CONSERVING THE STARHEAD TOPMINNOW *FUNDULUS DISPAR* IN WISCONSIN: 3. RE-ESTABLISHMENT SUCCESS!

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This article is Part 3 of a three-part series. Part 1 described the threats to, and current status of, the state-endangered Starhead Topminnow in Wisconsin and the value of a reintroduction project to increase its distribution. Part 2 covered efforts to culture large numbers of Starhead Topminnows for the reintroduction. Here in Part 3, we summarize results of the reintroduction project and conservation implications.

INTRODUCTION

Since 2018, we have been working on a project to reintroduce the Wisconsin state-endangered Starhead Topminnow (Figure 1), one of our favorite dickey fishes, back into a portion of its former range in the Wisconsin River. We discussed the rationale and plan for this project in our first Starhead Topminnow article of this series in the Spring 2021 issue of *American Currents* (Lyons et al. 2021). To start the project, we collected wild Starhead Topminnows from the lower Wisconsin River below the Prairie du Sac Dam. We brought them back to a small landlocked pond to breed them and raise their offspring for stocking into the Wisconsin River above the dam, which we discussed in our second article in the Summer 2021 issue (Marshall et al. 2021). In this final installment, we describe the process of stocking Starhead Topminnows above the Prairie du Sac Dam and evaluating their short and long-term survival and their reproductive success. We then discuss what our project means for the conservation of this and other rare and at-risk fish species in the Wisconsin River and elsewhere.

SELECTING STARHEAD TOPMINNOW STOCKING SITES

Our first step was to determine where to stock the Starhead Topminnows that we had reared. We had some ideas going into the project about areas with habitat conditions conducive to topminnow survival and reproduction, but we needed to confirm and finalize specific locations. Our criteria were that stocking sites had to be (1) relatively large (over 2 acres); (2) located off the main river channel in bays, (3) backwaters, sloughs, or creek mouths; (4) have permanent good-quality water, extensive and diverse aquatic vegetation, and substantial groundwater input (either through springs and seeps or via small coldwater tributaries); and (5) drain land areas with largely natural vegetation and little or no agriculture that could contaminate groundwater and surface water inputs.

The selection process took many days. We pored over maps and aerial photos, talked extensively amongst ourselves and with knowledgeable colleagues and landowners, and then walked and boated around a variety of potential sites (Figure 2). Initially, we were excited that we might have a good spot not far from Aldo Leopold's "Shack," the centerpiece of his book and conservation masterpiece "*A Sand County Almanac*" (Leopold 1949). However, the slough, which looked great from a distance, proved to have difficult access and stagnant water with little spring input, and it didn't meet our criteria. Eventually, we ended up with seven sites that appeared wor-

Figure 1. Pond-raised male Starhead Topminnow, ready to be stocked.

Photos by the author unless otherwise indicated.

We are all retired WDNR biologists. John Lyons was a statewide Fisheries Research Scientist and Supervisor for WDNR and is now Curator of Fishes at the University of Wisconsin Zoological Museum in Madison. Dave Marshall was a Water Quality Biologist for WDNR covering southwestern Wisconsin and the Lower Wisconsin River, as was Jean Unmuth. Sue Marcquenski was the statewide WDNR Fish Health Specialist, and Tim Larson was the WDNR Fisheries Manager for Sauk and Columbia counties, including the Wisconsin River above the Prairie du Sac Dam.

thy of stocking. Four were bays of Lake Wisconsin (Figure 3) (the impoundment formed by the Prairie du Sac Dam); two were sloughs located at the upstream end of the lake; and one was a creek mouth (Rocky Run) not far above the sloughs (Figure 4).

Figure 2. Dave Marshall collecting water quality data from a slough upstream of Lake Wisconsin that was rejected as a potential Starhead Topminnow stocking site.

Figure 3. Gallus Slough, a bay of Lake Wisconsin, one of our successful Starhead Topminnow stocking sites.

STARHEAD TOPMINNOW STOCKING PROCEDURES

Once stocking sites had been selected, our next step was to capture, transport, and introduce the Starhead Topminnows from the breeding and rearing pond to these sites. We wanted to make sure we did this in a way that minimized stress and maximized their chances of successfully adapting to their new home. Toward this end, we made multiple stockings of relatively small numbers (dozens to hundreds) of fish over several weeks in late spring or summer rather than trying to release large numbers (thousands) all at once. We moved fish only on days when the weather was calm and air temperatures were at or below the mid 80s F. We captured Starhead Topminnows from the rearing pond mainly with small-mesh dip nets (Figure 5) in groups of one to five and held them in tubs next to the pond until we had about 50 fish. We avoided touching any fish with our hands, and we moved only fish over 0.8 inches in length, which were hardier than smaller fish and also more likely to be adults capable of reproduction post-release. Once we had caught 50, which usually took less than 15 minutes, we transferred them to a large clear plastic bag full of fresh water with an aerator (Figure 6). We checked that the water temperature was under 80 F and the dissolved oxygen concentration was above 7 parts per million; then we sealed the bag, leaving a large air space above the water. We stowed the bag in an insulated cooler in the shade until we were ready to leave, never more than 1.5 hours later. When we had five to ten full coolers spread among two to three vehicles, we headed for the stocking area about a one-hour drive away (Figure 7). To reach the actual stocking sites usually then required a boat ride of up to 20 minutes from the nearest launch (Figure 8). All told, the longest time interval from the first capture of fish from the pond to their arrival at the stocking site was a little over three hours and the shortest about 1.5 hours.

Capturing Starhead Topminnows for stocking was not without its risks. The pond was full of aquatic insects, including Hemip-

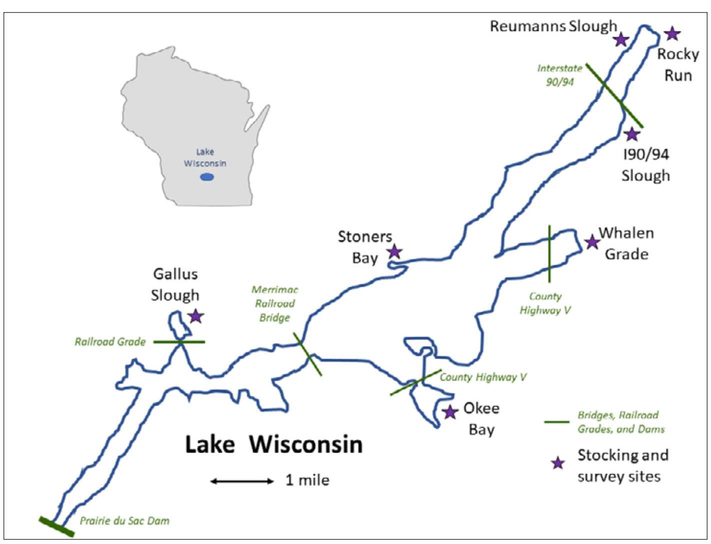

Figure 4. Map of Lake Wisconsin, formed by the Prairie du Sac Dam on the Wisconsin River, showing the seven sites stocked with Starhead Topminnows.

Figure 5. Dave and Tim Larson netting Starhead Topminnows from the rearing pond on a rainy black-fly-infested day.

terans (true bugs) with their fearsome proboscises that can inflict a painful "bite." We caught lots of these creatures along with the fish, and if we didn't remove them immediately, some would begin preying on the fish in the tubs and bags. But removing them was a tricky business, and if you weren't careful, you'd end up on the business end of that proboscis. The process of netting and sorting fish was regularly punctuated by loud yelps and curses, and after several days our fingers showed the damage (Figure 9).

Once we arrived at the stocking site, we followed a standardized procedure. First, we found a shallow area near aquatic vegetation. We then opened each bag and removed any moribund individuals. Fortunately, very few fish died in transit, and total transport mortality was always less than 1% and usually zero. We then placed the bags in the water so that the water temperatures in the bags could equilibrate with the water temperature at the site, a process that took 20–30 minutes (Figure 10). When the temperatures were within a few degrees, we began to gradually add site water to the bags so that the fish could equilibrate to the chemical conditions at the site. After about half the water in the bag was from the site and the bag water temperature was within 1–2 degrees of site water, we gently emptied the bags and released the fish at the site. The fish were on their own, and we wished them luck!

We ended up stocking 6,309 Starhead Topminnows during 22 stocking events at the seven sites from 2018–2021. Individual sites

Figure 6. Tim and Sue Marcquenski bagging up Starhead Topminnows for transport to a stocking site.

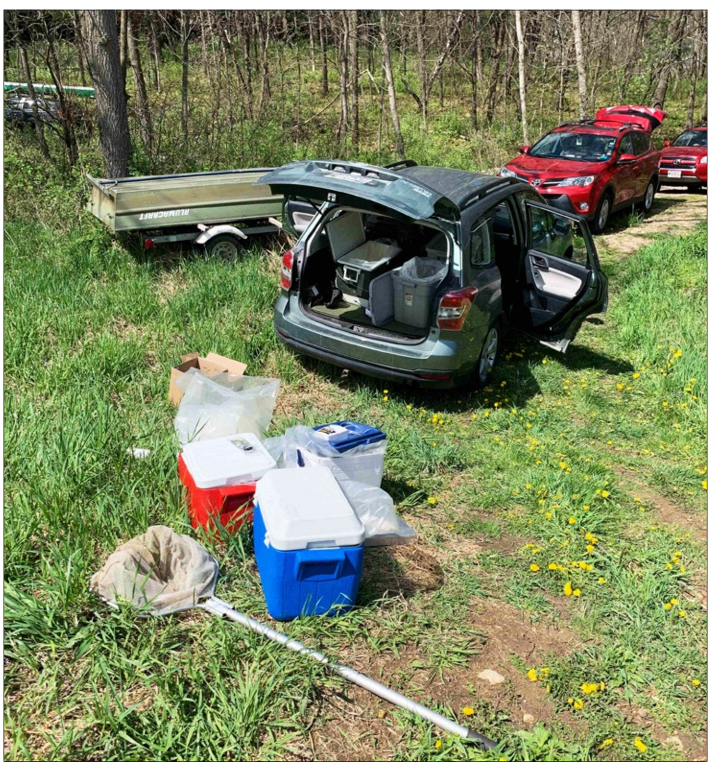

Figure 7. A caravan of cars and coolers full of Starhead Topminnows from the rearing pond almost ready to depart for Lake Wisconsin on a spring day.

Figure 8. Dave, Sue, and Tim in Tim's boat full of coolers of Starhead Topminnows, ready to cast off for a stocking site.

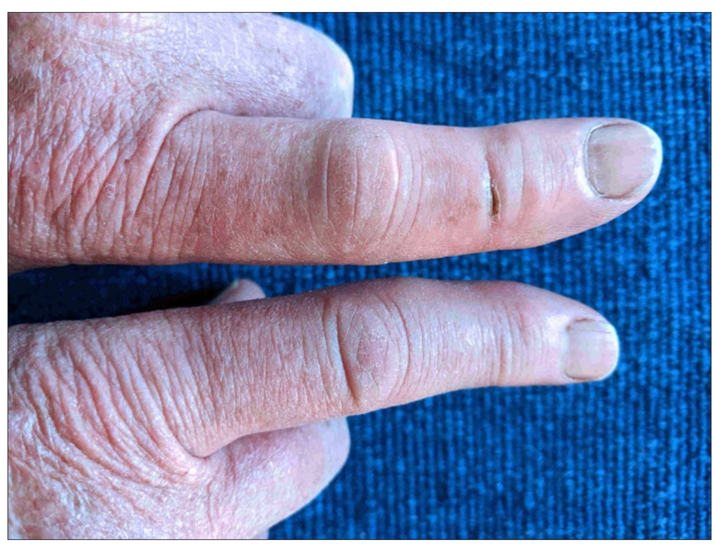

Figure 9. Dave's index fingers: The left is swollen from water bug bites after a day of netting and sorting.

Figure 10. Sue checking bags of Starhead Topminnows as their temperatures equilibrate with the water of Gallus Slough.

Figure 11. The view from the bow of John's boat during a survey of Whalen Grade.

Table 1. Stockings of Starhead Topminnow (SHTM) at seven sites in the Wisconsin River above the Prairie du Sac Dam from 2018–2021.¸

Site	Years Stocked	Number of Stockings	Total SHTM Stocked
I90/94 Slough	2018–2019	3	1,341
Reumanns Slough	2019	1	265
Rocky Run	2021	1	255
Gallus Slough	2019–2021	9	1,977
Stoners Bay	2020–2021	3	1,071
Whalen Grade	2020–2021	4	1,138
Okee Bay	2021	1	262
Totals	**2018–2021**	**22**	**6,309**

Table 2. Results of surveys for Starhead Topminnow (SHTM) in the Wisconsin River above the Prairie du Sac Dam at the seven sites stocked from 2018–2021.

Site	Years Surveyed	Number of Surveys	Total SHTM Observed
I90/94 Slough	2019–2021	4	42
Reumanns Slough	2020–2021	4	0
Rocky Run	None	0	Not applicable
Gallus Slough	2020–2021	3	>370
Stoners Bay	2020–2021	2	>200
Whalen Grade	2021	3	0
Okee Bay	2021	1	0
Totals	**2019–2021**	**17**	**>612**

received a total of 255 to 1,977 fish across one to nine stockings (Table 1).

SURVEYING STOCKED SITES FOR STARHEAD TOPMINNOWS

We began surveying our stocked sites in 2019, and we first surveyed each site at least three months after it was first stocked. We conducted surveys during mid to late summer in order to detect "young of the year" (YOY) Starhead Topminnows that had been locally reproduced earlier that year at the site. We completed 17 surveys at six of the seven sites from 2019–2021 (Figure 11). We were unable to survey the Rocky Run site, first stocked during higher flows in spring 2021, because low water and recently downed trees blocked access in late summer 2021. We plan to return there to do a survey in summer 2022.

Our surveys focused on small openings in otherwise heavily vegetated shallow areas, which is the type of habitat Starhead Topminnows normally frequented. Because thick vegetation and soft bottom materials made wading and deploying nets difficult in most places, we primarily made visual observations from a shallow-draft boat to document Starhead Topminnow occurrence. Once we saw what we thought were Starhead Topminnows, we captured a few specimens with a long-handled dip net or backpack electroshocker to confirm identification (Figure 12). With a little practice, Starhead Topminnows were easy to detect and classify as YOY (less than about 0.8 inches) or adults (greater than 0.8 inches) from the bow of a boat or kayak.

We found Starhead Topminnows post-release at three of the six survey sites (Table 2), and all three sites yielded YOY indicating successful natural reproduction and likely re-establishment. Starhead Topminnow YOY were too numerous to count completely during several surveys at Gallus Slough and Stoners Bay, but by extrapolating the numbers we saw in the small areas we assessed to the entire area of suitable habitat, we estimated that each site probably had many thousands of individuals by 2021. Stoners Bay and Whalen Grade also produced good numbers of Western Banded Killifish *Fundulus diaphanus menona* (Figure 13). The I90/94 Slough had fewer Starhead Topminnows and a much smaller area of suitable habitat, but we did observe YOY each year we visited the site from 2019–2021. Given that most Starhead Topminnows survive for just two years and reproduce only in their second year (Becker 1983; Taylor and Burr 1997), it seems likely that the 2021 YOY were offspring of fish that spawned in 2020 that were in turn the offspring either of the adults stocked in 2019 or of the offspring of the adults stocked in 2018. In other words, at least two and perhaps three generations of Starhead Topminnows had been produced at this site since the original stocking in 2018.

Of the three sites where Starhead Topminnows were not observed,

Figure 12. YOY Starhead Topminnows captured by dip net during a survey of Gallus Slough. (Photo by Dave Marshall)

Figure 13. Western Banded Killifish captured from Stoners Bay. (Photo by Dave Marshall)

Figure 14. Reumanns Slough, a site where Starhead Topminnow stocking appeared to be unsuccessful. Left photo, Jean Unmuth and Sue stocking Starhead Topminnows in the flooded forest during unusually high water in May 2019. Dave Marshall photo. The same stocking area, just behind the dead tree and now high and dry, during normal flows in August 2020. (Photo by John Lyons)

the lack of Starhead Topminnows at Reumanns Slough was not surprising in hindsight, but their absence at Whalen Grade and Okee Bay was puzzling. Reumanns Slough was scouted and stocked during an extended period of unusually high water (Figure 14). The water was so high that the fish had to be stocked in an area of flooded forest that would normally be dry and far from the river. When the waters receded and finally dropped back to normal, it became clear that this site had little groundwater input and limited aquatic vegetation and thus was poor Starhead Topminnow habitat. Although we never found Starhead Topminnows at this site, it did yield several interesting slough fishes including Blackstripe Topminnow *Fundulus notatus,* Grass Pickerel *Esox americanus vermiculatus,* Pirate Perch *Aphredoderus sayanus,* and Mud Darter *Etheostoma asprigene* (Figure 15).

However, both Whalen Grade and Okee Bay had large areas of what appeared to be excellent Starhead Topminnow habitat (Figure 16) that was similar to Gallus Slough and Stoners Bay. We only were able to visit Okee Bay once, and this site is worth further surveys in 2022 to make sure we didn't miss Starhead Topminnows that were actually present. But we thoroughly surveyed Whalen Grade three times in 2021 and, although we observed many Western Banded Killifish, we saw no Starhead Topminnows. Why our stockings didn't yield results there is a mystery, and we plan to recheck these sites in 2022.

CONCLUSIONS AND CONSERVATION IMPLICATIONS

Overall, our Starhead Topminnow reintroduction effort has been a success thus far. Starhead Topminnows have reproduced in multiple years and appear to be re-established in three discrete areas of the

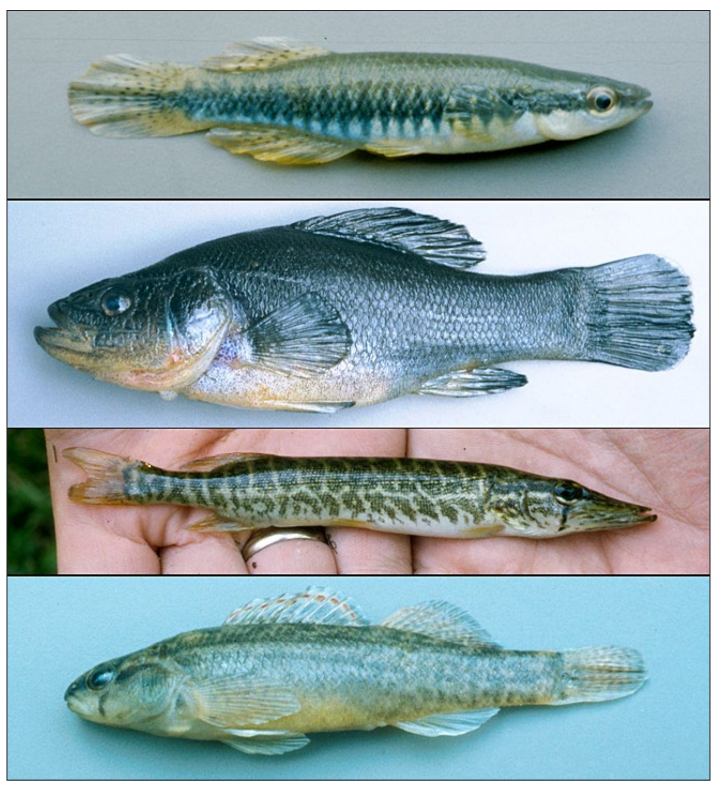

Figure 15. No Starhead Topminnows were observed in Reumanns Slough, but we collected four characteristic Wisconsin slough fish species. From top to bottom, Blackstripe Topminnow, Pirate Perch, Grass Pickerel, Mud Darter.

Figure 16. Lots of suitable habitat occurred at Whalen Grade, but we didn't observe any Starhead Topminnows there in three surveys in 2021.

Wisconsin River upstream of the Prairie du Sac Dam. Each of these sites were stocked with progeny derived from parents netted from several locations below the dam. Starhead Topminnow populations are large at two of these stocked sites, and two and perhaps three generations of offspring have been produced at the third. Three other stocked sites have not yet yielded Starhead Topminnows, but additional surveys are warranted. A seventh stocked site has not yet been visited but will be assessed for Starhead Topminnows in 2022. We hope to continue follow-up monitoring on a regular basis in future years

As far as we are aware, our project is the first to reintroduce Starhead Topminnows into a portion of their historical range. But it is hardly the first project to try re-establishing populations of rare, small-bodied, nongame fishes to aid in their conservation. In the past 25 years, a variety of minnow, darter, madtom, and killifish species have been successfully reintroduced in many parts of the central United States. Some reintroductions have involved translocations, which are direct transfers of fish from one natural habitat to another (e.g., Schmidt 1996, 2014; Huff et al. 2010), but this approach is impractical for many species in many areas for two reasons. First, because of the scarcity of rare species in the wild, capturing and moving the numbers of fish necessary for re-establishment could be challenging and could hurt existing natural populations. Second, many states have recently enacted regulations that restrict the transport and movement of wild fish in order to prevent the spread of disease and invasive species. For example, in Wisconsin, transfer of wild fish from one spot to another is almost completely prohibited by the 2009 "Invasive Species Rule" (NR40: https://dnr.wisconsin.gov/topic/invasives/classification.html). More commonly, the "conservation aquaculture" approach we followed (Marshall et al. 2021) is employed: wild fish are brought into a designated hatchery or pond (with appropriate permits, of course) and then bred and raised in large numbers for stocking into formerly occupied natural habitats (e.g., Shute et al. 2005; Schaeffer et al. 2012; Schumann et al. 2012; Bland 2013; Carlson et al. 2019; Rice and Zimmerman 2019, p. 264; Thiessen et al. 2019). Regardless of which approach is used—translocation or conservation aquaculture—multiple studies now demonstrate that the reintroduction of rare, small-bodied, nongame fishes can be an effective conservation strategy.

The challenge in any reintroduction effort is maintaining sufficient genetic diversity and quality for a fish species to have the capability to survive and adapt to changing future conditions in its new home (Neff et al. 2011). Unfortunately, only rarely do small-scale reintroduction projects of non-game fishes have the funding and expertise to assess genetic characteristics. Even when they do, the relatively small numbers of individuals used in a translocation or conservation aquaculture often do not contain the full range of genetic diversity present in the source populations (e.g., Bland 2013; Ozer and Ashley 2013). Furthermore, the small numbers may also lead to genetic drift and divergence between the source and the stocked populations. Projects often try to maximize genetic diversity by moving or breeding fish from a variety of different populations. However, if these populations are from different habitat types or widely separated areas, they may experience outbreeding depression in subsequent generations; that is, the loss of evolutionary fitness caused by interbreeding of distinctive genetic populations (Huff et al. 2011; Neff et al. 2011).

We were unable to conduct genetic analyses during our project, but we followed established guidelines to maximize reintroduction success and to ensure genetic diversity and quality (George et al. 2009; Houde et al. 2015). We recommend that anyone contemplating a similar fish reintroduction project follow these same guidelines. In particular, we used a combination of the "environment matching" and "multiple source population" approaches in selecting source fish for conservation aquaculture (Houde et al. 2015). We obtained Starhead Topminnows from specific habitats below the Prairie du Sac Dam that were similar to the habitats where we later stocked their offspring above the dam (environment matching), and we captured source fish from multiple sloughs and backwaters below the dam over two years (multiple source population) to increase genetic diversity (Lyons et al. 2021; Marshall et al. 2021). However, we only collected Starhead Topminnows from nearby (within 15 miles) and occasionally interconnected sloughs and backwaters below the dam, as opposed to from widely separated locations or different drainages, to minimize the chance of outbreeding depression. Time will tell if we were successful in encompassing enough genetic diversity and quality for our reintroduced Starhead Topminnow populations to survive and thrive.

While our recent success in re-establishing Starhead Topminnows above the Prairie du Sac dam is encouraging, the degradation of the Lower Wisconsin River floodplain, including the most important existing habitat for Starhead Topminnows in Wisconsin, unfortunately continues. Intensive row-crop agriculture and excessive application of fertilizer across the highly leachable Pleistocene sand terrace along the river persists, resulting in increasingly poor water quality in adjacent sloughs and backwaters. Although Starhead Topminnows remain locally common in some portions of the lower river, their future, and that of their companion fauna and flora, is at risk. And sadly, at the moment, little is being done to halt or reverse the damage that is occurring.

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Figure 17. Cap that the authors and volunteers wore to highlight the re-introduction project.

Riverway for sponsoring and receiving the grants on our behalf. We appreciate the help Lisie Kitchel, Naomi Rivers, Bradd Sims, and Nate Nye, WDNR, gave us in navigating the paperwork and obtaining the permits necessary for capturing, raising, and stocking state-endangered Starhead Topminnows. Merel Black, Don Golembiewski, Brian Goodman, Cheryl Goodman, Ron Grasshoff, Lisa Hartman, Mike Mossman, Steve Swenson, Pat Trochlell, Will Wawrzyn, Wendy K. Weisensel, and Timm Zumm graciously assisted us in scouting for sites and/or capturing, transporting, stocking, and surveying for Starhead Topminnows. We rewarded each of them with a special hat until we ran out (Figure 17). We also thank Sunnyside Rod and Gun Club for allowing us to access Stoners Bay through their property.

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