# Effects of ambient oxygen and size-selective mortality on growth and maturation in guppies

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**Lay summary**

Human activities, e.g., nutrient enrichment (resulting in low oxygen) and selective fishing (i.e., higher mortality on large fish) affect growth and maturation (key characteristics determining fish life history). We investigated combined effects of oxygen and size-dependent mortality on growth and maturation to provide informed management decision for these events.

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## Summary

Growth, onset of maturity and investment in reproduction are key traits for understanding variation in life-history strategies. Many environmental factors affect variation in these traits, but for fish hypoxia and size-dependent mortality have become increasingly important due to human activities, such as increased nutrient enrichment (eutrophication), climate warming, and selective fishing. Here we experimentally study the effect of oxygen availability on maturation and growth in guppies *Poecilia reticulata* from two different selected lines, one subjected to positive and the other negative size-dependent fishing. This is the first study to jointly assess the effects of reduced ambient oxygen and size-dependent mortality in fish. We show that reduced ambient oxygen led to stunting, early maturation and high reproductive investment. Similarly, lineages that had been exposed to high mortality on larger-sized individuals displayed earlier maturation at smaller size, greater investment in reproduction and faster growth. These life-history changes were particularly evident for males. The widely reported trends towards earlier maturation in wild fish populations are often interpreted as resulting from size-selective fishing. Our results highlight that reduced ambient oxygen, which has received little experimental

investigation to date, can lead to similar phenotypic changes. Thus, changes in ambient oxygen levels can be a confounding factor that occurs in parallel with fishing, complicating the causal interpretation of changes in life-history traits. We believe that better disentangling the effects of these two extrinsic factors, which increasingly affect many freshwater and marine ecosystems, is important for making more informed management decisions.

**Key words** Fishing selection, hypoxia, eutrophication, life history, *Poecilia reticulata*, water management

## Introduction

Maturation determines the beginning of the reproductive part of an individual's life cycle and is costly in terms of survival and energy. The age and size at which an individual matures are therefore key life-history traits. Growth determines the relationship between age and size, the latter being also a key determinant of survival and fecundity. Thus, studying the effects of different extrinsic factors on growth and maturity is important for understanding the variation in life-history strategies (Roff, 1992; Stearns, 1992; 2000).

Many different environmental factors, such as food availability, temperature, oxygen and presence of predators, affect the acquisition and allocation of resources to growth, maturation and reproduction (Berner and Blanckenhorn, 2007; Enberg *et al.*, 2012). Two factors affecting life-history traits are of particular interest in fishes: oxygen and size-dependent mortality. Oxygen is one of the most critical physical constraints for aquatic animals (Ross, 2000; Pauly, 2010): water is a dense, viscous medium that

contains little oxygen compared to air, only small quantities of oxygen can be dissolved, and respiratory areas do not grow as fast as body weight (Pauly, 1981, 2010). Oxygen demand is proportional to rate of metabolism and increases with, e.g., body size and stress. Low-oxygen conditions occur naturally in many closed water bodies and in the oxygen minimum zones of the World Ocean, but oxygen depletion is also getting increasingly prevalent in freshwater and marine ecosystems due to increasing eutrophication and temperature (Diaz and Rosenberg, 2008; Doney *et al.*, 2012; Jenny *et al.*, 2016). Importantly, temperature plays a dual role: increasing temperature reduces the solubility of oxygen, while in ectotherms, it also increases the metabolic demand for oxygen (Pörtner and Knust, 2007; Holt and Jørgensen, 2015).

Similar to the oxygen depletion, size-dependent mortality occurs naturally but can be influenced by human activities. Size-dependent natural mortality is driven by the presence of predators that commonly prey more heavily on smaller size-classes, i.e., is negatively size-selective (Sogard, 1997; Lorenzen, 1996; Gislason *et al.*, 2010). In contrast, fishing most often targets large-sized fish (i.e., is positively size-selective). Fishing pressure has increased since the middle of the past century, mainly targeting large individuals and higher trophic levels (Pauly *et al.*, 2002; Kolding *et al.*, 2016). Importantly, reduced oxygen levels and increased size-selective fishing co-occur in many aquatic ecosystems, for instance in lake Victoria (Kolding *et al.*, 2008b), Swedish west coast (Kattegat and Skagerrak; Cardinale and Svedäng, 2004), and the northern Benguela system (Utne-Palm *et al.*, 2010).

Reduced oxygen and overexploitation cause reduced abundance and recruitment in demersal fish (Diaz and Rosenberg, 2008). Low oxygen saturation in water is a

proximate factor driving reduced asymptotic maximum size, as the limited oxygen available is allocated to maintenance rather than somatic growth (Pauly, 2010; 1981; van Dam and Pauly, 1995; Chabot and Claireaux, 2008). Little is known on the effect of hypoxia on reproduction, but extreme levels of hypoxia can impair it (Wu *et al.*, 2003; Landry *et al.*, 2007; Chabot and Claireaux, 2008). However, it is predicted that at moderate levels of hypoxia, stunting is caused by earlier maturation and increased reproductive investment at early ages (Kolding, 1993; Kolding *et al.*, 2008a). However, similar changes in maturation and post-maturation growth are expected from evolutionary change caused by fisheries-induced selection (Heino *et al.*, 2015).

Despite the fact that low oxygen level and fishing may co-occur and drive similar changes in life-history traits, little effort has been made in studying their joint effect (Kolding *et al.*, 2008b). Studying the combined effect of several factors is crucial to better understand and inform management and conservation plans of natural resources and fish populations in particular (Jackson *et al.*, 2016). For instance, Kolding *et al.* (2008b) concluded that low oxygen, rather than overfishing, was the most important threat for Nile perch, *Lates niloticus*, in lake Victoria. Similarly, the reduction in individual size and maturation observed in Nile perch (Mkumbo and Marshall, 2015) and Dagaa, *Rastrineobola argentea*, (Sharpe *et al.*, 2012) in Lake Victoria could be driven by hypoxia. Crucially, mitigating actions depend on the driver. If reduced oxygen is the culprit, then changing the environment is needed (Rabalais *et al.*, 2007), and in the best case, the management response is rapid (Beutel and Horne, 1999). On the other hand, if dwarfing reflects evolutionary adaptation to fishing, then the fishing pattern needs to be changed, and even in the best case, response is likely slow (Law, 2000; Heino *et al.*, 2015).

Here we test how oxygen level affects maturation schedules and growth in fish populations exposed to different size-selective mortality regimes. We expect that both low oxygen and exposure to positive size-selective fishing result in earlier maturation and reduced growth. This is the first study to jointly assess the effects of reduced ambient oxygen and size-dependent mortality in fish. Thus, little is known about their relative importance in driving changes in key life-history traits. For this purpose we utilized populations of guppies, *Poecilia reticulata* in laboratory conditions. This model species was also used to demonstrate Bertalanffy's theory of growth Bertalanffy (1938), study the effect of fishing on population dynamics (Silliman and Gutsell, 1958), and assess the effect of predatory size-selection mortality in life-history traits (Reznick and Ghalambor, 2005). Moreover, similar laboratory experiments have been shown useful to inform conservation and management plans (see e.g., Stockwell and Weeks, 1999; Conover and Munch, 2002; Reznick and Ghalambor, 2005; Diaz Pauli and Heino, 2014).

## Materials and methods

We used guppies from a life-history experiment designed to study evolutionary consequences of size-selective fishing (Diaz Pauli *et al.*, 2014). The test fish were first generation (F1) offspring from six replicate laboratory populations that had experienced size-selective mortality for 3.1 years (approximately 4 generations). These populations represent two treatments, with three replicates each: 1) positive size-selected line, where large individuals (above 16 mm standard length SL) were removed from the population every $6^{th}$ week and 2) negative-size selected line where individuals smaller than 16 mm were removed at equal intervals.

Twenty females per population were housed together in 10-L tanks and feed ad lib with newly hatched *Artemia salina* in the morning and fish flakes (tetraMin, Tetra) in the afternoon. Tanks were checked twice a day for new-borns, which were collected and immediately transferred to 2-L individual isolation aquaria where they were randomly assigned one of two oxygen treatments: 1) high oxygen with 95 ± 5% oxygen saturation, corresponding to about 7.9 ± 0.4 mg $L^{-1}$, and 2) low oxygen with 64% ± 6% oxygen saturation (5.3 ± 0.5 mg $L^{-1}$). All tanks were covered with a tightly-fitting piece of Styrofoam covering the whole water surface. The cover prevented surface breathing and minimized the gas exchange with the atmosphere. In the high-oxygen treatment, high oxygen saturation was maintained with an air stone. This resulted in a 2x2 full factorial experiment with oxygen level and inherited background (past size-selective mortality) as the treatments. Ten males and ten females from each of the six populations were assigned to each oxygen treatment, resulting in a total of $N = 240$ fish (1:1 sex ratio).

Test fish were maintained in individual isolation at constant temperature 25 ± 0.5°C and under a 12:12 light regime. During the first two weeks each fish was fed daily 38 ± 6 µl of 3% solution of living filtered *Artemia salina*, at 2-weeks of age this was increased to 76 µl per day, and at 4-weeks of age it was increased to 114 µl, which was maintained until the fish reached maturation and the experiment was terminated. Fish were anaesthetized in a 0.3 g $L^{-1}$ solution of metacaine, measured for standard length (SL) and weight, and assessed for maturation weekly. Non-invasive assessment of maturation is reliable only in males; this is achieved by following the development of the gonopodium (modified anal fin used in insemination). Initiation of maturation

is indicated by the increase from nine to ten segments in the third ray of the anal fin, while complete maturation is marked by the growth of the fleshy hood over the tip of the gonopodium and the number of segments in the third ray being over 27 (Turner, 1941; Reznick, 1990). Gonopodium development correlates with the development of the gonadotrophic zone in the adenohypophysis and the maturation of the testis (Kallman and Schreibman, 1973; Schreibman and Kallman, 1977; Greven, 2011). The initiation of maturation stage correlates with initial enlargement of the testis, proliferation of spermagonia and possibly spermatocytes (van den Hurk, 1974; Koya *et al.*, 2003), while at the completion stage there are several layers of spermagonial cysts, sperm cells and developed testicular ducts with enzyme activity and spermatozeugmata (sperm bundles) are present (Schreibman *et al.*, 1982; Koya *et al.*, 2003). We consider initiation of maturation to be a better representation of male maturation 'decision' in guppies; it is the time when they commit to maturation, reflecting more accurately the factors that affect maturation (Tobin *et al.*, 2010; Harney *et al.*, 2012; Diaz Pauli and Heino, 2013). Therefore, in the present study we assess the effect of oxygen and size-selection on initiation of maturation, from now on referred as maturation. Female maturation cannot be assessed non-invasively, thus from them we only obtained growth data, from which we later estimated maturation (see below). Females were kept in the experiment until two weeks after a male from the same brood reached the last stage of maturation.

## STATISTICAL ANALYSIS

### *Growth*

All analyses were performed in R (version 3.2.4; R Core Team 2016). To assess treatment effects on individual growth we used the biphasic growth model of Boukal *et al.* (2014), which is derived from the model by Quince *et al.* (2008), within the

192 "nlme" R package (version 3.1-125; Pinheiro *et al.*, 2016). The model mechanistically
193 describes somatic growth pre- and post-maturation, based on the principles of
194 allometry and energy allocation. Surplus energy acquisition rate, which is equal to
195 maximum potential somatic growth, is related to somatic weight $W$ by the coefficient
196 $c$ and the allometric exponent $ß$:

197 $$\frac{dW}{dt} = cW^{ß} \quad (1)$$

198 Assuming that juveniles allocate surplus energy only to growth (reproductive
199 investment $r_a = 0$), the juvenile growth curve for weight at age $a$ is as follows:

200 $$W_a = \sqrt[1-ß]{W_0^{1-ß} + c(1-ß)a} \quad (2)$$

201 The post-maturation (adult) growth curve takes into account reproductive investment
202 $r$ for mature individuals, i.e., for $a \geq a_{mat}$:

203 $$W_a = \sqrt[1-ß]{R^{a-a_{mat}}\left(W_0^{1-ß} + Hb^{1-ß}a_{mat}\right) + \frac{RHb^{1-ß}}{1-R}\left(1-R^{a-a_{mat}}\right)}, \quad (3)$$

204

205 where $H = c(1-ß)b^{-(1-ß)}$, $R = 1/[1+(1-ß)\,r]$ and $W_0$ is weight at birth.

206

207 Growth curves were estimated for males and females separately. Weight at birth was
208 affected neither by sex ($F_{213,\,1} = 1.68$, $p = 0.19$) nor by size-selection treatment ($F_{4.3,\,1}$
209 $= 0.07$, $p = 0.79$) according to a linear mixed effect model with population as random
210 factor. These linear mixed models were performed with lme4 R package (version 1.1-
211 11; Bates *et al.*, 2015). *P*-values and degrees of freedom are obtained with the R
212 package "lmerTest" (version 2.0-29; Kuznetsova *et al.*, 2015). Therefore, weight at
213 birth $W_0 = 0.007$ g was used for both males and females. In males, age at maturation

214 $a_{mat}$ is included in the model as a known individual-specific variable (age at which
215 initiation of maturation occurs), but in females it is estimated as a model parameter.
216 Reproductive investment *r* and the coefficient in allometric growth rate-weight
217 relationship *c* were estimated for both males and females, while the allometric
218 exponent in growth rate-weight relationship *ß* is estimated for males but kept constant
219 for females as *ß* = 0.8 because simultaneous estimation of *ß* and $a_{mat}$ was not possible.
220 Initial exploration of our data showed that *ß* = 0.8 was the most appropriate value for
221 our data and similar values have been suggested by Boukal *et al.* (2014).
222
223 The parameters were estimated with a non-linear mixed effect model in the R package
224 "nlme" (Pinheiro *et al.*, 2016) with fish ID as random factor for *r* and *c* for both males
225 and females. Including fish ID as random factor for *ß* and $a_{mat}$ for males and females,
226 respectively, did not improve the models (males: ΔAIC = 6.1, likelihood ratio statistic
227 = 0.09, $p$ = 0.99; females: ΔAIC = 6.0, likelihood ratio statistic = 0.0002, $p$ = 1).
228 Oxygen, size-selection line and their interaction were tested as fixed effects on *r*, *c*
229 and *ß* for males and *r*, *c* and $a_{mat}$ for females. The model that yielded the lowest AIC
230 (Akaike Information Criterion) is considered the best approximating model, i.e. the
231 model that best describes the data. We also discuss models that differ from the best
232 ranked-model with AIC values smaller than 2 ($\Delta_i$ = AIC $_i$ -$AIC_{best}$), as these are
233 considered essentially as good as the best model (Burnham and Anderson, 1998). We
234 also calculated the probabilities of a model being the best model, referred as Akaike
235 weights ($w_i$). Notice that the approach chosen here does not involve significance
236 testing of the model parameters.
237
238 *Maturation*

Maturation in males is described by the probabilistic maturation reaction norm (PMRN; Heino *et al.*, 2002), estimated with generalized linear mixed models with binomial error distribution using the lme4 package in R (version 1.1-11; Bates *et al.*, 2015). Fish ID nested within population was included as random factor, while age, weight, oxygen, size-selection line, and all their first order interactions were included as fixed effects. As for the growth models, we used AIC to select the final model. The logistic curve for the probability of maturation is given by equation:

$$\text{logit}(p) \sim c_0 + c_1 a + c_2 w + c_3 o + c_4 s + \ldots c_n, \quad (4)$$

where $\text{logit}(p) = \log_e[p/(1-p)]$ is the link function, $c_0$ is the intercept and $c_1$ to $c_n$ are the regression parameters of the model for the different explanatory variables (age *a*, weight *w*, oxygen *o*, size-selection line *s*, interactions, etc.). To facilitate the interpretation of the model coefficients, weight and age were standardized to zero mean and unity standard deviation (SD). In males, mean age was $\bar{X} \pm \text{SD} = 87.6 \pm 27$ days and mean weight was $\bar{X} \pm \text{SD} = 0.055 \pm 0.012$ g. The PMRN midpoints (i.e. the estimated age-specific weight at which the probability of maturing is 50%; also referred as $W_{p50}$) were used to illustrate the estimated reaction norms and are roots of equation (4) for weight *w*.

For females, maturation cannot be assessed non-invasively and age at maturation $a_{mat}$ was estimated from the biphasic growth model. This implies a definition of maturation that is purely energetic and corresponds to the (assumed) abrupt start of allocating significant proportion of energy to reproduction; it is not possible to link this definition to male maturation based on different criteria.

## Results

## MALES

Growth in males showed high inter-individual variability (Fig. 1a). Nevertheless, growth models suggested significant effects of both oxygen treatment and parental size-selection line (Fig. 1b, Table 1). No single model was superior, but all highest-ranking models were broadly similar and suggested significant effects of oxygen and/or size selection on all parameters (Table 1). The model that explained the data best (M1) included oxygen and size-selection effects on reproductive investment $r$ and on the coefficient $c$ in growth rate-weight relationship, while there was an effect of size-selection line only on the allometric exponent $ß$ of growth rate-weight relationship (Table 1). This model was superior to the model that did not include any treatment effect (M0; $\Delta$AIC = 25.99, likelihood ratio test statistic = 35.99, $P < 0.001$). Males under low ambient oxygen from each selection line reached lower predicted weights at age 210 days than their counterparts with high ambient oxygen (Fig. 1b; Table 1), but their size-specific maximum potential growth rate was higher (growth rate theoretically attained in the absence of reproduction, Fig. 2a). Similarly, males that descended from the positive size-selection lines reached higher predicted weights at age 210 days (Fig. 1b) and presented higher size-specific maximum potential growth rate than those descending from the negative size-selection lines (Fig. 2a). Results are similar for the other models with high probability for explaining our data (M2–M4); also these models showed effect of our treatments on the growth parameters, particularly with an effect of size-selection line on allometric growth and oxygen in reproductive investment (Table 1). Only one model (M3) included an interaction effect between oxygen and size-selection, suggesting that the effect of oxygen on reproductive investment $r$ was reversed for the negatively compared to positively size-selected lines (Table 1).

Under high oxygen conditions, males from the lines exposed to negative size-selective mortality matured at 0.065 ± 0.010 g (mean ± SD) and 111 ± 25 days old, while those from positive size-selective mortality matured at 0.060 ± 0.008 g and 97 ± 23 days (Fig. 3). Under low oxygen availability, males matured at 0.048 ± 0.008 g and 75 ± 19 days old, and 0.044 ± 0.007 g and 66 ± 12 days old for negative and positive size-selection, respectively. Thus, both low oxygen and positive size-selective mortality resulted in earlier maturation at smaller size, but the effect of oxygen was larger than that of size-selective mortality.

Mean age and size at maturation are also influenced by growth. Maturation tendency can be expressed independently from growth by calculating age- and size-dependent maturation probabilities, i.e., probabilistic maturation reaction norms (PMRNs). Nearly horizontal PMRNs (Fig. 3) show that maturation is primarily determined by size, with only a weak, positive effect of age. The size (weight) at 50% maturation probability at a given age was significantly smaller under low oxygen conditions and for positive size-selection lines (Fig. 3). The oxygen availability had the strongest effect, with the odds of maturation under low oxygen about 61 times higher than under high oxygen conditions (estimate ± SE = 4.11 ± 0.9 in log(odds), $z = 4.68$, df = 1, $P < 0.001$). This is in line with the results obtained from analyzing growth curves showing that males under low oxygen also invested more in reproduction (higher $r$) than those reared in high oxygen. Descending from the positive size-selection line had a weaker positive effect, increasing the odds of maturation compared to negative size-selection by a factor of 3.1 (estimate ± SE = 1.12 ± 0.5 in log(odds), $z = 2.33$, df = 1, $P = 0.02$).

The effect of oxygen availability on maturation was strong also in comparison to the effect of growth. An increase in weight by 1 SD (0.012 g) corresponds to an increase in odds of maturing by a factor of 11.0 (estimate ± SE = 2.41 ± 0.5 in log(odds), $z$ = 5.12, df = 1, $P < 0.001$). Age influenced maturation only through its interaction with weight; the effect was weak but significant (odds ratio = 0.59 for 1 SD increase in weight and age, estimate ± SE = -0.53 ± 0.1 in log(odds), $z$ = -3.75, df = 1, $P < 0.001$), which resulted in a decreasing PMRN for older ages (Fig. 3).

### Females

As with males, inter-individual variability in female growth was high but contained significant effects related to oxygen availability and parental size-selection line (Fig. 4a). The best ranked-model (F1) showed an effect of oxygen level, selection line and their interaction on age at maturation, and effect of oxygen and size selection on reproductive investment and on the growth coefficient (Table 2). Females reared under low oxygen conditions showed lower predicted weight at age 190 days relative to females reared under high oxygen conditions (Fig. 4b). Similarly to the males, this was probably a result of a higher investment in reproduction and earlier age at maturation (Table 2), rather than size-specific maximum potential growth rate that was higher under low oxygen (Fig. 2b). While females from the positive size-selection line reached bigger predicted weight at 190 days (Fig. 4b), their size-specific maximum potential growth rate was lower than that of females in negative size-selection lines (Fig. 2b, Table 2). Females from positive size-selection lines presented lower reproductive investment and older age at maturation (Table 2) relative to females from negative size-selection lines. The model showing these treatment effects

(F1) was superior to the null model considering no treatment effects (F0; ΔAIC = 48.11, likelihood ratio test statistic = 62.10, $P < 0.001$). Similar results are obtained with the second-ranked model (F2; Table 2). Both best-ranked models suggest an interaction effect between oxygen and size-selection, either for age at maturation (F1) or reproductive investment (F2; Table 2).

Age at maturation for females could not be directly observed, but the estimates from the growth model show a pattern similar to the one obtained for males (Table 2). Mean age at maturation is lower under low-oxygen conditions compared to high-oxygen conditions. However, females from lines with negative size-selective mortality have lower age at maturation than those from lines with positive size-selective mortality. The highest mean age at maturation is from females under high-oxygen conditions for females from lines with positive size-selective mortality (65 days). These estimates are lower than the observations for males (treatment-specific mean 66–111 days), but the estimates are not directly comparable as they are based on different ways of defining and estimating maturation.

## Discussion

Oxygen saturation in ambient water and prior ancestral exposure to size-selective mortality affected maturation, growth and reproductive investment in similar ways. Reduced ambient oxygen led to stunting, early maturation and high reproductive investment. Fish exposed to high mortality on larger-sized individuals displayed earlier maturation at smaller size, greater investment in reproduction and faster growth. These results were clearer for male guppies compared to females.

## OXYGEN

Exposure to low oxygen saturation resulted in lower size at age and higher investment in reproduction relative to exposure to normoxic conditions, as expected if reduced oxygen supply triggers the shift from somatic growth to maturation (Pauly, 1984; Kolding, 1993; Kolding *et al.*, 2008a). Both males and females also matured at earlier age and smaller size when reared under low oxygen relative to high oxygen conditions. The low oxygen treatment was not severe enough to hamper fish maturation as observed in some other studies (e.g., Wu *et al.*, 2003; Landry *et al.*, 2007; Chabot and Claireaux, 2008).

Low oxygen resulted in faster juvenile size-specific maximum growth rate. Iles (1973) predicted such an increase in juvenile growth rate of wild tilapia due to low oxygen availability, although his prediction might be a result of lack of standardization of the growth rates. In any case, it should be noticed that a reduction in growth rate associated with low oxygen levels is only detectable after maturation (van Dam and Pauly, 1995; Pauly, 1981). Other studies of adult growth in guppies did see a decrease in growth rate due to oxygen limitation (Weber and Kramer, 1983). The lack of decrease in growth rate in our experiment was not due to surface respiration, as our experimental set-up prevented it. Aquatic surface respiration (ASR) is initiated in guppies at around 30% oxygen saturation (Kramer and Mehegan, 1981), and hence even if it had been allowed in our study it might not have been important. Thus, our modest reduction in oxygen availability led to slightly faster juvenile growth rate and triggered earlier maturation and increased reproductive allocation, which resulted in stunting in both males and females—despite higher maximum potential growth rates.

## SIZE-SELECTIVE MORTALITY

Positive size-selective mortality implies a higher mortality risk for large individuals relative to small individuals. In the present study the size limit for culling was set at 16 mm SL, slightly under normal guppy maturation length (Magurran, 2005). Positive size-dependent mortality favours fast life-history strategies involving early maturation, high investment in reproduction and, in many cases, faster growth rate prior to maturation (Charlesworth, 1994; Law, 2000; Enberg *et al.*, 2012; Réale *et al.*, 2010).

Our results agree with these expectations, particularly in the case of male guppies. Males descending from lines exposed to positive size-selective mortality had higher probability of maturing at a given age and size, which led to maturation at smaller size and younger age compared to males from the lines subjected to negative size-selection. Males also had higher investment in reproduction. Our estimates are comparable with earlier studies on guppies and other poeciliids for reproductive investment (Baatrup and Junge, 2001; Schlupp *et al.*, 2006) and size and age at maturation (Magurran, 2005; Reznick and Bryga, 1987); it should be noticed that most studies considered completion of maturation, rather than initiation of maturation (but see Diaz Pauli and Heino, 2013). Similar directional changes in maturation and reproductive investment have been observed in several exploited fish populations (Heino *et al.*, 2015) and in other selection experiments (van Wijk *et al.*, 2013; Uusi-Heikkilä *et al.*, 2015).

Males presented faster maximum potential and realized growth rates in lines exposed to positive size-dependent mortality. Studies on the effect of (positive) size-selective fishing mortality have often concluded that growth rates decreased rather than increased, but in most cases such reduction was a secondary effect from increased allocation in reproduction (reviewed in Enberg *et al.*, 2012; Heino *et al.*, 2015) and applies to post-maturation growth. This contrasts with the simplistic expectation that killing large fish should always favour smaller fish and thus slower growth. While this expectation is largely warranted for adult fish, expectations for juvenile growth are less straightforward (Enberg *et al.*, 2012). Dunlop *et al.* (2009) concluded that one key factor that determines whether positively size-selective fishing favours increased or decreased juvenile growth rate is the size limit at which the harvesting takes place. When the minimum size is set under the size at maturation, as it occurred in our experiment, juvenile growth is expected to accelerate to reach maturation earlier in life (Dunlop *et al.*, 2009). Positive size-selection also led to faster juvenile growth rate in zebrafish (*Danio rerio*; Uusi-Heikkilä *et al.*, 2015).

Males from the lines exposed to positive size-selective mortality had larger predicted size at age 210 days (the maximum age in the experiment). This occurred because of their high maximum potential growth rate, and despite their earlier maturation and higher investment in reproduction. This result is contrary to theoretical expectations (Heino *et al.*, 2015) and other experimental studies (Walsh *et al.*, 2006; van Wijk *et al.*, 2013; Uusi-Heikkilä *et al.*, 2015). A possible explanation is that because we sacrificed our fish soon after maturation, we have little information on how their realized growth and reproductive allocation would have developed through their adulthood, which was estimated in former studies (Walsh *et al.*, 2006; van Wijk *et al.*,

2013; Uusi-Heikkilä *et al.*, 2015; Heino *et al.*, 2015). Ultimate size at adulthood is affected by the maximum potential somatic growth rate as well as the continued investment in reproduction in this iteroparous species and might have resulted in smaller-sized individuals later in life in positive size-selected lines. Our estimates of realized growth rate are similar to those of Auer *et al.* (2010). The values of *ß* estimated from our model are on the lower range of the great variation in the values of the allometric exponent *ß* (Killen *et al.*, 2010; Boukal *et al.*, 2014), which is associated with determinate/indeterminate growth. Male poeciliids are typically considered to have determinate growth, although they do not completely cease growth after maturation (Snelson, 1982). Nevertheless, because fish were sacrificed well before reaching their maximum sizes, our estimates of *ß* might be downward biased. In practice, the estimations of *ß* and reproductive investment *r* are confounded, and the truncated adult life span may have aggravated this problem.

Whether the differences between size-selected lines represent evolutionary (i.e., genetic) change is ambiguous, as our experimental set-up only controlled for environmental differences among the fish subjected to the oxygen treatments, but not those of their parents. It is generally accepted that lines should be maintained for at least two generations in common garden conditions to be able to clearly discern genetic changes using phenotypic data (Reznick and Ghalambor, 2005). The differences could therefore represent parental effects, genetic differences, or—perhaps most likely—a combination of both. Nevertheless, the phenotypic changes were in agreement with the predictions from life-history theory.

Estimates for reproductive investment, growth rate and age at maturation in females are comparable with values obtained in other studies (Magurran, 2005; Auer *et al.*, 2010; Rocha *et al.*, 2011). Still, since maturation in females could not be visually determined, the study of life-history changes in them was not as thorough as with males. Exposure to positive size-selective mortality led to estimated maturation at older, rather than younger ages, and to a lower investment in reproduction. This is opposite to what was observed in males in this study and earlier selection experiments (Walsh *et al.*, 2006; Uusi-Heikkilä *et al.*, 2015). However, these results refer to age at maturation inferred with the growth model and which might be inaccurate, rather than to directly observed maturation, as with males. In addition, females in the positive size-selected line presented lower maximum potential size-specific growth rates, but higher realized growth, contrary to what observed in males. The estimation of maximum growth rate was only based on one parameter (*c*, coefficient in growth rate-weight relationship), while the allometric exponent *ß* was kept constant. For males, it was the allometric exponent *ß* that showed the strongest effect of size-selection line and the parameter that affected growth rate the most. If the growth model for females is performed to estimate *ß* by keeping *c* constant at 0.01 $g^{1-ß}day^{-1}$, the results remain very similar (results not shown). Nevertheless, the differences between positive and negatively size-selected lines were smaller for females than for males, despite being significant in all cases.

### Interplay of effects on life history traits and implications

Manipulating oxygen level resulted in bigger changes in reproductive investment and maturation compared to manipulating size-selective mortality in parental generations. Positive culling led to an estimated increase in reproductive investment of 33%

relative to negative culling (under high oxygen conditions), while low oxygen led to an increase of more than 100% relative to high oxygen. Similar results were obtained for age and size at maturation; the odds of maturing were 60 times higher under low oxygen compared to high oxygen, but only 3 times higher for positive lines compared to negative lines.

However, direct comparison of the importance or strength of these two different drivers is difficult for two reasons. First, the two treatments are conceptually very different: the oxygen treatment was affecting the ambient environment of the very same fish that we observed during the experiment, whereas the size-selective mortality treatment represented conditions that the parental generations of the test fish had experienced over the course of three years (approximately 4 generations). The actual treatments levels are in both cases somewhat arbitrary (i.e., the specific oxygen saturation level, and the duration and intensity of past size selection). Second, the mechanisms through which the treatments affect life histories are different. Oxygen is a strong proximate driver of phenotypic change in maturation and growth, triggering direct plastic responses (Kolding *et al.*, 2008a; Pauly, 1984), while the effect of size-selective mortality on life histories occurs through both genetic change (evolution) and phenotypic plasticity, including inter-generational plasticity (parental effects). Although hypoxia could also lead to evolutionary changes in life history (Riesch *et al.*, 2010), this was not considered in our experiment that followed only a single generation of fish.

Our results do not suggest strong interactions between ambient oxygen and prior size selection under controlled laboratory conditions, that is, that the effects of oxygen

level would depend on adaptations to contrasting size selectivity regimes. For males, only one of the four top-ranking growth models included an interaction between size selection and oxygen (affecting a single parameter), whereas for females, both top-ranking models contained a single interaction each. These provide some evidence for the oxygen depletion-induced increase in reproductive investment being stronger in the lines that had been subjected to negative size-selective mortality. Most effects, however, were simply additive.

We believe it is essential to consider both proximate and ultimate factors to gain a better understanding of life-history variation and how populations evolve under the influence of these factors. Hypoxia and size-dependent mortality—including that induced by fishing—not only co-occur, but can also drive similar life-history changes. Thus, investigating the interplay of fishing- and hypoxia-induced changes is necessary to perform ecosystem-based predictions on the sustainability of the fishery (Kolding *et al.*, 2008b). To our knowledge this is the first study looking at the combined effect of oxygen and size-dependent mortality on life-history traits. Despite being an experimental study, our results illustrate the risks of trying to infer the process from patterns. This is a well-known problem, much discussed in the context of using observational field data to study life-history changes in exploited fish populations (e.g., Kraak, 2007; Dieckmann and Heino, 2007; Browman *et al.*, 2008; Kuparinen and Merilä, 2008; Jørgensen *et al.*, 2008). The potential role of low oxygen levels in driving phenotypic change, however, has until now been overlooked (e.g., Sharpe *et al.*, 2012). We encourage performing further studies linking these factors to changes in life-history, behavioural and physiological traits, and considering the confounding

effect of oxygen along with other environmental factors when studying the effects of size-selective fishing in exploited populations.

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## Tables

Table 1. Male biphasic growth model estimates for reproductive investment, *r*, growth coefficient, *c*, and allometric exponent, *ß*. Support for a particular model is given by the change in the Akaike Information Criterion (AIC) relative to the model with the lowest AIC ($\Delta_i$), and by the Akaike weights ($w_i$). All models follow equations (2)–(3) but differ in which of the parameters (if any) are affected by the treatment(s) as well as presence of treatment interactions (denoted with "*" in the model formulae). Results are shown for the four best-ranked non-linear mixed effect models (M1–4; the model with the lowest AIC and all models for which $\Delta_i<2$) as well as for the null model (M0) without any effects of experimental treatments (formula "~1" means that parameter is unaffected by the treatments).

| Model | Effects | Support | | Parameter estimates | | | |
|---|---|---|---|---|---|---|---|
| | | $\Delta_i$ | $w_i$ | Treatment | $r$ | $c$ ($g^{1-ß}day^{-1}$) | ß |
| M1 | $r$ ~ size-selec.+ $O_2$ | 0 | 0.28 | High $O_2$ & neg. s-s. | 0.0006 | 0.0009 | 0.16 |
| | $c$ ~ size-selec.+ $O_2$ | | | High $O_2$ & pos. s-s. | 0.0009 | 0.0013 | 0.25 |
| | ß ~ size-selec. | | | Low $O_2$ & neg. s-s. | 0.0025 | 0.0010 | 0.16 |
| | | | | Low $O_2$ & pos. s-s. | 0.0028 | 0.0014 | 0.25 |
| M2 | $r$ ~ $O_2$ | 0.07 | 0.27 | High $O_2$ & neg. s-s. | 0.0007 | 0.0009 | 0.17 |
| | c ~ size-selec. | | | High $O_2$ & pos. s-s. | 0.0007 | 0.0013 | 0.25 |
| | ß ~ size-selec.+ $O_2$ | | | Low $O_2$ & neg. s-s. | 0.0023 | 0.0009 | 0.14 |
| | | | | Low $O_2$ & pos. s-s. | 0.0023 | 0.0013 | 0.22 |
| M3 | $r$ ~ size-selec.* $O_2$ | 0.11 | 0.26 | High $O_2$ & neg. s-s. | 0.0002 | 0.0009 | 0.16 |
| | c ~ size-selec.+ $O_2$ | | | High $O_2$ & pos. s-s. | 0.0014 | 0.0013 | 0.24 |
| | ß ~ size-selec. | | | Low $O_2$ & neg. s-s. | 0.0029 | 0.0010 | 0.16 |
| | | | | Low $O_2$ & pos. s-s. | 0.0020 | 0.0014 | 0.24 |
| M4 | $r$ ~ $O_2$ | 0.81 | 0.19 | High $O_2$ & neg. s-s. | 0.0007 | 0.0009 | 0.16 |
| | $c$ ~ size-selec.+ $O_2$ | | | High $O_2$ & pos. s-s. | 0.0007 | 0.0013 | 0.24 |
| | ß ~ size-selec. | | | Low $O_2$ & neg. s-s. | 0.0026 | 0.0010 | 0.16 |
| | | | | Low $O_2$ & pos. s-s. | 0.0026 | 0.0014 | 0.24 |
| M0 | $r$ ~ 1 | 25.99 | 0.00 | n.a. | 0.0013 | 0.001 | 0.18 |
| | c ~1 | | | | | | |
| | ß ~ 1 | | | | | | |

Table 2. Female biphasic growth model estimates for reproductive investment, $r$, growth coefficient, $c$, and age at maturation, $a_{mat}$. Results are shown for the two best-ranked non-linear mixed effect models (F1–2, i.e. the model with the lowest AIC and the only other model for which $\Delta_i<2$) as well as for the null model (F0) without any effects of experimental treatments. See Table 1 for further explanations.

| Model | Effects | Support | | Parameter estimates | | | |
|---|---|---|---|---|---|---|---|
| | | $\Delta_i$ | $w_i$ | Treatment | $r$ | $c$ ($g^{1-ß}day^{-1}$) | $a_{mat}$ (day) |
| F1 | $r$ ~ size-selec.+ $O_2$ | 0 | 0.48 | High $O_2$ & neg. s-s. | 0.011 | 0.013 | 53.7 |
| | $c$ ~ size-selec.+ $O_2$ | | | High $O_2$ & pos. s-s. | 0.009 | 0.012 | 65.2 |
| | $a_{mat}$ ~ size-selec.* $O_2$ | | | Low $O_2$ & neg. s-s. | 0.014 | 0.014 | 53.6 |
| | | | | Low $O_2$ & pos. s-s. | 0.012 | 0.013 | 62.8 |
| F2 | $r$ ~ size-selec.*$O_2$ | 0.95 | 0.30 | High $O_2$ & neg. s-s. | 0.010 | 0.012 | 54.4 |
| | $c$ ~ $O_2$ | | | High $O_2$ & pos. s-s. | 0.009 | 0.012 | 63.7 |
| | $a_{mat}$ ~ size-selec.+ $O_2$ | | | Low $O_2$ & neg. s-s. | 0.012 | 0.013 | 53.5 |
| | | | | Low $O_2$ & pos. s-s. | 0.014 | 0.013 | 62.9 |
| F0 | $r$ ~ 1 | 48.11 | 0.00 | n.a. | 0.011 | 0.013 | 60.6 |
| | c ~1 | | | | | | |
| | $a_{mat}$ ~1 | | | | | | |

## Figures

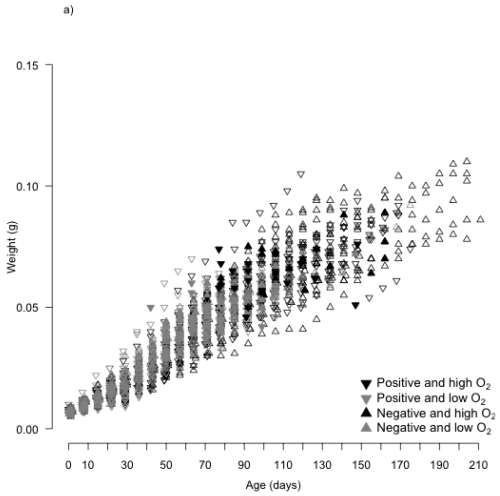
Figure 1: Growth trajectories for males from a) raw data and b) biphasic growth model estimates. In a) symbol type represents the size-selection treatment and colour the oxygen treatment. Filled symbols depict the observations when initiation of maturation was scored. In b) colour represents oxygen treatments, respectively and line type refers to size-selection line. Growth curves are based on the best-ranked model (M1) and growth parameter values are given in Table 1.

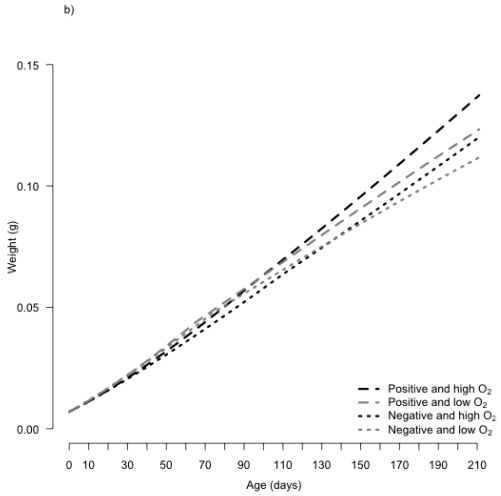
Figure 2: Maximum potential size-specific growth rates for a) males and b) females in high (black lines) and low (grey lines) oxygen treatments and that belonged to the positive size-selection lines (dashed lines) or the negative size-selection lines (dotted lines). Growth rates are based in a) males on the allometric exponent *ß* and coefficient *c* in growth rate-weight relationship estimated with the best-ranked model (M1, Table 1), while in b) females on the allometric coefficient *c* in growth rate-weight relationship estimated with the best-ranked model (F1) and the exponent *ß* had the value of 0.8 for all treatments (Table 2). Realized growth rates are lower when energy is allocated to reproduction; the predicted growth curves in Figs 1b and 4b account for this, for males and females respectively.

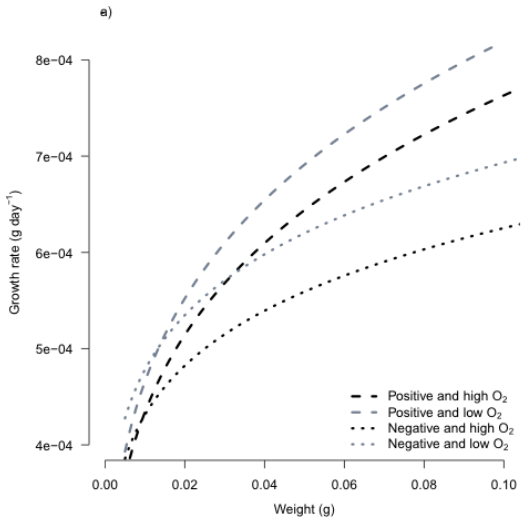
Figure 3: Weight and age-based probabilistic maturation reaction norms for males represented by the midpoints (weight with 50% maturation probability, Wp50) at high (black line) and low (grey line) oxygen conditions and for positive (dashed line) and negative (dotted line) size-selected lines. Black and grey triangles (negative size-selection line) and inverted triangles (positive size-selection line) represent the

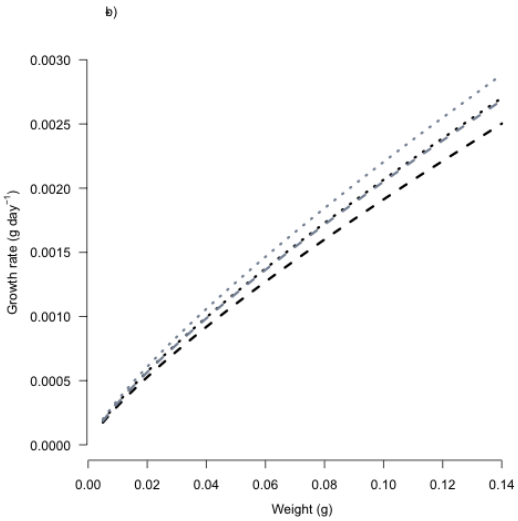
observed weights (g) and ages (days) at maturation for high and low oxygen, respectively.

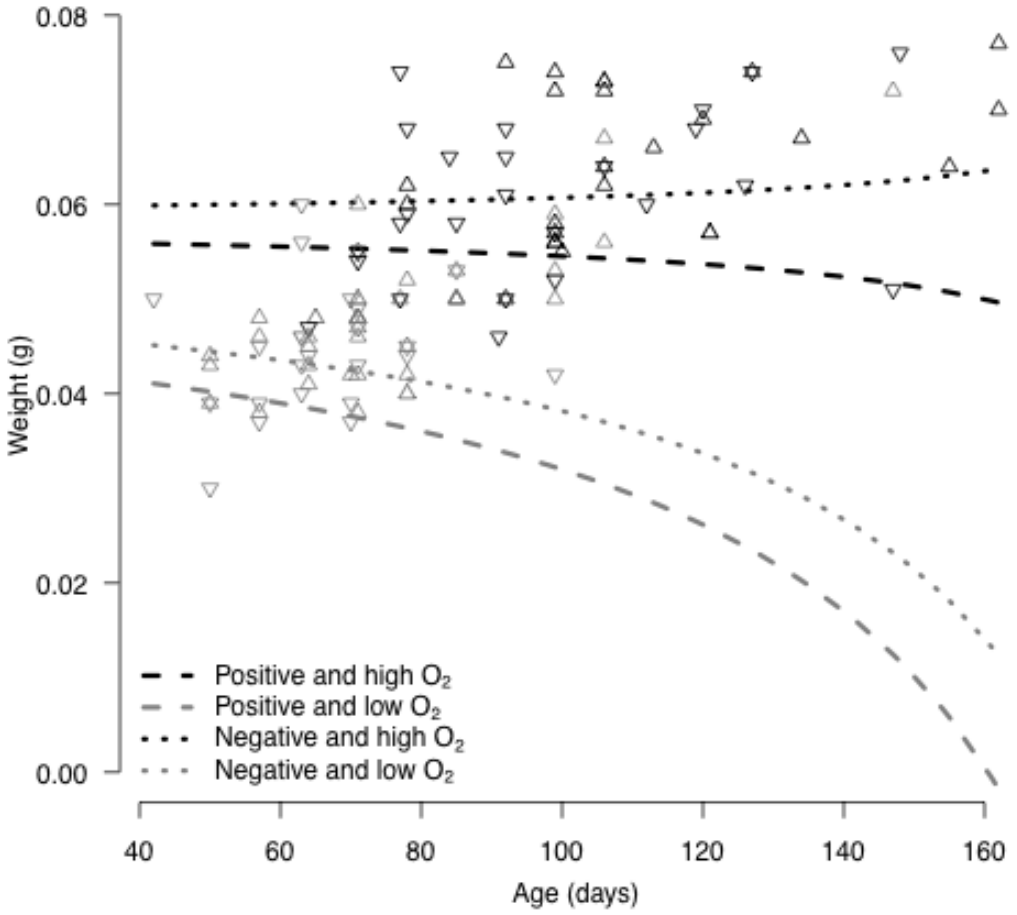
Figure 4: Growth trajectories for females from a) raw data and b) biphasic growth model estimates. In a) inverted triangles represent the positive size-selected line and triangles the negative size-selected line, while black refers to high oxygen and grey to low oxygen. In b) black and grey lines represent high and low oxygen treatments, respectively; and dashed lines refer to males that belonged to the positive size-selection line while dotted lines, the negative size-selection line. Growth curves are based on the best-ranked model (F1) and growth parameter values are given in Table 2.

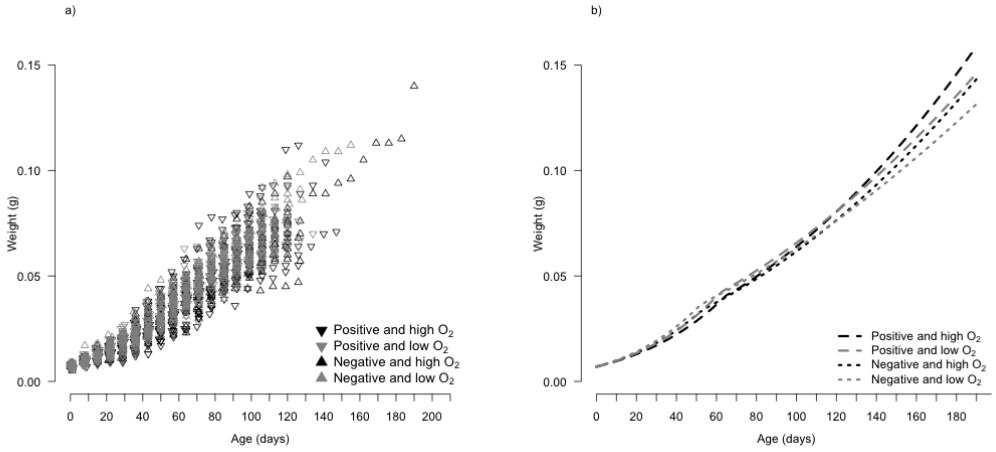

a)
Weight (g)
Age (days)
Positive and high O2
Positive and low O2
Negative and high O2
Negative and low O2


b)
Weight (g)
Age (days)
Positive and high O2
Positive and low O2
Negative and high O2
Negative and low O2


a)
Growth rate (g day−1)
Weight (g)
Positive and high O2
Positive and low O2
Negative and high O2
Negative and low O2


b)
Growth rate (g day−1)
Weight (g)


0.08
0.06
0.04
0.02
0.00
Weight (g)
Positive and high O2
Positive and low O2
Negative and high O2
Negative and low O2
40
60
80
100
120
140
160
Age (days)


a)
b)
0.15
0.10
0.05
0.00
Weight (g)
Positive and high O2
Positive and low O2
Negative and high O2
Negative and low O2
0
20
40
60
80
100
120
140
160
180
200
Age (days)