TITLE: Does reproduction and parenting influence personality traits? Insights from threespine stickleback

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ABSTRACT

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 (reproduced and parented) or control (did not reproduce and parent) group, and repeatedly measured a personality trait ('boldness') and 11-ketotestosterone (11-kT, the major androgen in fishes) in individual males. In the control group, males became more bold over time. However, in the experimental group, boldness did not change. Further, 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 a major adult event – reproduction and parenting.

KEYWORDS: paternal care, boldness, hormones, individual differences, behavioural syndrome, fathers

INTRODUCTION

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 2015).

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 et al. 2012; Royle et al. 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 one 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 (Montgomerie & Weatherhead 1988; Clark 1994). Indeed, on average, risk-taking behaviour is higher at the end of the breeding season than at the beginning (fish: Magnhagen & Vestergaard 1991, Candolin & Voigt 2003; birds: Pugesek 1983; insects: Rosenheim et al. 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 (Russell et al. 2001; Franssen et al. 2011) and endocrine (Wingfield et al. 1990; Saltzman & Ziegler 2014) changes that accompany reproduction and parenting have long-lasting effects on subsequent behaviour (see also Macbeth & Luine 2010; Cost et al. 2014; Logan et al.

2014). For example, physiological changes associated with parenting might influence personality traits if individuals do not return to a pre-parenting hormonal state. This hypothesis assumes that changes in physiology are more dramatic in individuals that parent versus those that do not, and 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 if they reflect confounding effects such as time, age, or seasonality. We first confirmed that our measures of boldness were personality traits, and 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 et al. 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 et al. 2002), non-androgen steroids remain at similar levels (Sebire et al. 2007).

METHODS

Adult threespine stickleback were collected from Putah Creek, California 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. On days when risk taking behaviour was measured, males were not fed outside the trials. All assays were conducted from May – July 2013. None of the males in this experiment were infected with *Schistocephalus solidus*, a tapeworm known to influence risk-taking behaviour (Giles 1987, Barber & Dingemanse 2010).

Fish were kept at 20 degrees Celsius on a summer (16L:8D) photoperiod. Water was cleaned via a recirculating flow-through system that consists of a series of particulate, biological, and UV filters (Aquaneering, San Diego, USA). 10% of the water volume in the tanks was replaced each day. Fish were fed a mixed diet consisting of frozen bloodworm, brine shrimp 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 x 33 x 24 cm) with a 5x2 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 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, a single male was transferred into the observation tank. Thirty seconds later, we added 10 live bloodworms directly under the egret skull. If the male did not approach the bloodworms within five minutes ($N=35$ of 169 trials), he was given a score of one greater than the maximum *latency to eat* (301 seconds) and these trials were not used in analysis of *pecks at food* and *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 (Bell 2005; Alvarez & Bell 2007). 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 the individual's head passed into a new square (activity under risk, *squares moved*) for five minutes from behind a blind.

Each male was observed three times with 24 hours between trials and measured for standard length and weight after the third trial.

Experimental and control groups

Following all three “before” boldness trials, males were randomly assigned to either the experimental or 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

complete a clutch. Males from both control and experimental groups were kept in individual 9.5L tanks containing a refuge, an open plastic box filled with fine sand, gravel, and filamentous algae for nest building.

Once both the control and experimental males within a pair had built nests, a gravid female was selected at random, weighed, placed in a long-necked flask and introduced to the paired control male for five minutes. 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 weight after spawning from weight 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 attempted to separate the two, we would have had to experimentally depredate the control males' nests, which could have influenced their subsequent behaviour. Due to variation in time to build a nest, the average number of days that elapsed between the Before trials and spawning was 12.7 (range = 2 – 43 days). The average number of days that elapsed between the Before and After trials was 35 (range = 18 – 65 days). We found no effect of days elapsed between trials on any behaviour or 11-kT (Table S1).

Five days after fry hatched, we transferred the experimental male and his paired control male to new separate housing tanks. Five days post-hatching 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 three days (the "After" trials). In total, we measured boldness of $N=10$ experimental and $N=10$ control males that completed both the Before and After trials ($N=6$ trials per individual).

Measuring 11-kT

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

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

11-kT was collected between 900 and 1100 CST. Water samples were frozen at -20°C until extraction. Freeze storage of water samples does not influence steroid concentrations (Ellis et al. 2004). Hormone was extracted from the water samples using C18 solid phase extraction columns (Waters Inc., Milford, MA) fitted to a 20-port manifold (Waters Inc., Milford, MA). Columns were primed once using 5 mL HPLC grade MeOH and then rinsed with a 5 mL wash of UltraPure water (EMD Millipore, Billerica, MA). A 50 mL container was then attached directly to the column. The vacuum was engaged and the water sample was added to the container, passed through the column and the wastewater was collected in a bin inside the manifold. Columns were then rinsed with 4 mL UltraPure water and two consecutive 2 mL washes with HPLC grade MeOH. Hormone was then eluted from the columns into 13x100 mm borosilicate vials via 4 mL of diethyl ether. The 4 mL of eluted solution was then evaporated overnight and

the resulting hormone pellet resuspended in 50 µl of enzyme-immunoassay (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) according to the manufacturer's protocol. Samples were diluted 10x 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 inter-assay coefficient of variation (CV) between all EIA plates ($N = 3$ plates) was 5.46%; intra-assay CVs averaged $15.62 \pm 17.94\%$ (mean $\pm$ standard deviation). We calculated 11-kT release rate as 11-kT sample concentration/mass/time in flask. 11-kT release rates 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 or 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 if 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 (Hadfield 2010, Dingemanse & Dochtermann 2013). 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 & Schielzith 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 non-informative 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 auto-correlation 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, and 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 et al. 2008).

ETHICAL NOTE

Steps were taken to maximize animal welfare at all steps of this experiment. Individuals were handled as little as possible and non-invasive measurements were used to determine hormone concentrations. Upon completion of the experiment, fish were maintained in the lab 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 (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 is *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). There was no evidence that the increase in boldness reflects 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 (paired t-test, $t_9 = -1.72$, $P = 0.12$, Fig 2C). Indeed, the general pattern is that control males became more bold over time, while the average behaviour of experimental males did not differ between the Before and After trials.

11-kT release rates were higher in the experimental group compared to 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 compared to males in the control treatment, and this difference was maintained after parenting ceased (Fig 3).

Hormonal changes and boldness

The boldness of experimental males after reproducing and parenting was related to the hormonal changes they exhibited (Fig 4). Specifically, males that exhibited an increase in 11-kT release rate foraged more under predation risk (*pecks at food*), while males that exhibited 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 (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 (Pugesek 1983; Magnhagen & Vestergaard 1991; Candolin & Voigt 2003; Rosenheim et al. 2008; Dammhahn 2012) 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 (Montgomerie & Weatherhead 1988; Clark 1994).

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 compared to Before. However, according to two other measures of boldness (*pecks at food* and *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 as measuring 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 risk, but this can be mediated if the animal quickly darts under cover after consuming the food item.

Why didn't the boldness of males in the experimental group 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 et al. 2013). It is possible that the experience of parenting in sticklebacks might do something similar, and reveal cryptic underlying individual variation.

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 (Rodgers et al. 2006; Pradhan et al. 2014), although this is not a universal pattern in fish with paternal care (Kindler et al. 1989; Magee et al. 2006). Importantly, on average parents did not return to a pre-parenting baseline, suggesting that they did not fully “recover” physiologically from the increase in 11-kT during parenting for at least one week and providing a potential mechanism to explain behavioural differences between males that reproduced and parented and those that did not. However, it is important to note that we measured 11-kT at the Before and After timepoints 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 et al. 1987), there is little evidence to suggest that 11-kT changes in immediate response to predation risk, especially over short time-scales (Lastein et al. 2008).

The extent to which a male exhibited dramatic hormonal fluctuations was related to foraging under risk. Males that exhibited 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 is 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 et al. 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, parenting, etc. influence personality traits (Jeronimus et al. 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.) can 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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FIGURE LEGENDS

Figure 1: Overview of experimental design. Experimental and control males 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 trials (Before). Once both males in a pair had built nests, all behavioural and 11-kT measurements were conducted on the same day for both the experimental and control males. This allowed males in the control group to account for variation in time to spawn and nesting cycle duration among males in the experimental group.

Figure 2: Differences in the mean $\pm$ 1 SE in a) latency to eat, b) number of pecks at food, and c) squares moved of experimental and control fish between the Before and After phases of the experiment.

Figure 3: 11-kT release rate over time in the experimental group (solid line) versus the control group (dotted line). Error bars $\pm$ 1 SE.

Figure 4: Shifts in 11-kT and its correlation with number of pecks at food in the experimental group (closed circles) and control group (open circles). The dashed line indicates no change; points above the line indicate males with greater 11-kT After and points below the line indicate males with lower 11-kT After.

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Figure 2

Figure 3

Figure 4

Table 1. Variance component (among- and within-individual) and repeatability estimates (R) of *latency to eat*, *pecks at food*, *squares moved* and *11-kT* in experimental and control males across the Before and After trials. 11-kT shows repeatability across all time points (Before, eggs, fry, After). Numbers in brackets indicate 95% credibility intervals. Bold indicates significant repeatability.

	<i>Control</i>	<i>Experimental</i>
<i>latency to eat (s)</i>		
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]
<i>pecks at food</i>		
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]
<i>squares moved</i>		
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]
<i>11-kT release rate (ng/g/min)</i>		
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]

Table 2. Linear mixed model results for all behaviours and 11-kT. Bold indicates significant effects ($P<0.05$).

<i>Factor</i>	<i>latency to eat (s)</i>		<i>pecks at food</i>		<i>squares moved</i>		<i>11-kT release rate (ng/g/min)</i>	
	<i>F(df)</i>	<i>P-value</i>	<i>F(df)</i>	<i>P-value</i>	<i>F(df)</i>	<i>P-value</i>	<i>F(df)</i>	<i>P-value</i>
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 [†]	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	-	-
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

[†]Stage has two levels for latency to eat, pecks at food, and number of squares moved (Before and After) and four levels for 11-kT (before, with eggs, with fry, and after).