Are personality variables reliable in fish? – the effects of varying personality test conditions on spontaneous behavior in a cyprinid fish species

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The aim of the present study was to test whether spontaneous movement traits usually adopted in fish personality measurement are repeatable or vary among different contexts in a common Chinese cyprinid fish species, i.e., the pale chub. The median swimming speed, percent time spent moving (PTM), head-turning rate and distance to the center of an arena with a novel object nearby (i.e., exploration measurement context) showed no difference from those measured under the control condition (i.e., activity context), suggesting that pale chubs might ignore the existence of a so-called 'novel' object. The pale chubs evaluated with a shelter nearby (i.e., under boldness measurement conditions) spent approximately 46% of their time in a shelter (the shelter occupied 20% of the total area of the arena), whereas those fish individuals also spent more time moving and had a higher median speed and head-turning rate in the open area (i.e., outside the shelter), suggesting that boldness might be a more reliable variable than exploration. Further analysis comparing the distance and time latency to explore the novel object between the exploration condition (with the novel object nearby) and control condition (in which we created a fictitious object at the same position as the same individual under the exploration condition) showed that only the amount of time it took for the fish individuals to first reach the object (or fictitious object) for exploration significantly differed between the two contexts and hence might be a potential variable indicating exploration in the pale chub in the future. All measured variables were quite repeatable across the three contexts, which suggests that the personality of pale chubs is quite conservative and that future studies might use such variables during exploration or boldness measurements and may not need to include activity measurements.

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Running title: spontaneous activity and personality

Abstract

The aim of the present study was to test whether spontaneous movement traits usually adopted in fish personality measurement are repeatable or vary among different contexts in a common Chinese cyprinid fish species, i.e., the pale chub. The median swimming speed, percent time spent moving (PTM), head-turning rate and distance to the center of an arena with a novel object nearby (i.e., exploration measurement context) showed no difference from those measured under the control condition (i.e., activity context), suggesting that pale chubs might ignore the existence of a so-called 'novel' object. The pale chubs evaluated with a shelter nearby (i.e., under boldness measurement conditions) spent approximately 46% of their time in a shelter (the shelter occupied 20% of the total area of the arena), whereas those fish individuals also spent more time moving and had a higher median speed and head-turning rate in the open area (i.e., outside the shelter), suggesting that boldness might be a more reliable variable than exploration. Further analysis comparing the distance and time latency to explore the novel object between the exploration condition (with the novel object nearby) and control condition (in which we created a fictitious object at the same position as the same individual under the exploration condition) showed that only the amount of time it took for the fish individuals to first reach the object (or fictitious object) for exploration significantly differed between the two contexts and hence might be a potential variable indicating exploration in the pale chub in the future. All measured variables were quite repeatable across the three contexts, which suggests that the personality of pale chubs is quite conservative and that future studies might use such variables during exploration or boldness measurements and may not need to include activity measurements.

Keywords personality measurement context, pale chub, behavior, activity, boldness, exploration, sociability

The personality of an animal is the consistent differences among individuals in behaviors such as activity, exploration, boldness and sociability (Réale et al., 2010; Mazué and Godin, 2015; Jolles et al., 2019). The existence of personality has been assumed to have large fitness consequences and a wide range of ecological and evolutionary implications (Smith and Blumstein, 2008; Réale et al. 2010; Sih et al. 2012); hence, it has recently attracted much attention from fish behaviorists (Jolles et al., 2017; Tang and Fu, 2019). For activity measurements, fish biologists usually use the characteristics of spontaneous activities in an arena, such as the median swimming speed, percent time moving (PTM) and total distance moved (TDM) during a given period (Brown and Irving 2014; Liu and Fu 2017). The tendency to leave a refuge and search an open environment is traditionally referred to as boldness (Jolles et al. 2017), and studies have used inspection latency and the number of inspection behaviors as indicators of this characteristic (Brown and Irving 2014; Tang, Wu and Fu, 2018). Exploration is usually evaluated in terms of the distance or time latency associated with the observation of a novel object (Liu and Fu, 2017). It has been long recognized that many animals exhibit different personality types and that bold individuals are typically more explorative and active than other individuals (Sih, Bell, Johnson, 2004; Sih et al., 2012; Martins and Bhat, 2014), i.e., personalities are highly correlated, at least in regard to activity, exploration and boldness. A study involving principal component analysis also suggested that most variables can be reduced to the so-called 'activity component', which is distinctly different from the 'sociability component' (Tang, 2019). However, the measurement protocol used in some studies might also artificially reinforce the relationship among personality trauts. For example, one might argue that active fish (individuals that spend more time moving and with a faster speed) could also be the first to enter an open area and hence spend more time in the open area and might also spend less time reaching a novel object independent of their boldness and exploration tendency. Thus, the present study aimed to test whether the spontaneous movement traits of fish individuals are repeatable or relevant among different personality measurement contexts. We hypothesize that the tested movement traits should differ under exploration (with a novel object nearby) or boldness (with a shelter available) contexts from those under the activity context (control condition with no novel object or shelter). The present study also

has another potential aim: if the variables are repeatable across different measurement contexts or show a positive correlation between different contexts, researchers may acquire data for variables related to different personalities from one measurement. For example, one can measure all variables mentioned above from a spontaneous movement trajectory of fish in an experimental arena with both a novel object and shelter presented.

To achieve our goals, we selected the pale chub (*Zacco platypus*), a common small cyprinid fish species distributed across east Asia, as an experimental model. We recorded videos of the experimental fish individuals under different contexts (with shelter, with a novel object, and under control conditions) with a webcam. The variables we selected for comparison are among the most frequently used personality indicators, e.g., median swimming speed, PTM, head-turning rate (which usually serves as an indicator of exploration tendency but is also closely correlated with activity), and the distance to the center of the arena (which has frequently been used as an indicator of boldness) (Couzin et al., 2011; Ioannou, Singh and Couzin, 2015; Sumpter et al., 2018).

Materials and methods

Source of the fish and their care

A total of 80 juvenile pale chubs (body mass: 6.42 ± 0.14 g) were purchased from the local fisherymen. All fish were maintained in a 250 L recirculating system filled with dechlorinated, well-aerated tap water. Two-tenths of the water in each tank was replaced daily with fresh water to maintain good water quality. The recirculating system was fitted with mechanical and biological filters and ultraviolet sterilizers. The water temperature was maintained at 25 ± 1 °C. The fish were fed to satiation once daily (at 8:00 am) for four weeks with Tubifex. The uneaten food and feces were collected with a siphon 1 h after feeding. Throughout the experimental period, the water was constantly aerated to ensure that the dissolved oxygen level was maintained at a minimum of 90% saturation. The photoperiod was 12 h light: 12 h dark. After four weeks of acclimatization, all fish were tagged intraperitoneally with passive integrated transponder (PIT; Guangzhou Hongteng Barcode Technology Company) tags under anesthesia with neutralized tricaine methane sulfonate (MS222, 50 mg L^{-1}). Then, all fish were allowed to recover for an additional two weeks; the maintenance and feeding

95 procedures were in accordance with those during the former rearing period. At the end of the recovery
96 period, all individuals were used to measure personality.
97 This study was approved by the Animal Care and Use Committee of the Key Laboratory of Animal
98 Biology of Chongqing (permit number Zhao-20161122-04) and performed in strict accordance with the
99 recommendations of the Guide for the Care and Use of Animals at the Key Laboratory of Animal
100 Biology of Chongqing, China.
101 **Experimental setup**
102 A rectangular tank (length × width × height: 70 × 20 × 20 cm) was used to measure personality
103 behavior, such as activity, exploration and boldness, as in previous studies (Liu and Fu, 2017; Fig. 1).
104 To minimize disturbance from the observers during the experiments, the tank was surrounded with an
105 opaque canvas. The tank was divided into two compartments by a removable opaque plastic partition
106 (ensuring an undisturbed acclimatization period) that was used to separate the open area (length ×
107 width × height: 55 × 20 × 20 cm) from a hidden area with shelter (length × width × height: 15 × 20 ×
108 20 cm). The water depth was maintained at 10 cm during the experiment. The behaviors of the test
109 fish were recorded using a webcam (Logitech Pro 9000; Logitech Company, Suzhou, China)
110 connected to a remote monitor, and the experimental tank was illuminated by properly placed
111 fluorescent lights.
112 **Measurement of spontaneous activities under different contexts**
113 All 80 individuals underwent spontaneous movement observation in three contexts. The replicate
114 number varied among the different personality traits because some files could not be read by the
115 software due to technical problems.
116 ***Activity context*** The fish were individually transferred to the middle of the open area of the aquarium
117 and acclimated for 10 min (the fish could not access the hidden area due to the partition between the
118 open and hidden area). Then, the movements of the fish were recorded by the webcam for 10 min (at
119 15 frames per second).
120 ***Exploration context*** The protocol was exactly the same as that for the activity context except that a
121 novel object was placed in the open area.

Boldness context A fish was transferred into the shelter area and acclimated for 10 min; then, the partition was gently opened (i.e., a small door), and the movement of the fish in the open area was recorded for 10 min.

For the data analysis, the videos were converted from .wmv to .avi format using Format Factory (http://format-factory.softonic.cn) and imported into an automated tracking program, i.e., EthoVision XT9 (EthoVision XT 9, Nodus, Netherlands). The program automatically tracked the position of the experimental fish in each frame and converted the x and y coordinates from pixels to cm. Then, the median speed during the 10 min of measurement, PTM, distance to the center of the open area and head-turning rate were calculated for all three contexts.

The swimming speed (v, cm s^{-1}) was calculated as follows:

$$v(t) = \sqrt{(x(t) - x(t-1))^2 + (y(t) - y(t-1))^2}/d \quad (1)$$

where $x(t)$ and $x(t\text{-}1)$ and $y(t)$ and $y(t\text{-}1)$ are the x and y coordinates, respectively, of the measured fish at time t and the time of the previous frame (t-1), and d is the length of the time interval (Miller and Gerlai, 2012).

The PTM was calculated as the percentage of time when the swimming speed was above 1.75 cm s^{-1} for 10 min (Tang et al., 2017).

The distance from the center of the arena was calculated as

$$D_i = \sqrt{(x_i - x_0)^2 + (y_i - y_0)^2}) \quad (2)$$

where x_i and y_i denote the coordinates of the fish, and x_0 and y_0 denote the coordinates of the arena center. In open fields, a large distance from the center often indicates low boldness in individual fish (Sumpter et al., 2018).

The head-turning rate of an individual was calculated using the absolute change in heading (see details in Herbert-Read et al., 2013). This measure can be used to quantify exploratory as opposed to goal-directed behavior in fish (Sumpter et al., 2018).

Measurement of spontaneous activities under different contexts

All four variables related to spontaneous activity were almost identical between the activity and exploration contexts. We assume that the pale chubs in the present study did not respond to the

presence of the so-called 'novel' object. To test this hypothesis, we calculated three variables that are commonly used in personality measurements of fish, i.e., the mean distance to the object, minimum distance to the object and first time exploring the novel object under the exploration context. The distance was calculated as

$$D_i = \sqrt{(x_i - x_n)^2 + (y_i - y_n)^2} \quad (3)$$

where x_i and y_i denote the coordinates of the fish, and x_n and y_n denote the coordinates of the object. The first time exploring the novel object is defined as the amount of time it took for the fish to first swim within 7 cm (approximately 1 body length) of the object.

Then, for the same fish measured under the activity context, we assumed the presence of a non-existent fake object with the same coordinates as the fish under the exploration context. We analyzed three variables to determine whether they were the same between the two contexts.

Data analysis

The program SPSS 17 was used for data analysis. P values < 0.05 were considered statistically significant, and all the data are presented as the means ± S.E. The four variables involved in spontaneous movement were compared among the three measurement contexts by one-way analysis of variance (ANOVA) followed by post hoc comparison with Duncan's multiple range test. The three variables associated with exploration under the exploration and activity contexts with a fake (i.e., non-existent) novel object were compared with paired t-tests. The repeatability of the four variables among the three contexts was first tested with the intraclass correlation coefficient (ICC), and then the relationships of each of the behavioral variables between any two contexts were tested with Pearson correlation analysis.

Results

Effect of measurement context on spontaneous movement traits

The median speed (R = 0.676, P < 0.001), PTM (R = 0.606, P < 0.001), head-turning rate (R = 0.676, P < 0.001) and distance to center (R = 0.430, P = 0.003) all showed repeatability across the three contexts. Measurement context had a significant effect on all four variables (Fig. 2). All variables measured with a novel object (i.e., under the exploration context) showed no significant difference

from those under the control condition. Fish spent 46% time in the shelter under the boldness context, whereas in the open area, fish showed a significantly higher median speed, PTM, and head-turning rate but a similar distance to the center of the open area.

The Pearson correlation analysis indicated that the median speed, PTM and head-turning rate measured under the activity context were positively correlated with the speed measured under the other two contexts (Table 1; Fig. 3). The distance to the center measured under the activity context was only correlated with that measured under the exploration context.

Difference in exploration variables between the exploration context and activity context with a fake novel object

Both the mean and minimum distance to the novel object in the exploration context showed no significant difference from the mean and minimum distance to the fictitious object in the activity context (Fig. 4a, b). However, it took a much shorter amount of time for the fish to first reach the novel object under the exploration context than for the fish to reach the fictitious object under the activity context ($P < 0.001$) (Fig. 4c).

Discussion

Effect of a novel object on movement traits

All variables measured under the control activity context were almost the same as those with the object nearby, i.e., under the exploration context. Thus, we assumed that the pale chubs might not have cared about the presence of the so-called novel object in the present study. If so, variables such as distance to the object and time latency to reaching the object might not be reliable indicators of exploration in fish species such as the pale chub. The fact that previous studies found a positive relationship between activity variables such as speed (or PTM) and exploration variables might have occurred because more active fish spend more time swimming at higher speed than in corners and hence show a shorter distance to the object and exploration latency in comparison to less active individuals (Heg, Schuürch and Roghenberger, 2011; Liu and Fu, 2017; Tang and Fu, 2019). This is reinforced by the results indicating that the fish showed almost identical values in terms of the mean and minimum distance to novel objects in the same location when measured under the activity context

(i.e., a fictious object). It was also interesting to find that the time it took for the fish to first reach the object was much shorter than that under the activity context, i.e., the fish purposefully encountered the object earlier for exploration. This suggests that only time latency can be used as an exploration indicator in fish species such as the pale chub. Thus, fish behaviorists should be cautious regarding explanations pertaining to exploration indicators previously used in other personality studies, such as the distance to an object (Wang et al., 2019; Wang, 2019). The testing of other variables under different experimental setups, such as the number of times individual guppies (*Poecilia reticulata*) traveled between containers connected by pipes (Brown and Irving, 2014), might be more appropriate for the measurement of exploration tendency. Researchers at least need to test the reliability of such measurements, for example, by comparing spontaneous movement trajectories in fish between activity and exploration contexts, as in the present study.

Effect of shelter on spontaneous activity in an open area

The measurements of all variables except distance to the center of aquarium differed between the boldness and activity contexts. The area of the shelter represented approximately 21% of that of the total aquarium. Pale chub individuals showed large variation in terms of the percentage of time spent in the open area (varying from 18 to 97%) and time latency to enter the open area (ranging from nearly zero to 23 min). The average value in the shelter area was approximately 46%, which was much higher than the proportion of the total area represented by the shelter (21%), suggesting that pale chubs prefer to stay in a shelter over the open area. Fish in the open area showed higher values of the PTM, speed and the head-turning rate, suggesting that they mainly rest in shelter areas but perform activities indicative of boldness, such as searching for food or potential predators. Thus, the results of the present study suggest that variables that have been frequently used in previous studies, such as the percentage of time spent outside of a shelter (Alain et al., 2015; Mazué and Godin, 2015), might be reliable indicators of boldness in fish species such as the pale chub.

The repeatability of variables across measurement contexts

All variables in the present study were quite similar across the different contexts. This agrees with the results of a previous study that showed that variables such as median speed and head-turning

rate were quite repeatable (Herbert-Read et al., 2013). Thus, the values of activity variables, such as the median speed and PTM, measured under the activity context were closely correlated with those measured under the exploration and boldness contexts. In fact, we also found that the median speed measured under the activity context was even positively correlated with that measured under the sociability context (i.e., with a stimulus shoal nearby, data not published). These results suggest that researchers can only evaluate personality traits associated with activity under boldness or exploration contexts and do not need to perform activity measurements separately.

In conclusion, the pale chubs showed high repeatability in all measured variables between the activity and exploration contexts, suggesting that the traditionally used variables may not be appropriate indicators of exploration, at least in regard to distance to a novel object. Further analysis revealed that the latency to an object might be a reliable indicator of exploration. The fish preferred to remain for a longer amount of time in a shelter than in the open area, whereas when swimming in the open area, the fish had a higher swimming speed, head-turning rate and PTM than those under the activity context, suggesting that variables associated with boldness, such as the amount of time spent in an open area or latency to the open area, might be reliable indicators of boldness. Moreover, all variables involved in activity are quite repeatable across different contexts, which suggests that fish behaviorists can use such traits from either boldness or exploration measurement tests rather than conduct additional activity personality measurements.

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Table 1(on next page)

Table 1 Correlations between measurements conducted under the activity context and those conducted under the other two conditions

Each data set indicates statistical result of correlation.

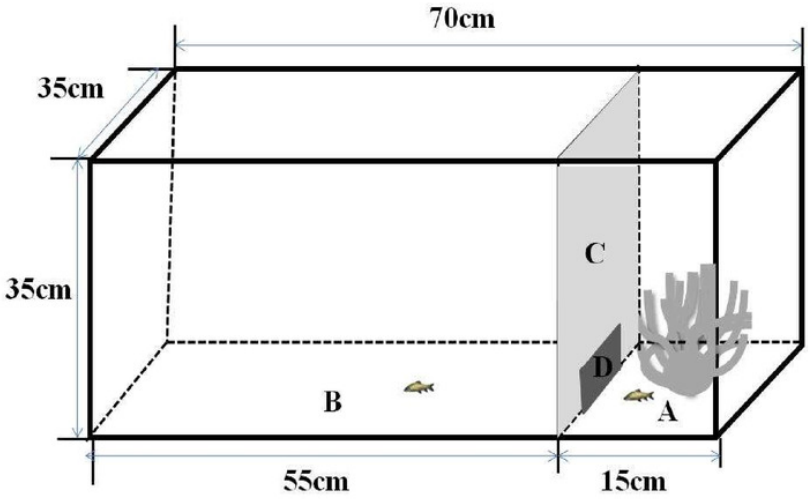
Table 1 Correlations between measurements conducted under the activity context and those conducted under the other two conditions

		Context	
		Exploration	Boldness
Median speed			
	N	72	67
	R	0.665	0.566
	P value	<0.001	<0.001
PTM			
	N	72	67
	R	0.691	0.396
	P value	<0.001	0.001
Turning rate			
	N	72	67
	R	0.604	0.636
	P value	<0.001	<0.001
Distance to center			
	N	72	67
	R	0.440	0.017
	P value	<0.001	0.978

Figure 1

Fig. 1 Design of the experimental aquarium used for the measurement of activity, exploration and boldness in the study.

A) hidden area (length × width × height: 15 × 35 × 35 cm); B) open area (length × width × height: 55 × 35 × 35 cm); C) removable opaque PVC divider; and D) small door



1

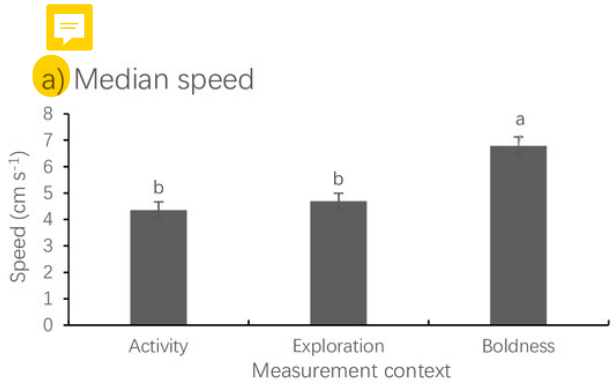
1

Figure 2

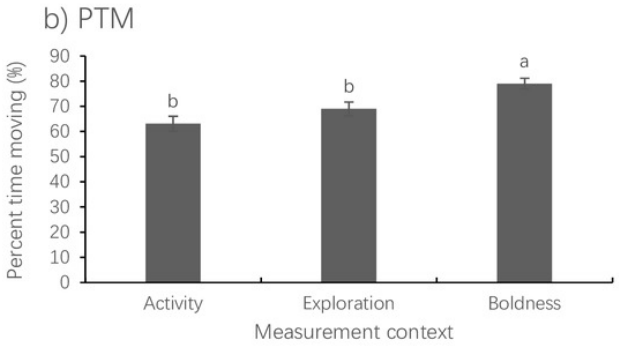
Fig. 2 Median speed (a),percent time moving (b), head-turning rate (c) and distance to the center ofaquarium (d) of pale chubs measured under different contexts .

Each data are means ± S.E., N = 72 under activity and exploration contexts and N= 67 under boldness context.

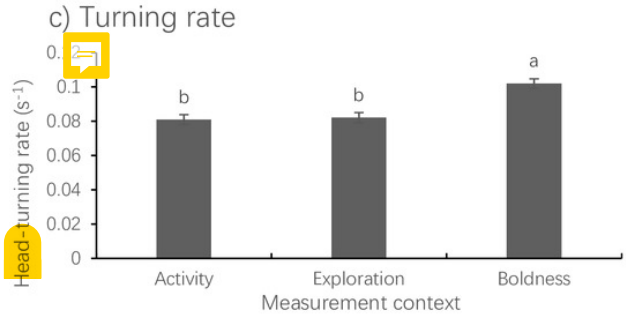
a, b values not sharing the same letter are significantly different ($P < 0.05$).


a) Median speed
Speed (cm s-1)
0
1
2
3
4
5
6
7
8
b
b
a
Activity
Exploration
Boldness
Measurement context

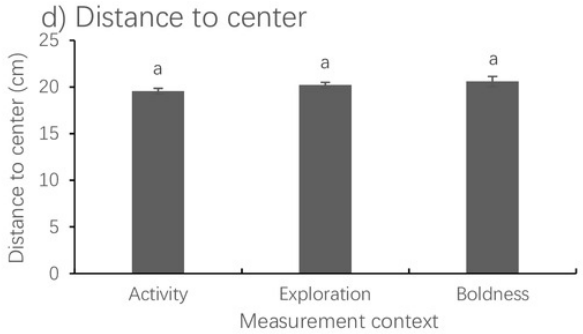
1


b) PTM
Percent time moving (%)
0
10
20
30
40
50
60
70
80
90
b
b
a
Activity
Exploration
Boldness
Measurement context

2


c) Turning rate
Head-turning rate (s-1)
0
0.02
0.04
0.06
0.08
0.1
b
b
a
Activity
Exploration
Boldness
Measurement context

3

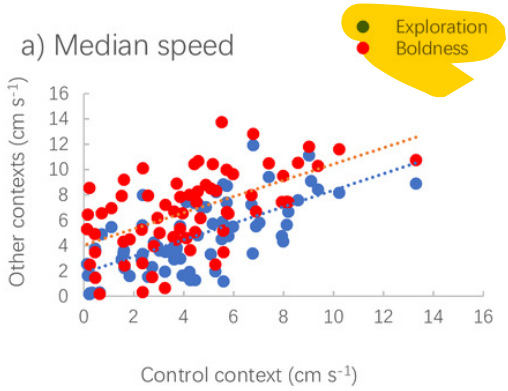

d) Distance to center
Distance to center (cm)
0
5
10
15
20
25
a
a
a
Activity
Exploration
Boldness
Measurement context

4

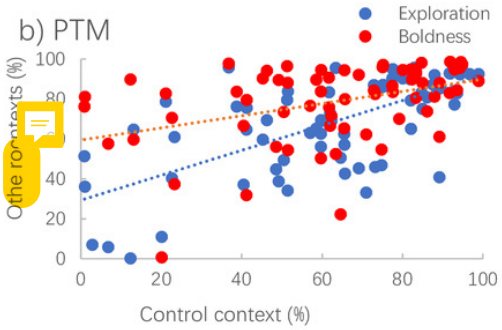
Figure 3

Fig. 3 Correlations of four measured variables between the activity context andexploration (or boldness) context. See the statistical results in Table1.

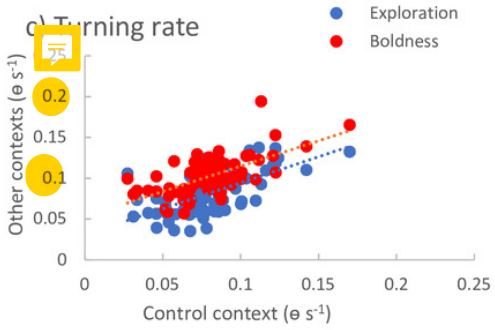
Each data point is the value under control and another context. See the statistical results in Table 1.


a) Median speed
Exploration
Boldness
Other contexts (cm s-1)
Control context (cm s-1)

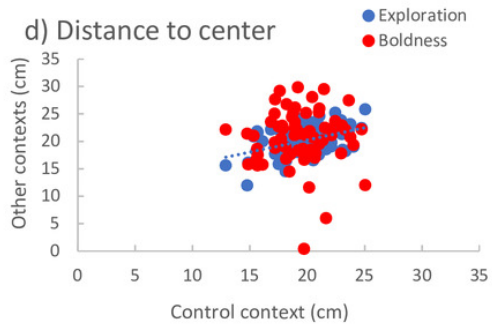
1


b) PTM
Exploration
Boldness
Control context (%)

2


Exploration
Boldness
Other contexts (ɵ s-1)
Control context (ɵ s-1)

3

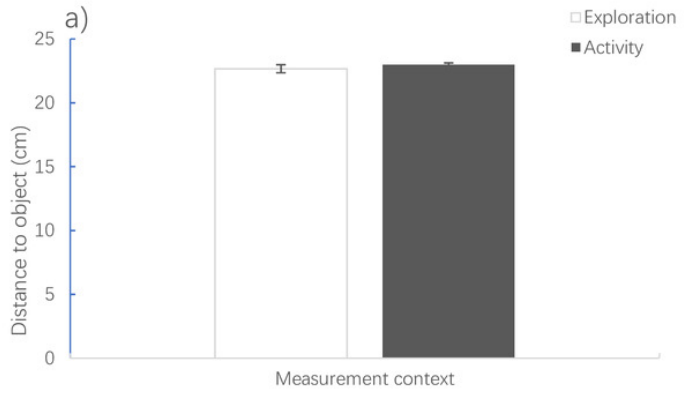

d) Distance to center
Exploration
Boldness
Other contexts (cm)
Control context (cm)

4

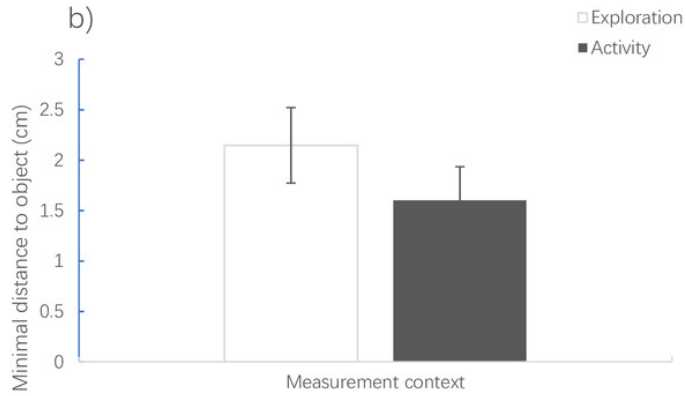
Figure 4

Fig. 4 Mean distance, minimum distance and amount of time it took for the fish tofirst approach the novel object under the exploration context and thefictitious object under the activity context (mean ± S.E., N = 72).

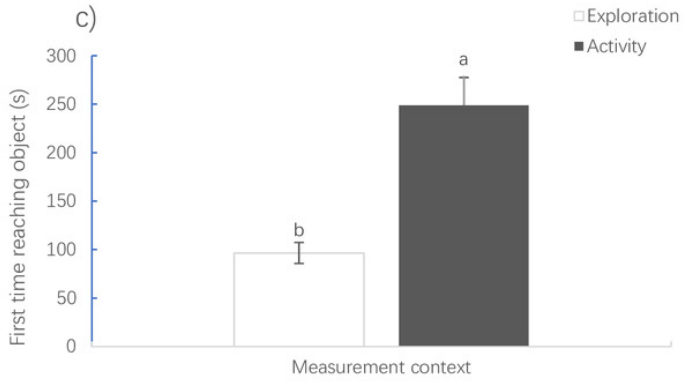
a, b values not sharing the same letter are significantly different ($P < 0.05$).


a)
Exploration
Activity
Distance to object (cm)
25
20
15
10
5
0
Measurement context

1


b)
Exploration
Activity
Minimal distance to object (cm)
3
2.5
2
1.5
1
0.5
0
Measurement context

2


c)
Exploration
Activity
First time reaching object (s)
300
250
200
150
100
50
0
a
b
Measurement context

3