

Response to ISRP Comments for Project ID: 35028

Evaluate White Sturgeon Nutritional Needs & Contaminant Effects
Influenced by the Hydroelectric System

Principal Investigator: Eugene Foster: PSU

ISRP Comment: There are inherent difficulties in field ecotoxicological studies. One difficulty is having some certainty that physiological and biological changes translate into biologically meaningful reductions in growth and reproduction. The second difficulty is knowing that physiological and biological changes are a result of exposure to contaminants and not some other environmental factor or density related phenomenon. In addition, how will the problems of extrapolating physiological and biochemical responses to growth and reproduction be addressed?

Response: There are inherent difficulties with any field study in that inferences and predictions that can be made using traditional cause and effect experimental designs are difficult in field situations because, among other things, significant confounding variables may not be controlled or recognized therefore creating a situation where there are no true control populations for comparison to “treatment” groups (Hilborn and Ludwig 1993). Field studies rely on: 1) comparison between groups that appear dissimilar but may have overlapping covariables that could affect the measured endpoint, 2) correlations between a dependent variable and independent variables, and 3) associations of variables through coordinates or patterns.

Ecotoxicological studies have the added complexity of using different levels of biological organization (hierarchical approach) which introduces potential error when extrapolating from a lower level effect to a higher level effect (Suter 1995; Perez 1995). Studies in ecotoxicology have traditionally used the hierarchical approach because specific effects at lower levels of biological organization can be associated with exposure to chemicals while higher levels of biological organization are more ecologically meaningful but are responsive to a variety of stressors (Landis and Yu, 1995). Field ecotoxicological studies are useful for indicating pollutant effects on organisms through this hierarchical, weight of evidence approach, and when used in combination with laboratory studies, can be powerful tools for identifying contaminant effects at organ, individual, and population levels. This approach is especially important to use when the species of concern is either not easily cultured, there is not a suitable surrogate species, or the organism is long lived making experiments at critical advanced life stages difficult. White sturgeon fall into this category because of their life history strategy in which they are long lived, slow to grow, delayed reproductive maturation, and individual spawning does not occur annually.

Biochemical and physiological endpoints in conjunction with growth, reproduction, or survivorship can be used to show associations between lower levels of biological organization and the important ecological endpoints (Adams et al., 1989). Some biochemical and physiological endpoints respond only to a specific contaminant or class

of contaminants and are therefore good indicators of exposure or effects from these type of chemicals (Walker et al., 1996). The enzyme, cytochrome P450 1A, is induced from exposure to Ah-receptor agonists such as co-planar dioxin, furans, PCBs, and PAHs. The enzyme activity for cytochrome P450 1A can be measured by the EROD assay. Fish basal levels of EROD activity are nearly zero. Therefore, when EROD activity is above zero this is an indication that the organism was exposed to an Ah-receptor agonist. Similarly, metallothionein is induced with exposure to certain types of metals, such as cadmium and copper. Other enzyme systems are inhibited by exposure to specific chemicals. Acetylcholinesterase (AChE) activity is inhibited by exposure to organophosphate and carbamate insecticides. However, AChE activity can be modulated by temperature and when measuring this endpoint it is important to know the temperature at the different locations in order to correct for temperature differences. This shows that when using biochemical and physiological effects it is important to know the other factors that could affect the endpoint and to monitor for those factors.

The lower levels of biological organization (biochemical, molecular, physiological, histological) can be used to show exposure to contaminants and that those exposures are at high enough concentrations to cause a biological response. These responses can also be used as an early warning signal of potential effects at higher levels of biological organization (individual and population). In addition, the combination of biochemical and physiological endpoints can be used to put contaminant tissue concentrations into context. That is, whether the tissue concentration is sufficient to cause a deleterious response. This is especially important when analytical chemistry is able to detect smaller and smaller amounts of chemicals in the environment. There has been a great deal of work done on tissue concentrations and the risk to human health from eating contaminated fish but much less work has been done on understanding the effect of these contaminant levels on the fish itself.

There have been ecotoxicology studies used in a variety of locations to identify contaminant effects on fishery resources. Studies have been done in the Great Lakes identifying effects of PCBs on reproduction in salmonids (Mac and Edsall, 1991) and lake trout early life stage mortality from dioxins (Walker & Peterson 1990). Field studies have used a weight of evidence approach utilizing biochemical, physiological, and histological endpoints in conjunction with growth, reproduction, and survivorship for determining if it might be reasonable to assume that contaminants were having an adverse affect on fishery resources (Adams et al., 1996). Controlled laboratory experiments could then be used to show toxicological relevance of environmental concentrations of the contaminant and establish cause and effect relationships (Spitsbergen et al., 1991; Walker et al., 1991). Laboratory studies are useful for identifying potential mechanisms of toxicity. However, laboratory studies usually lack the complexity of multiple stressors for evaluating exposure to multiple chemicals, environmental and density dependent stressors that occur in the environment. We are at these initial stages with Columbia River white sturgeon in our understanding of contaminant tissue concentrations, biochemical and physiological responses, and potential effects at higher levels of biological organization.

Through our studies we have shown that:

- 1) Condition factor (CF) was lower in white sturgeon from Bonneville Pool and that CF was negatively correlated with liver organochlorines such as PCBs.
- 2) Plasma androgens were negatively correlated with liver p,p'-DDE. Altered plasma androgen concentration can negatively affect growth and reproductive maturation.
- 3) Organochlorine concentrations in liver and gonads were lower in the estuary than above Bonneville dam. This is in contrast to studies done on other river estuary systems that show an increasing trend of contaminants downstream.
- 4) Liver enzymes, such as EROD activity were higher in white sturgeon from Bonneville and The Dalles Pool.
- 5) Vitellogenin (Vtg), an indicator of exposure to xenoestrogens, was found in the plasma of male white sturgeon in The Dalles and John Day Pool.
- 6) Triacylglycerides (TAG) were lower in white sturgeon from Bonneville Pool.
- 7) Intersexed fish and irregular ovarian development was observed in several female white sturgeon and consistent with exposure to environmental contaminants.
- 8) Liver histology that was consistent with contaminant exposure in several white sturgeon.

The correlation between organochlorine concentration and CF may not be the causative factor for reduced condition factor, poor growth, or late maturation of white sturgeon in the Bonneville Pool. There are a number of factors that would affect CF including food availability and quality, and competition. However, Ah-receptor agonists, such as chlorinated dioxins, can reduce growth in fish (Weiden et al., 1992) and cause wasting syndrome in mammalian models (Seefeld et al., 1984). Co-planar PCBs would be expected to act through the same mechanism of action as chlorinated dioxins. In addition, Vtg is a large molecule that is energetically expensive for an animal to produce. Energy used to produce Vtg could have been used for somatic growth or gonad maintenance. Elevated level of Vtg in white sturgeon from John Day may be playing a role in poor growth of juvenile white sturgeon from that reservoir. However, food availability and quality can not be ruled out.

The information from this study would be used to evaluate whether the fish had recently eaten (stomach content), the quality of the food (caloric content), and the nutritional status of the animal (CF, plasma TAG, gonad and liver lipid content, and length to age). This information would then be compared to tissue concentrations for trace elements and organochlorines, enzymatic markers for specific exposures to classes of chemicals (EROD activity, Vtg, and cytochrome P450 3A), intersexed fish, and histological indicators of chemical stress (macrophage aggregates and neoplasia). This data would be evaluated using ANOVA/ANCOVA for location differences, regression and multivariate methods will be used to examine associations between endpoints. Using these multiple endpoints we will be able to better understand the amount of nutritional variability that can be explained by food availability and quality versus contaminants.

ISRP Comment: There needs to be more justification of the consequences of the research if it is to be useful to managers.

This study would provide information for evaluating if the contaminant levels in white sturgeon are high enough to contribute to the poor growth and nutritional status of white sturgeon from the Columbia River.

The lower CF and slow growth for white sturgeon in the Bonneville Pool has been attributed to poor food availability and too many sturgeon in that reservoir. One of the fishery management options would be to reduce the numbers of white sturgeon in that reservoir. However, if contaminant levels are playing an important role then reducing the number of white sturgeon in the reservoir may not improve CF and growth and would only reduce the number of fish in the reservoir.

White sturgeon do not have migratory access to the ocean because of the dams. Historically, the ocean may have been an important source of trace elements for growth and reproduction. Data from this study would be used for determining if essential trace elements could be playing a role in CF and nutritional status. If they are then this information may be useful to fishery managers in determining the need for white sturgeon passage between dams.

This information would be useful to managers of water quality for determining if the Columbia River should be listed as water quality limited for classes of chemical contaminants. These listing would trigger the Total Maximum Daily Load (TMDL) process and lead to waste load and load allocations for reducing pollutant loads being discharged by point and nonpoint sources of pollution.

ISRP Comment: There needs to be clear mechanistic hypothesis and methods for data analysis that are specific to testing the hypothesis.

Response:

1) We propose that fish with elevated levels of organochlorines will have reduced CF, TAG, plasma androgens, lipid reserves which would be modulated by food quality.

The observed correlations between organochlorine concentration and CF, TAG, and GSI may not be the causative factor for reduced CF, poor growth, and delayed maturation of white sturgeon in the Bonneville Pool. Diet has been shown to significantly affect white sturgeon fatty acid composition (Xu et al., 1993).

There are a number of factors that would affect CF and growth including food availability and quality, and competition. However, Ah-receptor agonists, such as chlorinated dioxins, can reduce growth in fish (Weiden et al., 1992) and cause wasting syndrome in mammalian models (Seefeld et al., 1984). Co-planar PCBs would be expected to act through the same mechanism of action as chlorinated dioxins.

This information will be used to evaluate whether the fish had recently eaten (stomach content), the quality of the food (caloric content), and the nutritional status of the animal (CF, plasma TAG, gonad and liver lipid content, and length to age). This information would then be compared to tissue concentrations for trace elements and organochlorines, enzymatic markers for specific exposures to classes of chemicals (EROD activity and cytochrome P450 3A), intersexed fish, and histological indicators of chemical stress (macrophage aggregates and neoplasia). This data would be evaluated using ANOVA/ANCOVA for location differences, regression and multivariate methods will be used to examine associations between endpoints.

2) We propose that indicators of xenoestrogen exposure will be associated with lower lipid reserves and nutritional status.

Poor growth has recently been reported for white sturgeon from the John Day Pool. Elevated levels of Vtg were observed in white sturgeon from the John Day Pool but may not be the causative factor (Feist et al., 2002). Xenoestrogens have caused decreased levels of vitamins in fish (Palace et al., 2001), decreased GSI (Jobling et al., 1996), and elevated levels of Vtg (Folmar et al., 1996). Vtg induction would be energetically costly for fish to produce and would be expected to utilize resources that could have been used for somatic growth..

The food quality endpoints and Vtg will be compared to the nutritional endpoints using regression and multivariate methods to identify associations between the endpoints. Location differences will be examined using ANOVA and ANCOVA for determining differences between the estuary and reservoirs for the potential of pooling the data between reservoirs.

3) We propose that fish above Bonneville dam will have lower essential trace elements than fish from the estuary,

The Columbia River Basin is deficient in certain essential trace elements necessary for maintenance of health and reproduction. Deficiency in selenium, iodine and other essential trace elements can lead to physiological impairment (National research Council, 1993). Historically, white sturgeon were able to access ocean resources that were rich in essential trace elements. These essential trace elements may have been important in the life history strategy for these fish. The construction of the hydropower system has isolated white sturgeon from these ocean resources.

Measured levels of essential trace elements will be compared to the nutritional endpoints using regression and multivariate methods. Analysis of location differences will be done using ANOVA and ANCOVA. The comparisons will provide information on the relative importance of essential trace elements to fish health.

ISRP Comment: How will nutritional status be assessed? How will the information from stomach content and caloric value be used to determine if the nutritional needs are being met? How will the relationship between stomach contents, caloric content, and nutritional status be assessed and what will it mean?

Response: Nutritional status will be assessed by measuring CF, plasma TAG, gonad and tissue lipid, length and weight versus age, food item taxonomy (FIT), and stomach caloric content (SCC). The CF is a common descriptor used for fish growth but this approach ignores energetic factors, such as lipid content (Mommensen 1998). Lipid reserves and age of fish will give important information for energetic status of the fish and growth. Regression and multivariate techniques will be used to evaluate the variability explained by endpoints such as trace elements, organochlorines, enzyme activities, and histology for the nutritional endpoints. Whether nutritional needs are being met will be evaluated by comparison of nutritional endpoints

ISRP Comment: How will the data from measuring contaminant levels in sediments, stomach contents, and fish tissues and used to assess the association between nutritional status and contaminant concentration, and to use sediment and water chemistry to evaluate location differences and associations with tissue chemistry be performed? What type of analysis will be done?

Response: Correlations between sediment concentrations and tissue concentrations would be used to begin understanding the biota – sediment accumulation factors (BSAF). A major route of uptake into aquatic organisms for persistent bioaccumulative chemicals, such as PCBs, dioxins, furans, and chlorinated pesticides, is from the sediment into the food chain and then into the target organism. The BSAFs are used for determining the tissue chemical concentrations that would result from sediment chemical concentration. Knowing the tissue chemical concentration of concern would allow back calculation to a sediment concentration using the BSAF for determining the sediment chemistry levels necessary for the protection of the organism. In addition, sediment chemistry data will be evaluated for differences between the estuary and reservoirs using ANOVA/ANCOVA to begin understanding the spatial variability of these contaminants in the Columbia River.

Knowing if xenoestrogenic chemicals are in the water column would be useful in determining if the plasma Vtg observed in white sturgeon from The Dalles and John Day reservoirs were a result of pollutant discharge or potentially from dietary phytoestrogens. Water samples will be collected and analyzed for xenoestrogenic chemicals such as alkylphenolic compounds. These are chemicals commonly found in detergents and other household products. However, these chemicals have rarely been analyzed for in the Columbia River. Water chemistry data will be used for comparison between reservoirs and associations with plasma Vtg in white sturgeon to determine if the plasma Vtg could be a result of pollutant discharge.

ISRP Comment: Justify using CF and GSI as indicators of effects. What would be an adverse effect? What range?

Response: The CF is used as a descriptor of fish growth but this approach ignores energetic factors and should be used in combination with other indicators of fish growth and nutritional status that would be measured in this study, such as plasma TAG, lipid reserves, and length and weight versus age.

The gonado-somatic index (GSI) is a ratio of the gonad to total weight of the fish that has been used in studying the effect of endocrine disrupting chemicals in fish (Jobling et al., 1996). Lower GSI indicates that less resources are being used for reproduction by the fish. Lower GSI would indicate that fewer gametes would be released by the fish causing lower reproductive success. In addition, it appears in white sturgeon that the gonad tissue is a depot for lipid reserves that may be used by the fish for reproduction and could be an important trigger for determining if the fish is energetically ready for reproductive maturation.

We don't know what a "normal" range for CF and GSI are in wild Columbia River white sturgeon. However, we do know the ranges for CF and GSI for white sturgeon from the estuary where growth and reproductive success has been strong and in the reservoirs where CF is lower and growth is slow and reproductive maturation is delayed. This historic data from the Columbia River would be used for guiding our assessment of suitable CF and GSI ranges for white sturgeon. In addition, this data would be used for looking for associations between GSI, CF, other nutritional endpoints, and food quality and chemical contaminants to determine if contaminants are playing a role or if food quality is the dominant factor.

ISRP Comment: What new knowledge would be gained from this proposal?

Response: This study would provide information for determining whether contaminant levels reported in white sturgeon are high enough to contribute to the reduced nutritional status and growth of white sturgeon in the Columbia River.

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