The cestode community in northern fur seals (Callorhinus ursinus) on St. Paul Island, Alaska

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ABSTRACT
The diversity and ecology of cestodes from the northern fur seals, Callorhinus ursinus (NFS), were examined using newly collected material from 756 humanely harvested subadult males between 2011 and 2014. NFSs were collected from five different haul-outs on St. Paul Island, Alaska. A total of 14,660 tapeworms were collected with a prevalence of 98.5% and intensity up to 107 cestodes per host (mean intensity 19.7 ± 16.5 SD). Three species of tapeworms were found: Adenocephalus pacificus (Diphyllobothriidea) was the most prevalent (prevalence 97.4%), followed by Diplogonoporus tetrapterus (49.7%), and 5 immature specimens of Anophryocephalus cf. ochotensis (Tetabothriidea) (0.5%). Most of the cestodes found in the NFS were immature (69.7%). However, only 0.9% of cestodes were in larval (plerocercoid) stages. The species composition, prevalence and intensity of cestodes from these NFSs were not statistically different between the five separate haul-outs. Significant increases in the intensity of NFS infections were observed during the study period.

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1. Introduction

The northern fur seal, Callorhinus ursinus (Linnaeus) (NFS), has been documented as a host for more than 35 species of helminths (Delyamure, 1961; Dailey and Brownell, 1972; Margolis and Dailey, 1972; Yurakhno, 1998; Atrashkevich, 2008). The first tapeworm described from NFSs was Bothrioccephalus sp. by Stiles and Hassall (1899) from the Pribilof Archipelago (St. George Island) (Fig. 1). Nybelin (1931) later described that species as Adenocephalus septentrionalis Nybelin 1931, as the second species of his newly erected genus Adenocephalus Nybelin 1931 with the type species A. pacificus Nybelin 1931, from the Juan Fernández fur seal Arctocephalus philippii Peters. Stunkard (1948) studied NFS cestodes from St. Paul Island, Alaska, and recorded two unidentified species (reported as species Nos. 2 and 3) which corresponded with A. pacificus and Diplogonoporus tetrapterus (Siebold, 1848) (Delyamure et al., 1985; Hernández-Orts et al., 2015). Later, several authors reported A. pacificus from NFSs on St. Paul and St. George Islands, Alaska (Markowski, 1952; Margolis, 1956), but also from Honshu Island (Sanriku), Japan (Yamaguti, 1951; Machida, 1969) and Far East Russia from Sakhalin and Tyuleniy Islands (Krotov and Delyamure, 1952; Chupakhina, 1971) (Fig. 1). Adenocephalus pacificus was also reported under different names as Diphyllobothrium glacieale (Cholodovsky, 1915), Diphyllobothrium pacificum, Diphyllobothrium hians (Diesing, 1850) or Diphyllobothrium krotovi Delyamure, 1955 (Rausch et al., 2010), but recently, Hernández-Orts et al. (2015) transferred D. pacificum back to the genus Adenocephalus based on molecular and morphological evidence. Diplogonoporus tetrapterus was reported rarely in NFSs off St. Paul Island and Valdez, Alaska (Stunkard, 1948; Rausch, 1964) (Fig. 1). Yurakhno (1986) described a new species, Diplogonoporus violettae Yurakhno, 1986, collected from a Steller sea lion, Eumetopias jubatus (Schreber), from Karaginsky Gulf, Russia, and concluded that diplogonids reported from NFSs by Wardle et al. (1947), Margolis (1956) and Rausch (1964) belonged to this new species (Fig. 1).
Additionally, four other tapeworm species have been reported from NFSS: Anophyrocephalus ochotensis (Delyamure, 1955), Diphyllobothrium hians, Diphyllobothrium lanceolatum (Krabbe, 1865), and Pyramicocephalus phocarum (Fabricius, 1780), as well as phyllobothriidean plerocercoids Scolex pleuronectis Müller, 1788 (Delyamure, 1955; Dailey and Brownell, 1972; Margolis and Dailey, 1972; Yurakhno and Taikov, 1986; Yurakhno, 1998; Kamo, 1999). However, most of these taxa are not specific parasites of NFSS (such as D. hians, D. lanceolatum, or P. phocarum), and may represent misidentifications of the morphologically variable A. pacificus and D. tetrapterus (Delyamure et al., 1985; Hernández-Orts et al., 2015).

Studies of several aspects of NFS biology on the Pribilof archipelago have been undertaken regularly since 1944 (Scheffer et al., 1984; Ream, 2008), but only few parasitological surveys, mainly on hookworms, anisakid nematodes and acanthocephalans, have been published (Stiles and Hassall, 1899; Olsen and Lyons, 1962; Keyes, 1964, 1965; Lyons et al., 2000, 2003, 2011; Ionita et al., 2008; Kuzmina et al., 2012, 2014). Cestodes have been superficially mentioned in a few reports (Stiles and Hassall, 1899; Stunkard, 1948; Yamaguti, 1951; Krotov and Delyamure, 1952; Markowski, 1952; Delyamure, 1955; Machida, 1969; Kamo et al., 1982; Scheffer et al., 1984; Yurakhno, 1998; Ionita et al., 2008). Despite the thousands of seals slaughtered every year from the Pribilof archipelago (Scheffer et al., 1984; Ream, 2008), detailed studies on the diversity and prevalence of cestodes from NFSS have not been performed.

The objective of the present study was to characterize the species composition of cestodes from NFSS based on newly collected material from St. Paul Island, Alaska, including re-examination of type- and voucher material from several helminthological collections (see Hernández-Orts et al., 2015). Comparison of the species composition, prevalence and intensity of cestodes among five subpopulations of NFSS inhabiting different rookeries on St. Paul Island also was performed (Fig. 1).

2. Materials and methods

This study was carried out on St. Paul Island, Alaska (57°09′ N, 170°13′ W) in July–August, 2011 to 2014 (Fig. 1). The gastrointestinal tracts of 756 humanely harvested subadult NFS males (3–4 years old) were collected during four annual Aleut subsistence harvests at haul-out areas on five rookeries: Polovina (n = 164), Morjovi (n = 120), Zapadni (n = 182), Lukarin (n = 165) and Gorbacht (n = 125) (Fig. 1). The research was done under the authority of the Marine Mammal Protection Act Permit Number 14327 issued to the National Marine Mammal Laboratory.

The gastrointestinal tracts of freshly killed NFSS were collected in the field and placed in separate plastic bags, delivered to the laboratory, dissected and examined by classical methods (Bowman and Lynn, 1995). The age of NFS examined was estimated according to the size of their stomachs – stomachs were significantly larger in 4-year-old NFSS than in 3-year-old seals. All helminthes (more than 7700 specimens of nematodes, 14,660 cestodes, 1200 acanthocephalans and 3700 trematodes) were collected manually from the intestines. The cestodes were washed in saline and fixed in hot (>90 °C) tap water and stored in 70% ethanol. The cestodes were identified based on morphological characters according to Delyamure (1955,1985) and Hernández-Orts et al. (2015), and compared with type- and voucher specimens deposited in several helminthological collections [see Hernández-Orts et al. (2015) for material of A. pacificus; type material of D. tetrapterus from the Natural History Museum, London, UK (BMNH Nos. 51.7.24.58 and 51.7.24.27); and one specimen from type series of D. violettae deposited at the Helminthological collection of the Institute of Parasitology, Czech Academy of Sciences, České Budějovice, Czech Republic (IPCAS C-683)]. Current identifications were also supported by phylogenetic analyses based on molecular data (LSU and cox1) (Hernández-Orts et al., 2015; Kuchta unpublished data).

Whole-mounted specimens were stained with Schuberg’s hydrochloric carmine and mounted in Canada balsam. A total of 45 scolices and segments were prepared for scanning electron microscopy (SEM) following the procedure outlined by Kuchta and Caira (2010).

Studied material has been deposited in the parasitological collection of the Schmalhausen Institute of Zoology NAS of Ukraine, Kyiv (Nos. SAM 0-1-SAM 22-16), IPCAS (Nos. IPCAS C-487 and C-482) and National Museum of Natural History, Smithsonian Institution (Nos. 1241612 and 1241613).
Data summaries and descriptive analyses were calculated using the Microsoft Excel. The Palaeontological Statistics Software (PAST) (Hammer et al., 2001) was used for statistical analysis of the results obtained. Mann–Whitney and Chi-square tests were used to analyze differences in prevalence and intensity of NFS infection and from separate haul-outs on the island. Infection parameters were estimated according to Bush et al. (1997) and Rózsa et al. (2000). The prevalence, mean abundance and mean intensity are provided with 95% confidence intervals (CI). Sterne’s exact 95% CI was calculated for prevalence (Reiczigel, 2003). Mean abundance and mean intensity 95% CIs were estimated with 20,000 bootstrap replications with the statistical software Quantitative Parasitology v. 3.0 (Rózsa et al., 2000).

### 3. Results

A total of 14,660 cestodes were collected from 745 of 756 NFSs with a total prevalence of 98.5% (Table 1). The prevalence of infection was up to 107 (mean intensity 19.7 ± 16.5 SD; median intensity 16) cestodes per host (Table 1). Significant differences in prevalence and intensity of NFS infection from separate haul-outs were not observed (Mann–Whitney test; p > 0.05).

Intensity of infection within seals varied widely (Table 1). Distribution of cestodes in NFS populations on St. Paul Island were overdispersed; most of the seals (n = 260) harbored from 1 to 10 cestodes, while just a few hosts harbored several dozen parasites (Fig. 2A). Similar patterns of cestode distribution were observed from different rookeries (Fig. 2B).

Significant differences in the prevalence and intensity of NFS infections were observed between different years (Table 2) (Mann–Whitney test; p < 0.05). The intensity of infection significantly increased from 2011 to 2014 (Mann–Whitney test; p < 0.05). However, we did not find statistically significant differences in prevalence and intensity of infections between seals of two age groups (3 and 4 years old) (Mann–Whitney test; p > 0.05).

From the 14,660 cestodes collected, 99.9% belong to the two diphyllobothrid tapeworms; *A. pacificus* (n = 12,940, i.e. 88.3% of total number of cestodes reported) and *D. tetrapterus* (n = 1559, 10.6%). In addition, only five sexually immature individual Anophryocephalus cf. ochotensis (Tetraphyllidea) were found (Table 3). Most of the cestodes found were immature; specimens with gravid segments represented only 30.3% of the total number of cestodes found (from 14.9% to 40.8% in separate haul-outs). Statistically significant differences between proportions of mature/immature cestodes were observed between the five sub-populations of the NFSs (Chi squared = 12.9; p = 0.01).

Identification of most specimens to the species level was possible with the exception of the 156 plerocercoids (1.1% of the total number of tapeworms) in 10.5% of the NFSs. The shape of the scolex of *A. pacificus* is mostly lanceolate, with a short neck area and with short sharply craspedote anterior segments; whereas the scolex of *D. tetrapterus* is more oval, without a differentiated neck region, and without sharply craspedate anterior segments (Fig. 3). These characteristics seem to be quite stable and are used for identification of immature specimens, even though the variability of the shape of the scolex was high (Fig. 3).

### Table 1

| Haul-out areas (rookeries) | No. of seals | P % (95% CI) | MA (95% CI) | Median intensity | Range |
|---------------------------|--------------|--------------|--------------|-----------------|-------|
| Polovina                  | 164          | 98.2 (94.6–99.5) | 20.7 (18.4–23.6) | 20.3 (17.9–23.1) | 16    |
| Morjovi                   | 120          | 99.2 (95.6–100)  | 19.4 (16.7–22.5) | 19.3 (16.6–22.4) | 14    |
| Zapadni                   | 182          | 99.5 (96.9–100)  | 21.7 (19.4–24.1) | 21.5 (19.3–24.0) | 18    |
| Lukain                   | 165          | 97.0 (93.1–98.8) | 18.3 (16.1–21.0) | 17.7 (15.5–20.4) | 13    |
| Gorbatch                  | 125          | 99.2 (95.7–100)  | 17.4 (15.0–20.5) | 17.3 (15.0–20.4) | 13.5  |
| **Total**                 | **756**      | **98.5 (97.4–99.2)** | **19.7 (18.5–20.9)** | **19.4 (18.3–20.6)** | **16** |

### Table 2

| Year | No. of seals | Cestodes | Adenocephalus pacificus | Diplogonoporus tetrapterus |
|------|--------------|----------|-------------------------|---------------------------|
|      | P % (95% CI) | MA (95% CI) | Range | P % (95% CI) | MA (95% CI) | Range |
| 2011 | 105          | 99.0 (95.0–100) | 13.2 (11.3–15.3) | 1–47 | 98.1 (93.1–99.7) | 12.1 (10.2–14.1) | 1–45 | 37.1 (28.1–47.1) | 3.0 (2.5–3.6) | 1–9 |
| 2012 | 301          | 98.3 (96.1–99.3) | 18.1 (16.3–20.1) | 1–96 | 97.0 (94.4–98.5) | 16.4 (14.7–18.4) | 1–96 | 42.5 (37.0–48.2) | 4.2 (3.5–5.2) | 1–21 |
| 2013 | 203          | 98.0 (94.9–99.2) | 21.2 (19.3–23.3) | 1–80 | 96.6 (92.3–98.8) | 18.1 (16.1–20.2) | 1–80 | 51.7 (44.8–58.6) | 5.6 (4.7–6.8) | 1–29 |
| 2014 | 147          | 99.3 (96.4–100) | 25.3 (22.5–28.5) | 1–107 | 98.6 (95.1–99.8) | 23.1 (20.3–26.4) | 1–107 | 34.7 (27.2–42.8) | 6.2 (5.1–7.8) | 1–23 |

### Table 3

| Haul-out (rookeries) | Adenocephalus pacificus | Diplogonoporus tetrapterus | Anophryocephalus cf. ochotensis |
|---------------------|--------------------------|-----------------------------|--------------------------------|
|                     | P % (95% CI) | MA (95% CI) | P % (95% CI) | MA (95% CI) | P % (95% CI) |
| Polovina            | 97.0 (93.1–98.8) | 18.5 (16.1–21.2) | 47.0 (39.3–54.7) | 4.8 (3.9–6.1) | 0.6 (0.4–3.5) |
| Morjovi             | 97.5 (92.7–99.3) | 17.7 (15.0–20.8) | 43.3 (34.5–52.5) | 4.1 (3.3–5.1) | 0.8 (0.5–4.5) |
| Zapadni             | 97.8 (94.4–99.2) | 19.0 (16.8–21.5) | 47.8 (40.6–55.2) | 5.5 (4.5–6.9) | 0.5 (0.3–3.2) |
| Lukain              | 95.8 (91.3–98.0) | 16.4 (14.1–18.9) | 36.4 (28.3–44.0) | 5.3 (4.1–6.9) | 0.6 (0.4–3.5) |
| Gorbatch            | 99.2 (95.7–100) | 15.9 (13.6–19.1) | 37.6 (29.5–46.4) | 3.7 (2.9–5.0) | – | – |
| **Total**           | **97.4 (95.9–98.3)** | **17.6 (16.5–18.8)** | **42.7 (39.2–46.3)** | **4.8 (4.3–5.4)** | **0.5 (0.2–1.4)** | **1.3** |
were not observed between separate haul-outs (Chi squared = 3.93; p = 0.42). *Diplogonoporus tetraapterus* was less common (42.7%; maximum intensity 29 cestodes per host) (Table 3). Statistically significant differences were not found in the prevalence and intensities of *D. tetraapterus* at the five haul-outs (Chi squared = 6.24; p = 0.18). This species showed a high morphological variability in the shape of scolex, and also in the number of genital pores per segment. The typical double genital pores were observed in 75% of gravid specimens (studied on 40 mature specimens) (Fig. 3).

At the infracommunity level, the richness of tapeworm species ranged from 1 to 3 species. A total of 430 NFSs (57.7%) were infected with one species, 312 seals (41.9%) with two species and 3 seals (0.4%) had all three species. Most of the NFSs (98.2%) were infected with *A. pacificus*, while only 8 seals (1.8%) were infected exclusively with *D. tetraapterus*. A single seal (0.1%) was infected with *A. pacificus* and *Anophyrocephalus cf. ochotensis*. Significant differences were not found in the proportion of seals infected by one, two or three cestode species between the five NFS sub-populations (Chi squared = 13.1; p = 0.35).

4. Discussion

Tapeworms of the orders Diphyllobothriidea and Tetrabothriidea have been reported as common parasites of several species of marine mammals (Delyamure, 1955; Dailey and Brownell, 1972; Delyamure et al., 1985; Kamo, 1999; Hoberg and Adams, 2000). However, data addressing their ecology, host specificity, prevalence and distribution are fragmented or incomplete. Thus, this is the first ecological study on tapeworms from the northern fur seal based on a large amount of a freshly collected material confirmed by morphological and molecular data (see Hernández-Orts et al., 2015). Moreover, these data were obtained during a large scale study over four consecutive seasons and with a high number of NFSs examined from the same area. This allowed a comprehensive analysis on the ecology of tapeworms from the NFSs on St. Paul Island, and an examination of the current state of the helminth fauna on this otariid species.

Most of the previous studies were focused on material collected sporadically from a small number of NFSs in different regions (Cholodkovsky, 1914; Nybelin, 1931; Afanasyev, 1941; Stunkard,
Fig. 3. Scanning electron micrographs of cestodes from the northern fur seal (*Callorhinus ursinus*). Scoleces of adults of *Adenocephalus pacificus* (A–D, F). Scoleces of adults of *Diplogonoporus tetrapterus* (I–P). Gonopores of *D. tetrapterus* (O). Unidentified plerocercoids (E, G, H, R).
Detailed studies of the parasite fauna using large numbers of disected NFSs were first performed in Alaska by Stiles and Hassall (1899) and later in the Russian Far East, on the Kuril and the Commander archipelagos (Bering and Medny Islands) (Chupakhina, 1971; Kovalenko, 1975; Yurakhno and Taikov, 1986; Yurakhno, 1998) (Fig. 1). The first eco-parasitological study on different NFS populations was performed on Medny Island in the Bering Sea (Yurakhno and Taikov, 1986; Yurakhno, 1998) (Fig. 1). However, despite the thousands of NFSs harvested annually on the Pribilof archipelago during the commercial harvests until 1984, extensive studies on helminths, including cestodes, were not performed. Our results represent the first comprehensive data on the diversity and ecology of cestodes of the NFSs from St. Paul Island identified to the species level. Therefore we cannot compare our current findings with any previous data collected in the Alaskan part of the Bering Sea, but only with data collected from other NFS populations in the North Pacific (Kovalenko, 1975; Yurakhno and Taikov, 1986; Yurakhno, 1998). The population studies of the NFSs in the North Pacific indicate that the seals from the Commander, Kuril and the Pribilof archipelago populations can freely mix on feeding sites (Arsyev et al., 1973; Kajimura, 1980, 1984; Gentry, 1998; Sokolov et al., 1998). As the most of helminths parasitize NFSs, including cestodes, are transmitted through food webs (fish, crustacean or squids), using the same forage areas undoubtly facilitates the exchange of parasites between different groups of seals as well as even phylogenetically unrelated hosts, such as other marine mammals and seabirds (Delyamure, 1955; Hoberg, 1995, 1996; Yurakhno, 1998; Hoberg and Adams, 2000).

The high prevalence of cestodes in NFSs (98.6%) observed in our study was similar to previous data obtained from the Commander archipelago (92.3–93.6%) provided by Yurakhno and Taikov (1986) and Yurakhno (1998). These authors also did not find any significant differences in prevalence of cestodes at different haul-outs, as was observed in the present study. The intensity of infection in our study was also extremely high (mean intensity 19.7 specimens per host) (Table 1), which was significantly higher than in previous reports in NFSs from the Commander archipelago (10.9) (Yurakhno and Taikov, 1986; Yurakhno, 1998). Moreover, the high intensity of NFS infection with cestodes was consistently observed during all 4 years of the study, and the intensity of infection significantly increased from 2011 to 2014. Probably this increase in cestode intensity in NFSs is affected by changes in the ecological and oceanographic conditions in the Bering Sea and North Pacific related to climate change in Arctic during the last decades (Benson and Trites, 2002; Hoberg et al., 2012). However, the short duration of our study does not allow us to draw a reliable conclusion. The intensity of the cestodes infections in other otariid species differs in other regions of the world (Hernández-Orts et al., 2013 and references therein) which may be associated with different seasons of the studies, as well as with variations in diet of these otariid species. Nevertheless, this is the highest intensity of cestode infections reported in otariids so far.

Life cycles of cestodes in pinnipeds are still unknown; however, we can assume that diphyllobothriids that parasitize NFSs have similar life cycles to other species from the genera Diphyllobothrium (Cobbold, 1858) or Diplagonoporus (Lömbarg, 1892), and that their first intermediate hosts are planctonic crustaceans (copepods), with marine fish serving as the second intermediate hosts (Delyamure et al., 1985; Kuchta et al., 2015). The life cycles of diphyllobothriids also may be extremely complex, with several additional paratenic or reservoir hosts, such as predatory fish that may accumulate plerocercoids in their body (Delyamure et al., 1985). Therefore, any changes in the cestode fauna as well as in other helminths transmitted through food chains are a reflection of changes in diet of the hosts. Recent studies on diet and feeding behavior of NFSs revealed significant changes in NFS diet during the last decades (Trites, 1992; Sinclair et al., 2008; Sterling, 2009). Significant decrease in NFS infection with anisakid nematodes and acanthocephalans was observed during the last 50 years (Kuzmina et al., 2012, 2014). We suggest that the biodiversity and ecology of diphyllobothriids also have changed over this time. Current tendencies of increasing intensity of NFSs infection with cestodes observed in our study also may support this assumption. Many ecological factors, including physical environmental conditions (climate changes, oceanic regimes), biotic and anthropogenic (commercial fisheries) factors influence marine food-webs in the Bering Sea and North Pacific (Sinclair et al., 2008). However, the absence of retrospective data from the eastern part of the North Pacific does not allow us to clarify these changes and associate them with changes in the prey availability and feeding behavior of the seals.

The tetrabothriids of the genus Anoplycephalus Baylis, 1922 are well known parasites of phocids and otariids with narrow host and geographic distribution at high latitudes of Holarctic Region (Hoberg and Adams, 1992). Anoplycephalus ochotensis Delamure et Krotov, 1955 was described from the Steller sea lion, Eumetopias jubatus and in the NFS was reported only once on the Kommandorskii Islands by Yurakhno (1987, 1998). In the present study, we found only few immature specimens tentatively identified as Anoplycephalus cf. ochotensis.

The finding of a large number of immature cestodes (69.7%) in the present study may correspond with recent infection of the NFSs, but most probably is caused by hyper-infection (“crowding effect”) that may delay the growth and development of these cestodes, as has been observed in other cestodes (Halvorsen and Andersen, 1974; Bush and Lotz, 2000; Roberts, 2000).

Detailed morphological studies have shown high variability in the shape and size of the scolex in diphyllobothriid cestodes, however their specific identification was possible based to the shape of the first segments and the presence of the neck (Fig. 3) (Hernández-Orts et al., 2015). This extreme variability may explain the previous records of additional diphyllobothriid tapeworms reported in NFSs (Delyamure, 1955; Dailey and Brownell, 1972; Margolis and Dailey, 1972; Yurakhno and Taikov, 1986; Yurakhno, 1998). However, we concluded that these four additional species identified in NFSs were dubious records (see Hernández-Orts et al., 2015).

We also proposed that D. violettae may represents a junior synonym of D. tetrapterus, because the metrical data reported by Yurakhno (1986) overlap with the data obtained in the present study: body length (20–150; holotype of D. violettae 62 vs. D. tetrapterus 30 cm), scolex (1.21–1.88 × 1.18–1.57; 2.35 × 2.60 vs. 1.2 × 0.54 cm) and eggs (66–86 × 41–52; 64–80 × 42–58 vs. 43–46 × 33–40). Additionally, metrical data of D. violettae overlap with the data reported for D. tetrapterus by Margolis (1956) and Rausch (1964). These morphological characters are not useful to differentiate species of diphyllobothriid tapeworms as was shown recently for A. pacificus by Hernández-Orts et al. (2015). However, further studies with a combination of classical morphological and molecular methods are needed to clarify this issue.

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Conflict of interest

The authors declared that there is no conflict of interest.

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