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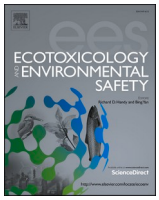

Differing behavioral changes in crayfish and bluegill under short- and long-chain PFAS exposures: Field study in Northern Michigan, USA☆

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ABSTRACT

The emergent contaminant family, per- and poly-fluorinated alkyl substances (PFAS) has gained research attention due to their widespread detection and stability within the environment. Despite the growing amount of research on perfluorooctanesulfonic acid (PFOS) and perfluoro-*n*-octanoic acid (PFOA) in aquatic organisms, investigations detailing behavioral and physiological effects of aquatic organisms exposed to a mixture of PFAS analytes in the wild have been limited. The objective of this study was to evaluate the potential behavioral and histological effects of environmental exposure to PFAS compounds within multiple trophic levels of aquatic ecosystems. The current study investigates effects of environmentally relevant PFAS concentration exposures in crayfish (*Faxonius immunis, F. rusticus, F. virilis*) and bluegill (*Lepomis macrochirus*) sourced from four water bodies in Northern Michigan. Antipredator response and foraging behavioral assays were used to investigate potential effects on crayfish; a swimming speed behavioral assay and liver and gill histology analysis were used to investigate potential effects on fish. Linear mixed model and multiple regression analyses resulted in significant relationships between tissue accumulation levels of long chain PFAS compounds and crayfish foraging and fish critical swimming speed responses. Crayfish foraging decreased and fish critical swim speeds increased with PFAS exposure which may lead to energetic and population concerns. Antipredator response in crayfish and liver and gill histology in fish were not significantly related to PFAS tissue or water concentrations. The sensitivity of crayfish and bluegill behavior contributes to the growing body of research regarding the differential toxicity of short-chain and long-chain PFAS compounds. The sensitivity of some aquatic organism behaviors to PFAS accumulated in tissue may have implications for PFAS transfer and alterations to ecosystem functioning; based on the results of this field study, further laboratory research is recommended to further evaluate these relationships.

1. Introduction

Per- and polyfluoroalkyl substances (PFAS) are a family of synthetic

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compounds that have been widely used in commercial products and industrial processes since the 1950 s (Munoz et al., 2015). There are over 4000 unique PFAS compounds, with applications including use in surfactants, lubricants, agrochemicals, cleaning products, cookware, food packaging, and firefighting foams (Gaballah et al., 2020). The stability of these compounds, their ubiquitous use, and persistence within the environment has led to large scale contamination (Hu et al., 2016). There are multiple pathways for PFAS-containing products to enter aquatic and terrestrial ecosystems and media (e.g., groundwater, surface water, and soil) (Filipovic et al., 2015). PFAS have been found to have a global distribution within aquatic ecosystems serving as important sinks for PFAS compounds. Since PFAS are proteinphilic, PFAS compounds have also been detected in the tissues and blood serum of a large range of biota including fish, birds, and mammals, including humans (Taniyasu et al., 2003). Animal exposure to and accumulation of PFAS is influenced by many factors, including biotransformation and biomagnification.

PFAS persistence in biota is due to the bioaccumulative properties associated with PFAS analytes (Kudo et al., 2001). Long-chain sulfonate groups have been found to exhibit greater bioaccumulation relative to PFAS containing shorter carbon chains and carboxylate groups (Conder et al., 2008). In the U.S., short-chain PFAS are defined as perfluorosulfonic acids with less than six carbons and perfluorocarboxylic acids with less than eight carbons (Li et al., 2020). Contrastingly, long-chain PFAS refers to compounds with greater carbon chain lengths, e.g., PFOA and PFOS. In some locations, the highest PFOS concentrations have been found in prey species, such as bluegill and aquatic insects, potentially due to differential contamination source signatures between trophic levels (Bush et al., 2015). In addition to the bioaccumulation of PFAS compounds, substances can also biotransform within aquatic food webs.

Polyfluoroalkyl substances are less stable or persistent relative to perfluoroalkyl substances; many polyfluoroalkyl substances can degrade and are precursors to perfluoroalkyl substances. At higher trophic levels, biotransformation is assumed to be more likely relative to lower trophic levels, which can confound bioaccumulation patterns (Tomy et al., 2004). Biotransformation, along with recently increased use of short chain PFAS in industry, may also be responsible for the wide distribution of these compounds, such as PFBS and perfluorobutanoic acid (PFBA), in the environment (Li et al., 2020). PFAS biomagnification and biotransformation are not only important to examine at the community-level, but also inform how organisms may be exposed and affected.

Toxic effects of various PFAS analytes have been documented, primarily in fish, however the fate, transport, accumulation, and full range of toxicity exhibited by PFAS analytes are not completely understood. PFAS have been associated with a variety of effects on fish physiology or behavior, as seen in altered body length, spinal curvature, and swimming performance after the exposure period ended (Hagenaars et al., 2014; Jantzen et al., 2016). PFAS contamination can also influence fish tissue health by causing increased liver lesions and presumably decreased liver function (Du et al., 2008). Specifically, among the investigations into the physiological impacts of long-chain PFAS compounds, PFOS and PFOA have gained particular research focus. PFOS and PFOA have been documented to have hepatotoxic, carcinogenic, developmental toxic, reproductive toxic, and neurotoxic effects in animals (Valdersnes et al., 2017). Changes in acute toxicity values with differing chain length, such as between short-chain PFAS (e.g., PFBA and perfluorohexanoic acid [PFHxA]) relative to PFOA, has been demonstrated with *Daphnia magna*, with toxicity decreasing with decreasing carbon chain length (Barmentlo et al., 2015). Long-chain perfluoroalkyl acid compounds have been shown to inhibit the bioconcentration of short-chain compounds in aquatic organisms (e.g., zebrafish) (Wen et al., 2017).

The degree of impacts to behavior and physiology may vary with individuals of various species as different patterns of bioaccumulation and biomagnification may lead to differing tissue concentrations within individuals., The primary research site in the current study is near the former Wurtsmith Air Force Base (the Base) in Oscoda, Michigan, USA. PFAS have migrated to the adjacent Clark's Marsh, Au Sable River, and surrounding areas from numerous different groundwater plumes. High PFOS levels in water and fish tissues in both the marsh and river resulted in a 'do not eat fish' consumption advisory from the State of Michigan. Previously sampled pumpkinseed and bluegill exhibited mean PFOS concentrations of nearly 5500 μg/kg ww, several times more than the highest consumption advisory thresholds of 300 μg/kg from Michigan's Department of Health and Human Services. Surface water PFAS concentrations have ranged from 3.23 to nearly 5100 ng/L PFOS at these sites (Bush et al., 2015). While broad effects of high level PFAS exposure

Nomenclature

8:2 diPAP	8:2 Fluorotelomer phosphate diester.
8:2 FTCA	8:2 Fluorotelomer carboxylic acid.
AIC	Akaike information criterion.
ASR	Au Sable River.
BAF	Bioaccumulation factor.
CLM	Clark's Marsh.
DoD ELAP	Department of Defense Environmental Laboratory Accreditation Program.
EtFOSAA	2-(N-Ethylperfluorooctanesulfonamido) acetic acid.
FTS	Fluorotelomer sulphonic acid.
LEH	Lamellar epithelial hyperplasia.
LMM	Linear mixed models.
MDEQ	Michigan Department of Environmental Quality.
MeFOSAA	2-(N-Methylperfluorooctanesulfonamido) acetic acid.
PET	Polyethylene terephthalate.
PFAA	Perfluoroalkyl acid.
PFAS	Per- and poly-fluorinated alkyl substances.
PFBA	Perfluorobutanoic acid.
PFBS	Perflurobutanesulfonic acid.
PFDA	Perfluorodecanoic acid.
PFDoA	Perfluorododecanoic acid.
PFDS	Perfluorodecanesulfonic acid.
PFHpA	Perfluoroheptanoic acid.
PFHpS	Perfluoroheptanesulfonic acid.
PFHxA	Perfluorohexanoic acid.
PFHxA	Perfluorohexanoic acid.
PFNA	Perfluorononanoic acid.
PFNS	Perfluorononanesulfonic acid.
PFOA	Perfluoro-*n*-octanoic acid.
PFOS	Perfluorooctanesulfonic acid.
PFPeA	Perfluoropentanoic acid.
PFPeS	Perfluoropentanesulfonic acid.
PFTeDA	Perfluorotetradecanoic acid.
PFTrDA	Perfluorotridecanoic acid.
PFUnA	Perfluoroundecanoic acid.
PIL	Pickerel Lake.
PMA	Pigmented macrophage aggregates.
PVC	Polyvinyl chloride.
SUL	Susan Lake.
Ucrit	Critical swimming speed.

are known, behavioral and physiological effects from PFAS mixtures at environmentally relevant levels have not been well studied. The objective of this study was to evaluate the potential behavioral and physiological effects of environmental exposure to PFAS compounds within multiple trophic levels of an aquatic ecosystem.

2. Methods

2.1. Experimental design

We collected wild animals (crayfish and bluegill) to investigate the effects of environmental exposure to PFAS on tissue accumulation, tissue lesions, and fitness-related behaviors. Both crayfish and bluegill were collected from four different waterbodies (Clark's Marsh, Au Sable River, Susan Lake, and Pickerel Lake) across Emmet, Charlevoix, and Iosco counties in Michigan, USA. Differences in foraging behavior and antipredator response of crayfish, and histological lesions and critical swimming speed of fish were compared across environments. Tissue PFAS concentrations were analyzed and used to investigate the relationship between behavior, physiology, and accumulation. A total of 17 crayfish (14 male, 3 female) and 36 bluegill from the four field sites were analyzed for this study (Table 1).

Table 1
Locations of bluegill (*Lepomis macrochirus*), crayfish (*Faxonius rusticus, F. virilis*, *F. immunis*), and surface water collection in July-September 2019 (animal) and February 2020 (water). Surface water sampling size was two independent samples for every surface water site. Crayfish behavior sample size is the number of crayfish per site that completed foraging and antipredator assays. Swimming sample size is the number of fish per site that completed the swimming trial. The histology sample size indicates the number of liver and gill samples per site. Fish and crayfish samples were pooled among locations at the same body of water.

Site Name	Latitude, Longitude	Animal Species or Surface Water	Behavior Sample Size (*n*)	Fish Histology Sample Size (*n*)
Clark's Marsh – Site 1	44.43783, −83.38853	*Faxonius immunis*	7	–
Clark's Marsh – Site 2	44.438076, −83.390103	*Lepomis macrochirus*	5	1 liver; 2 gills
Clark's Marsh – Site 3	44.437707, −83.38994	*Lepomis macrochirus*, Surface Water	5	2 livers; 1 gill
Au Sable River – Site 1	44.418956, −83.337097	*Lepomis macrochirus*	1	1 liver; 1 gill
Au Sable River – Site 2	44.43560, −83.439190	*Lepomis macrochirus*	6	2 livers; 2 gills
Au Sable River – Site 3	44.43209, −83.39156	*Faxonius rusticus*	6	–
Au Sable River – Site W	44.435878, −83.434643	Surface Water	–	–
Susan Lake – Site 1	45.329179, −85.179604	*Lepomis macrochirus*	8	3 livers; 3 gills
Susan Lake – Site W	45.328504, −85.180517	Surface Water	–	–
Pickerel Lake – Site 1	45.390555, −84.754775	*Lepomis macrochirus*, Surface Water	11	4 livers; 4 gills
Pickerel Lake – Site 2	45.38631, −84.75935	*Faxonius rusticus; Faxonius virilis*	3 (*F. rusticus);* 1 *(F. virilis)*	–

2.2. Study sites

Clark's Marsh and the Lower Au Sable River (i.e., in Oscoda, MI) were sampled as high contamination sites (Bush et al., 2015), and Susan Lake, near Charlevoix, Michigan, USA was chosen as a low contamination site (TOMWC, 2019). Clark's Marsh consists of three ponds and is adjacent and downgradient from a fire training area on the former Base. The marsh empties into the Au Sable River. Pickerel Lake, near Petoskey, Michigan, USA, served as a reference site, characterized with no urban and industrial land uses within its watershed and was assumed to have little to no contamination from historic uses. Two water samples were also collected at each water body (Table 1).

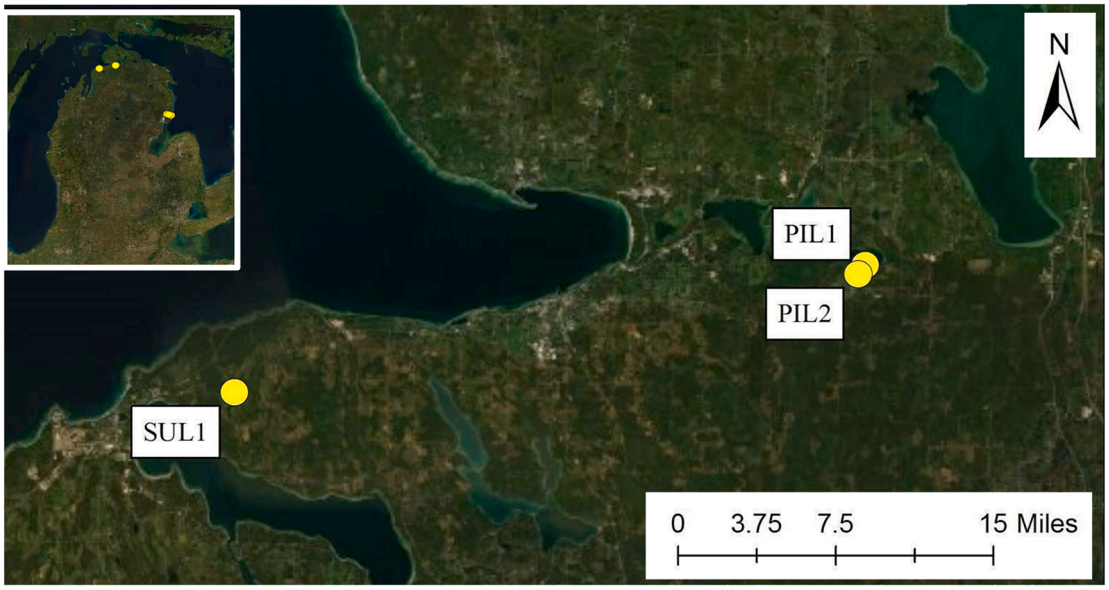
2.3. Water collection

Water samples were collected in late February 2020. Duplicate water samples (500 mL each) were collected in a 2 L stainless-steel Kemmerer bottle and split into two 250 mL HDPE bottles from Vista Analytical Laboratory (El Dorado, California, USA). Sampling followed the Michigan Department of Environmental Quality (MDEQ) Surface Water PFAS Sampling Guidance document (MDEQ, 2018). For additional information regarding how the contamination of the samples was minimized, see the Supplementary Information. Between each sample, the Kemmerer bottle was cleaned with Alconox® Powdered Precision Cleaner (Alconox, Inc; White Plains, New York, USA) mixed with Milli Q water in a Rubbermaid® 48-quart cooler (Wooster, Ohio, USA) and the crew changed to new gloves. At Pickerel Lake, Susan Lake, and the Au Sable River, water samples were collected one meter below the water's surface, with augering through ice preceded water sampling at lake sites. Due to safety concerns at Clark's Marsh, samples were collected via bottles from a shallow effluent stream between ponds. Field blanks were collected at two sampling locations.

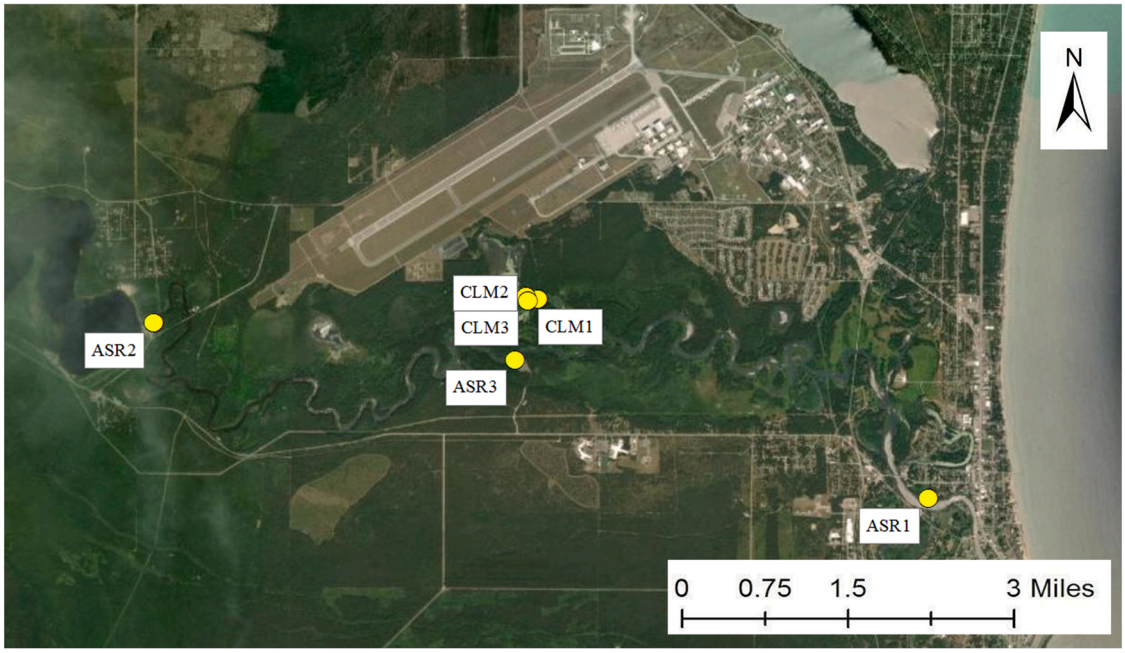
In addition to water samples for PFAS analysis, during each round of sampling temperature, pH, dissolved oxygen, conductivity, and turbidity were collected at 0.62 ± 0.10 m (mean depth ± SEM) from the surface of all the sites within 30 days of animal sampling using a Eureka Amphibian 2 handheld PC (Austin, Texas, USA) with a Manta 2 sub 3 Hydrolab probe or a YSI Professional Plus multiparameter meter (Yellow Springs, Ohio, USA).Fig. 1.

2.4. Animal collection and housing

Crayfish species, *F. rusticus, F. virilis,* and *F. immunis* (postorbital carapace length: 2.9 ± 0.13 cm, mean ± SEM; chelae length: 3.1 ± 0.37 cm, mean ± SEM), were collected from sampling locations between early July to early August 2019 (See *Study sites* section; Table 1). Crayfish were collected using minnow traps baited with sardines. Bluegill, *Lepomis macrochirus* (14.9 ± 0.43 cm; total length ± SEM), were collected using hook and line sampling under the University of Michigan IACUC Protocol: PRO00009089, between early July 2019 and mid-September 2019. Following collection, crayfish were placed in Ziploc® bags with aerated water sourced from the sampling site. Fish housing and transport were based on the MDEQ Fish Tissue PFAS Sampling Guidance Document (MDEQ, 2019). Bluegill were placed into one of three aerated coolers (Coleman Xtreme 70-quart cooler: Wichita, Kansas, USA; Coleman 48-quart performance cooler: Model 5286B; or Rubbermaid 80-quart cooler: Atlanta, Georgia, USA) with water sourced from the site. All animals were transported to the University of Michigan Biological Station Stream Research Facility, Pellston, Michigan, USA (45.563973, −84.751218). Animals were placed in flow-through artificial stream systems (crayfish streams: 284 ×81.2 ×40.6 cm, interior L x W x H; fish streams: 260 ×80×30 cm, interior L x W x H) composed of cement blocks lined in 0.1 mm thick polyethylene sheeting. Streams were fed by unfiltered stream water from the East Branch of the Maple River (Pellston, Michigan), non-detect for PFOA and PFOS, which entered the holding streams through polyvinyl chloride (PVC) piping



(A)



(B)

Fig. 1. (Color needed: yes; Columns: 1). SITE MAP (A) The sampling sites in Northwest Michigan, USA near Petoskey, and Charlevoix. PIL indicates Pickerel Lake and SUL indicates Susan Lake (Esri). (B) The sampling sites in Northeast Michigan near Oscoda. ASR indicates the Au Sable River and CLM indicates Clark's Marsh. The former Wurtsmith Air Force Base, heavily contaminated with PFAS, is shown North of the marsh and river.

(unpublished Michigan Department of Environment, Great Lakes, and Energy [formerly MDEQ] data). Crayfish were isolated in polyethylene terephthalate (PET) plastic containers (18 ×16×7 cm, L x W x H). Flow into the fish stream was 0.995 L/s, fish were housed for at least 2 h, and the stream was covered with polyethylene snow fence to prevent predation and escape of fish. Materials for animal housing were chosen to reduce potential leaching of PFAS; materials used followed the PFAS guidelines established by the MDEQ (MDEQ, 2019). All animals started behavioral trials a maximum of 55 h after being placed into the artificial stream, which is less than the depuration half-lives which range from 3 to 35 days in fish tissues, depending on the PFAS compound (Martin et al., 2004). During the holding period, animals were exposed to an ambient water temperature of approximately 19° C and a natural light: dark cycle of approximately 15:9 hr.

2.5. Behavioral and physiological assays

2.5.1. Crayfish foraging assay

A series of eight artificial flow-through streams (40.6 × 40.6 × 40.6 cm, interior L x W x H) were constructed using cinder blocks and 0.1 mm thickness polyethylene sheeting to assess foraging behavior of crayfish (Fig. 2). A high-density polypropylene, constant head tank (208 L) was placed at the upstream end of the artificial streams and was fed by water from the East Branch of the Maple River. Water entering the head tanks was minimally filtered using fine nylon mesh. Stream input hoses consisted of polyethylene irrigation tubing and were attached to

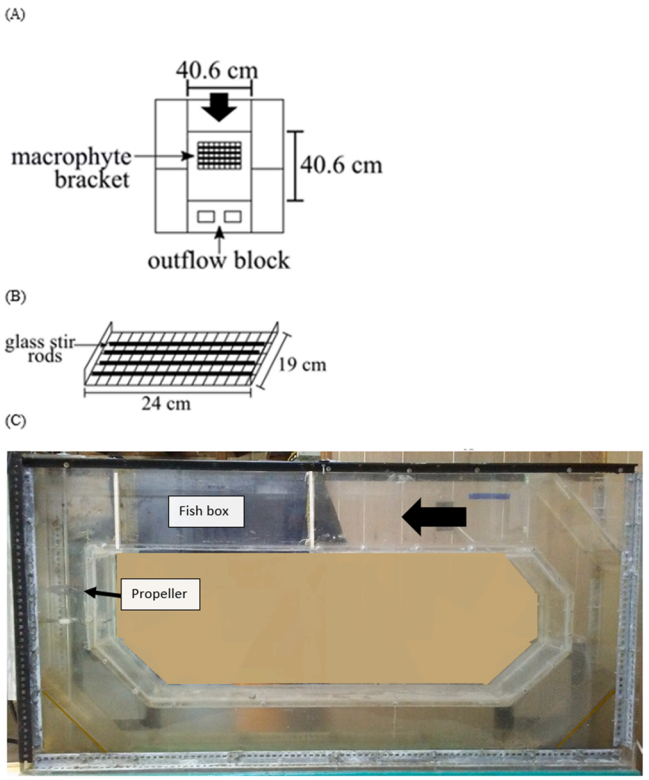


Fig. 2. (color needed: no; columns: 1.5). DIAGRAMS OF ASSAY MATERIALS. (A) Schematic of artificial stream (40.6 ×40.6 ×40.6 cm; interior L x W x H) constructed to conduct foraging assay with crayfish. Crayfish were tethered in the center of each artificial stream and allowed to consume *Chara* spp. attached to the macrophyte bracket for a total of 23 h. (B) Macrophyte bracket used to attach *Chara* spp. for foraging assay. The bracket was constructed from hardware cloth (24 ×19 cm; L x W) and four glass stir rods. *Chara* spp. were distributed throughout the stir rods and attached using floral wire. One bracket was placed in an artificial stream with a crayfish. (C) A picture of the recirculating flume used for swimming trials. The fish were in the box, enclosed by metal grates on either side, during the swimming trial. The thick, bold black arrow indicates the direction of water flow which flowed to the top left in a counterclockwise direction and around the flume in the white and striped areas.

the head tanks using 1.9 cm diameter polypropylene fittings. River water entered the artificial streams at a mean flow of 107 ± 38.1 mL/s (mean flow ± SEM) and was held at an average depth of 28.1 ± 0.4 cm (mean depth ± SEM). Outflow blocks (40.6 ×20.3 ×20.3 cm, L x W x H) at the downstream end of the artificial streams were used to control the volume of water flowing from the streams, and therefore water depth. Each stream had a 3 cm layer of sand substrate.

Chara spp. macroalgae was collected from North Fishtail Bay, Douglas Lake, Pellston, Michigan, USA (45.586311, −84.659420) and stored in an artificial stream until use in the foraging assay. *Chara* spp. was chosen due to the preference displayed by several crayfish species for this macrophyte species (Cronin et al., 2002). Douglas Lake was chosen as the source lake for macroalgae collection due to the absence of industrial and agricultural influences (i.e., potential PFAS sources) around the lake. *Chara* spp. was collected using a macrophyte sampling rake, cast from a boat into submerged *Chara* mats.

To determine the mass of macrophytes consumed by crayfish, mass of macrophytes was recorded before and after placement in the artificial stream using an Ohaus® Scout® STX (Model: SPX123) scale. Before mass was recorded, excess water was removed from each macrophyte sample using a salad spinner (20 revolutions per sample; Farberware Basics, Item No. 5158683). Crayfish were allowed to forage on 5.00 ± 0.0005 g (mean *Chara* spp. mass ± SEM) of *Chara* spp. during foraging trials. Macrophytes were attached to four glass stir rods (25 ×0.6 cm; L x D) using floral wire and placed in a hardware cloth bracket (24 ×19 cm; L x W) within each artificial stream (Fig. 2). Individual crayfish were tethered for 23 h within an artificial stream using 2.54 cm^2 Velcro® squares attached to tile weights (3.5 ×5.0 cm, W x L) and the carapace of each crayfish using superglue and connected with fishing line (20 cm, length) to prevent escape during trials (Neal and Moore, 2017).

2.5.2. Crayfish anti-predator assay

Crayfish anti-predator response was not found to be sensitive to PFAS exposure; therefore, details on predator odor preparation, assay methodology, and data collection for the crayfish anti-predator assay are included in the Supplementary Information.

2.5.3. Fish swimming speed assay

2.5.3.1. Swimming flume design. A recirculating flume was used to measure the fish's swimming ability. The swimming area of the flume (55.9 ×17.6 ×24.6 cm, L x W x H) was enclosed with 0.64 cm hardware cloth. Prior to the trials, the hardware cloth was soaked in acetone, isopropyl alcohol, and water to remove oils from the metal and prevent potential PFAS contamination. Black curtains surrounded the front of the swimming area (46.4 ×24.6 cm, L x H, curtained area) to prevent outside influences. A Motor Master 200 (Minarik Electric Company, Los Angeles, California, USA) with a Blue Chip II™ Adjustable Speed Motor (180 V DC, 2400 RPM, 1 HP; Minarik Electric Company) attached to a propeller created the flume's flow (Fig. 2).

A mirror was placed under the swimming area which faced both the swimming area and a handheld Panasonic High-Definition Video Camera (Model No.: HDC-HS250P; Osaka, Japan) which recorded the swimming trials. A Hach flow meter (Hach FH950, Loveland, Colorado, USA) was used to determine the flow at specific revolutions per minute (RPMs) and a linear regression found the RPM's needed for the desired flow rates.

2.5.3.2. Swimming performance testing protocol. The temperature of the flume water (19.3 ± 0.69 °C, mean ± SEM) was measured before each trial. An aerator, placed in front of the swimming area, ran for the entire trial to prevent oxygen limitation. The acclimation period of 150 min at 20 cm/s caused the fish to swim in a combination of two gaits: median and paired fins swimming and body/caudal fin swimming. After the acclimation period, the flow increased quickly to determine the potential relationship between PFAS and anaerobic swimming. After the acclimation period, the flow increased 4 cm/s every minute until the fish's tail rested on the back grate and its body wavered laterally, while facing upstream. If the fish was unable to stay off the back grate during the acclimation period, the fish was nudged with a 2.54 cm PVC pipe to ensure the bluegill was exhausted. If no swimming response followed three nudges or if the fish was unable to stay off the back grate following the acclimation period, the trial ended.

2.5.4. Histological analysis

Fish gill samples and liver samples were used to examine potential differences in histology (Table 1). Fish histology was not found to be sensitive to PFAS exposure; therefore, details on tissue preparation and histological analysis methodology are included in the Supplementary Information.Table 2.

2.6. PFAS quantification

For water, crayfish hepatopancreas tissue, and bluegill liver tissue analysis, samples were shipped to Vista Analytical Laboratory. For details on tissue sample preparation, see Supplementary Information. Vista Analytical Laboratory, a Department of Defense Environmental Laboratory Accreditation Program (DoD ELAP) certified laboratory for PFAS

Table 2
Range (average ± SEM) tissue concentration (ng/g w.w.), range of reporting limits (ng/g), and associated bioaccumulation factor (BAF; L/kg) for crayfish and bluegill. Calculated using tissue concentrations (ng/kg ww) and water concentrations (ng/L) of PFAS. Non-detect concentrations are reported as concentrations of 0 ng/g w.w. Asterisk indicates that bioaccumulation factors that could not be calculated due to analytes being non-detect in water across all sampling locations.

PFAS Analyte	*Crayfish Tissue Concentration (ng/g w.w.) Range (average ± SEM) (n = 8 pooled samples)*	*Crayfish Tissue Reporting Limit (ng/g)*	*Crayfish BAF*	*Bluegill Tissue Concentration (ng/g w.w.) Range (average ± SEM) (n = 14 pooled samples)*	*Bluegill Tissue Reporting Limit (ng/g)*	*Bluegill BAF*
PFBA	0.00 – 4.18 (1.27 ± 0.61)	0.476 – 2.98	200	0.00 – 0.00 (0.00 ± 0.00)	0.476 – 2.63	–
PFOA	2.66 – 146 (40.7 ± 19.7)	0.952 – 5.95	958	0.00 – 0.00 (0.00 ± 0.00)	0.952 – 5.26	–
PFOS	0.0 – 482 (151 ± 72.9)	0.476 – 2.98	–	47.7 – 13,600 (3990 ± 1340)	0.505 – 19.2	18,600
PFNA	0.0 – 4.23 (1.39 ± 0.52)	0.476 – 2.98	1110	0.00 – 0.00 (0.00 ± 0.00)	0.476 – 2.63	–
PFDA	0.00 – 2.44 (0.31 ± 0.31)	0.476 – 2.98	NA*	0.00 – 2.34 (0.93 ± 0.23)	0.476 – 2.63	NA*
PFUnA	0.00 – 2.17 (0.27 ± 0.27)	0.476 – 2.98	NA*	0.00 – 3.37 (1.57 ± 0.24)	0.476 – 2.63	NA*
PFDoA	0.00 – 5.64 (1.00 ± 2.05)	0.476 – 2.98	NA*	0.00 – 2.39 (0.60 ± 0.22)	0.476 – 2.63	NA*
Total PFAS	5.04 – 2440 (718 ± 370)	–	1325	52.2 – 13,900 (4090 ± 1370)	–	–

analyses, used EPA Method 537 modified for all samples to quantify 24 PFAS analytes (Table S1). For water samples, Method 537 was modified most substantially by the inclusion of 19 isotopically labelled standards and the isotope dilution quantitation method. To perform crayfish hepatopancreas and fish liver tissue analysis, tissue samples from each location were pooled for a total of eight crayfish and nine fish samples. Analyzed fish samples were pooled only if they had similar critical swimming speeds. Five samples were analyzed separately as they were of sufficient sample weight to provide a reasonable reporting limit for analysis (two from Au Sable River; three from Clark's Marsh). For tissue samples, Method 537 was also modified by homogenizing tissue samples prior to extraction. In both media, sample analysis and quantification included branched and linear isomers for PFHxS, PFOA, PFOS, 2-(N-Methylperfluorooctanesulfonamido) acetic acid (MeFOSAA), and 2-(N-Ethylperfluorooctanesulfonamido) acetic acid (EtFOSAA), whereas all other analytes were only analyzed for linear isomers. In addition to standard quality control measures, an additional lab blank was also analyzed prior to extracting each tissue type.

2.7. Data analysis

2.7.1. Crayfish behavior assays

For the foraging assay, the percent of plant material consumed was calculated using the difference of pre-trial macrophyte mass and post-trial macrophyte mass normalized by the pre-trial macrophyte mass:

$$Percent\ \ consumed\ \ (\%) = ([Mi\ \ -\ \ Mf]/Mi)\ \ \times\ \ 100$$

where M_i indicates pre-trial macrophyte mass and M_f indicates post-trial macrophyte mass (Wood et al., 2018).

2.7.2. Fish swimming speed assay

Critical swimming speed (U_{crit}) was calculated from the flow step and time information from each trial (Brett, 1964):

$$Ucrit = last\ \ successful\ \ flow\ \ step + (time\ \ swam\ \ in\ \ last\ \ step * flow\ \ interval)$$

Critical swimming speed was then standardized by the fish's length, which led to a measurement of body lengths swam per second. Only fish that had liver samples sent to Vista Analytical Laboratory for PFAS quantification were used in the analysis. The remaining fish were analyzed for histological lesions.

2.7.3. Histological data

Histological alterations identified in the photomicrographs were analyzed for their severity (Table S2). A fish's lesions for a specific tissue were categorized for each lesion type into four severity scores: minimal, mild, moderate, and severe (Wolf et al., 2010). The scoring methods were not standardized for all lesion types due to the ranges of size and severity of each lesion type. Some lesions, like lamellar clubbing, were scored based on the area of tissue altered by this lesion type (i.e., minimal: <25%, mild: 25–50%, moderate: 50–75%, severe: 75–100%). However, other lesion types, like lamellar fusion, were categorized by their number of discrete lesions (i.e., minimal: 1 −3 lesions, mild: 4 −6 lesions, moderate: 7 −9, severe: >9).

2.7.4. PFAS accumulation

For both crayfish and bluegill, bioaccumulation (L/kg) of PFAS was quantified for each sampling location for each PFAS analyte detected using the equation:

Bioaccumulation Factor (BAF) = $PFAS(t)/PFAS(w)$.

where PFAS(t) and PFAS(w) represents average PFAS concentration in tissue (ng/kg ww) and water (ng/L) across sampling sites, respectively.

2.8. Statistical analysis

2.8.1. Data conditioning

Crayfish dependent variables (percent plant matter consumed and total time to exit) were assessed for outliers and collinearity using methods described by Zuur et al. (2009). Behavior of males and females was not found to be significantly different, therefore data for both sexes were combined (T-test p-value > 0.1). Fish swimming speed was also assessed for outliers. The 24 PFAS analyte concentrations in water and tissue were also assessed for collinearity. PFAS variables in water were eliminated from further analysis due to collinearity or being non-detect; therefore, only total PFAS (i.e., sum of PFAS compounds) was used in further analysis of behavioral response variables. PFBA, PFDoA, PFDA, perfluorononanoic acid (PFNA), PFOA, PFUnA, and total PFAS were included in further analysis of crayfish tissues, with the remaining PFAS eliminated due to high collinearity or being non-detect. Perfluorohexanoic acid (PFHxA), PFHxS, PFHpS, PFDA, 8:2 FTS, PFUnA, PFDoA, and PFTrDA were included in further analysis for fish tissues, while the remaining PFAS analyses were eliminated due to high collinearity or were not detected.

2.8.2. Linear mixed model analysis

Linear mixed models (LMM) with a Maximum-Likelihood fit were used to determine the effect of PFAS exposure and PFAS accumulation on crayfish responses (percent plant matter consumed and total time to exit) using R statistical software (version 4.0.1) (Anon, 2020) and the "lme4″ package (Bates et al., 2019). PFAS analytes quantified in tissue (ng/g), PFBA, PFDoA, PFDA, PFNA, PFOA, PFUnA, 6:2 FTS, and total PFAS were entered as fixed effects, with crayfish species used as a random effect. Likelihood ratio tests and Akaike Information Criterion (AIC) were used to eliminate predictor variables and produce the best fitted model to predict the observed behavior of crayfish. The final reduced model was checked for assumptions of homoscedasticity and normality. Effect size analysis on the final reduced model was performed in R statistical software using "MBESS" and "effectsize" packages

(Ben-Shachar et al., 2020; Kelley, 2020). Considerations of the total sample size ($n = 17$) were given when interpreting effect size results, as lower sample size may result in greater effect sizes. All PFAS variables were log + 1 transformed prior to statistical modelling. Full linear mixed models included tissue PFAS analytes (ng/g ww), PFBA, PFDoA, PFDA, PFNA, PFOA, PFUnA, 6:2 FTS, and total PFAS, and water total PFAS (ng/L) with crayfish species as a random effect for each crayfish response variable.

Lmer (response ~ Tissue PFBA * Tissue PFDoA * Tissue PFDA * Tissue PFNA * Tissue PFOA * Tissue PFUnA * Tissue 6:2 FTS * Tissue Total PFAS * Water Total PFAS + (1|species)).

Analytes were dropped from the full model using a step method (Venables and Ripley, 2002). Each drop model was compared to the full model in a likelihood ratio test using the ANOVA function in the car package (Fox and Weisberg, 2019). When model comparison resulted in a significant difference ($p < 0.05$) with the drop model having a lower AIC value compared to the full model, the dropped analyte was determined to be a significant indicator of the crayfish response variable. When model comparison resulted in no significant differences ($p > 0.05$), the dropped analyte was removed from further analysis. This resulted in the final linear mixed model for the foraging assay to include the fixed effects, tissue PFBA, PFDoA, PFDA, PFNA, PFOA, PFUnA and total PFAS. The full interactive model and the non-interactive version of the model were also compared using the ANOVA function.

2.8.3. Regression analysis

Histological severity scores were analyzed using R statistical software (version 4.0.2) (Anon, 2020). Variables were reordered into the four categories using the factor function: not evident, minimal, mild, and moderate. Several ordered logistic regressions were completed, using the "polr" function (package: MASS) (Ripley et al., 2021) between lesions that were frequently found, and sampling site and the sex of the bluegill, combined and separately.

A multiple regression model, using the "lm" function determined if PFAS analytes in tissues, which were frequently detected and not collinear, explained critical swimming speed.

2.8.4. Principal component analyses

Two PCAs were performed with animal physical and behavioral characteristics, and tissue and surface water PFAS concentrations. This crayfish PCA included sex, species, reproductive form, carapace and chelae lengths, total weight of crayfish, hepatopancreas weight, foraging, anti-predator responses, total PFAS in surface water, and non-collinear PFAS analytes in crayfish tissues. The fish PCA included the sex of the fish, total length, weight, relative liver weight, critical swimming speed, total PFAS in surface water, and non-collinear PFAS analytes in fish tissues. Fish whose entire swimming trial (including the acclimation period) lasted less than 10 min were deemed as non-participating and were not included in the PCA. All variables were converted to numerical values and normalized by subtracting the variable's mean and dividing the result by the standard deviation of that variable. The PCAs and subsequent plots were performed using the "prcomp" function and "fviz_contrib" function in the "factoextra" package (Kassambara and Mundt, 2020).

3. Results

3.1. PFAS tissue concentrations and accumulation

Analysis of PFAS concentrations ranged from non-detected (individual analyte) to 2440 ng/g (maximum total PFAS) in crayfish tissues and non-detected (individual analyte) to 13,900 ng/g (maximum total PFAS) in fish tissue.

3.2. Crayfish behavior

The crayfish PCA had four dimensions explaining 84.9% of the variation in the dataset (Fig. 3). Crayfish species, total PFAS (tissue), PFOA (tissue), total PFAS (surface water), chelae length, and PFNA (tissue) contribute to dimension 1, which explained 46.3% of the variation. PFDoA (tissue), PFDA (tissue), PFUnA (tissue) and chelae length contributed to dimension 2, which explained 18.0% of the variation. In the biplot displaying dimensions 1 and 2, Clark's Marsh, the Au Sable River, and Pickerel Lake data were distinct as shown by the non-overlapping ellipses, with Clark's Marsh separated from the other sites along dimension 1 (Fig. 3).

Given no significant differences between the full interactive and non-interactive models for the foraging assay, the non-interactive model was chosen as the final foraging model. The final reduced LMM with tissue PFBA, PFDoA, PFDA, PFNA, PFOA, PFUnA and total PFAS produced the best fit to predict percent plant material consumed and indicated a significant effect on percent plant material consumed (LMM: AIC 131.2, Table 3), with PFBA the only analyte not found to be significant. Coefficients for each PFAS analyte varied: PFBA (3.08), PFDA (−132.2), PFDoA (31.9), PFNA (71.9), PFOA (−97.9), PFUnA (−10.8), and total PFAS (44.8). When each individual PFAS analyte is regressed against percent *Chara* consumed, each regression trended negative except for PFUnA, where crayfish sampled from environments with greater concentrations of PFAS displayed a decrease in consumption of *Chara* spp. This relationship is also exemplified within the crayfish PCA (Fig. 3). Using the methods described above, it was determined that no significant model was produced for the crayfish antipredator response.

Absolute fixed effects results are listed in Table 3, with PFBA having an effect size of 0.12 and a p-value of > 0.05 indicating no significant relationship of this variable and foraging response. Effects sizes of PFDoA, PFDA, PFNA, PFOA, PFUnA, and total PFAS in tissues (ng/g w. w.) demonstrated a significant relationship between each of these analytes and crayfish foraging response.

3.3. Fish behavior and physiology

The fish PCA had four dimensions explaining 81.4% of the variation in the dataset (Fig. 3). PFHpS (tissue), total PFAS (surface water), PFHxS (tissue), PFDA (tissue), 8:2 FTS (tissue), relative temperature between the water temperature at the sampling site and in the swimming trial, and PFHxA (tissue) contributed to dimension 1, which explained 43.8% of the variation. Fish weight, total length, PFTrDA (tissue), relative liver weight (standardized liver weight with fish weight), and relative temperature contributed to dimension 2, which explained 16.3% of the variation. In the biplot with dimension 1 and 2, Susan Lake and Pickerel Lake data overlap (Fig. 3). Au Sable River data is relatively close to these two sites but is distinct both dimensions. Clark's Marsh data is distinct along dimension 1.

Critical swimming speed in bluegill was explained by PFAS concentrations. A multiple regression model, including PFOS, PFDA, PFUnA, and PFDoA concentrations in fish tissues explained critical swimming speed (adj. $R^2 = 0.983$, p-value < 0.0001). Coefficients varied for each PFAS analyte: PFOS (-6.99×10^{-5}), PFDA (−6.990), PFDoA (17.94), and PFUnA (−0.2901).

While lamellar clubbing had minimal severity scores and was rarely found in bluegill gill samples, lamellar epithelial hyperplasia and lamellar fusion, ranging from minimal to moderate severity scores, were found in all samples (Table S2). These histological alterations are nonspecific effects, which are present due to exposure to irritants (i.e., lambda-cyhalothrin, cyphenothrin, chlorpyrifos), not specifically to PFAS exposure (Velmurugan et al., 2007). Gill damage in the form of epithelial lifting and lamellar fusion, can decrease oxygen uptake (Skidmore, 1970). The sex and sampling site of bluegill did not explain lamellar epithelial hyperplasia (LEH) or lamellar fusion severity scores in ordered logistic regressions. Abundance of gill lesions was not

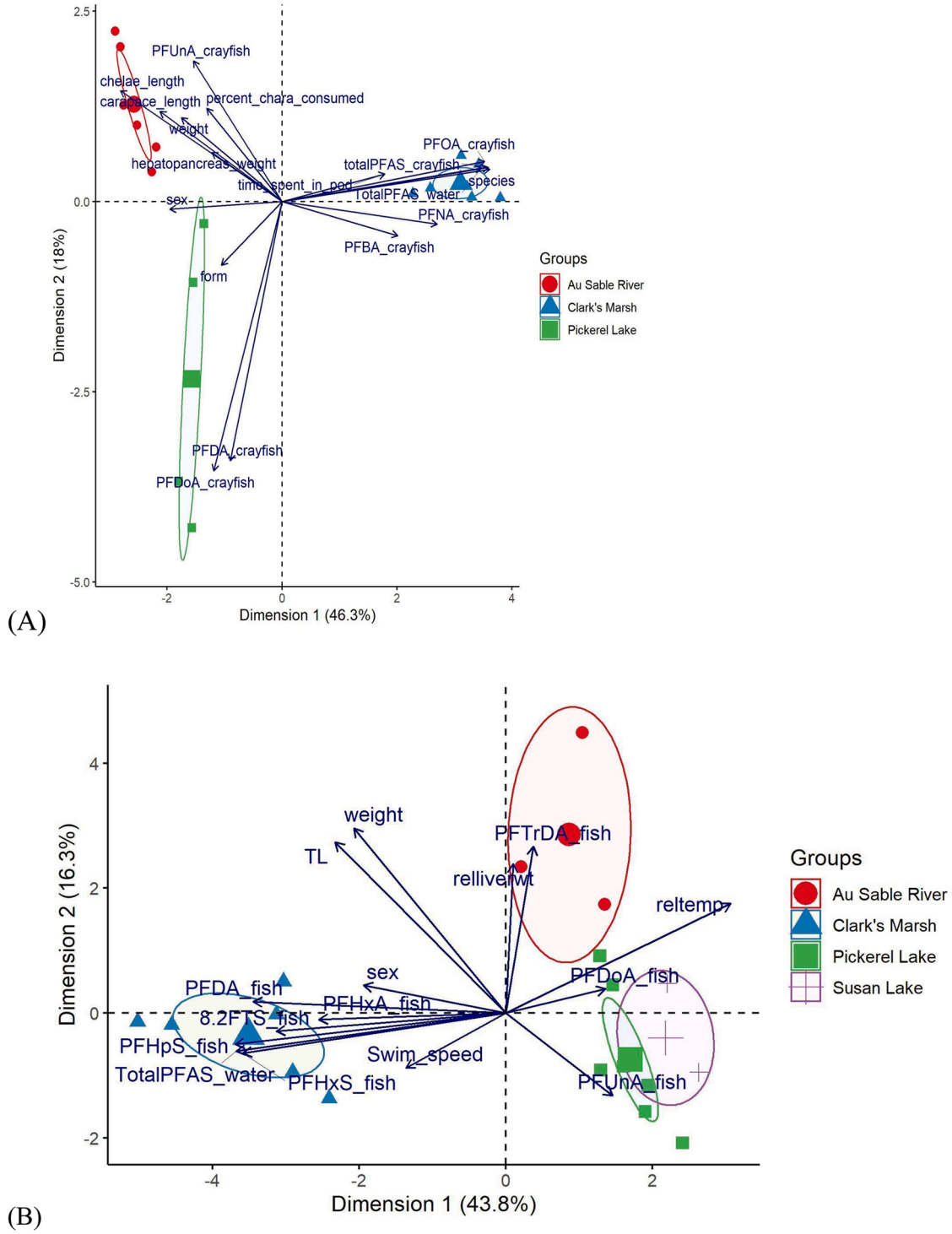


Fig. 3. (color needed: yes; columns: 1.5). PCA BIPLOTS. (A) PCA biplot showing crayfish PFAS concentrations (n = 8), surface water PFAS concentrations (n = 6), and crayfish physical and behavioral characteristics (n = 17). (B) PCA biplot showing bluegill PFAS concentrations (n = 14), surface water PFAS concentrations (n = 6), and bluegill physical and behavioral characteristics (n = 19).

associated with site (i.e., PFAS concentration).

In liver samples, increased glycogen vacuolation, ranging from minimal to moderate severity scores, was the most common lesion, while minimal severity scores of pigmented macrophage aggregates (PMA), hepatocyte hypertrophy, and sinusoidal congestion were rarer (Table S2). PMA or glycogen vacuolation severity scores were not explained by the sampling site or by the sex of the bluegill. Abundance of liver lesions was not associated with site (i.e., PFAS concentration).

4. Discussion

The broad effects of high level PFAS exposure have been of significant study, while behavioral and physiological effects from PFAS mixtures at environmentally relevant levels have not been well studied. This study provides evidence of the potential behavioral and physiological effects of environmental exposure to PFAS compounds within multiple trophic levels of an aquatic ecosystem. Exposure to PFAS and other

Table 3
Statistical significance and absolute effect size for final foraging Linear Mixed Model.

PFAS Analyte	F-value	p-value	Absolute effect size
PFBA	0.579	0.46	0.12
PFDoA	30.4	< 0.001	1.03
PFDA	83.9	< 0.001	2.51
PFNA	93.1	< 0.001	2.13
PFOA	40.6	< 0.001	7.23
PFUnA	5.54	0.03	0.41
Total PFAS	24.8	< 0.001	5.18

contaminants can potentially cause ecosystem effects extending past individual organisms by affecting the ecological role of exposed organisms. Further, the results of this study show differential toxicity of short-chain and long-chain PFAS compounds.

This study demonstrated differential distribution of PFAS within aquatic environments of Iosco, Charlevoix, and Emmet Counties (MI, USA). Relative to other sampling locations (i.e., Pickerel Lake [reference site], Susan Lake, and the AuSable River), the Clark's Marsh sampling location had greatest PFAS concentrations in both water and tissue media (e.g., Fig. S1). This study also demonstrates potential sensitivity of crayfish and bluegill behaviors and histological changes to PFAS exposure. Crayfish foraging behavior was significantly related to tissue accumulation of environmental concentrations of PFAS (i.e., PFDoA, PFDA, PFNA, PFOA, PFUnA, and total PFAS; Table 3). Generally, crayfish with greater tissue accumulation of PFAS demonstrated decreased consumption of *Chara* when compared to crayfish with lesser PFAS tissue levels. The antipredator response of crayfish exposed to environmental concentrations of PFAS was not found to be a sensitive behavior ($p > 0.05$). Bluegill swimming speed was significantly related to PFAS tissue concentrations detected (i.e., PFOS, PFDA, PFUnA, and PFDoA). Bluegill with greater tissue concentrations of PFAS demonstrated increased critical swimming speed when compared to bluegill with lesser PFAS tissue levels. Liver and gill histology were not significantly altered in bluegill exposed to environmental concentrations of PFAS ($p > 0.05$; Table S2). Due to the nature of this field study, further laboratory research is needed to further evaluate these relationships.

Behavioral endpoints have been shown to be 10–10,000 times more sensitive than LC_{50} measures and offer information on the altered ecological role of an organism that may occur at sublethal exposure levels to a contaminant (Hellou, 2011). All PFAS analytes included in the final LMM in tissue contributed significantly to the change in crayfish foraging behavior, except for PFBA (Table 3). PFBA is classified as a short-chain PFAS, while all other PFAS analytes included in the final model are classified as long-chain PFAS. The magnitude of relationships in the final model were also exemplified through fixed effect analysis, where the absolute effect size for the PFBA analyte was 0.12 while all other analytes had an effect size ranging between $0.4 - 7.1$ (Table 3). These analyses reflect a greater influence of long-chain PFAS in driving the changes in foraging behavior. Due to the nature of this field study and limited sample sizes, a controlled, laboratory study evaluating the relationship between PFAS chain length and animal behavior should be conducted to further elucidate these findings. Since only long-chain PFAS concentrations in tissues were included in the multiple regression (due to amount of non-detects for short-chain compounds), long-chain PFAS were also driving the changes in fish swimming behavior. The sensitivity of crayfish and bluegill behavior contributes to the growing body of research regarding the differential toxicity of short-chain and long-chain PFAS compounds.

Our study also demonstrates the importance of evaluating tissue concentrations of contaminants, in addition to environmental concentrations, to fully capture the associated toxicity and consequences of exposure on animal's behavior. Changes in animal behavior appeared to be more strongly correlated with tissue concentrations than the surrounding water concentrations. Models with the sum of PFAS concentrations in water (i.e., total PFAS in water) had significantly lower AIC values, when compared to models without concentrations of PFAS in water. Therefore, increased prediction of PFAS impairment to behavior is driven by using tissue samples rather than water samples. For crayfish, BAFs, used to express the relationship between concentrations of PFAS in water and tissue, were highest (i.e., greater than 958) for long-chain PFAS relative to short chain PFAS (PFBA) (BAF was ~200). For bluegill, PFOS was the only analyte included in the multiple regression which had a BAF calculated. For the other analytes in the model, the associated BAF value was not calculated due to PFAS concentrations being non-detect in surface water.

BAFs varied between locations and PFAS analytes. The BAFs found in the current study indicate that many analytes were not present at detectable levels in surface water but were detectable in tissues. Thus, the lack of a BAF may also indicate bioaccumulation. Overall, BAFs increased as carbon chain length increased, indicating higher levels of bioaccumulation for longer chain PFAS. Previously reported BAFs for other game fish species and crustaceans have varied widely. In the current study, bluegill had a BAF for PFOS at 18,600 (L/kg ww) within the range of previously reported values of Great Lakes lake trout and walleye PFOS BAFs of $7690 - 33{,}700$ (De Silva et al., 2011). Limited investigation of PFAS BAFs for crustacean species report PFOA, PFNA, PFDA, and PFBS BAFs ranging from $29.5 - 110$ for estuarine crabs (Naile et al., 2013). Within the current study, crayfish were found to have greater BAFs than these previously reported values; however, values are comparable to the above mentioned BAFs for fish species. These previous investigations have largely focused on marine crustacean species. In addition, for both crustaceans and fish, these studies have focused on long-chain PFAS compounds. Additional research is necessary to further address potential differences in BAFs of short- and long-chain PFAS in other species.

Previous studies support the differential sensitivity and toxicity of exposure to long-chain PFAS relative to short-chain and may be related to structural differences between PFAS analytes (e.g., chain length, end group) (Jantzen et al., 2016). Hagenaars et al. (2011) demonstrated differential toxicity of four PFAS compounds to zebrafish, with the lowest toxicity associated with PFBA followed by PFBS, PFOA, and PFOS. Significant malformations in recently hatched embryos resulted following exposure to all four PFAS. Delayed hatching, decreased length, and altered heart rates also resulted from PFBS, PFOA and/or PFOS exposure, while these changes were not found in embryos exposed to PFBA (Hagenaars et al., 2011). The carbon chain length of the PFAS compound as well as the end group type have also been shown to alter the bioaccumulation potential of PFAS analytes. Long-chain PFAS have been frequently found to biomagnify in aquatic communities (Xu et al., 2014); while, some short-chain PFAS do not biomagnify or bioaccumulate, such as perfluorocarboxylic acids (Xu et al., 2014).

Few studies have investigated the behavioral effects of PFAS exposure in animals. PFNA has been found to alter activity in exposed *D. rerio*, while neurobehavioral effects of PFOA were documented as spontaneous behavior or hyperactivity (Jantzen et al., 2016). Khezri et al. (2017) appears to be the only study which focuses on behavioral responses to PFAS-containing mixtures. Their study found increased swimming speed in PFAS mixture- and PFOS-only exposed fish, similar to the results of the current study. Exposure to PFOS has frequently demonstrated altered swimming performance, however, there is no consensus in the literature as to the manner that PFAS exposure affects swimming speed in fish. Furthermore, exposed larval zebrafish studies have shown increased and decreased swimming speed in separate studies with varying methodology and concentrations (Hagenaars et al., 2014; Khezri et al., 2017).

Long-chain PFAS are found to have negative effects on a wide variety of physiological systems including the reproductive, endocrine, immune, and nervous systems of experimental organisms (Han and Fang, 2010; Khezri et al., 2017). Documented developmental effects of PFOS have included uninflated swim bladders, spinal curvature, head, tail,

and heart malformations, and delayed and reduced hatching rates in zebrafish (Hagenaars et al., 2011; Huang et al., 2010), as well as spinal curvature and altered sexual development in *Xiphophorus helleri* and *Xenopus laevis*, respectively (Han and Fang, 2010; Lou et al., 2013). The toxicity and bioaccumulation potential of long-chain PFAS compounds may influence the distribution of PFAS found throughout an aquatic food web on a larger scale.

Known factors that contribute to biomagnification in aquatic organisms are diet, the tendency of the compound to stay in the water column or attach to the sediment, the organism's location in the environment (e.g., benthic or pelagic), and the tissue type to which the contaminant binds (Ahrens et al., 2015; Kannan et al., 2005). In addition, behavioral changes, such as increased success in predator evasion due to increased swimming speed, may alter the biomagnification potential in highly contaminated sites. Some studies have indicated that aquatic organisms are exposed to PFAS and other contaminants both by gill uptake and diet (Ahrens et al., 2015; Kannan et al., 2005). However, there are mixed results in the literature, suggesting biomagnification does not occur at all sites (Bush et al., 2015). In some instances, like in the Au Sable River, the highest PFOS concentrations were found in the lower trophic levels potentially due to differential contamination source signatures (Bush et al., 2015; Martin et al., 2004). Specifically, the mean PFOS level in bluegill and pumpkinseed sunfish fillets was nearly ten times greater than the mean PFOS level for largemouth bass and smallmouth bass fillets (Bush et al., 2015). PFAS results in the current study vary from the results of Bush et al. (2015): PFOS concentrations detected in surface water of Clark's Marsh in the current study were approximately five times lower than those reported by Bush et al. However, PFOS concentrations in Au Sable River surface water were similar between these two studies. The difference in surface water concentrations detected at Clark's Marsh could be due to specific sampling locations, the pump-and-treat system near Clark's Marsh installed after research by Bush et al. (2015), and the time difference between surveys. Bluegill concentrations in Clark's Marsh and the Au Sable River were up to double the concentrations Bush et al. (2015) reported, however, it is known that PFAS accumulate in the liver more than the fillets so this increase is expected (Martin et al., 2004). Although all the factors influencing biomagnification have yet to be determined, there are differences between ecosystems regarding the extent of PFAS biomagnification and in which species this occurs.

5. Conclusion

Exposure to PFAS and other anthropogenic contaminants can potentially cause wide reaching effects extending past individual organisms. Crayfish and bluegill hold ecological roles that have the potential to influence population and community structures of aquatic ecosystems. Crayfish are within the shredder functional feeding group which serve a critical role in transferring the energy from terrestrial detritus to aquatic food webs (Weinländer and Füreder, 2011). Therefore, the direct effects of PFAS contamination on crayfish foraging behavior reflected in this study may have ecosystem level implications. Crayfish are also polytrophic in nature and are prey to a variety of aquatic and terrestrial predators (Dorn and Wojdak, 2004). The prevalence and known uptake of per- and polyfluorinated compounds by aquatic organisms may offer crayfish as a vector to transfer PFAS throughout aquatic and terrestrial food webs. Bluegill are also expected to be a source of PFAS to higher trophic levels; however, significant variation exists between tissue accumulation of environmental PFAS concentrations and biomagnification. The increased critical swimming speeds may aid in the survival of contaminated fish, due to the increased chance of predator evasion, securing a mate, and opportunity for foraging. However, high critical swim speeds may also increase energy expenditure, therefore reduce energy available for other functions such as detoxification. With altered swimming speed resulting in impacts on survival, contaminants such as PFAS may alter population dynamics. Exposure to PFAS may have widespread consequences on the physiology, behavior, and ecosystem dynamics in aquatic systems, of which vary upon chain length of the PFAS compounds present.

CRediT authorship contribution statement

Carrie O. Coy: Conceptualization, Methodology, Formal analysis, Investigation, Writing – original draft, Writing – review & editing, Visualization, Funding; **Alexandra N. Steele:** Conceptualization, Methodology, Formal analysis, Investigation, Writing – original draft, Writing – review & editing, Visualization, Funding; **Sara A. Abdulelah:** Formal analysis; **Rachelle M. Belanger:** Methodology, Formal analysis, Writing – review & editing; **Karen G. Crile:** Formal analysis; **Louise M. Stevenson:** Methodology, Writing – review & editing, Funding; **Paul A. Moore:** Conceptualization, Methodology, Validation, Writing – review & editing, Visualization.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.ecoenv.2022.114212.

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