Final Study Plan for Mummichog Fecundity and Oocyte Condition in the Lower Passaic River

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U.S. Department of the Interior
U.S. Fish and Wildlife Service
and,
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National Oceanic and Atmospheric Administration

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Executive Summary

The lower Passaic River (LPR), flowing through Bergen, Essex, Hudson, and Passaic Counties in New Jersey, is contaminated through the past and ongoing release of hazardous substances at or from the Diamond Alkali Superfund Site (DASS), including 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), polychlorinated biphenyls (PCBs), chlorinated pesticides, polycyclic aromatic hydrocarbons (PAHs), and heavy metals. The DASS Natural Resource Federal Trustees – the U.S. Department of the Interior, acting by and through the United States Fish and Wildlife Service, and the U.S. Department of Commerce, acting by and through the National Oceanic and Atmospheric Administration (collectively, the "Federal Trustees"), acting under their authority as natural resource trustees pursuant to the Comprehensive Environmental Response, Compensation, and Liability Act (CERCLA), are conducting a natural resource damage assessment (NRDA) to determine and quantify the natural resources injured and services lost in the LPR, which is part of the DASS, due to the release of those hazardous substances. The final Natural Resource Damage Assessment Plan (DAP) provides additional detail regarding the environment in this area, the presence of hazardous substances, the role of the Federal Trustees, and the plan for moving forward with the NRDA (Federal Trustees 2020a).

The LPR provides habitat for freshwater and estuarine fish species of multiple feeding guilds. Fish surveys conducted over the course of one year in 2009 and 2010 identified 45 fish species throughout the lower 17.4 miles of the Passaic River (Windward 2019). The Federal Trustees intend to conduct both field and laboratory studies to evaluate injuries to fish in the LPR due to exposure to hazardous substances, and also support the quantification of any such injury.

Pursuant to the DAP, the Federal Trustees have developed this ***Final Study Plan for Mummichog Fecundity and Oocyte Condition in the Lower Passaic River*** (Final Study Plan), for a fish injury assessment effort. This Final Study Plan describes a field study the Federal Trustees intend to undertake to meet the following objectives:

1. evaluate mummichog fecundity in the LPR to determine, and as appropriate, quantify, injury caused by exposure to hazardous substances; and
2. evaluate mummichog oocyte condition in the LPR to determine, and as appropriate, quantify, injury caused by exposure to hazardous substances.

This study will support efforts to accurately evaluate and quantify potential fish injury by providing further information on the reproductive fitness of mummichog in the LPR, in addition to the currently-planned mummichog fish toxicity study released in 2020 (Federal Trustees 2020b). The Federal Trustees may propose additional work to supplement this effort in the future.

The Federal Trustees made a draft of this Study Plan available for review and comment for a period of 30 calendar days, ending April 14, 2021. The Federal Trustees did not receive any public comments during this time period.

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1.0 Background

In 1983, environmental sampling by the State of New Jersey and the U.S. Environmental Protection Agency (EPA) at and near the Diamond Alkali Company facilities, located on the Passaic River at 80-120 Lister Avenue in Newark, New Jersey, revealed high levels hazardous substances including 2,3,7,8-tetrachlordibenzo-*p*-dioxin (TCDD), polychlorinated biphenyls (PCBs) chlorine pesticides, polycyclic aromatic hydrocarbons (PAHs), heavy metals and other hazardous substances in the soil and groundwater. TCDD, PCBs, metals, PAHs and various pesticides were also found in sediment of the Passaic River. Additional sampling revealed hazardous substances throughout Newark Bay and its tributaries, the Hackensack River, the Arthur Kill and Kill Van Kull tidal straits. Subsequently, in September 1984, the EPA through its authorities under the Comprehensive Environmental Response, Compensation, and Liability Act (CERCLA), designated the area as the Diamond Alkali Superfund Site (EPA ID# NJD980528996) (DASS).

CERCLA also stipulates that "*natural resources*" (*i.e.*, land, fish, wildlife, biota, air, water, groundwater, drinking water supplies, and other such resources managed by or otherwise controlled by the United States, any state or local government) be restored to the state that they were at before being adversely impacted, or lost due to the release of a hazardous substance. To this end, CERCLA authorizes Natural Resource Trustees, in the instant matter currently designated as the U.S. Department of the Interior (DOI), acting by and through the U.S. Fish and Wildlife Service (USFWS) and the U.S. Department of Commerce, acting by and through the National Oceanic and Atmospheric Administration (NOAA), hereinafter referred to as the "Federal Trustees," to act on behalf of the public for the purpose of preparing an "*injury*" claim to recover "*damages*" from potential responsible parties necessary to restore or replace injured natural resources. In accordance with the Natural Resource Damage Assessment (NRDA) regulations (43 C.F.R. Part 11), the Federal Trustees developed a Natural Resource Damage Assessment Plan (DAP) (Federal Trustees 2020a) that presents an array of potential studies to identify the scope and scale of injury and service losses to natural resources. This ***Final Study Plan for Mummichog Fecundity and Oocyte Condition in the Lower Passaic River*** (Final Study Plan), is one such study. Ultimately, this and other studies are intended to help the Federal Trustees determine and quantify injuries to natural resources, and select the appropriate scope and scale of restoration projects to restore natural resources.

The DAP identified biological injuries to fish as an area the Federal Trustees planned to investigate to determine and quantify potential injury, and understand exposure pathways. Because many of the hazardous substances present at the DASS have been associated with endocrine disruption, the Federal Trustees propose to conduct both field and laboratory studies to determine potential adverse reproductive effects to fish in the lower Passaic River (LPR) (Bugel *et al.* 2010; Bugel *et al.* 2011; Walker and Peterson 1991; Walker *et al.* 1996).

2.0 Introduction

In accordance with the DAP, the Federal Trustees have developed this Final Study Plan, in addition to the currently-planned mummichog fish toxicity study released in 2020 (Federal Trustees 2020b), to provide further information on the reproductive fitness of mummichog (*Fundulus heteroclitus*) in the LPR. This information will be used to more comprehensively evaluate and accurately quantify potential injury to a lower trophic level fish species.

Estimates of fecundity (reproductive capacity of a single individual or a population) are important to determine the reproductive potential of mature fish in a population, and to predict trends in species abundance (Nitschke *et al.* 2001; Sullivan *et al.* 2019). The level of recruitment in a fish population may also be influenced by the variation of size and quality of eggs, in addition to fecundity (Friedland *et al.* 2005). Variations in egg number, size, and quality are highly dependent on fish size, age, and spawning cycle (Ware 1975; Chambers and Waiwood 1996; Marteinsdottir and Begg, 2002). Mummichog, however, employ a predictable reproductive strategy, with females reaching maturity during the spring of their second year at a size of 38 mm (Coad 1995). Oocytes (immature egg cells in the ovary) remain small through the fall and winter before enlarging and maturing during April, in preparation for the onset of spawning in May (Wang and Kernehan 1979). Mummichog are cyclic spawners, with gonadal readiness synchronized to high spring tides associated with new and full moon phases (Taylor *et al.* 1979). Mid-Atlantic populations are able to spawn as many as eight times between May and September (Taylor *et al.* 1979) with fecundity varying from several dozens to several hundred mature ova (eggs) per spawn (Scott and Crossman 1973). Gonadosomatic index (GSI), and the number of mature ova released per spawn, typically peak in June before steadily decreasing through the summer (Taylor *et al.* 1979).

Previous egg productivity measurements were conducted on five gravid female mummichog collected in the lower 8 miles of the LPR in May 2010. The average estimated egg count per gravid mummichog was 227 eggs and the average egg mass was 1.4 g (CPG 2011). Egg counts and weights were body-normalized for comparison to data from the scientific literature as part of the Baseline Ecological Risk Assessment (BERA) (USEPA 2019) to assess possible adverse effects to reproductive potential. Three studies were used to compare mummichog egg data: two laboratory studies (Bosker *et al.* 2010; Gutjahr-Gobell 1998), and one observational field study in a Florida salt marsh (Hsiao *et al.* 1994). Briefly, average egg count for LPR mummichog (41 eggs/g bw) was greater than the maximum egg counts from one laboratory study (8 eggs/g bw; Gutjahr-Gobell 1998) and the field study (19 eggs/g bw; Hsiao *et al.*1994). However, weights of LPR mummichog eggs (0.27 g egg/g bw) were less than those measured in the laboratory (0.38 g egg/g bw) (Bosker *et al.* 2010; USEPA 2011).

Two additional studies have been conducted comparing reproductive health and fecundity between mummichog from the Newark Bay, New Jersey and reference population from Tuckerton, New Jersey. The first study, conducted by Bugel *et al.* 2010, found Newark Bay females to have a significantly greater percent of pre-vitellogenic follicles (43% at Tuckerton, 64% at Newark Bay) and a significantly lower percent of follicles at the mid-vitellogenic and mature stages (25% mature at Tuckerton and 3% at Newark Bay). The second study, also conducted by Bugel *et al.*, found female mummichog from the Newark Bay produced 11 eggs per female, in comparison to 140 eggs per female in the Tuckerton reference location (Bugel *et al.* 2011).

While traditional methods of fecundity estimation have been time consuming, requiring long-term sample storage and manual counting of all maturing oocytes, recent advances have led to the use of scanners and image analysis software to effectively estimate fish fecundity (Friedland *et al.* 2005). This Final Study Plan describes the methods used to assess the fecundity and oocyte condition of female mummichog fish in the LPR. This study will inform future injury quantification, damages determination, and restoration planning in the LPR.

3.0 Public Review and Participation

Public participation and review are an integral part of any NRDA. Public comments help the Federal Trustees plan and conduct a NRDA that is scientifically valid, cost effective, and that incorporates a broad array of perspectives. The Federal Trustees made a draft of this Study Plan available for review and comment for a period of 30 calendar days, ending April 14, 2021. The Federal Trustees did not receive any public comments during this time period.

4.0 Study Purpose and Objectives

The purpose of this study is to inform the Federal Trustees' assessment of injury to fish caused by the release of hazardous substances from the DASS, and to guide their future efforts to evaluate contaminant exposure pathways and natural resource injuries, as defined in the Natural Resource Damage Assessment regulations (43 C.F.R. Part 11). The results of this study will also be used to help determine the nature and scope of future studies conducted in accordance with the DAP.

The objectives of this study are to:

1. evaluate mummichog fecundity in the LPR to determine, and as appropriate, quantify, injury caused by exposure to hazardous substances; and
2. evaluate mummichog oocyte condition in the LPR to determine, and as appropriate, quantify, injury caused by exposure to hazardous substances.

5.0 Methods

5.1 Study Location

Mummichog will be captured from the portion of the Passaic River that constitutes the 17.4-mile tidal portion from RM 0 to Dundee Dam (RM 17.4), and potentially other tributaries where suitable mummichog habitat is found.

The Wye River, Queenstown, Queen Anne's County, Maryland, will serve as the study reference river. The Wye River was selected as an appropriate reference location due to the documented robust and uncontaminated population of mummichog, and previous use as a reference site for mummichog toxicity studies (Hartzell *et al.* 2017; Hartzell *et al.* 2018).

5.2 Study Organism

As described by Abraham (1985), mummichog, also known as Atlantic killifish, are not commercial or sport fish; however, their distribution and abundance make them important in nearshore and estuarine food webs. They are among the most common killifishes on the Mid-Atlantic coast, and although rarely taken in full seawater, they are tolerant of salinity and temperature fluctuations. Mummichog attain sexual maturity and spawn during their second year. They have a semilunar spawning periodicity during the spawning season; eggs incubate in air and are not submerged until the next spring tide after they are laid. Young mummichog remain on the marsh for 6-8 weeks, then begin to move off with the tides, with the adults. Mummichog are able to subsist on wide variety of prey including diatoms, amphipods, insect larvae, and detritus.

5.3 Field Collections

Female mummichog of reproductive size will be collected from both the LPR and the Wye River. Mummichog spawn during flood tides associated with new and full moons. Given this, gravid female mummichog will be collected from the LPR and Wye River during the 48 hour period, immediately prior to new and/or full moon phases, of the common spawning season of May through September 2021. Priority will be given to occasions corresponding to new moon phases, with full moon phases as alternatives if weather or other conditions impede collection. Whenever possible, sampling will coincide with collections of adult mummichog for the currently-planned mummichog fish toxicity studies (Federal Trustees 2020b). Sampling locations will ideally be a sub-set of the LPR and Wye River locations targeted for the mummichog toxicity studies. Sampling from analogous locations will provide for possible correlations between the results of this study and potential adverse toxicological effects and measured concentrations of hazardous substances in eggs found in the mummichog toxicity studies. Results of the toxicity study, including concentrations of hazardous substances in sediment and toxicological effects, will guide efforts to determine collection locations of female mummichog. However, collection locations will ultimately be dependent upon where mummichog are found in both the LPR and Wye River. Lastly, seine and/or cast nets will be used for collections to minimize the duration of stress on captured fish before preservation.

5.4 Histological Assessment

Once collected, fish will be humanely euthanized by immersion in buffered tricaine methanesulfonate before being weighed (to the nearest ±0.01 g) and measured (to the nearest ±1.0 mm; total length). An abdominal incision will be made from vent to operculum to facilitate preservation of the ovary before placement in a pre-labeled sample jar containing a tissue fixative. Fixed ovaries will be removed from abdominal cavities, photographed for gross appearance and weighed (along with any expressed eggs) for calculation of GSI as follows:

$$GSI = \frac{Ovary\ weight}{Total\ fish\ weight} \times 100$$

Fecundity

For each collection event, ovaries from half of the fish at a given location will be used to estimate fecundity according to automated image analysis methods of Friedland *et al*. (2005). Briefly, whole ovaries will be vortexed in tubes, and eggs will be rinsed through sieves to facilitate disaggregation. Separated eggs will be loaded to petri dishes and photographed. Images will be imported into ImageJ software (version 1.47; National Institute of Health, Bethesda MD) for analysis. Using this imaging-based technique, total numbers of eggs and, where possible, egg size will be determined.

Oocyte Condition

Ovaries from the remaining half of the collected fish will be histologically examined for oocyte maturity and oocyte condition. Briefly, entire fixed ovaries will be transversely cut into 5 or more segments, 2-3mm thick, using a single-edge razor blade. Tissue segments will be place into histology cassettes for paraffin embedding, subsequent 5μm sectioning and slide mounting. Fixed and deparaffined slides will then be processed using a hematoxylin and eosin staining protocol (Luna 1992). Often referred to as the “Routine Stain” in histology laboratories, hematoxylin and eosin staining procedures can be used to stain any tissue specimen to reveal the underlying tissue structures and conditions. Prepared tissue slides will be examined by light microscopy to determine the proportion of oocytes at various levels of maturation; document the presence/abundance of atretic ovarian follicles (follicles that have degenerated before coming to maturity); and any other histopathological conditions. Additional staining procedures may be conducted to obtain or more detailed information of the tissues, as appropriate.

Adjustments may be necessary during field collections and histological assessments efforts to best accomplish the objectives of the study. The Federal Trustees and Field Team Leader/Principal Investigator may make *in situ* decisions regarding locations, sample numbers, or laboratory methods based on unforeseen circumstances. Any such decisions, and explanation for necessary changes, will be described in the report of assessment.

6.0 Quality Assurance/Quality Control

A detailed Quality Assurance Project Plan (QAPP) is being developed, in collaboration with the field contractor for this study. The QAPP will be consistent with the project management, data generation and acquisition, assessment and oversight, and data validation and usability objectives defined in Appendix A of the final DAP (Federal Trustees 2020a), and will be included in the Report of Assessment.

6.1 Project Management

The U.S. Fish and Wildlife Service (USFWS) is managing this study on behalf of the Federal Trustees. The project management organization for this Final Study Plan is consistent with that presented in Appendix A of the DAP (see Exhibit A-1, Federal Trustees 2020a). The Field Team Leader/Principal Investigator will report directly to the USFWS Assessment Managers. The Quality Assurance (QA) Coordinator at Industrial Economics, Incorporated (IEc) will oversee Quality Assurance and Quality Control (QC) of the study, including whether specified QA/QC procedures are followed as described. The QA Coordinator will discuss any identified issues with the Field Team Leader/Principal Investigator and the USFWS Assessment Managers as necessary.

6.2 Data Generation and Acquisition

It is important ensure that data of sufficient and known quality are generated through the implementation of this study. The Field Team Leader/Principal Investigator will submit written Standard Operating Procedures (SOPs) detailing respective methods and QC procedures. The USFWS Assessment Managers will review and approve the SOPs, and the QA Coordinator will review aspects related to QC. The approved SOPs will be kept on file.

Modifications to the approved SOPs will be documented in the final study report describing the field collection activities and laboratory results. Minor modifications will be made at the discretion of the Field Team Leader/Principal Investigator. For more significant modifications, the USFWS Assessment Managers will be consulted before modifications are implemented.

6.3 Data Quality Objectives

Data quality objectives (DQOs) are statements including narrative and quantitative criteria that specify the quality and quantity of data to be collected to support a project's goals. The methods and procedures for this study are being developed to achieve the DQOs quickly, safely, and cost-effectively (Exhibit 1).

Exhibit 1 Data Quality Objectives

STEP	DESCRIPTION
Step 1: State the Problem	Contaminants from upland industrial facilities (e.g., Diamond Alkali chemical manufacturing plant) have been released to the DASS. Potential injuries to fish resulting from these releases have not been quantified.
Step 2: Identify the Decision(s)	A mummichog toxicity investigation is currently being conducted to assess injury to mummichog due to contaminants currently in the LPR in advance of scheduled remedial dredging of the lower eight miles of the LPR. Additional data on exposure pathways will be necessary to quantify injury to mummichog in the LPR.
Step 3: Identify the Inputs to the Decisions	Data on the effect of LPR contamination on mummichog fecundity and oocyte condition will be collected using automated imaging of histological samples from female mummichog of the LPR.
Step 4: Define Study Boundaries	The LPR is the study area and the Wye River (Queenstown, MD) is the reference area for the purposes of this Final Study Plan.
Step 5: Develop Decision Rules	Collect and analyze histological samples using automated imaging software to assess, and as appropriate, quantify potential injury to mummichog in the LPR compared to the Wye River.
Step 6: Specify Tolerable Limits on Errors	Appropriate sampling techniques will be utilized to obtain an optimum sample quantity and to identify sampling locations that will meet study objectives. Data review will be performed by the QA Coordinator. Full data packages will be preserved for future review as needed.
Step 7: Optimize Sampling Design	Locations and numbers of samples will be based on a review of existing data combined with professional judgement.

7.0 Literature Cited

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