COLUMBIA RIVER NORTHERN PIKE - INVESTIGATING THE ECOLOGY OF BRITISH COLUMBIA'S NEW APEX INVASIVE FRESHWATER PREDATOR

by

DANIEL JAMES DOUTAZ

B.Sc. Cellular, Molecular, And Microbial Biology, Thompson Rivers University, 2014

A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS

FOR THE DEGREE OF

MASTER OF SCIENCE IN ENVIRONMENTAL SCIENCES

in the Department of Natural Resource Sciences

Thesis examining committee:

Brian Heise (PhD), Associate Professor and Thesis Supervisor, Natural Resource Sciences

Jonathan Van Hamme (PhD), Professor and Committee Member, Biological Sciences

Matthias Herborg (PhD), Adjunct Faculty and Committee Member, Natural Resource Sciences

J. Mark Shrimpton (PhD), Professor and External Examiner, Ecosystem Science and Management, University of Northern British Columbia

April 2019

Thompson Rivers University

Thesis Supervisor: Associate Professor Dr. Brian Heise

Abstract

Northern pike (*Esox lucius*) is a widely distributed freshwater fish in the northern hemisphere, holding both recreational and commercial value in many countries including Canada. Pike are apex predators and are well regarded as a sport fish due to their size and voraciousness, leading to a long history of introductions outside their native range in North America. Invasive pike populations can impose significant top-down pressure on native fish community structure through predation and competition for resources, and have been linked with significant reductions of a variety of native species. Pike have recently invaded the Columbia River in British Columbia downstream of the Hugh L. Keenleyside Dam, threatening important sport fisheries, local ecosystems, and Species at Risk recovery work currently being conducted in the Columbia and its tributaries. In response to the invasion, the province implemented a pike suppression program, yet several questions remain regarding the ecology and behaviour of the recently colonized population. This research aimed to answer these questions and aid in the development of a long term management strategy for the Columbia River. Four years of data collected through the suppression program were analyzed to investigate the potential risk the invasion poses to native fish communities in B.C., the status of pike in the B.C. Columbia River, and the efficacy and impacts of current efforts. A total of 352 northern pike were captured through the suppression program by gill-netting (n=323) and angler incentives (n=29), ranging in length from 260 mm to 1050 mm. Cleithra age estimates indicate pike from within this population are achieving sizes in excess of 1 m in five to six years, and are in better than average body condition (relative weight W_r used) when compared to other North American populations. A total of 584, 659, 407, and 676 gill-net hours were set in 2014, 2015, 2016, and 2017 respectively, and an overall reduction in pike catch per unit effort (CPUE) was observed from the initial year (0.19 pike/hr) to the final year (0.05 pike/hr). This suggests gill-netting has been effective at reducing the number of adult pike in B.C. and should be conducted annually. The capture of several juvenile pike (n = 14) near Castlegar in 2017 indicates spawning is occurring in B.C. and future years of suppression should include controlling the juvenile cohort. Salmonids, particularly whitefish spp., appear to be at the greatest risk from pike predation making up approximately 50% of pike diet overall. Acoustic tracking of pike indicated the majority of individuals from within this population remain relatively sedentary, yet one individual migrated in excess of 100 km between B.C. and Washington. Microchemical analysis of pike otoliths revealed significant differences in ratios of Sr and Ba to Ca between pike caught in the Columbia and Pend d'Oreille Rivers, and that a small portion of the current Columbia population in B.C. appears to have originated in the Pend d'Oreille River. Fluctuations in otolith elemental Mg concentrations also appear to provide information that can be used to estimate the age of northern pike and potentially other esocid species. Lastly, the use of environmental DNA (eDNA) sampling as a tool to monitor the presence and spread of northern pike in B.C. was explored with minimal success; the protocol used and results obtained are discussed.

keywords: *Esox lucius*, northern pike, invasive species, fisheries management, acoustic telemetry, otolith microchemistry

Acknowledgements

I would first like to thank Dr. Brian Heise for giving me the opportunity to be part of this project. This experience has taught me a myriad of things about the natural world and myself as a person, and I am extremely fortunate to have gone through it. For giving me this opportunity, I will be forever thankful.

Secondly, I would like to thank my partner Mindy for the unwavering support over the last 3 years, I couldn't imagine having gone through this roller coaster without you. I would also like to thank my parents, Mike and Carol, whose unconditional love and support (plus a few well needed smacks upside the head) over the years has helped me more than I could ever express. Without you this wouldn't have been possible, so with sincerest gratitude, thank you and I love you both.

There are so many additional people who made this project possible, so I will do my best in addressing everyone:

Thank you Richard Bailey for convincing me to take the plunge into the next step of my career, for the many years of mentorship (and good fishing), and for always having an open door for me.

A very special thanks to my field assistant Nick Coutu for the long hours, camping for weeks at a time in the field, and keeping my lack of organizational skills in check. Couldn't have done it without you, cheers!

Thank you, Jon Van Hamme and Matthias Herborg, for taking the time to sit on my graduate committee and for your contributions to this project, it was a pleasure working with you both.

Thank you, Jeremy Baxter at Mountain Water Research, for the continual assistance and collaboration on the project. Working together was a pleasure, hope we can again in the future.

Thank you, Matt Neufeld and the B.C. Ministry of Forests, Lands and Natural Resource Operations, for the field training, repeated equipment loans, and financial contributions. A large portion of this project would not have been possible without your support.

Thank you, James Crossman and BC Hydro, for permitting me to use your equipment and providing technical support, your contribution played a huge role in this project and led to important findings.

Thank you, Jamie Shippit, for your assistance generating maps for the project, your help saved me a lot of time and grief. Best of luck in your future endeavors.

Thank you to all at the Habitat Conservation Trust Foundation (HCTF) for providing funding for this project. This research has led to important findings that are currently being used to guide northern pike suppression efforts in B.C. Sincerest thanks.

Lastly, I would like to thank my graduate cohort for making this experience as entertaining as it was, you know who you are.

This work was conducted under permit number CB17-270126 granted by the British Columbia Ministry of Forests, Lands & Natural Resource Operations, and permit number 16-HPAC-00425 from the Department of Fisheries and Oceans Canada. The work was approved by the Thompson Rivers University Animal Care Committee, protocol number 100937.

Table of Contents

List of Figures

List of Tables

Chapter 1: History and implications of northern pike introductions in the Columbia Basin.

Northern pike (*Esox lucius*) is a widely distributed freshwater fish species native to much of the northern hemisphere, holding both recreational and commercial value in many countries. The native distribution of pike in Canada spans the majority of the country with the exception of the Maritimes and most of British Columbia; in B.C. they are restricted to the northeastern portion of the province contiguous with the Peace, Liard, Yukon, Alsek, Taku and Mackenzie River systems (Harvey 2009). Northern pike is one of few representatives of the ancient Holarctic family Esocidae, with *Esox* being the single living genus (Harvey 2009). Other members of this genus include muskellunge (*Esox masquinongy*), chain pickerel (*Esox niger*), southern pike (*Esox cisalpinus*), Amur pike (*Esox reichertii*), American pickerel (*Esox americanus*), and the newly identified Aquitanian pike (*Esox aquitanicus*) (Denys *et al.* 2014), with several hybrid species such as the tiger muskellunge. Despite being a ubiquitous species in the northern hemisphere, when introduced outside their native range pike have the potential to significantly alter native fish community structure through predation and competition for resources, the impacts of which are widely documented (Scott and Crossman 1973, Patankar *et al*. 2006, Bystrom *et al.* 2007, Johnson *et al*. 2008, Spens and Ball 2008, Haught and von Hippel 2011, Sandlund *et al.* 2016).

Pike are voracious ambush predators with an elongated body and anal and dorsal fins far back on the body allowing for rapid acceleration (Hubbs and Lagler 2004) with burst speeds in excess of 7 m/s (Harper and Blake 1990). Due to their size and aggressiveness pike are a highly sought-after sport fish for anglers, which has led to a long history of public pressure for intentional stocking and illegal introductions (McMahon and Bennett 1996). Introductions dating back to as early as the 1950's (Brown 1971) have resulted in the establishment of invasive populations throughout the Pacific Northwest, with pike present in major river systems such as the Flathead, Clark Fork, Spokane, Pend d'Oreille, and most recently the Columbia River in both B.C. and Washington. Northern pike were first detected in the Columbia River in 2007 in the upper reach of Lake Roosevelt near Alder Creek (Hunters, WA), and in 2009 a northern pike was captured in B.C. downstream of the Hugh L.

Keenleyside Dam (HLK) near Castlegar (Baxter and Neufeld 2015). Following their detection in the Columbia River, Fisheries and Oceans Canada conducted a biological risk assessment for northern pike in B.C. and were deemed to present a high level of risk to native biota if they were to spread further into B.C. (Bradford *et al.*2008). Pike have since colonized a significant area near Castlegar, likely due to an abundance of resources and habitat, and are also present throughout the approximate 56 km stretch of river extending from the confluence of the Pend d'Oreille to the HLK, hereby referred to as the B.C. Lower Columbia River (LCR) (Figure 2.1).

The Columbia River houses a wide array of fish species that comprise an important fishery for B.C and Washington with both economic and cultural significance, including members of the Salmonid (Salmonidae), Sturgeon (Acipenceridae), Minnow (Cyprinidae), Sucker (Catostomidae), Sculpin (Cottidae), Cod (Gadidae), Stickleback (Gasterosteidae), Lamprey (Petromyzontidae), and Trout-perch (Percopsidae) families (Dauble 2009). The detection of pike in the Columbia River sparked concern within provincial and state fisheries authorities as to their potential negative impact on native species, particularly native salmonids and those listed under the Species at Risk Act (SARA) including Columbia sculpin (*Cottus hubbsi*), Umatilla dace (*Rhinichthys umatilla*), and white sturgeon (*Acipencer transmontanus*) (Department of Fisheries and Oceans, Canada 2019).

The suspected source of the Columbia River invasion is the Pend d'Oreille River, which flows approximately 200 km from Lake Pend Oreille, Idaho, to its confluence with the Columbia in B.C. at the Canada-U.S. border. Two hydroelectric facilities reside in the B.C. portion of the Pend d'Oreille River; Seven Mile and Waneta dams, the latter of which is the last downstream dam on the Pend d'Oreille, just upstream of the U.S. border (Figure 2.1). Extensive hydroelectric development on the Pend d'Oreille River caused a shift from to lotic to lentic conditions in the resultant reservoirs, significantly altering native fish community structure and facilitating the colonization of a variety of non-native species including smallmouth bass (*Micropterus dolomieu*), yellow perch (*Perca flavescens*), walleye (*Sander vitreus*), pumpkinseed (*Lepomis gibbosus*), black crappie (*Pomoxis nigromaculatus*), tench (*Tinca tinca*), black bullhead (*Ameiurus melas*), brown bullhead (*Ameiurus nebulosus*), brown trout (*Salmo trutta*), and northern pike (HTI 2009). Many of these species are now present in the Columbia River due to movement through the dams or through human introduction, although it is unclear if the Pend d'Oreille is a significant input of northern pike into the LCR.

In the Box Canyon section of the Pend Oreille River (WA) estimates of northern pike population size increased from 665 in 2006 to greater than 5,500 in 2010 and 10,000 in 2011 (Bean 2014; Baxter and Neufeld 2015; King and Lee 2016), leading the Washington Department of Fish and Wildlife (WDFW) to implement a vigorous gill-netting suppression program in 2012. This response in the U.S., paired with increasing pike catch rates in the LCR prompted the provincial government to take action and in 2013, the B.C. Ministry of Forests, Lands and Natural Resource Operations (MFLNRO) implemented an angler incentive program to encourage the removal of pike from the Columbia River and to obtain information about their distribution (Baxter and Neufeld 2015). The following year, the MFLNRO and industry partner Teck Metals Ltd. initiated a 4-year gill-net suppression program in the Columbia River to reduce the number of mature adults in the Columbia, with a heavy focus on the Castlegar population due to its increasing density and proximity to HLK (Baxter and Neufeld 2015). This work generated numerous questions regarding the movement, population recruitment, and spawning behaviour of northern pike within the Castlegar population.

This research aimed to assist the provincial northern pike management effort by filling in these knowledge gaps through the study of pike diet, body condition, movements, population recruitment, spawning behaviour, habitat use, and risk of spread using both physical and biochemical means. Chapter 2 analyzes past and current efforts for pike management in the LCR, and summarizes all data collected through these efforts to investigate the status of this newly established population. Diet, body condition, size and growth rates of pike from within the LCR are compared to the longer established and suspected source population in the Pend d'Oreille River, in addition to other populations in North America using previously published data. The main goal of these analyses were to determine the efficacy and potential long-term effects of pike management in the LCR (i.e., gill-net effort/bycatch), the potential impacts of pike predation on native species (i.e., prey selection/quantity) and how well the LCR population is doing in relation to others (i.e., body condition, growth rates). Chapter 3 investigates the movements and geographic life history of pike in the LCR through both acoustic tracking of individual fish and microchemical analysis of otoliths. Pike in the LCR were tagged with acoustic transmitters and monitored for a year and a half to investigate annual activity and spawning behaviour (i.e., movements, habitat location/residence, migratory behaviour). Otolith microchemistry of pike caught in the LCR was compared to pike from the Pend d'Oreille River, the suspected source of the LCR invasion, to determine if distinguishable elemental signatures existed within otoliths from either river that could be used to infer the natal origin of individuals or movement between systems. In Chapter 4 a potentially useful technique for aging northern pike and other esocid species was discussed, correlating fluctuations in otolith trace element concentrations to changes in metabolic activity throughout the year. In Chapter 5 the use of environmental DNA (eDNA) sampling was explored as a potential monitoring tool for northern pike in the LCR and its tributaries. Lastly, in Chapter 6 all data collected through this study are summarized and recommendations for future years of northern pike management in B.C. are made, with the hopes of preserving an important and valuable fishery.

Literature Cited

Baxter, J.T.A., Neufeld, M. (2015). Lower Columbia River invasive northern pike suppression and stomach analysis – 2014. Mountain Water Research, Silverton, B.C. and Ministry of Forest, Lands and Natural Resource Operations, Nelson B.C. Prepared for Teck Metals Ltd., Trail B.C.

Bean, N. (2014). Kalispel non-native fish suppression project. 2014 Annual Report. Bonneville Power Administration Project 2007-149-00.

Bradford, M.J., Tovey, C.P., Herborg, L.M. (2008). Biological Risk Assessment for Northern Pike (*Esox lucius*), Pumpkinseed (*Lepomis gibbosus*), and Walleye (*Sander vitreus*) in British Columbia. Fisheries and Oceans Canada.

Brown, C.J.D. (1971). Fishes of Montana. Big Sky Books, Bozeman, MT.

Bystrom, P., Karlsson J., Nilsson P., V. and Kooten, T. (2007). Substitution of top predators: effects of pike invasion in a subarctic lake. *Freshwater Biology* 52(7): 1271 - 1280.

Dauble, D. (2009). Fishes of the Columbia Basin. Keokee Co Pub Inc., Sandpoint Idaho.

Denys, G.P.J., Dettai, A., Persat, H., Hautecoeur M., Keith, P. (2014). Morphological and molecular evidence of three species of pikes *Esox* spp. (*Actinopterygii, Esocidae*) in France, including the description of a new species. *Comptes rendus Biologies*, 337: 521 - 534.

Department of Fisheries and Oceans, Canada [Internet]. (2019). Aquatic species at risk. Accessed from: http://www.dfo-mpo.gc.ca/species-especes/sara-lep/index-eng.html

Harper, D.G., Blake, R.W. (1990). Fast-start performance of rainbow trout *Salmo gairdneri* and northern pike *Esox lucius*. *Journal of Experimental Biology,* 150: 321 - 342.

Harvey, B. (2009). A biological synopsis of northern pike (*Esox lucius*). *Canadian Manuscript Report of Fisheries and Aquatic Sciences,* 2885: 31 pp.

Haught, S., von Hippel, F.A. (2011). Invasive pike establishment in Cook Inlet Basin lakes, Alaska: diet, native fish abundance and lake environment. *Biological Invasions* 13: 2103 - 2114.

Hubbs, C.L., Lagler, K.F. (2004). Fishes of the Great Lakes Region. Rev. G.R. Smith. University of Michigan Press. 276 pp.

HTI (Hydroacoustic Technology Inc.). (2009). Boundary hydroelectric project (FERC No. 2144), Study No. 12 Fish entrainment and habitat connectivity study. Final report prepared for Seattle City Light. 114 p + 4 App.

Johnson, B.M., Martinez, P.J., Hawkins, J.A., Bestgen, K.R. (2008). Ranking predatory threats by nonnative fishes in the Yampa River, Colorado, via bioenergetics modeling. *North American Journal of Fisheries Management* 28: 1941 - 1953.

King, L., Lee, C. (2016). Evaluation of northern pike *Esox lucius* in Upper Lake Roosevelt and the Lower Kettle River, Washington, February 2016. Report to the Spokane Tribe, for Bonneville Power Administration Project No. 1994-043-00.

McMahon, T. E., Bennett, D.H. (1996). Walleye and northern pike: boost or bane to northwest fisheries? Fisheries 21(8): 6 - 13.

Patankar, R., Von Hippel, F., Bell, M. (2006). Extinction of a weakly armoured threespine stickleback (*Gasterosteus aculeatus*) population in Prator Lake, Alaska. *Ecology of Freshwater Fish* 15: 482 - 487.

Sandlund, O.T., Museth, J., and Øistad, S. (2016). Migration, growth patterns, and diet of pike (*Esox lucius*) in a river reservoir and its inflowing river. *Fisheries Research,* 173(1): 53 - 60.

Scott, W.B., Crossman, E.J. (1973). Freshwater Fishes of Canada. Bulletin 184. Fisheries Research Board of Canada. 966 pp.

Spens, J., Ball, J. (2008). Salmonid or nonsalmonid lakes: predicting the fate of northern boreal fish communities with hierarchical filters relating to a keystone piscivore. *Canadian Journal of Fisheries and Aquatic Sciences*, 65: 1945 - 1955.

Chapter 2: Ecology and population suppression of an invasive top predator, northern pike (*Esox lucius*) in the B.C. Lower Columbia River.

keywords: *Esox lucius*, northern pike, invasive fish, fisheries management

Abstract

This study investigates the ecology of invasive northern pike in the B.C. Lower Columbia River, in addition to analyzing the efficacy of a four-year pilot suppression program (2014-2017) employed to control the newly established population. Also explored is the status of northern pike in the B.C. section of the Pend d'Oreille River, the suspected source of the invasion, to compare the newly established Columbia population to a long-established one. The suppression program led to the capture of 352 pike by gill-netting (n=323) and angler incentives (n=29), with captured fish ranging in length from 260 mm to 1050 mm. A total of 584, 659, 407, and 676 gill-net hours were set in 2014, 2015, 2016, and 2017, respectively, and an overall reduction in pike catch per unit effort (CPUE) was observed from the initial year (0.19 pike/hr) to the final year (0.05 pike/hr). High live release rates (75.4%) indicate the current gill-netting methods (≤ 4-hour sets, ≤ 4 m depth, close to shore, no overnight sets, continuous monitoring of nets) have been successful in minimizing impacts on native fish. Approximately 50% of prey items found in Columbia River pike stomachs consisted of salmonid species (39% whitefish spp., 5% kokanee, 4% rainbow trout), with the remainder consisting of sculpin spp. (17%) sucker spp. (11%), redside shiner (11%), northern pikeminnow (7%), dace spp. (3%) and invertebrate spp. (3%). Relative weight was used as an index for body condition (i.e., fatness in relation to length), and the majority of this population (85.1%) was in better than average condition, suggesting this invasive population is thriving in its newly established territory. Gill-netting in the Pend d'Oreille River led to the capture of 43 northern pike ranging in length from 350 mm to 970 mm. Diet composition of Pend d'Oreille northern pike differed considerably from Columbia River pike, with yellow perch (48.4%) and smallmouth bass (45.2%) comprising the majority of prey items. A single rainbow trout and leech were observed suggesting salmonids and invertebrates make up a small proportion of pike diets in the Pend d'Oreille River. Over half (54.4%) of all fish captured by gill-netting in the Pend d'Oreille were invasive species and only a single salmonid was encountered. Pend d'Oreille River northern pike were found to have much higher variation in body condition in smaller fish, and only 44.2% of individuals were above average weight. Age estimates through analysis of cleithra (n=108) indicate pike from within the Columbia population attain lengths of up to 950 cm in 5 to 6 years of growth. Growth rates were similar in Pend d'Oreille River northern pike despite differences in body condition. Reductions in pike captures since the initial year of the suppression program suggest gill-netting is a viable technique for suppressing northern pike in the B.C. lower Columbia River, and continuation of the program is advisable to control this population. Gill-netting in the Pend d'Oreille should also be considered for future years of suppression to reduce the potential influx of invasive fish species into the Columbia River.

Introduction

Northern pike (*Esox lucius*) is a widely distributed freshwater species in North American and Eurasian waters, holding both recreational and commercial value in many countries including Canada. Native distribution of pike in Canada spans the majority of the country with the exception of the Maritimes and most of British Columbia (B.C.), where they are restricted to the Northern portion of the province contiguous with the Peace, Liard, Yukon, Alsek, Taku and Mackenzie River systems (Harvey 2009). Pike are a highly sought-after game fish for anglers due to their size and voraciousness, which has led to a long history of illegal introductions and public pressure for intentional stocking (McMahon and Bennett 1996).

Northern pike are an apex freshwater predator that, when introduced outside their native range, can impose significant trophic effects on native fish communities through predation and competition for resources, the impacts of which are widely documented in the literature (Scott and Crossman 1973, Patankar *et al*. 2006, Bystrom *et al.* 2007, Johnson *et al*. 2008, Spens and Ball 2008, Haught and von Hippel 2011, Sandlund *et al.* 2016). Adult pike are visual ambush predators (Bradford *et al.* 2008) and preferentially prey on soft-rayed fishes, but will also select spiny-rayed fishes when favourable prey species are at low density (Eklöv and Hamrin 1989). While pike are highly piscivorous, they are opportunistic feeders with other prey including invertebrates, frogs, crayfish, mice, muskrat, waterfowl up to one half their body length (Scott and Crossman 1973), and are known to become cannibalistic at sizes as small as 21 mm when preferred prey are at low densities (Eklöv and Hamrin 1989). Pike are a largely sedentary fish, yet are known to expand their range when conditions permit (Bradford *et al.* 2008). Pike also have relatively wide physiological tolerances allowing them to persist in a range of environmental conditions (Bradford *et al.* 2008), likely contributing to their expansion into non-native territory,

Northern pike were first detected in the Columbia River in 2007 in the upper reach of Lake Roosevelt near Alder Creek (Hunters, WA), and in 2009 a northern pike was captured in B.C. downstream of the Hugh L. Keenleyside Dam (HLK) near Castlegar (Baxter and Neufeld 2015). Following their detection in the Columbia River, Fisheries and Oceans Canada conducted a biological risk assessment for northern pike in B.C. and were deemed to present a high level of risk to native biota if they were to spread further into B.C. (Bradford *et al.*2008). Northern pike have since colonized a significant area near Castlegar, likely due to an abundance of resources and habitat, and are also present throughout the approximate 56 km stretch of river extending from the confluence of the Pend d'Oreille to the HLK, hereby referred to as the B.C. Lower Columbia River (LCR) (Figure 2.1). In Washington, the distribution of pike spans a considerably greater area between the Canada-U.S. border and Grand Coulee Dam (approximately 240 km) in addition to populations in sections of the Kettle and Spokane River systems. In 2017, nearly 5,000 pike were removed from Lake Roosevelt, the reservoir created following construction of the Grand Coulee Dam, through a variety of suppression techniques and an angler incentive program (Elliott Kittel, Fisheries Biologist, Spokane Tribe of Indians, pers. comm. 2017).

The suspected source of the Columbia invasion is the Pend d'Oreille River, where reports of pike captures date back to the 1980's (King and Lee 2016). The Pend d'Oreille extends from Lake Pend Oreille, Idaho, through Washington and into B.C. where it drains into the Columbia River at the Canada-U.S. border. Extensive hydroelectric development on the Pend d'Oreille/Pend Oreille caused a shift from lotic to lentic conditions in the resultant reservoirs, significantly altering native fish community structure and facilitating the colonization of non-native species including northern pike, smallmouth bass (*Micropterus dolomieu*), yellow perch (*Perca flavescens*), walleye (*Sander vitreus*), pumpkinseed (*Lepomis gibbosus*), black

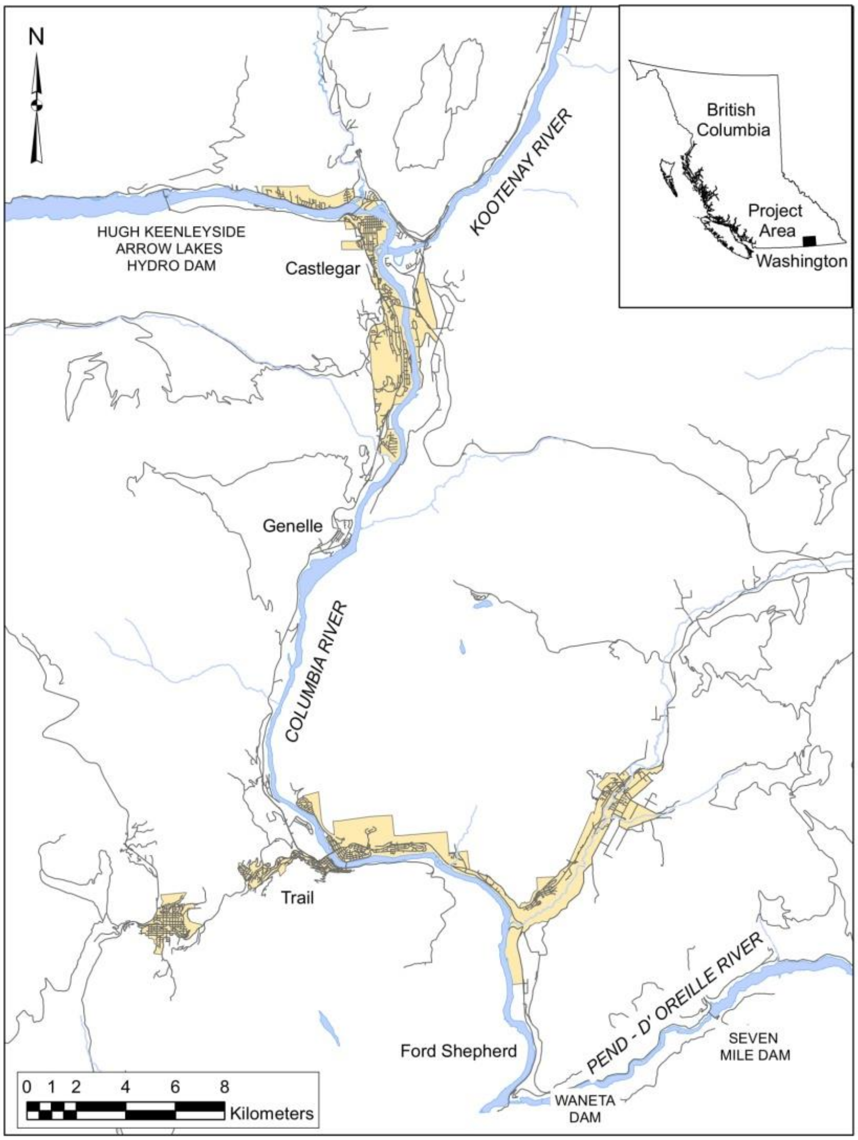

Figure 2. 1. B.C. section of the Columbia River downstream of the Hugh L. Keenleyside Dam (HLK), recently colonized by invasive northern pike, also referred to as the B.C. Lower Columbia River (LCR).

crappie (*Pomoxis nigromaculatus*), tench (*Tinca tinca*), black bullhead (*Ameiurus melas*), brown bullhead (*Ameiurus nebulosus*), and brown trout (*Salmo trutta*) (HTI 2009).

In 2012, the Washington Department of Fish and Wildlife (WDFW) initiated a vigorous gill-netting suppression program, which to date has been deemed successful by reducing catch rates of 12.2 pike/net night in the initial year to 0.18 pike/net night in 2015 (Bean 2014; Harvey and Bean 2016). While northern pike encounters have been reported in the B.C. section of the Pend d'Oreille (Waneta and Seven Mile Reservoirs) by both anglers and river indexing programs, no suppression efforts have been employed to date and as a result the size and status of these populations in not well known.

Increasing reports of northern pike captures in the Columbia River, particularly in the Castlegar area, sparked concern within the province about potential negative impacts on native fish populations in the LCR. Compounding risks to native B.C. fish is the presence of the navigation lock at HLK that provides access between the LCR and the Arrow Lakes Reservoir, extending approximately 230 km north to the Revelstoke Dam. The lock operates daily for both commercial (Zelstoff Celgar mill) and pleasure craft, and is often left open on the downstream side for extended periods of time as a result of dam operations (J. Crossman, Fish Ecologist, B.C. Hydro, pers. comm. 2015), providing potential access for pike to spread into the Arrow Lakes. Growing concern prompted the provincial government to intervene, and in 2013, the B.C. Ministry of Forests, Lands and Natural Resource Operations (MFLNRO) implemented a pilot gill-netting program, amended angling regulations (unlimited bag limit), and initiated an angler incentive program to encourage the removal of pike from the Columbia River and to obtain information about their distribution (refer to Appendix A, Figure A1 for example of provincial signage). In 2014, the MFLNRO and industry partner Teck Metals Ltd. initiated a four year gill-net suppression program in the Columbia to reduce pike numbers, with a heavy focus on the Castlegar population due to its increasing density and proximity to HLK.

The goal of this study was to assess the effectiveness of the current provincial suppression program in the LCR over a four year period and investigate pike predation on native fish species, particularly the aforementioned SARA listed species in the Columbia. The data collected through the program between May 2014 and September 2017 was used to investigate the diet, size, growth rates, and body condition of northern pike in the Columbia River, in addition to the effectiveness of employing the use of gill-netting as a management tool for this invasive population. Also explored is the ecology of northern pike in the Pend d'Oreille River, the suspected source of the invasion, to compare the newly established Columbia population to a long-established one.

Methods

In 2013, the provincial government released 30 Passive Integrated Transponder (PIT)-tagged northern pike in the Castlegar area as part of the angler incentive program, with an additional 11 tagged pike released in 2015. Anglers who captured a pike were encouraged to bring the head into the ministry office in Castlegar for a chance to win a $500 reward if it contained a PIT tag. Details of the incentive program were disseminated through media outlets in B.C., in addition to the deployment of signage at public boat launches in the Kootenay region.

Gill-netting was conducted primarily downstream of HLK near Castlegar, as this reach contains an abundance of preferable prey items (soft-bodied fishes, i.e., trout spp., char spp., whitefish spp., sculpin spp., minnow spp.), habitat (shallow water bays, abundant aquatic vegetation) and suitable hydrological conditions (slow currents, eddies) as a result of the reservoir created downstream of HLK. Several of these habitats were shown to have increases in aquatic vegetation (primarily Eurasion watermilfoil, *Myriophyllum spicatum*) leading up to the start of the B.C. suppression program, and the frequency of pike capture also increased in these areas (Golder 2014). Suitable pike habitat was identified at the Zelstoff Celgar mill located downstream of HLK, where an abundance of sunken logs and debris potentially serve as habitat and cover. These areas were heavily targeted by gill-netting due to their habitat characteristics and observed concentrations of northern pike. While pike encounters have been reported throughout the LCR, there is limited habitat for pike outside of the Castlegar area as much of the river between HLK and the border experiences fast currents along rocky or sandy shorelines. In the first year of the suppression program, areas with similar habitat characteristics to that of the Castlegar area were targeted with no success, and subsequent gill-netting was focused in the Castlegar area only.

Gill-nets used followed specifications which had been successful in U.S. pike suppression efforts, and consisted of 5 monofilament panels each measuring 9.1 m in length by 1.8 m deep with mesh sizes ranging between 2.5 and 5.0 cm. Nets were set in shallow water bays (0.5 m - 4 m) with aquatic vegetation. Gill-netting was conducted during daylight hours, and nets were set for no longer than 4 hours and checked regularly. In the initial year two night net sets were tested and deemed unsuitable due to lower catch rates of pike and increased bycatch and mortality of native species. Data collected from these net sets were not included in this study. All pike captured were immediately euthanized and when possible measured for fork length (mm), weight (g), sex, maturity, stomach contents, and scanned for the presence of a PIT tag. Pike heads were retained for removal of cleithra that were used for age estimation. Species present in gut samples were identified and measured either in the field by members of the suppression team or preserved in 95% ethanol and analyzed in the Freshwater Ecology Lab at Thompson Rivers University. Prey items were identified to the genus level, and when possible, to the species level. All bycatch fish were assessed for overall body condition, and if deemed fit for release they were returned to the river and recorded as a live release. Any fish deemed unfit for release were euthanized and were recorded as a mortality.

Due to the exploratory nature of the suppression program, gill-netting effort varied across season and year. A total of 584, 659, 407, and 676 total gill-net set hours were conducted in 2014, 2015, 2016, and 2017, respectively (Figure 2.2). Gill-netting was conducted in May of each year coinciding with pike spawning, with additional netting done in June (2016), August (2014, 2015), September (2015, 2017), and November (2014) to explore habitat use outside of the pike spawning season. Total effort (net hours) was used to determine the catch per unit effort (CPUE) of pike by gill-netting. All gill-netting in the Columbia River was conducted by Mountain Water Research (Trail, B.C.).

Gill-netting was conducted in the B.C. section of the Pend d'Oreille River in both Waneta and Seven Mile reservoirs to compare pike body size, condition and diet to the newly established Columbia River population. Efforts were mainly focused in the Waneta reservoir as it is the last section of the Pend d'Oreille before its confluence with the Columbia. Gill-nets were set in shallow sections with aquatic vegetation at depths from 0 (shore) to 4 m, and when possible, were set for 4 hours and checked regularly. This did, however, prove challenging due to changing water depths (hydro operations) which often resulted in nets having to be pulled and re-set. A total of 110.0 hours of net sets was deployed in the Waneta Reservoir in 2016 (25.4 in June, 14.3 in July, 70.4 in August), and 26.78 hours in May of 2017. A total of 20.1 hours of net sets were deployed in the Seven Mile reservoir in June of 2016, and 15.63 and 19.47 hours were set in May and August of 2017, respectively. Fish were euthanized and processed in the same manner as for the Columbia River suppression

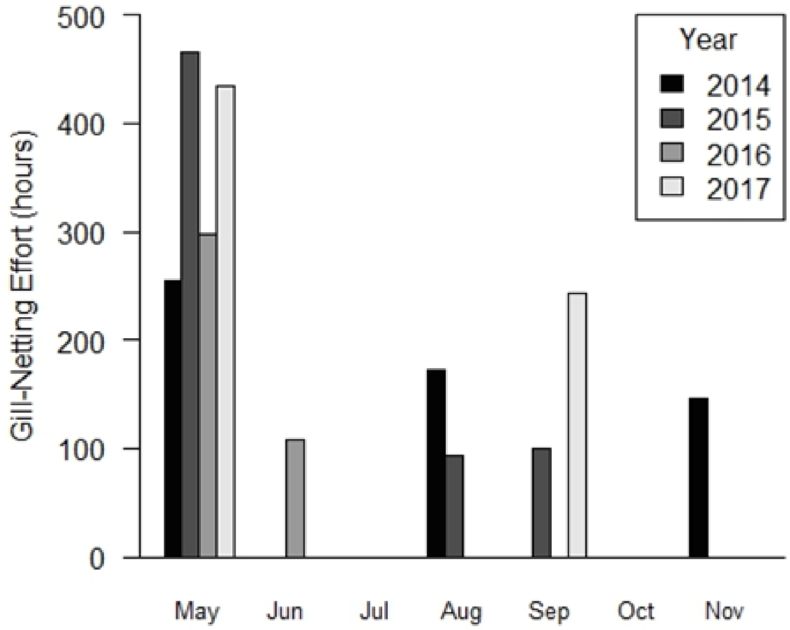

Figure 2. 2. Gill-netting effort by month between May 2014 and September 2017 in the B.C. Lower Columbia River. Sampling occurred every May to coincide with northern pike spawning time following spring freshet, in addition to summer and fall sampling in 2014, 2015, and 2017 and late spring sampling in 2016. A total of 584, 659, 407, and 676 gill-net set hours were completed in 2014, 2015, 2016, and 2017, respectively.

program. Gill-netting conducted in the Pend d'Oreille was conducted by myself and research assistant Nick Coutu (TRU B.Sc. candidate)Relative weight was used to compare the condition of Columbia and Pend d'Oreille River northern pike to other populations in North America. This method was used as it is more sensitive for analyzing changes in body condition across lengths than linear weight-length regression (Fisher and Fielder 1998). Relative weight is the ratio of observed fish weight to the "standard weight" of a fish predicted by a standard weight equation for that species. This ratio can be used to determine the condition or fitness of individual fish from within populations or to assess and compare populations as a whole. Wege and Anderson (1978) introduced the following equation for relative weight (W_r):

$$W_r = (W/W_s) \times 100,$$

where W is observed weight and W_s is the expected weight for a fish at that length using the standard weight equation. Ratios of greater than 100 may indicate that the observed weight of a fish is higher than would be expected for a typical fish at that length, potentially indicating superior conditions or resource availability for that population. Conversely, ratios below 100 may indicate poor environmental conditions or limited resource availability for that population. The standard weight equation proposed by Willis (1989) was used for calculating relative weight for Columbia and Pend d'Oreille northern pike, as the equation is considered the accepted standard (Anderson and Neumann 1996):

$$W_s = 3.096(\mathit{Total\ Length}) - 5.437mm$$

As this formula uses total length, fork length data for Columbia and Pend d'Oreille River northern pike were converted to total length using the following conversion reported by Mallete and Morgan (2005):

$$\mathit{Total\ Length} = 1.0431(\mathit{Fork\ Length}) + 10.448\ mm$$

The majority of relative weight equations have minimum applicable lengths due to differences in growth forms between juveniles and adults, in addition to potential errors when weighing small fish in the field (Murphy *et al.* 1991; Blackwell 2000). In the case of northern pike, minimum length was determined to be 336.6 mm (13.25 in), therefore all observations below this threshold (n = 17) were omitted from analyses. The five length category system proposed by Gabelhouse (1984) was used to categorize size data. Minimum stock (>336.6 mm), quality (>562.6 mm), preferred (>729.0mm), memorable (>897.9 mm) and trophy (>1082.0 mm) lengths were selected based on percentages of world-record lengths (converted from inches). An average was taken of the range reported in Gabelhouse (1984) to obtain minimum lengths for each category (Table 2.1).

The calculation of a mean relative weight across populations is not suggested without first determining whether statistical differences exist across sample lengths (Blackwell 2000), as this could mask important length-related trends in fish condition and biased assessments (Murphy *et al.* 1991). Two-way analysis of variance was used to compare mean relative weights between the 2 river systems and among length categories, followed by Tukey's multiple comparisons test to determine if significant differences in mean W_r were present between length categories. All data analysis was conducted using RStudio (v. 1.1.383).

Northern pike (n = 108) ages were estimated using cleithra following recommendations by Euchner (1988). Cleithra were removed from pike heads (collected through the provincial suppression program) and immersed in 95 °C water for up to a minute to loosen any tissue remaining on the sample. Samples were then cleaned with a coarse nylon brush and air dried for 48 hours prior to analysis. Cleithra were placed in a Petri dish filled with enough vegetable oil to cover the sample and inspected using a microscope under 10x magnification. Annuli were counted outwards from the origin to the anterior tip of the cleithra to obtain age estimates. Euchner (1988) defines an annulus as a prominent translucent zone that can be traced from the anterior of the cleithra blade around the entire blade and cannot be traced into the posterior. A portion (n = 25) of samples were sent to an external laboratory (North/South Consulting, Manitoba) for quality control.

Results

A total of 352 northern pike were removed from the LCR through the suppression program between May 2014 and September 2017, with 323 captured by gill-netting (91.8%) and 29 through the angler rewards program (8.2%). In the initial year 133 pike were captured by gill-netting, followed by a decrease in numbers in 2015 (n = 116), 2016 (n = 39), and 2017 (n=35) (Figure 2.3). CPUE increased from 0.19 pike/hr in 2014 to 0.20 pike/hr in 2015, followed by a decrease in 2016 (0.13 pike/hr) and 2017 (0.05 pike/hr) (Figure 2.3). The angler incentive program resulted in the collection of 33 pike throughout the study, with 29 of those from the LCR and the remaining 4 from the Pend d'Oreille River. In September 2017, 14 young-of-the-year (YOY) northern pike were identified during boat surveys and captured by seining in less than 30 cm of water in habitat with little to no flow.

A total of 1,452 fish were caught as bycatch from gill-netting through the suppression program, 1,095 of which were recorded as live releases (75.4%). Species captured included mountain whitefish (*Prosopium williamsoni*), lake whitefish (*Coregonus clupeaformis*), sucker spp. (*Catostomus* spp.), rainbow trout (*Oncorhynchus mykiss*), kokanee (*Oncorhynchus nerka*), northern pikeminnow (*Ptychocheilus oregonensis*), walleye (*Sander vitreus*), eastern brook trout (*Salvelinus fontinalis*), white sturgeon, smallmouth bass, tench, sculpin spp. (*Cottidae* spp.), yellow perch, bull trout (*Salvelinus confluentus*), and cutthroat

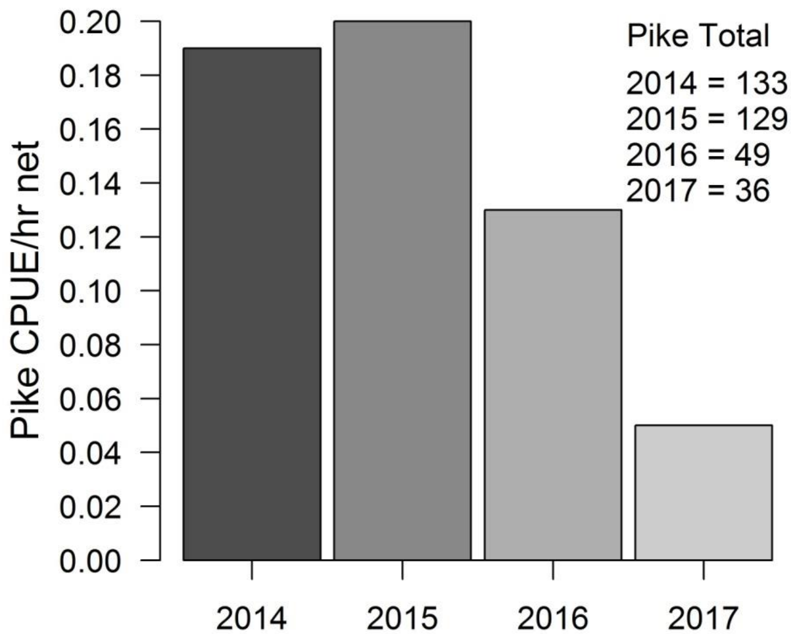

Figure 2.3. Northern pike gill-net catch per unit effort (CPUE) per 8 hour day in the B.C. Lower Columbia River. A total of 584, 659, 407, and 676 total gill-net set hours were completed in 2014, 2015, 2016, and 2017, respectively.

trout (*Oncorhynchus clarkii*) (Figure 2.4).

A total of 43 northern pike were caught in the Pend d'Oreille River in both Waneta (n = 32) and Seven Mile (n = 17) reservoirs. A total of 206 fish were caught as bycatch in the Pend d'Oreille, only 94 of which were native species (45.6%). These included sucker spp., northern pikeminnow, and a single mountain whitefish. Of the 94 native species encountered 80 were removed from the net and recorded as live releases (85.1%). Non-native species included tench, yellow perch, smallmouth bass, pumpkinseed, and walleye (Figure 2.5).

Of the 323 LCR northern pike stomachs analyzed 187 contained prey items (58%) (Figure 2.6). The most prevalent prey items identified were whitefish spp. (n = 70), followed by sculpin spp. (n = 30), sucker spp. (20), redside shiner (n = 20), northern pikeminnow (n = 13), kokanee (n = 9), rainbow trout (n = 7), dace spp. (n = 6), and invertebrates (n = 5).

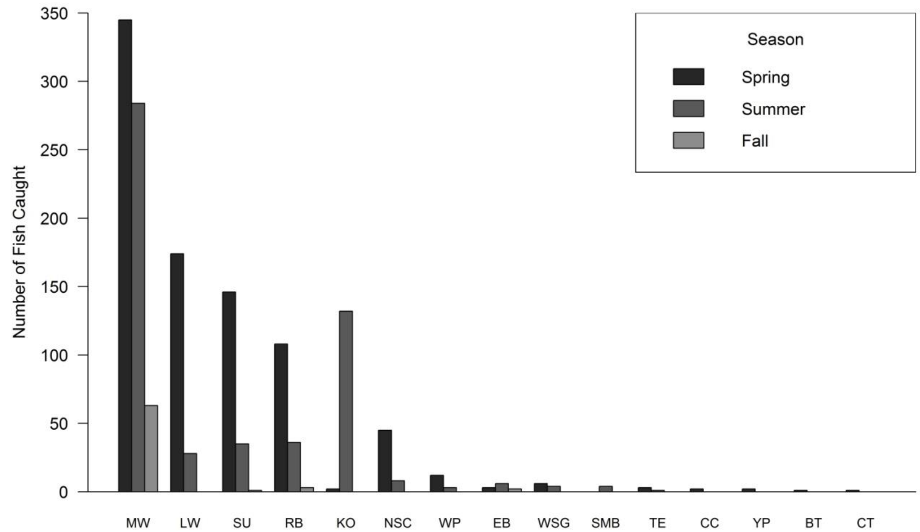

Figure 2.4. Bycatch caught in gill-nets between May 2014 and September 2017 in the B.C. Lower Columbia River. The number of species captured was separated by season to determine which species were most likely to be encountered while sampling throughout the year. Species captured (total) included mountain whitefish (MW) (n = 689), lake whitefish (LW) (n = 199), sucker spp. (SU) (n = 178), rainbow trout (RB) (n = 147), kokanee (KO) (n = 134), northern pikeminnow (NSC) (n = 53), walleye (WP) (n = 15), eastern brook trout (EB) (11), white sturgeon (WSG) (n = 9), smallmouth bass (SMB) (n = 7), tench (TE) (n = 4), sculpin spp. (CC) (n = 2), yellow perch (YP) (n = 2), bull trout (BT) (n = 1), and cutthroat trout (CT) (n = 1).

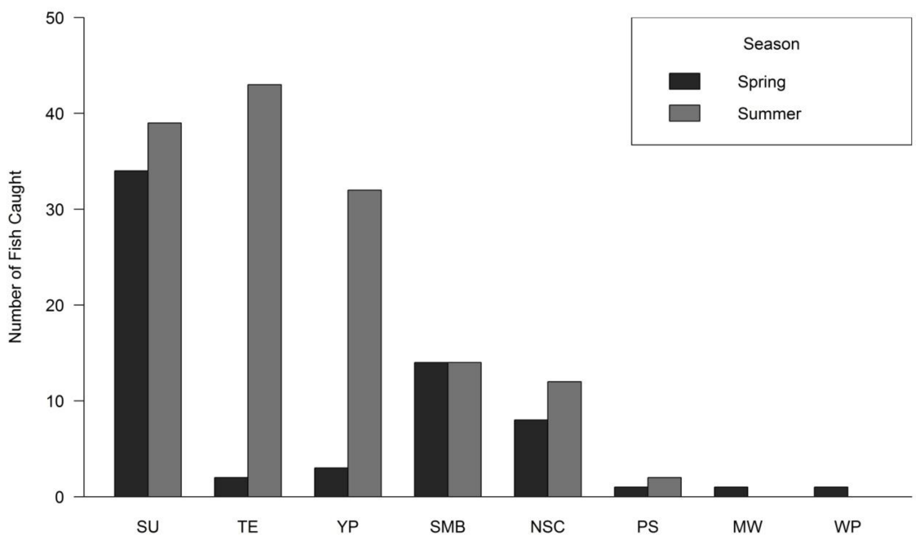

Figure 2.5. Gill-net bycatch in the Pend d'Oreille River between June 2016 and August 2017. A total of 206 fish were caught including sucker spp. (SU) (n = 73), tench (TE) (n = 45), yellow perch (YP) (n = 35), smallmouth bass (SMB) (n = 28) northern pikeminnow (NSC) (n = 20), pumpkinseed (PS) (n = 3), mountain whitefish (MW) (n = 1), and walleye (WP) (n = 1).

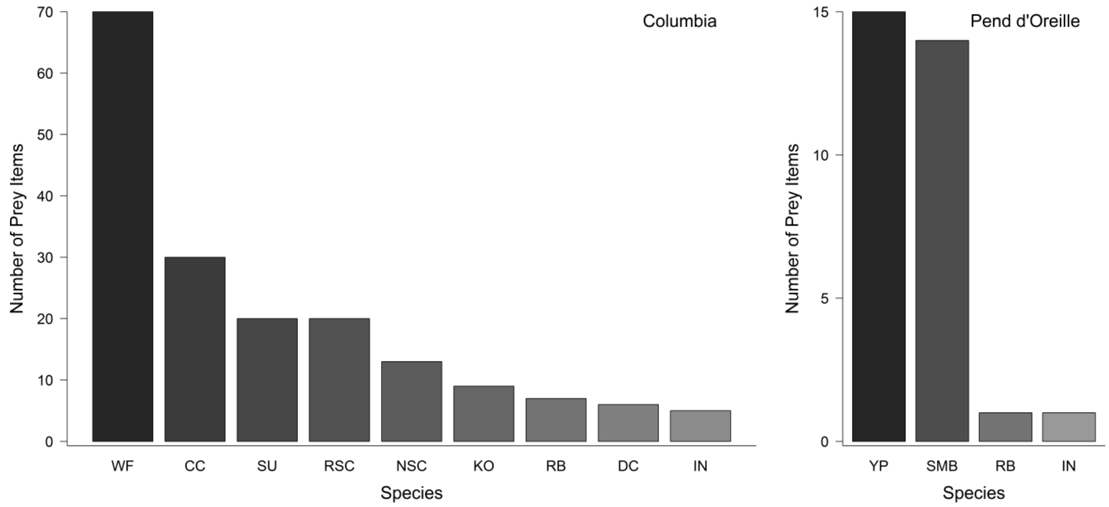

Figure 2.6. Prey items identified in stomachs of northern pike captured in the Columbia (n = 323) and Pend d'Oreille (n = 43) Rivers. Prey species in Columbia pike stomachs included whitefish spp. (WF), sculpin spp. (CC), sucker spp. (SU), redside shiner (RSC), northern pikeminnow (NSC), kokanee (KO), rainbow trout (RB), dace spp. (DC), and invertebrates. Pend d'Oreille northern pike stomachs contained yellow perch (YP), smallmouth bass (SMB), rainbow trout (RB) and invertebrates.

Similarly, 58% of Pend d'Oreille northern pike stomachs contained prey. The majority of prey items identified were yellow perch (n = 15) and smallmouth bass (n = 14), in addition to a single rainbow trout (n = 1) and leech spp. (n = 1) (Figure 2.6).

Northern pike caught in the Columbia River (n = 293) ranged from 260 mm to 1,050 mm in length and 200 g to 11,100 g in weight (Figure 2.7). The sample size analyzed was reduced from the number of pike caught by gill-netting (n = 323) as weight was not recorded for some individuals in the initial year. Pend d'Oreille pike (n = 43) ranged from 350 mm to 970 mm in length and 350 g to 7,000 g in weight (Figures 2.7, 2.8). Sexual maturity was observed in pike as small as 490 mm in the Columbia River and 500 mm in the Pend d'Oreille River.

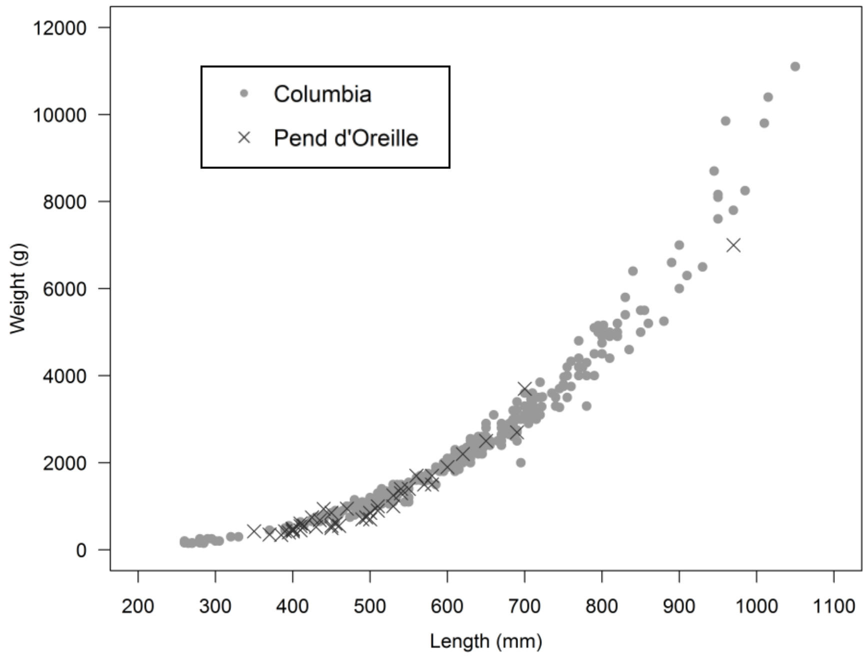

Figure 2.7. Length and weight of northern pike caught in the Lower Columbia (n = 293) and Pend d'Oreille (n = 43) Rivers in B.C. between May 2014 and September 2017.

Length-categorization of Columbia River northern pike (n = 293) yielded individuals in all five categories, with 76, 116, 83, 17, and 1 pike in the stock, quality, preferred, memorable, and trophy categories, respectively (Table 2.1).

Of these pike, 250 (85.3%) had relative weights equal to or greater than 100, with 30 of those individuals (10.2%) exceeding relative weights of 120 (Figure 2.9). Length categorization of Pend d'Oreille northern pike (n = 43) yielded 28, 12, 2, and 1 pike in the stock, quality, preferred, and memorable categories, respectively (Table 2.1). Nineteen of 43 (44.2%) pike

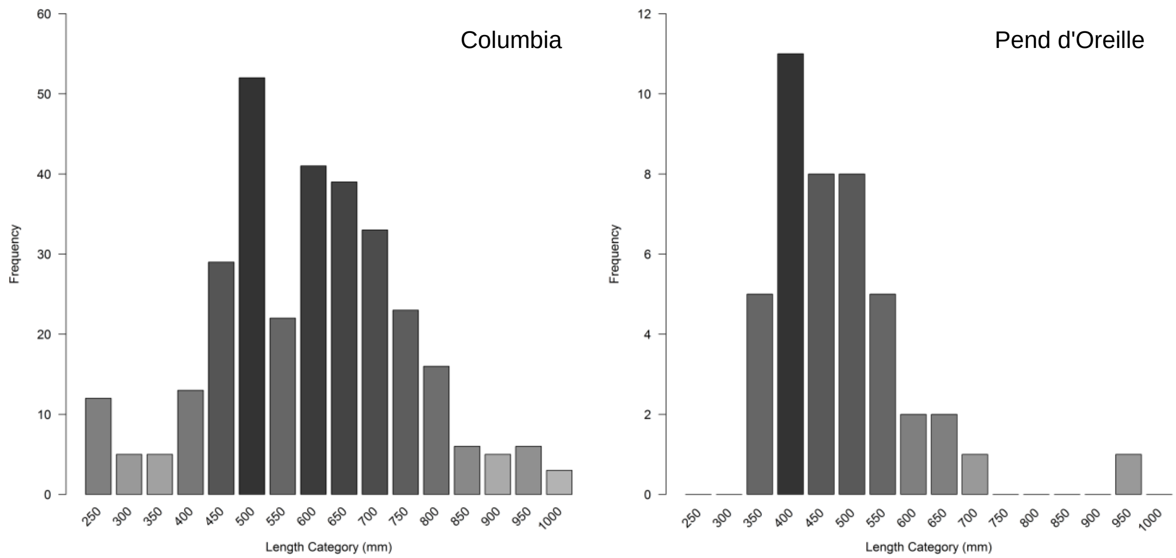

Figure 2. 8. Length-frequency of northern pike caught in the Lower Columbia (n = 293) and Pend d'Oreille (n = 43) Rivers in B.C. between May 2014 and September 2017.

Table 2. 1. Length Categorization for northern pike captured in the Columbia (n = 293) and Pend d'Oreille (n = 43) Rivers.

Length Category	Min Length (mm)	Columbia	Pend d'Oreille
Stock	336.6	76	28
Quality	562.6	116	12
Preferred	729.0	83	2
Memorable	897.9	17	1
Trophy	1082.0	1	0

captured in the Pend d'Oreille had relative weights equal to or greater than 100, with 4 individuals (9.3%) exceeding relative weights of 120 (Figure 2.9).

No significant difference in mean relative weight across length categories was observed for Columbia pike, therefore a mean W_r was calculated for the population as a whole (mean W_r = 109.6) (Figure 2.10). The mean relative weights of Pend d'Oreille pike within the stock category were significantly lower than the quality ($p = 0.003$) and preferred ($p = 0.017$) groups in the same river, therefore a mean W_r was not calculated for this population. As only a single pike in the memorable category was caught in the Pend d'Oreille River no population mean could be calculated.

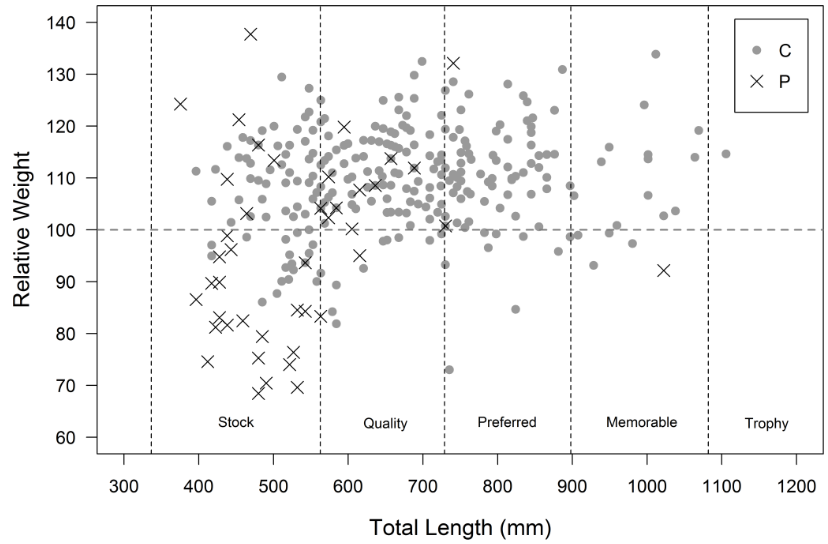

Figure 2.9. Relative weights calculated for northern pike captured in (C) the Columbia (n = 293) and (P) the Pend d'Oreille (n = 43) Rivers. Minimum stock (>336.6 mm), quality (>562.6 mm), preferred (>729.0 mm), memorable (>897.9 mm) and trophy (>1082.0 mm) lengths were selected based on percentages of northern pike world-record lengths reported in Gabelhouse (1984). The standard weight equation proposed by Willis (1989) was used in calculating relative weights: W_s = 3.096(Total Length) - 5.437.

A total of 77, 19, 6, 1, and 4 northern pike were estimated to be within the 1+, 2+, 3+, 4+, and 5+ age categories, respectively (Figure 2.11).Many of the samples presented easy to moderate interpretation problems for age estimates, potentially resulting in erroneous estimates. The external laboratory graded samples according to their characteristics (pattern clarity, repeatability) and found the majority (76%) to be fair in quality (estimations were within 1 year for most fish younger than 10 years), with the remainder graded as good quality (estimations would be the same for most fish younger than 10 years) (refer to Appendix A, Table A1 for grading characteristics).

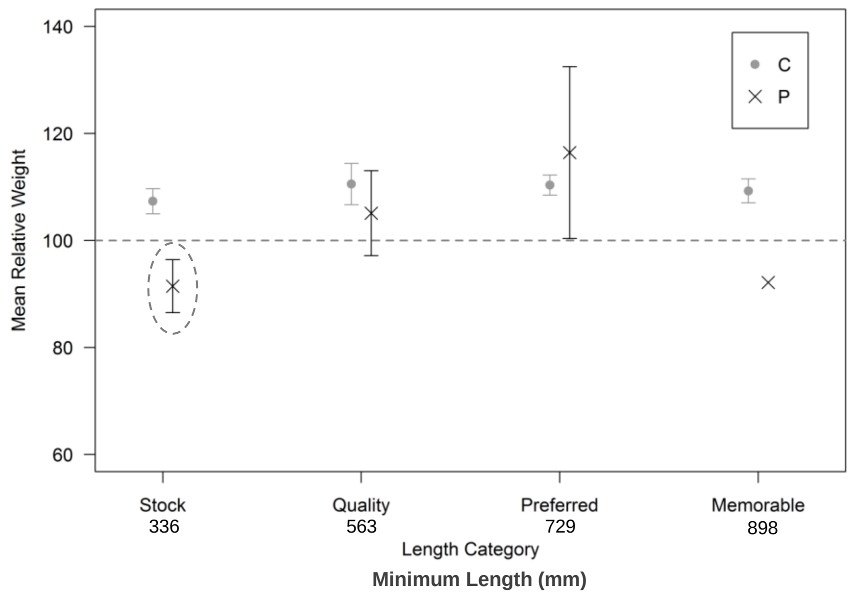

Figure 2.10. Mean relative weight (with 95% CI's) for individual length categories of northern pike captured in (C) the Columbia (n = 292) and (P) the Pend d'Oreille (n=43) Rivers. A total of 76, 116, 83, and 17 Columbia pike and 28, 12, 2, and 1 Pend d'Oreille pike represent each respective length category. As only a single pike in the memorable category was caught in the Pend d'Oreille River, no mean could be calculated. Mean relative weights of northern pike in the stock category (circled) were significantly lower than the quality ($p = 0.003$) and preferred ($p = 0.017$) groups in the Pend d'Oreille River, and the stock ($p<0.001$), quality ($p<0.001$), preferred ($p<0.001$) and memorable ($p<0.001$) groups in the Columbia River.

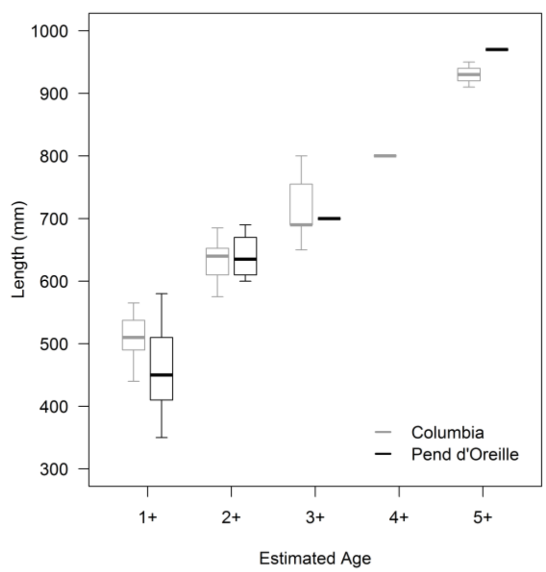

Figure 2.11. Northern pike cleithra age estimates and corresponding lengths for 108 pike captured in the Columbia and Pend d'Oreille Rivers.

Discussion

These results suggest gill-netting has been an effective means of reducing the number of adult northern pike in the LCR. The number of pike removed by gill-nets in 2017 (n = 35) was similar to 2016 (n = 39), yet considerably lower than the number removed in 2015 (n = 116) and 2014 (n = 133). The lower catch-rates in 2016 and 2017 (0.13 pike/hr and 0.05 pike/hr respectively) imply that the adult population is being controlled, a supposition supported by a basic mark-recapture population estimate conducted by Baxter and Lawrence (2018). A population estimate was generated during each year of the suppression program, which was based initially on the recapture of the pike caught and PIT-tagged for the provincial angler incentive program. In 2016 and 2017 the estimate incorporated an additional 15 pike that were tagged for an acoustic telemetry study (discussed in detail in Chapter 3) in spring 2016. Using a simple Lincoln-Petersen mark and recapture model they estimated the population to be at 725 individuals (95% CI, 478 - 2,759) in 2014, and in 2017 was estimated to have been reduced to 99 individuals (95% CI, 25 - 172). The authors identified violations of the Lincoln-Petersen mark-recapture model assumptions when generating these estimates, but were able to provide evidence of an overall reduction in adult pike numbers over the 4 years of suppression. The capture of juvenile life stages during the provincial suppression program indicate northern pike are successfully recruiting within the LCR and therefore also need to be controlled in subsequent years. High live release rates (75.4%) indicate the current gill-netting methods (≤ 4 hour sets, ≤ 4 m depth, close to shore, no overnight sets, continuous monitoring of nets) have been successful in minimizing impacts on native fish. A major concern of gill-netting in the LCR was the incidental capture of SARA-listed white sturgeon, 9 of which were encountered in gill-nets. All of these individuals were released unharmed, indicating the potential long-term impacts of pike gill-net suppression is minimal. The most vulnerable to the effects of gill-netting appears to be whitefish spp., comprising approximately 60% of total bycatch. Catch rates of whitefish spp. were highest for all seasons suggesting the presence of considerable habitat overlap with pike in the LCR. Golder (2014) reported LCR subadult mountain whitefish densities were greatest in the 10 km reach between HLK and the Kootenay River confluence, likely due to the presence of large bays and backwater areas which are preferred habitats for both whitefish and northern pike. This habitat overlap was also reflected in stomach contents of pike captured in this reach with 44% overall diet consisting of mountain and lake whitefish. Despite the initial impact on whitefish spp. in the LCR through high gill-net bycatch rates, the long-term effects of gill-netting may be offset by reduced pike predation. Spens and Ball (2008) suggest it is improbable northern pike can coexist with self-sustaining populations of salmonids, a suggestion that has been demonstrated in numerous invasive pike populations in south-central Alaska. These invasive populations were found to preferentially prey on juvenile salmonid species (Rutz 1999) and have been linked with significant declines in systems previously known to have abundant native and stocked anadromous salmonid populations (Haught and von Hippel 2011). Haught and von Hippel (2011) reported salmonids were a rare prey item in northern pike stomachs sampled from lakes in south-central Alaska post pike invasion, suggesting they are likely reduced in abundance or extirpated more rapidly than other native species. They also reported macroinvertebrates became a more important constituent of pike diet as years of pike residence increased and preferred prey items declined, with over 60% of stomach samples containing members of the odonates (dragonflies and damselflies) later in their study. Likely as a result of the Columbia River pike invasion being relatively recent (≈10 years) this has not yet been observed, with approximately 50% of Columbia River pike diets consisting of salmonid species (39% whitefish spp., 5% kokanee, 4% rainbow trout), with the remainder consisting of sculpin spp. (17%) sucker spp. (11%), redside shiner (11%), northern pikeminnow (7%), dace spp. (3%) and invertebrate spp. (3%). No SARA-listed species (Columbia sculpin, Umatilla dace, juvenile white sturgeon) were observed in pike stomachs. Other sculpin and dace spp. did, however, account for 20% of prey items and were mostly found in juvenile stomachs, suggesting these species may be a major constituent of juvenile pike diets in the Columbia. As juvenile northern pike were only captured in 2017 it is difficult to determine the extent of predation on sculpin and dace species, illustrating the need for future study of the juvenile pike cohort in the LCR.

Length and weight data collected through the suppression program suggest individuals from the Columbia population have higher than average body condition (overall mean W_r = 109.6). There are shortcomings, however, in the reliability of these values as sampling was conducted at different times of year in both systems over a 4 year period. Sampling effort was disproportionately higher in the spring during and before pike spawning when fish (particularly females) are in the highest condition (Pope and Willis 1996), potentially skewing the results. Additional sampling through all seasons is therefore needed to accurately investigate the seasonal dynamics of W_r for LCR northern pike. Nonetheless, approximately 85% of Columbia River pike had W_r values greater than 100 indicating the condition of this invasive population is equal to or greater than average populations. Numerous observed individuals in the "memorable" category (n=17) and one "trophy" classed northern pike also indicate current conditions in the LCR supports large northern pike that reach these sizes rapidly (5-6 years), yet these conditions will likely not persist as the LCR pike invasion is still in its infancy. By comparison, in the heavily invaded Pend d'Oreille system large pike are relatively rare, highlighting the long-term disincentive for intentional introductions. Pike as small as 490 mm in the LCR and 500 mm in the Pend d'Oreille were found to be sexually mature indicating spawning is occurring in 1+ year-old fish, which is consistent with other warm-water populations in some southern U.S. reservoirs (Crabtree 1969, Schryer *et al.* 1971; Vasey 1974). This underscores the necessity for controlling the juvenile cohort in order to prevent spawning in the first year.

Gill-netting and boat surveys in the Pend d'Oreille River led to the capture of 43 northern pike and the identification of several potential habitats in both B.C. reservoirs, with most effort focused in the Waneta Reservoir due to its proximity to the Columbia River. The majority (80%) of gill-net sampling in the Waneta Reservoir was conducted in the summer in shallow, heavily vegetated areas that likely served as nursery habitat for smaller pike. All pike captured in these locations were under 580 mm. Subsequent spring sampling was conducted at the same locations resulting in the capture of six pike in excess of 600 mm, all of which were sexually mature. Three of these fish were captured less than 1.5 km upstream of the Waneta Dam at the confluence of the Columbia River, highlighting the proximity of reproducing northern pike to the Columbia River confluence and the potential contribution this may have made to the Columbia invasion. No juvenile northern pike were captured in either reservoir as the gill-nets used were of the same specification as the LCR suppression program targeting adult pike (i.e., mesh sizes were too large).

Native bycatch in the Pend d'Oreille included sucker spp. (35.4%), northern pikeminnow (9.7%) and a single mountain whitefish (0.005%), with a live release rate of 85.1% indicating the impacts of gill-net suppression on native fish communities was minimal. It is worth noting that over half (54.4%) of all fish captured by gill-netting in the Pend d'Oreille were invasive species and only a single salmonid (mountain whitefish) was encountered. Sanderson *et al.* (2009) suggest the effect of nonindigenous species on salmonids could equal or exceed that of habitat alteration, harvest, hatcheries, and the hydrosystem, and that managing these species is a top priority for recovery. The capture of only a single salmonid in the Pend d'Oreille during this study his highlights the negative impacts of biological invasions on river systems such as the Pend d'Oreille, which was a historically productive salmonid fishery in southern B.C. (Nellestijn and Ells 2008).

While stomach samples were not available from pike captured throughout each season, the results suggest that yellow perch (48.4%) and smallmouth bass (45.2%) likely comprise the majority of pike diets in the Pend d'Oreille. Interestingly, only a single invertebrate (leech spp.) was observed in stomach samples from the Pend d'Oreille, suggesting prey fish densities are sufficiently high that pike do not target macroinvertebrates as seen in invasive Alaskan populations (Haught and von Hippel 2011). The body condition of stock category northern pike (336.6 mm - 562.6 mm) in the Pend d'Oreille River was significantly lower than other categories ($p = 0.003$ quality, $p = 0.017$ preferred) which may be partly due to diet composition or prey availability in the Pend d'Oreille paired with high competition for habitat and food resources from other invasive fish species. Northern pike are known to exhibit high variability in growth rates both within and among populations and can vary considerably within a single cohort (Inskip 1982). Carbine (1945) reported stocked northern pike fry in the first 17 months of growth varied from 8.3 to 44.6 cm in length (cited in Inskip 1982). Limited habitat and high competition with other invasive species in the Pend d'Oreille is likely a contributing factor to the high variation in 1+ year northern pike, which ranged from 350 mm to 580 mm in length. Age estimates of Pend d'Oreille pike indicate similar growth rates to the LCR population despite differences in body condition, yet with so few large pike captured (6 pike ≥ 600 mm) it is difficult to compare large fish between populations. The small sample size ($n = 43$) paired with disproportionally high sampling effort in the summer also makes it difficult to determine the condition of Pend d'Oreille pike within the other size categories, yet the results of this study suggest this system is not as productive for pike as the LCR. This study also highlights that despite differences in diet and competition pike can survive in both heavily and minimally invaded territory.

Conclusions

Reductions in pike captures since the initial year of the suppression program suggest gill-netting is a viable technique for suppressing northern pike in the LCR, and continuation of the program is advisable to keep the LCR population under control. With these reductions in catch rates, however, future consideration will have to be given to the amount of net effort conducted annually so as to limit the impact of gill-netting on native fish communities in the LCR while still effectively removing a maximum number of pike. Juvenile habitat surveys, combined with juvenile gill-net suppression is needed to control the juvenile cohort in future years. Gill-net suppression of northern pike should also be expanded to both B.C. reservoirs of the Pend d'Oreille River to reduce the influx of not only northern pike, but other invasive fish species present.

Literature Cited

Anderson, R.O., Neumann, R.M. (1996). Length, weight, and associated structural indices. Pages 447 - 482 in B. R. Murphy and D. W. Willis, editors. Fisheries techniques, 2nd edition. American Fisheries Society, Bethesda, Maryland.

Baxter, J.T.A., Lawrence, C. (2018). Lower Columbia River invasive northern pike suppression - 2017 Update. Prepared for Teck, Trail, B.C. 16 p.

Baxter J.T.A., Neufeld, M. (2015). Lower Columbia River invasive northern pike suppression and stomach Analysis – 2014. Mountain Water Research, Silverton, B.C. and Ministry of Forest, Lands and Natural Resource Operations, Nelson B.C. Prepared for Teck Metals Ltd., Trail B.C.

Bean, N. (2014). Kalispel non-native fish suppression project. 2014 Annual Report. Report. Bonneville Power Administration Project 2007-149-00.

Blackwell, B.G., Brown, M.L., Willis, D.W. (2000). Relative weight (W_r) status and current use in fisheries assessment and management. *Reviews in Fisheries Science*, 8(1): 1 - 44.

Bradford, M.J., Tovey, C.P., Herborg, L.M. (2008). Biological Risk Assessment for Northern Pike (*Esox lucius*), Pumpkinseed (*Lepomis gibbosus*), and Walleye (*Sander vitreus*) in British Columbia. Fisheries and Oceans Canada.

Brown, C. J. D. (1971). Fishes of Montana. Big Sky Books, Bozeman, MT.

Bystrom, P., Karlsson, J., Nilsson, P., Kooten, T.V., Ask, J., Olofsson, F. (2007). Substitution of top predators: effects of pike invasion in a subarctic lake. *Freshwater Biology*, 52(7): 1271 - 1280.

Carbine, W. F. (1945). Growth potential of the northern pike (*Esox lucius*). *Papers of the Michigan Academy of Science, Arts and Letters*, 30(1944): 205 - 220.

Crabtree, J. E. (1969). Region 1A fisheries studies. Walleye and northern pike study. Dingell - Johnson Project. F-7-R-17. Job 17. Progress report. Texas Parks Wildlife Department, 12 p.

Dauble, D. (2009). Fishes of the Columbia Basin. Keokee Co Pub Inc., Sandpoint Idaho.

Department of Fisheries and Oceans, Canada [Internet]. (2019). Aquatic species at risk. Accessed from: http://www.dfo-mpo.gc.ca/species-especes/sara-lep/index-eng.html

Eklöv, P., Hamrin, S. (1989). Predatory efficiency and prey selection: interactions between pike *Esox lucius*, perch *Perca fluviatilis* and rudd *Scardinus erythrophthalmus*. *Oikos*, 56: 149 - 156.

Euchner, R.B. (1988). Collection, preparation and use of northern pike (*Esox lucius*) cleithra for age determination. Ministry of Environment and Parks Recreational Fisheries Branch. Report No. PCE. 20.

Fisher, S. J., Fielder, D.G. (1998). A standard weight equation to assess the condition of North American lake herring (*Coregonus artedi*). *Journal of Freshwater Ecology*, 13: 269 - 277.

Gabelhouse, D.W. Jr. (1984). A length-categorization system to assess fish stocks. *North American Journal of Fisheries Management,* 4: 273 - 285.

Golder Associates Ltd. and Poisson Consulting Ltd. (2014). CLBMON-45 Lower Columbia River fish population indexing surveys – 2013 investigations. Report prepared for BC Hydro Generations, Water License Requirements, Castlegar, BC. Golder Report No. 1014920102-R-Rev0: 65 p. + 8 app.

Harvey, S., Bean, N. (2016). Kalispel non-native fish suppression project. 2015 Annual report. Bonneville Power Administration Project 2007-1 49-00.

Harvey, B. (2009). A biological synopsis of northern pike (*Esox lucius*). *Canadian Manuscript Report of Fisheries and Aquatic Sciences* 2885: v + 31 p.

Haught, S., von Hippel, F.A. (2011). Invasive pike establishment in Cook Inlet Basin lakes, Alaska: diet, native fish abundance and lake environment. *Biological Invasions* 13: 2103 - 2114.

HTI (Hydroacoustic Technology, Inc) (2009). Boundary hydroelectric project (FERC No. 2144), study No. 12 fish entrainment and habitat connectivity study. Final report prepared for Seattle City Light. 114 p + 4 App.

Inskip, P.D. (1982). Habitat suitability index models: northern pike. U.S. Department of the Interior, Fish and Wildlife Service, FWS/OBS-82/10.17. 40 p.

Johnson, B.M., Martinez, P.J., Hawkins, J.A., Bestgen, K.R. (2008). Ranking predatory threats by nonnative fishes in the Yampa River, Colorado, via bioenergetics modeling. *North American Journal of Fisheries Management*, 28: 1941 - 1953.

King, L., Lee, C. (2016). Evaluation of Northern Pike *Esox lucius* in Upper Lake Roosevelt and the Lower Kettle River, Washington, February 2016. Report to the Spokane Tribe, for Bonneville Power Administration Project No. 1994-043-00.

Malette, M. D., Morgan, G. E. (2005). Provincial summary of northern pike life history characteristics based on Ontario's fall walleye index netting (FWIN) program 1993 to 2002. Publication of the Cooperative Freshwater Ecology Unit, Department of Biology, Laurentian University, Sudbury, Ontario.

McMahon, T.E., Bennett. D.H. (1996). Walleye and northern pike: boost or bane to northwest fisheries? *Fisheries,* 21(8): 6 - 13.

Murphy, B.R., Willis, D.W., Springer, T. A. (1991). The relative weight index in fisheries management: status and needs. *Fisheries*, 16(2): 30 - 38.

Nellestijn, G., Ells, B. (2008). A History of the Salmo River Watershed: An overview of events that have had an effect on our river ecology. Report written for the Salmo Watershed Streamkeepers Society, Department of Fisheries and Oceans, Canada.

Patankar, R., von Hippel, F., Bell, M. (2006). Extinction of a weakly armoured threespine stickleback (*Gasterosteus aculeatus*) population in Prator Lake, Alaska. *Ecology of Freshwater Fish,* 15: 482 - 487.

Pope, K.L. Willis, D.W. (1996). Seasonal influences on freshwater fisheries sampling data. *Reviews in Fisheries Science*, 4: 57 - 73.

Rutz, D.S. (1999) Movements, food availability and stomach contents of northern pike in selected Susitna River drainages, 1996–1997. Alaska Department of Fish and Game, Fishery Data Series, No. 99 - 5.

Sanderson, B.L., Barnas, K.A., Rub, M.W. (2009). Nonindigenous Species of the Pacific Northwest: An Overlooked Risk to Endangered Salmon? *Bioscience*, 59(3): 245 - 256.

Sandlund O.T., Museth J., Øistad, S. (2016). Migration, growth patterns, and diet of pike (*Esox lucius*) in a river reservoir and its inflowing river. *Fisheries Research,* 173(1): 53 - 60.

Schryer, F., Ebert, V., Dowlin, L. (1971). Statewide fisheries surveys. Determination of conditions under which northern pike spawn naturally in Kansas reservoirs. Dingell-Johnson Project F-15-R-6, Job C-3. Final Report, Kansas Records of the Forestry, Fish and Game Commission, 37 p.

Scott, W.B., Crossman, E.J. (1973). Freshwater Fishes of Canada. Bulletin 184. Fisheries Research Board of Canada, 966 p.

Spens, J., Ball, J. (2008). Salmonid or nonsalmonid lakes: predicting the fate of northern boreal fish communities with hierarchical filters relating to a keystone piscivore. *Canadian Journal of Fisheries and Aquatic Sciences*, 65:1945 - 1955.

Vasey, F.W. (1974). Life history of introduced northern pike in Thomas Hill Reservoir. Dingell - Johnson Project F-1-R-23, Study 1-5, Job 2. Final Report. Missouri Department of Conservation,14 p.

Wege, G. J., Anderson, R.O. (1978). Relative weight (W_r): a new index of condition for largemouth bass. Pages 79 - 91 in G. Novinger and J. Dillard, editors. New approaches to the management of small impoundments. American Fisheries Society, North Central Division, Special Publication 5, Bethesda, Maryland.

Willis, D.W., Scalet, C.G. (1989). Relation between proportional stock density and growth and condition of northern pike populations. North American Journal of Fisheries Management, 9: 488-492.

Chapter 3: Investigating movements and geographic life history of invasive northern pike in the B.C. Lower Columbia River using acoustic telemetry and otolith microchemical analysis.

keywords: *Esox lucius*, northern pike, invasive fish, acoustic telemetry, otolith microchemistry

Abstract

Northern pike (*Esox lucius*) is a widely distributed freshwater predator in the northern hemisphere that has gained notoriety in recent years as a problematic invasive species, most recently in the Columbia River. Since initial detection in 2007, pike have spread throughout approximately 275 km of the Columbia River between the Grand Coulee Dam in Washington and the Hugh L. Keenleyside (HLK) Dam in British Columbia and threaten further expansion. The section of the Columbia River in B.C. currently inhabited by pike extends approximately 56 km from the confluence of the Pend d'Oreille River to the Hugh L. Keenleyside Dam near Castlegar. Both acoustic telemetry and otolith microchemical analysis were employed to investigate the movement and behaviour of pike from within this reach, and to determine the natal origin of individuals captured in the Columbia River. Fifteen mature northern pike were tagged with acoustic transmitters prior to spawning in 2016 and tracked for 16 months. Fourteen of 15 (93.3%) northern pike remained within a 10.5 km home range, with six of those (37.5%) remaining within 4 km. One individual migrated in excess of 50 km downstream to Washington where it was undetected for approximately 6 months before returning to its capture location near Castlegar. Microchemical analysis of otoliths from pike captured in the Columbia ($n = 50$) and Pend d'Oreille ($n = 10$) Rivers revealed otolith Sr:Ca ratios (measured at the outer surface of the otolith) were significantly higher in Columbia River pike with overall means of 556.5 µmol/mol compared to 251.3 µmol/mol in Pend d'Oreille River pike ($t = 6.581$, $p < 0.0001$). Conversely, otolith Ba:Ca ratios of Columbia River pike were significantly lower than Pend d'Oreille River pike with overall means of 3.61 µmol/mol and 8.33 µmol/mol, respectively ($t = -3.690$, $p = 0.0045$). All but one pike analyzed (98%) appeared to have originated in the Columbia River, with the exception appearing to have moved from the Pend d'Oreille to the Columbia in its second

year of growth. This study provides the first evidence of pike movement from the Pend d'Oreille into the Columbia River, in addition to indicating a proportion (albeit small) of pike in the B.C. Columbia River exhibit long-range migratory behaviour which has implications for management of this introduced apex predator.

Introduction

Northern pike have recently colonized the Columbia River, the largest river system in the Pacific Northwest, sparking concern in both the province of B.C. and Washington state as to the potential impacts on native fisheries. The suspected source of the invasion is the Pend Oreille River, which flows approximately 200 km from Lake Pend Oreille in Idaho to its confluence with the Columbia in B.C. at the Canada-U.S. border. Extensive hydroelectric development on the Pend d'Oreille/Pend Oreille has caused a shift from lotic to lentic conditions in the resultant reservoirs, significantly altering native fish community structure and facilitating the colonization of a variety of introduced non-native species. Many of these species are now present in the Columbia River due to either movement through the Waneta Dam or illegal introduction, although it is unclear whether the Pend d'Oreille is a significant source of northern pike for the B.C. section of the Columbia. Two hydroelectric facilities reside in the B.C. portion of the Pend d'Oreille River: the Seven Mile and Waneta dams, the latter of which is the last downstream dam on the Pend d'Oreille immediately upstream of the U.S. border (Figure 3.1).

Pike are a voracious freshwater predator and can impose significant trophic effects on native fish species through predation and competition for resources, the impacts of which are widely documented in the literature (Scott and Crossman 1973, Patankar *et al*. 2006, Bystrom *et al.* 2007, Johnson *et al*. 2008, Spens and Ball 2008, Haught and von Hippel 2011, Hesthagen *et al.* 2015, Sandlund *et al.* 2016). Pike are a highly piscivorous species, yet can be opportunistic feeders with prey including invertebrates, frogs, crayfish, mice, muskrat, and waterfowl up to one half their body length (Scott and Crossman 1973), and are known to become cannibalistic at sizes as small as 21 mm when preferred prey are at low densities (Eklöv and Hamrin 1989). Pike are generally associated with the near-shore zone in shallow (< 4 m) and moderately productive waters dominated by flooded vegetation (Harvey 2009);

however, pike are highly adaptable and can tolerate a wide range of environmental conditions (Casselman and Lewis 1996). Although they are not adapted for life in strong currents (Inskip 1982) pike can be found in rivers, generally seeking out shallow side channels, sloughs and other backwaters (Harvey 2009). Spawning occurs in the spring following freshet when water temperatures have increased to 8-12°C (Casselman and Lewis 1996). Optimal spawning habitat for northern pike is within grasses and sedges in shallow and sheltered areas which serve to suspend eggs in oxygenated waters above anoxic organic sediments (Casselman and Lewis 1996), and lack of suitable vegetation can inhibit spawning (Inskip 1982).

Northern pike were first detected in the B.C. portion of the Columbia River in 2009, downstream of the Hugh L. Keenleyside Dam (HLK) near Castlegar. This stretch of the Columbia River in B.C. flows approximately 56 km from HLK Dam to the Canada-U.S. border where the Pend d'Oreille drains into the Columbia (Figure 3.1). Although pike have been reported throughout this stretch of river, there is limited habitat for pike as much of the river between HLK and the border is fast-flowing with a rocky or sandy shoreline. Most suitable pike habitat exists near Castlegar where lentic conditions downstream of HLK and the abundance of prey likely facilitated their establishment and concentrated numbers. One of the major concerns with the Castlegar pike population is its proximity to HLK Dam, which houses a navigation lock allowing access to and from the Arrow Lakes Reservoir above the dam. The lock opens on a daily basis for both commercial and pleasure craft and is often left open for extended periods of time. The Arrow Lakes extend approximately 230 km from HLK Dam north to Revelstoke and makes up an important sport fishery for the province, and potential fish movement through the lock and colonization of the reservoir could have significant long term implications.

This research aimed to assist the B.C. provincial suppression program with the following questions in mind: (1) is the Pend d'Oreille River a significant input of pike into the Columbia? (2) are pike from within the Castlegar population spawning and overwintering in the area? (3) are pike passing through the navigation lock at HLK? (4) where are pike spawning? A multifaceted approach was taken to answer these questions, using both physical tracking of individual fish using implanted transmitters to investigate movements within the

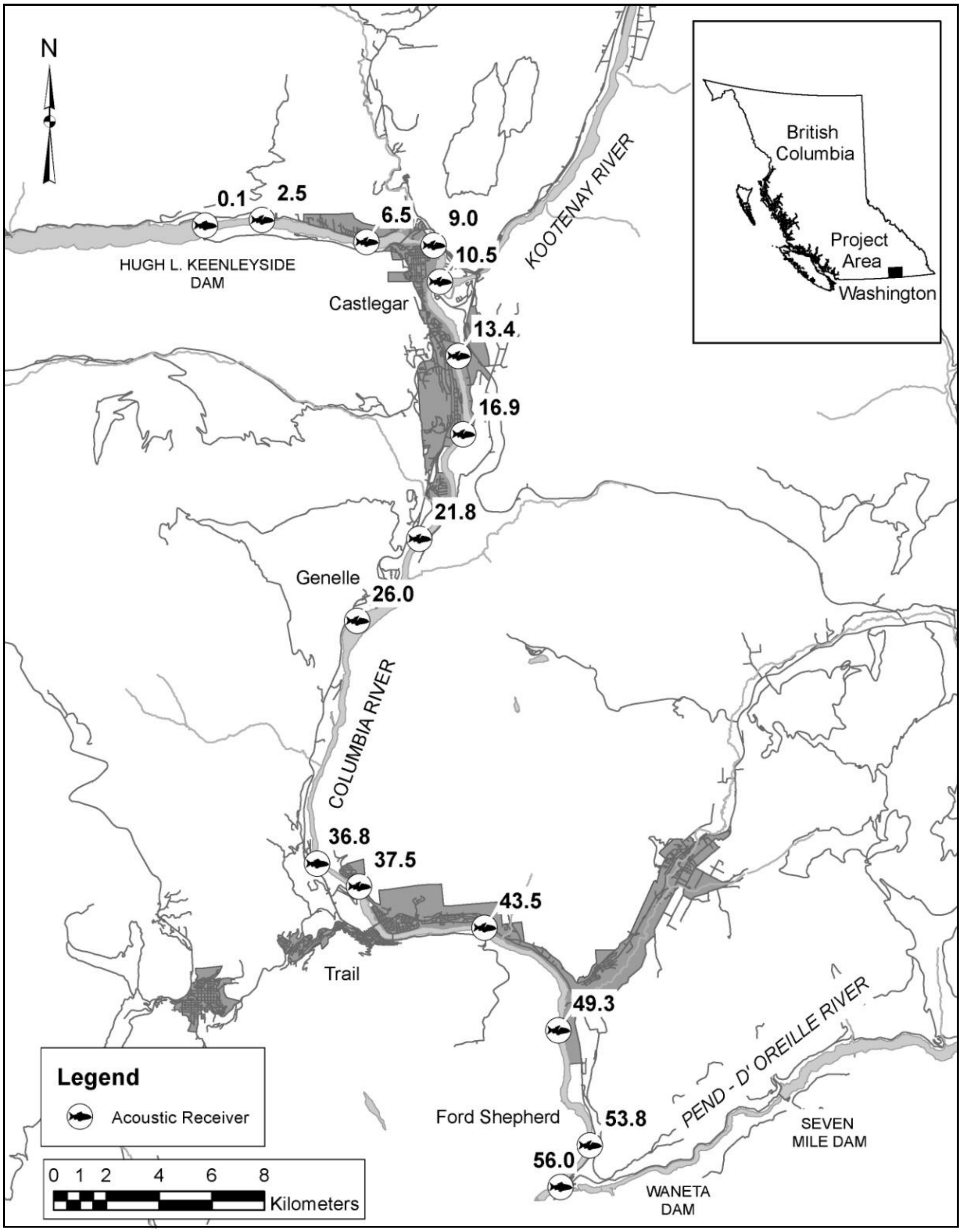

Figure 3.1. B.C. Lower Columbia River (LCR) downstream of the Hugh L. Keenleyside Dam (HLK). Numeric labels are the river kilometer downstream of HLK where B.C. Hydro-operated acoustic receivers are stationed.

Castlegar population, and microchemical analysis of otoliths to attempt to retrace the geographic life history of pike captured through the provincial suppression program.

Otoliths are paired calcified structures within the inner ear of all teleost fish that aid in hearing and orientation, formed by continuous crystallization of endolymphatic fluid (Bath *et al.* 2000). Fish endolymph contains bicarbonate, calcium, and trace elements that are mainly derived from the environment via branchial uptake (Farrell and Campana 1996, Bath *et al.* 2000), providing a life-long chemical record of ambient water conditions deposited within the otoliths (Thorrold *et al.*, 1997). The calcium carbonate structure of the otolith is also acellular and metabolically inert, permanently retaining trace elements and compounds incorporated into the matrix throughout the entire life of the fish (Campana 1999). This property allows researchers to investigate changes in environmental conditions a fish has experienced from the point of natal origin to time of death. This information can be used to identify the location of natal origin (Clarke *et. al* 2005, Engstedt *et al.* 2010) and movements of individual fish (Clarke *et al.* 2007, Shrimpton *et al.* 2014) if sufficient chemical differentiation exists between areas. Otolith elemental composition was analyzed using laser ablation inductively-coupled plasma mass spectrometry (LA-ICP-MS) to determine if pike captured in the Columbia and Pend d'Oreille rivers had identifiable elemental signatures to discriminate between populations, and if any pike captured through the Columbia River pike suppression program had moved from the Pend d'Oreille River.

To track individual movements of pike, acoustic transmitters were selected for use due to the existing array of acoustic receivers in the lower Columbia River operated by BC Hydro for white sturgeon recovery work. Acoustic receivers in the Columbia are fixed to anchored buoys at regular increments downstream of the HLK Dam to the U.S. border (Figure 3.1), as well as upstream of the dam in the Arrow Lakes Reservoir allowing us to observe any movement of pike through the navigation lock. Passive acoustic telemetry such as this was ideal as it requires limited labour once tags are deployed and an array of receivers is established, and allows for continuous automated data collection over long periods of time until movement data are downloaded.

Methods

Acoustic Telemetry

To monitor the movement of pike from within the Castlegar population, 15 mature pre-spawn pike were tagged with acoustic transmitters (Vemco V13, 69 kHz, 140s pulse rate, 11 g total weight) and monitored between May 2016 and September 2017. Movements were recorded using a previously deployed array of acoustic receivers (Vemco VR2W, 69 kHz) managed by BC Hydro, with receivers at regular increments downstream of HLK to the U.S. border (Figure 3.1). Northern pike were captured by gill-netting in early May, 2016 when water temperatures were approaching 8 ^{o}C downstream of HLK in areas of known pike abundance (near river kilometer 6.5) as surveyed by the provincial suppression team in previous years (Baxter and Neufeld 2015; Baxter 2016). The gill-nets used consisted of 5 panels each measuring 9.1 m in length by 1.8 m deep, with 2.5 cm, 3.2 cm, 3.8 cm, 4.5 cm, and 5 cm mesh sizes. Depth of sets ranged between 0.5 m and 4.0 m; no deeper sets were deployed due to concern over incidental capture of white sturgeon, a SARA listed species in the Columbia River. Nets were set for no longer than 145 minutes and continually monitored to limit bycatch and stress to pike prior to implantation of transmitters. Pike were assessed for sex, maturity, length, and overall body condition (i.e., physical injury from nets) upon capture, and 15 mature pre-spawn pike (9 male, 6 female) were selected for tagging with acoustic transmitters. Fish weight was not measured to reduce handling stress prior to surgery; approximate weights of fish were calculated using the length-weight relationship of Columbia River pike ($W = 0.1017e^{0.0478(FL)}$) reported by Baxter (2016). Following Vemco recommendations all tagged pike exceeded the minimum weight requirement (550 g, tag weight < 5% body weight). Table 3.1 summarizes data collected from the tagged fish.

Pike were anaesthetized for the tagging procedure using an 80 ppm eugenol (clove oil) solution (1:9 in 95% EtOH) in a 50 L plastic holding tank. Stage III of anesthesia (loss of gross body movement, cessation of opercular movement) was reached in most cases within 150 seconds, and no fish were exposed to the anesthetic longer than 200 seconds. Once anesthetized, fish were transferred to a surgery trough lined with foam and covered with sterile plastic sheeting to minimize mucous loss, and the gills irrigated using a hand pump

Table 3.1. Tag number, length, estimated weight and sex recorded for all northern pike tagged with Vemco V13 acoustic transmitters (69kHz, 140s pulse rate, 11g total weight). The length-weight relationship used to calculate weight estimates was reported in Baxter (2016): $W = 0.1017e^{0.0478(FL)}$.

Tag Number	**Length (cm)**	**Est. Weight (kg)**	**Sex**
A69-1601-54266	68	2.62	Male
A69-1601-54267	67	2.50	Male
A69-1601-54268	90	7.51	Female
A69-1601-54269	73	3.33	Male
A69-1601-54270	70	2.89	Male
A69-1601-54271	98	11.01	Female
A69-1601-54272	58	1.63	Male
A69-1601-54273	63	2.07	Male
A69-1601-54274	64	2.17	Male
A69-1601-54275	75	3.67	Female
A69-1601-54276	63	2.07	Male
A69-1601-54277	66	2.38	Female
A69-1601-54278	63	2.07	Female
A69-1601-54279	56	1.48	Male
A69-1601-54280	74	3.50	Female

throughout the process. An approximate 3 cm incision was made in the body cavity of each fish between the anus and pectoral girdle, slightly offset to minimize irritation of the wound on the river bottom during recovery. Acoustic transmitters were implanted into the body cavity, and the incisions were closed with 2 dissolving sutures. Surgeries were completed within an average time of 180 seconds with none exceeding 250 seconds. Tagged fish were marked with numbered Floy tags for identification and placed in a 50 L recovery tank filled with fresh water to monitor physical condition for approximately 30 minutes prior to release.

Acoustic receivers were downloaded via Vemco's VUE Software (version 2.2.7), and the raw detection data transferred into R (version 3.4.0) for analysis. Detection data was converted into residence events to determine the proportion of time tagged fish spent near each receiver within the array. A residence event was initiated if a transmitter was detected more than once at a receiver and terminated if the transmitter was detected by another receiver or not detected for more than 12 consecutive hours. All data analysis was performed in R using the VTrack software package.

Otolith Microchemistry

Otoliths from 60 Northern Pike caught in the Columbia (n = 50) and Pend d'Oreille rivers (n = 10) were used for microchemical analysis. Columbia River pike captured in Castlegar (2015) were provided through the provincial pike suppression program, and pike collected from the Pend d'Oreille River were gill-netted in both Seven Mile (n = 5) and Waneta (n = 5) reservoirs between June and August, 2016. Otoliths were removed and cleaned with deionized water and air dried prior to storage in plastic vials until preparation. Otoliths were placed sulcus-side down in a plastic mold using fine forceps, and covered with epoxy resin (Buehler Epoxy-Cure 2 Resin). The otoliths were turned over to ensure no air bubbles were trapped on the underside and cured for at least 8 hours. Otoliths were removed from the molds once the epoxy was cured, labeled and then polished to expose the core. Waterproof adhesive-backed lapping papers with 320, 600 and 1200 grit sizes (Allied, Silicon Carbide) were mounted on a plastic tray, and the samples were polished using a smooth circular hand motion on the wetted grit paper beginning with the 320 grit size. When the core appeared to be near, 600 grit paper was used to reach it followed by 1200 grit paper for smoothing. The samples were rinsed in distilled water between each size of grit paper to prevent the transfer of debris. Once the core was reached samples were rinsed with deionized water and ultrasonically cleansed for 2 minutes to remove all loose particles. Otoliths were then polished using 0.25 μm diamond suspension spray (Buehler, Metadi Supreme) on 2500 grit polishing pads (Allied, PanB) for approximately 10 minutes to achieve a highly polished surface. Lastly, the samples were rinsed and ultrasonically cleansed in deionized water for 1 minute and allowed to dry prior to being analyzed.

Northern pike otoliths were analyzed using laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS) with a Thermo X-Series 2 Quadrupole ICP-MS (Thermo Electron Corporation) coupled to a New Wave UP-213 UV laser ablation system. All otolith line scans were conducted from left to right across the sample with the rostrum oriented upwards (spot diameter of 15 µm). PlasmaLab (ver. 2.6.1.335, Thermo Electron 2011) software was used for all data collection and reduction. Elements measured included Ca, Sr, Ba, Mg, and Mn, and the limit of detection for each element was determined using certified National Institute of Standards and Technology (NIST) reference material prior to analysis. Otolith sample preparation and LA-ICP-MS was conducted at TrichAnalytics Inc. (North Saanich, B.C.).

Duplicate water samples were collected in the Columbia (n = 2) and both Waneta (n = 1) and Seven Mile (n = 1) reservoirs of the Pend d'Oreille where pike used for otolith analysis were captured. Columbia River northern pike otoliths used for analysis were harvested from fish captured in May 2015; however, water samples were not taken until the following year in May, 2016. Water samples taken from the Pend d'Oreille were collected in June 2016. Samples were collected following a syringe filtration method for trace element sampling published by Shiller (2003) with minor modifications suggested by Clarke *et al.* (2007). Fifty mL high-density polyethylene and 50 mL polyethylene/polypropylene syringes were rinsed with ultrapure water and filled with a 2% (v/v) high purity nitric acid solution for 10 days. Bottles and syringes were rinsed 5 times each with ultrapure water and left to dry in a laminar flow hood. Syringe filters were cleaned by running 40 mL of a 2% high-purity nitric acid solution through each filter followed by 20 mL of ultrapure water. Filters were then dried using high-pressure clean air and stored in a laminar flow hood until packaging. Field sampling kits were packaged by placing 2 bottles, 2 syringes, and 2 syringe filters in a sterile powder free nitrile glove, which was tied at the end and placed into a Ziploc bag until use. Duplicate 40 mL water samples were taken from each location and acidified immediately using a 2% solution of high purity nitric acid (800 µL HNO_3 per 40 mL sample). Water samples were analyzed using inductively coupled plasma optical omission spectrometry (ICP-OES) at an external lab (Activation Laboratories Ltd., Kamloops, B.C.), targeting all elements measured in northern pike otoliths.

Results

Acoustic Telemetry

A total of 9 transmitters were still active when last downloaded in September 2017; 3 tagged pike (transmitter 54266, 54277, 54279) were recorded as mortalities by members of the

Columbia River pike suppression team, 2 (54275, 54280) reported by anglers, and 1 unknown termination (54267) (Appendix B, Figure B1). Tagged pike were limited to a home range of less than 10.5 km in the Castlegar area (4 within 4 km, 5 within 6.5 km, 2 within 9 km, and 2 within 10.5 km) with the exception of one fish (54273). The receiver at RKM 6.5 was the highest activity area with approximately 77% of all logged residence time, followed by 20% at RKM 2.5 and approximately 1% or less each at RKM's 0.1, 9.0, and 10.5. Detections of tagged pike at RKM 9.0 were logged only in the spring and early summer of both 2016 and 2017, and detections at RKM 10.5 were limited to the spring and summer of 2017. Detections at RKM 0.1 (HLK Dam) were infrequent throughout the study and no residence events exceeding 1 day were recorded. The proportion of total residence time calculated for each receiver in the Castlegar area is displayed in Figure 3.2 (refer to Appendix B, Figure B2 for residence events over time at each receiver).

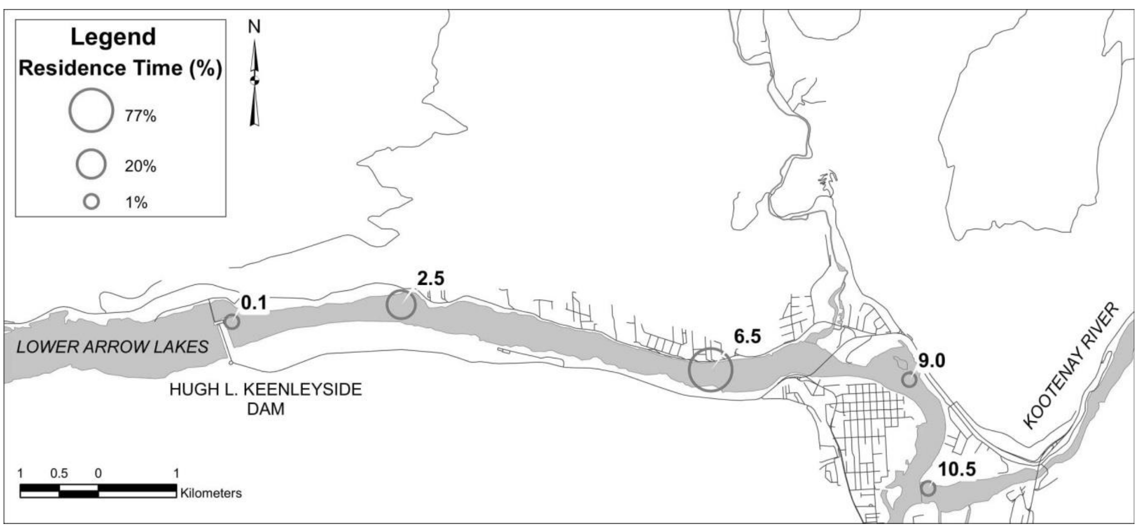

Figure 3.2. Total proportion of residence time logged by each VR2W acoustic receiver in the Castlegar area for tagged northern pike between May 2016 and September 2017. All but one tagged fish (transmitter 54273) stayed within this area for the duration of the study. Numeric labels are the river kilometer at which acoustic receivers are stationed downstream of HLK Dam.

Transmitter ID 54273 (63 cm, male) was the only fish detected outside of the Castlegar area (RKM 0.1 - RKM 10.5) between May, 2015 and September, 2017 (Figure 3.3). This pike stayed within a limited home range (approx. 4 km) for 9 months before beginning its migration south over 50 km into the U.S. in February, 2017. On February 2 (0214), pike 54273 began its downstream migration from RKM 6.5, reaching RKM 16.9 in just over 18 hours (2022) where it stayed until February 5 (1826). Pike 54273 was next detected at RKM 21.8 on February 8 (0116), followed by RKM's 26.0 (0332) and 43.5 (1959) of the same day (21.7 km, approx. 1.14 km/hr). Pike 54273 remained near this receiver until February 18 (1739) before continuing to RKM's 49.3 and 53.8 over a period of 4 days, and was last detected at RKM 51.5 on February 22 (0828).

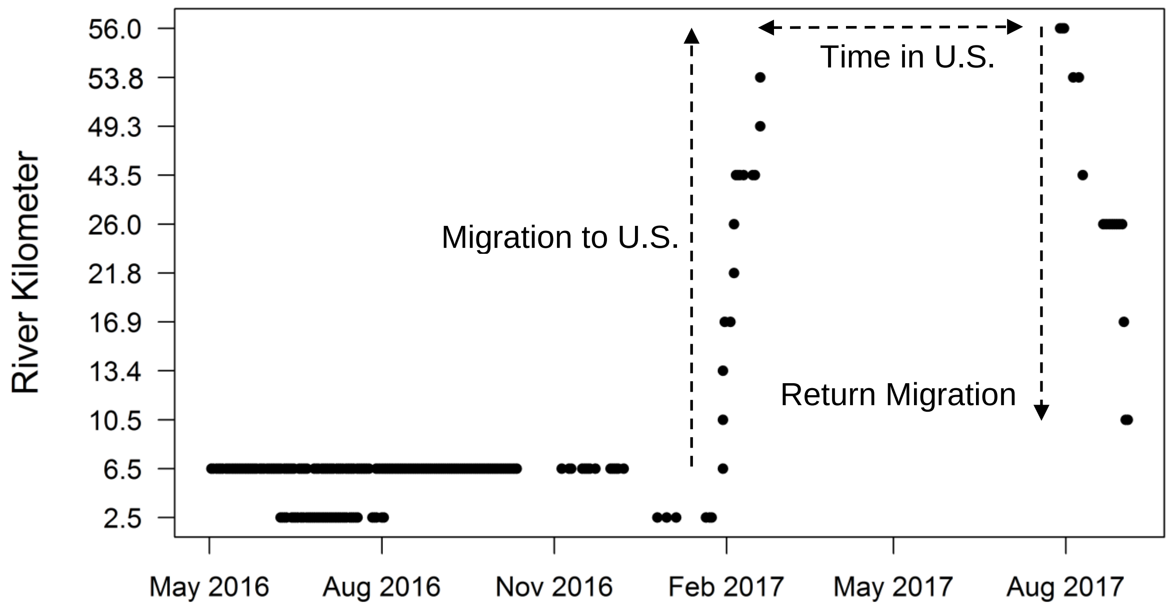

Figure 3.3. Detections for acoustic transmitter 54273 (63 cm, male) in the B.C. lower Columbia River between May 2016 and September 2017. This pike was the only tagged pike to travel downstream beyond RKM 10.5 in the LCR.

Pike 54273 was not detected again until August 01 (1356) at RKM 56.0, having been gone for nearly 6 months through the spring and much of the summer. It began its upstream migration on August 3 (0030), reaching RKM 53.8 on August 8 (0209) where it stayed until August 11 (0850). It was next detected a single time at RKM 43.5 on August 13 (1047), and was not detected again until August 24 (0822) at RKM 26.0 where it stayed until September 2 (1950). On September 4, it was detected at RKM's 16.9 (0353) and 10.5 (2030) where it was last picked up before receiver data was downloaded at the conclusion of the study on September 6 (1409) (refer to Appendix B, Figures B3 and B4 for residence time at each receiver). It is unclear how far south this pike travelled as no acoustic receivers are located in the northern section of the Columbia River in Washington.

Otolith Microchemistry

Water Sr was considerably higher in Columbia River samples, with averages of 110 µg L^{-1} compared to 40 µg L^{-1} in both Waneta and Seven Mile Reservoirs in the Pend d'Oreille. Water Ba was below the limit of detection (20 µg L^{-1}) for Columbia River samples, compared to 50 µg L^{-1} at both sample locations on the Pend d'Oreille. Mg differed to a lesser extent between the two systems, with averages of 3.4 mg L^{-1} in the Columbia River, 4.9 mg L^{-1} in Waneta Reservoir and 5.0 mg L^{-1} in the Seven Mile Reservoir. Water Mn was below the limit of detection (< 0.01 mg L^{-1}) and Zn was near or below the limit of detection (5 µg L^{-1}) for all samples taken in both rivers, therefore analysis of these elements was not possible.

Water element to Ca ratios at the location of fish capture were compared to otolith element to Ca ratios measured at the outer 15 µm of each otolith, as this presumably best reflects the water chemistry each fish was captured in. Otolith Sr:Ca ratios from pike captured in the Columbia and Pend d'Oreille rivers were highly correlated with water Sr:Ca ratios at the location of capture (R^2 = 0.837, p = <0.0001) (Figure 3.4). Water Ba was below the limit of detection (< 20 µg L^{-1}) for all Columbia River samples, therefore determining an accurate relationship between otolith and water Ba:Ca was not possible. A simple substitution was used of one half the limit of detection for Ba (10 µg L^{-1}) for all non-detects to get an approximation, and found otolith Ba:Ca to be highly correlated with water Ba:Ca at the locations of capture (R^2 = 0.756, p < 0.0001). No significant relationship was observed between otolith and water Mg, Mn or Zn to Ca ratios, and were subsequently omitted from further analysis.

Element to Ca ratios measured at the outer 15 µm of each otolith were used for comparison between pike captured in the Columbia (n = 50) and Pend d'Oreille (n = 10) Rivers. Otolith Sr:Ca ratios were significantly higher in Columbia River pike with overall means of 556.5 mmol/mol compared to 251.3 mmol/mol in Pend d'Oreille River pike (t = 6.581, p < 0.0001). Conversely, otolith Ba:Ca ratios of Columbia River pike were significantly lower than Pend d'Oreille River pike with overall means of 3.61 µmol/mol and 8.33 µmol/mol, respectively (t= -3.690, p = 0.0045). Ba:Ca ratios of Pend d'Oreille pike were elevated from the center of the otolith before decreasing towards the outer edge, which was not observed in Columbia River pike otoliths.

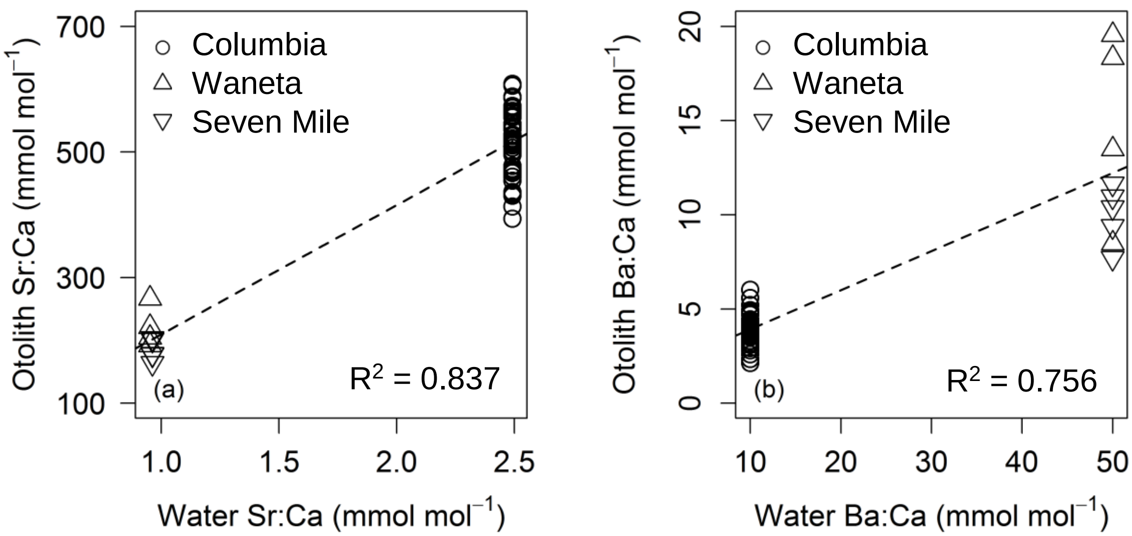

Figure 3.4. Water and otolith element (Sr and Ba) to Ca ratios for pike captured in the B.C. Columbia (Castlegar, n = 50) and Pend d'Oreille Rivers (Waneta (n = 5) and Seven Mile (n = 5) Reservoirs). Water Ba was below the limit of detection (20 µg L^{-1}) for Columbia River samples therefore a simple substitution of 1/2 the limit of detection was used.

Of the 50 Columbia River pike otoliths analyzed, one fish showed a distinct shift in Sr:Ca and Ba:Ca ratios throughout the otolith matrix. Otolith Sr and Ba to Ca ratios measured by LA-ICP-MS line scans of a Columbia River pike (57 cm male, 2 years old), a Pend d'Oreille pike (53 cm male, 2 years old), and a pike captured in the Columbia (76 cm male, 3 years old) with the observed shift in otolith chemistry are shown in Figure 3.5 (refer to Appendix B, Figure B5 for all otolith line scans). The first two pike were selected for comparison as they were the closest in length and weight between the Columbia and Pend d'Oreille Rivers. The difference in size resulted in minor differences in the length of otolith line scans as seen in Figure 3.5. Sr:Ca ratios of the third pike were consistent with that of Pend d'Oreille pike for the majority of its life (approximately 2 years), before rapidly increasing to average ratios observed in Columbia River pike. Elevated otolith Ba:Ca ratios in the center region of the otolith were also observed in the third pike before steadily decreasing to approach average ratios observed in Columbia River pike.

No significant patterns in elemental Mg, Mn, or Zn to Ca ratios were apparent between Columbia and Pend d'Oreille pike. There were, however, reoccurring fluctuations in otolith

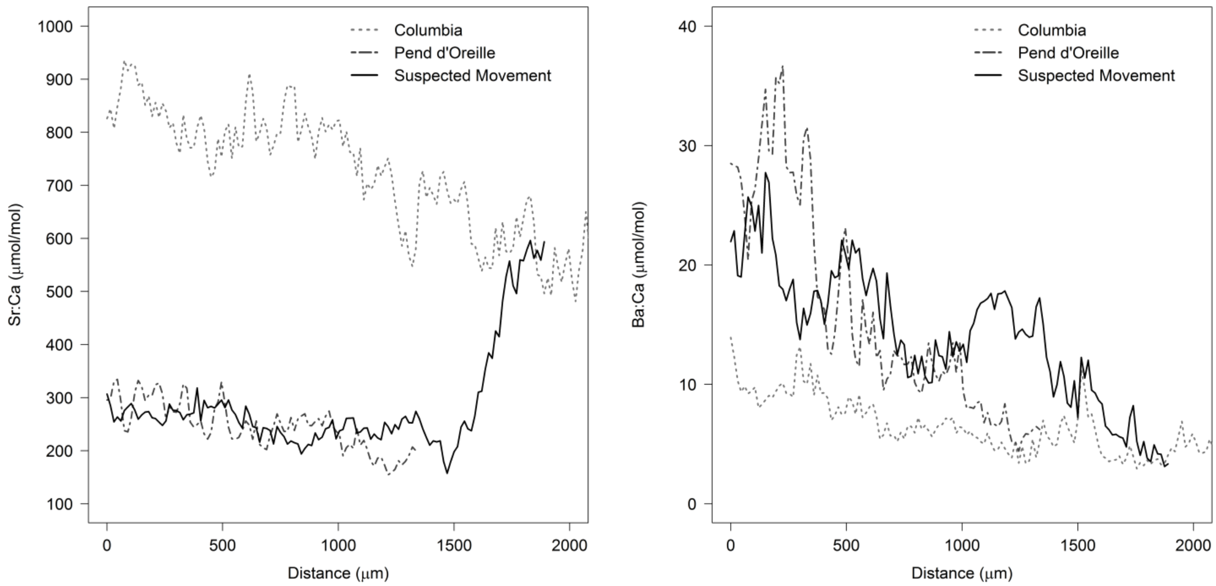

Figure 3.5. Otolith Sr:Ca and Ba:Ca ratios of three northern pike captured in the Columbia River, the Pend d'Oreille River, and a pike with suspected movement captured in the Columbia with an observed shift in otolith microchemistry. Element to Ca ratios were determined through LA-ICP-MS line scans of otoliths from the origin to the outer edge.

elemental Mg concentrations that appear to occur seasonally with highs in the spring, summer and fall months followed by a rapid decrease during winter. Similar fluctuations in otolith trace element Mg, Cu, Rb, Zn, Pb, and P have been used to estimate the age of Baltic Cod (Hussy *et al.* 2015; Serre *et al.* 2018), suggesting the observed fluctuations in pike otolith Mg may be a useful indicator of age through counting the number of distinct minima in concentration. The use of this method for estimating pike age was compared to cleithra age estimates reported in Chapter 2, and is discussed in detail in Chapter 4.

Discussion

Previous research has reported variable behaviour within pike populations, with some individuals remaining relatively sedentary throughout the year and others moving long distances or frequenting open water (Langford 1979, Chapman and Mackay 1984, Jepsen *et al.* 2001, Masters 2003, Ovidio and Phillipart 2005, Vehanen *et al.* 2006, Kobler *et al.* 2009, Sandlund *et al.* 2016). The results of this study are consistent with these reports with

Columbia River pike exhibiting a range of behaviours from remaining relatively inactive to embarking on migrations in excess of 50 km (each way). Of the 15 tagged pike monitored in this study, 4 remained within an approximate 4 km range between RKM's 6.5 and 2.5, and all but one stayed within the 10.5 km reach downstream of HLK Dam. The vast majority of detections within the Castlegar area were between the receivers at RKM's 2.5 and 6.5 (> 90%), with pike likely residing in areas of known pike abundance targeted annually for gill netting by the provincial suppression program (Baxter and Lawrence 2018). Within this 4 km stretch, over 75% of total northern pike detections occurred near the receiver at RKM 6.5 where pike were initially tagged. The south bank between RKM's 6.5 and 2.5 contains several shallow bays with riparian vegetation that floods during spring freshet, likely serving as spawning habitat for the Castlegar population. Paired with a high abundance of soft-bodied prey (whitefish spp., trout spp., sucker spp., sculpin spp., minnow spp.), an energetically favourable food source for northern pike (Beyerle and Williams 1968), this habitat likely limits the movement of the majority of pike from the area.

Detections at HLK Dam (RKM 0.1) were infrequent throughout the study (< 0.1% total) with no residence events recorded exceeding 1 day, and no tagged pike appearing to have entered the navigation lock at the dam. Eight out of fifteen tagged fish were detected near the dam at least once, indicating pike within the Castlegar population utilize most of the available reach downstream of the dam and periodically come within close proximity to the navigation lock. As previously mentioned, a major concern of the Castlegar pike population is the potential movement through the navigation lock at HLK Dam and colonization of the Arrow Lakes. While the infrequent and brief detections at the dam suggest the present risk of pike movement through the navigation lock is currently low, potential expansion of the Castlegar population and its proximity to the dam poses a significant long-term threat for the Columbia system. Parasiewicz *et al.* (2016) reported high efficiency (93.8-98.2 %) in preventing movement of a variety of fish species using a low-voltage electric fish guidance system coined "NEPTUN", with northern pike being one of the species tested. The lock at HLK Dam is relatively small in size (< 10 m in width), therefore implementation of such a system may be a feasible option for preventing movement of not only northern pike into the Arrow Lakes, but a variety of other invasive fish species currently in the Columbia including smallmouth bass, yellow perch, walleye, and tench, among others.

The majority of detections at the dam (RKM 0.1) occurred between May and July of both 2016 and 2017, coinciding with increases in detections at RKM 2.5 in front of the Zelstoff Celgar pulp mill. It was hypothesized that some pike may use sunken logs and woody debris in front of the mill (RKM 2.5) as a substrate for egg attachment during spawning, as submerged vegetation is limited in the reach downstream of the dam. These results suggest this may in fact be occurring as the majority of residence time at the mill (> 75%) was logged between April and June of both 2016 and 2017, with infrequent or no detections in the fall and winter. Pike have also been observed congregating in numbers near the Celgar mill in the Spring by members of the pike suppression program (J. Baxter, Fisheries Biologist, Mountain Water Research, pers. comm. 2016), and the capture of a juvenile pike in July of 2016 and several young-of-the-year pike in 2017 confirms there is reproductive success in the Castlegar area. This does not, however, indicate with any certainty the location or use of particular habitat within this reach due to limitations in the tracking methods used. A major advantage to using passive telemetry over that of cable or radio-linked systems is the continuous monitoring of fish throughout the year over a large geographical area, although this technology comes at the expense of location accuracy giving only presence or absence of fish within range of a particular receiver (Heupel *et al.* 2006). In addition, the receivers within the Castlegar area are in general placed in deep and open water to maximize detections of fish between both the north and south bank within the reach downstream of HLK Dam. The original deployment of acoustic receivers catered to the life strategies of white sturgeon, a SARA listed species in the Columbia River, maximizing detections of tagged fish in both the thalweg and near shore habitat. While this was advantageous for tracking sturgeon, a fish with a drastically different biological strategy, the accurate movement of northern pike may not be represented by this study.

While this research answered several questions regarding the behaviour and movement of pike from within this population, further investigation is required to determine the location(s) of spawning habitat and ultimately, the level of reproductive success within the reach downstream of HLK Dam. Further investigation into downstream populations in the U.S. is also required to determine the extent of transboundary movement and whether this movement is a significant input into the Castlegar population where suppression efforts are focused.

The single pike (54273) that migrated from the Castlegar area in February moved in excess of 50 km both to the U.S. and back, returning to within 4 km of the initial tagging site 6 months after departure. Also of interest, is the distance this pike travelled downstream into the U.S. where it was out of range from February to August, 2017, presumably to spawn. Previous studies have shown long-range movement of individual pike yet the majority of distances reported were under 30 km (Kaukoranta and Lind 1975, Vostradovsky 1975, Chapman and Mackay 1984, Ovidio and Phillipart 2005, Sandlund *et al.* 2016). There are exceptions to this, however, as a smaller proportion of studies have reported distances in excess of what was seen in this research; Moen and Henegar (1971) recaptured northern pike in a Missouri River reservoir up to 332 km from their initial capture site, yet most remained within a 32 km home range. Unfortunately, as no acoustic receivers are managed in the upper portion of the Columbia in Washington state the distance in which this pike travelled is unknown, which may have been comparable to extremes such as this.

The speed at which this fish traveled downstream is also of interest. While the total displacement downstream took approximately 20 days, pike 54273 traveled 40 km downstream in the first six days with 21.7 km of that travelled in one day. It is difficult to compare this movement to other reports, as this individual was moving downstream in its migration undoubtedly aided by river currents. Previous studies have reported seasonal spawning migrations of pike in rivers (Masters 2003, Ovidio and Phillipart 2005) yet distances reported are considerably less than observed in this study, and the direction of migration is generally upstream into flooded tributaries, marshes and wetlands during spring freshet. Ovidio and Phillipart (2005) observed variable upstream migrations of 6 radio-tagged pike in the rivers Ourthe and Amblève (Belgium), with displacements ranging from 0.75 to 15.7 km to reach spawning grounds. The team also reported variable daily migration rates, ranging from one individual moving 11.3 km upstream in 4 days to another moving only 4.5 km in 13 days. Pauwels *et al.* (2014) reported pike migrations of generally less than 500 m per day in a 44 km stretch of the River Yser (France, Belgium) and its tributaries, with infrequent displacements of up to 2 km and 4 km per day, the majority of which occurred between February and March. Langford (1979) reported sonic-tagged pike in the River Thames (United Kingdom) rarely exceeded 1.0 km in daily movements. Masters et al. (2003) described a continuum of spatial behaviours in 15 radio-tagged pike in the River Frome

(United Kingdom) ranging from individuals remaining within a home span of a few hundred meters to pike that made repeated seasonal journeys of several kilometers. In a telemetry study conducted by Chapman and Mackay (1984), pike in Seibert Lake (72.4 km^2), Alberta, were reported to make long distance displacements between habitats in a period of hours, and once in a new area they remained for up to several days making only short movements. The observations reported in this study are consistent with these findings as both downstream and upstream migration were characterized by short intervals of movement followed by a holding period of up to 10 days.

The downstream migration of this single pike could indicate natal homing to spawning grounds in the U.S. portion of the Columbia River or the lower reach of the Kettle River, a tributary that meets the Columbia approximately 35 km south of the Canada/US border where northern pike have been reported to be successfully reproducing (H. Maclellan, Fisheries Biologist, Colville Confederated Tribes, pers. comm. 2017). Previous studies have shown natal homing in northern pike (Miller *et al.* 2001, Vehanen *et al.* 2006, Engstedt *et al.* 2013). It is possible that pike from the Pend d'Oreille River initially seeded populations downstream of the U.S. border that eventually expanded upstream as a result of intraspecific competition for limited resources. Due to the abundance of prey species and habitat downstream of HLK Dam, pike born in the U.S. may overwinter in the Castlegar area and migrate back downstream to spawn. With limited habitat in the Castlegar area it is also reasonable to assume a percentage of pike seek out resources elsewhere outside of the spawning period.

One potential explanation for the initial movement is attraction to upstream chemical signals. Conspecific chemical odours have been shown to elicit aggregation, both reproductive and non-reproductive, in a variety of fish species, in some cases from great distances (Sorensen and Stacey 2004). Some fish species also release chemical alarm pheromones in response to physical stressors, serving as chemical attractants to other predators. Mathis *et al.* (1995) reported the alarm pheromone released by the fathead minnow served as an attractant to northern pike in laboratory trials. The fathead minnow is a member of the superorder Ostariophysi which includes the minnows, characins, catfishes, loaches and suckers, most of which are known to release chemical alarm signals (Chivers and Smith 1998). There are a multitude of species from within this superorder in the Columbia River, some of which (sucker spp., dace spp.) have been observed as prey items in northern pike stomachs during the provincial suppression program (Baxter and Lawrence 2018). The high abundance of prey fish in the Castlegar area may therefore elicit a predatory response in downstream pike, resulting in upstream migration outside of the spawning season. Regardless of what drove the initial upstream movement to Castlegar, the telemetry data presented in this study indicates a portion of LCR pike exhibit migratory behaviour, yet the admittedly small sample size of 15 was inadequate to infer the extent of transboundary movement between the Canadian and U.S. sections of the Columbia River.

Otoliths of northern pike captured in the Columbia and Pend d'Oreille rivers were found to have sufficient differences in chemical composition to discriminate between populations. Both Sr and Ba were good indicators of geographic location supporting previous otolith microchemistry studies investigating fish populations and movement (Kennedy *et al.*, 1997, Clarke *et. al* 2005, Clarke *et al.*, 2007, Engstedt *et al.* 2010, Shrimpton *et al.* 2014). Otolith Sr and Ba concentrations were both in proportion to their respective concentration in water samples collected at the site of capture; however, as Ba was below the limit of detection for Columbia River water samples this relationship could not accurately be defined. As previously mentioned a substitution of LOD/2 was used for Ba non-detects to approximate the relationship between otolith and water Ba concentrations. While the use of substitution methods (specifically LOD substitution methods) are widely criticized for introducing bias and erroneous results (She 1997; Helsel 2005), in this case it was not necessary to have high precision water chemistry results as sufficient chemical differentiation existed between the two systems as to be reflected in the otoliths alone.

While differences in otolith Mg were observed at the outer edge of the otolith between pike captured in the Columbia and Pend d'Oreille Rivers, they were likely due to fish being captured at different times of the year as the majority of fish from the Castlegar area were captured in May, while Pend d'Oreille fish were captured in July and August. When comparing Mg concentrations throughout the whole otolith, however, similar seasonal fluctuations were evident that appear to correspond to pike age estimates reported as part of the study in Chapter 2 through analysis of cleithra, a commonly used bone within the pectoral girdle used for aging esocids.

The single fish captured in Castlegar that appears to have moved from the Pend d'Oreille River suggests the system is not a significant source for recruitment into the Castlegar population (2% total). From a management perspective in B.C. these findings were positive; however, the Pend d'Oreille River could potentially be a significant contributor to downstream pike populations in the U.S. In time, the expansion of U.S. populations may in turn become a significant input of pike into B.C. waters. Analysis of otolith element concentrations of pike captured elsewhere in the Columbia was not possible as the provincial suppression effort is currently focused on the Castlegar population alone, and the little exploratory gill net sampling conducted in B.C. was unsuccessful. Future research should aim to compare otolith chemistry of Castlegar pike to other members of potential sub-populations downstream, both in B.C. and Washington.

The findings presented in this study provide a good framework for future pike research in the lower Columbia River which should focus heavily on downstream populations and the extent of movement between them, targeting areas of significant residence time observed in the single pike that migrated from Castlegar to the U.S. and back (transmitter ID 54273). Further additions to the Columbia River acoustic receiver array are also needed to locate habitat and spawning grounds in Washington, allowing fisheries managers to focus suppression efforts in the future.

Conclusions

The data presented in this study are but a piece of the larger management concern for northern pike in the Columbia River and its tributaries. They do, however, provide a framework for future collaboration between provincial and state authorities to better manage and control pike both in B.C. and Washington. Preemptive measures such as the implementation of a non-physical fish guidance system or deterrent is advisable to prevent the colonization of northern pike farther north in the Columbia. Due to the small sample size of acoustically tagged northern pike it is difficult to infer the proportion of fish that move between the Canadian and U.S. sections of the Columbia River. It is possible downstream populations are a significant source for recruitment into the Castlegar population, with pike migrating upstream from the U.S. to overwinter where resources are plentiful in the reservoir habitat downstream of the HLK Dam. The small percentage of Castlegar pike that appear to have originated in the Pend d'Oreille suggests if pike are in fact breaching Waneta Dam, they are traveling downstream to habitat in the U.S., potentially contributing to increasing numbers in Washington. This also suggests that if the input of pike from the Pend d'Oreille into the Columbia is significant, the majority of those fish are spreading further downstream and not into the LCR. Further long-term northern pike tracking studies and analysis of otolith microchemistry in the upper reaches of the U.S. Columbia River are required to determine the extent of transboundary movement between populations.

Literature Cited

Bath, G.E., Thorrold, S.R., Jones, C.M., Campana, S.E., McLaren, J.W., Lam, J.W.H. (2000). Strontium and barium uptake in aragonitic otoliths of marine fish. *Geochimica et Cosmochimica Acta*, 64: 1705–1714.

Baxter, J.T.A. (2016). Lower Columbia River Invasive Northern Pike Suppression – 2015 Update. Mountain Water Research, Trail, B.C. Prepared for Teck Metals Ltd., Trail, B.C. 16 p.

Baxter, J.T.A., Lawrence, C. (2018). Lower Columbia River Invasive Northern Pike Suppression - 2017 Update. Prepared for Teck, Trail, B.C. 16 p.

Baxter, J.T.A., Neufeld M. (2015). Lower Columbia River Invasive Northern Pike Suppression and Stomach Analysis – 2014. Mountain Water Research, Silverton, B.C. and Ministry of Forest, Lands and Natural Resource Operations, Nelson B.C. Prepared for Teck Metals Ltd., Trail B.C. 22 p.

Beyerle, G. B., Williams, J. E. (1968). Some observations of food selectivity by northern pike in aquaria. Transactions of the American Fisheries Society 97: 28 - 31

Brown, C.J.D. (1971). Fishes of Montana. Big Sky Books, Bozeman, MT.

Bystrom, P., Karlsson, J., Nilsson, P., V., Kooten, T. (2007). Substitution of top predators: effects of pike invasion in a subarctic lake. *Freshwater Biology* 52(7): 1271 - 1280.

Campana, S.E. (1999). Chemistry and composition of fish otoliths: pathways, mechanisms, and applications. *Marine Ecology Progress Series*, 188: 263 - 297.

Casselman, J.M., Lewis, C.A. (1996). Habitat requirements of northern pike (*Esox lucius*). *Canadian Journal of Fisheries and Aquatic Sciences*, 53(Suppl.1): 161 - 174.

Chapman, C.A., Mackay, W.C. (1984). Versatility in habitat use by a top aquatic predator, *Esox lucius* L. *Journal of Fisheries Biology*: 25: 109 - 115.

Chivers, D.P., Smith, R.J.F. (1998). Chemical alarm signaling in aquatic predator–prey systems: a review and prospectus. *Ecoscience*, 5: 338 - 352.

Clarke, A.D., Telmer, K.H., Shrimpton, J.M. (2005). Population structure and habitat use by Arctic grayling (*Thymallus arcticus*) in tributaries of the Williston Reservoir using natural elemental signatures. Peace/Williston Fish and Wildlife Compensation Program Report No. 300. 62p. + 1 app.

Clarke, A.D., Telmer, K.H., Shrimpton, J. M. (2007). Using natural elemental signatures to determine habitat use and population structure for a fluvial species, the Arctic grayling, in a watershed impacted by a large reservoir. *Journal of Applied Ecology*, 44: 1156–1165.

Dauble, D. (2009). Fishes of the Columbia Basin. Keokee Co Pub Inc., Sandpoint Idaho.

Eklöv, P., Hamrin, S. (1989). Predatory efficiency and prey selection: interactions between pike *Esox lucius*, perch *Perca fluviatilis* and rudd *Scardinus erythrophthalmus*. *Oikos*, 56: 149 - 156.

Engstedt, O., Engkvist, R., Larsson, P. (2013). Elemental fingerprinting in otoliths reveals natal homing of anadromous Baltic Sea pike (*Esox lucius L*.). *Ecology of Freshwater Fish*, 23: 313 - 321.

Engstedt, O., Stenroth, P., Larsson, P. Ljunggren, L., Elfman, M. (2010). Assessment of natal origin of pike (*Esox lucius*) in the Baltic Sea using Sr:Ca in otoliths. *Environmental Biology of Fishes*, 89: 547 - 555.

Farrell, J., Campana, S.E. (1996). Regulation of calcium and strontium deposition on the otoliths of juvenile tilapia, *Oreochromis niloticus*. *Comparative Biochemistry and Physiology - Part* A, 115: 103 - 109.

Harvey, B. (2009). A biological synopsis of northern pike (*Esox lucius*). *Canadian Manuscript Report of Fisheries and Aquatic Sciences* 2885: v + 31 p.

Haught, S., von Hippel, F.A. (2011). Invasive pike establishment in Cook Inlet Basin lakes, Alaska: diet, native fish abundance and lake environment. *Biological Invasions* 13: 2103 - 2114.

Helsel, D.R. (2005). More Than Obvious: Better Methods for Interpreting Nondetect Data. *Environmental Science & Technology*, 419A - 423A.

Hesthagen, T., Sandlund, O.T., Finstad, A., Johnsen, B.O. (2015) The impact of introduced pike (*Esox lucius* L.) on allopatric brown trout (*Salmo trutta* L.) in a small stream. *Hydrobiologia*, 744: 223 - 233.

Heupel, M. R., Semmens, J. M., and Hobday, A. J. (2006). Automated acoustic tracking of aquatic animals: scales, design and deployment of listening station arrays. *Marine and Freshwater Research*, 57: 1 - 13.

HTI (Hydroacoustic Technology, Inc.) (2009). Boundary Hydroelectric Project (FERC No. 2144), Study No. 12 Fish Entrainment and Habitat Connectivity Study. Final Report Prepared for Seattle City Light. 114 p + 4 App.

Hussy, K., Gröger, J., Heidemann, F., Henrichsen H.-H., Morohn, L. (2015). Slave to the rhythm: seasonal signals in otolith microchemistry reveal age of eastern Baltic cod (*Gadus morhua*). J*ournal of Marine Science*, 73(4): 1019 - 1032.

Inskip, P.D. (1982). Habitat suitability index models: northern pike. U.S. Department of the Interior, Fish and Wildlife Service, FWS/OBS-82/10.17. 40 p.

Jepsen, N., Beck, S., Skov, C., Koed, A. (2001). Behaviour of pike (*Esox lucius* L.) >50 cm in a turbid reservoir and in a clearwater lake. *Ecology of Freshwater Fish*, 10: 26 - 34.

Johnson, B.M., Martinez, P.J., Hawkins, J.A., Bestgen, K.R. (2008). Ranking predatory threats by nonnative fishes in the Yampa River, Colorado, via bioenergetics modeling. *North American Journal of Fisheries Management*, 28: 1941 - 1953.

Kaukoranta, E., Lind, E.A. (1975). The pike, *Esox lucius* L., in the estuary of the Oulujoki river. *Ichthyology Fennica Borealis*, 1972: 1 - 40.

Kennedy, B. P., Blum, J. D., Folt, C. L. (1997). Natural isotope markers in salmon. *Nature,* 387: 766 - 767.

Kobler, A., Klefoth, T., Mehner, T., Arlinghaus, R. (2009). Coexistence of behavioural types in an aquatic top predator: a response to resource limitation? *Oecologia*, 161: 837 - 847.

Langford, T.E., (1979). Observations on sonic tagged coarse fish in rivers. In Proceedings of the 1st British Freshwater Fisheries Conference 106- 114 pp. Liverpool UK, University of Liverpool.

Masters, J.E.G. (2003). Temporal variation in the behaviour of pike *Esox lucius* L. University of St. Andrews, Fife. (PhD Thesis).

Masters, J.E.G., Hodder, K.H., Beaumont, W.R.C., Gozlan, R.E., Pinder, A.C., Kenward, R.E., Welton, J.S. (2003). Spatial behaviour of pike *Esox lucius* L. in the River Frome, UK. Proceedings of the Fifth Conference on Fish Telemetry held in Europe. Aquatic telemetry: advances and applications: 179–190.

Mathis A., Chivers D.P., Smith R.J.F. (1995). Chemical alarm signals— predator deterrents or predator attractants. *The American Naturalist*, 145: 994 - 1005.

McMahon, T. E., and D. H. Bennett. (1996). Walleye and northern pike: boost or bane to northwest fisheries? *Fisheries*, 21(8): 6–13.

Miller, L.M., Kallemeyn, L., Senanan, W. (2001). Spawning-site and natal-site fidelity by northern pike in a large lake: Mark-recapture and genetic evidence. *Transactions of the American Fisheries Society* 130: 307–316.

Moen, T., and D. Henegar. (1971). Movement and recovery of tagged northern pike in Lake Oahe, South and North Dakota, 1964-68. *American Fisheries Society, Special Publications*, 8: 85 - 93.

Ovidio, M., Philippart, J.C. (2005). Long range seasonal movements of northern pike (*Esox lucius* L.) in the barbel zone of the River Ourthe (River Meuse basin, Belgium). In: Spedicato, M.T., Lembo, G., Marmulla, G. (Eds.), AquaticTelemetry: Advances and Applications. Proceedings of the fifth Conference on Fish Telemetry held in Europe. Ustica, Italy, 9 - 13 June 2003. FAO/COISPA,Rome, p. 191 - 202.

Parasiewicz, P., Wiśniewolski, W., Mokwa, M., Zioła, S., Prus, P., Godlewska, M. (2016). A low-voltage electric fish guidance system—NEPTUN. *Fisheries Research*, 181: 25 - 33.

Patankar, R., von Hippel, F. Bell, M. (2006). Extinction of a weakly armoured threespine stickleback (*Gasterosteus aculeatus*) population in Prator Lake, Alaska. *Ecology of Freshwater Fish*, 15: 482 - 487.

Pauwels, I.S., Goethals, P.L.M., Coeck, J., Mouton, A.M. (2014). Movement patterns of adult pike (*Esox lucius* L.) in a Belgian lowland river. *Ecology of Freshwater Fish,* 23: 373 - 382.

Sandlund, O.T., Museth, J., Øistad, S. (2016). Migration, growth patterns, and diet of pike (*Esox lucius*) in a river reservoir and its inflowing river. *Fisheries Research*, 173(1): 53 - 60.

Scott, W.B., Crossman, E.J. (1973). Freshwater Fishes of Canada. Bulletin 184. *Fisheries Research Board of Canada*. 966 p.

She, N. (1997). Analyzing censored water quality data using a nonparametric approach. *Journal of the American Water Resources Association*, 33: 615 - 624.

Shiller, A. M. (2003). Syringe filtration methods for examining dissolved and colloidal trace element distributions in remote field locations. *Environmental Science and Technology*, 37: 3953 - 3957.

Shrimpton, J.M., Warren, K.D., Todd, N.L., McRae, C.J., Glova, G.J., Telmer, K.H., Clarke, A.D. (2014). Freshwater movement patterns by juvenile Pacific salmon *Oncorhynchus* spp. before they migrate to the ocean: Oh the places you'll go! *Journal of Fish Biology*, 85: 987 - 1004.

Serre, S.H., Nielsen, K.E., Fink-Jensen, P., Thomsen, T.B., Hüssy, K. (2018). Analysis of cod otolith microchemistry by continuous line transects using LA-ICP-MS. *Geological Survey of Denmark and Greenland Bulletin,* 41: 91–94.

Sorensen, P.W., Stacey, N.E. (2004). Brief review of fish pheromones and discussion of their possible uses in the control of non-indigenous teleost fishes, *New Zealand Journal of Marine and Freshwater Research*, 38(3): 399 - 417.

Spens, J., Ball, J. (2008). Salmonid or nonsalmonid lakes: predicting the fate of northern boreal fish communities with hierarchical filters relating to a keystone piscivore. *Canadian Journal of Fisheries and Aquatic Sciences*, 65: 1945 - 1955.

Thorrold, S.R., Jones, C.M., and Campana, S.E. (1997). Response of otolith microchemistry to environmental variations experienced by larval and juvenile Atlantic croaker (*Micropogonias undulatus*). *Limnology and Oceanography*, 42: 102 - 111.

Vehanen, T., Hyvarinen, P., Johansson, K., Laaksonen, T. (2006). Patterns of movement of adult northern pike (*Esox lucius* L.) in a regulated river. *Ecology of Freshwater Fish*, 15: 154 - 160.

Vostradovsky, J. (1975). Horizontal distribution of individually tagged fish in the Lipno reservoir (English). EIFAC Technical Papers 23 (Suppl. 1, V.2), 651 - 655.

Chapter 4: Age estimation in northern pike (*Esox lucius*) using seasonal fluctuations in otolith magnesium.

Abstract

A technique for aging northern pike and potentially other esocid species is described through analysis of seasonal patterns in otolith Mg. Analysis of otoliths from 60 northern pike suggest otolith Mg is incorporated into otolith aragonite in proportion to metabolic activity, and distinct minima in otolith Mg correspond to the number of winters a fish has experienced, thus allowing the estimation of fish age. These minima were more distinct and easier to interpret than inspection of cleithra annuli. Estimates from the two techniques were in agreement 65% of the time, but this result may be due to the challenge of accurately extrapolating fish age from cleithra.

keywords: *Esox lucius*, northern pike, otolith microchemistry, fish aging

Introduction

Numerous calcified structures produce periodic growth increments that can be used to estimate the age of fish, including scales, vertebrae, fin rays, otoliths and cleithra (Campana 2001). However, age estimates often present interpretation problems for the reader as growth sequences may not be complete within the structure or not visible on all axes (Beamish 1979). For esocids such as northern pike (*Esox lucius*) or muskellunge (*Esox masquinongy*), accurate age estimates can be difficult to obtain through analysis of scales (Fitzgerald *et al*. 1997) and fin rays (Brenden *et al.*, 2006), and otoliths have not been validated for aging (Faust *et al.* 2013). It has been suggested that cleithra, paired flat bones within the pectoral girdle, provide the most reliable age estimates for esocids, yet still may present interpretation problems for readers resulting in inaccurate estimates (Faust *et al.* 2013). A potential technique for aging northern pike (and possibly other esocid species) is described that warrants further investigation and validation - the analysis of otolith microchemistry.

Otoliths are formed by continual crystallization of endolymphatic fluid within the inner ear canal of fish (Bath *et al.* 2000). Fish endolymph contains bicarbonate, calcium, and trace elements which are mainly derived from the environment via branchial uptake (Bath *et al.* 2000), providing a life-long chemical record of ambient water conditions deposited within otoliths (Thorrold *et al.* 1997). Otolith microchemistry has gained considerable momentum in recent years as a technique for investigating a variety of ecological questions in fisheries research including movement patterns (Shrimpton *et al.* 2014), anadromy (Hart *et al.* 2015), and natal origin (Lazartigues *et al.* 2017). A lesser explored aspect of otolith microchemistry is the potential for age estimation using seasonal fluctuations in otolith trace element concentrations. Hussy *et al*. (2015) reported the age of Baltic cod could be estimated through analyzing seasonal patterns of trace elements such as Mg, Cu, Rb, Zn, and Pb in otoliths. Seasonal patterns in P concentration were also reported to be a potential indicator of age in the same species, with the total number of minima occurring in the profiles from birth to death corresponding to the number of winters the fish has experienced (Serre *et al.* 2018). A similar scenario is discussed with northern pike from invasive populations in the Columbia and Pend d'Oreille Rivers in British Columbia, Canada, with distinct fluctuations in otolith Mg that appear to correspond to ages estimated through analysis of cleithra annuli.

Methods

Refer to Chapter 3 (pg. 37 and 38) for otolith preparation and LA-ICP-MS specifications. The number of clear decreases in Mg concentration from the otolith origin outwards were used to estimate northern pike ages, then compared to cleithra age estimates for the same fish. Two additional readers with no prior knowledge of northern pike size and growth rates were asked to estimate ages by identifying Mg minima using enlarged, paper-printed (8.5" x 11") plots of otolith Mg for each fish. All 60 fish were aged in less than 5 minutes by all readers.

Results

Northern pike age estimates were in agreement with cleithra estimates approximately 65% of the time (Figure 4.1). Both additional readers obtained similar results, with 68% and 63% in

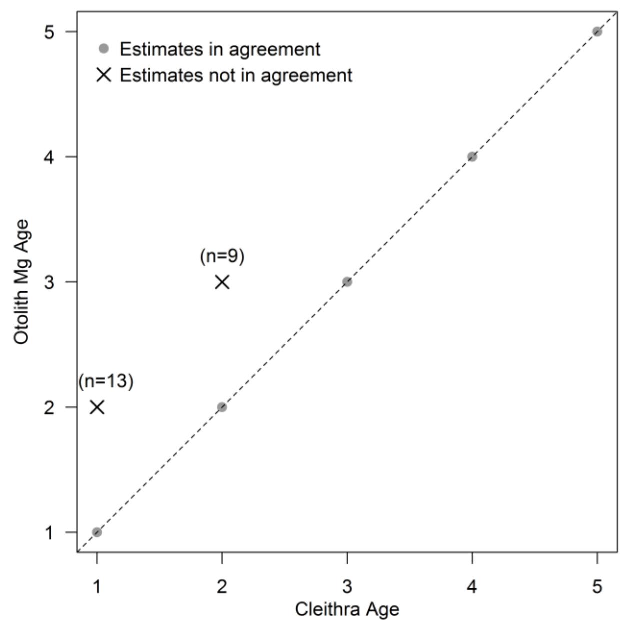

Figure 4. 1. Comparison of northern pike age estimates (n=60) through use of cleithra (x-axis) and analysis of otolith Mg (y-axis) for age determination, with the dotted line plotted through estimates in agreement. Estimates were in agreement 65% of the time, and all estimates not in agreement were in fish in the 1 (n=13) or 2 (n=9) year categories and were higher Mg estimates than cleithra estimates for the same fish.

accordance with cleithra ages. All estimates not in agreement were in the 1+ (n=13) or 2+ (n=9) age categories, and all disparities were in pike estimated to be younger using cleithra yet showed an additional distinct decrease in otolith Mg leading to an older estimate. A portion of cleithra samples (n = 20) were sent to an external laboratory (North South Consulting, Winnipeg, MB) and were graded according to their characteristics (pattern clarity, repeatability), the majority of which (76%) were found to be fair in quality where readers would be within 1 year most of the time for fish less than 10 years old (refer to Appendix A, Table A1 for grading characteristics). Taking this into account, a portion of the younger fish may have been erroneously aged through inspection of cleithra annuli, yet it cannot be said with certainty that other factors are not influencing the fluctuations in otolith Mg leading us to differing estimates. Otolith Mg fluctuations in five northern pike estimated to be 1+, 2+, 3+, 4+, and 5+ years of age using this technique is depicted in Figure 4.2 with their respective lengths.

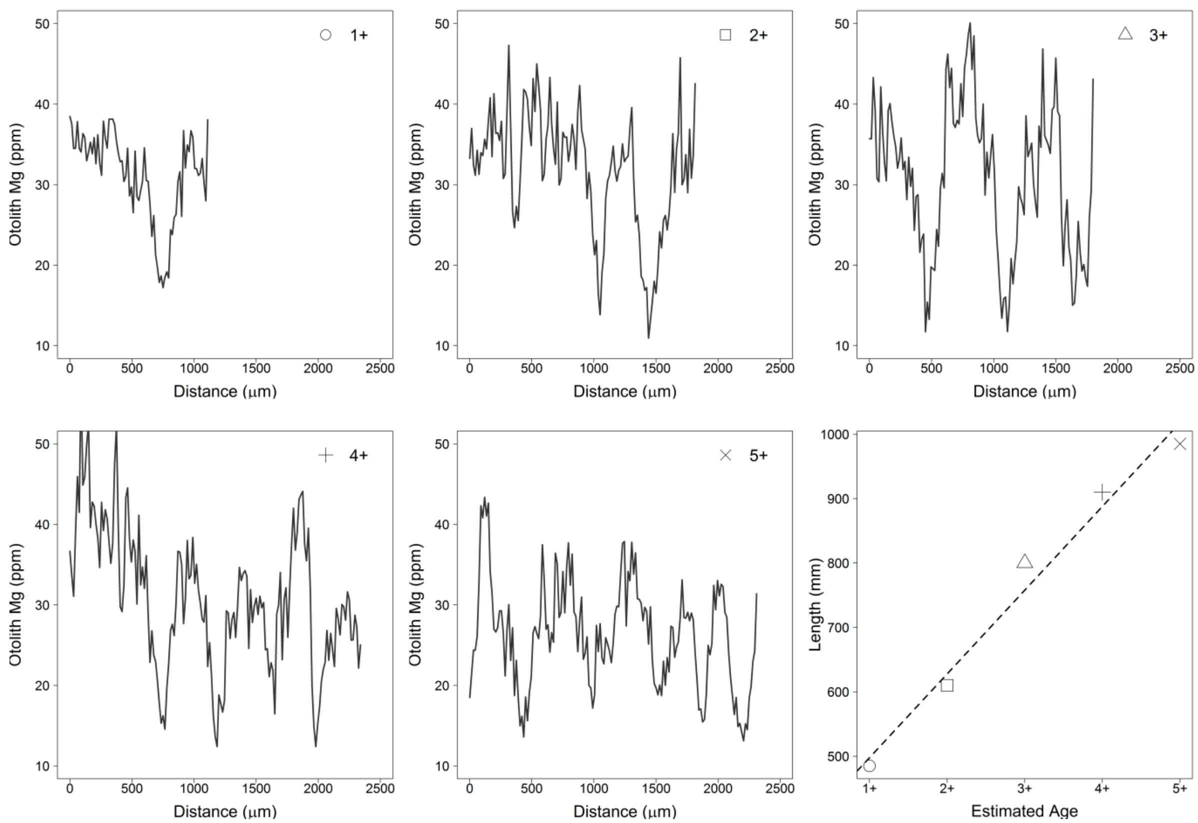

Figure 4.2. Northern pike otolith Mg measured by LA-ICP-MS line scans of five fish estimated to be 1+ (485 mm), 2+ (610 mm), 3+ (800 mm), 4+ (910 mm), and 5+ (985 mm) years of age. The bottom right plot depicts the linear relationship between estimated age and the length of those fish. Fluctuations in otolith Mg appear to correspond to cleithra age estimates, suggesting this may be a useful tool for researchers to age northern pike and possibly other esocids when analyzing otolith microchemistry.

Discussion

Several studies have indicated Mg is associated with somatic growth, and that this element is incorporated into otolith aragonite at a rate proportional to metabolic activity (Grammer *et al.* 2017; Limburg *et al.* 2018). This is supported by the presented results with reoccurring decreases in otolith Mg appearing to correspond to slow metabolic activity during the winter seasons, thus allowing the estimation of fish age. There are, however, numerous factors that require further investigation to validate the use of trace element aging. The uptake of elements from the environment and their incorporation into the otolith matrix is a result of multiple physiological pathways that each serve as barriers which alter the rate at which elements are transferred (Campana, 1999). Mg is involved in an array of physiological processes including reproduction, growth, and development, and has been shown to be regulated by both physiological and environmental factors (Grammer *et al.* 2017). Limburg *et al.* (2018) outline in detail a proposed mechanism of Mg entry and incorporation into otolith aragonite, but comment testing this mechanism experimentally presents a challenge as observing otolith depositional processes *in vivo* is still not achievable. Variations in factors such as species growth and maturation also need to be explored, and the development of a statistical approach for subjective signal interpretation in otolith element concentrations is required (Hussy *et al.* 2015). With respect to northern pike in the Columbia and Pend d'Oreille Rivers, only 65% of cleithra and otolith Mg age estimates were in agreement, and 100% of those not in agreement were in younger (1-2 year) pike aged a year older using otolith Mg. This may indicate cleithra annuli from smaller pike in these populations can present interpretation problems that lead to underestimation of fish age, which can potentially be resolved through analysis of otolith Mg. Further investigation is required to validate both cleithra and otolith Mg age estimates and needs to include considerably more individuals, especially from older age categories (3+ and older). The results of this study suggest that, in a highly metabolically active fish species such as northern pike, analysis of otolith Mg may prove to be a useful technique for estimating age that requires little to no training for interpretation. In addition, the rate at which a large number of fish can be aged at once is considerably higher than using cleithra for age estimates.

Conclusions

Cleithra are currently thought to provide the most reliable age estimates for esocids, yet they can still present interpretation problems for the experienced reader. While the cost of otolith microchemical analysis for estimating age is considerably higher than that of visual inspections of cleithra, the other information (natal origin, migration/movements, environmental conditions) attained through the study of otolith microchemistry paired with the rapid rate of reading samples warrants further validation for this technique.

Literature Cited

Bath, G.E., Thorrold, S.R., Jones, C.M., Campana, S.E., McLaren, J.W., Lam, J.W.H. (2000). Strontium and barium uptake in aragonitic otoliths of marine fish. *Geochimica et Cosmochimica Acta,* 64: 1705 - 1714.

Beamish, R.J. (1979). New information on the longevity of Pacific ocean perch (*Sebastes alutus*). *Journal of the Fisheries Research Board of Canada,* 36: 1395 - 1400.

Brenden, T.O., Hallerman E.M., Murphy B.R. (2006). Sectioned pelvic fin ray ageing of muskellunge *Esox masquinongy* from a Virginia river: comparisions among readers, with cleithrum estimates, and with tag–recapture growth data. *Fisheries Management and Ecology,* 13: 31–37.

Campana, S.E. (1999). Chemistry and composition of fish otoliths: pathways, mechanisms, and applications. *Marine Ecology Progress Series,* 188: 263–297.

Campana, S.E. (2001). Accuracy, precision and quality control in age determination, including a review of the use and abuse of age validation methods. *Journal of Fish Biology,* 59: 197 - 242.

Faust, M.D., Breeggemann, J.J., Bahr, S., Graeb, B.D.S. (2013). Precision and bias of cleithra and sagittal otoliths used to estimate ages of northern pike. *Journal of Fish and Wildlife Management,* 4(2): 332 - 341.

Fitzgerald, T.J., Margenau, T.L., Copes, F.A. (1997). Muskellunge scale interpretation: the question of aging accuracy. *North American Journal of Fisheries Management,* 17: 206 - 209.

Grammer, G.L., Morrongiello, J.R., Izzo, C., Hawthorne, P.J., Middleton, J.F., Gillanders, B. M. (2017). Coupling biogeochemical tracers with fish growth reveals physiological and environmental controls on otolith chemistry. *Ecological Monographs,* 87: 487 - 507.

Hart, L.M., Bond, M.H., May-McNally, S.L., Miller, J.A., Quinn, T.P. (2015). Use of otolith microchemistry and stable isotopes to investigate the ecology and anadromous migrations of Northern Dolly Varden from the Egegik River, Bristol Bay, Alaska. *Environmental Biology of Fishes,*98(6): 1633 - 1643.

Hussy, K., Gröger, J., Heidemann, F., Henrichsen H.-H., Morohn, L. (2015). Slave to the rhythm: seasonal signals in otolith microchemistry reveal age of eastern Baltic cod (*Gadus morhua*). J*ournal of Marine Science*, 73(4): 1019 - 1032.

Lazartigues, A., Girard, C., Brodeur, P., Lecomte, F., Mingelbier, M., Sirois, P. (2017). Otolith microchemistry to identify sources of larval yellow perch in a fluvial lake: an approach towards freshwater fish management. *Canadian Journal of Fisheries and Aquatic Sciences*, 75(3): 474 - 487.

Limburg, K.E., Weunschel, M.J., Hüssy, K., Heimbrand, Y., Samson, M. (2018). Making the otolith magnesium chemical calendar-clock tick: plausible mechanism and empirical evidence. *Reviews in Fisheries and Aquaculture,* 26(4): 479 - 493.

Serre, S.H., Nielsen, K.E., Fink-Jensen, P., Thomsen, T.B., Hüssy, K. (2018). Analysis of cod otolith microchemistry by continuous line transects using LA-ICP-MS. *Geological Survey of Denmark and Greenland Bulletin,* 41: 91–94.

Shrimpton, J.M., Warren, K.D., Todd, N.L., McRae, C.J., Glova, G.J., Telmer, K.H., Clarke, A.D. (2014). Freshwater movement patterns by juvenile Pacific salmon *Oncorhynchus* spp. before they migrate to the ocean: Oh the places you'll go! *Journal of Fish Biology,* 85: 987 - 1004.

Thorrold, S.R., Jones, C.M., and Campana, S.E. (1997). Response of otolith microchemistry to environmental variations experienced by larval and juvenile Atlantic croaker (*Micropogonias undulatus*). *Limnology and Oceanography*, 42: 102 - 111.

Chapter 5: Potential use of environmental DNA (eDNA) as a monitoring tool for invasive northern pike in the Columbia River, B.C.

Abstract

The use of environmental DNA was explored as a tool to detect and monitor the spread of invasive northern pike in the B.C. Columbia and Pend d'Oreille Rivers. An aquaria trial was conducted and pike DNA was successfully isolated from water samples and amplified using a previously developed quantitative PCR (qPCR) assay. Using the same protocol, surface water samples were taken from 13 locations of known northern pike abundance in the Columbia and Pend d'Oreille Rivers. No positive detections of northern pike DNA were observed. Potential factors leading to negative detections and recommendations for modifications to the sampling protocol are discussed.

keywords : environmental DNA, eDNA, *Esox lucius*, northern pike, invasive fish, fisheries management

Introduction

The introduction of invasive species is a driving force impacting biodiversity in freshwater ecosystems, which are among the most threatened habitats worldwide (Dudgeon *et al.* 2006). While inhibiting the introduction of nonnative species is a main goal for preserving freshwater ecosystem biodiversity, preventing or slowing the secondary spread of already established invasive populations play an important role in long-term management (Vander Zanden and Olden 2008). Detection of biological invasions in their early stages is challenging when population densities are at a minimum, and conventional surveying techniques such as gill-netting require considerable resources to conduct and have the potential to negatively impact non-target species in addition to having questionable effectiveness when target species abundance is low (Olsen *et al.* 2015). The use of environmental DNA (eDNA) sampling has gained considerable interest since its inception nearly a decade ago (Ficetola *et al.* 2008) as a non-invasive technique to detect and monitor invasive or rare freshwater species, requiring minimal effort in the field and eliminating potential negative impacts on non-target species. Environmental DNA sampling involves isolating DNA from the environment, the predominate source of which is thought to be derived from faeces, urine and shed epidermal cells (Haile *et al.* 2009), and species present are identified via their genetic material. Environmental DNA sampling was used to detect northern pike, a newly establish invasive species in the Columbia River, and to determine its efficacy as a potential monitoring tool for the Columbia and its tributaries.

Methods

In environments where DNA is present in low concentrations (i.e. low population density of target species), mitochondrial DNA (mtDNA) markers are often used due to the considerably higher concentration in cells than that of nuclear DNA (Rees *et al.* 2014). The assay used was developed by Olsen *et al.* (2015), which targets a 94 base pair sequence of the cytochrome oxidase subunit I gene (COI) of northern pike mtDNA. This assay was tested for specificity against 15 co-occurring fish species including Muskellunge, a closely related esocid species, and was found to have high specificity and sensitivity at low DNA concentrations. The assay consists of a primer set,

F - 5' CCTTCCCCCGCATAAATAATATAA 3',

R - 5' GTACCAGCACCAGCTTCAACAC 3',

and a FAM dye-labeled minor groove binding non-fluorescent quencher (MGB-NFQ) probe,

P - 5' CTTCTGACTTCTCCCC 3'.

An aquaria trial was first conducted to determine if the proposed field sampling and DNA extraction methods were sound. Two 285 L aquaria were cleaned with a 10% bleach solution and placed in an enclosure constructed out of poly sheeting with zippered doors. The aquaria were filled with tap water and fitted with a chiller unit to maintain water temperatures near 12 ^{o}C, the approximate water temperature in the Columbia at the proposed time of sampling. One aquarium was left empty, and the other stocked with tissue (10 g) sampled from a northern pike captured in the Columbia River near Castlegar, B.C. Tissue was removed using a new sterile scalpel blade, and all tools were washed with a 10% bleach solution before each sample was taken. After one hour, 1 L of surface water was sampled from each aquaria using a sterile Whirl-Pak bag and filtered through a Nalgene analytical test filter funnel (100 ml, 0.45 µm cellulose nitrate filter) attached to a simple filtering apparatus consisting of a Buchner flask and hand operated vacuum pump. Filters were then removed from the funnel apparatus using sterile forceps and placed in 50 mL tubes filled with 95 ethanol until DNA extractions were carried out. Qiagen's DNeasy Blood and Tissue Kit was used for filter DNA extractions following recommendations from Diener *et al.* (2015), in which they report maximal efficacy when using eukaryotic organisms and targeting the COI gene.

The TaqMan assay proposed by Olsen *et al.* (2015) was performed with slight modifications. All sample preparation was conducted in a Labconco Purifier Class II Biosafety Cabinet to prevent contamination of samples. PCR standards were created using a synthetic EluCOI gene (Integrated DNA Technologies, sequence obtained from BOLD database: ANGBF5760-12 - *Esox lucius* [COI-5P:651]), ranging in concentration from 10,000 copies/reaction to 10 copies/reaction. Reactions were conducted in 10 µL volumes consisting of 5 µL TaqMan Universal Master Mix, 0.5 µL of assay mix, 2.5 µL of nuclease free water, and 2 µL of either aquaria DNA filter elutions (x3), the synthetic EluCOI gene (in varying concentrations), or deionized water (negative control, x3). PCRs were run in Standard Curve mode on a Illumina Eco^{TM} Real-Time PCR System, with conditions set at 1 cycle of 50 ^{o}C/2 min, 95 ^{o}C/10 min followed by 50 cycles of 95 ^{o}C/15s, 60 ^{o}C/1 min. DNA amplification occurred for all standard concentrations, and negative controls behaved as expected (no amplification). The lowest concentration standard (10 copies/reaction) took approximately 1 hour for amplification to begin in the initial trial, leading us to change thermo-cycling parameters for field sample assays to 1 cycle of 50 ^{o}C/2 min, 95^{o}C/10 min followed by 60 cycles of 95 ^{o}C/15s, 60^{o}C/1 min. DNA was successfully amplified from aquaria DNA filter eluates suggesting the proposed testing protocol was sound.

Duplicate surface water samples were collected from 13 locations of known northern pike abundance in the Columbia and Pend d'Oreille Rivers in B.C. The majority of samples were collected from shore (n = 10) with the remainder (n = 3) collected from the bow of a boat approaching from the downstream side. Water samples were immediately stored in a cooler until processing, which occurred within 8 - 24 hours from when the sample was collected. Filters were processed in the same manner as in the aquaria trials with the exception of the increased duration of time before filtering water samples. PCR assays were also conducted under the same parameters as the laboratory trials.

Results

No DNA amplification occurred for any of the field samples despite being taken from known pike habitat.

Discussion

A variety of factors influence detection probability of eDNA including (but not limited to) density of target species, environmental conditions (variation in rates of dilution and diffusion, temperature), microbial communities, and rate of DNA degradation (Bohmann *et al.* 2014). A Lincoln-Peterson mark recapture estimate conducted by Baxter and Lawrence (2018) estimated northern pike numbers to be as low as 107 (59 lower CI, 155 upper CI) in the 10.5 km reach downstream of the Hugh L. Keenleyside Dam at the time of sampling, where pike densities are highest in the LCR. The low northern pike density in the LCR is likely the primary reason for reducing eDNA detection probability, yet a number of other factors are likely contributors. Samples were taken in late May and early June when Spring freshet is approaching a maximum, and changes in flow regimes through hydroelectric dam operation during this period could have led to significant fluctuations in water elevation and river currents. Peak flows between HLK Dam and Trail reached 4,247 cubic meters per second (m^3/s) in June not long after sampling occurred (BC Hydro 2018). While some habitat in the Castlegar area where pike have previously been captured is somewhat protected from fast currents, the considerable amount of water flowing through this reach likely reduced detection probability considerably. Turbidity as a result of high flows also proved to be a challenge for filtering water samples, with filters clogging with sediments and debris rapidly and taking considerable time to process the entire 1 L (some in excess of 30 minutes). The amount of water sampled also likely influenced detection of pike. Maximizing DNA detection probabilities depends largely on sampling a sufficient volume of water in the field, in addition to analyzing a sufficient proportion of the total DNA extracted from an environment (Wilcox *et al.* 2018). In a large fluvial system such as the Columbia River, water samples of considerably more than 1 L may therefore be necessary in addition to extracting DNA from the entire sample. Surface sampling may have also contributed to reduced detection probability. As a result of fluctuating water elevations pike are not always present near the shoreline, and much of the occupied habitat extends a considerable distance from shore and often in depths in excess of 4 meters. Sub surface sampling by boat may therefore be a more appropriate for these habitats.

Conclusions

In a large fluvial system such as the Columbia River, eDNA monitoring may not be appropriate for detecting rare or invasive species with such low population densities without modifications to the outlined protocol. Conventional sampling techniques such as netting or electrofishing may therefore be a better means of monitoring the status of northern pike in the LCR.

Literature Cited

Baxter, J., Lawrence, C. (2018). Lower Columbia River invasive northern pike suppression - 2017 update. Report prepared for Teck, 12 p.

BC Hydro [Internet] (2018). Columbia River Operations Summary. Accessed from: https://www.bchydro.com/content/dam/BCHydro/customer-portal/documents/corporate/community/columbia-river-operations-summary.pdf.

Bohmann, K., Evans, A., Gilbert, M.T.P., Carvalho, G.R., Creer, S., Knapp, M., Yu, D.W., de Bruyn, M., (2014). Environmental DNA for wildlife biology and biodiversity monitoring. *Trends in Ecology and Evolution,* 29(6): 358 - 367.

Diener, K., Walser, J.C., Mächler, E., Altermatt, F. (2015). Choice of capture and extraction methods affect detection of freshwater biodiversity from environmental DNA. *Biological Conservation,* 183: 53 - 63.

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

Ficetola, G.F., Miaud, C., Pompanon, F., Taberlet, P. (2008). Species detection using environmental DNA from water samples. *Biology Letters,* 4: 423 - 425.

Haile, J., Froese, D.G., MacPhee, R.D.E., Roberts, R.G., Arnold, L.J., Reyes, A.V., Rasmussen, M., Neilsen, R., Brook, B.W., Robinson, S., Demuro, M., Gilbert, M.T.P., Munch, K., Austin, J.J., Cooper, A., Barnes, I., Möller, P., Willerslev, E., (2009). Ancient DNA reveals late survival of mammoth and horse in interior Alaska. *Proceedings of the National Academy of Sciences of the United States of America*, 106: 22352 - 22357.

Olsen, J.B., Lewis C.J., Massengill R.L., Dunker K.J., Wenburg J.K. (2015). An evaluation of target specificity and sensitivity of three qPCRassays for detecting environmental DNA from Northern Pike (*Esox lucius*). *Conservation Genetics Resources*, 7: 615 - 617.

Rees, H.C., Maddison, B.C., Middleditch, D.J., Patmore, J.R.M., Gough, K.C. (2014). The detection of aquatic animal species using environmental DNA – a review of eDNA as a survey tool in ecology. *Journal of Applied Ecology*, 51(5): 1450 - 1459.

Vander Zanden, M.J., Olden, J.D. (2008). A management framework for preventing the secondary spread of aquatic invasive species. *Canadian Journal of Fisheries and Aquatic Science.*, 65: 1512 - 1522.

Wilcox, T.M., Carim, K.J., Young, M.K., McKelvey K.S., Franklin, T.W., Schwartz, M.K., (2018). Comment: the importance of sound methodology in environmental DNA sampling. *North American Journal of Fisheries Management,* 38:592–596, 2018.

Chapter 6: Conclusions and management recommendations

This study has demonstrated the adaptability of northern pike when introduced outside its native range, with pike in the Columbia River and connected tributaries utilizing an array of prey resources, habitat, and home to rapidly expand their territory in B.C. and Washington. Since initial detection in the Columbia River in 2007, pike have been detected throughout approximately 275 km of river between the Hugh L. Keenleyside (HLK) and Grand Coulee Dams, and pose the risk of further expansion both upstream and downstream of their current range. Fisheries managers in both B.C. and Washington were fortunately quick to respond to the invasion in the Columbia River, drawing from lessons learned by the widely documented introduction and spread of northern pike in Southcentral Alaska in which significant declines in a variety of salmonid species have been linked to expanding pike populations (Rutz 1999; Sepulveda *et al.* 2015, Dunker *et al.* 2018). This rapid response led to the deployment of the LCR pike suppression program in 2014, which to date appears to have been effective in reducing the number of adult northern pike in the LCR. Annual gill-net suppression will be required in subsequent years to continue controlling the adult cohort, yet future years of the suppression program need to include significant investigation into the juvenile life stage.

The capture of juvenile pike in 2017 (n=14) indicates spawning success in the LCR but it is currently unknown to what extent this is occurring. One of the major concerns with the presence of juvenile northern pike is the potential predation on salmonid species. Invasive juvenile northern pike in Southcentral Alaska have been shown to preferentially prey on juvenile salmonid spp., and that juvenile pike consumed more of these fish than adult pike (Sepulveda *et al*. 2015). This is especially concerning with the proximity of salmonid spawning habitat in the LCR that overlaps with pike habitat, such as Pass Creek in Castlegar which supports important spawning and rearing habitat for Columbia River rainbow trout, kokanee and mountain whitefish (Audy and Zimmer 2015). Another concern is potential predation on the SARA listed Umatilla dace (threatened) and Columbia sculpin (special concern) present in the LCR. Sculpin and dace spp. accounted for 20% of prey items and were mostly found in juvenile stomachs, suggesting these species may be a major constituent of juvenile pike diet in the Columbia. Juvenile habitat surveys and suppression (electrofishing, nets, quatrefoil light traps) should be high on the scale on management priorities for future years of pike control.

Current gill-netting protocols in the LCR (≤ 4 hours, ≤ 4 m depth) appear to have been successful in minimizing impacts on native species, with an approximate 75% live release rate across all species. The biggest concern with regards to gill-netting in the LCR was the incidental capture of SARA listed white sturgeon, 9 of which were encountered over the first 4 years of suppression. All sturgeon encountered in nets were released unharmed and deemed fit for release, indicating the overall risk for sturgeon is minimal. Future years of the suppression program will need to give some consideration to annual gill-netting effort in order to limit impacts on native fish species. Pike catch rates have decreased following the initial year of suppression resulting in high percentages of non-target species bycatch. The most vulnerable to the effects of gill-netting appears to be whitefish spp., comprising approximately 60% of total bycatch. Catch rates of whitefish spp. were highest for all seasons highlighting considerable habitat overlap with pike in the LCR throughout the year. This habitat overlap was reflected in stomach contents of pike captured in this reach with 44% overall diet consisting of mountain and lake whitefish. Paired with smaller numbers of rainbow trout and kokanee, LCR pike diets consisted of 50% salmonid species. This preference for salmonids has been thought to be the primary driver for declines of once healthy Chinook salmon, Coho salmon, Sockeye salmon, and rainbow trout stocks in Southcentral Alaska (Rutz 1999; Sepulveda et al. 2015). While the Columbia River pike invasion is still in its infancy and significant reductions in salmonid populations have not been observed, one can draw on examples from the Southcentral Alaskan pike invasion to gain insight into the long term outcome in the LCR if pike numbers are not controlled.

One can also draw on comparisons to the Pend d'Oreille River, a stark example of the long term effects of freshwater biological invasions (both plant and animal) where once plentiful trout and whitefish spp. are dominated by a variety of invasive species including yellow perch, smallmouth bass, tench, pumpkinseed, black crappie, black/brown bullhead, walleye and northern pike. Increased competition from the multitude of invasive fish species, paired with differences in prey availability is reflected in the body condition of Pend d'Oreille pike, which was in general lower than that of Columbia River pike feeding largely on salmonids and sculpin species. Pike diet in the Pend d'Oreille was made up primarily of yellow perch and smallmouth bass (>90%) and approximately 50% of all fish caught by gill-netting were invasive species. Native bycatch consisted almost exclusively of sucker spp. and northern pikeminnow with the exception of a single mountain whitefish, providing a snapshot of what fisheries in the Columbia River could look like if left unmanaged.

Microchemical analysis of otoliths suggests movement of pike from the Pend d'Oreille River to the Castlegar area is limited, with only one of fifty fish appearing to have moved from the Pend d'Oreille. From a management standpoint for B.C. this is positive as it suggests the influx of pike from the Pend d'Oreille is minimal, yet the extent of movement downstream into Lake Roosevelt is unknown and could potentially be a significant input into rapidly expanding numbers in Washington. Gill-netting in Waneta reservoir in May 2017 led to the capture of several sexually mature pike within approximately 1 km of Waneta Dam, highlighting the proximity of pike to the dam and potential for movement into the Columbia. Future years of suppression in B.C. should include the lower reaches of the Pend d'Oreille, Waneta Reservoir in particular, as it is the last reach before draining into the Columbia.

Acoustic tracking of fish showed a portion of Columbia River pike travel considerable distances between B.C. and Washington, demonstrating the need for collaboration between provincial and state fisheries managers, and indigenous groups and governments to effectively control pike on both sides of the border. Further investigation into the movements of pike in the Columbia should be considered for future years, including the deployment of additional receivers in Washington to locate source populations and potential habitat to target for suppression. The current distribution of pike in the Columbia spans a considerably greater area in Washington, with pike present in approximately 220 km of river between the border and Grand Coulee Dam. A northern pike was recently (Nov 2018) captured within 10 miles of the Grand Coulee Dam indicating there is a real threat of pike continuing to spread downstream in the Columbia (Francovich 2018). If northern pike breach the Grand Coulee Dam they can spread into systems such as the Okanogan River, posing the risk of impacting anadromous salmonid stocks as seen in Southcentral Alaska. The number of northern pike in B.C. also pale in comparison to numbers in Washington, with over 8,000 northern pike captured through suppression in Lake Roosevelt since 2015 (Francovich 2018) compared to fewer than 400 captured in B.C. since 2014. This could, however, rapidly change if northern pike enter the Arrow Lakes via the navigation lock at HLK Dam, which extends 232 km north of the dam to Revelstoke. Current dam operations pose a serious risk of northern pike passage through the lock as the downstream gate is often left open for extended periods of time awaiting return traffic. Acoustic telemetry data collected through this study showed pike in the Castlegar area occasionally come within close proximity to the navigation lock, with multiple detections recorded at the dam acoustic receiver (RKM 0.1). As a first line of defense it would be advisable to alter lock operations to minimize the risk of pike passage into the Arrow Lakes, simply by closing the gate immediately after traffic exits the lock. Additionally, the implementation of a non-physical barrier at the lock to prevent fish movement would also be advisable to further deter pike movement. The rapid expansion of northern pike in Lake Roosevelt over the last decade is a good indicator of the potential outcome of pike entering the Arrow Lakes, highlighting the need for pre-emptive control measures in British Columbia.

In conclusion, this study provides a framework for future years of northern pike control in the Columbia River in B.C., and demonstrates the need for collaboration between provincial and state fisheries authorities to preserve a culturally and economically important fishery in the province.

Literature Cited

Audy, N., Zimmer, M. (2015). Fish habitat structure monitoring in Pass (Norns) Creek 2013. Report prepared for the Columbia Basin Fish & Wildlife Compensation Program, Castlegar, B.C.

Dunker, K., P. Bradley, C. Jacobson. 2018. Operational Plan: Threemile Lake invasive northern pike population assessment. Alaska Department of Fish and Game, Regional Operational Plan ROP.SF.2A.2018.11, Anchorage.

Francovich, E. [Internet] (2018). Invasive northern pike found 10 miles from Grand Coulee Dam, Spokane Tribe catches 45-inch fish. The Spokesman Review (Newspaper). Accessed from: http://www.spokesman.com/stories/2018/nov/15/invasive-northern-pike-found-10-miles-from-grand-c/.

Rutz, D.S. (1999) Movements, food availability and stomach contents of northern pike in selected Susitna River drainages, 1996–1997. Alaska Department of Fish and Game, Fishery Data Series, No. 99 - 5.

Sepulveda, A.J., Ruts, D.S., Dupuis, A.W., Shields, P.A., Dunker, K.J. (2015). Introduced northern pike consumption of salmonids in Southcentral Alaska. *Ecology of Freshwater Fish*, 24(4): 519 - 531.

Appendix A

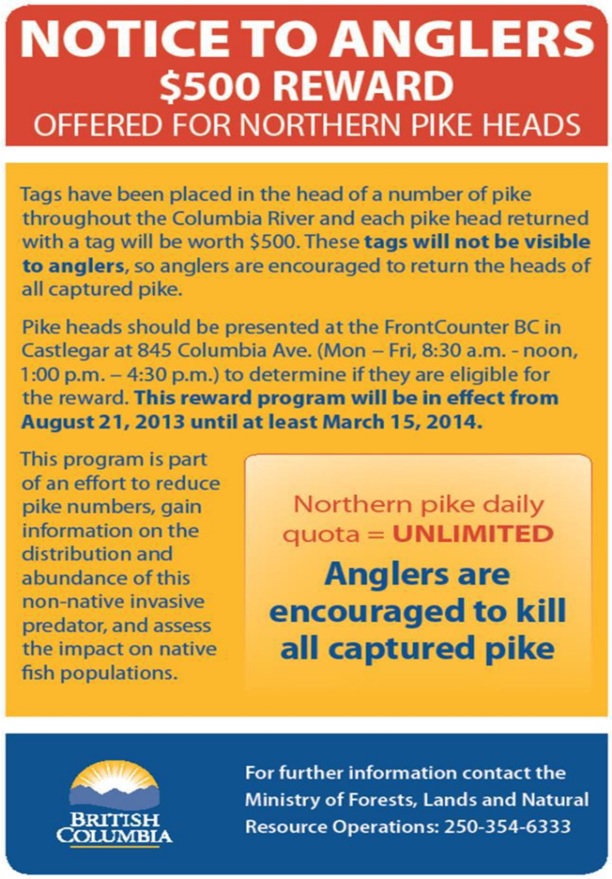

Figure A 1. Northern pike angler incentive signage created by the BC Ministry of Forests, Lands & Natural Resource Operations.

Table A 1. Grading scale for northern pike cleithra used for age estimates (provided by North/South Consultants Inc., MB)

Cleithra Quality	**Attributes**	**Aging Result**
Very Good (VG)	Annuli are clear with no interpretation problems	Reader always gets the same age
Good (G)	Annuli are clear with a few easy interpretation problems	Reader would get the same age most of the time for fish <10 years, within one year for fish 11-20 years
Fair (F)	Annuli are fairly clear with some areas presenting easy and moderate interpretation problems	Reader would be within 1 year most of the time for fish<10 years and 2-3 years for fish >10 years
Poor (P)	Annuli are fairly unclear presenting a number of difficult interpretation problems	Reader would be within 2-3 years most of the time for fish <10 years and 4-5 years for fish >10 years
Very Poor (VP)	Annuli are very unclear presenting significant interpretation problems	Reader has little confidence in repeatability of age within 4-5 years

Appendix B

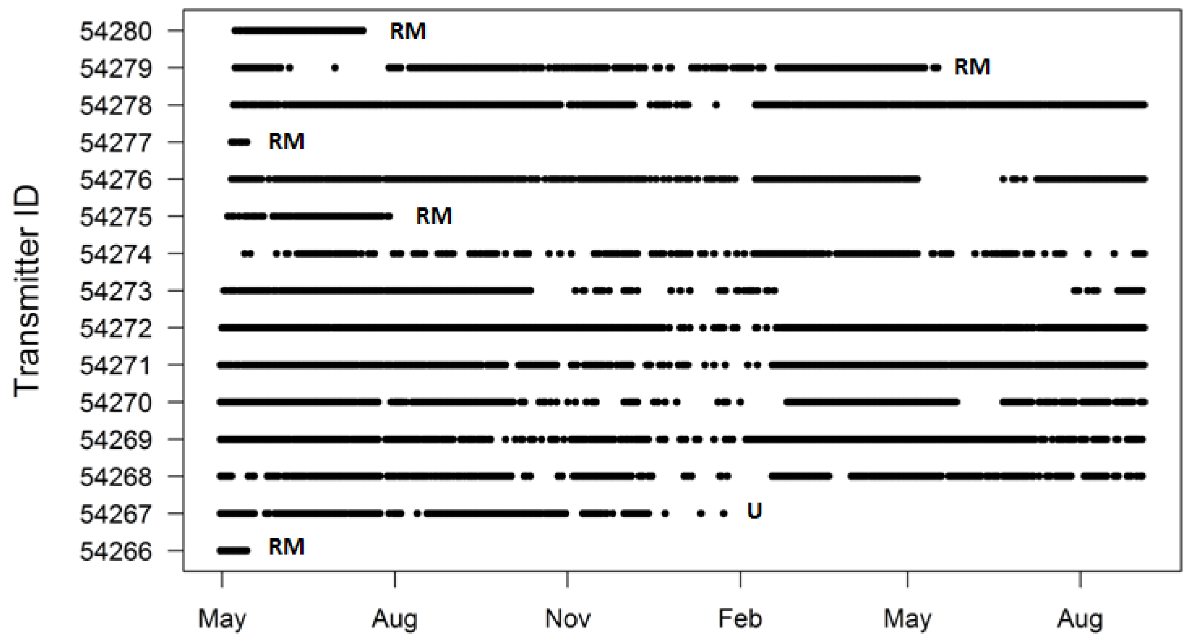

Figure B 1. Detections recorded for pike tagged with acoustic transmitters between May 2016 and September 2017, with each point representing a detection of the respective transmitter logged by any receiver within the Columbia River VR2W acoustic receiver array in B.C. (Figure 2). A total of 9 transmitters were still active at the conclusion of the study; 3 tagged pike were recorded mortalities (RM) by members of the Columbia River pike suppression team (54266, 54277, 54279), 2 RM's by anglers (54275, 54280), and 1 unknown termination (U) (54267). Transmitter 54273 was not detected for approximately 6 months between February and August, 2017.

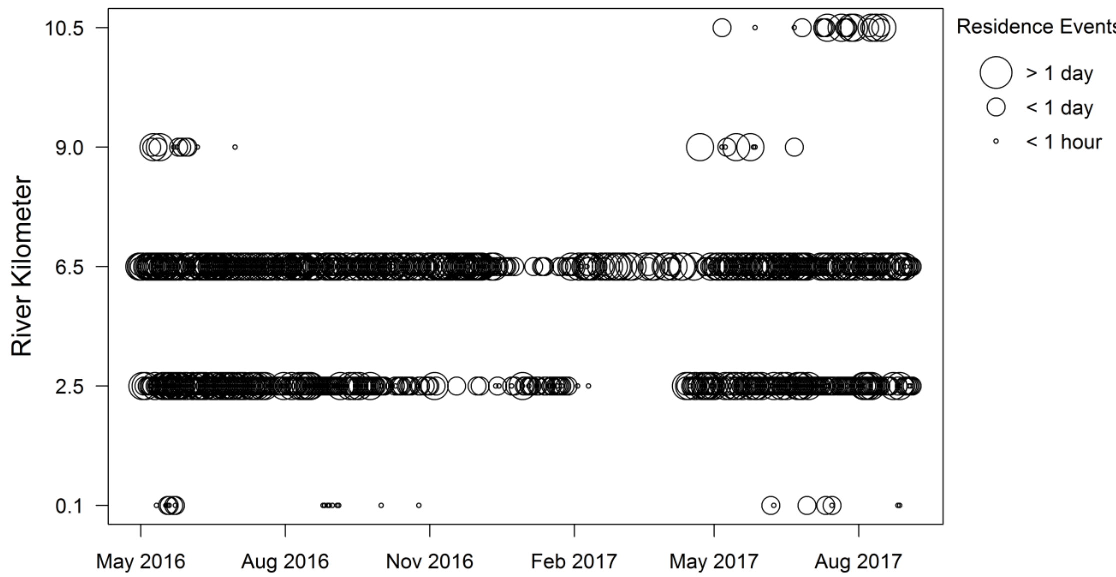

Figure B 2. Residence events calculated for VR2W acoustic receivers in the Castlegar area. A residence event was initiated if a transmitter was detected more than once at a receiver, and terminated if the transmitter was not detected for more than 12 consecutive hours or was detected by another receiver. Residence events were scaled by time spent at each receiver and grouped into 3 categories: < 1 hour; < 1 day; > 1 day. All but one tagged pike stayed within the range of these receivers for the duration of the study.

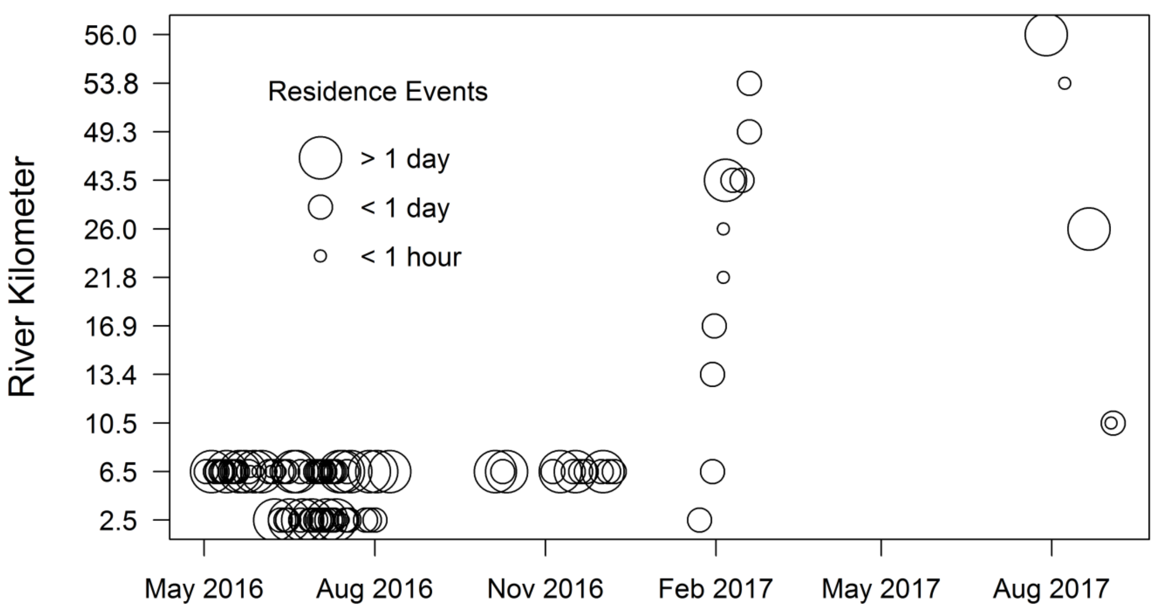

Figure B 3. Residence events calculated for transmitter ID 54273 throughout the LCR. A residence event was initiated if a transmitter was detected more than once at a receiver, and terminated if the transmitter was not detected for more than 12 consecutive hours. Residence events were scaled by time spent at each receiver and grouped into 3 categories: < 1 hour; < 1 day; > 1 day.

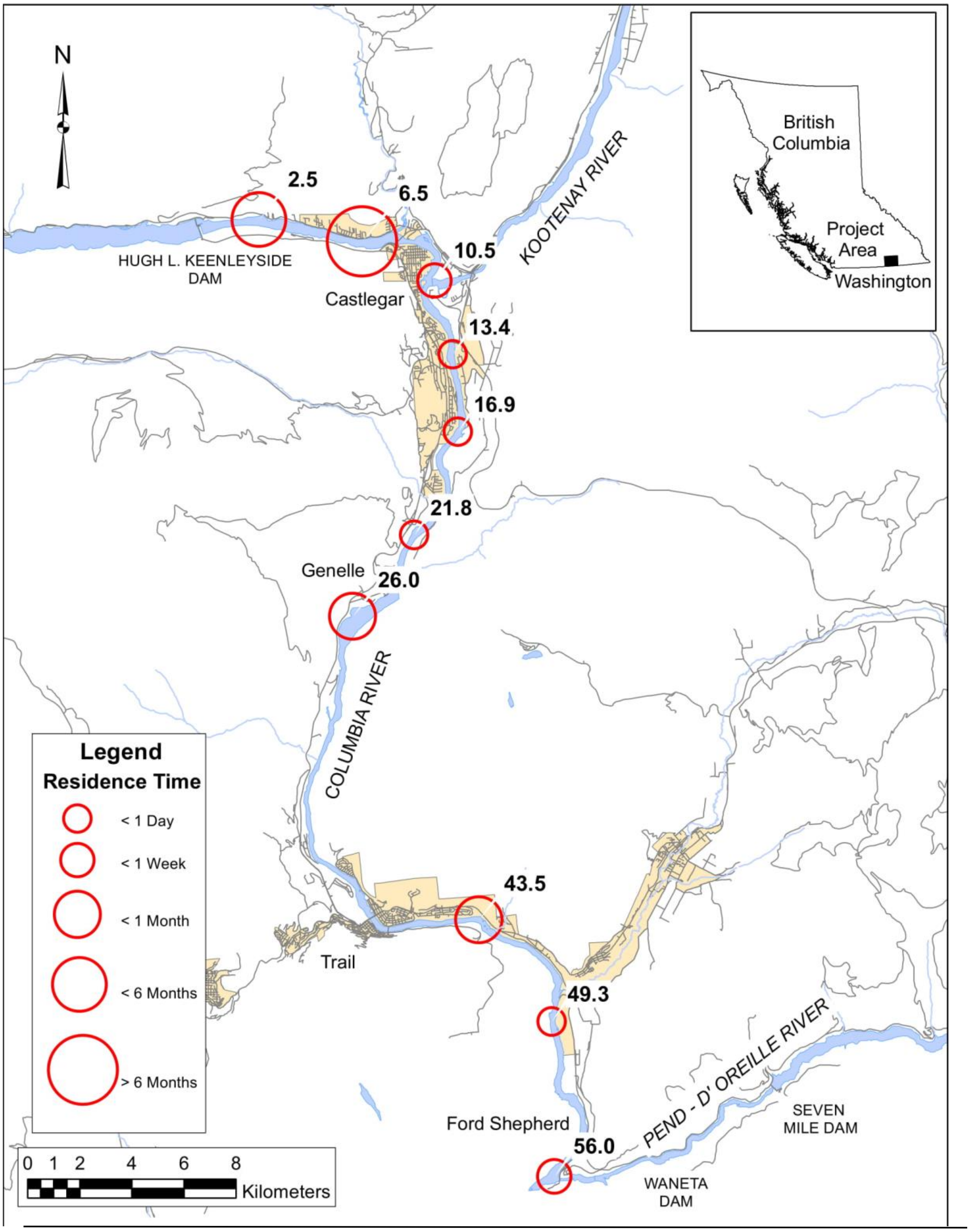

Figure B 4. Total residence time logged in the lower Columbia River for transmitter ID 54273 between May 2016 and September 2017. Residence time includes time spent during both the downstream and upstream migrations of this fish.

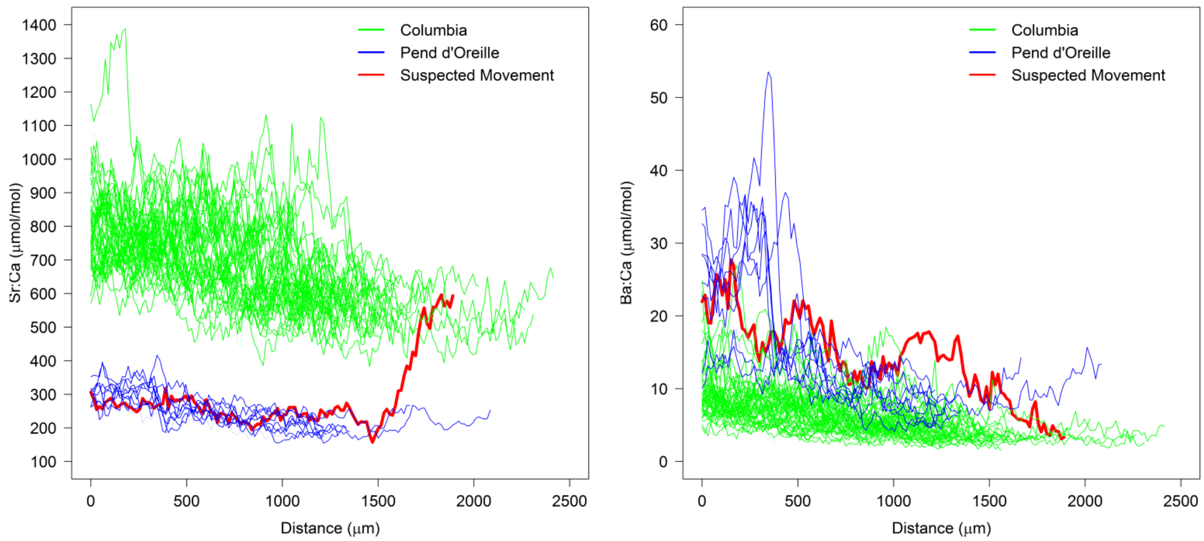

Figure B 5. Otolith Sr:Ca and Ba:Ca ratios (µmol/mol) of northern pike captured in the (n=50) and Pend d'Oreille (n=10) Rivers. Highlighted in red is a shift in otolith chemistry observed for a single pike captured near Castlegar, B.C., indicating potential movement from the Pend d'Oreille River.