Short-term stocking survival of yearling Muskellunge raised in a recirculating aquaculture system

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Jonathan R Meerbeek
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Table of Contents

Executive Summary

Culture of sportfish for wild stock enhancement via a recirculating aquaculture system (RAS) has large advantages over traditional flow-through culture systems and has increased in popularity amongst agencies that rear and stock sportfish. However, the methodology to maximize fish growth, condition, and health within a RAS system has not been explored for many sportfish nor have their overall contribution to the fishery been evaluated. We evaluated the short-term stocking survival of yearling Muskellunge *Esox masquinongy* raised in a RAS by implanting telemetry tags into 36 fish (mean total length = 419 mm) and tracking their movements, depth, and habitat usage for up to 158 days poststocking (DPS). We also evaluated if finishing (1-2 weeks prior to stocking) RAS Muskellunge yearlings in hatchery raceways via offering them either dry pellets or Fathead Minnows *Pimephales promelas* improved survival in the wild.

A presumptive diagnosis of *Aeromonas* based on RAS case history and clinical symptoms was reported for Muskellunge reared in the RAS system prior to transferring to the Spirit Lake Fish Hatchery for final grow-out. In addition, fish had moderate to severe fin erosion and outward signs of bacterial infection that contributed to poor health and quality of Muskellunge reared in the grow-out RAS system. Fish were treated and allowed to recover for 4-d prior to stocking. Fish appeared healthy and were stocked via hauling truck in Spirit Lake in late April 2020. All 36 fish were located alive one DPS within or near emerging Cattail (*Typha* spp.)/Bulrush (*Scirpus* spp.) stands. Between 2-21 DPS, 14 Muskellunge were consumed by either American White Pelicans *Pelecanus erythrorhynchos* or Great Blue Herons *Ardea herodias*. Piscivorous birds consumed or fatally injured 22 (61.1%) of the RAS yearling Muskellunge by 110 DPS. Fish (n = 4) and other non-avian terrestrial (n = 1) predators also consumed yearling RAS Muskellunge and two died due to the bacterial infection. Another five fish died of unknown reason > 57 DPS. Two (5.6%) fish survived ≥ 153 DPS and no differences were detected in the survival rate minnow or pellet-finished yearling Muskellunge.

Yearling RAS Muskellunge preferred shallow depths with silt-sand substrate and had high affinity to the Cattail/Bulrush stands up to 40 DPS. Habitat and depth usage after 40 DPS was more complex and varied by individual fish, but fish continued to maintain high affinity to aquatic vegetation, but most of these encounters were < 3.0 m. Timing (late April) and location (< 1.0 m water depth) of fish stocked, fish condition, water transparency (> 5.0 m), and preference of shallow water habitats likely contributed to the high rate of mortality of yearling RAS Muskellunge observed in this study.

Recommended best management practices from this research were as follows:

- The bacterial infection may have contributed to the poor initial survival (via observed bacterial infection mortalities and poor performance/predator avoidance) of stocked yearling RAS Muskellunge. For a meaningful evaluation of stocked yearling RAS Muskellunge survival in the wild, techniques and procedures used to maximize fish fin condition and improve fish health of fish raised in a RAS system need to be explored.
- The yearling Muskellunge raised in a RAS were considerably larger (mean TL = 383 mm for the 655 reared fish) than their traditionally raised counterparts stocked in May (mean TL = 320 mm). Research Study 7053 found significant differences in survival rate of traditional yearling Muskellunge stocked in Spirit Lake with larger fish surviving better. Muskellunge were a strong candidate for RAS culture and potential solutions to improve fish health and condition during future RAS culture experiments were identified.
- The final grow-out procedures used in this experiment (RAS-pellet; RAS-minnow) were found to be ineffective in providing fish with alternative foraging opportunities, likely due to the transfer of fish from a static to a dynamic temperature environment. Therefore, comparisons of survival and/or habitat preferences for these two groups during this experiment were not practical. Future final grow-out procedures should either be completed in the RAS system or delayed until water temperatures are conducive for fish foraging.
- Our results suggest that there is no practical size that yearling Muskellunge can be reared that will completely avoid potential mortality resulting from avian predation. Strong preference of yearling Muskellunge to shallow water habitats make them susceptible to avian predation for extended periods throughout the growing season. Techniques to reduce piscivorous bird mortality may include: avoid stocking during major piscivorous bird migration periods, stock fish offshore, scatter fish stockings around lake periphery to eliminate large concentrations of Muskellunge, and delay fish stocking until water transparency decreases.

- Our findings suggest that yearling RAS Muskellunge can survive up to 158 DPS, but overall survival was poor. Modifications to the RAS culture, finishing techniques, and stocking practices are warranted in future studies evaluating the yearling RAS Muskellunge product in Iowa.

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Introduction

Research Study 7053 recently identified that short-term (< 100 d) survival of pellet-started, minnow finished yearling Muskellunge *Esox masquinongy* stocked into Spirit Lake was significantly related to size at stocking and that larger fish are needed to produce consistent year classes. Subsequently, the Iowa DNR experimented with an additional 30-d grow-out period from May to June to improve yearling Muskellunge size and condition and found much improved short-term survival rates (Research Study 7053, unpublished data). However, the additional grow-out period necessary to achieve improved size and condition of yearling Muskellunge increases fish production costs, increases fish handling/transporting stress, requires valuable tank/pond space for both Muskellunge and Fathead Minnows *Pimephales promelas*, and increases the chances of fish contracting viral, bacterial, and/or protozoan pathogens. One solution to these problems may be use of recirculating aquaculture system (RAS) technology to raise yearling Muskellunge. The advantages of the RAS system over traditional single-pass culture systems are: 1) reduced water/chemicals; 2) self-cleaned by circular current; 3) reduced probability of fish mortality due to disease; 4) ability to maintain consistent water temperatures; 5) invasive species free; and 6) ability to grow fish through winter so they can be stocked in the spring at a larger size (Annual Assessment 7058). Although yearling Muskellunge have not been raised in a RAS system in Iowa previously, projected growth rates of RAS Muskellunge raised until April-May would exceed minimum size at stocking thresholds established in Research Study 7053 (e.g., ≥ 330 mm).

Yearling Muskellunge reared in a RAS system are fed a pellet-only diet and previous studies have observed poor survival of fall stocked pellet-only Muskellunge (Szendrey and Wahl 1995; Larscheid et al. 1999), thus leading to concerns of RAS fish survival and overall contribution to the fishery. Although these studies found poor survival of pellet-only Muskellunge, the size at time of stocking for pellet-only reared Muskellunge was substantially smaller (229-256 mm) than their minnow-only reared Muskellunge counterparts (≥ 256 mm), which experienced higher survival rates (Larscheid et al. 1999). Based off recent data collected from Research Study 7053, size at time of stocking was the most influential factor regarding Muskellunge stocking survival up to 100 d poststocking. Therefore, our primary objective was to evaluate the short-term (< 100 d) stocking survival of yearling Muskellunge raised in a RAS and stocked into Spirit Lake, Iowa. We also evaluated if finishing (1-2 weeks prior to stocking) RAS Muskellunge yearlings in hatchery raceways via offering them either dry pellets or Fathead Minnows improved survival in the wild. Our final objective was to evaluate the movement, depth, and habitat usage of RAS yearling Muskellunge during the 100 d stocking evaluation.

Methods

Muskellunge rearing - Broodstock Muskellunge were captured from Spirit Lake and East/West Okoboji lakes in early April 2019 via gill nets (1.8 m × 97.5 m × 6.35 cm bar mesh) and transported to the Spirit Lake Fish Hatchery where they were held in covered indoor concrete raceways (4.8 m × 1.3 m × 1.0 m). In mid-April, fish culturist spawned Muskellunge and fertilized eggs were placed in hatching jars until eggs hatched. Once the larvae absorbed their yolk sac, fish were transported to concrete hatchery raceways and fed a dry feed until they reached approximately 100 mm total length (TL). On July 18th 2019, 1,178 fingerling Muskellunge were transported to the Rathbun Fish Hatchery. From this point forward, fish were raised in a RAS system (*see* Annual Assessment 7058 - *Evaluation of recirculating aquaculture systems for sportfish culture* - for detailed information regarding RAS culture). Briefly, fingerling Muskellunge were reared in a larval RAS for 127 days. On November 20th 2019, fingerling Muskellunge (mean TL = 274.5 mm) were transported to the grow-out RAS. Fish were fed a dry pellet diet (Walleye Grower 9206; 3 mm and 4 mm; Skretting, Tooele, UT) throughout the RAS experiment. On April 15th 2020, yearling RAS Muskellunge were transported back to the Spirit Lake Fish Hatchery. At the hatchery, fish were separated into two concrete raceways with approximately equal number of fish per tank. Yearling Muskellunge were offered a dry pellet diet in one raceway (pellet finished) and Fathead Minnows in a second raceway (minnow finished) and reared for an additional 9 d.

Muskellunge transmitter implantation and Passive Integrated Transponder tagging - Prior to stocking (7-8 d), a representative subsample of yearling RAS Muskellunge were selected from each raceway (pellet finished; minnow finished). Fish were anesthetized with tricaine methanesulfonate (MS-222) by placing fish in a metal trough (1.5 m × 0.46 m × 0.30 m) filled with approximately 94.6 L of water containing 18 g of MS-222. Once immobilized, fish were examined for physical impairments (fin erosion, abrasions, eye or body deformities, etc.), measured for TL (mm), weighed to the nearest g, and a 12.5-mm Passive Integrated Transponder (PIT; Electronic Identification Devices, Ltd., Santa Barbara, California, Model FDX; 134.2 KHZ frequency) tag was implanted in the dorsal musculature. Each fish was then fitted with a radio tag (Advanced Telemetry Systems, Isanti, Minnesota; Model F1440; 2.1 g in water; 158-d battery life; 0.4-0.9% of body weight) following similar procedures as outlined in Ball and Weber (2015). More specifically, fish were placed ventral side up on a homemade surgery cradle (sponge mounted on plastic tote; Figure 1) and a plastic tube was placed in the mouth or on top of the isthmus (depending on fish TL). A water pump was used to recirculate a low-dose (< 10 g/L) of MS-222 laden water onto the gills of the fish to facilitate oxygen transfer and maintain immobilization of the fish. Prior to surgery, all surgical tools and the transmitter were placed in a chlorhexidine solution (2% Chlorhexidine Gluconate). A scalpel was used to make an initial incision (approximately 25 mm in length) anterior to the pelvic girdle. Then, an exit hole for the transmitter whip antenna was created by inserting a 16-gauge hypodermic needle into the incision and exiting anterior to the anus (Figure 2). The whip antenna was threaded through the 16-gauge hypodermic needle and the tag was carefully inserted into the fish. Size 4/0 nylon monofilament sutures (Scientify Global) were used to close the incision (2 to 3 sutures per incision; Figure 3). The incision was sprayed with iodine prior to placing the fish back into the raceway to recover. Initially, fish were implanted with transmitters 7-8 d prior to stocking. However, a presumptive diagnoses (based on fish and RAS case history and clinical symptoms; Alan Johnson, Iowa DNR; personal communication) of *Aeromonas* in conjunction with tagging stress caused mortality on 27 of the 40 initially tagged fish. Therefore, fish were treated by hatchery staff and PIT tagging and telemetry tagging was repeated when fish health improved (3-7 d prior to stocking). After fish were allowed to recover (3-8 d), fish were loaded onto a hatchery truck and transported 6.1 km to the stocking location (Hales Slough boat ramp; Spirit Lake). At the boat ramp, the hatchery truck backed up until sufficient water depth at the rear of the truck was obtained. Hatchery discharge lines were then opened, and yearling RAS Muskellunge were forced out of the truck via water pressure and stocked into the lake (Figure 4).

Radiotelemetry and survival - Telemetry began the day after stocking and was continued daily for 7 days. Telemetry was then conducted 1-2 times per week until transmitters had expired (158 d). Muskellunge tracking consisted of using an Advanced Telemetry Systems Model R4000 receiver connected to a three-element folding Yagi antenna. To improve Muskellunge detectability, an omnidirectional antenna (a 4.3 m aluminum rod with attached three-element folding Yagi) was used to locate Muskellunge while traversing the lake. More specifically, the worker set the receiver to user specific volume and gain (either maximum volume and gain or set at lower level to avoid interference) to scan for each tag frequency. Each tag frequency was scanned for 2 s before advancing to the next tag frequency. Tracking first consisted of slowly (< 16 kph) scanning the perimeter of the lake (antenna pointed to shore, approximately 100 m from shore) until a fish was detected. If a fish was not detected in near shore habitats during a tracking event, transects (typically 100-150 m apart aided via use of a Lowrance™ Gen 2 HDS-7 unit) were run in open water habitats encompassing the northeastern bay in Spirit Lake (hereafter referred to as Angler's Bay) and along the periphery (approximately 100 m from shore) of the lake with the antenna pointed towards offshore habitats. After detection, the direction of the tagged fish was determined by rotating the omnidirectional antenna until the signal was maximized. As the worker approached the tagged fish, the gain and volume was slowly reduced until the fish signal was faint. Then, a hand-held Yagi antenna was used to pinpoint the location of the fish by reducing the receiver gain to the lowest achievable setting, while maintaining strong signal strength. Fish locations were recorded with a handheld Garmin GPSMAP 78SC and waypoints were transferred using DNRGPS 6.0 software. On occasion, wind and weather conditions or vegetation density at the time of tracking were not conducive to obtaining exact fish locations. In these instances, fish locations were recorded to the nearest possible location (usually within < 20 m of fish location). At the office, daily and weekly movements were reviewed and fish that displayed no or little movement over three or more tracking events were identified. During the subsequent tracking event, attempts were made to force movement of these fish using a push pole, modified vegetation pole, or a pole with an attached magnetic strip. The fish was considered a mortality if no movement occurred or the tag was recovered and the data were reviewed to determine the fish's last live location (Wagner and Wahl 2011). In instances where fish movement ceased in water depths > 3.0 m, at least five consecutive tracking events with no movement were used to classify fish as a mortality. Due to the high detection rates observed in this study, survival throughout the experiment was simply expressed using the known ratio of live fish to those that died (verified mortality) or were assumed dead (non-verified mortality).

Movement, depth, and habitat usage - Once a fish was located, water depth (ft), and surface water temperature (°F) was recorded using a boat mounted Lowrance™ Gen 2 HDS-7 unit. Visual observations of habitat type (e.g., open water or edge habitat), substrate type (silt; silt/clay/little sand; mostly sand; gravel or rock), emergent/submergent vegetation percent (0%; 1-19%; 20-49%; ≥ 50%) coverage, emergent/submergent vegetation density (sparse or dense) and structure (wood laydowns, docks, weedline, large rock) were recorded. In addition, percent submergent vegetation biovolume (i.e., percent of water column with submersed vegetation at a given point) was estimated for fish that moved offshore by recording the height of submersed vegetation from the bottom via images provided by the Lowrance™ Gen 2 HDS-7 unit. Muskellunge minimum in water movement (m) between two consecutive locations was calculated using distance tools provided in Google Earth Pro V7.3 and minimum daily movement rates were calculated using this distance divided by the number of days lapsed between tracking events.

Results

Fish Health, radio tag implantation, and stocking - Visual inspections of the RAS fish upon arrival to the Spirit Lake Fish Hatchery on April 15th 2020 indicated moderate to severe fin erosion (Figure 5), particularly on the caudal, pelvic, and pectoral fins. However, fish health initially appeared normal (Figure 6). Twenty telemetry tags were implanted to RAS-minnow group the evening of April 16 and 20 telemetry tags were implanted to the RAS-minnow group on April 17. Moderate to severe fin erosion was noted on all fish and several fish had eye or body deformities (Figure 7). All but four of the RAS-minnow and nine of the RAS-pellet fish perished between April 16 and April 24 from these initial tagging's. An earlier presumptive diagnosis of *Aeromonas* likely caused additional fish stress and mortality of telemetry tagged fish (Alan Johnson, personal communication). Fish were treated by hatchery staff and telemetry tags were reinserted in four RAS-minnow Muskellunge on April 17 and 20 Muskellunge (10 RAS-minnow; 10 RAS-pellet) on April 21. Of these, only one fish died (RAS-minnow) on April 21. Therefore, a total of 17 RAS-minnow and 19 RAS-pellet yearling Muskellunge were effectively tagged via radio transmitters (Table 1). It was noted by hatchery staff that during the 9-d holding period RAS yearling Muskellunge did not attempt to strike Fathead Minnows nor consume pellet feed (Figure 8). Prior to stocking on April 24th, each fish was anesthetized (MS-222) and the tag incision and health of each fish were examined. All telemetry fish appeared healthy and were stocked at the Hales Slough boat ramp. The mean TL of stocked yearling RAS-minnow and RAS-pellet Muskellunge were similar (mean TL 419 mm) and lengths ranged from 372 to 473 mm (Table 1).

Radiotelemetry and survival up to 21 days poststocking (DPS) - All fish were located 1 DPS near the Hales Slough boat ramp and 14 of the 36 fish were visually observed. It was noted that the behavior (on top of water column or porpoising) or condition (coloration/visually appeared sick) of three of these fish was abnormal 1 DPS. Five RAS Muskellunge were known to be consumed by American White Pelicans *Pelecanus erythrorhynchos* by 2 DPS. By 21 DPS, a total of nine (25% of stocked fish) RAS Muskellunge (mean 413 mm; range 396-428 mm) were known to be consumed by American White Pelicans (Table 2). Four RAS yearling Muskellunge (mean TL 397 mm; range 372-422 mm) were found at a Great Blue Heron *Ardea herodias* rookery located approximately 14.5 km from stocking location and one RAS Muskellunge (432 mm) died from wounds sustained via a Great Blue Heron attack (a total of 5 or 13.9% died from Great Blue Herons by 21 DPS). Three RAS fish (mean TL 403 mm; range of 385-414 mm) died via fish (adult Walleye and Muskellunge) predation. Two of these tags were expelled by predators in dense submersed vegetation (Curlyleaf Pondweed *Potamogeton crispus* and/or Coontail *Ceratophyllum demersum*) stands (2.7-3.3 m deep) and one (1.222) was recovered in < 1.3 m water depth at the upper end of East Okoboji Lake near the Spirit Lake spillway outlet. Two fish were found dead near shore between 3 and 5 DPS and mortalities and observation during tracking events were consistent with those of fish that had died from bacterial infections at the hatchery. The fate of five RAS Muskellunge was unknown within the first 21 DPS (range of 1-20 DPS). Extensive tracking for these individual fish occurred throughout the first week(s) following disappearance and it was concluded that the movement and timing associated with no fish detections for four of these fish coincided with American White Pelican migrations and these fish were ultimately considered dead from avian predators (i.e., non-verified avian mortalities; Table 2). Fish 1.162 was considered a non-verified fish mortality based-off movement patterns of the fish observed during 14 DPS. Therefore, mortality of RAS yearling Muskellunge was 66.7% (24 of 36; 11 RAS-minnow; 13 RAS-pellet; mean TL = 413 mm; range = 372-457 mm) within the first 21 DPS.

*Radiotelemetry and survival 21 to 158 DPS - T*welve RAS yearling Muskellunge (6 RAS-minnow; 6 RAS-pellet; mean TL = 431 mm; range TL = 378-473) survived at least 56 DPS. Six fish died between 57-89 DPS by a variety of mortality sources. Fish 1.180 entered Sandbar Slough by 6 DPS, was alive on 56 DPS in the slough, but by 82 DPS the tag was located next to a rock where American White Pelicans frequently gathered (Figure 9). Fish 1.212 was located in Hales Slough 10 DPS. The slough was disconnected from the main lake by July and the fish was determined to be dead from unknown reasons by 82 DPS (Figure 10). Fish 1.452 was visually observed on May 24 2020 (30 DPS) and an anterior-dorsal head wound from a Great Blue Heron was noted. The fish went missing 56 DPS and the transmitter was later recovered in a Cattail (*Typha* spp.) clump in the extreme Northeast Bay (locally referred to as Little Angler's Bay) where Great Blue Herons were commonly observed feeding throughout the study at the Cattail-waterline interface (Figure 11). Fish 1.421 was located on the west-southwest side of Spirit Lake by 6 DPS, was visually observed 41 DPS; however the tag was recovered 68 DPS within the front door landscaping of a lake home (non-avian terrestrial predator; Figure 12). Fish 1.172 was visually observed during two tracking events < 7 DPS and on both occasions, the fish was noted as sick. The fish was declared dead by 68 DPS and the tag was recovered 112 DPS (Figure 13). Fish 1.343 was visually observed 41 DPS and displayed wounds consistent from a Great Blue Heron attack. The fish was later visually observed 68 DPS in shallow water near shore woody debris. By 82 DPS, the tag was located on shore near the water's edge, at a location where Great Blue Herons were frequently observed hunting, and was declared a Great Blue Heron mortality (Figure 14). Six fish were known to survive > 100 DPS. Of those, four died by 139 DPS. Fish 1.200 died between 103-109 DPS from unknown reasons (Figure 15). Fish 1.411 was visually observed 30 DPS and a Great Blue Heron wound/scar was observed near the head of the fish. During this tracking event, the reaction of the fish to stimuli was tested by using a push pole in attempt to frighten the fish. The fish was not responsive to external stimuli and required physically prodding to force it to move. The fish survived to 109 DPS, however, the tag was located on the shore near Great Blue Heron feces 116 DPS and died between 110-115 DPS (Figure 16). Fish 1.442 died between 117-123 DPS based on movements (unknown cause of mortality), however, when the tag was located with a magnet near shore 123 DPS, the tag had Zebra Mussels *Dreissena polymorpha* attached to it (Figure 17). Fish 1.393 was visually observed 41 DPS within < 0.5 m of the shore-water interface. During this tracking event, the reaction of the fish to stimuli was tested by slowly approaching the fish with the boat and a push pole. The fish did not react to stimuli and the worker was able to get within < 0.5 m of physically touching the fish. The fish died between 131 and 139 DPS from unknown reasons (Figure 18). Two fish (Fish 1.292; Fish 1.130) survived 151-158 DPS (Figure 19; Figure 20). Fish 1.292 was visually observed on 6 tracking events between 2-41 DPS, went missing between 83-125 DPS, and was later located in deeper water within Angler's Bay (126-153 DPS; Figure 19). Fish 1.130 was located in Sandbar Slough 7 DPS, remained in that location until at least 102 DPS. Between 103-123 DPS, the fish moved to Little Angler's Bay until the transmitter expired post-158 DPS (Figure 20).

*Radiotelemetry and survival to 158 DPS - C*umulatively, avian predators consumed or fatally injured 22 (22 of 36; 61.1%) RAS yearling Muskellunge. Of those, at least 10 were known to be consumed by American White Pelicans and Great Blue Herons consumed or fatally injured eight fish (four of the 22 fish were non-verified avian mortalities). Most of the observed avian mortality (18 of the 22) occurred within the first three weeks poststocking, however, avian predators consumed RAS yearling Muskellunge up to at least 110 DPS. All verified (n = 3) and non-verified (n = 1) fish predator mortality (4 of 36; 11.1%) and mortality resulting from bacterial infection (n =2; 5.6%) occurred within the first three weeks poststocking. One fish (2.8%) was consumed by a non-avian terrestrial predator between 42-68 DPS. Verified unknown mortalities occurred for five RAS yearling Muskellunge between 57-131 DPS (13.9%; Table 1). Therefore, cumulative survival to 158 DPS was 5.6% (2 of 36; 1 of 17 RAS-minnow [5.9%]; 1 of 19 RAS-pellet [5.3%]).

Movement and depth - Individual yearling RAS Muskellunge mean minimum daily movements ranged from 28.9 m (Fish 1.172) to 290.5 m (Fish 1.222) and minimum daily movements as little as 0.3 m (Fish 1.150) and as much as 2,409.2 m (Fish 1.421) were observed (Table 3). Collectively (i.e., all live fish encounters combined), mean daily movement one DPS was 172.9 m (95% CI = 120.5-225.3) and remained > 80 m (range of 81.4 to 110.3) up to four DPS (Figure 22). A strong northeast wind at five DPS did not allow for tracking to be conducted on that day. By six DPS, mean daily movement was the highest (377.2 m; 95% CI = 155.2-599.2) observed compared to any other day or period tracked (≤ 115.8 m). Mean daily movement decreased substantially (≤ 19.1 m) between 30-41 DPS (5/24/2020 - 6/4/2020), increased at 82-95 DPS (7/15/2020 - 7/28/2020), and was ≤ 59.4 m after 100 DPS (8/2/2020; Figure 22). Trends in mean daily movements between yearling RAS Muskellunge consumed by avian predators (verified and non-verified) and those known to live at least 56 DPS were similar; however, those that were not consumed by avian predators typically moved more earlier (i.e., first week poststocking) and occupied deeper habitats after 60 DPS (Figure 23; Figure 24). More specifically, the mean daily movement from four DPS to six DPS was 213.9 m for those consumed by avian predators and 686.3 m for those that lived at least 56 DPS (Figure 23; Figure 24). Individual yearling RAS Muskellunge mean depth ranged from 0.38 m (Fish 1.272) to 2.18 m (Fish 1.292) and depths as shallow as 0.15 m (multiple fish) and as deep as 5.73 m (Fish 1.292) were observed (Table 3). Collectively, mean depths were ≤ 2.1 m throughout 109 DPS (Figure 22). Mean depth of live encounters of yearling RAS Muskellunge between 116 and 158 DPS were between 2.7-3.7 m (Figure 22).

Habitat usage - Yearling RAS Muskellunge nearly exclusively were found either within or near the emerging Cattail spp. and Bulrush spp. (*Scirpus* spp.) stands that encompass the fringe of shoreline east and west of the Hales Slough boat ramp between one and four DPS (Table 4; Figure 21). The substrates in these areas were predominately silt-sand (Table 5). Most fish were associated with aquatic vegetation (1-19% coverage), but some fish did utilize near shoreline habitat such as woody debris or rock (Table 4, Table 5). By six DPS, 77% of contacts were in the Cattail/Bulrush stand(s) and fish used a more diverse range of habitat types (dock-rock; rock; wood; wood-rock; no physical habitat; Table 4; Table 5). From seven to 41 DPS, between 50.0 and 76.2% of occurred in the Cattail/Bulrush stands (Figure 21). During this period, fish were often associated with some form of aquatic vegetation, but also used a variety of other near-shore habitat (Table 4; Table 5). A declining trend in Cattail/Bulrush usage was observed from 48-95 DPS, with no fish using this habitat type ≥ 102 DPS (Figure 21). In addition, fish were progressively more associated with offshore aquatic vegetation and dock habitats during this period (Table 4; Table 5).

Discussion

Culture of sportfish for wild stock enhancement via a recirculating aquaculture system has large advantages over traditional flow-through culture systems and has increased in popularity amongst agencies that rear and stock sportfish. However, the methodology to maximize fish growth, condition, and health within a RAS system has not been explored for many sportfish nor have their overall contribution to the fishery been evaluated. Annual Assessment 7058 found that survival was high and fin erosion was low for Muskellunge reared in a larval RAS system from 100 mm to 275 mm; however, once fish were moved to the grow-out RAS system, survival decreased due to cannibalism and fin condition progressively deteriorated (Annual Assessment 7058). In addition, a presumptive outbreak of *Aeromonas* prior to transferring to the Spirit Lake Fish Hatchery for final grow-out (i.e., RAS-pellet; RAS-minnow) substantially impacted the overall health and quality of Muskellunge reared in a RAS. Upon arrival to the Spirit Lake Fish Hatchery, yearling RAS Muskellunge appeared healthy, however, it became quickly apparent that an internal bacterial pathogen was contributing to increased stress and decreased survival of telemetry tagged individuals as many of the fish died within one day post-tagging. Once fish were treated and appeared healthy (4/21/2020), a subsample of 50 fish were PIT tagged in addition to the 20 fish that were implanted with both PIT and telemetry tags to observe their health after subsequent handling/tagging stress. All PIT tagged fish survived the three day holding period prior to stocking and only one of the telemetry tagged fish died. Therefore, it was concluded that fish health was much improved, however, based on telemetry findings poststocking, the bacterial infection may have contributed to the poor initial survival (via observed bacterial infection mortalities and poor performance/predator avoidance) of stocked yearling RAS Muskellunge. For a meaningful evaluation of stocked yearling RAS Muskellunge survival in the wild, techniques and procedures used to maximize fish fin condition and improve health need to be explored. Annual Assessment 7058 found that Muskellunge were a strong candidate for RAS culture and their experimentations have identified potential solutions to improve fish health and condition during future RAS culture experiments. In addition to these improvements, the final grow-out procedures used in this experiment (RAS-pellet; RAS-minnow) were found to be ineffective in providing fish with alternative foraging opportunities, likely due to the transfer of fish from a static to a dynamic temperature environment. Therefore, comparisons of survival and/or habitat preferences for these two groups during this experiment were not practical. Future final grow-out procedures should either be completed in the RAS system or delayed until water temperatures are conducive for fish foraging.

Despite the poor fin condition and bacterial infection, the yearling Muskellunge raised in a RAS were considerably larger (mean TL = 383 mm for the 655 reared fish; mean of 419 mm for the telemetry tagged fish) than their traditionally raised counterparts stocked in May (mean TL ~ 320 mm). Research Study 7053 identified that size at stocking was the single most important variable influencing the survival of traditional reared Muskellunge (pellet-started, minnow-finished spring stocked yearlings) and that an additional 30 d grow-out period may improve survival rates of inadequate (< 330 mm) sized fish by 30% or more. Based off total length alone, the probability of survival from TLs of RAS telemetry tagged fish was >99% using the probability curve developed from Research Study 7053. However, as previously identified, total length as a predictor of survival in this experiment may have been compromised by factors independent of TL.

Short-term telemetry evaluations provide critical information regarding stocking success and provide managers with immediate results on the factors that may be contributing to bottlenecks that prevent establishment or maintenance of successful fisheries. In this study, all fish were located alive one DPS within or near the fringe of emerging Cattail/Bulrush stands that encompass the bay where Muskellunge were stocked. Movement rates the first few days poststocking were consistent with those observed during yearling Muskellunge telemetry evaluations where 100-d survival rates were exceptional (Research Study 7053; unpublished data). However, by two DPS, five Muskellunge were known to be consumed by American White Pelicans and an additional five were later known to be consumed by American White Pelicans. The timing of stocking during this evaluation coincided with migration of American White Pelicans and the combination of fish condition, stocking location (< 1.0 m), and water transparency (> 5.0 m) may have contributed to the high rate of mortality resulting from American White Pelican consumption (38.9%; 10 confirmed; 4 non-verified) observed here. Although it is not uncommon for American White Pelicans to consume large quantities of fish (Graham et al. 2019), the TL of RAS yearling Muskellunge were thought to be large enough to avoid most avian predation. However, our results suggest that there is no practical size that yearling Muskellunge can be reared that will completely avoid potential mortality resulting from avian predation. For example, during our tracking event on two DPS, a transmitter (fish 1.381; Appendix 1) was located in an American White Pelican near the entrance of Trickles Slough. The American White Pelican was approached and the bird flew for approximately 50 m and landed. It repeated this process during three consecutive approaches. Eventually the bird was able to leave and the location of the bird where it took off was examined due to the presence of a radio tag signal. Five regurgitated yearling RAS Muskellunge were found at this location (including the telemetry tagged fish) ranging from 368-394 mm (Appendix 1). Although previous telemetry studies conducted in Spirit Lake have never documented yearling Muskellunge mortality resulting from American White Pelican consumption, it has been speculated that fish of unknown fate may have been consumed by American White Pelicans and deposited elsewhere (Research Study 7053; unpublished data). Nevertheless, American White Pelicans are opportunistic piscivores that prey on a variety of popular sportfish and their range and population abundance has increased substantially (King and Anderson 2005), causing a growing concern for fisheries managers.

Other piscivorous birds have also been documented to have substantial impacts on poststocking Muskellunge survival (Warren 2013; Owensby et al. 2017; Weber and Weber 2020). In particular, Great Blue Herons have been the most frequently documented avian predator for recently stocked juvenile Muskellunge in Iowa and elsewhere. Eight of the 36 (22%) yearling Muskellunge in this study were consumed or fatally injured by Great Blue Herons. In two central Iowa reservoirs, 13.2% (8 of 61) stocked yearling Muskellunge were confirmed to be consumed by Great Blue Herons (Weber and Weber 2020), whereas 8% were consumed by Great Blue Herons in a Tennessee river (Warren 2013). In recent studies evaluating poststocking survival of traditionally reared yearling Muskellunge (pellet minnow-finished) stocked in Spirit Lake, 10% (4 of 40; 320-338 mm) of radio transmitter fish stocked in May 2017 were known to be consumed by Great Blue Herons (Research Study 7053). In 2018 and 2019, small (< 330 mm) yearling Muskellunge held for an additional 30-d grow-out period and stocked in late June in Spirit Lake (Hales Slough boat ramp) had high (≥ 75%) 100-d poststocking survival and negligible mortality resulting from avian predation (3.3%; 1 of 30). Initially, improved survival was attributed to their improved size and condition since most published and observed documentation of Muskellunge poststocking mortality via Great Blue Heron consumption/attacks predominately occurred on small (< 330 mm) fish (Warren 2013; Owensby et al. 2017; Research Study 7053). However, the results from this study demonstrated that Great Blue Herons were effective at consuming or fatally injuring yearling Muskellunge up to 454 mm. Four of the 10 yearling RAS Muskellunge consumed were found approximately 14.5 km from Spirit Lake at a Great Blue Heron rookery and their TLs at time of stocking ranged from 372-422 mm.

In 2020, a side-by-side comparison of hatchery products was conducted to provide more insight on yearling Muskellunge short-term survival. More specifically, in addition to the RAS culture and poststocking survival study, a subsample of traditional yearling Muskellunge sorted by TL (> 330 mm) and stocked in May (direct-large cohort) and a subsample of small (< 330 mm) traditional yearling Muskellunge raised for an additional 32 d and stocked in late June (grow-out cohort) were fitted with radio transmitters and their short-term poststocking survival was evaluated (see Research Study 7053 2021 annual report). Great Blue Herons consumed 47% of the direct-large telemetry group (338-355 mm) by 13 DPS and 18.2% of grow-out telemetry tagged fish by 7 DPS (Research Study 7053; no fish were consumed by American White Pelicans). Cumulatively, in 2020, Great Blue Herons consumed 22 of the 75 (29.3%) telemetry tagged yearling Muskellunge stocked in Spirit Lake and many of these (n = 16) were later located at the rookery located 14.5 km from stocking location. Hodgens et al. (2004) estimated that nearly 50,000 catchable-sized Rainbow Trout *Oncorhynchus mykiss* per year were consumed annually by a relatively small (n =227) Great Blue Heron population in an Arkansas tailwater. Although estimating the adult Great Blue Heron population at the rookery was outside of the scope of this project, it was not uncommon to encounter 50-75 adults during tag recovery attempts at the rookery. Predation of yearling Muskellunge by Great Blue Herons may be a learned behavior during nesting and prior to young fledging the nest as predation from Great Blue Herons declined substantially after the initial one to three weeks poststocking and as water transparency decreased (< 2.8 m). It is hypothesized that habitat preference of yearling Muskellunge up to 40 DPS (e.g., affinity to Cattail/Bulrush habitat) and water clarity (> 5 m) facilitated exceptional foraging efficiency via piscivorous birds in 2020. This observation was partially supported by examining movement, depth usage, and habitat preferences of yearling RAS Muskellunge consumed by piscivorous birds and those that survived at least 56 DPS. Those fish that were eventually consumed by avian predators on average moved less and preferred shallow water within the Cattail/Bulrush habitat.

Several other sources of yearling RAS Muskellunge mortality were identified during the 158-d evaluation. Fish predators (e.g., Walleye *Sander vitreus* and Muskellunge) were known to consume four yearling RAS Muskellunge (385-414 mm; one non-verified) within the first three weeks poststocking. No other yearling Muskellunge mortalities from fish predation were known to occur, although several fish were declared mortalities via unknown reasons after 100 DPS. These mortalities were consistent with fish predator mortalities based off tag deposition in deeper water habitats. One fish was consumed by a non-avian terrestrial predator, as evidenced by the tag deposition and fraying of the antenna. Bacterial infection was known to cause acute mortality of two yearling RAS Muskellunge within 5 DPS, but it is unknown how long the bacterial infection persisted or if those that died after 56 DPS were result of chronic bacterial disease, fish predation (deposited tags), inefficiency to convert to natural forage, or a combination of the three variables. In a controlled experiment, European Perch *Perca fluviatilis* infected with *Aeromonas sobria* experienced higher initial mortality rates up to 8 d post-infection and cumulative mortality rates up to 70% at 30 day post-infection (Wahli et al. 2005). Therefore, it is possible that the yearling Muskellunge mortality observed in this study post-56 DPS was a result of this infection or a cumulative effect that weakened the fish enough to allow for other pathogens and/or proper foraging and behavior to be modified. Although significant mortality was observed throughout the study period, two yearling Muskellunge of contrasting size (396 and 473 mm) and movement behaviors (Figure 19; Figure 20) were known to survive up to 153 DPS. In addition to those two fish, at least one other stocked RAS fish was known to survive 167 DPS based off angler recapture information. This fish was stocked at 400 mm and was approximately 450 mm upon recapture. These results suggest that Muskellunge raised in a RAS can survive and contribute to the Muskellunge fishery, despite being raised on a pelleted diet. Others that have examined poststocking survival of fingerling Muskellunge reared on a pellet-only died either found 0% contribution or that pellet-only fish were naïve and more vulnerable to predation and this practice has largely been disregarded as a rearing practice for Muskellunge (Szendrey and Wahl 1995; Larscheid et al. 1999). Although RAS yearling Muskellunge survival in this study was also very low, it is unknown what survival rates may have been given more favorable health, condition, and stocking scenario.

As previously noted, yearling Muskellunge in this study preferred shallow depths and had high affinity to the Cattail/Bulrush stands up to 40 DPS, thus the substrate frequently utilized and percent vegetation coverage was consistent with this habitat type during spring (silt-sand; 1-19% coverage). Habitat and depth usage after 40 DPS was more complex and varied by individual fish, but fish continued to maintain high affinity to aquatic vegetation throughout the study. During the first three weeks poststocking, all live fish encounters were at depths < 2.0 m. Fish were observed in < 2.0 m during 86% of encounters 56 DPS, but some fish preferred deeper water habitats. Only four fish contacts (< 2%) were made where fish used depths > 4.0 m. Several other studies have found that age-0 Muskellunge prefer shallow water habitats (Hanson and Margenau 1992; Craig and Black 1986; Farrell and Werner 1999; Owensby et al. 2017). Limited information is available on yearling Muskellunge habitat preferences, but studies conducted in Iowa's reservoirs and natural lakes found that stocked yearling Muskellunge also prefer shallow (< 3.0 m) water habitats consisting of aquatic vegetation and/or coarse woody habitat (this study, Research Study 7053; Weber and Weber 2020).

To our knowledge, this is the first study that has evaluated poststocking survival of Muskellunge cultured within a recirculating aquaculture system and stocked into a natural lake. Others have used similar aquaculture systems to cold-bank juvenile Walleye (held overwinter to be stocked in spring) and observed accelerated growth in recaptured individuals 2 and 3 years poststocking in an 8-ha lake (Harder et al. 2013). However, this, nor any other RAS experiment, to our knowledge, evaluated poststocking survival rates, thus it is relatively unknown if sportfish cultured or finished via these techniques contribute to wild populations. Our findings suggest that yearling RAS Muskellunge can survive up to 158 DPS, but overall survival was poor. We also found no evidence that Muskellunge finished 9 d via pellets or Fathead Minnows had improved survival. Modifications to the RAS culture, finishing techniques, and stocking practices are warranted in future studies evaluating the yearling RAS Muskellunge product in Iowa.

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Tables and Figures

Table 1. Number, mean total length (TL), and stocking technique of yearling Muskellunge raised in a recirculating aquaculture system (RAS) and stocked in Spirit Lake (Hales Slough access) on April 24, 2020.

Group	N Tagged	Mean TL	Min TL	Max TL	SE
RAS-minnow	17	419	372	473	6.5
RAS-pellet	19	419	378	457	4.8

Table 2. Fish telemetry tag identification (Fish ID), technique used to finish yearling Muskellunge raised in a recirculating aquaculture system (RAS; RAS-pellet finished; RAS-minnow finished), status (alive or dead), number (N) of days known to survive, number of times fish was detected with telemetry equipment, and fish mortality comments (verified = observed mortality source and/or recovered tag; non-verified = suspected mortality based off tracking data and personal observation) from 36 radio tagged yearling Muskellunge stocked into Spirit Lake on April 24 and tracked to September 29, 2020.

Fish ID	Group	TL	Wt	Status	N Days Known to Survive	N Detections	Mortality Comments
1.112	RAS - Pellet	449	500	Dead	4	4	non-verified avian mortality
1.120	RAS - Pellet	393	324	Dead	1	1	non-verified avian mortality
1.130	RAS - Pellet	396	366	Alive	158	18	
1.140	RAS - Minnow	411	396	Dead	20	10	non-verified avian mortality
1.150	RAS - Pellet	402	330	Dead	10	7	Verified Pelican Mortality
1.162	RAS - Minnow	400	318	Dead	10	8	non-verified fish mortality
1.172	RAS - Pellet	423	348	Dead	61	18	Verified Unknown Mortality
1.180	RAS - Minnow	430	398	Dead	56	10	Verified Pelican Mortality
1.190	RAS - Minnow	395	298	Dead	13	8	Verified Heron Mortality
1.200	RAS - Minnow	424	427	Dead	102	24	Verified Unknown Mortality
1.212	RAS - Minnow	435	424	Dead	56	12	Verified Unknown Mortality
1.222	RAS - Minnow	414	364	Dead	18	9	Verified Fish mortality
1.230	RAS - Minnow	400	306	Dead	18	9	Verified Heron Mortality
1.240	RAS - Minnow	372	298	Dead	7	6	Verified Heron Mortality
1.252	RAS - Minnow	385	316	Dead	7	6	Verified Fish mortality
1.272	RAS - Minnow	404	345	Dead	2	2	Verified Pelican Mortality
1.292	RAS - Minnow	473	532	Alive	153	25	
1.311	RAS - Pellet	422	374	Dead	1	1	Verified Pelican Mortality
1.322	RAS - Pellet	420	400	Dead	1	1	Verified bacterial infection mortality
1.330	RAS - Minnow	418	374	Dead	2	2	Verified Pelican Mortality
1.343	RAS - Pellet	426	432	Dead	74	20	Verified Heron Mortality
1.352	RAS - Pellet	428	410	Dead	18	9	Verified Pelican Mortality
1.360	RAS - Pellet	414	357	Dead	1	1	Verified Pelican Mortality
1.373	RAS - Pellet	457	524	Dead	13	8	non-verified avian mortality
1.381	RAS - Pellet	396	360	Dead	1	1	Verified Pelican Mortality
1.393	RAS - Pellet	426	468	Dead	130	26	Verified Unknown Mortality
1.401	RAS - Pellet	417	378	Dead	1	1	Verified Pelican Mortality
1.411	RAS - Minnow	439	442	Dead	109	25	Verified Heron Mortality
1.421	RAS - Pellet	378	250	Dead	61	16	Verified non-avian terrestrial mortality
1.442	RAS - Minnow	472	516	Dead	116	24	Verified Unknown Mortality
1.452	RAS - Pellet	454	486	Dead	56	17	Verified Heron Mortality
1.462	RAS - Pellet	432	480	Dead	6	5	Verified Heron Mortality
1.473	RAS - Minnow	422	370	Dead	20	10	Verified Heron Mortality
1.482	RAS - Pellet	420	376	Dead	1	1	Verified Pelican Mortality
1.491	RAS - Pellet	411	374	Dead	4	4	Verified Fish mortality
1.502	RAS - Minnow	422	386	Dead	4	4	Verified bacterial infection mortality

Table 3. Minimum daily movement (m, n = number of times located; SE = standard error; min = minimum; max = maximum; CI = confidence interval) and depth (m) statistics for live encounters of yearling Muskellunge (Fish ID = fish telemetry tag identification number) raised in a recirculating aquaculture system and stocked in Spirit Lake, April 24 to September 29, 2020.

Fish ID	Minimum Daily Movement					Depth				
	n	Mean (SE)	Min	Max	95% CI	n	Mean (SE)	Min	Max	95% CI
1.112	4	50.6(4.6)	20.6	84.0	18.4-82.7	4	0.66(0.11)	0.46	0.98	0.44-0.87
1.120	1	83.7	83.7	83.7		1	1.22	1.22	1.22	
1.130	17	77.3(16.5)	1.6	570.3	8.2-146.4	16	0.82(0.08)	0.15	1.37	0.66-0.98
1.140	9	69.2(8.4)	7.0	188.5	23.6-114.8	10	0.96(0.10)	0.46	1.43	0.77-1.16
1.150	7	57.3(6.7)	0.3	159.8	19.6-95.0	7	0.56(0.15)	0.15	1.07	0.27-0.84
1.162	7	96.3(6.9)	47.6	243.5	45.8-146.8	7	1.30(0.13)	0.61	1.68	1.04-1.56
1.172	18	28.9(7.0)	0.6	116.4	11.5-46.3	17	1.18(0.08)	0.46	1.52	1.03-1.33
1.180	10	113.8(11.2)	7.7	320.3	39.6-187.9	9	0.94(0.10)	0.61	1.46	0.74-1.14
1.190	8	37.7(5.0)	2.4	89.7	16.5-58.9	8	0.77(0.11)	0.15	1.16	0.56-0.98
1.200	24	37.1(6.6)	1.8	154.5	20.9-53.3	24	1.22(0.13)	0.30	2.56	0.97-1.47
1.212	12	81.4(12.8)	0.4	348.0	16.3-146.5	10	0.67(0.11)	0.15	1.22	0.46-0.88
1.222	8	290.5(11.9)	45.0	537.3	149.7-431.3	8	0.53(0.16)	0.15	1.25	0.23-0.84
1.230	9	74.7(11.7)	9.9	320.1	8.6-140.9	9	1.03(0.14)	0.30	1.65	0.76-1.30
1.240	6	44.6(3.6)	13.6	73.8	25.4-63.9	6	0.53(0.20)	0.15	1.49	0.13-0.93
1.252	6	95.9(6.7)	26.7	190.5	43.3-148.4	6	0.70(0.07)	0.46	0.91	0.56-0.84
1.272	2	146.0(13.9)	27.4	264.5	0.0-378.3	2	0.38(0.23)	0.15	0.61	0.00-0.83
1.292	25	59.4(12.1)	3.1	482.7	22.9-95.9	25	2.18(0.33)	0.15	5.73	1.53-2.83
1.311	1	132.0	132.0	132.0		1	1.31	1.31	1.31	
1.322	1	335.6	335.6	335.6		1	0.15	0.15	0.15	
1.330	2	71.9(5.7)	37.8	106.0	5.1-138.7	2	1.39(0.05)	1.34	1.43	1.30-1.48
1.343	20	49.9(9.8)	1.5	244.9	19.7-80.1	20	0.92(0.07)	0.15	1.52	0.79-1.06
1.352	9	97.8(9.1)	8.8	279.4	38.9-156.7	9	0.81(0.11)	0.49	1.52	0.60-1.02
1.360	1	635.7	635.7	635.7		1	0.91	0.91	0.91	
1.373	8	176.1(19.5)	9.7	735.7	0.0-355.2	8	0.96(0.21)	0.30	1.86	0.54-1.38
1.381	1	97.1	97.1	97.1		1	1.28	1.28	1.28	
1.393	26	142.7(20.2)	1.4	1,043.9	50.0-235.4	24	1.52(0.26)	0.15	3.75	1.00-2.04
1.401	1	72.6	72.6	72.6		1	0.4	0.40	0.40	
1.411	25	63.7(8.3)	0.4	233.3	37.6-89.8	25	1.11(0.06)	0.61	1.55	0.99-1.22
1.421	16	194.1(42.5)	0.8	2,409.2	0.0-484.5	16	0.59(0.12)	0.15	2.01	0.34-0.83
1.442	24	86.5(16.5)	3.5	765.3	25-147.9	24	1.27(0.22)	0.15	3.02	0.83-1.71
1.452	17	85.3(9.3)	2.1	297.4	44.4-126.3	17	1.06(0.14)	0.15	2.90	0.78-1.34
1.462	5	159.8(13.6)	40.2	457.4	9.1-310.4	5	1.10(0.14)	0.79	1.49	0.82-1.38
1.473	10	77.1(9.0)	11.6	272.4	28.3-126	10	0.83(0.14)	0.15	1.49	0.55-1.10
1.482	1	366.0	366.0	366.0		1	1.25	1.25	1.25	
1.491	4	86.6(11.0)	4.5	232.9	0.0-187	4	0.92(0.26)	0.30	1.37	0.41-1.43
1.502	4	245.4(5.4)	178.9	368.3	163.1-327.7	4	1.03(0.20)	0.79	1.62	0.64-1.41
Total	349	93.5(18.5)	0.3	2,409.2	74.7-112.4	343	1.08(0.04)	0.15	5.73	1.00-1.17

Table 4. Habitat characteristics (vegetation (veg) = submergent and/or emergent stands) where yearling Muskellunge raised in a recirculating aquaculture system and stocking into Spirit Lake were located between 1 and 158 days poststocking.

DPS	Rock	Veg	Veg-Rock	Dock	Dock-Rock	Dock-Veg	Dock-Veg-Rock	Wood	Wood-Rock	Wood -Veg	No physical Habitat
1	1(2.8)	30(83.3)						2(5.6)		2(5.6)	1(2.8)
2	1(3.4)	24(82.8)						1(3.4)		1(3.4)	2(6.9)
3		27(100)									
4		25(96.2)								1(3.8)	
6	1(4.5)	14(63.6)			4(18.2)			1(4.5)	1(4.5)		1(4.5)
7	1(4.8)	13(61.9)			4(19)			2(9.5)			
10-13	1(2.7)	27(73)		1(2.7)	5(13.5)			1(2.7)			1(2.7)
18-20	3(10.7)	21(75)			4(14.3)						
25-26	1(8.3)	9(75)		1(8.3)	1(8.3)						
30-33		15(83.3)		1(5.6)	2(11.1)						
38-41	1(5.6)	15(83.3)		1(5.6)	1(5.6)						
48-49		6(66.7)		2(22.2)							
55-56		9(75)		2(16.7)	1(8.3)						
61		6(75)		1(12.5)	1(12.5)						
68-70		4(66.7)		1(16.7)					1(16.7)		
74-77		5(83.3)				1(16.7)					
82		5(83.3)					1(16.7)				
89		4(100)									
95		3(75)				1(25)					
102		4(80)	1(20)								
109		2(66.7)				1(33.3)					
116		2(100)									
123		1(100)									
130-133		3(100)									
139-145		2(50)									1(25)
151-158		2(66.7)									1(33.3)
Total	10(2.9)	278(79.4)	1(0.3)	10(2.9)	23(6.6)	3(0.9)	1(0.3)	7(2)	2(0.6)	4(1.1)	7(2)

Table 5. Frequency (percentage in parentheses) of yearling Muskellunge raised in a recirculating aquaculture system and stocked in Spirit Lake and encountered in various densities of aquatic vegetation (of the fish found in or near aquatic vegetation; 0% = associated with vegetation stand but not within; 1-19%, 20-49%, or >49% vegetation) and substrate (all fish; soft-silt, silt-sand, or hard-gravel/rock substrates) between 1 and 158 days poststocking.

DPS	Vegetation Cover				Substrate		
	0%	1-19%	20-49%	> 49%	Soft-Silt	Silt-Sand	Hard-Gravel/Rock
1	2(6.3)	27(84.4)	3(9.4)		1(2.8)	34(94.4)	1(2.8)
2	2(8.0)	23(92.0)				27(93.1)	2(6.9)
3		27(100)				26(96.3)	1(3.7)
4	1(3.8)	25(96.2)				25(96.2)	1(3.8)
6		14(100)				16(72.7)	6(27.3)
7		13(100)				16(76.2)	5(23.8)
10-13		24(88.9)		3(11.1)	3(8.1)	24(64.9)	10(27.0)
18-20		18(85.7)	2(9.5)	1(4.8)	3(10.7)	18(64.3)	7(25.0)
25-26		6(66.7)		3(33.3)	2(18.2)	7(63.6)	2(18.2)
30-33		11(73.3)	4(26.7)		3(16.7)	13(72.2)	2(11.1)
38-41		12(80.0)		3(20.0)	3(16.7)	13(72.2)	2(11.1)
48-49		6(100)				8(100)	
55-56		6(66.7)	2(22.2)	1(11.1)	2(22.2)	5(55.6)	2(22.2)
61		3(50.0)	3(50.0)			5(83.3)	1(16.7)
68-70			4(100)			1(50.0)	1(50.0)
74-77			6(100)				
82			6(100)				
89			4(100)				
95		2(50.0)	2(50.0)				
102			5(100)				
109		1(33.3)	2(66.7)				
116		1(50.0)	1(50.0)				
123			1(100)				
130-133			3(100)				
139-145			2(100)				
151-158			2(100)				
Total	5(1.7)	219(76.3)	52(18.1)	11(3.8)	17(5.5)	249(80.6)	43(13.9)

Figure 1. Homemade surgery cradle and water pump system used to internally implant radio tags into yearling Muskellunge raised in a recirculating aquaculture system, April 2020.

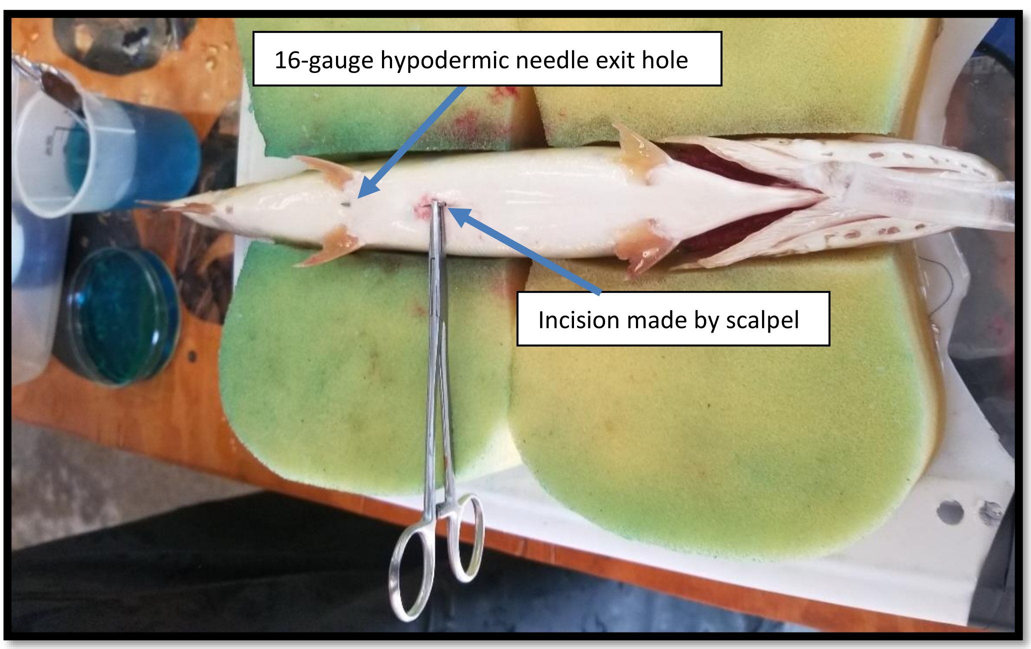


Figure 2. Location of incision made by scalpel and typical insertion location and exit hole of the 16-gauge hypodermic needle used to thread the whip antenna past the telemetry tag for yearling Muskellunge raised in a recirculating aquaculture system and stocked into Spirit Lake, April 2020.

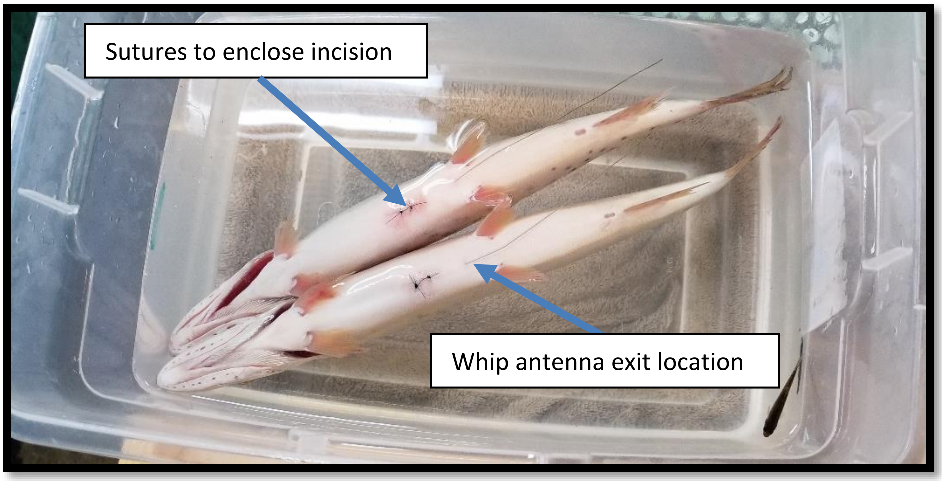


Figure 3. Sutures and whip antenna location on a telemetry tagged yearling Muskellunge raised in a recirculation aquaculture system and stocked into Spirit Lake, April 2020.

Figure 4. Stocking yearling Muskellunge raised in a recirculating aquaculture system at Hales Slough boat ramp, Spirit Lake, Iowa on April 24, 2020.

Figure 5. Typical fin erosion observed in yearling Muskellunge raised in a recirculating aquaculture system and stocked into Spirit Lake on April 24, 2020.

Figure 6. Physical appearance of yearling Muskellunge raised in the recirculating aquaculture system and transported to the Spirit Lake Fish Hatchery on April 15, 2020.

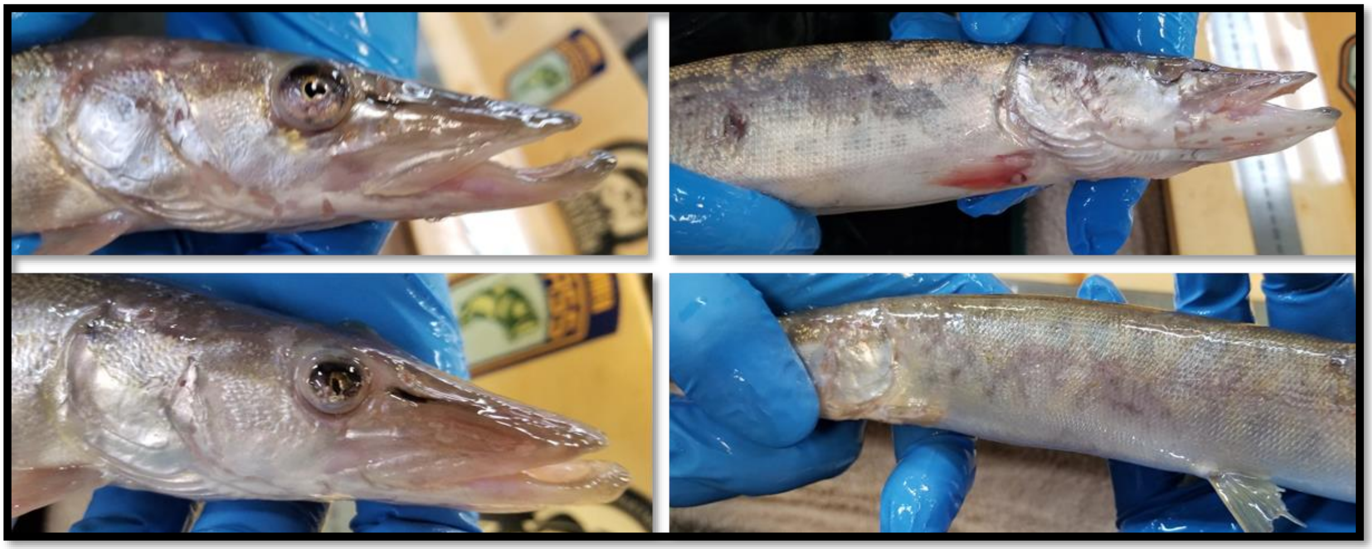


Figure 7. Typical deformities observed on yearling Muskellunge raised in a recirculating aquaculture system and transported to the Spirit Lake Fish Hatchery on April 15th 2020. Photos on the left show eye deformities and the right photos show fin erosion and abrasions. It was not uncommon to observe fish that had at least one blind eye (upper right).

Figure 8. Treatment groups (left = pellet finished; right = minnow finished) of yearling Muskellunge raised in a recirculating aquaculture system (RAS) and finished at the Spirit Lake Fish Hatchery 9-d prior to stocking in Spirit Lake.

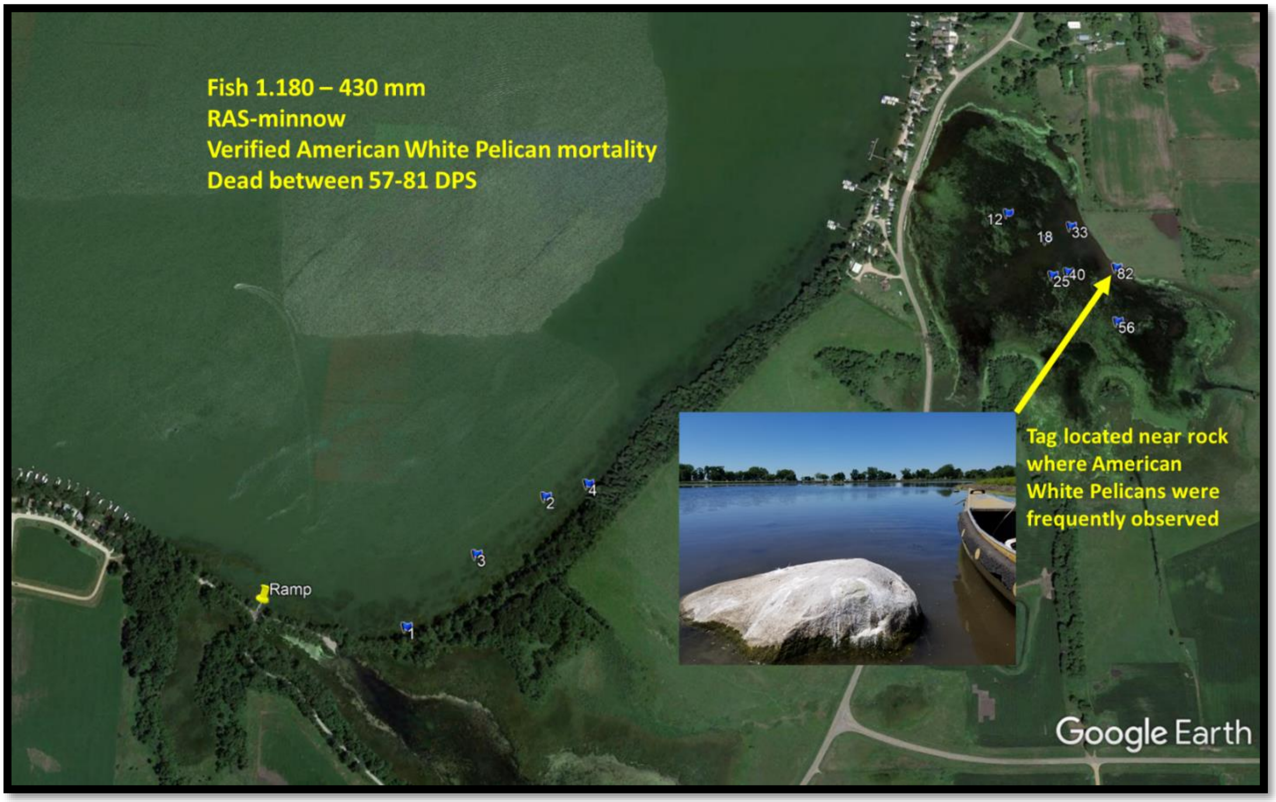


Figure 9. Location and day poststocking (DPS) encountered (numbered white flags) of radio transmitter implanted yearling Muskellunge fish 1.180 between 1 and 56 DPS. Yearling Muskellunge were cultured in a recirculating aquaculture system (RAS) and stocked at Hales Slough boat ramp (Ramp) in Spirit Lake on April 24, 2020 and tracked until September 29, 2020.

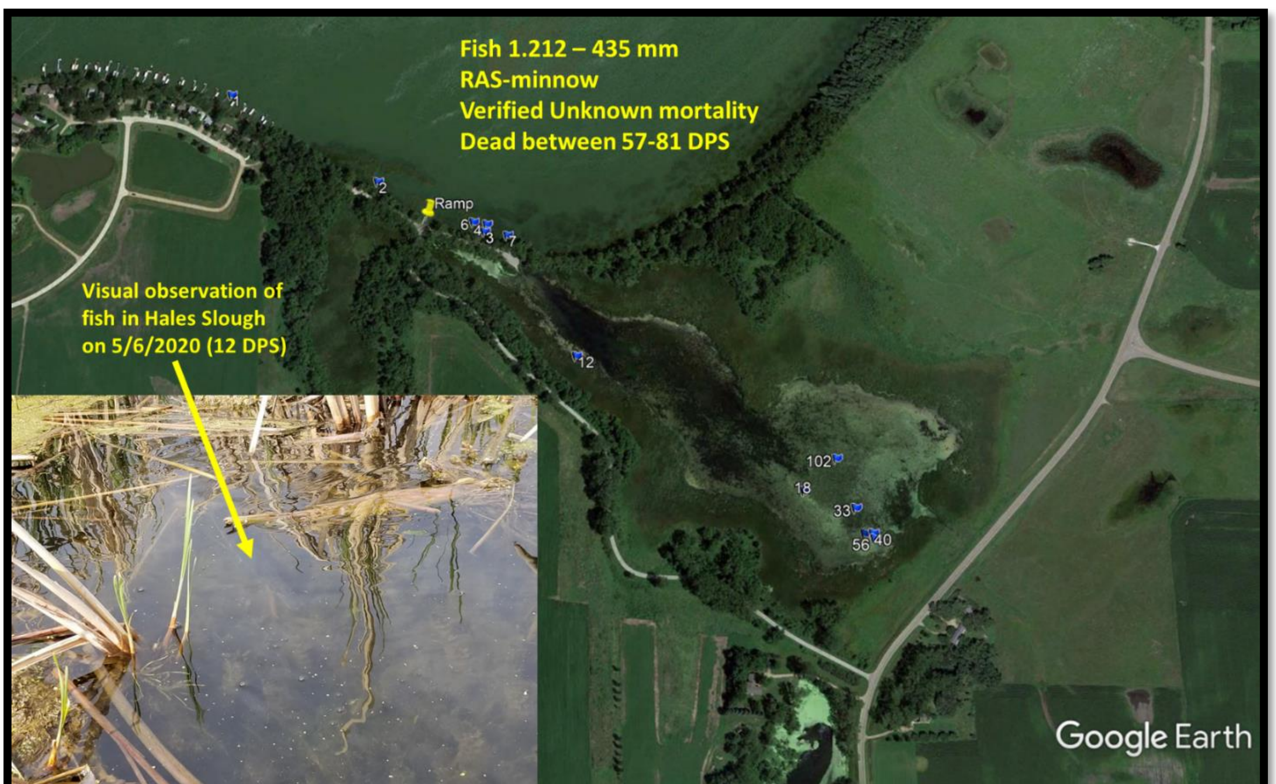


Figure 10. Location and day poststocking (DPS) encountered (numbered white flags) of radio transmitter implanted yearling Muskellunge fish 1.212 between 1 and 56 DPS. Yearling Muskellunge were cultured in a recirculating aquaculture system (RAS) and stocked at Hales Slough boat ramp (Ramp) in Spirit Lake on April 24, 2020 and tracked until September 29, 2020.

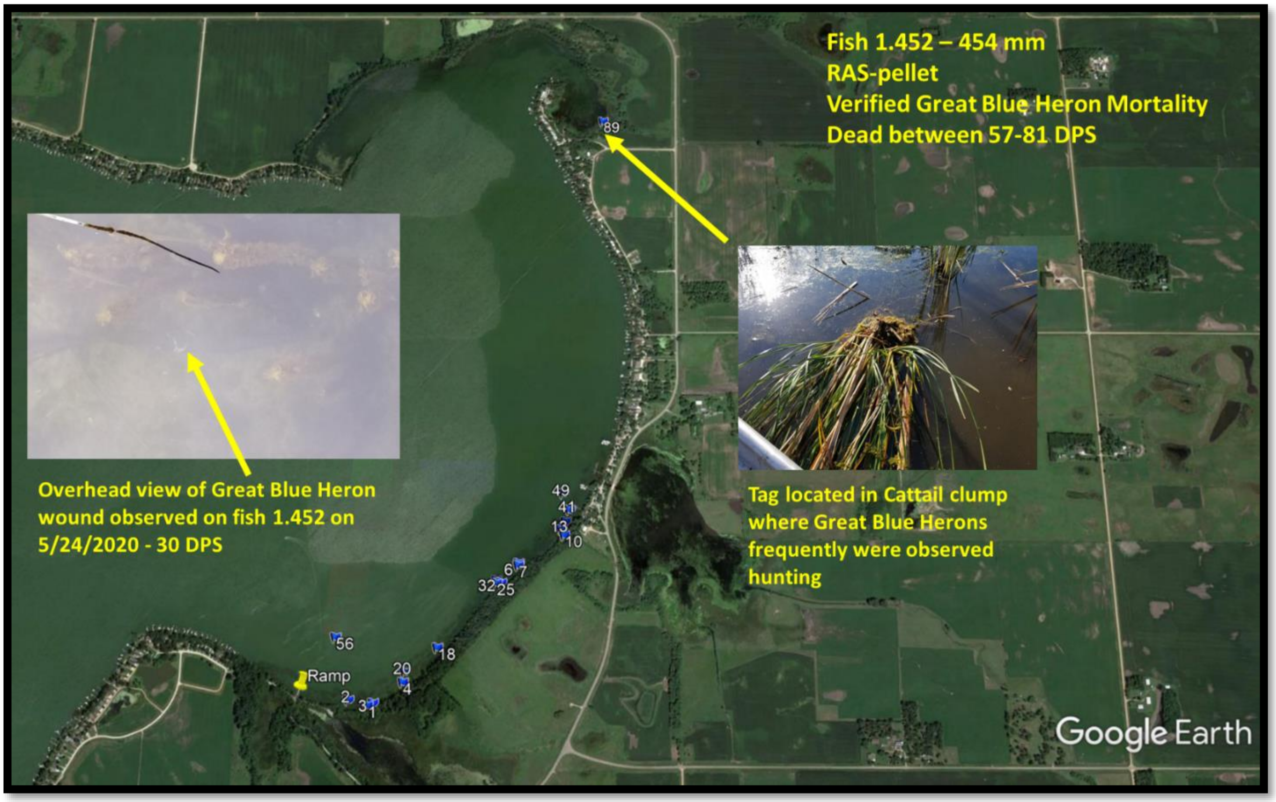


Figure 11. Location and day poststocking (DPS) encountered (numbered white flags) of radio transmitter implanted yearling Muskellunge fish 1.452 between 1 and 56 DPS. Yearling Muskellunge were cultured in a recirculating aquaculture system (RAS) and stocked at Hales Slough boat ramp (Ramp) in Spirit Lake on April 24, 2020 and tracked until September 29, 2020.

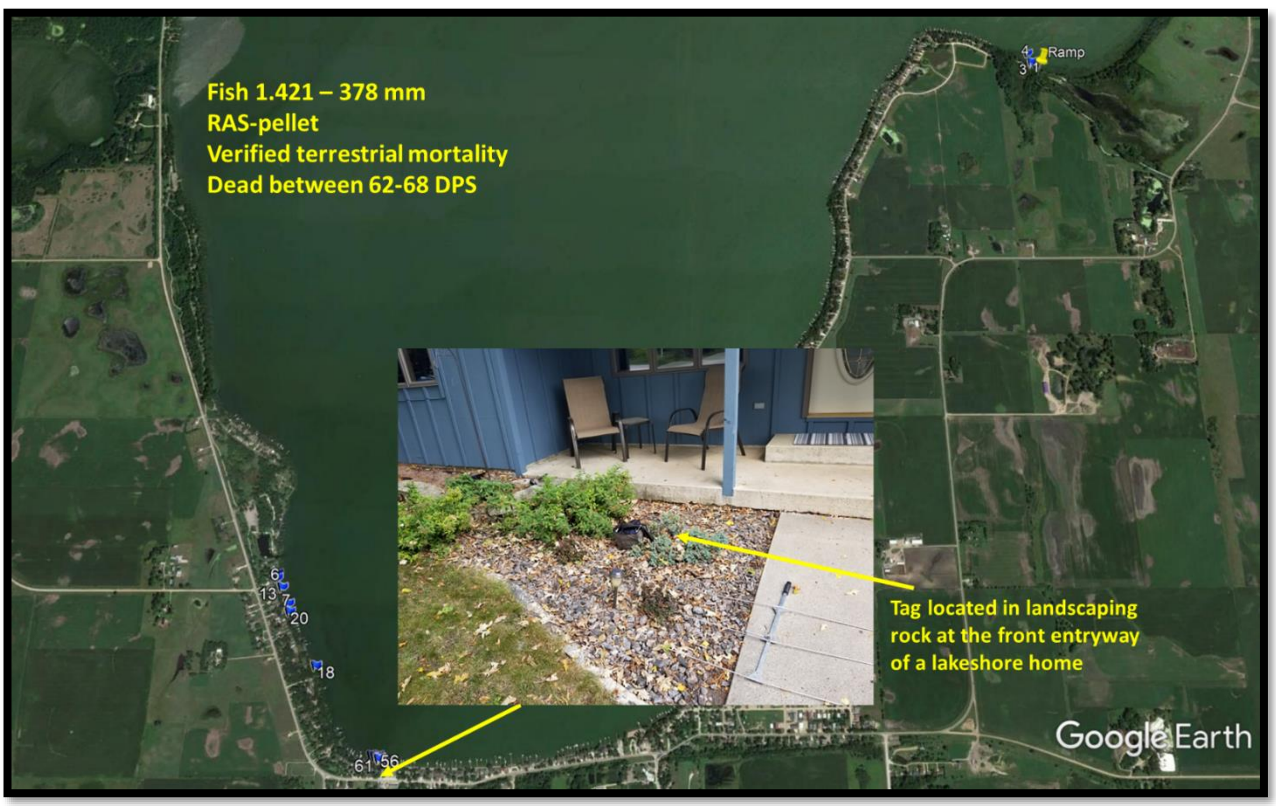


Figure 12. Location and day poststocking (DPS) encountered (numbered white flags) of radio transmitter implanted yearling Muskellunge fish 1.421 between 1 and 61 DPS. Yearling Muskellunge were cultured in a recirculating aquaculture system (RAS) and stocked at Hales Slough boat ramp (Ramp) in Spirit Lake on April 24, 2020 and tracked until September 29, 2020.

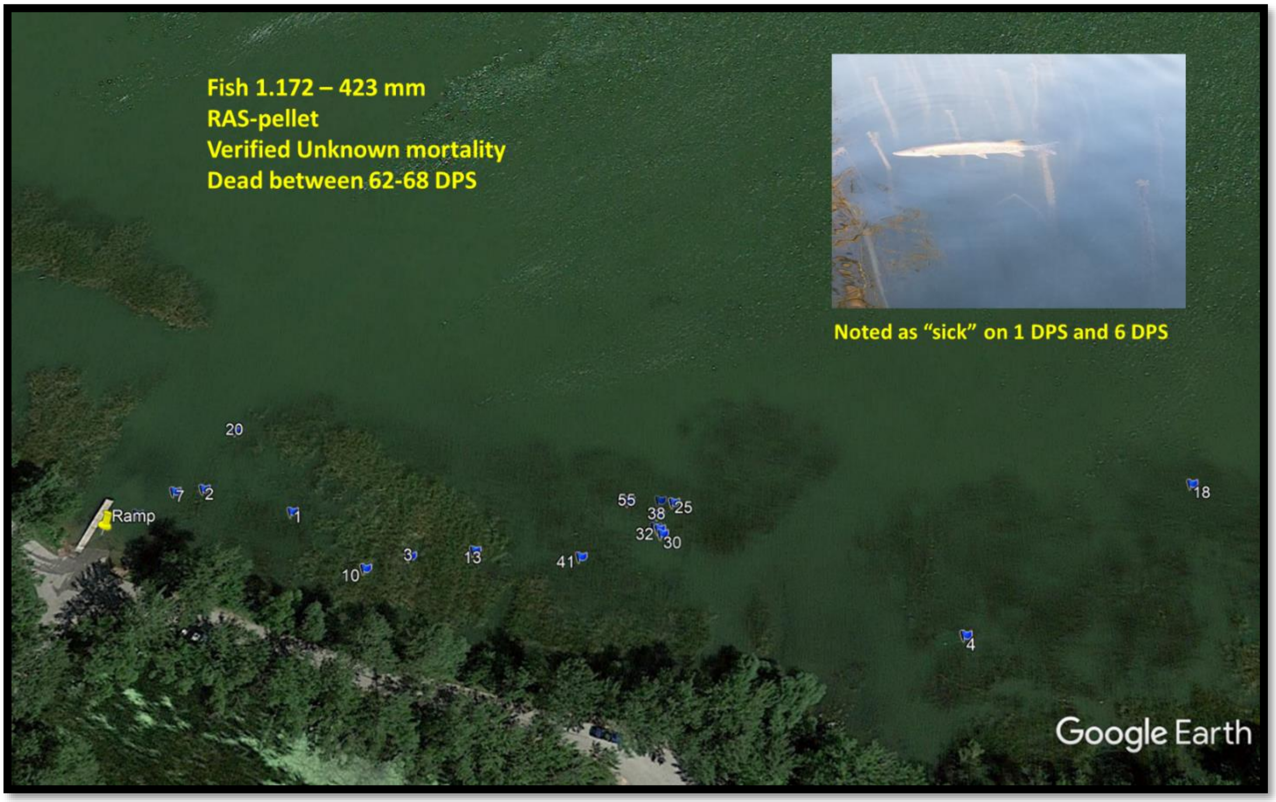


Figure 13. Location and day poststocking (DPS) encountered (numbered white flags) of radio transmitter implanted yearling Muskellunge fish 1.172 between 1 and 56 DPS. Yearling Muskellunge were cultured in a recirculating aquaculture system (RAS) and stocked at Hales Slough boat ramp (Ramp) in Spirit Lake on April 24, 2020 and tracked until September 29, 2020.

Figure 14. Location and day poststocking (DPS) encountered (numbered white flags) of radio transmitter implanted yearling Muskellunge fish 1.343 between 1 and 74 DPS. Yearling Muskellunge were cultured in a recirculating aquaculture system (RAS) and stocked at Hales Slough boat ramp (Ramp) in Spirit Lake on April 24, 2020 and tracked until September 29, 2020.

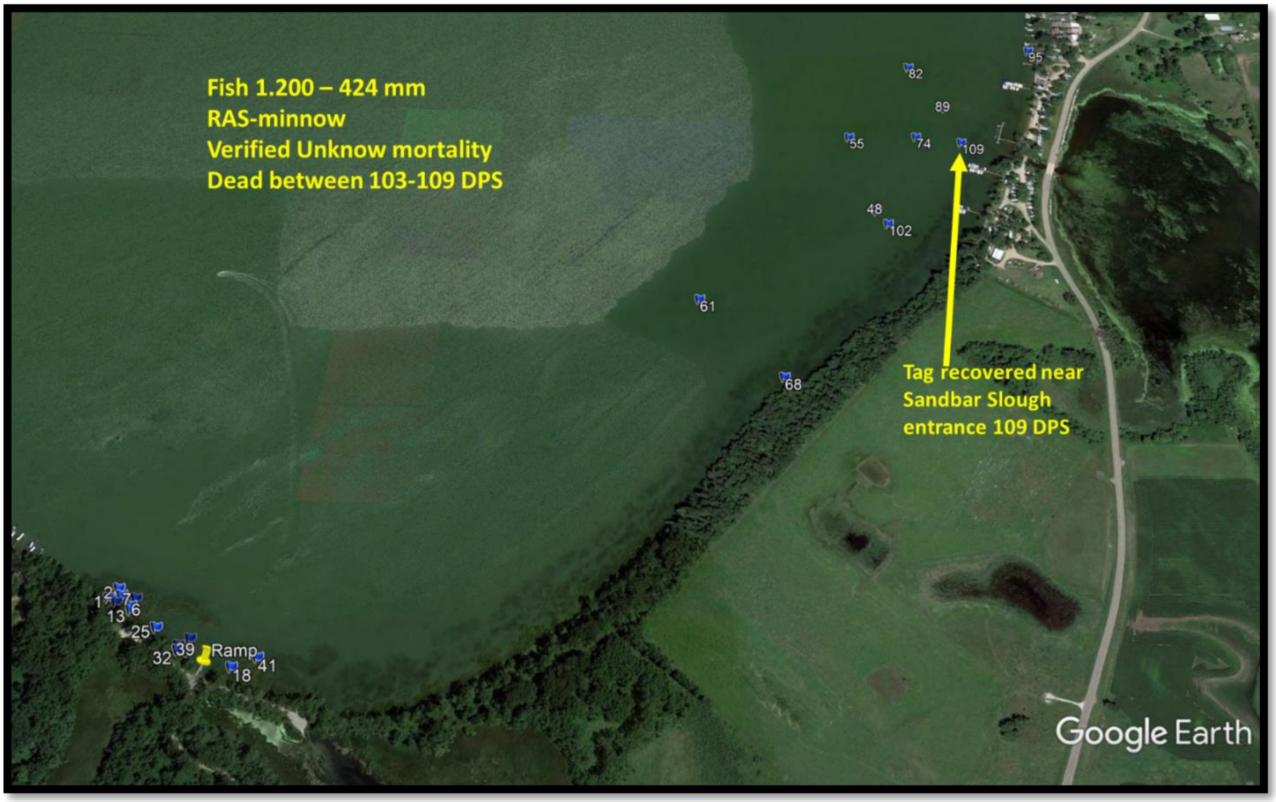


Figure 15. Location and day poststocking (DPS) encountered (numbered white flags) of radio transmitter implanted yearling Muskellunge fish 1.200 between 1 and102 DPS. Yearling Muskellunge were cultured in a recirculating aquaculture system (RAS) and stocked at Hales Slough boat ramp (Ramp) in Spirit Lake on April 24, 2020 and tracked until September 29, 2020.

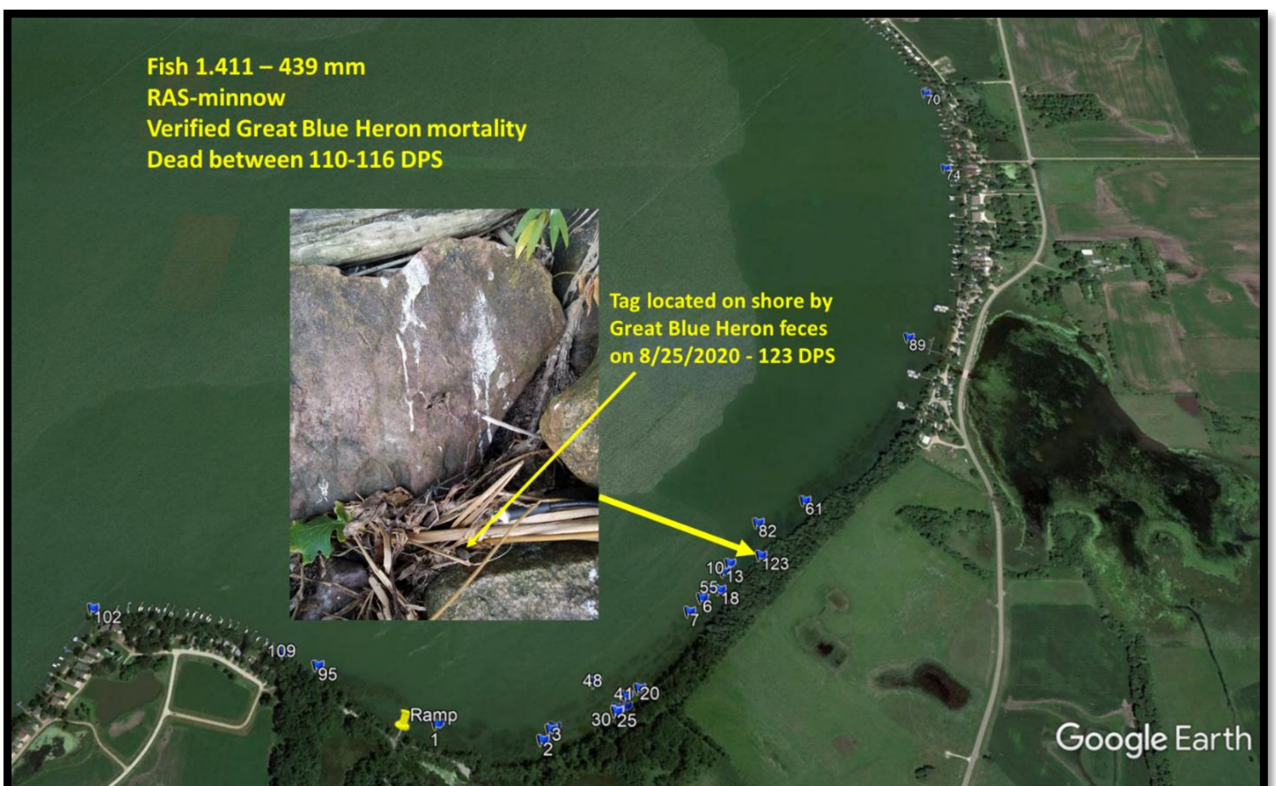


Figure 16. Location and day poststocking (DPS) encountered (numbered white flags) of radio transmitter implanted yearling Muskellunge fish 1.411 between 1 and 109 DPS. Yearling Muskellunge were cultured in a recirculating aquaculture system (RAS) and stocked at Hales Slough boat ramp (Ramp) in Spirit Lake on April 24, 2020 and tracked until September 29, 2020.

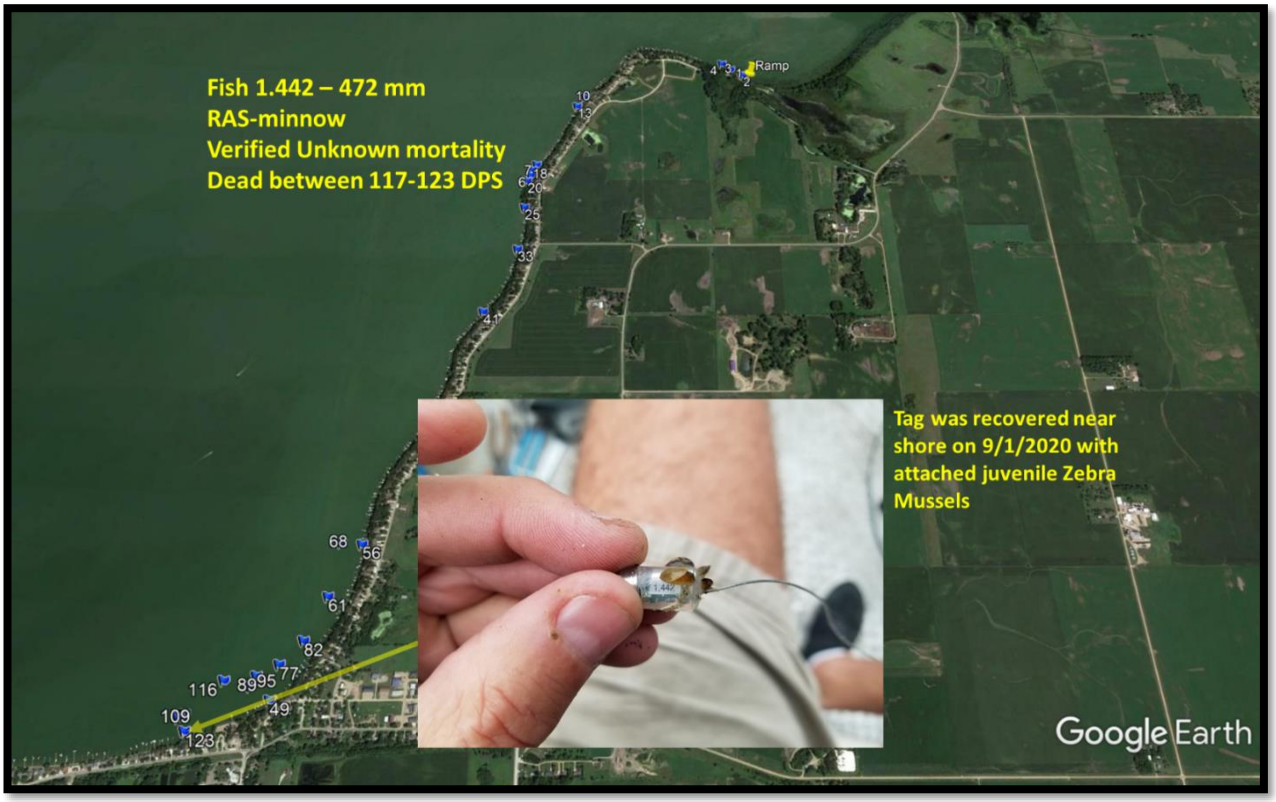


Figure 17. Location and day poststocking (DPS) encountered (numbered white flags) of radio transmitter implanted yearling Muskellunge fish 1.442 between 1 and 116 DPS. Yearling Muskellunge were cultured in a recirculating aquaculture system (RAS) and stocked at Hales Slough boat ramp (Ramp) in Spirit Lake on April 24, 2020 and tracked until September 29, 2020.

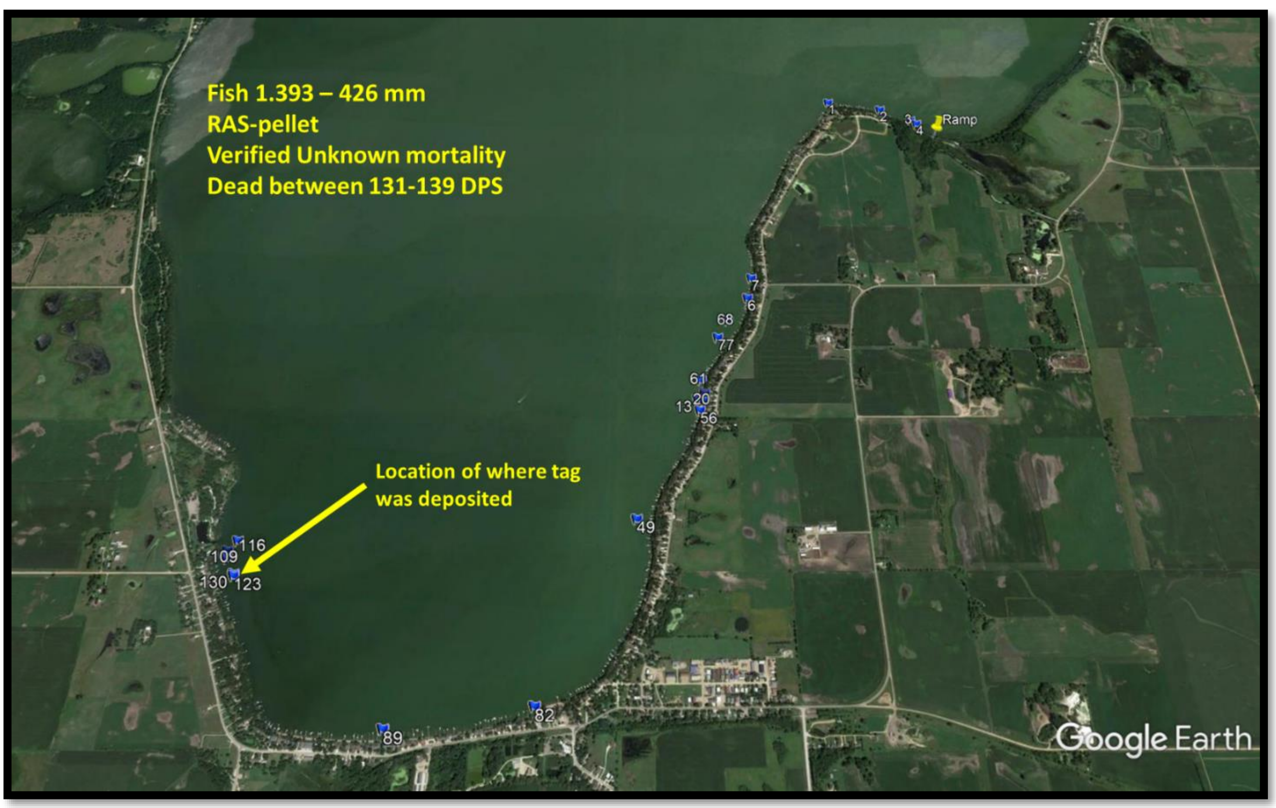


Figure 18. Location and day poststocking (DPS) encountered (numbered white flags) of radio transmitter implanted yearling Muskellunge fish 1.393 between 1 and 130 DPS. Yearling Muskellunge were cultured in a recirculating aquaculture system (RAS) and stocked at Hales Slough boat ramp (Ramp) in Spirit Lake on April 24, 2020 and tracked until September 29, 2020.

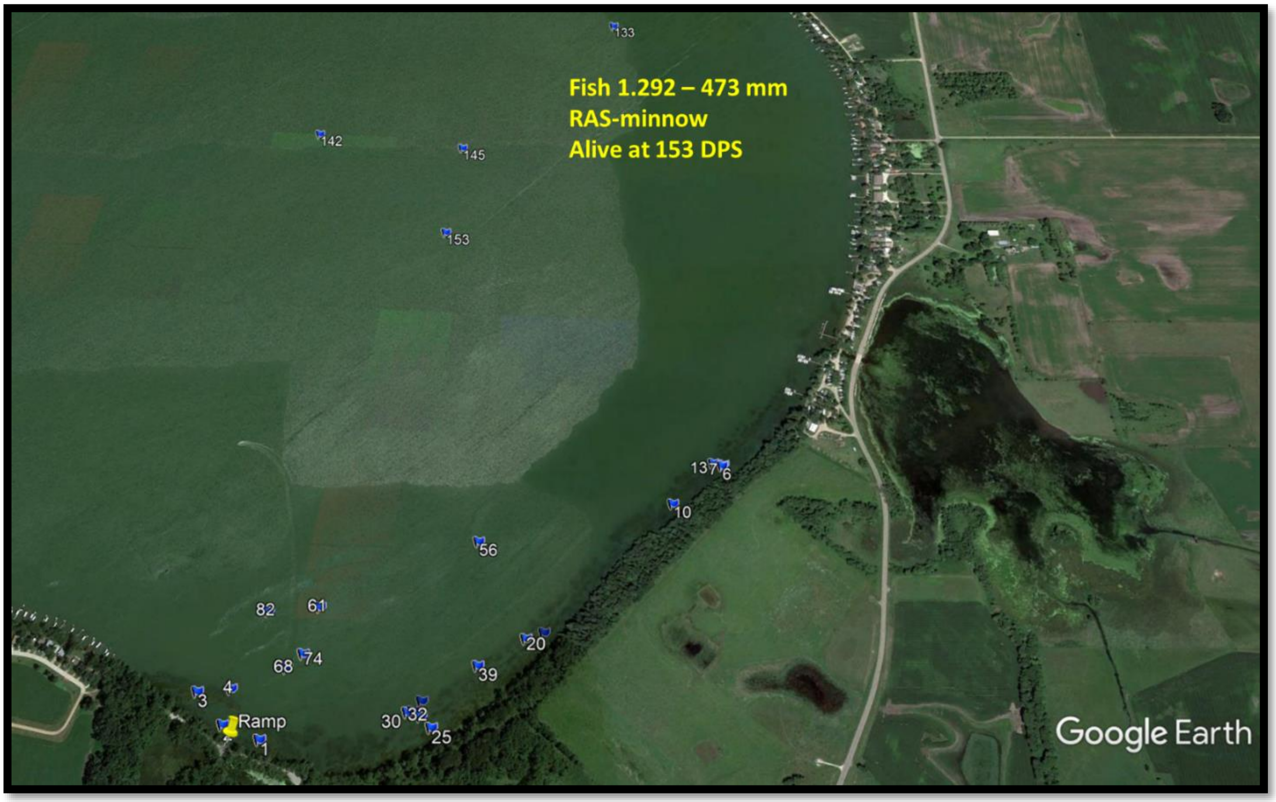


Figure 19. Location and day poststocking (DPS) encountered (numbered white flags) of radio transmitter implanted yearling Muskellunge fish 1.292 between 1 and 153 DPS. Yearling Muskellunge were cultured in a recirculating aquaculture system (RAS) and stocked at Hales Slough boat ramp (Ramp) in Spirit Lake on April 24, 2020 and tracked until September 29, 2020.

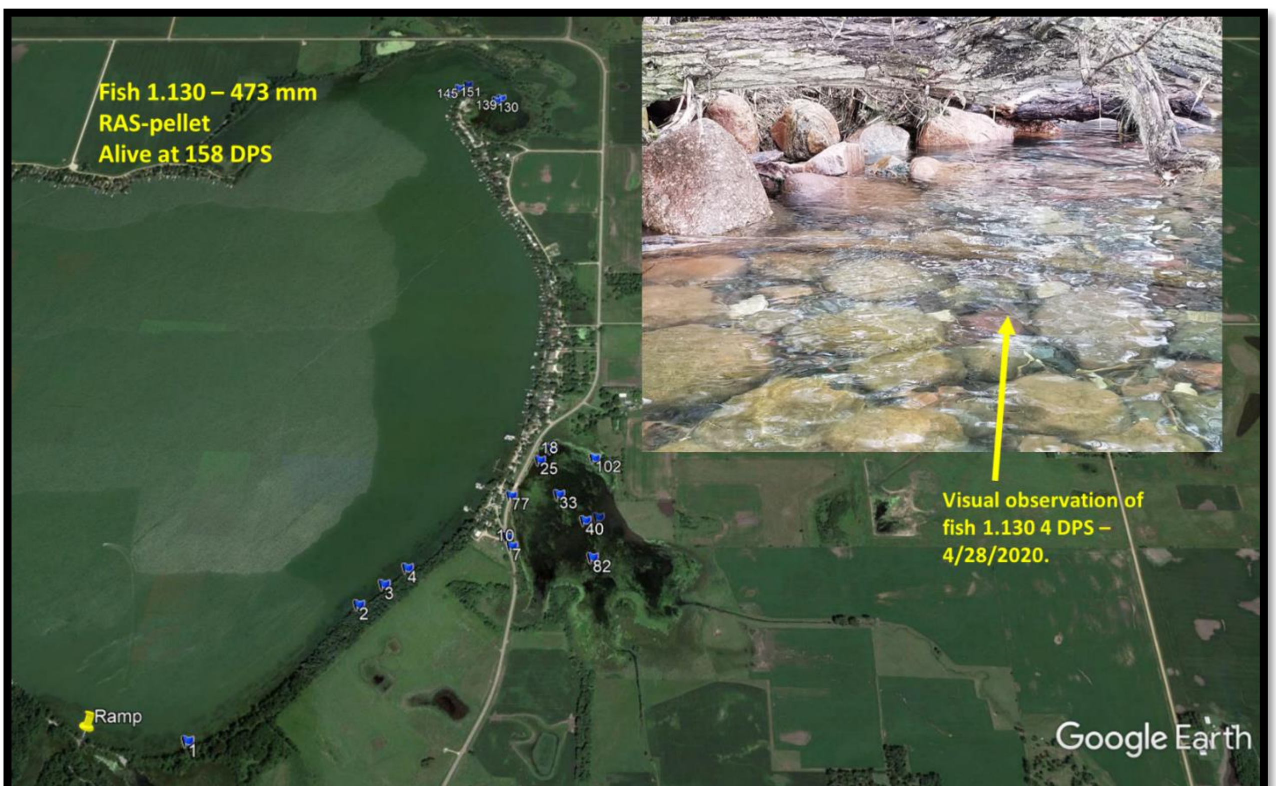


Figure 20. Location and day poststocking (DPS) encountered (numbered white flags) of radio transmitter implanted yearling Muskellunge fish 1.130 between 1 and 158 DPS. Yearling Muskellunge were cultured in a recirculating aquaculture system (RAS) and stocked at Hales Slough boat ramp (Ramp) in Spirit Lake on April 24, 2020 and tracked until September 29, 2020.

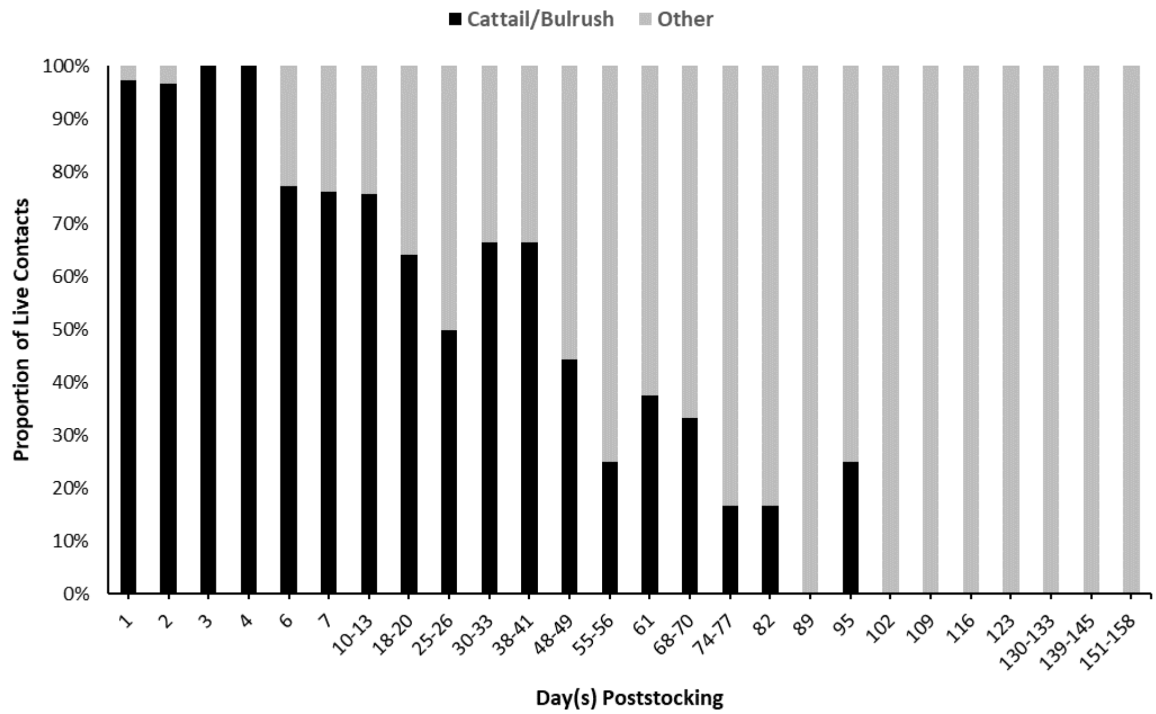


Figure 21. Proportion of radio telemetry live yearling Muskellunge contacts that were located within Cattail/Bulrush habitat compared to those found in open water, nearshore dock habitat, and submersed aquatic vegetation stands (collectively referred to as "other") for Muskellunge raised in a recirculating aquaculture system and stocked in Spirit Lake, April 24, 2020 and tracked for 158 days poststocking (September 29, 2020).

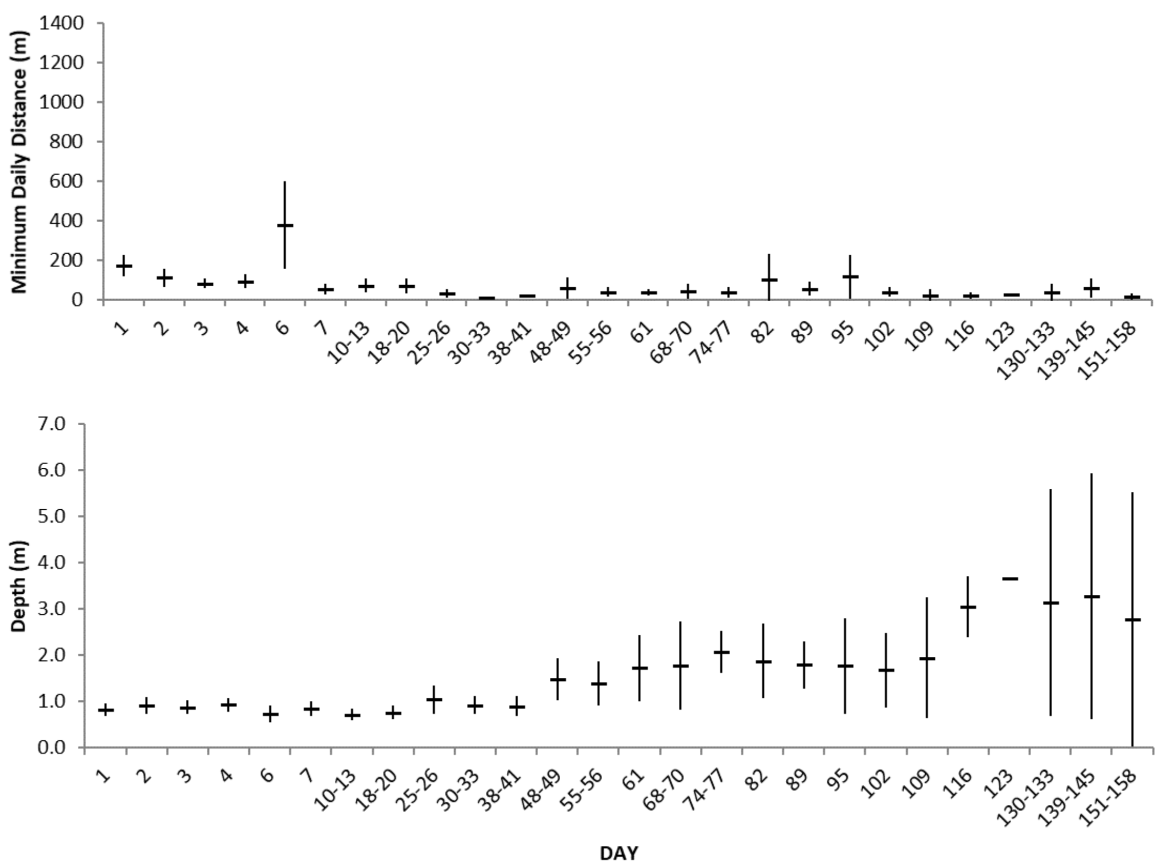


Figure 22. Mean (horizontal bar) minimum daily distance moved (m; top graph; 95% confidence interval represented by vertical bar) and water depth (m; bottom graph; 95% confidence interval represented by vertical bar) for live encounters of yearling Muskellunge in Spirit Lake between 1 and 158 days poststocking.

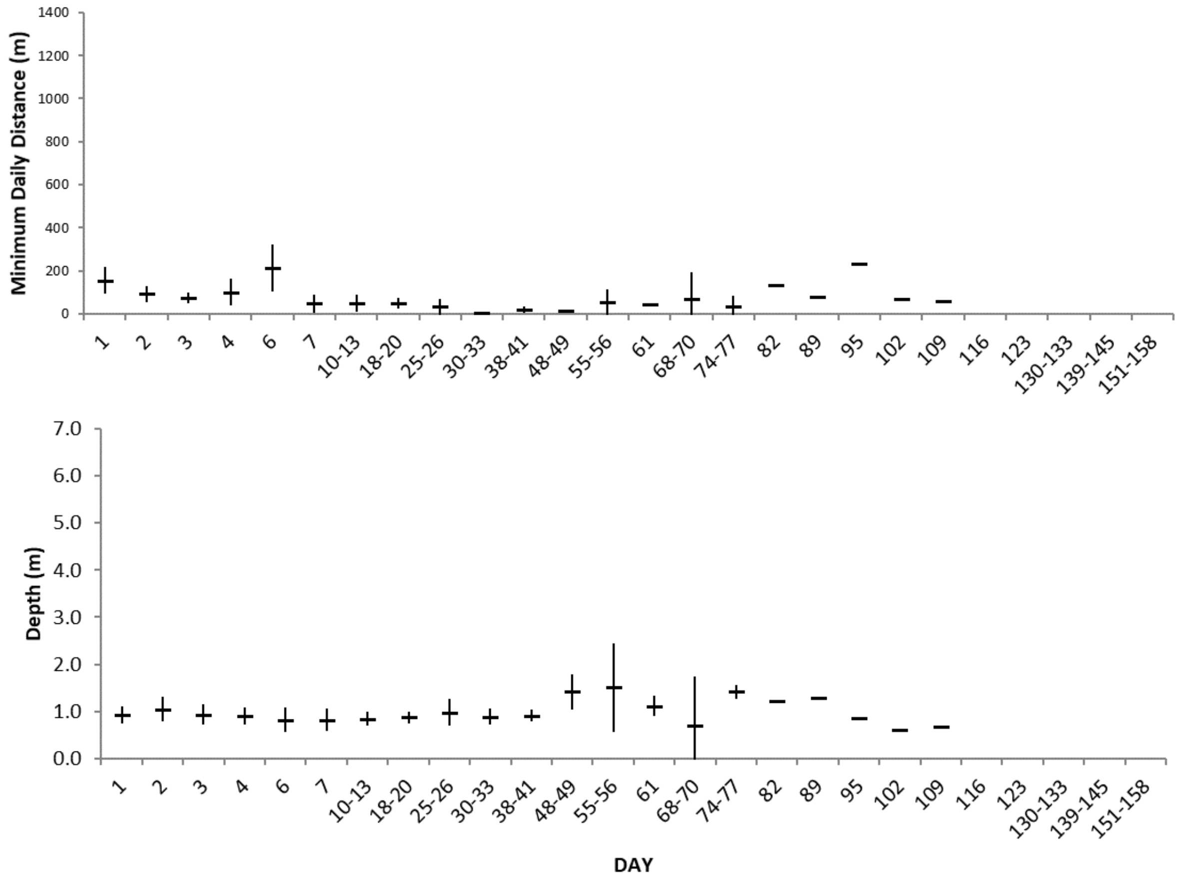


Figure 23. Mean (horizontal bar) minimum daily distance moved (m; top graph; 95% confidence interval represented by vertical bar) and water depth (m; bottom graph; 95% confidence interval represented by vertical bar) for live encounters of yearling Muskellunge that were later found to be consumed by avian (American White Pelican or Great Blue Heron) predators in Spirit Lake between 1 and 158 days poststocking.

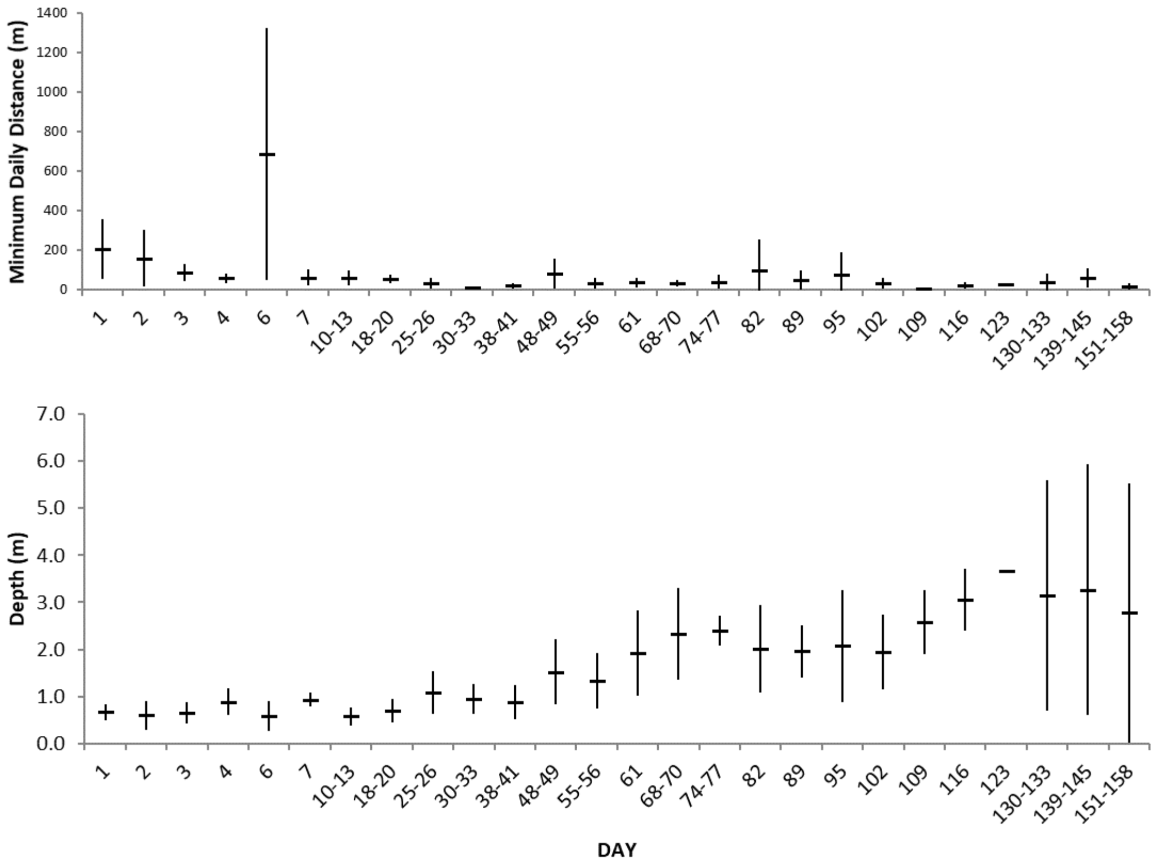
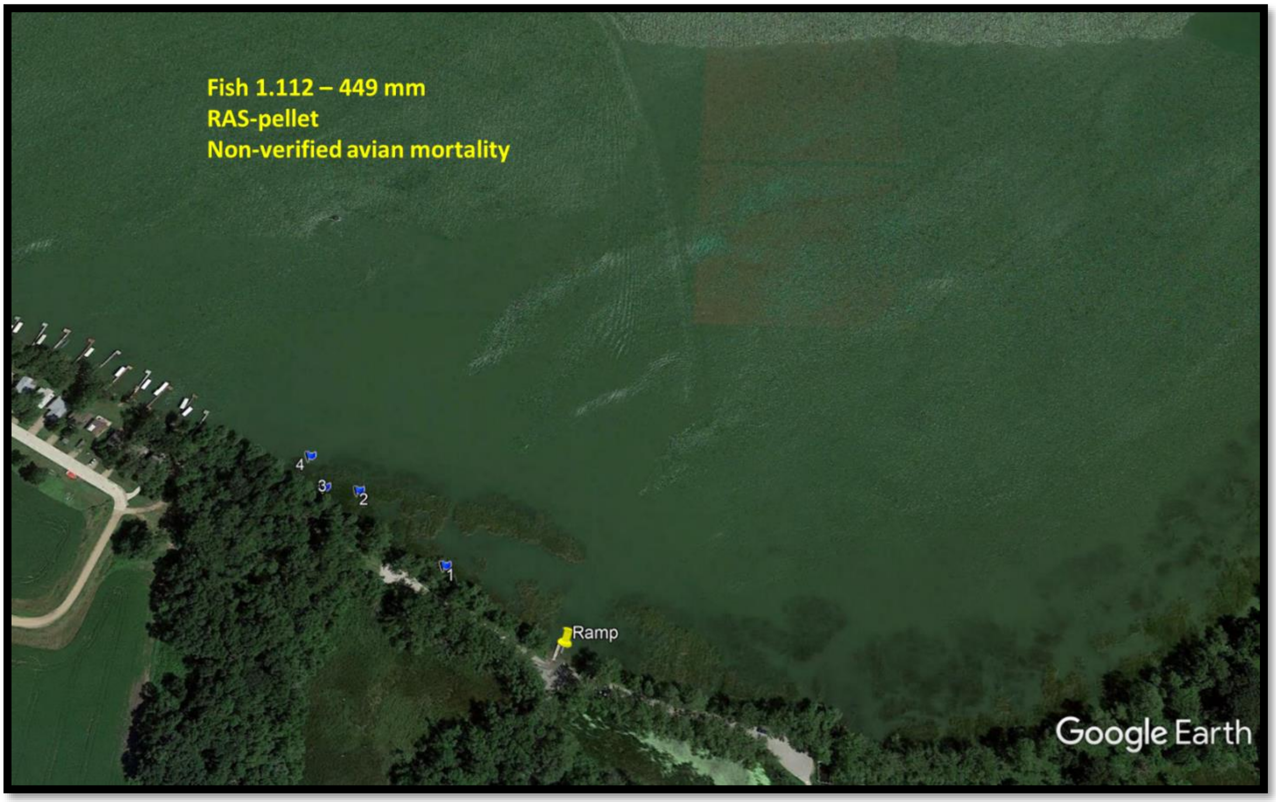
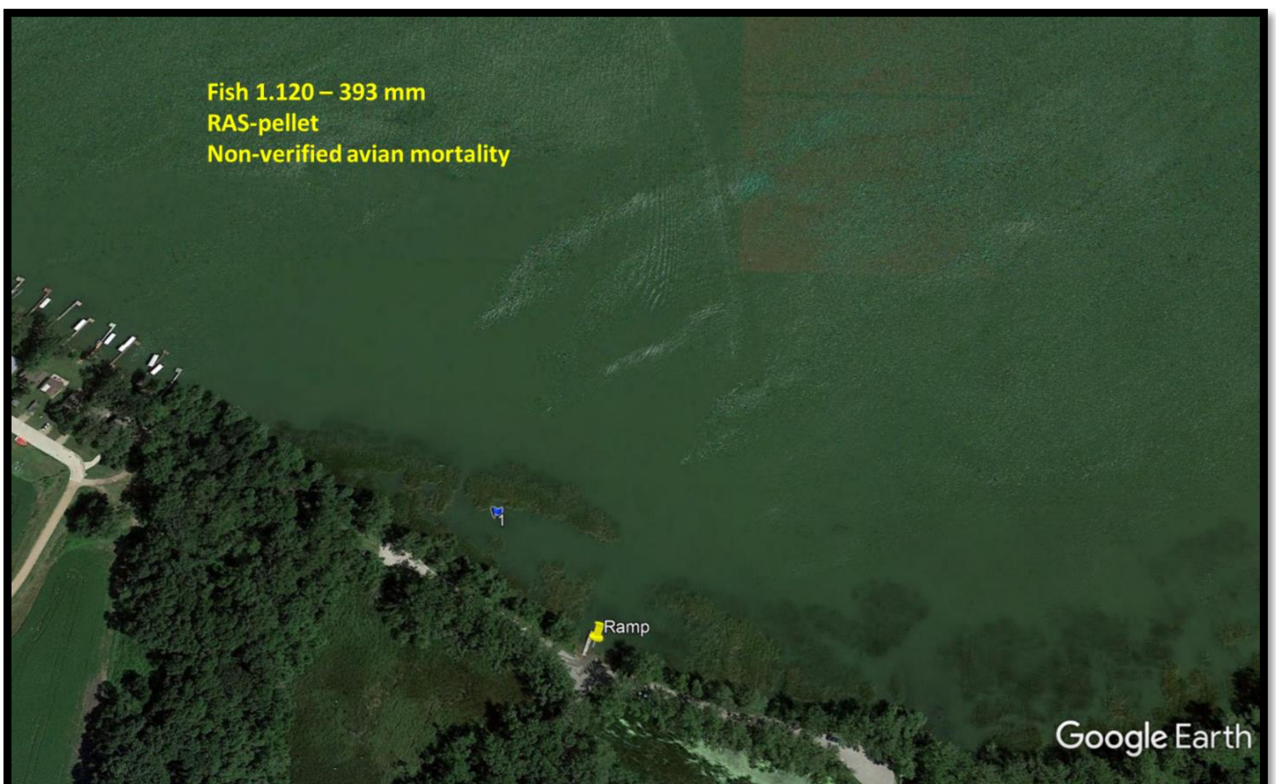


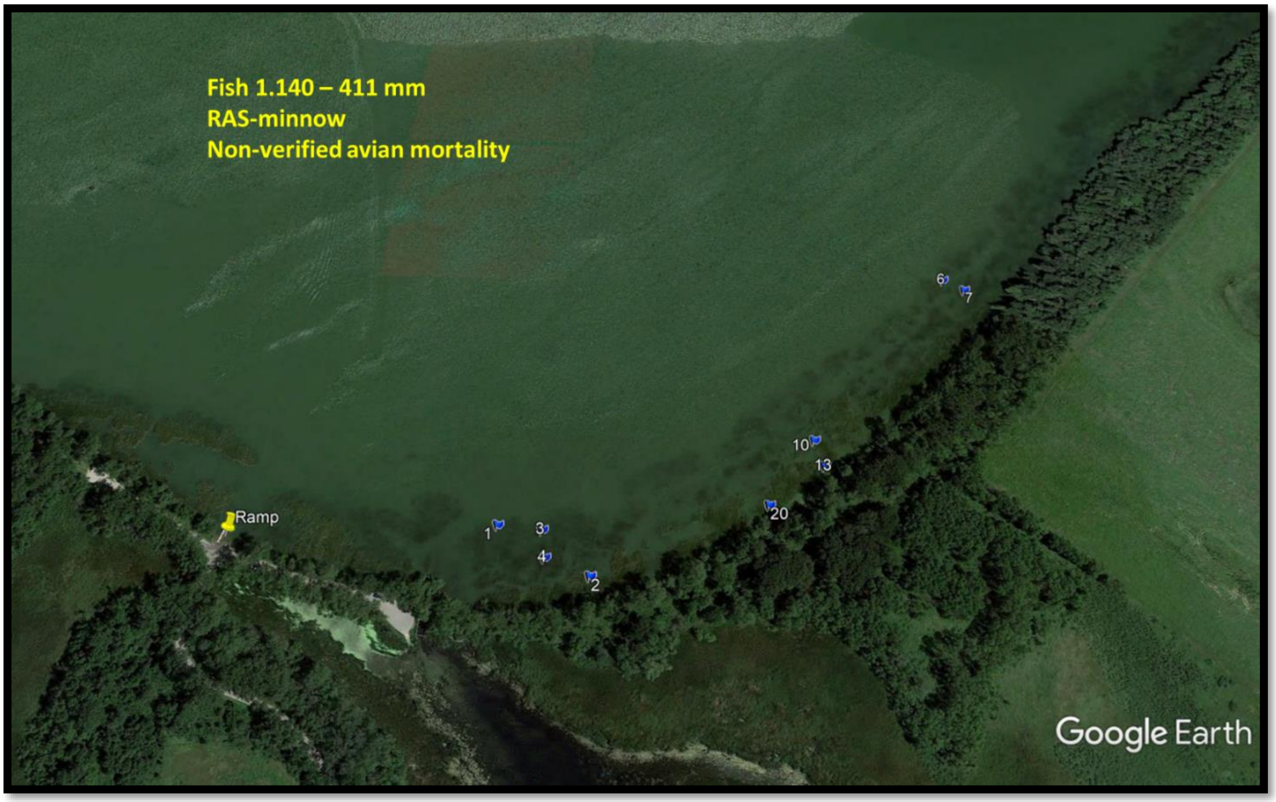
Figure 24. Mean (horizontal bar) minimum daily distance moved (m; top graph; 95% confidence interval represented by vertical bar) and water depth (m; bottom graph; 95% confidence interval represented by vertical bar) for live encounters of yearling Muskellunge that that survived at least 56 days in Spirit Lake poststocking.

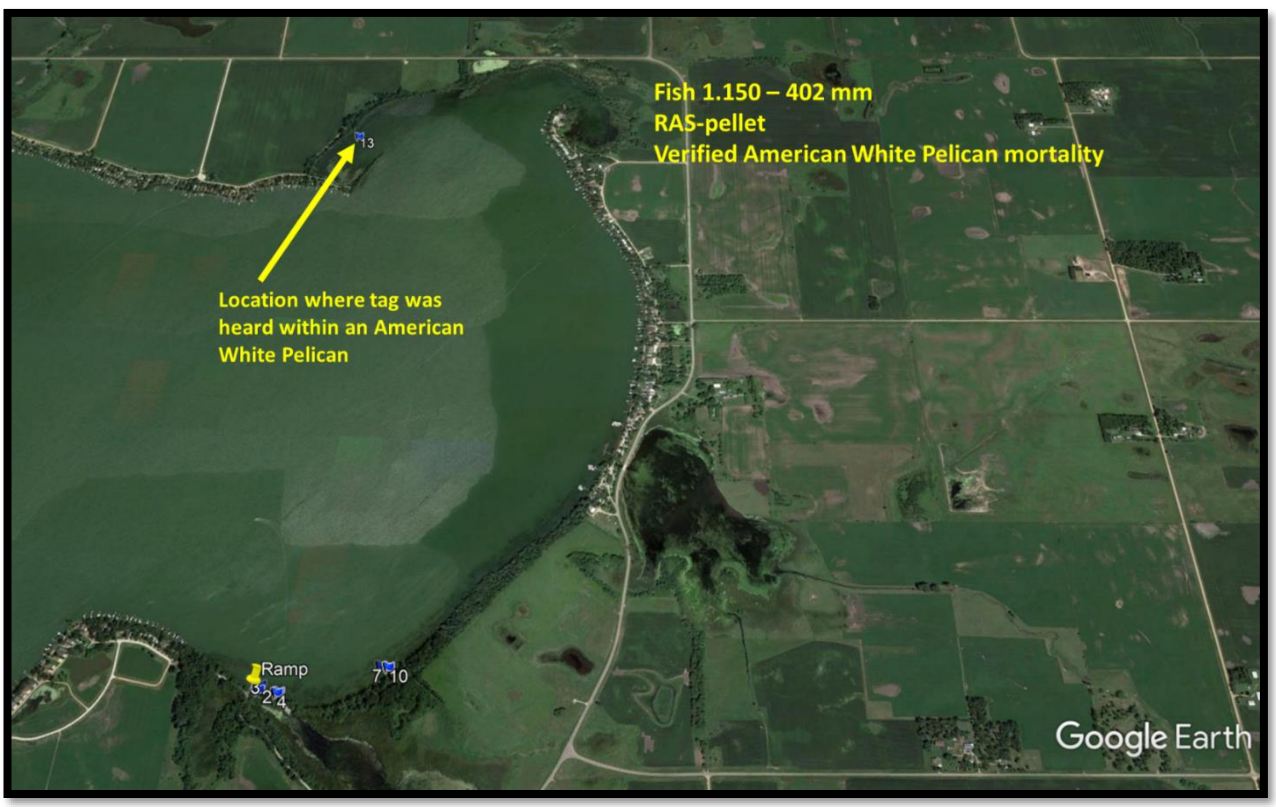
Appendices

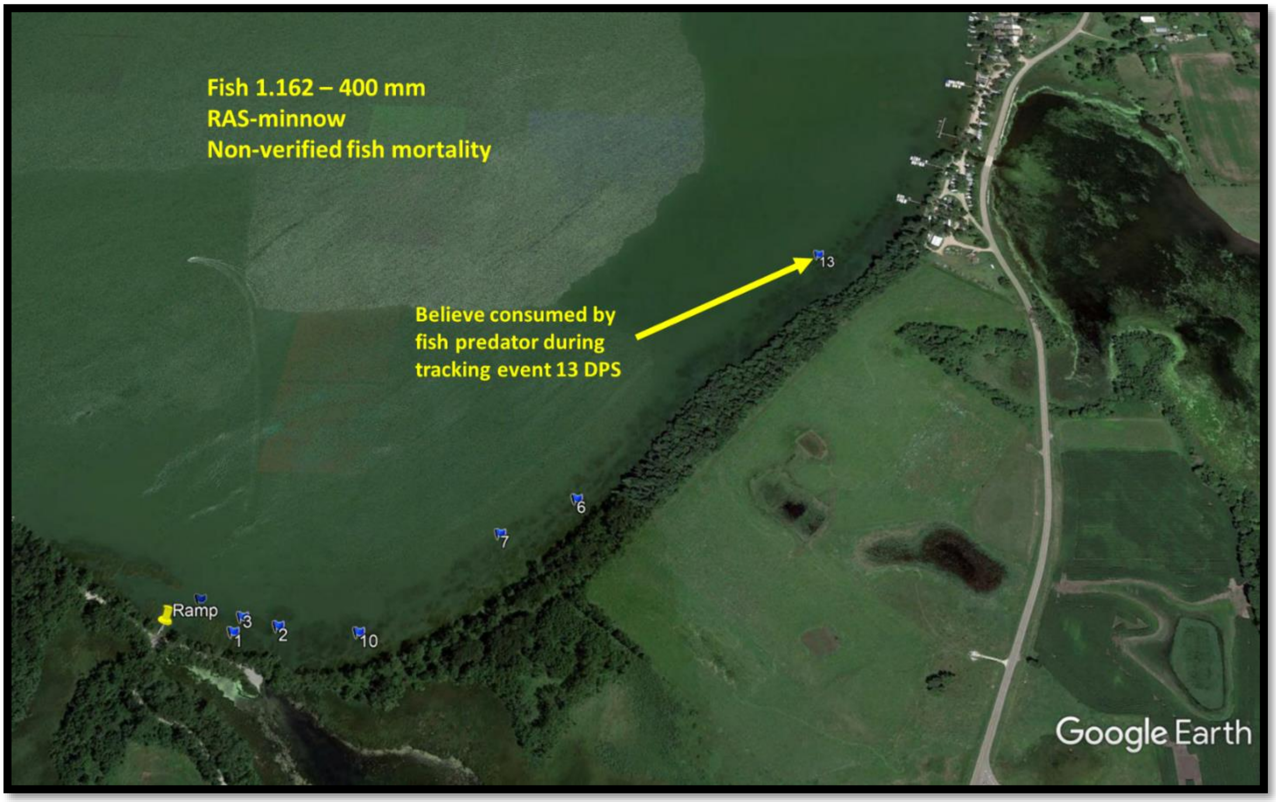
Appendix 1. Location and day poststocking (DPS) encountered (numbered white flags) of radio transmitter implanted yearling Muskellunge (n = 24). Yearling Muskellunge were cultured in a recirculating aquaculture system (RAS) and stocked at Hales Slough boat ramp (Ramp) in Spirit Lake on April 24, 2020 and tracked until September 29, 2020.



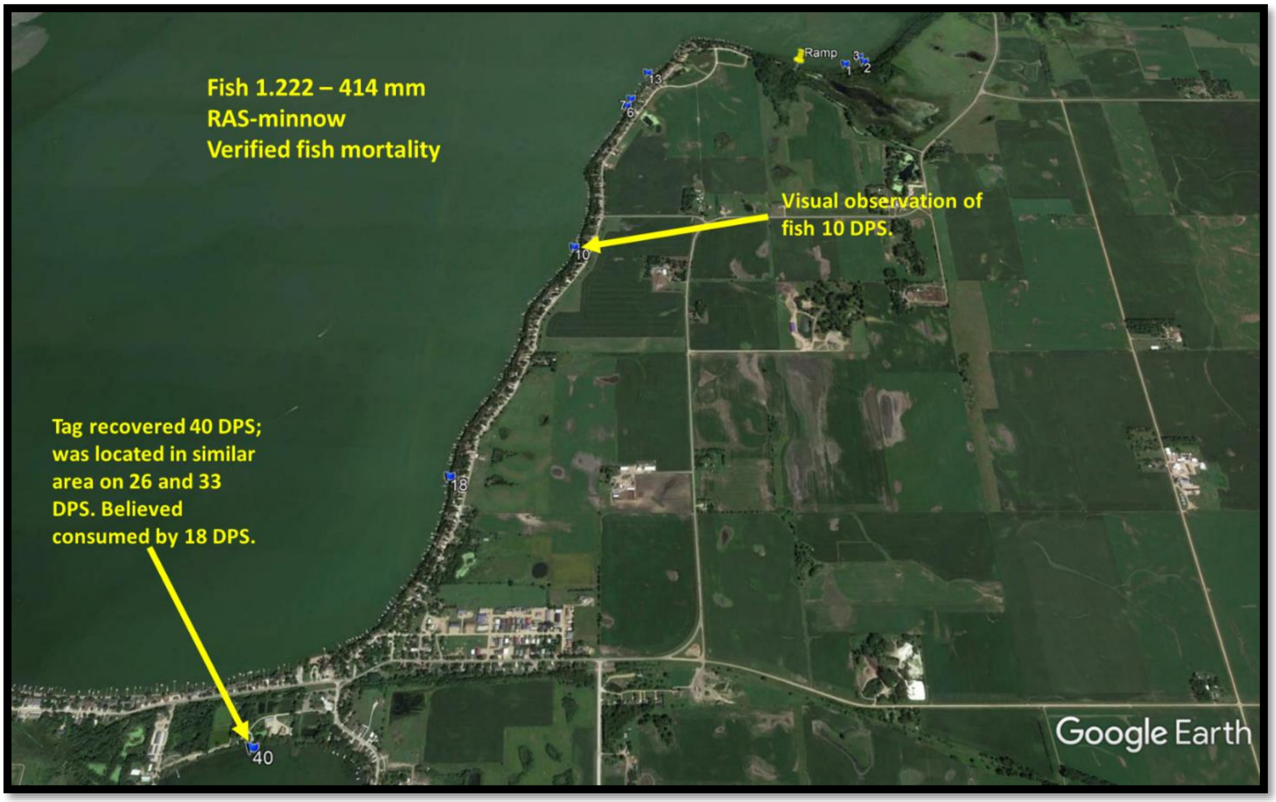


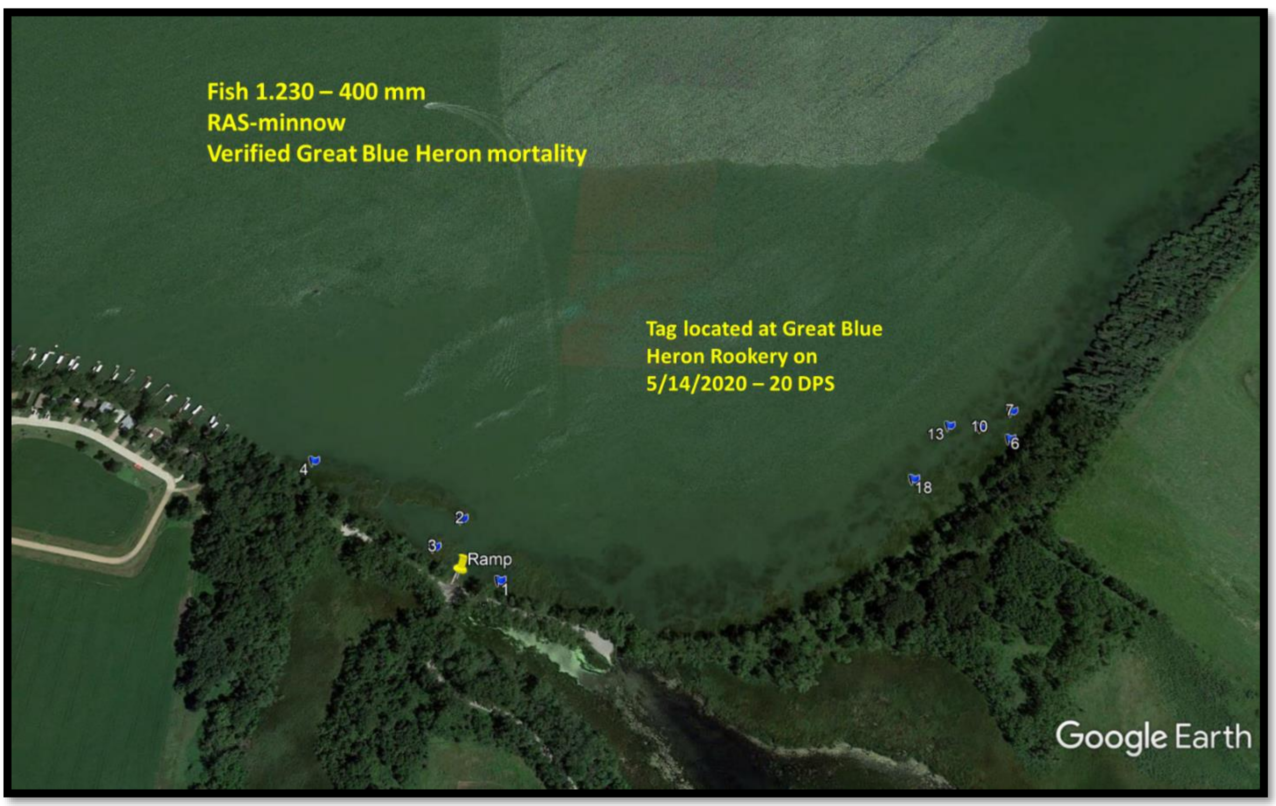

Fish 1.140 – 411 mm
RAS-minnow
Non-verified avian mortality
Ramp
1
2
3
4
6
7
10
13
20
Google Earth

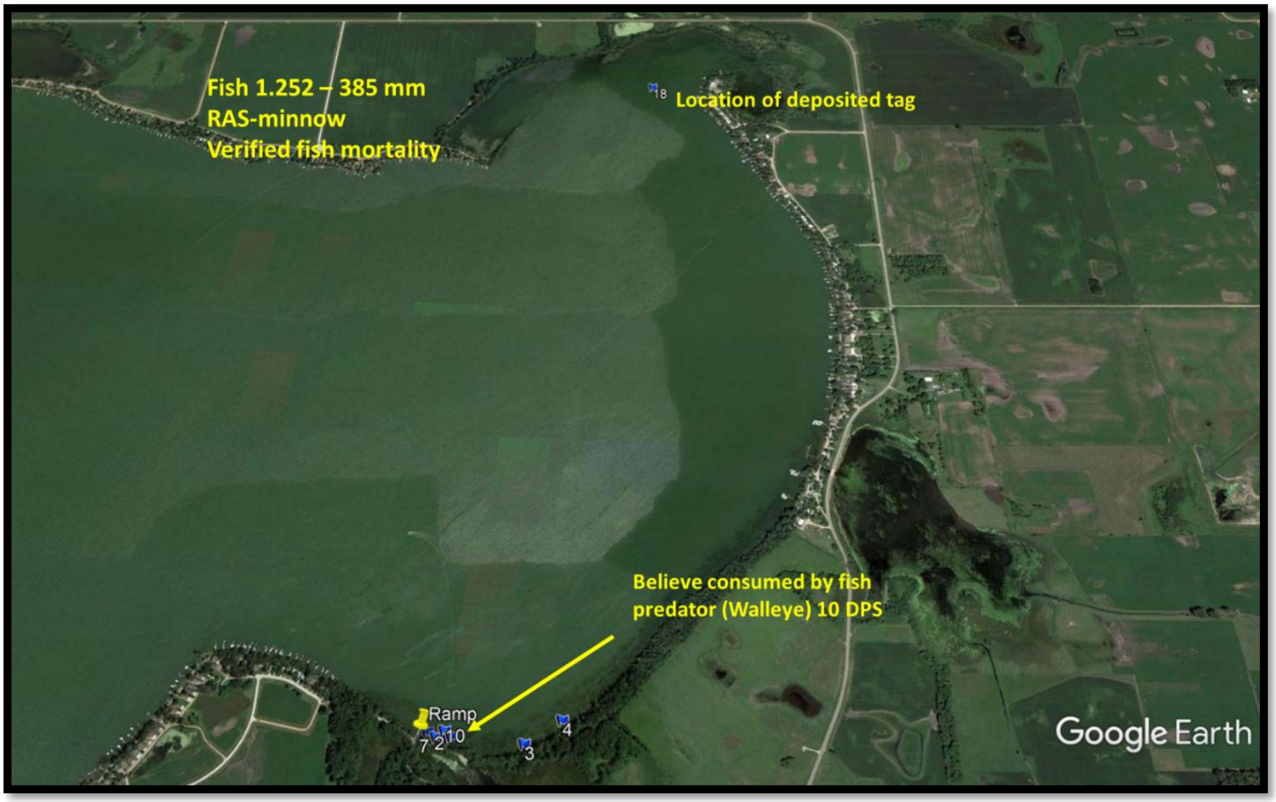

Fish 1.150 – 402 mm
RAS-pellet
Verified American White Pelican mortality
Location where tag was heard within an American White Pelican
13
Ramp
7
10
2
4
Google Earth

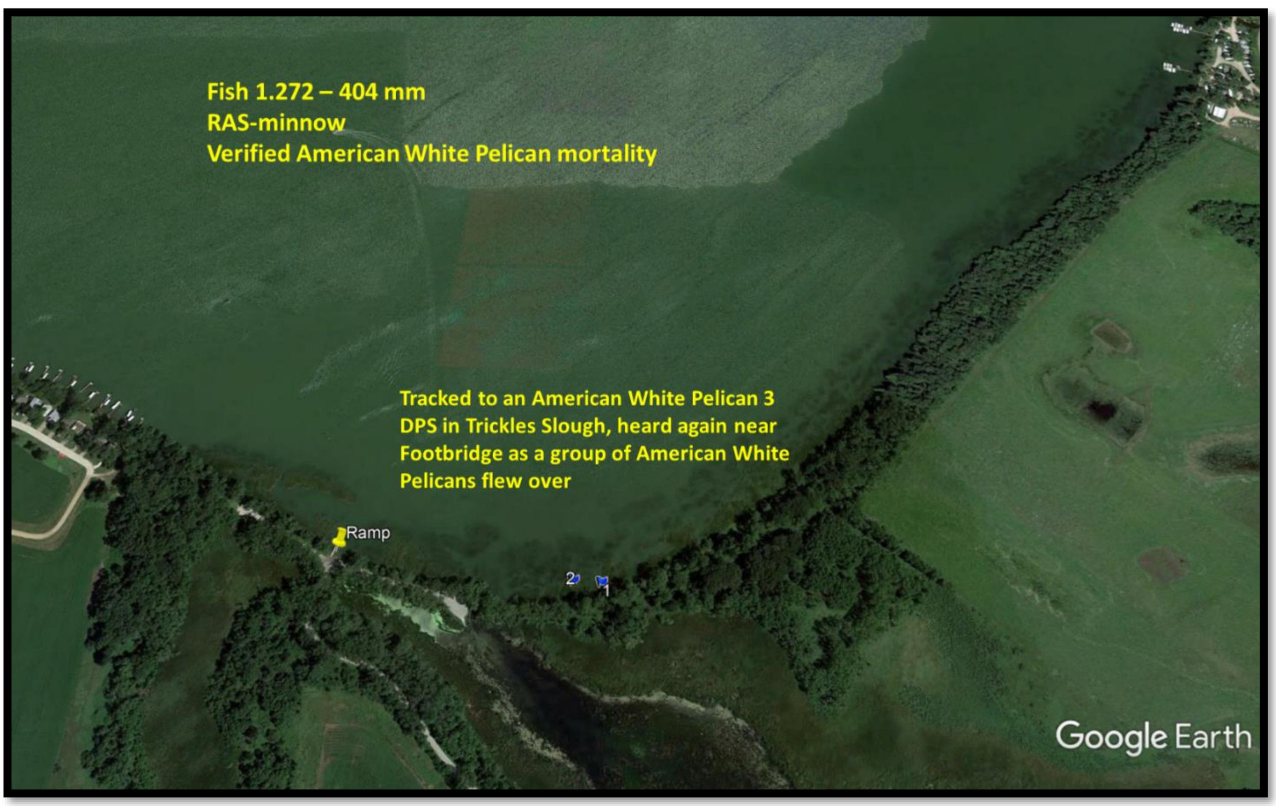

Fish 1.162 – 400 mm
RAS-minnow
Non-verified fish mortality
Believe consumed by
fish predator during
tracking event 13 DPS
Ramp
Google Earth

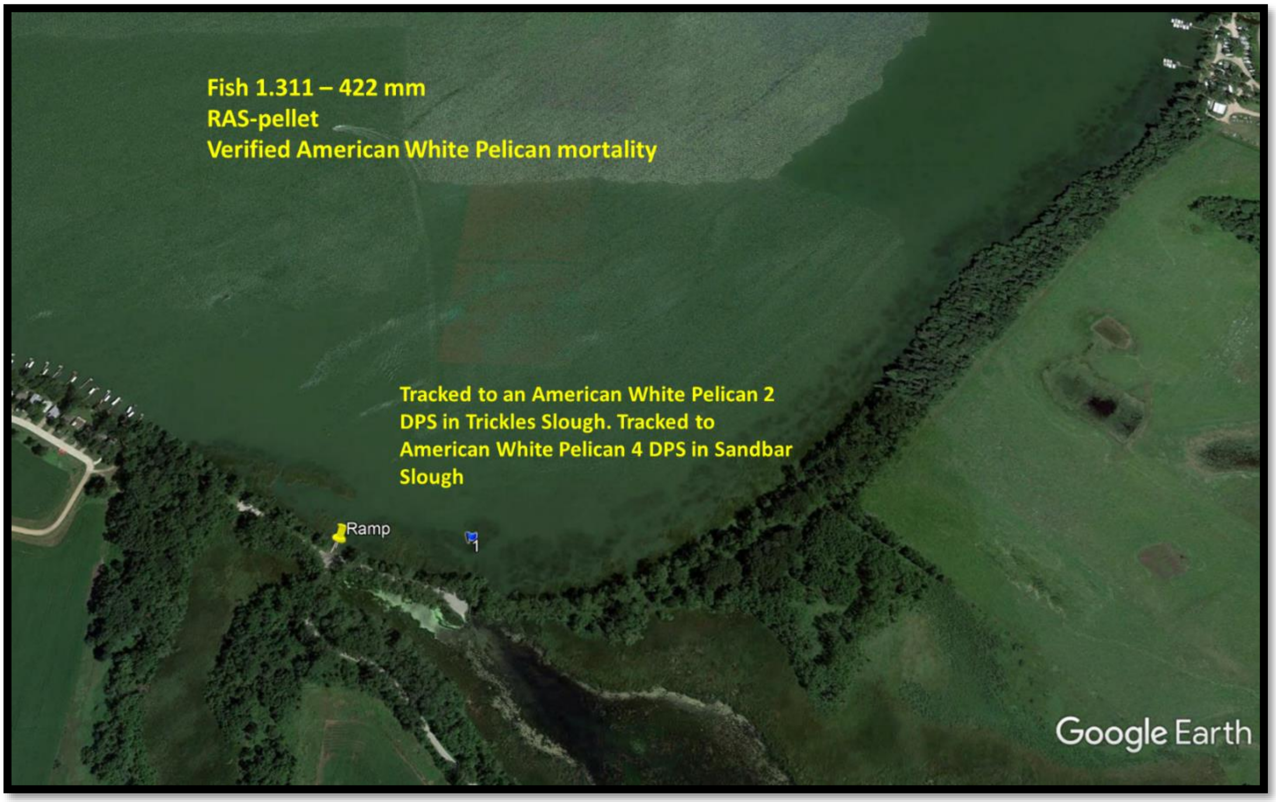

Fish 1.190 – 395 mm
RAS-minnow
Verified Great Blue Heron mortality
Tag located at Great Blue
Heron Rookery on
5/12/2020 – 18 DPS
Ramp
Google Earth

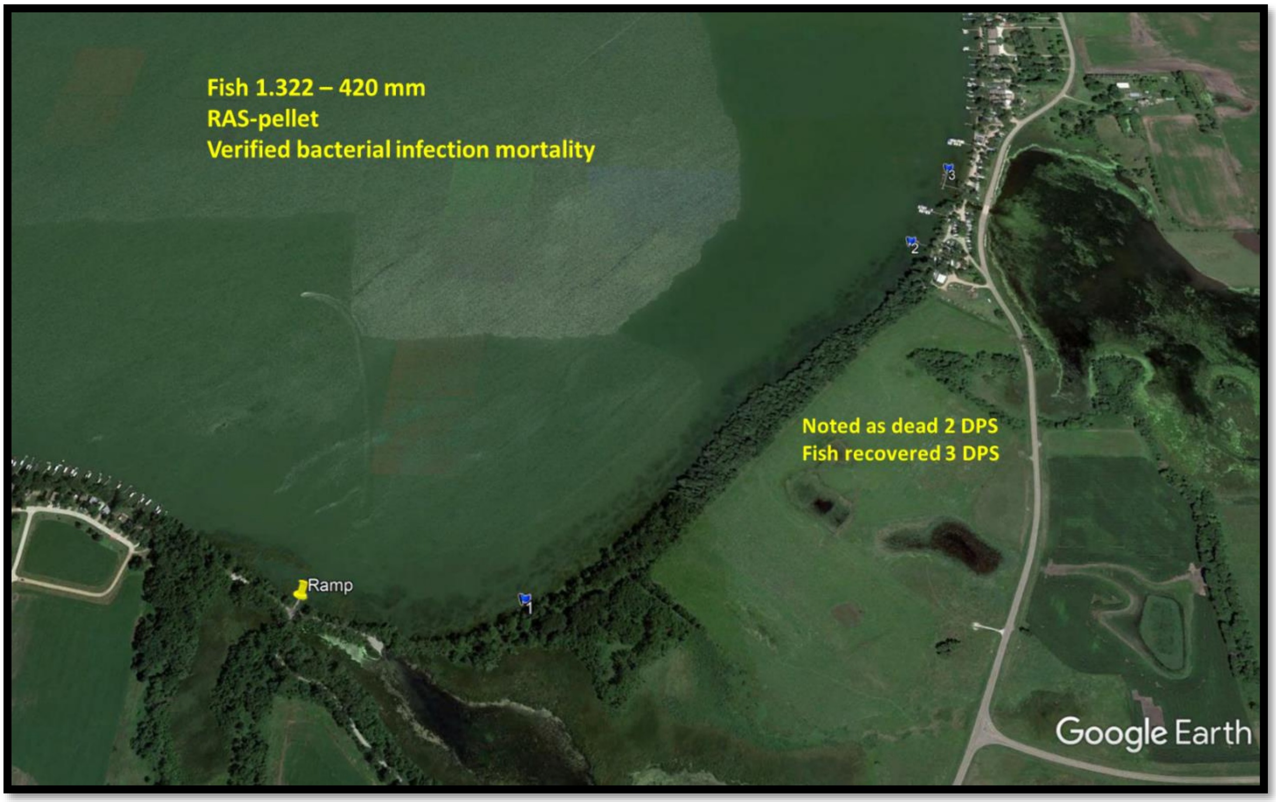

Fish 1.222 – 414 mm
RAS-minnow
Verified fish mortality
Visual observation of fish 10 DPS.
Tag recovered 40 DPS; was located in similar area on 26 and 33 DPS. Believed consumed by 18 DPS.
Ramp
Google Earth

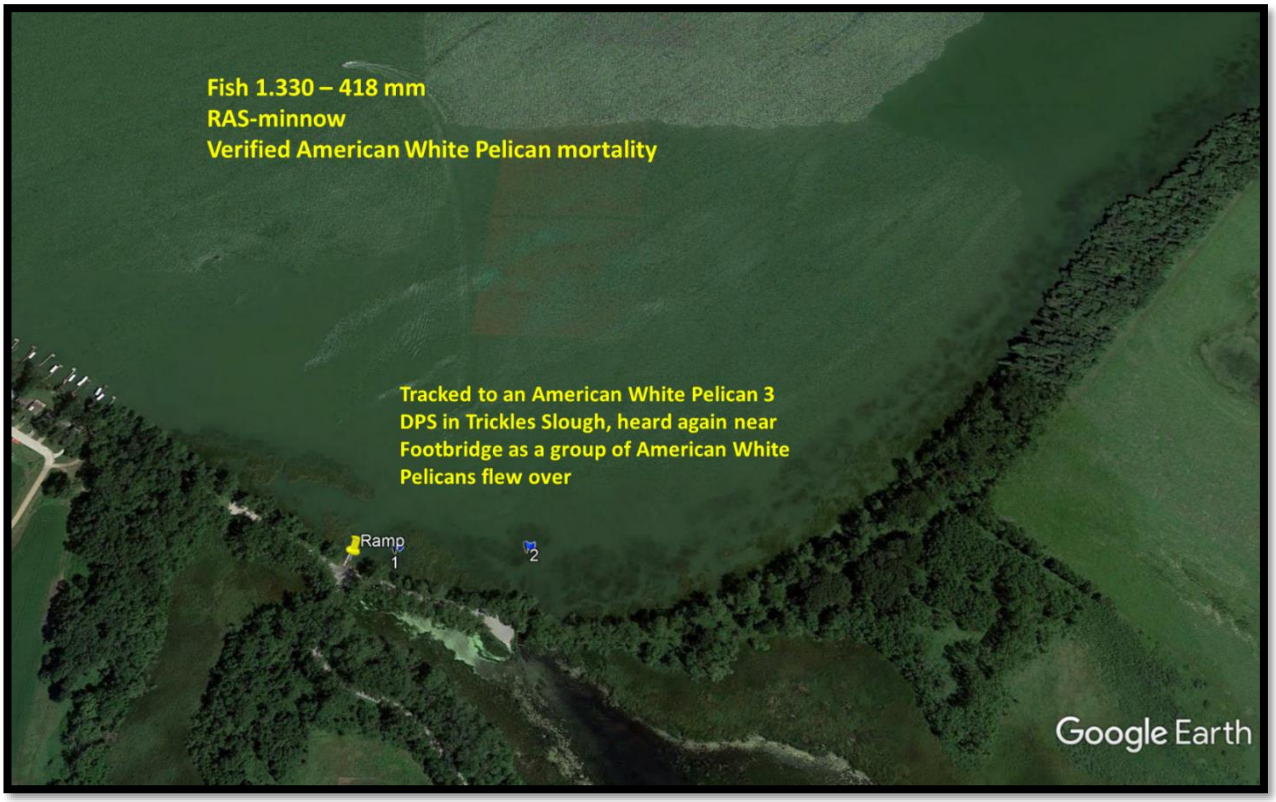

Fish 1.230 – 400 mm
RAS-minnow
Verified Great Blue Heron mortality
Tag located at Great Blue Heron Rookery on 5/14/2020 – 20 DPS
Ramp
Google Earth

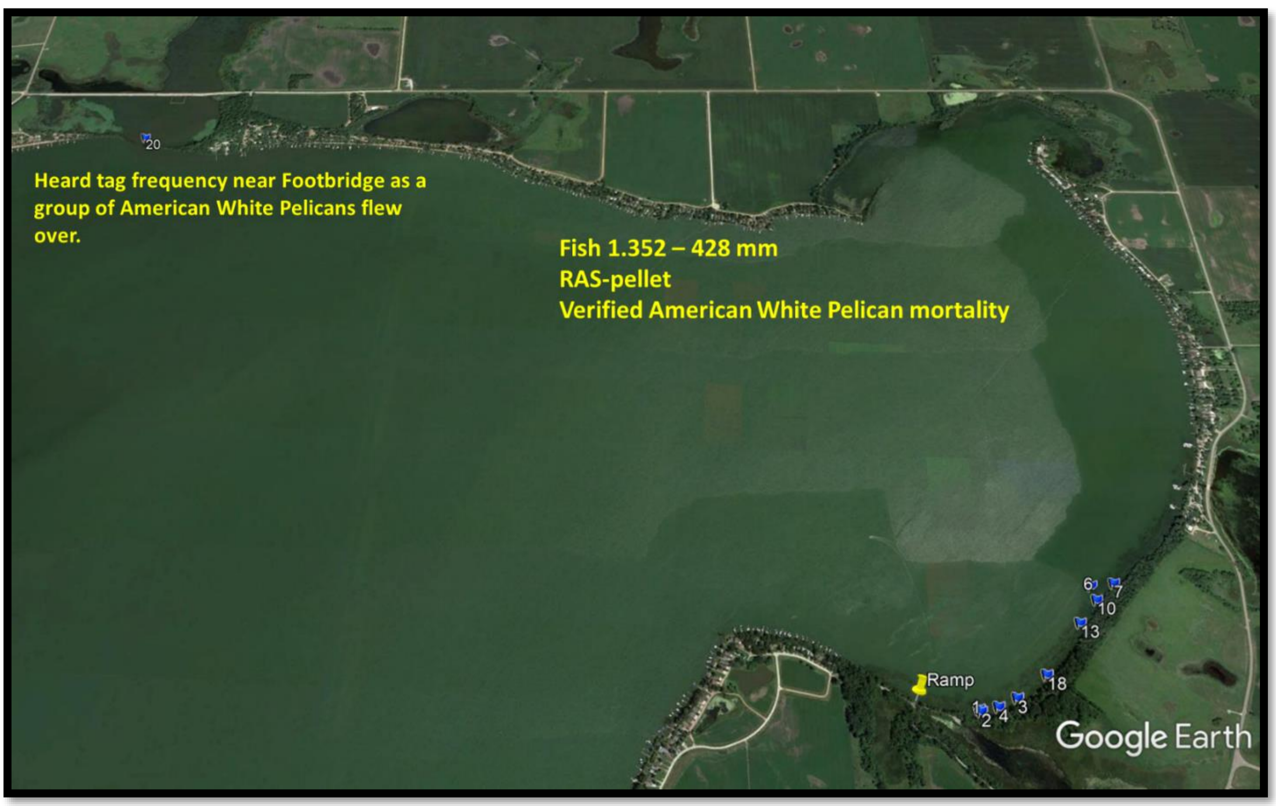

Fish 1.252 – 385 mm
RAS-minnow
Verified fish mortality
18
Location of deposited tag
Believe consumed by fish predator (Walleye) 10 DPS
Ramp
7 2 10
3
4
Google Earth

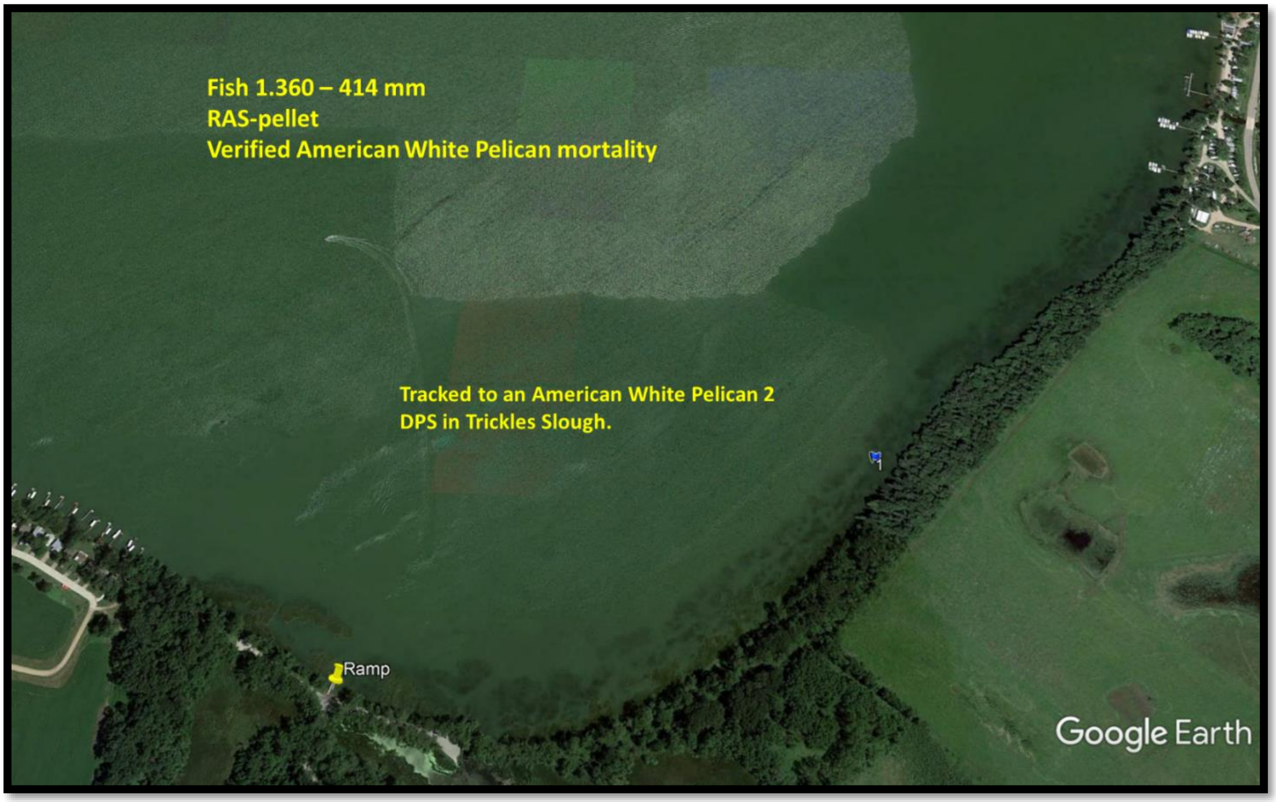

Fish 1.272 – 404 mm
RAS-minnow
Verified American White Pelican mortality
Tracked to an American White Pelican 3 DPS in Trickles Slough, heard again near Footbridge as a group of American White Pelicans flew over
Ramp
2
1
Google Earth


Fish 1.311 – 422 mm
RAS-pellet
Verified American White Pelican mortality
Tracked to an American White Pelican 2 DPS in Trickles Slough. Tracked to American White Pelican 4 DPS in Sandbar Slough
Ramp
1
Google Earth

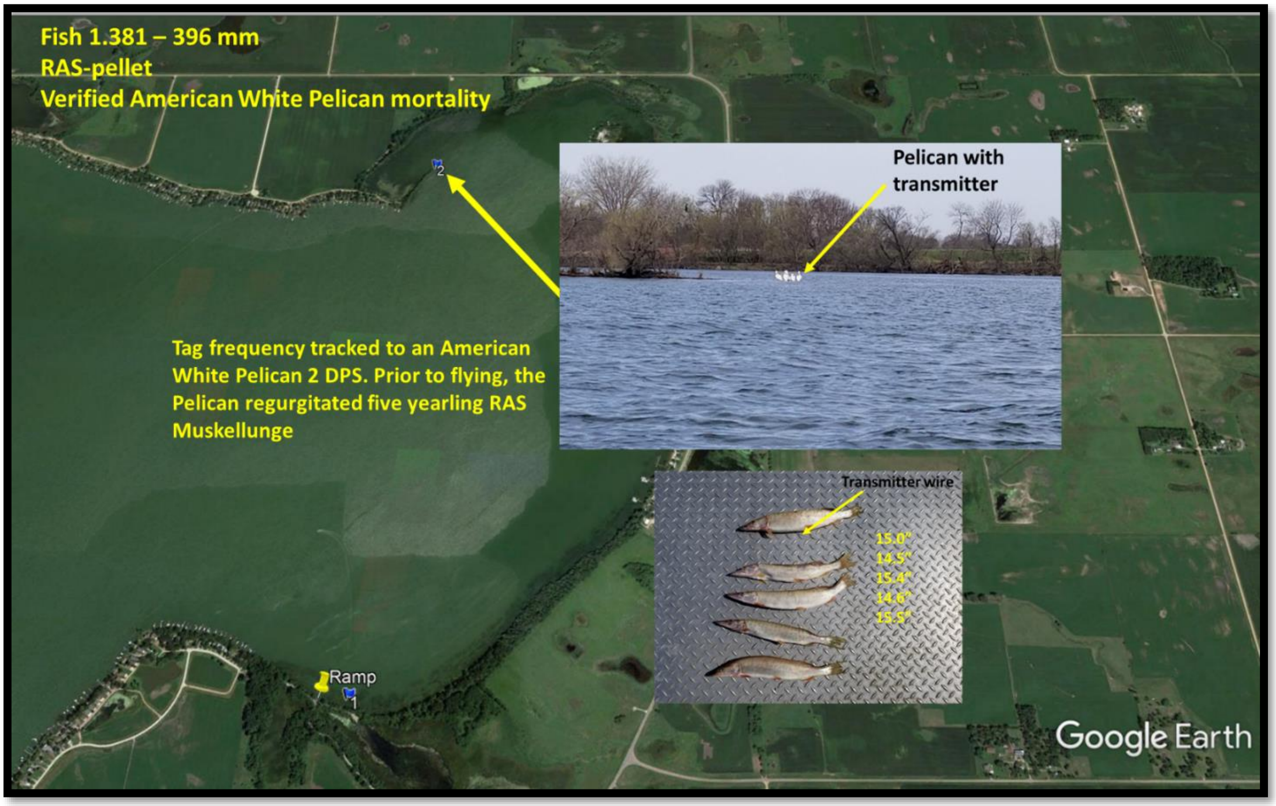

Fish 1.322 – 420 mm
RAS-pellet
Verified bacterial infection mortality
Noted as dead 2 DPS
Fish recovered 3 DPS
Ramp
1
2
3
Google Earth

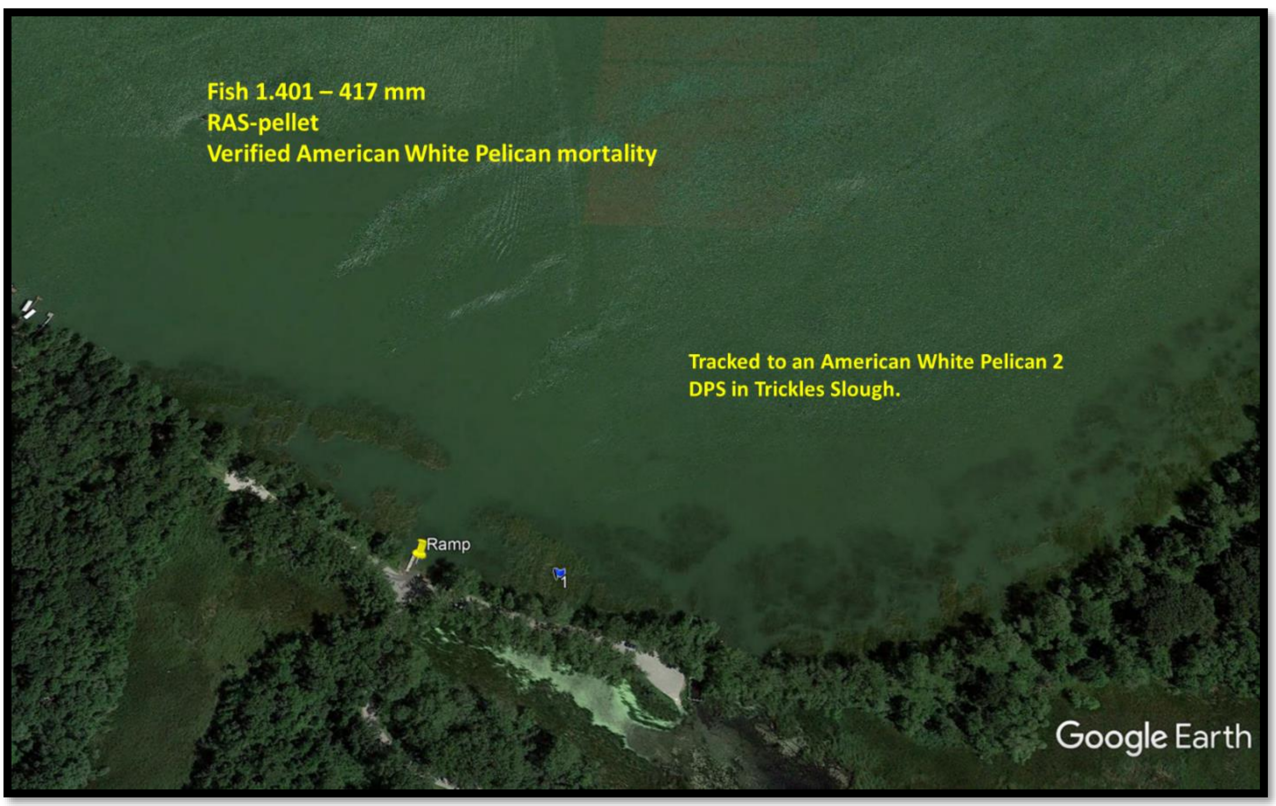

Fish 1.330 – 418 mm
RAS-minnow
Verified American White Pelican mortality
Tracked to an American White Pelican 3 DPS in Trickles Slough, heard again near Footbridge as a group of American White Pelicans flew over
Ramp
1
2
Google Earth


20
Heard tag frequency near Footbridge as a group of American White Pelicans flew over.
Fish 1.352 – 428 mm
RAS-pellet
Verified American White Pelican mortality
6
7
10
13
Ramp
18
1
2
4
3
Google Earth

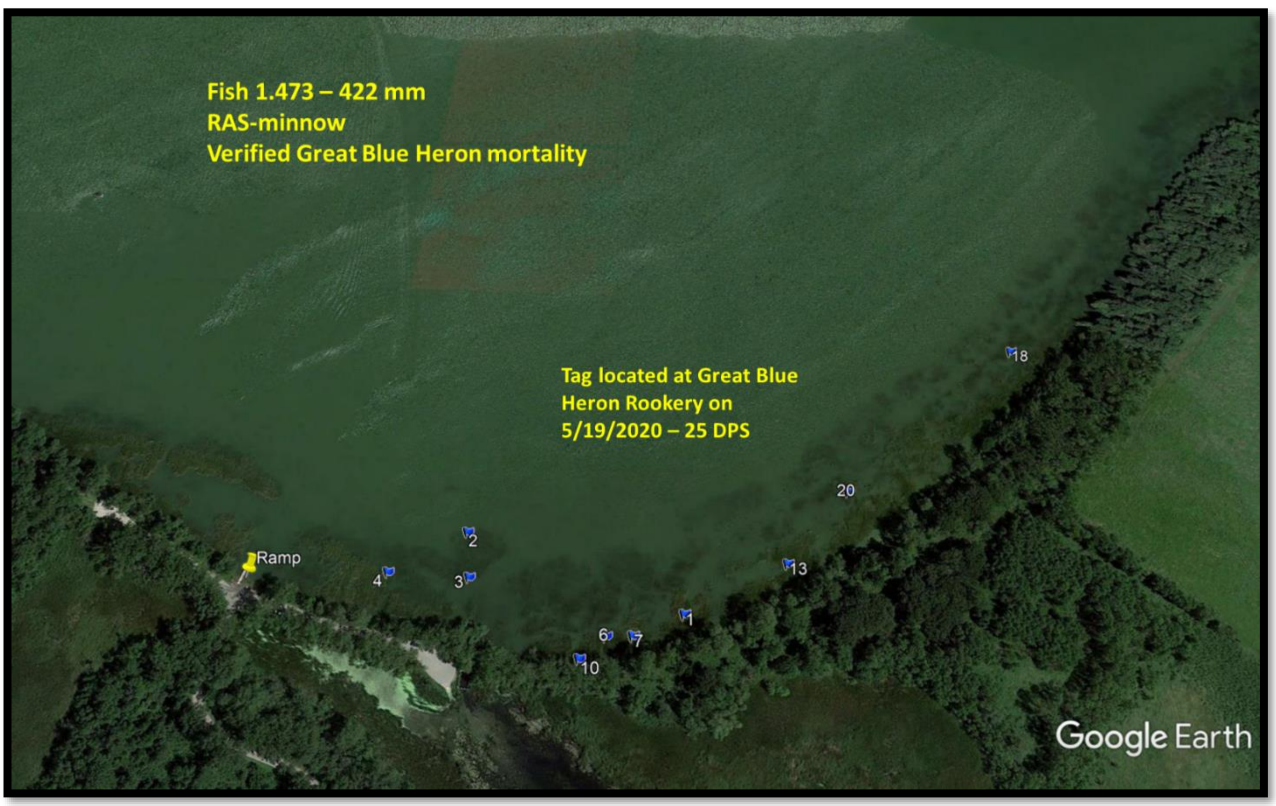

Fish 1.360 – 414 mm
RAS-pellet
Verified American White Pelican mortality
Tracked to an American White Pelican 2 DPS in Trickles Slough.
1
Ramp
Google Earth

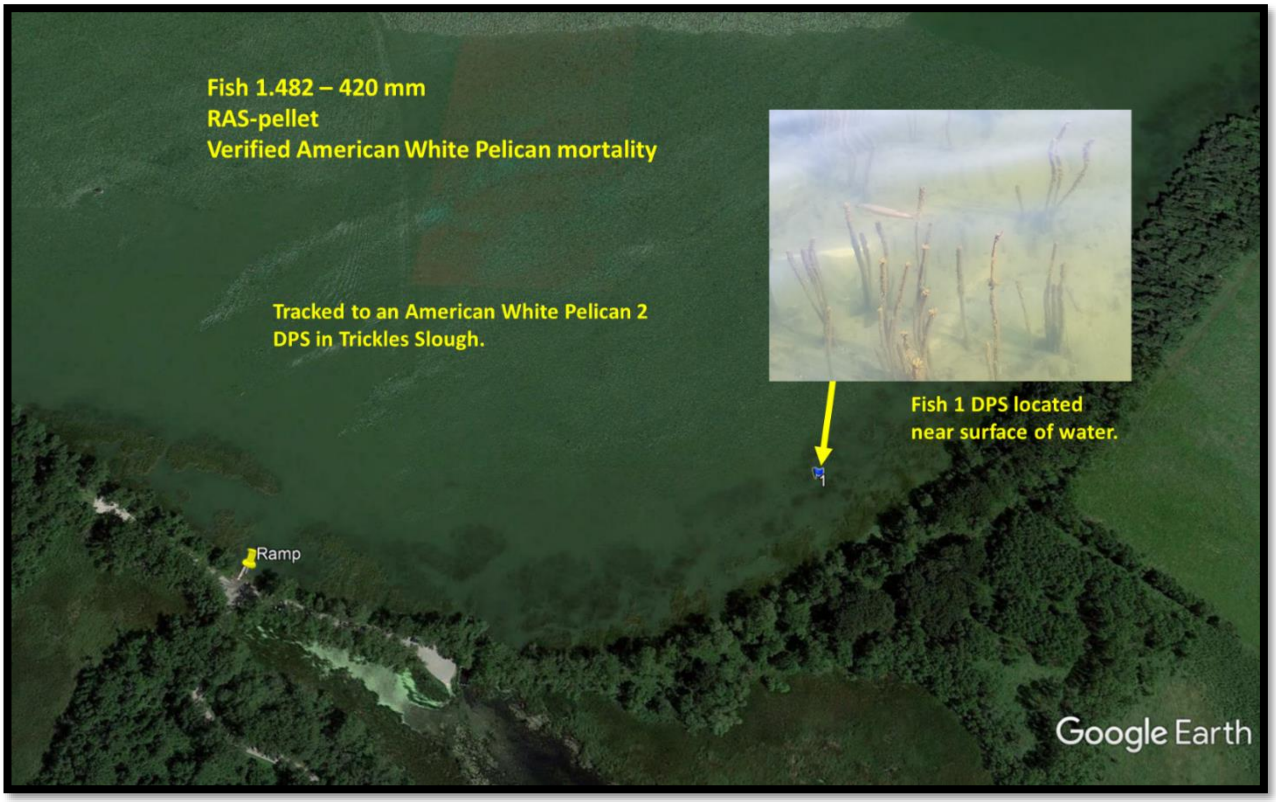

Fish 1.373 – 457 mm
RAS-pellet
Non-verified avian mortality
10
13
Ramp
2
1
4
3
7
6
Google Earth

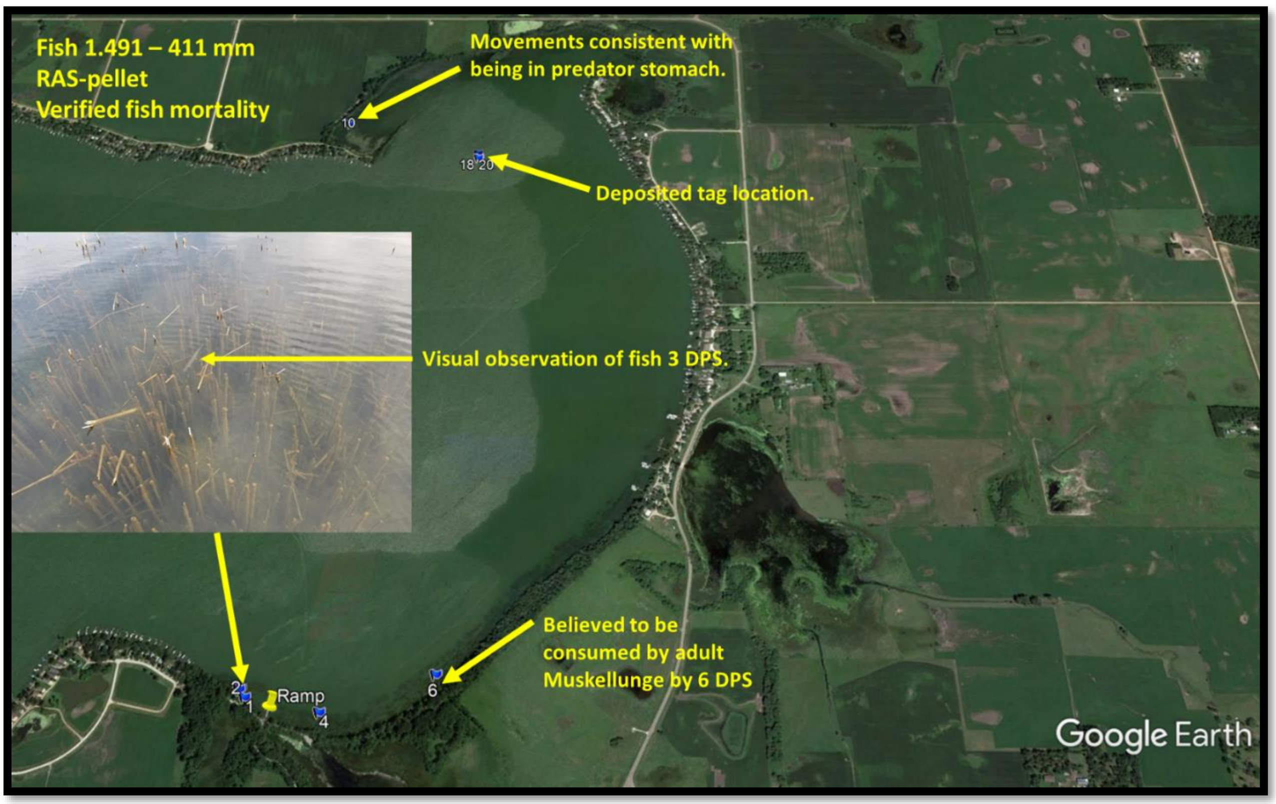

Fish 1.381 – 396 mm
RAS-pellet
Verified American White Pelican mortality
Pelican with transmitter
Tag frequency tracked to an American White Pelican 2 DPS. Prior to flying, the Pelican regurgitated five yearling RAS Muskellunge
Transmitter wire
15.0"
14.5"
15.4"
14.6"
15.5"
Ramp
Google Earth

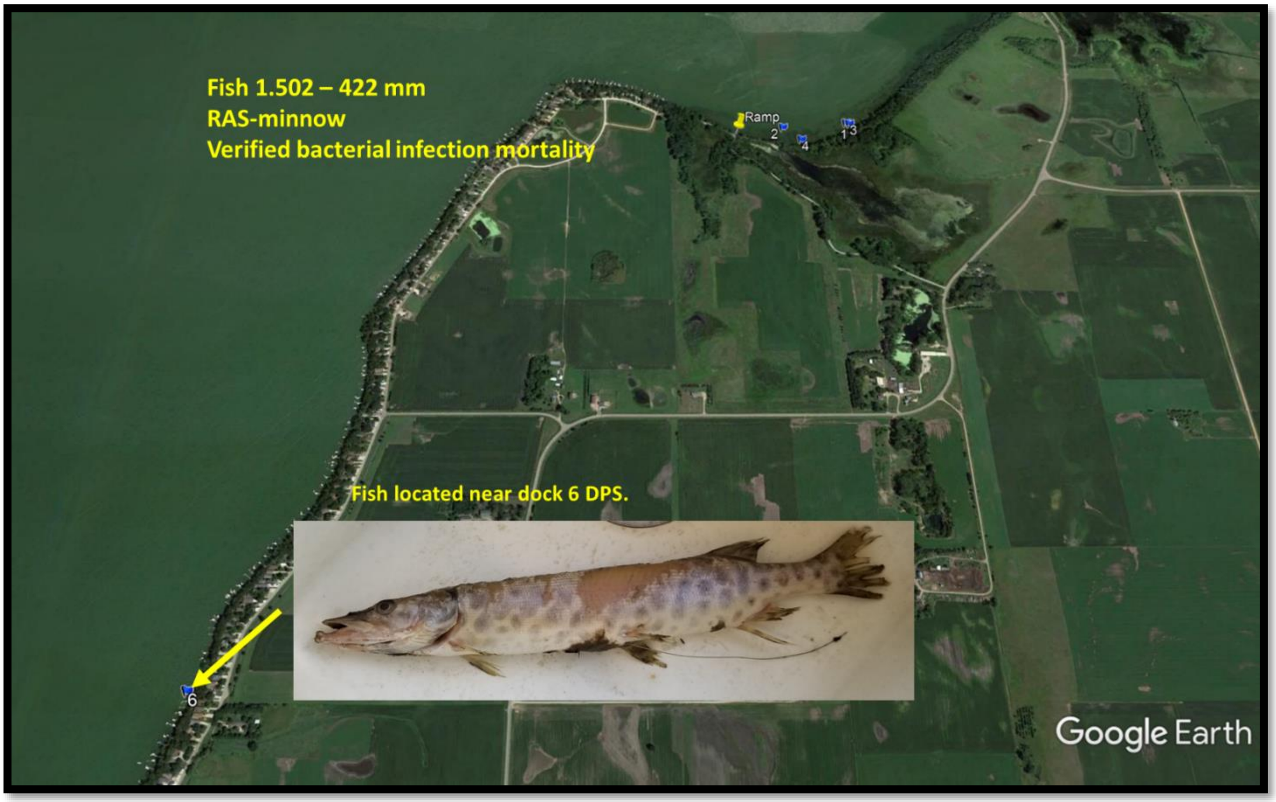

Fish 1.401 – 417 mm
RAS-pellet
Verified American White Pelican mortality
Tracked to an American White Pelican 2 DPS in Trickles Slough.
Ramp
Google Earth


Fish 1.462 – 432 mm
RAS-pellet
Verified Great Blue Heron mortality
Fish recovered dead on
5/1/2020 (7 DPS) with
wounds sustained via
Great Blue Heron attack.
4
6
7
Ramp
1
2
3
Google Earth


Fish 1.473 – 422 mm
RAS-minnow
Verified Great Blue Heron mortality
Tag located at Great Blue
Heron Rookery on
5/19/2020 – 25 DPS
18
20
Ramp
2
4
3
13
1
6
7
10
Google Earth


Fish 1.482 – 420 mm
RAS-pellet
Verified American White Pelican mortality
Tracked to an American White Pelican 2
DPS in Trickles Slough.
Fish 1 DPS located
near surface of water.
Ramp
Google Earth


Fish 1.491 – 411 mm
RAS-pellet
Verified fish mortality
Movements consistent with
being in predator stomach.
Deposited tag location.
Visual observation of fish 3 DPS.
Believed to be
consumed by adult
Muskellunge by 6 DPS
Ramp
Google Earth


Fish 1.502 – 422 mm
RAS-minnow
Verified bacterial infection mortality
Ramp
2
4
1
3
Fish located near dock 6 DPS.
6
Google Earth

Appendix 2. Data sheet used for tracking yearling Muskellunge raised in a recirculating aquaculture system and stocked into Spirit Lake on April 24, 2020.

Investigators ________________________

Date __________ **GPS Unit** __________

										Structure				
Fish ID	Way point	Time	Depth (ft)	Temp (F)	Secchi (ft)	Sub. Type	Hab. Type	Veg Cover	Veg Dens	Wood	Dock	Weedline	Rock	Additional Comments

Habitat Type: OP - open water; ED - edge
Substrate Type: 1=Silt(Soft), 2=Silt/Clay/Little Sand, 3=Sand/Mostly Sand, 4=Gravel/Rock/Hard Clay
Emergent/Submersed Vegetation Percent Coverage: 0=0%, 1=1-19%, 2=20-49%, 3=>50%
Emergent/Submersed Vegetation Density: 1=Sparse, 2=Dense
Structure (Wood laydowns/debris, Docks, Weedline, large rock): X if present