

## Predator-inspection behaviour in female three-spined sticklebacks *Gasterosteus aculeatus* is associated with status of gravidity

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Differences in predator-inspection behaviour between gravid and non-gravid female as well as between male and female three-spined sticklebacks *Gasterosteus aculeatus* were investigated. Gravid females confronted with a live rainbow trout *Oncorhynchus mykiss* showed bolder inspection behaviour than non-gravid ones. The behaviour of gravid females was comparable with that of males, maybe because both face a high risk of predation. The results indicate that antipredator behaviour in female *G. aculeatus* is not fixed but adjusted to their reproductive state.

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Key words: boldness; fish; gravidity; inspection behaviour; predation risk; pregnancy.

### INTRODUCTION

Reproduction is known to be costly. These costs are often especially high in females, which not only produce energetically costly eggs but also carry the eggs or the developing embryos. As a consequence, females often face a reduced manoeuvrability leading to a higher risk of predation (Brodie, 1989; Magnhagen, 1991). Furthermore, gravid females might be detected more readily in a group, *e.g.* because of their conspicuous morphology, amongst non-gravid, relatively uniform conspecifics ('oddy effect'; Landeau & Terborgh, 1986). These costs have been demonstrated in several animal groups. For example, female striped scorpions *Centruroides vittatus* carrying young suffer a reduced running speed (Shaffer & Formanowicz, 1996), as do gravid broad-headed skinks *Eumeces laticeps* (Cooper *et al.*, 1990). Swimming performance is influenced by gravidity in female northern death adders *Acanthophis praelongus* (Webb, 2004). Female European starlings *Sturnus vulgaris* show a decrease in escape performance before and during deposition (Lee *et al.*, 1996), whereas wolf spiders *Pardosa milvina* carrying egg sacs face a higher predation risk. Pregnant Utah prairie dogs *Cynomys parvidens* have a higher risk of being caught by red foxes *Vulpes vulpes* probably because their running speed is reduced (Hoogland *et al.*, 2006).

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In fishes, gravidity is known to impair females' swimming ability. In shorthorn sculpins *Myoxocephalus scorpius* (L.) swimming performance and the contractile properties of fast muscle fibres are reduced in gravid fish (James & Johnston, 1998). In guppies *Poecilia reticulata* Peters velocity and distance travelled decline rapidly over the course of pregnancy (Ghalambor *et al.*, 2004; Evans *et al.*, 2007), leading to a functional trade-off between reproduction and swimming performance (Ghalambor *et al.*, 2004). Gravid three-spined sticklebacks *Gasterosteus aculeatus* L. show a reduced probability to escape from an artificial predator attack (Rodewald & Foster, 1998). Consequently, gravid females often show changes in their antipredator behaviour. For example, bighorn sheep *Ovis canadensis* change habitats to reduce risk of predation, even though this change is associated with reduced foraging opportunities (Ruckstuhl & Festa-Bianchet, 1998). Gravid prairie rattlesnakes *Crotalus viridis* reduce their rattling behaviour and, thus, reduce the risk of being detected by a predator (Kissner *et al.*, 1997). Additionally, altered escape behaviours are demonstrated. Here, gravid females try to escape a possible predation threat earlier (Brown & Shine, 2004) or add additional escape elements (Qualls & Shine, 1998; Rodewald & Foster, 1998). These altered escape behaviours might lead to an increase in energy and time expenditure (Brown & Shine, 2004). In contrast, female crucian carp *Carassius carassius* (L.), show a decrease in fright reaction during the later stages of sexual maturation, maybe allowing uninterrupted spawning (Lastein *et al.*, 2008). A further behavioural change that would be expected in gravid females is a change in readiness to show high-risk behaviours like predator inspection. Such changes in boldness during the reproductive cycle, however, have seldom been demonstrated.

## THE MODEL SYSTEM

In this study *G. aculeatus* were used as a model species. Gravid *G. aculeatus* develop a distended body caused by the comparatively large eggs. A female's clutch might comprise >30% of the total body mass (Wootton & Evans, 1976; Wootton, 1984; Mehliis, 2007) leading to a strong reduction of swimming ability (Milinski & Bakker, 1992; Rodewald & Foster, 1998). Indeed, gravid females have been shown to modify their habitat use and escape behaviour, though this varied between populations (Rodewald & Foster, 1998).

The antipredator behaviour of *G. aculeatus* is multifarious, ranging from fast swimming, dashing and freezing (Wootton, 1984), to the formation of tighter shoals in the presence of a predator (Krause *et al.*, 1998). Furthermore, males reduce courtship intensity under high predation risk (Candolin, 1997). An often-described behaviour is predator inspection, where one or more individuals approach a potential predator, remain near the predator for some seconds and then move back to the group or hiding place (Dugatkin & Godin, 1992). By doing so they gain information about the posed threat (Milinski *et al.*, 1990, 1997). This behaviour has been shown to be dangerous, as the predation risk increases with decreasing distance to the predator (Milinski *et al.*, 1997). The inspecting individual, however, might not only gain information but also increase its social status or attractiveness as a mate (Dugatkin & Godin, 1992; Pitcher, 1992). Thus, the benefits of inspection behaviour might outweigh the costs. Furthermore, not all inspectors might face the same predation risk. Faster swimmers should, for example, have better chances to escape than slower ones. The predator-inspection behaviour of *G. aculeatus* varies at both the intrapopulation

(McLeod & Huntingford, 1994) and interpopulation levels (Huntingford *et al.*, 1994; Dingemanse *et al.*, 2007). Predator inspection furthermore depends on the presence of conspecifics (Milinski *et al.*, 1997), parasite load (Giles, 1987; Barber *et al.*, 2004) and nutritional state (Giles, 1987; Godin & Crossman, 1994). In males, reproductive state has been shown to be of importance (Huntingford, 1976). How the reproductive state is related to female's boldness, however, remains unknown thus far.

The aims of the present study were: first, to investigate the effect of female reproductive state and second, to consider the effect of sex on boldness. These aims were achieved by measuring the readiness of gravid and non-gravid females as well as sexually active males to inspect a predator.

## MATERIALS AND METHODS

### EXPERIMENTAL SUBJECTS

*Gasterosteus aculeatus* were caught in the pond Stadtweiher near Euskirchen, Germany (50° 38' N; 6° 47' E) on 27 March 2008 using minnow traps and brought to the laboratory. Here, they were kept in large outdoor tanks (750 l) with air ventilation and a constant supply of tap water at a flow rate of 3 l min<sup>-1</sup>. Fish were fed with *Chironomus* sp. larvae each evening in excess. At the start of the experiments, fish were *c.* 1 year old. All experiments were conducted during the breeding season. Thus, males and females were easily distinguishable by the presence or absence of male nuptial colouration (red throat region and blue eyes). Rainbow trout *Oncorhynchus mykiss* (Walbaum) were used as predators. Thirty *O. mykiss* were obtained from a local dealer and housed in the same way as the *G. aculeatus*. During the experiments, they were fed daily with *Chironomus* sp. larvae. To habituate them to the new surroundings (Milinski, 1997), the *O. mykiss* were moved from the outdoor tank to a smaller aquarium (100 cm × 40 cm × 40 cm) located in the experimental room (summer conditions: 16L:8D, temperature 17° C, range ± 1° C) 1 day before the respective trial. Standard length ( $L_S$ ) of the *O. mykiss* varied between 15.5 and 19.5 cm and did not differ significantly between treatments (Kruskal–Wallis test,  $n = 79$ ,  $P > 0.05$ ). Some *O. mykiss* were used in up to four experiments. There was, however, an interval of at least 5 days between the trials to reduce stress.

### EXPERIMENTAL DESIGN

Experiments took place in an aquarium measuring 100 cm × 40 cm × 40 cm (Fig. 1). The aquarium was divided into a predator compartment (25 cm × 40 cm) and a test fish compartment (75 cm × 40 cm) using perforated clear plexiglas. The predator compartment was additionally separated from the test fish compartment by a sheet of opaque plastic that could be lifted by a string using a pulley system. The test fish compartment was divided into a starting zone (15 cm) and a choice compartment using perforated clear plexiglas. This partition could also be lifted by a string. In the middle of the starting zone, a plastic plant was placed as a hiding place. Lines drawn on the bottom of the tank divided the choice compartment into three zones measuring 20 cm each (Fig. 1). The whole set-up was illuminated by a fluorescent lamp (36 W) placed 70 cm above the aquarium. Below the lamp, a web cam (Logitech, QuickCam Pro 9000; [www.logitech.com](http://www.logitech.com)) was installed, which was connected to a laptop (Fujitsu Siemens; <http://de.ts.fujitsu.com>). The whole set-up was surrounded by a black curtain to exclude disturbances from outside. The pulley systems to lift the partitions and the computer were placed outside the curtain. In half of the experiments, a small Petri dish containing two *Chironomus* sp. larvae was placed in each choice zone. Aside from two test fish (a gravid and a non-gravid female), however, all other fish ignored the food completely. Therefore, food was not supplied in the other half of the trials. The two females that fed on the *Chironomus* sp. larvae were excluded from analyses.

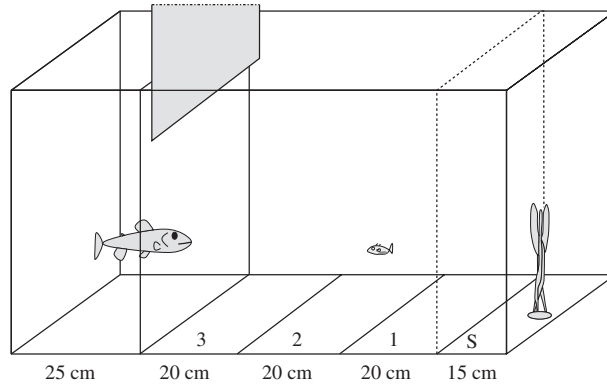


FIG. 1. The experimental tank with the predator compartment to the left, the starting zone (S) with a plant to the right and the three zones 1, 2 and 3 in between.

Experiments were carried out between June and August 2008. Shortly before the start of the experiment, the aquarium was filled with 1 day-old tap water up to a height of 16.5 cm. All partitions were lowered. Two minutes after the predator had been placed in its compartment a haphazardly chosen test fish was put into the starting compartment from the outdoor tank. Test fish were either gravid or non-gravid females, or males. Gravid females ( $n = 32$ ) were identified by their swollen abdomen and eggs shining through the skin near the cloaca. Non-gravid females ( $n = 30$ ) were identified by the lack of nuptial colouration, whereas males ( $n = 17$ ) could easily be spotted by their reddish throat and blue eyes. After introducing the test fish into the tank, they were allowed to settle for 10 min. Later, the opaque partition hiding the *O. mykiss* and the clear partition separating the starting compartment from the choice compartment were lifted. The behaviour of the test fish was then recorded for 30 min using the webcam. Later,  $L_S$  (cm) and body mass ( $M$ ; g) of all fish was taken and the body condition ( $K$ ) was calculated as  $K = 100 ML_S^{-3}$  following Bolger & Connolly (1989). After each trial, the test aquarium was rinsed with clear tap water to exclude confounding effects of remaining olfactory cues.

## DATA ANALYSES

The time the test fish spent in each choice zone was quantified from the digital film recordings. From these time values, a preference index ( $I_P$ ) was calculated as  $I_P = 0 \times \text{time (zone S)} + 1 \times \text{time (zone 1)} + 2 \times \text{time (zone 2)} + 3 \times \text{time (zone 3)}$ . Additionally, the time it took until the test fish left the starting zone for the first time was noted. Furthermore, as predator inspection behaviour is characterized by repeated moves towards a predator, alternating with moves away from the predator (Dugatkin & Godin, 1992), the number of visits to choice zone three was noted. For analyses, the SPSS 15.0 statistical package was used ([www.spss.com](http://www.spss.com)). As most time values significantly deviated from normal distributions according to Shapiro–Wilk tests, non-parametric statistics were applied. To compare the three groups (gravid females, non-gravid females and males), Kruskal–Wallis tests were conducted first. When they revealed significant differences, Mann–Whitney  $U$ -tests were used to compare the groups.  $P$ -values are two-tailed throughout.

## RESULTS

The preference index differed significantly between gravid and non-gravid females and between non-gravid females and males (Table I). The detailed analyses of the

TABLE I. Statistical analyses of predator-inspection behaviour of gravid (Gf;  $n = 32$ ) and non-gravid (F;  $n = 30$ ) female and reproductive male (M,  $n = 17$ ) *Gasterosteus aculeatus* towards an *Oncorhynchus mykiss*. The preference index, the time spent in the starting zone, and in zones one, two and three (in the order of decreasing distance from the predator) as well as the time span until first leaving the starting zone, and the number of visits in zone three (closest to the predator) were compared among the three groups (Kruskal–Wallis test) as well as pair-wise with Mann–Whitney  $U$ -tests (M–W  $U$ ). Significant ( $P < 0.05$ ) differences are given in boldface;  $P$ -values after sequential Bonferroni correction are given in parentheses

	Kruskal–Wallis test		M–W $U$ : Gf v. F		M–W $U$ : Gf v. M		M–W $U$ : F v. M	
	$\chi^2$	$P$	$z$	$P$	$z$	$P$	$z$	$P$
Preference index	8.05	<b>&lt;0.05</b>	-2.63	<b>&lt;0.01</b>	-0.49	>0.05	-2.0	<b>&lt;0.05</b>
Time starting zone	6.95	<b>&lt;0.05</b> (0.093)	-2.49	<b>&lt;0.05</b>	-0.33	>0.05	-1.80	>0.05
Time zone one	3.67	>0.05 (0.16)	—	—	—	—	—	—
Time zone two	4.96	>0.05 (0.16)	—	—	—	—	—	—
Time zone three	7.81	<b>&lt;0.05</b> (0.08)	-2.53	<b>&lt;0.05</b>	-0.42	>0.05	-2.08	<b>&lt;0.05</b>
Time till leaving starting zone	1.34	0.51	—	—	—	—	—	—
Number of visits zone three	19.98	<b>&lt;0.001</b>	-3.08	<b>&lt;0.001</b>	-0.10	>0.05	-3.69	<b>&lt;0.001</b>

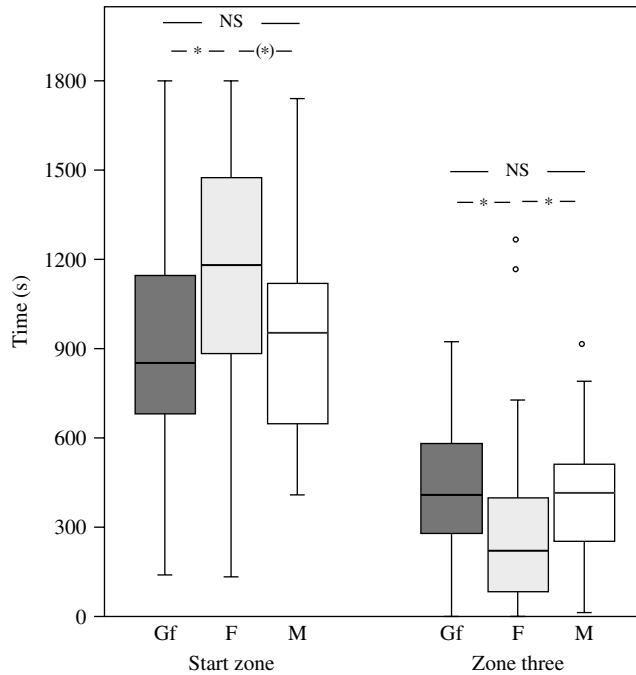


FIG. 2. The time test *Gasterosteus aculeatus* (Gf, gravid females; F, non-gravid females; M, reproductive males) spent in the starting zone furthest from the predator and in zone three closest to the predator during the 1800 s test period. Plotted are medians, quartiles, percentiles and outliers. \*,  $P < 0.05$ ; (\*),  $0.05 < P < 0.10$ ; NS,  $P > 0.10$ .

respective choice zones revealed significant differences in time test fish spent in the starting zone and in the choice zone next to the predator (zone three) between the three groups (Table I and Fig. 2). Gravid females spent significantly less time in the starting zone than non-gravid females (Table I and Fig. 2). The result of males pointed in the same direction, although it failed significance (Table I and Fig. 2). Gravid females and males did not differ significantly (Table I and Fig. 2). Furthermore, gravid females and males spent significantly more time in choice zone three than non-gravid females (Table I and Fig. 2). Again, gravid females and males did not differ significantly in their behaviour (Table I and Fig. 2). There were no significant differences in the median time the three groups spent in choice zones one and two or in the time until they left the starting zone for the first time (Table I and Fig. 2). The number of visits in zone three differed significantly between gravid females and non-gravid females and between non-gravid females and males (Table I and Fig. 3).

The three groups differed significantly in  $L_S$  (ANOVA,  $F_{2,74}$ ,  $P < 0.01$ ). Both gravid (mean  $\pm$  s.d.:  $5.7 \pm 0.5$  cm) and non-gravid females ( $5.5 \pm 0.4$  cm) were significantly larger than the males ( $5.2 \pm 0.3$  cm) (Tukey's test, both  $P < 0.05$ ), whereas they did not differ significantly from one another ( $P > 0.05$ ). Furthermore, groups differed significantly in  $K$  ( $F_{2,74}$ ,  $P < 0.001$ ) with non-gravid females possessing the lowest condition (gravid females  $1.6 \pm 0.2$  v. non-gravid females

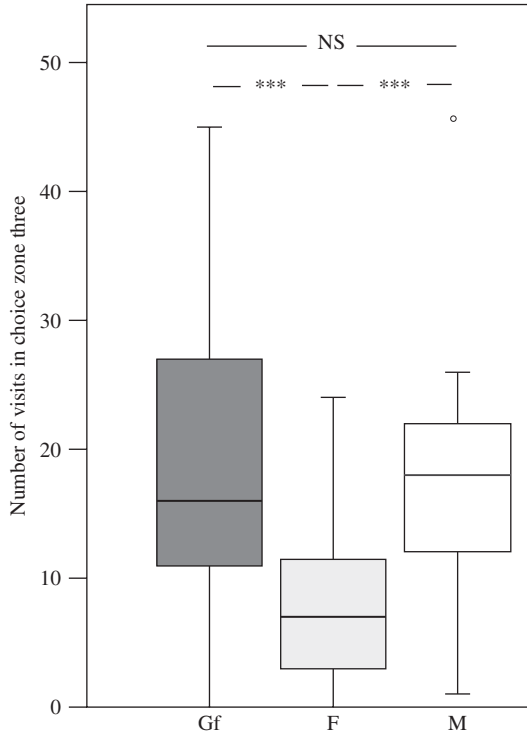


FIG. 3. The number of visits of test *Gasterosteus aculeatus* (Gf, gravid females; F, non-gravid females; M, reproductive males) in zone three closest to the predator. Plotted are medians, quartiles, percentiles and outliers. \*\*\*,  $P < 0.001$ ; NS,  $P > 0.05$ .

$1.3 \pm 0.3$ ,  $P < 0.001$ ; gravid females *v.* males  $1.4 \pm 0.1$ ,  $P < 0.001$ ; non-gravid females *v.* males,  $P > 0.05$ ).

## DISCUSSION

Gravid females are known to face a higher risk of predation, mainly because their manoeuvrability is reduced and they are more conspicuous than non-gravid females. Accordingly, they often show an altered antipredator behaviour. As predator inspection is a behaviour known to be risky (Milinski *et al.*, 1997), it was expected in this study that gravid *G. aculeatus* would show a reduced inspection behaviour and would hide longer in the plant (Rodewald & Foster, 1998). The complete opposite, however, was the case: compared with non-gravid females they spent significantly less time in the starting zone and remained significantly longer in the choice zone next to the predator. Additionally, they visited the choice zone next to the predator more often than their non-gravid conspecifics. An explanation for this pattern might be that there is a trade-off between staying in the safety of a hiding place at some distance from the predator receiving limited information about it, and leaving the hiding place gathering more detailed information about the predator's identity, precise location or motivational state. As gravid females are slower swimmers, it might



be of importance for them to have more detailed information about the predator than non-gravid ones. A comparable result was found in *P. reticulata*, where females show an increased predator-inspection behaviour compared with males, which is understood to be a result of their higher risk of being preyed upon (Magurran & Nowak, 1991). Alternatively, an inspecting individual might demonstrate its alertness, defensive mechanisms or flight abilities to the predator ('attack deterrence hypothesis'; Hasson, 1991). Godin & Davis (1995) showed that acara cichlids *Aequidens pulcher* (Gill) were significantly less likely to prey upon *P. reticulata* that showed inspection behaviour than on those that did not. This pattern, however, was not found in pike *Esox lucius* L. hunting on *G. aculeatus* (Milinski *et al.*, 1997). In the present study, the presentation of improved escape behaviour is hard to imagine, as gravid females show reduced manoeuvrability.

As egg production is energetically costly (Wootton & Evans, 1976), it is probable that gravid females were more willing to take risks because their metabolic rate and nutritional requirements are higher. Indeed, it has been shown that hungry fishes are willing to take higher risks while foraging than satiated ones (Godin & Crossman, 1994; Barber *et al.*, 1995). The food provided in half of the experiments, however, was ignored by both gravid and non-gravid test fish, thus contradicting the increased nutritional requirements of gravid females under the test conditions. A further explanation might be that gravid females have to lay their eggs within a short time frame in a male's nest, otherwise, eggs are laid spontaneously without being fertilized by a male. Thus, they have to find a mate within a couple of hours. Consequently, the price of being cautious might be a loss of the present clutch. Finally, the reason for gravid females to be bolder might be that in their original pond bolder individuals were more likely to be successful in food competition and were, thus, more likely to come into reproductive condition. To avoid that potential problem, all fish were caught before the production of eggs started. In their holding tanks, fish were fed in excess, which means that unconsumed food had to be removed the next day. Consequently, shy females had the same access to food as bold ones. As food quality was standardized (only *Chironomus* sp. larvae), the possibility that shy individuals only fed on low quality food was also excluded. Therefore, there is no reason to believe that only bold fish produced eggs. Furthermore, experiments took place during a time span of 3 months, which covers a large part of the breeding season of *G. aculeatus*. It can, thus, be excluded that only females, which were able to produce a clutch early in the season (because they were able to produce eggs faster as a result of any correlation between egg production and boldness), were used as gravid test fish. *Gasterosteus aculeatus* used in the experiments were taken from an annual population. During the experiments, gravid and non-gravid females were equally close to the end of their lives, making age an improbable explanation for the results.

Boldness of gravid females was comparable with that of reproductive males. As males show a high level of aggression (Bakker, 1986) and an increase in boldness (Huntingford, 1976) during their reproductive phase, this result was somewhat unexpected. Both males and gravid females, however, face a higher risk of predation than adult, non-reproductive fish (Frommen *et al.*, 2009). Therefore, it is conceivable that both might aim at receiving a comparable amount of information about the predator and, thus, show a similar amount of inspection behaviour. Another important point might be that gravid females and reproductive males are solitary, whereas non-reproductive males and non-gravid females form shoals (Wootton, 1984). In shoals,



predator inspections are often done in pairs (Milinski *et al.*, 1990, 1997), thereby reducing the predation risk per individual. Gravid females and reproductive males do not have this option and have to perform the predator inspection visits alone. They are thus 'forced' to take higher risks when they want to collect information about the predator.

In summary, this paper shows that reproductive state relates to the predator-inspection behaviour of female *G. aculeatus*. Gravid females were significantly bolder than non-gravid ones.

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