



# Stoichiometric consequences of size-selective mortality: An experimental test using the Japanese medaka (*Oryzias latipes*)

Charlotte Evangelista<sup>a,\*</sup>, Beatriz Diaz Pauli<sup>a,1</sup>, Leif Asbjørn Vøllestad<sup>a</sup>, Eric Edeline<sup>b,c</sup>

<sup>a</sup> Centre for Ecological and Evolutionary Synthesis (CEES), Department of Biosciences, University of Oslo, Oslo, Norway

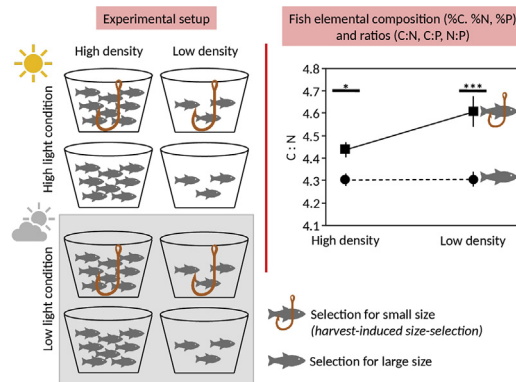
<sup>b</sup> Sorbonne Université, Université Paris Diderot, UPEC, CNRS, INRAE, IRD, Institut d'Ecologie et des Sciences de l'Environnement de Paris (iEES-Paris), F-75252 Paris, France.

<sup>c</sup> ESE, Ecology and Ecosystem Health, INRAE, Agrocampus-Ouest, Rennes, France

## HIGHLIGHTS

- We studied the consequences of size-selective mortality on stoichiometric variation.
- We used a mesocosm experiment and fish selected for large or small size.
- Size-selection had an effect on fish body N content.
- Stoichiometric variation due to size-selection was also density- and sex-dependent.
- Size-selective mortality was for the first time found to shape organismal stoichiometry.

## GRAPHICAL ABSTRACT



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## ABSTRACT

The determinants of intraspecific stoichiometric variation remain difficult to elucidate due to their multiple origins (e.g. genetic vs. environmental) and potential interactive effects. We evaluated whether two size-selected lines of medaka (*Oryzias latipes*) with contrasted life-history strategies (small- and large-breeder lines with slow growth and early maturity vs. fast growth and late maturity) differed in their organismal stoichiometry (percentage and ratios of carbon [C], nitrogen [N] and phosphorus [P]) in a mesocosm experiment. We also tested how size-selection interacted with environmental conditions (i.e. two levels of fish density and light intensity), body condition and sex. Results showed that large-breeder fish were significantly N-enriched compared to small-breeders, while the two size-selected lines did not differ in body P composition. Size-selection interacted with density – high density only affected small-breeders leading to decreasing %C and C:N – and with sex – large-breeder females had higher %C and C:N values than large-breeder males. Finally, C:P and N:P ratios increased with body condition due to decreasing %P. Overall, our results show that the ecological consequences of size-selective mortality extend to organismal stoichiometry and may, from there, change nutrient cycling and ecosystem functioning.

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\* Corresponding author.

E-mail address: [charlotte.evangelista@ibv.uio.no](mailto:charlotte.evangelista@ibv.uio.no) (C. Evangelista).

<sup>1</sup> Present address: Department of Biological Sciences, University of Bergen, Bergen, Norway.

## 1. Introduction

Environmental changes have long been recognized to affect evolutionary processes, while the effects of evolution on ecosystem functioning – *evo-to-eco pathways* – have been until now little explored empirically (Brunner et al., 2019). Recent studies have highlighted the role of intraspecific trait variation in shaping ecosystem functioning (see review in Des Roches et al., 2018), emphasizing its importance for modulating the interactions between ecological and evolutionary processes (Brunner et al., 2017; Matthews et al., 2016). Human activities can drastically modulate intraspecific trait variations due to increased mortality of large-sized individuals (Darimont et al., 2009; Sullivan et al., 2017), and thus likely affect *evo-to-eco pathways*. For instance, size-selective mortality due to harvest often leads to a rapid evolution towards reduced body size (Sharpe and Hendry, 2009), which may reduce the top-down control on lower trophic levels through changes in the assemblage of top consumer (Shackell et al., 2009). This is likely because small-bodied top consumers have lower *per-capita* consumption rates and reduced ranges of prey body size (DeLong et al., 2015; Renneville et al., 2016). Although such body size-dependent consequences of size-selective mortality are now being investigated, a full understanding of its ecological effects requires considering how other traits such as organismal stoichiometry (i.e. elemental composition and elemental ratios in a consumer's body) respond to size selection.

Ecological stoichiometry (ES) studies the balance of energy and multiple chemical elements in organisms and how these are shaped by various biological factors (Sternner and Elser, 2002). Changes in fitness-related traits drive stoichiometric changes, indicating that ES is a powerful framework for investigating the ecological importance of evolutionary changes (Kay et al., 2005). The theory of ES posits that individuals assimilate nutrients and sequester a part of these to meet their requirements for growth and reproduction (Hessen et al., 2013; Sternner and Elser, 2002), while nutrients in excess are excreted as by-products and can subsequently enhance the primary production (Bassar et al., 2010; Palkovacs et al., 2009). Stoichiometric traits (i.e. “individual measures of elemental composition, assimilation, allocation, or excretion that are usually defined by their elemental content or ratios between elements”; Leal et al., 2017) not only reflect balances between an individual's nutritional composition, energetic allocation and resource composition, but also provide information on the functional role of an individual within its ecosystem in terms of nutrient dynamics (Boros et al., 2014; McIntyre and Flecker, 2010). All these findings suggest that organismal stoichiometry may be a useful tool to quantify the ecological consequences of size-selective mortality, in which a suite of fitness-related traits is often modulated (e.g. somatic growth rate, reproduction; Kuparinen and Festa-Bianchet, 2017).

Recent advances have documented the ubiquitous nature of variation in organismal stoichiometry within fish species (see review in Jeyasingh et al., 2014). Such variability can be shaped by environmental factors (e.g. biotic and abiotic conditions such as competition, predation, temperature and light availability; Dalton and Flecker, 2014; Kay et al., 2005) and individual genetic background (El-Sabaawi et al., 2016), but their interactions on organismal stoichiometry remain poorly elucidated (Leal et al., 2017). Environmental factors influence organismal stoichiometry through direct effects on individuals (e.g. the predator-induced stress paradigm, Dalton and Flecker, 2014), or by altering the quantity and quality of the resources consumed. For instance, phytoplankton is expected to have lower nutritional quality (i.e. low nutrient content relative to carbon or high C:nutrient molar ratios) with increasing light intensity (Sternner et al., 1997) and this effect can spread to upper trophic levels, ultimately altering the body nutrient content of top consumers such as fish (Dickman et al., 2008). This effect on the organismal stoichiometry of top consumers can be dampened by several mechanisms, including access to a larger amount of resources and/or selection towards traits that have low nutrient demand (e.g. slow growth rate; Sternner and Elser, 2002). In the context of size-selective mortality

induced by fisheries, changes in fish organismal stoichiometry can occur via (1) increased resource availability due to relaxed competition from removal of fish biomass (Law, 2000), and (2) selection for earlier maturation and slower somatic growth due to the removal of large-bodied and fast-growing individuals (Heino et al., 2015). However, these environmental and evolutionary effects of size-selective mortality on intraspecific variability in organismal stoichiometry remain largely overlooked (Palkovacs et al., 2018).

The aim of the present study was to investigate how size-selection induced life-history changes can modulate intraspecific variability in organismal stoichiometry (percentage and ratios of carbon [C], nitrogen [N] and phosphorus [P]). We used two lines of medaka (*Oryzias latipes*) originating from a size-selection experiment performed under controlled laboratory conditions over 10 generations (Renneville et al., 2020). The two lines presented divergent life-history strategies: the small-breeder line was composed of individuals with slow growth and early maturity, and the large-breeder line was composed of individuals with fast growth and late maturity. We also investigated how environmental conditions may interact with size-selection to modulate organismal stoichiometry. Fish density was chosen as a treatment due to its role in competition release that comes with size-selective mortality, and light intensity was chosen because it is an important factor impacting on resource availability in aquatic ecosystems; both treatments can influence the stoichiometric traits of top consumers (Dickman et al., 2008; Zandonà et al., 2011).

We predicted that large-breeder fish would have higher body N and P content compared to small-breeder fish due to evolution towards faster somatic growth rate favoring the development of a P-rich skeleton and N-rich muscle (McIntyre and Flecker, 2010; Pilati and Vanni, 2007). However, because the somatic growth of adult fish is expected to rely more on muscle development compared to bone and scale production (Tanner et al., 2000), we predicted that adults of the large-breeder line would have higher body N:P ratios. We also predicted that the early-maturing small-breeder line would display high body C content because reproduction largely relies on storing and mobilizing C-rich fat tissue (Sternner and Elser, 2002). Finally, we predicted that high body condition (i.e. high body “plumpness”) would be associated with high C-rich lipid storage, and that females would be C-enriched because they invest more in reproduction (e.g. egg storage and development of adipose tissue).

## 2. Material and methods

### 2.1. Experimental fish

Native to East Asian countries, the medaka is a small omnivorous fish species (adult length = 32 mm) that preferentially feeds on zooplankton and benthic invertebrates. The experimental fish originated from two experimental size-selected lines bred for 10 generations under controlled laboratory conditions (temperature: 26 °C, photoperiod: 14 h Light/10 h Dark, density: 14–17 fish per 3L-tank, feeding: ad libitum with mixed diet of dry food and living *Artemia salina* nauplii) to ensure that differences between lines were genetically- rather than environmentally-induced. The selection procedure consisted of removing the largest or the smallest breeders, hence producing two lines with distinct life-history strategies: the small-breeder line (i.e. slower growth rate and earlier maturation) and the large-breeder line (i.e. faster growth and delayed maturation), where only small-bodied and large-bodied individuals were allowed to reproduce, respectively. Specifically, at 60 day-post-hatching (dph), among a total of at least 20 families per line, 10 families with the largest (large-breeder line) and smallest (small-breeder line) average standard body length were kept. At 75 dph, individuals within each family were measured and the largest-bodied (large-breeder line) or the smallest-bodied (small-breeder line) mature males ( $n = 2$  or  $3$ ) and females ( $n = 2$  or  $3$ ) were used as breeders for the next generation. After 7 generation,

the small-breeder fish were 5.5% shorter in standard length at 75 day-post-hatch, had 3 times higher odds of being mature, and also had larger eggs than their large-breeder conspecifics (Renneville et al., 2020).

On 27th June 2017, fish from generation F11 were checked for maturity according to secondary sexual characters (Kinoshita et al., 2009). For each line, 180 mature fish (initial fork length  $\pm 1$  mm FL<sub>i</sub>; mean large-breeder = 19.4 mm  $\pm 1.4$  SD; mean small-breeder = 18.9 mm  $\pm 1.3$  SD; ANOVA:  $F_{1,358} = 13.70$ ,  $P < .001$ ) were chosen to generate 24 populations subsequently transferred to outdoor mesocosms. Each population ( $n = 48$  in total) was composed of individuals from the same line, but from distinct families to limit inbreeding (mean kinship coefficient =  $0.23 \pm 0.1$  and  $0.17 \pm 0.1$  SE in large- and small-breeder populations, respectively). Fish were anesthetized with MS-222 (tricaine methanesulfonate) and marked using visible implant elastomer (VIE; Northwest Marine Technology, Shaw Island, WA, USA) to render each fish individually identifiable within each population and to allow the calculation of individual somatic growth rate (Appendix S5).

## 2.2. Outdoor mesocosm experiment

In early April 2017, 48 outdoor mesocosms (500 L, 0.8 m deep, 1.0 m diameter) were arranged in 5 blocks at the CEREEP-Ecotron Ile de France (Saint-Pierre-les-Nemours, France; [www.foljuif.ens.fr](http://www.foljuif.ens.fr)) and filled with a mix of dechlorinated tap water (100L) and filtered oligotrophic pond water (300 L; after filtering out larger zooplankton and debris through 150  $\mu$ m mesh). The mesocosms were supplied with 2 L of mature sediment mixture including benthic invertebrates (mainly Ephemeroptera and Chironomidae larvae, Planorbidae, Hydrachnidia, Nematoda and Ostracoda) and 2 L of homogenized mixture of zooplankton (Copepoda and Cladocera) collected from local ponds. In each mesocosm, two floating shelters made of wool threads (30 cm length) provided spawning substrate and two floating brushes made of plastic threads provided protection for larvae. Each mesocosm was then covered with a shading net (see details below) and given 3 months to mature before fish were introduced.

On 4th July 2017, large- and small-breeder fish were released into the outdoor mesocosms under contrasting environmental conditions. Specifically, we applied two densities (high density, HD: 12 fish/mesocosm or 3.2 mg fish L<sup>-1</sup>  $\pm 0.3$  SD; low density, LD: 3 fish/mesocosm or 0.9 mg fish L<sup>-1</sup>  $\pm 0.1$  SD) and two light intensities using shade nets with different mesh size that allowed the passage of 92% (high light, HL) and 70% of ambient light (low light, LL), resulting in a total of 8 treatments combinations (i.e. SB-HD-HL, SB-HD-LL, SB-LD-HL, SB-LD-LL, LB-HD-HL, LB-HD-LL, LB-LD-HL, LB-LD-LL), each with 6 replicates. In each medaka population, sex ratio was 2 females per male.

On 22nd September 2017, marked fish were recaptured with hand nets (i.e. survival rate = 92%), measured for final fork length (FL<sub>f</sub>  $\pm 1$  mm), weighed ( $W_f \pm 1$  mg), and euthanized using MS-222. All the surviving marked fish from LD treatments, and a subsample of 4 marked individuals per mesocosm (2 females and 2 males, randomly selected) from HD treatments were used for subsequent stoichiometric analyses (Appendix S1). Finally, body condition of each selected individual ( $n = 161$ ) was calculated using the residuals of the relationship between  $\log_{10} W_f$  and  $\log_{10} FL_f$ . One fish had an extremely low body condition ( $-0.31$ ) compared to the others (mean =  $0.002 \pm 0.052$  SD) and was thus removed from the subsequent analyses.

## 2.3. Elemental analysis

Organismal stoichiometry was quantified from gutted fish (Pilati and Vanni, 2007), while reproductive tissues were kept. After dissection, fish were stored at  $-20$  °C, freeze-dried, ground into a fine homogeneous powder and finally analyzed for C, N and P content. For each fish, %C and %N of dry mass were measured by dry combustion with a CHN analyser (Flash 200, Thermo Fisher Scientific, Waltham, MA, USA;

method ISO 10694:13878). %P was quantified following the molybdate-blue method using a spectrophotometer (Uvi Light XT5, Secomam, Alès, France; absorbance measured at 880 nm) after an acid digestion in autoclave. For each element, one sample was analyzed per individual. Body nutrient contents were used to calculate molar ratios for C:N, C:P and N:P.

## 2.4. Statistical analyses

All statistical analyses were performed using R v.3.6.1 (R Development Core Team, 2019). Linear mixed effects models (LMMs) were used to assess the effect of size-selection (Line) and environmental conditions (Density and Light intensity) on individual body condition and stoichiometry (one for each stoichiometric trait: %C, %N, %P, C:N, C:P and N:P). "Tank identity" nested within "Block identity" were set as random effects. Full models also included the interactions of Density  $\times$  Line and Light intensity  $\times$  Line, as well as Sex, and the interaction of Sex  $\times$  Line as predictors. In addition, body condition was used as a predictor in the LMMs with stoichiometric traits as dependent variables. We did not provide any hypotheses for higher-order interactions and thus they were not tested.

LMMs were fitted using the "lme" function from the "nlme" R package (v.3.1.140; Pinheiro et al., 2019). The variance inflation factors ranged between 1.2 and 3.4, indicating no evidence for multicollinearity among the predictors (Zuur et al., 2009). The significance of each predictor was based on Type II Wald Chi-square statistics ( $\chi^2$ ) using the "Anova" function from the "car" R package (v.3.0.3; Fox and Weisberg, 2011). For each model, interactions were removed when non-significant using a backward selection procedure. When significant, the interactions were further investigated using post hoc Tukey's pairwise comparison of the estimated marginal means (Appendix S3) using the "emmeans" function from the "emmeans" R package (v.1.3.5.1; Lenth, 2019). Assumptions of linearity and homogeneity of variances on residuals of all LMMs were checked visually. All response variables were  $\log_{10}$  transformed to meet these assumptions. When appropriate (i.e. for LMM with C:N as response variable), a "weight" argument was included into the "lme" function to correct for Line heterogeneity in residuals variance (varIdent; Zuur et al., 2009). For each LMM, both the marginal  $R^2$  ( $R^2_M$ , variance explained by fixed factors) and conditional  $R^2$  ( $R^2_C$ , variance explained by fixed and random factors) were calculated (Nakagawa and Schielzeth, 2013).

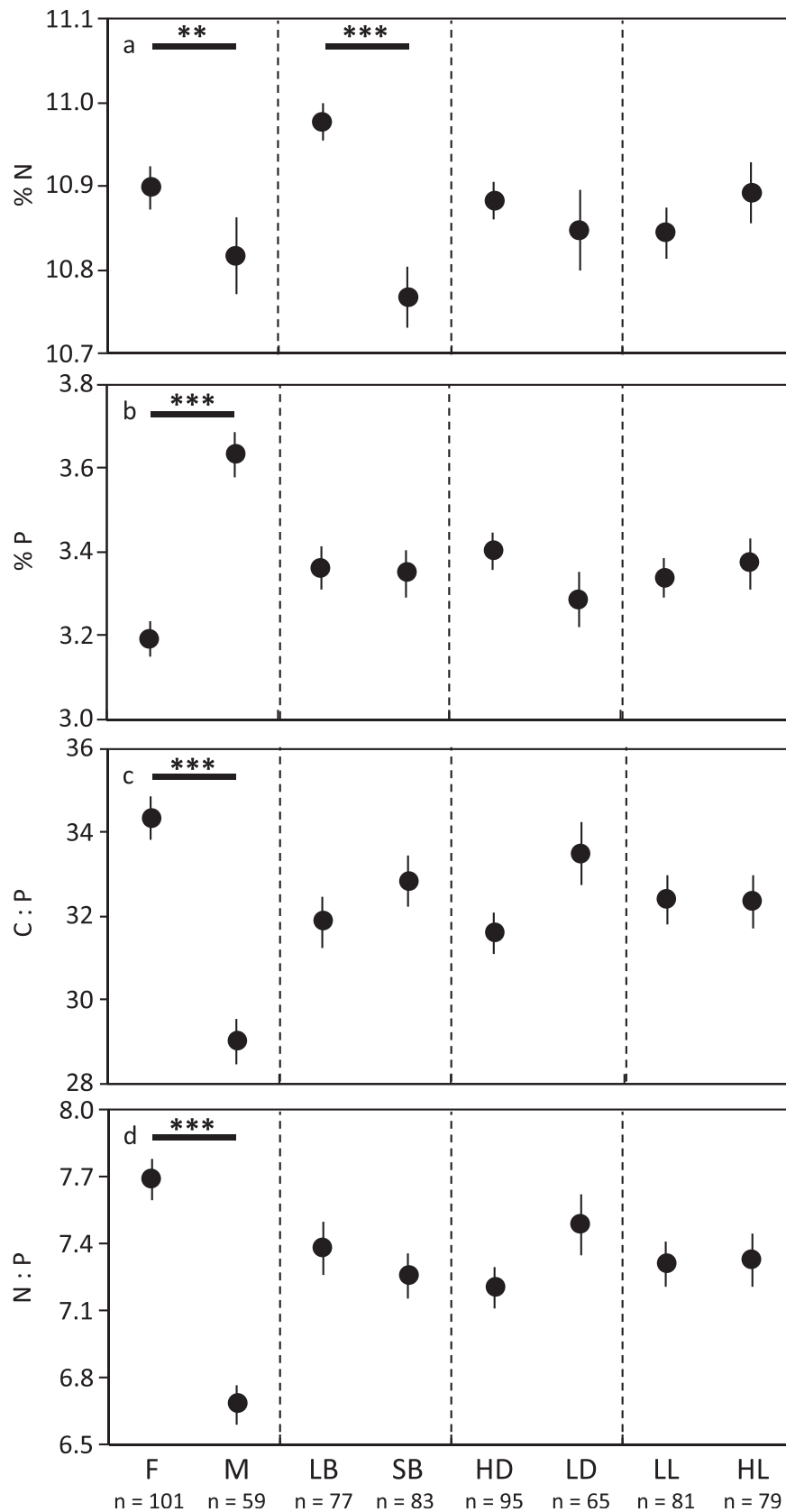
## 3. Results

Body P content (%P) was the most variable element among medaka (overall coefficient of variability = 14.4%) ranging from 2.3 to 5.6%. Percent C and N (CV = 4.2% and 2.7%, respectively) varied from 36.6 to 46.0% and from 9.6 to 11.4%, respectively (Appendix S2).

Individuals from the large-breeder line were significantly N-enriched (Fig. 1a) and had a lower C:N ratio (Table 1; Fig. 2c-d). Independently from other factors, selection line did not affect %C and %P, and density and light intensity had no significant effect on organismal stoichiometry (Table 1; Fig. 1).

We found, however, a significant density-by-line interaction. The large-breeder line was density-insensitive for %C or C:N, but increasing density decreased both %C and C:N in small-breeder medaka (Appendix S3, Fig. 2a,c). Small-breeder fish had a significantly higher %C compared to large-breeder medaka, but only in the low-density treatment (Appendix S3; Fig. 2a). The difference in C:N between the small- and large-breeder lines was more pronounced at low density (Appendix S3; Fig. 2c).

Females were significantly N-enriched and P-depleted compared to males (Table 1; Fig. 1a-b), resulting in significantly higher C:P and N:P ratios for females (Table 1; Fig. 1c-d). Sex differences in %C and C:N were only observed in the large-breeder line, with females having higher %C and C:N (Appendix S3; Fig. 2b,d). The small-breeder line



**Fig. 1.** Elemental composition (%N and %P; **a - b**) and ratios (C:P and N:P; **c - d**) of female (F) and male (M) medaka fish ( $n = 160$ ; mean  $\pm$  SE) originated from two opposite size-selected lines (large-breeder LB and small-breeder SB) and under contrasted density (high HD and low LD) and light intensity conditions (high light HL and low light LL). Asterisks highlight significant differences among lines within factors: \*\*\*  $P < .001$ ; \*\*  $P < .01$ .

**Table 1**

Analysis-of-deviance table derived from the linear mixed effects models used to test the effects of size-selection (Line), environmental conditions (Density and Light intensity), sex and body condition on medaka fish ( $n = 160$ ) elemental composition (%C, %N and %P) and elemental ratios (C:N, C:P and N:P). All stoichiometric traits were  $\log_{10}$  transformed prior to analysis. Marginal ( $R_M^2$ , effect of the fixed effects) and conditional ( $R_C^2$ , effect of the fixed and random effects)  $R^2$  are also provided. Two-way interactions were removed when non-significant (-). Significant  $P$  values are displayed in bold.

Predictors	Elemental composition (%)					
	%C		%N		%P	
	$\chi^2$	$P$	$\chi^2$	$P$	$\chi^2$	$P$
Sex	36.43	<0.001	5.27	<b>0.022</b>	39.16	<0.001
Body condition	3.15	0.076	0.42	0.518	4.42	<b>0.036</b>
Line	10.62	<b>0.001</b>	17.24	<0.001	0.36	0.547
Density	1.43	0.232	0.32	0.572	0.55	0.459
Light intensity	<0.01	0.964	0.89	0.345	<0.01	0.980
Sex $\times$ Line	10.67	<b>0.001</b>	-	-	-	-
Density $\times$ Line	4.45	<b>0.035</b>	-	-	-	-
Light intensity $\times$ Line	-	-	-	-	-	-
$R_M^2 - R_C^2$	0.31-0.53		0.15-0.33		0.21-0.44	
	Elemental ratios					
	C:N		C:P		N:P	
Sex	26.47	<0.001	43.35	<0.001	50.60	<0.001
Body condition	0.38	0.538	4.26	<b>0.039</b>	5.43	<b>0.020</b>
Line	20.03	<0.001	0.10	0.754	2.14	0.143
Density	0.04	0.835	0.99	0.321	0.42	0.515
Light intensity	0.83	0.363	<0.01	0.965	0.03	0.868
Sex $\times$ Line	7.49	<b>0.006</b>	-	-	-	-
Density $\times$ Line	6.43	<b>0.011</b>	-	-	-	-
Light intensity $\times$ Line	-	-	-	-	-	-
$R_M^2 - R_C^2$	0.36-0.59		0.24-0.46		0.26-0.47	

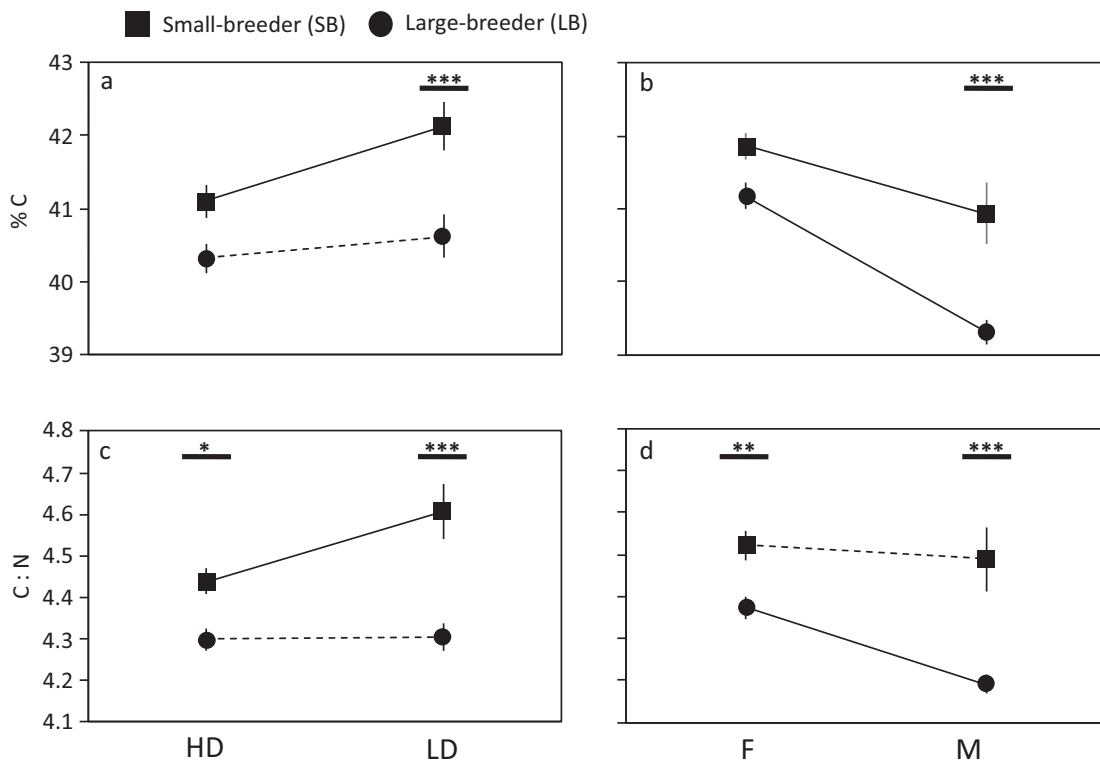
had higher %C than the large-breeder line, but only when considering males (Appendix S3; Fig. 2b). As a result, the overall difference in C:N between the two lines was higher in males compared to females (Fig. 2d).

Body condition of medaka was significantly higher in females compared to males, but only in the small-breeder line (Appendix S4). Small-breeder females had higher body condition than large-breeder ones, but body condition did not differ between small- and large-breeder males (Appendix S4). Body P content (%P) was negatively affected by body condition (Table 1; Fig. 3c), while %C and %N did not change with body condition (Table 1; Fig. 3a-b). Therefore, body C:P and N:P increased with increasing body condition (Fig. 3e-f), but C:N remained constant (Fig. 3d).

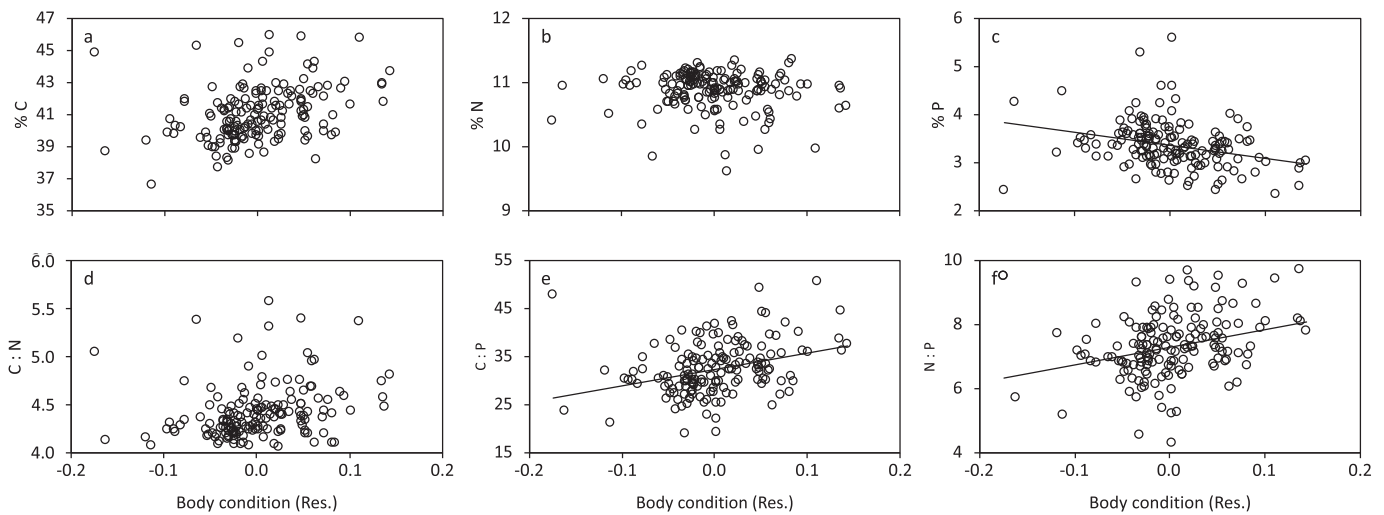
**4. Discussion**

Recent studies have observed substantial intraspecific variability in organismal stoichiometry (e.g. El-Sabaawi et al., 2012; El-Sabaawi et al., 2016; González et al., 2011) and revealed its potential to shed light on the *evo-to-eco pathways* (Kay et al., 2005; Leal et al., 2017). However, the underlying mechanisms driving such variation are still poorly understood. Here, we have shown that fish stoichiometry differed between individuals originated from two size-selected lines with contrasted life-history strategies. We also uncovered that the stoichiometric differences between the size-selected lines depended on density and sex, but not on the light treatment. Overall, our findings suggest that intraspecific variation in organismal stoichiometry might arise, not solely from organisms' genetic background, but also from interaction between genetic background and environmental conditions (Leal et al., 2017).

Our results confirm that fish display wide intraspecific variability in their organismal stoichiometry. McIntyre and Flecker (2010) found that %P and %C variability (i.e. coefficient of variability expressed as a percentage) across 100 species of freshwater fish were 29.8% and 8.3%, respectively. The level of intraspecific variability that we observed in our experiment represents almost 50% of the interspecific variability (14.1% compared to 29.8% and 4.1% compared to 8.3%; Appendix S2). Therefore, in agreement with El-Sabaawi et al. (2012), our results



**Fig. 2.** Density- (high HD and low LD) and sex-dependent (female F and male M) effects of size-selected lines (large-breeder LB and small-breeder SB) on (a - b) %C composition and (c - d) C:N ratio of medaka fish ( $n = 160$ ; mean  $\pm$  SE). Solid lines highlight significant interactions among factors within size-selected lines and dotted lines non-significant interactions (post hoc analyses). Asterisks highlight significant differences among lines within factors: \*\*\*  $P < .001$ ; \*\*  $P < .01$ ; \*  $P < .05$ .



**Fig. 3.** Relationship between fish body condition (residuals from the  $\log_{10}(W_t) - \log_{10}(FL_t)$  regression;  $n = 160$ ) and (a) %C, (b) %N, (c) %P, (d) C:N, (e) C:P and (f) N:P. Significant relationships are displayed using regression lines.

clearly show that conspecific fish are not homogeneous in their organismal stoichiometry and this variability likely accounts for a significant portion of interspecific variation (El-Sabaawi et al., 2014). In addition, body P content was the most variable element in medaka, partly due to variability in individual body condition. Indeed, medaka with higher condition had higher C:P and N:P ratios due to decreased body P content, highlighting that fish with high body condition stored relatively more fat reserves and muscle than bones compared to individuals with low body condition (Leal et al., 2017).

We observed that large-breeder medaka had higher %N than small-breeders, whereas %P did not differ between the two lines. Previous results showed that under laboratory conditions large-breeder medaka grew faster relative to their small-breeder conspecifics (Diaz Pauli et al., 2019; Renneville et al., 2020). Therefore, differences in %N between the two lines could indicate that large-breeder fish accumulate more muscle than small-breeders, perhaps because adult somatic growth rely more on the development of muscle than bones and scales (Tanner et al., 2000). However, during the present experiment, large- and small-breeder adult medaka did not differ in somatic growth rate (Appendix S5), suggesting that outdoor conditions alleviate, at least partly, the phenotypic consequences of size-dependent selection performed under controlled laboratory conditions (Biro and Post, 2008). This highlights that further investigations are required to assess the mechanisms explaining differences in body N content between the two lines. For instance, higher N content in large-breeder medaka might reflect their increased ability to acquire resources, which is expected from earlier studies (Walsh et al., 2006; Diaz Pauli et al., 2019).

We found that size-selection interacted with fish density to determine body %C and the C:N ratio in medaka, but the pattern of this relationship did not match our predictions. Indeed, high medaka density (i.e. low resource quantity) led to decreased %C and C:N of small-breeder medaka, but it did not affect the stoichiometry of large-breeder medaka. Usually, at high density, intraspecific competition is increased and this may impede the selective consumption of high-quality food items (Zandonà et al., 2011), leading to high C:N ratios (i.e. high C relative to N; Dalton et al., 2017). Our results suggest that this trophic niche shift towards high-quality diet under low density did not occur for either line. These results suggest that small-breeder medaka have lower lipid levels when competition is strong, perhaps because under limited competition pressure, they required more C relative to N due to their larger egg sizes (Renneville et al., 2020). Large-breeder medaka seem to tolerate the effect of competition because their body N content was not affected by increasing density, possibly due to their higher capacity to acquire and/or assimilate resources (Diaz Pauli et al., 2019).

However, the mechanisms of changes in energy allocation between individuals remain poorly understood, making it difficult to provide a more detailed interpretations of our results.

We found that females had lower %P and higher %N and N:P compared to males, perhaps due to their higher somatic growth rate (Appendix S5), which is expected to rely more on muscle development relative to bone and scale production (Tanner et al., 2000). In addition, female investment into low phosphorus reproductive tissues might also explain their lower %P relative to males (Durstun and El-Sabaawi, 2019), but this plausible explanation requires further investigations. Sex-based differences in %C and C:N ratio occurred in the large-breeder line, with females having higher relative content of C-rich lipids than males. In addition, small-breeder males had a higher %C than large-breeder males, suggesting a higher energy allocation to C-rich (i.e. energy-rich) lipids. Previous results showed that in laboratory conditions small-breeder medaka matured earlier and had larger egg sizes relative to their large-breeder conspecifics (Renneville et al., 2020), early maturity being often associated with higher fecundity in certain circumstances (e.g. positive size-selective mortality; Wright, 2007). Therefore, although further investigations will be needed, notably by measuring investment into reproduction, we speculate that energy storage in the form of lipid could be attributed to higher investment in reproduction in females, which required large amount of fat for egg development. It could also be due to increased reproductive activity in small-breeder males, such as courtship and fighting to gain access to females (Clark and Grant, 2010), rather than investment into storage. Indeed, body condition can measure the ability of fish to tolerate starvation, but here, differences in body condition were not associated with differences in body C content.

Basal resources are expected to be nutrient-enriched under low light condition, which can subsequently enhance the compositional quality of higher trophic levels such as fish (Dickman et al., 2008). We found no effect of the light intensity treatment on organismal stoichiometry, perhaps due to three non-mutually exclusive mechanisms. First, perhaps the potential nutrient-richer resource under low light conditions did not compensate for the potentially reduced quantity of resources. Second, heterotrophs tend to be more homeostatic (i.e. they keep a constant C:nutrient ratio) than primary producers (Persson et al., 2010), which could dampen stoichiometric changes in consumers. Finally, our light manipulation was potentially not sufficiently contrasted to produce any effects on the stoichiometry of basal resources. Indeed, our light treatment only varied over 1.3-fold, while other light manipulations varying over 3- to 10-fold showed significant effects of light on the elemental composition of both primary producers and fish

(Dickman et al., 2008; Downs et al., 2016). Given the absence of data regarding the C:N:P composition of basal resources, it is difficult to distinguish between these mechanisms. Having information about limiting nutrients in the mesocosms would have helped to provide a better understanding of the mechanistic causes of organismal stoichiometric variability, since the degree of elemental imbalance between the nutrient demand by a consumer and its diet can have consequences for consumer's stoichiometry (Vanni, 2002).

## 5. Conclusion

Our study has illuminated the relevance of studying the interplay between life histories and organismal stoichiometry (Jeyasingh et al., 2014). We highlighted that size-selective mortality may be a potential driver of intraspecific variation in organismal stoichiometry, both directly and through modulating the effects of sex and population density. The organismal stoichiometry of consumers often influences the stoichiometry of excreted material (Vanni, 2002), which can ultimately affect lower trophic levels (Taylor et al., 2015; Evangelista et al., 2017). Further studies should investigate the genetic basis of stoichiometric trait variation within species (Durstion and El-Sabaawi, 2017; Jeyasingh et al., 2014). This is particularly true in the context of human-induced selective forces that impinge on both genetic and phenotypic variability of conspecific individuals.

## CRedit authorship contribution statement

**Charlotte Evangelista:** Conceptualization, Methodology, Data curation, Formal analysis, Writing - original draft. **Beatriz Diaz Pauli:** Data curation, Writing - review & editing. **Leif Asbjørn Vøllestad:** Writing - review & editing. **Eric Edeline:** Methodology, Writing - review & editing. All authors approved the final version of the manuscript.

## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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## Permits

The experiment was approved by the Darwin Ethical committee (case file #Ce5/2010/041) from the French Ministry of Education, Higher Education and Research.

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## Data accessibility

Data that support the findings of this study are available in the figshare repository: 10.6084/m9.figshare.12029667.

## Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.scitotenv.2020.138193>.

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