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The effects of elevated carbon dioxide and temperature levels on tilapia (*Oreochromis mossambicus*): Respiratory enzymes, blood pH and hematological parameters

Hasan Kaya [a,*], Olcay Hisar [a], Sevdan Yılmaz [b], Mert Gürkan [c], Şükriye Aras Hisar [d]

[a] Department of Basic Sciences, Marine Sciences and Technology Faculty, Çanakkale Onsekiz Mart University, Çanakkale, Turkey
[b] Department of Aquaculture, Marine Sciences and Technology Faculty, Çanakkale Onsekiz Mart University, Çanakkale, Turkey
[c] Department of Biology, Faculty of Arts and Sciences, Çanakkale Onsekiz Mart University, Çanakkale, Turkey
[d] Department of Food Engineering, Engineering Faculty, Çanakkale Onsekiz Mart University, Çanakkale, Turkey

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ABSTRACT

Oreochromis mossambicus were exposed to two different temperature and carbon dioxide partial pressure levels for about two weeks, as the ambient (Control; 25 °C, 3.3 mg/L CO_2), high CO_2 (25 °C, 14 mg/L CO_2), high temperature (30 °C, 3 mg/L CO_2) and combined (30 °C, 14.1 mg/L CO_2) groups. No mortality was observed during the experiments. As a result of the study, elevated CO_2 concentrations cause negative effects on the hematological parameters. At the end of the study, while the blood Carbonic Anhydrase (CA) activity, in the high CO_2 group (25 °C, 14 mg/L CO_2), statistically increased at the 7th day compared to the control group, it decreased at the 14th day ($p < 0.05$). In addition, the blood CA activity, in the combined (30 °C, 14.1 mg/L CO_2) group, showed a decrease at the 14th day compared to the control group ($p < 0.05$). At the end of study, unlike the blood CA activity, gill, liver and kidney CA activity showed an increase in the tissues compared to the control groups ($p < 0.05$). Furthermore, the Na^+, K^+-ATPase activities were stimulated significantly in the gills in both high CO_2 and temperature groups at day 7, but it showed a significant amount of inhibition at the 14th day compared to the control groups. Overall, increasing carbon dioxide concentration in different temperatures has negative effects on the hematological parameters and respiratory enzyme of the tilapia fish. In addition, it is observed that the fish survive at negative conditions with adaptation mechanisms.

1. Introduction

Due to anthropogenic activities, the level of atmospheric CO_2 has raised by 280 mg/L from industrial revolution and today reached to 390 mg/L and it's estimated to reach 421–936 mg/L by the year 2100 (IPCC, 2013). Approximately 1/3 of the excess of CO_2 in the atmosphere will be absorbed by surface ocean waters, leading to an estimated drop in pH of 0.3–0.4 units globally by the end of 21st century (IPCC, 2013). Because of global warming, the rise in atmospheric temperatures will range between +1 °C (0.3 – 1.7 °C) and +3 °C (2.6 – 4.8 °C) (IPCC, 2013) over the next decades, affecting freshwater ecosystems too (Rosset and Oertli, 2011; Foucreau et al., 2014). This increase may induce thermal stress, which would mean alterations in metabolism of organisms especially fish (Pörtner, 2002).

Temperature changes have a strong impact not only on oxygen consumption of poikilothermic organisms and oxygen ligation of hemoglobin but also on the oxygen status of an environment. This essential environmental factor affects metabolism activities of all poikilothermic animals and, thus draws their life boundaries. Studies being conducted today report that global warming in waters has severe effects on the distribution and reproduction of fish species (Perry et al., 2005; Brander, 2007). The tolerance of fish to temperature changes may increase the need for oxygen or affect the oxygen supply capacity to tissues even in low levels apart from the high concentrations (Lannig et al., 2003).

The studies reveal that environmental stress sources affect organisms through operating effect mechanisms (synergistic and antagonistic etc.) (Schiedek et al., 2007; Gooding et al., 2009). Possible rises in the levels of temperature and CO_2, as a result of climate change in water, may lead to biochemical changes and, thus may bring irrevocable effects on aquatic organisms. In studies that researched the acidification resulted from CO_2 in waters, important calcifying organisms were mostly used. However, the

* Corresponding author.
E-mail address: hasankaya@comu.edu.tr (H. Kaya).

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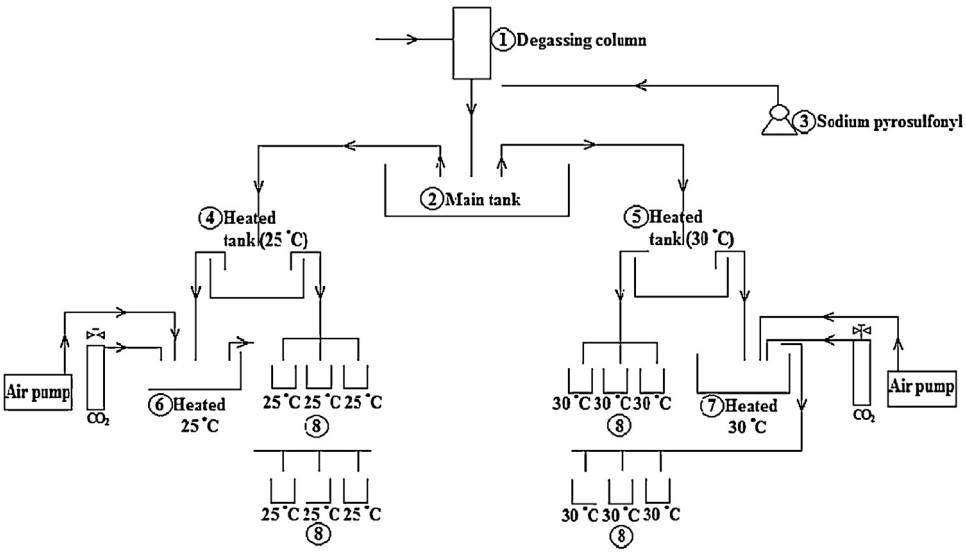

Fig. 1. Experimental system.

knowledge about this effect on fish physiology is limited. In most aquatic invertebrates, oxygen consumption falls despite increasing additional energy use due to elevated CO_2 levels. Fish, on the other hand, sustain their oxygen consumption on elevated CO_2 levels. Nevertheless, the effects on the reproduction, early development, growth, behaviors and physiologies of fish exposed to CO_2 for a long term are of importance and take place among the prioritized issues researched. This study aims to investigate the effects of possible elevations in temperature and CO_2 levels in waters, being associated with the scenarios on global warming, on respiration and blood physiologies using tilapia(*O. mossambicus).* In experiments conducted within this scope, changes in hematology, blood pH and respiration enzymes (Na^+, K^+-ATPase and Carbonic Anhydrase) of tilapia fish exposed to elevated CO_2 concentrations in two different temperature values were investigated.

2. Material and methods

2.1. Experimental setup

In this study, tilapia fish are preferred as test material since they easily adapt into environmental conditions, have more tolerance to changing temperature and oxygen conditions compared to other species. In the present study, a total of 144 tilapia fish (*O. mossambicus*) weighing at 13–15 g are used. Tilapia has been supplied from the Department of Aquaculture in the Faculty of Marine Sciences and Technology at Çanakkale Onsekiz Mart University. The fish were adapted into environment conditions for 30 days under 12:12 photoperiod regime at 26 °C within 12 adaptation tanks where the test was conducted. During the test, 12 fish were placed in each tank and the test was conducted for 3 replicates for each test group. Throughout the test lasted for 14 days, the fish were fed twice a day at the rate of 2% of each fish's body weight. A total of 4 different groups were made in order to detect the effect of carbon dioxide and temperature on fish in the test.

2.2. Experimental system

In this study, stabilized tap water which was injected with CO_2 under heating and pressure was used (Fig. 1). Initially, tap water has passed through the degassing column (1) and stored in the main tank (2) (Hisar et al., 2007). Afterwards, sodium pyrosulfuryl was added through a peristaltic pump (3) into the tank with the purpose of removing chlorine in water and total chlorine level was determined via color test (o-tolidine). The water was taken from the main tank into two different tanks (4–5) via pipes. The temperature was set to 25 °C in the first tank and to 30 °C in the second one through 40 kW heaters. CO_2 gas was injected to two different main tanks (6–7) (which were heated again with heaters and aerated with air pump) through ceramic diffuser in order to stabilize CO_2 concentration at 14 mg/L. The water taken from these tanks was transferred into 100-L aquaculture tanks (8) [3 tanks for each group; Control (25 °C, 3.3 mg/L CO_2), high CO_2 (25 °C, 14 mg/L CO_2), high temperature (30 °C, 3 mg/L CO_2) and combined (30 °C, 14.1 mg/L CO_2)]. During the tests, water quality in tanks (pH, water temperature, dissolved oxygen and carbon dioxide level) was measured three times a day. In addition, behaviors (time allocated for swimming, irregular movements and sudden bounces and so on) and external findings of the fish were observed prior to every feeding session.

2.3. Sampling

In the experiment, twelve fish on the first day (fish from the stock), six fish from each aquarium on the 7th and 14th day were used for blood pH and hematology analyses, and tissues were dissected for the determination of Na^+, K^+-ATPase and CA activities analysis.

2.4. Blood analysis

For blood sampling, ethyl 3-aminobenzoate methanesulfonate (MS-222, Sigma Aldrich Chemical Co., St. Louis, MO, USA) was used to anesthesia the fish (Kaya et al., 2015). They were wiped clean to avoid mucus being mixed into the blood, and then the blood was collected from the caudal vein using a 2.5 mL plastic syringe without harming the fish. An aliquot of blood (200 μL) was transferred to EDTA tubes (BD, Oxford, UK) for hematological analysis, and the remainder (300 μL) to plastic biochemistry tubes (Vacutest Kima s.r.l., Piove di Sacco, Italy). The tubes were centrifuged at 4000*g* for 10 min in a centrifuge for serum separation and stored at −80 °C. The serum was used for CA analysis.

2.5. Hematological parameters

RBCs (10^6 mm^3) were counted with a hemocytometer (Glaswarenfabrik Karl Hecht KG,Rh∈on, Germany) using Dacie's