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The Effects of Aqueous Nitrate on Freshwater Crayfish

By Erik Hartman

Introduction

Globalization and a rising need for greater production from the agricultural system have increased the use of fertilizer by farms across the entire country. With more fertilizer being used, more chemicals are entering aquatic environments as a result of runoff. In many of these fertilizers, a principle ingredient is potassium nitrate. In the United States, the concentration of aqueous nitrate in areas that do not use fertilizers has been measured at 0-4 mg/L, while it has been measured as high as 44 mg/L in areas that use fertilizers (World Health Organization, 2011). The increased concentration of nitrate in areas exposed to these fertilizers can have various effects on local wildlife, some of which can have severe impacts on fishing industries.

The primary action of nitrate on aquatic organisms is via a conformational change in the oxygen-carrying pigments in the circulatory fluid (whether it be blood or hemolymph), which decreases their affinity for binding oxygen (Camargo, Alonso, & Salamanca, 2005). By interfering with the organism’s ability to transport oxygen throughout its body, tissues will not be adequately perfused, and the activity of the organism will decrease. In addition to this, the resulting hypoxia in these tissues will cause cells to shift from oxidative phosphorylation to anaerobic respiration, which should cause an accumulation of lactate in the body (Lee et al., 2015).

The purpose of this experiment was to observe the effects of varying concentrations of aqueous nitrate over extended periods of time on the metabolic rate and hemolymph lactate concentration of freshwater crayfish (Procambarus clarkii),
which serve as a model organism for many different types of aquatic environments.

While there has been research on the effect of nitrate on the metabolic rate of freshwater crayfish over short periods of time (Meade and Watts, 1995), the effect of nitrate on hemocyanin (Camargo, Alonso, & Salamanca, 2005), and the effect of poor oxygen conditions on lactate accumulation (Lee et al., 2015), there has not been any research on the effect of nitrate on metabolic rate over extended periods of time or the direct effect of increasing nitrate concentrations on hemolymph lactate concentration. In the previous research on metabolic rate, *Cherax quadricarinatus* (Australian freshwater crayfish) were exposed to various nitrate concentrations over a period of 48 hours, after which their metabolic rate via oxygen consumption was recorded (Meade and Watts, 1995). From the data collected, there was no significant difference among any of the groups. In addition to this, another experiment focused on the rate of uptake of nitrate ions in another species of freshwater crayfish (*Astacus astacus*) (Jensen, 1996). In this research, it was found that crayfish have a low branchial permeability to nitrate ions. Based on this, crayfish would have to be exposed to nitrate for extended periods of time in order for any significant effects to be seen.

Regarding the effects of nitrate on respiratory pigments, nitrate-induced oxidation of hemocyanin (the major respiratory pigment in freshwater crayfish) results in a conformational change to a form that cannot bind oxygen, impeding oxygen transport (Walsh and Wright, 1995). In addition to this, there has been research on the effects of nitrate on lactate concentration in rainbow trout, which also serve as a model organism for aquatic environments (Stormer, Jensen, & Rankin, 1996). In this experiment, there was no significance found among the groups exposed to nitrate and the control.
Based on past research and the proposed mechanism of nitrate on hemocyanin, it is hypothesized that as the concentration of aqueous nitrate increases, the metabolic rate will decrease, and the concentration of hemolymph lactate will increase.

**Materials and Methods**

In this experiment, three groups of crayfish were tested: a control group that was not exposed to HNO$_3$, a group that was exposed to 15 mg/L HNO$_3$ (simulating an environment with moderate fertilizer usage), and a group that was exposed to 30 mg/L HNO$_3$ (simulating an environment with heavy fertilizer usage) (N = 14 for each group). After one week of exposure to these conditions, the metabolic rate of each crayfish was calculated from the amount of oxygen consumed over a two hour period (measured by a dissolved oxygen probe), and the hemolymph lactate concentration was measured for half of the crayfish via a Lactate Pro meter. After an additional week of exposure, the hemolymph lactate concentration of the remaining crayfish was measured.

Once all of the measurements had been taken and normalized by dividing by the weight of the crayfish, a one-way analysis of variance (ANOVA) test along with a Tukey post-hoc test was used to determine if there was any difference among the three groups.

**Results**
**Figure 1:** Mean normalized oxygen consumption per minute after one week of exposure (error bars represent standard error).

Based on **Figure 1**, there is no statistical difference between the three groups in terms of metabolic rate ($p > 0.05$).

**Figure 2:** Mean normalized hemolymph lactate concentrations after each week (error bars represent standard error).
Figure 3: Mean normalized hemolymph lactate concentrations (error bars represent standard error).

There was no statistical difference between the measurements of week 1 and week 2 for any of the three groups (Figure 2, p > 0.05 for each). Because of this, the measurements between week 1 and week 2 were pooled for each group when determining significance among the three groups. As can be seen in Figure 3, both the 15 mg/L and 30 mg/L groups had a significantly lower hemolymph lactate concentration than the control group (p = 0.002, 0.001). Compared to each other, however, there was no difference between the two exposure groups (p > 0.05).

**Discussion**

It was hypothesized that as the ambient levels of nitrate increased, the metabolic rate of the crayfish would decrease as a result of a decreased level of oxidative phosphorylation, causing a reduction in the amount of ATP available for the organism to use. A reduction in ATP would have caused a decrease in metabolic rate in order to
balance ATP use with synthesis (Wheaton & Chandel, 2011). However, based on the data (Figure 1), there was no change in metabolic rate among the three groups. It is possible that exposure to nitrates had a positive effect on the catabolism of stored biomolecules in the experimental groups, which would have generated ATP for the organism to use (Voet and Pratt, 2008). This addition of ATP would have countered the loss of ATP resulting from the exposure, leading to no change in metabolic rate.

In addition to an increase in metabolic rate, it was hypothesized that there would be an increase in the concentration of hemolymph lactate. With pigments having a decreased affinity for binding oxygen, cells would enter hypoxic conditions, shifting from oxidative phosphorylation to anaerobic respiration (Lee et al., 2015). This would then cause an upregulation of lactate dehydrogenase synthesis, resulting in more pyruvic acid being converted into lactate, resulting in the hypothesized increase in hemolymph lactate concentration in the groups exposed to nitrate (Firth, Ebert, & Ratcliffe, 1995). Despite this, the data presented in Figure 2 and Figure 3 support a decrease rather than an increase in hemolymph lactate levels as nitrate levels increase, going against the proposed physiological mechanism. It is possible that the increased nitrate levels had an inhibitory effect on lactate dehydrogenase synthesis, or that exposure to nitrate caused them to become ill and not produce as much lactate as when they are healthy; however, further testing would be required to determine the actual cause of this decrease.

While further experimentation could identify the mechanism at work behind the lactate results, it is possible that a longer exposure period and groups exposed to higher concentrations would generate better results for both the lactate measurements and the
metabolic rate measurements, which may then support the proposed hypotheses. It would also be beneficial to test other chemicals used in common fertilizers to see if they have similar effects on the physiology of aquatic organisms such as crayfish. The results of further testing could show specifically how increased fertilizer use could affect neighboring aquatic ecosystems. Once analyzed, these results could lead to measures that will lessen contamination of aquatic ecosystems, allowing them to thrive and grow to what they were before the use of fertilizers containing harsh chemicals.
References


