

Female guppies shorten brood retention in response to predator cues

Jonathan P. Evans · Clelia Gasparini · Andrea Pilastro

Received: 24 April 2006 / Revised: 24 October 2006 / Accepted: 29 October 2006 / Published online: 30 November 2006
© Springer-Verlag 2006

Abstract Predation risk influences the duration of offspring development in many species where embryos develop from externally shed eggs. Surprisingly, such predator-mediated effects on offspring development have rarely been explored in live-bearers. In this paper, we use the guppy (*Poecilia reticulata*), a live-bearing freshwater fish, to test whether the duration of brood retention (the time from mating to parturition) is influenced by experimental changes in the perceived level of predation. Because the swimming performance of female guppies is impaired during late pregnancy, we predicted that females would withhold broods for shorter periods when they are exposed to cues that signal a heightened risk of predation on adults rather than on juveniles. We therefore simulated increased risk of predation on adults by using a combination of pike-shaped models (resembling natural predators that prey on adult guppies) and ‘alarm substances’ derived from the skin extracts of adult conspecific females. Our results revealed that, under simulated predation risk, female guppies produced broods significantly more quickly than their counterparts assigned to a control group where predator cues were absent. A subsequent evaluation of offspring swimming performance revealed a significant positive

association between neonate swimming speeds and the duration of brood retention, suggesting that by accelerating parturition, females may produce offspring with impaired locomotor skills. These findings, in conjunction with similar results from other live-bearing species, suggest that the conditions experienced by gestating females can generate significant variation in the timing of offspring development with potentially important implications for offspring fitness.

Keywords Phenotypic plasticity · Gestation · Pregnancy · Predator · Brood development

Prey animals frequently exhibit phenotypic plasticity in their responses to temporal or spatial variability in the risk of predation, for example by altering their foraging behaviour, mating strategies or habitat use (Lima and Dill 1990; Lima 1998). A number of studies have also demonstrated that variation in the risk of predation can lead to changes in the duration of offspring development. For example, predator-mediated changes in offspring hatching times have been reported in numerous amphibian species, although the direction of the effect can vary according to the nature of the predator–prey relationship. In the salamander *Ambystoma barbouri*, for example, hatching is delayed when fertilized eggs are exposed to predators (or chemical cues that signal their presence) presumably because offspring hatched at advanced stages of development are less susceptible to predators (Sih and Moore 1993). By contrast, in the frog *Rana sphenocéphala*, eggs appear to be more susceptible to predation than hatchlings (Richter 2000), and therefore, hatching times are accelerated when developing embryos are exposed to egg predators (see also Warkentin 1995; Saenz et al. 2003). Likewise, in the egg-carrying spider *Scytodes pallida*,

Communicated by J. Krause

J. P. Evans (✉)
Centre for Evolutionary Biology, School of Animal Biology,
University of Western Australia,
Nedlands, WA 6009, Australia
e-mail: jonevans@cyllene.uwa.edu.au

C. Gasparini · A. Pilastro
Dipartimento di Biologia,
Università di Padova,
via U. Bassi 58/B,
35131 Padova, Italy

females accelerate egg hatching in response to chemical cues from their predators (Li 2002), and similar accelerated hatching effects have been reported in fishes after the exposure of externally shed eggs to predator odours (Kusch and Chivers 2004) or other ecological cues that signal high-risk hatching environments (e.g. Wedekind and Muller 2005).

Despite the well-documented effects of predation on hatching times in externally developing embryos, few studies have examined the influence of predation risk on brood retention (and therefore potentially offspring development) in viviparous species. Yet brood retention times can be highly variable in viviparous species (Shine and Downes 1999; Evans and Magurran 2000; Wapstra 2000), and this can influence offspring performance as well as the gestating parent's own susceptibility to predators. For example, after prolonged pregnancies, neonate alpine skinks have better locomotor skills (running speed) and exhibit enhanced survival compared to their earlier born counterparts (Shine and Olsson 2003). In these ovoviviparous (i.e. lecithotrophic) species, such effects are probably due to the increased opportunity for continued embryo differentiation and neuromuscular development after prolonged pregnancies rather than elevated placental nutrient transfer from mothers to developing offspring (Shine and Olsson 2003). From the gestating parent's perspective, studies spanning a broad range of species have revealed that the physical burden of carrying unborn young, coupled with the associated physiological costs incurred during pregnancy, can impair the females' locomotor performance and increase their susceptibility to predation (Sinervo et al. 1991; Shaffer and Formanowicz 1996; Plaut 2002; Shine 2003; Ghalambor et al. 2004; Webb 2004). Consequently, we speculate that selection may favour the ability of viviparous females to vary the length of brood retention according to temporal or spatial changes in predation risk.

Natural populations of Trinidadian guppies (*Poecilia reticulata*) have provided some of the most compelling examples of life-history evolution in response to selection imposed by predators (reviewed by Magurran 2005). Guppies are small live-bearing ovoviviparous fish native to freshwater rivers throughout Trinidad (Magurran and Phillip 2001). Populations exhibit marked differences in mating behaviour, male colour patterns and life-history traits, all of which can be attributed to the presence or absence of predators (Endler 1995). In the (south-flowing) downstream river sites of Trinidad's Northern Range mountains, guppies typically co-occur with a suite of piscivorous fish including the pike cichlid *Crenicichla alta*, a major predator on guppies in these communities. Both laboratory and field experiments have confirmed that females inhabiting these sites rapidly evolve earlier maturity, higher fecundity, shorter interbrood intervals and

therefore a higher reproductive allotment compared to their upstream (low-predation) counterparts (Reznick and Endler 1982; Reznick et al. 1990, 1997). As a result of these differing selection pressures, females from high-predation populations tend to carry significantly heavier reproductive loads during late-term pregnancy than similarly sized fish from low-predation environments (Ghalambor et al. 2004), with potentially important implications for female survival in dangerous habitats. Indeed, recent studies have reported that pregnancy decreases swimming performance in guppies and other poeciliids, especially as females approach parturition (Plaut 2002; Ghalambor et al. 2004).

In this paper, we tested the hypothesis that female guppies adjust the duration of brood retention according to the perceived level of predation risk. We know from previous work that the interval between copulation and offspring production can be highly variable in virgin female guppies, possibly due to the high variation in the timing from insemination to fertilization, which may, to some extent, be under the female's facultative control (Evans and Magurran 2000). Nevertheless, we have anecdotal evidence that parturition can be induced by exposing late-term pregnant (previously non-virgin) females to changed temperature regimes (Evans, unpublished observations). This serendipitous finding suggests a degree of facultative control over the timing of offspring release (rather than control over the timing of fertilization) and provides the stimulus for the current investigation. We predicted that, under experimentally heightened predation risk, females would accelerate parturition. This prediction is based on the assumption that the indirect costs of losing offspring to predators would not outweigh the direct costs of the mother herself being predated upon. To simulate predation on adults rather than juveniles, we therefore used a combination of visual stimuli in the form of pike-shaped models and non-visual cues in the form of 'alarm substances' derived from the skin extracts of adult conspecific females. The models were chosen because they resemble the natural predator *Crenicichla* (see Evans et al. 2002), which feeds on adult guppies (Johansson et al. 2004) and preferentially selects females (rather than males) as they are larger and therefore likely to be more profitable as prey items (Mattingly and Butler 1994; Pocklington and Dill 1995). We chose to use a combination of visual and olfactory cues because freshwater fish can exhibit heightened responses to visual stimuli when they are accompanied by chemical alarm cues (Brown et al. 2004). We also asked whether any observed reduction in the duration of brood retention would have a detrimental effect on early juvenile performance (e.g. Shine and Olsson 2003). Our analysis of offspring performance focused on locomotor skills such as schooling, escape velocity (during a simulated avian predation threat) and the ability of neonates to evade capture, as these traits

are likely to influence the ability of newborn fish to evade predators and will therefore affect the probability of juvenile survival in the wild (Magurran 2005).

Materials and methods

The study population and its maintenance

The guppies used in this experiment were descendants of wild-caught fish collected in 2002 from the Tacarigua River in Trinidad (Trinidad national grid reference, PS 787 804; coordinates, 10°40.736'N, 061°19.168'W). This is a high-predation locality where guppies coexist with a variety of predators, including the pike cichlid *C. alta* (Magurran and Seghers 1994). Laboratory stock and all experimental fish were maintained under controlled temperature and lighting conditions (26°C±1°C; 12:12 h light/dark cycle provided by Philips TLD 36W fluorescent lamps) and fed twice daily on a mixed diet of brine shrimp nauplii (*Artemia salina*) and commercially prepared dry food (DuplaRin). Virgin females (4 months old, *N*=40) were reared for the experiment in single-sex groups under the same temperature, light and food regimes as the stock fish.

Experimental design

Our experiment had two broad aims. First, we determined whether female guppies are able to adjust the duration of brood retention according to their perception of predation risk, which we manipulated using a combination of visual and olfactory cues. We timed the 'predation pulse' to ensure that it coincided with the latter stage of brood development (between days 10 and 30 of the females' ca. monthly interbrood cycle; Houde 1997) so that females would have perceived a heightened risk of predation close to parturition (i.e. when they are likely to be most vulnerable to predators; see Ghalambor et al. 2004). Our second aim was to determine whether any observed influence of the predation treatment on the timing from mating to parturition subsequently affected offspring traits, including schooling behaviour, swimming speed and escape performance (see below).

We performed 20 replicate trials between March and July 2004. Experimental aquaria (37×44×51 cm) contained gravel, natural and artificial weed and an air filter. Opaque partitions prevented visual access between neighbouring tanks and minimized outside disturbance to the fish. At the start of the experiment, two virgin females were placed in each tank and left to settle overnight. Virgin females were used because they are usually sexually receptive (Houde 1997) and do not contain stored sperm from previous males. On the following morning, we placed a single non-

virgin male (male 1) in each tank and left him to interact with the females for 48 h. To maximize the chances that both females successfully mated, we added a second male (male 2) immediately after the first one was removed. In each replicate, both females therefore interacted with two stock males (sequentially) throughout a 4-day period. After the 4-day mating trials, pairs of females in each replicate tank were randomly assigned to one of two experimental treatments: (1) a simulated predator treatment (hereafter termed 'predator treatment') or a (2) sham (i.e. predator-free) exposure (hereafter termed 'control').

Simulated predation trials

In the predator treatment, we simulated predation risk every second day during a 20-day period, commencing 10 days after the females' initial encounter with male 1 (i.e. predator treatments ran on days 10, 12, 14 and so on until day 30). For the visual stimuli, we used three pike-shaped fish models of a brand (Abu Garcia products, Spirit Lake, IA) known to elicit strong anti-predator responses in females from the Tacarigua River (Evans et al. 2002). Although these models were similar to each other in shape and size, they differed in their specific (hand-painted) markings. During each exposure, female pairs were presented with one (of the three) model, which was replaced by a different model on subsequent exposure days. By alternating models this way, and using models that resembled a natural predator in the Tacarigua River (*C. alta*), we minimized the possibility that females habituated to any particular model over the course of the experiment (see Magurran and Girling 1986). Similar to the methods described previously (Evans et al. 2002), an observer moved a model (predator treatment) or empty steel line (control) through each tank from a remote position (i.e. not visible to the test females). In these trials, the model (or steel wire) was moved repeatedly through the tank at intervals of approximately 1 min during the 20-min trial. During each exposure, we measured the duration that females spent schooling within two body lengths of each other (Pitcher and Parrish 1993). Schooling is known to offer an effective defence against many types of predator (Magurran 1990; Pitcher and Parrish 1993). We also measured schooling (exactly as described above) by females 4 h after the visual exposure trials to determine whether any observed difference in schooling between treatments persisted in the absence of the models.

During the third (day 14), sixth (day 20) and ninth (day 26) simulated predator exposure, we strengthened the perceived threat of predation by adding filtered extracts of 'wounded' females and filtered water to the predator and control treatments, respectively. Filtered skin extract is known to elicit a strong anti-predator response (increased

cohesiveness, dashing, freezing and reduced foraging) in guppies and other poeciliid fishes (Nordell 1998; Brown 2003) presumably because conspecific fish associate the chemical signal with danger. We used adult females (size not recorded but approximately matched for size) for the skin extracts because we were attempting to simulate predation on adult females rather than males. Although the reproductive status of the females used to obtain skin extracts was not known, it is unlikely that they were virgin as they came from mixed-sex stock tanks. We chose to introduce the olfactory cues during only three of the ten exposures to minimize the number of females killed for the experiment and because chemical alarm cues are known to persist for prolonged periods in enclosed tanks (Nordell 1998). We used the method described by Nordell (1998) for extracting skin extracts from female guppies. Briefly, on each of the three exposure days, two non-focal females were taken from our stock population and euthanized in an overdose of the anaesthetic MS222 (250 mg l⁻¹). We then removed the epidermis from each female and placed it in a tube containing 2 ml of tank water. This solution was mixed thoroughly, centrifuged and filtered through 0.2- μ l paper to produce a clear supernatant. For the control treatment, we used the same technique with filtered tank water. Fresh samples were prepared on each of the three exposure days, and therefore, a total of 12 females were killed to produce sufficient samples for this experiment.

We monitored all females (twice daily) until they produced their first brood. When the first female produced her brood, we removed her (she was easily recognizable as a newly post-partum female) and measured her standard length to the nearest 0.5 mm. We calculated the duration of brood retention as the time (in days) taken to produce her brood after first encountering male 1. Offspring were removed from their natal tank and placed in holding aquaria overnight before taking part in the offspring performance trials (below). The post-partum female was returned to her tank until the second female produced her brood, at which point, we measured the second female's standard length and removed her brood for testing on the following day. After both females had produced broods, they were moved to post-experimental aquaria and not reused for future experiments.

Offspring performance

The swimming performance (schooling, swimming speed and anti-predator responses) of juvenile guppies was assessed approximately 24 h after their birth. All broods were tested 'blind' of experimental treatment (i.e. without a priori knowledge of each brood's treatment status). Following previous work (Evans et al. 2004), we estimated schooling by pairs of offspring over a 10-min period,

intersected by a simulated predation threat by an avian predator (see below). This enabled us to measure schooling by pairs of offspring during the trial period (Evans et al. 2002) and estimate escape velocities during the simulated threat (Evans et al. 2004). We chose to simulate an avian rather than a fish attack for two reasons. First, our ability to measure an escape response by offspring depended on video footage taken during the offspring performance trials (see below). Because this footage was taken from above the schooling arena, we had to impose a maximum water depth (2.5 cm) to ensure that the juvenile guppies were in focus for the videos. This constraint made it technically difficult to simulate an attack using any of our fish models, which required greater depth to function properly. Second, our previous work has shown that simulated attacks by avian predators elicit a strong anti-predator response in newborn fish, characterized by sudden bursts of 'darts' and erratic swimming as the bird model moved over the schooling arena (Seghers 1974; Evans et al. 2004; Templeton and Shriner 2004 for discussion about the role of avian predators in wild guppies; see also Magurran 2005). Following previous work (Evans and Magurran 2000), we also measured 'capture time'—the time taken for a single naïve (but experienced) observer to catch both offspring sequentially with a hand net—to further assess the escape abilities of the juvenile fish.

The schooling arena was constructed from a circular disc of white plastic sheeting (35 cm diameter) marked with 2-cm gridlines. The walls of the arena (6.5 cm) were attached to the base with silicon sealant, which was placed inside a circular plastic tray (44 cm diameter) for support. Illumination was provided by two 60-W bulbs positioned 40 cm above the arena. Before each schooling trial, the test arena was filled with conditioned fresh water (depth=2.5 cm) and labelled with a code that subsequently identified the brood and the individuals being tested. At the start of each trial, a pair of sibling juveniles was caught using a hand net and transferred to the arena where they were allowed to acclimate for 10 min before recording commenced. After the settlement period, the schooling behaviour of both fish was video recorded until 5 min elapsed, at which point, the observer pulled the model bird over the arena using a system of pulleys operated from behind a blind. The model bird (10.5 cm in length, wingspan of 12.5 cm, constructed of black plastic) was suspended on a length of monofilament line 26 cm above the arena. The line was held taut by two 43-cm-long vertical poles clamped to the desk. A second length of monofilament line tied to the front of the model bird allowed the observer to control its movement from behind the blind. Two plastic platforms were constructed at either end of the arena so that the fish were unable to see the model bird when it was not in use. After the simulated bird flight, a further 5 min of schooling

behaviour was recorded before the offspring were captured with a net (see above) and photographed (each photograph included a section of ruler for calibration). Offspring body size (snout to base of caudal fin=standard length) was measured to within 0.1 mm from these photographs. The water in the arena was changed between consecutive trials (both within and among different broods) to control for the possibility that chemical cues released by frightened fish affected the behaviour of fish taking part in the subsequent trials (Wisenden et al. 1995; Mirza and Chivers 2002). For each family, schooling, swimming speed and capture times were expressed as mean values for all sibling pairs (where broods contained uneven numbers of offspring, one individual was therefore not tested in these trials).

Schooling and escape responses by newborn fish were analysed from the videos using Zoom Player software (available online at: http://www.inmatrix.com/files/zoom_player_download.shtml). Our criterion for schooling was similar to that for adult fish (see above). In each trial, the percentage of time that the two siblings spent swimming in synchrony within two body lengths of each other was estimated for the 10-min trial period (see Evans and Magurran 2000). The response by each offspring to the simulated predation threat was then estimated in two ways using video footage taken during the trials. First, we estimated mean response time for each brood. To do this, we recorded the time (in seconds) taken for each fish to respond to the model bird. Such responses were obvious, as they involved the cessation of normal schooling or exploratory behaviour and commencement of a sudden flight response. Second, we estimated mean swimming speeds for each brood, specifically focussing on the period from the start of the flight response until the model bird had passed over the schooling arena (thus excluding the ‘response time’ period). To estimate swimming speeds, we viewed the section of video during which the bird moved over the arena frame by frame (at 25 frames/second) and measured the distance travelled by each offspring during the allotted period (using Pesci2 software, developed at University of Padova, Italy). After estimating the total distance travelled by each individual offspring (using the gridlines on the arena) and the number of frames it took for the bird to pass (at 1/25 s/frame), it was possible to calculate each offspring’s speed (cm/s) during the simulated bird flight. These two offspring-response measures are presented (and analysed) as mean values for each brood.

Statistical analyses

Before the statistical analyses, we checked all data for normal distributions, and variances were checked for homogeneity. Only offspring capture times (for the offspring performance measures, see below) deviated signifi-

cantly from normal; a log (x) transformation significantly improved their distribution. To compare the time from mating to parturition between treatments, we used the mean value for both females in each tank. This avoided pseudo-replication because the two females in each tank could not be treated as independent samples given their shared environment and simultaneous exposure to the treatment. We used an independent sample t -test to compare our two response variables (time to parturition and schooling) between treatments. Where only one of the two females in each tank produced a brood, we used this single value in the analysis of parturition times. Of the 40 females initially used, 28 produced a brood from $n=16$ of the original 20 replicate trials.

To test whether the predator treatment and the duration from mating to parturition influenced offspring performance traits, we performed a multivariate analysis of covariance (MANCOVA) in which treatment was entered as the fixed categorical factor (predator or control), the duration from mating to parturition as the covariate and the four offspring performance traits (schooling duration, swimming speed, capture time and response time) as dependent variables. In this analysis, we tested for homogeneity of slopes by including the interaction between the categorical predictor (predator treatment) and the covariate (days to parturition) in our initial model. We found no significant interaction for any of the dependent variables (schooling duration, $F_{1,20}=0.20$, $P=0.66$; swimming speed, $F_{1,20}=0.45$, $P=0.84$; capture time, $F_{1,20}=0.38$, $P=0.85$; response time, $F_{1,20}=0.18$, $P=0.68$) and therefore report the results from a reduced model with no interaction term. In this analysis, we used mean values for each female’s brood (rather than mean values for female pairs), and therefore, the degrees of freedom in the analysis of offspring performance is based on the number of broods ($n=28$) rather than the number of ‘replicate’ female pairs ($n=16$). Similar results were obtained when we used mean offspring traits from replicate female pairs (analysis not presented). All statistical tests were two-tailed and performed using SPSS (v. 13).

Results

Effect of simulated predation on female schooling behaviour

We initially confirmed that schooling by female pairs did not change over the course of the ten exposures by performing an analysis of covariance, where schooling duration was entered as the dependent variable, female pair identity as a random factor, time of the day (initial observation=morning; subsequent observation 4 h later=

afternoon) as a fixed factor and exposure number (from 1 to 10) as the covariate. The two treatment groups were analysed separately. Female pair identity was significant in both groups (i.e. some female pairs schooled more than others; predator, $F_{9,165}=5.85$, $P=0.007$; control, $F_{9,175}=9.38$, $P=0.001$). Furthermore, in the predator treatment, there was a significant difference in schooling when the model predator was present (morning) compared to the afternoon when the model was absent (predator, $F_{1,165}=8.33$, $P=0.017$; control, $F_{1,175}=0.25$, $P=0.63$), demonstrating that the females reacted to the presence of the model. Day of exposure was not significant in either of the groups (predator, $F_{1,165}=0.77$, $P=0.38$; control, $F_{1,175}=1.866$, $P=0.17$), indicating that females did not habituate to the models over time. Our subsequent analysis compared grand means (for female pairs over the entire exposure period) and confirmed that females responded to the model predators and odour cues; those assigned to the predator treatment spent significantly more time schooling than those in the control group, both during the simulated predator exposures ($t_{18}=4.61$, $P<0.0001$) and 4 h after the trials ($t_{18}=2.46$, $P=0.024$) when only olfactory cues (predator treatment) would have been present (Fig. 1).

Simulated predation and the timing from mating to parturition

Our analysis of parturition times was based on the 28 females that produced offspring. Of the 12 females that failed to produce a brood, two died (one from each treatment group) and ten failed to produce a brood. Among the females that produced offspring, we detected a significant influence of predator treatment on the timing

to parturition. After confirming that there was no significant difference in the size of females initially assigned to the two treatment groups ($t_{18}=0.95$, $P=0.35$), we found that the time to parturition was significantly shorter in females that were assigned to the predator treatment compared to their counterparts in the control group (mean±SD number of days for females to produce their first brood; predator treatment=32.00±5.09; control treatment=38.71±6.31; $t_{14}=2.36$, $P=0.033$; Fig. 2).

Offspring traits

Although the above analysis revealed an influence of predator treatment on duration from mating to parturition, we found no direct effect of the treatment on offspring performance traits (MANCOVA with timing to parturition entered as the covariate, treatment as the fixed factor and mean offspring performance traits [schooling duration, swimming speed, capture time and response time] entered as dependent variables: treatment effects on all offspring traits $F_{4,21}=0.72$, $P=0.585$). However, the model did suggest an effect of the covariate (days to parturition) on offspring traits (covariate: $F_{4,21}=2.15$, $P=0.110$), driven by the influence of the covariate on neonate swimming speed (univariate analysis of variance, covariate: $F_{1,24}=4.53$, $P=0.044$). A subsequent pairwise correlation test indicated that the relationship between brood retention times and neonate swimming speed was significant and positive (Pearson correlation: $r=0.41$, $n=27$, $P=0.036$; Fig. 3). This latter finding suggests that by shortening the duration of brood retention, females produce offspring with impaired locomotor skills, although we note that, when we excluded broods with development times greater than 45 days from

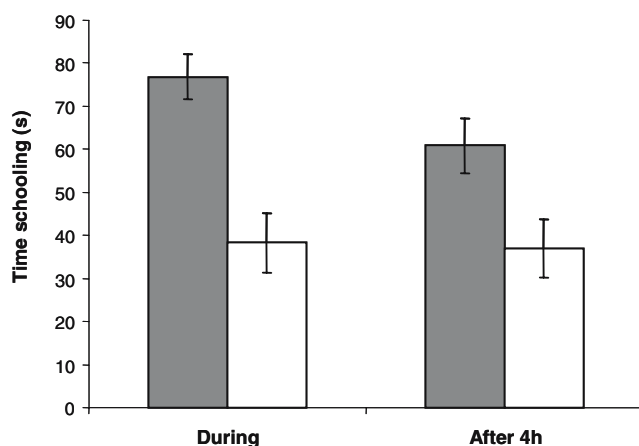


Fig. 1 Mean (±SE) duration of schooling by females assigned to the predator treatment (filled bars) and control treatment (clear bars) during and after the predator exposure trials. The analysis of schooling times (see main text) was based on the comparison of grand means for schooling times over the ten predator exposures

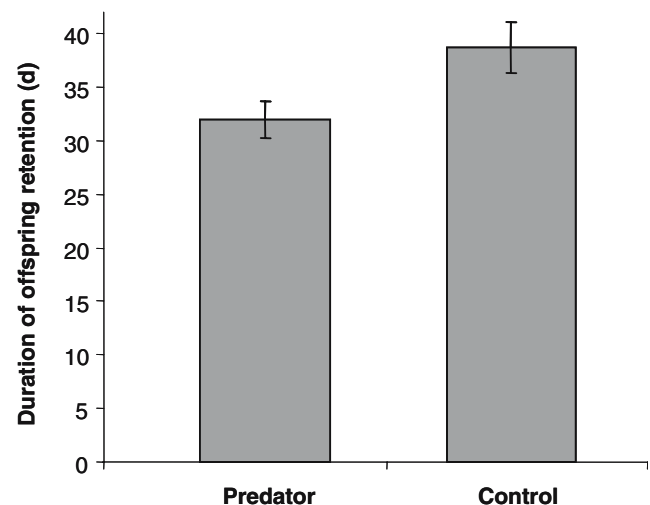


Fig. 2 Mean (±SE) duration of offspring retention (the number of days between copulation with the first male and parturition) in females assigned to the predator and control treatments

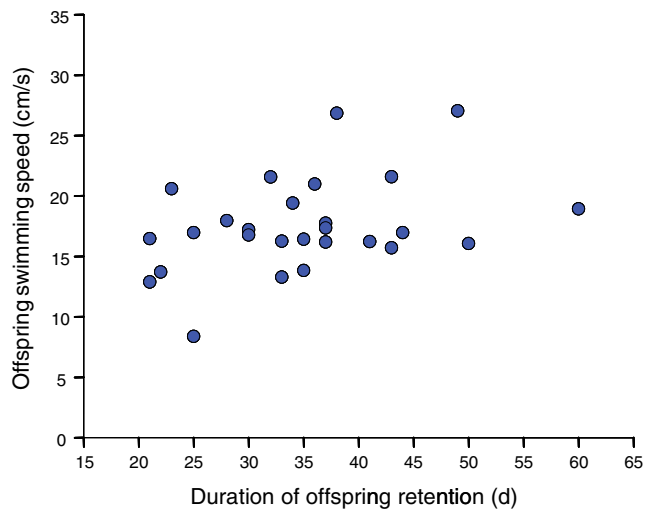


Fig. 3 The association between the duration of offspring retention and offspring swimming performance when juveniles were exposed to a simulated threat by an avian predator. See text for details

the analysis ($n=3$), the relationship between offspring development time and mean neonate swimming speeds was no longer significant ($r=0.36$, $n=24$, $P=0.08$). Broods with development times >45 days may have been second broods (e.g. where a reproductive cycle was skipped). We detected no significant effect of time to parturition on either offspring capture ($F_{1,24}=0.66$, $P=0.799$) or schooling times ($F_{1,24}=1.81$, $P=0.191$). Interestingly, response times were negatively correlated with mean swimming speed ($r=-0.44$, $n=27$, $P=0.023$) and capture times ($r=-0.58$, $n=27$, $P=0.002$), indicating that individuals that responded most quickly to the simulated threat exhibited the fastest swimming speeds and were consequently most difficult to catch.

Finally, our analyses revealed no significant difference in mean offspring size (mean standard length \pm SD: predator treatment= 8.04 ± 0.41 mm; control group= 8.16 ± 0.35 mm; $t_{26}=0.81$, $P=0.423$) or the mean number of offspring produced (mean \pm SD: predator treatment= 16.53 ± 10.18 ; control treatment= 18.18 ± 11.31 ; $t_{26}=0.4$, $P=0.691$) between the predator and control treatments.

Discussion

Our principal finding that female guppies accelerate parturition in response to a perceived increase in the level of predation supports our prediction that females can manipulate the timing of offspring release. As in other live-bearing species (e.g. Sinervo et al. 1991; Shine 2003; Webb 2004), pregnancy reduces the mobility of female guppies, particularly during the latter stages of brood development when predator-evasion skills, such as ‘fast-start’ swimming performance, are impeded (Ghalambor et al. 2004). Our finding that females responded to the

predator treatment by accelerating parturition is consistent with the hypothesis that cues signalling elevated risk to adults (rather than juveniles) stimulate females to expel offspring more quickly. Indeed, our behavioural observations made during the predator exposures confirmed that females responded to the perceived level of predation by engaging more in schooling (see also Evans et al. 2002) and other anti-predator behaviours such as inspections and avoidance manoeuvres (not analysed here but see Evans et al. 2002 for analysis of these responses by *Tacarigua* guppies to the same models). In guppies, like other freshwater fishes, the primary function of schooling is predator evasion (see reviews by Krause and Ruxton 2002; Magurran 2005); our results therefore strongly suggest that the females assigned to the predation treatment would have perceived that they were at risk during the treatment phase of the experiment.

A corollary of the argument that females respond to adult threats by expelling offspring more quickly is that the opposite pattern should occur when predation cues indicate a heightened risk of juvenile rather than adult mortality. This hypothesis could be tested by experimentally increasing the perceived threat to juveniles (for example by using models that resemble gape-limited predators such as Hart’s killifish *Rivulus hartii*) and determining whether females extend the duration of brood retention, thus potentially allowing further intra-uterine development of offspring. Our finding that the duration of brood retention was positively correlated with offspring swimming speed bodes well for this idea because it suggests that by delaying parturition, females may enhance their offspring’s swimming performance (see Shine and Olsson 2003). However, we found no evidence that the duration of offspring retention influences the ability of neonates to evade capture, which contrasts with our previous findings for this population where we reported improved predator-evasion skills in later born offspring (Evans et al. 2004). This may be due to the fact that different observers (performing offspring captures) took part in the two studies.

Our main response variable—time to parturition—is actually a composite measure broadly comprising two phases: (1) the interval between insemination and fertilization (which may also include a yolk-loading phase), and (2) the ensuing interval between fertilization and the production of offspring. In the absence of dissections in which fertilization status and embryo growth can be assayed at various intervals (Matthews and Magurran 2000), we cannot definitively disentangle the relative importance of variation in the timing of either of these two phases in influencing the timing of parturition, although for the reasons stated in the introduction, we suspect that females exert some degree of control over the timing of offspring release. Moreover, our experimental design involved

exposing experimental females to predation cues from the tenth day after they first interacted with a male (i.e. after fertilization was likely to have occurred). Nevertheless, we cannot rule out the possibility that variation in fertilization times influenced our findings.

In conclusion, we have shown that female guppies are able to tailor the timing of parturition according to experimentally manipulated predation cues, thus providing further support to a growing body of evidence that predation can generate an important source phenotypic plasticity in offspring development. In conjunction with the recent work reporting similar results for domesticated guppies (exposed to predation cues from exotic African cichlids; Dzikowski et al. 2004), this finding provides experimental evidence that viviparous females can facultatively adjust the duration of brood retention (and possibly influence offspring phenotypes) in response to ecological cues that signal high-risk environments. Similarly, Shine and Downes (1999) reported that in the placental Australian lizard, *Pseudemona pagenstecheri*, the increased opportunity for basking by pregnant females resulted in shorter gestation times and the production of larger neonates with slower running speeds (see also Wapstra 2000). Intriguingly, Shine and Downes (1999) also found that, when females were repeatedly exposed to predator odours during pregnancy, they gave birth to larger offspring with improved anti-predator responses compared to neonates from control females. Taken together, these findings provide evidence that the conditions experienced by gestating females can generate significant variation in the timing of offspring development with potentially important implications for offspring fitness.

Acknowledgement We thank Ferdinando Benatelli for the help with the experiments and Jennifer Kelley and two anonymous referees for comments on a previous draft of the manuscript. This work was supported by grants from the Italian Research Ministry (AP) and the Australian Research Council (JPE). The research conformed to the relevant Italian laws governing the care of animals in research (D.L. 116/27-01-92, C.M.S. 8/22-04-94) and was authorized by the Istituto Superiore di Sanità (National Health Institute).

References

- Brown GE (2003) Learning about danger: chemical alarm cues and local risk assessment in prey fishes. *Fish Fish* 4:227–234
- Brown GE, Poirier J-F, Adrian JC (2004) Assessment of local predation risk: the role of subthreshold concentrations of chemical alarm cues. *Behav Ecol* 15:810–815
- Dzikowski R, Hulata G, Harpaz S, Karplus I (2004) Inducible reproductive plasticity of the guppy *Poecilia reticulata* in response to predation cues. *J Exp Zool* 301A:776–782
- Endler JA (1995) Multiple-trait coevolution and environmental gradients in guppies. *Trends Ecol Evol* 10:22–29
- Evans JP, Magurran AE (2000) Multiple benefits of multiple mating in guppies. *Proc Natl Acad Sci US Am* 97:10074–10076
- Evans JP, Kelley JL, Ramnarine IW, Pilastro A (2002) Female behaviour mediates male courtship under predation risk in the guppy. *Behav Ecol Sociobiol* 52:496–502
- Evans JP, Kelley JL, Bisazza A, Finazzo E, Pilastro A (2004) Sire attractiveness influences offspring performance in guppies. *Proc R Soc Lond B Biol Sci* 271:2035–2042
- Ghalambor CK, Reznick DN, Walker JA (2004) Constraints on adaptive evolution: the functional trade-off between reproduction and fast-start swimming performance in the Trinidadian guppy (*Poecilia reticulata*). *Am Nat* 164:38–50
- Houde AE (1997) Sex, color, and mate choice in guppies. Princeton University Press, Princeton NJ
- Johansson J, Turesson H, Persson A (2004) Active selection for large guppies, *Poecilia reticulata*, by the pike cichlid, *Crenicichla saxatilis*. *Oikos* 105:595–605
- Krause J, Ruxton GD (2002) Living in groups. Oxford University Press, Oxford
- Kusch RC, Chivers DP (2004) The effects of crayfish predation on phenotypic and life-history variation in fathead minnows. *Can J Zool* 82:917–921
- Li DQ (2002) Hatching responses of subsocial spitting spiders to predation risk. *Proc R Soc Lond B Biol Sci* 269:2155–2161
- Lima SL (1998) Stress and decision making under the risk of predation: recent developments from behavioral, reproductive, and ecological perspectives. *Adv Study Anim Behav* 27:215–290
- Lima SL, Dill LM (1990) Behavioral decisions made under the risk of predation: a review and prospectus. *Can J Zool* 68:619–640
- Magurran AE (1990) The adaptive significance of schooling as an anti-predator defence in fish. *Ann Zool Fenn* 27:51–66
- Magurran AE (2005) Evolutionary Ecology: the Trinidadian guppy. Oxford University Press, Oxford
- Magurran AE, Girling SL (1986) Predator model recognition and response habituation in shoaling minnows. *Anim Behav* 34:510–518
- Magurran AE, Seghers BH (1994) Sexual conflict as a consequence of ecology: evidence from guppy, *Poecilia reticulata*, populations in Trinidad. *Proc R Soc Lond B Biol Sci* 255:31–36
- Magurran AE, Phillip DAT (2001) Evolutionary implications of large-scale patterns in the ecology of Trinidadian guppies, *Poecilia reticulata*. *Biol J Linn Soc* 73:1–9
- Matthews IM, Magurran AE (2000) Evidence for sperm transfer during sneaky mating in wild Trinidadian guppies. *J Fish Biol* 56:1381–1386
- Mattingly HT, Butler MJ (1994) Laboratory predation on the Trinidadian guppy: implications for the size-selective predation hypothesis and guppy life history evolution. *Oikos* 69: 54–64
- Mirza RS, Chivers DP (2002) Behavioural responses to conspecific disturbance chemicals enhance survival of juvenile brook charr, *Salvelinus fontinalis*, during encounters with predators. *Behaviour* 139:1099–1109
- Nordell SE (1998) The response of female guppies, *Poecilia reticulata*, to chemical stimuli from injured conspecifics. *Environ Biol Fishes* 51:331–338
- Pitcher TJ, Parrish JK (1993) Functions of shoaling behaviour in teleosts. In: Pitcher TJ (ed) Behaviour of teleost fishes. Chapman & Hall, London, pp 363–439
- Plaut I (2002) Does pregnancy affect swimming performance of female mosquitofish, *Gambusia affinis*? *Funct Ecol* 16:290–295
- Pocklington R, Dill LM (1995) Predation on female or males: who pays for bright male traits? *Anim Behav* 49:1122–1124
- Reznick DN, Endler JA (1982) The impact of predation on life history evolution in Trinidadian guppies (*Poecilia reticulata*). *Evolution* 36:125–148
- Reznick DN, Bryga H, Endler JA (1990) Experimentally induced life-history evolution in a natural population. *Nature* 346:357–359

- Reznick DN, Shaw FH, Rodd FH, Shaw RG (1997) Evaluation of the rate of evolution in natural populations of guppies (*Poecilia reticulata*). *Science* 275:1934–1937
- Richter SC (2000) Larval caddisfly predation on the eggs and embryos of *Rana capito* and *Rana sphenoccephala*. *J Herpetol* 34:590–593
- Saenz D, Johnson JB, Adams CK, Dayton GH (2003) Accelerated hatching of southern leopard frog (*Rana sphenoccephala*) eggs in response to the presence of a crayfish (*Procambarus nigrocinctus*) predator. *Copeia* 646–649
- Seghers BH (1974) Geographic variation in the responses of guppies (*Poecilia reticulata*) to aerial predators. *Oecologia* 14:93–98
- Shaffer LR, Formanowicz DR (1996) A cost of viviparity and parental care in scorpions: reduced sprint speed and behavioural compensation. *Anim Behav* 51:1017–1023
- Shine R (2003) Effects of pregnancy on locomotor performance: an experimental study on lizards. *Oecologia* 136:450–456
- Shine R, Downes SJ (1999) Can pregnant lizards adjust their offspring phenotypes to environmental conditions? *Oecologia* 119:1–8
- Shine R, Olsson M (2003) When to be born? Prolonged pregnancy or incubation enhances locomotor performance in neonatal lizard (Scincidae). *J Evol Biol* 16:823–832
- Sih A, Moore RD (1993) Delayed hatching of salamander eggs in response to enhanced larval predation risk. *Am Nat* 142:947–960
- Sinervo B, Hedges R, Adolph SC (1991) Decreased sprint speed as a cost of reproduction in the lizard *Sceloporus occidentalis*—variation among populations. *J Exp Biol* 155:323–336
- Templeton CN, Shriner WM (2004) Multiple selection pressures influence Trinidadian guppy (*Poecilia reticulata*) antipredator behavior. *Behav Ecol* 15:673–678
- Wapstra E (2000) Maternal basking opportunity affects juvenile phenotype in a viviparous lizard. *Funct Ecol* 14:345–352
- Warkentin KM (1995) Adaptive plasticity in hatching age—a response to predation risk trade-offs. *Proc Natl Acad Sci US Am* 92:3507–3510
- Webb JK (2004) Pregnancy decreases swimming performance of female northern death adders (*Acanthophsis praelongus*). *Copeia* 357–363
- Wedekind C, Muller R (2005) Risk-induced early hatching in salmonids. *Ecology* 86:2525–2529
- Wisenden BD, Chivers DP, Smith RJF (1995) Early warning in the predation sequence—a disturbance pheromone in Iowa darters (*Etheostoma exile*). *J Chem Ecol* 21:1469–1480