PAIN IN THE BASS: DIET AND DISTRIBUTION OF INVASIVE SMALLMOUTH BASS (*MICROPTERUS DOLOMIEU)* IN CULTUS LAKE, BRITISH COLUMBIA

by

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ABSTRACT

Invasive species are widely recognized as the second greatest threat to biodiversity loss. With rapid international trade, unpredictable human activity, and a lack of preventative action, these introduced species are spreading more each year. Invasive predatory fish can have devastating consequences on native species and are near impossible to eradicate from a waterbody without the use of chemicals. As a result, effective measures for fish suppression needs to be creative, sustainable, and well-planned. Since their illegal introduction in 2017, smallmouth bass (*Micropterus* dolomieu) have proliferated in Cultus Lake, British Columbia and impacts on two species-at-risk, Cultus Sockeye salmon (*Oncorhynchus nerka*) and Cultus pygmy sculpin (*Cottus aleuticus*) are unknown. By completing a diet analysis and monitoring bass movement, we documented their impacts and laid the groundwork for a sustainable suppression plan. For the diet analysis, bass (*n* = 204) were sampled in spring/summer 2020-2021 in Cultus Lake. DNA barcoding (*n* = 145) and a visual analysis (*n* = 204) of diet was completed. Diet composition, factors influencing the predation of species-at-risk, and dietary shifts were analyzed using R. DNA analysis identified 32 more taxa at the family level than morphological analysis. Multiple logistic regression showed that bass were more likely to predate on salmon within the spawning grounds, and over 90% of bass had sculpin in their diets. Diet composition did not shift as bass size changed, demonstrating a strong predation on fish from 100 mm to > 300 mm total length. Then, to monitor bass movement, 43 bass were tagged with acoustic transmitters, and a receiver array was constructed in the lake. The bass were tracked for 16 months, and snorkel surveys supplemented the movement data. Bass are spawning in a 1 km stretch along the north shore of Cultus Lake, starting in early May until mid-June. They remain above the thermocline throughout the summer until water temperature drops, and they migrate offshore to deeper water for a less active winter period, eventually resurfacing in April for spawning. The ideal timing for suppression is during this congregated spawning period when adult male bass remain on the nests in 0.5 – 2.5 m of water for 6 weeks. We recommend trialing spearfishing and nest destruction in a controlled setting to suppress the population for the following reasons (1) bass are congregated in a 1 km stretch, and nests are not deeper than 2.5 m (an easy depth for snorkeling) (2) after snorkel surveys in 2021, we know that male bass guarding the nests do not move until snorkelers are closer than 1 – 2 m (3) adult spawning males are large enough for spearfishing (4) nests with eggs/fry can then be destroyed via burial, electrofishing, or natural predation from white suckers. We also recommend continuing outreach programs to aid in a preventative approach to further reduce the spread of invasive species.

Keywords: British Columbia, Invasive, *Micropterus dolomieu*, Acoustic Telemetry, Species-at-Risk, Diet

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CHAPTER 1
INTRODUCTION

In 2017 Smallmouth bass (*Micropterus dolomieu*; SMB) were detected in Cultus Lake; the first confirmed establishment of the species within the lower mainland of British Columbia, Canada. This is of particular concern given the presence of Sockeye salmon (COSEWIC status Endangered) and Cultus Lake coastrange sculpin (SARA and COSEWIC status Threatened) in Cultus Lake. SMB are voracious predators and have been documented predating on both salmon and sculpin. A working group of government, academic, and local stewards was established to create a plan for monitoring and future suppression work. This thesis is a summary of the research conducted from 2020 – 2021 on the invasive population of smallmouth bass in Cultus Lake.

SMALLMOUTH BASS ECOLOGY

Smallmouth bass (*Micropterus dolomieu)* (SMB) are a medium sized fish, with a deep-bodied shape and a slightly forked caudal fin (Figure 1.1). Their colouration can range from golden brown through to olive green, fading to a white underside. Often the fish exhibit 8-15, patchy, vertical stripes along their body, slightly darker than their base colour. The anal fin is slightly smaller than the dorsal fin, which has both leading spiny rays, and posterior smooth rays. The jawline does not pass the eye, which is a distinguishing feature between small and largemouth bass. The fork length of this species rarely exceeds 50 cm, and they typically weigh less than 2 kg (Brown et al. 2009).

Figure 1.1. Smallmouth bass caught in Cultus Lake, BC. Source: Wendy Margetts.

Smallmouth bass reach maturity between 3 – 6 years old and live approximately 13 years (Becker 1983; Brown et al. 2009). There are four key stages at the start of development for smallmouth bass: egg, egg-sac, swim-up, and free-swimming fry (Cooke et al. 2002). The egg and egg-sac period of the bass life is typically between 1-2 weeks. The remaining time, in the free-swimming fry stage, can be between 2-3 weeks (Ridgway 1987), during which time male bass fiercely defend the fry (Cooke et al. 2002). The free-swimming fry are darkly pigmented for 1 week and are referred to as black fry (Gillooly and Baylis 1999), until they develop scales and their green/brown colour (Ridgway 1987). By the end of the first growing season, young of the year typically reach between 40-100 mm. Young smallmouth bass will also have a discrete orange marking on the base of their caudal fin (Tovey et al. 2008). In Canada, bass can live up to 15 years old.

Native Range and Recreational Use

The native range of smallmouth bass in North America extended throughout the Great Lakes and St Lawrence River System in Canada, and throughout Ohio, Tennessee, and the upper Mississippi rivers in the United States (Scott and Crossman 1973). They are seen as a prized sport fish for anglers due to the fighting nature during angling. Much of the current literature on the species is based in northern Ontario Lake systems (Ridgway et al. 1991; Rejwan et al. 1997; MacRae and Jackson 2001; Cooke et al. 2003), where populations are stable.

Diet

Smallmouth bass are carnivorous, eating a variety of foods directly correlated with their body size. At the larval stage, fish are still consuming the yolk sac attached to their bodies. As they grow into independent fry, they begin their predatory lifestyle feeding on copepods, water fleas and other zooplankton, but staying close to the well-guarded nest. As the fish transform into juveniles, they start eating larger aquatic insects and small crayfish (Weidel et al. 2000). Once the fish have reached 50 mm, they become heavily piscivorous, opportunistically consuming small fish, proportionate with their growing size (Pflugr and Pauley 1984). A second noticeable diet shift occurs when the bass start choosing more and larger fish species. This occurs when the bass reach 150 mm (Weidel et al. 2000).

As adults, smallmouth bass are aggressive carnivores, preying on a wide variety of aquatic species throughout both the pelagic and littoral zones of the lake (Weidel et al. 2000). Besides their dominantly piscivorous diet (Beck 2013), bass also consume species such as crayfish and frogs (Pflugr and Pauley 1984; Weidel et al. 2000; Berra 2007), potentially causing a negative impact on the amphibian community (Kiesecker and Blaustein 1998). The bass have also been documented exhibiting cannibalistic behaviours, with large adult bass consuming juveniles (Fisheries and Oceans Canada 2013). Additionally, bass have been observed taking advantage of seasonal pulses of food availability, rapidly altering their diet to maximize predation during fry migration (Beck 2013).

Because of the bass' ability to survive and thrive in a wide variety of environments, there are only a few times when feeding becomes an issue for the species. For example, if the lake becomes turbid due to wind or recreation, this can significantly decrease the probability of a fish's ability to react to an item of prey (Sweka and Hartman 2003). Bass feeding is also greatly reduced when water temperatures are below 8.5 – 10°C (Keast 1968; Shuter et al. 1980). If juveniles have not sufficiently grown by this time, their winter reserves may be too low, which may lead to winter kill (Keast 1968).

Movement and Habitat

Strong indicators of bass habitat are water depth and water temperature (Ettinger-Dietzel et al. 2016). Studies using ultrasonic tracking have shown that smallmouth bass generally remain in the littoral zone throughout warmer months. Their depth range is typically between 0 – 5 m, especially during the spawning season (Fayram and Sibley 2000; Suski and Ridgway 2009), and rarely deeper than 12 m (Tabor et al. 2010). In the winter they often migrate farther offshore to greater depths, typically between 12-15 m depending on the lake size (Ettinger-

Dietzel et al. 2016). Movements throughout the water column are closely linked to the seasonal changes in the thermocline's presence and position, staying above the thermocline in summer months and below it in the winter (Suski and Ridgway 2009).

Bass have been found in greater abundances in unvegetated areas, demonstrating their lack of need for vegetative cover (Bryan and Scarnecchia 1992). This in turn shows the bass ability to flourish in developed areas where vegetation has been removed for recreational or commercial purposes. Bass prefer shorelines composed of sand, gravel, pebbles, and large boulders (Wiegmann et al. 1992). They appear to have little preference in lake depth and size, adapting easily to a diversity of lakes across North American (Brown et al. 2009), as long as temperatures reach 15°C in summer months to initiate spawning (Kaemingk et al. 2011a). Bass also thrive in river environments, such as the Ozark River (Ettinger-Dietzel et al. 2016), Wolf Rivers (Langhurst and Schoenike 1990), Columbia River (Tabor et al. 1993), and many more.

Smallmouth bass frequently exhibit both seasonal and daily migrations, depending on the structure of the watershed. With access to river systems, bass have been observed making long seasonal migrations, demonstrating their ability to spread easily through new environments. By age 2, fish are able to migrate up to 87 km during the fall and spring from spawning grounds to their over-winter locations (Langhurst and Schoenike 1990). Bass also migrate within large lake systems, spending winters in deeper lakes, and migrating back to spawning grounds in the spring (Tabor et al. 2010). On a smaller timescale, bass have been observed to complete diel vertical migrations, often moving closer to the surface at night and slightly deeper during the day (Suski and Ridgway 2009).

INVASIONS OF SMALLMOUTH BASS

Invasive Characteristics

The impacts of aquatic invasive species are well documented, and known to cause a loss of biodiversity, alter ecosystem functions, and disrupt trophic structure (Pimentel et al. 2005; Sanderson et al. 2009; Wainright and Muhlfeld 2021). The spread of these species is becoming more prevalent, with humans causing both intentional and unintentional introductions (Loppnow et al. 2013). Rational for introducing a non-native species often includes anthropogenic motives such as enhancing sport fishing, commercial fishing, and the release of baitfish (Carey et al. 2011; Drake and Mandrak 2014). Once established, invasive species use resources, occupy habitat, and outcompete individuals, displace native species, and disrupt the natural system balance (Kerr 2000).

Understanding the characteristics of good invasive species, has been a long-debated topic. Much of this arose in the 1990's with Williamson and Fitter (1996), and the propagule pressure concept. This concept states that the first trait of a successful invader is the frequency and intensity at which they are introduced to the ecosystem. Since then, extensive literature has been published on invasive species characteristics. Some of these include the species' physiological tolerance to new environments or generic habitat-modeling approach (Marchetti et al. 2004; Marvier et al. 2004; Sol et al. 2012). There is also a host of environmental conditions that may assist in the establishment of invaders such as lack of predators, abundance of prey, disturbed areas, and new associations between parasites and host (Pimentel et al. 2005).

Some more specific characteristics of invasive species that enhance their ability to establish themselves are as follows: large brain to body size ratio, high reproduction rate, and similarities between their original and new environment. In past research, there has been a focus on the high fecundity trait of invasive species; however, Sol et al. (2012) suggest that good invaders can be characterized with life-history strategies that distribute reproductive effort across a number of reproductive stages (i.e., with longer survival time, the individual has more time to learn the ecosystem and adjust their behaviour towards more adapted reproductive strategies). Another shift in invader characteristic concepts is the idea that invasion is a process with three distinct steps, transport, establishment and spread, and species need specific traits to help them with each stage (Williamson and Fitter 1996; Kolar and Lodge 2001). Overall, there is no single strategy to a good invader. Instead, a combination of characteristics and environmental factors lead to a successful establishment (Sol et al. 2012).

Smallmouth bass (*Micropterus dolomieu*) are a spiny-rayed fish that exhibit numerous adaptable traits, making them an ideal competitor for resources in new habitats (Tovey et al. 2008). One trait is their spiny rays, that act as a defense mechanism against curious predators. SMB can survive, in a wide range of temperatures (Cooke et al. 2003), as long as temperatures reach 15°C for spawning (Kaemingk et al. 2011), are opportunistic carnivores, preying on groups such as insects, fish, crustaceans, and amphibians (Pflugr and Pauley 1984; Kiesecker and Blaustein 1998; Weidel et al. 2000; Berra 2007), and are known to extirpate small-bodied fish from lakes (MacRae and Jackson 2001).

Current Distribution and Impacts on Native Habitats

The first human aided relocation of SMB occurred in the mid-1800s through the Erie Canal to New York State. Soon after they were moved to New England, New Brunswick and Nova Scotia (Fisheries and Oceans Canada 2009). Since then, SMB have been spread internationally (Loppnow et al. 2013), and their current distribution has been aided through many means such as illegal introductions, fisheries stocking, and natural migration through drainage networks (Funnell 2012). Smallmouth bass are now present in almost every state and province of the United States and Canada. In the United States, smallmouth bass can be found in every state except for Alaska, Florida, and Louisiana (Brown et al. 2009; Fuller et al. 2019). In Canada, the species has been extensively spread to provinces including Nova Scotia, New Brunswick, Quebec, Ontario, Manitoba, Saskatchewan, and British Columbia (Tovey et al. 2008). Although native to Ontario, their distribution has extended throughout the province to an additional 823 lakes (Kerr 2000) and as far north as Timmins, ON (Brown et al. 2009).

SMB were first introduced to British Columbia in 1901 through an authorized introduction aimed at improving local sport fishing (Fisheries and Oceans Canada 2010). Since then, they have been introduced into five regions of British Columbia: Columbia, Thompson, Upper Fraser, Lower Mainland, and Vancouver

Island (Tovey et al. 2008) (Figure 1.2). In recent years, these introductions have been through illegal means, often to boost sport fishing (Carey et al. 2011). The population in Cultus Lake is the first established population in the Lower Mainland. In 2010 Fisheries and Oceans Canada completed a risk assessment of smallmouth bass in British Columbia (BC). In the assessment they concluded that lakes in the Chilliwack area (Lower Mainland) had a suitability rating of 81-90% for SMB habitation. In general, due to factors such as climate and availability of prey, the lower mainland is expected to be highly impacted by the spread of smallmouth bass (Fisheries and Oceans Canada 2010). More broadly speaking, most of the central and southern portions of British Columbia are suitable habitat for smallmouth bass (Mandrak et al. 2010).

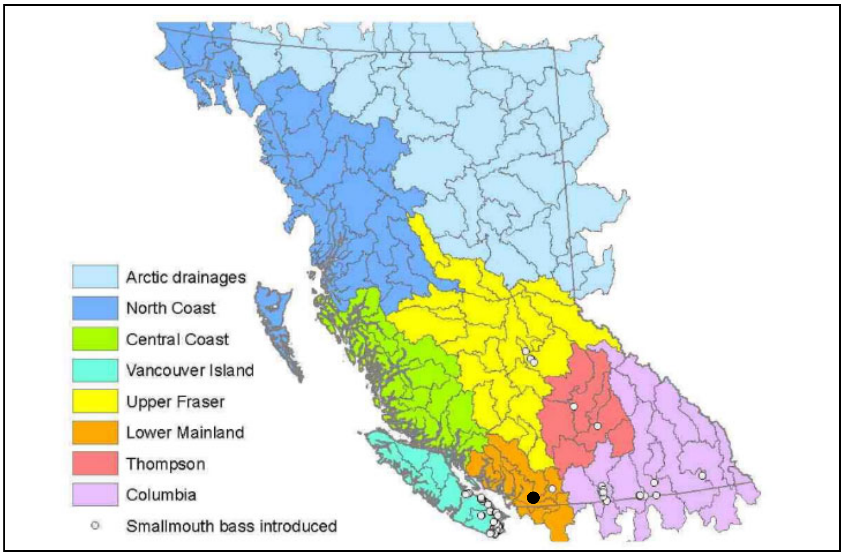

Figure 1.2. Known locations of smallmouth bass in British Columbia as of 2008 (Tovey et al. 2008). Black dot represents Cultus Lake.

A major concern surrounding the continual spread of smallmouth bass throughout British Columbia is their effects on local salmon populations. Many studies have shown that smallmouth bass feed on salmon juveniles, especially during smolt outmigration when habitats overlap (Fayram and Sibley 2000; Tabor et al. 2007; Emingway et al. 2019) and can lower the total energetic densities of diets in competing native fish (Beck 2013). However, the impacts that smallmouth bass have on overall salmon mortality vary greatly depending on the study region. The fact that bass feed on salmon appears to be undeniable, but often their habitats only overlap during outmigration, minimizing the impact on the salmon (Rieman et al. 1991; Tabor et al. 2007). Since Cultus Lake contains an endangered population of sockeye salmon that spawn within the lake, the two species' habitats may overlap for longer periods of time, increasing the risk of predation on juvenile salmon.

A second concern with smallmouth bass introductions is their cumulative effects on the ecosystem. Bass ferociously consume smaller fish, can out-compete species in similar habitat ranges, and can quickly become a dominant player in the food web (Fisheries and Oceans Canada 2009). Smallmouth bass also have negative effects on small-bodied fish populations. Lakes invaded with bass show an average of 2.3 fewer small-bodied species, as well as extirpation of some groups such as sticklebacks, minnows, and dace. The bass can also alter the habitats of the small-bodied species, forcing them to live in complex shoreline zones, hiding from the voracious predator (MacRae and Jackson 2001). Cultus Lake supports 19 species of native fish, many of which are small bodied (Schubert et al. 2002).

Finally, Cultus Lake is connected to major salmon spawning rivers (Vedder River, Fraser River) via Sweltzer Creek. Since bass are known to thrive in both lakes and rivers, there is great concern over their migration into neighbouring tributaries. There is a fish fence on Sweltzer Creek that is closed during the spring to count migrating salmon, and SMB have been found at the fence, showing their interest in moving downstream. The November 2021 flooding that occurred in the Lower Mainland may have also allowed for easier transportation of SMB through enlarged streams. The total effects of this event on the SMB will be apparent in years to come.

Suppression and Species Management

The challenge for fisheries managers in finding reliable removal methods for invasive fish populations is the lack of information on the effectiveness of techniques (Rytwinski et al. 2019). Also, their popularity in competitive sport fishing events (Funnell 2012), creates additional difficulties in the management of the species. Fisheries agencies must balance the demand of the sport fishing industry with the threat that smallmouth bass pose to migrating salmon and other native species. Management practices have included increasing the allowable catch for size and number and implementing mandatory kill policies (Carey et al. 2011). Halfyard (2010) wrote a comprehensive report for Fisheries and Oceans Canada reviewing containment and eradication methods for smallmouth bass in Canada.

One highly effective method of species removal is rotenone, a naturally occurring chemical substance found in the roots of some plants in the *Fabaceae* Family (American Fisheries Society 2000). When sprayed, it interferes with the electron transport chain in the fish's mitochondria and inhibits metabolic processes (Colpo et al. 2017). An example of a successful rotenone treatment on smallmouth bass is the Rondegat River in Cederberg, South Africa. In 2012, 500 bass were killed in the first treatment, and in 2013 only 1 was found and killed. Snorkel surveys in the following summer detected zero bass post-application (van der Walt 2013). Before the rotenone application, bass had contributed to the eradication of four out of the five native fish species in the river (Woodford et al. 2005). The successful treatment showed immediate results, with an instantaneous increase in native species populations (Weyl et al. 2014).

In many lake systems, chemicals are not a viable option and can be publicly controversial (Simberloff et al. 2005). If lakes are too large, contain endangered species, or are highly popular for recreational users, rotenone may not be used (Ling 2003) (as is the case with Cultus Lake). Without rotenone, a combination of removal methods leads to the greatest decrease in population size. Boat electrofishing and beach seining appear to be two of the most successful methods of suppression (Burdick 2008; Biron et al. 2014; Rytwinski et al. 2019). An example of this comes from an eradication program on the Yampa River, Colorado (2003-2007) where boat electrofishing and beach seining was used to suppress the population of SMB. They found that after intensive removal, the abundance of smallmouth bass decreased but the population was not completely removed, and that immigration from separate populations was a contributing factor to recruitment (Hawkins et al. 2009).

If viable, barriers can be an effective method of stopping the migration of smallmouth bass during spring and fall. Bass have been documented migrating through watersheds (Langhurst and Schoenike 1990; Tabor et al. 2010) and creating barriers can physically stop these movements. A study in Oxford County, Maine quickly found that with the large size of the waterbody, eradication of the species was not feasible. Instead, they constructed a barrier which successfully restricted the spread of the bass into nearby tributaries (Boucher 2007). The location of the fish fence at Sweltzer Creek may be a viable option to slow the migration of SMB into neighbouring water bodies.

There have been a few major eradication efforts in Canada using mixed method techniques to remove smallmouth bass. Ministry of Forests, Lands and Natural Resource Operations and Rural Development (FLNRORD) discovered smallmouth bass in the Cariboo Region, British Columbia in 2003. By 2007 the population had spread throughout the Beaver Lake System, posing a threat to all central British Columbia. A combination of methods were used in 2007, 2008 and 2009 to control the population. Some of these techniques included gillnetting, beach seining, physical barriers, and dip netting larva from rocky nests. Rotenone was considered as a potential treatment solution but was deemed unfeasible due to the size and complexity of the system. The cost alone to purchase rotenone for the Beaver Lake System was estimated at $2,500,000 (Gomez and Wilkinson 2008). None of these methods were fully successful in suppressing the bass and management wrote that future control efforts should focus on removing juveniles (Gomez and Wilkinson 2008).

A second example of mixed method eradication efforts in Canada comes from Lake Miramichi in New Brunswick. In 2008, smallmouth bass were detected in Lake Miramichi, and were assessed to pose a high risk for the Atlantic Salmon population if they spread throughout the watershed (Fisheries and Oceans Canada 2009). Barriers were first installed at the lake’s outlets and monitored daily to allow for other species to pass during yearly migrations. Boat electrofishing was the most efficient method for removing young of the year, followed by beach seining (Fisheries and Oceans Canada 2013). Few bass were found reproducing in years following the eradication efforts, and continued fishing pressure is necessary to keep the population low (Biron et al. 2014).

To succeed in eradication or control of the population a combination of high intensity, several continuous years and multiple control methods is necessary (Simberloff 2007). Managers have found that early intervention with invasive fish, increases the probability of successful eradication or containment of the species (Halfyard 2010). Creating initial models to better understand the system and variables affecting smallmouth bass recruitment can also streamline the suppression method selection process (Sharma et al. 2009). Often, the most successful non-chemical eradications use low-tech solutions and intensive manual labour. Many projects in the United States rely heavily on volunteers to perform eradication tasks, and some initiatives have started programs where convicts use community service time to help with invasive species management (Simberloff 2007).

Another method for stopping the spread of invasive species may be to take proactive rather than reactive measures (Finnoff et al. 2006). It is well-known that once an aquatic species is introduced into a watershed, it becomes very difficult to fully eradicate. However, cost-benefit analysis of investing in proactive measures often leave large areas of scientific uncertainty (Finnoff et al. 2006). This risk can leave policymakers and economists unenthused, and more likely to invest once the issue is present, rather than before (Simberloff 2007). The precautionary principle to environmental science is a long-standing approach that focuses on preventative methods (Cooney 2004). It explores all possible alternatives to environmentally harmful activities and highlights the importance of increasing public participation in policy making (Kriebel et al. 2001). These methods should be incorporated into aquatic invasive species government policy.

Prevention, in terms of invasive species, can be implemented using different methods including public engagement, education (Pimentel et al. 2005), and monitoring. By informing resource users of the harm that invasive species cause, they can make more informed decisions in their daily lives. For example, anglers who introduce invasive fish into new lakes may not understand that those species could negatively affect populations of other popular sporting fish in the lake. For this reason, engagement through newsletters, events, social media platforms and educating local leaders are all viable options. Wide-spread monitoring for early detection and rapid response, and intense localized effort during the early stages of establishment for high priority invasive species (Chai et al. 2016) are additional preventative actions that can be taken. However, these efforts are often stifled by vague policies, insufficient funding and a lack of ecosystem knowledge (Simberloff et al. 2005).

In the planning stages of this project, there was great enthusiasm to develop a stewardship program and hold outreach events. The project is in collaboration with multiple government and non-governmental organizations, and so there were resources to spread awareness about the invasive SMB. However, the start of the project in Spring 2019, coincided with the beginning of COVID 19 and so all events had to be put on hold. We were able to install permanent signage around the lake informing users of the issue and the project. We also held a well-attended online seminar and recruited volunteers from the local Cultus Lake Stewardship Society (CLASS) to help with various aspects of the project. Additionally, we had posters around the community and in a local tackle shop with information regarding the impacts of the bass, and how to become a steward for the lake.

STUDY SITE

Cultus Lake (49.054910, -121.987446) is in the southwestern corner of British Columbia, near the city of Chilliwack, approximately 80 kilometers east of Vancouver (Figure 1.3). The lake sits at 47 m in elevation, has an area of 631.1 ha, a perimeter of 13.5 km, and a maximum depth of 42 m. The Chilliwack River flows 2.4 km north of Cultus Lake and connects to the lake through the outflow of Sweltzer Creek. Several other creeks flow into the lake including Frost, Windfall, Redtail, and Reservoir Creek. The biogeoclimatic zone is Coastal Western

Hemlock (CWH) with subzones Dry Maritime (dm) and Very Dry Maritime (xm) (Pojar et al. 1991).

Cultus Lake is surrounded by mixed use zoning including two residential areas on the north and south ends of the lake, and Cultus Lake Provincial Park. The Park was established in 1948 and spans 2729 ha (BC Parks 2019). The lake's shoreline drops off steeply in most areas, except along the north shore, and a small area around Spring Bay. The littoral zone includes areas of soft mud, organics (invasive Eurasian milfoil: *Myriophyllum spicatum*), gravel, and large cobble. Both depth and substrate are important indicators of smallmouth bass spawning areas (Wiegmann et al. 1992; Ettinger-Dietzel et al. 2016). Due to its proximity to a major city and multiple attractions, Cultus Lake is used heavily for recreational activities throughout the summer.

Cultus Lake (Swí:lhcha) also sits on the ancestral territory of the Soowahlie (The'wá:lí) First Nation, a band government of the Sto:lo people. The initial goal was to collaborate with members of Soowahlie and hire a first nation technician. This communication was attempted in spring/summer 2019, before Thompson Rivers University was involved in the project. Ministry of Environment and Climate Change Strategy, and FLNRORD team members oversaw this communication and building of relationships. The team had brief contact with the band and continued to invite a band representative to all meetings but did not hear back. FLNRORD hired Garrett Martindale, a member of the Sts'ailes band as the technician for the project. Garrett had worked contracts for FLNRORD in the past and was a great asset to this project.

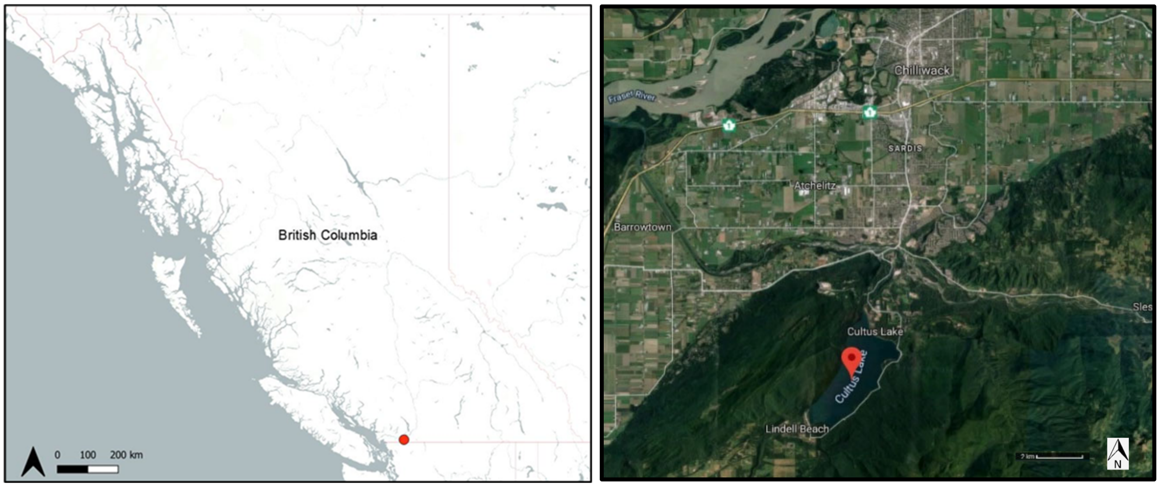

Figure 1.3. Location of Cultus Lake in reference to British Columbia (left), and Cultus Lake (right). Google Maps, 2020, maps.google.com

THESIS OBJECTIVES

The goal of the project was to document the diet and movement of smallmouth bass in Cultus Lake to lay the groundwork for an effective suppression plan. The data collected throughout two years of fieldwork (2020 – 2021) will inform fisheries managers of the best next steps in suppressing the smallmouth bass population in Cultus Lake.

In this thesis, we documented the predation of SMB on native Cultus Lake species including *Oncorhynchus nerka* and *Cottus aleuticus,* and factors that influenced their predation on these species. We completed DNA and visual analysis of the gut content of 145 SMB stomachs to identify diet throughout spring and summer in Cultus Lake. Additionally, we conducted an acoustic telemetry study and completed snorkel surveys, documenting the movements of SMB throughout the year to determine spawning and over wintering locations. The research objectives of my thesis were to:

1. Identify the diet of smallmouth bass in Cultus Lake to determine if they are feeding on species-at-risk, and the factors associated with their food choices.
2. Document the movements of smallmouth bass throughout the 2-year study to identify possible areas for effective suppression.

In Chapter 2 of the thesis, we present the diets of smallmouth bass, differences in DNA vs visual analysis, and how the diets change with age and season. In Chapter 3, we map out the movements of smallmouth bass, identify spawning grounds, and determine bass depths throughout the year. Finally, in Chapter 4, we summarize the key findings from this research and discuss the significance of the results for suppression of the species. We conclude with suggestions for further research and management strategies for smallmouth bass in Cultus Lake.

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CHAPTER 2

SMALL MOUTH, BIG APPETITE: DIET ANALYSIS OF INVASIVE SMALLMOUTH BASS (*MICROPTERUS DOLOMIEU*) IN CULTUS LAKE, AND THE POTENTIAL RISKS TO ENDANGERED CULTUS SOCKEYE SALMON (*ONCORHYNCHUS NERKA*) AND CULTUS LAKE PYGMY SCULPIN (*COTTUS ALEUTICUS*)

INTRODUCTION

The impacts of aquatic invasive species are well documented, and known to cause a loss of biodiversity, alter ecosystem functions, and disrupt trophic structure (Pimentel et al. 2005; Sanderson et al. 2009; Wainright and Muhlfeld 2021). The spread of these species is becoming more prevalent, with humans causing both intentional and unintentional introductions (Loppnow et al. 2013). Rational for introducing a non-native species often includes anthropogenic motives such as enhancing sport fishing, commercial fishing, and the release of baitfish (Carey et al. 2011; Drake and Mandrak 2014). Once established, invasive species use resources, occupy habitat, and outcompete individuals, displacing native species, and disrupting the natural system balance (Kerr 2000).

Smallmouth bass (SMB) (*Micropterus dolomieu*) are a spiny-rayed fish that exhibit numerous adaptable traits, making them an ideal competitor for resources in new habitats (Tovey et al. 2008). SMB can survive in a wide range of temperatures (Cooke et al. 2003), as long as temperatures reach 15°C for spawning (Kaemingk et al. 2011a), are opportunistic carnivores, preying on groups such as insects, fish, crustaceans, and amphibians (Pflugr and Pauley 1984; Kiesecker and Blaustein 1998; Weidel et al. 2000; Berra 2007), and are known to extirpate small-bodied fish from lakes (MacRae and Jackson 2001). Native to eastern Canada and the United States, these fish now have established populations in both Western North America, and internationally (Loppnow et al. 2013).

The introduction of SMB into the Pacific Northwest has raised concern about Pacific salmon populations (Sanderson et al. 2009; Carey et al. 2011). Bass feed on salmon juveniles, especially during smolt outmigration when habitats overlap and temperatures are above 15°C (Fayram and Sibley 2000; Tabor et al. 2007; Emingway et al. 2019). However, the overall impacts of bass on salmon mortality vary greatly depending on the study region and spatial overlap during migration (Rieman et al. 1991; Tabor et al. 1993). Cultus Lake is located in the southwestern corner of British Columbia, and SMB were detected in the lake in 2017, with immediate concern about their impacts on the endemic populations of Cultus Lake sockeye salmon (*Oncorhynchus nerka*) (COSEWIC status Endangered) and coastrange sculpin (Cultus population) (*Cottus aleuticus*) (SARA status Threatened), given their known predation on both species (Fayram and Sibley 2000; Brown et al. 2009), and potentially overlapping habitat (Schubert et al. 2002; Chiang et al. 2015). The population of Cultus Lake sockeye has been in decline for years (Cultus Sockeye Recovery Team 2009) with an average of 254 natural-origin adult spawners entering the lake from 2015-2018 (DFO 2019). Results from this study will be a factor in the decision to list Cultus Lake sockeye as threatened under the Federal Species at Risk Act.

Metabarcoding analysis of stomach content is not able to distinguish between sockeye salmon and kokanee. Cultus Lake has anadromous sockeye, non-anadromous kokanee, and a ‘residual’ group of sockeye, which are presumably the progeny of anadromous parents (Ricker 1938). Distinguishing among the three can often be done visually on mature individuals and using DNA of fin clips. It has not been attempted using digested DNA, and most diet studies group these species into *Oncorhynchus nerka* (Beauchamp et al. 1995). Both sockeye and kokanee are semelparous, have similar rates of alevin development (Wood and Foote 1990), typically overlap both spatially and temporally during spawning (Foote and Larkin 1988; Wood and Foote 1996), and behave similarly in Cultus Lake (G. Lidin (DFO), e-mail message, July 13, 2021). For these reasons, we assume that smallmouth bass are opportunistically feeding on both fish equally.

The use of presence-absence identification of stomach contents provides a simple and robust measure to identify diet composition (Buckland et al. 2017). Since no method has been universally adopted for diet analysis (Amundsen and Sánchez-Hernández 2019), the method chosen should reflect the objectives of the project and be closely linked to the research questions. We investigated the relative impacts of a predator population on prey, and therefore the fast and easy to use (Hyslop 1980) presence-absence is an appropriate measure, especially due to the highly digested nature of bass stomachs (Baker et al. 2014). This method has been criticized for giving the same ‘importance’ to all prey (Ahlbeck et al. 2012) and cannot be used to determine relative prey abundance. However, due to the objectives of the study, and the importance to accurately identify the two species at risk, using presence-absence in morphological and DNA analysis was appropriate.

The use of barcoding for diet analysis is a relatively new technique that is gaining popularity within the field. This method has many benefits including a high level of species identification (Nelson et al. 2017), the ability to identify prey in juvenile predators (Jo et al. 2014), the accurate identification of soft-bodied and small prey items (Carreon-Martinez et al. 2011; Sakaguchi et al. 2017), and the removal of subjectivity biasing in prey identification (Leray et al. 2012). However, there are issues with barcoding such as secondary consumption identification (overestimating richness of prey) (Jakubavičiūtė et al. 2017; Sakaguchi et al. 2017), and the inability to detect highly digested prey (Paquin et al. 2014; O’Dell et al. 2020). With these factors taken into consideration, a combination of morphologic and DNA analysis appears to be a complementary and comprehensive method (Taguchi et al. 2014; Barbato et al. 2019).

Our main objective was to assess the overall diet of invasive smallmouth bass in Cultus Lake, specifically whether they prey on *Oncorhynchus nerka* and *Cottus aleuticus*. The use of DNA metabarcoding allowed us to capture diversity in bass diet and identify the species-at-risk. In this study we evaluated the impact of smallmouth bass by (1) assessing their diet using presence-absence and a combination of morphological identification and DNA analysis (2) using multiple logistic regression to determine the probability of predation given factors such as predator total length (TL), time, and location, and (3) determining the effects of bass body size and seasonality on dietary shifts. We hypothesized that DNA analysis would show high diversity in species composition and greatly increase the detection rate of operational taxonomic units (individual detections of species/genera). This study is part of a larger project we have undertaken to understand the impacts of SMB in Cultus Lake, and to discover potential management solutions to suppress the population (van Poorten and Beck 2021). By understanding their timing, location, and size classes for feeding on endemic species, management can more effectively target bass, potentially relieving pressure on sensitive native species.

Our study will make a significant contribution to the growing body of literature focusing on impacts to species-at-risk and invasive species management (Rytwinski et al. 2019). We can also offer valuable data for fisheries managers by studying the invasive species' movements (Chapter 3 of Thesis) and informing them of the dietary impacts on the sockeye and sculpin, before investing extensive time and money into suppression. Using baseline data, a sustainable and effective method of suppression can be developed to target different life stages of SMB (Loppnow et al. 2013) in Cultus Lake.

METHODS

Study Site and Fish Collection

Sampling was conducted in Cultus Lake, British Columbia (49°03'00"N, 121°53'52"W), located near the city of Chilliwack, 80 kilometers east of Vancouver. This mesotrophic, monomictic lake (Shortreed 2007) has an area of 6.3 km^2, and outflows via Sweltzer Creek to the Chilliwack River. Figure 2.1 shows the location of Cultus Lake, as well as the known spawning ground of the bass. This area was identified through extensive snorkel surveys during spring 2019 - 2021. Because of its proximity to a major city and multiple attractions, Cultus Lake is used heavily for recreational activities throughout the summer.

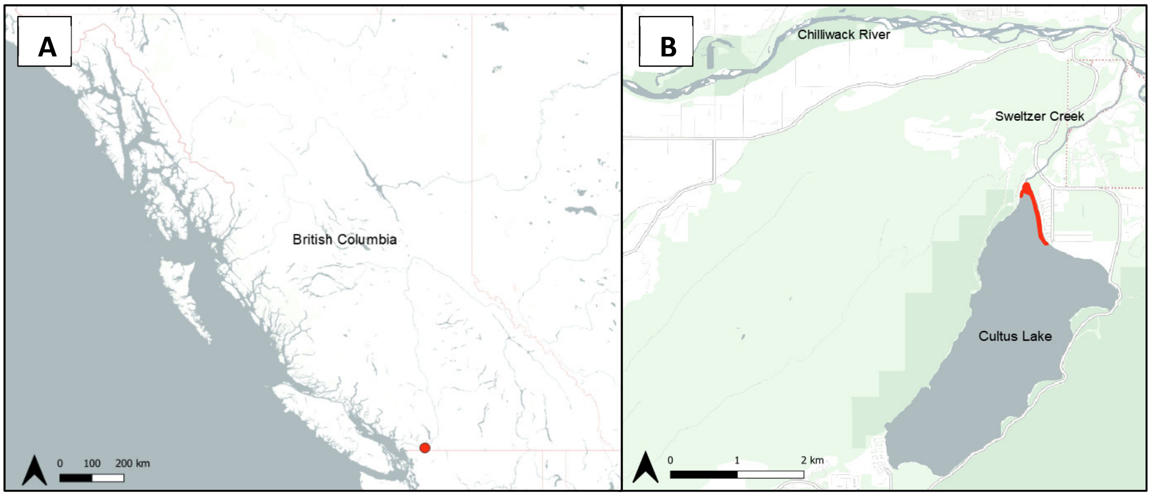

Figure 2.1. British Columbia with location of Cultus Lake represented by the red dot (A), Cultus Lake with smallmouth bass spawning area (red) and outflow into the Chilliwack River via Sweltzer Creek (B).

Using targeted angling, bass were collected from May – September 2020 and April/May 2021. Bass feeding is greatly reduced when water temperatures are below 8.5°C – 10°C (Keast 1968; Shuter et al. 1980), which in Cultus Lake occurs from October – April. Sampling from May – September gave us an accurate representation of diet when the bass are regularly feeding. To account for bias in sampling, bass were caught throughout the lake. Different methods of sampling were considered (such as gillnetting); however, due to the endangered species in the lake, permitting was challenging. Anglers fished from 7:30 am – 1:00 pm using a mixture of crank baits and worms and caught 204 fish for the study in 29 days over 2 years.

Bass were immediately killed using ethical practices approved by the Thompson Rivers University Animal Care Committee (file no. 102400), following Canadian Council on Animal Care practices, and placed on ice (Scientific Collecting Permit No. SU21-623149 and SU20-605014). Each hour, anglers brought the bass to shore, where they were weighed, measured to total length (TL), fork length (FL), and sexed. Full digestive tracts (stomachs and intestines) were dissected using a clean scalpel, then injected and submerged in 95% ethanol.

Otoliths and scales were removed from SMB, and aging was done at the BC Provincial Aging Lab after the 2020 (*n=50*) and 2021 (*n=26*) field seasons. Whole otoliths have been shown to give the most precise estimate of age in smallmouth bass (Long and Fisher 2001; Rude et al. 2012; Starks and Rodger 2020). Scales were removed from below the lateral line, posterior to the operculum and collected as a secondary means of aging and can be used for fish less than 7 years old (Blackwell et al. 2019). Confidence in age reading was recorded, and only one record was removed due to low confidence.

Visual Analysis

All 204 bass stomach samples were visually analyzed for the morphological identification of prey items. Digestive tracts were dissected and flushed with 95% ethanol to remove all stomach contents. Stomach contents were then examined under a Leica MZ 6 stereo microscope and separated into their lowest identifiable operational taxonomic units (OTU). Frequency of occurrence was estimated by calculating the percentage of stomachs in which each OTU was present, based on all stomachs. Stomach contents were then transferred to 50 mL centrifuge tubes and mixed with 95% ethanol for subsequent DNA analysis.

DNA Sequence Processing

145 samples were sent to the Canadian Centre for DNA Barcoding (CCDB) at the University of Guelph for barcode sequencing and taxonomic identification using the BOLD (Barcode of Life Data System) reference library (Ratnasingham and Hebert 2007). Samples were not selected for DNA barcoding if the stomachs were empty. The barcoding results were aggregated into unique taxonomic categories and identifications were only accepted as correct if they were supported by at least 100 reads that matched a reference sequence, with at least 95% identity across at least 100 base pairs. See Moran et al. (2019) for the CCDB’s full methods of DNA analysis.

One issue with this process is its inability to distinguish the difference between widespread coastrange sculpin (*Cottus aleuticus*) and the unique Cultus Lake population (pygmy sculpin). Although there is some level of genetic diversity between the two, it can only be detected using 8 microsatellite analysis loci (Woodruff and Taylor 2013), a process beyond the scope of this project. Therefore, analysis was done using the species level identification of *Cottus aleuticus*, with consideration to their differences in habitat and migrational feeding. Bait items (worms: *Lumbricus terrestris*) were omitted from the analysis, as these are not a natural prey item.

Statistical Analysis

Statistical analyses were completed using R software (R Core Team 2020). Boxplots were created to determine differences in total length (mm) between male and female smallmouth bass. Total lengths of males and females were compared using a Welch's two sample t-test and a 95% confidence interval. To determine if bass were larger within the spawning area, a linear model was created, accounting for the differences in length based on sex. Tukey's post hoc comparison test was then used to compare female total length of bass caught in the spawning vs non-spawning area, and vice versa for males.

A cumulative prey curve was used to determine whether a sufficient number of samples had been collected to accurately determine SMB diet (Ferry and Cailliet 1996). The number of randomly selected (Gotelli and Colwell 2001) stomachs was plotted against the cumulative number of prey types and a nonlinear Lomolino model (Lomolino 2000; Dengler 2009) was fitted to the data, where *Asym* is the asymptotic maximum number of species, *slope* is the maximum slope of increase of richness, and *xmid* is the area where half of the maximum richness is achieved (1). The asymptotic stabilization of the curve represents the number of stomachs needed to accurately analyze the data (Cortes 1997).

$$n = \frac{Asym}{1 + slope^{\log\left(\frac{xmid}{area}\right)}} \tag{1}$$

To determine the probability of SMB predation on the two species-at-risk, we used a multiple logistic regression with binary variables. SMB length (mm), month, and location caught were all used as fixed effects on the predation of either sockeye salmon or coastrange sculpin, and interactions were analyzed for their significance using a Pearson's chi-squared test with Yate's continuity correction.

An age-length key was used to summarize the relationship between age and length in a population of fish by using an aged subsample of fish and applying the key to the total population to get an unbiased estimate for the mean length-at-age ($\bar{L}_j$) (2) for *N* fish (Bettoli and Miranda 2001), and standard deviation-at-age ($SD(L_j)$), the following formulas were used, where $\bar{L}_{ij}$ is the mean length of a fish, and p_{ij} is the proportion of fish from the aged sample in the *i*th length interval that were age *j* (3)(Ogle 2016).

(2) $$\bar{L}_j = \frac{N}{N_j}\sum_{i=1}^{L} p_{ij}\,\bar{L}_{ij}$$

(3) $$SD(L_j) = \sqrt{\frac{N}{N_j - 1}\sum_{i=1}^{L} p_{ij}\,(\bar{L}_{ij} - \bar{L}_j)^2}$$

To estimate the ages of fish when otoliths were not collected, we used the method described in Isermann and Knight (2005), where all unaged fish are assigned an age based on the age-length key. The means and standard deviations of each age group was calculated, and a plot of individual lengths-at-age with mean lengths-at-age overlaid was constructed (Ogle 2016).

Schoener's Index of dietary overlap was used to determine similarities in diet between size classes. The index ranges from α = 0 (no overlap) to α = 1 (complete overlap), and overlap is considered significant at > 0.6 (Schoener 1970). In equation (4), *p* is the proportion of sequences from the *i*th prey genera, and x and y represent different size classes.

(4) $$\alpha = 1 - 0.5x(\sum |p_{xi} - p_{yi}|)$$

RESULTS

Smallmouth Bass Physical Characteristics

The mean total length (mm) ± SD and weight (g) ± SD of the 204 smallmouth bass used for the visual analysis was 239 ± 70 mm and 263 ± 234 g. 56 females, 112 males, and 36 juveniles were caught, with males dominating most catches in the spring. Table 2.1 represents the collection data from the 2020 – 2021 field seasons.

Table 2.1. Sizes of Smallmouth bass collected from 2020 – 2021 in Cultus Lake, BC.

Year	Month	Sample Size	Mean TL (mm) ± SD	Mean Weight (g) ± SD
2020	May	18	255 ± 31	249 ± 117
2020	June	7	278 ± 28	331 ± 117
2020	July	66	234 ± 58	240 ± 194
2020	August	39	192 ± 66	155 ± 220
2020	September	20	164 ± 39	70 ± 81
2021	April	6	305 ± 70	515 ± 422
2021	May	48	291 ± 57	423 ± 236
	Total	*n* = 204	238 ± 69	262 ± 234

Male smallmouth bass caught in this study were significantly (p<0.00) larger (total length) than female smallmouth bass (Figure 2.2).*Figure* With a 95% confidence level, males are on average 51mm longer than females. There was also a significant difference (p=0.0036) in size between bass caught in the spawning vs non-spawning areas, irrespective of sex. The difference in total length was significant between females caught in the spawning vs non-spawning area, but not between males caught in either area (Figure 2.2). These total lengths do not account for ages; however, male bass (based on our sample population) were only 0.58 years older on average than female bass, based on otolith aging.

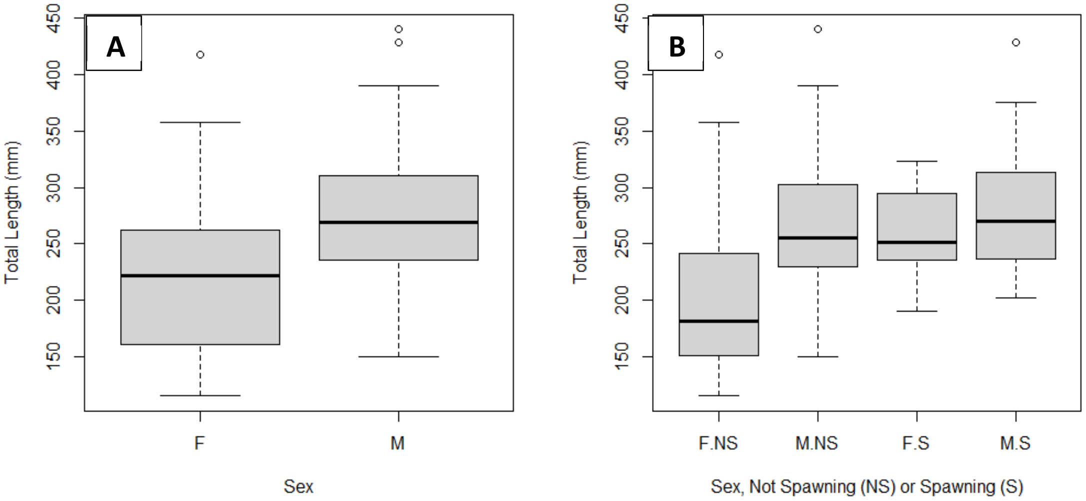

Figure 2.2. Boxplot of smallmouth bass male and female total length (mm) (A), and boxplot of bass male and female total length (mm), and whether they were caught in the spawning grounds (S) or not in the spawning grounds (NS) (B) (n=170) from Cultus Lake, BC. Juveniles not included

DNA Analysis

DNA sequencing successfully detected prey in 144 of the 145 stomachs analyzed. Results identified 998 reads within 82 species, belonging to 48 families, and 26 orders (Figure 2.3). Although many taxa were identified to the species level, confidence of identification was often at the genus level only due to the short length of molecular markers used. The orders with the highest sequencing detections and the average proportional contribution of count data combined were sculpin/stickleback (Cottidea/Gasterosteidae: Scorpaeniformes) (26%), crayfish (Astacidae: Decapoda) (15%), and mayflies (Baetidae: Ephemeroptera) (5%).

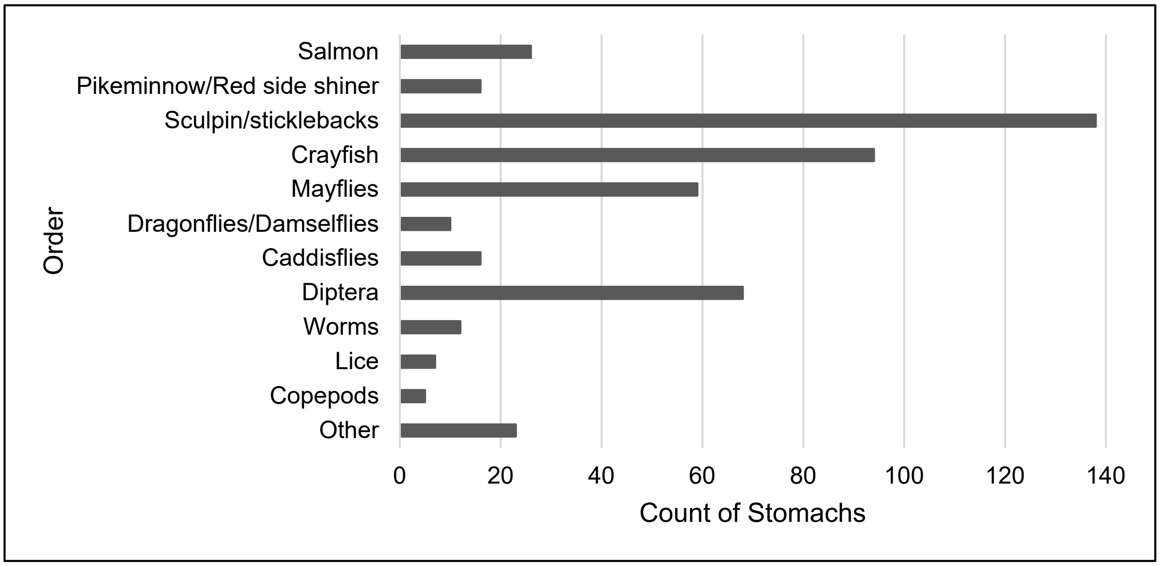

Figure 2.3. Number of smallmouth bass stomachs with each order present, based on DNA analysis from 2020-2021 Cultus Lake, BC. Scientific order names were converted to common names for ease of understanding. The 'other' category represents orders with less than 5 counts.

Seven fish species were identified during metabarcoding. They were (including count): Prickly sculpin (*Cottus asper*) (110), coastrange sculpin (*Cottus aleuticus*) (98), three-spined stickleback (*Gasterosteus aculeatus*) (34), sockeye salmon/kokanee (*Oncorhynchus nerka*) (26), redside shiner (*Richardsonius balteatus*) (10), Northern pikeminnow (*Ptychocheilus oregonensis*) (8), and peamouth chub (*Mylocheilus caurinus*) (1). These species are all known residents of Cultus Lake. DNA analysis was not able to distinguish *Oncorhynchus nerka* into subcategories of kokanee and sockeye salmon (see discussion). There were two additional species identified, bullhead sculpin (*Cottus gobio*) and Reticulate sculpin (*Cottus perplexus*), with one count each. Bullhead sculpin has not been found in North America, leading to an assumption of misidentification during analysis. Reticulate sculpin are found in the Pacific Northwest, and so it is possible that the identification was correct.

Cumulative Prey Curve

The cumulative prey curve using a nonlinear Lomolino line (equation 1) showed that more SMB needed to be collected to provide a full description of diet (Figure 2.4). The curve used genera as the level of taxonomic detail identified in the stomachs, and the function was run with 100 permutations. Confidence intervals were calculated using standard deviations, and the asymptotic point of the model was estimated at 167.96. Therefore, an additional 23 samples would be needed to better describe the diet of SMB in Cultus Lake.

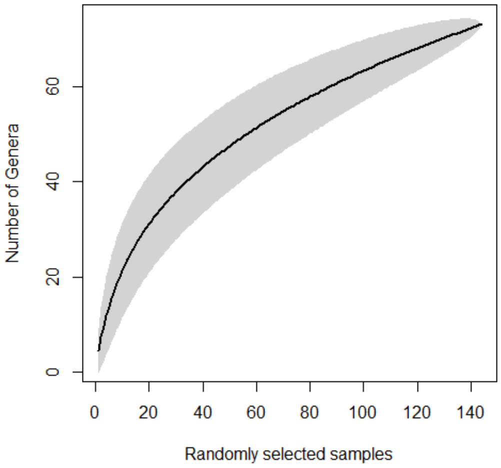

Figure 2.4. Cumulative prey curve (n=145) of prey from smallmouth bass, using a Limono fitted line, and confidence intervals calculated using standard deviations.

DNA Versus Visual Diet Analysis

The data showed the effectiveness of using DNA barcoding over morphological analysis, as a tool for species identification (Figure 2.5). The stomach contents were heavily digested, making morphological distinctions difficult, especially for juvenile or small fish species, and daphnia. Morphological analysis, using the 145 samples also sent for metabarcoding, did result in the identification of 10 (Cottidae, Gasterosteidae, Cyprinidae, Ephemeridae, Baetidae, Caenidae, Chironomidae, Hyallidae, Astacidae, Leptoceridae) of the top 12 most frequently detected families from DNA analysis, which used the BOLD reference library (n = 145). The two missing families, not detected in visual analysis were Daphniidae and Salmonidae. There were many unidentifiable pieces of fish during the visual analysis, due to the high level of digestion. Many of these prey items are now assumed to be Salmonidae (see ‘Unknown Fish’ category in Figure 2.5). Overall, DNA analysis detected 32 more families than visual identification, and 84% of those additional families detected had counts ≤ 3.

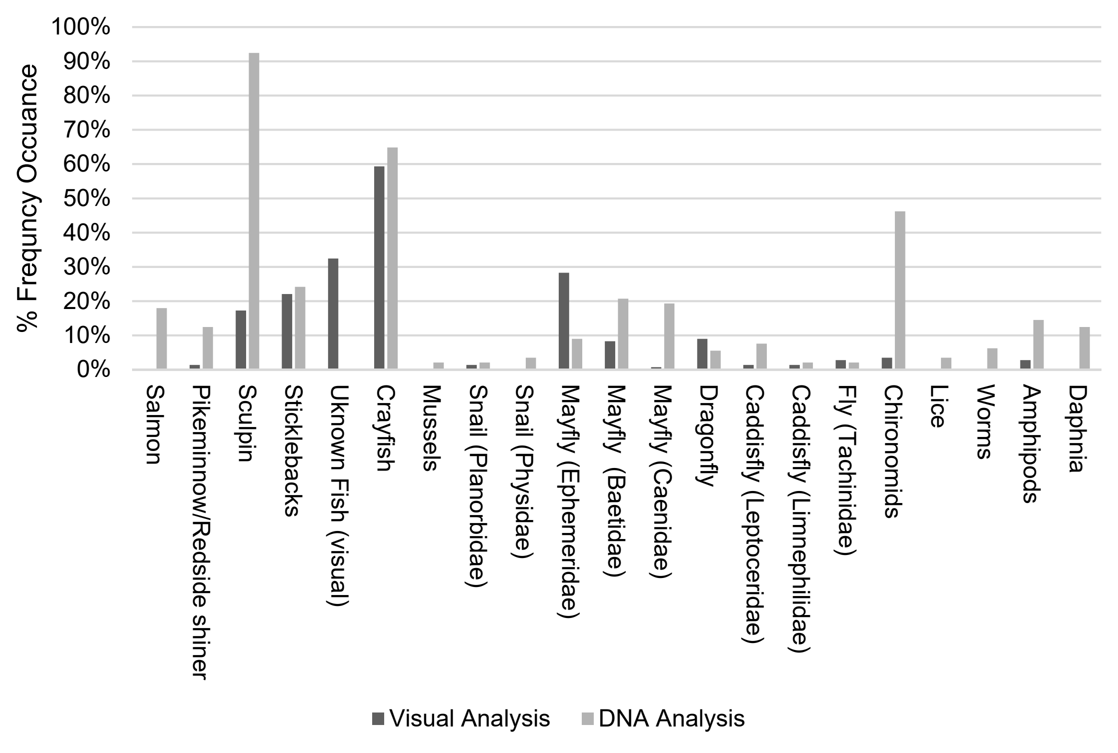

Figure 2.5. DNA vs visual analysis of smallmouth bass diet (n = 145) using frequency of occurrence. Families (common names) shown were detected in more than 2 stomach. Stomachs not used in both visual and DNA analysis were omitted from this graph.

Probability of Predation

Oncorhynchus nerka (sockeye/kokanee) were found in 17.8% of the smallmouth bass stomachs analyzed using metabarcoding. Variables listed in Table 2.2 were used to determine the probability of smallmouth bass predation on sockeye/kokanee. A multiple logistic regression run with binary presences or absence variables of *Oncorhynchus nerka* showed that there was a significant interaction between location caught and presence of the fish. Further analysis showed strong evidence of a statistical difference between the presence/absence of sockeye/kokanee in stomachs and whether the SMB were caught in the spawning grounds ($p<0.001$). A proportion table (Table 2.3Table) summarizes the findings that 30% of bass caught in the spawning ground were eating salmon, and 8% of bass caught outside of the spawning ground were eating salmon.

Table 1.2. Factors used in smallmouth bass multiple logistic regression.

Variable	Purpose	Description
Total Length (mm)	Categorized into size class bins	100 – 149 mm (I), 150 – 199 mm (II), 200 – 249 mm (III), 250 – 299 mm (IV), ≥ 300 mm (V)
Time	Month caught	April, May, June, July, August, September (combined data from 2020 with 2021)
Location	Categorize location	Spawning grounds OR non-spawning grounds (west or east of the marina/remainder of lake)
Presence of *Oncorhynchus nerka*	Presence/absence of salmon/kokanee in SMB stomach	0 or 1
Presence of *Cottus aleuticus*	Presence/absence of coast range sculpin in SMB stomach	0 or 1

Cottus aleuticus was found in 67.6% of the smallmouth bass stomachs analyzed using metabarcoding. A multiple logistic regression was also used to analyze the presence/absence of *Cottus aleuticus* in the stomachs of the bass. Results showed some interaction between the size class category and presence/absence of bass with sculpin in their diet. Upon further analysis, the two smallest size categories had the highest proportions of bass with sculpin in their stomachs. A log likelihood test of independence showed there was a significant difference between size classes ($p<0.05$), and Table 2.4 shows a proportional comparison of the size classes and the percentage of bass found with and without sculpin.

Table 2.3. Proportion table of bass caught with salmon present in stomachs in either the spawning or non-spawning areas of Cultus Lake.

Salmon Present	Non-Spawning Area	Spawning Area
0	0.916	0.694
1	0.084	0.306

Table 2.4. Proportion table of bass caught with sculpin present in stomachs in size classes I-V in Cultus Lake.

Sculpin Present	I	II	III	IV	V
0	0.083	0.188	0.370	0.519	0.340
1	0.917	0.813	0.630	0.481	0.660

Total Length, Age, and Diet Shifts

An age-length key was created using 76 otoliths, and total lengths (mm) from the additional 128 SMB used for diet analysis (*n = 204*) (Figure 2.6). Mean total lengths (mm) and standard deviations (SD) were calculated using both the aged fish and predicted values for unaged fish. Mean length (mm) ± SD were age 1 (150.44 ± 15.39), age 2 (224.65 ± 25.27), age 3 (295.06 ± 29.86), age 4 (377.29 ± 36.57), and age 5 (440.00 ± NA). For the age 5 category there was only 1 fish present, and so no SD was calculated. Ages are calculated according to spring spawning and whether they were caught in 2020 or 2021. No aged bass were less than 1 year old; hence all age 1 bass were either born in 2019 (caught in 2020) or 2020 (caught in 2021).

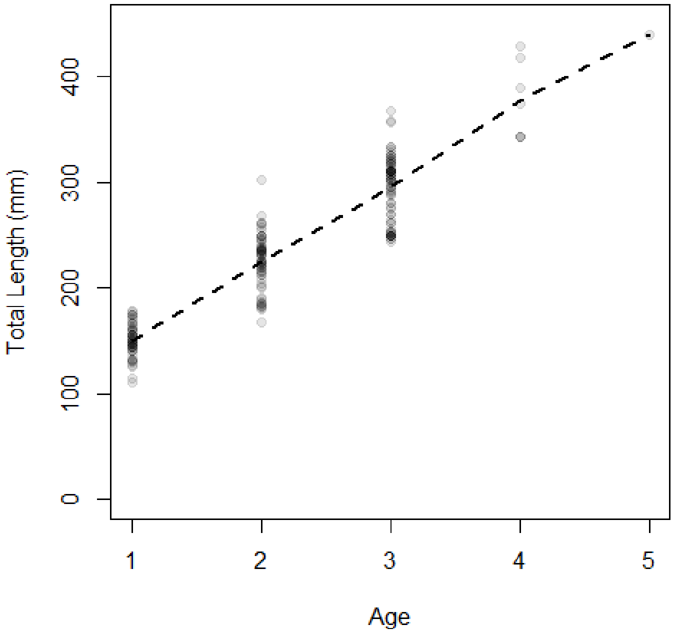

Figure 2.6. Age-length key for smallmouth bass in Cultus Lake. The dashed line represents the mean age-at-length. n = 50 otoliths from 2020; n = 26 from 2021. Key represents combined ages from both years.

Bass were categorized into 5 groups (Table 2.5) based on their total lengths (mm). There was very little dietary shift among size classes. The three dominant prey items were fish, crayfish, and insects*,* followed by molluscs, copepods, and daphnia (Figure 2.7). SMB become piscivorous once they reach 100 mm and continue to eat larger fish as they grow. Surprisingly, the diet percentage composition of the size class I (100 – 149 mm) bass was similar to that of the largest group. Calculations of Schoener's index resulted in α values all above the significance threshold of 0.6 (Schoener 1970). Overlap (α) between size classes were 0.805 (I – II), 0.732 (II – III), 0.860 (III – IV), and 0.858 (IV – V), indicating that all size classes had a significant amount of dietary overlap.

Table 2.5. Total length classes for smallmouth bass dietary shift analysis (n = 145).

TL range (mm)	Size Class	n	TL Mean (mm) ± SD
100 – 149	I	12	137.7 ± 10.5
150 – 199	II	32	167.5 ± 13.0
200 – 249	III	27	228.8 ± 12.6
250 – 299	IV	27	265.6 ± 16.2
≥ 300	V	47	326.7 ± 31.8

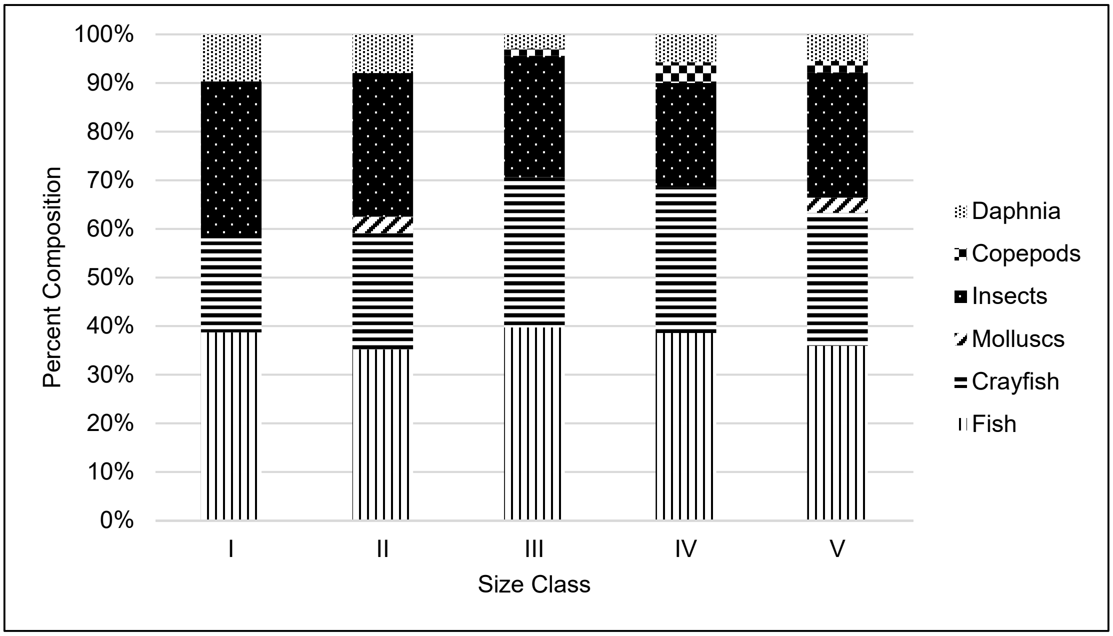

Figure 2.7. Diet composition of smallmouth bass in Cultus Lake, based on size class (I-V).

A time-series was created to identify any shifts in seasonal diet (Figure 2.8) using the six most dominant prey items. Data points represent the count of bass with each taxon present in their diet divided by the total bass caught for each month. Due to low catches of bass in some Spring months, April – June was combined for Spring 2020, and Spring 2021. Frequency of occurrence decreased in July in all taxa except Ephemeroptera and Odonata in July, which coincides with their emergence peaks (Weidel et al. 2000). Curiously, % occurrence was higher for fish and chironomids in Spring 2021 than in 2020.

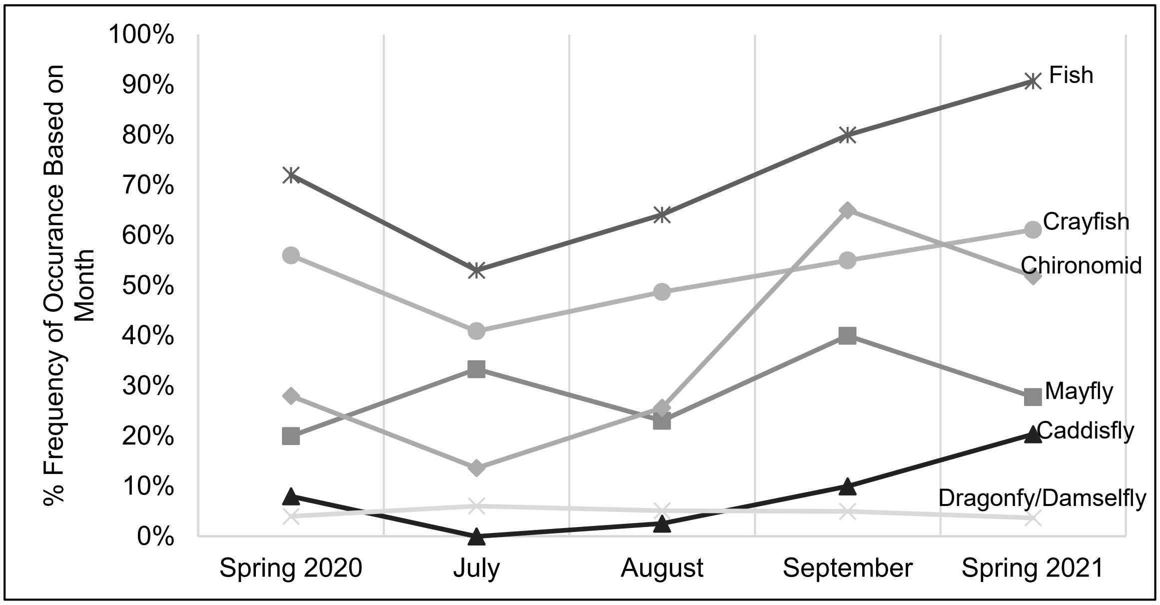

Figure 2.8. % Frequency of prey occurrence in smallmouth bass, caught in Cultus Lake, BC. Month: Spring 2020 (n = 25), July (n = 66), August (n = 39), September (n = 20), Spring 2021 (n = 54).

DISCUSSION

Our analysis shows the value in using metabarcoding analysis as a tool in identifying prey items of aquatic invasive fishes, and the impacts SMB are having on native species in Cultus Lake. Invasive bass have the potential to extirpate native species, change the behaviour of prey species (MacRae and Jackson 2001) and drive changes in the trophic structure of freshwater food webs (Wainright and Muhlfeld 2021). This study confirmed that SMB are predating on *Oncorhynchus nerka* in the bass spawning grounds, and that over 90% of bass caught had *Cottus spp.* in their stomachs. It also supports the underlying assumption of bass as opportunistic feeders. Bass are impacting every trophic level, possibly outcompeting species for prey, creating an imbalance in the lake overall ecology. This baseline information is critical for developing sustainable and effective management strategies for the suppression of SMB.

Smallmouth Bass Physical Characteristics

The significant size difference between SMB inside and outside the spawning area, is indicative of bass behaviour. There is limited research on SMB females, with most studies focusing on guarding male behaviour during spawning. Our data shows that there was no size difference in males caught within the spawning ground versus those outside of the spawning ground. Males that successfully breed are typically larger due to the high energetic cost of territorial guarding behaviours (Winemiller and Taylor 1982; Iguchi et al. 2004; Dunlop et al. 2005), but smaller males will also attempt to spawn, even if unsuccessful. In comparison, females were significantly larger in the spawning ground. Smaller, sexually immature females have no reason to enter the spawning area, and so it's only larger mature females that were caught. These larger females may spawn multiple times (Winemiller and Taylor 1982) before moving farther offshore into the pelagic zone (Kaemingk, Galarowicz, et al. 2011).

DNA and Visual Diet Analysis

Morphological identification of prey gave us supplementary data to confirm the presence of most taxa during DNA analysis. Without visually inspecting the diet, the amount of secondary prey items in the stomachs would be unknown. Visual analysis was challenging, with the vast majority of fish items being unidentifiable, resulting in low confidence in identification (Baker et al. 2014). Due to the high levels of digested content, presence-absence was the most robust measure for documentation (Buckland et al. 2017).

Some of the discrepancies between the morphological and DNA analysis (Figure 2.5) can be explained. Sculpin and salmon were detected at a much higher rate during DNA analysis. During visual analysis, there were many unidentifiable fish pieces, which were left uncategorized (see Unknown Fish in Figure 2.5) due to their high-level decay during digestion. Mayflies (Ephemeridae*)* resulted in a higher count from the visual analysis but there was a lower count in Caenidae and Baetidae. Many mayflies were identified using individual body parts and may have been misidentified at the family level. The last major difference was with Chironomidae. SMB eat chironomids (Beck 2013); however, the head capsules of chironomid larvae are highly scleratinized, so these should have been identifiable during visual analysis, unless the SMB were feeding on adult chironomids at the surface. It is most likely that the identified chironomids were secondary prey.

Even though DNA is becoming a popular alternative for diet analysis, there are still some limitations, especially when it comes to metabarcoding homogenized stomach samples. False positives can occur from factors such as amplification bias, contamination, and false negatives from sample degradation (Darling and Mahon 2011), but increasing the number of replicated PCRs, and excluding taxa identified only once during sequencing can limit these errors (Ficetola et al. 2015). Other factors that could influence results are the degree of digestion among different prey morphological structures, the unavailability of some organisms in gene databases (Sakaguchi et al. 2017), and the detection of secondary predation (Sheppard and Hardwood 2005; O’Rorke et al. 2012). Despite these limitations, DNA analysis is still being used increasingly for its numerous benefits such as increased detection rates, removal of subjectivity biasing in prey identification, and accurate identification of small and soft bodied prey (Leray et al. 2012; Jo et al. 2014; Nelson et al. 2017).

As previously mentioned, metabarcoding was not able to differentiate between coastrange sculpin (*Cottus aleuticus*) and pygmy sculpin (C*ottus aleuticus*: Cultus Population). Pygmy sculpin are a neotenic form of coastrange sculpin, endemic to Cultus Lake, BC (Chiang et al. 2015), and very little research has been done on the species. They typically inhabit deeper water, and have similar morphologic traits to *C. aleuticus*, only maturing at a smaller size (Ricker 1960). Genetic differences between the two is modest and only identifiable using microsatellite analysis (Woodruff and Taylor 2013). No research has been done on whether the two species would be identifiable using mitochondrial DNA once they have been partially digested through predation.

The high diversity of prey consumed by the smallmouth bass was not surprising, given their known opportunistic feeding behaviours (Pflugr and Pauley 1984). Most of the frequently found items in their stomachs (crayfish, insects, fish) were similarly found in other feeding studies (Dunlop et al. 2005), and we assume that if studied, bass length would correlate to an increase in the number of prey items/size (Weidel et al. 2000). One difference in diet was the diversity of fish taxa found from their western to eastern range. SMB in the Pacific Northwest appear to predate heavily on *Cottus sp.* (Pflugr and Pauley 1984; Tabor et al. 1993) and were identified in 134/145 stomachs analyzed, whereas the dominant taxa in SMB in Eastern North America included sunfish, darters*,* and gobies (Olson and Young 2003; Nelson et al. 2017; Waraniak et al. 2019). This is most likely due to the availability of prey in each geographic region.

Probability of Predation

One concern was whether SMB predation on sockeye would increase during their smolt outmigration in the spring (Pflugr and Pauley 1984; Tabor et al. 1993; Fayram and Sibley 2000). Additional phases of this project involved snorkel surveys and acoustic telemetry analysis to identify bass spawning locations in Cultus Lake (Chapter 3 of this thesis). With these data, we know the spawning area for SMB spatially and temporally overlaps with the outmigration route of sockeye salmon via Sweltzer Creek. There was no increase in the consumption of salmon during the smolt outmigration in the spring; however, there were significantly more bass with salmon/kokanee in their diets in the SMB spawning ground.

The concern over the dwindling population of Cultus Lake sockeye is significant. The most recent (2015-2018) average of natural-born adult spawners returning to the lake was 254, compared to a historical average of 19,890 spawners (DFO 2019). In addition to migratory pressures, these sockeye are also facing the eutrophication of Cultus Lake (Gauthier et al. 2020), the spread of invasive Eurasian watermilfoil rendering habitat unsuitable for spawning, and now a predatory invasive species. Positive identification of salmon redds have not been documented since 2009, making management of these areas more difficult.

The high frequency of detection of sculpin found in the stomachs of bass is concerning. Coastrange sculpin were found in 68% of the stomachs, while prickly sculpin were found in 76%. Although we couldn’t discern between pygmy sculpin and coastrange sculpin, it is probable the bass are feeding on both due to the bass' wide distribution in the lake during summer and fall. There is no current population estimate for pygmy sculpin (Chiang et al. 2015), and the most current sampling survey completed in 2005 showed pygmy sculpin distributed throughout the lake with a congregation in the south (Woodruff and Taylor 2013). Bass have been known to extirpate small-bodied fish (MacRae and Jackson 2001), so there is great concern for the continued survival of pygmy sculpin in Cultus Lake, and further research is recommended.

Collecting characteristic data on the predator species during diet analysis can be important information to factor into the understanding of predation. Sample site, sex, and predator total length can significantly influence prey selectivity (Mychek-Londer et al. 2020), and so sample design should be considered carefully before collecting samples. In our design, sampling heavily within the spawning ground during the spring may have led to bias in the diet analysis. There is also little to no sexual dimorphism between SMB males and females (as size differences could be accounted for by age) and could therefore only be accounted for after euthanasia. Length categories were considered when sampling, but because SMB were recently introduced, older and therefore larger size classes were difficult to collect.

Total Length, Age, and Diet Shifts

Most bass caught were between ages 1 – 3, with only a few ages 4, and one bass aged 5. From these ages, we assume the bass were introduced into Cultus Lake between 2016-2017. The age-length key depicted surprisingly high growth rates compared to other populations in North America (Pflugr and Pauley 1984; Dunlop et al. 2005), especially for the age 1 category (see summary table in Becker 1983). This may be because bass were caught from May – Sept, and so fall catches were almost 1.5 years old. Growth rates were most similar to Starks and Rodger (2020) study of lotic-dwelling smallmouth bass. Age at maturity appears to vary based on location and lake temperatures (Becker 1983; Dunlop et al. 2005), with Cultus Lake SMB maturing at ages 3 – 4. Cultus Lake is relatively warm (avg 23°C July – September) and the bass have little competition due to their recent establishment, possibly causing early maturity.

There was little to no ontogenetic shift in diet among size classes. For all 5 size categories, SMB ate mostly fish*,* crayfish, and insects. Many studies have seen a diet shift from insectivory to piscivory at around 150 mm (Tabor et al. 1993; Weidel et al. 2000), while this study shows SMB as small as 115 mm feeding on fish. Schoener's index also showed a high level of dietary overlap, with $\alpha > 0.7$ for all size classes. These patterns of dietary similarity may have shown more difference had prey length or weight been calculated. The pattern for fish and chironomids matches fairly well, which could indicate that SMB prey fish (sculpin and sticklebacks) are feeding on chironomids (larvae and/or pupae). This supports our theory that chironomids are secondary prey for SMB.

Management

The management of invasive smallmouth bass is complicated, expensive, and difficult to sustain (Halfyard 2010; Carey et al. 2011; Rytwinski et al. 2019). In this study, we suggest monitoring a population's diet before investing large sums of money into suppression. In this 'walk before you run' technique, baseline data can be gathered (such as diet and distribution) to help inform policy, enact efficient management of the species, and create community awareness of the issue. We know that smallmouth bass are feeding on salmon, especially at the mouth of Sweltzer Creek. This information will a) leverage funds due to the known impacts SMB are having on salmon and b) enable managers to target the SMB population where they are impacting salmon the most. As more of British Columbia (and Canada) is becoming increasing habitable to smallmouth bass due to Climate Change (Sharma and Jackson 2008), management of the species needs to happen quickly and effectively.

Another consideration is the movement of SMB from Cultus Lake into neighbouring water bodies (Langhurst and Schoenike 1990; Tabor et al. 2010). SMB are known to thrive in both lakes and rivers (Tabor et al. 1993; Hawkins et al. 2009; Ettinger-Dietzel et al. 2016) and therefore the potential for them to migrate into the Chilliwack River, via Swetlzer Creek is high. This risk is heightened due to November 2021 extreme flooding in the BC Lower Mainland that connected many isolated waterbodies surrounding Cultus Lake. There is currently a fish fence at a Department of Fisheries and Oceans Salmon Research Lab on Sweltzer Creek; however, this is only maintained throughout the Spring and Summer. Fences have been shown to be effective barriers to the migration of smallmouth bass (Boucher 2007), but considerations would need to be made for migrating salmon.

Smallmouth bass were illegally introduced into Cultus Lake between 2016 – 2017. Since then, their population has increased, and they have been feeding on endangered and endemic species. The bass may also be outcompeting native species for food such as mayflies and damselflies. Smallmouth bass spawning areas are spatially and temporally overlapping with the outmigration of sockeye salmon via Sweltzer Creek, and SMB are disproportionately feeding on sockeye/kokanee in the bass spawning grounds. There is also a high possibility that smallmouth bass are currently migrating into neighbouring water bodies. The framework for a successful project has been laid, and management of this species can be possible with public awareness, and immediate and intensive suppression efforts.

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CHAPTER 3

SPATIAL DISTRIBUTION AND SPAWNING PATTERNS OF INVASIVE SMALLMOUTH BASS (*MICROPTERUS DOLOMIEU*) IN CULTUS LAKE, BC

INTRODUCTION

Identifying invasive fish movement patterns is essential for successful management (Tabor et al. 2010; Bajer et al. 2011; Gutowsky et al. 2020), and to minimize impacts on native and endangered species (Hegna et al. 2020). In the last 10 years, acoustic telemetry studies have made their way to the forefront of tracking aquatic animal behaviour in both marine and freshwater environments (Hays et al. 2016; Lennox et al. 2021). With advancements in technology, scientists have been able to monitor species post habitat reclamation (Marsden et al. 2016), during economic fisheries evaluations (Matley et al. 2020), to assess the effects of urbanization on critical habitat (Veilleux et al. 2018), and to study the reintroduction of extirpated species (Klinard et al. 2020). The use of acoustic telemetry to monitor and target invasive species is relatively new but has potential to aid in efficient targeted suppression of a species. In this study, we tracked invasive smallmouth bass (*Micropterus dolomieu*) (SMB) to document their movements and spawning congregations in Cultus Lake, British Columbia (BC).

Smallmouth bass movements have been well documented in both lake and river habitats. Water depth/temperature and habitat type are the two strongest predictors of bass locations (Ettinger-Dietzel et al. 2016). Bass migrate to shallower, warmers waters in the spring for spawning (May and June), and stayed at depths between 1.5 to 4 m (Suski et al. 2009; Tabor et al. 2010). Temperatures during this time are typically between 13 to 20 °C (Neves 1975; Pflugr and Pauley 1984), but bass have the highest success rate with a minimum temperature of 15°C (Kaemingk, Clem, et al. 2011). Throughout the summer, bass remain above the thermocline (Suski et al. 2009), but may move offshore, with females migrating farther than males (Kaemingk, Galarowicz, et al. 2011). In larger aquatic systems, bass can migrate far distances, with fish moving out of harbours (Carter et al. 2012), into adjoining lake systems (Kaemingk, Galarowicz, et al. 2011), or throughout rivers (Langhurst and Schoenike 1990). SMB are least active during the winter months (Suski et al. 2009; Watson et al. 2019), when they inhabit deeper water, and feeding is greatly reduced (Keast 1968; Shuter et al. 1980).

Male bass create nests every spring to attract mates and protect their eggs and fry. Nests are typically constructed using substrates such as sand, gravel and pebbles with minimal macrophyte vegetation (Wiegmann et al. 1992; Cooke et al. 2002; Funnell 2012). Most frequently, nests are constructed 1 m below the surface (Neves 1975; Rejwan et al. 1997) but can range from 1-4 meters and are often built on a plateau before a drop-off contour (Pflugr and Pauley 1984). The distribution of bass nests within the littoral zone is influenced by environmental factors and interactions between aggressive, territorial males (Scott 1996; Iguchi et al. 2004). Larger males are able to select and defend the optimal nesting sites, with subordinate males settling on less desired locations (Winemiller and Taylor 1982). If a male bass fails to attract a mate with their first nest, they will often construct a second within the same breeding season (Goff 1985). Throughout the lake, nests are often created in clumps or patches and not evenly distributed (Rejwan et al. 1997). This may be due to preferential spawning areas being specific to the substrate and depth, with only parts of the lake containing such characteristics.

Acoustic telemetry is a spatial technique used to locate aquatic vertebrates, where acoustic tags implanted in the animal send out unique pings and are identified by receivers. Receiver arrays can provide valuable three-dimensional data to the researchers, but there are many factors that can influence the performance of the arrays. Environmental factors such as wind and rain can have a strong impact on the receiver's ability to detect the tagged animals (Gjelland and Hedger 2013). The range of the receivers is also dependent on the degree of water column stratifications (Singh et al. 2009), depth of receiver deployment (Klinard et al. 2019), and biofouling (Heupel et al. 2008). If too many tags are in the same location at the same time, this can cause collisions, affecting both the range and ability of the receiver to recognize uniquely coded IDs (Simpfendorfer et al. 2008). All these factors need to be taken into consideration when designing the study and when analyzing the data using receiver diagnostics.

Acoustic telemetry fish studies provide a unique spatial component to management discussions and planning (Brooks et al. 2019). This baseline spatial data creates a ‘walk-before-you-run’ model, where fisheries managers have a greater understanding of where the invasive individuals are throughout the year, before attempting to suppress the population. The invasive smallmouth bass project at Cultus Lake currently has stakeholders from many groups including government, non-government organizations, and local community members (van Poorten and Beck 2021). Spatial data provides a powerful, visual tool to inform and engage all stakeholders in order to collaborate more efficiently (Brooks et al. 2019). Using acoustic telemetry, we were able to track the movements and aggregations (Bajer et al. 2011) of invasive smallmouth bass during different seasonal migrations. This information can now be used to facilitate suppression activities in an effective manner (Gutowsky et al. 2020).

Understanding the movements and habitat use of smallmouth bass will help fisheries management groups target the species for suppression at Cultus Lake. The objectives of this study were to 1) characterize seasonal spatial distribution of SMB within Cultus Lake 2) analyze SMB depth preference, specifically over winter 3) locate SMB spawning areas and duration of nesting behaviours. A combination of acoustic telemetry and snorkel surveys were used to meet our objectives and map the movements of SMB in Cultus Lake. We hypothesized that from April to June, SMB will be found in higher densities, with many SMB spawning along the north shore of Cultus Lake. Then SMB will then migrate offshore to deeper waters throughout the winter, where they will be mostly sedentary.

This study is part of a larger project to understand the impacts of SMB in Cultus Lake (both diet and movement), especially on the two species-at-risk, Cultus Lake sockeye salmon (*Oncorhynchus nerka*) (COSEWIC status

Endangered) and Pygmy sculpin (Cultus population) (*Cottus aleuticus*) (SARA status Threatened). By understanding their movements and spawning aggregations, management can more effectively target bass, potentially relieving pressure on endangered native species. Our study will make a significant contribution to the growing body of literature focusing on freshwater acoustic telemetry (Lennox et al. 2021) and invasive species management (Rytwinski et al. 2019). By studying the invasive species' movements and diet before investing extensive time and money into suppression, we can set a standard for invasive species management. Using baseline data, a sustainable and effective method of suppression can be developed to target different life stages of SMB (Loppnow et al. 2013) in Cultus Lake.

METHODS

Study Site

Cultus Lake (49.054910, -121.987446) is in the southwestern corner of British Columbia, near the city of Chilliwack, and approximately 80 kilometers east of Vancouver (Figure 3.1). Originally an oligotrophic lake (Ricker 1937), the now oligo-mesotrophic, peri-urban lake experiences significant anthropogenic nutrient loading (Putt et al. 2019). The lake sits at 47 m in elevation, has an area of 631.1 ha, and a perimeter of 13.5 km. The Chilliwack River flows 2.4 km north of Cultus Lake and connects to the lake via the outflow of Sweltzer Creek. Several other creeks flow into the lake including Frost, Windfall, Redtail, and Reservoir Creek. The biogeoclimatic zone is Coastal Western Hemlock (CWH) with subzones Dry Maritime (dm) and Very Dry Maritime (xm) (Pojar et al. 1991).

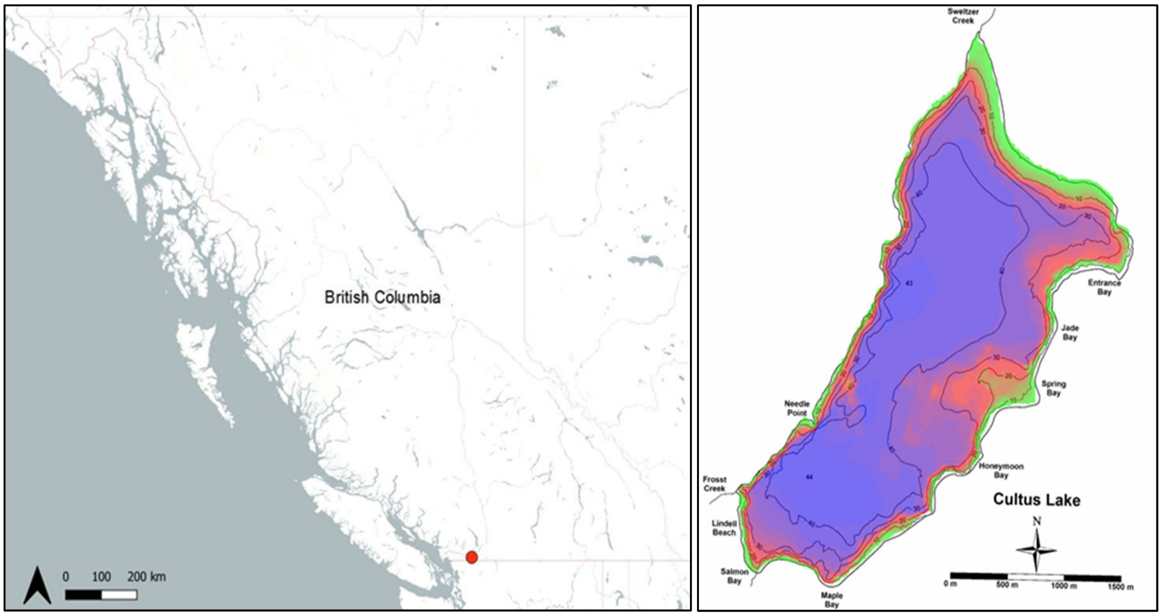

Figure 3.1. Location of Cultus Lake within British Columbia (left). Bathymetric map of Cultus Lake with green indicating shallow areas and purple indicating the deepest areas, sourced from the DFO Cultus Lake Lab (right).

Cultus Lake is surrounded by mixed use zoning including two residential areas on the north and south ends of the lake, and Cultus Lake Provincial Park on the eastern shore. The Park was established in 1948 and spans 2729 ha (BC Parks 2019). The lake's shoreline drops off steeply in most areas, except along the north shore, and a small area around Spring Bay. The littoral zone includes areas of soft mud, organics (i.e., invasive milfoil, detritus), gravel, and large cobble. Due to its proximity to a major city and multiple attractions, Cultus Lake is used heavily for recreational activities throughout the summer.

Receiver Array

To collect and store data, 10 InnovaSea Systems Inc receivers were deployed in Cultus Lake (Figure 3.2). All receivers were VR2Tx-69kHz coded acoustic receivers with transponders that sent out uniquely coded identifiers. Eight receivers were mounted to metal frames and attached to braided lines with a small float. They were then deployed between 6-8 m depth, with the sunken float just visible from the surface. Two receivers were attached to existing data collection lines, placed at depth (20 – 25 m), in the centre of the lake. The eight receivers on metal frames were deployed in May 2020, and the remaining two were deployed in September 2020. Receiver locations remained constant other than for data downloading every 4 – 6 months and replacing batteries once a year. The scheduled maintenance was frequent to avoid the loss of data if the receiver went missing or if batteries died.

Receiver locations were determined based on snorkel surveys, substrate type, littoral zone depth, and to maximize coverage on the lake. In 2019, snorkel surveys indicated that bass nests were primarily at the north end of Cultus Lake, between Sweltzer Creek and Entrance Bay. Additionally, for spawning smallmouth bass prefer shallow (1 – 2 m depth) (Fayram and Sibley 2000; Suski and Ridgway 2009), rocky substrate (Bryan and Scarnecchia 1992; Wiegmann et al. 1992), with little vegetation. Therefore, five receivers were placed along the north shore, and three in rocky, shallow areas towards the south end of the lake. The additional two receivers at depth were placed to document over wintering habits. Overlapping of receiver detection range was expected, especially within the spawning grounds. This overlap allowed us to determine the detection range of the receivers and disregard any possibility of undetected zones within the spawning grounds (Peat et al. 2016).

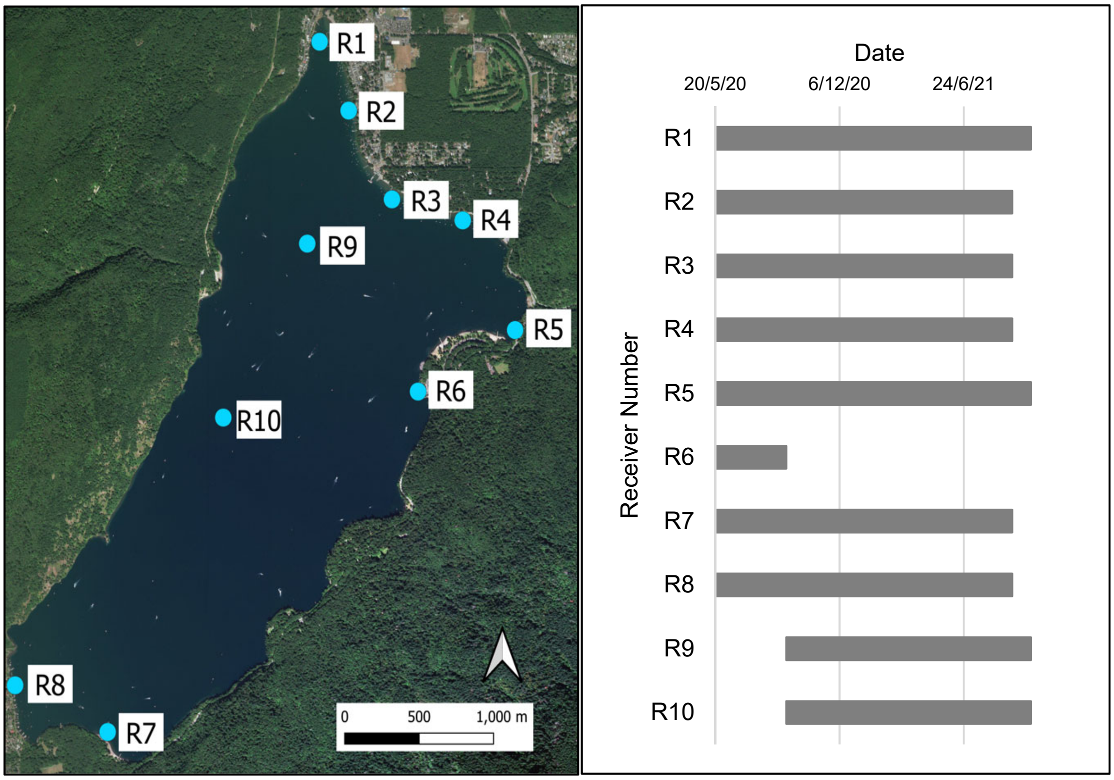

Figure 3.2. Receiver locations in Cultus Lake, BC (left). Deployment and removal dates of receivers (R1 - R10) (right).

Fish Surgery

All acoustic tags and receivers were ordered through InnovaSea Systems Inc. Tags were ordered based on fish size, never exceeding 5% of the total body weight, and included both V9 and V13 69 kHz coded tags. Battery life ranged from 520 to 699 days, with pings sent out every two to three minutes, once deployed. Some tags were also able to record temperature, which could then be correlated to water temperature profiles to determine fish depths. To verify that transmitters were functioning properly post-implantation, signal transmission was confirmed with a VR-100 portable receiver. 43 tags were surgically implanted in the bass in May 2020 and May 2021.

All angling and surgeries took place at Cultus Lake, BC to minimize handling time before release. Two local anglers (Nick Basok and Peter Buck) were hired using project funding to catch smallmouth bass throughout the length of the study. The anglers targeted bass on the northeast shore (near Main Beach) since this was the known spawning area and bass congregated in this location. The anglers started fishing at 7:30 am each day and would stop at 1:00 pm. This seemed to be the ideal timeframe for catching smallmouth bass in the spring. Once caught, the anglers transferred the bass to a live well on their boat, replenishing water every 5 minutes. The bass were held for a maximum of 30 minutes in the live well until they were transferred to shore for surgery.

There is a potential for bias in catching bass exclusively in the north end of the lake, since this could be their home range. However, in extensive snorkel surveys, we did not find nesting bass in any other location except the north end. Additionally, the anglers did attempt to catch fish in other areas, but were only successful in the north end in the spring. This supports the findings of bass currently spawning exclusively in the north. Other methods of capture were considered (gillnetting, electrofishing) but were either too expensive or had the potential to harm the fish before surgery.

Once on shore, the bass were immediately transferred to a holding tank filled with ambient lake water, a bubbler, and shade cover. Water in this bin was also changed frequently, depending on the daily temperature. In preparation for a surgery, all surfaces and instruments were disinfected. To anesthetize the bass 35 mL pure clove oil was emulsified with ETOH and added to 50 L of water. As soon as the bass were placed in the clove oil mixture, we timed their progress to immobilization and stage-4 anesthesia, sufficient for tag surgery (total loss of equilibrium; regular and slow opercular rates (Gutowsky et al. 2020)). Depending on the size of the bass, movement stopped between 4 and 9 minutes, and bass were then placed on the surgical table.

Biological data (fork length (mm), weight (g), and scale removal) was taken immediately following anesthesia. Bass were then inverted, ventral side up in a V-shaped surgery trough lined with nonslip matting. Gills were continuously aerated with cool, oxygenated water to maintain normal respiration. Using a clean scalpel, a small incision was made slightly off center of the fish’s ventral line, anterior to their anus. An acoustic transmitter was then inserted and gently massaged until it lay flush in the body cavity. Incisions were then closed with 1-2 absorbable monofilament sutures. The fish was also tagged using an external spaghetti tag (Floy Manufacturing, Seattle, Washington), between the dorsal pterygiophores, and printed with the researcher's phone number, a unique ID, and 'Please return to lake'. External pink tags signalled the presence of an internal acoustic tag and messaging was delivered to anglers to release these fish.

Fish were then placed in cool, oxygenated water for 10 minutes of recovery. If after 10 minutes, body orientation was prone and at equilibrium, the fish were released from shore, within 400 m of their capture location. All fish recovered quickly and swam away immediately after placement in the lake. Surgical processes were approved by the Thompson Rivers University Animal Care Committee (102400), and performed by a vet-trained individual. Surgeries were performed by the same individual in both study years to maintain consistency. Scientific Fish Collections Permits were obtained through the province of British Columbia in both 2020 (SU20-605014) and 2021 (SU21-623149)

Water Temperature Measurements

Lake characteristics were measured twice a month to document temperature (°C) and oxygen (% and mg/L). Throughout the summer months (May – Sept), these measurements were taken every meter to 42 m, using a YSI multi-meter. In the winter (Oct – April), data were collected using the Department of Fisheries and Oceans permanent data logger lines. These are the same mooring lines that the two project receivers were attached to. This temperature data was graphed and used to compare with temperatures recoded from the tagged fish, resulting in information on the vertical movements of the bass.

Snorkel Surveys

Snorkel surveys were conducted every 2 – 3 weeks during the spawning season (April – June) in 2021. The objectives of the surveys were to visually identify and quantify nesting sites, and to document timing of nesting behaviour. Depth and substrate are important indicators of smallmouth bass spawning areas (Wiegmann et al. 1992; Ettinger-Dietzel et al. 2016) and were considered when choosing snorkel transects. Two snorkelers swam a 400 m meandering transect and repeated this over 3 days. Due to the cold-water, snorkelers stayed in the water for a maximum of two hours at a time. An accompanying kayaker recorded general nest location, and the presence/absence of a guarding male, eggs, and/or fry. Once a nest was located, snorkelers hovered for approximately one minute to identify if a guarding male was present. Additional exploratory snorkel surveys were conducted in similar habitats throughout the lake, but nesting was not documented anywhere except the northeast shore of Cultus Lake.

Data Management and Analysis

Data was downloaded twice a year via Bluetooth and analyzed using R (R Core Team 2020), QGIS (QGIS Development Team 2022), as well as Innovasea's software VUE and Fathom Central (Innovasea 2020). Using VUE, a time correction analysis was run as well as a search for false detections. False positive detections can occur where there is too much noise, either from the surrounding environment, or from too many tags in the same locations, causing transmissions to collide and resulting in random erroneous tag IDs (Binder et al. 2017; Brooks et al. 2019). These detections were deleted from the data set. Data was then plotted on an individual fish basis to check for bass mortality. Any bass that were suddenly missing from the data or spent an indefinite amount of time at one receiver at a consistent temperature were assumed dead and removed from the data set (Klinard et al. 2020).

Receiver data files were then uploaded to the Fathom Central software to check for receiver logistics. Receivers collect data on noise level, tilt, temperature, and number of detections. This information can then be used to determine the accuracy of the receiver's listening capability. Noise levels above 650 mV are considered challenging, and fewer detections are expected on the receiver during these conditions.

RESULTS

43 SMB were tagged over the 16 months of the study. Weight (g), fork length (mm), and processing times of the tagged bass can be seen in (Table 3.1). 30

tags were implanted in May, and of those, ten bass appeared to die or been removed from the system, most likely via fishing. Five of those ten SMB were caught by the project's anglers. The external spaghetti tags seemed to occasionally be pulled out. This may be due to them falling out naturally, or anglers pulling them out. The project did have opposition from the bass fishing community, with some anglers threatening to remove the pink tags. The five SMB tags were re-implanted into new bass, two in July 2020 and three in May 2021. An additional eight new tags were implanted in the bass in May 2021, for a total of 43 bass over the course of the study.

Table 3.1. Descriptive statistics on tagged smallmouth bass in Cultus Lake, BC.

	Mean ± SD	**Minimum**	**Maximum**
Weight (g)	499 ± 252	228	1405
Fork Length (mm)	306 ± 42	242	425
Processing time (mm:ss)	04:42	02:60	08:30

Receiver Array

Receivers were downloaded twice a year to ensure data was not lost due to a missing receiver or a dead battery. Eight receivers were deployed in May 2020 (Figure 3.2Figure). One receiver (R6) went missing in September 2020 and was never found. This receiver was removed from the analysis. Two receivers (R9 & R10) were placed on data logger lines owned and managed by Department of Fisheries and Oceans Canada (DFO). These were deployed in September 2020 and downloaded in February 2022, as they were subject to DFO's work schedule. R3 and R4 had the most detections with over 1 million each. This may be due to 1) their position in relationship to the morphology of the lake and 2) their location within the spawning grounds.

Detections ranges were determined based on the receiver's ability to hear one another. Each receiver sends out a unique ping that can be heard by other receivers within range. Using this technique, we determined that the receiver's detection range varied greatly throughout the duration of the study. During the winter, (December – March), detection ranges appeared to be much greater. This may be due to the lack of activity on the lake, and the lessoning of temperature stratification (Singh et al. 2009). Detection ranges also appeared greater in Summer 2021, than in Summer 2020. In Spring/Summer 2020 Cultus Lake was much murkier than in Spring/Summer 2021 with Secchi depth readings of 2.7 m is 2020 vs 8 m in 2021. The additional particulates in the water might have led to the decreased detection range in 2020. Figure 3.3Figure 3.31 shows a conservative and liberal example of detection ranges in the lake depending on time of year. R1 and R5 were the most limited in their ranges due to their position on the shoreline.

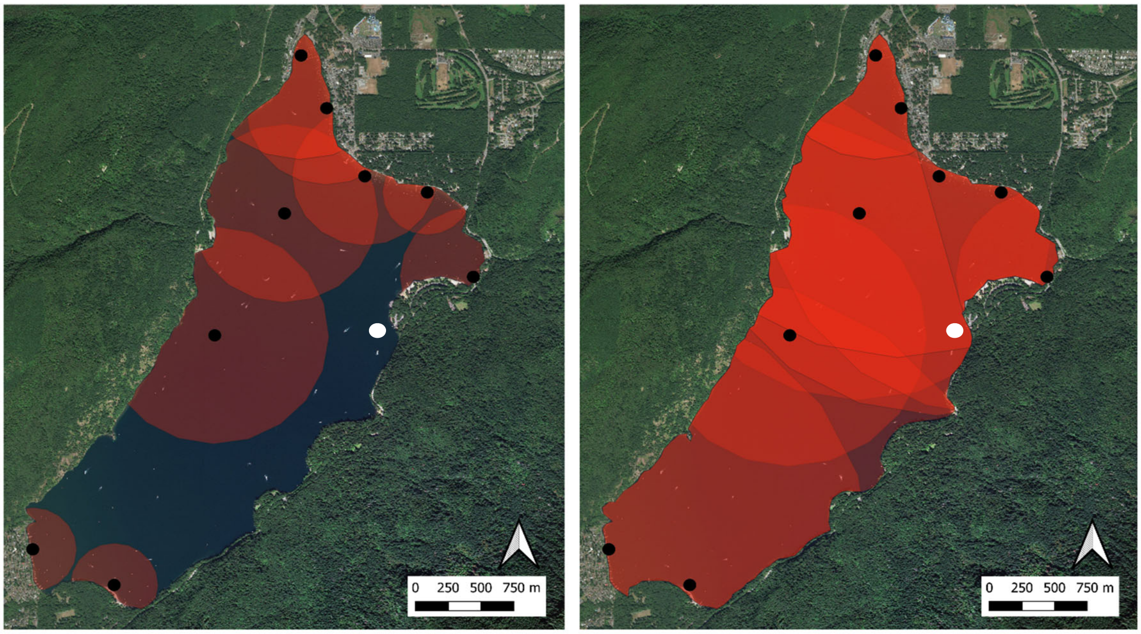

Figure 3.31. Conservative (left) and liberal (right) approximate detections ranges of acoustic receivers 1 – 9 in Cultus Lake, BC. The 10th missing receiver is shown with a white dot. Map created using QGIS (QGIS Development Team 2022).

Receiver diagnostics were analyzed using InnovaSea's Fathom Central software (Innovasea 2020). Factors such as water temperature, ambient noise, and tilt of the receiver all influence the receiver's ability to hear SMB tags. Figure 3.4 shows an overview of four (R1, R2, R4, and R9) receiver diagnostics. Note that the time scale is slightly different for all receivers, depending on when they were deployed. R1 had the lowest ambient noise, possibly due to its location, tucked into the bay, with no direct wind action. There is also a clear increase in detections during the April – June spawning time, which was expected. R2 and R4 had more ambient noise, due to their positioning. The wind on Cultus Lake typically travels along the fetch from south to north, and so these receivers were most likely impacted from the creation of wind bubbles (Gjelland and Hedger 2013). R2 had a spike in tilt on September 09, 2020. Someone had dragged the receiver to shore and this spike was from us retrieving, downloading, and replacing the receiver.

R9 was attached to DFO's data logger line and was placed at 23 m depth. At this depth, the receiver was consistently below the thermocline, and therefore had consistent temperatures throughout the study. R9 also had significantly more noise than expected. One possible explanation is the receiver's attachment to the data logger line. The receiver was attached via zip ties to a 42 m long line that can move around significantly, based on water currents, and there may have been some friction between the line and the receiver, causing noise. There was a noticeable increase in detections in the winter months on R9, indicating an increase in bass presence.

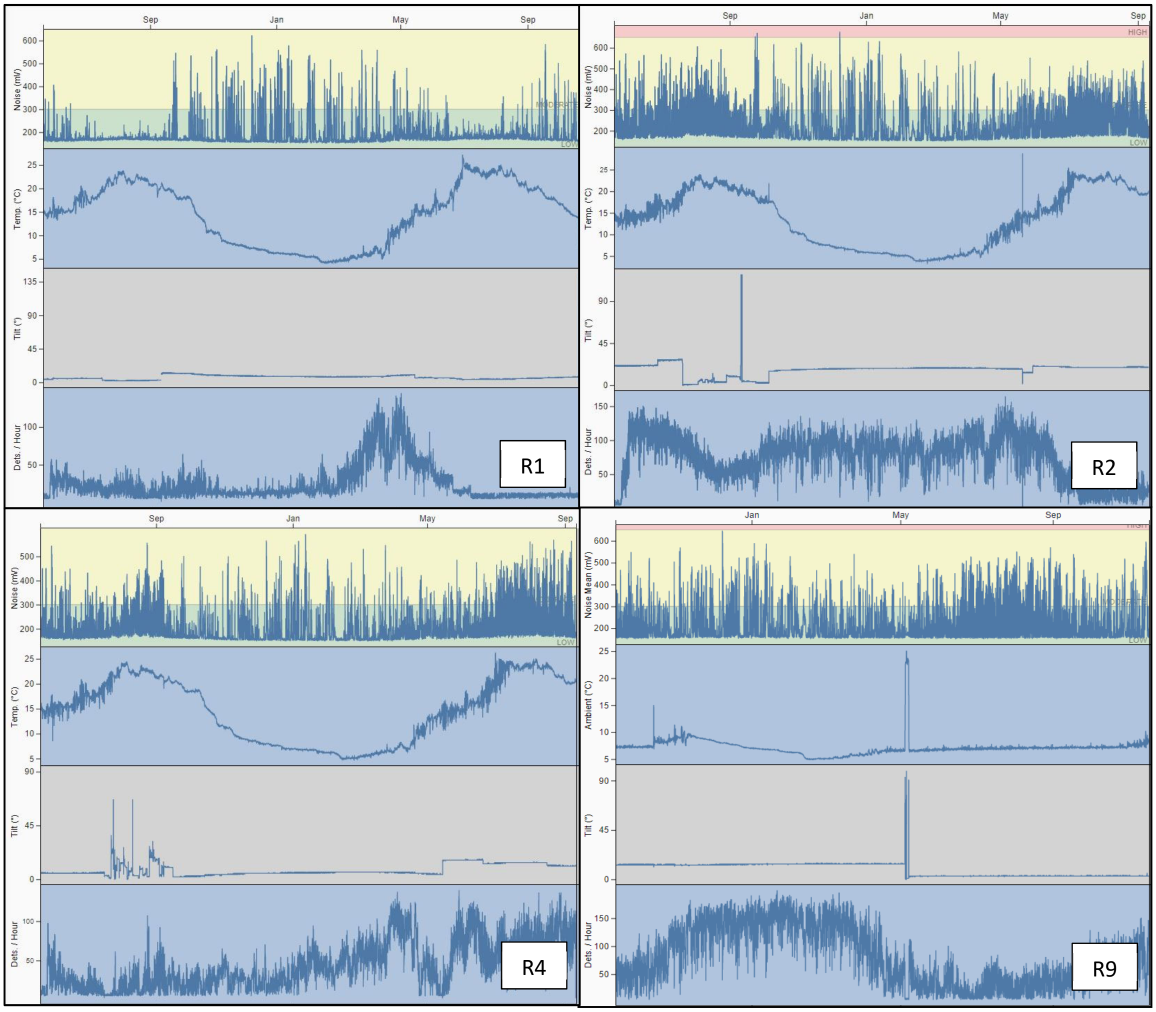

Figure 3.4. Receiver diagnostics for R1, R2, R4, and R9 (top left to bottom right) in Cultus Lake, BC, created using InnovaSea Fathom Software (Innovasea 2020). Diagnostics were calculated using InnovaSea's Fathom software, and are used to determine the noise, temperature, tilt, and detections/hour on each receiver. Noise levels are separated into three categories: low noise (green), medium noise (yellow), and high noise (red). Once noise levels are in the red, the receivers are no longer able to detect tags at an acceptable level.

Tag Detections

Once detections were filtered for false positives, there were 5,566,286 unique detections heard on the receivers. Figure 3.5 demonstrates the lifetime of each bass, with black bars indicating which bass were alive at the end of the study. Only one fish (SMB 19) perished within a few days after surgery. The increase in mortality in the Spring is possibly due to the increase of anglers and ease of catching bass during this time. Mortality was determined if we were notified by the angler of the catch or if the bass did not move from one receiver/depth for an extended period (more than 1 week).

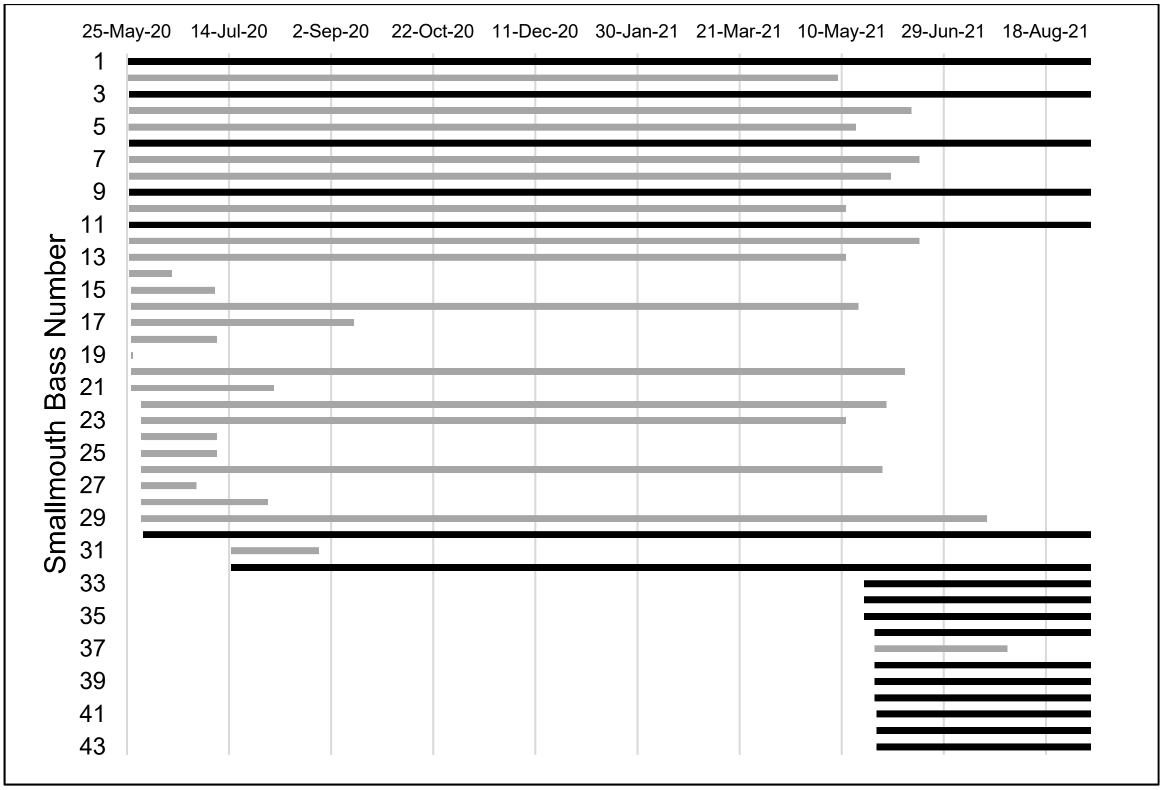

Figure 3.5. Smallmouth bass tagging and mortality in Cultus Lake, BC. Black bars indicate bass that were alive at the end of the study.

Seasonal bass movements were identified based on receiver detection rates. Seasons were divided into Spring (April – June), Summer (July – September), Fall (October – December), and Winter (January – March). Figure 3.6 Figure depicts these seasonal changes using the 9 receivers placed in the lake. Summer 2020 map does not include R9 and R10 since these receivers were added to the array in September. The gradient of colours represents detection rates, with lighter-coloured receivers detecting less fish and darker coloured receivers detecting more. The maximum number of pings on a receiver within a season was 252,283.

Spring detections showed a high level of activity along the north shore of Cultus Lake. This was expected since snorkel surveys identified this area as a spawning site. There were almost no detections at the south end of the lake during this time. Summer detections showed slightly more detections in the south and northeast corner of the lake. During this time the bass are finished with parental obligations and are feeding. In fall, there was a noticeable densification of pings at R3, R9 and R10. This could indicate that the bass are moving offshore and into deeper waters. Winter detections were similar to Fall detections, in that R3 and R9 were pinged the most. A few other receivers on the north shore had an increased number of detections in winter, this could be from bass coming back to shallower, more near-shore waters in March (See Discussion).

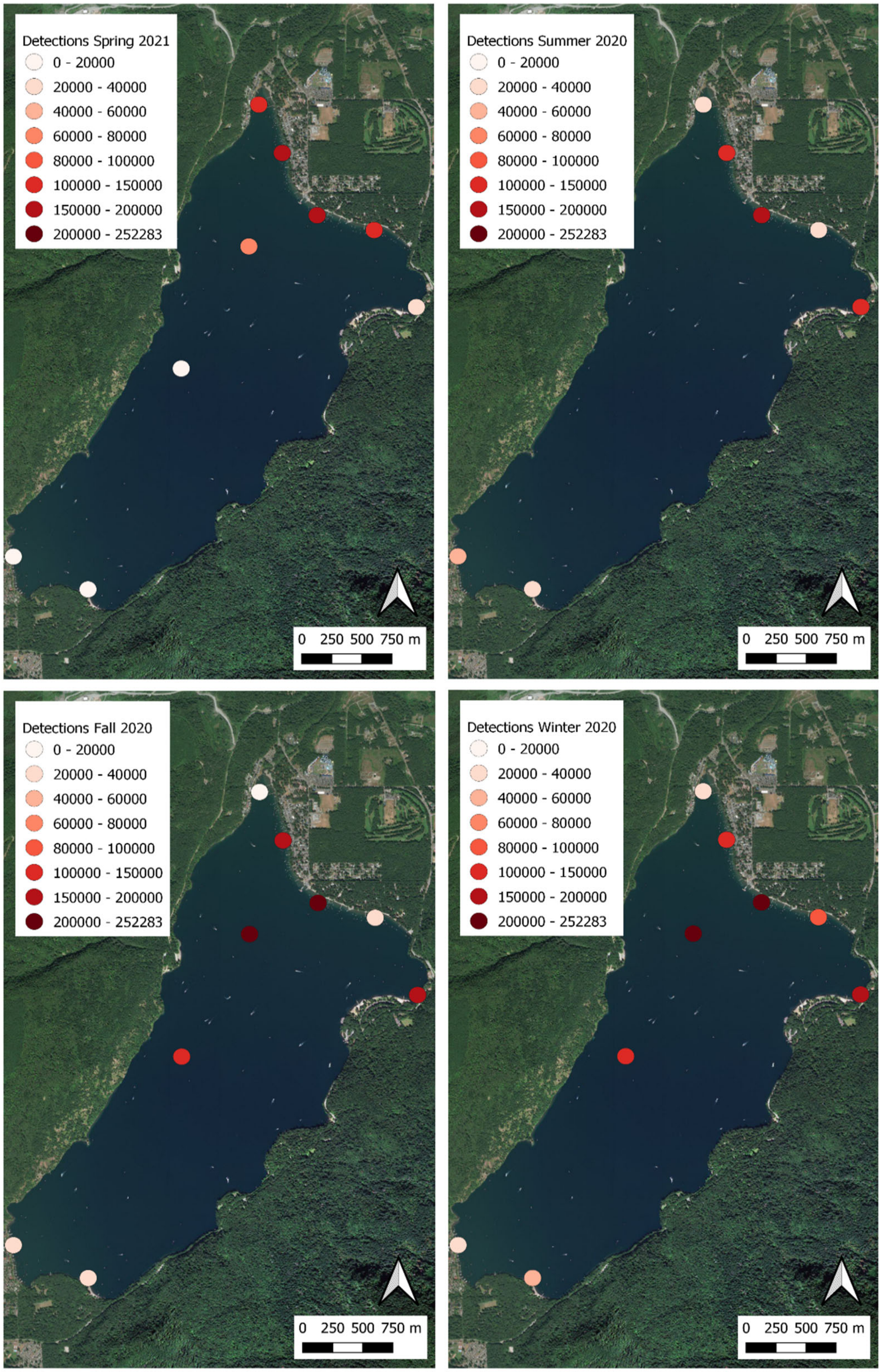

Figure 3.6. Frequency of smallmouth bass detections on receivers 1 - 10 for Spring 2021, Summer, Winter, and Fall 2020 (clockwise) in Cultus Lake, BC. R6 went missing. Map created using QGIS (QGIS Development Team 2022).

SMB residency periods were calculated using the InnovaSea VUE software. Residency periods were determined if a SMB was heard on a receiver for 48 hours (960 unique pings) with a maximum absence threshold of 1 hour. Figure 3.7 shows the residency periods for SMB 1, SMB 3, SMB 6, and SMB 11. These bass were chosen to be represented in the figure because they were alive for the complete duration of the study (Figure 3.5). The numbers above the bars represent the number of days that the bass were present at the receivers without more than 1 hour away. There are periods of time when the bars are overlapping between receivers, this is due to overlapping detection ranges, and the scale of the figure itself.

Residency periods can be used to interpret bass behaviour and predict aggregations. SMB 1 was heard for almost the entirety of the study on R3 and had a long (75 day) residency period during the spawning season in May/June. SMB 1, SMB 3, and SMB 11 were heard consistently on R9 (offshore receiver) throughout the winter. SMB 3 was the one bass with no spawning period on a receiver. This may indicate that this was a female SMB and did not stay to guard a nest. SMB 6 spent much of the fall and winter at the south end of the lake. This was consistent with 5 – 6 other bass in the study, while all others stayed north. SMB 6 and 11 both had distinct spawning periods in the Spring and were detected on R1 and R3 respectively. SMB 11 spent much of its time in the northeast corner of the lake where there is an abundance of large logs and variable boulder sizes.

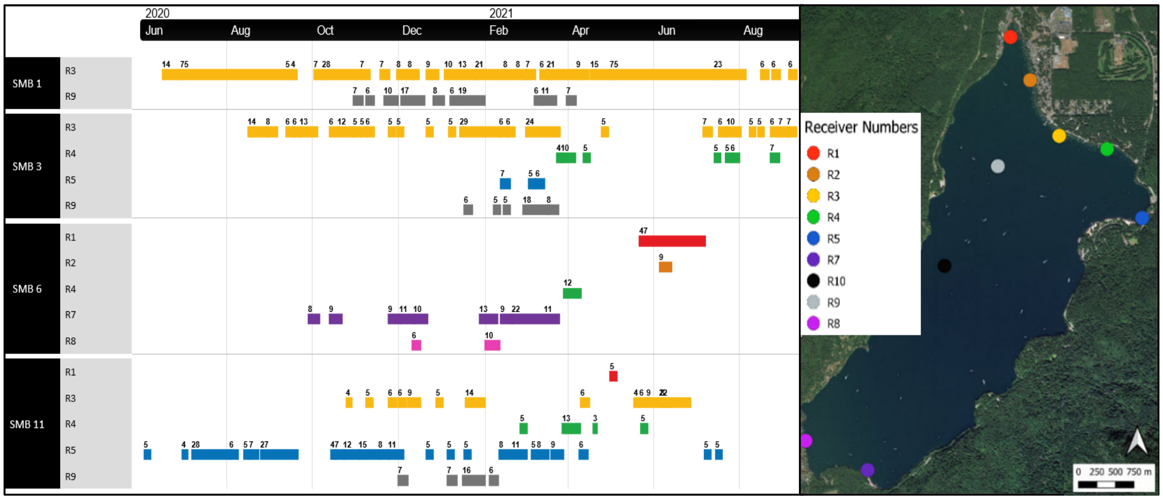

Figure 3.7. Residency periods for smallmouth bass 1, 3, 6, and 11 in Cultus Lake, BC. Residency periods were defined as a 48-hour periods with detections absence gaps never exceeding one hour. Numbers above coloured bars represent number of days of residency.

Water Temperature Analysis

Acoustic tag data was analyzed in R (R Core Team 2020) to determine average (±SD) bass temperatures each month for the study period. Bass appeared to stay above the thermocline in waters 15°C or higher until October (Figure 3.8). Water temperatures cooled off in November and tag sensors indicated bass between 5 – 10 °C from November to March. The standard deviation (SD) during this time was also significantly less than bass SD temperatures from April – October. This could be a combination of a homogenous water temperature column, and the inactivity of bass in the winter. Bass temperature peaked in July 2021 at an average of 23.6 °C.

Approximate bass depth was difficult to determine, due to the lack of temperature stratification in the winter. Average bass temperatures were compared to water temperatures on the 15th of each month (Figure 3.8). Then, a range of depths were approximated using the two readings. In January and February, the water column only fluctuated by 0.1°C, and so winter bass depths could not be determined. SMB do appear to move to shallow water in May and stay above 10 m depth throughout Spring and Summer. They then move offshore to deeper waters in October and November, and so this trend may continue into the winter.

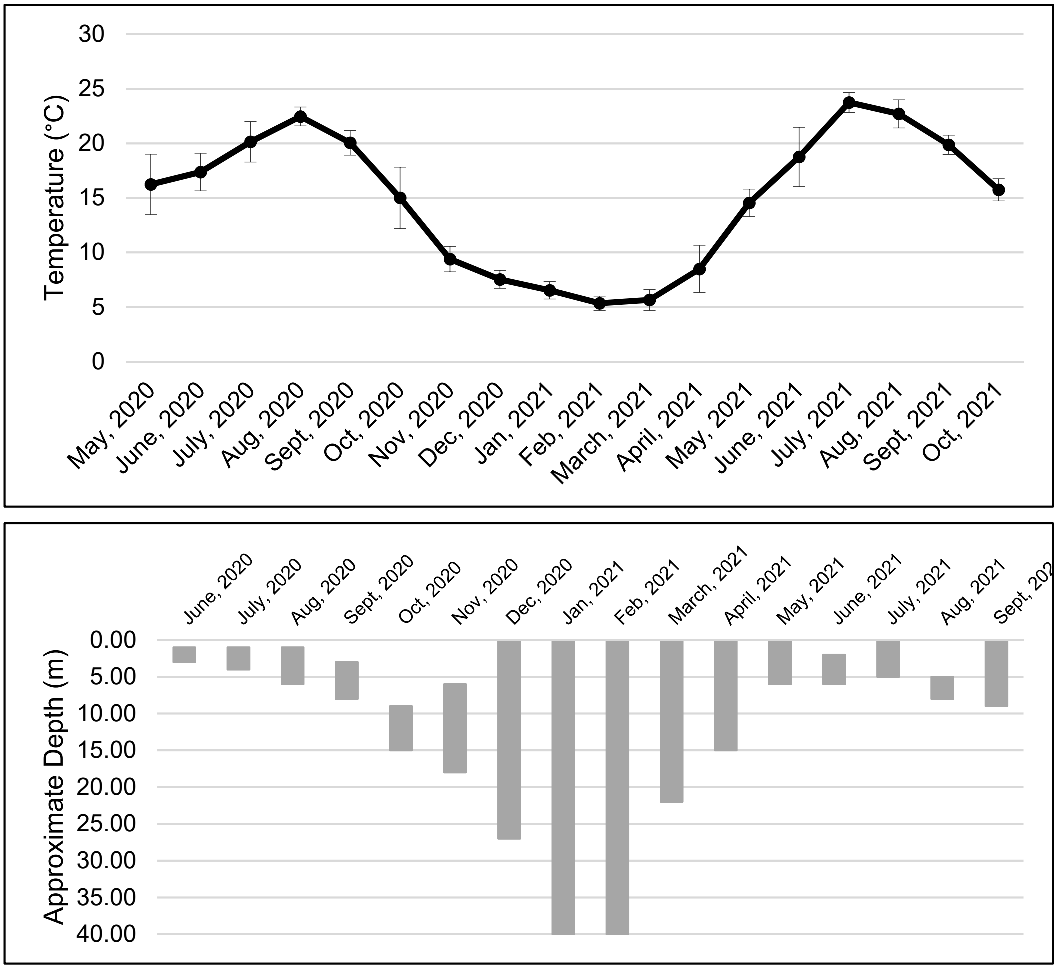

Figure 3.82. Average tagged smallmouth bass temperature, summarized by month (above). Correlated smallmouth bass depths, based on monthly temperature profiles and average bass temperature (below). Temperature data curtesy of Dr. Dan Selbie, DFO and John Axford, CLASS.

Snorkel Surveys

Four snorkel surveys were completed in Spring 2021 along the north shore of Cultus Lake. The first survey was completed on April 28th, when surface water temperatures were 12°C. No signs of bass presence or bass nesting were seen during this survey. The next survey was completed on May 12th – 14th (surface water temp: 14.5°C) resulted in the identification of 100 nests, 57 of which had a guarding male, and 19 had eggs present. Only one nest was found to have eggs and no guarding male. The following two surveys were completed on May 26th – 28th (surface water temp: 15°C), and June 10th – 11th (surface water temp: 16.5°C). On May 26th, one nest was found with SMB fry, and during the final survey 31 nests had fry. Figure 3.9 shows the nest locations and a corresponding heat map for the second (May 12th – 14th) and fourth (June 10th – 11th) snorkel survey.

Based on observations during snorkel surveys, nesting locations can be predicted in Cultus Lake using a few key factors. Locations with the highest density of nests had cobble substrate with no vegetation and little detritus. Nests were found between 0.5 – 2.5 m deep, but most often at 1.5 m depth. Surveys were extended east past the mapping extent, along Sunnyside campground; however, only 6 nests were ever found. One possible explanation is the male's affinity to large cement blocks, used to secure boat buoys. The area where nests were found is mostly a developed, residential stretch of beach, with many personal boat buoys.

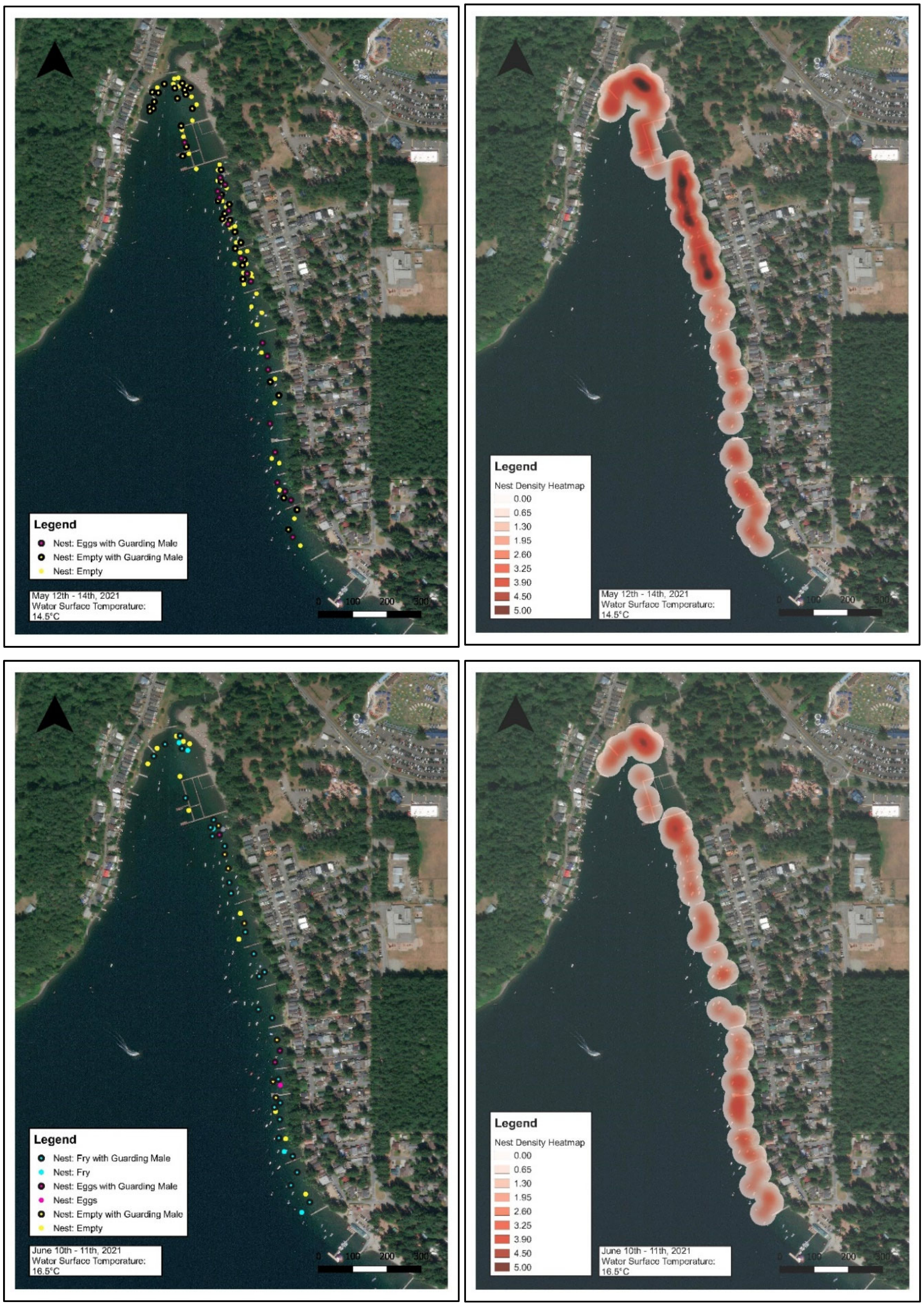

Figure 3.9. Nest Locations (left) and heatmap (right) of smallmouth bass nest sites found during a May 12th - 14th snorkel survey (top), and a June 10th - 11th snorkel survey (bottom). Nest locations were documented as 'between dock x/z', and were then plotted randomly between identified docks, within 0.5 – 2.5 m

depth. Map projection from QGIS using WGS 84 UTM zone 10N. Map created using QGIS (QGIS Development Team 2022).

DISCUSSION

Smallmouth Bass Seasonal Movements

Strong indicators of bass location are water depth and temperature (Ettinger-Dietzel et al. 2016), both of which are evident in Spring (April – June) SMB movements at Cultus Lake. SMB appear to congregate at the north end of the lake in the Spring. There was a clear shift from deeper water (0 to 22 m) to shallower water (0 to 6 m) in May when spawning begins, aligning with current literature (Fayram and Sibley 2000; Suski et al. 2009). Temperatures of the bass during this time averaged 15°C, the ideal spawning temperature (Kaemingk et al. 2011a). Residency periods in the spring ranged from 6 to 10 weeks, during which time the male bass will invest large amounts of energy choosing a nest location, creating the nest, attracting a mate, and protecting the young (Steinhart 2004). Due to the nature of the surgery, it was not possible to distinguish between males and females. However, using the residency periods we can estimate which SMB were females due to their lack of extended spring residency periods.

SMB appeared to disperse throughout the lake in the Summer (July – September). Detection rates were less intense at individual receivers, indicating a more evenly distributed population. Females are known to move offshore into the pelagic zone post-breeding (Kaemingk et al. 2011b). During this time, the bass stayed in warm (19 – 23°C), shallow (1 – 10 m) water. Movements throughout the water column are closely linked to the seasonal changes in the thermocline's presence and position, with bass staying above the thermocline in summer months (Suski and Ridgway 2009). Cultus Lake has warm, monomictic and thermally stratified water from May to November each year (Shortreed 2007). Bass also have a high tolerance to temperature increases (Mckinley et al. 2000; Cooke et al. 2003), and this ability to acclimate to increasing temperatures could forecast easier adaptation for bass during climate change (Middaugh and Magoulick 2018). This temperature change has been documented at Cultus

Lake, with a significant increase in both shallow and deep-water temperature from 1930 – 2000 (Ricker 1937; Shortreed 2007). Summer months also indicated fewer residency periods for the bass, demonstrating movement and higher activity levels.

SMB typically migrate offshore to greater depths during the winter season (Tabor et al. 2010; Ettinger-Dietzel et al. 2016). At Cultus Lake, this migration started in our 'fall' period. In October, bass were found between 9 – 15 m, and in cooler temperatures. The thermocline at this time was between 10 – 14 m deep, and so the shift from above to below this stratification was evident. In November, bass continued their trend downward averaging 6 – 18 m depth and 9.4°C. Unfortunately, the lake became less stratified in November and so depth estimates became less precise. The average temperature of the bass in December was 7.5°C, with an even larger estimate of depth (0 – 27 m). Even though we cannot estimate depths during this time, the trend from October – December indicates the bass are moving deeper, and so we assume the bass remain at depths during this period.

We estimate that bass remained at depth throughout the winter period (December – March). The lake remained unstratified until late April, making depth calculations impossible. Bass temperatures remained between 5 – 6°C for the duration of winter, with the lowest average temperature in February (5.3°C). There was also an increase in residency periods throughout December – March, with many bass spending extended periods of time within the range detection of R9. This was supported by the seasonal detection maps, where there appeared to be a densification of bass during this time around R9 and R3. There are two deep (43 m) areas of the lake, and one is located close to R9. Between the increased detections at R9, the increasing depths from Oct – Dec, the minute standard deviation on average temperature, and the residency periods, we assume the bass are at depth in this lake depression.

The second seasonal migration for the bass occurs in April, when average bass temperatures start increasing, and the lake begins to stratify (Shortreed 2007). Thermal stratification of the lake starts in March (Ricker 1937), and a clear thermocline was observed in April. Average bass temperature increased from 8.5°C in April to 14.5°C in May, and bass depths changed from 1 – 15 m in April, to 0 – 6 m in May. This migration to shallow warmer waters, and the start of the spawning period is the ideal opportunity for suppression. Overall, the bass spend the majority of their time in the north end of the lake, with only a few individuals migrating south.

Study Limitations: Public Interference

Overall, the receiver array worked well and covered the targeted areas of the lake, with only minor setbacks. One issue with the placement of the receivers was the public. The sunken floats attached to the receivers were meant to be far enough down that a snorkeler could not retrieve the device. However, on two occasions, receivers were found dragged up on shore. Another issue was the ability of strong fishing lines to hook the floats and pull them down the steep drop-off contour. This happened with multiple receivers; two were located via scuba diving and one was never found. The popularity of Cultus Lake made it difficult to conduct a study with zero interference from the public and this should be considered in future studies. Diagnostics of the receivers did show that noise levels were within a reasonable range. This was a concern going into the study considering the high boat traffic, wind (Gjelland and Hedger 2013), and degree of water temperature stratification in the summer (Singh et al. 2009). With the noise levels detected, the receiver's range and accuracy of ID detections was sufficient for the study.

The mortality of tagged SMB was expected at Cultus Lake due to the popularity of sport fishing in the area. SMB are present in five regions of British Columbia, but their establishment in Cultus Lake is a first in the Lower Mainland, a densely populated area with highly suitable habitat for the bass (Mandrak et al. 2010). A great effort was made to spread awareness about the issue and our study methods, but challenges included a high number of visitors, the COVID-19 pandemic, and some opposition from the bass fishing community. This problem is one of the most challenging aspects of invasive species management (Drake et al. 2015), especially due to the large size of Cultus and the substantial angling activity at the lake (Drake and Mandrak 2014). To change angler behaviour, we need to alter perceptions of a healthy ecosystem (Drake et al. 2015) through proactive, preventative education (Finnoff et al. 2006).

Nesting Patterns

Snorkel surveys completed in Spring 2021 showed a distinct area of spawning aggregation. Nests were found along a 1 km stretch from the north corner (Sweltzer Creek) east towards the Cultus Lake Marina. They were built between 0.5 – 2.5 m depth on the shoreline plateau, before a drop-off contour (Pflugr and Pauley 1984). Nests were created in clumps or patches and were not evenly distributed throughout the lake (Rejwan et al. 1997). The location of the nests is ideal for egg success. Wind patterns on Cultus Lake travel along the fetch from south to north and can cause an upwelling of cold water, killing developing offspring (Steinhart et al. 2005), and scattering the eggs throughout the littoral zone (Goff 1985). The nests in Cultus are along a west facing shoreline and are less heavily impacted than if nests were created along the south facing shoreline. The first survey was completed on April 28th, when surface water temperatures were 12°C, and no bass activity was found. The following surveys (May 12th – June 14th) showed the progression of nests from eggs to fry.

Bass nests were often found adjacent to the cement buoy anchorages. One hypothesis is that the cement blocks act as a shield from predators. In this sense, the bass only have to guard three directions from the nest, instead of four. Snorkel surveys were also completed at the south end of the lake along Lindell Beach. No nests were ever found, possibly due to the large amount of accumulated detritus on the substrate.

The location and timing of SMB nesting in Cultus Lake overlaps considerably with the migration of endangered sockeye salmon smolts. Sockeye return to Cultus Lake by way of the Fraser, Sumas, Vedder, and Chilliwack River via Sweltzer Creek (Cultus Sockeye Recovery Team 2009) every fall with a historical 4-year average abundance of 19,890 spawners. The population now (2015 –

2018) has a generational average of 254 natural-born, and 941 hatchery-born fish (DFO 2019). The smolts of these spawners then leave the lake in April/May, again via Sweltzer Creek. This outmigration overlaps both spatially and temporally with the spawning of smallmouth bass.

Management

The data used in this study will be beneficial for future suppression of smallmouth bass in Cultus Lake. Similar to Cultus Lake, other studies have used spatial data to target invasive fish populations and identify their spaciotemporal congregations for more effective suppression (Bajer et al. 2011; Gutowsky et al. 2020). The spatial and temporal data gives us a clear idea of where and when the bass are congregating in the spring, an ideal time for suppression. Methods such as electrofishing (Weidel et al. 2007; Burdick 2008; Biron et al. 2014) and beach seining (Hawkins et al. 2009) are popular suppression techniques, with similar objectives of targeting multiple life stages (Loppnow and Venturelli 2014) with intensive effort over multiple years (Rytwinski et al. 2019). Targeting the most productive males and enhancing native nest predators (Loppnow et al. 2013) are two additional, feasible methods due to our knowledge of nest locations and the presence of predatory white suckers (*Catostomus commersonii*). Regardless of the suppression methods chosen by fisheries managers, they should consider the broader goals and unintended outcomes (Prior et al. 2018) of smallmouth bass suppression.

Suppression of the bass is not only important for the overall ecosystem health, but also to mitigate further declines in populations of two species-at-risk, Cultus Lake sockeye salmon (*Oncorhynchus nerka*) (COSEWIC status Endangered) and coastrange sculpin (Cultus population) (*Cottus aleuticus*) (SARA status Threatened). Sockeye smolts leave Cultus Lake in April/May via Swetlzer Creek (Cultus Sockeye Recovery Team 2009). This temporally and spatially overlaps with smallmouth bass spawning. Additionally, in Chapter 2 of the thesis, we discussed the increased presence of *Oncorhynchus nerka* in the stomachs of bass caught on the spawning ground. The bass may also have the opportunity to impact additional native populations, with their potential migration through Swetlzer Creek, and into neighbouring water systems. With all this information, it is critical that bass are suppressed with immediate and intensive action. Unfortunately, there have not been any recently published studies on the endemic pygmy sculpin locations (Woodruff and Taylor 2013).

Based on the data collected here, we recommend spearfishing and physical nest destruction as a creative (Loppnow et al. 2013) and targeted method of SMB suppression. Spearfishing is a selective technique with zero bycatch (Morris and Whitfield 2009), that has shown some successful eradication efforts (Hill and Sowards 2015; Hickerson et al. 2021) and has even been used by Indigenous groups for sustainable marine management (Tsai 2020). Historically used in marine environments (Morris and Whitfield 2009; Harris et al. 2020; Michailidis et al. 2020), the technique is now being adopted into freshwater systems for invasive control (Blanton et al. 2020). The Oregon Department of Fish and Wildlife (ODFW) recently opened spearfishing on the Coquille River as a suppression method for invasive SMB (G. Vonderohe (Oregon Department of Fish and Wildlife), personal communication, August 31, 2021) and are currently conducting snorkel surveys to document the effectiveness of the method. We recommend trialing spearfishing and nest destruction in a controlled setting for the following reasons (1) bass are congregated in a 1 km stretch, and nests are not deeper than 2.5 m (an easy depth for snorkeling) (2) male SMB guarding the nests do not move until snorkelers are closer than 1 – 2 m (3) adult spawning males are large enough for spearfishing (4) nests with eggs/fry can then be destroyed via burial, electrofishing, or natural predation from white suckers. Finally, both of these methods are low cost, and can be implemented from a community level without relying on government involvement. In addition to these methods, we recommend the continuation of snorkel surveys to detect if spawning is spreading to different locations or depths.

The outcomes from this paper also suggest a need for preventative education. Cultus Lake has a unique situation where there is a passionate group of volunteers (Cultus Lake Stewardship Society), and a large influx of tourists throughout the summer. With this combination, there is potential for a sustainable long-term awareness program at Cultus Lake, to inform users of the dangers of spreading invasive species, and the impacts SMB are having on the native fauna. This collaboration between locals, academia, and fisheries managers could lead to co-production of knowledge and allow for more efficient implementation of management strategies (N’Guyen et al. 2016). There is also the potential for SMB to migrate via Sweltzer Creek into the surrounding water bodies. Here, the precautionary principle should be enacted in order to avoid serious harm (Cooney 2004) to the neighbouring fish populations. With community outreach, rapid response in early detections of the species is a possible, and cost-effective preventative action (Finnoff et al. 2006).

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CHAPTER 4
CONCLUSION AND MANAGEMENT RECOMMENDATIONS

The smallmouth bass study at Cultus Lake was successfully completed over two field seasons and resulted in some key findings and management recommendations. The importance of this study is relevant in academic fields such as species-at-risk, acoustic telemetry, and invasive species management. This final chapter will recount some of those key findings and the overall significance of the research. It will then go over some study limitations and end with management and scholarly recommendations.

KEY FINDINGS

Diet

Smallmouth bass are eating a wide variety of species in Cultus Lake, which is in line with our underlying assumption of them as opportunistic feeders. The DNA analysis was helpful in identifying highly digested prey items and resulted in the identification of 26 different prey orders. Bass began their piscivorous diet in Cultus Lake once they reached 115 mm total length and continued to feed on fish throughout their lives. There was little difference in diet composition, based on size class; however, this may have been different if prey items were counted or weighed. There were noticeable seasonal shifts in bass diets, often correlating to invertebrate emergence timing.

Smallmouth bass were feeding on both *Oncorhynchus nerka* and *Cottus aleuticus*. Coastrange/pygmy sculpin were found in 134/145 stomachs and were consumed more heavily by bass in smaller size classes. We were unable to distinguish between coastrange and pygmy sculpin, as they are too closely related to identify using basic DNA barcoding. The diet samples are being held and may undergo further analysis in the future. Sockeye salmon/kokanee were found in 18% of bass stomachs. Bass were also feeding more heavily on salmon within the bass spawning grounds, which overlap spatially and temporally with the outmigration of salmon fry via Sweltzer Creek.

Distribution

Seasonal movements of bass were documented using 10 receivers and 43 tagged bass from May 2020 – October 2021. Bass spent the Spring (April – June) congregated along the north shore of Cultus Lake, where they spawn. During this time, they remained above the thermocline between 0 – 6 m deep, and bass temperature increased from an average of 8°C in April to 15°C in May, which is ideal SMB spawning temperature. Then in the Summer (July – September) the bass migrated to other areas of the lake where they were actively feeding post-spawning. Bass depths during this time remained shallow until September when the bass began to move deeper, to cooler temperatures. In Fall (October – December), the bass continued to move offshore and to deeper waters. They started congregating around R9 (northwest corner), with average temperatures dropping from 15°C to 7°C. SMB moved to noticeably deeper water in October and November; however, in December the water column became homogenous, and depth could not be determined. Finally, in Winter (January – March), bass remained in cooler temperatures (5°C - 6°C) in the northwest corner of the lake. Based on the temperature/depth profiles, bass may start to move shallower in March (0 – 22 m), with shallower movements further in April (0 – 15 m), and May (0 – 6m).

Bass spawning locations and timing are now known and can be used to create a suppression plan. In 2021, SMB started spawning in the first two weeks of May when the surface water temperature was 14.5°C. 100 nests were identified during the May 12th – 14th survey, all between Sweltzer Creek and Cultus Lake Marina. Additional surveys were completed at Lindell Beach (south end) and Sunnyside Beach (east of Cultus Lake Marina), with few or no nests identified. Snorkel surveys continued to show dense SMB nesting throughout May and June when both eggs and fry were observed. Nests were typically between 0.5 m and 2.5 m depth and could often be seen from the surface.

OVERALL SIGNIFICANCE OF STUDY

The overall significance of the study is relevant within academics, management, and policy. As smallmouth bass are introduced into an increasing number of western waterbodies (Fayram and Sibley 2000; Tabor et al. 2007; Fisheries and Oceans Canada 2010; Emingway et al. 2019), their impacts are important to publish and can be applied to similar ecosystems. As well, although this thesis focuses mainly on the diet and movement of smallmouth bass, the methods were developed with suppression in mind. The data collected laid the groundwork for potential suppression techniques and therefore, the study can be used to cite the benefits of studying a population before investing millions of dollars into suppression. This relates to the relevance of this study for fisheries management. The project was in collaboration with regional management (FLNRORD), the local Cultus Lake Laboratory (DFO), and the aquatic invasive unit at the provincial level (MOE). Together, they helped develop the methods of this study, to better inform their next steps in management of SMB. Finally, the project had great importance to a current review of the federal listing (Species at Risk Act: SARA) of Cultus Lake sockeye salmon. The results of this study were presented to a review board and taken into consideration, in efforts to list the first ever Pacific salmon species.

The other significant aspect of this study was its collaborative efforts. As previously mentioned, the project was developed and executed in partnership with FLNRORD, DFO, BC’s Ministry of Environment and Climate Change Strategy (MOE), Thompson Rivers University, volunteers, and the local stewardship group CLASS (Cultus Lake Stewardship Society). With all these partners, it meant that the project was well funded, had a multitude of experts helping with project development, and the awareness of the issue was spread at regional, provincial, and federal levels. The people involved in this collaborative work often volunteered time to help with snorkel surveys and outreach events, lent equipment, offered space for boat storage, and supplied supplemental data. The fieldwork and team meetings offered an opportunity for people from different backgrounds to get to know one another, creating a great framework for future work in the community.

LIMITATIONS

As with any project, there were limitations and challenges with the study. One of the initial setbacks, was the project's lack of involvement with the local Soowahlie First Nation, whose territory is adjacent to Cultus Lake. Originally the project proposal had asked for the involvement of the Band and stipulated a First Nation Technician. Unfortunately, the formation of the project also coincided with the outbreak of COVID-19, which left many communities and their resources stretched thin, and DFO was unable to secure band participation. Luckily, we were able to hire Garrett Martindale, who was an active member of the Sts'ailes Band, and a great asset to the project. The second challenge was the COVID-19 pandemic throughout the entirety of the project. One of the original objectives of the project was to hold outreach events to bring awareness to the issue and gather support from the community for future volunteer work. Due to restrictions, these events could not take place. We did however host an online information session, speak at multiple conferences, and join the monthly CLASS meeting on many occasions.

A third challenge was the large number of visitors to Cultus Lake. With so many people, and a multitude of different management areas around Cultus Lake (BC Parks, Soowahlie, Cultus Lake Park, residential) it was difficult to inform all users of the study. This meant that on occasion, our receivers were removed from the lake and dragged up on shore. There is also a vocal group of anglers in BC who are pro-bass in Cultus Lake. These anglers would engage in online forums, and discuss the destruction of the study, specifically by pulling out the pink tags from the bass. Ultimately, there was no way to monitor this behaviour and so it is possible that they tampered with the study. To try and dissuade these actions, we posted signage around the lake, and posted a bulletin on the FLNRORD website informing anglers of the dangers of smallmouth bass, and methods used throughout the study.

The many stakeholders involved in the project both helped and hindered the work. Different governments have individual restrictions to follow, timelines, and resource availability. Funding came from DFO and was funneled through MOE to TRU. However, this did mean that the project was well funded and had expert advice from professionals. There is also a difference in accountability when there are so many partners in that they each assume someone else will take on more responsibility. We found that much of that additional responsibility fell to TRU to manage. When there are many stakeholders involved in a project, they often have different objectives. In the future, we suggest creating a clear organizational chart with responsibilities, and objectives of each group to uphold team accountability. In addition to the government stakeholders, we had anglers that were both pro and con SMB in Cultus Lake. Some were supportive of the project's efforts, while others actively sought to destroy it by fishing out the tagged fish. This dispute between anglers and resource managers at Cultus Lake needs to be addressed with more education on the ecological dangers of such an adaptable, piscivorous, invasive fish.

The project also faced challenges with some specific data analysis. The first was with InnovaSea's receiver data. InnovaSea provides the physical equipment, and basic software to view receiver diagnostics and general patterns in tag/receiver detections. For any in-depth analysis, there is very little support, and the only available option appears to be two packages in R called GLATOS and V-Track. GLATOS was developed specifically for the Great Lakes System and so has little use in other water bodies. The second package (V-Track) was developed by an independent researcher; however, most details on how to use the package have been removed from the internet. For future work, we would recommend establishing a partnership with an experienced V-Track researcher. The second analysis issue was during the DNA barcoding, and the inability to distinguish between kokanee/sockeye salmon and pygmy/coastrange sculpin. We knew going into the analysis that there would be no distinguishing between the sculpins, since this has been established (Woodruff and Taylor 2013). In personal communications with a DFO geneticist (Ben Sutherland), distinguishing salmon from a homogenized sample is not possible. It may be possible with individual prey samples but due to the inbreeding of Cultus sockeye and kokanee this might still be challenging.

MANAGEMENT AND SCHOLARLY RECOMMENDATIONS

The outcomes from this project are informative for some specific management recommendations. The first recommendations are associated with the timing and sizes of hatchery releases. It appears that the hatchery released larger sockeye fry (87.7 – 99.7 mm length) into Cultus Lake in 2019 – 2020 than in previous years (2015 – 2018) (G. Lidin (DFO), e-mail message, August 25, 2021). They also released these fry in January instead of the spring/summer months. We recommend continuing this release strategy, as bass can consume sockeye fry between 34 – 130 mm total length (Tabor et al. 1993; Fayram and Sibley 2000; Tabor et al. 2007), and they are less active in winter (Suski and Ridgway 2009; Watson et al. 2019), giving the sockeye more of an opportunity to survive before the bass resurface in the spring. Even though growing the fry to a larger size will take more time and resources, it may have a greater payoff for survivorship. The second recommendation for DFO is for monitoring of the active fish fence in Sweltzer Creek. This fence is operational from late-March to May for juvenile salmon and late-July to November for adults (D. Klassen (DFO), personal communication, February 17, 2022). Most bass have been found at the fence in the spring (possibly looking for spawning habitat) and were euthanized by DFO staff. This should continue, and a data sheet kept, documenting the number of bass caught in the fence.

The next set of recommendations are for regional management (FLNRORD). Since there is a strong possibility of SMB migrating into neighbouring water bodies via Sweltzer Creek, we recommend implementing an early detection program in the Lower Mainland. This may include signage or online information about the appearance of smallmouth bass and where to report any findings. With early alerts on the invasive bass, action can be taken quickly, and costly damages could be avoided (Kaiser et al. 2010). This outreach could also include visiting local angling shops and monitoring online fishing forums to inform active angers of the SMB problem.

In terms of suppression, our recommendation is for FLNRORD to trial the use of spearfishing and nest destruction. Spearfishing is a selective method with zero bycatch (Morris and Whitfield 2009), that has shown some successful eradication efforts (Hill and Sowards 2015; Hickerson et al. 2021), and has even been used by Indigenous groups for sustainable marine management (Tsai 2020). Historically used dominantly in marine environments (Morris and Whitfield 2009; Harris et al. 2020; Michailidis et al. 2020), the technique is now being adopted into freshwater systems for invasive control (Blanton et al. 2020). The Oregon Department of Fish and Wildlife (ODFW) recently opened spearfishing on the Coquille River as a suppression method for invasive SMB (G. Vonderohe (Oregon Department of Fish and Wildlife), personal communication, August 31, 2021) and are currently conducting snorkel surveys to document the effectiveness of the method. We recommend trialing spearfishing and nest destruction in a controlled setting for the following reasons (1) bass are congregated in a 1 km stretch, and nests are not deeper than 2.5 m (an easy depth for snorkeling) (2) after snorkel surveys in 2021, we know that male SMB guarding the nests do not move until snorkelers are closer than 1 – 2 m (3) adult spawning males are large enough for spearfishing (4) nests with eggs/fry can then be destroyed via burial, electrofishing, or natural predation from white suckers. Finally, both methods are low cost, target multiple age structures, and can be implemented from a community level without relying on government involvement.

The final management recommendation would be to implement a sustainable community stewardship program via CLASS. The members of CLASS live in the Cultus Lake community and are actively searching for ways to increase stewardship at the lake. The first action would be to implement bi-weekly nest counts via kayak. When the water is clear, most nests can be observed from the surface, and a count would aid in populations estimates. The second action is for more involvement in the yearly Cultus Lake Derby. This event was initially held to suppress the population of Northern pikeminnow, but could easily be expanded to document SMB catches, again aiding in the population estimate. Since this event attracts large numbers of anglers, it’s also a great opportunity for community outreach and education about invasive smallmouth bass.

There is potential at Cultus Lake to continue the SMB project and expand the scope to focus on sustainable suppression methods. Cultus Lake has an ideal setup for trialing different suppression methods due to the location of the nests, and the evenly spaced docks, which create natural treatment segments. Suppression methods may include spearfishing, electrofishing, localized gillnetting, physical nest destruction, or a combination of methods. The study could also look at whether white suckers are feeding on the eggs once the male bass are removed and if they could aid in further suppressing the species. There is a great need for new creative suppression methods for invasive fish (Loppnow et al. 2013) and future research at Cultus Lake could produce additional insight into sustainable suppression of SMB.

CONCLUSION

Smallmouth bass are a highly adaptable fish, making their way through British Columbia, but with more research and collaboration, we can understand their impacts and efficiently target populations for suppression. The information collected during this project not only lays the groundwork for an effective suppression plan, but also has created inter-organizational relationships that may give the project the leverage and sustainability it needs to continue. This research would not have been possible without the funding and expertise lent by professionals and passionate Cultus Lake community members. With more collaboration, the conversation around invasive species reaches a wider breadth of people, hopefully educating and inspiring ecosystem stewardship.

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APPENDIX A

Output from the multiple logisic regression (Table 2.2 and Table 2.3):

```
glm(formula = Salmon.Present ~ Size.Class + Sex + Month.Caught +
   Location.Caught..S.NS., data = MLR.Salmon)
Deviance Residuals:
    Min       1Q    Median       3Q       Max
-0.49711  -0.21221  -0.10335  -0.00127   1.00836
Coefficients:
                    Estimate Std. Error t value Pr(>|t|)
(Intercept)            0.161940   0.237254   0.683  0.49608
Size.ClassII            0.024876   0.138350   0.180  0.85758
Size.ClassIII          -0.013456   0.171581  -0.078  0.93761
Size.ClassIV            0.126974   0.180020   0.705  0.48185
Size.ClassV            -0.003822   0.175928  -0.022  0.98270
SexJuv                -0.096936   0.128083  -0.757  0.45051
SexM                  -0.052178   0.087605  -0.596  0.55246
Month.CaughtAug        -0.080240   0.202509  -0.396  0.69258
Month.CaughtJuly        0.038348   0.204245   0.188  0.85136
Month.CaughtJune         0.049435   0.240048   0.206  0.83716
Month.CaughtMay        -0.104667   0.196739  -0.532  0.59561
Month.CaughtSept       -0.081998   0.216210  -0.379  0.70511
Location.Caught..S.NS.S  0.210941   0.080170   2.631  0.00952 **
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
(Dispersion parameter for gaussian family taken to be 0.1400852)
   Null deviance: 21.338  on 144  degrees of freedom
Residual deviance: 18.491  on 132  degrees of freedom
AIC: 140.87
Number of Fisher Scoring iterations: 2
```

```
glm(formula = Salmon.Present ~ Location.Caught..S.NS.)
Deviance Residuals:
    Min       1Q    Median       3Q       Max
-0.30645  -0.30645  -0.08434  -0.08434   0.91566
Coefficients:
                      Estimate Std. Error t value Pr(>|t|)
(Intercept)            0.08434    0.04062   2.076 0.039678 *
Location.Caught..S.NS.S  0.22211    0.06212   3.575 0.000478 ***
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
(Dispersion parameter for gaussian family taken to be 0.1369724)
    Null deviance: 21.338  on 144  degrees of freedom
Residual deviance: 19.587  on 143  degrees of freedom
AIC: 127.22
Number of Fisher Scoring iterations: 2
```

APPENDIX B

Below is example R code used in Chapter 2 of the thesis. For complete R code and data files please contact the author at wendymargetts1@gmail.com

Bass Length, Sex, and Location

```
BASS.STATS <- read.csv("C:/Users/Wendy/OneDrive - Thompson Rivers University/Documents/Thesis/Data/Bass Statistics/Bass Descriptive Statistics.csv", header = T)
library (DAAG)
attach(BASS.STATS)
BASS.STATS[1:3,]
names(BASS.STATS)
library(psych)

## Total Length ~ Sex
boxplot(Total.Length..mm.~Sex)
bartlett.test(Total.Length..mm.~Sex,data = BASS.STATS)
BASS.SEX.AOV=aov(Total.Length..mm.~Sex)
anova(BASS.SEX.AOV)
summary.lm(BASS.SEX.AOV)
library(psych)
describeBy(Total.Length..mm.,Sex)

##No Juveniles##
BASS.STATS2 <- read.csv("C:/Users/Wendy/OneDrive - Thompson Rivers University/Documents/Thesis/Data/Bass Statistics/Bass Descriptive Statistics_NO Juv.csv", header = T)
boxplot(Total.Length..mm.~Sex, xlab = "Sex", ylab = "Total Length (mm)")
detach(BASS.STATS)
attach(BASS.STATS2)
t.test(Total.Length..mm.~Sex)
```

```
## Total Length ~ N/NS Area
boxplot(Total.Length..mm.~S.NS)
t.test(Total.Length..mm.~S.NS)
bartlett.test(Total.Length..mm.~S.NS,data = BASS.STATS)
BASS.AOV=aov(Total.Length..mm.~S.NS)
anova(BASS.AOV)
summary.lm(BASS.AOV)

# Other
boxplot(Total.Length..mm.~Month.Caught)
BASS.AOV2=aov(Total.Length..mm.~Sex+S.NS)
anova(BASS.AOV2)
summary.lm(BASS.AOV2)
boxplot(Total.Length..mm.~Sex+S.NS, xlab = "Sex, Not Spawning (NS) or Spawning (S)", ylab = "Total Length (mm)")



BASS.AOV3=aov(Total.Length..mm.~Sex*S.NS)
anova(BASS.AOV3)
TukeyHSD(BASS.AOV3)
```

Cumulative Prey Curve

```
library(vegan)   # Load vegan package
CPC <- read.csv("C:/Users/Wendy/OneDrive/Documents/Thesis/Data/Stomach Dissections/Cumulative Prey Curve/CPC.csv", header = T) # Read data
head(CPC)
Samples <- CPC$Samples
Genus <- CPC$Genus
Samples <- sort(unique(Samples))
```

```
Genus <- sort(unique(Genus))
wBCI <- matrix(NA, nrow = length(Samples), ncol = length(Genus))
for(i in 1:length(Samples)){
  for(j in 1:length(Genus)){
#    wBCI[i, j] <- ifelse(any(Genus[j] == CPC$Genus[CPC$Samples == Samples[i]]), 1, 0)
    wBCI[i, j] <- ifelse(any(Genus[j] == CPC$Genus[CPC$Samples == Samples[i]]), sum(Genus[j] == CPC$Genus[CPC$Samples == Samples[i]]), 0)
  }
}
wBCI[1:10, 1:10]
colnames(wBCI) <- Genus
#data(wBCI)
sp1 <- specaccum(wBCI)
sp2 <- specaccum(wBCI, "random")
sp2
summary(sp2)


plot(sp1, ci.type="poly", col="black", lwd=2, ci.lty=0, ci.col="lightgray", xlab = "Randomly selected samples", ylab = "Number of Genera")
boxplot(sp2, col="gray", add=TRUE, pch="+")
boxplot(sp2, col = "gray", border = "black", lty=1, cex=0.3)

## Fit Lomolino model to the exact accumulation
mod1 <- fitspecaccum(sp1, "lomolino")
coef(mod1)
fitted(mod1)
plot(sp1, xlab = "Randomly selected samples", ylab = "Genus")


## Add Lomolino model using argument 'add'
```

```
plot(mod1, add = TRUE, col=2, lwd=2)
```

Schoener's Index

```
library(droglenc)
library(FSAmisc)
if (!require('devtools')) install.packages('devtools'); require('devtools')
devtools::install_github('droglenc/FSAmisc')
install.packages("remotes")
remotes::install_github("droglenc/FSAmisc")
SCH <- read.csv("C:/Users/Wendy/OneDrive - Thompson Rivers
University/Documents/Thesis/Data/Stomach Dissections/Schoeners
Index/Schoeners Index.csv", header = T)
names(SCH)
dietOverlap(Size.Class I, Size.Class II
=rownames(Size.Class),prey=names,type="Schoener")
```

Multiple Logistic Regression

```
MLR.Salmon <-
read.csv("C:/Users/Wendy/OneDrive/Documents/Thesis/Data/Stomach
Dissections/Multiple Logistic Regression/MLR.Salmon.csv", header = T)
names (MLR.Salmon)
attach(MLR.Salmon)
MLR.SMB=glm(Salmon.Present~Size.Class+Sex+Month.Caught+Location.Caug
ht..S.NS.,data=MLR.Salmon)
summary(MLR.SMB)
MLR.SMB.S=glm(Salmon.Present~Location.Caught..S.NS.)
summary(MLR.SMB.S)
```