

## Current prevalence of adult *Uncinaria* spp. in northern fur seal (*Callorhinus ursinus*) and California sea lion (*Zalophus californianus*) pups on San Miguel Island, California, with notes on the biology of these hookworms

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### Abstract

A prevalence survey for hookworms (*Uncinaria* spp.) was done in northern fur seal (*Callorhinus ursinus*) and California sea lion (*Zalophus californianus*) pups on San Miguel Island, CA, in 2000. Intestines of dead pups were examined for adult hookworms in July. These parasites were found in 95% of 20 fur seal pups and 100% of 31 sea lion pups. The number of hookworms varied from 4 to 2142 ( $\bar{x} = 760$ ) in fur seal pups and from 20 to 2634 ( $\bar{x} = 612$ ) in sea lion pups. A direct relationship was evident between body condition and number of hookworms in the pups; that is, pups in poor condition had fewer hookworms than those in good condition. There was a decline in the number of hookworms in sea lion pups in 2000 compared to collections in 1996. Eggs of *Uncinaria* spp. were found in rectal feces (collected in late September and early October) of none of 35 (0%) live fur seal pups and 41 of 48 (85%) live sea lion pups. Packed cell volume values, determined for most of the same live pups, were essentially normal for *C. ursinus* but were much lower than normal for most *Z. californianus*. Hookworm larvae were not found in blubber of fur seal and sea lion pups or in rookery sand in July. Rookery sand, positive for live hookworm larvae when put in a refrigerator, was negative at removal 2.5 years later. The average number of eggs in utero of

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female hookworms was 285 for three specimens from a fur seal pup and 281 from three specimens from a sea lion pup. One hookworm larva was recovered from milk stripped from the teats of a stranded *Z. californianus* female at The Marine Mammal Center, Sausalito, CA. © 2001 Published by Elsevier Science B.V.

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## 1. Introduction

Pinnipeds are marine carnivores separated into three families: Otariidae or eared seals, Phocidae or earless seals, and Odobenidae or walruses (Reeves et al., 1992).

Monitoring diseases in pinnipeds permits a better understanding of their health. Hookworms (*Uncinaria* spp.) are common parasites of the otariids (fur seals and sea lions), uncommon in phocids (e.g. southern elephant seals) (George-Nascimento et al., 1992), and unreported in odobenids. Only two species, *Uncinaria lucasi* and *Uncinaria hamiltoni*, have been described but intermediate types have been observed (Olsen, 1958; Dailey and Hill, 1970). Hookworms in all otariids are considered by George-Nascimento et al. (1992) to be one species, *U. lucasi*. Recent research on hookworms from northern fur seal (*Callorhinus ursinus*) and California sea lion (*Zalophus californianus*) pups on San Miguel Island (SMI), CA, demonstrated, by molecular and morphometric characteristics, distinctive differences between *Uncinaria* spp. in these two hosts (Nadler et al., 2000). This indicated that these host-associated hookworms are separate species (Nadler et al., 2000). Uncinariasis has been associated with morbidity and mortality of fur seal and sea lion pups (Lucas, 1899; Olsen, 1958; T. Spraker, personal communication).

The life cycle of *Uncinaria* spp., as determined mostly in northern fur seals in Alaska (Lyons, 1963; Olsen and Lyons, 1965), is briefly as follows: (1) free-living third-stage larvae (L<sub>3</sub>) hatch from eggs passed in feces of pups on the rookery; (2) free-living L<sub>3</sub> enter seals, orally and percutaneously, and do not mature but go to tissues, especially ventral abdominal blubber; (3) parasitic L<sub>3</sub> in the tissues are at a dead end except in females which pass them at parturition in the first milk to their pups; and (4) adult hookworms are typically only found in pups.

The main purpose of the present investigation was to ascertain the current prevalence of adult *Uncinaria* spp. in *C. ursinus* and *Z. californianus* pups on SMI for comparison to the high prevalence found there in 1996 (Lyons et al., 1997). A few other aspects of these parasites were investigated to complement some recent observations (Lyons et al., 2000a): (1) examination for hookworm larvae in milk from lactating female sea lions, in blubber from dead fur seal and sea lion pups in July, in rookery sand in July, and in rookery sand, positive for hookworm larvae when put in a refrigerator 2.5 years previously; (2) determination for hookworm eggs in the feces and of the packed cell volume values of live fur seal and sea lion pups in late September/early October; (3) enumeration of eggs in female hookworms; and (4) comparison of numbers of hookworms: (a) relative to male and female specimens in *Z. californianus* pups; (b) to specimens in male and female pups of each host species; and (c) to specimens in pups of both hosts.

## 2. Materials and methods

### 2.1. Dead *C. ursinus* and *Z. californianus* pups

#### 2.1.1. Location of research-hosts-prevalence-intensity-adult hookworms

Hookworm research was done on San Miguel Island (SMI) (34°2'N, 120°26'W) in the California Channel Islands in 2000. Adult hookworms were collected between 25 and 31 July from the small and large intestines of freshly dead *C. ursinus* pups ( $n = 20$ ) and *Z. californianus* pups ( $n = 25$ ) to establish the current prevalence and intensity of infection. All *C. ursinus* pups were from the Adams Cove Rookery. The *Z. californianus* pups were from four rookeries, Point Bennett ( $n = 10$ ), Northwest Point ( $n = 5$ ), Northwest Cove ( $n = 5$ ) and Northeast Point ( $n = 5$ ). All dead pups in the current and 1996 study were collected randomly without determination of physical condition until necropsy. Fixed intestinal contents from each pup were put in a sealed plastic bag; those from each host species were put in separate liquid-tight plastic containers. During transportation, there was leakage of some of the bags and, although all specimens (24 from fur seals and 114 from sea lions) were recovered from the shipping container of material from each host, it is not known from which pups they originated. Data for "leaked specimens" were used for calculating total hookworms and mean number of hookworms per pup but not for other intensity values. From an additional six dead *Z. californianus* pups, specimens were collected and considered for the prevalence study; they were not counted because of utilization in a non-related study.

This research was conducted under Marine Mammal Protection Act Research Permit Number 977, issued to the National Marine Mammal Laboratory, Seattle, Washington, USA.

#### 2.1.2. Enumeration of eggs in female hookworms

Gravid female hookworms ( $n = 6$ ), three each from one fur seal pup and one sea lion pup, were individually shredded in a plastic dish (marked with a 13 mm grid) under a dissecting microscope, and all eggs per worm counted.

#### 2.1.3. Body condition-examination of blubber

Body condition, based mainly on visual determination of the amount of subcutaneous ventral blubber of the dead pups, was classified into three categories (poor, fair, and good) when the pup carcasses were opened. Ventral abdominal blubber from four *C. ursinus* and six *Z. californianus* pups was examined for parasitic L<sub>3</sub> hookworms.

### 2.2. Live *C. ursinus* and *Z. californianus* pups

#### 2.2.1. Fecal samples and hematocrits

Fecal samples were obtained from the rectum of live fur seal pups ( $n = 35$  on 4 October) and sea lion pups ( $n = 48$  on 30 September and 1 October) for detection of hookworm eggs. In addition, blood samples for packed cell volume (PCV) determination were taken from most (28 fur seal and 40 sea lion) of the same pups.

### 2.3. Rookery sand

Five sand samples, about 100 g each, were collected on 31 July from different areas of South Cove Rookery for examination for free-living L<sub>3</sub> hookworms. In addition, SMI

rookery sand, positive for free-living L<sub>3</sub> hookworms in January 1998, was re-examined in August 2000, after being refrigerated (45°F) since the time of collection.

#### 2.4. Milk from *Z. californianus* females

In addition to research on SMI, milk samples were collected on 8 and 12 July 2000 from teats of nine stranded lactating female California sea lions (parturition date unknown) at The Marine Mammal Center (TMMC), Sausalito, CA. These milk samples were examined for parasitic L<sub>3</sub> hookworms.

#### 2.5. Methods

Techniques for examinations for hookworms were similar to that previously published (Lyons, 1963; Olsen and Lyons, 1965; Lyons et al., 1997).

#### 2.6. Statistical analysis

Statistical methods used for analysis of data were (1) Mann–Whitney Rank Sum Test (Snedecor and Cochran, 1989) for (a) number of hookworms in male versus female pups of both species and in fur seal versus sea lion pups and (b) comparison of PCV values for live fur seal and sea lion pups; and (2) Kruskal–Wallis Test (Randles and Wolfe, 1979) for number of hookworms in sea lion pups on the four rookeries and in pups of both species relative to body condition. Differences in 1996 and 2000 infection levels were tested using analysis of variance of the log-transformed numbers of hookworms with body condition as a blocking factor. Additionally, to avoid possible misinterpretation resulting from non-normality, we used analysis of variance on the rank-transformations (Zar, 1996) of the number of hookworms within each body condition.

### 3. Results

#### 3.1. Dead *C. ursinus* and *Z. californianus* pups

##### 3.1.1. *C. ursinus*

**3.1.1.1. Prevalence-intensity-adult hookworms.** Nineteen of the 20 (95%) dead fur seal pups harbored adult hookworms. All pups were from Adams Cove Rookery (Table 1). Intensity of hookworms in the 19 infected pups was 2–897 ( $\bar{x}$  = 351) for male specimens, 2–1245 ( $\bar{x}$  = 409) for female specimens, and 4–2142 ( $\bar{x}$  = 760) for all specimens. There was no significant difference (Wilcoxon rank sum;  $P$  = 0.79) in the number of hookworms in male and female pups.

**3.1.1.2. Body condition and worm burden.** Body condition of the majority of the infected fur seal pups ( $n$  = 13) was good (Table 1). These pups harbored much higher numbers of

Table 1  
Hookworm intensity and body condition of infected northern fur seal pups ( $n = 19$ ) found dead on a rookery (Adams Cove) on San Miguel Island (California, USA) in July 2000

Body condition	Number examined	Number of hookworms		
		Range	Mean	$\pm$ S.D. <sup>a</sup>
Poor	3	4–192	71	$\pm$ 105
Fair	3	29–553	319	$\pm$ 267
Good	13	228–2142	1021	$\pm$ 491
Combined	19	4–2142	760	$\pm$ 575

<sup>a</sup> Standard deviation.

hookworms (Kruskal–Wallis;  $P = 0.0042$ ) than pups in fair ( $n = 3$ ) and poor ( $n = 3$ ) condition. There was no difference in infection intensity in fur seals between years 2000 and 1996 (ANOVA on log transformed values;  $P = 0.237$ , ANOVA based on ranks;  $P = 0.124$ ) (Fig. 1).

### 3.1.2. *Z. californianus*

3.1.2.1. *Prevalence-intensity-adult hookworms.* All 25 sea lion pups were positive for adult hookworms (Table 2). Although all the specimens were adult (L<sub>5</sub>), some were much smaller than others. Counts for male specimens were 10–1280 ( $\bar{x} = 299$ ), for female

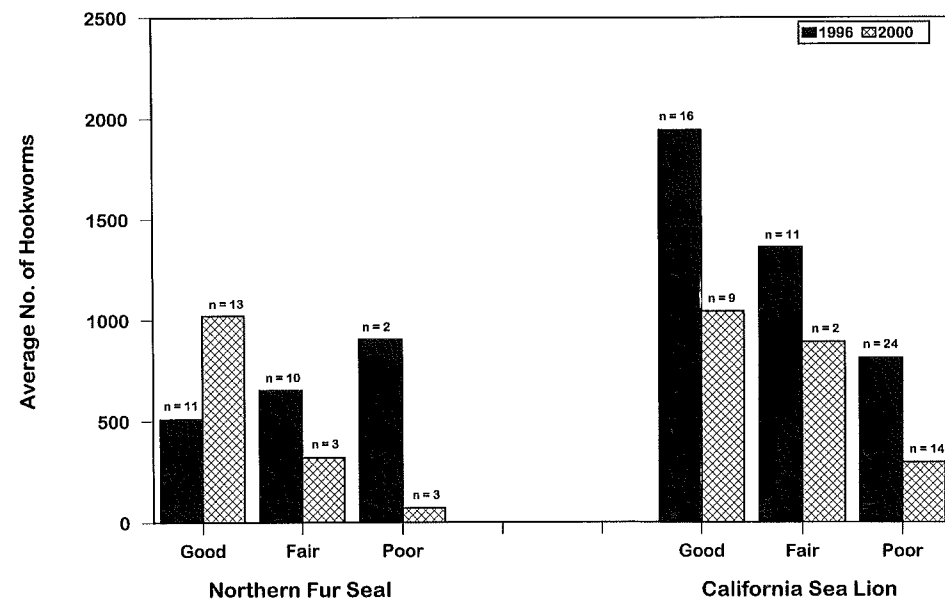


Fig. 1. Average number of hookworms in northern fur seal and California sea lion pups categorized by condition in 1996 and 2000.

Table 2

Hookworm intensity and body condition in infected California sea lion pups ( $n = 25$ ) found dead on four rookeries<sup>a</sup> on San Miguel Island (California, USA) in July 2000

Body condition	Number examined	Number of hookworms		
		Range	Mean	±S.D. <sup>b</sup>
Poor	14	20–2037	297	±524
Fair	2	683–1097	890	±293
Good	9	77–2634	1040	±880
Combined	25	20–2634	612	±737

<sup>a</sup> Point Bennett, Northwest Point, Northwest Cove, and Northeast Point.

<sup>b</sup> Standard deviation.

specimens were 10–1354 ( $\bar{x} = 313$ ), and for all specimens were 20–2634 ( $\bar{x} = 612$ ). The number of hookworms in male and female pups was not statistically different ( $P = 0.26$ ). Counts of hookworms in pups on the four rookeries revealed no significant difference ( $P = 0.79$ ) between any of the locations (Table 3). Six additional pups were examined for hookworms, but counts were not made; all were infected. Therefore, 100% of the 31 pups examined were positive.

**3.1.2.2. Body condition and worm burden.** Body condition of the majority of sea lion pups ( $n = 14$ ) was poor (Table 2). These pups had fewer adult hookworms ( $P = 0.0079$ ) than did pups with fair ( $n = 2$ ) or good ( $n = 9$ ) body condition (Fig. 1).

**3.1.2.3. 2000 versus 1996 infection intensity.** When the 1996 and 2000 data on numbers of worms in each pup were combined and compared, there was a significant decrease (ANOVA (log of total worms);  $P = 0.03$ , ANOVA (ranks of total worms);  $P = 0.003$ ) in infection intensity (numbers of worms) in pups in 2000 (Fig. 1).

**3.1.2.4. Sex ratio of worms.** When the 2000 data were combined with the 1996 data (Lyons et al., 1997) to increase sample size there was a significantly greater (Wilcoxon signed rank;  $P = 0.011$ ) number of female than male worms in sea lions.

Table 3

Hookworm intensity in infected California sea lion pups ( $n = 25$ ) found dead on four rookeries on San Miguel Island (California, USA) in July 2000

Rookery	Number examined	Number of hookworms		
		Range	Mean	±S.D. <sup>a</sup>
Point Bennett	10	39–1310	458	±474
Northwest Point	5	32–2634	1184	±1151
Northwest Cove	5	20–2093	594	±869
Northeast Point	5	118–996	365	±363

<sup>a</sup> Standard deviation.

### 3.1.3. Both hosts

**3.1.3.1. Interspecific differences.** There was not a significant difference in numbers of worms in fur seal and sea lion pups in 2000 (Wilcoxon rank sum;  $P = 0.44$ ). However, when the 2000 worm counts were combined with those from 1996 (Lyons et al., 1997) and tested, there was a significantly greater (Wilcoxon ranked sum;  $P = 0.05$ ) number of worms in California sea lions than in northern fur seals.

**3.1.3.2. Enumeration of eggs in female hookworms.** Total number of hookworm eggs was counted in six female specimens (three from one pup of each host). The number of eggs in specimens from the fur seal pup varied from 225 to 369 ( $\bar{x} = 285$ ) and from the sea lion pup from 262 to 312 ( $\bar{x} = 281$ ).

**3.1.3.3. Examination of blubber.** Ventral abdominal blubber from dead fur seal and sea lion pups, examined in July, was negative for hookworm parasitic L<sub>3</sub>.

### 3.2. Live *C. ursinus* and *Z. californianus* pups

#### 3.2.1. Fecal samples

Rectal fecal samples, collected in late September and early October and examined for hookworm eggs, were negative for 35 fur seal pups but positive for 41 of 48 (85%) sea lion pups.

#### 3.2.2. Hematocrits

Packed cell volume (%) values varied from 32 to 49 ( $\bar{x} = 42$ ; S.D.  $\pm 3.62$ ) for fur seal pups and 10 to 41 ( $\bar{x} = 30$ ; S.D.  $\pm 6.66$ ) for sea lion pups. Sea lions had significantly lower ( $P < 0.0001$ ) PCV than fur seals.

### 3.3. Rookery sand

Free-living hookworm L<sub>3</sub> were not recovered from rookery sand collected and examined in July. Likewise, rookery sand, positive for free-living L<sub>3</sub> at time of collection in January 1998, and refrigerated until re-examination in August 2000, was negative for these larvae.

### 3.4. Milk from *Z. californianus* females

One *Uncinaria* spp. parasitic L<sub>3</sub> was recovered from a milk sample from one of nine lactating females at TMMC.

## 4. Discussion

The current high prevalence of adult *Uncinaria* spp. in northern fur seal and California sea lion pups on SMI is similar to that reported in these hosts at the same time of year on this island in a survey in 1996 (Lyons et al., 1997). Whereas, intensity for fur seal pups

was slightly lower in 2000. The number of hookworms in sea lion pups was significantly less than found in 1996. It is not clear as yet whether this observed difference represents inter-annual variability or a trend in infection intensity.

There was a significant difference in the number of hookworms in *C. ursinus* and *Z. californianus* pups when the 1996 and 2000 data are combined. The fact that there was not a difference in numbers of hookworms in the two host species when only the 2000 sample was analyzed may be a reflection of the much smaller sample size for *Z. californianus* pups in the 2000 collection. Lack of a difference in intensity of hookworms in male versus female pups was as found previously (Lyons et al., 1997).

Body condition of fur seal and sea lion pups was directly related to the number of hookworms present. That is, pups in poor condition had significantly fewer hookworms than the pups in good condition. A similar relationship was found in the 1996 study on SMI for *Z. californianus* pups but not *C. ursinus* pups (Lyons et al., 1997). It was also evident for *C. ursinus* pups on St. Paul Island (SPI), AK in previous studies (Lucas, 1899; Olsen, 1958) and for Juan Fernandez fur seal (*Arctocephalus philippii*) pups in Chile (Sepúlveda, 1998). This occurrence is possibly associated with transmammary transmission of the hookworm larvae which is known to occur in *C. ursinus* (Lyons, 1963; Olsen and Lyons, 1965). Limited data indicate it also takes place in *A. philippii* (Sepúlveda and Alcaino, 1993) and *Z. californianus* (the present research). Pups in poor condition may nurse less, and therefore, acquire fewer larvae than the pups in better condition.

Relationship between numbers of hookworms and clinical hookworm disease is unclear. Olsen (1958), in his research in *C. ursinus* pups on SPI, AK, reported a correlation of presence of 100 or more hookworms and anemia. Keyes (1965), in research on *C. ursinus* on the Pribilof Islands, AK, describes some of his findings on infections of hookworms in dead pups as: "Acute uncinariasis was characterized by extremely pale organs, watery blood, and usually over 100 worms and free blood in the small intestine and colon".

Both incidence and intensity of *Uncinaria* spp. infections in *C. ursinus* pups on SMI are much higher than are currently recorded in the same host on SPI (Lyons et al., 2000b). In the Alaska fur seal herd, definite explanation for the precipitous decline in hookworm infections is unclear; however, the hosts have also decreased in recent years (Lyons et al., 2000b).

The number of eggs (about 300), counted in utero per female hookworm in both species of pups on SMI, may be an indicator of the 24 h egg production for an individual specimen. Faust and Russell (1964) quoted Soper (1927), who stated that an ancylostome (*Ancylostoma* spp.) hookworm female contains, in utero, about 5% of the daily egg output. *Ancylostoma duodenale* females reportedly produce about 10,000–30,000 eggs each per day (Faust and Russell, 1964). If *Uncinaria* spp. in *C. ursinus* and *Z. californianus* pups are similar to *Ancylostoma* spp., it would mean, based on the few specimens examined, that a single female hookworm has the ability to produce about 30,000 eggs a day. Theoretically, 100 *Uncinaria* spp. females could contaminate rookeries on SMI with about 3,000,000 eggs daily. Dutch researchers (Rep and Bos, 1979), in the English summary of their paper, state that the highest number of eggs produced by a female *Uncinaria stenocephala* per day in dogs is "estimated at well over 5000".

Absence of parasitic L<sub>3</sub> in the blubber of pups in July in the present research reflects the lack of recovery of free-living L<sub>3</sub> from sand on rookeries during the same time period.



This replicates previous results on SMI, which showed free-living L<sub>3</sub> hookworms were not recovered from sand on rookeries in the summer. However, they were present in fall and winter months (Lyons et al., 2000a). Similar findings were evident many years ago on SPI with free-living L<sub>3</sub> hookworms being nonexistent on the fur seal rookeries during summer months but abundant in the fall; they usually first appeared in late August (Olsen and Lyons, 1965). Rookery sand, positive for *Uncinaria* spp. free-living L<sub>3</sub> at the time of collection on SMI in January 1998, but negative after 2.5 years under refrigeration, indicates lack of survival of the larvae for this long period of time.

Results of examination of rectal fecal samples from live pups on SMI in the early fall months paralleled earlier observations where *C. ursinus* pups were devoid of hookworms in the fall months, but *Z. californianus* pups were still infected at that time (Lyons et al., 2000a). Other research on SMI and coastal California showed loss of adult hookworms in fur seal pups at about 2 to 3 months of age and in sea lion pups at about 6–8 months of age (Lyons et al., 2000a).

Packed cell volumes in live pups, in the fall of 2000 on SMI, followed a pattern of being essentially normal for *C. ursinus* pups compared to overall lower values for *Z. californianus* pups. Thus, high PCVs in fur seals were consistent with the absence of hookworms and the lower PCVs in sea lions were consistent with the evident hookworm-induced anemia. These PCV values ( $\bar{x} = 42\%$ ) for *C. ursinus* were markedly higher than reported for 12 “sick” fur seal pups (PCV = 11–27%) on SPI in hookworm research in the summer months over 40 years ago (Olsen, 1958). In the same study, Olsen reported that for eight apparently healthy, nonhookworm-infected pups, PCVs were 22 and 23% for two of them and 36 to 40% for the other six. Sweeney (1974) lists a mean PCV of 45% for eight *Z. californianus*, which is much higher than the mean (30%) for the 40 live pups on SMI in the present research.

Milk from one stranded *Z. californianus* cow at TMMC, containing a parasitic hookworm L<sub>3</sub>, further supports indication of transmammary transmission of *Uncinaria* spp. in this host. This type of transmission of *Uncinaria* spp. has been suggested strongly by recovery of parasitic hookworm L<sub>3</sub> from ventral abdominal blubber of *Z. californianus* (Lyons et al., 2000a). It may have been the way a *Z. californianus* pup, born in a zoo, was infected with hookworms (Twisleton-Wykeham-Fiennes, 1966; Lyons and Keyes, 1984).

The present research on *C. ursinus* and *Z. californianus* provided description of an apparent decrease in the intensity of *Uncinaria* spp. infections in sea lion pups on SMI compared to 1996. However, the high prevalence of hookworm infections in fur seal and sea lion pups was similar in both the 1996 and 2000 studies. Several aspects on the biology of these parasites were determined.

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