DOI: https://doi.org/10.36094/sc.v91.2025.Impact_of_Variable_Water_Flow_Rates.Bhattacharya.462

Research Communication

Sci. and Cult. 91 (7–8) : 462-466 (2025)

Impact of Variable Water Flow Rates on Rheotactic Ability: A Live Simulation Study Using Zebrafish Model

***Abstract*: Flow rate of surrounding water is a crucial factor that affects various physiological and biophysical sensory responses of aquatic animals like fish. In recent past zebrafish has emerged as an effective animal model for studying various aspects of sensory responses. In the present study the effect of variable flow rate of surrounding water on positive rheotactic response has been investigated in zebrafish model through development of a customized artificial water flow swimming tunnel set up.**

Keywords: Rheotaxis, Zebrafish, Mechanoception, Sensory Physiology.

Flow rate of surrounding water is an important hydromechanical factor for aquatic organisms like fish[1]. In nature, water flow rate is a variable element in terms of time and space[2]. In hydrodynamics, water flow rate is defined as the volume of water flowing through a given cross-sectional area per unit time. Fishes' morphological and life-history traits are associated with the water flow regime and habitat of their environment and are correlated with swimming modes[3-5]. Fish body shape, particularly the shape and length of the caudal fin, is important for swimming, acceleration and manoeuvrability in different water flows, as well as for catching prey or escaping predators[6-8]. It also affects fish culture systems[9]. Water flow rate is also known to influence metabolic rates of fish. For example, tropical reef fish living in high-flow areas have higher maximum swimming speeds and aerobic scopes, and quicker fast-start escape responses, while those in lower flow areas have lower metabolic rates, aerobic scopes and maximum swimming speeds[10, 11].

Apart from the mentioned aspects, flow rate of water is considered as an important quantifiable parameter for studying fish sensory physiology and the corresponding motor output[12-15]. Water flow patterns are detected along the entire length of the body via sensory hair cells called neuromasts of the lateral line system. In this way, the fish detects the flow field as it swims forward and detects disturbances in the flow field made by prey and predators. For example, when a fish detects the accelerating flows of suction or ram actions of predators, it will instinctively make a turn or C-shaped body bend and move in an opposite direction[16.] The reaction occurs within 10 milliseconds. Thus, flow rate of water is a crucial parameter to be considered for research studies on sensory biology using aquatic model animals. Among several aquatic animal models, zebrafish (*Danio rerio*), a freshwater teleost is considered as an emerging model for studying sensory physiology and motor responses[17,18]. In light of this scenario, an experimental research has been undertaken using zebrafish model to study the effect of changes in water flow rate of the immediate environment on the swimming behavior of the fish. To perform the live simulation study a customized swimming apparatus was designed in the laboratory that allowed generation of controllable, consistent and repeatable water flow during the experimentation.

Methodology

Designing of the Artificial Water Flow Swimming Apparatus: The Artificial Water Flow Swimming Apparatus consisting of a modified submersible water pump (SOBO WP 1000F AC 220V, 50Hz, 15W, F. Max: 650 $l.h^{-1}$) and a 2-side open cylindrical glass tunnel having a diameter of 2.5 cm and length of 14 cm (Fig. 1) was developed at the laboratory for the present study. The rotor blades of the submersible pump were modified for generating controllable and quantifiable water currents. The inlet terminal of the pump was fitted to the glass tunnel and the outlet terminal of the pump was connected to an outlet vertical connector pipe for maintaining a continuous and almost stream line water flow within the tunnel and the bath chamber. Both the ends of the 2-side open glass tunnel were covered with nets to confine the swimming area of the fish and to avoid accidental injury of the fish from the rotor blades. The whole setup, i.e., the glass tunnel fitted to the pump was immersed inside the rectangular glass bath chamber

having a dimension of 30 cm × 18 cm × 18 cm. The water flow rate inside the swimming tunnel was regulated by adjusting the height of the water column through vertical extension of the outlet-connector pipe, as per the necessity. In order to control the flow rate of the water two identical metal plates were attached in between the jaws of the clamp holding the vertically extended pipe (Fig. 1). These metal plates allowed uniform lateral compression of the outlet pipe which in turn maintained a laminar flow of water and also regulated the rate of the flow.

The flow rate (*Q)* was calculated using the expression (1) as the volume of fluid (*V*) per unit time (*t*) flowing past a point through the area (A)[19].

$$Q = \frac{V}{\pi r^2 t} \text{cm.s}^{-1} \quad (1)$$

To ascertain whether the water flow pattern was laminar or not the Reynold's number (R) was computed using the following equation (2):

$$R = \frac{\rho VD}{\mu} \quad (2)$$

where ρ = fluid density, V = fluid velocity, D = pipe diameter, μ = fluid viscosity.

It is assumed that a Reynold's number less than or equal to 2100 indicates laminar flow, and a Reynold's number greater than 2100 indicates turbulent flow[20]. In the study the R was computed to be 1910. Thus, the water flow pattern in the study was laminar in nature.

Experimental procedure: For the present study a total of 15 adult zebrafish of mixed gender were chosen and divided equally into three groups – F1, F2 and F3. In the three groups, i.e., F1, F2 and F3 the flow rates of the surrounding water were maintained at 5.5 $cm.s^{-1}$, 7.69 $cm.s^{-1}$ and 11.9 $cm.s^{-1}$, respectively throughout the experiment. The different flow rates selected on the present study were based on the habitable water flow rate range (3.5 – 13.9 $cm.s^{-1}$) of wild zebrafish[21]. For tracking the locomotion of zebrafish inside the swimming tunnel the Animal Tracking plugin of the imageJ software was used[22]. The position of the fish of the three groups inside the tunnel was tracked based on the X-axis pixel value only. For the ease of calculation, the pixel values were converted to cm scale. The data were then statistically analysed through ANOVA testing computed using Python language-based library function. After the onset of hydrodynamic flow cue, the fish was left swimming for a habituation period of 5 min inside the swimming tunnel[23]. Following the brief acclimatization period, the position of the fish of the three groups inside the rheotaxis tunnel was tracked based on the X-axis pixel value. The video clips used for analysis were tracked for 10 sec (s) and were shot at 30 frames per second (fps) with a resolution of 1080 x 1920 pixels (px)[24,25]. The important physiological parameters like water temperature (25 °C) and water pH (7) were maintained within the physiological range throughout the experiment26. During experimentation submersible aerator was used to maintain the oxygen supply of the surrounding water within the apparatus.

Results

The results indicated that upon varying the flow rate of the surrounding water there was a variation in the X-axis position of fish inside the swimming tunnel. In case of group F1 the grand mean value of the X-axis position of fish was significantly higher ($P<0.05$) in F1 comparison to that of groups F2 and F3 (Fig. 2).

From the tracked path of fish (Fig. 3) it was noted that with increase in water flow rate the fluctuations in the X-axis position of fish inside the tunnel also increased.

Discussion

The variable water flow rate stimulus was used to study the mechanoception of the model fish and how mechanosensory perception regulates the swimming behaviour of the fish. Mechanoreception in fish includes tactile perception, pressure gradients in surrounding water, water currents and vibration[27]. The results indicated that with variation in artificially generated water flow rate there was a prominent variation in the locomotory pattern of the fish inside the swimming tunnel. It was also found that with increase in flow rate of the surrounding water there was a significant ($P < 0.05$) decrease in the X-position of the fish within the swimming tunnel. The change in X-axis position of fish with increase in artificially generated water flow rate coincides with a recent study conducted on larval zebrafish where a variation in horizontal position of fish was reported with alterations in water flow rate inside an artificial microfluidic device[28].

Earlier studies have mentioned that in aquatic animals two types of rheotactic response are found, one is positive rheotaxis (maintenance of position and orientation, directed prey capture), swimming in a direction opposite to fluid flow direction and the other is negative rheotaxis (migratory behaviour), swimming along the same direction to that of

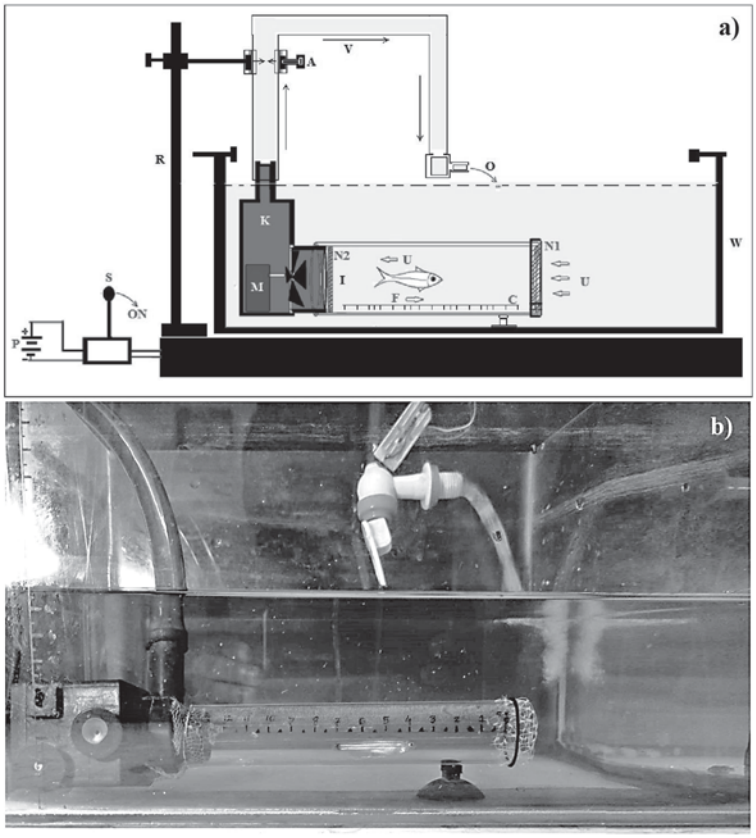

Fig. 1: a) Schematic representation of the Artificial Water Flow Swimming Apparatus

b) Image of the swimming tunnel with the flow generator pump and fish placed within the water chamber

S – power source, M – modified motor, K – flow generator submersible pump, N1-N2 – protection nets, I – pump inlet, O – pump outlet, F – swimming direction of fish in presence of water flow, U – direction of water flow, C – scale, R – height adjustable stand, A – screw with metal plates for uniform lateral compression, V – vertical connector pipe, W – water chamber

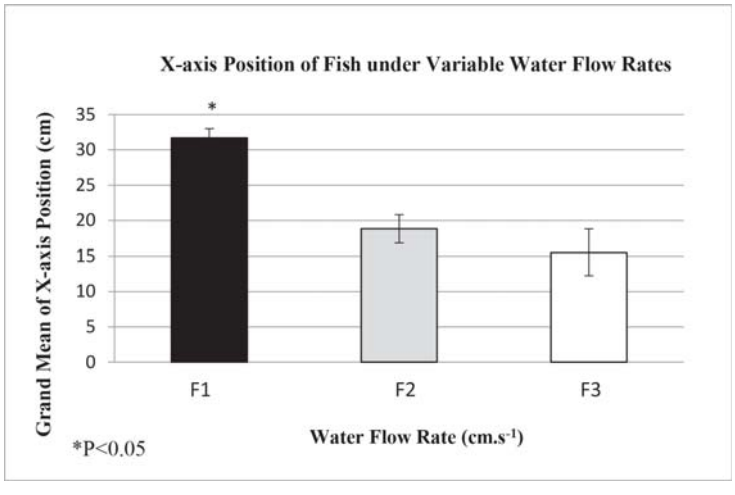

Fig. 2: Graphical Representation of the X-Axis Position of Fish under Three Variable Water Flow Rate Conditions – F1 (5.5 $cm.s^{-1}$), F2 (7.69 $cm.s^{-1}$) and F3 (11.9 $cm.s^{-1}$)

water flow[29]. It has been reported by several research studies that in nature, zebrafish respond to incoming water current by swimming in a direction opposite to that of the water flow in order to maintain position and alignment with minimum energy expenditure[30]. Similarly, in the present study it was noted that when zebrafish were exposed to an environment with artificial water flow the fish exhibited positive rheotactic response. Studies have shown that the mechanoreceptive ability of teleost group of fish, like zebrafish is modulated by a specialized mechanosensory system called the lateral line system[31]. Recent studies have reported that the lateral line sensory system plays a significant role in regulating the rheotaxis of fish[32]. The functional unit of lateral line system is the neuromast[32]. An individual mechanoreceptor is either located on the surface of the animal (superficial neuromast (SN)) or inside fluid-filled lateral line canals (canal neuromast (CN))[33,34]. The SNs respond to the surrounding water's flow velocity and CNs are sensitive to fluid motion caused by pressure gradients between adjacent pores that connect lateral line canals to the surrounding water[35]. The neuromast consist of

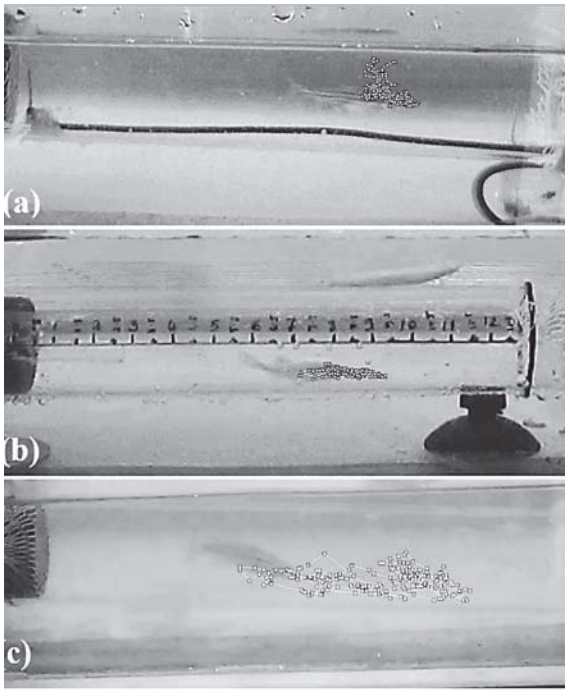

Fig. 3: The 10 s Tracked Path of Fish inside the Swimming Tunnel as obtained from the Tracking Software under Three different Water Flow Rates

(a) Flow rate 5.5 $cm.s^{-1}$, (b) Flow rate 7.69 $cm.s^{-1}$, (c) Flow rate 11.9 $cm.s^{-1}$

bundles of hair cells. The hair bundles are oriented in same axis but in opposing directions (staircases of increasing length) – like either antero-posterior or dorso-ventral36. The rationale for such arrangement is that half of the hair cells respond to stimuli from one direction (deflection of hair cells towards the posterior) and the other half responds to stimuli from the other direction (deflection towards the anterior). Thus, the arrangement determines the directionality of stimulus sensitivity[37].

In the present study to understand how the variation in rate of water flow affects the positive rheotactic ability of the zebrafish model, the X-axis position of the fish of each of the groups were computed and statistically analysed through ANOVA testing. When the water flow rate was maintained at 5.5 $cm.s^{-1}$ the grand mean of the X-axis position of the fish during 10 s tracking period was 31.70 cm. The X-axis position under flow rate 7.69 $cm.s^{-1}$ was 18.82 cm. The X-axis position under flow rate 11.9 $cm.s^{-1}$ was 15.52 cm. Thus, the data pattern indicated a maximum value of X-axis position under minimum water flow rate of 5.5 $cm.s^{-1}$. Therefore, it is evident that with increase in flow rate of surrounding water the ability of the fish to maintain rheotactic behavior significantly reduces ($P<0.05$). Thus, zebrafish perceives the variation in the water flow rate through activation of the neuromasts of the lateral line system which in turn, modulates the rheotactic ability.

Conclusion

The present study thus indicates that variation in water flow rate of the immediate environment affects the swimming behaviour of the zebrafish model, quantified in terms of positive rheotactic response. It was also evident that with increase in rate of water flow there was a significant reduction ($P<0.05$) in the grand mean value of X-axis position of fish within the swimming tunnel. Thus, it may also be inferred that there exists an inverse relationship between water flow rate and positive rheotactic output.

Acknowledgement

The authors acknowledge the Electrical Workshop Unit of Indian Statistical Institute, Kolkata.

Conflicts of Interest

The authors declare no conflicts of interest. ❐

***BARNINI BHATTACHARYA[1,2]**
SHIBSANKAR ROY[1,2]
BIJAY BAL[3]
SHANKARASHIS MUKHERJEE[4]
KUNTAL GHOSH[1,5]

[1]Indian Statistical Institute
(Laboratory for Cognitive Systems and Cybernetics Research, Center for Soft Computing Research), Kolkata 700108, India

[2]University of Calcutta
(Department of Physiology), Kolkata, India

[3]Saha Institute of Nuclear Physics (Retired), Kolkata, India

[4]Department of Food and Nutrition, West Bengal State University, India

[5]Indian Statistical Institute
(Machine Intelligence Unit), Kolkata, India

*Corresponding author: barnibhattacharya@gmail.com

Received: 18 June, 2024

Accepted: 17 March, 2025

References

1. J.C. Liao, *Philosophical Transactions of the Royal Society B: Biological Sciences.* **362**(1487), 1973-93 (2007).
2. D. Cortese, A. Crespel, S.C. Mills, T. Norin, S.S. Killen, R. Beldade, *Functional Ecology.* **36**(12), 2983-94 (2022).
3. C.J. Fulton, D.R. Bellwood, P.C. Wainwright, *Marine Biology*. **139**(1), 25–33 (2001).
4. R. Langerhans, C.A. Layman, A.K. Langerhans, T.J. Dewitt, *Biological Journal of the Linnean Society*. **80**(4), 689–698 (2003).
5. P.W. Webb, *American Zoologist*. **24**(1), 107–120 (1984).
6. P. Domenici, R.W. Blake, *Journal of Experimental Biology*. **200**(8), 1165–1178 (1977).
7. R.B. Langerhans, *Integrative and Comparative Biology*. **48**(6), 750–768 (2008).
8. J.J. Videler, Fish swimming (Springer Science & Business Media, 1993).
9. M.D. Krom, A. Neori, J. Van Rijn, *Israeli Journal of Aquaculture/Bamidgeh*. **41**(1), 23-33 (1989).
10. S.A. Binning, D.G. Roche, C.J. Fulton, *Oecologia*. **174**(3), 623–630 (2014).
11. L.E. Nadler, S.S. Killen, P. Domenici, M.I. McCormick, *Biology Open*. **7**(10), bio031997 (2018).
12. D.A. Burbano-L, M. Porfiri, *PLoS Computational Biology*. **17**, 1–24 (2021).
13. G.P. Arnold, *Journal of Experimental Biology*. **75,** 147 – 169 (1977).
14. A. Suli, G.M. Watson, E.W. Rubel, D.W. Raible, *PLoS One*. **7**, 1 – 6 (2012).
15. J. Bak-Coleman, S. Coombs, *Journal of Experimental Biology*. **217,** 2338 – 2347 (2014).
16. M. Mirjany T. Preuss, D.S. Faber, *Journal of Experimental Biology*. **214,** 3358–3367 (2011).
17. M. Privat, S.A. Romano, T. Pietri, *Current Biology.* **29** (23), 4010-23 (2019).

18. H.A. Burgess, M. Granato, *Journal of Neuroscience*. **27**(18), 4984-94 (2007).

19. H Oertel, C.I. Technik, Introduction to Fluid Mechanics Fundamentals and Applications (Springer, New York, 2001).

20. F.M. White, Fluid Mechanics (McGraw Hill, 2011).

21. S. Cartner, J.S. Eisen, S.F. Farmer, K.J. Guillemin, M.L. Kent, G.E. Sanders, The Zebrafish in Biomedical Research: Biology, Husbandry, Diseases, and Research Applications (Academic Press, USA, 2019).

22. J.E. Franco-Restrepo, D.A. Forero, R.A. Vargas, *Zebrafish*. **16**, 223-232 (2019)

23. V. Mwaffo, P. Zhang, S.R. Cruz, M. Porfiri, *PeerJ*. **5**, e4041 (2017).

24. H. Zhang, S.C. Lenaghan, M.H. Connolly, L.E. Parker, 12th International Conference on Machine Learning and Applications, IEEE. **1**, 161-166 (2013).

25. M.D. Barreiros, D.D. Dantas, L.C. Silva, S. Ribeiro, A.K. Barros, *Scientific reports*. **11**(1), 3219 (2021).

26. A. Avdesh, M. Chen, M.T. Martin-Iverson, A. Mondal, D. Ong, S. Rainey-Smith,...R.N. Martins, *Journal of Visualized Experiments*. **69**, e4196 (2012).

27. K.A. Sloman, Encyclopedia of Fish Physiology (Academic Press, 2011) 783-789.

28. A. Schmitz, H. Bleckmann, J. Mogdans, *Journal of Morphology*. **269**, 751-61 (2008).

29. K.J. Painter, *Journal of The Royal Society Interface*. **18**, 20210582 (2021).

30. E. Han, Y.J. Choi, S. Park, Y.C. Rah, H.C. Park, S.H. Lee, J. Choi, *Expert Systems with Applications*. **237**, 121496 (2024).

31. R. Manuel, A.B. Iglesias Gonzalez, J. Habicher, H.K. Koning, H. Boije H, *Frontiers in Neuroanatomy*. **15**, 666109 (2021).

32. M. Beckmann T. ErQs, A. Schmitz, H. Bleckmann, *International Review of Hydrobiology*. **95**, 273-84 (2010).

33. S. Coombs, P. Görner, H. Münz. Neurobiology and Evolution (Springer, New York, 1989) 725.

34. S. Coombs, Bleckmann H, R.R. Fay, A.N. Popper, The Lateral Line System (Springer, New York, 2013) 17–72.

35. H. Herzog, B. Klein, A. Ziegler, *Journal of the Royal Society Interface*. **14**, 20160898 (2017).

36. E.D. Thomas, I.A. Cruz, D.W. Hailey, D.W. Raible, *Wiley Interdisciplinary Reviews: Developmental Biology*. **4**, 1-6 (2015).

37. Y.R. Ji, S. Warrier, T. Jiang, D.K. Wu, K.S. Kindt, *Elife*. **7**, e35796 (2018).