

PRELIMINARY RESEARCH PROPOSAL (COE) (FY05)

TITLE: Potential neurobehavioral mechanisms of latent mortality among salmon smolts migrating through the Columbia River hydrosystem

PROJECT LEADERS: Nathaniel L. Scholz
NOAA Fisheries
Northwest Fisheries Science Center
Environmental Conservation Division
2725 Montlake Blvd. E.
Seattle, WA 98112
Voice: (206) 860-3454
Fax: (206) 860-3335
Nathaniel.Scholz@noaa.gov

Arthur N. Popper
University of Maryland
Department of Biology
College Park, MD 20742
Voice: (301) 405-1940
Fax: (301) 314-9358
apopper@umd.edu

STUDY CODES: TPE-W-04-06

PROJECT DURATION: One year (beginning FY05)

SUBMISSION DATE: August 2004

PROJECT SUMMARY

The proposed project is a one-year pilot study with the following specific aim:

Investigate potential mechanisms of latent mortality for juvenile salmon that migrate through the Columbia River hydrosystem. Specifically, determine whether conventional fish passage operations damage sensory systems that are required by salmon for migration, orientation, predator detection, and predator avoidance when they enter the estuary below Bonneville Dam.

It is now well established that survival rates are relatively high for stocks of fish that migrate through the Columbia River hydropower system as juveniles en route to the ocean. This is true for fish that are transported around the dams in barges and also for fish that navigate the hydrosystem as in-river migrants [cite draft Tech memo?]. Paradoxically, however, multi-year analyses of smolt-to-adult returns (SARs) have shown that adult return rates are consistently lower than would be expected from the juvenile survival data. This has led to the hypothesis that different hydrosystem operations cause varying degrees of “delayed” or latent mortality, and that this latent mortality manifests at some point between the entry of fish to the estuary and their subsequent return from the ocean as adults.

Possible sources of latent mortality include increased disease susceptibility and increased predation. With respect to predation, potential mechanisms of latent mortality are poorly understood. There are several reasons for this uncertainty. First, hydrosystem-associated stressors are by definition sublethal, and thus would be difficult if not impossible to diagnose via casual observations or simply counting PIT-tagged fish along different routes through the hydrosystem. Second, the effects of fish passage operations on salmon physiology and behavior have not been systematically evaluated. Finally, “ecological death” via increased predation in the estuary or the ocean is almost impossible to monitor directly.

To advance our understanding of latent mortality, it will be necessary to specifically evaluate the physiological condition of fish that have different passage histories through the hydrosystem. One mechanistic approach is to focus on the impairment of behaviors that underlie predator-prey interactions, including orientation, habitat use, schooling, predator detection, and predator avoidance. Importantly, the expression of these behaviors depends on a properly functioning nervous system and, in particular, properly functioning sensory systems. Salmon sensory organs (i.e., the ear, nose, and lateral line) are vulnerable to a wide range of environmental stressors, and sublethal injury to any of these neurobehavioral systems during juvenile outmigration could increase predation losses when affected smolts reach the Columbia River estuary.

Here we propose a one-year pilot study to evaluate the ultrastructural integrity and functional performance of the auditory, olfactory, and lateral line systems of juvenile salmon exposed to different stressors via transport (i.e., barging) or in-river migration (i.e., spill and turbines). The experiments proposed will provide a very detailed

anatomical overview of the peripheral sensory organs. In addition, we will use neurophysiological recording techniques to measure the functional properties of the auditory system in response to natural environmental stimuli. Overall, this study will be the first to systematically evaluate the sensory condition of salmon with different routes of passage through the hydrosystem.

BACKGROUND

Large scale and long-term investigations are increasingly highlighting the importance of sublethal effects in terms of estimating the impacts of anthropogenic stressors at the scale of natural salmon populations (Peterson et al., 2003). In the specific context of predation, it is typically easier for predators to capture prey if the latter are in a substandard condition because of an exposure to an environmental stressor (Mesa et al., 1994;

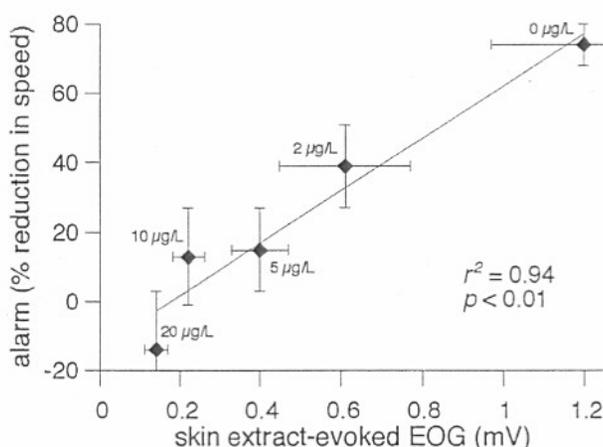


Figure 1. Relationship between a neurophysiological indicator of anthropogenic stress (copper exposure; concentrations range from 0 – 20 µg/L), as indicated by odor-evoked electro-olfactograms (EOGs) from the olfactory sensory epithelium, and effects on predator avoidance behavior, as indicated by alarm pheromone-induced antipredator (freezing) responses in juvenile coho salmon. The physiological and behavioral measures were obtained in a paired manner (i.e. both measurements were obtained from each fish) and were highly correlated. Each point is the mean of the 6-8 fish in the treatment group indicated by the label. Error bars represent one standard error of the mean. Sandahl and Scholz, unpublished results.

Temple, 1987). For juvenile salmon, a key determinant of predation vulnerability is the proper functioning of the nervous system. The nervous system serves as the physiological basis for detecting and avoiding predators, and stressors that impact sensory or motor function have been shown to increase predation vulnerability in juvenile salmon (Scholz et al., 2000; Mesa and Warren, 1997; Kruzynski and Birtwell, 1994).

The sensory organs of salmon and other fish are particularly sensitive to environmental injury. For example, underwater noise has recently been shown to cause hearing loss in fish (Smith et al., 2004), and high intensity sound damages the hair cells of the inner ear (McCauley et al., 2003). In turn, a loss of hearing could diminish a salmon's ability to detect predators or determine the

structure of the acoustic environment in the estuary (Popper, 2003). Chemical stressors, including copper and other heavy metals which may be released into the barge environment during transport from plumbing and holding tanks, have also been shown to damage primary sensory neurons in the olfactory system (Hansen et al. 1999) as well as the lateral line (Linbo and Scholz, unpublished results; see Fig. 4 below). For the salmon olfactory system, short-term (3 hr) exposures to copper at relatively low concentrations

interferes with the detection of chemical signals in the environment (Baldwin et al., 2003), as well as olfactory-mediated predator avoidance behaviors (Sandahl and Scholz, unpublished results; see Fig. 1).

Depending on the nature of the stressor, one or more of these key sensory systems (auditory, olfactory, or lateral line) may be vulnerable to sublethal injury from typical hydrosystem-associated fish passage operations. For example, all three systems could be impaired by the underwater noise and chemical contamination that may be associated with transporting fish. Conversely, the auditory system and lateral line could be affected by the shear, turbulence, or noise associated with passage through turbines.

To explore these possibilities, we are proposing to survey the sensory neurobiology of juvenile salmon exposed to transport, turbine, and/or spill environments. This one-year pilot study will be a collaborative effort between NOAA's Northwest Fisheries Science Center (Scholz, PI) and the University of Maryland's Center for Comparative and Evolutionary Biology of Hearing (Popper, PI). The two research groups have extensive experience in terms of evaluating the structure and function of sensory systems in salmon and other fish species. The study will be conducted by a staff research scientist (NWFSC) and a postdoctoral associate (UMD), with project oversight from the two PIs and technical support from research laboratories at the two institutions. This preliminary proposal identifies a general experimental approach that can be used to evaluate the condition of juvenile salmon, independent of passage history. If a full proposal is invited, we intend to work with the Corps of Engineers to identify the highest priority question(s) with respect to latent mortality (e.g., barges vs. turbines) and to develop a sampling and analysis plan that fit within the scope of a one-year pilot study.

To ensure that transportation does not impair the sensory capacity of juvenile salmon, we will compare the condition of the major sensory organs (the eye, lateral line, ear, and nose) in barged fish vs. in-river migrants. The structure and integrity of each sensory organ will be evaluated using histology and scanning electron microscopy (as appropriate). In addition, we will use neurophysiological recording techniques to measure the responsiveness of each sensory system to natural environmental stimuli. In the event that nervous system damage is found to occur in transported salmon, the information gained from this study could be used to: (1) identify and mitigate the source of the damage, and (2) investigate the consequences of sublethal nervous system injury for post-release behavior and survival.

OBJECTIVES

There are two basic (and complementary) approaches to evaluating the structural integrity and functional performance of salmon sensory systems. The first is to perform an ultrastructural analysis of the ciliated receptor neurons of the nose, ear, or lateral line using a combination of scanning electron microscopy (SEM), laser scanning confocal microscopy, and conventional histology. The advantages of this approach are that we could determine 1) whether hydrosystem-related stressors are sufficient to damage

sensory neurons during transport or in-river migration, 2) whether there are latent effects on sensory organs that manifest later in time (see, for example, McCauley et al., 2003), and 3) whether sensory neurons are repaired or replaced in the event of injury. The only major drawback to the ultrastructural approach is that it typically reveals only the most extensive damage, and there may be important losses of neural function that would not be evident from an anatomical screen alone (e.g., Sandahl et al., 2004). A second and more sensitive approach is to assess the functional performance and responsiveness of sense organs to natural environmental stimuli (Baldwin et al., 2003). In the case of auditory function, for example, the auditory brainstem response (ABR) is a non-invasive technique that is commonly used in fish to measure hearing sensitivity and how it changes as a result of noise exposure (Higgs et al., 2003). Recent studies using this approach have shown that even moderate amounts of noise can result in substantial hearing loss that lasts for weeks or longer in goldfish (Smith et al., 2004) and rainbow trout (A.N. Popper, unpublished results). Similar to the auditory system, odor-evoked field potential recordings are commonly used to monitor a loss of sensitivity in the olfactory system (Sandahl et al., 2004). The primary drawback to neurophysiological recordings is that they are more logistically difficult to conduct, particularly in the field.

For this pilot study, we are proposing a compromise approach. This will be to conduct an ultrastructural analysis of all three sensory systems, and to perform ABR recordings to monitor the hearing sensitivity of potentially affected juvenile salmon. Underwater noise is likely to be a significant stressor of concern, and the ABR method is straightforward and amenable to on-site data collection at appropriate points within the hydrosystem. Also, the equipment needed for ABR recordings is relatively inexpensive.

Objective 1 - Evaluate the ultrastructure of primary receptor neurons in the auditory, olfactory, and lateral line systems of outmigrating juvenile salmon.

Neuroanatomical screens have previously been used to diagnose peripheral nervous system damage in salmonids (e.g. Mesa et al., 1997) and, in particular, cell death in primary sensory neurons (e.g. Hansen et al., 1999). Although damage to sensory structures can be severe, it can also be difficult to detect because the affected fish appear healthy to the casual observer. For example, transported fish may look relatively normal while in a holding tank, and yet they may be cut off from their sensory environment and thus more vulnerable to predation when released below Bonneville Dam.

We propose to use conventional anatomical methods to directly evaluate the condition of sensory structures from smolts moving through the Columbia River hydrosystem. This will include sensory tissue from the otolithic end organs and the cristae of the semicircular canals, the ciliated sensory epithelium of the olfactory rosette, and free and canal neuromasts of the lateral line. Anatomical examples of these three sensory systems are shown in Figs. 2-4. As discussed above, a complete description of the sampling protocol will be given in the full proposal, but the general aim of the ultrastructural studies will be to collect reference and potentially affected fish (i.e., sample from a group of animals before, during, and after barging, or before and after passage through a turbine). Groups of fish will also be held for 4-6 weeks and

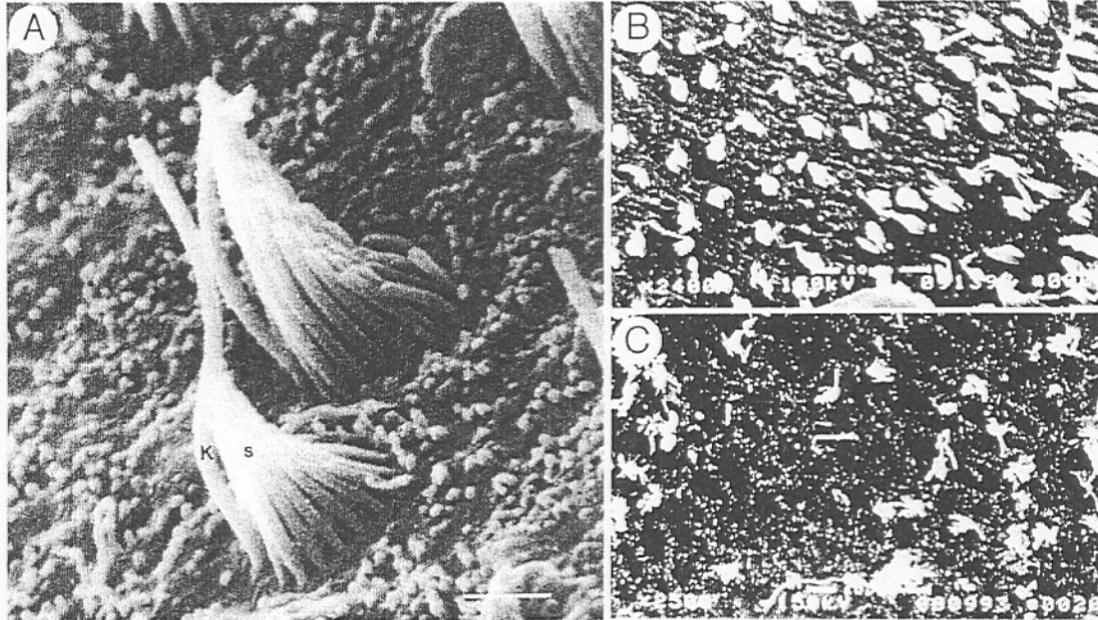


Figure 2. Scanning electron micrographs of apical ciliary bundles on the sensory epithelium of the fish inner ear. *A*, High magnification image from a goldfish (*Carassius auratus*) showing kinocilia (K) and stereocilia (S), the two types of ciliary bundles that extend from primary sensory neurons in the ear. *B*, Lower magnification image from the oscar (*Astronotus ocellatus*) showing the normal appearance of sensory ciliary bundles. *C*, In the oscar, ciliary bundles are damaged by a one hour exposure to 300 Hz continuous-wave stimulation at 180 dB (*re*: 1 μ Pa). *A* adapted from Platt and Popper (1981); *B* & *C* adapted from Hastings, et al. (1996).

subsampled to monitor for delayed degeneration and/or recovery. We will collect tissue samples for all three sensory systems from each fish.

For conventional histology, we will embed the target organs in plastic, section the tissues on a microtome, and then screen the sections using standard histopathological markers in tandem with light microscopy and a high-resolution digital imaging system. The advantage of this approach is that it clearly outlines the complex anatomy of certain sensory structures – for example, the psuedostratified columnar structure of the olfactory epithelium (Fig. 3). In addition, it is relatively straightforward to identify neurons that are dying via necrotic or apoptotic pathways.

Histological screens of sensory tissues will be conducted to examine sensory neurons and associated supporting cells. Just prior to tissue collection, fish will be euthanized and tissues immediately removed and placed into a formalin-based fixative. The fixed tissues will be shipped to the NWFSC or UMD for further processing. Tissues will be dehydrated through a graded series of ethanol, and infiltrated with a plastic resin (methyl-methacrylate). Plastic embedded tissues will be sectioned 1 to 4 microns thick, and stained with standard stains such as Hemotoxylin and Eosin, or methyl blue. Specialized stains may also be used as needed. Sections will be examined with a Nikon E600 compound light microscope for necrotic lesions, abnormalities of cellular architecture, or

disruption of sensory structures such as cilia and microvilli, as seen in cross section. Images of sections will be captured using a SPOT-RT digital camera and stored on digital media.

For the purposes of imaging, we will use a scanning electron microscope to visualize the anatomy of primary receptor neurons. Scanning electron microscopy (SEM) provides three-dimensional images of cell surface structures at magnifications much higher than can be seen with a standard stereo light microscope. This allows observation of surface features such as the cilia and microvilli that are prominent on sensory cell surfaces.

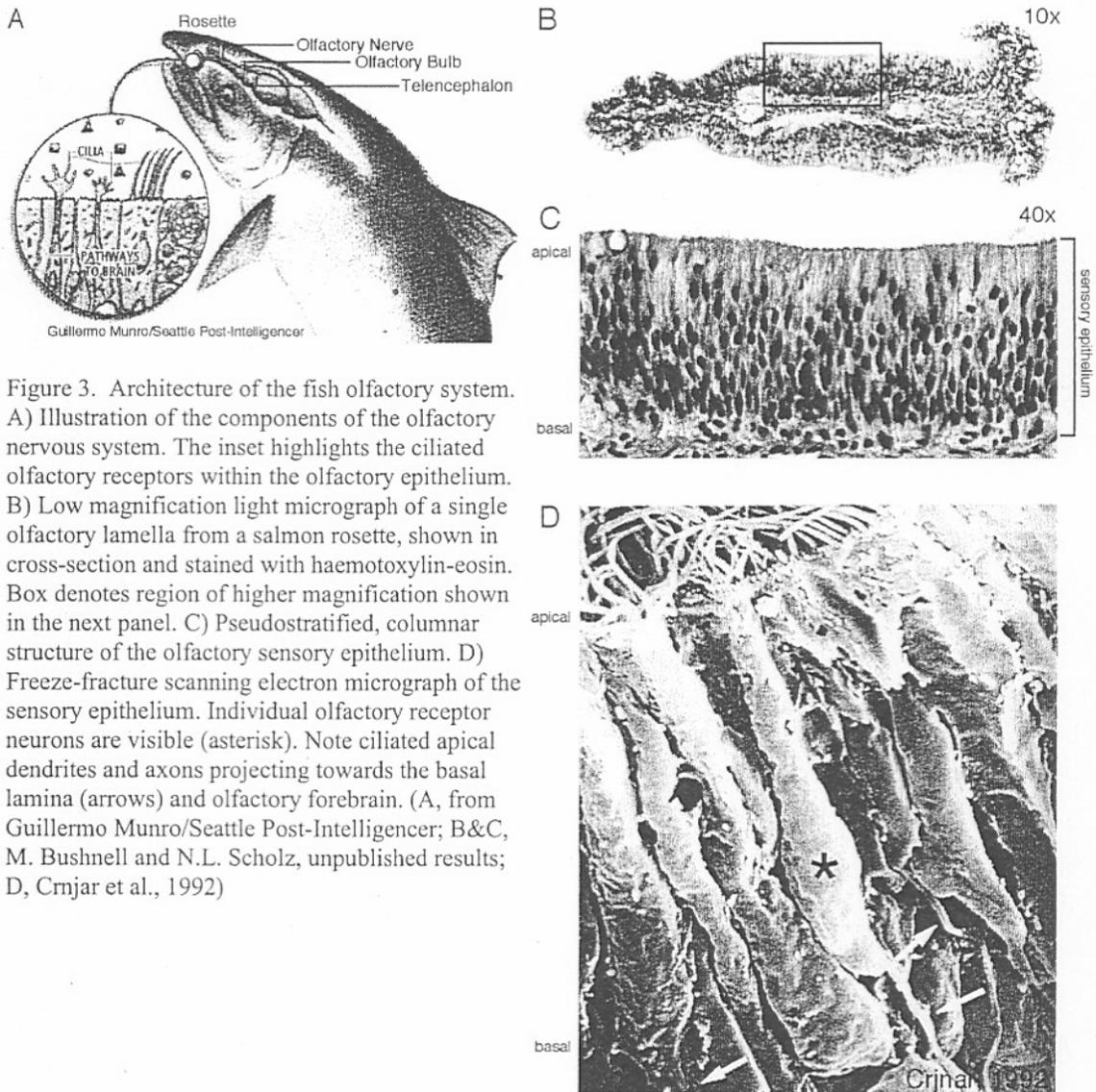


Figure 3. Architecture of the fish olfactory system. A) Illustration of the components of the olfactory nervous system. The inset highlights the ciliated olfactory receptors within the olfactory epithelium. B) Low magnification light micrograph of a single olfactory lamella from a salmon rosette, shown in cross-section and stained with haematoxylin-eosin. Box denotes region of higher magnification shown in the next panel. C) Pseudostratified, columnar structure of the olfactory sensory epithelium. D) Freeze-fracture scanning electron micrograph of the sensory epithelium. Individual olfactory receptor neurons are visible (asterisk). Note ciliated apical dendrites and axons projecting towards the basal lamina (arrows) and olfactory forebrain. (A, from Guillermo Munro/Seattle Post-Intelligencer; B&C, M. Bushnell and N.L. Scholz, unpublished results; D, Crnjar et al., 1992)

Scanning electron microscopy (SEM) has been widely used to evaluate the condition of auditory, olfactory, and lateral line receptors in fish. In these three sensory systems, the receptor neurons are ciliated, and the integrity of the cilia can be directly examined using

SEM. This is important because the cilia are particularly sensitive to environmental

stressors and sensory function is lost if the cilia are damaged or destroyed. An example of hair cell damage is shown in Fig. 2.

Thus, the advantage of SEM is that it can be used visualize the three-dimensional architecture of sensory neurons. Surface features of sensory organs will be examined using a JEOL JSM-5610LV scanning electron microscope. Tissue will be collected as for histology and placed into a formalin-based fixative appropriate for SEM. After 24 hrs, fixative will be removed and tissues will be placed into a buffer solution until samples can be returned to the laboratory for processing.

Tissues will be dehydrated in a graded series of ethanol, followed by a graded series replacing ethanol with acetone. Tissues will be then processed in a critical point dryer, which gradually removes the acetone, leaving the tissue completely dry with minimum artifacts. Tissues will adhere to stubs, then coated with a conductive metal (gold/palladium) to provide a conductive surface for optimum viewing with SEM. Images will be recorded and stored with digital imaging software (JEOL).

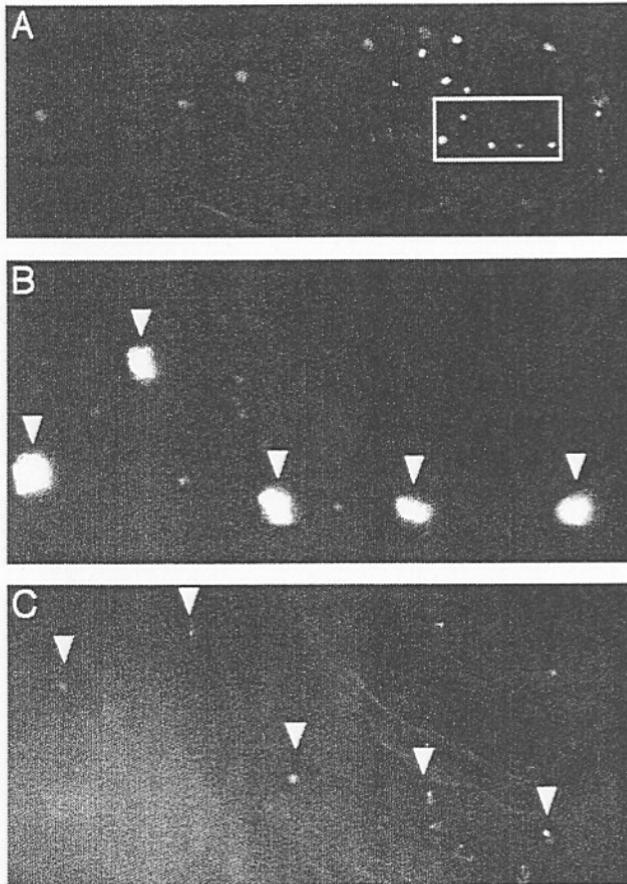


Figure 4. Epifluorescent micrographs showing lateral line neuromasts in a zebrafish larvae. The receptor neurons of the lateral line system have been labeled with a fluorescent vital dye (DASPEI). *A*, Low magnification image showing the distribution of neuromasts along the body of a control animal. The box indicates the region shown in *B*. *B*, Higher magnification image of the suborbital neuromasts from the same fish. Note that numerous sensory neurons are labeled within each neuromast. *C*, Suborbital neuromasts in an animal exposed to an environmental stressor (50 µg/L copper). Note the marked reduction in labeled cells within each neuromast. Kao and Scholz, unpublished results.

Objective 2 – Monitor hearing changes using the auditory brainstem response (ABR).

While anatomical observations (Objective 1) can reveal physical damage to salmon sensory organs, there could also be impacts on sensory physiology that would not be evident from an anatomical screen. To address this possibility for the auditory system, we will use a conventional neurophysiological technique (the ABR) to measure the

hearing sensitivity in juvenile salmon with different passage histories through the hydrosystem. This technique has been described previously (Smith et al., 2004). In brief, each fish will be restrained in a mesh sling and suspended in a 19-l plastic bucket filled with water. The fish will be suspended so that the top of the head will be approximately 3 cm below the water surface and 25 cm above a UW-30 underwater speaker. A reference electrode will be placed on the dorsal surface of the fish's head along the midline between the anterior portion of the eyes while a recording electrode will be placed on the dorsal midline surface of the fish approximately halfway between the anterior insertion of the dorsal fin and the posterior edge of the operculae, directly over the brainstem. A ground electrode will be placed in the water near the body of the fish.

To determine hearing thresholds, sound stimuli will be presented and ABR waveforms will be collected using a physiology apparatus using SigGen and BioSig software [Tucker-Davis Technologies (TDT) Inc., Gainesville, FL, USA]. Sounds will be computer generated *via* TDT software and passed through a power amplifier connected to the underwater speaker. Tone bursts will have a 2 ms rise and fall time, and will be 10 ms in duration and will be gated through a Hanning window – similar to the conditions of other ABR studies. Responses to each tone burst at each SPL will be collected using the BioSig software package, with 400 responses averaged for each presentation. The calibration of each frequency will be done using a calibrated Model 902 Interocean Systems, Inc. (San Diego, CA, USA) underwater hydrophone (calibration sensitivity of -195 dB re $1 \text{ V}/\mu\text{Pa}$; ± 3 dB, 0.02-10 kHz, omnidirectional).

Note that tissue and electrophysiological samples can be collected from the same animals. Thus, Objectives 1 & 2 will be carried out concurrently.

SCHEDULE

This is a one-year pilot study that will commence in 2005.

DATA ANALYSIS AND STATISTICS

For anatomical analyses, the severity of the observed effects will be examined and ranked (*e.g.* sensory cilia absent, highly eroded, slightly eroded, intact). Significant differences among groups (transported and in-river) will be determined using a non-parametric test (Mann Whitney or Kruskal Wallis, depending on the number of groups). The observed effects will be documented with digital reproductions of the specimens.

For the electrophysiological experiments, the results will be analyzed using conventional multifactor ANOVA methods. The response data will be transformed if needed (*e.g.* log transformation) to normalize the data. A significant effect ($p < 0.05$) of passage route will be used to indicate a significant effect of anthropogenic stress.

FISH REQUIREMENTS

The number of samples, as well as the time and location of sampling, will be worked out in cooperation with the Corps of Engineers. We will also coordinate to the extent possible with other fish passage projects that will be ongoing in 2005.

EXPECTED RESULTS AND APPLICABILITY

The experiments proposed here specifically address the transportation question and the informational needs highlighted by TPE-W-04-06. However, a similar approach could be used to evaluate the sensory capacity of fish moving through other passage routes where lower than expected survival has been observed – for example, turbines and spillways. Thus, the results from a small and focused transportation study will demonstrate a technology that could be used to address related management concerns in the hydrosystem.

If, during the course of this study, sensory injury is observed, additional studies can be designed to determine: 1) the source of the injury and strategies for mitigation, and 2) the consequences for post-release survival and potential causative links between nervous system damage and latent mortality.

RELEVANCE

Information from this study can be used to directly or indirectly address actions in the NMFS 2000 Biological Opinion – notably, RPA 49. 51, 52, 189, and 195. For example, action 189 stipulates that the causes of discrepancies in adult return rates for juvenile salmonids that have different passage histories through the hydrosystem be investigated.

PROJECT IMPACTS, FACILITIES, AND EQUIPMENT

There are no anticipated impacts on threatened or endangered salmonids in the Columbia River hydrosystem from this study. We can address the core hypothesis (i.e., that barging damages the sensory biology of smolts) using hatchery fish.

All of the experiments under Objective 1 will be conducted in the Ecotoxicology and Environmental Fish Health Program at the NWFSC Montlake facility, or at the University of Maryland's Center for Comparative and Evolutionary Biology of Hearing. Both PIs supervise research laboratories with standard equipment for neurobiology research. This includes a full range of conventional laboratory equipment (e.g. refrigerators, freezers, centrifuges, etc.) as well as microscopes (stereo, compound, and epifluorescent), cameras for low-light digital imaging, and computers and software for image and data analysis. Both institutions have a scanning electron microscope at the Center and a laser scanning confocal microscope.

The experiments under Objective 2 will be conducted at a suitable facility in the Columbia River hydrosystem.

PROJECT PERSONNEL AND DUTIES

1. Project co-leaders – Nat Scholz (NOAA-F/NWFSC) and Art Popper (UMD).
2. Histopathology and microscopy – NOAA-F staff scientist.
3. ABR recordings – UMD postdoc.
4. Data analysis – all.
5. Report writing – all.

TECHNOLOGY TRANSFER

Technology transfer will be in the form of written and oral research reports as required. A draft report will be provided to the COE at the completion of the project. Results will also be published in appropriate scientific journals and presented at scientific forums.

REFERENCES

- Baldwin, D.H., Sandahl, J.F., Labenia, J.S., and Scholz, N.L. (2003). Sublethal effects of copper on coho salmon: impacts on non-overlapping receptor populations in the peripheral olfactory nervous system. *Environ. Toxicol. Chem.* 22:2266-2274.
- Boston, J.R. (1981). Modeling the effects of stimulus frequency and intensity on hair cell potentials. In: *Hearing and Sound Communication in Fishes* (Tavolga, W.N., Popper, A.N. and Fay, R.R. eds.). Springer-Verlag, pp 507-513.
- Crnjar, R., Scalera, G., Bigiani, A., Barbarossa, I.T., Magherini, P.C. and Pietra, P. (1992). Olfactory sensitivity to amino acids in the juvenile stages of the European eel *Anguilla anguilla* (L.). *J. Fish Biol.* 40:567-576.
- Evans, R.E. and Hara, T.J. (1985). The characteristics of the electro-olfactogram (EOG): its loss and recovery following olfactory nerve section in rainbow trout (*Salmo gairdneri*). *Brain. Res.* 330:65-75.
- Hansen, J.A., Rose, J.D., Jenkins, R.A., Gerow, K.G. and Bergman, H.L. (1999b). Chinook salmon (*Oncorhynchus tshawytscha*) and rainbow trout (*Oncorhynchus mykiss*)

exposed to copper: Neurophysiological and histological effects on the olfactory system. *Environ. Toxicol. Chem.* 18:1979-1991.

Hastings, M.C., Popper, A.N., Finneran, J.J. and Lanford, P.J. (1996). Effects of low-frequency underwater sound on hair cells of the inner ear and lateral line of the teleost fish *Astronotus ocellatus*. *J. Acoust. Soc. Am.* 99:1759-1766.

Higgs, D.M., Rollo, A.K., Souza, M.J. and Popper, A.N. (2003). Development of form and function in peripheral auditory structures of the zebrafish (*Danio rerio*). *J. Acoust. Soc. Am.* 113:1145-1154.

Kruzynski GM, Birtwell IK. 1994. A predation bioassay to quantify the ecological significance of sublethal responses of juvenile chinook salmon (*Oncorhynchus tshawytscha*) to the antisapstain fungicide TCMTB. *Can J Fish Aquat Sci* 51:1780-1790.

McCauley, R.D., Fewtrell, J. and Popper, A.N. (2003). High intensity anthropogenic sound damages fish ears. *J. Acoust. Soc. Am.* 113:638-642

Mesa, M.G. and Warren, J.J. (1997). Predator avoidance ability of juvenile chinook salmon (*Oncorhynchus tshawytscha*) subjected to sublethal exposures of gas-supersaturated water. *Can. J. Fish. Aquat. Sci.* 54:757-764.

Mesa, M.G., Poe, T.P., Gadomski, D.M., and Peterson, J.H. 1994. Are all prey created equal? A review and synthesis of differential predation on prey in substandard condition. *J. Fish. Biol.* 45:81-96.

Peterson CH, Rice SD, Short JW, Esler D, Bodkin JL, Ballachey BE, Irons DB. 2003. Long term ecosystem response to the Exxon Valdez oil spill. *Science* 302:2082-2086.

Platt, C. and Popper, A.N. (1981). Fine structure and function of the ear. In: *Hearing and Sound Communication in Fishes* (Tavolga, W.N., Popper, A.N. and Fay, R.R. eds.). Springer-Verlag, pp 3-38.

Popper, A.N. (2003). Effects of anthropogenic sound on fishes. *Fisheries* 288:24-31.

Sandahl, J.F., Baldwin, D.H., Jenkins, J.J., and Scholz, N.L. (2004). Odor-evoked field potentials as indicators of sublethal neurotoxicity in juvenile coho salmon exposed to copper, chlorpyrifos, or esfenvalerate. *Canadian Journal of Fisheries and Aquatic Sciences*, 61:404-413.

Scholz, N.L., Truelove, N.K., French, B.L., Berejikian, B.A., Quinn, T.P., Casillas, E. and Collier, T.K. (2000). Diazinon disrupts antipredator and homing behaviors in chinook salmon (*Oncorhynchus tshawytscha*). *Can. J. Fish. Aquat. Sci.* 57:1911-1918.

Smith, M.E., Kane, A.S. and Popper, A.N. (2004). Noise-induced stress response and hearing loss in goldfish (*Carassius auratus*). *J. Exp. Biol.* 207:427-435.

Temple, S.A. (1987). Do predators always capture substandard individuals disproportionately from prey populations? *Ecology*, 68:669-674.