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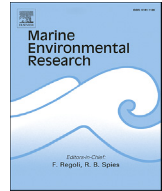

Influence of ontogeny and environmental exposure on mercury accumulation in muscle and liver of male Round Stingrays

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ABSTRACT

Mercury tissue distribution and its dynamics are poorly understood in elasmobranchs. Total mercury was measured in liver and muscle of male Round Stingrays (*Urobatis halleri*) from Seal Beach, California, an anthropogenically impacted site, and from the offshore island of Santa Catalina, a less impacted site. Stable isotope analysis was also performed on the muscle and red blood cells (RBCs) of a subset of rays over a range of age classes to investigate mercury accumulation with respect to trophic ecology. Mercury in both tissues was found to be significantly greater in adults than juveniles in mainland rays; however, liver mercury accumulation drastically increased after maturity and was significantly greater in mainland adult rays than Catalina rays. There were no patterns in $\delta^{15}N$ or $\delta^{13}C$ with size in muscle; however, there were indications of seasonal changes in RBC $\delta^{15}N$, suggesting short term shifts in diet or behavior is likely linked to reproductive status as is mercury accumulation.

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1. Introduction

Mercury is released into the environment both by natural (e.g. volcanic eruptions) and anthropogenic routes (e.g. burning of fossil fuels, artisanal small-scale gold mining), with wide-ranging and important effects on animal and ecosystem health (Driscoll et al., 2007). While mercury is released in its inorganic form (devoid of an alkyl group) into the environment, mercury can be converted into its organic, methylated form (methylmercury) through microbial activities. Methylmercury readily accumulates in organisms' tissues over time (bioaccumulation) and magnifies in concentration (biomagnifies) through food webs as well. Certain life history characteristics of animals can further exacerbate mercury bioaccumulation. For example, animals that are long lived or inhabit areas with high anthropogenic inputs of mercury may be more prone to bioaccumulate mercury (Burger et al., 2001; Mol et al., 2001). In particular, marine fishes that occupy higher trophic levels are well documented to accumulate large amounts of mercury in their muscle (Bergés-Tiznado et al., 2015; Bosch et al., 2016; Monteiro and Lopes, 1990). Ontogenetic changes in an animal's trophic ecology or habitat use can, therefore, change its potential to accumulate mercury. Because patterns of mercury accumulation are influenced by a variety of biological and ecological factors, an integrative approach that combines mercury analysis with other analytical tools provides a better pathway to understanding dynamics of mercury accumulation in organisms. Stable isotope analysis (SIA) is a tool that is often used to detect shifts in diet, trophic level, and habitat use in marine species (Michener et al., 2007), and since mercury accumulation is closely tied to diet, SIA has been a useful tool for studying mercury accumulation (Atwell et al., 1998; Jarman et al., 1996).

Due to methylmercury's potent neurotoxic effects (Castoldi et al., 2001), research into mercury levels in wildlife, and fish in particular (Chan et al., 2003), has generally been biased towards topics related to human consumption concerns. While this focus has provided much insight into how mercury accumulates in many species, it has been fairly focused on accumulation in muscle, as this tissue is mainly consumed by people. Because of methylmercury's affinity for proteins (Clarkson and Magos, 2006), the manner in which mercury accumulates in the different tissues can be quite complicated and has been generally understudied. In particular, the role that the liver plays in influencing organismal mercury dynamics is poorly understood, which is an issue as it functions as a mercury depot and plays an important role in mercury excretion. While other tissues can assist with mercury excretion (e.g. hair, nails, urine; Magos and Clarkson, 2008; Morton et al., 2004), the

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liver is the main organ equipped to demethylate mercury and process it for excretion or detoxification (Clarkson and Magos, 2006). During demethylation, the liver can form inert mercury crystals by conjugating inorganic mercury to selenium (Palmisano et al., 1995) or to other peptides and proteins such as glutathione or metallothionein, respectively, (Ballatori and Clarkson, 1985; Hogstrand and Haux, 1991), all of which can accumulate in the liver. In these situations the liver can drastically increase its mercury concentration, despite the fact that mercury is in a less harmful chemical state, making the liver a potentially important organ to consider in mercury accumulation studies.

Mercury accumulation in k-selected elasmobranchs (sharks, skates, and rays) has long been known (Walker, 1976). The high levels found in these animals is in part related to their long life span, giving mercury the opportunity to bioaccumulate for longer periods, and their often upper trophic level position in food webs, allowing mercury the potential to biomagnify from prey to predators. However, many of these studies focus on muscle accumulation and few have examined dynamics between muscle and liver (Endo et al., 2016, 2015). While some studies have previously documented changes in liver and muscle mercury accumulation with ontogeny in sharks (Boush and Thieleke, 1983; Endo et al., 2008; Horvat et al., 2014), much remains unknown about the factors that influence these patterns. In addition, sharks have been the focus of most mercury studies because they are most frequently consumed by humans, leaving other elasmobranchs, such as rays, understudied.

In the present study, we used the Round Stingray (*Urobatis halleri*) to examine mercury accumulation dynamics in muscle and liver (the two largest tissues in these animals) throughout ontogeny to identify important life history events that may influence accumulation. SIA was used as a tool to investigate the role that changes in habitat or trophic ecology may play in influencing patterns of mercury accumulation. We also sampled stingrays from two local populations of varying anthropogenic influence within the southern California region to examine the potential effect of location on mercury accumulation. Finally, we examined selenium-mercury relationships with respect to location and size to generate hypotheses about how stingrays biochemically deal with mercury contamination.

2. Methods

2.1. Study sites

Stingrays were sampled from two different sites with varying anthropogenic influence (i.e. high and low contamination levels) within southern California (Fig. 1). Mainland stingrays (n = 77) were sampled from a known aggregation area at Seal Beach (Hoisington and Lowe, 2005) that represented our site of high anthropogenic influence (i.e. contaminated site, hereafter "mainland") due to the large amount of human activity in the Los Angeles and Orange County area. For our site of low anthropogenic influence (i.e. uncontaminated), stingrays (n = 10) were sampled at Empire Landing on Santa Catalina Island (hereafter "Catalina"), located approximately 35 km offshore from Los Angeles, California. Stingrays from this island location have been shown to be genetically distinct (Plank et al., 2010) and the deep trench between the island and the mainland is thought to be a barrier to stingray movements. Catalina is a relatively pristine environment with localized human activity, and Empire Landing is located between two of its main villages (Avalon and Two Harbors). Only males were examined in this study to prevent the effects that maternal offloading (Horvat et al., 2014; Lyons and Lowe, 2013) would have on altering mercury accumulation patterns.

2.2. Tissue sampling

Male stingrays were captured via beach seine or hook and line in conjunction with a previous study (Lyons et al., 2014) over a range of their life history stages from juveniles to adults (disk width 11.4–25 cm, n = 77), and only adults were sampled from Catalina (n = 10). Note that Round Stingrays are born at a size of approximately 8–9 cm disk width. Stingrays were sampled in May to June (n = 23), and October (n = 3) of 2010, May to October (n = 57) of 2011 and February of 2012 (n = 4). Upon capture, stingrays were immediately brought back to California State University Long Beach where they were subsequently euthanized (IACUC protocol #273) and samples of muscle and liver were collected and frozen at −20 °C until analysis. Where feasible, samples of red blood cells (RBCs) were obtained via cardiac puncture and subsequent separation from plasma through centrifugation.

2.3. Elemental analysis

Total mercury was determined by first digesting a subsample (~0.5 g) of muscle or liver in a 15 mL 9:5:1 mixture of water, trace metal grade nitric acid and hydrochloric acid, respectively, in a MARS 5 microwave reaction system (CEM Corporation, Matthews, NC). Samples were preserved in 2% nitric acid until analysis. Samples were analyzed for total mercury using a Hydra AF Gold + Automated Mercury Analyzer (Teledyne Leeman Labs Inc, Hudson, NH) and accompanying WinHg software using EPA mercury analysis method 245.7. Blanks, duplicates, and a certified reference material (DOLT-3 [3.37 ± 0.14 mg/kg] and DORM-2 [4.64 ± 0.26 mg/kg]) were run in tandem with samples to ensure data quality control. The coefficient of variation in replicate samples was relatively low for muscle (13.9 ± 13.6%) and liver (9.9 ± 7.9%). CRM recoveries for DOLT and DORM were within 9.2 ± 5.1% and 10.2 ± 5.9% of certificate values and within acceptable ranges defined by the US Environmental Protection Agency (EPA). Blanks had mercury levels below detection or <1.6% of sample values.

A subset of liver (n = 36) and muscle samples (n = 9) analyzed for mercury were also analyzed for selenium (^{78}Se) in rays sampled at Seal Beach (n = 27) and Catalina Island (n = 10). Similar to above, a subsample of tissue (~0.5 g) was digested in an acid-water mixture and analyzed using an Agilent 7500CE ICPMS after 22x dilution with ^{103}Rh and ^{169}Tm used as the internal standards. Blank spikes were within 0.99 ± 0.72% of spiked value (i.e. 100 ppb) and replicates were within 3.0 ± 1.8% of each other. Measured DORM-2 values slightly exceeded reported values (18% of actual), but was within acceptable EPA range of under 20% (US EPA, 1991).

2.4. Stable isotope analysis

Samples of RBCs and muscle were taken from a subset of stingrays over nine 0.5 cm disk width (DW) bins from 11.5 to 22.4 cm DW, with approximately three stingrays per bin. Where possible, RBCs and muscle samples were taken from the same individual (n = 32), but some stingrays were only sampled for RBCs (n = 7) or muscle (n = 7). RBCs analyzed for SIA were sampled from stingrays collected in May (n = 19), June (n = 16), August (n = 3), and September (n = 1) 2010 and 2011. Muscle samples were sampled during the months of May (n = 19), June (n = 19), July (n = 1), and September (n = 1) 2010 and 2011. Two adult male stingrays from Catalina had paired samples of RBC and muscles analyzed sampled in October, 2010, and August, 2011. RBCs and muscle were chosen to make short-term and long-term trophic ecology inferences, respectively, due to the difference in turnover rate between these two tissues.

Deionized water rinses were used to extract urea from muscle

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