Quantifying a potential mechanism between ice cover and cisco recruitment success: what role does light play in cisco embryonic development?

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**ABSTRACT:**

**INTRODUCTION:**

Freshwater whitefishes, Salmonidae Coregoninae (hereafter coregonines) have played important economic and ecological roles throughout the northern hemisphere (REFS). Over the past 35 years, coregonine populations worldwide have experienced declines due to highly variable and weak year-class strengths (Nyberg et al. 2001, Myers et al. 2015). Historical coregonine declines have been attributed to overfishing, invasive species, habitat alterations, and competition (Stockwell et al. 2009, Rosinski et al. 2020, Lucke et al. 2020). However, actual reasons for contemporary declines remain unknown, but climate-induced changes in early-life stage environments have been hypothesized, and winter ice and water temperature regimes have changed over the past 20 years or more (Austin and Colman 2007, O’Reilly et al. 2015). In the Laurentian Great Lakes, native coregonine conservation and restoration efforts are at the forefront of fisheries management efforts (REFS). Future climate change predictions show that the timing and physical characteristics of winter and ice regimes are likely to drive some of the most important biological changes (REFS).

Year-class strength in most fish species, including coregonines, is thought to be established prior to the end of the first season of growth (Hjort 1914, Cushing 1990). Unlike larvae, embryos are static, leaving this early-life stage vulnerable to predation (Stockwell et al. 2014) and unable to evade inter-annual variation in winter conditions (REFS). Most coregonines are autumn spawners whose embryos incubate under ice throughout the winter and hatch in spring (REFS). Changes in winter severity and ice cover could alter developmental rates, embryo survival, and time of hatching (REFS). Potential mechanisms by which ice cover might influence cisco development include the reduction of physical wave action (REFS), lower and more stable winter and spring water temperatures (REFS), and less sunlight reaching the lake bottom (REFS). Recent changes in ice cover coupled with poor coregoninerecruitment has led to speculation about the relationship between ice cover and embryo survival for decades with limited rectification. In Lake Superior, all known cisco spawning locations are estimated to be covered when lakewide ice cover reaches 15% (Figure 1, Goodyear 1982), and nearly all lakes with known populations of coregonines are seasonally ice-covered (REFS). Therefore, understanding how variable and declining ice regimes may impact coregonine early-life history is an important aspect of coregonine conservation and restoration efforts.

Lake ice cover can also play a critical role in physical and ecological processes (Sharma et al. 2020). The formation and breakup of ice are important to signal seasonal events (REFS), such as the onset of spring plankton (REFS). As ice thins in the spring, increasing levels of sunlight under the ice can drive plankton blooms suspended in the photic zone (REFS, Kelley 1997, Yang et al. 2020). Earlier ice-off has been shown to lead to earlier spring plankton bloom (Adrian et al. 2006, Sommer et al. 2012, Yang et al. 2016), and emphasizes the role winter and ice regimes can have on seasonal succession.

Increases in spring water temperature and ice breakup cues the onset of hatching in autumn-spawning coregonines (Häkkinen et al. 2002, Urpanen et al. 2005, Karjalainen et al. 2015). Synchronization of coregonine hatching and spring plankton blooms is critical to match optimal nursery feeding conditions (Hjort, Myers et al. 2015). Larval coregonines can withstand extended periods after hatching without feeding, but the time until exogenous feeding is relative to the amount of available maternal yolk for endogenous feeding (Lucke et al. 2020, Fuiman 2002). However, increased metabolic rates during embryogenesis can compromise the amount of yolk retained (REFS).

As ectotherms, fish metabolism and nearly all other biological rates increases exponentially with temperature (Brown et al. 2004, Gillooly et al 2002). Ontogenetic development and coregonines are no exception to this rule. However, light intensity has been shown to be an additive effect to temperature in regulating embryo development and growth rates (Kwain 1975). Ice thickness and snow impact light penetration into the water column (Hampton et al. 2015), and can reduce light transmittance from 83% in open water to 62% under ice coverage, and to ≤ 10% under snow and ice coverage (Bolsenga and Vanderploeg 1992). Consequently, increased light during winter, as a result of reduced ice and snow cover, could lead to an additional stressor during coregonine embryo development. Understanding the role ice has, given the recent high variability observed in coregoninerecruitment, can help predict what the future of these species may look like under current climate trends to inform restoration efforts.

We experimentally measured how cisco embryos responded to different ice regimes. Our objective was to identify to what extent light, as a proxy for ice, influences cisco embryo survival, incubation duration, yolk-sac volume and length at hatching. We hypothesized that exposure to elevated light intensity (low ice cover) would accelerate embryogenesis, resulting in smaller yolk-sacs and lower larval survival. Populations adapted to lower light levels are expected to experience more negative impacts from increasing light intensity.

**METHODS:**

Study Species and Locations

Cisco were collected from Lake Superior, near Bayfield, Wisconsin, and Lake Ontario, Chaumont Bay, New York, in December 2019. Lakes Superior and Ontario were chosen as they have differential spawning habitat for cisco. Lake Superior cisco spawn at deep depths, likely below the photic zone, with no known preference in habitat. Lake Ontario cisco spawn in shallow, protected bays on rocky shoals. Ice conditions over spawning grounds between the two lakes vary based on depth, with shallower and more protected spawn grounds likely to have more consistent ice coverage than deeper, open waters. Lakes Superior and Ontario provide a contrast in ice cover and subsequent light exposure to coregonine embryos.

Crossing Design and Fertilization

Eggs and milt were stripped from 12 females and 16 males and artificially fertilized under a blocked, nested full-sib, half-sib fertilization design to create 48 families from each lake. The crossing design maximized the amount of genetic variation and minimized the potential loss of multiple families if a female or male produced poor quality gametes, compared to a full-factorial design. Adults used in the experiment were divided into four fertilization blocks. A single block consisted of four males each paired with three unrelated females, where all offspring of a given dam were full siblings. Fertilizations were performed block by block to ensure germ cell survival.

Approximately 200 eggs per female were fertilized with an equal amount of milt (5-15 μl) from each male in the block. After the addition of milt, water was added to activate the germ cells and gently mixed for one minute. The embryos were rinsed with water 2-3 times until the water was clear. Reconstructed fresh water was used during fertilizations (OECD ISO 6341:2012) to standardize the chemical properties of the water used between populations. Embryos were transported in coolers either by shipping overnight for Lake Superior or driven same-day for Lake Ontario. A temperature logger recorded air temperature inside the cooler during transport (Lake Superior: mean (SD) = 2.80°C (0.21); Lake Ontario: mean (SD) = 3.28°C (0.37)). Demographic data (e.g., total length, mass, and egg diameter) were collected on adults. Fertilization success was determined by haphazardly 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.

Rearing Conditions

Embryos from successfully fertilized families were individually distributed into 24-well cell culture microplates and incubated in 2 ml of reconstructed fresh water. Reconstructed fresh water was used during incubation to maintain sterility, prevent bacterial growth in the wells, and eliminate the need for fungicide treatments on the embryos. A total of 36 embryos per family were used for each of Lake Ontario and Lake Superior cisco. Families were randomly distributed across three microplates (*i.e.,* 12 eggs per family per microplate and two families per 24-well microplate).

Microplates from each population were incubated under three experimental light treatments to represent the light intensity under 90-100, 40-60, and 0-10% ice cover (Table X), and followed mean weekly photoperiods with gradual sunrise and sunset transitions. Light treatments were chosen to mimic *in situ* winter, lakebed light measurements recorded from Lake Superior at 10 m of water in 2016-17. Remote-sensing ice data (U.S. National Ice Center) was used to quantify the daily percentage of ice cover above the light sensor (Figure X). Embryos were incubated at a constant target water temperature of 4.0°C in a climate-controlled chamber (Conviron® E8; Table X). Forced airflow was used in the climate-controlled chamber to ensure equal air circulation around the microplates and opaque, plastic sheeting was used to separate light treatments.

All microplates were covered to minimize evaporation. Microplate orientation and position were rotated weekly to eliminate any temperature heterogeneity within the chamber. Water temperature and light intensity were recorded hourly with loggers (HOBO® Water Temperature Pro v2 and JFE Advantech Co., Ltd. DEFI2-L) and daily mean values calculated. Microplates were checked weekly for dead eggs and the eye-up stage. During the hatch period, microplates were checked on a three-day cycle for newly hatched larvae. All newly hatched larvae were photographed for life-history and morphological traits and preserved in 95% ethanol.

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 assessed by two variables: the number of days from fertilization to hatching (days post-fertilization; DPF) and the sum of the degree-days (accumulated degree-days; ADD). 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. Yolk-sac volume 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).

Statistical Analyses and Estimation of Variance Components

Embryo survival was analyzed as a binomial response variable, and incubation period, length-at-hatch, and yolk-sac volume at hatching as continuous response variables. Early embryo mortality induced from fertilization failure produced inequalities in the number of offspring among families and an unbalanced design. The sample size for incubation period is a function of embryo survival and subsequently resulted in an unbalanced design. Therefore, binary data (i.e., embryo survival) were analyzed with binomial generalized linear mixed-effects models (LMM) and normally distributed data (i.e., incubation period, length-at-hatch, and yolk-sac volume) were analyzed with restricted maximum likelihood LMMs with the lme4 package (Bates et al. 2015). Population and incubation light treatment were included as fixed effects and female, male, family (female and male combination), and fertilization block as random effects. Because embryos were raised independently, the replication unit in the statistical models is the individual embryo. All traits were examined for population and incubation light effects in addition to individual parental effects (female, male, and family effects), fertilization block, and all possible interactions with backward, stepwise effect-selection using the buildmer package (Voeten 2020). The maximal model for each trait was selected by comparing a model including or lacking the term of interest to the reference model based on changes in log-likelihood, Akaike information criterion, Bayesian information criterion, and change in explained deviance. The mixed-effects model output does not produce significance values for model effects; therefore, significance for population, species, incubation temperature, interaction effects, and any random-effects selected were determined using a likelihood ratio test between the maximal model and reduced models with the model effect of interest removed.

**RESULTS:**

**DISCUSSION:**

**ACKNOWLEDGMENTS:**

**LITERATURE CITED:**

**TABLES:**

Table X. Mean (± SD) water temperatures during embryo incubations from each light treatment for Lake Superior and Lake Ontario.

|  |  |  |  |
| --- | --- | --- | --- |
|  | Light Treatment | | |
| Lake | High | Medium | Low |
| Superior | 4.25 ± 0.24 | 4.28 ± 0.28 | 4.34 ± 0.34 |
| Ontario | 4.24 ± 0.25 | 4.28 ± 0.28 | 4.36 ± 0.36 |

Table X. Mean (± SD) photon flux (μmol m-2 s-1) for three ice regimes from Lake Superior and corresponding laboratory experimental conditions.

|  |  |  |  |
| --- | --- | --- | --- |
|  | Ice Cover (Light Treatment) | | |
| Location | > 90% (Low) | 40-60% (Medium) | < 10% (High) |
| Lake Superior | 1.96 ± 1.07 | 3.35 ± 2.54 | 5.45 ± 5.88 |
| Laboratory | 0.62 ± 0.06 | 3.85 ± 1.88 | 6.15 ± 0.99 |

Table X. Likelihood ratio test output for each model selected for embryo survival (%), incubation period (number of days post-fertilization; DPF), incubation period (accumulated degree-days; ADD), length-at-hatch (mm), and yolk-sac volume (mm3) from Lakes Superior and Ontario cisco (*Coregonus artedi*). pop indicates population. The full model that was selected is bolded for each trait.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Trait | Model | Effect Tested | df | χ2 | p-value |
| Embryo Survival | **light+ pop** |  |  |  |  |
| pop | light | 2 | 181.92 | < 0.001 |
|  | light | pop | 1 | 95.00 | < 0.001 |
| Incubation Period (DPF) | **light + pop + light:pop + dam:sire + dam + sire** |  |  |  |  |
| pop + dam:sire + dam + sire | light | 2 | 11.12 | 0.004 |
| light + dam:sire + dam + sire | pop | 1 | 2974.54 | < 0.001 |
|  | light + pop + dam:sire + dam + sire | light:pop | 2 | 10.75 | 0.005 |
|  | light + pop + light:pop + dam + sire | dam:sire | 1 | 84.36 | < 0.001 |
|  | light + pop + light:pop + dam:sire + sire | dam | 1 | 25.07 | < 0.001 |
|  | light + pop + light:pop + dam:sire + dam | sire | 1 | 10.78 | 0.001 |
| Incubation Period (ADD) | **light + pop + light:pop + dam:sire + dam + sire** |  |  |  |  |
| pop + dam:sire + dam + sire | light | 2 | 56.01 | < 0.001 |
| light + dam:sire + dam + sire | pop | 1 | 3,041.75 | < 0.001 |
|  | light + pop + dam:sire + dam + sire | light:pop | 2 | 17.39 | < 0.001 |
|  | light + pop + light:pop + dam + sire | dam:sire | 1 | 84.44 | < 0.001 |
|  | light + pop + light:pop + dam:sire + sire | dam | 1 | 10.76 | 0.001 |
|  | light + pop + light:pop + dam:sire + dam | sire | 1 | 25.03 | < 0.001 |
| Length-at-Hatch | **pop + dam + sire** |  |  |  |  |
| dam + sire | pop | 1 | 334.33 | < 0.001 |
|  | pop + sire | dam | 1 | 74.32 | < 0.001 |
|  | pop + dam | sire | 1 | 6.80 | 0.009 |
| Yolk-sac Volume | **light + pop + light:pop + dam + sire** |  |  |  |  |
| pop + dam + sire | light | 2 | 7.18 | 0.028 |
| light + dam + sire | pop | 1 | 712.29 | < 0.001 |
|  | light + pop + dam + sire | light:pop | 2 | 26.84 | < 0.001 |
|  | light + pop + light:pop + sire | dam | 1 | 130.50 | < 0.001 |
|  | light + pop + light:pop + dam | sire | 1 | 2.87 | 0.090 |

**FIGURES:**

Chart, histogram

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Figure X.

**Chart

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