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Assessing Impacts of Rising pCO2 on Rainbow Trout (Oncorhynchus mykiss) Swimming Mechanics

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Abstract

Concern over the impacts of water acidification on fishes is growing due to their value commercially and ecologically. Although research has found that acidification negatively affects fish development, growth, and their ability to take up oxygen, little is known about how these effects translate to biomechanical changes in swimming behavior. We sought to determine whether freshwater acidification altered the performance and biomechanics of *Oncorhynchus mykiss* (rainbow trout) swimming using a circulating swim tunnel. Due to the COVID-19 pandemic, we were unable to complete our experiment, but we will provide our preliminary results, as well as a description of the experimental challenges we encountered and how we sought to overcome them.

Introduction

Due to growing atmospheric carbon dioxide levels, surface waters are becoming increasingly acidic. This poses a threat to the development of marine organisms, especially those that calcify, such as shellfish and corals. As the ocean acidifies, less carbonate ions are available in the water, leading to degradation of calcium carbonate structures made by these organisms (Doney et al. 2009). It was previously thought that fish would be robust to such changes in water chemistry due to their physiological capacity to handle changes in acid-base composition (Heuer & Grosell, 2014), but concern over the potentially negative impacts on fish is increasing due to their economic, cultural, and recreational value. Increases in pCO_2 cause hemoglobin to have a reduced affinity for oxygen in fish, known as the "Root effect" (Randall & Brauner, 1991), and researchers found that this translates to an overall decrease in oxygen uptake in fish (Basu 1959). Several studies have revealed that increasingly acidic water is leading to negative physiological changes in some fishes, including changes in olfactory response (Dixson et al., 2010; Munday et al., 2012; Leduc et al., 2013) reduced growth (Baumann et al., 2012; Ou et al., 2015), and increased anxiety (Ou et al., 2015), but little research has been done to understand the effects of increased carbon dioxide levels on swimming behavior of fish. Ye et al. (1991) found that acidic or alkaline conditions negatively impact the swimming speed of rainbow trout; still, no research has assessed the impacts that acidic water could have on the mechanics of fish movement. A more thorough analysis of swimming behavior could shed light on physiological changes caused by increased pCO_2, such as variation in muscle use, oxygen consumption, and stamina.

Changes in swimming behavior could alter predator-prey dynamics, food web relationships, and the and population of individual fish species in the Pacific Northwest region. Despite an increased understanding of ocean acidification, very little is known about the

significance of freshwater acidification, in large part due to the substantial variation in chemistry across freshwater systems throughout the world. Studies are beginning to find that various parts of the globe are experiencing freshwater acidification, including rivers in the Pacific Northwest (Leduc et al., 2013; Weiss et al., 2018), revealing a need for increased research in this area of ecosystem disturbance. The lack of understanding related to these environmental changes and their effects on behavior of fish provide an opportunity to forecast trends in ecophysiology, dispersion, and species interactions.

Our objective was to determine the effect of increased carbon dioxide levels on the swimming stamina and mechanics of *Oncorhynchus mykiss* (rainbow trout). We expected that the swimming speed and duration would decrease under exposure to elevated CO_2 levels, as well as cause reduced mechanical efficiency such as fewer fin beats/minute, greater tail amplitude, and an earlier gait transition. Using this research, we hoped to provide insight when making management and policy decisions for salmonids. During the first pilot study, I encountered various challenges in our equipment and experimental design. I made revisions to improve the study, but due to the COVID-19 crisis, I was unable to implement these revisions. Here, I will highlight these challenges, how I sought to overcome them, and how future researchers could successfully carry out this study.

Methods

Obtaining specimens

Juvenile rainbow trout were obtained from the Washington Department of Fish and Wildlife (WDFW) Arlington Commercial Hatchery. The fish were eggs in December, and they ranged in size from 53 to 82 mm (total length) at the time of testing. Fish were transported under constant temperature to Shannon Point Marine Center (SPMC) from the hatchery, where they were acclimated overnight. Fish were held in 114 L tanks in a cold room during the period of experiments. Individuals not yet tested were moved to a new 114 L tank placed within a larger tank of circulating seawater for a maximum of 9 days.

Swimming tests

Experiments were conducted over a two-week period. I intended to divide 75 individuals across three pCO_2 treatments: 400 ppm (ambient), 800 ppm (predicted for the end of this century), and 1200 ppm (worst-case scenario for next century), in accordance with other studies that have examined the effects of projected levels of atmospheric pCO_2 levels. We ran into several challenges that led us to halt the experiment before completing the 800-ppm treatment; thus, we do not have any data for that treatment.

Each individual was measured according to length (fork length and total length) and mass (Table S1). We used a 242 L PVC circulating swim tunnel to conduct our swimming trials. We measure flow velocity using a flow meter. For each swimming trial, flow velocity in the swim tunnel was increased from 0 cm/sec to 5 cm/sec so that the fish could orient to the direction of flow for five minutes. Some fish were oriented to flow at a velocity of 3 cm/sec due to issues with the flow meter, and we altered our statistical analysis accordingly. Following adjustment to

swim tunnel conditions, velocity was rapidly increased in a stepwise fashion at uniform increments of 5 cm/sec every five minutes until final swimming speed was reached (Figure 1). Exhaustion was determined as the time at which the fish was no longer able to swim against the current as indicated by its impingement against the downstream screen of the swimming chamber (based on Lee et al. 2003). The impinged fish was then removed and placed into a quiescent tank. Each individual was tested only once.

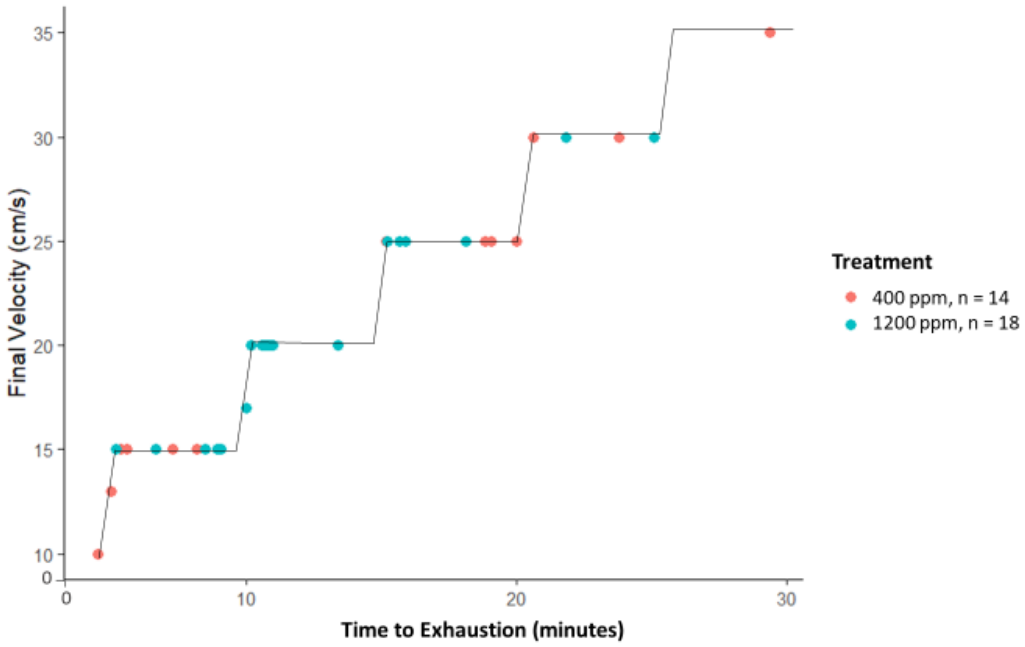

Figure 1. Stairstep showing velocity increments over time. Each point represents the point of exhaustion for one individual.

During swimming trials, we attempted to record high speed videos using a FastCam HiSpec Lite camera to analyze swimming kinematics. At each velocity increment, fish were allowed to adjust to the new flow velocity for 30 seconds. We then attempted to record video for 30 seconds at 150 frames/sec. We experienced challenges with this, which we will describe in later sections of this paper.

CO_2 Measurements

We ran a benchtop titration analysis to calculate alkalinity of the water in our swim tunnel and tanks to ensure that carbon dioxide levels were at the desired treatment level. We measured the initial pH of our 100-mL water samples. We added drops of 0.02 N H_2SO_4 (sulfuric acid) to the sample until a pH of 4.5 was reached. The volume of H_2SO_4 was recorded. We then calculated total alkalinity using the equation: Amount of acid used to reach pH 4.5 (ml) * Normality of acid (eq/L) * 50,000 (mg CaCO3/eq) / sample volume (ml). We then used a program provided by Dr. Brooke Love that uses pH and alkalinity to determine the pCO_2 in a sample.

Data Analysis

Because a couple of the individuals were given a different acclimation velocity than the other individuals, I chose to run statistical tests using change in velocity (cm/sec) rather than final velocity (cm/sec). Change in velocity was calculated as the final velocity subtracted by the initial velocity. I ran a Levene's test to determine whether the assumption of equal variance was met with our data. The result was narrowly non-significant ($F_{1,30}$=3.9, p=0.06), and transformations of the data did little to alter this. Given this result and my knowledge of the abnormalities in the data, I chose to run a non-parametric Kruskall-Wallis test. Because some individuals would not orient to flow and thus would not swim, I removed them from analysis. Therefore, 14 individuals are included in the 400-ppm treatment, and 18 individuals are included in the 1200-ppm treatment.

I intended to use Matlab software (Matlab, 2010) and code developed by Dr. Cassandra Donatelli at the University of Ottawa to analyze biomechanical factors, including tail beat amplitude (distance from midline), tail beats/minute, and time of gait transition.

Experimental Challenges

CO_2

We encountered two significant problems while using the CO_2 system in our experiment. First, our swim tunnel required a large volume of water, which resulted in the time to equilibration being much longer than expected. I was challenged to accurately measure the pCO_2 in our swim tunnel. The pH probe gave highly varied pH values in duplicate samples of the swim tunnel, which led to considerable variation in the calculated pCO_2. Therefore I could not be assured of whether the amount of pCO_2 in the tunnel had reached the desired level. Because of this, we halted our experiment to find a solution to the pH probe problem which prevented testing of fish at 800 ppm.

Fish orienting to flow

I intended to use impingement of fish on the back grate as a sign that the fish had reached exhaustion. This is common practice for most fish swimming studies. However, we experienced difficulty keeping fish off the back grate throughout the duration of each test, which prevented us from accurately determining when a fish truly reached exhaustion. Other studies used a glass rod to prod fish off the back grate, but we found that this strategy created more problems. First, fish seldom stayed off the back grate after being prodded, thus requiring us to disrupt the test multiple times. Secondly, it led to evasive behaviors and likely increased stress, which likely impacted the quality of my results.

Camera

The high-speed camera was new, and we were the first to use it. As such, we were not surprised to find that we had some unanswered questions about how to use it. First, we struggled to save the videos on any computer without the program crashing. We needed to film fish at a speed of at least 100 frames/second, but software appeared to not be able to handle such large file sizes on any computer we used. Secondly, the image tended to pixelate above 150

frames/sec, which is well below the capability of this camera This indicates that some settings need to be changed or that there could be something wrong with the camera.

Results and Discussion

Results of the Kruskall-Wallis test found no significant effect of CO_2 treatment on change in swimming speed by the point of exhaustion (chi-squared = 0.14555, df = 1, p-value = 0.7028, Figure 2). Due to the difficulties with our experiment and our limited sample size, it is impossible to say whether this result reflects the true behavioral and physiological effects of CO_2. Randall and Brauner (1991) did find that changes in swimming speed were noticed most at extreme pH levels, suggesting that changes in swimming ability might be more subtle than can be detected using a simple swimming experiment. Recent studies examining the effects of CO_2 on other groups of fishes found that skeleton mineralization and swimming mechanics under long-term exposure to high CO_2 can be impacted (Di Santo 2016, 2019). Because of the variability in measures of stamina even among similar-sized fishes within treatments future experiments might consider looking at more subtle measures such as changes in swimming mechanics in salmonid species.

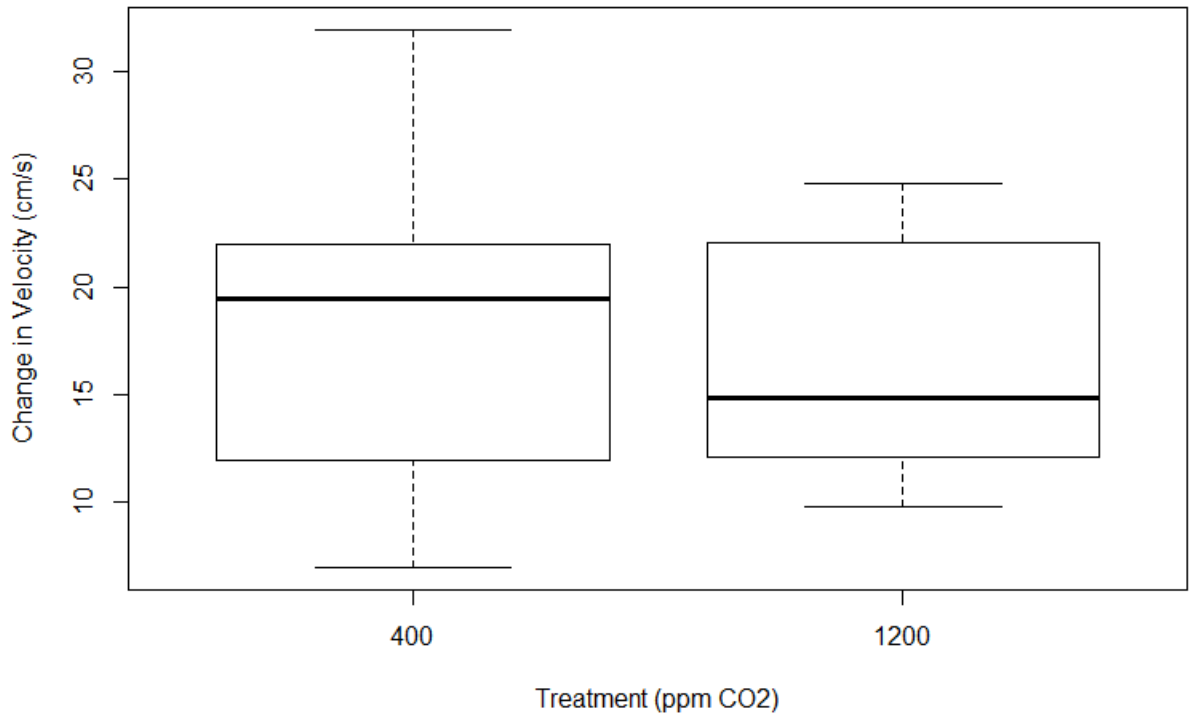

Figure 2. Boxplot showing change in velocity by point of exhaustion (cm/s) for each CO_2 treatment. N= 14 for the 400 ppm treatment. N= 18 ppm for the 1200 ppm treatment.

Revisions to experiment

In order to improve our use of the CO_2 system, we first planned to modify our methods by turning on the CO_2 system 4 to 5 days in advance of swimming trials. This would allow the tunnel more time to equilibrate to the desired pCO_2 level in time for the experiment. In order to measure pCO_2 more accurately, we chose to use the DIC LI-7000 CO_2/H_2O Analyzer Apollo/Scitech in further experiments. I was trained to use this machine by Dr. Brooke Love. Measurements of pH would still be involved in the calculation, but using this method would provide a more precise, accurate measurement of pCO_2. The next step we planned to take in order to use this method was to develop a freshwater standard, as the standards at SPMC are all designed to be used for saltwater samples.

We developed several possible solutions to help fish orient to flow. After discussing our methods with other scientists who have done similar studies, we intended to modify our methods by increasing acclimation time for each swimming test to 15 minutes. Typically, researchers will use this time to determine if an individual will orient to flow or not. If the fish will not swim against the current within those 15 minutes, the researchers do not use it in the experiment. We also began developing a new method to stimulate the fish to swim. We obtained a Harvard Apparatus Stimulator to electrify the back grate in order to provide a small electrical stimulus when the fish touched it. By the end of winter quarter, we were able to develop a prototype and planned to test the stimulator on fish in spring quarter before using it in the experiment.

Our solution to the camera problems was to develop a simpler test by using a GoPro camera instead of the FastCam camera. Because the other issues in our study required more attention, we chose to simplify our biomechanical analysis rather than attempt to make the FastCam camera suit our needs. Using a GoPro camera would still allow us to obtain simple biomechanical data, such as fin beats/minute. However, its fish-eye lens prevents us from looking at any variable that need to be to scale. To run this study in the future, we recommend devoting a substantial amount of time to solving issues related to the FastCam camera, while keeping the GoPro camera as a backup. The FastCam camera is a valuable piece of equipment that could provide useful data.

Acknowledgements

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Sources

Basu, S.P., 1959. Active Respiration of Fish in Relation to Ambient Concentrations of Oxygen and Carbon Dioxide. J. Fish. Res. Bd. Can. 16, 175–212. https://doi.org/10.1139/f59-016

Dixson, D.L., Munday, P.L. and Jones, G.P., 2010. Ocean acidification disrupts the innate ability of fish to detect predator olfactory cues. Ecology letters, 13:1, 68-75.

Di Santo, V., 2016. Intraspecific variation in physiological performance of a benthic elasmobranch challenged by ocean acidification and warming. J Exp Biol 219, 1725–1733. https://doi.org/10.1242/jeb.139204

Di Santo, V., 2019. Ocean acidification and warming affect skeletal mineralization in a marine fish. Proc. R. Soc. B 286, 20182187. https://doi.org/10.1098/rspb.2018.2187

Doney, S.C., Fabry, V.J., Feely, R.A., Kleypas, J.A., 2009. Ocean Acidification: The Other CO2 Problem. Annual Review of Marine Science 1, 169–192. https://doi.org/10.1146/annurev.marine.010908.163834

Heuer, R.M., Grosell, M., 2014. Physiological impacts of elevated carbon dioxide and ocean acidification on fish. American Journal of Physiology-Regulatory, Integrative and Comparative Physiology 307, R1061–R1084. https://doi.org/10.1152/ajpregu.00064.2014

George, H.C.P.H., Miles, G., Bemrose, J., White, A., Bond, M.N., Cameron, T.C., 2019. Intergenerational effects of CO2-induced stream acidification in the Trinidadian guppy (Poecilia reticulata). Ecology and Evolution 9, 12836–12845. https://doi.org/10.1002/ece3.5761

Leduc, A.O.H.C., Munday, P.L., Brown, G.E., Ferrari, M.C.O., 2013. Effects of acidification on olfactory-mediated behaviour in freshwater and marine ecosystems: a synthesis. Phil. Trans. R. Soc. B 368, 20120447. https://doi.org/10.1098/rstb.2012.0447

Lee, C.G., Farrell, A.P., Lotto, A., MacNutt, M.J., Hinch, S.G., Healey, M.C., 2003. The effect of temperature on swimming performance and oxygen consumption in adult sockeye (Oncorhynchus nerka) and coho (O. kisutch) salmon stocks. Journal of Experimental Biology 206, 3239–3251. https://doi.org/10.1242/jeb.00547

MATLAB, 2010. version 7.10.0 (R2010a), Natick, Massachusetts: The MathWorks Inc.

Ou, M., Hamilton, T.J., Eom, J., Lyall, E.M., Gallup, J., Jiang, A., Lee, J., Close, D.A., Yun, S.-S., Brauner, C.J., 2015. Responses of pink salmon to CO2-induced aquatic acidification. Nature Clim Change 5, 950–955. https://doi.org/10.1038/nclimate2694

Peake, S.J., Farrell, A.P., 2004. Locomotory behaviour and post-exercise physiology in relation to swimming speed, gait transition and metabolism in free-swimming smallmouth bass (Micropterus dolomieu). Journal of Experimental Biology 207, 1563–1575. https://doi.org/10.1242/jeb.00927

Randall, D. and Brauner, C., 1991. Effects of environmental factors on exercise in fish. Journal of Experimental Biology, 160(1), 113-126.

Weiss, L.C., Pötter, L., Steiger, A., Kruppert, S., Frost, U., Tollrian, R., 2018. Rising pCO2 in Freshwater Ecosystems Has the Potential to Negatively Affect Predator-Induced Defenses in Daphnia. Current Biology 28, 327-332.e3. https://doi.org/10.1016/j.cub.2017.12.022

Ye, Xuemin, Randall, D.J., 1991. The effect of water pH on swimming performance in rainbow trout (Salmo gairdneri, Richardson). Fish Physiology and Biochemistry 9, 15–21.

Supplemental Tables and Figures

Table S1. Raw data for each individual.

ID	Treatment	Mass (g)	Total Length (mm)	Fork Length (mm)	Initial Speed (cm/s)	Final Speed (cm/s)	Final Time (min)
1A	400	3.8	69.0	66	3	13	5.00
2A	400	4.3	74.0	71	3	35	29.37
3A	400	70.0	3.4	67	3	15	5.38
6A	400	64.0	2.4	60	3	25	18.85
7A	400	73.0	4.2	69	3	15	8.17
8A	400	80.0	4.8	76	3	15	5.58
11A	400	69.0	3.2	66	3	10	4.50
16A	400	78.0	4.6	75	3	25	19.07
17A	400	74.0	5.1	70	3	20	10.88
19A	400	4.6	66.0	63	3	15	7.30
20A	400	4.0	71.0	68	3	25	15.17
21A	400	3.0	63.0	60	3	25	20.00
22A	400	4.5	76.0	73	3	30	20.63
24A	400	4.1	69.0	65	3	30	23.80
1C	1200	2.0	58.0	56	3	20	10.87
3C	1200	3.5	73.0	70	3	20	10.98
5C	1200	5.2	81.0	78	3	25	15.67
6C	1200	2.0	61.0	59	3	15	8.50
10C	1200	2.8	64.0	60	3	25	18.12
11C	1200	5.1	79.0	75	3	25	15.25
12C	1200	1.5	53.0	51	3	17	10.00
13C	1200	6.6	82.0	77	5	15	8.93
14C	1200	2.4	63.0	61	5	15	6.67
15C	1200	4.3	74.0	71	5	25	15.90
16C	1200	5.1	88.0	84	5	20	10.70
17C	1200	4.5	76.0	72	5	30	21.83
18C	1200	2.6	62.0	59	5	30	25.08
19C	1200	4.3	76.0	72	5	15	9.08
21C	1200	5.1	77.0	74	5	20	10.58
22C	1200	4.9	74.0	71	5	15	5.20
23C	1200	4.2	74.0	72	5	20	10.20
24C	1200	3.9	76.0	73	5	20	13.38