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Does predation risk affect body size and shape ontogeny in the silver barb *(Barbonymus gonionotus)*?

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Abstract

We studied here the effect of predation risk on size and shape during the development of the Cyprinid fish *(Barbonymus gonionotus)*. In the experiment, juvenile silver barbs (*Barbonymus gonionotus)* were developing either together or not together with the predator snakehead fish *(Channa striata)* during 25 days. Predation was limited by isolating the predator from the silver barb with a net. In replicated trays, 60 fish were randomly selected and compared before and after the experiment in presence and absence of the predator. The experiment was replicated three times. Fourteen landmarks were recorded on the fish body and a generalized Procrustes superimposition was performed. Analyses of variance and linear discriminant analyses were used to detect effects of the predator presence on body shape and growth pattern. Results show that if presents, effect of the predator on size and shape evolution in silver barbs is very subtle. A small increase of size and a decrease in relative caudal peduncle height could be reported in all cases suggesting either that the predator could exert directional selection or that developmental plasticity induced by the predator was present. In the case of shape, this developmental plasticity appears to be maladaptive in the experiment because the predator may have selected for these shape attributes in the tank showing the highest predation rate. Finally we found that shape variation decreased with development suggesting that phenotypic canalisation was acquired during the ontogeny of the fish but that size differences among individuals were accumulating with ontogeny.

Keywords: developmental plasticity, predation, fish

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1. Introduction

It has long been known that adaptive phenotypic plasticity might be advantageous when it allows a genotype to have a broader tolerance to environmental conditions and hence higher fitness across multiple environments including interaction with predators (see review in [1]). Several examples in plants or animals have shown that predation risk could induce phenotypic changes during development due to adaptive phenotypic plasticity or local adaptation [2 – 6]. Trinidadian guppy fish (*Poecilia reticulata*) are well studied in this regard. For instance, it was reported that male fish that lived upstream and exposed to low predation risk were more colourful or brighter and larger in size than the male fish living downstream exposed to high predation environment [7]. In this fish species, predation risk can also alter metabolic rate and growth rate [8]. It has been shown that predation risk can indeed generate change in body shapes for the Trinidadian guppy [9]. However, few studies have been addressed in the laboratory, where predation and generations can be controlled, and it is invariably difficult to attribute the relative importance of phenotypic plasticity and local adaptation for understanding the role of predation risk.

In this study, we investigated whether the presence of a predator was altering size and shape changes in the silver barb (*Barbonymus gonionotus*). *Barbonymus gonionotus* is a common native Cyprinid fish in South-East Asia [10, 11] and it is found in rivers, streams, floodplains and occasionally in reservoirs, although it is likely that this Cyprinid species prefers standing water habitats to flowing waters [12]. Furthermore, this fish is one of the most important freshwater species in commercial, aquaculture and inland fisheries [13, 14]. In Thailand, reintroduction program of this fish species has been implemented ev-

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ery year in river and natural habitat by Department of Fisheries when there are special occasions [15]. In nature, the silver barbs, especially juveniles, are exposed to several predators (both terrestrial vertebrate predators and aquatic fish predators), including the common and native snakehead fish (*Channa striata*) [16]. In the laboratory, it was shown that the snakehead fish was a predator for the juvenile silver barb [17]. If adaptive phenotypic plasticity as response to predation risk is present in the juvenile silver barb (*Barbonymus gonionotus*), we would expect changes in size and shape depending on the presence of the snakehead fish (*Channa striata*) as a predator.

2. Method

2.1. Experimental design

About 1800 juvenile silver barb fish were obtained from commercial farm (Chalong Pun Pla, Chachoengsao Province, Thailand WGS84: N 13° 34’ 13.7”E 101° 7’ 23.0”) in October 2017. Because fertilisation is under control in the farm by using a few males and females for mating over one night, age of the fish was the same for all fish when they arrived at the laboratory, Department of Aquatic Science, Faculty of Science, Burapha University (27 days after fecundation). Furthermore, because fertilisation is external in Cyprinids and several males were used during the breeding process, the obtained population was outbred. Six pools of fish were obtained from the same stock of the farm and then placed in six circular independent tanks with 300 litres of water and with air pumps to aerate each tank. The number of fish at the beginning of the experiment was approximately the same in each tank (around 300). In three of these tanks (treatment: T1, T2 and T3), fish were exposed to a predator (the snakehead fish) whereas the other three tanks had no predators (control: C1, C2 and C3). Fish were fed ad libitum during the whole experiment twice a day in the morning and in the afternoon with commercial dried artemia. For avoiding active predation on fish, the predator was isolated from the silver barb by a net. However, the net was large enough to allow the silver barb to go in the compartment of the predator. Therefore, it was not impossible that a few fish were eaten by the predator. Sixty fish were taken at random in every tank and anaesthetized with clove oil and digitized at equal focal distance at the beginning and at the end of the experiment (25 days after). Since photographed fish were exposed to clove oil, they were removed from the original sample. At the end of the experiment, the number of remaining fish was counted to provide an estimate of mortality rates (Table 1). Fish were photographed after the experiment and timing of the photography was recorded to know whether there was potential variation within the day.

2.2. Quantitative shape analysis and statistical methods

In order to quantify shape variation among tanks and changes before and after the experiment, fourteen landmarks were digitized on the fish body (Figure 1). In total, 720 fish were digitized. In addition, 12 fish were photographed anaesthetized and photographed two times in order to have an idea of percentage measurement error due to digitization bias (anaesthesia, photography and digitization) by comparison to interindividual variation within a tank. Shape components and size were later obtained by the use of a partial generalized Procrustes analysis on digitized coordinates [18]. In this analysis, all fish configurations of landmarks were scaled to unit centroid size, translated on their centroid and rotated in order to remove information due to scaling, position and orientation. The superimposed coordinates were later projected onto the tangent shape space so that they could serve as shape variables in further analyses [18]. Procrustes superimposition and projection was done in R [19] using the functions developed by Claude [20].

Percentage measurement error for size and shape was obtained by following the ANOVA procedure [21] adapted for Procrustes data (see [20, 22]). More precisely, for size, expected variance due to error and individual variation was assessed from the ANOVA and was summed up; the ratio of expected variance due to error on the sum of expected variances provided the percentage of measurement error. Shape measurement error was obtained similarly by using a Procrustes ANOVA [23]. In that case, traces of expected variances were taken into account.

Pairwise proportion tests (Chi squared test) were used to estimate different mortality between tanks at the end of the experiment. In order to have an idea of selection, we used the mortality rate in control tanks (tanks without predators) and compared that mortality rate with the one estimated in the tanks containing a predator.

In order to estimate size and shape changes before and after the experiment, we performed mixed linear models [24]. For size, which is univariate, we tested whether differences could be reported before and after the experiment, whether size changes was greater or smaller in the group exposed to the predator by comparison to the control group, and whether these differences are replicable among groups. In the model, time was therefore considered as a fixed effect (before and after the experiment), treatment (with or without predator) as a fixed effect, the interaction between these two effects as a fixed effect, and tank as a random effect. The full model is, therefore, on the form size ∼ time x treatment (fixed effects) + tank (random effect). Significance of effects was assessed by using Akaike Information Criterion (AIC). Differences at the end of the experiment were tested by AIC and LRT test using group as random effect and models were run with the

Table 1. Initial number of fish in each tank and mortality during the experiment.

	Initial number of fish	Mortality during the experiment
tank C1	298	12
tank C2	298	55
tank C3	298	12
tank T1	300	29
tank T2	291	46
tank T3	290	18

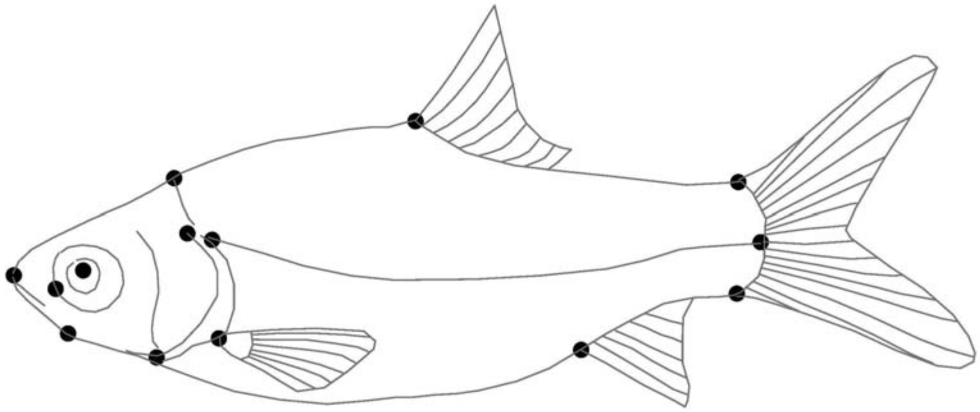


Figure 1: Location of the fourteen landmarks used to quantify shape variation.

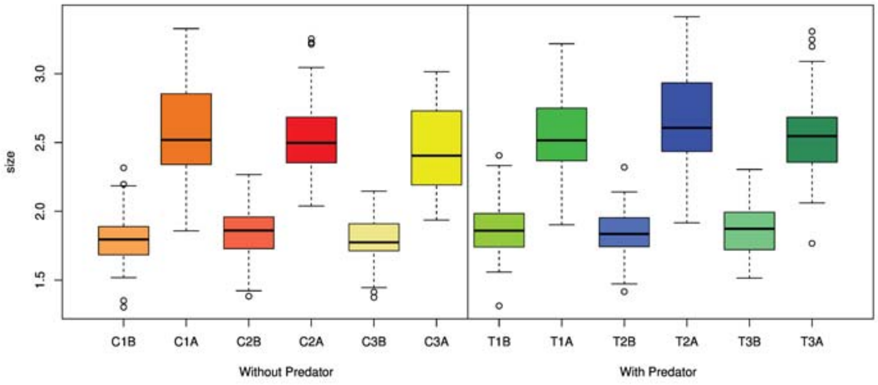


Figure 2: Box plot of size variation among tanks and with and without the predator effect. Letter C refers to tanks without a predator and letter T refers to tanks with a predator. Letter B refers to measurement before the experiment and letter A refers to measurement after the experiment. The numbers correspond to individual tanks. Size is expressed in centroid size (unit is cm.)

package lme4 in R [24]. We also checked for differences at the end of the experiment by comparing size between each tank receiving a predator at the end and control (for this we pooled size in the 3 tanks that were not receiving any predator). For shape, because there are much more variables than tanks, differences were estimated similarly by using an ADONIS based on mean shape for each tank (permutational MANOVA using euclidean distances among groups). We first computed a mean-shape for every tank and then analysed whether there was any effect of time, treatment or interaction on shape variation with the package vegan [25]. We also tested for shape differences between each tank that received a predator and pooled control tanks at the end of the experiment.

In addition to these statistical analyses, we performed canonical variate analysis on shape data and between group PCAs. In these analyses, tanks were used as group and the ordination between groups gave an idea about the importance of treatment and treatment x time interaction. We expected to find less variation among groups at the beginning of the experiment than at the end since fish were naive to presence of the predator at that time. Related shape variation was reconstructed on PC or CVA axes following procedures of Claude [20, 22]. In CVA, to obtain sketches of shape variation, linear discriminant function had to be rescaled according to intragroup variation. In addition, we pooled all the fish in the control tank from the end of the experiment to compare them to each of the tank that was receiving a predator in terms of size and shape. This last strategy allowed us to check whether the predator could have an effect on shape variation for each tank. We performed ANOVAs for size and MANOVAs for shape. For shape, since matrices were not singular, we used the non-null PCs as variable instead of Procrustes coordinates [20, 22].

We finally estimated any role of mortality rate on shape and size by applying regression (univariate for size, multivariate for shape) on tank mean size and mean shape or on tank mean size variance or tank mean shape variance (taken as the trace of variance covariance matrices for shape in every tank).

3. Results

Size and shape measurement errors were reaching 0.5% and 48.5% respectively. In both cases, interindividual variation was larger than intraindividual variation. For shape, that value means that on average difference between two individuals was about two times larger than difference between replicates on the same individual.

Mortality rates among control experiments showed no difference between tank C1 and C3 but there was an increase in tank C2 (p-value < 0.0001). Mortality in tank T2 was stronger than in tank T1 (p-value = 0.019) and T3 (p-value <0.001), whereas there was no significant difference between T1 and T3. When comparing mortality in tanks with a predator with mortality rate in all control tanks (7.8%), we found an increase only in tank T2; which showed that selection could play a role in this tank only.

When considering all fish, the treatment and time had an effect on size but the interaction were not significant (Figure 2). However, treatment was found to decrease the AIC when considering only tanks at the end (AIC=196.84 against 197.58) although the LRT test failed to be significant (p-value = 0.098). In average, sizes were increasing when fish were exposed to the predator. Significant size differences among tanks were noticed at the beginning of the experiment, showing a slight increase of size for fish that were also exposed to the predator. By analysing the timing of the pictures, we could notice that fish measured at the beginning of the experiment were just receiving food in the control group by comparison to the treatment, and that fish measured in the group exposed to the predator were measured about 12 hours after the control. Since centroid size summarizes increase of size in all directions, this result rather suggested that size variation among groups at the beginning of the experiment was more an artifact coming from the timing of fish feeding (fish just fed were larger just after their meal) and from the fact that tested fish had a bit more time to grow (the difference in size was about 15 times smaller than the averaged difference in size between the beginning of the experiment; therefore that interpretation can make sense.). Because fish were measured in the same order at the end of the experiment, a similar effect could play also at the end of the experiment, but it is more unlikely since growth was much more advanced at that time. When pooling the size of all control fish together and comparing it with fish in every tank containing a predator, we found an increase of size in all tanks but that increase was significant only in tank2 (Table 2). We did not find significant relationship between size and mortality rate (r = 0.54, p-value = 0.27) even if the relationship was positive. There was no relationship between size variance and mortality rate as well (r = 0.10, p-value = 0.85). Finally, we found a significant increase of size variance in average for every tank from the beginning to the end of experiment (Table 6, Figure 7).

We found significant shape differences between the beginning and the end of the experiment (certainly resulting from allometric growth) (Table 3). These shape changes concerned changes in head to body proportion as well as change in the position of the deeper body (more backward with age) as seen on the first axes of the between group PCA (Figure 3) or on the canonical variate analysis (Figure 4). The ADONIS performed on mean shape among tanks showed a significant effect of time, a marginal effect of treatment (probably artifact of time in the day of picture -see before-), but there was no interaction meaning that the

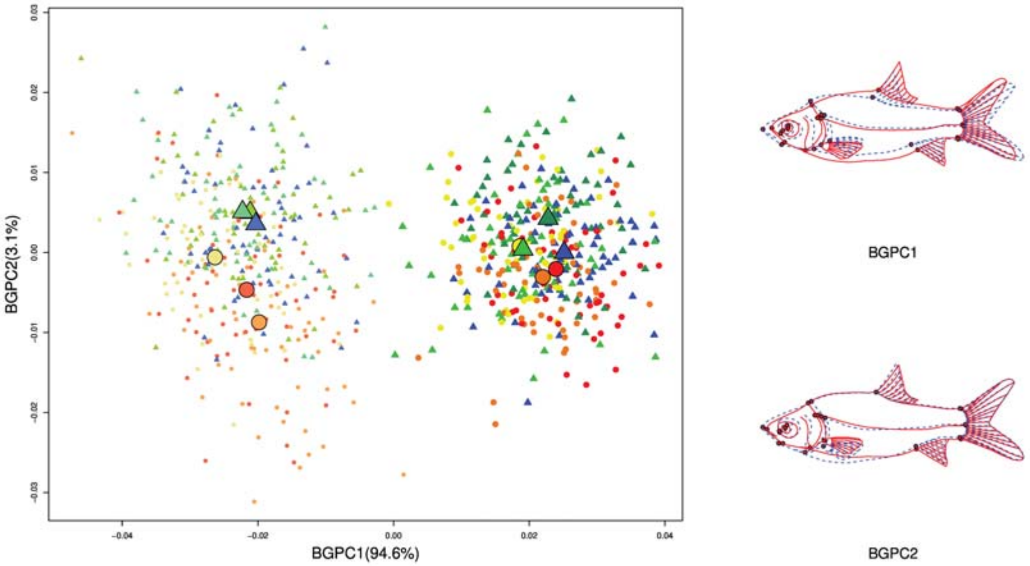


Figure 3: Between-group PCA. Colours of symbol are defined as for figure 2: upper triangles are for fish shapes in the presence of the predator, circles when the predator was absent. On the lateral side of the plot, we represented extreme shape change on each axis. The blue shape corresponds to the warped shape for the minimal value on the axis, and the red one to the maximal value on the axis

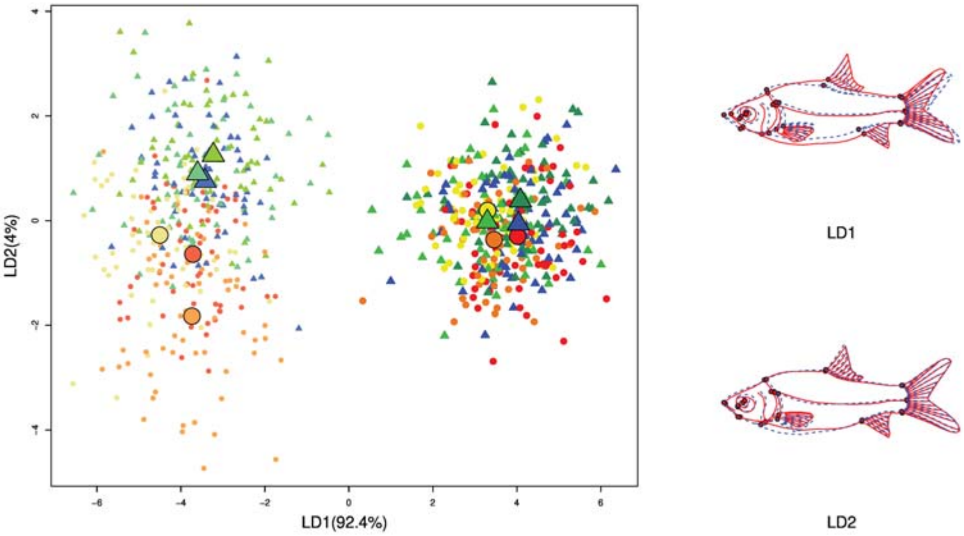


Figure 4: Canonical variate analysis among tanks before and after the analsysis: colours and symbols as for figure 3. The sketch on the lateral side of the plot shows extreme shape differences along each axis (red: maximal score; blue: minimal score).

Table 2. Relationship with size against time, presence or absence of a predator (treat), and tank as random variable. We provide parameter degrees of freedom as well as AIC (Akaike Information Criterion) for each model.

model	DF	AIC
Size ~ time × treat + (1|group)	6	94.734
Size ~ time + treat + (1|group)	5	93.08
Size ~ treat + (1|group)	4	859.2
Size ~ time + (1|group)	4	95.81
Size ~ 1 + (1|group)	3	860.26

Table 3. Pairwise differences in size between tanks that received a predator and tank without a predator (control).

Group	n	Average size after the experiment	ANOVA (control versus treated tank) F value	P-value
Control	180	2.515	-	-
Versus Tank 1	60	2.549	0.54	0.465
Versus Tank 2	60	2.643	7.11	0.008
Versus Tank 3	60	2.558	0.85	0.357

Table 4. ADONIS on fish shape against time (beginning/end of the experiment), treat (with or without the predator). In this analysis, shape was averaged for every tank to avoid pseudoreplicaiton bias.

effect	DF	SSQ	F value	P-value
Time	1	0.00577	207.61	< 0.001
Treat	1	0.00012	4.291	0.09
time:treat	1	0.00005	1.89	0.14
Residuals	8	0.00003		

Table 5. Pairwise MANOVA between control and each tank that contained a predator at the end of the experiment.

	Pillai	Approx F	P-value
Tank 1 and control	0.216	2.467	<0.001
Tank 2 and control	0.165	1.77	0.02
Tank 3 and control	0.358	4.994	<0.001

exposure to the predator had no significant effect on shape changes or that it was subtle by comparison to error variation (Table 3). The between group PCA (Figure 3) and CVA (Figure 4) showed that there was more differences among tanks in the beginning of the experiment than at the end; suggesting that the presence of the predator was not altering shape variation in the fish. Variation at the beginning of the experiment was mostly found on the second axes. Shape variation here corresponded to change in belly curvature, which was certainly related with the timing related to fish feeding. Furthermore, a PCA on all individuals was performed (not shown) and suggested that interindividual variation decreased with age, possibly as resulting from a canalisation effect. This was later confirmed by a crossed two factor ANOVA on shape variance for each tank (Figure 8, Table 6).

When applying a CVA on the 6 tanks at the end of the experiment, differences between tanks with and without the predator appeared on the first axis but there were considerable overlaps (Figure 5). Fish exposed to the predator tended to show a narrower caudal peduncle and a smaller head by comparison to body (Figure 5).

Taken individually, we looked at differences between each tank that received a predator and all the fish coming from tanks without predators (Figure 6). We found significant differences between all tanks and the control (Table 5). Interestingly, in all cases, we found some common patterns associated with the presence of the predator; this included the shape of the caudal peduncle (narrower when a predator was present), and the relative length of the head compared to the body (shorter when the predator was present). Levels of significance or percentages of good reclassification (not given here) were not related with predation or mortality rate. For instance, in tank 2 where the predator was more active, differences were more subtle than in the two other tanks with the predators. This indicates that there was a potential shape change induced by the predator but that shape phenotypic plasticity, if present was not adaptive. In other words, the predation went against phenotypic plasticity, which means that the observed phenotypic plasticity did not confer any adaptive advantage when there was some predation. Furthermore, if phenotypic plasticity was adaptive, the predation would have been against fish which were not exhibiting a strong phenotypic plasticity response. There was no relationship between final shape and mortality rate (ADONIS: F=1.022, p-value = 0.44) and no correlation between final shape variation and mortality rate (r= 0.4117, p-value = 04173).

4. Discussion

When taking tank as a random effect, size changed when the predator was present butit was not signif-

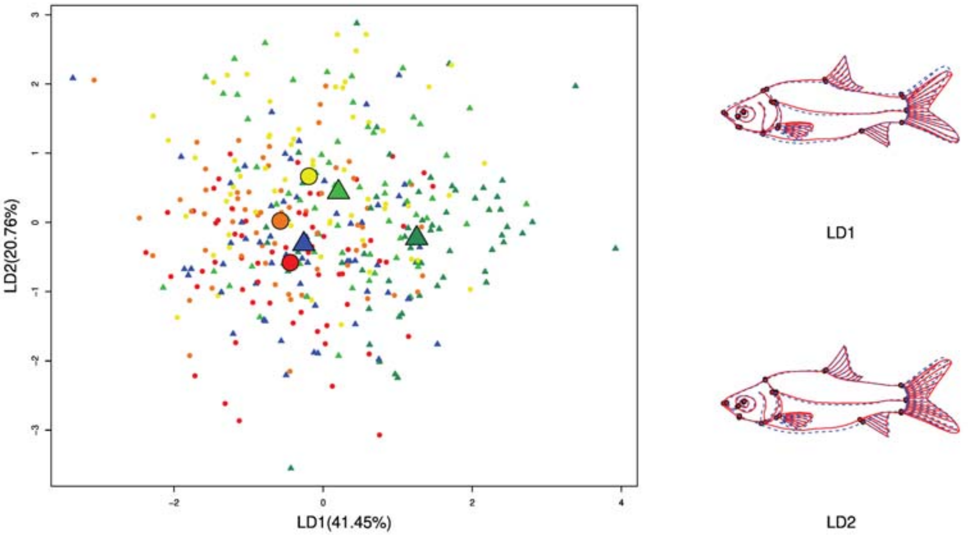
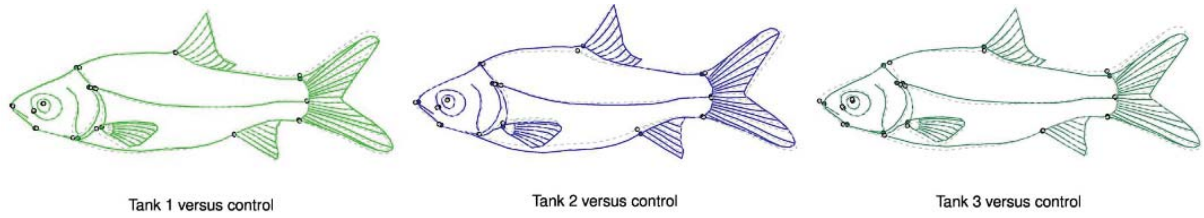


Figure 5: Canonical variate analysis among the fish sampled from the six tanks at the end of the experiment.


Tank 1 versus control

Tank 2 versus control

Tank 3 versus control

Figure 6: Warped mean shape for fish that received a predator (dotted line) against warped mean shape for fish that received a predator for tanks T1 to T3 at the end of the experiment. Colour chart follows previous figures.

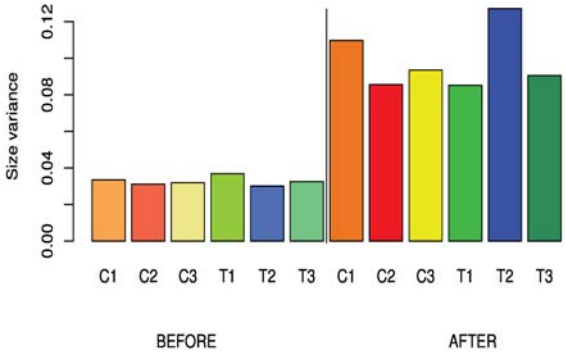


Figure 7: Barplot of size variance (in squared cm) for every tank from the beginning to the end of experiment: letters and colours as for figure 2.

Table 6. Crossed factor two way ANOVA on shape and size variance for each tank. Effects considered are the presence or absence of predator (treat), the beginning or end of the experiment (time), and the interaction (treat:time).

Size	**DF**	**SS**	**F value**	**P-value**
Treat	1	0.00002	0.13	0.72
Time	1	0.01307	76.06	< 0.001
treat:time	1	0.00001	0.06	0.81
Residuals	8	0.00017		
Shape	**DF**	**SS * 1000000**	**F value**	**P-value**
Treat	1	0.00034	0.29	0.61
Time	1	0.15234	130.03	< 0.001
treat:time	1	0.00325	2.77	0.13
Residuals	8	0.00937		

icantly different than when it was absent. Nonetheless, there was a small increase in size in all tanks exposed to the predator, but it was significant only in tank T2. In this tank, the selective pressure exerted by the predator was higher than in other tanks and size was increased. There was therefore a potential adaptive phenotypic plasticity for size [26], which involved a faster size increase when the predator was present. This effect was, however, subtle. Since the increase was not found significant in tank 1 and 3, it might also mean that predators in these tanks were also predating on the smallest fish (although it did not seem that they were involved in an increase of mortality in these tanks) and that rather to observe adaptive phenotypic plasticity, we just observed natural selection against the smallest fish. In order to know if phenotypic changes are really related to the predation risk or to the active selection made by the predator, future experiments should manage to isolate completely predator and prey.

ADONIS failed to identify a significant effect of the predator on body shape changes (no interaction) when fish body shape were averaged by tank (Table 4). This result should be eventually interpreted with caution because the number of replicates was low and the power of the analysis might be low. However, by comparing each tank containing a predator with the control, significant shape differences were discovered. These differences seemed to involve the head proportion (smaller in fish exposed to the predator) and the caudal peduncle (narrower in fish exposed to the predator). It seems therefore that during the experiment, some body parts evolved similarly. We know few results about shape phenotypic plasticity for fish and predator cues. However, there was a study that found similar patterns compared to our results [27]. In this previous study in the Trinidadian guppies, it was found that the guppies were reared with predator cues developed longer and shallower heads than fish reared without predator cues. In other words, when the guppies reared in the presence of predator cues, they developed a more fusiform head shape.

These changes could have functional grounds and improve the maneuverability [28] and show morphological adaptation for fish with steady swimming [29]. Increasing maneuverability could be an adaptive response to escape attacks from the predator. The patterns for shape were again subtle but might show that the fish display some plasticity to respond to the predator. Interestingly, the differences between control and presence of a predator were not found in the tank having displaying the more important predation rate. If developmental plasticity is indeed induced by the predator, it seems therefore maladaptive in this case. Once again, further experiments, controlling that predation is 0 in all tanks or/and controlling the shape of the predated fish could be confirmatory. We noted an interesting patterns of size and shape variation with age in all tanks. While shape variance decreased, size variance increased suggesting that shape changes were buffered while difference in size among was individuals were increasing. The decrease in shape variation would be an evidence for developmental canalisation in shape with age [30]. The introduction of the predator had no effect on these patterns, suggesting that this pattern was likely under genetic control rather than resulting from natural selection exerted by predators.

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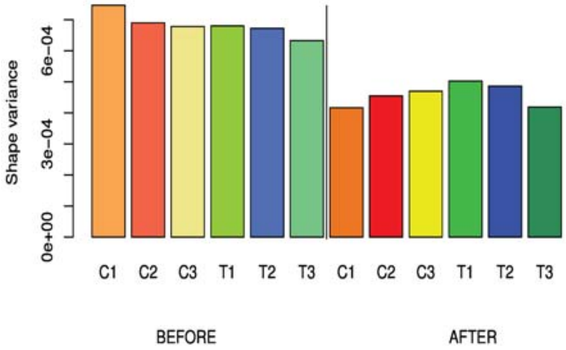

Figure 8: Barplot of shape variance for every tank from the beginning to the end of experiment: letters and colors as for figure 2.

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