Update: Fish Injury Studies in the Lower Passaic River (April 2022)

Diamond Alkali Superfund Site Federal Natural Resource Trustees

U.S. Department of the Interior, U.S. Fish and Wildlife Service

U.S. Department of Commerce, National Oceanic and Atmospheric Administration

This update serves as a summary for the public of several of the ongoing 2022 field and laboratory fish injury assessment activities being conducted by the U.S. Fish and Wildlife Service, acting by and through the U.S. Department of the Interior, and the U.S. Department of Commerce, acting by and through the National Oceanic and Atmospheric Administration (collectively the Federal Natural Resource Trustees ("Federal Trustees")) for the Lower Passaic River ("LPR") portion of the Diamond Alkali Superfund Site. These activities are being conducted in connection with the objectives of the Federal Trustees' existing injury studies, for both lower and upper trophic level fish, as part of the ongoing Diamond Alkali Natural Resource Damage Assessment being performed by the Federal Trustees. Any such assessment work performed by the Federal Trustees will be done in accordance with the Natural Resource Damage Assessment Plan for the Diamond Alkali Superfund Site (January 2020).

UPPER TROPHIC FISH INJURY STUDY

In April 2021, the Federal Trustees finalized and initiated a "Study to Evaluate Upper-Trophic Fish Injury in the Lower Passaic River". The objectives of this fish injury study are threefold:

1. Identify and, as appropriate, quantify injury in adult white perch of the LPR compared to the Mullica River (MR), the reference area for this study.
2. Identify and, as appropriate, quantify potential injury in the sensitive early life-stage (ELS) of offspring of white perch from the LPR and MR populations.
3. Use contaminant-exposure experiments in ELS white perch to determine relative contribution to potential injuries from maternal transfer of contaminants to eggs versus direct egg exposure to site contaminants.

The 2021 fish sampling achieved several elements but did not meet collection targets for gravid white perch from the LPR and will require a subsequent season of sampling and associated laboratory studies to achieve Objectives 2 and 3. White perch sampling will take place in both the LPR and MR beginning in spring 2022. In preparation for this additional fish collection, the Federal Trustees collected additional sediment samples from the LPR and MR in January 2022 to use for studies in which white perch eggs are exposed to several different sediment samples in the laboratory. The use of different sediment samples provides a range of environmentally relevant sediment contaminant exposures.

Consistent with Objective 1, to identify and, as appropriate, quantify injury in adult white perch, the Federal Trustees will be collecting adult female white perch from each of the two rivers in spring 2022. These adult female white perch will be assessed for fecundity to further the laboratory components in the original study. Also, the Federal Trustees will continue genomic

sequencing to identify and evaluate molecular level changes in specific tissues that may link contaminant exposure in the LPR with potential adverse effects on reproduction and ELS development.

Any additional or modified protocols will be incorporated as addenda to the final Sampling and Analysis Plan for Evaluating Upper-Trophic Fish Injury in the Lower Passaic River and included in the Report of Assessment.

LOWER TROPHIC LEVEL FISH INJURY STUDIES

In July 2020, the Federal Trustees finalized and initiated a fish toxicity study entitled "Final Study Plan Fish Injury of the Lower Passaic River" (referred to in this update as the "Fish Toxicity Study") to assess potential injury to, and quantification of such injury, to lower trophic level fish species in the LPR.

Building on the objectives of the Fish Toxicity Study, the Federal Trustees finalized and initiated a subsequent study plan in 2021 to gather further information on the reproductive fitness of lower trophic level fish in the LPR entitled "Final Study Plan for Mummichog Fecundity and Oocyte Condition in the Lower Passaic River" (referred to in this update as the "Lower Trophic Fish Reproductive Fitness Study").

In 2022, the Federal Trustees will continue to collect mummichog from sites throughout its range in the LPR. Based on prior field collections, mummichogs were found to be absent or infrequent in certain areas of the LPR associated with lower salinity levels. Therefore, the Federal Trustees may also collect banded killifish (*Fundulus diaphanous*), a close freshwater relative of mummichog, in areas where banded killifish are likely to be present and more abundant than mummichog.

Consistent with the Fish Toxicity Study and the Lower Trophic Fish Reproductive Fitness Study (collectively referred to in this update as the "Lower Trophic Fish Studies"), in 2022, the Federal Trustees intend to conduct the field fish collection and laboratory analyses as described below.

Any additional or modified sampling or laboratory protocols associated with the activities described below will be included in the Federal Trustees' Report of Assessment.

Fish Toxicity Study

The work performed by the Federal Trustees as outlined in the Fish Toxicity Study will continue with additional fish collection and associated laboratory work in 2022 to assess potential injury to LPR fish, including embryo/larval ELS and adults. These activities will include, but not be limited to, assessment of gamete fertilization success and viability assessment, sediment exposure ELS developmental toxicity, and potentially, genomic sequencing of potentially affected tissues.

Assessment of Gamete Fertilization Success and Viability

As part of the ongoing evaluation of embryo-larval life stages of resident lower trophic level fish in the LPR, in 2022, the Federal Trustees intend to perform in-site and cross-site fertilization to continue the assessment of injury and contribution to ELS developmental injury in LPR fish.

In addition to continuing fertility assessments, in 2022, the Federal Trustees will also utilize cross-site (also known as "out-crossing") fertilization to further study the contribution of sperm and egg to developmental injury of lower trophic fish in the LPR. For this cross-fertilization analysis, the Federal Trustees will perform out-cross fertilization of egg and sperm from sites in the LPR that have been found to produce poor ELS viability (low or unsuccessful fertilization results) with reference sites, as well as other LPR sites. These studies will determine whether the egg or sperm quality is primarily responsible for impacts on fertilization impairments observed in prior testing.

In 2022, the same type of in-site and cross-site fertilization laboratory tests and analyses conducted on mummichog may be applied to banded killifish collected and used by the Federal Trustees for locations in the LPR for the reasons described above.

Sediment Exposure

In 2022, the Federal Trustees will continue the sediment exposure and toxicity assessment described in the Fish Toxicity Study. In 2021, the Federal Trustees' sediment toxicity assessment focused on evaluating LPR sediments for ELS developmental effects in laboratory-raised sheepshead minnow (*Cyprinodon variegatus*) embryos. In 2022, this sediment injury assessment will be performed using reference mummichog, and banded killifish where mummichog are not available, to assess developmental toxicity to these lower trophic fish species due to exposure to LPR contaminants.

Genomic Sequencing

In 2022, the Federal Trustees intend to, as part of the Lower Trophic Fish Studies, use genomic sequencing to identify molecular changes in fish associated with the exposure to contaminants in the LPR and concurrent reproductive and developmental injury identified in other assessment studies for the Diamond Alkali Superfund Site.

<u>Lower Trophic Fish Reproductive Fitness Study</u>

In 2022, the Federal Trustees will expand upon collection efforts started in 2021 to gather additional data assessing fecundity and oocyte health in lower trophic fish as outlined in the Lower Trophic Fish Reproductive Fitness Study. To further the study objectives for understanding the reproductive fitness of lower trophic fish in the LPR, the Federal Trustees' 2022 assessment activities aim to better understand LPR contaminant effects on male reproductive health. These field and laboratory studies will include the collection of adult male mummichog (and adult male banded killifish in freshwater areas of the LPR, for the reasons described above) to examine testes histopathology, as well as to evaluate sperm morphology,

motility, and viability. The Federal Trustees' histological examination of ovaries in lower trophic fish (primarily mummichog, or banded killifish where mummichog are not available) will also continue in 2022.