How Do You Spell Success? The Rare Fish Variety, That Is

Part I: Grading Success in Rearing Threatened and Endangerd Species

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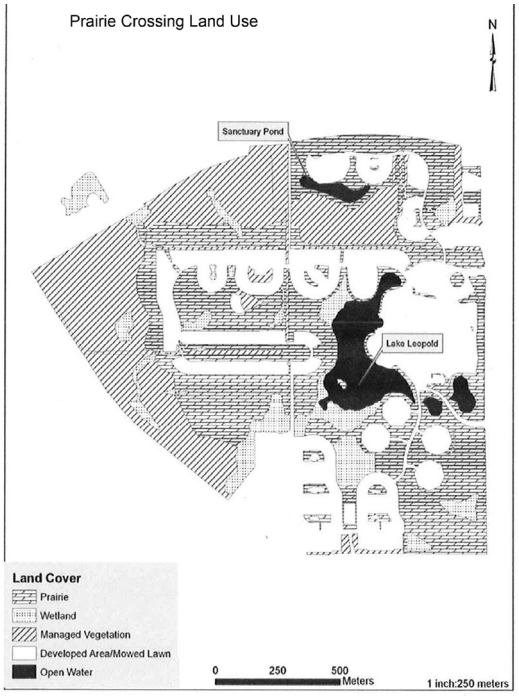
Introduction

I find it surprising that we are still talking about this project despite the fact that it was initiated 15 years ago in 1998. It's appropriate, however, if you feel that the success of environmental projects need to be interpreted over the long run and not with short-duration results. Grading our results, I would call the project largely successful with some qualifications. Our mistakes are probably just as instructive as some of the things that went well. In 1998 we (Jim Bland and Integrated Lakes Management [ILM] staff) were tasked with doing some design work, water quality monitoring, and fish stocking for a residential complex called Prairie Crossing located in Grayslake, Illinois. Prairie Crossing is unique in the country in being one of the first residential developments focused on environmental design and sustainability. The 630-acre development has been configured to retain prairie, wetland, and farmland on site. One lake and three ponds are also part of the complex. Lake Leopold is a 28-acre lake with a maximum depth of 15 feet and Sanctuary Pond is 2.8 acres with a maximum depth of six feet. Applied Ecological Services (AES) was principally responsible for the landscape design elements of Prairie Crossing. Critical to insuring good water quality and high clarity was the incorporation of a concept which AES refers to as a "treatment train." Stormwater is handled by minimizing impervious surfaces, and routing runoff sequentially through grassed swales, prairies, wetlands, and ultimately stormwater detention ponds (Afelbaum et al. 1995). Impervious surfaces include roofs, walkways, roads, and those surfaces that don't let water infiltrate into the soil. Additionally, the homeowners association was "tolerant" of the presence of rooted aquatic plant populations in densities that other associations would find weedy and objectionable. Typical stocking mixes for our region included bass, Bluegill, and Fathead Minnows. However, we did not want to do what was typical. Given its environmental mandate and homeowners' covenant, ILM proposed that Prairie Crossing embark on a project to establish a sanctuary for endangered and threatened (E/T) Illinois fishes.



Toward that end we consulted with Dr. Larry Page, formerly of the Illinois Natural History Survey, and Dr. John Janssen of the University of Wisconsin. Dr. Page's assessment of the Illinois fish fauna and his discussion of rearing ponds is what suggested the project to begin with (Page 1991). Dr. Page also had poor experience with trying to stock E/T species into a situation where predators still existed; for our project he insisted that all potential predators be removed. The Prairie Crossing lake and ponds which were the focus of our project were essentially brand new and thus, hypothetically, there shouldn't have been any fish in them. Much to our surprise, however, Green Sunfish (*Lepomis cyanellus*) had undergone "spontaneous generation" and were present in both Lake Leopold and Sanctuary Pond. It was cost-prohibitive to use fish toxicants in Lake Leopold and thus we decided to stock the upper 2.8-acre pond (later to be called Sanctuary Pond) after we had rotenoned it to remove the Green Sunfish. Fathead Minnows in a minnow cage were used as a bioassay to determine when it was safe to reintroduce fish.

Regional data suggested that Blacknose Shiners (*Notropis heterolepis*), Blackchin Shiners (*Notropis heterodon*), Banded Killifish (*Fundulus diaphanus*), and Iowa Darters (*Etheostoma exile*) had disappeared or were disappearing from the Des Plaines drainage in northeastern Illinois. They also typically occur together and thus problems of competing populations would seem less likely. The Blacknose Shiner is endangered in Illinois while the other three species have a threatened status. All of these species were present in two lakes, Deep Lake and Cedar Lake, in the Fox River drainage. One of the first problems which we encountered was determining how many fish to transfer; we consulted literature to try to determine the minimum viable population (MVP is the minimum number of fish that could be transferred to preserve genetic variability and prevent genetic bottlenecks). Numbers from the literature were not very helpful as they varied from 50 to 1,500. Dr. Janssen, with the cooperation of the Illinois Department of Natural Resources (IDNR), negotiated a figure of 200. While we had a target figure of 200 fish, our collections fell short of that number. Original collection numbers were:

Blackchin Shiner: 200 Iowa Darter: 150
Blacknose Shiner: 116 Banded Killifish: 80

With a small crew of ILM staffers, graduate students, and Dr. Janssen we collected the four species from Deep Lake and Cedar Lake. A 30-foot x 6-foot x 1/8-inch mesh bag seine was used to collect the fish for transfer. Notably both of the shiner species experience net shock very easily; the killifish and the Iowa Darter don't seem to experience the same type of mortality with handling. There are apocryphal stories intimating that killifish can be sent through the mail in wet newsprint! Certainly they are hardier to handle in the net. Fish were transferred to an aerated cooler lined with a black plastic garbage bag. At Prairie Crossing the bag was removed from the cooler and allowed to sit in the water to acclimate temperatures; fish were then removed with an aquarium net, and the black garbage bag flushed to the ground. Minimal amounts of water were thus transferred from one water body to another.

Results

From the outset it was our intention to do three things:

1. Complete a transfer of the E/T species to build their populations within Sanctuary Pond and to subsequently effect transfers to other parts of the Des Plaines drainage; additionally to monitor water quality and pond biology across an extended period of time. All of this to be done in acknowledgement of protocols from the American Fisheries Society.
2. To get detailed life history studies prepared for the two shiners and the Banded Killifish.
3. To get genetic work done to substantiate that variability had been sustained in the receiving ponds.

None of these goals would have been possible were it not for exceptional support from the Prairie Crossing Homeowners Association (PCHA) and their environmental staff. The fish were transferred in the fall of the year and in the subsequent summer it was clear that we had hundreds of thousands of shiners and Banded Killifish in Sanctuary Pond. The Iowa Darters were present in far lower numbers but we had young of the year for all four species. Monitoring of lakes typically involves a standard set of parameters which include ortho- and total phosphorus, ammonia, nitrates, chlorides, chlorophyll a, alkalinity, pH, turbidity, conductivity, and temperature and dissolved oxygen (DO) measured as depth profiles. Both Lake Leopold and Sanctuary Pond were monitored in this way five to

six times across the monitoring season. This type of monitoring was continued for both water bodies for over 10 years. Additionally we were able to deploy a multiparameter Sonde that took hourly readings for several sampling seasons for Sanctuary Pond and later for Lake Leopold. After the fish translocation we did seining of Sanctuary Pond approximately four times per year but the seining was not time controlled and no efforts were directed at estimating population size for any of the species. Our collection records do give a description of the relative numbers of each of these species across time and they document breeding success. Rooted aquatic plant populations were identified, densities estimated, and on several occasions were mapped. We were able to do more limited collection and characterization of zooplankton and phytoplankton for Sanctuary Pond. Two years after our first translocation we actively moved a collection of all four species from Sanctuary Pond into Lake Leopold. Lake Leopold was actively managed as a recreational fishery and had a variety of sunfish and bass. The E/T species continue to survive in Lake Leopold and additional records exist for the stream drainage directly downstream from Prairie Crossing.

Our Sonde results were particularly notable. One of the legitimate concerns of the IDNR was the possibility of winter kill or summer kill for the E/T species. The Sonde did document some dramatic loss of DO in the water column and underneath the ice for some winter and summer weather episodes. The low dissolved oxygen in the winter continued for over a month's period of time, however no mortality was evident for any of the four species. Summer reductions in DO were largely diurnal and did not last for extended periods. It appears that these particular species are far more tolerant of low winter DO values than larger fish fauna. Shuter et al. (2012) describes various types of winter survival strategies: "*Lakes that are subject to frequent winter kill events typically support a unique community of fish species that possess a range of specialized behaviors and physiological strategies for tolerating winter oxygen deficit.*" Another significant observation with the Sonde data was the pond and lake response to rain events. Urban detention ponds typically get pretty murky in response to stormwater runoff. By contrast both the Lake and Sanctuary Pond had minimal spikes in turbidity, even when subjected to very large rain events. As follow-up translocations to other lakes and ponds in the Des Plaines drainage were undertaken, even the hint of turbidity seemed enough to kill off both the shiners and the killifish. Less critical but still worrisome was a spike in chloride levels due to winter salting of roadways. Chloride values got as high as 300 parts per million. Prairie Crossing, in response to the elevated chloride levels, incorporated a salt control program.

Shoreline seining was initiated in 1999 but only presence/absence data were recorded in 1999. In 2000 total catches and estimates of effort were recorded but shiners were lumped into a single category (in part because we were concerned about shiner sensitivity to handling). Seining was undertaken four times per season and sufficient numbers collected to develop size/frequency data. Detailed information concerning the results can be found in *Fisheries* (Schaeffer et al. 2012). Field methods have changed across time and more fish were collected for purposes of profiling the population. All four fish species continue to be present in Sanctuary Pond although Iowa Darters have from time to time disappeared from seasonal collections. This is more likely an artifact of collection techniques and the difficulty of collecting in heavy plant growth. Percentage of total catch from 2003 to 2010 is given below.

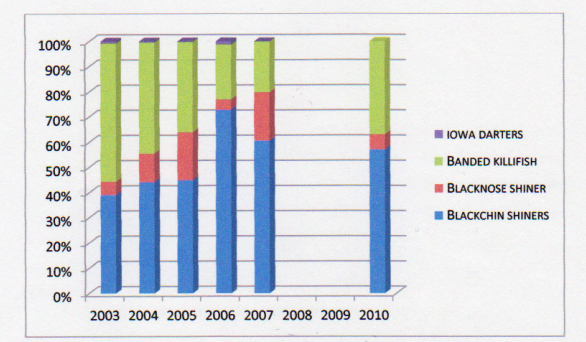


Figure 2 Percentage of Total Catch from 2003 to 2010

General population trends mirror relative population numbers found in other regional lakes. So, Blackchin Shiners are found in larger numbers, Blacknose Shiners in far fewer numbers, Banded Killifish as a substantial presence, and Iowa Darters as a 1% presence. Fish have moved from Sanctuary Pond to Lake Leopold and from Lake Leopold, they have moved down the watershed to Almond Marsh and still further to Bulls Brook just above the junction with the mainstem of the DesPlaines River. While sampling

has been limited, fish appear to have spread throughout the Bulls Brook watershed. A significant element for the project is that a ten-foot drop structure exists at the street immediately to the east of Prairie Crossing. Carp and other exotic species cannot make their way into the lakes and ponds of Prairie Crossing. Fish can, however, be exported downstream into the Bulls Brook watershed and the DesPlaines drainage (Figure 3).

In addition to spreading within the immediate watershed there have been approximately six different trials where different combinations of the four species were moved from Sanctuary Pond to other locations within the greater DesPlaines drainage. These trials were not monitored as closely as the original translocation. Shiners were translocated to a zoological society pond but the algal burden of the pond and the presence of sunfish meant that no surviving fish were found in the subsequent season. Transfers to ponds with even a modest amount of turbidity resulted in no survivorship for the shiners. Concerns for those places where the shiners have survived are genetic variability and founder effects. The founder effect describes a condition where the genetic profile is different enough that it has the possibility of creating a new species.

Literature Cited

Afelbaum, S., J.D. Eppich, T.H. Price, and M. Sands. 1995. The Prairie Crossing Project: Attaining Water Quality and Stormwater Management Goals in a Conservation Development. *In* Proceedings of National Symposium on Using Ecological Restoration to Meet Clean Water Act Goals, pp. 33-38. Chicago, Illinois, March 14-16. USEPA Conference.

Page, L.M. 1991. Streams of Illinois .Illinois Natural History Survey Bulletin 34(4): 439-446.

Schaeffer, J.S., J.K. Bland,and J. Janssen. 2012. Use of a Storm water Retention System for Conservation of Regionally Endangered Fish. Fisheries Vol.37 (2): 66-75.

Shuter, B.J.,A.G. Finstad, I.P. Helland, I. Zweimöller, and F. Hölker. 2012. The role of winter phenology in shaping the ecology of freshwater fish and their sensitivities to climate change, Aquatic Sciences Vol. 74(4): 637-657.

Part II: Life History Studies and Conservation Status

Arrangements were made with the Illinois DNR, Southern Illinois University (SIU), and Max McGraw Wildlife Foundation (MWF) to sponsor two graduate students to determine the conservation status and life history profiles of the two shiners and the Banded Killifish (Burr et al. 2005). Dr. Brooks Burr of SIU and Vic Santucci of MWF oversaw the work of Matt Roberts and Adrienne Davis. One of the first finds of their field studies was that the Pugnose Shiner (*Notropis anogenus*) was present in Lake Leopold. Apparently, we had collected some Pugnose along with the other shiners as part of our original translocation. Pugnose consistently showed up in the graduate student collections at Lake Leopold but only in very limited numbers. Life history traits were compared between the original source lakes, Deep Lake and Cedar Lake in Northeastern Illinois, and the Prairie Crossing location in an effort to determine similarities and differences in stocked populations versus natural populations.

Presence/absence records across North America and for Illinois lakes and rivers were reviewed and field sampling undertaken for as many historical Illinois collection sites as possible. In Illinois the Blacknose Shiner was present at only one of 21 historical stream locations and in eight of 12 historical lake sites. The Blackchin Shiner was found in seven of the 18 historical sites; six of these sites are lakes and only a single stream site had a recent record. Extant populations of the Pugnose Shiner were present at four of 10 historical sites. The Pugnose is a particularly difficult species to characterize because of its low numbers, association with high density plant beds, and difficulties with collecting conditions. The graduate students found Banded Killifish at four of 11 historical sites and no new localities were found. Banded Killifish were no longer found in Cook, McHenry, or McClean counties; only Lake County had extant populations. Reductions in ranges thus exist for all of the species in Illinois. Major conservation concerns were the absence of most stream populations for any of these species and the need for active conservation management in the lakes where remnant populations still exist. The following section abstracts the life history and global distribution data compiled by Matt Roberts and Adrienne Davis (Burr et al. 2005).

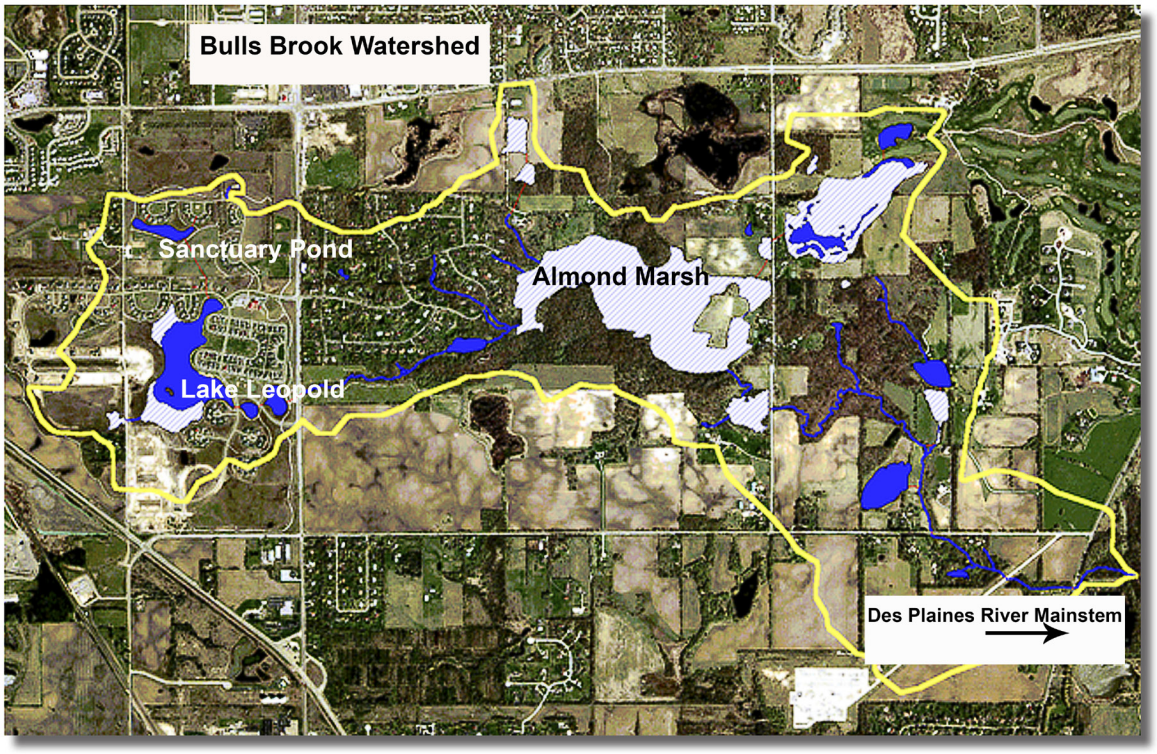


Figure 3. Bulls Brook Watershed and relative location to the DesPlaines River Mainstem. (This figure was left out of the original article in *American Currents*).

Blacknose Shiner *Notropis heterolepis*

The Blacknose Shiner shows a pattern of range reduction along the southern border of its range but it is secure in the northern part of its distribution in Canada and the United States. According to the SIU study, it's typically found in lakes close to shore over sand and in low-to-moderate cover. The species was characterized as having an "*opportunistic life history producing multiple clutches of eggs over a period extending from April to early July*…." Males and females were reproductively mature at 1+ age class; males developed breeding tubercles on the dorsal surface of their pectoral rays from June through October. The mean number of ova present in a clutch was 167. Feeding followed a diel pattern with morning and evening peaks. Their principal diet consisted of zooplankton; mainly chydorid and bosminid water fleas and ostracods. Feeding is heavier in the spring than summer or fall. Life span is short (1+) years and very few individuals survive into a second year, according to field studies conducted by other researchers. Data from Ohio indicate that eggs are scattered over vegetation with no subsequent parental care. The Blacknose was recorded as a fossil from the last glaciations in one ice-dammed lake in northern North Dakota (Newbrey and Ashworth 2004). The Blacknose Shiner is listed as state endangered in Illinois.

Blackchin Shiner *Notropis heterodon*

The Blackchin Shiner showed a 61% reduction in its range across Illinois. The SIU students identified sand and the presence of vegetation from their field sites as important physical components sustaining regional populations. Males and females reach approximately the same size at maturity. Breeding season is from late May through early August and multiple clutches are part of the reproductive pattern. Similar to the Blacknose, the Blackchin was found to be an opportunistic strategist—early maturation, small clutch size, egg diameters, and production of multiple clutches. The Blackchin is also a diel feeder and forages over vegetation, in the open water and at the surface. It is primarily a planktivore, feeding on water fleas and ostracods. It would seem as though the two shiner species should be in competition; their occurrence together is somewhat of a surprise.

Banded Killifish *Fundulus diaphanus*

The most immediate condition that ties all four translocated species together is the requirement for high clarity water and vegetation. A seemingly hardy species, Banded Killifish is resistant to low dissolved oxygen and can withstand a wide variety of temperatures. They can be found over substrates ranging from silt to gravel; the Prairie Crossing ponds and lakes all have a silt substrate. Many of the recreational lakes in northeastern Illinois employ active management to reduce rooted aquatic plant populations and this may have had a bearing on its contracted distribution. It favors shallow, still waters of lakes and ponds. Spawning occurs from late spring to early summer. Adhesive eggs are produced that stick to vegetation. It is described as a generalized feeder and will feed on micro-crustaceans, insect larva, and broad assortment of zooplankton (Becker1983).

Iowa Darter *Etheostoma exile*

The SIU students did not do conservation or life history studies for the Iowa Darter since substantive work had already been done by others. Unlike the other three species, Iowa Darters were still present at DesPlaines sites. Once listed as Endangered, it is currently listed as Threatened in Illinois as a consequence of updated distributional data. The Iowa Darter is one of a handful of darters that can commonly be found in lakes. After its translocation to Prairie Crossing, it was found in all of the Prairie Crossing ponds and as part of the greater Bulls Brook drainage. It lives up to three years, achieves a size of up to 2.75 inches, disperses eggs against the available substrate, and does not protect its young after spawning. Males establish breeding territories in shallow water as water temperatures moderate in early spring. They don't, however, create nests. As with the other Prairie Crossing species, Iowa Darters are able to survive low levels of oxygen. Food sources range from micro-crustaceans, aquatic insect larva to various types of zooplankton. Recent sampling of headwater stream assemblages in Northeastern Illinois has found Iowa Darters as a common occurrence.

Pugnose Shiner *Notropis anogenus*

The Pugnose Shiner is rare throughout its range (Burr et al. 2005). In northeastern Illinois, at the southern limit of its distribution, it is found in only a handful of glacial lakes. SIU students found the Pugnose at five of the 12 sites it had been collected historically. The habitat association of Pugnose Shiners,

(Continued on Page 20)

Fishes of Prairie Crossing

Blacknose Shiner Jim Bland

Blacknose Shiner Uland Thomas
Elkhart River system, Indiana

Blackchin Shiner Uland Thomas
Elkhart River system, Indiana

Pugnose Shiner Konrad Schmidt
Crooked Creek, Crow Wing County, Minnesota

Iowa Darter (male) Uland Thomas
Elkhart River system, Indiana

Iowa Darter (female) Uland Thomas
Elkhart River system, Indiana

Banded Killifish Jim Bland

dense weed cover, made it hard to collect. Therefore, backpack shocking and nearshore seining have not always been effective in confirming their presence. SIU sampling teams noted that Pugnose also seem to have preferred locations within the larger habitat. They have speculated that the localization within lakes and the association with dense weed beds may have contributed to biases in collection data. Jen Porterfield and Dr. Patrick Ceas of St. Olaf studied the life history traits of the Pugnose Shiner in Minnesota (Ceas 2012). They document a shift from the use of deeper water environments (4 to 6 feet) in early spring to nearshore shallows (3 to 4 feet) where aquatic vegetation is abundant. They also infer from feeding studies that Pugnose feed on both filamentous algae and micro-crustaceans. Year 2 females and males are sexually mature by mid-may while Year 1 males were sexually mature by July and thus represent potential spawning later in the summer. Scientists from Canada are doing microsatellite DNA profiles for different populations. Preliminary results distinguish significant differences between western stocks and eastern stocks. Canada has also prepared a recovery plan for this species (Lyons 2012)

Literature Cited

Becker, G.C.1983. *Fishes of Wisconsin*. University of Wisconsin Press. 1,052 pp.

Burr, B.M.,V.J. Santucci, M.E. Roberts, A.M. Davis, and M.R. Wiles. 2005. Conservation status and life history characteristics of the blacknose shiner *Notropis heterolepis*, blackchin shiner *Notropis heterodon* (Cyprinidae),with conservation evaluations of the pugnose shiner *Notropis anogenus* (Cyprinidae), and banded killifish *Fundulus diaphanus* (Fundulidae), in Illinois. Max McGraw Wildlife Foundation, Final Report.

Ceas, P., and J. Porterfield. 2012. Life histories of the northern longear sunfish (*Lepomis megalotis peltastes*) and pugnose shiner (*Notropis anoge nus*) in Minnesota, with examinations of other rare non-game fishes. Final Report. Minnesota State Wildlife Report T-32-R-1.

Illinois Department of Natural Resources. 2011. Checklist of Endangered and Threatened Animals and Plants of Illinois. Illinois Endangered Species Protection Board.

Lyons, J. Editor. 2012. *Fishes of Wisconsin* E-book.. Wisconsin Department of Natural Resources, Madison and U.S. Geological Survey, Middleton, Wisconsin. http://www.fow-ebook.us

Newbrey, M.G., and A.C. Ashworth. 2004. A fossil record of colonization and response of lacustrine fish populations to climate change, Canadian Journal of Fisheries and Aquatic Sciences 61(10): 1807-1816.

Roberts, M.E, B.M. Burr, M.R. Whiles, and V.J. Santucci, Jr. 2006. Reproductive Ecology and Food Habits of the Blacknose Shiner, *Notropis heterolepis,* in Northern Illinois, American Midland Naturalist 155: 70-83

Part III: Preserving Genetic Variability: The Real Measure of Successful Translocations

The last part of this story has taken perhaps the longest time. The results are important however for anyone looking to effect translocations of imperiled fish fauna. Dr. Mary Ashley of the University of Illinois Chicago Circle and her graduate student, Fuson Ozer, performed microsatellite analysis of the two shiner species. Microsatellites are short sequences of DNA that repeats themselves frequently; they are used as molecular markers. Fish samples were taken from the source lakes, Cedar Lake and Deep Lake in the Fox River drainage, and from the receiving lake and pond, Lake Leopold and Sanctuary Pond (Upper Pond in their publication). Initial genetic analysis occurred between 2001 and 2005, after the breeding season. Given a generation time of 1- to- 2 years, these samples were taken between two and seven generations post-translocation. Success would require that genetically diverse and representative populations were created in the sanctuary lake/pond. Preliminary results were hopeful; however, more detailed analysis determined that there were short-falls in the project (Ashley and Ozer 2013).

The research objectives of the UIC team were: 1) compare microsatellite profiles between the source lake and the sanctuary lake/pond; 2) assess levels of divergence between Blackchin Shiners and Blacknose Shiners and insure that hybridization was not taking place; 3) quantify levels of genetic losses if in fact there were losses; 4) test whether close kin (siblings) occurred in the sample; and 5) estimate the effective

size for sampled populations for microsatellite studies.

Microsatellite data showed that the two species are quite distinct and there was no evidence of hybridization in either the source or translocated samples. Moderate levels of heterozygosity were sustained by both species as part of the translocation. When thinking about heterozygosity you might think about the alternate traits on Mendel's pea plants. If a dominant and recessive trait exists at one gene allele site we speak of it as heterozygous. On a population level if animals have low heterozygosity they may be at risk; examples include Cheetahs and Black-footed Ferrets. High levels of heterozygosity imply that genetic variability has been preserved. For the Prairie Crossing project many alleles observed in the source populations were not observed in the translocated populations, indicating that some genetic diversity had not been preserved. Surprising also was the finding that full sibs (brothers and sisters) occurred with half sibs (cousins) within the source lake samples. This has the effect of reducing the effective population size of the reintroduced stock.

The loss of genetic diversity implies that taking fish from Sanctuary Pond or Lake Leopold for additional reintroductions risks the possibility of genetic bottlenecks (populations eventually die back) or founder effects (we're busy creating new species). In the future we may do additional translocations from Cedar Lake and Deep Lake into Sanctuary Pond and/or Lake Leopold. In the short-run however, Dr. Ashley has suggested that any stock for reintroductions come directly from the original source lakes. It is also implied that we took too few fish from the source lakes for the creation of the sanctuaries. As we collect fish, Dr. Ashley would also suggest that we cast a larger net, i.e., look to sample a broader range of environments in the lake so that we are not sampling close relatives (sibs). In the initial translocation we were not able to obtain the 200 fish per species that was our target. According to Ashley and Ozer (2013): *"While this study does not preclude the use of small, man-made ponds and lakes in management plans, it does suggest that to maintain both heterozygosity and allelic diversity, sanctuary populations will need to be established using several hundred fish from multiple sites, compared to 200 or fewer collected from single sites, as used for the Prairie Crossing sanctuary."*

Literature Cited

Ashley,M., and F. Ozer. 2013. Genetic evaluation of remnant and translocated shiners *Notropis heterodon* and *Notropis heterolepis*. Journal of Fish Biology 82:1281-1296.

The George Maier Fund is now accepting grant requests

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Request for Applications (RFA) No. GMF A-13 Please refer to this number in all correspondence

Key Dates:
Application Deadline: Applications are due by December 31, 2013 Grant Awards: Grants will be awarded no later than January 31, 2014.

Relevant Procedures:
GMF P-1 Grant Proposal Requirements
Proposals not in accordance with this document will not be evaluated. Copies of this procedure are available via e-mail request to the address noted below.

2013 Grant Summary:

Total Available Funding:	$7,500
Length of Project Period:	Up to 2 years
Estimated Number of Awards:	Up to three

(3)a.Multiple grants for lesser amounts may be considered whose sum is equal to or less than the maximum amount.

Charles A. Nunziata	Anthony Terceira Ph.D
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