# Antipredator defences of young are independently determined by genetic inheritance, maternal effects and own early experience in mouthbrooding cichlids

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## Abstract

1. Predation is a prime force of natural selection. Vulnerability to predation is typically highest early in life, hence effective antipredator defences should work already shortly after birth. Such early defences may be innate, transmitted through non-genetic parental effects, or acquired by own early experience.

2. To understand potential joint effects of these sources of antipredator defences on phenotypic expression, they should be manipulated within the same experiment. We investigated innate, parental and individual experience effects within a single experiment. Females of the African cichlid *Simochromis pleurospilus* were exposed to the offspring predator *Ctenochromis horei* or a benign species until spawning. Eggs and larvae were hand-reared, and larvae were then exposed to odour cues signalling the presence or absence of predators in a split brood design.

3. Shortly after independence of maternal care, *S. pleurospilus* undergo a habitat shift from a deeper, adult habitat to a shallow juvenile habitat, a phase where young are thought to be particularly exposed to predation risk. Thus maternal effects induced by offspring predators present in the adult habitat should take effect mainly shortly after independence, whereas own experience and innate anti-predator responses should shape behaviour and life history of *S. pleurospilus* during the later juvenile period.

4. We found that the manipulated environmental components independently affected different offspring traits. (i) Offspring of predator-exposed mothers grew faster during the first month of life and were thus larger at termination of maternal care, when the young migrate from the adult to the juvenile habitat. (ii) The offspring's own experience shortly after hatching exerted lasting effects on predator avoidance behaviour. (iii) Finally, our

results suggest that *S. pleurospilus* possess a genetically inherited ability to distinguish dangerous from benign species.

5. In *S. pleurospilus* maternal effects were limited to a short but critical time window, when young undergo a niche shift. Instead, own environmental sampling of predation risk combined with an innate predisposition to correctly identify predators appears to prepare the young best for the environment, in which they grow up as juveniles.

**Keywords:** predator recognition, developmental plasticity, maternal effects, innate predator defences, growth, cichlids

## Introduction

Predation is one of the most important selective forces in nature. Evolving efficient anti-predator strategies is thus a pivotal component of Darwinian fitness (e.g. Lima & Dill 1990). In many species, the vulnerability to predation is particularly high at very early life stages, when young can become an easy treat for predators because of their small size, limited body strength or constrained escape potential (e.g. Gosselin & Qian 1997; Sogard 1997). Therefore it is not surprising that young often possess efficient antipredator defences already at or shortly after birth, as shown in a number of vertebrates (e.g. Laurila, Kujasalo, & Ranta 1997; Veen *et al.* 2000; Goth 2001; Vilhunen & Hirvonen 2003; Fendt 2006; Hawkins, Magurran, & Armstrong 2007), and invertebrates (e.g. Abjörnsson, Hansson, & Brönmark 2004; Storm & Lima 2010).

Causal mechanisms that have been proposed to be responsible for the expression of early antipredator responses include genetic predisposition for predator recognition (Magurran 1990; Abjörnsson *et al.* 2004; Scheurer *et al.* 2007), non-genetic maternal effects (Dzialowski et al. 2003, Sheriff, Krebs, & Boonstra 2010; Coslovsky & Richner 2011; Giesing *et al.* 2011; McGhee *et al.* 2012; Segers & Taborsky 2012), learning before (Mathis *et al.* 2008; Colombelli-Negrel *et al.* 2012) or shortly after birth (Brown, Ferrari, & Chivers 2011), and the use of cues from the diet of predators (rev. in Ferrari *et al.* 2007). Studies comparing the relative influence of non-genetic maternal effects and own early predator experience on the phenotype of young animals showed that maternal effects can act in isolation (Dzialowski et al. 2003), or interactively with own experience (Tollrian 1995, Kaplan & Phillips 2006). To obtain a sound understanding of the action of the sources of anti-predator responses, these sources should be studied jointly . Here we present a factorial experiment allowing us to

identify separate or joint effects of three main sources of phenotypic variation, namely of innate, non-genetically inherited and individually acquired early-life anti-predator defences, and to test for short and long-term consequences of these effects on behaviour and life history traits. If parents can reliably predict the conditions of their offspring's environment, they may adjust offspring phenotypes via anticipatory parental effects potentially enabling their offspring to better cope with these conditions (e.g. Uller 2008). In contrast if parents cannot sample the offspring environment, or if conditions are strongly fluctuating, anticipatory parental effects may be of little use to offspring, in which case offspring may do better to solely rely on innate information and own experience.

We chose the African mouthbrooding cichlid fish *Simochromis pleurospilus* as model species (Fig. 1), as it is one of the few species in which the parents' possibilities to predict the offspring environment in the wild (Kotrschal *et al.* 2012) and to adjust offspring traits to the environment (Taborsky 2006a,b) has been explicitly studied. These earlier studies addressed the resource availability for young. In this study, we focused on environmental cues elicited by offspring predators. Adult and juvenile *S. pleurospilus* occupy different, slightly overlapping niches along the depth gradient of Lake Tanganyika with juveniles inhabiting shallower depth than adults. When young become independent of maternal care at an age of four weeks, they start to move from the adult to the juvenile depth range. While we cannot entirely exclude that some brooding mothers actively deliver their offspring to the juvenile habitat, field observations of the distribution of brooding females and the sizes of young held in the mouths suggest that juveniles are released in the adult habitat and then migrate to the shallow zone on their own (A. Kotrschal pers. obs; B. Taborsky, pers. obs). The main offspring predators of *S. pleurospilus* are other cichlids fish, which either feed on

small invertebrates and on cichlid fry opportunistically, or which represent specialized pisicivores, such as *Ctenochromis horei*, the predator used in this study. The distribution of *C. horei* is predominantly bound to the existence of underwater vegetation, which occurs patchily in the lake at various depths (Oshi 1993). Therefore, large, dangerous *C. horei* can occur in the juvenile or in the adult niches of *S. pleurospilus,* or both (Ochi 1993, Sefc et al. 2009). Thus we should expect that maternal effects induced by offspring predators take effect mainly shortly after the release of young, i.e. while the young still travel to their juvenile habitats, whereas both innate and acquired anti-predator responses should shape the behaviour and life history of S. pleurospilus during the entire juvenile period, or at least until they reach a size above the gape-size of their main predators.

We performed a factorial experiment with three levels of manipulations. (1) To test for predator-induced non-genetic maternal effects, we exposed mothers either to an environment with perceived offspring predation threat or to a control environment during the egg formation phase until spawning. (2) To test for innate anti-predator responses, we hand-reared eggs individually under highly controlled conditions, preventing any environmental maternal effects or own experience to take effect after egg-laying. (3) To test for acquired anti-predator responses, we either exposed predator-naïve offspring to a chemical predator experience shortly after birth, or to control cues. This setup allowed us to test jointly for innate, maternally-mediated or acquired effects on offspring growth and long-term anti-predator behaviour in a single experiment.

## Materials and Methods

**Study species.** *Simochromis pleurospilus* (subfamily Tropheini) is a mouthbrooding cichlid endemic to Lake Tanganyika, East Africa. It lives along the rocky shores of the lake down to a depth of 12 m (Taborsky 2006a, Kotrschal et al. 2012), where it feeds on epilithic algae. *S. pleurospilus* reproduce year-round. Males defend small breeding territories against conspecific and heterospecific food competitors (Kotrschal & Taborsky 2010). Females visit these breeding sites for mating and leave directly after spawning with the clutch held in their buccal cavity (Taborsky 2006b; Kotrschal & Taborsky 2010). Females brood the clutches in their buccal cavities continuously for two weeks during which the young use up most of their yolk reserves. During the following two weeks the mothers release the offspring temporarily allowing the young and herself to feed, but she takes them back in case of danger or disturbance (Segers, Gerber, & Taborsky 2011). Four weeks after spawning the young are independent.

**Animal husbandry.** The study was conducted at the University of Bern, Switzerland, under license 21/08 of the Veterinary Office of the Kanton Bern. The experimental animals were second and third generation offspring from fish originating from Nkumbula Island, Lake Tanganyika, Zambia. Parental fish were kept in four 400-l tanks until spawning. Offspring were reared individually in 20-L tanks. All fish were kept under standard housing conditions described in (Segers & Taborsky 2011). All adult cichlids used in this study were fed twice per day on 6 days per week with commercial tropical fish flake food (4 days) or a mixture of frozen zooplankton (2 days).

**Female treatment.** To induce potential non-genetic maternal effects via perceived offsping predation risk on offspring phenotypes, we exposed adult *S. pleurospilus* females to an

offspring predator ('predator treatment') or a non-predatory fish ('control treatment') during the phase when they form the egg for their next clutch. As stimulus offspring predator, we chose adult *Ctenochromis horei*, which are a dangerous predator of small juvenile *S. pleurospilus*, but pose no threat to adult females as they are of similar body size. This approach contrasts previous work, where the perceived predation risk for adult females rather than for offspring was manipulated (e.g. McCormick 1998; McCormick 2006; Coslovsky & Richner 2011; Giesing *et al.* 2011; McGhee *et al.* 2012). We did so, because where we were explicitly interested in whether mothers adjust offspring phenotype to the expected offspring environment ('anticipatory maternal effects'; sensu Uller 2008) In the control treatment we exposed adult females to individuals of a non-predatory algae eater (*Ophtalmotilapia ventralis*) of a similar body size to the adult *S. pleurospilus* females. Both the predatory and the control stimulus species are endemic to Lake Tanganyika and occur sympatrically with *S. pleurospilus*.

The experimental clutches were produced in four 400-L tanks, two assigned to the predator treatment and two assigned to the control treatment, inhabited by groups of six to nine *S. pleurospilus* females, one male and one heterospecific cichlid (a *C. horei* or an *O. ventralis*). Adult *S. pleurospilus* were captured from their home tanks and were randomly assigned to the four breeding tanks. At introduction in the experimental tanks and also directly after spawning, we measured the females' total lengths (TL; tip of mouth to end of caudal fin) on a measuring board with 1-mm grid, estimating their length to the nearest of 0.5 mm, and we weighed them to the nearest of 0.01 g on an electronic balance.

153 The tanks were checked daily for females with clutches in their buccal cavity. As soon as a
154 female had spawned, the eggs were collected by slightly pressing her cheeks. Then the
155 female was placed in a 20-L tank for recovery for 50 days. Afterwards the 50 days, she was
156 transferred to a 400-L tank of the opposite treatment to produce a second clutch. To
157 maintain stable densities in the breeding tanks the removed female was replaced by a new
158 female. In total we obtained eight clutches from the control treatment and ten clutches
159 from the predator treatment. Only three females produced one clutch each in both
160 treatments, and only in one case both clutches of a female hatched; all other females
161 contributed just one clutch to this experiment.
162
163 We exchanged the males several times during the experiment (3 males produced offspring in
164 the predator treatment and 3 males did so in the control treatment). Our treatment aimed
165 at inducing environmental *maternal* effects, and maternal effects are indeed more likely to
166 occur than paternal effects in this species, which has a very high maternal reproductive
167 investment (females produce very large, energy rich eggs and exhibiting a long female-only
168 care period), but only a small paternal investment. We are aware, however, that our
169 experimental design does not allow the exclusion of the possibility of environmental
170 paternal effects.
171
172 **Rearing of experimental broods.** Each single egg of the 18 clutches was individually reared
173 in a 250-ml Erlenmeyer flask filled with clean tap water that was mounted in a self-
174 constructed egg tumbler described in (Segers & Taborsky 2011). Each flask was individually
175 oxygenated by an air flow. Eggs take five days to hatch (Segers & Taborsky 2011). At
176 experimental day 8 (see experimental timeline, Table S1; 'experimental days' correspond to

177 days after hatching) the larvae were moved individually to net cages (16.5x12x13.5 cm)
178 placed in individual 20-L tanks. From day 18, when yolk sacs were absorbed, juveniles were
179 released in the 20-L tanks and fed a near *ad libitum* ration (12% of their body mass) of fine-
180 grained 'Tetramin Baby'® flake food 6 days per week with adjustment to increasing body
181 mass every 2 weeks (see Taborsky 2006b).
182
183 **Length, mass and growth.** Eggs were placed individually on a moistened cotton pad and
184 weighed to the nearest of 0.1 mg on an electronic balance. We obtained egg weights from
185 17 of the 18 experimental clutches. Offspring lengths and weights were taken every 4 weeks
186 from day 28 until day 168. Body condition was calculated using Fulton's index *F* as *F*= mass
187 [g]/TL[cm]$^3$ x 100. Length growth of individual larvae was modelled as larval length
188 controlled for individual egg mass. Larval mass growth was directly calculated as the
189 differences between individual larval mass at day 28 and individual egg mass. For juveniles,
190 we calculated the specific length growth rate for each 4-weekly measuring interval as (Ln
191 $TL_2$-Ln$TL_1$)/28d*100, where $TL_1$ and $TL_2$ are the two successive measurements. Specific
192 growth rates give the percentage of daily growth.
193
194 **Opercular beat rates and offspring treatment.** Predator naïve larvae of both maternal
195 treatments were exposed to a chemical predator cue (*C. horei*) or to a control cue (tap
196 water) in a split-brood design. We used olfactory cues to elicit responses in larvae, as in fish
197 olfaction is an essential source of information about predators (Ferrari, Wisenden, & Chivers
198 2010)). The heterospecific cues were produced by confining an adult *C. horei,* an *O. ventralis*
199 or several snails for 1h in 700 ml water taken from its holding tank. Afterwards the 700-ml
200 sample was filled in 2-ml Eppendorf tubes and kept at -20°C until use.

We tested for the strength of responses to the chemical cues by recoding the opercular beat rates (OBR) of larvae. Under standardized conditions ventilation frequency is known as a sensitive measure of response to disturbance (Brown, Gardner, & Braithwaite 2005). Repeated exposures were done at days 8, 13 and 18. Two fish per brood each were haphazardly assigned to the predator cue and to the control cue, except in five broods where only three individuals survived until day 8 (individuals in total: predator cue=36; control cue=33). To measure opercular beat rates (OBR), a larva was placed in a small glass tube (1.2 cm diameter, 4 cm length), filled with water from its holding container. The tube was placed upright under a binocular microscope connected to a video camera. The tube holding the fish and containers holding the water cues were kept at a constant temperature of 28°C by a thermostat-controlled water bath. After 5 min of acclimatization, OBR was video-recorded for 40 sec ('baseline 1'; see "Supporting Information", Appendix S1, Fig. S1). Then the fish holding water was removed with a pipette such that the larva was still covered with water, and the water was quickly replaced by the respective treatment cue water (control cue, i.e. tap water, or heterospecific cue), followed by another 40 sec video recording of OBR ('treatment'). Finally, the treatment water was exchanged against tap water ('baseline 2') followed by a third 40-sec video recording. 'Baseline 2' was mainly done to detect a potential fear response to the control cue (tap water) in the 'treatment' recording.

To test whether OBR changes after exposure to *C. horei* odour represent a specific anti-predator response or a general neophobic fear response towards other fish (Hirvonen *et al.* 2000), we exposed additional larvae obtained from those broods with sufficient surviving

young (N=5 broods) to the odours of the herbivorous cichlid *O. ventralis*. To test further whether larvae are able to discriminate between fish and non-fish odour, we exposed additional larvae of these 5 large broods to the odour of an aquatic snail of the family Thiraidae, a gastropod family occurring in Lake Tanganyika. *C. horei* and *O. ventralis* used to produce the odour cues were fed on identical standard diets (see above), thereby controlling for potential clues larvae may obtain about the danger exerted by a stimulus species only based on the species' diet (rev. in Ferrari *et al.* 2007).

For data analysis we compared the absolute values of beat rates between treatments, and we compared the differences of OBR between treatment and baseline 1 ('difference 1') and between treatment and baseline 2 ('difference 2'), respectively.

**Long-term effects on behaviour.** We tested for long-term effects of our treatments on behaviour both in generally threat-related contexts (novel object, unspecific startle stimulus) and in a predation context (presentation of predator). We conducted (i) a novel object test of general explorative or neophobic tendencies in a non-predatory context; (ii) a startle response test recording recovery from a fear response induced by non-predator stimulus; and (iii) a visual and olfactory exposure to the offspring predator *C. horei*.

The novel object test was performed at day 94 in the home tank of a focal fish. We removed the filter temporarily, and placed a shelter (flowerpot half) in a distance of 15 cm to both front corners of the tank. After letting a test fish acclimatize to this set-up for 15 minutes and verifying that it stayed in the shelter, a novel object, randomly chosen from either a blue-red rubber eraser or a blue or a red clothes peg, was gently placed in one of the

249 corners. We recorded the latency until first emergence from the shelter and the closest
250 distance the fish approached the novel object. Observation time was 10 min.
251
252 The startle response test was done twice, at days 33 and 84, to capture possible
253 developmental changes of risk-related behaviour as has been previously observed in this
254 species (Segers & Taborsky 2011). In this test we measured the time until fish resume
255 feeding after being startled by a short but strong threat stimulus. The home tank of a focal
256 fish was divided by a partition into two equally sized compartments; the test was performed
257 in the frontal compartment. A shelter (a short PVC tube) was placed at the right screen of
258 the compartment. Near the centre of the frontal screen, standard flake food diluted with
259 water was supplied with a pipette directly to the sandy bottom creating a food patch of
260 approximately 0.5 cm diameter. Immediately after the fish started feeding, a glass marble of
261 2 cm diameter was dropped next to the patch, and the response of the focal fish was video-
262 recorded. From the videos, we recorded the behavioural responses (fleeing, freezing or no
263 response; none of the fish entered the shelter), and we analysed the time until fish resumed
264 feeding after being startled.
265
266 The presentation of *C. horei* was conducted at day 140. We presented visual and chemical
267 cues of an adult male *C. horei* (11.5 cm TL) to *S. pleurospilus* juveniles. For each trial a focal
268 juvenile was transferred to a 20-L tank placed directly to the left or right of another 20-L
269 tank containing the *C. horei*. A shelter (flowerpot half) was provided at the furthest possible
270 distance to the predator's tank. The testing order of siblings tested the same day and the
271 testing position (left or right of predator) was balanced with regard to maternal and
272 offspring treatments. Before the test, the tanks were visually separated by an opaque

divider. After 5 min of acclimatization of the focal fish, 15 ml of *C. horei* holding tank water were added to the focal's tank, the opaque divider was removed and the behaviours of focal fish and predator were observed for 10 min. Afterwards the opaque divider was put in place again and the test fish was transferred back to its home tank.

Every 30 s during the 10-min recording (n = 20 observations per trial and fish) we noted the position of focal fish and predator, and whether they were active (i.e. moving around). To record the positions we divided the volume of each 20-L tank in 18 virtual, three-dimensional sections of 13.3×8.3×8.3 cm by applying marks at the tank screens. We simultaneously determined the two sections where the predator and the focal fish were located and calculated the distances between the mid-points of these sections by applying Pythagoras' law. For statistical analysis we used the mean distances of the 20 observations, and the percentages of observations the focal was active. The activity of the predator was included as covariate.

Furthermore we recorded any actions of the focal fish towards the predator on an all-occurrence basis, as these actions were rare. We ranked them from most defensive to most offensive: fleeing (rank 1), freezing (rank 2), inspection of predator (rank 3) and aggression (rank 4). For each fish we calculated the weighted mean of ranks by multiplying the frequency of an action by its rank, summing these products for the four actions and dividing by the total number of actions in 10 min. Additionally we counted the total occurrence of all above-mentioned actions without distinguishing the type of the response. This gives only a coarse measure of responsiveness, but it allows the inclusion of fish which showed zero responses.

297

298 **Statistical analysis.** When the data structure fulfilled the conditions for parametric testing,
299 we analyzed our data by linear mixed-effects models (LME) with identity link functions and
300 by AN(C)OVAs; otherwise we used non-parametric tests. Some variables were log-
301 transformed to allow for parametric analyses (transformed variables are indicated in the
302 respective result tables). All main fixed factors and covariates included in the models are
303 explicitly listed in the main text and results tables. In the initial models we also fitted all
304 interactions between fixed factors. To simplify our models we used stepwise backward
305 elimination of non-significant interaction terms of the fixed factors (Bolker *et al.* 2009), but
306 the main fixed factors were always kept in the models even if non-significant. We
307 determined the variance components of all potentially relevant random factors for each of
308 these models (percentages of variance are given in the Appendix S1, Tables S2, S3 and S4),
309 namely breeding tank, male identity, female identity, clutch identity and for the repeated
310 measures in the OBR analysis, also individual identity. None of these random factors
311 accounted for a significant amount of variance in the random term, except for clutch identity
312 in some of the models on offspring size and growth (see Table S3). In order to avoid
313 overparametrization of our models, for the final models presented in Tables 1, 2 and 3 we
314 kept only clutch identity in the random term and, for the repeated OBR analyses, we also
315 kept individual identity in the random term to account for the repeated measures. All
316 analyses were done using SPSS 17, SPSS Inc., Chicago, USA.

317

318

319 ## Results

320 **Predator responses of predator-naïve larvae.** Larvae receiving the offspring-predator cue
321 had strongly reduced OBRs compared to larvae receiving the control cue (Fig. 2a, Table 1a).

OBRs before the presentation (baseline 1) did not differ between larvae assigned to predator and control treatment (Figure 2a, Table 1a), whereas OBRs after the presentation (baseline 2) were still slightly lower after the predator cue than after the control cue (Table 1a, Factor "O"). The reduction of OBR in response to the predator cue was particularly strong on the first experimental day, whereupon the effect decreased gradually (Fig. 2b, Table 1a). In addition, OBRs during both baselines and the during the olfactory treatment decreased over the experimental days 8, 13 and 18 (Table 1a, Factor "Day"). The female treatment did not influence OBR significantly (Table 1a).

In the five broods tested with cues of three different species and the control, the OBR responses differed significantly between the four presented cues both when analyzing the differences to the first ('difference 1'; Fig. 2c) and to the second baseline ('difference 2'). Again the responses were strongest in naïve fish (day 8), and declined afterwards (significant effects of 'day'; Table 1b). Pairwise analyses of the initial responses of naïve fish (i.e. 'difference 1' at day 8; Table 1c) revealed that OBRs were significantly more reduced in response to the cues of the offspring predator and, unexpectedly, also towards the snail odour compared to tap water or the herbivorous cichlid. The responses to tap water and the herbivore did not differ. Interestingly, naïve larvae reduced OBR even more strongly when exposed to the snail cue than to the predator cue. This difference vanished, however, when taking all three exposure days into account, whereas all other differences remained significant in an analysis including all days (results not shown).

**Body size, clutch traits and growth.** Offspring of mothers that had been exposed to an offspring predator during clutch production were larger (Fig. 3a) and heavier 28 days after

346 hatching than offspring from females exposed to a herbivorous control fish (Table 2),
347 whereas their condition factor did not differ (Table 2). The treatment effect on offspring size
348 cannot be explained by variation in female total length, as female size and mass after
349 spawning did not differ significantly (there was even a weak tendency of females being
350 shorter in the predator treatment: total length: $F_{1,15}$=3.23, $p$=0.090, body mass: $F_{1,15}$=1.89,
351 $p$=0.19; ANOVAs). The treatment effect can also not be explained by differences in clutch
352 size ($F_{1,14}$=0.24, $p$=0.63; female TL included as covariate: $F_{1,14}$=5.24, $p$=0.038 as larger females
353 lay more eggs; ANCOVA) or egg mass between treatments (Female treatment: $F_{1,14}$=0.073,
354 $p$=0.79; clutch size included as covariate: $F_{1,14}$=56.74, $p$= 0.021 as there is an egg
355 size/number trade-off; ANCOVA). Instead, young produced by predator-exposed mothers
356 grew faster during their first 4 weeks of life (Table 2; raw mass-based growth rates are
357 shown in Fig. 3b). This effect was reversed during the following 4 weeks (wk 5-8), when
358 offspring of mothers exposed to the control treatment had higher growth rates (Table 2, Fig.
359 3c). After wk 8, until an age of 24 weeks, female treatment no longer influenced growth. At
360 the day of final measurement, the TL of fish did not differ anymore (Table 2). Larval growth,
361 juvenile growth and size at final measurement were not influenced by the offspring
362 treatment (Table 2).
363
364 **Long-term effects on behaviour.** Neither female nor offspring treatment influenced (i) the
365 latency until fish first emerged from their shelters after insertion of a novel object in their
366 tank or (ii) the closest distance to which juveniles approached a novel object (Table 3). Also
367 in the startle response test female and offspring treatment did not influence (i) the
368 distributions of the three occurring types of behavioural responses (fleeing, freezing or no

response) towards the startle stimulus or (ii) the time until feeding was resumed after being startled (Table 3).

Long-term effects on behaviour were detected, however, during the presentation of the predator *C. horei*. Focal fish that had received the chemical predator cue during the offspring treatment kept a greater distance from the predator than young that had experienced the control cue, irrespective of female treatment (Table 3, Fig. 4). The activity, the type of behavioural responses and the frequency of responses were not affected by offspring or female treatment (Table 3).

## Discussion

Our results suggest that *S. pleurospilus* young possess an innate ability to recognize predators and to distinguish these from cichlids that pose no risk to them, based on chemical information obtained from odour cues. This ability was not modulated by the perceived threat of offspring predation experienced by mothers. Instead environment-induced maternal effects influenced offspring growth; offspring of predator-exposed females grew faster during their first month of life. Early experience, but not maternal effects, exerted a long-term effect the distance kept towards predators, but not on specific anti-predator behaviours.

Predator naïve *S. pleurospilus* larvae responded strongly to the odour of an offspring predator, but neither to a control cue nor to the cue of a benign herbivorous cichlid. This shows that *S. pleurospilus* have a sophisticated ability to distinguish different odour cues already at birth. The maternal exposure to an offspring predator or to a benign species

393 during egg production did not affect the predator recognition abilities of larvae as judged
394 from the intensity of their ventilation response. Moreover, due to hand-rearing none of our
395 tested fish had the possibility to experience heterospecific odour cues prior to the first
396 behavioural test, and they also could not obtain clues about the potential danger of the
397 stimulus species by the latters' diet as all fish were fed the same type of food. It is
398 furthermore highly unlikely that genetic maternal or paternal effects induced predator
399 recognition, because the maternal and paternal identity did not explain a significant amount
400 of variance in the models testing for responses to odour cues (the variance explained by the
401 identity of mother or father never exceeded 2% in the models testing for OBR responses;
402 Table S2). Together these results suggest that *S. pleurospilus* possess a genetically inherited
403 predisposition to distinguish predatory from non-predatory species. Previous reports of
404 innate predator recognition (e.g. invertebrates: Abjörnsson *et al.* 2004; fish: Magurran 1990;
405 Hawkins *et al.* 2007; Scheurer *et al.* 2007; amphibians: Laurila *et al.* 1997; birds: Veen *et al.*
406 2000; Bize, Diaz, & Lindstroem 2012) could not exclude (i) that predator recognition was
407 induced by environmentally mediated maternal effects because they tested offspring of
408 wild-born, unmanipulated mothers, or (ii) that predators were recognized by odour cues of
409 their piscivorous or carnivorous diet (e.g. Vilhunen *et al.* 2003, Fendt 2006; see Ferrari *et al.*
410 2007 for discussion of diet effects). Thus, to the best of our knowledge here we report the
411 first unconfounded evidence for a genetically inherited ability in animals to distinguish a
412 dangerous from a benign species.
413
414 Unexpectedly, the odour of an aquatic snail elicited the strongest response in larval *S.*
415 *pleurospilus*. There are three alternative explanations for this result. (i) The larvae might
416 have recognized the snail odour and responded strongly, because snails may pose a real

417 threat to larvae; in our laboratory, F. Segers (pers. comm.) found a snail that ate a yolk-sac
418 larva while staying within the mouth of a brooding *S. pleurospilus* female. (ii) Alternatively,
419 snail odour might represent a novel, unrecognized stimulus to the larvae eliciting a strong
420 neophobic response. Strong neophobic responses can be adaptive, because they can help to
421 survive first encounters with unknown potential dangers before an individual had the
422 opportunity to collect information about a novel stimulus (Hirvonen *et al.* 2000). (iii) Finally,
423 snails might emit odours that are chemically similar to odours of natural predators of *S.*
424 *pleurospilus* young.
425
426 Typically, fish increase their ventilation rate in face of danger (e.g. Brown *et al.* 2005;
427 Hawkins *et al.* 2007). In contrast, *S. pleurospilus* young reduced their OBR, a response which
428 has only recently been reported for the first time in a fish species (Kempster, Hart, & Collin
429 2013). Reducing the ventilation rate might be part of a freezing response in the face of
430 danger which is well known from young mammals, where it is accompanied by bradycardia
431 (Smith & Woodruff 1980; Espmark & Langvatn 1985). Freezing rather than preparing for
432 flight may be the most appropriate anti-predator response by *S. pleurospilus* larvae, which
433 still have huge yolk sacs particularly during the first two weeks of life preventing an effective
434 escape. If by accident a mouthbrooding female drops a larva, freezing and reducing
435 ventilation is likely to induce visual and chemical crypsis. Furthermore, mouthbrooding
436 mothers might also benefit from a reduced larval OBR and thus reduced oxygen expenditure
437 of offspring in the mouth when the female is threatened by a predator.
438
439 At first exposure, predator naïve *S. pleurospilus* larvae showed the highest baseline OBRs
440 and the strongest responses towards predator odour, and baselines and responses then

441 gradually declined with age. This decline is most likely an effect of increasing body size and a
442 corresponding decrease of metabolic rate (Jones 1971) rather than a habituation response,
443 as in the latter case baseline OBRs should not change with age as well.
444
445 Offspring from predator-exposed mothers grew faster during the first four weeks after
446 hatching, and consequently were larger and heavier at day 28 compared to offspring from
447 control mothers. In small fish, mortality is strongly negatively size-dependent, as the most
448 important predators are other fish species, which are gape-size limited (Sogard 1997). Faster
449 growth will allow the young to outgrow the time window of highest juvenile mortality more
450 quickly (Sogard 1997, Segers & Taborsky 2011). Moreover, even slightly larger body sizes
451 allowed for higher burst speeds of juvenile mouthbrooders (Schürch & Taborsky 2005;
452 Segers & Taborsky 2011), which should enable them to better escape predation (e.g. Husak
453 2006). Interestingly, offspring of predator-exposed females reached their larger size at about
454 the age when they would become independent of maternal care in nature and start
455 migrating from the deeper adult habitats to the shallow juvenile habitat (Kotrschal et al.
456 2012). Thus our results suggest that the maternally mediated growth boost detected in our
457 experiment prepares those offspring born into a predator-dense adult surrounding to better
458 cope with predator attacks during their highly dangerous migration to the juvenile habitat.
459 Moreover, it may reflect a general tendency of maternal effects to vanish quickly with time
460 (Lindholm, Hunt, & Brooks 2006). Soon after this initial growth boost, juveniles of predator-
461 exposed mothers grew more slowly than offspring of control mothers, so that already at an
462 age of three month offspring sizes did not differ anymore between the two maternal
463 treatments. This decrease of growth rates after a growth boost might help to buffer

potential negative effects resulting from possible costs incurred during the phase of fast growth (Metcalfe & Monaghan 2001).

In contrast to previous results in fish (Giesing *et al.* 2011; Segers *et al.* 2012), egg size was not affected by the maternal treatment. Currently, we can only speculate about the possible mechanisms underlying the maternal effect on growth. In *S. pleurospilus*, larval growth depends on the post-hatching expression of the gene coding for growth hormone receptor (GHR) (Segers, Berishvili, & Taborsky 2012), and it is possible that females can influence the larval expression of this gene.

In the long term, juvenile traits were only influenced by own experience. Juveniles that had experienced *C. horei* odour five months ago kept a greater distance from this predator than did control fish, indicating that early predator experience induces an adjustment of risk-taking behaviour. In contrast, none of the specific behaviours we recorded at this age were affected by early predator exposure. This overall rather weak long-term effect may reflect the fact that at this advanced age and body size, *C. horei* does not pose a severe life-threatening risk to *S. pleurospilus* anymore. The absence of treatment effects on the behaviours measured during the novel object and the startle response tests suggests that the early odour exposure induced behavioural changes specific to a predation context, rather than altering the general fearfulness of fish. This adds to previous findings showing that behavioural syndromes present in a predation context do not necessarily match behavioural syndromes in non-predatory contexts (Coleman & Wilson 1998, Dingemanse et al. 2007, Ardiaenssens & Johnson 2009).

In summary, maternal effects were effective for a short, critical time window after birth, and they might have a specific, but important effect on offspring survival by increasing body size during offspring migration. Own predator odour experience modulated the behavioural response of fish in the long-run, in particular the 'wariness' of juveniles towards large predatory individuals. Own environmental sampling of predation risk combined with innate predisposition to correctly identify predatory species is likely to reveal the best possible prediction of environmental risk for juveniles in *S. pleurospilus*, a species in which adults and juveniles occupy different ecological niches. In order to understand the general principles of separate and joint action of parental and individual environmental effects we would like to encourage further factorial experiments in species where the environmental predictability is known both from the perspective of adults and of offspring.

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Supporting Information

Additional supporting information may be found in the online version of this article.

Appendix S1: Additional methodological and statistical information

638 **Table 1.** Treatment effects on opercular beat rates

639

| Dependent variable | Fixed factors | d.f. | *F* | *p* |
|---|---|---|---|---|
| *(a) Predator vs. control treatment* | | | | |
| Baseline 1 | F | 16.08 | 0.07 | 0.79 |
| | O | 189.13 | 1.88 | 0.17 |
| | Day | 186.22 | 47.65 | **< 0.001** |
| Baseline 2 | F | 31.60 | 0.04 | 0.84 |
| | O | 50.63 | 4.99 | **0.030** |
| | Day | 136 | 47.25 | **< 0.001** |
| Treatment | F | 15.85 | 0.15 | 0.71 |
| | O | 50.55 | 71.48 | **< 0.001** |
| | Day | 134 | 26.51 | **< 0.001** |
| | O x Day | 134 | 7.62 | **0.001** |
| Difference 1 | F | 15.01 | 0.08 | 0.78 |
| | O | 50.94 | 155.05 | **< 0.001** |
| | Day | 134 | 4.37 | **0.014** |
| | O x Day | 134 | 4.73 | **0.010** |
| Difference 2 | F | 14.12 | 0.67 | 0.43 |
| | O | 50.72 | 45.76 | **< 0.001** |
| | Day | 134 | 5.53 | **0.005** |
| | O x Day | 134 | 9.59 | **< 0.001** |
| *(b) Treatment with four odour cues* | | | | |
| Difference 1 | O | 36 | 24.25 | **< 0.001** |
| | Day | 72 | 14.64 | **< 0.001** |
| | O x Day | 72 | 2.31 | **0.043** |
| Difference 2 | O | 32.66 | 7.67 | **0.001** |
| | Day | 72 | 23.39 | **< 0.001** |
| | O x Day | 72 | 3.18 | **0.008** |

*(c) M*ultiple comparisons (day 8)

| | Herbivore | Predator | Snail |
|---|---|---|---|
| Tap water | 0.16 | **< 0.001** | **< 0.001** |
| Herbivore | | **0.023** | **0.004** |
| Predator | | | **0.027** |

640 'F' refers to female treatment, 'O' refers to offspring treatment; 'Day' refers to the
641 experimental day 8, 13 or 18 after hatching; 'Difference 1' and Difference 2' refer to the
642 differences between 'Treatment' and 'Baseline 1' and between 'Treatment' and 'Baseline 2',
643 repectively; significant *p*-values are marked in bold; in *(c)* all bold p-values are significant
644 after accounting for false discovery rate using the Benjamini-Hochberg method (Verhoeven
645 et al. 2005)

646

**Table 2.** Treatment effects on offspring body size and growth; 'F' refers to female treatment, 'O' refers to offspring treatment, TL is total length.

| Dependent variable | Fixed factors | d.f. | *F* | *p* |
|---|---|---|---|---|
| TL at day 28 | F | 16.31 | 6.07 | **0.025** |
| | O | 43.73 | 1.02 | 0.19 |
| Weight at day 28 | F | 15.88 | 8.47 | **0.010** |
| | O | 45.68 | 0.042 | 0.84 |
| Condition factor *K* | F | 16.13 | 0.44 | 0.52 |
| | O | 47.63 | 0.25 | 0.62 |
| TL at day 28[a] | F | 15.34 | 6.97 | **0.018** |
| | O | 42.62 | 1.91 | 0.17 |
| | egg mass | 54.93 | 0.054 | 0.82 |
| Juvenile specific growth rate | F | 262 | 14.73 | **<0.001** |
| | O | 262 | 0.33 | 0.57 |
| | age | 262 | 136.98 | **<0.001** |
| | F x age | 262 | 9.70 | **0.002** |
| Final TL | F | 13.55 | 0.68 | 0.42 |
| | O | 35.35 | 0.17 | 0.68 |

[a]this model fits larval length at day 28 corrected for the individual egg size of this larvae; as larger larvae hatch from larger eggs (Segers & Taborsky 2011), this analysis actually models larval growth

658 **Table 3.** Results of Mann-Whitney U-tests ('Distance to object'), Chi-square tests ('Response
659 types') and LME models (all other results) of the experimental tests for long-term effects of
660 female and offspring treatments on behaviour; 'F': female treatment; 'O': offspring
661 treatment; 'Predator': Predator activity.

662
663

| Dependent variable | Fixed factors | d.f. | *F* | *U* | $\chi^2$ | *p* |
|---|---|---|---|---|---|---|
| *Novel object test* | | | | | | |
| Latency to 1st emergence[a] | F | 13.51 | 0.55 | | | 0.47 |
| | O | 45.44 | 2.08 | | | 0.16 |
| Distance to object | F | | | 389 | | 0.81 |
| | O | | | 401 | | 0.97 |
| *Startle response tests* | | | | | | |
| Time to feed (day 33)[a] | F | 15.25 | 0.35 | | | 0.56 |
| | O | 46.07 | 0.05 | | | 0.82 |
| Time to feed (day 84) | F | 22.93 | 0.48 | | | 0.50 |
| | O | 42.99 | 0.62 | | | 0.43 |
| Response type (day 33, n=65) | F | | | | 0.90 | 0.64 |
| | O | | | | 0.34 | 0.84 |
| Response type (day 84, n=50) | F | | | | 3.44 | 0.18 |
| | O | | | | 0.62 | 0.73 |
| *Response to 'familiar' predator C. horei* | | | | | | |
| Mean distance (cm) | F | 10.26 | 0.43 | | | 0.52 |
| | O | 32.33 | 13.72 | | | **0.001** |
| | Predator | 40.84 | 3.47 | | | 0.070 |
| Activity | F | 9.37 | 1.05 | | | 0.33 |
| | O | 32.66 | 0.33 | | | 0.57 |
| | Predator | 43.25 | 0.57 | | | 0.45 |
| Type of behaviour | F | 6.97 | 0.43 | | | 0.53 |
| | O | 12.31 | 0.046 | | | 0.83 |
| | Predator | 14.94 | 0.88 | | | 0.36 |
| Frequency of any behaviour[a] | F | 9.48 | 0.45 | | | 0.52 |
| | O | 30.69 | 0.011 | | | 0.92 |
| | Predator | 40.84 | 0.13 | | | 0.72 |

664 [a]Variables $Log_{10}$-transformed
665

**Figure legends**

Fig. 1: Female Lake Tanganyika cichlid *Simochromis pleurospilus* recalling her young to the mouth (Photo by Christoph Grüter).

Fig. 2: Opercular beat rates (OBR; beats per 40 sec) of larvae during the exposure to different odour cues, shown for the combined predator and control female treatments, as the female treatments did not affect OBR (see Table 1); means (±SE). (a) OBR of predator-naïve larvae (i.e. at first exposure, day 8) during exposure to baseline and treatment cues; (b) difference in OBRs between treatment cue and baseline 1 ('difference 1') on all three exposure days; (c) OBRs towards the control cue (tap water), offspring predator (*C. horei*), herbivore (*O. ventralis*) and a Thiraid snail, averaged over all three observation days

Fig. 3: Size and growth rates of juveniles; means (±SE). (a) Differences in total length at first size measurement (day 28); (b) Individual larval mass increase (weight at day 28 minus egg weight); (c) specific growth rate of juveniles; data of the two offspring treatments are combined, as offspring treatment did not affect growth. Open bars in (a) and (b): offspring control treatment, closed bars: offspring predator treatment; grey bars in (c): female control treatment; black bars: female predator treatment

Fig. 4: Mean distance (cm) (±SE) between focal fish and an offspring predator *C. horei* during predator presentation trials. Open bars in (a) and (b): offspring control treatment, closed bars: offspring predator treatment

**Fig. 1**

**Fig. 2**

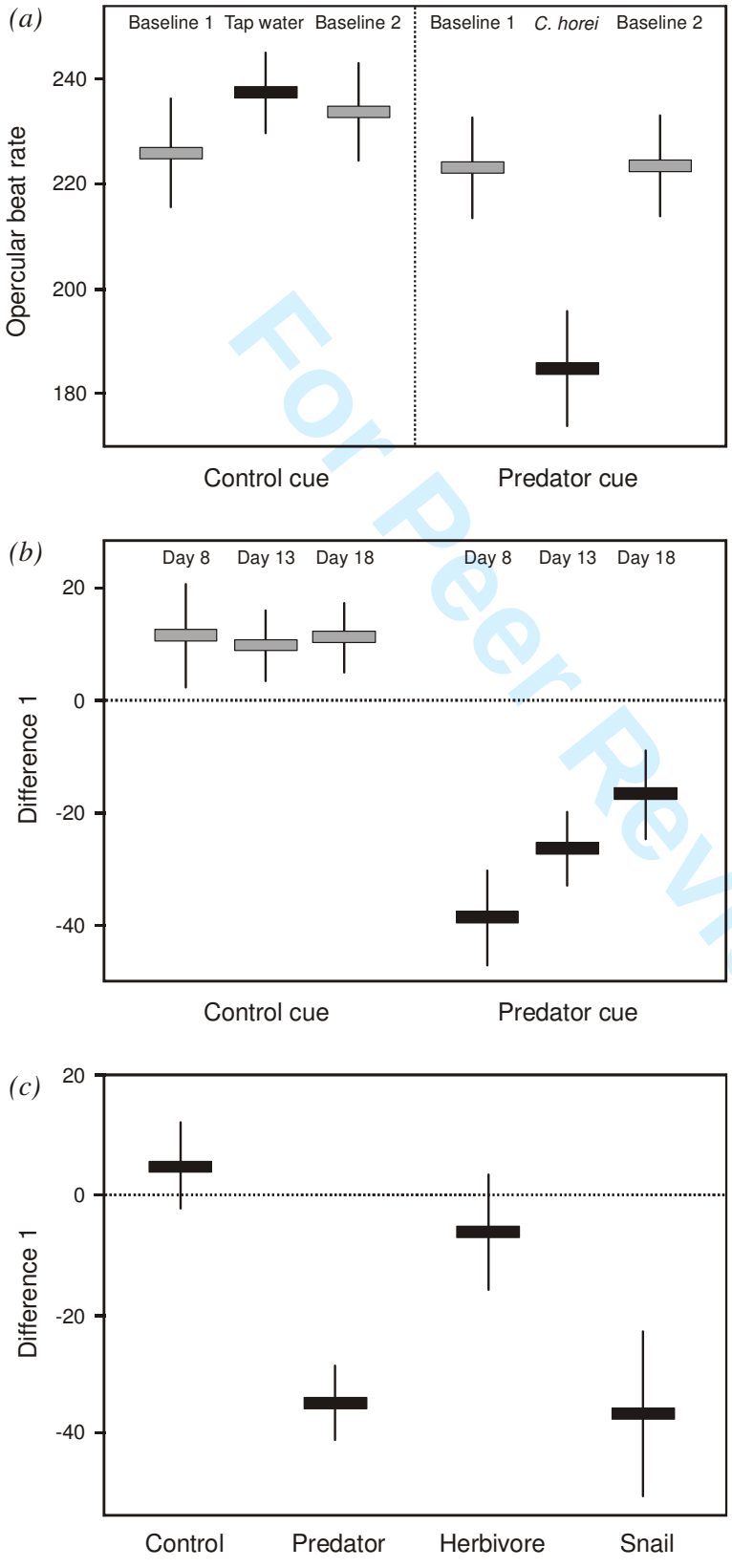


**Fig. 3**

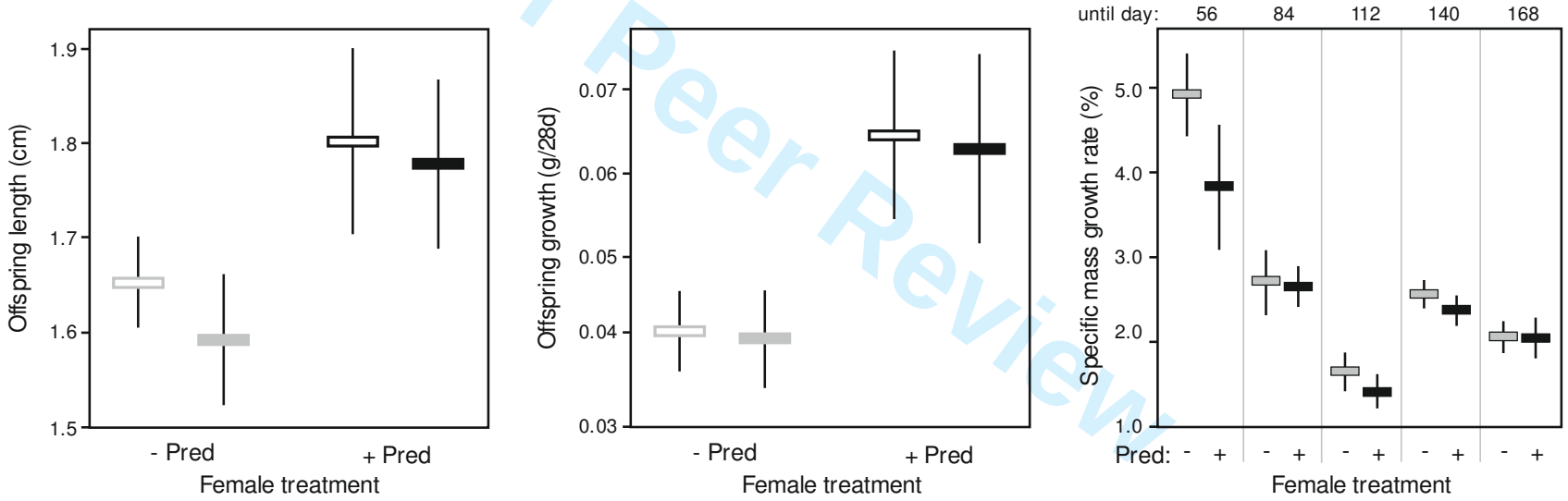
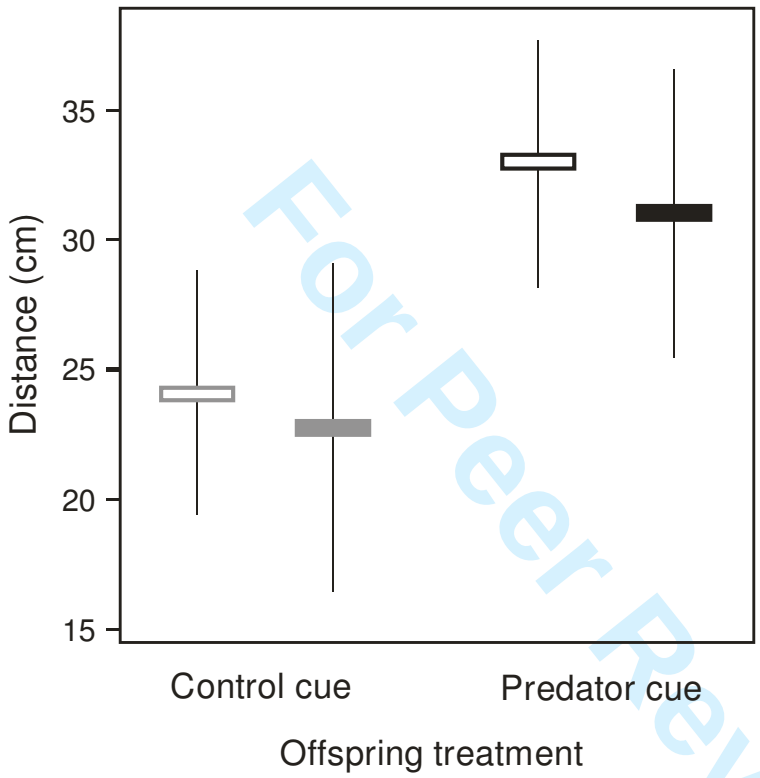
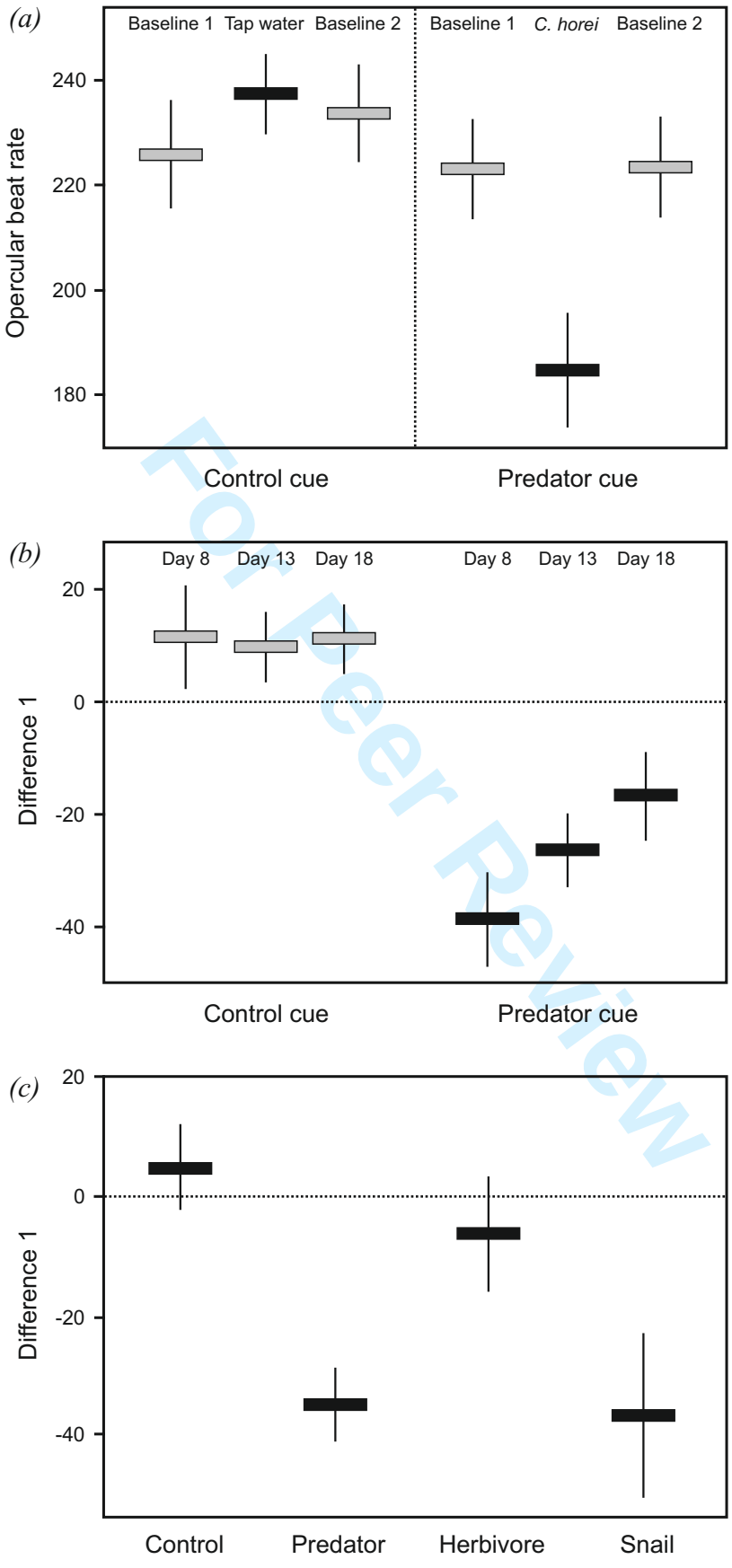


**Fig. 4**





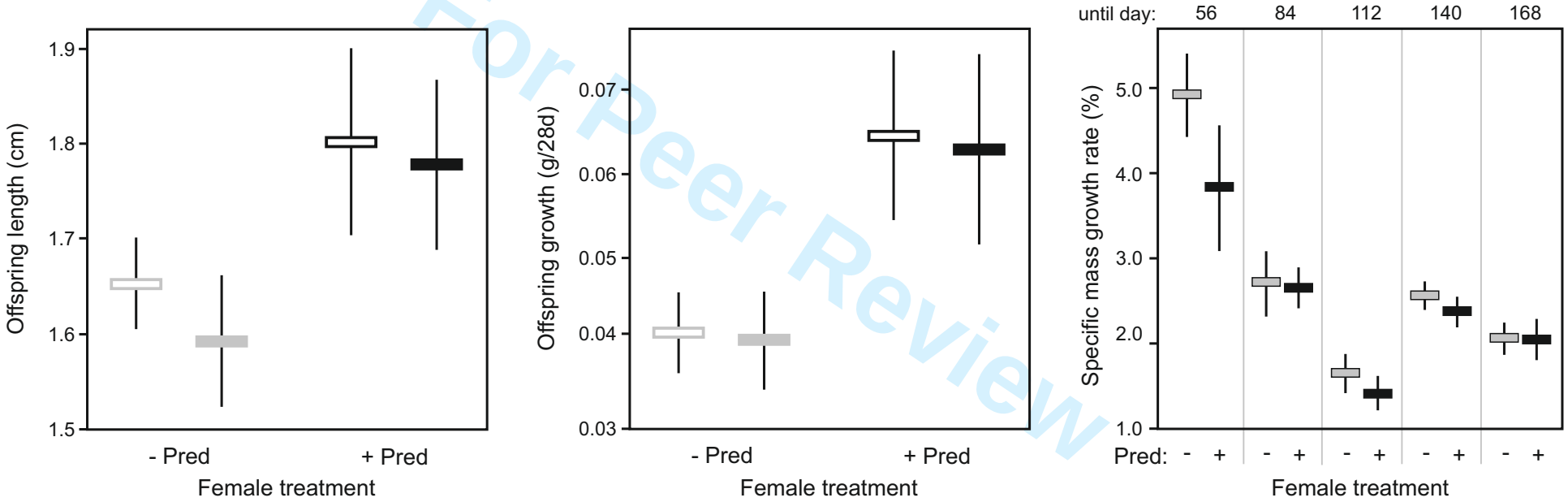





Fig. 2

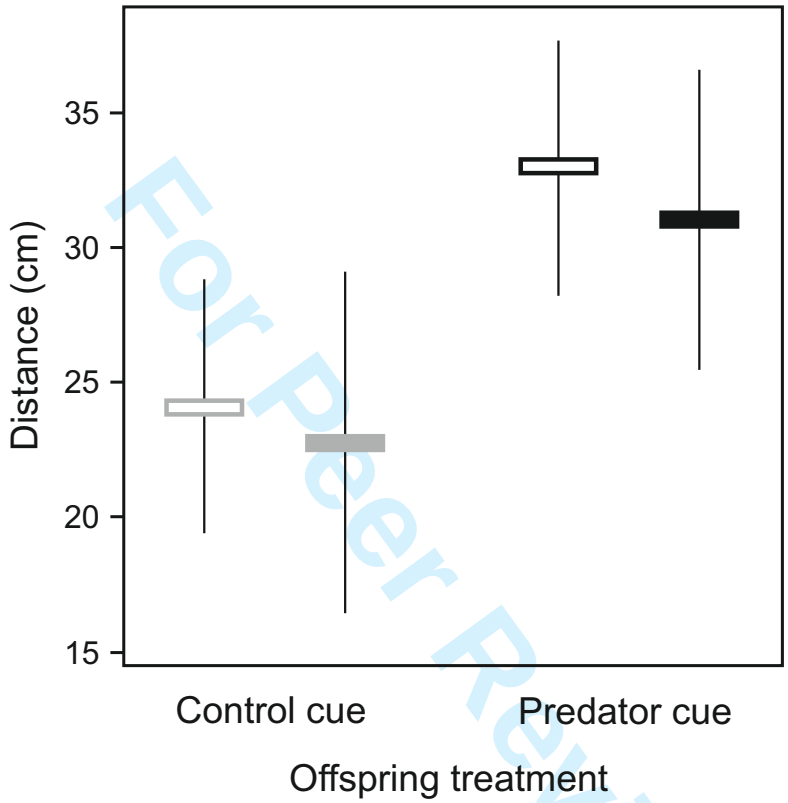





Fig. 3

**Supporting Information**

**Antipredator defences of young are independently determined by genetic inheritance, maternal effects and own early experience in mouthbrooding cichlids (A. Stratmann & B. Taborsky)**

**Appendix S1: Additional methodological and statistical information**

**Table S1.** Timeline of experiment including maternal treatment, offspring treatment and behavioural tests. "Day" refers to day of experiment, with day of hatching of larvae being defined as day 0; OBR: Opercular beat rate measurement.

| Day | Experimental event |
|---|---|
| -74 | Mean onset of maternal treatment (±14.7 SEM) |
| -5 | Spawning of experimental clutch |
| 0 | Hatching of larvae |
| 8 | Odour experience 1 and OBR 1 |
| 13 | Odour experience 2 and OBR 2 |
| 18 | Odour experience 3 and OBR 3; onset of flake food feeding |
| 28 | First measurements of weight and length (repeated every 4 weeks) |
| 33 | Startle response test 1 |
| 84 | Startle response test 2 |
| 94 | Novel object test |
| 140 | Response to *C. horei* |
| 196 | Last measurement of weight and length |

**Figure S1.** Scheme of odour cue presentation to measure opercular beat rates (OBR) of larvae. OBR was recorded in direct succession for three periods of 40 sec each. First period (baseline 1): both treatments received water from their own holding tank; second period: odour of tap water (control treatment) or of different heterospecific cues were presented was presented; third period (baseline 2): both treatments received tap water

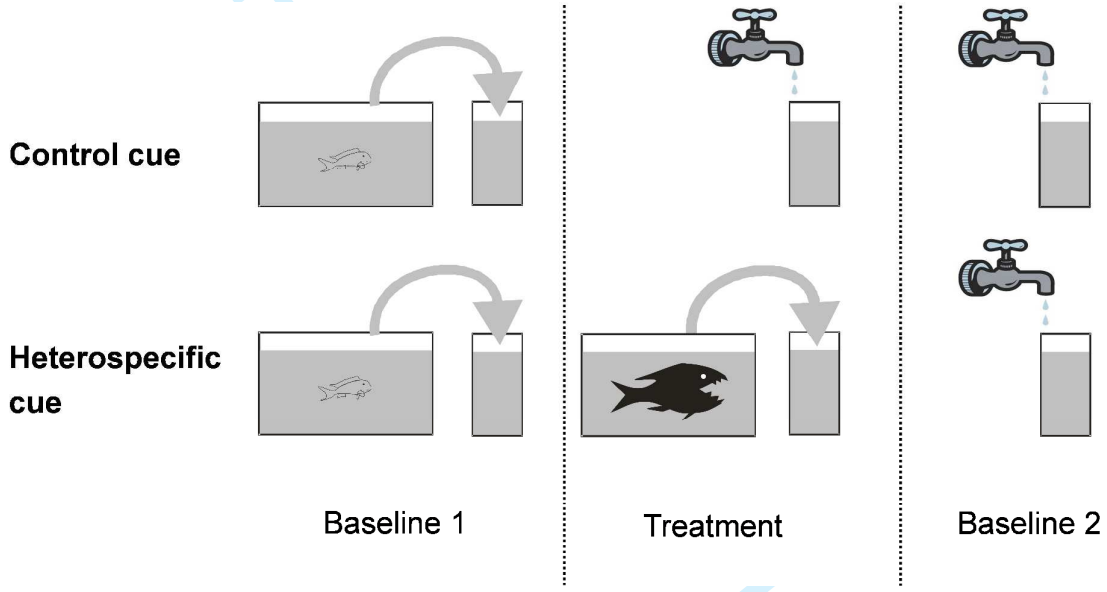


**Table S2.** Variance components of the random term when all possible random effects were included in models testing for treatment effects on opercular beat rate (OBR) differences. Percentage of the total variance each random factor accounted for is given. None of the random factors accounted for a significant proportion of variance in these models.

| Dependent variable | Random factor | % variance |
|---|---|---|
| *(a) Predator vs. control treatment* | | |
| Difference 1 | residual | 92.44 |
| | tank | 0.00 |
| | male | 1.57 |
| | female | 0.00 |
| | clutch | 5.21 |
| | individual | 0.79 |
| Difference 2 | residual | 86.64 |
| | tank | 3.66 |
| | male | 0.80 |
| | female | 0.00 |
| | clutch | 0.93 |
| | individual | 7.98 |
| *(b) Treatment with four odour cues* | | |
| Difference 1 | residual | 96.19 |
| | tank | 0.00 |
| | male | 0.00 |
| | female | 0.00 |
| | clutch | 0.00 |
| | individual | 3.81 |
| Difference 2 | residual | 76.84 |
| | tank | 6.65 |
| | male | 0.00 |
| | female | 0.00 |
| | clutch | 0.00 |
| | individual | 16.51 |

**Table S3.** Variance components of the random term when all possible random effects were included in models testing for treatment effects on offspring body size and growth. Percentage of the total variance each random factor accounted for is given. Random effects accounting for a significant amount of variance are highlighted in bold.

| Dependent variable | Random factor | % variance |
|---|---|---|
| TL at day 28 | residual | 41.61 |
| | tank | 0 |
| | male | 0 |
| | female | 0 |
| | **clutch** | **58.39** |
| Weight at day 28 | residual | 47.29 |
| | tank | 0 |
| | male | 0 |
| | female | 0 |
| | **clutch** | **52.71** |
| Condition factor *K* | residual | 75.92 |
| | tank | 0 |
| | male | 0 |
| | female | 0 |
| | clutch | 24.08 |
| TL at day 28 | residual | 40.81 |
| | tank | 0 |
| | male | 0 |
| | female | 0 |
| | **clutch** | **59.19** |
| Juvenile specific growth rate | residual | 100.00 |
| | tank | 0 |
| | male | 0 |
| | female | 0 |
| | clutch | 0 |
| | individual | 0 |
| Final TL | residual | 42.36 |
| | tank | 0 |
| | male | 0 |
| | female | 0 |
| | **clutch** | **57.64** |

**Table S4.** Variance components of the random term when all possible random effects were included in models testing for long-term treatment effects on behaviour. Percentage of the total variance each random factor accounted for is given. None of the random factors accounted for a significant proportion of variance in these models.

| Dependent variable | Random factor | % variance |
|---|---|---|
| *Novel object test* | | |
| Latency to 1st emergence | residual | 97.79 |
| | tank | 0 |
| | male | 0 |
| | female | 0 |
| | clutch | 2.21 |
| *Startle response tests* | | |
| Time to feed (day 33)[a] | residual | 96.67 |
| | tank | 0 |
| | male | 0 |
| | female | 0 |
| | clutch | 3.33 |
| Time to feed (day 84) | residual | 98.86 |
| | tank | 0 |
| | male | 0 |
| | female | 1.14 |
| | clutch | 0 |
| *Startle response tests* | | |
| Mean distance (cm) | residual | 71.36 |
| | tank | 25.77 |
| | male | 0 |
| | female | 2.87 |
| | clutch | 0 |
| Activity | residual | 76.50 |
| | tank | 4.45 |
| | male | 0 |
| | female | 0 |
| | clutch | 19.05 |
| Type of behaviour | residual | 70.80 |
| | tank | 0.00 |
| | male | 20.03 |
| | female | 9.17 |
| | clutch | 0.00 |
| Frequency of any behaviour | residual | 74.78 |
| | tank | 0 |
| | male | 0 |
| | female | 20.18 |
| | clutch | 5.04 |