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Phenotypic plasticity during external embryonic development is affected more by maternal effects than multiple abiotic factors in brook trout

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ABSTRACT

Purpose: Maternal (transgenerational) effects and phenotypic plasticity are important in early life history. However, most of the literature focuses on one or the other. We examined the contribution of maternal effects and context-dependent phenotypic plasticity on early life-history traits (hatch time and size) in developing brook trout (*Salvelinus fontinalis*) embryos.

Hypotheses: (1) Environmental effects have more of an impact on hatch time than maternal effects. (2) Maternal effects have more of an impact on hatchling size than environmental effects. (3) Egg size affects the degree of plasticity of hatch time and hatchling size.

Methods: In a split-brood common-garden experiment, brook trout embryos were individually incubated in four treatments consisting of two temperatures: stable (5°C) and fluctuating (range 2 to 8°C, mean 5°C), and two pH values: benign (6.5) and stressful (5.25).

Results: The environmental variables affected hatch time in that the fluctuating-temperature/benign-pH treatment combination had a significantly longer hatch range (decreased hatching synchrony) than the other treatment combinations. However, maternal effects (egg size) overshadowed environmental effects on hatch size. Larger eggs produced longer hatchlings. In addition, larger eggs were more plastic, suggesting maternal effects influenced the degree of plasticity.

Keywords: hatch size, hatch time, maternal effects, pH, *Salvelinus fontinalis*, temperature.

INTRODUCTION

Phenotypic plasticity is a genotype's differing responses to variable environmental conditions (Schlichting and Pigliucci, 1998; West-Eberhard, 2003). Plasticity is an important aspect of evolutionary ecology because although it can be neutral or maladaptive, it often offers a protective, adaptive mechanism in times of environmental change (Hutchings, 1996; Crispo and Chapman, 2010; Chevin and Lande, 2015). Reaction norms are a measure or function of the potential

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phenotypic responses of a genotype to different environments (Woltereck, 1909; Gupta and Lewontin, 1982; Pigliucci, 2001). While plasticity studies usually examine two or three levels of an environmental variable, reality is much more complicated and oversimplification may result in misunderstanding important aspects of phenotypic plasticity.

When considering the impacts of multiple environmental variables on a phenotypic trait, it should be noted that phenotypic plasticity to one environmental variable can interact with other environmental variables. This type of plasticity can be considered context-dependent or multidimensional phenotypic plasticity, where the complexity of multiple reaction norms can be combined to create an *n*-dimensional reaction surface (Pigliucci, 2001, 2005). This can complicate our interpretation of environmental effects, but is more realistic than single, isolated environmental gradients. For example, temperature and moisture gradients both vary, and their combination can affect the phenotype of lizard embryos (Flatt *et al.*, 2001). Many studies have examined phenotypic plasticity to one environmental variable (Stearns, 1989; DeWitt *et al.*, 1998; Pigliucci, 2005), however it is likely due to logistic complications and difficulties with interpretation that there are few studies on context-dependent phenotypic plasticity (Pigliucci *et al.*, 1995; West-Eberhard, 2003; Brooks *et al.*, 2010).

Complicating matters further, the environment can also include biotic interactions. Transgenerational plasticity, more commonly known as non-genetic parental effects, is the impact of the parent's phenotype on their offspring's phenotype (Mousseau and Fox, 1998; Ezard *et al.*, 2014). Such effects can include biomolecules (nutrients and hormones transferred to offspring), environmental (natal environment, timing of reproduction) and behavioural (parental care) factors (Crean and Bonduriansky, 2014). Due to similar environmental factors (potential spatial and temporal autocorrelation) between parents and offspring, it has been shown that transgenerational plasticity can be adaptive through adjusting offspring phenotype to better match the likely environmental conditions (Uller, 2008; Ezard *et al.*, 2014).

Both context-dependent and transgenerational phenotypic plasticity have been examined most thoroughly in plants (Pigliucci *et al.*, 1995; Sultan, 2000, 2003, 2004; Valladares *et al.*, 2007; Brooks *et al.*, 2010; Herman and Sultan, 2011) and vertebrates (Wimberger, 1992; Bashey, 2006; Westneat *et al.*, 2009; Xu *et al.*, 2010; Scordato *et al.*, 2012; Salinas and Munch, 2012; Burton *et al.*, 2013; AbGhani and Merilä, 2014; Berejikian *et al.*, 2014; Donelson *et al.*, 2016; Jonsson and Jonsson, 2016). Most of the work conducted has been on transgenerational plasticity to one environmental variable (Burgess and Marshall, 2011; Ezard *et al.*, 2014); no study has examined whether these parental signatures can influence the next generation's plastic response to multiple abiotic factors.

While we acknowledge that paternal effects can be important, maternal effects tend to have more of an impact than paternal contributions, especially in very early life (Crean and Bonduriansky, 2014), therefore maternal effects are the focus of this paper. However, when mothers make a large investment into each offspring (large eggs), maternal effects may overshadow the effects of abiotic variation. Plasticity in developing animal embryos is usually best studied using species with external development, because the embryonic microenvironment can be manipulated and recreated relatively easily. For internal developers, the environment is relatively stable, and there is little opportunity to observe mechanisms that impact development. Working with externally spawning fish, for example, allows repeatable studies, and precise control and manipulation of the abiotic environment. Hatch timing (Witzel and MacCrimmon, 1983) and size (Xu *et al.*, 2010; Régnier *et al.*, 2013; Leblanc *et al.*, 2014) are traits that can predict growth and survival and can be influenced by several variables, including abiotic factors [such as temperature (Jensen *et al.*, 1989) and water chemistry (Leduc *et al.*, 2009; Purchase, 2018)], non-genetic parental effects (Bagenal, 1971; Einum and Fleming, 1999),

and parental behaviour [e.g. nest-site selection and spawning time (Sternecker *et al.*, 2014; Beer and Steel, 2018)].

Using four populations of brook trout (*Salvelinus fontinalis*) from Cape Race, Newfoundland, Canada, that have been studied since the late 1980s (see Hutchings, 1991), we focused on two potentially maternally influenced early life-history characteristics (hatch time and size) of these trout, which produce very large eggs that develop externally over an extended period of time. While previous work on the Cape Race populations has been conducted on early phenotypic plasticity, its focus was on post-hatch development (Hutchings, 1991). The objective of the present study was to determine the relative contributions of maternal and environmental effects on embryonic development, and whether degree of plasticity is influenced by maternal effects. We examined how maternal effects (variation in egg size) influence the plastic response of embryos to environmental conditions. To test this we used a split-brood approach that tracked half-sibling families and then manipulated two environmental factors: (1) temperature (stable or fluctuating), as fluctuating temperatures may affect hatch synchronization and is rarely examined in plasticity studies (Post *et al.*, 2001; Dammerman *et al.*, 2016); and (2) acidity (stressful or benign), as pH can affect resource conversion efficiency due to metabolic stress (Jordahl and Benson, 1987; Kamler, 2008).

We wished to test three hypotheses: (1) environmental effects have more of an impact than maternal effects on hatch time because previous work in salmonids has shown that there is a weak relationship between egg size and hatch time but there is a strong positive relationship between egg size and hatch size (Beacham *et al.*, 1985; Hutchings, 1991); (2) maternal effects have more of an impact than environmental effects on hatch size (dry weight, yolk volume, hatchling length) due to the large investment (i.e. yolk) salmonid mothers make in their offspring (Einum and Fleming, 1999; Berejikian *et al.*, 2014); and (3) egg size affects the degree of plasticity of hatch time and hatchling size.

MATERIALS AND METHODS

Gamete collection and embryo fertilization

We captured sexually mature brook trout in October 2012 (♀ = 24, ♂ = 48) by electrofishing four populations on Cape Race, Newfoundland, Canada: Freshwater River (FW), Watern Cove River (WN), Cripple Cove River (CC), and Ouananiche Beck River (OB) (see Table 1 for stream details). Fish were held overnight (~12 hours) in flow-through cages in their home streams until gamete collection took place the following morning, and were then promptly released unharmed.

Eggs (FW and WN) and sperm (FW, WN, CC, and OB) were collected into 50 mL plastic containers and 1.5 mL Eppendorf tubes, respectively. They were held below 4°C and transported back to Memorial University, where fertilization took place 8–11 hours after gamete collection. We ensured that sperm from each male used were viable by microscopic examination. Previous work on the same maternal populations showed that Cape Race females have low fecundity [FW: 47 ± 25 eggs; WN: 55 ± 28 eggs; mean ± SD (Hutchings, 1991)]. As in the present study we had few eggs per female (FW: 29–77; WN: 22–80), each cross was small (11–40 embryos). Egg size was used to quantify maternal effects (see below), and the relationship between mother's fork length and egg volume is shown in Fig. 1.

Each female's brood was split into two and fertilized with sperm from two sources: one random male from her native stream and one random male from a foreign stream.

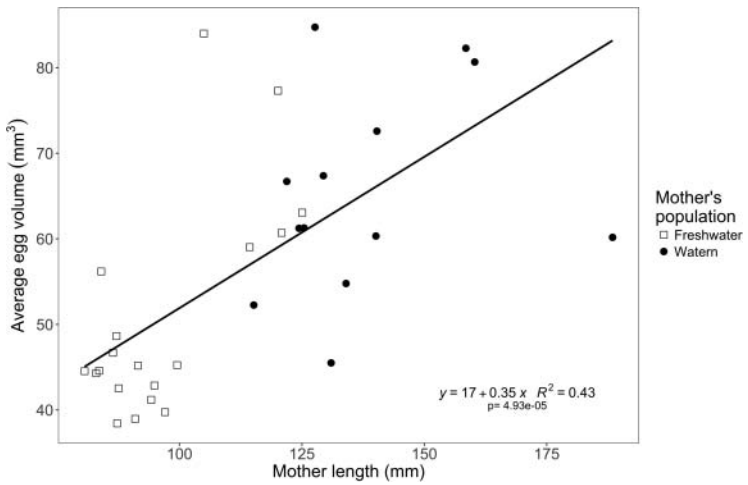


Fig. 1. The relationship between mother fork length (mm) and her average egg volume (mm^3) for Freshwater (FW) and Watern (WN) populations.

Although these trout spawn in consistent locations (Purchase and Hutchings, 2008), they have natural differences in spawning phenology in the different rivers; therefore, conducting a full reciprocal factorial cross-design was not possible because when we were sampling females from FW and WN, the females from OB and CC were not in spawning condition. We crossed each female with males from two sources as our initial plan was to evaluate population hybridization on long-term development (Fig. 2). However, due to logistical problems with adequate samples, this second planned experiment was not conducted, thus we ignored native/foreign status. Of the 48 crosses, 45 produced embryos that hatched (FW♂ × FW♀ = 12; CC♂ × FW♀ = 11; WN♂ × WN♀ = 12; and OB♂ × WN♀ = 10). The three unsuccessful crosses were due to issues with the sperm, as the mother had eggs

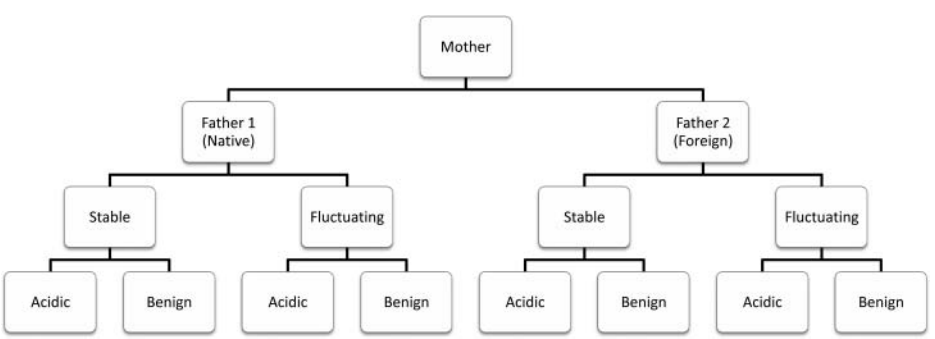


Fig. 2. Cross design and the split-brood, common-garden experiment set-up. Half of each female's brood (FW and WN) was crossed with a male from her native stream (FW × FW; WN × WN) and half with a male from a foreign stream (FW × CC; WN × OB). Each cross was then split into two temperature treatments and two acidity treatments.

hatch from the cross with the other male (for family details, see Table A1: evolutionary-ecology.com/data/3116Appendix.pdf). Each brood was then split evenly into the four environmental treatments (see 'Incubation set-up' below).

Yolk size measurements

We refer to eggs and egg size as unfertilized maternal components (oocytes) that may influence developmental timing and size of embryos. Each individual was photographed twice, immediately after fertilization (as an embryo) to measure egg size and within 24 hours post-hatch (as a hatchling), with a camera (Leica DFC420) mounted on a dissecting microscope (Leica M80). The yolk in each photograph was measured (two perpendicular width measurements) to a known scale using ImageJ (Schneider *et al.*, 2012). Yolk volumes for each individual egg and hatchling (not an average per brood) were then calculated using: length $\times$ height^2 $\times$ (π/6) (Koskinen *et al.*, 2002).

Incubation set-up

We examined phenotypic plasticity during embryonic development using a split-brood experiment among siblings, which controls for genetic effects of family (Fig. 2). Each embryo (n = 1162) was placed in an individual 50 mL tube, which contained a gravel bottom (embryo sat on top of the gravel to be visible). Embryos were incubated in the dark in incubators with a programmable temperature cycle (ThermoScientific Precision Model 818 Incubator) in their assigned temperature treatment. Incubator and position were randomized every 2 days to avoid a potential incubator or position effect. Water changes were made once or twice a week until hatch. Once hatched, the hatchlings (n = 299) were euthanized using an overdose of clove oil.

Influences of two environments on phenotype

We were interested in the plasticity of two early life-history traits (hatch time and size, where size was measured by three metrics: dry weight, yolk volume, and hatchling length). Each individually incubating embryo was examined every 24 hours to establish hatch time, which was converted to thermal summed units (sum of daily mean degrees Celsius). Hatchlings were photographed within 24 hours after hatching (see above), and hatch length was measured from the tip of the snout to the end of the notochord (in millimetres). Hatchlings (including unabsorbed yolk) were then placed in a drying oven for a minimum of 48 hours at 60°C to obtain a dry weight (milligrams).

To investigate the plasticity of these traits, we manipulated two environmental variables (Fig. 2): temperature and acidity [stable-benign (SB), stable-acidic (SA), fluctuating-benign (FB), and fluctuating-acidic (FA) treatments]. We used a stable (S; 5°C) or fluctuating (F; mean 5°C, range 2–8°C) temperature. Temperature changed every 18 hours, in a cyclical pattern: 2°C to 5°C to 8°C to 5°C to 2°C to 5°C to 8°C, and so on. This cycle was chosen so that both treatments had the same number of thermal units each week. Thermal units are important in ectothermic animals because it factors in time and temperature, which combine to affect development.

We produced water with a conductivity of 0.8 mS$\cdot$cm^{-1} by adding aquarium salt to deionized water and then adding sulphuric acid (H_2SO_4), which has been shown to be a

source of surface water acidification in freshwater streams (Rodhe *et al.*, 1995; Leduc *et al.*, 2004), to achieve the required acidity. We created stressful [acidic (A), 5.25 pH] and non-stressful [benign (B), 6.5 pH] conditions based on the pH of the mothers' home rivers. A pH of 5.25 was chosen as an acidic pH, because it would be stressful but not cause deformities or mortalities (Fiss and Carline, 1993; Leduc *et al.*, 2004). Data collected in 2010 and 2011 by Wood *et al.* (2014) indicated the average pH for Freshwater River (pH 6.59) and Watern Cove (pH 6.51) was 6.55 (see Table 1).

Statistical analyses

General approach

For all analyses, α was set at 0.05. Residuals were examined to test for normality and heteroscedasticity, and no deviations were observed. All graphs were created in base plot or ggplot2 in R v.3.2.2. We conducted generalized linear models, and generalized linear mixed effects models on our data using factors described in Table 2.

Mother's population (Mpop) and father's population (Ppop) were considered random factors for our main model (model 4, see below). However, they were treated as fixed effects in models 1 and 2 because we wanted to examine differences among populations for generalization purpose.

Testing for survivorship differences

To ensure that there were no survivorship differences among the four treatments (percent hatch: SB = 18.6%, SA = 23.8%, FB = 16.4%, FS = 21.4%), populations (FW = 21.9%, WN = 17.9%) or egg sizes that could potentially bias further analyses, we conducted an analysis of deviance on a general linearized mixed effects model with a binomial error structure:

$$\text{model 1: HS} \sim \text{pH} + \text{T} + \text{pH} \times \text{T} + \text{ES} + \text{Mpop} + (1|\text{Mother}) + \text{error},$$

where hatch success (HS, binomial 'yes/no') is explained by fixed effects – egg size (ES), mother's population (Mpop), the pH and temperature (T) treatments with a two-way interaction – and the random effect of mother's ID (1|Mother). We determined that the pattern of hatch success differed between the two pH treatments ($\chi^2_{(1)} = 6.52$, $P = 0.01$), with the benign pH treatments (17.4%) having a slightly lower hatch success than the acidic treatments (22.6%). However, hatch success did not differ among temperature treatments ($\chi^2_{(1)} = 1.47$, $P = 0.22$), or by the pH–temperature interaction ($\chi^2_{(1)} = 0.06$, $P = 0.91$), maternal population ($\chi^2_{(1)} = 3.12$, $P = 0.41$) or egg size ($\chi^2_{(1)} = 0.09$, $P = 0.77$).

Testing for differences in maternal and paternal populations

As our key interest was related to maternal effects, to determine if we should treat the two maternal populations separately in further analyses we conducted an analysis of deviance on model 2:

$$\text{model 2: AES} \sim \text{Mpop} + \text{ML} + \text{Mpop} \times \text{ML} + \text{error},$$

where AES is average egg size per mother, Mpop (fixed factor) is mother's population, and ML (covariate) is mother's length. The interaction between mother's length and mother's population was not significant ($F_{1,28} = 3.77$, $P = 0.06$).

To determine if there were differences among fathers' populations, we evaluated if the slopes and intercepts differed significantly between male populations by performing

Table 1. River locations (GPS), pH values (average of 2010 and 2011), temperature (°C) from our loggers placed in spawning areas (ground water seeps) during incubation (7 November 2012 to 2 May 2013), population size estimate (95% CI for 2011), mean length, and mean egg size (volume in mm^3) of spawning fish (standard deviation is shown in brackets)

					Average size in mm (SD)						
					Male		Female			Egg size (mm^3)	
	GPS[a]	pH[b]	Temp.[a] (SD)	Pop. size[b]	Our study[a]	n^a	Previous work[c]	Our study[a]	n^a	Previous work[c]	Our study[a]
FW	46°38.760N, 53°13.304W	6.59	7.4 (0.6)	5076–5743	99 (16.1)	31	109 (15)	96 (14.0)	19	57.9 (0.01)	51.3 (14.3)
OB	46°38.944N, 53°11.137W	5.90	NS	3355–6269	131 (18.9)	18		NS	NS		NS
WN	46°37.942N, 53°09.546W	6.51	5.3 (1.5)	7225–10255	129 (20.0)	18	129 (11)	138 (20.0)	13	55.1 (0.01)	66.6 (13.5)
CC	46°38.750N, 53°06.164W	6.05	NS	2231–2632	153 (34.8)	24		NS	NS		NS

Abbreviations: FW = Freshwater River, OB = Ouananiche Beck, WN = Watern Cove River, CC = Cripple Cove River, NS = not sampled.
[a] Present study.
[b] Data extracted from Wood *et al.* (2014).
[c] Data extracted from Hutchings (1991) (*Note*: egg diameters have been converted to egg volume).

Table 2. List of the factors, description, levels and type of factor used for our models

Factor	Description	Levels	Type of factor
pH	pH	stressful, benign	Fixed
Temp	Temperature	stable, fluctuating	Fixed
Mpop	Maternal population	FW, WN	Fixed in models 1, 2 Random in models 4, 5
Ppop	Paternal population	FW, WN, CC, OB	Fixed in model 3 Random in model 4
ES	Egg size	Continuous variable	Covariate
ML	Mother length	Continuous variable	Covariate
Mother	Mother's ID code	FW 1–12, WN 1–12	Random
Father	Father's ID code	FW 1–12, WN 1–12, CC 1–11, OB 1–10	Random

analyses of covariance on each linear model (Figs. 3a, 4, 5a, 6a, 7a, and 8) with the following format:

$$\text{model 3: DV} \sim \text{ES} + \text{Ppop} + \text{ES} \times \text{Ppop} + \text{error},$$

where the DV is each dependent variable, ES (covariate) is the individual egg size, and Ppop (fixed factor) is the paternal population. If the main effects or interactions were significant, we plotted separate lines and presented separate equations for each paternal source (see Appendix Table A2). *Note*: in all of the remaining analyses we treated mother's population and father's population as random factors. We could not treat parental populations as a random factor in models 1, 2, and 3 because we needed to include them in the interaction term to determine how to plot the equations.

Main model

For hatch timing and size, we conducted analyses using the following model:

$$\text{model 4: DV} \sim \text{ES} + \text{pH} + \text{T} + \text{pH} \times \text{T} + (1|\text{Mpop}) + (1|\text{Ppop}) + (1|\text{Mother}) + (1|\text{Father}) + \text{error},$$

where the dependent variable (DV) is explained by fixed effects – egg size (ES), the pH and temperature (T) treatments with a two-way interaction – and included random effects: mother's population (1|Mpop), father's population (1|Ppop), mother (1|Mother), and father (1|Father) IDs (Table 3). Using parental IDs as random effects allowed us to control for additive genetic effects, in the repeated sibling groups across environmental conditions. A model including the interaction terms with egg size did not change qualitative conclusions, so a simplified version is presented here.

Analysing hatch characteristics

To test our first hypothesis – that environmental effects have more of an impact than maternal effects on hatch time – we conducted a Cox proportional hazards mixed effects model (Coxme; using the 'coxme' R package) on model 4, which is a common method to analyse time-to-event data (Rich *et al.*, 2010) but can also include random effects. Our second hypothesis was that maternal effects have more of an impact than environmental effects on hatch size characteristics. To determine whether there were differences in our main effects,

Table 3. A summary of the Cox proportional hazards mixed effects model for hatch time and the analyses of deviance on the fixed effects from linear mixed effects (LME) models for dry weight, yolk volume, and hatch length

Dependent variable	Effect	Variance	SE	χ^2	Estimate	z	df	P
Hatch time	Random							
	Father	0.14						
	Mother	0.0004						
	Mpop	0.34						
	Ppop	0.02						
	Fixed							
	Intercept							
	pH					5.03	1	**<0.00001**
	Temp					8.40	1	**<0.00001**
	Egg size					0.76	1	0.45
	pH × Temp					−3.40	1	**<0.0001**
Dry weight	Random							
	Father	0.14						
	Mother	9.53						
	Mpop	8.89						
	Ppop	0.004						
	Residuals	1.19						
	Fixed							
	Intercept		2.27		13.31	5.85		
	pH		0.18	0.80	−0.06	−0.34	1	0.37
	Temp		0.17	0.50	0.15	0.86	1	0.48
	Egg size		0.01	1.84	0.01	1.36	1	0.17
	pH × Temp		0.26	0.24	−0.13	−0.49	1	0.62
Yolk volume	Random							
	Father	0.00						
	Mother	168.96						
	Mpop	177.82						
	Ppop	0.00						
	Residuals	46.87						
	Fixed							
	Intercept		10.42		44.91	4.31		
	pH		1.17	0.51	0.66	0.56	1	0.53
	Temp		1.12	2.94	1.51	1.36	1	0.09
	Egg size		0.06	0.11	0.02	0.30	1	0.77
	pH × Temp		1.68	0.09	−0.23	−0.14	1	0.89
Hatch length	Random							
	Father	0.36						
	Mother	0.05						
	Mpop	0.53						
	Ppop	0.02						
	Residuals	2.44						
	Fixed							
	Intercept		0.78		11.66	15.50		
	pH		0.26	0.07	0.08	0.26	1	0.78
	Temp		0.25	0.73	−0.04	−0.19	1	0.39
	Egg size		0.01	9.07	0.03	3.01	1	**0.003**
	pH × Temp		0.38	0.48	−0.27	−0.69	1	0.49

Note: χ^2 or z values, degrees of freedom, and the corresponding P-value are given for each effect.

we conducted an analysis of deviance on the linear mixed effects model (LME, model 4) (lme4 package in R) for each dependent variable: dry weight, yolk volume, and hatchling length. There was more inter- than intra-brood variability in egg size (see variance estimates in Table 3), thus the variance from the random effect of 'mother' encompassed most of the variation in egg size.

Analysing magnitude of phenotypic plasticity

We analysed plasticity at the half-sibling level because we did not have enough hatchlings to run the analysis at the full-sibling level (i.e. collapsed father's population). We included families that had embryos hatch in at least two treatments ($n = 23$ families: Freshwater $n = 12$, Watern $n = 11$). While many studies assess reaction norms by analysing line slopes (e.g. Purchase and Moreau, 2012; Fuhrman *et al.*, 2018), it has to be examined differently when the environments are categorical or do not have a natural gradient (i.e. are plotted in an arbitrary order on the x-axis), thus plasticity is measured in terms of character states (de Jong, 1995; Van Leeuwen *et al.*, 2015) or comparing the mean for each environment to the overall mean (Via *et al.*, 1995). Therefore, we determined plasticity for each mother's brood for each dependent variable as follows: (1) we calculated a grand mean of all embryos for all treatments ($\bar{x}$) for each mother; (2) we determined the average trait (x_i) for each treatment (SA, SB, FA, FB) for each mother; (3) for each average trait (x_i) we obtained an absolute value for each of the four treatments from the formula: $\Delta x_i = x_i - \bar{x}$; and (4) we used an average of the four treatments ($\Delta x_{(SA,SB,FA,FB)}$) to get an overall plasticity score for each mother:

$$\text{model 5: } \Delta x_{(SA,SB,FA,FB)} \sim \text{ES} + (1|\text{Mpop}) + \text{error.}$$

For each dependent variable we ran an analysis of deviance on a linear mixed effects model (model 5) with the averages of $\Delta x_{(SA,SB,FA,FB)}$ for each dependent variable (hatch time, dry weight, yolk volume, hatch length), with egg size (ES) as the fixed factor and mother's population (1|Mpop) as a random factor.

RESULTS

Egg size

We found that the majority of the variance in egg size came from inter-brood not intra-brood variation. We examined the variation (standard deviation, SD) in egg yolk size measurements as a whole (mean = 58.5 mm^3, SD = 15.9), and among mothers within each population (FW: mean = 51.3 mm^3, SD = 14.3; WN: mean = 66.6 mm^3, SD = 13.5). We obtained an average of the intra-brood standard deviations to determine the difference in standard deviation within (average SD = 6.1 mm^3) and among broods (average SD = 15.9 mm^3). The average egg volume per mother's length [egg volume (mm^3)/mother's length (mm)] was very similar to previous work conducted by Hutchings (1991) for both Freshwater (current study: 0.53; Hutchings: 0.53) and Watern mothers (current study: 0.48; Hutchings: 0.43). Thus while Watern females had larger eggs, Freshwater females produced larger eggs for their body size.

Hatch time

Across all parental populations, and all four treatments, on average it took 425.6 TSUs to hatch. The Cox proportional hazards mixed effects model (Coxme) showed that hatch time was not affected by egg size ($z = 0.76$, $P = 0.45$). However, there was a significant interaction between temperature and pH ($z = -3.40$, $P < 0.0001$; Table 3). Upon examining Fig. 3a, it is clear that the fluctuating-benign (FB) treatment was less synchronous, in that hatching began earlier but finished around the same time as in the other three treatments. This means that the FB treatment had a wider hatch range (more variability), which may be driving the significant relationship for the Coxme rather than a directional difference in mean hatch time.

Hatch size

For hatch size, we measured hatchling yolk size (mean: 47.25 mm^3), dry weight (mean: 14.16 mg), and length (mean: 13.64 mm); we compared all three metrics to each other (Fig. 4a–c). Salmonids hatch with a large amount of yolk left, thus unsurprisingly, we found that yolk size and dry weight were very strongly correlated (Pearson's $r = 0.91$, $P < 0.0001$). Dry weight and hatchling length were mildly correlated (Pearson's $r = 0.35$, $P < 0.0001$), as were hatchling length and yolk volume (Pearson's $r = 0.48$, $P < 0.0001$).

The analysis of deviance on the LME showed that for both dry weight (Fig. 5) and yolk volume (Fig. 6), a large amount of variance was explained by the random effects of mother's population and Mother ID but no main effects were significant (Table 3). This indicates that the maternal variables encompass much of the variance for dry weight and yolk volume (more variation in egg size among mothers than within mothers).

According to the analysis of deviance on the LME, hatch length was affected by egg size ($\chi^2_{(1)} = 9.1$, $P = 0.003$), but no other factors were significant, including the pH and temperature treatments which had no effect on length at hatch. There was also a large amount of variance explained by mother's population and Mother ID. Overall, the pattern showed that bigger eggs tended to produce longer hatchlings (Fig. 7).

Phenotypic plasticity

The degree of phenotypic plasticity of hatch time and all three size metrics was affected by egg size, indicating that there was a strong maternal effect impacting plasticity, where larger eggs were more plastic. Generalized linear mixed effects models were run on the plasticity scores (mean Δx_i) for hatch time, dry weight, yolk volume, and length (Fig. 8). An analysis of covariance was conducted on each model (Table 4). For the Δx_i among the treatments, there was a significant effect of egg size on the plasticity of hatch time ($\chi^2_{(1)} = 4.52$, $P = 0.22$), dry weight at hatch ($\chi^2_{(1)} = 17.97$, $P < 0.0001$), yolk volume at hatch ($\chi^2_{(1)} = 3.22$, $P = 0.07$), and hatch length ($\chi^2_{(1)} = 8.51$, $P = 0.004$).

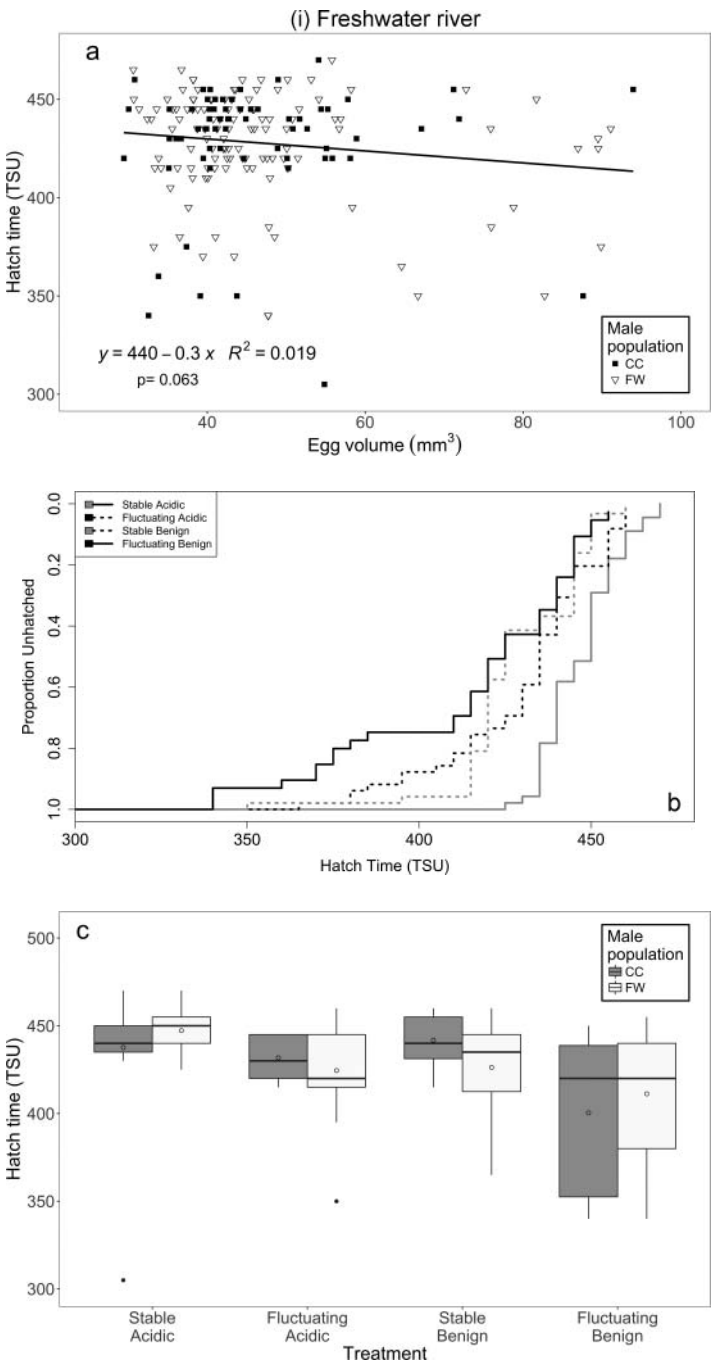
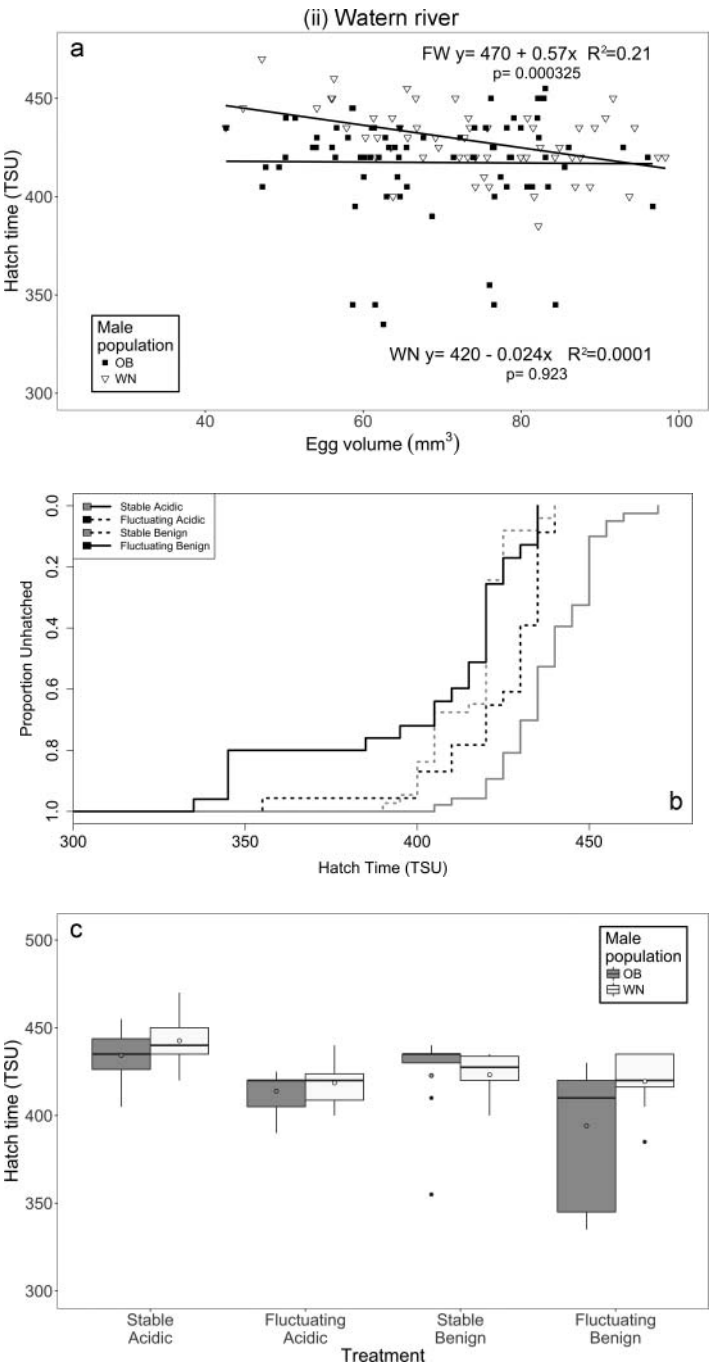


Fig. 3. Hatch time in thermal summed units (TSU) for each full-sibling family. (a) Relationship between egg volume (individual egg) and hatch time for native and foreign male (father) sourced embryos from (i) Freshwater and (ii) Watern mothers. (b) Hatch time for each pH and temperature treatment in the Cox proportional hazards mixed effects model analysis; different lines represent



different treatment combinations. (c) Hatch time for each pH and temperature treatment from (i) Freshwater and (ii) Watern. The boxplot shows the median (line) and interquartile range (IQR, 25% and 75%), whiskers represent the next quartile of the data (1.5 *IQR), and outliers are represented by dots. Open circles represent the mean for each group.

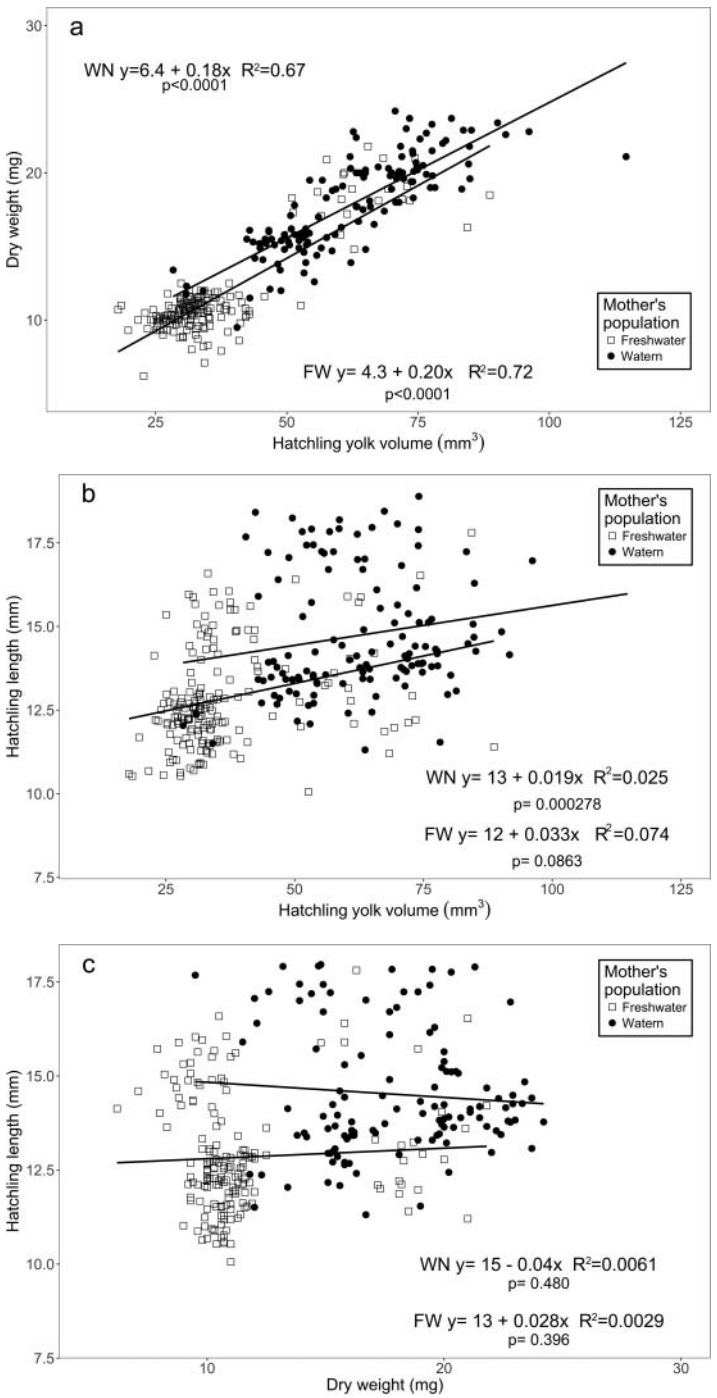


Fig. 4. Relationship between (a) hatchling yolk volume (mm^3) and dry weight (mg); (b) hatchling yolk volume (mm^3) and length (mm); and (c) hatchling dry weight (mg) and length (mm) for each population.

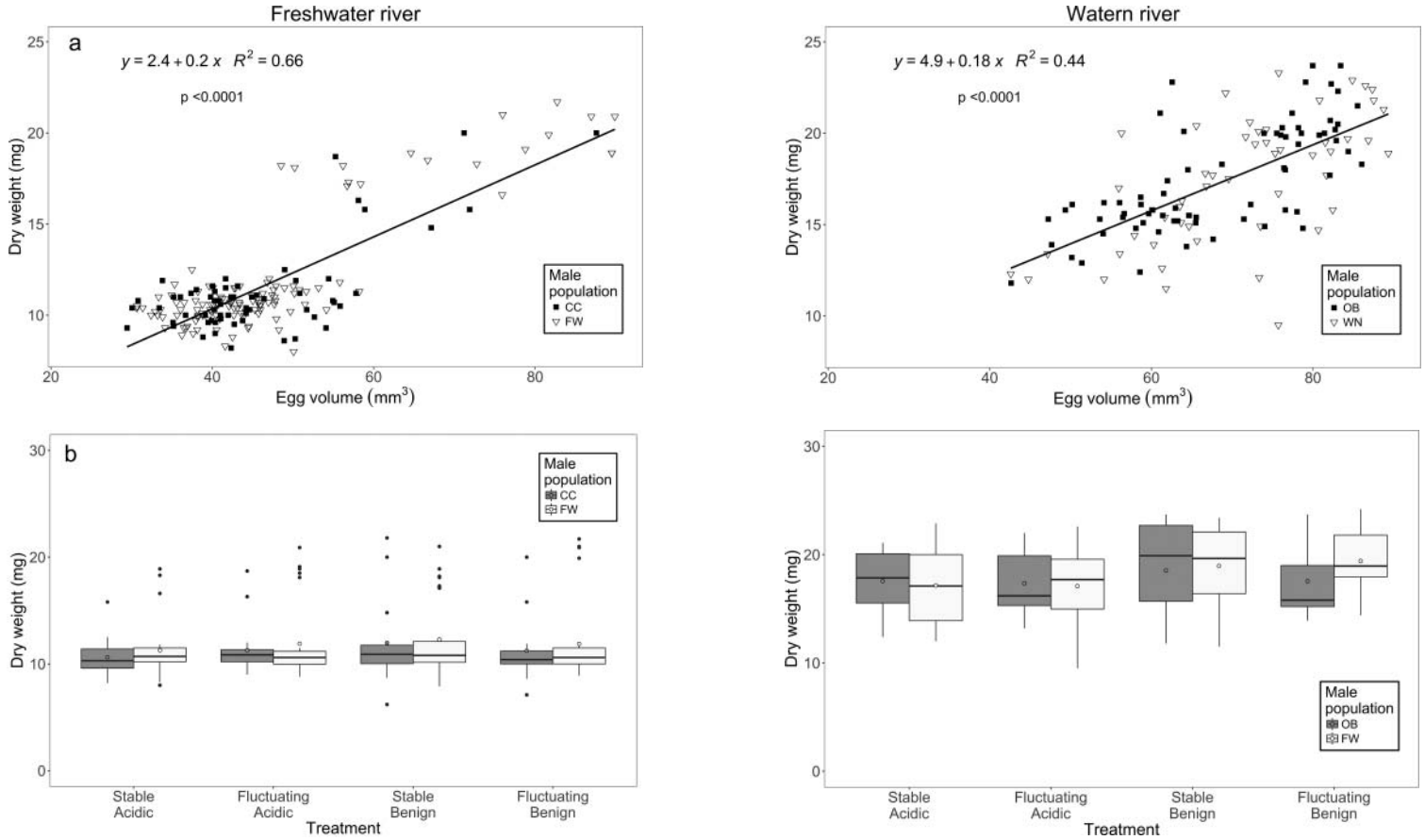


Fig. 5. (a) Relationship between egg volume (individual egg) and hatchling dry weight (mg) for native and foreign male (father) sourced embryos from (i) Freshwater and (ii) Watern mothers. (b) Hatchling dry weights for each pH and temperature treatment from (i) Freshwater and (ii) Watern. The boxplot shows the median (line) and interquartile range (IQR, 25% and 75%), whiskers represent the next quartile of the data (1.5 *IQR), and outliers are represented by dots. Open circles represent the mean for each group.

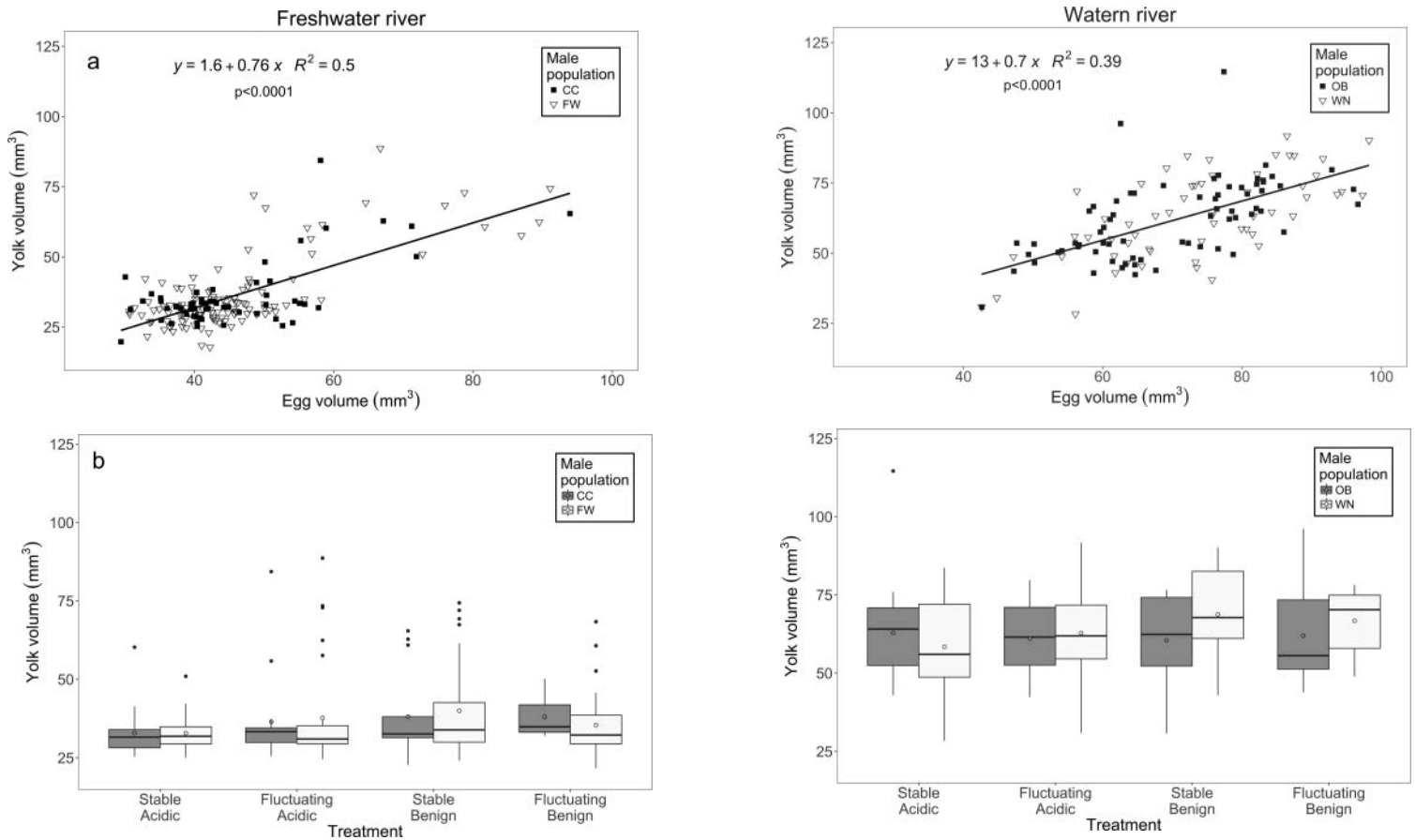


Fig. 6. (a) Relationship between egg volume (individual egg) and hatchling yolk volume (mm^3) for native and foreign male (fathers) sourced embryos from (i) Freshwater and (ii) Watern mothers. (b) Hatchling yolk volume for each pH and temperature treatment from (i) Freshwater and (ii) Watern. The boxplot shows the median (line) and interquartile range (IQR, 25% and 75%), whiskers represent the next quartile of the data (1.5 *IQR), and outliers are represented by dots. Open circles represent the mean for each group.

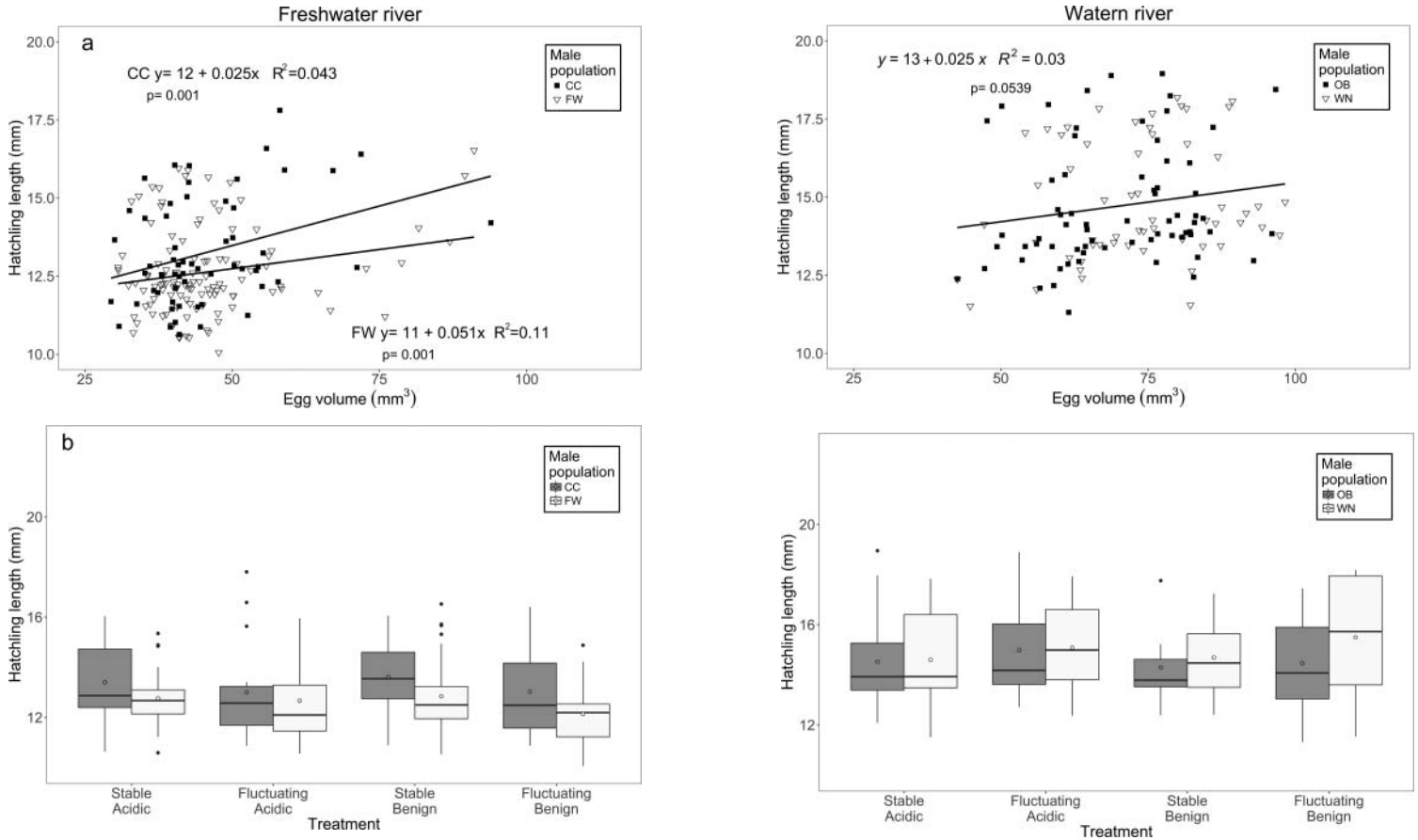


Fig. 7. (a) Relationship between egg volume (individual egg) and hatchling length (mm) for native and foreign male (father) sourced embryos from (i) Freshwater and (ii) Watern mothers. (b) Hatchling length for each pH and temperature treatment from (i) Freshwater and (ii) Watern. The boxplot shows the median (line) and interquartile range (IQR, 25% and 75%), whiskers represent the next quartile of the data (1.5 *IQR), and outliers are represented by dots. Open circles represent the mean for each group.

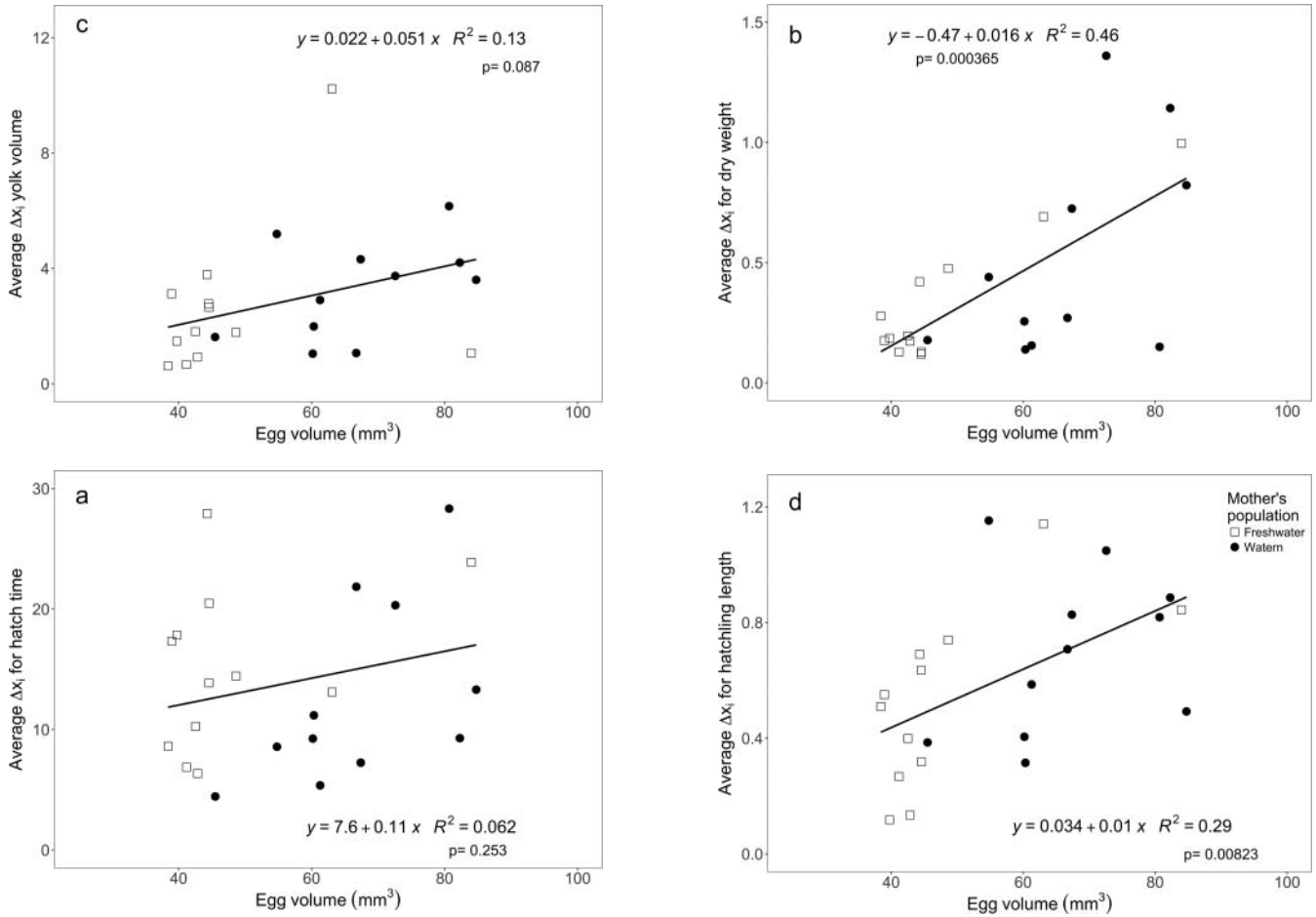


Fig. 8. Relationship between egg size (average egg volume for a mother) and the average of the absolute Δx_i for each family for (a) hatch time, (b) dry weight, (c) yolk volume, and (d) length, for mothers from Freshwater (FW) and Watern (WN).

Table 4. A summary of the analysis of deviance on the linear mixed effects models on plasticity of hatch time (thermal summed units), dry weight (mg), yolk volume (mm^3), and hatch length (mm) among environmental treatments (average Δx_i)

Dependent variable	Effect	Variance	SE	χ^2	*df*	*P*
Δx_i **Hatch time**	Random					
	Mpop	21.53				
	Residuals	42.48				
	Fixed					
	Intercept		7.08			
	Egg size		0.11	4.52	1	**0.03**
Δx_i **Dry weight**	Random					
	Mpop	0.008				
	Residuals	0.07				
	Fixed					
	Intercept		0.25			
	Egg size		0.004	17.97	1	**< 0.0001**
Δx_i **Yolk volume**	Random					
	Mpop	0.00				
	Residuals	4.39				
	Fixed					
	Intercept		1.66			
	Egg size		0.03	3.22	1	**0.07**
Δx_i **Hatch length**	Random					
	Mpop	0.00				
	Residuals	0.07				
	Fixed					
	Intercept		0.20			
	Egg size		0.003	8.51	1	**0.004**

Note: The standard error, χ^2-values, degrees of freedom, and the corresponding *P*-value are given for the fixed effects.

DISCUSSION

Maternal (transgenerational) (Burton *et al.*, 2013) and environmental effects (Wood and Budy, 2009) have been shown to be important in early development. However, this is the first study to compare the contributions of maternal effects and context-dependent phenotypic plasticity (both acidity and fluctuating temperatures) during embryonic development. We found evidence for at least partial support for all three of our hypotheses: (1) environmental effects have a larger impact than maternal effects on hatch time; (2) maternal effects have more of an impact than environmental effects on hatch size – one out of three of our size metrics (length) was significantly affected by egg size; and (3) egg size affects the degree of plasticity of hatch time and all three measures of hatchling size.

Hatch time was not affected by egg size, supporting previous work that has shown no relationship between egg size and hatch time in the same two maternal populations examined in our study (Hutchings, 1991). The environmental effects of pH and temperature had

a significant effect on hatch time. Our results suggest that there is a difference in hatch synchrony (variability), rather than differences in mean hatch time, among the different groups. This result is largely driven by the fluctuating-temperature/benign-pH treatment group, in that they began hatching earlier so had a wider hatch range. Fluctuating temperatures seem to have been attracting more research attention recently (Richter-Boix *et al.*, 2015; Jeuthe *et al.*, 2016; Beer and Steel, 2018), likely because of impacts of climate change (Paaijmans *et al.*, 2013), but have not been examined in terms of context-dependent phenotypic plasticity. Even moderate fluctuations in temperature (in the present study, 6°C of variation) can affect hatch time, and a high degree of asynchrony may have negative consequences for competition in establishing feeding territories (Einum and Fleming, 2000) and avoiding predators (Mirza *et al.*, 2001).

On the other hand, the variance in hatch size (dry weight, yolk volume, hatch length) was mostly encompassed by maternal impacts (random effects of maternal population and Mother ID) and there were no effects of the pH and temperature treatments (context-dependent abiotic influences). Our effect sizes ranged from small to medium, which implies not only statistical but at least some biological significance. As with previous work (Solberg *et al.*, 2014), larger eggs produced longer hatchlings. Other work has shown that maternal effects are very strong in early life and decrease over time, when environmental and potentially paternal effects also play a much larger role (Heath *et al.*, 1999). Previous work has shown effects of pH (Fiss and Carline, 1993) and temperature (Dammerman *et al.*, 2016; Beer and Steel, 2018; Fuhrman *et al.*, 2018) on developing fish embryos and juveniles. However, our manipulations of pH and temperature were well within the natural ranges, so the environmental variables may have simply had no effect, or the magnitude of the maternal effects (transgenerational signature) may have masked the environmental effects. When we ran our models, the random effects of 'mother' and 'mother's population' explained the majority of the variance, so much so that it overshadowed the effect of individual egg size for dry weight and hatchling's yolk volume. The term 'mother' encompasses several different variables within a sibling brood, including genetic, epigenetic, maternal size, egg size, and yolk composition effects. Although the relative components are beyond the scope of this study, most of the egg size variation was across broods, not within a brood.

The most interesting finding in our study was the evidence for an interaction between maternal (transgenerational) and environmental (context-dependent) effects on the degree of plasticity of hatch length: mothers with larger eggs (maternal effect) had families whose siblings showed more plasticity to multiple abiotic conditions than mothers with smaller eggs. However, it is unclear if the result is due to plasticity or constraint. In salmonids, a portion of the yolk is retained in a yolk sac and absorbed while the hatchling is in the gravel nest. In general, if a hatchling is heavier during the absorption stage, it is likely that it is at an earlier developmental stage, where conversely being longer is indicative of being more developed. Each egg has a fixed amount of nutritional material that the embryo and hatchling can convert into growth (length and weight), meaning that at theoretical 100% conversion efficiency there is a fixed maximum dry weight prior to exogenous feeding. Smaller eggs are constrained by a lower maximum size (some combination of length and weight depending on developmental stage at hatch) that affects the size range that a hatchling can be, while larger eggs have more flexibility depending on environmental conditions (i.e. differences in nutrients available and conversion efficiency). Thus, maternal investment affects how susceptible embryos are to environmental conditions, where a large investment allows for more flexibility. This novel finding could show that if embryos from larger eggs

are more plastic, they may have potential for a greater range of expression, which may provide advantages to fit their environment. If the plasticity for size continues through from hatchling and juvenile stages to adults, it may confer benefits and be one explanation for increased survival in larger juveniles (Einum and Fleming, 2000; Xu *et al.*, 2010; Pess *et al.*, 2011).

This is the first study of its kind to attempt to tease apart maternal effects from multiple environmental effects on embryonic development and we found that maternal effects observed in this study were so strong, particularly for hatch size, that environmental effects could not be detected. We have known for decades that maternal effects may override potential environmental effects during embryonic development, which has implications for future studies on early life phenotypic plasticity. While a lot of research has been conducted on maternal effects or environmental plasticity, we need to pay special attention to each type of plasticity due to the interactive effects between transgenerational and environmental impacts. Therefore, carefully designed experiments are required to control for maternal effects when testing questions on phenotypic plasticity.

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