

1 **Effect of the early social environment on behavioural and genomic responses to a social**  
2 **challenge in a cooperatively breeding vertebrate**

3  
4

5 Cecilia Nyman, Stefan Fischer<sup>2</sup>, Nadia Aubin-Horth<sup>3</sup> and Barbara Taborsky<sup>1</sup>

6  
7

8 Running head: Effects of social rearing on genomic response

9  
10

11 Institutional addresses of all authors

12 <sup>1</sup> Institute for Ecology and Evolution, Behavioural Ecology, University of Bern, CH-3032  
13 Hinterkappelen, Switzerland

14  
15 <sup>2</sup> Institute of Integrative Biology, University of Liverpool, Leahurst Campus, Chester High  
16 Road, Neston, CH64 7TE, UK

17  
18 <sup>3</sup> Département de Biologie et Institut de biologie intégrative et des systèmes, Université  
19 Laval, Quebec, G1V 0A6 , Canada

20

21 Address for correspondence

22 C. Nyman, Division of Behavioural Ecology, Institute of Ecology and Evolution, University  
23 of Bern, Wohlenstrasse 50A, CH-3032 Hinterkappelen, Switzerland.

24 E-mail address: [cecilia.wikstroem@iee.unibe.ch](mailto:cecilia.wikstroem@iee.unibe.ch) (C. Nyman).

25  
26

27 **Abstract**

28 The early social environment can have substantial, lifelong effects on vertebrate social  
29 behaviour, which can be mediated by developmental plasticity of brain gene expression. Early  
30 life effects can influence immediate behavioural responses towards later-life social challenges  
31 and can activate different gene expression responses. However, while genomic responses to  
32 social challenges have been reported frequently, how developmental experience influences the  
33 shape of these genomic reaction norms remains largely unexplored. We tested how  
34 manipulating the early social environment of juvenile, cooperatively-breeding cichlids,  
35 *Neolamprologus pulcher*, affects their behavioural and brain genomic responses when  
36 competing over a resource. Juveniles were reared either with or without a breeder pair and a  
37 helper. Fish reared with family members behaved more appropriately in the competition than  
38 when reared without. We investigated whether the different social rearing environments also  
39 affected the genomic responses to the social challenge. A set of candidate genes, coding for  
40 hormones and receptors influencing social behaviour, were measured in the telencephalon and  
41 hypothalamus. Social environment and social challenge both influenced gene expression of  
42 *egr-1* (early growth response 1) and *gr1* (glucocorticoid receptor 1) in the telencephalon and  
43 of *bdnf* (brain derived neurotrophic factor) in the hypothalamus. A global analysis of the 11  
44 expression patterns in the two brain areas showed that neurogenomic states diverged more  
45 strongly between intruder fish and control fish when they had been reared in a natural social  
46 setting. Our results show that same molecular pathways may be used differently in response  
47 to a social challenge depending on early life experiences.

48

49 **Keywords**

50 Developmental plasticity, behavioural flexibility, social competence, early social  
51 environment, genomic reaction norm, neurogenomic state, cooperative breeder, brain gene  
52 expression, social challenge.

53

54

55

56

57

58

59

60

## 61 **Introduction**

62 The early social environment can have important and persisting effects on the development of  
63 an animal's emotional (reviewed in Champagne 2010) and behavioural phenotype (reviewed  
64 in Kasumovic & Brooks 2011). Long-term effects of the early social environment have been  
65 reported in all vertebrate classes (mammals: e.g. Harlow & Zimmermann 1959; Mireault &  
66 Bond 1992; Liu *et al.* 1997; Bastian *et al.* 2003; Branchi & Alleva 2006; birds: Adkins-  
67 Regan & Krakauer 2000; Ruploh *et al.* 2013; Ruploh *et al.* 2014; Schmidt *et al.* 2014;  
68 reptiles: Ballen *et al.* 2014; amphibians: Nicieza & Metcalfe 1999; fish: e.g. Arnold &  
69 Taborsky 2010; Taborsky *et al.* 2012). The social conditions experienced early in life can  
70 affect a remarkably broad array of traits including life history traits and reproductive  
71 schedules (Kasumovic & Brooks 2011), coloration (Ballen *et al.* 2014) or learning and  
72 memory (Liu *et al.* 2000), but most often it affects behaviours in the social domain (reviewed  
73 in Taborsky 2016a). For instance, variation in the amount of received maternal care can affect  
74 maternal care behaviour of the next generation (Liu *et al.* 1997; Francis *et al.* 1999) or the  
75 ability to use social information in effective hierarchy formation (Branchi *et al.* 2006). The  
76 sex composition of littermates or social groups during rearing can affect later mate choice  
77 decisions (Adkins-Regan & Krakauer 2000) or aggressive tendencies (Benus & Henkelmann  
78 1998).

79

80 Lasting effects induced by the early social environment on social behaviours are thought to  
81 result from developmental plasticity in the brain (e.g. Fischer *et al.* 2015) and can be mediated  
82 by organizational effects of hormones or epigenetic modifications. Organizational effects of  
83 the hormonal system (Phoenix *et al.* 1959; Soares *et al.* 2010) impact the neural structural  
84 level, are slow and involve mechanisms such as neurogenesis, apoptosis and synaptic  
85 plasticity (reviewed in Soares *et al.* 2010). Organizational effects are considered non-  
86 reversible and they usually affect a phenotype during specific sensitive periods of  
87 development, for example in the perinatal period or during puberty (Rice & Barone Jr 2000;  
88 Romeo 2003). Furthermore, early adversity can result in socially driven epigenetic  
89 modifications (Champagne 2008). These lasting effects can often be measured by persistent  
90 alterations of gene expression profiles in different brain areas, including effects on hormonal  
91 ligands and receptors related to the stress response and social recognition (e.g. corticosteroids,  
92 serum oxytocin, and oxytocin and estrogen receptors; (Zimmer *et al.* 2013, Cao *et al.* 2014);  
93 glucocorticoid receptors (*gr*, *gr1*, Zimmer *et al.* 2014) and corticotropin-releasing factor (*crf*);  
94 (Liu *et al.* 1997; McGowan *et al.* 2009; Banerjee *et al.* 2012; Taborsky *et al.* 2013)). The

95 early social environment might also have long lasting consequences for the individual by  
96 influencing and modulating neuronal plasticity of the brain and related gene expression  
97 pathways [brain-derived neurotrophic factor (*bdnf*) and nerve growth factor (*ngf*); (Zhang *et*  
98 *al.* 2002; Roceri *et al.* 2004)].

99

100 Behavioural flexibility, a form of plasticity that should be distinguished from developmental  
101 plasticity, is expressed as a response to an environmental trigger, and is immediate and  
102 reversible (Taborsky & Oliveira 2012). For example, in the social domain, individuals  
103 perceive and use social information to flexibly adjust their behaviour to the present social  
104 context ('social competence', Taborsky & Oliveira 2012). Behavioural flexibility is mediated  
105 in part by the activational effects of the hormonal system (Soares *et al.* 2010). Activational  
106 effects work at the functional level by changing the activity of neural circuits and are rapid  
107 and transient. Social challenges and opportunities can activate different patterns of gene  
108 expression in specific brain areas, which can be measured as genomic reaction norms (Aubin-  
109 Horth & Renn 2009). For example, when previously subordinate cichlid fish, *Astatotilapia*  
110 *burtoni*, change their social rank, changes in behaviour and colouration are accompanied by  
111 an activation of different brain areas through expression of the immediate early genes (IEG's)  
112 *egr-1* and *c-fos* (Burmeister *et al.* 2005; Maruska *et al.* 2013) and changed expression of  
113 genes coding for hormones and their receptors in different brain areas associated with social  
114 behaviour (Huffman *et al.* 2012a, 2015).

115

116 We predict that developmental plasticity and behavioural flexibility will jointly shape social  
117 behaviour, resulting in different slopes of behavioural reaction norms dependent on social  
118 rearing conditions (e.g. Dettling *et al.* 2002). This means that the shape of an immediate  
119 behavioural response towards a social challenge (e.g., the slope between baseline and  
120 challenged condition) would differ depending on the early rearing environment. For example,  
121 rhesus monkeys separated from their mothers early in life respond to peer presence with much  
122 lower frequencies of affiliation behaviour than do mother-reared peers, even after years of  
123 living in normal social conditions (Feng *et al.* 2011). This difference in short-term  
124 behavioural response of individuals that experienced divergent rearing environments should  
125 correspond to changes in components of the underlying control mechanisms, in particular  
126 long-term and short-term alterations of gene expression. At the molecular level, this can best  
127 be studied by measuring brain genomic reaction norms in response to an environmental  
128 challenge (behavioural flexibility) of individuals reared in different environmental conditions

129 (developmental plasticity). With genomic reaction norms, we measure how an individual of a  
130 particular phenotype responds to a specific situation at the gene expression level, within a  
131 specific tissue, brain area or cell type, depending on the question asked. As a hypothetical  
132 example, an individual that experienced benign early life conditions might respond by high  
133 brain glucocorticoid receptor (*gr*) expression toward a social stimulus, whereas an individual  
134 that grew up under adverse conditions may mount a much smaller *gr* response (or might not  
135 respond at all). Individuals reared in socially more complex early environments generally  
136 behave more socially competent in a range of different social challenges compared to when  
137 reared in more simple environments (reviewed in Taborsky 2016a). Furthermore, in order to  
138 capture the change in the overall pattern of expression after a social challenge in individuals  
139 from the two contrasting early rearing environments, a neurogenomic state can be defined  
140 using the expression of all genes in all surveyed brain regions at once (Robinson et al. 2008).  
141 Such differences in molecular responses to a behavioural challenge between individuals that  
142 faced different early social environments have so far been only demonstrated in laboratory  
143 strains of rodents (measured at the mRNA or protein level, Plotsky & Meaney 1993; Wigger  
144 & Neumann 1999; Ago *et al.* 2013). For example, male mice reared in isolation show higher  
145 *c-fos* protein levels in the cortex when faced with a social challenge than group-reared males  
146 (Ago *et al.* 2013). There is however no published explicit test of the effect of the early rearing  
147 environment on gene expression levels in response to a short term challenge.

148  
149 Finally, the consistent finding that variation in the early social environment of animals results  
150 in different behavioural responses to social challenges and opportunities (Taborsky 2016a)  
151 gives rise to the question whether changes in behaviour relate to changes in gene expression  
152 patterns. Testing such a relationship is an important first attempt to decipher the functional  
153 significance of this variation at the gene expression level (Williams 2008). For instance  
154 Cummings *et al.* 2008 show that specific genes are turned on in the females swordtail fish,  
155 *Xiphophorus nigrensis*, interacting with attractive males but then turned off when interacting  
156 with other females. Further aggressive behaviour in threespine stickleback, *Gasterosteus*  
157 *aculeatus*, was shown to be positively correlated with gene expression of glucocorticoid  
158 receptors (Aubin-Horth *et al.* 2012). However, whether one always expects a linear  
159 relationship between a phenotype and the underlying endocrine pathways, or whether  
160 individuals from different context (age, sex, status, environment) should show the same  
161 relationship is less certain (Williams 2008).

162

163 In order to understand how brain genomic reaction norms have evolved in the social domain  
164 under natural conditions when confronted with biologically relevant challenges, we need  
165 information from a broader array of taxonomic groups and, in particular, also from natural  
166 study organisms (as opposed to organisms artificially selected for a certain purpose), because  
167 they can be expected to display naturally evolved reaction norms (Groothuis & Taborsky  
168 2015). Here we chose a highly social fish species as study system, the cooperatively breeding  
169 cichlid *Neolamprologus pulcher*. This species, which has become a key organism for the  
170 study of vertebrate social evolution (e.g. Wong & Balshine 2011; Taborsky 2016b), is now  
171 also studied within an ecological genomics framework (Aubin-Horth *et al.* 2007; Taborsky *et*  
172 *al.* 2013; Brawand *et al.* 2014; O'Connor *et al.* 2015, 2016; Reddon *et al.* 2015, O'Connor *et*  
173 *al.* 2016). We investigated the association between behavioural and genomic reaction norms  
174 in this species by comparing the response to a social challenge (a contest over a resource) of  
175 individuals whose early rearing environment differed in levels of social complexity. Since  
176 previous experiments showed that *N. pulcher* reared in different social environments display  
177 altered behavioural responses to social challenges (Arnold & Taborsky 2010; Taborsky *et al.*  
178 2012), we predicted that social rearing and social challenge would jointly influence genomic  
179 reaction norms in the brain of these fish.

180  
181 We aimed to answer two questions: (i) How do genomic reaction norms measured in fish  
182 exposed to a social challenge or a control situation differ between fish reared in different  
183 social environments? (ii) Is the observed behaviour and the early social environment related  
184 to the genomic response? To answer the first question, we measured gene expression in the  
185 telencephalon and hypothalamus of socially challenged and control fish. These two brain  
186 areas play a key role in social behaviour and decision-making in fish (O'Connell & Hofmann  
187 2011) and in their hypothalamic-pituitary-interrenal (HPI) stress axis. The HPI is homologous  
188 to the mammalian hypothalamic-pituitary-adrenal (HPA) stress axis, which has been shown to  
189 be strongly impacted by the early social environment across different vertebrate classes  
190 (Meaney & Szyf 2005; Banerjee *et al.* 2012; Taborsky *et al.* 2013). In the telencephalon we  
191 measured expression of *egr-1*, *bdnf*, *gr1*, *crf*, and *neuroserpin*, and in the hypothalamus the  
192 expression of *egr-1*, *bdnf*, *gr1*, *crf*, *avt* and its V1a2 receptor (*avtr*). The product of these  
193 genes are known to be involved in the modulation of social behaviour or social dominance  
194 relationships and/or to be affected by early social experience in vertebrates (Liu *et al.* 1997;  
195 Young *et al.* 1999; Zhang *et al.* 2002; Madani *et al.* 2003; Burmeister *et al.* 2005; Branchi *et*  
196 *al.* 2006; Aubin-Horth *et al.* 2007). To answer our second question, we analysed the

197 relationship between social behaviours expressed during the social challenge and gene  
198 expression.

199

## 200 **Methods**

201

### 202 *Study species*

203 *Neolamprologus pulcher* is a cooperatively breeding cichlid endemic to Lake Tanganyika,  
204 East Africa. It lives in large family units of up to 25 fish consisting of a dominant breeder  
205 pair, one or several related or unrelated helpers and fry from recent broods. Subordinates  
206 provide help in form of direct brood care of the dominants' offspring and of territory defence  
207 and maintenance. In turn they remain accepted by dominants at a territory, at which they have  
208 access to critical resources ('pay-to-stay'; Taborsky 1985; Balshine-Earn *et al.* 1998;  
209 Bergmüller & Taborsky 2005; Stiver *et al.* 2005; Heg & Taborsky 2010; Zöttl *et al.* 2013b;  
210 Fischer *et al.* 2014). By being accepted at a territory, helpers benefit particularly from  
211 protection from predators and access to high quality shelters (Balshine-Earn *et al.* 1998; Heg  
212 *et al.* 2004), and they might eventually get a chance to inherit a breeder position (Stiver *et al.*  
213 2004). *N. pulcher* groups are organized in size-based linear hierarchies (Dey *et al.* 2013) and  
214 the fish have a large, fine-scaled repertoire of social behaviours to establish and maintain  
215 these hierarchies (Taborsky 1984). Higher ranking fish show an array of open and restrained  
216 aggressive displays towards lower ranking fish, which in turn show different submissive  
217 behaviours.

218

219 The early social environment influences the development of social behaviour and social  
220 competence of *N. pulcher*. When young are reared either with the breeding pair, a helper, and  
221 their siblings (+F treatment), or with their siblings only (-F treatment), +F fish show more  
222 adequate social behaviour and solve social conflicts more efficiently than -F fish (Arnold &  
223 Taborsky 2010; Taborsky *et al.* 2012). Analysis of whole brain gene expression in adult  
224 individuals has shown that the stress axis of these fish is stably reprogrammed by the early  
225 social rearing treatment. +F fish had a lower expression of *gr1* and *crf* compared to fish from  
226 the -F treatment (Taborsky *et al.* 2013).

227

### 228 *Housing conditions*

229 The experiment was carried out at the 'Ethologische Station Hasli' of the Institute of Ecology  
230 and Evolution, University of Bern, Switzerland, under licence number 52/12 of the Veterinary

231 Office of the Kanton Bern. The breeding pairs used to generate the experimental fish were 2<sup>nd</sup>  
232 and 3<sup>rd</sup> generation offspring of wild caught *N. pulcher* from Kasakalawe Point, Mpulungu,  
233 Zambia. Rearing tanks of 200 L were equipped with a 2 cm sand layer, and eight clay pot  
234 halves and two PET bottles serving as shelters. The light:dark cycle was set to 13:11 h with a  
235 10 min dimmed light period in the morning and evening to simulate the light conditions of  
236 Lake Tanganyika. Fish were fed ad libitum 6 days a week (5 days commercial flake food, 1  
237 day frozen zooplankton). Water temperature was held constant at 27±1 °C.

238

### 239 *Early social environment treatments*

240 We used two early social environments: being reared (i) with parents, one helper and same-  
241 aged siblings (+F treatment), or (ii) with same-aged siblings only, but no older family  
242 members (-F treatment). We first created the experimental broods, by forming 20 social  
243 groups in separate 200-L tanks, consisting of a breeder male, a breeder female and an  
244 immature helper by haphazardly selecting unfamiliar fish from the institute's breeding stock.  
245 Ten days after a breeder pair had spawned a clutch, the offspring had reached the free  
246 swimming stage and were used to form 20 experimental groups. Each experimental group was  
247 placed in a 100-L compartment of a 200-L tank, separated from neighbouring groups by an  
248 opaque PVC sheet. Offspring of each experimental group were assigned randomly to one of  
249 the two early social environment treatments. Mean group size was 32.6 fish ± 3.8 SEM in the  
250 +F treatment and 35.4 fish ± 5.1 SEM in the -F treatment. Groups receiving the +F treatment  
251 were moved to an empty 100-l compartment together with their parents and helper, whereas  
252 groups receiving the -F treatment were moved to another empty 100-l compartment without  
253 their parents and the helper. The early social environment treatment lasted for 62 days in  
254 accordance with earlier studies (Arnold & Taborsky 2010; Taborsky *et al.* 2012, 2013;  
255 Fischer *et al.* 2015). Afterwards the parents and the helper were removed from the +F  
256 treatment and were transferred back to the institute's breeding stock. During the following 72  
257 ± 2 days ('neutral phase'), the sibling groups of both treatments were kept in their original  
258 100-L compartments under identical, standard housing conditions (following Taborsky *et al.*  
259 2012).

260

### 261 *Social challenge test*

262 As a social challenge, we chose a test situation that juvenile fish encounter regularly in natural  
263 territories, where they have to defend a private shelter against other juvenile family members  
264 (Taborsky 2016b). On day 134 (± 2 days), four individuals per experimental group were used



265 in this social challenge test. Two fish were assigned to the challenge treatment and two fish to  
266 a control treatment. Behavioural data were collected from a total of 80 fish (36 +F individuals  
267 from 9 groups and 44 – F individuals from 11 groups). Brain samples were taken from a total  
268 of 71 fish; 36 challenged individuals (16 +F and 20 –F fish) and 35 controls (15 +F and 20 –F  
269 fish). We staged an asymmetric contest over a shelter (for details see Arnold & Taborsky  
270 2010). Briefly, a 20-L test tank (30 x 20 x 20cm) was divided into two compartments by an  
271 opaque PVC wall. One compartment was empty and the other compartment contained a small  
272 clay pot half placed in the centre, which served as a shelter. The focal individual of the  
273 challenge test was always assigned the role of a territory intruder, that is, initially it did not  
274 own the shelter. Twenty-four hours before testing, a focal juvenile (2.303 cm  $\pm$  0.012 SEM)  
275 was removed from its home tank, measured, weighed and placed into the empty compartment  
276 of the test tank (balanced between right and left side between trials). At the same time, an  
277 unfamiliar *N. pulcher* of the same age was placed in the compartment with shelter to become  
278 the pre-assigned shelter owner (2.303 cm  $\pm$  0.645 SEM) and, thereby, the territory owner.  
279 Sizes were matched between the two individuals as close as possible (size difference 0.038  
280 cm  $\pm$  0.006 SEM). The shelter owner, which served only as an opponent for the focal fish,  
281 was always a fish reared in a social group consisting of a breeder pair and a helper (+F  
282 condition). Each shelter owner was used only once. In the control treatment, juveniles were  
283 exposed to the same handling procedures as the challenged fish and placed in the empty  
284 compartment of tanks equally equipped as the test tanks of the challenged fish, but without  
285 any opponent present.

286

287 The asymmetric competition trials were carried out on the day after the fish had been placed  
288 in the experimental tank, between 12:00 and 14:00 h. Previous studies have shown that 24 h is  
289 sufficiently long for *N. pulcher* individuals to occupy a novel shelter and defend it as its core  
290 territory (Arnold & Taborsky 2010; Taborsky *et al.* 2012). Before the start of a trial, the  
291 divider between the compartments was lifted so that the pre-assigned intruder and the shelter  
292 owner could interact. The starting point of the trial was set to the moment when either of the  
293 two fish crossed the virtual, vertical border between the two compartments (the place where  
294 the PVC divider had been before) for the first time. From that moment onwards, the  
295 behaviour of the focal individual was recorded for 20 min from behind a black curtain with an  
296 observation slit. The observer (CN) was blind to the rearing treatment of the focal fish. The  
297 behaviour of both fish (submission, overt aggression, restrained aggression, hiding in shelter  
298 and swimming activity) was recorded continuously using the Observer 5.0 software (Noldus,

299 The Netherlands). After 20 min the winner and loser of the contest were determined. A fish  
300 was considered as winner when it stayed in or close ( $< 3$  cm) to the shelter and when it was  
301 not attacked by its conspecific. Conversely, it was regarded as loser when it was evicted from  
302 the vicinity of the shelter and showed submission, but no overt aggression, towards the other  
303 fish, or if it stayed close to the water surface ( $< 5$  cm; see Taborsky *et al.* 2012). In seven  
304 cases (2 +F fish and 5 -F fish) there was no clear winner or loser after 20 min, in which case  
305 this contest was rated as ‘undecided’ and these trials were excluded from further behavioural  
306 analysis. After 20 min the two fish were separated again by the divider and the winner was  
307 allowed to use the shelter for 10 min. For the control trials, we followed the same procedures  
308 as in the challenge test, but the focal fish in the control situation was not exposed to a shelter  
309 owner. In these trials, after the divider had been removed, the control fish could swim freely  
310 in the test tank for 20 min while we recorded its activity (swimming or in pot). At the end of  
311 the observation the opaque wall was put back in and the control fish was left 10 min on the  
312 side with the shelter if it had entered the shelter during the experiment, otherwise it was left  
313 on the opposite side in the aquarium.

314

#### 315 *Tissue sampling*

316 A 30-min interval from the start of the trial to brain collection was chosen since this protocol  
317 has been used successfully before (Cummings *et al.* 2008). It could thus safely be assumed  
318 that changes in gene activation patterns could be measured after this time. After the opaque  
319 divider was put back in place following the 20-min behavioural recording, a 10-min period  
320 without social contact followed for both the challenged and the control fish before the brain  
321 tissue was sampled. In the challenge treatment, only brains of the intruder fish (the focal fish)  
322 were sampled. In the control treatment, all control fish were sampled. Individuals were  
323 sacrificed with an overdose of buffered Tricaine methanesulfonate (MS222; Sandoz,  
324 Switzerland) within 30 s of catching and the brain was quickly dissected under a binocular  
325 microscope (magnification: 16x). The brain was divided into five brain areas, telencephalon,  
326 hypothalamus, cerebellum, optic tectum and hind brain. After the dissection each part was put  
327 into a 1.5 ml vial and immersed in RNAlater (Ambion). Further analysis focused on the  
328 telencephalon and hypothalamus regions. Samples in RNA later were left overnight at  $+6$  °C  
329 and then moved to  $-20$  °C for permanent storage. The sex of the individuals could not be  
330 determined since in *N. pulcher* the sex can only be determined when the fish start to become  
331 sexually mature, which occurs around lengths of 3.5 cm, while our test subjects ranged  
332 between 2.1 - 2.4 cm standard length.

333

#### 334 *Sample preparation*

335 We performed RNA extraction from telencephalon and hypothalamus, for each brain part  
336 separately, using a miRNeasy micro kit (Qiagen) using a modified manufacturer protocol (see  
337 supplementary material) so that the miRNAs were discarded. The RNA concentration and  
338 sample composition was checked with a Nanodrop microvolume spectrophotometer (samples  
339 ranged between 27-139 ng/ul). Reverse transcription was done using the same amount of  
340 RNA from each sample (200 ng RNA from hypothalamus and 304 ng RNA from  
341 telencephalon) using a standard Superscript protocol (Invitrogen). To confirm the expression  
342 of each candidate gene and success of RT, a small amount of cDNA from random samples  
343 from both treatments was used in a PCR using all the different candidate genes and visualised  
344 using an electrophoretic gel.

345

#### 346 *Candidate genes*

347 We measured the expression of five genes in the telencephalon (*egr-1*, *bdnf*, *gr1*, *crf* and  
348 *neuroserpin*) and six genes in the hypothalamus (*egr-1*, *bdnf*, *gr1*, *crf*, *avt* and *avtr*) of *N.*  
349 *pulcher*. We were interested in the reaction norm of these genes, that is, if their expression  
350 level is different in fish facing a control versus a challenge condition, and if these reaction  
351 norms differed between fish reared in +F or -F social conditions. The gene 18S was used as a  
352 control gene. *egr-1* (early growth response 1, also known as NGFI\_A, Krox-24, zif268,  
353 ZENK and TIS8) is an immediate early gene coding for a transcription factor used as a  
354 marker for neuronal activity (Desjardins & Fernald 2010) and plasticity (Morgan & Curran  
355 1995). The gene is activated in different brain areas in response to a novel or changing social  
356 cue (Burmeister *et al.* 2005), and this property has been used to determine which brain areas  
357 respond to a certain stimulus. In the lateral part of the dorsal telencephalon (DI), which is  
358 thought to be the fish homologue of the mammalian hippocampus (Folgueira *et al.* 2004), *egr-*  
359 *1* has been proposed to act as a transcription factor targeting later-acting genes involved in  
360 stress responses (Desjardins & Fernald 2010). *bdnf* (brain-derived neurotrophic factor) is a  
361 molecule influencing neuronal proliferation, differentiation and synaptogenesis (McAllister *et*  
362 *al.* 1999) and is therefore assumed to impact brain function and structure (Branchi *et al.*  
363 2004). Rat pups facing repeated maternal deprivation show persistently altered *bdnf*  
364 expression in the hippocampus and prefrontal cortex compared to control (undisturbed) pups  
365 (Roceri *et al.* 2004). In *A. burtoni* a higher *bdnf* expression was observed in the DI of fish  
366 learning a task (finding shelter and a female) compared to non-learners (Wood *et al.* 2011).

367 *gr1* (glucocorticoid receptor 1) is a ligand-activated nuclear receptor that is part of the HPI  
368 stress axis in fish and is activated by glucocorticoids. Acting as a transcription factor, it is  
369 involved in modulating stress responses in different tissues and in the negative feedback of  
370 corticosteroids on stress responses taking place in the hippocampus (Jacobson & Sapolsky  
371 1991; Kloet *et al.* 1998). Previous work showed that adult *N. pulcher* reared in -F conditions  
372 have higher *gr1* expression in whole brain samples than +F individuals (Taborsky *et al.*  
373 2013). *crf* (corticotropin-releasing factor) plays a role in activating the stress response, and in  
374 modulating social behaviours associated with parental care, social memory, as well as  
375 prosocial and affiliative behaviours (review in Hostetler & Ryabinin 2013). *crf* was higher  
376 expressed in whole brain samples of *N. pulcher* reared in -F conditions (Taborsky *et al.*  
377 2013). Neuroserpin is a serine protease inhibitor that is assumed to play a role in synaptic  
378 plasticity and is most prominently expressed in areas of the brain that participate in learning,  
379 memory and behaviour (review in Miranda & Lomas 2006). Thus this gene might be  
380 implicated in plastic behavioural responses in fish. The neuropeptide arginine vasotocin (*avt*),  
381 the fish homologue to the mammalian arginine vasopressin (AVP), is involved in  
382 osmoregulation, the regulation of the stress response, and in reproductive and social  
383 behaviours (reviewed in Godwin & Thompson 2012). Aubin-Horth *et al.* (2007) showed that  
384 dominant individuals of *N. pulcher* had higher levels of whole brain *avt* gene expression,  
385 compared to subordinate conspecifics, and its expression is higher in wild-caught males of the  
386 social cichlid *N. pulcher* than of the non-social cichlid *Telmatochromis temporalis* (O'Connor  
387 *et al.* 2015), but this difference was not repeated in a laboratory study (O'Connor *et al.* 2016).  
388 The V1a2 receptor for *avt* (*avtr*) is implicated in social behaviour in fish by mediating  
389 aggressive and mating behaviour (Lema 2010; Kline *et al.* 2011; Huffman *et al.* 2012b;  
390 Oldfield *et al.* 2013; Huffman *et al.* 2015).

391

#### 392 *Quantitative real time PCR*

393 Primers for *gr1* and *crf* were as in Taborsky *et al.* 2013, the *avt*, *avtr* and 18S primers were as  
394 in O'Connor *et al.* 2015, while primers for the other genes were designed using the sequences  
395 available from the genome of *N. brichardi*  
396 (<http://cichlid.umd.edu/cichlidlabs/kocherlab/bouillabase.html>). The sequences are as follows:  
397 *egr-1* (using the *A. burtoni* sequence as a search template, NCBI database ID number:  
398 AY493348.1, *N. brichardi* NCBI database ID number XM\_006781510.1, for-  
399 CGGCGATATATCCTAAAATC; rev-TCCCATGCCTATAAACACT), *bdnf* (using the *A.*  
400 *burtoni* sequence as a template, NCBI database ID number: HQ398161.1, *N. brichardi* NCBI

401 database ID number XM\_006780270.1, for-GGGTGACAGCTGTGGATAAAA; rev-  
402 GGGGTTGCATTTGGTCTCATA) and *neuroserpin* (using the *Oreochromis mossambicus*  
403 sequence as a template, NCBI database ID number: HQ667766.1, *N. brichardi* NCBI  
404 database ID number XM\_006799864, for-GGATGGACCCTGTTCTCC; rev-  
405 TTGCCCTGACCAGGACTCT). To determine amplification efficiency, the absence of  
406 primer dimers and the specificity of amplification for each primer pair, qPCR experiments  
407 and melting curves (50 to 90 Celsius) were run using standard curves consisting of 5 x 10-fold  
408 dilutions (of pooled samples) in duplicates (Aubin-Horth *et al.* 2012). The primers (Eurofins)  
409 and 5 µl of sample cDNA were prepared on a 384-well plate (axigen) using an epMotion  
410 liquid handler (Eppendorf) and used for a quantitative real-time PCR experiment following  
411 the scaled-down version of the Quantitect SYBRGreen PCR kit manufacturer's protocol  
412 (Qiagen) using a 384-well plate qRT-PCR machine (Light Cycler, Roche). Each sample for  
413 hypothalamus and telencephalon was run in triplicate for a given gene together with no  
414 primers and no template controls. To verify that only a single amplified product was present  
415 and that no primer dimers were produced, a melting curve was also performed on each  
416 replicate. Relative gene expression for each individual-brain area combination was calculated  
417 using the expression of a control gene (18S) (Pfaffl 2001).

418

#### 419 *Data analysis*

420 We used two different data sets to answer our questions. To analyse genomic reaction norms  
421 and neurogenomic states of individuals from the different early social environment and social  
422 challenge treatments, we included all intruder and all control fish (data set 1). To analyse (i)  
423 the expressed behaviours during the challenge of intruders and owners and (ii) the  
424 relationship between intruder behaviour and gene expression, we only analysed intruder fish  
425 that either won or lost the contest over the shelter (data set 2). Furthermore we analysed only  
426 the interactions between the start and the end of a contest. Contests were considered to be  
427 terminated when the loser did not aim to gain access to the shelter and retreated either to the  
428 upper parts of the water column or to a distant corner of the tank. As the duration of these  
429 periods varied between trials, we analysed behavioural rates (per min). We used this subset of  
430 the data (data set 2) for two reasons. (i) Controls could not be included because they could not  
431 show any social behaviour; (ii) Contests which were still undecided after 20 min observation  
432 time were excluded, because behavioural frequencies are expected to vary with the eventual  
433 fight outcome (e.g., the loser should show submission). By including fights that were ongoing

434 at the end of the observation time behaviours would be biased towards higher aggression  
435 relative to submission rates.

436

437 Statistical analyses were conducted with R 3.0.2 (R Core Development team 2013) including  
438 the package ‘lme4’ (Bates *et al.* 2013) and ‘afex’ (Singmann *et al.* 2015). Linear mixed  
439 models (LMM) were built to analyse the influence of the two rearing treatments (+F and –F  
440 fish) on fish behaviour. We used intruder behaviour as our dependent variable and rearing  
441 treatment (+F / -F) as our independent variable. In the LMM with intruder submission as  
442 dependent variable owner aggressive behaviour was included as covariate, as in *N. pulcher*  
443 submission is often an immediate response to received aggression. In the LMMs with intruder  
444 overt aggression and restraint aggression as dependent variable the contest outcome  
445 (winning/losing) was included as covariate. In a further set of LMMs, we analysed the  
446 influence of the two rearing treatments (+F and –F fish), the social challenge treatments  
447 (intruder vs control fish) and their interactions on the expression levels of each single gene. If  
448 the interaction term “rearing treatment x social challenge“ was significant we conducted post  
449 hoc analyses by testing for gene expression differences between the two social challenge  
450 situations, separately within +F fish and –F fish, respectively. For all post-hoc analyses we  
451 present adjusted P-values after applying the Benjamini-Hochberg false-discovery rate method  
452 (Benjamini & Hochberg, 1995) to correct for multiple testing. For some individuals, gene  
453 expression data were missing for one or more genes because the coefficient of variation (CV)  
454 of the three replicates was too large. A CV cutoff of 5% was used for all genes. The sample  
455 sizes for each gene are as follows: in telencephalon: *egr-1*, *bdnf*, *gr1*, *crf*, *neuroserpin* N=57  
456 (of them –F control = 14, –F intruder = 16, +F control = 13 and +F intruder = 14) and in  
457 hypothalamus: *egr-1* N=40 (of them –F control =12, –F intruder = 12, +F control = 6 and +F  
458 intruder = 10), *bdnf*, *gr1*, *crf*, *avtr* N=56 (of them –F control =17, –F intruder = 17, +F control  
459 = 10 and +F intruder = 12) and *avt* N=54 (of them –F control = 17, –F intruder = 17, +F  
460 control = 9 and +F intruder = 11). In addition, a principal component analysis (PCA) was  
461 performed in order to reduce the complexity of the gene dataset and thus to obtain a  
462 “neurogenomic state” (Robinson *et al.* 2008) for each individual that summarises the  
463 information on all genes in both brain areas. The PCA was done with 70 individuals as  
464 observations and expression levels of 7 different candidate genes, with a total of 11 measures  
465 of gene expression (5 in telencephalon, 6 in hypothalamus) as variables using the R package  
466 “psych” (function “principal”). A correlation matrix for the 11 measures of gene expression  
467 was used as input (Pearson correlation coefficients). To be able to include individuals with

468 missing data (see above) in the analysis, the mean gene expression of that gene for a given  
469 combination of rearing environment and social challenge was used in the data analysis for  
470 these individuals (Zar 1999). A varimax rotation was applied to the data. Loadings of  
471 individual genes on each principal component (PC) were determined and the PC scores for  
472 individual fish were calculated. LMMs were built to analyse the influence of the early social  
473 environment and social challenge treatments and their interactions on the first two principal  
474 components (see below). All models assumed a Gaussian error structure, which was validated  
475 by visual inspection of the distributions of residuals, predicted vs. fitted values and Quantile-  
476 Quantile (Q-Q)-plots. Some variables were log-transformed in order to achieve a normally  
477 distributed error structure. Experimental group was included as random factor in each model.  
478 To account for possible effects of intruder size, the intruder standard length (I\_SL) was  
479 included as covariate in all behavioural models. For significance testing each term was singly  
480 removed from the model and the reduced model was compared to the full model. To do so we  
481 used the command 'mixed' in the R package 'afex', which calculates type 3 p-values using a  
482 Kenward-Roger approximation for degrees-of-freedom (Singmann *et al.* 2015). Models were  
483 fitted with sum contrasts. These are orthogonal contrasts, where every level of a factor is  
484 compared to the overall factor mean, which is represented by the intercept.

485

#### 486 *Ethical note*

487 Fish interacted directly with each other in the asymmetric competition. We observed carefully  
488 that no fish was injured during the experiment, in which case the trial would have been  
489 immediately interrupted. This never happened. Some fish showed overt aggression towards  
490 each other (i.e. aggression that involves body contact, Taborsky 1984). Probably due to the  
491 small size and low weights of the fish, these direct body contacts never caused any injuries in  
492 the opponent. A fish subject to overt aggression usually responded by showing submissive tail  
493 quivering and/or by retreating out of reach of the aggressor, which stopped aggression  
494 immediately.

495

## 496 **Results**

497

### 498 *Effect of early social environment on behavioural phenotype*

499 To test whether our early social environment treatment was effective to influence the  
500 phenotypic development of the fish, we tested whether the rearing treatment influenced the  
501 later-life social behaviour of our experimental fish. Intruder fish of the +F treatment displayed

502 more submissive behaviour relative to the amount of received owner aggression than did fish  
503 from the -F treatment (Fig. 1, LMM, interaction term:  $F = 7.2413$ ,  $P = 0.013$ , treatment:  $F =$   
504  $1.269$ ,  $P = 0.270$ , received aggression:  $F = 22.599$ ,  $P < 0.0001$ ,  $N = 31$ ). In contrast, intruder  
505 overt aggression did not differ between the rearing treatments but winners showed more overt  
506 aggression than losers (LMM, treatment:  $F = 0.759$ ,  $P = 0.397$ , contest outcome:  $F = 4.381$ ,  $P$   
507  $= 0.048$ ,  $N = 31$ ). Intruder restraint aggression (i.e., threat displays towards the opponent  
508 without body contact) was not influenced by the rearing treatment or by contest outcome  
509 (LMM, treatment:  $F = 0.203$ ,  $P = 0.658$ , contest outcome:  $F = 0.001$ ,  $P = 0.992$ ,  $N = 31$ ).

510

#### 511 *Genomic reaction norms in response to early social environment and social challenge* 512 *treatments*

513 Telencephalon. The early social environment (+F/-F) and the social challenge  
514 (intruder/control) treatments interactively influenced the expression of *egr-1* and *gr1* in the  
515 telencephalon (Fig. 2, table 1). Post hoc analysis revealed that -F fish had a lower *egr-1*  
516 expression in the control than in the intruder situation (LMM, -F fish:  $F = 11.372$  adjusted  $P$   
517  $= 0.006$ ,  $N = 30$ ), whereas in +F fish there was no difference in *egr-1* expression with respect  
518 to the social challenge (LMM, +F fish:  $F = 0.215$ , adjusted  $P = 0.648$ ,  $N = 27$ ). In +F fish *gr1*  
519 expression tended to be lower in the intruder than in the control situation (LMM, +F fish:  $F =$   
520  $5.355$ , adjusted  $P = 0.063$ ,  $N = 27$ ), whereas -F fish did not differ with respect to the social  
521 challenge (LMM, -F fish:  $F = 0.124$ , adjusted  $P = 0.728$ ,  $N = 30$ ). The early social  
522 environment and social challenge did not significantly influence gene expression levels of  
523 *bdnf*, *crf* and *neuroserpin* in the telencephalon (Fig. 2, table 1 and S1, Supplementary  
524 material).

525

526 Hypothalamus. The early social environment (+F/-F) and the social challenge  
527 (intruder/control) treatments interactively influenced the expression of *bdnf* in the  
528 hypothalamus (Fig. 3, table 1). Post hoc analysis showed that +F fish had a higher *bdnf*  
529 expression in the control than in the intruder situation (LMM, +F fish:  $F = 5.815$ , adjusted  $P =$   
530  $0.029$ ,  $N = 22$ ), whereas the reverse was found in -F fish, which had a higher *bdnf* expression  
531 in the intruder than in the control situation (LMM, treatment:  $F = 15.007$ , adjusted  $P = 0.001$ ,  
532  $N = 34$ ). Moreover, fish reared in the +F social environment had a higher expression of *egr-1*  
533 than in the -F condition, whereas the social challenge did not influence its expression (Fig. 3,  
534 table 1). The early social environment and social challenge did not influence the expression of  
535 *gr1*, *CFR*, *avt* and *avtr* in the hypothalamus (Fig. 3, table 1 and S1, Supplementary material).



536

### 537 *Neurogenomic states*

538 We used a PCA analysis to define a neurogenomic state that synthesises gene expression  
539 patterns in the two brain areas studied for each individual. The first two principal components  
540 of the PCA accounted for a total of 45% of the variance in gene expression (PC 1: 27 %; PC  
541 2: 18%, table 2). All genes analysed in the telencephalon (*egr-1*, *bdnf*, *gr1*, *crf*  
542 and *neuroserpin*) loaded positively on PC1. The genes analysed in the hypothalamus loaded  
543 negatively (*egr-1*, *gr1*) or positively (*bdnf*, *crf*, *avt* and *avt*) on PC2 (table 2). We extracted the  
544 individual PC scores for each fish for the two first principal components and investigated the  
545 effects of early social environment and social challenge treatment on these two components  
546 by LMMs (Fig. 4). For example, a positive score for an individual on PC1 indicates higher  
547 expression in the telencephalon of the five genes studied. The early social environment and  
548 the social challenge jointly influenced PC1 and PC2 (table 3). This significant interaction was  
549 reflected in a larger divergence of neurogenomic state (PC scores) between control and  
550 intruder fish from the +F rearing treatment as compared to -F fish, along both PC axes (Fig.  
551 4).

552

### 553 *Behaviour and gene expression*

554 The expression levels of two of the analysed genes were associated with behavioural variation  
555 among individuals (table 4). In the telencephalon, *crf* expression was interactively influenced  
556 by the early social environment and intruder submission. In +F intruders the expressed *crf*  
557 levels decreased with increasing amounts of displayed submissive behaviours, whereas no  
558 such relationship was present in -F intruders (Fig. 5a). In the hypothalamus, *gr1* expression  
559 decreased with intruder submission, with no effect of early social environment (Fig. 5b). Gene  
560 expression was not influenced by intruder overt and restrained aggression. Winning or losing  
561 the contest did not impact expression of any of the genes, nor was gene expression of winners  
562 vs. losers influenced by the social treatment. None of the other analysed genes were  
563 significantly related to any social behaviour.

564

## 565 **Discussion**

566 In this experimental study, we aimed to understand how the early social rearing environment  
567 of a cooperatively breeding fish species influences brain genomic responses to a short-term  
568 social challenge. We found that early social environment and social challenge treatments  
569 interactively influenced the expression of an immediate early gene (*egr-1*) and a

570 glucocorticoid receptor (*gr1*) in the telencephalon, and of a neural plasticity gene (*bdnf*) in the  
571 hypothalamus. Moreover, *egr-1* in the hypothalamus was more expressed in fish reared in the  
572 +F environment, independently of their exposure to a social challenge. A global analysis of  
573 the 11 measures of gene expression patterns in the brain showed that the neurogenomic state  
574 diverged more between intruder fish and control fish from the +F rearing treatment than in -F  
575 fish. Finally, we showed that with increasing submissive behaviour of intruders the expression  
576 of *crf* in the telencephalon decreased, but only in fish from the +F rearing treatment. In the  
577 hypothalamus, *gr1* expression decreased with increasing amounts of submissive behaviour of  
578 the intruder.

579

580 We first established that the behavioural response of a fish to a social challenge was markedly  
581 affected by the rearing treatment. During the social challenge, intruder fish reared with  
582 parents and a helper showed more submissive behaviour per received aggression. If in a  
583 natural context an intruder cannot monopolize its own shelter, the adequate response is to  
584 submit towards other shelter owners (Taborsky 1985, Zöttl *et al.* 2013a). The latter are then  
585 willing to tolerate the subordinate fish close to the shelter (Taborsky *et al.* 2012), which  
586 would enable the subordinate to share the access to the shelter in case of a predator attack.  
587 Our result therefore suggests that +F fish showed better social competence, confirming earlier  
588 findings by Arnold & Taborsky (2010) from a similar behavioural experiment.

589

590 The early rearing environment influenced the gene expression response to a social challenge  
591 of several genes in both the telencephalon and the hypothalamus. First, the telencephalon  
592 expression of *egr-1* was relatively high in +F fish in both social situations (control or  
593 intruder), while in -F fish this gene was highly expressed only after taking part in the contest  
594 over a shelter. Environmental stimulation activates the expression of *egr-1* (Burmeister &  
595 Fernald 2005, Goerlich *et al.* 2012). Higher *egr-1* expression of -F intruders after the  
596 challenge compared to the -F control suggests a short term response to the challenge, while  
597 there is a lack of an *egr-1* response to the challenge in the +F intruders which keep a higher  
598 baseline *egr-1* expression. Similarly, isolation-reared, but not group-reared, male mice had a  
599 significant rise in expression levels of *c-Fos*, another immediate early gene, in the prefrontal  
600 cortex two hours after facing a social challenge (Ago *et al.* 2013). Together, these studies  
601 suggest that the transcription response of *egr-1* to a social challenge can be affected by the  
602 early social environment in vertebrates. These changes can have far-ranging consequences.  
603 Since *egr-1* is a transcription factor mediating the expression of downstream genes belonging

604 to many different pathways, it is likely that entirely different networks are activated under the  
605 two social rearing conditions. Higher *egr-1* expression measured in +F fish and in challenged  
606 –F fish could increase their behavioural and neuronal plasticity (Donovan *et al.* 1999),  
607 activate effector genes downstream (for example by regulating GR expression by binding to  
608 its promoter (Weaver *et al.* 2007; Weaver *et al.* 2014) and increase learning and memory  
609 capabilities (Joëls *et al.* 2006; Roozendaal & McGaugh 2011).

610

611 Second, like *egr-1* expression, expression levels of *gr1* in the telencephalon were influenced  
612 by the combined effect of rearing environment and social challenge treatments. In +F fish, *gr1*  
613 was downregulated in the intruder challenge group compared to the control situation, whereas  
614 in –F fish, *gr1* expression was generally low and unaffected by the social challenge. Fewer  
615 glucocorticoid receptors in specific brain regions are known to reduce the efficiency of  
616 negative feedback to return cortisol levels to normal, pre-stress levels (Ladd *et al.* 2004). In  
617 rats, for instance, decreased quality of maternal care leads to life-long reduction of *gr*  
618 expression (the functional homologue of the *gr1* gene in fish, Bury *et al.* 2003) in the  
619 hippocampus and prefrontal cortex (telencephalon in fish), impairing their negative feedback  
620 inhibition of the HPA axis (Liu *et al.* 1997; Ladd *et al.* 2004; Navailles *et al.* 2010).  
621 Interestingly, after the social challenge, +F and –F fish had similarly low *gr1* levels. Post-  
622 stress down-regulation of glucocorticoid receptor gene expression has been recently  
623 quantified in mammals. A 15-min forced swim test in rats quickly resulted in lower levels of  
624 *gr* mRNA in the hippocampus, which was suggested to be a mechanism protecting neurons  
625 from repeated stress (Mifsud *et al.* 2016). The response to the social challenge observed in +F  
626 fish is similar suggesting that this could be a “normal” vertebrate-wide transcriptional  
627 response to challenging situations, which is disturbed by early rearing in a socially-deprived  
628 environment, as seen in –F fish.

629

630 Finally, *bdnf* expression levels in the hypothalamus showed crossing reaction norms, as there  
631 were both developmental and short-term environmental effects. After the contest, +F fish had  
632 a lower *bdnf* expression than in the control situation, whereas the reverse pattern was present  
633 in –F fish. Thus the response in –F individuals was opposite to that of +F fish, suggesting that  
634 the same activational pathways were used differently in the same situation by fish from the  
635 two rearing treatments. *bdnf* is implicated in several important functions, including the stress  
636 response. Rats subjected to stress show increased hypothalamic *bdnf* mRNA levels (Smith *et al.*  
637 *al.* 1995) and conversely, strong cerebral *bdnf* inhibition decreases HPA activity in mice

638 (Naert *et al.* 2015). Our results would thus suggest that –F fish may be subject to a higher  
639 stress response when socially challenged. Moreover, +F fish might have been more stressed  
640 while being alone in the control situation. However, increased *bdnf* expression is also  
641 expected to enhance synaptic plasticity (Alder *et al.* 2003). Therefore we would have  
642 predicted +F fish, which are known to behave more flexibly in social encounters (Taborsky &  
643 Oliveira 2012, this study), to show higher expression when socially challenged. +F  
644 individuals had a higher *bdnf* expression only in the control situation, suggesting that their  
645 basic state, that is, before a social challenge, may be inherently more amenable to plasticity.  
646 However, the fact that we found lower expression after the challenge may mean that the role  
647 of *bdnf* in the stress response is more prominent in this system. Measuring *bdnf* levels after a  
648 non-social stress could help disentangle these two effects.

649

650 Gene expression was not always influenced by both the early rearing environment and the  
651 short term social challenge. In the hypothalamus, *egr-1* was only influenced by the rearing  
652 treatments. The hypothalamus is a key area regulating many different social behaviours,  
653 including aggression, parental care, sexual behavior and social cognition, and the activity of  
654 the HPA axis (O’Connell & Hofmann 2011; Wolkers *et al.* 2015). Because of the broad effect  
655 of *egr-1* on many different pathways the higher *egr-1* hypothalamus expression in +F fish  
656 compared to –F fish might indicate that +F fish are able to show a greater extent of plasticity  
657 than –F fish in a wide array of social behaviours and social contexts. Furthermore, contrary to  
658 our expectations, the early social environment and social challenge did not influence gene  
659 expression of *crf*, *bdnf* and *neuroserpin* in the telencephalon, or *gr1*, *crf*, *avt* and *avtr* in the  
660 hypothalamus. There are several possible reasons to explain the lack of treatment difference  
661 in expression of these genes. First the timing of sampling is crucial (see Liu *et al.* 2000). If we  
662 sample the brain too early, some later acting genes have possibly not been activated yet,  
663 whereas when sampling too late we might miss the window for early-activated genes.  
664 Furthermore, it is possible that differential gene expression in opposite directions in different  
665 sub-regions of the complex 'social decision making (SDM) network' might have masked an  
666 effect (Greenwood *et al.* 2008). The telencephalon contains six important nodes of the SDM  
667 network and the hypothalamus holds two nodes (O’Connell & Hofmann 2011). Since we  
668 sampled the whole telencephalon and hypothalamus, we might have lost some valuable  
669 information on gene expression at the level of the subregions (Wood *et al.* 2011) Finally,  
670 while the control fish in our experiment did not meet an opponent in the control situation, we  
671 nevertheless cannot exclude that they perceived the control environment as novel experience,

672 which influenced brain gene expression.

673

674 The pattern of expression of several genes can define the neurogenomic state associated with  
675 a particular behaviour (Robinson *et al.* 2008, Aubin-Horth *et al.* 2009). In addition to our  
676 analysis of effects on single genes, we investigated the neurogenomic state of fish reared in  
677 each type of environment. Fish reared in the more natural +F environment showed a larger  
678 shift in neurogenomic state when faced with a social challenge compared with fish that  
679 experienced a -F rearing environment. The principal component analysis suggests that the  
680 expression of candidate genes is strongly coordinated within each of the targeted brain areas.  
681 The larger overall change observed in fish reared in the natural, +F environment thus suggest  
682 that the social challenge we chose has significant consequences for the coordinated activation  
683 of the molecular networks of these genes. This result also raises the intriguing possibility that  
684 -F fish do exhibit a genomic response, but that it is delayed. Quantifying such a potential time  
685 shift in genomic response was beyond the scope of the study but could also potentially result  
686 in the altered behavioural response observed in these fish. In any cases, these concerted  
687 genomic modifications may be linked to the modulation of behaviour in response to the social  
688 challenge (reviewed in Robinson *et al.* 2008, Taborsky & Oliveira 2012).

689

690 The observation that a behavioural response to a social challenge is accompanied by changes  
691 in the average level of gene expression can reasonably lead to the prediction that behaviour  
692 and gene expression will covary at the individual level (Williams 2008). This is supported by  
693 our results on the link between gene expression and the expression of submissive displays, a  
694 social behaviour, which is of particular importance for *N. pulcher* to maintain the stability of  
695 its social system. The amount of submissive displays by intruders decreased with the  
696 expression of *crf* in the telencephalon, and *gr1* in the hypothalamus. Showing more  
697 submissive displays represents an adequate behavioural response when being in the intruder  
698 role, as most intruders were not able to take over the shelter. For *crf* the interaction between  
699 social rearing and amount of submission was significant; intruders of +F treatments showing  
700 more submission had lower *crf* expression, while in -F intruders this trend was absent. For  
701 *gr1*, intruders from both rearing treatments showed more submission with a lower expression  
702 of the gene. It is possible that the amount of submission an intruder shows influences the  
703 expression of these genes, or that the gene expression itself regulates the submissive  
704 behaviour. The lower *crf* expression in intruders showing more submission could be related to  
705 social defeat stress (SDS) as seen in rats (Panksepp *et al.* 2007), as submissive intruders are

706 the defeated contestants in our social challenge test. Rats facing SDS have lower hippocampal  
707 *crf* mRNA expression 6 hours after an encounter compared to non-defeated rats (Panksepp *et*  
708 *al.* 2007). *N. pulcher* intruders with higher *gr1* expression might be more bold and risk-prone,  
709 as it has been observed in sticklebacks (Aubin-Horth *et al.* 2012), which might explain their  
710 lower submission tendencies.

711

712 In conclusion, our results highlight the importance to incorporate the environmental  
713 conditions experienced during development when we aim to understand the genomic basis of  
714 social behaviour. Furthermore it shows how integrative biology approaches can help  
715 understanding the evolution of complex social behaviour, by jointly investigating molecular,  
716 neuroendocrine and behavioural responses to environmental conditions in ecologically  
717 relevant contexts (Aubin-Horth & Renn 2009; Taborsky & Taborsky 2015). Future studies  
718 should aim to obtain a more complete picture of the genes and the gene networks involved in  
719 the development and regulation of social behaviour.

720

#### 721 Acknowledgements

722 We would like to thank François Olivier Hébert, Chloé Berger, Lucie Grecias and Caroline  
723 Côté for comments on an earlier version of this manuscript. We are grateful to Sergio Cortez  
724 Ghio for providing critical advice on sample preparation, Leif Engqvist for statistical advice  
725 and Evi Zwygart for logistic support. This research was supported by the Swiss National  
726 Science Foundation (SNSF grant 31003A\_156881 to BT), the Natural Sciences and  
727 Engineering Research Council of Canada (NSERC) Discovery program (to NAH) as well  
728 as by Ressources Aquatiques Québec (RAQ) international fellowship program and Ella och  
729 Georg Ehrnrooths Stiftelse fund (to CN). CN, BT and NAH designed the study. CN  
730 performed the behavioural experiments. CN and NAH did the gene expression laboratory  
731 work. CN performed the statistical analysis. CN, BT and NAH drafted the manuscript. All  
732 authors have approved the content of the manuscript.

733

734

735

736

737 **References:**

- 738 Adkins-Regan E, Krakauer A (2000) Removal of adult males from the rearing environment increases  
739 preference for same-sex partners in the zebra finch. *Animal Behaviour*, **60**, 47–53.
- 740 Ago Y, Araki R, Tanaka T *et al.* (2013) Role of social encounter-induced activation of prefrontal  
741 serotonergic systems in the abnormal behaviors of isolation-reared mice.  
742 *Neuropsychopharmacology*, **38**, 1535–1547.
- 743 Alder J, Thakker-Varia S, Bangasser DA *et al.* (2003) Brain-derived neurotrophic factor-induced gene  
744 expression reveals novel actions of VGF in hippocampal synaptic plasticity. *Journal of*  
745 *Neuroscience Research*, **23**, 10800–10808.
- 746 Arnold C, Taborsky B (2010) Social experience in early ontogeny has lasting effects on social skills in  
747 cooperatively breeding cichlids. *Animal Behaviour*, **79**, 621–630.
- 748 Aubin-Horth N, Deschênes M, Cloutier S (2012) Natural variation in the molecular stress network  
749 correlates with a behavioural syndrome. *Hormones and Behavior*, **61**, 140–146.
- 750 Aubin-Horth N, Desjardins JK, Martei YM, Balshine S, Hofmann HA (2007) Masculinized dominant  
751 females in a cooperatively breeding species. *Molecular Ecology*, **16**, 1349–1358.
- 752 Aubin-Horth N, Letcher B, Hofmann H.A. (2009) Gene-expression signatures of Atlantic salmon’s  
753 plastic life cycle. *General and Comparative Endocrinology*, **163**, 278–284.
- 754 Aubin-Horth N, Renn SCP (2009) Genomic reaction norms: using integrative biology to understand  
755 molecular mechanisms of phenotypic plasticity. *Molecular Ecology*, **18**, 3763–80.
- 756 Ballen C, Shine R, Olsson M (2014) Effects of early social isolation on the behaviour and performance  
757 of juvenile lizards, *Chamaeleo calyptratus*. *Animal Behaviour*, **88**, 1–6.
- 758 Balshine-Earn S, Neat FC, Reid H, Taborsky M (1998) Paying to stay or paying to breed? Field  
759 evidence for direct benefits of helping behavior in a cooperatively breeding fish. *Behavioral*  
760 *Ecology*, **9**, 432–438.
- 761 Banerjee SB, Arterbery AS, Fergus DJ, Adkins-Regan E (2012) Deprivation of maternal care has  
762 long-lasting consequences for the hypothalamic-pituitary-adrenal axis of zebra finches.  
763 *Proceedings of the Royal Society B, Biological Sciences*, **279**, 759–66.
- 764 Bastian ML, Sponberg AC, Suomi SJ, Higley JD (2003) Long-term effects of infant rearing condition  
765 on the acquisition of dominance rank in juvenile and adult rhesus macaques (*Macaca mulatta*).  
766 *Developmental Psychobiology*, **42**, 44–51.
- 767 Bates D, Maechler M, Bolker B (2013) lme4: Linear mixed-effects models using S4 classes. R  
768 package version 0.999999-2.
- 769 Benjamini Y, Hochberg Y (1995) Controlling the false discovery rate: a practical and powerful  
770 approach to multiple testing. *Journal of the Royal Statistical Society B*, **57**, 289–300.
- 771 Benus R, Henkelmann C (1998) Litter composition influences the development of aggression and  
772 behavioural strategy in male *Mus domesticus*. *Behaviour*, **135**, 1229–1249.
- 773 Bergmüller R, Taborsky M (2005) Experimental manipulation of helping in a cooperative breeder:  
774 Helpers “pay to stay” by pre-emptive appeasement. *Animal Behaviour*, **69**, 19–28.
- 775 Branchi I, Alleva E (2006) Communal nesting, an early social enrichment, increases the adult anxiety-  
776 like response and shapes the role of social context in modulating the emotional behavior.  
777 *Behavioural Brain Research*, **172**, 299–306.
- 778 Branchi I, D’Andrea I, Fiore M *et al.* (2006) Early social enrichment shapes social behavior and nerve  
779 growth factor and brain-derived neurotrophic factor levels in the adult mouse brain. *Biological*  
780 *Psychiatry*, **60**, 690–696.
- 781 Branchi I, Francia N, Alleva E (2004) Epigenetic control of neurobehavioural plasticity: the role of  
782 neurotrophins. *Behavioural Pharmacology*, **15**, 353–362.
- 783 Brawand D, Wagner CE, Li YI *et al.* (2014) The genomic substrate for adaptive radiation in African

- 784 cichlid fish. *Nature*, **513**, 375-381.
- 785 Burmeister SS, Fernald RD (2005) Evolutionary conservation of the *egr-1* immediate-early gene  
786 response in a teleost. *The Journal of Comparative Neurology*, **481**, 220–32.
- 787 Burmeister SS, Jarvis ED, Fernald RD (2005) Rapid behavioral and genomic responses to social  
788 opportunity. *PLoS Biology*, **3**, e363.
- 789 Bury NR, Sturm A, Le Rouzic P *et al.* (2003) Evidence for two distinct functional glucocorticoid  
790 receptors in teleost fish. *Journal of Molecular Endocrinology*, **31**, 141–156.
- 791 Cao Y, Wu R, Tai F *et al.* (2014) Neonatal paternal deprivation impairs social recognition and alters  
792 levels of oxytocin and estrogen receptor  $\alpha$  mRNA expression in the MeA and NAcc, and serum  
793 oxytocin in mandarin voles. *Hormones and Behavior*, **65**, 57–65.
- 794 Champagne FA (2008) Epigenetic mechanisms and the transgenerational effects of maternal care.  
795 *Frontiers in Neuroendocrinology*, **29**, 386–397.
- 796 Champagne FA (2010) Early adversity and developmental outcomes: Interaction between genetics,  
797 epigenetics, and social experiences across the life span. *Perspectives on Psychological Science*,  
798 **5**, 564–574.
- 799 Cummings ME, Larkins-Ford J, Reilly CRL *et al.* (2008) Sexual and social stimuli elicit rapid and  
800 contrasting genomic responses. *Proceedings of the Royal Society B, Biological Sciences*, **275**,  
801 393–402.
- 802 Desjardins JK, Fernald RD (2010) What do fish make of mirror images? *Biology Letters*, **6**, 744–7.
- 803 Dettling AC, Feldon J, Pryce CR (2002) Early deprivation and behavioral and physiological responses  
804 to social separation/novelty in the marmoset. *Pharmacology Biochemistry and Behavior*, **73**,  
805 259–269.
- 806 Dey CJ, Reddon AR, O'Connor CM, Balshine S (2013) Network structure is related to social conflict  
807 in a cooperatively breeding fish. *Animal Behaviour*, **85**, 395–402.
- 808 Donovan KJO, Tourtellotte WG, Milbrandt J, Baraban JM (1999) The EGR family of transcription-  
809 regulatory factors : progress at the interface of molecular and systems neuroscience. *Trends in*  
810 *Neurosciences*, **22**, 167–173.
- 811 Feng X, Wang L, Yang S, Qin D, Wang J, Li C, Lv L, Ma Y, Hu X. (2011) Maternal separation -  
812 produces lasting changes in cortisol and behavior in rhesus monkeys. *PNAS*, **109**, 14312–14317.
- 813 Fischer S, Bessert-Nettelbeck M, Kotrschal A, Taborsky B (2015) Rearing-group size determines  
814 social competence and brain structure in a cooperatively breeding cichlid. *The American*  
815 *Naturalist*, **186**, 123–140.
- 816 Fischer S, Zöttl M, Groenewoud F, Taborsky B (2014) Group-size-dependent punishment of idle  
817 subordinates in a cooperative breeder where helpers pay to stay. *Proceedings of the Royal*  
818 *Society B, Biological Sciences*, **281**, 1–9.
- 819 Figueira M, Anadón R, Yáñez J (2004) Experimental study of the connections of the telencephalon in  
820 the rainbow trout (*Oncorhynchus mykiss*). II: Dorsal area and preoptic region. *The Journal of*  
821 *Comparative Neurology*, **480**, 204–233.
- 822 Francis D, Diorio J, Liu D, Meaney MJ (1999) Nongenomic transmission across generations of  
823 maternal behavior and stress response in the rat. *Science*, **286**, 1155–1158.
- 824 Godwin J, Thompson R (2012) Nonapeptides and social behavior in fishes. *Hormones and Behavior*,  
825 **61**, 230–238.
- 826 Goerlich VC, Nätt D, Elfving M, Macdonald B, Jensen P (2012) Transgenerational effects of early  
827 experience on behavioral, hormonal and gene expression responses to acute stress in the  
828 precocial chicken. *Hormones and Behavior*, **61**, 711–8.
- 829 Greenwood AK, Wark AR, Fernald RD, Hofmann HA (2008) Expression of arginine vasotocin in  
830 distinct preoptic regions is associated with dominant and subordinate behaviour in an African  
831 cichlid fish. *Proceedings of the Royal Society B, Biological Sciences*, **275**, 2393–402.



- 832 Groothuis TGG, Taborsky B (2015) Introducing biological realism into the study of developmental  
833 plasticity in behaviour. *Frontiers in Zoology*, **12**, S6.
- 834 Harlow HF, Zimmermann RR (1959) Affectional response in the infant monkey. *Science*, **130**, 421–  
835 432.
- 836 Heg D, Bachar Z, Brouwer L, Taborsky M (2004) Predation risk is an ecological constraint for helper  
837 dispersal in a cooperatively breeding cichlid. *Proceedings of the Royal Society B, Biological  
838 Sciences*, **271**, 2367–74.
- 839 Heg D, Taborsky M (2010) Helper response to experimentally manipulated predation risk in the  
840 cooperatively breeding cichlid *Neolamprologus pulcher*. *PLOS ONE*, **5**.
- 841 Hostetler CM, Ryabinin AE (2013) The CRF system and social behavior: A review. *Frontiers in  
842 Neuroscience*, **7**, 1-15.
- 843 Huffman LS, Hinz FI, Wojcik S, Aubin-Horth N, Hofmann HA (2014) Arginine vasotocin regulates  
844 social ascent in the African cichlid fish *Astatotilapia burtoni*. *General and Comparative  
845 Endocrinology*, **212**, 106-113 .
- 846 Huffman LS, Mitchell MM, O’Connell LA, Hofmann HA (2012a) Rising StARs: behavioral,  
847 hormonal, and molecular responses to social challenge and opportunity. *Hormones and  
848 Behavior*, **61**, 631–41.
- 849 Huffman LS, O’Connell LA, Kenkel CD *et al.* (2012b) Distribution of nonapeptide systems in the  
850 forebrain of an African cichlid fish, *Astatotilapia burtoni*. *Journal of Chemical Neuroanatomy*,  
851 **44**, 86–97.
- 852 Jacobson L, Sapolsky R (1991) The role of the hippocampus in feedback regulation of the  
853 hypothalamic-pituitary-adrenocortical axis. *Endocrine Reviews*, **12**, 118–134.
- 854 Joëls M, Pu Z, Wiegner O, Oitzl MS, Krugers HJ (2006) Learning under stress: How does it work?  
855 *Trends in cognitive sciences*, **10**, 152–158.
- 856 Kasumovic MM, Brooks RC (2011) It’s all who you know: The evolution of socially cued anticipatory  
857 plasticity as a mating strategy *The Quarterly Review of Biology*, **86**, 181–197.
- 858 Kline RJ, O’Connell LA, Hofmann HA, Holt GJ, Khan IA (2011) The distribution of an AVT V1a  
859 receptor in the brain of a sex changing fish, *Epinephelus adscensionis*. *Journal of Chemical  
860 Neuroanatomy*, **42**, 72–88.
- 861 Kloet E De, Vreugdenhil E, Oitzl M (1998) Brain corticosteroids receptor balance in health and  
862 disease. *Endocrine Reviews*, **19**, 269–301.
- 863 Ladd CO, Huot RL, Thiruvikraman K V, Nemeroff CB, Plotsky PM (2004) Long-term adaptations in  
864 glucocorticoid receptor and mineralocorticoid receptor mRNA and negative feedback on the  
865 hypothalamo-pituitary-adrenal axis following neonatal maternal separation. *Biological  
866 Psychiatry*, **55**, 367–75.
- 867 Lema SC (2010) Identification of multiple vasotocin receptor cDNAs in teleost fish: sequences,  
868 phylogenetic analysis, sites of expression, and regulation in the hypothalamus and gill in  
869 response to hyperosmotic challenge. *Molecular and Cellular Endocrinology*, **321**, 215–30.
- 870 Liu D, Diorio J, Day JC, Francis DD, Meaney MJ (2000) Maternal care, hippocampal synaptogenesis  
871 and cognitive development in rats. *Nature Neuroscience*, **3**, 799–806.
- 872 Liu D, Diorio J, Tannenbaum B *et al.* (1997) Maternal care, hippocampal glucocorticoid receptors,  
873 and hypothalamic-pituitary-adrenal responses to stress. *Science*, **277**, 1659–1662.
- 874 Madani R, Kozlov S, Akhmedov A *et al.* (2003) Impaired explorative behavior and neophobia in  
875 genetically modified mice lacking or overexpressing the extracellular serine protease inhibitor  
876 neuroserpin. *Molecular and Cellular Neuroscience*, **23**, 473–494.
- 877 Maruska KP, Zhang A, Neboori A, Fernald RD (2013) Social opportunity causes rapid transcriptional  
878 changes in the social behaviour network of the brain in an African cichlid fish. *Journal of  
879 Neuroendocrinology*, **25**, 145–57.

- 880 McAllister AK, Katz LC, Lo DC (1999) Neurotrophins and synaptic plasticity. *Annual Review of*  
881 *Neuroscience*, **22**, 295–318.
- 882 McGowan PO, Sasaki A, D'Alessio AC *et al.* (2009) Epigenetic regulation of the glucocorticoid  
883 receptor in human brain associates with childhood abuse. *Nature Neuroscience*, **12**, 342–8.
- 884 Meaney MJ, Szyf M (2005) Maternal care as a model for experience-dependent chromatin plasticity?  
885 *Trends in Neurosciences*, **28**, 456–463.
- 886 Mifsud KR, Saunderson EA, Spiers H, Carter SD, Trollope AF, Mill J, Reul JMHM (2016), Rapid  
887 down-regulation of glucocorticoid receptor gene expression in the dentate gyrus after acute stress  
888 in vivo : Role of DNA methylation and microRNA activity. *Neuroendocrinology*. DOI:  
889 10.1159/000445875
- 890 Miranda E, Lomas D a (2006) Neuroserpin: a serpin to think about. *Cellular and Molecular Life*  
891 *Sciences*, **63**, 709–22.
- 892 Mireault GC, Bond LA (1992) Parental death in childhood: perceived vulnerability, and adult  
893 depression and anxiety. *American Journal of Orthopsychiatry*, **62**, 517–524.
- 894 Morgan JI, Curran T (1995) Review : The immediate-early gene response and neuronal death and  
895 regeneration. *The Neuroscientist*, **1**, 68–75.
- 896 Naert G, Zussy C, Ba CT Van *et al.* (2015) Involvement of endogenous brain-derived neurotrophic  
897 factor in hypothalamic-pituitary-adrenal axis activity neuroendocrinology. *Journal of*  
898 *Neuroendocrinology*, **27**, 850–860.
- 899 Navailles S, Zimnisky R, Schmauss C (2010) Expression of glucocorticoid receptor and early growth  
900 response gene 1 during postnatal development of two inbred strains of mice exposed to early life  
901 stress. *Developmental Neuroscience*, **32**, 139–48.
- 902 Nicieza AG, Metcalfe NB (1999) Costs of rapid growth: The risk of aggression is higher for fast-  
903 growing salmon. *Functional Ecology*, **13**, 793–800.
- 904 O'Connell LA, Hofmann HA (2011) The vertebrate mesolimbic reward system and social behavior  
905 network: a comparative synthesis. *The Journal of Comparative Neurology*, **519**, 3599–639.
- 906 O'Connor CM, Marsh-Rollo SE, Aubin-Horth N, Balshine S (2016) Species-specific patterns of  
907 nonapeptide brain gene expression relative to pair-bonding behaviour in grouping and non-  
908 grouping cichlids. *Hormones and behavior*, **80**, 30-38
- 909 O'Connor CM, Marsh-Rollo SE, Ghio SC, Balshine S, Aubin-Horth N (2015) Is there convergence in  
910 the molecular pathways underlying the repeated evolution of sociality in African cichlids?  
911 *Hormones and Behavior*, **75**, 160–168.
- 912 Oldfield RG, Harris RM, Hendrickson DA, Hofmann HA (2013) Arginine vasotocin and androgen  
913 pathways are associated with mating system variation in North American cichlid fishes.  
914 *Hormones and Behavior*, **64**, 44–52.
- 915 Panksepp J, Burgdorf J, Beinfeld MC, Kroes RA, Moskal JR (2007) Brain regional neuropeptide  
916 changes resulting from social defeat. *Behavioral Neuroscience*, **121**, 1364–1371.
- 917 Pfaffl MW (2001) A new mathematical model for relative quantification in real-time RT-PCR. *Nucleic*  
918 *Acids research*, **29**, 2002-2007.
- 919 Phoenix CH, Goy RW, Gerall AA, Young WC (1959) Organizing action of prenatally administered  
920 testosterone propionate on the tissues mediating mating behavior in the female guinea pig.  
921 *Endocrinology*, **65**, 369–382.
- 922 Plotsky PM, Meaney MJ (1993) Early, postnatal experience alters hypothalamic corticotropin-  
923 releasing factor (CRF) mRNA, median eminence CRF content and stress-induced release in adult  
924 rats. *Molecular Brain Research*, **18**, 195–200.
- 925 Reddon AR, Connor CMO, Marsh-Rollo SE *et al.* (2015) Brain nonapeptide levels are related to social  
926 status and affiliative behaviour in a cooperatively breeding cichlid fish. *Royal Society Open*  
927 *Science*. 2: 140072.

- 928 Rice D, Barone Jr S (2000) Critical periods of vulnerability for the developing nervous system:  
929 evidence from humans and animal models. *Environmental Health Perspectives*, **108**, 511-533.
- 930 Robinson GE, Fernald RD, Clayton DF (2008) Genes and social behavior. *Science*, **322**, 896-900.
- 931 Roceri M, Cirulli F, Pessina C *et al.* (2004) Postnatal repeated maternal deprivation produces age-  
932 dependent changes of brain-derived neurotrophic factor expression in selected rat brain regions.  
933 *Biological Psychiatry*, **55**, 708-14.
- 934 Romeo RD (2003) Puberty: A period of both organizational and activational effects of steroid  
935 hormones on neurobehavioural development. *Journal of Neuroendocrinology*, **15**, 1185-1192.
- 936 Roozendaal B, McGaugh J (2011) Memory modulation. *Behavioral Neuroscience*, **125**, 797-824
- 937 Ruploh T, Bischof H-J, von Engelhardt N (2013) Adolescent social environment shapes sexual and  
938 aggressive behaviour of adult male zebra finches (*Taeniopygia guttata*). *Behavioral Ecology and*  
939 *Sociobiology*, **67**, 175-184.
- 940 Ruploh T, Bischof H-J, von Engelhardt N (2014) Social experience during adolescence influences  
941 how male zebra finches (*Taeniopygia guttata*) group with conspecifics. *Behavioral Ecology and*  
942 *Sociobiology*, **68**, 537-549.
- 943 Schmidt KL, Macdougall-Shackleton EA, Soma KK, Macdougall-Shackleton SA (2014)  
944 Developmental programming of the HPA and HPG axes by early-life stress in male and female  
945 song sparrows. *General and Comparative Endocrinology*, **196**, 72-80.
- 946 Singmann H, Bolker B, Westfall J (2015) Analysis of factorial experiments, package 'afex'.  
947 <https://github.com/singmann/afex>
- 948 Smith M., Makino S, Kim SY, Kvetnansky R (1995) Stress increases brain-derived neurotrophic  
949 factor messenger ribonucleic acid in the hypothalamus and pituitary. *Endocrinology*, **136**, 3743-  
950 3750.
- 951 Soares MC, Bshary R, Fusani L *et al.* (2010) Hormonal mechanisms of cooperative behaviour.  
952 *Philosophical transactions of the Royal Society of London. Series B, Biological sciences*, **365**,  
953 2737-50.
- 954 Stiver KA, Dierkes P, Taborsky M, Balshine S (2004) Dispersal patterns and status change in a co-  
955 operatively breeding cichlid *Neolamprologus pulcher*: Evidence from microsatellite analyses and  
956 behavioural observations. *Journal of Fish Biology*, **65**, 91-105.
- 957 Stiver KA, Dierkes P, Taborsky M, Gibbs HL, Balshine S (2005) Relatedness and helping in fish:  
958 examining the theoretical predictions. *Proceedings of the Royal Society B, Biological Sciences*,  
959 **272**, 1593-1599.
- 960 Taborsky M (1984) Broodcare helpers in the cichlid fish *Lamprologus brichardi*: their costs and  
961 benefits. *Animal Behaviour*, **32**, 1236-1252.
- 962 Taborsky M (1985) Breeder-Helper Conflict in a cichlid fish with broodcare helpers: an experimental  
963 analysis. *Behaviour*, **95**, 45-75.
- 964 Taborsky B (2016a) Opening the black box of developmental experiments: behavioural mechanisms  
965 underlying long-term effects of early social experience. *Ethology*, **122**, in press.
- 966 Taborsky M (2016b) Ecology and evolution of cooperative breeding in cichlid fish. In: *Cooperative*  
967 *Breeding in Vertebrates: Studies of Ecology, Evolution, and Behavior*. (eds Koenig W,  
968 Dickinson J). Cambridge University Press.
- 969 Taborsky B, Arnold C, Junker J, Tschopp A (2012) The early social environment affects social  
970 competence in a cooperative breeder. *Animal Behaviour*, **83**, 1067-1074.
- 971 Taborsky B, Oliveira RF (2012) Social competence: an evolutionary approach. *Trends in Ecology and*  
972 *Evolution*, **27**, 679-688.
- 973 Taborsky M, Taborsky B (2015) Evolution of genetic and physiological mechanisms of cooperative  
974 behaviour. *Current Opinion in Behavioral Sciences*, **6**, 132-138.
- 975 Taborsky B, Tschirren L, Meunier C, Aubin-horth N, B PRS (2013) Stable reprogramming of brain

976 transcription profiles by the early social environment in a cooperatively breeding fish.  
977 *Proceedings of the Royal Society B, Biological Sciences*, **208**: 1-7.

978 Weaver ICG, D'Alessio AC, Brown SE *et al.* (2007) The transcription factor nerve growth factor-  
979 inducible protein a mediates epigenetic programming: altering epigenetic marks by immediate-  
980 early genes. *The Journal of Neuroscience*, **27**, 1756–68.

981 Weaver ICG, Hellstrom IC, Brown SE *et al.* (2014) The methylated-DNA binding protein  
982 transcriptional activation of the glucocorticoid receptor. *Philosophical transactions of the Royal  
983 Society of London B, Biological Sciences*, **369**, 1–11.

984 Wigger A, Neumann ID (1999) Periodic maternal deprivation induces gender-dependent alterations in  
985 behavioral and neuroendocrine responses to emotional stress in adult rats. *Physiology and  
986 Behavior*, **66**, 293–302.

987 Williams TD, (2008) Individual variation in endocrine systems: moving beyond the ‘tyranny of the  
988 Golden Mean’. *Philosophical Transactions of the Royal Society B: Biological Sciences* **363**,  
989 1687–1698.

990 Wong M, Balshine S (2011) The evolution of cooperative breeding in the African cichlid fish,  
991 *Neolamprologus pulcher*. *Biological Reviews of the Cambridge Philosophical Society*, **86**, 511–  
992 530.

993 Wood LS, Desjardins JK, Fernald RD (2011) Effects of stress and motivation on performing a spatial  
994 task. *Neurobiology of Learning and Memory*, **95**, 277–85.

995 Young LJ, Nilsen R, Waymire KG, Macgregor GR, Insel TR (1999) Increased affiliative response to  
996 vasopressin in mice expressing the V1a receptor from a monogamous vole. *Nature*, **400**, 766–  
997 768.

998 Zar JH (1999) *Biostatistical Analysis. 4th ed., Prentice Hall, Upper Saddle River, New Jersey.*

999 Zhang L, Levine S, Dent G *et al.* (2002) Maternal deprivation increases cell death in the infant rat  
1000 brain. *Developmental Brain Research*, **133**, 1–11.

1001 Zimmer C, Boogert NJ, Spencer KA (2013) Developmental programming: Cumulative effects of  
1002 increased pre-hatching corticosterone levels and post-hatching unpredictable food availability on  
1003 physiology and behaviour in adulthood. *Hormones and Behavior*, **64**, 494–500.

1004 Zimmer C, Spencer KA, (2014) Modifications of glucocorticoid receptors mRNA expression in the  
1005 hypothalamic-pituitary-adrenal axis in response to early-life stress in female Japanese quail.  
1006 *Journal of Neuroendocrinology*, **26**, 853-860.

1007 Zöttl M, Frommen J, Taborsky M (2013a) Group size adjustment to ecological demand in cooperative  
1008 breeder. *Proceedings of the Royal Society B, Biological Sciences*, **280**, 1-9.

1009 Zöttl M, Heg D, Chervet N, Taborsky M (2013b) Kinship reduces alloparental care in cooperative  
1010 cichlids where helpers pay-to-stay. *Nature Communications*, **4**, 1-9.

1011

1012 **Data accessibility**

1013 - Behavioural observation files and gene expression values have been deposited to Dryad,  
1014 doi:10.5061/dryad.9c2j1

1015

1016 - Information on primers is provided in the Methods section

1017

1018 **Supplementary material**

1019 - RNA extraction protocol

1020 - Results of the full linear mixed models including non-significant interactions testing the  
1021 effect of rearing environment and social challenge on the expression of candidate genes.

1022

1023

1024

1 **Figure legends**

2

3 Fig. 1: Intruder submission (log transformed) in relation to received owner aggression (log  
4 transformed). Behaviours are expressed as rates per minute. Circles and black lines represent  
5 the –F treatment; triangles and red lines represent the +F treatment.

6

7 Fig. 2: Gene expression for control and intruder fish for 5 genes in the telencephalon. (A)  
8 immediately early gene *egr-1*, (B) brain-derived neurotrophic factor (*bdnf*), (C) glucocorticoid  
9 receptor (*gr1*), (D) corticotropin releasing factor (*crf*) and (E) *neuroserpin*. Gene expression  
10 of *egr-1* is log-transformed as it was done in the linear mixed model. Black circles represent –  
11 F treatment, red triangles represent +F treatment. Figures display means± SE.

12 Fig. 3: Gene expression for control and intruder fish for 6 genes in the hypothalamus. (A)  
13 immediately early gene *egr-1*, (B) brain-derived neurotrophic factor (*bdnf*), (C) glucocorticoid  
14 receptor (*gr1*), (D) corticotropin releasing factor (*crf*) (E) arginine-vasotocin (*avt*) and (F)  
15 arginine-vasotocin receptor V1a2 (*avtr*). Gene expression of *egr-1*, *gr1*, *crf* and *avt* is log-  
16 transformed as it was done in the linear mixed models. Black circles represent –F treatment,  
17 red triangles represent +F treatment. Figures display means±SE.

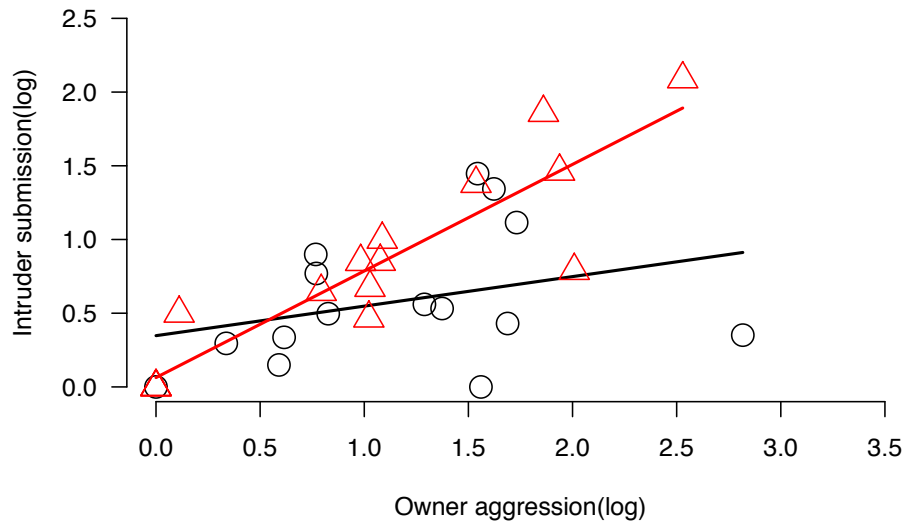
18 Fig. 4. Relationship between individual PC1 and PC2 scores representing the neurogenomic  
19 states of individuals from each combination of early social environment and social challenge.  
20 Triangles represent +F rearing treatment fish and circles –F individuals. Open symbols  
21 represent control individual in the social challenge and filled symbols represent intruders.

22

23 Fig. 5: Association of intruder submission and gene expression of (A) *crf* in the telencephalon  
24 and (B) *gr1* in the hypothalamus. Gene expression of *gr1* is log-transformed as it was done in  
25 the linear mixed model. Sample sizes *crf*: N=22, *gr1*: N=21. Circles and black lines represent  
26 –F treatment; triangles and red lines represent +F treatment.

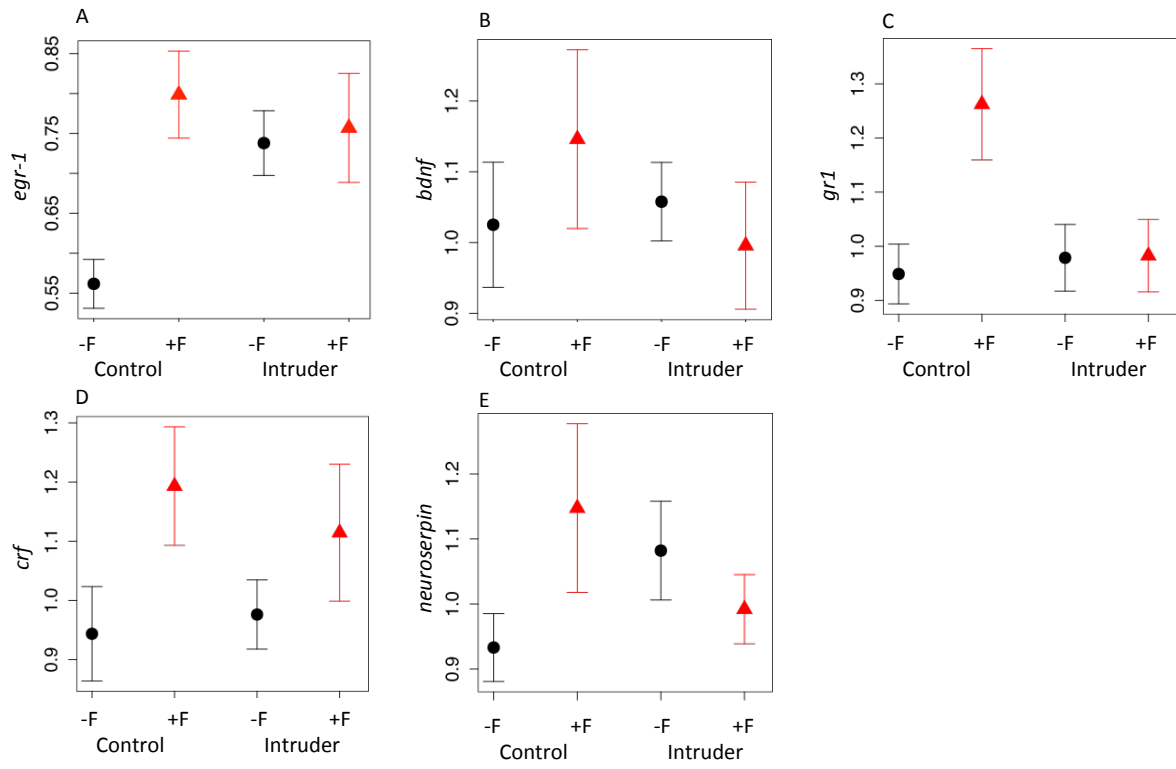
27

28  
29  
30  
31



32  
33

34



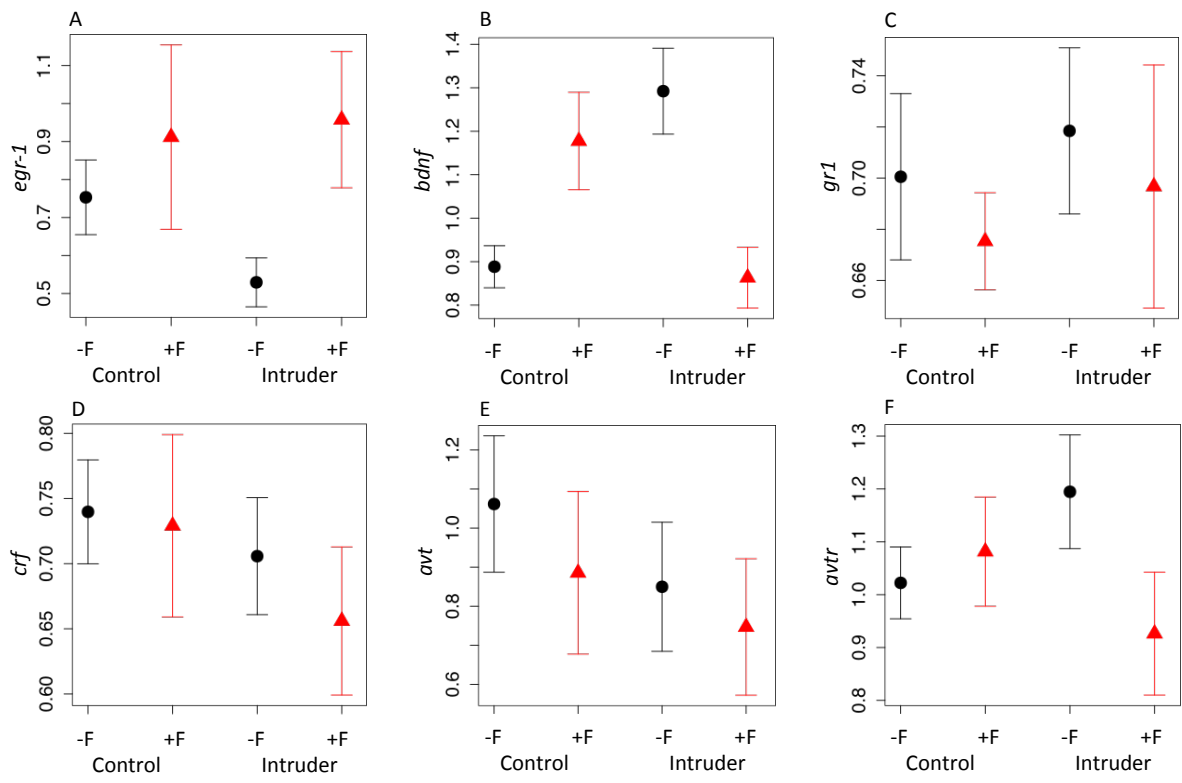
35

36

37



38

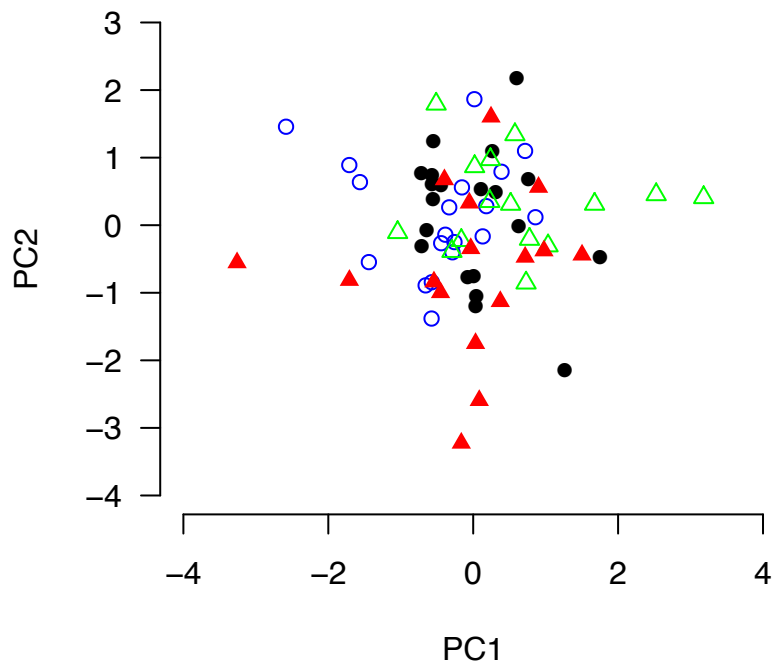


39

40

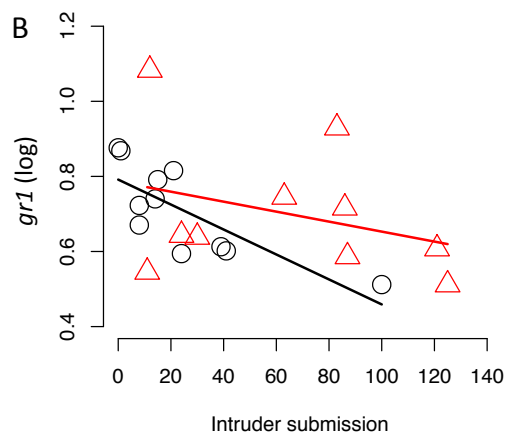
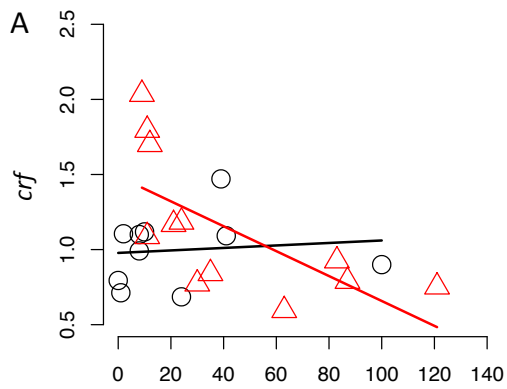
41

42  
43  
44



45  
46  
47  
48

49  
50  
51  
52



53  
54  
55  
56  
57  
58  
59  
60  
61  
62  
63

Table 1: Results of the linear mixed models testing the effect of rearing environment (–F or +F) and social challenge (intruder or control situation) on the expression of candidate genes in *N. pulcher*. For sample sizes see section “Data analysis”. P-values <0.05 are highlighted in bold.

Brain area	Factors	Estimate ± SE	F-value	p-value
Telencephalon	<b><i>egr-1 (log)</i></b>			
	Rearing	- 0.064± 0.025	6.473	<b>0.023</b>
	Challenge	- 0.034±0.025	1.787	0.189
	Rearing x Challenge	-0.055±0.025	4.684	<b>0.036</b>
	<b><i>bdnf</i></b>			
	Rearing	-0.016±0.049	0.101	0.756
	Challenge	0.030±0.044	0.457	0.503
	<b><i>gr1</i></b>			
	Rearing	-0.080±0.037	4.627	<b>0.048</b>
	Challenge	0.062± 0.036	2.959	0.093
	Rearing x Challenge	-0.077±0.036	4.577	<b>0.038</b>
	<b><i>crf</i></b>			
	Rearing	-0.101±0.056	3.260	0.090
	Challenge	0.022±0.040	0.315	0.578
	<b><i>neuroserpin</i></b>			
	Rearing	-0.028±0.043	0.425	0.524
Challenge	-0.001±0.041	0.001	0.980	
Hypothalamus	<b><i>egr-1 (log)</i></b>			
	Rearing	-0.157±0.070	4.880	<b>0.044</b>
	Challenge	0.060±0.068	0.756	0.392
	<b><i>bdnf</i></b>			
	Rearing	0.036±0.045	0.643	0.435
	Challenge	-0.023±0.042	0.281	0.600
	Rearing x Challenge	-0.181±0.042	18.195	<b>0.0001</b>
	<b><i>gr1 (log)</i></b>			
	Rearing	0.011±0.019	0.360	0.557
	Challenge	-0.010±0.017	0.352	0.556
	<b><i>crf (log)</i></b>			
	Rearing	0.015±0.029	0.181	0.676
	Challenge	0.025±0.025	1.077	0.3055
	<b><i>avt (log)</i></b>			
	Rearing	0.076±0.123	0.379	0.547
	Challenge	0.084±0.076	1.199	0.280
<b><i>avtr</i></b>				
Rearing	-0.057± 0.052	1.196	0.291	
Challenge	-0.022± 0.050	0.191	0.665	

Table 2: Factor loadings for the 7 different candidate genes, with a total of 11 measures of gene expression (5 in telencephalon, 6 in hypothalamus) on the first two principal components (PC). The respective higher loadings among the two PCs are highlighted in bold. N=70.

<b>Brain area</b>	<b>Gene</b>	<b>PC1</b>	<b>PC2</b>
<b>Telencephalon</b>	<i>egr-1</i>	<b>0.66</b>	-0.04
	<i>bdnf</i>	<b>0.80</b>	-0.13
	<i>gr1</i>	<b>0.79</b>	0.05
	<i>crf</i>	<b>0.74</b>	-0.14
	<i>neuroserpin</i>	<b>0.83</b>	-0.11
Variance explained		27%	
<b>Hypothalamus</b>	<i>egr-1</i>	0.11	<b>-0.22</b>
	<i>bdnf</i>	0.15	<b>0.78</b>
	<i>gr1</i>	0.08	<b>-0.21</b>
	<i>crf</i>	0.03	<b>0.43</b>
	<i>avt</i>	-0.14	<b>0.56</b>
	<i>avtr</i>	-0.03	<b>0.86</b>
Variance explained			18%

Table 3: Results of the linear mixed models testing the effect of rearing environment (-F or +F) and social challenge (intruder or control situation) using the PC scores of the first two principal components. N= 70. P-values <0.05 are highlighted in bold.

<b>Factors</b>	<b>Estimate ± SE</b>	<b>F-value</b>	<b>p-value</b>
<b>PC1</b>			
Rearing	-0.234±0.128	3.337	0.09
Challenge	0.059±0.109	0.290	0.59
Rearing x challenge	-0.313±0.109	8.262	<b>0.006</b>
<b>PC2</b>			
Rearing	0.156±0.117	1.766	0.2
Challenge	0.250±0.114	4.794	<b>0.03</b>
Rearing x challenge	-0.231±0.114	4.126	<b>0.05</b>

Table 4: Effect of rearing environment, submissive behaviour and size of intruders on brain gene expression in fish facing a social challenge. *crf*: N=22, *gr1*: N=21. P-values <0.05 are highlighted in bold.

Brain area	Factors	Estimate ± SE	F-value	p-value
<b><i>Telencephalon</i></b>				
<b><i>crf</i></b>				
	Rearing	-0.318±0.101	9.002	<b>0.009</b>
	Submission	-0.003±0.002	2.832	0.128
	Intruder size	-1.241±0.968	1.381	0.263
	Rearing x submission	0.006± 0.002	8.995	<b>0.014</b>
<b><i>Hypothalamus</i></b>				
<b><i>gr1</i></b>				
	Rearing	-0.060±0.042	1.917	0.191
	Submission	-0.003±0.001	8.121	<b>0.012</b>
	Intruder size	0.171±0.494	0.097	0.759