

Anthropogenic degradation of endangered Coastrange Sculpin (*Cottus aleuticus*, Cultus Lake population) critical habitat: nutrient and climatic stresses threaten the persistence of multiple species at risk in Cultus Lake, British Columbia

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ABSTRACT

Loudon, K.L., Pon, L.B., Block, G.S., Lidin, G.W., and Selbie, D.T. 2022. Anthropogenic degradation of endangered Coastrange Sculpin (*Cottus aleuticus*, Cultus Lake population) critical habitat: nutrient and climatic stresses threaten the persistence of multiple species at risk in Cultus Lake, British Columbia. Can. Tech. Rep. Fish. Aquat. Sci. 3514: viii + 62 p.

Cultus Lake, British Columbia contains habitat that species at risk depend on for survival, yet is currently experiencing cultural eutrophication and climate-induced warming. In this study, assessments of lake water quality near the sediment-water interface, found hypoxic to near-anoxic conditions during the late-stratified period (i.e. late-summer to fall). Indicators of anaerobic microbial decomposition and internal loading were evident near the sediment-water interface, particularly in the deep profundal region of the lake. As well, high concentrations of fluoride, exceeding water quality guidelines, were observed near the sediment-water interface. Stratified minnow trapping, targeting Coastrange Sculpin (*Cottus aleuticus*, Cultus Lake population; *Endangered*, COSEWIC), otherwise known as Cultus Pygmy Sculpin, indicated extensive use of the profundal zone of the lake. Individuals were largely distributed between 30 and 40 m depth, with a propensity for deeper depths, except during late-lake-stratification (October and November) when the majority of the catch shifted to a depth of 30 m along the lake margin. Threats associated with declines in water quality not only impact the aquatic species and their habitat, but may also alter and shift species distribution to less suitable habitats, which for species that are already geographically restricted, can potentially increase the risk of extinction significantly. Lake management and watershed- to airshed-scale mitigation of nutrient loadings, coupled with further monitoring and research are therefore required and prescribed.

RÉSUMÉ

Loudon, K.L., Pon, L.B., Block, G.S., Lidin, G.W., and Selbie, D.T. 2022. Anthropogenic degradation of endangered Coastrange Sculpin (*Cottus aleuticus*, Cultus Lake population) critical habitat: nutrient and climatic stresses threaten the persistence of multiple species at risk in Cultus Lake, British Columbia. Can. Tech. Rep. Fish. Aquat. Sci. 3514: viii + 62 p.

Le lac Cultus, en Colombie-Britannique, abrite un habitat dont la survie des espèces en péril dépend, mais il subit actuellement une eutrophisation due aux cultures et un réchauffement induit par le climat. Dans la présente étude, les évaluations de la qualité de l'eau du lac près de l'interface sédiments-eau ont révélé des conditions hypoxiques à quasi-anoxiques pendant la fin de la période de stratification (c.-à-d. de la fin de l'été à l'automne). Des indicateurs de décomposition microbienne anaérobie et de charge interne étaient évidents près de l'interface sédiments-eau, en particulier dans la partie profonde du lac. De plus, on a observé des concentrations élevées de fluorure, dépassant les valeurs recommandées pour la qualité de l'eau, près de l'interface sédiments-eau. La pêche stratifiée au piège à méné ciblant le chabot de la chaîne côtière (*Cottus aleuticus*, population du lac Cultus; espèce en voie de disparition selon le COSEPAC), aussi appelé chabot pygmée, a révélé une utilisation extensive de la zone profonde du lac. Les individus étaient largement répartis entre 30 et 40 m de profondeur, avec une propension pour les profondeurs plus importantes, sauf pendant la fin de la période de stratification du lac (en octobre et novembre), lorsque la majorité des prises se sont déplacées vers une profondeur de 30 m le long du bord du lac. Les menaces associées au déclin de la qualité de l'eau n'ont pas seulement une incidence sur les espèces aquatiques et leur habitat, mais elles peuvent également modifier et déplacer la répartition des espèces vers des habitats moins adaptés, ce qui, pour les espèces restreintes sur le plan géographique, peut potentiellement augmenter le risque d'extinction de manière importante. La gestion des lacs et l'atténuation des charges en éléments nutritifs à l'échelle du bassin hydrographique ou du bassin atmosphérique, associées à une surveillance et à des recherches supplémentaires, sont donc nécessaires et prescrites.

INTRODUCTION

Cultus Lake, British Columbia is the sole freshwater habitat for two endemic species at risk of extinction in Canada. The Coastrange Sculpin (*Cottus aleuticus*, Cultus Lake population), otherwise known as the Cultus Pygmy Sculpin, was listed as *threatened* under Schedule 1 of Canada's *Species at Risk Act* (SARA 2002) in 2003. Subsequently, the Committee on the Status of Endangered Wildlife in Canada (COSEWIC) re-assessed Cultus Pygmy Sculpin as *Endangered* in 2019 due to its small distribution range and increasing threats to the species and its habitat. Due to the higher risk of extinction, a decision by Governor in Council to list Cultus Pygmy Sculpin as *Endangered* under Schedule 1 of SARA is currently pending. SARA contains provisions that allow for the protection of certain listed species at risk, their residences (i.e. dwelling places) as well as their critical habitat, or the habitat that is necessary for the survival or recovery of a wildlife species, on which they depend directly or indirectly in order to carry out their life processes (SARA 2002). Consequently, the whole of Cultus Lake up to its wetted boundary is identified as the geographic extent of the critical habitat for Cultus Pygmy Sculpin while their nests are considered residences (DFO 2017). Yet current, on-going threats are impacting both the species and its habitat, despite the legal protections afforded under SARA. This species also largely occupies the profundal and benthic zones of the lake (deep water and sediment-associated habitats) making forays into the upper water column to feed; Ricker 1960; McPhail 2007; Woodruff 2010). The highly-limited range of the Cultus Pygmy Sculpin makes it susceptible to regional anthropogenic stressors and global climate change, which are impacting Cultus Lake, critical habitat and residence for this species (Chiang et al. 2015; DFO 2017; Sumka 2017; Putt et al. 2019; Selbie et al. 2022).

Cultus Lake Sockeye Salmon (*Oncorhynchus nerka*, Cultus-L population) also spawn, incubate their eggs, and rear in Cultus Lake, spending approximately one half of their life histories within the system before emigrating to the Pacific Ocean to mature (Selbie et al. 2022). The Cultus Lake Sockeye Salmon population was first designated as *Endangered* by COSEWIC in 2002 in an emergency assessment (COSEWIC 2003), but was not listed under Schedule 1 of the *Species at Risk Act* (SARA 2002), due to potential socioeconomic consequences associated with the Fraser River mixed-stock fishery (SARA 2005). This status was re-examined and confirmed in 2003 and 2017, due to declining number of mature individuals and increasing threats to the species and the deterioration of its habitat within Cultus Lake. As a result, this population is under re-evaluation for listing under Schedule 1 of SARA by Governor in Council (Selbie et al. 2022). Cultus Lake Sockeye Salmon undergo diel vertical migrations that span the entire water column during the open-water rearing phase (Selbie et al. 2022). Cultus Pygmy Sculpin and Sockeye Salmon share common freshwater habitat and exhibit a trophic overlap (Ricker 1960; COSEWIC 2010), with both species likely to be

deleteriously affected by ongoing water quality degradation, climate change, spread of invasive species, and associated effects on food webs and frequented habitats (i.e. the profundal zone; DFO 2017; Selbie et al. 2022).

Cultus Lake is warming in response to climate change (Shortreed 2007; Sumka 2017), and is subject to excess loadings of nitrogen and phosphorus from atmospheric deposition, agricultural land uses, septic leaching and migratory gull guano, amongst other sources (Putt et al. 2019; Selbie et al. 2022). Cultus Lake was historically oligotrophic (Shortreed 2007), but has been reclassified as oligo-mesotrophic with the potential to transition into a mesotrophic state in the near-term, should nutrient loadings not be abated (Putt et al. 2019). Seasonal depletion of hypolimnetic oxygen over the stratified period has doubled between the 1920–1930s and 2001–2003 (Shortreed 2007). Since 2003, seasonal hypolimnetic oxygen depletion has increased, resulting from deep aerobic decomposition of increasing autochthonous organic matter loads associated with eutrophication (Putt et al. 2019; Selbie et al. 2022). Hypoxic to anoxic conditions at the sediment-water interface not only impact fish survival directly, but can promote internal loading of nutrients and metals (Mortimer 1941, 1971) and can induce alternative anaerobic microbial processes (Wetzel 2001; Burgin and Hamilton 2007) that produce toxic by-products (Smith and Oseid 1972, Keimer et al. 1995). The deep profundal zone and sediment-water interface are the most frequented portions of the Cultus Pygmy Sculpin critical habitat. Similarly, Cultus Lake Sockeye Salmon reside near the lake sediments for long day-lit periods of their diel vertical migrations (Selbie et al. 2022). Cultus Lake sediments, in some cases, also exhibit concentrations of metals and organic pollutants that exceed the Canadian Council for the Ministers of Environment (CCME) guidelines for the protection of aquatic life (Tovey et al. 2008). Moreover, preliminary limnological evaluation indicates elevated profundal water concentrations of faunal toxicants (e.g. As, MeHg, Fe, Mn) late in the stratified period, some of which can induce interactive stresses for fish at low oxygen levels, increasing the chances of mortality (Siegel 2021; Selbie et al. 2022).

The maintenance of deep-water oxia and lake water quality that support functional lake ecosystem characteristics ensuring spawning, feeding and rearing of Cultus Pygmy Sculpin have been deemed core attributes and functions of their critical habitat, tied to survival (Chiang et al. 2015; DFO 2017). However, the spatial extent of seasonal deep-water oxygen depletion within Cultus Lake, the prevalence of anaerobic microbial activity at depth, and internal loading of nutrients and other sediment constituents into the water column have not been documented, and thus are key foci of this study. From 2012 to 2017, Fisheries and Ocean Canada (DFO) Lakes Research Program conducted integrated trapping and limnological monitoring, specifically designed to study Cultus Pygmy Sculpin and its critical habitat. This report synthesizes this monitoring and research, to better define spatiotemporal trends in Cultus Pygmy

Sculpin critical habitat use, assess potential degradation of its critical habitat over the late-stratified period, and characterize key stressors potentially limiting species at risk survival. Given shared use of the deep water zones of Cultus Lake, and links to survival, the findings of this study and recommendations for mitigation are directly relevant to the persistence of *Endangered* Cultus Lake Sockeye Salmon (Selbie et al. 2022).

Ongoing deleterious water quality changes within Cultus Lake will likely imperil both species at risk, should their ultimate drivers not be addressed (Putt et al. 2019). Our findings affirm the current whole-lake boundary of Cultus Pygmy Sculpin critical habitat (Chiang et al. 2015; DFO 2017) and provide new insights into the urgent necessity for management of nutrient loadings responsible for lake eutrophication to support the persistence of the lake's species at risk (see Putt et al. 2019; Selbie et al. 2022). Suggestions for future monitoring and research are proposed, as are general recommendations for improving land-use management within the Cultus Lake watershed, and management of the regionally-contaminated airshed.

MATERIALS AND METHODS

Study Area

Cultus Lake is a warm monomictic aquatic ecosystem, typically thermally stratified from late-March to mid-December each year (Shortreed 2007; Sumka 2017). The lake has a surface area of ~6.3 km², with a simple morphometry, characterized by a broad profundal region, steep sides, and a limited littoral area (12% of total surface area). The entire wetted area of the lake has been identified as critical habitat for Cultus Pygmy Sculpin (Chiang et al. 2015; DFO 2017) and is similarly used for spawning, incubation, and rearing by Cultus Lake Sockeye Salmon (Selbie et al. 2022). Cultus Lake has a mean depth of 31 m and maximum depth of 44 m, the latter occurring within a limited depression at the south end of the lake (Shortreed 2007). More than 70% of the littoral area is colonized by invasive Eurasian Watermilfoil (*Myriophyllum spicatum*; COSEWIC 2010; Chiang et al. 2015). Cultus Lake exhibits a trophic status that is classified within the upper oligotrophic and lower mesotrophic range, based upon total phosphorus concentrations, and is actively undergoing cultural eutrophication from several identified sources of nutrient loading (Shortreed 2007; Putt et al. 2019; Selbie et al. 2022).

Cultus Lake is one of the most intensively-used recreational destinations in British Columbia's Lower Mainland, with annual visits exceeding 1.5–3 million, as recorded in 2003 (FVRD 2011). There are five major municipal and provincial campgrounds along the eastern shoreline with numerous day-use beaches, and residential communities along the northeastern and southern shorelines. The

campgrounds and residences rely largely upon within-catchment septic systems for sewage disposal (Urban Systems 2012).

The Cultus Lake watershed is ~69 km², and transboundary, with the majority (82%) contained within Canada (18% in Washington State, USA; Putt et al. 2019). Surface hydrology is dominated by eleven major tributaries, with additional substantial groundwater intrusions and contributions (Putt et al. 2019; Figure 1). Cultus Lake is bounded by International Ridge to the east, Vedder Mountain to the west, and the Smith Falls Creek sub-watershed to the northeast, which are underlain by sandstone and/or argillite shale (Putt et al. 2019). The Columbia Valley bounds the lake to the south, and is characterized by extensive agricultural lands and diffuse rural development. The Columbia Valley sub-watershed is situated upon glaciofluvial sand and gravel outwash sediments that are greater than 120 m deep (Zubel 2000, Putt et al. 2019) which form an unconfined aquifer spanning from near the US-Canada border to Cultus Lake (Zubel 2000). The majority of water flowing into Cultus Lake arises from the Columbia Valley, with 38% delivered as surface runoff and 9.5% as groundwater inflows (Putt et al. 2019). Frosst Creek, the largest tributary, flows through the Columbia Valley, and is seasonally heavily-influenced by agriculturally-contaminated groundwater (Zubel 2000; Putt et al. 2019). The second largest water source arises from the hydrology of International Ridge, which contributes 13.5% as surface runoff and 10.5% as groundwater inflow (Putt et al. 2019). The only surface outlet, Sweltzer Creek, is situated at the north end of the lake (Figure 1).

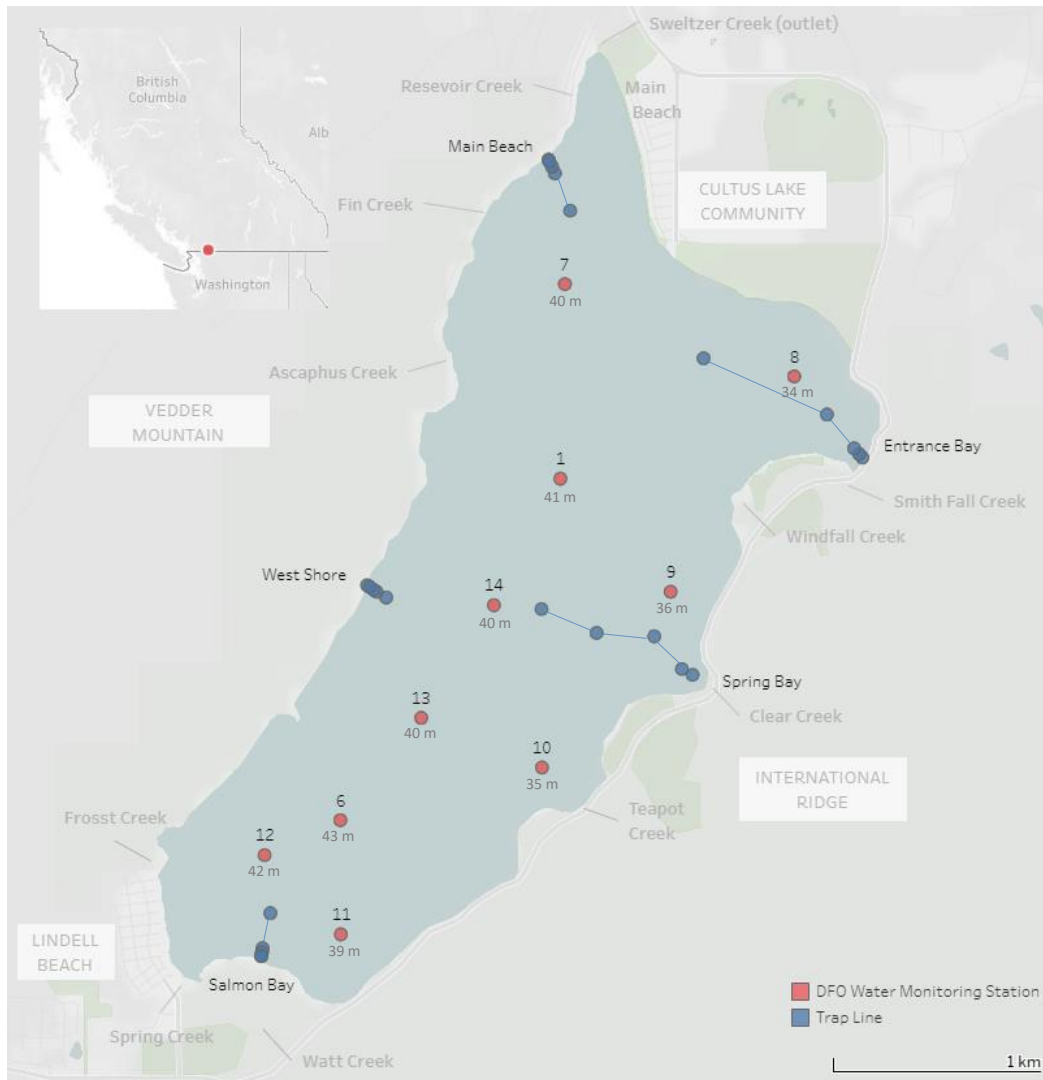


Figure 1. Map of Cultus Lake, British Columbia and surrounding lands and creeks (not full watershed boundary). Fisheries and Oceans Canada's Lakes Research Program water monitoring stations and associated depths, and minnow trap lines are shown. The sole outlet, Sweltzer Creek, is located at the north end of the lake.

Study Species

Coastrange Sculpin, Cultus Lake population (Cultus Pygmy Sculpin)

The Cultus Pygmy Sculpin is a neotenic (i.e. dwarf) form of the ‘typical’ Coastrange Sculpin (*Cottus aleuticus*, Gilbert), endemic and adapted to a lentic existence in Cultus Lake, BC, with divergent morphological features and life history traits (McPhail 2007). While ‘typical’ Coastrange Sculpin grow to more than 140 mm, Cultus Pygmy Sculpin only grow to ~65 mm (COSEWIC 2019). Unlike ‘typical’ stream-dwelling Coastrange Sculpin, the Cultus Pygmy Sculpin is believed to carry out its entire lifecycle within the wetted boundary of Cultus Lake, its critical habitat (Chiang et al. 2015; DFO 2017). Cultus Pygmy Sculpin mostly occupy the profundal and benthic zones of the lake (deep water and sediment-associated habitats), but also actively transit upward into the limnetic zone (upper water column) to feed on abundant zooplankton at night (Ricker 1960; McPhail 2007). The duration and frequency of these vertical movements by individuals is unknown, but like ‘typical’ Coastrange Sculpin, Cultus Pygmy Sculpin lack a swim bladder, making vertical movements, and specifically maintaining an upper water column position, energetically-costly. Although British Columbia has experienced repeated glacial advances and retreats, providing numerous opportunities for speciation, Cultus Pygmy Sculpin and parapatric ‘typical’ Coastrange Sculpin share a postglacial origin, and only recently diverged from one another within the last 8,000 to 10,000 years (Woodruff and Taylor 2013).

The Cultus Pygmy Sculpin was first discovered within the stomachs of lake piscivores in 1934, and while the known ecology of this species is limited, most biological information comes from two studies (Ricker 1960; Woodruff 2010), and recent monitoring by Fisheries and Oceans Canada’s (DFO) Lakes Research Program at the Cultus Lake Salmon Research Laboratory. Ricker (1960) developed a size-age classification matrix to confirm the dwarf morphology, documented historical predator-prey relationships, and approximated the ecological niche of Cultus Pygmy Sculpin based upon these characteristics.

The existence and continuance of nocturnal vertical feeding behaviours are supported by consistent incidental captures of Cultus Pygmy Sculpin within the pelagic zone during nighttime midwater trawls targeting juvenile Sockeye Salmon (COSEWIC 2010; DFO *unpublished data*). Genetic and behavioural studies conducted by Woodruff (2010) and Woodruff and Taylor (2013), support classification of the Cultus Pygmy Sculpin as an evolutionary Designatable Unit (DU), or population discrete from the ‘typical’ Coastrange Sculpin.

There are only a few other known occurrences of freshwater limnetic sculpin, including a population of vertically migrating dwarf pelagic sculpin within Lake

Washington, USA (*C. aleuticus*), a similar population in Lake Sammamish, USA (*C. aleuticus*), which is connected to Lake Washington by the Sammamish River (Larson and Brown 1975), and three species of highly-divergent and larger pelagic sculpin within Lake Baikal, Russia (*Comephorus baikalensis*, Pallas, *Comephorus dybowskii*, Korotneff, and *Cottocomephorus inermis*, Yakovlev; Kontula et al. 2003). Limnetic neotenic Coastrange Sculpin may also exist in Seton Lake, BC, within the Fraser River Basin, and Tuya Lake, BC, within the Stikine River drainage (COSEWIC 2010). Woodruff (2010) discovered genetic divergence between the Cultus and Washington populations, and although the selective forcings are unknown, this unusual limnetic life history strategy is considered a unique example of parallel evolution that may be ongoing (McPhail 2007). Large and fundamental gaps exist regarding the basic ecology of Cultus Pygmy Sculpin, including but not limited to spatiotemporal trends in critical habitat use, spawning and parenting behaviour, hypoxia tolerance, and population size and stability.

Cultus Lake Sockeye Salmon

Cultus Lake Sockeye Salmon (*Oncorhynchus nerka*, Cultus-L population) are a genetically distinct salmon Designatable Unit (DU) or population within the Fraser Watershed (COSEWIC 2017). As with other Fraser Sockeye populations, Cultus Sockeye Salmon are anadromous; rearing in freshwater for typically one year, migrating to sea, and returning in their fourth year of life to spawn and die (i.e. semelparity; Burgner 1991). Cultus Lake Sockeye Salmon spawn exclusively within the bounds of Cultus Lake in November and December (CSRT 2009). Upon hatching and emergence in spring, juveniles eventually make their way off-shore to rear in the limnetic zone of the lake. As is typical of Sockeye Salmon, Cultus fry exhibit diel vertical migration behaviour (Clark and Levy 1988); spending daylight hours in the darker, colder depths of the lake, and only moving up in the water column at night to feed on zooplankton (primarily *Daphnia* spp.; Shortreed 2007). In the following spring, most juveniles migrate to sea as age-1 smolts. Cultus Sockeye Salmon typically spend 2–3 years growing in the ocean before returning as adults to their natal freshwater spawning grounds.

Cultus Lake Sockeye Salmon are designated by COSEWIC as *Endangered* due to declining number of mature individuals and increasing threats to the species and the deterioration of its habitat within Cultus Lake (COSEWIC 2003; 2017; CSRT 2009). However, in the last two decades, numerous conservation efforts have been pursued including hatchery and broodstock supplementation, and predator control. Despite these conservation efforts however, Cultus Sockeye have continued to decline in abundance.

Water Quality Instrumental Sampling

Guided by long-term sampling of Cultus Lake by DFO's Lakes Research Program (Shortreed 2007; Selbie et al. 2022), spatio-temporally enhanced limnological sampling was conducted from August to December 2017, at 10 sites around the lake (DFO stations 1, 6–14; Figure 1). This work was conducted to constrain profundal lake habitat conditions during a seasonally-known period of hypolimnetic oxygen minima (Shortreed 2007; Putt et al. 2019). During each sampling event, at each site, a cage-mounted, multi-parameter YSI EXO2 sonde was lowered through the water column to a depth of ~1–1.5 m above the sediment-water interface to characterize the Cultus Lake water column. This effort produced depth-constrained profiles of instrument-inferred temperature, dissolved oxygen (DO), oxidation-reduction potential (ORP), pH, and specific conductance (normalized to 25 °C; Figure 1; Appendix 1). Sampling occurred one to two times per month during the stratified and turnover periods (late-summer and fall 2017 (August 25, September 11, October 6, October 27, November 8, December 1, and December 21, 2017). Sonde sensors were calibrated prior to each sampling event, following manufacturer-recommended sensor calibration, storage, and deployment methods (YSI 2018). The 'default' averaging setting was used, which recorded a rolling average over 5 to 40 seconds, with shorter duration averages automatically implemented when the sonde detected sharp gradients in the parameters being measured (YSI 2018). Additional deep dissolved oxygen measurements were made monthly at DFO Station 1 from January to August 2017 (Figure 1), using a YSI Pro optical dissolved oxygen meter, following methods detailed in Shortreed (2007). This broader yearly data provides important context for interpretation of the deep oxygen concentrations observed during the late-season sampling.

Water Chemistry Sampling And Analyses

Cultus Lake water samples were collected during each sampling event (August to December 2017) at three depths (surface, 30 m and ~1–1.5 m above the sediment-water interface) across ten DFO sampling stations (Figure 1). Water samples were collected via an acid-washed, ethanol-treated, lake water-rinsed Van Dorn sampler, and stored in acid-washed and deionized- and lake water-rinsed opaque, insulated 1 L polyethylene bottles. Replicate lake water quality samples were filtered into clean, rinsed polyethylene bottles or glass vials (analysis-dependent) using a Swinnex filtering unit, with pre-ashed Advantec glass fiber filters (GF-75; 0.3 µm pore size), and rinsed and flushed with deionized and sample water. Samples held in polyethylene bottles were frozen and the glass vials were refrigerated at 4°C until analysis. The samples were analysed for nitrate (NO_3^-), total ammonia-nitrogen ($\text{NH}_4^+ + \text{NH}_3$), sulfate (SO_4^{2-}), fluoride (F^-), chloride (Cl^-), manganese (Mn^{2+}), soluble reactive phosphorus (SRP) and

total dissolved phosphorus (TDP). Anions and cations were analysed via ion chromatography using a Dionex ICS-5000 ion chromatograph. TDP and SRP were analysed via a Seal AA3 AutoAnalyser, based upon the modified methods from Murphy and Riley (1962). For surface and 30 m samples, only one of the two analytical replicates was analysed, while both analytical replicates were analysed for deep samples, which showed a high-level of reproducibility across all analyses (< 1% mean variance).

Climate Data

Regional climate data were analysed for the purpose of placing 2017 air temperatures within historical climate trends and variability. In 2012, a Meteorological Service of Canada-DFO partnered climate station was installed at the Cultus Lake Salmon Research Laboratory by DFO's Lakes Research Program. Given the relatively short time series for evaluating trends and variability, 2012–2017 historical daily mean air temperatures from the Cultus Lake station were regressed against those of proximal meteorological stations at Abbotsford and Agassiz (Environment Canada 2018a). Strong positive daily air temperature relationships were established with the Cultus station (Agassiz, 2012–2017, $r^2 = 0.97$, $p < 0.0001$, $n = 1975$; Abbotsford, 2012–2017, $r^2 = 0.97$, $p < 0.0001$, $n = 1973$). Long-term homogenized surface air temperature data (1893 to 2016) were available at Agassiz, BC (Environment Canada 2018b), which provide surrogate long-term climate conditions for the Cultus Lake area.

At the time of analysis, the 2017 adjusted and homogenized climate data were not available from Environment Canada for use. In lieu, the monthly averages of mean daily air temperatures were calculated from the historical daily Cultus Lake meteorological station data, using the above relationship, and both seasonal and annual averages added to the existing adjusted and homogenized climate time series (i.e. 1893 to 2016). This data averaging method was tested on previous years, where both sets of data were available, and was demonstrated to be robust.

Cultus Pygmy Sculpin Trapping

An ongoing trapping program targeting Cultus Pygmy Sculpin was implemented by DFO's Lakes Research Program in May 2012, July and October 2014, July and October 2015, February, April and November 2016 and April 2017. Trapping did not occur during August or September, to avoid any potential prolonged exposure of fish to warm waters above the hypolimnion. Minnow (Gee) traps (~3 mm mesh size) were baited with commercial food-grade salmon meat and 12-hour glow sticks, as was

previously successful for Woodruff (2010). Traps were typically set out between mid-morning and early-afternoon of the first day and were collected approximately 20 hours later in the morning of the second day. This schedule allowed the traps to fish overnight, when Pygmy Sculpin are known to feed in the water column. Traps were set along five transects, from shore towards the centre of the lake until a depth of 40 m was reached. Trapping sites included Salmon Bay, the West Shore, the Main Beach area, Entrance Bay and Spring Bay (Figure 1, Appendix 1); however, the May 2012 trapping event did not include West Shore or Main Beach. Due to morphometric variation around the lake, transect lengths varied substantially. Traps were attached along each vertical line, ranged between 0 m and 40 m in depth (Figure 2). A total of 20 traps were deployed along each transect for a total of 60 to 100 traps in each trapping event.

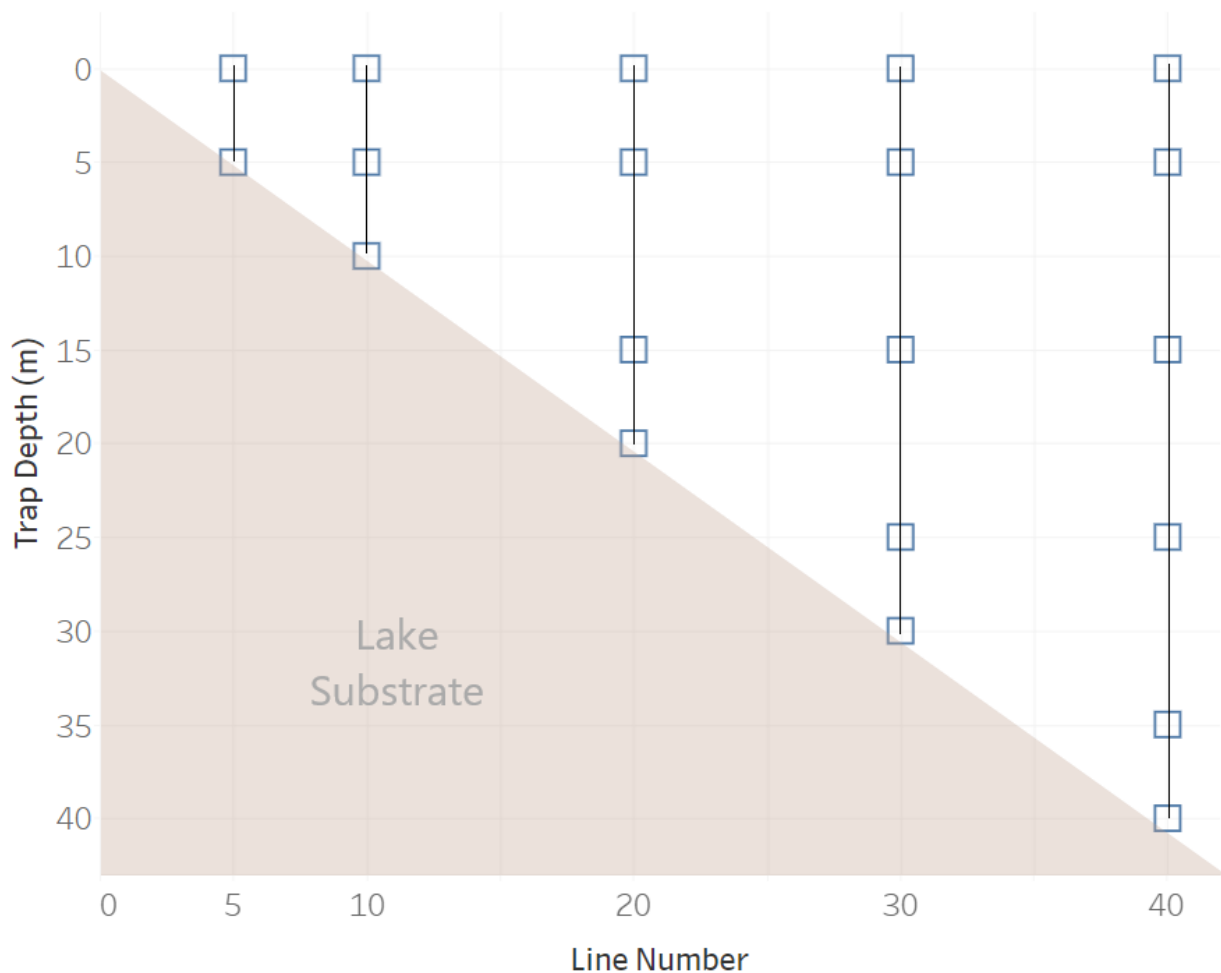


Figure 2. Conceptual cross section of the minnow trap arrangements along each sampling transect in Cultus Lake, ranging from the 5 m line (left) to the 40 m line (right). Each trap line consisted of a trap (represented by a blue square) at the surface and 5 m below the surface, at the substrate-water interface and 5 m above the substrate-water

interface, and, where applicable, at 15 and 25 m. Each transect included a total of 20 traps.

Sculpin Identification

Multiple sculpin species are known to inhabit Cultus Lake, including the Prickly Sculpin (*Cottus asper*), the stream-resident 'typical' Coastrange Sculpin (*Cottus aleuticus*, typical form), and the Cultus Pygmy Sculpin. 'Typical' Coastrange Sculpin may periodically be found within Cultus Lake, possibly seeking refuge near the shoreline in the summer when tributaries are dry or lacking flow (Woodruff 2010). Prickly sculpin, a piscivore and predator of Cultus Pygmy Sculpin, are believed to primarily occur within the littoral (Ricker 1960; Woodruff 2010) and benthic habitats of the lake (Woodruff 2010), congruent with our findings.

In order to minimize handling stress, fish captured in minnow traps were rapidly identified using morphological characteristics to the species level. Fish identified as Coastrange Sculpin were assumed to be Cultus Pygmy Sculpins based on the findings of Woodruff and Taylor (2013), which noted that Coastrange Sculpin captured from tributaries of Cultus Lake were genetically distinct from those trapped within the lake (i.e. Cultus Pygmy Sculpin). At lengths below 25 mm, sculpin could not always be visually confirmed as either Coastrange or Prickly sculpin. Therefore, sculpin under 25 mm were classified as 'juvenile sculpin'. According to the size-age matrix developed by Ricker (1960), Cultus Pygmy Sculpin of 25 mm or larger are likely older than 1 year.

RESULTS

Climate Data

Eastern Fraser Valley (Agassiz, BC) temperature data over the period 1890 to 2017 exhibit a long-term warming trend across all four seasons (Figure 3). In 2017, the region experienced a relatively warm summer and a cold winter, compared to historical trends (Figure 3). Spring air temperatures were 1.5 °C (\pm 1.0 °C) colder in 2017 than the previous four years. On an annual basis, 2017 was slightly colder than the trend and notably colder than the previous four years. As such, 2017 can be considered a relatively cold year within a long-term warming trend.

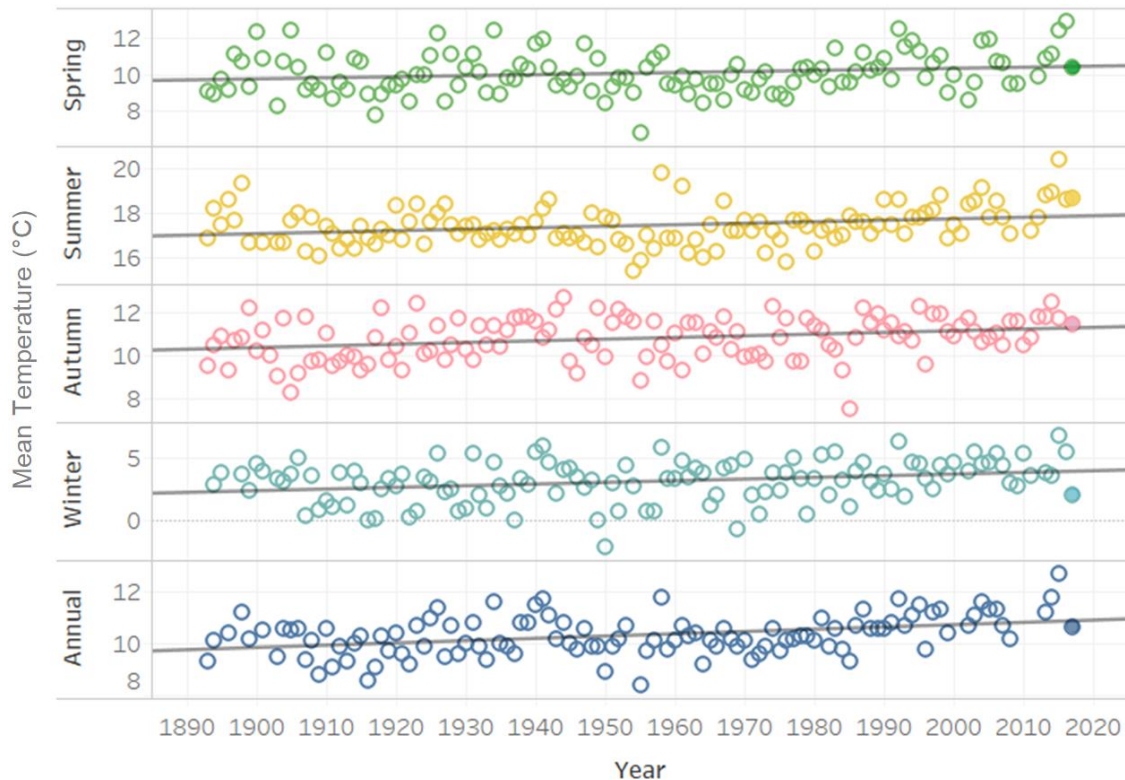


Figure 3. Monthly mean of daily mean temperatures at Agassiz from 1893 to 2016 (Environment Canada 2018b) and 2017 (Environment Canada 2018a). 2017 data are represented by solid dots.

2017 Late-Season Water Quality and Chemistry

Water Temperature

Cultus Lake water temperatures on August 25 and September 11, 2017 averaged 21.8 °C (± 0.2 SD) at the surface, 5.6 °C (± 0.1 SD) at 30 m, and 5.3 °C (± 0.1 SD) at 40 m, indicative of strong thermal stratification and a high level of hypolimnetic isolation from surface waters. Mean metalimnetic depths ranged from approximately 7 to 20 m depth over the sampling period, until lake destratification (Figure 4). Mean surface temperatures decreased to 16.9 °C (± 0.1 SD) by early-October 2017, 8.9 °C (± 0.04 SD) by early-November, and 7.6 °C (± 0.03 SD) by early-December 2017. The lake was fully mixed with an average temperature of 6.4 °C (± 0.04 SD) by December 21, 2017, verifying sampling occurred through lake turnover (Figure 4). Thermal profiles revealed little spatial variation between the ten DFO lake sampling stations, with the exception of November 8, 2017 (a particularly windy day) which exhibited pronounced variation across the metalimnion, likely the product of oscillatory vertical displacement associated with lake internal seiche (Figure 4).

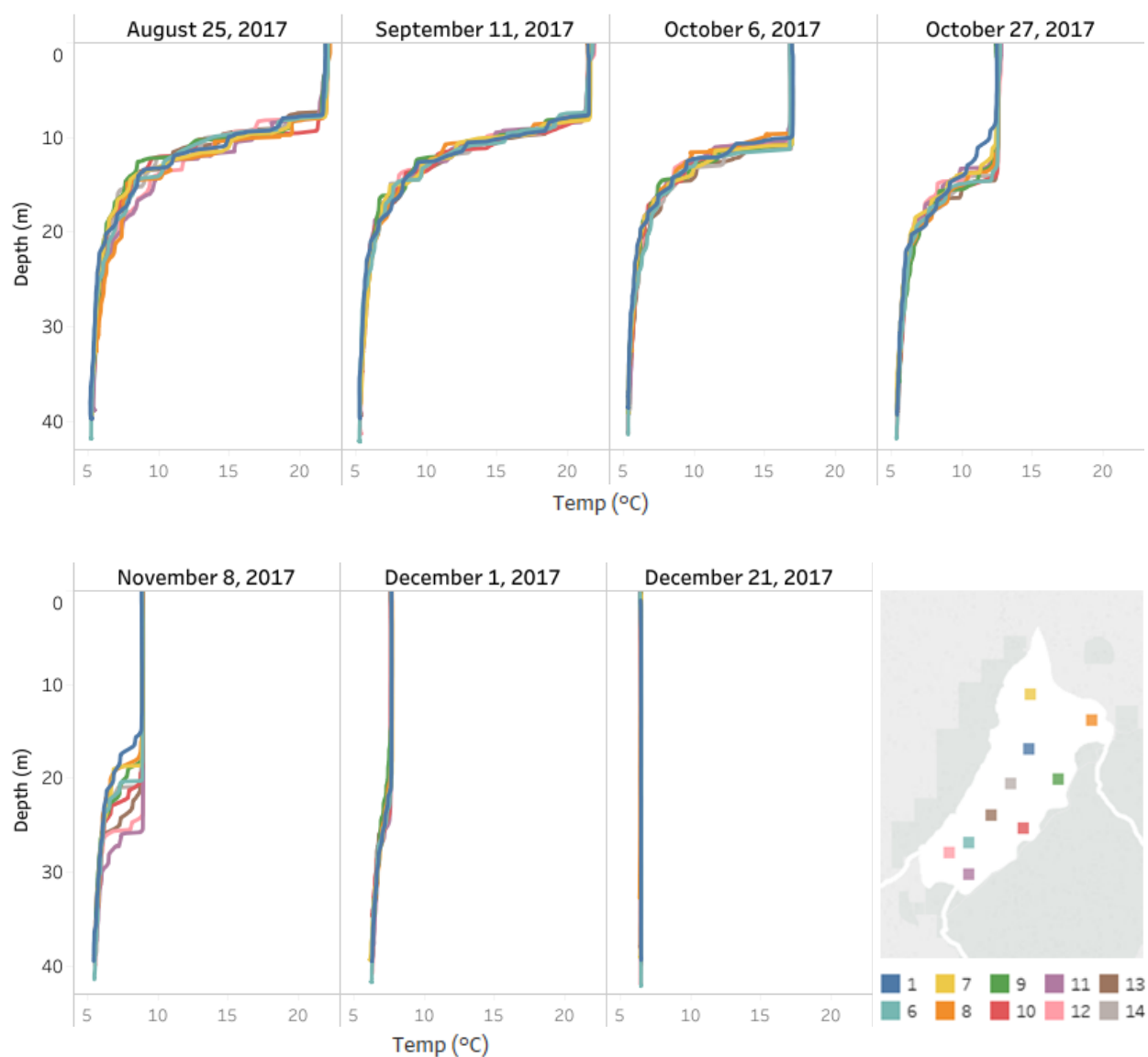


Figure 4. Depth profiles of water temperature for each sampling day in 2017, with each DFO Water Monitoring Station represented by a colour.

Dissolved Oxygen

The upper 30 m of the Cultus Lake water column remained well oxygenated throughout late-2017 (Figures 5 and 6). Dissolved oxygen (DO) concentrations remained above 9.6 mg/L at the surface and above 7.3 mg/L at 30 m throughout the 2017 late-season sampling period. When sampling commenced in August 2017, a deep chlorophyll maximum (DCM) was already well established, as inferred from pronounced DO concentrations spanning 7 to 22 m depth (Figure 5), and confirmed by growing

season chlorophyll *a* (algal surrogate) and photosynthetic rate (PR) profiles from Station 1 (data not shown). DCM effects on water column oxygen concentrations were weakened by late-October 2017 and were non-existent by November 2017.

2017 early-season deep water sampling at Station 1 revealed a slow decline in profundal oxygen concentrations, progressing from 10.7 mg/L DO in January to 5.1 mg/L DO in early-August. Our enhanced late-season sampling revealed significant oxygen depletion over the late-stratified period, across all ten DFO water quality sampling stations (Figure 6). Dissolved oxygen concentrations near the sediment-water interface were positively associated with the depth of the substrate at each station. Deeper profundal zone sites exhibited more pronounced oxygen depletion with the strongest oxy-spatial correlation occurring on September 11, 2017 ($n = 10$, $r^2 = 0.90$, $p < 0.0001$). When expanded late-season sampling commenced on August 25, 2017, profundal DO concentrations at Station 1 (40 m substrate depth) and Station 6 (deepest station; 43 m substrate depth) had already decreased to 3.3 and 0.76 mg/L DO, respectively, and four of the six stations with depths greater than 39 m had DO profiles exhibiting profundal hypoxic concentrations (< 5 mg/L DO; Figures 5 and 6). By October 6, 2017, eight of ten stations (all stations with a sampling depth greater than 34 m) had water column DO minima below 5 mg/L, with the two deepest stations, 6 and 12, exhibiting concentrations below 1 mg/L DO near the sediment-water interface (Figure 6). Profundal DO concentrations averaged 3.3 mg/L (± 2.2 SD) over all stations on October 6, 2017. Profundal DO recovered in November, and by December 1, 2017, just prior to lake turnover, all deep samples had concentrations > 5 mg/L DO. By December 21st (soon after turnover), profundal DO concentrations throughout the lake averaged 9.2 mg/L DO (± 0.3 SD), suggesting relatively rapid re-oxygenation of much of the profundal zone following lake turnover.

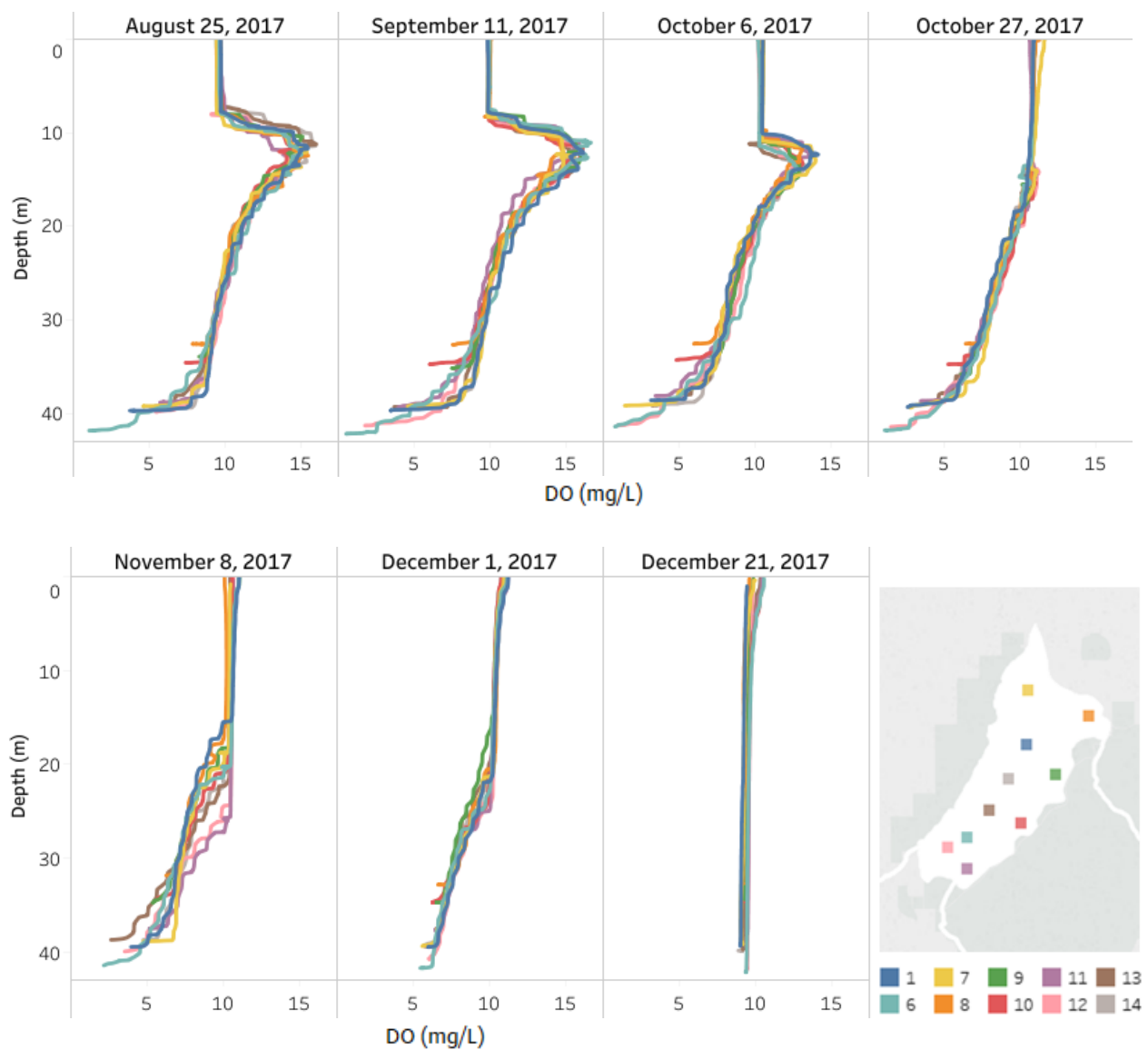


Figure 5. Depth profiles of dissolved oxygen (DO) for each sampling day in 2017, with each DFO Water Monitoring Station represented by a colour.

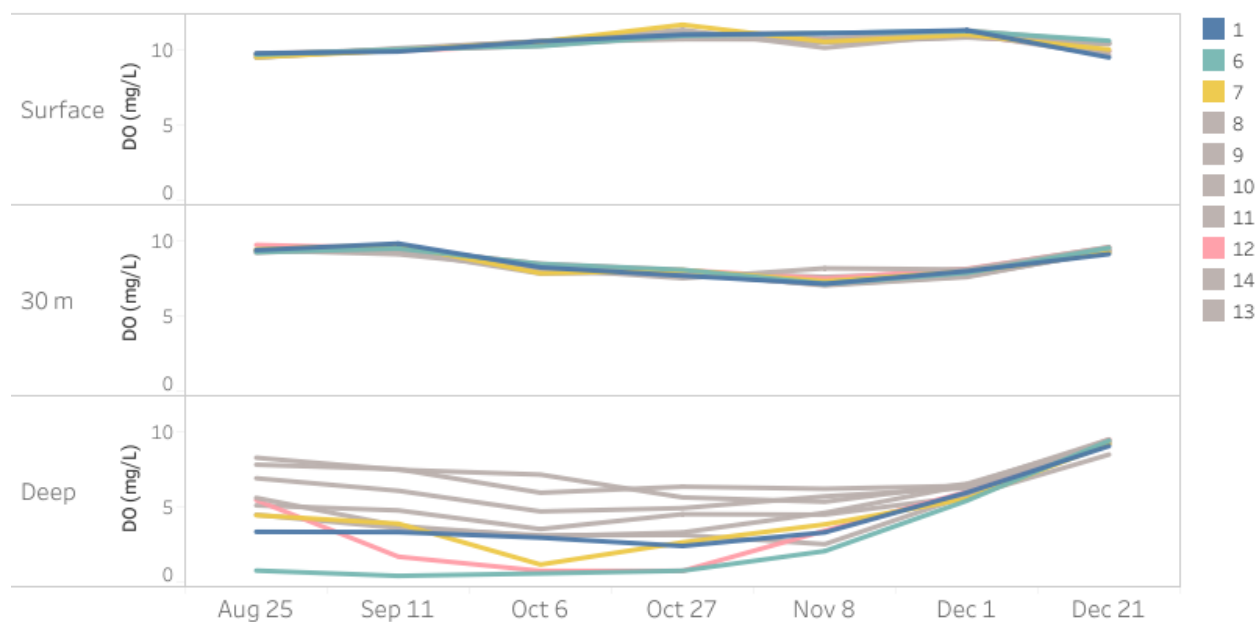


Figure 6. Time series of dissolved oxygen (DO) for the surface, 30 m, and deep samples in 2017. Station 1, 6, 7, and 12 are shown in colour throughout this report, as the deep sample results from these stations vary notably from that of other stations.

Oxidation-Reduction Potential

Oxidation-reduction (redox) potential (ORP) in Cultus Lake averaged 243 mV (\pm 68 SD) for all sampling days, including surface, 30 m and deep values ($n = 210$). Decreases in redox potential were observed at ~12 m depth during August, September and early-October, associated with the peak of the deep chlorophyll maximum (Figure 7). Marked reductions in redox potential were observed near the sediment-water interface, late in the stratified period (Figure 8). Redox potential and DO were largely unrelated above ~ 1 mg/L DO (i.e. profundal zone), but marked ORP drops were observed below this threshold (Figure 9). In particular, marked reductions in profundal zone redox potential occurred at Station 6 (the deepest station) in August, September and early-October, and at Station 12 (second deepest station) in early-October. Inter-site spatial variation in ORP (Figure 7) likely reflects a variety of co-occurring processes or environmental conditions affecting this nonspecific measurement.



Figure 7. Depth profiles of redox potential (ORP) for each sampling day in 2017, with each DFO Water Monitoring Station represented by a colour.

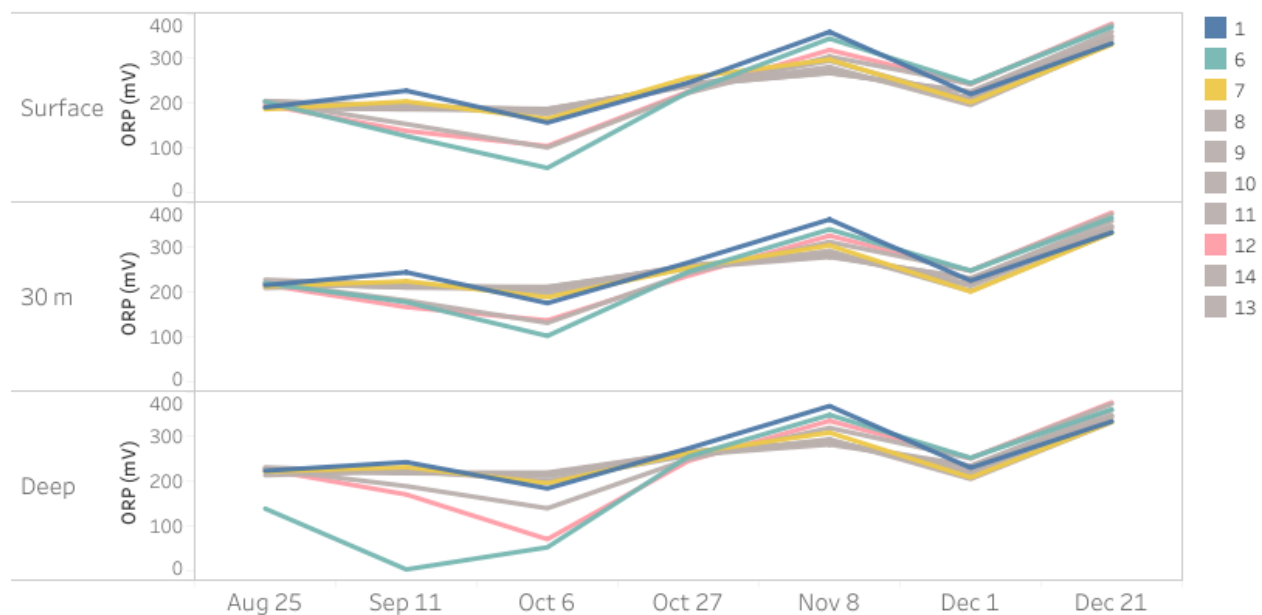


Figure 8. Time series of redox potential (ORP) for the surface, 30 m, and deep samples in 2017, with Station 1, 6, 7, and 12 shown in colour.

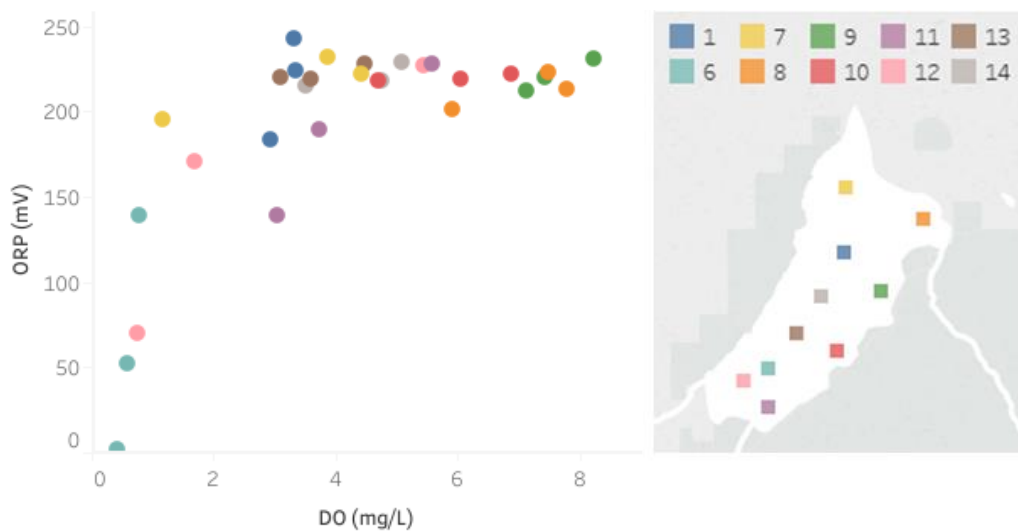


Figure 9. Redox potential (ORP) regressed against dissolved oxygen (DO), including data from deep samples from all ten stations from August, September and early-October 2017 ($n = 30$).

pH

2017 water samples from the surface and 30 m were alkaline, across all sampling stations, on all sampling dates (Figures 10 and 11). On average, the pH of surface waters were highest (8.4 ± 0.1 SD) in August, September, and early-October, although notable increases were observed within the depths influenced by the seasonal deep chlorophyll maximum (Figure 10). Late-season lake water pH reached a maximum of 8.8, at ~ 11 m depth, corresponding with the deep chlorophyll maximum (DCM; Figure 10). Water pH was lowest (circumneutral) in the deep samples in August, September and October. By turnover, water column pH averaged $7.6 (\pm 0.03$ SD).

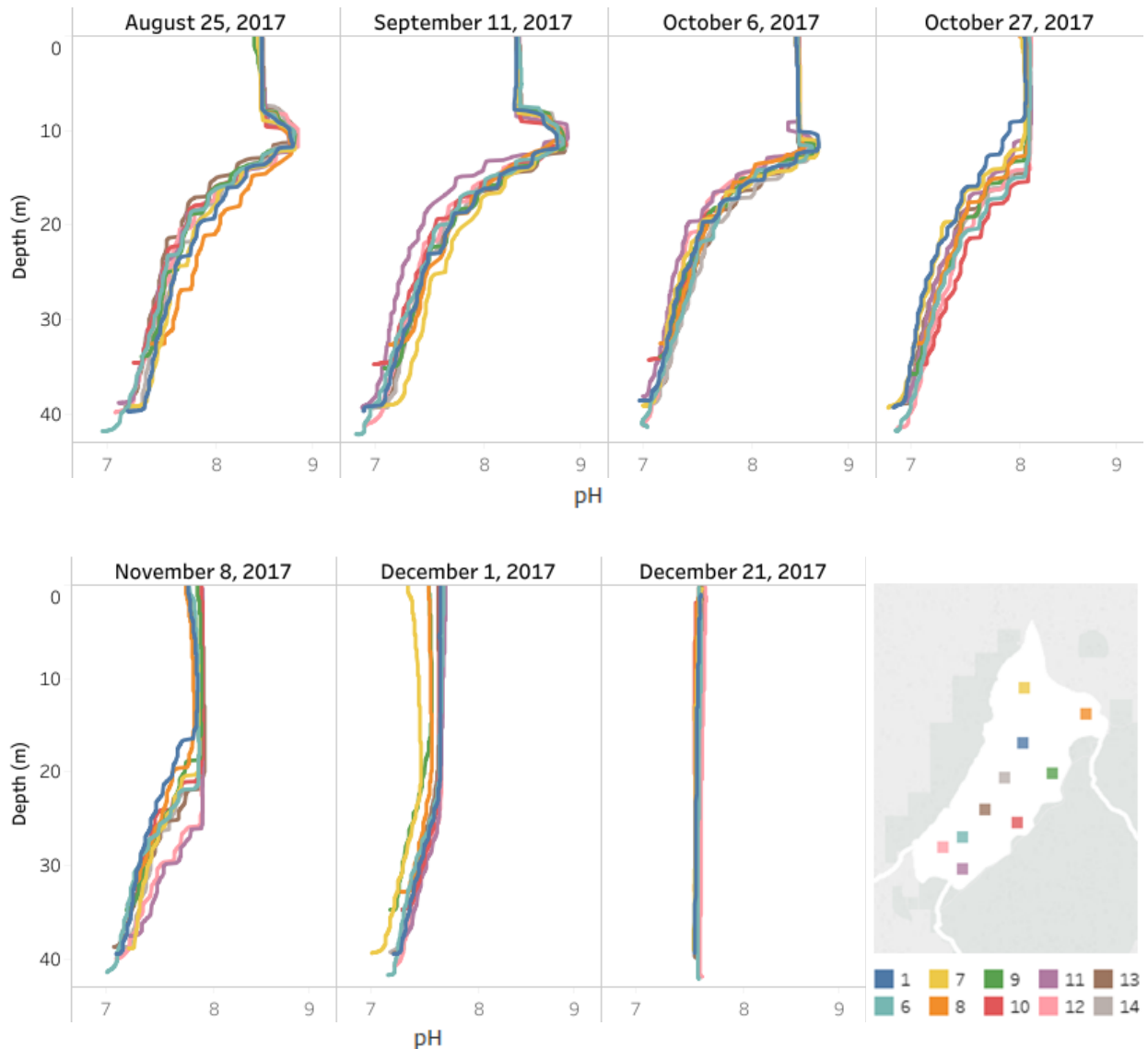


Figure 10. Depth profiles of pH for each sampling day in 2017, with each DFO Water Monitoring Station represented by a colour.

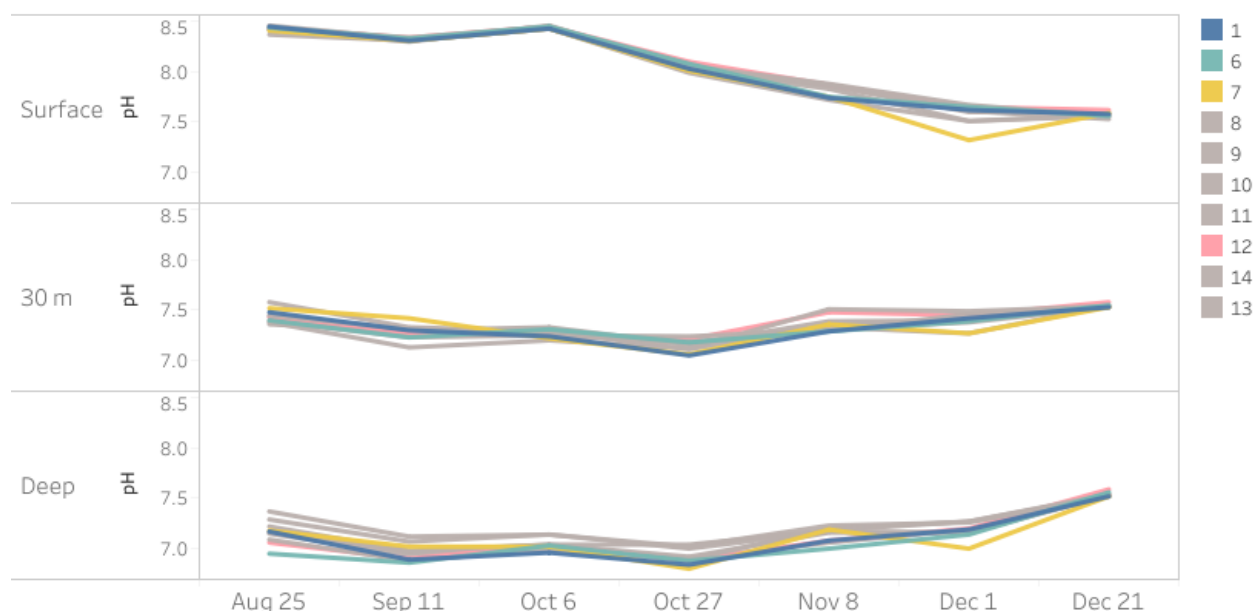


Figure 11. Time series of pH for the surface, 30 m, and deep samples in 2017, with Station 1, 6, 7, and 12 shown in colour.

Nitrogen

Nitrate-N: From August to October 2017 nitrate ($\text{NO}_3\text{-N}$) concentrations remained low at the surface ($9.5 \mu\text{g/L NO}_3\text{-N} \pm 10.7 \text{ SD}$) and relatively high at 30 m ($161.5 \mu\text{g/L NO}_3\text{-N} \pm 5.1 \text{ SD}$), with little variation between stations (Figure 12). By December 21st, post-turnover water column nitrate concentrations (i.e. water column homogenization) averaged $120 \mu\text{g/L NO}_3\text{-N}$ ($\pm 3.0 \text{ SD}$) with negligible inter-station variation.

Profundal nitrate concentrations ranged between 2.5 and $178.5 \mu\text{g/L}$, with notable spatial and temporal variation (Figure 12). Dissolved oxygen showed little relationship with nitrate until oxygen levels dropped below $\sim 2 \text{ mg/L DO}$, at which point consistent reductions in nitrate concentrations were observed (Figure 13). Profundal nitrate concentrations at Station 6 (deepest) were consistently low in August, September and October, averaging $14.8 \mu\text{g/L NO}_3\text{-N}$ ($\pm 10.4 \text{ SD}$), coinciding with dissolved oxygen below 1 mg/L (Figure 13). The second lowest deep nitrate concentrations were observed at Station 12 (second deepest station) in September and October, averaging $92.7 \text{ mg/L NO}_3\text{-N}$ ($\pm 16.2 \text{ SD}$), when dissolved oxygen was at or below 1.68 mg/L DO . Notable reductions in profundal nitrate were also observed at Station 1 on September 11th, and at Station 7 on October 6th. Excluding these four stations, profundal $\text{NO}_3\text{-N}$ concentrations were similar throughout the late-stratified

period (August - October), averaging $159.3 \mu\text{g/L}$ (± 12.7 SD), similar to the overlying hypolimnion.

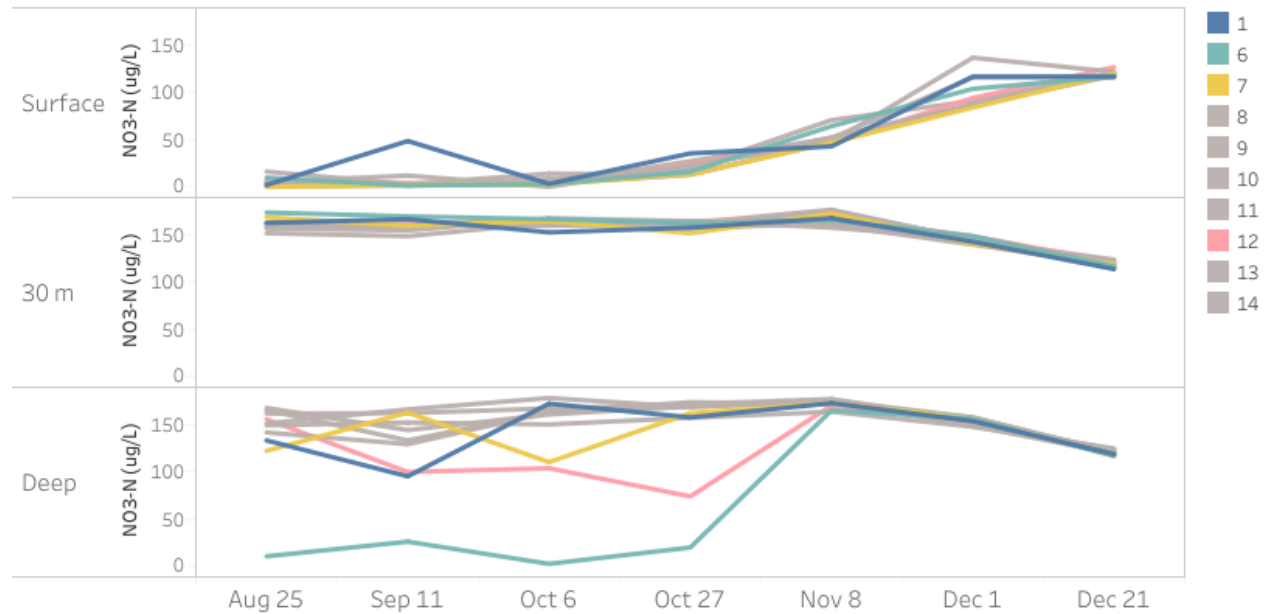


Figure 12. Time series of nitrate ($\text{NO}_3\text{-N}$) for the surface, 30 m, and deep samples in 2017, with Station 1, 6, 7, and 12 shown in colour.

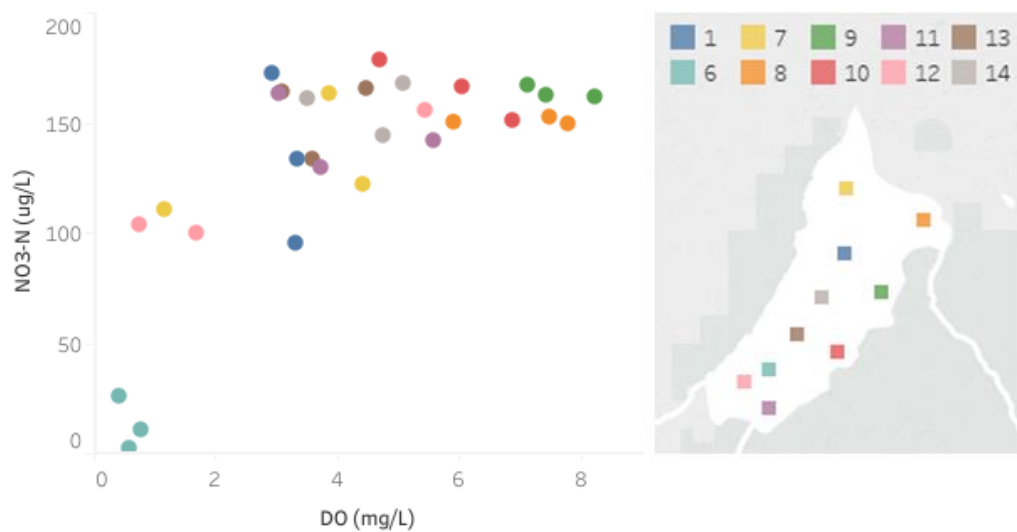


Figure 13. Nitrate ($\text{NO}_3\text{-N}$) regressed against dissolved oxygen (DO), including data from deep samples from all ten stations in August, September and October 2017 ($n = 40$).

Ammonium-N + Ammonia-N: Our analytical process for total ammonia-nitrogen (TAN) converts ammonia and ammonium to ammonium for measurement. The relative amounts of TAN in the form of un-ionized ammonia (ammonia, NH_3), which is toxic to aquatic life, versus ionized ammonia (ammonium, NH_4^+), which is less toxic and used as a nutrient, is dictated by ambient pH levels and, to a lesser extent, temperature (CCME 2010). The proportion of toxic un-ionized ammonia increases with pH and temperature.

Late-season Cultus Lake TAN concentrations at the surface and at 30 m averaged $1.2 \mu\text{g/L}$ (± 1.2 SD), with negligible variation between stations or sampling days. As both temperature and pH varied greatly within the upper 30 m of the water column, the proportion of ammonium to ammonia also varied, but their summed and individual concentrations remained low. Elevated TAN levels were detected across all profundal sampling locations, except Station 9, and were most pronounced at Station 6, 12, 1 and 7 (Figure 14), coinciding spatiotemporally with dissolved oxygen minima. Under observed hypolimnetic conditions (pH ~ 7 ; $\sim 5^\circ\text{C}$), the vast majority ($\sim 99.9\%$) of TAN likely existed as ammonium ($\text{NH}_4\text{-N}$). TAN reached a maximum near-sediment concentration of $349 \mu\text{g/L}$ TAN at Station 6 on October 6th (pH 7.0; 5.3°C), yielding a maximum calculated value of $\sim 0.4 \mu\text{g/L}$ of ammonia-N and $\sim 348.6 \mu\text{g/L}$ of ammonium-N. TAN concentrations in deep samples from Station 6 were consistently high in August, September and October, averaging $303.9 \mu\text{g/L}$ TAN (± 31.1 SD). The second highest concentrations were recorded in profundal samples from Station 12, averaging $165.0 \mu\text{g/L}$ TAN (± 23.0 SD) in September and October. TAN concentrations rose to $\sim 150 \mu\text{g/L}$ at Station 1 on September 11th and Station 7 on October 6th. Across the remaining five stations (DFO Stations 8, 10, 11, 13, 14), profundal TAN averaged $25.4 \mu\text{g/L}$ (± 25.0 SD) during August, September and October. By December 21, 2017, profundal TAN concentrations decreased to an average of $1.9 \mu\text{g/L}$ TAN (± 0.9 SD) across all stations. A strong negative linear regression relationship ($n = 40$, $r^2 = 0.96$, $p < 0.0001$) was observed between nitrate depletion and the appearance of TAN during stratification (Figure 15).

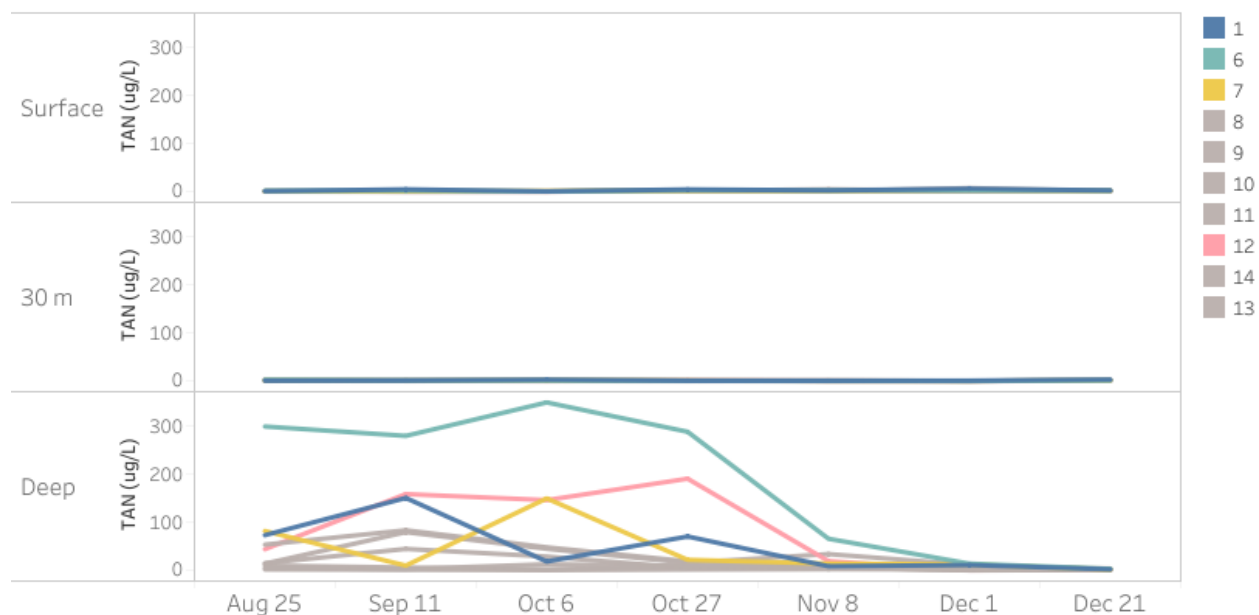


Figure 14. Time series of total ammonia-nitrogen (TAN) for the surface, 30 m, and deep samples in 2017, with Station 1, 6, 7, and 12 shown in colour.

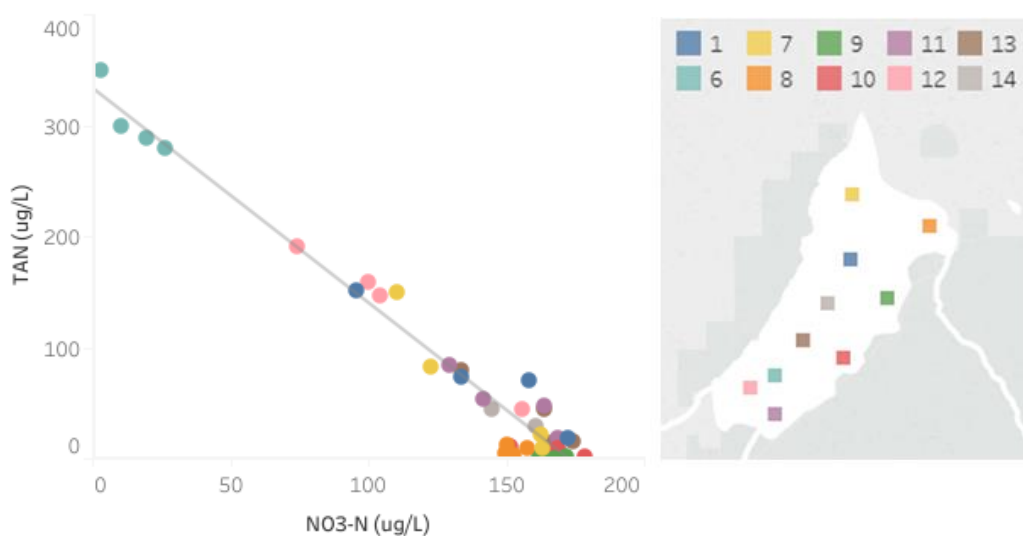


Figure 15. Total ammonia-nitrogen (TAN) regressed against nitrate ($\text{NO}_3\text{-N}$), including data from deep samples from all ten stations in August, September and October 2017 ($n = 40$, $r^2 = 0.96$, $p < 0.0001$).

Phosphorus

Cultus Lake soluble reactive phosphorus (SRP) and total dissolved phosphorus (TDP) at the surface and 30 m exhibited little monthly variation with no distinct spatial trends (Figure 16). Mean SRP and TDP at the lake surface and 30 m were slightly depleted in September, averaging $0.9 \mu\text{g/L}$ (± 0.3 SD) and $2.7 \mu\text{g/L}$ (± 0.9 SD), respectively, which was approximately half of the concentrations observed during other months. Elevated profundal concentrations of SRP and TDP were observed at Station 6 from August to early-October, and at Station 7 in early-October. Profundal SRP and TDP concentrations at Station 6 and 7 reached respective maxima of 16.5 and $30.9 \mu\text{g/L}$ in early-October. Slight increases in profundal TDP were also observed at Station 1 and 12 from September to late-October, while slight increases in profundal SRP were observed at Station 1 from September through late-October and at Station 12 in September 2017.

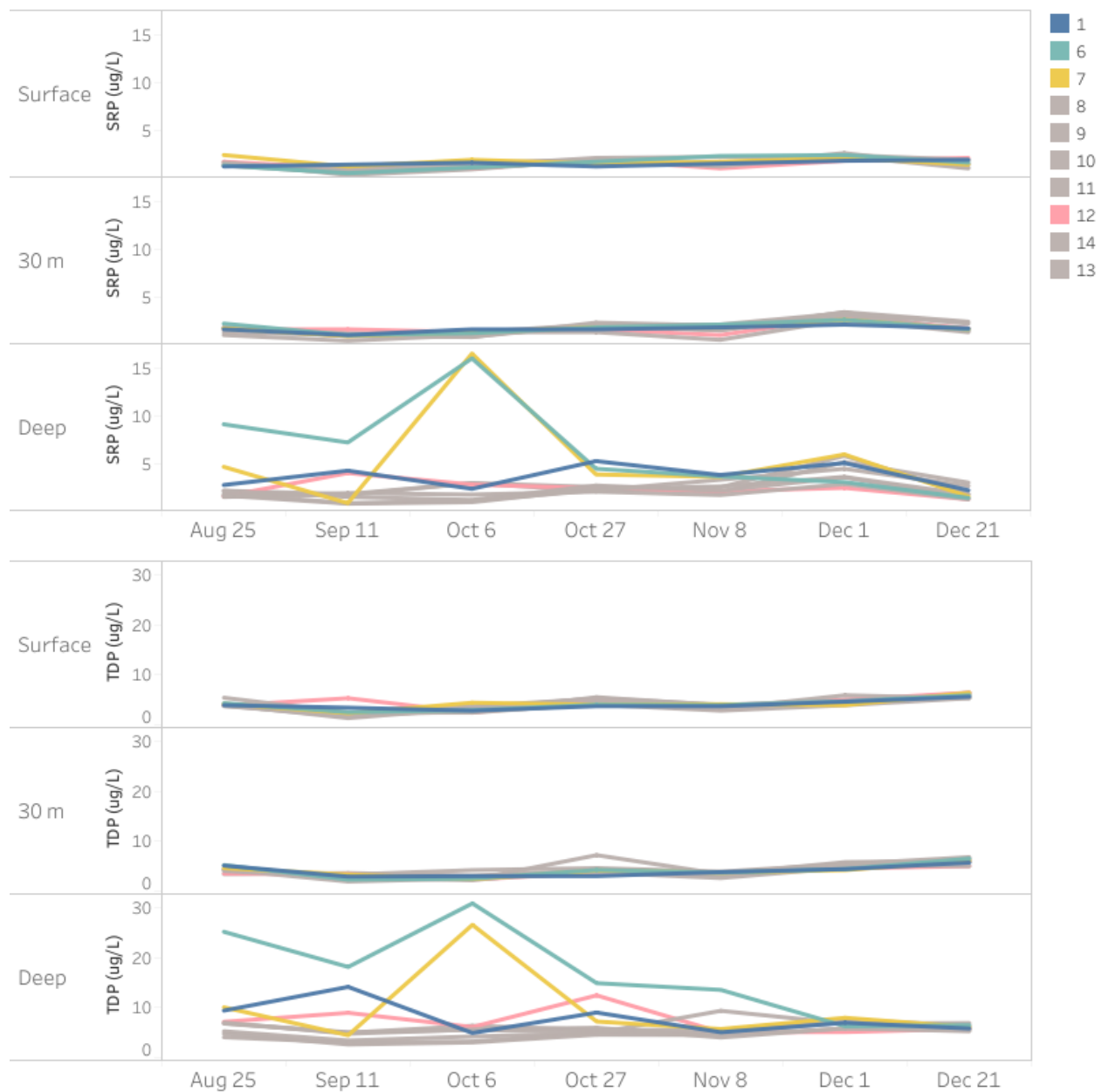


Figure 16. Time series of soluble reactive phosphorus (SRP) and total dissolved phosphorus (TDP) for the surface, 30 m, and deep samples in 2017, with Station 1, 6, 7, and 12 shown in colour.

Manganese

Manganese (Mn^{2+}) was not detected within the surface and 30 m water samples. It was, however, detected within near-sediment samples from 9 of the 10 stations (all except Station 9; Figure 17). Station 6 had the highest profundal concentrations of manganese, for the longest duration, followed by Station 12, then Station 1 and 7 (Figure 17). Manganese concentrations averaged 2.3 mg/L (± 0.3 SD) at Station 6 from August to October, and 1.5 mg/L (± 0.3 SD) at Station 12 from September to October, and reached 1.9 mg/L at Station 1 (September), and Station 7 (early-October; Figure 17). Manganese concentrations demonstrated a negative curvilinear relationship with oxygen concentrations ($n = 28$, $r^2 = 0.71$, $p < 0.0001$) (Figure 18).

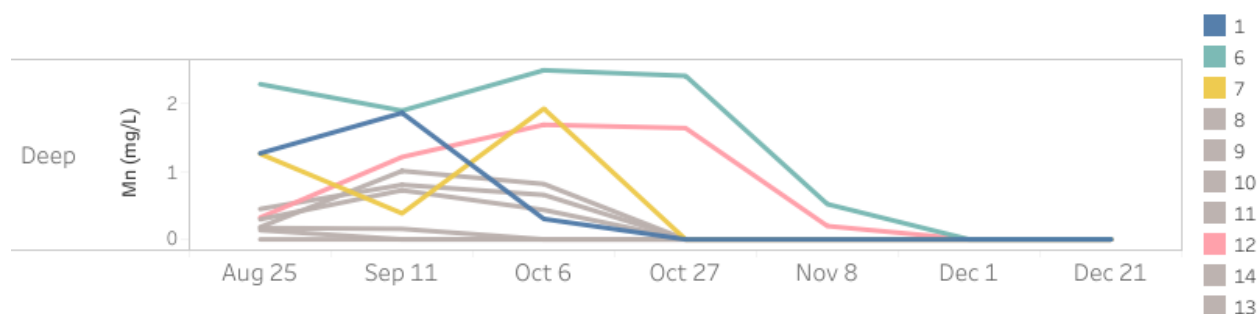


Figure 17. Time series of manganese (Mn) for deep samples in 2017, with Station 1, 6, 7, and 12 shown in colour.

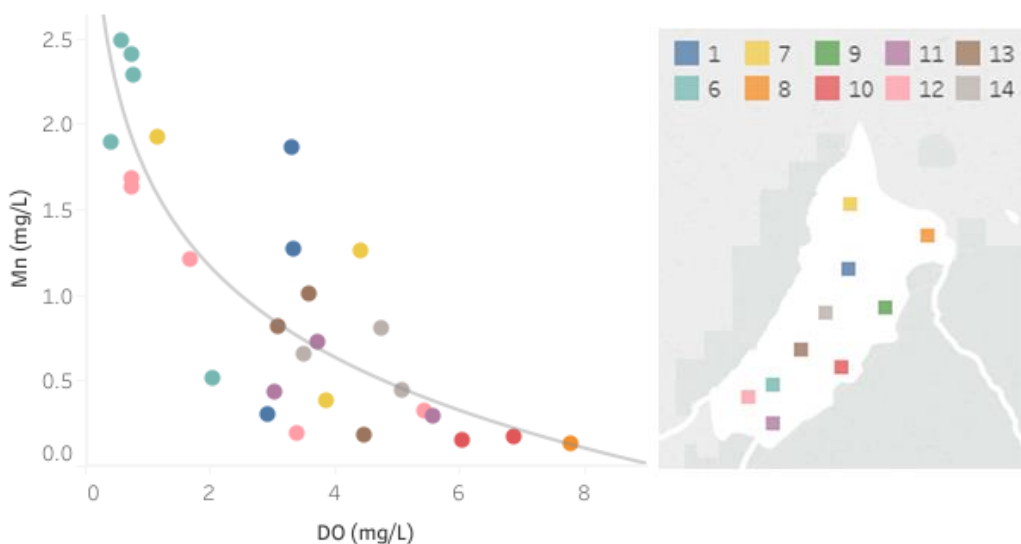


Figure 18. Manganese (Mn) regressed against dissolved oxygen (DO), including data from all samples which exhibited elevated concentrations of manganese (which include deep samples spanning from August to mid-November 2017 ($n = 28$, $r^2 = 0.71$, $p < 0.0001$)).

Sulfate

Cultus Lake late-season sulfate (SO_4) concentrations averaged 19,600 $\mu\text{g/L}$ (± 958 SD) at the surface and 19,000 $\mu\text{g/L}$ SO_4 (± 645 SD) at 30 m across the lake from late-August to late-December, with little spatial or temporal variation (Figure 19). A small spike in sulfate was observed in surface waters at Station 10 on October 6th and at 30 m at Station 11 on November 8th. Moderate profundal sulfate depletion was observed from August to October. Similar to nitrate-N, profundal sulfate concentrations had a positive and non-linear relationship with near-sediment oxygen levels ($n = 20$, $r^2 = 0.91$, $p < 0.0001$) (Figure 20). Station 6 exhibited the most notable decreases in profundal sulfate (14,600 $\mu\text{g/L}$ SO_4 , October 6, 2017), averaging 15,900 $\mu\text{g/L}$ SO_4 ($\pm 1,250$ SD) from August to October. The second lowest profundal sulfate concentrations were observed at Station 12 throughout September and October, averaging 16,600 $\mu\text{g/L}$ SO_4 (± 860 SD). A decrease in deep sulfate was also observed at Station 7 on October 6, 2017 (16,300 $\mu\text{g/L}$ SO_4).

A strong rotten-egg smell was detected in the deep samples from Station 6 and 11 on October 6, 2017, which, although not explicitly measured, may reflect the presence of hydrogen sulfide (H_2S), which exists within lake sediments and is redox sensitive (Wetzel 2001). Station 6 is the deepest DFO Monitoring Station and, on this day had the lowest recorded dissolved oxygen and sulfate concentrations. Station 6 also had the lowest recorded profundal redox potentials.

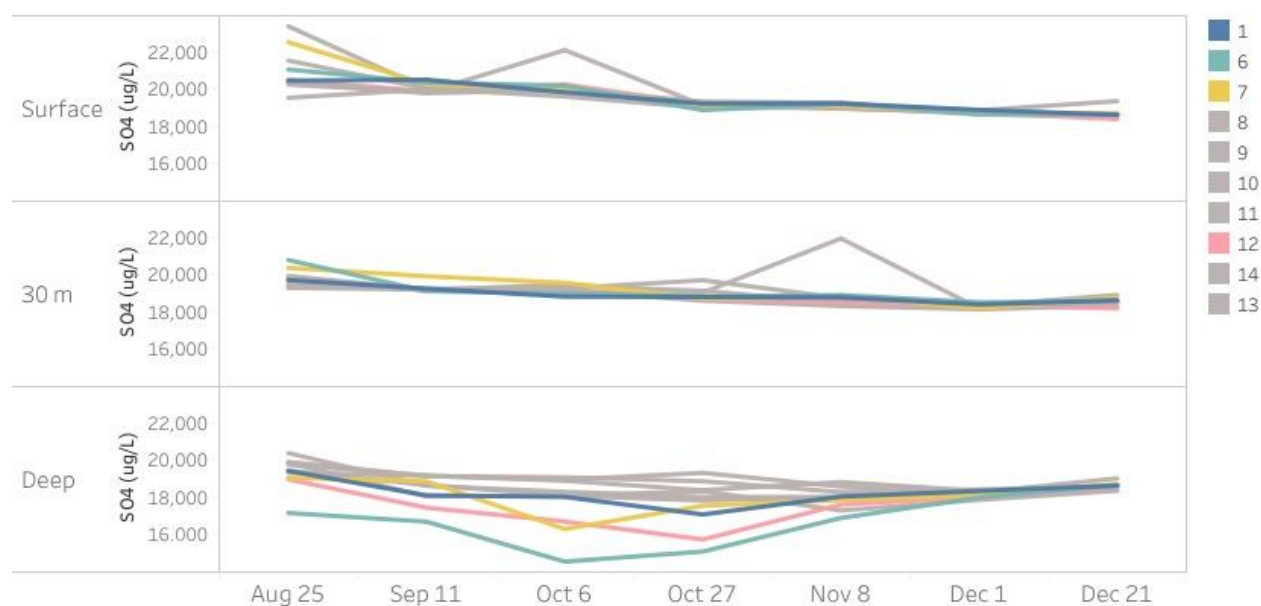


Figure 19. Time series of sulfate (SO_4) for the surface, 30 m, and deep samples in 2017, with Station 1, 6, 7, and 12 shown in colour.

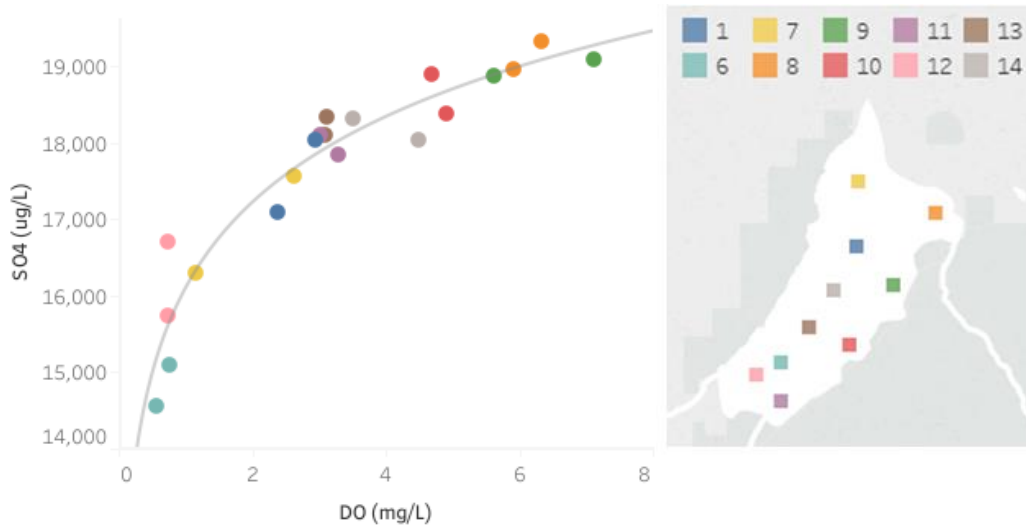


Figure 20. Sulfate (SO₄) regressed against dissolved oxygen (DO), including data from deep samples from all ten stations in early- and late-October 2017 ($n = 20$, $r^2 = 0.91$, $p < 0.0001$).

Specific Conductance

Cultus Lake profile data revealed notable spikes in specific conductance between 6 and 20 m water column depth, coinciding with the metalimnetic DCM, from August to October, which migrated downward and diminished as the stratified period concluded (Figure 21). Specific conductance readings at the surface, 30 m and within the profundal zone showed slight month-to-month variation, ranging from 165 to 188 $\mu\text{S}/\text{cm}$. Limited variation was observed in specific conductance between stations and depths, with the exception of deep observations at DFO Station 6, 12, 7 and 1, which were elevated to varying degrees from August through October, reaching a maximum value of 227 $\mu\text{S}/\text{cm}$ at Station 6 in September (Figure 22).

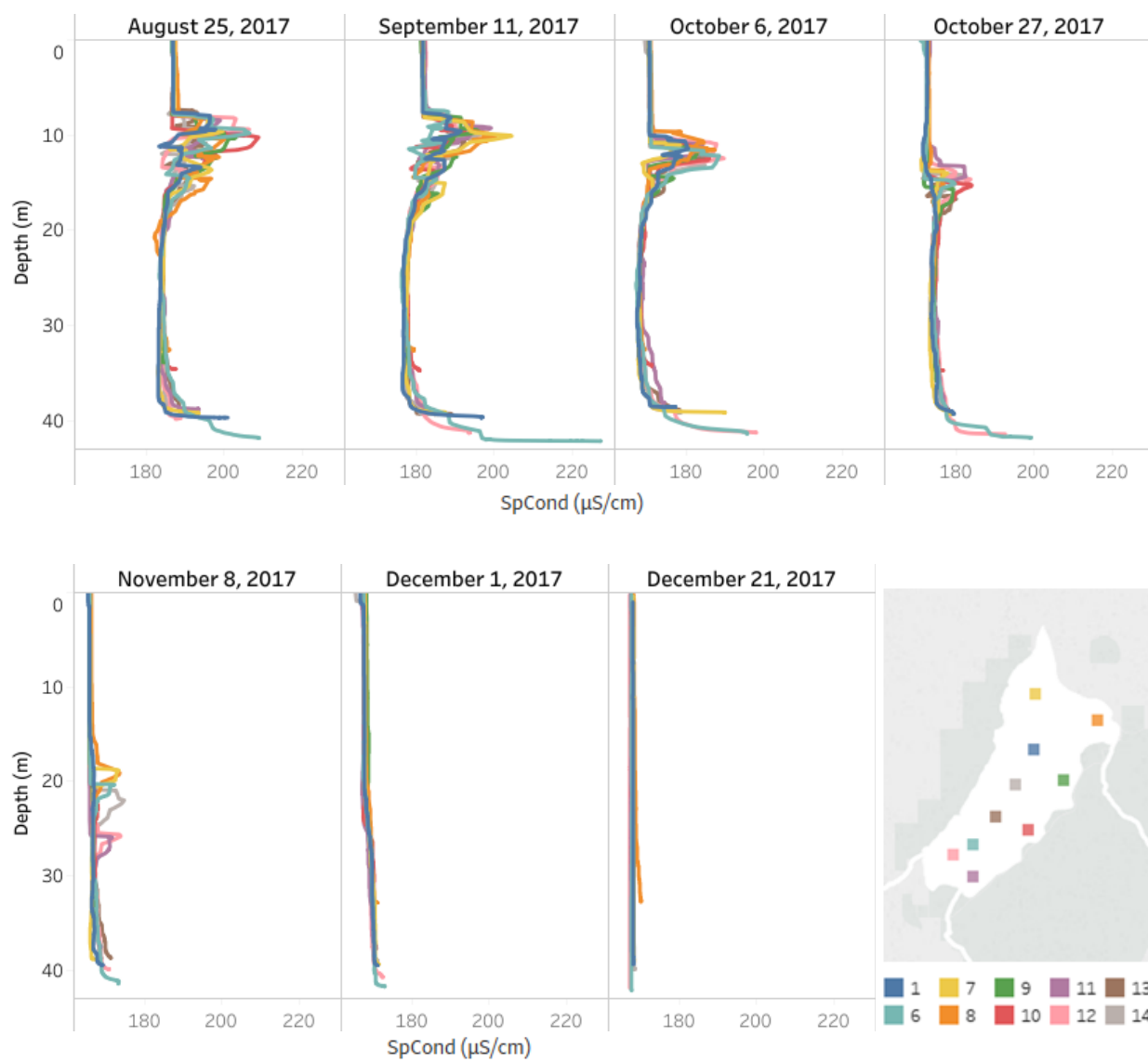


Figure 21. Depth profiles of specific conductance (SpCond) for each sampling day in 2017, with each DFO Water Monitoring Station represented by a colour.

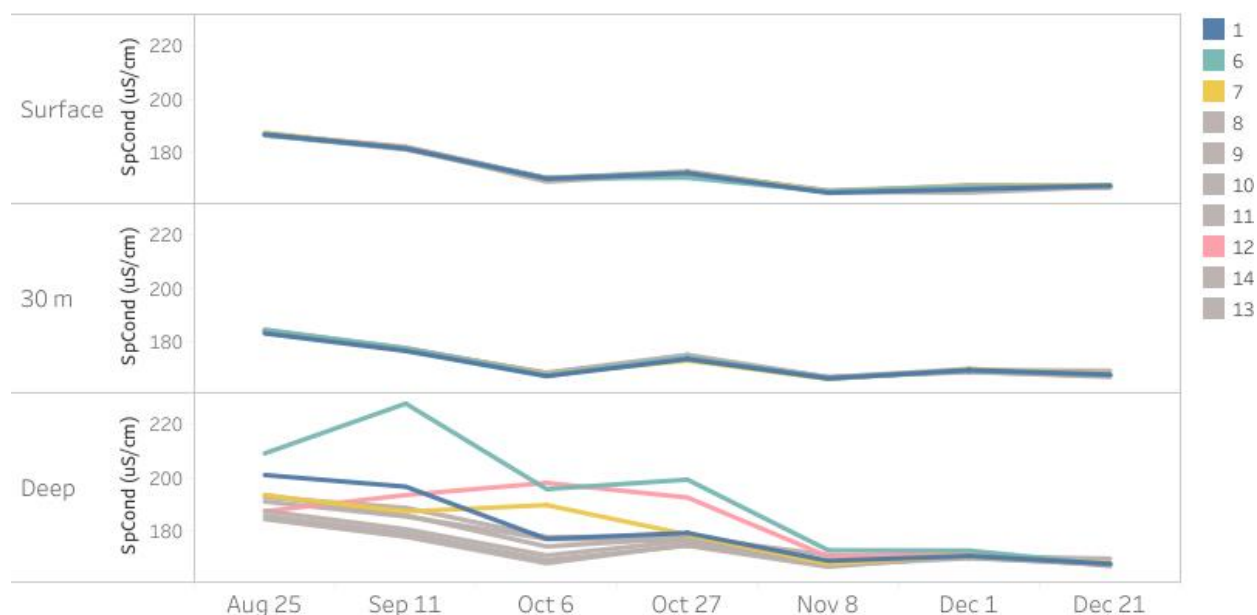


Figure 22. Time series of specific conductance (SpCond) for the surface, 30 m, and deep samples in 2017, with Station 1, 6, 7, and 12 shown in colour.

Fluoride

Cultus Lake fluoride (F^-) concentrations ranged from 40 to 66 $\mu g/L$ at all stations, all depths, and all dates, with the exception of profundal samples taken from Station 6 (deepest station) on September 11 and October 6, 2017, which reached mean concentrations of 121 and 193 $\mu g/L$ respectively (Figure 23). The Canadian Council of Ministers of the Environment (CCME) Guideline for the Protection of Aquatic Life for fluoride in freshwater is 120 $\mu g/L$ (interim), noting that anadromous salmonids are more sensitive to fluoride, with effects observed as low as 50 $\mu g/L$ (CCME 2002). Exceedances of the CCME guideline are detailed in Table 1.

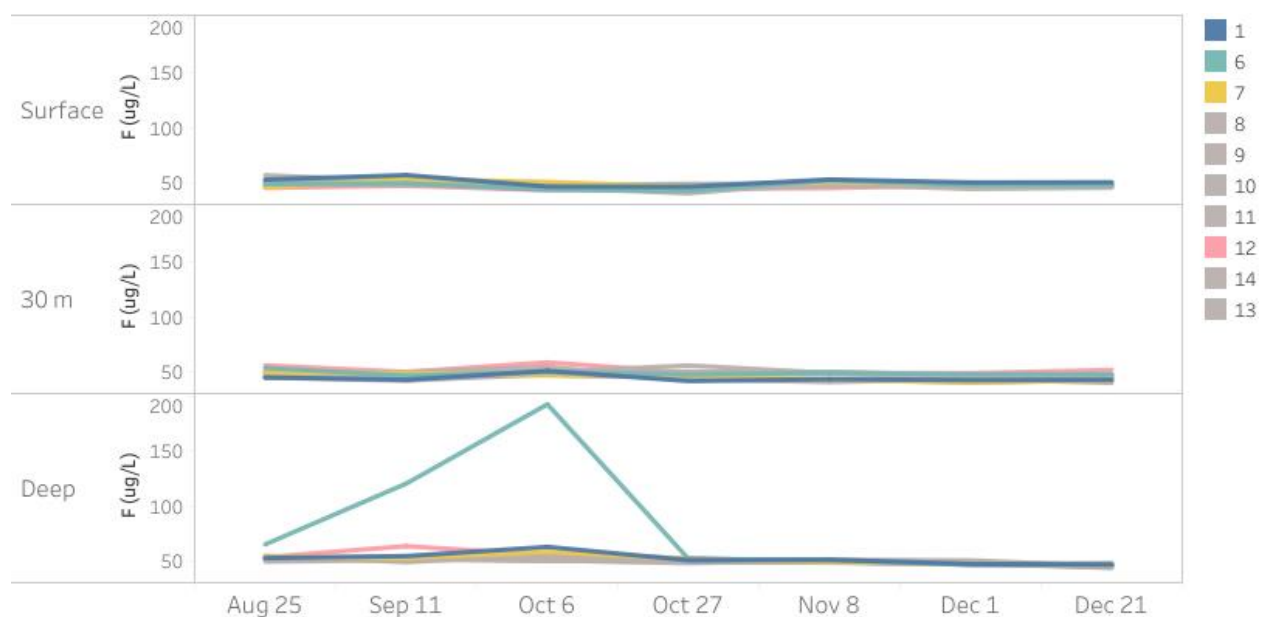


Figure 23. Time series of fluoride (F) for the surface, 30 m, and deep samples in 2017, with Station 1, 6, 7, and 12 shown in colour.

Table 1. Fluoride samples that exceeded CCME Guidelines for Protection of Aquatic Life (120 µg/L), including the results from replicate 1, replicate 2 and the mean value.

Date	Station	Depth (m)	Fluoride (µg/L)		
			Rep. 1	Rep. 2	Mean
September 11, 2017	6	41.9	117	125	121
October 6, 2017	6	41.3	188	198	193

Fish Capture Data

A total of 350 Cultus Pygmy Sculpin were captured during 9 minnow trapping events between 2012 and 2017. Catch-per-unit-effort (CPUE) was calculated as the number of individuals caught per minnow trap. The highest catch-per-unit-effort for minnow trapping occurred in the summer (July; Figure 24). The minnow trapping data ($n = 350$) exhibited a decrease in CPUE in September and October, late in the stratification period.

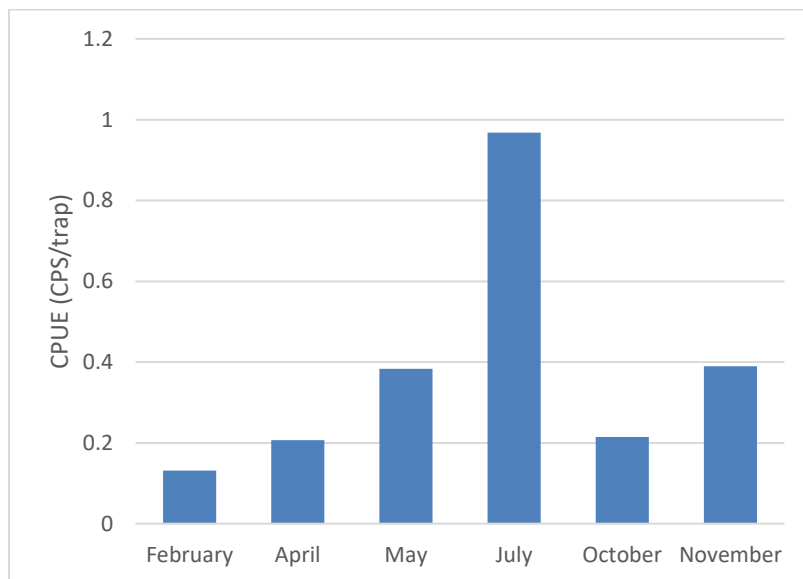


Figure 24. Catch-per-unit-effort (CPUE) by month for minnow trapping. Cultus Pygmy Sculpin were visually identified as such and were ≥ 25 mm. Sculpin under 25 mm were classified as juvenile sculpin and not included in the analysis.

Depth of Captures

Of the 350 Cultus Pygmy Sculpin captured by minnow trapping, 333 (95%) were captured near the sediment-water interface, 13 (4%) were captured 5 m above the substrate, and 4 (1%) were captured higher in the water column (Figure 25). Of the 333 benthic captures, the majority were relatively evenly distributed between 30 m (141 captures or 42%) and 40 m (122 captures or 37%), with fewer occurring at 20 m (66 captures or 20%) and very few occurring near shallower substrates.

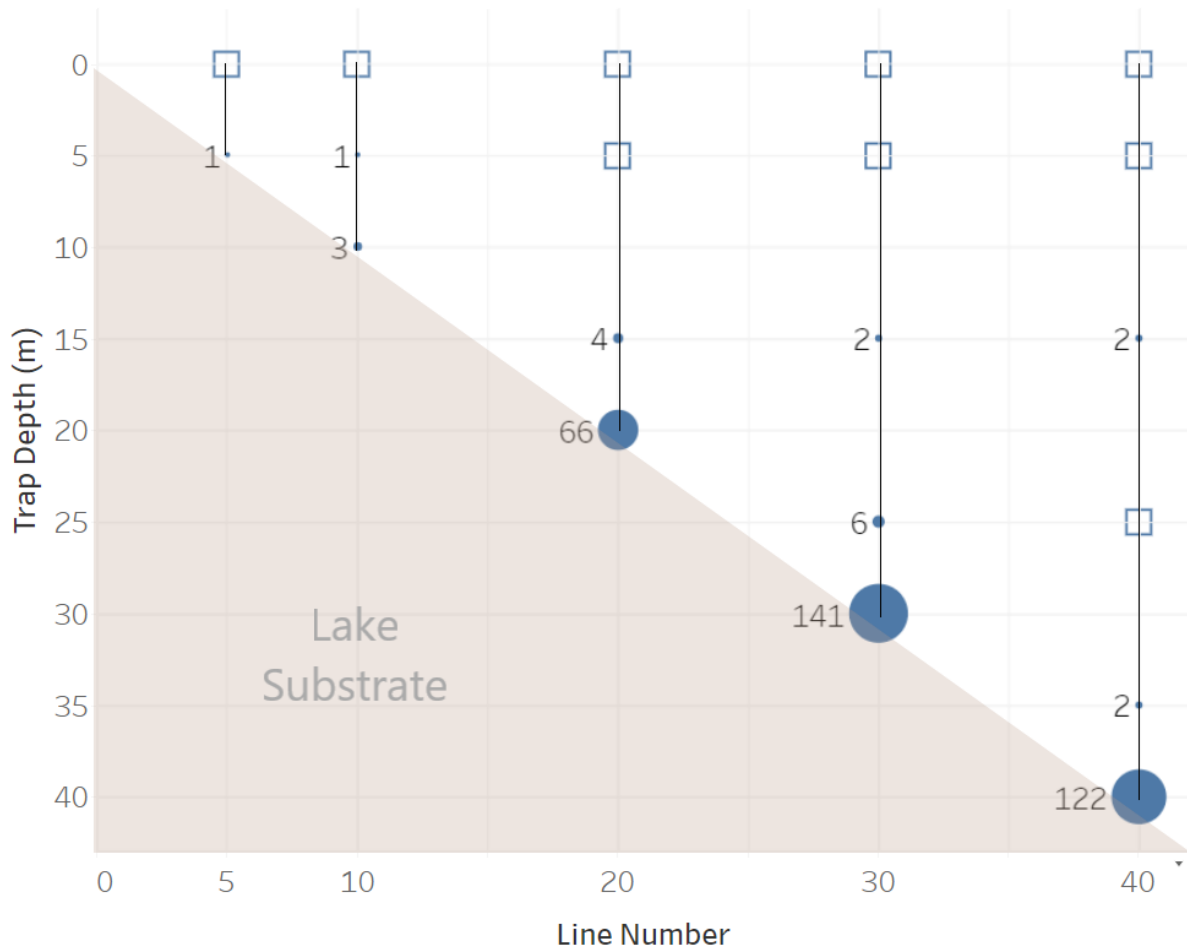


Figure 25. Cross section of the minnow trap arrangement, showing captures as blue circles ($n = 350$), with 333 captures occurring near the sediment-water interface.

A distinct shift in the depth of maximum minnow trapping captures was observed in early-fall, coinciding with a sharp decline in trap CPUE. The majority of Cultus Pygmy Sculpin trap captures transitioned from 40 m in July (51%) to 30 m in October (70%) and November (59%), returning to a relatively even distribution between 20 and 40 m for the remainder of the year (Figure 26). Although dissolved oxygen above the substrate interface was not measured for all substrate depths or on all minnow trapping dates, the defined upward shifts in minnow trap CPUE, during the late stratified period (October-November), contrasted with all other sampling periods, and coincided with pronounced profundal hypoxia both spatially and temporally (Figure 26).

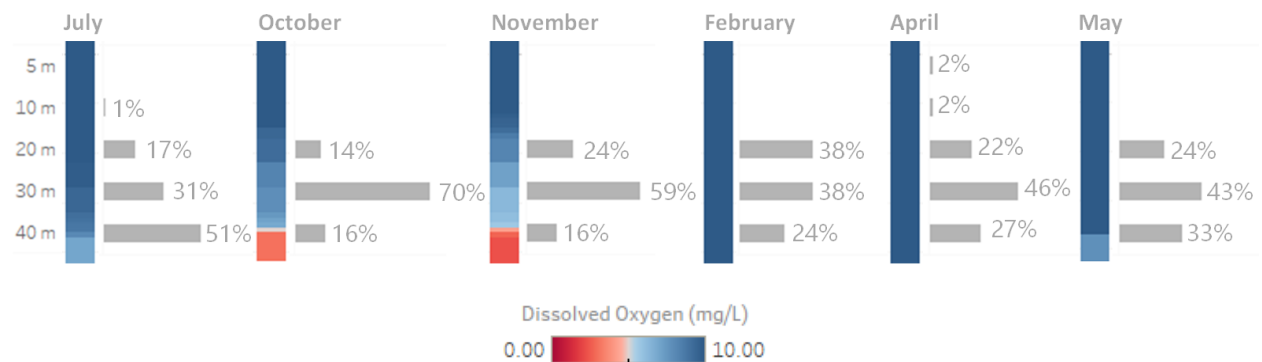


Figure 26. The vertical bars show dissolved oxygen profile data from Station 1 limnology monitoring in 2017 which transition from dark blue (oxygenated) to red (hypoxic) with light blue representing 5 mg/L. The horizontal bars show the proportion of the total CPUE of Cultus Pygmy Sculpin by depth for each month that trapping occurred ($n = 350$).

Spatial Variations in CPUE

Our minnow trapping data suggest spatial and temporal variations in Cultus Pygmy Sculpin residence within Cultus Lake, marked by higher CPUE at the northern end the lake (Main Beach and Entrance Bay; Figure 27). By contrast, CPUE was the lowest at Salmon Bay, at the south end of the lake. CPUE was also notably higher at the north end of Cultus Lake in February, May, and October, compared to the rest of the year (Figure 27).

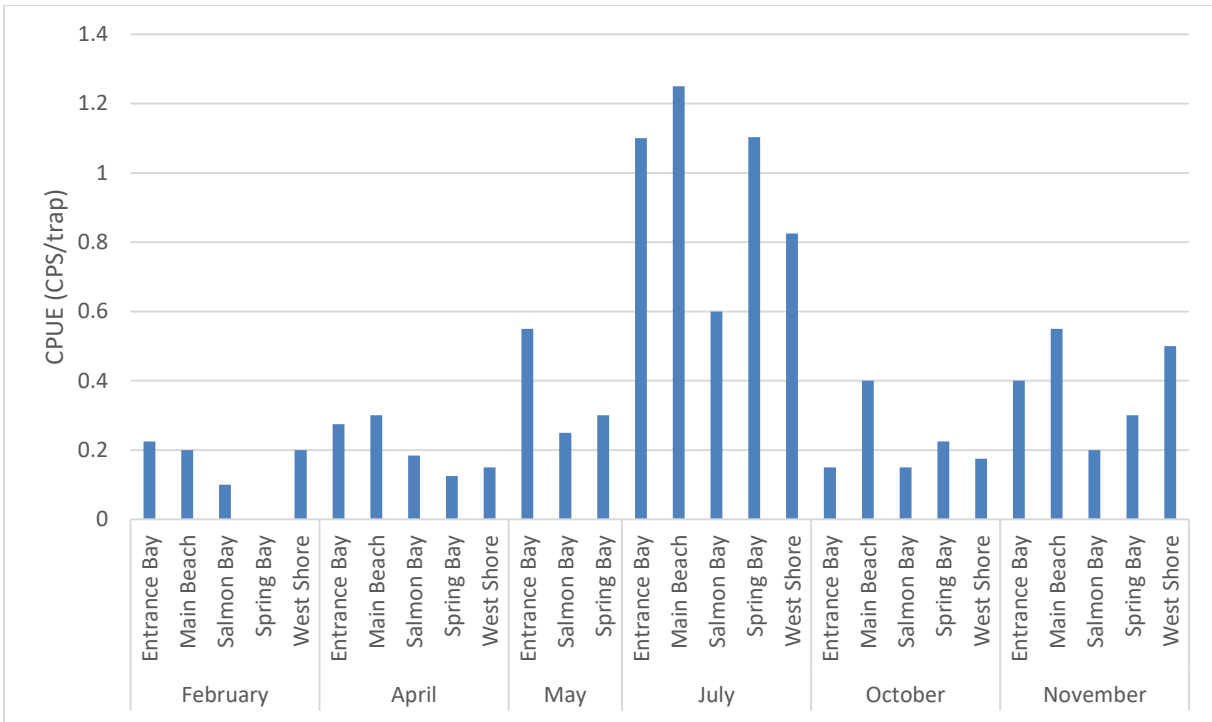


Figure 27. Catch-per-unit-effort by location and month from minnow trapping data ($n = 350$). Note that the West Shore and Main Beach (2 of the 5 sites) were not sampled in May.

DISCUSSION

Anthropogenic nutrient loadings to Cultus Lake, British Columbia, coupled with climatic warming of the water column, have increased annual primary productivity and exacerbated seasonal hypolimnetic and profundal oxygen depletion, as a consequence of enhanced organic matter decomposition at depth (Shortreed 2007; Sumka 2017; Putt et al. 2019; Gauthier et al. 2020; Selbie et al. 2022). Low oxygen concentrations near the sediment-water interface can impact fish survival directly, but can also induce anaerobic microbial decomposition pathways (Wetzel 2001; Burgin and Hamilton 2007) and internal loading of sediment constituents (Mortimer 1941; Mortimer 1971), which can further degrade deep-water lake habitats. In Cultus Lake, profundal habitats are extensively used by two endemic species at elevated risk of extinction, the Cultus Pygmy Sculpin and Cultus Lake Sockeye Salmon. This study further characterizes critical habitat use by the Cultus Pygmy Sculpin, and evaluates degradation of profundal habitats within Cultus Lake, a focal threat to the habitat and survival of both species at risk (DFO 2017; Selbie et al. 2022).

Spatio-temporal patterns of habitat use by Cultus Pygmy Sculpin

COSEWIC considers this species to be facing imminent extinction due to its small distribution and increasing threats to the species and its habitat, and therefore designated this species *Endangered* in 2019. Threats and limiting factors directly and indirectly impact species and the habitat that is necessary for their survival. This can alter and shift species distribution to less suitable habitats, which for species that are already geographically restricted, can potentially increase the risk of extinction. Primary limitations and threats for Cultus Pygmy Sculpin include its sole endemic lineage within Cultus Lake, BC, its limited and degrading critical habitat (Chiang et al. 2015), and in particular seasonal hypoxia-anoxia in the lake profundal zone, which arises from interactions between ongoing eutrophication and climate change (DFO 2017; Putt et al. 2019; Gauthier et al. 2020; COSEWIC 2021; Selbie et al. 2022). Habitat threats are compounded by increasing urbanization and recreation use of Cultus Lake and the invasion of a non-native piscivore, Smallmouth Bass (*Micropterus dolomieu*), first detected in Cultus Lake in 2018 (COSEWIC 2019). Smallmouth Bass are anticipated to prey on Cultus Pygmy Sculpin which are displaced by degraded habitat conditions and are more vulnerable to predation due to altered vertical distribution.

DFO Lakes Research Program sculpin trapping within identified critical habitat for the species has yielded novel information on the seasonal distribution of the Cultus Pygmy Sculpin. Over the period 2012–2017 a strong prevalence of benthic and profundal catches (~ 95%) relative to those in pelagic zone (~ 5%), indicates that Cultus Pygmy Sculpin likely principally reside near the sediment-water interface, deep within Cultus Lake, spending comparatively less time in the water column. While this inference assumes roughly equal catchability at depth, and this may not be fully true for a species that lacks a swim bladder across a ~44 m water column, this benthic tendency is supported by direct diver observations (ManFish 2014) and previous trapping (Woodruff 2010). Sustained interceptions of Cultus Pygmy Sculpin in nighttime midwater trawling substantiate the persistence of nocturnal vertical movements into the overlying water column to forage (COSEWIC 2010; DFO *unpublished data*).

Our findings indicate a seasonality to the distribution and/or swimming activity of Cultus Pygmy Sculpin within Cultus Lake, over the annual cycle. Minnow trapping yielded the highest catch–per-unit-effort (CPUE) in July, consistent with the initial findings of Woodruff (2010), followed by June and August, and were marked by sharp decreases in the late-stratified period (September and October). Near-sediment captures continuously dominated trap interceptions, but were marked by a seasonal shift, following the lake bathymetry along its periphery, from captures at 40 m in July to 30 m in October and November, returning to a relatively even distribution of captures between 30 and 40 m for the remainder of the year. This apparent shift in habitat use coincides with the sharp decline in trap CPUE, and decreasing profundal and

hypolimnetic oxygen, late in the stratified period (see subsequent discussion). The reason(s) for these CPUE-inferred seasonal residence shifts are not fully known, but we hypothesize they may reflect several potential mechanisms that are not necessarily mutually-exclusive:

- 1) Cultus Pygmy Sculpin mortality in the late-stratified period in response to seasonal deep-water habitat degradation, largely occurring below 30 m depth (e.g. hypoxia/anoxia, redox-related toxin exposure); and/or
- 2) Detection and avoidance of degrading deep-water habitat conditions, inducing seasonal repositioning of Cultus Pygmy Sculpin from preferred deep benthic and profundal habitats to substrate interfaces above 30 m, along the lake periphery; and/or
- 3) Natural (e.g. temperature and food web effects on metabolism) and/or stressor-induced suppression (e.g. hypoxia, exposure to redox toxins, predator avoidance) of profundal zone use and/or swimming behaviour impacting trap capture efficiencies in deep and degraded environments; and/or
- 4) Some unknown aspect of their behavioural ecology affecting seasonal captures (e.g. suspected deep summer spawning; Ricker 1960).

The 2012–2017 areal spatial distribution of Cultus Pygmy Sculpin capture frequencies, along the lake axis, directly contrasted with historical observations. Higher trap CPUE observed at the north end of the lake in the recent period ($n = 350$) distinctly contrasts with earlier sampling in 2007 and 2008 by Woodruff (2010), who recorded the majority of captures towards the south end ($n = 167$). Over this period there have been significant declines in hypolimnetic and profundal oxygen concentrations, which have been most pronounced toward the southern extent of the lake (Selbie et al. 2022). Such stark contrast is unlikely the result of random variation, and may reflect recent habitat degradation and some level of habitat avoidance or exclusion.

Regardless of the mechanism(s) underlying the apparent seasonal spatiotemporal shifts in Cultus Pygmy Sculpin residence, it is clear that profundal oxygen depletion is rendering portions of Cultus Pygmy Sculpin critical habitat largely uninhabitable in the later stages of stratification (i.e. hypoxic to anoxic), as appears to be the case for endangered Sockeye Salmon (Selbie et al. 2022). The aerobic tolerance of Cultus Pygmy Sculpin is not yet known, nor is their ability to detect and avoid hypoxic and/or toxic environments. Several studies have investigated the critical oxygen tension (P_{crit} ; an indicator of hypoxia tolerance) of various other freshwater and marine sculpin

(e.g. Henriksson et al. 2008; Mandic et al. 2009a; Mandic et al. 2009b). In a recent experiment, Prickly Sculpin from Little Campbell River (estuarine habitat) and Kamloops Lake (freshwater habitat) were exposed to low oxygen concentrations of 6.4 Torr (0.37 mg/L), and had a time-to-LOE (time to loss of dorsal-ventral equilibrium) of 4.5 and 2.5 hours, respectively (S. Liu, pers. comm., February 20, 2018, *unpublished data*). Such experimental dissolved oxygen minima are comparable to those observed in deep portions of Cultus Lake, during the late-stratified period (Selbie et al. 2022). Hypoxia tolerance varies between sculpin species, and is broadly related to oxygen levels in their typical habitats, with higher tolerance occurring in those that occupy intertidal areas inherently subjected to diurnal variations of oxygen concentrations, and lower tolerance in sculpin found in stable sub-tidal zones and freshwater (Mandic et al. 2009a).

Cultus Pygmy Sculpin have evolved from Coastrange Sculpin over the millennia since post-deglaciation (Woodruff and Taylor 2013), a lotic species that typically inhabits highly-oxic environments (McPhail 2007). As Cultus Lake appears to have been ultra-oligotrophic to oligotrophic prior to anthropogenic nutrient loading of the 20th century (Putt et al. 2019; Gauthier et al. 2020), and thus likely oxic in the deep waters, it is unclear whether the Cultus Pygmy Sculpin's physiological tolerance to hypoxia is sufficient to endure modern profundal dissolved oxygen minima.

Upper Water Column

The upper water column of Cultus Lake (i.e. metalimnion, epilimnion), is an important feature of the habitat for both Cultus Pygmy Sculpin and Sockeye Salmon, as they are known to occupy this lake region periodically for foraging (Woodruff 2010; Chiang et al. 2015; DFO 2017; Selbie et al. 2022). Limnological processes emerging within this lake region strongly influence water quality conditions deeper within the lake (i.e. hypolimnion, profundal and benthic zones) over the seasonal cycle (Wetzel 2001; Selbie et al. 2022).

Characteristic of numerous freshwater lakes, seasonal epilimnetic nitrate ($\text{NO}_3\text{-N}$) depletion was observed in Cultus Lake during the 2017 growing season, reaching late-summer and fall minima consistent with previous observations (Shortreed 2007; Selbie et al. 2022). Depletion of limiting nutrients in the upper euphotic zone (i.e. above the metalimnion) following the spring bloom, coupled with relatively high light penetration, likely drives the annual development of a growing season deep chlorophyll maximum (DCM) at the metalimnion and/or upper hypolimnion, where limiting nutrients remain abundant (Shortreed 2007; Leach et al. 2018; Selbie et al. 2022). DCM water quality in Cultus Lake is characterized by a dissolved oxygen maximum (partly a by-product of

autotrophic photosynthesis; Wilkinson et al. 2015), a spike in specific conductance, and a decrease in oxidation-reduction potential. DCM-localized reducing conditions occur despite elevated dissolved oxygen concentrations, likely due to photosynthetic reduction of carbon dioxide, and oxidation of H₂O during organic matter production; a process which produces reduced states (Wetzel 2001). The spike in DCM specific conductance reflects higher ion concentrations in this lake region, possibly due to physical mechanisms affecting accumulation within the metalimnion, but also likely higher rates of *in situ* organic matter remineralization and/or zooplankton grazing and excretion (Pilati and Wurtsbaugh 2003). Elevated pH, as observed in surface waters and throughout the DCM, is likely related to photosynthetic influences on dissolved inorganic carbon speciation (i.e. bicarbonate equilibrium; Wetzel 2001).

The seasonal development of a DCM within Cultus Lake is a persistent feature of the ecosystem, as inferred from comparisons of oxygen profiles in the 1930s and early-2000s (Shortreed 2007). However, pronounced increases in the metalimnetic maxima of the lake's positive heterograde oxygen profile, during the growing season (Shortreed 2007; Selbie et al. 2022), suggests DCM primary production has likely increased substantially through time, as the lake transitioned from oligotrophy to near-mesotrophy in response to cultural eutrophication (Putt et al. 2019; Gauthier et al. 2020; Selbie et al. 2022). The cumulative effects of a pronounced spring bloom, followed by sustained and elevated primary production at depth within the DCM (Shortreed 2007), occurring over longer annual stratified periods forced by climate change (Sumka 2017), have likely significantly enhanced annual autochthonous organic matter loads and commensurate decomposition within Cultus Lake (Putt et al. 2019; Gauthier et al. 2020). This interpretation is supported by increasing algal production and lake sedimentation rates within the Cultus Lake paleolimnological record, particularly over the latter half of the past century (Gauthier et al. 2020). Sedimentation of excess organic matter produced within the water column drives microbial decomposition particularly within the hypolimnion, the profundal zone, and at the sediment-water interface (Wetzel 2001; Foley et al. 2012; Steinsberger et al. 2020).

Deep Hypolimnion & Profundal Zone

The deepest regions of Cultus Lake (i.e. hypolimnion, profundal and benthic zones) are essential features of the critical habitat for the lake's species at risk. Cultus Pygmy Sculpin are known to largely exist within the lake profundal and benthic zones (Ricker 1960; Woodruff 2010; ManFish 2014), as substantiated by our minnow trapping results. Similarly, sub-annual DFO Lakes Research Program hydroacoustics and trawl surveys indicate juvenile Sockeye Salmon frequent the profundal zone for protracted periods during their diel vertical migrations, residing in very close association with the lake

sediments (Selbie et al. 2022) and demonstrate genomic signatures of significant hypoxia stresses when profundal dissolved oxygen low (Akbarzadeh et al. 2021). Consequently both species at risk are likely seasonally exposed to stresses associated with water quality degradation within this critically-impacted lake region.

Profundal Zone Dissolved Oxygen and Redox Conditions

Cultus Lake currently experiences hypoxic to anoxic profundal conditions in the late-summer and early-fall (Shortreed 2007; Selbie et al. 2022), with dissolved oxygen concentrations near the sediment-water interface well below the range required for aquatic life (Davis 1975; CCME 1999). Minima averaged 3.3 mg/L (\pm 2.2 SD) across all stations on October 6, 2017. As the deep measurements from our study were made 1–1.5 m above the sediment-water interface, and steep dissolved oxygen and redox gradients occur within a few millimeters of this horizon (Mortimer 1941), oxygen depletion at the boundary layer, influential to internal loading, is likely much more pronounced than that represented by our profundal sampling. Concentrations of dissolved oxygen have little impact on oxidation-reduction potentials until ambient conditions approach anoxia, resulting in concurrent reductions in redox, and associated microbial utilization of a series of alternate electron acceptors, that facilitate ongoing organic matter decomposition through anaerobic pathways (Orihel et al. 2017).

Induction of Anaerobic Microbial Decompositional Pathways

Aerobic decomposition of organic matter is a prevalent and important recycling process in aquatic ecosystems (Wetzel 2001). However, under hypoxic to anoxic conditions at the sediment-water interface of lakes, and within the sediments, bacterial communities shift to anaerobic metabolic pathways, making use of alternative electron acceptors in a relatively fixed sequence: reduction of nitrate \rightarrow manganese \rightarrow iron \rightarrow sulfate \rightarrow carbon dioxide (Laanbroek 1990; Balistrieri et al. 1992a; Davison 1993; Orihel et al. 2017). These processes can produce toxic by-products (Wetzel 2001), including hydrogen sulfide evolution (H_2S ; Smith and Oseid 1972; Kiemer et al. 1995). Internal loading of sediment constituents also occurs under anoxia at the sediment-water interface, releasing phosphorus, co-precipitated iron, manganese (Wetzel 2001), and metals (Mortimer 1941; Mortimer 1971) into the overlying water column in quantities that can ultimately impact aquatic life and lake nutrient budgets.

Indicators of anaerobic decomposition in freshwaters, in general order of occurrence, include nitrate loss, the appearance of manganous and ferrous ions, sulfate loss, and the appearance of methane (Laanbroek 1990). Co-indicators include

increases in carbon dioxide, TAN, phosphorus, pH and specific conductance, and reductions in dissolved oxygen and redox potential (Mortimer 1941; Mortimer 1971). In our study, indicators of microbial decomposition and internal loading were evident near the sediment-water interface, particularly at DFO stations 6, 12, 1 and 7, which characterize the deep profundal region of Cultus Lake (Figure 1). Of these parameters, we measured dissolved oxygen, ORP, nitrate, TAN, manganese, sulfate, total dissolved phosphorus and specific conductance (see Figure 28).

Consistent with seasonal induction of anaerobic microbial decomposition, profundal nitrate depletion, relative to the overlying water column, was observed at DFO Station 6, and to a lesser extent at DFO stations 12, 1 and 7 (Figure 28). Potential mechanistic pathways include respiratory denitrification (Burgin and Hamilton 2007), fermentative dissimilatory nitrate reduction to ammonium (DNRA; Silver et al. 2001, Kelso et al. 1997), and sulfur-driven nitrate reduction (Brunet and Garcia-Gil 1996, Zopfi et al. 2001). These complex reactions are capable of converting nitrate into ammonium, likely in part explaining the late-season disappearance of nitrate and appearance of total ammonium-N (TAN) in these deep hypoxic environments. Additionally, moderate profundal sulfate depletion was subsequently observed, and negatively associated with oxygen, depth, and redox potential (Figure 28). Sulfate depletion was greatest at Station 6, followed by Station 12 and 7. A strong rotten-egg smell was detected in profundal samples at Station 6 (and Station 11), late in the stratified period, likely indicative of the presence of hydrogen sulfide (H_2S ; Mortimer 1941). These findings highlight the likely onset of sulfate reduction pathways in these deep environments, which can be associated with the release of the faunal toxicant hydrogen sulfate (H_2S).

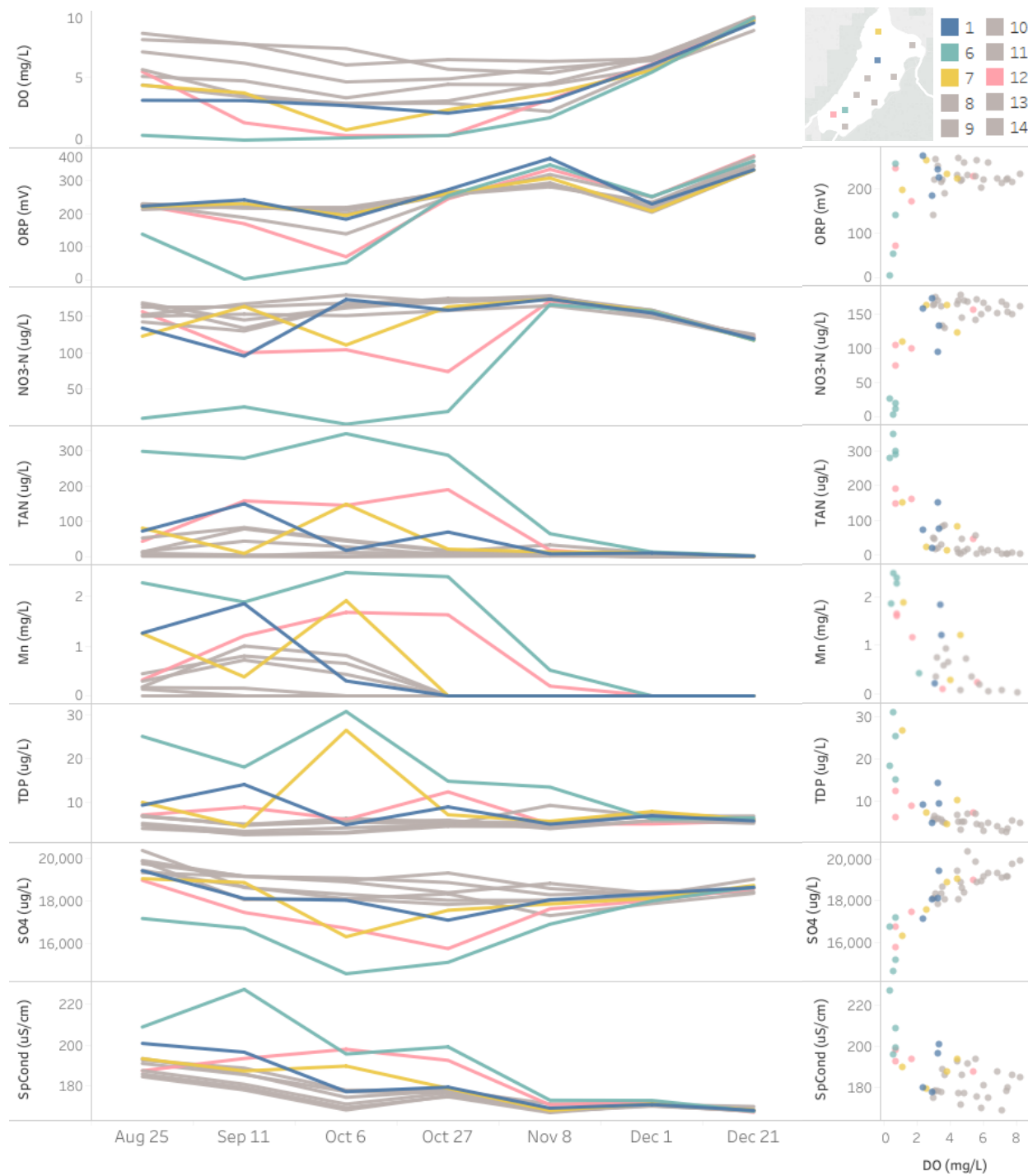


Figure 28. 2017 late-season profundal time series (left) of dissolved oxygen (DO), redox potential (ORP), nitrate (NO₃-N), total ammonia-nitrogen (TAN), manganese (Mn), total dissolved phosphorus (TDP), sulfate (SO₄), and specific conductance (SpCond). The biplots on the right show the relationship between deep dissolved oxygen versus other deep redox-sensitive parameters during August, September and October 2017. All biplots have $n = 40$, with exception for the DO vs. Mn plot which has $n = 28$. Station 1, 6, 7, and 12 are shown in colour.

The late-season evolution of apparent anaerobic microbial decomposition was prominent across the Cultus Lake profundal zone (DFO stations 6,12,1, and 7), with the most pronounced changes in the deepest lake regions, towards the southern extent of Cultus Lake (e.g. DFO stations 6,12). This observed spatial pattern may be influenced by a variety of factors including lake morphometry, heterogeneity in sediment composition, localized watershed nutrient and organic matter loadings and associated organic matter accumulation, and earlier profundal oxygen depletion within the southern deep bathymetric depression, which may have hydrologic connectivity to oxygen-depleted, nutrient-contaminated groundwater from the Columbia Valley Aquifer (Figure 1; Zubel et al. 2000; Putt et al. 2019). Nutrient loadings to Cultus Lake largely arise from watershed runoff, which is enriched by deposition from the nutrient-contaminated Fraser Valley airshed, and inputs from localized septic leaching, agricultural runoff and migratory gull guano (Putt et al. 2019).

Although the various nutrient loadings to Cultus Lake identified by Putt et al. (2019) cumulatively impact overall lake water quality, spatially- and temporally-localized loadings may have more direct influence on proximal receiving areas, particularly during thermal stratification (Wetzel 2001; Jankowski et al. 2014). Nitrogen and phosphorus sources specific to the south end of the lake (DFO stations 6, 12) include intrusions of groundwater enriched by septic leaching from the Columbia Valley and the community of Lindell Beach, and watershed runoff and groundwater enriched by local agricultural activities (Putt et al. 2019). DFO stations 1 and 7 characterize the northern lake profundal zone towards the sole outlet, Sweltzer Creek, receiving localized nitrogen and phosphorus sources, including intrusions of groundwater likely enriched by septic leachate from the Cultus Lake community and proximal communal campgrounds (Putt et al. 2019). Hydraulic flushing toward the outlet may deliver enriched waters from Vedder Mountain (west) and International Ridge (east) creeks, overland flow, and groundwater, towards the north end of the lake (Putt et al. 2019).

Internal Loading: A Positive Feedback Accelerating Lake Eutrophication & Mobilization of Contaminants

Internal loading of sediment constituents into the overlying water column can be a pronounced, deleterious, and self-reinforcing effect of lake eutrophication, with potentially transformative effects on biota and lake ecosystems that can be difficult to reverse once entrenched (Orihel et al. 2017). For instance, redox-sensitive reversals of lake sediments from sinks to sources of limiting nutrients available for autotrophic production can accelerate and/or reinforce eutrophication through induction of feedbacks (i.e. profundal anoxia → enhanced internal nutrient loading from sediments → enhanced primary production → enhanced profundal organic matter

decomposition/anoxia → enhanced internal nutrient loading). Moreover, similar upward evolution of redox-sensitive contaminants under these conditions, can lead to direct stresses on profundal-dwelling fauna (Hamilton-Taylor and Davison 1995; Chandra Sekhar et al. 2004; Zamparas and Zacharias 2014).

The increases in ammonium, manganese and phosphorus near the sediment-water interface observed in our study likely reflect the seasonal evolution of internal loading in Cultus Lake (Figure 28). Observed late-season profundal increases in ammonium ($\text{NH}_4\text{-N}$) are approximately double the decreases in nitrate ($\text{NO}_3\text{-N}$), indicating that nitrate reduction is not the only process contributing to Total Ammonia Nitrogen (TAN) evolution at depth. TAN may also be derived directly from the sediments via internal loading. When the sediment-water interface is oxic, ammonium is adsorbed on sediment particles; however, upon loss of the oxidized microzone, a marked release can occur (Wetzel 2001). Under existing hypolimnetic conditions (i.e. circumneutral pH; relatively cold temperatures), a minimal proportion of total ammonia-nitrogen exists as ammonia; therefore, our observed ammonium concentrations are likely not directly toxic to fish, but rather an important tracer of sediment-profundal exchanges. This relationship may change in the future under climate change, which is warming the deep waters of Cultus Lake (Sumka 2017; Kerker 2020).

When the sedimentary microzone nears anoxia (<1 mg/L), and nitrate becomes depleted, manganese and then iron can be rapidly internally loaded from lake sediments, diffusing into the overlying water column, along with previously-bound phosphate (Mortimer 1941, Mortimer 1971, Laanbroek 1990). Although iron was not measured as part of our study, it is enriched in Cultus Lake sediments (Tovey et al. 2008), and pronounced releases of manganese (at Station 6, 12, 1 and 7) and phosphorus (at Station 6 and 7) were observed in profundal samples during the late-stratified period, coinciding with low oxygen concentrations, indicative of internal loading (Orihel et al. 2017). While additional anthropogenic phosphorus sources including septic leaching, agricultural activities and atmospheric deposition may be contributory to lake enrichment during stratification (Putt et al. 2019), disparity between disproportionate accumulation of these constituents in the profundal region versus the overlying hypolimnion, suggest the lake sediments are the primary source of observed profundal enrichment.

Phosphorus, ultimately the chronic limiting nutrient to primary production across many of the world's lakes (Wetzel 2001; Sterner 2008), is mobilized in association with the redox-sensitivity of iron (Fe), and its supply is a principle concern for accelerated cultural eutrophication of Cultus Lake (Putt et al. 2019; Selbie et al. 2022). While internal loading of phosphorus was clearly evident and pronounced at DFO Station 6, the deepest part of the lake, and at Station 7 during the late-season (Figure 28), only slight increases in SRP and TDP were observed at Station 1 and 12, despite significant

depletion of oxygen at both stations. The lack of clear internal P loading at Station 12 may reflect localized spatial heterogeneity in sedimentary or watershed-delivered sources, and/or influential limnological processes, combined with incomplete depletion of profundal nitrate reserves. The presence of nitrate can keep iron in its oxidized form (Søndergaard et al. 2003), thus preventing the release of iron-bound phosphorus from the sediments, and the contaminated and proximal Columbia Valley aquifer is a potential groundwater nitrate source to southern Cultus Lake (Zubel 2000; Putt et al. 2019). Conversely, the ultimate loss of any such suppressive effect, as eutrophication and climate change proceed, could result in non-linear and amplified internal loading of phosphorus to Cultus Lake, likely ultimately a catastrophic scenario for lake ecosystem structure and functioning supporting multiple species at risk, and societal values alike.

Phosphorus is the principle limiting macro-nutrient for primary production in Cultus Lake, although seasonal co-limitation by P and N occurs (Shortreed 2007; Gauthier et al. 2020). Elevated phosphorus bio-availability is anticipated to lead to even higher phytoplankton production, water turbidity, organic matter delivery to the hypolimnion and associated oxygen depletion, and undesired biological changes such as biodiversity and fish species losses (Søndergaard et al. 2003; Moss et al. 2011; Putt et al. 2019). Phosphorus loading can result in runaway eutrophication (a positive feedback loop), which can persist for decades after nutrient loading has ceased. Even if deep waters remains oxygenated, oxic sediments that have become saturated with phosphorus can continue to release it into the overlying water column, a potentially significant impediment to lake recovery (Søndergaard et al. 2003).

The localized redox state also determines the bioavailability of toxic trace metals (e.g. lead, mercury; Kalff and Downing 2016). Hypolimnetic redox-cycling of manganese and iron can impact the release of other metals from the sediments (Balistrieri et al. 1992b, Morfett et al. 1988), as can detrital sedimentation and decomposition (Wetzel 2001). Concentrations of iron, manganese, zinc, copper, nickel, chromium, barium, cadmium and selenium in the sediments of Cultus Lake all exceed guidelines for sediment quality and protection of aquatic life (Tovey et al. 2008). Of these metals, iron, manganese, zinc, copper and nickel, are micronutrients required for plant and animal growth, but like most metallic micronutrients, are very toxic at elevated concentrations and their toxicity may change markedly with ambient environmental conditions (Wetzel, 2001). Profundal mobilization of metals (i.e. redox solubility, desorption, organic matter decomposition) within the deep waters of Cultus Lake has not yet been fully characterized, but preliminary sampling indicates relationships between declining near-sediment dissolved oxygen and the evolution of methylmercury, arsenic, and manganese in the Cultus Lake profundal zone (Siegel 2021). Given the high degree of organic matter decomposition in the hypolimnion and profundal zone, established redox-sensitive metal and metalloid concentrations within the sediments, and seasonal

limnological conditions conducive for internal loading, it is conceivable that both Cultus Pygmy Sculpin and Sockeye Salmon could be encountering toxins within this most frequented portion of their habitat.

Evidence of profundal sulfate depletion in Cultus Lake may indicate worrisome outcomes for species at risk, should lake eutrophication not be mitigated, as the processes involved can yield evolution of hydrogen sulfide (H_2S), a potent faunal toxicant even at low concentrations (Torrans and Clemens 1982). A strong “rotten-egg” smell was detected in profundal samples at Station 6 (and Station 11), late in the stratified period, likely indicative of the presence of H_2S (Mortimer 1941). These findings likely indicate the onset of sulfate reduction pathways in these deep environments. Hydrogen sulfide is highly toxic to many forms of aquatic life, and often goes undetected in lakes, as it has a limited environmental half-life and is often most pronounced at the sediment-water interface, a portion of the water column that is seldom sampled (Smith and Oseid 1972). Hydrogen sulfide can harm and/or kill adult fish (Kierner et al. 1995), eggs and fry, with toxic effects increasing as oxygen concentrations decrease (Smith and Oseid 1972). If present in substantial amounts, hydrogen sulfide could have severe adverse effects on Cultus Pygmy Sculpin and rearing Sockeye Salmon directly, and/or trophically. Hydrogen sulfide concentrations have not yet been characterized in Cultus Lake, but given the observations of this study, are recommended.

The final process within the sequence of alternative redox reactions is the reduction of carbon dioxide, which can be detected through the appearance of methane (Laanbroek 1990). Neither carbon dioxide nor methane was measured in this study, so there are no indicators to suggest the presence or absence of this process. Given the evidence of precursory microbial decomposition pathways, however, further investigation of methane- CO_2 dynamics at depth are warranted.

Early Seasonal Recovery of Hypolimnetic Oxygen

In 2017, deep oxygen concentrations began recovering in November and early-December, before lake turnover, suggesting alternative delivery mechanism(s) at depth. Annual precipitation maxima occur in November, with modelled watershed recharge maximized through November and December (Holding and Allen 2012). Groundwater contributes significantly to the annual water balance of Cultus Lake (30%), with many shallow, rapidly-flushing, oxic sub-surface inputs occurring along the eastern margin of the lake (Putt et al. 2019). Thus, fall groundwater flushing may, in part, drive fall pre-mixis hypolimnetic/profundal oxygen recovery within Cultus Lake. Moreover, as mean late-fall tributary water temperatures (measured December 1, 2017), were colder than in the lake epilimnion and hypolimnion, and marginally colder than mean lake profundal

temperatures (DFO *unpublished data*), it is also highly likely that plunging inflows from abundantly-fed, high-gradient tributaries deliver cold, highly-oxic water to the deep portions of Cultus Lake during this period. Such seasonal lake cooling and oxygen recharge mechanisms will likely diminish with climate change, potentially intensifying and extending the influence of seasonally-deleterious habitat conditions.

CCME Water Quality Guidelines For the Protection of Aquatic Life Exceedances

The Canadian Council of Ministers of the Environment (CCME) establishes Water Quality Guidelines for the Protection of Aquatic Life (CCME 2018a). Of all the water chemistry parameters measured in this study, guidelines exist for total ammonia, un-ionized ammonia (NH_3), chloride, dissolved oxygen, fluoride, and nitrate. Of these, only fluoride and dissolved oxygen transgressed the guideline thresholds during our sampling window. The life-stage-specific water quality guidelines for dissolved oxygen are a minimum of 9.5 mg/L (early-life stages) to 6.5 mg/L (later life stages) in cold freshwaters (CCME 1999). Despite noted hypolimnetic oxygen recovery prior to destratification, profundal dissolved oxygen concentrations observed during this period remained well below the guideline, and thus are interpreted to be persistently harmful to fish using these habitats.

Mean fluoride measurements from deep samples at Station 6 in September and early-October (i.e. 121 and 193 $\mu\text{g/L}$ respectively) exceed the 120 $\mu\text{g/L}$ CCME guideline for long-term exposure in freshwater environments. The guideline specifically notes that exposure effects levels are significantly lower for anadromous fish species (e.g. Pacific salmon) in freshwaters ($\sim 50 \mu\text{g/L}$; CCME 2002). Anthropogenic fluoride sources are dominated by aluminium smelting and phosphate fertilizer production, with lesser amounts arising from sewage effluents where drinking water is fluoridated (Warrington 1990, Government of Canada 1993) and agricultural runoff containing insecticides and herbicides containing fluorides (Warrington 1990). The most significant natural source arises from fluoride-containing minerals which are leached from bedrock by groundwater, specifically via the weathering of alkalic and silicic igneous and sedimentary rocks (e.g. shales; Warrington 1990). Concentrations of fluoride in creeks may rise during dry seasons when proportional contributions to the hydrograph are derived from groundwater (McNeely et al. 1979 in Warrington 1990).

Seasonally-increased profundal fluoride levels in Cultus Lake may be attributed to multiple sources, but the distinct localization of their emergence in the deepest waters (i.e. southern Cultus Lake), proximal to potential human sources (i.e. community septic/treatment plant, agricultural sources, nutrient-contaminated groundwater) imply that they may, in part, be of anthropogenic provenance. A significant portion of the

watershed is underlain by sandstone and argillite shales, and groundwater directly and indirectly contributes a sizeable portion of the lake's inflow (30%; Putt et al. 2019). Groundwater contributions to Frosst Creek in the Columbia Valley, nearest Station 6, are proportionally higher during the dry season (~100%; Putt et al. 2019), which likely extended to early-October in 2017, based upon the meteorological context. Notably, elevated concentrations of fluoride were not observed Cultus Lake in August 2017, during the peak of dry season, and results from regular monitoring of the Sardis-Vedder aquifer indicate concentrations <100 ug/L (City of Chilliwack 2017), although fluoride and other groundwater constituents may largely be mobilized through substrates via flushing associated with fall precipitation events.

Multiple environmental sources may contribute to observed profundal fluoride enrichment. Agricultural contributions of fluoride to Cultus Lake are possible. The use of fluorinated pesticides have more than doubled in the first two decades of the 21st Century, now accounting for more than half of those in use globally (Alexandrino et al. 2022). Approximately 48% of Cultus Lake's inflows arise from the agricultural Columbia Valley (Putt et al. 2019); however, it is not known to what degree nor or historically, fluoride-containing insecticides or herbicides are used. An aluminium processing plant is located approximately 20 km east of Cultus Lake, but this location does not smelter aluminium (Clayburn Industries Ltd., pers. comm., March 1, 2018), and thus is unlikely an ongoing source. Given the localized nature and timing of the fluoride exceedances, human sewage may be a contributory source. Residential and communal, discharge-to-ground septic systems and treatment plants, of various tenures and loadings, service the Lindell Beach community and increasingly expansive southern Cultus Lake developments, with a history of using "dry pits" (subsurface diffusion boxes) at lower residential densities, and more recently communal rapid infiltration basins (Urban Systems 2012). Significant residential development has occurred in this watershed region over the past decade, which overlies the unconstrained Columbia Valley Aquifer terminating proximal to, and possibly within Cultus Lake (Zubel 2000; Holding and Allen 2012). Any associated increases in septic leachate loadings from discharge-to-ground systems likely represent new leachate exports to Cultus Lake, not captured in the budget modeling of Putt et al. (2019), nor recent sewerage estimations (Urban Systems 2012). Legacy and ongoing fluoride point or diffuse source contributions to the Columbia Valley aquifer may be important sources to Cultus Lake and its species at risk, given their proximity and the broad groundwater intrusions at this locale.

Eutrophication x Climate Warming Interactions: A Focal Threat to Species at Risk Persistence

Climate change and eutrophication are highly-interactive forcings, with warming generally exacerbating the impacts of nutrient enrichment on lake ecosystems (Moss et al. 2011). Cultus Lake is responding to intensifying climate warming (Shortreed 2007, Sumka 2017; Selbie et al. 2022) and nutrient loadings, exhibiting typical symptoms of cultural eutrophication (Shortreed 2007, Putt et al. 2019, Sumka 2017; Selbie et al. 2022; Gauthier et al. 2020; Kerker 2020). Cumulatively these interactive drivers reduce annual deep-water oxygen reserves through prolongation of thermal stratification (i.e. deep-water atmospheric isolation), enhanced aquatic productivity, and associated deep organic matter loading and decomposition (Sumka 2017; Putt et al. 2019; Gauthier et al. 2020; Kerker 2020; Selbie et al. 2022). Lake hydrodynamic models, forced by a regionally down-scaled global climate model (GCM) predict a shift from the current warm monomictic regime (i.e. one mixing period per year) toward oligomixis (i.e. inconsistent inter-annual mixing) over the next ~ 80 years (Sumka 2017). Associated limnological models predict significant and worrisome water column “habitat squeezes” for cold-water fish species in Cultus Lake (Kerker 2020). Specifically, in-season warming and inter-annual heat storage are forecasted to yield inhospitable summer epilimnetic temperatures, and anticipated primary production and associated decomposition at depth are predicted to fuel increasingly exclusionary hypoxic to anoxic conditions within deep cold-water refugia, should lake eutrophication not be reversed (Kerker 2020; Selbie et al. 2022).

While directional climate change is occurring within Pacific North America, the coupled ocean-atmospheric climate system is highly dynamic, marked by quasi-periodic climate oscillations of varying frequencies (e.g. El Nino-Southern Oscillation, Pacific Decadal Oscillation) that enhance (warm phases) or dampen (cold phases) regional warming trends (BC MoE 2016). Placing our observations within the broader inter-annual climatic context, 2017 was a relatively cold year, characterized by a cool spring (Figure 3). Given autochthonous production dominates sedimentary organic matter loads within Cultus Lake (Gauthier et al. 2020), and primary production scales with temperature (Selbie et al. 2022), it stands to reason that the 2017 observations of lake habitat degradation (i.e. organic matter loading, microbial decomposition, hypolimnetic and profundal oxygen depletion, internal loading) were not as severe as they may have been during the preceding hot period (2014–2016), nor under conditions predicted with future climate change (Sumka 2017; Kerker 2020).

Contrasting profundal survey data from April to December of 2014 (DFO *unpublished data*) validate internal loading of nutrients and other sediment constituents as inter-annually-persistent, but that levels of seasonal oxygen depletion, internal loading, and the induction of anaerobic decomposition pathways may be novel and/or

intensifying (Selbie et al. 2022). Despite being a relatively warm year, with oxygen depletion comparable to 2017, there was little indication of profundal anaerobic microbial activity in Cultus Lake in 2014. By contrast, 2014 deep nitrate and TDP averaged 173 µg/L (\pm 31 SD) and 4.2 µg/L (\pm 1.4 SD), respectively, building at depth through the late-stratified period, with little evidence of profundal nitrate reduction (Selbie et al. 2022). Moreover, profundal total ammonia-nitrogen reached a maximum of 90 µg/L at Station 6 on September 5, 2014, compared to that of 349 µg/L on October 6, 2017, which, in the presence of nitrate, likely reflects significant progression of internal loading (Fukuhara and Yasuda 1989). These limnological conditions and their ecological outcomes are tied to rising water temperatures, and thus Cultus Lake will likely degrade in a non-linear fashion under future warming, particularly if the nutrient loadings responsible for lake eutrophication are not addressed in the near-term (Putt et al. 2019; Selbie et al. 2022).

Our study highlights that seasonal hypolimnetic and profundal oxygen depletion is inducing a relatively fixed seasonal sequence of anaerobic microbial processes and internal loading within Cultus Lake, yielding seasonally-deleterious conditions for aquatic fauna within its profundal and benthic zones. Such processes are predicted to intensify, without targeted action to abate identified nutrient loadings (Putt et al. 2019), and have serious potential to rapidly accelerate the lake eutrophication trajectory, through intensification of internal loading (Søndergaard et al. 2003; Orihel et al. 2017). Moreover, species at risk and other faunal exposures to hypoxic waters and fluoride levels deemed to be harmful to aquatic life, amongst other potentially redox-sensitive toxicants (i.e. hydrogen sulfide, metals) is occurring already, with significant potential jeopardize the survival of species at risk, and lake ecosystem services, if eutrophication proceeds unabated.

Freshwater survival of the endangered (COSEWIC assessed) Cultus Lake Sockeye Salmon is linked to temperature- and low oxygen-mediated profundal habitat degradation (Selbie et al. 2022). The exact impact of these environmental conditions on the Cultus Pygmy Sculpin is not yet known, however, spatiotemporal trends in capture data suggest this species at risk may be avoiding, dying or suppressing activity within hypoxic regions resulting from interactions between anthropogenically-forced lake eutrophication and climate change (Sumka 2017; Selbie et al. 2022). Cultus Pygmy Sculpin and Sockeye Salmon share significant overlaps in habitat and life histories, as such, efforts to mitigate stressors for either species at risk, should have mutual positive benefit.

The *Endangered* Cultus Pygmy Sculpin, its critical habitat and residence are all afforded protection under the *Species at Risk Act* (SARA). Cultus Lake Sockeye Salmon, also assessed as *Endangered* are under consideration for listing, which would afford this species the same protections under SARA (SARA 2021; Selbie et al. 2022).

Yet, continued degradation and destruction of critical habitat within Cultus Lake, particularly via cultural eutrophication, a resolvable stressor (Putt et al. 2019; Selbie et al. 2022), is in contravention of the *Species at Risk Act*, directly counters existing SARA implementation plans (i.e. DFO 2017) and Canada's Policy for the Conservation of Wild Pacific Salmon (DFO 2005), and likely jeopardizes the survival of both species at risk (DFO 2017, 2020).

Recommendations And Future Research

As detailed by Putt et al. (2019) and DFO (2020), cultural eutrophication is the primary threat to Cultus Lake and persistence of its species at risk, requiring local- to regional-scale actions to reverse. Lake eutrophication is a cumulative effects problem, arising from both point and diffuse source nutrient loadings, and thus the targeted abatement of each source, irrespective of magnitude, will contribute to overall water quality and critical habitat improvements (Schindler 2006; Selbie et al. 2022). Mitigation of within-watershed nutrient sources (e.g. agricultural runoff, septic leachate, gull guano) is critical, and steady-state models predict significant improvement in relevant water quality endpoints (i.e. phosphorus, nitrogen, chlorophyll) that will slow the rate of eutrophication advancement, but that reversal of this trajectory will ultimately require abatement of nutrient entrainment in the regionally contaminated airshed, requiring interdisciplinary and inter-jurisdictional efforts to resolve (Putt et al. 2019). Hereafter we propose several areas in which enhanced monitoring, research, and action are likely to reduce the degradation of Cultus Lake and improve the fate of its species at risk.

Targeted Research and Action to Abate Primary Nutrient Loadings to Cultus Lake

Significant and targeted efforts are required to address the complex nutrient loading to Cultus Lake. Putt et al. (2019) has quantified the various sources and loadings of nutrients from the watershed and airshed, and hind-cast modeling and paleolimnology provide informed ecological recovery states and water quality targets for mitigation (Putt et al. 2019; Gauthier et al. 2020). It is clear, however, that abatement of the primary nutrient sources (i.e. atmospheric deposition, avian guano loading, septic leachate,) will require inter-jurisdictional cooperation and interdisciplinary knowledge. As such, focussed coalescence and engagement of technical expertise (i.e. aquatic sciences, atmospheric sciences, agricultural sciences, problem bird controls, liquid waste management) and governance (e.g. municipal, provincial, federal regulators) are necessary to resolve the nutrient pollution of Cultus Lake. This interdisciplinary focus is required to formulate and enact feasible strategies to abate specific loadings, with several strategies already proposed in the literature (Putt et al. 2019). It should be noted

that with internal loading and climate change, however, the rate of lake eutrophication may increase rapidly, making near-term and substantive action to mitigate nutrient pollution to Cultus Lake increasingly important. Given this context, and the longer-term horizon to resolve large-scale and influential stressors like atmospheric loading of nutrients, interim habitat restoration efforts (e.g. hypolimnetic oxygenation) are recommended to sustain Cultus Lake species at risk, while such primary drivers can be addressed.

Further Research on the Biology and Ecology of Cultus Pygmy Sculpin

Given the challenges associated with observing the Cultus Pygmy Sculpin in its natural habitat, our understanding of its basic biology, ecology and susceptibilities to environmental stresses remain incomplete. Basic research into spawning behaviour and timing, predator-prey interactions, physiological and behavioural thresholds to changing habitat conditions (i.e. high temperature, low oxygen), and potentially other critical habitat needs, are required to further contextualize the impact of ongoing lake degradation across the various life history stages of the Cultus Pygmy Sculpin, and focus actions conserve them.

Continued Critical Habitat Monitoring and Research for Multiple Species at Risk

Cultus Lake, as critical habitat for Cultus Pygmy Sculpin and important freshwater habitat for Cultus Sockeye Salmon, has degraded significantly over the past century (Gauthier et al. 2020), yielding many potential contemporary ecological interactions threatening their persistence. Cumulative anthropogenic stressors, and their interactions, are evolving in complex ways. It is recommended that the ongoing vessel-based and autonomous monitoring of the lake, which have been instrumental in defining the imperilment context for both species, continue, providing indication of population abundances, critical habitat status, and tracking the efficacy of any mitigation efforts. Additionally, targeted monitoring and research that characterizes both Cultus Pygmy Sculpin and Cultus Sockeye Salmon behaviour within the lake, provides a meaningful population estimate/index for Cultus Pygmy Sculpin, and further elucidates emerging threats to persistence (i.e. profundal toxicants, future climate interactions) are recommended. Moreover, as predictive modeling on future habitat states evolve (Sumka 2017; Kerker 2020), it is advised that ecosystem models be developed, and linked to known drivers of habitat and populations, permitting informed prediction of habitat-species at risk interactions, particularly under climate change.

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APPENDICES

Appendix 1. Locational information for minnow trap transects and DFO water monitoring stations.

Table 1. Minnow trap line locations

Location	Alternate Name	Substrate Depth (m)	Latitude	Longitude
Salmon Bay	.	5	49.03680	-122.00390
Salmon Bay	.	10	49.03682	-122.00393
Salmon Bay	.	20	49.03698	-122.00387
Salmon Bay	.	30	49.03715	-122.00383
Salmon Bay	.	40	49.03867	-122.00332
West Shore	Needle Point	5	49.05288	-121.99692
West Shore	Needle Point	10	49.05282	-121.99675
West Shore	Needle Point	20	49.05268	-121.99648
West Shore	Needle Point	30	49.05260	-121.99628
West Shore	Needle Point	40	49.05235	-121.99565
Main Beach	.	5	49.07133	-121.98490
Main Beach	.	10	49.07125	-121.98485
Main Beach	.	20	49.07102	-121.98467
Main Beach	.	30	49.07073	-121.98448
Main Beach	.	40	49.06912	-121.98347
Entrance Bay	.	5	49.05842	-121.96412
Entrance Bay	.	10	49.05857	-121.96433
Entrance Bay	.	20	49.05882	-121.96467
Entrance Bay	.	30	49.06028	-121.96647
Entrance Bay	.	40	49.06272	-121.97463
Spring Bay	Jade Bay	5	49.04900	-121.97537
Spring Bay	Jade Bay	10	49.04925	-121.97607
Spring Bay	Jade Bay	20	49.05067	-121.97790
Spring Bay	Jade Bay	30	49.05082	-121.98172
Spring Bay	Jade Bay	40	49.05185	-121.98537

Table 2. DFO Water monitoring station locations

Location	Substrate Depth (m)	Station Depth (m)	Latitude	Longitude
Station 1	41	41	49.057500	-121.984117
Station 6	43	43	49.042700	-121.998683
Station 7	40	40	49.065933	-121.983817
Station 8	34	34	49.061933	-121.968633
Station 9	36	36	49.052600	-121.976817
Station 10	35	35	49.044983	-121.985333
Station 11	39	39	49.037750	-121.998650
Station 12	42	42	49.041183	-122.003700
Station 13	40	40	49.047133	-121.993333
Station 14	40	40	49.052017	-121.988517