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1 # Pathogen-induced hatching and population-specific life-history response to
2 # water-borne cues in brown trout (*Salmo trutta*)
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17
18 **Abstract**
19 Hatching is an important niche shift, and embryos in a wide range of taxa can either
20 accelerate or delay this life-history switch in order to avoid stage-specific risks. Such behavior
21 can occur in response to stress itself and to chemical cues that allow anticipation of stress. We
22 studied the genetic organization of this phenotypic plasticity and tested whether there are
23 differences among populations and across environments in order to learn more about the
24 evolutionary potential of stress-induced hatching. As a study species we chose the brown
25 trout (*Salmo trutta;* Salmonidae). Gametes were collected from five natural populations
26 (within one river network) and used for full-factorial *in vitro* fertilizations. The resulting
27 embryos were either directly infected with *Pseudomonas fluorescens* or were exposed to
28 water-borne cues from *P. fluorescens*-infected conspecifics. We found that direct inoculation
29 with *P. fluorescens* increased embryonic mortality and induced hatching in all host
30 populations. Exposure to water-borne cues revealed population-specific responses. We found
31 significant additive genetic variation for hatching time, and genetic variation in trait plasticity.
32 In conclusion, hatching is induced in response to infection and can be affected by water-borne
33 cues of infection, but populations and families differ in their reaction to the latter.
34
35 **Keywords**:
36 Additive genetic variation, fish embryo, induced hatching, niche shift, phenotypic plasticity,
37 reaction norm, salmonid
38
39

Introduction

The timing of life-history transitions and niche shifts is expected to be influenced by the risk-benefit ratio before and after the change (Werner and Gilliam 1984; Werner 1986; Rowe and Ludwig 1991). As such risk-benefit ratios often vary according to environmental conditions, some degree of phenotypic plasticity is expected (Gomez-Mestre et al. 2008; Reed et al. 2011). Hatching represents such a life-history transition and niche shift, and plasticity in this trait has been observed in a number of taxa (Warkentin 2007; Warkentin 2011). Induced hatching is often favored in response to egg-specific stressors (e.g. predators, pathogens) (Warkentin et al. 2001; Wedekind 2002; Moreira and Barata 2005; Vonesh 2005; Gomez-Mestre et al. 2006; Touchon et al. 2006), and conversely, delayed hatching is preferred when there is increased risk of larval mortality (Sih and Moore 1993; Moore et al. 1996; Martin 1999; Laurila et al. 2002; Schalk et al. 2002).

Notably, the physical presence of a threat is not always required to induce a plastic response in hatching time. Cues from both predators (Moore et al. 1996; Smith and Fortune 2009; Miner et al. 2010) and pathogens (Kiesecker et al. 1999; Wedekind 2002) have been observed to alter hatching time. These cues can be quite specific, allowing animals to distinguish between predators and non-predators, and even predator diets (Chivers et al. 2001; Kusch and Chivers 2004). Induced responses can equally be elicited from injured or infected conspecifics (Kiesecker et al. 1999; Chivers et al. 2001; Wedekind 2002; Miner et al. 2010). The ability to respond to a threat before physical contact is not only practical for slower hatching species (e.g. that require proteolytic cleavage of the egg membrane; Anderson and Brown 2009), but is particularly well suited for pathogen challenge as it decreases infection risk.

The ability for such inducible defenses to evolve in a population resides in the persistence of genetic variation in trait plasticity (reaction norms) (Pigliucci 2001); however, the genetic organization of a trait can change according to environmental condition, and not necessarily in a consistent direction. For example, genetic variation has been shown repeatedly to increase from favorable to unfavorable conditions (Hoffmann and Merilä 1999; Agrawal et al. 2002; Relyea 2005; Kraft et al. 2006), while other studies reported either a decrease (Merilä 1997; Laugen et al. 2005) or no change in heritable variation with increasingly stressing conditions (Merilä and Fry 1998; Pakkasmaa et al. 2003; Merilä et al. 2004). Taken together, these studies suggest that the expression of genetic variation in a trait is not only trait dependent but can also be reliant on the stressor. To add an additional level of complexity, the amount of genetic variation in a trait can also vary among populations, due to genetic drift and/or local adaptation (Einum and Fleming 2000; Gomez-Mestre and Warkentin 2007). Indeed, in the case of salmonids, life histories are typically finely tuned to local environmental conditions (Crozier et al. 2008). Consequently, differences in micro-ecological conditions often lead to population-specific reaction norms in early life history traits.

While much work has been conducted to characterize patterns of genetic variation in plastic traits at more advanced developmental stages, few have done so during the egg stage (Gomez-Mestre et al. 2008; Jensen et al. 2008). To our knowledge, no study to date has assessed genetic variation for induced hatching in response to water borne cues of infection. Here, we performed full-factorial *in vitro* fertilizations within five populations of brown trout (*Salmo trutta*), and infected resulting embryos with the opportunistic fish pathogen, *Pseudomonas fluorescens* (Austin and Austin 2007). As in Wedekind (2002), water-borne cues from acutely stressed embryos were then taken and, after sterile filtration, applied to conspecifics. By virtue of our experimental design, we were able to observe whether (i) brown trout embryos responded to water-borne cues, (ii) determine if populations differed in their response, (iii) estimate the amount of additive genetic variation for induced or delayed hatching, (iv) and assess whether these patterns differed by population.

90 **Material and Methods**
91 Fertilization protocol and embryo rearing
92 Adult male and female brown trout were caught by electro-fishing from the river network
93 between the cities of Bern and Thun; Switzerland, i.e. the river Aare (from 7°34'16.67"E/
94 46°49'09.58"N to 7°26'46.78"E / 46°56'39.00"N) and four of its tributaries (Kiese:
95 7°37'11.29" E / 46°50'55.85" N; Gürbe: 7°30'4.19" / 46°52'59.37"; Giesse: 7°32'44.00" /
96 46°53'05.33"; Amletenbach: 7°34'04.73" / 46°47'05.95") during the breeding season in
97 October to November 2009. Pairwise comparisons between these populations not only
98 showed significant genetic differentiation but also important morphological differences
99 (Stelkens et al. 2012). After capture, adults were kept at the cantonal hatchery until the start of
100 the experiment. Four females and six males from each population were haphazardly chosen,
101 anaesthetized (Tricaine mesylate MS-222), measured (total length), and stripped of their
102 gametes. These gametes were used for *in vitro* fertilizations (following methods described in
103 Jacob et al. 2007). Crossings were performed within populations in a full-factorial breeding
104 design, i.e. for each population all possible crosses of four females and six males (North
105 Carolina II; Lynch and Walsh 1998) yielding in total 120 sib-groups (24 per population).
106 Water used for fertilizations and thereafter was chemically standardized (OECD 1992),
107 aerated, and cooled to 6.5°C before use.
108 After water hardening, eggs were transported to a climate chamber (6.5°C) where they
109 were washed (as described in von Siebenthal et al. 2009). Eggs were then distributed singly to
110 24-well plates (Falcon, Becton Dickinson; 2ml water per well) in a block-wise design, such
111 that a set of five plates contained all types of siblings. They were examined 54 days post-
112 fertilization on a light table (Hama professional, LP 555) and with a stereo zoom microscope
113 (Olympus SZX9) to assess fertilization success.
114
115 Isolation of *Pseudomonas fluorescens*, identification, and infection protocol
116 To facilitate the collection of a pathogenic strain of the gram-negative bacterium *P.*
117 *fluorescens,* brown trout eggs from various origins were left in Petri dishes and incubated
118 until signs of infections could be observed. A plate was randomly selected (eggs originated
119 from Amletenbach), and 100µl of water was streaked onto a non-selective tryptic soy agar
120 media (TSA) (Sigma Aldrich) plate and incubated at 22°C for 48 hours. Non-selective media
121 is preferred for single colony selection as it allows for identification of other bacterial
122 contaminants (Holt et al.1994). A fluorescent colony was selected and restreaked onto a TSA
123 plate, which was allowed to incubate for 24 hours. This procedure was repeated twice to
124 ensure colony purity (Sambali and Mehrotra 2009). For identification, DNA was isolated
125 from the colony using the GenElute^{TM} Bacterial Genomic DNA Kit, according to the
126 manufacturer's instructions (Sigma-Aldrich). A PCR was performed using a *P. fluorescens*-
127 specific primer set: 16SPSEfluF (5'-TGCATTCAAAACTGACTG-3') and 16SPSER
128 (5'AATCACA -CCGTGGTAACCG-3') (Sparpellini 2004). *P. fluorescens* strain ATCC
129 17400 was used as the positive control. All PCR reactions were performed in a total volume
130 of 50 µl, and contained: ~50-100ng bacterial genomic DNA, 5 µl of 10X PCR buffer, 200 µM
131 of each dNTP, 2 mM of MgCl2, 0.5 µM of each primer, and 0.5 U of Taq polymerase
132 (Qiagen). The thermal profile was modified from Scarpellini et al. (2004): 2 min at 94°C; 5
133 cycles of 94 ^{o}C for 45s, 55 ^{o}C for 1 min, 72 ^{o}C for 2 min; 35 cycles of 92 ^{o}C for 45s, 60 ^{o}C for
134 45s, 72 ^{o}C for 2 min; final extension of 72 ^{o}C for 2 min; and final cooling at 12 ^{o}C. Following
135 the PCR, the amplified products were purified with the Wizard® SV Gel and PCR Clean-Up
136 System (Invitrogen) and sequenced in the forward and reverse directions with
137 16SPSEfluF/16SPSER on a ABI Prism 3100 genetic analyzer (Applied Biosystems).
138 To prepare the bacterial inoculum, 200 ml of tryptic soy broth (TSB) was inoculated
139 with a single colony of *P. fluorescens*. The flask was then placed on a shaker (200rpm) for 36

140 hours at 22°C, until reaching exponential growth phase. The contents were transferred to 50
141 ml conicals and centrifuged for 15 minutes at 4000 rpm. Pellets were washed twice with
142 sterile standardized water and pooled. Bacterial cells were counted with a Helber
143 hemocytometer using a phase contrast microscope (400x). The suspension was diluted such
144 that an inoculation with 100 µl/well would result in a concentration of 6.0×10^8 cells/ml.
145 Eight of the twenty replicates per sibgroup were treated with *P. fluorescens* 54 days
146 post-fertilization. The remaining 12 replicates received only standardized water. Nine days
147 later, 100µl of water were taken from each treated well, pooled, and sterile filtered (0.2µm) to
148 produce the water-borne cue (WBC). Eight of the 12 non-infected embryos per sibgroup were
149 then exposed to 100µl of WBC. The remaining four embryos served as controls and were
150 sham-treated with sterile-filtered standardized water. Following treatment, embryos were
151 monitored daily and mortality and hatching date were recorded.
152
153 Statistical analyses
154 Analyses were performed with Jmp7.0 (JMP 1989-2007) and R (R Development Core Team
155 2006) using the lme4 package for linear and logistic mixed effect model analyses (Bates and
156 Sarkar 2007). Eggs that were not eyed on day 54 post fertilization were assumed to be non-
157 fertilized and hence excluded from all further analyses. Survival was analyzed as a binomial
158 response variable in generalized linear mixed models (GLMM), and hatching time as a
159 continuous variable in linear mixed models (LMM). Treatment was considered as fixed effect,
160 and population, dam, sire, treatment x population, treatment x dam, and treatment x sire
161 interactions as random effects. Starting with a reference model that included treatment,
162 population, dam, and sire, we added or removed one effect at a time and compared the
163 reference and the changed model with likelihood ratio tests (LRT) using restricted maximum
164 likelihood (REML) (Zuur et al. 2009). Sire x dam interactions were not included in the
165 models to avoid low sample sizes in some experimental cells.
166 Because infection with *P. fluorescens* and inoculation with WBC were performed at
167 two different time points during embryo development, and reactions to stressors may be
168 conditional to developmental stage (Johnson et al. 2001; Schotthoefer et al. 2003; Sollid et al.
169 2003; E. S. Clark et al., unpubl. data), infected embryos were not directly compared to WBC-
170 exposed embryos. Instead, both treatments were compared to the controls only. Moreover,
171 because embryo survival was low in the infection treatment, survival but not hatching date
172 was analyzed in the corresponding mixed effect models. Analogously, because survival
173 turned out to be close to 100% in the WBC treatment (see Results), hatching date but not
174 survival was analyzed in the corresponding mixed effect models.
175 Variance components for hatching time were extracted from mixed models using
176 REML. With a full-factorial breeding design, assuming that epistasis is negligible, additive
177 genetic variance (V_A) is estimated as four times the sire variance (V_{SIRE}) (Lynch and Walsh
178 1998). Maternal environmental effects (V_M), i.e. the part of dam variance (V_{DAM}) not
179 explained by additive genetic effects, was calculated by subtracting V_{SIRE} from V_{DAM}.
180 Residual variance (V_{RES}) includes environmental variance as well as ½ V_A and ¾ dominance
181 variance (V_D) (Kearsey and Pooni 1996). Narrow sense heritability was estimated as V_A
182 divided by total variance, i.e V_{DAM}, V_{SIRE} and V_{RES}, as in Lynch and Walsh (1998). Standard
183 errors of variance components and heritabilities were calculated using a bootstrap procedure,
184 taking the standard deviation of 1000 estimates produced by reshuffled hatching dates
185 separately per population and treatment in R. To facilitate comparisons with other studies, we
186 also report the coefficients of additive genetic variation (CVA) (Houle 1992). Variance
187 components and heritability were not estimated for the binominal response variable survival,
188 because mortalities turned out to be either very low (controls, WBC) or very high (infected)
189 rendering heritability estimates from count data estimates spurious. Genetic correlations for

hatching time across two environments were estimated using Pearson's product moment correlations for sire sib-group means (Roff 1997; Relyea 2005; Gomez-Mestre et al. 2008).

Results

Embryo survival

Exposure to *P. fluorescens* significantly decreased mean survival until hatching from 98.8 ± 0.5% (SE) in the control to 11.6 ± 1.1% in infected eggs (Table 1). Populations did not differ significantly in average embryo viability or susceptibility to infection (Table 1). While sire x treatment or dam x treatment interactions were not significant (Table 1), there were significant overall dam and sire effects in offspring viability (Table 1). Survival in the WBC treatment was on average 97.3% ± 1.0% (SE) and not significantly different from the controls (GLMM z = -1.58, p = 0.12).

Treatment and population effects on hatching time

Embryos treated with *P. fluorescens* hatched significantly earlier than controls (comparing the hatching times of the first hatchling per sibgroup: median difference = 60 hours, Wilcoxon signed rank test, V = 561.5, p = 0.006; mean hatching time per sibgroup: median difference = 57 hours, V = 587, p = 0.002; Fig. 1). This median difference in the timing of first hatching per sibgroup corresponded to a median hatching acceleration of 18% since exposure to *P. fluorescens* and did not significantly among the populations (Kruskal-Wallis $\chi^2 = 4.7$, d.f. = 4, p = 0.32). Reaction to WBC treatment varied among populations (Table 1; Fig. 1). Treatment with WBC induced hatching in the Gürbe population, delayed hatching in Giesse and Amletenbach, and appeared to have no significant overall effect in Kiese and Aare (Fig. 1).

We found significant overall dam and sire effects on hatching time (the main effects in Table 1). We also found significant genetic variation for hatching plasticity (the sire x treatment and dam x treatment interaction in Table 1). In the control, sire effects accounted for a significant part of the variance in two populations, i.e. Aare (LRT: $\chi^2 = 7.85$, p = 0.005) and Gürbe ($\chi^2 = 17.53$, p < 0.0001), but were not significant in Kiese ($\chi^2 = 0.08$, p = 0.78), Giesse ($\chi^2 = 0.07$, p = 0.80) or Amletenbach ($\chi^2 = 0.00$, p = 1.00) (Table 2). However in the WBC treatment, sire effects were significant in four out of five populations, i.e. Aare ($\chi^2 = 13.97$, p = 0.0002), Gürbe (χ^2=19.30, p<0.0001), Giesse ($\chi^2 = 56.66$, p < 0.0001) and Amletenbach ($\chi^2 = 13.93$, p = 0.0002), but not Kiese ($\chi^2 = 0.56$, p = 0.45) (Table 2).

Cross-environmental trait correlations

As populations differed significantly in hatching response to treatments (Table 1), cross-environment genetic correlations between control and WBC were calculated separately for each population. For the Kiese and Giesse, there is no correlation in hatching time among the two environments (r = -0.01, p = 0.98; and r = 0.66, p = 0.15 respectively). However, hatching time was positively correlated among the two environments in Aare (r = 0.82, p = 0.04), Gürbe (r = 0.89, p = 0.02) and Amletenbach (r = 0.86, p = 0.03).

Discussion

In whitefish (*Coregonus* sp.), i.e. in another salmonid genus, exposure to *P. fluorescens* has been shown to cause significant embryonic mortality (Wedekind et al. 2004; von Siebenthal et al. 2009), as well as to induce hatching (Wedekind 2002). Brown trout embryos differ in many respects from whitefish embryos, including mean size, various characteristics of their typical habitat, and the behavior of freshly hatched alevins (Wedekind and Müller 2005). We nevertheless found that treatment with *P. fluorescens* increased mortality and also prompted precocious hatching. Sensitivity to *P. fluorescens* and the host's life-history response therefore appears to extend to brown trout embryos. This allowed us to compare populations

240 that live in different habitats (but still within the same catchment area) and for which Stelkens
241 et al. (2012) found significant morphological and genetic differentiation despite the rather
242 small geographic scale. Some of the previously observed morphological differentiation within
243 this catchment area could even be linked to habitat characteristics (Stelkens et al. 2012),
244 consistent with local adaptation.
245 Although experimental infection with *P. fluorescens* induced mortality and precocious
246 hatching in all study populations, we found that the populations varied significantly in their
247 hatching response to water-borne cues from infected embryos, i.e. we found significant
248 differences in the reaction norms. Such variation at the level of the population could
249 potentially indicate differential selection pressures (Laurila et al. 2002; Jensen et al. 2008). As
250 the microbial community composition can differ vastly between streams (Evans and Neff
251 2009), it is possible that our five study populations would typically be exposed to different
252 pathogen communities. However, differences between populations could also result, for
253 example, from genetic drift. We further found significant dam and sire by treatment
254 interactions, i.e. additive genetic variation for hatching plasticity. Such variability in reaction
255 norms could indicate a potential for adaptation in response to environmental changes
256 (Hutchings 2011). However, as dams and sires were nested in population, family effects
257 cannot fully be disentangled from potential population differences.
258 The study populations not only differed in their reaction to water-borne cues but also
259 in the genetic organization of hatching time. While our overall analysis showed significant
260 amounts of additive genetic and maternal environment variation in hatching time across
261 treatments, closer examination within populations revealed more variable patterns. Certain
262 populations seemed to have little genetic variation for hatching time under benign conditions.
263 This finding would meet traditional expectations for life-history traits, which anticipate
264 reduced genetic variation as a consequence of directional selection (Mousseau and Roff 1987)
265 or stabilizing selection (Fisher 1930). However, significant additive genetic variance for the
266 trait was found in some other populations, conforming with studies which have shown
267 considerable heritable variation in fitness-related traits (Houle 1992; Merilä and Sheldon
268 1999).
269 The water-borne cues we used likely consisted of a complex mixture of chemicals of
270 both pathogen and embryo origin. On the one hand, these chemicals could mainly convey an
271 imminent danger to embryos, as it did in Kiesecker et al. (1999) and Wedekind (2002). On the
272 other hand, they may also relay other kinds of information that may be important in other than
273 the host-parasite context, e.g. they could reveal that conspecifics were in the environment.
274 The presence or absence of conspecifics has been shown to induce or delay hatching time in
275 other species (Miner et al. 2010). It therefore remains unclear what aspect of a potential
276 multiple signal the embryos reacted to in our experiment. Additionally, it remains to be seen
277 how the difference in the timing of exposure to pathogen versus the water-borne cues may
278 have impacted the behavior response to treatment. Nevertheless, the population differences
279 we observed suggests that hatching time in brown trout is not only dependent on
280 environmental factors, but that these environmental factors are differently weighted by
281 embryos of different origins.
282 Despite significant overall dam and sire effects on embryo mortality, we found no
283 significant population x treatment effects on mortality, indicating a more or less uniform
284 susceptibility to infection. We further found no sire or dam by treatment interactions,
285 indicating a lack of genetic variability in survival reaction norms. Lack of variation in
286 reaction norms at both the family and population level could have important implications on
287 the species' ability to cope with infectious disease—a stressor which is anticipated to increase
288 in occurrence due to anthropogenic activities (Dewitt et al. 1998; Harvell 1999; Dobson and
289 Foufopoulos 2001). However, significant gene by environment interactions on larval survival

could be found in other salmonids, e.g. in the Chinook salmon (*Oncorhynchus tshawytscha*) (Evans et al. 2010) or in whitefish (*Coregonus* sp.) (von Siebenthal et al. 2009), and even in other populations of brown trout (Wedekind et al. 2008; Jacob et al. 2010). Genetic variability in reaction norms may therefore depend on the kind of stressor, the developmental stage, and the species or population in question.

As with pathogen susceptibility, we found significant additive genetic variation for hatching time. In addition, we found genetic variation in hatching plasticity after exposing embryos to water-borne cues of infection. We further observed that hatching time was correlated across control and WBC treatments in the majority of the populations, indicating that trait expression is mediated by the same loci in different environments (Via 1984). Trait means, therefore, may not be able to evolve independently, which places a constraint on the evolutionary potential of the reaction norm (Pigliucci 2001).

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Ethical standards

Permissions for handling embryos were granted by the local authority (i.e. the Fishery Inspectorate of the Bern canton). The manipulations of the adults were part of the yearly hatchery program of the Bern canton. Experimental manipulations on embryos were performed prior to yolk sac absorption. All manipulations comply with the current law of the country in which they were performed (Switzerland). The authors declare that they have no conflict of interest.

References

Agrawal A, Conner J, Johnson M, Wallsgrove R (2002) Ecological genetics of an induced plant defense against herbivores: additive genetic variance and costs of phenotypic plasticity. Evolution 56:2206-2213

Anderson AL, Brown WD (2009) Plasticity of hatching in green frogs (*Rana clamitans*) to both egg and tadpole predators. Herpetologica 65:207-213

Austin B, Austin DA (2007) Bacterial fish pathogens: disease of farmed and wild fish, 4th edn. Springer Praxis Publishing Ltd, Chichester, UK

Bates D, Sarkar D (2007) lme4: Linear mixed-effects models using S4 classes (R package); http://cran.r-project.org/web/packages/lme4

Chivers DP, Kiesecker JM, Marco A, De Vito J, Anderson MT, Blaustein AR (2001) Predator-induced life history changes in amphibians: egg predation induces hatching. Oikos 92:135-142

Crozier LG, Hendry AP, Lawson PW, Quinn TP, Mantua NJ, Battin J, Shaw RG, Huey RB (2008) Potential responses to climate change in organisms with complex life histories: evolution and plasticity in Pacific salmon. Evol Appl 1:252-270

Dewitt TJ, Sih A, Wilson DS (1998) Costs and limits of phenotypic plasticity. Trends Ecol Evol 13:77-81

Dobson A, Foufopoulos J (2001) Emerging infectious pathogens of wildlife. Philos T Roy Soc B 356:1001-1012

340 Einum S, Fleming I (2000) Selection against late emergence and small offspring in Atlantic
341 salmon (*Salmo salar*). Evolution 54:628-639
342 Evans ML, Neff BD (2009) Major histocompatibility complex heterozygote advantage and
343 widespread bacterial infections in populations of Chinook salmon (*Oncorhynchus*
344 *tshawytscha*). Mol Ecol 18:4716-4729
345 Evans ML, Neff BD, Heath DD (2010) Quantitative genetic and translocation experiments
346 reveal genotype-by-environment effects on juvenile life-history traits in two
347 populations of Chinook salmon (*Oncorhynchus tshawytscha*). J Evol Biol 23:687-698
348 Fisher RA (1930) The genetical theory of natural selection. Clarendon Press, Oxford, England
349 Gomez-Mestre I, Touchon JC, Saccoccio VL, Warkentin KM (2008) Genetic variation in
350 pathogen-induced early hatching of toad embryos. J Evol Biol 21:791-800
351 Gomez-Mestre I, Touchon JC, Warkentin KM (2006) Amphibian embryo and parental
352 defenses and a larval predator reduce egg mortality from water mold. Ecology
353 87:2570-2581
354 Gomez-Mestre I, Warkentin KM (2007) To hatch and hatch not: similar selective trade-offs
355 but different responses to egg predators in two closely related, syntopic treefrogs.
356 Oecologia 153:197-206
357 Harvell CD (1999) Emerging marine diseases - climate links and anthropogenic factors.
358 Science 285:1505-1510
359 Hoffmann A, Merilä J (1999) Heritable variation and evolution under favourable and
360 unfavourable conditions. Trends Ecol Evol 14:96-101
361 Holt JA, Krieg NR, Sneath PHA, Staley JT, Williams ST (1994) Bergey's manual of
362 determinative bacteriology, 9th edn. Williams & Wilkins, Baltimore, USA, p 5
363 Houle D (1992) Comparing evolvability and variability of quantitative traits. Genetics
364 130:195-204
365 Hutchings JA (2011) Old wine in new bottles: reaction norms in salmonid fishes. Heredity
366 106:421-437
367 Jacob A, Evanno G, von Siebenthal BA, Grossen C, Wedekind C (2010) Effects of different
368 mating scenarios on embryo viability in brown trout. Mol Ecol 19: 5296-5307
369 Jacob A, Nusslé S, Britschgi A, Evanno G, Müller R, Wedekind C (2007) Male dominance
370 linked to size and age, but not to 'good genes' in brown trout (*Salmo trutta*). BMC
371 Evol Biol 7:207
372 Jensen LF, Hansen MM, Pertoldi C, Holdensgaard G, Mensberg K-LD, Loeschcke V (2008)
373 Local adaptation in brown trout early life-history traits: implications for climate
374 change adaptability. Proc R Soc Lond B 275:2859-2868
375 JMP V (1989-2007) SAS Institute Inc., Cary, NC
376 Johnson PTJ, Kellermanns E, Bowerman J (2011) Critical windows of disease risk:
377 amphibian pathology driven by developmental changes in host resistance and
378 tolerance. Funct Ecol 25:726-734.
379 Kearsey M, Pooni H (1996) The genetical analyses of quantitative traits. Chapman and Hall,
380 London
381 Kiesecker J, Skelly D, Beard K, Preisser E (1999) Behavioral reduction of infection risk. P
382 Natl Acad Sci USA 96:9165-9168
383 Kraft P, Wilson R, Franklin C, Blows M (2006) Substantial changes in the genetic basis of
384 tadpole morphology of *Rana lessonae* in the presence of predators. J Evol Biol
385 19:1813–1818
386 Kusch RC, Chivers DP (2004) The effects of crayfish predation on phenotypic and life-
387 history variation in fathead minnows. Can J Zool 82:917-921

388 Laugen AT, Kruuk LEB, Laurila A, Rasanen K, Stone J, Merilä J (2005) Quantitative
389 genetics of larval life-history traits in *Rana temporaria* in different environmental
390 conditions. Genet Res 86:161-170
391 Laurila A, Karttunen S, Merilä J (2002) Adaptive phenotypic plasticity and genetics of larval
392 life histories in two *Rana temporaria* populations. Evolution 56:617-627
393 Lynch M, Walsh B (1998) Genetics and analysis of quantitative traits. Sinauer Associates Inc,
394 Sunderland, Massachusetts
395 Martin KLM (1999) Ready and waiting: delayed hatching and extended incubation of
396 anamniotic vertebrate terrestrial eggs. Integr Comp Biol 39:279
397 Merilä J (1997) Expression of genetic variation in body size of the collared flycatcher under
398 different environmental conditions. Evolution 51:526–536
399 Merilä J, Fry JD (1998) Genetic variation and causes of genotype-environment interaction in
400 the body size of blue tit (*Parus caeruleus*). Genetics 148:1233–1244
401 Merilä J, Sheldon BC (1999) Genetic architecture of fitness and nonfitness traits: empirical
402 patterns and development of ideas. Heredity 83:103-109
403 Merilä J, Söderman F, O'Hara R, Räsänen K, Laurila A (2004) Local adaptation and genetics
404 of acid-stress tolerance in the moor frog, *Rana arvalis*. Conserv Genet 5:513-527
405 Miner BG, Donovan DA, Andrews KE (2010) Should I stay or should I go: predator- and
406 conspecific-induced hatching in a marine snail. Oecologia 163:69-78
407 Moore R, Newton B, Sih A (1996) Delayed hatching as a response of streamside salamander
408 eggs to chemical cues from predatory sunfish. Oikos 77:331-335
409 Moreira PL, Barata M (2005) Egg mortality and early embryo hatching caused by fungal
410 infection of Iberian rock lizard (*Lacerta monticola*) clutches. Herpetol J 15:265-272
411 Mousseau TA, Roff DA (1987) Natural selection and the heritability of fitness components.
412 Heredity 59:181–197
413 OECD (1992) OECD guideline for the testing of chemicals 203 (fish acute toxicity test),
414 Annex 2. 9; http://www.oecd-ilibrary.org, Paris, France
415 Pakkasmaa S, Merilä J, O'Hara RB (2003) Genetic and maternal effect influences on viability
416 of common frog tadpoles under different environmental conditions. Heredity 91:117-
417 124
418 Pigliucci M (2001) Phenotypic plasticity beyond nature and nurture. The Johns Hopkins
419 University Press, Baltimore
420 R Development Core Team (2006) R: A language and environment for statistical computing.
421 In. R Foundation for Statistical Computing; http://www.R-project.org, Vienna, Austria
422 Reed TE, Schindler DE, Waples RS (2011) Interacting effects of phenotypic plasticity and
423 evolution on population persistence in a changing climate. Conserv Biol 25:56-63
424 Relyea RA (2005) The heritability of inducible defenses in tadpoles. J Evol Biol 18:856-866
425 Roff DA (1997) Evolutionary quantitative genetics. Chapman & Hall, London
426 Rowe L, Ludwig D (1991) Size and timing of metamorphosis in complex life cycles: time
427 constraints and variation. Ecology 72:413-427
428 Sambali G, Mehrotra R (2009) Principles of microbiology. Tata McGraw Hill, Delhi, India
429 Scarpellini M, Franzetti L, Galli A (2004) Development of PCR assay to identify
430 *Pseudomonas fluorescens* and its biotope. FEMS Microbiol Lett 236:257-260
431 Schalk G, Forbes M, Weatherhead P (2002) Developmental plasticity and growth rates of
432 green frog (*Rana clamitans*) embryos and tadpoles in relation to a leech (*Macrobdella*
433 *decora*) predator. Copeia 2002:445-449
434 Schotthoefer AM, Koehler AV, Meteyer CU, Cole RA (2003) Influence of *Ribeiroia*
435 *ondatrae* (Trematoda : Digenea) infection on limb development and survival of
436 northern leopard frogs (*Rana pipiens*): effects of host stage and parasite-exposure
437 level. Can J Zool 81:1144-1153

438 Sih A, Moore RD (1993) Delayed hatching of salamander eggs in response to enhanced larval
439 predation risk. Am Nat 142:947-960
440 Smith GR, Fortune DT (2009) Hatching plasticity of wood frog (*Rana sylvatica*) eggs in
441 response to mosquitofish (*Gambusia affinis*) cues. Herpetol Conserv Biol 4:43-47
442 Sollid SA, Lorz HV, Stevens DG, Bartholomew JL (2003) Age-dependent susceptibility of
443 Chinook salmon to *Myxobolus cerebralis* and effects of sustained parasite challenges.
444 J Aquat Anim Health 15:136-146.
445 Stelkens RB, Jaffuel G, Escher M, Wedekind C (2012) Genetic and phenotypic population
446 divergence on a microgeographic scale in brown trout. Mol Ecol 21:2896-2915
447 Touchon J, Gomez-Mestre I, Warkentin K (2006) Hatching plasticity in two temperate
448 anurans: responses to a pathogen and predation cues. Can J Zool 84:556-563
449 Via S (1984) The quantitative genetics of polyphagy in an insect herbivore. II. Genetic
450 correlations in larval performance within and among host plants. Evolution 38:896-
451 905
452 von Siebenthal BA, Jacob A, Wedekind C (2009) Tolerance of whitefish embryos to
453 *Pseudomonas fluorescens* linked to genetic and maternal effects, and reduced by
454 previous exposure. Fish Shellfish Immunol 26:531-535
455 Vonesh JR (2005) Egg predation and predator-induced hatching plasticity in the African reed
456 frog, *Hyperolius spinigularis*. Oikos 110:241-252
457 Warkentin KM (2007) Oxygen, gills, and embryo behavior: mechanisms of adaptive plasticity
458 in hatching. Comp Biochem Physiol A 148:720-731
459 Warkentin KM (2011) Environmentally cued hatching across taxa: embryos respond to risk
460 and opportunity. Integr Comp Biol 51:14-25
461 Warkentin KM, Currie CR, Rehner SA (2001) Egg-killing fungus induces early hatching of
462 red-eyed treefrog eggs. Ecology 82:2860-2869
463 Wedekind C (2002) Induced hatching to avoid infectious egg disease in whitefish. Curr Biol
464 12:69-71
465 Wedekind C, Müller R (2005) Risk-induced early hatching in salmonids. Ecology 86:2525-
466 2529
467 Wedekind C, Walker M, Portmann J, Cenni B, Müller R, Binz T (2004) MHC-linked
468 susceptibility to a bacterial infection, but no MHC- linked cryptic female choice in
469 whitefish. J Evol Biol 17:11-18
470 Wedekind, C., Jacob, A., Evanno, G., Nusslé, S. & Müller, R. (2008) Viability of brown trout
471 embryos positively linked to melanin-based but negatively to carotenoid-based colours
472 of their fathers. Proc R Soc Lond B 275: 1737-1744
473 Werner EE (1986) Amphibian metamorphosis: Growth-rate, predation risk, and the optimal
474 size at transformation. Am Nat 128:319-341
475 Werner EE, Gilliam JF (1984) The ontogenetic niche and species interactions in size
476 structured populations. Annu Rev Ecol Syst 15:393-425
477 Zuur AF, Ieno EN, Walker NJ, Saveliev AA, Smith GM (2009) Mixed effects models and
478 extensions in ecology with R. Springer, New York
479

480 Table 1 Likelihood ratio tests on mixed model logistic regressions on (a) embryo survival,
481 and (b) hatching time. Two treatments are compared each, i.e. control vs. infected and control
482 vs. WBC (see main text). The Akaike information criterion (AIC) indicates the goodness of
483 fit of a model. P-values were obtained from comparisons between the respective model and
484 the reference model (in bold)

Model	Effect tested	DF	AIC	X^2	P
(a) Survival					
Control vs Infected					
T+P+D+S		5	520.9		
T+T|P+D+S	Treat. x Population	7	524.5	0.4	0.81
T+P+T|D+T|S	Treat. x Dam	7	523.3	1.6	0.44
T+P+D+T|S	Treat. x Sire	7	520.4	4.5	0.10
T+D+S	Population	4	518.9	0.0	1.00
T+P+S	Dam	4	580.3	61.3	<0.0001
T+P+D	Sire	4	608.8	89.9	<0.0001
P+D+S	Treatment	4	1754.2	1235.3	<0.0001
(b) Hatching time					
Control vs WBC					
T+P+D+S		6	9423.6		
T+T|P+D+S	Treat. x Population	8	9371.9	55.6	<0.0001
T+P+T|D+S	Treat. x Dam	8	9393.2	34.4	<0.0001
T+P+D+T|S	Treat. x Sire	8	9389.1	38.4	<0.0001
T+D+S	Population	5	9424.5	2.9	0.09
T+P+S	Dam	5	9835.2	413.6	<0.0001
T+P+D	Sire	5	9544.5	122.9	<0.0001
P+D+S	Treatment	5	9422.0	0.4	0.52

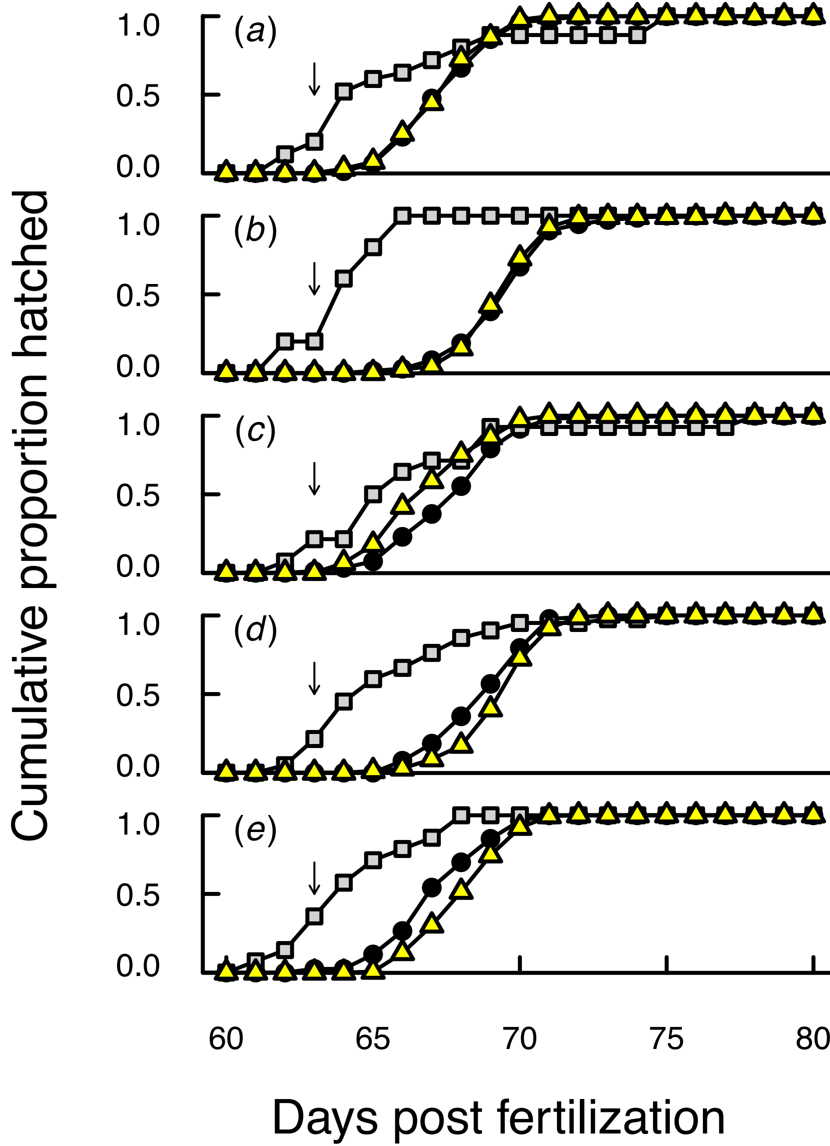
485

486 Table 2 Within-environment variance components (V_A: additive genetic variance, V_M:
487 maternal effect variance, V_{RES}: residual variance), narrow sense heritability estimate (h^2) and
488 coefficients of additive genetic variation (CV_A) for hatching time in the control and the WBC
489 treatment. Numbers in parentheses indicate standard errors; asterisks indicate whether males
490 explain a significant part of the variance (see main text)
491

Population	V_A	V_M	V_{RES}	h^2	CV_A
Hatching time					
(a) *Control*					
Kiese	6.2 (14.2)	40.9 (5.0)	80.9 (16.9)	0.05 (0.12)	0.56 (0.52)
Aare	108.8 (17.3)**	-2.7 (6.1)	85.4 (24.8)	0.79 (0.14)	2.30 (0.56)
Gürbe	132.7 (19.2)***	31.8 (6.3)	81.4 (26.0)	0.74 (0.12)	2.60 (0.60)
Giesse	4.5 (13.4)	31.0 (4.5)	82.6 (13.2)	0.04 (0.12)	0.47 (0.49)
Amletenbach	0.0 (15.7)	38.5 (5.2)	96.6 (17.4)	0.00 (0.12)	0.00 (0.54)
(b) *WBC*					
Kiese	4.7 (6.3)	49.1 (2.1)	63.0 (9.8)	0.04 (0.06)	0.49 (0.34)
Aare	67.2 (6.6)***	-13.1 (2.3)	70.7 (17.8)	0.74 (0.08)	1.81 (0.35)
Gürbe	63.0 (7.9)***	47.6 (2.6)	78.0 (11.2)	0.40 (0.06)	1.82 (0.39)
Giesse	107.4 (5.1)***	-2.6 (1.7)	47.3 (10.4)	1.09 (0.06)	2.29 (0.30)
Amletenbach	28.2 (5.7)***	48.9 (1.9)	45.6 (8.1)	0.26 (0.06)	1.20 (0.33)

492 *p<0.05; **p<0.01; ***p<0.001
493

494 **Fig. 1** Cumulative proportion hatched per population (a: Kiese ; b: Aare; c: Gürbe; d: Giesse;
495 e: Amletenbach) and treatment (control: round symbols; *P. fluorescens*-treated: squares;
496 WBC-treated: triangles). The arrows indicate the timing of the WBC treatment
497



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