DR. TOM JOHNSTON (Orcid ID : 0000-0003-2414-1724)

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Maternal effects on embryonic development and survival in walleye of Lake Nipissing, Ontario

T.A. Johnston [1*], A.J. Harry [2], R.D. Montgomerie [3], M.D. Wiegand [4], G.A. Spiers [5], J.M. Casselman [3], and W.C. Leggett [3]

[1] Ontario Ministry of Northern Development, Mines, Natural Resources and Forestry, Cooperative Freshwater Ecology Unit, Laurentian University, Sudbury, ON P3E 2C6, Canada

[2] Department of Biology, Laurentian University, Sudbury, ON P3E 2C6, Canada

[3] Department of Biology, Queen's University, Kingston, ON K7L 3N6, Canada

[4] Department of Biology, University of Winnipeg, Winnipeg, MB R3B 2E9, Canada

[5] Department of Earth Sciences, Laurentian University, Sudbury, ON P3E 2C6, Canada

* Corresponding author: Tom Johnston, Tel: 705-671-3828, E-mail: tom.johnston@ontario.ca

Abstract

Life history theory predicts that females may increase reproductive allocation with advancing age as the probability for future reproduction diminishes. In iteroparous fishes, increasing age is usually accompanied by increasing fecundity but evidence of increasing offspring quality is less consistent. We examined the developmental rate and survival of walleye embryos with respect to female age, size, condition, and various ova traits in an exploited stock dominated by young spawners. Embryo batches from individual females were collected in the field on multiple dates and reared to hatch under controlled conditions in both flow-through and static incubation systems. Survival and thermal inputs to hatch (TU_{50}) were analyzed with respect to spawn date, incubation method, and both maternal and ova traits. Spawning date had a relatively strong effect on embryo survival but only a minor effect on TU_{50}, whereas incubation method had a small effect on survival but a stronger effect on TU_{50}. Embryos reared in static incubators required greater thermal inputs to reach hatch. Using a model selection approach to assess the effects of maternal and ova traits, we found that neither maternal age nor size were strong predictors of survival or TU_{50}. Instead, survival was more strongly related to egg size and fatty acid composition, and TU_{50} was not strongly related to any maternal or ova trait. Our results suggest that the nature and magnitude of maternal effects on early development and survival may vary among walleye stocks, modified by physical conditions during incubation.

Keywords: reproduction, egg quality, growing degree-days, age, fatty acids, metals

Introduction

Many economically valuable fish populations exhibit considerable temporal variability in abundance, a phenomenon that both intrigues population ecologists and challenges fisheries managers (Hilborn and Walters 1992). These fluctuations in abundance are often attributed to variation in recruitment, the net effect of births, survival, and growth of young individuals into the adult stock. Recruitment variability has been associated with a wide range of biotic and abiotic factors that are purported to influence population dynamics at various levels (Johnston et al. 2016). For many fish species the relationship between recruitment and the number of offspring produced each year (the latter usually inferred from female abundance) tends to be highly variable, suggesting that recruitment may be influenced more by offspring survival than the total numbers produced. In particular, factors influencing survival early in life are believed to be key (Houde 1987; Chambers and Trippel 1997). Survival during the earliest life stages may be related to the quality of eggs produced by the females, an influence known as a maternal effect (Bernardo 1996a; Green 2008). If the quality of eggs produced is in turn related to characteristics of the female, then recruitment may depend on the composition of the spawning stock. It is thus of interest to know which females are producing the highest quality eggs.

There is a growing recognition that the older and larger females in spawning stocks may be contributing disproportionately to recruitment because of the relatively higher quantity and/or quality of eggs and offspring they produce (Venturelli et al. 2009; Hixon et al. 2014; Barneche et al. 2018), sometimes called the BOFFFF (big old fat fecund female fish) effect (Hixon et al. 2014). This has clear implications for fisheries management because harvest is often size-selective and can shift spawning stock composition towards younger and smaller fish (Longhurst 2002; Hsieh et al. 2010), potentially destabilizing population dynamics (Anderson et al. 2008). In iteroparous species, an increasing reproductive investment with successive spawning seasons, and hence age, is expected as the probability for future reproduction declines (Pianka and Parker 1975; Clutton-Brock 1984; Belk and Tuckfield 2010). But is this theory supported by empirical evidence? Maternal fitness ultimately depends on the number of offspring produced and the mean fitness of the offspring (Stearns 1992;

Roff 2002), with the trade-off in quantity vs quality presumably somewhat flexible. Females could increase their reproductive investment through their adult years by increasing fecundity, egg quality, or both. Though positive relationships between fecundity and female size and age have been found to be strong and consistent across many fish stocks, relationships between egg and offspring quality and female size and age appear to be somewhat more variable (Kamler 2005). Because offspring survival in fishes tends to be positively related to egg size (Miller et al. 1988), there has been considerable research on the relationship between egg size—and to a lesser extent egg biochemical composition—and female age and size (Chambers and Leggett 1996; Kamler 2005; Rollinson and Hutchings 2010; Johnston 2018). Studies that have directly measured the relationship between early life survival and maternal age and size are less common (Trippel 1998; Keckeis et al. 2000; Berkeley et al. 2004; Johnston et al. 2007).

Walleye, *Sander vitreus,* is the most targeted and economically valuable freshwater fish species in Canada. It is an iteroparous, relatively r-selected (high fecundity, small eggs), broadcast-spawning piscivore that uses shallow gravel and rock substrates in rivers and lakes as spawning grounds in the spring (Colby et al. 1979; Bozek et al. 2011). Growth and maturation in walleye vary widely over its native range, with southern populations growing faster and maturing younger than northern populations (Colby and Nepszy 1981; Baccante and Colby 1996). Male and female walleye in central Ontario generally reach maturity at two to three years old, and four or five years old, respectively (Henderson and Morgan 2002). Recruitment in many walleye populations can show wide temporal variation and recent studies suggest that stronger recruitment may be associated with higher proportions of older and larger females in the spawning stock (Venturelli et al. 2010; Shaw et al. 2018). Older and larger female walleye tend to produce larger eggs, and hence larger offspring, though this trend is quite variable among populations (Johnston and Leggett 2002; Wang et al. 2012; Feiner et al. 2016). Similarly, survival through the embryonic period has been found to be positively related to maternal age and/or size in some walleye populations (Johnston 1997; Johnston et al. 2007) but not in others (Czesny et al. 2005; Gatch et al. 2020). Though not well-studied, maternal effects leading to the production of higher quality eggs might also influence recruitment.

The objective of this study was to build upon earlier research on the relationship between offspring performance and maternal characteristics of walleye using controlled laboratory incubation

of egg batches from individual females sampled from the wild. Our working hypothesis was that embryonic survival would be positively related to maternal age, and to a lesser extent, related to other indices of maternal and ova quality. Based on earlier research that found the maternal age effect on survival was most pronounced among young spawners in walleye stocks from Lake Manitoba and Lake Ontario (Johnston et al. 2007), we chose to focus on a walleye stock primarily composed of younger and smaller females for the current study. In addition to embryo survival, we also examined the influence of maternal effects on embryo developmental rates as this may influence the timing of hatch and post-hatch survival. We also tested two different rearing environments to explore the potential for methodological influences on our results.

Methods

Study site and spawn collection

Lake Nipissing is a large (surface area 873 km^2) mesotrophic lake in central Ontario, Canada. Long fetches and shallow depth (mean 4.5 m) allow extensive mixing such that the lake is isothermal over most of its area (Neary and Clark 1992). The Lake Nipissing fish community includes over 40 fish species with walleye as the dominant apex piscivore (Morgan 2013). The fish community, and walleye in particular, has a long history of supporting subsistence, recreational, and commercial fisheries (Anthony and Jorgensen 1977; Morgan 2013). Lake Nipissing's walleye population has been subjected to heavy and sustained exploitation, such that it has been overfished in recent decades (Morgan 2013; Zhao and Lester 2013). Fisheries-independent surveys of the lake have shown that the walleye population has a relatively truncated age structure, and the spawning stock is dominated by younger age classes (Ontario Ministry of Natural Resources and Forestry, North Bay District, *unpubl. data*).

Mature walleye were sampled from the Wasi Falls spawning site at the eastern end of Lake Nipissing (46° 17' N, 80° 00' W). The Wasi River descends over a steep waterfall (impassable to fish) as it enters Lake Nipissing, and spring-spawning species use rubble substrate in the river plume at this site. Thus, though the spawning site is within the lake, the spawning habitat is considered

riverine. Ovulated female (ova free-flowing) and ripe male (semen free-flowing) walleye used for this study were sampled on 28 April, 30 April, and 2 May 2005 (total 23 females and 30 males). Water temperatures in the river plume over this period ranged from 6 to 8˚C. Fish were captured in an 8-foot trapnet that was set near the base of the waterfall in the evening and emptied the following morning. Both ovulated and unovulated female walleye were in the trap net catches on each of these dates, indicating that the timing of sampling was near the middle of the walleye spawning period.

Both males and females used for our experiment were selected haphazardly, except with respect to body size; an attempt was made to cover the full size range of ovulated females and ripe males from the trap net catch on each sampling date. Selected individuals of both sexes were dried with a towel and then stripped of gametes by applying gentle pressure along their flanks. Gametes were collected into sterile containers (plastic petri dishes for semen, 100 mL plastic screw-cap jars for ova) taking care to avoid contamination with water, faeces, or epithelial slime. Two unique semen pools (no males common between them) were created on each sampling date by pipetting 2 mL of semen from each of five males into a common sterile petri dish then swirling gently for 1 min to homogenize. These semen pools were used for all fertilizations on the date of collection. Ova samples from each female were immediately subdivided into those used for fertilization and those used for analysis of size and composition. On 28 April, a single batch of ova (20 mL) from each female was retained for fertilization, but on 30 April and 2 May two batches of ova (20 mL each) from each female were retained for fertilization. These ova batches were placed into sterile 100-mL plastic screw-cap jars that served as fertilization containers. The remaining ova from each female were transferred into two plastic Whirl-pak bags (~30-40 mL in each). All collected gametes were placed over (but not touching) wet ice in a cooler and transferred along with Wasi River water to a nearby laboratory for fertilization within 2 - 4 h of collection. All male and female walleye that were sampled for gametes were euthanized by a sharp blow to the head, packed on wet ice and transported to the laboratory for further processing.

Fertilization and water-hardening were performed as follows. Each batch of 20 mL of ova in a 100-mL jar received 0.5 mL of semen delivered by pipette, followed by 40 mL of Wasi River water. The jar was then immediately capped, gently swirled for 5 s, and allowed to stand. After 15 min, the

fertilized eggs were poured into a stainless steel bowl, another 100 mL of Wasi River water was added, the eggs were gently stirred with a feather, then left to stand again. Thereafter, the eggs were gently stirred at 15-min intervals to reduce adhesion and clumping as they hardened, and an additional 250 mL of Wasi River water was added at each of 30 min and 60 min post-fertilization. Two hours after fertilization, the eggs were drained and rinsed in Wasi River water, and ~20 mL of water-hardened eggs from each batch were transferred to 250-mL plastic screw-cap jars along with 200 mL of Wasi River water. These jars were placed in a cooler and transported to the incubation facility. Eggs were checked several times during transport to ensure no further adhesion or clumping.

Embryo incubation

Embryos were incubated to hatch in a controlled-environment (CE) room at the Department of Biology Animal Holding Facility of Queen's University (Kingston, Ontario). The CE room was supplied with dechlorinated municipal water, and lighting was maintained at low levels on a 16:8 light:dark cycle to approximate seasonal environmental conditions at Lake Nipissing. To test the effects of water circulation on embryos, we used two incubation systems, flow-through and static, both set up in the same CE room. The flow-through system was composed of a long, wide, and shallow stainless steel trough with water maintained at a depth of ~8 cm by a stand-pipe at the outflow. The receiving reservoir for the trough outflow was aerated and contained a submersible pump that recirculated water back to the opposite end of the trough. Fresh, dechlorinated water was added to the system at a replacement rate of 50% per day, and water temperature was controlled by room temperature (10-12˚C throughout experiment). Embryos were placed in incubation tubes constructed of 10-cm long sections of 7.5-cm diameter PVC pipe placed vertically in the trough. The submerged end of the tube was covered with 1.0 mm Nitex mesh (on which the eggs rested) and had three 2-cm PVC legs attached to keep it suspended above the bottom of the trough to allow water to circulate. One hundred, individually-numbered tubes were placed in the trough in a grid pattern. The static incubation system was composed of 120 individually numbered, glass dishes (~6-cm diameter) placed in a grid pattern on the bottom of an empty trough, identical to the one used for the flow-through system. Water from the aeration reservoir of the flow-through system was added manually to

these dishes to a depth approximately twice that of walleye egg diameter and was replaced once daily throughout the incubation period.

Jars of fertilized and water-hardened eggs brought from the field site were placed in the incubation room and allowed to acclimate (~10 ˚C). Two random subsamples (~200 each) of eggs were examined under a dissecting scope and fertilization success was estimated as the mean percentage of eggs that had a normally-developing blastula. From each egg batch 70 successfully-fertilized eggs were counted into each of two randomly selected incubation tubes (flow-through system), and two randomly selected incubation dishes (static system) at ~24 h post-fertilization. Embryos were monitored and mortalities counted and removed daily until hatch. Embryo survival was estimated as the percentage of embryos in each incubation chamber that successfully hatched. Hatchling viability was estimated as the percentage of hatched embryos in each incubation chamber that exhibited normal body form and swimming behaviour 24 h after hatch.

Laboratory analyses

Adult walleye and unfertilized ova samples were processed as follows. For each female, one bag of ova was frozen at –20˚C for determinations of egg size, total lipid content, and metal composition. The second bag of ova was frozen at –70˚C for analysis of fatty acid composition. Adults were processed following previously published protocols (Johnston et al. 2007; Moles et al. 2008). Briefly, fork length (±1 mm), somatic mass (body minus gonads, ±1 g), and liver mass (±1 g) were measured for all fish. Sagittal otoliths were removed for age determinations, and a subsample of liver was frozen in Whirl-pak plastic bags at –20˚C for lipid analysis. Carcasses (soma) were frozen following processing but were later thawed, cut into pieces and homogenized using a commercial meat grinder. Aliquots of the soma homogenate of each fish were frozen in Whirl-pak plastic bags at –20˚C for lipid analysis. All frozen tissue samples, except ova frozen at –70˚C, were freeze-dried (Labconco FreeZone 12) for seven days. Three replicates of 30 freeze-dried ova from each female were weighed (±0.1 mg) to determine mean egg sizes. All freeze-dried tissues were then ground to a fine powder in a ball mill (Retsch MM 400).

Most of the tissue analysis methods for the female walleye of this study have been described previously. Walleye ages were determined at Queen's University by counting annuli on polished otolith sections (Wiegand et al. 2007). Lipid concentrations of all freeze-dried tissues (ova, liver, soma homogenate) were determined at Laurentian University (Sudbury, ON, Canada) using a gravimetric chloroform-methanol extraction procedure (Moles et al. 2008). Fatty acid compositions of total lipid extracts from the ova samples stored at –70˚C were determined at the University of Winnipeg (Winnipeg, MB, Canada) by flame ionization gas chromatography, and individual fatty acids were expressed as relative abundances (proportion of total fatty acids) (Wiegand et al. 2007). Metal concentrations of freeze-dried ova were determined at Laurentian University by inductively-coupled plasma mass spectrometry (ICP-MS) following hot-block digestion of ~0.5-g subsamples in nitric acid and hydrogen peroxide (Ashoka et al. 2009) and were expressed as dry mass concentrations.

Statistical analyses

Our primary dependent variables of interest were percent embryo survival from fertilization to hatch and thermal units to 50% hatch (TU_{50}). 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 before hatching began. The duration of incubation was then estimated as the number of days from fertilization to the date of 50% hatch. Our index of developmental rate (thermal units to 50% hatch, TU_{50}) was estimated as the cumulative growing-degree-days above 5°C by summing mean daily water temperatures above 5°C from 24-h post-fertilization to the date of 50% hatch. Embryo survival was transformed as logit (survival – 1) in all statistical analyses to normalize residuals.

We selected predictor variables for our models based on previous research indicating their potential importance in female reproductive allocation, oogenesis, and embryo development and survival. Maternal traits included ontogenetic factors (age, fork length, soma mass), and indices of

energetic condition (hepatosomatic index, liver lipid concentration, soma lipid concentration). Ova traits included egg size (mean mass per ovum) and lipid concentration, as well as measures of egg lipid fatty acid composition and metal concentrations. Fatty acid variables included the relative abundances of three long-chain polyunsaturated fatty acids (PUFA) — arachidonic acid (ARA, 20:4*n*-6), eicosapentaenoic acid (EPA, 20:5*n*-3), and docosahexaenoic acid (DHA, 22:6*n*-3) — and two ratios derived from these fatty acids (EPA:ARA and DHA:EPA). These fatty acids are essential for development and growth of all teleosts (Sargent et al. 1999; Tocher 2010). We also included the relative abundance of linoleic acid (LNA, 18:2*n*-6) based on preliminary examination of bivariate scatter plots between dependent variables and individual fatty acids. Metals analyses of the walleye ova provided concentrations for 15 metals of which we considered seven to be nutritionally essential (Cr, Cu, Fe, Mg, Mn, Se, Zn; (Lall 2002; Wood et al. 2011).

Most statistical analyses were carried out using SAS/STAT® procedures (SAS Institute Inc 2013). We first tested for semen pool effects on embryo survival and TU_{50} using mixed-effects ANOVAs (GLM procedure) with semen pool and incubation treatment (flow-through, static) as fixed effects and female identity as a random effect, with separate analyses for each spawning date. Effects of spawning date (categorical variable) were tested by one-way ANOVA with separate analyses for each incubation treatment. Finally, effects of incubation treatment were tested by paired-comparison tests.

Analysis of embryo survival and TU_{50} in relation to maternal and ova traits followed a model selection approach (Johnson and Omland 2004; Anderson 2008; Symonds and Moussalli 2011). We first examined the nature and strength of variation in dependent variables with respect to independent variables by examining bivariate scatter plots and simple linear regressions (GLM procedure). Based on this preliminary analysis some predictors were transformed to linearize relationships, and some were eliminated if they were deemed weak, or too strongly correlated with other predictors. Predictor variables were converted to Z-scores (STANDARD procedure) prior to model fitting to reduce scaling effects. We then fitted and ranked linear models of female means of the dependent variables as functions of all one and two predictor combinations (GLMSELECT procedure). Models with more than two predictors were not considered, given the relatively low sample size of the data set (n = 23

females). Fitted models were ranked by the corrected Akaike's Information Criterion (AICc). We compared models with and without spawn collection date (categorical variable) included as an additional fixed effect and retained this effect if it resulted in higher rank for most models. Relative strengths of models were inferred from Akaike weights (w_i), and relative strengths of predictors were inferred from means and 95% confidence limits of standardized model coefficients calculated by full model averaging using the MuMIn package (Bartoń 2020) in R version 4.0.1 (R Core Team 2021).

Results

Experimental summary

Females used for the breeding experiment ranged from 5 to 11 years old, and from 382 to 569 mm FL (Table 1). Sampled males were of a similar age (range 3 – 12 years) but generally smaller than the females (range 314-500 mm FL). Our sample of females was skewed towards younger age classes, with six 5-year-olds, eight 6-year-olds, and only two females of age 10 or greater. With respect to ova traits, egg size was more variable among females than egg lipid concentration, and EPA was the most variable egg essential fatty acid among females (Table 1). Following preliminary analyses we reduced the number of female and ova traits used in subsequent analyses, omitting female soma mass and lipid concentration, and retaining only two of the essential metals, Mn and Zn. Correlation analysis among the 14 predictors retained for model building indicated that indices of fatty acid composition were the only ova traits that correlated significantly with maternal age and size (Table 2). Egg size was significantly correlated only with indices of liver status and egg Zn content, whereas egg total lipid content was not significantly correlated with any maternal or ova traits (Table 2).

For the two spawn dates where duplicate semen pools were compared, differences in embryo survival (logit-transformed) between pools were negligible. There was no significant interaction between semen pool and incubation treatment on either the 30 April (ANOVA, $F_{1,9} = 0.27$, $P = 0.62$) or the 2 May ($F_{1,18} = 0.40$, $P = 0.53$) spawn dates, and following removal of this term, there was no significant effect of semen pool on either the 30 April (ANOVA, $F_{1,10} = 2.24$, $P = 0.17$) or the 2 May

($F_{1,19}$ = 3.45, P = 0.08) spawn dates. Similarly, for analyses of TU_{50}, there was no significant semen pool by incubation treatment interaction on either spawn date (P > 0.39), and there were no significant semen pool effects (P > 0.53) following interaction term removal. Data were combined across semen pools for all subsequent analyses.

Embryonic survival

Embryo survival (mean 80%, range 29 – 99%) was quite variable among females. By comparison, both fertilization success and hatchling viability were generally higher and less variable. Fertilization success (mean 97%, range 81 – 99%) was moderately positively correlated with embryo survival in both flow-through (r = 0.40, n = 23, P = 0.06) and static (r = 0.50, n = 23, P = 0.01) incubation systems. Similarly, hatchling viability (mean 95%, range 79 – 100%) was also positively correlated with embryonic survival in both flow-through (r = 0.62, n = 23, P = 0.002) and static (r = 0.40, n = 23, P = 0.06) incubation systems. Hatchling viability was significantly higher than embryo survival in both flow-through and static incubation systems (Wilcoxon Signed Rank test, S > 130, n = 23, P < 0.001). Subsequent analyses focused on embryo survival only.

We observed lower and more variable embryo survival rates among females spawned on 28 April than on the latter two spawning dates (Fig. 1a). There was an overall significant effect of spawning date on embryo survival in both flow-through (ANOVA, $F_{2,20}$ = 15.4, P < 0.001) and static (ANOVA, $F_{2,20}$ = 14.4, P < 0.001) incubation systems, with survival significantly lower on 28 April than on the other two dates (Tukey's HSD test, P < 0.05). Embryo survival differed slightly between static and flow-through incubation systems (Fig. 2a). There was a strong positive correlation between embryo survival in these two systems (r = 0.93, n = 23, P < 0.001) and overall survival was 2% higher in the static system (paired-comparisons t-test, t = 2.20, n = 23, P = 0.04).

Embryo survival was more strongly related to ova traits than female traits. All embryo survival models that contained spawn date in addition to female and ova traits ranked higher than corresponding models without spawn date (Table 3). Adjusted R^2 for models with only spawn date were 0.55, and the addition of female and ova traits increased adjusted R^2 to as high as 0.69 and 0.72

for models based on flow-through and static systems, respectively (Table 3). Relative support for the top-ranked models was weak (w_i = 0.13 for flow-through, w_i = 0.16 for static) and a slow decline of Akaike weights with decreasing model rank suggested multiple plausible models (Table 3). Survival did not increase with maternal age or size for embryos reared in either incubation system (Table 3). Mean model coefficients for those two maternal traits were both negative and did not differ significantly from 0 (Table 4). For embryos incubated in both systems, survival was positively related to relative abundance of ova LNA (Fig. 3a), and LNA was the strongest predictor of embryonic survival, both on its own and in combination with other traits (Tables 3 and 4). Similarly, survival was negatively related to egg size for embryos incubated in both systems (Fig. 3b, Table 3). Most top-ranking models (ΔAICc < 2) for embryos reared in the flow-through system included LNA, whereas top-ranking models for embryos from the static incubation system included one of several fatty acid predictors (Table 3). For embryos from flow-through incubators, LNA was the only predictor with a coefficient differing significantly from 0 in full model averaging, whereas for embryos from static incubators multiple predictors, including LNA, ARA, EGGSZ, DHA, and HSI had coefficients that differed significantly from 0 (Table 4).

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

The pattern of variation in TU_{50} among spawn dates differed between embryos incubated in flow-through and static incubation systems (Fig. 1b). There was an overall significant effect of spawning date on TU_{50} in flow-through (ANOVA, $F_{2,20}$ = 4.1, P = 0.03) but not in static (ANOVA, $F_{2,20}$ = 0.24, P = 0.79) incubation systems. In the flow-through system, mean TU_{50} declined by approximately 6 degree-days >5°C over the three spawning dates with a significant difference between the 28 April and 2 May spawning dates (Tukey's HSD test, P < 0.05). We measured higher TU_{50} for embryos incubated in static compared to flow-through incubation systems (Fig. 2b) and there was no correlation between TU_{50} estimates from these two systems (r = –0.01, n = 23, P = 0.97). Ranges in TU_{50} among females were 144 – 167, and 127 – 144 degree-days >5°C in the static and flow-through systems, respectively, and mean TU_{50} was 22 degree-days >5 °C higher in the static system (paired-comparisons t-test, t = 13.6, n = 23, P < 0.001).

Female and ova traits were not strong predictors of TU_{50}. Spawning date was included in all highly ranked TU_{50} models for embryos in flow-through incubators, but not for embryos in static incubators (Table 5). In both cases, the highest ranked model contained no female or ova traits, and relative support for highly-ranked models was weak ($w_i < 0.10$; Table 5). For analysis of embryos from the flow-through system, several models with female and ova traits had relatively high adjusted R^2 (0.26 – 0.34) and Akaike weights approaching that of the top-ranked model with spawn date only (Table 5). But, for highly-ranked models containing maternal and ova traits there was little consistency in the traits appearing in the models between analyses for the two systems (Table 5), and none of these predictors differed significantly from 0 in full model averaging.

Discussion

This study builds upon our growing knowledge of the role of maternal effects in early development and survival in walleye, while also demonstrating the potential modification of these effects by environmental conditions. Our focus was on among-female variation in offspring quality for an exploited walleye stock with an age distribution skewed towards younger spawners. We examined offspring quality from the perspective of survival as well as rate of development (inferred from thermal inputs required to attain hatch, TU_{50}). Though we found evidence of maternal effects on the embryo stage, they were not related to female size and age as expected. Embryo survival for Lake Nipissing walleye varied considerably among individual females, consistent with many (Hurley 1972; Johnston 1997; Johnston et al. 2005; Johnston et al. 2007; Gatch et al. 2020) but not all (Czesny et al. 2005) studies on other walleye populations. Survival was more strongly related to the size and chemical composition of the female's ova. We also observed that the date of spawn collection had a relatively strong effect on embryonic survival but only a minor effect on TU_{50}, whereas differences in incubation method (flow-through vs static) had a small effect on survival but a stronger effect on TU_{50}. Taken in the context of earlier research (Czesny et al. 2005; Johnston et al. 2007), our results suggest that the factors influencing embryonic development and survival may vary among walleye stocks, and that maternal effects may interact with environmental factors.

We predicted that older and/or larger females would produce higher quality eggs, resulting in embryos with higher survival, based on both life history theory (Clutton-Brock 1984; Belk and Tuckfield 2010) and earlier results from similar studies on walleye and other fishes (Johnston 1997; Trippel 1998; Berkeley et al. 2004; Johnston et al. 2007). Some of these earlier studies also reported that the greatest increase in embryo survival with increasing female age occurred among the youngest age classes, particularly between recruit and older spawners (Johnston 1997; Trippel 1998). Though we studied a spawning stock with a high proportion of young spawners, many of which were probably first-time spawners, we did not detect a positive effect of maternal age or length on embryonic survival for Lake Nipissing walleye. Several other studies of walleye embryo survival have also found no significant relationship to maternal age or size (Czesny et al. 2005; Johnston et al. 2005; Gatch et al. 2020). It thus appears that the nature and strength of this relationship may vary among different walleye stocks or be sensitive to experimental conditions.

In addition to age and size which reflect a female's progress along their reproductive lifespan, we also examined the possible effects of body condition which is believed to represent a female's current energetic status. Our expectation was that females in better body condition would produce higher quality eggs and embryos, as the body condition of large females was found to have a positive influence on walleye recruitment in northern Wisconsin lakes (Feiner et al. 2019). Relationships between indices of egg quality and maternal body condition have been reported in some walleye populations (Moles et al. 2008), as well as in populations of other marine and freshwater species (Marteinsdottir and Steinarsson 1998; Lambert et al. 2000; Bunnell et al. 2005; Muir et al. 2014). We measured three indices of female condition – somatic lipid concentration, hepatosomatic index (HSI), and liver lipid concentration – and none were strong predictors of embryo survival or TU_{50}. Similarly, Johnston et al. (2007) did not find indices of maternal body condition to be strong predictors of walleye embryo survival, but did not examine indices of liver status. We included liver-based indices in the current study because of the important role of this organ in the production and transfer of nutrients to the developing ova (Brooks et al. 1997). Our results indicate that liver status is not a strong indicator of offspring quality in the Lake Nipissing walleye stock, at least during the embryo stage, though this may reflect the energetic status of the stock at the time of our study. The strength and nature of relationships between egg quality and maternal body condition in lake whitefish

(*Coregonus clupeaformis*) have been shown to vary among populations that differ in overall body condition (Muir et al. 2014).

Egg size is a commonly used index of the maternal investment per offspring (Bernardo 1996b). Because it is a strong determinant of size at hatch in fishes, including walleye (Johnston 1997), it is believed to primarily influence fitness through post-hatch survival (Miller et al. 1988; Pepin and Myers 1991). Egg size is positively related to female age and size in many walleye populations (Johnston and Leggett 2002), but not in the Lake Nipissing population (Wiegand et al. 2007). Previous controlled-incubation experiments with walleye have generally found weak or no effect of egg size on embryonic survival (Moodie et al. 1989; Johnston 1997; Johnston et al. 2007; Gatch et al. 2020). However, walleye egg size was found to have a positive effect on survival to hatch in an experiment where large volumes of eggs were incubated in an upwelling bell jar system (Johnston et al. 2005), suggesting a possible methodological influence on this relationship. In the current study, we found that embryo survival was negatively related to egg size, but TU_{50} was not related to egg size. The mechanisms behind this pattern are not clear. Smaller eggs have higher surface area to volume ratios which improves gas exchange, though it is unlikely that this was a limiting factor in our well-oxygenated incubation systems.

Relationships between egg quality and the quantity and quality of egg lipids have been a key focus in fish culture research. In particular, the three long-chain fatty acids ARA, EPA, and DHA are essential because they have critical structural and metabolic roles and cannot be produced by the female so must be ingested (Sargent et al. 1995; Tocher 2010). Thus, the PUFA provided during oogenesis originate from the female's diet, and shifts in PUFA composition of that diet can influence various aspects of egg and embryo quality (Fernández-Palacios et al. 1995; Navas et al. 1997; Rodríguez et al. 1998; Mazorra et al. 2003). In previous studies of walleye embryo survival, fatty acid profile was found to be an important predictor in some cases (Czesny and Dabrowski 1998; Johnston et al. 2007; Mejri et al. 2014) but not others (Czesny et al. 2005; Johnston et al. 2007). Furthermore, the particular indices of lipid or fatty acid composition that have accounted for the observed variability have also differed among studies. In the current study, the strongest link that we observed to fatty acid profile was a positive relationship between embryonic survival and relative abundance of

ova LNA, a non-essential fatty acid. Embryonic survival was also positively related to ova ARA and negatively related to ova DHA, though these relationships were only significant for embryos reared in the static incubation system. Relative abundances of some ova fatty acids are significantly correlated with maternal age in the Lake Nipissing population though the correlation with LNA is weak (Table 2). Interestingly, we found that models containing egg total lipid concentration, fatty acid ratios or two fatty acid measures as predictors were not among the highly ranked models. Thus, our results suggest that embryo survival in Lake Nipissing walleye is related to some aspect of egg fatty acid composition, represented by a single predictor, that complements other maternal or ova traits. The key aspects of how this fatty acid composition influences survival are not clear. Most of the variability among females in the total lipid content and fatty acid proportions of ova is found in the neutral lipid fraction (Wiegand et al. 2004) which is primarily in the oil globule, and this is not consumed until after hatching (Moodie et al. 1989).

Metals are also passed on to the developing ova through vitellogenin and relative abundances of these minerals may also affect the quality of eggs produced (Watanabe et al. 1997; Wang and Wang 2018). Metal concentrations in ova can vary considerably among walleye populations but do not appear to covary strongly with female age or size within populations (Johnston et al. 2008b). In an earlier study, walleye embryo survival was found to be significantly related to ova concentrations of essential minerals such as Zn, Cu, and Co (Johnston et al. 2007). Among the essential minerals, we found that Zn appeared to have the strongest relationship with embryo survival and development for Lake Nipissing walleye. Embryo survival was negatively associated with ova Zn concentration in several high-ranking models, though the overall effect of this predictor based on model averaging was not significant. All essential metals can be toxic at higher concentrations (Wood et al. 2011) and must be regulated both within the female body and during provisioning to the ova. Thus, it may be expected that the relationship between embryo survival and essential metal concentrations in ova would be positive at low concentrations, negative at high concentrations, and relatively flat at intermediate concentrations. The significance of the observed negative influence of ova Zn concentration on walleye embryo survival in the current study is unknown.

We found that embryo survival, and to a lesser extent TU_{50}, varied significantly with the date of spawn collection in our study. Previous studies have also found that spawning date can account for a significant amount of variation in walleye embryo survival (Johnston et al. 2005, Johnston et al. 2007). Walleye spawning at our sampling site on Lake Nipissing has historically started as early as mid-April and ended as late as mid-May, with spawning activity typically lasting 10-14 days within these extremes. We sampled over a relatively narrow period (5 days) at roughly the mid-point of the historical spawning period. The variation we observed in embryo quality among dates may represent an interaction between the maternal effects of ovulation timing and preferred spawning conditions, and shifting environmental conditions following ovulation. Changing water temperature and photoperiod are believed to trigger ovulation and spawning (Colby et al. 1979) but individual females vary somewhat in their ovulation timing. Environmental conditions during the spring spawning period can shift abruptly from day to day, and this may create a gap in time between ovulation and preferred spawning temperatures. Fertilization success for walleye ova and survival of resultant embryos declines as the time between ovulation and fertilization increases (Rinchard et al. 2005; Johnston et al. 2008a). It is possible that females of the first sampling date in this study may have ovulated earlier relative to our sampling than females of the latter two sampling dates.

We found that the type of incubation system used in our experiments, flow-through or static, had a small effect on embryo survival but a larger effect on TU_{50}. Furthermore, as discussed above, the relative ranking of models relating survival to maternal and ova traits differed slightly between the two systems, suggesting a possible interaction between incubation conditions and maternal influences. On natural spawning beds where water flow as well as temperature show greater spatio-temporal variability, embryo survival and TU_{50} may also show greater variation than what we observed under controlled conditions. Walleye spawn in a variety of habitats including rivers, shorelines and offshore reefs, and it is possible that the selective pressures on egg quality may also differ among these habitat types. Other environmental factors influencing walleye embryo survival at natural spawning sites include siltation (Gatch et al. 2020) and egg predation (Roseman et al. 2006). The strong effect of incubation conditions on TU_{50} we observed was unexpected, and the underlying mechanism is unknown. The sensitivity of TU_{50} to flow could influence both overall hatch timing and hatch

synchrony within the cohort. How these ultimately influence post-hatch survival may depend on the particular environmental conditions into which the hatchlings emerge (Rutherford et al. 2016).

The nature and magnitude of maternal effects on early life survival in walleye and many other iteroparous fishes remain poorly understood. Studies examining the fate of offspring from individual females representing a wide diversity of age and size classes within spawning stocks provide new insights and allow us to assess the prevalence and generality of these effects. The current study, using a heavily exploited and age-truncated walleye spawning stock, found that embryo survival and developmental rate were not strongly dependent on maternal age or size but were instead more strongly related to indices of egg size and composition. The implication for size-selective harvest regulations is that protection of the spawning stock for this population may only need to consider its total biomass and not its age and size composition. Studies of the relationship between early life survival and maternal traits in wild fishes are still relatively uncommon, and results from such studies thus far appear to be mixed; positive relationships are found in some cases but not others. Expanding such studies to other walleye populations, including those supported by different food web structures and experiencing different exploitation pressures would help to determine under what conditions maternal effects are most evident. Given the effect of spawning date on embryo performance observed in this and several earlier studies we also recommend further examination of maternal effects over the full spawning period. Finally, in light of the variability of physical conditions at walleye spawning sites in spring, we suggest that future studies of maternal effects consider the potential interactive effects of temperature or flow regimes.

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Table 1. Attributes of female walleye (*Sander vitreus*) and their ova sampled during spawning from 28 April to 2 May 2005 at Wasi Falls, Lake Nipissing, Ontario. All statistics calculated among 23 females. All attributes except soma mass and soma lipid content were used as predictor variables in subsequent modeling (LNA = Linoleic acid, 18:2*n*-6; ARA = Arachidonic acid, 20:4*n*-6; EPA = Eicosapentaenoic acid, 20:5*n*-3; DHA = Docosahexaenoic acid, 22:6*n*-3)

Predictor	Attribute description (units)	Range	Mean ± SD
	Female traits		
AGE	Age (years)	5 - 11	6.9 ± 1.9
FL	Fork length (mm)	382 - 569	455 ± 50
SOMA	Somatic mass (g wet)	593 - 2029	1075 ± 380
SOMLIP	Soma lipid content (% dry)	13.1 - 30.2	21.6 ± 4.8
HSI	Hepatosomatic index (% wet)	2.05 - 3.42	2.46 ± 0.41
LIVLIP	Liver lipid content (% dry)	8.4 - 14.0	11.8 ± 1.7
	Ova traits		
EGGSZ	Egg size (mg dry)	0.67 - 1.07	0.88 ± 0.09
EGGLIP	Ova total lipid content (% dry)	32.8 - 36.0	34.3 ± 0.9
LNA	LNA content of ova fatty acids (%)	3.82 - 6.24	5.15 ± 0.59
ARA	ARA content of ova fatty acids (%)	3.50 - 5.96	4.33 ± 0.58
EPA	EPA content of ova fatty acids (%)	3.95 - 10.26	7.25 ± 1.54
DHA	DHA content of ova fatty acids (%)	15.0 - 20.9	17.46 ± 1.38
EPA:ARA	EPA:ARA ratio in ova fatty acids (unitless)	0.90 - 2.79	1.72 ± 0.52
DHA:EPA	DHA:EPA ratio in ova fatty acids (unitless)	1.70 - 4.39	2.52 ± 0.61
ZN	Ova zinc content (ug g^{-1} dry)	48.0 - 75.2	60.0 ± 7.3
MN	Ova manganese content (ug g^{-1} dry)	2.02 - 8.46	4.80 ± 1.80

Table 2. Pearson correlation coefficients (r) for correlations between maternal and ova traits (defined in Table 1) used as predictors in models of embryonic survival and TU_{50} for female walleye sampled during spawning from 28 April to 2 May 2005 at Wasi Falls, Lake Nipissing, Ontario (n = 23 females). Significant correlations ($P < 0.05$) are indicated *.

	log_eAGE	FL	HSI	LIVLIP	EGGSZ	EGGLIP	LNA	ARA	EPA	DHA	EPA: ARA	DHA: EPA	ZN	MN
log_eAGE	1	.	.	.	.	.	.	.	.	.	.	.	.	.
FL	**0.81***	1	.	.	.	.	.	.	.	.	.	.	.	.
HSI	-0.25	-0.09	1	.	.	.	.	.	.	.	.	.	.	.
LIVLIP	-0.12	-0.03	-0.38	1	.	.	.	.	.	.	.	.	.	.
EGGSZ	0.36	0.37	**0.44***	**-0.66***	1	.	.	.	.	.	.	.	.	.
EGGLIP	-0.12	-0.17	0.17	-0.09	-0.02	1	.	.	.	.	.	.	.	.
LNA	0.21	0.12	-0.01	-0.24	-0.09	-0.22	1	.	.	.	.	.	.	.
ARA	**0.61***	0.29	**-0.44***	-0.02	0.02	-0.29	**0.49***	1	.	.	.	.	.	.
EPA	**-0.61***	**-0.51***	0.35	-0.09	-0.13	0.24	0.17	-0.37	1	.	.	.	.	.
DHA	-0.34	-0.10	0.05	0.19	-0.14	0.27	-0.34	**-0.57***	0.33	1	.	.	.	.
EPA: ARA	**-0.70***	**-0.50***	**0.48***	-0.08	-0.09	0.32	-0.07	**-0.71***	**0.91***	**0.49***	1	.	.	.
DHA: EPA	**0.47***	**0.55***	-0.27	0.21	0.08	-0.21	-0.30	0.11	**-0.90***	0.03	**-0.72***	1	.	.
ZN	0.19	0.29	0.27	**-0.64***	**0.68***	0.06	-0.05	-0.22	-0.03	-0.09	0.06	-0.02	1	.
MN	0.31	0.23	-0.15	0.02	0.12	-0.18	-0.17	-0.04	**-0.50***	0.17	-0.33	**0.59***	-0.11	1

Table 3. Ranking of linear models for walleye (*Sander vitreus*) embryo survival (logit-transformed) as a function of various combinations of female and ova traits (defined in Table 1). A selected subset of the 106 fitted models, including the highest ranked 1 and 2 trait models, are shown for embryos reared in both flow-through and static incubation systems. All models contained spawning date (DATE) as a fixed effect. AIC_c = Akaike's Information Criterion corrected for small sample size, Δ_i = difference between AIC_c value of a given model and the top-ranked model, w_i = Akaike weight, Adj-R^2 = adjusted R^2 for each model.

Model predictors (standardized coefficient)	Rank	AIC_c	Δ_i	w_i	Adj-R^2
Embryo survival in flow-through incubation system					
DATE, LNA (+0.46), FL (-0.29)	1	16.70	0.00	0.127	0.69
DATE, LNA (+0.39), EGGSZ (-0.27)	2	16.93	0.23	0.113	0.69
DATE, LNA (+0.41)	3	17.39	0.69	0.090	0.65
DATE, LNA (+0.40), ZN (-0.25)	4	17.57	0.87	0.082	0.68
DATE, LNA (+0.48), log_eAGE (-0.26)	5	17.71	1.01	0.076	0.68
DATE, EGGSZ (-0.35), DHA (-0.33)	6	19.12	2.43	0.038	0.66
DATE, EGGSZ (-0.31)	9	20.33	3.63	0.021	0.60
DATE	13	20.93	4.23	0.015	0.55
Embryo survival in static incubation system					
DATE, LNA (+0.47), HSI (-0.39)	1	20.21	0.00	0.158	0.72
DATE, ARA (+0.46), EGGSZ (-0.37)	2	20.87	0.66	0.113	0.72
DATE, DHA (-0.48), EGGSZ (-0.43)	3	21.03	0.82	0.105	0.71
DATE, LNA (+0.45), ZN (-0.32)	5	22.50	2.29	0.050	0.70
DATE, DHA (-0.45), ZN (-0.38)	6	22.81	2.60	0.043	0.69
DATE, LNA (+0.47)	7	23.26	3.05	0.034	0.65
DATE, ARA (+0.45)	8	23.30	3.09	0.034	0.65

DATE	30	26.89	6.68	0.006	0.55

Table 4. Means and 95% confidence limits (CL) of standardized coefficients for each of the female and ova traits (predictors, Table 1) used in walleye (*Sander vitreus*) embryo survival models for flow-through and static incubation systems. Values were calculated by full model averaging across all models that contained the predictor and spawn date as a fixed effect. Predictors are ranked from highest to lowest absolute mean coefficient. Confidence intervals that do not overlap zero are indicated in bold text.

Flow-through system survival models			***Static system survival models***		
Predictor	**Mean**	**95% CL**	**Predictor**	**Mean**	**95% CL**
LNA	**0.271**	**0.083, 0.767**	**LNA**	**0.183**	**0.082, 0.837**
EGGSZ	-0.076	-0.699, 0.054	**ARA**	**0.137**	**0.050, 0.883**
FL	-0.049	-0.632, 0.066	**EGGSZ**	**-0.115**	**-0.744, -0.025**
ZN	-0.040	-0.624, 0.085	**DHA**	**-0.104**	**-0.836, -0.029**
DHA	-0.036	-0.677, 0.099	**HSI**	**-0.092**	**-0.741, -0.018**
$\log_e$AGE	-0.028	-0.656, 0.155	ZN	-0.046	-0.711, 0.044
ARA	0.013	-0.280, 0.707	FL	-0.013	-0.746, 0.235
DHA:EPA	-0.009	-0.654, 0.286	DHA:EPA	-0.007	-0.825, 0.402
LIVLIP	-0.009	-0.744, 0.433	EPA:ARA	-0.006	-1.038, 0.706
HSI	-0.004	-0.484, 0.266	EPA	0.003	-0.768, 0.964
EGGLIP	-0.004	-0.506, 0.285	$\log_e$AGE	-0.003	-0.784, 0.638
EPA	0.003	-0.494, 0.661	LIVLIP	0.002	-0.457, 0.599

MN	-0.002	-0.448, 0.344	EGGLIP	-0.002	-0.522, 0.363
EPA:ARA	-0.001	-0.584, 0.536	MN	-0.001	-0.473, 0.415

Table 5. Ranking of linear models for thermal units to 50% hatch (TU_{50}) of walleye (*Sander vitreus*) embryos as a function of various combinations of female and ova traits (defined in Table 1). A selected subset of the 106 fitted models, including the highest ranked 1 and 2 trait models, are shown for embryos reared in both flow-through and static incubation systems. Spawning date was included as a fixed effect in all models for the flow-through system, but not the static system. AIC_c = Akaike's Information Criterion corrected for small sample size, Δ_i = difference between AIC_c value of a given model and the top-ranked model, w_i = Akaike weight, Adj-R^2 = adjusted R^2 for each model.

Model predictors (standardized coefficient)	**Rank**	**AIC_c**	**Δ_i**	**w_i**	**Adj-R^2**
TU_{50} in flow-through incubation system					
DATE	1	98.71	0.00	0.065	0.22
DATE, MN (-1.49)	2	99.42	0.72	0.045	0.27
DATE, log_eAGE (-4.16), FL (+3.56)	3	99.53	0.82	0.043	0.34
DATE, ARA (-1.35)	4	99.57	0.86	0.042	0.26
DATE, LIV_L (+2.18), ZN (+2.47)	5	99.98	1.27	0.034	0.32
TU_{50} in static incubation system					

None (intercept only)	1	107.14	0.00	0.080	0.00
HSI (+1.10)	2	108.93	1.79	0.033	< 0.01
DHA:EPA (+0.81)	3	109.34	2.20	0.027	< 0.01
ZN (-0.80)	4	109.34	2.20	0.026	< 0.01
$\log_e$AGE (-1.95), EPA (-1.92)	16	110.59	3.45	0.014	< 0.01

Figure Captions

Figure 1. Variation among embryos produced on three dates during the 2005 Lake Nipissing walleye (*Sander vitreus*) spawn based on a) survival to hatch, and b) thermal units to 50% hatch (TU_{50}). Symbols are means (±1 SE) among females for embryo batches reared in the laboratory in flow-through (closed circles) and static (open circles) incubation systems.

Figure 2. Comparison between walleye (*Sander vitreus*) embryos reared in the laboratory in flow-through and static incubation systems based on a) survival to hatch, and b) thermal units to 50% hatch (TU_{50}). Symbols are means for individual females, solid lines are fitted model II regressions, and dashed reference lines represent 1:1 agreement between the two incubation systems.

Figure 3. Scatter plots of walleye (*Sander vitreus*) embryonic survival to hatch vs a) relative abundance of linoleic acid (LNA, 18:2*n*-6) in ova lipid fatty acids, and b) egg size. Plots are for embryos raised in flow-through (solid circles, solid lines) and static (open circles, dashed lines) incubation systems. Symbols are means ±1 SD for embryo batches of individual females and trend lines are fitted OLS regressions.

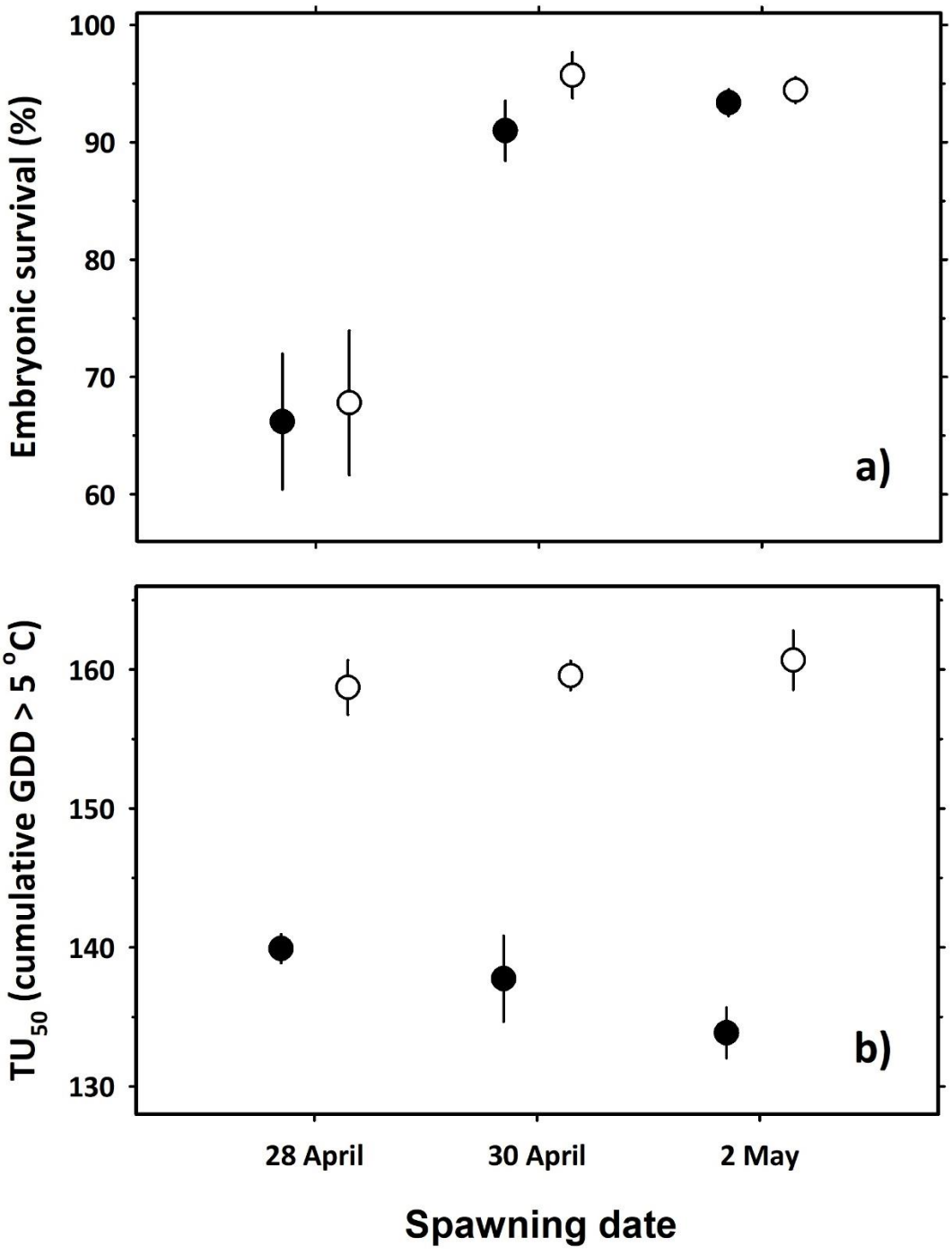

Figure 1

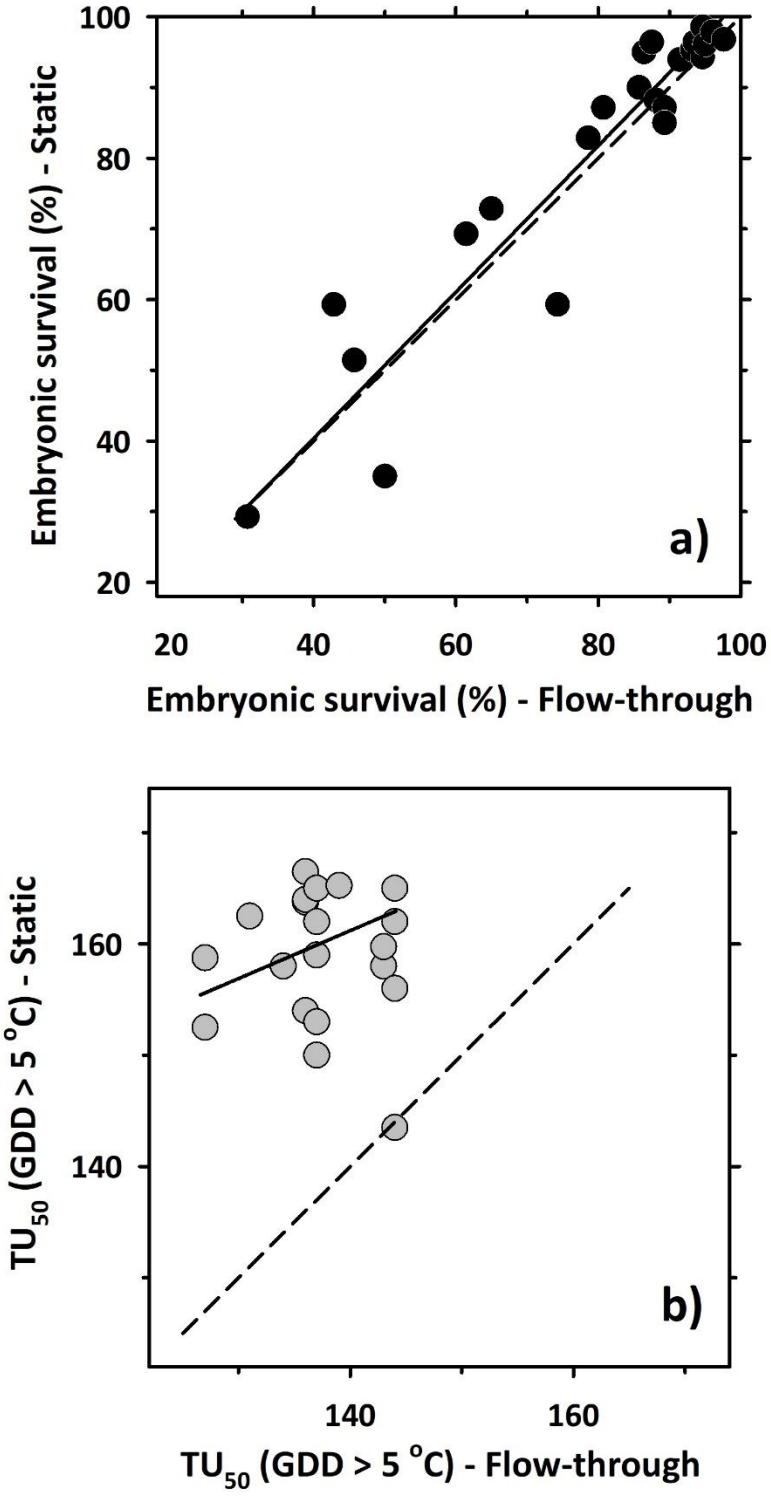

Figure 2

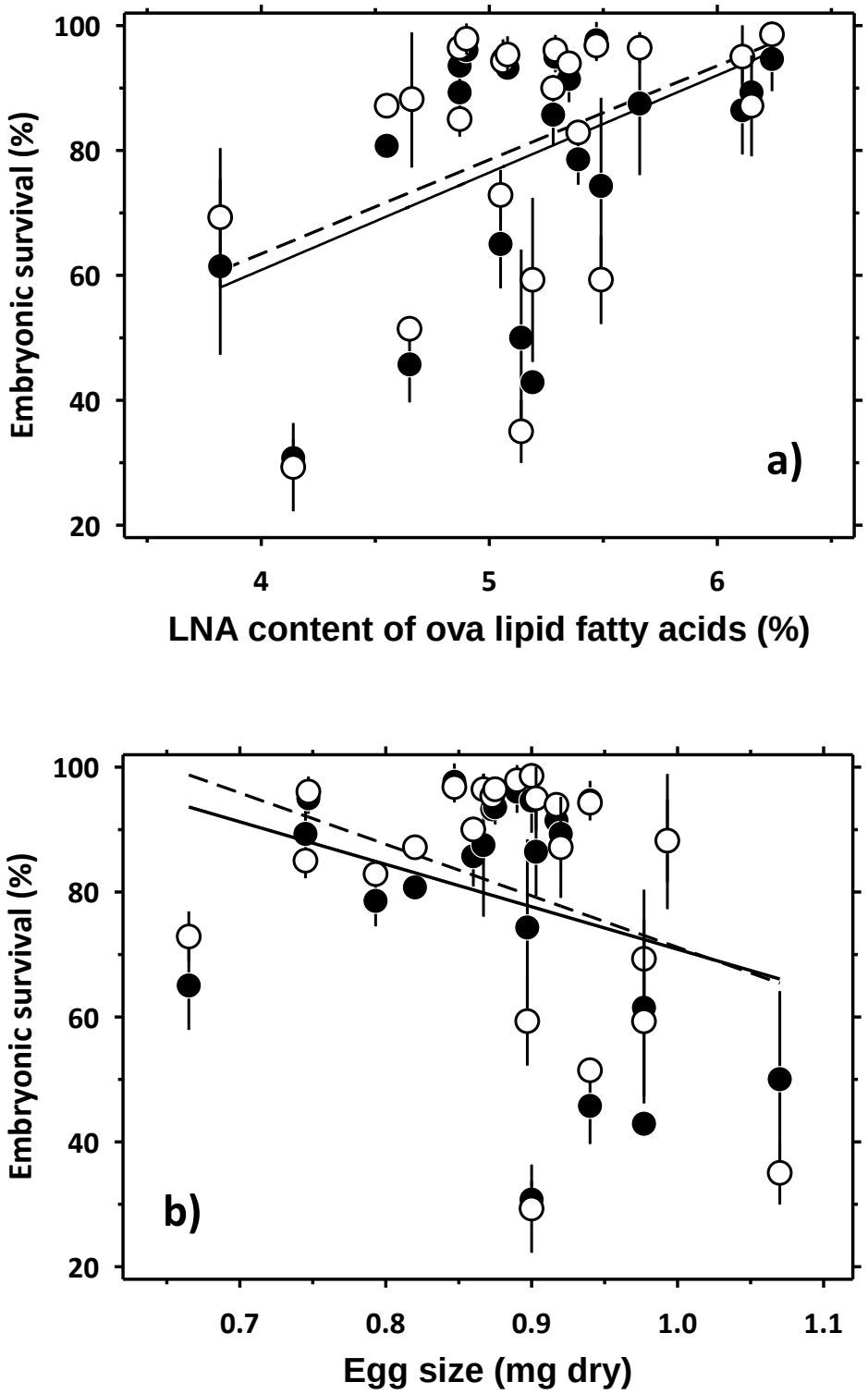

Figure 3