Unidirectional response to bidirectional selection on body size.

I. Phenotypic, life history and endocrine response.

Running headline: Evolvability of body size in medaka

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ABSTRACT

Anthropogenic perturbations such as harvesting often select against a large body size, and are predicted to induce rapid evolution towards smaller body sizes and earlier maturation. However, the evolvability of body size and size-correlated traits remains seldom evaluated in wild populations. Here, we use a laboratory experiment over 6 generations to measure the ability of wild-caught medaka fish (*Oryzias latipes*) to evolve in response to bidirectional size-dependent selection mimicking opposite harvest regimes. Specifically, we imposed selection against a small body size (Large line), against a large body size (Small line) or random selection (Control line), and measured correlated responses across multiple phenotypic, life-history and endocrine traits. As expected, the Large line evolved faster somatic growth and delayed maturation, but also evolved smaller body sizes at hatch, with no change in average levels of pituitary gene expressions of luteinizing, follicle-stimulating or growth (GH) hormones. In contrast, the Small medaka line was unable to evolve smaller body sizes or earlier maturation, but showed marginally-significant signs of increased reproductive investment, including larger egg sizes and elevated pituitary GH production. Natural selection on medaka body size was too weak to significantly hinder the effect of artificial selection, indicating that the asymmetric body-size response to size-dependent selection reflected an asymmetry in body-size evolvability. Our results show that trait evolvability may be contingent upon the direction of selection, and that a detailed knowledge of trait evolutionary potential is needed to forecast population response to anthropogenic change.

Key words: Anthropogenic selection, Body size, Evolvability, Fisheries, Life history.

INTRODUCTION

Human activities often converge towards selecting against large-bodied individuals in animal populations, mainly through harvesting, habitat fragmentation and climate warming (Edeline, 2016). In this context, the dynamics of wild populations may critically rely on their capacity to evolve in response to this selection pressure.

Whether and how wild populations can respond to anthropogenic size-dependent selection has been mostly explored in the context of fisheries, which are often highly size-selective (Lagler, 1968; Law, 2000; Carlson *et al.*, 2007; Kuparinen *et al.*, 2009). Harvesting large-bodied individuals is predicted to induce adaptive evolution towards earlier maturation through reduced life expectancy and, at the same time, towards slower somatic growth through selection against a large body size at a given age (Heino *et al.*, 2015). Paradoxically, however, selection for an earlier maturation may also result in evolution of faster somatic growth, which allows for an earlier maturation (Dunlop *et al.*, 2009; Eikeset *et al.*, 2016; Diaz Pauli *et al.*, 2017). This result highlights the importance of considering trait correlations and multivariate phenotypes in evolutionary biology.

In the wild, fishing has been associated with phenotypic changes towards earlier maturation at a smaller body size and/or towards slower growth rates (see reviews by Trippel, 1995; Law, 2000; Kuparinen & Merilä, 2007; Fenberg & Roy, 2008; Heino et al., 2015). Yet, cases of stocks with no phenotypic response to fishing are also reported (Devine & Heino, 2011; Silva *et al.*, 2013; Marty *et al.*, 2014), suggesting that harvested populations might not always be able to respond to harvest-induced selection. Studies based on data from the wild, however, are often criticized for problems in measuring actual selection pressures (but see Carlson et al., 2007; Edeline et al., 2007; Kendall et al., 2009), in disentangling the effects on mean trait values of size-selective mortality *vs.* evolutionary changes (Hairston *et al.*, 2005), or in controlling for the confounding effects of phenotypic plasticity (Heino *et*

al., 2002). Hence, there is still debate as to whether changes (or absence thereof) towards earlier maturation and slower somatic growth in exploited populations are genetic (Borrell, 2013), or are occurring rapidly enough to influence population dynamics and thus probability of population persistence (Diaz Pauli & Heino, 2014). Experimental harvesting experiments in the laboratory are potentially free of such problems because they make it possible to accurately target the traits under selection, to fully control the pattern and intensity of artificial selection, as well as to standardize environmental variation so that the effects of phenotypic plasticity are alleviated.

Size-selective experiments have been performed on model organisms such as *Drosophila melanogaster* (e.g., Partridge et al., 1999), chicken *Gallus gallus* (Dunnington *et al.*, 2013) or mice *Mus musculus* (e.g., Macarthur, 1949). Often, selection is bidirectional, i.e., is performed at random (Control line), against a small body size (Large line) and against a large body size (Small line, mimicking the effects of harvesting). Results from these experiments show that body-size response to selection may sometimes be asymmetric, with either the Large or Small lines showing slower, or sometimes no or halted response to selection (Falconer & Mackay, 1996 and references therein; Dunnington et al., 2013; Lynch & Walsh, 2018 and references therein). Additionally, selection on body size may be associated with changes in other traits. For instance, selection for increased thorax length in *D. melanogaster* was associated with an increase in larval development time and no change in somatic growth rate, while selection for reduced thorax length was associated with reduced growth rate but no change in duration of larval development (Partridge *et al.*, 1999). Similarly, experiments specifically designed to simulate harvesting on wild populations of model or non-model organisms have shown that size-at-age or size at maturity in populations subject to small- vs. large-sized harvesting may (Edley & Law, 1988; Conover & Munch, 2002; Amaral & Johnston, 2012; Cameron *et al.*, 2013; van Wijk *et al.*, 2013), or may not (Uusi-Heikkilä *et al.*, 2015) evolve in the direction imposed by selection (see the Discussion for a more

detailed treatment of these harvest-simulating experiments). Hence, so far our knowledge of whether and how exploited populations can respond to size-selective harvesting remains limited.

To contribute filling this gap in our knowledge, we examined the ability of a wild population of medaka fish (*Oryzias latipes*) to respond to bidirectional size-dependent harvesting in the laboratory. Specifically, we selected medaka randomly (Control line), against a large body size (Small line), and against a small body size (Large line) during 2.5 years (30 months, 6 medaka generations), measuring at each generation a total of 14 phenotypic, life-history and neuroendocrine traits (Table 1).

We made three specific predictions for medaka response to size-dependent selection: (1) compared to the Control line, medaka from the Small line should evolve slower somatic growth rates. We predicted an opposite pattern in the Large medaka line. (2) Selection on body size has often been shown to induce correlated responses of reproductive traits and larval viability (e.g., Walsh et al., 2006). Therefore, we predicted that evolution of somatic growth in the Small medaka line should be paralleled by evolution towards increased reproductive investment, which may result in earlier maturation and/or higher fecundity at a given body size and/or larger egg sizes (Roff, 1992), and/or towards reduced size at hatch and larval survival (Walsh *et al.*, 2006). We predicted an opposite response in the Large medaka line. (3) The neuroendocrine control of vertebrate body growth and reproduction involves production of the growth (GH), luteinizing (LH) and follicle-stimulating (FSH) hormones in the pituitary (Rousseau & Dufour, 2007; Zohar *et al.*, 2010). Hence, compared to Control line we predicted altered GH, LH, and FSH expression levels in the pituitary, with potentially opposite alteration patterns in the Small and Large medaka lines. Our results validate prediction (1), but in the Large medaka line only, because the Small line did not show *any* body-size response to selection. Prediction (2) was validated in the Large line, but only partially in the Small line that did not mature earlier but showed

signs of increased reproductive investment. Finally, prediction (3) was mainly not supported since only the pituitary expression GH showed a marginally-significant response to size-dependent selection.

MATERIALS AND METHODS

Fish origin and maintenance

Our start medaka population descended from 100 wild-caught individuals sampled in Kiyosu (Toyohashi, Aichi Prefecture, Japan) in June 2011. The genome of the Kiyosu population is free of any significant structure and shows a high degree of polymorphism, indicating no recent population bottleneck (Spivakov *et al.*, 2014). These 100 breeders were maintained in five 20 L aquariums and eggs were collected daily from July to September 2011. Hatched larvae were stocked in six 10 m^3 outdoor ponds.

In 2013, around 100 adult fish were transferred from outdoor ponds to the laboratory where all the 9 subsequent generations (dubbed F_{-1} to F_7) were maintained under constant environmental conditions (common garden): 3 L aquariums connected to a continuous flow-through system ensuring good water quality, cycle of 14h of light - 10h of darkness, temperature maintained between 26 and 27.5°C. Fish were fed *ad libitum* with a mixed diet of dry food (Marin Start, Le Gouessant Aquaculture) delivered 4 times per day using automatic microfeeders (Eheim 3581), and live food (*Artemia salina* nauplii and/or *Turbatrix aceti*) manually delivered once a day, 5 days per week. These light, temperature and food conditions provide optimal growth and maturation conditions to medaka (Kinoshita *et al.*, 2009).

Larvae were initially introduced in their aquariums at a controlled density of 19.6 ± 1.6, 19.2 ± 1.9, 19.8 ± 1.0 (mean ± SD) larvae per aquarium in the Control, Small and Large lines, respectively. Densities were manually homogenized as much as possible at 15 days-post-hatch (dph) to reach 17.0 ±

2.3 , 16.1 ± 2.1, 17.7 ± 2.0 individuals per aquarium and, at 75 dph, were 15.0 ± 2.4, 14.2 ± 2.1, 15.6 ± 2.4 in the Control, Small and Large lines, respectively.

Selection procedure

We provide a schematic diagram of the experimental design in Fig. S1. A size-dependent selection differential was applied both on families at 60 dph and on mature individuals at 75 dph, an age at which 86% of the fish were mature on average (for dynamics of maturity in each line, see Companion Paper II). At 60 dph, we discarded families of less than 10 individuals to avoid confounding density effects on phenotypes. This procedure generated significant selection for a higher fecundity (overdispersed Bernoulli GLM, discarded ~ fecundity, p-value < 0.001), but not for a larger or smaller body length (discarded ~ mean parent body length, p-value = 0.352). Among the remaining families, we kept 10 families at random (Control line) or that had the smallest (Small line) or largest (Large line) average standard body length. At 75 dph, we individually-selected breeders among mature fish based on their individual standard body length.

Specifically, we kept 4 mature fish as breeders (2 males and 2 females) in each of 10 families per line to form the subsequent generation (20 breeding pairs/line/generation, Fig. S1). Each generation, selection was performed on 636 fish on average (212 fish/line), and the selection procedure resulted in keeping on average 12% of individuals per line (number of breeders / total number of fish before selection at 75 dph). We calculated the resultant selection differentials as the difference in maturity probability (i.e., proportion of mature fish) and standard body length after and before selection. Selection differentials across generations F_1 to F_6 were: Control line: +0.13 (0.12 SD) maturity proportion and +0.68 mm (0.18 mm SD); Small line: +0.10 (0.08 SD) maturity proportion and -1.06

mm (0.55 mm SD); and Large line: +0.13 (0.08 SD) maturity proportion and +2.05 mm (0.55 mm SD). For selection gradients, see Companion paper II.

Breeding design, pedigree and fish numbers

Prior to starting selection, we bred medaka during two generations in the laboratory to alleviate maternal and grand maternal effects (Fig. S1). Fish initially transferred from outdoor ponds to the laboratory were allowed to mate randomly in groups of 3-6 fish per aquarium to produce the F_{-1} generation. In F_{-1} and F_0, we randomly mated 54 (F_{-1}) and 56 (F_0) pairs, respectively (Fig. S1), to break any genetic structure or linkage disequilibrium that could remain from possible assortative mating in the wild population (Lynch & Walsh, 2018). During the subsequent 30 months (February 2014 to August 2016) we proceeded with selection (see above) on the F_1 to F_7 generations. Each generation, eggs from each breeding pair were pooled for incubation in the same jar in a common recirculation system, and larvae from the same clutch were transferred to the same growth aquarium so as to form sibling families. This way, we were able to keep track of individual pedigrees and to estimate individual inbreeding rate as 2k-1, where k is one's kinship coefficient with oneself (as calculated from the pedigree data using the kinship2 R package, Sinnwell et al., 2014).

Phenotyping and hormonal measurements

Eggs from each breeding pair were collected during a period corresponding to mother’s 88 to 92 dph. Eggs were counted and photographed, and ImageJ was then used to measure their individual egg perimeters (9795 eggs measured from F_1 to F_7). Hatched larvae were collected during a 5-day time window so as to synchronize hatching dates as much as possible. Birthdate was the median hatching date of each sibling family, and all siblings were thus assigned the same age.

At 0 (hatching), 15, 60 and 75 dph each single individual was photographed, and then ImageJ was used to measure standard body length (from the tip of the snout to the base of the caudal fin) using ImageJ (16808 individual measurements from F_1 to F_7). Additionally, each individual at each phenotyping was sexed as immature, female or male according to their secondary sexual characters (Yamamoto, 1975), which was a non-destructive proxy for the onset of maturity. All fish manipulations were performed after anaesthesia in tricaine methane sulfonate (MS222), except at 0 and 15 dph when larvae and juveniles were manipulated with a Pasteur pipette and photographed in a droplet.

In addition to phenotyping, a subsample of fish were individually measured for pituitary mRNA levels of β-subunits of gonadotropin hormones (LHβ and FSHβ) and GH. At about 40 dph in each generation from F_1 to F_7, 10 to 15 fish per line were randomly sampled and dissected for endocrine measurements (233 fish measured for all three hormones from F_1 to F_7). F_0 preliminary data indicated that the onset of secondary sexual characteristics occurred roughly between 40 and 60 dph, and we chose to dissect fish at 40 dph so as to sample fish at the initiation of puberty. Fish were phenotyped as described above, sacrificed and dissected under a binocular microscope for the pituitary which was immediately immersed in 250 µL Trizol (Ambion) and stored at -20°C. Pituitary mRNA levels of LHβ, FSHβ and GH were measured using reverse transcription quantitative real-time polymerase chain reaction (RT-qPCR). Further details on the RT-qPCR procedure are provided in Supplementary Methods.

Data analyses

Analysis of trait dynamics in response to selection is the purpose of Companion Paper II. In the present companion paper I, we rather adopted a “static” perspective asking whether medaka responded to selection or not. The aim of our statistical analyses, therefore, was to estimate and test for an overall effect of the selected lines on traits. A visual appreciation of time series response to selection on body length (Fig. 2B) shows that the divergence between the three selected lines somehow stabilized from

generation F_3. Hence, we pooled data from generations F_3 to F_7 and treated generation as a random effect. Briefly, we modelled a line effect (Small and Large vs. Control) on univariate traits using (generalized) linear mixed-effects models, and we modelled individual neuroendocrine profile using a multivariate linear mixed-effects model, which accounted for the line effect on both mean and residual variances-covariances of mRNA levels. All models controlled for the effects of inbreeding and, when relevant, of body size also. A detailed description of the statistical models is provided as Supplementary Methods.

We visualized the effect of anthropogenic selection on the maturation process using probabilistic maturation reaction norms (PMRNs), and approach developed to account for the plastic effects of juvenile somatic growth rate on the maturation process, such that a shift in the maturation reaction norm may be interpreted as an evolutionary shift in maturation (Stearns & Koella, 1986; Heino *et al.*, 2002; Heino & Dieckmann, 2008). PMRNs classically account for the effects of age and body length on maturation, but they may also be "higher dimensional" to account for the effects of body mass or individual somatic growth rate (e.g., Morita & Fukuwaka 2006). Here, however, we neither weighed individual medaka nor followed individual growth trajectories. Therefore, we used classical age- and length-dependent PMRNs, which have been demonstrated to be as efficient as higher-dimensional PMRNs to detect evolutionary trends (Dieckmann & Heino, 2007).

For each medaka line, we computed age- and length-dependent PMRNs, defined as the age- and length-dependent 50% probability for an immature medaka to initiate maturity (as informed by the onset of secondary sexual characteristics), using the methods of Barot et al. (2004) and Van Dooren et al. (2005). Briefly, the methods consisted in (1) computing *maturity* "ogives", (2) computing *maturation* probabilities and (3) computing line-specific PMRNs. More details are provided as Supplementary Methods.

Our analyses also included measurement of natural selection, which often opposes the effects of artificial selection (e.g., Carlson et al., 2007). In medaka in the laboratory, natural selection may act on the standard body length of the selected parents through affecting their reproductive success or through the survival of their progeny. We visualized these potential effects of natural selection using quadratic regressions of daily egg number (fecundity), hatching rate, number of progeny reaching age 75 dph, and number of progeny kept as breeders for the next generation on mean parental body length. We used generalized linear mixed models similar as those used to estimate line effects on traits, except that line effects were replaced by the quadratic effect of mean parent body length (see Supplementary Methods for more details). All models were fitted using Markov Chain Monte Carlo (MCMC) in JAGS (Plummer, 2003) through the jagsUI R package (Kellner, 2019).

RESULTS

Effect sizes for responses to selection of the 14 measured traits are presented in Table 1, while quantitative statistical results are provided in Supplementary Table S2.

In line with our first prediction, the Large medaka line evolved towards a larger standard body length at 75 dph in both mature (Figs. 1A and S2A; Model 1 in Table S2) and immature fish (Figs. 1B and S2B). This effect was identical in females, males and immatures at 75 dph (+1.23 mm, MCMC p-value = 0.000, results shown for females only in Table S2). However, in contrast with our first prediction, body size in the Small medaka line did *not* respond to selection (Figs. 1 and S2, Table S2). This lack of response was consistent in females, males and immatures (-0.02 mm, MCMC p-value > 0.800, results shown for females only in Table S2). Therefore, medaka presented a unidirectional response to bidirectional size-dependent selection.

Our second prediction was that evolution of body-size should be paralleled by evolution of correlated traits, and in particular of age and size at maturation, size-specific fecundity, egg sizes, size at hatch and larval survival. Only maturity probability at 75 dph responded as expected (Table 1, Model 2 in Table S2), and more sharply so in the Large than in the Small line. Specifically, maturity probability at an average age and body length decreased in the Large medaka line (Model 2 in Table S2). This change was associated with an upward shift the probabilistic maturation reaction norm (PMRN) for the Large medaka line compared to the PMRN for the Control line (Fig. 2).

In the Small medaka line, however, maturity probability at an average age and body length did not respond to selection (Model 2 in Table S2) and, accordingly, PMRNs for the Small and Control lines largely overlapped (Fig. 2). Noticeably, however, there were some signs of an increased reproductive investment in the Small medaka line: the length-corrected maturity probability decreased less fast with an increasing age than in the Control line (Model 2 in Table S2), and egg sizes increased (Table 1, Model 5 in Table S2, see also results on GH below).

In contrast with our second prediction, we found that body length at hatch was significantly decreased in *both* the Large and Small medaka lines, as compared to the Control line (Table 1, Model 7 in Table S2). This result suggests that larvae might have had larger yolk sacs in these two lines, owing to their similar and larger eggs sizes, respectively. We did not photograph yolk sacs and can not test this hypothesis. Noticeably, body length at hatch was also the only of the 14 monitored traits that was significantly influenced by inbreeding, more inbred individuals having a larger size at hatch (Table 1, Model 7 in and S2). Hatch rate marginally decreased in the Large line compared to the Control line (Table 1, Model 3 in Table S2), but we found no effect of selection on survival at later development stages (Table 1, Model 3 in Table S2).

Our third prediction was that evolution of body size and maturation should be associated with changes in pituitary production of the growth hormone (GH), and of the β subunits of luteinizing (LH) and follicle-stimulating (FSH) hormones. Mean pituitary expression levels of GH marginally increased in males (but not females) in the Small (but not Large) medaka line compared to the Control line (Fig. 3, Table 1, Model 8 in Table S2). There was a trend towards mean pituitary expression levels of LH and FSH to increase in the Small line, and to decrease in the Large line (Fig. 3). However, these trends were not statistically significant (Table 1, Model 8 in Table S2), highlighting a probable lack of statistical power. Interestingly, residual pituitary gene expressions for the three hormones did not trade off, but were instead highly positively correlated (Model 8 in Table S2). Finally, the residual correlation between LH and GH significantly increased in the Large line compared to the Control line (Fig. S3).

We detected significant natural selection on medaka body length during our experiment. Specifically, a longer mean parental body length was associated with increased fecundity (Fig. 4A), but with a decreased egg hatch rate (Fig. 4B, Table 1, Model 3 in Table S2). Despite density normalization at 15 dph, longer-bodied medaka parents still had an increased number of progeny reaching 75 dph (Fig. 4C) and, despite controlled pairing at 75 dph, stabilizing natural selection on parental body length remained present in terms of number of progeny being selected as breeders for the next generation (Fig. 4D). Therefore, natural selection opposed the effects of artificial selection on medaka body size during our experiment.

An accurate quantification of how opposition from natural selection reduced the strength of artificial selection on medaka body size is provided in Companion Paper II. Briefly, we compared at each generation the selection gradients generated on body size by artificial selection with effective selection gradients, which resulted from the combined action of both artificial and natural selection (Lynch & Walsh, 2018). In the Small line, negative artificial selection gradients were on average shifted up by

+15%. In the Large line, positive artificial selection gradients on body size were on average shifted down by -7%. Therefore, we conclude that natural selection reduced the strength of artificial selection on medaka body size only marginally.

DISCUSSION

We measured in the laboratory the realized evolvability of body size in response to size-dependent selection in wild-caught medaka fish. We show that medaka responded to selection for a large body size, but not to selection for a small body size. Before discussing this unexpected result, we start with a mini review of previous harvest-simulating experiments and how their results and designs compare to ours.

Laboratory harvesting experiments, line replication and effective population sizes

Size-selection experiments are a classic in evolutionary biology, and have been conducted multiple times on model organisms such as mice (e.g., Macarthur, 1949; Falconer, 1973), chicken (Dunnington *et al.*, 2013) or drosophila (e.g., Hillesheim & Stearns, 1991; Partridge et al., 1999). More recently, problems with overexploitation have renewed the interest in size-selective experiments mimicking size-selective harvesting. In a pioneering study, Edley & Law (1988) have applied small *vs*. large harvesting during a 150 day period to six clonal populations of *Daphnia magna*. About 200 individuals were left in each clonal population after each round of harvesting (unknown effective population sizes). Populations of clones exposed to small-harvesting (Large lines) evolved rapid somatic growth through small size classes and delayed maturation, while populations of clones exposed to large-harvesting (Small lines) evolved slow growth through small size classes and earlier maturation. Computation of reproductive values showed that evolution resulted in a redistribution of reproductive investment towards size classes that were not harvested.

Conover & Munch (2002) applied small, large or random harvesting at 190 days postfertilization (dpf) during five generations in six experimental populations of the Atlantic silverside *Menidia menidia* maintained in 700L tanks (about 100 breeders/generation/population). The Atlantic silverside is an annual fish, and it was assumed that all individuals were mature at selection such that selection was imposed on body size only. Conover & Munch (2002) found that the mean weight of fish evolved in the expected direction and, by generation F_5, an average fish aged 190 dpf weighted 4.5 g in the Large lines, 2.5 g in the Small lines, and 3.5 g in the Control lines. These differences were due to differences in somatic growth rate and underlying traits (Walsh *et al.*, 2006).

Amaral & Johnston (2012) applied small, large or random harvesting at 90 dpf on six populations of zebra fish *Danio rerio* maintained in 25 L tanks (24 to 78 breeders/generation/population). After four generations, the selected lines changed in the expected directions with the Small and Large lines evolving mean standard body lengths 2% lower and 10% larger than in the Control line, respectively (actual body length values not presented).

Cameron et al. (2013) exposed soil mites *Sancassania berlesei* to juvenile or adult harvesting during 70 weeks (i.e., harvesting was stage- but not directly size-dependent). There were 6 populations per harvest treatment, plus six unharvested populations (hundreds of individuals per population). In accordance with theoretical predictions (Heino *et al.*, 2015), juvenile harvesting induced evolution towards earlier maturation, while adult harvesting induced evolution towards delayed maturation. Interestingly, the amplitude of harvest-induced evolution was overwhelmed by evolution to delayed maturation in all treatments. This change was interpreted by authors as a response to the captive environment, in which density and competition for resources were increased compared to the natural environment from where mites were initially sampled.

van Wijk et al. (2013) applied small, large or random harvesting in the guppy *Poecilia reticulata* during a 3-generation experiment in five experimental populations maintained in 120L aquariums (125 breeders/generation/population). Male guppy stop growing at maturation, and selection was applied on the body length of mature males only. After 3 generations of selection, body lengths of mature male guppy were on average 21 mm in the Large lines *vs*. 18 mm in the Small lines (19 mm in the Control line). However, the age of males was not standardized, such that it is unclear whether selection acted on male age at maturation, on male somatic growth rate or on both traits simultaneously.

Finally, Uusi-Heikkilä et al. (2015) applied small, large or random harvesting during 5 generations on six experimental populations of zebra fish that were maintained in 320L tanks (120 breeders/generation/population, mating by groups of 2 or 4 fish). Zebra fish were harvested at an age corresponding to 50% of mature fish in the Control line and breeders were mated 14 days later. Response to selection was contingent upon both the trait considered and upon the direction of selection. Compared to the Control line, the Large line showed no change in juvenile somatic growth rate or asymptotic length but matured at a later age (but not size), while the Small line showed no change in juvenile somatic growth rate but evolved lower asymptotic length and maturation at a smaller size (but not age).

All the above-listed designs, and ours as well, imposed truncation selection on body size, which may or may not accurately reproduce the form of fishing-induced selection depending on the fishing gear. Towed gears and long-lining catch all individuals above a threshold body size, and their effects are thus accurately simulated by truncation selection. In contrast, gillnets or traps selectively target medium-long individuals (Lagler, 1968; Millar & Fryer, 1999; Carlson *et al.*, 2007; Kendall *et al.*, 2009; Kuparinen *et al.*, 2009), and thus generate at the same time disruptive selection and directional selection against a large body size (Carlson *et al.*, 2007; Edeline *et al.*, 2009). Truncation selection does

not reproduce the disruptive component of gillnet-induced selection, but it still does capture the directional component. Hence, on the whole truncation selection provides a simple and relatively inclusive selection framework to simulate fishing-induced selection on body size.

Another key feature of all previous laboratory harvesting experiments is that they used a mass-selection design with replication of the selected lines, but no control over effective population sizes, inbreeding rate or natural selection. To avoid these problems, we isolated selected pairs and raised their offspring in individual tanks, keeping track of the pedigrees along the experiment. This made it possible to control for the number of offspring per individuals, to maximize effective population sizes, to limit inbreeding throughout the selection procedure, and to measure natural selection. To our knowledge, this is the first time that such a high level of control is achieved in a size-selection experiment on fish.

However, because the number of individuals included in such an experiment is limited, line replication trades off with increasing effective population size N_e. Maximizing N_e should prime, because a large N_e decreases genetic drift, limits the effect of linkage disequilibrium on selection limits, and delays the unavoidable increase in inbreeding (Robertson, 1960; Hill & Robertson, 1966), see e.g. Weber & Diggins (1990) for experimental evidence. In particular, avoiding genetic drift and inbreeding is crucial when studying the evolution of correlated characters (Phillips *et al.*, 2001). Therefore, we chose to derive three large-population lines (N_e = 30 in each, see Companion Paper II) rather than replicating small-population treatments.

This N_e = 30 is likely to compare favourably with most of previous mass-selection designs. It is possible to compute expected N_e from adult number N based on a median N_e/N = 0.23 in random-mating populations (Palstra & Fraser, 2012). On average, expected N_e in the Small and Large lines was

less than 10 in Amaral & Johnston (2012), less than 30 in Conover & Munch (2002) and in (van Wijk *et al.*, 2013), and more than 26 in Uusi-Heikkilä et al. (2015), who mated fish by groups of 2 or 4. In all of these experiments, line duplicates responded similarly to selection, indicating no significant influence of genetic drift (see also similar results of Falconer 1973 in mice using 16 breeders per line). Hence, we were also expecting limited effects of genetic drift in our non-replicated medaka lines. In agreement with this expectation, a pedigree-based quantitative genetic model shows that medaka trait dynamics in our experiment were not compatible with random drift, and instead reflected deterministic evolutionary processes. This model and results are presented extensively in the Companion Paper II.

Medaka phenotypic and life-history response to bidirectional selection on body size

At the end of our experiment (F_7), body sizes of mature medaka at 75 days-post-hatch were 20.5 vs. 22.0 mm (7% difference) in the Control vs. Large lines, respectively. This difference is modest, but is in the range of responses to selection observed in other fish harvesting experiments for the Control *vs*. Large lines: 62.3 vs. 76.1 mm (22% difference) in the Atlantic silverside (Conover & Munch, 2002, mean lengths estimated from a mass-length relationship based on data from Duffy et al., 2013), 10% (raw data not available) in zebra fish *Danio rerio* (Amaral & Johnston, 2012), 19.3 vs. 20.8 mm (7.5%) in the guppy *Poecilia reticulata* (van Wijk *et al.*, 2013), and 29.2 vs 29.5 mm for asymptotic length (<1% difference) or 22.6 vs. 22.9 mm for length at maturity (1.2% difference) in zebra fish (Uusi-Heikkilä *et al.*, 2015).

In contrast, medaka body size did not respond to selection in the Small line. Such an unidirectional response to bidirectional selection was not found in previous experiments on Atlantic silverside (Conover & Munch, 2002), zebra fish by Amaral & Johnston (2012) or guppy (van Wijk *et al.*, 2013), but compares with the results of Uusi-Heikkilä et al. (2015) in zebra fish, who show that the magnitude of response to size-dependent selection was trait-specific and contingent upon the direction of selection

(see above). The qualitative agreement between our results and those of Uusi-Heikkilä et al. (2015) might possibly come from a convergence among our respective selective designs. The selection procedure by Uusi-Heikkilä et al. (2015) involved mating the fish 14 days after that 50% of the population reached maturity, a delay that was possibly not long enough to allow for 100% of the fish to reach maturity, in which case selection was applied both on body size and for maturity (similar to our own design). For a further modelling and discussion of response to such bivariate selection, see Companion Paper II.

In our experiment, lack of body-size response to selection in the Small medaka line could not be ascribed to an absence of artificial selection, which was strong and consistent (see Companion Paper II), nor due to the counteracting effects of natural selection, which remained weak compared to the strength of artificial selection, nor due to inbreeding which was by F_7 identical among the random- and large-harvested lines. Instead, the absence of evolution in the Small medaka line suggests that medaka are at a lower evolutionary limit for body size. This particular functional constraint (sensu Arnold, 1992) might be due to millions of years of natural selection for a small body size. In the wild, small-bodied juvenile medaka competitively exclude their larger-bodied parents, because a small body size provides fish with a strong advantage in exploitative competition for food (Edeline et al. 2016 and references therein). This natural selection regime in the wild was reversed in our laboratory experiment, where large-bodied medaka parents had increased absolute fitness compared to smaller ones. This reversal of the natural selection regime was possibly due to increased interference competition under tank conditions, just as for soil mites (Cameron *et al.*, 2013).

Our results show that evolution towards faster somatic growth rates in the Large medaka line was paralleled by evolution towards delayed maturation, as indicated by an upward shift of their age- and size-dependent probabilistic maturation reaction norm. Importantly, this upward shift of medaka

PMRN in the Large line occurred despite that we applied size-dependent selection on mature fish only, i.e., despite that we applied a positive selection differential on maturity in all the selected lines (see Methods and Companion Paper II). However, selection differentials are not a proper measure of selection on multiple correlated traits, which should instead be measured using selection *gradients* in multiple linear regressions of relative fitness on traits (Lande & Arnold, 1983; Phillips & Arnold, 1989).

Computation of selection gradients for medaka body size in our experiment was performed in Companion Paper II. In the Large medaka line, selection gradients on maturity were *negative*, in opposition with selection differentials on maturity which were positive. Additionally, the most parsimonious quantitative genetic models suggest that medaka body size and maturity were environmentally- but not genetically-correlated (see Companion Paper II). Therefore, evolution towards delayed maturation in the Large medaka line may be ascribed to the sole action of the artificial selection gradient generated on maturity by our design. In the Small medaka line, selection differentials and gradients on maturity were in the same direction, and selection for a smaller body size reinforced the strength of selection for an earlier maturation (see Companion Paper II). Generalizing this finding implies that trends towards earlier maturation observed in a number of exploited fish stocks may be not only the result of fishing-induced selection for earlier reproduction, but also of the parallel selection for a smaller body size.

Medaka neuroendocrine response to bidirectional selection on body size

As a first approach to uncovering the molecular regulation of adaptive life-history evolution in medaka, we measured mRNA levels of candidate genes in the pituitary. This RTqPCR approach allowed us to assay multiple hormones in each individual pituitary. It would have not been possible to assay circulating hormones, because no enzyme-linked immunosorbent assay (ELISA) is available for

medaka GH. Additionally ELISAs are much less sensitive than RTqPCR and require plasma volumes that are too large to allow individual measurements in medaka.

We specifically targeted genes known to play a central role in the regulation of somatic growth and reproduction. In teleosts, growth hormone (GH) is a pleiotropic pituitary hormone that stimulates not only somatic growth rate (Reinecke *et al.*, 2005; Canosa *et al.*, 2007) but also maturation, and also mediates osmoregulation and the stress response (Le Gac *et al.*, 1993; Wendelaar Bonga, 1997; Rousseau & Dufour, 2007).

We expected pituitary mRNA GH levels to be altered in parallel with body-size and maturation response to selection in the Large medaka line. However, pituitary mRNA GH levels were similar in the Large and Control lines. Instead, pituitary GH expression increased marginally significantly in the Small medaka line, which body size did not respond to selection. Specifically, the increase in GH was marginally significant in males only (+0.450, Table S2) but was of a similar amplitude in females (+0.448, results not shown). This counter-intuitive result may, in fact, be explained by the pleiotropic effects of GH on both somatic growth and maturation. In the Large medaka line, evolution towards faster somatic growth was probably mediated by increased pituitary production of GH but, at the same time, evolution towards delayed maturation was probably sustained by decreased pituitary GH production. The net result was that pituitary GH production was not significantly increased in the Large line compared to the Control line.

In contrast, in the Small medaka line the absence of body size evolution did not counteract evolution towards an increased pituitary production of GH, which was possibly associated with an increased reproductive investment. This hypothesis is supported by both results from the maturity probability model, in which the slope of the age effect on maturity probability was marginally significantly less

negative in the Small compared to the Control line (Table S2, Model 2, see also Companion Paper II), and by increased egg size in the Small medaka line. Anyway, many of these effects in the Small line were weak or marginally significant, and further studies are needed to test whether reproductive traits do respond to selection for a smaller body size in the medaka.

Together with GH, we measured pituitary mRNA levels of the β subunits of the gonadotropins, the luteinizing (LH) and follicle-stimulating (FSH) hormones, which are known to stimulate steroidogenesis and gametogenesis and are involved in the onset of puberty in teleosts as in other vertebrates (Zohar *et al.*, 2010). We could not detect any significant effect of selection on pituitary gonadotropins in either the Large or Small medaka lines, suggesting than LH and FSH are less critical than GH to the evolution of life-history traits in the medaka. Interestingly, however, pituitary activity of the somatotropic and gonadotropic axes were highly positively correlated, suggesting that they are synergistic in their effects on medaka development. Similar results were previously found in the rainbow trout *Oncorhynchus mykiss* (Gomez *et al.*, 1999). Finally, the LH-GH correlation significantly increased in the Large medaka line, indicating that size-dependent selection may alter patterns of hormonal synergies. Future transcriptomic approaches on central and peripheral tissues will provide a deeper understanding of the molecular regulation of response to size-dependent selection in the medaka.

Conclusions

Inability of medaka to respond to selection for a smaller body size is a warning signal that calls for increasing research efforts to assess life-history evolvability in wild populations. A crucial line of work in achieving this goal will consist in accurately measuring the multivariate components of selection that act on correlated life-history traits such as body size and maturity (Lande & Arnold, 1983, Companion Paper II), both in the wild and in laboratory experiments. The other key element of this effort will rely

on developing diagnosis tools to evaluate potential for (and signature of) adaptive response to size-dependent, anthropogenic selection (Therkildsen *et al.*, 2019). In the future, comprehensive approaches melting wide-spectrum candidate genes, transcriptomics and genome scans of experimentally- and wild-selected populations will probably be needed to finely decipher the molecular architectures that regulate the adaptive evolution of life histories and that ultimately support the maintenance of biodiversity and ecosystem productivity.

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Author contributions

EE, ALR, GM and SD designed the study. CR, DC, AM, SA maintained the fish and performed selection, breeding, phenotyping and data collection. CR and GM performed mRNA measurements. EE and ALR made final analyses and finalized paper writing.

Ethical statement

The protocols used in this study were designed to minimize discomfort, distress and pain of animals, and were approved by the Darwin Ethical committee (case file #Ce5/2010/041). The committee also confirmed that our methods were performed in accordance with the relevant guidelines and regulations on animal research.

Data archiving statement

All data and codes used in this paper will be archived online.

Competing interests

The authors declare no competing financial and/or non-financial interests.

Supplementary information

Supplementary Methods.

Table S1. Primers for RT-qPCR.

Table S2. Quantitative statistical results.

Supplementary Figures S1, S2 and S3.

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Table 1. Effect sizes (%) of bidirectional selection on body size on phenotypic, life-history and neuroendocrine traits in medaka. Effects sizes were computed as $\mu_S - \mu_C$, where μ_S and μ_C are mean trait values in Small/Large and Control lines, respectively. Shaded columns show "raw" effect sizes computed from a simple line contrast. Non-shaded columns show effects sizes corrected for the effect of covariates in the models presented in the SI Appendix. We tested for significance of the 28 corrected effect sizes by applying a Bonferonni correction in which the significance cut off was $\alpha = 0.05/28 = 0.002$. Statistically significant values are highlighted in bold. Marginally significant values ($\alpha < 0.05$) are italicized.

Trait	Model (SI Appendix)	Small line		Large line	
Standard body length at 75 dph (mm)[1]	1	0.13	0.15	1.12	**1.14**
Maturity probability[2]	2	0.30	0.17	0.05	**-1.00**
Egg-to-larvae survival (hatch rate)[2]	3	-0.12	-0.10	-0.22	*-0.44*
Larvae-to-15 dph survival[2]		-0.34	-0.36	0.10	0.30
15-to-60 dph survival[2]		0.07	0.08	0.00	-0.02
60-to-75 dph survival[2]		0.13	0.52	0.20	0.14
Fertility[2]	4	0.49	-0.58	-0.19	0.58
Non-zero fecundity[3]		-0.25	-0.14	0.06	-0.12
Egg size (perimeter mm)	5	0.13	**0.08**	0.06	0.03
Mean incubation time (days)	6	0.24	0.18	-0.05	0.12
Standard body length at hatch (mm)	7	-0.07	**-0.07**	-0.11	**-0.12**
Pituitary LHβ[1,4]	8	0.14	0.20	-0.07	-0.26
Pituitary FSHβ[1,4]		0.24	0.28	-0.16	-0.30
Pituitary GH[1,4]		0.43	*0.44*	0.22	0.20

1: Effects averaged across sexes, 2: Logit scale, 3: Natural-log scale, 4: Log hormone-to-actin ratio.

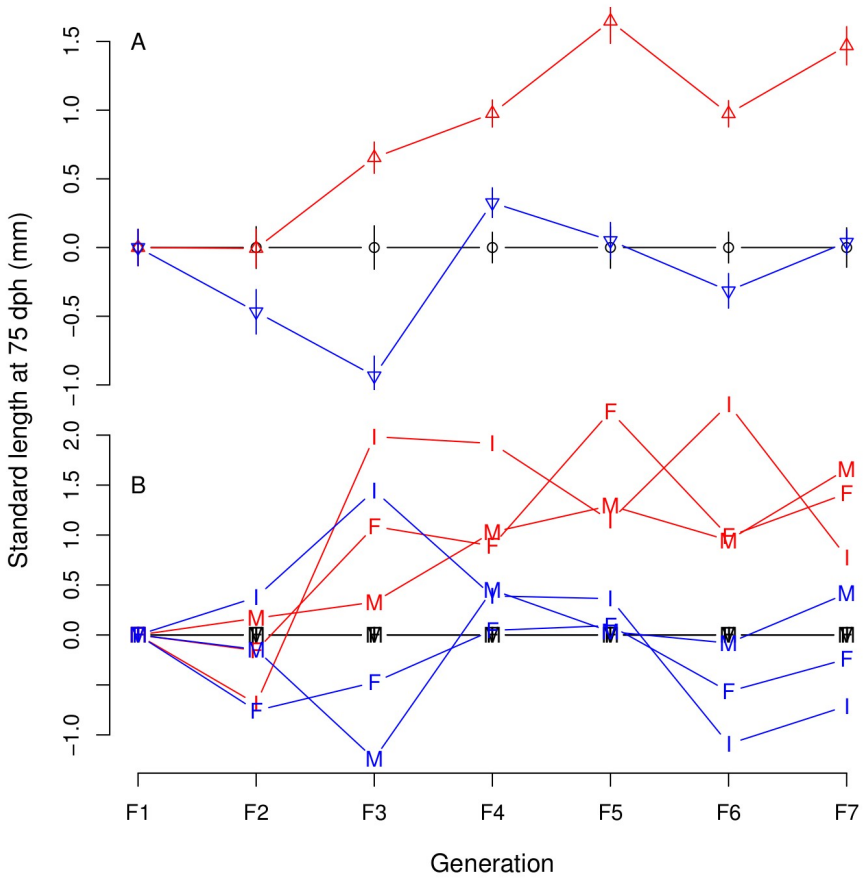


Fig. 1. Medaka body-size time series response to bidirectional selection on body size. A: mean standard body length of mature fish (± SE) at 75 dph. Black circles: Control (random size-selected) line; Blue bottom-pointing triangles: Small line; Red, top-pointing triangles: Large line. B: same as A but separately for immature (I), male (M) and female (F) fish and without error bars. Data were centred on the mean of the random-harvested line (for raw data, see Fig S2).

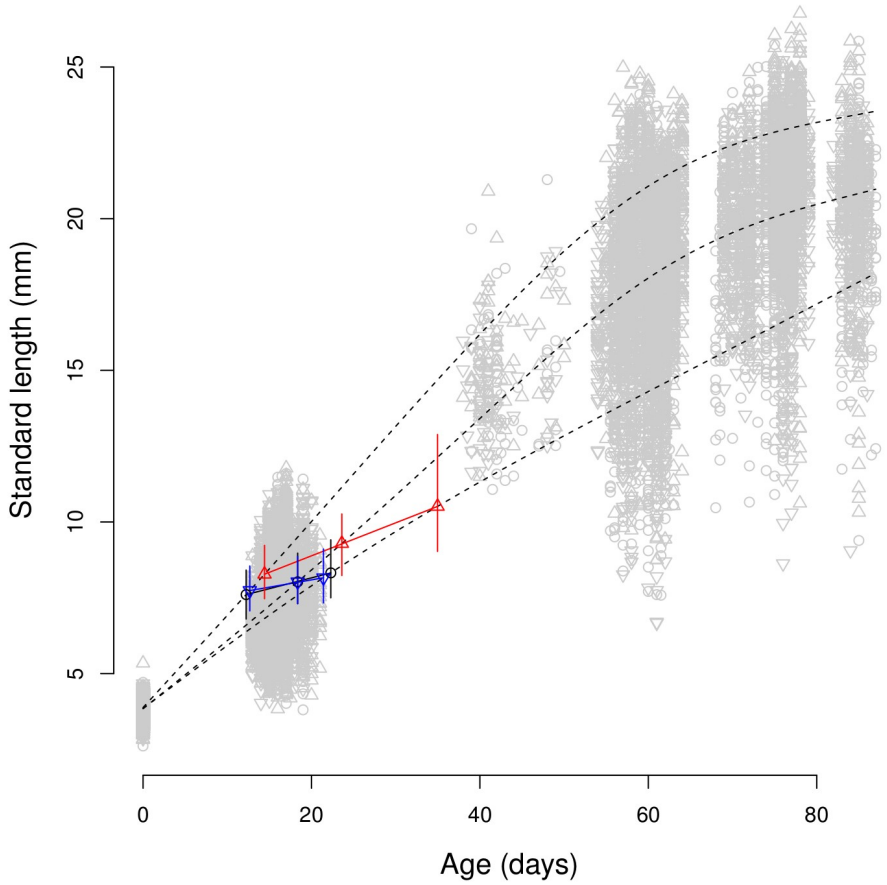
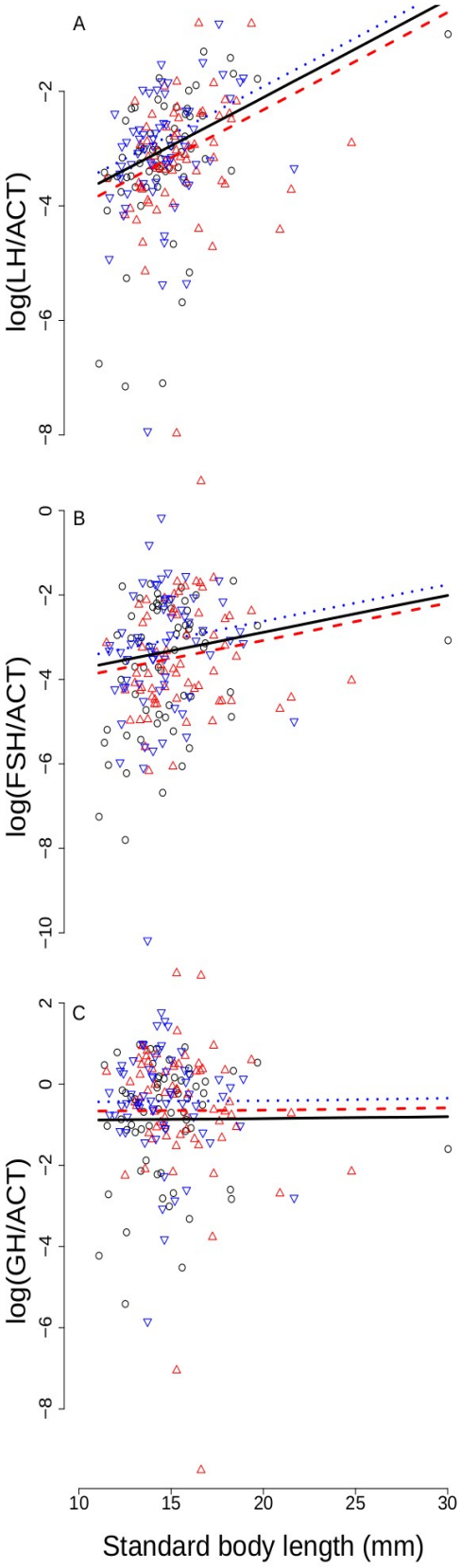


Fig. 2. Medaka probabilistic maturation reaction norm (PMRN) response to bidirectional selection on body size. Light grey dots are raw data. Black dotted curves represent simulated slow, medium and fast growth trajectories. Coloured solid lines and dots represent 50% PMRNs and their intersection with the simulated growth curves, respectively. Black circles: Control (random size-selected) line; Blue bottom-pointing triangles: Small line; Red, top-pointing triangles: Large line. Error bars around the coloured dots represent 95% MCMC confidence intervals.

Fig. 3. Medaka endocrine response to bidirectional selection on body size. Pituitary mRNA levels for A: the luteinizing hormone (LH, β subunit), B: the follicle-stimulating (FSH, β subunit) and C: growth hormone (GH) were standardized by actin β (ACT) levels and log-transformed. Dots represent raw data. Black circles: Control (random size-selected) line; Blue bottom-pointing triangles: Small line; Red, top-pointing triangles: Large line. Lines represent mean MCMC model predictions. Black solid lines: Control medaka line; Blue dotted lines: Small medaka line; Red dashed lines: Large medaka line. For clarity, only model predictions for males are represented.



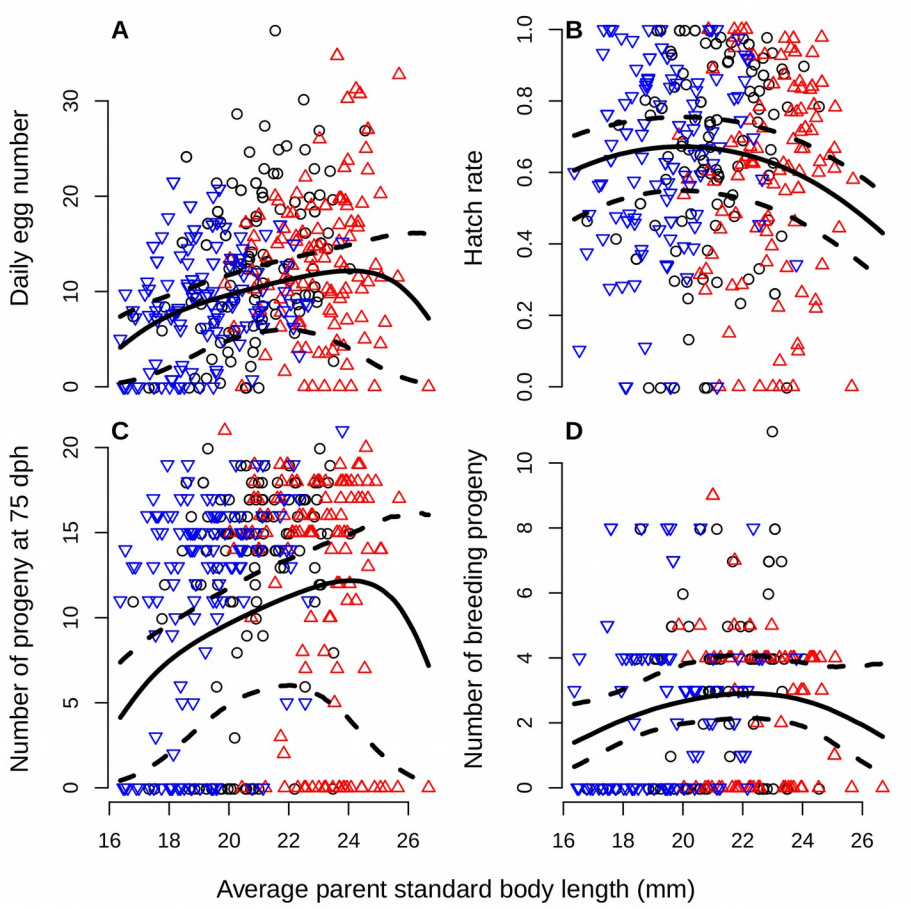


Fig. 4. Natural selection on medaka body size. Solid lines show mean MCMC predicted values and dashed lines 95% credible intervals. Dots represent raw data. Black circles: Control (random size-selected) line; Blue bottom-pointing triangles: Small line; Red, top-pointing triangles: Large line. A: Daily fecundity as a function of average standard body length of the parental pair. B: Hatch rate of the aggregated clutches as a function of mean parent standard body length. C: Number of progeny reaching an age of 75 days-post-hatch as a function of mean parent standard body length. D: Same as C but after the progeny was selected as breeder to produce the next generation, i.e., to produce the second generation after parents were measured for standard body length and mated.