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An evaluation of mercury offloading in two Central California elasmobranchs

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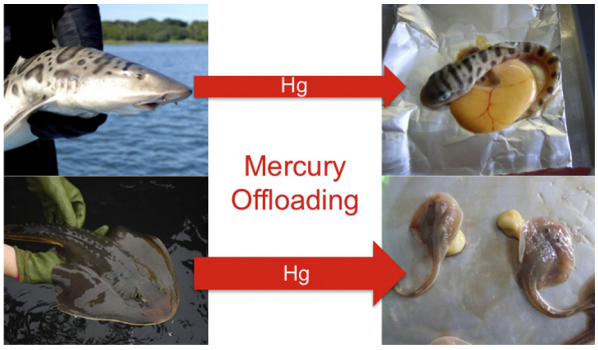
HIGHLIGHTS

- An investigation of maternal mercury transfer in sharks and batoids, and influencing factors
- Behavior of mercury in adult tissues differed between the two species.
- Embryos of both species had potentially harmful mercury concentrations.
- Female mercury concentration may be a significant factor for offloading.

GRAPHICAL ABSTRACT



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ABSTRACT

Elasmobranchs occupy high trophic levels, accumulate high concentrations of mercury in their tissues, and have high energetic levels of maternal investment to offspring, which may cause embryos to be exposed in utero to harmful concentrations of mercury. We investigated the maternal transfer of mercury in two common coastal elasmobranch species, *Triakis semifasciata* and *Platyrhinoidis triseriata*, to determine which reproductive parameters may influence mercury offloading, and whether embryos are at risk to mercury toxicity. Mercury concentration was measured in female muscle, female liver, and embryonic tissues. The behavior of mercury in adult female tissues differed between species, as liver mercury concentration was significantly correlated to muscle mercury concentration in *P. triseriata* but not in *T. semifasciata*. Embryos of both species were found with potentially harmful mercury concentrations in their muscle tissues. Embryo mercury concentration increased with female muscle mercury concentration, but the relationship to female liver mercury was more variable. The rate of mercury transfer and overall offloading potential were significantly greater in *P. triseriata* than *T. semifasciata*. It appears that female mercury concentration, either in muscle or liver, is an important influencing factor for mercury offloading, but the impact of the differing reproductive modes in these two species was less clear. More study on this subject will continue to elucidate the factors influencing mercury offloading in sharks and rays, and how contaminant risk affects populations on a whole.

1. Introduction

Mercury (Hg) is a toxicant found in marine environments that can be transformed to methylmercury (MeHg) via bacterial methylation

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and then rapidly absorbed into the tissues of organisms through their diet (Mason et al., 1995; Wiener et al., 2003; Lavoie et al., 2013). This biomagnification of methylmercury can result in high concentrations in the tissues of predators, such as marine mammals (Brookens et al., 2007; Loseto et al., 2015) and sharks (Storelli et al., 2003; Mull et al., 2012). In sharks, the proportion of methylmercury in total mercury in muscle tissue is over 90%, possibly leading to a greater neurotoxicity risk (Pethybridge et al., 2010a; De Carvalho et al., 2014). High methylmercury, herein mercury, exposure during embryonic developmental stages can reduce foraging and reproductive success, alter sex ratios, cause morphological defects, and increase mortality (Fjeld et al., 1998; Matta et al., 2001; Gelsleichter and Walker, 2010). Embryos can be exposed to mercury from maternal offloading, which has been demonstrated in a variety of taxa (Knott et al., 2012; Sackett et al., 2013; Frías-Espericueta et al., 2015). This is especially a concern in species that occupy high trophic levels, accumulate high concentrations of mercury in their tissues, and have high energetic levels of maternal investment.

Elasmobranchs (sharks and batoids) invest high levels of maternal energy, producing well-developed neonates with gestation times ranging from a few months to more than a year (Callard et al., 1988; Wourms and Demski, 1993; Hussey et al., 2010). During energy provisioning of the embryo, developing oocytes take up vitellogenin, a lipophosphoprotien yolk-granule precursor that is produced in the female's liver (Hamlett and Koob, 1999; Pethybridge et al., 2010a). During this process, toxins including mercury that bind to the vitellogenin are transferred to developing embryos (Pethybridge et al., 2010a, Pethybridge et al., 2011; Lyons and Lowe, 2013a). Since sharks and batoids are mostly high trophic level predators (Cortes, 1999; Ebert and Bizzarro, 2007), mercury often accumulates in concentrations over 1.0 μg g^{-1} in muscle tissue (Hueter et al., 1995; Storelli et al., 2011; Rumbold et al., 2014), with reports of concentrations higher than 10.0 μg g^{-1} in the muscle tissues of White Sharks (Mull et al., 2012) or hammerhead sharks (Storelli et al., 2003). These high levels, combined with high maternal investment, may cause embryos to be exposed in utero to concentrations of mercury that could cause morphological defects or reduced survival (Fjeld et al., 1998; Matta et al., 2001; Sandheinrich and Wiener, 2011). However, studies have shown that transfer rates can be variable between species (Hueter et al., 1995; Adams and McMichael, 1999). Work has been done with organic contaminants to determine total offloading potential, or the maximum proportion of contaminants females are able to eliminate via reproduction (Lyons and Lowe, 2013a), but this parameter has not been calculated for mercury.

Sharks and batoids employ a variety of reproductive modes, from lecithotrophy where embryos derive nutrition solely from a yolk sac inside the uterus, to matrotrophy where females provide additional nutrition such as in oophagy, histotrophy or placental viviparity (Hamlett et al., 2005). These differences in the amount of nutrition invested in embryos could affect contaminant transfer as well. Female mercury tissue concentration, and by extension trophic position may also affect mercury offloading. Offspring of marine mammals and shark species that feed at higher trophic levels have been shown to have higher levels of mercury and other contaminants in their tissues (Lyons et al., 2013; Ylitalo et al., 2001). Additionally, a female's reproductive history could have an effect on the transfer of mercury to offspring. Females have been shown to offload the greatest proportion of lipid-associated contaminants, such as PCBs and DDTs, during their first reproductive event (Larsson et al., 1993; Nakata et al., 1995; Lyons and Lowe, 2013a). While this relationship may be different for potentially toxic elements such as mercury, it may still be an impacting factor. It is currently unclear which characteristics are the most important in determining the maternal transfer of mercury in elasmobranchs.

This study investigated the maternal transfer of mercury in two common coastal elasmobranch species, *Triakis semifasciata* (Carcharhiniformes: Triakidae) (leopard sharks), and *Platyrhinoidis triseriata* (Torpediniformes: Platyrhinidae) (thornback rays). These species have similar diets consisting mainly of crustaceans and small teleost fishes, and gestation periods of 10–12 months (Ebert, 2003). *Triakis semifasciata* litters consist of 4 to 37 pups with pupping occurring during the spring, while *P. triseriata* litters consist of 1 to 17 pups with pupping occurring during the summer (Ebert, 2003; Ebert et al., 2013). The reproductive modes of these species differ slightly. While both species are considered yolk-sac viviparous, *T. semifasciata* also exhibits limited histotrophy. Reproductive modes in the family Triakidae evolved to placental viviparity, but this has been lost in a few species, including *T. semifasciata*, where limited histotrophy remains (Musick and Ellis, 2005; López et al., 2006). Egg envelopes typical of placental species have been observed in *T. semifasciata* (Ebert and Ebert, 2005), and in this form of reproduction the embryos absorb a mucous secretion produced by the uterus (Musick and Ellis, 2005). This limited or mucoid histotrophy differs from the lipid histotrophy observed in some Myliobatid rays where embryos receive a substantial protein and lipid rich investment from the mother in addition to the yolk sac (Musick and Ellis, 2005). The amount of additional material that *T. semifasciata* embryos receive from limited histotrophy is unknown, as it is likely species specific. However, as reproduction in *P. triseriata* is considered to be yolk sac viviparous (Ebert, 2003; McEachran and Aschliman, 2004; Musick and Ellis, 2005) without additional nutrient input from the female, this difference may have an effect on overall contaminant transfer.

The goals of this study were to 1) establish mercury concentrations in the embryos of *T. semifasciata* and *P. triseriata* throughout the gestation period of both species to understand mercury partitioning in offspring during development, 2) calculate the percent transfer of mercury and the total offloading potential of each species, and 3) determine whether female parameters such as liver mercury, muscle mercury, or female size influence this transfer.

2. Materials and methods

2.1. Sample collection and processing

Pregnant female *T. semifasciata* ($n = 10$) and pregnant female *P. triseriata* ($n = 11$) were collected by gill net from March 2013 through July 2014 in Elkhorn Slough, a shallow, tidally influenced estuary near Moss Landing, California, USA (36° 49′ 33.24″N 121° 45′ 24.84″W). Elkhorn Slough is a pupping/nursery ground for both species. Pregnant females were euthanized according to SJSU IACUC protocol #991 and transported to Moss Landing Marine Laboratories (MLML) for processing. Specimens were dissected in the lab for tissue samples on a 1% micro-cleaned surface with 1% micro-cleaned stainless steel tools to prevent mercury contamination. Total length measurements and weights for total body, liver, ovaries, and uteri were obtained. Samples of white muscle tissue were taken from the right dorsal side of the sharks just below the dorsal fin origin and from the right wing close to the midline of the body in rays. Liver samples were taken from the center of the left lobe of the liver of each individual, or if the liver was <100 g, the entire liver was retained. Reproductive tissue was separated into ova from the ovaries, and yolk sacs with/without developing embryos from the uterus. The number of embryos found in *T. semifasciata* ranged from 14 to 31, with an average of 26, while the number of embryos found in *P. triseriata* ranged from 2 to 17, with an average of 10. All samples were wrapped in aluminum foil to prevent contamination and stored in a −20 °C freezer until further processing.

2.2. Sample preparation

Liver and muscle tissue of adult female *T. semifasciata* and *P. triseriata* were analyzed for total mercury (THg) using a Milestone® Direct Mercury Analyzer 80 (DMA) housed at the Marine Pollution Studies Laboratory at MLML. Liver tissues were homogenized prior to mercury analysis. For embryo tissue types (outlined below), embryos from a

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