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 (Nyberg et al. 2001, Ebener et al. 2008b, 2008a, Vonlanthen et al. 2009, 2012, Lynch et al. 2015, 2016). 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). In the Laurentian Great Lakes, native coregonine conservation and restoration efforts are at the forefront of fisheries management efforts (Favé and Turgeon 2008, Zimmerman and Krueger 2009, Bronte et al. 2017). 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, reasons for failing to recover remain unknown. Climate-induced changes in early-life stage environments have been hypothesized as reasons for declining recruitment. Most coregonines are autumn spawners whose embryos incubate under ice throughout the winter (Karjalainen et al. 2000, Stockwell et al. 2009), 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, Sharma et al. 2019). 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 (Sharma et al. 2007, Sharma et al. 2019, Woolway et al. 2020).

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 (Pepin 1991). Changes in winter severity and ice cover could alter developmental rates, embryo survival, and time of hatching (Karjalainen et al. 2015). Potential mechanisms by which ice cover might influence cisco development include the reduction of physical wave action (Walter et al. 2006, Austin and Colman 2007, Wang et al. 2010, Nguyen et al. 2017), more stable winter and spring water temperatures (Magnuson et al. 1997, Winslow et al. 2017), and less sunlight reaching the lake bottom (Bolsenga and Vanderploeg 1992, Hampton et al. 2015). Recent changes in ice cover coupled with poor coregoninerecruitment has led to hypotheses about the relationship between ice cover and embryo survival for decades without testing. Therefore, testing whether 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). 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). Light can serve not only as a source of energy for aquatic ecosystems, but also can regulate fish phenology, behavior, and physiology (Ruchin 2007, Villamizar et al. 2011). The length of photoperiods characterize circadian rhythms and ensure that biological processes are synchronized with the environment (Marchesan et al. 2005, Gaston et al. 2013, Ruchin 2020). Additionally, the intensity of light exposure can alter the development, survival, and growth of fish (Villamizar et al. 2011, Ruchin 2020).

The impact of light exposure during fish embryogenesis varies across species (Ruchin 2020). Embryos from some salmonid species, including European whitefish *Coregonus lavaretus*, incubated under elevated light levels have higher mortality and deformity rates, slower formation of cartilaginous skeletal elements, decreased time to hatching, and smaller size-at-age; with development after organogenesis accelerated (Eisler 1958, 1961, McCrimmon & Kwain 1969, Kwain 1975, Chernyaev 2007, Lyutikov 2012). However, other teleost species have had opposing responses, or no response, to high illumination (Mangor-Jensen & Waiwood 1995, Iglesias et al. 1995, Seth et al., 2014). Consequently, increased light intensity during winter incubations, as a result of reduced ice and snow cover, may act as an additional stressor during coregonine embryo development for populations with seasonal ice cover. Understanding the impact ice and light have on coregonine embryogenesis, 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 photoperiod intensities, as a proxy for different ice regimes. Our objective was to identify to what extent light influences cisco embryo survival, incubation duration, length and yolk-sac volume at hatching. We hypothesized that exposure to elevated light intensity (low ice cover) would accelerate embryogenesis, resulting in smaller yolk-sacs and lower embryo survival. We also hypothesized that populations adapted to lower light levels (high ice cover) are expected to experience more negative impacts from increasing light intensity.

# METHODS:

## Study Species and Locations

Cisco were collected from the Apostle Islands, Lake Superior (46.85, -90.55) and Chaumont Bay, Lake Ontario (44.05, -76.20) in December 2019. Lakes Superior and Ontario were chosen because the cisco populations sampled have different spawning habitats. Lake Superior cisco were aggregated and sampled at deeper depths, with little or no known expected preference in habitat (Dryer and Beil 1964, Paufve 2019). Lake Ontario cisco were aggregated in a shallow, protected bay and sampled on rocky shoal (Pritchard 1931, Paufve 2019). Ice conditions over the spawning locations sampled from each lake vary based on bathymetric depth, with shallower, more protected Lake Ontario having more consistent ice coverage than the deeper, open location in Lake Superior (Figure 1). However, light transmittance in deeper water is less than shallow water (Secchi 1864, Preisendorfer 1986, Ramus et al. 1976, Fleming-Lehtinen and Laamanen 2012). Consequently, the cisco populations we sampled from Lakes Superior and Ontario provide a contrast in ice cover and subsequent light exposure to coregonine embryos.

## Crossing Design and Fertilization

Gametes 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 (Stewart et al. 2021).

Approximately 500 eggs per female were fertilized with an equal amount of milt (5-15 μl) from each male in the block and water used to activate the germ cells. The embryos were rinsed with water until the water ran clear. Reconstructed fresh water was used during fertilizations (OECD ISO 6341:2012) to standardize the chemical properties of the water used between lakes. Embryos were transported in coolers by shipping overnight for Lake Superior samples and driven same-day for Lake Ontario samples. 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 assessing 10 embryos under microscopy within 72-hours post-fertilization (Oberlercher and Wanzenböck 2016). If fertilization was low (<30%), the family was removed from the experiment (Stewart et al. 2021).

## 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 (Stewart et al. 2021). 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 1), 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 2). Embryos were incubated at a constant target water temperature of 4.0°C in a climate-controlled chamber (Conviron® E8; Table 2). 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 and rotated (*i.e.,* orientation and position) weekly. 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 embryos. All hatched embryos were photographed and immediately preserved in 95% ethanol.

## Life-History and Morphological Traits

Embryo survival was estimated as the percent of embryos surviving between the eye-up and post-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

To characterize the life-history and morphological traits, 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. Because embryos were raised independently, the replication unit in the statistical models is the individual embryo and the design was unbalanced from different levels of embryo mortality. 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. All traits and possible interactions were examined 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 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.

To allow for population comparisons, the response to temperature for each trait was standardized to what we assumed was the optimal light treatment - the low light treatment (Table 1). For each trait, the within-family mean was calculated for each light treatment and the percent change from the optimal light intensity estimated. Standard error was calculated as the among-family variation in percent change.

All analyses were performed in R version 4.0.3 (R Core Team 2020).

# RESULTS:

## Spawning Adults

Lake Superior spawning adults used for gamete collection were larger in total length and fresh mass than Lake Ontario adults. However, Lake Ontario females had larger egg diameters than Lake Superior females (Table 3).

## Life-History and Morphological Traits and Variance Components

All traits, except embryo survival and LAH, had significant interaction effects between population and light treatments (maximum *P* = 0.005; Table 4). The interaction effects precluded any interpretation of main effects, but did suggest contrasting norms of reaction between populations. Below we describe the interaction effects and the light pairwise comparisons for embryo survival. All random effects (*i.e.,* female, male, and family) were significant (maximum *P* = 0.009) except female for embryo survival, male for embryo survival and YSV, and family for embryo survival, LAH and YSV (Table 4). All statistical model results can be found in Table 4.

### *Embryo Survival*

Embryo survival was highest for both populations at the medium light treatment, but lowest at the low light treatment for Lake Ontario and at the high light treatment for Lake Superior (Figure 3). Light and population main effects were significant, and all pairwise light treatment comparisons were significant (maximum *P* < 0.005). Embryo survival was higher for Lake Ontario at the high (98.4%) and medium (99.6%) light treatments than Lake Superior (85.3 and 89.3%, respectively) but not different between populations (0.9%) at the low light treatment (Figure 3).

### *Incubation Period (days post-fertilization)*

The number of days post-fertilization to hatching was highest for Lake Ontario at the low light treatment (115.47 days) and for Lake Superior at the high light treatment (101.22 days; Figure 3). Incubation period (DPF) was higher for Lake Ontario than Lake Superior across all light treatments (mean (SD) = 13.9 (0.8) days). Lake Ontario cisco had a decrease in DPF from the low light to the high light treatments (-0.7%), while Lake Superior had an increase from the low light to the high light treatments (1.9%; Figure 3).

### *Incubation Period (accumulated degree-days)*

The effect of population depended on light because the difference in ADD between populations was less pronounced at the high light treatment (60.8 ADD), while ADD was higher for Lake Ontario (497.7 and 485.9 ADD) than Lake Superior (427.5 and 420.8 ADD) at the low and medium light treatments, respectively (Figure 3). Lake Ontario ADD had a negative response from the low to high light treatments (-2.5%), while ADD for Lake Superior did not change from the low to high light treatments (0.05%; Figure 3).

### *Length-at-Hatch*

Light was not a component returned in the stepwise-selected model, but the population main effect between Lake Ontario and Lake Superior was significant (*P* < 0.001; Table 4). Lake Ontario had a higher LAH than Lake Superior across all light treatments (Figure 4). Lake Superior and Lake Ontario responded to increasing light intensity with a 3.2 and 0.2% respective decrease in LAH from the low to high light treatments (Figure 4).

### *Yolk-sac Volume*

Yolk-sac volume had a different response to light intensity between populations (Figure 4). The effect of population depended on light because the difference in YSV between populations was less pronounced at the low light treatment (0.22 mm3), while YSV was lower for Lake Ontario (0.35 and 0.37 mm3) than Lake Superior (0.67 and 0.63 mm3) at the high and medium light treatments, respectively (Figure 4). Lake Superior YSV had a positive response from the low to high light treatments (15.3%), while YSV for Lake Ontario had a negative response from the low to high light treatments (-5.5%; Figure 4).

# DISCUSSION:

# ACKNOWLEDGMENTS:

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# LITERATURE CITED:

# TABLES:

Table 1. Mean ± SD photon flux (μmol m-2 s-1) for three ice regimes from Lake Superior and corresponding laboratory experimental light 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 2. Mean ± SD water temperatures (°C) during embryo incubations from each light treatment for Lakes Superior and 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 3. Mean ± SD total length (TL) and fresh mass (FM) of the female and males and egg diameters (ED) of the females from Lakes Superior and Ontario cisco (*Coregonus artedi*) used in the experiment.

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
|  | Lake Superior | | |  | Lake Ontario | | |
| Sex | TL (mm) | FM (g) | ED (mm) |  | TL (mm) | FM (g) | ED (mm) |
| Female | 428.92  ± 44.40 | 676.02  ± 181.51 | 2.14  ± 0.12 |  | 380.33  ± 24.18 | 567.59  ± 122.89 | 2.30  ± 0.08 |
| Male | 400.25  ± 34.35 | 523.82  ± 134.65 | -- |  | 366.56  ± 25.30 | 443.29  ± 103.16 | -- |

Table 4. 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 + female:male + female + male** |  |  |  |  |
| pop + female:male + female + male | light | 2 | 11.12 | 0.004 |
| light + female:male + female + male | pop | 1 | 2974.54 | < 0.001 |
|  | light + pop + female:male + female + male | light:pop | 2 | 10.75 | 0.005 |
|  | light + pop + light:pop + female + male | female:male | 1 | 84.36 | < 0.001 |
|  | light + pop + light:pop + female:male + male | female | 1 | 25.07 | < 0.001 |
|  | light + pop + light:pop + female:male + female | male | 1 | 10.78 | 0.001 |
| Incubation Period (ADD) | **light + pop + light:pop + female:male + female + male** |  |  |  |  |
| pop + female:male + female + male | light | 2 | 56.01 | < 0.001 |
| light + female:male + female + male | pop | 1 | 3,041.75 | < 0.001 |
|  | light + pop + female:male + female + male | light:pop | 2 | 17.39 | < 0.001 |
|  | light + pop + light:pop + female + male | female:male | 1 | 84.44 | < 0.001 |
|  | light + pop + light:pop + female:male + male | female | 1 | 10.76 | 0.001 |
|  | light + pop + light:pop + female:male + female | male | 1 | 25.03 | < 0.001 |
| Length-at-Hatch | **pop + female + male** |  |  |  |  |
| female + male | pop | 1 | 334.33 | < 0.001 |
|  | pop + male | female | 1 | 74.32 | < 0.001 |
|  | pop + female | male | 1 | 6.80 | 0.009 |
| Yolk-sac Volume | **light + pop + light:pop + female + male** |  |  |  |  |
| pop + female + male | light | 2 | 7.18 | 0.028 |
| light + female + male | pop | 1 | 712.29 | < 0.001 |
|  | light + pop + female + male | light:pop | 2 | 26.84 | < 0.001 |
|  | light + pop + light:pop + male | female | 1 | 130.50 | < 0.001 |
|  | light + pop + light:pop + female | male | 1 | 2.87 | 0.090 |

# FIGURES:

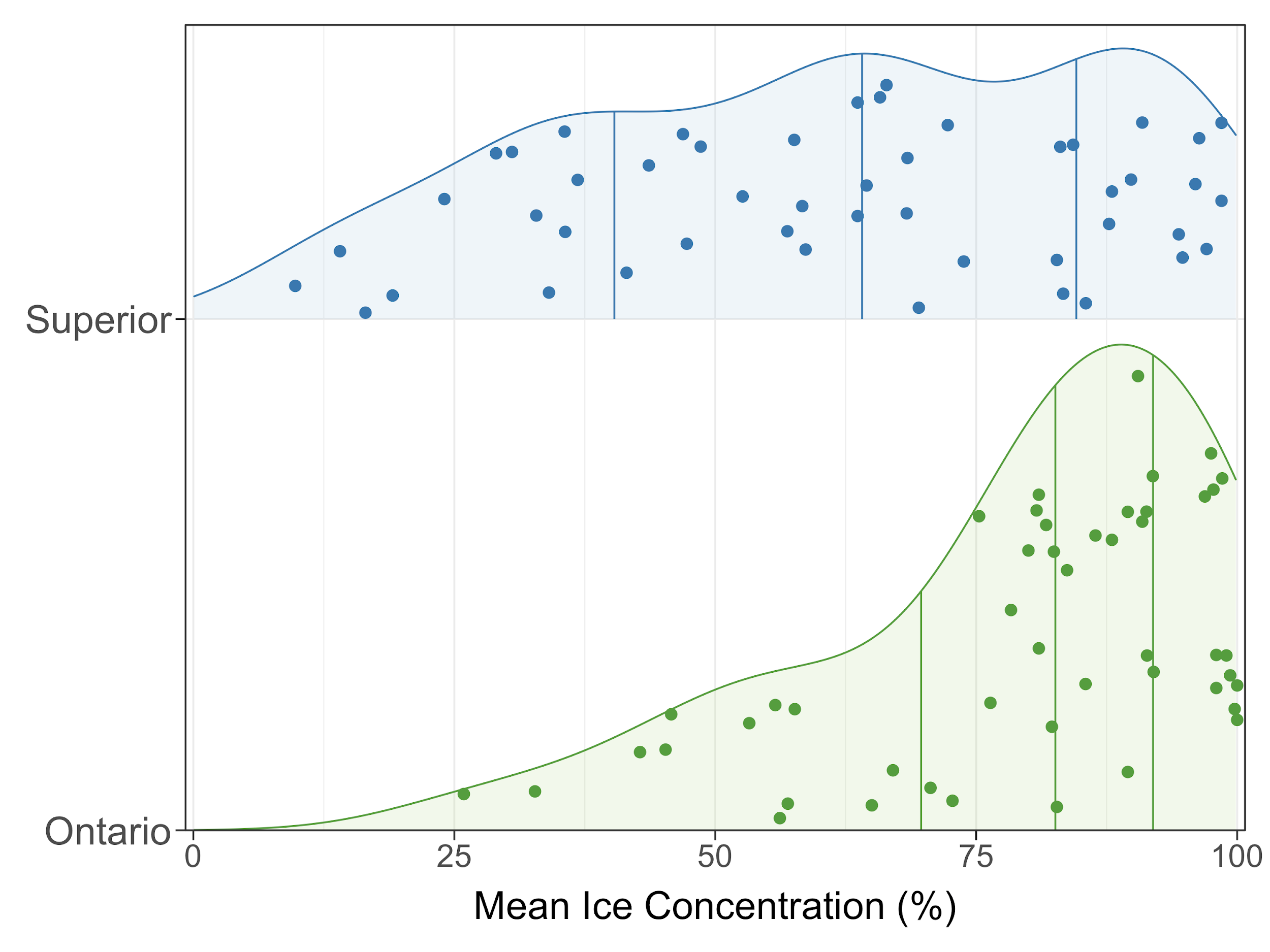
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Figure 1. Kernel density estimate of annual mean ice concentration between 1-Jan and 15-Mar from 1973-2020 for each sampling location in Lake Superior (top; blue) and Lake Ontario (bottom; green). The height of the density curve is proportional between lakes and the vertical lines represent quartiles. Points represent each year (n = 48) and were jittered vertically to show distribution. Ice coverage data was collected from the U.S. National Ice Center.

Chart, histogram

Description automatically generated

Figure 2. Daily ice coverage (%; blue line) and light intensity (μmol m-2 s-1) relationship based on light sensors set at 10 m depth off Sand Island, Lake Superior. Ice coverage data above the sensor was collected from the U.S. National Ice Center.

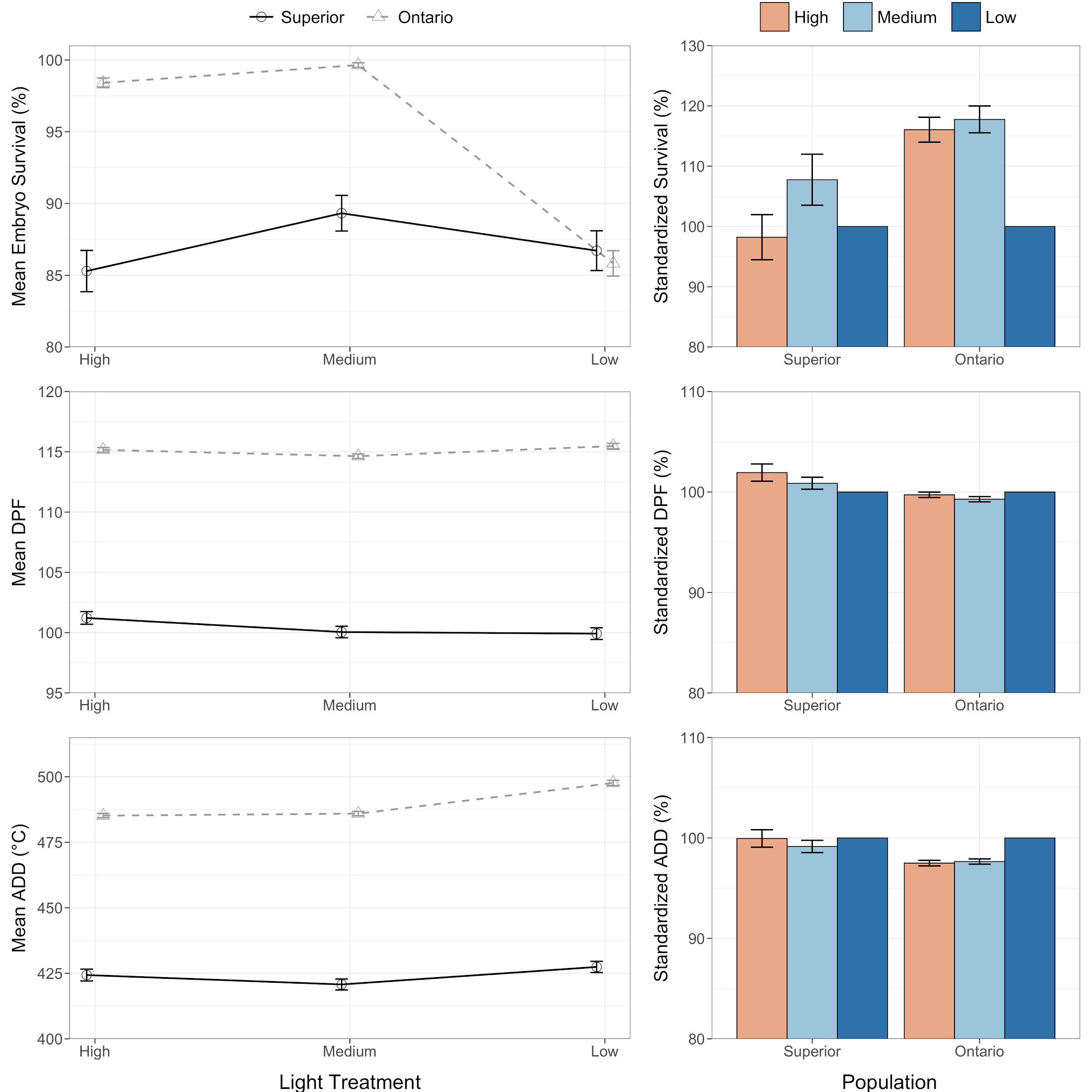
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Figure 3. Mean embryo survival (%), incubation period (number of days post-fertilization; DPF), and incubation period (accumulated degree days (°C); ADD) at each incubation light treatment (left) and standardized responses to light within each population (right) from Lake Superior and Lake Ontario cisco (*Coregonus artedi*). Error bars indicate standard error.

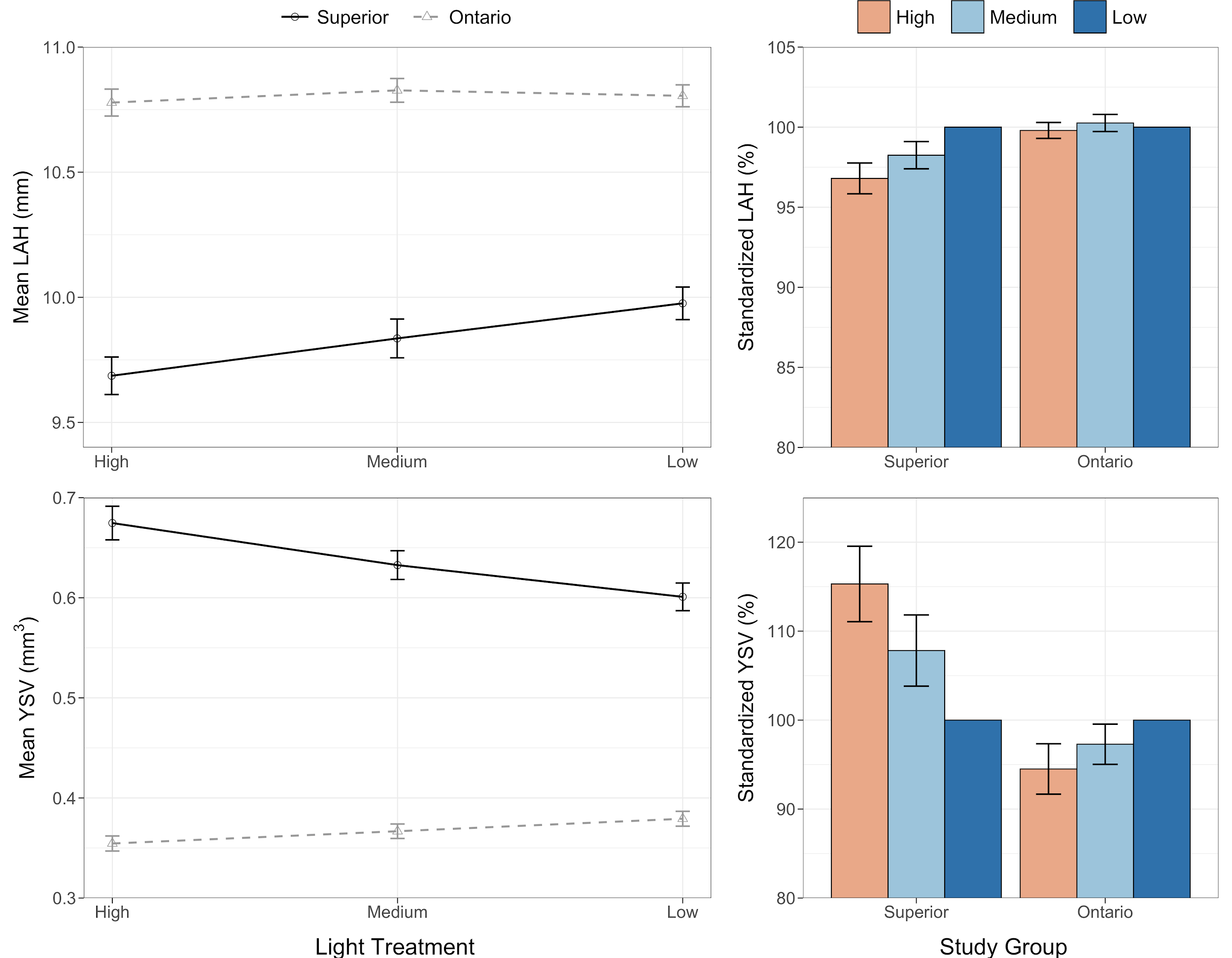
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Figure 4. Mean length-at-hatch (mm; LAH) and yolk-sac volume (mm3; YSV) at each incubation light treatment (left) and standardized responses to light within each population (right) from Lake Superior and Lake Ontario cisco (*Coregonus artedi*). Error bars indicate standard error.