POPULATION DEMOGRAPHY OF A GLACIAL-RELICT STREAM FISH MEDIATED VIA ANTHROPOGENIC ALTERATION

By

Joseph M. Spooner

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Joseph M. Spooner, M.S.

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Advisor: Jonathan J. Spurgeon

Northern Pearl Dace *Margariscus nachtriebi* are a small-bodied glacial-relict fish species of greatest conservation need (SGCN) found throughout Canada and the northern United Sates. Their distribution within the Nebraska Sandhills Ecoregion is isolated from the northern core distribution of the species following the last glaciation period approximately 18,000 years ago. Headwater streams within the Nebraska Sandhills Ecoregion are predominately groundwater fed and provide the cool water temperatures needed to support Northern Pearl Dace and other glacial-relict SGCN. Headwater streams within the Nebraska Sandhills Ecoregion have been geomorphically altered through anthropogenic processes such as channelization whereby habitat homogenization has occurred. Evidence of stream habitat changes stemming from channelization directly influencing fish population demographic parameters is limited. Capture-mark-recapture studies used to estimate population demographic parameters may provide insight into the linkages between stream habitat alteration and influences on demographic parameters of fishes. However, limited methods exist to individually mark small-bodied fish <100mm that do not alter behavior or reduce survival of marked individuals. Here, we investigated 1) small-bodied fish survival and tag retention using p-Chip microtransponder tags, and 2) annual survival of Northern Pearl Dace in channelized stream sites within the Nebraska Sandhills Ecoregion. We found tag retention of p-Chip microtransponder tags

was high in small-bodied fish and did not affect fish survival. Northern Pearl Dace annual survival significantly differed between channelized and non-channelized stream sites. These results highlighted the utility of p-Chip microtransponder tags as effective marks for use in small-bodied fish where individual identification is needed. Further, these results indicate channelization may reduce Northern Pearl Dace survival when assuming complete site fidelity. As such, extensive channelization practices within the Nebraska Sandhills Ecoregion may ultimately influence distribution patterns of Northern Pearl Dace populations. Management efforts to increase Northern Pearl Dace populations may benefit from mitigation of channelized streams. Further research assessing spatiotemporal responses of Northern Pearl Dace to channel restoration practices may further refine specific habitat manipulation techniques and spatial distribution of habitat patches needed within watersheds.

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CHAPTER 1. THREATS TO FISH SPECIES OF GREATEST CONSERVATION NEED INHABITING HEADWATER STREAMS

Ecological Roles of Headwater Streams within River Networks

Freshwater systems contribute disproportionately to global biodiversity, including > 50% of known fish diversity, despite comprising approximately 3% of total aquatic resources. Lotic systems account for 0.0001% of freshwater and support diverse and unique fish assemblages across a gradient of stream sizes. For instance, large-river systems including the Amazon, Congo, and Mekong River basins hold approximately 33% of the globe's freshwater fish species (Winemiller et al. 2016). Further, headwater streams located within large-river basins comprise 70%-80% of global stream miles and contribute to maintaining and promoting biological diversity across the landscape (Downing et al. 2012, Su et al. 2021). Headwater streams support numerous biota that rely on specific habitat conditions during discrete periods or across their entire life history (Meyer et al. 2007).

Headwater streams include first and second-order stream segments and are characterized by either intermittent or perennial flow (Gomi et al. 2002, Meyer et al. 2007). Headwater streams originate where surfacing groundwater (e.g., springs, swales, etc.) or surface runoff maintain fluvial characteristics including erosion, transportation, or deposition of sediment (Richardson 2019). The spatial extent of a headwater stream is difficult to discern (Wohl 2017; Richardson and Dudgeon 2020). Alexander et al. 2007 defines a headwater stream by various topographic, hydrologic, and geometric properties through the Horton-Strahler classification (Horton 1945; Strahler 1957). In contrast, Gomi et al. (2002) defines the lower limits of a headwater stream by a tributary connection to another perennial stream. Further, Gomi et al. (2022) suggested potential problems with using Horton-Strahler's classification as demarcations for headwater streams. Specifically, stream orders depend on scales of maps, are modified by basin-scale topography (e.g., steep mountains versus plains) and are not suitable for explaining hydrologic, geomorphic, and biological processes, or the importance of headwater streams.

The diversity of headwater streams across the landscape facilitates multiple ecosystem processes and support unique biotic communities (Meyer et al. 2007, Richardson 2019). Finn et al. (2011) showed headwater streams in arid regions had greater relative isolation and lower species dispersal capabilities than headwater streams in wetter regions. The biological, geochemical, and physical processes that occur within headwater streams support the ecological health of entire drainage basins (Colvin et al. 2019). Alexander et al. (2007) showed that headwater streams account for 45% of the nitrogen load transported through the watershed. Allochthonous production dominates in headwater streams and contributes energy sources (e.g., detritus) downstream (Vannote et al. 1980). Headwater streams enhance flood protection and flood mitigation. Wahren et al. (2012) showed that riparian land-use practices such as forestation and increased catchment size of headwater streams can reduce downstream flooding when compared to urbanized headwater streams. Headwater streams provide both habitat and food resources for fish and other aquatic and riparian organisms across the drainage basin (Meyer et al. 2007; Richardson and Danehy 2007; Sullivan 2012; Hill et al. 2014; Richardson 2019). Furthermore, headwater streams contain many endemic and threatened fish species with distinct habitat needs (Colvin et al. 2019). For example, Hubbs (1995) described springs found in headwater streams which contained endemic fish species such as *Gambusia* in Texas due to thermal refugia. Headwater streams are diverse across the landscape and promote unique biotic communities due to distinct habitat characteristics such as low temperature variation, groundwater related water-chemistry, high edge area, and low disturbance frequency (Richardson 2019).

Headwater Stream Habitat and Fish Demographics

The distribution, abundance, and suitability of habitat drive population demographics of fish (Schlosser 1991). Habitat is defined as an area where an organism lives under a range of physical, chemical, and biological variables (e.g., the environment; Hudson et al. 1992). Habitat variables can be classified as consumable and non-consumable (Hayes et al. 1996). The availability of consumable habitat (cover, oxygen, prey resources, etc.) is density dependent and can be depleted by fish use and increased abundance (Hayes et al. 1996). Non-consumable habitat (temperature, turbidity, water velocity, sediment type, etc.) is not depleted by fish-use or abundance and is density independent. Both consumable and non-consumable habitat variables dictate presence or absence of fish species and may drive fish survival (Hayes et al. 1996). For example, pool depth and volume increased survival in Coho Salmon *Oncorhynchus kisutch* and Steelhead Trout *Oncorhynchus mykiss* (Woelfle-Erskine et al. 2017). Smiley et al. (2008) showed that instream habitat (macrophytes, wetted width, velocity, substrate, water depth, etc.) strongly influenced fish communities when compared to riparian habitat. A positive relation between the spatial and temporal availability of habitat and successful completion of different life stages facilitates the maintenance and expansion of a species' distribution (Rosenfeld and Hatfield 2006). Knowledge of habitat needs at different life stages may refine predictions regarding the influence of habitat management practices on fish demographics.

Habitat Alterations in Headwater Streams and the Influence on Fishes

Streams exist in a state of dynamic equilibrium where geomorphic, physical, and biotic conditions vary within a bounded range (Hack 1975). However, geomorphic, physical, and biotic changes to headwater streams, beyond the bounds of naturally occurring conditions, may negatively influence population demographics of stream fishes (Vandenberghe et al. 2011). Alteration of headwater streams is widespread (Richardson 2019). Fragmentation and channelization are two of many processes that have significantly changed ecosystem processes of headwater streams with implications for fishes (Richardson 2019; Richardson and Dudgeon 2020).

Fragmentation reduces habitat amount, increases number of habitat patches, decreases habitat patch size, and increases isolation of habitat patches (Fahrig 2003). Causes of stream fragmentation stem from both natural and anthropogenic processes. Fragmentation occurs naturally from fluvial-geomorphic (e.g., waterfalls) and biotic (e.g., beaver dams) features. Anthropogenic causes of fragmentation include—but are not limited to—dams and road crossings (Wilcove et al. 1986; Fagan 2002; Fuller et al. 2015; Bouwes et al. 2016). A predominate consequence of fragmentation is the decrease in movement by stream fishes (Fagan 2002; Colvin et al. 2019; Richardson and Dudgeon 2020). For example, Walters et al. (2014) showed that dams significantly decreased Flathead Chub *Platygobio gracilis* upstream spawning movement. Reducing movement may create an isolation of populations and enhance the chance of extirpation of species from a once native range by cutting off a potential source population.

Channelization furthers stream habitat alteration and may act synergistically with fragmentation (Smiley et al. 2008; Richardson and Dudgeon 2020). Channelization is the straightening and deepening of existing stream channels. Headwater streams are particularly vulnerable to alteration via channelization due to their relative size and position in the watershed (Richardson 2019). Further, headwater streams are likely to be both channelized and highly fragmented via culvert-type stream crossings with implications for distribution of stream fishes. The ecological ratchet concept in stream systems suggests changes to fish community composition are due to the synergistic effects of fragmentation and hydrologic disturbance through time. Pelagic fishes may eventually become extirpated from an area caused by interactions between habitat fragmentation and drought (Perkin et al. 2014).

Channelization effects stream hydrologic functions and habitat heterogeneity that combine to change fish populations and assemblage structure. Channelization alters fluvial geomorphic features of a stream including stream width, depth, slope, and sinuosity (Wyllie et al. 1985). Changes to fluvial geomorphic features may negatively affect floodplain connectivity, average depth, water velocity, water temperature, and instream physical structure all of which are drivers for fish population demographics and assemblage structure (Brooker 1985; Smiley et al. 2008; Smiley et al. 2009; Smiley et al. 2017). Brown et al. (2008) showed that habitat features including bank height, bank width, plant richness, and percent of canopy cover of channelized streams significantly differed from non-channelized streams. Increased water velocity from slope and sinuosity changes decreases mesohabitat heterogeneity and may result in extended run habitat with few pools.

Species of Greatest Conservation Need in Nebraska Including Northern Pearl Dace

Headwater streams in Nebraska, USA provide suitable habitat for fish species of greatest conservation need (SGCN) at-risk of extinction or extirpation from a significant portion of their distribution. For instance, the state listed and federally endangered Topeka Shiner *Notropis topeka*, as well as many other threatened species such as the Blacknose Shiner *Notropis heterolepis*, Northern Pearl Dace *Margariscus nachtriebi*, Northern Redbelly Dace *Chrosomus eos*, Finescale Dace *Chrosomus neogaeus*, and Plains Topminnow *Fundulus sciadicus* exist within headwater streams of Nebraska. There are thirty-two fish SGCN in Nebraska and 16 are in the Cyprinidae family. Three genera of dace including *Rhinichthys, Margariscus, and Chrosomus* are represented by a total of five species comprised of four fish SGCN. Dace species inhabit cool, clear, and slow-moving water predominately restricted to only a few headwater streams within select ecoregions of Nebraska. If these streams were to degrade substantially, these species may be extirpated due to their restricted ranges, distinct habitat needs, and connections to other populations.

Northern Pearl Dace *Margariscus nachtriebi* are a small-bodied glacial-relict fish SGCN found throughout Canada and the northern U.S.A. Their distribution within the Nebraska Sandhills Ecoregion was isolated from the northern core distribution during the last glaciation period 18,000 years ago to create a unique subpopulation (Lee et al. 1980). Northern Pearl Dace in Nebraska reside in prairie streams of the Sandhills Ecoregion. Headwater streams in the Sandhills Ecoregion are often highly connected to groundwater (Pasbrig 2013). As such, Sandhills Ecoregion headwater streams exhibit cool temperatures throughout the year and largely maintain perennial flow. Northern Pearl Dace are an important indicator species that is intolerant of degradation including decreased macrophyte coverage, incision of the stream channel, and sedimentation caused by stream geomorphic changes via channelization (Pasbrig 2013). Northern Pearl Dace use a diversity of habitat types including slow-moving, cool-water streams with meandering channels, well-vegetated undercut banks, and pool habitats (Tallman 1979; Tallman and Gee 1982; Cunningham 1995; Cunningham 2006; Hrabik et al. 2015). Northern Pearl Dace use different habitats based on age and season where individuals older than age 2 used pools greater than 50cm deep and velocities less than 5cm s^{-1} and age-0 individuals used shallow pools and riffles until the fall when they shift towards deeper pools (Tallman 1979).

Capture-Mark-Recapture of Small-bodied Fish to Obtain Demographic Estimates

Estimating population demographics of small-bodied fish SGCN is challenging, and using capture-recapture data can be difficult given limited tagging techniques and minimal recapture data. Individual marking techniques for small-bodied fish may bias parameter estimates given assumptions of capture-mark-recapture (CMR) study designs. For instance, violation of assumptions in CMR study designs including increases in fish mortality due to the tagging process or fish losing tags when estimating fish demographic rates may bias parameter estimates lower than expected. Individual tags such as 8mm PIT tags are commonly used for tagging small-bodied fish species. However, the use of 8mm PIT tags may not affect fish survival (Ficke et al. 2012; Pennock et al. 2016; Swarr et al. 2021) or may negatively influence fish survival (Musselman 2007; Schumann et al. 2020). Furthermore, 8mm PIT tags may have high tag retention (Bolland et al. 2009; Swarr et al. 2021) or low tag retention (Johnston and Smithson 1999; Pennock et al. 2016) prompting the need to specifically study small-bodied fish species of interest.

The p-Chip microtransponder tag (PharmaSeq 2017) may serve as a new alternative method for individual identification of small-bodied fishes (Moore and Brewer 2021). P-Chip microtransponders have been used in two small-bodied fish lab-studies including Arkansas River Shiner *Notropis girardi* (Moore and Brewer 2021) and larval European Sea Bass *Dicentrarchus labrax* (Faggion et al. 2020). Moore and Brewer (2021) showed that p-Chip microtransponder tags were retained significantly higher than 8mm PIT tags in Arkansas River Shiners. Further, Arkansas River Shiners with p-Chip microtransponder tags showed similar survival rates to control fish. However, limited data exists on the overall performance in field-based experiments and across multiple fish species for p-Chip microtransponder tags. Therefore, an assessment of p-Chip microtransponder tags for use on Northern Pearl Dace including their influence on survival and tag retention rates is needed to alleviate concerns of fish mortality due to tagging and potential low tag retention assumptions within CMR study designs.

There exists limited understanding regarding influences of stream geomorphic change on Northern Pearl Dace population demographics within Sandhills Ecoregion streams. Quantifying population demographic response to geomorphic alterations including channelization can inform understanding how degradation and restoration of distinct habitat features at multiple spatial scales could impact Northern Pearl Dace populations. Due to the lack of knowledge on p-Chip microtransponders for tagging small-bodied fish and survival of Northern Pearl Dace in geomorphically altered streams, this study has addressed the following questions and objectives:

1) Do p-Chip microtransponder tags serve as an alternative individual marking technique for small-bodied fish?

   a. Estimate survival and tag-retention following p-Chip microtransponder tag implantation in Creek Chub and Northern Pearl Dace in a 90-day laboratory environment.
   b. Estimate tag retention of p-Chip microtransponder tags in Northern Pearl Dace over a year-long field trial.

2) How do varying fluvial-geomorphic conditions and changes to instream habitat resulting from channelization influence population demographics including survival, movement, and abundance of Northern Pearl Dace in Sandhills Ecoregion headwater streams?
   a. Determine if differences occur in geomorphic characteristics, and instream habitat including mesohabitat, depth, and macrophyte coverage in channelized verses non-channelized stream sites in the Nebraska Sandhills Ecoregion.
   b. Estimate annual survival of Northern Pearl Dace in channelized and non-channelized sites in the Nebraska Sandhills Ecoregion.

Literature Cited

Alexander, R. B., E. W. Boyer, R. A. Smith, G. E. Schwarz, and R. B. Moore. 2007. The role of headwaters streams in downstream water quality. JAWRA Journal of the American Water Resources Association 43(1):41–59.

Bolland, J. D., Cowx, I. G., & Lucas, M. C. 2009. Evaluation of VIE and PIT tagging methods for juvenile cyprinid fishes. Journal of Applied Ichthyology 25:381–386.

Bouwes, N., N. Weber, C. E. Jordan, W. C. Saunders, I. A. Tattam, C. Volk, J. M. Wheaton, and M. M. Pollock. 2016. Ecosystem experiment reveals benefits of natural and simulated beaver dams to a threatened population of Steelhead (*Oncorhynchus mykiss*). Scientific Reports PMC4931505.

Brooker, M. P. 1985. The ecological effects of channelization. The Geographical Journal 151(1):63–69.

Brown, T. T., T. L. Derting, and K. Fairbanks. 2008. The effects of stream channelization and restoration on mammal species and habitat in riparian corridors. Journal of the Kentucky Academy of Science 69(1):37–49.

Colvin, S. A. R., S. M. P. Sullivan, P. D. Shirey, R. W. Colvin, K. O. Winemiller, R. M. Hughes, K. D. Fausch, D. M. Infante, J. D. Olden, K. R. Bestgen, R. J. Danehy, and L. Eby. 2019. Headwater streams and wetlands are critical for sustaining fish, fisheries, and ecosystem services. Fisheries 44(2):73–91.

Cunningham, G. R. 1995. Life history traits and reproductive ecology of the Pearl Dace, *Margariscus margarita* (Pisces: Cyprinidae) in the Sandhills of Nebraska. M.A. Thesis. University of Nebraska-Omaha, Omaha, Nebraska.

Cunningham, G. R. 2006. Pearl Dace (*Margariscus margarita):* a technical conservation assessment. USDA Forest Service, Rocky Mountain Region. Available: https://www.nrc.gov/docs/ML1004/ML100481436.pdf. (December 2021)

Downing, J. A., J. J. Cole, C. M. Duarte, J. J. Middleburg, J. M. Melack, Y. T. Prairie, P. Kortelainen, R. G. Striegl, W. H. McDowell, and L. J. Tranvik. 2012. Global abundance and size distribution of streams and rivers. Inland Waters 2(4):229–236.

Fagan, W. F. 2002. Connectivity, fragmentation, and extinction risk in dendritic metapopulations. Ecology 83(12):3243–3249.

Faggion, S., P. Sanchez, M. Vandeputte, F. Clota, A. Vergnet, M. O. Blanc, and F. Allal. 2020. Evaluation of a European Sea Bass (*Dicentrarchus labrax L.*) post-larval tagging method with ultra-small RFID tags. Aquaculture 520:734945–734956.

Fahrig, L. 2003. Effects of habitat fragmentation on biodiversity. Annual Review of Ecology, Evolution, and Systematics 34:487–515.

Ficke, A. D., Myrick, C. A., & Kondratieff, M. C. (2012). The effects of PIT tagging on the swimming performance and survival of three nonsalmonid freshwater fishes. Ecological Engineering 48:86–91.

Finn, D. S., N. Bonada, C. Murria, and J. M. Hughes. 2011. Small but mighty: Headwaters are vital to stream network biodiversity at two levels of organization. Freshwater Science 30(4):963–980.

Fuller, M. R., M. W. Doyle, and D. L. Strayer. 2015. Causes and consequences of habitat fragmentation in river networks: river fragmentation. Annals of the New York Academy of Sciences 1355(1):31–51.

Gomi, T., R. C. Sidle, and J. S. Richardson. 2002. Understanding processes and downstream linkages of headwater systems. BioScience 52(10):905–916.

Hack, J. T. 1975. Dynamic equilibrium and landscape evolution. Pages 87–1021 *in* W. N. Melhorn, editor. Theories of landform development. State University of New York, Albany New York.

Hayes, D. B., C. P. Ferreri, and W. W. Taylor. 1996. Linking fish habitat to their population dynamics. Canadian Journal of Fisheries and Aquatic Sciences 53(S1):383–390.

Hill, B. H., R. K. Kolka, F. H. McCormick, and M. A. Starry. 2014. A synoptic survey of ecosystem services from headwater catchments in the United States. Ecosystem Services 7:106–115.

Hrabik, R. A., S. C. Schainost, R. H. Stasiak, and E. J. Peters. 2015. The fishes of Nebraska. University of Nebraska-Lincoln, Lincoln, NE.

Hubbs, C, 1995. Springs and spring runs as unique aquatic systems. Copeia 1995(4):989-991.

Hudson, P. L., R. W. Griffiths, and T. J. Wheaton. 1992. Review of habitat classification schemes appropriate to streams, rivers, and connecting channels in the Great Lakes drainage basin. Pages 73–107 *in* W. D. N. Busch and P. G. Sly, editors. The development of an aquatic habitat classification system for lakes. CRC Press, Ann Arbor, Michigan.

Johnston, C. E., and E. B. Smithson. 1999. Retention of passive integrated transponder (PIT) tags for individual identification of warmwater stream fishes. Proceedings of the Southeastern Fishes Council 38:1–4.

Lee, D. S., C. R. Giber, C. H. Hocutt, R. E. Jenkins, D. E. McAllister, and J. R. Stauffer, Jr. 1980. Atlas of North American freshwater fishes. The North Carolina Biological Survey, North Carolina.

Meyer, J. L., D. L. Strayer, J. B. Wallace, S. L. Eggert, G. S. Helfman, and N. E. Leonard. 2007. The contribution of headwater streams to biodiversity in river networks. JAWRA Journal of the American Water Resources Association 43(1):86–103.

Moore, D. M., and S. K. Brewer. 2021. Evaluation of visual implant elastomer, PIT, and p-chip tagging methods in a small-bodied minnow species. North American Journal of Fisheries Management 41:1066–1078.

Musselman, W. C. 2007. The importance of maintaining shallow-water habitats for the movement and survival of stream fishes. (Master's thesis. University of Missouri, Columbia, MO). Retrieved from The importance of maintaining shallow-water habitats for the movement and survival of stream fishes - ProQuest

Pasbrig, C. 2013. The glacial relict fishes of South Dakota’s sandhills. American Currents 39(1):29–31.

Pennock, C. A., B. D. Frenette, M. J. Waters, and K. B. Gido. 2016. Survival of and tag retention in Southern Redbelly Dace injected with two sizes of PIT tags. North American Journal of Fisheries Management 36:1386–1394.

Perkin, J. S., K. B. Gido, K. H. Costigan, M. D. Daniels, and E. R. Johnson. 2014. Fragmentation and drying ratchet down Great Plains stream fish diversity. Aquatic Conservation: Marine and Freshwater Ecosystems 25(5):639–655.

PharmaSeq. 2017. Adult Zebrafish p-Chip® Implantation Protocol. Available: https://www.isenet.it/wp-content/uploads/2017/01/PharmaSeq_Zebrafish_Tagging_Protocol-1.pdf. (October 2023)

Richardson, J. S. 2019. Biological diversity in headwater streams. Water 11:366–385.

Richardson, J. S., and D. Dudgeon. 2020. Headwater stream ecosystems: an initial evaluation of their threat status. In: Earth Systems and Environmental Sciences. Elsevier.

Richardson, J. S., and R. J. Danehy. 2007. A synthesis of the ecology of headwater streams and their riparian zones in temperate forests. Forest Science 53(2):131–147.

Rosenfeld, J. S., and T. Hatfield. 2006. Information needs for assessing critical habitat of freshwater fish. Canadian Journal of Fisheries and Aquatic Sciences 63:683–698.

Schlosser, I. J. 1991. Stream fish ecology: a landscape perspective. BioScience 41(10):704–712.

Schumann, D. A., Graeb, K. N., Wagner, M. D., Graeb, B. D. S., Prenosil, E., & Hoekwater, J. 2020. Suitability of surgically implanted 8-mm passive integrated transponder tags for small-bodied fishes. Journal of Applied Ichthyology 36:682–692.

Smiley, P. C., R. B. Gillespie, K. W. King, and C. Huang. 2008. Contribution of habitat and water quality to the integrity of fish communities in agricultural drainage ditches. Journal of Soil and Water Conservation 63(6):218A–219A.

Smiley, P. C., R. B. Gillespie, K. W. King, and C. Huang. 2009. Management implications of the relationships between water chemistry and fishes within channelized headwater streams in the midwestern United States. Ecohydrology 2:294–302.

Smiley, P. C., K. W. King, and N. R. Fausey. 2017. Influence of different habitat factors on Creek Chub (*Semotilus atromaculatus*) within channelized agricultural headwater streams. Northeastern Naturalist 24(8):18–44.

Su, G., M. Logez, J. Xu, S. Tao, S. Villeger, and S. Brosse. 2021. Human impacts on global freshwater fish biodiversity. Science 371(6531):835–838.

Sullivan, S. M. P. 2012. Geomorphic-ecological relationships highly variable between headwater and network mountain streams of northern Idaho, United States. Journal of the American Water Resources Association 1(12):1221–1232.

Swarr, T. R., C. A. Myrick, and R. M. Fitzpatrick. 2021. Tag retention in and effects of passive integrated transponder tagging on survival and swimming performance of a small-bodied darter. Journal of Fish Biology 100(3):705–714.

Tallman, R. F. 1979. Environments occupied, indices of maturity, feeding ecology, shoaling behavior and interactions with other species by Pearl Dace, *Semotilus margarita* (Cope) in Manitoba. Master's thesis. University of Manitoba, Winnipeg.

Tallman, R. F., and J. H. Gee. 1982. Intraspecific resource partitioning in a headwaters stream fish, the Pearl Dace, *Semotilus margarita* (Cyprinidae). Environmental Biology of Fishes 7:243–249.

Vandenberghe, J., G. Venhuizen, and J. de Moor. 2011. Concepts of dynamic equilibrium of interest for river management in the lower Maas catchment. Geographia Polonica 84(Special Issue Part 2):141–153.

Vannote, R. L., G. W. Minshall, K. W. Cummins. J. R. Sedell, and C. E. Cushing. 1980. The river continuum concept. Canadian Journal of Fisheries and Aquatic Sciences 37:130–137.

Wahren, A., K. Schwärzel, and K. H. Feger. 2012. Potentials and limitations of natural flood retention by forested land in headwater catchments: evidence from experimental and model studies. Journal of Flood Risk Management 5(4):321–335.

Walters, D. M., R. E. Zuellig, H. J. Crockett, J. F. Bruce, P. M. Lukacs, and R. M. Fitzpatrick. 2014. Barriers impede upstream spawning migration of Flathead Chub. Transactions of the American Fisheries Society 143:17–25.

Wilcove, D. S., C. H. McLellan, and A. P. Dobson. 1986. Habitat fragmentation in the temperate zone. Pages 237–256 *in* M. E. Soulé, editor. Sinauer Associates, Incorporated, Sunderland, Massachusetts.

Winemiller, K. O., P. B. McIntyre, L. Castello, E. Fluet-Chouinard, T. Giarrizzo, S. Nam, I. G. Baird, W. Darwall, N. K. Lujan, I. Harrison, M. L. J. Stiassny, R. A. M. Silvano, D. B. Fitzgerald, F. M. Pelicice, A. A. Agostinho, L. C. Gomes, J. S. Albert, E. Baran, M. Petrere, C. Zarfl, M. Mulligan, J. P. Sullivan, C. C. Arantes, L. M. Sousa, A. A. Koning, D. J. Hoeinghaus, M. Sabaj, J. G. Lundberg, J. Armbruster, M. L. Thieme, P. Petry, J. Zuanon, G. T. Vilara, J. Snoeks, C. Ou, W. Rainboth, C. S. Pavanelli, A. Akama, A. van Soesbergen, and L. Sáenz. 2016. Balancing hydropower and biodiversity in the Amazon, Congo, and Mekong. Science 351(6269):128–129.

Woelfle-Erskine, C., L. G. Larsen, and S. M. Carlson. 2017. Abiotic habitat thresholds for salmonid over-summer survival in intermittent streams. Ecosphere 8(2):1–23.

Wohl, E. 2017. The significance of small streams. Frontiers of Earth Science 11(3):447–456.

Wyllie, P. J., D. K. Wojcik, P. Raven, F. G. Charlton, J. Fitzsimons, R. D. Hey, P. B. Spillett, G. Petts, W. B. Harris, and K. S. Richards. 1985. The impact of river channelization: discussion. The Geographical Journal 151(1):69–74

CHAPTER 2. RETENTION OF P-CHIP MICROTRANSPONDERS AND POST-TAGGING SURVIVAL OF SMALL-BODIED STREAM FISHES

Abstract

Obtaining demographic rates often requires complex open-population capture-mark-recapture (CMR) study designs. Conducting such studies for small-bodied fishes has been limited in part by excessive mortality following tagging procedures and poor tag retention. As new tag types emerge, information regarding fish survival and tag retention over varying timescales may benefit resource managers to effectively plan future CMR studies. The p-Chip microtransponder tag has been used on a limited number of small-bodied fishes with relatively high rates for fish survival and tag retention compared to other tag types. However, information pertaining to post-tagging survival and tag retention of p-Chip microtransponders across a range of small-bodied fish species and tagging locations is needed to inform their effectiveness in future CMR studies. The objectives of this study were to estimate 1) survival and tag-retention following p-Chip microtransponder tag implantation in Creek Chub and Northern Pearl Dace in a 90-day laboratory environment, and 2) tag retention of p-Chip microtransponder tags in Northern Pearl Dace over a year-long field trial. Survival for Creek Chub was 85% (SE = 5.9) and did not significantly differ from control fish (95%; SE = 3.2) in the 90-day laboratory experiment. Survival for Northern Pearl Dace was 89% (SE = 11.0) and did not significantly differ from control fish (100%) in the 90-day laboratory experiment. Tag retention was 89% (SE = 4.6) for Creek Chub and 100% for Northern Pearl Dace in the 90-day laboratory experiment. The p-Chip microtransponder performed well during the CMR field study with tag retention for Northern Pearl Dace at 94% across 374 days. The

p-Chip microtransponder tag may be appropriate for use on small-bodied fishes where individual identification is needed in a CMR study.

Introduction

Freshwater fishes are greatly threatened from extensive anthropogenic alteration to freshwater habitats and need direct interventions that can target key demographic rates including survival and movement at different life stages. For instance, many species of Great Plains stream fishes are critically imperiled due to dewatering and fragmentation (Perkin and Gido 2012; Perkin et al. 2014; Perkin et al. 2015). Anthropogenic mediated fragmentation of streams has occurred in many forms including road crossings and channelization (Warren and Pardew 1998; Smiley et al. 2008; Bouska et al. 2010). The frequency and magnitude of these changes to the stream channel, especially in headwater stream reaches, has intensified through time with corresponding negative effects on fish community characteristics (i.e., species diversity, density, and biomass; Brooker 1985; Fahrig 2003). However, studies explicitly linking demographic rates of stream fishes to habitat alteration are limited (Labbe and Fausch 2000). Quantification of key demographic rates using empirically derived data is needed to set baselines from which to assess effectiveness of management actions including habitat restoration.

Capture-mark-recapture (CMR) efforts for small-bodied fishes have relied on a variety of mark types (i.e., batch mark and individual tags) including Visual Implant Elastomer (VIE), Passive Integrated Transponder (PIT) tags, and T-bar anchor tags (Pine et al. 2012). Batch marking using VIE or fin clips is inexpensive, and techniques exist to use them as individual identification marks when sample sizes are low to moderate (Ficke and Myrick 2009). Individual tagging methods, although more expensive, allow for broader applications to estimate demographic parameters when sampling large numbers of fishes (Pine et al. 2012). For example, PIT tags and T-bar anchor tags have been used to estimate population demographic parameters in larger fish species such as Channel Catfish *Ictalurus punctatus* and Pallid Sturgeon *Scaphirhynchus albus* (Steffensen et al 2012; Blank et al. 2017; Steffensen et al 2017). Although multiple options exist for large-bodied fishes, individual tags for small-bodied fishes (i.e., <100mm) are limited. Reliable individual tagging methods are needed to gain knowledge of demographic rates under CMR study designs for small-bodied fishes in pursuance of more informed conservation and management decisions.

Different marking techniques (i.e., batch marks and individual tags) for small-bodied fishes have confounding issues that may bias or cause misinterpretations of the parameter estimates given assumptions of CMR study designs. Studies have reported high retention and survival when using VIE marks (Leblanc and Noakes 2012; Szczepkowski et al. 2015; Mamer and Meyer 2016), but individually marking large numbers of fish becomes strenuous and may limit VIE to batch-marking study designs (Ficke and Myrick 2009). For example, ambiguity among colors of multiple VIE marks may alter the assignment of the individual fish (Ficke and Myrick 2009). However, the use of 8mm PIT tags may not affect fish survival (Ficke et al. 2012; Pennock et al. 2016; Swarr et al. 2021) or may negatively influence fish survival (Musselman 2007; Schumann et al. 2020). Furthermore, PIT tags may have both high tag retention (Bolland et al. 2009; Swarr et al. 2021) or low tag retention (Johnston and Smithson 1999; Pennock et al. 2016) prompting the need to specifically study small-bodied fish species of interest. A Type I error occurs when the null hypothesis is rejected that truly describes the population. For instance, violation of assumptions in CMR study designs including increases in fish mortality due to the tagging process or fish losing tags may enhance the chance for a Type I error when estimating fish demographic rates by biasing the estimate lower than expected. Assessment of novel tag types for use on small-bodied fishes including their influence on survival and tag retention rates is needed to alleviate concerns of fish mortality due to tagging and potential low tag retention assumptions within CMR study designs.

The p-Chip microtransponder tag (PharmaSeq 2017) may be a potential alternative method for individual identification of small-bodied fishes (Moore and Brewer 2021). The small (500 x 500 x 100 µm) tag contains a 9-digit unique identification number and uses photocell technology powered by a laser wand connected to a computer to read and display tag information. P-Chip microtransponders were first used in laboratory mice *Mus musculus* (Gruda et al. 2010) and have since expanded to two small-bodied fishes including Arkansas River Shiner *Notropis girardi* and larval European Sea Bass *Dicentrarchus labrax* (Faggion et al. 2020; Moore and Brewer 2021). P-Chip microtransponder tags had a minimal effect on Arkansas River Shiner survival and had comparable or greater retention (72%) to VIE marks and PIT tags (Moore and Brewer 2021). Furthermore, the p-Chip microtransponder tag had a minimal effect on larval European Sea Bass survival (82-98%) and high retention (76.2%; Faggion et al. 2020). However, low tag reading success rate (50%) has been attributed to sub-cutaneous tag movement (Faggion et al. 2020).

There exists limited evidence regarding survival and tag retention following p-Chip microtransponder tags implanting across species. Further, the method has not been applied extensively in field environments. Here, we assessed the effectiveness of p-Chip microtransponder tags on two stream fish including the Creek Chub *Semotilus atromaculatus* and the Northern Pearl Dace *Margariscus nachtriebi* in laboratory and field environments. The objectives of this study were to estimate 1) survival and tag-retention following p-Chip microtransponder tag implantation in Creek Chub and Northern Pearl Dace in a 90-day laboratory environment, and 2) tag retention of p-Chip microtransponder tags in Northern Pearl Dace over a year-long field trial.

Methods

Study Species

The Creek Chub is a widely distributed species throughout the eastern half of the United States. Creek Chub occurs across a wide range of habitat conditions but is commonly found in clear, cool streams with pools and abundant cover (McMahon 1982; Hrabik et al. 2015). The Northern Pearl Dace is distributed in the Northern United States and Canada with relict populations located in South Dakota, Nebraska, and Iowa (Lee et al. 1980; Hrabik et al. 2015). Northern Pearl Dace uses slow-moving, cool-water streams with meandering channels, well-vegetated undercut banks, and pool habitats (Tallman 1979; Tallman and Gee 1982; Cunningham 1995; Cunningham 2006; Hrabik et al. 2015).

Laboratory Experiment Fish Collection

Creek Chub (n = 113) and Northern Pearl Dace (n = 18) were collected by backpack electrofishing (60Hz, 25% duty cycle, and 180V) from Bone Creek in Brown County, Nebraska in December 2021. A single pass upstream was performed with 3 netters. Fish were tempered from the stream temperature (9°C) to the tank temperature (12°C) at a rate of 2°C hr^{-1}. A 265 L tank was filled with well water treated for chlorine with sodium thiosulfate (118mL of product to 757 L of water) and used to transport fish from the field to the laboratory. Oxygen was supplied during transport (15.73cm^3 s^{-1}). A second tempering occurred upon transfer from the tank truck (12°C) to the experimental tank (16°C) at a rate of 2°C hr^{-1}. No mortalities occurred during transport from the stream to the experimental tanks.

Laboratory Experimental Design

The study occurred at Schramm Outdoor Education Center in Gretna, Nebraska. Daily fish care occurred under direct supervision of Nebraska Game and Parks Commission staff. Fish were fed to satiation each day using Artemia (i.e., Brine Shrimp; Bulk Reef Supply 2022). Two independent systems were used to house fish given laboratory space constraints. The first system consisted of fifteen 38 L tanks, where each tank had a consistent temperature of 16°C maintained via a single chiller unit. Consistent water quality was maintained across tanks via a closed flow-through system. Tanks housed six fish each with one tank having seven fish for a total of 91 individuals. Creek Chub of similar size were grouped to reduce bias of survival estimates given potential predation and dominance behaviors. Northern Pearl Dace of similar size were housed in three of the fifteen tanks. The second system was a 946 L recirculating circular tank without a chiller where Creek Chub (n = 40) of different sizes were housed with a temperature range from 18.6-19.8°C.

Each tank received double tagged (i.e., p-Chip microtransponder tag and VIE mark) and control (not tagged) fish of similar length (Table 2.1). Tagged fish received a p-Chip microtransponder behind the left dorsal fin (Figure 2.1) using a 0.8mm-diameter injection needle (PharmaSeq 2017). Tagged fish were additionally marked with VIE to boost detection of p-Chip microtransponders. A VIE mark was injected dorsally on the right side using a 0.3mL syringe and a 0.36mm-diameter needle (Northwest Marine

Technology 2021). Control fish were not tagged or injected with a blank needle but were manually manipulated (i.e., scanned for tags) similar to tagged fish. Individual fish were randomly selected from each tank for inclusion in the tagged or control group (system 1: 3 tagged and 3 control; system 2: 20 tagged and 20 control). Random selection was performed by blindly netting three of the six fish in each tank of system 1 and twenty of the forty fish in system 2 to be tagged. Fish were placed in a tank and anesthetized with Aqui-s (Aqui-s 2022) treated water at 27ppm prior to being randomly assigned to the tagged or control group. Fish were measured for total length (mm TL) and given tags and marks if selected for the tagging group. All fish were placed in a recovery tank until equilibrium was regained and active swimming commenced. All fish were retained for analysis of survival (Moore and Brewer 2021). If mortality occurred during the experiment, the fish were frozen with a recording of the date, tank number, experimental group, and length of the fish. Tag retention was confirmed by a successful tag reading every fifteen days for the first two months followed by every thirty days to reduce handling stress and to better observe short-term tag loss. Fish capture and housing during the experiment followed approved procedures laid out by the Institutional Animal Care and Use Committee at the University of Nebraska-Lincoln (Project Number: 2070).

Statistical Analyses

Length distributions for each species-specific group were assessed graphically to ensure equal representation among tagged and control fish (Figure 2.2). Unpaired Two-Sample t-tests were conducted to test whether mean length for each species differed between tagged and control fish. Kaplan-Meier curves were constructed to estimate the probability of survival and tag retention (Goel et al. 2010). Kaplan-Meier curves use time-at-event data to estimate the probability of an event (e.g., survival or tag loss).

Survival curves were constructed using days post tagging and fish that survived the study were right-censored. Tag-retention curves were created with the same methods as the survival curves except mortalities were censored from the study at the time of death. We tested the null hypothesis that survival or tag retention did not differ between groups by computing a log-rank test to compare the survival and tag retention for each species. We used the survival package (Therneau 2022) in program R version 4.2.0 (R Core Team 2022) to develop models and test for significant differences between tagged and control groups.

Field Application

Northern Pearl Dace (≥40mm TL) were collected June 2022, September 2022, October 2022, and June 2023 by backpack electrofishing (60Hz, 25% duty cycle, and 100W). Multiple sites (n = 28) were sampled along four headwater streams including Willow Creek, Clifford Creek, Sandy-Richards Creek, and Gordon Creek in Cherry County, Nebraska (Figure 2.3). A robust sampling design was employed where each site was sampled three instances in an assumed closed-population time frame of 1-4 days each season. Sampled Northern Pearl Dace were placed in a large holding tank with two battery operated aerators and individuals ≥40mm TL were double tagged with a p-Chip microtransponder tag and VIE mark following the tagging procedures used in the laboratory study. Northern Pearl Dace on first encounter were given a season-specific VIE color mark (June-red; September-yellow; October-orange). For each following site visit, Northern Pearl Dace not previously captured were given season specific VIE marks and p-Chip microtransponder tags while recaptured fish were scanned and recorded as a recapture or shed tag based on the assumption of 100% VIE retention from the laboratory study. Data recorded for this analysis included date, site, season, sequential sample number, VIE color, and days at large. The binary response variable was whether the p-Chip microtransponder tag was shed. If the fish shed a tag within a season (e.g., sample 2 of 3 in spring) then the exact days at large for the shed tag could be recorded. If the shed tag was noticed during a different season (i.e., October sample with a June VIE mark) or sample 3 within a sequential season sample, then the second sample date of the VIE marking period for that season was used so that days at large was either exact or ±1-4 days.

Field Application Analyses

Tag retention in Northern Pearl Dace was estimated by using two instantaneous tag retention models described by McCormick and Meyer (2018). The estimates where the probability of retaining a single tag (Q) following time at large (t) calculated as:

$$Q(t) = \alpha e^{-Lt},$$

where α is the immediate tag retention and L is the instantaneous rate of tag shedding (Barrowman and Myers 1996; McCormick and Meyer 2018). The second model assumed that the initial tag retention was 100% (i.e., α = 1). The probability that an individual Northern Pearl Dace retained both tags (VIE and p-Chip microtransponder) was calculated as:

$$P_{AA}^{AA} = Q(t)^2,$$

and the probability that an individual Northern Pearl Dace would retain one tag (p-Chip microtransponder) was calculated as:

$$P_{A}^{AA} = 2Q(t)[1-Q(t)].$$

The probability of observing either outcome (i) (i.e., one or two tags) was defined as:

$$\frac{p_i(t)}{\sum_{i=1}^{2} p_i(t)}.$$

The model parameters α and L were estimated by using the maximum likelihood through minimizing the negative of the log-likelihood function (l) conditioned on the observed time at large by using:

$$l = \sum_{j-1}^{N_{AA}} log_e \frac{Q(t_j)^2}{Q(t_j)^2 + 2Q(t_j)[1 - Q(t_j)]}$$

$$+ \sum_{k-1}^{N_A} log_e \frac{2Q(t_k)[1 - Q(t_k)]}{Q(t_k)^2 + 2Q(t_k)[1 - Q(t_k)]},$$

where t_j is the time at large of individual Northern Pearl Dace that were recaptured with both tags and t_k is the time at large for individual Northern Pearl Dace that were recaptured with a single tag (Barrowman and Myers1996; McCormick and Meyer 2018). Akaike information criterion corrected for small sample sizes (AIC_c) was used to assess model fit between both instantaneous models. All statistical analyses were performed in program R (R Core team 2022).

Results

Lab Results

System 2 had 6 fish that shed tags within the first 30 days and were retagged and included as new tagged fish. Creek Chub and Northern Pearl Dace mean length did not differ between tagged and control groups (Creek Chub, t = 0.267, df = 111, P = 0.79; Northern Pearl Dace, t = -1.46, df = 16, P = 0.16). Creek Chub survival did not differ between systems (χ^2 = 1.2, P = 0.3) and individuals from the two systems were combined to assess survival over the course of the laboratory study. Survival did not differ between tagged and control groups for Creek Chub (χ^2 = 2.7, P = 0.1, Figure 2.4) and Northern

Pearl Dace (χ^2= 1.0, P = 0.3, Figure 2.5). Tagged Creek Chub (n = 56) had 85% survival with 8 deaths 33-90 days post tagging and control Creek Chub (n = 57) had 95% survival with 3 deaths 33-87 days post tagging. Tagged Northern Pearl Dace (n = 9) had 89% survival with 1 death 80 days post tagging and control Northern Pearl Dace (n = 9) had 100% survival with no deaths over 90 days post tagging. Tag retention did not differ between Creek Chub and Northern Pearl Dace (χ^2 = 2.7, P = 0.1, Figure 2.6). Creek Chub (n = 62) had 89% tag retention with 7 tag losses 15-45 days post tagging and Northern Pearl Dace (n = 9) had 100% tag retention with no tag loss over 90 days. VIE marks had 100% retention over the 90-day study, which suggested its suitability as a permanent mark for the field-based study.

Field Application Results

A total of 1,990 individual Northern Pearl Dace (41-145mm TL) were double tagged with VIE and p-Chip microtransponder tags. There were 846 individuals recaptured during the 374-day field study (Table 2.2). A total of 805 (95%) individuals were recaptured with both tags and 41 (5%) individuals were recaptured with the VIE tag only. VIE tag retention was assumed 100%. Time at large ranged from 1 to 374 days. The top-ranked instantaneous tag loss model suggested initial tag retention was not 100% ($\alpha \neq 1$; AICc of 608.86 verses AICc of 1780.66). Tag retention of p-Chip microtransponder tags in over the 374-day field study was estimated at 94% (SE = 0.00056) (Figure 2.7).

Discussion

The p-Chip microtransponder tag may be a useful addition to tagging strategies for small-bodied fishes in study designs that rely on individual identification. The tagging process implemented during this study did not significantly affect survival rates in Creek Chub or Northern Pearl Dace and supports initial assessments performed on Arkansas

River Shiner (Moore and Brewer 2021). In general, p-Chip microtransponder tag retention remained high in Creek Chub (89%) and Northern Pearl Dace (100%) compared to Arkansas River Shiner (72%, Moore and Brewer 2021). Tagger experience likely influenced tag retention as 5 of the first 6 fish tagged lost p-Chip microtransponder tags within the first 30 days. Compared to studies assessing other individual tagging methods (e.g., t-bar tags, 94.3% in Huhn et al. 2014; pit tags, 40% in Pennock et al. 2016, 96% in Allan et al 2018, 100% in Swarr et al. 2021; VIE-alpha, 84% in Osbourn et al. 2011, 91% in Turek et al. 2014), p-chip microtransponder tags combined with VIE marks had similar tag retention.

This study may be the first to assess tag retention of p-Chip microtransponder tags in the field. We found that tag retention in Northern Pearl Dace across the 374-day field study was relatively high (94%) when compared to other field-based tag retention studies with different individual tag methods (e.g., t-bar tags, 88% in Spurgeon et al. 2020; pit tags, 74% in Bateman et al 2009). Approximately 31% of our shed tags occurred quickly and may have been caused by tags flipping or improper tag placement due to harsh environmental conditions on the tagger. Faggion et al. (2020) mentioned that tag readability was low due to p-Chip microtransponder tags shifting position post-injection. Further, during our summer sample period, air temperatures reached up to 37^{o} C and resulted in a more rushed tagging process to reduce fish stress.

The ability to individually mark small-bodied fishes is critical to estimate demographic rates under certain study designs and inform management of their populations. Many native stream fishes are considered species of greatest conservation need with minimal knowledge on how changing local-scale habitat through channelization, anthropogenic barriers, and system degradation influences demographic rates (e.g., survival, movement) and population characteristics (e.g., abundance). With many small-bodied fishes at-risk, it becomes important to implement study designs to directly quantify status and demographic responses to management actions. Our results indicate there were minimal negative effects on wild small-bodied fishes tagged with p-Chip microtransponders. Furthermore, with tagging experience, double-tagged small-bodied fishes with VIE and p-Chip microtransponders can aid in meeting mark-recapture analysis assumptions including tags remaining readable and not overlooked. Since the p-Chip microtransponder tag relies on light activation and only reads one-sided, studies on tag readability in fast growing fishes may provide insight regarding tag readability over time. Studies evaluating various tag locations and the influences of fish pigmentation on p-Chip microtransponder readability may also benefit future tag placement. Studies of length effects of fish on survival and tag retention of p-Chip microtransponders may help determine size boundaries and further usage into other settings such as fish hatcheries. Studies of survival and tag retention of p-Chip microtransponders in other aquatic taxa (e.g., mussels, crayfish) may also benefit study designs that require mark-recapture.

Literature Cited

Allan, H., P. Unmack, R. P. Duncan, and M. Lintermans. 2018. Potential impacts of pit tagging on a critically endangered small-bodied fish: a trial on the surrogate Mountain Galaxias. Transactions of the American Fisheries Society 147(6):1078–1084.

Aqui-s New Zealand LTD. 2022. Aqui-s Tech Sheet. Available: https://www.aquis.com/products/aqui-s-20e. (March 2022)

Barrowman, N. J., and R. A. Myers. 1996. Estimating tag shedding rates for experiments with multiple tag types. Biometrics 52:1410–1416.

Bateman, D. S., R. E. Gresswell, and A. M. Berger. 2009. Passive integrated transponder tag retention rates in headwater populations of coastal Cutthroat Trout. North American Journal of Fisheries Management 29(3):653–657.

Blank, A. J., M. J. Hamel, J. J. Spurgeon, and M. A. Pegg. 2017. Assessment of a channel catfish population in a large open river system. Fisheries Management and Ecology 24(6):460–468.

Bolland, J. D., Cowx, I. G., & Lucas, M. C. 2009. Evaluation of VIE and PIT tagging methods for juvenile cyprinid fishes. Journal of Applied Ichthyology, 25:381–386.

Bouska, W. W., T. Keane, and C. P. Paukert. 2010. The effects of road crossings on prairie stream habitat and function. Journal of Freshwater Ecology 25(4):499–506.

Brooker, M. P. 1985. The ecological effects of channelization. The Geographical Journal 151(1):63–69.

Bulk Reef Supply. 2022. Bio-Pure Brine Shrimp-Hikari. Available: https://www.bulkreefsupply.com/bio-pure-frozen-brine-shrimp-hikari.html?queryID=61124d3481346fb44f48bf8b31037086&objectID=5210&indexName=brs_prod_m2_default_products. (December 2022)

Cunningham, G. R. 1995. Life history traits and reproductive ecology of the Pearl Dace, *Margariscus margarita* (Pisces: Cyprinidae) in the Sandhills of Nebraska. M.A. Thesis. University of Nebraska-Omaha, Omaha, Nebraska.

Cunningham, G. R. 2006. Pearl Dace (*Margariscus margarita):* a technical conservation assessment. USDA Forest Service, Rocky Mountain Region. Available: https://www.nrc.gov/docs/ML1004/ML100481436.pdf. (January 2023)

Faggion, S., P. Sanchez, M. Vandeputte, F. Clota, A. Vergnet, M. O. Blanc, and F. Allal. 2020. Evaluation of a European Sea Bass (*Dicentrarchus labrax L.*) post-larval tagging method with ultra-small RFID tags. Aquaculture 520:734945–734956.

Fahrig, L. 2003. Effects of habitat fragmentation on biodiversity. Annual Review of Ecology, Evolution, and Systematics 34:487–515.

Ficke, A. D., and C. A. Myrick. 2009. A method for monitoring movements of small fishes in urban streams. North American Journal of Fisheries Management 29:1444–1453.

Ficke, A. D., Myrick, C. A., & Kondratieff, M. C. (2012). The effects of PIT tagging on the swimming performance and survival of three nonsalmonid freshwater fishes. Ecological Engineering, 48:86–91.

Goel, M. K., P. Khanna, and J. Kishore. 2010. Understanding survival analysis: kaplan-meier estimate. International Journal of Ayurveda Research 1(4):274–278.

Gruda, M. C., A. Pinto, A. Craelius, H. Davidowitz, W. M. Kopacka, J. Li, J. Qian, E. Rodriguez, E. Kuspiel, and W. Mandecki. 2010. A system for implanting laboratory mice with light-activated microtransponders. Journal of the American Association for Laboratory Animal Science 49(6):826–831.

Horton, R. E. 1945. Erosional development of streams and their drainage basins; Hydrophysical approach to quantitative morphology. Bulletin of the Geological Society of America 56:275–370.

Hrabik, R. A., S. C. Schainost, R. H. Stasiak, and E. J. Peters. 2015. The fishes of Nebraska. University of Nebraska-Lincoln, Lincoln, NE.

Huhn D., T. Klefoth, T. Pagel, and P. Zajicek. 2014 Impacts of external and surgery-based tagging techniques on small northern pike under field conditions. North American Journal of Fisheries management 34:322–334.

Johnston, C. E., and E. B. Smithson. 1999. Retention of passive integrated transponder (PIT) tags for individual identification of warmwater stream fishes. Proceedings of the Southeastern Fishes Council 38:1–4.

Labbe, T. R., and K. D. Fausch. 2000. Dynamics of intermittent stream habitat regulate persistence of a threatened fish at multiple scales. Ecological Applications 10(6):1774–1791.

Leblanc, C. A., and D. L. Noakes. 2012. Visible implant elastomer (VIE) tags for marking small Rainbow Trout. North American Journal of Fisheries Management 32(4):716–719.

Lee, D. S., C. R. Giber, C. H. Hocutt, R. E. Jenkins, D. E. McAllister, and J. R. Stauffer, Jr. 1980. Atlas of North American freshwater fishes. The North Carolina Biological Survey, North Carolina.

Mamer, E. R. J. M., and K. A. Meyer. 2016. Retention rates of passive integrated transponder tags, Visible Implant Elastomer tags, and Maxillary Marks in wild trout. North American Journal of Fisheries Management 36(5):1119–1124.

McCormick, J. L., and K. A. Meyer. 2018. Sensitivity of exploitation estimates to tag loss estimation methods in Idaho sport fisheries. North American Journal of Fisheries Management 38(1):170–179.

McMahon, T. E. 1982. Habitat Suitability Index Models: Creek Chub. U.S.D.I Fish and Wildlife Service FWS/OBS-82/10.4.

Moore, D. M., and S. K. Brewer. 2021. Evaluation of visual implant elastomer, PIT, and p-chip tagging methods in a small-bodied minnow species. North American Journal of Fisheries Management 41:1066–1078.

Musselman, W. C. 2007. The importance of maintaining shallow-water habitats for the movement and survival of stream fishes. (Master's thesis. University of Missouri, Columbia, MO). Retrieved from The importance of maintaining shallow-water habitats for the movement and survival of stream fishes - ProQuest

Northwest Marine Technology. 2021. Instructions for Visible Implant Elastomer (VIE) Tags. Available: https://www.nmt.us/wp-content/uploads/2021/01/VIE-Manual-Kit-Instructions-.pdf. (April 2022)

Osbourn, M. S., D. J. Hocking, C. A. Hocking, W. Peterman, and R. D. Semlitsch. 2011. Use of fluorescent visible implant alphanumeric tags to individually mark juvenile ambystomatid salamanders. Herpetological Review 42(1):43–47

Pennock, C. A., B. D. Frenette, M. J. Waters, and K. B. Gido. 2016. Survival of and tag retention in Southern Redbelly Dace injected with two sizes of PIT tags. North American Journal of Fisheries Management 36:1386–1394.

Perkin, J. S., and K. B. Gido. 2012. Fragmentation alters stream fish community structure in dendritic ecological networks. Ecological Applications 22(8):2176–2187.

Perkin, J. S., K. B. Gido, A. R. Cooper, T. F. Turner, M. J. Osborne, E. R. Johnson, and K. B. Mayes. 2015. Fragmentation and dewatering transform Great Plains stream fish communities. Ecological Monographs 85(1):73–92.

Perkin, J. S., K. B. Gido, K. H. Costigan, M. D. Daniels, and E. R. Johnson. 2014. Fragmentation and drying ratchet down Great Plains stream fish diversity. Aquatic Conservation: Marine and Freshwater Ecosystems 25(5):639–655.

PharmaSeq. 2017. Adult Zebrafish p-Chip® Implantation Protocol. Available: https://www.isenet.it/wp-content/uploads/2017/01/PharmaSeq_Zebrafish_Tagging_Protocol-1.pdf. (December 2022)

Pine, W. E., J. E. Hightower, L. G. Coggins, M. V. Lauretta, and K. H. Pollock. (2012). Design and analysis of tagging studies. Pages 521-572 *in* A.V. Zale, D. L. Parrish, and T. M. Sutton, editors. Fisheries techniques, 3rd edition. American Fisheries Society, Bethesda, Maryland.

R Core Team. 2022. A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna.

Schumann, D. A., Graeb, K. N., Wagner, M. D., Graeb, B. D. S., Prenosil, E., & Hoekwater, J. 2020. Suitability of surgically implanted 8-mm passive integrated transponder tags for small-bodied fishes. Journal of Applied Ichthyology 36: 682–692.

Smiley, P. C., R. B. Gillespie, K. W. King, and C. Huang. 2008. Contribution of habitat and water quality to the integrity of fish communities in agricultural drainage ditches. Journal of Soil and Water Conservation 63(6):218A–219A.

Spurgeon, J. J., M. A. Pegg, S. F. Siddons, and H. H. Hansen. 2020. Retention of t-bar anchor tags for Channel Catfish in the Red River of the North. North American Journal of Fisheries Management 40(2):330–334.

Steffensen, K. D., L. A. Powell, and M. A. Pegg. 2012. Population size of hatchery-reared and wild Pallid Sturgeon in the Lower Missouri River. North American Journal of Fisheries Management 32(1):159–166.

Steffensen, K. D., L. A. Powell, and M. A. Pegg. 2017. Using the robust design framework and relative abundance to predict the population size of Pallid Sturgeon *Scaphirhynchus albus* in the Lower Missouri River. Journal of Fish Biology 91(5):1378–1391.

Strahler A. N. 1957. Quantitative analysis of watershed geomorphology. Transaction, American Geophysical Union 38(6):913–920

Swarr, T. R., C. A. Myrick, and R. M. Fitzpatrick. 2021. Tag retention in and effects of passive integrated transponder tagging on survival and swimming performance of a small-bodied darter. Journal of Fish Biology 100(3):705–714.

Szczepkowski, M., Z. Zakęś, B. Szczepkowska, I. Piotrowska, M. Kozłowski, K. Wunderlich and A. Kapusta, 2015. Tag retention, growth rate, and survival of juvenile pike tagged with visible implant elastomer and coded wire tags. Bulg. J. Agric. Sci., Supplement 1, 21:12–16

Tallman, R. F. 1979. Environments occupied, indices of maturity, feeding ecology, shoaling behavior and interactions with other species by Pearl Dace, *Semotilus margarita* (Cope) in Manitoba. Master's thesis. University of Manitoba, Winnipeg.

Tallman, R. F., and J. H. Gee. 1982. Intraspecific resource partitioning in a headwaters stream fish, the Pearl Dace, *Semotilus margarita* (Cyprinidae). Environmental Biology of Fishes 7:243–249.

Therneau, T. 2022. A package for survival analysis in R. R package version 3.2-7. Available: https://cran.r-project.org/web/packages/survival/index.html. (December 2022).

Turek, K. C, M. A. Pegg, and K. L. Pope. 2014. Short-term evaluation of visible implant alpha tags in juveniles of three fish species under laboratory conditions. Journal of Fish Biology 84:971– 981.

Warren, M. L., and M. G. Pardew. 1998. Road crossings as barriers to small-stream fish movement. Transactions of the American Fisheries Society 127:637–644.

Table 2.1—System, experimental group, number of individuals (n), mean total length (Mean TL), standard deviation (SD), minimum TL, and maximum TL for Creek Chub and Northern Pearl Dace. Fish were marked with a p-Chip microtransponder on the left dorsal and VIE on the right dorsal.

Species	**System**	**Experimental Group**	**n**	**Mean TL(mm) ± SD**	**Min-Max TL(mm)**
Creek Chub	1	Control	37	89±27	44-150
Creek Chub	1	Tagged	36	91±27	54-168
Creek Chub	2	Control	20	96±26	50-137
Creek Chub	2	Tagged	26*	96±24	54-149
Northern Pearl Dace	1	Control	9	106±7	97-120
Northern Pearl Dace	1	Tagged	9	101±6	90-113

*6 Creek Chub were retagged at day 45

Table 2.2—The number of Northern Pearl Dace that retained both tags (AA: VIE mark + p-Chip microtransponder tag) or a single tag (A: VIE mark only) at recapture. Fish counts are grouped by days at large during the field-based application. The exact time at large (days) was used in the modeling when captured the second sample within season. Otherwise, an approximate time at large (±1-4 days) was used for all shed tags between seasons.

Tag Outcome	**Days at Large**			
	1-45	**46-100**	**101-135**	**136-374**
AA	593	97	59	56
A	27	5	2	7

Figure 2.1—Marking locations on Northern Pearl Dace. Left photo depicts VIE mark on fishes right. Right photo depicts p-Chip Microtransponder tag on fishes left.

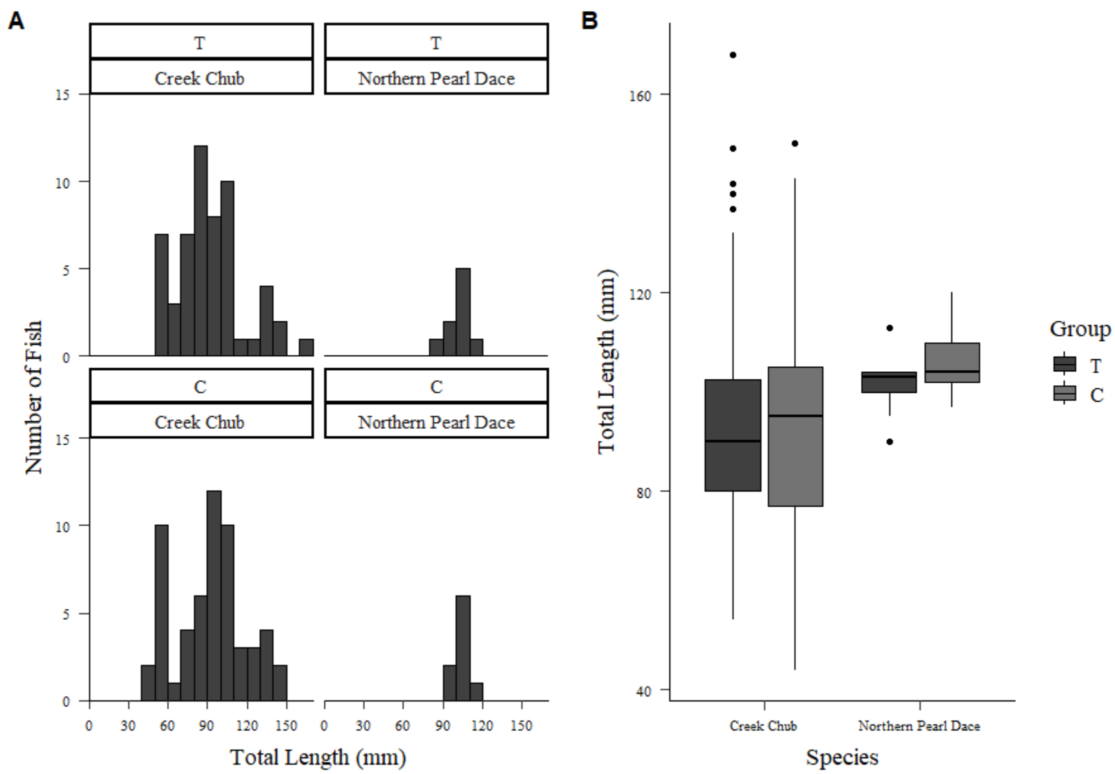

Figure 2.2—Length-frequency distribution (A) for experimental groups (T-Tagged; C-Control) of Creek Chub and Northern Pearl Dace and box plot (B) for experimental groups of Creek Chub and Northern Pearl Dace used in the laboratory experiment. The horizontal mid-line in the box is the median value. The box ends include the interquartile range. The whiskers include maximum and minimum values, and the dots indicate outliers.

Figure 2.3—Field-based application sampling sites within the Cherry County Biological Unique Landscape, Nebraska.

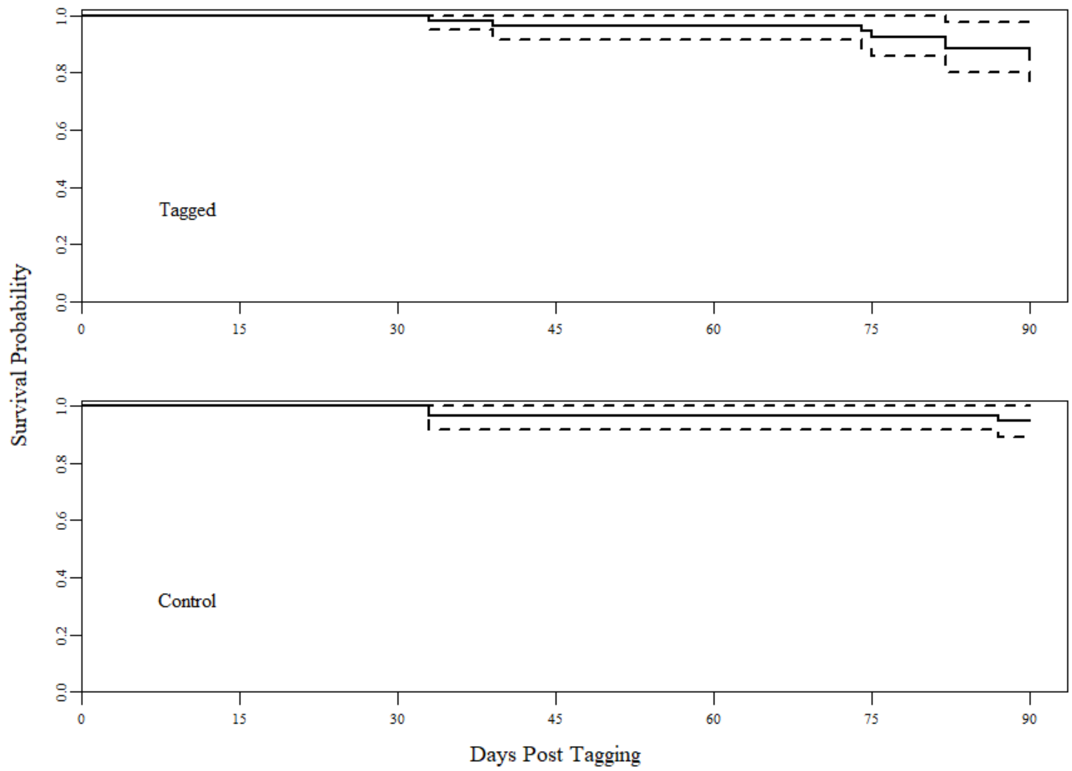

Figure 2.4—Kaplan-Meier survival curves developed for survival of tagged and control Creek Chub. The survival probability with 95% confidence interval (dashed lines) are shown over 90 days post tagging. Mortality occurred 33-90 days post tagging.

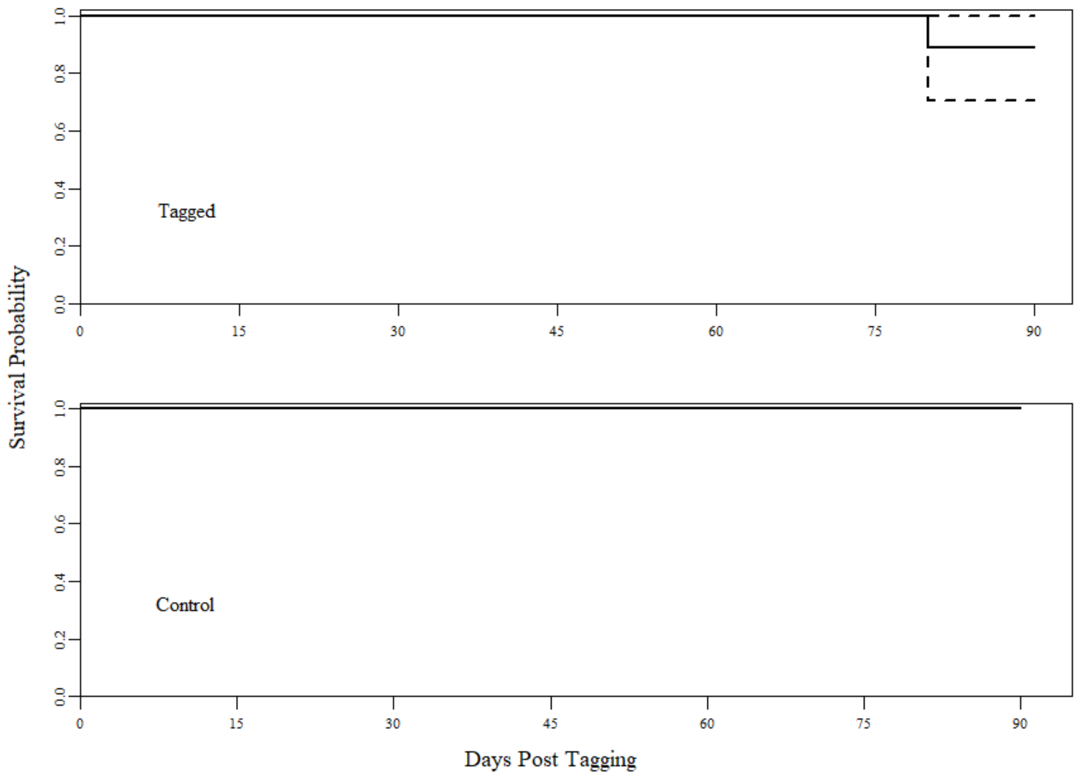

Figure 2.5—Kaplan-Meier survival curves developed for survival of tagged and control Northern Pearl Dace. The survival probability with 95% confidence interval (dashed lines) are shown over 90 days post tagging. Mortality occurred 80 days post tagging.

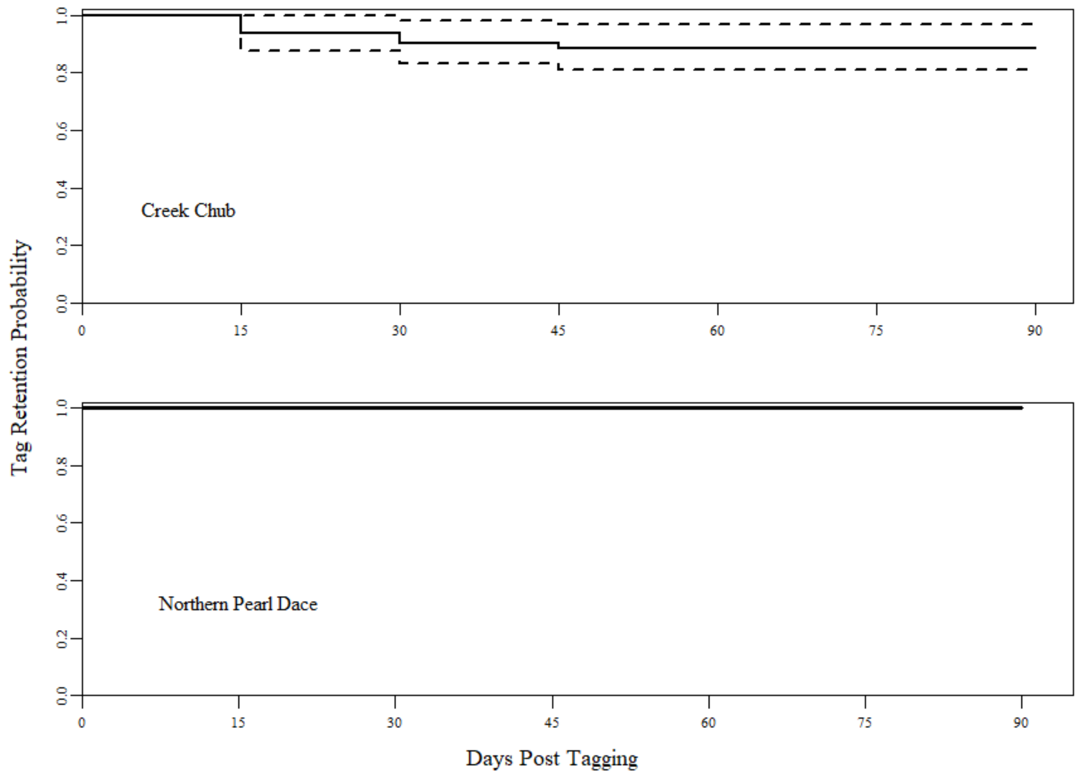

Figure 2.6—Kaplan-Meier time-at-event curves developed for tag retention of all Creek Chub and Northern Pearl Dace. The tag retention probability with 95% confidence interval (dashed lines) are shown over 90 days post tagging. Tag loss occurred 15-45 days post tagging.

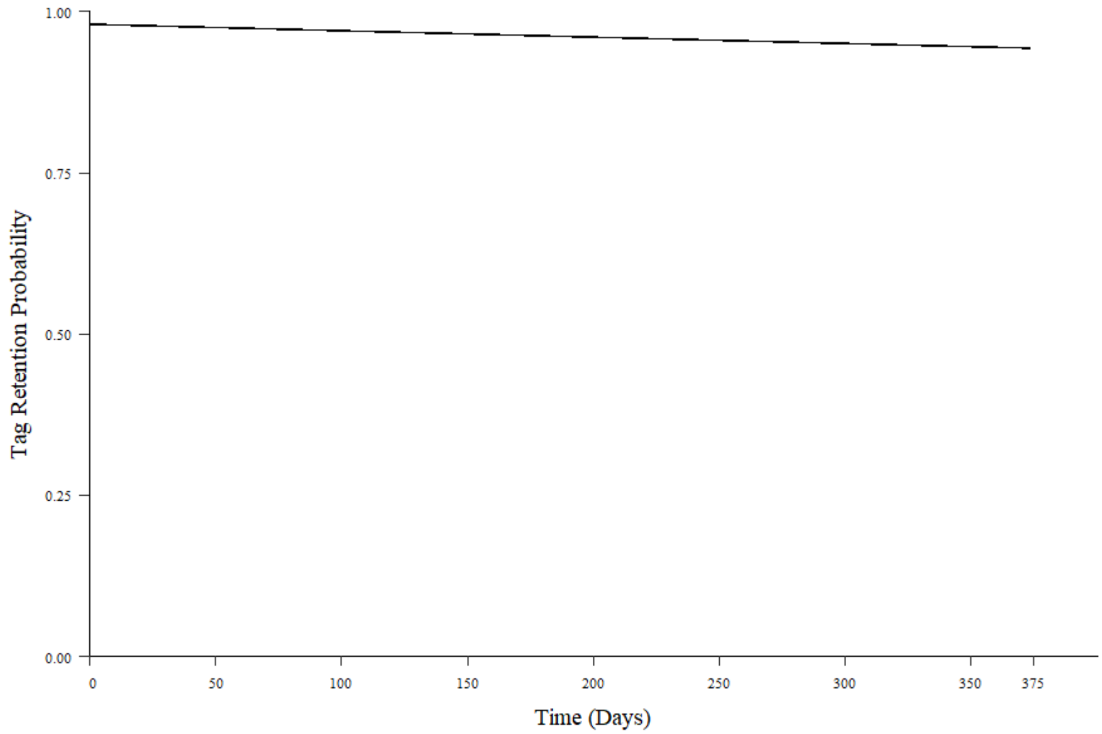

Figure 2.7—Top ranked instantaneous tag retention model with 95% confidence intervals (dashed lines) for tag retention of p-Chip microtransponders in Northern Pearl Dace in the 374-day field-based application.

CHAPTER 3. SURVIVAL OF A GLACIAL RELICT FISH IN ANTHROPOGENICALLY ALTERED STREAMS OF THE NEBRAKSA SANDHILLS ECOREGION

Abstract

A current knowledge gap limiting management of small-bodied fish species of greatest conservation need (SGCN) and reducing global biodiversity loss is empirical evidence of changes in demographic rates in response to environmental perturbations. Extensive alteration of headwater streams influences the habitat template on which small-bodied fish are dependent to carry out distinct life stages and maintain or increase population growth. Northern Pearl Dace *Margariscus nachtriebi* is a small-bodied fish SGCN in the Nebraska Sandhills Ecoregion and isolated from its core northern distribution. Channelization is extensive in streams of the Nebraska Sandhills Ecoregion and limited evidence exists regarding its influence on fish SGCN demographic rates including Northern Pearl Dace survival. The objectives of this study were to 1) determine differences in geomorphic characteristics, and instream habitat including mesohabitat, depth, and macrophyte coverage in channelized and non-channelized stream sites in the Nebraska Sandhills Ecoregion, and 2) estimate annual survival of Northern Pearl Dace in channelized and non-channelized sites in the Nebraska Sandhills Ecoregion. Environmental parameters in channelized and non-channelized stream sites differed (Pillai's Trace = 0.9181, $F(8, 6) = 8.4040$, $p = 0.0091$). Specifically, mean sinuosity index ($F(1, 13) = 16.6400$, $p = 0.0013$) and the percent of pool mesohabitat ($F(1, 13) = 5.3848$, $p = 0.0372$) differed between channelized and non-channelized stream sites. A capture-mark-recapture robust-design study was conducted where a total of 1,949 Northern Pearl Dace individuals were tagged. A total of 853 individual Northern Pearl Dace were

recaptured over the 374-day field study. Estimated survival ($\hat{S}$) varied by time and between channelized and unchannelized sites. Seasonal monthly survival was lower in channelized sites ($\hat{S}_{\text{Spring to Summer}} = 0.340$; SE = 0.064; $\hat{S}_{\text{Summer to Fall}} = 0.425$; SE = 0.112; $\hat{S}_{\text{Fall to Spring}} =0.703$; SE = 0.040) compared to non-channelized sites ($\hat{S}_{\text{Spring to Summer}} = 0.928$; SE = 0.025; $\hat{S}_{\text{Summer to Fall}} =0.545$; SE = 0.046; $\hat{S}_{\text{Fall to Spring}} =0.764$; SE = 0.020). Channelization in headwater streams appeared to reduce Northern Pearl Dace survival in headwater streams of the Sandhills Ecoregion. Mitigation of channelized stream sites may benefit the persistence of Northern Pearl Dace populations.

Introduction

Freshwater fishes are threatened, in part, by anthropogenic alteration of freshwater habitats and may benefit from management interventions that target life-stage specific demographic rates (Allan and Flecker 1993; Dudgeon et al. 2006). Anthropogenic alteration of headwater streams has intensified resulting in reduced habitat heterogeneity with direct and indirect influences on fish demographics (Brooker 1985; Smiley et al. 2008; Fischer and Paukert 2009; Smiley et al. 2009; Smiley et al. 2017). Brown et al. (2008) suggested channelization negatively affected environmental parameters such as bank height, bank width, plant richness, and percent canopy cover associated with habitat conditions used by fishes within headwater streams. The distribution, abundance, heterogeneity, and suitability of habitat drive population demographics of fishes (Labbe and Fausch 2000; Bond et al. 2015). A positive relation between the spatial and temporal availability of habitat and successful completion of different life stages facilitates the maintenance and expansion of a fish's distribution (Rosenfeld and Hatfield 2006). As such, knowledge of habitat needs throughout the life history of fishes as well as the influence on population demographics may refine the prescription of habitat management practices.

Headwater streams are the first stream segments in a watershed and originate where surfacing groundwater (e.g., springs, swales, etc.) or surface runoff maintain fluvial characteristics including erosion, transportation, or deposition of sediments (Gomi et al. 2002; Richardson 2019). Headwater streams are highly dynamic and include greater edge area, less variable water temperatures, and greater response to precipitation events compared to downstream streams (Richardson 2019). Ecological functions of headwater streams for fish include provisioning of predator-free refuge, seasonal environmental refuge, breeding sites, thermal refuge, and a heavily detritus-based food web (Richardson 2019). Anthropogenic alteration resulting in reduced habitat suitability and ecological function is pervasive in headwater streams due to their position in the watershed and size. Substantial degradation of headwater streams may result in extirpation or extinction of fish species of greatest conservation need (SGCN).

Channelization of headwater streams influences hydrologic function, habitat heterogeneity, and fish-assemblage characteristics (Brooker 1985; Fausch et al. 1990; Casatti et al. 2006; Smiley et al. 2008; Brown et al. 2008; Smiley et al. 2009; Smiley et al. 2017). King et al. (2009) showed significantly higher bankfull discharge capacities in channelized headwater streams which created faster peak discharge times and quicker runoff post-precipitation. Further, channelization may fragment existing habitats and homogenize habitat diversity (e.g., creation of a single channel with elevated stream flow; Fahrig 2003) leading to decreased fish survival. Studies have suggested the effects of channelization of headwater streams on fish community structure including changes to species diversity, density, and biomass (Brooker 1985; Fahrig 2003; Richardson 2019). However, specific knowledge regarding demographic parameters of fish SGCN remains limited. Employing effective monitoring strategies aimed at quantifying demographic parameters of fish SGCN in channelized headwater streams may reduce such a knowledge gap.

Northern Pearl Dace *Margariscus nachtriebi* is a small-bodied glacial relict fish SGCN isolated in the Nebraska Sandhills Ecoregion from its core distribution in the northern United States and Canada (Lee et al. 1980; Pasbrig 2013). Northern Pearl Dace occupy slow-moving, cool-water streams with meandering channels, well-vegetated undercut banks, and pool habitats (Tallman 1979; Tallman and Gee 1982; Cunningham 1995; Cunningham 2006). As such, Northern Pearl Dace are an important indicator species that is intolerant of degradation including decreased macrophyte coverage, incision of the stream channel, sedimentation, and loss of pool mesohabitat caused by stream geomorphic changes (Pasbrig 2013). Changes to these habitats through anthropogenic alteration including channelization may affect survival and ultimately the persistence of this and other glacial relict species in isolated distributions within prairie streams. However, estimates of survival and population size of Northern Pearl Dace with respect to stream habitat and stream alteration are lacking. Quantification of key demographic rates using empirically derived data is needed to set baselines from which to determine if management actions including habitat restoration influence demographic rates and promote persistence of the species across the landscape. As such, the objectives of this study were to 1) determine if differences occur in geomorphic characteristics, and instream habitat including mesohabitat, depth, and macrophyte coverage in channelized and non-channelized stream sites in the Nebraska Sandhills Ecoregion, and 2) estimate annual survival of Northern Pearl Dace in channelized and non-channelized sites in the Nebraska Sandhills Ecoregion.

Methods

Study Area

The Sandhills Ecoregion is in north-central Nebraska and spans 49,000 km^2 of grass-stabilized sand dunes (Hayford and Baker 2011). The Sandhills Ecoregion is characterized by mixed-grass prairies with a sandy soil. There exists a limited human population density in the Sandhills Ecoregion. The human population of the approximately 15,563 km^2 Cherry County, Nebraska located in the Sandhills Ecoregions is 5,779. Land coverage is primarily grasses used for cattle grazing and hay production (Kuzelka et al. 1993). The connection between the Ogallala Aquifer and surface waters within the Sandhills Ecoregion maintains perennial headwater streams. Headwater streams within the Sandhills Ecoregion are characterized as clear and slow moving with cool water temperatures (< 25ºC max). Within the Sandhills Ecoregion exists the Cherry County Wetlands Biologically Unique Landscapes described as an area in need of conservation (Schneider et al. 2018). Historically, headwater streams within the Sandhills Ecoregion were highly connected to the floodplain providing ample feeding and spawning habitat for residing fish species. The hydrological characteristics (e.g., groundwater to surface water connection) and cool-water temperatures of Sandhills Ecoregion streams may afford a level of ecological resilience to changing climate conditions not found elsewhere in the Great Plains. However, channelization is a popular practice in the Sandhills Ecoregion to efficiently move water out of wet meadows and promote haying opportunities (Ducey 1991). Channelization homogenizes environmental parameters such as sinuosity and instream habitat such as channel depth and mesohabitat (e.g., pools and runs) diversity.

Four headwater streams within Cherry County, Nebraska known to contain Northern Pearl Dace include Willow Creek, Clifford Creek, Sandy Richards Creek, and Gordon Creek. Willow Creek is a 21 km long tributary to Clifford Creek. Clifford Creek is a 32 km long tributary to the Snake River. Sandy Richards Creek is a 17 km long tributary to Gordon Creek. Gordon Creek is 241 km and is the largest Sandhills Ecoregion stream in Nebraska that flows into the Niobrara River. These four headwater streams are spatially close in terms of overland straight-line distance. However, there is an estimated 482 km linear stream miles from Willow Creek to the headwaters of Gordon Creek.

Experimental Design

Capture-mark-recapture (CMR) data were collected via the robust design experimental framework and used to estimate survival, temporary emigration, capture probability, recapture probability, and the population size of Northern Pearl Dace. The robust design consists of a hierarchical sampling scheme where multiple secondary samples occur within primary periods (Pollock 1982; Kendall et al. 1995; Kendall et al. 1997; Powel and Gale 2015). Estimates of survival and movement were obtained between primary periods whereas estimates of the super population were obtained for each secondary period. The super population was defined spatially based on movement assumptions. For example, if no movement was assumed then the super population estimates were spatially confined to the specific sites sampled. Conversely, if random movement was assumed then the super population estimates were multiplied by the estimated temporary immigration rate which allows a larger spatial inference such as the entire headwater stream. The robust design model has the same assumptions as the Cormack-Jolly-Seber model including (a) every animal has the same chance of capture, (b) every animal has the same probability of surviving to the next sampling period, (c) tags are not lost or overlooked, (d) samples are instantaneous and the animal is released immediately, (e) all emigration from the sample area is permanent, (f) fates of animals are independent of other animals (Powel and Gale 2015). Additional assumptions of the robust design model include (g) the population is assumed closed to additions and deletions across each secondary sampling session within a primary period, (h) survival rates are assumed constant for all animals in the population regardless of availability for capture, (i) permanent emigration out of the super-population influences the survival estimate (Powel and Gale 2015).

A major advantage of the robust design is its ability to estimate temporary emigration rates. Kendall et al. (1997) defined the parameters γ’ and γ” as the probability of an animal being off the study area and unavailable for capture during primary trapping session. The parameter γ’ represents an animal absent on the study area during primary trapping session (i − 1) and survived to trapping session (i). The parameter γ” represents an animal present during primary trapping session (i-1) and survived the trapping session (i). The parameters γ’ and γ” can be assumed equal (γ’ = γ”) where temporary emigration on and off the study area is the same, not equal (γ’ ≠ γ”) where temporary emigration on or off the study area is greater than the other, or fixed (γ’ = 1 γ” = 0) where temporary emigration on or off the study area is assumed to not occur (Kendall et al. 1997).

Site Selection

A total of 28 sites were selected from Willow Creek, Clifford Creek, Sandy-Richards Creek, and Gordon Creek (Figure 3.1). Each site was visually delineated using

ArcGIS to determine whether it was channelized or non-channelized. Sites were classified by using Rosgen's sinuosity index (SI) as either channelized (SI = 1.0-1.2) or non-channelized (SI > 1.2; Rosgen 1994). An even number of channelized sites (Willow Creek = 4; Clifford Creek = 4; Sandy-Richards Creek = 2; and Gordon Creek = 3) and non-channelized sites (Willow Creek = 4; Clifford Creek = 4; Sandy-Richards Creek = 2; and Gordon Creek = 5) were evenly distributed on each stream with consideration of proximity and time needed to sample for independence assumptions for closed-population time periods. Sites selected included a randomly plotted location in ArcGIS across each stream. Due to unforeseen circumstances (i.e., sites went dry or changes in landowner permission), 4 lower sites on Willow Creek, 4 lower sites on Clifford Creek, 2 sites on Sandy-Richards Creek, and 1 site on Gordon Creek were excluded from the population demographics analysis for Northern Pearl Dace. The total length of stream sampled at each site was 40 times stream width with a minimum distance of 150m and a maximum of 300m (Kaufmann et al. 1999). Latitude and longitude were recorded at the beginning and end of each site. Each site was visited three times each season (n = 12 total samples). A 2-to-4-day period elapsed between successive site visits during secondary periods wherein closure of the population was assumed.

Stream Geomorphometry and Instream-Habitat Collection

A multi-probe meter (Xylem YSI; PRO-DSS) was used to measure the water quality parameters for each sample occasion that included temperature (ºC), dissolved oxygen (mg/L), pH, conductivity (µS/cm), and turbidity (NTU). The multi-probe meter was calibrated 3 times per season at the beginning of each sample round. Discharge (m^3/s) was calculated from a cross-section of depth (m) and velocity (m/s) readings along a single transect. A minimum of ten readings were recorded depending on depth change for each sample occasion. For example, if a 5m cross-section had a flat bottom with minimal depth changes, ten recordings evenly distributed would be taken. However, if the same 5m cross-section had multiple depth changes, more than ten recordings would be needed to accurately calculate discharge. Physical stream measurements were collected for each site in August 2022 using standard protocols for assessing wadeable streams (US EPA 2019). Each variable selected was hypothesized to influence population dynamics of Northern Pearl Dace (Table 3.1). Each site was divided into eleven transects spaced approximately 15m apart. Mesohabitats were visually assigned for each transect in the field by the following criteria: riffle—swiftly flowing with a high proportion of its water surface broken; pool—slow flowing with a smooth water surface; and run—intermediate between a pool and riffle with a wavy water surface (Jowett 1993). Depth (m) and velocity (m/s) measurements were collected across each of the 11 transects every 0.5m to calculate Froude's number:

$$Fr = V/\sqrt{(gY)}$$

where water column velocity at 60% depth (V) divided by square root of the water depth (Y) multiplied by the acceleration due to gravity (g).

Fish Collection

Sites were sampled for Northern Pearl Dace June 2022, September 2022, October 2022, and June 2023. Each sample occasion included deploying a block net, calibrating the backpack electrofisher, and backpack electrofishing. A block net (7.6m length; 3mm mesh) was deployed at the upstream stop point anchored to the bank with t-posts. Northern Pearl Dace were sampled via backpack electrofishing (Midwest Lake Electrofishing System; 60Hz, 25% duty cycle, and 100W). The 100 Watt-method was used whereby a pool and run mesohabitat were electrofished just off the sample site to calibrate the output to average 100W between the mesohabitat types by adjusting the volts on the backpack electrofisher at each site during each sample occasion (Meyer et al. 2020).

Backpack electrofishing was conducted with a single pass upstream with 3 netters. All fish were collected and placed into a 19L bucket. In some instances, fish were moved to an aerated holding tank (114L) to reduce stress if numerous individuals were captured. Northern Pearl Dace were sorted into a separate holding tank (114L) containing two battery operated aerators. Northern Pearl Dace length (mm TL) was measured and individuals ≥ 50mm TL were given double tags (i.e., visual implant elastomer-VIE-right dorsal and p-Chip microtransponder-left dorsal) following protocols utilized in Spooner and Spurgeon (In Review; Chapter 2). Northern Pearl Dace on first encounter were given a season specific VIE color tag (June 2022, red; September 2022, yellow; October 2022, orange; June 2023, blue). For each following site visit, Northern Pearl Dace not previously captured were scanned and given season specific VIE tags and p-Chip microtransponders. Northern Pearl Dace not previously captured were sedated with 2.5mL Aqui-s (Aqui-s 2022) in a 19L bucket. Recaptured Northern Pearl Dace were measured (mm TL), scanned for a p-Chip microtransponder tag, and recorded as a recapture or shed tag based on the assumption of 100% VIE retention (Spooner and Spurgeon, In Review; Chapter 2). Processed Northern Pearl Dace were put into a recovery tank (114L) with Vidalife (Syndel 2023) treated water at 1ml to 15L of water. A YSI was used to monitor oxygen and temperature in the recovery tank. Oxygen via battery operated air stones and new water was added to the recovery tank based on fish behavior such as gasping at the surface. Northern Pearl Dace were returned to the middle of the sample site after they were processed and achieved equilibrium. Northern Pearl Dace unable to recover were noted and removed from the study. Due to extreme air temperatures > 35 ºC during the summer and fall seasons, only Northern Pearl Dace ≥ 50mm total length were tagged to minimize tagging mortality likely due to fish stress.

Statistical Analysis

Raw data from the environmental parameters were first converted into z-scores prior to any analysis by subtracting the mean from each observation and dividing by the standard deviation. A principal component analysis (PCA) was conducted using the 'factoextra' package (Kassambara and Mundt 2022) in program R version 4.3.1 (R Core Team 2022) to visualize multidimensional data in two reduced dimensions which we could expect changes in geomorphic and instream habitat in channelized and non-channelized stream sites. The 'corrr' package (Kuhn et al. 2022) was used to create a Pearson's correlation matrix and determine which environmental parameters were highly correlated. A Levene's test was performed to test for homogeneity of variance for each environmental parameter between channelized and non-channelized stream sites. A Multivariate Analysis of Variance (MANOVA) test was used to determine differences using reduced environmental parameters between channelized and non-channelized stream sites. MANOVA outputs the results using Pillai's Trace test and effect size was measured with a Partial Eta Squared test. Analysis of Variance (ANOVA) was used to determine differences in specific environmental parameters between channelized and non-channelized sites. Box plots were created to visually inspect environmental parameters for differences.

Northern Pearl Dace length distributions for channelized and non-channelized sites were created to assess potential bias in tagged individuals between channel types

(Figures 3.2 and 3.3). Individual capture histories were created for Northern Pearl Dace in Program R (R Core Team 2022). All secondary periods (dates sampled) were included in encounter histories. An example capture history of an individual fish across the 4 primary sampling occasions is expressed as:

100 101 000 000 0 1

where each number represents a sampling occasion, and the last two numbers represents a binary group covariate. The first “1” represents the occasion the individual Northern Pearl Dace was first captured, and the second and third “1” represents occasions that the Northern Pearl Dace was recaptured. Primary sessions were grouped by season in that the first “100” represents the first three days of data collection in June 2022. A grouping covariate as a binary response of channelized “0 1” or non-channelized “1 0” was added at the end of each capture history.

Program MARK (White and Burnham 1999) was used to build models (Pollock 1982). Robust design models are parameter-rich meaning the estimable number of parameters can be limited with small sample sizes (Kendall et al. 1997). Therefore, individual covariates were not added to the models. Competing models were built to assess the influence of channelization on survival ($\hat{S}$), movement (γ’ and γ”), capture probability (p), recapture probability (c), and the population size (N). Models were created assuming temporal variation or time-constant survival [i.e., $\hat{S}(t)$ or $\hat{S}(.)$] within or between group covariates. The delta method was used to calculate the variance of annual survival when the temporal scale was changed from monthly survival (Seber 1982; Williams et al. 2002; Skalski et al. 2005; MacKenzie et al. 2006; and Cooch and White 2014). Models were created assuming temporal variation or time-constant equal or unequal capture and recapture probabilities (i.e., pt = ct or pt ≠ ct; p. = c. or p. ≠ c.) within or between group covariates. Population estimates (N) for each group covariate were defined as the number of Northern Pearl Dace ≥50mm in the effective sampling area that included the area from which fish could have been captured by our sampling gear. The super population (Nsp) was calculated from the population estimate (N) where emigration rates were assumed to be 0 based on the no movement model. An information theoretic approach was used whereby competing models were developed and ranked using Akaike's information criterion corrected for small sample size (AICc; Burnham and Anderson 2002). No conditional or unconditional model averaging was utilized if ΔAICc was > 4 from the top ranked model (Burnham and Anderson 2002).

Results

Habitat Differences Between Channelized and Non-channelized Stream Sites

A scree plot suggested the first two principal components in the PCA would adequately represent variability in our environmental variables (Figure 3.2). The first two principal coordinates explained 62.7% of the variability in our environmental dataset (Table 3.2). The first axis explained 42.3% (Table 3.3) of the variability and was driven by habitat composition variables. The second axis represents 20.4% of the variability and was driven by flow variables based on loadings. Ellipses were displayed as 95% confidence intervals for channelized and non-channelized stream sites (Figure 3.3). The Levene's test suggested that the homogeneity of variance assumption was violated by sinuosity index ($F(1, 13) = 6.3613$, $p = 0.0255$) and the percent of pools ($F(1, 13) = 6.11773$, $p = 0.0273$; Table 3.4). The correlation matrix of the a-priori selected environmental parameters suggested that mean discharge in spring 2022, summer 2022, fall 2022, and spring 2023 were highly correlated ($r > 0.75$; Figure 3.4). Further, the mean, max, and variance of pool and run depth environmental parameters were highly correlated within mesohabitat type. Therefore, mean discharge in spring 2022, fall 2022, and spring 2023 were selected to remove for the MANOVA and ANOVA analyses. Further, the variance and max of pool and run mesohabitat were also removed. The MANOVA test revealed a significant difference in the means of at least one post-PCA selected environmental parameter between channelized and non-channelized stream sites (Pillai's Trace = 0.9181, $F(8, 6) = 8.4040$, $p = 0.0091$). The measure of effect size (Partial Eta Squared; η_p^2) was 0.92 and suggested that there was a large effect of channelized and non-channelized stream site classification on post-PCA selected environmental parameters. The one-way ANOVA revealed that there was a statistically significant difference in mean sinuosity index ($F(1, 13) = 16.6400$, $p = 0.0013$) and the percent of pool mesohabitat ($F(1, 13) = 5.3848$, $p = 0.0372$) between channelized and non-channelized sites (Table 3.5). Box plots of all hypothesized environmental parameters were examined (Figures 3.5-3.19) and showed visual differences in variance and max of pool depth, the percent of pools, sinuosity index, and spring 2023 mean discharge.

Survival Differences Between Channelized and Non-channelized Stream Sites

A total of 1,949 Northern Pearl Dace (50-145mm total length; Figure 3.20) were double-tagged across channelized (n = 836 individuals; 50-135mm total length) and non-channelized sites (n = 1,113 individuals; 50-145mm total length; Table 3.6). Visualization of box plots for tagged Northern Pearl Dace revealed no difference in size structure (Figure 3.21). A total of 811 individual Northern Pearl Dace were recaptured across channelized (n = 164) and non-channelized sites (n = 647; Table 3.7). There were 41 Northern Pearl Dace that shed p-Chip microtransponders which led to 5% tag loss over the 374-day field study (Spooner and Spurgeon, In Review). Therefore, the estimates given are conservative estimates with minimal violations to assumptions of perfect tag retention.

The model with the most support, given the data, suggested survival (Ŝ) varied by time and site classification, movement (y' and y") did not occur, and capture and recapture (p and c) rates were equal but varied by time and site classification (Table 3.8). Survival estimates were significantly lower in channelized sites (Ŝ = 0.34; SE = 0.06) compared to non-channelized sites (Ŝ = 0.93; SE = 0.03) from spring to summer 2022 (Figure 3.22). Survival estimates were not significantly different between channelized sites (Ŝ = 0.43; SE = 0.11) and non-channelized sites (Ŝ = 0.54; SE = 0.05) from summer to fall 2022. Survival estimates were not significantly different between channelized sites (0.70; SE = 0.04) and non-channelized sites (Ŝ = 0.76; SE = 0.02) from fall 2022 to spring 2023. Annual survival estimates, derived from the monthly survival estimates, differed between channelized sites (Ŝ = 0.001; SE = 0.009) and non-channelized sites (Ŝ = 0.047; SE = 0.024; Figure 3.23). Capture and recapture probabilities varied by time and site classification (Table 3.9). Population estimates for channelized sites remained relatively constant among seasons. Population estimates for non-channelized sites increased from the spring 2022 to summer 2022 post-spawning period and slowly declined throughout fall 2022 and spring 2023 (Table 3.10). The super population estimates were the same as the population estimates in the top ranked model since no emigration was assumed.

Discussion

Geomorphic and instream habitat changes to headwater streams via channelization influences population demographic parameters of small-bodied fish. Specifically, channelization may reduce survival of small-bodied fish. Northern Pearl

Dace exhibited reduced annual survival in channelized compared to non-channelized headwater stream sites. Low annual survival of Northern Pearl Dace differed from higher annual survival on another short lived (4-5 years) small-bodied fish, the Slimy Sculpin *Cottus cognatus* (Keeler et al. 2007). However, the Slimy Sculpin is a bottom-dwelling predator while Northern Pearl Dace are a pelagic prey species which may affect annual survival estimates based on position in the food web. Monthly survival was highest from spring to summer for non-channelized sites and was comparable to another study on Southern Redbelly Dace *Chrosomus erythrogaster* and Cardinal Shiner *Luxilus cardinalis*, SGCN, in Kansas and New Mexico (Siller et al. 2023). Given lower annual and monthly survival of Northern Pearl Dace in channelized headwater streams, delineation of watershed geomorphology at a larger spatial scale may provide insight to the overall impact to their populations. For example, if 75% of the stream length is channelized then mitigation may be warranted.

Channelization altered local scale habitat conditions within headwater streams of the Nebraska Sandhills. A difference was shown between channelized and non-channelized headwater streams when considering selected environmental parameters post-correlation. Specifically, differences were determined in mean sinuosity and the percent of pool mesohabitat between channelized and non-channelized headwater stream sites. The findings of the effects of channelization on reducing pool habitat and sinuosity were similar to other studies (Lau et al. 2006, Lennox and Rasmussen 2016). However, macrophyte coverage was not different between channelized and non-channelized headwater stream sites. Although Rambaud et al. (2009) showed differences in channelized from non-channelized stream site macrophyte communities (e.g., tolerant species presence and species richness). Anecdotally, observations were noted in the field that sites defined as channelized were beginning to self-repair given macrophyte presence which may positively influence environmental parameters such as pool depth or the percent of pools in a site. O'Briain et al. (2017) showed that macrophyte presence in channelized streams showed strong fine-scale relationships in repairing heterogeneity in stream velocity and depth. However, Lennox and Rasmussen (2016) showed that even after 80 years post channelization streams did not restore themselves naturally. Therefore, local scale habitat conditions in channelized headwater streams could be monitored over time to determine if a rehabilitation project would be justified to improve favorable conditions for fish present.

Small-bodied fish of the Nebraska Sandhills in channelized headwater streams may exhibit decreased residence time compared to non-channelized headwater streams and may negatively influence survival estimates. Mushet et al. (2023) suggested Pearl Dace *Margariscus margarita* in Canada displaced from ~220m to ~300m. Further, Walker et al. (2013) suggested adult Southern Redbelly Dace in Arkansas displayed an average displacement of 88m in streams. There was an instance where approximately 150 Northern Pearl Dace were marked in a channelized site during a single sampling occasion in our study with subsequent sampling occasions during the closed period containing few Northern Pearl Dace. Movements by Northern Pearl Dace may be indicative of searching for suitable habitat (Albanese et al. 2004). Sampling sites were 150m to 200m in length and may not have encompassed the distance Northern Pearl Dace travel within a 24hr sampling period. Therefore, utilizing a multistate CMR study design on a larger spatial scale (e.g., 1000m including 10; 100m sites) may provide insight to residence time and spatially defining closed sampling reach size.

Channelization of headwater streams and reduced pool habitat in the Nebraska Sandhills may affect small-bodied fish abundance. The estimated population size of Northern Pearl Dace in non-channelized sites was greater in summer 2022 and fall 2022 compared to the spring in 2022 and 2023. An increase in in the number of Northern Pearl Dace was observed in non-channelized sites in summer 2022 which may suggest increased recruitment due to favorable refugia habitat. The estimated population size of Northern Pearl Dace in the channelized stream sites were similar across all seasons and may indicate no increase in abundance possibly due to less favorable conditions. Northern Pearl Dace, at various ages, are more abundant in pool habitat compared to other mesohabitats throughout all seasons (Tallman 1979; Cunningham 1995). The presence of pools in a headwater stream increases the variability in depth and decreases velocity which small-bodied fish depend on for refugia from high disturbance events (Labbe and Fausch 2000). Therefore, increasing the percentage of pools in channelized sites may increase abundance of small-bodied fish in headwater streams.

The use of the robust design to link the influence of anthropogenic alteration in headwater streams on population demographics and evaluate future restoration efforts may benefit the preservation of small-bodied and SGCN fish. The robust design has been used to monitor population trends over time in other fish SGCN such as the Pallid Sturgeon (Steffenson et al. 2017). The Cormack-Jolly-Seber (CJS) design has previously been used in small-bodied and SGCN fish survival estimates (Steffenson et al. 2010; Chiotti et al. 2023; Kahn et al. 2023). For example, Siller et al. (2023) used the CJS design to estimate survival of two small-bodied fish SGCN. The CJS design only incorporates open population data and yields an apparent survival estimate. The robust design allows for more accurate estimates of survival by incorporating temporary emigration parameters. Choosing a spatial location and scale of a stream restoration project to target distinct environmental parameters with the goal of benefiting fish SGCN are one of many considerations for project planning. For example, Pretty et al. (2003) suggested spatial proximity to source populations and non-disturbed stream reaches were important considerations for restoration designs. A long-term monitoring study in Indiana on a channelized stream restoration project where increases on pool prevalence and stream length were targeted, indicated a positive response in fish community relative abundance and biomass (~300% increase) over seventeen years (Shirey et al. 2016). The prevalence of pool mesohabitats and stream length (i.e., greater sinuosity index) were similar to the environmental parameters that differed in the channelized sites of our study. The use of the robust sampling design provided a baseline survival estimates that may be used to compare across years and to evaluate future channelized headwater stream mitigation projects.

Violated assumptions for both the habitat and survival analysis of Northern Pearl Dace warrant broad interpretation of the results. First, we did violate the assumption of homogeneous variances for both sinuosity and the percent of pool mesohabitat. Olson (1974) suggested the Pillai’s Trace test to use when homogeneity of variance is violated in a MANOVA as it is the most robust to minimize a type 1 error and was applied to our MANOVA. Secondly, a violation of an assumption of the robust design that all tagged fish must remain on the study site for each secondary sampling period may have occurred. Further, it is unknown if these fish died or found more suitable habitat off the study site. For example, tagged fish were never resampled on other sampling sites. It is unknown if the assumption of fish remaining on the study site for secondary periods was violated. If we did violate the assumption, then our survival estimates would be biased low since fish that emigrate from the site are considered lost to the population. Therefore, our annual estimate of Northern Pearl Dace survival should be considered a conservative estimate prior to further research on their movement.

Literature Cited

Albanese, B., P. L. Angermeier, and S. Dorai-Raj. 2004. Ecological correlates of fish movement in a network of Virginia streams. Canadian Journal of Fisheries and Aquatic Sciences 61:857–869.

Allan, J. D., and A. S. Flecker. 1993. Biodiversity conservation in running waters. BioScience 43(1):32–43.

Bond, N. R., S. R. Balcombe, D. A. Crook, J. C. Marshall, N. Menke, and J. S. Lobegeiger. 2015. Fish population persistence in hydrologically variable landscapes. Ecological Applications 25(4):901–913.

Brooker, M. P. 1985. The ecological effects of channelization. The Geographical Journal 151(1):63–69.

Brown, T. T., T. L. Derting, and K. Fairbanks. 2008. The effects of stream channelization and restoration on mammal species and habitat in riparian corridors. Journal of the Kentucky Academy of Science 69(1):37–49.

Burnham, K. P., Anderson, D.R., 2002. Model Selection and Multimodel Inference: a practical information-theoretic approach, 2nd edition. Springer, New York.

Casatti, L., F. Langeani, and C. P. Ferreira. 2006. Effects of physical habitat degradation on the stream fish assemblage structure in a pasture region. Environmental Management 38(6):974–982.

Chiotti, J. A., J. C. Boase, A. S. Briggs, C. Davis, R. Drouin, D. W. Hondorp, L. Mohr, E. F. Roseman, M. V. Thomas, and T. C. Wills. 2023. Lake Sturgeon population trends in the St. Clair–Detroit River system, 2001–2019. North American Journal of Fisheries Management 43(4):1066–1080.

Cooch, E., and G. White. 2014. Appendix B: The 'Delta method' In Program MARK: a gentle introduction, 12th edition, Cooch, E. and G. White, eds. Online: http://www.phidot.org/software/mark/docs/book/pdf/app_2.pdf

Cunningham, G. R. 1995. Life history traits and reproductive ecology of the Pearl Dace, *Margariscus margarita* (Pisces: Cyprinidae) in the Sandhills of Nebraska. M.A. Thesis. University of Nebraska-Omaha, Omaha, Nebraska.

Cunningham, G. R. 2006. Pearl Dace (*Margariscus margarita):* a technical conservation assessment. USDA Forest Service, Rocky Mountain Region. Available: https://www.nrc.gov/docs/ML1004/ML100481436.pdf. (December 2021)

Ducey, J. E. 1991. Ditching of wetlands in the Nebraska Sandhills: a case study of Grant County. Transactions of the Nebraska Academy of Sciences and Affiliated Societies 145:1–9.

Dudgeon, D., A. H. Arthington, M. O. Gessner, Z. I. Kawabata, D. J. Knowler, C. Lévêque, R. J. Naiman, A. H. Prieur-Richard, D. Soto, M. L. J. Stiassny, and C. A. Sullivan. 2006. Freshwater biodiversity: importance, threats, status, and conservation challenges. Biological Reviews of the Cambridge Philosophical Society 81:163–182.

Fahrig, L. 2003. Effects of habitat fragmentation on biodiversity. Annual Review of Ecology, Evolution, and Systematics 34:487–515.

Fausch, K. D., J. Lyons, J. R. Karr, P. L. Angermeier. 1990. Fish communities as indicators of environmental degradation. American Fisheries Society Symposium 8:123–144

Fischer, J., and C. Paukert. 2009. Effects of sampling effort, assemblage similarity, and habitat heterogeneity on estimates of species richness and relative abundance of stream fishes. Canadian Journal of Fisheries and Aquatic Sciences 66:277–290.

Gomi, T., R. C. Sidle, and J. S. Richardson. 2002. Understanding processes and downstream linkages of headwater systems. BioScience 52(10):905.

Hayford, B., and D. Baker. 2011. Lakes of the Nebraska Sandhills. Lakeline (Winter): 26–30.

Jowett, I. G. 1993. A method for objectively identifying pool, run, and riffle habitats from physical measurements. New Zealand Journal of Marine and Freshwater Research 27(2):241–248.

Kassambara, A., and F. Mundt. 2022. A package to extract and visualize the results of multivariate data analyses. R. R package version 1.0.7. Available: https://cran.r-project.org/web/packages/factoextra/index.html. (October 2023).

Kaufmann, P. R., P. Levine, E. G. Robinson, C. Seeliger, and D. V. Peck. 1999. Quantifying physical habitat in wadable streams, EPA 620/R–99/003. Environmental Monitoring and Assessment Program, U.S. Environmental Protection Agency, Corvallis, Oregon.

Keeler, R. A., AndréR. Breton, D. P. Peterson, and R. A. Cunjak. 2007. Apparent survival and detection estimates for PIT-tagged Slimy Sculpin in five small New Brunswick streams. Transactions of the American Fisheries Society 136(1):281–292.

Kendall, W. L., J. D. Nichols, and J. E. Hines. 1997. Estimating temporary emigration using capture–recapture data with Pollock’s robust design. Ecology 78(2):563–578.

Kendall, W. L., K. H. Pollock, and C. Brownie. 1995. A likelihood-based approach to capture-recapture estimation of demographic parameters under the robust design. Biometrics 51:293–308.

King, K. W., P. C. Smiley, and N. R. Fausey. 2009. Hydrology of channelized and natural headwater streams / Hydrologie de cours d'eau recalibrés et naturels de tête de bassin. Hydrological Sciences Journal 54(5):929–948.

Kahn, J., C. Hager, J. Watterson, N. Mathies, A. Deacy, and K. Hartman. 2023. Population and sex-specific survival estimates for Atlantic sturgeon: addressing detection probability and tag loss. Aquatic Biology 32:1–12.

Kuhn, M., S. Jackson, and J. Cimentada. 2022. A package for correlations. R. R package version 0.4.4. Available: https://cran.r-project.org/web/packages/corrr/index.html. (October 2023).

Kuzelka, R. D., C. A. Flowerday, R. N. Manley, B. C. Rundquist, and S. J. Herrin. 1993. Flat water: a history of Nebraska and its water. Resource Report (12):1–291.

Labbe, T. R., and K. D. Fausch. 2000. Dynamics of intermittent stream habitat regulate persistence of a threatened fish at multiple scales. Ecological Applications 10(6):1774–1791.

Lau, J. K., T. E. Lauer, and M. L. Weinman. 2006. Impacts of channelization on stream habitats and associated fish assemblages in East Central India. The American Midland Naturalist 156:319–330.

Lee, D. S., C. R. Giber, C. H. Hocutt, R. E. Jenkins, D. E. McAllister, and J. R. Stauffer, Jr. 1980. Atlas of North American freshwater fishes. The North Carolina Biological Survey, North Carolina.

Lennox, P., and J. Rasmussen. 2016. Long Term Effects of Channelization on a Coldwater Stream Community. Canadian Journal of Fisheries and Aquatic Sciences 73:1–8.

Mackenzie, D. I., J. D. Nichols, J. A. Royale, K. H. Pollock, L. L. Bailey, and J. E. Hines. 2006. Occupancy estimation and modeling: inferring patterns and dynamics of species occurrence. Elsevier Academic Press, Burlington, MA.

Meyer, K. A., L. V. Chiaramonte, and J. B. Reynolds. 2020. The 100-watt method: a protocol for backpack electrofishing in small streams. Fisheries 46(3):125–130.

Mushet, G. R., C. Yee, S. Bergson, and P. J. Blanchfield. 2023. Abundance and movement of fishes among nearshore habitat types in small boreal shield lakes. Hydrobiologia 850:1985–2003.

O'Briain, R., S. Shephard, and B. Coghlan. 2017. Pioneer macrophyte species engineer fine-scale physical heterogeneity in a shallow lowland river. Ecological Engineering 102:451–458.

Olson, C. L. 1974. Comparative robustness of six tests in multivariate analysis of variance. Journal of the American Statistical Association 69:894–908.

Pasbrig, C. 2013. The glacial relict fishes of South Dakota's sandhills. American Currents 39(1):29–31.

Pollock, K. H. 1982. A capture-recapture design robust to unequal probability of capture. The Journal of Wildlife Management 46(3):752–757.

Powell, L. A., and G. A. Gale. 2015. Estimation of parameters for animal populations: a primer for the rest of us. Caught Napping Publications, Lincoln, NE.

Pretty, J. L., S. S. C. Harrison, D. J. Shepherd, C. Smith, A. G. Hildrew, and R. D. Hey. 2003. River rehabilitation and fish populations: assessing the benefit of instream structures. Journal of Applied Ecology 40(2):251–265.

Rambaud, M. P., N. Machon, J. Moret, and I. Combroux. 2009. Does channelization alter spatial and temporal dynamics of macrophyte communities and their physical habitat? Fundamental and Applied Limnology 174(2):159–172.

R Core Team. 2022. A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna.

Richardson, J. S. 2019. Biological diversity in headwater streams. Water 11:366–385.

Rosenfeld, J. S., and T. Hatfield. 2006. Information needs for assessing critical habitat of freshwater fish. Canadian Journal of Fisheries and Aquatic Sciences 63:683–698.

Rosgen, D. L. 1994. A classification of natural rivers. Catena 22(3):169–199.

Schlosser, I. J. 1991. Stream fish ecology: a landscape perspective. BioScience 41(10):704–712.

Schneider, R., M. Fritz, J. Jorgensen, S. Schainost, R. Simpson, G. Steinauer, and C. Rothe-Groleau. 2018. Revision of the tier 1 and 2 lists of species of greatest conservation need: a supplement to the Nebraska Natural Legacy Project State Wildlife Action Plan. The Nebraska Game and Parks Commission, Lincoln, Nebraska.

Seber, G. A. F. 1982. The estimation of animal abundance and related parameters. 2nd ed. Chapman, London and Macmillan, New York.

Siller, M. K., P. J. Pfaff, E. Wild, and K. B. Gido. 2023. Apparent survival and detection probability of PIT-tagged small-bodied stream fishes using multi-pass wand antenna surveys. Environmental Biology of Fishes 106(6):1371–1381.

Shirey, P. D., M. A. Brueseke, J. B. Kenny, and G. A. Lamberti. 2016. Long-term fish community response to a reach-scale stream restoration. Ecology and Society 21(3):11

Skalski, J. R., K. E. Ryding, and J. J. Millspaugh. 2005. Wildlife demography: analysis of sex, age, and count data. Elsevier Academic Press, Burlington, MA.

Smiley, P. C., R. B. Gillespie, K. W. King, and C. Huang. 2008. Contribution of habitat and water quality to the integrity of fish communities in agricultural drainage ditches. Journal of Soil and Water Conservation 63(6):218A–219A.

Smiley, P. C., R. B. Gillespie, K. W. King, and C. Huang. 2009. Management implications of the relationships between water chemistry and fishes within channelized headwater streams in the midwestern United States. Ecohydrology 2:294–302.

Smiley, P. C., K. W. King, and N. R. Fausey. 2017. Influence of different habitat factors on Creek Chub (*Semotilus atromaculatus*) within channelized agricultural headwater streams. Northeastern Naturalist 24(8):18–44.

Spooner, J. M., and J. J. Spurgeon. In Review. Retention of p-Chip microtransponders and post-tagging survival of small-bodied stream fishes. North American Journal of Fisheries Management.

Steffensen, K. D., L. A. Powell, and J. D. Koch. 2010. Assessment of hatchery-reared Pallid Sturgeon survival in the Lower Missouri River. North American Journal of Fisheries Management 30(3):671–678.

Steffensen, K. D., L. A. Powell, and M. A. Pegg. 2017. Using the robust design framework and relative abundance to predict the population size of pallid sturgeon *Scaphirhynchus albus* in the lower Missouri River. Journal of Fish Biology 91(5):1378–1391.

Syndel. 2023. Vidalife. Available: https://syndel.com/wp-content/uploads/2019/01/Vidalife-Info-Sheet.pdf. (April 2023)

Tallman, R. F. 1979. Environments occupied, indices of maturity, feeding ecology, shoaling behavior and interactions with other species by Pearl Dace, *Semotilus margarita* (Cope) in Manitoba. Master's thesis. University of Manitoba, Winnipeg.

Tallman, R. F., and J. H. Gee. 1982. Intraspecific resource partitioning in a headwaters stream fish, the Pearl Dace, Semotilus margarita (Cope). Environmental Biology of Fishes 7:243–249.

United States Environmental Protection Agency. 2019. Field operations manual wadeable. Available: https://www.epa.gov/sites/default/files/2019-05/documents/nrsa_1819_fom_wadeable_version_1.2_0.pdf. (May 2022)

Walker, R. H., G. L. Adams, and S. Reid Adams. 2013. Movement patterns of southern redbelly dace, *Chrosomus erythrogaster* , in a headwater reach of an Ozark stream. Ecology of Freshwater Fish 22(2):216–227.

White, G. C., and K. P. Burnham. 1999. Program MARK: survival estimation from populations of marked animals. Bird Study 46(Supplement 1):S120–S139.

Williams, B. K., J. D. Nichols, and M. J. Conroy. 2002. Analysis and management of animal populations. Academic Press, San Diego.

Table 3.1—Summary of environmental parameters of physical habitat characterization (United States EPA 2019). Each environmental parameter was assessed at each stream site of Willow Creek, Clifford Creek, and Gordon Creek in summer 2022 and used to differentiate channelized and non-channelized sites.

Environmental Parameter	**Description**	**Variable Type**
Maxpooldepth	Maximum pool depth (cm)	Continuous
Meanpooldepth	Average pool depth (cm)	Continuous
Variancepooldepth	Variance of the pool depth	Continuous
Maxrundepth	Maximum run depth (cm)	Continuous
Meanrundepth	Average run depth (cm)	Continuous
Variancerundepth	Variance of the run depth	Continuous
Meanmac	Average macrophytes (%)	Categorical
SI	Sinuosity index = stream length/valley length	Continuous
Spring2022_Meandischarge	Average discharge spring, 2022	Continuous
Summer2022_Meandischarge	Average discharge summer, 2022	Continuous
Fall2022_Meandischarge	Average discharge fall, 2022	Continuous
Spring2023_Meandischarge	Average discharge spring, 2023	Continuous
Percent_Pool	Percentage pool mesohabitat	Continuous
Habitat_Frequency	Number of changes in mesohabitat (pool-run or run-pool)	Continuous
Meanfroudes	Average Froude’s number from the max depth at each of the 11 transects	Continuous

Table 3.2—Principal component analysis dimensions defined by the eigenvalue and the variance explained of dimensions for channelized and non-channelized stream sites in summer 2022 of Willow Creek, Clifford Creek, and Gordon Creek.

Component	**Eigenvalue**	**Variance Percent**	**Cumulative Variance Percent**
Dimension 1	6.3496	42.3307	42.3307
Dimension 2	3.0573	20.3823	62.7130
Dimension 3	2.1340	14.2268	76.9397
Dimension 4	1.2026	8.0170	84.9567
Dimension 5	0.8535	5.6901	90.6468
Dimension 6	0.7333	4.8891	95.5359
Dimension 7	0.3108	2.0717	97.6076
Dimension 8	0.1940	1.2934	98.9010
Dimension 9	0.0742	0.4947	99.3958
Dimension 10	0.0582	0.3881	99.7838
Dimension 11	0.0228	0.1520	99.9358
Dimension 12	0.0061	0.0404	99.9762
Dimension 13	0.0035	0.0236	99.9998
Dimension 14	3.07E-05	0.0002	100.0000

Table 3.3—Principal component analysis loadings of environmental parameters of the first two components for channelized and non-channelized stream sites in summer 2022 of Willow Creek, Clifford Creek, and Gordon Creek. See Table 3.1 for parameter definition.

	PC1	**PC2**
Maxpooldepth	-0.346552255	-0.03698737
Meanpooldepth	-0.362793728	-0.10457275
Variancepooldepth	-0.252863209	-0.17454473
Maxrundepth	-0.336290315	-0.17951105
Meanrundepth	-0.322027319	-0.14400104
Variancerundepth	-0.304083031	-0.23970326
Meanmac	-0.231679709	-0.30501942
SI	-0.065377213	0.24915176
Spring2022_Meandischarge	-0.212885437	0.37523261
Summer2022_Meandischarge	-0.272309623	0.38045983
Fall2022_Meandischarge	-0.268586403	0.38147016
Spring2023_Meandischarge	-0.272541642	0.37458733
Percent_Pool	0.006787344	0.13863715
Habitat_Frequency	0.165766574	0.06116304
Meanfroudes	0.154008273	0.31201519

Table 3.4—Levene's test results on homogeneity of variance for each environmental parameter between channelized and non-channelized stream sites in Willow Creek, Clifford Creek, and Gordon Creek summer 2022. See Table 3.1 for parameter definition. Bold values are significant.

Environmental Parameter	**df1**	**df2**	**F-value**	**p-value**
Maxpooldepth	1	13	0.6103	0.4486
Meanpooldepth	1	13	0.0225	0.8832
Variancepooldepth	1	13	1.7953	0.2032
Maxrundepth	1	13	2.5558	0.1339
Meanrundepth	1	13	2.0133	0.1794
Variancerundepth	1	13	0.0174	0.8970
Meanmac	1	13	0.8667	0.3688
SI	1	13	6.3613	**0.0255**
Spring2022_Meandischarge	1	13	5.9095	**0.0303**
Summer2022_Meandischarge	1	13	1.1885	0.2954
Fall2022_Meandischarge	1	13	1.4771	0.2458
Spring2023_Meandischarge	1	13	1.7120	0.2134
Percent_Pool	1	13	6.1773	**0.0273**
Habitat_Frequency	1	13	0.3859	0.5452
Meanfroudes	1	13	0.7365	0.4063

Table 3.5—One-way Analysis of Variance (ANOVA) test results for each environmental parameter between channelized and non-channelized stream sites in Willow Creek, Clifford Creek, and Gordon Creek summer 2022. See Table 3.1 for parameter definition. Bold values are significant.

Response Variable	**Group**	**Df**	**Sum of Squares**	**Mean Square**	**F-value**	**p-value**
Meanpooldepth	Between	1	1.6624	1.66243	1.7517	0.2085
	Within	13	12.3376	0.94904		
Meanrundepth	Between	1	0.0888	0.08881	0.0830	0.7778
	Within	13	13.9112	1.0701		
Meanmac	Between	1	0.2844	0.28445	0.2696	0.6123
	Within	13	13.7156	1.05504		
SI	Between	1	7.8597	7.8597	16.6400	**0.0013**
	Within	13	6.1403	0.4723		
Summer2022_meandischarge	Between	1	1.2953	1.2953	1.3254	0.2704
	Within	13	12.7047	0.97729		
Percent_Pool	Between	1	4.1005	4.1005	5.3848	**0.0372**
	Within	13	9.8995	0.7615		
Habitat_Frequency	Between	1	2.4680	2.46798	2.7821	0.1192
	Within	13	11.5320	0.88708		
Meanfroudes	Between	1	1.1731	1.17313	1.1890	0.2953
	Within	13	12.8269	0.98668		

Table 3.6—Total number of individual Northern Pearl Dace tagged in the spring 2022, summer 2022, fall 2022, spring 2023, and across all seasons in channelized and non-channelized sites of Willow Creek, Clifford Creek, Sandy-Richards Creek, and Gordon Creek.

		2022		**2023**	
Site Classification	Spring	Summer	Fall	Spring	All Seasons
Channelized	116	295	157	268	836
Non-channelized	130	537	262	184	1113
Total	246	832	419	452	1949

Table 3.7—Total number of Northern Pearl Dace recaptured in the spring 2022, summer 2022, fall 2022, spring 2023, and across all seasons in channelized and non-channelized sites of Willow Creek, Clifford Creek, Sandy-Richards Creek, and Gordon Creek.

		2022		**2023**	
Site Classification	Spring	Summer	Fall	Spring	All Seasons
Channelized	6	85	46	27	164
Non-channelized	48	236	285	78	647
Total	54	321	331	105	811

Table 3.8—Comparison of competing models used to describe Northern Pearl Dace population demographic estimates in channelized and non-channelized sites in Willow Creek, Clifford Creek, Sandy-Richards Creek, and Gordon Creek. Models include survival (Φ), temporary emigration (γ'and y''), capture probability (p), recapture probability (c). Subscripts include "t" (time variation), "." (constant across time), and "g" (channelized and non-channelized variation). Models were ranked by corrected Akaike's information criterion (AICc). ΔAICc is the difference between a model's AICc value and that of the highest-ranked model. Weight is the Akaike weight (sum of all weights = 1.00). K is the number of parameters.

Model	**AICc**	**ΔAICc**	**Weight**	**K**
{Φ(t*g) (y'g = 1) (y"g = 0) (pt*g) = (ct*g)}	8356.378	0	0.99921	30
{Φ(t*g) (y'g = 1) (y"g = 0) (pt*g) (ct*g)}	8370.661	14.2824	0.00079	46
{Φ(.*g) (y'g = 1) (y"g = 0) (pt*g) = (ct*g)}	8417.405	61.0269	0	26
{Φ(t) (y'g = 1) (y"g = 0) (pt*g) = (ct*g)}	8440.002	83.624	0	27
{Φ(.) (y'g = 1) (y"g = 0) (pt*g) = (ct*g)}	8454.079	97.7004	0	25
{Φ(t) (y'g = 1) (y"g = 0) (pt) = (ct)}	8756.223	399.8449	0	15
{Φ(t*g) (y'g = 1) (y"g = 0) (p.*g) = (c.*g)}	8930.222	573.8441	0	14

Table 3.9—Capture probability and standard error of Northern Pearl Dace in spring 2022, summer 2022, fall 2022, spring 2023 in channelized and non-channelized sites of Willow Creek, Clifford Creek, Sandy-Richards Creek, and Gordon Creek.

Secondary Period	Site Classification	Season	Estimate	SE
1	Channelized	Spring 2022	0.05	0.02
2	Channelized	Spring 2022	0.04	0.02
3	Channelized	Spring 2022	0.07	0.03
1	Non-channelized	Spring 2022	0.44	0.05
2	Non-channelized	Spring 2022	0.30	0.04
3	Non-channelized	Spring 2022	0.16	0.03
4	Channelized	Summer 2022	0.40	0.04
5	Channelized	Summer 2022	0.25	0.03
6	Channelized	Summer 2022	0.12	0.02
4	Non-channelized	Summer 2022	0.27	0.02
5	Non-channelized	Summer 2022	0.24	0.02
6	Non-channelized	Summer 2022	0.21	0.02
7	Channelized	Fall 2022	0.18	0.04
8	Channelized	Fall 2022	0.06	0.02
9	Channelized	Fall 2022	0.03	0.01
7	Non-channelized	Fall 2022	0.27	0.02
8	Non-channelized	Fall 2022	0.21	0.02
9	Non-channelized	Fall 2022	0.15	0.02
10	Channelized	Spring 2023	0.29	0.05
11	Channelized	Spring 2023	0.03	0.01
12	Channelized	Spring 2023	0.06	0.01
10	Non-channelized	Spring 2023	0.21	0.03
11	Non-channelized	Spring 2023	0.20	0.03
12	Non-channelized	Spring 2023	0.12	0.02

Table 3.10—Population estimates and standard error of Northern Pearl Dace in the spring 2022, summer 2022, fall 2022, spring 2023 in channelized and non-channelized sites of Willow Creek, Clifford Creek, Sandy-Richards Creek, and Gordon Creek.

Site Classification	**Season**	**Estimate**	**SE**
Channelized	Spring 2022	804	306
Channelized	Summer 2022	494	36
Channelized	Fall 2022	752	172
Channelized	Spring 2023	779	138
Non-Channelized	Spring 2022	212	18
Non-Channelized	Summer 2022	1076	55
Non-Channelized	Fall 2022	871	60
Non-Channelized	Spring 2023	490	57

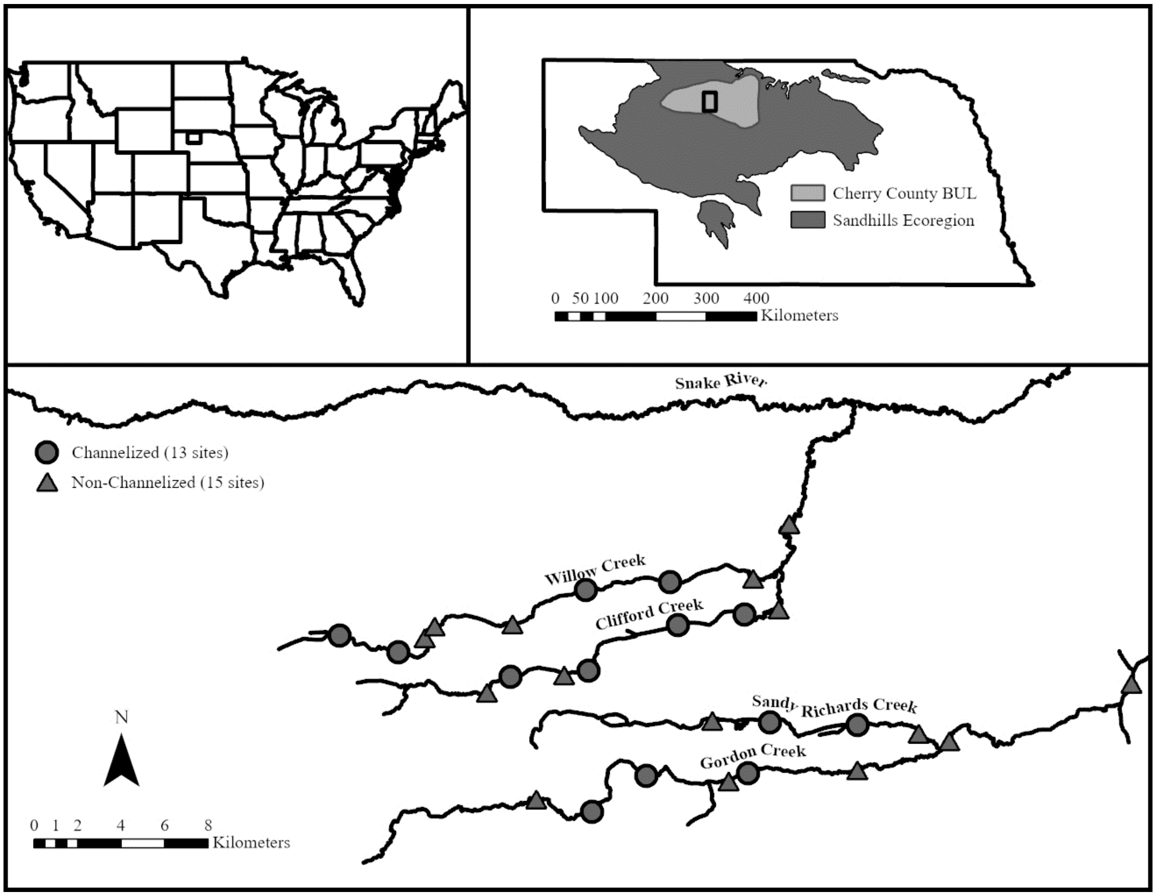

Figure 3.1—Sampling channelized and non-channelized sites in the Cherry County Wetlands Biologically Unique Landscape within the Nebraska Sandhills Ecoregion on Willow Creek, Clifford Creek, Sandy-Richards Creek, and Gordon Creek spring 2022 to spring 2023.

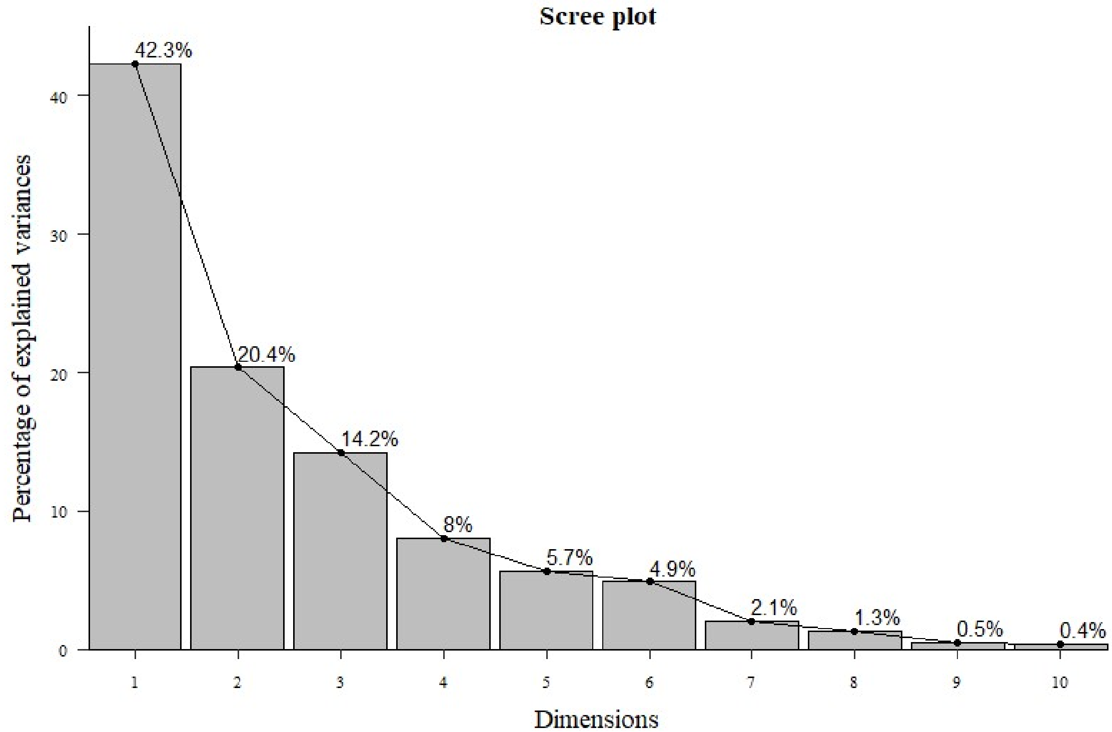

Figure 3.2—Eigenvalues explaining percent variability of the top ten principal component axes for environmental parameters between channelized and non-channelized stream sites in Willow Creek, Clifford Creek, and Gordon Creek summer 2022.

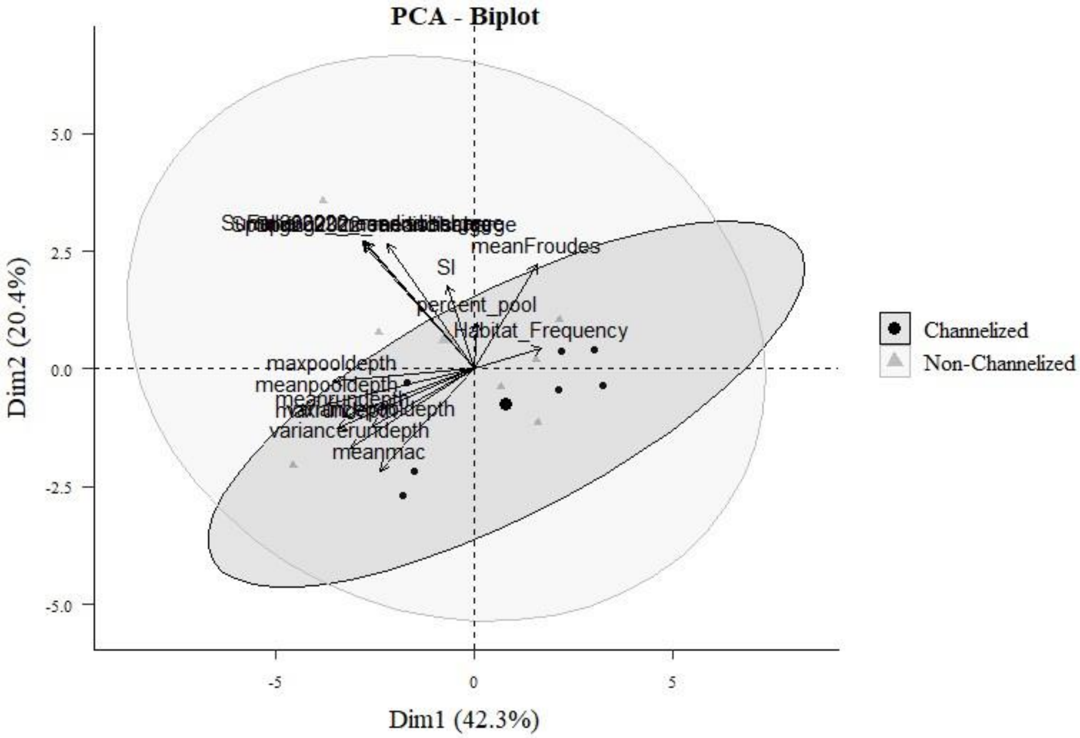

Figure 3.3—Principal component correlation biplot of environmental parameters using the top two variable axes between channelized and non-channelized stream sites in Willow Creek, Clifford Creek, and Gordon Creek summer 2022. Proportion of variability by each axis is shown in parenthesis. See Table 3.1 for parameter definition.

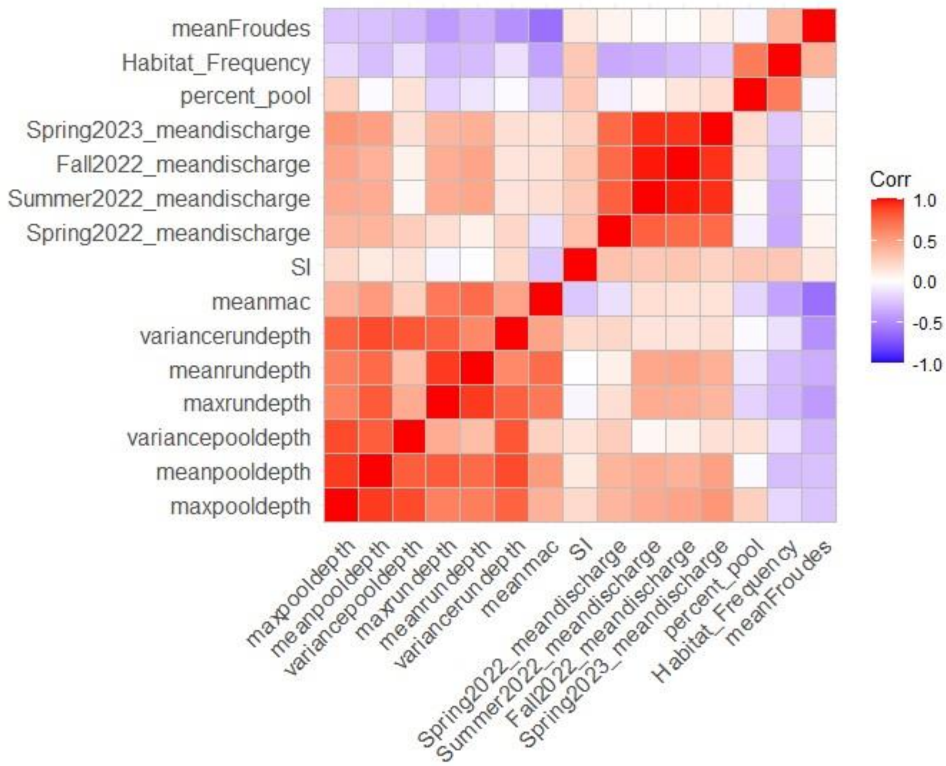

Figure 3.4—Pearson's correlation matrix of environmental parameters in Willow Creek, Clifford Creek, and Gordon Creek summer 2022. Darker red squares are highly correlated. See Table 3.1 for parameter definition.

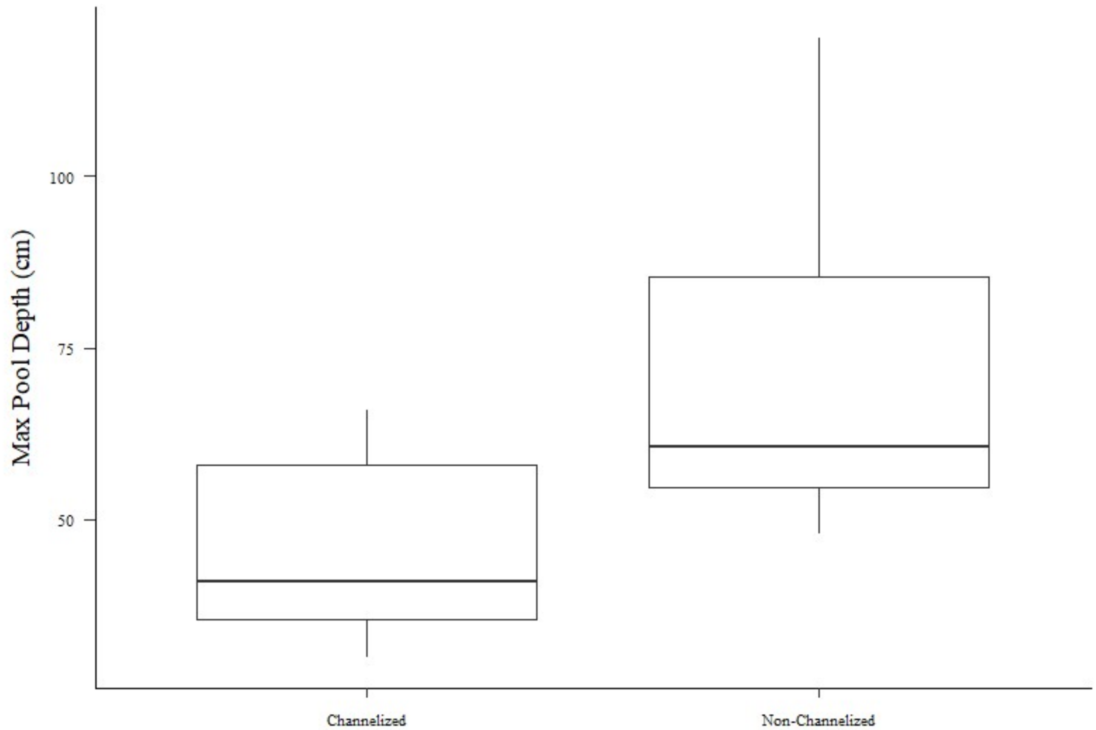

Figure 3.5—The difference in maximum pool depth between channelized and non-channelized sites in Willow Creek, Clifford Creek, and Gordon Creek summer 2022. The horizontal mid-line in the box is the median value. The box ends include the interquartile range. The whiskers include maximum and minimum values. See Table 3.1 for parameter definition.

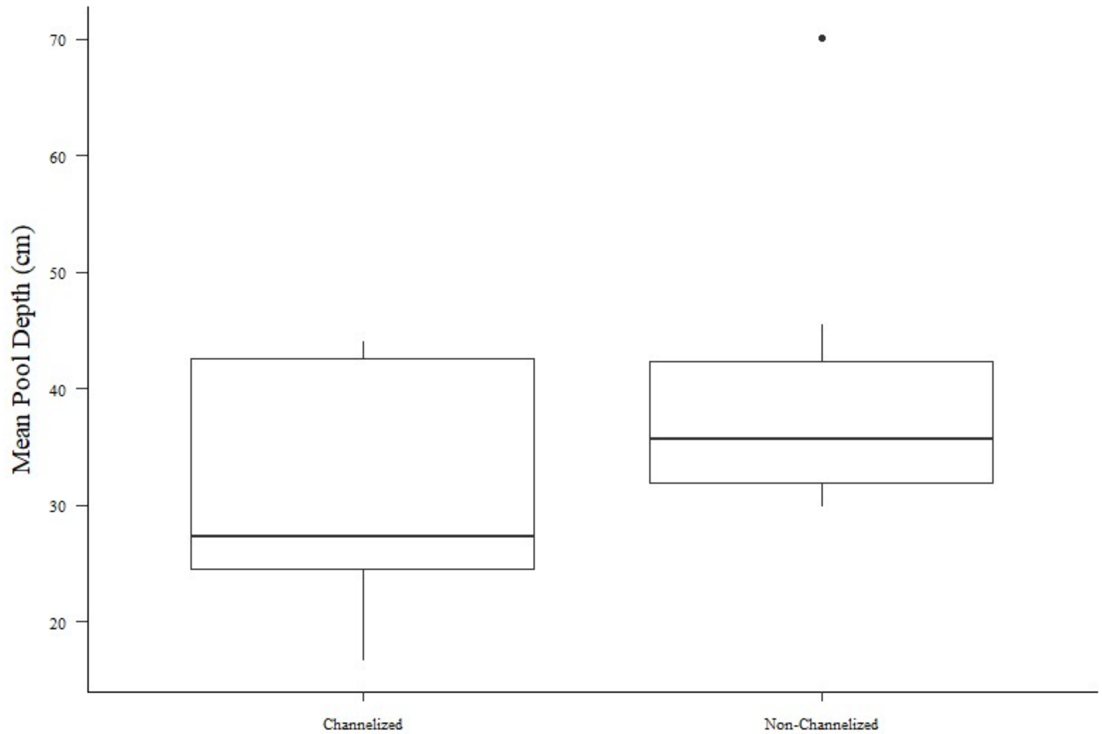

Figure 3.6—The difference in mean pool depth between channelized and non-channelized sites in Willow Creek, Clifford Creek, and Gordon Creek summer 2022. The horizontal mid-line in the box is the median value. The box ends include the interquartile range. The whiskers include maximum and minimum values, and the dots indicate outliers. See Table 3.1 for parameter definition.

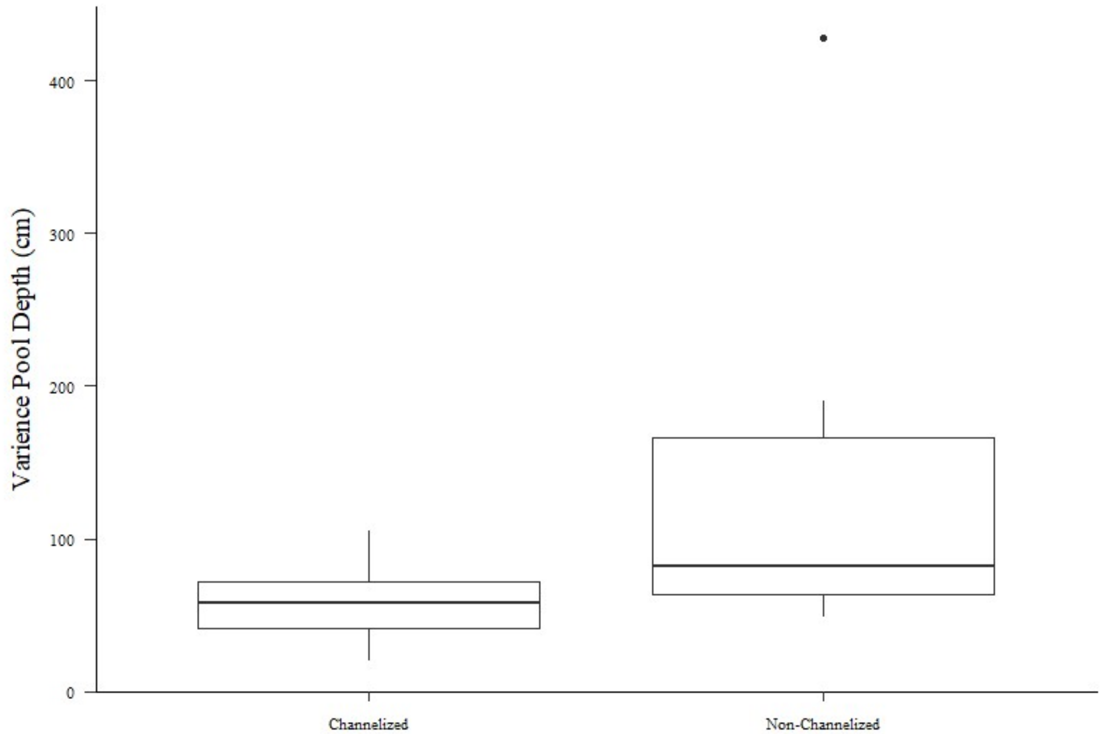

Figure 3.7—The difference in the variance of pool depth between channelized and non-channelized sites in Willow Creek, Clifford Creek, and Gordon Creek summer 2022. The horizontal mid-line in the box is the median value. The box ends include the interquartile range. The whiskers include maximum and minimum values, and the dots indicate outliers. See Table 3.1 for parameter definition.

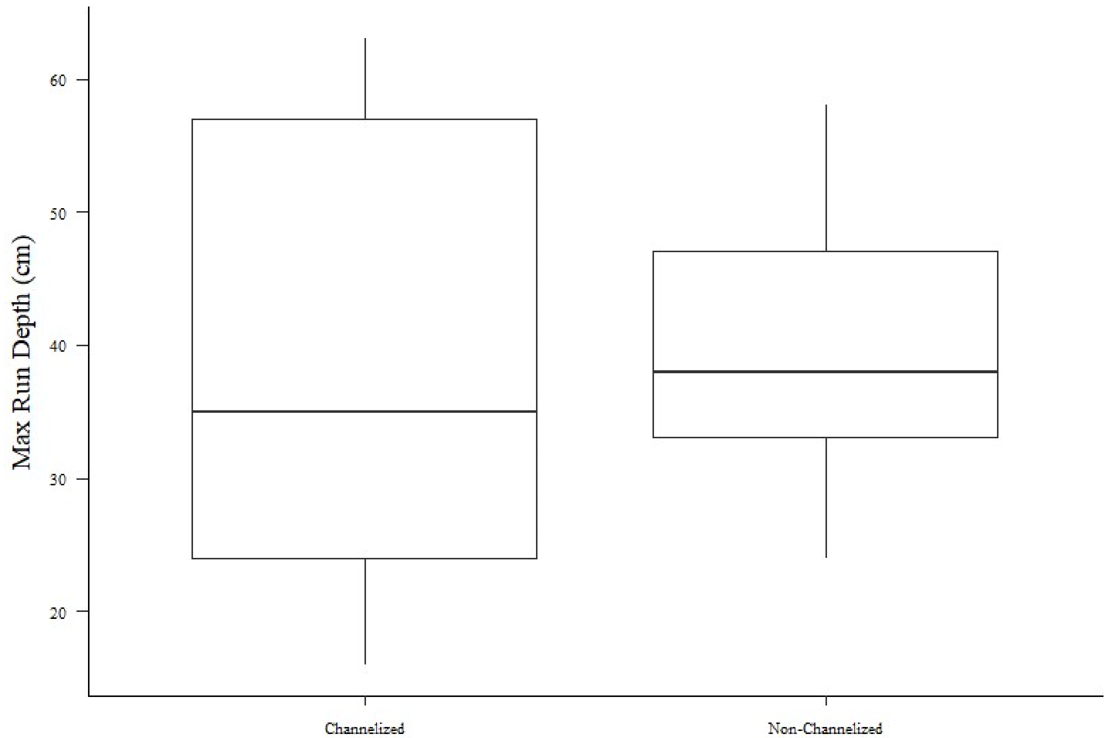

Figure 3.8—The difference in maximum run depth between channelized and non-channelized sites in Willow Creek, Clifford Creek, and Gordon Creek summer 2022. The horizontal mid-line in the box is the median value. The box ends include the interquartile range. The whiskers include maximum and minimum values. See Table 3.1 for parameter definition.

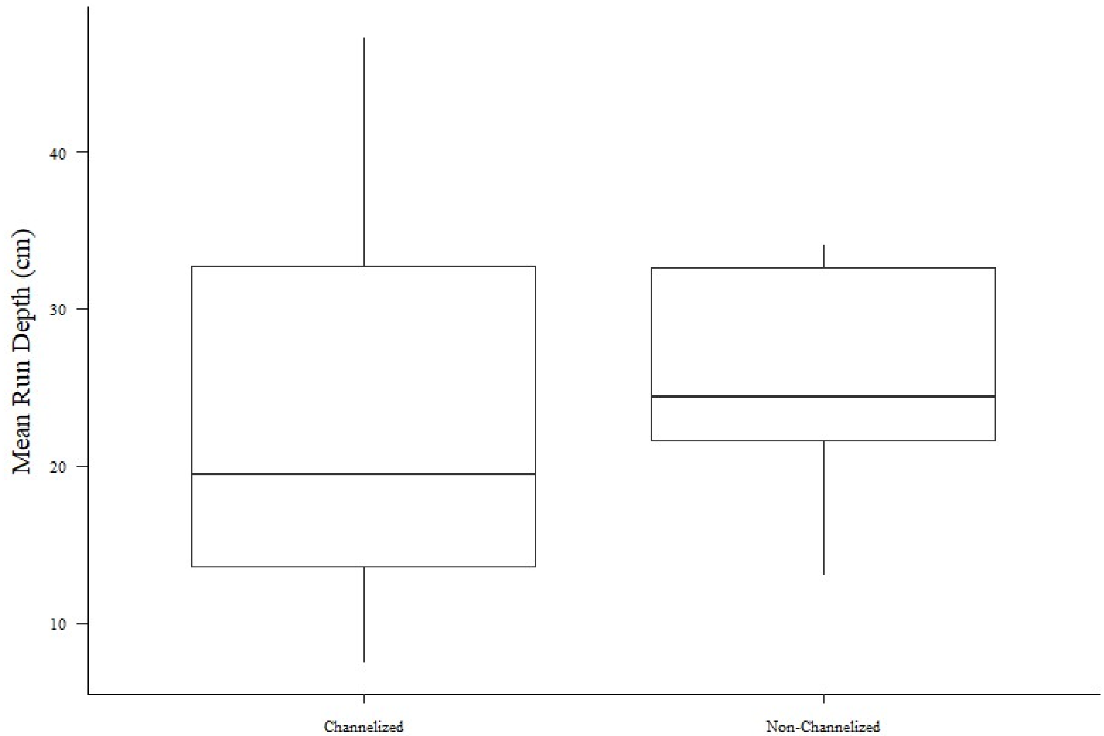

Figure 3.9—The difference in mean run depth between channelized and non-channelized sites in Willow Creek, Clifford Creek, and Gordon Creek summer 2022. The horizontal mid-line in the box is the median value. The box ends include the interquartile range. The whiskers include maximum and minimum values. See Table 3.1 for parameter definition.

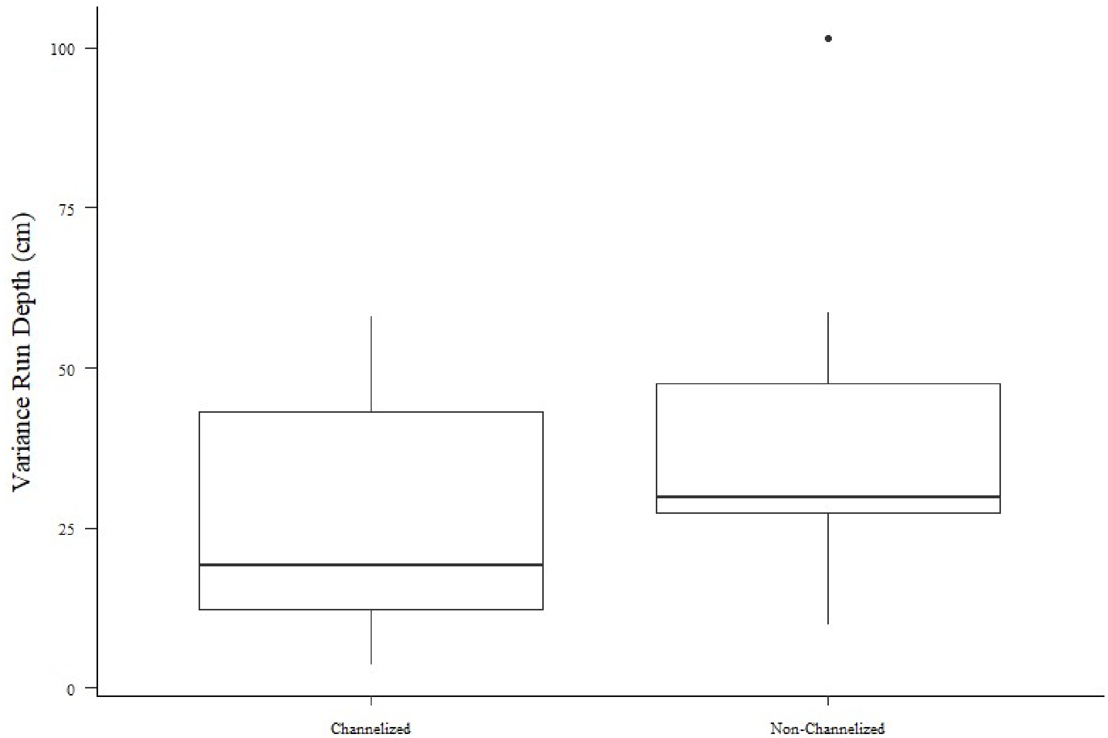

Figure 3.10—The difference in the variance of run depth between channelized and non-channelized sites in Willow Creek, Clifford Creek, and Gordon Creek summer 2022. The horizontal mid-line in the box is the median value. The box ends include the interquartile range. The whiskers include maximum and minimum values, and the dots indicate outliers. See Table 3.1 for parameter definition.

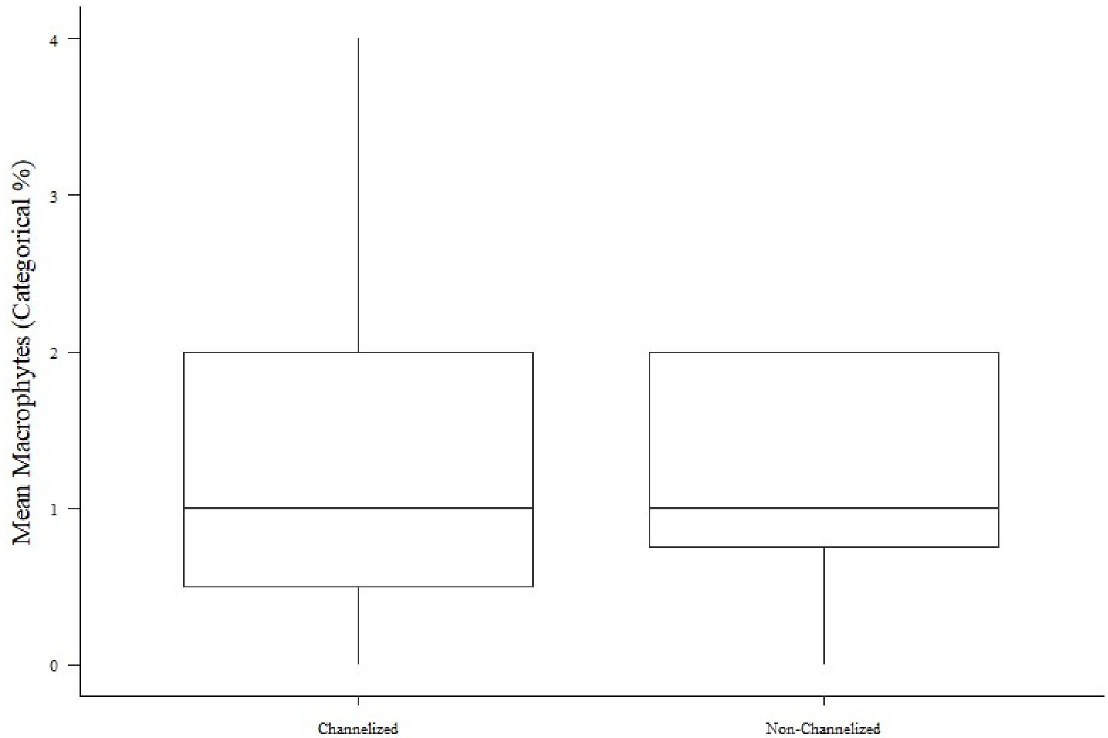

Figure 3.11—The difference in mean macrophytes by categorical variable (1 = 0-25%; 2 = 26-50%; 3 = 51-75%; 4 = 76-100%) between channelized and non-channelized sites in Willow Creek, Clifford Creek, and Gordon Creek summer 2022. The horizontal mid-line in the box is the median value. The box ends include the interquartile range. The whiskers include maximum and minimum values. See Table 3.1 for parameter definition.

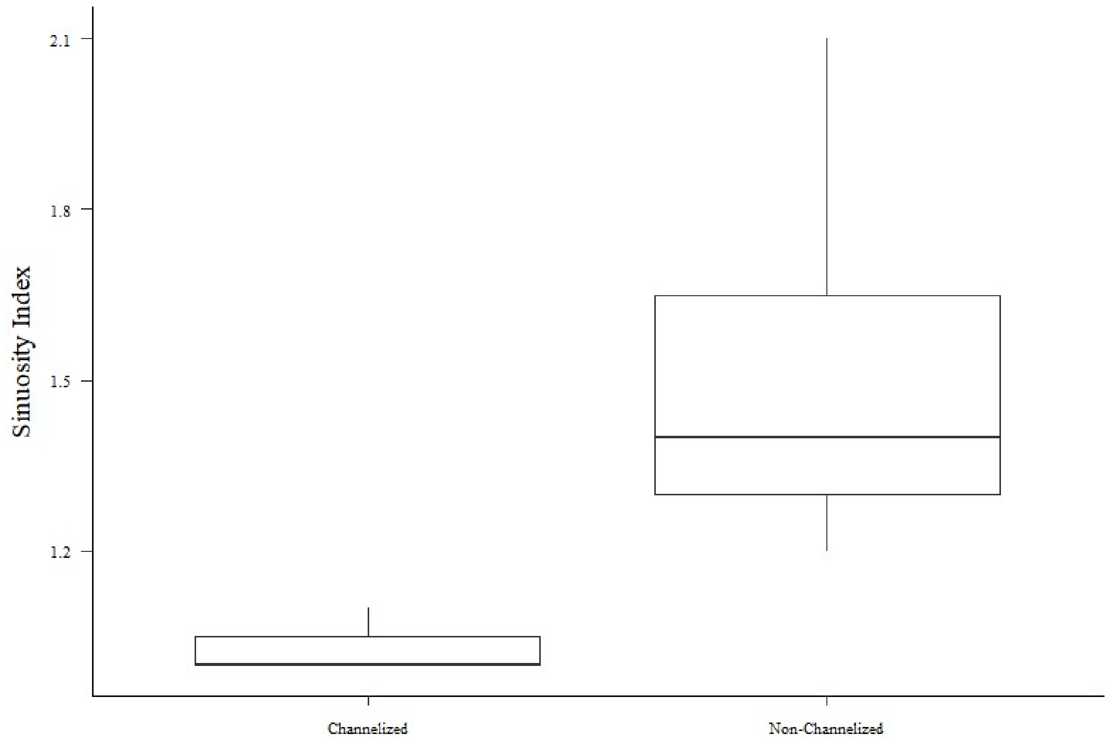

Figure 3.12—The difference in sinuosity index between channelized and non-channelized sites in Willow Creek, Clifford Creek, and Gordon Creek summer 2022. The horizontal mid-line in the box is the median value. The box ends include the interquartile range. The whiskers include maximum and minimum values. See Table 3.1 for parameter definition.

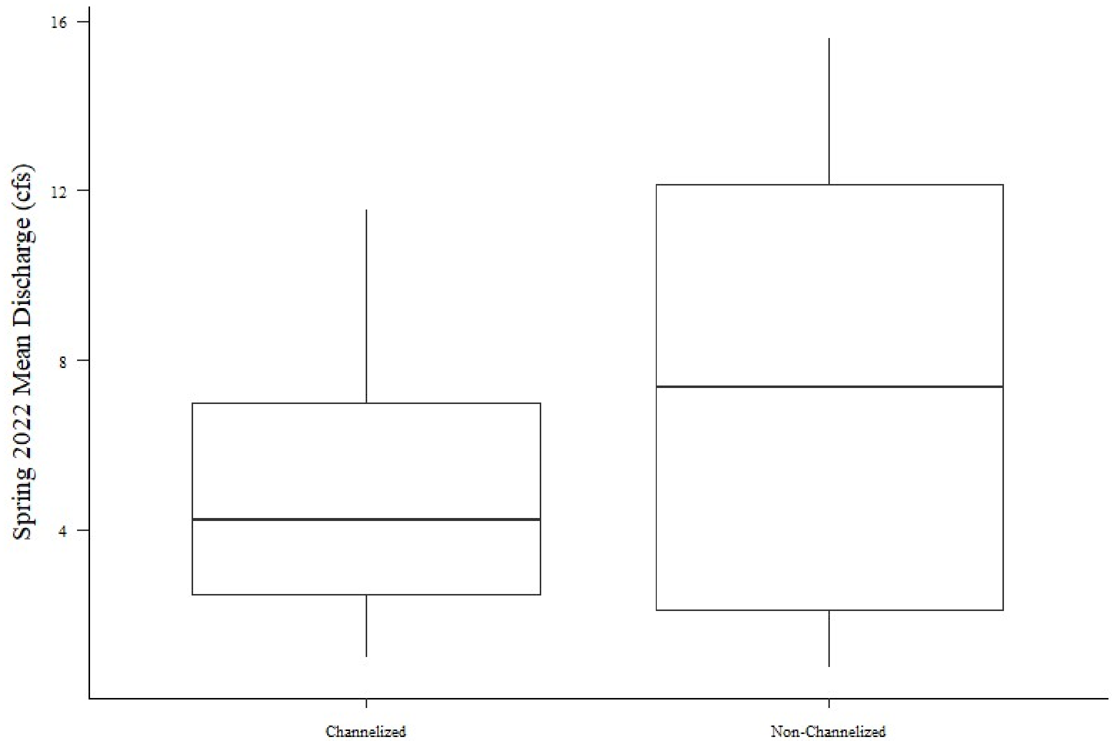

Figure 3.13—The difference in mean discharge between channelized and non-channelized sites in Willow Creek, Clifford Creek, and Gordon Creek spring 2022. The horizontal mid-line in the box is the median value. The box ends include the interquartile range. The whiskers include maximum and minimum values. See Table 3.1 for parameter definition.

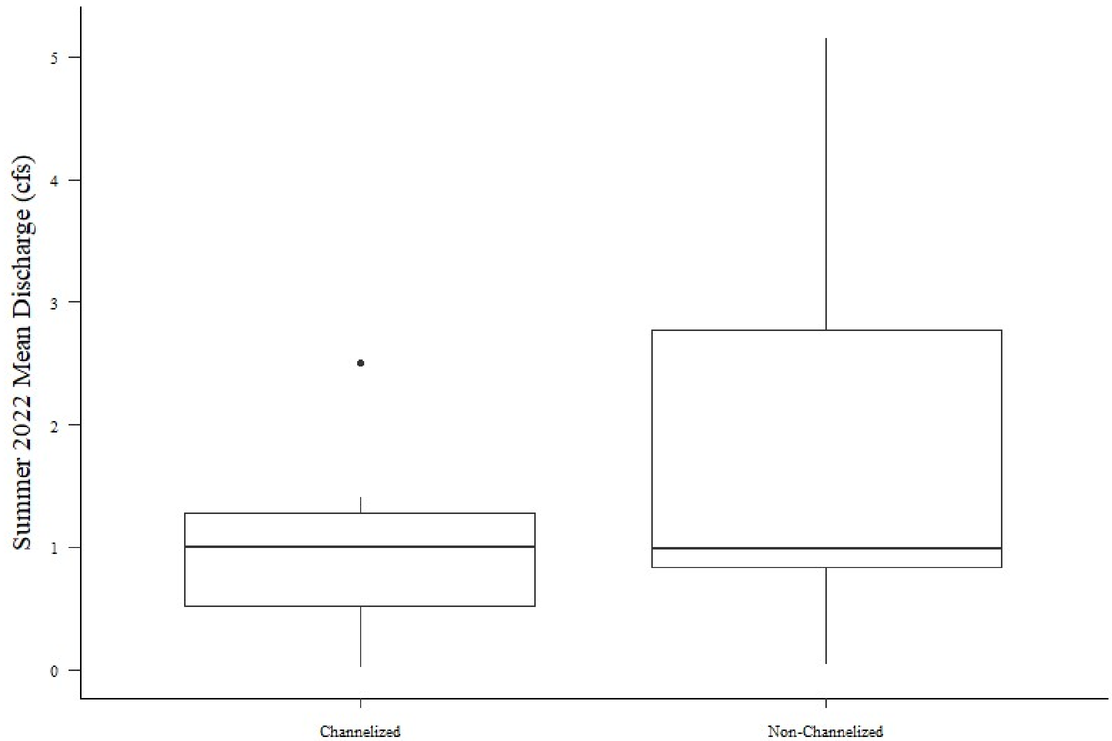

Figure 3.14—The difference in mean discharge between channelized and non-channelized sites in Willow Creek, Clifford Creek, and Gordon Creek summer 2022. The horizontal mid-line in the box is the median value. The box ends include the interquartile range. The whiskers include maximum and minimum values, and the dots indicate outliers. See Table 3.1 for parameter definition.

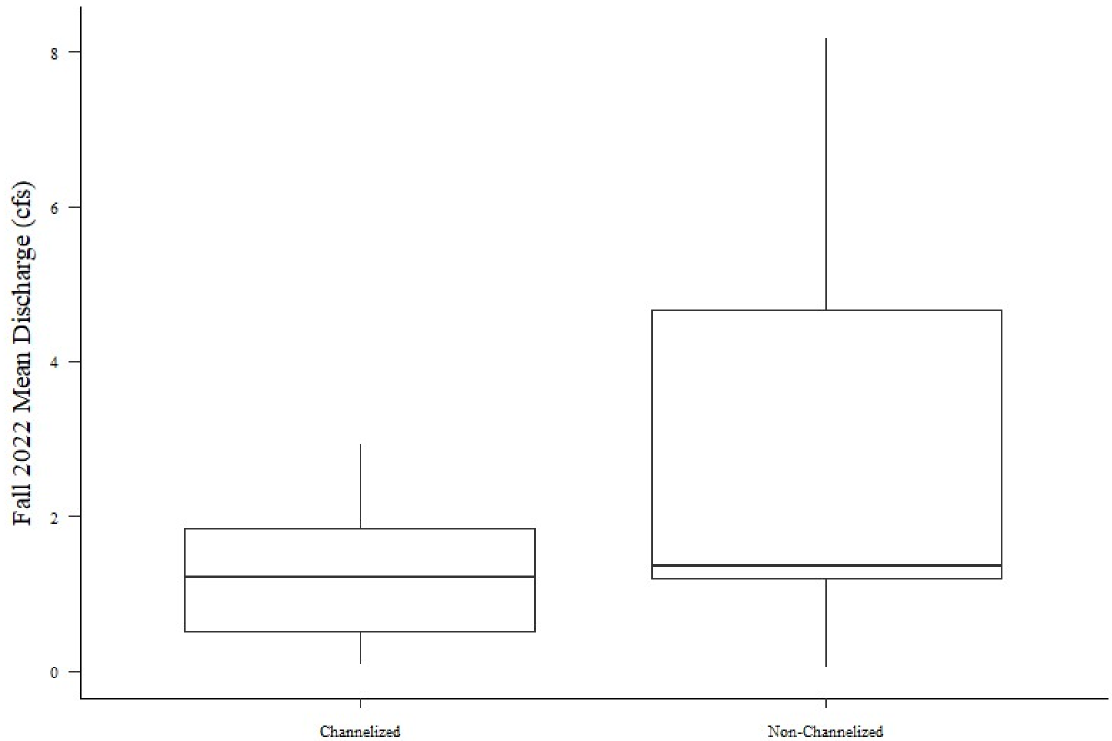

Figure 3.15—The difference in mean discharge between channelized and non-channelized sites in Willow Creek, Clifford Creek, and Gordon Creek fall 2022. The horizontal mid-line in the box is the median value. The box ends include the interquartile range. The whiskers include maximum and minimum values. See Table 3.1 for parameter definition.

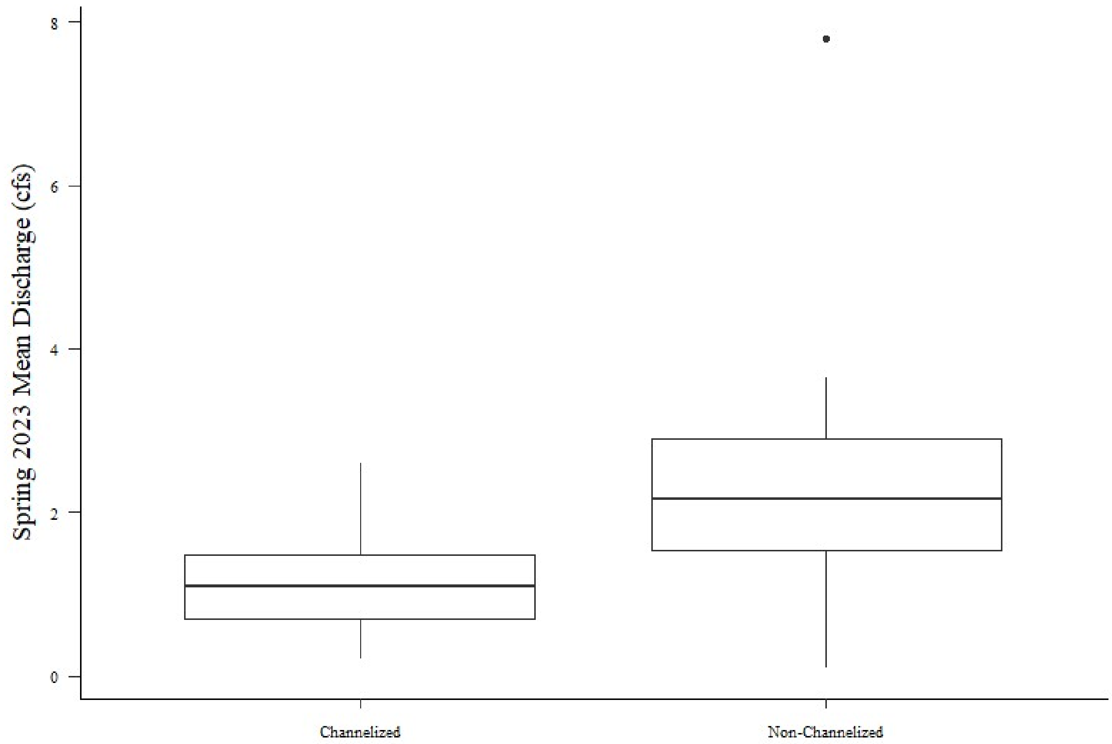

Figure 3.16—The difference in mean discharge between channelized and non-channelized sites in Willow Creek, Clifford Creek, and Gordon Creek spring 2023. The horizontal mid-line in the box is the median value. The box ends include the interquartile range. The whiskers include maximum and minimum values, and the dots indicate outliers. See Table 3.1 for parameter definition.

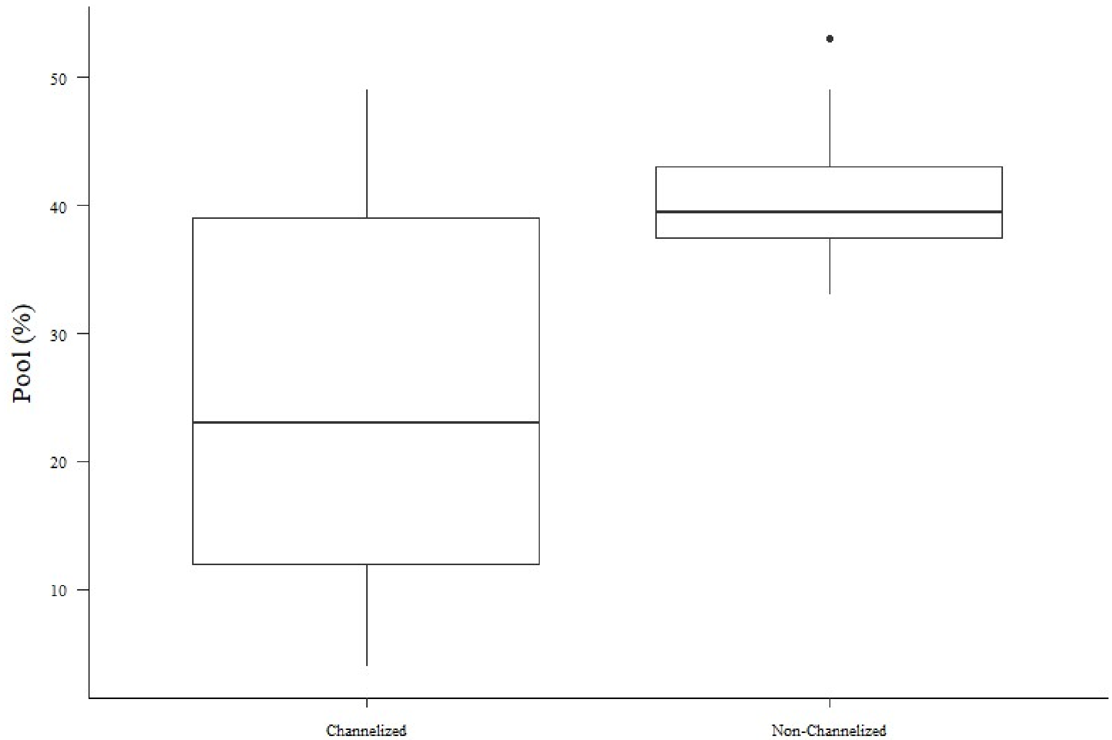

Figure 3.17—The difference in the percent of pool mesohabitat between channelized and non-channelized sites in Willow Creek, Clifford Creek, and Gordon Creek summer 2022. The horizontal mid-line in the box is the median value. The box ends include the interquartile range. The whiskers include maximum and minimum values, and the dots indicate outliers. See Table 3.1 for parameter definition.

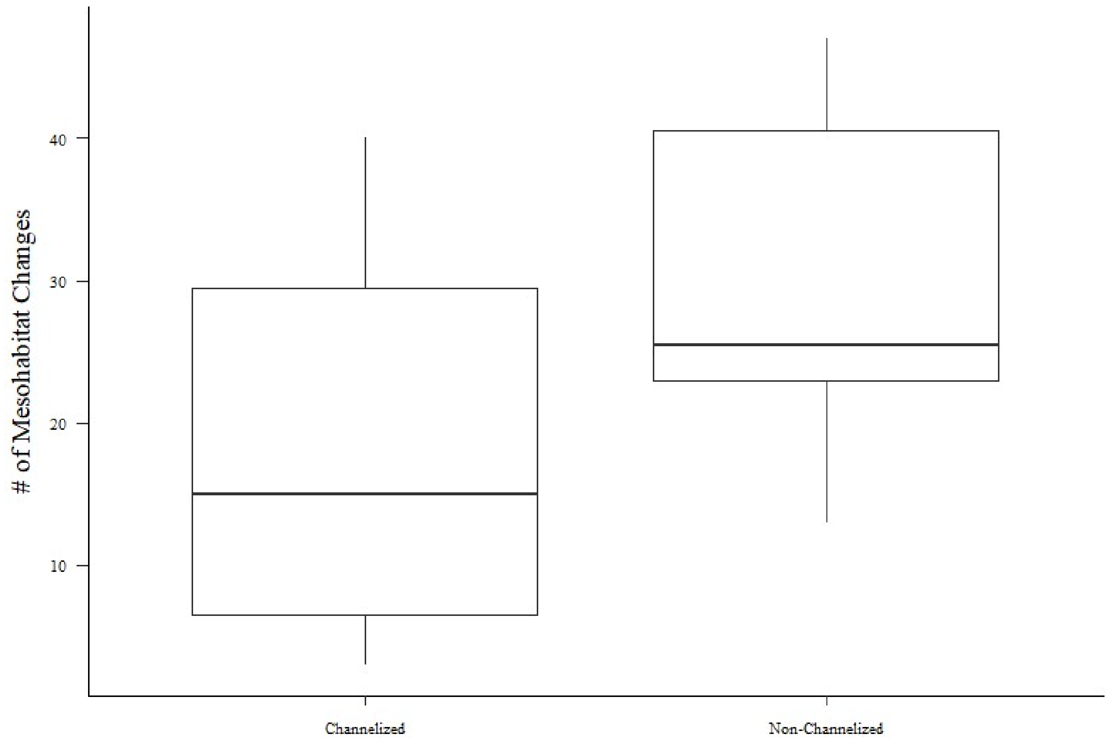

Figure 3.18—The difference in number of mesohabitat changes between channelized and non-channelized sites in Willow Creek, Clifford Creek, and Gordon Creek summer 2022. The horizontal mid-line in the box is the median value. The box ends include the interquartile range. The whiskers include maximum and minimum values. See Table 3.1 for parameter definition.

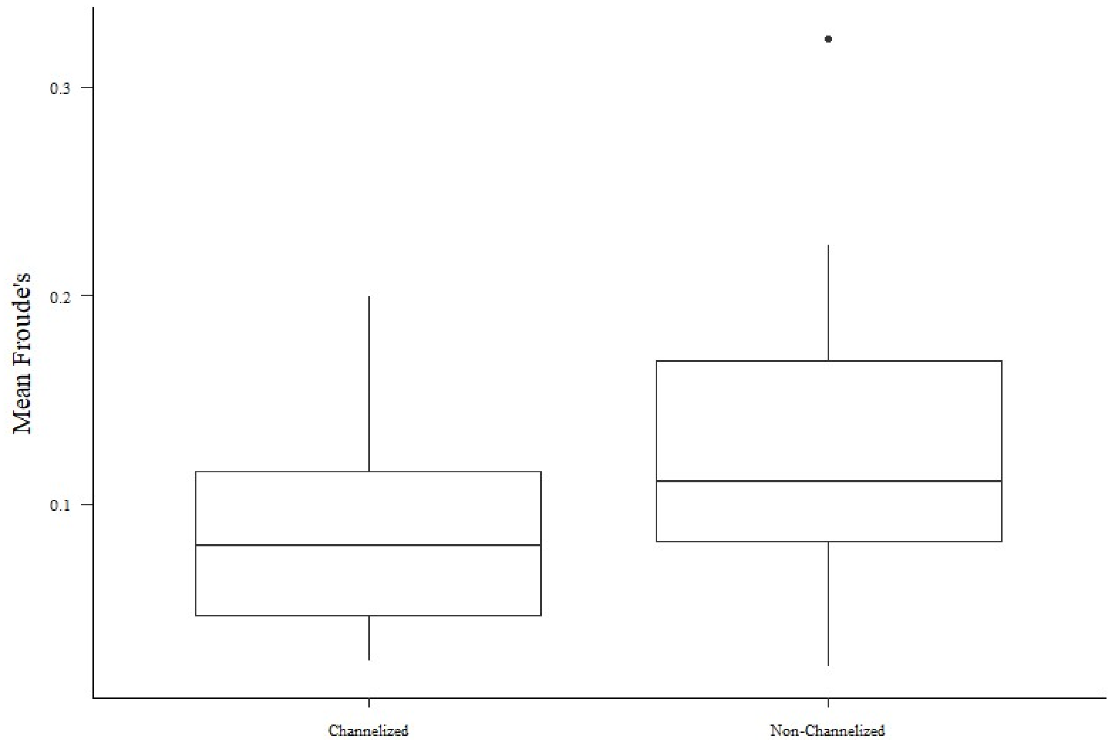

Figure 3.19—The difference in mean Froude's number between channelized and non-channelized sites in Willow Creek, Clifford Creek, and Gordon Creek summer 2022. The horizontal mid-line in the box is the median value. The box ends include the interquartile range. The whiskers include maximum and minimum values, and the dots indicate outliers. See Table 3.1 for parameter definition.

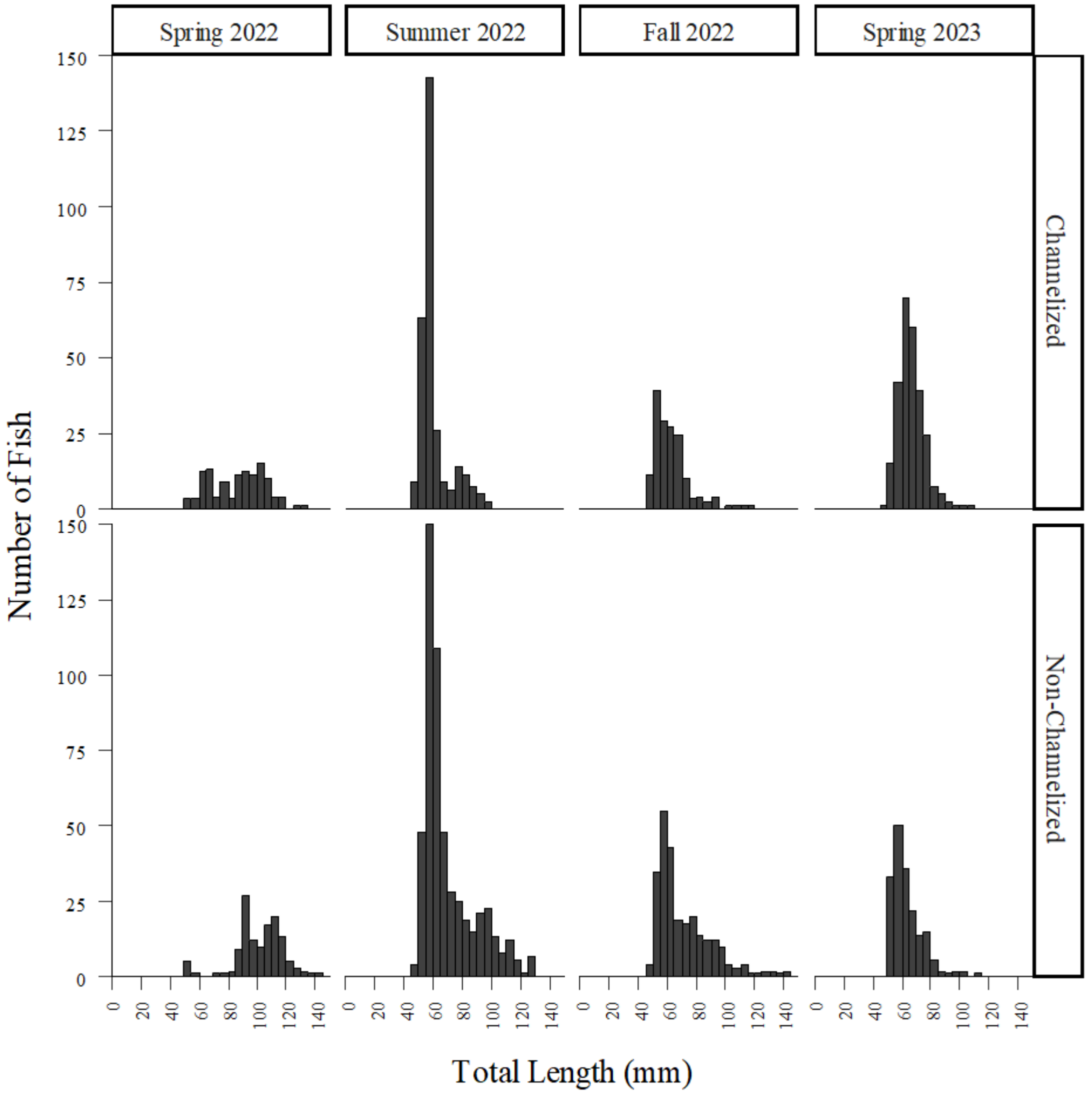

Figure 3.20—Number of tagged Northern Pearl Dace by the total length (mm) for channelized and non-channelized sites in Clifford Creek, Sandy Richards Creek, and Gordon Creek in spring 2022, summer 2022, fall 2022, spring 2023.

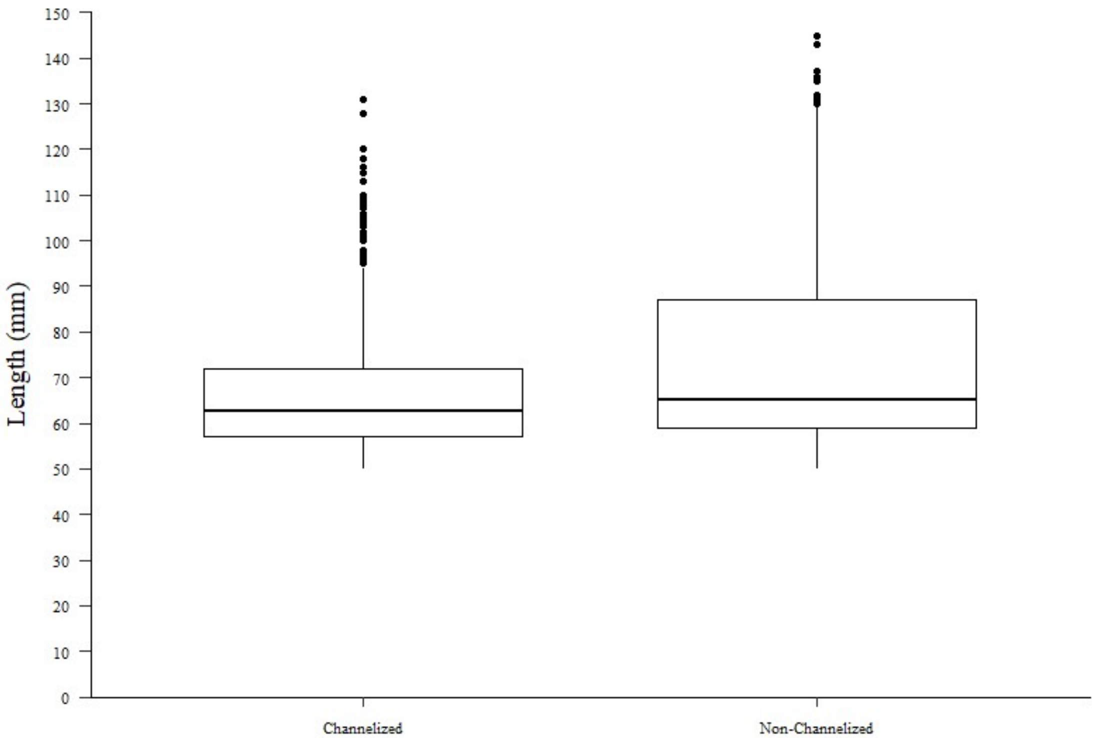

Figure 3.21—Northern Pearl Dace length distribution for channelized and non-channelized sites in Clifford Creek, Sandy Richards Creek, and Gordon Creek from spring 2022 to spring 2023. The horizontal mid-line in the box is the median value. The box ends include the interquartile range. The whiskers include maximum and minimum values, and the dots indicate outliers.

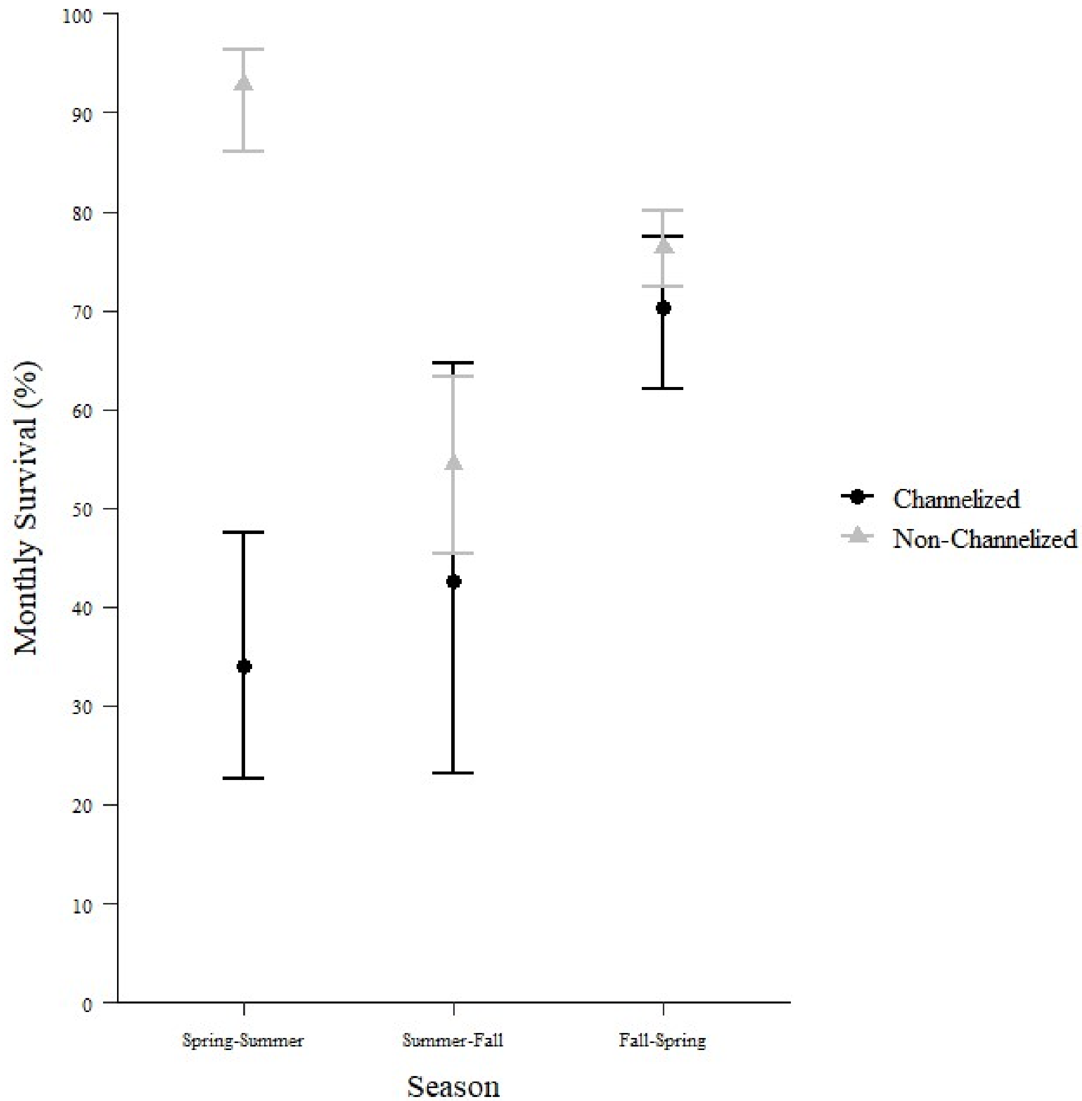

Figure 3.22—Percent monthly survival estimates in channelized and non-channelized sites in each season with 95% confidence intervals for Northern Pearl Dace ≤50mm in Clifford Creek, Sandy Richards Creek, and Gordon Creek from spring 2022 to spring 2023.

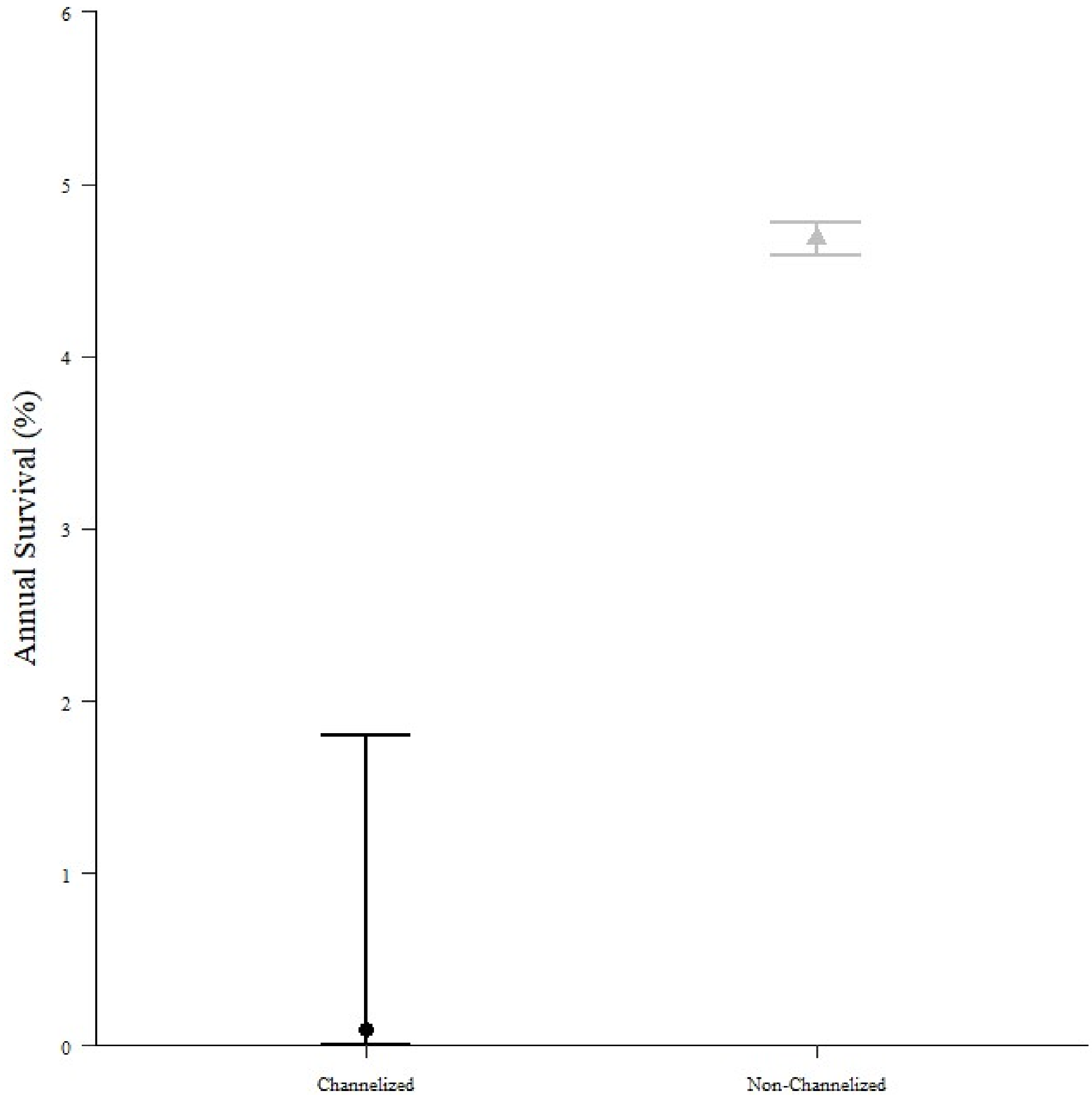

Figure 3.23—Percent annual survival estimates in channelized and non-channelized sites with 95% confidence intervals for Northern Pearl Dace ≤50mm in Clifford Creek, Sandy Richards Creek, and Gordon Creek from spring 2022 to spring 2023.

CHAPTER 4. FUTURE RESEARCH AND MANAGEMENT RECOMENDATIONS

Project Overview

Cool headwater streams across the nation and within the Nebraska Sandhills Ecoregion are threatened by anthropogenic alterations and provide refugia for many understudied small-bodied species of greatest conservation need SGCN including Northern Pearl Dace (Schneider et al. 2018; Colvin et al. 2019). Channelization of headwater streams in the Nebraska Sandhills Ecoregion is a common anthropogenic alteration where haying operations occur (Ducey 1991). Estimating demographic parameters such as survival for small-bodied fish SGCN is difficult due to reliable individual tagging methods which do not affect fish survival and are retained for the duration of the study. The goals of this study were to find a reliable tagging method for Northern Pearl Dace and evaluate annual survival in channelized headwater streams in the Nebraska Sandhills Ecoregion. The specific objectives of this study were:

1. Estimate survival and tag-retention following p-Chip microtransponder tag implantation in Creek Chub and Northern Pearl Dace in a 90-day laboratory environment (Chapter 2).
    - Survival for Creek Chub was 85% (SE = 5.9) and did not significantly differ from control fish (95%; SE = 3.2).
    - Survival for Northern Pearl Dace was 89% (SE = 11.0) and did not significantly differ from control fish (100%).
    - Tag retention was 89% (SE = 4.6) for Creek Chub and 100% for Northern Pearl Dace.

2. Estimate tag retention of p-Chip microtransponder tags in Northern Pearl Dace over a year-long field trial (Chapter 2).
    - Tag retention for Northern Pearl Dace was 94%.
3. Determine differences in geomorphic characteristics, and instream habitat including mesohabitat, depth, and macrophyte coverage in channelized and non-channelized stream sites in the Nebraska Sandhills Ecoregion (Chapter 3).
    - Channelized and non-channelized stream site classification had a statistically significant association with post-Principal Component Analysis (PCA) selected environmental parameters (Pillai's Trace = 0.9181, $F(8, 6) = 8.4040$, $p = 0.0091$).
    - Specifically, mean sinuosity index ($F(1, 13) = 16.6400$, $p = 0.0013$) and the percent of pool mesohabitat ($F(1, 13) = 5.3848$, $p = 0.0372$) differed between channelized and non-channelized stream sites.
4. Estimate annual survival of Northern Pearl Dace in channelized and non-channelized sites in the Nebraska Sandhills Ecoregion (Chapter 3).
    - Annual survival estimates were significantly different between channelized sites ($\hat{S} = 0.001$; SE = 0.009) and non-channelized sites ($\hat{S} = 0.047$; SE = 0.024).

P-Chip microtransponder tags appear to be an adequate tagging method for small-bodied fish studies where individual identification is required. The tagging process implemented during this lab study did not significantly affect survival rates in Creek Chub or Northern Pearl Dace and supports initial assessments performed on Arkansas River Shiner (Moore and Brewer 2021). In general, p-Chip microtransponder tag retention remained high in Creek Chub (89%) and Northern Pearl Dace (100%) compared to Arkansas River Shiner (72%, Moore and Brewer 2021). To our knowledge, this study is the first to assess tag retention of p-Chip microtransponder tags in the field. We found that tag retention in Northern Pearl Dace across the 374-day field study was relatively high (94%) when compared to other field-based tag retention studies with different individual tag methods (e.g., t-bar tags, 88%-Spurgeon et al. 2020; pit tags, 74%-Bateman et al 2009). As new tagging methods emerge, I believe it is critical to evaluate and compare p-Chip microtransponders to other tagging methods with the study species of interest so that confident parameter estimates can occur.

Northern Pearl Dace annual survival overall was low and was significantly affected by channelization for this 1-year study. Small-bodied fish SGCN survival studies are rare with minimal adequate individual tagging methods, limited recaptures, and the time commitment for the robust sampling design. However, there was a recent study that used the less robust Cormack-Jolly-Seber design to estimate a comparable monthly survival for Southern Redbelly Dace *Chrosomus erythrogaster* and Cardinal Shiner *Luxilus cardinalis*, SGCN, in Kansas (Siller et al. 2023). Sinuosity index and the percent of pool mesohabitat differed between channelized and non-channelized stream sites. My evaluation of the above objectives led to the following recommendations for future research and management.

Research Recommendations

1. We only estimated tag retention and survival of p-Chip microtransponder tags. Therefore, I recommend that a future research study would compare across other individual identification tags. Moore and Brewer (2021) compared tag retention

of p-Chip microtransponders to 8mm passive integrated transponder (PIT) tags and found p-Chip microtransponders to be better suited to their specific species of interest. However, that is only one study on one species and there needs to be replication on other fish species for more support to use for induvial marks in capture-mark-recapture (CMR) studies.

2. With a new tagging technology emerging to provide individual identification, I recommend to research tag retention and survival effects on other small-bodied fish, particularly SGCN. For example, Musselman et al. (2017) provided an overview claiming that fish survival and tag retention of PIT tags appear to be species specific. CMR study designs assume that tags remain readable and are not lost over the course of the study. Therefore, researching other fish species may give insight to others wanting to utilize p-Chip microtransponder tags in CMR studies.
3. This study only considered some environmental parameters, therefore measuring other parameters should be considered. Water temperature measured and averaged monthly throughout the year would be a parameter to consider for a future survival study. Climate change effects most ecosystems already and cool water streams may slowly be increasing in water temperature over time (Heino et al. 2009). Species distribution modeling has been used to study the effects of climate change on fish distributions (Makki et al. 2023). Therefore, considerations of climate change and effects to more highly connected groundwater-fed perennial streams of the Nebraska Sandhills Ecoregion may benefit knowledge gained to monitor Northern Pearl Dace population persistence. Another variable to consider

would be predator presence. We had one stream site with Northern Pike *Esox lucius* and further monitoring of this site over time should be considered to observe any survival or fish community level effects such as decreased abundance or species richness.

4. If a chance to restore a channelized reach of stream arises, I believe monitoring the response in survival will be important for the perseverance of Northern Pearl Dace in Nebraska. Pending landowner interests, some areas that are highly degraded due to past channelization may be able to be restored. If that opportunity arises it would be beneficial to monitor fish survival in those areas over time and compare them to our studies results. Depending on the restoration funding and area, different alterations to mesohabitat could be made such as adding more pool mesohabitat in the defined reach and fish survival could be tested against other restored and non-restored areas.
5. This study took place over a severe drought year. Therefore, I believe it will be critical to repeat this study during a non-drought year as well as more years so that a comparison in survival variability can be made. Most studies on the effects of drought on fish populations are at the local or site scale (Matthews and Marsh-Matthews 2023). Most studies in the literature review by Matthews and Marsh-Matthews (2023) found decreased abundance in streams affected by drought. It is hypothesized that fish in perennial streams would be greater affected by a drought event then an intermittent stream due to evolutionary adaptation in the local area. Therefore, survival in extreme 10+ year drought events may negatively influence Northern Pearl Dace populations in their current distribution.

6. During our study it was noted that many of our marked fish were possibly moving out of our study area within a 24hr period. This is a violation of the assumption of a closed population sample and may negatively influence survival estimates. I hypothesize that fish in channelized stream sites displace greater distances or have a decreased residence time compared to non-channelized stream sites. Therefore, I would suggest that another study be completed on looking at the displacement of Northern Pearl Dace through seasons. This can be accomplished by using a multi-state sampling approach. For example, I would choose 10-100m streams sites back-to-back (total = 1000m) and label them A-K for channelized and non-channelized reaches. This would allow for better site selection for future survival studies that may be applied to other small-bodied fish SGCN with similar life-history characteristics.

Management Recommendations

1. I recommend that p-Chip microtransponder tags would be a good alternative to PIT tags for use in small-bodied fish. Although, p-Chip microtransponder tags are less versatile than PIT tags since they are only readable via one side. Tagger experience may reduce this versatility issue. P-Chips microtransponders are comparable in price to 8mm PIT tags. A study species of interest should always consider testing the tagging procedure before applying it to a CMR study design.
2. I recommend consideration in design to a channelized habitat restoration project to include increasing sinuosity index above 1.2 and increasing the percent of pools to > 40% of the stream site. Based on our results stream length and the percentage of pools were the defining differences in environmental parameters between channelized and non-channelized stream sites. According to past

literature, pools are a critical habitat type for Northern Pearl Dace to carry out life-history needs and should be managed (Tallman 1979; Tallman and Gee, 1982). This may benefit Northern Pearl Dace populations by increasing annual survival and encouraging population growth.

Literature Cited

Bateman, D. S., R. E. Gresswell, and A. M. Berger. 2009. Passive integrated transponder tag retention rates in headwater populations of coastal Cutthroat Trout. North American Journal of Fisheries Management 29(3):653–657.

Colvin, S. A. R., S. M. P. Sullivan, P. D. Shirey, R. W. Colvin, K. O. Winemiller, R. M. Hughes, K. D. Fausch, D. M. Infante, J. D. Olden, K. R. Bestgen, R. J. Danehy, and L. Eby. 2019. Headwater streams and wetlands are critical for sustaining fish, fisheries, and ecosystem services. Fisheries 44(2):73–91.

Ducey, J. E. 1991. Ditching of wetlands in the Nebraska Sandhills: a case study of Grant County. Transactions of the Nebraska Academy of Sciences and Affiliated Societies. 145:1–9.

Heino J., R. Virkkala, and H. Toivonen. 2009. Climate change and freshwater biodiversity: detected patterns, future trends and adaptations in northern regions. Biological Reviews 84(1):39–54.

Makki, T., H. Mostafavi, A. Matkan, H. Aghighi, R. Valavi, Y. Chee, and A. Teimori. 2023. Impacts of climate change on the distribution of riverine endemic fish species in Iran, a biodiversity hotspot region. Freshwater Biology 68(6):1007–1019.

Matthews, W. J., and E. Marsh-Matthews. 2003. Effects of drought on fish across axes of space, time and ecological complexity. Freshwater Biology 48(7):1232–1253.

Moore, D. M., and S. K. Brewer. 2021. Evaluation of visual implant elastomer, PIT, and p-chip tagging methods in a small-bodied minnow species. North American Journal of Fisheries Management 41:1066–1078.

Musselman, W. C., T. A. Worthington, J. Mouser, D. M. Williams, and S. K. Brewer. 2017. Passive integrated transponder tags: review of studies on warmwater fishes with notes on additional species. Journal of Fish and Wildlife Management 8:353–364.

Schneider, R., M. Fritz, J. Jorgensen, S. Schainost, R. Simpson, G. Steinauer, and C. Rothe-Groleau. 2018. Revision of the tier 1 and 2 lists of species of greatest conservation need: a supplement to the Nebraska Natural Legacy Project State Wildlife Action Plan. The Nebraska Game and Parks Commission, Lincoln, Nebraska.

Siller, M. K., P. J. Pfaff, E. Wild, and K. B. Gido. 2023. Apparent survival and detection probability of PIT-tagged small-bodied stream fishes using multi-pass wand antenna surveys. Environmental Biology of Fishes 106(6):1371–1381.

Spurgeon, J. J., M. A. Pegg, S. F. Siddons, and H. H. Hansen. 2020. Retention of t-bar anchor tags for Channel Catfish in the Red River of the North. North American Journal of Fisheries Management 40(2):330–334.

Tallman, R. F. 1979. Environments occupied, indices of maturity, feeding ecology, shoaling behavior and interactions with other species by Pearl Dace, *Semotilus margarita* (Cope) in Manitoba. Master's thesis. University of Manitoba, Winnipeg.

Tallman, R. F., and J. H. Gee. 1982. Intraspecific resource partitioning in a headwaters stream fish, the Pearl Dace, Semotilus margarita (Cope). Environmental Biology of Fishes 7:243–249.

APPENDIX A

Supplemental tables and figures pertaining to Chapter 3

Table A-1— Total numbers of each fish species (common name) captured in the spring 2022 (sample occasion: 1, 2, 3), summer 2022 (sample occasion: 4, 5, 6), fall 2022 (sample occasion: 7, 8, 9), spring 2023 (sample occasion: 10, 11, 12), and across all sample occasions in Willow Creek site locality 1. See Figure A-1 for locality details.

	Sample Occasion												
Species	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**Total**
Bigmouth Shiner	1	2	3	0	0	0	3	0	0	0	0	0	9
Black Bullhead	1	3	2	0	1	0	0	0	2	0	0	0	9
Brassy Minnow	0	9	3	23	1	13	18	9	3	3	7	1	90
Brook Stickleback	9	6	2	3	5	6	4	16	8	4	2	1	66
Chrosomus Hybrid	0	0	0	0	0	0	0	0	0	14	5	1	20
Fathead Minnow	122	54	44	14	11	4	27	7	3	20	16	3	325
Finescale Dace	53	58	79	10	20	14	9	19	2	13	6	16	299
Green Sunfish	0	0	2	2	0	0	0	0	0	0	0	0	4
Longnose Dace	3	0	0	0	1	0	0	1	1	0	0	0	6
Northern Redbelly Dace	86	69	26	28	6	44	27	24	9	14	20	2	355
Plains Topminnow	24	11	17	6	14	12	1	8	4	2	1	0	100
Total	299	212	178	86	59	93	89	84	32	70	57	24	1283

Table A-2—Total numbers of each fish species (common name) captured in the spring 2022 (sample occasion: 1, 2, 3), summer 2022 (sample occasion: 4, 5, 6), fall 2022 (sample occasion: 7, 8, 9), spring 2023 (sample occasion: 10, 11, 12), and across all sample occasions in Willow Creek site locality 2. See Figure A-1 for locality details.

	Sample Occasion												
Species	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**Total**
Bigmouth Shiner	0	0	1	0	1	0	0	1	0	0	0	0	3
Black Bullhead	1	2	0	4	9	4	0	1	1	0	0	0	22
Brassy Minnow	30	33	24	43	8	10	82	57	19	5	17	3	331
Brook Stickleback	6	0	4	0	0	0	0	0	0	0	0	0	10
Chrosomus Hybrid	0	0	0	0	0	0	0	0	0	9	6	0	15
Creek Chub	0	0	0	0	0	1	0	0	0	0	0	0	1
Fathead Minnow	63	57	48	54	30	25	38	20	3	34	59	12	443
Finescale Dace	70	103	49	76	17	26	16	11	6	11	27	17	429
Green Sunfish	0	3	4	1	3	5	1	0	1	0	0	0	18
Northern Redbelly Dace	79	25	10	7	19	12	25	9	7	3	9	1	206
Plains Topminnow	22	16	25	31	22	20	11	25	5	2	10	1	190
White Sucker	0	0	0	0	0	0	0	0	0	1	0	0	1
Total	271	239	165	216	109	103	173	124	42	65	128	34	1669

Table A-3—Total numbers of each fish species (common name) captured in the spring 2022 (sample occasion: 1, 2, 3), summer 2022 (sample occasion: 4, 5, 6), fall 2022 (sample occasion: 7, 8, 9), spring 2023 (sample occasion: 10, 11, 12), and across all sample occasions in Willow Creek site locality 3. See Figure A-1 for locality details.

	Sample Occasion												
Species	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**Total**
Bigmouth Shiner	15	5	6	8	13	17	6	5	2	28	19	5	129
Black Bullhead	0	0	0	1	0	1	0	0	0	0	0	0	2
Blacknose Dace	0	0	0	2	21	20	3	1	1	18	13	26	105
Brassy Minnow	9	1	0	5	7	6	6	0	6	0	0	0	40
Brook Stickleback	0	0	0	0	0	0	0	1	0	1	0	0	2
Chrosomus Hybrid	0	0	0	0	0	0	0	0	0	3	11	1	15
Creek Chub	0	0	0	0	2	0	5	2	4	0	0	0	13
Fathead Minnow	10	4	8	3	3	3	0	5	0	1	4	1	42
Finescale Dace	23	20	20	5	13	3	2	2	4	10	8	12	122
Longnose Dace	79	56	44	8	9	4	8	8	6	47	35	4	308
Northern Redbelly Dace	18	15	16	4	4	3	2	0	4	12	8	4	90
Plains Topminnow	0	5	6	4	2	5	0	3	2	8	5	1	41
Sand Shiner	0	5	0	0	0	0	0	0	1	0	0	0	6
Total	154	111	100	40	74	62	32	27	30	128	103	54	915

Table A-4—Total numbers of each fish species (common name) captured in the spring 2022 (sample occasion: 1, 2, 3), summer 2022 (sample occasion: 4, 5, 6), fall 2022 (sample occasion: 7, 8, 9), spring 2023 (sample occasion: 10, 11, 12), and across all sample occasions in Willow Creek site locality 4. Missing sample occasions denote no sample was taken. See Figure A-1 for locality details.

	Sample Occasion						
Species	**7**	**8**	**9**	**10**	**11**	**12**	**Total**
Bigmouth Shiner	14	7	4	12	5	6	48
Blacknose Dace	7	8	2	55	36	48	156
Chrosomus Hybrid	0	0	0	7	1	2	10
Fathead Minnow	0	0	0	1	0	2	3
Finescale Dace	0	0	0	12	1	6	19
Longnose Dace	31	36	12	23	19	7	128
Northern Redbelly Dace	0	0	0	4	0	0	4
Total	52	51	18	114	62	71	368

Table A-5—Total numbers of each fish species (common name) captured in the spring 2022 (sample occasion: 1, 2, 3), summer 2022 (sample occasion: 4, 5, 6), fall 2022 (sample occasion: 7, 8, 9), spring 2023 (sample occasion: 10, 11, 12), and across all sample occasions in Willow Creek site locality 5. Missing sample occasions denote no sample was taken. See Figure A-1 for locality details.

	Sample Occasion						
Species	**1**	**2**	**3**	**4**	**5**	**6**	**Total**
Bigmouth Shiner	16	22	82	283	149	273	825
Blacknose Dace	0	0	0	0	0	12	12
Brassy Minnow	3	3	15	73	10	33	137
Brook Stickleback	1	3	0	2	4	1	11
Fathead Minnow	51	20	36	85	8	27	227
Finescale Dace	5	0	8	8	1	2	24
Longnose Dace	106	133	280	607	230	329	1685
Northern Redbelly Dace	1	2	4	0	0	0	7
Sand Shiner	7	22	47	156	14	25	271
Total	190	205	472	1214	416	702	3199

Table A-6—Total numbers of each fish species (common name) captured in the spring 2022 (sample occasion: 1, 2, 3), summer 2022 (sample occasion: 4, 5, 6), fall 2022 (sample occasion: 7, 8, 9), spring 2023 (sample occasion: 10, 11, 12), and across all sample occasions in Willow Creek site locality 6. Missing sample occasions denote no sample was taken. See Figure A-1 for locality details.

	Sample Occasion						
Species	**1**	**2**	**3**	**4**	**5**	**6**	**Total**
Bigmouth Shiner	18	15	43	71	123	42	312
Blacknose Dace	0	0	0	0	0	3	3
Brassy Minnow	0	1	2	5	6	4	18
Brook Stickleback	3	5	0	4	5	4	21
Fathead Minnow	33	16	11	23	18	6	107
Finescale Dace	0	1	1	4	5	4	15
Green Sunfish	0	1	0	0	0	0	1
Longnose Dace	114	20	117	304	256	231	1042
Northern Pearl Dace	1	0	0	0	0	0	1
Plains Topminnow	1	0	1	5	6	6	19
Sand Shiner	33	5	50	91	91	54	324
White Sucker	0	0	0	3	2	1	6
Total	203	64	225	510	512	355	1869

Table A-7—Total numbers of each fish species (common name) captured in the spring 2022 (sample occasion: 1, 2, 3), summer 2022 (sample occasion: 4, 5, 6), fall 2022 (sample occasion: 7, 8, 9), spring 2023 (sample occasion: 10, 11, 12), and across all sample occasions in Willow Creek site locality 7. Missing sample occasions denote no sample was taken. See Figure A-1 for locality details.

	Sample Occasion						
Species	**1**	**2**	**3**	**4**	**5**	**6**	**Total**
Bigmouth Shiner	0	1	34	52	103	55	245
Black Bullhead	0	0	1	1	1	0	3
Brassy Minnow	0	1	0	45	52	27	125
Brook Stickleback	0	22	1	1	1	6	31
Creek Chub	3	1	0	6	5	0	15
Fathead Minnow	38	3	21	23	20	23	128
Finescale Dace	0	1	0	8	17	6	32
Green Sunfish	2	0	2	0	0	0	4
Longnose Dace	13	3	19	13	9	14	71
Northern Redbelly Dace	0	0	0	6	0	9	15
Plains Topminnow	1	0	0	15	26	50	92
Sand Shiner	198	16	96	247	193	219	969
White Sucker	2	0	5	2	4	3	16
Total	257	48	179	419	431	412	1746

Table A-8—Total numbers of each fish species (common name) captured in the spring 2022 (sample occasion: 1, 2, 3), summer 2022 (sample occasion: 4, 5, 6), fall 2022 (sample occasion: 7, 8, 9), spring 2023 (sample occasion: 10, 11, 12), and across all sample occasions in Willow Creek site locality 8. Missing sample occasions denote no sample was taken. See Figure A-1 for locality details.

	Sample Occasion						
Species	**1**	**2**	**3**	**4**	**5**	**6**	**Total**
Bigmouth Shiner	0	1	2	41	63	16	123
Black Bullhead	0	0	0	0	3	0	3
Blacknose Dace	0	0	0	1	0	1	2
Brassy Minnow	0	0	3	43	51	18	115
Creek Chub	0	1	1	1	0	0	3
Fathead Minnow	2	0	2	8	8	17	37
Finescale Dace	0	0	0	3	6	1	10
Green Sunfish	0	0	0	0	1	0	1
Longnose Dace	9	5	11	45	33	19	122
Northern Redbelly Dace	0	0	0	0	0	1	1
Plains Topminnow	4	3	4	16	5	1	33
Sand Shiner	9	6	18	89	64	64	250
White Sucker	2	0	2	1	1	0	6
Total	26	16	43	248	235	138	706

Table A-9—Total numbers of each fish species (common name) captured in the spring 2022 (sample occasion: 1, 2, 3), summer 2022 (sample occasion: 4, 5, 6), fall 2022 (sample occasion: 7, 8, 9), spring 2023 (sample occasion: 10, 11, 12), and across all sample occasions in Clifford Creek site locality 9. See Figure A-1 for locality details.

	Sample Occasion												
Species	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**Total**
Brassy Minnow	0	0	0	15	13	12	8	9	9	0	0	0	66
Brook Stickleback	15	3	6	95	244	142	92	101	126	41	53	39	957
Chrosomus Hybrid	0	0	0	0	0	0	0	0	0	16	28	4	48
Fathead Minnow	7	12	1	282	59	29	34	30	23	16	2	2	497
Finescale Dace	16	16	13	187	234	204	106	171	79	12	35	29	1102
Green Sunfish	0	0	0	2	0	0	2	0	2	0	0	0	6
Northern Pearl Dace	93	63	33	206	308	344	288	378	244	55	56	33	2101
Northern Redbelly Dace	24	10	7	15	56	116	38	60	33	3	2	2	366
Plains Topminnow	0	0	0	1	0	5	7	5	4	0	1	0	23
Total	155	104	60	803	914	852	575	754	520	143	177	109	5166

Table A-10—Total numbers of each fish species (common name) captured in the spring 2022 (sample occasion: 1, 2, 3), summer 2022 (sample occasion: 4, 5, 6), fall 2022 (sample occasion: 7, 8, 9), spring 2023 (sample occasion: 10, 11, 12), and across all sample occasions in Clifford Creek site locality 10. See Figure A-1 for locality details.

	Sample Occasion												
Species	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**Total**
Bigmouth Shiner	11	8	48	9	11	2	28	49	2	13	26	4	211
Black Bullhead	0	0	3	0	0	0	0	0	0	0	0	0	3
Blacknose Dace	0	0	0	0	1	0	0	0	0	9	38	11	59
Brassy Minnow	0	0	5	6	4	0	0	0	0	0	0	0	15
Brook Stickleback	0	0	0	2	2	3	1	1	0	0	1	0	10
Fathead Minnow	1	4	8	7	1	0	0	0	0	0	0	0	21
Finescale Dace	1	1	5	5	5	3	1	1	0	10	1	0	33
Green Sunfish	2	5	4	2	1	1	4	1	0	0	0	0	20
Longnose Dace	9	63	38	13	68	18	11	34	11	24	9	0	298
Northern Pearl Dace	2	1	0	0	35	8	2	3	1	2	15	0	69
Northern Redbelly Dace	2	3	4	0	1	0	0	0	0	0	0	0	10
Plains Topminnow	0	1	0	0	0	0	0	1	0	0	0	0	2
Sand Shiner	0	21	0	0	0	0	0	0	0	0	0	0	21
Total	28	107	115	44	129	35	47	90	14	58	90	15	772

Table A-11—Total numbers of each fish species (common name) captured in the spring 2022 (sample occasion: 1, 2, 3), summer 2022 (sample occasion: 4, 5, 6), fall 2022 (sample occasion: 7, 8, 9), spring 2023 (sample occasion: 10, 11, 12), and across all sample occasions in Clifford Creek site locality 11. See Figure A-1 for locality details.

	Sample Occasion												
Species	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**Total**
Bigmouth Shiner	8	2	7	19	36	26	63	18	5	46	19	6	255
Black Bullhead	0	0	1	0	0	0	0	0	0	1	0	0	2
Blacknose Dace	0	0	0	9	0	12	0	0	0	127	131	93	372
Brassy Minnow	1	2	14	15	25	14	65	14	3	4	1	1	159
Brook Stickleback	0	0	0	0	0	0	0	1	0	1	2	0	4
Chrosomus Hybrid	0	0	0	0	0	0	0	0	0	16	10	4	30
Creek Chub	1	0	0	0	0	0	0	0	0	0	0	0	1
Fathead Minnow	3	6	11	21	4	3	17	2	1	2	2	0	72
Finescale Dace	1	1	6	6	11	16	36	19	12	15	15	12	150
Green Sunfish	0	1	3	0	1	0	2	1	2	0	1	0	11
Longnose Dace	16	42	65	12	26	9	28	33	21	20	63	8	343
Northern Pearl Dace	10	2	3	7	11	13	12	7	2	12	6	8	93
Northern Redbelly Dace	0	3	2	0	1	3	8	14	8	14	8	5	66
Plains Topminnow	0	0	0	1	2	1	2	0	0	0	1	0	7
Sand Shiner	0	0	2	9	5	0	10	0	0	0	2	0	28
White Sucker	1	0	0	0	0	0	0	0	0	0	0	0	1
Total	41	59	114	99	122	97	243	109	54	258	261	137	1594

Table A-12—Total numbers of each fish species (common name) captured in the spring 2022 (sample occasion: 1, 2, 3), summer 2022 (sample occasion: 4, 5, 6), fall 2022 (sample occasion: 7, 8, 9), spring 2023 (sample occasion: 10, 11, 12), and across all sample occasions in Clifford Creek site locality 12. Missing sample occasions denote no sample was taken. See Figure A-1 for locality details.

	Sample Occasion						
Species	**7**	**8**	**9**	**10**	**11**	**12**	**Total**
Bigmouth Shiner	84	29	32	28	10	1	184
Blacknose Dace	2	0	0	89	52	25	168
Brassy Minnow	8	1	0	0	0	0	9
Brook Stickleback	0	1	0	0	1	1	3
Chrosomus Hybrid	0	0	0	1	0	0	1
Fathead Minnow	6	2	1	8	1	4	22
Finescale Dace	30	2	2	0	0	0	34
Green Sunfish	1	0	0	0	1	0	2
Longnose Dace	57	22	5	51	1	3	139
Northern Pearl Dace	9	5	2	20	8	1	45
Northern Redbelly Dace	5	2	1	0	0	0	8
Plains Topminnow	1	2	0	0	0	0	3
Sand Shiner	9	2	1	0	0	0	12
Total	212	68	44	197	74	35	630

Table A-13—Total numbers of each fish species (common name) captured in the spring 2022 (sample occasion: 1, 2, 3), summer 2022 (sample occasion: 4, 5, 6), fall 2022 (sample occasion: 7, 8, 9), spring 2023 (sample occasion: 10, 11, 12), and across all sample occasions in Clifford Creek site locality 13. Missing sample occasions denote no sample was taken. See Figure A-1 for locality details.

	Sample Occasion						
Species	**1**	**2**	**3**	**4**	**5**	**6**	**Total**
Bigmouth Shiner	23	5	18	76	20	20	162
Black Bullhead	0	1	0	0	0	0	1
Blacknose Dace	0	0	0	0	0	2	2
Blacknose Shiner	0	0	0	1	0	0	1
Brassy Minnow	1	0	4	59	70	14	148
Brook Stickleback	1	3	0	2	1	1	8
Fathead Minnow	20	13	25	4	7	0	69
Finescale Dace	12	3	6	15	14	4	54
Green Sunfish	2	3	4	1	2	0	12
Longnose Dace	81	31	47	63	46	30	298
Northern Pearl Dace	6	0	5	7	4	3	25
Northern Redbelly Dace	0	0	0	4	4	4	12
Plains Topminnow	0	1	0	4	7	10	22
Sand Shiner	9	4	24	29	8	7	81
Total	155	64	133	265	183	95	895

Table A-14—Total numbers of each fish species (common name) captured in the spring 2022 (sample occasion: 1, 2, 3), summer 2022 (sample occasion: 4, 5, 6), fall 2022 (sample occasion: 7, 8, 9), spring 2023 (sample occasion: 10, 11, 12), and across all sample occasions in Clifford Creek site locality 14. Missing sample occasions denote no sample was taken. See Figure A-1 for locality details.

	Sample Occasion						
Species	**1**	**2**	**3**	**4**	**5**	**6**	**Total**
Bigmouth Shiner	1	0	0	3	2	0	6
Black Bullhead	1	1	4	2	4	2	14
Blacknose Dace	0	0	0	6	0	2	8
Brassy Minnow	2	2	1	132	208	136	481
Brook Stickleback	4	3	2	0	0	0	9
Creek Chub	10	5	7	1	2	0	25
Fathead Minnow	23	21	26	25	40	55	190
Finescale Dace	47	58	61	52	57	40	315
Green Sunfish	2	5	1	7	11	6	32
Longnose Dace	33	20	52	67	83	112	367
Northern Pearl Dace	4	0	0	0	0	0	4
Northern Redbelly Dace	4	13	19	0	5	7	48
Plains Topminnow	13	5	11	2	1	3	35
Sand Shiner	13	17	30	24	22	21	127
White Sucker	19	10	9	5	2	3	48
Total	176	160	223	326	437	387	1709

Table A-15—Total numbers of each fish species (common name) captured in the spring 2022 (sample occasion: 1, 2, 3), summer 2022 (sample occasion: 4, 5, 6), fall 2022 (sample occasion: 7, 8, 9), spring 2023 (sample occasion: 10, 11, 12), and across all sample occasions in Clifford Creek site locality 15. Missing sample occasions denote no sample was taken. See Figure A-1 for locality details.

	Sample Occasion						
Species	**1**	**2**	**3**	**4**	**5**	**6**	**Total**
Bigmouth Shiner	0	0	16	19	37	56	128
Black Bullhead	0	0	0	2	0	1	3
Brassy Minnow	0	2	8	56	44	33	143
Creek Chub	0	0	1	1	1	0	3
Fathead Minnow	0	4	1	13	8	0	26
Finescale Dace	4	8	13	11	7	1	44
Green Sunfish	0	1	2	2	6	2	13
Longnose Dace	3	34	60	83	62	61	303
Northern Pearl Dace	0	0	0	0	1	0	1
Northern Redbelly Dace	0	0	0	0	4	0	4
Plains Topminnow	0	2	3	0	4	1	10
Sand Shiner	3	20	97	128	167	182	597
White Sucker	0	3	2	9	8	4	26
Total	10	74	203	324	349	341	1301

Table A-16—Total numbers of each fish species (common name) captured in the spring 2022 (sample occasion: 1, 2, 3), summer 2022 (sample occasion: 4, 5, 6), fall 2022 (sample occasion: 7, 8, 9), spring 2023 (sample occasion: 10, 11, 12), and across all sample occasions in Clifford Creek site locality 16. Missing sample occasions denote no sample was taken. See Figure A-1 for locality details.

	Sample Occasion			
Species	**4**	**5**	**6**	**Total**
Bigmouth Shiner	38	55	21	114
Black Bullhead	1	0	0	1
Blacknose Dace	0	0	1	1
Brassy Minnow	41	40	9	90
Creek Chub	3	1	1	5
Fathead Minnow	16	15	1	32
Finescale Dace	3	0	0	3
Green Sunfish	1	0	0	1
Longnose Dace	35	58	27	120
Northern Redbelly Dace	0	1	1	2
Plains Topminnow	1	1	0	2
Sand Shiner	74	194	114	382
White Sucker	11	11	4	26
Total	224	376	179	779

Table A-17—Total numbers of each fish species (common name) captured in the spring 2022 (sample occasion: 1, 2, 3), summer 2022 (sample occasion: 4, 5, 6), fall 2022 (sample occasion: 7, 8, 9), spring 2023 (sample occasion: 10, 11, 12), and across all sample occasions in Sandy Richards Creek site locality 17. Missing sample occasions denote no sample was taken. See Figure A-1 for locality details.

	Sample Occasion									
Species	**1**	**2**	**3**	**7**	**8**	**9**	**10**	**11**	**12**	**Total**
Bigmouth Shiner	1	0	10	0	0	0	0	0	0	11
Black Bullhead	0	0	1	3	0	0	0	0	0	4
Blacknose Shiner	0	1	4	0	0	0	0	0	0	5
Brassy Minnow	0	3	0	0	0	0	12	22	10	47
Brook Stickleback	0	0	0	0	0	0	2	3	9	14
Chrosomus Hybrid	0	0	0	0	0	0	2	3	3	8
Fathead Minnow	0	1	2	0	0	0	1	0	1	5
Finescale Dace	5	39	42	4	0	0	5	6	14	115
Northern Pearl Dace	0	0	1	0	0	0	7	2	13	23
Northern Redbelly Dace	2	6	12	0	0	0	0	0	0	20
Plains Topminnow	4	38	39	19	3	1	6	9	17	136
Sand Shiner	0	2	0	0	0	0	0	0	0	2
Total	12	90	111	26	3	1	35	45	67	390

Table A-18—Total numbers of each fish species (common name) captured in the spring 2022 (sample occasion: 1, 2, 3), summer 2022 (sample occasion: 4, 5, 6), fall 2022 (sample occasion: 7, 8, 9), spring 2023 (sample occasion: 10, 11, 12), and across all sample occasions in Sandy Richards Creek site locality 18. Missing sample occasions denote no sample was taken. See Figure A-1 for locality details.

	Sample Occasion						
Species	**1**	**2**	**3**	**7**	**8**	**9**	**Total**
Black Bullhead	7	5	3	0	0	0	15
Blacknose Shiner	0	1	0	0	0	0	1
Brassy Minnow	0	1	0	0	0	0	1
Fathead Minnow	1	0	0	0	0	0	1
Finescale Dace	20	16	21	0	0	3	60
Northern Redbelly Dace	4	0	1	0	0	0	5
Plains Topminnow	34	13	53	0	1	0	101
Total	66	36	78	0	1	3	184

Table A-19—Total numbers of each fish species (common name) captured in the spring 2022 (sample occasion: 1, 2, 3), summer 2022 (sample occasion: 4, 5, 6), fall 2022 (sample occasion: 7, 8, 9), spring 2023 (sample occasion: 10, 11, 12), and across all sample occasions in Sandy Richards Creek site locality 19. See Figure A-1 for locality details.

	Sample Occasion												
Species	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**Total**
Bigmouth Shiner	3	5	1	1	2	1	9	0	0	2	3	6	33
Black Bullhead	2	6	1	5	26	24	14	4	2	3	2	3	92
Blacknose Shiner	6	5	8	2	1	1	30	7	3	0	0	4	67
Brassy Minnow	7	7	10	189	78	24	209	35	42	6	2	11	620
Brook Stickleback	0	0	0	0	0	0	0	0	1	0	0	0	1
Chrosomus Hybrid	0	0	0	0	0	0	0	0	0	0	1	0	1
Fathead Minnow	11	6	2	34	10	5	25	3	1	2	1	0	100
Finescale Dace	39	8	33	95	104	66	265	68	50	0	1	0	729
Northern Pearl Dace	6	6	5	3	14	17	89	13	25	5	3	4	190
Northern Redbelly Dace	13	19	11	0	0	0	19	3	12	0	0	1	78
Plains Topminnow	12	10	42	32	2	2	4	1	3	0	1	3	112
Total	99	72	113	361	237	140	664	134	139	18	14	32	2023

Table A-20—Total numbers of each fish species (common name) captured in the spring 2022 (sample occasion: 1, 2, 3), summer 2022 (sample occasion: 4, 5, 6), fall 2022 (sample occasion: 7, 8, 9), spring 2023 (sample occasion: 10, 11, 12), and across all sample occasions in Sandy Richards Creek site locality 20. Missing sample occasions denote no sample was taken. See Figure A-1 for locality details.

	Sample Occasion									
Species	**1**	**2**	**3**	**7**	**8**	**9**	**10**	**11**	**12**	**Total**
Bigmouth Shiner	1	0	6	25	7	8	16	1	1	65
Black Bullhead	0	1	1	0	0	0	0	0	0	2
Brassy Minnow	3	0	0	0	0	1	0	0	0	4
Chrosomus Hybrid	0	0	0	0	0	0	0	0	2	2
Finescale Dace	10	1	4	3	0	0	0	5	0	23
Longnose Dace	1	0	0	1	0	1	0	0	0	3
Northern Pearl Dace	0	0	0	0	0	3	4	7	0	14
Plains Topminnow	1	2	1	0	0	0	0	0	0	4
Total	16	4	12	29	7	13	20	13	3	117

Table A-21—Total numbers of each fish species (common name) captured in the spring 2022 (sample occasion: 1, 2, 3), summer 2022 (sample occasion: 4, 5, 6), fall 2022 (sample occasion: 7, 8, 9), spring 2023 (sample occasion: 10, 11, 12), and across all sample occasions in Gordon Creek site locality 21. Missing sample occasions denote no sample was taken. See Figure A-1 for locality details.

	Sample Occasion			
Species	**1**	**2**	**3**	**Total**
Black Bullhead	3	0	2	5
Brassy Minnow	5	0	6	11
Finescale Dace	2	5	5	12
Northern Redbelly Dace	16	2	40	58
Plains Topminnow	5	3	7	15
Total	31	10	60	101

Table A-22—Total numbers of each fish species (common name) captured in the spring 2022 (sample occasion: 1, 2, 3), summer 2022 (sample occasion: 4, 5, 6), fall 2022 (sample occasion: 7, 8, 9), spring 2023 (sample occasion: 10, 11, 12), and across all sample occasions in Gordon Creek site locality 22. See Figure A-1 for locality details.

	Sample Occasion												
Species	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**Total**
Bigmouth Shiner	0	0	0	48	43	13	36	25	21	15	16	5	222
Black Bullhead	1	1	0	0	0	0	0	0	0	0	0	0	2
Blacknose Dace	0	0	0	0	0	0	0	0	0	12	4	0	16
Blacknose Shiner	0	0	0	0	0	0	2	0	0	0	0	0	2
Brassy Minnow	5	5	13	91	21	5	44	10	3	35	1	58	291
Brook Stickleback	0	0	0	1	2	0	0	0	0	0	0	0	3
Chrosomus Hybrid	0	0	0	0	0	0	0	0	0	48	13	0	61
Fathead Minnow	2	0	4	7	4	0	10	2	1	14	0	1	45
Finescale Dace	11	2	6	196	130	52	73	81	41	64	11	31	698
Green Sunfish	0	0	0	1	2	2	2	0	0	0	0	0	7
Longnose Dace	2	1	3	0	16	4	9	4	9	11	0	5	64
Northern Pearl Dace	19	18	50	210	105	46	80	29	4	227	113	304	1205
Northern Redbelly Dace	7	4	12	0	12	13	75	16	1	16	2	0	158
Plains Topminnow	0	0	0	0	5	5	13	27	13	0	1	2	66
Total	47	31	88	554	340	140	344	194	93	442	161	406	2840

Table A-23—Total numbers of each fish species (common name) captured in the spring 2022 (sample occasion: 1, 2, 3), summer 2022 (sample occasion: 4, 5, 6), fall 2022 (sample occasion: 7, 8, 9), spring 2023 (sample occasion: 10, 11, 12), and across all sample occasions in Gordon Creek site locality 23. See Figure A-1 for locality details.

	Sample Occasion												
Species	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**Total**
Bigmouth Shiner	0	0	3	202	56	103	97	162	80	93	17	49	862
Blacknose Dace	0	0	0	0	0	0	0	0	1	106	47	56	210
Brassy Minnow	0	0	0	1	1	0	1	1	1	0	0	0	5
Fathead Minnow	0	1	0	1	1	2	0	1	0	0	0	0	6
Finescale Dace	1	0	0	6	7	3	0	1	2	5	2	1	28
Longnose Dace	1	13	32	61	106	98	8	58	33	42	7	5	464
Northern Pearl Dace	0	1	0	4	6	4	2	4	1	36	6	13	77
Northern Pike	0	0	0	0	0	0	0	0	0	1	1	0	2
Plains Topminnow	0	0	0	7	2	1	11	20	11	2	0	0	54
Sand Shiner	0	2	0	0	15	0	0	0	0	0	0	0	17
Total	2	17	35	282	194	211	119	247	129	285	80	124	1725

Table A-24—Total numbers of each fish species (common name) captured in the spring 2022 (sample occasion: 1, 2, 3), summer 2022 (sample occasion: 4, 5, 6), fall 2022 (sample occasion: 7, 8, 9), spring 2023 (sample occasion: 10, 11, 12), and across all sample occasions in Gordon Creek site locality 24. See Figure A-1 for locality details.

	Sample Occasion												
Species	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**Total**
Bigmouth Shiner	0	0	5	0	0	0	0	0	0	0	7	1	13
Black Bullhead	0	5	1	0	5	2	6	6	2	0	0	1	28
Blacknose Shiner	0	0	0	0	0	0	0	0	0	2	0	0	2
Bluegill	0	0	1	1	1	1	0	0	0	0	0	0	4
Northern Pearl Dace	0	0	1	0	0	0	0	0	0	0	0	0	1
Northern Pike	0	0	0	2	0	3	4	5	2	1	1	1	19
Sand Shiner	0	1	2	0	0	0	0	0	0	5	0	0	8
Total	0	6	10	3	6	6	10	11	4	8	8	3	75

Table A-25—Total numbers of each fish species (common name) captured in the spring 2022 (sample occasion: 1, 2, 3), summer 2022 (sample occasion: 4, 5, 6), fall 2022 (sample occasion: 7, 8, 9), spring 2023 (sample occasion: 10, 11, 12), and across all sample occasions in Gordon Creek site locality 25. See Figure A-1 for locality details.

	Sample Occasion												
Species	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**Total**
Bigmouth Shiner	0	0	0	0	0	1	0	0	0	0	1	0	2
Black Bullhead	0	0	0	0	0	0	0	0	1	0	0	1	2
Bluegill	0	0	0	1	0	0	0	0	0	0	0	0	1
Finescale Dace	0	1	0	0	0	0	0	0	0	0	0	0	1
Northern Pearl Dace	0	1	0	0	0	0	0	0	0	0	0	0	1
Northern Pike	0	0	0	2	0	0	1	0	1	0	0	1	5
Total	0	2	0	3	0	1	1	0	2	0	1	2	12

Table A-26—Total numbers of each fish species (common name) captured in the spring 2022 (sample occasion: 1, 2, 3), summer 2022 (sample occasion: 4, 5, 6), fall 2022 (sample occasion: 7, 8, 9), spring 2023 (sample occasion: 10, 11, 12), and across all sample occasions in Gordon Creek site locality 26. See Figure A-1 for locality details.

	Sample Occasion												
Species	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**Total**
Bigmouth Shiner	0	0	5	29	67	33	105	182	65	13	14	13	526
Black Bullhead	0	0	1	0	0	0	0	2	0	2	2	2	9
Blacknose Dace	0	0	0	1	0	5	0	0	0	12	19	29	66
Blacknose Shiner	1	0	0	1	0	1	0	0	0	0	0	0	3
Brassy Minnow	3	0	0	0	0	0	4	0	3	2	0	1	13
Chrosomus Hybrid	0	0	0	0	0	0	0	0	0	0	3	0	3
Fathead Minnow	0	0	0	1	0	0	1	0	0	0	0	0	2
Finescale Dace	2	1	13	1	1	2	2	4	0	0	0	2	28
Longnose Dace	3	4	19	60	32	52	51	51	41	4	0	0	317
Northern Pearl Dace	0	0	4	4	2	2	15	10	4	14	14	7	76
Northern Pike	0	0	0	0	2	2	1	0	0	0	0	0	5
Plains Topminnow	0	0	0	0	0	0	5	3	3	0	0	0	11
Sand Shiner	0	1	0	0	0	0	0	0	0	0	0	0	1
Total	9	6	42	97	104	97	184	252	116	47	52	54	1060

Table A-27—Total numbers of each fish species (common name) captured in the spring 2022 (sample occasion: 1, 2, 3), summer 2022 (sample occasion: 4, 5, 6), fall 2022 (sample occasion: 7, 8, 9), spring 2023 (sample occasion: 10, 11, 12), and across all sample occasions in Gordon Creek site locality 27. See Figure A-1 for locality details.

	Sample Occasion												
Species	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**Total**
Bigmouth Shiner	35	1	6	190	174	175	488	186	101	78	27	48	1509
Black Bullhead	0	0	0	0	4	5	9	4	2	0	0	1	25
Blacknose Dace	0	0	0	9	0	1	0	0	1	73	92	102	278
Brassy Minnow	1	0	0	3	0	0	0	0	0	0	0	1	5
Chrosomus Hybrid	0	0	0	0	0	0	0	0	0	1	2	5	8
Fathead Minnow	0	0	0	3	0	0	0	1	0	1	1	0	6
Finescale Dace	6	1	11	92	4	7	6	4	2	8	15	6	162
Longnose Dace	3	4	26	303	313	226	171	82	79	104	4	0	1315
Northern Pearl Dace	1	0	1	107	71	44	36	28	16	10	5	14	333
Northern Redbelly Dace	0	1	3	6	2	1	3	0	2	0	1	1	20
Plains Topminnow	0	0	0	0	1	2	5	1	3	0	2	0	14
Total	46	7	47	713	569	461	718	306	206	275	149	178	3675

Table A-28—Total numbers of each fish species (common name) captured in the spring 2022 (sample occasion: 1, 2, 3), summer 2022 (sample occasion: 4, 5, 6), fall 2022 (sample occasion: 7, 8, 9), spring 2023 (sample occasion: 10, 11, 12), and across all sample occasions in Gordon Creek site locality 28. Missing sample occasions denote no sample was taken. See Figure A-1 for locality details.

	Sample Occasion									
Species	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**Total**
Bigmouth Shiner	9	50	53	65	50	55	72	72	62	488
Black Bullhead	11	7	12	6	11	5	1	0	2	55
Blacknose Dace	1	0	2	0	4	0	80	124	96	307
Blacknose Shiner	0	0	0	6	4	4	1	0	0	15
Brassy Minnow	18	74	79	182	22	10	5	7	2	399
Fathead Minnow	2	0	0	1	0	0	0	0	0	3
Finescale Dace	0	0	0	0	0	0	2	1	0	3
Longnose Dace	46	97	172	141	156	109	7	0	1	729
Northern Pearl Dace	26	44	44	49	45	20	14	26	14	282
Northern Pike	2	1	0	1	0	0	0	0	0	4
Northern Redbelly Dace	0	0	0	1	2	0	0	0	0	3
Pumpkinseed	0	0	2	0	0	0	0	0	0	2
Sand Shiner	3	0	0	0	0	0	0	0	0	3
Total	118	273	364	452	294	203	182	230	177	2293

Figure A-1—Sampling channelized and non-channelized sites in the Cherry County Wetlands Biologically Unique Landscape within the Nebraska Sandhills Ecoregion on Willow Creek, Clifford Creek, Sandy-Richards Creek, and Gordon Creek spring 2022 to spring 2023. The numbers by each site correspond to sample tables in Appendix A.