2013 Wisconsin DNR analysis of sucker tumors

Scope of Work - University of Wisconsin Center for Limnology

Background

Analysis of tumor frequencies in white suckers collected in the Sheboygan River in April 2012 indicate substantial incidence rates of liver tumors (~8.3%). Given that incidence rates exceed accepted thresholds suggesting the presence of toxic contaminants (5%), it is essential to understand whether the individual fish being assessed for tumors reside within or outside the limits of named Areas of Concern (AOC). If fish reside within the AOC, the removal of sediments or other remediation strategies in that area may be expected to reduce beneficial use impairments (BUI). However, if fish reside elsewhere, then the linkage between contamination within the AOC and indicators such as fish tumor BUIs may be weak.

White suckers are a major native fish species in the Great Lakes, and are widely used to assess contaminant burdens via tumor analysis. Every year, they perform spring migrations in March-May to reach spawning habitats within tributary rivers. Capturing these fish is easiest during the migration, when they aggregate and move upstream *en masse*. There is little information about the size of the area used by white suckers outside the breeding season; for most populations, we lack data to resolve whether they reside primarily within or near rivers, or instead travel widely outside those areas. This aspect of the biology of white suckers is fundamental to interpreting data on their tumor rates because most AOCs in Wisconsin are designated within large tributary rivers and associated harbors or embayments.

Stable isotope analysis enables tracking the movement of fishes when they shift between habitats that differ in their stable isotope signatures. This is because the tissues of all animals possess a similar and predictable stable isotope ratio compared to their diet. Thus, if all potential diet items in one area differ in stable isotope ratios from all potential diet items in another area, the tissues of fishes residing in each area will reflect these differences.

We measured stable isotope ratios of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) in 50 white suckers collected in the Sheboygan River in 2012. The results clearly indicate that these fish do not reside within the Sheboygan River *per se* or the associated harbor, both of which would have much higher $\delta^{15}N$ and $\delta^{13}C$ isotope ratios than nearby habitats in Lake Michigan based on comparisons among likely diet items. However, there were slight differences in stable isotope ratios between fish with and without tumors, which is likely a reflection of the physiological costs of bearing a tumor. Comparison of the Sheboygan suckers to counterparts collected in 12 other Wisconsin tributaries suggests that sucker populations are localized and distinct along the shore of Lake Michigan, however we cannot yet resolve the spatial scale at which individual movement might occur.

These results demonstrate that stable isotope analysis of every individual sampled for tumor incidence will assist in interpreting results with regard to habitat use, and thereby inferences about likely history of exposure to contaminants within designated AOCs. For

instance, if fish sampled within an AOC area represent a mixture of individuals that reside within and outside that area, isotope ratios can be used to separate these two spatial contexts, enabling strong tests for differential likelihood of tumors. Similarly, if fish are sampled from outside an AOC area (i.e., a reference site such as the Kewaunee River), stable isotope analysis of those individuals would reveal whether any were likely to have spent time within an AOC river or other comparably-polluted tributary.

Our initial results from the Sheboygan River represent one of the first tests of isotopic tracking of migratory fishes in the Great Lakes. We have proven the value of the stable isotope assay for testing residency, as well as the feasibility of collecting this data in parallel with direct assessment of tumor incidence, body condition, and other measurements. However, it would be unwise to assume that they are directly applicable to white suckers in other tributaries without further evidence.

To further the goal of Wisconsin DNR to understand whether white sucker tumor incidence in the Sheboygan and Milwaukee Estuary AOCs are aberrant relative to comparable river systems, we will expand the sampling of suckers for stable isotope analysis in 2013. The Kewaunee River will be used as a reference site to assess background incidence of tumors in non-AOC rivers. The Milwaukee River in the Milwaukee Estuary AOC will be sampled as a second AOC where white sucker tumor incidence may be enhanced by exposure to contaminants. In each case, we need to sample both invertebrate food resources and migrating white suckers to test whether these fishes reside primarily within the river or in Lake Michigan broadly.

Specific objectives

1) Comparability of Residency

Stable isotope data from the Sheboygan River collected in 2012 demonstrate that white suckers in that population reside primarily in Lake Michigan rather than the river or associated harbor where contaminant concentrations are expected to be highest. However, it is not yet clear whether this represents a general pattern that applies to all tributaries. Thus, we will collect tissue samples from invertebrate diet items (benthic insects, zebra mussels) in the river and estuary habitats of the Kewaunee and Milwaukee Rivers, as well as nearby sites to the north and south in Lake Michigan. In parallel, we will collect muscle samples from every white sucker sampled for tumors in both rivers (~200/river). All samples will be stored, processed, and analyzed for stable isotope ratios following standard protocols. Baysian mixing models will be used to assess the residency of each fish. These results will be compared to those from the Sheboygan River and other reference sites where suckers were sampled in 2012.

2) Relationship between Residency and Tumor Development

To date, the relationship between residency within an AOC and tumor development has either been assumed or assessed qualitatively. Stable isotope analysis offers the

possibility of inferring residency within an AOC area in a quantitative way on a continuous scale (i.e., % of diet derived from river vs. lake habitats). Our 2012 results from the Sheboygan River indicate comparable residency among all the fish sampled for both stable isotopes and tumors (~50 individuals). However, most of the fish sampled for isotopes that lacked tumors, and only 25% of fish analyzed for tumors were also analyzed for stable isotopes. Thus, additional data are needed to test for a gradient in residency that would allow stronger inferences about the association between AOC residency and tumor incidence rates. Analyzing all fish from both a second AOC (Milwaukee Estuary) and a reference site (Kewaunee River) will ensure that relative residency of those fish with liver tumors will be known. The relationship between residency and tumor incidence rate will be examined using logistic regression.

Specific tasks to be completed:

- Travel to Kewaunee and Milwaukee Rivers to assist WDNR with fish collection and USGS team with tumor analysis (Spring 2013)
- Sampling and analysis of muscle tissue from 200 white suckers from each river (Spring 2013)
- Sampling and analysis of invertebrates from river, estuary, and lake habitats of the Kewaunee and Milwaukee Rivers (Spring 2013)
- Statistical analysis of tumor incidences (comparing Sheboygan, Milwaukee, and Kewaunee Rivers) and stable isotope data (comparing fish to invertebrate results from each habitat) (Summer-Fall 2013)
- Compilation and distribution of all data on fish size/age, tumor categorization, and stable isotopes to WDNR research team (Fall 2013)
- Preparation of a report detailing all of the above tasks, and providing summary inferences and recommendations to WDNR based on the results of the 2013 work (Winter 2013-2014)
- Project Duration: January 2013-March 2014

Budget

Sheboygan

-Diet item collection and analysis in Kewaunee River, Estuary, and Lake: $1,000
-White sucker tissue collection: $1,000
-Isotopic analysis for tumor positive white suckers: $3,400
-Data management and analysis: $12,500

Milwaukee

-Diet item collection and analysis in Milwaukee River, Estuary, and Lake Michigan: $1,000
-White sucker tissue collection: $1,000
-Isotopic analysis for tumor positive white suckers: $3,400
-Data management and analysis: $12,500

Total: $35,800

Title:	Monitoring for white sucker tumors in the Milwaukee AOC: testing spatial history using stable isotopes
PI:	Pete McIntyre
Project Period:	1/1/1/13-12/31/13
Sponsor:	DNR (EPA pass-through)

Salary	
Post Doc (2.5 mo @ $44K/yr)	9,167
Grad ($40,800 full-time rate; 50% appt for 5 mo)	8,500
Subtotal	*17,667*
Fringe	
Post Doc (26.7%)	2,448
Grad (28%)	2,380
Subtotal	*4,828*
Travel	1,417
Stable isotope fees	2,323
Supplies	1,418
Tuition (set at $4K/semester)	4,000
Total Direct	31,652
Indirects*	4,148
Total	**35,800**

*If funded with State GPR funds or other nonfederal funds which by program or policy do not allow, F&A costs should be excluded from the award.