White Sucker Tumor BUI Assessment – Final Report
Sheboygan River Area of Concern
University of Wisconsin Center for Limnology
8 February 2013

The 2012 assessment of white sucker tumor incidence in the Sheboygan River AoC involved three partner organizations: Wisconsin Department of Natural Resources (WDNR), United States Geological Survey (USGS), and the University of Wisconsin-Madison (UW). The three groups led a joint field sampling effort on 31 March – 1 April 2012 with a goal of sampling 200 migrating adult white suckers from the Sheboygan River AoC. The UW-Madison team was responsible for coordinating field work, assisting with collecting and dissecting fishes, collecting and analyzing tissue samples for stable isotopes, collating all data from all partners, and reporting all project results and inferences.

Heavy rains just before the field sampling dates resulted in high, turbid water. This made net-based fish sampling techniques difficult and ultimately ineffective. Fortunately, WDNR staff were available to assist with an electrofishing barge and boats, and the team was able to collect roughly the number of fishes desired. The USGS team led the dissection and tumor assays while UW assisted as needed in field collections, tumor assays, data collection, and led stable isotope tissue preparations. Tissue samples were analyzed by USGS for tumor characterization and by UW for carbon (C) and nitrogen (N) isotope ratios over the six months following field sampling. Fish age was also calculated from otoliths by USGS.

A total of 193 white suckers were analyzed for tumors, of which 50 were also analyzed for stable isotope ratios. These fish spanned a wide range of size (**Figure 1**), age (**Figure 2**), and body mass (**Figure 3**). All results for each individual fish are compiled in the file "UW summary of Sheboygan sucker results 2012.xlsx".

Tumor rates

A Chi-Square test is a commonly used test statistic to compare an observed frequency of tumors to a benchmark. Our team observed a liver tumor incidence rate of 8.3% among white suckers sampled. The benchmark for comparison is a 5 % tumor rate, which is considered to be the upper end of the normal range in uncontaminated sites, and thus the threshold for identifying a Beneficial Use Impairment (BUI) in the sampled population of white suckers.

The Chi-square test indicates that the observed tumor rate was statistically higher than the benchmark ($p=0.0169$, two tailed test). The contingency table and p-value associated with the Chi-Square test are shown in **Table 1**. This was a two-tailed test, meaning that we were not explicitly testing for a directional difference from the benchmark tumor rate. Thus, the test statistic is conservative, and this result is considered robust. ***Therefore, we conclude that white suckers in the Sheboygan River do not meet the BUI threshold of <.05% tumor incidence***. Logistic regression of tumor presence against age, length, and weight are all statistically significant ($p<0.01$, $p<0.01$, and $p<0.05$ respectively). This indicates that older, larger fish are more likely to have tumors than younger, smaller counterparts.

Residency of white suckers based on stable isotopes

Surveying the C and N stable isotope ratios among a wide range of Wisconsin tributaries of Lake Michigan, we observed broad, consistent patterns among sucker populations (**Figure 4**). Sucker migrants in tributaries to Green Bay are significantly enriched in $\delta^{15}N$ and depleted in $\delta^{13}C$ relative to those migrating into tributaries on the main Lake Michigan shoreline. Along the Lake Michigan shore, suckers from further North/East tend to be more depleted in $\delta^{15}N$ and $\delta^{13}C$. This regional variation in isotope ratios is intriguing and has not been reported previously at such large spatial scales. If these fish were mixing randomly within Lake Michigan, no such differences would be possible. Muscle isotope ratios reflect months of incorporation of dietary isotope ratios, therefore the observed differences must reflect long-term differences in the location of residence. ***The consistent differences among sucker populations strongly suggest that these fish do not migrate long distances within the lake, but rather remain relatively close to the mouth of the tributary where they spawn.***

Stable isotope analysis also offered insights at a finer spatial scale in the Sheboygan River population. Isotope ratios of an animal are related in predictable ways to those of its dietary resources, which are benthic invertebrates in the case of white suckers. Specifically, $\delta^{15}N$ is expected to be roughly 3.0-3.5 units higher in a consumer than its diet, while $\delta^{13}C$ is expected to be comparable between a consumers and its diet. Thus, when food sources vary in characteristic stable isotope ratios among sites, we can draw strong inferences about where consumers have been feeding. In the study region, we characterized stable isotope signatures for invertebrates collected from the Sheboygan River and Lake Michigan for comparison to white suckers captured during spawning migrations (**Figure 5)**. Samples collected 1 km north and 1 km south of the mouth of the Sheboygan River have very distinct isotopic signatures from Sheboygan River samples. Invertebrates collected in the Sheboygan River harbor show intermediate isotope ratios, but were more like counterparts from the Sheboygan River than those from Lake Michigan. The strong agricultural influence in the Sheboygan River watershed likely drives the enriched $\delta^{15}N$ levels in the river and harbor. There is substantial variability in the carbon isotopes within the lake samples (**Figure 5**), suggesting that carbon sources differ among invertebrate taxa. However, almost all of the lake samples are clearly enriched in $\delta^{13}C$ relative to the river and harbor samples.

The close concordance between the isotope signature of suckers and that expected for a consumer of lake invertebrates indicates that suckers collected in the Sheboygan River derived their diet and growth from lake-based food resources (**Figures 6-7**). The small amount of variation among suckers suggests little variation in residency, with the exception of three small suckers (**Figure 6**). These small fish appear to spend considerably more time in the Sheboygan River or harbor than any of the full-size adults. These small fishes were below the size threshold for tumor analysis, so no tumor characterization is available for them. ***Despite collection of adult suckers in the Sheboygan River during the spawning migration, these fish reside primarily in the lake itself, not in the river or the river mouth.***

Tumor rates and isotopic signatures

There is not enough variation in Sheboygan River residency among samples suckers to draw any link to tumors, but there were still some interesting but subtle patterns (**Figures 7-8).** Tumor-bearing fish were slightly different in stable isotope ratios than their non-tumor counterparts. Fish with "Hep. Cell. Neo" tumors are enriched in $\delta^{15}N$ compared to non-tumor fish and those with "BD neoplasia." tumors. Welch's two-sample t-test confirm these differences are statistically significant ($p<0.05$). Fish with "BD Neoplasia" tumors are significantly enriched in $\delta^{13}C$ compared to non-tumor fish and those with "Hep. Cell. Neoplasia" tumors ($p<0.05$). ***The isotopic differences are very small, and most likely represent the physiological cost of the infection rather than differences in habitat use or diet***.

Furthermore, we assessed the C and N elemental content of the muscle tissue in relation to the body condition of each fish (**Figure 9)**. High elemental C:N ratio indicates high fat content in the muscle, while high $\delta^{15}N$ is typical of animals that are starving. There was no correlation between body condition and $\delta^{13}C$, $\delta^{15}N$, or elemental C:N ratio, or any relationship with tumor incidence (all $p>0.10$). Only one major outlier was observed; its low body condition, low C:N elemental ratio, and high $\delta^{15}N$ suggest that it was starving, perhaps due to an ailment.

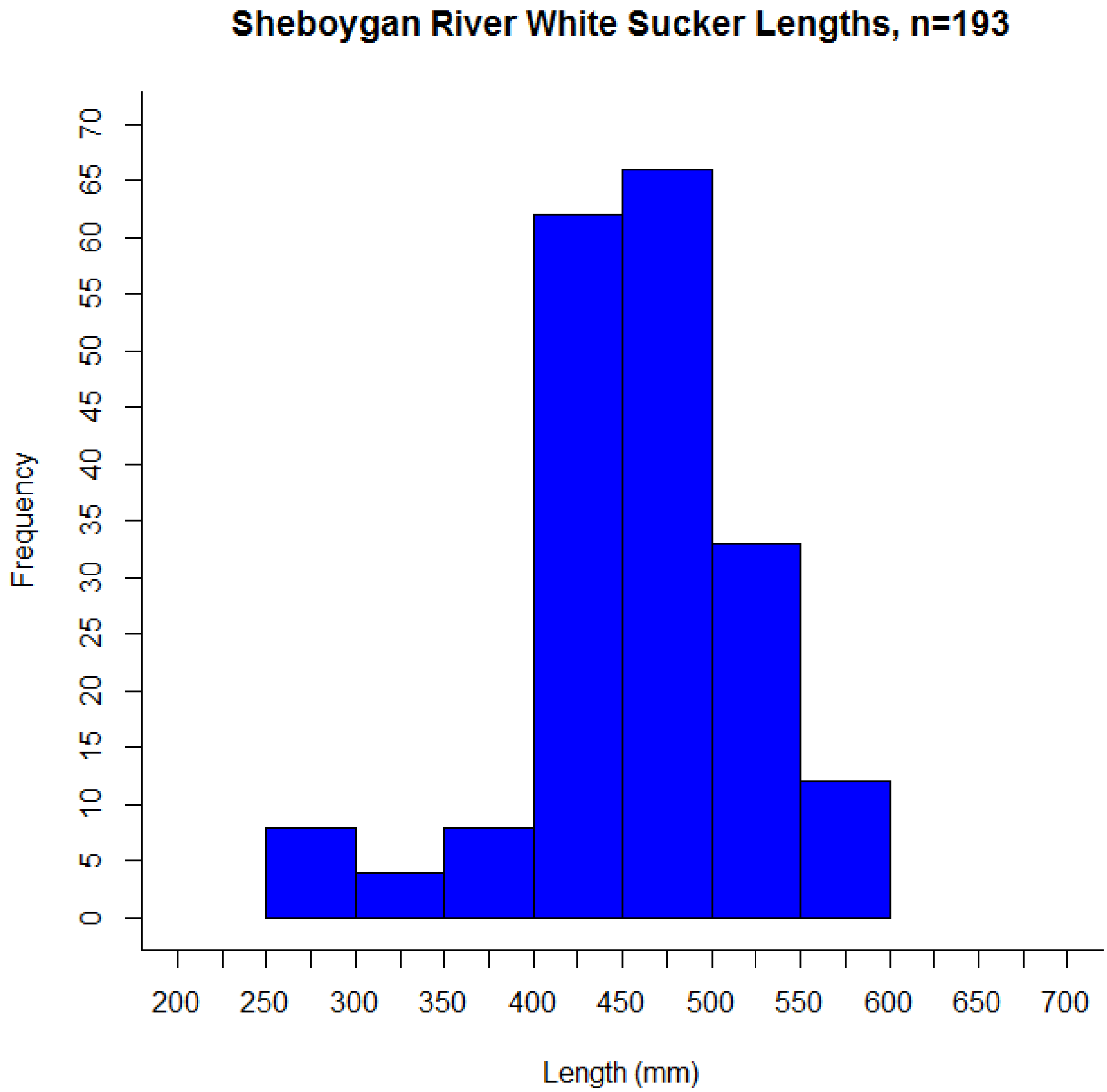

Figure 1. Frequency distribution of body length (as total length) of white suckers collected in the Sheboygan River.

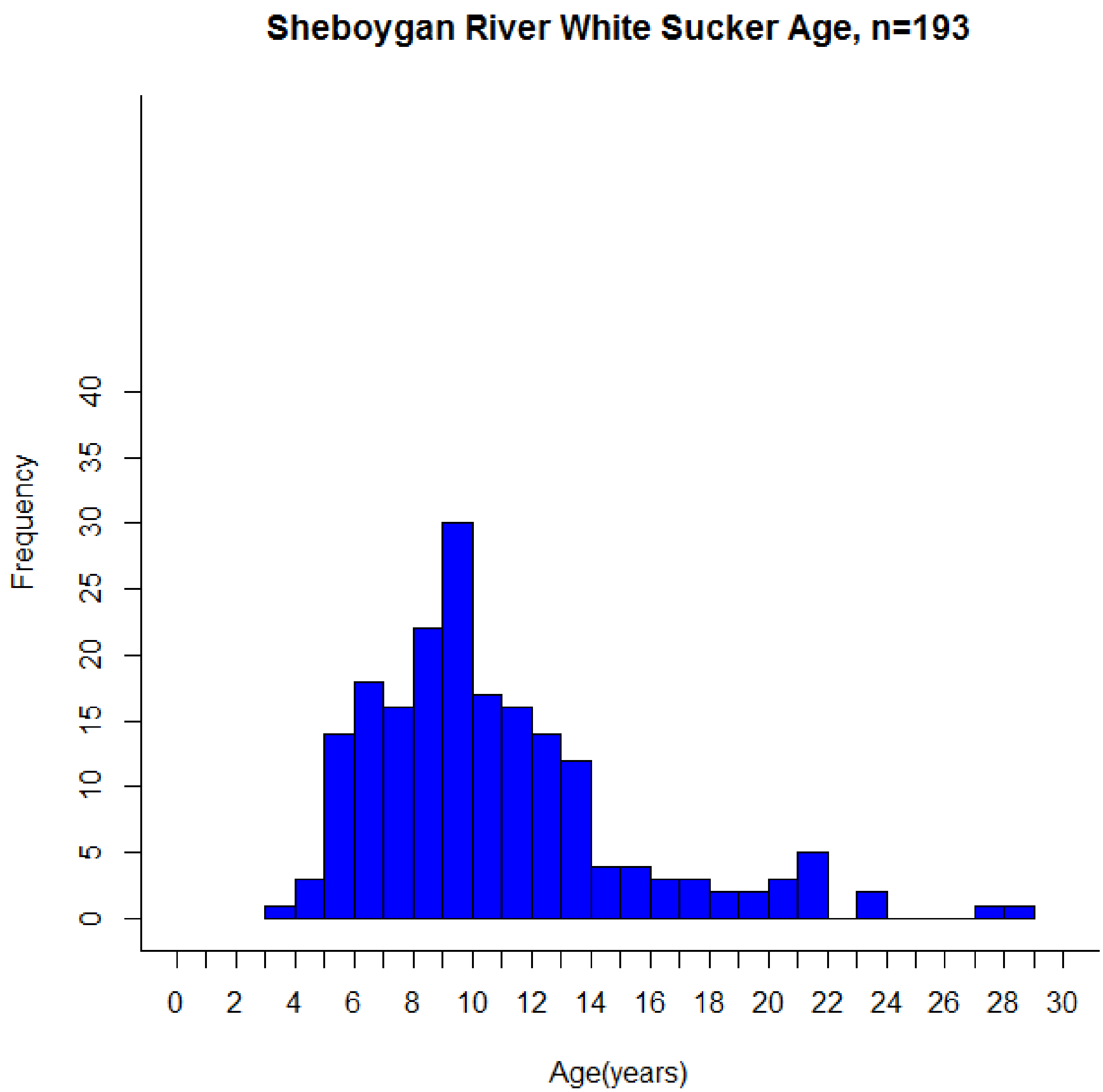

Figure 2. Frequency distribution of age of white suckers collected in the Sheboygan River.

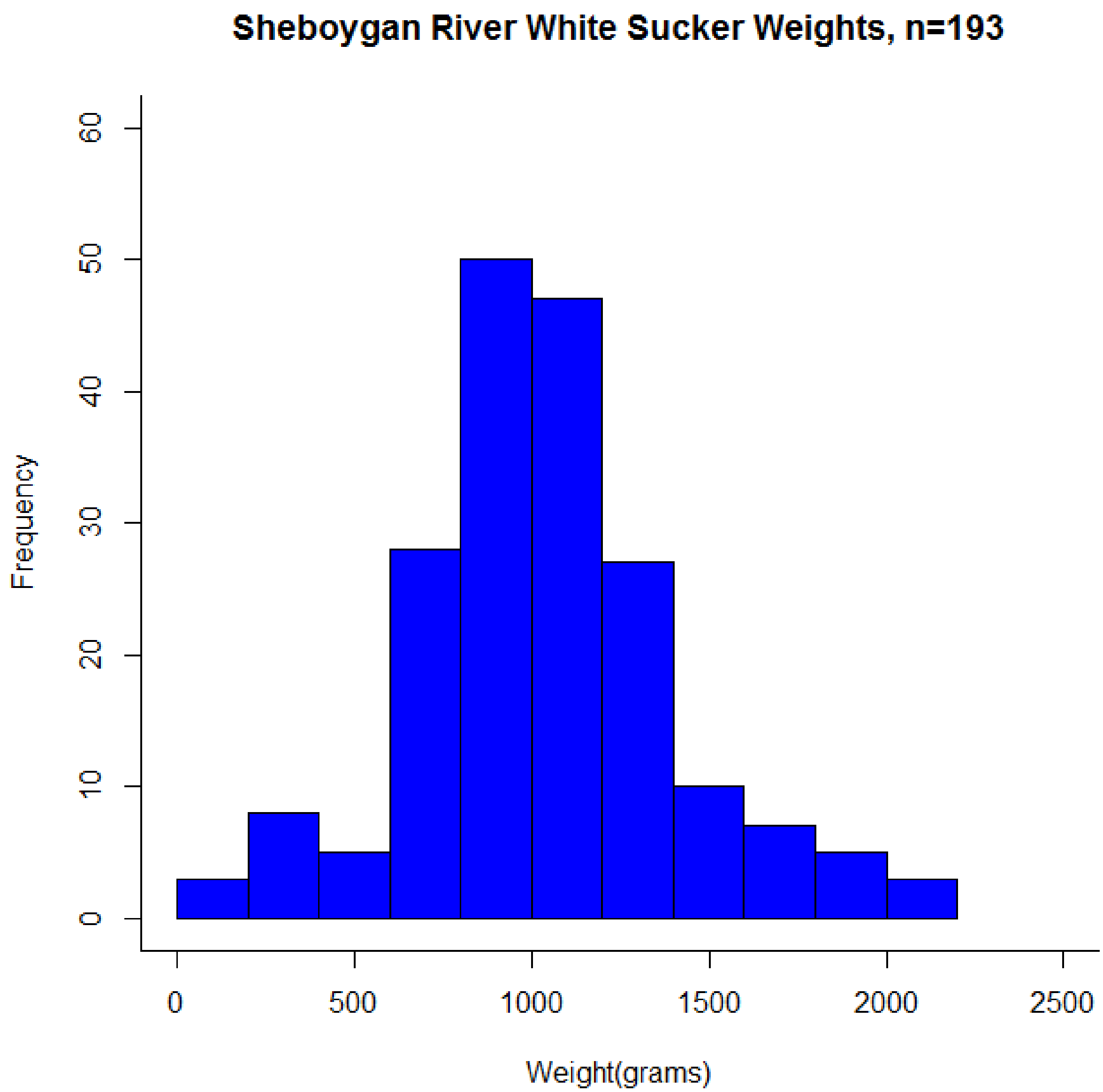

Figure 3. Frequency distribution of body mass (as wet mass) of white suckers collected in the Sheboygan River.

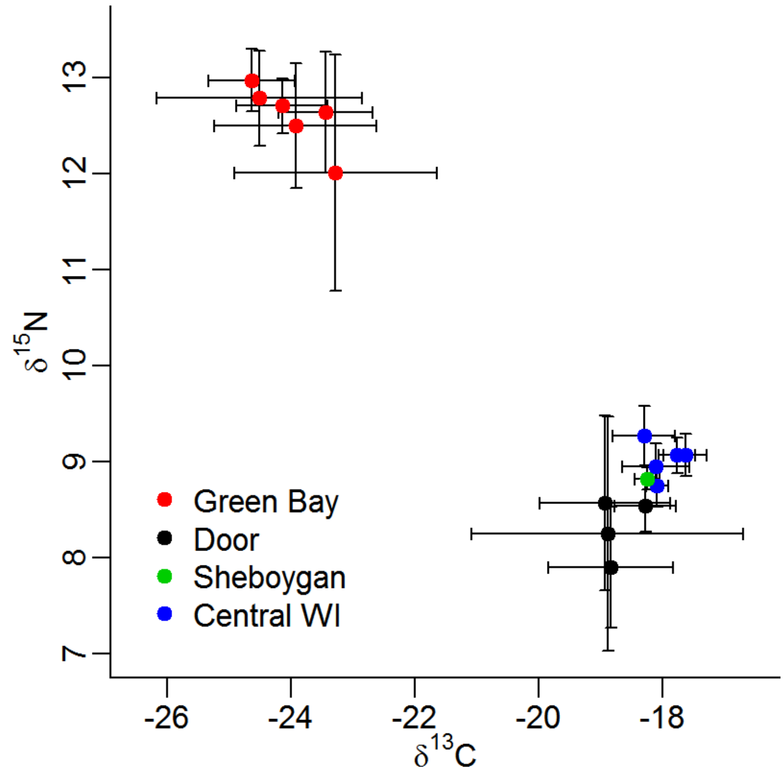

Figure 4: $\delta^{15}N$ and $\delta^{13}C$ isotopic signatures for different populations of white suckers collected in Lake Michigan and Green Bay tributaries along the Wisconsin shoreline.

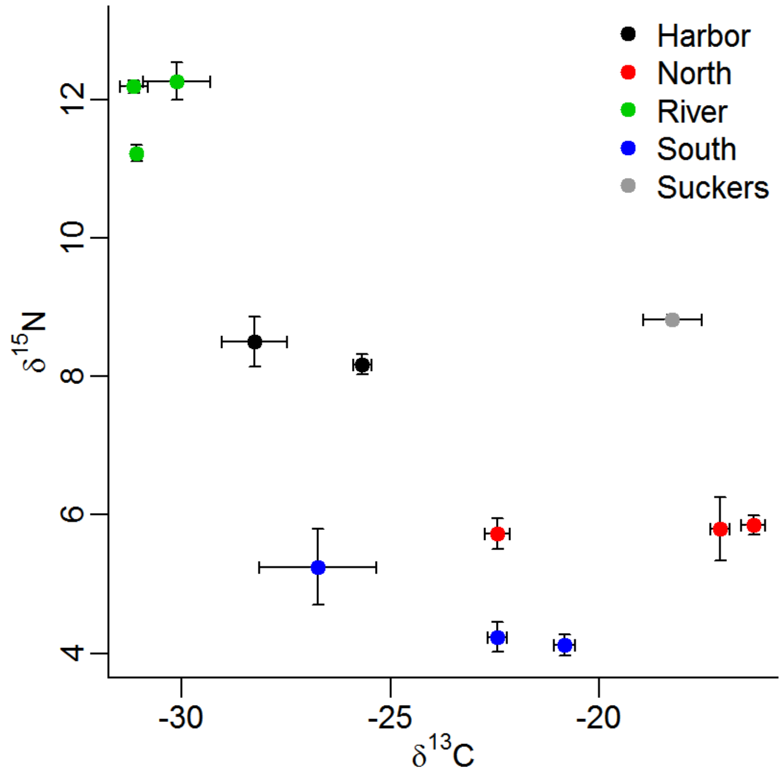

Figure 5: $\delta^{15}N$ and $\delta^{13}C$ isotopic signatures from invertebrates captured in and around Sheboygan River compared to white suckers.

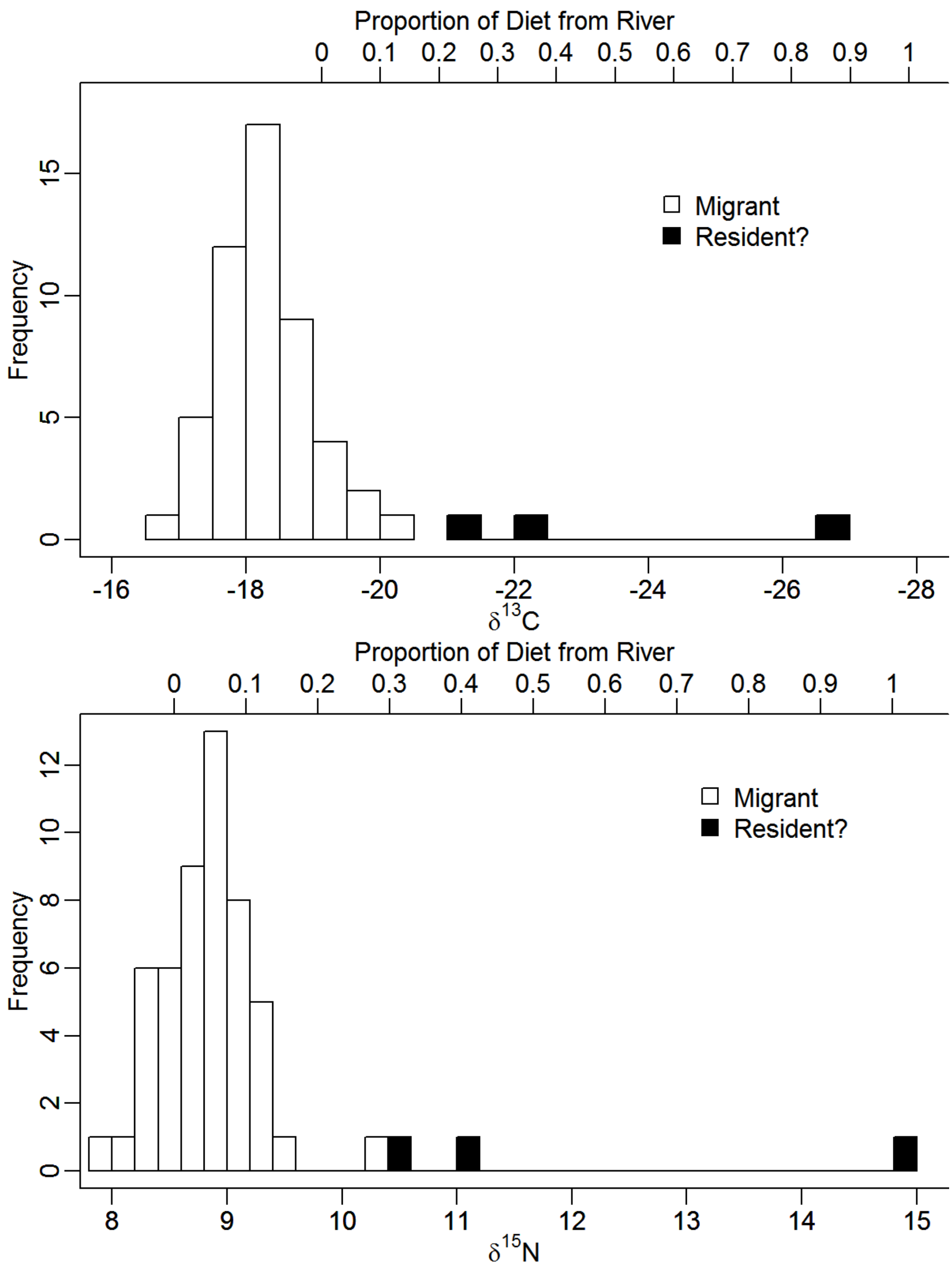

Figure 6. Frequency distributions of $\delta^{15}N$ and $\delta^{13}C$ among suckers collected in the Sheboygan River, and interpretation of river residency based on $\delta^{15}N$ and $\delta^{13}C$ of invertebrates collected in the river, harvor, and lake. Three fish indicated by black markers were small (~20cm) and appear to be juvenile residents.

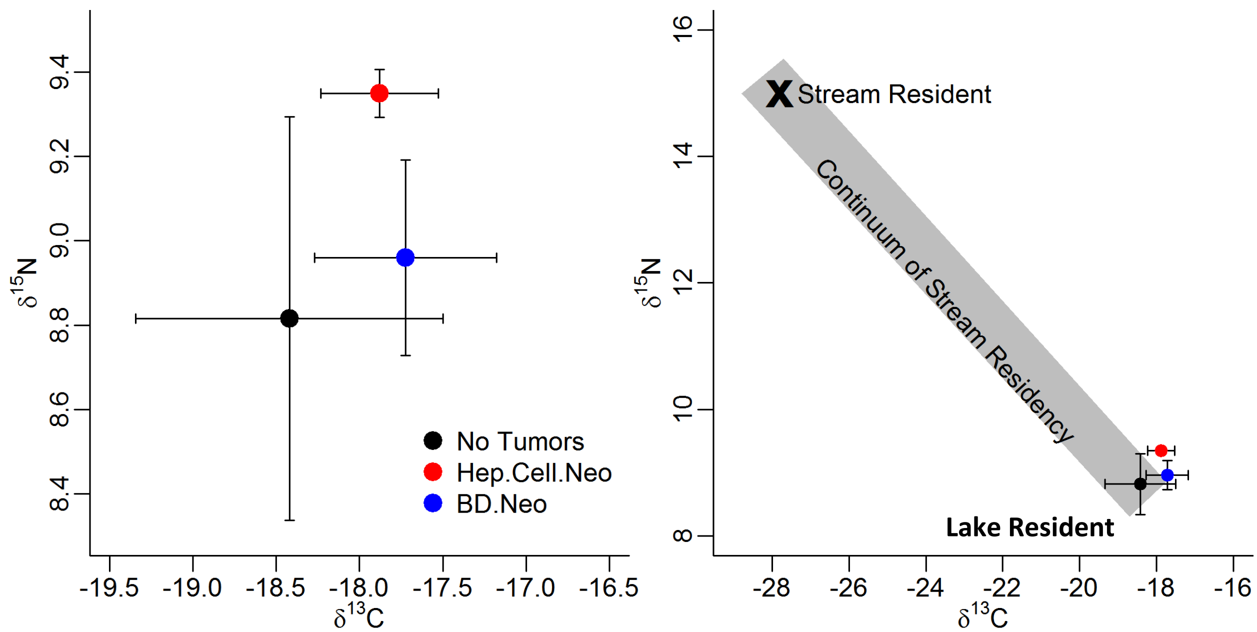

Figure 7: $\delta^{15}N$ and $\delta^{13}C$ isotopic signatures of Sheboygan River suckers relative to their tumor classification (left panel), and compared to the range of variation in $\delta^{15}N$ and $\delta^{13}C$ observed among invertebrates sampled in the Sheboygan River ("stream resident") versus Lake Michigan (right panel). The isotopic variation among suckers based on tumor status is trivial compared to spatial differences in food resources.

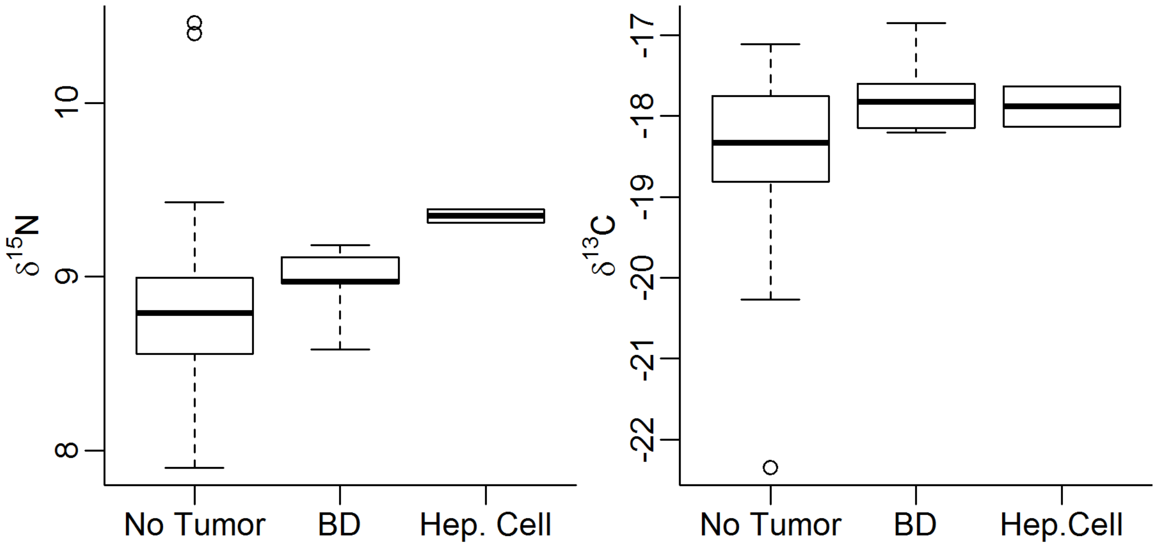

Figure 8: Differences in $\delta^{15}N$ and $\delta^{13}C$ isotope ratios among Sheboygan River suckers without no tumors, BD tumors, and "Hep. Cell" tumors. Note that most tumor-bearing fish are within the range of $\delta^{15}N$ and $\delta^{13}C$ observed in suckers without tumors.

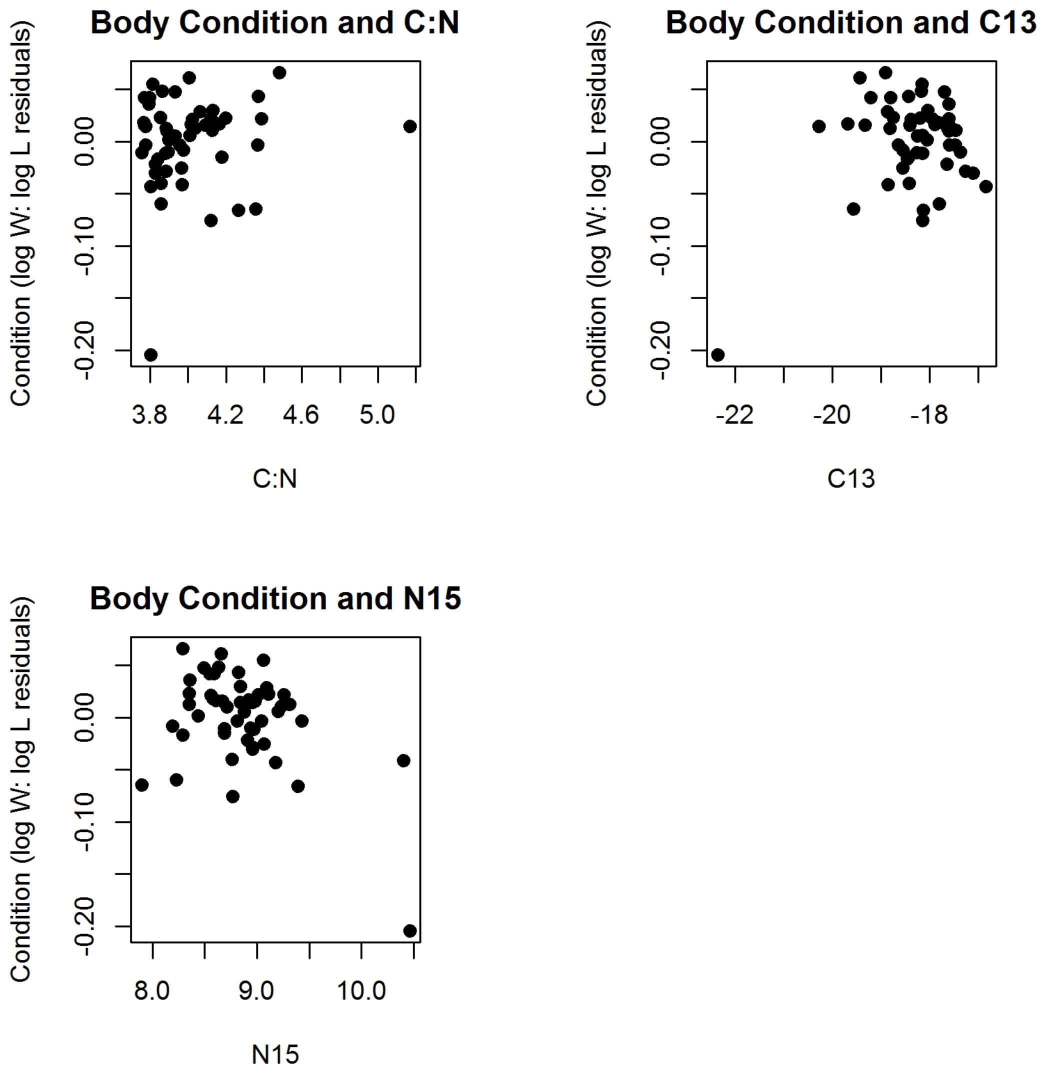

Figure 9: Body condition of white suckers collected in the Sheboygan River relative to their C:N elemental ratio, δ^{15}N (15N) and δ^{13}C (13C).

	tumors	no tumors	total	proportion w/ tumors	proportion w/o tumors
expected (5%)	9	184	193	0.046632124	0.953367876
observed(8.3%)	16	177	193	0.082901554	0.917098446
Total	25	361	386		
		Chi Squared Test p-value =		**0.016861346**	(two-tailed test)

Table 1: Chi-Square test contingency table and p-value.