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Ecotoxicoparasitology of the gastrointestinal tracts of pinnipeds: the effect of parasites on the potential bioavailability of total mercury (THg)

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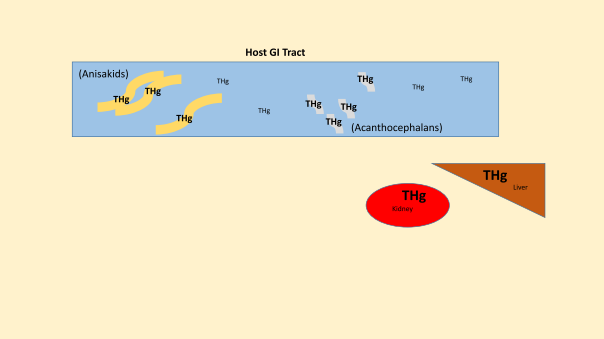
HIGHLIGHTS

- [THg] in the GI tract and parasites was determined for seals and sea lions.
- The goal was to determine the toxicant-parasite relationships within the GI tract.
- [THg] and stable isotopes provide insight on host-parasite-Hg interactions.
- [THg] varies within the GI tract and may be influenced by the presence of parasites.
- [THg] are highest in acanthocephalans compared to other parasitic groups.

GRAPHICAL ABSTRACT



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ABSTRACT

Acanthocephalans, cestodes, and some species of nematodes acquire nutrients from the lumen contents in the gastrointestinal (GI) tract of their definitive host. These parasites are exposed to toxicants, such as mercury (Hg), through passive or active feeding mechanisms; therefore, the focus of this study was to determine if there is an effect of parasites on the dietary availability of total mercury (THg) within piscivorous pinniped hosts. THg concentrations ([THg]) in selected host tissues, parasites, and GI lumen contents from 22 California sea lions (*Zalophus californianus*), 15 ringed seals (*Phoca hispida*), and 4 spotted seals (*Phoca largha*) were determined. Among all pinnipeds, [THg] in acanthocephalans of the large intestine were significantly higher than concentrations in other samples (host lumen contents, other parasites and host intestinal wall), irrespective of location within the host GI tract. $\delta^{15}N$ values of parasites depended both on parasite group and location within the GI tract. $\delta^{15}N$ values were consistently higher in parasites inhabiting the large intestine, compared to elsewhere in the GI tract, for both sea lions and seals. $\delta^{13}C$ values in parasites did not differ significantly from host GI tissues. Based on both [THg] and stable isotope values, parasites are likely affecting the Hg bioavailability within the GI lumen contents and host tissues, and toxicant-parasite interactions appear to depend on both parasitic taxon as well as their location within the host intestine.

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1. Introduction

Few investigations have explored the ecotoxicoparasitology of marine systems, the study of the relationships among host organisms, their parasite populations, and the toxicants to which both are exposed (McGrew et al. 2015). Effective approaches exist for studying these relationships, and well established tools such as stable isotope analysis and the measurement of toxicant distributions in host-parasite systems, can contribute to a better understanding of feeding ecology, food web structure, and contaminant transfer (Campbell et al. 2005; Finger et al. 2017; McGrew et al. 2015; McHuron et al. 2016).

Mercury (Hg) is a ubiquitous, non-essential element. Natural sources (e.g. volcanic activity, forest fires, natural weathering of rocks), as well as anthropogenic sources, contribute Hg to the environment and associated biota. Mercury is subject to long-range transport by winds and ocean currents, and its occurrence in marine food webs has been, in part, attributed to anthropogenic sources from distant regions (Muir et al. 1999). Consumer exposure to Hg in marine systems is of concern because of the widespread nature of this contaminant, its ability to biomagnify in food webs, and the potential for long range atmospheric transport (Alava et al. 2017; AMAP 2011). Hg has a wide spectrum of effects on health, depending upon the chemical forms and modes of exposure (Hansen and Gilman 2005; Satoh 2003), and increased exposure can have important implications on human and wildlife populations (Van Hoomissen et al., 2017; Rea et al. 2013; Sonne et al., 2009). Monomethylmercury ($MeHg^{+}$), produced by microbial methylation of inorganic Hg, becomes integrated into the food web at lower trophic levels, and is then subject to biomagnification in higher trophic-level organisms such as fish (Kruzikova et al., 2008) and marine mammals (Dehn et al. 2006; McHuron et al. 2014). As top predators in marine food webs, piscivorous marine mammals generally have elevated total mercury concentrations ([THg]) in their tissues (Braune et al. 2005; McHuron et al. 2014; Moses et al., 2009).

It is well established that parasites play an important role in food webs (Lafferty et al. 2008), but host-parasite interactions, in the context of contaminants, have received far less attention. Often, parasites are transmitted trophically to their host through their food source. Many helminths, including nematodes, cestodes, and acanthocephalans, that live in the gastrointestinal (GI) tracts of their definitive hosts, feed on GI lumen contents through passive or active mechanisms as nutrients from the host lumen contents are ingested or taken up across parasite's tegument. These organisms are also exposed to toxicants (i.e. Total Hg (THg)) present in lumen contents. Intestinal helminths occupy distinct niches within the host GI tract, and are known to compete for host nutrients in instances of co-infection (Holmes 1961). They can also alter resource availability and trophic niche of their hosts (Britton and Andreou 2016); conversely, host diet can alter or influence the specific composition of parasite communities (Friesen and Roth, 2016).

Some toxicants have been shown to bioaccumulate in parasites at concentrations orders of magnitude higher than host tissues (Sures et al. 1999). The accumulation of non-essential elements in the parasites of marine organisms has been studied (Courtney-Hogue 2016; Monteiro et al. 2016; Van Hees and Ebert, 2017). Separately, associations between [THg] and stable N isotopes in marine mammals have been explored (Brookens et al. 2008; Friesen and Roth 2016), but the relationships among toxicants, stable isotopes and marine mammal parasites have not been investigated to date.

The purpose of this study was to compare [THg] in multiple matrices (i.e. GI tissues, lumen contents, parasites), and to determine the toxicant-parasite relationships within the GI tracts of pinnipeds from two locations: California, USA (California sea lions (*Zalophus californianus*)), and Alaska, USA (ringed seals (*Phoca hispida)* and spotted seals (*Phoca largha*)). While the intent was not to make direct comparisons between these pinniped species, the opportunity to explore ecotoxicoparasitological relationships and Hg exposure in two distinct geographical regions, and among three host species, provided insight into these host-parasite-toxicant systems. In order to better define the ecological relationships between Hg exposure and uptake by parasites and their hosts, we compared [THg] and stable isotope values (C and N) in parasites, GI tissues, and GI lumen contents, based on parasite group, and their location within the host GI tract. Liver and kidney [THg] were used to provide relative estimates of overall host exposure to Hg. Bioaccumulation factors were used to demonstrate whether or not parasites were bioaccumulating THg. We hypothesized that parasites are capable of THg uptake in pinniped hosts, and that [THg] varies based on location within the host GI tract due to complex host-parasite interactions. Interactions between host exposure to THg, host diet, GI parasite community composition, and interspecies competition are factors likely contributing to [THg] within the pinniped host. THg bioaccumulation by parasites may potentially be of benefit to infected host populations with a subsequent decreased bioavailability of THg to the parasitized pinniped.

2. Materials and methods

2.1. Sample collection

Twenty two California sea lions that stranded along the central coast of California, were transported to The Marine Mammal Center (TMMC) (Sausalito, CA) in June 2010. Individuals either stranded dead, died in transport, or were alive upon stranding and underwent rehabilitative efforts at TMMC for up to 15 days, prior to death. They were then necropsied and samples were collected within 48 h of death. This work was conducted under the authorization of NOAA Fisheries. Ice seals were harvested by subsistence hunters in the fall of 2009 (10 ringed seals and 2 spotted seals) and 2010 (5 ringed seals and 2 spotted seals) in Kotzebue Sound, Alaska (USA). Seals were sampled with support provided by the Native Village of Kotzebue under permit No. 932-1905-00IMA-009526 issued by the National Marine Fisheries Service (NMFS) and the U.S. Fish and Wildlife Service (USFWS) under the authority of the Marine Mammal Protection Act (MMPA) and Endangered Species Act (ESA).

Sex was determined for each animal, and liver, kidney, and GI tissue samples were collected. During GI processing, stomach, large intestine (LI) and small intestine (SI) were opened longitudinally, using stainless steel instruments, and all macroparasites were manually removed with forceps from the GI tract, sorted by helminth type, and weighed. Nematodes and acanthocephalans were enumerated. Mean intensity and prevalence were determined as defined by Bush et al. (1997). Parasites were frozen at −20 °C for future [THg] determination, and C and N stable isotope analysis. Lumen contents and GI tissue sections (2 × 2 cm, full-thickness) were collected from stomach, proximal and distal small intestine (dSI), and colon. Representative acanthocephalans and nematodes were fixed in 10% buffered formalin, and later identified based on morphological criteria (Anderson et al. 1974; Rausch et al. 2010; Van Cleave 1953).

2.2. Total mercury (THg) analysis

Host samples were thawed at room temperature. Tissues were subsampled (70–150 mg) using stainless steel forceps and scissors. Instruments were washed with ultrapure water and dried between each sample. [THg] is reported on a wet weight (ww) basis as ng/g, but also converted to dry weight (dw) to ensure that observed differences were not driven by variations in moisture content. Parasite [THg] were measured on a dw basis and converted to ww concentrations, as freeze drying the parasites allowed them to be fully homogenized with a mortar and pestle prior to THg and stable isotope analyses. Weights were obtained before and after the freeze-drying process in order to calculate percent moisture. Samples were analyzed on a Milestone DMA-80 instrument (Butala et al. 2006; EPA 600-R-04-012) following procedures