Intraspecific phenotypic variation in life history traits of *Daphnia galeata* populations in response to fish kairomones

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Phenotypic plasticity is the ability of a genotype to produce different phenotypes depending on the environment. It has an influence on the adaptive potential to environmental change and the capability to adapt locally. Adaptation to environmental change happens at the population level, thereby contributing to genotypic and phenotypic variation within a species. Predation is an important ecological factor structuring communities and maintaining species diversity. Prey developed different strategies to reduce their vulnerability to predators by changing their behavior, their morphology or their life history. Predator-induced life history responses in *Daphnia* have been investigated for decades, but intra-and inter-population variability was rarely addressed explicitly. We addressed this issue by conducting a common garden experiment with 24 clonal lines of European *Daphnia galeata* originating from four populations, each represented by six clonal lines. We recorded life history traits in the absence and presence of fish kairomones. Additionally, we looked at the shape of experimental individuals by conducting a geometric morphometric analysis, thus assessing predator-induced morphometric changes. Our data revealed high intraspecific phenotypic variation within and between four *D. galeata* populations, the potential to locally adapt to a vertebrate predator regime as well as an effect of the fish kairomones on morphology of *D. galeata*.

1 **Intraspecific phenotypic variation in life history traits of *Daphnia galeata* populations in**

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9 **Abstract**

10 Phenotypic plasticity is the ability of a genotype to produce different phenotypes depending on

11 the environment. It has an influence on the adaptive potential to environmental change and the

12 capability to adapt locally. Adaptation to environmental change happens at the population level,

13 thereby contributing to genotypic and phenotypic variation within a species. Predation is an

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16 behavior, their morphology or their life history. Predator-induced life history responses in

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18 addressed explicitly. We addressed this issue by conducting a common garden experiment with

19 24 clonal lines of European *Daphnia galeata* originating from four populations, each represented

20 by six clonal lines. We recorded life history traits in the absence and presence of fish kairomones.

21 Additionally, we looked at the shape of experimental individuals by conducting a geometric

22 morphometric analysis, thus assessing predator-induced morphometric changes. Our data

23 revealed high intraspecific phenotypic variation within and between four *D. galeata* populations,

24 the potential to locally adapt to a vertebrate predator regime as well as an effect of the fish

25 kairomones on morphology of *D. galeata*.

Introduction

27   Intraspecific phenotypic variation is crucial for the persistence of a population, since low

28   intra-population variation increases the risk of extinction (Bolnick et al. 2011; Scheiner and Holt

29   2012; Forsman 2014). Loss of phenotypic variation can be caused by the reduction of genetic

30   variation e.g. due to genetic drift (random loss of alleles) (e.g., Vanoverbeke and De Meester

31   2010; Bolnick et al. 2011), inbreeding depression (e.g., Lynch 1991; Swillen et al. 2015) or positive

32   selection (e.g., Biswas and Akey 2006). On the contrary, phenotypic variation can increase as a

33   consequence of environmental change (biotic and/or abiotic) as well as through an increase in

34   genetic variation, which in turn occurs through gene flow (migration), mutation and

35   recombination (Griffiths et al. 2000). Phenotypic variation 'is the fuel that feeds evolutionary

36   change' because natural selection acts on it (Stearns 1989). Phenotypic plasticity describes the

37   ability of genotypes to produce different phenotypes depending on the environment, helping

38   organisms to survive and reproduce in heterogeneous environment (e.g., Stearns 1989; Agrawal

39   2001). Phenotypic plasticity implies an adaptive potential to locally adapt to a changed

40   environment (Stearns 1989). If the phenotypically plastic organism produces a modified and

41   successful phenotype whose fitness (higher reproductive success) is higher than an unmodified

42   phenotype, the underlying genotype contributes more to the genetic make-up of the whole

43   population.

44   Predation structures whole communities (Werner and Peacor 2003; Beschta and Ripple

45   2009; Boaden and Kingsford 2015; Aldana et al. 2016), drives natural selection within populations

46   (Morgans and Ord 2013; Kuchta and Svensson 2014) and maintains species diversity (Estes et al.

47   2011; Fine 2015). Aquatic predators release chemical substances, so called kairomones, into the

48   surrounding waters which can be detected by their prey. Both vertebrates (e.g., Schoeppner and

49   Relyea 2009; Stibor 1992) and invertebrates (e.g., Machacek 1991; Stibor and Lüning 1994)

50   release kairomones, triggering specific phenotypic plastic responses such as morphological or

51   behavioral changes (e.g., Dodson 1989; Schoeppner and Relyea 2009). The predator-induced

52   defenses can be highly variable within a species, depending on factors such as the predator and

53   colonization histories (e.g., Eklöv and Svanbäck 2006; Kishida et al. 2007; Edgell and Neufeld

54   2008).

55   Invertebrate as well as vertebrate predator kairomones have been shown to cause

56   phenotypic plastic responses in *Daphnia*. These induced responses are predator specific and vary

57   across *Daphnia* species. Behavioral changes such as diel vertical migration (DVM) (Effertz and von

58   Elert 2015) and the associated metabolic costs (Dawidowicz and Loose 1992), aggregation and

59   escape response (Pijanowska 1997), reduction of ingestion and filtration rates (Rose et al 2003;

60   Beckerman et al 2007; Pestana et al 2013), depth selection (Cousyn et al. 2001), increased

61   alertness (Boersma et al. 1998) and diapause (production of resting eggs = ephippia) (Pijanowska

62   and Stolpe 1996) were reported for different daphnids exposed to vertebrate predator

63   kairomones (fish). Diverse morphological changes have been shown to occur in the presence of

64   kairomones of the invertebrate predator *Chaoborus*, such as the production of neck teeth in *D.*

65   *pulex* (Lüning 1995; Tollrian 1995) or the famous helmets of *D. longispina* (Brett 1992) and *D.*

66   *cucullata (*Agrawal et al. 1999). Recently Herzog *et al.* (2016) observed a remarkable

67   morphological change of *D. barbata* exposed to *Triops* kairomones. *D. barbata* changes its whole

68   body symmetry to an S-shape, presumably to impede ingestion by their invertebrate predator.

69   Apart from morphology, physiology and behavior, predator kairomones were also shown to

70   influence life history traits in different *Daphnia* species. Among others, size and fecundity, two

71   important traits for population survival, were affected, resulting in earlier maturation (Riessen

72   1999; De Meester and Weider 1999; Weber 2003; Castro et al. 2007) and smaller size (Stibor and

73   Lüning 1994; Castro et al 2007). Size is a very important factor for survival in the face of fish

74   predation, since small individuals are more likely to go undetected. These predator-induced

75   responses are the result of phenotypic plasticity and their magnitude might play a role in

76   adaptation.

77   The ability of *Daphnia* to locally adapt to different stressors has been demonstrated

78   before, e.g. for fish as a vertebrate predator (Boersma et al. 1998; Cousyn et al. 2001; Declerck

79   and Weber 2003) and pesticides (Jansen et al. 2011). Differences in phenotypic responses

80   between *Daphnia* populations can further happen because of the high genetic divergence

81   between lakes, which is turn due to specific colonization patterns in *Daphnia* (De Meester et al.

82   2002), monopolization effects, and overall lack of gene flow. Hence, we expect the investigated

83   populations to exhibit population specific responses. Clonal variation of *Daphnia* has been

84   regularly reported (e.g., Machacek 1991; Castro et al. 2007; Beckerman et al. 2010). Some

85   investigators evaluated clonal variation within one population (Cousyn et al. 2001; De Meester

86   and Weider 1999) or among populations of *Daphnia* (Boersma et al. 1998; Declerck and Weber

87   2003; Boeing et al. 2006; Hamrová et al. 2011; Lind et al. 2015). Others rarely used more than

88   one or two clonal lines per population, drawing conclusions based on single clonal lines. Although

89   intra-population variation or lack thereof is relevant to population maintenance in the face of

90   predation pressure, the relative importance of the intra- and inter-population variation was

91   rarely measured. We are aware of only a few studies which addressed this aspect of predator-

92   induced responses in *Daphnia*. Boersma et al. (1998) used four clonal lines of *D. magna* for each

93   of the four population showing that the strength and combination of responsive traits can differ

94   across genotypes (clonal lines). The clonal variation in *D. pulex* exposed to vertebrate (fish) and

95   invertebrate (*Chaoborus*) predator kairomones was assessed for migration behavior (Boing et al.

96   2006) and life history traits (Lind et al. 2015) at the inter- and intrapopulation level. Recently,

97   Reger et al. (2018) showed that predation drives local adaptation in phenotypic plasticity in 70

98   clonal lines of *D. pulex*.

99   In the present study, we assess the intraspecific phenotypic variation of European

100 *Daphnia galeata* in the presence of fish kairomones, by measuring shifts in life history traits as

101 well as morphological changes in a total of 24 clonal lines (six clonal lines within each of the four

102 populations). We expect that (i) there is intraspecific phenotypic variation, evidenced by

103 interclonal variation within as well as among populations. We hypothesize that (ii) local

104 adaptation and population divergence caused by drift and limited gene flow shape the observed

105 phenotypic variation, and is evidenced by population specific responses. Finally, we expect that

106 (iii) the exposure to fish kairomones affects the morphology in *D. galeata*. We hypothesize that

107 (iv) a correlation between life history change and morphology exists. Specifically, we hypothesize

108 that females which increased their total number of offspring in the presence of fish kairomones,

109 change their morphology towards a bulkier shape to accommodate more eggs.

110 **Materials and methods**

111 Experimental organisms and lakes of origin

112   This study integrated 24 *D. galeata* clonal lines from four different locations: Lake Constance
113   (popLC), Germany; Greifensee (popG), Switzerland; Müggelsee (popM), Germany and Jordan
114   Reservoir (popJ), Czech Republic. These are all permanent lakes with a large water body and
115   varying densities of planktivorous fish (Table 1).

116   Clonal lines were established from dormant eggs from sediment cores and have been used in
117   previous studies (Henning-Lucass et al. 2016; Herrmann et al. 2017). These ephippia originate
118   from sediment layers of the recent decade (2000-2010) and were subjected to hatching stimuli
119   described in detail in Henning-Lucass et al. (2016). The clonal lines were maintained in lab
120   cultures (18°C, 16h light / 8h dark cycle, food: *Acutodesmus obliquus*, medium: Aachener
121   Daphnien Medium (ADaM) (Klüttgen et al. 1994)) for up to 5 years and no less than 3 years prior
122   to the present experiment.

123   Fish densities and hence predation pressures are not easy to measure very precisely due
124   to seasonal variation (e.g., Fischer and Eckmann 1997), variation between years (e.g., Kruuk et
125   al. 1991) as well as differences between lakes (e.g., Mehner et al 2005). We provide here
126   estimates of fish biomass available from the literature corresponding as closely as possible to the
127   time period the resting eggs came from. The highest estimate of fish biomass was for the Jordan
128   Reservoir, which is stocked for recreational purposes and the lowest for Greifensee (Table 1).
129   Fish belonging to the *Leuciscus* genus were found in all lakes from which our clonal lines originate
130   (Baudirektion Kanton Zürich 2006; Kubecka and Bohm 1991; Alexander et al. 2016; Fischereiamt
131   Berlin 2013).

132   Media preparation

133   The basic medium was ADaM for fish and *Daphnia* cultures. Two types of media were used for
134   breeding and experimental conditions: fish kairomone and control medium. In total forty ide
135   (*Leuciscus idus*) were maintained in an aerated, separate 200L aquarium, in which they were fed
136   frozen *Daphnia* cubes and dry food. The ide or closely related species are present in all the
137   studied lakes (Table 1). Previous studies showed that ide elicit plastic responses in *D. galeata*
138   clonal lines from Lake Constance (Sakwinska 2002) and Greifensee (Wolinska et al 2007). Fish
139   medium was obtained by keeping 5 randomly chosen ide in an aerated 20L aquarium for 24h to

140   produce fish kairomone medium. The fish were not fed in the fish medium production tank to

141   avoid *Daphnia* alarm cues to be mixed with the fish kairomones. The fish kairomone media

142   imitates a scenario of high fish density (Cousyn et al. 2001; Swillen et al. 2015). Control medium

143   was produced in an aerated, separated aquarium and handled first, before handling of fish and

144   fish medium. All media was filtered before use to remove feces from predators and bacteria

145   larger than 1.2µm (Whatman, membrane filters, ME28, Mixed cellulose-ester, 1.2µm). All media

146   were supplemented with 1.0 mg C L-1, P rich *Acutodesmus obliquus* before use and exchanged

147   daily (1:2) to guarantee a nutrient rich environment and a constant fish kairomone concentration.

148   The algae concentration was calculated from photometric measurement of the absorbance rate

149   at 800 nm.

150   Because fish was used to produce fish kairomone media, this experiment was subject to approval

151   through the "Behörde für Gesundheit und Verbraucherschutz" of the City of Hamburg. It was

152   approved under the number 75/15 and the corresponding document can be found in the

153   supplementary materials.

154   Experimental design and procedures: life table experiment

155   Prior to the experiment, each clonal line was bred in kairomone-free water (control environment)

156   and in kairomone water (fish environment) for two subsequent generations to minimize inter-

157   individual variances. To this end, 10-15 egg-bearing females per clonal line were randomly

158   selected from mass cultures. From these females of unknown age, neonates were collected and

159   raised under experimental conditions and served as grandmothers (F0) for the experimental

160   animals (F2). Neonates of the 3rd to 5th brood carried by the F0 animals were used as breeding

161   (F1) animals. Neonates of the 3rd to 5th brood carried by the F1 animals were used in turn as

162   experimental individuals (F2). A pair of neonates was introduced in the experimental vessels (50

163   mL glass tube) at the start of the experiment to compensate for eventual mortality. One of the

164   individuals was randomly discarded when necessary at day 4 (t4), so that one individual remained

165   in each vessel. This procedure was applied to F1 and F2 individuals. Fifteen replicates were used

166   per environment and per genotype (clonal line). Sister neonates of F2 (n=15) were collected in

167   70% ethanol for size measurements at day 0 (t0). Life history parameters were recorded daily

168   during the experiment. Before media renewal, females were checked for maturation and

169   neonates were counted, removed and preserved in ethanol every day. Adults were preserved in

170   ethanol as well at the end of the experiment. The experiment lasted for 14 days (t14) for each

171   experimental individual to monitor the performance of each clonal line within a fixed period of

172   time.

173   Cetyl alcohol was used to break the surface tension of the media during breeding and the

174   experiment to reduce juvenile mortality (Desmarais 1997). Breeding and experimental phases

175   were conducted at a temperature of 20°C and a 16h light / 8h dark cycle in a brood chamber with

176   a light intensity of 30% (Rumed, Type 3201D).

177   The experiment was conducted in three experimental rounds due to logistic reasons, and clonal

178   lines from all four populations were includedin each round (Table S1). Previous pilot studies

179   showed that ensuring synchronicity of so many clonal lines at once were extremely difficult.

180   Data collection and analysis

181   Life history traits

182   Life history parameters such as age at first reproduction ('AFR') [d], number of neonates per

183   brood per female, total number of broods per female ('broods'), total numbers of neonates per

184   female ('offspring'), size of first clutch ('brood1') [number of neonates per female], 'survival' [%]

185   and somatic growth rate ('SGR') [$\mu m\ d^{-1}$] were recorded. Age at first reproduction was the day of

186   releasing the first brood from the brood pouch, with neonates swimming in the vessel. For further

187   analysis the average value of the 15 individuals per clonal line (genotype) per environment

188   (treatment) was calculated for each life history trait to estimate the clonal response to a

189   kairomone (fish) vs. kairomone-free (control) environment. Survival rate was defined as the

190   proportion of females surviving from the day of separation (t4) until the end of the experiment

191   (t14). Reproductive rate was calculated by dividing the total number of offspring per female by

192   the total number of broods per female. Relative fitness (w) was calculated by multiplying survival

193   and reproductive rate of a clonal line before dividing by the maximum survival and reproductive

194   rate of the other clonal lines within population and among all populations. Some clonal lines

195   produced male offspring during breeding and the experiment. Males occurred at very low

196   frequencies and were excluded from the data analysis. We aimed to test a total of 720 individuals

197   in this experiment (24 clonal lines x 2 treatments x 15 replicates). In total we measured life history

198   traits for 684 experimental individuals (Table S1).

199   Digitizing of experimental animals for 'size' and 'shape' analysis

200   Digital photographs of *Daphnia* preserved in ethanol were taken with a stereomicroscope (Nikon

201   SMZ800N) at a magnification of 60x for neonates (t0) and 40x for adults (t14) with NIS-elements

202   4.3 software. All experimental individuals were photographed in lateral view (left body side up).

203   Measurement of body length ('size')

204   Body length was measured from the top of the head through the middle of the eye to the ventral

205   basis of the spine, excluding the spine itself. Somatic growth rate ('SGR', µm/day) was calculated

206   by subtracting the average length of neonates at the beginning of the experiment (t0; n=15) from

207   the length of each adult individual at the end of the experiment (t14), divided by the complete

208   experimental time in days. The measurement error of digitizing and measuring the length of the

209   same individual 10 times was +/-3.24 µm (SD). The measurement error of measuring 10 times

210   the length of an individual using the exact same picture was +/-1.67 µm (SD).

211   Geometric morphometric analysis of the 'shape' of the body

212   Since the morphology of *Daphnia* does not allow the assignment of many landmarks, we decided

213   to use the semilandmark approach. Semilandmarks are a set of individual landmarks which are

214   interpolated to represent the curve of a structure (Zelditch et al. 2004). Landmarks and

215   semilandmarks were assigned on a subset of digital images of adult experimental individuals

216   (max. n=10 per clonal line and environment, with a total of 459 individuals; Table S1) according

217   to Zelditch et al. 2004. In total three landmarks and 115 semilandmarks were assigned on each

218   individual photograph. The first landmark was appointed to the tip of the rostrum, the second in

219   the middle of the eye and the third at the ventral basis of the spine. In our study the first curve

220   consisted of 70 interpolated landmarks (=semilandmarks) along the dorsal body outline, starting

221   at the first landmark and ending on the dorsal basis of the spine. The second set of semilandmarks

222   consisted of 45 semilandmarks along the ventral body outline, starting at landmark three and

223   ending opposite of the dorsal basis of antenna. After the assignment of landmarks and
224   semilandmarks, X and Y coordinates were recorded using TpsDig2 (Rohlf 2015). A General
225   Procrustes Analysis (GPA) was performed using the package '*geomorph*' in R (Adams and Otárola-
226   Castillo 2013). The measurement variance for assigning landmarks and semilandmarks of an
227   individual using the exact same picture was <0.0001. Investigators of 'shape' measurements
228   worked with a blind data set, not knowing which individual belongs to which group (environment,
229   genotype and population).

230   Statistical analysis

231   All statistical analyses for life history traits were performed and all figures were created using R
232   version 3.3.1 (R Core Team 2016). For the generalized linear mixed models (GLMM) the package
233   '*lme4*' was used (Bates et al. 2015). Visualization of life history traits were performed by using
234   the package '*ggplot2*' (Wickham 2009). For the geometric morphometric analysis the package
235   '*geomorph*' was used (Adams and Otárola-Castillo 2013). The visualization of shape differences
236   was performed with the R package '*shapes*' (Dryden 2017). R scripts are provided in
237   supplementary materials.

238   To compare life history traits between the different populations in the presence and absence of
239   fish kairomones, we applied generalized linear mixed effect models for each trait, except 'shape'.
240   Visual inspection of residual plots as well as the Shapiro-Wilk-Test revealed deviations from
241   homoscedasticity for each trait, supporting the decision to use nonparametric models for
242   statistical analysis. Hence, error distributions were assigned individually per trait. We used
243   'Environment x Population' and 'Genotype x Environment' as fixed categorical factors in our
244   models. To account for genotype differences among populations, we included 'Clone' nested
245   within 'Population' as a random factor. To account for experimental rounds, we added 'round' as
246   a random factor. We checked for the necessity of random slopes and intercepts by the application
247   of likelihood ratio tests of the full model with the effect in question against the model without
248   the effect in question, finally resulting in a general random slope and intercept model for
249   'Environment x Population' (response ~ treatment*pop + (treatment|pop/clone) + (1|round))
250   and 'Genotype x Environment' (response ~ treatment*clone + (1|round)). Statistical significances

251   for life history traits were obtained by using the function Anova(model,type=2)) which performs

252   a Wald Chi-Square test.

253   To assess shape variation we used the principal component analysis (PCA) after the General

254   Procrustes Analysis (GPA) in the R package '*geomorph*'. Subsequently the statistical analysis was

255   done with Procrustes ANOVA and pairwise tests to reveal statistically relevant 'shape' differences

256   between groups.

257   **Results**

258   Effect of genotype origin on predator-induced response in life history traits: 'Environment x

259   Population' effect

260   Reaction norms of life history traits per population (Figure 1) as well as their boxplots (Figure 2)

261   show intra- and inter-population variation of life history traits. The statistical analysis revealed

262   that the factor 'Environment' affected the life history traits 'AFR' and 'broods' in a significant

263   manner. The factor 'Population' affected 'offspring', 'brood1', 'relnest' and 'relclone', but there

264   was no significant interaction effect of 'Environment x Population' for any life history trait (Table

265   2A). The visualization of growth differences between environments and populations (dSGR,

266   Figure 3) showed that all clonal lines from popG had a negative growth rate in the fish-exposed

267   environment, resulting in a smaller body size. Four out of six clonal lines from popJ had a negative

268   growth rate, while clonal lines from popLC and popM vary in growth rates across environments.

269   The fittest population in control environment was popJ (w=1), followed by popM (w=0.83), popLC

270   (w=0.78) and popG (w=0.67). In fish environment a small change of positions occurred for popLC

271   and popM. Here the decreasing order was popJ (w=1), followed by popLC (w=0.80), popM

272   (w=0.77) and popG (w=0.63) among all populations. Further details of relative fitness for each

273   clonal line within their population can be found in Table 3A and 3B.

274   Effect of 'Genotype x Environment' interaction on life history traits

275   In the model comprising the factors 'genotype' and 'environment', most of the traits were

276   significantly affected by both factors as well as their interaction: 'AFR', 'broods', 'offspring', 'SGR',

277   and 'length' (Table 2B). The traits 'brood1', 'relnest' and 'relclone' were only affected by the

278   factor 'Genotype' (Table 2B). Reaction norms for each life history trait of each clonal line can be

279   found in the supplementary material (Figure S2 to Figure S7).

280   Effect of fish kairomones on the morphological trait 'shape'

281   A total of 83% of 'shape' variation was explained by the first four principal components (PC1=

282   42%, PC2=24%, PC3=11% and PC4= 6%) (Figure S1).

283   The geometric morphometric analysis revealed a significant 'Environment' effect on 'shape'

284   (Df=455, F=5.93, Pr(>)F=0.001). Differences in shape occurred within each population except for

285   popG (Table 4A) as well as among populations (Table 4B). There was a significant interaction

286   effect of 'Environment x Population' on 'shape' (Df=3, F=1.9163, Pr(>)F=0.019). The *p*-value

287   matrix revealed a statistical significance difference within popLC between environments

288   (p=0.035; Table 4C). There was a statistical significant 'Genotype x Environment' effect on 'shape'

289   (Df=411, F=3.2947, Pr(>)F=0.047). Experimental 'round' was included in the geometric

290   morphometric analysis revealing its statistical significant effects on the morphological trait

291   'shape'.

292   The shape of females with many offspring (n>22 = upper quartile of total number of offspring)

293   differed significantly among populations in the control environment, but not in the fish

294   environment. There was no association between 'shape' and a high number of 'offspring' in the

295   fish environment. Further analysis revealed that the 'shape' of females with many offspring did

296   not differ significantly between environments within each population.

297   Visualization revealed an overall 'shape' change towards a smaller body. In detail, a homogenous

298   change from all directions to a smaller body form was found for popG (Figure 4B). Within popJ

299   the overall shape change towards a smaller body size was shown with the strongest change in

300   the head area (bending of the TPS) and an anterior-posterior direction (Figure 4C). Within popLC

301   the head position changed from dorsal to ventral direction, while a small change of the tail area

302   from a ventral to dorsal direction (Figure 4D) occurred. Within popM the overall shape change

303   towards a smaller body size was shown in the head area from a dorsal to ventral direction and in

304   the tail area from a ventral to dorsal direction (Figure 4E).

305 **Discussion**

306 Intraspecific phenotypic variation of life history traits in *D. galeata*

307 Our study revealed a significant 'Environment', 'Genotype' as well as 'Genotype x Environment'

308 effect for the life history traits 'AFR', 'broods', 'offspring', 'SGR' and 'size'. Concordant to previous

309 studies (Boersma et al. 1998; Stibor and Lüning 1994), our results showed a decrease of age at

310 first reproduction, a decrease of somatic growth rate and a decrease of body length in the

311 presence of fish kairomones in *Daphnia galeata.* Out of the 24 studied genotypes, 13 matured

312 earlier (Figure S2) and 17 reduced their body length (Figure S7) in the presence of fish

313 kairomones. Indeed, early maturation and a reduced size of *Daphnia* in the presence of

314 vertebrate predators have been reported before (Machacek 1991; Weider and Pijanowska 1993;

315 Lampert 1993; Gliwicz and Boavida 1996). The ecological benefit lies in a successful reproduction

316 before reaching a body size making the individual vulnerable to fish predation (Lynch 1980;

317 Lampert 1993).

318 The 'Genotype x Environment' effect for most of the life history traits implies that the presence

319 or absence of certain clonal lines within one population might have an effect on overall

320 population survival, depending on environmental factors such as predation risk. Hence, if the

321 phenotypic diversity within one population is reduced and the majority produces relatively less

322 offspring in a fish environment, the result could be an overall low number of offspring in the

323 following cohorts, which would threaten the persistence of the whole population. Notably,

324 individuals of popG produced less offspring and less broods compared to the other three

325 populations regardless of the environment and their relative fitness was comparatively low.

326 Potential explanations for this relative low performance of popG could be genetic drift and

327 inbreeding depression which have a negative effect on genetic diversity (Vanoverbeke and De

328 Meester 2010). However, low genetic variation for *D. galeata* in Greifensee was not identified

329 (Herrmann et al. 2017), making these two explanations unlikely at first glance. On the contrary,

330 most clonal lines in Greifensee (four out of six) had a higher heterozygosity than expected,

331 perhaps as a result of hybridization, which is known to occur in this population (e.g. Brede et al

332   2009). Therefore, outbreeding depression could explain lower fitness in popG and should be

333   further investigated in a future study.

334   Our experimental design allowed us to assess the distribution of variance at clonal as well as

335   population level. We thus detected phenotypic variation within each as well as among several

336   populations independent of the environment. We identified two different strategies of

337   phenotypic plastic responses of *Daphnia galeata* by comparing the 'Environment' effect within

338   as well as among the populations. In popJ, the variation of a trait itself, not the change in the trait

339   median value as a response was extremely reduced for two life history traits, 'AFR' and total

340   number of 'broods' (Figure 2C). Almost all individuals of popJ started to reproduce at the same

341   age and produce the same amount of broods in fish environment, showing a striking

342   homogeneity under stress. On the contrary, in popM the variation for 'AFR' increased, resulting

343   in a broader range of ages at first reproduction in fish environment. The phenotypic variation

344   between clonal lines was best visualized by plotting the differences of somatic growth rate (dSGR)

345   between the environments (Figure 3), unifying the environmental and genotype effect. All six

346   clonal lines of popG and four out of six clonal lines of popJ decreased their somatic growth in fish

347   environment, while the direction of response varies for popLC and popM. Overall our study with

348   a total of 24 clonal lines revealed a broad spectrum of phenotypic variation of life history traits

349   in European *D. galeata.*

350   To our surprise we did not find an 'Environment x Population' effect on the life history response,

351   although we observed intra- and inter-population differences, especially between the two

352   extremes popG and popJ. A significant 'Population' effect was found for the interdependent traits

353   total number of offspring ('offspring'), size of first brood ('brood1'), relative fitness within

354   ('relnest') and among populations ('relclone'). This observed significant population divergence

355   could be due to the extreme difference of total number of offspring between popG and popJ. In

356   general, genotypes in popJ produced the highest number of offspring among all populations. In

357   contrast, the total number of offspring of genotypes in popG was overall lower compared to the

358   other three populations, regardless of the environment. Even the increased number of offspring

359   for clonal lines of popG in fish environment is lower than the numbers of offspring for clonal lines

360   of popJ in control environment. Hence, the genotype origin ('Population') itself had little to no

361   effect on life history traits in *Daphnia* implying that the identity of a clonal line ('Genotype') within

362   a population seems to be more important than the origin of the clonal line *per se*.

363   In the end, we were not able to identify one main driving force influencing the phenotypic

364   variation of life history traits in *D. galeata*, and could not infer a population specific response.

365   Instead our study displays the complexity of the interacting factors 'Environment' and 'Genotype'

366   to produce a variety of phenotypes within one species, thereby contributing to the understanding

367   of intraspecific phenotypic variation.

368   Potential for local adaptation

369   Our findings allow the conclusion that there is potential for local adaptation to predation risk in

370   the investigated European populations of *D. galeata*. This conclusion was based on two outcomes

371   of our study.

372   On one hand, we observed an extreme predator-induced life history response for popJ. The range

373   of variation of the phenotypic response was reduced to a minimum in popJ, so that almost all

374   individuals of the six clonal lines and 15 replicates reproduce at the very same age when exposed

375   to fish (Figure 2A). On top of that, we observed a similar reduction of variation for the life history

376   trait total number of broods (Figure 2C). These strong responses could be explained by local

377   adaptation to the presence of fish. The Jordan Reservoir is an artificial inner city water reservoir,

378   used for recreational purposes such as fishing since 1900 (Kubecka and Bohm 1991) and had been

379   regularly stocked with fish (Seda et al 2000). Therefore, *D. galeata* of Jordan reservoir had the

380   possibility to adapt to an environment with a higher predation risk for more than a century. Such

381   micro-evolutionary changes for *Daphnia* species have been described in other contexts before.

382   For instance, Jansen et al. (2011) showed that *D. magna* was able to evolve resistance to a

383   pesticide (carbaryl) within experimental time. Further, Declerck et al. (2001) as well as Reger et

384   al (2018) showed that populations were able to locally adapt to fish kairomones (*D. galeata* and

385   *D. pulex,* respectively). Alternatively, since the reservoir, unlike the other lakes in this study, has

386   been created specifically with fishing in mind, differential colonization might also be the source

387   of the observed pattern. This habitat might have been colonized only by *Daphnia* pre-adapted to

388   fish, with very specific life-histories, leading to the present-day striking pattern.

389   On the other hand, the relative fitness of individuals of popJ suggests that females exposed to

390   fish kairomones are fitter, concurring with results obtained by Castro et al (2007) and Jansen et

391   al. (2011). Since local adaptation to a certain stressor implies a better performance in the stressful

392   environment than without this stressor (Lenormand et al. 1999; Joshi et al. 2001) we suggest that

393   the local adaptive potential exists for at least three populations because the relative fitness in

394   the presence of fish kairomones increased overall for 14 out of 24 clonal lines (popG=3, popJ=4,

395   popLC=4, popM=3) (Table 3A). Our results are in line with earlier studies showing the adaptive

396   potential of phenotypic plasticity in *Daphnia* exposed to different stressors (e.g., Yin et al. 2011;

397   Altshuler et al. 2011; Reger et al 2018; Hesse et al. 2012).

398   Predation risk and morphological changes

399   In general, we did not observe any predator-induced extreme morphological changes such as the

400   formation of helmets for fish kairomone exposed *Daphnia* as those reported for *D. lumholtzi*

401   (Laforsch and Tollrian 2004). We presented here the first study using the geometric

402   morphometric analysis to measure morphometric changes to an environmental factor in *D.*

403   *galeata*, hence complementing the traditional approaches (life history traits and behavior)*. Our*

404   morphometric analysis revealed that the presence of fish kairomones had an effect on the body

405   shape of *D. galeata*. However, no overall pattern was recognizable among the populations and

406   no effect was observed at all for popG. Instead we observed different changes of 'shape' in each

407   population. We suggest that the morphological trait 'shape' is phenotypically plastic due to high

408   clonal variation, which is consistent with the results reported by Dlouhá et al. (2010) and Zuykova

409   et al (2012). The difference between experimental rounds for 'shape' could be attributed to this

410   observed high clonal variation which we observed in all life history traits as well.

411   We hypothesized that life history change and morphological change are correlated, meaning that

412   females with a higher number of offspring (n>22, upper quartile of observed total number of

413   offspring) would change their 'shape' towards a bulkier body form to accommodate a greater

414   number of offspring within their brood pouch. This correlation was found only in individuals in

415   control environment and not for individuals in fish environment. Changing shape of the body

416   might come along with some drawbacks: the bulkier the shape, the higher the detection risk by

417   the predator and the slower the swimming ability due to drag. In fact, fish prey size-selectively

418   on *Daphnia,* meaning that larger *Daphnia* are preyed upon more often than smaller *Daphnia*

419   (e.g., Weber and Van Noordwijk 2002; Beckerman et al 2010). Since fish prey on faster swimming

420   individuals of *Daphnia* (O'Keefe et al 1998), being a slow swimming *Daphnia* would be beneficial.

421   Alternatively, accommodating more offspring without changing the shape of the body might be

422   achieved through the production of smaller offspring, as was shown by Castro et al (2007), among

423   others (e.g., Lampert 1993). In line with previous studies showing a predator-induced reduction

424   in neonate size, we can speculate that this is also the case in our experiment and plan to further

425   explore this dimension.

426   **Conclusions**

427   The study presented here focused on the assessment of intraspecific phenotypic variation in *D.*

428   *galeata.* By comparing the range of phenotypic response of 24 clonal lines originating from four

429   populations, we contribute to the understanding of the effect of environmental change (shifts in

430   predator regime) on intraspecific phenotypic variation at the population level. We observed high

431   clonal variation in all studied life history traits at the intra- and interpopulation level, leading to

432   the suggestion that single genotype studies on *Daphnia* might deliver biased conclusions.

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676

Figure 1

Reaction norms for selected life history traits showing population differences (mean +/- SE).

Population Greifensee (popG, yellow), population Jordan reservoir (popJ, black), population Lake Constance (popLC, magenta) and population Müggelsee (popM, green). (**A**) Age at first reproduction ('AFR'). (**B**) Total number of offspring first brood ('brood1'). (**C**) Total number of broods ('broods'). (**D**) Total number of offspring ('offspring'). (**E**) Somatic growth rate ('SGR'). (**F**) Body length ('size').

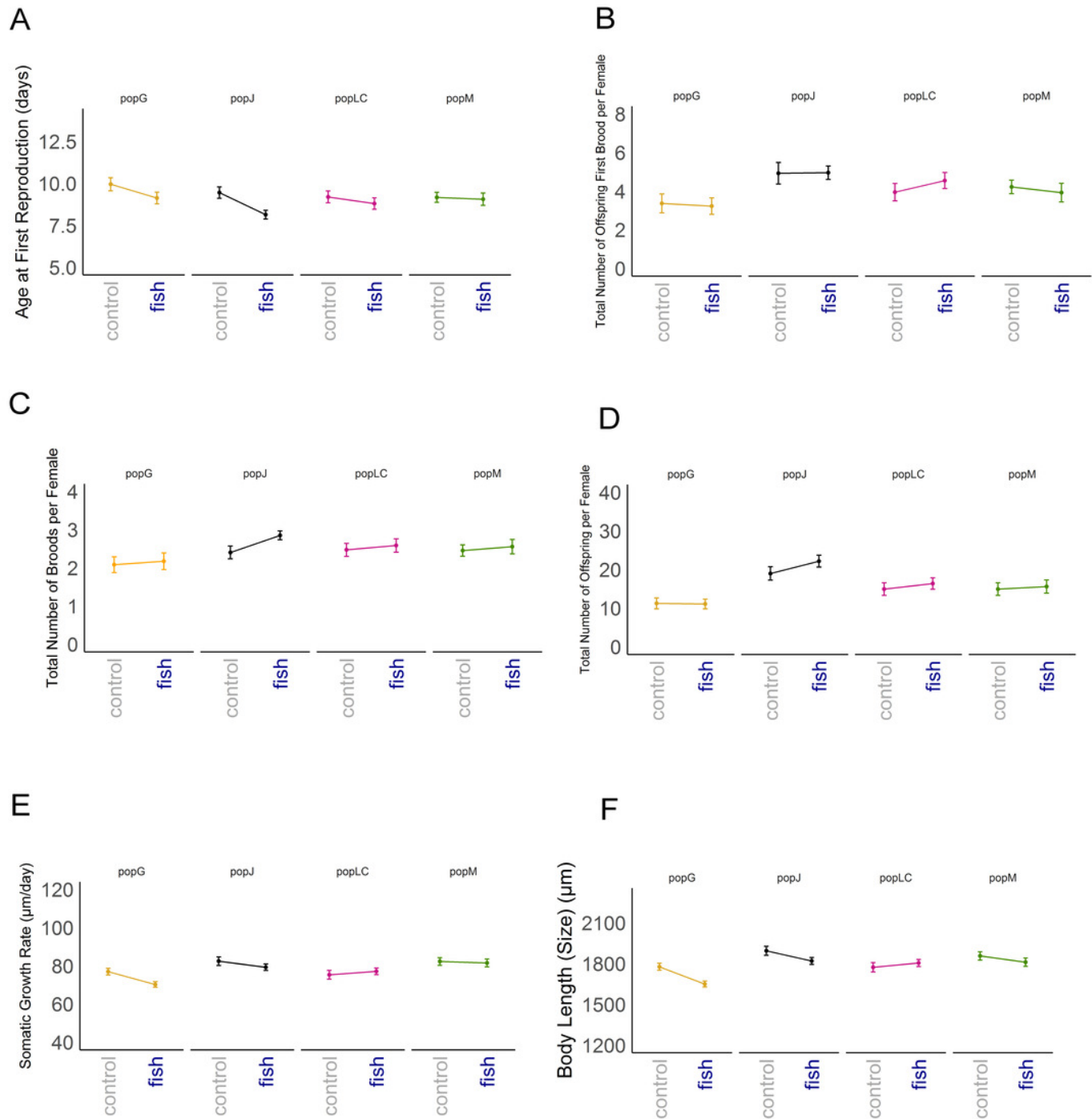



Figure 2

Boxplots for selected life history traits showing population differences (median +/- SD).

(**A**) Age at first reproduction ('AFR'). (**B**) Total number of offspring first brood ('brood1'). (**C**) Total number of broods ('broods') (**D**) Total number of offspring ('offspring'). (**E**) Somatic growth rate ('SGR'). (**F**) Body length ('size').

A

Age at First Reproduction

B

Clutch Size First Brood

C

Total Number of Broods

D

Total Number of Offspring

E

Somatic Growth Rate

F

Body Length (Size)

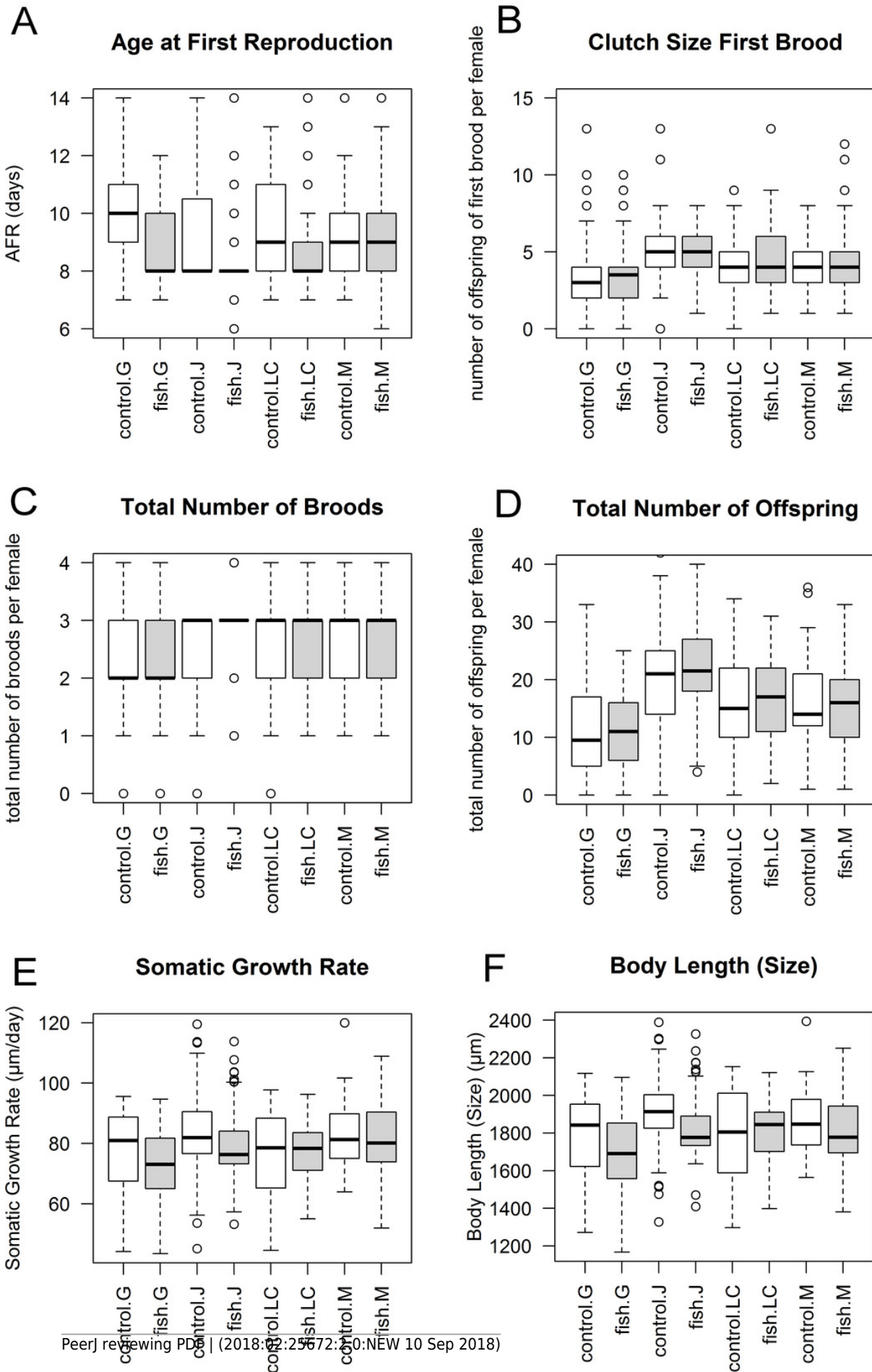



Figure 3

Differences of somatic growth rate (dSGR).

Differences of somatic growth rate (dSGR) as μm per day (mean +/-SD); calculated as: mean of SGR (fish) minus mean SGR (control) equals dSGR per genotype, sorted by populations.

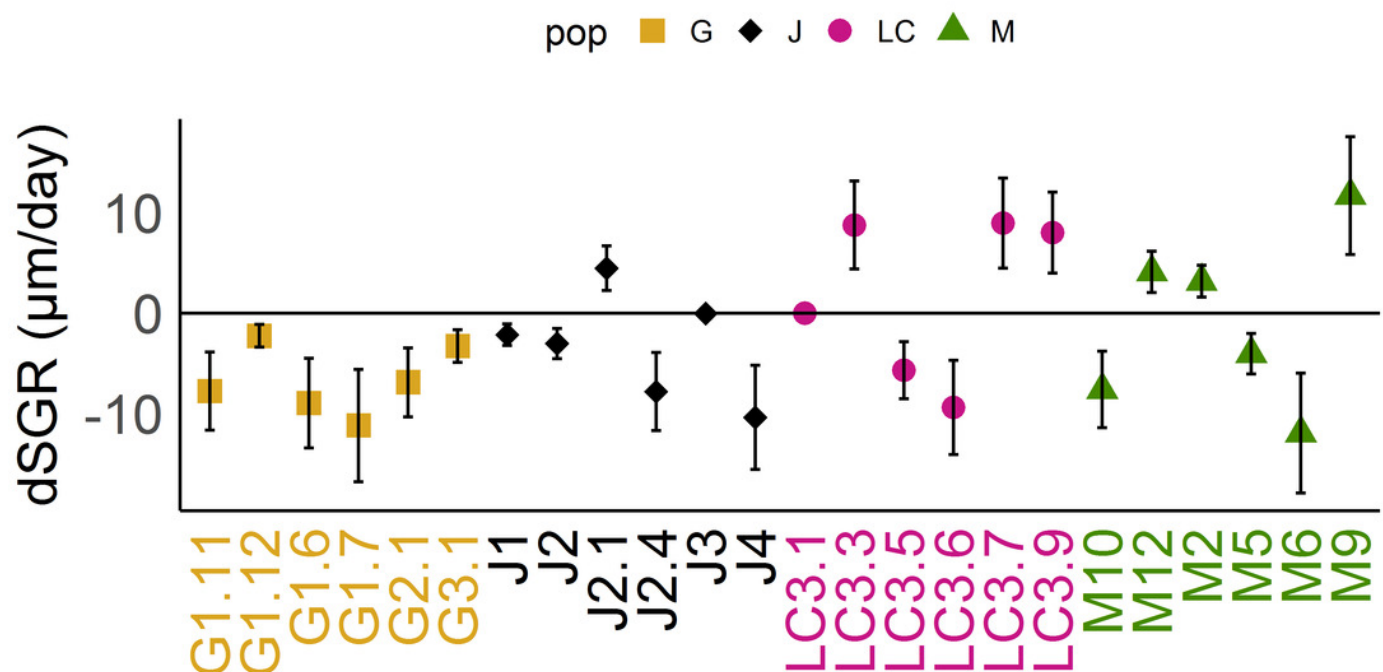
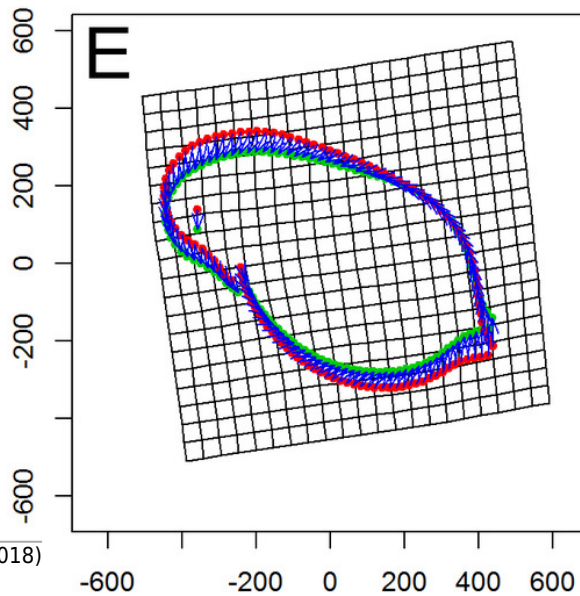
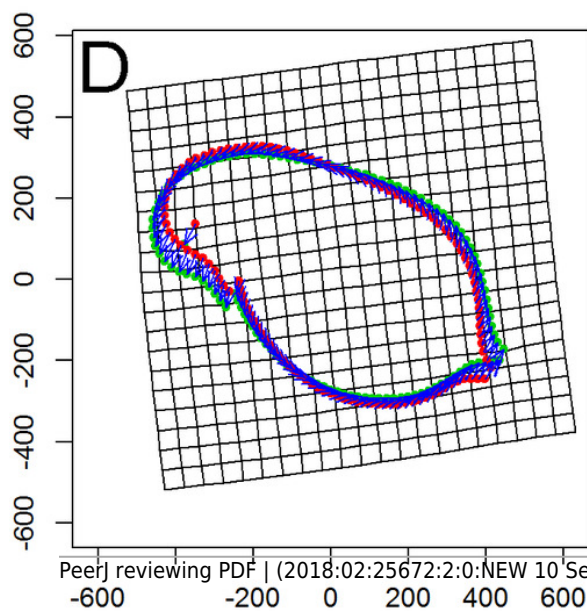
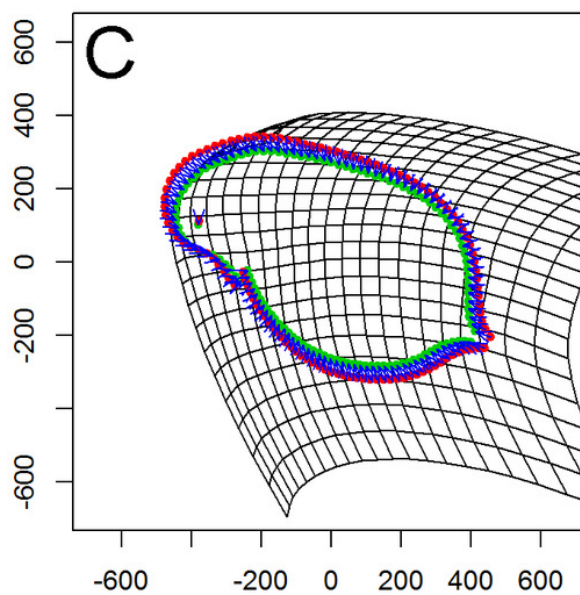
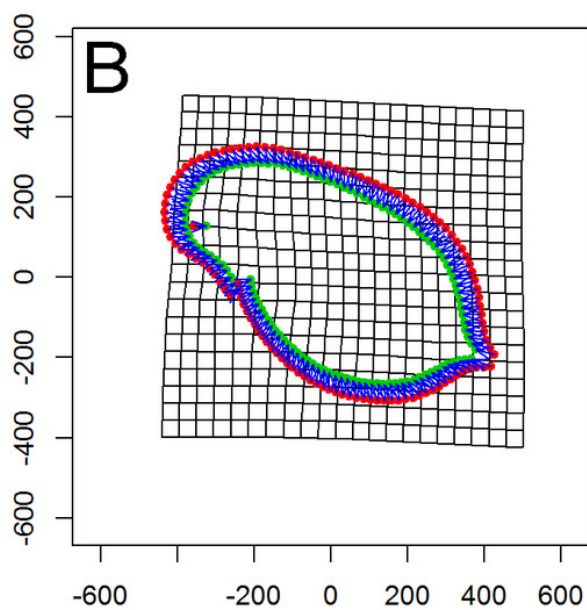
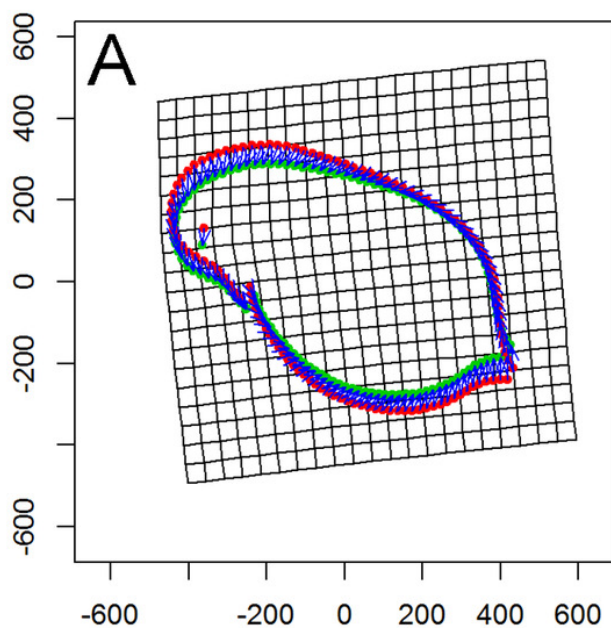



Figure 4

Thin Plate Spline (TPS) grids of consensus shapes of superimposed Procrustes coordinates. Control (red). Fish (green).

(**A**) All specimens. (**B**) Population Greifensee (popG). (**C**) Population Jordan Reservoir (popJ).

(**D**) Population Lake Constance (popLC). (**E**) Population Müggelsee (popM).

Background information of ecological aspects of the four European lakes of which experimental clonal lines originate from.

Number of genotypes (N). Altitude (Alt.). Volume (Vol.). Maximum depth (Dep.). Average depth (Av. Dep.).

Lake	Greifensee	Jordán Reservoir	Lake Constance	Müggelsee
Abbreviation	popG	popJ	popLC	popM
Location	Switzerland	Czech Republic	Austria, Germany, Switzerland	Germany
GPS coordinates	47° 21'20'' N, 8° 40'10'' E	49° 24'55'' N, 14° 39'49'' E	47° 37'21'' N, 9° 26'24'' E	52° 26'6'' N, 13° 38'6'' E
N	6	6	6	6
Alt. [m]	435	437	395	32
Vol. [km3]	0.1485	0.0027	48	0.0366
Dep. [m]	34	14	254	8
Av. Dep. [m]	18	4.5	90	4.9
Stratification	dimictic	dimictic	monomictic	polymictic
Fish biomass [kg/ha]	32	607.5	54	70-100
Presence of *Leuciscus* sp.	yes	yes	yes	yes

Results of general linear mixed effect models (GLMM) on various life history traits.

A. the effect of individual origin ('Environment x Population') **B.** the effect of 'Genotype x Environment' interaction. Significant values (p<0.05*, p<0.01**, p<0.001***) are highlighted in bold. Values are rounded.

(A)

Life history trait		Environment x Population		
		Chisq	Df	Pr(>Chisq)
Age at first reproduction ('AFR')	Environment	5.0261	1	**0.0250**
	Population	3.5870	3	0.3097
	Environment x Population	1.9912	3	0.5742
Total number of broods ('broods')	Environment	3.9718	1	**0.0463**
	Population	7.5636	3	0.0560
	Environment x Population	1.4309	3	0.6983
Total number of offspring ('offspring')	Environment	1.5044	1	0.2200
	Population	17.1803	3	**0.0006**
	Environment x Population	1.9182	3	0.5860
Total number of offspring first brood ('brood1')	Environment	0.0065	1	0.9358
	Population	10.3740	3	**0.0156**
	Environment x Population	0.6623	3	0.8031
Survival ('surv')	Environment	0.2127	1	0.6447
	Population	0.2403	3	0.9708
	Environment x Population	0.0000	3	1
Relative fitness within populations ('relnest')	Environment	0.2608	1	0.6096
	Population	9.8864	3	**0.0196**
	Environment x Population	0.1236	3	0.9889
Relative fitness among populations ('relclone')	Environment	0.3751	1	0.5402
	Population	13.6158	3	**0.0035**
	Environment x Population	0.3250	3	0.9553
Somatic growth rate ('SGR')	Environment	3.3442	1	0.0674
	Population	5.2943	3	0.1514
	Environment x Population	5.7855	3	0.1225
Body length ('size')	Environment	3.5277	1	0.0604
	Population	2.1413	3	0.5436
	Environment x Population	5.2355	3	0.1553

(B) Life history trait		Genotype x Environment		
		Chisq	Df	Pr(>Chisq)
Age at first reproduction ('AFR')	Environment	41	1	**<0.001**
	Genotype	253	23	**<0.001**
	Genotype x Environment	146	23	**<0.001**
Total number of broods ('broods')	Environment	11	1	**<0.001**
	Genotype	114	23	**<0.001**
	Genotype x Environment	64	23	**<0.001**
Total number of offspring ('offspring')	Environment	5	1	**<0.001**
	Genotype	988	23	**<0.001**
	Genotype x Environment	175	23	**<0.001**
Total number of offspring first brood ('brood1')	Environment	0.0267	1	0.870
	Genotype	116	23	**<0.001**
	Genotype x Environment	34	23	0.060
Survival ('surv')	Environment	3	1	0.0944
	Genotype	28	23	0.1991
	Genotype x Environment	8	23	0.9987
Relative fitness within populations ('relnest')	Environment	2.6	1	0.1076
	Genotype	302	23	**<0.001**
	Genotype x Environment	NA	NA	NA
Relative fitness among populations ('relclone')	Environment	2.6	1	0.1041
	Genotype	235	23	**<0.001**
	Genotype x Environment	NA	NA	NA
Somatic growth rate ('SGR')	Environment	20	1	**<0.001**
	Genotype	1289	23	**<0.001**
	Genotype x Environment	147	23	**<0.001**

Body length ('size')	Environment	50	1	**<0.001**
	Genotype	1613	23	**<0.001**
	Genotype x Environment	179	23	**<0.001**

Relative fitness (w) within and among populations.

A. relative fitness within populations for genotype means. **B.** range of relative fitness among populations for genotype means. Fittest genotype or population (w=1.0) is highlighted in bold.

…

population	clone	w within population (relnest)		w among populations (relclone)	
		control	fish	control	fish
G	G1.11	0.53	0.84	0.36	0.50
	G1.12	0.35	0.66	0.24	0.40
	G1.6	0.46	0.31	0.32	0.19
	G1.7	0.95	0.86	0.65	0.51
	G2.1	0.81	0.86	0.56	0.52
	G3.1	**1.00**	**1.00**	0.69	0.60
J	J1	0.73	0.75	0.73	0.75
	J2	0.64	0.68	0.64	0.68
	J2.1	0.50	0.69	0.50	0.69
	J2.4	**1.00**	**1.00**	**1.00**	**1.00**
	J3	0.67	0.70	0.67	0.70
	J4	0.63	0.55	0.63	0.55
LC	LC3.1	0.73	0.59	0.55	0.45
	LC3.3	0.56	0.63	0.42	0.47
	LC3.5	0.78	0.96	0.59	0.72
	LC3.6	**1.00**	**1.00**	0.75	0.75
	LC3.7	0.46	0.54	0.35	0.41
	LC3.9	0.78	0.95	0.59	0.71
M	M10	0.72	0.97	0.43	0.66
	M12	0.87	0.71	0.52	0.48
	M2	0.98	0.86	0.59	0.59
	M5	0.95	**1.00**	0.57	0.69
	M6	0.82	0.88	0.50	0.60
	M9	**1.00**	0.78	0.60	0.54

1   (A)

population	w control	w fish
G	0.24-0.69	0.19-0.60
J	**0.50-1.00**	**0.55-1.00**
LC	0.35-0.75	0.41-0.75
M	0.43-0.60	0.54-0.69

2   (B)

Results of geometric morphometric analysis.

A. p-values of 'Environment' effect on 'shape' differences within populations. **B.** p-value Matrix of 'Environment' effect on 'shape' among populations. **C.** p-value Matrix of the interaction of 'Environment x Population' on 'shape'. Statistical significant F-values (Pr(>F)<0.05) are displayed in bold.

1   (A)

Population	Df	F	Pr(>F)
G	1	0.39	0.843
J	1	5.87	**0.001**
LC	1	3.79	**0.007**
M	1	3.16	**0.009**

2   (B)

-	G	J	LC	M
G	-	**0.013**	0.167	**0.013**
J	**0.013**	-	0.143	**0.001**
LC	0.167	0.143	-	**0.003**
M	**0.013**	**0.001**	**0.003**	-

3   (C)

-	G:control	G:fish	J:control	J:fish	LC:control	LC:fish	M:control	M:fish
G:control	-	0.848	0.589	0.364	0.137	0.473	0.420	0.932
G:fish	0.848	-	0.913	0.671	0.470	0.454	0.418	0.421
J:control	0.589	0.913	-	0.534	0.731	0.352	0.629	0.614
J:fish	0.364	0.671	0.534	-	0.172	0.290	0.892	0.290
LC:control	0.137	0.470	0.731	0.172	-	**0.035**	0.103	0.248
LC:fish	0.473	0.454	0.352	0.290	**0.035**	-	0.743	0.876
M:control	0.420	0.418	0.629	0.892	0.103	0.743	-	0.399
M:fish	0.932	0.421	0.614	0.290	0.248	0.876	0.399	-