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PREVALENCE OF HOOKWORMS, *UNCINARIA LUCASI* (ANCYLOSTOMATIDAE), IN NORTHERN FUR SEALS (*CALLORHINUS URSINUS*) ON ST. PAUL ISLAND, ALASKA

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Prevalence of Hookworms, *Uncinaria lucasi* (Ancylostomatidae), in Northern Fur Seals (*Callorhinus ursinus*) on St. Paul Island, Alaska. Lyons, E. T., Kuzmina, T. A., Carie, J. L., Tolliver, S. C., Spraker, T. R. — Review of main studies on biology and ecology of the hookworm *Uncinaria lucasi* Stiles, 1901 performed on St. Paul Island, Alaska, is presented. Current data on prevalence of adult hookworms parasitizing northern fur seals (NFS), *Callorhinus ursinus* Linnaeus, 1758, were obtained based on the examination of the intestines of dead NFS pups and subadult 3–4 year-old males in July and August of 2011–2013. In addition, blubber samples collected from subadult NFS males were examined for parasitic third stage hookworm larvae (L_3). All current data were compared with previously published studies performed in 1950s –1960s. Current prevalence of *U. lucasi* in dead pups collected from Reef Rookery was 4.9 % in 2011, 0 % in 2012 and 10.5 % in 2013. This rookery has a rocky substrate. On sandy rookeries prevalence was up to 75 % on Morjovi Rookery and 50 % on Vostochni Rookery. Parasitic L_3 were recovered in 2.5 % of subadult males examined in 2013. Decreasing prevalence of hookworm infection of dead pups and subadult males during the last several years follows the tremendous decline in the number of fur seals in the herd on St. Paul Island during last several decades.

K e y w o r d s: hookworms, *Uncinaria lucasi,* northern fur seal, *Callorhinus ursinus,* St. Paul Island, Alaska.

Распространение анкилостом, *Uncinaria lucasi* (Ancylostomatidae), у северных морских котиков (*Callorhinus ursinus*) на острове Св. Павла, Аляска. Лайонс Е. Т., Кузьмина Т. А., Карие Дж. Л., Толливер Ш. К., Спрейкер Т. Р. — Представлен обзор основных исследованний по биологии и экологии анкилостомы *Uncinaria lucasi* Stiles, 1901, проведенных на о. Св. Павла, Аляска. Данные об экстенсивности инвазии северных морских котиков (NFS), *Callorhinus ursinus* Linnaeus, 1758, взрослыми анкилостомами были получены при исследовании кишечников погибших щенков северного морского котика, а также молодых самцов (возраст 3–4 года) с июля по август 2011–2013 гг. Кроме кишечников, на наличие паразитических личинок унцинарий 3-й стадии (L_3) были собраны и исследованы пробы брюшного жира от молодых самцов. Экстенсивность инвазии (ЭИ) щенков котиков унцинариями на лежбище Риф с каменистым субстратом составляла 4,9 % в 2011 г., 0 % — в 2012 г. и 10,5 % — в 2013 г.; в то время как на песчаных лежбищах ЭИ была намного больше (75 % на лежбище Моржовое и 50 % на лежбище Восточное). Паразитические личинки L_3 были обнаружены в 2013 г. в 2,5 % проб от молодых самцов. Результаты сравнения наших данных с данными исследований 1950–1960-х гг. показали, что продолжающаяся тенденция по снижению ЭИ унцинарий в кишечниках погибших щенков и в брюшном жире молодых самцов северного морского котика является результатом снижения численности популяции северных морских котиков на о. Св. Павла за последние десятилетия.

К л ю ч е в ы е с л о в а: анкилостомы, *Uncinaria lucasi,* северный морской котик, *Callorhinus ursinus,* о. Св. Павла, Аляска.

Introduction

Hookworms *Uncinaria lucasi* Stiles, 1901 (fig. 1) were first found in the small intestine of northern fur seal (NFS) (*Callorhinus ursinus* Linnaeus, 1758) pups by F. A. Lukas in 1896 in his investigation of fur seal pup mortality on the Pribilof Islands, Alaska (Lucas, 1899). Later C. W. Stiles and A. Hassall partially described these nematodes (Stiles, Hassall, 1899; Stiles, 1901) and named them *Uncinaria lucasi* in honor of Lucas (Lyons et al., 2011). Full description of this species was made more then 40 years later (Baylis, 1947).

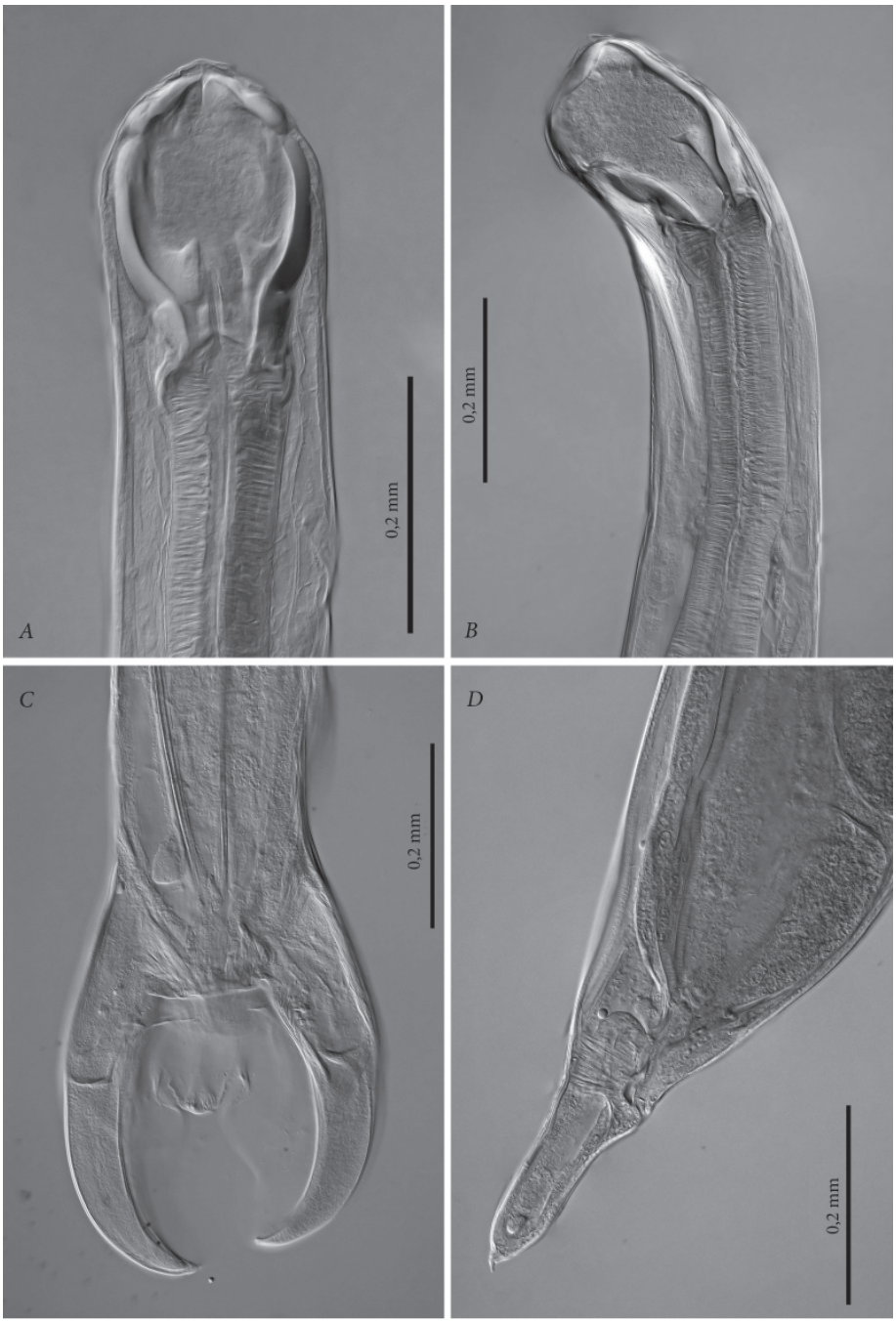

Fig. 1. *Uncinaria lucasi* adults from a northern fur seal pup *Callorhinus ursinus*: *A* — anterior end, dorsal view; *B* — anterior end, lateral view; *C* — male tail; *D* — female tail.

Рис. 1. Взрослая нематода *Uncinaria lucasi* из щенка северного морского котика *Callorhinus ursinus*: *A* — передний коней, дорсальный вид; *B* — передний конец, вид сбоку; *C* — хвост самца; *D* — хвост самки.

Despite high NFS pup mortality from uncinariosis on the Pribilof Islands, studies on biology and ecology of this parasite have not been performed until the 1950s, when O. W. Olsen and C. F. Dixon started a multiyear investigation in 1951 on St. Paul Island. Their aim was to reduce morbidity and mortality caused by these parasites in pups (Lyons et al., 2011). These researchers explored several aspects of biology and ecology of *U. lucasi* (Olsen, 1958); however the life cycle and transmission of this parasite in the NFS population on St. Paul Island were unknown at that time.

Extensive research concentrating on the biology of *U. lucasi*, mainly on St. Paul Island, was carried out in the 1960s by O. W. Olsen and E. T. Lyons, who identified the source of infection of fur seal pups and discovered the life cycle (Olsen, Lyons, 1962, 1965; Lyons, 1963). *Uncinaria lucasi* has a life cycle unique among nematodes (fig. 2).

NFS pups become infected from their mothers. Parasitic third-stage larvae (L_3) pass in the first milk of females to their nursing pups in which they mature in the intestines in about 2 weeks. Eggs are passed from the pups' intestines with feces and develop to larvae of 1st (L_1), 2nd (L_2) and then 3rd (L_3) stages, but L_3 do not begin hatching until late August (according to researches in the early 1960s: Olsen, Lyons, 1962, 1965; Lyons, 1963). Then, the L_3 enter pups and, possibly, older seals predominately via penetration of the flippers but also orally; next, they migrate to parenteral tissues, especially ventral blubber — a tissue phase of the life cycle. These larvae, now called parasitic L_3 (fig. 3), do not escape except through the milk of pregnant females. Thus, the only way that adult hookworms can be produced in pups is by transmammary transmission of larvae (Lyons et al., 2011).

After the late 1960s, periodically there has been an opportunity to study the parasites in dead NFS pups on St. Paul Island, Alaska (Lyons et al., 2012). The main aim was to necropsy dead pups and to follow the prevalence pattern of *U. lucasi* in them over time (Lucas 1899; Olsen 1958; Lyons, 1963; Lyons et al., 2000, 2011, 2012).

Previously, uncinariosis was an extremely important cause of mortality of NFS pups (Olsen, 1958; Lyons, 1963; Lyons et al., 2000, 2011) on the Pribilof Islands. Recent research has documented the tremendous decline in prevalence of these parasites since the 1950s and 1960s (Lyons et al., 2011). There has been a simultaneous huge decrease in the number of pups born on St. Paul Island for this period – from about 300 to 400 thousands in the 1950s and early 1960s to a little more than 120,000 in the early 2000s (Melin et al., 2006).

In subsequent years (2011, 2012, and 2013) the authors of the present paper have had the opportunity to continue monitoring studies of NFS pups and subadult 3–4 year old males infection with separate groups of parasites. The records on hookworms recovered in 2011 (Lyons et al., 2012), acanthocephalans in 2011 (Kuzmina et al., 2012) and filariids from subadult males (SAMs) in 2011 and 2012 (Kuzmina et al., 2013) were published.

Some current findings on the prevalence and intensity of NFS infection with *U. lucasi*, especially in the rookeries with different environmental conditions (Lyons et al., 2013), provide new data for the analysis of the current state of prevalence and intensity of NFS infection by these parasites, and for the assessment of future trends in host–parasite relationship between *U. lucasi* and NFSs on St. Paul Island.

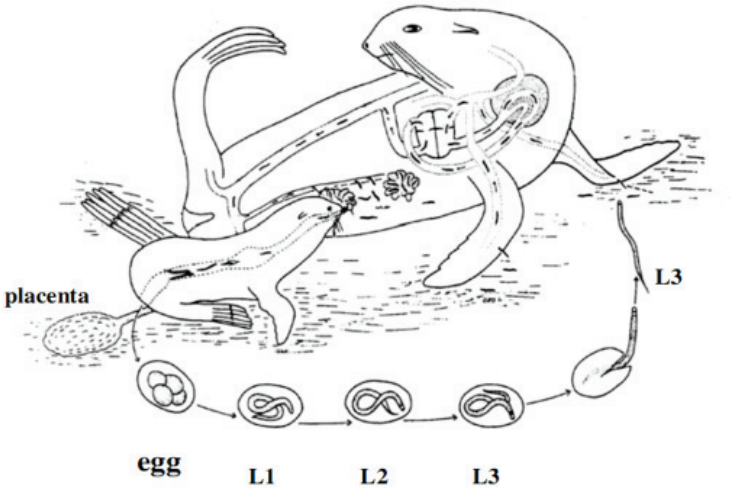

Fig. 2. Life cycle of *Uncinaria lucasi* (drawing adapted after Olsen, 1962): L1, L2 and L3 — free-living larvae of 1st, 2nd and 3rd stages.

Рис. 2. Жизненный цикл *Uncinaria lucasi* (по: Olsen, 1962): L1, L2 и L3 — свободноживущие личинки 1-й, 2-й и 3-й стадий.

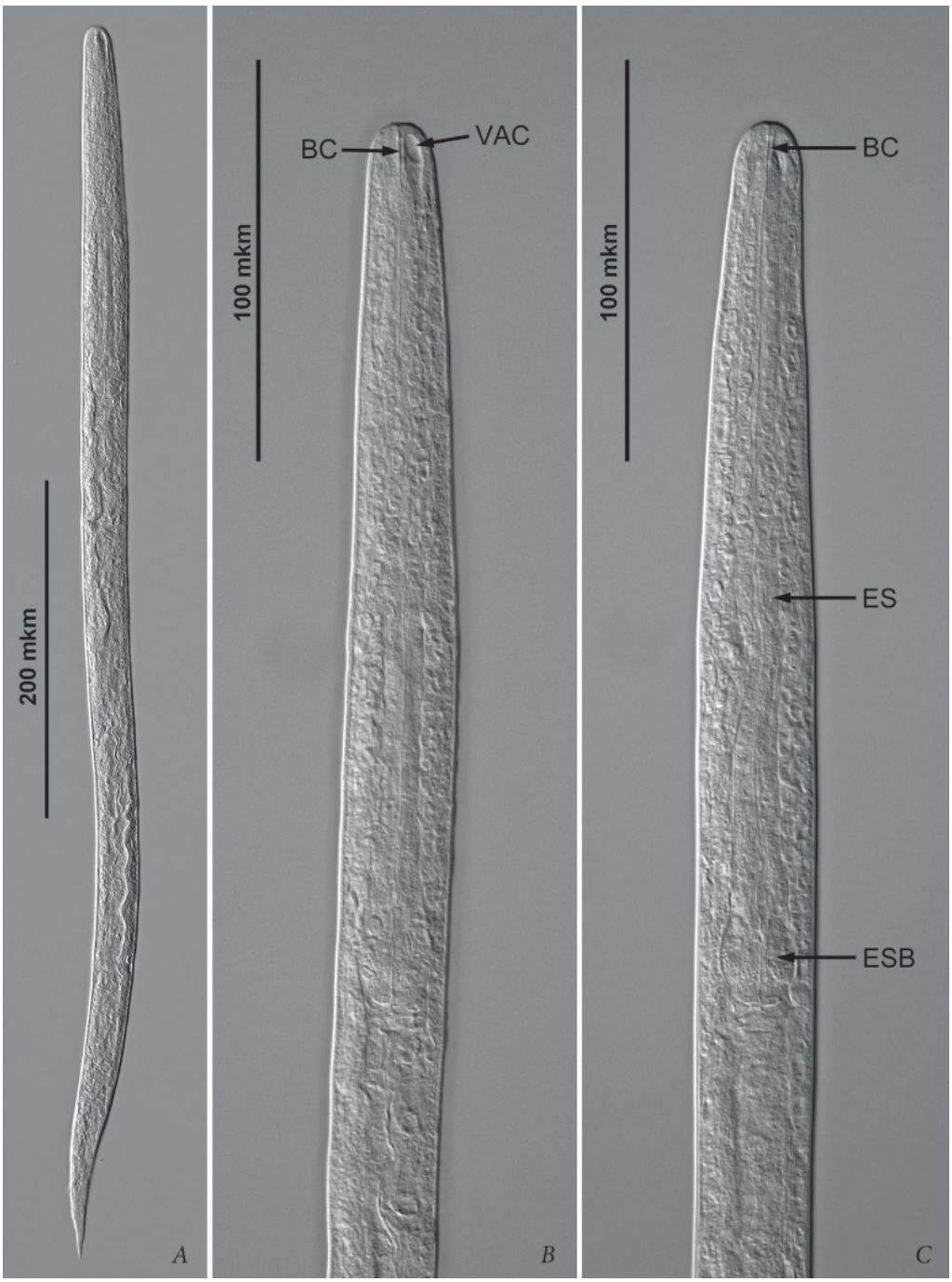

Fig. 3. Parasitic third stage hookworm larvae (L_3) from the blubber of a northern fur seal male: *A* — general view of entire L_3; *B* — anterior part of L_3, lateral view; *C* — anterior part of L_3, dorsal view.
BC — buccal cavity; ES — esophagus; ESB — esophagus bulb; VAC — vacuole — provisional buccal capsule of fourth stage larva.

Рис. 3. Паразитические личинки анкилосктом (L_3) из брюшного жира северного морского котика: *A* — общий вид личинки L_3; *B* — передний конец личинки L_3, вид сбоку; *C* — передний конец личинки, дорсальный вид).
BC — ротовая капсула; ES — пищевод; ESB — бульбус пищевода; VAC — вакуоль — зачаток ротовой капсулы личинки четвертой стадии.

Material and methods

The present research was conducted on St. Paul Island, Alaska (57°15′ N, 170°20′ W) during three field seasons in July–August 2011–2013. Collection of northern fur seal (NFS) dead pups was done as described previously (Lyons et al., 2012) from four rookeries (Reef, Morjovi, Vostochni and Zapadni) (fig. 4).

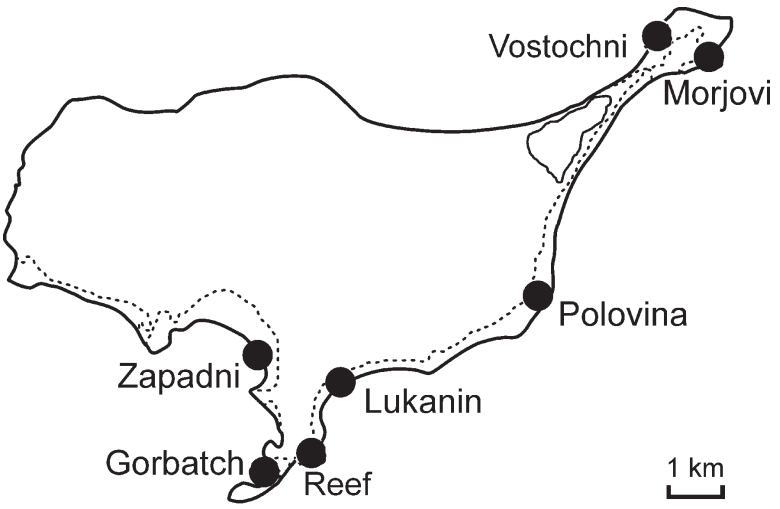

Fig. 4. Sampling locations (rookeries) on St. Paul Island.

Рис. 4. Расположение мест сбора проб (лежбища) на о. Св. Павла.

Reef rookery (rocky substrate), situated in the southern part of the island, is the main site where dead NFS pups have been collected for necropsy for many years. Occasionally, a few freshly dead pups were collected at three sandy rookeries — Morjovi (4 pups), Vostochni (2 pups) and Zapadni (1 pup) and examined for presence of hookworms. Samples (609 — intestines and 293 — blubber) were collected also from NFS subadult males during the annual Aleut harvests on five rookeries (Gorbatch, Lukanin, Morjovi, Polovina and Zapadni) in 2011–2013. Contents of both the small and the large intestines of the pups and sub-adult males were examined specifically for adult hookworms by methods published previously (Lyons et al., 2005, 2012). Blubber was collected and examined for parasitic hookworm larvae (L_3) from subadult males according to published procedures (Lyons et al., 2012).

The Paleontological Statistics Software (PAST) (Hammer et al., 2001) was used for the analysis of the data obtained. The images of helminthes were made with microscope Zeiss AxioImager M1 using DIC method at the Center of collective use of scientific equipment "Animalia" (Institute of Zoology NAS of Ukraine).

Results and discussion

Data from the studies on the prevalence of hookworms in the intestines of NFS pups examined on St. Paul Island in 2012 and 2013 are summarized in table 1.

Fewer dead pups were collected from the Reef Rookery in 2012 and 2013 than in 2011. This was due to factors including fewer pups born in 2012 and 2013 (Fur…, 2013) as well as decreased access to this rookery, because a large storm destroyed some catwalks on the Reef Rookery resulting in reduced possibility to collect dead pups. Prevalence of adult hookworms in pups was negative in 2012 but about twice as high in 2013 comparing to 2011; although the number of hookworms per pup was lower in 2013 than in 2011. Statistically significant differences in NFS pups infected with *U. lucasi* between these years was not observed (Mann–Whitney test, $p > 0.05$).

Although a low number of dead pups was available for necropsy from two sandy rookeries (Morjovi and Vostochni) in 2012, the prevalence of intestinal infections of hookworms was much higher than that for Reef Rookery in the past three years and for several other more recent years before (Lyons et al., 2000). The number of hookworm specimens in pups from Morjovi greatly exceeded the number in pups from Reef for the

Table 1. Examination for hookworms (*Uncinaria lucasi*) in blubber and intestines of northern fur seal (*Callorhinus ursinus*) subadult males on St. Paul Island, Alaska

Таблица 1. Исследование брюшного жира и кишечника щенков северного морского котика (*Callorhinus ursinus*) на о. Св. Павла, Аляска, на наличие анкилостом (*Uncinaria lucasi*)

Rookery	Number of pups examined	Number of pups infected (prevalence, %)	Number of adult hookworms / infected pup
2011[1]			
Reef	61	3 (4.9 %)	1–13
2012			
Reef	17	0 (0 %)	0
Morjovi	4	3 (75 %)	2–154
Vostochni	2	1 (50 %)	1
Total in 2012	23	4 (17.4 %)	1–154
2013			
Reef	19	2 (10.5 %)	1
Zapadni	1	0 (0 %)	0
Total in 2013	20	2 (10.0 %)	1
Total data 2011–2013	104	9 (8.7 %)	1–154

[1] Data for 2011 already published by Lyons et al. (2012).

past several years. Documented reports for examination of dead pups from Morjovi for hookworms were not found. In earlier studies on the sandy rookery Vostochni (Northeast Point) in 1977 (Lyons et al., 1978), prevalence of hookworms from dead pups was found to be high (over 90 %). It is unknown for certain if there are foci of hookworms presently being transmitted to NFS on the two rookeries. There is an impression that this is happening. Also, it is possible that the females which gave birth and infected their pups on these rookeries, relocated there from other islands (Commandor Islands, Russia, and San Miguel Island, California, USA) where hookworm prevalence is still quite high in pups (Kolevatova et al., 1998; Lyons et al., 2011). No matter what was the geographical source of females infecting their pups with parasitic L_3 through the milk, hookworm eggs passed in the pup feces in the environment undergoes development to free-living L_3 which infect the tissues of seals, thus spreading hookworm populations in these hosts. Level of NFS pup infections with *U. lucasi* (prevalence and intensity) found in sandy rookeries (Morjovi and Vostochni) in this study suggests that despite the low prevalence of these parasites observed on St. Paul Island in previous years (Lyons et al., 2012), uncinariosis still can be one of the main causes of NFS pup mortality on sandy rookeries. It would be quite beneficial if a hookworm prevalence study, even a limited one, could be made in pups on these two rookeries. This would help to determine the extent of hookworm prevalence on sandy rookeries as compared to rocky rookeries.

Comparison of current findings on prevalence of *U. lucasi* in dead NFS pups with published data from similar studies (Olsen, 1958; Lyons, 1963; Lyons et al., 2000; Ionita et al., 2008; Lyons et al., 2012) revealed decrease of hookworm prevalence during last decades (fig. 5).

Data from the studies on hookworm prevalence in NFS sub-adult males examined on St. Paul Island in 2011–2013 are summarized in table 2.

Numbers of subadult males from which blubber samples were examined in 2011 and 2013 are comparable, as well as the number of males infected with *U. lucasi* parasitic L_3 found in these years. The negligible prevalence of hookworm larvae in the blubber of subadult males parallels the current similar low level of adult hookworms in the intestines of pups. Significant differences in number of subadult males infected with the hookworm L_3 were not observed in these years (Mann–Whitney test, $p > 0.05$).

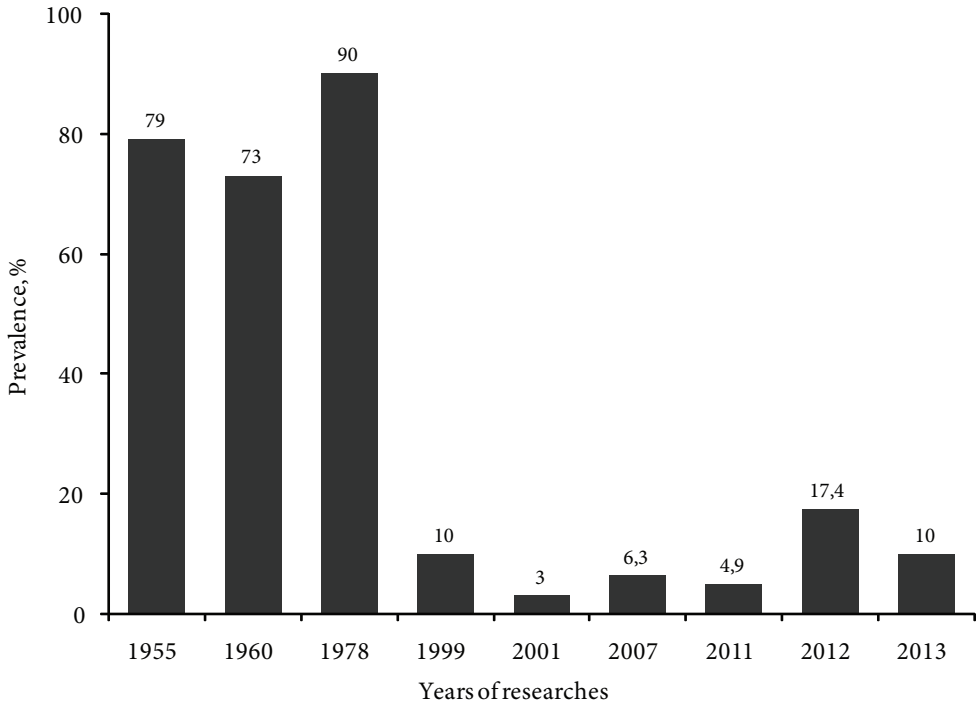

Fig. 5. Prevalence of *Uncinaria lucasi* adult nematodes observed in dead NFS pups on St. Paul Island since 1955.

Рис. 5. Экстенсивность инвазии северных морских котиков половозрелыми нематодами *Uncinaria lucasi* на о. Св. Павла с 1955 г.

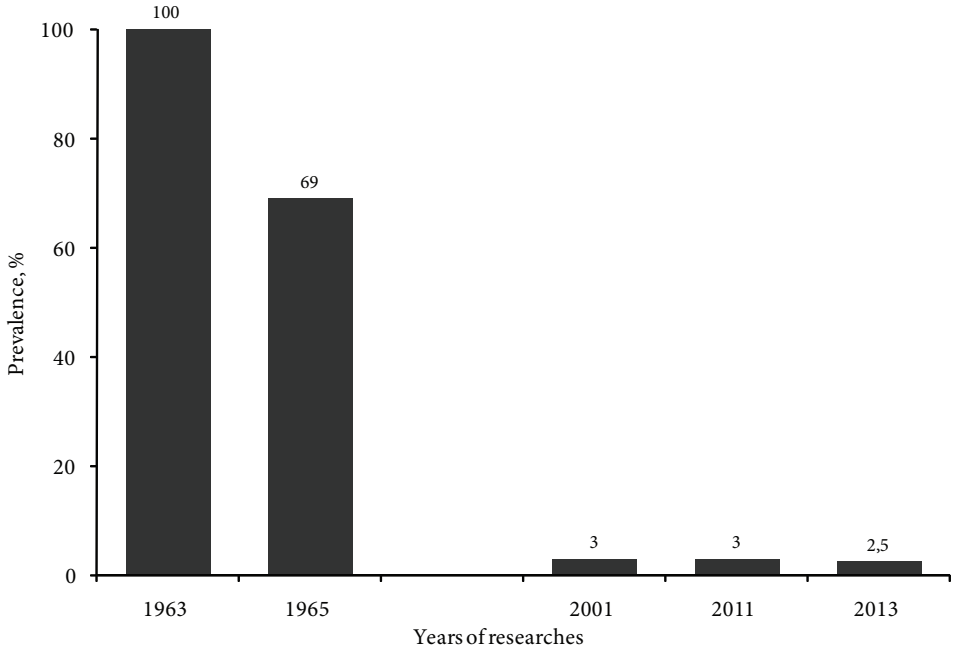

Fig. 6. Prevalence of *Uncinaria lucasi* parasitic L_3 observed in the blubber of subadult NFS males on St. Paul Island since 1963.

Рис. 6. Экстенсивность инвазии северных морских котиков паразитическими личинками L_3 *Uncinaria lucasi*, обнаруженными в брюшном жире котиков на о. Св. Павла с 1963 г.

Table 2. Examination for hookworms (*Uncinaria lucasi*) in blubber and intestines of northern fur seal (*Callorhinus ursinus*) subadult males (SAMs) on St. Paul Island, AK

Таблица 2. Исследование брюшного жира и кишечника молодых самцов северных морских котиков (*Callorhinus ursinus*) на о. Св. Павла (Аляска) на наличие анкилостом (*Uncinaria lucasi*)

Body part	Number of samples examined	Number positive (prevalence, %)	Number hookworms/ infected NFS
2011[1]			
Blubber	133	4 (3 %)	1 parasitic L_3
Intestines	105	1 (0.95 %)	0–3 adults
2012[2]			
Intestines	301	0 (0 %)	0 adults
2013			
Blubber	160	4 (2.5 %)	1–11 parasitic L_3
Intestines	203	0 (0%)	0 adults
Total data from 2011–2013			
Blubber	293	8 (2.7 %)	1–11 parasitic L_3
Intestines	609	1 (< 1 %)	3 adults

[1] Data for 2011 already published by Lyons et al. (2012).
[2] The blubber was not examined in 2012.

All *U. lucasi* parasitic L_3 (fig. 4) found in blubber of subadult NFS males, corresponded morphologically to their description by Lyons (1963) and Olsen and Lyons (1965). Some of the identification characters on the anterior end of the parasitic L_3 are the following: 1) the buccal capsule appears as two spear-like parallel projections and 2) a vacuole which is the provisional buccal capsule of the fourth stage larva.

Comparison of the prevalence of *U. lucasi* parasitic L_3 in blubber of subadult NFS males during last decade with data collected more then 50 years ago (Lyons, 1963; Olsen, Lyons, 1965) also showed dramatic decrease of the number of infected fur seals (fig. 6).

The huge decrease of the number of subadult NFS males infected with parasitic hookworm L_3 found in their blubber in the present study, if compared with data from previous studies, was presumably caused by the enormous reduction in density of the NFS populations on various rookeries of St. Paul Island during the last several decades (Melin et al., 2006). This reduction effects the possibility of the parasite transmission.

Three adult hookworms (1 ♂ and 2 ♀) were found in the small intestine of one subadult NFS male in 2011 (table 2). The finding of adult hookworms in the intestines of NFS male in 2011 is interesting. Olsen (1958) examined 1,426 yearling and older NFS for intestinal infections with hookworms and found none of them infected. Apparently he did gross examination of the entire small intestines in about 500 to 600 of these NFS and for the other half of the NFS, he looked at only the ileoceal junction (typical site of concentration of hookworms in NFS) or just did flotation of feces for presence of eggs. Low numbers of hookworms could have been missed by examination using these methods which were not as precise as in the present research of washing and sieving all the intestinal contents plus scraping and looking at the mucosa. However, it is evident that adult hookworms are rarely present in NFS older than pups; the same was also reported for Steller sea lions (*Eumetopias jubata*) (Olsen 1958; Olsen, Lyons 1965).

The present research shows the continuing significant reduction of hookworm prevalence in the intestines of NFS pups and blubber of subadult males compared to the data from several decades ago. This current pattern of low hookworm prevalence coincides with the huge decline of NFS. Hookworm infection in NFS appears to be related to the host density. Thus, if very few hosts are infected, low parasite transmission occurs. Current research also shows the value of constant monitoring of hookworm infections in NFS on St. Paul Island. This means not only to look for any changes in prevalence in pups on Reef Rookery but also to take advantage of examination of pups from other rookeries, if

possible. Further monitoring of *U. lucasi* on the Pribilof Islands is necessary to ascertain whether these parasites may still be a factor causing decline of the NFS population in the Bering Sea. Monitoring the hookworm prevalence in NFS on the Pribilof Islands is not only of scientific interest from the parasite aspect, but it is also an indicator on a broader level of the result of the great decline of NFS.

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