Combined Effects of Maternal Traits and Spring Warming Patterns on Spawning Success of Walleye

by

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Abstract

Factors influencing survival through the earliest life stages are believed to be strong drivers of recruitment variability in many fishes. Previous research on walleye (*Sander vitreus*) has suggested that early life survival and subsequent year-class strength may be related to the age composition of spawners through maternal influences on egg quality, as well as environmental conditions during spawning and early rearing periods. My objective was to examine survival and developmental rates for embryo batches of individual female walleye of Lake Nipissing, Ontario, in relation to the combined influences of maternal effects and incubation temperature.

Female walleye were spawned on multiple dates in each of three consecutive years (2017 – 2019) and their embryos incubated under three spring warming regimes (slow, seasonal, and rapid warming; approximately 4 °C range in mean daily temperature) in a controlled laboratory setting. Embryo survival to hatch and thermal units to hatch (TU_{50}; cumulative growing degree-days) were determined for each egg batch. Maternal traits measured included female age and length, ovum (egg) size and total lipid content, and relative abundances of essential fatty acids in ova lipids. For each year, embryo survival and TU_{50} were modelled as functions of spawning date, incubation temperature treatment and maternal and ova traits using standard frequentist statistics and a model selection approach based on AICc ranking.

Embryo survival varied among spawning dates in 2017, the year with the earliest spawning period, but not in 2018 or 2019. Embryo survival did not vary with respect to incubation warming rate in any year. Relationships between embryo survival and maternal and ova traits varied among spawning years but did not appear to be related to incubation temperature treatments within years. Contrary to my predictions, embryo

survival was more consistently related to ova characteristics than to female age or size, and relationships between embryo survival and indices of ova quality were not always positive. Mean TU_{50} was higher in 2017, the year with the earliest spawning period, than in 2018 and 2019. Spawning date and incubation warming rate had interactive effects on TU_{50}; there was generally a positive relationship between TU_{50} and spawning date in all years in the seasonal and slow-warming incubation treatments but not in the rapid-warming treatment. Similarly, relationships between TU_{50} and maternal and ova traits were strongest in the slow-warming treatment and weakest in the rapid-warming treatment. TU_{50} tended to decline with increasing female age and size, and egg size. Time to 50% hatch ranged from 13 to 33 days across all years, spawn dates, treatments and females in my experiments. Based on my laboratory results and temperature measurements at natural spawning sites I estimated that walleye would hatch 3 to 5 days earlier at river inflow sites than at shoreline spawning sites in Lake Nipissing.

My results provide new and unique information on reproduction and the recruitment process in walleye and have implications for size-selective harvest guidelines used in fisheries management. It appears that the older and larger females of spawning stocks may not always produce the eggs with highest survival. Also, it appears that temperature could influence early life survival more indirectly, through its effects on hatch timing, this is an aspect that merits further investigation.

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Introduction

Global fish populations support important subsistence, commercial and recreational fisheries. Variability in abundance of these valuable renewable resources makes sustainable fisheries management an ongoing challenge. Fish populations can vary in abundance due to natural ecological processes and environmental forces coupled with anthropogenic stressors such as exploitation, climate change, habitat alteration, introduction of invasive species, and pollution (Evans et al. 1987; Dextrase and Mandrak 2006; Ryan et al. 2019). Though the relative importance of each of these factors on fish abundance is not always known, it is believed that they often act through the recruitment process. Many economically valuable fish species are very r-selected, producing large numbers of small offspring with low survival (Winemiller and Rose 1992). Relatively small variations in survival during the vulnerable, early life stages of such species can potentially have large influences on future year-class strength, recruitment and ultimately population dynamics. Thus, research on the factors that drive recruitment variability through early life survival is central to the understanding of fish population dynamics.

Early life survival in fishes can be linked to a number of density-dependent and density-independent factors, with the latter considered more important in r-selected species (Johnston et al. 2016). Density-independent factors may include aspects of habitat quantity and quality, as well as prey and predator abundances (Chevalier 1977; Magnuson et al. 1979; Miller et al. 1988; Bailey and Houde 1989; Sogard 1997; Johnston et al. 2016). Factors affecting early life survival can also be categorized as biotic or abiotic in nature. A key biotic factor is egg quality, which in turn affects offspring quality (Brooks et al. 1997; Kamler 2005). Egg quality in fishes is largely determined by the maternal phenotype and thus represents a maternal effect (Bernardo 1996; Green 2008). Life history theory predicts that individuals should invest relatively more in reproduction

with increasing age and this may extend to both the quantity and quality of eggs (Belk and Tuckfield 2010). There is increasing empirical evidence that older and larger females within iteroparous spawning stocks may be producing higher quality eggs (Johnston 1997; Trippel 1998; Berkeley et al. 2004; Johnston et al. 2007; Venturelli et al. 2010). This is important because fisheries tend to be selective for larger size classes of fish and thus, may select against the best egg producers (Longhurst 2002; Hsieh et al. 2010; Hixon et al. 2014). The maternal effect of egg quality on subsequent offspring performance may be modified by environmental conditions in the spawning or nursery habitats, but such potential interactions remain largely unexplored.

Walleye, *Sander vitreus,* is the most targeted and economically valuable freshwater fish species in Canada and is the focus of ongoing fisheries research. It is a member of the family Percidae and a keystone predator of lakes and rivers across central North America. The walleye is an iteroparous (capable of spawning multiple times in their life), relatively r-selected (high fecundity, small eggs), broadcast-spawning species that uses rivers and rocky shorelines as spawning grounds in the spring (Colby et al. 1979; Bozek et al. 2011). Growth rates decline and ages of maturity increase moving from south to north across the walleye native range (Baccante and Colby 1996). Female age of maturity is generally four or five years old in central Ontario (Henderson and Morgan 2002), and longevity can reach 30 years in some lightly-exploited northern populations (T.A.Johnston, unpubl. data). As with many other exploited species, walleye harvest tends to be size-selective such that larger fish are removed first, and heavily exploited stocks often exhibit increased growth rates, reduced age of maturity, and truncated age and size distributions (Spangler et al. 1977). The implications of exploitation-induced shifts in spawning stock structure and abundance for walleye recruitment and population dynamics are not completely understood.

Early life survival is considered a key factor in year-class strength and recruitment in walleye (Colby et al. 1979; Bozek et al. 2011). Recent research has explored how egg and offspring quality varies among different age and size classes of female walleye from various perspectives. Older and larger walleye tend to produce eggs that are larger (Johnston and Leggett 2002), and in some cases eggs that contain more favourable lipid compositions (Wiegand et al. 2007). Experimental rearing of eggs from individual females has found that embryo survival is positively related to maternal age and/or size in some cases (Johnston 1997; Johnston et al. 2007), but not others (Czesny et al. 2005). A similar experiment examining walleye early life survival to a later juvenile stage found that survival was more strongly related to egg size than maternal age or size (Venturelli et al. 2010). Long-term analysis of walleye recruitment in Escanaba Lake, Wisconsin, found that early life survival was positively related to the contribution of larger females to annual egg production (Shaw et al. 2018), suggesting a maternal effect on egg and offspring quality. Another maternal factor that may influence spawning success is the timing of ovulation and spawning, as this will determine environmental conditions experienced in the early embryonic period, and possibly the duration of incubation. Again, variation among females in hatching success of their eggs has been found to be related to spawning date in some studies (Johnston 1997; Johnston et al. 2005; Johnston et al. 2007; Harry 2015), but not others (Czesny et al. 2005). Further research is required to determine how maternal effects may be influencing early life survival in walleye, and how their role may be modified by or interact with environmental conditions.

Spawning success and recruitment in walleye have often been associated with environmental factors, particularly environmental conditions during spawning, egg incubation, and the early growth period (Colby et al. 1979; Bozek et al. 2011). Stronger walleye year-class strengths have been linked to rapid spring warming rates at reef spawning sites (Busch et al. 1975) and higher spring flow rates in spawning rivers

(Johnston et al. 1995) both of which are believed to influence embryonic survival. Walleye embryos develop more rapidly when incubating at warmer temperatures (Colby et al. 1979) and this may reduce their exposure to siltation or predation on the spawning beds. Spring warming patterns vary among spawning years and may become more variable in future. Recent climate change is already causing significant changes in snow accumulations, the volume and timing of spring runoff in streams, and the timing of ice-off on lakes (Magnuson et al. 2000; Mote et al. 2005; Stewart et al. 2005). Changes in seasonal temperature regimes could further alter the timing and duration of spawning, and embryonic development in fishes (Pankhurst and Munday 2011; Farmer et al. 2015). Earlier and warmer springs are already causing walleye to spawn earlier in many lakes of northern Minnesota (Schneider et al. 2010). The implications of these shifts on walleye spawning success have yet to be studied in detail.

Though the roles of maternal and environmental effects have been studied on several walleye populations, research comparing the relative effects of these factors, and their potential interaction, is still in the early stages. The objective of my study was to examine the combined effects of these factors on the embryonic development and survival of walleye. My working hypothesis was that embryo quality would increase with female age under most incubation conditions, but that this effect would be least pronounced under conditions of rapid warming during incubation. Based on earlier research suggesting that older and larger females produce higher quality eggs and more robust embryos, I predicted that embryonic survival would be positively related to female age and size, and possibly also to indices of their egg quality, such as size and lipid composition. Based on results of earlier field-based research, I also tested the prediction that walleye embryo survival would be higher under rapidly-warming incubation temperature regimes. Furthermore, I predicted that these two factors may interact such that the relationship between embryonic survival and maternal traits would be weaker

under rapidly-warming incubation conditions. Specifically, I predicted that rapid-warming during incubation would improve survival most for embryos of younger females. Regarding developmental rates and the timing of hatch, I also predicted that the duration of the incubation period (time to hatch) would decline with increasing incubation temperature, but that the thermal units (temperature x time) required to develop and hatch would be relatively invariant with respect to both maternal traits and the incubation temperature regime.

I conducted this research on a walleye spawning stock from Lake Nipissing, Ontario. Lake Nipissing is the third largest freshwater lake wholly contained in Ontario, supports valuable subsistence, commercial and recreational walleye fisheries, and has been subjected to various anthropogenic stressors including exploitation, habitat alteration and introduction of invasive species (Morgan 2013). Each year over 60 000 people and 125 tourist establishments use the lake for recreational purposes (Morgan 2013). Heavy and sustained exploitation of Lake Nipissing's walleye population has reduced its abundance and altered its demographics. It is estimated that by 2010 the adult walleye population of Lake Nipissing was being reduced 30-55% every year through fisheries harvest (Morgan 2013; Zhao and Lester 2013). Stock assessments prior to 2008 indicated the population was impacted but not decimated by overexploitation (Morgan 2013). However, in the past decade, the population has shown a more drastic decline than expected, leading scientists to believe that other ecosystem changes are affecting the population (Morgan 2013; Zhao and Lester 2013). Recent fisheries-independent surveys of the lake indicate that the walleye population has a relatively truncated age structure, and the spawning stock is dominated by younger age classes (Ministry of Natural Resources and Forestry, North Bay District, unpubl. data).

Methods

Study site and field sampling

Spawning walleye were sampled at Wasi Falls (46° 12’ N, 79° 22’ W) in Callander Bay on the southeast corner of Lake Nipissing. The Wasi River enters the lake at this location, dropping over a steep, impassable falls at the lake’s edge. Walleye and other spring-spawning species, particularly white sucker (*Catostomus commersoni*), spawn on rubble substrate in the river plume that extends into the lake. Ovulated female (ova free-flowing) and ripe male (semen free-flowing) walleye used for the breeding experiments were sampled over multiple dates in each of 2017, 2018, and 2019. For each year, sampling dates were selected to cover as much of the spawning period as possible. Water temperatures in the river plume on these sampling dates ranged from 3 to 11 °C.

Fish were captured using an eight-foot trap net that was set near the base of the falls. The trap net was set in the evening and lifted the following morning. Ripe males were abundant in catches over most dates and were not a limiting factor. The first date of spawn collection in each year occurred on the earliest date when a sufficient number of ovulated females (generally ≥ 5) were captured in an overnight set. Both ovulated females and ripe males used for the experiments were selected from the catch haphazardly, except with respect to body size; an attempt was made to cover the full-size range of ovulated females observed in the trap net catch on each date. Selected individuals of both sexes were placed in tubs of river water or in mesh holding pens near the river plume to await processing.

All selected fish were processed on shore near Wasi Falls and males were processed first. Each male was dried with a towel and then stripped of semen by applying gentle pressure along the flanks. Semen was expelled into a clean, dry glass cup taking

care to avoid contamination with water, faeces, or epithelial slime. Males that could not provide at least 1 mL of semen were not used and were released immediately. One mL of clean semen was transferred by pipette into a 100-mL sterile tissue culture flask. Fork length was then measured (FL, ± 1mm), the second and third dorsal spines were removed by cutting at their base, and the fish was released. Dorsal spines were placed in paper envelopes and allowed to air dry. This protocol was repeated for six males on each spawn collection date with all semen placed in the same tissue culture flask. This semen pool was gently swirled for several minutes to homogenize then placed in a cooler at ambient river temperature. Similarly, each female was dried with a towel and approximately 50-80 mL of ripe ova were stripped into a clean, dry glass cup. Females that could not provide at least 50 mL of ova were not used and were released immediately. Ova were poured into individual Whirl-pak bags and placed in a cooler at ambient river temperature. The female was then measured and had dorsal spines removed before being released. This was repeated for up to five females on each spawn collection date.

Fertilizations were initiated within 1 h of completing gamete collection. For each female, 20 mL of ova were poured from the Whirl-pak into a stainless-steel mixing bowl, 0.5 mL of semen was pipetted from the pooled semen flask onto the ova, and the two were gently mixed with a feather. Once all females' ova were mixed with semen, 40 mL of Wasi River water was added to each bowl to activate the sperm. Each mixture was gently stirred with a feather for 15 min before another 100 mL of Wasi River water was added. Thereafter, the eggs were intermittently stirred as required to reduce adhesion and clumping as they hardened. An additional 250 mL of Wasi River water was added to each bowl at 30 min and again at 60 min post-fertilization. Two hours after fertilization, the fertilized eggs were drained, rinsed and transferred to 500-mL plastic screw-cap bottles filled to the top with Wasi River water. Bottles were placed in a cooler at ambient river temperature and transported to the incubation facility.

Embryo incubation

Embryos were incubated under controlled conditions in re-circulating Heath tray systems at the Vale Living with Lakes Centre (Laurentian University, Sudbury, Ontario, Canada). The base of each system consisted of a reservoir tank (270 L) with freshwater inflow, an aerator, a water chiller coil, an Onset HOBO® TidbiT v2 water temperature data logger (continuous recording at 1-h intervals), and a submersible pump to move reservoir water to the top of the Heath tray stack above the reservoir. Fresh water was obtained from Ramsey Lake, filtered to remove coarse particulates, and input to each reservoir at a rate of 50 % of the system volume per day. Each Heath stack had four egg incubation trays, and each of these had an inner egg tray with a fixed mesh bottom and removable mesh lid, both made of 1 mm square fiberglass mesh. The inner tray was subdivided into 16 compartments by a plexiglass divider, thus providing 64 compartments per system (4 trays x 16 compartments in each). Three such systems (hereafter stack A, B and C) were constructed to allow independent temperature control and all were held in a common lab with seasonal photoperiod. Water temperatures in each system were controlled by the chillers.

Upon returning to the lab from the spawn collection site, bottles with the water-hardened eggs were placed into the stack B reservoir. At approximately 24 h post-fertilization, subsamples of the embryos (3 replicates of ~ 100 each) were examined under a dissecting microscope to determine percent viability. Those with a clear perivitelline space, and symmetrical blastodisc development were deemed viable. For each female, 70 viable embryos were added into each of two randomly assigned compartments within each of stacks A, B and C (70 eggs x 2 compartments x 3 stacks = 420 total embryos per female). In 2018 only, embryo addition to the stacks had to be

delayed to a later developmental stage due to problems with the tray dividers. In this year, embryo viability was also assessed at 24-h post-fertilization, but embryos were then left in their 500-mL transport bottles (water changed daily) in reservoir B roughly to the pre-epiboly stage (for 3 - 6 days or 5 – 15 growing-degree-days > 5 °C) before viability was assessed a second time, and viable embryos were counted (n = 50) into the tray compartments.

Water temperatures in systems A, B and C were held at near ambient Wasi River temperatures until one day after embryos from the final spawn collection were counted into the trays. Afterwards, temperatures were gradually shifted to different warming trajectories. System B temperature was regulated to roughly continue tracking seasonal spawning site temperatures, system A was raised to 2 °C warmer than B, and system C was lowered to 2 °C cooler than B. These three unique temperature regimes were maintained until all embryos were hatched. Embryos were initially checked every 2 days, and mortalities (eggs appearing opaque) were counted and removed from each compartment. After the period of heaviest mortality had passed, mortalities were counted and removed every 3-4 days, but the schedule returned to every 2 days after the first signs of hatching. Once hatching was detected in a compartment, both the number of egg mortalities and the number of remaining unhatched eggs were recorded at each interval until hatching was completed. After hatching was completed, water temperature loggers were removed, and data were downloaded.

Lab analyses

Unfertilized ova samples brought back from the field were separated into two subsamples, with one frozen at -20 °C and the other at -70 °C. Those frozen at -20 °C were later freeze-dried and used for determinations of egg size and total lipid content.

Three replicates of 30 freeze-dried ova were weighed (± 0.1 mg) for each female to determine mean egg size (mg dry per ovum). The freeze-dried ova were then ground to a fine powder in a ball mill (Retsch MM 400). Lipid concentrations of the freeze-dried and ground ova tissue samples were determined gravimetrically using a chloroform-methanol extraction procedure (Moles et al. 2008). Ova frozen at -70 °C were analyzed for fatty acid composition at the University of Winnipeg in 2017 and 2018 following the methods of Wiegand et al. (2004), and at Ryerson University in 2019 following the methods of Strandberg et al. (2017). Fatty acid composition of extracted total lipids was determined by flame ionization gas chromatography (FIGC). Relative abundances of each fatty acid were expressed as percentages of total fatty acids normalized to 100%.

Ages of the female walleye were determined by counting annular growth rings on dorsal spine sections at the Northwest Fisheries Ageing Lab (Ontario Ministry of Natural Resources and Forestry, Dryden, ON). Spines were set in epoxy, thin transverse sections were cut from the proximal end with a jeweler's saw, and the sections were mounted on glass microscope slides and lightly polished to make the annuli more visible. Annuli were counted under a dissecting scope using reflected light. Because the fish were sampled at the time of spawn, near their birth dates, the outer edge of the section was always counted as a complete annulus.

Data analyses

Embryonic survival was estimated for each incubation chamber as the percentage of viable embryos at 24-h post-fertilization that successfully hatched. Because embryo addition to the incubation trays was at a later developmental stage in 2018, calculations for this year were adjusted for survival between the first and second viability estimates in order to be comparable with 2017 and 2019 calculations. The duration of the incubation

period was estimated as the number of days from fertilization to 50% hatch. The date of 50% hatch was interpolated from the relationship between percent unhatched embryos and incubation day, where the former was estimated from the number of unhatched embryos counted on a given date divided by the total number of live embryos remaining on the last date before hatching was detected. Thermal units to 50% hatch (TU_{50}) was estimated as the cumulative growing-degree-days above 5 °C by summing mean daily temperatures above 5 °C from 24-h post-fertilization to the date of 50% hatch.

All independent variables used in analyses are summarized in Table 1. The overall incubation temperature regime experienced by each batch of embryos was a function of both the female spawn date and the temperature treatment applied in the lab. Spawn date was included as a fixed effect in initial analyses of spawn date and temperature treatment, and as a random effect in subsequent analyses of maternal and ova traits. Incubation temperature treatment was included as a fixed effect with three levels (A – rapid warming, B – seasonal, C – slow warming). Because all fish within a year received the exact same application of the three temperature treatments, they were, strictly speaking, pseudo-replicates. However, they were treated as replicates within the context of my statistical analysis. Independent variables representing characteristics of the females and their ova were chosen based on previous research indicating their potential importance for embryonic development and survival (Table 1). These included female age and fork length as ontogenetic variables, and egg size (dry mass) and egg total lipid concentration as indices of maternal investment per offspring. Quality of egg nutrients was represented by relative abundances of three nutritionally significant polyunsaturated fatty acids - arachidonic acid (ARA; 20:4(*n*-6)), eicosapentaenoic acid (EPA; 20:5(*n*-3)), and docosahexaenoic acid (DHA; 22:6(*n*-3)) – and two additional fatty acids that contributed to a high proportion of the observed variation in egg lipid fatty acid

profiles among females – palmitoleic acid (PLA; 16:1(*n*-7)) and linoleic acid (LNA; 18:2(*n*-6)).

Table 1. Independent variables used in analyses of embryonic survival and TU_{50} for Lake Nipissing walleye embryos reared in controlled-breeding experiments in 2017, 2018, and 2019.

Variable	Description
DATE	Spawning day of year; 4 - 6 dates per year
TEMP	Incubation temperature treatment; A = rapid warming, B = seasonal warming, C = slow warming
Female traits	
AGE	Female age (years) determined from dorsal spine sections
FL	Female fork length (mm)
Ova Traits	
EGGSZ	Mean egg (ovum) size (mg dry)
GON_L	Gonad (ova) lipid content (% of dry mass)
ARA	Percentage of ova fatty acids as Arachidonic acid, 20:4(*n*-6)
EPA	Percentage of ova fatty acids as Eicosapentaenoic acid, 20:5(*n*-3)
DHA	Percentage of ova fatty acids as Docosahexaenoic acid, 22:6(*n*-3)
PLA	Percentage of ova fatty acids as Palmitoleic acid, 16:1(*n*-7)
LNA	Percentage of ova fatty acids as Linoleic acid, 18:2(*n*-6)

All statistical analyses were carried out using R studio v3.6.1 procedures. Scatter plots of dependent vs independent variables and of model residuals vs predicted values were examined to determine the nature of relationships and identify outliers. A single female from the 2018 experiment produced embryos with unusually low survival and data from this fish were excluded from subsequent analyses. Some variables were transformed to normalize residuals and/or linearize observed relationships, as required. This included arcsine-square-root transformation of embryonic survival and $\log_e$ transformation of female age. Because both the distribution of spawn dates and the three temperature treatment trajectories were anchored to environmental conditions within their particular spawning years (see Results), I could only make statistical comparisons within years, not across years. For this reason, analyses proceeded separately for each year's experiment.

Analyses of embryonic survival and TU_{50} were carried out in a similar fashion. First, I modelled each as functions of spawn date, temperature treatment and female identity as a random effect nested within spawn date using linear mixed-effects (LME) models (lmer function in lme4 package) using data from individual incubation chambers (female reps within treatments). These analyses provided tests for overall spawn date and temperature treatment effects. For tests of spawn date, partial-F statistics (type III SS) were calculated using the female-nested-in-spawn-date term as the denominator, rather than the full model residual term. Second, I examined maternal and ova effects using a model selection approach (Burnham and Anderson 2002; Johnson and Omland 2004). These analyses were conducted separately for each temperature treatment, and data for dependent variables were the within-treatment means for each female, weighted by the inverse of the within-treatment variances. All models included spawn date as a random effect, and various combinations of the maternal and ova traits (Table 1) converted to standardized z-scores (standardize procedure in R psycho package). Models were fitted

for spawn date only and for spawn date with all combinations of one and two continuous variables (46 models in total) and were ranked according to Akaike's Information Criterion adjusted for small sample size (AICc). More complex models with three or more continuous variables were not considered, given the relatively low sample sizes of the data sets ($n < 30$ females per year). Fitted models were compared using AICc rankings and Akaike weights, and the nature (positive or negative) and strength of maternal and ova trait effects on embryonic survival and TU_{50} were interpreted from standardized model coefficients. Predictor variables in high-ranking models were also tested for their statistical significance based on partial-F (type III SS) tests.

Results

Overview of field and laboratory conditions

Spring warming (to end of April, day 120) proceeded much more slowly in 2018 and 2019 compared to 2017 (Fig. 1) and this influenced spawn timing. Based on recent spawning stock assessments at Wasi Falls, the seasonal timing of the 2017 spawn would be considered typical whereas the 2018 and 2019 spawns would be considered late. Ovulated females were sampled, and embryo batches produced on five dates from 19 April to 3 May in 2017, on four dates from 6 to 14 May in 2018, and on six dates from 29 April to 11 May in 2019 (Fig. 2). Variation in river temperatures among spawn collection dates was lowest in 2017 and highest in 2019 (Fig.2).

Mean ages and sizes of females sampled in the three study years were very similar (Table 2). Females ranged in age from 5 to 19 years, 7 to 16 years and 5 to 13 years in 2017, 2018 and 2019, respectively. These age ranges are representative of mature female age ranges seen previously in this spawning stock. In all years, the size and age of

sampled females tended to decline over the spawning period. Ova size was similar in 2017 and 2018, but somewhat lower in 2019, whereas ova lipid content was very similar among years (Table 2). Key ova lipid fatty acid compositions were similar to those reported previously for Lake Nipissing walleye (Wiegand et al. 2007) (Table 2). A full summary of ova lipid fatty acid composition is presented in Appendix 2. Fatty acid profiles in 2019 showed higher proportions of monounsaturates and lower proportions of polyunsaturates compared to 2017 and 2018 (Table 2; Appendix 2), though this may be due, in part, to changes in analytical procedures (see Methods).

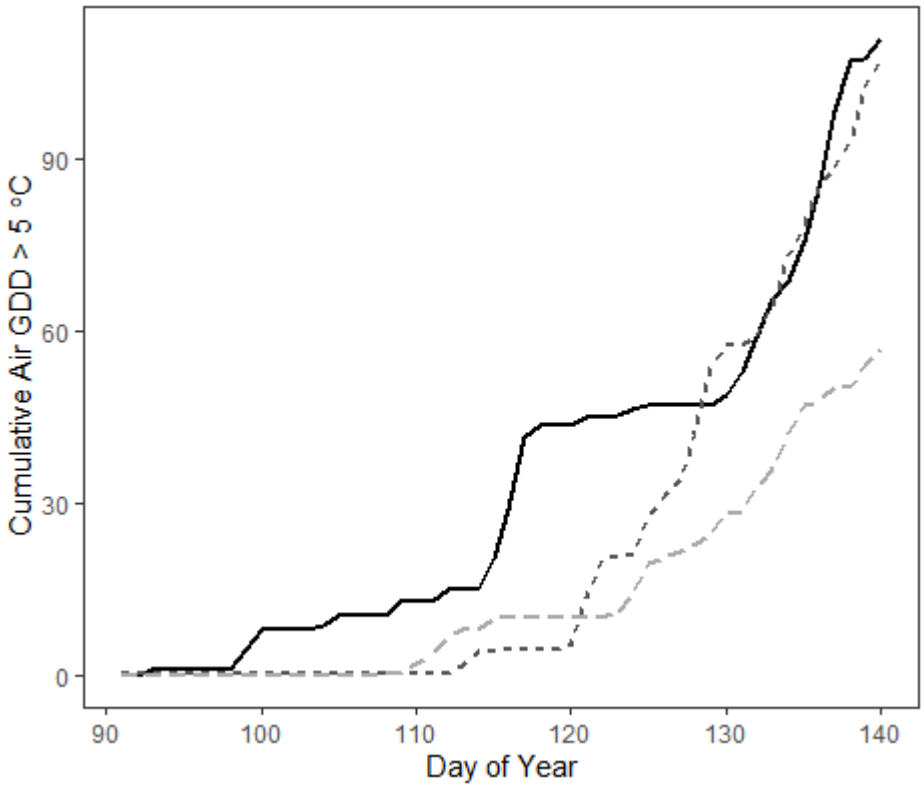

Figure 1. Cumulative growing-degree days above 5° C (GDD > 5 °C) from April 1st based on mean daily air temperatures recorded by Environment Canada at North Bay, Ontario, vs day of year (120 = 30 April) for each of 2017 (solid line), 2018 (black short-dash), and 2019 (grey medium-dash).

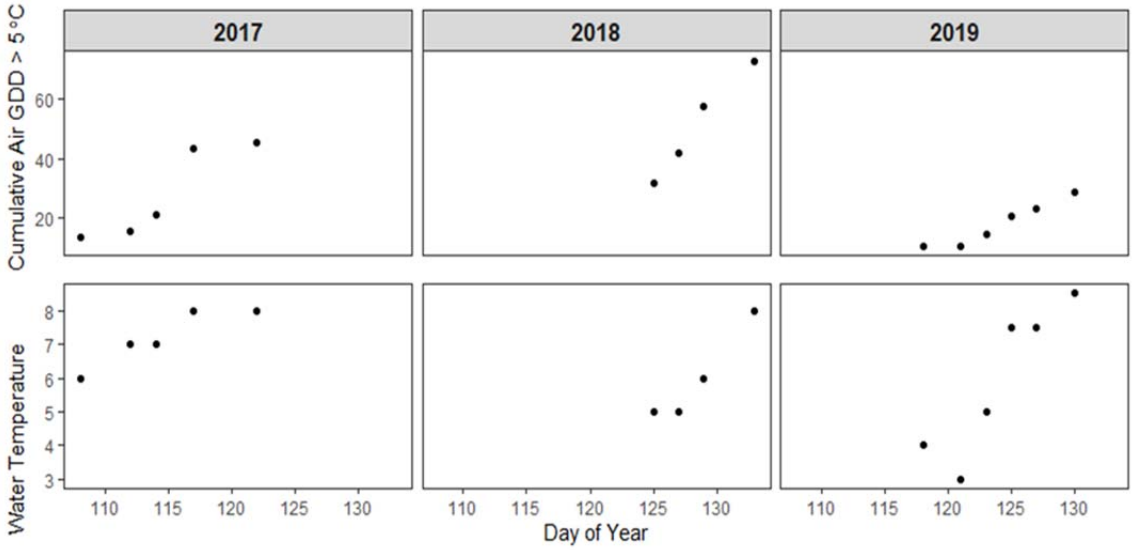

Figure 2. Cumulative growing-degree days above 5° C (GDD > 5 °C) from April 1st based on mean daily air temperatures recorded by Environment Canada at North Bay, Ontario (top) and water temperature (° C) in the Wasi Falls plume, Lake Nipissing (bottom) vs day of year (120 = 30 April) for each spawn collection date over the three study years.

Table 2. Summary of maternal and ova characteristics for mature female walleye (n = number per year) from Lake Nipissing, Ontario, used for controlled-breeding experiments in 2017, 2018 and 2019. Variables are defined in Table 1. Values are unadjusted means (± 1 SE).

	Study Year		
Variable	**2017 (n = 25)**	**2018 (n = 20)**	**2019 (n = 28)**
Female traits			
AGE (years)	8.68 ± 0.32	8.74 ± 0.22	7.86 ± 0.13
FL (mm)	485 ± 10.5	480 ± 10.2	482 ± 5.2
Ova traits			
EGGDW (mg)	0.86 ± 0.02	0.88 ± 0.02	0.80 ± 0.01
GON_L (%)	34.0 ± 0.3	34.0 ± 0.4	34.0 ± 0.3
PLA (%)	11.8 ± 0.23	12.1 ± 0.31	16.7 ± 0.37
LNA (%)	4.40 ± 0.14	4.27 ± 0.26	3.55 ± 0.14
ARA (%)	5.39 ± 0.09	4.94 ± 0.17	3.84 ± 0.10
EPA (%)	5.55 ± 0.22	5.29 ± 0.26	4.35 ± 0.29
DHA (%)	18.9 ± 0.30	19.9 ± 0.46	18.2 ± 0.22

Incubation warming regimes in the laboratory differed among years (Fig. 3), both in response to the unique spring warming trends at the field site which were used to guide the seasonal regime, and to challenges associated with controlling water temperature in the laboratory. Incubation warming patterns were most variable in 2017 and trajectories of the seasonal- and slow-warming treatments (B and C, respectively) overlapped for part

of the incubation period (Fig. 3). Temperature control in the laboratory improved in subsequent years and incubation temperature trajectories were smoothest in 2019 (Fig. 3). Warming rates over the incubation period were most rapid in 2018, with temperatures rising from 5 to 15 °C in 16 days in the seasonal (B) treatment, whereas this same temperature increase took approximately 30 days in 2017 and 2019 (Fig. 3). As a measure of accuracy, I compared daily mean temperatures between data loggers in the seasonal (B) incubation treatment in the laboratory and in the river plume at Wasi Falls (Appendix 1) in 2018. Wasi Falls temperatures were slightly more variable compared to the laboratory incubation treatment, as expected, but the mean daily deviation between them from incubation to hatch was only 0.65 °C. Thus, my laboratory seasonal temperature regime tracked the spawning site temperature regime relatively well.

Egg viability and embryo survival to hatch

Egg viability determined at 24 h post-fertilization varied among females but was generally high. For the 72 females used in my analyses, 59 females (82 %) had egg viability > 90 %, 11 females (15 %) had egg viability between 80 and 90 %, and only two females (3 %) had egg viability < 80 %. Embryo survival from 24 h to hatch was much more variable among females than egg viability at 24 h post-fertilization and the two were not strongly correlated. Their relationship was strongest in 2017 ($r = 0.48$, $n = 25$, $P = 0.015$) but weaker in 2018 ($r = 0.04$, $n = 19$, $P = 0.87$) and 2019 ($r = 0.26$, $n = 28$, $P = 0.19$).

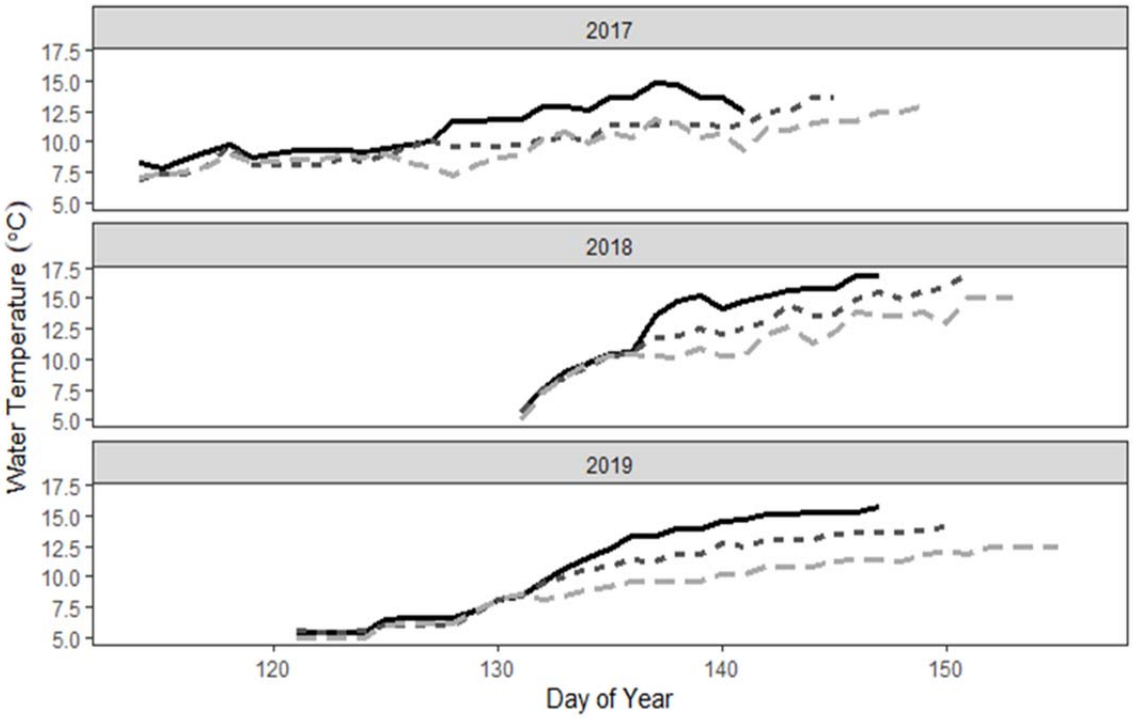

Figure 3. Water temperature vs day of year (135 = 15 May) in three laboratory egg incubation systems in 2017, 2018 and 2019. In each year incubation systems were regulated for rapid warming (treatment A, black solid line), seasonal warming (treatment B, black dashed line), and slow warming (treatment C, grey dashed line).

Embryonic survival from 24 h post-fertilization to hatch varied both among and within years. Mean embryonic survival for individual females ranged from 46 to 96 % in 2017, 43 to 71 % in 2018, and 48 to 86 % in 2019. There was no significant interaction between the effects of spawn date and temperature treatment on embryonic survival in any of the three study years (partial-$F < 1.15$, $df_{error} > 86$, $P > 0.34$) and this term was removed from the models. Variation in embryonic survival among spawn dates was greatest in 2017 and lowest in 2018 (Fig. 4), but only statistically significant in 2017 (Table 3). Embryonic survival tended to be highest for females sampled during the middle of the spawning period in 2017, but this pattern was not apparent in 2018 or 2019 (Fig. 4). Mean embryonic survival adjusted for spawn date effects in the rapid-, seasonal- and slow-warming incubation treatments for each of the three study years was: 69, 69 and 68 %, respectively, in 2017; 56, 56 and 56 %, respectively, in 2018; 65, 65, and 64 %, respectively, in 2019. Differences in survival among the three incubation temperature treatments were not statistically significant in any study year (Table 3).

Models relating embryonic survival to maternal and ova traits were fitted and ranked for nine subsets of data (three years x three temperature treatments) (Tables 4 – 6). Embryonic survival generally showed stronger relationships with ova traits than with female age or size. The highest-ranked single-predictor models were based on ova traits in eight of nine cases, and the highest-ranked two-predictor models contained two ova traits in six of nine cases. Akaike weights were low for most top-ranked models ($w_i < 0.5$ in seven of nine cases) and declined slowly moving down in rank, suggesting that multiple models were plausible (Tables 4 – 6). The ova traits most commonly appearing in high-ranked models were egg size, egg lipid content and ARA in 2017 (Table 4), ARA and EPA in 2018 (Table 5), and DHA, EPA and egg size in 2019 (Table 6). Interestingly, the relationship between embryonic survival and these ova traits tended to be negative in 2017 and 2018 (Tables 4 and 5) but positive in 2019 (Table 6). Female FL and age were

part of several highly-ranked models in each of 2018 and 2019 (Tables 5 and 6) but not 2017 (Table 4). Similar to the case with ova traits, the relationship between embryonic survival and maternal FL and age tended to be negative in 2018 (Table 5) but positive in 2019 (Table 6). There was no consistent pattern in how the models ranked with respect to temperature treatment over the three study years.

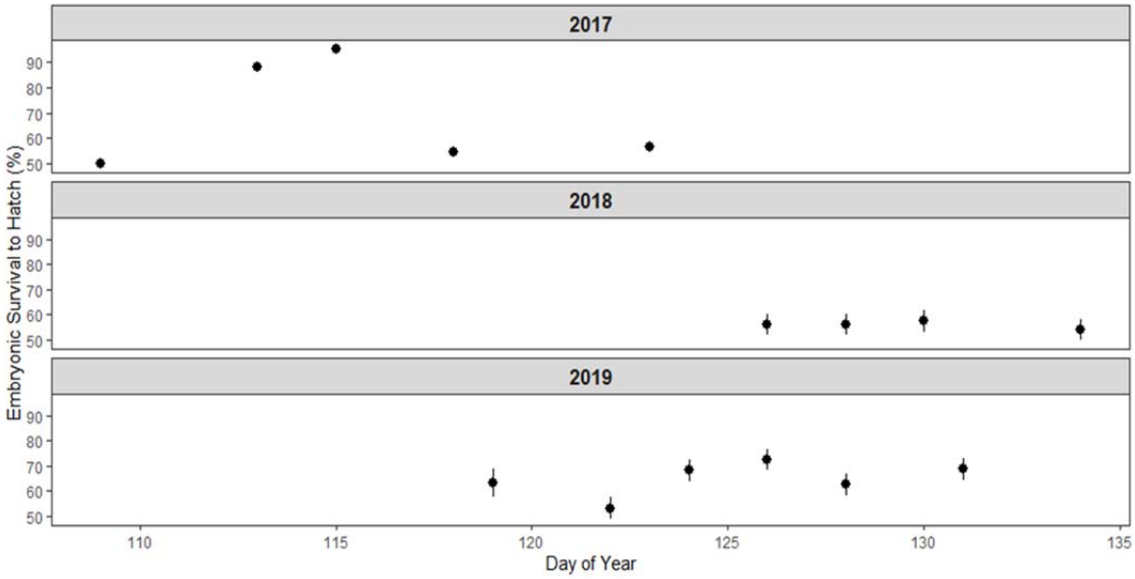

Figure 4. Plot of embryo survival to hatch vs spawning day of year for each of the three study years. Symbols are means adjusted for incubation temperature treatment ± 1 SE.

Table 3. Results of tests for main effects in linear models relating walleye embryonic survival to incubation temperature treatment (TEMP; fixed), spawning date (DATE; fixed) and female identity nested within spawning date (random) for each study year. Interaction between TEMP and DATE was not significant in any year ($P > 0.34$) and was removed from the models.

Variables	partial-F	df	P-value	Conditional r^2
2017				0.91
TEMP	0.41	2, 123	0.66	
DATE	135.6	4, 20	< 0.001	
2018				0.86
TEMP	0.28	2, 93	0.76	
DATE	0.11	3, 15	0.95	
2019				0.72
TEMP	0.28	2, 138	0.76	
DATE	2.47	5, 22	0.064	

Table 4. Models ranked by second order Akaike Information Criterion (AICc) of embryo survival to hatch as a function of egg and female characteristics (fixed effects; defined in Table 1) for the 2017 walleye controlled-breeding experiment (n = 25 females). Model fitting and ranking were carried out separately for each incubation temperature treatment. Spawning date was included as a random effect (intercept) in all models. Models were fitted to all combinations of 1 and 2 fixed effects and only top-ranked models are displayed. Δ_i = Akaike differences, W_i = Akaike weights.

Fixed Effects in Model (standardized coefficients)	**Rank**	**AIC_c**	**Δ_i**	**W_i**
Treatment A - rapid warming				
EGGSZ (-0.32), GON_L (-0.12)	1	-33.70	0	0.30
EGGSZ (-0.24), PLA (-0.05)	2	-31.90	1.77	0.12
EGGSZ (-0.35)	3	-31.67	2.04	0.11
EGGSZ (-0.28), DHA (0.04)	4	-29.99	3.71	0.05
EGGSZ (-0.30), EPA (0.05)	5	-29.97	3.73	0.05
Intercept	30	-23.56	10.14	0
Treatment B - seasonal warming				
ARA (-0.24)	1	-36.45	0	0.24
ARA (-0.22), DHA (-0.04)	2	-36.11	0.34	0.20
ARA (-0.21), GON_L (-0.06)	3	-35.21	1.24	0.13
ARA (-0.22), PLA (0.05)	4	-34.66	1.8	0.09
ARA (-0.24), EPA (-0.05)	5	-34.38	2.07	0.09
Intercept	30	-20.23	16.2	0
Treatment C - slow warming				
GON_L (-0.46), EGGSZ (-0.29)	1	-2.41	0	0.40
ARA (-0.33), PLA (0.20)	2	-0.97	1.44	0.20
PLA (0.29), GON_L (-0.16)	3	-0.06	2.35	0.12
ARA (-0.50), log AGE (0.23)	4	0.81	3.21	0.08
PLA (0.20)	5	1.90	4.3	0.04
Intercept	42	23.70	26	0

Table 5. Models ranked by second order Akaike Information Criterion (AICc) of embryo survival to hatch as a function of egg and female characteristics (fixed effects; defined in Table 1) for the 2018 walleye controlled-breeding experiment (n = 19 females). Model fitting and ranking were carried out separately for each incubation temperature treatment. Spawning date was included as a random effect (intercept) in all models. Models were fitted to all combinations of 1 and 2 fixed effects and only top-ranked models are displayed. Δ_i = Akaike differences, W_i = Akaike weights.

Fixed Effects Model (Standardized Coefficients)	**Rank**	**AIC_c**	**Δ_i**	**W_i**
Treatment A - rapid warming				
ARA (-0.24), LNA (0.15)	1	33.75	0	0.48
ARA (-0.22), EPA (0.08)	2	32.64	1.10751	0.27
ARA (-0.17)	3	30.06	3.69266	0.08
PLA (0.19)	4	27.72	6.02768	0.02
ARA (-0.13), PLA (0.05)	5	26.93	6.81966	0.02
Intercept	40	15.35	18.3946	0.00
Treatment B - seasonal warming				
ARA (-0.34), FL (-0.27)	1	38.01	0	0.91
ARA (-0.34), log AGE (-0.34)	2	32.76	5.24874	0.07
EPA (-0.18), ARA (-0.14)	3	27.31	10.6958	0.00
LNA (-0.25), FL (-0.18)	4	27.30	10.7037	0.00
ARA (-0.23)	5	26.83	11.1772	0.00
Intercept	32	14.21	23.796	0.00
Treatment C - slow warming				
EPA (-0.29), FL (-0.16)	1	20.54	0	0.23
EPA (-0.34), LNA (0.22)	2	18.70	1.83678	0.09
log AGE (-0.47), ARA (-0.30)	3	18.62	1.9169	0.09
FL (-0.34), ARA (-0.30)	4	18.28	2.25727	0.08
EPA (-0.24), log AGE (-0.19)	5	18.01	2.52489	0.07
EPA (-0.29)	9	16.41	4.12776	0.03
Intercept	42	16.33	4.20504	0.03

Table 6. Models ranked by second order Akaike Information Criterion (AICc) of embryo survival to hatch as a function of egg and female characteristics (fixed effects; defined in Table 1) for the 2019 walleye controlled-breeding experiment (n = 28 females). Model fitting and ranking were carried out separately for each incubation temperature treatment. Spawning date was included as a random effect (intercept) in all models. Models were fitted to all combinations of 1 and 2 fixed effects and only top-ranked models are displayed. Δi = Akaike differences, Wi = Akaike weights.

Fixed Effects and Standardized Coefficients	**Rank**	**AIC_c**	**Δ_i**	**W_i**
Treatment A - rapid warming				
FL (0.33), DHA (0.26)	1	-8.82	0	0.92
FL (0.30), EPA (0.18)	2	-2.55	6.261599	0.04
GON_L (0.35), FL (0.31)	3	-1.25	7.563166	0.02
DHA (0.35)	4	1.67	10.48812	0.00
EPA (0.25)	5	3.12	11.93318	0.00
Intercept	27	22.79	31.6042	0.00
Treatment B - seasonal warming				
EPA (-0.37), DHA (0.35)	1	-28.88	0	0.39
FL (0.20)	2	-27.54	1.340064	0.20
FL (0.19), DHA (0.08)	3	-26.14	2.737045	0.10
FL (0.23), ARA (-0.06)	4	-25.59	3.285364	0.08
FL (0.19), EPA (-0.06)	5	-25.20	3.677911	0.06
Intercept	13	-18.77	10.10833	0.00
Treatment C - slow warming				
EGGSZ (0.19)	1	-27.34	0	0.33
EGGSZ (0.21), EPA (0.06)	2	-25.63	1.704917	0.14
EGGSZ (0.18), GON_L (0.04)	3	-24.99	2.345662	0.10
EGGSZ (0.16), L_AGE (0.05)	4	-24.98	2.352633	0.10
EGGSZ (0.20), DHA (0.02)	5	-24.42	2.91953	0.08
Intercept	18	-15.26	12.07874	0.00

Thermal units to 50% hatch (TU_{50})

The TU_{50} for walleye embryos appeared to be both higher and more variable among females in 2017 than in 2018 and 2019 (Fig. 5). Least-square means of TU_{50} for individual spawn dates ranged from 124 to 134 degree-days in 2017, from 105 to 118 degree-days in 2018, and from 107 to 117 degree-days in 2019. Within all study years, there was significant interaction between the effects of spawn date and temperature treatment on TU_{50} (Table 7). In the full models containing the interaction term both spawning date and incubation temperature treatment were also statistically significant. The general pattern between TU_{50} and spawn date was a positive relationship, and this was fairly consistent in the seasonal- and slow-warming treatments (Fig. 5). However, the relationship between TU_{50} and spawn date was more variable among years for the rapid-warming treatment (Fig. 5). There was also no consistent pattern with respect to temperature treatment; TU_{50} was highest in the rapid-warming treatment on most spawn dates in 2017, but highest in the slow-warming treatment on most spawn dates in 2019 (Fig. 5).

Models relating TU_{50} to maternal and ova traits were also fitted and ranked for the nine subsets of data (three years x three temperature treatments) (Tables 8 - 10). Overall, relationships between TU_{50} and the maternal and ova traits appeared to be weaker than those seen between embryonic survival and maternal and ova traits, particularly for embryos reared in the rapid-warming treatment. The intercept-only model (containing no maternal or ova traits) was the top-ranking model for the rapid-warming treatments of 2017 (Table 8) and 2018 (Table 9). It was also the second highest-ranked model for the rapid-warming treatment and highest-ranked model for the seasonal-warming treatment of 2019 (Table 10). Akaike weights were low for most top-ranked models ($w_i < 0.4$ in

eight of nine cases) and declined slowly moving down in rank, suggesting that multiple models were plausible (Tables 4 – 6). High-ranking models for embryos of the seasonal- and slow-warming treatments in each year tended to contain female age, fork length and egg size and TU_{50} exhibited negative relationships with all three of these predictors (Tables 8 – 10). Indices of ova lipid content and composition were less prominent in high-ranking TU_{50} models (Tables 8 – 10).

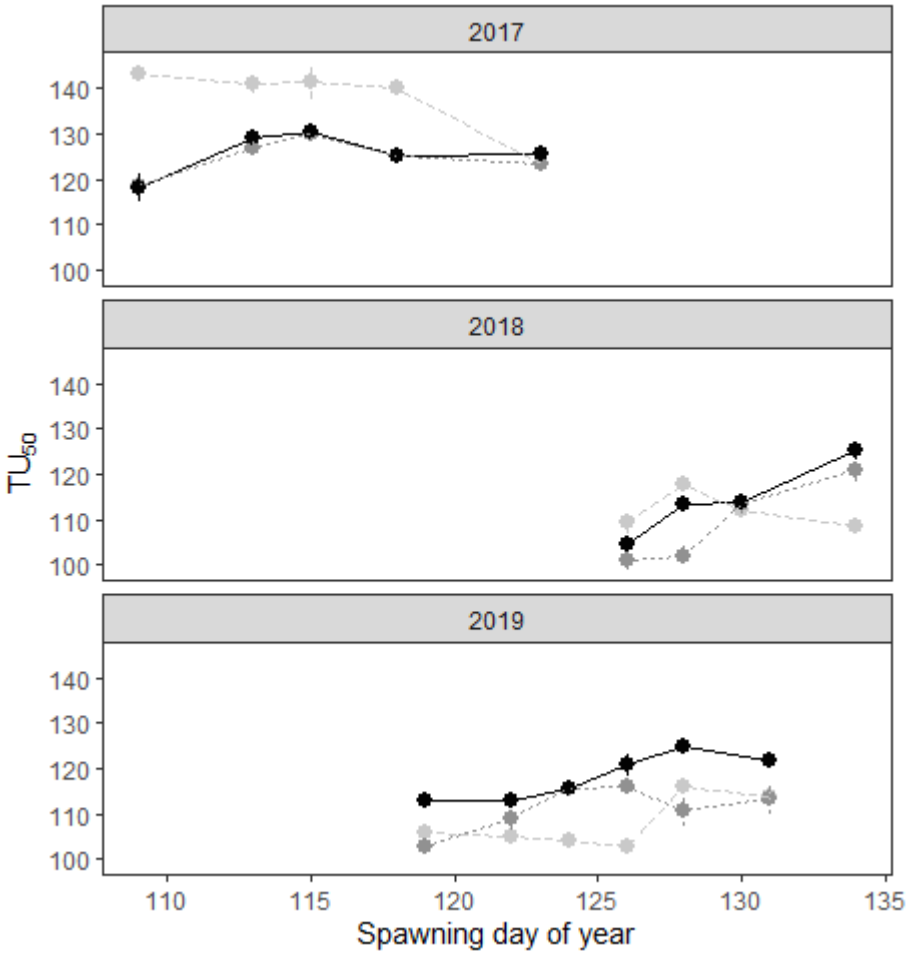

Figure 5. TU_{50} (cumulative degree-days >5°C) vs spawning date for each of the three incubation temperature treatments in each of the three study years. Treatments are rapid-warming (light grey, dashed line), seasonal (dark, dashed line) and slow-warming (black, solid line). Symbols are means ± 1 SE.

Table 7. Results for tests of main effects in linear models relating TU_{50} for walleye embryos to incubation temperature treatment (TEMP; fixed), spawning date (DATE; fixed), their interaction and female identity nested within spawning date (random) for each study year.

Variable	partial-F	df	P-value	Conditional r^2
2017				0.89
TEMP	227	2, 115	< 0.001	
DATE	11.5	4, 20	< 0.001	
DATE x TEMP	25.6	8, 115	< 0.001	
2018				0.72
TEMP	8.38	2, 87	< 0.001	
DATE	26.3	3, 15	< 0.001	
DATE x TEMP	15.5	6, 87	< 0.001	
2019				0.71
TEMP	65.4	2, 128	< 0.001	
DATE	19.1	5, 22	< 0.001	
DATE x TEMP	6.97	10, 128	< 0.001	

Table 8. Models ranked by second order Akaike Information Criterion (AICc) of TU_{50} for walleye embryos as a function of egg and female characteristics (fixed effects; defined in Table 1) for the 2017 walleye controlled-breeding experiment (n = 25 females). Model fitting and ranking were carried out separately for each incubation temperature treatment. Spawning date was included as a random effect (intercept) in all models. Models were fitted to all combinations of 1 and 2 fixed effects and only top-ranked models are displayed. Δ_i = Akaike differences, W_i = Akaike weights.

Fixed Effects in Model (standardized coefficients)	**Rank**	**AIC_c**	**Δ_i**	**W_i**
Treatment A - rapid warming				
Intercept	1	166.12	0	0.12
DHA (5.82)	2	166.88	0.76544	0.08
LNA (-3.65)	3	167.78	1.6608	0.05
ARA (-4.04)	4	167.88	1.75962	0.05
FL (-2.58)	5	168.20	2.08602	0.04
DHA (5.86), ARA (-4.04)	9	168.80	2.68321	0.03
Treatment B - seasonal warming				
log AGE (-4.51), DHA (4.31)	1	134.12	0	0.18
log AGE (-3.61), LNA (-3.33)	2	134.92	0.79447	0.12
log AGE (-4.18)	3	136.30	2.18288	0.06
LNA (-3.83)	4	136.45	2.32817	0.06
LNA (-3.43), FL (-2.31)	5	136.82	2.70083	0.05
Intercept	14	138.51	4.38388	0.02
Treatment C - slow warming				
EGGSZ (-12.86), LNA (-4.99)	1	148.98	0	0.18
EGGSZ (-10.60), log AGE (-5.15)	2	149.41	0.43516	0.14
EGGSZ (-12.86), GON_L (5.40)	3	149.87	0.88927	0.11
EGGSZ (-11.89)	4	150.21	1.2279	0.10
EGGSZ (-11.26), FL (-2.95)	5	151.54	2.56398	0.05
Intercept	13	153.83	4.85489	0.02

Table 9. Models ranked by second order Akaike Information Criterion (AICc) of TU_{50} for walleye embryos as a function of egg and female characteristics (fixed effects; defined in Table 1) for the 2018 walleye controlled-breeding experiment (n = 19 females). Model fitting and ranking were carried out separately for each incubation temperature treatment. Spawning date was included as a random effect (intercept) in all models. Models were fitted to all combinations of 1 and 2 fixed effects and only top-ranked models are displayed. Δ_i = Akaike differences, W_i = Akaike weights.

Fixed Effects and Standardized Coefficients	Rank	AIC_c	Δ_i	W_i
Treatment A - rapid warming				
Intercept	1	33.01	0.00	0.33
ARA (-0.00)	2	36.27	3.26	0.07
GON_L (-0.00)	3	36.27	3.26	0.07
FL (-0.00)	4	36.27	3.26	0.07
DHA (0.00)	5	36.27	3.26	0.07
ARA (-0.00), GON_L (-0.00)	9	40.02	7.01	0.01
Treatment B - seasonal warming				
FL (-21.83), ARA (-14.02)	1	167.68	0.00	0.51
FL (-15.25), GON_L (-10.93)	2	168.95	1.27	0.27
FL (-13.91)	3	172.31	4.62	0.05
FL (-11.80), EPA (7.69)	4	173.77	6.08	0.02
FL (-19.17), EGGSZ (9.31)	5	174.12	6.44	0.02
Intercept	17	178.61	10.93	0.00
Treatment C - slow warming				
log AGE (-7.09)	1	145.23	0.00	0.14
EGGSZ (-8.62)	2	145.42	0.19	0.12
FL (-6.79)	3	145.47	0.25	0.12
Intercept	4	146.10	0.87	0.09
EPA (5.79)	5	147.73	2.50	0.04
log AGE (-6.28), GON_L (-3.68)	8	148.23	3.00	0.03

Table 10. Models ranked by second order Akaike Information Criterion (AICc) of TU_{50} for walleye embryos as a function of egg and female characteristics (fixed effects; defined in Table 1) for the 2019 walleye controlled-breeding experiment (n = 28 females). Model fitting and ranking were carried out separately for each incubation temperature treatment. Spawning date was included as a random effect (intercept) in all models. Models were fitted to all combinations of 1 and 2 fixed effects and only top-ranked models are displayed. Δ_i = Akaike differences, W_i = Akaike weights.

Fixed Effects and Standardized Coefficients	Rank	AIC_c	Δ_i	W_i
Treatment A - rapid warming				
ARA (-6.61)	1	180.25	0.00	0.09
Intercept	2	180.48	0.23	0.08
ARA (7.27), FL (-4.52)	3	180.56	0.31	0.08
GON_L (-4.67)	4	180.88	0.63	0.07
ARA (-6.19), GON_L (-4.26)	5	180.95	0.70	0.06
EPA (-5.63), FL (-5.34)	6	181.06	0.81	0.06
Treatment B - seasonal warming				
Intercept	1	176.17	0.00	0.19
EGGSZ (2.63)	2	177.66	1.49	0.09
ARA (-2.95)	3	177.82	1.65	0.08
GON_L (2.67)	4	178.29	2.11	0.06
EPA (-1.16)	5	178.65	2.48	0.05
ARA (-3.24), GON_L (3.11)	9	180.00	3.83	0.03
Treatment C - slow warming				
L_AGE (-5.22), EGGSZ (-4.03)	1	151.85	0.00	0.33
EGGSZ (-5.95), EPA (-3.44)	2	153.82	1.97	0.12
log AGE (-7.29)	3	153.96	2.11	0.11
EGGSZ (-5.66)	4	154.50	2.65	0.09
EPA (-2.84), log AGE (7.27)	5	154.54	2.69	0.09
Intercept	14	160.82	8.97	0.00

Time to 50% hatch

The combined effects of variation in spawn date and incubation temperature regime resulted in wide variation in time to 50% hatch. In all study years the mean time to 50% hatch was shortest for embryos in the rapid-warming treatment from females of the final spawn date, and longest for embryos in the slow-warming treatment from females of the first spawn date. The ranges of mean time to 50% hatch were 18 to 32 days in 2017, 13 to 24 days in 2018, and 15 to 33 days in 2019. These results were compared with estimated incubation times in the field based on temperature monitoring at natural spawning sites (Appendix 1).

Discussion

Despite considerable research, the factors that affect walleye embryonic development and survival are just beginning to be understood. Research examining among-spawner variation in offspring quality could help shed new light on which age and size classes of fish are contributing most to recruitment, yet such studies are still relatively uncommon for wild fish populations. To my knowledge, this is the first experimental study to examine the combined effects of environmental conditions and maternal traits on spawning success of individual female walleye. I assessed embryonic development and hatching success of individual females from a single population of walleye over three consecutive spawning years and my key findings can be summarized as follows. First, contrary to my prediction and earlier research, older and larger females in the spawning stock aren't always producing the eggs with highest survival. Second, the timing of ovulation and spawning may affect embryonic survival in some, but not all

years. Third, moderate changes in spring warming rates don't have much direct effect on embryo survival. Finally, developmental rates in relation to thermal inputs seem to be more variable than expected, suggesting that temperature may influence hatch timing more than survival. These results provide new and unique information on reproduction and the recruitment process in walleye, have implications for size-selective harvest guidelines used for fisheries management, and help us to understand the potential effects of climate change on walleye spawning.

Embryonic survival to hatch

The three study years, from 2017 to 2019, provided an excellent contrast in spring warming patterns, and in the timing and progression of walleye spawning at Wasi Falls. The spawn timing in 2017 was closest to the long-term pattern for this site (MNRF, unpubl. data), whereas spawn timing was shifted later in 2018 and 2019. In addition, warming proceeded more rapidly in the post-spawn period in 2018 than in 2017 or 2019. Interestingly, variation in embryonic survival among spawn dates was only significant in 2017. This suggests a potential environmental influence on egg quality that may be dampened by later ovulation and spawning. Previous experimental research has also reported variation in embryonic survival among spawn collection dates in some stocks of walleye (Johnston et al. 2005; Johnston et al. 2007) including Lake Nipissing (Harry 2015). A similar controlled-breeding experiment using an Ohio reservoir walleye stock found no significant effect of spawning date on embryonic survival (Czesny et al. 2005). However, this latter study pooled survival estimates among adjacent days which may have obscured some of the day-to-day variability. Nevertheless, it is possible that the

mechanisms behind the observed spawning date variability are not consistent among walleye stocks, or among spawning years. Walleye ovulation and spawning are influenced by both photoperiod and water temperature (Scott and Crossman 1973; Becker 1983; Malison and Held 1996). In some stocks walleye will commence spawning at warmer temperatures when spring arrives early, but at cooler temperatures when spring starts late (Rawson 1957), apparently a photoperiod effect modifying the temperature effect. The timing of spawn relative to ovulation may be a factor as ova can become less viable over time following ovulation (Aegerter and Jalabert 2004; Johnston et al. 2007). However, it is not clear if or why females captured on some dates in my study may have been delaying spawn following ovulation. Egg quality typically declines with successive spawnings in batch-spawning species such as cod (*Gadus morhua*) (Petersen et al. 2016). However, studies of variation in embryo viability over the spawning season in synchronous spawning species, such as walleye, are uncommon. Slight methodological differences in spawn collection among sampling dates in my study may have added some variability to the embryo survival estimates, though why such variability might be greater in 2017 than in the other two years is unknown.

I found that walleye embryo survival in the laboratory was not strongly affected by differences in incubation warming trajectories spanning approximately 4 °C. This result did not differ between 2018, when the rate of temperature increase was most rapid, and the other two study years. This suggests that the embryonic stage is tolerant of a range of warming regimes. Differences among years in winter snowpack, spring melting rates, river flows and air temperatures probably result in considerable variation in water temperatures at the spawning sites. Previous research has also indicated that walleye

embryo survival under laboratory conditions is not strongly influenced by temperature fluctuations during incubation, though wide fluctuations in temperature can induce developmental abnormalities that reduce post-hatch survival (Schneider et al. 2002). In contrast, survival of walleye embryos reared in incubators at a natural river spawning site was found to be higher under conditions of warmer temperatures and lower discharge (Rutherford et al. 2016). The range of incubation temperatures provided by my study was usually within the optimal range identified for walleye embryos (Koenst and Smith Jr 1976; Hokanson 1977). My incubation system was regulated to track mean daily temperature, but it did not follow the diurnal temperature cycles expected at natural spawning habitats. However, it is possible that daily water temperature fluctuations are lower in the interstitial spaces of spawning substrates where eggs generally settle than in the water mass above the spawning beds (Shepherd et al. 1986).

The effects of incubation temperature on embryonic survival appear to vary among species. For example, lake trout (*Salvelinus namaycush*) embryos, which incubate through fall and winter, can experience lower survival if winter temperatures remain elevated (Ostergaard 1987) or if they are subjected to prolonged warmer temperatures prior to the descent into winter temperatures (Casselman 1995). In lake whitefish (*Coregonus clupeaformis*), another species with winter incubation, embryo survival appears to be optimized at different incubation temperature regimes for different stocks (Brooke 1975; Mueller et al. 2015). A study assessing embryonic development in lake whitefish at constant incubation temperatures over a 6°C range found that the warmest treatment elicited higher mortality (Lim et al. 2017), suggesting that effects of incubation temperature on embryonic survival may be evident across wider contrasts in temperature.

I found that walleye embryonic survival varied considerably among female egg batches in all study years, and this is consistent with findings from several earlier studies (Hurley 1972; Johnston 1997; Johnston et al. 2005; Johnston et al. 2007; Harry 2015; Gatch et al. 2020). However, at least one study reported fairly uniform and high embryonic survival for all female walleye spawned over a single breeding season (Czesny et al. 2005), suggesting that among-female variability in embryo performance is not universal across all populations and/or spawning years. Offspring fitness in walleye depends on the genetic contributions of both parents, as well as the phenotypic contributions of the female, termed maternal effects. In fishes, male genotype effects have generally been found to be weaker than female effects (genotype + phenotype) during the embryonic period (Nagler et al. 2000) though some parental crosses are clearly less viable than others (Wedekind et al. 2001; Rideout et al. 2004). Because my focus was on maternal effects, I attempted to reduce the paternal influence by using pooled semen from a random set of six males on each spawn collection date. This approach was previously found to be effective in reducing variation due to semen source (T.A. Johnston, unpubl. data). After accounting for spawning date and temperature treatment effects, I attributed the remaining variation in embryo survival to maternal effects, though my experimental design did not allow me to separate phenotypic and genotypic contributions.

I predicted that older and/or larger female walleye would produce higher quality eggs, and thus, embryonic survival would be positively related to maternal age and size. This prediction was based on both life history theory (Belk and Tuckfield 2010) and the results of previous studies on walleye and other species (Johnston 1997; Trippel 1998;

Berkeley et al. 2004; Johnston et al. 2007). However, consistent with an earlier, smaller-scale study of Lake Nipissing walleye (Harry 2015), I found that embryonic survival was more strongly related to indices of ova size and composition than to maternal age and size. Positive relationships between embryonic survival and maternal age and size were only evident in the 2019 study year. I also found no evidence that the relationships between embryo survival and maternal and ova traits varied with respect to incubation temperature treatment in any consistent manner. The particular ova traits that accounted for the most variability in embryonic survival varied somewhat among study years and temperature treatments, but egg size, and relative proportions of ARA and EPA figured prominently.

Egg size and fatty acid composition are indices of the quantity and quality of resources, respectively, provided to individual offspring by the female. The mechanisms by which these particular traits may be influencing embryonic survival in walleye are not completely understood. Egg size tends to be positively related to female age and size in walleye, but the strength of this relationship as well as the population mean egg size are highly variable among walleye populations (Johnston and Leggett 2002). Egg size is not strongly correlated with female age or size in the Lake Nipissing walleye population (T.A. Johnston, unpubl. data). Egg size is a strong determinant of size at hatch in fishes (Miller et al. 1988) including walleye (Johnston 1997) and is believed to exert its greatest effect on post-hatch survival (Miller et al. 1988). Within walleye populations, smaller eggs have been found to have smaller yolk volumes and poorer fatty acid compositions, and to yield smaller larvae with lower post-hatch survival (Moodie et al. 1989). In earlier walleye incubation experiments, embryo survival to hatch was found to be negatively

related to egg size for Lake Nipissing walleye (Harry 2015) but positively related to egg size for a Lake Ontario walleye stock (Johnston et al. 2005). I found that the relationship between embryo survival and egg size for Lake Nipissing walleye was negative in 2017, non-existent in 2018, and positive in 2019. The reason for this shift in the egg size effect across years is not clear. Similarly, the effects of some fatty acid relative abundances also shifted among study years; negative effects of ARA and EPA were most evident in 2017 and 2018, but positive effects of DHA were most evident in 2019. Though the importance of *n*-3 and *n*-6 polyunsaturated fatty acids (PUFA) to early development in fishes is well-recognized (Sargent et al. 1995), their influence on embryonic survival is not always apparent (Czesny et al. 2005; Johnston et al. 2007). Fatty acids in walleye eggs are included in both the polar lipids, which primarily serve a structural role, and the neutral lipids, which primarily serve as energy storage, and the fatty acid composition tends to be more variable in the neutral fraction (Wiegand et al. 2004). As with egg size, walleye egg fatty acid composition is more strongly related to female age and size in some populations than in others (Wiegand et al. 2007). Because my study is, to my knowledge, the first to conduct egg rearing experiments with the same walleye population over multiple years it suggests that the effects of egg size and fatty acid compositions on embryo hatching success are highly variable.

Thermal units to 50% hatch (TU_{50})

The strong positive relationship between walleye embryonic development rate and water temperature has been recognized for over 50 years (Johnson 1961; Priegel 1970; Hurley 1972; Koenst and Smith Jr 1976; McElman and Balon 1979; Jones et al. 2003). I chose to examine developmental rates of walleye embryos with respect to thermal energy inputs using an index that combines both time and temperature. Growing degree-days (GDD), or thermal units in my study, is a measure that has been widely used in analysis of growth and development in plants and insects, and is increasingly being applied in research with other ectotherms, including fish (Neuheimer and Taggart 2007; Chezik et al. 2014). Previous research has indicated that the GDD required to complete embryonic development in fish may be relatively stable over the range of incubation temperatures typically encountered by a population (Neuheimer and Taggart 2007). I predicted this would also be the case for Lake Nipissing walleye, but found that my metric, TU_{50}, was more variable than expected. My results indicated that the nature of the incubation warming regime may influence the thermal energy needed to develop to hatch.

Both spawning date and the incubation temperature treatment influenced TU_{50} in my experiments in a somewhat complex manner. Overall, TU_{50} was lower in the later-spawning years, 2018 and 2019, than in 2017, and TU_{50} tended to increase with spawning date within years, except for embryos of the rapid-warming treatment. My TU_{50} estimates in 2017 were similar to what was recorded for Lake Nipissing walleye embryos incubated in a similar system with a relatively stable temperature regime (10-12 ˚C) in 2005 (T.A.Johnston, unpubl. data). Thus, it appears that the required TU_{50} is less in years when spawning begins late, but increases with spawning date within years, unless temperatures

warm rapidly. These patterns seem contradictory, but they are at different temporal scales (between years vs within years). One possible explanation for both patterns is that hatch is regulated in part by photoperiod, such that embryos will tend to hatch out at a lower or higher TU_{50} to more closely approach a preferred photoperiod, provided there are no developmental limitations to doing so. A rapid accumulation of GDDs seems to have more diverse outcomes; TU_{50} was most variable among years in the rapid-warming treatment. Reported GDD to hatch for lake trout embryos can also vary considerably among incubation temperatures both in the laboratory (Dwyer 1987) and at natural spawning sites (Casselman 1995).

I also found that TU_{50} was related to maternal and ova traits, though the strength and nature of these relationships differed from the analyses of embryo survival. Maternal and ova traits appeared to have their strongest effects on TU_{50} at slow- and seasonal-warming rates, and very little effect at rapid-warming rates. Furthermore, maternal age and size and egg size appeared to have stronger effects on TU_{50} than indices of egg lipid composition. The dominant trends were negative relationships between TU_{50} and maternal age, size, and egg size and these were consistent among study years. It is not clear why the embryos of older or larger females, or of females that produce larger eggs may require less thermal energy input to reach hatch, nor why rapid-warming conditions seem to obscure this effect. Previous studies reporting GDD to hatch for wild fish embryos have not analyzed results with respect to maternal and ova traits. Among-female variability in TU_{50} has the potential to modify patterns in hatch timing for a cohort.

The duration of incubation and hatch date may influence post-hatch survival and the year-class strength of a cohort. By sampling numerous females over a wide range of

spawn dates in each of three years and rearing their embryos over a range of temperatures I was able to demonstrate the potential variability in walleye hatch timing. Newly-hatched larval fishes are very susceptible to both predation and starvation mortality (Houde and Hoyt 1987) and the timing of hatch may influence both. Hatching that coincides with the spring increase in zooplankton production can enhance growth and reduce starvation mortality (Cushing 1990), and hatching in synchrony with many other fishes may provide some protection from predation by swamping the functional response of larval fish predators. At a finer temporal scale, hatching and dispersing from spawning beds at night appears to be a common strategy in fishes to avoid visual predators (Johnston et al. 1995; Bradbury et al. 2004).

Many organisms exhibit some control over hatch timing, responding to environmental cues presumably to optimize post-hatch survival (Warkentin 2011). The among-female variability that I observed in TU_{50} may reflect the inherent ability of walleye embryos to modify their hatch timing in response to environmental conditions. Hatch and emergence may vary between different types or locations of spawning habitat. On Lake Nipissing, I found that water temperatures warmed more slowly at a lake spawning site (Iron Island) than at a river spawning site (Wasi Falls) (Appendix 1, Table A2). Though the mean difference between sites was only 1 – 2 °C during the spawning and incubation periods, I estimated that this would result in embryos hatching 3 to 5 days earlier at the river spawning site (Appendix 1, Table A3). It is unknown if such a difference in hatch timing could influence the relative post-hatch survival from eggs spawned at these two sites, or if the relative success between sites may vary among years

according to particular environmental conditions. It is also unknown if TU_{50} of embryos may differ between sites, possibly modified by other environmental cues.

Other factors influencing embryonic survival and development and post-hatch survival

My focus was to examine the combined effects of temperature and maternal and ova traits on walleye embryo development and survival using an experimental approach. Numerous other factors may influence the fate of walleye offspring, both pre- and post-hatch, in experimental and natural settings. I reared walleye embryos in small batches on screened trays in a flow-through system to approximate conditions on natural spawning beds. Some previous experiments assessing maternal effects have reared all females' embryos combined in upwelling hatchery jar systems and determined the relative success of individual females through offspring genotyping (Johnston et al. 2005; Venturelli et al. 2010). It is not known if these different incubation methods impart different selective pressures upon females' embryos that may affect the experimental outcomes and interpretation of maternal effects. Water quality is another consideration. I reared Lake Nipissing walleye embryos in water from Ramsey Lake (Sudbury, Ontario) which is known to have elevated concentrations of Na and Cl from municipal road salting and metals from local smelter emissions. Fish embryo survival may be affected by dissolved salts, but generally only at concentrations higher than those observed in Ramsey Lake (Burnham and Peterka 1975; Weirich and Tiersch 1997). The effect of elevated waterborne metals concentrations (mostly Ni and Cu) on walleye embryos in my experiment is unknown, but by water-hardening the eggs using the original spawning site water I feel that I reduced any potential effects. I used filtered water in my incubation

experiments, but on natural spawning beds siltation may be a factor as relatively small silt accumulations can reduce walleye embryonic survival (Gatch et al. 2020). It is unknown if river or shoreline spawning sites differ in their susceptibility to siltation. Embryos on natural spawning sites may also be vulnerable to changes in flow rates and water currents, as well as a variety of egg predators (Ivan et al. 2010). Finally, it is possible that some maternal effects on embryonic development and survival in walleye are associated with maternal and ova traits not examined in my study. This may include various aspects of ova composition that contribute to ova quality (Brooks et al. 1997; Kamler 2005). However, it is also possible that the suite of maternal and ova traits most closely associated with offspring performance may differ among walleye spawning stocks according to the particular life history and nutritional trade-offs they make to meet the combined challenges of reproducing successfully and surviving to reproduce again.

Conclusions and Recommendations for Future Research

This study, using a Lake Nipissing walleye population, has provided insight into how seasonal variability, coupled with many female and egg attributes impact the hatching success and embryo development of walleye. My results suggest that embryo survival is not strongly dependent on maternal age or size but is strongly related to egg traits in this population, though clearly older and larger females produce more eggs (i.e. have higher fecundity). The implication for size-selective harvest regulations is that protection of the Lake Nipissing walleye spawning stock may only need to consider its total biomass and not its age and size composition. However, results from my analysis of

TU_{50} suggest that development and hatch timing are related to female age and size, and this may have implications for post-hatch survival. Because studies of the relationship between embryonic survival and maternal traits in wild fishes are relatively uncommon, and because results thus far appear to be mixed, future studies such as experiments with other walleye populations, including those supported by different food web structures and exploitation pressures should be used to determine under what conditions maternal effects are most evident. I also suggest using more in-situ incubation studies to determine if the patterns observed in the lab studies would be seen at natural spawning sites. I was able to show that embryonic survival is resilient to a variety of warming regimes within walleye's natural temperature range. Future studies, using a wider range of warming rates may be able to demarcate the limits of this resilience. My observation that spawn timing influenced embryonic survival, but only in the earliest spawn year, is interesting from a climate change perspective. The implication is that the variability in embryo success among spawn dates may increase if climate warming leads to earlier spawning periods. My results indicated that hatch timing as related to thermal inputs may not be as predictable under rapidly warming conditions. Future research should continue to investigate the relationships among embryonic development, incubation warming patterns and female and ova traits to determine how they may influence both timing and synchrony of hatching in a cohort. Overall, my research highlights the importance of interactive studies surrounding recruitment variability and supports the need for more research in early life history to ensure best management practices moving forward.

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Appendix 1. Thermal conditions and timing of walleye hatch at river and shoreline spawning sites on Lake Nipissing, 2016 - 2019

Background

The timing of spring hatch may influence the fate of larval fishes in various ways. Much of larval fish mortality is attributed to predation and starvation, and both of these processes could be affected by hatching time. Hatching early allows larval fish to begin growing sooner, thus giving them an earlier start over other competitors and predators. This may be particularly important for the offspring of piscivorous fish that must have a size advantage to switch their diet over to other young-of-the-year fishes early in the growing season. But, hatching early also carries risks. The availability of food organisms, primarily zooplankton, is low early in the growing season and this increases the probabilities of both starvation mortality and predation mortality for those larvae that emerge early. However, embryos on the spawning beds are also subject to predation mortality, and hatch timing may involve a trade-off between the relative risks of pre-hatch vs post-hatch mortality. Though the broad patterns of early life history are shaped by these ecological forces, environmental factors, particularly temperature, clearly influence early development at a finer scale.

My laboratory rearing experiments demonstrated that walleye embryonic development and the timing of hatch can vary considerably with both the spawning date and the incubation temperature regime. These may be related in part to the spawning habitat selected by the adults. Walleye will utilize riverine or lacustrine spawning habitats, and walleye populations of some larger lakes will spawn in both types of habitat. The ability to successfully reproduce in a diversity of habitats suggests that walleye may have a stronger ability to cope with natural fluctuations in environmental conditions. Depending on the hydrology of the waterbody river and lake spawning sites may reach

the preferred spawning temperature at different times in the spring and may provide different temperature regimes during incubation. This may lead to differences in the timing of emergence of walleye larvae from these spawning habitats.

In Lake Nipissing, walleye use both inflowing rivers and shoreline spawning locations, though it is unknown if the walleye spawning at these sites represent separate reproductive stocks. In most years, inflowing rivers are ice-free well before the main body of the lake, and spawning adults appear at the river spawning sites before ice has left the lake. This suggests that river sites warm faster than shoreline sites in the spring. River sites are also presumably more mixed, due to current, though lacustrine spawning grounds may also receive wind-generated currents. To my knowledge, there have not been any studies that have directly compared thermal regimes during walleye egg incubation between river and lake spawning sites.

My objective was to determine how temperature conditions during incubation may vary with spawning depth and spawning habitat and how this variability may influence the timing of walleye hatch. I achieved this objective by monitoring spring water temperatures at two natural spawning sites of Lake Nipissing walleye – a riverine site and a lake shoreline site – and interpreting the chronology of spawning, embryonic development and hatch from these temperature records and my incubation experiment results. I predicted that water temperatures would be warmer but also show greater diel variation at shallower depths and that this would be similar at both river and lake spawning sites. I also predicted that water temperatures would be warmer and more variable at the river spawning site than at the lake spawning site. Finally, using observed temperature trajectories in the field I estimated spawn and hatch timing at both sites.

Methods

Spawning site temperature data were obtained from a river-spawning site, Wasi Falls (46° 12’ N, 79° 22’ W), and a lake-spawning site, Iron Island (46° 26’ N, 79° 87’W) on Lake Nipissing. Continuous-recording (30-minute interval), Onset HOBO® TidbiT v2 water temperature data loggers were attached inside the cavity of concrete blocks and placed on spawning substrates at both locations from 2016 to 2019. In all years, two loggers were deployed at each site, at 2 m and 3 m depths in 2016 and 2017, and at 1 m and 2 m depths in 2018 and 2019. The loggers were placed in their respective locations soon after ice break-up; in all years this occurred earlier at Wasi Falls than at Iron Island. The loggers were retreived at least one week after the predicted completion of walleye hatch (generally, after 15 June) and data were downloaded. Loggers deployed at Wasi Falls at the 3 m depth in 2017, and at both the 1 m and 2 m depths in 2019 could not be found later and data were lost for these three placements.

Temperature data were first condensed to daily means and CVs for each logger time series. The range of dates used in subsequent analyses was selected to represent one hypothetical incubation period (spawn to hatch) at each individual logger placement. This was determined as follows. The first date (spawn date) was selected as the day when the mean daily temperature first exceeded 7 °C. This is roughly the median temperature at spawn for walleye sampled at Wasi Falls as part of my incubation experiments (Fig. 2). Cumulative growing degree-days (GDD) > 5 °C was then estimated daily from the spawn date forward. The final date (hatch date) was selected as the day when the cumulative GDD surpassed 120. This is roughly in the middle of the TU_{50} estimates I derived from my laboratory incubation experiments (Fig. 5). The duration of incubation was then estimated as the number of days from spawn to hatch.

I assessed the effects of spawning depth and spawning site on the daily mean and CV of water temperature over the incubation period using a paired-comparisons approach. Tests involved data from specified pairs of loggers; each test used data from all days (reps) that were common to both estimated incubation periods for the pair. Where distributions of paired-differences were found to be normal (based on Shapiro-Wilk test) I used a paired-comparisons *t*-test, and where found to be non-normal I used a Wilcoxon signed-rank test. These tests were carried out with R Studio 3.6.1, using t.test and wilcox.test in the 'PairedData' package. To assess spawning depth effects, comparisons were made between loggers at different depths at the same site for all site x year combinations. To assess spawning site effects, comparisons were made between loggers at different sites but the same depth for all depth x year combinations.

Results

Mean daily temperatures differed significantly with depth at both riverine and shoreline spawning sites (Table A1). With one exception (Iron Island site, 2016), temperature was greater at the shallower depth in the comparison, though the mean temperature difference over a 1 m depth gradient was generally small, ranging from 0.14 to 0.50 °C (Table A1). Contrary to my prediction, I found that daily variability in water temperatures, estimated as CV, did not vary significantly with depth at either the Wasi Falls or the Iron Island spawning sites (Table A1).

Temperature variation was more pronounced in comparisons between spawning sites than between depths within sites. As predicted, daily mean temperatures were significantly higher at the Wasi Falls riverine spawning site than at the Iron Island shoreline spawning site for all depths and years (Table A2). Mean daily temperatures at Wasi Falls were 1 – 2 °C warmer than at Iron Island on the same dates during the

incubation period (Table A2). However, contrary to my prediction, daily variation in water temperatures was not higher at the Wasi Falls site compared to the Iron Island site. In fact, there was a consistent trend of lower daily variation at the Wasi Falls site and this was statistically significant for the 2 m depth in both 2016 and 2017 (Table A2).

Differences in incubation temperatures between Wasi Falls and Iron Island led to differences in estimated spawn and hatch dates at these spawning sites. For my model incubation period (spawn at 7 °C, hatch after 120 GDD above 5 °C) spawn and hatch dates tended to be earlier at Wasi Falls than at Iron Island (Table A3). This was most pronounced in 2018. However, temperature loggers were deployed later than usual at Iron Island in 2018 and may not have captured the earliest date of mean daily temperatures above 7 °C, possibly leading to overestimation of spawn and hatch dates, and underestimation of incubation duration. Apart from this instance, the incubation period was generally 19 – 21 days in 2016, 2018 and 2019, but 26 – 29 days in 2017 (Table A3). For the three years with data from both sites, 2016 to 2018, walleye embryos were predicted to hatch 3 – 5 days earlier at Wasi Falls than at Iron Island (Table A3).

Table A1. Comparison of daily mean temperatures and daily CVs of temperature between different depths at two walleye spawning sites on Lake Nipissing, ON. Comparisons were made over the estimated incubation period common to both depths (16 - 29 days, depending on site and year) using a paired-comparisons t-test (paired-t) or a Wilcoxon signed-rank test (WSR).

Site	Year	Depth trend	Mean difference	Test	Statistic	df	P
			Daily mean temperature (°C)				
Wasi Falls	2016	2 m > 3 m	0.41	WSR	231	20	<0.001
Wasi Falls	2018	1 m > 2 m	0.28	paired-*t*	7.12	18	<0.001
Iron Island	2016	2 m < 3 m	-0.50	WSR	-5.23	20	<0.001
Iron Island	2017	2 m > 3 m	0.14	paired-*t*	4.96	28	<0.001
Iron Island	2018	1 m > 2 m	0.35	WSR	136	15	<0.001
Iron Island	2019	1 m > 2 m	0.28	paired-*t*	6.91	20	<0.001
			Daily CV of temperature (%)				
Wasi Falls	2016	2 m > 3 m	0.10	WSR	110	20	0.86
Wasi Falls	2018	1 m > 2 m	0.28	paired-*t*	0.79	18	0.44
Iron Island	2016	2 m > 3 m	0.98	paired-*t*	1.44	20	0.17
Iron Island	2017	2 m > 3 m	0.34	WSR	1.93	28	0.12
Iron Island	2018	1 m < 2 m	-0.29	WSR	60	15	0.71
Iron Island	2019	1 m > 2 m	0.78	WSR	162	20	0.11

Table A2. Comparisons of daily mean temperatures and daily CVs of temperature between two walleye spawning sites on Lake Nipissing, ON. Wasi Falls (Wasi) is a river spawning site and Iron Island (Iron) is a shoreline spawning site. Comparisons were made at fixed depths over the estimated incubation period common to both sites (11 - 26 days, depending on depth and year) using paired-comparisons t-tests.

Depth	Year	Site trend	Mean difference	*t*	df	P
			Daily mean temperature (°C)			
2 m	2016	Wasi > Iron	2.00	8.69	16	<0.001
3 m	2016	Wasi > Iron	1.03	4.35	18	<0.001
2 m	2017	Wasi > Iron	0.96	8.06	25	<0.001
1 m	2018	Wasi > Iron	1.67	5.62	10	<0.001
2 m	2018	Wasi > Iron	1.68	5.19	11	<0.001
			Daily CV of temperature (%)			
2 m	2016	Wasi < Iron	-2.32	-3.70	16	0.002
3 m	2016	Wasi < Iron	-1.16	-1.33	18	0.20
2 m	2017	Wasi < Iron	-1.54	-3.64	25	0.001
1 m	2018	Wasi < Iron	-1.70	-1.31	10	0.22
2 m	2018	Wasi < Iron	-1.83	-1.62	11	0.13

Table A3. Spawn date, hatch date and incubation time of walleye embryos estimated using temperature monitoring data collected at a riverine spawning site (Wasi Falls) and a shoreline spawning site (Iron Island) on Lake Nipissing, 2016 - 2019. Spawn date was estimated as the first day of the year with a mean water temperature > 7 °C, and hatch date was estimated as the date where cumulative growing degree-days > 5 °C following spawn surpassed 120 (see text).

Year	Depth	Spawn Site	Estimated spawn date (Day of year)	Estimated hatch date (Day of year)	Estimated days to hatch
2016	2 m	Wasi Falls	2 May (123)	22 May (143)	20
2016	2 m	Iron Island	6 May (127)	27 May (148)	21
2016	3 m	Wasi Falls	2 May (123)	23 May (144)	21
2016	3 m	Iron Island	5 May (126)	26 May (147)	21
2017	2 m	Wasi Falls	27 April (117)	23 May (143)	26
2017	2 m	Iron Island	28 April (118)	26 May (146)	28
2017	3 m	Iron Island	28 April (118)	27 May (147)	29
2018	1 m	Wasi Falls	7 May (127)	26 May (146)	19
2018	1 m	Iron Island	16 May (136)	31 May (151)	15
2018	2 m	Wasi Falls	8 May (128)	27 May (147)	19
2018	2 m	Iron Island	16 May (136)	31 May (151)	15
2019	1 m	Iron Island	11 May (131)	1 June (152)	21
2019	2 m	Iron Island	12 May (132)	2 June (153)	21

Conclusions

My analysis of spring water temperatures at walleye spawning sites on Lake Nipissing has provided new information on the spatio-temporal variability of environmental conditions at embryonic habitats and this has implications for understanding potential variability in walleye embryonic development and hatch timing.

The observed variation in mean daily temperatures was greatest between the river and shoreline spawning sites, and less between depths within spawning sites. The pattern of higher temperatures at the river site confirmed my expectation that river inflows warm faster than shoreline sites in Lake Nipissing. The warmer temperatures at river sites probably result from radiant warming of the drainage basin landscape supplying the surface runoff to the river. The landscape warming, and runoff warming, probably accelerates after snow cover is lost in the spring. Thus, it is possible that the difference in warming patterns between these sites may depend on snow and ice conditions in the drainage basin at the end of winter. For example, a cold but dry winter could result in heavy ice on the lake but limited snowpack on the drainage basin, leading to more rapid warming in the river but slower warming in the lake and a greater temperature differential between river and shoreline spawning sites. The pattern of river warming may also depend on characteristics of its drainage basin. Catchments with extensive forest cover may experience slower snow melt and more gradual warming of their spring run-off than those that have been partially cleared. Future research monitoring temperature trends at multiple river and shoreline spawning sites around Lake Nipissing would be useful for determining the generality of my results.

In addition to differences in mean daily temperatures, I also predicted that the daily variation in temperature would be stronger at the river spawning site. This prediction was based on the expectation that the warming of the water flowing off the

drainage basin landscape would vary more from day to night than the warming of the lake's surface. Contrary to my prediction, I found that daily temperature fluctuations were slightly higher at the Iron Island shoreline spawning site than at the Wasi Falls river spawning site. It is possible that the mixing dynamics of the river provide greater homogeneity in temperatures over the course of a 24-h cycle. Walleye embryos would be expected to tolerate typical diel variations in temperature, but it is unknown how their development may be affected by more drastic fluctuations in temperature.

The observed differences in mean daily temperature between the river and shoreline spawning sites were 1 to 2 °C. Although these are relatively small temperature differences, my incubation scenario calculations indicated that they would be accompanied by differences in hatch timing of 3 to 5 days between sites. Though these simple calculations are useful for illustrating the potential variability in incubation trajectories between these sites, the situation is likely more complex. As my spawn collection records have shown, in a given year walleye will spawn over 1 to 2 weeks over a wide range of water temperatures, not just 7 °C, and as my incubation experiments have shown, walleye embryos will hatch over a wide range of thermal inputs, not just 120 GDD. This diversity in spawn timing and embryonic development, combined with observed temperature differences between spawning sites, could generate considerable diversity in hatch timing.

There are other possible differences between river and lake shoreline spawning sites that could influence the fate of developing embryos. First, other environmental factors such as water movement and siltation probably differ between these habitats and this could differentially influence embryo development and hatch. Second, there may be biological differences between these sites such as in the abundance of egg predators. Another interesting possibility is that the subsets of adult walleye that choose to spawn at

these two types of habitat may exhibit subtle ecological differences, such as in spawning temperature preferences, and the developmental rates of their embryos.

Appendix 2. Fatty acid compositions of ova total lipids for Lake Nipissing walleye, 2017 - 2019

Table A4. Summary of relative abundances of selected fatty acids (percentages of total fatty acids by weight) and fatty acid ratios in ova lipids of individual female Lake Nipissing walleye sampled at Wasi Falls in spring 2017. Analyses completed at University of Winnipeg (Murray Wiegand Lab).

	Individual FAs					Ratios	
Female #	**PLA, 16:1(n-7)**	**LNA, 18:2(n-6)**	**ARA, 20:4(n-6)**	**EPA, 20:5(n-3)**	**DHA, 22:6(n-3)**	**EPA / AA**	**DHA / EPA**
251	13.65	3.35	5.39	4.27	18.65	0.79	4.37
252	10.83	4.72	6.26	5.81	17.72	0.93	3.05
253	12.17	5.08	5.46	4.49	18.37	0.82	4.09
254	11.12	5.57	4.85	4.81	18.74	0.99	3.89
255	10.88	4.55	5.58	5.38	18.19	0.96	3.38
256	12.59	5.46	4.93	4.47	16.32	0.91	3.65
257	12.57	4.10	5.13	6.07	20.67	1.18	3.40
258	10.44	4.91	5.92	6.09	18.83	1.03	3.09
259	11.13	4.78	5.68	5.65	19.65	1.00	3.48
260	13.63	3.41	4.42	4.62	18.28	1.04	3.96
262	11.60	5.31	5.48	4.53	17.52	0.83	3.87
263	11.98	4.07	5.09	5.89	20.52	1.16	3.48
264	10.69	4.90	5.48	6.04	18.64	1.10	3.09
265	12.28	3.96	4.57	4.88	19.26	1.07	3.94
266	11.47	4.77	5.66	6.26	17.96	1.10	2.87
267	11.98	3.66	5.88	5.14	22.83	0.87	4.44
268	11.44	3.52	4.89	4.88	20.76	1.00	4.25
269	11.92	4.49	5.36	5.42	17.86	1.01	3.29
270	12.97	4.39	6.10	5.41	17.23	0.89	3.18
271	13.17	3.90	5.19	4.86	17.15	0.94	3.53
272	8.92	5.27	5.48	6.93	20.31	1.27	2.93
273	11.15	4.67	5.51	6.50	19.59	1.18	3.01
274	12.75	3.19	5.07	9.36	17.61	1.85	1.88
275	12.96	3.81	5.67	4.80	17.99	0.85	3.75
276	10.42	4.34	5.61	6.27	20.76	1.12	3.31
Means	**11.79**	**4.41**	**5.39**	**5.55**	**18.86**	**1.04**	**3.49**

Table A5. Summary of relative abundances of selected fatty acids (percentages of total fatty acids by weight) and fatty acid ratios in ova lipids of individual female Lake Nipissing walleye sampled at Wasi Falls in spring 2018. Analyses completed at University of Winnipeg (Murray Wiegand Lab).

	Individual FAs					Ratios	
Female #	**PLA, 16:1(n-7)**	**LNA, 18:2(n-6)**	**ARA, 20:4(n-6)**	**EPA, 20:5(n-3)**	**DHA, 22:6(n-3)**	**EPA / AA**	**DHA / EPA**
251	13.75	2.35	3.98	4.80	18.70	1.21	3.89
252	12.98	3.55	4.86	5.23	23.98	1.08	4.58
253	12.53	4.98	5.37	6.50	18.89	1.21	2.90
254	11.68	3.78	5.61	4.62	20.68	0.82	4.48
255	13.77	3.29	4.39	2.91	19.89	0.66	6.83
256	14.55	2.74	3.48	5.65	17.97	1.62	3.18
257	10.29	5.96	5.72	6.50	16.36	1.14	2.52
258	12.42	3.77	4.31	5.12	19.93	1.19	3.90
259	11.77	3.94	4.85	4.84	20.94	1.00	4.33
260	10.27	5.81	5.88	6.76	17.13	1.15	2.54
261	11.85	3.42	4.30	6.01	20.43	1.40	3.40
262	11.25	4.33	5.68	6.48	19.48	1.14	3.01
263	10.67	5.74	5.92	6.29	20.63	1.06	3.28
264	12.56	3.55	4.52	6.07	20.21	1.34	3.33
265	12.27	3.68	4.41	4.35	18.40	0.99	4.23
266	10.47	6.60	5.72	6.33	17.75	1.11	2.80
267	14.46	4.08	5.13	4.74	22.54	0.92	4.75
269	10.86	4.16	4.54	7.86	22.57	1.73	2.87
270	12.08	3.95	4.27	4.59	21.90	1.07	4.77
271	11.24	4.99	5.33	5.29	19.48	0.99	3.68
Means	**12.09**	**4.23**	**4.91**	**5.55**	**19.89**	**1.14**	**3.76**

Table A6. Summary of relative abundances of selected fatty acids (percentages of total fatty acids by weight) and fatty acid ratios in ova lipids of individual female Lake Nipissing walleye sampled at Wasi Falls in spring 2019. Analyses completed at Ryerson University (Michael Arts Lab).

	Individual FAs					Ratios	
Female #	**PLA, 16:1(n-7)**	**LNA, 18:2(n-6)**	**ARA, 20:4(n-6)**	**EPA, 20:5(n-3)**	**DHA, 22:6(n-3)**	**EPA / AA**	**DHA / EPA**
251	19.35	3.34	4.27	3.19	13.86	0.75	4.34
252	18.32	3.25	4.40	4.56	17.82	1.04	3.91
253	15.55	3.37	4.37	4.19	19.68	0.96	4.70
254	16.20	5.02	4.55	6.26	12.95	1.38	2.07
255	17.33	4.13	4.55	4.76	16.86	1.05	3.54
256	17.08	3.87	4.71	4.94	20.30	1.05	4.11
257	17.10	4.35	3.63	3.69	18.69	1.02	5.06
258	20.91	3.73	3.58	2.27	15.89	0.63	7.00
259	17.73	3.27	2.78	2.64	15.50	0.95	5.86
260	12.80	2.80	4.29	5.02	23.51	1.17	4.68
261	15.47	2.51	3.00	3.24	17.89	1.08	5.51
262	17.73	4.33	3.47	3.32	15.70	0.96	4.73
263	18.86	3.35	3.25	2.85	15.02	0.88	5.27
264	16.58	3.53	3.84	3.77	18.47	0.98	4.90
265	18.18	1.99	3.83	2.73	17.80	0.71	6.52
266	17.93	3.61	3.06	2.93	16.01	0.96	5.46
267	16.38	4.55	4.17	7.96	23.36	1.91	2.93
268	17.49	2.84	3.62	3.63	17.90	1.00	4.93
269	16.57	3.60	3.04	3.23	16.92	1.06	5.25
270	14.24	3.79	3.70	6.23	22.11	1.68	3.55
271	18.03	2.32	3.13	2.90	16.53	0.93	5.70
272	17.32	3.55	4.32	3.75	17.19	0.87	4.58
273	18.27	2.98	3.57	3.29	16.96	0.92	5.15
274	15.49	4.88	4.27	7.26	19.38	1.70	2.67
275	16.96	3.40	3.97	6.57	19.00	1.65	2.89
276	12.72	4.69	4.27	6.24	23.97	1.46	3.84
277	12.41	3.80	3.84	6.22	23.63	1.62	3.80
278	15.74	2.69	4.11	4.22	17.52	1.03	4.15
Means	**16.74**	**3.55**	**3.84**	**4.35**	**18.23**	**1.12**	**4.54**