RESEARCH REVIEW JOURNALS
Volume-04
Issue-05
May-2019

ISSN: 2455-3085 (Online)
RESEARCH REVIEW International Journal of Multidisciplinary
www.rrjournals.com[UGC Listed Journal]

Study of Respiratory Behaviour Under Different Climate Conditions of Zebra Fish

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ARTICLE DETAILS

Article History
Published Online: 25 May 2019

Keywords
Investigation, Global warming, Kinematic, Respiratory, Climate, Temperature.

ABSTRACT

In this research, a series of exposure tests were conducted to investigate behavioral changes of adult zebrafish (Danio rerio) exposed to deltamethrin (DM) in six concentrations of 0, 0.15, 1.5, 3.75, 7.5 and 15 µg/L. Swimming changes in zebrafish were detected at a concentration as low as 1% of the LC50-24h within five hours. Climate change can have a pronounced impact on the physiology and behaviour of fishes. Notably, many climate change stressors, such as global warming, hypoxia and ocean acidification (OA), have been shown to alter the kinematics of predator– prey interactions in fishes, with potential effects at ecological levels. Here, we review the main effects of each of these stressors on fish escape responses using an integrative approach that encompasses behavioural and kinematic variables. Elevated temperature was shown to affect many components of the escape response, including escape latencies, kinematics and maximum swimming performance, while the main effect of hypoxia was on escape responsiveness and directionality.

1. Introduction

Zebrafish is a member of the genus Danio of the family Cyprinidae. It was referred to in the scientific literature as Brachydanio rerio for many years until its redesignation as the genus Danio (Westerfield, 2000). Zebrafish are a highly valued model organism in developmental biology, genetic studies, and drug screening. Adult and larval zebrafish offer many perspectives in neuroscientific studies because they are a vertebrate species with high physiological and genetic homology to humans. Zebrafish are considered a useful species for investigating central drug effects, psychiatric diseases the immune system, behavioral effects and neurotoxicity. Adult zebrafish were evaluated for general activity, exploratory/motor behavior, climbing to the water surface, tremors, and erratic movements. The gills were evaluated because they are a metabolically active and readily available organ that is commonly used for bio-monitoring analyses in fish, and such analyses can reveal respiratory system impairments. The aim of the present study was to determine the validity of behavior as early sign of toxicity and respiratory injuries induced by cadmium acute exposure in adult zebrafish (Danio rerio). In addition, correlations between behavioral and morphological effects in fishes may open protocols for respiratory system studies that are related to toxicology.

A rapid increase in atmospheric carbon dioxide combined with anthropogenic pollutants is causing major changes in the physical and chemical properties of the ocean, with major impacts on its inhabitants. Climate change models project that ocean pCO2 will exceed 900 ppm by 2100 from current levels of ~ 400 ppm, leading to a decrease in ocean pH. Elevation of this primary greenhouse gas will also lead to a predicted 3–5◦C increase in ocean temperatures over the same time period. The temperature dependence of chemical reactions means that levels of available oxygen within the shallow ocean will decrease, particularly when combined with increased levels of nutrient discharge along the shores and eutrophication, and these have already led to an increase in the size and number of hypoxic zones in coastal and oceanic waters. In the aquatic environment, global warming, ocean acidification (OA) and hypoxia are intrinsically linked with each other and with an array of other stressors that are predicted to increase over time due to accelerating global economies. Global warming can increase the release of CO_2 from terrestrial and marine sinks, thereby creating a positive feedback causing a further increase in temperatures. Similarly, global warming is contributing to deoxygenation, as a result of a decrease in oxygen solubility and an increase in oxygen consumption in marine organisms. OA is predicted to be amplified by hypoxia in coastal areas, where much higher pCO_2 values than predicted for other areas of the ocean can be expected, because of the production of CO_2 related to heterotrophic degradation of organic material. Furthermore, although the levels of each of these three fundamental stressors are known to show a natural daily and seasonal variation, the extent of their variation is predicted to increase, particularly for CO_2.

Global warming is having a major effect on the structure and functionality of aquatic communities, affecting the abundance and geographical distribution of aquatic organisms. Ecological effects at the population, community and ecosystem level have been claimed to be largely related to the physiological responses of aquatic organisms to temperature. Fishes, like all other ectotherms, are particularly vulnerable to temperature changes and therefore, it is important to understand how temperature affects their overall performance as well as their inter-specific interactions, in order to allow predictions of how climate change will affect aquatic communities.

Many organismal functions are temperature dependent, and thermal response curves have been derived that illustrate the relationship between various fundamental biological rates and temperature. In addition to such basic physiological traits, swimming performance and kinematics are known to be affected by temperature due to alterations in aerobic activity and endurance, cardiac output, muscle development as well as power output for anaerobic swimming (burst) through changes in the contractile properties of the swimming muscles. Here, we discuss how temperature may specifically affect escape swimming performance and kinematics, which are relevant for predator–prey interactions. In addition, temperature can affect other traits that are key in predator–prey interactions, such as those related to brain and sensory functions, e.g. the timing of the response. As a result, temperature is likely to change the balance of predator and prey performance, which can have a major effect on aquatic communities at large.

2. Literature Review

Sara J. Abdallah (2015) Severe hypoxia elicits aquatic surface respiration (ASR) behaviour in many species of fish, where ventilation of the gills at the air–water interface improves

O2 uptake and survival. ASR is an important adaptation that may have given rise to air breathing in vertebrates. The neural substrate of this behaviour, however, is not defined. We characterized ASR in developing and adult zebrafish (Danio rerio) to ascertain a potential role for peripheral chemoreceptors in initiation or modulation of this response. Adult zebrafish exposed to acute, progressive hypoxia (PO2 from 158 to 15 mmHg) performed ASR with a threshold of 30 mmHg, and spent more time at the surface as PO2 decreased. Acclimation to hypoxia attenuated ASR responses. In larvae, ASR behaviour was observed between 5 and 21 days postfertilization with a threshold of 16 mmHg. Zebrafish decreased swimming behaviour (i.e. distance, velocity and acceleration) as PO2 was decreased, with a secondary increase in behaviour near or below threshold PO2 . In adults that underwent a 10-day intraperitoneal injection regime of 10 μg g−1 serotonin (5-HT) or 20 μg g−1 acetylcholine (ACh), an acute bout of hypoxia (15 mmHg) increased the time engaged in ASR by 5.5 and 4.9 times, respectively, compared with controls. Larvae previously immersed in 10 μmol l−1 5-HT or ACh also displayed an increased ASR response. Our results support the notion that ASR is a behavioural response that is reliant upon input from peripheral O2 chemoreceptors. We discuss implications for the role of chemoreceptors in the evolution of air breathing.

Matthias Messerli (2020) In order to study the adaptation scope of the fish respiratory organ and the O_2 metabolism due to endurance training, we subjected adult zebrafish (*Danio rerio*) to endurance exercise for 5 weeks. After the training period, the swimmer group showed a significant increase in swimming performance, body weight and length. In scanning electron microscopy of the gills, the average length of centrally located primary filaments appeared significantly longer in the swimmer than in the non-trained control group (+6.1%, 1639 *μ*m vs. 1545 *μ*m, p = 0.00043) and the average number of secondary filaments increased significantly (+7.7%, 49.27 vs. 45.73, p = 9e-09). Micro-computed tomography indicated a significant increase in the gill volume (p = 0.048) by 11.8% from 0.490 mm^3 to 0.549 mm^3. The space-filling complexity dropped significantly (p = 0.0088) by 8.2% from 38.8% to 35.9%., i.e. making the gills of the swimmers less compact. Respirometry after 5 weeks showed a significantly higher oxygen consumption (+30.4%, p = 0.0081) of trained fish during exercise compared to controls. Scanning electron microscopy revealed different stages of new secondary filament budding, which happened at the tip of the primary lamellae. Using BrdU we could confirm that the growth of the secondary filaments took place mainly in the distal half and the tip and for primary filaments mainly at the tip. We conclude that the zebrafish respiratory organ—unlike the mammalian lung—has a high plasticity, and after endurance training increases its volume and changes its structure in order to facilitate O_2 uptake.

Allan V. Kalueff, (2013) Zebrafish (*Danio rerio*) are rapidly gaining popularity in translational neuroscience and behavioral research. Physiological similarity to mammals, ease of genetic manipulations, sensitivity to pharmacological and genetic factors, robust behavior, low cost, and potential for high-throughput screening contribute to the growing utility of zebrafish models in this field. Understanding zebrafish behavioral phenotypes provides important insights into neural pathways, physiological biomarkers, and genetic underpinnings of normal and pathological brain function. Novel zebrafish paradigms continue to appear with an encouraging pace, thus necessitating a consistent terminology and improved understanding of the behavioral repertoire. What can zebrafish '*do*', and how does their altered brain function translate into behavioral actions? To help address these questions, we have developed a detailed catalog of zebrafish behaviors (Zebrafish Behavior Catalog, ZBC) that covers both larval and adult models. Representing a beginning of creating a more comprehensive ethogram of zebrafish behavior, this effort will improve interpretation of published findings, foster cross-species behavioral modeling, and encourage new groups to apply zebrafish neurobehavioral paradigms in their research. In addition, this glossary creates a framework for developing a zebrafish neurobehavioral ontology, ultimately to become part of a unified animal neurobehavioral ontology, which collectively will contribute to better integration of biological data within and across species.

Dimitris G. Sfakianakis (2010) It is widely known that water temperature affects the swimming capacity of fish. But the effect of the rearing temperature on the swimming ability of the fish at later stages, has not had similar attention. In this study, four populations of zebrafish, were reared in different water temperatures (22, 25, 28 and 31°C) and after being acclimatized in a common temperature (26.5°C) for over a month, they were subjected to swimming trials in order to evaluate the maximum relative critical velocity (RUcrit) in each case. Fish that were reared in 22°C showed statistically significant lower performance than the ones reared in 31°C (7.72±0.17 vs. 8.79±0.28, means ± S.E.). Possible explanations for the observed differentiation could be the effect of early life temperature on fish muscle ontogeny or on body shape.

Yi Huang (2014) Alterations of fish behavioral responses are sensitive indicators to identify accidental chemical pollution. In this research, a series of exposure tests were conducted to investigate behavioral changes of adult zebrafish (Danio rerio) exposed to deltamethrin (DM) in six concentrations of 0, 0.15, 1.5, 3.75, 7.5 and 15 μg/L. Swimming changes in zebrafish were detected at a concentration as low as 1% of the LC50-24h within five hours. Hyperactivity was the first response, followed by a second response of fish surfacing. The change patterns of swimming speed in zebrafish were similar in all exposure groups, but the degree increased with increasing concentrations. Swimming speed and depth were altered within the first two hours after exposure, which was regarded as the most vital phase for water quality monitoring. The duration of hyperactivity and the time of zebrafish surfacing were both logarithmically correlated with exposure concentrations, which was helpful to distinguish the level of pollution.

Rowena Spence (2007)The zebrafish *Danio rerio*, is an important model organism in developmental genetics, neurophysiology and biomedicine, but little is known about its natural ecology and behaviour. It is a small, shoaling cyprinid, native to the flood-plains of the Indian subcontinent, where it is found in shallow, slow-flowing waters. Zebrafish are group spawners and egg scatterers, although females are choosy with respect to sites for oviposition and males defend territories around such sites. Laboratory studies of zebrafish behaviour have encompassed shoaling, foraging, reproduction, sensory perception and learning. These studies are reviewed in relation to the suitability of the zebrafish as a model for studies on cognition and learning, development, behavioural and evolutionary ecology, and behavioural genetics.

3. Material And Methods

Test Species and Test Chemical

Zebrafish were obtained from the Institute of Hydrobiology and kept under a constant photoperiod of 10:14 (L:D) and fed three times per day. Dechlorinated water with a dissolved oxygen concentration of 6.8 ± 0.2 mg/L was used and the temperature was maintained at 28.5 ± 1 °C. Adult zebrafish of similar lengths (30 ± 2 mm), age (6–8 months) and body weight (300 ± 2 mg) were selected and acclimated for three weeks in a glass tank before experiments. Deltamethrin, ((S)-α-cyano-3-phenoxybenzyl (1R,3R)-3 -(2,2-dibromovinyl)-2,2-dimethylcyclopropan-1-carboxylate, CAS Registry No.: 52918-

63-5), was obtained from the Shanghai Pesticides Research Institute.

Behavioral Response Monitoring

Subsequently, six groups of fish (n = 5) was exposed to 0, 0.15, 1.5, 3.75, 7.5 and 15 µg/L of DM for behavior monitoring. Measurements were performed at the same period of the day, under similar illuminance level (200 lx) and without noise. Three male and two female adult zebrafish were selected in each experiment and then transferred into the rectangular test tank (400 × 75 × 300 mm). A real-time CCD camera (SDZ-371P; Samsung, Tianjin, China) was placed in the front of the test tank to record the swimming trajectories of the five zebrafish (Figure 1).

Temperature

Concerning the temperature range in which zebrafish live and reproduce in the environment, more than one suggestion exists. Froese and Pauly (2010) argue that zebrafish's natural habitat temperature ranges from 18°C to 24°C, whereas Engeszer et al. (2007) report that the observed temperature range for zebrafish in the wild lies between 24.6°C and 38.6°C. Spence et al. (2008) on the other hand, mention that temperature in D. rerio's habitats ranges from 6°C in the winter to 38°C in the summer. Our personal observations throughout many rearing trials showed that the rearing temperatures of 22°C and 31°C are the lower and upper limit values for successful rearing in the laboratory. At lower or higher – respectively – temperatures, the risk of unsuccessful hatching, mass larvae mortality and severe skeletal malformations throughout the populations, is extremely high. The purpose of this article is to study the effect of four different rearing temperatures (ranging from 22°C to 31°C) on the swimming performance of adult zebrafish.

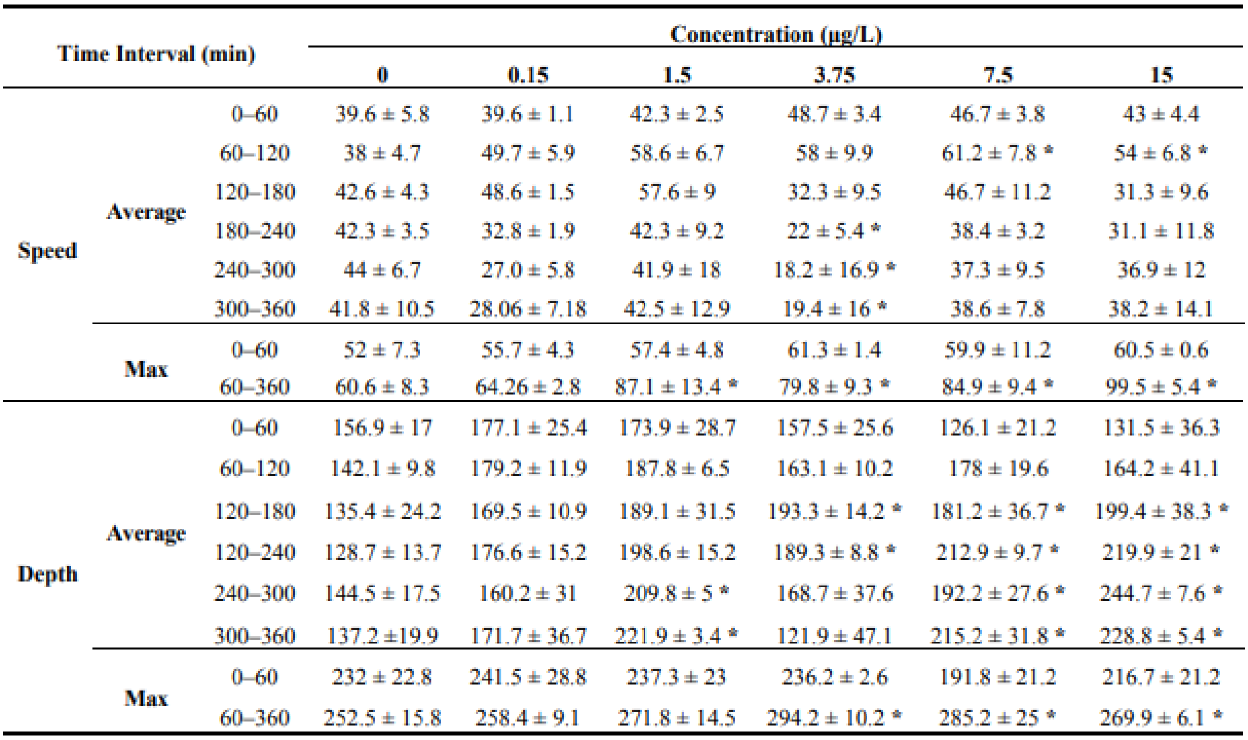
4. Data Analysis

We performed statistical analysis of fish responses under exposed and unexposed conditions at 1 min intervals for behavioral parameters. The values during each interval were compared to the values during the initial 60 min of the exposure test before exposure (unexposed conditions). Differences in endpoints were checked for assumptions of homogeneity of variance across treatments by Levene's test, analysed by one-way analysis of variance (ANOVA) and then tested by Dunett's test. When homogeneity was not observed, nonparametric statistical comparisons (Wilcoxon-test) were used to detect differences between unexposed and exposed conditions. Statistical tests were done using the SPSS 13.0 computer program (SPSS Inc. Chicago, IL, USA). Differences between means were considered significant when $p < 0.05$.

5. Result

Although variations existed between individuals, the data obtained from our behavioral monitoring system showed that fish behavior in the control group remained stable over the experimental period. The behavior did not vary significantly between the control group and unexposed condition in the toxic exposure tests (Table 2, $p > 0.05$). The speed of zebrafish varied mainly in a certain range of 25–60 mm/s (Figure 2).

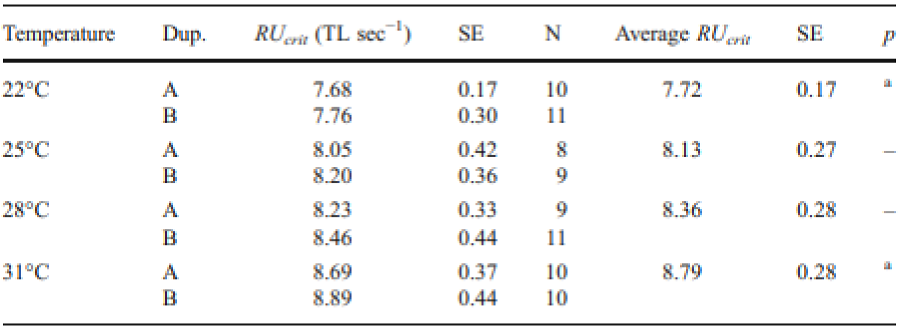
Table 1: statistical comparisons of fish responses under unexposed conditions and under exposure to DM



Time Interval (min)			**Concentration (µg/L)**					
			0	**0.15**	**1.5**	**3.75**	**7.5**	**15**
Speed	**Average**	0–60	39.6 ± 5.8	39.6 ± 1.1	42.3 ± 2.5	48.7 ± 3.4	46.7 ± 3.8	43 ± 4.4
		60–120	38 ± 4.7	49.7 ± 5.9	58.6 ± 6.7	58 ± 9.9	61.2 ± 7.8 *	54 ± 6.8 *
		120–180	42.6 ± 4.3	48.6 ± 1.5	57.6 ± 9	32.3 ± 9.5	46.7 ± 11.2	31.3 ± 9.6
		180–240	42.3 ± 3.5	32.8 ± 1.9	42.3 ± 9.2	22 ± 5.4 *	38.4 ± 3.2	31.1 ± 11.8
		240–300	44 ± 6.7	27.0 ± 5.8	41.9 ± 18	18.2 ± 16.9 *	37.3 ± 9.5	36.9 ± 12
		300–360	41.8 ± 10.5	28.06 ± 7.18	42.5 ± 12.9	19.4 ± 16 *	38.6 ± 7.8	38.2 ± 14.1
	Max	0–60	52 ± 7.3	55.7 ± 4.3	57.4 ± 4.8	61.3 ± 1.4	59.9 ± 11.2	60.5 ± 0.6
		60–360	60.6 ± 8.3	64.26 ± 2.8	87.1 ± 13.4 *	79.8 ± 9.3 *	84.9 ± 9.4 *	99.5 ± 5.4 *
Depth	**Average**	0–60	156.9 ± 17	177.1 ± 25.4	173.9 ± 28.7	157.5 ± 25.6	126.1 ± 21.2	131.5 ± 36.3
		60–120	142.1 ± 9.8	179.2 ± 11.9	187.8 ± 6.5	163.1 ± 10.2	178 ± 19.6	164.2 ± 41.1
		120–180	135.4 ± 24.2	169.5 ± 10.9	189.1 ± 31.5	193.3 ± 14.2 *	181.2 ± 36.7 *	199.4 ± 38.3 *
		120–240	128.7 ± 13.7	176.6 ± 15.2	198.6 ± 15.2	189.3 ± 8.8 *	212.9 ± 9.7 *	219.9 ± 21 *
		240–300	144.5 ± 17.5	160.2 ± 31	209.8 ± 5 *	168.7 ± 37.6	192.2 ± 27.6 *	244.7 ± 7.6 *
		300–360	137.2 ±19.9	171.7 ± 36.7	221.9 ± 3.4 *	121.9 ± 47.1	215.2 ± 31.8 *	228.8 ± 5.4 *
	Max	0–60	232 ± 22.8	241.5 ± 28.8	237.3 ± 23	236.2 ± 2.6	191.8 ± 21.2	216.7 ± 21.2
		60–360	252.5 ± 15.8	258.4 ± 9.1	271.8 ± 14.5	294.2 ± 10.2 *	285.2 ± 25 *	269.9 ± 6.1 *

The relative critical velocities measured for the individuals of each duplicate of the four temperature groups are presented in Table 1. Statistical analysis showed a significant influence of the factor, rearing temperature, on the swimming performance of the juveniles ($p < 0.05$, Mann-Whitney). Fish cultured in 25 and 28°C achieved intermediate critical swimming speeds although not significantly different from the other.

Table 2 RUcrit (SE, standard error) for each duplicate (A and B) and for each temperature group (22, 25, 28 and 31°C)



Temperature	Dup.	RU_{crit} (TL sec^{-1})	SE	N	Average RU_{crit}	SE	p
22°C	A	7.68	0.17	10	7.72	0.17	^{a}
	B	7.76	0.30	11			
25°C	A	8.05	0.42	8	8.13	0.27	–
	B	8.20	0.36	9			
28°C	A	8.23	0.33	9	8.36	0.28	–
	B	8.46	0.44	11			
31°C	A	8.69	0.37	10	8.79	0.28	^{a}
	B	8.89	0.44	10			

6. Discussion

Fish are known to react to their environment's fluctuations in all possible ways and it is an evolutionary imperative to try and adjust to these changes. Zebrafish, although considered to be a tropical fish, inhabits ecosystems with "monsoon climate", that present an extraordinary seasonal variation of temperatures that can range from as low as 6°C in winter to over 38°C in summer (Spence et al. 2008). With the phenomenon of global warming being more and more imminent, the effects that such a wide range of temperatures can have on many important processes and aspects of the fish life are certainly worth of extensive study. Developmental temperature plays an important role on the subsequent life of fish. On the present study the role of early rearing temperature on the swimming capacity of zebrafish juveniles was examined. The results showed that fish reared at 31°C have a higher swimming ability (8.79 TL sec−1) than fish reared at 22°C (7.72 TL sec−1). Fish raised at the two intermediate temperatures (25 and 28°C), although not statistically different from either one of the extreme temperatures, they seem to perform better than the fish of 22°C and worse than the fish of 31°C (8.13 and 8.36 TL sec−1 respectively).

7. Conclusion

In the present study, we tried to investigate whether there is a direct correlation between a single morphometric character and RUcrit. The fact that the examined morphometric characters do not significantly vary among the four temperature groups leads us to conclude that there is no such correlation between these size-related values and the observed differentiation of swimming performance. On the other hand, unpublished results of our team indicate that zebrafish grown in different temperatures tend to differ in both body shape and meristic character count, which is in agreement with the results of Georgakopoulou et al. (2007). It is logical to assume therefore that it is a combination of those characters (body shape in general) and it's variation among the different temperature regimes rather than the effect of a single one that eventually influences the swimming ability of a fish.

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