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Host characteristics and infection level of an intestinal parasite *Corynosoma strumosum* (Acanthocephala) in the Kuril harbor seal of Erimo Cape, Hokkaido, Japan

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ABSTRACT

The Kuril harbor seal around Hokkaido is presently recovering from a resource crisis while conflicts with local fisheries have become a concern. However, its feeding habits, which are fundamental information for taking proper preventive measures, are still poorly understood. We thus examined the infection status of a trophically-transmitted parasite, *Corynosoma strumosum* in the seals of Erimo Cape, to assess the host's feeding habits with a practical view of the parasite as a biological indicator. A total of 2802 worms were found from 20 male and 20 female by-caught animals in salmon set nets within local fisheries during August to November 2014. The parasite abundance was explained mainly by the host's developmental stage and intestinal length while weakly affected by gender and body size, through an estimation of generalized linear models combined with hierarchical partitioning. Considering the past records that demersal fishes are the probable main sources of infection, the infection level may owe to individual host differences regarding these sources and/or feeding grounds with relating the host characteristics. This supports that the resource management of Kuril harbor seals requires careful consideration of the individual differences in feeding behavior.

1. Introduction

The Kuril harbor seal *Phoca vitulina stejnegeri* Allen, 1902 is a subspecies of *P. vitulina* Linnaeus, 1758, with a distribution from the tip of the Alaskan Peninsula, through the Aleutian Islands, Kamchatka, Kuril Islands and down to the Erimo Cape in south-eastern Hokkaido, Japan [1–3]. This seal is known to exhibit sedentary habitation at a preferred landing place (i.e. haulout site) [4], where individuals use it for resting, reproduction and molting [5]. On the shores of Hokkaido, 8 of 11 haulout sites have so far been recognized as breeding sites (two haulout sites were already collapsed) [6–7], and population censuses have been conducted every year since the 1970's [8] (see also [7]). According to an outdated record by Inukai [9], a total of 1500–4800 individuals were thought to have been originally distributed in Hokkaido [8], but they had declined to less than a few hundred in the early 1970's due to anthropogenic disturbances (e.g. overhunting) [8,10]. Thereafter, population size in Hokkaido has gradually been increasing over the last four decades [7], in large part due to protection under the Revised Birds and Mammals Protection Law of 2002 (Ministry of the Environment, Government of Japan; http://law.e-gov.go.jp/htmldata/H14/H14F18001000028.html).

With a recovering population size, however, conflicts between the seals and local fisheries have become a serious issue [12]; indeed, economic losses have been calculated for salmon set net fishing (e.g. [13–16]). A triune consensus among scientists, administrators and local people was thus required for the proper management (see [11,17–19]), but it has not reached a conclusion as of yet because insufficient information is currently available for ecological features of this seal under wild conditions. Most of the information, concerning these animals such as predator-prey interactions and behavior around the haulout sites, are showing little progress despite a surge in the conservation awareness from the late 1980's (see [6]). To construct a better compromise between seal conservation and local economic activities, further investigation, especially on the feeding habits of these seals are needed.

Parasites are sometimes used as tags or indicators to provide biological information on their hosts [20–21]. In particular, endoparasite species, which have trophically-transmitted life cycles, infecting via

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predator-prey relationships, can provide useful insights into the feeding habits of the targeted hosts in a given food web [20,22–23]. Under the circumstances in which quantitative data of a host's feeding habits is hard to be obtained due to difficulty of field sampling, the parasitological approach has the potential to support stomach content surveys and stable isotope analysis as an alternative method.

Corynosoma strumosum (Rudolphi, 1802) (Acanthocephala: Polymorphidae) is an intestinal parasite commonly found in pinnipeds in the northern hemisphere [24–27]. This species has also been reported in the seals off the coast of Japan (see [28–29]), including the Kuril harbor seal [30–31]. Its life cycle is known as a complex type, using marine amphipods as its first intermediate host (e.g. [32]) and coastal fishes as paratenic hosts (e.g. [33–34]); seals are definitive hosts for this parasite, with infection via feeding on the intermediate host organisms.

We recently investigated infection status of *C. strumosum* in the Kuril harbor seals by-caught from Erimo Cape of Hokkaido, Japan, while considering the practical aspects of parasites as biological indicators. This paper describes the details of its host utilization and relationships between the parasite infection and host's characteristics. Based on these findings, feeding habits of this seals are discussed.

2. Material and methods

2.1. Study site and host collection

Erimo Cape (41°55′28″N, 143°14′57″E) is a locality corresponding to the southwestern limit of the distribution in the Kuril harbor seal [6]. This is the largest haulout site in Hokkaido with a population size up to 500 individuals [7]. The details of this site are available elsewhere (e.g. supplemental article in Kobayashi et al. 2014); the seal population at this site is about 200 km away from other haulout sites in the eastern Hokkaido, and due to this geographic distance, it is isolated from other sites [35–38].

In this area, many seals are accidentally caught with salmon set nets during the commercial fishing season [15]. The individuals examined in our survey (i.e. 20 males and 20 females) were randomly obtained as a subset of individuals from these by-caught ones during 19 August 2014 to 17 November 2014. All dead-seals were immediately measured for total length (TL), weight (W) and thickness of dorsal blubber (B), sampled their whole skulls for age determination and dissected to remove the digestive tract for the parasite examination. The digestive tracts were further separated into stomach and intestine and preserved in a freezer. The frozen intestines were brought to laboratories in Abashiri city (i.e. Tokyo University of Agriculture) or Sapporo city (i.e. Hokkaido University) where they were examined for parasites at a later date.

2.2. Age determination

For age determination, upper right canine teeth were taken from each skull, sectioned 10 μm by cryostat (Leica co. Ltd) and stained by Delafield's hematoxylin, following the fundamental methods in the textbook [39–40]. Ages were estimated based on a count of cemntum annuli [41]. The assumed mean birth date was set in May [42]. Ages were calculated to add the 0, 0.5 or 1 year old to accommodate differences in the timing of collections. After age determination, we classified their growth stage (i.e. yearlings were 0 and 0.5 year olds, sub-adults were from 1 to 2 year olds and adults were 5 or older in males and 4 or older in females [43]) by estimated age.

2.3. Parasite sampling

After thawing the frozen intestines, their length (IL) was measured in meters (m). Each sample of the intestine was subdivided equally into 10 sections, and numbered from stomach side to anal side. Each section was opened in turn using forceps, and parasite specimens were collected using tweezers under visual check. To prevent overlooking any parasite specimens, each section was subsequently washed with tap water and the intestinal contents were placed in plastic bottles; after the sediment settled at the bottom, it was extracted and put into a petri dish, and checked under a stereomicroscope. For confirmation of the species identification, some specimens were randomly collected, fixed with 70% ethanol under slight pressure and kept as flattened specimens with made transparent by 70% glycerin-ethanol solution or stained with alum carmine. The stained samples were subsequently dehydrated in a series of ascending concentrations of ethanol, cleared in xylene and mounted in Canada balsam. The remaining specimens were preserved in a bottle filled with 70% ethanol, and separated according to intestinal section.

Identification was based on the morphological character of proboscis shape, number of hook rows and hooks per row on the proboscis, hook size and covering range of the trunk spine, following the previous descriptions [44–48], by using an Olympus BX51 light microscope with phase contrast optics. Drawings of specimens were made with the aid of a drawing tube. Measurements were taken using an ocular micrometer; all measurement values were provided as the mean followed by the range in parenthesis.

2.4. Statistical analyses

Infection indices, i.e. prevalence (percentage of infected host individuals), abundance (number of individual parasites from examined host), intensity (number of individual parasites from infected host) and abundance (number of individual parasites from examined host), were used in accordance with the definition of Bush et al. [49].

To elucidate the relationship between parasite infection and host characteristics, hierarchical partitioning (HP) and generalized linear models (GLMs) were applied to the abundance data. In these analyses, eight explanatory variables were initially assumed as candidates; those were gender (male or female), developmental stage (yearling, sub-adult and adult), age (year), TL (cm), W (kg), IL (m), B (cm) and Fulton's condition factor (K) calculated as $W/TL^3 \times 10^6$ (see [50]). Negative binomial error distribution with log link function was employed for the model fitting, by following the known law that macroparasites generally represent aggregated distribution in a given host population (e.g. [51]).

HP was firstly performed to check independent and conjoint importance of explanatory variables in explaining variance of the response variable (i.e. the parasite abundance) [52–54]. This analysis jointly uses all possible models, represented as combinations of explanatory variables, in GLMs, and averages the improvement in fit for each variable, both independently and jointly, across all these models [52–55]. Independent and conjoint contribution of each variable is provided as percentage explaining variation in the response variable; conjoint values can be negative when the effect of the variables is suppressed by the presence of other variables [52–53]. A shape parameter (i.e. theta) of the negative binomial distribution, obtained from the full model fitting the parasite abundance, was tentatively postulated in our estimation. Significance of the independent effect was estimated as *Z*-score, with upper 95% confidence limit > 1.65, using 1000 times of randomization procedure [56].

Since HP approach just provides the contribution of explanatory variables to the response variables, detail relationships between the effective variables and the parasite abundance were supplied by GLMs fitting; the accuracy of the independent contribution in HP was additionally confirmed by a model selection in GLMs. Due to a possibility of multi-collinearity between these candidate variables, we checked their correlations and variance inflation factor (VIF) prior to applying GLMs. The candidate models were ranked by Akaike's information criterion (AIC; [57]). The difference in AIC value (ΔAIC) between a constructed model and an optimal model with the lowest AIC value was