BIOLOGICAL INTERACTIONS BETWEEN HOSTS, PARASITES, AND MERCURY

By

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Abstract

Coastal communities, including regions along the North Carolina coast, have seen impacts of sea level rise. These impacts include wetland salinization, loss of nursery habitat, and decreased protection from major storm events. Wetlands provide ecosystem services for our environment, including filtration of runoff from land pollutants. One of those major pollutants which affects North Carolina biota is the heavy metal, mercury.

Mercury pollution in wetlands is mainly due to anthropogenic sources, including the burning of fossil fuels, and also natural sources, such as volcanic activity. Inorganic mercury is released into the atmosphere, where it eventually settles to the ground. Mercury can also be taken up by plants and stored in leaves which eventually fall to the ground. These processes of mercury deposition can ultimately make their way to wetlands through surface water runoff. Inorganic mercury, which is deposited in wetlands, can be converted through sulfate reducing bacteria within the sediment to a bioavailable form known as methyl-mercury. Methyl-mercury (MeHg) production may also depend on salinity concentrations in wetlands; therefore, sea level rise could affect mercury levels in wetlands, possibly leading to increases in MeHg in some locations. In

general, mercury is a major health concern for North Carolina residents, especially those living near the coast. In particular, mercury poses a major health concern to animals, and can bioaccumulate and biomagnify through trophic levels, eventually reaching humans.

Previous work has shown that parasites can take in pollutants, like mercury, from their host tissues and store those pollutants within their own tissues, thus acting like a sponge to pollutants in the host, and in some cases, lowering the levels of pollutants in the host. However, salinity may also have an impact on the amount of MeHg which gets produced within wetland sediments. One of my objectives looked at the mercury levels of uninfected host tissues compared to infected host tissues, and how this might change over a salinity gradient. In order to determine this, parasite life-cycles were taken into consideration and how parasites and mercury might transfer to different hosts.

This study took place in two North Carolina estuaries, the Pamlico and Neuse Rivers. I had five sites along each river, from oligohaline to mesohaline localities. Over a one-year period, I collected three host species (two resident species: naked gobies and white-fingered mud crabs, and one mobile species: blue crabs) and recorded their parasite diversity and abundance. I then preserved host and parasite tissues and analyzed them for total mercury (THg) content. In addition, water samples were collected to measure DOC levels because DOC has also been shown to correlate with THg in biota. Also, sediment samples from each site were measured for THg and MeHg. Finally, I took abiotic measurements (most importantly salinity and temperature) as these measures were predicted to be important physical factors of MeHg concentrated in my study system.

I predicted that hosts which were parasitized would have lower levels of THg in their tissues compared to hosts which were unparasitized, and that number of parasites would positively

increase with salinity. For one type of parasite in the mud crabs, this prediction was upheld, but I did not observe this pattern for the other parasites in my study. In fact, results from THg analysis showed higher mercury levels in hosts which were parasitized compared to unparasitized hosts. This result could suggest that parasites are influencing the health of these hosts making them more prone to infection by parasites (i.e., susceptibility is higher when mercury levels are higher). In addition, I found THg and MeHg levels in sediments were higher at lower salinities than at higher salinities. In naked gobies, THg levels in tissues from the Neuse River were highest at the lowest salinity site. In the Pamlico River, THg in naked goby tissues showed a slight increase with increasing salinity. With increasing salinity, DOC decreased. Finally, for naked gobies in the Neuse River, parasites were found consistently throughout my sites, and in the Pamlico River parasites were most abundant in the second lowest salinity and at the highest salinity site. Altogether, this study provides important information for how parasite communities and THg levels change throughout an estuary; however, more investigation is needed to determine the THg levels of uninfected and infected hosts, and their parasites.

Biological Interactions between Hosts, Parasites, and Mercury

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In Partial Fulfillment

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By

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Biological Interactions between Hosts, Parasites, and Mercury

Master of Science in Biology

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Introduction

A major human-mediated impact on coastal wetlands is heavy metal pollution, such as by mercury. Mercury is one of the leading pollutants of water bodies throughout the United States (EPA 2004). The deposition of atmospheric mercury is one of the major contributing sources, accounting for 40–60% of the total mercury found in the environment (Sorensen et al. 1990; Fitzgerald et al. 1991). Inorganic mercury is a pollutant known for its impacts on public health (Driscoll et al. 2013). It is a dangerous neurotoxin, and eating fish contaminated with mercury may cause brain and kidney damage (ATSDR 1999). Children are especially sensitive to mercury poisoning, and exposure can cause medical issues (e.g., mental retardation, incoordination, physical disabilities) (ATSDR 1999). Yet, North Carolina residents consume a considerable amount of fish that are listed on mercury advisory lists, including: tuna, catfish, and largemouth bass (N.C. Health and Human Services 2018). Women of childbearing age, pregnant women, and nursing mothers are advised to limit their consumption of "shark, swordfish, king mackerel, or tilefish" because these species contain high amounts of mercury (EPA 2004).

Anthropogenic sources, such as burning coal for energy and burning biomass, can release mercury into the atmosphere, eventually leading to ground deposition (Mitchell et al. 2008). In addition, other sources of mercury can come from dust, soils, and waste materials (Deonarine et al. 2015). Particulate mercury can be discharged via anthropogenic sources through dry and wet deposition (Carpi 1997) (Figure 1). The extent and contribution of these sources vary based on factors like location, and land use practices (Deonarine et al. 2015). Mercury is recognized as one of the most ubiquitous pollutants in aquatic ecosystems (EPA 1997), and chronic exposure to its organic form, methyl-mercury (MeHg), can negatively impact enzymes which regulate: cell membrane function, amino acid transport, and cellular migration of growing human brains

(Sager and Matheson 1988). Inorganic mercury can be converted to its organic form within sediments, through the process of methylation to methyl-mercury (Windom et al. 1976) or by gaseous dimethylmercury (Kolb et al. 1973). Within sediments of wetlands, swamps, and marshes, atmospherically deposited inorganic mercury can undergo a conversion to MeHg, allowing the organic and highly toxic form to be taken up and concentrated in animal tissues. Virtually all (>95%) of the mercury present in nature is in the form of MeHg (Bloom 1992), and field measurements have found that >90% mercury in fish muscles was in the MeHg form (Bloom 1992). As in humans, MeHg enters the blood, brain, and organs of fish, but it is primarily stored and accumulated in skeletal muscle (Giblin and Massaro 1973). Fish embryos can be affected by just a small amount of MeHg, with the greatest risk coming from trace amounts of MeHg transferred from the mother (Weiner and Spry 1996).

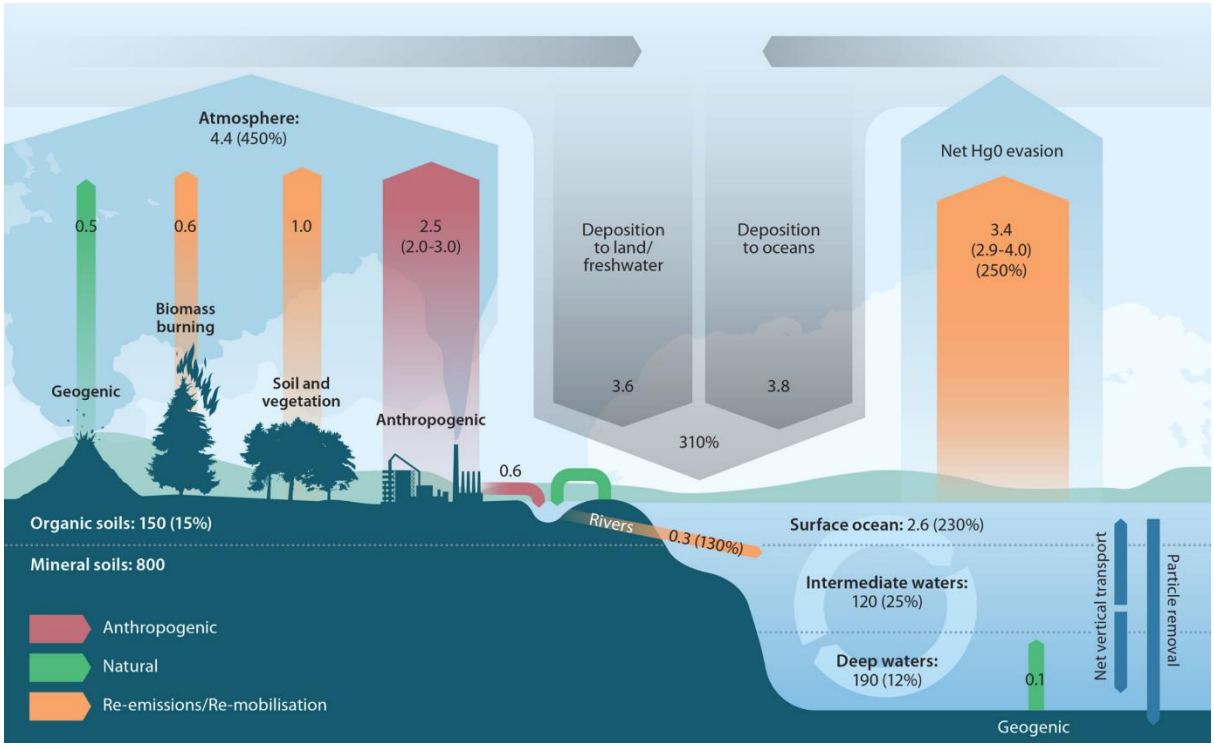

Figure 1: Anthropogenic and natural sources of mercury within the environment. Mass units in kilotonnes (kt) (Outridge et al. 2018).

Environmental conditions play a major role in the conversion of inorganic mercury to MeHg because the conversion is accomplished by anaerobic bacteria, most commonly sulfate-reducing and iron-reducing bacteria (Mallin et al. 2011; Parks et al. 2013) (Figure 2). Research has shown that as water acidity increases, the process of mercury methylation also amplifies (Lange et al. 1993). At a lower pH, sulfur can bind with mercury and this makes it more available to the sulfur-reducing bacteria responsible for methylation (Gilmour et al. 1992). Sulfate is often a limiting source in sediment for mercury methylation in freshwater wetlands (Jeremiason et al. 2006). With saltwater intrusion, sulfate can increase (Herbert et al. 2015).

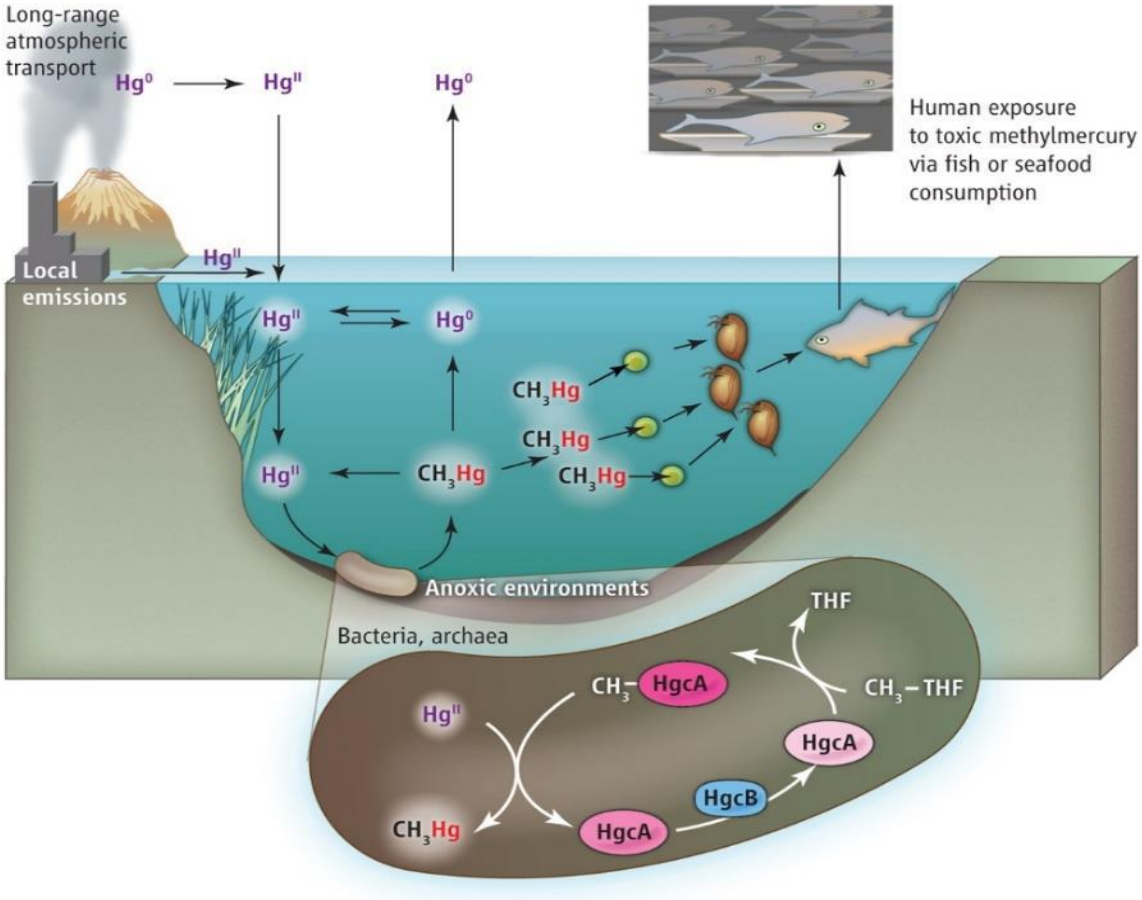

Figure 2: Mercury/methyl mercury cycle within the sediment and conversion by bacteria. (K. Sutliff/Science).

Dissolved organic carbon (DOC) will bind with mercury, making inorganic mercury unavailable to the bacteria which methylate mercury (Jeremiason et al. 2006). DOC has a less negative charge in environments with a low pH, and will not easily bind to inorganic mercury. This leads to the availability of more of mercury to be methylated (Miskimmin et al. 1992; Barkay et al. 1997; Ravichandran 2004). At lower DOC levels, bacteria which methylate mercury may do so more efficiently (Zhao et al. 2017). Additionally, research has shown with a slight increase in salinity, bacteria can eliminate this sulfate limitation (Driscoll et al. 2013). Therefore, increases in salinization can decrease wetland DOC concentrations (Ardon et al. 2016) and impact mercury levels in estuaries and wetlands (Driscoll et al. 2013).

In recent years, climate change has led to enhanced salinization in coastal wetlands, due to sea level rise, storm surge, and other human-induced modifications (Herbert et al. 2015). The North Carolina coast and wetlands have seen, and will continue to see, significant impacts from rising seas; between 1961 and 2009, sea levels rose 1.9 ± 0.4 mm year^{-1}, enhancing salinities of coastal estuaries and wetlands (Church and White 2011). Drought and surface water withdrawals are also major factors leading to enhanced salinization of wetlands (Ardón et al. 2013; Herbert et al. 2015). Paleochannels, permeable ground formations, can serve as potential channels for saltwater to intrude into freshwater aquifers (Barlow and USGS, 2003). In North Carolina, a paleochannel lays under the Neuse River, near Cherry Point Marine Corps station (Cardinell, 1999).

In marine ecosystems, salinity is one of the most vital factors contributing to biotic linkage structuring (Berger and Kharazova, 1997; Ingole and Parulekar, 1998). As described above, salinity increases can also influence MeHg levels in coastal wetlands. For example, in a recent study, MeHg was found to be 6 times higher in wetland soils with an increase of just 1 ppt saltwater (Figure 3) (Ku et al. 2018). This demonstrates that salinity can play a strong role in MeHg conversion in wetland soils, and that this could also influence MeHg levels in wetland biota. Wetland sediments serve as a sink and source for the mercury cycle (Oliveira et al. 2007).

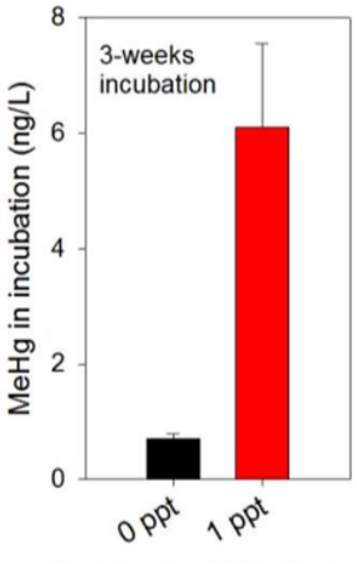

Figure 3: Results of MeHg levels from 3-week soil incubation experiment at 0 and 1 ppt. The soil was collected from coastal plain wetlands in Georgetown, SC. Error bars are SD (n=3). (Tsui et al., unpublished)

In a scenario of changing salinity levels in wetlands and estuaries, the impact of mercury contamination on North Carolina biota could be very detrimental. This is because wetland organisms can serve as effective conduits of MeHg through food webs and can efficiently bio-magnify up trophic levels (Mitchell et al. 2008; Fry and Chumchal 2012; Gilmour et al. 2013). Heterotrophic animals, which consume their food at higher trophic levels, will be put at the highest risk for mercury contamination (Vander Zanden and Rasmussen 1996). Biomagnification is the accumulation of certain non-essential chemicals that are persistent in the environment which are insoluble in water, but highly soluble in fats (Freedman 2014). Biomagnification of mercury in aquatic food chains is mainly in the form of MeHg which is easily taken up by organisms and transferred to higher trophic levels (Watras and Bloom 1992; Waltras et al. 1998). The trophic transfer factor for MeHg is >1, supporting the idea of biomagnification as

exemplified in freshwater zooplankton, and this is largely due to its high assimilation efficiency (Tsui and Wang 2004).

Methyl-mercury biomagnifies across successive trophic levels, resulting in elevated MeHg concentrations in larger/older organisms and top-level consumers (Andres et al. 2002; Wiener et al. 2003; Olivero-Verbel et al. 2008; Reichmuth et al. 2010). Methyl-mercury also comprises a small percentage of the THg in lower trophic level species (Chen et al. 2009), including molluscs, polychaetes, and shrimp. When species at lower tropic levels are consumed, mercury can easily accumulate in species of higher trophic levels, such as carnivorous fishes, and birds (Xu et al., 2013; Xu and Wang, 2015).

In both freshwater and marine systems, MeHg concentrations have the potential to biomagnify, and studies indicate that MeHg may have a greater potential to biomagnify in aquatic systems compared to inorganic mercury (Suedel et al. 1994). Since MeHg and inorganic mercury are biomagnified in food chains, organisms feeding lower within the food web would be expected to have lower mercury concentrations, which is exemplified in Figure 4 (Suedel et al. 1994). Additionally, gross growth efficiency decreases with age (Pauly, 1986), and older

organisms can accumulate MeHg to higher levels than younger more rapidly growing organisms (Pauly 1986).

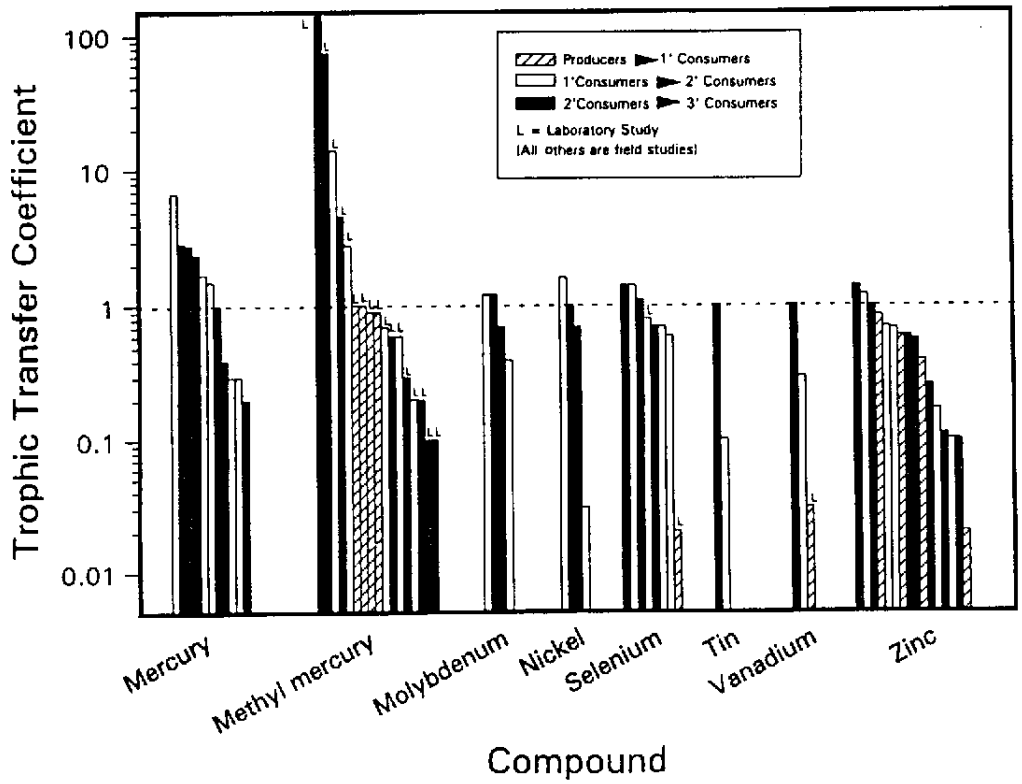

Figure. 4: Trophic transfer coefficients from metals examined in this study by Seudel et al. 1994. (TCC values >1 indicate a potential for bioaccumulation in aquatic ecosystems).

Another biotic component of coastal wetlands that could also be affected by mercury and salinity levels is parasite and disease transmission. Estuarine species are already under pressure to evolve ways to defend against parasites (Morran et al. 2011). Given that enhanced salinization may increase the formation of toxic MeHg in coastal wetlands and thereby increase MeHg uptake in benthic biota and fish, salinity changes may also influence parasite life cycles and disease transmission in these intermediate hosts. This may result in environmental consequences, such as parasites and mercury transferring to higher tropic levels throughout parasite lifecycles. Moreover, past research has shown strong connections between metal

concentrations in host tissues and infection by tropically transmitted parasites like tapeworms and flukes (Figure 5) (Mackenzie 1999; Sures et al. 2017). Helminth parasites which dwell in the intestinal tract of their hosts are in rivalry for nutrients (Coop and Kyriazakis, 1999). As a result, intestinal parasites of fishes, like acanthocephalans (Sures et al. 2005), can reduce metal levels within tissues of their hosts, helping to remove pollutants from the host (Sures and Siddall 1999). Acanthocephalans cannot synthesize their own cholesterol and other essential fatty acids, and they must acquire them from the intestine of their hosts (Sures and Siddall 1999).

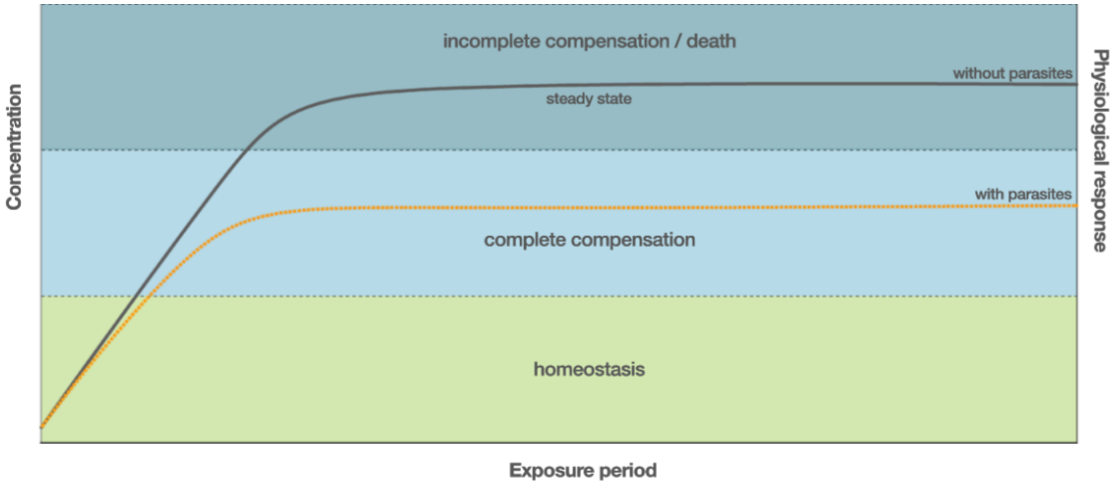

Figure 5: This accumulation model shows the concentration of a toxic substance in tissues of infected vs uninfected hosts. There is a difference in homeostasis with the addition of toxic substances. Hosts with parasites exhibit a lower concentration of the toxic chemical compared to the hosts without parasites. (Sures et al. 2017).

Additionally, past research has shown that heavy metals may affect the immune system, and in some cases, hosts may be more susceptible to infection by parasites, with more polluted sites demonstrating higher prevalence and intensities of infection in hosts (Sures et al. 2017). For example, in the freshwater fish, blue gourami, short-term exposure to 9 ppb (ng/g) of MeHg cause a decrease in the immune response of the fish (Roales and Perlmutter 1977).

As salinity increases, this may also affect parasite transmission among estuarine species, potentially allowing parasites to access hosts that were previously inaccessible to them. For example, a parasitic barnacle *Loxothylacus panopaei*, of native mud crabs along the East Coast cannot tolerate salinities lower than ~10 ppt (Tepolt et al. 2019; Blakeslee et al., 2021), This suggests that water bodies lower than ~10 ppt could provide a refuge from parasitism for the host mud crabs (Reisser and Forward, 1991, Walker and Clare, 1994). Changing salinity levels can allow this parasite to access locations that were formerly inaccessible due to salinity levels that existed below that threshold (Blakeslee et al. 2021). Moreover, past research has found that shellfish, like the eastern oyster, can be negatively impacted by diseases at higher salinities (Ford and Haskin 1982). Although low salinity regions in an estuary provide oysters with a refuge from parasites, lower salinities can negatively impact reproduction and resistance to diseases sublethal impacts of diseases (Hoffman et al 2009).

Parasites are integral parts of food webs (Lafferty et al. 2006). Numerous estuarine parasites have multi-host life cycles, which link multiple trophic levels together (Rohde 2005) (Figure 6). Enhanced salinization may increase the formation of toxic MeHg in coastal wetlands. Therefore, salinity changes may also influence parasite life cycles and disease transmission in species which serve as intermediate hosts for parasites. This may result in environmental consequences, such as parasites and mercury being transferred to higher tropic levels.

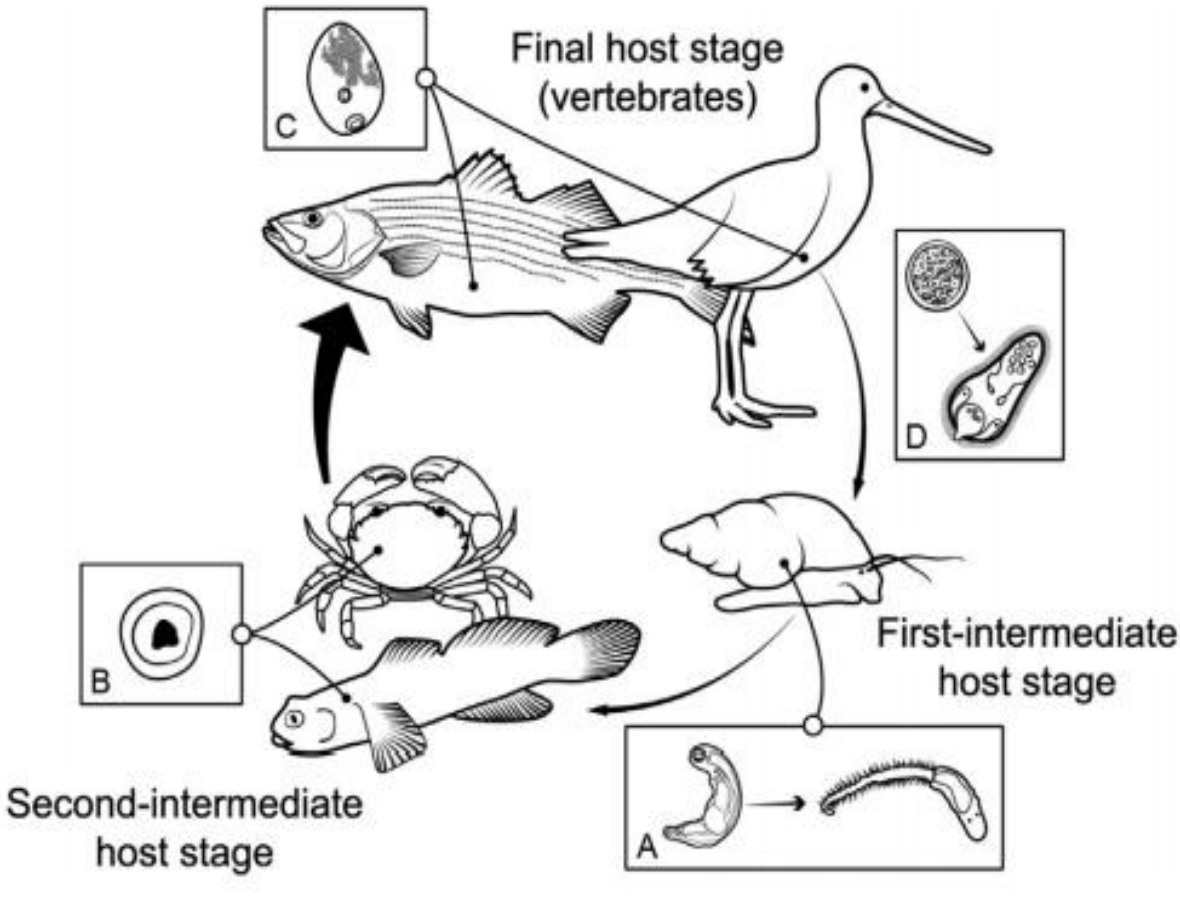

Figure 6: An example of a multi-host lifecycle of a digenean parasite. This diagram exemplifies target specimens as second-intermediate hosts, and are hosts of metacercarial cysts at this stage of the lifecycle (B). Trophic transfer of diseases may be occurring between the second-intermediate host stage and the final host stage. (Moore et al. 2020)

In summary, mercury movement within coastal wetlands have multiple environmental and human health concerns. It is critical to understand the sources, concentrations and transformations of mercury, which includes concentrations of MeHg, in the environment and biota, and how this is influenced by salinity. In my study, I had two objectives. My first objective focuses on the influence of mercury and DOC at my sites and in resident host species. My second objective focuses on the influence of salinity on parasite communities and on mercury

levels in parasitized and unparasitized hosts, within two North Carolina estuaries. To help understand these objectives, I asked three questions:

(1) *How does Hg change with salinity and with DOC in resident biota and sediments in North Carolina estuaries?*

(2) *How does parasite diversity change with salinity in North Carolina estuaries?*

(3) *What are the environmental drivers of Total Hg in resident biota (naked gobies and mud crabs) in North Carolina estuaries?*

My predicted outcomes for this study included: parasite prevalence increases with an increase in salinity, and uninfected host species will have higher levels of THg within their tissues compared to species which were infected. To determine the *in situ* impact of salinity on mercury levels and parasites in estuarine biota, I sampled THg concentrations, which serve as a proxy for MeHg (i.e. total Hg levels are between one and two times higher than levels of MeHg; Driscoll et al. 2007), and parasite prevalence/abundance of host organisms along a salinity gradient in two North Carolina rivers (Pamlico and Neuse Rivers). Wetland biota in my study included the naked goby fish (*Gobiosoma bosc*), Panopeid mud crabs (*Rhithropanopeus harrisii* and *Eurypanopeus depressus*), and a small subset of blue crabs (*Callinectes sapidus*). These species were selected based on their positions in both food webs and parasite life cycles, thus serving as conduits of mercury and parasites to higher trophic levels, as well as their life history location throughout an estuary. In coastal estuaries, species which exhibit homage to specific areas include my target mud crab and goby species (Toscano et al. 2014; Harding et al. 2020). In addition, these selected biota function as an important trophic link between benthic and pelagic communities (Markle and Grant 1970; Breitburg et al. 1995; Breitburg 1999; Palmer et al. 1995).

I also included blue crabs in my study because they are dominant, opportunistic scavengers, and active predators which can impact community structure (Hines et al. 1990). These crabs are epibenthic omnivores and prey for species at higher trophic levels which makes blue crabs critical for energy transfer across food webs in estuarine and coastal communities (Hines 2007). This suggests they might greatly influence the transfer of mercury in their environment (Taylor and Calabrese 2018).

Results from this study will help in decision making for coastal agencies. This study will also add to what we already know about saltwater intrusion in coastal regions of North Carolina, and watersheds draining into coastal areas. I am focusing on the interplay of enhanced salinization and mercury contamination in coastal wetlands, where recreational fishing, fisheries management, and aquaculture of marine species occurs.

Methods

Field Sampling

The location of this study focused on two large North Carolina estuary systems. Five sites along the Pamlico River and five sites along the Neuse River were chosen for their ranges in salinity (Figure 7). The sites were chosen to assess parasite richness and abundance, diversity, as well as mercury levels in Panopeid mud crabs, naked gobies, and blue crabs along a salinity gradient. Salinity at these sites range from fresh and oligohaline (0-5.0 ppt) to mesohaline (5.0-18.0 ppt). The sites represent previously established field sites by the Blakeslee lab.

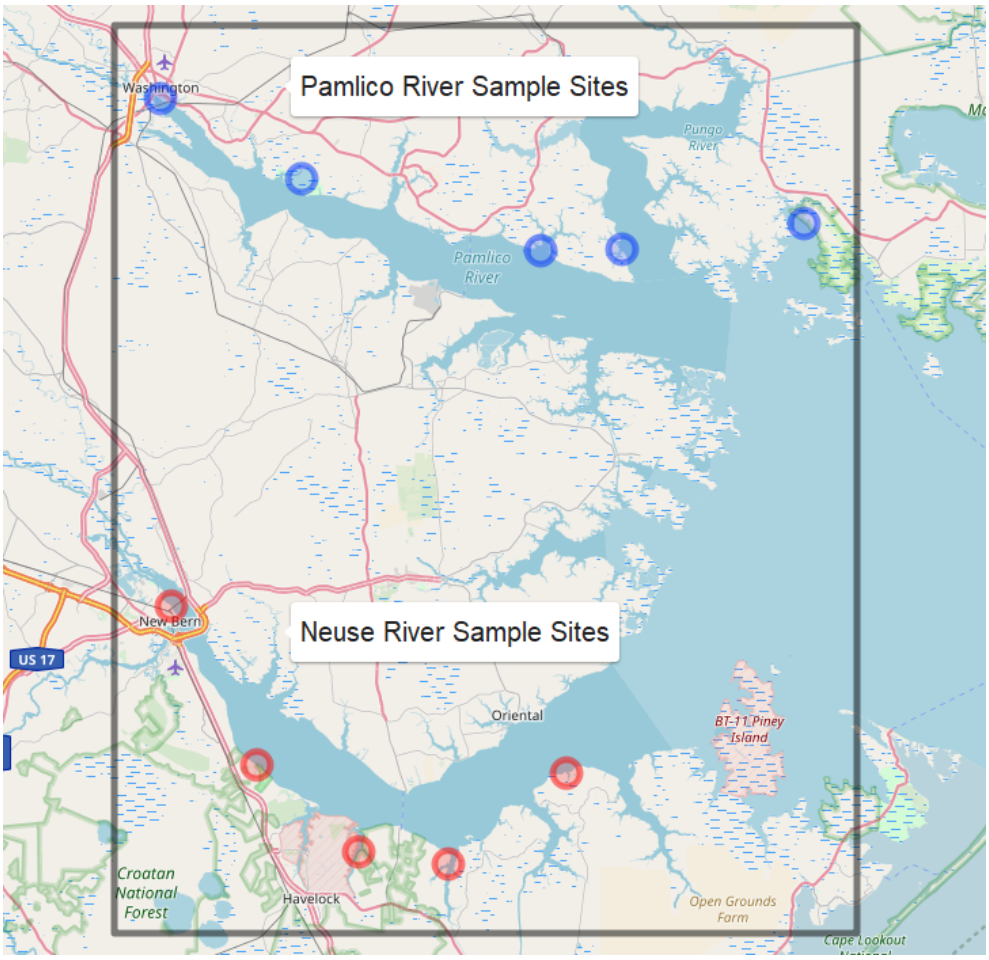

Figure 7: Map of Eastern North Carolina. Five sites are on the Pamlico River to the North, and five sites are on the Neuse River to the South.

Readings of salinity, water temperature, air temperature, and dissolved oxygen were taken at the time of passive sampler (see below) deployment and collection of specimens. At three sites per river (lowest, mid, and highest salinities; Figure 7), salinity was be measured every 30 minutes using a HOBO U24 Conductivity Logger. In addition, at all sites, point measurements of salinity (units ppt) were taken using a handheld YSI: EcoSense EC 300A. For water temperature (°C), a HOBO Pendent Temp/Light logger was deployed at each site and measured temperature every 30 minutes; in addition, point measurements of temperature were taken using a handheld YSI: EcoSense EC 300A. Finally, a YSI Pro DO IP67 meter was used to measure dissolved oxygen in units of mg/L at each site.

Sediment samples were collected at every site, once per season (approximately every 12 weeks), using a gloved hand, from a depth between 0-10 cm to fill a 6-ounce container, using Dr. Tsui's methods for collection (personal communication). Additionally, some sediment was visually quantified for sediment composition and recorded. In locations too deep to stand, a swing sampler was used to collect soil samples. Samples were placed in Ziplock bags, and transported in a cooler, on ice, until reaching ECU where they were frozen in a -20°C freezer, until Hg analysis at Dr. Tsui's lab (see below).

In addition to soil samples, water samples were collected at each site, once per season (approximately every 12 weeks, excluding fall) using Dr. Ardón's methods for collection (personal communication). Water was collected in pre-cleaned plastic bottles. Samples were filtered in the field using a glass fiber filter (Whatman GF/F 0.7µm). Samples were transported to the Blakeslee lab in a cooler with ice packs. Once at the lab, samples were stored at 4°C until they could be analyzed at Dr. Ardón's lab. See below for analysis of water samples for: ions, nutrients, and DOC at Dr. Ardón's lab at NC-State University.

Specimen collections occurred every 4 – 6 weeks, for a total of two collection events every season (excluding one collection event in the Fall). To collect resident mud crabs and naked gobies, two passive sampling devices, composed to small plastic milk crates (19 cm × 22 cm × 16 cm) filled with (3 lbs, 8 oz) of autoclaved oyster shell. Each sampler sat on the top of the sediment, roughly 0.2 to 2.5 meters below the surface. These samplers provide structure which attracts small fishes and invertebrates including my target species (Roche and Torchin 2007; Blakeslee et al. 2021). Sampling was done in a way to ensure that a minimum of ten parasitized individuals and ten unparasitized individuals per target species were sampled for total mercury analyses (described below). The comparison of infected and uninfected species should give insight as to how parasites may differentially affect the uptake of Hg from host tissues compared to unparasitized conspecifics (Sures et al. 2017).

Adult male blue crabs were collected using three collapsible traps no larger than 18 inches in diameter, in accordance with the SEAP (permit #706671) from the North Carolina Department of Marine Fisheries. Raw chicken legs were used as bait. Traps were placed at three sites per river (lowest, mid, and highest salinity sites Figure 7), and sampled once per season. Blue crabs were placed in Tupperware containers with damp paper towels, and kept in a cooler while being transported back to ECU.

Below is a visualization of my collection events throughout the year (Table 1). The original plan was to have two collection events each season; however, the second fall sampling event was cancelled. For every sampling event, each collection of biotic and abiotic data sampled is blocked out in blue.

Table 1. A tentative plan for sampling at my sites on the Pamlico and Neuse rivers over the course of one year.

	Biotic Data Collected			Abiotic Data Collected		
Collection Dates	Collection of Naked Gobies	Collection of Mud Crabs	Collection of Atlantic Blue Crabs	Environmenal Parameters	Collection of Sediment	Collection of Water
May 27th - 28th, 2020 1st collection event						
June 24th - 25th, 2020 2nd collection event						
August 19th - 22nd, 2020 3rd collection event						
September 26th - 27th, 2020 4th collection event						
November 6th - 7th, 2020 5th collection event						
February 6th - 10th, 2020 6th collection event						
March 10th - 13th, 2020 7th collection event						

Lab Processing of Biota

After collection, naked gobies (*G. bosc*) were transported back to ECU in a five-gallon bucket filled with water from their collection sites and aerated using two battery powered bubblers. Crabs (Panopeid spp. and C. sapidus) were kept alive in small Tupperware containers with damp paper towels, and kept in a cooler while being transported back to ECU. After arrival at ECU, the fish were euthanized and dissected using approved IACUC guidelines (IACUC AUP #D346). Before dissection, naked goby length (head to end of caudal fin) was measured with calipers and recorded in millimeters (Moore, unpublished). Fish and crabs were frozen in a -20°C freezer upon arrival at ECU, until dissection.

Naked gobies were dissected using an established protocol (Moore, unpublished). Specifically, tissues were wet mounted on glass slides with a glass coverslip and observed under a compound microscope at 4x magnification. Parasites were identified to their lowest taxonomic

level using a morphology identification guide (Moore, unpublished). Parasites large enough to be separated from the host's tissue were placed in a 1.5mL tube and stored until mercury analysis at Dr. Tsui's lab at UNC-Greensboro.

Panopeid spp. mud crabs and blue crabs were dissected in a similar manner to assess tissues for parasites, using the following adapted protocol (Blakeslee et al. 2015; Blakeslee et al. 2021). Tissues were wet mounted on glass slides with a coverslip, and observed at 4x magnification, under a compound microscope. Blue crab tissues were wet mounted using a glass compressorium. Parasites present in the tissues were identified to their lowest taxonomic level using a morphology identification guide (Blakeslee, unpublished). Parasites large enough to be separated from the host's tissue were placed in an additional 1.5mL tube and kept in a -20°C freezer until Hg analysis at Dr. Tsui's lab.

Commonly seen fish parasites included: trematode cysts (8A) and trematode adults (Figure 8B (Class Digenea)), tapeworm (Class Cestoda) larvae (Figure 8C), acanthocephalan (Phylum Rotifera) cystocanths (Figure 8D) and adults, and nematodes (Phylum Nematoda). After dissection, parasites large enough to separate were placed in a 1.5mL tube and kept in a -20°C freezer. The rest of the body, composed mostly of bones and white muscle tissue, were placed in a separate 1.5mL tube and frozen in a -20°C freezer until total mercury analysis at Dr. Tsui's lab.

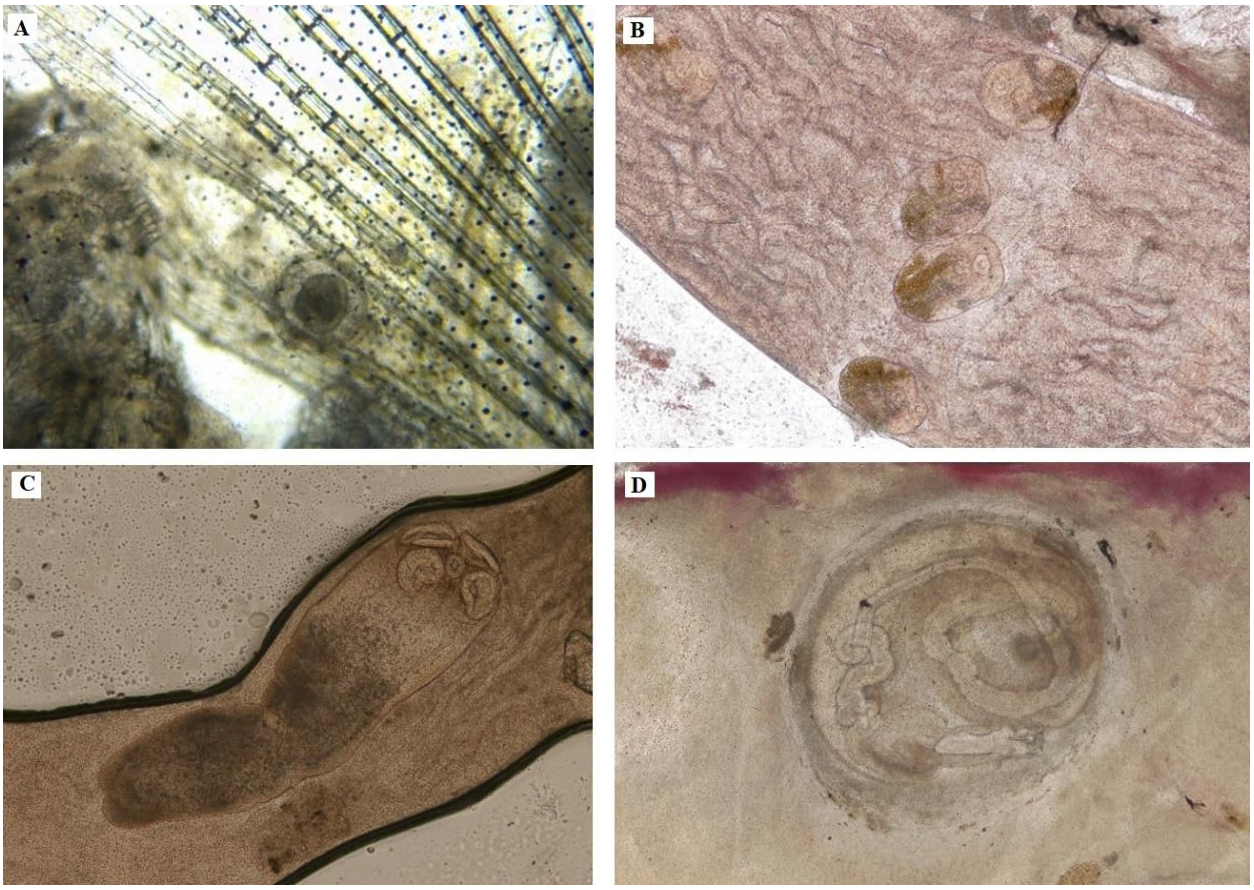

Figure 8: microscopic photos of parasites in naked gobies. A) trematode cyst on fin ray 4x B) trematode adults in intestines 5x C) larval tapeworm in intestines 5x D) cystocanth 5x. Photo credit: Christopher Moore.

Crab parasites present in the tissues were identified to their lowest taxonomic level using a morphology identification guide (Blakeslee unpublished). Parasites large enough to be separated from the host's tissue were placed in an additional 1.5mL tube and kept in a -20°C freezer until Hg analysis at Dr. Tsui's lab. Commonly observed parasites included: parasitic adult barnacles (Figure 9A) (Superorder Rhizocephala), parasitic adult isopods (Figure 9B - male) (Family Entoniscidae), larval trematodes (Figure 9C) (Class Digenea), larval tapeworms (Class Cestoda), larval acanthocephalans (Phylum Rotifera), and nematodes (Phylum Nematoda)

(Figure 9D). After dissection, all wet mounted tissue was be placed in a 1.5mL tube and kept in a -20°C freezer, until Hg analysis in Dr. Tsui's lab.

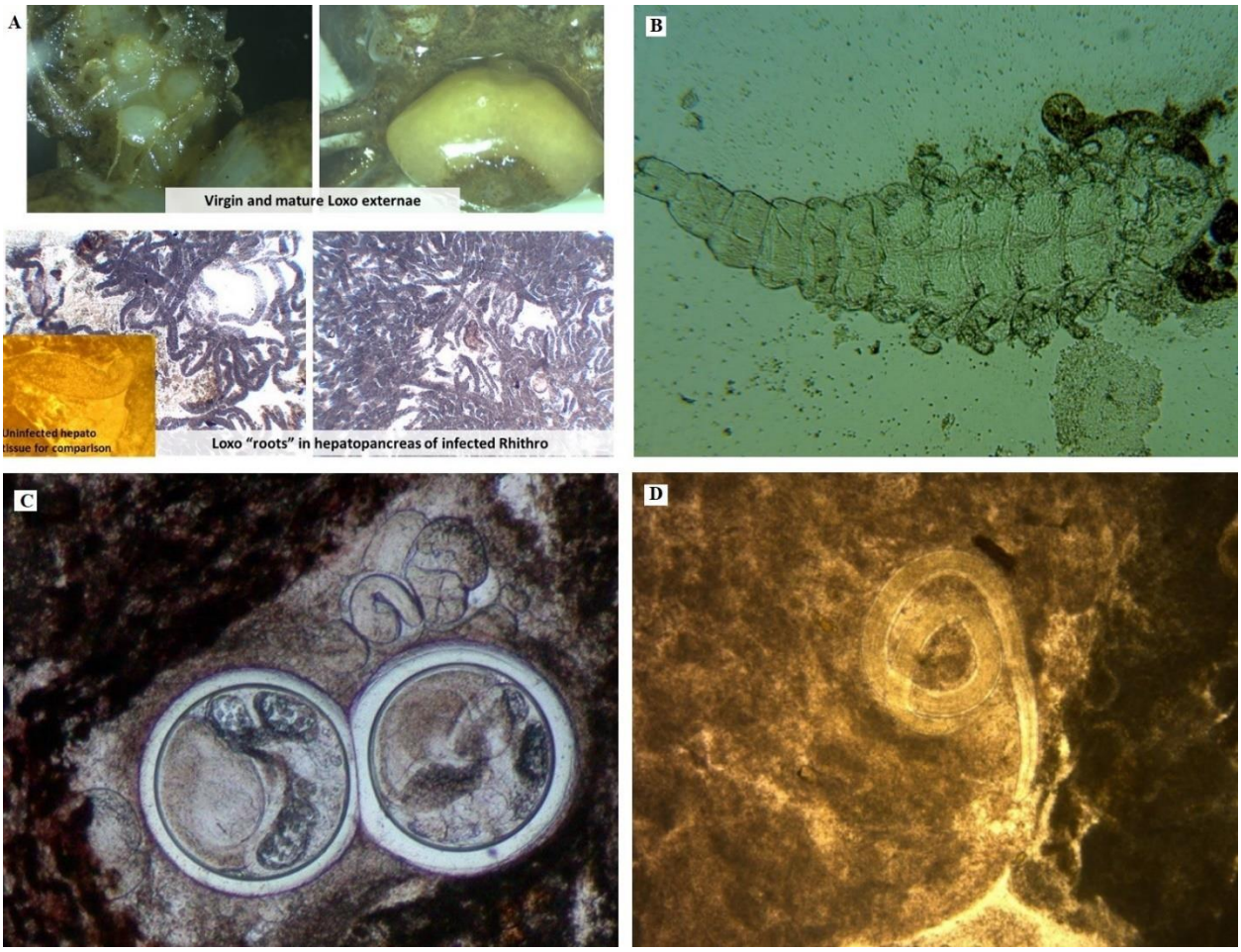

Figure 9: microscopic photos of parasites in mud crabs. A) rhizocephalan externa and roots 4x. B) entonicid isopod (male) 10x. C) trematode cysts 10x. D) adult nematode 10x. Photo credit: April Blakeslee.

Mercury Analyses

Selected animal and parasite tissues, and sediment samples were analyzed for mercury in the Tsui lab at UNC- Greensboro. Samples maintained at -20°C were initially freeze dried. Samples were then homogenized and sieved (for sediment), and weighed, and placed in an acid-cleaned borosilicate glass vial. Biota samples were added to a concentration of concentrated trace-metal grade nitric acid and ~30% reagent grade hydrogen peroxide. Sediment samples were added to hydrochloric acid and nitric acid. Samples were left overnight at room temperature. Bromine monochloride (5%) was added to sediment samples, and placed in a heated water bath (80°C) overnight. Gold traps were connected to soda lime traps to collect gaseous Hg, then stannous chloride (20%) was added to the samples and purged with Hg-free and high purity N_2 gas for 15 minutes. Mercury was quantified with a Brooks Rand Model III Cold Vapor Atomic Fluorescence Spectrophotometer (CVAFS) detector.

Biota samples were weighed and digested with 4.6 M nitric acid (60°C) for 12 hours (Hammerschmidt and Fitzgerald 2005). For THg analysis, dilute nitric acid digests were spiked into Barnstead nanopure water in a glass bubbler; the pH was adjusted with potassium hydroxide; and was buffered by sodium acetate. Samples were then ethylated with ice-cold sodium tetraethylborate (1%) for 25 mins while mixing every 5 minutes. Gold traps were connected to bubblers for collection of gaseous mercury. Total Hg was quantified using a Brooks Rand Model III CVAFS after being separated by a GC column held at 36°C and pyrolyzed at 700-800°C (Brooks Rand GC and Pyrolysis Module). The actual MeHg concentration were regularly verified against in-house total-Hg standard using the method outlined by the EPA (1998).

Sediment samples were analyzed for both THg and MeHg. For extraction of MeHg I used a distillation approach (Hammerschmidt and Fitzgerald 2005). Distillate was buffered with sodium acetate and ethylated by sodium tetraethylborate (1%) in a glass bubbler. The actual MeHg concentration was verified against in-house total-Hg standard using the method outlined by EPA (1998). For analysis of THg, sediments were analyzed using the same method (above) which was used for the analysis of biota samples.

Water Sample Analyses

Water samples, which were collected once per sampling season (approximately every 12 weeks), were taken to NC State University in Raleigh for analysis by the Ardón lab. At Dr. Ardón's lab, the protocol below (Ardón et al. 2010) was followed for water quality analysis, to test samples for: ions, nutrients, and DOC. Nitrate, Nitrite, NH4-N, and PO4-P were analyzed with a Segmented Flow Analyzer. Anions were analyzed using a Dionex ICS-2000 ion Chromotograph with an AS-18 column (Dionex Corporation, Sunnyvale, California, USA). NPOC and TDN were analyzed with a TOC total carbon combustion analyzer with a TNM-1 nitrogen module (Shimadzu Scientific Instruments, Columbia Maryland, USA.

Statistical Analyses

The following statistical analyses were used to answer the three main questions in my system:

(1) *How does Hg change with salinity and with DOC in resident biota and sediments in North Carolina estuaries?*

I used a bar graph to visualize the mercury levels in sediments at all of my sites. In addition, tables were created to assess salinity levels from all of my sites. I made a DOC and salinity regression to determine the relationships of DOC with increasing salinity. Additionally, for naked gobies, I had enough mercury data across spring and summer to create box plots to visualize changes in THg levels across all sites.

(2) *How does parasite diversity change with salinity in North Carolina estuaries?*

I used R-Studio version 3.6.2 to create data tables which visualized parasite communities in the Pamlico and Neuse River estuaries. Two tables were constructed. One table was created for naked gobies and included: parasite abundance and prevalence at five sites in the Neuse River and five sites in the Pamlico River. The sites were arranged from low to high salinity in each river, thus providing a salinity gradient. The second table was created for mud crabs and included the same information listed above.

(3) *What are the environmental drivers of Total Hg in resident biota (naked gobies and mud crabs) in North Carolina estuaries?*

I used JMP 15.0 to analyze Hg as a response variable for gobies and mud crabs to look for drivers of Hg along salinity gradients. Because total Hg in gobies did not significantly differ from a normal distribution, I used a linear model (identity link) in these models. The following were included as fixed effects: salinity, DOC, DO, sample month, goby sex, infection abundance (i.e., counts of parasites, including zeros), and parasite richness (i.e., the number of parasite species). Temperature was not included as a fixed effect because it was found to positively correlate with DOC. For mud crabs, total Hg also conformed to a normal distribution, and so a linear model (identity link) was used in these models. Because infection abundance was limited

in mud crabs, only infection status (infected or uninfected) was used in analyses with mud crabs. Other fixed effects included salinity, DOC, dO, and crab sex. For both gobies and mud crabs, an information criterion approach was taken to determine the model with the highest AICc weight. This provided a way to determine the combination of fixed effects that best explained our total Hg data in each host organism. Linear regressions were used to analyze and visualize important numerical relationships with total Hg in both gobies and mud crabs, blue crabs, and box plots were used to visualize categorical data with Hg in gobies, mud crabs, and some parasite taxa.

Results/Discussion

My study questions below are used to organize my results:

(1) *How does Hg change with salinity and with DOC in resident biota and sediments in North Carolina estuaries?*

Abiotic Measurements Across Sites

The conversion of MeHg from inorganic mercury is dependent on multiple abiotic parameters. Abiotic data from my sites were recorded throughout the year. Salinity levels from my sites ranged from oligohaline to mesohaline (Figure 10). Point measurements taken at the time of each sampling event, for a total of 7 times, over a one-year period are provided in Tables (2A-B). Overall, the average salinity levels from the sites at the Pamlico River had higher measurements of ppt than average salinity levels from the sites at the Neuse River. The water temperature remained relatively similar between sites at the Neuse and Pamlico Rivers. There were no trends in dissolved oxygen data from both rivers. However, in both rivers the sites highest in salinity were lowest in dissolved oxygen. (Note: there was no data collection from Swan Quarter in March 2021, due to a vehicle mechanical issue).

Table 2: Salinity, water temperature, and DO readings for the Pamlico and Neuse rivers. N refers to the total times each site was visited through one year. Mean with standard deviation (SD) was calculated for all parameters, as well as median with min. and max. values. A) data from my 5 sites on the Pamlico River B) Data from my 5 sites on the Neuse River.

Table 2A

	New Bern (N=7)	Flanners Beach (N=7)	Cahooque Creek (N=7)	Matthews Point (N=7)	Pin Oak (N=7)
Salinity (ppt)					
Mean (SD)	0.143 (0.0787)	1.56 (1.59)	1.93 (2.04)	4.27 (2.93)	6.61 (4.90)
Median [Min, Max]	0.100 [0.100, 0.300]	1.00 [0.100, 4.70]	0.900 [0.100, 5.30]	3.00 [0.300, 7.60]	5.30 [0.400, 13.3]
Temperature (°C)					
Mean (SD)	20.6 (7.21)	20.2 (7.48)	26.2 (8.16)	21.0 (7.47)	20.3 (6.77)
Median [Min, Max]	21.8 [8.30, 28.2]	23.7 [6.70, 28.2]	24.8 [17.1, 39.7]	24.1 [7.50, 27.7]	22.1 [8.50, 27.4]
Dissolved Oxygen (mg/L)					
Mean (SD)	5.64 (1.80)	6.79 (1.51)	7.67 (3.20)	4.91 (1.51)	4.42 (1.18)
Median [Min, Max]	4.64 [4.56, 7.71]	6.06 [5.78, 8.52]	7.04 [4.84, 11.1]	4.76 [3.48, 6.49]	4.15 [3.40, 5.71]

Table 2B

	Estuarium (N=7)	Mallard Creek (N=7)	North Creek (N=7)	Wrights Creek (N=7)	Swan Quarter (N=6)
Salinity (ppt)					
Mean (SD)	0.0857 (0.146)	4.32 (2.84)	7.76 (3.69)	9.50 (3.36)	13.2 (3.07)
Median [Min, Max]	0 [0, 0.400]	6.00 [0.200, 6.70]	8.90 [1.90, 12.5]	9.80 [4.20, 13.5]	14.3 [9.20, 16.1]
Temperature (°C)					
Mean (SD)	17.9 (6.39)	22.4 (6.55)	21.3 (7.60)	21.4 (7.69)	21.6 (7.55)
Median [Min, Max]	19.5 [8.70, 26.1]	22.9 [14.1, 30.4]	23.4 [9.50, 30.0]	25.2 [8.60, 29.5]	24.2 [8.30, 28.2]
Dissolved Oxygen (mg/L)					
Mean (SD)	5.30 (2.80)	6.11 (2.43)	5.91 (3.31)	7.49 (4.81)	4.24 (1.94)
Median [Min, Max]	4.98 [2.68, 8.25]	5.46 [4.07, 8.80]	6.85 [2.23, 8.64]	8.73 [2.18, 11.6]	4.30 [2.27, 6.14]

Salinity Measurements Across Sites

Salinity measurements (ppt) were taken at every sampling event. To determine average salinities at the site level, salinities per site were averaged over one year of sampling. Salinity measurements are displayed along a salinity gradient (Figure 10). Sites from the Neuse and Pamlico Rivers were combined.

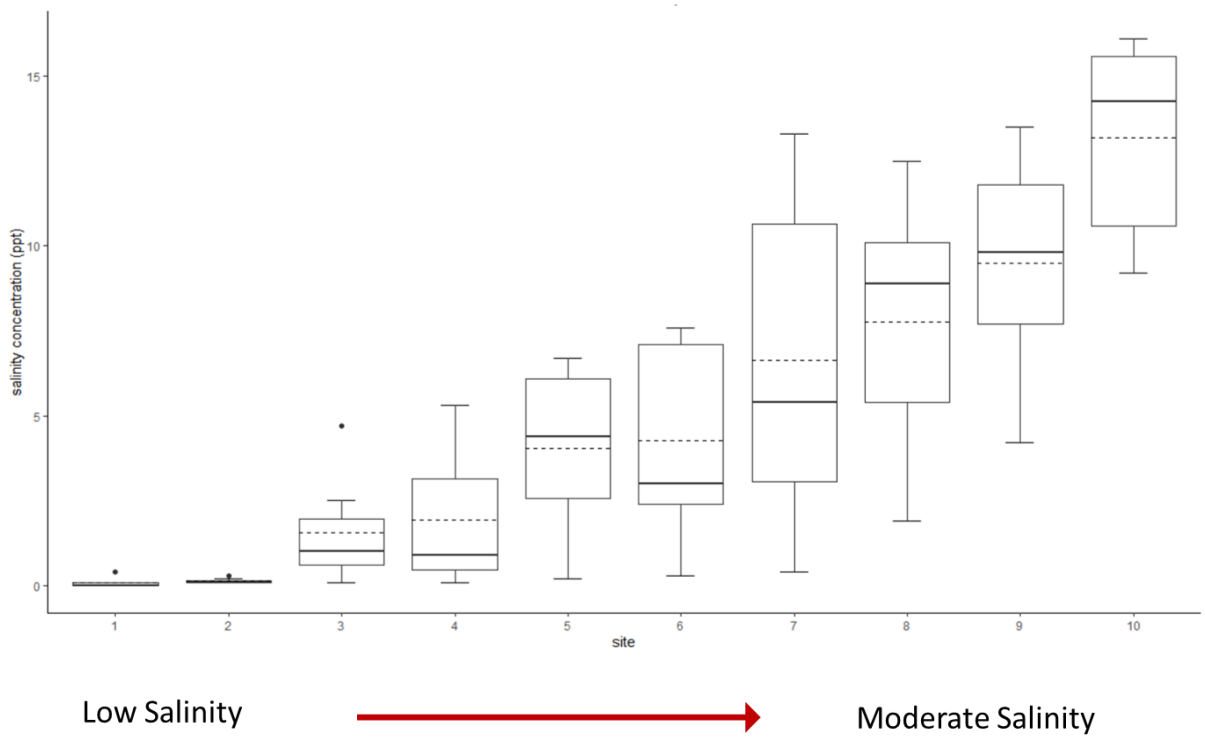

Figure 10: Salinity measurements (ppt) are measured from all ten of my sites. My Neuse River sites include: 1 – New Bern, 3 – Flanners Beach, 4 – Cahooque Landing, 6 – Matthew's Point Marina, 7 – Pin Oak Court (Merrimon). My Pamlico River sites include: 2 – Washington Estuarium, 5 – Mallard Creek, 8 – North Creek Landing, 9 – Wrights Creek, 10 – Swan Quarter.

Mercury in Sediments

Sediment samples collected during the spring and summer were analyzed separately for THg and MeHg. Figure 11 includes the averages of THg and MeHg from spring and summer sampling events, and the sites from each river are arranged from low salinity to high salinity (left to right). Interestingly, sites in the Pamlico River had higher amounts of mercury compared to sites at similar positions in the Neuse River. In general, the lower salinity sites had higher amounts of THg in sediments, than sites at higher salinities. This concurs with what Driscoll et al. (2013) found, suggesting the change in salinity impacts mercury levels.

It should be noted however, that the sediment composition across all sites was not the same. Sandy sediment was the most common type found, at the following sites: New Bern, Flanners Beach, Cahooque Landing, and Matthews Point Marina. Sites with a mix of detritus and sand included: Washington Estuarium, Wrights Creek, and Pin Oak Court. Lastly, sediment which was composed mostly of loose organic debris included: Mallard Creek and North Creek Landing. Washington Estuarium, Mallard Creek, and North Creek Landing had the highest amounts of MeHg.

Previous work by Hyland et al. (2004) described sediment composition in the Pamlico and Neuse Rivers. In the Pamlico River, they had collections near all my Pamlico River sites. At Washington they found oligohaline sand, near Mallard Creek they found mesohaline mud, near North Creek Landing and Wrights Creek they found mesohaline sand, and near Swan Quarter National Wildlife Refuge they found polyhaline sand. They collected near one of my sites on the Neuse River, Pin Oak Court, and found polyhaline sand. Hyland et al. (2004) sampled sites in the middle of both rivers, in mesohaline and polyhaline waters (5.0-18.0 ppt to 18.0 30.0 ppt); these sites were characterized by mud with impaired benthos. This may be due to the high movement

of water in the estuary center, which is different from my sheltered collection sites. Opposite to what I observed, Hollweg et al. (2010) found the ratio of MeHg to THg in estuary sediments to increase with salinity. Kannan et al. (1997) found that MeHg levels did not correlate with THg.

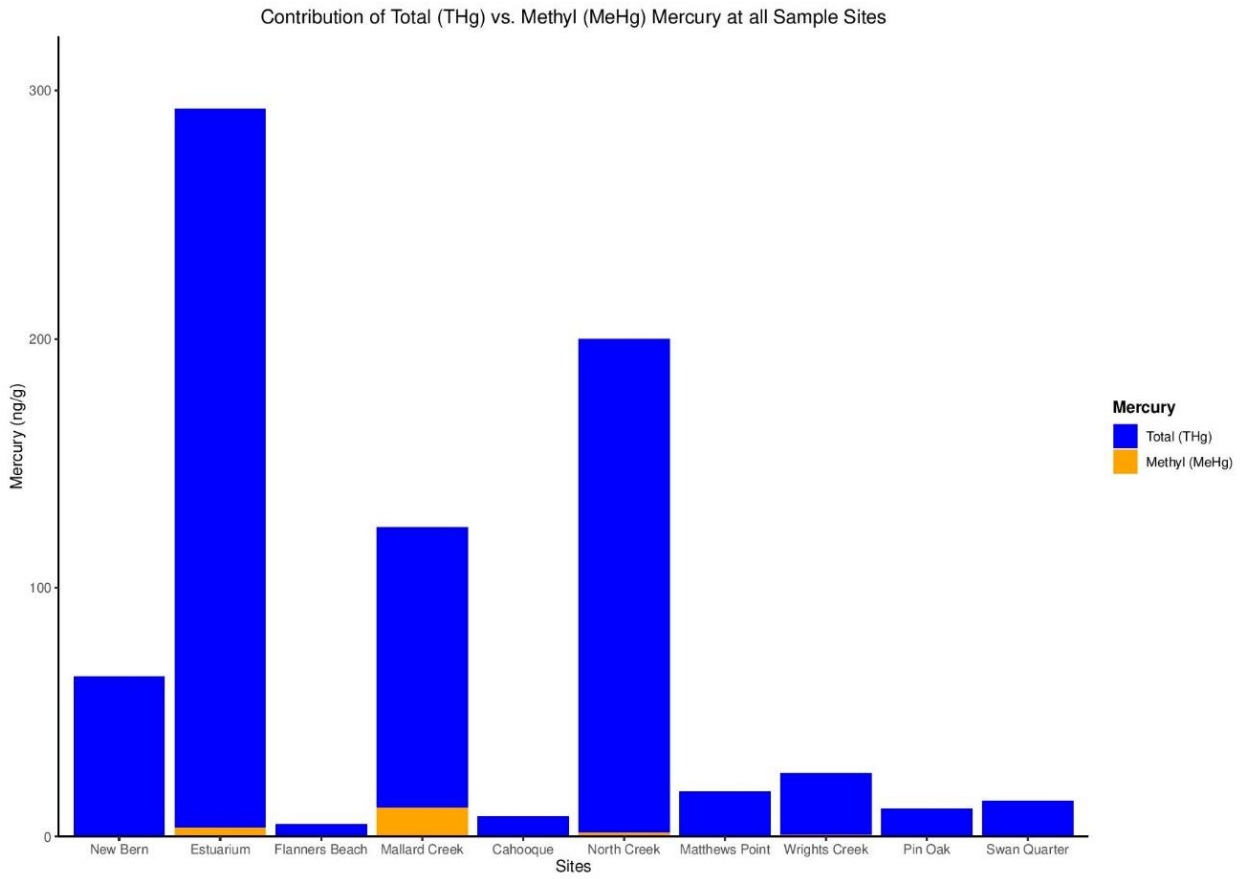

Figure 11: The total THg (blue) and (MeHg) (orange) levels ranging from oligohaline (left) to mesohaline (right). Sites from the Neuse River included: New Bern, Flanners Beach, Cahooque Landing, Matthew's Point Marina, and Pin Oak Court. Sites from the Pamlico River included: Washington Estuarium, Mallard Creek, North Creek Landing, Wrights Creek, and Swan Quarter.

DOC and salinity

When determining the association between salinity and DOC, I found DOC to show a negative relationship with salinity. As seen in Figure 12, DOC has a strong, significant negative association with salinity, and becomes very low at my higher salinity sites. These results were in agreement with work by Ardón et al. (2016), that with an increase in salinity, DOC decreases. Unfortunately during this study I did not measure pH; as reported in previous work, the influence pH has on the electric charge of DOC, and at a lower pH, bacteria will methylate mercury at a higher rate (Gilmour et al. 1992). A large source of DOC in estuaries comes from terrestrial sources (Bauer and Druffel, 1998). My results have combined DOC and salinity levels from both the Neuse and Pamlico Rivers.

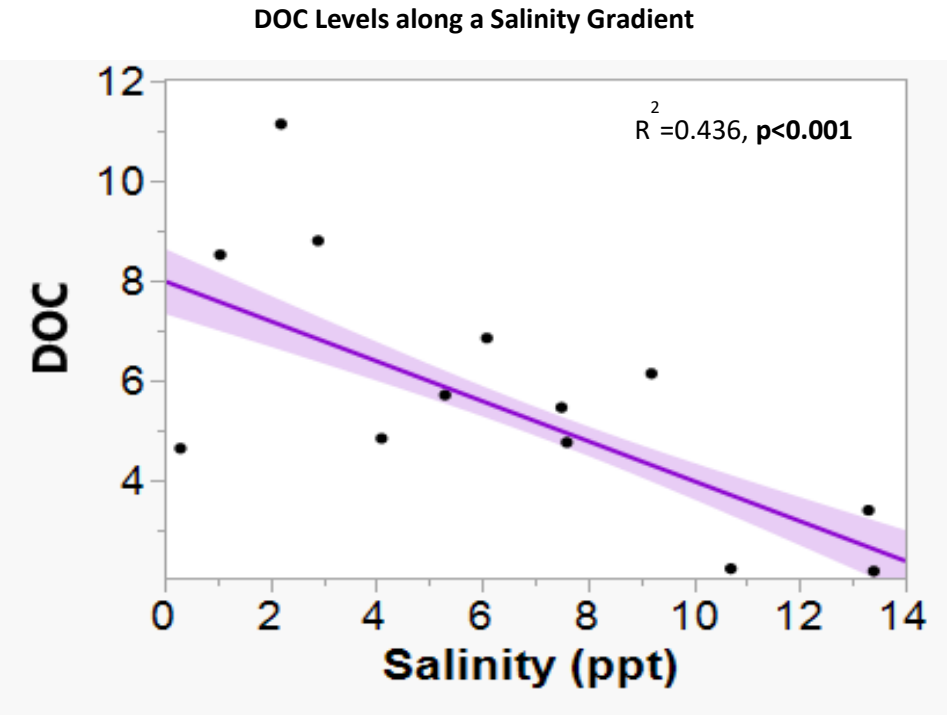

Figure 12: DOC levels and salinities. R^2 refers to close the data fit the regression line. The p-value in bold shows the data as significant, as it is below 0.05.

Mercury and DOC in Host Organisms

In addition to the influence of salinity on mercury, I examined the influence of DOC in the water on mercury levels in the gobies and the mud crabs. With comparison of water DOC which proceeds the production of MeHg, I compared THg levels in both uninfected and infected naked gobies (Figure 13A) and mud crabs (Figure 13B). In naked gobies, the highest levels of mercury were found when DOC values were between 5 and 6 mg C/L. For mud crabs, there was an inverse DOC trend. Figures 13A-B show significant results. The results of both figures are combined from both the Neuse and Pamlico Rivers.

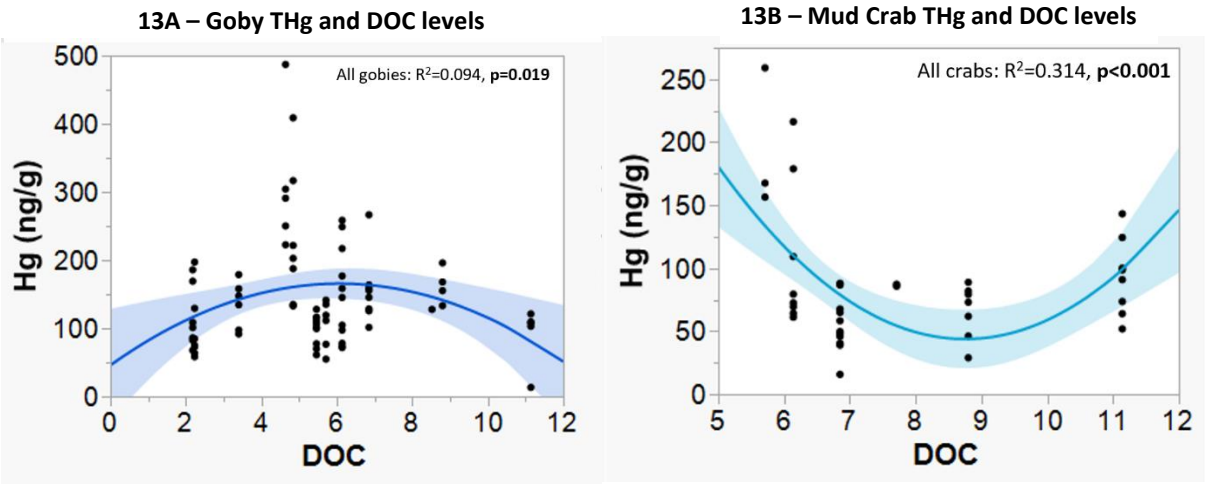

Figure 13: Comparing THg levels with DOC of water and salinity. R^2 refers to close the data fit the regression line. The p-value in bold shows the data as significant. A) The THg values of all naked gobies and the values of DOC from the Neuse and Pamlico rivers. B) The THg values of all mud crabs and the DOC values from the Neuse and Pamlico Rivers.

Mercury in resident biota and salinity

In Figures 14A-B uninfected naked gobies and mud crabs are displayed by how much mercury was in their tissues across the salinity levels from where they were collected. In the uninfected naked gobies, there is no significance between the THg in their tissues and the salinity levels of collection. However, there was a slight negative trend in THg from low to high salinity (Figure 14A). For infected naked gobies, there is a weak but significant negative trend, (p-value =0.032). My results are combined from the Neuse and Pamlico River sites. These results concur with previous work, which found that in lower salinities of estuaries THg in fishes tend to be lower than in higher salinities (Farmer et al., 2010, Fry and Chumchal, 2012).

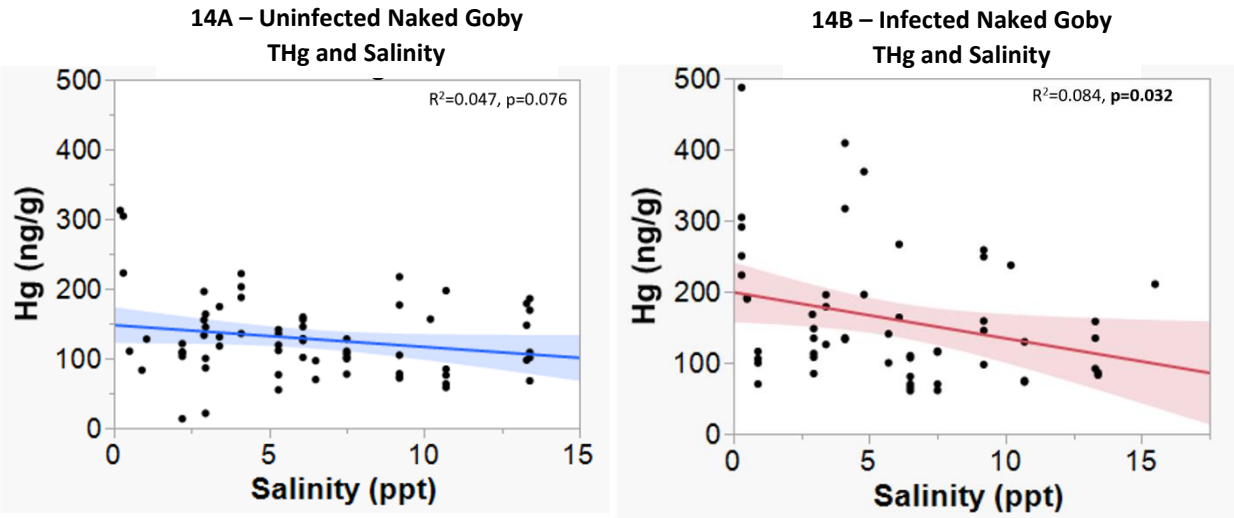

Figure 14: Comparing THg levels with salinity. R^2 refers to close the data fit the regression line. The p-value in bold shows the data as significant. A) The THg levels of only uninfected naked gobies along a salinity gradient from the Neuse and Pamlico rivers. B) The THg levels of only infected naked gobies along a salinity gradient from the Neuse and Pamlico rivers.

In Figure 15, uninfected and infected mud crabs are displayed together. For both uninfected and infected hosts, there was no significant relationship between the amount of THg observed in their tissues and salinity levels. Although, uninfected mud crabs did show a slight

increase in THg levels with increasing salinity. For both uninfected and infected mud crabs at the lowest salinity sites, THg levels were similar, between 75 and 80 ng/g. My results are combined from sites at the Neuse and Pamlico Rivers.

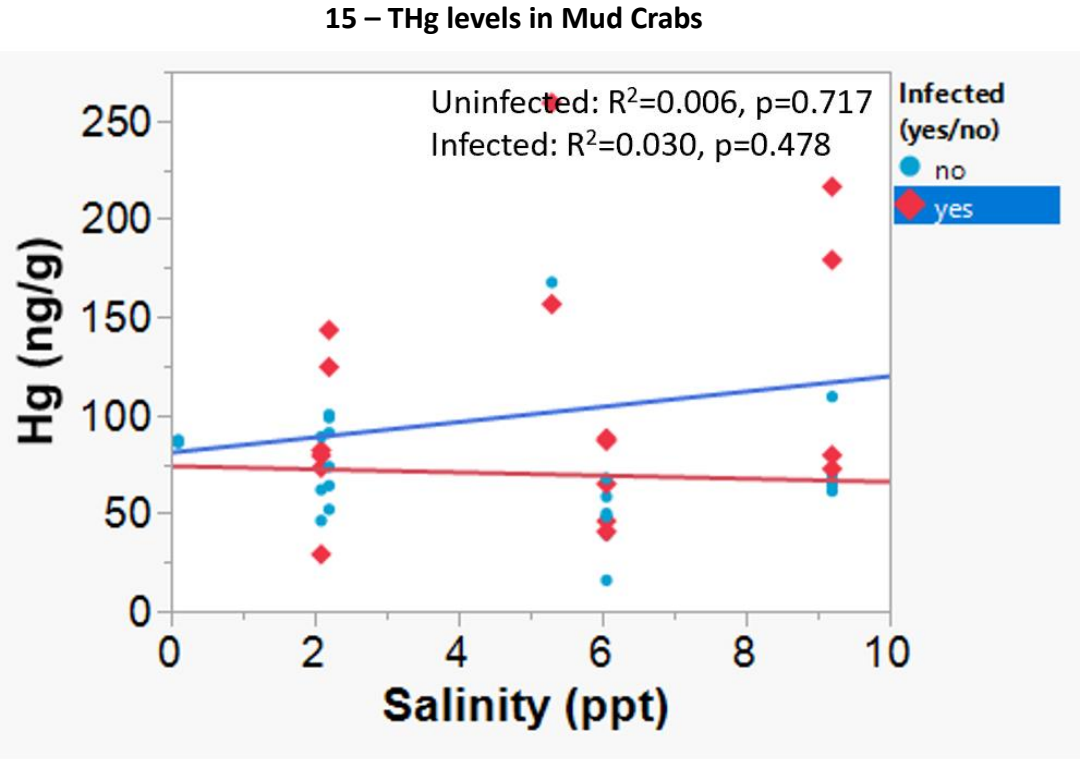

Figure 15: Comparing THg levels with salinity. R^2 refers to close the data fit the regression line. The p-values show the data is not significant.) The THg levels of uninfected mud crabs are represented in blue, and the THg levels of infected mud crabs are represented in red.

(2) *How does parasite diversity change with salinity in North Carolina estuaries?*

For my second question, I have created data tables for my two resident organisms to examine the influence of salinity gradients on parasite communities in these hosts. Naked goby parasites are displayed by abundance and prevalence at different sites in the Neuse and Pamlico Rivers (Table 3). The sites are arranged by increasing salinity, so we can infer which parasites

are found at different salinity ranges. On average, the Pamlico River had a greater range in salinity compared to the Neuse River. The data for parasite diversity occurred over the course of one year.

Table 3 details the parasites from naked goby hosts, which included: cestodes, acanthocephalans, trematodes, and nematodes. Cestodes were more abundant in the Pamlico River, and at higher salinity sites. In the Neuse River, cestodes were observed at lower salinity sites. Larval acanthocephalans were abundant at lower salinity sites in the Neuse River, and were more abundant at middle sites in the Pamlico River. Adult acanthocephalans were only found at my oligohaline sites in the Neuse River. In the Pamlico River, adult acanthocephalans were found at mid-salinity sites, and my highest salinity site. Trematode larvae were observed at all sites in the Neuse River except for Pin Oak Court. In the Pamlico River, larval trematodes were observed at mid salinity sites only.

Overall, parasite prevalence for naked gobies was highest at the New Bern site (0.774). The site with the lowest parasite prevalence was at Matthew's Point Marina (0.0650). There were no trends in parasite prevalence in the Neuse River from low to high salinity. In the Pamlico River, the site with the highest parasite prevalence was at Mallard Creek (0.431), and previous work in the Blakeslee lab has shown this area to be a parasite hotspot with high infections. The lowest site for parasite prevalence was at the Washington Estuarium site (0). There were no trends in parasite prevalence with increasing salinity.

Table 3: Parasite diversity for naked gobies from the Neuse and Pamlico rivers. The number of times a site was visited is represented by (N). Mean with standard deviation (SD) was calculated for all parasites, as well as median with min. and max. values.

Naked Goby Parasite Taxa: Mean Abundance

(in order of increasing salinity by river)

	Neuse					Pamlico				
	New Bern	Flanners Beach	Cahooque	Matthews Point	Pin Oak	Estuarium	Mallard Creek	North Creek	Wrights Creek	Swan Quarter
	(N=7)	(N=7)	(N=7)	(N=7)	(N=7)	(N=7)	(N=7)	(N=7)	(N=7)	(N=6)
Cestodes										
Mean (SD)	5.00 (7.07)	0 (0)	1.80 (2.17)	0 (0)	0 (0)	0 (0)	1.50 (2.26)	0.857 (1.21)	4.00 (6.25)	0.500 (0.707)
Median [Min, Max]	5.00 [0, 10.0]	0 [0, 0]	1.00 [0, 5.00]	0 [0, 0]	0 [0, 0]	0 [0, 0]	1.00 [0, 6.00]	0 [0, 3.00]	1.00 [0, 15.0]	0.500 [0, 1.00]
Larval Acanthocephalans										
Mean (SD)	1.00 (1.41)	0 (0)	0.600 (1.34)	0 (0)	0 (0)	0 (0)	1.67 (4.08)	0.143 (0.378)	0 (0)	0 (0)
Median [Min, Max]	1.00 [0, 2.00]	0 [0, 0]	0 [0, 3.00]	0 [0, 0]	0 [0, 0]	0 [0, 0]	0 [0, 10.0]	0 [0, 1.00]	0 [0, 0]	0 [0, 0]
Adult Acanthocephalans										
Mean (SD)	5.00 (7.07)	0.500 (0.707)	0.600 (1.34)	0 (0)	0 (0)	0 (0)	5.50 (4.46)	0.286 (0.756)	0 (0)	0.500 (0.707)
Median [Min, Max]	5.00 [0, 10.0]	0.500 [0, 1.00]	0 [0, 3.00]	0 [0, 0]	0 [0, 0]	0 [0, 0]	4.50 [2.00, 14.0]	0 [0, 2.00]	0 [0, 0]	0.500 [0, 1.00]
Larval Trematodes										
Mean (SD)	3.00 (4.24)	1.00 (1.41)	6.20 (8.50)	1.00 (1.00)	23.8 (47.5)	0 (0)	14.0 (17.3)	0.571 (1.51)	0 (0)	0 (0)
Median [Min, Max]	3.00 [0, 6.00]	1.00 [0, 2.00]	0 [0, 16.0]	1.00 [0, 2.00]	0 [0, 95.0]	0 [0, 0]	7.50 [0, 46.0]	0 [0, 4.00]	0 [0, 0]	0 [0, 0]
Adult Trematodes										
Mean (SD)	0 (0)	0.500 (0.707)	0 (0)	0 (0)	0.250 (0.500)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Median [Min, Max]	0 [0, 0]	0.500 [0, 1.00]	0 [0, 0]	0 [0, 0]	0 [0, 1.00]	0 [0, 0]	0 [0, 0]	0 [0, 0]	0 [0, 0]	0 [0, 0]
Nematodes										
Mean (SD)	10.5 (7.78)	4.50 (4.95)	2.80 (3.35)	1.33 (1.53)	4.25 (6.55)	2.00 (3.46)	3.33 (3.33)	0.429 (1.13)	1.00 (0.707)	8.00 (11.3)
Median [Min, Max]	10.5 [5.00, 16.0]	4.50 [1.00, 8.00]	2.00 [0, 8.00]	1.00 [0, 3.00]	1.50 [0, 14.0]	0 [0, 6.00]	3.50 [0, 7.00]	0 [0, 3.00]	1.00 [0, 2.00]	8.00 [0, 16.0]

Mean Infection Prevalence

	Neuse					Pamlico				
	New Bern	Flanners Beach	Cahooque	Matthews Point	Pin Oak	Estuarium	Mallard Creek	North Creek	Wrights Creek	Swan Quarter
	(N=7)	(N=7)	(N=7)	(N=7)	(N=7)	(N=7)	(N=7)	(N=7)	(N=7)	(N=6)
Naked Goby Infection Prevalence										
Mean (SD)	0.774 (0.0841)	0.299 (0.221)	0.249 (0.219)	0.0650 (0.0568)	0.242 (0.239)	0 (0)	0.431 (0.137)	0.0719 (0.0673)	0.137 (0.0900)	0.235 (0.257)
Median [Min, Max]	0.774 [0.714, 0.833]	0.299 [0.143, 0.455]	0.238 [0, 0.600]	0.0900 [0, 0.105]	0.234 [0, 0.500]	0 [0, 0]	0.459 [0.167, 0.550]	0.118 [0, 0.130]	0.125 [0.0480, 0.286]	0.235 [0.0530, 0.417]

Mud crab parasite data was organized using abundance and prevalence table (Table 4), similar to Table 3. Parasite abundance was separated by each of the 5 sites on the Neuse and Pamlico rivers. The parasites of mud crabs included: cestodes, acanthocephalans, entoniscid isopods, nematodes, and rhizocephalan barnacles.

Cestode larvae were most notable at North Creek Landing in the Pamlico River; however, I did not find them in the Neuse River. Only one acanthocephalan was observed at North Creek Landing. Entoniscid isopods were more commonly observed in the Pamlico River. Entoniscids in the Pamlico River were found at all sites, but they were most abundant at my middle site, North Creek Landing. In the Neuse River, entoniscids were found at two sites, Cahooque Landing and Matthew's Point Marina. Cahooque Landing had the highest abundance of entoniscids. Nematodes were not seen in the Neuse River. In the Pamlico River, nematodes were observed at the two highest salinity sites: Wrights Creek and Swan Quarter National Wildlife Refuge. Rhizocephalans were only observed at the two highest salinity sites in the Neuse River, and at the highest site in the Pamlico River. There was surprisingly low diversity of parasites in the Neuse River. In the Pamlico River, entoniscid isopods were more commonly found at the mid salinity sites.

Parasite prevalence increased at the higher salinity sites in the Neuse River. However, in the Pamlico River parasite prevalence was higher at the mid-oligohaline sites and at the highest salinity site. Entoniscids were more commonly found at oligohaline sites while rhizocephalans were observed at mesohaline sites. Entoniscids are a type of parasite which has a multi-host life cycle (Adkison 1990), and it is dependent on hosts other than mud crabs present in the community in order to reproduce. While on the other hand, rhizocephalans have a direct life cycle (Lützen et al. 2018), they only require a mud crab to reproduce. For mud crabs, we do see

the disappearance of rhizocephalan infection at lower salinities, which was what Tepolt et al. (2019) and Blakeslee et al. (2021) found. The entoniscids however were still common at the lowest salinity site in the Pamlico River, and their crab hosts likely do not find refuge at lower salinities. In the mud crabs, it should be noted that the two most dominating parasites were castrating parasites, meaning that hosts can no longer reproduce when they are infected by these parasites (Lafferty and Kuris 2009).

Table 4: Parasite diversity for mud crabs from the Neuse and Pamlico rivers. The number of times a site was visited is represented by (N). Mean with standard deviation (SD) was calculated for all parasites, as well as median with min. and max. values.

Mudcrab Parasite Taxa: Mean Abundance

(in order of increasing salinity by river)

	Neuse					Pamlico				
	New Bern	Cahooque	Flanners Beach	Matthews Point	Pin Oak	Estuarium	Mallard Creek	North Creek	Wrights Creek	Swan Quarter
	(N=7)	(N=7)	(N=7)	(N=7)	(N=7)	(N=7)	(N=7)	(N=7)	(N=7)	(N=6)
Cestodes										
Mean (SD)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	6.17 (8.13)	0.857 (1.57)	0 (0)
Median [Min, Max]	0 [0, 0]	0 [0, 0]	0 [0, 0]	0 [0, 0]	0 [0, 0]	0 [0, 0]	0 [0, 0]	3.00 [0, 21.0]	0 [0, 4.00]	0 [0, 0]
Acanthocephalans										
Mean (SD)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0.333 (0.516)	0 (0)	0 (0)
Median [Min, Max]	0 [0, 0]	0 [0, 0]	0 [0, 0]	0 [0, 0]	0 [0, 0]	0 [0, 0]	0 [0, 0]	0 [0, 1.00]	0 [0, 0]	0 [0, 0]
Entoniscids										
Mean (SD)	0 (0)	0.571 (0.976)	0 (0)	0.143 (0.378)	0 (0)	1.00 (1.26)	7.00 (4.43)	11.8 (7.22)	5.00 (3.65)	0.600 (0.894)
Median [Min, Max]	0 [0, 0]	0 [0, 2.00]	0 [0, 0]	0 [0, 1.00]	0 [0, 0]	0.500 [0, 3.00]	7.00 [2.00, 13.0]	9.50 [6.00, 26.0]	4.00 [1.00, 10.0]	0 [0, 2.00]
Nematodes										
Mean (SD)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0.143 (0.378)	0.200 (0.447)
Median [Min, Max]	0 [0, 0]	0 [0, 0]	0 [0, 0]	0 [0, 0]	0 [0, 0]	0 [0, 0]	0 [0, 0]	0 [0, 0]	0 [0, 1.00]	0 [0, 1.00]
Rhizocephalans										
Mean (SD)	0 (0)	0 (0)	0 (0)	2.86 (4.88)	7.86 (3.53)	0 (0)	0 (0)	0 (0)	0 (0)	9.00 (2.83)
Median [Min, Max]	0 [0, 0]	0 [0, 0]	0 [0, 0]	0 [0, 10.0]	9.00 [1.00, 12.0]	0 [0, 0]	0 [0, 0]	0 [0, 0]	0 [0, 0]	10.0 [4.00, 11.0]

Mean Infection Prevalance

	Neuse					Pamlico				
	New Bern	Flanners Beach	Cahooque	Matthews Point	Pin Oak	Estuarium	Mallard Creek	North Creek	Wrights Creek	Swan Quarter
	(N=7)	(N=7)	(N=7)	(N=7)	(N=7)	(N=7)	(N=7)	(N=7)	(N=7)	(N=6)
Mudcrab Infection Prevalence										
Mean (SD)	0 (0)	0 (0)	0.0230 (0.0395)	0.211 (0.249)	0.344 (0.160)	0.114 (0.0989)	0.408 (0.135)	0.553 (0.246)	0.198 (0.120)	0.440 (0.0655)
Median [Min, Max]	0 [0, 0]	0 [0, 0]	0 [0, 0.0870]	0.0480 [0, 0.500]	0.400 [0.0530, 0.500]	0.166 [0, 0.176]	0.375 [0.250, 0.584]	0.524 [0.231, 0.846]	0.214 [0.0670, 0.385]	0.476 [0.344, 0.500]

(3) *What are the environmental drivers of Total Hg in resident biota (naked gobies and mud crabs) in North Carolina estuaries?*

The influence of parasites on Hg concentrations

Opposite to what I originally predicted, THg levels in naked gobies increased with increasing parasite abundance (Figure 16). I found that at lower infection abundance, THg levels were lower compared to higher infection abundance, which had higher THg levels. These results are significant (p-value <0.001). Most of the data is clustered between an infection abundance of 0-6 individuals. Research has shown that mercury is known as an endocrine disruptor; therefore, it can weaken the immune system (Martin et al. 2010). Mercury, more specifically MeHg is an environmental stressor (Evers et al. 2008). This could make species like the naked goby more susceptible to parasites or impair their overall health (Sures et al. 2017). Olivero-Verbel and Caballero-Gallardo (2013) suggests a relationship between parasitism by nematodes in fish and their respective mercury levels. However, because we were unable to assess toxicity in our host organisms, it is unclear whether immunosuppression has or has not occurred in this system. More research is needed to determine this.

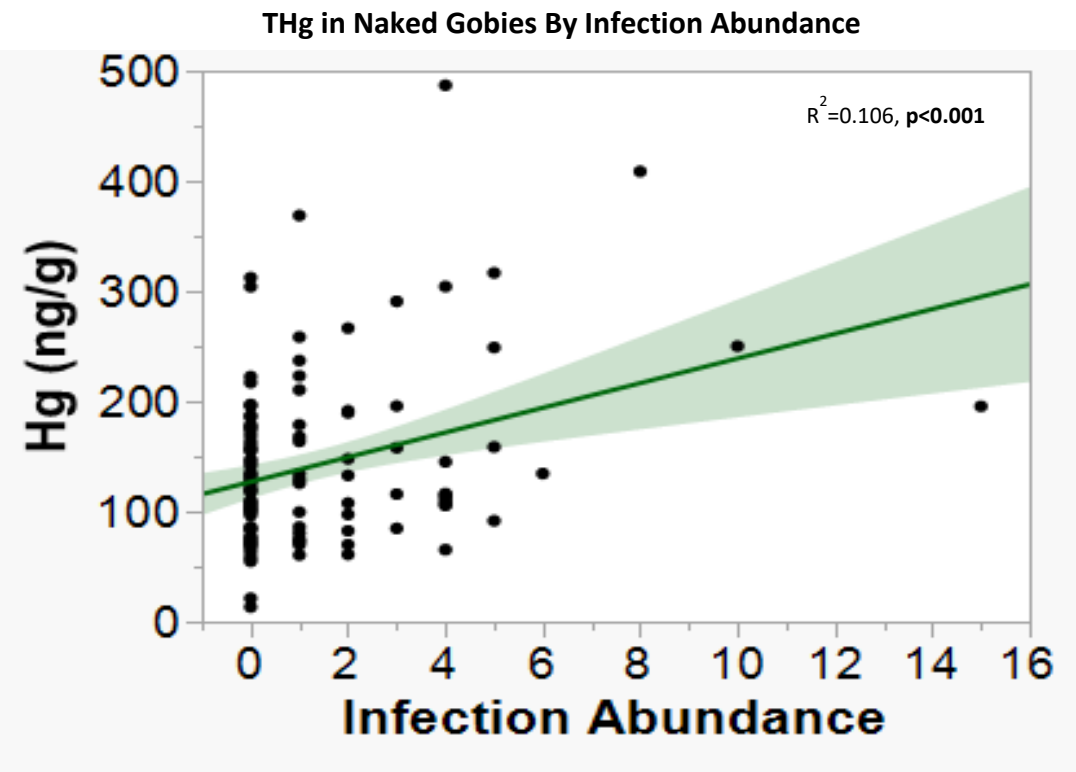

Figure 16: Total mercury levels are shown for naked with their infection abundance.

Opposite to my prediction, THg levels in naked gobies (Figure 17A) and mud crabs (Figure 17B) were lower in host organisms which were parasitized compared to those which were parasitized. Uninfected naked gobies had on average 125 ng/g of THg in their muscle tissue compared to the infected fish which had on average 160 ng/g of THg in their muscle tissue. Uninfected mud crabs had on average 65 ng/g of THg in their tissues compared to the infected crabs which had on average 75 ng/g of THg in their tissues. Additionally, mud crabs overall had about half the THg levels compared to the naked gobies. This may partly be due to the different tissue types of the hosts. Vertebrates tend to sequester mercury within the liver, kidneys, and muscles (Wolfe et al. 1998; Kenow et al. 2007), and the hepatopancreas in crabs is the target organ of mercury (Cheng et al. 2009). It could also be due to the different types of parasite taxa

which infect the hosts. For example, most of the parasites of naked gobies I observed were helminths, which are usually found in host intestines (Coop and Kyriazakis 1999), while in the mud crabs I mostly found are castrating parasites (Høeg 1995; Williams and Boyko 2012). Both figures suggest that parasites may not be taking in as much THg from their hosts as previously reported (Sures and Siddall 1999). A study by Jankovská et al. (2012) reported THg levels of infected and uninfected tissue from perch and found like my study, that infected fish had higher levels of THg compared to uninfected fish.

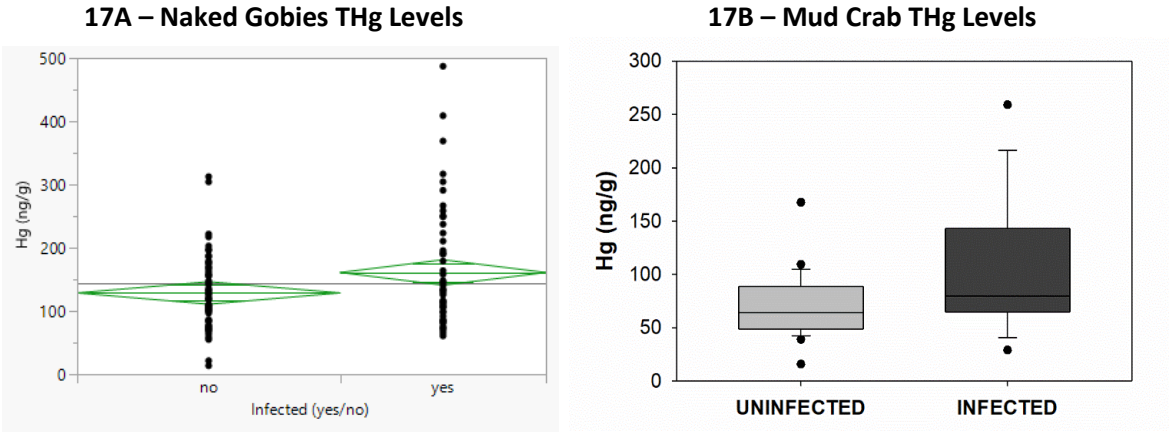

Figure 17: THg levels for uninfected and infected naked gobies and mud crabs. A) The THg levels of naked gobies are shown with uninfected host tissues on the left, and infected host tissues on the right. B) The THg levels of mud crabs are shown with uninfected host tissues on the left, and infected host tissues on the right.

When adding the blue crab data next to the resident host species, the same trend as above was observed: THg levels were lower in uninfected blue crab host tissues compared to infected host tissues (Figure 18). Infected naked gobies had the highest THg levels (175 ng/g), mud crabs had the lowest THg levels (130 ng/g), and blue crab THg levels were in between (100 ng/g). Host species were arranged relative to their predicted trophic levels. Size criteria needs to be considered when studying trophic dynamics (Garrison and Link, 2000). “Dietary analysis is one

way in which to determine trophic levels in estuarine invertebrates, however other ways are needed to determine isotope discrimination when assessing trophic levels (Bui and Lee 2014; Kristensen et al. 2017). Future analyses of $\delta^{15}N$ will be conducted to better understand their trophic links of my three host species.

Blue crabs are important in the energy transfer within estuaries, as they are prey for top-level consumers (Hines 2007). The US EPA (1997) reports THg levels for blue crabs at 300 ng/g as a threshold for safe consumption. The blue crabs I collected from the Neuse and Pamlico rivers are below this limit, which shows safe mercury levels for consumption.

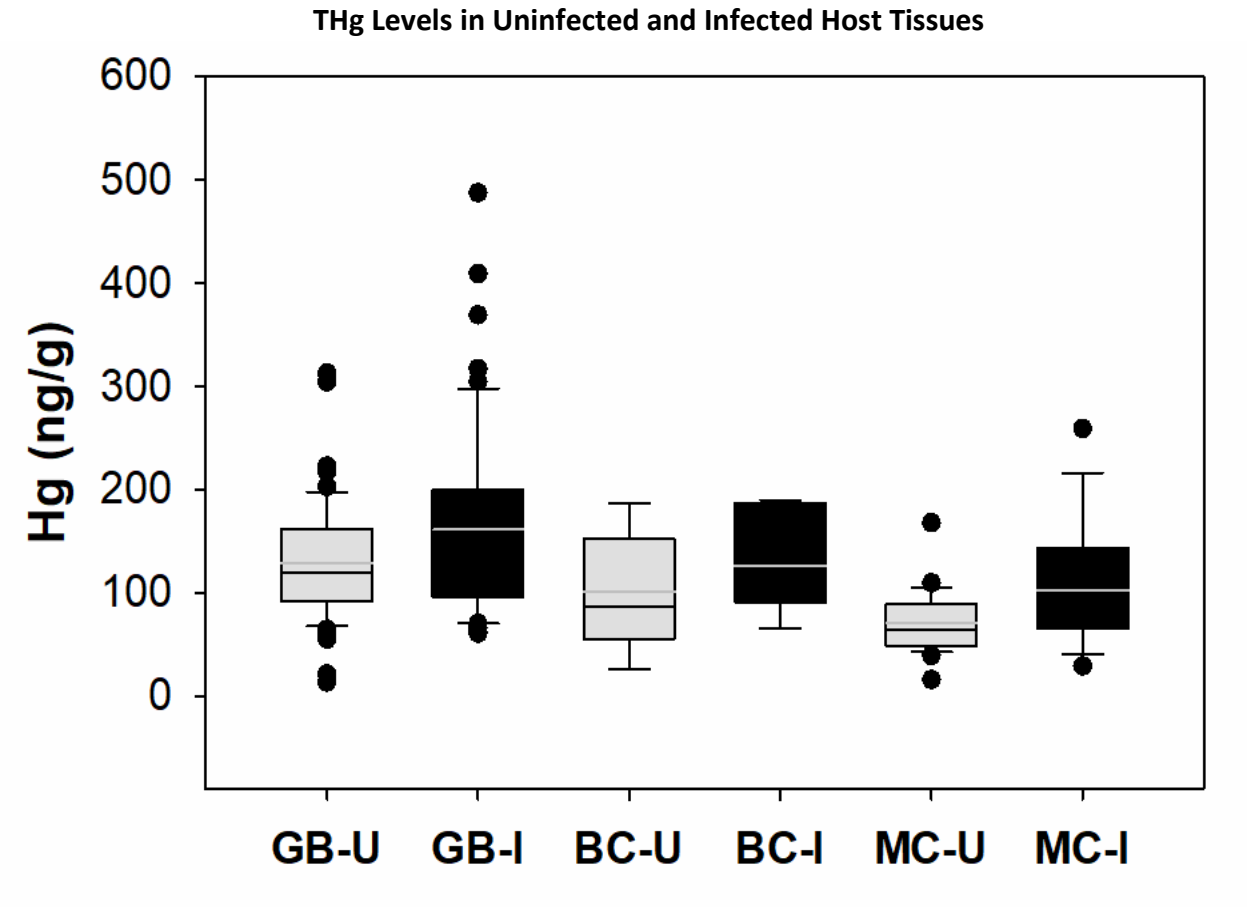

Figure 18: The THg levels of uninfected and infected host tissues. GB refers to naked gobies, BC refers to BC blue crabs , and MC refers to mud crabs. U is uninfected, and I is infected.

Parasites which were large enough to separate from the host tissues were analyzed for THg content. Parasites with a large enough sample size were arranged by their hosts' trophic levels (Figure 19A). The most commonly observed parasites in the naked gobies were helminth parasites. Acanthocephalans had an average level of 65 ng/g THg within their tissues. Jankovská et al. (2012) found that acanthocephalans from perch had on average 9.9 ng/g of THg. In my study, I found the THg levels in the acanthocephalan to be much higher. Sures et al. (2005) found that acanthocephalans can extract metal levels from their hosts. However, it should be noted that the acanthocephalans from Jankovská et al. (2012) came from a freshwater fish, likely exposed to lower MeHg levels. When comparing parasite THg levels to host THg (Figure 19B), we see that although parasites are lower in mercury levels, they still contain a substantial amount of the metal. Similar to my average THg results in cestodes (80 ng/g), McGrew et al. (2018) found cestodes from pinnipeds to have THg levels of 87.5 ng/g. Mud crabs had crustacean parasites; however, there were no previous studies for mercury levels in entoniscid isopods. My study documents the first known levels of THg for this taxa. These parasites may be taking up mercury within their hosts differently, and this should be investigated further in the future.

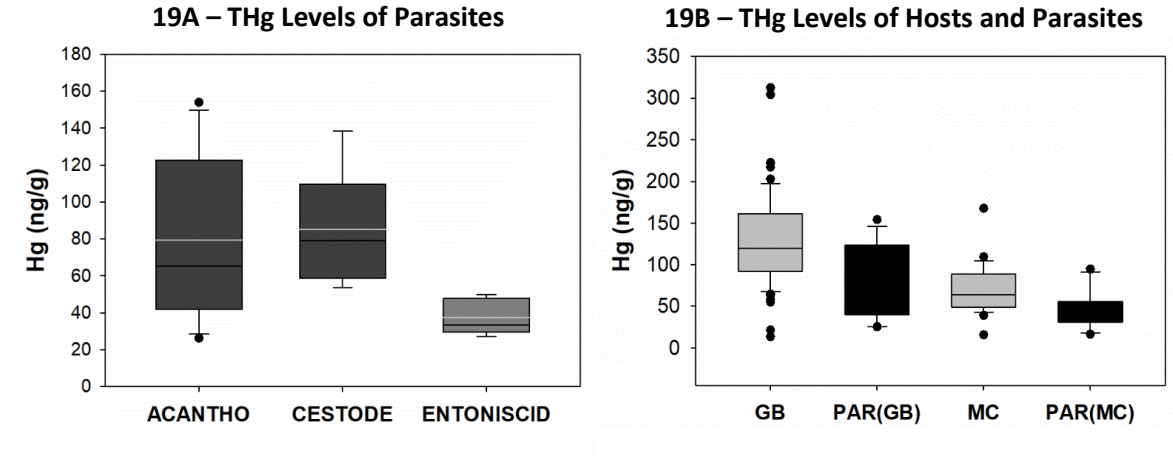

Figure 19: THg levels of different parasite taxa and hosts. A) The THg levels from naked goby parasites (acanthocephalan and cestode) and THg levels from mud crab parasties (cestode and entoniscid). B) THg levels from host naked gobies (GB) and parasite tissues PAR(GB), and THg levels from host mud crabs (MC) and parasite tissues PAR(MC).

Naked gobies were the only species which were analyzed for THg over the course of 4 sampling events (2 in spring and 2 in summer) (Figure 20). There was not enough time to analyze naked gobies for THg from all seasons, so a small subset was measured from only two seasons. I have separated the naked gobies by the rivers from which they were collected. The sites are a proxy for salinity and are arranged from oligohaline on the left to mesohaline on the right (increasing numbers). In the Neuse River, the THg levels were found to decrease from the first site in both uninfected and infected gobies (Figure 20A), although the THg leveled out at higher salinities. There was no trend in data from the Pamlico River for naked gobies which were uninfected (Figure 20B). The infected naked gobies from the Pamlico River showed a trend of increasing THg with the increase in salinity (Figure 20B), suggesting that infected fish take in more mercury at higher salinities.

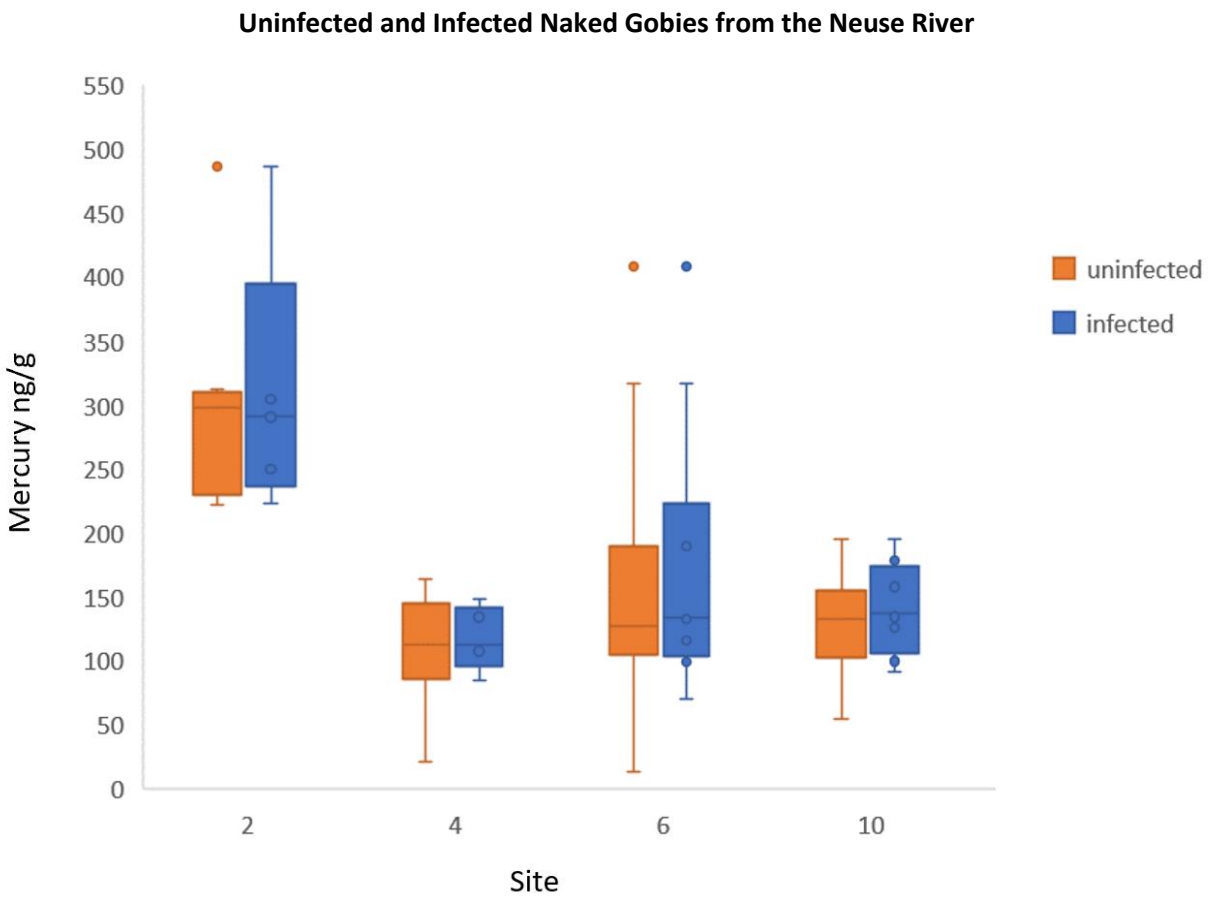

Figure 20A: Naked goby THg levels with increasing salinity. Site is a proxy for salinity and the increase in site represents an increase in salinity. In the Neuse River, sites are as followed: 2 – New Bern, 4 – Flanners Beach, 6 – Cahooque Landing, and 10 – Pin Oak Court.

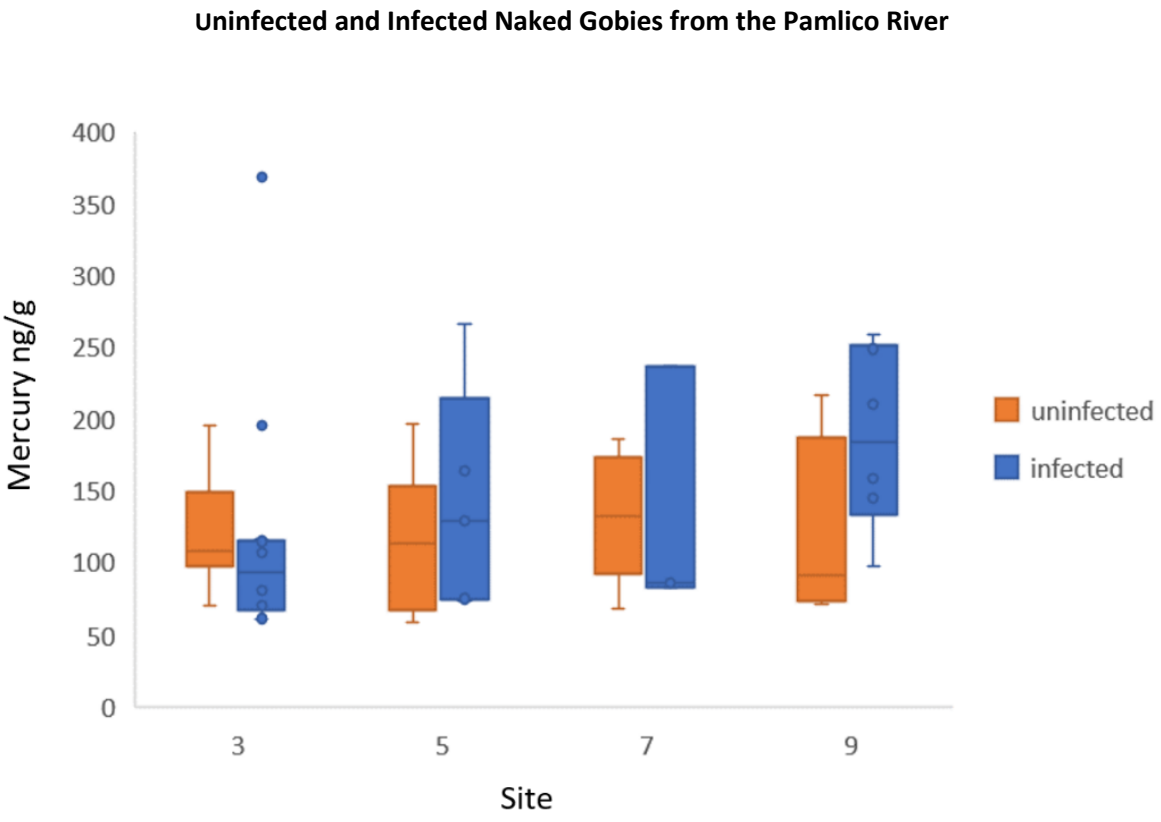

Figure 20B: Naked goby THg levels with increasing salinity. Site is a proxy for salinity and the increase in site represents an increase in salinity. In the Pamlico River, sites are as followed: 3 – Mallard Creek, 5 – North Creek Landing, 7 – Wrights Creek, and 9 – Swan Quarter National Wildlife Refuge.

There were multiple parameters which were taken into account during this study, and AICc scores were used to determine which parameters were the most influential on levels of mercury in naked gobies and mud crabs (Table 5A & Table 5B). The best models were selected based on lowest weights. AIC is known to sometimes overfit models and favors those with more parameters (Staniczenko et al. 2014). Rather AICc is a better choice for smaller sample size (Hurvich and Tsai 1995). Environmental research often exhibits overdispersion which usually leads to overly complex models (Song et al. 2017). The different predictors, as seen in the tables below, were used in various combinations to determine the model with the highest model weight for the naked gobies and mud crabs. In naked gobies, multiple environmental parameters (sample month, DOC, DO, and the interaction of salinity and DOC), along with the infection abundance, had the highest model weight, with DOC, salinity*DOC, and infection abundance being significant predictors at $p<0.05$. For mud crabs, these same environmental parameters (except for sample month) were represented in the top model, along with parasite presence/absence information. For naked gobies, when parasite richness was included, the model weight actually went down from 0.663 to 0.313. Without including any parasite data, the model weight was much lower (0.0000449) compared to the other models. For mud crabs, the model which included parasites had a weight of 0.827. Without parasites the weight goes down to 0.058. This demonstrates how important parasites are and that they should be taken into account for future ecological studies.

Table 5: AICc models and weights. Bolded parameters are significant at $p < 0.05$. A) The two top models for naked gobies, with the environmental model without parasites displayed for comparison purposes. B) The top three models for mud crabs, demonstrating that the model which includes parasite status has a high model weight.

Table 5A, naked gobies

Model Name	Model Parameters	Model Weight (w_i)
Environmental + Infection Abundance	Month, Salinity, **DOC**, dO, **Salinity*DOC**, **Infection Abundance**	0.663
Environmental + Infection Abundance + Parasite Richness	Month, Salinity, **DOC**, dO, **Salinity*DOC**, **Infection Abundance**, Parasite Richness	0.313
Environmental Only	Month, salinity, DOC, dO, Salinity*DOC	0.0000449

Table 5B, mud crabs

Model Name	Model Parameters	Model Weight (w_i)
Environmental + Parasite Presence/Absence	**Salinity, DOC, dO, Salinity*DOC, Parasite Present/Absent**	0.827
Environmental + Crab Sex	**Salinity, DOC, dO, Salinity*DOC, Crab Sex**	0.079
Environmental	**Salinity, DOC, dO, Salinity*DOC**	0.058

Conclusion

For coastal communities a major impacting factor for the environment is sea level rise. Sea level rise can potentially affect parasite communities and methyl-mercury production, and I have looked at both over the course of one year. NOAA records sea level trends from around the world, and their closest data point to my sites in the Neuse and Pamlico Rivers is located in Beaufort, North Carolina. Sea level rise in Beaufort is expected to change by an additional 3-6 mm/yr (Sea Level Trends - NOAA). Changes in salinity can affect sulfate reducing bacteria, and inorganic mercury which is available for methylation (Boyd et al. 2017). Moreover, at lower DOC/higher salinity levels, sulfate reducing bacteria methylate mercury more efficiently (Zhao et al. 2017). With that, Cossa et al. (2014) has noted that DOC concentration is not an absolute predictor of MeHg levels in estuaries. Multiple factors are at play for mercury levels in estuaries and wetlands, including run-off from various cities upriver.

To answer my first objective, water samples collected from my sites were analyzed for DOC; all of which showed a negative relation between salinity and DOC (Figure 12). My higher salinity sites had lower DOC, compared to my lower salinity sites which had higher DOC. At my lower salinity sites THg and MeHg levels in sediments were higher than THg and MeHg in sediments from higher salinity sites (Figure 11). Lower salinity sites may be exposed to higher DOC levels and a slight influx of salinity as sea levels rise. In the Neuse River, New Bern had the highest amount of THg while higher salinity sites remained lower in THg (with the exception of Matthew's Point Marina, which could be due to pollution, as this is a public marina with a number of moored boats). The Pamlico River had much higher THg levels within sediments compared to the Neuse River. This may be due to higher salinity levels or high DOC, and mercury runoffs from upriver. These rivers should continue to be monitored to see if these trends

are held steady, or if they are changing. Higher levels of MeHg in estuaries will bioaccumulate to commercially important species which may eventually be consumed by humans.

Uninfected naked gobies and mud crabs did not present any strong relationships between THg levels along a salinity gradient (Figures 14 & 15). The naked gobies did show a slight decrease in THg levels with increasing salinity. In a study by Rumbold et al. (2018) the THg levels of predatory fishes show the same trend. Mud crabs had a slight increase in THg with an increased in salinity. Again, the sample size for naked gobies was larger than the sample size of mud crabs, which could have played a role in the results. These results are very important because these species are benthic dwelling, and mercury bioaccumulation is a bottom up process (Chasar et al. 2009; Rolfhus et al. 2011). Biota samples, which were analyzed from spring and summer sampling events, were selected based upon research by Cesário et al. (2016), that the increase in temperature during summer months encourages mercury methylation because of increased microbial activity.

To answer my second objective, which focuses on the influence of salinity on parasite communities and on mercury levels in parasitized and unparasitized hosts within two North Carolina estuaries, I created tables showing parasite abundance and prevalence in my resident hosts species (Tables 3 & 4). In mud crabs, I observed an increase of infection prevalence with increasing salinity. This was the case for the rhizocephalan parasite; I expected prevalence would decrease at lower salinities, thus providing refuge for hosts as observed in other studies (Tepolt et al. 2019; Blakeslee et al. 2021). Having refuge from parasites is helpful, but it may come with trade-offs like lower reproductive success in less favorable salinities (Gelin et al. 2001). Rhizocephalan were known to infect crabs at salinities ~10 ppt and above (Tepolt et al. 2019), and I observed this at my sites, where rhizocephalans infected mud crabs at Swan Quarter

National Wildlife Refuge in the Pamlico River and Pin Oak Court in the Neuse River, both sites had average salinities surpassing 10 ppt. This expected declining parasite abundance was not the case in the Pamlico River, due to entoniscid isopod parasites being observed at all sites (most commonly observed at oligohaline sites). I found that parasite abundance in naked gobies positively correlated with THg levels in host tissues (Figure 16), which may be due to immune suppression (Roales and Perlmutter 1977). Additionally, simply having parasites can also hinder the host's immune system (Rigaud and Moret 2003), making hosts prone to even greater parasite infections. Although, more research needs to be conducted to determine what the threshold is in my host organisms for which mercury and parasitism affect their hosts' immune systems.

Interesting when comparing the results of THg levels in uninfected hosts to infected hosts, mud crabs, naked gobies, and blue crabs showed opposite results to what I had initially predicted. The mercury levels of uninfected hosts had lower levels of THg compared to infected hosts (Figures 17 & 18). This suggests that parasites may not be taking in as much mercury from their hosts as previously believed, and there may be other factors impacting the THg levels in uninfected and infected hosts. More research is needed to determine the pathways which may affect how much mercury is taken in by parasites.

There were no trends in parasite abundance or prevalence of naked gobies when comparing the data to salinity. Naked gobies hosts were rarely seen at my lowest salinity sites, and this is also what Moore et al. (2018) observed in the Pamlico and Neuse Rivers in North Carolina. For future studies, host diet should be considered, as it can potentially have an influence on parasite communities (Friesen and Roth 2016). More research needs to be done with the naked gobies to be able to determine how parasite communities change with salinity. While

the sample size for blue crab parasite data was too small to draw any conclusions, I did observe some cestodes and nematodes in their tissues.

The majority of mud crab parasites detected in my investigation were parasitic castrators, which use their host as a vessel to reproduce their own offspring (Lafferty and Kuris 2009). Entoniscid isopods take up almost half the interior space of the crab. This is not the case for naked gobies, which I observed most often in the intestinal tract, or specific to one organ in the fish. Yet Figure 19A shows the average THg in entoniscids have little more than half the levels seen in the naked goby parasites. There were not enough rhizocephalans parasite from the mud crabs to see trends in the data. The only two rhizocephalan externa (parasite reproductive structure) analyzed for mercury, had THg levels of 16.5 ng/g and 37.0 ng/g.

To help understand the importance of my data, AICc models were very informative as to what predictors were most impactful. The strongest model for naked gobies and mud crabs included parasite infection abundance or infection status (Tables 5A-B). This is important because parasites play an integral role in communities and food webs (Lafferty et al. 2008). With the addition of parasites in food web analyses the length of trophic chains extends (Williams and Martinez 2004). Parasites are often ignored in studies on natural systems, but my study is one of the many that continues to show that parasites should be considered in future research.

This study provides insight to better understand parasite diversity in estuary systems. I have presented data which has contradicted past work on parasites' potential to remove mercury from their hosts. However, more research is still needed to understand this relationship. I have also demonstrated that in some cases mud crabs may find refuge from their rhizocephalan hosts at lower salinities, which concurs with the literature; I have found that mud crabs may not escape

entoniscid isopod parasites as they were seen at all sites. Although they may seem insignificant, parasites make up a large portion of biomass in an estuary (Kuris et al. 2008).

My results can be used for understanding parasite communities in coastal regions around the world. This work will be important to coastal communities and management agencies, as it helps to understand the distribution of mercury in an estuary, and the bioaccumulation of mercury for commercially important/consumed species. Mercury levels in an estuary should be monitored through multiple trophic levels to better understand where mercury is bioaccumulating most. By doing this, mercury pollution can be mitigated and toxic levels in fish consumed by humans can be prevented.

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