

Tactics of Fish Behavior in the Water Flow during Starvation

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Abstract—Individual movement parameters for the zebrafish *Danio rerio* and the Prussian carp *Carassius gibelio* during starvation for 12 days have been determined in an experimental hydrodynamic installation *circular tank*. The fishes represent two tactics of behavioral responses on starvation. The tactics of unidirectional responses have been manifested in the movement of individuals in one direction relative to the water flow on days 2–5 of starvation. The tactics of multidirectional responses have been manifested on days 10–12 of starvation as movements in different directions relative to the water flow. The population advantages of such behavioral tactics of fish response to an unfavorable factor are considered.

Keywords: fish, starvation, behavioral tactics, zebrafish *Danio rerio*, Prussian carp *Carassius gibelio*

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INTRODUCTION

Trophic factor is one of the most important environmental factors that determine fish behavior in the water flow. A lack or total absence of food leads to the changes in physiological state of fish, and they change their habitats. In river systems such changes in the habitats occur in the form of migrations or denatant/contratant movements (Pavlov, 1979; Olsson et al., 2006; Flecker et al., 2010; Ferguson et al., 2019).

The movements of fish in the water flow are determined by their rheoreaction, i.e. the attitude of the fish to the water current (Pavlov et al., 2020b). The rheoreaction, along with the orientation and locomotor components, also includes a motivational one, which determines the selection of direction of movement in the water current (Pavlov et al., 2010). One of the indicators of the motivational component of rheoreaction is the ratio of its own types. Fish exhibit four types of rheoreaction: positive (PTR), the movement against the current; negative (NTR), movement with the water current; compensatory, resistance to the current while preserving the habitat; and facultative, leaving the current in quiet areas (MacLean and Gee, 1971; Pavlov, 1979; Pavlov et al., 2010, 2020b; Johnston et al., 2017). Preference for one of the first two types of rheoreaction is characteristic of the migratory behavior of fish. Changes in the motivational component of the rheoreaction are the main behavioral mechanisms for the start, cessation, and/or finish of fish migrations (Pavlov et al., 2010, 2019, 2020b; Zvezdin, 2016). Under experimental conditions, the indicators of this component determine the migratory or resident nature of the rheoreaction.

Previously (Pavlov et al., 2021), using the *Fishay* installation, it was experimentally shown that many fish species have two phases of behavioral response to starvation. The first phase, on days 2–5 of starvation, is characterized by fish movements with the current (NTR), that is, the fish exhibit migratory behavior. The second phase (on days 10–12 of starvation) is characterized by no preferences for a particular direction of movement (the frequencies of PTR and NTR are equal). However, these experiments were carried out using a method that allowed the calculation of average rheoreaction rates only for the sample as a whole, but not for individual fish. Therefore, the same average rheoreaction rates were possible with different individual behavior of the fish. For example, during resident behavior, each fish individual equally often moves with and against the current, ultimately remaining at the starting point. In another case, some individuals move against the current, and some move with the current. However, in this case, the average result will be the same as in the first example. That is, there can be different behavioral tactics:

(1) tactics of unidirectional behavioral responses of fish to starvation, which is manifested in the movement of individuals in one direction relative to the water current;

(2) tactics of multidirectional behavioral responses of fish to starvation, which is manifested in the formation of several groups of fish that move in different directions relative to the current.

The opportunity to experimentally test whether, during the process of starvation, fish are divided into groups with different preferences for the direction of movement, or whether such division into groups does

not occur, appeared after the development of a technique for individually tracking the behavior of fish in a circular tank with a limnozone (Pavlov et al., 2020b). The circular tank makes it possible to assess individual variability in the indicators of the motivational component of the rheoreaction and to record several additional parameters of the behavior of individuals in the water flow.

The aim of this work is to determine the presence or absence of different tactics of behavioral response to starvation in fish in a water flow.

MATERIALS AND METHODS

The objects of the study were the Prussian carp *Carassius gibelio* with an average standard body length (*SL*) of 94.6 (78–104) mm and the zebrafish *Danio rerio* with *SL* of 26.5 (24–33) mm. Prussian carps were caught in ponds of Yaroslavl Oblast (Russia); zebrafish were aquarium fish taken from the aquarium of A.N. Severtsov Institute of Ecology and Ecology of the Russian Academy of Sciences. The fish were kept in aerated aquariums at a stocking density of ~1 specimen/5 L of water. The water temperature was maintained at 22°C. Artificial lighting was turned on from 10:00 to 19:00, the illumination was 450–500 lux. Before the experiments, the fish were kept for 5 days and fed once a day with Tropical Fish Flakes aquarium fish food (Prodac, Italy).

The results of determining the phases of response of the Prussian carps and zebrafish to starvation were published previously (Pavlov et al., 2021). In this work, experiments on individual variability in rheoreaction parameters were carried out both in the first phase of the fish's response to starvation (5 days of starvation) and in the second phase (10 and 12 days of starvation for the Prussian carp and zebrafish, respectively). Previously (Pavlov et al., 2020a) it was shown that the tested rheoreaction parameters do not depend on the nature and experience of fish individuals (whether they were naive (a new batch of fish on each test day) or previously used in the experiments). All tested individuals (both among naive and experienced fish) were tested only once a day. Therefore, in this work, the same batch of fish (60 specimens) was used throughout the entire starvation period.

The circular tank with a limnozone (Fig. 1) is a ring-shaped channel with a wall height of 15 cm, the inner wall above 8 cm is made of a nylon sieve (mesh size 0.9 mm). Through this sieve, water flowed from the working channel to the center of the installation, from where an Atman AT-107 pump (Chuangxing Electrical Appliances, China) returned it to the working channel of the device. The height of the overflow layer is 2 cm; the depth of water in the channel is 10 cm. At regular intervals, 1 cm wide lines were drawn on the bottom of the device, indicating the boundaries

of eight sectors. The limnozone is located on the inner side of the channel.

The parameters of the circular tank varied depending on the size of the fish. For zebrafish, we used a setup with a diameter of 80 cm; a working corridor width was 10 cm, and a limnozone area was 630 cm²; for Prussian carp these parameters were 94, 15, and 800 cm², respectively.

According to the methodology for determining the indicators of the motivational component of rheoreaction (Pavlov et al., 2010), operating speed rates in installations should be within 0.2–0.7 of the critical current speed (V_c) for the fish. Before conducting experiments, V_c levels were preliminarily determined for 20 individuals of each species according to the standard method (Pavlov, 1979) in a hydrodynamic 1 m long tube with a diameter of 22 or 47 mm (for zebrafish or Prussian carp, respectively). These fish individuals were not further used in the tests.

The current velocities in the circular tank were measured with a hydrometric micro-spinner (blade diameter 8 mm) with a controller for communication with a computer. In total, 14 measurements were taken at depths of 5 and 9 cm in seven sectors 1 cm from the outer wall of the canal. The average current speed was found to be $0.4V_c = 22.7$ cm/s for the Prussian carp and $0.5V_c = 17.1$ cm/s for the zebrafish. The water temperature and illumination in the installation were the same as in fish keeping aquariums.

Previously (Pavlov et al., 2020b) it was shown that the frequency of NTR significantly increased in single zebrafish compared to the group of fish. Zebrafish and Prussian carps are schooling fish, so their placing and keeping alone in a device is naturally supposed to be stressful for them. To exclude this, tests on the Prussian carps and zebrafish were carried out in groups of 6 individuals on the 5th, 10th, and 12th days of starvation. The fish started from the limnozone, which was isolated from the channel with a grid before placing the test fish in it. The acclimation time was 20 min, after which the starting sector grid was removed. The movement of fish was recorded for 30 min using an HD Webcam C270 (Logitech, China), located above the installation and connected to a laptop. During the revisal of video recordings, the direction and moment of complete (whole body) crossing of the border between sectors was recorded for each fish individual separately. The movements of fish within the sector and limnozone were not recorded.

Based on the results of the experiments, the following indicators were calculated.

1. The total path (S) is the product of the number of sectors passed to the length of the central arc of the sector. Additionally, we similarly calculated the length of the path along the current (S_d) and against it (S_u). To calculate the indicators of the fish path, the starting point was the border between the first and second sectors.

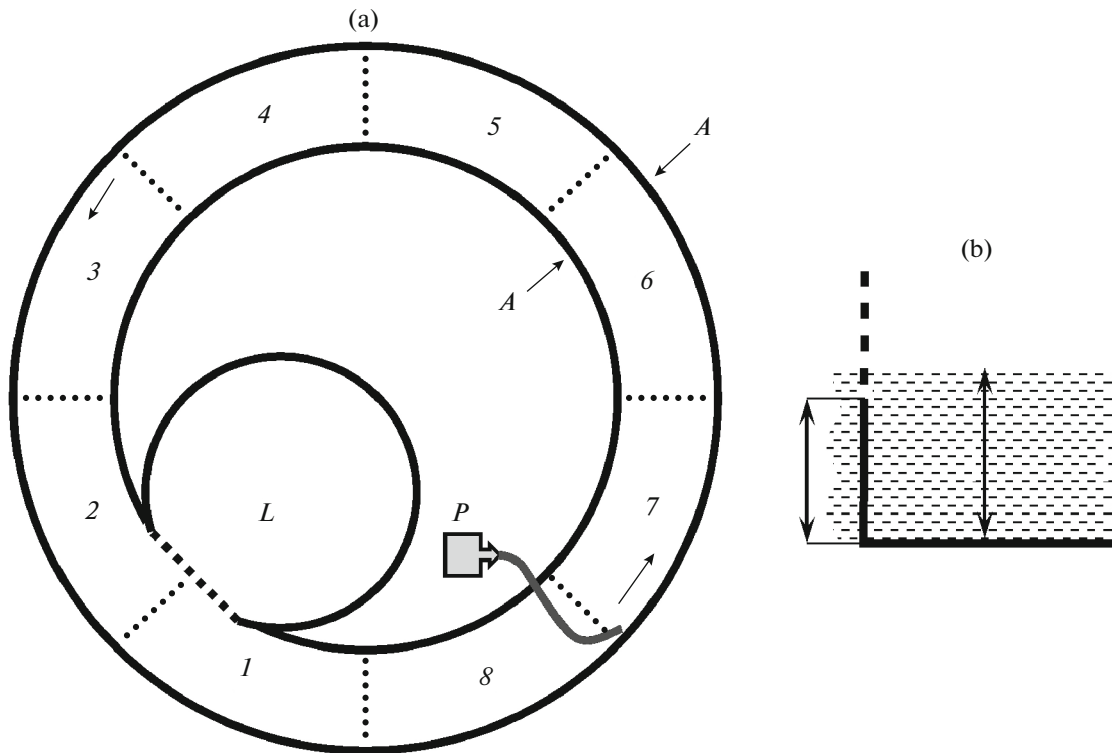


Fig. 1. Hydrodynamic installation “Circular tank with limnozone”: (a) top view; (b) section A–A; *P*, pump; *L*, limnozone; 1–8, installation sectors, (•••), boundaries of sectors and limnozone; (■ ■ ■), part of the wall made of nylon sieve; (---), filled with water part of the installation; (→), current direction. Linear dimensions are given in cm.

2. Movement of an individual ($S_u - S_d$) is the difference between the path against the current and along the current.

3. To compare the obtained results with the results of other studies, the displacement index (Id) was calculated using the formula: $Id = (S_u - S_d)/S$. The index value varies from 1 (when the fish moves only against the current) to -1 (the fish moves only with the current).

In total, 20 tests were carried out for the Prussian carp and 20 for the zebrafish in a circular tank and 60 individuals of each species were used in the experiments. In total, 40 h of video materials were revised and processed.

Statistical analysis of the data was carried out using the STATISTICA 10.0 software package (StatSoft, Inc., USA). Identification of groups of fish according to the preferred direction of movement was carried out using the frequency distribution of individual Id values. Samples of identified Id values were tested for differences between the empirical frequency distribution and the theoretical ones: unimodal (normal), bimodal, and trimodal (the sum of two and three normal distributions, respectively) using the Lilliefors test from the specified software package. To calculate bi- and trimodal distributions, the sample was divided

into two or three groups using the least squares method (Pecherovyi, 2005; Borovkov and Savyolova, 2007). Comparison of theoretical frequencies of uni-, bi-, and trimodal distributions was carried out using the likelihood functions of the frequency distribution using the χ^2 criterion (Gurskii, 1971).

RESULTS

In the first phase of the fish’s response to starvation (5 days), the frequency distribution of individual Id values did not differ from normal both for the Prussian carp and zebrafish (Table, Fig. 2). Thus, it is not possible to cluster fish into groups with different preferred types of rheoreaction; their behavior was similar. This indicates the tactics of unidirectional movements of fish during relatively short periods of starvation. In our experiments, these movements were directed along the current.

With further starvation (in the second phase of the response), the energy capabilities of the fish decrease and the incentive to migrate increases. At the same time, the rheoreaction indicators change: the empirical distribution of Id in the second phase significantly differs from normal (Table) and the uniformity of fish behavior disappears (Fig. 3). The frequency distribution of individual Id values is approximated by a tri-

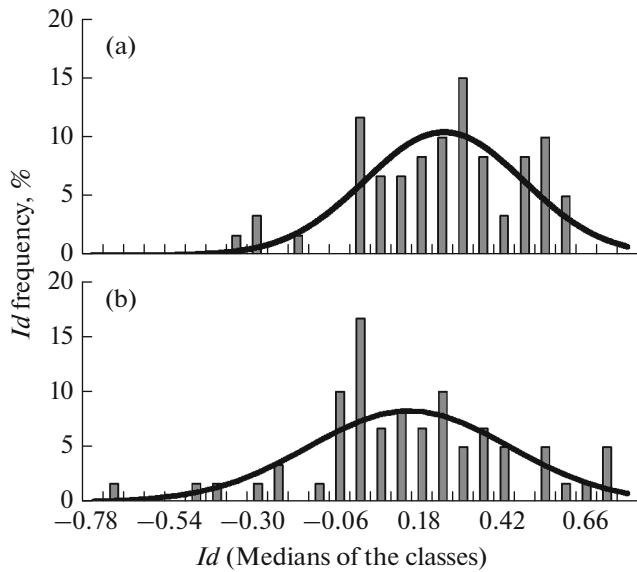


Fig. 2. Frequency distribution of individual values of the displacement index (Id) in the 1st phase of fish response to starvation (5 days): (a) Prussian carp *Carassius gibelio*; (b) zebrafish *Danio rerio*; (■), actual frequencies; (—), normal distribution density. On the 5th day of starvation, distribution of Id in the Prussian carp and zebrafish does not differ from normal according to the Lilliefors criterion.

modal distribution, which reflects the empirical distribution of Id significantly better than the bimodal one. The significance level for differences in tri- and bimodal distributions for the Prussian carp was 0.002 and for zebrafish, <0.0001.

Analysis of the data represented on Figure 3 identified three groups of fish (the sample volume of 60 specimens for each species is taken as 100%) that move:

- 1) downstream ($Id \leq -0.2$): Prussian carp 50%; zebrafish 37%;
- 2) upstream ($Id > 0.3$): Prussian carp 17%; zebrafish 25%;
- 3) almost equally downstream and upstream, and thus remaining at the starting point ($-0.2 < Id \leq 0.3$): Prussian carp 33%, zebrafish 38%.

The results indicate that in the second phase of starvation, fish exhibit tactics of multidirectional behavioral responses. Most fish (67% of Prussian carps and 62% of zebrafish) exhibit migratory behavior: they are still moving, but the direction of their movement changes. One part of the individuals moves with the current, and the other part, despite decreased energy reserves, begins to move against the current. A minority of individuals exhibit resident behavior, moving with the current and then against it, thereby remaining in their original location. This is exact manifestation of the tactics of multidirectional movements of fish during starvation.

DISCUSSION

Thus, we experimentally revealed two tactics of fish movements during starvation, determined by individual Id values: the tactics of unidirectional responses and the tactics of multidirectional responses. In the first phase of the fish's response to starvation, fish exhibit the tactics of unidirectional responses, i.e. all fish individuals move in the most energetically favorable direction (downstream) thereby providing themselves with an equal probability of individual survival. These results are consistent with literature data: many authors show that lack of food stimulates fish to downstream migrations (Pavlov, 1979; Olsson et al., 2006; Flecker et al., 2010; Ferguson et al., 2019).

The second phase of the fish's response to starvation is characterized by the tactics of multidirectional movements: some fish continue to move with the current, while others, in search of food, move in the most energy-consuming direction, against the current, and the probability of their individual survival may decrease. Movements of fish in two opposite directions lead to an increase in the search area for food and thereby to an increase in the probability of population persistence.

Various strategies of the so-called partial migration, noted by many authors, also lead to expansion of the feeding grounds (Hutchings, 1986; Jonsson and Jonsson, 1993; Falconer and Mackay, 1996; Pavlov and Savvaitova, 2008; Shaw and Levin, 2011; Chapman et al., 2011, 2012; Dodson et al., 2013). Such partial migration is characteristic for different stages of

Table 1. Significance level (p) for the differences between the distribution of individual movement index values for the tested fish from the normal distribution according to the Lilliefors criterion and the duration of fish starvation (T , days)

Species	Phase of the fish response to starvation			
	1		2	
	p	T	p	T
Prussian carp <i>Carassius gibelio</i>	>0.20	5	<0.05	10
Zebrafish <i>Danio rerio</i>	>0.15	5	<0.05	12

Significance levels indicating the reliability of the difference are highlighted in bold.

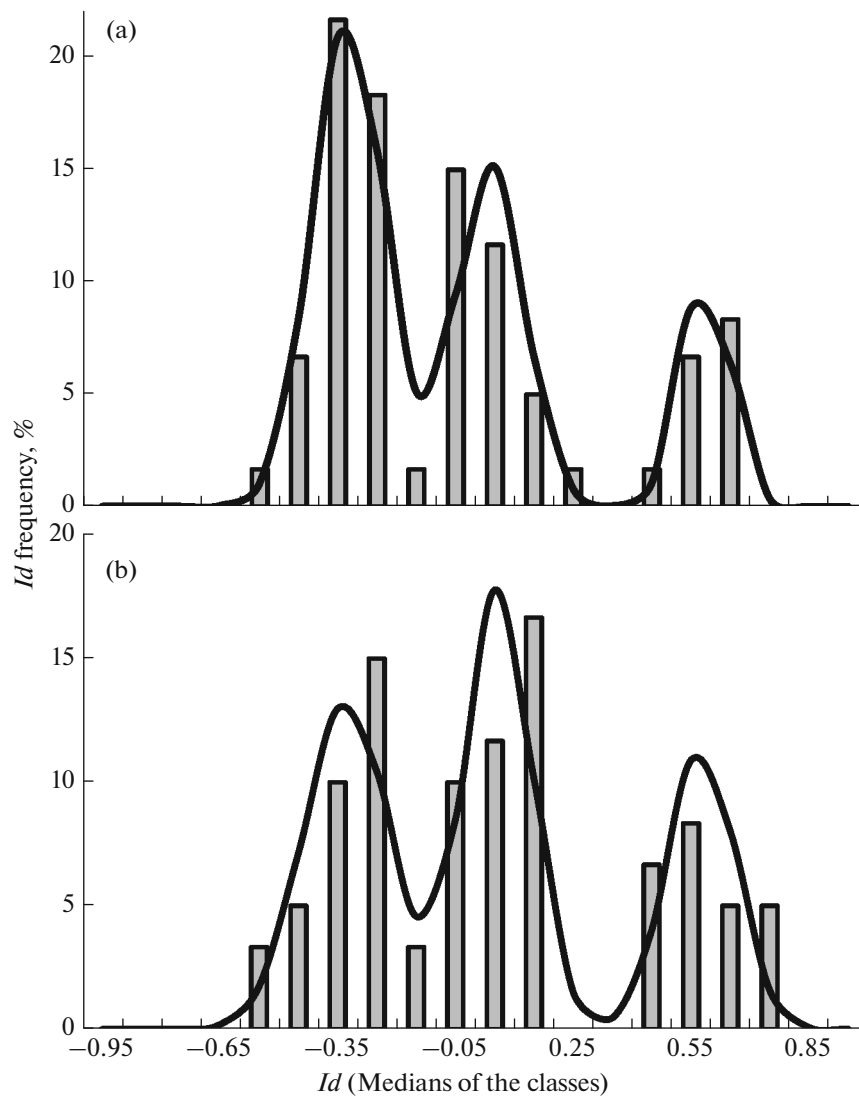


Fig. 3. Frequency distribution of individual values of the displacement index (Id) in the 2nd phase of the fish response to starvation: (a) Prussian carp *Carassius gibelio* (10 days of starvation); (b) zebrafish *Danio rerio* (12 days of starvation); (■), actual frequencies; (—), total density of three theoretical normal distributions constructed using the sample parameters. Trimodal distribution according to the Lilliefors criterion differs significantly from bimodal in the Prussian carp at $p = 0.002$, in zebrafish at $p < 0.0001$.

ontogenesis in many fish species and is manifested in the implementation of a migratory life strategy by some individuals, and a resident one by others. In addition, migratory behavior is formed not only under the influence of starvation, but also under the influence of other unfavorable factors of various natures. Identification of different behavioral tactics of fish response to such impacts, as well as the species-specific tactics may be relevant directions for future research.

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ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Animal-related experiments were conducted in accordance with the NIH Guidelines for the care and use of laboratory animals (<http://oacu.od.nih.gov/regs/index.htm>). Animal protocols were approved by the Bioethics Commission of the Federal State Budgetary Institution of Science, A.N. Severtsov Institute of Ecology and Evolution of the Russian Academy of Sciences, Moscow, Russia (approval ID: No. 79 of November 23, 2023).

CONFLICT OF INTEREST

The authors of this work declare that they have no conflicts of interest.

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