FEATURES OF MOVEMENTS OF CLIMBING PERCH *Anabas testudineus* IN WATER FLOW AND ON LAND

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1. INTRODUCTION

Fish movements in different directions and distances (migrations) are an evolutionary adaptation directly related to population survival. In contrast to most fish, climbing perch *Anabas testudineus* is able to move not only in water but also on land. This species is found in various freshwater bodies in India, Bangladesh, Vietnam, China, and Southeast Asia. Fish often inhabits small and shallow rivers, streams, ditches, canals, reservoirs, ponds, paddy fields [1 - 3]. Habitat conditions in these water bodies are highly variable due to seasonality (rainy or dry season) and anthropogenic pressure. Unfavorable habitat conditions manifest in a decrease in oxygen levels, an increase in temperature and decrease in water levels, and chemical water pollution with fertilizers and pesticides. The response of climbing perch to unfavorable conditions shows the high plasticity of the species. That fish can live in the water with high toxic pollution by pesticides [4 - 6]. This species can remain outside the water for a long time due to air-breathing in a three-phase cycle of air exchange, which does not require water [7, 8]. Correction of the body position on the land and movement by opening and closing gill covers allows climbing perch to overcome the distance of about 90 m and obstacles in its path [2]. Increased adaptability contributes to survival in difficult habitat conditions and is essential during its dispersal or migration to the new water bodies. The data on movements of climbing perch in water and on land and what factors initiate the crawling are insufficient.

In this work, we aimed to determine the indicator of the directional movement of the climbing perch in the water flow (rheoreaction), which helps to identify the features and dynamics of movements and the behavioral response of fish to the different factors [9]. We aimed to evaluate the locomotor activity of fish during the day, the effect of decreasing water level, and water pollution with urea and thiourea on movements. Urea is highly soluble in the water and has a toxic effect, like thiourea [10]. Urea is often used as an inexpensive fertilizer for many cultivated crops in Vietnam, which contributes to its pollution and accumulation in water bodies.

2. MATERIALS AND METHODS

The studies were carried out in the Coastal Branch of Joint Vietnam-Russia Tropical Science and Technology Research Centre (Nha Trang, Khanh Hoa province, Vietnam). The fishes were captured in ponds (depth 70 cm, water transparency up to 30 cm, temperature 24°C) included in the system of paddy fields, near Ninh Hoa City (12°30’34”N; 109°09’40”E) in January-February 2020 to evaluate the movement behavior in the water flow and on the land. The fish body length (TL) was 71 ± 1.9 mm, and body weight was 12 ± 1.2 g. Fish were captured in irrigation canal Am Chua (12°17’26”N 109°06’04”E) in January 2022 to evaluate the diurnal locomotor activity. The mean body length of these fish was 98 ± 2.4 mm, and the body weight was 16 ± 1.4 g. Fish capturing was used by artificial shelters-traps.

In the laboratory, individuals were kept in four 100 L aquariums at a water temperature of 25-26°C, with 25 specimens each. The aquariums were filled with water with a half (50 L) and covered with lids. Water exchange in aquariums was once a day. The fish were fed twice daily (at 07:00 and 19:00) with dry pelleted food Humpy Head (Singapore). Most fish began to consume pelleted food within the first three days.

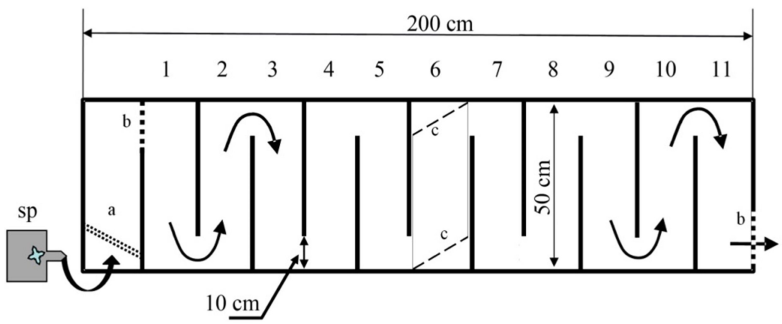

Figure 1. The experimental hydrodynamic test chamber: sections (1-11); starting section (6), mesh for flow laminarization (a), permanent barrier net (b), removable nets (c), submersible pump (sp), flow direction (→)

Experiments of fish movements in water flow were carried out in the hydrodynamic test chamber [9]. The test chamber is a tray divided into 11 sections (50 × 16 cm each) with 15 cm high walls; the width of the passage between the sections was 10 cm. The height of the perimeter walls of test chamber reached 60 cm. The water level in the test chamber varied from 6 to 8 cm. The water flow was made by a submersible pump and was 20 cm/s (Global Water FP211 flow tracker). The average illumination in the test chamber sections during the experiment was 180 Lux (Amtast LX1330B luxmeter). Above the test chamber, a GoPro Hero 7 Black video camera was installed to register fish movements. Video recording was controlled remotely. Fish (10 specimens) were placed in the middle (starting) section no. 6, closed on both sides with removable lattices. After 20 minutes, the lattices of the starting section were removed, and during the next 20 minutes, the movements of the fish were recorded. The position of each individual was monitored on the basis of video recordings every minute. The direction moving index (I_d) is a movement vector calculated in the test chamber sections and normalized to values from -1 to +1. The direction moving index of fish indicates the direction relative to the current and how far (by the number of transferring covered sections) the fish moved on average in the test. If all the fish move from the starting section against the water current to the upper compartment 1st, $I_d = +1$. If all the fish move from the starting section along with the water current to the lower section 11th, $I_d = -1$. The index was calculated using the formula: $I_d = (\sum[n_i (6-i)])/((6-i)\sum n_i)$, where: direction moving index (I_d); fish number in the i-th section of the test chamber (n_i); the number of the starting section (6); the maximum number of sections from the starting section, to which an individual can move in the test chamber (6-i); section number (i) (Fig. 1).

The behavior of fish in clean water (control) and under the influence of 0.05% solution of urea (U) or thiourea (TU) was evaluated for 12 days. The fish movements were determined in the 2nd, 5th, 8th, and 12th days after placing the fish in solutions to U and TU. A break of 2-3 days is necessary to reduce the manipulation stress of individuals. Forty-nine experiments were performed (11—control, 19 each exposed to U and TU).

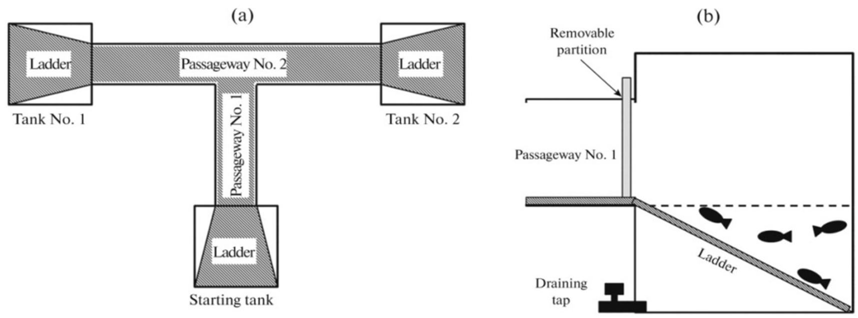

Figure 2. Test chamber "dry maze": (a) top view, (b) starting tank, side view; (□) space accessible for climbing perch *Anabas testudineus* during the experiment; (----) the water level in the starting tank at the beginning of the experiment.

Experiments of decreasing water level influence on fish crawling and movement on the land were carried out in the "dry maze" test chamber (Fig. 2a). The unit consisted of three tanks 0.4 m long and wide and 0.5 m high. The water level was ≥32 cm under the height of the wall. The ladders from the bottom to the entrance of the horizontal passageway were mounted in each tank. The inclination of the ladders was 27°. The horizontal passageway no. 1 has a length of 0.8 m, going up to the intersection with passageway no. 2 with a length of 1.8 m. There was a tap to drain the water at the bottom of each tank, under the ladder. The passageways were placed at the height of 0.2 m relative to the bottom of the tanks. The ladders and the bottom of the passageways were covered with a fabric to increase the adhesion of the climbing perch to the surface. The starting tank was filled almost to the passageway level (18 cm height), and ten fish were placed (Fig. 2b). A removable partition blocked the exit from the starting tank to the passageway. The same water level was in one of the other two tanks; the last remained without water, but the fabric on the ladder was moistened. After 20 minutes, the partition was removed, and the tap was opened to drain water from the starting tank. The water level in the basin decreased from 18 to 0 cm in 5 minutes. The experiments were conducted from 7 am to 4 pm; the mean of illumination was 153 Lux.

The fish movements in the passageways were recorded during the experiment (30 min) using a GoPro Hero 7 Black video camera with remote control. The video records were used to determine the number of air swallowed by the fish and the number of jumps out of the water; the time required for the fish to enter the passageways; the duration of stay in tanks No. 1 and 2; the direction of movements. The speed of fish movements along the passageways was calculated. Ten experiments were conducted.

Experiments evaluating the diurnal locomotor activity were carried out in three aquariums (56×38×38 cm) with top-mounted video cameras. Six fish were placed in each aquarium, and for 24 hours, the video cameras recorded the movements of the fish. The beginning of experiments varied from 8 am to 5 pm. We used this experimental design for the decreasing influence of the fish stress on the results. The aquarium illumination matched the room's natural light and was 0-80 Lux. Fifteen experiments were conducted with 90 fishes. The diurnal locomotor activity was detected in video files using open source code (Python Software Foundation). Verification of the automatic software detection showed that precision was not less than 95%.

The data were processed statistically, applying nonparametric analysis of variance (Kruskal-Wallis H-test), Mann-Whitney U-test, Student's t-test, Student's test for fractions.

3. RESULTS AND DISCUSSION

3.1. Fish behavior in water flow

After transferring fish to the starting section of the hydrodynamic test chamber, they did not avoid the water current. Often some fishes jumped from the start section to other sections before opening the lattices. Accordingly, the climbing perch also moved in the test chamber in groups (≥ 3 individuals): the movement of one individual against the water current initiated a similar reaction in other fish.

The control individuals (clean water) more often (Student's test for the fractions: $p < 0.05$) than the experimental ones (polluted water with U or TU) jumped over the barriers, as evidenced by the proportion of fish remaining in the starting section before opening the lattices: 17.9% (control), 38.4% (U), 47.9% (TU). At the initial stage (to 8-9 minutes), the control individuals predominantly moved downstream; as a rule, they reached section no. 11 and stayed there for some time. Then, the fish began to move upstream. The I_d in control fish significantly depends on the registration period (Kruskal-Wallis H-test: $p < 0.01$) (Fig. 3).

Climbing perch movement in the water flow relates (Kruskal-Wallis H-test: $p < 0.01$) to its belonging to fish from clean water or fish exposured in U or TU. The fish from polluted water more often than individuals from clean water moved upstream: the mean value of the I_d for the whole recording period span at exposure to urea and thiourea solutions compared with the control is higher (−0.40 and −0.45 versus −0.56, respectively). During the entire registration period, no significant differences ($p > 0.05$) were found in per-minute I_d values between climbing perch exposed to U and TU (Fig. 3). No changes in the dynamics of I_d values with time (20 min) were revealed ($p > 0.05$) in fish under the influence of U and TU. The values of the I_d in fish under U influence begin to differ (Mann-Whitney U-test: $p < 0.01$) from the control fish already on the 2nd day of exposure. In the group of fish contained in thiourea, differences ($p < 0.01$) appear only on the 5th day between fish under the influence of TU and control fish. Control fish more often (Student's t-test for fractions: $p < 0.05$) than the individuals under the influence of U and TU moved along with the group (85.1 versus 71.8 and 73.9%, respectively).

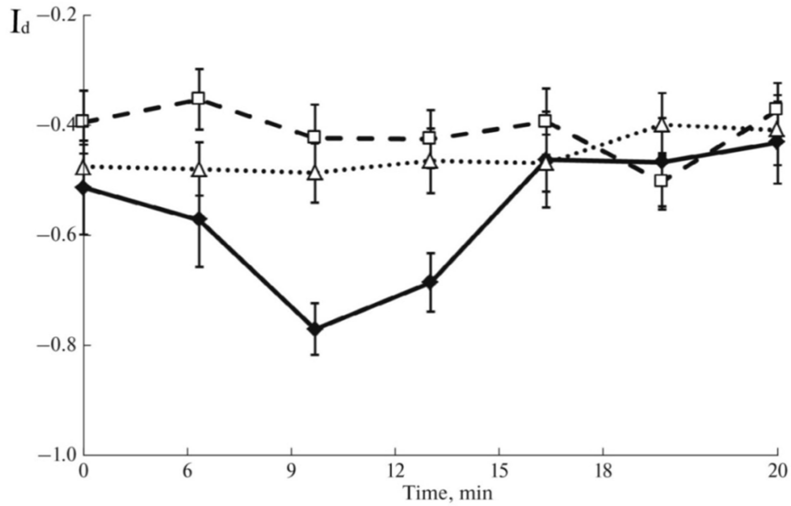

Figure 3. Dynamics of values of direction moving index (I_d) in climbing perch *Anabas testudineus* of the control group (in clean water) (---), at exposure to urea (- - -) and thiourea (·······) (polluted water); (|) - standard error of the mean.

3.2. The fish crawling and movements on the land

The fish swam freely during the 20-minute after placing to the starting tank of "dry maze". Fish lied at its bottom or on the underwater part of the ladder with their heads up. From time to time, climbing perch made jumps, directed mainly toward the tank's walls, but rarely jumped onto the ladder. Decreasing the water level in the starting tank from 18 to 16 cm caused anxiety in the fish. They were swimming in the tank more active, more often moved to the water surface and swallow air, and approached the water's edge on the ladder. At a water level of 16-8 cm, the number of fish moving to the water surface to swallow air increased and decreased from 6 cm below. On average, when the water level dropped from 18 to 4 cm, five moves to the surface of the water were recorded with a group of 10 specimens. Climbing perch jumps out of the water were observed at 14 cm and below. The jumping frequency of fish significantly increased with a decreasing water level (Kruskal-Wallis H-test: $p < 0.001$).

Two types of exit to the ladder were typical of climbing perch. The first was crawling with body and operculum movements. The second was a jump in the direction of the ladder, usually achieved with a hit on the first third of it. Individuals easily exited the ladder when the water in the tank was present or completely absent. When the fish jumped onto the ladder, most of the fish went out into the maze while the rest of the fish rolled back into the water. Fish began to emerge on the ladder at a water level of <3 cm (4th min of the experiment), and most intensively when it dropped to 0 cm: during the 5-6th minutes, more than half of the fish were crawling from the tank and left it during the entire experiment, or 36% of the total number of specimens. Individuals significantly less often reached the trap (Student's t-test for fractions: $p = 0.0047$) individually (40% of all exited individuals) than in a group (2-3 specimens, less often 4), forming a chain in which the next fish rushed after the first one. The distance between neighboring specimens moving in a group did not exceed 1.5 lengths. The speed of fish movement in the passageways was 4.3 ± 0.24 (1.6-10.6) cm/s (n = 71).

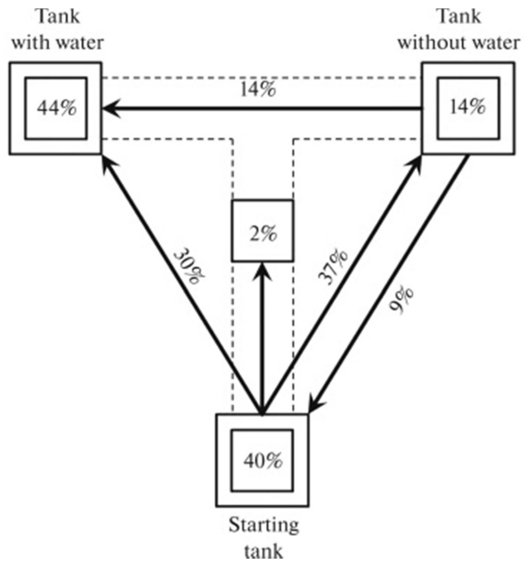

Figure 4. Directions (→) of movements of climbing perch *Anabas testudineus* in the "dry maze" and the proportion of fish in the tanks and passageways at the end of the experiments (□). Next to the arrows, the proportion of individuals moving during the investigation is indicated.

By the end of the experiment, significantly more fish left the starting tank (69%, Student's test for fractions: $p < 0.001$) than remaining in it (31%). The remaining specimens were lying at the bottom of the tank either individually or in groups, forming compact immobile aggregations. During the experiment, some fish that entered the maze could return to the starting tank. In some cases (16% of all specimens), the fish that entered the passageways returned to the tank they left (starting tank or a tank without water). Individuals in approximately equal proportions (Student's test for fractions: $p = 0.30$) reached the tank with water (30%) or without water (37%). Differences in the illumination of the left and right parts of passageways No. 2 did not affect (Kruskal-Wallis H-test: $p = 1.0$) the direction of specimens' movement. Two-thirds of the fish that got into the dry tank left it moved along the passageways to the starting tank or tank with water. While moving from the dry tank, they sometimes met in the passageways with fish that had not reached this tank yet. At the same time, neither one nor the other individuals encountered changed the direction of movement. By the end of the experiments, 40% of the fish were in the starting tank, 44 and 14% were in the tank with and without water, respectively. Only 2% of the fish remained in the passageways. Significantly (Student's test for fractions: $p = 0.0001$), more fish (44%, or 2/3 of the specimens that left the starting tank) were in the tank with water (Fig. 4).

3.3. Diurnal locomotor activity of fish

The locomotor activity significantly increased (Student's t-test: $p < 0.001$) from 12 pm to 5 pm and decreased ($p < 0.01$) at night (0 am to 5 am) (Fig. 5).

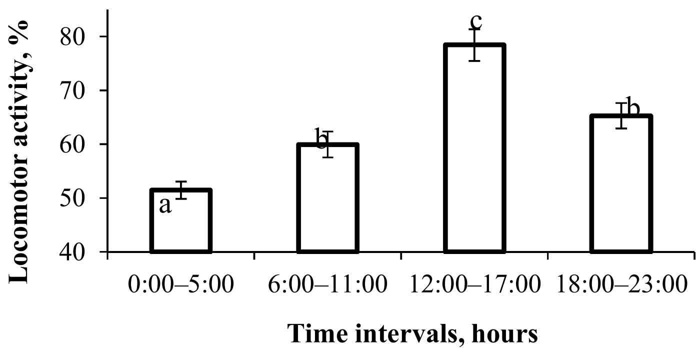

Figure 5. Locomotor activity (% of total time of interval) of climbing perch *Anabas testudineus* at a different time of the day. Different letters (a, b, c) indicates significant differences ($p < 0.05$ according Student's *t*-test)

Our results showed that the climbing perch has common behavioral features when moving in water or on land. In both cases, fish often move into groups (≥2 specimens). The groups of individuals in the water flow can decrease or increase over time. Fish formed the groups when they crawled to the land, but unlike the aquatic environment, these groups never raised during the movements over land. Another common feature is jumping out of the water, which may be an element of fish exiting to land. The frequency of these jumps reflects the increasing influence of an unfavorable factor; it increased by a decreasing water level.

Climbing perch from clean water (control) had a pronounced dynamics of movements during 20 minutes in hydrodynamic test chamber. Unlike these fish, the individuals under the influence of urea and thiourea observed chaotic movements without predominant direction. Changes in movements in the climbing perch under the influence of urea and thiourea may be important in the natural environment within migration and the distribution of fish in water bodies and streams. Urea and thiourea significantly decreased jumping from the water and the frequency (on 10%) of group formation of fish. Group lifestyle is a characteristic of climbing perch [11]. Under the influence of testing substances, the group-directed movement of fish is replaced by an individual multidirectional one. Probably, the substances used generally suppress the natural behavioral response of the fish. The effect of urea was noted already on the 2nd day of exposure; of thiourea, only on the 5th day, which is consistent with the data concerning thiourea that we obtained earlier [12]. In water, urea is less chemically stable than thiourea. The study indicates that a shorter period of urea activity is compensated by its faster effect on the fish behavior. The widespread use of urea in agriculture in Vietnam can negatively affect fish's physiological state and behavior.

Fish movements in the "dry maze" revealed that a decreasing water level is one of the most critical factors stimulating climbing perch to move overland. Water decreasing to almost zero within 5 minutes stimulates the fish to quickly respond: to move overland in searching for new water bodies. The water level ≤3 cm may be considered critical in the experimental conditions at which the climbing perch begins to crawl on land.

During a period of drought, climbing perch can burrow into the ground [2, 13] or crawl overland into new water bodies [14]. In the present experiments, the fish reacted to a decreased water level: waiting or crawling and moving on land to search for new habitat conditions. Specimens waiting in unfavorable conditions commonly formed compact groups at the bottom of the tank that apparently allowed them to maintain body moisture longer. Such fish aggregation, as well as burrowing into the ground during drought, has an adaptive implication. Burrowing of climbing perch is possible only on wet land. Our *in situ* experiments [15] did not reveal any attempt by climbing perch to burrow into dry land. The success of the behavior with critical decreasing water completely depends on the environmental conditions that are formed further. An active search could be more favorable than waiting at high air humidity and a nearby location of a new water body. At the same time, waiting allows fish to preserve energy resources for a long time since the movement over land has a higher energetic value [16].

The average speed of the climbing perch move in the "dry maze" was 4.3 cm/s (0.6 TL/s); the maximal rate was 10.6 cm/s. In nature, climbing perch overcomes over 90 m of a challenging route in about 30 min (5 cm/s) [2]; in a laboratory experiment, the maximal speed of larger individuals (TL 14.3 cm) approached 25.6 cm/s [17]. Our data is consistent with these results. The speed of climbing perch overland mostly depends on its physiological state and the composition of the ground.

Climbing perch searched a tank with water randomly in our experiments. Some specimens changed the direction of movement in the passageways to the opposite. That indicated an absence of specific landmarks for orientation of fish. Moving inside the maze, as a rule, preceded until the fish found a tank with water: most of the specimens that initially got into the dry tank continued to search. The fish in the tank with water did not attempt to leave it (mainly). Consequently, the search for water determined the climbing perch's terrestrial movement (migration). Experiments *in situ* [15] confirmed the random searching for a water body by climbing perch. Fish were placed near 4 m from the pond and moved both towards and away from it. Higher illumination from the side of the pond increased the chances of fish reaching the aquatic environment. The survival rate of climbing perch when moving on land seems depends not so much on its ability to navigate on land but on external factors such as illumination, the type of the ground and vegetation, the temperature and humidity of the air, and the distance to the nearest water body, etc.

The diurnal locomotor activity of climbing perch preliminary connects with the probability of the active movements of fish at certain times of the day. The fish had the lowest locomotor activity at night and the highest during the day. Consequently, the probability of fish movements in the water and on land is higher in the daytime. The data obtained are consistent with our experiments in the "dry maze": 70-100% of cases, the fish do not move and remain in the starting pool in the darkness [15].

4. CONCLUSION

- Climbing perch exposed in clean water has behavior in water flow in which individuals first move downstream and then upstream. The exposition of fish in 0.05% solutions of urea or thiourea modifies their behavior, which is expressed in the chaotic movement of fish relative to the current.

- Water decreasing stimulates climbing perch to crawl and move on land. There are two behavioral responses to water decrease: waiting for adverse conditions or moving over land searching for optimal habitat conditions. Under the experimental conditions, searching for a new water body is random.

- The probability of climbing perch movements in the water and on land is higher during daylight than in the dark.

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REFERENCES

1. Das B. K., *The bionomics of certain air-breathing fishes of India, together with an account of the development of their air-breathing organs*, Philos. Trans. R. Soc., *B*, 1928, **216:**183-216.
2. Smith H. M., *The freshwater fishes of Siam, or Thailand*, Bull. US Natl. Mus., 1945, **188:**1-622.
3. Rainboth W. J., *Fishes of the Cambodian Mekong. FAO species identification field guide for fishery purposes*, 1996. FAO, Rome, 265 tr.
4. Binoy V. V., Job N., Thomas K. J., *Influence of dicofol on the behavior of the climbing perch, Anabas testudineus*, Indian J. Fish., 2004, **51**(3):345-351.
5. Nordin I. L., Ibrahim N., Hamidin N., et al., *Acute toxicity of endosulfan to Anabas testudineus*, Adv. Environ. Biol., 2015, **9**(27):341-345.

6. Velmurugan B., Cengiz E. I., Yolcu M., et al., *Cytological and histological effects of pesticide chlorpyriphos in the gills of Anabas testudineus*, Drug. Chem. Toxicol., 2018, **43**(4):409-414.

7. Liem K. F., *Functional design of the air ventilation apparatus and overland escursions by teleosts*, Fieldiana Zool., 1987, **37**:1-29.

8. Kasumyan A. O., Pashchenko N. I. and Oan L. T. K., *Morphology of the olfactory organ of the climbing perch Anabas testudineus (Anabantidae, Perciformes)*, Zool. Zh., 2021, **100**(1):40-56.

9. Pavlov D. S., Kostin V. V., Zvezdin A. O., Ponomareva V. Yu., *On methods of determination of the rheoreaction type in fish*, J. Ichthyol., 2010, **50**(11):977-984.

10. Ziegler-Skylakakis K., Kielhorn J., Könnecker G., et al., *Concise International Chemical Assessment Document 49: Thiourea*, World Health Org.: Geneva, 2003, p. 1-37.

11. Binoy V. V. and Thomas, K. J., *The climbing perch (Anabas testudineus Bloch), a freshwater fish, prefer larger unfamiliar shoals to smaller familiar shoals*, Curr. Sci., 2004, **86**(1):207-211.

12. Pavlov E. D., Zvezdin A. O. and Pavlov D. S., *Effect of thiourea on migratory activity of climbing perch Anabas testudineus and food consumption*, J. Ichthyol., 2019, **59**(5):810-814.

13. Datta N. C., Saha A. K. and Das A., *Investigation on the morphology of the olfactory apparatus of Anabas testudineus (Bloch)*, J. Inland Fish. Soc. India, 1976, **8**:13-18.

14. Linke H., *Labyrinth Fish, the Bubble Nest Builders: Their Identification, Care and Breeding*, Tetra: Melle, 1992.

15. Pavlov E. D., Tran Duc Dien, Ganzha E. V., Vo Thi Ha, *Feuters of migration behavior of fish in waterbodies of Southern Vietnam*, Scientific report of the program Ecolan3.2 "Taxonomic Diversity, Ecology, and Behavior of Freshwater Aquatic Organisms", Vietnam, Nha Trang, 2022, 60 pp.

16. Sayer M. D. J. and Davenport J., *Amphibious fish: why do they leave water?* Rev. Fish Biol. Fish., 1991, **1**(2):159-181.

17. Davenport J. and Matin A. K. M. A., *Terrestrial locomotion in the climbing perch, Anabas testudineus (Bloch) (Anabantidea, Pisces)*, J. Fish Biol., 1990, **37**:175-184.

SUMMARY

Climbing perch *Anabas testudineus* in contradistinction to most fish is able to move not only in water but also on land. In the experiments, we study the diurnal locomotor activity of fish, the features of their movements in flow of clean water and polluted water by urea and thiourea, and the effect of decreasing water level on the crawling and moving on land. The behavior of fish in clean water aimed to move downstream at the beginning of the test and moving upstream in the end of the test.

Fish exposition (12 days) under 0.05% urea and thiourea change their behavior in the water flow, which is expressed in the chaotic movement of fish relative to the current. Decreasing water level to the critical value (<3 cm) initiates climbing perch to crawling and moving on land. Two types of fish responses to decreasing water level were found: waiting, or crawling to land and moving. Based on the study diurnal locomotor activity, we found that the probability of climbing perch movements is higher in the daytime than in the dark.

Keywords: *Climbing perch Anabas testudineus, movements, locomotor activity, water flow, water level, urea.*

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