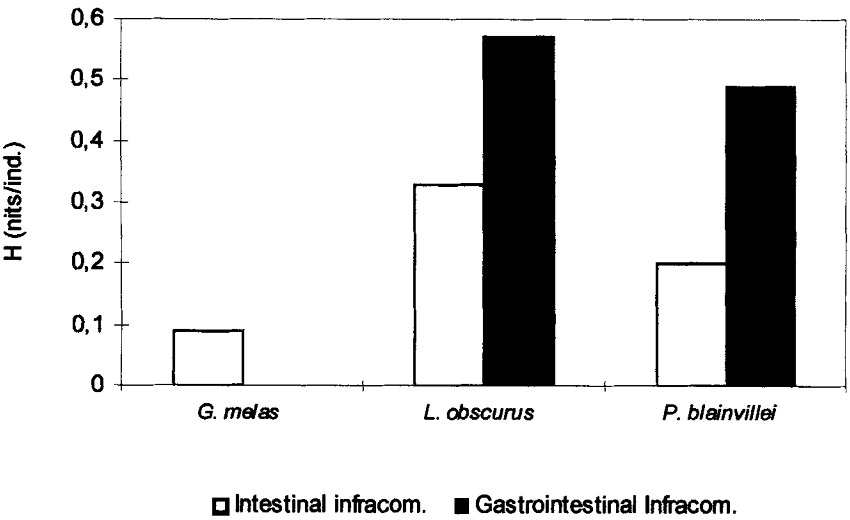

Figure 2. Brillouin's diversity values of helminth infracommunities of pilot whales, dusky dolphins, and franciscanas. Diversity values from Balbuena and Raga (1993) for *Globicephala melas*, Aznar *et al.* (1994) for *Pontoporia blainvillei*, and the present study for *Lagenorhynchus obscurus*.

fish and squid species (Koen Alonso *et al.* 1998) and possibly live in a less-parasitized pelagic community, they prey on a wide range of intermediate host species over a large area, leading to more diverse helminth communities but with low mean total abundance of helminths.

Comparison with other populations or stocks of dusky dolphins is difficult because information on gastrointestinal helminths is very scanty. In any case, some differences seem to exist with the Peruvian population (Van Waerebeek 1992, Van Waerebeek *et al.* 1993). *Anisakis* sp. and *B. cordiformis* have been recorded in Peru but with lower prevalence (*Anisakis* sp. 40%, *B. cordiformis* 8.5%) and intensity (*Anisakis* sp. 1–17, $\bar{x} \pm$ SD = 2.96 ± 3.41; *B. cordiformis* between 1 and 10, 2.17 ± 2.26) than observed for *A. simplex* and *B. cordiformis* in Patagonia. No campulids or acanthocephalans were reported from Peru, whereas cestodes occurred in 3.7% of the Peruvian dolphins but not in the Argentine dolphins (see Table 1). Differences in age structure between the Peruvian and Argentinean samples might account for some of these differences, because host-related and temporal factors are known to influence the structure of helminth communities in mammals. However, species occurrence tends to be more stable over time than prevalence and abundance (Pence 1990) and presence/absence data have been used to differentiate stocks of cetaceans (Dailey and Vogelbein 1991, Aznar *et al.* 1995). Therefore, the qualitative differences presented here may indicate segregation between the Peruvian and Argentine dolphins. However, additional data on the host's biology and parasite faunas are needed to reach a definitive conclusion about the stock identity of dusky dolphins in South America.

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