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Impacts of freshwater aquaculture on fish communities: A whole-ecosystem experimental approach

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Abstract

1. Aquaculture is a growing global industry; freshwater aquaculture has significant potential for expansion in Canada, but growth of the freshwater sector has been slow due to concerns over potential environmental impacts and a lack of information on potential impacts to native fish communities.
2. To provide guidelines and target variables for evaluating aquaculture impacts on freshwater fish communities, we operated an experimental aquaculture farm as a whole-lake experiment where 10,000 rainbow trout (*Oncorhynchus mykiss*) were raised annually from 2003 to 2007. Impacts were assessed using up to 8 years of pre-impact and 8–10 years of post-impact data.
3. Prey fish abundance increased dramatically during aquaculture but declined sharply following the experiment. High abundance of littoral minnows in autumn was not observed during spring and, combined with size distribution data, suggests high overwinter mortality of adult minnows. White sucker (*Catostomus commersonii*) abundance and body condition declined during and after aquaculture, with evidence of overwinter juvenile recruitment failure in the last 2 years of operation, although size-at-age increased.
4. The adult abundance of lake trout (*Salvelinus namaycush*) doubled during aquaculture, due to a combination of (a) increased growth rates of young trout and (b) earlier age and larger sizes at maturation. Within 2 years following aquaculture, lake trout abundance declined by nearly 50% to background levels, suggesting a large increase in lake trout mortality once operations ceased. These changes were not observed in nearby reference lakes.
5. While aquaculture appeared to benefit some species (slimy sculpin [*Cottus cognatus*], minnows, lake trout), prolonged declines in white sucker abundance and condition and continued depression of *Mysis* densities and optimal oxythermal habitat availability nearly a decade following operations suggest potentially long-term impacts at this magnitude. Importantly, this experiment highlights important indicator species and life history traits for monitoring of freshwater aquaculture impacts on native fish communities.

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KEYWORDS
BACI design, fish farm, life history changes, mark–recapture, nutrient loading, size distribution shifts

1 | INTRODUCTION

Aquaculture is a major and growing industry around the world. In Canada, over 90% of aquaculture production is focused on finfish, and the value of the industry has grown in a near-linear fashion since 1986, worth $877 million dollars in 2015 (Government of Canada, 2015). Freshwater aquaculture contributes only 5–10% of this total value, and freshwater aquaculture production has remained relatively stable since 1996, while marine aquaculture has tripled over the same timeframe. Freshwater aquaculture in Canada is primarily based on rainbow trout (*Oncorhynchus mykiss*) production, for which Ontario is the primary Canadian producer (generating 66% of trout production across Canada on average, 1986–2015). Ontario trout production is focused mainly in aquaculture facilities in Lake Huron, which have been generating steady production levels of 4,500 tonnes of fish on average since 1996. Concerns over environmental impacts have limited the expansion of freshwater aquaculture in Canada (Yan, 2005).

Increased nutrient loading is one of the primary environmental concerns surrounding impacts of aquaculture in freshwater ecosystems (Ackefors & Enell, 1994; Bristow, Morin, Hesslein, & Podemski, 2008; Enell, 1995). Aquaculture-related nutrient release can be dramatic and cause significant increases in primary productivity in smaller or more enclosed systems (Bristow et al., 2008; Findlay, Podemski, & Kasian, 2009), but more muted responses have been observed in practice on the Great Lakes (Johnston, Keir, & Power, 2010; Milne, Marvin, Yerubandi, McCann, & Moccia, 2017). By comparing the initial results of a whole-lake aquaculture experiment at the IISD-Experimental Lakes Area (IISD-ELA or ELA hereafter, www.iisd.org/ela) to previous nutrient addition experiments conducted at the ELA, Bristow et al. (2008) concluded that aquaculture impacts on total phosphorus, ammonia, and chlorophyll *a* trends within the water column best reflected hypolimnetic nutrient deposition experiments (in the North Basin of Lake 302) where most added nutrients underwent sedimentation. Under sustained additions, as might be observed with aquaculture (to either the epilimnion or hypolimnion), concern exists over the potential for hypolimnetic oxygen depletion and habitat limitation and/or overwinter mortality for cold water fish species (Yan, 2005).

Nutrient loading from aquaculture at sufficient levels is expected to affect fish communities. In a well-known nutrient addition experiment at the ELA, productivity of lake whitefish (*Coregonus clupeaformis*, a coldwater benthivore) increased during 7 years of epilimnetic phosphorus additions, but responded only slightly to epilimnetic additions of carbon and nitrogen only (Mills, 1985; Mills & Chalanchuk, 1987). Where hypolimnetic phosphorus was added to the North Basin of Lake 302, lake whitefish demonstrated an immediate increase in growth rates (Rennie, 2013) and recruitment (Mills, Chalanchuk, Findlay, Allan, & McCulloch, 2002), leading to a lagged increase in abundance (Mills et al., 2002). However, impacts of hypolimnetic nutrient additions on other species, including warm water taxa such as minnows and white sucker (*Catostomus commersonii*) have not been characterised during these previous ELA experiments and are unknown.

Both lake trout (*Salvelinus namaycush*) and the freshwater shrimp *Mysis* (*Mysis diluviana*) are sensitive to coldwater oxygen depletion (Evans, 2007; Nero & Davies, 1982) and therefore likely to be affected by aquaculture-related declines in oxygen availability. In many Ontario lakes, nutrient targets are dictated by cold water oxygen requirements for lake trout (Nelligan, Jeziorski, Rühland, Paterson, & Smol, 2016; Nicholls, 1997; Young, Winter, & Molot, 2011), which are a highly sought after recreational fish species across Canada and the U.S.A. (Evans, Nicholls, Allen, & McMurtry, 1996; Gunn & Mills, 1998; Gunn, Steedman, & Ryder, 2003). Additionally, *Mysis* is an important prey species to lake trout (France & Steedman, 1996; Trippel & Beamish, 1993), and *Mysis* abundance can greatly influence lake trout growth and life history dynamics (Devlin et al., 2017; Ellis et al., 2011; Mills, Chalanchuk, Mohr, & Davies, 1987). Moderate nutrient increases can have some positive impacts on the growth and condition of coldwater fish species (Lienesch, McDonald, Hershey, O'Brien, & Bettez, 2005; Mills & Chalanchuk, 1987) but potentially also long-term negative impacts on recruitment (Lienesch et al., 2005).

To date, there have been relatively few evaluations of aquaculture impacts on freshwater fish communities. There is some evidence of aquaculture cage sites attracting certain fish species (Charles, Blanchfield, & Gillis, 2017) and of increasing fish abundance locally around cage operations (Johnston et al., 2010 and references therein). Studies investigating impacts of aquaculture wastes on fish communities have been mixed, with studies from smaller systems reporting significant incorporation of wastes into the food web (Grey, Waldron, & Hutchinson, 2004; Kullman, Kidd, Podemski, Paterson, & Blanchfield, 2009; Wellman, Kidd, Podemski, Blanchfield, & Paterson, 2017), while others from larger systems report no significant impact (Johnston et al., 2010). Impacts on large fish species such as white sucker and top predator species such as lake trout are also not well known.

To provide management guidelines and identify target species for monitoring of potential aquaculture impacts in freshwaters, the goal of this study was to evaluate the impacts of an experimental aquaculture operation on the abundance, growth rates and life history strategies of the native fish community, as well as the response of the fish community to the cessation of aquaculture operations. Previous work from this study has demonstrated increased nutrients

TABLE 1 Physical limnological parameters of lakes under study at the IISD Experimental Lakes Area

	Lake			
Parameter	**224**	**442**	**373**	**375**
Area (ha)	26.2	16.0	27.4	23.2
Maximum depth (m)	27.3	17.8	21.2	26.5
Volume (m^3)	3,066,672	1,440,605	3,107,474	2,658,813

Note. Lake 375 was subjected to aquaculture from 2003 to 2007. Lakes 373, 442 and 224 represent reference lakes to which data from lake 375 were compared to evaluate aquaculture impacts.

(Bristow et al., 2008) and phytoplankton biomass (Findlay et al., 2009) in response to aquaculture, as well as significant uptake of aquaculture nutrients in fish (Wellman et al., 2017). In contrast, declines in profundal benthos (Rooney & Podemski, 2009) and lasting suppression of densities of the opossum shrimp *M. diluviana* and of oxygenated coldwater habitat were also observed (Kennedy et al., In press; Paterson, Podemski, Wesson, & Dupuis, 2011). Lake trout condition and early growth rates also increased in this study (Kennedy et al., In press), which raises the possibility of potential top-down influences on food webs influenced by aquaculture. We advance this body of research by: (1) extending data sets and time series for fish, their habitat, and their prey both pre- and post-manipulation; (2) providing the most comprehensive quantitative evaluation of fish community responses to aquaculture to date; and (3) describing seasonality for several variables important for understanding fish community responses. Impacts of aquaculture were evaluated against similar parameters measured in nearby undisturbed reference lakes. Based on previous work at the ELA on the effects of both epilimnetic and hypolimnetic nutrient additions on fishes, we predicted that individual growth rates of fish might increase, and that abundance of fish might also increase due to increased nutrient loads from aquaculture.

2 | METHODS

2.1 | Location

Lake 375 was selected for an experimental aquaculture operation in 2001 (Table 1). The experiment was scaled to compare phosphorus loading from aquaculture to previous whole lake nutrient addition experiments at the ELA (Bristow et al., 2008). This resulted in a very high ratio of fish biomass to water volume but allowed us to demonstrate clear pathways of effects and provide potential parameters for evaluating aquaculture impacts in freshwater ecosystems elsewhere. Previous lake monitoring was intensified to characterise background conditions for all ecosystem components. Nearby Lake 373 (immediately upstream from Lake 375) was selected as a primary reference system to evaluate changes due to aquaculture versus regional influences but additional data from reference lakes 224 and 442 were also utilised (Table 1). Additional details on lakes 373 and 375 can be found in Bristow et al. (2008) and Paterson et al. (2011). Each year from 2003 to 2007, approximately 10,000 rainbow trout (at a mean stocking size of 127 g) were raised in the Lake 375 aquaculture cage, measuring 10 m × 10 m × 10 m and anchored over water 16 m deep (Bristow et al., 2008). The experiment simulated commercial aquaculture operations; fish were fed twice daily (1%–3% of body mass as a daily ration, depending on water temperatures) with Martin Mills Profishent© feed (Blanchfield, Tate, & Podemski, 2009; Findlay et al., 2009). Fish grew to marketable size by the end of the growing season (*c*. 1 kg) and were harvested annually in autumn. On average, 8,200 kg of rainbow trout biomass was produced from 9,800 kg of feed (Bristow et al., 2008). Aquaculture waste generated approximately 81 kg phosphorus per year on average, increasing the total phosphorus load to Lake 375 by 5 times background levels (Bristow et al., 2008).

Monitoring was conducted for various parameters related to fish and their habitat. Lakes 375 and 373 are both dimictic and support populations of *Mysis*, minnows (family Cyprinidae), slimy sculpin (*Cottus cognatus*), white sucker and lake trout. The minnow community in Lake 375 is dominated by fathead minnow (*Pimephales promelas*, Supporting Information Figure B1) which are absent from Lake 373. Two other reference lakes, Lake 442 and Lake 224, were included in the study to evaluate changes in fathead minnow size spectra. Lakes 375 and 373 both support Northern Redbelly Dace (*Chrosomus eos*, which dominate the Lake 373 minnow community), Finescale Dace (*Chrosomus neogaeus*), and Pearl Dace (*Margariscus margarita*).

2.2 | Temperature, oxygen

Oxygen (O_2) and temperature profiles were taken bi-weekly at the deepest points of lakes 375 and 373 using a YSI Model 6562 probe; prior to 2001, O_2 concentrations were measured using Winkler titrations and temperature was measured using a Flett Research Mark II digital telethermometer (see Paterson et al., 2011 for additional details). We focused on profiles taken only in late summer (mid-August to mid-September) to capture the maximum extent of oxygen limitation for coldwater organisms (Quinlan, Paterson, Smol, Douglas, & Clark, 2005). We estimated optimal habitat volume for coldwater organisms (*Mysis*, lake trout) as the volume of lake bound by 4 mg/L O_2 and 15°C (Guzzo & Blanchfield, 2017; Plumb & Blanchfield, 2009).

2.3 | Mysis collections

During 2002–2008, *Mysis* were collected monthly as described in Paterson et al. (2011), and opportunistically during late summer/

autumn from 2009 to 2017. Briefly, *Mysis* were collected at least 1 hr after sunset using vertical hauls from 1.5 m above the lake bottom to the surface using a 0.75 m diameter net with 500 μm mesh at 4–11 stations in each lake. Stations were fixed and located along a transect, each station representing each of the 5 m depth strata included in the study (5–10, 10–15, 15–20, >20 m). Average lake-wide densities during autumn were determined as a weighted average using *Mysis* densities in each depth zone and the relative contribution of that depth zone to overall lake area (see Paterson et al., 2011 for details).

2.4 | Fish collections

Fishes were sampled in spring and autumn using various methods. To estimate abundance and age distribution, white sucker were targeted during spring spawning (May to mid-June) whereas lake trout were targeted during autumn spawning (mid-September to October). Trap-netting and spring sampling ceased in 2011 in Lake 375, whereas sampling targeting lake trout abundance only (no trap-netting, gillnetting on spawning shoals only; see below for details) was conducted during autumn of 2013 and 2016. Otherwise, during both spring and autumn sampling periods, fishes from all lakes were captured with 2–3 Beamish-style trap nets (Beamish, 1973) as described in Guzzo, Rennie, and Blanchfield (2014). Trap nets were of two types that either contained a central lead set perpendicular to shore (Beamish, 1973) or without a central lead, where one wing of the net was tied to shore and set roughly parallel to the shoreline in order to capture fish travelling in a single direction. Nets of both designs had holding pots suspended by floats and pots and wings were anchored in place. Holding pots ranged in volume from 2.2 to 6.1 m^2 and were set in *c.* 3–4 m of water. The length of leads varied depending on the steepness of the lake bottom and the distance from shore to *c.* 3–4 m depth, as traps were targeting the littoral area (≤3 m depth) of the lakes. The height of the pots and leads was 90 cm. Importantly, the same nets and locations were fished annually (although sites differed between spring and autumn to target white sucker and lake trout, respectively). This standardisation of gear and locations within a season allowed for catches to be meaningfully standardised within a lake over time. Mesh sizes of trap nets used in all lakes was approximately 3 mm, and the smallest fish retained in nets was approximately 19 mm fork length.

Trap nets were typically set for 4–6 weeks and emptied every 2–5 days depending on water temperatures. All fish captured in trap nets were enumerated and measured; volumetric subsamples to estimate counts and size distributions were performed when catches of small fish (mainly minnows) were high (>1 L in volume). Minnow counts (fathead minnow and all species combined) were summed daily and a catch-per-unit-effort (CUE) from trap nets (number of fish per net day, or number of fish divided by the product of the number of nets set and days that nets fished) was estimated for each spring and autumn sampling period. All captured fish were live-released.

During autumn, trap net samples were augmented with short-set (10–20 min) 38 mm gillnets targeting lake trout on spawning shoals shortly after sunset. lake trout were typically entangled by their teeth and mandibles. Following removal from nets, fish were held in a large (2 m × 1 m × 1 m) mesh pen overnight and were sampled the next morning. Handling mortality by either trap netting or gillnetting was negligible.

White sucker >100 mm in fork length were given a dorsal fin nick (batch mark) that corresponded to the capture period, measured, and weighed. Lake trout were handled similarly, except all individuals >200 mm were given individual identifiers (either numbered Carlin-style sew-on tags, visual implant tags, or passive integrated transponder tags). Lake trout were also given a dorsal fin nick corresponding to the capture period. Dorsal fin nicks (white sucker) and tags (lake trout) were the primary data used to facilitate mark–recapture population estimates (see below). In both species, sex determination was evaluated externally by expression of gametes; white sucker sex was determined during spring sampling and lake trout during autumn sampling. Fin rays were taken from white sucker (during spring) and lake trout (during autumn) to determine age. Typically, the first few pectoral rays on the left or right side of the fish were taken, cut as close to the insertion to the body as possible, and placed in an envelope to dry. Fins were later mounted in epoxy and cross-sectioned using an isomet low-speed saw, mounted on slides and read for age determination (Mills & Chalanchuk, 2004).

White sucker abundance was determined using a Schnabel census based on the numbers of marked and unmarked fish over multiple capture events within each sampling period in both lakes. Lake trout abundance was estimated using the POPAN Jolly–Seber model (Schwarz & Arnason, 1996) in Program MARK (White & Burnham, 1999) using the RMark() package in R (R Core Team, 2017). Briefly, the POPAN formulation uses the Jolly-Seber model to estimate abundance, allowing for births and deaths in the population between sampling periods. Individual capture histories from tagged lake trout were used as the basis for estimation of real and derived parameters from the model. Real parameters of interest were interval survival and catchability estimates, and derived estimates of interest were gross births (Schwarz, Bailey, Irvine, & Dalziel, 1993) and abundance. Additional details regarding model estimation and selection procedures are outlined in Supporting Information Appendix A.

Because sampling targeted mature (spawning) lake trout and white sucker, we estimated size and age at maturity as the smallest and youngest 20% of male or female fish captured in each sampling period (Cruz Font, Shuter, Blanchfield, Minns, & Rennie, In press).

Body condition of lake trout and white sucker was estimated using the relative weight method and expressed as a percentage of the observed weight divided by the estimated standard weight for the species. The standard weight equation for lake trout was from Piccolo, Hubert, and Whaley (1993) and for white sucker from Bister et al. (2000). For white sucker, we evaluated condition changes in both small (100–150 mm) and large (≥350 mm) individuals seperately. Only lake trout >280 mm were considered for relative weight (Piccolo et al., 1993).

2.5 | Statistical analysis

Statistical analyses were conducted to compare trends over time between aquaculture and reference ecosystems. To evaluate the degree to which aquaculture operations have continued to impact coldwater habitat, we examined changes in the 4 mg/L oxycline, 15°C isocline and volume of coldwater habitat in both lakes using a before-after control-impact (BACI) design ANOVA (Green, 1979; Smokorowski & Randall, 2017). Years were classified as either before aquaculture (prior to 2003) during aquaculture (2003–2007) or after aquaculture (2008 and later). A significant interaction term indicated a difference in direction and/or magnitude of effects between the lake undergoing aquaculture and reference lake. Due to limited availability of pre-impact data for oxygen and thermal profiles, we used all data available to evaluate prior conditions in these lakes (1983–2002). Oxycline data were log-transformed to normalise residuals. We also evaluated the relationship between coldwater habitat volume availability and area-weighted mean densities of *Mysis* during the late summer/autumn period using linear regression. To evaluate the impact of aquaculture on minnow and sculpin abundance (mean CUE in either spring or autumn), we used the same BACI ANOVA as described above. Minnow and sculpin abundance were both square-root transformed to normalise and homogenise residual distributions. We included pre-impact minnow and sculpin abundance from 1989–2002 to provide sufficient sample sizes. Separate ANOVAs were conducted for minnows and sculpin in each season (spring and autumn). Visual plots and Tukey post hoc tests were used to determine the significance and directionality of reported differences.

Fathead minnow and white sucker in the aquaculture and reference lakes were examined using an independent mixture model—hereafter referred to as *mixture model*—to estimate mean young-of-year (YOY) size in these fishes using the mix() function in the depmixS4 package (Visser & Speekenbrink, 2010) for R. We used a Gaussian distribution to classify groups. Initially, fish were modelled using either two or three hidden states, but in some cases the underlying distributions represented more than three states or rarely, a single state (one age group). Once the models were created using the mix() function, we used the fit() function to optimise the parameters of the mix() model. Models were run until convergence, and the best candidate model was determined using Akaike's information criteria and Bayes' information criteria, and then used to identify YOY fishes. In complex cases, we used manual cut-offs for maximum size to help identify peaks associated with YOY fish. Preliminary analyses demonstrated that in Lake 373, white sucker identified from mixing models as spring age 1 were not significantly different in size from those identified as autumn YOY in the year immediately previous (paired t test, $t_5 = 2.2614$, $p = 0.07$). Due to this similarity in size between spring and autumn periods, and lacking autumn YOY data for certain years, we used mixing model results for spring age 1 sizes from lake 373 during 2002–2004 as an estimate of autumn YOY sizes during 2001–2003. Trends in time for YOY size in lakes were analysed using linear regression during 2002–2009.

We evaluated trends in 2002–2009 spring white sucker abundance using linear regression. Changes in white sucker mean size (fork length) and body condition were evaluated using linear regression over the period of aquaculture operation (2003–2007) and 2002–2011, respectively. We also examined length-frequency distributions of fathead minnow and white sucker to evaluate potential overwinter mortality in Lake 375 during aquaculture (due e.g. to predation or body size effects sensu Post & Evans, 1989; Supporting Information Appendices C, D). Trends in lake trout mean size were evaluated using linear regression. Lake trout size at maturity was compared pre- and post-aquaculture using a BACI-design ANOVA as described for coldwater habitat (above). Residuals of maturation sizes showed some evidence of heterogeneous variance that could not be corrected with log or square-root transformation but statistical conclusions were consistent with visual presentations of data. Patterns in lake trout survival, catchability, and gross births across time periods (pre-aquaculture, during aquaculture, and post-aquaculture) were evaluated for both lakes 373 and 375 using ANOVA.

3 | RESULTS

3.1 | Changes in cold water habitat availability and invertebrate prey

The availability of optimal oxythermal habitat (>4 mg/L O_2 and <15°C) declined significantly in Lake 375 after aquaculture, but not in Lake 373 (ANOVA, lake by period interaction, $F_{2,37} = 3.3$, $p = 0.0497$; Figure 1a; Lake 375 Tukey HSD test pre versus post, $p = 0.02$; Lake 373 Tukey HSD test pre versus post, $p = 0.99$). This reduction in habitat appeared to be due largely to reductions in the extent of hypolimnetic oxygen in Lake 375 (ANOVA, lake by period interaction, $F_{2,37} = 2.5$, $p = 0.09$; Lake, $F_{1,37} = 45.4$, $p < 0.0001$; Period, $F_{2,37} = 18.4$, $p < 0.0001$; Figure 1b). Lake means of the depth of 4 mg O_2/L were similar before aquaculture (Tukey, $p = 0.4$) but differed after (Tukey, $p < 0.0001$). Both lakes showed declines in the depth of 4 mg O_2/L, (Tukey p-values, Lake 373 = 0.03, Lake 375, <0.0001) but the decline was greatest in Lake 375 (above 10 m in 2007, 2008; Figure 1b). The depth of the 15°C isotherm was not different over time (ANOVA, interaction $F_{2,37} = 1.6$, $p = 0.21$; Period $F_{2,37} = 1.46$, $p = 0.24$) but was consistently shallower in Lake 375 than in Lake 373 (ANOVA, Lake $F_{1,37} = 34.9$, $p < 0.0001$, Figure 1c).

Following declines in hypolimnetic O_2 in Lake 375, there was a dramatic decline in *Mysis* density, and a shift from most *Mysis* being found in the deepest portion of the lake (>20 m in 2002, the only pre-aquaculture year sampled) to a preference for more shallow regions (Paterson et al., 2011; Figure 2). *Mysis* continue to be concentrated in the shallower depth strata at impaired densities in 2017 compared with conditions prior to aquaculture (Figure 2). *Mysis* in Lake 373 did not show a similar pattern in depth distribution (Paterson et al., 2011).

Mysis densities were well predicted by optimal oxythermal habitat (Figure 3). The relationship was significant for *Mysis* in Lake 375 (regression, $F_{1,7} = 15.94$, $p = 0.005$, $R^2 = 0.69$) and for *Mysis* over

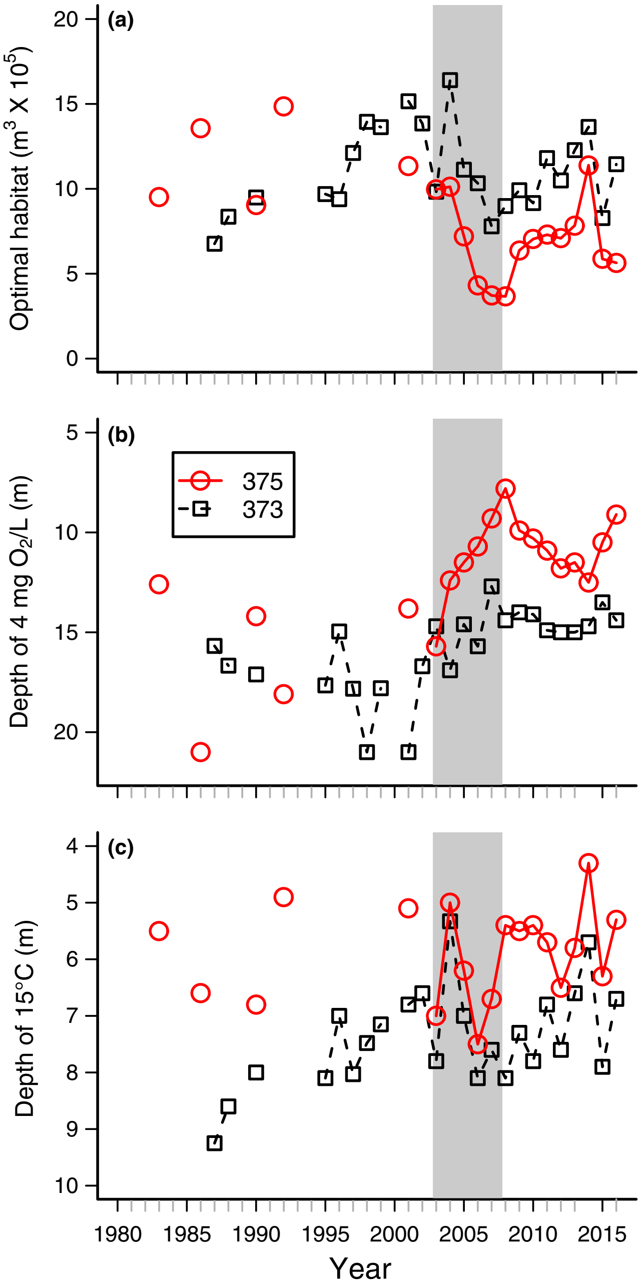

FIGURE 1 Shifts in optimal oxythermal habitat over time. (a) Changes in late summer (*c.* 1 September) habitat volume (oxygen ≥ 4 mg O_2/L, temperature ≤15°C) in lakes 375 (aquaculture, circles and solid lines) and 373 (reference lake, squares and dashed lines). (b) Depth of oxygen ≥ 4 mg O_2/L over time in both lakes; (c) the depth of the 15°C isocline. Grey shading represents the period of aquaculture in Lake 375 [Colour figure can be viewed at wileyonlinelibrary.com]

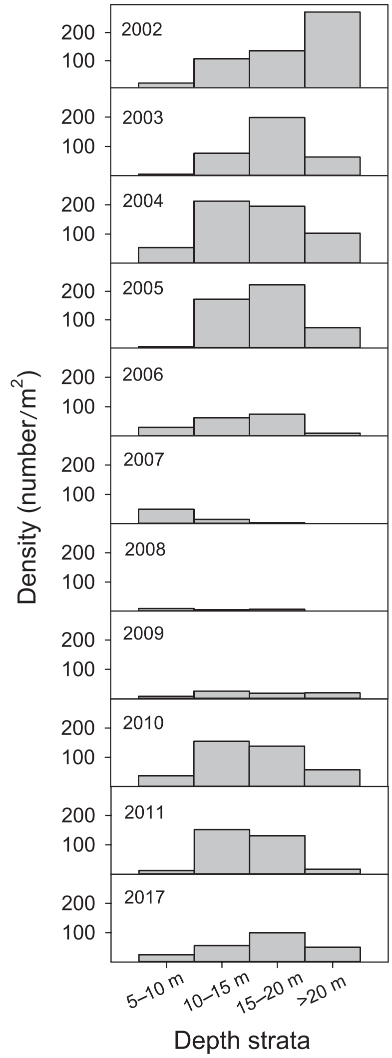

FIGURE 2 Late summer *Mysis* densities within different depth strata in Lake 375, 2002–2017. Data for 2002–2008 are represented by multiple sampling events in June–July, reproduced with permission from Paterson et al. (2011). Data from 2009 to 2017 are from a single night of sampling in July of each year, except 2017, when they were collected in September. Aquaculture operations ran from 2003 to 2007

both lakes ($F_{1,14}$ = 15.3, p = 0.002, R^2 = 0.52) but not when considering Lake 373 alone ($F_{1,5}$ = 2.4, p = 0.18).

3.2 | Changes in prey fish abundance

Prey fish abundance varied with aquaculture and by season (Figure 4). Minnow abundance (mean seasonal CUE) in autumn increased sharply in Lake 375 during aquaculture, a pattern that was not observed in Lake 373 (ANOVA, lake by period interaction, $F_{2,32}$ = 4.8, p = 0.015; Figure 4a). During aquaculture, autumn minnow abundance in Lake 375 was significantly higher than before (Tukey, p = 0.0004) and after aquaculture (Tukey, p = 0.04). Minnow abundance in Lake 375 was significantly greater than in Lake 373 during aquaculture (Tukey, p = 0.005), but not different from 373 before and after aquaculture (Tukey, $p \gg 0.05$). By contrast, there were no significant differences in minnow abundance between aquaculture and non-aquaculture years among lakes during spring (ANOVA, $F_{2,32}$ = 0.3, p = 0.7; Figure 4b), suggestive of significant overwinter mortality of minnows. Increases in minnow abundance in Lake 375 were almost entirely driven by an increase in fathead minnow (Supporting Information Appendix B). Sculpin abundance in Lake 375 was significantly elevated during aquaculture relative to non-aquaculture years in both spring and autumn, a pattern not observed in Lake 373 (autumn, $F_{2,31} = 6.7$, $p = 0.004$, Figure 4c; spring, $F_{2,32} = 3.5$, $p = 0.04$, Figure 4d). Autumn sculpin abundance remained elevated in Lake 375 into 2008, 1 year after aquaculture operations ceased (Figure 4c).

Changes in fathead minnow size distributions in Lake 375 suggest that larger fatheads were most susceptible to overwinter mortality during aquaculture (Supporting Information Appendix C). Prior to aquaculture, size distributions of fatheads in Lake 375 were similar to those observed in reference lakes (Supporting Information Appendix C), which were typically left-skewed in autumn (dominated by YOY minnows), and right-skewed in spring (loss of smaller YOY minnows over winter). During aquaculture operations in 2004–2007, this pattern reversed; autumn minnow size distributions became right-skewed (more large individuals) rather than left-skewed, a pattern which persisted until 2009, returning to a left-skew pattern in 2010. The changes in shape and magnitude of Lake 375 spring size distributions during 2005–2009 (closer to normally-distributed and less skewed; Supporting Information Appendix C) suggests a loss of larger minnows over winter in this lake.

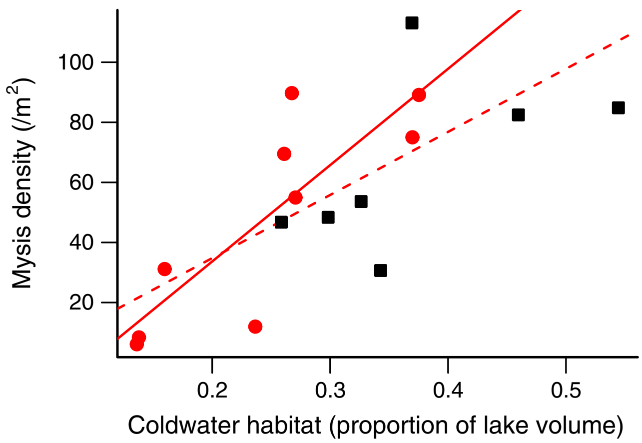

FIGURE 3 Relationships between mean area-weighted *Mysis* densities and the proportion of coldwater habitat (as defined in Figure 1, text) in Lake 375 (circles, solid line) and Lake 373 (squares). Dashed line represents the relationship including data over both lakes [Colour figure can be viewed at wileyonlinelibrary.com]

The autumn size of YOY fishes in Lake 375 (i.e. at the end of the growing season) declined during and immediately following aquaculture (Figure 5). From 2002 to 2009, fathead minnow YOY size as estimated from mixing models declined significantly ($F_{1,4} = 9.3$, $p = 0.038$) as did that of white sucker ($F_{1,5} = 7.03$, $p = 0.045$). No similar trends were observed in reference lakes for fathead minnow (Lake 442, Lake 224; Figure 5a) or for white sucker (Lake 373; Figure 5b). YOY average sizes of fathead minnows, but not white suckers, recovered to pre-aquaculture sizes by 2012 in Lake 375.

3.3 | Population changes in white sucker

White sucker abundance declined significantly in Lake 375 over the course of the aquaculture experiment, remained low for 2 years post-recovery ($F_{1,5} = 13.5$, $p = 0.014$; Figure 6a) and returned to pre-manipulation levels in 2010. Mean size of white sucker increased by over 150 mm during the aquaculture experiment ($F_{1,3} = 58$, $p = 0.047$; Figure 6b), and did not return to pre-manipulation levels until 2009, 2 years after aquaculture operations ceased. Age at maturity in male white sucker delayed steadily from age 3 to age 5 during the experiment and recovered to pre-aquaculture levels by 2008 (Figure 6c), whereas female age at maturity did not change in any clear fashion (Figure 6d). Size at maturity of white sucker increased by nearly 20 cm in both sexes during aquaculture (Figure 6e,f). Body condition in small white sucker did not change in any predictable pattern ($p = 0.5$, Figure 6g), but the condition of large white sucker

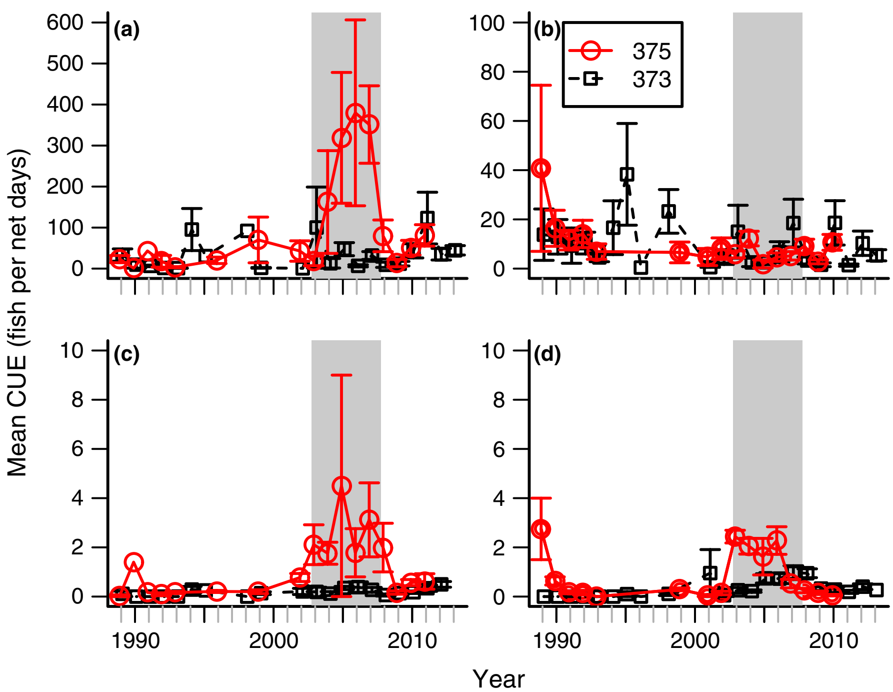

FIGURE 4 Changes in prey fish communities in lake 375 (aquaculture) and 373 (reference). Symbols as in Figure 1. Mean relative abundance of all minnow species (trap net catch per unit effort, CUE) in autumn (a) and spring (b). Relative abundance of sculpin in autumn (c) and spring (d). Note differences in axis scaling between spring and autumn minnow abundance (a, b), and between minnows (a, b) and slimy sculpin abundance (c, d). Note slight horizontal offset in data points for each lake. Grey shading represents the period of aquaculture in Lake 375. Error bars are ±1 *SE* [Colour figure can be viewed at wileyonlinelibrary.com]

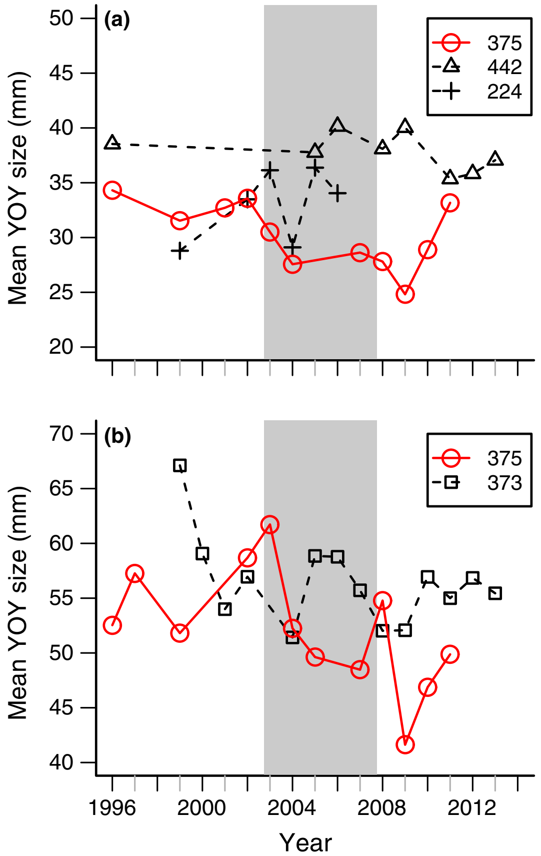

FIGURE 5 Size of young-of-year (YOY) fishes captured in trap nets at the end of their first growing season. (a) Mean size (fork lengths) of autumn YOY fathead minnow during aquaculture in Lakes 375 (aquaculture) and reference lakes 224 and 442. (b) Mean size (fork lengths) of YOY white sucker in Lake 375 during aquaculture and in reference Lake 373 [Colour figure can be viewed at wileyonlinelibrary.com]

declined over the course of aquaculture and remained depressed until 2011, returning to pre-aquaculture levels between 2012 and 2013 ($F_{1,8}$ = 63.4, p < 0.0001, Figure 6h). No similar trends in abundance (p = 0.3), mean size (p = 0.7), maturity or body condition (small, p = 0.9; large, p = 0.28) were observed in our Lake 373 reference population of white sucker (Figure 6a–h).

Size distributions of white sucker suggest an increase in overwinter mortality in juveniles (Supporting Information Appendix D). In both lakes 373 and 375, abundant YOY (<70 mm fork length) and occasionally yearling white sucker (<100 mm) dominated autumn catches (Supporting Information Appendix D). Throughout the time series in Lake 373, there is evidence of some overwinter survival of these youngest age classes from autumn to the following spring. In Lake 375, this was true until 2005, after which the size distribution of the population became heavily right-skewed in spring catches, indicating that only the largest individuals survived the winter. Overwinter survival of juvenile white sucker was again evident in 2008 after the cessation of aquaculture operations.

Size-at-age of Lake 375 white sucker appeared to increase for younger age classes during aquaculture, increasing by nearly 100 mm in age classes 4–6 (Figure 7a). No similar increases were observed in the reference population (Figure 7b) over a similar age range.

3.4 | Population changes in lake trout

Abundance of adult lake trout increased during aquaculture; population estimates from spring 2006 to autumn of 2007 (725 adult lake trout) were more than double those during pre-manipulation (340 adult lake trout; Figure 8a). This increase in abundance was observed despite no difference in catchability estimates between pre-manipulation and aquaculture periods (Supporting Information Appendix A, Figure A2). Abundance declined sharply after aquaculture ceased (Figure 8a), despite an increase in catchability in Lake 375 (Supporting Information Appendix A). Our reference lake (Lake 373) showed a steady but modest increase over time, very different from the changes observed in Lake 375 (Figure 8a). The increase in population size observed during aquaculture appears to have been largely a consequence of increased immature growth rates combined with advancing age at maturity and increased body condition (detailed below). Additional results from POPAN model fits for lake trout in Lakes 373 and 375 are summarised in Supporting Information Appendix A.

Mean size of lake trout increased sharply in 2006 and remained elevated until 2010 (well past aquaculture ended), not returning to values close to pre-manipulation levels until 2011; by contrast, mean size of lake trout in the reference lake declined over time ($F_{1,17}$ = 14.1, p = 0.0016).

In the last year of aquaculture operations, age at maturity in both male and female lake trout was approximately half of historical values. Male age at maturity decreased from 7–8 years pre-aquaculture to age 4 in the last 2 years of the experiment, increasing steadily again after the cessation of aquaculture activities (Figure 8c). Female age at maturity decreased from 9–11 years pre-aquaculture to age 7–6, continuing to decline to age 5 in the year following aquaculture operations before increasing steadily afterwards (Figure 8d). No clear trend was apparent in age at maturity for either sex in reference Lake 373 lake trout. There was a significant interaction between lake and time period (pre- versus post-aquaculture) for lake trout size at maturity for both males ($F_{2,27}$ = 21.4, p < 0.0001) and females ($F_{2,26}$ = 20.9, p < 0.0001). Size at maturity in both male and female lake trout in Lake 375 increased during aquaculture (Figure 8e,f) and was much higher following aquaculture (402 and 409 mm for males and females, respectively) compared to pre-aquaculture (378 and 387 mm for males and females, respectively), an increase of about 20 mm for both sexes. By contrast, size at maturity for male and female lake trout in reference Lake 373 decreased by approximately 20 mm.

Body condition of lake trout collected in autumn increased sharply in 2005 during aquaculture and remained well outside the envelope of observed variation pre-manipulation or for that of reference Lake 373 until the end of aquaculture operations in 2007. Body condition then declined sharply and was lower than pre-aquaculture values in 2009–2010 before returning to pre-manipulation levels in

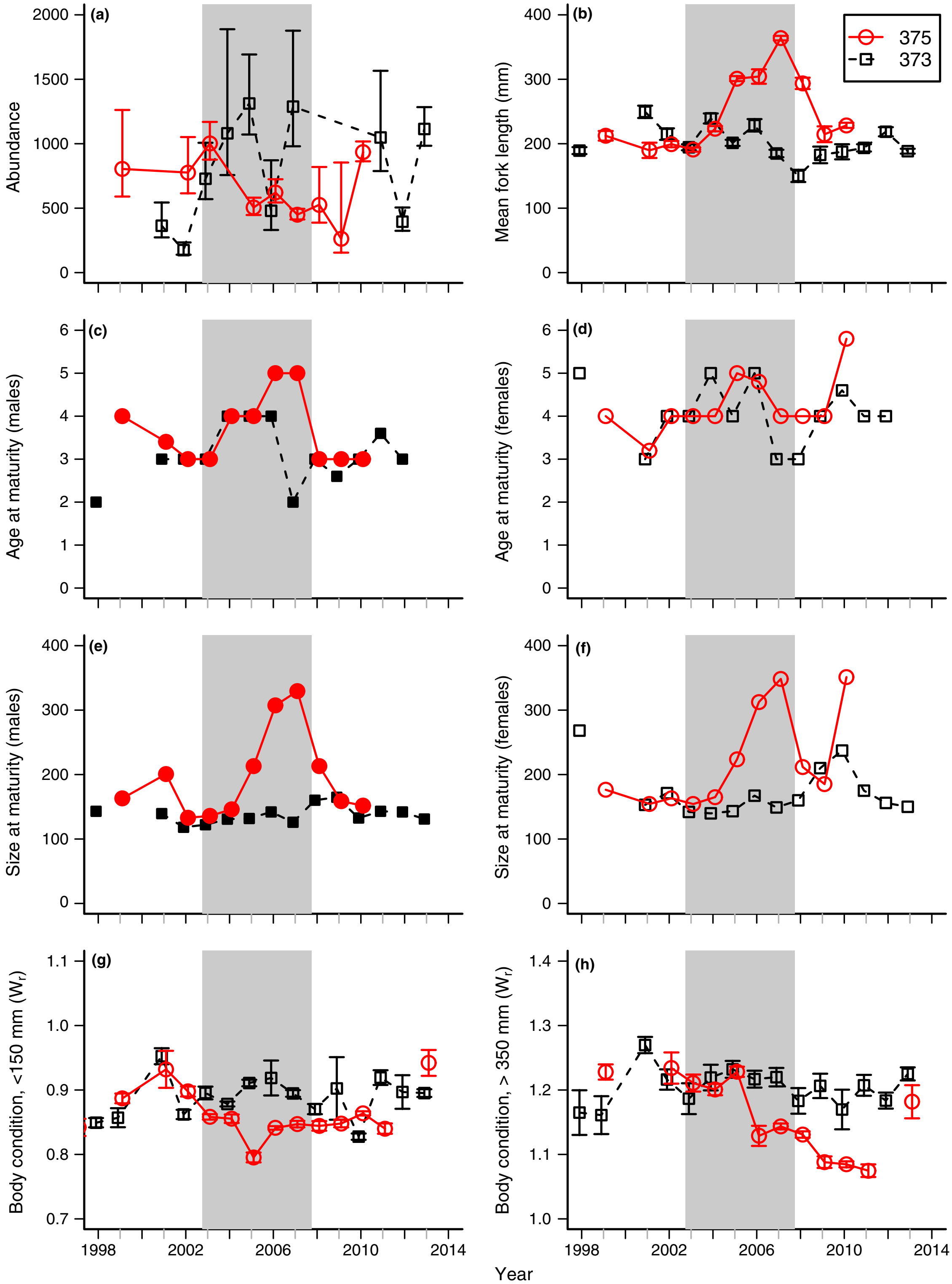

FIGURE 6 Changes in white sucker in lakes 375 (aquaculture) and 373 (reference). (a) Abundance estimated using Schnabel census; (b) mean size (fork length); (c, d) age at maturity (20% quantile of aged fish captured during spawning) for males and females, respectively; (e, f) size at maturity (20% quantile of measured fish captured during spawning) for males and females; and body condition (relative weight, W_r) of small (<150 mm, g) and large (>350 mm, h) white sucker. All metrics except body condition are for spring-collected fish only; body condition combines information from spring and autumn. Error bars in (a) are 95% confidence intervals, otherwise are ±1 *SE* [Colour figure can be viewed at wileyonlinelibrary.com]

2011. Body condition in Lake 373 was variable, but followed a similar pattern as observed in Lake 375 except for the period during and immediately after aquaculture (Figure 8g).

Lake trout size-at-age generally increased in Lake 375 during aquaculture operations, particularly in younger age classes (ages 2–4), reaching asymptotic sizes more rapidly (Figure 9a). Following

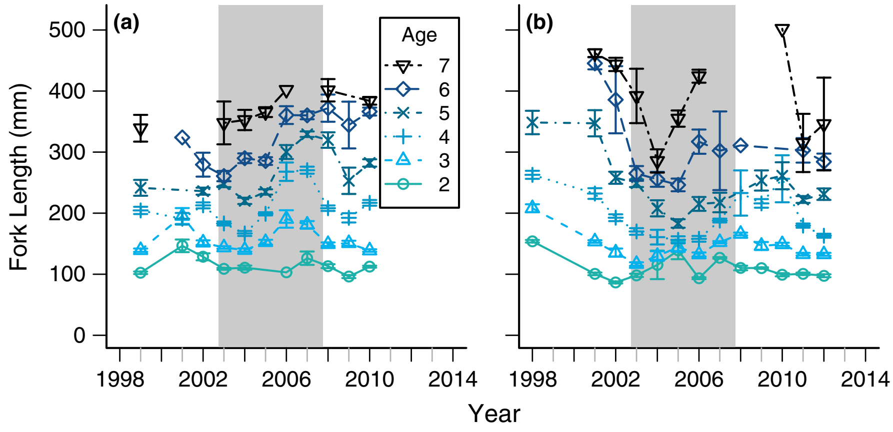

FIGURE 7 Growth (size at age) of select age classes of spring-caught white sucker in lake 375 (aquaculture, a) and 373 (reference, b). Data are for male and female fish combined, and include fish where sex was undetermined [Colour figure can be viewed at wileyonlinelibrary.com]

the cessation of aquaculture, size-at-age returned to background levels between 2008 and 2011, the recovery being more rapid in younger age classes. Growth in older age classes of fish (5–12) also increased during aquaculture, but much less dramatically, and declined more slowly to background levels (Figure 9a). By contrast, size-at-age of all age classes in reference Lake 373 declined during the period of investigation (Figure 9b).

4 | DISCUSSION

We observed significant and dramatic effects of aquaculture on fish populations, largely consistent with expectations of effects due to nutrient additions that are probably associated with added trout feed and production of waste from cultured fish (Azevedo et al., 2011; Bristow et al., 2008; Wellman et al., 2017). In nearly all cases where changes were observed during aquaculture operations in Lake 375, there were no corresponding changes (or changes were in the opposite direction) in our reference lakes. Most increases in fish abundance occurred 2–3 years after aquaculture began, which is consistent with observations from nutrient addition experiments in other ELA lakes (Mills & Chalanchuk, 1987; Mills et al., 2002). Further, given the relative insensitivity of water nutrient parameters versus those expected via aquaculture nutrient bioenergetics models (Azevedo et al., 2011), it appears likely from the results of this study that a large proportion of these nutrients ended up as either increased fish biomass or deposited in the hypolimnion.

Optimal oxythermal habitat and *Mysis* densities declined dramatically during aquaculture in Lake 375, and both show continued impairment a decade following the end of the experiment. Indeed, *Mysis* densities were significantly related to the availability of coldwater habitat both within Lake 375 and across both lakes 373 and 375. As such, we may not expect a full recovery of the *Mysis* population in Lake 375 until coldwater habitat availability there improves.

Slimy sculpin and minnows (during autumn) showed a significant and positive numeric response to aquaculture. The increased catch of slimy sculpin in spring and autumn may reflect either an increase in density, or their inability to occupy deeper habitats during these time periods due to oxygen limitation (therefore forcing a higher density of slimy sculpin into smaller, more shallow areas where they are more susceptible to trap net capture). Nutrient enrichment has been shown in other small, boreal lakes to increase egg deposition and hatching, as well as growth rates (larger end of season YOY size) and abundance of YOY fish in autumn (Grant & Tonn, 2002). In contrast, we observed *smaller* YOY size of fathead minnow and white sucker in autumn, suggesting slower YOY growth rates in response to aquaculture. If nutrient additions were localised around the cage and were not effectively transported to nearshore environments, then increased minnow abundance (and therefore increased competition nearshore) may have resulted in reduced rates of growth of YOY fishes, which would be more limited to nearshore habitats than adult minnows. Offshore invertebrates seemed to change more dramatically than nearshore invertebrates during aquaculture (Wellman et al., 2017), suggesting that, while incorporation of aquaculture feed occurred throughout the lake, transport to nearshore environments may have been lower compared to offshore environments.

Despite increased autumn catches, spring minnow abundance showed no significant changes due to aquaculture, suggesting considerable overwinter mortality. This is further supported by declining sizes of fall YOY fathead minnow during and immediately after aquaculture. An examination of size distributions of fathead minnow (the dominant minnow species in Lake 375) revealed that large individuals were probably also suffering overwinter mortality during the last 2 years of aquaculture; large minnows dominated the autumn catches 2005–07, whereas they were far less represented in the following spring. This is opposite to what was seen in other (non-aquaculture) years in Lake 375 or in other ELA lakes where small fathead minnow YOY dominate the autumn catches and are typically the size class that is less well represented the following spring (Supporting Information Appendix C). In other nutrient enrichment studies, overwinter mortality was mostly concentrated on smaller size classes of minnow (Grant & Tonn, 2002) and overwinter mortality was typically highest in small-bodied YOY fish with relatively lower energy reserves (Post & Evans, 1989). There is some evidence that egg deposition and hatching in fathead minnow may be extended under nutrient enrichment (Kiesling, 1999). As a species that spawns continuously through the warm water season, this could have negative impacts on the overwinter survival of adult fathead

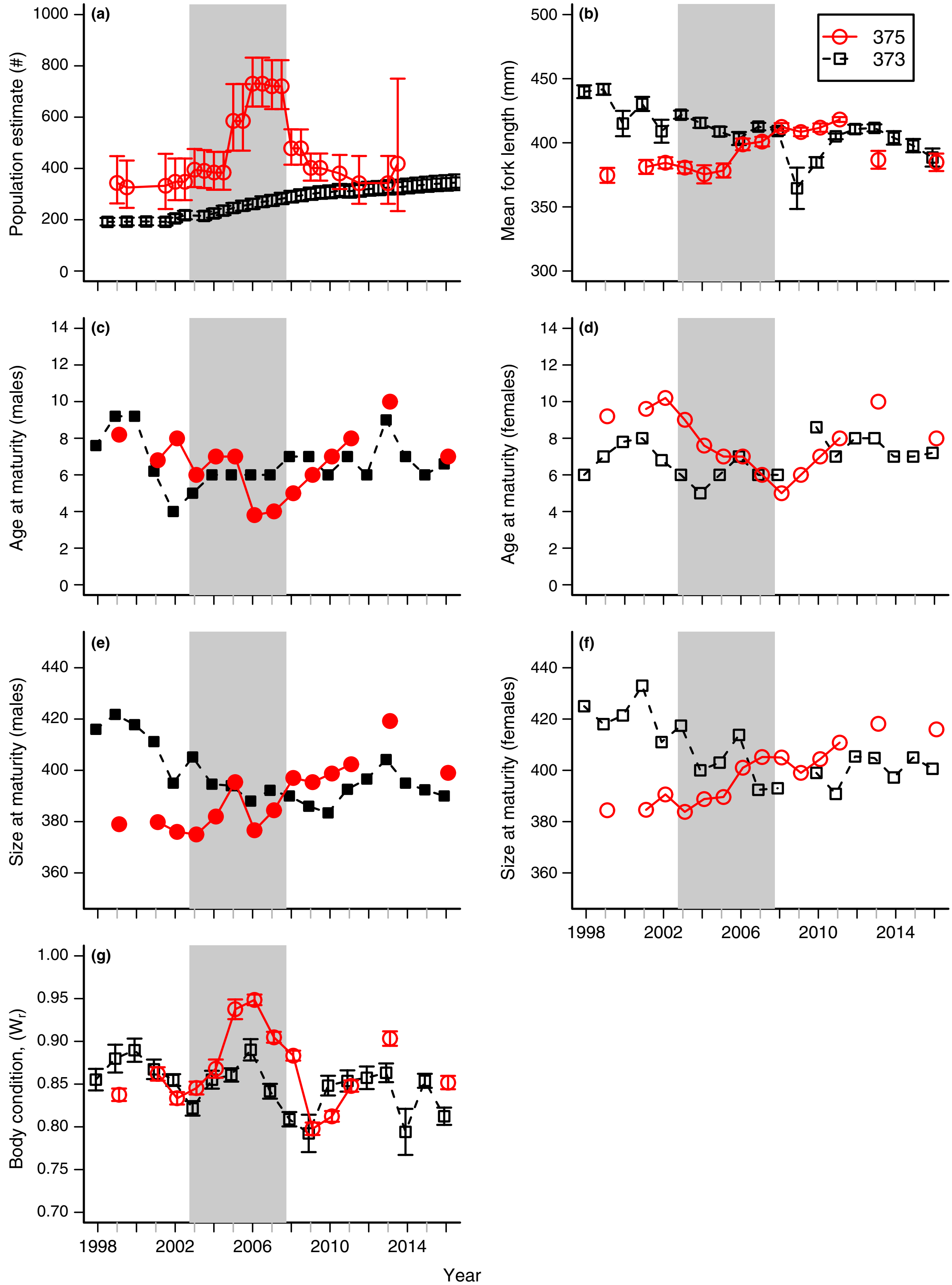

FIGURE 8 Changes in adult lake trout in lakes 375 (aquaculture) and 373 (reference). (a) Abundance estimated using a POPAN model applied to lake trout mark–recapture data in each lake; (b) mean size (fork length); (c, d) age at maturity (20% quantile of aged fish captured during spawning) for males and females, respectively; (e, f) size at maturity (20% quantile of measured fish captured during spawning); and (g) body condition (relative weight, W_r) of lake trout >280 mm total length. Error bars in (a) are 95% confidence intervals, otherwise are ±1 *SE* [Colour figure can be viewed at wileyonlinelibrary.com]

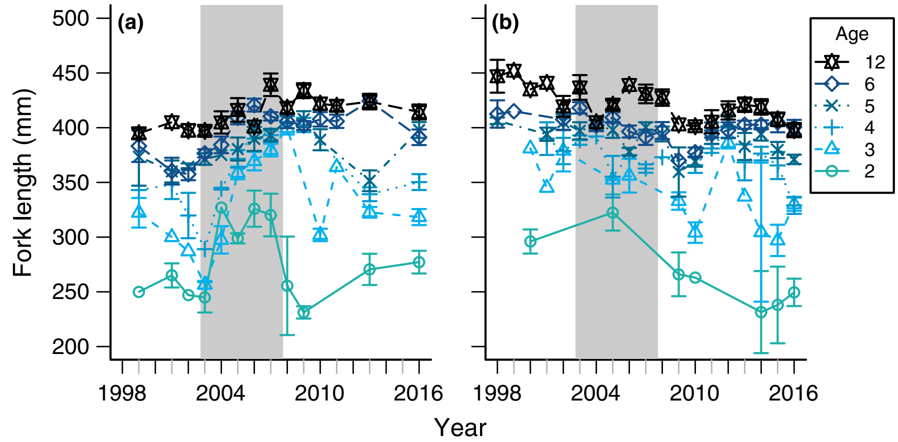

FIGURE 9 Growth (size at age) of select age classes of autumn-caught lake trout in lake 375 (aquaculture, a) and 373 (reference, b). Data are for male and female fish combined and include fish where sex was undetermined [Colour figure can be viewed at wileyonlinelibrary.com]

minnow if spawning occurs too late in the year to secure sufficient somatic energy to permit overwinter survival. Additionally, overwinter mortality of small fishes could be due not just to small size and insufficient somatic provisioning, but also to predation from increased abundance of adult lake trout; minnows are important prey species for lake trout when surface water temperatures facilitate nearshore feeding (e.g. during the shoulder seasons and winter; Guzzo & Blanchfield, 2017; Guzzo, Blanchfield, & Rennie, 2017).

Of all fishes investigated, white sucker were the most negatively affected by aquaculture. Estimated abundance of white sucker >100 mm declined from approximately 800 individuals to approximately 500 during the last 3 years of aquaculture operations and achieved the lowest observed abundance of 250 individuals in 2009, 2 years after operations ceased. Abundance returned to pre-impact levels in 2010. This dramatic decline in abundance probably played a significant role in increased growth rates observed in Lake 375 white sucker. Despite increased growth and reduced competition, body condition of large white sucker declined throughout aquaculture and for 4 years after operations ceased. Increases in mean size, increased size, and age at maturity and declines in abundance strongly suggest overwinter mortality and recruitment failure during the later years of aquaculture. Although the abundance of small white sucker in autumn was high, YOY white sucker size declined as the experiment progressed and were greatly underrepresented in spring catches in the last 2 years of aquaculture operations. Successful recruitment of young white sucker from 2008 and 2009 year classes (Supporting Information Appendix D) appeared to be largely responsible for increased abundance estimates in 2010. By contrast, body condition of large white sucker remained depressed until 2013.

The decline in overwinter survival and reduced YOY sizes of both minnows and white sucker during nutrient enrichment associated with aquaculture is unexpected and opposite the impacts of eutrophication observed elsewhere (Grant & Tonn, 2002; Post & Evans, 1989). Increased nutrients should provide additional resources for increased growth rates of YOY fish and permit increased overwinter survival for larger bodied fishes. Additionally, size of age 2 white sucker was stable throughout the period of study, suggesting that growth rates of smaller fish (aged 0–2) may not have benefitted from aquaculture operations. It is possible that the high abundance of YOY white sucker along with the large increases in minnow abundance in autumn caused significant competition among small fishes sharing similar habitats (nearshore), preventing growth rates above critical size thresholds to permit overwinter survival. white sucker typically feed on benthic algae, detritus, zooplankton, and invertebrates (Saint-Jacques, Harvey, & Jackson, 2000; Scott & Crossman, 1998). Benthic invertebrate densities immediately under and near the cage declined during operations (Rooney & Podemski, 2009), although white sucker were not likely to be using this region for foraging; acoustic telemetry data from Lake 375 suggest that white sucker are typically found at depths 5–10 m and very rarely occupy benthic habitats at depths observed under the cage (16 m; P. Blanchfield, unpublished data).

Declines in white sucker abundance and body condition could plausibly be due to food limitation. Both fathead minnow and white sucker can exhibit zooplanktivory in small lakes (Saint-Jacques et al., 2000; Tallman, Mills, & Rotter, 1984). Although zooplankton biomass did not increase with aquaculture (Paterson, Podemski, Findlay, Findlay, & Salki, 2010), increased abundance of fish with potential to forage on zooplankton would result in a lower per capita availability of prey for zooplanktivorous fishes during aquaculture. Additionally, recent work has demonstrated that benthic habitat availability can limit secondary production of invertebrates, either through light limitation (Finstad, Helland, Ugedal, Hesthagen, & Hessen, 2014; Karlsson et al., 2009) or oxythermal habitat (Craig, Jones, Weidel, & Solomon, 2015). In Lake 375, mean secchi depth in the last year of aquaculture was 1–2 m shallower than in 2002 (C. Podemski, unpublished data). This reduction in light penetration combined with our observed reduction in well-oxygenated benthic habitat during aquaculture suggests that both processes could be responsible for an overall reduction in benthic algal and invertebrate production in the lake (Craig et al., 2015). As white sucker are a primarily zoobenthivorous fish, this reduction in suitable habitat for zoobenthos during aquaculture operations could provide a plausible explanation for negative impacts of aquaculture on this species, as well as for reduced growth in YOY sucker and minnows, which all tend to occupy nearshore benthic environments. Direct predation of lake trout on white sucker is also a possibility, but currently lacks direct evidence (Guzzo, 2018; Kennedy et al., In press).

Lake trout appeared to respond positively to aquaculture operations, despite reductions in coldwater habitat availability and reduction in *Mysis* densities. Abundance of adult lake trout more than doubled during aquaculture, accompanied by increased growth rates, maturation at earlier ages and larger sizes and improved body condition, probably in response to increased prey availability (Kennedy et al., In press) facilitated by increased nutrient loading (Bristow et al., 2008). Increased growth rates of lake trout in response to nutrient enrichment has been reported elsewhere (Lienesch et al., 2005). Recent work demonstrated that increased size at age reported here correspond well with back-calculated size at age in Lake 375, which was based on a greater number of observations and arguably better reflect true early growth patterns in the lake (Kennedy et al., In press). More rapid growth combined with earlier maturation resulted in larger mean sizes overall and larger sizes at maturity in younger fish. As fish matured earlier, this would have resulted in additional (younger) cohorts arriving on spawning shoals over time. Based on a maturation age of 9 for female lake trout pre-aquaculture and 6 in the last year of aquaculture operations, three additional cohorts of fish would be using spawning shoals by the end of the experiment compared to before aquaculture. This likely caused the large increase in lake trout abundance estimates, which measures adult population size based on fish collected primarily from spawning shoals. Because fecundity is dependent on body size (Shuter et al., 2005; Trippel, 1993; Wootton & Smith, 2014), larger mature lake trout during aquaculture were probably also depositing a greater number of eggs on spawning shoals. This suggests that future collections of lake trout from Lake 375 should show 2003–2007 as strong recruitment years. Furthermore, body condition of lake trout improved during aquaculture. Condition increases in young fish would probably also improve survival to maturity during aquaculture operations. The simultaneous increase in abundance, growth rates, and body condition would suggest that aquaculture operations increased the carrying capacity for lake trout in the ecosystem. The rapid decline in lake trout abundance at the end of aquaculture operations in 2008 is likely to be associated with food limitation; sculpin and minnow abundance declined following operations in 2008 and 2009, *Mysis* densities were dramatically depressed during 2007–2009, and body condition of lake trout declined dramatically in 2009 and 2010. Although not significantly different among time periods, lake trout annual survival was consistently below 1 and recruitment was consistently low immediately following aquaculture (Supporting Information Figure A1). Size at age of smaller age classes of lake trout (ages 2 and 3) declined sharply after aquaculture ceased.

Increased growth of lake trout may have also been related to an increase in spatial overlap with key prey items (specifically *Mysis* and slimy sculpin) during the summer when all are thermally limited, as indicated by an increased reliance on offshore prey during aquaculture (Kennedy et al., In press). As such, reductions in *Mysis* densities during aquaculture may have been at least partially due to increased predation by lake trout as the oxythermal habitat of both species became more restricted, resulting in increased encounter rates between the two. Only when *Mysis* densities became critically low in 2007 was there evidence of summer feeding on minnows (Kennedy et al., In press).

The differences in patterns between spring and autumn minnow CUE observed here (probably due to significant overwinter mortality) may also help explain observed patterns in lake trout resource use during aquaculture (Kennedy et al., In press). Lake trout isotopic signatures in fall suggested reduced use of nearshore/fish prey resources during aquaculture in summer, despite significant increases in mean annual minnow abundance (Kennedy et al., In press). However, we demonstrate here that although minnow abundance in autumn was greatly enhanced during aquaculture, spring minnow abundance was not affected. Recent work demonstrated the importance of minnow CUE in spring on lake trout feeding patterns, and that autumn minnow CUE had little impact on lake trout feeding behaviour (Guzzo, Blanchfield, & Rennie, 2017). Furthermore, fish often reduce their feeding rates in advance of spawning (Fordham & Trippel, 1999; Hung, Eder, Javidmehr, & Loge, 2014; Krumsick & Rose, 2012), which may limit resource use of abundant minnows in autumn by lake trout. Foraging on small fish prey by lake trout (e.g. minnows, juvenile white sucker) during winter months may be significant.

Impacts on many of the metrics reported in this study persisted for years after aquaculture operations ceased. Autumn minnow and slimy sculpin abundance took 2 years to return to background levels. Many metrics associated with white sucker (abundance, mean size) took 2 years to return to background levels, and body condition took 4–6 years to recover. Lake trout abundance returned to background levels in 2009, and mean size and body condition was outside pre-aquaculture ranges until 2011. Some metrics, including lake trout size at maturity, *Mysis* densities and coldwater habitat appear to be impaired into our last years of data collection (2016–17), and may require further monitoring to assess recovery.

The results of this study reflect aquaculture impacts on a small boreal lake; on a larger lake (as in Lake Diefenbaker or on the Great Lakes, both of which support freshwater aquaculture activities), it is unlikely that aquaculture operations would scale similarly to lake size. However, our results provide some guidance as to which species appear to be most sensitive to aquaculture, and therefore would make good indicator species for assessing aquaculture impacts in larger systems (e.g. lake trout, white sucker, slimy sculpin, minnows, and *Mysis*), as well as the importance of the timing of monitoring (spring versus autumn time periods).

In conclusion, the results from this experiment indicated significant changes in the fish community during aquaculture, and that these changes were largely facilitated by changes in prey availability via changes in primary productivity. Fish species that responded most strongly to aquaculture were lake trout, white sucker, fathead minnow, and slimy sculpin, consistent with other whole-lake manipulations at the ELA (Kidd et al., 2014; Mills, Chalanchuk, & Allan, 2000; Mills, Chalanchuk, Mohr, & Davies, 1987). Metrics associated with growth and maturation schedules, body condition, abundance, and overwinter mortality were most sensitive to aquaculture. The ongoing restriction of deep-water habitat and continued depression of *Mysis* densities in Lake 375 is suggestive of ongoing impacts that have carried over from aquaculture (possibly internal loading), although most species appear to be in a general state of recovery 9 years after operations ceased. While it is highly unlikely that aquaculture operations would ever be licensed or conducted in lakes as small as Lake 375, this study provides important information on identifying key species and traits of fishes that are likely to respond to operations in freshwater ecosystems elsewhere that could be used to develop effective monitoring programmes.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

DATA ACCESSIBILITY

All data presented are available by request via e-mail to the first author, where they are archived with the IISD-ELA.

AUTHOR CONTRIBUTIONS

C.P., K.M., M.P., and P.B. conceived the idea and designed the study; all authors collected data; C.R., L.H., and S.C. organised data for analysis; M.P., C.R., C.C., M.D.R., and P.K. analysed data; M.D.R., P.K., L.H., C.C., and M.P. prepared figures and tables and wrote the manuscript. All authors contributed critically to drafts and gave final approval for publication.

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REFERENCES

Ackefors, H., & Enell, M. (1994). The release of nutrients and organic matter from aquaculture systems in Nordic countries. *Journal of Applied Ichthyology*, *10*, 225–241. https://doi.org/10.1111/j.1439-0426.1994.tb00163.x

Azevedo, P. A., Podemski, C. L., Hesslein, R., Kasian, S. E. M., Findlay, D., & Bureau, D. (2011). Estimation of waste outputs by a rainbow trout cage farm using a nutritional approach and monitoring of water quality. *Aquaculture*, *311*, 175–186. https://doi.org/10.1016/j.aquaculture.2010.12.001

Beamish, R. J. (1973). Design of a trapnet with interchangeable parts for the capture of large and small fishes from varying depths. *Journal of the Fisheries Research Board of Canada*, *30*, 587–590. https://doi.org/10.1139/f73-104

Bister, T. J., Willis, D. W., Brown, M. L., Jordan, S. M., Neumann, R. M., Quist, M. C., & Guy, C. S. (2000). Proposed standard weight (W_s) equations and standard length categories for 18 warmwater nongame and riverine fish species. *North American Journal of Fisheries Management*, *20*, 570–574. https://doi.org/10.1577/1548-8675(2000)020<0570:pswwse>2.3.co;2

Blanchfield, P. J., Tate, L. S., & Podemski, C. L. (2009). Survival and behaviour of rainbow trout (*Oncorhynchus mykiss*) released from an experimental aquaculture operation. *Canadian Journal of Fisheries and Aquatic Sciences*, *66*, 1976–1988. https://doi.org/10.1139/f09-127

Bristow, C. E., Morin, A., Hesslein, R. H., & Podemski, C. L. (2008). Phosphorus budget and productivity of an experimental lake during the initial three years of cage aquaculture. *Canadian Journal of Fisheries and Aquatic Sciences*, *65*, 2485–2495. https://doi.org/10.1139/f08-155

Charles, C., Blanchfield, P. J., & Gillis, D. M. (2017). Site fidelity of escaped rainbow trout to an experimental freshwater aquaculture facility and habitat overlap with native fish fauna. *Aquaculture Environment Interactions*, *9*, 415–428. https://doi.org/10.3354/aei00243

Craig, N., Jones, S. E., Weidel, B. C., & Solomon, C. T. (2015). Habitat, not resource availability, limits consumer production in lake ecosystems. *Limnology and Oceanography*, *60*, 2079–2089. https://doi.org/10.1002/lno.10153

Cruz Font, L., Shuter, B. J., Blanchfield, P. J., Minns, C. K., & Rennie, M. D. (In press). Life at the top: Lake ecotype influences the metabolic costs and life history of an apex fish predator. *Journal of Animal Ecology*, https://doi.org/10.1111/1365-2656.12956.

Devlin, S. P., Tappenbeck, S. K., Craft, J. A., Tappenbeck, T. H., Chess, D. W., Whited, D. C., … Stanford, J. A. (2017). Spatial and temporal dynamics of invasive freshwater shrimp (*Mysis diluviana*): Long-term effects on ecosystem properties in a large oligotrophic lake. *Ecosystems*, *20*, 183–197. https://doi.org/10.1007/s10021-016-0023-x

Ellis, B. K., Stanford, J. A., Goodman, D., Stafford, C. P., Gustafson, D. L., Beauchamp, D. A., … Hansen, B. S. (2011). Long-term effects of a trophic cascade in a large lake ecosystem. *Proceedings of the National Academy of Sciences of the United States of America*, *108*, 1070–1075. https://doi.org/10.1073/pnas.1013006108

Enell, M. (1995). Environmental impact of nutrients from Nordic fish farming. *Water Science and Technology*, *31*, 61–71. https://doi.org/10.2166/wst.1995.0364

Evans, D. O. (2007). Effects of hypoxia on scope-for-activity and power capacity of lake trout (*Salvelinus namaycush*). *Canadian Journal of Fisheries and Aquatic Sciences*, *64*, 345–361. https://doi.org/10.1139/f07-007

Evans, D. O., Nicholls, K. H., Allen, Y. C., & McMurtry, M. J. (1996). Historical land use, phosphorus loading, and loss of fish habitat in Lake Simcoe, Canada. *Canadian Journal of Fisheries and Aquatic Sciences*, *53*, 194–218. https://doi.org/10.1139/f96-012

Findlay, D. L., Podemski, C. L., & Kasian, S. E. M. (2009). Aquaculture impacts on the algal and bacterial communities in a small boreal forest lake. *Canadian Journal of Fisheries and Aquatic Sciences*, *66*, 1936–1948. https://doi.org/10.1139/f09-121

Finstad, A. G., Helland, I. P., Ugedal, O., Hesthagen, T., & Hessen, D. O. (2014). Unimodal response of fish yield to dissolved organic carbon. *Ecology Letters*, *17*, 36–43. https://doi.org/10.1111/ele.12201

Fordham, B. S. E., & Trippel, E. A. (1999). Feeding behaviour of cod (*Gadus morhua*) in relation to spawning. *Journal of Applied Ichthyology*, *15*, 1–9. https://doi.org/10.1046/j.1439-0426.1999.00098.x

France, R., & Steedman, R. (1996). Energy provenance for juvenile lake trout in small Canadian Shield lakes as shown by stable isotopes. *Transactions of the American Fisheries Society*, *125*, 512–518. https://doi.org/10.1577/1548-8659(1996)125<0512:epfjlt>2.3.co;2

Government of Canada S.C. (2015). Aquaculture Statistics. Retrieved from http://www.statcan.gc.ca/pub/23-222-x/23-222-x2015000-eng.htm

Grant, S. C., & Tonn, W. M. (2002). Effects of nutrient enrichment on recruitment of age-0 fathead minnows (*Pimephales promelas*): Potential impacts of environmental change on the Boreal Plains. *Canadian Journal of Fisheries and Aquatic Sciences*, *59*, 759–767. https://doi.org/10.1139/f02-054

Green, R. (1979). *Sampling design and statistical methods for environmental biologists*. Chichester, UK: Wiley Interscience.

Grey, J., Waldron, S., & Hutchinson, R. (2004). The utility of carbon and nitrogen isotope analyses to trace contributions from fish farms to the receiving communities of freshwater lakes: A pilot study in Esthwaite Water, UK. *Hydrobiologia*, *524*, 253–262. https://doi.org/10.1023/b:hydr.0000036144.49795.dd

Gunn, J. M., & Mills, K. H. (1998). The potential for restoration of acid-damaged lake trout lakes. *Restoration Ecology*, *6*, 390–397. https://doi.org/10.1046/j.1526-100x.1998.06409.x

Gunn, J. M., Steedman, R. J., & Ryder, R. A. (2003). *Boreal shield watersheds: lake trout ecosystems in a changing environment*.

Guzzo, M. M. (2018). *The effects of climate change on north-temperate lake trout (*Salvelinus namaycush*) populations*. Winnipeg, MB: University of Manitoba.

Guzzo, M. M., & Blanchfield, P. J. (2017). Climate change alters the quantity and phenology of habitat for lake trout (*Salvelinus namaycush*) in small Boreal Shield lakes. *Canadian Journal of Fisheries and Aquatic Sciences*, *74*, 871–884. https://doi.org/10.1139/cjfas-2016-0190

Guzzo, M. M., Blanchfield, P. J., & Rennie, M. D. (2017). Behavioral responses to annual temperature variation alter the dominant energy pathway, growth, and condition of a cold-water predator. *Proceedings of the National Academy of Sciences of the United States of America*, *114*, 9912–9917. https://doi.org/10.1073/pnas.1702584114

Guzzo, M. M., Rennie, M. D., & Blanchfield, P. J. (2014). Evaluating the relationship between mean catch per unit effort and abundance for littoral cyprinids in small boreal shield lakes. *Fisheries Research*, *150*, 100–108. https://doi.org/10.1016/j.fishres.2013.10.019

Hung, T.-C., Eder, K. J., Javidmehr, A., & Loge, F. J. (2014). Decline in feeding activity of female cultured delta smelt prior to spawning. *North American Journal of Aquaculture*, *76*, 159–163. https://doi.org/10.1080/15222055.2014.886650

Johnston, T. A., Keir, M., & Power, M. (2010). Response of native and naturalized fish to salmonid cage culture farms in northern Lake Huron, Canada. *Transactions of the American Fisheries Society*, *139*, 660–670. https://doi.org/10.1577/t09-064.1

Karlsson, J., Byström, P., Ask, J., Ask, P., Persson, L., & Jansson, M. (2009). Light limitation of nutrient-poor lake ecosystems. *Nature*, *460*, 506–509. https://doi.org/10.1038/nature08179

Kennedy, P., Blanchfield, P. J., Kidd, K. A., Paterson, M., Podemski, C. L., & Rennie, M. D. (In press). Changes in the diet, early growth, and condition of lake trout (*Salvelinus namaycush*) in response to an experimental aquaculture operation. *Canadian Journal of Fisheries and Aquatic Sciences*, https://doi.org/10.1139/cjfas-2017-0578.

Kidd, K. A., Paterson, M. J., Rennie, M. D., Podemski, C. L., Findlay, D. L., Blanchfield, P. J., & Liber, K. (2014). Direct and indirect responses of a freshwater food web to a potent synthetic oestrogen. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *369*, 20130578. https://doi.org/10.1098/rstb.2013.0578

Kiesling, S. (1999). *Effects of nutrient enrichment on recruitment of age-0 fathead minnows (*Pimephales promelas*)*. Edmonton, AB: University of Alberta.

Krumsick, K. J., & Rose, G. A. (2012). Atlantic cod (*Gadus morhua*) feed during spawning off Newfoundland and Labrador. *ICES Journal of Marine Science*, *69*, 1701–1709. https://doi.org/10.1093/icesjms/fss158

Kullman, M. A., Kidd, K. A., Podemski, C. L., Paterson, M. J., & Blanchfield, P. J. (2009). Assimilation of freshwater salmonid aquaculture waste by native aquatic biota. *Canadian Journal of Fisheries and Aquatic Sciences*, *66*, 1965–1975. https://doi.org/10.1139/f09-128

Lienesch, P. W., McDonald, M. E., Hershey, A. E., O'Brien, W. J., & Bettez, N. D. (2005). Effects of a whole-lake, experimental fertilization on lake trout in a small oligotrophic arctic lake. *Hydrobiologia*, *548*, 51–66. https://doi.org/10.1007/s10750-005-3620-9

Mills, K. H. (1985). Responses of lake whitefish (*Coregonus clupeaformis*) to fertilization of lake-226, the experimental lakes area. *Canadian Journal of Fisheries and Aquatic Sciences*, *42*, 129–138. https://doi.org/10.1139/f85-016

Mills, K. H., & Chalanchuk, S. M. (1987). Population dynamics of lake whitefish (*Coregonus clupeaformis*) during and after the fertilization of lake-226, the experimental lakes area. *Canadian Journal of Fisheries and Aquatic Sciences*, *44*, 55–63. https://doi.org/10.1139/f87-280

Mills, K. H., & Chalanchuk, S. M. (2004). The fin-ray method of aging lake whitefish. *Annales Zoologici Fennici*, *41*, 215–223.

Mills, K. H., Chalanchuk, S. M., & Allan, D. J. (2000). Recovery of fish populations in Lake 223 from experimental acidification. *Canadian Journal of Fisheries and Aquatic Sciences*, *57*, 192–204. https://doi.org/10.1139/f99-186

Mills, K. H., Chalanchuk, S. M., Findlay, D. L., Allan, D. J., & McCulloch, B. R. (2002). Condition, recruitment and abundance of lake whitefish (*Coregonus clupeaformis*) in a fertilized acid lake. *Advances in Limnology*, *57*, 423–433.

Mills, K. H., Chalanchuk, S. M., Mohr, L. C., & Davies, I. J. (1987). Responses of fish populations in lake-223 to 8 years of experimental acidification. *Canadian Journal of Fisheries and Aquatic Sciences*, *44*, 114–125. https://doi.org/10.1139/f87-287

Milne, J. E., Marvin, C. H., Yerubandi, R., McCann, K., & Moccia, R. D. (2017). Monitoring and modelling total phosphorus contributions to a freshwater lake with cage-aquaculture. *Aquaculture Research*, *48*, 283–297. https://doi.org/10.1111/are.12881

Nelligan, C., Jeziorski, A., Rühland, K. M., Paterson, A. M., & Smol, J. P. (2016). Managing lake trout lakes in a warming world: A paleolimnological assessment of nutrients and lake production at three Ontario sites. *Lake and Reservoir Management*, *32*, 315–328. https://doi.org/10.1080/10402381.2016.1203844

Nero, R. W., & Davies, I. J. (1982). Comparison of two sampling methods for estimating the abundance and distribution of *Mysis relicta*. *Canadian Journal of Fisheries and Aquatic Sciences*, *39*, 349–355. https://doi.org/10.1139/f82-048

Nicholls, K. H. (1997). A limnological basis for a Lake Simcoe phosphorus loading objective. *Lake and Reservoir Management*, *13*, 189–198. https://doi.org/10.1080/07438149709354310

Paterson, M. J., Podemski, C. L., Findlay, W. J., Findlay, D. L., & Salki, A. G. (2010). The response of zooplankton in a whole-lake experiment on the effects of a cage aquaculture operation for rainbow trout (*Oncorhynchus mykiss*). *Canadian Journal of Fisheries and Aquatic Sciences*, *67*, 1852–1861. https://doi.org/10.1139/f10-106

Paterson, M. J., Podemski, C. L., Wesson, L. J., & Dupuis, A. P. (2011). The effects of an experimental freshwater cage aquaculture operation on *Mysis diluviana*. *Journal of Plankton Research*, *33*, 25–36. https://doi.org/10.1093/plankt/fbq096

Piccolo, J. J., Hubert, W. A., & Whaley, R. A. (1993). Standard weight equation for lake trout. *North American Journal of Fisheries Management*, *13*, 401–404. https://doi.org/10.1577/1548-8675(1993)013<0401:sweflt>2.3.co;2

Plumb, J. M., & Blanchfield, P. J. (2009). Performance of temperature and dissolved oxygen criteria to predict habitat use by lake trout (*Salvelinus namaycush*). *Canadian Journal of Fisheries and Aquatic Sciences*, *66*, 2011–2023. https://doi.org/10.1139/f09-129

Post, J. R., & Evans, D. O. (1989). Size-dependent overwinter mortality of young-of-the-year yellow perch (*Perca flavescens*): Laboratory, in situ enclosure, and field experiments. *Canadian Journal of Fisheries and Aquatic Sciences*, *46*, 1958–1968. https://doi.org/10.1139/f89-246

Quinlan, R., Paterson, A. M., Smol, J. P., Douglas, M. S. V., & Clark, B. J. (2005). Comparing different methods of calculating volume-weighted hypolimnetic oxygen (VWHO) in lakes. *Aquatic Sciences*, *67*, 97–103. https://doi.org/10.1007/s00027-004-0717-6

R Core Team (2017). *R: A language and environment for statistical computing*. Vienna, Austria: R Foundation for Statistical Computing. Retrieved from http://www.R-project.org/

Rennie, M. D. (2013). Quagga and zebra mussels: Biology, impacts and control. 2nd ed. In T. F. Nalepa & D. W. Schloesser (Eds.), *Context-dependent changes in lake whitefish populations associated with dreissenid invasion* (pp. 661–680). Boca Raton, FL: CRC Press.

Rooney, R. C., & Podemski, C. L. (2009). Effects of an experimental rainbow trout (*Oncorhynchus mykiss*) farm on invertebrate community composition. *Canadian Journal of Fisheries and Aquatic Sciences*, *66*, 1949–1964. https://doi.org/10.1139/f09-130

Saint-Jacques, N., Harvey, H. H., & Jackson, D. A. (2000). Selective foraging in the white sucker (*Catostomus commersoni*). *Canadian Journal of Zoology*, *78*, 1320–1331. https://doi.org/10.1139/z00-067

Schwarz, C. J., & Arnason, A. N. (1996). A general methodology for the analysis of capture-recapture experiments in open populations. *Biometrics*, *52*, 860–873. https://doi.org/10.2307/2533048

Schwarz, C. J., Bailey, R. E., Irvine, J. R., & Dalziel, F. C. (1993). Estimating salmon spawning escapement using capture–recapture methods. *Canadian Journal of Fisheries and Aquatic Sciences*, *50*, 1181–1197. https://doi.org/10.1139/f93-135

Scott, W. B., & Crossman, E. J. (1998). *Freshwater fishes of Canada*. Oakville, ON: Galt House Publications Ltd.

Shuter, B. J., Lester, N. P., LaRose, J. K. L., Purchase, C. F., Vascotto, K., Morgan, G., … Abrams, P. A. (2005). Optimal life histories and food web position: Linkages among somatic growth, reproductive investment, and mortality. *Canadian Journal of Fisheries and Aquatic Sciences*, *62*, 738–746. https://doi.org/10.1139/f05-070

Smokorowski, K. E., & Randall, R. G. (2017). Cautions on using the before-after-control-impact design in environmental effects monitoring programs. *FACETS*, *2*, 212–232. https://doi.org/10.1139/facets-2016-0058

Tallman, R., Mills, K. H., & Rotter, R. G. (1984). *The comparative ecology of pearl dace (*Semotilus margarita*) and fathead minnow (*Pimephales promelas*) in Lake 114, the Experimental Lakes Area, northwestern Ontario, with an appended key to the cyprinids of the Experimental Lakes Area*. Fisheries and Oceans Canada.

Trippel, E. A. (1993). Relations of fecundity, maturation, and body size of lake trout, and implications for management in northwestern Ontario lakes. *North American Journal of Fisheries Management*, *13*, 64–72. https://doi.org/10.1577/1548-8675(1993)013<0064:rofmab>2.3.co;2

Trippel, E. A., & Beamish, F. W. H. (1993). Multiple trophic level structuring in *Salvelinus coregonus* assemblages in boreal forest lakes. *Canadian Journal of Fisheries and Aquatic Sciences*, *50*, 1442–1455. https://doi.org/10.1139/f93-165

Visser, I., & Speekenbrink, M. (2010). depmixS4: An R package for hidden Markov models. *Journal of Statistical Software*, *1*(7), 2010.

Wellman, S., Kidd, K. A., Podemski, C. L., Blanchfield, P. J., & Paterson, M. J. (2017). Incorporation of wastes by native species during and after an experimental aquaculture operation. *Freshwater Science*, *36*, 387–401. https://doi.org/10.1086/692028

White, G. C., & Burnham, K. P. (1999). Program MARK: Survival estimation from populations of marked animals. *Bird Study*, *46*, S120–S139. https://doi.org/10.1080/00063659909477239

Wootton, R. J., & Smith, C. (Eds.). (2014). Reproduction and life-history evolution. In *Reproductive biology of teleost fishes* (pp. 323–356). Oxford, UK: John Wiley & Sons, Ltd.

Yan, N. D. (2005). Research needs for the management of water quality issues, particularly phosphorus and oxygen concentrations, related to salmonid cage aquaculture in Canadian freshwaters. *Environmental Reviews*, *13*, 1–19. https://doi.org/10.1139/a05-001

Young, J. D., Winter, J. G., & Molot, L. (2011). A re-evaluation of the empirical relationships connecting dissolved oxygen and phosphorus loading after dreissenid mussel invasion in Lake Simcoe. *Journal of Great Lakes Research*, *37*, 7–14. https://doi.org/10.1016/j.jglr.2010.12.008

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