Mercury in parasitic nematodes and trematodes and their double-crested cormorant hosts: Bioaccumulation in the face of sequestration by nematodes

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A B S T R A C T

Endoparasites can alter their host’s heavy metal concentrations by sequestering metals in their own tissues. Contracaecum spp. (a nematode), but not Drepanecephalus spathans (a trematode), were bioaccumulating mercury to concentrations 1.5 times above cormorant hosts. Nematodes did not have significantly greater stable nitrogen isotope values (δ15N) than their hosts, which is contradictory to prey–predator trophic enrichment studies, but is in agreement with other endoparasite–host relationships. However, Contracaecum spp. δ13C values were significantly greater than their hosts, which suggest that nematodes were consuming host tissues. Nematodes were accumulating and thus sequestering some of their cormorant hosts’ burden of methyl mercury; however, they were not dramatically reducing their hosts’ accumulation of methyl mercury.

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1. Introduction

It is now evident that there can be relationships between pollution and parasitism. Originally, investigations looked at the effects of pollution on the distribution and abundance of parasites in a particular host or ecosystem (Lafferty, 1997; Vidal-Martinez et al., 2010). However, this avenue of research resulted in very few generalizable patterns between parasites and pollution; in fact, many studies demonstrate positive associations between pollution and parasitism, whereas others show no relationship or inverse relationships (Lafferty, 1997; Vidal-Martinez et al., 2010). Notwithstanding, researchers continue to try and understand interactions between pollution and parasites as such relationships might be important for understanding constraints on biodiversity or wildlife health for particular hosts. One area of evolving research is directed at determining the ability of parasites to alter heavy metal levels in their hosts, either by changing the hosts’ physiology and its ability to accumulate heavy metals (e.g., Evans et al., 2001; Bergey et al., 2002) or by accumulating and therefore sequestering heavy metals in their own tissues (Sures et al., 1999; Sures, 2001, 2003).

Several examples warrant mention. Molluscs (Littorina littorea) parasitized by trematodes (Evans et al., 2001), grass shrimp (Palaemonetes pugio) parasitized by isopods (P. proboscis pandalicola) and killifish (Fundulus heteroclitus) parasitized by nematodes (Eustrongylides sp.; Bergey et al., 2002) all accumulated lower concentrations of heavy metals than their unparasitized counterparts. It has been suggested that behavioural and physiological changes, resulting from parasitism, could alter a host’s feeding and metabolism which would affect the accumulation of heavy metals (Bergey et al., 2002). However, it has also been recognized that the parasites causing these changes in their host are themselves potentially accumulating the heavy metals and therefore reducing the hosts’ exposure. Adult intestinal helminths, particularly acanthocephalans and cestodes, accumulate heavy metals to orders of magnitude above their fish hosts (Sures and Siddall, 1999), mammalian hosts (Sures et al., 1998; Sures et al., 2003) and avian hosts (Barus et al., 2000a,b; Barus et al., 2001). This ability of parasites to accumulate heavy metals thereby reduces the accumulation in fish host tissues (Sures and Siddall, 1999; Sures et al., 2003).

Whether or not nematodes biomagnify heavy metals, such as methyl mercury, is not well studied (Bergey et al., 2002). Mercury is a natural metal in the environment that has no known biological function, but is highly toxic to wildlife, especially in its methylated form (Weiner et al., 2003). Methyl mercury biomagnifies with trophic level; therefore, high concentrations are found in top consumers (Weiner et al., 2003). As consumers, nematodes possess a complete digestive system and can actively feed on host tissues whereas trematodes, acanthocephalans and cestodes absorb host digested nutrients across their tegument (Roberts and Janovy, 2005). Therefore, nematodes should occupy higher trophic positions than their hosts and accumulate greater concentrations of biomagnifying heavy metals.

Such trophic relationships and their importance to heavy metal dynamics can be studied by various means, including the use of stable carbon and nitrogen isotopes. Trophic relationships using stable
carbon and nitrogen isotopes are measured using the ratio of heavy to light isotopes (\(^{13}\text{C}/^{12}\text{C}\) and \(^{15}\text{N}/^{14}\text{N}\), respectively) because with each interaction between consumer and its diet there is an increase in the ratio (Kelly, 1999). Typically, \(^{13}\text{C}\) values increase between 0 and 1‰ with each trophic transfer and \(^{15}\text{N}\) values increase by ~3.4‰ (Kelly, 1999 and references therein). Furthermore, a positive relationship occurs between methyl mercury concentrations and \(^{15}\text{N}\) values; therefore, top consumers have high \(^{13}\text{C}\) values and high mercury concentrations (Cabana and Rasmussen, 1994).

In a previous study, we found that male cormorants with high nematode abundance had lower concentrations of mercury than male cormorants with low nematode abundance (Robinson et al., 2009). Therefore, we were interested in determining if mercury concentration in cormorants is reduced by the accumulation of mercury in nematodes. To this end, here we investigate the bioaccumulation of mercury in a bird host, the double-crested cormorant (Phalacrocorax auritus) and its nematode parasite, Contracaecum spp. We determine the likelihood of the nematodes reducing the mercury concentration in the male cormorants responsible for the male bias in parasitism by calculating the percentage of the host body burden of mercury sequestered by nematodes in these male cormorants and comparing it to the difference in mercury concentration between these male cormorants and the male cormorants responsible for the male bias in mercury concentration. We also determine Contracaecum spp. feeding ecology and compare that to the feeding ecology of the cormorant host, using stable carbon and nitrogen isotopes (\(^{13}\text{C}\) and \(^{15}\text{N}\)). Finally, we compare mercury accumulation and feeding ecology between Contracaecum spp. and Drepanecephalus spathans (a trematode) which represent two different parasite feeding strategies (i.e., ingestion vs. absorption). We compare stable carbon and nitrogen isotope values and mercury concentrations between host and parasites to address if nematodes are more likely to be accumulating mercury from the host (greater \(^{13}\text{C}\) and \(^{15}\text{N}\) values in nematode than host) or from the prey fish the cormorant consumed (similar \(^{13}\text{C}\) and \(^{15}\text{N}\) values between nematode and host).

2. Methods

2.1. Sample collection and preparation

We collected 30 double-crested cormorants (15 males and 15 females) from Presqu’ile Provincial Park (43° 59′ N, 77° 42′ W) on Lake Ontario during May 2006. These birds were from a larger study, where details regarding sample collection, preparation, parasite enumeration and identification are described (Robinson et al., 2008). The birds were selected from the larger study based on their high abundance of Contracaecum spp. Parasites were stored in chemically-clean glass jars at ~40 °C until processed for total mercury and carbon and nitrogen (\(^{13}\text{C}\) and \(^{15}\text{N}\)) isotope analysis. Samples were thawed and from each of the 30 cormorants 5 to 25 adult male Contracaecum spp. (body length > 13 mm and presence of spicules) and adult female Contracaecum spp. (body length > 25 mm, spicules absent and eggs in uterus) were selected (number of Contracaecum spp. from each bird depended on the number of nematodes available per sample).

Ten cormorants (7 of which were represented in the samples described above used to obtain nematodes) were selected for additional study. These birds were selected for their high abundance of D. spathans (range from 10 to 131 D. spathans). This ‘select sample’ enabled comparisons between host and parasite species for mercury and carbon and nitrogen isotopes. Each sample of parasite tissue was stored in an acid-washed polypropylene vial, homogenized and wet mass recorded. Parasite tissue samples were freeze-dried for a minimum of 48 h, dry mass was recorded and % moisture content was calculated (88.7% moisture for nematodes and 78.3% for trematodes).

In a previous study, samples of breast muscle from cormorants were analysed for total mercury concentration and \(^{13}\text{C}\) and \(^{15}\text{N}\) values, details of sample preparation, lipid normalization of \(^{13}\text{C}\) values and analysis are described elsewhere (Robinson et al., 2009). Mercury in avian breast muscle tissue is predominately methyl mercury; therefore measuring total mercury well represents the biomagnification of the toxic form of mercury (e.g., Houserova et al., 2007).

2.2. Mercury analyses

Freeze-dried parasite tissue was crushed into a powder and total mercury was measured using a Milestone DMA-80 direct mercury analyzer (Shelton, Connecticut, USA). We also measured methyl mercury in 10 samples of nematode tissue (6 female nematode samples and 4 male nematode samples) which were selected based on the amount of tissue in the sample. We measured methyl mercury in nematodes to determine the percentage of total mercury that was bioaccumulating and was the toxic form; this information is not available in the literature. We extracted methyl mercury from nematode tissue using a modification of the method of Callum et al. (1981) as described in Scheuhammer et al. (1998) and measured the concentration of methyl mercury using the Milestone DMA-80.

Quality control was assured by processing and analysing procedural blanks, certified reference material (dogfish liver certified reference material for trace metals [DOLT-3], Metals Toxicology Laboratory, National Wildlife Research Centre, Ottawa, ON, Canada) and sample replicates. The average accuracy was within 10% of certified values for all analyses and analytical precision (percent relative standard deviation (%RSD) of replicate samples) averaged <10% for all analyses. All values were expressed as a dry mass concentration in pg/g (unless stated otherwise as wet mass) and mean values were used for sample replicates. We calculated wet mass total mercury concentration by multiplying (1 − % moisture of tissue/100) by the dry mass total mercury concentration of the tissue.

2.3. Stable isotope analyses

We calculated \(^{13}\text{C}\) and \(^{15}\text{N}\) values to investigate the trophic position of Contracaecum spp. and D. spathans compared to their cormorant host and relationships between carbon and nitrogen signatures and total mercury concentration. Half a milligram (±0.2 mg) of parasite tissue powder was encapsulated in tin. Isotope analysis was accomplished using a Vario EL III (Elementar, Hanau, Germany) followed by gas chromatograph separation and on-line analysis using a DeltaPlus Advantage isotope ratio mass spectrometer (Thermo Scientific) coupled with a Conflo III (G.G. Hatch Isotope Laboratories, University of Ottawa, Ottawa, ON, Canada). Data quality was assured using international standards for calibration (IAE-CH-6, IAEA-NBS22, IAEA-N1, IAEA-N2, USGS-40, and USGS-41) and further quality control was maintained through sample duplicates. Values are reported in delta notation in parts per thousand (‰, per mil) relative to an atmospheric air standard. Mean values are reported for samples analysed in duplicate. Analytical precision, based on repeat measures of a standard (C-55), was ±0.2‰.

2.4. Data analyses

We used non-parametric tests for all analyses to ensure all comparisons between sexes and host and parasite were consistent in testing the same null hypothesis; thereby allowing the results to be easily compared. We used Wilcoxon tests to determine differences in median values (i.e., abundance of Contracaecum spp., total mercury concentration, \(^{13}\text{C}\) and \(^{15}\text{N}\) values) between sexes and between host and parasite. We used Spearman’s Rho correlations to determine relationships between parasite and host mercury concentrations and \(^{13}\text{C}\) and \(^{15}\text{N}\) values. For the non-parametric analyses we reported median values with 25% and 75% interquartile ranges. All analyses

We performed a series of calculations to determine whether the nematode burden in heavily parasitized male cormorants could account for their lower methyl mercury burden compared to lightly parasitized male cormorants. We used equations adapted from Lewis et al. (1993) to calculate the percentage of host body burden of mercury sequestered by nematodes in cormorants. First, we calculated male cormorant mercury body burden using Braune and Gaskin’s (1987) estimate that approximately 36% of the mercury body burden in birds was contained in the liver. We relied on liver mass and total mercury concentrations from a Lake Erie cormorant sample to calculate cormorant mercury body burden because we did not have this information for the Lake Ontario cormorants; however, all other values used in the subsequent calculations are from the Lake Ontario cormorant sample. We expect that liver mercury concentrations are similar between the two sampling locations because we found a strong positive correlation between breast muscle and liver total mercury concentrations for male cormorants from the Lake Erie sample ($R_{Pearson} = 0.9$, $P < 0.001$, S.A. Robinson, unpublished data) and the median mercury concentration in breast muscle was similar between Lake Ontario and Lake Erie male cormorants (Lake Ontario: median of 1.7 μg/g (dry mass; interquartile range from 1.3 to 2.8 μg/g); Lake Erie: median of 1.8 μg/g with interquartile range from 1.5 to 2.5 μg/g ($Z = -0.5, P = 0.58$; S.A. Robinson, unpublished data)). Therefore, the liver mercury concentrations are reflective of the breast muscle concentrations and since the breast muscle values are similar between Lake Erie and Lake Ontario we expect that the liver values are also similar.

3. Results

The 15 male cormorants had 2.5 times more Contracaecum spp. compared to the 15 female cormorants ($Z = 3.9, P < 0.001$). Median number of worms per infected male and female host (or median intensity of Contracaecum spp.) was 127 (interquartile range of 91 to 285) and 51 (interquartile range of 36 to 84), respectively. Male cormorants had 1.7 times more mercury than female cormorants ($Z = 3.7, P < 0.001$, Table 1). Subsequent analyses pertaining to mercury concentration were done separately by sex because of the significant difference in these values between male and female cormorants.

Sufficient quantities of tissue (>5 μg) were available to determine total mercury concentrations in 59 of 60 Contracaecum spp. samples. There were significant positive correlations between total mercury concentration in Contracaecum spp. (males and females analysed separately) and their male and female cormorant hosts (Spearman’s Rho ranged from 0.8 to 0.9, all $P < 0.001$, Fig. 1). Furthermore, the median total mercury concentrations for both male and female Contracaecum spp., were significantly different between male and female cormorants (greater if found in male cormorants: $Z = 3.4$ for both, all $P < 0.001$, Table 1). The difference in total mercury concentration in both male and female Contracaecum spp. sampled from male and female cormorants resembled the difference in total mercury concentration in the breast muscle of male and female cormorants (i.e., same ratio). Male and female Contracaecum spp. had up to 1.5 times greater median total mercury concentrations than their male and female cormorant hosts (Table 1). However, the difference in median total mercury concentration between Contracaecum spp. and cormorant hosts was significant only for female cormorants ($Z$ ranged from 2.2 to 2.5, all $P < 0.03$, whereas for male cormorants $Z$ ranged from 1.2 to 1.3, all $P > 0.19$). Importantly, the median percentage of total mercury that was methyl mercury in female nematodes was 91.2% (interquartile range of 84.8 to 105.8%) and for male nematodes it was 96.9% (interquartile range of 88.2 to 106.7%) (Table 1). Therefore, most of the mercury in nematode tissue is the toxic methylated form.

Only 4 of 10 D. spathans samples had sufficient quantities of tissue (>5 μg) available to determine total mercury concentrations. The median total mercury concentration for D. spathans was not significantly different from the median total mercury concentration of their four female cormorant hosts (median for the four female cormorants was 1.3 μg/g (dry mass) with an interquartile range of 0.9 to 1.7 μg/g (dry mass); $Z = 1.0, P = 0.31$, Table 1). Furthermore, two female cormorants had co-infections of D. spathans and Contracaecum spp. where total mercury concentrations were measured and found to be within a similar range for both parasite species (mean ± S.E.) total

![Fig. 1. Positive correlation between male (open symbol) and female (closed symbol) double-crested cormorant total mercury concentration in breast muscle and the total mercury concentration in their male (inverted triangle) and female (circle) Contracaecum spp. (μg/g dry mass).](image)

Table 1 Total mercury (TotHg) concentrations for male and female cormorant breast muscle, male and female nematode tissue and trematode tissue are reported as median values in μg/g (dry mass) with their 25% and 75% interquartile range (IQR). The ratio of methyl mercury (MeHg) to total mercury concentrations are reported as median values in μg/g (dry mass) with their 25% and 75% interquartile range (IQR). Percentages over 100% are a result of analytical uncertainties. Sample size (n) is indicated for each analysis.

<table>
<thead>
<tr>
<th></th>
<th>TotHg (median (IQR))</th>
<th>n</th>
<th>MeHg/TotHg (median (IQR))</th>
<th>n</th>
<th>%MeHg (median (IQR))</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male cormorant</td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Host breast muscle</td>
<td>2.0 (1.8–5.5)</td>
<td>15</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Female nematode</td>
<td>2.8 (2.1–6.0)</td>
<td>14</td>
<td>5.2 (2.8–7.0)/6.0 (3.4–7.5)</td>
<td>3</td>
<td>86.0 (81.4–91.8)</td>
<td>3</td>
</tr>
<tr>
<td>Male nematode</td>
<td>2.7 (2.4–6.7)</td>
<td>15</td>
<td>6.5 (3.3–7.8)/7.4 (3.1–8.9)</td>
<td>3</td>
<td>88.4 (88.2–105.4)</td>
<td>3</td>
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<tr>
<td>Female cormorant</td>
<td></td>
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<tr>
<td>Host breast muscle</td>
<td>1.2 (1.1–1.4)</td>
<td>15</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Female nematode</td>
<td>1.7 (1.3–2.3)</td>
<td>15</td>
<td>2.5 (1.3–2.6)/2.3 (1.4–2.5)</td>
<td>3</td>
<td>105.7 (88.6–106.2)</td>
<td>3</td>
</tr>
<tr>
<td>Male nematode</td>
<td>1.7 (1.4–2.3)</td>
<td>15</td>
<td>(3.3)/(3.1)</td>
<td>1</td>
<td>107.2</td>
<td>1</td>
</tr>
<tr>
<td>Trematode</td>
<td>1.5 (1.3–1.7)</td>
<td>4</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>
Contracaecum was no difference between the median δ13C and δ15N values of female  
and 75% interquartile range (IQR) are reported as per mil (‰) provided for each  
tissue analysed. We determined the mercury sequestered in nematode tissue by multiply-
ing the concentration of total mercury in nematodes (wet mass) by the liver mass (wet mass) 
by the total mass of the nematodes (wet mass) found in the male cormorants  
responsible for the male bias in parasitism, which resulted in mean mercury content in 
nematode tissue of 0.6 μg (wet mass; range from 0.1 to 3.2 μg). Therefore, using our calculated total amount of mercury in 
nematode tissue and cormorant tissue we determined that the percentage of cormorant mercury body burden sequestered by 
nematodes in the male cormorants responsible for the male bias in parasitism was 0.6/672.2 + 0.6) = 0.1% (range from 0.003% to 2.2%). 

Male cormorants also had similar median δ13C values (Z = 0.0, P = 0.40); however, female cormorants had greater median 
δ13N values than male cormorants (Z = −3.4, P < 0.001, Table 2), similar to our previous findings (Robinson et al. 2009). Subsequent 
analyses pertaining to δ13N values were done separately by sex because of the significant difference in these values between male and  
female cormorants. We measured δ13C and δ15N values of female Contracaecum spp. because there was sufficient tissue available for  
isotope analysis for 29 of 30 female Contracaecum spp. samples but not for most male Contracaecum spp. samples. Furthermore, we expected that male and female Contracaecum spp. from the same host would have similar isotopic values because their mercury concentrations were also similar. To confirm this, we measured the δ13C and  
δ15N values of 5 samples of male Contracaecum spp. and found significant positive correlations between male and female Contracaecum  
spp. δ13C and δ15N values from the same five hosts (Spearman’s Rho was 0.9 and 1.0, both P < 0.04, respectively). Furthermore, there  
was no difference between the median δ13C and δ15N values of male and female Contracaecum spp. (δ13C: male: −20.5% (interquartile range  
from −21.6 to −19.0%), female: −21.3% (interquartile range from −22.1 to −20.1%), Z = 0.8, P = 0.40; δ15N: male: 13.9% (interquartile range from  
13.1 to 14.5%), female: 13.8% (interquartile range from 13.2 to 14.5%), Z = 0.0, P = 1.0, Table 2).  

There was a significant positive correlation between female Contracaecum spp. δ13C values and cormorant δ13C values (Spear-
man’s Rho = 0.6, P < 0.001). The female Contracaecum spp. had more positive median δ13C values than their cormorant hosts (Z = 2.5,  
P = 0.01, Table 2). There was no significant correlation between δ15N values of female Contracaecum spp. and the δ15N values of their male or  
female hosts (Spearman’s Rho was 0.08 and 0.3, all P > 0.29, respectively). However, median δ15N values of female Contracaecum  
spp. were significantly lower than those in their male and female hosts (male cormorants: Z = −3.5, P < 0.001, female cormorants: Z =  
−2.9, P = 0.004, Table 2).  

We measured the δ13C and δ15N values of D. spathans in 8 cormorants and found no correlation between δ13C or δ15N values in D. spathans and their cormorant hosts (Spearman’s Rho ranged from 0.6 to 0.7, all P > 0.06). D. spathans appear to have slightly greater  
δ15N values than their male hosts and female hosts and similar δ13C values as their male and female hosts (Table 2). We also report δ13C and  
δ15N values in D. spathans and Contracaecum spp. that were co-


<table>
<thead>
<tr>
<th>Table 2</th>
<th>Stable carbon (δ13C) and nitrogen isotope (δ15N) values for male and female 13 cormorants and their female nematodes and trematodes. Median values with their 25% and 75% interquartile range (IQR) are reported as per mil (‰, dry mass). Sample size (n) is provided for each tissue analysed.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>δ13C values (‰)</td>
</tr>
<tr>
<td>Male cormorant</td>
<td></td>
</tr>
<tr>
<td>Host breast muscle</td>
<td>−21.3 (−20.2 (−20.4))</td>
</tr>
<tr>
<td>Female nematode</td>
<td>−20.1 (−19.8 (−19.8))</td>
</tr>
<tr>
<td>Trematode</td>
<td>−21.6 (−21.2 (−21.2))</td>
</tr>
<tr>
<td>Female cormorant</td>
<td></td>
</tr>
<tr>
<td>Host breast muscle</td>
<td>−21.0 (−20.6 (−20.6))</td>
</tr>
<tr>
<td>Female nematode</td>
<td>−20.4 (−19.6 (−19.6))</td>
</tr>
<tr>
<td>Trematode</td>
<td>−21.2 (−21.3 (−20.7))</td>
</tr>
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</table>

4. Discussion  
We found that Contracaecum spp. bioaccumulate mercury to concentrations above their cormorant hosts. Both male and female  
Contracaecum spp. exceeded the mercury concentration in the muscle of their hosts; however, the difference was not statistically significant in  
male hosts. No other studies have looked at mercury accumulation in nematodes of birds, and there have only been three other studies that have  
analysed mercury concentration in nematodes, where the host was the European eel (Anguilla anguilla; Palikova and Barus, 2003; Eira et al.,  
2009) and the killfish (Bergey et al., 2002). The eel nematode (Anguillicola crassus) is found in the swimbladder and had a lower  
concentration of mercury than the hosts muscle, liver and kidney (Palikova and Barus, 2003; Eira et al., 2009). The killfish nematode is  
found encysted in the abdominal cavity of its fish host and was also found to have lower mercury concentrations than its host muscle tissue  
(Bergey et al., 2002). Other studies on nematode bioaccumulation have looked at non-biomagnifying heavy metals, such as lead and cadmium in  
fishes, birds, and mammals (Sures, 2004; Barus et al., 2007 and references therein). In these studies, nematodes can exceed their host  
tissue concentrations (Barus et al., 2007), but not as often or to the magnitude of cestodes and acanthocephalans (Sures, 2003, 2004).  

Adult trematodes have rarely been studied for their bioaccumulation potential (Sures, 2003); however, they are similar to cestodes and  
acanthocephalans in that they absorb nutrients across their tegument from the hosts’ intestinal lumen because they lack a complete  
intestinal system (Roberts and Janovy, 2005). One of the few studies
that have analysed trematode bioaccumulation found that adult *Fasciola hepatica* had greater lead concentrations than bovine host muscle, kidney and liver (Sures et al., 1998). In comparison, yellow perch (*Perca flavescens*) muscle tissue and *Apophallus brevis* metacercaiae had similar concentrations of methyl mercury (Ryan et al., 2008). In our study, *D. spathans* can bioaccumulate mercury but not to concentrations that are detectably different than host tissues or co-infesting male and female *Contracaecum* spp.

Methylmercury biomagnifies with trophic level (Weiner et al., 2003) and we predicted that *Contracaecum* spp. would have higher mercury concentrations than their host because they could be feeding on host tissues. Methylmercury is lipophilic and therefore can accumulate in lipid rich tissues; however, it will preferentially bind to sulphydryl groups in proteins (Weiner et al., 2003). Parasites are generally depleted in lipids compared to their hosts (Barrett, 1981, Sures, 2004 and references therein); therefore we did not expect lipid content to influence the accumulation of methylmercury in *Contracaecum* spp. We found that *Contracaecum* spp. total mercury concentration was predominately methyl mercury, which means that they were accumulating the most readily available and toxic form of mercury and had the potential to reduce mercury exposure to the cormorant host. However, based on our calculations the percentage of methyl mercury sequestered in nematode tissue (~0.1%) cannot explain the 20% difference in mercury concentration between the male cormorants responsible for the trematode burden of the nematode tissue (~0.1%) cannot explain the 20% difference in mercury concentration between the male cormorants responsible for feeding on host tissues. Furthermore, there is evidence of selective feeding and excretion of nitrogenous compounds (Barrett, 1981). Although the dynamics of nitrogen utilisation and excretion have not been fully determined for parasites there are some biochemical explanations for 15N depletion in some endoparasites (see Power and Klein, 2004). In particular, excretion of nitrogen (i.e., ammonia vs. urea) was found as a significant source of variation in δ15N values, where ammonotelic organisms had lower δ15N values than ureotelic/uricotelic organisms (Vanderklift and Ponsard, 2003). Parasitic helminths excrete 80% of their nitrogenous waste as ammonia (Barrett, 1981); furthermore, there is evidence of selective feeding of 15N depleted amino acids by parasites (Hare et al., 1991). In addition to the physiological and biochemical explanations for 15N depletion in parasites, *Contracaecum* spp. could also be feeding on fish tissue consumed by the cormorants (Anderson, 2000) and not relying on host tissues for nutrients thus further reducing the potential 15N enrichment between host and parasite. However, we would expect δ15C values to be similar between host and parasite if they were both assimilating the same nutrient source (i.e., fish; Power and Klein, 2004). The more positive δ13C values in *Contracaecum* spp. suggest that they are consuming some host tissues. The fishes that cormorants tend to consume during the pre-chick period in which we sampled have δ13C values that range from −24 to −22‰. (C.E. Hebert, unpublished data). Therefore, we would expect cormorant δ13C values to be at the values we found (−1% above fishes) and *Contracaecum* spp. are −1% above the cormorant’s δ13C values which suggest that *Contracaecum* spp. were feeding on host tissues. We do not expect lipid content in cormorant breast muscle to be significantly influencing the difference between host and parasite δ13C values because we have lipid normalized the δ13C values (see methods and Robinson et al., 2009).

Similar 13C enrichment was found in nematodes known to feed on their host rabbit tissues where they had more positive δ13C values than their host’s diet and host tissues (Boag et al., 1998). Furthermore, cestodes known to rely on host digested nutrients had similar δ13C values as their host because both were relying on the same food source (Boag et al., 1998; Persson et al., 2007). Therefore, stable carbon isotopes in *Contracaecum* spp. more consistently follow the conventional enrichment pattern found between prey and predator (DeNiro and Epstein, 1978) than stable nitrogen isotopes.

We cannot explain the slightly greater δ15N values found in *D. spathans* compared to their hosts or co-occurring *Contracaecum* spp. We would expect trematodes like *D. spathans* to have δ15N values similar to their host because both host and trematode are exposed to similar digested food contents or trematodes may be depleted compared to the host for the biochemical reasons mentioned above. However, δ13C values were slightly lower in *D. spathans* compared to *Contracaecum* spp. (Table 2) which suggest that *D. spathans* were relying on host digested nutrients more so than *Contracaecum* spp. Our ability to interpret these results is limited because of small samples sizes for *D. spathans*.

Finally, in cormorants, we did not find the predicted positive relationship between mercury concentration and trophic level as inferred from δ15N values (Cabana and Rasmussen, 1994). The only significant relationship was negative, where greater mercury concentration related to lower δ15N values in female cormorants. In our previous study using the larger sample we found a similar negative relationship but only when the data were analysed with the male and female cormorant data sets combined (Robinson et al., 2009). A negative correlation between δ15N values and mercury concentration was found for several species of toothed whales from Japan (Endo et al., 2010). They indicated that the negative correlation was likely a result of the geographical variation in mercury and δ15N values in the foraging areas of the toothed whales (Endo et al., 2010). We do not have an explanation for the negative correlation in female cormorants, but have previously suggested that it might be a result of sex differences in consumption of round gobies (*Apollania melanostoma*) and alewife (*Alosa pseudoharengus*; see Robinson et al., 2009).

5. Conclusion

In summary, *Contracaecum* spp. bioaccumulate mercury and therefore could be reducing the hosts’ exposure to some dietary methyl mercury. However, the magnitude of mercury bioaccumulation by *Contracaecum* spp. is much less than what acanthocephalans were found to bioaccumulate when they reduced lead concentrations in their fish host (Sures and Siddall, 1999). Furthermore, we found that *Contracaecum* spp. likely occupy a higher trophic position than their hosts based on δ13C values but not δ15N values, which is in agreement with other host-parasite studies where nematodes are not enriched in 15N compared to their host (e.g., Pinnegar et al., 2001). Of course, the possibility remains that male and female parasites, and indeed different parasite species, assimilate and excrete nitrogenous compounds at different rates and this accounts for some of the differences in isotopic signature.

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