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Perfluorinated alkyl acids and fecundity assessment in striped mullet (*Mugil cephalus*) at Merritt Island national wildlife refuge

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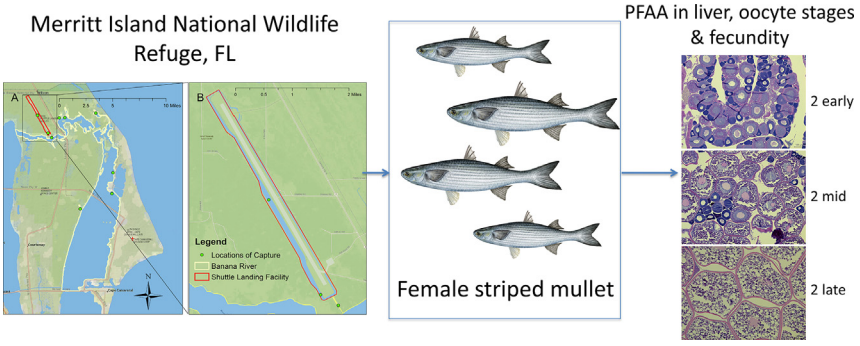
HIGHLIGHTS

- High liver PFOS in Striped mullet (median, 124 /g; range, 12.6–2770 ng/g)
- Liver PFOA, PFNA, & PFTriA increase with increasing oocyte development.
- Liver PFOS and PFOSA decrease with increasing oocyte development.
- No significant negative impacts of liver PFAA on wild-caught, mullet fecundity

GRAPHICAL ABSTRACT



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ABSTRACT

This study investigated wild caught striped mullet (*Mugil cephalus*) at Merritt Island National Wildlife Refuge (MINWR) for levels of 15 perfluoroalkyl acids (PFAA) in tandem with individual fecundity measurements (Oocyte sub-stage 2 late, $n = 42$) and oocyte reproductive stages (Stages 1–5, $n = 128$). PFAA measurements were quantified in striped mullet liver ($n = 128$), muscle ($n = 49$), and gonad ($n = 10$). No significant negative impacts of liver PFAA burden on wild-caught, mullet fecundity endpoints were observed in this study; however, changes in PFAA were observed in the liver as mullet progressed through different sub-stages of oocyte development. Of the PFAA with significant changes by sub-stage of oocyte development, the carboxylic acids (perfluorooctanoic acid, perfluorononanoic acid, and perfluorotridecanoic acid) increased in the liver with increasing sub-stage while the sulfonic acid and its precursor (perfluorooctanesulfonic acid (PFOS) and perfluorooctanesulfonamide, respectively) decreased in the liver with increasing sub-stage of oocyte development. This is a unique find and suggests PFAA change location of compartmentalization as mullet progress towards spawning. Investigations also revealed higher than expected median muscle and gonad levels of PFOS in striped mullet collected at MINWR (9.01 ng/g and 80.2 ng/g, respectively).

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1. Introduction

Perfluoroalkyl acids (PFAA) are a commonly studied family within the larger group of chemicals known as perfluoroalkyl substances (PFAS). PFASs are organic chains (branched and linear) in which all hydrogen atoms attached to the carbon backbone have been substituted for a fluorine atom creating a carbon fluoride (C—F) bond. Two subclasses of the PFAA family that will be investigated in this study are perfluoroalkyl carboxylic acids (PFCAs) and perfluoroalkyl sulfonic acids (PFSAs). Structurally, PFCAs and PFSAs have the general chemistry formula $C_nF2_{n+1}COOH$ and $C_nF_{2n+1}SO_3H$, respectively (Buck et al., 2011).

With numerous applications in waterproofing, stain proofing, and firefighting products (Moody and Field, 2000; Kärrman et al., 2011; de Solla et al., 2012; Place and Field, 2012; Laitinen et al., 2014), PFAA have found their way into the environment (de Solla et al., 2012), humans (*Homo sapiens*) (Laitinen et al., 2014), and wildlife (Houde et al., 2011) across the globe. Recent investigations of PFAA levels in the American alligator (*Alligator mississippiensis*) in Florida and South Carolina revealed variations in PFAA burden by site, noting that alligators residing at Merritt Island National Wildlife Refuge (MINWR) maintained the highest PFAA burden compared to alligators present at other southeastern sampling sites (Bangma et al., 2017a). This would suggest that wildlife around MINWR is at higher risk to potential exposure to PFAA in comparison to other investigated sites within Florida and South Carolina. Within wildlife, studies have shown the highest levels of PFAA reside in protein heavy matrices such as the liver, kidney, and plasma (Kudo, 2015).

PFAA have shown a variety of health effects such as immunotoxicity (DeWitt et al., 2012), neurotoxicity (Liao et al., 2009), and reduced fertility and fecundity. These reduced fecundity rates, due to PFAA exposure, have been observed in human (Fei et al., 2009; Velez et al., 2015), copepod (*Tigriopus japonicas*) (Han et al., 2015), nematode (*Caenorhabditis elegans*) (Tominaga et al., 2004), and freshwater flea (*Hyalella azteca*) (Lee et al., 1986) studies, while some human (Whitworth et al., 2012) and zebra fish (*Danio rerio*) (Wang et al., 2011) studies have shown no adverse effects of the investigated PFAA on fecundity.

The pathways for possible mechanisms of action are still being elucidated for many of these effects. Some are peroxisome-proliferator activating receptor alpha (PPARα) dependent (Ren et al., 2009) and some are PPARα independent (Ren et al., 2009; Rosen et al., 2010). While PPARα is expressed in grey mullet (*Chelon labrosus*) liver and gonad tissue (Raingeard et al., 2006), potential PPARα independent mechanisms for changes in fecundity and fertility in teleosts have begun to be investigated as well. Changes in liver histology has been recorded in both male and female zebra fish exposed to perfluorooctanesulfonic acid (PFOS) (Cui et al., 2017), as well as changes in expression of vitellogenic genes recorded in tilapia (*Oreochromis niloticus*) hepatocytes (Liu et al., 2007) and zebra fish (*Brachydanio rerio*) livers (Cheng et al., 2012). In the case of the tilapia hepatocytes, the changes in expression of vitellogenic genes depended upon co-exposures with estrogen. While most of these studies have been conducted in a controlled laboratory setting, it is possible PFOS and other PFAA may impact or change a female teleost's fecundity through impacts on the liver and gonad in the wild.

To date, no study published has attempted to measure potential fecundity effects in a wild population. MINWR is ideally suited to investigate potential wildlife fecundity effects of PFAA due to the higher levels of PFAA measured in organisms (American alligators) compared to other locations in Florida and South Carolina (Bangma et al., 2017a). Therefore, this study aimed to investigate PFAA levels and fecundity measures in a locally abundant marine species that is a prey species of alligators at MINWR and is also consumed by local fishermen in the surrounding areas outside of MINWR. Of the several fish species present at MINWR that met both of these criteria, the striped mullet (*Mugil cephalus*), was among the earliest to mature and was also one of the few species that undergoes isochronal spawning. These qualities ensure minimal effect of sampling on the population and provide highly accurate fecundity measurements. Overall, this study aimed to investigate PFAA burden and fecundity endpoints in sexually mature, female striped mullet early in the spawning season at MINWR.

2. Materials and methods

2.1. Sample collection

Collections of striped mullet were conducted at MINWR under the protocol GRD-06-044 reviewed by the Institutional Animal Care and Use Committee (IACUC). Sampling occurred during October 24–28 ($n = 83$) and December 4–7, 2016 ($n = 45$) to ensure that samples were collected during the time period where reproductive development was occurring for the spawning season (McDonough et al., 2003). Striped mullet were obtained from numerous locations throughout the Banana River (BR), as well as from the drainage ditch that runs the length of the Shuttle Landing Facility (SLF) at Kennedy Space Center (Supplemental Information (SI), Fig. S1). Unlike the fish in the Banana River, that were free to move about the entirety of the estuarine system, the fish within in the SLF were trapped within the surrounding SLF drainage ditch and were unable to move outside of that area for years at a time (only during infrequent large flood events can mullet move in and out of the SLF). Fish were caught using a cast net ($n = 125$) as the primary form of sampling gear with a few adult mullet ($n = 3$) obtained using a 183-m haul seine. Samples obtained using a 183-m haul seine are a result of collaborations with FWC's Fish and Wildlife Research Institute (FWRI). Of the mullet captured, only adult female mullet larger than 30 cm were collected for this study to ensure that a high percentage of sampled mullet had reached sexual maturity (McDonough et al., 2005). Sex was assessed in the field by applying pressure to the abdomen and looking for the extrusion of milt or eggs (Kucherka et al., 2006).

All mullet identified as female were necropsied within 12 h of capture. Standard morphological measurements taken were total length (TL), standard length (SL), fork length (FL), total height (TH), and fish girth (FG) in cm, and fish weight (FW), liver weight (LW), and gonad weight (GW) in grams (g) (SI, Fig. S2). Fish girth was taken as fish circumference at the same location fish height was measured. Any subsequent mention of fish length in the remaining text will be total length unless otherwise noted. Sagittal otoliths were removed for estimating fish age (See Section 2.6. Aging). Livers were removed, using a clean stainless steel scalpel, stored in methanol rinsed foil, and frozen a −20 °C for later PFAA analysis. Gonads were collected and divided for analysis. One large section from the distal end of the left gonad was wrapped in methanol rinsed foil and frozen at −20 °C for later PFAA analysis. The whole right lobe of the gonad was weighed separately and preserved in 10% neutral buffered formalin (NBF) for fecundity counts. Additionally, a small section (~1 cm^3) from the posterior portion of the gonad, where the lobes were joined, was removed and fixed in 10% NBF for histological confirmation of sex and reproductive stage. Muscle was also removed, using a clean stainless steel scalpel, stored in methanol rinsed foil, and frozen a −20 °C for later PFAA analysis.

2.2. Chemicals

Two solutions, National Institute of Standards and Technology (NIST) Reference Materials (RMs) 8446 Perfluorinated Carboxylic Acids and Perfluorooctane Sulfonamide in Methanol and RM 8447 Perfluorinated Sulfonic Acids in Methanol were combined to create calibration solutions for liquid chromatography-tandem mass spectrometry (LC-MS/MS). analysis. The final solution comprised of 15 PFAA as follows: perfluorobutyric acid (PFBA), perfluoropentanoic acid (PFPeA), perfluorohexanoic acid (PFHxA), perfluoroheptanoic acid