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Do reproduction and parenting influence personality traits? Insights from threespine stickleback

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Although one of the hallmarks of personality traits is their consistency over time, we might expect personality traits to change during life history shifts. Becoming a parent is a major life history event, when individuals undergo dramatic behavioural and physiological changes. Here we employ a longitudinal experiment to ask whether personality changes in response to the experience of parenting in male threespine sticklebacks, *Gasterosteus aculeatus*. Life history theory predicts that males should be less risk averse after successfully parenting, and the neuroendocrinology of parenting suggests that parenting could reorganize the hormonal landscape and behaviour of fathers. We randomly assigned males to either an experimental group (reproduced and parented) or a control group (did not reproduce and parent), and repeatedly measured a personality trait ('boldness') and 11-ketotestosterone levels (11-kT, the major androgen in fishes) in individual males. In the control group, males became bolder over time. However, in the experimental group, boldness did not change. Furthermore, 11-kT changed dramatically in the experimental group, and changes in 11-kT in parents were associated with boldness after parenting ceased. Our study is one of the first to assess proximate and ultimate explanations for changes in personality as a function of reproduction and parenting.

The experience of reproducing and becoming a parent is one of the most important life history events for most organisms. Although there is a rich literature documenting physiological and behavioural changes that organisms undergo as they become parents, there are few data in either humans or nonhuman animals that test the intuitive hypothesis that becoming a parent influences personality traits (behaviours that are variable among individuals and consistent within individuals over time; Stamps & Groothuis, 2010). Understanding the robustness of personality traits across critical lifetime events can shed light on their plasticity, causation and evolution (Duckworth, in press).

It is reasonable to suppose that personality traits might change as a function of reproduction and parenting because we know that parenting can have long-term effects on behaviour. For example, the experience of being a parent influences parenting behaviour during subsequent breeding attempts (Reichert, Cattau, Fletcher, Kendall, & Kitchens, 2012; Royle, Smiseth, & Kolliker, 2012). What has not been explored, however, is whether the experience of becoming a parent influences 'personality traits' (i.e. behaviours that are variable among individuals and consistent within individuals over time).

Here, we investigate the effects of reproduction and parenting on personality (boldness) in threespine sticklebacks, *Gasterosteus aculeatus*. In this species, all of the parental care necessary for offspring survival is provided by the father, and parenting is an energetically costly (Smith & Wootton, 1999) yet critical experience for males that strongly influences fitness (Wootton, 1984). Most freshwater sticklebacks live for 1 year and are seasonal breeders. Boldness is an important source of behavioural variation in this species: some individual sticklebacks are consistently relatively timid while others are bolder (Huntingford, 1976), and this variation influences fitness (Bell & Sih, 2007). Here, we measure boldness as willingness to forage under predation risk.

There are at least two nonmutually exclusive hypotheses to explain how and why boldness might change as a function of reproduction and parenting. First, according to life history theory, investment in current reproduction often comes at a cost to future reproduction; therefore, as the probability of future reproduction decreases, we might expect boldness to increase (Clark, 1994; Montgomerie & Weatherhead, 1988). Indeed, on average, risk-taking behaviour is higher at the end of the breeding season than

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at the beginning (fish: Candolin & Voigt, 2003; Magnhagen & Vestergaard, 1991; birds: Pugesek, 1983; insects: Rosenheim, Jepsen, Matthews, Smith, & Rosenheim, 2008; mammals: Dammhahn, 2012; but see Ukegbu & Huntingford, 1988). However, cross-sectional studies that do not repeatedly measure the same individuals cannot tell us whether individuals change their behaviour as a function of experience, or if changes reflect factors such as selection or dispersal, for example. Moreover, work to date has been observational (rather than manipulative); therefore, we do not know the causal factors driving changes in boldness (e.g. experience, age, seasonality).

Another hypothesis (the 'physiological remodelling hypothesis') supposes that the dramatic neural (Franssen et al., 2011; Russell, Douglas, & Ingram, 2001) and endocrine (Saltzman & Ziegler, 2014; Wingfield, Hegner, Dufty, & Ball, 1990) changes that accompany reproduction and parenting have long-lasting effects on subsequent behaviour (see also Cost, Lobell, Williams-Yee, Henderson, & Dohanich, 2014; Logan, Hill, Jones, Holt-Lunstad, & Larson, 2014; Macbeth & Luine, 2010). For example, physiological changes associated with parenting might influence personality traits if individuals do not return to a preparenting hormonal state. This hypothesis assumes that changes in physiology are more dramatic in individuals that parent versus those that do not, and it predicts that hormonal changes occurring over the course of parenting are associated with personality traits after parenting has ceased. Importantly, the life history and physiological hypotheses are not mutually exclusive; while the former offers an ultimate explanation, the latter offers a proximate one.

We evaluate evidence for the life history and physiological remodelling hypotheses by repeatedly measuring boldness before and after reproduction and parenting in male sticklebacks. A control group was also repeatedly measured for boldness but did not rear offspring. By comparing individuals that reproduced and parented (experimental) with the control group, we could ask whether changes experienced by males in the experimental group were specifically due to reproduction and parenting, or whether they reflect confounding effects such as time, age or seasonality. We first confirmed that our measures of boldness were personality traits, then asked how the experience of becoming a parent influences boldness by comparing the average risk-taking behaviour between males in the experimental and control groups. To test the physiological remodelling hypothesis, we repeatedly measured excreted 11-ketotestosterone (11-kT), the main androgen in fishes associated with courtship and parenting (Pradhan, Solomon-Lane, Willis, & Grober, 2014) and examined how changes in 11-kT levels were related to boldness. We chose to measure 11-kT as opposed to other steroids (e.g. cortisol) as it has been previously established in stickleback that while 11-kT changes over the nesting cycle and is important in reproduction and parenting (Pall, Mayer, & Borg, 2002), nonandrogen steroids remain at similar levels (Sebire, Katsiadaki, & Scott, 2007).

METHODS

Adult threespine stickleback were collected from Putah Creek, California, U.S.A. in April 2013. At this time, adults in this population begin showing nuptial coloration but have not yet begun breeding. Therefore, it is unlikely the males in this study had previous parenting experience. Fish were shipped to the University of Illinois at Urbana-Champaign (Champaign, IL, U.S.A.). On days when risk-taking behaviour was measured, males were not fed except during the trials. All assays were conducted from May to July 2013. None of the males in this experiment were infected with *Schistocephalus solidus*, a tapeworm known to influence risk-taking behaviour (Barber & Dingemanse, 2010; Giles, 1987).

Fish were kept at 20° C on a summer photoperiod (16:8 h light:dark cycle). Water was cleaned via a recirculating flow-through system that consisted of a series of particulate, biological and UV filters (Aquaneering, San Diego, CA, U.S.A.). Ten per cent of the water volume in the tanks was replaced each day. Fish were fed a mixed diet consisting of frozen bloodworm (*Chironomus* spp.), brine shrimp (Artemia spp.) and *Mysis* shrimp in excess each day.

Boldness Assay

Males were introduced into separate housing tanks. One week later, individuals were phenotyped for boldness (the 'Before' trials) in an observation tank (53 × 33 × 24 cm) with a 5 × 2 grid drawn on the front, a gravel bottom and plastic plants for refuge. A model great egret, *Casmerodius albus*, skull was attached over the observation tank. The egret skull was situated so that when it was released via a lever from behind a blind, the tip of the egret's bill splashed the water surface (Fig. 1). This stimulus simulated the sudden overhead attack of an egret searching for prey (Giles & Huntingford, 1984).

To phenotype boldness for each fish, we transferred a single male into the observation tank, and 30 s later, we added 10 live bloodworms directly under the egret skull. If the male did not approach the bloodworms within 5 min ($N = 35$ of 169 trials), he was given a score of one greater than the maximum 'latency to eat' (301 s), and these trials were not used in analysis of 'number of pecks at food' and 'number of squares moved' (see below).

When the male approached within one body length of the bloodworms, we released the egret skull to splash the water twice in quick succession, and then affixed the skull so that it remained above the water (Alvarez & Bell, 2007; Bell, 2005). Following the simulated attack, we recorded three behaviours: time to resume eating following the predator attack ('latency to eat'), number of pecks at the bloodworms (foraging under risk, 'pecks at food') and total number of times that the individual's head passed into a new square (activity under risk, 'squares moved') for 5 min from behind a blind.

We observed each male three times, with 24 h between trials, and measured males for standard length and body mass after the third trial.

Experimental and Control Groups

Following all three 'Before' boldness trials, we randomly assigned males to either the experimental or the control group. Males in the experimental group were randomly assigned to be 'paired' with a male from the control group (Fig. 1). While males were kept individually, males in paired groups were measured for all behaviours and 11-kT at the same time. This experimental design allowed us to control for variation among experimental males in time to spawn and time to complete a clutch. Males from both control and experimental groups were kept in individual 9.5-litre tanks containing a refuge, an open plastic box filled with fine sand and gravel, and filamentous algae for nest building.

Once both the control and experimental males within a pair had built nests, we selected a gravid female at random and weighed her, then placed the female in a long-necked flask inside the paired control male's tank for 5 min. This allowed the male to interact with and court the female but not spawn. We then placed the same female directly into the tank of the experimental male. We subtracted female body mass after spawning from female body mass prior to spawning to estimate egg mass. We acknowledge that the experience of reproduction and parenting were confounded in this experiment. However, if we had

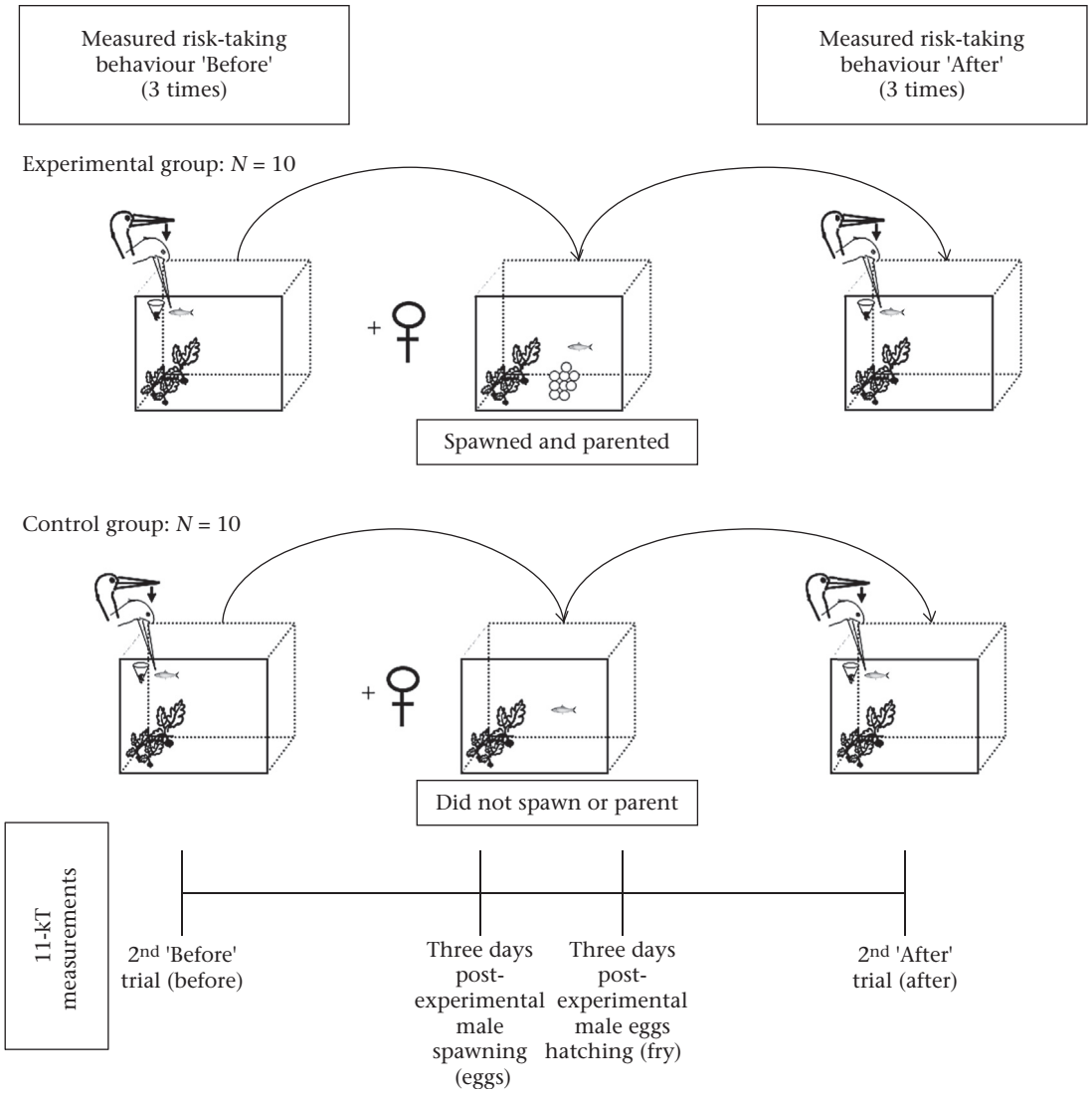

Figure 1. Overview of the experimental design. Experimental and control male sticklebacks experienced the same assays, with the exception that males in the control treatment were not allowed to spawn with a female. Each control male was paired with an experimental male upon finishing the first three boldness trials (risk-taking behaviour in response to a model predator before reproduction and parenting by experimental males, Before). Once both males in a pair had built nests, we conducted all behavioural and 11-ketotestosterone (11-kT) measurements on the same day for both experimental and control males (i.e. 3 days post-experimental male spawning (eggs), 3 days post-experimental male eggs hatching (fry), and in response to a model predator after reproduction and parenting by experimental males (After)), which allowed us to control for variation among experimental males in time to spawn and time to complete a clutch.

attempted to separate the two, we would have had to experimentally depredate the control males' nests, which could have influenced their subsequent behaviour. Because of variation in time to build a nest, the average number of days that elapsed between the Before trials and spawning was 12.7 days (range 2–43 days). The average number of days that elapsed between the Before and After trials was 35 days (range 18–65 days). We found no effect of days elapsed between trials on any behaviour or on 11-kT levels (Supplementary Table S1).

Five days after fry hatched, we transferred the experimental male and his paired control male to new separate housing tanks. Five days posthatching is the time period where fry naturally disperse and males abandon their nests in this population; therefore, males had completely ceased parenting at this time. One week later, males were measured for boldness once per day for 3 days (the 'After' trials). In total, we measured boldness of 10 experimental and 10 control males that completed both the Before and After trials ($N = 6$ trials per individual).

Measuring 11-kT

We measured 11-kT excreted in water at four time points using enzyme immunoassay (EIA): Before (immediately following the second Before trial), with eggs in the nest (3 days after spawning), with fry in the nest (3 days after fry hatched), and After (immediately following the second After trial). We measured control males for 11-kT at the same time as their paired experimental males.

We placed individual males in 500 ml long-necked glass flasks filled with 100 ml of tank water for 30 min, as in Sebire et al. (2007); all flasks had been rinsed with ethanol and distilled water beforehand. We then placed the flask in a covered bucket to minimize stress to the focal male. Following the 30 min collection period, we transferred 50 ml of the holding water into a 50 ml sterile polypropylene conical tube.

We collected 11-kT between 0900 and 1100 hours Central Standard Time. Water samples were frozen at −20 °C until

extraction. Freeze-storage of water samples does not influence steroid concentrations (Ellis, James, Stewart, & Scott, 2004). We extracted hormone from the water samples using C18 solid-phase extraction columns (Waters Inc., Milford, MA, U.S.A.) fitted to a 20-port manifold (Waters Inc.). We primed columns once using 5 ml of HPLC grade MeOH and then rinsed them with a 5 ml wash of UltraPure water (EMD Millipore, Billerica, MA). We then attached a 50 ml container directly to the column, engaged the vacuum and added the water sample to the container. The water sample passed through the column and wastewater was collected in a bin inside the manifold. We then rinsed the columns with 4 ml of UltraPure water and two consecutive 2 ml washes with HPLC grade MeOH. We eluted hormone from the columns into 13 × 100 mm borosilicate vials via 4 ml of diethyl ether, evaporated the 4 ml of eluted solution overnight and resuspended the resulting hormone pellet in 50 μl of EIA buffer supplied with the kits.

We determined the concentration of 11-kT in our samples via 11-kT EIA kits (Cayman Chemicals Inc., Ann Arbor, MI, U.S.A.) according to the manufacturer's protocol. Samples were diluted 10× to fit on the standard curve. All samples were assayed in duplicate and samples from both treatments and all time points were represented on each of the plates. The interassay coefficient of variation (CV) between all EIA plates ($N = 3$ plates) was 5.46%; mean ± SD intra-assay CV was 15.62 ± 17.94%. We calculated 11-kT release rate as 11-kT sample concentration per mass per time in flask. Release rates of 11-kT correlate strongly ($r^2 = 0.72$) with plasma 11-kT (Sebire et al., 2007).

Data Analysis

To confirm that our measures of boldness were personality traits, we estimated the repeatability of boldness during the Before trials. We first measured repeatability for both control and experimental groups separately to confirm that both groups showed similar patterns prior to assigning males to either the control group or the experimental group. For further analysis, we combined data from males in the control and experimental groups for the Before trials, as they had all received the same experience at this time. To test whether parenting influenced rank-order stability (among-individual variation) in boldness, we estimated repeatability across the Before and After trials for the two treatment groups separately. We used generalized linear mixed models with Markov chain Monte Carlo (MCMC) estimation for all repeatability analyses using MCMCglmm (Hadfield, 2010) in R v.3.0.1 (http://www.r-project.org). MCMC is a Bayesian statistical method that is powerful for fitting non-Gaussian distributions and partitioning variance among random effects (Dingemanse & Dochtermann, 2013; Hadfield, 2010). MCMCglmm returns 95% credibility intervals for random effects. We then used these variance components to estimate repeatability as the proportion of total variation attributable to among-individual variation. We corrected all repeatability estimates as appropriate for each behaviour's distribution (Poisson with additive overdispersion for latency to eat; Gaussian for pecks at food, number of squares moved and 11-kT release rate (Nakagawa & Schielzeth, 2010)). We determined whether there were consistent individual differences by visually inspecting the posterior distribution of the repeatability estimate: if the estimate (and its 95% CI) was not pressed against zero, we interpreted this as evidence of personality traits.

Throughout, we used noninformative priors (Hadfield, 2010) appropriate for the relative error distributions, and preliminary analyses indicated that our results were not sensitive to changes in prior settings (data not shown). We ensured convergence and adequate chain mixing by comparing the posterior distributions and autocorrelation plots of five independent chains with 500 000 iterations, a 1000 burn-in period and thinning every 100 iterations for each model.

To determine whether reproduction and parenting influenced mean-level stability of personality traits, we used linear mixed models (LMMs). Models examining mean-level change over time included treatment group, stage and their interaction as fixed effects, trial nested within stage as a fixed effect and length as a covariate. We originally included egg mass as a covariate for males in the experimental group, but this was never significant and was thus removed for all subsequent analyses. All models included individual as a random effect to account for multiple measurements. For 11-kT release rate, we tested for the effect of treatment, time point (Before, eggs, fry and After) and their interaction, with individual and EIA plate as random effects.

We examined whether the change in 11-kT release rate was correlated with latency to eat, pecks at food or number of squares moved both Before and After. We computed the difference in 11-kT release rate before and after parenting, as we were interested in long-term impacts of hormonal changes after parenting had ceased. We used Spearman rank correlations, as boldness behaviours were not normally distributed. To avoid pseudoreplication, we first averaged each behaviour across the three trials within each stage (Before and After). We ran separate correlations for males from the experimental and control treatment groups and used sequential Bonferroni correction to account for multiple correlation tests (Rice, Schork, & Rao, 2008).

Ethical Note

We took steps to maximize animal welfare at all stages of this experiment. Individuals were handled as little as possible and noninvasive measurements were used to determine hormone concentrations. Upon completion of the experiment, fish were maintained in the laboratory for use in future projects. The experiments were approved by the Institutional Animal Care and Use Committee at the University of Illinois Urbana-Champaign (Protocol number 09204, approved on 1 September 2009).

RESULTS

Repeatability of Boldness and 11-kT

During the Before trials, there were consistent individual differences in all behaviours measured in both control and experimental males (Supplementary Table S2), confirming these behaviours can be considered personality traits.

In general, males that were relatively bold during the Before trials were also relatively bold during the After trials (repeatability across Before and After; Table 1). A notable exception was latency to eat, which in the control group showed little among-individual variation and was not repeatable between the Before and After trials.

There were also consistent individual differences in 11-kT release rate (Table 1). Repeatability estimates of 11-kT did not statistically differ between the experimental and control treatment groups. However, relative to control males, males in the experimental group had both greater among-individual variation (i.e. were more different from one another) and greater within-individual variation in 11-kT.

Effects of Parenting on Change in Behaviour and 11-kT Levels

Boldness changed over time. Regardless of treatment, males were quicker to resume eating following a simulated predator attack during the After trials compared to the Before trials (Fig. 2a).

Table 1
Variance component (among- and within-individual) and repeatability estimates (R) of latency to eat, number of pecks at food, number of squares moved and 11-ketotestosterone (11-kT) levels for male threespine sticklebacks in experimental (reproduced and parented) and control (did not reproduce and parent) groups across boldness trials (Before vs After reproduction and parenting)

	Control	Experimental
Latency to eat (s)		
Among	0.008 (0, 3.99)	2.56 (0.81, 11.95)
Within	3.22 (2.17, 5.96)	2.51 (1.50, 4.08)
R	0.003 (0, 0.52)	**0.53 (0.14, 0.82)**
Pecks at food		
Among	3.84 (0.95, 16.99)	3.13 (0.95, 19.88)
Within	20.27 (12.91, 32.95)	16.48 (10.72, 29.23)
R	**0.14 (0.04, 0.46)**	**0.18 (0.05, 0.56)**
Squares moved		
Among	191.40 (38.33, 791.60)	67.77 (12.35, 319.71)
Within	306.70 (193.90, 518.75)	200.77 (126.93, 366.01)
R	**0.41 (0.14, 0.75)**	**0.25 (0.06, 0.63)**
11-kT release rate (ng per g per min)		
Among	8.86 (2.08, 41.91)	216.58 (61.00, 906.00)
Within	67.72 (37.72, 112.10)	259.27 (155.78, 558.32)
R	**0.11 (0.04, 0.40)**	**0.61 (0.19, 0.80)**

11-kT showed repeatability across all time points (Before, with eggs, with fry, After; see Fig. 1). Numbers in parentheses indicate 95% credibility intervals. Bold values indicate significant repeatability.

There was no evidence that the increase in boldness reflected habituation to the assay, as there was no effect of trial on behaviour (Table 2).

The experience of reproduction and parenting also influenced boldness (Table 2). Specifically, there were significant treatment∗ stage interactions for pecks at food (Fig. 2b) and squares moved (Fig. 2c). Males in the control group increased activity (squares moved) in the After trials compared to the Before trials (paired t test: $t_9 = -2.61$, $P = 0.03$), while males in the experimental group did not ($t_9 = -1.72$, $P = 0.12$; Fig. 2c). Indeed, the general pattern was that control males became bolder over time, while the average behaviour of experimental males did not differ between the Before and After trials.

Release rates of 11-kT were higher in the experimental group than in the control group (Table 2). Specifically, when experimental males had eggs or fry in the nest, they had higher 11-kT release rates on average and showed greater among-individual variation than males in the control treatment, and this difference was maintained after parenting ceased (Fig. 3).

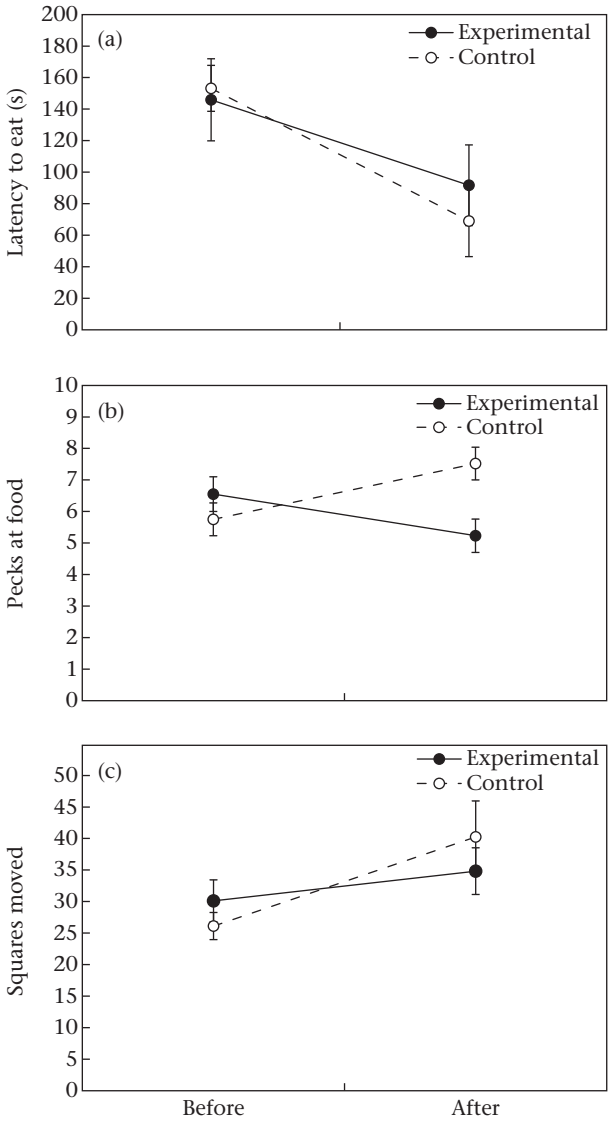

Figure 2. Mean ± SE (a) latency to eat, (b) number of pecks at food and (c) number of squares moved by male threespine sticklebacks in response to a model predator before (Before) vs after (After) reproduction and parenting by experimental males.

Hormonal Changes and Boldness

Boldness of experimental males after reproducing and parenting was related to changes in the males' hormone levels (Fig. 4). Specifically, males that showed an increase in 11-kT release rate foraged more under predation risk (pecks at food), while males that showed a decrease in 11-kT release rate foraged relatively little ($r_S = 0.80$, $N = 10$, $P = 0.009$). Foraging under risk was not related to hormonal changes in control males ($r_S = -0.44$, $N = 10$, $P = 0.19$). Hormonal changes were not significantly related to either latency to eat or squares moved in either treatment (Supplementary Table S3).

DISCUSSION

We provide experimental evidence that personality traits can change, even in adults. According to all three measures of boldness, the control group became bolder over time. This result is consistent with correlative studies (Candolin & Voigt, 2003; Dammhahn, 2012; Magnhagen & Vestergaard, 1991; Pugesek, 1983; Rosenheim et al., 2008) showing that risk-taking behaviour increases over the course of the season, and is predicted by life history theory: animals take more risks as the end of the breeding season approaches because there are fewer opportunities for future reproduction (Clark, 1994; Montgomerie & Weatherhead, 1988).

We also provide experimental evidence that the experience of becoming a parent can influence personality traits. As in the control group, the experimental group resumed foraging faster (latency to eat) during the After trials than during the Before trials. However, according to two other measures of boldness (pecks at food, squares moved), the behaviour of the experimental group did not change over time. We interpret quickly foraging after an attack, foraging under predation risk and activity to reflect differences in levels of risk taking. For example, because foraging activity can be associated with an increased risk of mortality to predation (Godin & Smith, 1988; Krause & Godin, 1996), continuing to forage under predation risk and maintaining high activity levels are both conspicuous (Lima & Dill, 1990) and require males to remain within reach of a sit-and-wait predator such as egrets. In contrast, quickly returning to eat after a predator attack puts the male at immediate

Table 2
Linear mixed model results for all behaviours and 11-ketotestosterone (11-kT) levels in male threespine sticklebacks

Factor	Latency to eat (s)			Pecks at food			Squares moved			11-kT release rate (ng per g per min)		
	F	*df*	*P*	*F*	*df*	*P*	*F*	*df*	*P*	*F*	*df*	*P*
Treatment	0.18	1, 17.0	0.67	0.12	1, 18.0	0.74	0.06	1, 16.7	0.81	**5.11**	**1, 21.5**	**0.03**
Stage[1]	**9.88**	**1, 91.1**	**0.002**	0.52	1, 69.4	0.48	**11.98**	**1, 66.9**	**0.001**	2.35	3, 49.4	0.08
Treatment∗stage	0.15	1, 91.1	0.70	**4.70**	**1, 69.5**	**0.03**	**4.97**	**1, 66.8**	**0.03**	0.80	3, 49.4	0.50
Trial (Stage)	0.78	4, 91.1	0.54	1.55	4, 68.9	0.20	0.08	4, 66.3	0.99	–		–
Standard body length	0.001	1, 17.2	0.99	3.18	1, 17.7	0.09	0.05	1, 16.2	0.83	0.73	1, 21.0	0.40

Bold indicates significant effects ($P < 0.05$).
[1] Stage has two levels for latency to eat, number of pecks at food and number of squares moved (Before vs After reproduction and parenting by experimental males) and four levels for 11-kT (Before, with eggs, with fry, and After). See Fig. 1 for details.

risk, but this can be mediated if the animal quickly darts under cover after consuming the food item.

Why did the boldness of males in the experimental group not change over time? One possibility is that there was positive feedback in males that had successfully raised a clutch: as their behavioural strategy had produced a successful clutch in the past, there was no reason to change that strategy. Male sticklebacks often have multiple breeding attempts in a single season, and males that have one successful clutch are more likely to have another successful clutch (Wootton, 1984). Another possibility is that neural and hormonal changes that occur during parenting might act as a proximate constraint on personality traits, channelling males along different trajectories. Although experimental males did not change boldness over time, on average, the experience of reproducing and parenting made males more different from one another: males that had parented showed greater among-individual variation in latency to eat and 11-kT release rates than control males. There is evidence in humans that personality stability increases with age and experience, which is thought to reflect an increase in options that allow the expression of naturally occurring individual variation (Roberts & DelVecchio, 2000). This can lead to individuals actively choosing environments that appeal to their personality (humans: Roberts & DelVecchio, 2000; flies: Saltz, 2011; stickleback: Pearish, Hostert, & Bell, 2013). It is possible that the experience of parenting in sticklebacks might do something similar and reveal cryptic underlying individual variation.

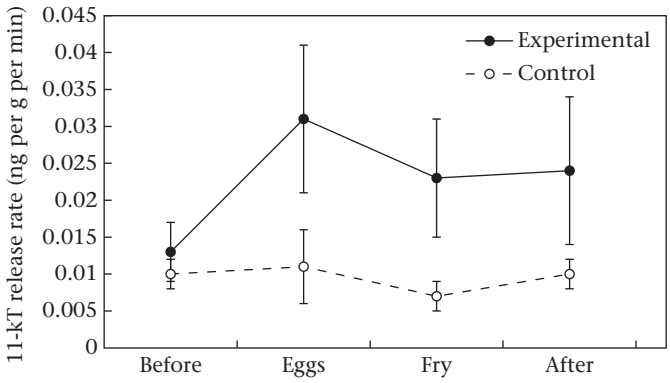

Figure 3. Mean ± SE release rate of 11-ketotestosterone (11-kT) over time in experimental and control groups of male threespine sticklebacks.

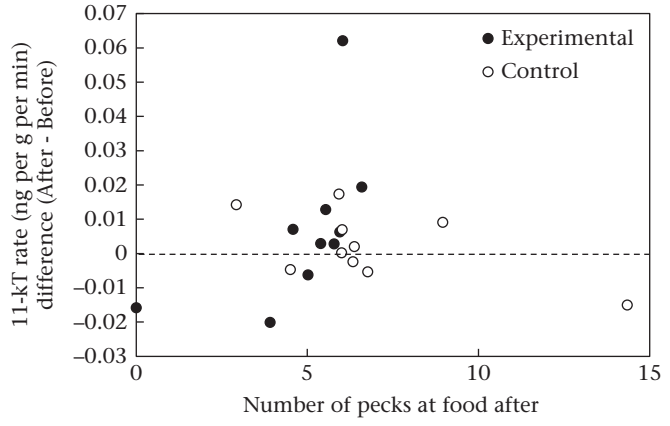

Figure 4. Shifts in 11-ketotestosterone (11-kT) and its correlation with number of pecks at food in experimental and control groups of male threespine sticklebacks before (Before) vs after (After) reproduction and parenting by experimental males. The dashed line indicates no change After vs Before; points above the line indicate males with greater 11-kT After and points below the line indicate males with lower 11-kT After.

We also found support for the physiological remodelling hypothesis. One of the assumptions of this hypothesis is that parenting is associated with dramatic physiological change. In the experimental group, 11-kT release rate increased 1.4-fold after there were eggs in the nest. In contrast, 11-kT release rate remained relatively stable over time in the control group. Other studies, including in sticklebacks (Pall et al., 2002), found that 11-kT levels peak while males care for offspring (Pradhan et al., 2014; Rodgers, Earley, & Grober, 2006), although this is not a universal pattern in fish with paternal care (Kindler, Philipp, Gross, & Bahr, 1989; Magee, Neff, & Knapp, 2006). Importantly, on average, parents did not return to a preparenting baseline, suggesting that they did not fully 'recover' physiologically from the increase in 11-kT during parenting for at least 1 week, providing a potential mechanism to explain behavioural differences between males that reproduced and parented and those that did not. Note, however, that we measured 11-kT at the Before and After time points immediately following the boldness assay; therefore, it is possible that our measurement of 11-kT after parenting could also reflect predator-induced changes in 11-kT. Although there is some evidence in fishes that 11-kT decreases as cortisol increases (Pickering, Pottinger, Carragher, & Sumpter, 1987), there is little evidence to suggest that 11-kT changes immediately in response to predation risk, especially over short timescales (Lastein, Hoglund, Mayer, Overli, & Doving, 2008).

The extent to which a male showed dramatic hormonal fluctuations was related to foraging under risk. Males that showed a drop in 11-kT release rate following reproduction and parenting were more timid (foraged less under risk). In contrast, if a male showed an increase in 11-kT release rate, he was more bold (foraged more under risk). For males that did not parent, changes in 11-kT release rates were not associated with foraging under risk. It is unlikely that this was due to a 'ceiling' effect in control males, as there was

still variation in whether the control group experienced an increase or decrease in 11-kT release rate, albeit not as extreme as in the experimental group. In other fish species, males with high levels of androgens have greater paternity (Neff & Knapp, 2009), and higher 11-kT levels are associated with larger brood sizes (Ros, Fagundes, & Oliveira, 2009). Therefore, males with higher 11-kT levels after parenting may be more likely to resume breeding, and may therefore be more willing to take risks. Further studies examining the links between breeding experience, boldness and hormone profiles are needed to elucidate mechanisms underlying changes in personality traits following the experience of parenting.

We found evidence suggesting both proximate and ultimate causes of change in personality traits in stickleback as a function of a major life history event: the experience of reproducing and becoming a parent. While studies in humans have suggested that experiences such as marriage, divorce or parenting influence personality traits (Jeronimus, Ries, Sanderman, & Ormel, 2014), such studies are by necessity correlational, and are often not longitudinal. By using a repeated measures design in which the same males were measured before and after a formative experience, and by comparing them to a control group that did not have that experience, we report here that reproduction and parenting can influence personality traits. It will be fascinating for future longitudinal and experimental studies to test whether other major adult experiences (pregnancy, dispersal, acquiring a territory, food shortage, etc.) influence personality traits in nonhuman animals and to develop proximate and ultimate explanations for how and why personality traits might change over time.

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Supplementary Material

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