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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 population variability was rarely addressed explicitly. We addressed this issue by conducting a common garden experiment with four European *Daphnia galeata* populations, each represented by six genotypes. 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 all 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*.

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

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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
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13 level, thereby contributing to genotypic and phenotypic variation within a species. Predation is
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21 shape of experimental individuals by conducting a geometric morphometric analysis, thus
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23 phenotypic variation within and between all four *D. galeata* populations, the potential to locally
24 adapt to a vertebrate predator regime as well as an effect of the fish kairomones on
25 morphology of *D. galeata*.

Introduction.

Intraspecific phenotypic variation is crucial for the persistence of a population, since low within-population variation increases the risk of extinction (Bolnick et al. 2011; Scheiner and Holt 2012; Forsman 2014). Loss of phenotypic variation can be caused by the reduction of genetic variation e.g. due to genetic drift (random loss of alleles) (e.g. Vanoverbeke and De Meester 2010; Bolnick et al. 2011) or inbreeding depression (e.g. Lynch 1991; Swillen et al. 2015). On the contrary, phenotypic variation can increase as a consequence of environmental change (biotic and/or abiotic) as well as through an increase in genetic variation, which in turn occurs through gene flow (migration), mutation and recombination (Griffiths et al. 2000). Phenotypic variation 'is the fuel that feeds evolutionary change' because natural selection acts on it (Stearns 1989). Phenotypic plasticity describes the ability of genotypes to produce different phenotypes depending on the environment, helping organisms to survive and reproduce in heterogeneous environment (e.g. Stearns 1989, Agrawal 2001). Phenotypic plasticity implies an adaptive potential to locally adapt to a changed environment (Stearns 1989). If the phenotypically plastic organism produces a modified and successful phenotype whose fitness (higher reproductive success) is higher than an unmodified phenotype, the underlying genotype contributes more to the genetic set-up of the whole population.

Predation structures whole communities (Werner and Peacor 2003; Beschta and Ripple 2009; Boaden and Kingsford 2015; Aldana et al. 2016), drives natural selection within populations (Morgans and Ord 2013; Kuchta and Svensson 2014) and maintains species diversity (Estes et al. 2011; Fine 2015). Aquatic predators release chemical substances, so called kairomones, into the surrounding waters which can be detected by their prey. Both vertebrates

(e.g. Schoeppner and Relyea 2009, Stibor 1992) and invertebrates (Machacek 1991; Stibor & Lüning 1994) release kairomones, triggering specific phenotypic plastic responses such as morphological or behavioral changes (Dodson 1989; Schoeppner and Relyea 2009). The predator-induced defenses can be highly variable within a species, depending on factors such as the predator and colonization histories (e.g. Eklöv and Svanbäck 2006; Kishida et al. 2007; Edgell and Neufeld 2008).

Invertebrate as well as vertebrate predator cues have been shown to cause phenotypic plastic responses in *Daphnia*. These induced responses are predator specific and vary across *Daphnia* species. Behavioral changes such as diel vertical migration (DVM) (Effertz and von Elert 2015) and the associated metabolic costs (Dawidowicz & Loose 1992), depth selection (Cousyn et al. 2001), increased alertness (Boersma et al. 1998) and diapause (production of resting eggs = ephippia) (Pijanowska and Stolpe 1996) were reported for different *Daphnia* species exposed to vertebrate predator kairomones. Diverse morphological changes have been shown to occur in the presence of kairomones of the invertebrate predator *Chaoborus*, such as the production of neck teeth in *D. pulex* (Lüning 1995; Tollrian 1995) or the famous helmets of *D. longispina* (Brett 1992) and *D. cucullata* (Agrawal et al. 1999). Recently Herzog et al. (2016) observed a remarkable morphological change of *D. barbata* exposed to *Triops* kairomones. *D. barbata* changes its whole body symmetry to an S-shape, presumably to impede ingestion by their invertebrate predator. Apart from morphology, physiology and behavior, predator cues were also shown to influence life history traits in different *Daphnia* species. Among others, size and fecundity, two important traits for population survival, were affected, resulting in earlier maturation (Riessen 1999; De Meester and Weider 1999; Weber 2003; Castro et al. 2007) and

smaller size (Stibor and Lüning 1994; Castro et al 2007). Size is a very important factor for survival in the face of fish predation, since small individuals are more likely to go undetected. These predator-induced responses are the result of phenotypic plasticity and their magnitude might play a role in adaptation.

Although clonal variation of *Daphnia* within one population has been regularly reported (Cousyn et al. 2001; Machacek 1991; De Meester and Weider 1999; Castro et al. 2007; Beckerman et al. 2010), and many experimental studies compare several populations of *Daphnia* (Gliwicz and Boavida 1996; Boersma et al. 1998; Declerck and Weber 2003; Boeing et al. 2006; Hamrová et al. 2011; Lind et al. 2015) we are aware of only one study which addressed the intra-population level by using four clonal lines for each of the four populations (Boersma et al. 1998). Others rarely used more than one or two genotypes per population, drawing conclusions based on single genotypes. The relative importance of the intra and inter-population variation was thus rarely measured. In our experimental design we specifically considered the population level by using six genotypes per population. All of our genotypes stem from lakes with different fish predation pressures, and we therefore expect the populations to be locally adapted, which translates into a population specific response. The ability of *Daphnia* to locally adapt to different stressors has been demonstrated e.g. for vertebrate predators (Boersma et al. 1998; Cousyn et al. 2001; Declerck and Weber 2003) and pesticides (Jansen et al. 2011).

90 In the present study, we assess the intraspecific phenotypic variation among four
 91 European *Daphnia galeata* populations in the presence of fish kairomones, measuring shifts in
 92 life history traits as well as body shape changes. We expect that (i) there is intraspecific
 93 phenotypic variation within each population. Our experimental setup allows us to (ii) assess the
 94 relative importance of the factors (treatment, genotype, population or their interaction) driving
 95 phenotypic variation in the different populations. We hypothesize that (iii) the potential for
 96 local adaptation is reflected in phenotypic predator-induced life history responses. Finally, we
 97 expect that (iv) kairomones exposure affects body shape and that a correlation between life
 98 history change and morphology exist. We hypothesize that females which increase their
 99 number of offspring in the presence of vertebrate predator cues, change their shape towards a
 100 bulkier shape to accommodate more eggs.

Materials and methods.

Experimental organisms

This study integrated 24 *D. galeata* clones from four different locations: Lake Constance (popLC), Germany; Greifensee (popG), Switzerland; Müggelsee (popM), Germany and Jordan Reservoir (popJ), Czech Republic. These are all permanent lakes with a large water body and varying fish predation pressures (Table S1). Clonal lineages were established from dormant eggs from dated sediment cores which have been extracted from sediment layers corresponding to the same 5 years span in all locations and have been used in previous studies (Henning-Lucass et al. 2016; Herrmann et al. 2017). The clonal lineages were maintained in lab cultures (18°C, 16h light / 8h dark cycle, food: *Acutodesmus obliquus*, medium: Aachener Daphnien Medium (ADaM) (Klüttgen et al. 1994)) for up to 5 years and no less than 3 years prior to the present experiment.

Media preparation

The basic medium was ADaM for fish and *Daphnia* cultures. Two types of media were used for breeding and experimental conditions: fish kairomone and control medium. In total forty ide (*Leuciscus idus*) were maintained in an aerated, separate 200L aquarium, in which they were fed with frozen *Daphnia* cubes and dry food. The ide or closely related species are present in all the studied lakes and have been shown to elicit plastic responses in *D. galeata* clones from Lake Constance (Sakwinska 2002) and Greifensee (Wolinska et al 2007). Fish medium was obtained by keeping 5 randomly chosen ide in an aerated 20L aquarium for 24h to produce fish kairomone medium. The fish were not fed in the fish medium production tank to avoid *Daphnia*

alarm cues to be mixed with the fish kairomones. The fish kairomone treatment imitates a scenario of high fish density (Cousyn et al. 2001; Swillen et al. 2015). Control medium was produced in an aerated, separated aquarium and handled first, before handling of fish and fish medium. All media was filtered (Whatman, membrane filters, ME28, Mixed cellulose-ester, 1.2µm) before use to remove feces from predators and bacteria larger than 1.2µm. All media were supplemented with 1.0 mg C L⁻¹, P rich *Acutodesmus obliquus* before use and exchanged daily (1:2) to guarantee a nutrient rich environment and a constant fish kairomone concentration. The algae concentration was calculated from photometric measurement of the absorbance rate at 800 nm.

Because fish was used to produce the kairomones, this experiment was subject to approval through the “Behörde für Gesundheit und Verbraucherschutz” of the City of Hamburg. It was approved under the number 75/15 and the corresponding document can be found in the supplementary materials.

Experimental design and procedures: life table experiment

Prior to the experiment, each clone was bred in kairomone-free water (control) and in kairomone water (fish) for two subsequent generations to minimize inter-individual variances. To this end, 10-15 egg-bearing females per clone were randomly selected from mass cultures. From these females of unknown age, neonates were collected and raised under experimental conditions and served as grandmothers (F0) for the experimental animals (F2). Neonates of the 3rd to 5th brood carried by the F0 animals were used as breeding (F1) animals. Neonates of the 3rd to 5th brood carried by the F1 animals were used in turn as experimental individuals (F2). A

pair of neonates was introduced in the experimental vessels (50 mL glass tube) at the start of the experiment to compensate for eventual mortality. One of the individuals was randomly discarded when necessary at day 4 (t₄), so that one individual remained in each vessel. This procedure was followed for F1 and F2 individuals. Fifteen replicates were used per treatment and per genotype. Sister neonates of F2 (n=15) were collected in 70% ethanol for size measurements at day 0 (t₀). Life history parameters were recorded daily during the experiment. Before media renewal, females were checked for maturation and neonates were counted, removed and preserved in ethanol every day. Adults were preserved in ethanol as well at the end of the experiment. The experiment lasted for 14 days (t₁₄) for each experimental individual to monitor the performance of each genotype within a fixed period of time.

Cetyl alcohol was used to break the surface tension of the media during breeding and the experiment to reduce juvenile mortality (Desmarais 1997). Breeding and experimental phases were conducted at a temperature of 20°C and a 16h light / 8h dark cycle in a brood chamber with a light intensity of 30% (Rumed, Type 3201D).

The experiment was conducted in three experimental rounds due to logistic reasons. In each round clones from all four populations were present, see supporting material in the appendix (Table S2). We further conducted pilot studies showing it was extremely difficult to ensure synchronicity of so many clonal lines at once.

Data collection and analysis

Life history traits

Life history parameters such as age at first reproduction (AFR) [d], number of neonates per brood per female, total number of broods per female (broods), total numbers of neonates per female (offspring), size of first clutch (brood1) [number of neonates per female], survival [%] and somatic growth rate (SGR) [$\mu\text{m d}^{-1}$] were recorded. Age at first reproduction was the day of releasing the first brood from the brood pouch, with neonates swimming in the vessel. For further analysis the average value of the 15 individuals per genotype per treatment was calculated for each life history trait to estimate the clonal response to a kairomone (fish) vs. kairomone-free (control) environment. Survival rate was defined as the proportion of females surviving from the day of separation (t4) until the end of the experiment (t14). Reproductive rate was calculated by dividing the total number of offspring per female by the total number of broods per female. Relative fitness (w) was calculated by multiplying survival and reproductive rate of a genotype before dividing by the maximum survival and reproductive rate of the other genotypes within population and among all populations. Some genotypes produced male offspring during breeding and the experiment. Males occurred at very low frequencies and were excluded from the data analysis. We aimed to test a total of 720 individuals in this experiment (24 clones x two treatments x 15 replicates). In total we measured life history traits for 684 experimental individuals (see Table S2).

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

Digital photographs of *Daphnia* preserved in ethanol were taken with a stereomicroscope (Nikon SMZ800N) at a magnification of 60x for neonates (t0) and 40x for adults (t14) with NIS-elements 4.3 software. All experimental individuals were photographed in lateral view (left body side up).

Measurement of body length ('size')

Body length was measured from the top of the head through the middle of the eye to the ventral basis of the spine, excluding the spine itself. Somatic growth rate (SGR, $\mu\text{m}/\text{day}$) was calculated by subtracting the average length of neonates at the beginning of the experiment (t_0 ; $n=15$) from the length of each adult individual at the end of the experiment (t_{14}), divided by the complete experimental time in days. The measurement error of digitizing and measuring the length 10 times of the same individual was $\pm 3.24 \mu\text{m}$ (SD). The measurement error of measuring 10 times the length of an individual using the exact same picture was $\pm 1.67 \mu\text{m}$ (SD).

Geometric morphometric analysis of body shapes ('shape')

Since the morphology of *Daphnia* does not allow the assignment of many landmarks, we decided to integrate the semilandmark approach. Semilandmarks are a set of individual landmarks which are interpolated to represent the curve of a structure (Zelditch et al. 2004). Landmarks and semilandmarks were assigned on a subset of digital images of adult experimental individuals (max. $n=10$ per clone and treatment, with a total of 459 individuals, see Table S1) according to Zelditch et al. 2004. In total three landmarks and 115 semilandmarks were assigned on each individual photograph. The first landmark was appointed to the tip of the rostrum, the second in the middle of the eye and the third at the ventral basis of the spine. In our study the first curve consisted of 70 interpolated landmarks (=semilandmarks) along the dorsal body outline, starting at the first landmark and ending on the dorsal basis of the spine. The second set of semilandmarks consisted of 45 semilandmarks along the ventral body outline,

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

Statistical analysis

All statistical analyses for life history traits were performed and all figures were created using R version 3.3.1 (R Core Team 2016). For the generalized linear mixed models (GLMM) the package ‘lme4’ was used (Bates et al. 2015). Subsequent post-hoc tests were performed with the package ‘lsmeans’ (Lenth 2016). To account for multiple testing, strict Bonferroni correction was applied. Visualization of life history traits were performed by using the package ‘ggplot2’ (Wickham 2009). For the geometric morphometric analysis the package ‘geomorph’ was used (Adams and Otárola-Castillo 2013). The visualization of shape differences was performed with the R package ‘shapes’ (Dryden 2017). R scripts are provided in supplementary materials.

To compare life history traits between the different populations in the presence and absence of fish kairomones, we applied generalized linear mixed effect models for each trait, except ‘shape’. Visual inspection of residual plots as well as the Shapiro-Wilk-Test revealed deviations from homoscedasticity for each trait, supporting the decision to use nonparametric models for statistical analysis. Hence, error distributions were assigned individually per trait. We used

228 'Treatment' and the interaction of 'Treatment x Population' as fixed categorical factors in our
 229 models. To account for genotype differences among populations, we included 'Clone' nested
 230 within 'Population' as a random factor. We checked for the necessity of random slopes and
 231 intercepts, finally resulting in a general random intercept model for 'Treatment' (response ~ T +
 232 (1|pop/clone)) and 'Treatment x Population' (response ~ T*P + (1|pop/clone)). Statistical
 233 significances for life history traits were obtained by likelihood ratio tests of the full model with
 234 the effect in question against the model without the effect in question using the function
 235 (Anova(model,type=2)) which performs a Wald Chi-Square test.

236 To assess shape variation we used the principal component analysis (PCA) after the General
 237 Procrustes Analysis (GPA) in the R package 'geomorph'. Subsequently the statistical analysis
 238 was done with Procrustes ANOVA and pairwise tests to reveal statistically relevant shape
 239 differences between treatment groups.

Results.

Effects of predator kairomones on life history traits: 'Treatment' effect

Predator kairomones significantly affected age at first reproduction (AFR) ($\text{Chisq}=34.09$, $\text{Df}=1$, $\text{Pr}(>\text{Chisq})=5.26\text{e-}09$), total number of broods (broods) ($\text{Chisq}=11.085$, $\text{Df}=1$, $\text{Pr}(>\text{Chisq})=0.0008705$), somatic growth rate (SGR) ($\text{Chisq}=15.968$, $\text{Df}=1$, $\text{Pr}(>\text{Chisq})=6.442\text{e-}05$) and body length ($\text{Chisq}=37.976$, $\text{Df}=1$, $\text{Pr}(>\text{Chisq})=7.161\text{e-}10$) (Table 1, Figure 1, Figure 2). *D. galeata* exposed to fish kairomones matured 1.7 hours earlier compared to a mean of 9 days, grew $2.53\mu\text{m}$ less per day (± 0.63 SE) and were smaller by $59.82\mu\text{m}$ (± 9.71 SE) at the end of the experiment (day 14). The probability of having more than two broods decreased from 0.55 in the control to 0.43 in the treatment (Figure 3).

The population effect was small for total number of broods (4.11% of total random effect variation) and estimated to be zero for age at first reproduction, somatic growth rate and body length. The clone effect was small for age at first reproduction (0.12% of the total random effect variation) and estimated to be zero for body length, while it was high for total numbers of broods (47.46% of total random effect variation) and somatic growth rate (65.09% of total random effect variation).

The presence of fish kairomones did not affect the relative fitness of females within each population (relnest) as well as the relative fitness among all populations (relclone) (Table 1). There was no random population effect for the relative fitness of females within one population, since we did not compare across several populations, while there was a population effect of 19% of total random effect variation for the relative fitness of females among all

populations. The clone effect was substantial for the relative fitness of females within one population (109.4% of total random effect variation) and among populations (159.89% of total random effect variation). For further details of relative fitness for each genotype within their population see Table 2A and 2B. The fittest population in non-fish environment was popJ ($w=1$), followed by popM ($w=0.83$), popLC ($w=0.78$) and popG ($w=0.67$). In kairomone environment a small change of positions occurred for pop LC and popM. Here the decreasing order was popJ ($w=1$), followed by popLC ($w=0.80$), popM ($w=0.77$) and popG ($w=0.63$) among all populations.

Effect sizes of the factors 'Treatment', 'Genotype' and 'Population'

We summarized the effect sizes of the fixed factor 'Treatment' and the random factors 'Genotype' and 'Population' by plotting their effect sizes (Figure 4). Effect sizes were standardized by dividing the standard error (SE) of one trait by its residual, turning the effect size of residuals into 1 and thus allowing comparisons across the different data types.

Three traits (brood1, relnest and relclone) were not influenced by any of the three factors. 'Treatment' seemed to be the main driver for the two traits AFR and size. The two traits offspring and SGR seemed to be mainly influenced by 'Genotype', while the last trait broods was influenced by 'Treatment' and 'Genotype'. The random factor 'Population' had overall no to little effect on the predator-induced response.

Effects of genotype origin on predator-induced responses in life history traits: 'Treatment x Population' effect

A significant interaction effect of 'Treatment x Population' was revealed mainly for within-population differences in the population from Greifensee (popG) and Jordan Reservoir (popJ) as well as among those two populations.

In the presence of fish kairomones the age at first reproduction (AFR) differed significantly within popJ ($p < .0001$) and within popG ($p = 0.0023$). Additionally, AFR differed significantly between popG and popJ ($p = 0.0347$) in the absence of fish kairomones, meaning that genotypes of popG reproduce later compared to genotypes of popJ regardless of the treatment.

The total number of offspring (offspring) differed significantly between popG and popJ in the absence of predator kairomones ($p = 0.0198$) and the presence of predator kairomones ($p = 0.0023$), as well as between treatment groups (popG-fish vs. popJ-control ($p = 0.0311$)). Additionally, the number of offspring differed significantly between treatments within popJ ($p = 0.0243$) resulting in an increase of offspring for popJ exposed to fish.

In the presence of predator kairomones the somatic growth rate (SGR) differed significantly within popG ($p < .0001$) and popJ ($p = 0.0135$) (Figure 1E, Figure 2E). The visualization of growth differences between treatments and populations (dSGR, Figure 5) showed that all genotypes from popG had a negative growth rate in fish exposed environment, resulting in a smaller body size. Four out of six genotypes from popJ had a negative growth rate, while genotypes from popLC and popM vary in growth rate across treatments.

In the presence of predator kairomones body length (size) differed significantly within popG ($p < 0.001$), popJ ($p = 0.0002$) and popM ($p = 0.0042$) (Figure 1F, Figure 2F).

Genotype origin ('Population') had a significant effect on total number of offspring in first brood (brood1) ($\text{Chisq}=11.6722$, $\text{Df}=3$, $\text{Pr}(> \text{Chisq})=0.008595$). The trait brood1 differed significantly between popG and popJ in the absence ($p=0.0073$) and the presence ($p=0.0301$) of predator kairomones, meaning that the total number of offspring in the first brood for popG was overall smaller compared to popJ regardless of the treatment (Figure 1B, Figure 2B).

Effect of predator kairomones on the morphological trait 'shape'

A total of 83% of shape variation was explained by the first four Principal Components (PC1= 42%, PC2=24%, PC3=11% and PC4= 6%) (Figure S1). The geometric morphometric analysis showed that 'Treatment' was a meaningful factor for shape variation ($\text{Df}=454$, $F=3.4177$, $Z=3.1515$, $\text{Pr}(> F)=0.001^{**}$). Visualization revealed an overall shape change towards a smaller body. In detail, the head area changed to a ventral position, while the tail area changed to a dorsal position (Figure 6A).

A significant 'Treatment' effect on shape existed within populations (popJ: $\text{Df}=1$, $F=4.5402$, $Z=2.1303$, $\text{Pr}(> F)=0.001^{**}$; popLC: $\text{Df}=1$, $F=3.4311$, $Z=2.4901$, $\text{Pr}(> F)=0.011^{*}$; popM: $\text{Df}=1$, $F=2.4901$, $Z=3.6039$, $\text{Pr}(> F)=0.014^{*}$), except for popG ($\text{Df}=1$, $F=0.3246$, $Z=0.26184$, $\text{Pr}(> F)=0.897$) (see Table 3A). The visualizations showed a homogenous change from all directions to a smaller body form for popG (Figure 6B). Within popJ the overall shape change towards a smaller body size was shown with the strongest change in the head area (bending of the TPS) and an anterior-posterior direction (Figure 6C). Within popLC the head position changed from dorsal to ventral direction, while a small change of the tail area from a ventral to dorsal direction (Figure 6D) occurred. Within popM the overall shape change towards a smaller body size was shown in

the head area from a dorsal to ventral direction and in the tail area from a ventral to dorsal direction (Figure 6E).

The p-value matrix as well as the effect sizes (Z) matrix showed that the treatment effect differed among populations. The shape of individuals of popM differed compared to all the other three populations ($p=0.001$) and the shape of individuals differed between popG and popJ ($p=0.011$) (Table 3B).

There was a significant interaction effect of 'Treatment x Population' on shape ($Df=451$, $F=2.5725$, $Z=2.3747$, $Pr(>F)=0.004^{**}$). The p-value matrix revealed that there was a statistical significance difference within popLC between treatments ($p=0.043^{*}$; Table 3C).

Further analysis revealed significant shape differences among populations within each treatment group (control: $Df=3$, $F=2.1558$, $Z=1.9388$, $P=0.002$ $Pr(>F)=0.002^{**}$; fish: $Df=3$, $F=5.2562$, $Z=4.6072$, $Pr(>F)=0.001^{**}$).

The shape of females with lots of offspring ($n>22$ = upper quartile of total number of offspring) differed significantly among populations in the control group ($Df=1$, $F=2.3358$, $Z=1.8997$, $Pr(>F)=0.049^{*}$), but not in the fish exposed group ($Df=1$, $F=0.93$, $Z=0.72905$, $Pr(>F)=0.431$). There is no association of shape and a high number of offspring when exposed to fish kairomones. Further analysis revealed that the shape of females with lots of offspring did not differ significantly between treatments within each population.

Statistical analysis revealed no block effect for all traits in our experiment, except for total number of offspring first brood (brood1, GLMM: $Pr(>Chisq)=0.001867^{**}$), somatic growth rate (SGR, GLMM: $Pr(>Chisq)<0.001^{***}$) and shape (Procrustes ANOVA: $Pr(>F)=0.001^{**}$).

342 Detailed experimental information for each genotype can be found in the supporting material
 343 (appendix, Figure S2 to Figure S7).

344

Discussion.

Intraspecific phenotypic variation in life history traits within and among populations

Concordant to previous studies by Boersma et al. (1998) as well as Stibor and Lüning (1994), our results showed a decrease of age at first reproduction, a decrease of somatic growth rate and a decrease of body length in the presence of fish kairomones in *Daphnia galeata*. Our experimental design further allowed us to assess the distribution of variance at different levels, clonal and population level. We thus detected phenotypic variation within each as well as among several populations independent of the treatment. We identified two different strategies of phenotypic plastic responses of *Daphnia galeata* by comparing the ‘Treatment’ effect within as well as among the populations. In popJ, the variation of a trait itself, not the change in the trait median value as a response was extremely reduced for two life history traits, AFR and total number of broods (Figure 2C). Almost all individuals of popJ started to reproduce at the same age and produce the same amount of broods when exposed to fish kairomones, showing a striking homogeneity under stress. On the contrary, in popM the variation for AFR increased, resulting in a broader range of ages at first reproduction when exposed to kairomones. Overall our study with a total of 24 genotypes revealed a broad spectrum of intraspecific variation of phenotypes in European *Daphnia galeata*.

Driving forces of phenotypic variation (‘Effect Sizes’)

Our analysis brought to light that the effect size of the fixed factor ‘Treatment’ was largest for AFR and body length implying that the environment, here predation risk, influences the life history of its prey. In our study 13 out of 24 genotypes matured early (Figure S2) and 17 of 24

genotypes reduced their body length (Figure S7) in the presence of fish kairomones, which thus concur with previous findings. Indeed, early maturation and a reduced size of *Daphnia* in the presence of vertebrate predators have been reported before (Machacek 1991; Weider and Pijanowska 1993; Lampert 1993; Gliwicz and Boavida 1996). The ecological benefit lies in a successful reproduction before reaching a body size making the individual vulnerable to fish predation (Lynch 1980, Lampert 1993).

Although the effect of kairomones have been extensively studied in the context of predator-prey interactions (ecologically), they are not completely understood until now (chemically). Fish-associated bacteria seem to be involved in the fish kairomone production (Ringelberg & Van Gool 1998, Beklioglu et al 2006). One might argue that the genotypes exposed to fish water had a nutritional benefit due to introduced bacteria which they could have fed on. Since our model organism *Daphnia galeata* falls into the low efficiency bacteria feeders with a medium sized filter mesh of 1-1.6µm (Geller and Müller 1981), we cannot exclude this effect with certainty. However, we think this potential of nutritional benefit can be ignored, because if there had been a benefit of bacterial uptake in our experiment especially for the fish exposed group, we would have expected to find an increase of relative fitness for all clones exposed to this medium, which we did not observe (Table 2A).

We observed that the random factor 'Genotype' was the main driver for the observed phenotypic variation of the two traits total number of offspring and somatic growth rate. The phenotypic variation between genotypes was best visualized by plotting the differences of somatic growth rate (dSGR) between the treatments (Figure 5), unifying the environmental and clonal effect. All six clones of popG and four out of six genotypes of popJ decreased their

somatic growth in a fish environment, while the direction of response varies for popLC and popM. The main effect of 'Genotype' on the traits offspring and SGR implies that the presence or absence of certain genotypes within one population might have an effect on overall population survival, depending on environmental factors such as predation risk. Hence, if the phenotypic diversity within one population is reduced and the majority produces relatively less offspring in a predator environment, the result could be an overall low number of offspring in the following cohorts, which would threaten the persistence of the whole population. Notably, individuals of popG produced less offspring and less broods compared to the other three populations regardless of the treatment and their relative fitness was comparatively low. Potential explanations for this relative low performance of popG could be genetic drift and inbreeding depression which have a negative effect on genetic diversity (Vanoverbeke and De Meester 2010). However, no low genetic variation for *D. galeata* in Greifensee was identified (Herrmann et al. 2017), making these two explanations unlikely at first glance. Yet, Herrmann et al. (2017) showed that most genotypes in Greifensee (four out of six) had a lower heterozygosity than expected, perhaps as result of inbreeding in this population. Therefore, inbreeding depression could explain lower fitness in popG and should be further investigated in a future study.

For three life history traits we found a statistically significant block effect. The difference between experimental rounds for somatic growth rate, total number of offspring in first brood and shape could be attributed to the high clonal variation we observed in all life history traits. Since we did not find a significant 'Treatment' effect for total number of offspring first brood (brood1), we rule out that the block effect was connected to the presence of fish kairomones or

differences of effectiveness of fish kairomones between rounds which we accounted for by providing same treatment conditions (number and size of fish per l) in experimental rounds. For these reasons, we neglect the block effect although we are aware that we cannot completely rule out this constraint in our experimental design. It would be beneficial to change the strategy for follow up studies and prepare a single stock of kairomones solution to be used throughout experiments (see von Elert & Stibor, 2006 for details). Block effects due to variation in kairomone concentration inherent to our design could be thus avoided. However, synchronizing many different clonal lines from various populations was the main limitation in our case, and is difficult to avoid.

To our surprise the effect size of the random factor 'Population' was overall non-existent to small on the predator-induced response, although we observed population differences, especially between the two extremes popG and popJ. The effect size of population was large for two traits only: total number of offspring (offspring) and relative fitness among populations (relclone), while the latter was calculated based on the total number of offspring (Figure 4). The best explanation for the observed population difference could be the extreme difference of total number of offspring between popG and popJ. In general, genotypes in popJ produced the highest number of offspring among all populations. In contrast, the total number of offspring of genotypes in popG was overall lower compared to the other three populations, regardless of the treatment. This implies that even the increased number of offspring for genotypes of popG exposed to fish is less than the numbers of offspring for genotypes of popJ not exposed to fish. Hence, the genotype origin ('Population') itself had little to no main effect on life history traits

431 in *Daphnia* implying that the identity of a genotype within population seems to be more
432 important than the origin of the genotype per se.

433 In the end, we were not able to identify one main driving force influencing the phenotypic
434 variation in life history traits. Instead our study - displays the complexity of the interacting
435 factors environment and genotype to produce a variety of phenotypes within one species,
436 thereby contributing to the understanding of intraspecific phenotypic variation.

437 Potential for local adaptation to fish kairomones

438 Our findings allow the conclusion that there is potential for local adaptation to predation risk in
439 the investigated European populations of *D. galeata*. This conclusion was based on three
440 outcomes of our study. Firstly, an effect of the interaction of exposure to fish kairomones
441 ('Treatment') and genotype origin ('Population') was found for many of the measured traits:
442 age at first reproduction, total number of offspring, total number of offspring first brood,
443 somatic growth rate, body length, and body shape. Furthermore, we observed an extreme
444 predator-induced life history response for popJ. The variation of the phenotypic response was
445 reduced to a minimum in popJ, so that almost all individuals of the six genotypes and 15
446 replicates reproduce at the very same age when exposed to fish (Figure 2A). On top of that, we
447 observed a similar reduction of variation for the life history trait total number of broods (Figure
448 2C). These strong responses could be explained by local adaptation to the presence of fish. The
449 Jordan Reservoir is an artificial inner city water reservoir, used for recreational purposes such as
450 fishing since 1900 (Kubecka and Bohm 1991) and had been regularly stocked with fish (Seda et
451 al 2000). Therefore, *D. galeata* of Jordan reservoir had the possibility to adapt to an

environment with a higher predation risk for more than a century. Such microevolutionary changes for *Daphnia* species have been described in other contexts before. For instance, Jansen et al. (2011) showed that *D. magna* was able to evolve resistance to a pesticide (carbaryl) within experimental time. Further, Declerck et al. (2001) showed that populations of *D. galeata* were able to locally adapt to fish kairomones. Alternatively, since the reservoir, unlike the other lakes in this study, has been created specifically with fishing in mind, differential colonization might also be the source of the observed pattern. This habitat might have been colonized only by *Daphnia* pre-adapted to fish, with very specific life-histories, leading to the present-day striking pattern. Finally, the relative fitness within and among populations of individuals of popJ suggests that females exposed to fish kairomones are fitter, concurring with results obtained by Castro et al (2007) and Jansen et al. (2011). Since local adaptation to a certain stressor implies a better performance in the 'stress' environment than without this stressor (Lenormand et al. 1999; Joshi et al. 2001) we suggest that the local adaptive potential exists for at least three populations because the relative fitness in the presence of fish kairomones increased overall for 13 out of 24 genotypes (popG=2, popJ=4, popLC=4, popM=3) (Table 2A and 2B). Our results are in line with earlier studies showing the adaptive potential of phenotypic plasticity in *Daphnia* exposed to different stressors (e.g. Yin et al. 2011; Altshuler et al. 2011; Hesse et al. 2012).

Predation risk and body shape

In general, we did not observe any predator-induced extreme morphological changes such as the formation of helmets for fish kairomone exposed *Daphnia*, such as those reported for *D. lumholtzi* (Laforsch and Tollrian 2004). We presented here the first study using the geometric morphometric analysis, hence complementing the traditional approaches (life history traits and

behavior) by measuring morphometric changes to an environmental factor in an intraspecific context in *D. galeata*. Our morphometric analysis revealed that the presence of fish kairomones had an effect on the body shape of *Daphnia*. However, no overall pattern was recognizable among the populations and no effect was observed at all for popG. Instead we observed different changes of shape in each population. We suggest that the morphological trait shape is phenotypically plastic due to high clonal variation, which is consistent with the results reported by Dlouhá et al. (2010) and Zuykova et al (2012).

We hypothesized that life history change and morphological change are correlated, meaning that females with a higher number of offspring ($n > 22$, upper quartile of observed total number of offspring) would change their shape towards a bulkier body form to accommodate a greater number of offspring within their brood pouch. This correlation was found only for the control group and not for the fish-exposed group. Changing the body shape might come along with some drawbacks: the bulkier the shape, the higher the detection risk by the predator and the slower the swimming ability due to drag. In fact, fish prey size-selectively on *Daphnia* meaning that larger *Daphnia* are preyed upon more often than smaller *Daphnia* (e.g. Weber and Van Noordwijk 2002; Beckerman et al 2010). Since fish prey on faster swimming individuals of *Daphnia* (O'Keefe et al 1998), being a slow swimming *Daphnia* would be beneficial. Alternatively, accommodating more offspring without changing the body shape might be achieved through the production of smaller offspring, as was shown by Castro et al (2007, among others e.g. Lampert 1993). In line with previous studies showing a predator-induced reduction in neonate size, we can speculate that this is also the case in our experiment and plan to further explore this dimension.

Conclusion.

The study presented here focused on the intraspecific phenotypic variation among and within populations. By comparing the range of phenotypic response of four populations with six genotypes per population, we contribute to the understanding of the effect of environmental change on intraspecific phenotypic variation at the population level. We observed high clonal variation in all studied life history traits and identified high inter clonal variation, thus leading to the suggestion that single genotype studies on *Daphnia* might deliver biased conclusions.

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726

Figure 1

Reaction norms for selected life history traits showing population differences (mean \pm 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).

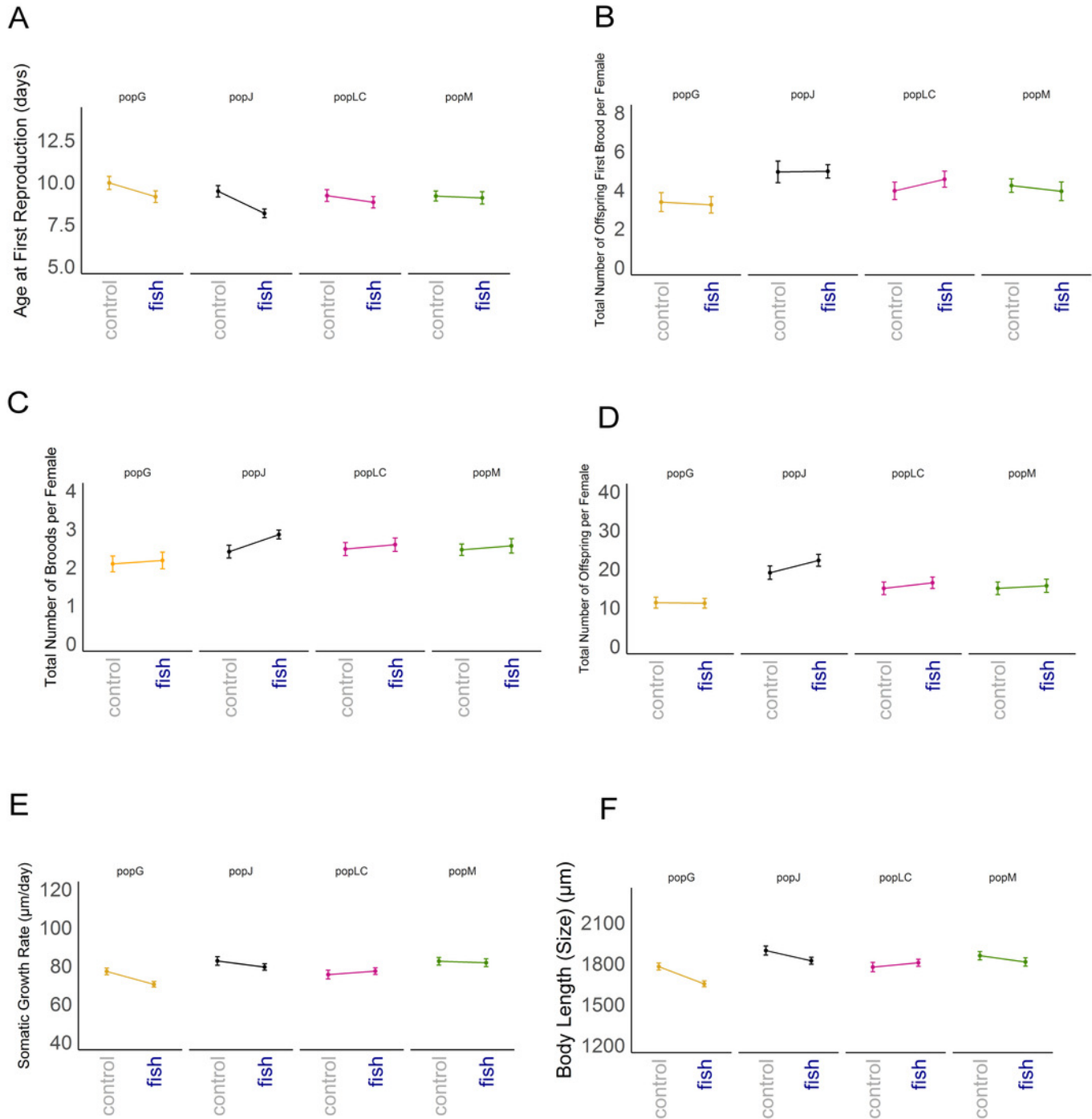


Figure 2

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).

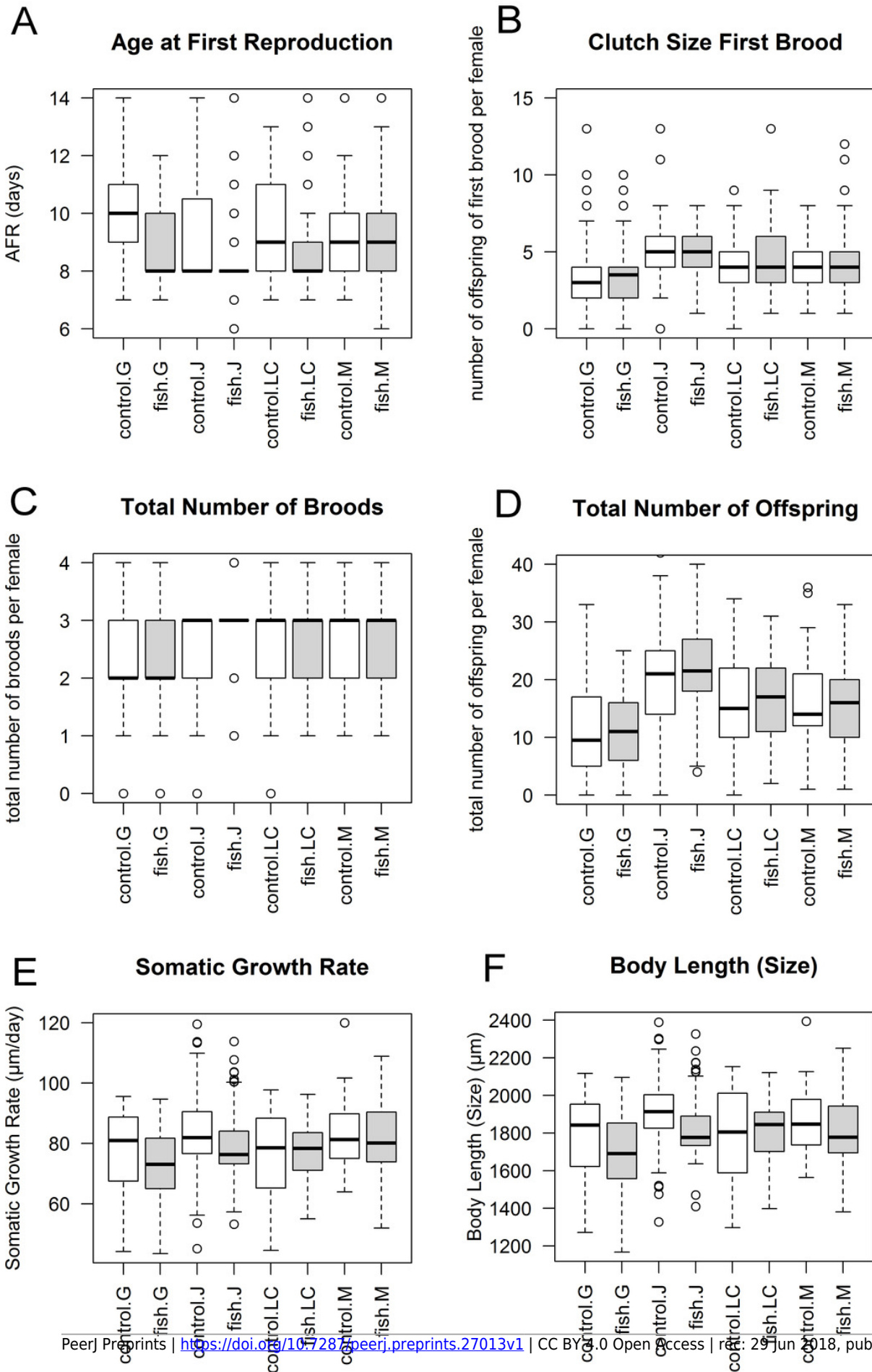


Figure 3 (on next page)

Probability plot showing the probability of having two broods within each treatment.

Treatment

Control

1 2 3 4

0.0

0.2

0.4

0.6

0.8

1.0

Probability

Figure 4

Visualization of standardized effect sizes.

Absolute values of the fixed effect 'Treatment' are plotted with black dots (± 1 SE). The effect of random factors are displayed in orange bars for 'Population' and blue bars for 'Genotype' (clone) nested in 'Population'. Effect sizes of random factors were standardized by dividing the trait standard error (SE) by its residual (all residuals become 1) to allow for comparisons across different data types. The life history traits are Size= body length, SGR= somatic growth rate, relclone= relative fitness among population, relnest= relative fitness within population, brood1= total number of offspring first brood, offspring= total number of offspring, broods= total number of broods, AFR= age at first reproduction.

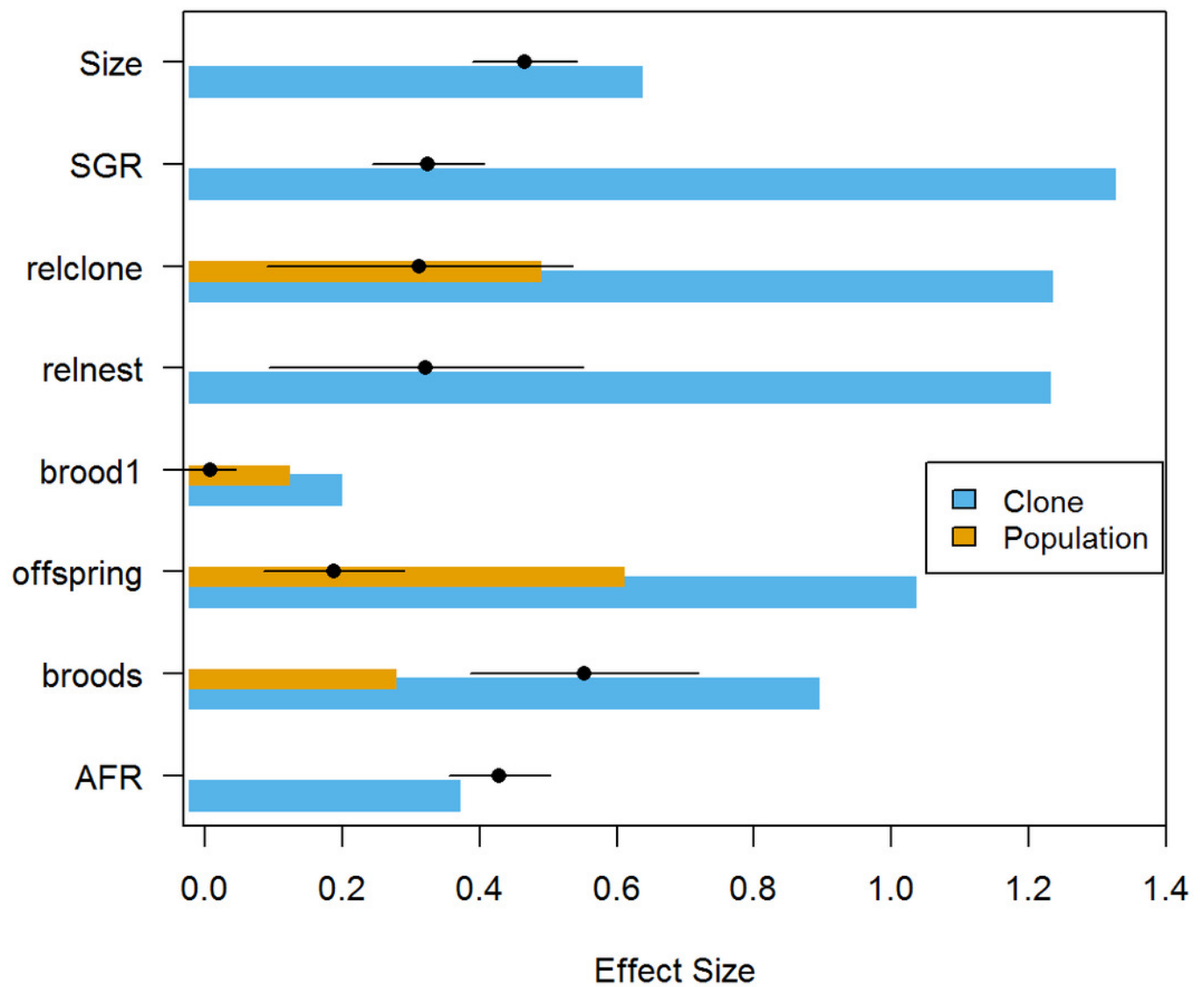


Figure 5

Differences of somatic growth rate (dSGR).

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

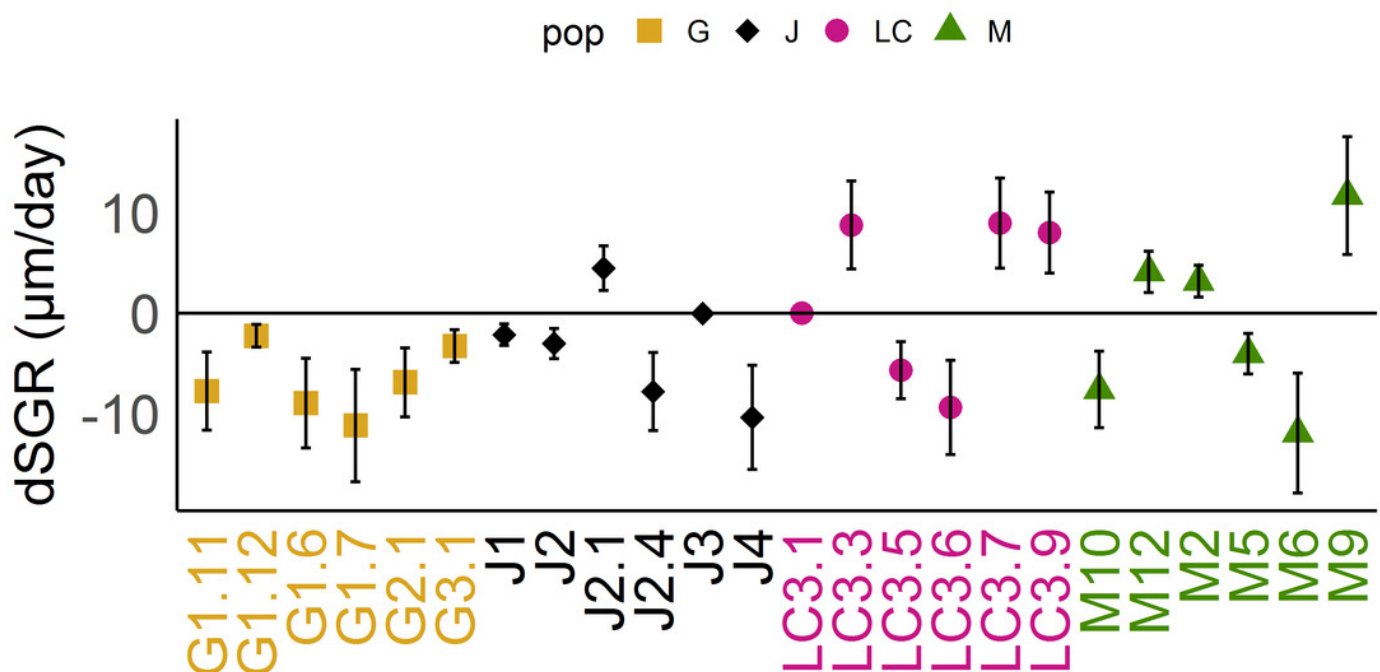


Figure 6

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).

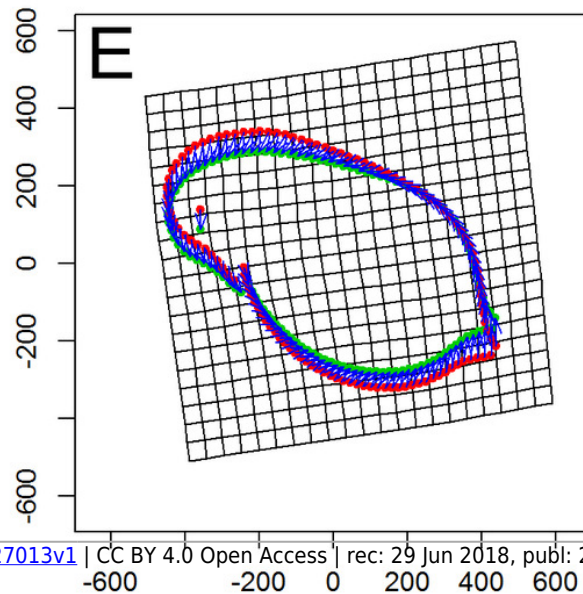
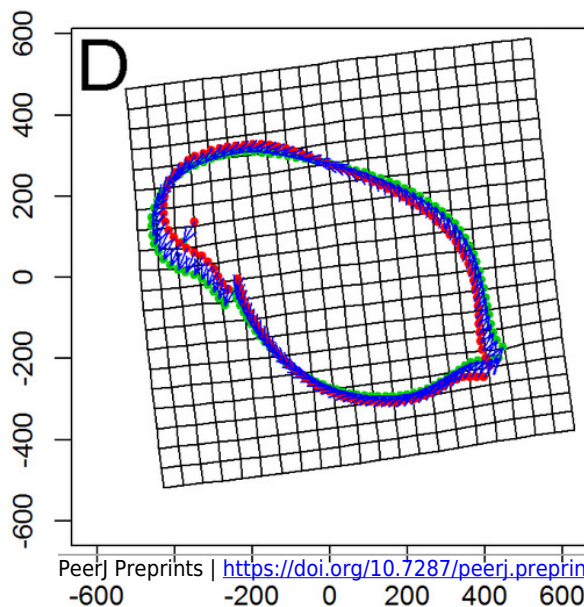
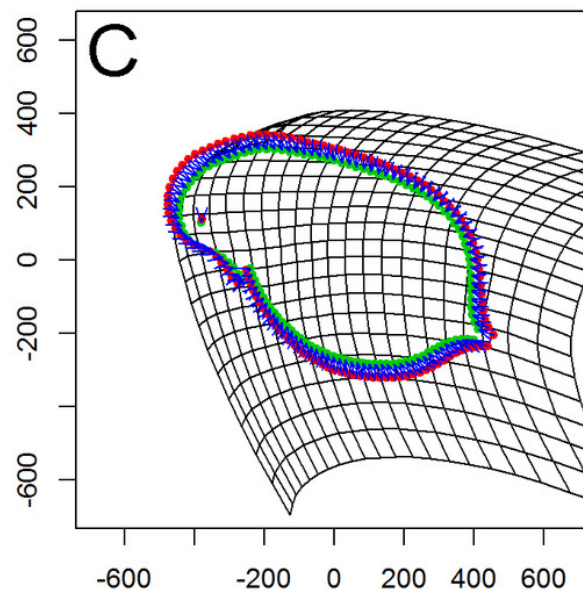
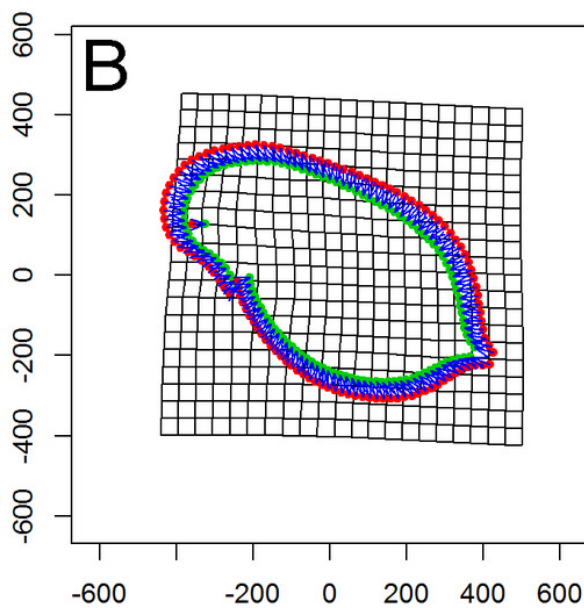
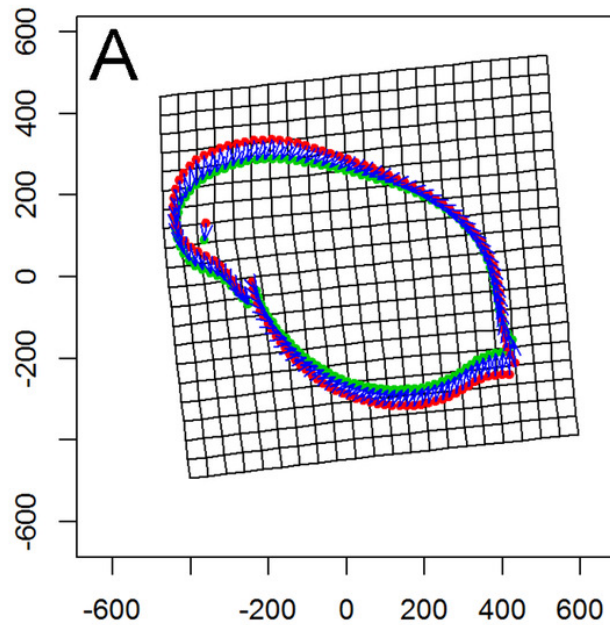


Table 1 (on next page)

General linear mixed effect model testing for the effect of presence/absence of fish kairomones (Treatment) and individual origin (Population) on various life history traits.

For the trait 'shape' Procrustes ANOVA/regression was used as a model to test for effects.

Significant values ($p < 0.05^*$, $p < 0.01^{**}$, $p < 0.001^{***}$) are highlighted in bold. Values are rounded.

	'Treatment'			'Treatment x Population'		
Model	Response ~ T + (1 pop:clone)			Response ~ T * P + (1 pop:clone)		
Life history trait	Chisq	Df	Pr(>Chisq)	Chisq	Df	Pr(>Chisq)
Age at first reproduction (AFR)	34	1	<0.001	20	3	<0.01
Total number of broods (broods)	11	1	<0.001	4	3	0.26
Total number of offspring (offspring)	3	1	0.65	9	3	0.05
Total number of offspring first brood (brood1)	0.04	1	0.84	4	3	0.24
Survival (surv)	3	1	0.07	0.06	3	0.70
Somatic growth rate (SGR)	16	1	<0.001	22	3	<0.001
Relative fitness within populations (relnest)	0.59	1	0.443	2	3	0.59
Relative fitness among populations (relclone)	0.09	1	0.76	2	3	0.64
Body length (size)	38	1	<0.001	35	3	<0.001
Morphological trait	F	Df	Pr(>F)	F	Df	Pr(>F)
Body shape (shape)	4	454	<0.001	3	451	0.004

Table 2 (on next page)

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)

Table 3 (on next page)

Results of geometric morphometric analysis.

(**A**) P-values of 'Treatment' effect on shape differences within populations. (**B**) P-value matrix of 'Treatment' effect on shape among populations. ([b]C[b]) P-value matrix of the interaction of 'Treatment 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	0.32	0.897
J	1	4.54	0.001
LC	1	3.43	0.011
M	1	2.49	0.014

2 (B)

-	G	J	LC	M
G	-	0.011	0.180	0.001
J	0.011	-	0.354	0.001
LC	0.180	0.354	-	0.003
M	0.001	0.001	0.003	-

3 (C)

-	G:control	G:fish	J:control	J:fish	LC:control	LC:fish	M:control	M:fish
G:control	-	0.512	0.569	0.398	0.077	0.614	0.666	0.972
G:fish	0.512	-	0.960	0.168	0.695	0.175	0.098	0.158
J:control	0.569	0.960	-	0.192	0.867	0.225	0.508	0.417
J:fish	0.398	0.168	0.192	-	0.083	0.463	0.964	0.313
LC:control	0.077	0.695	0.867	0.083	-	0.043	0.165	0.229
LC:fish	0.614	0.175	0.225	0.463	0.043	-	0.959	0.772
M:control	0.666	0.098	0.508	0.964	0.165	0.959	-	0.403
M:fish	0.972	0.158	0.417	0.313	0.229	0.772	0.403	-

4