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Gastrointestinal Helminths in the Hawaiian Monk Seal (*Monachus schauinslandi*): Associations with Body Size, Hematology, and Serum Chemistry

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Abstract

Gastrointestinal helminth parasites are found commonly in the Hawaiian monk seal (*Monachus schauinslandi*), the most endangered seal in the United States and one of the world's most endangered pinnipeds. We studied potential associations between gastrointestinal helminth infections and body size, and hematologic and serum chemistry variables in a sample of 282 monk seals captured between 1998 and 2002 as part of a population health assessment. Based on the presence of eggs in feces, the highest prevalence of infection (78%) was for cestodes belonging to a complex of several *Diphyllobothrium* spp. Infections with the nematode *Contracaecum turgidum* were found in 29% of samples tested. Eggs of the acanthocephalan *Corynosoma rauschi* were found in 4% of seals examined; and the feces of four weanling seals on the French Frigate Shoals contained eggs of the recently described trematode, *Heterophyopsis hawaiiensis*. We used a general linear model and analysis of variance techniques with adjustment for subpopulation and age to determine whether infections with *Diphyllobothrium* spp. or *C. turgidum* were associated with changes in hematologic or serum chemistry variables and found little evidence of an effect when we compared infected with negative seals or seals in the highest quartile of egg counts with negative seals. We also conducted analyses of associations between infection and morphometric values in adjusted, age-stratified data. Infection with *Diphyllobothrium* spp. was associated with a decrease in axillary girth and an increase in dorsal standard length/axillary girth ratio in seals less than 2 y of age, with the effects most pronounced in seals less than 1 y of age. After adjustment for *Diphyllobothrium* spp., *C. turgidum* infection was not associated with morphometric parameters. Co-infection with *Diphyllobothrium* spp. and *C. turgidum* was not associated with differences in body size greater than those found with diphyllobothriid tapeworm infection alone. These findings suggest that intervention strategies to reduce the prevalence of tapeworm infections in immature Hawaiian monk seals should be considered as a conservation measure for this highly endangered marine mammal.

Key Words: Hawaiian monk seal, *Monachus schauinslandi*, hematology, serum chemistry, *Diphyllobothrium* spp., *Contracaecum turgidum*, *Corynosoma rauschi*, *Heterophyopsis hawaiiensis*, gastrointestinal helminths

Introduction

The population of Hawaiian monk seals (*Monachus schauinslandi*) has declined in recent years to approximately 1,244 individuals, and the species is threatened with extinction (Carretta et al., 2005). Its distribution is limited to six primary subpopulations on the Northwestern Hawaiian Islands (NWHI) and an expanding subpopulation on the main Hawaiian Islands (Baker & Johanos, 2004). Several sources of mortality have been described in Hawaiian monk seals, including male aggression (Hiruki et al., 1993); starvation, primarily affecting young seals (Banish & Gilmartin, 1992); predation by sharks (Alcorn & Kam, 1986); entanglement by debris (Henderson, 1990); and disease and trauma (Banish & Gilmartin, 1992; Hiruki et al., 1993). Biotoxins such as ciguatoxin and mitotoxin have also been suspected as causes of mortality (Gilmartin et al., 1980).

The importance of gastrointestinal helminths as a cause of morbidity and mortality in Hawaiian monk seals is unknown, although the prevalence of infection is high. In an examination of fecal samples from four of the breeding sites in the NWHI, Dailey et al. (1988) reported finding the eggs of diphyllobothriid cestodes, ascaridoid nematodes (*Contracaecum turgidum* and *Anisakis* spp.), an acanthocephalan of the genus *Corynosoma*, and an unidentified trematode. Infections with adult helminths were reported as a pathological finding in 88% of monk seals necropsied from 1981 to 1985 (Banish & Gilmartin, 1992). Of significance was the finding of gastric ulcers with attached *Contracaecum* adults and the report of a similar finding during an investigation of monk seal mortality on Laysan Island (Gilmartin et al., 1980). A new trematode, *Heterophyopsis hawaiiensis*, was identified recently in Hawaiian monk seals (Dailey et al., 2004). Evidence of moderate to severe gastrointestinal helminthiasis was found in the alimentary system of all 21 juvenile and adult Hawaiian monk seals necropsied during studies of individual deaths and unusual mortality events in the NWHI between 1998 and 2002 (M. M. Kliks, unpub. data). Many of these seals were noted to have been emaciated at the time of death.

Gastrointestinal helminths could play a significant role in Hawaiian monk seal mortality as a direct contributor to emaciation and starvation by damaging the gastrointestinal mucosa and/or interfering with the processing of nutrients. Debilitation and impairment of the monk seal's physical condition could, in turn, result in an impaired ability to forage for prey and in an increased susceptibility to shark predation. This is the first study to assess the effects of gastrointestinal helminths on morphometric values, hematology, and serum chemistry in this highly endangered species.

Materials and Methods

Study Sites and Seal Selection

Monk seals were sampled at six major breeding colonies in the NWHI between 1998 and 2001. From 1998 to 1999, seals were sampled at three sites as part of a population health assessment: French Frigate Shoals (FFS: 23º 45' N, 166º 10' W), located approximately 1,520 km northwest of Oahu; Midway (MID: 28º 15' N, 177º 23' W); and Pearl and Hermes Reef (PHR: 27º 50' N, 175º 50' W). After 1999, seals were captured at Kure Atoll (KUR: 28º 25' N, 178º 10' W), Laysan (LAY: 25º 42' N, 171º 44' W), and Lisianski (LIS: 26º 02' N, 174º 00' W) for a variety of reasons (e.g., instrumentation with satellite-linked radio transmitters and investigation of unexplained mortality) as well as at sites previously sampled (FFS, MID, and PHR).

National Marine Fisheries Service guidelines for the capture and handling of Hawaiian monk seals were followed. Apparently healthy animals were selected for disease screening. Female-pup pairs, molting seals, females believed to be pregnant, and wounded and obviously emaciated seals were not sampled in order to avoid stress and potential adverse impacts.

Morphometric Measurements and Sample Collection

Monk seals were captured, sampled, and released using field and laboratory techniques described in detail elsewhere (Reif et al., 2004; Aguirre et al., in press). Briefly, seals were captured while hauled out on the beach, restrained, and sedated with intravenous diazepam (Steris Laboratories, Phoenix, AZ). During physical examination, dorsal standard length (DSL) and axillary girth were measured with a cloth tape. The DSL/girth ratio was calculated as an indicator of body condition.

Blood was collected from the extradural vein in silicone-treated glass tubes for serum and in tubes containing ethylenediaminetetra-acetic acid disodium salt (Na-EDTA) for hematology. Absolute white blood cell counts were performed using the Microcollection System Test (UNOPETTE, Becton Dickinson, Rutherford, NJ). Packed cell volume (PCV) was determined by microhematocrit centrifuge (16,000 RPM, 1,200 xg for 5 min) and reader (StatSpin, Norwood, MA). PCV was determined for most seals within 1 h of collection. Fresh blood smears were prepared on glass slides, air dried, and stained for differential counts of white blood cells with Wright's Giemsa. Hematology variables measured included PCV, white blood cell count, and white blood cell differential to obtain absolute numbers of neutrophils, lymphocytes, eosinophils, and monocytes.

Following serum centrifugation, total solids and glucose were measured with a refractometer and glucometer, respectively. Serum samples for additional chemistry analyses were placed on ice, processed in the field within 4 h after collection as described above, and frozen in liquid nitrogen at –68º C. Additional serum chemistry analytes were measured at a commercial veterinary diagnostic laboratory (IDDEX Veterinary Services, Sacramento, CA). Serum enzymes included alkaline phosphatase (ALP), alanine aminotransferase (ALT, formerly SGPT), aspartate aminotransferase (AST, formerly SGOT), creatine phosphokinase (CPK), gamma glutamyl transferase (GGT), and lactate dehydrogenase (LDH). Serum proteins measured were albumin, globulin, total protein,

and total solids. Renal function was assessed by measuring serum urea nitrogen and creatinine concentrations. Complete results for all parameters were not obtained for all animals sampled.

Evaluation for Helminth Eggs

Fecal material was collected from the rectum of each restrained seal with a fecal loop. We attempted to obtain approximately 5 g of feces, which were placed in pre-marked, 44.4-ml glass screw-top vials and immediately commutated in 10 ml of polyvinyl alcohol (PVA) fixative (Medical Chemical Corp, Torrance, CA). Upon arrival at the laboratory, the actual volume of feces and PVA in each sample vial were determined by visual inspection, and a correction factor was calculated for use in adjusting egg counts, as needed. For example, if it was estimated that only 2.5 g of feces had actually been added to the vial, the egg count was multiplied by a correction factor of 2.0; if it was estimated that only 5 ml of fixative was present in the vial, a correction factor of 0.5 was applied to the egg count. Helminth eggs were counted on a single standardized wet smear. Approximately 2 mg of fecal material were added to a drop of PVA fixative on a microscope slide, covered by a 22-mm square cover slip, and scanned across 12 sequential rows using the 40X objective on a binocular microscope. All helminth eggs present were counted and recorded. Occasionally, there were too many diphyllobothriid eggs to count accurately. In those cases where between 200 and 500 eggs were counted in the first row scanned, only four rows were scanned and the resulting counts multiplied by 3 to get a total for that smear. In those few cases where there were more than 500 diphyllobothhriid eggs in the first row scanned, only three rows were scanned and the results multiplied by 4 to obtain a total for that smear. A single technician prepared and read all fecal smears and established correction factors where required. Helminth eggs were identified using standard methods (Rausch, 1969; Dailey & Gilmartin, 1980; Dailey, 2001). Eggs were not measured, and identification to the species level was not performed.

Statistical Analyses

Statistical analyses were performed using *SAS, Version 9.1 for Windows* (SAS Institute, Cary, NC). Statistical significance was defined *a priori* as $p < 0.05$. Chi-square was used to test for differences in prevalence of parasitic infection across strata of age group and subpopulation. Hematology and serum chemistry variables were selected for analysis based on *a priori* hypotheses for potential effects of helminth infections. The mean values for infected and uninfected monk seals were compared in an analysis of variance (ANOVA), adjusting for age and subpopulation. Least-squared means were computed for each variable in PROC GLM. In an earlier analysis (Reif et al., 2004), we found substantial differences for some hematology and serum chemistry variables between subpopulations. Therefore, we adjusted the results for subpopulation to control for potential confounding. Conversely, we found no associations between sex and any of the hematology and serum chemistry variables in our previous analysis. Therefore, sex was not included in the multivariate analysis for these variables.

We performed age-stratified analyses to evaluate potential associations between infection with gastrointestinal helminths (using the presence of eggs in the feces as a surrogate for infection) and DSL, girth, and DSL/girth ratio, controlling for age, sex, and subpopulation in each ANOVA. Age was included as a covariate in order to eliminate potential confounding due to differences in age within strata between infected and uninfected monk seals. Initially, analyses were conducted among seals < 2 y of age, seals 2 to 4 y and seals 5 y and older. Further analyses were performed for associations with morphometric variables by partitioning the youngest age group into seals < 1 y old, and seals 1 to 2 y of age. We examined the relationship between helminth infection and morphometric values, incorporating the number of months elapsed between weaning and the date of sampling (months of exposure) as a covariate. Months of exposure were calculated from the known weaning date (or estimated from the birth date plus 6 wks) to the date of sampling. The potential effects of co-infection with *Contracaecum turgidum* and *Diphyllobothrium* spp. were explored in separate analyses in which we considered infection with each helminth as a potential confounder for the other, as well as in analyses in which we considered the possibility that co-infection might lead to an additive or synergistic association with body size.

To examine the effect of "intensity of infection," we used the magnitude of egg counts as an indicator of the relative number of adult female worms present in the host. We compared seals in the highest quartile of fecal egg counts with negative seals and evaluated this marker for associations with hematology, serum chemistry, and morphometric variables. Analyses of egg counts were performed for *C. turgidum* and *Diphyllobothrium* spp. The number of seals positive for *Corynosoma rauschi* or *Heterophyopsis hawaiiensis* eggs was too small to permit statistical analyses; no further analyses were conducted for these helminths.

Results

We studied 331 fecal samples from 312 Hawaiian monk seals captured between 1998 and 2001 for the presence of gastrointestinal helminth eggs; 302 seals also had a blood sample collected at the time of examination. Sixteen seals had been captured two or more times, leading to multiple records for each seal. The data were reduced to one record per seal by using the first capture that provided complete results for all the variables of interest; three seals had incomplete information for one or more key variables and were deleted, leaving data from 282 seals for analysis. The age of 240 of the 282 seals (85.1%) was known precisely from tagging of weaned pups; for 39 adult seals and three immature seals, age was estimated from tags originally placed on them as juvenile animals and assigned to an age class by size at the time of tagging. Monk seals were stratified by sex and grouped initially into age-groups comprised of seals < 2 y old, including weanlings; seals 2 to 4 y old, and adults ≥ 5 y of age (Table 1). The largest number of seals was sampled at FFS, which accounted for 37% of the sample.

Prevalence of Helminth Infection

The prevalence of infection with gastrointestinal helminths is shown in Table 2. The highest prevalence was for the tapeworm *Diphyllobothrium* spp., which infected 78% of the monk seals tested. The rates of infection were 75% among seals < 2 y of age, 95% among seals 2 to 4 y old, and 73% among adults. The differences in prevalence across age groups were statistically significant ($p = 0.003$). The difference in prevalence between subpopulations was also statistically significant ($p < 0.0001$). The highest prevalence of infection was found at PHR and the lowest at FFS.

Infection with *C. turgidum* was found in 29% of the monk seals sampled, with the highest prevalence in seals aged 2 to 4 y (49%). The differences in prevalence of *C. turgidum* infection between age groups and between subpopulations were not statistically significant. *Corynosoma rauschi* infections were found in 12 (4%) of the seals tested. *C. rauschi* positive seals were found in all subpopulations except KUR; the prevalence of infection was highest at FFS where 7% of the seals sampled were positive. Differences in the age and subpopulation distributions of *C. rauschi* infections were not statistically significant. Infection with *Heterophyopsis hawaiiensis* was found in four weanling seals from FFS.

Associations with Hematologic and Serum Chemistry Parameters

Results of analyses for associations between infection with *C. turgidum* and *Diphyllobothrium* spp. and hematologic and serum chemistry parameters are shown in Table 3. We compared age and subpopulation adjusted mean values for hematology and serum chemistry variables between seals with and without helminth eggs in their feces. Gastrointestinal helminth infections were not associated with any significant hematologic or serum chemistry alterations. Although the PCV was higher in seals infected with *Diphyllobothrium* spp. compared to negative seals ($p = 0.05$), the difference was not of biological significance (48.9% compared to 50.8%).

Table 1. Distribution of 282 Hawaiian monk seals sampled by age class, sex, and subpopulation in the Northwestern Hawaiian Islands, 1998-2001

Age class	FFS[1]	KUR[2]	LAY[3]	LIS[4]	MID[5]	PHR[6]	Total (%)
Immature (< 2 years)							
Male	31	3	11	9	8	4	66
Female	28	4	9	5	6	2	54
Subtotal							120 (42.6)
Juvenile (2-4 years)							
Male	7	5	7	2	4	6	31
Female	4	5	6	2	4	5	26
Subtotal							57 (20.2)
Adult (≥ 5 years)							
Male	19	4	15	4	6	8	56
Female	16	4	7	5	12	5	49
Subtotal							105 (37.2)
Total (%)	105 (37.2)	25 (8.9)	55 (19.5)	27 (9.6)	40 (14.2)	30 (10.6)	282 (100.0)

[1]French Frigate Shoals, [2]Kure Atoll, [3]Laysan, [4]Lisianski, [5]Midway, [6]Pearl and Hermes Reef

Table 2. Prevalence of *Contracaecum turgidum* and *Diphyllobothrium* spp. infections in Hawaiian monk seals by subpopulation and age group, 1998-2001

	Contracaecum turgidum					*Diphyllobothrium* spp.				
	Prevalence by age group			Overall prevalence		Prevalence by age group			Overall prevalence	
Subpopulation	< 2	2-4	≥ 5	Number	(%)	< 2	2-4	≥ 5	Number	(%)
French Frigate Shoals	13/59	2/11	10/35	25/105	23.8	34/59	8/11	26/35	68/105	64.8
Kure Atoll	2/7	6/10	3/8	11/25	44.0	7/7	10/10	7/8	24/25	96.0
Laysan	7/20	5/13	1/22	13/55	23.6	18/20	13/13	10/22	41/55	74.5
Lisianski	3/14	2/4	2/9	7/27	25.9	14/14	4/4	7/9	25/27	92.6
Midway	4/14	6/8	6/18	16/40	40.0	12/14	8/8	13/17	33/39	84.6
Pearl and Hermes Reef	2/6	2/11	6/13	10/30	33.3	5/6	11/11	12/12	28/29	96.6
Total	31/120	23/57	26/105	82/282	29.1	90/120	54/57	75/103	219/280	78.2
(%)	25.6	49.4	24.8			75.0	94.7	72.8		

Eosinophilia is a hallmark of parasitic infection with many helminth species. The relative (14.0% and 11.6%) and absolute eosinophil counts (1,249/ml and 1,122/ml) were higher among seals with *Diphyllobothrium* spp. infection ($p = 0.005$ and 0.20, respectively). The relative eosinophil percentage was similar in seals with and without *C. turgidum* infection (13.5% and 13.0%, $p = 0.55$), however, and the absolute eosinophil count was higher among negative seals (1,128/ml and 1,247/ml; $p = 0.14$).

Separate analyses were performed to evaluate the effects of magnitude of egg counts as an indicator of "infection intensity." We compared seals in the upper quartile of egg counts for *C. turgidum* and *Diphyllobothrium* spp. with negative seals. The results for hematologic and serum chemistry parameters were similar to those obtained comparing infected and negative seals; none of the comparisons was statistically significant, although a small difference in PCV was again noted among *C. turgidum* and *Diphyllobothrium* spp. infected seals (data not shown).

Associations with Morphometric Parameters

We evaluated potential associations between infection with *C. turgidum* and *Diphyllobothrium* spp. and DSL, girth, and the DSL/girth ratio (Table 4). Immature seals < 2 y of age and positive for *Diphyllobothrium* spp. eggs had a smaller girth ($p < 0.0001$) and a greater DSL/girth ratio ($p < 0.0001$) than negative seals. The mean difference in girth between infected and uninfected seals was 13.3 cm. A similar pattern was seen for seals infected with *C. turgidum*; the mean difference in girth was 4.5 cm, and the difference approached statistical significance ($p = 0.09$). The DSL/girth ratio was also higher among seals infected with *C. turgidum* than among negative seals ($p = 0.02$). Similar trends toward smaller girths were observed among juvenile seals 2 to 4 y of age infected with either helminth, but the differences were not statistically significant, and the number of negative seals available for analysis of *Diphyllobothrium* spp. was extremely small ($n = 3$). Morphometric parameters were similar for adult seals infected with *Diphyllobothrium* spp. and negative seals; adult seals infected with *C. turgidum* were longer and had larger girths than negative adults ($p = 0.03$ and $p = 0.07$, respectively).

Further analyses of the relationship between age and changes in morphometric parameters were conducted by examining seals < 1 y of age, seals 1 y of age, and 2-y-old seals. The strongest effects were found in seals < 1 y of age, a group that included weanlings (data not shown). In this age group, seals with eggs of *C. turgidum* or *Diphyllobothrium* spp. in feces were longer ($p = 0.08$ and $p = 0.03$, respectively), had smaller girths ($p = 0.02$ and $p < 0.0001$, respectively), and had a greater DSL/girth ratio ($p = 0.0009$ and $p < 0.0001$, respectively) than negative seals. Similar trends were found in 1-y-old seals, although none of the differences was statistically significant. Analyses of the effects of *Diphyllobothrium* spp. in 2-y-old seals were precluded since all 31 monk seals in that age group were infected. When the age-stratified data were analyzed by comparing seals in the highest quartile of egg counts to negative seals, the results were virtually unchanged.

We conducted a separate analysis for monk seals from FFS since this subpopulation has experienced poor juvenile survival and decreased size at weaning (Craig & Ragen, 1999). We found associations with the presence of eggs of *Diphyllobothrium* spp. and *C. turgidum* in seals from FFS similar to those described for the entire population, with reduced girth and increased DSL/girth ratio among positive seals < 5 y of age. Sample size limitations precluded conducting analyses of smaller age groups and other subpopulations.

Table 3. *Contracaecum turgidum* and *Diphyllobothrium* spp. infections and hematology and serum chemistry in Hawaiian monk seals

		Contracaecum turgidum					*Diphyllobothrium* spp.				
		Uninfected		Infected			Uninfected		Infected		
		Mean	SE	Mean	SE	p[1]	Mean	SE	Mean	SE	p[1]
Hematology units		n = 172		n = 76			n = 48		n = 201		
WBC	/ml	9,266.5	238.5	9,108.3	306.7	0.58	9,290.5	338.4	9,197.0	244.1	0.79
Neut (abs)	/ml	4,924.1	156.2	4,864.2	202.1	0.76	4,895.9	223.4	4,912.9	160.2	0.94
Lymph (abs)	/ml	2,529.7	106.8	2,483.8	138.2	0.73	2,677.3	152.1	2,455.3	109.1	0.16
Eos (abs)	/ml	1,246.9	65.5	1,128.2	84.8	0.14	1,122.2	93.8	1,249.0	67.3	0.20
Mono (abs)	/ml	578.7	34.5	588.9	44.7	0.81	629.5	49.2	563.0	35.3	0.20
Baso (abs)	/ml	29.7	6.0	33.8	7.8	0.58	28.7	8.6	31.7	6.2	0.74
PCV[2]	%	50.1	0.86	51.0	0.86	0.25	48.9	1.0	50.8	0.7	0.05
Serum chemistry		n = 183		n = 78			n = 56		n = 205		
Alk Phosphatase	IU/l	282.8	18.8	269.9	24.0	0.56	301.8	26.2	271.4	19.0	0.24
SGPT (ALT)	IU/l	85.1	5.9	95.1	7.6	0.34	84.3	8.3	87.9	6.0	0.65
SGOT (AST)	IU/l	96.1	5.8	102.6	7.5	0.34	97.8	8.2	97.9	5.9	0.99
LDH	IU/l	783.7	42.9	846.0	54.8	0.22	790.2	60.1	804.4	43.7	0.81
GGT	IU/l	6.3	0.4	6.42	0.5	0.83	5.78	0.52	6.55	0.38	0.14
CPK	IU/l	574.9	42.6	593.2	54.4	0.72	579.1	59.4	580.2	33.2	0.98
Albumin	g/dl	3.1	0.04	3.1	0.06	0.93	3.0	0.06	3.1	0.05	0.68
Globulin	g/dl	4.9	0.09	4.9	0.11	0.73	4.9	0.12	4.9	0.09	0.86
Total Protein	g/dl	7.9	0.10	8.0	0.13	0.73	7.9	0.14	7.9	0.10	0.98
Total Solids	g/dl	8.2	0.12	8.4	0.14	0.11	8.1	0.17	8.3	0.12	0.51
BUN	mg/dl	30.9	1.8	31.7	2.3	0.70	31.0	2.5	31.2	1.8	0.95
Creatinine	mg/dl	1.2	0.04	1.22	0.05	0.83	1.19	0.05	1.24	0.04	0.38
Glucose	mg/dl	92.8	2.0	91.5	2.6	0.58	95.1	2.8	91.5	2.0	0.20

[1] p values from analysis of variance; means adjusted for age and subpopulation

[2] n for PCV analysis = 148 infected, 77 uninfected

Table 4. *Contracaecum turgidum* and *Diphyllobothrium* spp. infections and body size in Hawaiian monk seals by age group

		Contracaecum turgidum					*Diphyllobothrium* spp.				
		Uninfected		Infected			Uninfected		Infected		
Age group	Variable[1]	Mean	SE	Mean	SE	p^2	Mean	SE	Mean	SE	p^2
		$n = 88$		$n = 31$			$n = 29$		$n = 90$		
Immature	DSL	137.7	1.15	139.5	1.70	0.37	136.3	1.85	138.6	1.04	0.22
(< 2 years)	Girth	88.4	1.54	83.9	2.27	0.09	98.4	2.16	85.1	1.21	< 0.0001
	DSL/Girth	1.58	0.02	1.66	0.03	0.02	1.40	0.03	1.63	0.02	< 0.0001
		$n = 34$		$n = 23$			$n = 3$		$n = 54$		
Juvenile	DSL	164.9	1.98	161.2	2.33	0.15	164.7	5.73	163.5	1.77	0.82
(2-4 years)	Girth	99.8	1.60	96.8	1.88	0.14	102.4	4.60	98.5	1.43	0.40
	DSL/Girth	1.66	0.03	1.67	0.03	0.65	1.61	0.07	1.66	0.02	0.49
		$n = 76$		$n = 27$			$n = 28$		$n = 75$		
Adult	DSL	204.1	2.07	211.0	2.87	0.03	205.8	3.20	206.2	2.21	0.92
(≥ 5 years)	Girth	132.3	1.81	137.1	2.51	0.07	132.5	2.76	134.1	1.91	0.60
	DSL/Girth	1.55	0.02	1.54	0.02	0.82	1.56	0.03	1.54	0.02	0.52

[1] Measurements in centimeters

[2] p values from analysis of variance; means adjusted for age, sex, and subpopulation

To evaluate the possibility that confounding from co-infection may have been responsible for differences in body size, we added the second major species to each model as a covariate (Table 5). When *Diphyllobothrium* spp. infection was added to the model for *C. turgidum* infection in seals < 2 years of age, the differences between positive and negative seals in DSL, girth, and DSL/girth ratio shown in Table 4 were no longer seen, indicating that the differences were due to confounding by *Diphyllobothrium* spp. (Table 5). Conversely, the addition of *C. turgidum* infection to the model for *Diphyllobothrium* spp. did not reduce the differences in morphometric values. The results remained statistically significant, indicating that the co-infection with *C. turgidum* was not a confounder. These analyses show that all of the effects of helminth infections on body size among monk seals < 2 y of age are associated with infection with *Diphyllobothrium* spp.

We also considered the possibility that co-infection with both species of helminths may have amplified the effects on body size through a synergistic process. To evaluate biological interaction, we repeated the analysis comparing seals < 2 y of age infected with both *Diphyllobothrium* spp. and *C. turgidum* to negative seals. The results were similar to those presented in Table 5 for data for *Diphyllobothrium* spp. infection controlled for *C. turgidum* ($p = 0.15$, $p < 0.0001$, and $p < 0.0001$ for DSL, girth, and DSL/girth ratio, respectively). When seals positive for *Diphyllobothrium* spp. but without *Contracaecum* eggs in their feces were compared to seals negative for both types of eggs, the results were again similar to those presented in Table 5 ($p = 0.27$, $p < 0.0001$, and $p < 0.0001$ for DSL, girth, and DSL/girth ratio, respectively). All seals positive for *Contracaecum* eggs were also positive for *Diphyllobothrium* spp. eggs.

Table 5. *Contracaecum turgidum* and *Diphyllobothrium* spp. infections and body size in Hawaiian monk seals < 2 y of age, controlling for co-infection

	Contracaecum turgidum					*Diphyllobothrium* spp.				
	Uninfected		Infected			Uninfected		Infected		
Variable[1]	Mean	SE	Mean	SE	p^2	Mean	SE	Mean	SE	p^2
	$n = 88$		$n = 31$			$n = 29$		$n = 90$		
DSL	137.3	1.24	138.3	2.10	0.63	136.8	2.13	138.7	1.09	0.33
Girth	91.6	1.46	92.2	2.44	0.80	98.7	2.48	85.2	1.28	< 0.0001
DSL/Girth	1.52	0.02	1.52	0.03	0.90	1.40	0.03	1.63	0.02	< 0.0001

[1] Measurements in centimeters

[2] p values from analysis of variance; means adjusted for age, sex, subpopulation, and co-infection

Finally, we explored the possibility that the associations between *Diphyllobothrium* spp. infection and girth and DSL/girth ratio in young seals may have been due to the time elapsed between weaning and sampling. Recently weaned seals are in generally good body condition and may not have had ample time to become infected with helminths by foraging on intermediate hosts. Several months after weaning, young seals may be less robust as the result of having had to forage on their own and may have acquired a helminth infection. Therefore, we used the months elapsed between the known or estimated (from the birth date) date of weaning and the date of sampling as a covariate in the ANOVA and repeated the analyses (Table 6). The findings were similar to those for the analyses shown in Table 4. Seals infected with *Diphyllobothrium* spp. had a smaller girth (5.8 cm) ($p = 0.06$) and higher DSL/girth ratio ($p = 0.004$), although the absolute differences in the adjusted means were smaller than those without months of exposure in the model, and the p value for girth was marginally significant.

Discussion

The work described here represents a unique effort to evaluate the role of gastrointestinal helminth infection on the health of an endangered pinniped population in a large sample of free-living animals. The Hawaiian monk seal is threatened with extinction due to its low abundance and restricted habitat. Beach counts of seals at FFS—historically the principal breeding colony for the monk seal—showed that this population was depleted in the 1950s but increased substantially in the 1960s and 1970s (Ragen & Lavigne, 1999). In the early 1980s, beach counts were relatively stable, but then they increased during the latter half of the decade. Beginning in 1990, the FFS colony declined rapidly, primarily due to a large decline in the survival of immature seals. A similar decrease in survival rates was reported at Laysan Island in the early 1990s (Lombard et al., 1996). Craig & Ragen (1999) studied the relationship between body size and survival of Hawaiian monk seals in response to the declines in population at two of the major breeding sites. The probability of survival from weaning to age 2 y was significantly related to weaning size measured by girth, DSL, and estimated mass in three birth cohorts of seals from FFS (1984-1987, 1988-1989, and 1990-1994). At Laysan Island, survival of weanling pups was also significantly related to girth and estimated mass in the youngest cohort. The authors hypothesized that the poor survival rate was due to reduced prey availability at both sites.

A complementary, but previously unevaluated hypothesis is that gastrointestinal helminth parasites contribute to mortality by one or more mechanisms. The interaction between host nutrition and parasitism has been described as a series of events that results in the allocation of scarce nutrient resources such as energy and protein between the competing body functions of the host. The direct absorption of nutrients by helminths within the gastrointestinal tract may compromise the host and lead to sub-optimal growth rates among juvenile animals. One of the key features of gastrointestinal nematode infection is an increased loss of endogenous protein due to leakage of plasma protein, increased mucoprotein production, and sloughing of epithelial cells into the alimentary tract (Coop & Kyriazakis, 1999). Helminth parasites such as *C. turgidum* that attach to the mucosa may cause gastric ulceration with blood loss (Whitlow et al., 1979; Banish & Gilmartin, 1992). Alterations in the nutrient balance in juvenile monk seals hosting large populations of gastrointestinal helminths, particularly *Diphyllobothrium* spp. (> 90,000 adults in one seal; M. M. Kliks, unpub. data) may account for the decreases in girth and the increases in DSL/girth ratio described in this paper. Further, *Diphyllobothrium latum* infections in humans are associated with decreased serum vitamin B12 levels (Nyberg et al., 1961). The fish

Table 6. *Contracaecum turgidum* and *Diphyllobothrium* spp. infections and body size in Hawaiian monk seals < 2 y of age, controlling for months elapsed between weaning and sampling

	Contracaecum turgidum					*Diphyllobothrium* spp.				
	Uninfected		Infected			Uninfected		Infected		
Variable[1]	Mean	SE	Mean	SE	p[2]	Mean	SE	Mean	SE	p[2]
	$n = 88$		$n = 31$			$n = 29$		$n = 90$		
DSL	139.8	1.24	139.6	1.55	0.89	138.5	2.26	140.0	1.13	0.54
Girth	88.8	1.56	86.7	1.94	0.35	92.7	2.80	86.9	1.40	0.06
DSL/Girth	1.59	0.02	1.62	0.02	0.33	1.51	0.03	1.62	0.02	0.004

[1] Measurements in centimeters

[2] p values from analysis of variance; means adjusted for sex, subpopulation, and months of exposure

tapeworm competes for vitamin B12 and is also thought to interfere with the absorption of the vitamin, leading to the development of anemia, neurological effects, confusion, and poor growth in children (Schantz et al., 2002).

Diphyllobothrium spp. was the most prevalent gastrointestinal helminth infection in this sample of monk seals. We found evidence of an association between infection with the adults of these cestodes and body size in monk seals < 2 y of age. The associations were strongest among seals < 1 y of age, including weanlings, the group at highest risk of mortality. Further analyses showed that the associations between *Diphyllobothrium* spp. and body size were not due to confounding by *C. turgidum* infection and that co-infection with *Diphyllobothrium* spp. and *C. turgidum* did not appear to act synergistically to enhance the effects found with *Diphyllobothrium* spp. alone. The time elapsed between weaning and sampling did not explain the results, although the associations were weaker when this variable was included in the analysis.

An important limitation of this study is that the sampling protocol precluded capturing monk seals that appeared to be emaciated. Inclusion of seals with poorer body conditions may have strengthened the associations observed if emaciated seals were more likely to be infected. Necropsy evidence from seals from these cohorts shows that single PVA-fixed fecal smears made from feces taken directly from the colon or rectum may be negative despite the presence of large numbers of either *Diphyllobothrium* spp. or *C. turgidum* adults in the gastrointestinal tract (M. M. Kliks, unpub. data). Therefore, it is probable that some of the seals that were considered as negative based on a single fecal smear were infected. Misclassification may also have diminished the magnitude of the associations observed in this study. Further, identification of *Diphyllobothrium* spp. cestodes to individual species was not performed. Because of this, we were unable to assess which of the multiple species of the parasite were responsible for the associations we identified.

There was little indication that gastrointestinal helminth infections significantly altered hematologic or biochemical analyte concentrations. Heavy helminth infection may result in anemia, eosinophilia, protein loss, and alterations in liver function. The absence of significant abnormalities suggests that helminth infection is not principally or directly altering organ function, but, rather, as our data suggest, infection contributes in a more insidious fashion by altering growth and body condition of young seals. Increases in PCV were small and probably not biologically significant, and they may be secondary to minor alterations in fluid balance contributing to hypovolemia and relative polycythemia. Interestingly, eosinophilia was not noted in spite of the fact that it is considered a hallmark of the tissue migration phase of gastrointestinal helminth infection. Stressed animals with increased plasma steroid concentrations consistently show decreased blood eosinophil concentrations, however (Stockham & Scott, 2002). Since the larval stages of diphyllobothriid cestode parasites do not invade visceral organs or muscle tissue, a vigorous eosinophilic response may not be triggered. Thus, these opposing factors on eosinophil concentration may contribute to the absence of eosinophilia in parasitized animals.

Incorporating the magnitude of egg counts as an indicator of "intensity of infection" did not change the findings appreciably. When seals in the highest quartile of egg counts were compared to uninfected seals, the results were similar to those comparing all infected seals with negative seals. The semi-quantitative method we used did not control for variability in the amount of feces and diluent used in each sample. In this host-parasite system, the correlation between the number of eggs per fecal smear and the number of egg-laying adult worms in the gastrointestinal tract is not known. Furthermore, the numbers of adult and larval helminth parasites present at a single point in time may not reflect historical burdens due to the natural senescence of the helminth population or to the hosts' immunologic responses that lead to clearing or reduction of parasite infections (Hayes et al., 2004).

Our findings lend credence to the hypothesis that helminth infections contribute to the impairment of juvenile monk seal growth that is a predictor of survival at this critical period in development. At FFS, survival of weaned pups was shown to be dependent on size for all cohorts studied (Craig & Ragen, 1999). Young monk seals that have impaired growth rates may not be able to forage successfully in an environment with reduced prey availability and may die from starvation. Alternatively, smaller seals or seals in poor condition may be more likely to become prey for sharks that inhabit the waters surrounding the NWHI. Heavy infections in younger monk seals may be due to their dependence on prey species that harbor infective stages of these helminths (Deardorff et al., 1982). Studies to determine the prevalence of helminth larvae among fauna in the reef environment could be useful to establish the source of these infections.

In view of the critical status of the monk seal population, intervention strategies to reduce the gastrointestinal helminth burdens in immature animals should be considered as a conservation measure. Rehabilitated pinnipeds, such as California sea lions (*Zalophus californianus*) and elephant seals

(*Mirounga angustirostris*), are commonly treated prophylactically with praziquantel (Dailey, 2001). Although the logistical details for restraint, method of administration, and dosage schedule need to be developed, clinical trials could be initiated to assess the effects of antihelminthic treatment on growth and survival during regularly scheduled field activities or in captive animals.

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