Effects of ageing and inbreeding on the reproductive traits in a cichlid fish II: the female perspective

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Inbreeding and ageing have substantial impact on an individual's fitness. Both can lead to an accumulation of deleterious alleles resulting in an addition of effects when inbreeding and age interact. The aim of this study was to investigate the separate and simultaneous effects of both factors on primary reproductive traits in females of the West African cichlid *Pelvicachromis taeniatus*, a socially monogamous cave breeder with biparental brood care that prefers kin as mating partner. We compared 1-year-old and 4-year-old lab-bred inbred and outbred females to reveal potential effects of inbreeding and age on ovary and egg traits. Inbreeding and degree of microsatellite heterozygosity had no significant effects on primary reproductive traits (ovary mass, gonadosomatic index, egg number, egg size, and egg color). However, inbred females were larger than outbred ones and individual heterozygosity correlated positively with female body size. The lack of inbreeding depression was expected considering the inbreeding preference of *P. taeniatus*, suggesting that the genetic load is purged from the examined population. Older females were larger than younger females and larger females had heavier ovaries containing a higher number and more intensively yellow colored eggs. The results indicate that age does not negatively affect reproductive traits in females of *P. taeniatus*. We discuss the results in comparison with male *P. taeniatus* in which negative ageing effects were found.

ADDITIONAL KEYWORDS: ageing – egg size – fecundity – gonad – inbreeding depression – microsatellite heterozygosity – ovary – senescence – West African cichlid.

INTRODUCTION

Age and inbreeding can affect individual reproductive traits, for example an individual's fertility and fecundity. As these two factors may interact and be additive, studies dealing with their interplay should contribute to a better understanding of their consequences. Such studies are few (Keller, Reid & Arcese, 2008; Decanini, Wong & Dowling, 2013).

Ageing is defined as the progressive loss of function associated with decreasing fertility and increasing mortality with advancing age (Kirkwood & Austad, 2000). Two classic theories try to explain ageing effects (Charlesworth, 1994): the mutation accumulation theory and the antagonistic pleiotropy theory. According to Medawar's mutation accumulation theory (1952),

deleterious alleles accumulate with age leading to a fitness reduction in late life (Medawar, 1952). William's antagonistic pleiotropy theory (Williams, 1957) predicts that pleiotropic genes cause selection for earlier maturity and increased reproduction early in life at costs of reproduction late in life.

Ageing is expected to affect male reproductive traits more seriously than those of females. Although the risk of mutations generally increases with age, and mutations accumulate in the germ line (Pizzari *et al.*, 2008), the germ line mutation rate is lower in females than in males because fewer germ cells are produced in oogenesis than in spermatogenesis (e.g. Ellegren, 2007). As a consequence, an age-related decline in sperm quality is often greater than a decline in egg quality.

In animals with indeterminate growth, such as fishes, age is correlated with body size, which in turn

influences fecundity (Berglund, Rosenqvist & Svensson, 1986; Howard, 1988). As a consequence, in such species higher fecundity of older and larger females may promote the evolution of delayed senescence compared to species with determinate growth, such as mammals and birds, in which fecundity does not increase with age (Reznick, Ghalambor & Nunney, 2002). In the marine fish *Sebastes melanops*, larger and older females produce offspring of higher quality (Berkeley, Chapman & Sogard, 2004). However, other fish studies report a decline of fecundity or egg quality (Shelton *et al.*, 2012). Thus although larger individuals seem to be preferred as mating partners in many species (Charlton, Reby & McComb, 2007; Chandler & Zamudio, 2008; Baldauf *et al.*, 2009; e.g. Côte & Hunte, 1989; Shine *et al.*, 2001), the positive effect of age and the negative effect of size need to be considered.

Inbreeding is another factor often influencing female reproductive success. It leads to an increased frequency of homozygotes (Hedrick, 2005) facilitating the expression of recessive deleterious alleles in a population. As a consequence, inbreeding often results in a decline of fitness (Slate *et al.*, 2000; Frommen *et al.*, 2008; Charlesworth & Charlesworth, 1987). The quality of ovaries and eggs as well as egg quantity can be negatively affected by inbreeding (in insects: Robert, Couvet & Sarrazin, 2005; Akimoto, 2006; Robinson, Kennington & Simmons, 2009; in birds: Congdon & Briskie, 2010; in fishes: Gallardo *et al.*, 2004). Due to inbreeding depression, most species avoid mating with relatives (reviewed in Pusey & Wolf, 1996). However, some species tolerate inbreeding and experience no disadvantages (reviewed in Szulkin *et al.*, 2013). Inbreeding tolerance or preference can evolve when the costs of inbreeding avoidance or the benefits of inbreeding override the costs of inbreeding (Kokko & Ots, 2006). Purging reduces the strength of inbreeding depression (Pusey & Wolf, 1996), when selection acts against deleterious, recessive alleles in inbred populations (Kristensen & Sørensen, 2005). Thus, further inbreeding can cause little or no fitness reduction (Keller & Waller, 2002), which has been shown in several studies (reviewed in Crnokrak & Barrett, 2002).

Egg quantity and quality are two important factors affecting female reproductive success. In fishes, egg quality is determined by a female's diet, nutrients within the yolk, the eggs' genes, and maternal mRNA transcripts, besides the endocrine status of a female during oocyte growth (reviewed in Brooks, Tyler & Sumpter, 1997). Once ovulated, fish eggs need only few nutrients such as water and water chemicals (Holliday & Jones, 1967), thus all important contents for egg quality have to be incorporated during oocyte growth (Brooks *et al.*, 1997). In several species, egg quality is often illustrated by the egg's color: colorful yellow-orange eggs contain more carotenoids, which are implemented in the egg yolk and act as antioxidants to protect vulnerable tissues against damage caused by free radicals during egg development (e.g. Blount, Houston & Møller, 2000). Furthermore, egg coloration is a signal for egg maturity with ripe eggs being more colorful (Rossoni *et al.*, 2010). Thus, age and inbreeding affect egg coloration and egg ripening.

In the present study, we examined the effects of inbreeding and age on female gonadal traits in *Pelvicachromis taeniatus*. This West African cichlid shows inbreeding preference in laboratory experiments with both sexes preferring full siblings as mating partners (Thünken *et al.*, 2007a, b, 2012). Inbreeding might be adaptive in this species because genetically related breeding pairs are better parents and inbreeding individuals may increase their inclusive fitness. Inbreeding does not seem to be associated with high costs in this species. For instance, there is no evidence for inbreeding depression in juvenile fish in terms of reduced survival or growth (Thünken *et al.*, 2007a). Furthermore, the findings of these studies are underlined by a population genetic analysis showing inbreeding in the wild Moliwe population (Cameroon, Africa) (Langen *et al.*, 2011). Here, we investigated the effects of ageing and inbreeding and their interplay on ovary and egg traits of adult females. For that purpose, we examined 1-year-old (young) and 4-year-old (aged) reproductively active inbred and outbred females. Additionally, we calculated heterozygosity values based on microsatellite data to reveal and relate the degree of heterozygosity to inbreeding effects. Furthermore, as fishes show indeterminate growth, we analyzed allometric relationships of the gonadal traits to study size effects in more detail. From previous studies, we expect little negative inbreeding effects in young females due to purging in this model species (Thünken *et al.*, 2007a; Langen *et al.*, 2011). As mutations of the germ line increase with advancing age, we expect negative ageing effects not only on egg quantity but also on ovary quality and egg quality. Furthermore, theory predicts that age and inbreeding should interact leading to stronger expressed inbreeding effects in old individuals compared to young ones (Charlesworth & Hughes, 1996; Goymer, 2008). In males of *P. taeniatus*, we found strong age effects on gonadal and sperm traits but little inbreeding effects, which were age-dependent, as they were only present in aged males (Langen *et al.*, 2016). Due to differences in the germ line mutation rate between females and males (e.g. Ellegren, 2007), we expect to find less effects in females compared to males.

MATERIAL AND METHODS

STUDY SPECIES AND EXPERIMENTAL FISH

Pelvicachromis taeniatus is a socially monogamous cave breeder with biparental brood care that shows

sexual size dimorphism and sexual dichromatism, the latter being most pronounced during reproduction (Baldauf *et al.*, 2011). Females have a purple belly coloration that represents information about female quality and maturation status (Baldauf *et al.*, 2011). While the male defends the territory against intruders, clutch and larvae are mainly parented by the female in the cave. Free-swimming fry are guarded by both parents for a few weeks.

In total, 38 1-year-old virgin females originating from 16 families (19 inbred and 19 outbred individuals of 8 families each) and 28 4-year-old virgin individuals from 12 families (14 inbred from 5 families and 14 outbred from 7 families) were examined. Second generation, reproductively active, and lab-bred females originating from F1-offspring of a wild-caught parent generation of the Moliwe River population in Cameroon (West Africa, 04°04′N/09°16′E) were used. In the years 2003 and 2006, adult breeding pairs of *P. taeniatus* were caught in the Moliwe River and transported to the Institute for Evolutionary Biology and Ecology in Bonn, Germany. First, F1-offspring of the wild parents were raised. To create the inbred and outbred fish, brother–sister pairs and unrelated pairs were bred in large tanks (50 cm × 30 cm × 30 cm, 45 L). Tanks were separated by opaque grey plastic partitions to avoid visual contact between families. The water temperature was kept at 25 ± 1 °C. Tanks were equipped with sand, Java moss, and an internal filter but without caves to avoid reproduction. In spring 2006 (4-year-old individuals) and summer 2009 (1-year-old individuals), experimental fish were produced from inbreeding (full-sib mating partners) and outbreeding (unrelated mating partners) pairs and raised in full-sib families in large tanks (50 cm × 30 cm × 30 cm, 45 L) that were separated by opaque grey plastic partitions to avoid visual contact between families. Tanks were equipped with sand, Java moss, and an internal filter but without caves to avoid reproduction. The water temperature was kept at 25 ± 1 °C.

In May and October of 2010, females were removed from group tanks and kept individually in small tanks (30 cm × 20 cm × 20 cm, water level 12 cm, 7.2 L), equipped with sand, an airstone, and half a flowerpot as breeding cave. Tanks were filled with 1-day-old tap water and lit with a fluorescent lamp (Lumilux de Luxe daylight, Osram, 36 W) with a light–dark cycle of 12L:12D. The water temperature was 25 ± 1 °C. To avoid visual contact between females to prevent female competition, tanks were separated by grey opaque plastic partitions. Fish were fed daily ad libitum on defrosted *Chironomus* larvae. Females were allowed to see a male that was placed in front of the female's tank in a transparent plastic box (18.5 cm × 11.5 cm × 13.5 cm) for 30 min per day over a period of 9 days to ensure production of eggs, that is ripening of eggs. Only brightly colored, non-stressed males of different families were chosen as stimulus fish to assure females' reaction. All females responded to the males' presence by showing courtship behaviour and swimming towards the male.

The standard length (SL) of each fish was measured and body mass (BM) was weighed (Sartorius LC 221S, Germany) after 9 days (mean ± SD: $SL_{INyoung}$ = 3.96 ± 0.31 cm, $SL_{OUTyoung}$ = 3.75 ± 0.19 cm, SL_{INaged} = 4.57 ± 0.27 cm, $SL_{OUTaged}$ = 4.41 ± 0.17 cm; $BM_{INyoung}$ = 1.71 ± 0.37 g, $BM_{OUTyoung}$ = 1.46 ± 0.20 g, BM_{INaged} = 2.48 ± 0.38 g, $BM_{OUTaged}$ = 2.33 ± 0.29 g). A fin clip of each female was stored in 99.8% ethanol in 1.5 mL tubes for later DNA extraction. Females were killed quickly by decapitation and stored in 4% formaline over a period of at least 2 weeks for fixation, followed by a graded ethanol series in 10% steps from 30 to 70%. After fixation, SL and BM were measured again to account for differences due to formaline storage (after storage individuals were significantly larger but lighter, *P* < 0.001). Thus, the second measurements were used for statistical analyses as ovary and egg traits were also measured after storage.

OVARY AND EGG TRAITS

After fixation, ovaries were extracted surgically. Ovary mass was weighed with an analytical balance (Explorer OHAUS E11140, error ± 0.1 mg) three times for each ovary and a mean was calculated. The total gonad mass and the gonadosomatic index (GSI) were calculated according to de Vlaming, Grossman & Chapman (1982) [GSI = (gonad mass [g]/body mass [g]) × 100]. Each ovary was put on an object slide plastered with Munsell-chips as color standard and scale paper as size standard, and photographed against a black background under a binocular microscope (Leica S8AP0, camera Hitachi HV-C20A 3CCD, software Discus v. 4.6). To ensure standardized illumination among photos, ovaries were lit with a cold light source (KL 1500; settings: stage 4, aperture C, color temperature 3200 K), which was the only light source in the darkened room and it was always placed in the same position. Before taking a photo, a white balance adjustment was conducted with a brightness of 0 and a contrast of 0.45.

Ovaries were dissected in a Petri dish with distilled water to keep ovaries and eggs wet for easier preparation. Eggs were counted and divided into three classes: large (ripe and yellow), medium (white), and small eggs (opaque). Eggs were placed in a Petri dish with Munsell-chips and scale paper and photos were taken using the same setup and light conditions as for ovaries. Egg length and width were measured three times from the photos using ImageJ v. 1.43. Out of the mean values, the effective egg diameter (for non-spherical

eggs) was calculated according to Coleman (1991) using the following equation: effective diameter $(d_e) = (ab^2)^{1/3}$, with the major axis a (egg length) and the minor axis b (egg width).

EGG COLORATION MEASUREMENTS

The female's ovary and egg coloration were measured on the photos using the software Adobe Photoshop (CS4). Photos were imported with the default color temperature. On the basis of the white Munsell-chip a tonal correction was conducted. The CIE L*a*b (Commision Internationale de l'Eclairage, 1976) color space was used for analysis. It is a device-independent, standardized, and perceptually uniform color space (Chen, Hao & Dang, 2004), commonly used in analyzing fish coloration partly including carotenoid-based coloration (e.g. Svensson *et al.*, 2009). The L*-value indicates the lightness, the a*- and b*-values the saturation of different colors (a: negative values = green, positive values = red; b: negative values = blue, positive values = yellow). Ovaries and eggs were cut out from images using the magnetic lasso tool and an average of the L*a*b was calculated out of the whole area. Six eggs per ovary of the largest egg class (ripe eggs to be spawned) were measured, eggs randomly chosen from each ovary. A chromaticity value, which entered the analyses, was then calculated according to the following formula by Robertson (1977) representing the color saturation:

$$\text{Chromaticity} = \sqrt{a^2 + b^2}$$

MICROSATELLITE ANALYSIS

A microsatellite analysis was conducted, on the one hand, to determine the effects of experimental inbreeding on heterozygosity and, on the other hand, to take into account the potential variation in individual heterozygosity using nine microsatellites already established in *P. taeniatus* through cross-species amplification (Langen *et al.*, 2011; Langen, Thünken & Bakker, 2013). Details on microsatellite analysis and microsatellite statistics are given in the electronic supplementary material and methods.

STATISTICAL ANALYSES

Statistics were performed with the R version 3.1.0 statistical software package (R Development Core Team, 2009). *P*-values are two-tailed throughout. Linear mixed effects models (lme's) were conducted that implement family identity of individual fish as random factor and are based on a likelihood ratio test (LRT) using the nonlinear mixed effects models (nlme)

package in R. Non-significant interactions ($P > 0.05$) and factors ($P > 0.05$) were removed from the model.

The effects of age class (1-year-old vs. 4-year-old) and breeding line (inbred vs. outbred) on body, ovary, and egg traits were examined (see Table 1). Additionally, SL was added as fixed factor to estimate its relative importance because age and SL are often correlated. To account for previous inbreeding, lme's with heterozygosity and age class and SL as fixed factors were conducted.

Reported *P*-values of models refer to the increase in deviance when the respective variable was removed. If data were not normally distributed according to the Shapiro-Wilk test, residuals were tested on normal distribution or data were transformed. Data of gonad mass were logarithmically (only for the lme with effective diameter as explanatory variable) and data of effective diameter were exponentially transformed to achieve normally distributed data. The sample size for gonad mass and GSI was only $N = 65$ instead of $N = 66$ because one 1-year-old outbred female had only a single ovary. For egg coloration measurements, two females had only non-ripe eggs that were not used leading to a sample size of $N = 64$.

RESULTS

EFFECTS OF AGE AND INBREEDING

Interactions between ageing and inbreeding were never significant (see Table 1). Aged females were significantly larger and heavier than young ones (SL: LRT, $N = 66$, $\chi^2 = 48.649$, $P < 0.001$; BM: LRT, $N = 66$, $\chi^2 = 39.868$, $P < 0.001$), and inbred females were larger and heavier than outbred ones (SL: LRT, $N = 66$, $\chi^2 = 5.166$, $P = 0.023$; BM: LRT, $N = 66$, $\chi^2 = 5.198$, $P = 0.023$). In aged females, ovaries were heavier than in young females (Table 1, Fig. 1A), but the GSI did not differ significantly between age groups (Table 1). Ovary color intensity was significantly greater in older females than in young ones (Table 1). Large females produced significantly more eggs than small females (Table 1, Fig. 1B). Egg coloration was more intense (more yellow) in old females than in young ones (Table 1). Age, SL, and breeding line did not significantly affect the egg's effective diameter (Table 1). Descriptive statistics of measured traits are given in Table 2.

EFFECTS OF HETEROZYGOSITY

According to microsatellite analyses, the inbred group exhibited high inbreeding values and low heterozygosity and deviated significantly from Hardy Weinberg equilibrium ($A = 4$, $H_{exp} = 0.456$, $H_{obs} = 0.350$, $p_{HWE} < 0.001$, $F_{IS} = 0.231$), while the outbred group did not

Table 1. Results of the linear mixed effects models examining the effect of age and inbreeding (or microsatellite heterozygosity, respectively) on different gonadal traits of female *P. taeniatus*.

Dependent variable	Effects		N	Δd.f.	χ^2	P
Ovary mass	Fixed	Breeding line × age	65	1	0.133	0.715
		Age	65	1	0.002	0.962
		Breeding line	65	1	0.039	0.844
		SL	65	1	13.916	**<0.001**
		Heterozygosity × age	65	1	0.035	0.853
		Age	65	1	0.0003	0.985
		Heterozygosity	65	1	0.572	0.450
		SL	65	1	13.916	**<0.001**
	Random	Family	65	1	9.587	**0.002**
Gonadosomatic index	Fixed	Breeding line × age	65	1	0.592	0.442
		Breeding line	65	1	0.024	0.876
		Age	65	1	0.336	0.562
		SL	65	1	0.052	0.819
		Heterozygosity × age	65	1	0.008	0.929
		SL	65	1	0.220	0.639
		Age	65	1	0.121	0.727
		Heterozygosity	65	1	0.488	0.485
	Random	Family	65	1	1.923	0.166
Egg number	Fixed	Breeding line × age	66	1	0.394	0.530
		Breeding line	66	1	0.419	0.517
		Age	66	1	2.766	0.096
		SL	66	1	30.338	**<0.001**
		Heterozygosity × age	66	1	0.018	0.893
		Heterozygosity	66	1	3.027	0.082
		Age	66	1	2.766	0.096
		SL	66	1	30.338	**<0.001**
	Random	Family	66	1	3.057	0.080
Effective egg diameter	Fixed	Breeding line × age	66	1	0.050	0.823
		SL	66	1	2.188	0.139
		Breeding line	66	1	1.686	0.195
		Age	66	1	2.522	0.112
		Heterozygosity × age	66	1	0.054	0.816
		Heterozygosity	66	1	0.061	0.806
		SL	66	1	0.747	0.388
		Age	66	1	2.522	0.112
	Random	Family	66	1	0.000	0.999
Egg color saturation	Fixed	Breeding line × age	64	1	1.521	0.218
		SL	64	1	0.038	0.845
		Breeding line	64	1	1.826	0.177
		Age	64	1	10.833	**0.001**
		Heterozygosity × age	64	1	0.264	0.608
		Heterozygosity	64	1	0.020	0.888
		SL	64	1	0.624	0.430
		Age	64	1	10.833	**0.001**
	Random	Family	64	1	0.181	0.671
Ovary color saturation	Fixed	Breeding line × age	66	1	1.450	0.229
		SL	66	1	0.157	0.692
		Breeding line	66	1	1.000	0.317
		Age	66	1	9.385	**0.002**

Table 1. Continued

Dependent variable	Effects		N	Δd.f.	χ²	P
		Heterozygosity × age	66	1	3.378	0.067
		SL	66	1	0.035	0.852
		Heterozygosity	66	1	1.558	0.212
		Age	66	1	9.385	**0.002**
	Random	Family	66	1	0.017	0.896

Standard length (SL) was included as covariate and family as random factor. The sample size (N), difference of degrees of freedom (Δd.f.), χ², and P-values are given. Significant differences (P < 0.05) are marked in bold
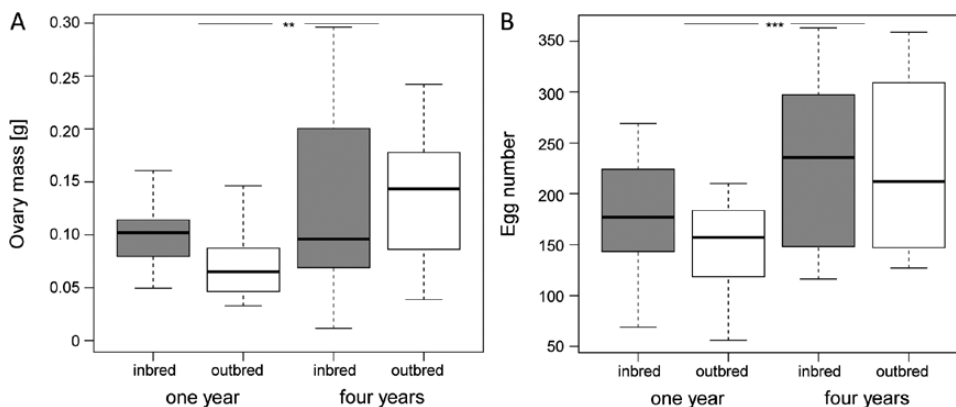

Figure 1. Boxplots of (A) ovary mass and (B) egg number of 1-year-old and 4-year-old inbred (grey) and outbred (white) females. Median, quartiles, and whiskers are given. **P < 0.01, ***P ≤ 0.001.

Table 2. Descriptive statistics of measured traits.

Trait	Young		Aged	
	Inbred	Outbred	Inbred	Outbred
Standard length (cm)	3.83 ± 0.29 (N = 19)	3.64 ± 0.18 (N = 19)	4.56 ± 0.25 (N = 14)	4.41 ± 0.15 (N = 14)
Body mass (g)	1.73 ± 0.41 (N = 19)	1.49 ± 0.22 (N = 19)	2.66 ± 0.45 (N = 14)	2.49 ± 0.33 (N = 14)
Ovary mass (mg)	94.03 ± 42.20 (N = 19)	72.46 ± 33.20 (N = 18)	123.25 ± 89.52 (N = 14)	135.66 ± 60.30 (N = 14)
Gonadosomatic index	5.29 ± 2.40 (N = 19)	4.84 ± 1.94 (N = 18)	4.38 ± 2.85 (N = 14)	5.29 ± 1.91 (N = 14)
Egg number	177.84 ± 56.09 (N = 19)	150.37 ± 42.90 (N = 19)	232.21 ± 82.92 (N = 14)	227.79 ± 86.74 (N = 14)
Effective egg diameter (mm)	0.84 ± 0.22 (N = 19)	0.89 ± 0.18 (N = 19)	0.77 ± 0.18 (N = 14)	0.84 ± 0.11 (N = 14)
Egg color saturation	40.95 ± 2.58 (N = 17)	43.53 ± 4.45 (N = 19)	46.02 ± 4.73 (N = 14)	45.73 ± 5.34 (N = 14)
Ovary color saturation	43.19 ± 4.98 (N = 19)	45.59 ± 3.63 (N = 19)	48.23 ± 5.72 (N = 14)	47.81 ± 4.65 (N = 14)

Mean ± standard deviation and sample sizes are given for young and aged inbred and outbred females

$(A = 4.11, H_{exp} = 0.471, H_{obs} = 0.487, p_{HWE} = 0.783, F_{IS} = –0.032)$. Heterozygosity was positively related to SL and BM (SL: LRT, $N = 66, \chi^2 = 6.491, P = 0.011$; BM: LRT, $N = 66, \chi^2 = 9.818, P = 0.002$). Heterozygosity did not affect any egg traits (Table 1). Age and/or SL explained ovary and egg traits better than heterozygosity (Table 1).

DISCUSSION

In the present study, we examined the effects of age and inbreeding on gonadal and egg traits in female *P. taeniatus*. We found no negative ageing effects on gonadal female traits, but instead larger females had a higher number of eggs as well as larger eggs, which were more intensively yellow colored. Inbreeding also had no negative effect on egg traits.

The results of our study contrast to the results of some other fish studies that report inbreeding depression affecting females' fecundity (e.g. Gallardo *et al.*, 2004; Fessehaye *et al.*, 2009). However, a study of Naish *et al.* (2013) also detected no significant correlation between inbreeding coefficient and female

fecundity, gonad mass or age at return in the rainbow trout (*Oncorhynchus mykiss*). In that study, only body size decreased significantly with increasing inbreeding coefficient, which is in contrast to our findings as inbred female *P. taeniatus* were larger than outbred ones. Because body size is an important mate choice criterion for males of *P. taeniatus* (Baldauf *et al.*, 2009; Thünken *et al.*, 2012), inbred females being larger than outbred females may have an advantage in mate choice. The present results underline previous findings (Thünken *et al.*, 2007a, b) by failing to find negative effects of inbreeding on primary reproductive female traits in this species, suggesting that deleterious alleles are probably purged from the population.

In the present study, age had an effect on egg and ovary coloration with older females having more colorful eggs and ovaries. Yellow eggs are assumed to be the ripest eggs in the ovary and thus the ones to be spawned (e.g. Rossoni *et al.*, 2010). The yellow coloration is often due to carotenoids that are important in egg development, influencing egg quality (Blount *et al.*, 2000), hatching success, and juvenile resistance (e.g. Watanabe & Vassallo-Agius, 2003). Carotenoids have to be absorbed as part of the diet (in fishes, e.g., through red mosquito larvae). There is increasing evidence that carotenoids act as powerful antioxidants, support the immune system, and reflect an animal's health status (Olson & Owens, 1998; von Schantz *et al.*, 1999). Thus, more colored eggs reflect the female's health status.

Larger females of *P. taeniatus* had heavier ovaries with more eggs compared to smaller ones. Such a fecundity–size relationship, which is most pronounced in species with indeterminate growth (Berglund *et al.*, 1986; Howard, 1988), has been reported in many fishes (e.g. cichlids: Galvani & Coleman, 1998; three-spined sticklebacks *Gasterosteus aculeatus*: Kraak & Bakker, 1998; Kolm *et al.*, 2006a, b; Baker *et al.*, 2008; salmonids: Naish *et al.*, 2013). Accordingly, a female's egg number should increase with a female's body size, thus males should prefer to mate with a larger female as she increases his fitness by producing more offspring (Kraak & Bakker, 1998; Einum & Fleming, 1999; Kolm, 2002). Egg diameter and egg size are important because they relate to larval size, growth rate, survival, adult fecundity, and behaviour (Katoh & Nishida, 1994; Segers & Taborsky, 2011).

In contrast to the positive age and size effects in females of *P. taeniatus* in the present study, we found negative age effects in a parallel study on males. Old males had less sperm than young males (Langen *et al.*, 2016). Furthermore, while we found generally no inbreeding depression in females, in males inbreeding effects were age-dependent and occurred in older males, with outbred males having a higher testis mass and a higher sperm number than inbred males (see Langen *et al.*, 2016). The differences between the sexes of *P. taeniatus* concerning age and inbreeding effects may result from higher mutation rates in the germ line of males compared to that of females. Therefore, female gonads should age more slowly and be less negatively affected by ageing than male gonads. Aged females in this study showed no typical symptoms of ageing, such as crooked backs, on body shape. Under natural conditions, predation is probably a major cause of mortality. Furthermore, in the present study females were prevented from reproduction, which is a highly energy demanding process (Baldauf *et al.*, 2011). Thus, ageing and lifespan, which are substantial fitness components, are expected to differ between natural and laboratory individuals of *P. taeniatus* (see Kawasaki *et al.*, 2008).

In short, we found no evidence for inbreeding depression and negative ageing effects on primary reproductive female traits in *P. taeniatus*. However, inbred females had a potential advantage over outbred females by being larger. Inbreeding did not negatively affect egg quality and quantity in young as well as in old females, probably due to purging. Quality and quantity of reproductive traits did not decrease with age and body size. In conclusion, large females of *P. taeniatus* may have fitness advantages by producing more, larger, and more intensively colored eggs and thus eggs of higher quality and quantity compared to smaller females. Overall, we report evidence for an increase of quality and quantity of reproductive traits with age in females of a short-lived species but no expression of inbreeding depression.

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