INDIANA-ILLINOIS SEA GRANT FINAL REPORT

Title: The effect of rain induced driven dissolved oxygen reductions on fish in an urban system.

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Introduction

Oxygen-depleting substances from point and non-point sources are one of the most prominent sources of pollution currently found in aquatic urban environments. During large rainfall events, waters that receive storm-water runoff accumulate oxygen-depleting substances from combined sewer overflow (CSO) discharges, as well as re-suspension of existing substances in sediments. These substances can cause conditions of hypoxia (or even anoxia) that can persist for several days. While the effect of these wet weather events on dissolved oxygen (DO) in urban environments is difficult to assess (complex nature of the system and associated interactions), it is assumed they negatively affect biota in the receiving waters. Federal and state regulations in the form of minimum concentrations and/or daily limits exist, and these regulations are intended to protect aquatic biota from these low oxygen conditions. To date, studies that quantify the impacts of storm-driven low oxygen events on aquatic ecosystems, and fishes in particular, are lacking, and regulations for oxygen limits have been based largely on laboratory-based studies. As human populations, and the size of urban environments both continue to grow, the impact of urban areas on aquatic ecosystems will also become more pronounced in the future. There is therefore a *critical need* to examine the impact of wet weather events on fishes in urban settings. Without data generated by such studies, regulations currently in place to protect fishes from hypoxia may conceivably be overly conservative (or overly liberal), resulting in either minimal protection for aquatic organisms, or else overly restrictive regulations.

The Chicago Area Waterway System (CAWS) is an urban environment that has a well-documented history of storm-driven hypoxia events, as well as a sizeable fish community, thereby presenting a unique environment to quantify the impacts of storm-driven hypoxia on urban environments. An improved understanding how fishes are able to survive in the CAWS despite regular occurrences of hypoxia will provide a basis for proper management applications to water systems in other highly urbanized areas. The implications of this study may help design new and innovative regulations to ensure optimal oxygen concentration guidelines, potentially enhancing ecological services and biodiversity.

Objectives

The *objective* of this study is to quantify the impacts of acute hypoxia events on fishes, using the Chicago Area Waterway System as a test environment. More specifically, the study will use (a) a *laboratory setting* to quantify the physiological responses of CAWS fish to acute hypoxic conditions, and (b) *automated acoustic telemetry* to quantify the movement and activity rates of free-swimming fishes in the CAWS following storm-driven hypoxia events. The behavior and physiology of fish residing in areas of the CAWS frequently exposed to storm-driven hypoxia will be compared to fish from other portions of the CAWS upstream of point-source sewage overflows that do not have a history of repeated hypoxic events. This combination of field- and laboratory-based studies will provide a robust assessment of how storm-driven hypoxia impacts fish, at scales ranging from molecular to whole-animal, and may identify 'thresholds' of dissolved oxygen concentrations that are challenging for aquatic organisms that can be used to guide policy formation to protect urban ecosystems.

The model organism for the proposed study is the largemouth bass, *Micropterus salmoides*. This species was chosen for its well-documented abundance throughout the CAWS, having well defined home ranges (Lewis and Flickinger 1967; Gerking 1953; Winter 1977), and do not exhibit large migratory patterns over great distances (Winter 1977; Fish and Savitz 1983; Warden and Lorio 1975). Largemouth bass are also sufficiently large to allow for the successful implantation of telemetry tags, permitting the coupling of field and laboratory analyses that would not be possible with smaller fish species.

The *null hypothesis* for this study is that there is no difference in either the physiological or behavioral response to hypoxia between fish regularly exposed to hypoxia compared to fish that rarely experience hypoxia. There are two alternate hypotheses. The *first alternative hypothesis* states that bass regularly exposed to hypoxia are able to survive in such environments through improved physiological conditions (beneficial acclimation) relative to fish that do not regularly experience hypoxia. The *second alternative hypothesis* is that bass regularly exposed to hypoxia are able to survive in such environments through behavioral adjustments and movements that causes them to seek out conditions of improved dissolved oxygen. Together, addressing these hypotheses will provide insight as to how fish communities can survive in conditions of low oxygen, and may define thresholds of oxygen concentration that can be used to guide policy on dissolved oxygen.

Methodology

Fourteen autonomous acoustic receivers (VR2W, Vemco, Inc.) will be strategically placed in the Sanitary and Shipping Canal section of the CAWS. The array will cover areas upstream and downstream of Bubbly, *the area of concern for fish movement in relation to low dissolved oxygen levels*. There is a large sewage outflow pumping station at the top of Bubbly Creek (figure 1). This pumping station engages regularly during large rain events and is responsible for significant declines in dissolved oxygen in our study area. Receiver density will be greater near Bubbly Creek to ensure sufficient coverage and optimize detections. Based on preliminary range testing, receivers will be placed approximately 600m apart.

Autonomous dissolved oxygen sondes will be deployed at 4 locations *within the study site* to monitor DO levels throughout the duration of the study. Dissolved oxygen data will continuously be sampled 24 times per day; previous studies have shown that this density of sondes and rate of data collection will provide sufficient DO data to assess the oxygen concentration in the water before, during, and after large rain events. These data will be used in conjunction with telemetry data with the purpose of relating movement to hypoxia events. Sondes will be deployed and managed by the Metropolitan Water Reclamation District of Greater Chicago (MWRDGC), and data will be shared with researchers at the University of Illinois for analyses. Quality assurance of dissolved oxygen data will be compared with Winkler-Titration for DO to ensure sondes are recording accurate data.

Following the deployment of the receivers and oxygen sondes, 20 largemouth bass will be collected for transmitter implantation using DC electrofishing efforts. Upon collection fish will be placed in an aerated holding tank. Fish will then be transferred to an anesthetic bath for induction before surgery. After reaching stage 4 anesthesia (total loss of muscle tone and equilibrium; slow but regular opercular rate; loss of spinal reflexes, (Summerfelt and Smith 1990)) fish will be implanted with an acoustic transmitter through surgical procedures. These transmitters will last for two years. Telemetry data will consist of presence/absence of individual tags any given receiver detects. Movement data collected throughout the tag life will be sufficient to assess the location, movement, and activity patterns of the largemouth bass relative to dissolved oxygen concentration within the study site. These detections across receivers will be used to track movement of the bass and determine residency, habitat occupancy and rate of movement/activity. Dissolved oxygen data can then be overlaid to quantify movement in relation to DO levels.

Phase 2: Laboratory trials
To determine the physiological effects of repeated acute hypoxia conditions on fishes in the CAWS, we will collect largemouth bass by boat electrofishing both from within the CAWS (test site) and 3 sites outside the influence of acute hypoxia events in the CAWS (control sites). Initially, 8 largemouth bass from each site will be immediately sampled and blood and tissues will be collected to quantify baseline physiological and nutritional parameters of these fish (hereafter referred to as field sampled fish). After collection, largemouth bass from both sites will be taken to the aquatic research facility at the University of Illinois and held in aerated tanks to recover from hauling stressors and acclimate to laboratory conditions. Fish from both sites will then be used in *two experiments*.

For the *first experiment*, bass from both sites will be acutely exposed to low DO concentrations that match DO levels observed in the study site for 6 hours. At the conclusion of this exposure period, fish will be euthanized and blood and tissue samples will be collected. These samples will be evaluated for stress indicators (e.g. cortisol, plasma lactate, glucose), ion concentrations (Na^+, K^+, Cl^-), and hematocrit and hemoglobin concentrations. Metabolic rate (oxygen consumption) will also be quantified following DO challenges.

In the *second experiment*, bass from both sites will first be held for 2 weeks at constant low DO levels (actual concentrations used to mimic conditions in the CAWS). After this prolonged holding at low DO levels, fish from both sites will be subjected to an acute low DO treatment identical to conditions in experiment 1. Bass would again be sampled for blood and tissue in a manner identical to *experiment 1*. Thus, the second experiment will quantify the potential for largemouth bass to exhibit physiological acclimation to low oxygen conditions, which may explain how populations of bass can thrive in the CAWS despite repeated exposure to low oxygen.
Comparisons between the physiological responses of bass from both sites across *both experiments* will (a) quantify the physiological differences in the response to hypoxia from both sites, (b) help identify thresholds of oxygen concentration that induce significant physiological disturbances for fish, and (c) allow us to determine the potential for fish to acclimate to low oxygen through regular exposure, likely through phenotypic plasticity. These results can help develop resource management strategies at an appropriate scale for hypoxia levels as well as

enhancing and protecting the ecosystem services and biodiversity of the CAWS and similarly impacted urban systems.

Key Findings

The results of the field and lab based experiments are summarized in this section. Tables and figures have been excluded to protect the status and originality of data for future submission to scientific journals.

(1) During the 2010 and 2011 study period, largemouth bass movement was observed and quantified in relation to periods of low DO. After identifying nine periods of low DO throughout the system, movement of these fish was assessed before the event and during the event. During those events, largemouth bass did not show a clear pattern of avoiding areas of low oxygen. While there were a couple instances of fish moving away from the area, largemouth bass typically remained, and paradoxically, there were instances where presence increased during the low DO event.

(2) The results of the field sampled fish indicate the baseline stress parameters (e.g. plasma cortisol, glucose, lactate, etc.) of the study site fish were not significantly different than fish from the three control sites. Additionally, the nutritional parameters of the study site fish (e.g. triglycerides, total plasma proteins, relative weight, etc.) were also not significantly different that control site fish. Together, these results indicate the fish from the study area are not chronically stressed or nutritionally deprived.

(3) Results from the oxygen shock trial indicated the magnitude of the stress response in the study site fish was similar to the control site fish. The oxygen trial was sufficient to elicit a stress response in the fish, as indicated by the elevated plasma glucose and lactate levels, however, no site specific differences were observed for any parameter measured. Analysis of metabolic rates, specifically P_{crit} values (definition: the oxygen tension at which an animal ceases to respire aerobically and begins anaerobic respiration. An animal with a lower P_{crit} value is said to be more hypoxia tolerant than an animal with a higher P_{crit} value), showed non-significant differences between metabolic rates and P_{crit} values of the study site fish and the control sites. Together, these results indicate the largemouth bass from the study site do not have an increased tolerance to hypoxia compared to control site populations and the magnitude of their stress response to hypoxia is similar to control site populations.

Conclusions and importance of the study

Dissolved oxygen is a resource that is essential for aquatic life. Exposures to low levels of DO can induce physiological changes to fish, while chronic, prolonged exposure to hypoxia can result in changes to community structure, alterations to movement patterns, or even death. Acute and chronic water quality criteria are established for waterways to prevent these adverse effects. Results from the current study did not demonstrate a clear threshold at which point largemouth bass regularly altered behavioral movement patterns, activity rates or residency within the study area. Furthermore, largemouth bass within the study area did not appear to experience negative consequences, based on the parameters measured. Current DO conditions do not appear to be having any substantial or chronic negative impacts on resident largemouth bass, despite regular and prolonged periods of hypoxia. In Bubbly Creek, largemouth bass appear to largely reside in habitats that are peripheral to the main channel that are somewhat sheltered from hypoxic episodes induced by rain events. Based on these results, it is difficult, if not impossible, to identify a 'threshold' DO concentration that should be avoided to benefit largemouth bass.

Results from this study have important, unique and meaningful implications for understanding the impact of DO concentrations on fish, for a number of reasons. From an 'applied science' perspective, results from this study are important for understanding the behavior of DO in aquatic systems. Regulatory authorities need to be aware of the variability in DO concentration within aquatic systems, as well as the ability of fish to actively select areas of differing DO concentration when assessing the impacts of low oxygen on biota. It is currently believed that this spatial heterogeneity is underappreciated and largely unexplored. Future studies can build upon this work for additional species in additional locations to both corroborate the results from the current study in other urban systems and document how fluctuations in oxygen concentration can impact fish in disparate systems. Similarly, many DO standards have been set to ensure maintenance of oxygen levels of approximately 2-4 mg/L. While this level is undoubtedly important for many fish species and also for many different life stages, the current study highlights the importance of species-specific quantification of oxygen preference as largemouth bass regularly inhabited DO concentrations below 2 mg/L for extended periods without apparent negative consequences. As climate warms, and as urban areas continue to expand, the impact on oxygen resources will likely grow, necessitating an improved understanding of both oxygen dynamics, and fish responses to this variable.

It is important to note that while the results from the current study do not show largemouth bass from the study area to respond differently physiologically compared to control populations, this does not indicate that CSOs are not a concern for this system. Indeed, the results show that fish avoid areas where CSO events occur, during and after those events. While absolute fish diversity in the study area is high (over 30 species have been recorded by MWRD sampling crews from 2001-2005 [data available at mwrd.org]), species compositions comprise generalists and/or hypoxia-tolerant species (e.g., sunfish, common carp, catfish) rather than habitat specialists or hypoxia-intolerant species that may exist elsewhere where oxygen levels are higher (e.g. salmon, walleye). Performance of largemouth bass in this system may not be representative of these other fishes, because of their physiological tolerance to hypoxia (Furimsky et al., 2003) and observed DO inhabitance (Hasler et al., 2009).

Impact of SEA-GRANT funding

The original proposal for this study only included sampling largemouth bass from a single control site. However, after being awarded IISG funding, we were able to include 2 additional control sites to the study. This was a *crucial* addition towards the success of this study as it allowed us to utilize replicate control sites. This provided us more confidence in the physiology results of comparing the study site fish to control populations. The IISG funding allowed us the additional travel, lodging, and equipment to collect the fish from the additional sites. Furthermore, the IISG funds also allowed us to purchase the additional assays, reagents, and other lab equipment required to quantify the physiological parameters we measured from the fish in the two additional control sites. Without the aid of ISSG funding, this study would not have such strong data to support our inferences and conclusions. Because of the added strength to the data, the funding allowed the principal investigator to successfully complete a significant portion of his thesis work to fulfill the requirements of his MSc. program.

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