Evaluating the Utility of a Point of Care Device to Assess Physiological Differences Among Wild Boreal Fishes

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Abstract

With the increasing risk of anthropogenic impacts in boreal ecosystems there is a need for tools to rapidly assess impacts on organisms and properly inform resource managers. Though well-researched, the i-STAT a portable Point-of-Care device has been minimally validated in species and environments, especially those that represent freshwater species in cold, boreal environments. To determine the accuracy of the i-STAT, blood from Lake Trout (*Salvelinus namaycush*) and White Sucker (*Catostomus commersoni)* was collected from the IISD Experimental Lakes Area (ELA) and analyses compared between traditional lab methods and results of the i-STAT POCD. The stability of i-STAT-based whole blood parameters was also evaluated over 3-4 hours for both ELA and captive Lake Trout. Finally, the i-STAT was tested in ambient conditions that are outside of its recommended usage (<16°C) to assess changes in physiological status in spawning Lake Trout across various capture and handling methods from five ELA lakes in October 2020. The time between capture method and blood sampling was also assessed for spawning Lake Trout.

The blood chemistry relationship between the i-STAT and traditional lab methods was significant for glucose, sodium, potassium (White Sucker only) and lactate (Lake Trout only). Lake Trout glucose and lactate maintained stability (e.g., non-significant change) in both wild-caught and lab-sourced blood samples over a 3-4-hour period, but haematocrit, sodium (lab-reared fish only), and calcium (wild-caught fish only) did not. The duration of time between capture and blood sampling Lake Trout had a significant on glucose (angling only), haematocrit (angling only), lactate and sodium a significant

increase in lactate and glucose, and a decrease in sodium was seen across almost all methods of capture.

The sex of the fish was found to be a significant additive factor, being significantly higher in males than in females for both glucose and haematocrit. Captive White Sucker showed an atypical change in physiological status indicating a lack of physiological stimulation. My thesis demonstrates that the i-STAT evaluated here has significant potential to be applied as an effective rapid assessment tool in Lake Trout outside of its intended application, but requires additional research for meaningful applications to White Sucker.

Lay Summary

The boreal forest is experiencing increasing development of mines, forestry, hydro dams, and oil pipelines that pose a risk to aquatic ecosystems. A tool is needed that can rapidly detect changes to fish health that can be used to assess the well-being of species when human industrial accidents or impacts occur. The purpose of my thesis was to determine if the i-STAT, a device that can rapidly tell you information about the blood chemistry of an organism using small amounts of whole blood, is accurate compared to traditional lab methods and if it can determine whether a fish is experiencing health issues in a cold boreal environment. Lake Trout and White Sucker were handled in both lake and lab environments, and small amounts of blood were taken from each. Lake Trout from these lakes and from a lab had blood samples analyzed by the i-STAT across three to four hours to determine how stable the blood values are. The i-STAT was found to be reliable when determining some of the blood values in Lake Trout and most blood values were determined to be stable for three to four hours when kept cold. Wild-caught White Sucker held in a lab did not show evidence of relevant physiological change when using the i-STAT, but Lake Trout captured and handled in their natural environment did. It was determined that the i-STAT can detect a physiological change in Lake Trout blood in a cold Boreal environment, it was not determined whether it was able to detect a relevant physiological change in White Sucker.

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Chapter 1 Background

Currently, fish community assessments use mark-recapture, bioindicator variables, and changes in growth rate to determine the status of an aquatic ecosystem and can give insight into how anthropogenic impacts influence fishes physiological status and in turn, overall health (Adams and Ryon, 1994; Attrill and Depledge, 1997; Huntingford *et al.*, 2006). However, these assessment methods often require destructive sampling, and/or require multiple sampling events and a great deal of time and effort (months to years) to quantify. If damage from resource development or other anthropogenic impacts has manifested in an ecosystem before the health assessment has concluded impacts, then the ability of regulators to respond appropriately to impacts is greatly reduced, and opportunities for immediate interventions that might help to avoid further damage are lost. Fisheries are under threat from several anthropogenic impacts, such as habitat loss through resource development (Baker et al., 2017), retention structure failure (Cooke et al., 2016; Sussbauer, 2017), transportation structure projects (TRB and NRC, 2005), and spills of environmentally dangerous materials (DFO, 2018).

Section 35 of the Canadian fisheries act clearly states that “No person shall carry on any work, undertaking or activity that results in serious harm to fish that are part of a commercial, recreational or Indigenous fishery, or to fish that support such a fishery.”, where “serious harm” is defined as “the death of fish or any permanent alteration to, or destruction of, fish habitat”. Ideally, fisheries monitoring methods should exist to detect physiological status that would allow for the alteration of activities such that “serious harm” as an endpoint can be minimized or avoided altogether. When damaging events occur in or near aquatic environments, they should be met with rapid assessments and

immediate intervention strategies to mitigate damage. As such, monitoring the health of fish communities in near-real-time is integral to responding appropriately to anthropogenic impacts on freshwater populations (Fausch, et al., 1990) and provides a means for rapid interventions not currently possible using existing monitoring methods (Dawkins, 2004**;** Morgan and Iwama, 1997; Huntingford *et al.*, 2006). While the need for non-destructive sampling and a rapid assessment in fisheries has been recognized, few technologies currently exist to support these activities.

Fish biomarkers via blood chemistry may represent a viable means of achieving rapid, non-destructive fish health assessments. Constituents commonly measured such as blood chemicals, enzymes, and haematological parameters can indicate a host of health complications (Table 1). For instance, cortisol is a commonly used indicator of stress in fish and can accurately reflect acute individual stress (Pottinger and Carrick, 1999; Porchas, 2009; Endo and Wu, 2019). Cortisol is an example of a biomarker that can be used to detect environmental contaminants or other anthropogenic impacts (Beliaeff and Burgeot, 2002). However, cortisol, by itself, is limited because it can also indicate activity, arousal, or poor welfare (Barton, 2002), and additional biomarkers are often required to differentiate among these responses (Adams and Ham, 2011). Additionally, changes in cortisol and other biomarkers (glucose, lactate, sodium etc.) are not synonymous with stress but indicators of a change in physiological status (Boonstra, 2012; MacDougall-Shackleton et al., 2019). Factors such as body size, spawning status, and how these factors influence physiological reactions in fishes are also important to consider when interpreting blood parameters (Pottinger and Carrick, 1999; Porchas, 2009; Endo and Wu, 2019). Blood parameters such as glucose (Abreu et al., 2009;

Harrenstien et al., 2005), haematological indices (haemoglobin and hematocrit; Li, 2003), plasma chloride, and plasma sodium (Carneiro and Urbinati, 2001) are all important physiological indicators and have been used in monitoring and research (Table 1). However, they currently all require different methods and assays/devices to produce accurate results (Borissov et al., 2019). Drawing blood from fish can be easily done in the field, and is only mildly invasive, however, the transportation of blood from the field to lab settings can present challenges concerning appropriate preservation methods, particularly in remote environments (Endo and Wu, 2019).

Recent point-of-care devices (POCD) that measure blood chemistry, though designed for humans and mammals (e.g., the i-STAT 1, hereafter referred to as the i-STAT or POCD) can provide rapid measurements of blood chemistry, enzyme, and haematological data within minutes in a range of organisms (Borissov et al., 2019; Stoot et al., 2014). Additionally, several of these devices are reasonably portable, permitting their use in field settings (Cooke et al., 2008). However, applications of these devices in non-target organisms require careful evaluation to facilitate meaningful interpretation of results. Many of the blood parameters that POCDs report can provide information on the health of fish, whether it be indicators of physical exertion, anaemia, presence of toxins or disease (Table 1; Roche and Boge, 1996; Baker et al., 2013; Barton, 2002). Assessments of the i-STAT tests on several teleosts, including *Albula vulpes, Sebastes melanops, Sebastes mystinus, Gadus morhua, and Oncorhynchus mykiss* do demonstrate high precision for several parameters, and significant correlations with lab-based estimates, but poor accuracy generally, with varying results among species (Harrenstien et al., 2005; Cooke et al., 2008; Harter et al., 2014; Borissov et al., 2019). For instance,

chloride, sodium, potassium ions, and haematocrit when measured with the i-STAT were found to be accurate with a slight deviation for Bonefish (*Albula vulpes*; Cooke et al., 2008) but not for Seminole Killfish (Fundulus seminolis; DiMaggio et al., 2010). Glucose and lactate measured with the i-STAT have shown reasonable accuracy when compared with standard laboratory practices, but only in marine fish, one of them being an elasmobranch (Harrenstien et al., 2005; Gallagher et al., 2010). The only study to evaluate the POCD for a freshwater fish to date has been Harter et al., (2014) using farm-raised Rainbow Trout, *Oncorhynchus mykiss* testing blood gas parameters, sodium, pH, and haematocrit, with only pH showing accuracy when compared to traditional assay methods. The i-STAT POCD has yet to be validated in cold or cool freshwater species collected from the wild, such as Lake Trout and White Sucker. If POCDs are to be applied meaningfully in natural settings, assessments of their performance on relevant species of wild-caught individuals are needed.

Changes to a physiological status where chemicals and hormones are raised or lowered to improve short-term survivability, as well as an extended version of this state without necessarily being a detriment to the overall health and longevity of fishes (Barcellos et al., 1999; Barton, 2002; Boonstra, 2013). During capture in the field (e.g., via angling, trap netting, gill netting, or other methods of capture), fish will undergo a physiological reaction during capture through handling, air exposure confinement and/or crowding during field capture. The longer capture or holding takes, the greater the potential physiological changes which will be reflected in blood parameters (e.g., glucose, haematocrit, lactate, sodium; Cooke et al., 2008; Roth and Rotabakk, 2010).

Lake Trout and White Sucker are widely distributed throughout Boreal regions and are both important species in Boreal aquatic ecosystems. Lake Trout are significant in First Nation subsistence fisheries as well as culturally, are ecologically important (as top predators), and are economically important as they are sought-after fish by anglers (Scott and Crossman, 1973). They are also important indicators of environmental changes, as they are sensitive to pH and oxythermal changes (Lange and Smith, 1995; Schindler et al., 1985; Plumb and Blanchfield, 2009). White Sucker are widely distributed in North America and are regarded as both an indicator species and an important aquatic and terrestrial food source for other organisms (Scott and Crossman, 1973; Inglis and Wilton, 1998; Munkittrick et al., 1991; Servos et al., 1992). The habitat of both species is being impacted currently by mining, forestry, oil extraction, hydroelectric development and transportation sectors, which are increasing in both scope and frequency, and often have long-term effects on both terrestrial and aquatic ecosystems alike (Wells et al., 2020; Willow, 2016). Understanding how these and other species are being impacted by these sectors is important to minimize the damage that may occur; large-scale impacts can be minimized or eliminated if physiological changes can be rapidly detected at the individual level, informing potential corrective measures to take place that might help to mitigate or altogether avoid impacts at the population level (Palace et al., 2009; Kidd et al., 2007; Kilgour et al., 2007; Kidd et al., 2014).

Although blood contains many parameters that can be valuable to understanding fish health, these values may change or drift up or down from the point of collection to the point of analysis if mishandled or stored incorrectly (Fazio et al., 2017), leading to potential misinterpretation of results. For instance, during field sampling, and in the

absence of a portable POCD, blood must be transported back to the laboratory for analysis, which can lead to stability complications (Agina, et al., 2020). Refrigeration at 4°C greatly helps to stabilize whole blood, reducing enzymatic reactions such as glycolysis (Fazio et al., 2017; Oddoze et al., 2012). However, long-term storage, even in cold conditions can still lead to altered fish blood chemistry values due to erythrocyte degradation (Fazio et al., 2017). If blood cannot be centrifuged and the plasma fraction separated and stored separately for later analysis, it is recommended that blood be analyzed within 6 hours to avoid the drifting of blood values (Faggio et al., 2013; Fazio et al., 2017). In remote locations, often days away from analytical laboratories, this can be difficult if not impossible to achieve. Thus, if assessed as providing reasonably accurate and interpretable results, portable POCDs like the i-STAT could facilitate rapid assessments of fish health in remote locations not easily serviced with regular and rapid courier or postal services.

Objectives

The overarching goal of this research was to (a) evaluate the i-STAT POCD as a tool for rapid fish health assessment when applied outside of its intended scope of use, and (b) to evaluate the accuracy of the POCD in detecting gradients of physiological change in both Lake Trout and White Sucker.

My specific objectives were:

1) To determine the accuracy of the i-STAT POCD when comparing its blood values in Lake Trout and White Sucker to traditional lab methods of

spectrophotometric biochemical assays and inductively coupled plasma (ICP) mass spectrometer results;

2) To determine the stability of Lake Trout whole blood parameters over time in a field and laboratory setting while being kept cold (4°C);
3) To determine the efficacy of the POCD on Lake Trout blood to detect differences across an imposed gradient of physiological challenges using methods of capture in a natural ecosystem when applied in environmental conditions outside of the POCD's intended scope of use;
4) To determine which blood parameters change in Lake Trout and White Sucker across imposed gradients of physiological change in Lake Trout and White Sucker; and
5) To evaluate what other factors may be influencing blood parameters (e.g. sex, different populations, handling time).

Chapter 2 Evaluation of a point-of-care device applied to boreal fishes

Abstract

With expanding development infrastructure and research occurring in more remote areas, the need grows for rapid assessment tools to assess the well-being of potentially impacted aquatic organisms that can be deployed in a broad range of field conditions. While some consideration has been given to point-of-care devices (POCD) to fill this need, there remains a need for their evaluation when applied to remote boreal species and environments, regions often impacted by mining, oil extraction and transportation. Here, the i-STAT POCD was used to analyse White Sucker and Lake Trout blood in both lab and field conditions to evaluate POCD results against traditional lab analyses. The stability of Lake Trout blood parameters for up to four hours after the collection was also assessed. Blood sodium and glucose results from the POCD showed significant relationships with standard measurement procedures in both White Sucker and Lake Trout. Potassium in White Sucker blood showed significant relationships with standard procedures, but not lactate. Conversely, in Lake Trout, lactate results from the POCD showed significant relationships with standard measurement procedures, but not potassium. Calcium levels reported from the POCD were not related to standard procedures in either species. Blood glucose, lactate, and sodium from wild-caught Lake Trout analyzed on the POCD appeared to be reasonably stable for at least four hours once drawn from live fish. In contrast, POCD-based haematocrit increased significantly with storage time although I was not able to compare results to traditional methods. While POCD-based calcium was stable in blood drawn from lab-held Lake Trout, these results are unreliable when using the i-STAT. Whereas sodium was stable in blood drawn from

lake-sourced Lake Trout, it declined in lab-held samples to a biologically meaningful degree. My results suggest that the i-STAT POCD demonstrates the potential for applications in accurately assessing certain Lake Trout and White Sucker blood parameters and that up to four hours of storage of whole blood on ice still provides reasonably stable results for several parameters, allowing for rapid, field-based assessments of these species.

Introduction

Analyzing blood samples is commonplace in veterinary and human medical practices as a means of assessing an individual's level of well-being, often interpreted as assessments of physiological change. Physiological change can be initiated through various means, such as toxic exposure, disease, and infections. In remote areas or away from a reasonably-equipped laboratory, transporting blood samples from where organisms are sampled for analysis is often impractical, as several blood parameters are known to be unstable even when kept cold (Korcock et al., 1988; Tavares-Dias and Sandrim, 1998; Oddoze et al., 2012; Faggio et al., 2013; Collicut, 2014; Fazio et al., 2017).

A variety of point-of-care devices (POCDs) have been tested to allow for the sampling of blood in a field setting (Stoot et al., 2014), as the ability to analyze blood at the point of sampling avoids issues related to sample stability. The i-STAT 1 (hereafter referred to as the i-STAT or POCD) is one of the more widely studied POCDs, particularly in fisheries applications (Stoot et al., 2014), likely due to the wide variety of

blood parameters that it can cover, and successful previous applications in hospitals and veterinary clinics for both humans and other mammals. Despite the potential for the application of POCDs such as the i-STAT in a broader range of organisms, few have evaluated their application in fish; indeed, of seven previous studies examining the application of the i-STAT in fishes (Table 1), varying levels of success have been reported. No study to date has evaluated lactate in ray-finned fishes, though it has been assessed in some elasmobranch species (Gallagher et al., 2014). Previous studies have also indicated that the analysis of blood gas parameters in fish using POCDs is generally impractical, given difficulties in preventing air contamination and certainty in the collection of exclusively arterial or veinous blood given typical sampling techniques (Borissovet al., 2019; Harter et al., 2015).

The stability of blood parameters once drawn from an organism when in a field or lab setting has also been tested in a variety of species by fish biologists (Joen et al., 1995; Brown et al. 2008; Collicut, 2014; Agina et al., 2020), as changes in these values from the point of sampling to the time of analysis can lead to false or spurious conclusions. Changes in blood chemistry after being drawn from organisms can result from a variety of processes, including hemolysis, glycolysis, swelling of red blood cells, mishandling by researchers (e.g. drawing blood with too much pressure, samples not kept sufficiently cold, samples shaken), and evaporation if improperly stored and transported (Mudge et al., 2004; Brill et al., 2008; Rummer et al., 2010; Oddoze et al., 2012; Fazio et al., 2017). Proper storage of blood parameters (4°C with an added anticoagulant) can help prevent drift in parameter values, but significant changes can occur over longer storage times at these temperatures (e.g., 8 hours; Korcock et al., 1988; Tavares-Dias and Sandrim, 1998;

Oddoze et al., 2012; Faggio et al., 2013; Collicut, 2014; Fazio et al., 2017). If blood values cannot rapidly be analyzed or centrifuged to attain and freeze plasma (which is more stable once separated from cellular matrices; Cohn et al., 2020), good practice dictates that stability should be tested under relevant study conditions by immediate and subsequent sampling (Ziobrowska-Bech et al., 2022). If this is not possible, researchers must turn to robust literature on a species' blood stability, which does not currently exist for many species, particularly those in the boreal region of North America.

Boreal forests make up ~30% of the global forests worldwide with more accessible freshwater than any other biome (Brandt et al., 2013; Gauthier et al., 2015; Mery et al., 2010). Mining, forestry, hydroelectric development, oil extraction and transportation are rapidly increasing industrial sectors in this region of the world and can have major and often long-term effects on surrounding terrestrial and aquatic ecosystems (Wells et al., 2020; Willow, 2016). The boreal aquatic ecosystem is also a major source of domestic and international tourism and serves important cultural and subsistence purposes for both indigenous and non-indigenous peoples in Canada, Alaska, Scandinavia, and Russia.

Several fish species in the boreal region are important and widespread; Lake Trout are a desirable sport fish, also used for subsistence fisheries by First Nations communities, and are ecologically important as top predators in many freshwater ecosystems across the boreal region, including the Great Lakes which roughly marks their southern native geographical extent (Scott and Crossman, 1973). They are also important indicator species due to their sensitivity to oxythermal changes and acid (Lange and Smith, 1995; Schindler et al., 1985; Plumb and Blanchfield, 2009). White Sucker are

an important sentinel species for environmental impacts as they are widely distributed throughout North America and an important food source for both terrestrial and aquatic species (Scott and Crossman, 1973; Inglis and Wilton, 1998; Munkittrick et al., 1991; Servos et al., 1992). Understanding the effects of anthropogenic disturbances on fish physiological status and well-being is important for maintaining healthy fish habitats and waterways. Detecting physiological changes at the individual level can often serve as an early warning system for anticipating larger population-level responses (Palace et al., 2009; Kidd et al., 2007; Kilgour et al., 2007; Kidd et al., 2014), and potentially permit more rapid corrective measures before large-scale impacts are realized.

Blood parameters that can be considered when using POCD technology depend on their relevance to fish health and their accuracy when compared to traditional assays. In particular, the blood parameters glucose, lactate, haematocrit, and sodium are all evaluated by the CHEM8+, CG4+, and CG8+ cartridges for the portable i-STAT POCD and are well known in the literature as indicators of change in physiological status in fish (Baker et al., 2013; Seibelet al., 2021). Limited evaluations of blood parameters and cartridges have previously been evaluated in fishes using this POCD, and agreement with traditional analytical methods in these assessments vary among species (Harrenstein et al., 2005; Cooke et al., 2008; DiMaggio et al., 2010; Harter et al., 2014; Borissov et al., 2019). As such, species-specific evaluations are required for a given species of interest when the use of POCDs is desired. Portable POCD devices such as the i-STAT have yet to be evaluated in either White Sucker or Lake Trout.

My goals in this chapter were to 1) evaluate the i-STAT POCD as an accurate method of evaluating blood parameters in both Lake Trout and White Sucker, with

particular emphasis on the biological significance of the parameters under investigation; and 2) assess the stability of chilled whole blood samples over time in both field and laboratory settings. To evaluate the accuracy of the blood parameters, White Sucker and Lake Trout POCD results were compared to results from standard laboratory analyses (assays for metabolites, and inductively coupled plasma mass spectrometry [ICP-MS] for anions). To evaluate the stability of my chosen suite of parameters, blood was sourced from both lab-held and wild-caught Lake Trout populations. The i-STAT was determined suitable for use in both species for assessing blood glucose and sodium. The i-STAT was also determined suitable for assessing blood lactate levels in Lake Trout and blood potassium levels in White Sucker. Blood parameters were determined to be stable over time for all parameters excluding haematocrit.

Methods

Fish Handling

White Sucker were originally taken from Lake 375 at the IISD Experimental Lakes Area (ELA) and brought to the Biology Aquatic Facility (BAF) at Lakehead University for a separate study. At the completion of this study, the destruction of held fish was required, providing an opportunity for blood collection just prior to euthanasia. Fish were held in three separate 132-gallon circular tanks in the Lakehead BAF. Fish were removed one at a time from tanks using a dip net and placed into a tricaine methanesulfonate (TMS) bath. Once anaesthetized, fish (n=21) were weighed (194-816g), measured (182-409mm), and blood was taken via the caudal vein. Fish were then

returned to the TMS bath for euthanasia. Whole blood from each fish was run on the i-STAT POCD using both CHEM8+ and/or the CG4+ cartridges. A subsample of whole blood from each fish was also centrifuged at 4000 rpm in an Eppendorf Minispin Plus for six minutes. Following centrifugation, the plasma fraction from the blood was extracted by pipette and frozen for later use in standard assays (for comparison to POCD results).

Lake Trout were captured by trap netting, gillnetting, or angling in lakes 223, 224, 260, 373, or 626 at the ELA. All fish were brought to shore by boat and stored in tubs of lake water that were continually refreshed. During sampling, air temperatures ranged from 0.6-11.2°C while water temperature was between 10-12.5°C. Gillnetted fish were kept in underwater pens overnight after capture and were sampled the next day. They were moved from the pen to bins to await anaesthesia. Fish were removed from trap nets or holding pens and stored temporarily in bins on the shore where they were quickly moved by a net. Angled fish were immediately placed in bins on the boat, transported back to shore and placed in bins awaiting anaesthesia Anaesthetized with TMS and taken out of temporary holding bins one by one to be weighed, measured, and have blood drawn. Fish were held in a wet cradle while blood was taken (≤0.1% of body weight, by CCAC guidelines; www.ccac.ca) from the caudal vein by a puncture to the ventral area behind the caudal peduncle which was cleaned with a Kimwipe™ and povidone-iodine. Sampled Lake Trout (n=107) ranged in weight from 508-1375g. Blood was then analyzed on the i-STAT POCD, which was kept warm by an incubator (hot water bottles in a cooler with a towel), using the CG4+, CHEM8+ and/or CG8+ cartridges. Once samples were run on the POCD, they were centrifuged at 4000 rpm by a Minispin Plus by Eppendorf for six minutes, after which plasma was separated by a pipette and stored on

ice. Plasma was then frozen at -20°C for later use in assays. All animals were handled under the approval of the Lakehead Animal Care Committee (AUP # 1466997).

To evaluate stability, blood was taken from four wild-caught fish (which were included in my Lake Trout sampled for physiological status) after anaesthesia. Blood samples were stored on ice and removed briefly when subsamples were extracted to run through the POCD. Blood samples were run between zero and four hours for wild-caught fish. A second lab-reared population of Lake Trout was also evaluated for use in blood stability analyses. Lab-held Lake Trout were reared with gametes sourced from Clearwater Lake, MB, Canada and reared at the University of Manitoba (UM project # 49588 and AUP #F19-022). A total of four lab-held Lake Trout were sampled for blood stability. These 4 Lake Trout were larger than ELA Lake Trout (1900 - 3300 g) and underwent sampling without the imposed physiological gradients of an extended capture event (angling or gill netting), experiencing only the physiological of netting from a tank, transfer to an anaesthetic tub and handling during anaesthesia. Lab fish were sampled similarly to ELA fish, where blood samples were taken with lithium heparin rinsed syringes (≤0.1% of body weight) after anesthetization in TMS water. Once taken, blood was immediately placed into the CG4+ and CHEM8+ cartridges and run on the POCD. The blood samples were placed on ice and run every hour for up to three hours.

Lab Analysis

For lactate assays, the L-lactate assay kit (MAK329, Sigma-Aldrich, MO, USA) was used in accordance with the manufacturer's instructions (Sigma-Aldrich; Christine and Giesy, 2021) and samples were measured at 565nm on a spectrophotometric multi-well plate reader (BioTek Epoch). Plasma samples were diluted 1:7 with milli-Q water to

dilute blood concentrations within the range of the standard curve. Each sample had a replicate with no active assay reagent which was subtracted from the final result to eliminate the influence of any plasma discolouration on spectrophotometric measurements. All but two samples (due to limited sample quantity) were run in duplicate or triplicate, and the mean value of replicate analyses was reported. The assay was validated by confirming that an increased sample quantity (2.5, 5, and 10µL) across three different plasma samples for each Lake Trout, and produced a corresponding proportional increase in measured lactate concentrations. For this validation procedure, each sample quantity was run in triplicate on three fish samples (9 samples per fish; n=27 total samples run). This confirmed that the measured value in the sample was proportional to the amount of sample added. The assay was also validated by testing for appropriate recovery of sample material. Using three random samples of defrosted frozen plasma from Lake Trout, a mix of 50% plasma (5µL) and 50% lactate standard (5µL, P+S) were run via the lactate assay in duplicate. The same three samples were also run with a mix of 50% of water (5µL) and 50% standard (5µL, W+S). The comparison of the three PS to replicates was within 10% of each other (with the exception of one sample being within 13%) when P+S results were subtracted by W+S results (Tables A2.1 and A2.2).

The glucose (HK) colourimetric assay kit (GAHK20) was used, but procedures were modified from the manufacturer's recommendation and the kit was instead run as an endpoint assay using the provided standard solution (1.0 unit/mL of glucose6-phosphate dehydrogenase) to establish a standard curve. This was done to expand the number of samples that could be run in a kit (move from standard to a multi-well

spectrophotometer) and to increase the accuracy of the reliability of the kit by removing variability from the time it takes to prep wells. The standard curve was then used to convert spectrophotometer absorbance values to glucose concentrations. Samples were run on a 96–well standard plastic flat bottom plate at 340 nm. Each plate (five plates total) had a standard curve with standards run in duplicate that used concentrations ranging from 0.0099-0.0566 mg/mL or 1-6 uL of glucose standard in 100 uL milli-Q water. Plasma samples were run in sextuplicate with 3 µL of plasma added to 100 µL of an assay reagent. Samples that were greater than 20% different between replicate means were discarded. The lowest r^2 value measured on any standard curve using this method was 0.97.

Anions (sodium, calcium, and potassium) were run using the Varian Vista Pro ICP-MS at the Lakehead University LUCAS lab. Samples were digested prior to analysis with 10% nitric acid using milli-Q water. Prepared samples were diluted to within the detection range of the instrument with a ratio of 20 µL of plasma per 5 mL of 10% nitric acid solution with a maximum of 60 µL of plasma per 15 mL of solution. The micro-haematocrit centrifuge intended for an independent measure of this variable did not arrive in time to analyze samples due to COVID-19 supply-chain delays. Therefore, haematocrit could not be evaluated for accuracy in this study.

Statistical analysis

All analyses were carried out in R, v. 4.0.5 (R Core Team 2022). To address objective 1 (evaluating POCD accuracy), Lake Trout and White Sucker blood parameters from both the POCD and lab analyses were compared using linear regression. Ionized calcium and potassium both showed outliers identified by Cook's distance; removal of

these outliers from the regression did not alter the significance of reported relationships (Figure 1c,d). Relationships between lab and POCD results were also compared for differences from the 1:1 line using Wald-test. Data transformations were made when necessary to meet assumptions of normality and homogeneity of variance.

To address objective 2 (stability of whole blood), the relationship between the stability of Lake Trout blood parameter values from whole blood samples with time, as well as the influence of the source of fish were evaluated using a linear mixed-effects model using the nlme package (Bates et al., 2012). Specifically, to evaluate the stability of blood parameters of interest over increasing time of storage, I modelled time (in minutes since blood was drawn from the fish originally) and source (lab or field-collected) as fixed effects, and blood from an individual fish as a random effect. The full model with an interaction term was first assessed:

$$\text{Response} \sim \text{time}*\text{source} + \text{time} + \text{source} + (1|\text{fish}) \qquad \text{(eq. 2.1)}$$

The significance of individual terms in the models was determined from likelihood ratio tests, comparing the full model against the model without the main effect of concern (Fincham et al., 2019). Model comparisons evaluating the significance of fixed effects were conducted on model fit using maximum likelihood estimators. Where the interaction between time and source was not significant (determined via comparison to the additive model, eq. 2);

$$\text{Response} \sim \text{time} + \text{source} + (1|\text{fish}) \qquad \text{(eq. 2.2)}$$

The significance of time was evaluated by comparing the additive model against a model with a source and a random fish effect only (eq. 3);

Response ~ source + (1|fish) (eq. 2.3)

Visual inspection of residual plots from final models did not reveal any obvious deviations from homoscedasticity or normality. The rate of change in blood parameters per hour was calculated by Slope * 60 minutes. Percent change was calculated by subtracting final (180 or 240 min) means by initial means (0 min).

Results

In White Sucker, traditional methods for evaluating sodium ($F_{1,15}$=9.15, p=0.004), glucose ($F_{1,16}$=81.47, p=0.001) and potassium ($F_{1,14}$=7.38, p=0.008) were all significantly related to measures reported from the POCD (Figure 2.1). Diagnostic plots indicated that residuals were normally distributed, and that variance of error terms was homogeneous for all response variables but potassium; heterogeneity of variance was observed in potassium results due to smaller values measured on the POCD being more variable when analyzed using ICP-MS. Comparisons between POCD and lab results for both ionized calcium ($F_{1,15}$=0.09, p=0.38) and lactate ($F_{1,6}$=0.15, p=0.34) were non-significant, though ICP-MS calcium results were consistently greater than those measured by the POCD, even when an outlier was excluded (Figure 2.1d). In addition, the POCD showed a more narrow range (0.6-1.4 mM) for ionized calcium compared to ICP-MS samples (1.8-3.8 mM). Over the range of values tested, slopes of calcium (t = -2.38, df = 15; p= 0.03) showed a significant difference to a slope of 1 when evaluated with a t-test (Figure 2.1). Sodium (t = 3.03, df = 15, p= 0.89), glucose (t = -2.6 , df = 16; p= 0.66), lactate (t =

-0.16, df = 6; p= 0.88), and potassium (t = -0.63, df = 13; p= 0.5) showed no significant difference to a slope of 1 (Figure 2.1).

As with White Sucker, traditional methods for evaluating Lake Trout sodium ($F_{1,42}$=12.4, p=0.001), lactate ($F_{1,20}$=4.44, p=0.048), and glucose ($F_{1,131}$=50.4, p<0.001) were significantly related to POCD measures (Figure 2.4.). Potassium and ionized calcium did not show a significant relationship between POCD and ICP-MS results (p>0.05). Over the range of values tested, all slopes tested for glucose (t = 1.51, df = 31; p= 0.55), lactate (t = -0.33, df = 20; p= 0.8), sodium (t = 0.43, df = 42; p= 0.7), and potassium (t = -2.01, df = 13; p= 0.07) were not significantly different from a slope of 1. Calcium (t = -3.28, df = 41; p<0.001) was different from a slope of 1. Generally, lab-based assays were similar between methods at lower values of glucose, but lab values were consistently higher than the POCD for glucose at higher concentrations. Sodium values for the POCD appeared greater than lab ICP-MS values below 150mM but less than lab values above 150mM, while lactate was closer to the 1:1 line overall (Figure 2). Even more so than with White Sucker, the POCD showed a narrow range (0.9-1.6 mM) for ionized calcium compared to ICP-MS results (2.9-8 mM). Diagnostic plots indicated that residuals were normally distributed, and that variance of error terms was homogeneous for all significant results.

A linear mixed-effects analysis to evaluate the stability of blood over time across Lake Trout blood parameters revealed a significant interaction between source and time for sodium (*LLR*=3.18, df=5,6 p=0.04) and calcium *(LLR*=3.84, df=5,6 p=0.03). Interactions showed a slight trend of decreasing blood sodium across time for lab-reared Lake Trout and almost no change was seen in wild-caught Lake Trout blood sodium

(Figure 2.3). Wild-caught Lake Trout blood calcium appeared to decrease with time, while lab-reared Lake Trout appear to increase (Figure 2.3). The interaction between location and time was not significant for lactate (*LLR*=1.37, df=5,6, *p*= 0.1) or glucose (*LLR*=0.02, df=5,6, *p*= 0.5). Time in the model explained a significant additional component for several blood parameters (Table 2.3 Figure 2.3), including lactate (*LLR*=4.16, df=3,4, *p*=0.02), haematocrit (*LLR*=5.17, df=3,4, *p*< 0.001), and glucose (*LLR*=1.37, df=4,5, *p*= 0.03) but not for sodium or calcium (*p*> 0.05). The results of time were reported even though an interaction was present because the location is a random effect while time is the fixed effect that I care about. Even though glucose and sodium showed high variability in wild-caught Lake Trout blood samples, the overall trends showed no significant shifts over time. Lactate showed little variability and relatively small increasing or decreasing trends over time. Haematocrit increased over time with blood from both lab-reared and wild-caught fish. Source of fish in the model explained a significant additional component for sodium (*LLR*=11.39, df=4,5, *p*< 0.001), lactate (*LLR*=32.20, df=4,5, *p*< 0.001), and glucose (*LLR*=4.89, df=4,5, *p*= 0.01); lab-reared fish values for sodium were higher than for wild-caught fish across time (Figure 2.3). This was the opposite for lactate and glucose, which had higher values in wild-caught fish compared to lab-reared fish over the entire evaluation period (Figure 2.3). Haematocrit and calcium levels were not different between the source of Lake Trout (lab-reared vs. wild-caught; *p*>0.05). The inclusion of fish as a random intercept explained significantly more variance in the full model for sodium (*LLR*=5.17, df=3,4, *p*= 0.2), lactate (*LLR*=29.74, df=6,5 *p*< 0.001), and glucose (*LLR*=1.37, df=5,6, *p*=<0.001) but not for haematocrit or calcium (*p*> 0.005).

Discussion

Based on my results, the POCD evaluated here appears to provide reasonably accurate descriptions of blood glucose and sodium in both Lake Trout and White Sucker, with significant relationships between POCD results and standard analytical methods for each of these blood analytes. The POCD also showed significant relationships in blood potassium with traditional methods in White Sucker, though not in Lake Trout; similarly, the POCD showed significant relationships for lactate in Lake Trout when compared to laboratory assays, but not in White Sucker. The POCD was not accurate for either species when evaluating calcium. This is the first time glucose, sodium, lactate (Lake Trout only) and potassium (White Sucker only) have been validated for the POCD in either a salmonid or catostomid. Now that a suite of parameters has been validated for the POCD in White Sucker and Lake Trout, the values produced for these variables within the range reported here can be used to estimate values using traditional methods for investigations involving these species when observing physiological change.

While this is the first time sodium has been validated as accurate in Lake Trout and White Sucker (Chapter 2) while using the i-STAT POCD, it has been evaluated in a variety of other fishes. Four other POCD studies evaluated sodium in marine teleosts (*Albula vulpes*; Cooke et al., 2008; *Gadus morhua*; Borissov et al., 2019; *Sebastes melanops and Sebastes mystinus*; Harrenstein et al., 2005) and freshwater teleost (*Fundulus seminolis*; DiMaggio et al., 2010; *Oncorhynchus mykiss*; Harter et al., 2014). The current study is the second to find sodium in the POCD to be a reliable blood parameter (Cooke et al., 2008), as DiMaggio et al. (2010) and Harrenstein et al. (2005) both found sodium to be significantly lower compared to traditional analyses. By

contrast, Borissov et al. (2019) and Cooke et al. (2008) found the opposite, with sodium measured by the POCD being higher than laboratory results (significantly higher in Borissov et al., 2019). All evaluation studies of the i-STAT used different traditional methods for determining accuracy, including this one. Interestingly, sodium has previously been shown to be less accurate at lower temperatures (Borissov et al., 2019) and all but lab-reared Lake Trout in my study had blood taken at low temperatures (0.6-11.2°C).

Although glucose has been evaluated using this POCD previously (Harrenstien et al., 2005), this is also the first time glucose has been validated in these taxonomic orders using this POCD (Salmoniformes and Cypriniformes; Hughes et al., 2018). Glucose has previously been validated for use in the marine species black rockfish (*Sebastes melanops*) and blue rockfish (*Sebastes mystinus*) using a POCD (Harrenstien et al., 2005). Similar to my study, the range of glucose values was large and there was a strong relationship between the POCD and lab results. In all cases, lab glucose assays were run on plasma samples while field glucose values (POCD) were based on whole blood, which could explain why assay-derived glucose samples tend to be consistently higher than POCD-derived samples (Cooke et al., 2007).

This is also the first time lactate has been evaluated and validated for the POCD in any ray-finned fish species (specifically in Lake Trout). Lactate has also been validated against the YSI StatPlus POCD in elasmobranchs (specifically sharks) in the field. Similar to Lake Trout in my study, POCD and laboratory analysis in elasmobranchs in Gallagher et al. (2010) showed no difference for lactate and found the POCD to be accurate in blood lactate determination for *Mustelus antarcticus, Carcharhinus plumbeus,*

and *Mustelus canis* (Gallagher et al., 2010). Lactate is a well-known indicator of exercise in the blood (Wood et al., 1983; Schindler et al., 2012) and may therefore be valuable in further Lake Trout research and monitoring, allowing for the use of this POCD for rapid assessments examining movement-related physiological changes.

Potassium has previously been validated as accurate in the POCD (Harrenstien et al., 2005; Cooke et al., 2008) as in this study for White Sucker, but it has been shown to underestimate this parameter in other studies (DiMaggio et al., 2010; Borissov et al., 2019). However, in Cooke et al., (2008) potassium was consistently higher in the POCD compared to lab analysis. This discrepancy is not likely due to a difference between methods of analysis, as both Harrenstien et al., (2005) and Cooke et al. (2008) used methods of ion-selective potentiometry. The ICP-MS (used in this study) ionizes the sample and detects atomic and polyatomic ions whereas ion-selective potentiometry measures the potential generation from an ion crossing a membrane barrier. Results from Harrenstien et al., (2005) and Cooke et al. (2008) are difficult to compare to this study as their sodium values were higher than mine (>165mmol/L). Potassium values in my study were generally overestimated in the POCD, which is the opposite of what has been seen in Harrenstien et al. (2005), DiMaggio et al. (2010), and Borissov et al. (2019).

While my study demonstrated significant relationships between POCD results and traditional analyses in the analytes/species described above, they were not related to others. Potassium was shown to be inaccurate in Lake Trout using the POCD compared to ICP-MS. This is in agreement with the majority of studies (DiMaggio et al., 2010; Borissov et al., 2019) evaluating the accuracy of potassium which all show a reduced value of potassium in the POCD relative to traditional analyses. Similarly, the i-STAT

POCD cannot be recommended for lactate results in White Sucker as assay results in this species showed no relationship with POCD results. White Sucker blood had low statistical power (n=7) which may explain why I failed to detect a relationship; however, most values are generally distributed around the 1:1 line which suggests that with a greater sample size, a relationship between the POCD and the ICP-MS results may yet be found. Ionized calcium showed much lower values in the POCD compared to laboratory analysis which was also seen in Borissov et al. (2019). This may be due to the difference in sample type (plasma and whole blood), pH, and temperature (Borissov et al. 2019). The lack of relationships between POCD and traditional analyses for lactate in White Sucker, potassium in Lake Trout, and calcium for both species suggests that these parameters measured from a POCD should be interpreted with caution in these contexts without additional investigation. Hematocrit is known to be inaccurate in this POCD and consistently underestimated due to fish cells being larger than mammalian cells (DiMaggio et al., 2010; Harter et al., 2014). Much of the deviation in lactate, potassium, and calcium can likely be attributed to the POCD not being intended for ectothermic species but endothermic mammals.

It was found that all blood parameters except haematocrit, sodium (lab-reared) and calcium (wild-caught Lake Trout) showed no biologically significant changes over four hours across both lake and lab-reared Lake Trout; even though there were some statistically significant shifts observed in glucose, sodium (wild-caught), and calcium (lab-reared), they were small. Here, biological significance was defined as a shift in blood parameter values that would imply a change in physiological status (Chapter 3, Table 3.1). For Lake Trout in laboratory settings, lactate, glucose, and calcium in whole blood

should produce consistent values for up to four hours as long as samples are actively cooled and insulated. The same is true for sodium, lactate, and glucose in field settings. However, some minor variability should be expected in sodium, glucose and calcium (lab-reared). A biologically significant decrease of Calcium (-0.06 mmol/L or -20% after four hours) was observed in wild-caught Lake Trout, but given that calcium was found to be inaccurate when evaluating with this POCD, the meaning of such shifts is difficult to interpret. A biologically significant decrease of sodium (-4.41 mmol/L or -3% after three hours) was observed in lab-reared Lake Trout; similar changes have previously been observed in another salmonid (Rainbow Trout) from chilled whole blood samples after 3 hours (-5 mmol/L, Korcock et al., 1988). The observed increase of haematocrit in Lake Trout over time observed here is a known phenomenon that has been observed in other species sampled with similar methods (Korcock et al., 1988; Tavares-Dias and Sandrim 1998; Fazio et al., 2017). Stability has been observed for haematocrit in some elasmobranch species (*Carcharhinus melanopterus* and *Negaprion acutidens*) for over 180 minutes (Schwieterman et al., 2019). However, the comparison between elasmobranch and teleost fish species haematocrit is unclear as elasmobranch red blood cells do not swell after taking blood as they do in teleosts (Brill et al., 2008; Rummer et al., 2010). Additionally, elasmobranchs typically have much lower blood haematocrit compared to those of teleost's (10-18% Packed Cell Volume [PCV], Baldwin and Wells, 1990) and therefore likely also have a reduced capacity for change in blood values. Based on my results, haematocrit should be expected to increase in value over time in Lake Trout at a relatively consistent rate.

In the current study, glucose, lactate, sodium (wild caught), and calcium in whole blood all showed biological stability when kept cold (4°C or on ice) which is supported by other studies, most with longer storage periods (Joen et al., 1995; Brown et al., 2008; Agina et al., 2020). Glucose and sodium in whole blood were stable when kept at 4°C in Rockfish (*Sebastes schlegeli*), Israeli Carp (*Cyprinus capio*), and Rainbow Trout (*Oncorhynchus mykiss*) for up to 30 days (Joen et al., 1995). Whole blood samples from African Catfish (*Clarias gariepinus*) have also been shown to be stable with regard to glucose for up to 72 days (Agina et al., 2020). Lactate values in whole blood have shown stability in Atlantic Cod (*Gadus morhua*; Brown et al., 2008) and Cownose Ray (*Rhinoptera bonasus*) for up to eight hours and 90 minutes respectively (Innis et al., 2020). Although a study of the stability of calcium in fish blood could not be found, a study in human blood found that at 4°C calcium significantly increased after four hours (van Balveren et al., 2017).

Biochemical and metabolic parameters in blood samples can change in value given enough time (eight hours or greater; Korcock et al., 1988; Tavares-Dias and Sandrim 1998; Collicut, 2014; Fazio et al., 2017) or when improperly stored (above 4°C) as red blood cells may leak intracellular constituents, glycolysis or hemolysis may occur, and water from plasma may evaporate, all of which may have influenced my haematocrit results (Oddoze et al., 2012; Ghirmai et al., 2020). Glycolysis, or the consumption of glucose which forms lactate, can be slowed by reduced temperatures (4°C) or it may be inconsequential if glucose values are obtained in less than one hour from blood sample acquisition (Chan et al., 1989; Oddoze et al., 2012; Agina et al., 2020). Time did influence the values of Lake Trout blood lactate, haematocrit, glucose, sodium, and

calcium after 240 minutes of chilled, even though changes were generally minor. For hematocrit, a significant increase of 1.83% PCV/hour (5.6% of change/hour) was observed in fish which can be considered a rapid change (-1-5%PCV/hour, Korcock et al., 1988; no significant change, Tavares-Dias and Sandrim, 1998; 0.015%PCV/hour, Fazio et al., 2017). This change in haematocrit would be enough to alter conclusions regarding physiological impacts after 2-3 hours of storage in males (physiological change of 4.2%, Ch 3, Table 1). Sodium increased by 0.1mmol/L (0.03%/hour) in wild-caught fish and decreased by 1.47mmol/L (1%/hour) hourly in lab-reared fish. This amount of change in lab-reared fish would cause enough drift to influence conclusions around fish physiological status at 2-3 hours (mean changes of -4.2mmol/L, Ch 3, Table 1). Lactate showed a small shift in the hourly value of only 0.06mmol/L (0.6%/hour) in wild-caught fish with even less of a shift in laboratory samples. This indicates that samples stored for four hours should not influence conclusions regarding the physiological change (mean changes from physiological of 1.6mmol/L, Ch 3, Table 1). Glucose increased hourly by 2.2mg/dL (2.2%/hour) in wild-caught fish and by 2 mg/dL (2.9%) in lab-reared. This indicates that, like lactate, four hours of cold storage should not influence conclusions regarding physiological changes for glucose (mean changes of 16.4mmol/L), but over eight hours following this trend, significant changes may occur (Chapter 3, Table 3.1). Ionized calcium showed the opposite drifts in the lake and lab samples with lab-reared samples increasing by 0.03mmol/L (2.3%) hourly and wild-caught samples decreasing by a steeper 0.06mmol/L (4.4%/hour) which could lead to significant shifts. Across all parameters, it was unclear whether these observed trends would have continued past the period examined here, but previous research shows that values can stabilize (sodium,

glucose, and lactate; Brown et al., 2008; van Balveren et al., 2017; Agina et al., 2020) or even alter direction (glucose; Joen et al., 1995) over longer durations. With this rate of change across all parameters measured, it is recommended that blood samples for haematocrit be analysed within <2 hours to maintain the stability of values.

Blood parameters of Lake Trout and White Sucker have been shown here to produce reliable results in glucose, sodium (both species), lactate (Lake Trout only), and potassium (White Sucker only), which can potentially be used for further research and assessments. Future research should look to validate other species with POCDs which can allow for reliable and accurate blood values in which researchers and government agencies can test and monitor species' health. The blood parameter values in Lake Trout glucose, lactate, sodium, and calcium displayed stability in this study when chilled, which can be used as a reference for future research if whole blood must be transported for analysis. Haematocrit was unstable even when chilled, therefore determining inaccuracy against the i-STAT or any other POCD should be done when minimizing storage time between blood drawing and analysis. This research should not dissuade further testing of lactate accuracy in other species as it was shown to be accurate in Lake Trout and was limited by sample size in White Sucker. In the absence of a point of care device, this and other research shows that most blood parameters are stable for at least four hours (≤1 hour for haematocrit) when kept cold, but future research should be cautiously interpreted if blood cannot be centrifuged or analyzed promptly, or if samples cannot be kept cold. Finally, the price of the POCD and cartridges should be compared to other less costly point-of-care devices such as glucose meters, lactate meters, micro-haematocrit tubes,

and battery-powered centrifuges (for stable transport of samples) and other methods of acquiring these values for the focal species of interest before purchasing.

Tables

Table 2.1. A review of literature that has performed i-STAT evaluation studies and species studied in each. Species are listed top to bottom then left to right for each parameter category.

Blood Parameter	i-STAT Below Lab Control	i-STAT Above Lab Control	No significant difference	Species
Glucose			Harrenstien et al., 2005	Rockfish (*Sebastes melanops* and *Sebastes mystinus*)
Lactate			Gallagher et al., 2010	Sharks (*Carcharhinus plumbeus* and *Mustelus canis*)
Haemoglobin	Harrenstein et al., 2005			Rockfish (*Sebastes melanops* and *Sebastes mystinus*)
Haematocrit	Harrenstein et al., 2005, DiMaggio et al., 2010, Harter et al., 2014, Borissov et al., 2019			Seminole killifish (*Fundulus Seminoles*), sandbar shark (*Carcharhinus plumbeus*), and Atlantic cod (*Gadus morhua*)
Sodium	DiMaggio et al., 2010, Harrenstein et al., 2005	Borissov et al., 2019, Harter et al., 2014	Cooke et al., 2008	Seminole killifish (*Fundulus Seminoles*), Rockfishlisted (*Sebastes melanops* and *Sebastes mystinus*), Atlantic cod (*Gadus morhua*), sandbar shark (*Carcharhinus plumbeus*), and bonefish (*Albula vulpes*)
Calcium	Borissov et al., 2019,			Atlantic cod (*Gadus morhua*)
Potassium	DiMaggio et al., 2010, Borissov et al., 2019		Harrenstein et al., 2005, Cooke et al., 2008	Seminole killifish (*Fundulus Seminoles*), Atlantic cod (*Gadus morhua*), sandbar shark (*Carcharhinus plumbeus*), and bonefish (*Albula vulpes*)

Table 2.2. Method of blood parameter determination for the i-STAT. Methods information was taken from the Procedure Manual for the i-STAT system (2012).

Sodium, Potassium, Chloride, Ionized Calcium	Ion-selective electrode potentiometry measures ions. The Nernst equation was used to calculate concentrations.
Glucose	Glucose is measured amperometrically. The glucose concentration is proportional to an electric current produced by liberated hydrogen peroxide that was oxidized at an electrode. The hydrogen peroxide was produced by the oxidation of glucose catalyzed by glucose oxidase.
Lactate	Lactate is measured amperometrically where lactate oxidase is converted to hydrogen peroxide, oxidized, and the current is proportional to the lactate concentration.
Hematocrit	Hematocrit is determined conductometrically after electrolyte concentration correction.

Table 2.3. Hourly rate of change in chilled wild-caught blood samples and lab-reared blood samples in Lake Trout. Due to no difference being found between lab-reared and wild-caught Lake Trout the total change and % changes were considered the same.

Blood parameter	Wild-caught sample		Lab-reared sample	
	Total change	Percent change	Total change	Percent change
Haematocrit (%PCV)	+1.83	+5.6%	+1.83	+5.6%
Sodium (mmol/L)	+0.1	+0.03%	-1.47	-1%
Lactate (mmol/L)	-0.06	-0.6%	+<0.01	+<0.8%
Glucose (mg/L)	+2.2	+2.2%	+2	+2.9%
Ionized calcium (mmol/L)	-0.06	-4.4%	+0.03	+2.3%

Figures

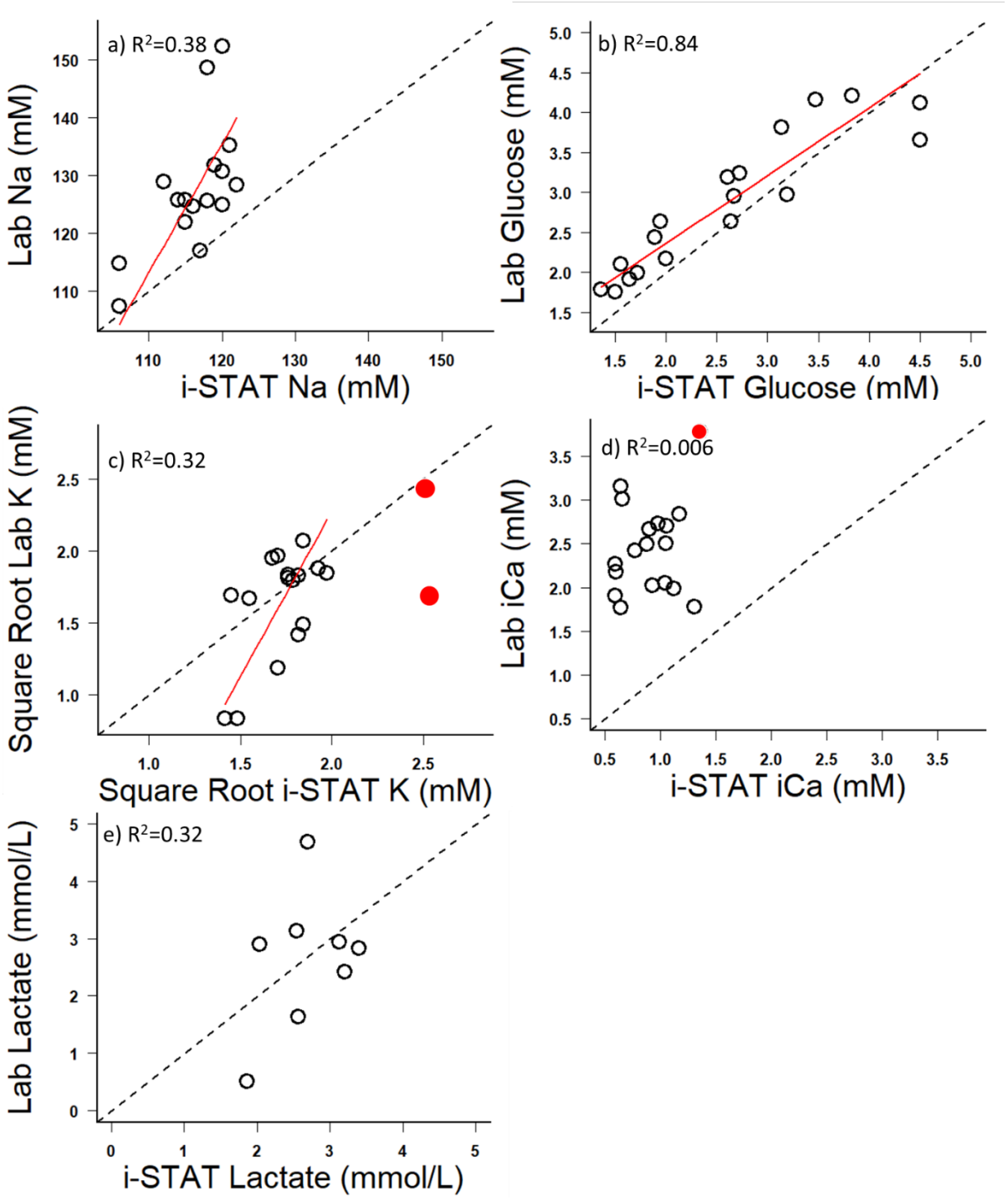

Figure 2.1. Linear regressions of POCD and lab assay results of White Sucker. The black dotted line represents a 1:1 relationship. Red lines represent significant regressions. Red solid dots represent outliers determined by Cook's distance.

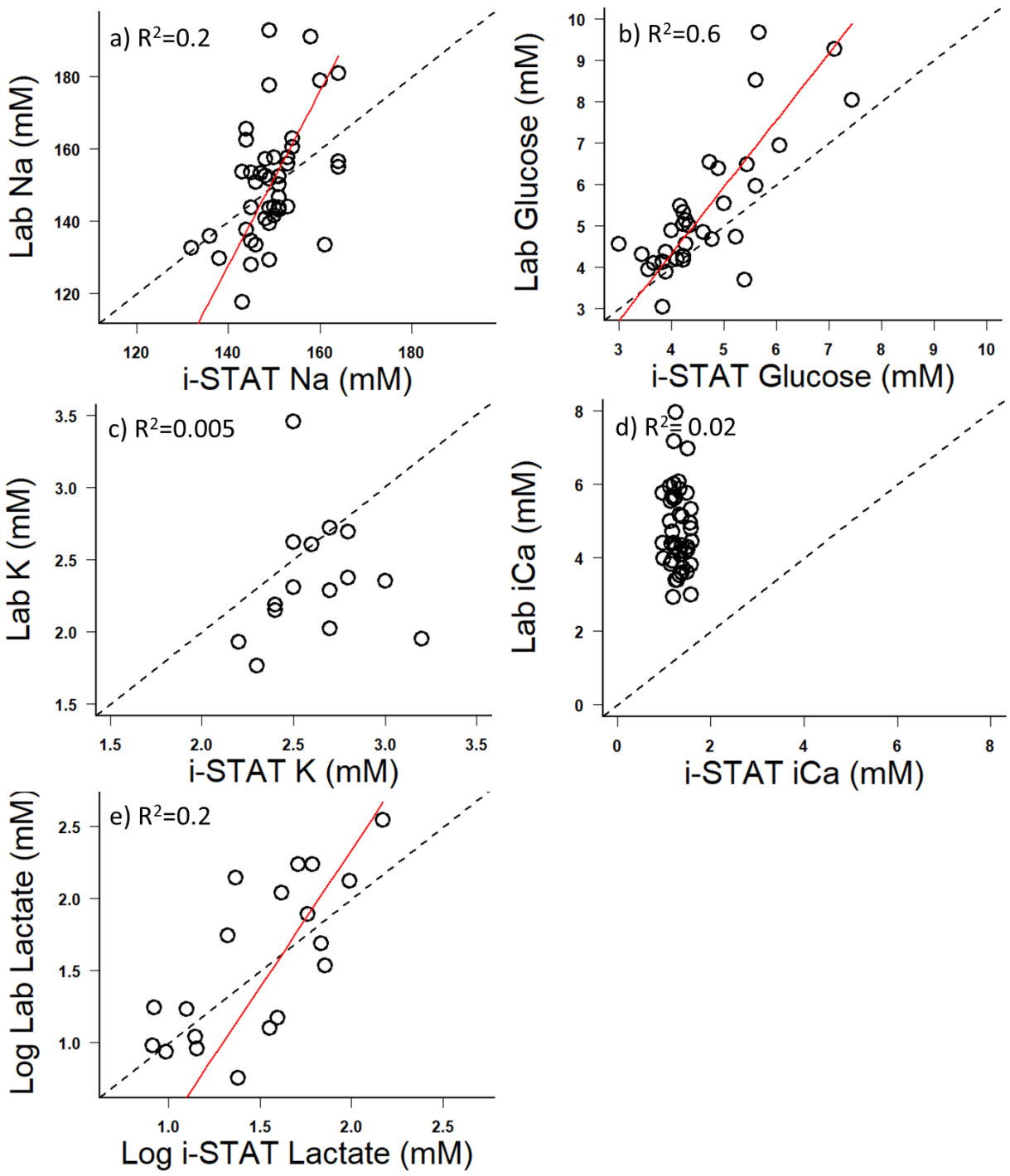

Figure 2.2. Linear regressions of POCD and lab assay results of Lake Trout. The black dotted line represents a 1:1 relationship. The red line represents significant regressions.

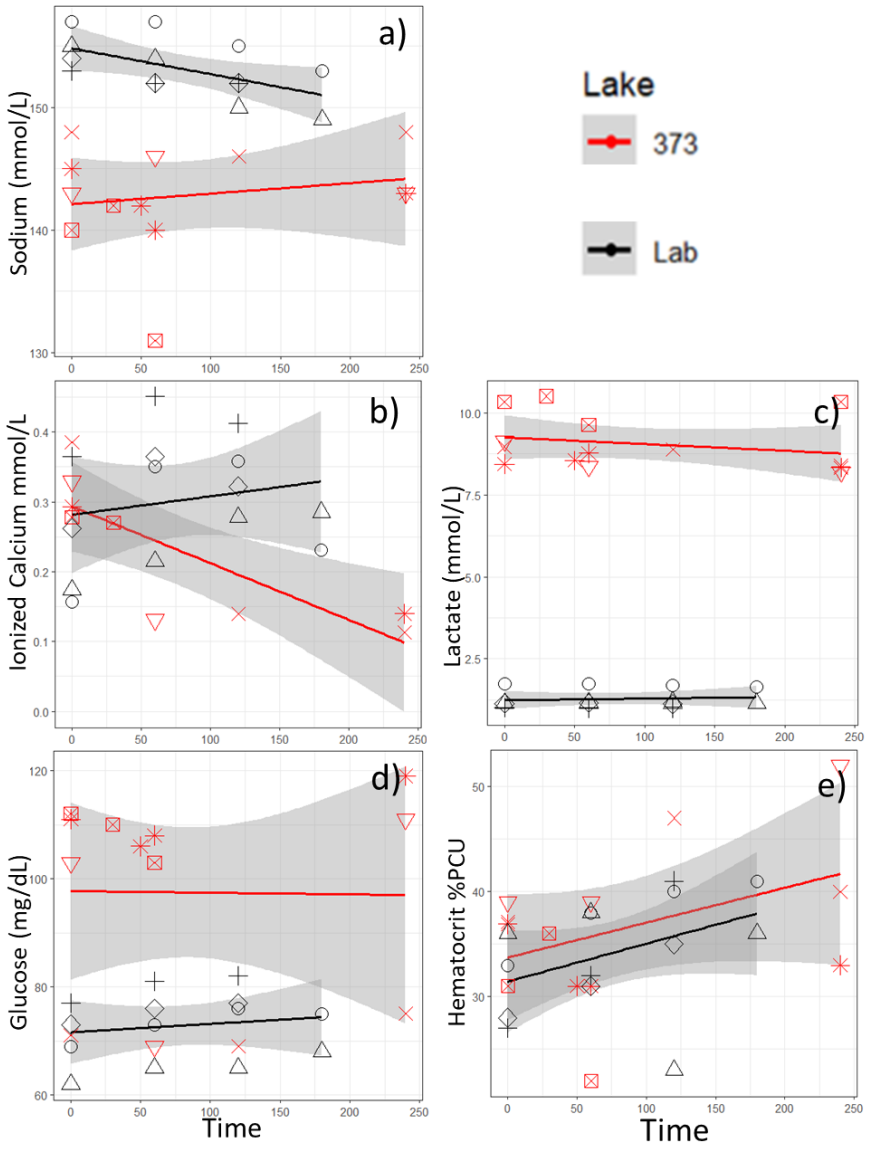

Figure 2.3. Blood parameter stability between the time of drawing and time of analysis among ELA lakes and a lab held a population of Lake Trout (see text). Individual fish were included as a random effect. There were significant interactions of time and source of Lake Trout for glucose (a), lactate (b), and ionized calcium (c). Significant changes over time were observed for sodium (d) and Haematocrit (e), and haemoglobin (f). A pairwise comparison of means showed differences between lakes and lab fish. The grey bars represent a 95% confidence interval.

Chapter 3 Evaluating Differences in Physiological Status Among Boreal Fishes using a Point of Care Device In a Natural Ecosystem

Abstract

Technologies for the rapid assessment of physiological status in fish can provide an important tool to resource managers, particularly in the face of sudden and unexpected environmental impacts. To field-test a proposed rapid assessment tool to evaluate physiological status in fish under typical field and lab conditions, I evaluated the ability of the i-STAT Point-of-Care device (POCD) to effectively detect a gradient of physiological change using blood from spawning Lake Trout (*Salvelinus namaycush*), where gradients of physiological change were induced by capture and handling methods increasing in their degree of invasiveness (angling, trap netting, and gillnetting followed by overnight caging). Treatments were applied to fish collected across 5 lakes. In addition, blood from wild-caught White Sucker (*Catostomus commersonii*) captured in the field and held in a lab was also evaluated against capture-induced physiological changes. Blood glucose, lactate, and haematocrit in Lake Trout increased with the level of physiological challange applied, while sodium decreased across the same gradient. Additionally, sex was a significant additive factor, with elevated levels of lactate and haematocrit in male fish compared to females across all lakes. I also found that the duration of time between capture and blood sampling for fish had a significant positive effect on glucose (angling only), haematocrit (angling only), lactate and sodium, highlighting the importance of minimizing the time between capture and blood sampling to obtain measurements. Unlike wild-caught Lake Trout, White Sucker showed significant decreases in calcium and haematocrit across the gradient of physiological change from capture, but no significant responses in other measured blood parameters.

These results provide support for the potential use of the i-STAT as a rapid assessment tool for the determination of physiological status in Lake Trout, but its use in White Sucker likely requires further investigation.

Introduction

Fish in boreal lakes experience sub-lethal stress from a multitude of natural and anthropogenic factors (Baker et al., 2013). High temperatures in lakes and rivers can cause physiological changes from heat in cool or cold-adapted fish species during summer, especially for salmonids in shallow water bodies (Scott and Crossman, 1973; Ackerman et al., 2000). Similarly, over-wintering warm-adapted fish experience physiological changes to the cold through low productivity and activity, especially minnows, carp, and bass (Scott and Crossman, 1973; Binder et al., 2015; McMeans et al., 2020). Lakes often have hypoxic zones in the hypolimnion during summer where fish (e.g. Lake Trout) are more limited in their habitat and experience changes to their physiologic status from thermal changes in the epilimnion zone or changes from anoxic environments in deeper waters (Plumb and Blanchfield, 2009; McBryan et al., 2013; Guzzo et al., 2017). Activities associated with spawning, including competition for mates, competition for spawning territory, gamete production, and potential lack of feeding, often cause seasonal change in physiological status (Crossman, 1973; Baker et al., 2013). Toxins in the aquatic environment, such as lead, copper, cadmium or zinc, primarily from anthropogenic sources, can often cause physiological or chemical stress in fish (Hontela et al., 1993; Diego, 1984; Gopal et al., 1997; Garfish and Belone, 2005). Sublethal stress impacts can be cumulative for fish, reducing their capacity to deal with

future stressors, thus highlighting the importance of understanding the impacts of anthropogenic impacts on fisheries (Barton et al., 1986; Sigismondi and Weber, 1988; Clements et al., 2002).

Understanding the response of fishes to major anthropogenic impacts often requires pre-and post-impact data, multiple sampling events, and destructive sampling methods, and can take months to years to process depending on resources available. The use of blood chemistry to more rapidly assess physiological status of fish has become an increasingly common method of assessment as it often does not require lethal sampling of fish (Wedemeyer, 1977). Chronic or acute stress reactions will often be reflected in the blood chemistry of fishes as a means of maintaining homeostasis (Chrousos, 1998). However, these changes to physiological status do not necessarily imply a stress reaction as they are natural and regular reactions to their environment and surroundings (Boonstra et al., 2013; MacDougall-Shackleton et al., 2019). It is additionally important to understand (and/or potentially control for) the influences of factors known to influence the interpretation of blood chemistry, such as species identity, size, temperature, sex, and season (Meka and McCormick, 2005; Baker et al., 2013; Hyatt et al., 2018).

Rapid assessment tools to evaluate the levels of physiological changes quickly and efficiently when ecologically catastrophic events occur (such as significant releases of oil or mine tailings) can be invaluable for providing rapid information on the impacts of such events on native fishes. Similarly, a rapid assessment tool can also allow for quick adjustments to research design to minimize negative impacts on animals. Many studies have observed changes in blood chemistry due to capture and/or purposefully

induced changes in physiological status that are often used as a proxy for general physiological responses (Clements et al., 2002; Baker et al., 2013).

Point-of-Care devices (POCDs) are typically used in hospital or laboratory settings to produce rapid assessments of blood chemistry. POCDs are also being adopted for use in animals to develop tools that can expand our ability to evaluate species' health, though not all devices are designed to be compatible with all species (Stoot et al., 2014). The most used portable POCD for vertebrates currently is the i-STAT 1 (hereafter referred to as the i-STAT or POCD) point-of-care whole blood analyzer (Stoot et al., 2014), which can produce up to 24 blood parameters using a host of cartridges requiring only 17–95µL of whole blood per cartridge. The i-STAT POCD has been evaluated in several marine warm water species under field conditions, including two species of rockfish (*Sebastes melanops* and *Sebastes mystinus*; Harrenstien et al., 2005), Bonefish (*Albula vulpes*; Cooke et al., 2008), Seminole Killifish (*Fundulus seminoles*; DiMaggio et al., 2010), Sandbar Shark (*Carcharhinus plumbeus*; Harter et al., 2015) and Atlantic Cod (*Gadus morhua*; Borissov et al., 2019). In freshwater, only the Rainbow Trout (*Oncorhynchus mykiss*; Harter et al., 2014) has been evaluated, and only under laboratory conditions. The i-STAT is relatively precise, often showing consistent results that are above (sodium, Borissov et al., 2019), below (haematocrit, haemoglobin, calcium, potassium, sodium; Harrenstein et al., 2005; Cooke et al., 2008; DiMaggio et al., 2010; Harter et al., 2014; Borissov et al., 2019; Chapter 2) or the same as (glucose, lactate, and potassium, Harrenstien et al., 2005; Cooke et al., 2008; lactate, Gallagher et al., 2010; Chapter 2) results from conventional laboratory methods (Harrenstien et al., 2005; Cooke et al., 2008; Borissov et al., 2019; Chapter 2, Table 2.1). Aquatic field studies have used

the i-STAT to study the physiological change in sharks (Frick et al, 2012; Gallagher et al., 2014; Hyatt et al., 2018; Anderson et al., 2012; Mandelman and Farrington, 2007; Mandelman and Skomal, 2009), Saithe (*Pollachius virens*; Roth and Rotabakk, 2012), Bonefish (Cooke et al., 2008), Dugongs (*Dugong dugon;* Wong, 2018), as well as in terrapins including Yellow-Bellied Sliders and River Cooters turtles (*Trachemys scripta*, *Pseudemys concinna*; Savo et al., 2018), however all of the above studies took place in warm, tropical marine environments (excluding Roth and Rotabakk, 2012). Although a boreal freshwater species have been evaluated with this POCD (Rainbow Trout; Harter et al., 2014), the fish were farm grown, and the important physiological indicators lactate and glucose were not evaluated. No research to date has evaluated the ability of the i-STAT POCD to detect gradients of physiological change in wild boreal fish species in their natural environment. Understanding whether portable POCDs like the i-STAT can accurately capture how natural populations respond to gradients of physiological change in a climate more commonly encountered in boreal environments will help in understanding the applicability of this device for rapid assessments of fish in regions with the potential for ecological disasters (presence of oil pipelines and mining).

Lake Trout (*Salvelinus namaycush*) are the largest of the freshwater charr, are widely distributed in the northern regions of North America, and are a species of economic, commercial and aboriginal importance in Canada (Royce, 1951; Deroche, 1969; Scott and Crossman, 1973). During fall, Lake Trout migrate from the deeper, colder waters occupied in the summer to the coarse gravel and cobbly shallower waters to spawn when waters reach ≤11°C (Slongo, 2022). Males enter the spawning ground first, followed by the females (Gunn et al., 2003). In some lakes during the summer (and

especially in small boreal lakes which make up a significant proportion of Lake Trout habitat on the landscape; Gunn et al., 2003), Lake Trout experience oxythermal habitat limitations when warm epilimnetic water from above (≥15°C) and anoxic water from below (≤4 mg/L) restrict their tolerable habitat (Redick, 1967; Guzzo et al., 2017).

Salmonid species' blood chemistry in physiologically stable and physiologically unstable states has been reported in the literature and validated with generally consistent patterns; when experiencing confinement, extremes in temperature, or capture, many salmonids show different levels of physiological changes between males and females, with females often showing elevated cortisol, lactate, glucose and calcium (Clark et al., 2011; Clements et al., 2002; Donaldson et al., 2014; Edsall, 1999; Hruska et al., 2010; Sopinka et al., 2016). However, blood glucose has also been reported as higher in migrating male rainbow trout compared to females (Clements et al., 2002). The same study found that angled post-spawn rainbow trout had lower glucose, lactate and cortisol than pre-spawn trapped trout (Clements et al., 2002). Compared to other species of salmonids, Lake Trout seem to be the least sensitive to transport and handling compared with other salmonids, with the lowest blood glucose and lactate concentrations after physiological challenges compared to rainbow trout (*Oncorhynchus mykiss*), brown trout (*Salmo trutta*), and brook trout (*Salvelinus fontinalis;* Barton, 2000). Thus, if Lake Trout shows changes in blood metabolites in response to physiological challenges, other charr species should show a similar or greater reaction. Current literature has found significant changes to fish blood parameters—especially lactate, hematocrit, sodium, and glucose—after stressful events across a wide range of species, including salmonids (Baker et al., 2013; Seibelet al., 2021; Table A1).

White Sucker are not a typically targeted sport fish and literature on their physiological response is much more limited compared to Lake Trout or other salmonids. However, due to their wide distribution and lack of angling pressure, they are an important sentinel species for environmental impacts in North American lakes (Munkittrick et al., 1991; Servos et al., 1992; Doherty et al., 2010). Toxic exposure and handling have been shown to increase White Sucker blood glucose and lactate (McMaster et al., 1994; Miller et al., 2009; Quinn et al., 2010), as well as decreased sodium, chloride, and potassium levels (Hobe et al., 1984).

My main goal in this study was to 1) evaluate the i-STAT as a reliable method for evaluating physiological change outside of its intended scope of operation (<16°C); 2) investigate how and which i-STAT blood parameters change in White Sucker (*Catostomus commersoni*) and Lake Trout across imposed gradients of physiological challenges; and 3) understand what other factors may have influenced blood parameter values (e.g. sex, proximity to peak spawn, and holding time between capture and blood removal). The blood parameters glucose, lactate, haematocrit, haemoglobin, sodium, and calcium were all assessed using the i-STAT due to their reported validity (or lack thereof) as indicators of physiological change in previous studies (Harrenstien et al., 2005; Cooke et al., 2008; Harter et al., 2014; Stoot et al., 2014; Borissov et al., 2019; Chapter 2).

The validity of the POCD as a rapid assessment tool in the field was confirmed with several Lake Trout blood parameters with increasing levels of imposed physiological change. In addition, differences between sexes, and the effect of time post-capture (e.g., time between capture and drawing of blood) were also assessed for several response variables. By contrast, blood chemistry from White Sucker held in the lab

exposed to increasing chasing did not shift as expected or did not significantly change for most parameters evaluated.

Methods

Lake Trout Sampling

Up to 10 Lake Trout (minimum five) were captured by each method of angling, gill net, and trap nets across each of the five lakes (Table A2.2; Figure A 3.3) included in the study. Fish were captured using three established methods used at the IISD Experimental Lakes Area for over 50 years and are consistent with many fisheries monitoring programs globally, which ranged in their anticipated induction of changes in physiological status. Angling was used as a capture method anticipated to cause the lowest impact on physiological change. In addition, holding time between capture and drawing of blood also varied for each capture method potentially having additional impacts on physiological status. Prior to sampling, fish were held in large plastic tubs (~7.5–10 gallons of water in each) replenished with lake water every 5–10 minutes to maintain temperatures equivalent to the lake and sufficient oxygen levels. When angled, fish were brought into the boat <1 minute from capture on the line and were sampled as quickly as possible (between 4 to 58 min after capture with an average of 19.8 minutes). Short-set (20–30 minutes) gill netting was done on known spawning shoals at night (17:05±5min-19:57±17min). Lake Trout captured via gill nets were subsequently held in a submerged cage overnight and were processed the next day anywhere between 5–120 minutes after removal from the cage, during which time they were held in tubs on shore

as described above. Trap netting was used as a third method of capture. Because trap nets were checked every three days, Lake Trout captured in trap nets were held at most for that duration of time, but may have been held for less. Lake Trout were removed from trap nets and were processed within 103 minutes or less from the removal of nets and held in tubs as outlined above in the interim. All fish captured and handled were under the approval of Lakehead Animal Use Protocol #1467807 and a scientific collection permit from the Ontario Ministry of Northern Development, Mines, Natural Resources and Forestry (permit # 1095715).

I sampled fish during Lake Trout spawning in the fall (Scott and Crossman 1973). Air temperature during sampling ranged from 0.6–11.2°C while the temperature of the water was between 10–12.5°C. These temperatures are outside of the i-STAT's operating range (16–30°C) but are typical of conditions during spring or fall in boreal regions. Sampling of blood occurred during October 4th–13th in 2020.

Lake Trout >500g were targeted for sampling to provide similar-sized fish across lakes, as well as sufficient volumes of blood to support all required analyses. Additional effort was made to try and select fish for blood sampling such that a balanced sex ratio could be achieved to evaluate potential differences in blood chemistry between male and female fish. Sex was assessed by the expression of gametes (sperm or eggs) when handled. Fish were moved one at a time to a basin of water with ~60 mg/L solution of tricaine methanesulfonate (TMS) buffered with ~120 mg/L sodium bicarbonate for approximately 2–4 min to achieve anaesthetization. Once immobility was achieved, fish were examined for existing tags (Passive Integrated Transponder or PIT tags), removed from the anaesthetic bath, weighed, and placed in a cradle on the wet mesh. During this

procedure, the gills of the fish were irrigated by pouring lake water over them using a 1L measuring cup. All participants involved in the blood drawing process wore nitrile gloves. The ventral area behind the caudal peduncle was cleaned with a Kimwipe™ and povidone-iodine and blood was drawn. Needles of 20-gauge, pre-rinsed with lithium heparin (7500 units/ml) were inserted under the skin of the ventral midline of the caudal peduncle at a 45°angle from the body until encountering the vertebral column. The needle was then pulled away from the ventral column slightly while applying light suction to the syringe plunger to draw up the pre-determined amount of blood (based on the body mass of the individual, ≤0.1% of body weight, in accordance with CCAC guidelines; www.ccac.ca) with the average drawing time lasting 29 seconds. As soon as possible, ~90uL of whole blood was added to a POCD cartridge with the needle taken off of the syringe to avoid blood lysing. Lactate, haematocrit, glucose, sodium, and ionized calcium blood parameters were acquired using the CG4+, CG8+, and CHEM8+ i-STAT cartridges. Haemoglobin was not evaluated in this research due to its dependence on haematocrit values making it redundant. All blood samples had at least some air in the syringe upon drawing the required volume; because the drawing of pure arterial or venous blood without fish destruction and no air exposure to the sample is impractical in most field settings, results for blood gas parameters were ignored (Harter et al., 2015, 2014). After drawing of blood, fork length and total length for each fish were measured and recorded, and untagged fish were injected with a Passive Integrated Transponder (PIT) tag subcutaneously following standard IISD-ELA procedures. To allow recovery, sampled fish were held in a basin of water with pressure on the wound until the blood was clotted (30sec–3min), and then observed for an additional 5–10 minutes to ensure

full recovery (e.g., recovery of equilibrium and vigour), after which they were released back into the lake with a mesh net.

In all cases, blood samples were run on the i-STAT at temperatures between ~0–12°C in the lithium heparin-washed syringes) after being drawn. Most times where Lake Tout were pulled from trap nets or pens was recorded but some start times were implied from recorded change in capture methods with a variability of ±5 minutes. Delays in analyses between the drawing of blood and analyses were typically due to cartridge failures, (~10% failure rate, Table A3.4) the need to reheat the i-STAT from ambient temperatures or a short backlog of samples.

For long-term storage (e.g. during travel and prior to the day of sampling), all POCD cartridges were held in cold conditions either insulated with icepacks or a 4°C environment in accordance with the manufacturer's specifications. On the day of analysis, POCD cartridges (i-STAT CHEM8+ and/or CG8+) were kept in incubators (a cooler with a towel and hot water bottles to maintain cartridges at the manufacturer's listed functioning temperature, 16–30°C), whereas the i-STAT CG4+ were left out in air temperatures ranging from ~0–5°C, following Borisov et al., (2019) who showed only infrequent (6.25%) cartridge failure for the CG4+ at 5°C. No temperature-related cartridge failures for the CG4+ occurred during this research, excluding instances where the i-STAT become too cold to produce results (uncertain how many times this occurred). The i-STAT was returned to the warm incubator while analyzing cartridges.

White sucker sampling

In the Biology Aquatic Facility (BAF) at Lakehead University, White Sucker that were scheduled for destruction due to a COVID-19 related lab shutdown had blood taken during euthanasia via overdose of Tricaine methanesulphonate (TMS). White Sucker were originally collected from Lake 375 of the ELA in the spring of 2019. Twenty-two fish were held in three separate 132-gallon tanks on a recirculating water system and were taken out in sequential order one at a time and placed into a TMS water bath. Each fish captured experienced a gradient of physiological change created by chase stress from the previous fish being netted from the tank, which was expected to cause an increasing gradient of physiological change over each round of removal. The white sucker ranged in size from 182mm (fork length) and 194g (weight) to 409mm and 816g. The sex of the white sucker was not determined. The method of drawing blood was the same as described above for Lake Trout.

Statistical analysis

All analyses were carried out in R, v. 4.0.5 (R Core Team, 2022). Data were log or square root transformed when necessary in order to meet assumptions of homogeneity of variance and normality, using the *car* (*LeveneTest* function) and *nortest (ad.test function)* packages, respectively.

To address objective 3 (understanding what other factors influenced blood parameter values), a linear regression analysis was used to evaluate whether lactate concentrations varied with Lake Trout fork length. Additionally, eight fish sampled during the study had previously had telemetry tags surgically implanted, five of which

were gill netted fish, one was angled, and two were trap netted. A series of *t*-tests were performed to determine if previous surgery resulted in different blood chemistry compared to fish without surgery scars using residual values from my models.

A correlogram was used to evaluate correlations among blood parameters, fish weight, and length and to identify potential outliers in the data. Haemoglobin was excluded from the analysis because the i-STAT uses haematocrit to calculate haemoglobin, making these two variables perfectly correlated ($r = 1$). A principal component analysis was then used to identify the grouping of variables. Ellipses (95% confidence) were drawn around groups to understand how they differ on the axis. PCA's were carried out using the *prcomp* and *ggplot* functions in the *tidyverse* packages.

To contribute to answering objectives 2 and 3 (understanding the impact gradients of physiological change, time-since-capture, and fish sex on blood chemistry, represented by a perceived change in physiological status), a linear mixed-effects analysis was conducted on the relationship between the Lake Trout blood parameters (lactate, haematocrit, glucose, sodium, ionized calcium) across gradients of physiological change, time-since-capture, and fish sex across five lakes. I modelled gradients of physiological change (capture method), time-since-capture, sex, and interaction terms between sex and capture method, as well as between time since capture and capture method as fixed effects. Three-way interaction terms among fixed effects were not included to preserve degrees of freedom in the models and to ensure that model results could be interpreted meaningfully. Interactions between sex and time since capture were also excluded for similar reasons. Because of its potentially modifying effect on all other parameters, I emphasized understanding the impact of time since capture for all response variables

first, before evaluating the impacts of other interaction terms. The lake from which fish were collected was included as a random intercept:

Response ~ capture method + sex + time since capture + sex * capture method + capture method*time since capture + (1|lake) (eq. 3.1)

Significance of individual terms in the models were determined from likelihood ratio tests comparing the full model against the model without the main effect of concern (Fincham et al., 2019). Model comparisons evaluating the significance of fixed effects were conducted on models fit using maximum likelihood estimators. The significance of the interaction between capture method and time since capture was first evaluated, and determined via comparison to an additive model:

Response ~ sex *capture method + sex + capture method + time since capture + (1|lake) (eq. 3.2)

If this interaction was found to be non-significant, the significance of time since capture as a main effect was then evaluated, comparing the following two models (eq. 3.2, 3.3):

Response ~ sex * capture method + capture method + sex + (1|lake) (eq. 3.3)

I then evaluated the interaction between sex and capture method (using either equation 3.1, 3.2, or 3.3, as appropriate depending on the significance of time since capture and its interaction with capture method) by comparing with models lacking the interaction term, as outlined above. Finally, main effects were evaluated using model comparisons with and without the presence of the main effect of interest.

When significant as a main effect or as part of an interaction with sex, a pairwise comparison using the Tukey's method in the "emmeans" R package was used to evaluate differences among capture methods. Visual inspection of residual plots from final models did not reveal any obvious deviations from homoscedasticity or normality.

To address objective 2 (understanding the impact of increasing disturbance on blood chemistry), a linear mixed effects analysis was conducted on the relationship between the White Sucker blood parameters (glucose, lactate, haematocrit, sodium, ionized calcium, and potassium), and rounds of removal. I modelled a round of removal, as a fixed effect. The tank from which fish were collected was included as a random intercept:

Response ~ round of removal + (1|tank) (eq. 3.4)

Significance of individual terms in the model were determined from likelihood ratio tests comparing the full model against the model without the main effect of concern (Fincham et al., 2019). Model comparisons evaluating the significance of fixed effects were conducted on models fit using maximum likelihood estimators. A pairwise comparison using the Tukey's method in the "emmeans" R package was used to evaluate differences among capture methods.

White Sucker hematocrit showed non-normal distribution, therefore a non-parametric generalized additive linear mixed effects model with a random effect was used. The models compared were a standard linear model, a linear model with a quadratic term and a GAMM using the "mgcv" R package:

Hct ~ round of removal + (1|tank) (eq. 3.5)

Hct ~ round of removal + (round of removal^2), random=list(tank=~1) (eq 3.6)

Hct ~ s(round of removal), random=list(tank=~1) (eq 3.7)

where the 's' function in equation 3.6 represents an implicit smoother matrix function to avoid over fitting or under fitting the data to better capture non-linear relationships. Smoothers are made of many basic functions that are multiplied by coefficients which are parameters in the model and fit the data.

Results

The recorded cartridge failure rate in the field was 10%, with most of the failures occurring in the first day of field sampling for Lake Tout (likely due to temperature, filling issues, and gaining familiarity with the operation of the POCD in a field setting), and most were from CHEM8+ or CG8+ cartridges (Table A4). Operating success was gained by ensuring the unit was kept warm in an incubator during analyses, improving the failure rate to be negligible after day 1 (Lake 224).

Fork length had a significant effect on blood lactate ($F_{1,75}$=7.02, p=0.001), as lactate values decreased with increasing fork length (Figure 3.1). Diagnostic plots indicated that the variance of error terms was homogenous and data appeared normal after a log transformation.

Several variables in my analysis were correlated (Figure A3.1). There were significant negative correlations between lactate and weight (r=0.29) and a significant positive correlation between lactate and glucose (r=0.45), and between lactate and haematocrit (r=0.39). A significant positive correlation was observed between sodium

and ionized calcium (r=0.55) and a significant negative relationship was seen between sodium and glucose (r=-0.27). It should be noted that there was a slight male bias in the data of 41 females to 66 males, across all lakes, with the exception of lake 626 where more females were sampled than males (Table A2.3).

A PCA among blood chemistry parameters and fish morphological attributes explained 51.9% of the total variance on the first two axes, with PC1 accounting for 31.2% of the total variance while PC2 explained 21.7% (Fig 3.2, a, b). Blood parameters grouped according to correlogram results, with glucose, haematocrit, and lactate grouping together, all correlating negatively with axis 1, and calcium, and sodium grouping together, correlating negatively with axis 2. Time since capture was also correlated negatively with axis 1. Fish size (as round weight) trended opposite blood chemistry parameters on both axes. Ellipses describing the three capture methods showed substantial overlap, however, gill netting and angling appeared to differentiate along axis 1, with gill netting grouping around increased glucose, haematocrit, and lactate compared with angling. The trap netting ellipse was by far the most variable, encompassing both gill netting and angling results. The ellipses around the sexes also overlapped, but males tended to group more around increased glucose, lactate and haematocrit along axis 1 compared to females.

A linear mixed-effects analysis to evaluate differences in physiological status across time-since-capture found significant interactions within my gradient of physiological change and time-since-capture for lactate (LLR=6.58, df=9,7 p=0.02) and sodium (LLR=5.96, df=7,9 p=0.03; Figure 3.2). For parameters where interactions were not significant, the inclusion of time-since-capture did not explain a significant additional

component of variability for glucose (LLR=3.48, df=11,9 p=0.9), haematocrit (LLR=1.82, df=11,9 p=0.2) or calcium (LLR=0.84, df=11,9 p=0.4), and were therefore excluded from further consideration.

Controlling for time since capture effects (where they existed), I found significant responses of individual blood parameters to physiological changes in Lake Trout, with trap netting values (physiological challenge 2) usually lower than or about equal to gillnetting values (physiological challenge 1; Figure 3.4). Males always appeared to have a great change in physiological status than females across all physiological changes, and physiological challenge 2 seems less harmful to females than physiological challenge 1. However, the gradient of physiological change in the model explained a significant additive component for glucose and sodium compared with models without the physiological changes present (glucose: LLR=20.13, df=4,6, p< 0.001; sodium, LLR=25.14, df=4,8, p< 0.001). Sodium levels were not different among sexes (LLR=4.85, df=5,8, p= 0.2). Pairwise comparisons among means were used to determine differences between the gradients of physiological change using the additive model (glucose) and the single factor model (sodium). For glucose, there was an increase from baseline to capture methods 1 and 2 (p= 0.005). For lactate, and haematocrit capture method 1 and 2 (gill netting and trap netting respectively), values were significantly elevated for all male Lake Trout compared to the baseline (lactate: p<0.001; glucose: p=0.01; haematocrit: p=0.003). Female trout only had significantly elevated physiological values for glucose and capture method 1 for lactate compared to the baseline. The inclusion of lake as a random intercept was significant for glucose (LLR=0.09, df=8,7 p=0.4). For ionized calcium, no models using sex, the gradient of

physiological change, time-since-capture or lake explained any significant component of variability in the data.

Lake Trout that had previously undergone surgery (n=8 for lactate and n=6 for all other parameters) had blood parameters that were not significantly different to those that had not undergone surgeries (t-test, all p>0.05).

A linear mixed-effects analysis to evaluate differences between rounds of removal (our gradient of physiological change) in lab-held White Sucker found that round of removal explained a significant additional component of variation for ionized calcium (LLR=9.82, df=4,6, p= 0.004). Haematocrit had a non-normal distribution and therefore a generalized additive mixed effect model was used with a random intercept. A standard linear model, a linear model with a quadratic term and a GAM all with tank as a random intercept were compared. The quadratic model with a random intercept was found to be the best fit for haematocrit, having a significant relationship with round of removal ($F_{3,16}$=9.04, =16, p<0.001). Haematocrit decreased from the first round of removal to the next two (Figure 6). Round of removal did not explain a significant additional component for lactate, glucose, sodium, or potassium (all p>0.05). The inclusion of tank (e.g., the original tank from which the fish was drawn) as a random intercept was not significant for any blood parameter (all p>0.05).

Discussion

The gradients of physiological status revealed by measured blood chemistry with this POCD shown across Lake Trout handling duration and an imposed gradient of physiological challenges (controlling for time since capture) provide support for the use of this device as a potential field tool for evaluating fish physiological status via blood chemistry in this species, including for use in conditions outside of the manufacturers intended parameters (i.e., using modified procedures in ambient temperatures <16°C). A suite of blood parameters (lactate, haematocrit, glucose, and sodium) were demonstrated as reliable indicators of physiological status in Lake Trout. Haematocrit results in this study should be interpreted with caution due to its lack of validation here (Chapter 2), though generally the i-STAT tends to not reflect hematocrit when evaluated in other studies (DiMaggio et al., 2010; Harter et al., 2014; Borissov et al., 2019). The values in my study changed to a lesser extent than observed in other studies that have challenged salmonid physiological status, and Lake Trout inherently showing lower physiological changes in blood glucose results (Barton, 2000). The changes due to my imposed gradient of physiological change were biologically significant for glucose, lactate, haematocrit and sodium relative to the baseline physiological challenge (Table 1; Clements et al., 2002; Olsen et al., 2005; Wells and Pankhurst, 1999). In other studies, blood parameters including glucose, haematocrit, sodium, and potassium have been shown to significantly change during exposure to toxic chemicals (e.g., metals found in mining tailings, pesticides, and crude oil). Thus, the results of the current study suggest that POCD technology could be a valuable rapid assessment tool for fish health assessments following catastrophic events such as oil or mining tailings spills (McCain et al., 1978; Mishra and Srivastava, 1980; Jyothi and Narayan, 1999; Wood et al., 1999;

Javed and Usmani, 2015), which have recently become a more common occurrence in boreal regions (e.g., Mount Polley, Husky Oil North Saskatchewan spill, proposed release of oilsands mine tailings into the Athabasca, etc). Exposures of salmonids to bitumen and other pollutants have been shown to contribute to increased lactate and/or reduced recovery time of lactate levels (Hodson, 1976; Goss and Wood, 1988; Folmar, 1993; Lin et al., 2022). Further, using a suite of blood parameters to evaluate physiological status may be a more powerful approach due to potential variation in response to physiological changes among any single parameter, depending on the nature of any particular physiological change (e.g. toxic exposure, exhaustion, disease, etc.; Martínez-Porchas et al., 2009). Further, the generation of rapid results on-site by use of a POCD avoids the need to transport and chill whole blood samples (and/or centrifuge to isolate plasma for freezing) like most fish blood studies must do when in the field, eliminating several logistic challenges that might otherwise exist in remote locations.

Though the i-STAT was not designed to function in cold temperatures (<16°C) or work on the blood of ectotherms, I was able to generate meaningful findings to measure Lake Trout physiological responses in this study. The techniques applied here to ensure functionality in cold temperatures (via active warming/incubation of the device in the field) can therefore potentially be used to expand the scope of operation of these units in more challenging environments (e.g. insulated container with a heat source) when using the POCD. Similarly, my methods suggest that higher ambient temperature ranges (30°C+) can likely also be combatted with active cooling in the field. Due to the limited operating temperatures of POCD units, it is recommended to keep a thermometer with the stored i-STAT in non-ideal temperature or humidity ranges. Further, blood chemistry

from only a limited number of fish species has been validated for the POCD, with varying success (Gallagher et al., 2010; Harter et al., 2014, 2015; Borissov et al., 2019) including the species examined here (Chapter 2). Based on those most recent findings, the values for some of the parameters provided by this POCD (calcium, lactate in White Sucker, and potassium in Lake Trout; Chapter 2) should still only be interpreted as relative and not absolute (Harrenstien et al., 2005; Cooke et al., 2008; Harter et al., 2014; Borissov et al., 2019). Though I was unable to evaluate it in my study (Chapter 2), several studies have indicated that haematocrit and haemoglobin are biased low using the i-STAT vs. traditional methods due to fish having larger nucleated red blood cells than mammals (DiMaggio et al., 2010; Harter et al., 2014). Also, while methodological issues with cartridge loading can initially be problematic (e.g., when filling i-STAT cartridges, errors generated due to the issue of air bubbles, under-filling or over-filling), these can be effectively mitigated with some practice by the user. As with all blood work, attention must be made not to take other tissue fluids (muscle; Bowen et al., 2010).

Changes in White Sucker blood chemistry observed here (reduction in both ionized calcium and hematocrit) were not consistent with increased levels of physiological change when compared to results for Lake Trout or with other previous studies that demonstrated changes in blood chemistry along an induced gradient of physiological change. Ionized calcium changes are not typically associated with increased physiological changes in fish, and haematocrit is expected to increase, rather than decrease with increased physiological changes (Ahmed et al., 2020; Olsen et al., 2005). As such, I conclude that the gradient of physiological change from chasing White Sucker with nets was likely not intense enough to cause a significant change to

physiological status over rounds of removal, and/or that handling was too short, and fish were anaesthetized too soon relative to the imposition of physiological change to generate a significant change to physiological status that would be reflected in blood chemistry (Lawrence et al., 2018). Haematocrit and glucose were much more variable for lab White Sucker (lab-reared) compared to Lake Trout (wild-caught), which is the opposite of what was expected for haematocrit and glucose in lab-reared vs. wild-caught fish (Edsall, 1999). This increased variability in white Sucker potentially obscured changes in glucose. In contrast, lactate and sodium were slightly more variable in Lake Trout compared to White Sucker.

The induced physiological change led to increasing glucose levels in Lake Trout blood compared to baseline levels in angled Lake Trout, which is consistent with the literature (Barton et al., 2000, Clements et al., 2002, Cooke et al., 2008). Initial plasma glucose increases tend to be from the release of catecholamine (Vijayan and Moon, 1992). However, the more likely cause of a sustained increased level of glucose is likely due to increased cortisol causing hyperglycaemia which is a common reaction in fish preparing themselves for threats (Rottmann et al., 1992). Stress-induced hyperglycaemia has been observed in most fish species but its intensity can fluctuate based on species, recent meals and physiological change intensity (Barton 2000; Martínez-Porchas et al., 2009). In my study, glucose continued to increase with time post angling capture, likely due to handling and transportation, followed by containment in bins on shore. Similar results were also observed in Rainbow Trout in another study (Wells and Pankhurst, 1999) that showed increased lactate and glucose due to handling and confinement stress.

My study found that time of sampling after capture significantly influence sodium but a did not cover up a significant change in my gradient of physiological change. Sodium decreased in Lake Trout for physiological challenge 1 (gillnetting), and physiological challenge 2 (trap netting) compared to my baseline challenge (angling), consistent with similar studies (McDonald, and Milligan 1997; McDonald and Robinson 1993; McDonald and Milligan, 1997; Hruska et al., 2010). Confinement was an important driver of physiological change observed as decreased sodium levels in Lake Trout blood, although duration of handling and severity of physiological change heavily influence these decreases in sodium seen in this and other freshwater species (McDonald, and Milligan 1997; McDonald and Robinson 1993; McDonald and Milligan, 1997; Hruska et al., 2010). By contrast, physiological change in marine species has been reported to result in increased blood sodium (e.g. *O. kisutch*, Farrell et al., 2001; *O. nerka*, Cooke et al., 2006b; *O. elongatus*, Milston et al., 2006; *S. acanthias*, Mandelman and Farrington 2007; *A. vulpes* Suski et al., 2007; *A. vulpes* Cooke et al., 2008). Stress in both freshwater and marine fishes is associated with increasing vasoconstriction and cardiac output from adrenaline (Mazeaud and Mazeaud, 1981). Adrenaline also leads to increased gill/lamellae perfusion which increases gill diffusion capacity, also increasing ion transfer in gill tissues, decreasing sodium in freshwater fish but increasing sodium in marine fish (Randall and Ferry, 1992; McDonald and Milligan, 1997). Rises in sodium due to the use of anticoagulant in syringes used to retrieve blood was not a concern in this study because lithium (rather than sodium) heparin was used to avoid influencing sodium ion values.

My study has also shown significant associations with physiological change (capture and handling) and increased haematological parameters (haematocrit and haemoglobin) which has been shown in several other freshwater species through capture, infection, toxicity, and medication overdose stress (*Esox masquinongy*, Beggs et al., 1980*; O. mykiss*, Wydoski et al., 1976; and *Oreochromis aureus, O. mossambicus, O. niloticus*; Silveira-Coffigny et al., 2004). Fish haematocrit can also be affected by age (sexually mature; Jamalzadeh and Ghomi, 2009), sex (Lusková, 1998), and temperature (Fazio et al., 2013). My study explicitly controlled for sex and temperature in these Lake Trout populations, as all sampled trout had gamete expression to confirm sex and that fish were mature (>6 years old; Scott and Crossman, 1973). Haematological parameters such as haematocrit and haemoglobin are sensitive to the physiochemical environment (salinity, toxicity, and oxygen) and therefore tend to be good indicators of environmental impacts on fishes (Silveira-Coffigny et al., 2004; Fazio et al., 2013; Bermejo-Nogales et al., 2014; Martos-Sitcha et al., 2017; Seibel et al., 2021).

Increased lactate levels in males for physiological challenge 2 (trap-netting), and physiological challenge 1 (gillnetting), compared to angling in my study provides clear evidence that Lake Trout were stressed and exerting more energy relative to baseline/angled fish. Blood lactate increases in the blood due to anaerobic activity such as strenuous exercise or burst swimming (associated with foraging or escape from predators; Driedzic and Kiceniuk, 1976; Kieffer, 2000). The significant negative relationship between lactate and fish size when stressed are consistent with findings reported in other studies (Ferguson et al., 1993) which have been linked to higher glycogen stores in larger fish. Lactate significantly increased with time between angling and processing in Lake

Trout, even within 4–10 minutes, similar to other studies (<2 minutes to >6 minutes; Meka and McCormick, 2005). A lack of a significant lactate response is a good indication of a fish that did not experience an exhaustive event, such as chasing, as was observed in the case of White Sucker used in this study (Wood et al., 1983).

The amount of time it took for Lake Trout to have blood taken during processing had no significant influence on glucose, potassium, or Ca but significantly influenced the blood parameters for lactate and sodium. Lactate is more often associated with physiological change due to intensive activity (burst swimming; Wells and Baldwin, 2006) and a significant decrease in lactate over time (due to recovery) would be expected during confinement, but was only seen in the angling treatment. Some fish being overly active in bins, which was observed in certain individuals, may partly explain why lactate increased over time during confinement in bins. Lake Trout sodium over time appeared to decrease in angling and gillnet captured Lake Trout which can be interpreted as increasing stress (McDonald, and Milligan 1997; McDonald and Robinson 1993; McDonald and Milligan, 1997; Hruska et al., 2010), but sodium increased in trap net captured fish. This trend is primarily driven by two Lake Trout with the lowest sodium results who were sampled at the beginning and near the end of sampling days at their respective lakes. The higher levels of lactate and lower levels of sodium found especially at earlier times was likely due to the original methods of capture and confinement (in the case of gill captured fish being held in pens). Results emphasize the importance of either minimizing or recording times between capture and handling in the field so can be controlled for statistically or the effect can be evaluated

Based on similar directions of impacts of stress on Lake Trout blood chemistry observed in the literature, I believe that the observations here based on physiological change induced by capture methods (gillnetting and trap-netting) can be extrapolated to other impacts causing similar alterations in blood chemistry that are often associated with negative environmental exposures. I expected that Lake Trout experiencing angling, short set gill-netting and confinement, or trap-netting capture all experienced varying degrees of air exposure, handling time, and confinement which are known to alter my suite of measured parameters (lactate, haematocrit, glucose, sodium, ionized calcium; Pickering et al., 1982; Edsall 1999; Barton 2000; Clements et al., 2002; Hruska et al., 2010; Clark et al., 2011; Donaldson et al., 2014; Sopinka et al., 2016). The blood glucose of salmonids has been shown to almost always increase in the presence of chemical contaminants or acidic conditions, taking into account the magnitude of contaminant exposure, intrinsic or extrinsic presents of contaminants, and delays in reactions (Zbanyszek and Smith, 1984; Goss and Wood, 1988; Aabel and Jarvi, 1990; Folmar 1993; Alkindi et al., 1996; Barton 1997; Begg and Pankhurst, 2004; Martínez-Porchas et al., 2009). Sodium has been shown to usually decrease in freshwater fish with exposure to acid, and other pollutants, and rapidly increase when exposed to bitumen (Morrow, 1974; Engelhardt et al., 1981; Baklien et al., 1986; Folmar, 1993; Goss and Wood, 1988). Fish hematocrit increases with copper, chromium, bitumen and acid exposure, with some exceptions (McCain et al., 1978; Goss and Wood, 1988), and decreases with cadmium and pulp effluent (McCain et al., 1978; Zbanyszek and Smith, 1984; Folmar, 1993). The cause of the physiological changes in this study compared to oil spills or pollutants may be different, but the direction of trends or significant changes seen in these blood

parameters in my study similar to that of published responses to environmental stressors provides support for the potential application of this tool to investigate environmentally-related physiological changes.

Results reported here support the validity of angled fish being a reliable relative baseline for most of my measured parameters. Though angling data was variable due to the time of sampling occurring 4–35 minutes after angling, significant differences between this group and those in my imposed stressor categories were significant for most parameters when controlling for this delay (or in several cases confinement time was found to not explain a significant component of variation). In another study, six freshwater species had blood metrics tested where haematocrit and lactate were elevated after ~2–5 minutes after stress, and glucose remained stable up to 3 minutes after stress events (Lawrence et al., 2018). However, some angling struggles (less than one minute), and other stressful events associated with captures such as air exposure and transportation stress cannot be avoided. Although my baseline group was variable, my mean values of parameters increased in most cases due to other stress treatments (or decreased in regard to sodium) despite this variability, as has been observed in other studies with less variable baselines (Graham, 1983; Olsen et al., 2005). Increasing the time of angling events (e.g., time between capture and drawing of blood) has been shown to increase haematological parameters which have been shown in other studies (Wydoski et al., 1976; Beggs et al., 1980), which is why this was minimized in the current study.

For most stress-relevant blood parameters observed in this study, the range of values expected for Lake Trout and other salmonids were well within the ranges of the i-STAT (excluding potassium; Table 3.2). The i-STAT is susceptible to cartridge or device

failures and the range of the blood parameters may not encompass range values for all species. The failure rate when using the i-STAT in the current study was 10%, primarily from out of range temperatures, over or under filling (five failures) with other errors also contributing to failed cartridges (20 failures; Table A4). Other studies have shown that for several parameters (glucose, sodium, potassium, chloride, and haematocrit), all were reproducible even when compared among multiple i-STAT units (Bingham et al., 1999; Harrenstien et al., 2005). In the current study, many results when using the point of care device were below the lowest range of 2mmol/L for potassium (only 20/107 results succeeded). For chloride (17/107 succeeded), most values were above the maximum range of 140mmol/L. This also resulted in few anion gap results as this calculation depends on useable results for all of potassium, chloride, sodium, and bicarbonate. Most blood urea nitrogen values (BUN) were below their ranges of 3mg/dL respectively, but BUN values typically have a weak relationship with stress in fish (Adeogun et al., 2020; Dawood et al., 2017). Creatinine was also consistently below the standard operating range of the i-STAT.

The results of this research may also help provide a better understanding of blood chemistry in Lake Trout blood during spawning, and how males' and females' reactions to environmental stress may differ. Males had higher blood chemistry values changes across several response variables (lactate, haematocrit, and glucose), compared to females, suggesting males experienced greater changes to physiological status during pre-spawning. The difference in stress indicators between fish sexes is thought to result from a higher metabolic (Raizada et al., 1983) and physiological activity (Rao et al., 1989) in males compared to female fish. Blood lactate values for trap-netted females were much

lower compared to trap-netted males, so much so that trap netted females showed no significant difference compared to angled (baseline) males or females. In other studies examining both Lake Trout (Edsall, 1999) and other salmonid species (Some during spawning; Clark et al., 2011; Charoo at al., 2013; Donaldson et al., 2014; Hruska et al., 2010; Sopinka et al., 2016), glucose was found to be higher in females compared to males. No significant difference was seen in this study between males and females for glucose at the 0.05 level, though visually the pattern appeared to show the opposite trend of previous research (i.e., higher in males vs. females). Female haematocrit showed no difference between baseline (angling) and imposed stress, however, time between capture and blood sampling showed that haematocrit was influenced by time between capture and blood drawing. As observed in my study, a previous evaluation across 53 freshwater and marine fish species found that haemoglobin was slightly higher in males than in females, particularly during periods of higher gonadal activity, such as spawning (Gelineo 1969). Studies on freshwater fishes' haematological parameters for *Alburnoides eichwaldii* (Kohanestani et al., 2013), *Gymnocypris eckloni* (Tang et al., 2015), *Synodontis membranacea* (Owalobi 2011), *Cyprinus carpio* (Baghizadeh and Khara 2015), and *Rhinogobio ventralis* (Zhao et al., 2018) all showed higher values of haemoglobin and percent haematocrit (excluding *Alburnoides eichwaldii* for haematocrit) in male compared to female fish (Ahmed et al., 2020), supporting the findings reported here for Lake Trout.

Future research on the field application of POCDs should seek its application in other broadly distributed boreal species and the application of other stressors such as toxic exposures or specific disruptive anthropogenic events. In particular, species that are

known to be sensitive to anthropogenic changes or those that are at a greater risk of extinction or extirpation and would benefit most from validated POCD approaches to have a rapid assessment tool available. Even though my understanding of how certain toxins affect certain species based on lab methods is arguably reasonably well developed, they are less well-develop for POCD devices such as the i-STAT, which have the potential to make assessments in a natural ecosystem more feasible. Future projects should also look to improve the i-STAT itself by potentially creating cartridges or algorithms programmed specifically for fish. The POCD can also be implemented as an important tool in assessing and improving fisheries practices as fish welfare can be observed in more remote areas, away from traditional laboratory bench equipment. Research should continue to explore fish stress and methods of evaluating stress to detect environmental impacts to be influence fisheries policy.

Tables

Table 3.1. Lake Trout baseline and stressors mean values

	Sex	**Baseline**	**Capture methods 1 and 2**

Sodium (mmol/L)	Male	150.9±14.5	144.6±12.5
	Female	148±8.5	146±11.5
Glucose (mg/dL)	Male	79±25.5	94.3±92
	Female	71.4±23.5	88.9±35.5
Haematocrit (%PCV)	Male	32.7±10	36.9±7.5
	Female	27.4±10	29±8.5
Lactate (mmol/L)	Male	4.5±2	7±4.4
	Female	3.8±1.4	5.5±3.9

Table 3.2. Lake Trout and other Salmonids range of blood parameters reported in the literature compared to reported i-STAT range.

	Literature Blood Parameter Range		**i-STAT Blood Parameter Range**	
Sodium (mmol/L)	135-170	(Rainbow Trout, *Oncorhynchus mykiss*; Wood et al., 1983)	100-180	(Abbott Point of Care, 2012)
Glucose (mg/dL)	64*-195*	(Edsall, 1999; Pottinger and Carrick, 1999)	20-700	
Haematocrit (%PCV)	29*-45*	(Jayaram and Beamish, 1992)	10-75	
Lactate (mmol/L)	0.3-18.6	(Atlantic salmon, *Salmo salar*; A. Foss et al., 2012)	0.3-20	
Ionized Calcium (mmol/L)	0.55*-2.2	(Edsall, 1999)	0.25-2.50	
Potassium (mmol/L)	2-8.2	(Rainbow Trout, *Oncorhynchus mykiss*; Wood, et al. 1983; Atlantic salmon, *Salmo salar*; Foss et al., 2007)	2-9	

* denotes the range was from a study on Lake Trout blood parameters

Figures.

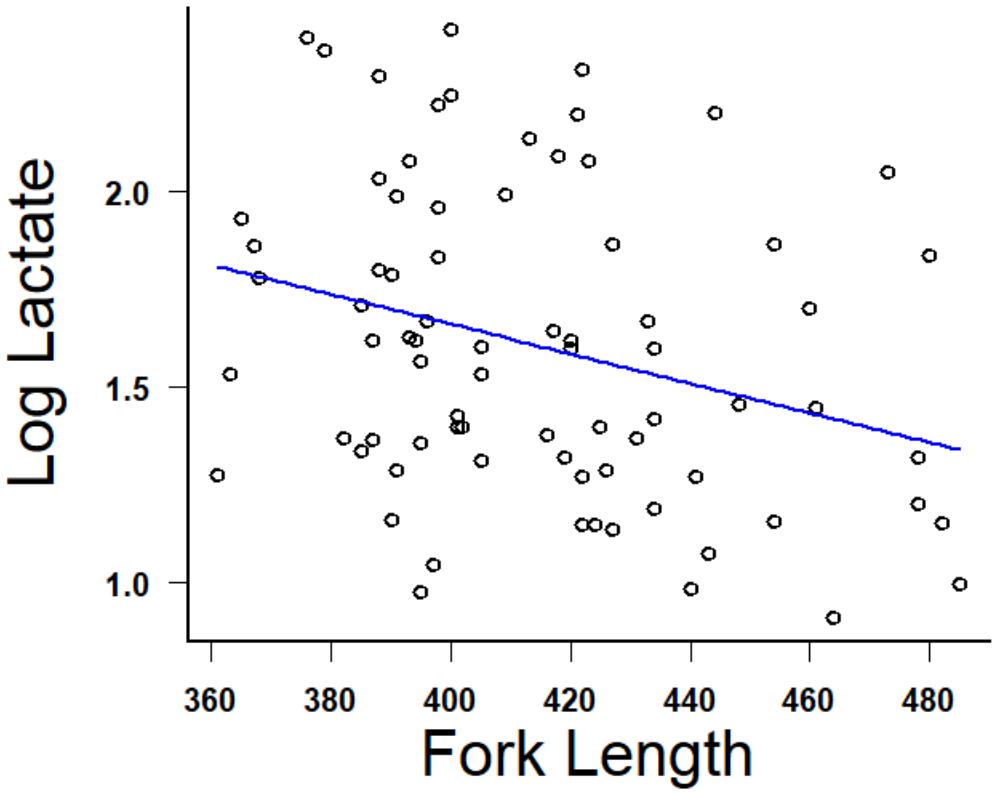

Figure 3.1. Linear regressions of blood lactate and fork length across all lakes and capture methods

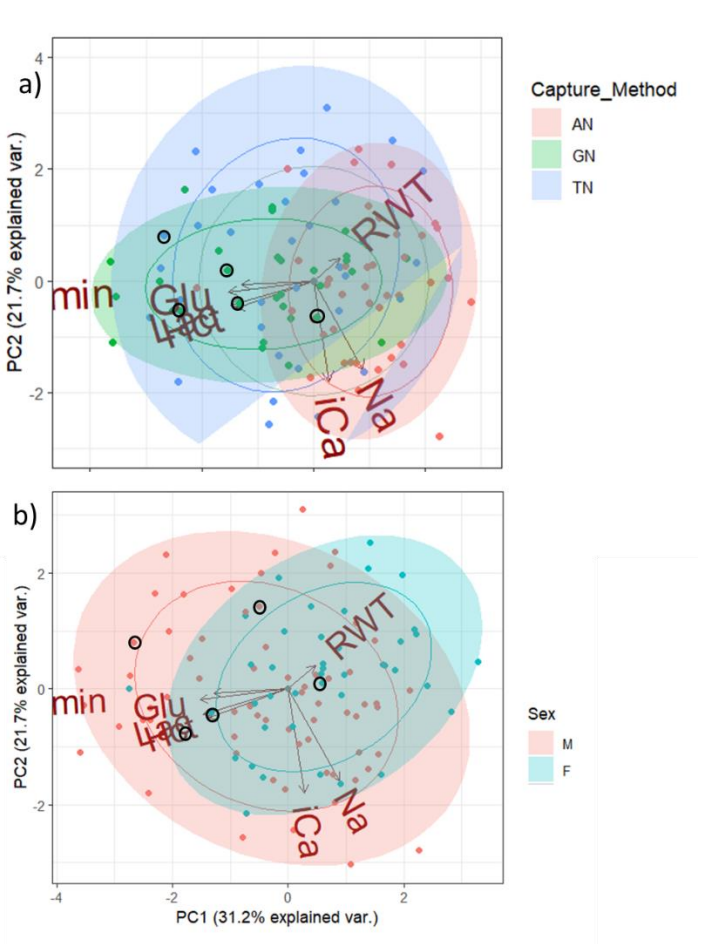

Figure 3.2. Principal component analysis of Lake Trout blood parameters, fork length and weight from the Point of Care i-STAT device grouped by gradient of physiological change (Panel a); AN = angling (pink, baseline stress), GN = gillnetting (green, high stress), and TN = trap netting (blue, chronic stress). Results of PCA showing grouping by sex (male, pink; female, green) appear in Panel b. Elipses are calcuated with a 95% confidance level. The black circles are the fish that underwent previous surgeries. Fish blood and physical parameters are Glu = glucose, Hct = Haematocrit, Lac = lactate, iCa = calcium, Na = sodium, Flen = fork length, RWT = round weight, and min = time since capture.

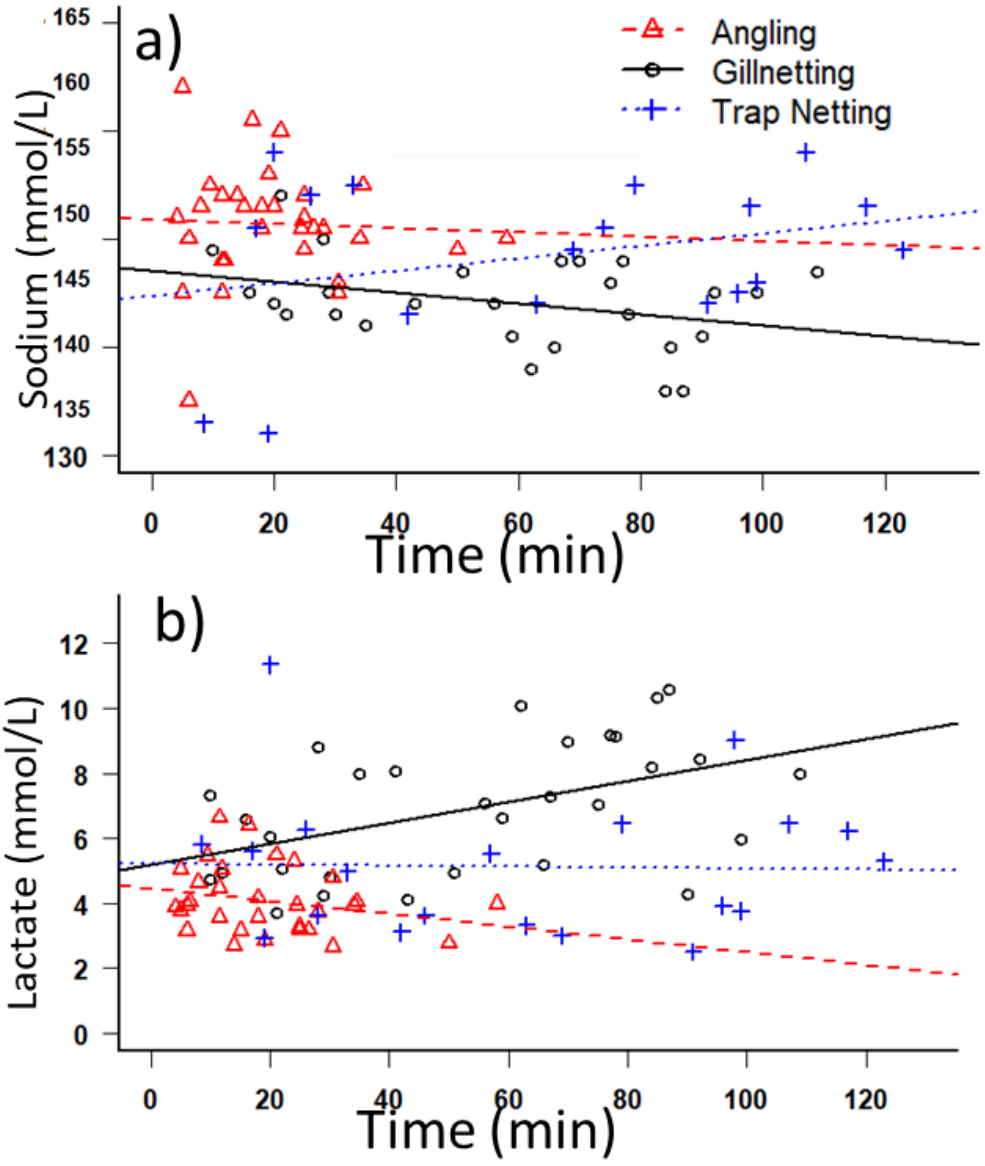

Figure 3.3. Plots of Lake Trout blood parameters over time-since-capture across a gradient of physiological change, obtained from the i-STAT point of care device. Gradient of physiological change from handling methods changed over time from baseline (angling), capture method 1 (gill netting), and capture method 2 (trap netting) across five experimental lakes. Panel a) is the change in sodium after capture Panel b) is the change in lactate after capture

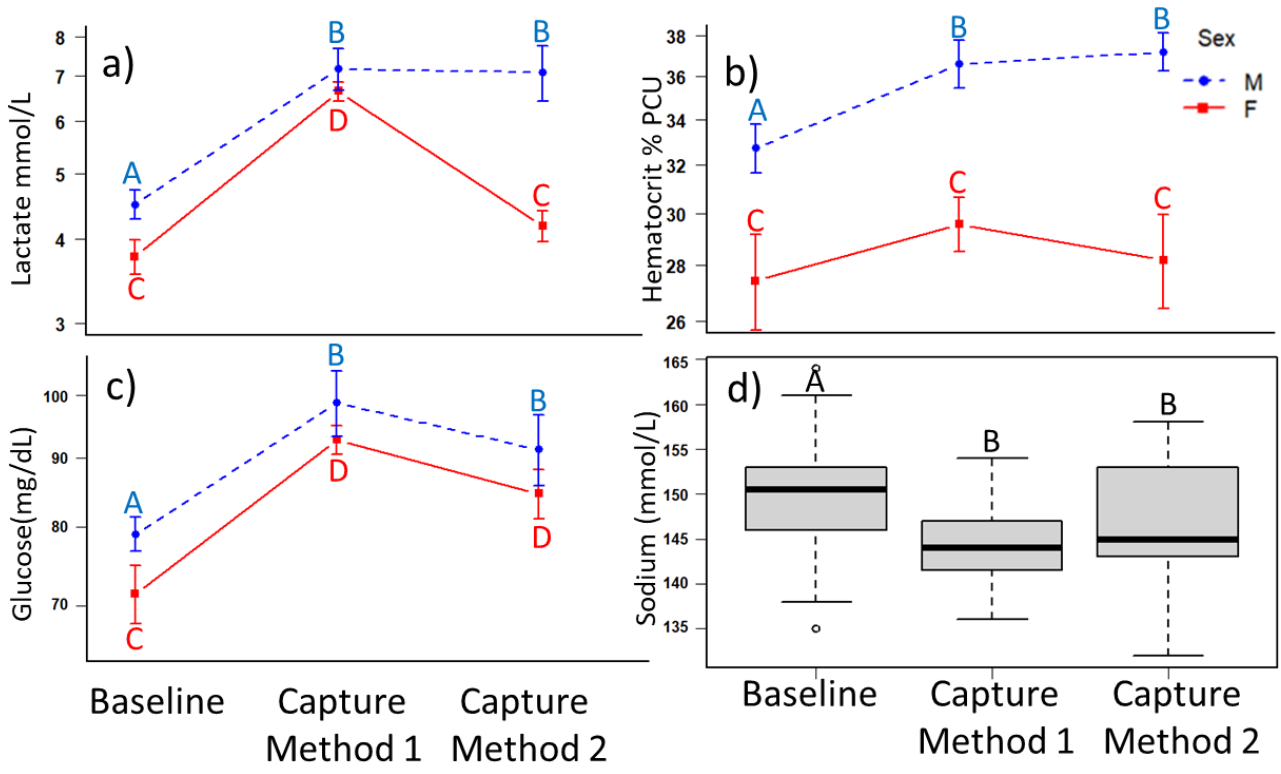

Figure 3.4. Interaction plots of Lake Trout blood across a gradient of physiological change, obtained from the i-STAT point of care device. Gradient of physiological change from handling methods increased from baseline (angling), capture method 1 (gill netting), and capture method 2 (trap netting) across five experimental lakes. Error bars are ±1 SE. Letters on figures refer to their differences determined by pairwise comparisons of means. Panel a) male and female Lake Trout across a gradient of physiological change for a) lactate, b) haematocrit, c) glucose d) sodium.

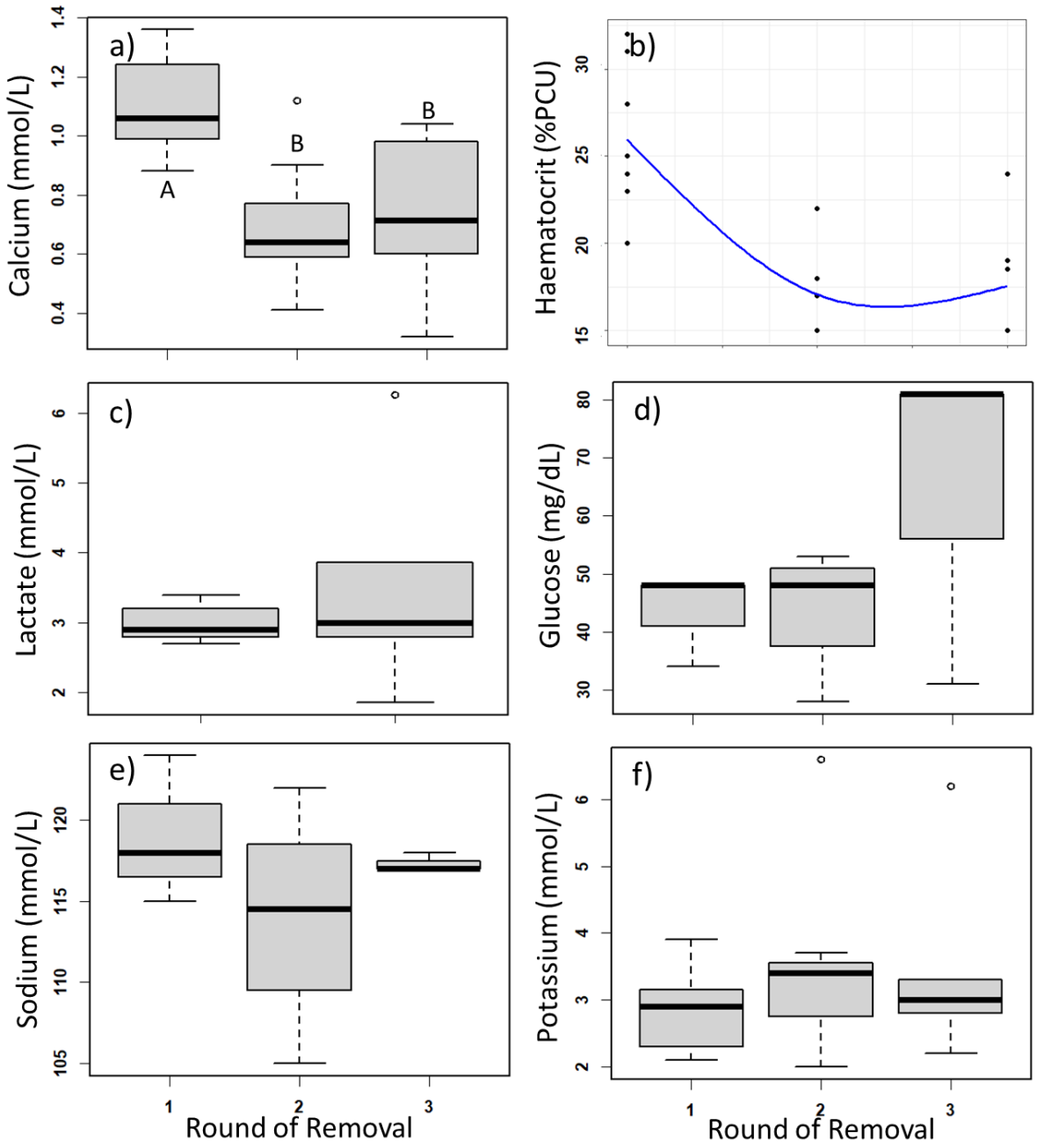

Figure 3.5. Box plots and GAMM of White Sucker blood across a gradient of physiological change, showing responses of (a), haematocrit (b), lactate (c), glucose (d), sodium (e) obtained from the i-STAT POCD. Gradient of physiological change from handling methods increased. Error bars are ±1 SE. Letters on a) refer to their differences determined by pairwise comparisons of means.

Chapter 4 Conclusion

The evaluation of the i-STAT 1 (hereafter referred toas the i-STAT or POCD) as a reliable POCD for determining fish blood parameters, specifically those related to changes in physiological status in a cold boreal setting provides the potential for it to be used as a tool for resource managers and researchers to make rapid assessments of fish health. The methods developed in this thesis demonstrate that the POCD can be used outside of its intended scope (<16°C), and may allow the device to be used in a wider range of environments and species. Specifically, this research will allow for the POCD to be used in Lake Trout with reliability assessments of physiological changes, with the potential to extend the relationships developed here to other charr species. The amount of time after capture influenced my results but did not eliminate trends created by my gradient of physiological change seen in the blood parameters

Tools like POCDs allow for the rapid health assessment of fish, particularly in examples of major impacts where immediate assessment is not otherwise feasible (e.g., spills of oil or mining tailings in their environment). These assessments at an individual level can potentially give insight into larger-scale impacts that have not yet presented themselves (Palace et al., 2009; Kidd et al., 2007; Kilgour et al., 2007; Kidd et al., 2014). With the expansion of oil pipelines and mining projects in boreal regions, a tool such as this would be useful to properly assess and mitigate damages (Wells et al., 2020; Willow, 2016).

The blood parameters of both Lake Trout and White Sucker were shown to be accurate when compared to assay and ICP analysis for both glucose and sodium, respectively. Lactate from the POCD was accurate in Lake Trout blood samples but not

White Sucker blood, whereas potassium was the opposite (accurate for White Sucker but not Lake Trout). This is the first time that lactate has been validated using this POCD on a ray-finned fish species (Lake Trout), and the first time glucose and sodium have been validated in a Salmoniform species in its natural setting using this POCD. This is also the first time this glucose, sodium, and potassium have been validated in a Cypriniform species. The POCD was shown to consistently undervalue calcium results compared to ICP results which is in agreement with other research using this POCD on Atlantic Cod (Borissov et al. 2019).

My study found the POCD to be an effective tool for evaluating changes in physiological status in Lake Trout, but I was not able to validate POCD for use in White Sucker. I expected White Sucker blood parameters to be similar to those of wild-caught ELA Lake Trout, in terms of changes in blood parameter values over imposed gradients of physiological change. The decrease in haematocrit and calcium observed in White Sucker are however not what would have been expected in a physiological response. Likely, the cumulative effect of chasing fish (before moving to be anesthetized) over time was not enough to elicit a physiological reaction from White Sucker, or fish were anesthetized before a physiological reaction could be detected in the blood.

While my research demonstrated that the i-STAT can be a reliable tool for assessing a variety of Lake Trout and White Sucker blood parameters, it may not be the most cost-effective method of collecting these data as the i-STAT ($3500+) and cartridges (CG4+ $259.58/10 pack, CG8+ $564.51/25 pack, CHEM8+ $330.75/10 pack) are considered expensive. Alternative, more affordable options might be a combination of methods to achieve this suite of parameters such as POCDs that are specific for a single

parameter, such as glucose ($30+ for the POCD and strips <$1) and lactate ($285+), microhematocrit tubes ($30+ and centrifuge $500+) and professional lab analysis for sodium, calcium, and potassium ($4.25 per samples for multiple ions tested). These methods require an estimated total of 150-400μL (depending on the methods of analysis blood) which is a greater volume required than the i-STAT, but an attainable amount for many large-bodied species. However, the advantage of this POCD is to obtain a more consolidated method for running these tests (several parameters on a single cartridge) with a small volume of blood (~90μL) and is therefore a more convenient method for blood assessment.

My research sought to determine what factors may influence the blood parameters of spawning Lake Trout when experiencing a gradient of physiological change. However, sex, size, and duration of capture were all also determined to influence these parameters. Sex was a significant additive factor in Lake Trout blood glucose, lactate and haematocrit, showing males consistently had higher values for all parameters across the gradient. This was likely due to male Lake Trout arriving at spawning shoals first, and having a generally higher metabolic rate (Raizada et al., 1983) and physiological activity (Rao et al., 1989) compared to females. This also suggests that females may be more resilient to induced physiological changes than males, as female LT showed more muted changes in lactate or haematocrit compared to males across the imposed gradient of physiological change. This finding indicates that the sex ratio of the fish being analyzed should be noted when researching blood, as different hormones and activity in the opposite sex could influence conclusions (Gelineo, 1969).

Time between capture and analysis of blood was also shown to have a significant effect on lactate, sodium, haematocrit (angling only), and glucose (angling only) values, which likely contributed to variation in baseline measurements of blood parameters for comparison to physiological changes. This is likely due to a continual increase of blood parameters during an ongoing physiological change(Cooke et al., 2008; Lawrence et al., 2018). Even with this added variability in my baseline (angling) data, there was still a significant gradient of physiological change found for most blood parameters as mentioned. This finding highlights the importance of sampling fish promptly with limited handling. The length of Lake Trout was also found to negatively change the values of lactate, something that should be taken into consideration when comparing populations or groups. Understanding what factors can influence blood parameters as illustrated here are important components of data interpretation and should be considered in future studies.

Blood parameters for Lake Trout (glucose, sodium, lactate, and calcium) were determined to be stable for up to four hours, when kept cool in a field setting and up to three hours in a laboratory setting. Therefore, if there is a delay in analyzing or centrifuging blood, my study shows that reliable results without a major change for up to four hours for glucose, lactate, sodium (wild-caught trout), and potassium (lab-raised). Glucose, lactate, and calcium appeared to shift less erratically and showed less variability in lab-sourced fish compared to lake-sourced fish, indicating blood from fish held in the lab may be more stable compared to wild-caught fish. This was not found however for haematocrit, which rapidly destabilized (1.5%PCV/hour in lab-sourced trout and 2.16%PCV/hour in field-sourced trout). Blood stability in laboratory settings has been well established (Korcock et al., 1988; Tavares-Dias and Sandrim, 1998; Fazio et al.,

2017; Agina et al., 2020); however, this study contributes novel information on the stability of blood samples when taken from wild-caught individuals, held and transported in the field.

Future research should look to validate this and other POCDs in a variety of species and environments to expand researchers' and managers' capabilities in remote and impacted environments. Other potentially more cost-effective POCDs (lactose meter, glucose meter, haematocrit tubes, external lab analysis) should also be compared to this POCD and traditional when considering use as other devices may be more inexpensive, even if multiple devices are required. This POCD can be further improved if work is done to develop a fish-specific algorithm or cartridge that can include important biomarkers (glucose, lactate, haematocrit, sodium, potassium, and pH) and calibrate to fish haematocrit levels. Making sure a sufficient sample size is secured is strongly recommended, as my White Sucker lactate results may have shown a better relationship with greater a sample size. My results show that this and potentially other portable POCDs have potential for use in the evaluation of animal welfare and environmental monitoring, and therefore, should continue to be evaluated for reliability to influence environmental and natural resources policy.

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Appendix

Table A2.1. Results from the proportional increasing of samples was tested by determining Lake Trout plasma samples quantity (2.5 µL, 5 µL, 10 µL) produced proportionally increasing absorbance values.

	2.5 µL	**5 µL**	**10 µL**
Lake Trout 15 Lake 626	0.53 a.u.	4.44 a.u.	4.20 a.u.
Lake Trout 4 Lake 626	0.57 a.u.	3.97 a.u.	3.61 a.u.
Lake Trout 3 Lake 626	0.87 a.u.	5.47 a.u.	3.84 a.u.

Table A2.2. Results from proportional recovery of sample using 5µL of Lake Trout plasma and 5µL of lactate standard, subtracted by a 5µL of Lake Trout plasma and 5µL of water solution.

	Duplicate 1	**Duplicate 2**
Lake Trout 15 Lake 626	2.409983 a.u.	2.351337 a.u.
Lake Trout 4 Lake 626	2.359715 a.u.	2.518897 a.u.
Lake Trout 3 Lake 626	3.459328 a.u.	3.068913 a.u.

Table A2.3. Sex distribution of fish collected from ELA lakes via different capture methods.

Lake Number	Angling (F:M)	Trap Netting (F:M)	Gill Netting (F:M)	Total Fish/Lake
223	4:5	2:5	4:6	26
224	2:8	1:9	0:0	20
260	2:7	5:6	0:0	20
373	1:4	0:0	5:5	15
626	6:4	4:3	5:5	27
Total	15:28	12:23	14:16	41:66

Table A3.1. Errors that occurred during IISD ELA Lake Trout field sampling and errors description. Bolded numbers are the ones that were displayed on the i-STAT

Error Number	Error Frequency	Error Description
31, 34, 44	3	**Unable to Position Sample / Use Another Cartridge.** The analyzer did not detect movement of sample across the sensors. This could be due to a clot in the sample (especially in neonates), to not closing the snap closure on the cartridge, or to an aberrant cartridge.
35, 36	3	**Sample Positioned Short of Fill Mark / Use Another Cartridge.** The cartridge was underfilled. The sample must reach the fill mark. Try another cartridge.
42, **43**	3	**Cartridge Error / Use Another Cartridge.** These codes indicate that the conductometric sensor (code 42) or the amperometric sensor (code 43) was out of specification. This could be caused by a pre-burst calibrant pack, dirty cartridge contact pads, or a dirty connector in the analyzer.
15	4	**Barcode Does Not Match Cartridge Type.** The barcode scanned by the user does not match the immunoassay cartridge type indicated by the identification chip in the cartridge. The user should run another cartridge, being careful to scan the barcode from the portion pack of the specific cartridge type being run on the analyzer.
20, **27**-29, 32, **33**, 40, **41**, 45, 87	6	**Cartridge Error / Use Another Cartridge.** These codes identify problems with the cartridge such as: calibrant fluid arriving too soon, too late, or not at all, or noise in the calibrant fluid signals. Codes 20, 27, 41, and 87 can be caused by poor contact that can sometimes be corrected by conditioning the pins in the analyzer using the ceramic conditioning cartridge.
79-81	1	**Cartridge Error / Use Another Cartridge.** Bad contact between the thermal probes in the analyzer and the metalization on the back of the chips in the cartridge trigger these codes. Causes are: poor metalization of the chips, dirt on the metalization, or bent or broken thermal probes in the analyzer.
21	2	**Cartridge Preburst / Use Another Cartridge.** This code indicates that the analyzer detected fluid on the sensors before it should have. Possible causes: mishandling of cartridges (putting pressure in the centre of the cartridge), poor storage conditions of cartridges (frozen), or rerunning used cartridges.
30, 37	2	**Sample Positioned Beyond Fill Mark / Use Another Cartridge.** The cartridge was overfilled. The sample was past the fill mark. Try another cartridge.
66	1	**Analyzer Error / See Manual**

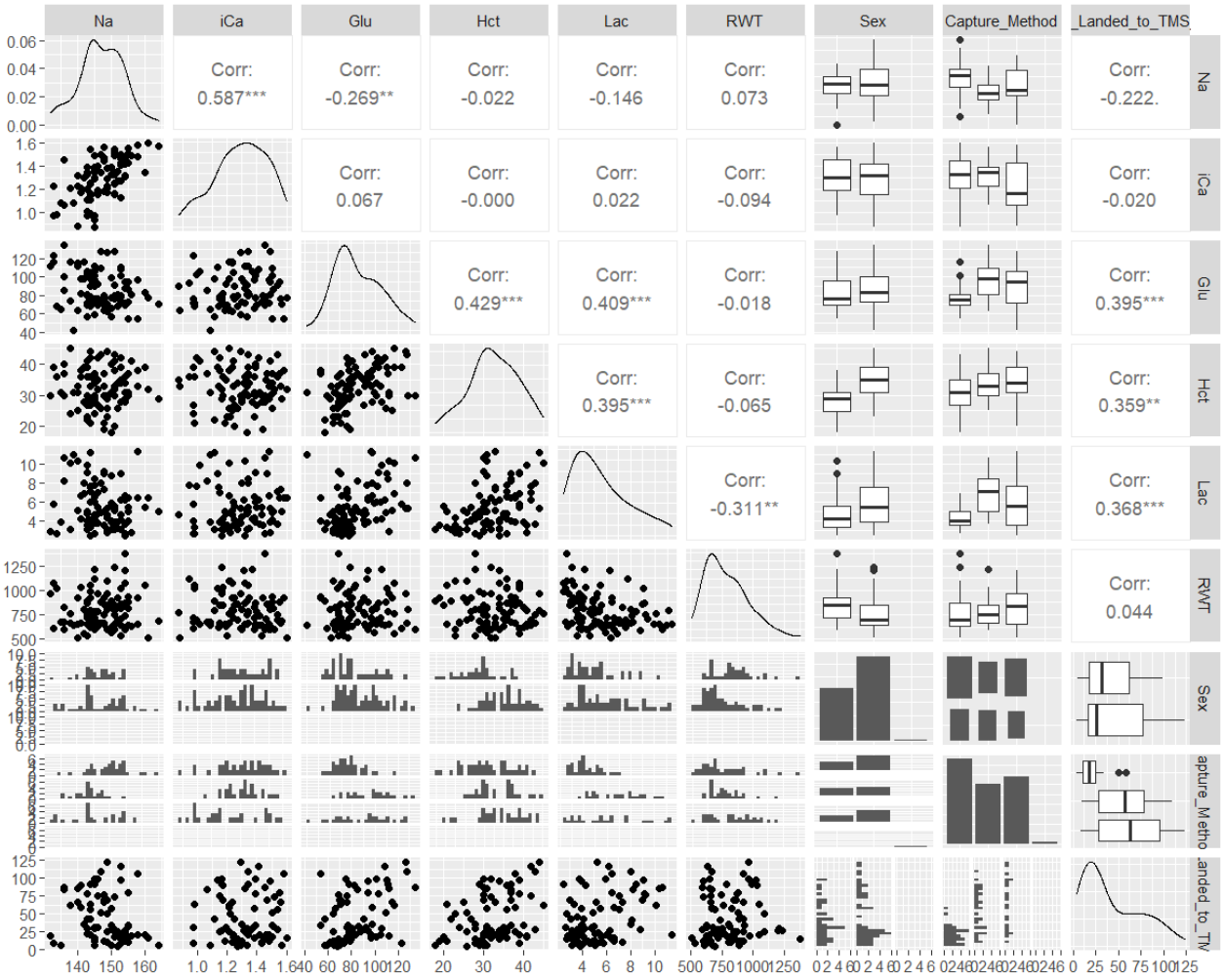

Figure A3.1. Correlogram among predictor variables. Na = sodium, iCa = calcium, Glu = glucose, Hct = haematocrit, Lac = lactate, RWT = weight, sex (Male or female), Capture_Method (angling, trap netting, gill netting), and landed_to_TMS = time-since-capture. Upper diagonal are correlation coefficients, boxplots on right and histograms on the bottom are the categorical parameters (sex and capture method) and numeric blood parameters, weight and length, scatterplots numeric parameters, and line graphs and bar graphs going top left to bottom right are parameters vs. themselves.

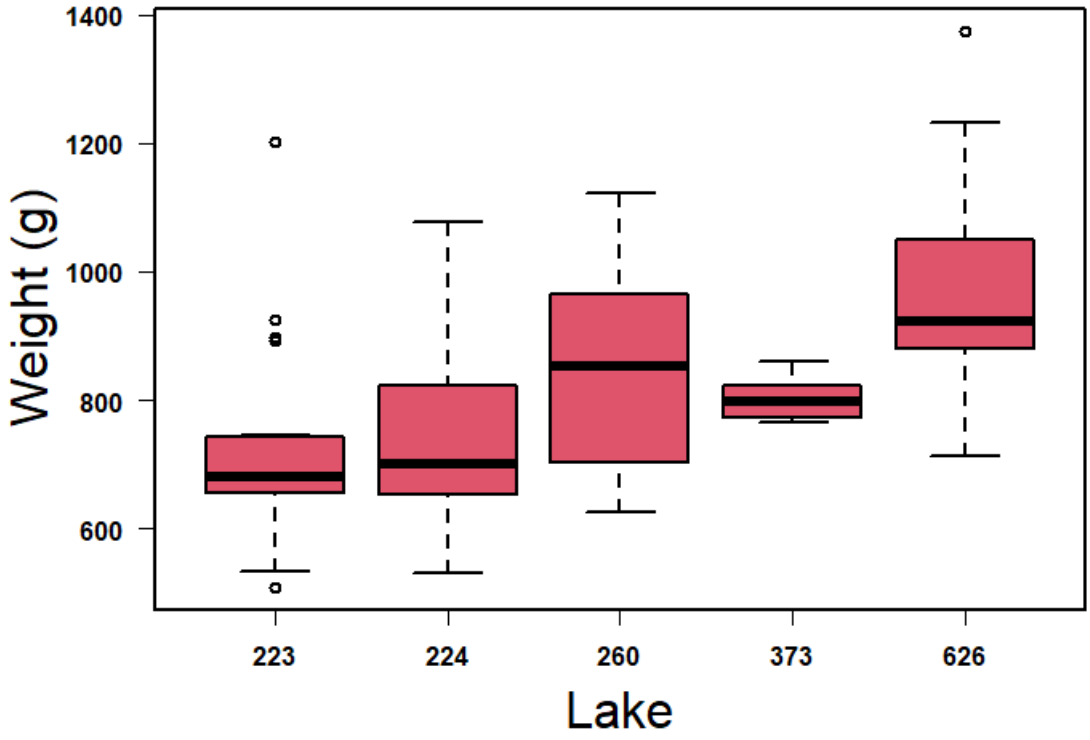

Figure A3.2. Boxplots of the mean weight of Lake Trout across lakes from which blood was drawn.

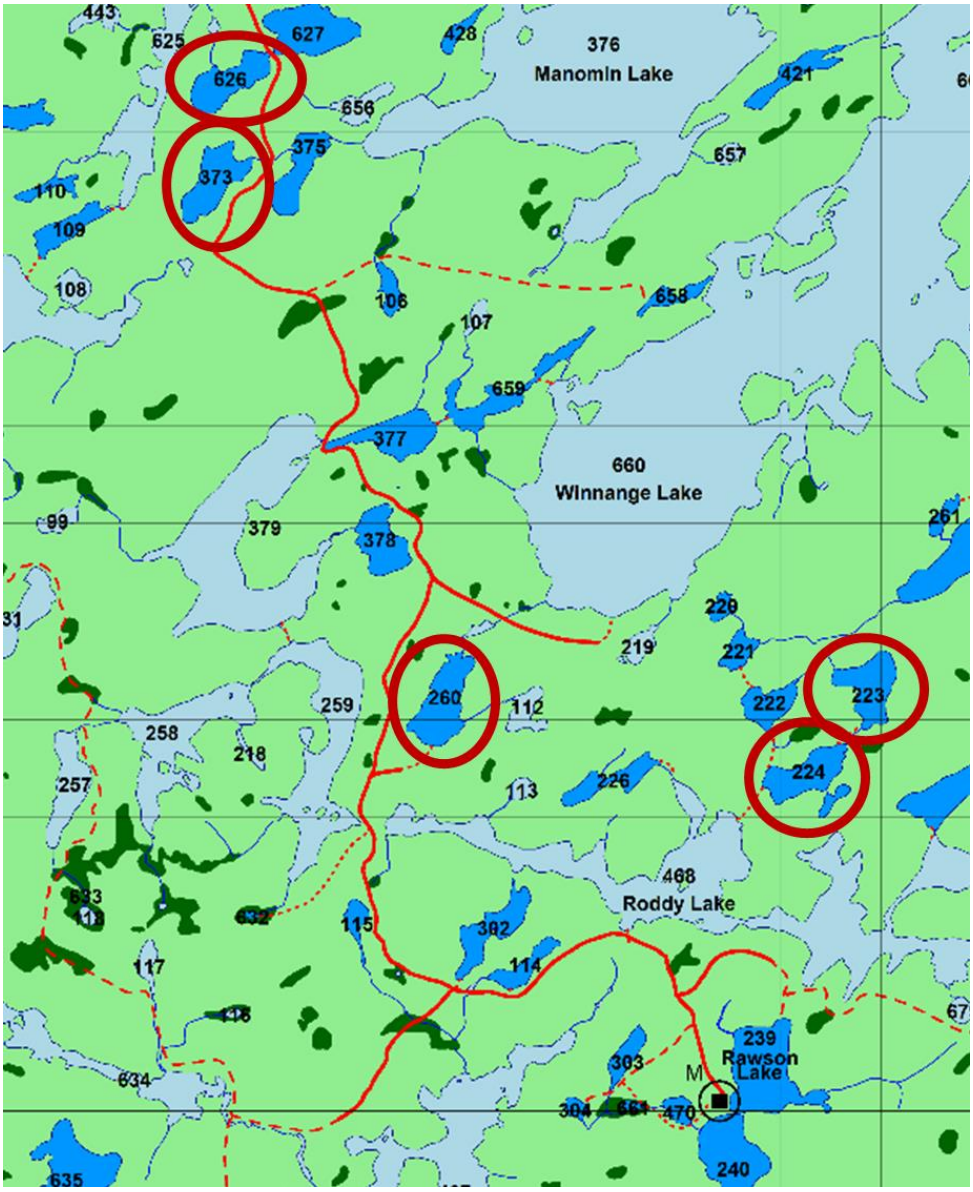

Figure A3.3. IISD ELA map of lakes sampled