Fundamental questions for evolutionary and conservation biologists in a global change context include how species will respond to environmental change and what mechanisms will be involved in the process. ﻿The persistence of populations and species in the face of environmental change is ultimately shaped by dynamic and complex feedbacks between ecology and evolution (Kinnison and Hairston 2007). Ecological factors include direct and indirect effects on reproduction, mortality, and species interactions, whereas evolutionary factors include changes in the genetic and resulting phenotypic composition of populations (Parmesan 2006). Typically, ecological and evolutionary responses to environmental change are considered separately (Dieckmann and Ferrière 2004, Kokko and López-Sepulcre 2007, Pelletier et al. 2009). However, the pace of evolution has been proven to occur rapidly in some species (Hendry and Kinnison 1999, Carroll et al. 2007) and simultaneously influence ecological dynamics (*i.e.,* eco-evolutionary dynamics; Hairston et al. 2005). An eco-evolutionary perspective advocates that we expand our focus beyond the problems of threatened populations and growing invasions to consider how contemporary environmental and micro-evolutionary changes contribute to such issues in the first place and affect restoration and conservation efforts (Kinnison and Hairston 2007, Pelletier et al. 2009).

Aquatic ecosystems present unique and difficult challenges for biodiversity conservation (Halpern et al. 2015). Fish compose a large fraction of standing biomass of aquatic ecosystems and constitute over half of all vertebrate species (Jennings et al. 2008). Fish contribute in numerous ways to the diversity and functioning of aquatic ecosystems, and to the health, well-being, and economies across all continents (Hughes 2014). Nevertheless, many marine and freshwater fishes are threatened by critical population declines and increased risk of local or global extinction (Craig 2015, Darwall and Freyhof 2015), and one response has been distributional shifts (Hickling et al. 2006, Cheung et al. 2009, Last et al. 2011). However, freshwater ecosystems are particularly vulnerable to the effects of climate change (O’Reilly et al. 2015, Hansen et al. 2017). For example, lake summer surface water temperatures are warming significantly, with a mean trend of 0.34°C per decade (Figure 1; O’Reilly et al. 2015). Species that reside in and are restricted to lake systems, such as coregonines, have few, if any, opportunities to migrate due to the isolated nature of lakes. Studies that project the effects of climate change on lake fishes have predicted declines in cold-water species (Fang et al. 2004, Mackenzie-Grieve and Post 2006, Jacobson et al. 2010, Herb et al. 2014) and increases in warm-water species (Lehtonen 1996, Chu et al. 2005, Sharma et al. 2007, Van Zuiden et al. 2016) as the climate warms (Comte et al. 2013, Hansen et al. 2017). ﻿However, predictions are not consistent across regions and species (Comte et al. 2013, Van Zuiden et al. 2016). Studies limited to local or regional scales may miss important mechanisms operating at larger scales (Conover and Present 1990, Jonassen 2000, Power et al. 2005). Large-scale studies can help provide context for local and regional dynamics and refine our understanding of and predictions for plastic or adaptive responses of populations to increasing water temperatures.

Identification of how fish have adapted to climatic gradients (*i.e.,* latitudinal and longitudinal) in environmental parameters, such as water temperature, may provide further insight to climate-driven shifts in the biological characteristics of fish populations. High-latitude populations which experience lower water temperatures and shorter growing seasons are expected to (1) have prolonged incubation periods and (2) exhibit slower egg development and smaller size-at-age than populations at lower latitudes (Colby and Brooke 1973; Edsall and Colby 1970; Karjalainen et al. 2015; Karjalainen et al. 2016; Oyadomari and Auer 2008; Urpanen et al. 2005). However, a number of species (*e.g.,* Arctic char *Salvelinus alpinus*, Atlantic cod *Gadus morhua*, Atlantic silversides *Menidia menidia*, Atlantic salmon *Salmo salar*, striped bass *Morone saxatilis*, and turbot *Scophthalmus maximus*) have demonstrated an ﻿inverse relationship between the length of the growing season and growth potential (*i.e.,* countergradient variation; Billerbeck et al. 2000; Chavarie et al. 2010; Conover and Schultz 1995; Conover and Present 1990; Jonassen 2000; Schultz et al. 1996, 1998; Yamahira et al. 2002), where higher-latitude populations have higher growth potential compared with lower-latitude populations. Countergradient variation suggests that higher-latitude populations compensate for a shorter growing season by evolving a higher overall capacity (efficiency) for growth; therefore, growing proportionally faster than those from lower latitudes across all temperatures (*e.g.,* Figure 2, middle panel). Conover and Present (1990) first suggested that life history traits in fishes can vary between latitudes and the driving selective force for this variation is size-dependent winter mortality in young-of-the-year (YOY). ﻿Such size-dependent winter mortality results in a strong and increasing selection pressure towards fast-growing fish with increasing latitude. In addition to north-south gradients, similar climatic gradients can be found at the same latitude across ecosystems. Longitudinal gradients across Europe and Asia provide a sharp contrast ranging from coastal, high-altitude mountains and deserts, to the tundra of Siberia. Such longitudinal adaptation might be categorized as local adaptation and ﻿indicate inter-population differences in growth (Figure 2, left panel). Understanding how environmental variables are associated with climatic-gradients can prove useful to determine the genetic, environmental, and ecological mechanisms involved in population declines and therefore aid efforts to better predict species responses to future conditions, and aid in conservation and restoration efforts.

Coregonines are of great socio-economic value but considered to be critically sensitive to the effects of climate change because they are cold, stenothermic fishes (Stockwell et al. 2009, Elliott and Bell 2011, Jeppesen et al. 2012, Isaak 2014, Jonsson and Jonsson 2014, Karjalainen et al. 2015, 2016b). Cisco (*Coregonus artedi*) and other related *Coregonus* species (*C. hoyi, C. kiyi, C. zenithicus, C. nigripinnis*) were once the most abundant and commercially valuable fish in the Laurentian Great Lakes (Baldwin et al. 2002, 2009, Schmidt et al. 2009). Through a combination of overfishing, invasive species, and habitat changes, Great Lakes coregonine fisheries collapsed in the 20th century (Lawrie and Rahrer 1972, Wells and McLain 1973, Christie 1974, Spangler and Peters 1995, Madenjian et al. 2008). Currently, large-scale commercial fisheries operate in all of the Great Lakes but in a lesser capacity than historical exploitation (Baldwin et al. 2002, 2009, Ebener et al. 2008). Failing coregonine populations are not restricted to the Great Lakes nor the past. Coregonine fisheries worldwide have experienced population declines due to highly variable and weak year-class strengths (Anneville et al. 2015; Myers et al. 2015; Nyberg et al. 2001; Vonlanthen et al. 2012). The reason for declining recruitment is unknown, but winter conditions appear to play a role in recruitment success (Karjalainen et al. 2015; Karjalainen et al. 2016; Marjomäki et al. 2004; Nyberg et al. 2001) and ice and water temperature regimes have changed over the past 20 years (Magnuson et al. 2000, Austin and Colman 2007, Jensen et al. 2007, O’Reilly et al. 2015). The greatest seasonal increase in water temperature of boreal lakes is projected to take place during the spring (Schindler et al. 1990, Winslow et al. 2017), and the greatest seasonal increase in air temperature will be during winter in northern Europe and North America (Christensen et al. 2007). Changes in spring conditions and increases in the frost-free season can prolong annual growing seasons with warmer summers, longer autumns, shorter ice-cover duration, and rapid spring water warming (*e.g.,* Meehl et al. 2007), and considerably affect the growth, survival, and reproduction of coregonines (Saloranta et al. 2009, Forsius et al. 2013, Karjalainen et al. 2015).

Climate change is a major challenge for the sustainable management of coregonines (Lynch et al. 2010, 2016). The identification of critical life stages and the mechanisms driving recruitment failure as thermal habitat and phenology continues to degrade and change for cold-water fishes is much needed. Year-class strength in most fish species, including coregonines, is thought to be established prior to the end of the first season of growth, with the first few weeks after hatching especially critical (Hjort 1914, Cushing 1990, Ludsin et al. 2011, 2014). The response to environmental change at the egg and larval stages is expected to play an important role in population persistence (Myers 1997, Karjalainen et al. 2000) and have major implications on ecosystem health as coregonines contribute significantly to ecosystem function and energy transfer (Gamble et al. 2011, Muir et al. 2014, Stockwell et al. 2014, Karjalainen et al. 2015). Large-scale common garden and experimental evolution studies, which integrate genetic and ecological approaches and examine adaptation and evolution of environmental resistance, will aid in understanding the response of coregonines to changing environments (Hoffmann and Sgrò 2011). We propose to experimentally identify genetic and ecological mechanisms driving latitudinal and longitudinal variation in egg and larval development, growth, and survival across a suite of congeners with similar and contrasting life history traits (*e.g.,* benthic and pelagic resource use). Our proposed work is part of an international scientific consortium (Table 1) with an interest to analyze the response of different coregonine species to changing thermal regimes across broad climatic gradients (*e.g.,* latitudinal and longitudinal). The expected response is (1) populations are locally adapted to their environment, where survival will be maximized in the native environment but reduced elsewhere, or (2) countergradient variation, where survival will be greater in higher latitude populations across all temperatures. Determining the evolutionary differences and mechanisms driving thermal resilience across populations, at critical early-life stages, will be important for future sustainable management practices of the ecologically and economically important coregonines.

*H1* – Local Adaptation Hypothesis (Figure 2, left panel): Populations are adapted to their local environment and will perform worse outside of those conditions. We predict that southern populations will perform best when incubated under warmer temperatures and northern populations will perform best when incubated under colder temperatures.

*H2* – Countergradient Variation Hypothesis (Figure 2, middle panel): Northern populations have evolved climate-driven shifts in performance to adapt to shorter growing seasons, colder summer water temperatures, and more extreme winter conditions. We predict that northern populations will perform better than southern populations across all incubation temperatures.

*H3* – Mixed-strategy Hypothesis (Figure 2, right panel): Populations are locally-adapted and exhibit countergradient variation. We predict that northern populations will have the highest performance but will be limited by local adaptation under warmer conditions.

*H4* – Null Hypothesis: Populations will perform equivalently across all populations and incubation temperatures. We predict that northern and southern populations will perform the same across all incubation temperatures.

**METHODS:**

Study Sites and Collections

We used a cross-lake, cross-continent, cross-species approach to evaluate the response and thermal tolerance of coregonine embryos and larvae to changing thermal regimes. Wild-caught populations of cisco in Lake Superior (USA/Canada) and Lake Ontario (USA/Canada) and vendace and European whitefish in Lake Konnevesi (Finland) were sampled using live-capture gear (Figure X). Adult field collections occurred during known coregonine spawning periods for Lake Ontario and Lake Superior. On Lake Konnevesi, adults were collected prior to spawning and stored in an aquaculture pool with water fed directly from the lake until spawning was initiated. A single laboratory in North America (University of Vermont (UVM), USA) and Europe (University of Jyväskylä (JYU), Finland) conducted all sampling, fertilization, and experimental work for populations on each continent.

Fertilization and Incubation

Eggs and milt were stripped from females (or dams) and males (or sires) from each population and artificially fertilized under a blocked, nested full-sib, half-sib fertilization design to create 36 or 48 full-sibling families nested within half-siblings per population (Table X). This fertilization design was used to maximize the amount of genetic variation captured within a population and allow for heritability estimates to be made. Pairing was performed by dividing the adults used in the experiment into three or four fertilization blocks. A single block was comprised of four sires each paired to three unrelated dams, where all offspring of a given dam were full siblings (Figure X). Fertilizations were performed block by block to ensure germ cell survival. This fertilization design minimized the potential loss of multiple families if a dam or sire produced poor quality gametes, compared to a full-factorial design.

Approximately 200 eggs per female were fertilized by approximately 10 μl of milt from each male in the block. After the addition of milt, water was added to the plates to activate the germ cells and the plate was mixed gently for one minute. The embryos were rinsed 2-3 times until the water was clear. Water used during fertilizations was reconstructed freshwater (OECD ISO 6341: 2012) to standardize the chemical properties of the water used among populations and between labs. Embryos were transported in coolers either by shipping overnight for Lake Superior or driven same-day for Lake Ontario. A temperature logger recorded air temperatures inside the cooler during transport. No embryo transport was required for Lake Konnevesi. Demographic data (*e.g.,* total length and weight) and fin clips were collected on adults.

Fertilization success was determined by taking 10 embryos from each family and assessing under microscopy within 72-hours post-fertilization (Oberlercher and Wanzenböck 2016). If fertilization was low (<30%), the family was removed from the experimental setup. Eggs from successful families were individually distributed into 24-well cell culture microplates and incubated in 2 ml of reconstructed freshwater. Reconstructed freshwater was used during incubation maintained sterility, prevented bacterial growth in the wells, and eliminated the need for harsh fungicide treatments on the embryos. A total of 36 embryos per family were used for Lake Konnevesi and 48 embryos per family for Lake Ontario and Lake Superior. Families were randomly distributed across three or four microplates (*i.e.,* 12 eggs per family per microplate). Microplates from each species and population were incubated at constant temperatures of 2.0, 4.5, 7.0, and 9.0°C and randomly placed in climate-controlled chambers at UVM (Memmert® IPP260Plus) and climate-controlled rooms at JYU (Brand). Airflow was used in both the climate-controlled chambers and rooms to ensure equal air circulation around the microplates. Microplate orientation and position were rotated weekly eliminate any temperature homogeneity within the chambers and rooms. Water temperatures were was recorded hourly with loggers (HOBO® Water Temperature Pro v2 at UVM and Escort iMini at JYU) and daily mean water temperatures calculated. Incubations took place in the dark, with the exception of short maintenance periods. Microplates were checked weekly for dead eggs and the eye-up stage. During the hatch period, microplates were checked on a two-day cycle for newly-hatched larvae. All newly hatched larvae were photographed for life-history traits and malformations.

Statistical Analyses

All statistical analyses were performed in R version XX (R Core Team 2020).

*Life-history and Morphological Traits*

Embryo survival was estimated as the percent of embryos surviving between the eye-up and hatch stages. Incubation period was considered by two variables: the number of days from fertilization to hatching and the sum of the degree-days. Total length-at-hatch (LAH; mm) and yolk-sac volume (YSV; mm3) were measured from five individuals per family at, or as close as possible to, 50% hatching for each family. YSV was calculated assuming the shape of an ellipse (Blaxter 1963):

where a = length of the yolk sac (mm) and b = height of the yolk sac (mm).

Embryo survival was analyzed as a binomial response variable in a generalized linear mixed-effects model fitted with maximum likelihood, and incubation period, length-at-hatch, and yolk sac volume at hatching as continuous response variables in linear mixed-effects models with the lme4 package (Bates et al. 2015). The reference models included population and incubation temperature treatment as fixed effects and sire and dam as random effects. The significance of the model terms was obtained by comparing a model including or lacking the term of interest to the reference model with Akaike information criterion (AIC). Because embryos were raised independently, the replication unit in the statistical models is each individual embryo.

Post-hoc least-squares means tests, adjusted for multiple comparisons using the Bonferroni method, were performed with the emmeans package (Lenth 2020).

*Heritability*

To estimate heritability and sire/dam variance components for life history (embryo survival and incubation period) and morphological (length-at-hatch and yolk-sac volume) traits, we fitted a random-effects model using the lme4 package to the data with sire, dam, sire:dam interaction, fertilization block, and microplate as random factors. Estimates were calculated for each population and incubation temperature treatment independently. Under our fertilization design, the variance among half-sib families represents one-fourth of the additive genetic variance (VA) and can therefore be used to estimate VA and the narrow-sense heritability (h2), assuming that epistasis is negligible (Lynch and Walsh 1998). Additive genetic variance was calculated as four times the sire component of variation and dominance genetic variance (VD) as four times the sire:dam component. Heritability was calculated by dividing VA by the total phenotypic variance (including sire, dam, sire:dam, block, microplate, and residual variances).

Early embryo mortality induced from fertilization failure produced inequalities in the number of offspring among families and an unbalanced design. The variance components and heritability estimates were assessed using non-parametric bootstrapping due to the unbalanced family sizes. ﻿We generated a bootstrap sample by randomly assigning, with replacement, a trait value, sire, dam, block, and microplate to a bootstrapped fish, for each of the fish in a population and incubation temperature treatment (i.e., the number of observations in the original sample). ﻿The random-effect model was fit to the bootstrapped sample, VA and VP components extracted, and heritability calculated from the bootstrapped variance components. The resampling procedure was repeated 5,000 times to create a distribution of bootstrapped variance components and heritability estimates. We calculated the mean variance components and heritability estimate with 95% confidence intervals for each trait from the bootstrapped distributions.

Assuming that epistasis is negligible, additive genetic variance (VA) was calculated as four times the sire component of variation and dominance genetic variance (VD) as four times the sire × dam component.