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Oxythermal habitat as a primary driver of ecological niche and genetic diversity in
cisco (*Coregonus artedii*)

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analysis

Abstract

Cisco (*Coregonus artedii*) are threatened by climate change and lake eutrophication, and their oxythermal habitat can be assessed with T_{DO3} , the water temperature at which dissolved oxygen equals 3 mg L⁻¹. We assessed the influence of T_{DO3} on cisco habitat use, genetic diversity, diets, and isotopic niche in 32 lakes ranging from oligotrophic to eutrophic. Results showed that as T_{DO3} increased cisco were captured higher in the water column, in a narrower band, with higher minimum temperatures and lower minimum dissolved oxygen. T_{DO3} was also negatively related to cisco allelic richness and expected heterozygosity, likely driven by summer kill events. Moreover, T_{DO3} influenced the isotopic niche of cisco, as fish captured deeper were more depleted in $\delta^{13}C$ and more enriched in $\delta^{15}N$ compared to epilimnetic baselines. Lastly, cisco in high T_{DO3} lakes consumed more *Daphnia*, had fewer empty stomachs, and achieved larger body size. Our work identifies specific characteristics of cisco populations that respond to climate change and eutrophication effects, and provides a framework for understanding responses of other cold-water species at the global scale.

Introduction

Cisco (*Coregonus artedii*) are a cold-water pelagic fish found in many North American lakes, and provide an important energetic link to pelagic energy sources for gamefish such as walleye (*Sander vitreus*; Kaufman et al. 2009), muskellunge (*Esox masquinongy*; VanderBloemen et al. 2020), lake trout (*Salvelinus namaycush*), and northern pike (*Esox lucius*; Kennedy et al. 2018). Cisco show a high degree of variability in their morphology, behavior, and diets, allowing them to inhabit diverse aquatic habitats (Turgeon et al. 2016; Eshenroder and Jacobson 2020). Within-lake variability in habitat use varies among smaller inland lakes in North America, with cisco displaying multiple patterns of diel migration. The most common pattern is

normal diel vertical migration (DVM), where cisco move deeper in the water column during the day and higher at night, yet other populations exhibit reverse DVM or no daily migration pattern at all (Ahrenstorff et al. 2013). Additionally, patterns of average depth utilized by cisco varies across lakes, with average cisco depths ranging from 7 to 30 m in mesotrophic lakes in Minnesota (USA; Ahrenstorff et al. 2013). Ahrenstorff (2013) concluded these differences in habitat use are driven by cisco size and abundance and types of prey available in each lake. Not surprisingly, cisco diets also vary among lakes, with some diets dominated by *Daphnia*, others showing high proportions of benthic invertebrates (*Chaoborus spp.*, *Hexagenia spp.*), and some focused on copepods (Ahrenstorff et al. 2013; Jacobson et al. 2018).

Phenotypic plasticity likely aided the selection pressures that enable Cisco to inhabit a wide range of lakes ranging from oligotrophic to eutrophic (Jacobson et al. 2020). In Minnesota, cisco are found in 648 lakes that range from oligotrophic to eutrophic moving from the northeastern to central part of the state (Jacobson et al. 2010). As cold-water species with relatively high requirements for oxygen, water temperature is a key factor for growth rate and metabolism for coregonines (Dabrowski 1985). Subsequently, cisco are threatened by increasing water temperatures from climate change, longer durations of stratification that reduce oxygenated-hypolimnetic volume, and by declining oxygen levels in the hypolimnion due to eutrophication (Magnuson et al. 1997, Stefan et al. 2001, Jacobson et al. 2010). Threats of both eutrophication and climate change for cisco habitat are further complicated by invasive species such as zebra mussels (*Dreissena polymorpha*) that can shift energy pathways towards lake littoral zones (McEachran et al. 2019; Morrison et al. 2021). Cisco in eutrophic lakes in the upper Midwest of the USA lie near the southern edge of their native range (Jacobson et al. 2013; Honsey et al. 2016; Renik et al. 2020), were most likely stressed by historic oxygen and

temperature patterns, and are subsequently among the first to be further stressed by anthropogenic driven changes in lake temperature and oxygen.

The dual effects of eutrophication and climate change across the lake productivity gradient causes cisco to inhabit the upper water column possessing higher temperatures in impacted lakes, which in turn has been shown to impact cisco morphology (Jacobson et al. 2020) and stable isotope patterns regardless of diets (Zimmer et al. 2020). A useful metric for quantitatively measuring thermal habitat quality for cold-water fishes is the water temperature at which dissolved oxygen drops below 3 mg L⁻¹ (T_{DO3}; Jacobson et al. 2010). This dissolved oxygen threshold is a useful benchmark because if levels drop below 3 mg L⁻¹ many cold-water fish species such as cisco, lake trout, and burbot (*Lota lota*) cannot meet their respiration demands (Jacobson et al. 2010). T_{DO3} is positively related to lake productivity (Zimmer et al. 2020) with lower T_{DO3} values indicating higher habitat quality (Jacobson et al. 2010). T_{DO3} has also been shown to influence both average capture depth and body shape of cisco across lakes (Jacobson et al. 2020), as well as likelihood of cisco summer kill when cisco are forced to inhabit waters that do not meet their respiration demand (Jacobson et al. 2012). An example of how T_{DO3} relates to oxythermal habitat and its influence on habitat use of cisco is shown in Figure 1.

Lake productivity gradients could also impact cisco diets through effects on both abundance and access to prey. Cisco diets are variable, but they often feed heavily on zooplankton with more limited consumption of benthic prey (Engel 1976; Ahrenstorff et al. 2013). Zooplankton abundance generally increases with lake productivity (Pace 1986), and increased hypoxia at higher nutrient levels may limit cisco access to benthic prey (Ahrenstorff et al. 2013). Thus, cisco diets may shift away from benthic prey to large-bodied zooplankton such as *Daphnia* as lake productivity increases. Aku and Tonn (1999) support this prediction; in a

divided lake experiment they showed that cisco in the half supplied with artificially oxygenated water fed on pelagic and benthic prey, while hypolimnetic hypoxia in the non-oxygenated side of the lake forced cisco to feed only on pelagic prey in the metalimnion and epilimnion.

Finally, cisco populations constrained into the warm water layers in eutrophic lakes with hypoxic hypolimnia (high T_{DO3} lakes) and experiencing more frequent summer kill events (Jacobson et al. 2012) could have increased levels of inbreeding and reduced genetic diversity at the population scale via bottleneck effects (Amos and Hardwood 1998; Vonlanthen et al. 2012; Hirsch et al. 2013; Alexander et al. 2017). In combination, these genetic effects could make populations more vulnerable to extirpation (Hellmair and Kinziger 2014; Frankham 2015) and reduce their ability to adapt to future environmental changes. Lyons et al. (2018) observed lower relative abundance of cisco in lakes with poor oxygen and temperature environments, and Jacobson et al. (2012) showed that climate change and eutrophication were principal causes of cisco decline in Minnesota lakes. Additionally, being forced into warmer water layers may also expose cisco to increased predation pressure from predators like northern pike, which could lead to declines in cisco abundance as well as changes to cisco life history strategies (Jacobson et al. 2012).

In this study we sampled cisco in 32 Minnesota lakes that ranged from oligotrophic to eutrophic. These lakes have been the focus of previous research that provided insights on the morphological response of cisco to productivity gradients (Jacobson et al. 2020), the influence of lake productivity on stable isotope patterns across lakes (Zimmer et al. 2020), and the ability of cisco to retain morphological and ecological traits when translocated from Lake Superior to inland lakes (Jacobson et al. 2018). The present study aims to build upon this body of work by assessing effects of the oxythermal habitat on cisco habitat use, isotopic patterns, diets, genetic

diversity, and their physical-chemical environment within and across lakes. We hypothesize that cisco habitat use, stable isotope values, diets, and genetic diversity will change in concert across lake oxythermal gradients on a broad geographic scale. In lakes containing low quality oxythermal habitat we expect cisco would be captured higher in the water column in warmer water with less oxygen, consume more epilimnetic zooplankton and less benthic prey, have isotopic values that reflect zooplankton prey and foraging higher in the water column, and have lower genetic diversity relative to cisco from systems with high quality oxythermal habitat.

Methods

Study Design

From 2013 to 2015 we sampled 32 lakes dispersed across 38,500 km² of Minnesota, USA, with the lakes ranging from oligotrophic to eutrophic with a wide range in physical characteristics (Table 1). Several lakes contained invasive species (Table 1), but preliminary analyses indicated no effects of invasive species on any of our response variables. Lakes spanned from the Canadian Shield in northeastern Minnesota, a region with large expanses of exposed igneous rock and shallow soils with lakes formed by direct glacial gouging, to the glacial till region of south-central Minnesota, a region with deeper soils with a mixture of end moraine and kettle lakes. Lakes on the shield are often deep (> 17 m) with few dissolved solids and low productivity (total phosphorus < 18.3 µg L⁻¹), while lakes in the till region are often shallow (< 17 m) with higher dissolved solids and productivity (total phosphorus > 18.3 µg L⁻¹, Jacobson et al. 2010). One lake was sampled on 25 June, while all others were sampled between 15 July and 26 August. Each lake was sampled once, and all sampling (water temperature and dissolved oxygen, zooplankton, mussels, and cisco) was conducted within a two-week span for each lake.

Additional sampling dates would have been useful for assessing temporal changes in T_{DO3} , but we were unable to do so. However, we chose our sampling window to bracket 11 August, which is the average date of maximum T_{DO3} in stratified Minnesota lakes (Jacobson et al. 2010). Thus, our study design was focused on the time period when T_{DO3} effects on cisco should be maximized. Logistical constraints prevented us from collecting all data on fish from all the lakes, which resulted in 32 lakes used in our genetic analysis, 21 lakes each for capture depth, dissolved oxygen-temperature at capture depth and diet data, and 20 lakes for our isotope data set (Table S1 and S2). All types of fish data (genetics, capture depth, diet, and isotope data) were collected for 18 lakes.

Field Methods

We measured temperature-oxygen profiles in 21 lakes at the deepest location in each lake using an optical YSI Dissolved Oxygen Pro to estimate T_{DO3} (Jacobson et al. 2010). T_{DO3} values at the deepest depth should have the lowest T_{DO3} value and thus represent the most optimal lake-wide habitat for cisco. T_{DO3} for our remaining sites was estimated from data collected by the Minnesota Department of Natural Resources or Pollution Control Agency ($n=8$) or taken from the literature using the same methods that we used ($n=3$; Fang et al. 2010; Jiang et al. 2017). The 11 lakes with T_{DO3} estimated by others were used for the genetic analysis only.

Cisco were collected in the field using vertical gill nets used by the Minnesota Department of Natural Resources to sample pelagic fish in deep lakes (Jacobson et al. 2018). The nets consist of seven 61 m deep panels of monofilament webbing (bar-measure mesh size x panel width): 10 mm x 0.9 m, 13 mm x 0.9 m, 19 mm x 1.2 m, 25 mm x 1.8 m, 32 mm x 3.0 m, 38 mm x 3.0 m, 44 mm x 3.0 m). The 10 mm and 13 mm panels were sewn together vertically into one

net, as were the 19 mm and 25 mm panels, while the 32 mm, 38 mm, and 44 mm panels were used individually for a total of five separate vertical nets. Each net was ganged together by a 2 m connecting rope and the gang (5 nets with 7 panels) was set as a unit in the deepest basins of a lake. One gang was usually set in simple lake basins with one deep area, while a maximum of three gangs were set in complex lake basins with multiple deep areas. Each net was deployed to sample the entire water column and was fished for 24 hrs. Up to 70 cisco were processed from each lake and visually selected by the Minnesota Department of Natural Resources to represent the size range of all cisco captured in the gill nets. Selected cisco were measured for total length, assigned a sex and maturity designation, and their capture depth was recorded. The cisco were then placed on ice. Dissolved oxygen and temperature at their capture depth was also recorded based on the temperature-oxygen profiles. In the lab diet samples were collected by removing the stomach and rinsing contents of the whole stomach into sample jars using 95% ethanol. Dorsal tissue muscle for isotope analysis was removed with a fillet knife and frozen. Fin clips were collected and preserved in 100% ethanol for genetic analysis. The abundance of zooplankton prey in the hypolimnion was estimated by vertically towing a 153 μ m closing plankton net from one meter off the bottom to the top of the hypolimnion at three of the deepest locations in each lake during the day, and the net contents were then preserved in 70% ethanol. Benthic invertebrates were sampled at the same three locations following the zooplankton tows using a ponar grab, rinsed through 500 μ m mesh, and preserved with 70% ethanol. We also collected up to five mussels from epilimnetic habitats in each lake to use for baseline corrections for our isotope analyses (Post 2002). Mussels were placed on ice and frozen upon return to the lab.

Capture Depth Analysis

We controlled for differences in lake depth on capture depths of cisco by calculating the metric relative capture depth (RCD; Jacobson et al. 2020), calculated as $[(\text{bottom depth} - \text{capture depth})/(\text{bottom depth} - \text{thermocline depth})]$ for fish below the thermocline, and $[(\text{thermocline depth} - \text{capture depth})/(\text{thermocline depth}) + 1]$ for fish above the thermocline. Therefore, fish captured on the lake bottom, thermocline, and lake surface have RCD values of 0, 1, and 2, respectively. Our previous work has shown that T_{DO_3} has a negative effect on lake-mean RCD of cisco (Jacobson et al. 2020). However, within-lake variability of cisco RCD and the role that fish body size may play is unknown. Thus, we used a mixed linear effects model to test for significant within-lake effects of cisco length (centered within each lake to control for differences in lengths among lakes) on RCD with lake as a random factor effect and length and length*lake as fixed effects. We subsequently tested whether lake intercepts were related to lake T_{DO_3} values, and used Akaike's information criterion corrected for sample size (AIC_C) to determine whether lake slopes were related to T_{DO_3} , lake maximum depth, and lake-mean proportion of benthic prey or Chironomidae in diets. We also used linear regression to test for effects of T_{DO_3} in each lake on minimum, maximum, and range of cisco RCD, actual capture depth below surface (m), dissolved oxygen at capture depth (mg L^{-1}), and water temperature at capture depth ($^{\circ}\text{C}$).

Genetic Diversity Analysis

Cisco were genotyped at eight microsatellite DNA loci to measure population genetic diversity. Genotyping methods were as described in Jacobson et al. (2018) except that two loci (ClaTet12 and ClaTet15) were removed because they had excessive deviations from Hardy-Weinberg equilibrium in this larger sample collection. For each lake sample, expected heterozygosities (H_e) were obtained using Genepop 4.2 (Raymond and Rousset 1995; Rousset

2008). Genepop was also used to test for deviations from Hardy-Weinberg equilibrium followed by a correction for multiple testing with a false discovery rate of 5% (Benjamini and Hochberg 1995). During this preliminary testing; significant deviations from Hardy-Weinberg equilibrium were indicated for 12 of 250 tests. Deviations were not associated with specific samples or loci, which might have indicated population or genotyping anomalies, so all data were deemed suitable for assessing diversity measures and we proceeded with our formal analyses. Allelic richness was calculated using HP-Rare (Kalinowski 2005), with a sample size of 16 alleles (the minimum sample size in the data set; i.e., twice the number of fish genotyped from St. Mary's Lake). This rarefaction method standardizes allelic diversity to a common sample size to allow comparisons among unequally sampled populations. We tested for significant effects of T_{DO3} on genetic diversity (both expected heterozygosity and allelic richness) using two approaches. First, we tested for a linear relationship with T_{DO3} using simple linear regression on both variables. Second, using Fang et al.'s (2012) classification of T_{DO3} values less than 11°C as cisco refuge lakes, we predicted that lakes with T_{DO3} less than 11°C would have higher expected heterozygosity and allelic richness than lakes with values higher than 11°C. We tested for differences in heterozygosity and allelic richness between these two groups of lakes using two-sample *t*-tests. We also tested for a significant relationship between lake size and expected heterozygosity and allelic richness. We then used AIC_C to assess a) whether our continuous or categorical T_{DO3} variable best fit the data, and b) whether the best T_{DO3} model could be improved by including lake size as both an additive and interaction effect with T_{DO3} .

Diet and Isotope Analysis

Contents of cisco diets were examined with a dissecting scope and prey items were identified to genus level for Cladocera, order for copepods, class for ostracods, and family for

235 insects. All prey were identified, counted, and morphometric length measurements were taken on
236 up to 30 individuals from each taxonomic group using an Olympus cellSens Image Analysis
237 System (Olympus Corporation). Individual dry weights were estimated using published length-
238 weight regressions (Smock 1980; McCauley 1984; Traina and von Ende 1992; Branstrator
239 2005), average dry weights were determined for each type of prey in each diet, and mean dry
240 weight of each prey was multiplied by number of prey to estimate total dry mass of each prey
241 type in each diet. Results for each taxa were expressed as proportion of the total dry mass of each
242 diet for statistical analysis; along with the composite variable benthic prey, which consisted of
243 the sum percentage of *Chaoborus* and non-emerging Chironomidae larva along with other rarer
244 taxa such as *Hexagenia* mayflies. Zooplankton in vertical lake tows were identified to the same
245 taxonomic level as diets and counted, and density estimates were corrected for water volume
246 towed and expressed as number L⁻¹ for each taxonomic group. Benthic invertebrates in ponar
247 samples were identified to the same taxonomic level as diets and expressed as number m⁻². We
248 used the average across the three vertical tows for zooplankton and the average across the three
249 ponar grabs for benthic invertebrates for each lake for statistical analyses.

250 The dorsal muscle tissue from cisco and the soft tissue from mussels were both dried to a
251 constant weight at 60°C, ground, and analyzed for carbon ($\delta^{13}\text{C}$) and nitrogen ($\delta^{15}\text{N}$) stable
252 isotopes by the UC-Davis Stable Isotope Facility using a PDZ Europa ANCA-GSL elemental
253 analyzer interfaced to a PDZ Europa 20-20 isotope ratio mass spectrometer (Sercon, Cheshire,
254 UK). Analytical precision (SD) was $\pm 0.2\text{‰}$ for $\delta^{13}\text{C}$ $\pm 0.3\text{‰}$ for $\delta^{15}\text{N}$, respectively. Final δ
255 values are given as ratios of $^{13}\text{C}: ^{12}\text{C}$ and $^{15}\text{N}: ^{14}\text{N}$ relative to the international standards Vienna
256 PeeDee Belemnite and air for carbon (C) and nitrogen (N), respectively (Peterson and Fry 1987).
257 $\delta^{13}\text{C}$ estimates were lipid corrected using equation 3 in Post et al. (2007).

For our isotope analysis, we accounted for natural variability in $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ among lakes by using mussel isotope values as a baseline correction where the mean lipid-corrected $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ for mussels in each lake were subtracted from the values of each cisco (Post 2002). Hereafter all reported isotope data are lipid and baseline corrected and are denoted as $\delta^{13}\text{C}_{\text{DF}}$ and $\delta^{15}\text{N}_{\text{DF}}$ (DF signifies difference between mussel and cisco isotope values). $\delta^{13}\text{C}_{\text{DF}}$ is usually analyzed to provide an estimate of littoral versus pelagic C use by lake organisms because pelagic C is more depleted relative to littoral C and only enriches 0.4‰ for each trophic transfer, while $\delta^{15}\text{N}_{\text{DF}}$ is usually used to estimate trophic position of organisms as it enriches 3.4‰ on average with each trophic transfer (Vander Zanden and Rasmussen 1999, 2001; Post 2002). Given that mussels feed on epilimnetic phytoplankton, cisco consuming epilimnetic zooplankton should have $\delta^{13}\text{C}_{\text{DF}}$ and $\delta^{15}\text{N}_{\text{DF}}$ values of 0.4‰ and 3.4‰, respectively. However, recent work has shown that $\delta^{13}\text{C}_{\text{DF}}$ and $\delta^{15}\text{N}_{\text{DF}}$ can vary for reasons beyond diet, including changes driven by depth use in organisms, with $\delta^{13}\text{C}_{\text{DF}}$ becoming more depleted and $\delta^{15}\text{N}_{\text{DF}}$ becoming more enriched with increasing depth (Vander Zanden and Rasmussen 1999). Our previous work has shown that overall lake productivity, through effects on lake biogeochemistry, influences lake-average cisco $\delta^{13}\text{C}_{\text{DF}}$ and $\delta^{15}\text{N}_{\text{DF}}$ values across lakes (Zimmer et al. 2020). However, within-lake isotope patterns remain unknown, specifically whether $\delta^{13}\text{C}_{\text{DF}}$ and $\delta^{15}\text{N}_{\text{DF}}$ of individual fish vary with RCD within lakes. Fish length may also be related to cisco isotope values given changes in optimal water temperature with body size in fish (Morita et al. 2010), such that larger fish may inhabit deeper, colder water. Benthic prey such as Chironomidae may also have strong effects on within-lake $\delta^{13}\text{C}_{\text{DF}}$ values as their tissue can be strongly depleted in $\delta^{13}\text{C}$ due to consumption of methanotrophic bacteria (Jones et al. 2008), while *Chaoborus* could increase cisco $\delta^{15}\text{N}_{\text{DF}}$ as they also feed on zooplankton and would add an extra step of $\delta^{15}\text{N}_{\text{DF}}$

enrichment to the cisco that consume them. We therefore tested for within-lake effects on both cisco $\delta^{13}\text{C}_{\text{DF}}$ and $\delta^{15}\text{N}_{\text{DF}}$ using mixed models on 18 lakes (with lake as a random factor effect), and used AIC_C to assess competing models comprised of combinations of centered log cisco length, RCD, proportion *Chaoborus* prey, and proportion Chironomidae prey as well as possible interactions between the variables.

We log transformed the following variables to increase homoscedasticity of residuals: T_{DO_3} , cisco length, and minimum, maximum, and range of depth of capture, RCD, temperature at capture depth, and dissolved oxygen at capture depth. All univariate analyses were performed with JMP version 15 (SAS 2019), and $P < 0.05$ was our significance threshold for all analyses.

Multivariate Analysis

Finally, we used a multivariate approach to test for significant effects of T_{DO_3} , maximum lake depth, and lake surface area at the lake-average scale for all variables we measured on individual cisco, including diet proportions (proportion of calanoids, cyclopoids, *Chaoborus*, Chironomidae, and *Daphnia*), proportion of empty stomachs, diet richness (# of different prey in diets), maximum cisco body size, RCD, temperature and dissolved oxygen at cisco capture depth, expected heterozygosity, allelic richness, $\delta^{13}\text{C}_{\text{DF}}$, and $\delta^{15}\text{N}_{\text{DF}}$. This analysis was conducted on the 18 lakes for which we had full data sets for all variables, and we used the lake-mean value for each response variable given our explanatory variables (T_{DO_3} , lake depth, and lake area) are whole-lake factors. A lake-by-lake breakdown of lake-mean values is provided in Table S1 and S2. We used redundancy analysis (RDA, Zuur et al. 2007) to test for effects of the predictor variables on the response variables, with the analysis conducted on the correlation matrix (standardized RDA) to account for different scales among the response variables. Statistical

significance was assessed with permutation tests (1,000 permutations): the reported P value is the proportion of times the explanatory variables in the randomly generated data explained more variance than did the original data set. We also assessed whether abundance of prey in the lake was related to diet proportions by using lake abundance of cycloid copepods, calanoid copepods, *Daphnia*, Chironomidae larva, and *Chaoborus* larva as supplementary variables in our RDA analysis. Supplementary variables are not used in the main RDA test with explanatory variables, but are subsequently assessed for their correlation relationships with lake scores on axis 1 and 2 derived in the main RDA analysis. RDA was performed using Canoco 5 (ter Braak and Šmilauer 2012).

Results

Relative Capture Depth

Our data set for cisco capture depth consisted of 21 lakes with an average of 21 cisco per lake (range 8 to 33). Cisco RCD was not related to differences in fish length among lakes ($P = 0.052$), but there was a significant fish length*lake interaction ($P < 0.001$) indicating a different relationship between RCD and fish length within lakes. Slopes ranged from one lake strongly positive, several lakes with approximately flat slopes, to many lakes with moderately negative slopes (Fig. 2A). Lake intercepts were positively related to T_{DO3} ($P < 0.001$), indicating that fish were caught higher in the water column as T_{DO3} increased (Fig. 2B). Lake slopes showed a significant negative relationship with lake-mean proportion of Chironomidae in diets ($P < 0.001$; Fig. 2C), and this variable had greater support than variables in all other candidate models (all $\Delta AIC_C \geq 6.7$; Table 2). Overall, our results indicate that negative slopes between cisco length

and capture depth within lakes occurred where the larger cisco in the population fed more heavily on benthic Chironomidae.

T_{DO3} had a significant positive effect on both minimum ($P < 0.001$; Fig. 3A) and maximum ($P = 0.014$; Fig. 3B) temperature at capture depth of cisco, but was not related to range of temperature at capture depth ($P = 0.406$; Fig. 3C), indicating that the distribution of cisco shifted to occupy warmer water in the upper water column as T_{DO3} increased but maintained a similar range of temperatures. Differences in maximum temperature across the T_{DO3} gradient were substantial, with predicted maximum water temperatures of 15°C at $T_{DO3} = 5^{\circ}\text{C}$ increasing to 24°C when $T_{DO3} = 22^{\circ}\text{C}$. Patterns for dissolved oxygen at capture depth differed from temperature, with minimum dissolved oxygen being negatively related to T_{DO3} ($P < 0.001$; Fig. 3D), maximum dissolved oxygen showing no relationship ($P = 0.379$; Fig. 3E), and range of dissolved oxygen being positively related ($P = 0.023$; Fig. 3F). These results demonstrate that cisco were captured in similar maximum levels of dissolved oxygen across the T_{DO3} gradient, but in high T_{DO3} lakes some cisco were also captured in much lower dissolved oxygen levels, increasing the range of dissolved oxygen exposure. Differences in minimum dissolved oxygen across the T_{DO3} gradient were also pronounced, with a minimum predicted value of 5.9 mg L^{-1} when $T_{DO3} = 5^{\circ}\text{C}$, decreasing to 0.4 mg L^{-1} at $T_{DO3} = 22^{\circ}\text{C}$.

T_{DO3} also had significant positive effects on both minimum ($P < 0.001$; Fig. 4A) and maximum ($P = 0.005$; Fig. 4B) RCD, but no effect on RCD range ($P = 0.478$; Fig. 4C), indicating cisco range shifted higher in the water column as T_{DO3} increased but maintained a similar width in the RCD metric. T_{DO3} was also negatively related to both minimum ($P < 0.001$; Fig. 4D) and maximum ($P < 0.001$; Fig. 4E) capture depth. However, in contrast to the results for RCD, the range of actual capture depth was negatively related to T_{DO3} ($P = 0.003$; Fig. 4F).

This difference was because RCD converts the hypolimnion depth from 0 to 1 and the epilimnion depth from 1 to 2, yet the actual hypolimnion is thicker than the epilimnion in our lakes. Cisco habitat use was sharply constrained at high T_{DO3} , as cisco capture depth ranged across 19 m of the water column at $T_{DO3} = 5^{\circ}\text{C}$ but fell to a range of just 5 m at $T_{DO3} = 22^{\circ}\text{C}$.

Genetic Diversity

Our final genetic data set consisted of 32 lakes with an average of 25 cisco per lake (range 8 to 70). T_{DO3} was inversely related to genetic diversity of our cisco populations. Treating T_{DO3} as a continuous variable showed a negative linear effect on both expected heterozygosity ($P = 0.004$) and allelic richness ($P = 0.009$), while treating T_{DO3} as a categorical variable similarly showed lakes with $T_{DO3} > 11^{\circ}\text{C}$ had significantly lower expected heterozygosity ($P < 0.001$) and lower allelic richness ($P = 0.003$). Lake surface area has also been shown to have significant, positive effects on genetic diversity of fish (Cena et al. 2006; Valiquette et al. 2014). For our lakes lake surface area was positively related to allelic richness ($P = 0.024$) but showed no relationship with expected heterozygosity ($P = 0.120$). AIC_C values indicated the most parsimonious model overall was categorical T_{DO3} + lake surface area for both expected heterozygosity and allelic richness (Table 3), showing genetic diversity was highest in large lakes with low T_{DO3} (Fig. 5A, B).

Diets and Stable Isotopes

Our data set for cisco diets was the same as our dataset for RCD but for cisco isotope data it consisted of 20 lakes with an average of 21 cisco per lake (range 8 to 45). The AIC_C chosen model for predicting $\delta^{13}\text{C}_{DF}$ in individual cisco was RCD ($P = 0.016$), proportion Chironomidae in diets ($P = 0.019$), centered cisco length ($P < 0.001$), and length*lake ($P < 0.001$), although it

369 had only marginally more support than the model with the second-lowest AIC_C score comprised
370 of the same variables minus proportion Chironomidae (Table 4A). The majority of lakes showed
371 a negative relationship between cisco length and $\delta^{13}\text{C}_{\text{DF}}$, with some lakes exhibiting steep slopes
372 where $\delta^{13}\text{C}_{\text{DF}}$ ranged from 0.3 to -5.7 from the smallest to largest fish, respectively (Fig. 6A).
373 $\delta^{13}\text{C}_{\text{DF}}$ was positively related to RCD (Fig. 6B) and negatively related to proportion of
374 Chironomidae in diets (Fig. 6C), but effect sizes were less pronounced than length effects. The
375 best-supported model for $\delta^{15}\text{N}_{\text{DF}}$ in cisco was RCD ($P = 0.015$), centered cisco length ($P <$
376 0.001), and cisco length*lake (fixed effect, $P < 0.001$), which had slightly more support than the
377 second-best model comprised of the same variables minus RCD (Table 4B). $\delta^{15}\text{N}_{\text{DF}}$ in cisco
378 showed the opposite pattern compared to $\delta^{13}\text{C}_{\text{DF}}$, with the majority of lakes showing a positive
379 relationship between cisco $\delta^{15}\text{N}_{\text{DF}}$ and length (Fig. 6D) and a negative relationship with RCD
380 such that cisco caught deeper in the water column were the most enriched in $\delta^{15}\text{N}_{\text{DF}}$ (Fig. 6E).
381 Cisco diets were dominated by *Daphnia* (53% of diet by mass), composite variable benthic prey
382 (13%), *Chaoborus* (9%), cyclopoid copepods (7%), calanoid copepods (2%), and non-emerging
383 Chironomidae larva (2%). A lake-by-lake breakdown of the cisco diet data is provided in Table
384 S2.

385 **Multivariate Analysis**

386 Our multivariate analysis was conducted on 18 lakes with complete data sets. RDA
387 indicated T_{DO_3} , maximum lake depth, and lake surface area had significant effects on the overall
388 pattern of cisco diets, genetic diversity, stable isotopes, RCD, and dissolved oxygen and water
389 temperature at cisco capture depth. Together, the three predictors explained 39% of the total
390 variance in the response variables ($P = 0.001$); analyzed individually, T_{DO_3} explained more
391 variation (23%) than either maximum lake depth (6%) or lake size (7%). The overall pattern

using all three predictor variables showed that lakes with high T_{DO3} had larger cisco, shallower RCD, higher temperature at capture depth, higher proportion of *Daphnia* in diets, lower allelic richness and expected heterozygosity, lower $\delta^{15}N_{DF}$, lower proportion of empty stomachs, lower dissolved oxygen at capture depth, and lower abundance of cyclopoids in diets (Fig. 7). Maximum lake depth showed a weak inverse relationship with T_{DO3} , and was more strongly associated with high dissolved oxygen at capture and cyclopoids in diets. Lake surface area was orthogonal to T_{DO3} , such that at a given level of T_{DO3} , larger lakes had more Chironomidae in diets, fewer calanoids in diets, and lower prey richness. *Chaoborus* in diets and $\delta^{13}C_{DF}$ showed the weakest relationships with the predictor variables, and supplementary variables indicated proportions of prey in diets were only marginally related to abundance in lakes. Taken together, the T_{DO3} gradient indicates cisco in more oligotrophic, low T_{DO3} lakes are caught at deeper depths in colder water with more oxygen, have higher genetic diversity, are more enriched in $\delta^{15}N_{DF}$, are more likely to have empty stomachs, and feed more heavily on cyclopoids and less often on *Daphnia*.

Discussion

Our results show that the oxythermal environment as measured by T_{DO3} has significant effects on habitat use by cisco, resulting in cisco using warmer water in the upper water column in high T_{DO3} lakes. Use of the upper water column, in turn, was associated with reduced genetic diversity, enriched $\delta^{13}C_{DF}$, and depleted $\delta^{15}N_{DF}$ relative to cisco in deeper water in low T_{DO3} lakes. Finally, high T_{DO3} lakes were characterized by larger cisco with diets dominated by *Daphnia* and less likely to have empty stomachs, while diets dominated by copepods and empty stomachs were more prevalent in low T_{DO3} lakes. Coupled with our previous work showing

effects of T_{DO3} on cisco morphometrics (Jacobson et al. 2020), the emerging picture is that oxythermal habitat is a primary driver of cisco behavior, habitat use, trophic niche, population genetic diversity, and overall ecology. Our previous work has shown that cisco populations are vulnerable to impacts of climate change and eutrophication via their effects on oxythermal habitat characteristics (Jacobson et al. 2008), and this study identifies specific cisco characteristics responding to changes in the oxythermal environment.

Our results document the “temperature-oxygen squeeze” faced by cisco as T_{DO3} increases. In low T_{DO3} lakes cisco were captured over a 19 m section of the water column, which decreased to 5 m at the highest levels of T_{DO3} . Moreover, cisco in high T_{DO3} lakes were caught in both higher minimum (16°C) and maximum (25°C) water temperature, with the minimum temperature in high T_{DO3} lakes actually warmer than the maximum temperature (15°C) for cisco in lakes with the lowest T_{DO3} values. Similarly, the average maximum capture depth in high T_{DO3} lakes (7.6 m) was nearly identical to the minimum capture depth in low T_{DO3} lakes (7.7 m), and at T_{DO3} values greater than 6.8°C mean cisco RCD was above the thermocline. Thus, the shift in distribution of cisco in the water column along the T_{DO3} gradient was pronounced, with subsequent impacts on their oxythermal environment.

T_{DO3} also influenced patterns of dissolved oxygen at capture depth, though patterns differed from that for temperature. Maximum temperature was positively related to T_{DO3} while maximum dissolved oxygen was not, and temperature range was unrelated to T_{DO3} while range of dissolved oxygen showed a positive relationship with T_{DO3} . These differences are driven by cisco capture depth and hypolimnetic oxygen levels having negative relationships with T_{DO3} . Cisco in low T_{DO3} lakes were in water with much higher minimum values of dissolved oxygen (6.2 mg L⁻¹) compared to high T_{DO3} lakes (0.4 mg L⁻¹), while maximum dissolved oxygen

(approximately 8.7 mg L^{-1}) was consistent across lakes and unrelated to T_{DO_3} . This pattern is likely driven by cisco making short-duration foraging trips into hypoxic depths in high T_{DO_3} lakes, resulting in lower minimum dissolved oxygen. In contrast, maximum dissolved oxygen is consistent as cisco in high T_{DO_3} lakes access high dissolved oxygen habitat in the epilimnion while cisco in low T_{DO_3} lakes are able to access similarly high dissolved oxygen levels at deeper depths as indicated by their deeper minimum depth of capture. Thus, the overall oxythermal pattern is that higher T_{DO_3} caused cisco to shift upwards into narrower bands of the water column with warmer water and lower minimum oxygen values. These fish had access to equally high dissolved oxygen levels as fish in low T_{DO_3} lakes, but at the cost of inhabiting water much warmer than cisco in low T_{DO_3} lakes.

Water temperature is a key determinant of fish metabolism and oxygen demand (Clarke and Johnston 1999), and the oxythermal habitat used by 78% of cisco ($n=172$) captured in high T_{DO_3} lakes ($T_{\text{DO}_3} > 11^\circ\text{C}$) was outside their optimal range, with some fish captured in potentially lethal combinations of temperature-dissolved oxygen. Edsall and DeSorcie (2002) estimated age-zero cisco had a preferred temperature of 16.5°C and an upper lethal temperature of 26°C , nearly identical to the minimum (16°C) and maximum (25°C) temperatures at capture depth in lakes with the highest T_{DO_3} values. Moreover, Jacobson et al. (2008) showed the lethal oxythermal niche boundary for cisco ranged from 8 mg L^{-1} at 24°C , 3.0 mg L^{-1} at 22°C , to 1.0 mg L^{-1} at 19.5°C . Based on oxythermal lethal thresholds reported by Jacobson et al. (2008), we captured 41 of our 442 cisco in potentially lethal habitat. These fish were found in just 6 lakes, but a t -test assuming unequal variance showed lakes with cisco in potentially lethal habitat had both significantly higher T_{DO_3} (21°C ; $P < 0.001$) and RCD (1.21 ; $P = 0.001$) compared to lakes with no cisco in lethal habitats (mean $T_{\text{DO}_3} = 7^\circ\text{C}$, mean RCD = 0.66). Additionally, 16 of 20 cisco

captured in Middle Spunk Lake were in lethal habitat, and this population had the lowest allelic richness (3.1) and expected heterozygosity (0.39) of all lakes. Lethal estimates from Jacobson et al. (2008) were estimated for longer-term exposure (likely several days to weeks) that induces summer kill, and cisco in this study may only have been in lethal habitat for short periods of time. Nonetheless, our results do show that lakes with cisco in potentially lethal oxythermal habitat have higher T_{DO3} and RCD values. Additional sampling dates would have been useful for estimating the length of time cisco were forced to use unsuitable habitat, but logistical constraints limited us to one sample date. However, Jacobson et al. (2010) showed 11 August was the mean date of maximum T_{DO3} in stratified Minnesota lakes. Thus, our sampling window occurred when T_{DO3} effects on cisco should be maximized. Jacobson et al. (2010) also showed that T_{DO3} values vary temporally within lakes, but there was no relationship between day of year and T_{DO3} in our study sites ($P = 0.179$), likely due to our relatively narrow sampling window. Instead, differences in T_{DO3} among lakes are driven by lake productivity (Zimmer et al. 2020).

T_{DO3} also had a negative effect on cisco genetic diversity, and we argue this pattern was driven by impacts of T_{DO3} on cisco habitat use. Our data show that as T_{DO3} increases cisco are forced to use water in the upper water column that is both warmer and contains less oxygen than their optimum, with some fish using habitat that borders on lethal values. The dual stress of water that is both too warm and too hypoxic likely contributes to summer kill events (Jacobson et al. 2008), which may function as bottleneck episodes causing reduced genetic diversity (Luck et al. 2003; Hellmair and Kinzinger 2014). Together, smaller populations sizes and reduced genetic diversity create a positive feedback loop whereby populations become susceptible to inbreeding depression and lack the genetic diversity to adapt to changing environments, resulting in further declines in population size (Gilpin and Soule 1986; Hellmair and Kinzinger 2014; Frankham

2015). Lake size, presumably serving as a proxy for population size, has been shown to influence genetic diversity in other species of fish (Cena et al. 2006; Valiquette et al. 2014), and was related to cisco allelic richness but not expected heterozygosity in our study when analyzed alone. Similarly, Heath et al. (2001) used suitable juvenile rearing habitat, rather than stream area, when demonstrating a positive relationship between habitat size and genetic diversity in a salmonid. However, our most parsimonious model was lake size combined with categorical T_{DO3} , therefore T_{DO3} and lake size appear to have independent effects on cisco genetics. In our study lakes, AIC_C indicated that T_{DO3} provided a stronger proxy than lake size for suitable habitat and thus population size, especially when summer kills cause recurrent bottlenecks. Overall, our results reinforce the importance of maintaining suitable oxythermal habitat when trying to sustain viable cisco populations. Lake warming will likely continue (Jacobson et al. 2017), which increases the importance of controlling lake eutrophication to protect oxythermal habitat for cisco and other cold-water fish.

Stocking and genetic population structure were unlikely to have confounded influences of habitat on genetic diversity. Three study populations in lakes with $T_{DO3} < 11$ were founded by translocations from Lake Superior over 90 years ago (Jacobson et al. 2018), so their genetic diversity would partly reflect the diversity of their source and possible founder effects. Regardless, removal of these samples would increase mean diversity slightly for lakes with $T_{DO3} < 11$, which would increase the observed differences between the T_{DO3} lake groups even further. The other lakes lack verified records of cisco stocking and stocking ciscoes has been uncommon in Minnesota in recent decades. Shared colonization history among populations in different major drainages could affect regional levels of genetic diversity and thus confound our results if T_{DO3} was correlated with drainages. However, native inland populations in Minnesota

all likely fall into a western cisco lineage (Turgeon and Bernatchez 2001). In addition, most populations are within two major drainages within Minnesota, each of which contained several high and several low T_{DO3} study lakes. Similar to the total dataset, low T_{DO3} lakes consistently had higher genetic diversity within drainages (average expected heterozygosity 0.02 and 0.04 higher, allelic richness 0.68 and 0.79 higher, in Hudson Bay and Mississippi drainages, respectively).

Body size was also a strong predictor of RCD in cisco, though its effect was not consistent across lakes. Other studies have found an inverse relationship between fish size and thermal preference (Morita et al. 2010), but our results showed some lakes where larger fish were not captured in deeper, colder water. Slopes for RCD versus length were not related to T_{DO3} , indicating the oxythermal habitat was not precluding larger fish from inhabiting deeper water. Instead, slopes for RCD versus length showed a strong negative relationship with proportion of Chironomidae in diets, suggesting the cisco size-depth pattern is driven by trophic relationships where larger cisco captured deeper are feeding more heavily on Chironomidae. There is precedent for benthic prey, such as Chironomidae, being an important alternate energy pathway for pelagic fishes (Vander Zanden and Vadeboncoeur 2002; Sierszen et al. 2003). Causes for the variability in Chironomidae in cisco diets among lakes are unknown, but higher consumption may require both moderate abundance of Chironomidae in benthic habitats and T_{DO3} values that facilitate access to benthic prey.

Our multivariate analysis showed that T_{DO3} had significant effects on cisco RCD, genetic diversity, isotope patterns, and diets. Cisco have been shown to have positive electivity for *Daphnia* and negative electivity for calanoid and cyclopoid copepods during summer (Olynyk et al. 2017), and our results show cisco in lakes with high T_{DO3} fed heavily on *Daphnia*. Positive

electivity coupled with *Daphnia* abundance positively related to T_{DO3} likely drives high consumption rates of *Daphnia* in high T_{DO3} lakes. As T_{DO3} and cisco RCD declined, cisco increased their consumption on cyclopoids, Chironomidae, and *Chaoborus*, with the two latter prey more important in shallower lakes. Thus, Chironomidae may increase in diets as alternative prey for cisco when *Daphnia* decline in abundance in low T_{DO3} lakes, and when these lakes have oxythermal habitats that provide cisco access to benthic prey. Aku and Tonn (1999) showed that cisco increased consumption of benthic prey in an artificially oxygenated lake, and oxythermal conditions likely influenced consumption of benthic prey by cisco in this study.

Cisco in high T_{DO3} lakes also had greater richness of prey in their diets, were less likely to have empty stomachs, and grew to greater maximum size. Jacobson et al. (2020) showed cisco body size was positively related to T_{DO3} , and hypothesized that attaining larger size was a response to greater predation pressure for cisco in high T_{DO3} lakes. Increases in northern pike abundance are known to be associated with decreased cisco abundance in Minnesota lakes and this relationship could drive cisco towards increased body sizes to escape predation (Jacobson et al. 2012). The relationship between increased body size and reduction of predation risk has also been documented the coregonine European whitefish (*Coregonus lavaretus*), which morphologically splits into dwarf/giant forms in response to predation pressure from northern pike over time (Öhlund et al. 2020). Diet and prey availability are two of several factors that influence fish growth rates and body size, and our results illustrate how differences in diets along the T_{DO3} gradient facilitate larger cisco size in high T_{DO3} lakes. Proportion *Daphnia* in diets was positively related to T_{DO3} , and consumed *Daphnia* were much larger (mean length = 1.44 mm) than either calanoid (0.98 mm) or cyclopoid (0.91 mm) prey. Kerr (1971) showed that growth rates of planktivorous fish are positively related to prey size, and Tabor et al. (1996) found that

consumption rates of rainbow trout (*Oncorhynchus mykiss*) were positively related to lake *Daphnia* biomass. Given that abundance of *Daphnia* and other cladocerans is positively related to lake productivity (Pace 1986), higher consumption rates of *Daphnia* in productive, high T_{DO3} lakes should help optimize cisco growth rates. It is worth noting that influences of lake productivity on abundance of different types of prey, rather than T_{DO3} per se, likely drives the shift in zooplankton prey, probability of empty stomachs, and maximum size of cisco along the T_{DO3} gradient. However, higher T_{DO3} forces cisco higher in the water column and increases the predation threat from cool-water predators such as northern pike (Jacobson et al. 2020). This behavior makes T_{DO3} a key component of the pattern among cisco habitat use, predation threat, body size, and diets.

T_{DO3} also influenced isotopic patterns in cisco through impacts on their capture depth. $\delta^{13}C_{DF}$ and $\delta^{15}N_{DF}$ are traditionally used to assess the proportion of littoral C and trophic position of organisms, respectively (Vander Zanden and Rasmussen 1999, 2001; Post 2002). However, Zimmer et al. (2020) showed that $\delta^{13}C_{DF}$ and $\delta^{15}N_{DF}$ in cisco varies across lakes due to differences in lake biogeochemistry. Here we build upon this previous work and show that cisco $\delta^{13}C_{DF}$ and $\delta^{15}N_{DF}$ can also vary systematically within individual lakes, with cisco captured deeper and feeding more on Chironomidae being more depleted in $\delta^{13}C_{DF}$, while deeper fish were also more enriched in $\delta^{15}N_{DF}$. Effects of body size on isotopes were particularly pronounced in many lakes, with $\delta^{15}N_{DF}$ in the most extreme lakes having the largest fish (total length = 241 mm) enriched 4.0‰ over the smallest fish (142 mm), larger than the 3.4‰ enrichment associated with a one-level trophic increase. Similarly, the most extreme example for $\delta^{13}C_{DF}$ was a 6.6‰ depletion from the smallest (142 mm) to largest cisco (295 mm), equivalent to the difference between littoral and pelagic primary consumers in many lakes (Post 2002). We

postulate the size-isotope patterns observed in many lakes are driven by changes in diet, which are in turn driven by ontogenetic dietary shifts, gape limitation, and use of deeper habitat by larger fish due to differences in thermal preferences (Kahilainen et al. 2014). Several studies have shown that invertebrate $\delta^{13}\text{C}_{\text{DF}}$ decreases and $\delta^{15}\text{N}_{\text{DF}}$ increases with depth in the same lake (Francis et al. 2011; Cummings and Schindler 2013), and our results show the same pattern in cisco where across lakes fish captured deeper were more depleted in $\delta^{13}\text{C}_{\text{DF}}$ and more enriched in $\delta^{15}\text{N}_{\text{DF}}$. Within lakes, cisco should also show the same isotopic pattern if larger individuals are feeding at deeper depths. Benthic Chironomidae may be especially important for cisco isotopes, as they can be especially depleted in $\delta^{13}\text{C}_{\text{DF}}$ (Jones et al. 2008) and were most prevalent in diets where cisco were captured deeper and where cisco were the most depleted in $\delta^{13}\text{C}_{\text{DF}}$. Thus, isotopic patterns in this study reflect cisco foraging behavior and trophic niche, which are ultimately driven by T_{DO3} effects on cisco habitat use. It is worth noting that the RDA indicated a weak negative relationship between $\delta^{13}\text{C}_{\text{DF}}$ and RCD at the whole-lake scale, while our univariate analysis showed a positive relationship between RCD and $\delta^{13}\text{C}_{\text{DF}}$. These relationships suggest lake depth counters the influence of T_{DO3} and RCD on $\delta^{13}\text{C}_{\text{DF}}$ at the whole-lake scale, resulting in weaker relationships between $\delta^{13}\text{C}_{\text{DF}}$ and other variables in the RDA.

In conclusion, our results show that oxythermal habitat, as measured by T_{DO3} , is a major predictor of cisco ecology through impacts on their behavior, trophic niche, and genetic diversity. The net result is a suite of cisco traits that change in concert along productivity gradients, with contrasting optimal responses at each end of the productivity spectrum. Cisco in more oligotrophic systems feed deeper in the water column, experience more optimal oxythermal environments, and have higher genetic diversity, yet feed on less optimal prey for growth, are more likely to have empty stomachs, and have smaller maximum body size. In more eutrophic

systems, cisco feed on a prey base with higher richness that includes large-bodied *Daphnia* and reach larger sizes, but are forced to use marginal oxythermal habitat resulting in population bottlenecks that lead to reduced genetic diversity. Our sampling along a lake productivity gradient serves as a “space-for time” design, with the more productive lakes in this study serving as surrogates for how climate change and eutrophication could change oxythermal environments in lakes. Previous work has shown that cisco populations are vulnerable to impacts of climate change and eutrophication via effects on oxythermal habitat in lakes (Jacobson et al. 2012), and this study identifies specific characteristics of cisco that respond to those habitat changes. Our results also provide a framework for understanding specific effects of climate change and eutrophication on other cold-water species at the global scale. Fisheries managers should consider these specific impacts of changing oxythermal environments when trying to identify and preserve refuge lakes for cisco and other cold-water species of fish.

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Tables

Table 1. Summary characteristics and the temperature at which the dissolved oxygen content of the water equals 3 mg L⁻¹ (T_{DO3}) for all 32 study lakes sampled. Minnesota Department of Natural Resources (MNDNR) lake identification number (ID), surface area, presence of invasive species (Invasives), and max depth were obtained from MNDNR Lakefinder. Invasive species present in each lake at the date sampled are indicated by letter: none (N), zebra mussels (*Dreissena polymorpha*, Z), Eurasian watermilfoil (*Myriophyllum spicatum*, E), spiny waterflea (*Bythotrephes longimanus*, W), and starry stonewort (*Nitellopsis obtusa*, S). Lake Superior was the source for the introduced category of associated population type (Type).

Lake	ID	T _{DO3} (°C)	Surface Area (ha)	Max Depth (m)	Invasives	Type
Bear Island	69011500	7.0	956	18.9	N	Native
Big Sand	29018500	7.0	662	41.1	N	Native
Blue	29018400	15.5	136	25.6	N	Native
Burgen	21004900	17.2	71	13.1	N	Native
Carlos	21005700	8.5	1054	49.7	Z, E	Native
Cedar	1020901	5.3	698	32.0	N	Native
Clearwater	16013900	5.6	536	39.6	N	Introduced
Elk	15001000	18.8	123	28.3	N	Native
Fall	38081100	19.4	914	9.8	W	Native
Farm	38077900	22.3	523	17.1	N	Native
Green	34007900	21.9	1201	40.2	N	Native
Greenwood	16007700	6.5	827	34.1	W	Introduced
Kabekona	29007500	8.8	985	40.5	N	Native
Koronis	73020002	21.8	2250	33.5	S	Native
Leech	11020300	23.9	41699	45.7	E	Native
Long	1008900	5.3	175	35.4	N	Native
Loon	16044800	5.3	443	61.6	N	Introduced
Middle Spunk	73012800	21.5	95	24.7	E	Native
Moose	38064400	7.9	526	19.8	N	Native
Newfound	38061900	7.6	260	13.7	N	Native
Ojibway	38064000	4.6	149	35.1	N	Native
Osakis	77021500	20.8	2586	22.3	N	Native
Prairie	69084800	19.3	321	14.3	N	Native

Rachel	21016000	11.7	181	19.8	N	Native
Round	1020400	5.7	297	38.1	N	Native
Shagawa	69006900	17.8	949	14.6	W	Native
Snowbank	38052900	6.8	1884	45.7	N	Native
St Mary's	69065100	9.3	95	13.4	N	Native
Sucker	38053000	10.5	159	9.4	N	Native
Ten Mile	11041300	7.2	2056	63.4	N	Native
Trout	69049800	8.7	3005	29.9	N	Native
West Sylvia	86027900	10.3	366	29.6	E	Native

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Table 2. Performance of four models and the intercept-only model predicting the slopes for relative capture depth (RCD) relative to fish length. Δ_i is the difference in AICc values between each model and the model with the lowest AICc value. Models are sorted in order of increasing Δ_i values, with smaller Δ_i values indicating more parsimonious models. Akaike weights (w_i) represent weight of evidence (out of 1.00) that each model is the best model in the set, and the evidence ratio shows multiplicative improvement of the best model over all other models. Parameter estimates and 95% confidence intervals (95% CI) are shown for the top model.

Model (Parameter)	Δ_i	w_i	Evidence ratio	Parameter estimate (95% CI)
Proportion Chironomidae prey (Chironomidae prey)	0	0.96		-14.0 (-20.9, -7.1)
Proportion benthic prey	6.7	0.04	29	
Intercept only	11.2	< 0.01	270	
Lake maximum depth	11.8	< 0.01	365	
T_{DO3}	13.7	< 0.01	944	

22 Table 3. Performance of five models and the intercept-only model for predicting cisco A) expected heterozygosity and B) allelic
23 richness. T_{DO3} is the temperature at which the dissolved oxygen content of the water equals 3 mg L⁻¹. For each variable, models are
24 sorted in order of increasing Δ_i values, with smaller Δ_i values indicating more parsimonious models. Parameter estimates and 95%
25 confidence intervals (95% CI) are shown for the top model, and the parameter for categorical T_{DO3} is the estimate for $[(T_{DO3} < 11^{\circ}\text{C}) -$
26 $(T_{DO3} > 11^{\circ}\text{C})]$.

Model	Δ_i	w_i	Evidence ratio	Parameter estimate
(Parameter)				(95% CI)
A) Expected heterozygosity				
T_{DO3} categorical + lake surface area	0	0.56		
(T_{DO3} categorical)				0.05 (0.02, 0.07)
(Lake surface area)				0.04 (0.001, 0.09)
T_{DO3} categorical	1.8	0.23	2	
T_{DO3} categorical*lake surface area	2.8	0.14	4	
T_{DO3} continuous	4.5	0.06	9	

Lake surface area	10.7	< 0.01	211
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Intercept only			
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B) Allelic richness

T _{DO3} categorical + lake surface area	0	0.69	
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(T _{DO3} categorical)			0.04 (0.02, 0.07)
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Lake surface area			0.06 (0.02, 0.11)
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T _{DO3} categorical * lake surface area	2.5	0.20	3
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T _{DO3} categorical	5.3	0.05	12
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T _{DO3} continuous	5.8	0.04	15
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Lake surface area	7.5	<0.02	35
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Intercept only			
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29 Table 4. Performance of the four top models for predicting cisco A) $\delta^{13}\text{C}_{\text{DF}}$ and B) $\delta^{15}\text{N}_{\text{DF}}$. For each variable, models are sorted in
30 order of increasing Δ_i values, with smaller Δ_i values indicating more parsimonious models. Parameter estimates and 95% confidence
31 intervals (95% CI) are shown for the top model, but for brevity are not given for interaction effects.

Model (Parameter)	Δ_i	w_i	Evidence ratio	Parameter estimate 95% CI
A) $\delta^{13}\text{C}_{\text{DF}}$				
cisco length + RCD + proportion Chironomidae prey + cisco length*lake	0	0.84		
(cisco length)				-4.81 (-5.84, -3.80)
(RCD)				0.38 (0.07, 0.69)
(proportion Chironomidae prey)				-0.93 (-1.70, -0.16)
cisco length + RCD + cisco length*lake	3.5	0.15	6	
cisco length + cisco length*lake	8	0.02	55	

cisco length + RCD + cisco length*lake +

RCD*lake 13.9 < 0.01 1,043

B) $\delta^{15}\text{N}_{\text{DF}}$

cisco length + RCD + cisco length*lake

0 0.66

(cisco length)

3.06 (2.29, 3.82)

(RCD)

-0.29 (-0.52, -0.06)

cisco length + RCD + proportion Chironomidae

prey + cisco length*lake

2.0 0.24 3

cisco length + cisco length*lake

4 0.09 7

cisco length + RCD + cisco length*lake +
RCD*lake

10.7 < 0.01 211

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1 Figures

Figure 1. Depth profiles for temperature (triangles) and dissolved oxygen (circles) for the two lakes with lowest (A, Cedar) and highest (B, Koronis) temperature at which dissolved oxygen equaled 3 mg L⁻¹ (T_{DO3}) in our study. T_{DO3} can be visualized by the vertical grey line at 3 mg L⁻¹ dissolved oxygen, which subsequently shows the water temperature that occurred at 3 mg L⁻¹. Boxplots show distribution of capture depths of cisco in each lake (Cedar median = 14.5 m, Koronis median = 5.5 m), reflecting the influence of T_{DO3} on cisco depth distribution.

14 Figure 2. Relationship in 21 lakes between A) centered log cisco length and predicted relative
15 capture depth (RCD) from a mixed model analysis, B) $\log T_{\text{DO3}}$ (temperature at which the
16 dissolved oxygen content of the water equals 3 mg L⁻¹) and the random lake effect from the
17 analysis in Panel A, and C) proportion benthic prey in diets versus slopes for RCD from the
18 analysis in Panel A.

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Figure 3. Results from 21 lakes for linear regression effects of $\log T_{D03}$ (temperature at which the dissolved oxygen content of the water equals 3 mg L⁻¹) on A) log minimum, B) log

22 maximum, and C) log range of temperature at capture depth of cisco, as well as D) log minimum,
23 E) log maximum, and F) log range of dissolved oxygen at capture depth of cisco.

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28 Figure 4. Results from 21 lakes for linear regression effects of $\log T_{D03}$ (temperature at which the

29 dissolved oxygen content of the water equals 3 mg L⁻¹) on A) minimum, B) maximum, and C)

range of cisco relative capture depth (RCD), as well as D) log minimum, E) log maximum, and
F) log range of actual cisco capture depth.

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Figure 5. Effects of T_{D03} (temperature at which the dissolved oxygen content of the water equals 3 mg L⁻¹) and log lake size on cisco A) expected heterozygosity and B) log allelic richness in 32 lakes.

Figure 6. Predicted values from the most parsimonious models from mixed model analyses of cisco isotopes from 20 lakes. The most parsimonious model for predicting cisco $\delta^{13}\text{C}_{\text{DF}}$ was lake*centered log cisco length (A), cisco relative capture depth (RCD) (B), and proportion of

63 Chironomidae in cisco diets (C), while the most parsimonious model for cisco $^{15}\text{N}_{\text{DF}}$ was
64 lake*centered log cisco length (D) and cisco RCD (E).

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Figure 7. Biplot from redundancy analysis of 18 lakes testing for significant effects of the predictor variables (bold text, bold arrows): temperature at which the dissolved oxygen content of the water equals 3 mg L⁻¹ (T_{DO3}), lake surface area (Surfarea), and maximum lake depth (Maxdepth) on the response variables (solid lines and arrows) cisco expected heterozygosity (Hexp), allelic richness (AR), $\delta^{13}\text{C}_{\text{DF}}$ (13C), $\delta^{15}\text{N}_{\text{DF}}$ (15N), relative capture depth (RCD),

77 temperature at capture depth (Fishtemp), dissolved oxygen at capture depth (Fishdo), maximum
78 body size (Maxsize), proportion of empty stomachs (PrEmpty), richness of diet prey (Richness),
79 and diet proportion of calanoids (Calapr), cyclopoids (Cyclpr), *Chaoborus* (Chaoprop),
80 Chironomidae (Chirprop), and *Daphnia* (Daphpr). The predictor variables explained a significant
81 amount of variation (39%, $P < 0.001$) in the response variables based on permutation tests. Also
82 shown are the supplementary variables (dashed lines, italic text) of lake abundance for cycloid
83 copepods (Cycloab), calanoid copepods (Calaab), *Daphnia* (Daphab), Chironomidae (Chirab),
84 and *Chaoborus* (Chaoab). For all variables, arrows point in the direction of increasing values,
85 and longer arrows represent stronger relationships with the predictor variables.

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