

**Habituation of Predator Inspection and Boldness in the Guppy
(*Poecilia reticulata*)**

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Abstract---This study examined habituation of the predator inspection behavior in the guppy (*Poecilia reticulata*) and its relationship with boldness (open field locomotion). Two different strategies were discovered: (1) initial inspection of a predator-like fish, correlated with boldness; (2) subsequent surveillance, governed by a random underlying process and unrelated with boldness. The surveillance inspection is probably linked with anti-predator vigilance. Possible implications to between-population variation in inspection behavior are discussed.

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The predator inspection behavior occurs when a fish approaches a potentially dangerous predator-like object. In a typical case, one or several individual fish leave the school, move to the predator, observe it, and then go back (Pitcher *et al.*, 1986; Magurran and Pitcher, 1987). Approaching predator involves high risk of being eaten, and the basic benefits of predator inspection include acquiring information about the predator (Magurran and Girling, 1986; Magurran, 1990), its motivational state (Licht, 1989), and deterring the predator's attacks (Godin and Davis, 1995), although there may be other benefits such as advertising the inspector's quality to potential mates (Godin and Dugatkin, 1997; see Dugatkin and Godin, 1992 for a costs-benefits review). Thus, inspection behavior plays an extremely important role as a predator avoidance mechanism in natural environments (Magurran, 1990). Indeed, fish from populations with high predation risk show higher tendency to inspect predators (Magurran and Seghers, 1990, 1994; Magurran *et al.*, 1992, 1995).

Because predator inspection is dangerous, it may be associated with boldness, i.e. propensity to take risks (Wilson *et al.*, 1994, Budaev, 1997a). However, the fish from populations with higher predation risk show many signs of shyness² (slower habituation of anti-predator responses, more pronounced responses to the alarm substance, higher schooling tendency, etc.) but simultaneously

² Shyness is the opposite of boldness, i.e. the propensity to avoid risk (Wilson *et al.*, 1994).

have higher tendency to inspect (reviewed by Magurran *et al.*, 1992, 1995). Also, sticklebacks (*Gasterosteus aculeatus*) of higher social rank (presumably bolder) showed as low tendency to inspect as subordinate (presumably the most timid) ones, and the best inspectors were fish with intermediate rank (McLeod and Huntingford, 1994). Individual differences in predator inspection were not consistent in the guppy (*Poecilia reticulata*) and correlated with exploration but not fear (Budaev, 1997b). Thus, the relationships between predator inspection and boldness are not completely clear: predator inspection might be associated with boldness or shyness. In this study we examined the relationships between predator inspection behavior and boldness in the guppy (*Poecilia reticulata* Peters). We tested how this relationship changed with habituation of the fish to the predator-like object during repeated encounters.

MATERIALS AND METHODS

Twenty-nine adult male guppies (average standard length 1.8 cm) were used in the present experiments. During the experiments, the fish were kept individually in four 100 l aquariums divided by partitions into twelve compartments. The fish were fed daily with commercial fish flakes.

Each individual was tested for boldness in the open field test. In

the guppy and several other fish species, open field behavior is correlated with both exploration and lack of fear (Budaev, 1997b; Budaev *et al.*, 1999). This test was performed in a hexagonal tank 0.9 m in diameter, and a 60 W lamp was suspended 1.5 m above. A coordinate grid 10x10 cm was marked on the bottom of the tank to record the locomotor activity of the fish. During the testing, the fish was gently released into a white bottomless opaque plastic cylinder, standing in the center of the open field. After two minutes of acclimation, the cylinder was lifted and the behavior of the fish was observed from above during 5 minutes. We recorded locomotor activity of the fish, that is, the number of crosses of the coordinate grid per minute.

One month after the open field test, the guppies were tested in a test of the inspection behavior. It was conducted in an aquarium (60x30x20 cm) divided into three unequal compartments. The model predator (convict cichlid *Archocentrus nigrofasciatus*), was placed into a small compartment at the flank of the aquarium, separated by a glass wall from the big central compartment. At the opposite end of the aquarium there was the third small compartment, separated by an opaque partition with a door. The tested guppy was released into the last compartment for five minutes, then we opened the door, and when the fish moved to the central compartment, we observed it for five minutes. Each guppy was tested in this test three times during three consecutive days. We

recorded (1) the number of predator inspections (frequency per minute), (2) the percentage of the time spent inspecting, (3) the percentage of the time spent in proximity (<6 cm) of the compartment with the model predator, and (4) the latency to the first predator inspection visit.

For the statistical analysis, we calculated Friedman ANOVA, sign test, and Spearman correlations (Sokal and Rohlf, 1981). In cases of behavioral latencies, we used statistical methods of survival analysis (Lee, 1992).

RESULTS

The percentage of time spent inspecting and the frequency of predator inspections exponentially reduced with repeated encounters of the cichlid (Fig. 1 a,b). This is the most commonly observed pattern of decline of a behavioral response to the constant stimulus (see Hinde 1970). The time spent in proximity of the model predator also decreased, but only in the last exposure (Fig. 1c): it did not differ significantly from the first to the second exposure, but significantly reduced in the third exposure (the differences with the first two days: sign test, $p < 0.05$). Thus, the guppies changed their behavior in a way indicating habituation of predator inspection, well documented in the

literature (Csanyi, 1985; Magurran and Girling, 1986; Csanyi *et al.*, 1989; Huntingford and Coulter, 1989).

The distribution of the latency to the first inspection significantly deviated from exponential distribution in the first and second days of testing, but not in the third day (Table 1). Kaplan-Meier analysis indicated, that during the first day, the fish tended to inspect the model predator very soon after they entered the central compartment (median latency to inspect = 32 s, i.e. almost 50% of guppies begun to inspect the convict cichlid within the first 1/2 min of the test). The exponential distribution of the inspection latency during the third day means that the probability of the decision to inspect (hazard rate) did not depend on the time elapsed since the guppy entered the compartment with the model predator. The estimates of the hazard rate reduced from the first to the third day of testing (Table 1). In other words, the guppies tended to inspect the convict cichlid rapidly during the first day, but at the third day, the decision to approach the model predator become governed by a single underlying random process, similar to radioactive decay with low probability.

Correlations between the percentage of time inspecting in different days were all non-significant ($p > 0.1$, the maximum correlation was between the second and third days, Spearman

$r_s=0.34$, exact $p=0.11$). Correlations between the frequency of inspections were significant only between the second and third days (Spearman $r_s=0.40$, exact $p=0.031$, all other $p_s>0.1$). The percentage of time spent near compartment with the model predator did not correlate across days (all $p_s>0.1$).

Open field locomotion (boldness) correlated with the inspection behavior measures only in the first day of the inspection testing (Table 2). The same pattern was also characteristic of the latency to inspect: during the first day of testing, there was a significant positive relationship between the inspection latency and open field locomotion (beta=7.3, $p=0.016$, Cox proportional hazard regression), but during the second and third days, this relationship was not significant ($p>0.1$, Cox proportional hazard regression model).

DISCUSSION

This study showed that the predator inspection is a relatively complex behavior, in which at least two strategies can be distinguished. The first is exploratory predator inspection, which is associated with boldness and appears first when a predator-like fish is found in proximity. The second strategy of predator inspection is not related to boldness and may be tentatively called surveillance. In the latter case, the fish does not

approach and inspect the predator-like fish shortly after it is detected. Rather, the inspection tendency is described by a random process similar to the radioactive decay. Apparently, surveillance looks similar to patrolling (Birke and Archer, 1983; Cowan, 1983) observed in cases of spatial exploration, when an animal is actively searching for changes in a known environment rather than explores a novel one. In this way, the inspection visits randomly elicited in habituated guppies would be directed to monitoring possible change of the current state of the potentially dangerous large cichlid, for example, its motivation (Licht, 1989). Also, it may serve the function of signaling that the possible predator has been discovered and still is not forgotten (Hasson, 1991; Godin and Davis, 1995).

The surveillance inspection behavior may suggest that the guppies really perceived the convict cichlid as a potentially dangerous predator. Cichlid predators looking similarly are sympatric with many guppy populations in their natural sites in Trinidad (Liley and Seghers, 1975; Reznick and Endler, 1982). It is why this species could be equipped with an inherited mechanism for the recognition of unique features of predators, such as characteristic body shape, which do not disappear even after artificial breeding for several generations (Csanyi, 1985; Altbacker and Csanyi, 1990). Thus, surveillance inspection may reflect anti-predator vigilance of the fish to the possible

predator (see Lima 1994, 1995 for a discussion on anti-predator vigilance). However, it was still not associated with boldness.

Consistency of individual differences in the inspection behavior also reflects the two strategies. The initial exploratory inspection, observed during the first day of the experiment and associated with boldness, did not correlate with the surveillance inspection during the subsequent days. However, individual differences in the surveillance inspection tended to be consistent over the last two days. This also suggests, that there may be two distinct mechanisms, governing approaching of the potential prey to the possible predator. In real situations, there may be complex interactions between the boldness-related initial inspection and vigilance-related surveillance inspection.

We hypothesize, that predator inspection would be initiated by the boldest individuals in the school. However, subsequent inspections, associated with vigilance, would not necessarily be performed by these individuals. This has important implications for between-population differences in boldness and predator inspection, found in many fish species (Magurran *et al.* 1992, 1995): fish in populations with high predation pressure have lower boldness but higher propensity to predator inspection. It is likely, that the high inspection tendency of fish from heavily predated populations is based on the motivational mechanisms,

linked with vigilance rather than with boldness (i.e. it may be unrelated to the propensity to take risks). The data, indicating that fish from high-risk populations show signs of shyness during their predator inspection visits (e.g. they avoid the dangerous mouth region of the predator, inspect mainly in groups rather than individually, keep at a greater distance from the predator, etc., see Magurran and Seghers, 1990, 1994) seems to agree with this hypothesis. However, further experiments, comparing boldness-related and surveillance predator inspection in fish from populations with different predation pressure are necessary.

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Table 1. Fitting exponential distribution to the latency of the first inspection

Day of testing	Lambda±SE
1	0.0066±0.0022, $\chi^2=23.99$, df=8, p=0.002
2	0.0028±0.0011, $\chi^2=19.90$, df=8, p=0.011
3	0.0008±0.0004, $\chi^2=4.84$, df=4, p=0.304

Lambda (hazard rate) is the parameter of the exponential distribution.

Table 2. Correlations between inspection behavior and open field locomotion

Measure	Day of testing		
	1	2	3
Percentage of time inspecting	0.44*	0.11	0.05
Frequency of inspections	0.48**	0.12	0.05
Percentage of time spent near predator	0.43*	-0.19	0.15

*p<0.05, **p<0.01

Figure Captions

Figure 1. Characteristics of the inspection behavior over the three days of testing. The percentage of time inspecting and the frequency of inspections are given in logarithmic scale.

