

FISH TUMOR BUI CRITERIA: DETERMINING NUMBERS FOR THE DELISTING PROCESS

Introduction

Appendix One of the 1987 Protocol amending the Great Lakes Water Quality Agreement of 1978 included elevated prevalence of tumors in native fish populations as one of the Beneficial Use Impairments considered in defining an Area of Concern (AOC). Such high occurrences of both external and internal tumors in fish (neoplasm epizootics) have been associated with carcinogens in sediment and water at a variety of AOCs on the Great Lakes and at a variety of other locations in North America (Baumann 1998). Over the years numerous field and laboratory investigations have demonstrated a cause and effect relationship between carcinogens (particularly polynuclear aromatic hydrocarbons or PAHs) and liver cancer in fish. One of these studies in Michigan linked high PAH metabolite levels in bile with elevated levels of DNA adducts in the livers of brown bullhead and walleye from the Detroit River (Maccubbin and Black 1990).

In the mid-1990s, US and Canadian researchers reviewed earlier Great Lakes studies that had published information on tumor prevalence in fish (Baumann *et al.* 1996). At the time the authors concluded that a liver tumor prevalence above 5 percent or a cutaneous papilloma prevalence above 25 percent should be “interpreted as indicators of environmental degradation”. However a 10 tributary survey conducted on Lake Erie from 1999 through 2001 combined with other recent investigations has indicated a need to reevaluate the data once more. Also the dependency of tumor incidence with age and questions concerning uniform diagnosis and statistical evaluation need to be addressed. In this short report I will use the Detroit River as an example in order to provide some perspective on the history of investigations of tumor incidence in fish, to examine some problems in data interpretation, and to suggest some site-specific criteria for the stages of delisting.

Technical Summary

Most of the problems associated with developing criteria for tumor prevalence are evident in a comparison of three sets of Detroit River data on brown bullhead tumors, both external and internal. The earliest set was collected from a variety of locations, including some in the Trenton Channel, between 1985 and 1987 (Maccubbin and Ersing 1991). Another set was collected in 1996 from three different locations in the Detroit River: in order of decreasing contamination, the Trenton Channel, the Amherstburg Channel and Peche Island (Leadley *et al.* 1998). The last set was collected in 2000, partly in the Trenton Channel and partly at the mouth of the Huron River below the mouth of the Detroit River as it enters Lake Erie (Baumann *et al.* unpublished data).

Table 1: Tumor prevalence in brown bullhead from the Detroit River. When external lip and skin tumors were reported as one percentage they are listed under “com” (combined). N represents sample size.

Date	External N	Lip %	Skin %	Com. %	Liver N	Liver %
85-87	River Wide 449			10	306	8.8
96	Trenton Channel 25	20	24		25	24
96	Amherstburg 23	17	4.3		23	14
96	Peche Island 27	7.4	7.4		27	4
00	River Wide 34			23	34	6

An examination of the data in the table, without reference to other information, indicates that fish in the Detroit River had a higher prevalence of tumors in 1996 than in 1985-87, and that skin tumors were more common in 2000 than in 1985-86. Since this is almost certainly not the case, these figures point out the need to compare “apples to apples” when discussing fish tumors. First, during the 1985-87 studies fish were collected from a variety of locations within the Detroit River, some more heavily contaminated than others. As we can see from the 1996 studies, tumor prevalence varies greatly depending on where fish are sampled. A tagging study in Presque Isle Bay found that bullheads did not move between the bay and Lake Erie, although they did travel within the bay. Thus while bullheads might move short distances in the Detroit River, it is expected that populations have a reasonably small home range. The bullhead therefore would be differentially exposed to contaminants depending on the area of river where they live. This expectation is consistent with the higher tumor prevalence found in Trenton Channel fish in the 1996 study. It is also confirmed by a comparison of PAH bile metabolites taken from the fish caught in 2000. Bile from fish caught in the Trenton Channel demonstrated a much higher PAH exposure than did the bile from the Huron River fish. Thus fish from the 2000 sample have a lower prevalence of liver tumors than the 1996 fish primarily because over half of the 2000 fish (18) were caught at the mouth of the less contaminated Huron River.

A second major influence on tumor prevalence in a population is the age distribution of the fish sampled. Tumor frequency increases with age. This happens both because of increasing exposure, and because initiated cells go through a latent period before promotion and development into neoplasms. Maccubin and Ersing (1991) present a table of tumor prevalence by age for fish captured in 1986. In that year, 36 percent of the fish examined for external tumors and 23 percent of those examined for liver tumors were age two or younger. Fish two years or younger in that study had an external tumor prevalence of 2 percent or less and a liver tumor prevalence of 5 percent or less, while by age four they had a prevalence of about 12 percent for liver tumors and 22 percent for external tumors. By age five both external and liver tumor prevalence exceeded 40 percent. Thus including these young fish in calculating an “average” tumor prevalence

creates a bias. The fish collected by Leadley *et al.* (1998) in 1996 were all either three or four years old, which helps explain the differences among the three data sets. Similarly, the fish from the 2002 study were all age three or older.

Two other variables that should be considered in developing criteria for tumor prevalence are species and season of collection. The 1985-87 study found liver tumor frequencies of 10 percent to 18 percent in redhorse, white sucker, walleye and bowfin. Comparison data sets for these species, however, are limited, with the exception of the white sucker. Over time the brown bullhead and white sucker have emerged as the most common sentinel species for tumors, and one or both of them should probably remain the species of choice.

Season can also influence apparent tumor prevalence. During the summer when fish are more metabolically active, tumors develop more quickly and become noticeable through histopathology. During the winter many older fish (particularly those with tumors) die. Thus tumor prevalence is usually lower in spring and higher in fall. Bullhead, however, are more active in spring and more vulnerable to gear when spawning. Thus collection during spring is still normally preferable, since greater numbers of bullhead (needed to statistically differentiate among tumor rates) can be taken then.

Recommendations Concerning Delisting Criteria

Preliminary data from around the Great Lakes (Baumann *et al.* unpublished) would support a liver tumor prevalence of about 5 percent in brown bullhead aged three and older as good criterion for an “Area of Recovery” as opposed to an “Area of Concern”. In my opinion, the external tumor criterion of 25 percent, as listed by Baumann *et al.* (1996), is no longer acceptable. Overall external tumor frequencies are much lower than this at a number of reference and remediated Great Lakes locations (Baumann, unpublished data). After an initial review of these data I recommended an external tumor criterion of about 12 percent (for age three and older brown bullhead) to the Remedial Action Committee of Presque Isle Bay and to the Detroit River Delisting Criteria Workshop (4/26/2002). Certainly the current database does not support a delisting criterion of one in every four fish with an external tumor. Twelve percent is still relatively high, and again should be used as a criterion for an Area of Recovery. These prevalence percentages (internal and external) would need to apply to fish from the more contaminated portions of the Detroit River, such as the Trenton Channel. The percentages are qualified by the word “about”, since samples of 100 fish at each site (or time) would only provide enough statistical power to differentiate a 7% difference in neoplasm frequency at $p < 0.05$. To reduce the criterion to even 5% at $p < 0.05$ would require that a total of 280 fish be examined between the two locations or at different times. Thus consideration should be given to using $p < 0.1$ as a sufficient level of certainty for real world regulatory purposes.

To delist an area completely should require a documented low tumor prevalence in the population over a series of years. At least three sampling periods spaced two to three years apart should be necessary. Final acceptable delisting criteria will require that researchers from North America pool their information and collaborate on joint analysis.

A start toward this end will be a fish tumor conference to be held at Erie, PA on January 21 and 22 of 2003. Liver tumors will probably be expressed in terms of the prevalence of hepatic and biliary cancers, neoplasms and altered foci, rather than in terms of the prevalence of "tumors" or even "neoplasms". Similarly, external tumor delisting criteria should reference papillomas and carcinomas associated with different body areas (particularly oral). Thus studies need to include the histopathology of all external lesions, as has been the case in recent years for liver lesions. In addition, further research should be undertaken on the development and adoption of criteria using external barbel (sensory receptors) lesions and blood parameters, both of which can be obtained without killing the fish. Leadley *et al.* (1998) found shortened barbels on bullhead, ranging from 4% at Peche Island and Amherstburg to 20% in the Trenton Channel. The 2000 study found shortened and missing barbels in 53% of all fish captured.

References

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