# CONSERVING THE STARHEAD TOPMINNOW *FUNDULUS DISPAR* IN WISCONSIN: 2. CONSERVATION AQUACULTURE

## David W. Marshall, John Lyons, Sue Marcquenski, Tim Larson, and Jean Unmuth

This article is Part 2 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 covers efforts to culture large numbers of Starhead Topminnows for the reintroduction. Part 3 will summarize results of the reintroduction project and conservation implications. Here, in Part 2, we also relate the three important lessons we learned from raising Starhead Topminnows in a semi-wild pond: 1. Expect the unexpected. 2. Forget any notions of laissez-faire pond management. 3. Monitor often and adjust as needed.

### INTRODUCTION

Conservation aquaculture is defined as the use of fish rearing to recover endangered or imperiled fish populations (Anders 1998, Ireland et al. 2002). Our discussions about potentially using conservation aquaculture techniques to protect the Wisconsin state-endangered Starhead Topminnow began in 2012. We had realized that the ongoing loss of this topminnow's primary habitat along the Lower Wisconsin State Riverway would continue since the core of the problem is intransigent agricultural policies and practices that allow nitrate pollution. In 2013, the Lake Ripley Management District in southern Wisconsin had sponsored a state grant to study conservation aquaculture as a way to restore nongame fishes (aka "dickey fishes") in their lake. That document (*Feasibility of Restoring Nongame Fish Species in Lake Ripley, Jefferson County, Wisconsin*) provided additional justification for our first-of-its-kind project to re-establish a rare nongame fish species in Wisconsin.

As we mentioned in Part 1, the Starhead Topminnow recovery effort includes introducing and expanding the fish's population beyond the zone of pollution and above the Prairie du Sac dam, which was part of the species' historic range. To have enough fish, we needed to raise large numbers via conservation aquaculture. We already had a site identified for this—an isolated, fishless pond on Dave Marshall and Wendy Weisensel's rural property—but we needed funds to raise and stock the fish. The project became a reality in 2018 thanks to a grant from the Prairie du Sac Dam Aquatic Resources Enhancement Fund, established by Alliant Energy as part of this utility's hydroelectric dam licensing under Section 408 of the Federal Energy Regulatory Commission. Because the Starhead Topminnow is a state-endangered species, to begin conservation aquaculture we also needed to obtain an Endangered Species Collection Permit from the Wisconsin Department of Natural Resources (WDNR) and a Class III Fish Farm License from the Wisconsin Department of Agriculture, Trade and Consumer Protection.

### THE POND

Our rearing pond, which we now call Topminnow Pond (Figures 1 and 2), was originally constructed in 1995 for wildlife habitat. It is located within a grassland bird conservancy known as Big Hill

**Figure 1. September 2018 Starhead topminnow collection in Topminnow Pond for stocking above the Prairie du Sac dam. Tim Larson and Dave Marshall used the small mesh seine to concentrate the fish at one end of the pond for dip netting. View facing north. (Photo by Wendy K. Weisensel)**

Photos by David Marshall unless otherwise indicated.

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.

**Figure 2. Topminnow Pond January 2021, facing south.**

Savanna in Iowa County. This small pond, only 0.1 acre in area with a maximum depth of 6 feet, was constructed as an embankment-excavation pond. Its only water source was infrequent surface runoff from the pond's 22-acre watershed of native grassland. The pond is productive given the relatively large watershed to pond ratio (220:1) but no significant nutrient sources exist. This landlocked pond did not require permits since it was constructed in a low-lying hay field as opposed to a floodplain or wetland. Floating Leaf Pondweeds *Potamogeton natans* and *P. nodosus* cover most of the pond surface during the growing season. Other plants include White Waterlily *Nymphaea odorata,* Spatterdock *Nuphar advena*, and Pickerel Weed *Pontederia cordata. Low densities of Muskgrass Chara* and the non-native Eurasian Watermilfoil *Myriophyllum spicatum* grow around the perimeter. The pond supports diverse planktonic and benthic invertebrates, along with American Toad *Anaxyrus americanus*, Green Frog *Lithobates clamitans*, Pickerel Frog *L. palustris*, Spring Peeper *Pseudacris crucifer*, Chorus Frog *P. triseriata*, Eastern Gray Treefrog *Hyla versicolor*, Cope's Gray Treefrog (*H. chrysoscelis*), Snapping Turtle *Chelydra serpentina*, occasional Painted Turtle *Chrysemys picta*, and Common Water Snake *Nerodia sipedon*.

We thought this wildlife pond could be suitable for raising Starhead Topminnows for three reasons. First, we had experience raising the closely related Blackstripe Topminnow *F. notatus* in the pond. Five adults, three males and two females, were introduced into the pond over a decade ago in what could be called a whim, certainly not a controlled experiment. The fish were collected as part of a routine WDNR survey in the spring of that year and were originally destined for an aquarium. By the end of the summer more than 100 Blackstripe Topminnows were easily seen swimming at the pond surface. They survived the winter and became far more numerous by the end of the next growing season. The topminnows were so abundant that the water surface boiled when observers approached the pond. However, by the end of the second winter all were found dead. The pond was not aerated or managed in any way. Anoxic winterkill was the likely cause. Since the sole source of water for the pond was the 22 acres of grassland, where surface runoff is often low, the combination of snow, ice, and low water likely contributed to anoxia.

Second, we were encouraged by NANFA member P. T. Johnson's (2012 *American Currents* 37:4–10) article and his reported success raising Starhead Topminnows in an artificial pond. Third and most importantly, our pond displayed many of the environmental characteristics of the Lower Wisconsin State Riverway oxbow lakes, where Starhead Topminnows occur naturally. What the pond lacked was groundwater input. Nearly all areas where we have found good Starhead Topminnow populations receive water from springs or groundwater seeps. To provide this important environmental feature, a contractor drilled a 160-foot well and a pump installer put in a DC solar well pump system to discharge calcareous groundwater into the pond. The pump system supplies the pond with groundwater to compensate for the limited runoff the pond typically receives. The 255-watt solar panel system does not include batteries and only pumps well water during daylight hours. The pump can deliver about six gallons of groundwater per minute but the rate declines under cloudy skies. The pond's groundwater source is chemically similar to the Lower Wisconsin River aquifer, though nitrate levels are much lower (Table 1).

We describe the pond as semi-wild since the fish receive no artificial food. The pond has natural predators including birds, reptiles and aquatic insects: Belastomidae – *Belastoma* and *Lethocerus grisea,* (giant water bugs), and Notonectidae (backswimmers). Introduced Starhead Topminnows are the only fish in the pond.

## YEAR 1

Our goal in Year 1 was to collect enough Starhead Topminnows from the Lower Wisconsin River in the spring to establish a ge-

**Table 1. Pond and Well water quality data collected during the project. Also presented are recent data from one of the Lower Wisconsin River (LWR) polluted oxbow lakes (Jones Slough) for comparison. ND indicates no data.**

| Location | Dissolved oxygen (mg/l) | pH | Alkalinity (mg/l) | Nitrate $NO_3$-N (mg/l) | Total Kjeldahl Nitrogen (mg/l) | Total Phosphorus (mg/l) | Turbidity (NTU) | Conductivity (uS/cm) |
|---|---|---|---|---|---|---|---|---|
| **Pond** | 0.9 – 19.5 | 7.1 – 8.2 | 144 - 300 | 0 – 0.4 | 0.48 – 0.52 | 0.028 – 0.379 | 2.2 – 5.6 | 235 - 430 |
| **Well** | 7 | 8 | 300 | 1.3 – 2.2 | ND | 0.015 | 0 | 559 |
| **Jones Slough LRW groundwater** | 1.5 – 11.8 | 6.9 -8.1 | ND | 17.4 mean | 0.4 – 1.3 | 0.11 mean | 2.5 mean | 422 mean |

netically diverse brood stock in the pond, then propagate topminnows the entire summer and stock all of them above the Prairie du Sac Dam by mid-October. We then planned to repeat the process in following years. In May and June 2018, we collected 119 Starhead Topminnows from seven different oxbows and sloughs along the river. Our brood stock consisted of 61 females, 44 males (1.4:1 ratio) and 14 immature fish. Taylor and Burr (1997) reported as part of their study of Illinois Starhead Topminnows that the female to male ratio was 1.5:1. We used long-handled small-mesh dip nets that are very effective for collecting Starhead Topminnows at the surface. Dip nets are also much easier to haul around than DC electroshocking gear in the plant-filled soft-bottom sloughs. We learned years ago to avoid seines that often result in fish mortalities in weedy environments. Starhead Topminnows and other small fishes are difficult to separate from dense clumps of plants in the seine and can suffocate. We also occasionally lost some fish that were stung by water bugs (Hemiptera) and we have also personally experienced that painful sting.

Collected fish were acclimated and transported in pond water that we brought to the river in coolers to minimize chances of introducing river "hitchhikers" into the pond. Once the topminnows were placed in the coolers, we added battery-powered aeration. By the time all of the brood stock were released into the pond, on June 5, 2018, the water column was teeming with large-bodied *Daphnia*, copepods, and phantom midge larvae (*Chaoborus*), all of which would be potential food for Starhead Topminnows. Backswimmers, potential predators, were also numerous in the pond. After the initial fish releases, we didn't see any topminnows until June 20 other than an occasional adult near shore. Starhead Topminnows are clutch spawners, releasing about 20 eggs per clutch several times from spring to fall. Females lay their eggs on submergent vegetation and fry hatch about 9–11 days later at water temps ca. 25° C. By June 27, we started seeing fry close to shore.

We were elated to see Starhead fry emerge in the pond, but that enthusiasm quickly turned to concern when dissolved oxygen levels began dropping daily. Very low concentrations persisted for most of the summer. We responded by setting up several 100-watt solar panels and deep-cycle batteries for continuous small-scale DC and AC inverter powered aeration (< 10 watts). However, the units were not powerful enough to increase dissolved oxygen levels beyond the immediate area of the air stones. Interestingly, we observed that the topminnows were not at all attracted to the aeration and remained dispersed across the pond even when dissolved oxygen levels dropped below 2 mg/l, and occasionally as low as 1.1 mg/l. The topminnow response to low dissolved oxygen wasn't a complete surprise since we routinely find Starheads in sloughs containing very low dissolved oxygen. Fundulidae species survival in low dissolved oxygen environments had been reported elsewhere (Killgore and Hoover 2001, Turko and Wright 2015).

Suppressed dissolved oxygen as a response to dense aquatic plant canopies is well-documented (Unmuth et al. 2000, Frodge et al. 1990). Topminnow Pond is loaded with plants but what took us by surprise was the extended periods of very low oxygen levels. More importantly, we were concerned that these conditions would be harmful to the Starhead Topminnow eggs and fry, which may be more vulnerable than adults.

Beginning in early July, we used a long-handled weed cutter to remove dense pondweeds from the middle of the pond. We hoped

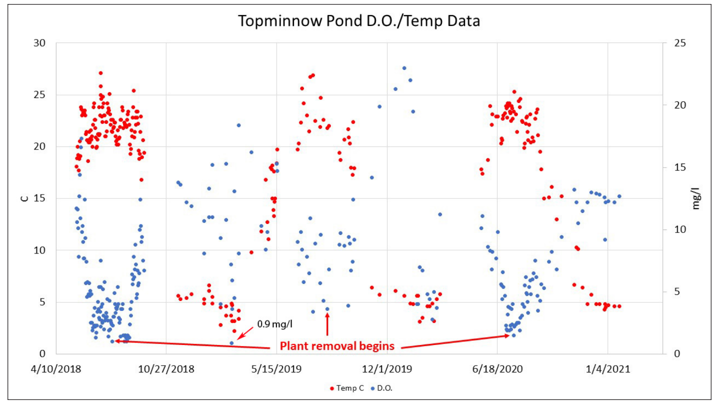


**Figure 3. Pond Dissolved Oxygen (blue dots) and Temperature (red dots) Data.**

to expand open water to increase atmospheric gas exchange, water circulation and algal photosynthesis. Instead, our data demonstrated that re-aeration was very slow (Figure 3). Wind-driven water circulation is minimal because the pond sits in a protected valley. Phytoplankton and filamentous algae did not increase significantly after the macrophyte harvesting, perhaps due to nutrient competition and chemicals that some rooted plants release (Lurling et al. 2006).

During our first aquatic plant harvest, we carefully inspected stems and leaves to ensure that we weren't also removing fish eggs. We did not see any eggs on pondweeds removed from the middle of the pond, but we did find fish eggs attached to milfoil leaflets in the nearshore area. The plants with eggs were placed in a bucket and later in a small aquarium. In spite of atmospheric exposure up to 10 minutes before being placed in the bucket, Starhead eggs hatched in the aquarium several days later (Figures 4 and 5). The newly hatched fry in the aquarium were attracted to ground commercial fish food, but whether they ate any was unclear. After approximately one week, we returned the fry to the pond where numerous other tiny fish were swimming in the nearshore areas.

We also observed numerous backswimmers and Starhead Topminnow young-of-year (YOY) constantly chase one another, but we didn't notice casualties. The opposite occurred when dip-netted Starheads were placed in stocking buckets containing backswimmers that had been unintentionally collected during the

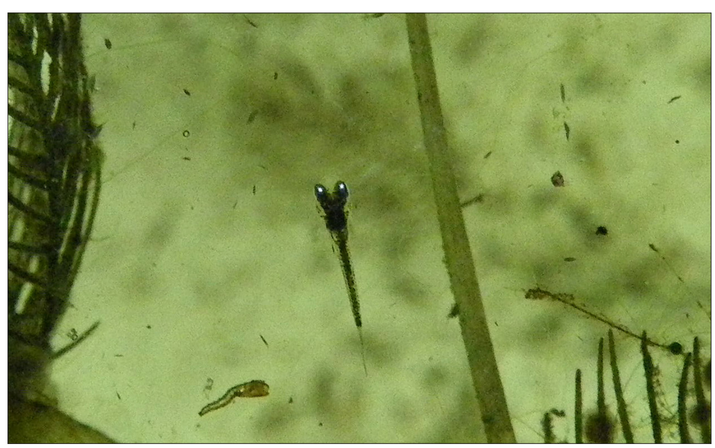

**Figure 4. In an aquarium, newly hatched Starhead Topminnow fry from an egg attached to Eurasian watermilfoil.**

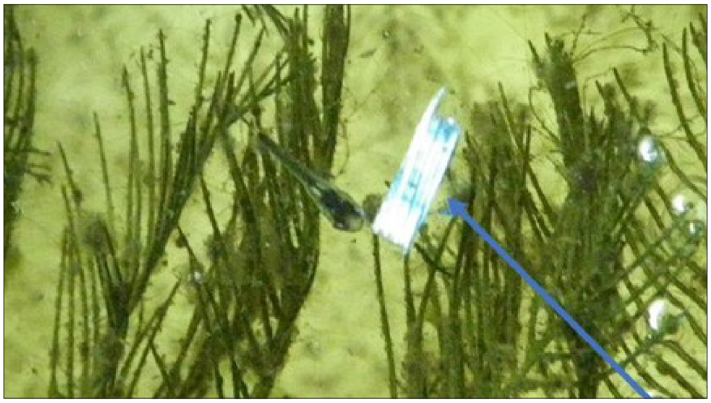

**Figure 5. A nearly week-old Starhead Topminnow fry next to a 5-mm float for scale.**

capture process. When confined in the bucket, the backswimmers could easily catch and kill the small Starhead Topminnows. NANFA member Charles Nunziata (2017 *American Currents* 42: 5–9) described the importance of careful and limited handling of Fundulidae species. He mentioned collecting the fish can be traumatic and expose them to infections. Respectful handling of the fish was always our priority, but we also learned to remove backswimmers immediately from the buckets of Starhead Topminnows that were destined to be stocked in the Wisconsin River.

## CLIMATE CHANGES EVERYTHING

Pond management included daily dissolved oxygen monitoring, aeration when needed, daily well pumping, and aquatic plant harvesting. This level of management was possible because Dave

**Figure 6. Jury-rigged system set up to catch escaping Starhead Topminnows, with Hatchery Manager Biscuit on duty.**

Marshall lives on the pond property. Beyond routine pond management, our original goal was to dewater the pond to remove all of the cultured fish and stock them by the end of the 2018, then restock the empty pond with wild fish the following spring. But thanks to the unexpected, we had to alter our plan by late summer due to a prolonged wet period. Continuous river flooding through October 2018 reduced stocking opportunities. And for the first time since the pond was constructed in 1995, late summer runoff water exceeded the pond capacity and led to a spillover. A metal screen had been installed in front of the outlet culvert to deter possible fish escape. As the pond reached its capacity, we added a small-mesh seine for additional outlet screening. On the morning after a September 5th storm, 73 fish somehow escaped through the culvert and ended up with nowhere to swim below the landlocked pond. We were able to save 47 fish found flopping in the grass and return them to the pond. The other 26 were found dead and were preserved for necropsy. During the storm, a 12-foot jon boat sat at the pond edge with its drain plug removed. After the pond level reached the drain, dozens of Starheads entered the boat. Perhaps these escape behaviors mimicked the migrations that occur along the Lower Wisconsin River during high river stages?

Throughout that fall we were able to keep all the fish in the pond by securely overlapping the small-mesh seine around the outlet culvert. As an additional response to the unexpected, we set up a jury-rigged system to capture fish alive in case of escape (Figure 6). From the initial 119 Starhead Topminnows introduced into the pond, we were able to stock 1,164 mostly YOY at two sites above the Prairie du Sac dam in 2018 (Figure 7). However, hundreds of topminnows remained in the pond that we were unable to stock in 2018. The next challenge was keeping them alive over the winter, something we hadn't planned for.

## YEARS 2 AND 3

Year 2, 2019, began with managing water supply and maintaining adequate dissolved oxygen levels in the pond over the winter. A few water-supply freeze-ups occurred in the shallowly buried pipe between the well's anti-siphon chamber (an air space to prevent backflow to the well) and pond. The buried pipe was replaced with an

**Figure 7. Starhead topminnows in transport cooler for stocking. (Photo by Wendy K. Weisensel)**

**Figure 8. We had to hire for snow removal after heavy snowstorms. This 9-inch snowstorm was way too much work for us senior-citizen biologists.**

overland pipe that can be detached and thawed if ice blocks water flow. Clearing snow off the pond was a priority to ensure light penetration and photosynthetic oxygen production (Figure 8). After one storm dumped 10 inches of snow on the pond, dissolved oxygen declined rapidly to 0.9 mg/l (Figure 3). The heavy snow also placed pressure on the ice. As holes were drilled for aeration, water rushed onto the ice and melted snow. We moved air stones around daily to distribute aeration and allow the open water to quickly freeze and create clear ice. The combination of snow melt and patches of clear ice, as opposed to cloudy white ice, increased light penetration, which then encouraged plant photosynthesis and increased dissolved oxygen (Prowse and Stephenson 1986). We only aerated when low dissolved oxygen threatened because continuous aeration in subzero conditions could quickly cool this shallow pond adding to ice thickness and reduced living space.

Ice-out occurred March 27, 2019, and by March 29 Starhead Topminnows were observed around the pond perimeter. Fish remained submerged and surface schooling did not begin until water temperatures reached 16.8° C (62.2° F) (Table 2). By early May, hundreds of Starhead Topminnows of varying sizes swam across the pond surface. We were able to stock 865 in the spring and another 858 in late summer. After the spring stocking, we added 15 more adults from the Lower Wisconsin State Riverway to increase genetic diversity and avoid genetic drift. We had intended to collect more fish, but spring flooding dispersed them, and we didn't find many.

We noticed one significant change in 2019 from previous years when the pond was fishless. The normally abundant zooplankton and phantom midge larvae had disappeared. We suspected that the Starhead Topminnows represented a form of biomanipulation

**Table 2. Notes on Starhead Topminnow behavior in Topminnow Pond**

| |
|---|
| Starhead Topminnow surface schooling declines significantly during spawning. |
| Spring surface schooling begins when water temperatures reach about 62° F. |
| Starhead Topminnows remained below the surface and inactive at night. The fish were not attracted to a night light. Only giant water bugs swarmed under a light hanging over the pond. |
| Giant water bugs and backswimmers pose a hazard if allowed in the same container with Starhead Topminnows. |
| Starheads were not attracted to aeration even when dissolved oxygen levels dropped to 1 ppm. |
| Seining is not recommended for Starhead Topminnow collection since they can be lost in dense clumps of plants and suffocate. |
| The female to male adult sex ratio (1.56:1) in Topminnow Pond was very similar to published data. |

that reduced the large-bodied zooplankton. This loss of zooplankton did not appear to affect Starhead growth and survival, perhaps due to the topminnow's omnivorous diet (Becker 1983). On top of that, we saw fewer backswimmers in the pond as well.

During the 2019 late summer stocking, we tried to collect mostly adults and larger juveniles. We thought that adult fish chances for survival in the wild were greater than the much smaller YOY fish that can be more vulnerable to predation. Smaller fish were certainly vulnerable to netting and far easier to capture than adults. We preferred to collect adults, but they avoided us by swimming to the middle of the pond as soon as we approached the bank. Their avoidance behavior reduced the total numbers of fish we hoped to stock in late summer 2019 and 2020.

Given our success with overwinter survival in 2018, we decided to overwinter YOY fish in the pond the next two years to increase growth and improve survival. We weren't confident about stocking the very small fish into new environments full of fish predators. Our surveys of Lower Wisconsin River Starhead Topminnows suggested annual population pulses with maximum numbers typically occurring in late summers following multiple clutches. We always observed far lower numbers each spring, suggesting predation or other forms of mortality over the winter.

We continued to remove rooted plants from the middle of the pond each year even though the environmental benefits remained dubious, given the Starhead Topminnow's tolerance of low dissolved oxygen. We felt that removing plants during the summer could reduce under-ice decomposition and oxygen depletion during the winter.

In Year 3, 2020, pond propagation continued, and we removed and stocked another 940 fish. We followed Covid-19 health precautions by wearing masks and maintaining social distancing. Those masks made it a lot harder to drink beer after we finished our work, but we managed. We tallied adults by sex and determined that the female to male ratio of stocked adults was 1.56:1, very close to the Taylor and Burr (1997) reported value. By the end of Year 3 we had stocked a total of 3,579 fish above the Prairie du Sac dam.


**Figure 9. Sue Marcquenski preparing aerated coolers and fry bags for Starhead Topminnow stocking above the Prairie du Sac dam. (Photo by Wendy K. Weisensel)**

## FISH HEALTH CERTIFICATE

As part of the Wisconsin Fish Farm License, a qualified fish health inspector must certify that fish stocked from hatcheries into the wild are healthy and do not pose a disease threat to other wild fish populations. Fortunately, our team includes a qualified fish disease specialist, Sue Marcquenski (Figure 9). Sue really loves this stuff. Who else has a Pirate Perch *Aphrododerus sayanus* liver parasite named after them (*Henneguya marcquenskiae*)? In addition to conducting annual health inspections for fish in the pond, Sue was able to perform necropsies on the few fish lost during stocking operations and the fish that died as part of the September 2018 pond overflow event. The fish lost during stocking events amounted to less than 1% of the total number of fish stocked.

As part of the health certification, live fish as well as preserved specimens were closely examined. Three annual health certificates were issued from 2018 through 2020 and each one stated that no signs of contagious or infectious disease were observed in a 60-fish sample from the pond. Using standard necropsy protocols for the fish that died due to flooding or during release gave us the opportunity to learn more about the anatomy and physiology of this state-endangered species. Fish were preserved in 10% formalin or frozen prior to necropsy. With technical help from the Wisconsin Veterinary Diagnostic Lab, preserved tissues were prepared for histopathology. This allowed us to examine internal organs at the cellular level and evaluate indicators of health such as gill quality, liver condition, ovary and testes development, amount of body fat, and presence of food in the gastrointestinal tract. The gills were generally parasite-free but were hyperplastic in some of the fish, with filament clubbing evident in some of the fish that died at the release sites. This gill pathology can occur when fish are exposed to very low dissolved oxygen levels or other water quality stresses. However, we don't know for sure what specifically caused this gill pathology in some of the Topminnow Pond Starheads. The species evolved to tolerate low dissolved oxygen and no wild fish gills were examined for comparison. Other notes of interest included degenerating livers in old adults and an astonishing observation of egg maturation in YOY fish as small as 22 mm. The frozen fish we preserved gave us the chance to learn more about growth rates, feeding activity, general gill quality, sex ratios, and fin quality.

## YEAR 4: 2021

The last year of the Starhead Topminnow conservation aquaculture program is underway. So far this winter the pond dissolved oxygen has been sustained near saturation and the ice has been mostly free of snow. After a recent seven-inch snowstorm, the dissolved oxygen dropped from 12.6 mg/l to 9.2 mg/l in just one day. The snow was removed right after the storm that evening and dissolved oxygen rebounded to 12.2 mg/l in less than 24 hours. We plan to stock all Starhead Topminnows from the pond this spring and conduct surveys to determine if we will meet our goal of establishing a population of state endangered Starhead Topminnows above the Prairie du Sac dam. Part 3 will present these findings.

## ACKNOWLEDGMENTS

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