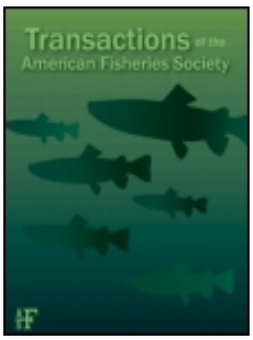

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ARTICLE

Behavioral and Physiological Responses of Largemouth Bass to Rain-Induced Reductions in Dissolved Oxygen in an Urban System

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Abstract

Waters in urban areas often experience hypoxic events due to combined sewer overflows, which have the potential to negatively affect aquatic biota. Despite these hypoxic events, many urban areas have diverse fish assemblages, suggesting hypoxia has a minimal impact. Data to quantify the impacts of aquatic hypoxia in urban systems are currently lacking. The current study sought to define how rain-induced hypoxia affected the movement, distribution, and physiology of individual Largemouth Bass *Micropterus salmoides* residing in the Chicago Area Waterway System (CAWS), an urban area prone to episodes of hypoxia. Following the onset of hypoxic events, the likelihood of Largemouth Bass remaining in hypoxic water was reduced, but fish did not completely avoid hypoxic areas. This suggests that hypoxia exerts only a moderate influence on the movement of Largemouth Bass. Field sampling showed that Largemouth Bass from the site prone to hypoxia were not in poor nutritional condition and were not suffering from chronic stress, relative to compared with those from reference sites. Field sampling also showed that fish from the CAWS displayed an improved capability to transport oxygen in the blood compared with individuals from control sites. Following a low-oxygen challenge in the laboratory, fish from the CAWS also displayed elevated levels of oxygen transport capabilities compared with fish from some control sites. Together, results suggest that hypoxic events have limited behavioral consequences for Largemouth Bass, and in fact, Largemouth Bass in our study may have developed an improved ability to tolerate hypoxia, which would allow them to persist in hypoxia-prone areas.

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Point and nonpoint discharges in urban areas from rain events and combined sewer overflow (CSO) discharges have long been identified as a significant cause of pollution inputs into watercourses resulting in poor water quality. In particular, wet weather events that result in CSO discharges can result in increased levels of sedimentation, high biological oxygen demand, increased prevalence of bacteria, and elevated concentration of heavy metals (Brombach et al. 2005; Casadio et al. 2010). More importantly, oxygen-depleting substances discharged into, or resuspended within, receiving waters during rain events are considered one of the leading pollutants affecting urban waters (Burton and Pitt 2001). Such oxygen depletion events may be associated with a single rain event, but reduced oxygen conditions can persist for days, or even weeks, following such events (Burton and Pitt 2001; Alp et al. 2007).

The adverse effects of hypoxia (dissolved oxygen [DO] concentrations < 2 mg/L: Diaz 2001) on aquatic life, such as fish, have been well studied in controlled laboratory settings (Kramer 1987; Pollock et al. 2007). When aquatic organisms experience severe reductions in DO, they may react by either working to extract additional oxygen from their environment (Timmerman and Chapman 2004), moving to more oxygen-rich habitats (Bogert 1949; Bell 2002; Craig and Crowder 2005), or otherwise mitigating the potential negative consequences of hypoxia for energy generation and homeostasis (Pollock et al. 2007; Mandic et al. 2009). Exposure of fish to repeated and/or prolonged periods of hypoxia can result in a suite of sublethal consequences that include chronic stress, reduced swimming speed, shifts in habitat use, altered availability of prey resources, and, potentially, reduced feeding opportunities (Kramer 1987; Pollock et al. 2007). Exposure to low-oxygen environments can also cause elevated bacterial and/or fungal infections, increased parasite loads, elevated levels of oxidative stress, and possibly endocrine disruption (Pickering and Pottinger 1989; Lushchak and Bagnyukova 2006). It is presently believed that these hypoxic events adversely affect the biota within receiving waters (Pollock et al. 2007). Examinations of the response of aquatic organisms to hypoxia and CSOs in field situations are lacking because of the complex physical and chemical interactions between discharge events, receiving waters and site-specific conditions, making it difficult to confidently define the sensitivity of urban organisms to rain-induced hypoxia in the wild. Thus, there exists a critical need to quantify the impact of rain events on aquatic organisms in the field and integrate this with laboratory-derived data.

Based on this background, the first goal of the current study was to define the movement patterns of Largemouth Bass *Micropterus salmoides* residing in an urban setting following rain-induced periods of hypoxia. To accomplish this goal, a stationary, autonomous telemetry array was deployed downstream of a major sewer discharge within the Chicago Area Waterway System (CAWS), and resident Largemouth Bass were outfitted with acoustic telemetry transmitters. Historically, the CAWS has not only experienced episodic periods of hypoxia and anoxia (DO = 0 mg/L) but has also maintained stable resident fish populations (Dennison et al. 1998). The second goal was to quantify the physiological response of Largemouth Bass from the CAWS that frequently experience prolonged rain-induced hypoxia to an acute hypoxia stressor and compare this response with those in Largemouth Bass from reference sites that do not experience episodic bouts of hypoxia. Our intent was to use the combined results to elucidate how fish inhabiting an urban environment respond, both behaviorally and physiologically, when exposed to frequent and extended periods of low oxygen.

METHODS

Study Site

The study site was located southwest of downtown Chicago, Illinois, within a formerly natural segment of the CAWS (41°50′N, 87°39′W), which has been highly modified, including dredging and hardening of banks (Figure 1). The study site spanned approximately 7 km of the South Branch Chicago River (Main Channel) at the confluence of the South Fork of the South Branch Chicago River (Bubbly Creek). Nearly 40 CSOs are located within the study area that may discharge effluent during rain events and contribute to reductions in DO following these events (Kollias 2008). Bubbly Creek itself historically experiences frequent periods of low DO (https://www.mwrd.org/irj/portal/anonymous/WQM; Waterman et al. 2011). From 2000 to 2009, CSOs within the study area discharged sewage an average of 15 times per year, with a minimum of five events and a maximum of 23 during a single year (Waterman et al. 2011). Dissolved oxygen was monitored within the study site through the use of three dissolved oxygen sondes (YSI 6600 and YSI 6920; YSI Instruments, Yellow Springs, Ohio) programmed to log DO data every hour. Sondes were located ~1 km downstream from Bubbly Creek, ~0.4 km upstream from Bubbly Creek, and within Bubbly Creek (Figure 1B). All three sondes were located approximately 1 m below the water surface. Start and stop dates and times of CSO discharges were recorded by the Metropolitan Water District of Greater Chicago to quantify discharges in relation to DO fluctuations.

Fish Movement

Fish collection and telemetry receiver deployment.—The fish species used to relate movement to DO concentrations was the Largemouth Bass. Largemouth Bass were chosen because Wasik et al. (2008) previously indicated they were abundant throughout the CAWS. Largemouth Bass also have well-defined home ranges (Gerking 1953; Lewis and Flickinger 1967; Winter 1977), do not exhibit large migratory

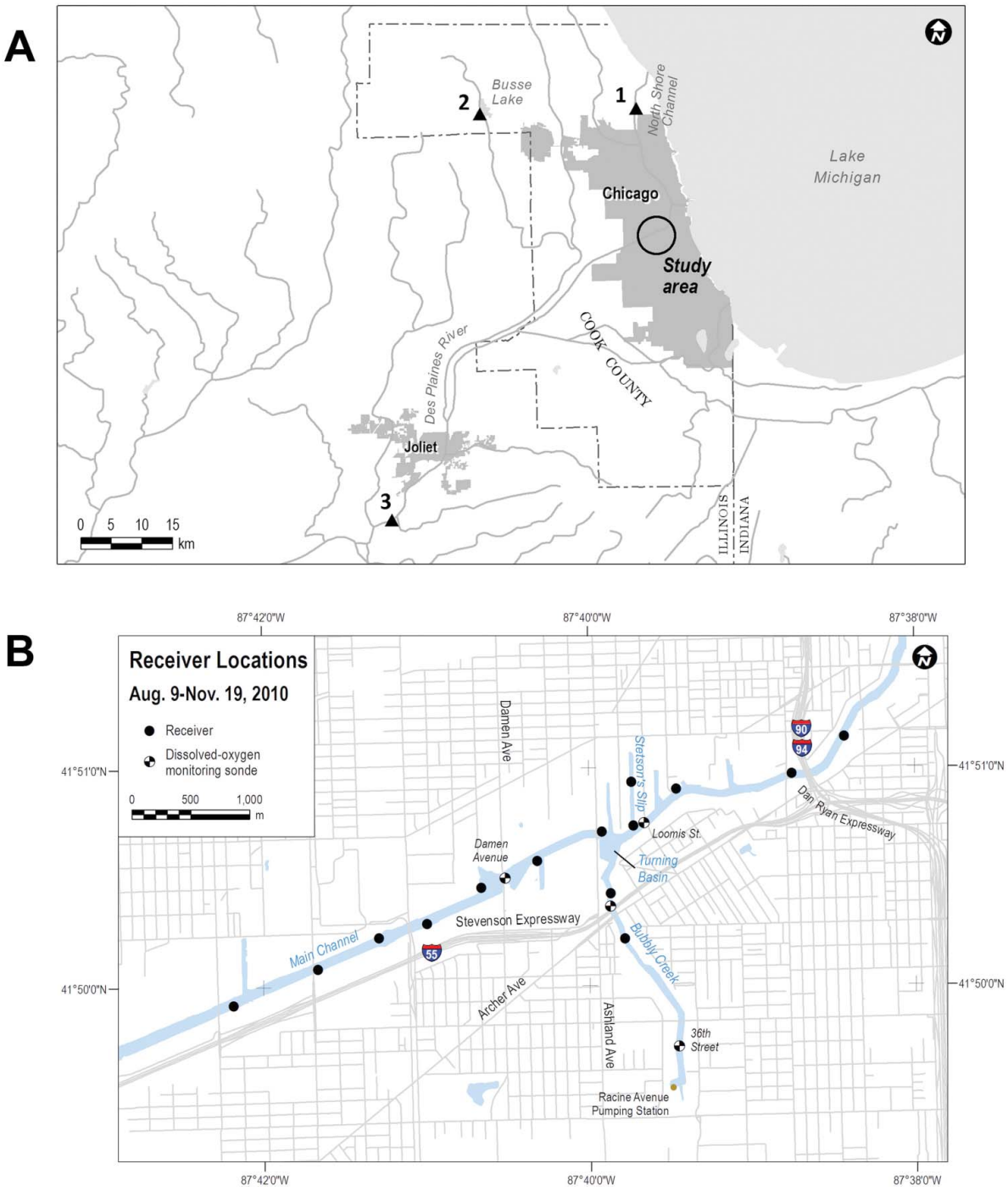

FIGURE 1. (**A**) Location of study site in relation to both the city of Chicago and Lake Michigan, and (**B**) the location of telemetry receivers (solid circles) and DO monitoring sondes (patterned circles) within the study site. Solid triangles in (A) show the location of the reference sites used for collection purposes (site 1 = North Shore Channel, site 2 = Busse Lake, site 3 = the Des Plaines River). [Color figure available online.]

movements over great distances (Warden and Lorio 1975; Winter 1977; Fish and Savitz 1983), and can reach sufficient size to permit implantation of long-term telemetry devices (Hasler et al. 2009). A total of 20 Largemouth Bass were collected for tagging between July 14 and 16, 2010, using standard boat DC electroshocking procedures. Collected Largemouth Bass ranged in size from 224 to 350 mm in length (274 ± 6 mm, mean ± SE) and ranged from 180 to 653 g in weight (326 ± 24 g). Another six Largemouth Bass were tagged in April 2011 using procedures identical to the 2010

tagging to supplement transmitter loss that occurred in 2010. Fourteen VR2W receivers (Vemco, Halifax, Nova Scotia) were deployed within the Main Channel and Bubbly Creek from July 2010 to October 2011 to record fish positions (Figure 1B). These receivers had a listening radius of approximately 250 m (defined using stationary range tests similar to Espinoza et al. 2011) that logged the presence of tagged Largemouth Bass within this radius, but did not provide three-dimensional positions or exact locations within the CAWS.

Surgical implantation of telemetry tags.—Two tanks were used for surgeries, each filled with 20 L of water taken from the CAWS. Each tank contained two air stones, one bubbling carbon dioxide (CO_2) and the other oxygen (O_2) (Summerfelt and Smith 1990). Carbon dioxide was released at 2.4 L/min from a regulator, and O_2 was released at 1 L/min. Both tanks were buffered with 3.0 mg/L of sodium bicarbonate ($NaHCO_3$). Fish were placed ventral side up on a V-cut foam pad, and a silicon tube was inserted into the fish's mouth to perfuse the gills with oxygenated water. A 15-mm incision was made parallel to and off the midline, anterior to the pelvic girdle, into the peritoneal cavity using a #10 scalpel blade. The transmitter (V7-4 L; 22.5 × 7 mm in size, 1.8 g in air, 1.0 g in water), programmed to emit a signal randomly every 110–250 s, was placed inside the body cavity and two sutures consisting of four double throws were used to close the incision (Mono-Dox absorbable synthetic monofilament 3/0 NFS-1; CP Medical, Portland, Oregon). Surgery times did not exceed 2–3 min. Fish were then placed into a recovery tank (150 L) for at least 1 h for postsurgical monitoring and then released into the CAWS if swimming behavior appeared normal. A total of 20 Largemouth Bass were released alive and seemingly healthy after surgery and recovery.

Physiology

Reference site selection.—In addition to collecting samples from fish residing in the CAWS study site for baseline physiological analysis, we selected three additional reference sites against which fish from the study site could be compared (Figure 1A). Requirements for identifying a suitable reference site included (1) proximity to the CAWS study site, and (2) higher annual DO profiles than the CAWS study site, based on data collected between 2007 and 2009 by the Metropolitan Water District of Greater Chicago. Based on these criteria, three locations were chosen as reference sites: (1) the North Shore Channel of the CAWS (42°1′N, 87°42′W) approximately 20 km upstream from the study site (reference site 1), (2) Busse Lake (42°1′N, 88°0′W), a 239-ha impoundment approximately 35 km northwest of the study site in the Salt Creek Watershed, Ned Brown Preserve, Chicago (reference site 2), and (3) a section of the Des Plains River (41°25′N, 88°10′W), south of Joliet, Illinois, and approximately 60 km southwest of the study site (reference site 3). All three sites were located within the greater Chicago area. Within each of the three reference sites, DO data were assessed by Metropolitan Water District of Greater Chicago to determine the percentage of time DO levels remained above and below 4.0 mg/L (https://www.mwrd.org/irj/portal/anonymous/WQM). During low DO events, temperatures remained fairly constant, ranging within 3–7°C for low DO events lasting <15 d. During low DO events lasting >15 d, temperature fluctuations were greater, likely a result of seasonal water temperature changes (https://www.mwrd.org/irj/portal/anonymous/WQM). The three reference sites chosen all remained above a DO level of 4.0 mg/L 95% of the time during the 3-year period, and a mean DO concentration of 4.0 mg/L was used instead of 2.0 mg/L to be confident these sites rarely experienced periods of moderate to low DO concentrations. In comparison, during the same 3-year period DO concentrations in the CAWS study site were above 4.0 mg/L only 39% of the time (https://www.mwrd.org/irj/portal/anonymous/WQM).

Field sampling.—Eight Largemouth Bass (between 200 and 300 mm TL) were collected from each of the four sites (CAWS study site and three reference sites) between June and August 2011 using standard boat electroshocking techniques. Water temperatures at the study site averaged 24.7°C and at reference sites 1, 2, and 3 averaged 17.7, 22.4, and 28.4°C, respectively. Dissolved oxygen was measured before and during the field sampling to ensure no hypoxic events were occurring. To avoid biased results, Largemouth Bass were not sampled in an area where an event was or potentially was occurring. The collected fish were immediately sacrificed by cerebral concussion and sampled for blood and tissues to quantify physiological and nutritional indicators, representing the condition of free-swimming Largemouth Bass at these sites. Blood was drawn from the caudal vessel with a 21-gauge needle and 1-mL syringe rinsed with lithium heparin. To quantify hematocrit (Hct; i.e., percent packed red cell volume), a small volume of whole blood was placed in a capillary tube and spun for 2 min using a hematocrit spinner; due to technical problems in the field, hematocrit data were collected from fish at only three of the four sites (none from reference site 2). An additional aliquot of whole blood was transferred to a microcentrifuge tube for the subsequent quantification of hemoglobin (Hb). The remaining whole blood was centrifuged for 2 min at 2,000 × *g* to separate plasma from the erythrocytes; the plasma was then transferred to an additional microcentrifuge tube. Plasma and whole blood were frozen and stored in the field in liquid nitrogen, and then transferred to an ultracold freezer (−80°C) until further laboratory analyses (Suski et al. 2003). A section of white epaxial musculature posterior to the operculum and above the lateral line was excised with a razor blade and stored in liquid nitrogen until further processing (Suski et al. 2003). To avoid any sampling-induced physiological disturbances, sampling typically was completed in less than 2 min (Romero and Reed 2005). Additionally, eight Largemouth Bass from each site were transported to the Aquatic Research Facility in Champaign,

Illinois, for the hypoxia challenge experiment described below, and an additional eight fish from each site were transported to the Kaskaskia Biological Station (Sullivan, Illinois) for the quantification of critical oxygen tension (P_{crit}), also described below.

Hypoxia challenge experiment.—Following transport to the aquatic research facility, Largemouth Bass from each of the four sites were allowed to recover from hauling stress for 48–72 h in outdoor holding tanks supplied with pond aerated, water. Immediately after the recovery period, 16 Largemouth Bass (randomly assigned using at least two sites) were placed into opaque sensory-deprived chambers continuously supplied with aerated freshwater from a central basin in a closed system, in which DO was maintained at 7.0 mg/L (Suski et al. 2006). Following a 24-h acclimation period to these chambers, 8 of the 16 Largemouth Bass were subjected to a hypoxia challenge of 2.0 mg/L (±0.1 SE) for 6 h by bubbling nitrogen gas (N_2) into the chambers to displace the oxygen in the water (Suski et al. 2006). Exposure to this DO concentration for this duration of time can elicit a physiological response in Largemouth Bass (VanLandeghem et al. 2010). To act as a control group, the second group of eight Largemouth Bass remained in identical chambers for 6 h with no change in DO (6.5 mg/L, ±0.1 SE). Following this 6-h period, all Largemouth Bass were sacrificed with an overdose of tricaine methanesulfonate (MS-222; 250 mg/L in solution buffered with 250 mg/L sodium bicarbonate) and removed from the chambers. Blood and tissue samples were collected using methods identical to the field sampling described above.

Critical oxygen tension (P_{crit}).—Seven Largemouth Bass from each site (study site and the three aforementioned reference sites) were subjected to decreasing DO levels, and both metabolic rates and P_{crit} were quantified to determine whether Largemouth Bass from the study site exhibited lower P_{crit} values compared with populations not exposed to frequent periods of hypoxia. For this, after being transported to Kaskaskia Biological Station, Largemouth Bass were allowed to recover from handling and hauling stressors for 48 h in outdoor, aerated tanks. The equipment used to generate P_{crit} data can process four fish simultaneously, and for P_{crit} analyses, fish from at least two study sites were randomly chosen and placed in one of four acrylic experimental chambers (355 mm long × 127 mm inner diameter; 4.5 L volume). Each chamber was outfitted with a fiber-optic oxygen probe (calibrated with oxygen-free water and fully aerated water continuously throughout the experiment), immersed in a 500-L tank maintained at 24°C (±0.5°C) and allowed to acclimate for 3 h at ambient temperatures and DO levels (7–8 mg/L). Treatments consisted of exposing Largemouth Bass to declining DO concentrations (6, 4, 3, 2, and 1 mg/L) over a 4-h period; reductions of DO were achieved by bubbling nitrogen gas (N_2) into the 500-L tank until the desired concentration was achieved. Automated software (AutoResp 2.0; Loligo Systems, Tjele, Denmark) was used to control DO concentration in the tank, as well as to record metabolic rates and generate P_{crit} values similar to methods described in Iversen et al. (2010). Briefly, the generation of each data point occurred with a trial loop consisting of a 10-min flush period, followed by a 1-min wait period (to allow for conditions to stabilize), and concluded with a 5-min measurement period in which the chamber was closed and oxygen decline was quantified every second. Trial loops were run in triplicate for each DO concentration (Iversen et al. 2010). Change in oxygen concentration (α) for each chamber was calculated as slope,

$$\alpha = \Delta O_{2saturation} \div \Delta time,$$

and oxygen consumption rate (MO_2; mg $O_2 \cdot kg^{-1} \cdot h^{-1}$) for each fish was calculated by

$$MO_2 = \alpha \cdot V_{resp} \cdot \beta \cdot M_b^{-1},$$

where V_{resp} is the volume of each experimental chamber minus the volume of the fish, β is oxygen solubility (mg $O_2 \cdot L^{-1} \cdot kPa^{-1}$, adjusted for temperature and barometric pressure), and M_b is the fish mass (kg) prior to placing it in the chamber. Standard metabolic rate was calculated as the average of the lowest 5% of the MO_2 values collected during the acclimation period (Nelson and Chabot 2011). The P_{crit} was determined for each individual fish by first fitting a linear regression of MO_2 values below the standard metabolic rate against the oxygen concentration, and P_{crit} was defined as the oxygen concentration where the regression line intersected with the previously determined standard metabolic rate (Schurmann and Steffensen 1997; Iversen et al. 2010). The coefficient of determination (r^2) for all slope measurements was >0.95, and oxygen concentration in the chambers was recorded every 1 s. Chambers were disinfected with iodine between trials to prevent bacterial growth. Background oxygen consumption of chambers was measured and subtracted from MO_2 values attained during the trials.

Data Analyses

Fish movement.—The movement of Largemouth Bass in response to hypoxia was quantified using two separate analyses. For the first analysis, daily mean DO readings recorded by each sonde were first calculated, and each sonde was assumed to be representative of the section of the study site in which it was deployed. If a sonde (i.e., section of the study site) showed a daily mean DO of below 2.0 mg/L for 4 d or more, these periods were defined as "hypoxia events" and used for analyses. To quantify movement of the Largemouth Bass in response to hypoxia events, the number of unique transmitter identifications (IDs; i.e., tagged Largemouth Bass) located in proximity of a sonde was summed for a period of 4 d before a hypoxia event and compared with the number of unique transmitter IDs located in proximity to that same sonde 4 d

immediately after the onset a hypoxia event. A 4-d observation duration was chosen to standardize the duration of examination and to exclude transient, brief hypoxia events that can occur at the Bubbly Creek site.

The second analysis to quantify the movement of the 20 telemetered Largemouth Bass in response to hypoxia used a logit choice model that assessed data from the summer of 2010 only. Logit choice models predict the probability of an event occurring based on a set of independent variables (Berkson 1944). For the current study, the model quantified the probability that a tagged Largemouth Bass inhabiting Bubbly Creek would leave Bubbly Creek based on DO concentrations recorded both inside and outside of Bubbly Creek, and was calculated as

$$P = 1/(1 + e^{-(\alpha + \sum \beta i Xi + u)}),$$

where α represents a constant intercept term, βi represents coefficients of independent variable Xi, and u represents unmeasured factors that influence behavior (e.g., barge traffic, pursuit of prey items; Berkson 1944). Logit was used to specify two distinct models of fish response to rain events: one model represented the behavior of fish currently in Bubbly Creek (the portion of the system experiencing the most frequent hypoxia events) and the other model represented the behavior of all fish in the system. The model accounted for autocorrelation of successive measurements from individual fish according to recommendations by (Fieberg et al. 2010).

Physiological analyses.—Plasma triglycerides were quantified using a commercially available colorimetric assay kit (BioAssay Systems, Hayward, California), and total plasma proteins (TPP) were quantified using a hand-held refractometer (Reichart VET 360; Reichert, Depew, New York). Plasma cortisol was quantified using an enzyme-linked immunosorbent assay (ELISA) kit (Kit 900-071; Enzo Life Sciences, Farmingdale, New York), and plasma glucose and plasma lactate were quantified enzymatically using methods described in Lowry and Passonneau (1972). Whole-blood hemoglobin concentration (Hb) was quantified using a commercially available kit (DIHB-250; BioAssay Systems). Mean cell hemoglobin concentration (MCHC) was calculated by

$$(Hb \div \%H_{ct}) \times 100$$

according to Houston (1990). Plasma sodium (Na^+) and potassium (K^+) were quantified using a flame photometer (model 2655-00; Cole-Parmer Instrument Company, Chicago, Illinois). Plasma chloride (Cl^-) was quantified using a chloridometer (model 4435000; Labconco Corporation, Kansas City, Missouri). Relative weight (W_r) was calculated as

$$W \div W_s \times 100,$$

where W is the actual weight (g) of the fish measured in the field, and W_s is a length-specific weight standard that is a proxy for fish health and condition; a relative weight of 100 is considered to be an ideal weight for a given length, and values below 100 indicate reduced condition (Anderson and Neumann 1996).

Statistical tests.—A *t*-test was used to determine whether the number of tagged Largemouth Bass in the study area in the CAWS 4 d prior to a hypoxia event differed significantly from the number of tagged Largemouth Bass observed in the same location 4 d after the onset of a hypoxia event (Sokal and Rohlf 1995). A one-way ANOVA was used to quantify differences in physiological variables, nutritional properties, and P_{crit} values between Largemouth Bass collected from the study site and the three reference sites (Sokal and Rohlf 1995). A Tukey–Kramer honestly significantly different (HSD) post hoc test was used to separate means when results from the ANOVA indicated significant differences across sites. A two-way ANOVA (main effects: oxygen exposure and site, and their interaction) was used to define differences in plasma response variables and morphometric measurements for fish in the hypoxia challenge experiment (Sokal and Rohlf 1995). If the interaction was significant, or if the interaction was not significant but at least one of the main effects was significant, a Tukey–Kramer HSD post hoc test was used to separate means. For all statistical analyses, normality was confirmed through visual analysis of fitted residuals using a normal probability plot (Anscombe and Tukey 1963), while homogeneity of variances was assessed using a Hartley's F_{max} test (Hartley 1950) combined with visual analysis of fitted residuals using a residual by predicted plot. Data were rank transformed, if necessary, to meet assumptions of normality and homogeneity of variances (Conover and Iman 1981; Iman et al. 1984; Potvin and Roff 1993). All tests were performed using JMP, version 9.0.3 (SAS Institute, Cary, North Carolina). The level of significance for all tests was set at $\alpha = 0.05$.

RESULTS

Movement Analyses

During 2010 and 2011, nine time periods were identified as hypoxia events as they experienced daily mean DO concentrations below 2 mg/L for 4 d or more (Table 1). Three of these events occurred downstream from Bubbly Creek and the remaining six occurred within Bubbly Creek. There was no significant difference between Largemouth Bass abundance in an area of the study site 4 d prior to the onset of hypoxia compared with the number of Largemouth Bass in that same area 4 d after the onset of hypoxia events (*t*-test: $t_{14.5} = 0.44$, $P = 0.67$). More specifically, during the nine events, there were only three instances where Largemouth Bass abundance in an area decreased after the onset of a low oxygen event (Table 1).

An analysis of bass movement over the full study period was also conducted to capture responses to DO fluctuations

TABLE 1. Largemouth Bass present during each low DO event 4 d before and 4 d after the onset of a hypoxia event from July 2010 to October 2011. Delta is the change in fish presence, with a negative number indicating fish movement out of the area, 0 indicating no change, and a positive number indicating fish presence increasing after the low DO event. For reference, the event length (days) for each low DO event and mean DO concentration during the whole event is included.

Event	Location	Before event	After event	Delta	Event length (d)	Mean DO (mg/L)
1	Bubbly Creek	4	2	−2	4	1.04
2	Bubbly Creek	1	1	0	6	1.76
3	Bubbly Creek	1	1	0	7	1.03
4	Downstream	1	2	1	5	1.39
5	Bubbly Creek	1	1	0	52	1.21
6	Downstream	0	3	3	19	0.96
7	Bubbly Creek	2	1	−1	22	0.96
8	Downstream	3	0	−3	9	1.82
9	Bubbly Creek	2	2	0	4	1.43

that may not have been fully captured by the event-based analysis. During 2010, only 4 Largemouth Bass of 19 (one transmitter was never recorded after implantation and release) inhabited Bubbly Creek at any time period, necessitating the use of these four individuals when generating the predictive models (Figure 2a). Dissolved oxygen concentration during this time varied from 0.1 to 14.6 mg/L. Results from predictive modeling analyses revealed that as the DO level decreased within Bubbly Creek, the probability of fish leaving this area increased significantly ($P < 0.0001$). However, even if the DO concentration approached anoxia (0.0–0.5 mg/L), there was a probability of less than 0.4 that a fish would leave Bubbly Creek, indicating that anoxic conditions were not predicted to drive all fish from the study area (Figure 2b).

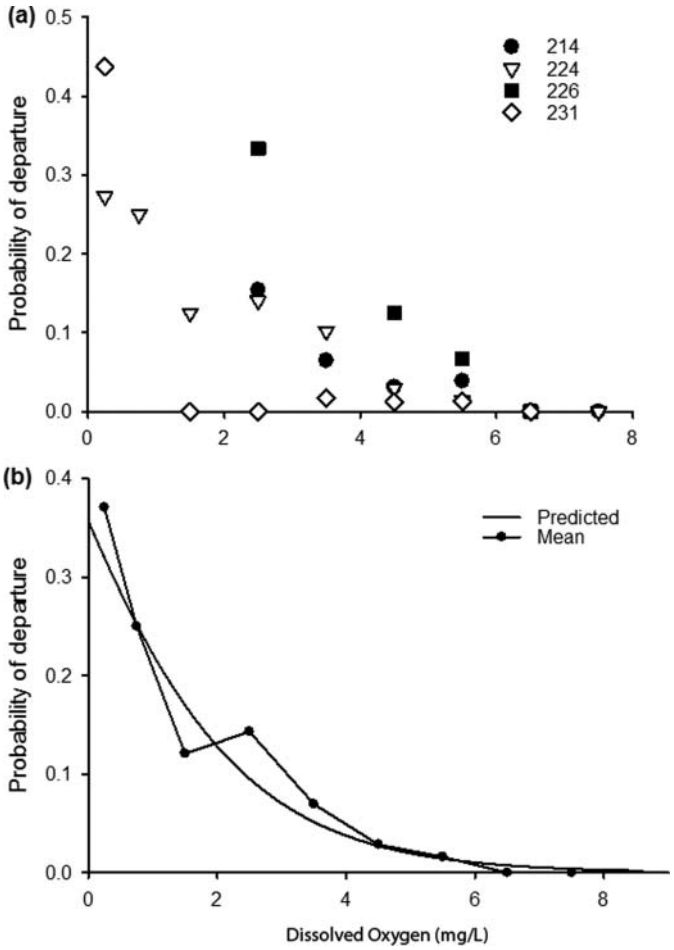

FIGURE 2. (**a**) The probability of each of four individual Largemouth Bass (designated by fish ID number) located within Bubbly Creek during the 2010 season to depart the area as DO levels decreased; symbols denote individual fish. (**b**) The results of the predictive model compared with the mean probabilities of departure for the four Largemouth Bass located within Bubbly Creek during the 2010 season.

Field Sampling

All plasma cortisol values from fish sampled in the field averaged at or below approximately 30 ng/mL (Table 2); differences in plasma cortisol concentrations existed across sample locations, but Largemouth Bass residing in the study site showed plasma cortisol concentrations that were intermediate relative to those at other sites. Plasma glucose concentrations were 33–77% higher for fish sampled in the field at reference site 1 relative to fish sampled from other locations (Table 2). Concentrations of plasma lactate in fish for all sites did not differ significantly and were below approximately 3.0 mmol/L (Table 2). Hematocrit was significantly higher in Largemouth Bass sampled from the study site compared with fish from reference site 1, while hemoglobin content was approximately one-third higher in Largemouth Bass residing in the study site compared with all other reference sites, but this difference was not statistically significant (Table 2). The MCHC was statistically similar among Largemouth Bass from all sites sampled, but fish residing in the study site showed MCHC values

TABLE 2. Morphological and physiological metrics for eight Largemouth Bass collected from the study site in the Chicago Area Waterway System (CAWS) and three reference sites (North Shore Channel of the CAWS, Busse Lake, Des Plaines River) sampled immediately after collection in the field. Data were compared across sites using a one-way ANOVA followed by a Tukey–Kramer HSD test to separate means. Data are presented as mean ± SE; significance was assessed at $\alpha = 0.05$ and indicated by bold text. Levels within a specific metric not sharing the same letter are significantly different.

Metric	Study site	Reference site 1	Reference site 2	Reference site 3	*P*-value
Length (mm)	244 ± 6	252 ± 15	248 ± 12	245 ± 10	0.96
Weight (g)	209 ± 20	270 ± 48	241 ± 41	217 ± 31	0.68
W_r	**102 ± 3** y	**114 ± 3** z	**106 ± 3** zy	**103 ± 3** zy	**0.03**
Plasma cortisol (ng/mL)	**14.9 ± 11** zy	**31 ± 17** z	**3.6 ± 0.6** y	**7.1 ± 2** zy	**0.03**
Plasma glucose (mmol/L)	**4.0 ± 0.3** y	**5.6 ± 0.7** z	**2.5 ± 0.2** x	**3.6 ± 0.2** yx	**<0.0001**
Plasma lactate (mmol/L)	1.9 ± 0.7	3.1 ± 1.2	1.5 ± 0.3	2.3 ± 0.5	0.79
Hematocrit (%)	**31.1 ± 1.4** z	**26.1 ± 0.6** y		**28.1 ± 0.8** zy	**0.02**
Hemoglobin (g/dL)	10.5 ± 1.5	6.9 ± 0.4	6.5 ± 0.4	7.7 ± 0.1	0.06
MCHC (g/dL)	34.3 ± 5.6	26.5 ± 1.6		27.6 ± 0.6	0.49
Water content (%)	**78.6 ± 0.1** y	**79.0 ± 0.2** zy	**78.6 ± 0.4** zy	**79.3 ± 0.1** z	**0.01**
Potassium (mEq/L)	3.78 ± 0.2	2.98 ± 0.4	3.74 ± 0.2	3.27 ± 0.2	0.16
Sodium (mEq/L)	148 ± 5.3	158 ± 8.0	144 ± 4.4	147 ± 5.7	0.58
Chloride (mEq/L)	93.4 ± 4.4	93.2 ± 5.7	101.8 ± 3	95.1 ± 1.7	0.29
Triglycerides (mmol/L)	2.6 ± 0.6	4.3 ± 1.1	3.9 ± 0.5	2.2 ± 0.7	0.13
Plasma protein (g/dL)	**7.0 ± 0.7** zy	**9.2 ± 0.9** z	**6.1 ± 0.5** y	**8.1 ± 0.6** zy	**0.02**

approximately 30% higher than in fish from the other sites sampled (Table 2). There were no significant differences in Largemouth Bass across sampling locations for plasma Na^+, plasma K^+, plasma Cl^- (Table 2), or plasma triglycerides, and plasma protein levels of fish from the study site were intermediate to all other sample locations (Table 2). Lengths and weights did not differ across sites (Table 2), W_r values of all fish in the study were greater than 100 (i.e., all fish were considered to be an appropriate weight for their length), and fish from reference site 1 had significantly higher W_r than those from all other sites (Table 2).

Hypoxia Challenge Experiment

Following exposure of Largemouth Bass to 2.0 mg/L DO for 6 h, hematocrit values in Largemouth Bass from reference sites 1 and 2 increased by 25% and 26%, respectively, compared with control values, but hematocrit concentrations in Largemouth Bass collected from the study site did not change compared with control fish (Figure 3a; Table 3). A 6-h exposure to 2.0 mg/L DO did not cause any significant change in hemoglobin concentration for any Largemouth Bass in this study, but hemoglobin levels in Largemouth Bass collected from the study site were significantly higher than in fish residing at reference site 1 (Figure 3b; Table 3). Similarly, MCHC did not differ significantly following hypoxia exposure or across sites, but MCHC levels in fish from the study site were significantly higher than in fish from reference sites 1 and 2 (Figure 3c; Table 3).

Exposure of Largemouth Bass to 2.0 mg/L DO for 6 h caused significant increases in plasma cortisol (compared with control values) only for reference site 1, and no change in plasma cortisol was observed for fish from the other three sites (Figure 4a; Table 3). Concentrations of plasma glucose in fish increased by approximately 41% across all sites following the low DO exposure, but site-specific increases did not occur (Figure 4b; Table 3). Plasma lactate concentrations for Largemouth Bass from all sites increased at least fourfold as a result of a 6-h exposure to a 2.0-mg/L low DO challenge, and the response of reference site 1 was larger than the response of fish from reference site 2 (Figure 4c; Table 3). Plasma Na^+ and K^+ did not increase or decrease significantly as a result of the hypoxia treatment, and only fish from reference site 2 showed a change in plasma Cl^- (a decrease of approximately 10%) (Figure 5; Table 3). Total lengths and weights of Largemouth Bass used in the oxygen challenge experiment did not differ across sites (Table 3). Relative weight differed significantly between sites ($P < 0.001$) and treatments ($P = 0.013$), but all W_r values were within the normal range for Largemouth Bass (average range, 91–108).

Critical Oxygen Tension (P_{crit})

Standard metabolic rates of Largemouth Bass for the study site and reference sites 1, 2, and 3 were 113.0 ± 9, 123.6 ± 8, 118.0 ± 6, and 121.0 ± 10 mg $O_2 \cdot kg^{-1} \cdot h^{-1}$, respectively. There was no significant difference in the standard metabolic rate between Largemouth Bass collected from the four sites ($F_{3, 28} = 0.3$, $P = 0.83$). Critical oxygen tension (P_{crit}) varied from 21.1% to 26.7% oxygen saturation across the four sites (Figure 6), but values did not differ significantly ($F_{3, 28} = 2.3$, $P = 0.10$; Figure 5).

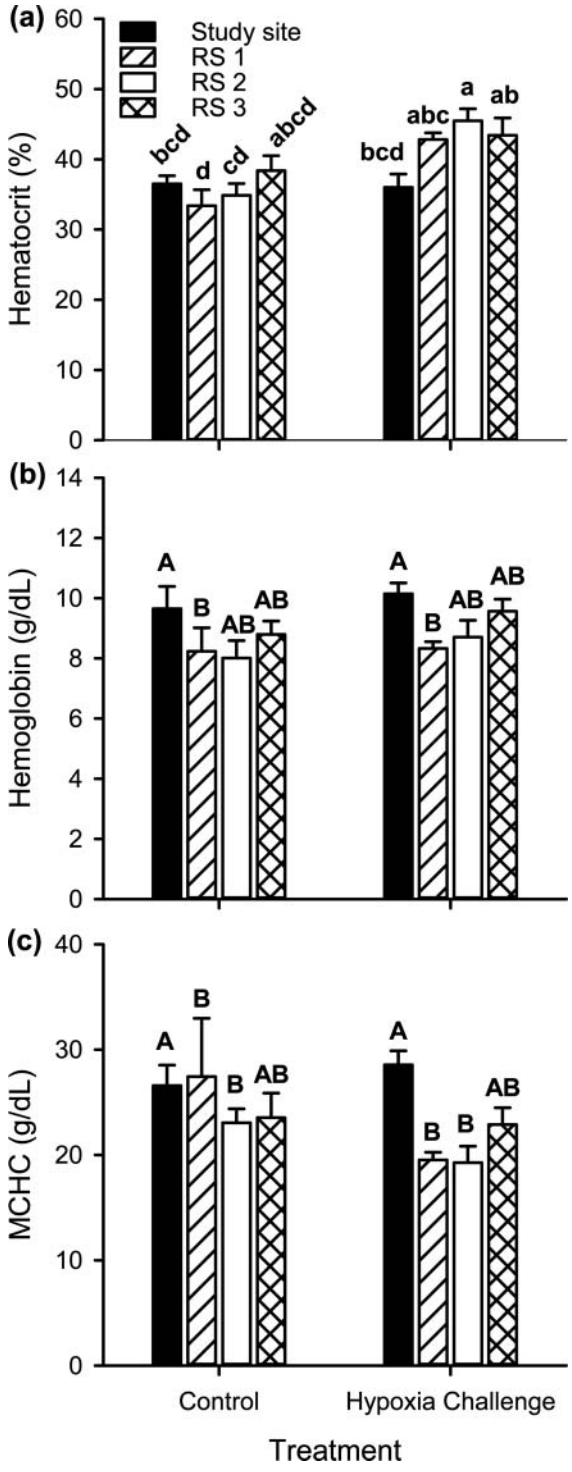

FIGURE 3. (**a**) Hematocrit, (**b**) hemoglobin, and (**c**) MCHC for Largemouth Bass from the study site in the Chicago Area Waterway System (CAWS) and three reference sites (RS; North Shore Channel of the CAWS, Busse Lake, Des Plaines River) subjected to a low DO challenge of 2.0 mg/L for 6 h. Control treatment fish were held for 6 h at 6.5 mg/L. Error bars show ±1 SE. Lowercase letters represent statistical differences between individual bars, while uppercase letters represent significant differences across sites only; bars not sharing the same letter are significantly different from others in that group ($\alpha = 0.05$). Sample sizes ranged from $n = 7$ to 11.

TABLE 3. Statistical results examining physiological and hematological differences in Largemouth Bass sampled from the study site in the Chicago Area Waterway System and three reference sites (North Shore Channel of the CAWS, Busse Lake, Des Plaines River) subjected to a hypoxia challenge of 2 mg/L for 6 h. Significance was assessed at $\alpha = 0.05$ and indicated by bold text.

Metric	Effect	df	*F*-value	*P*-value
Length (mm)	Site	3	2.3	0.08
	Treatment	1	0.1	0.74
	Site × Treatment	3	2.0	0.12
Weight (g)	Site	3	1.1	0.35
	Treatment	1	1.8	0.18
	Site × Treatment	3	1.0	0.39
W_r	Site	**3**	**8.1**	**<0.001**
	Treatment	**1**	**7.2**	**0.01**
	Site × Treatment	3	0.7	0.57
Hematocrit (%)	Site	3	2.4	0. 08
	Treatment	**1**	**20.0**	**<0.001**
	Site × Treatment	**3**	**3.5**	**0.02**
Hemoglobin (g/dL)	Site	**3**	**3.9**	**0.01**
	Treatment	1	2.9	0.09
	Site × Treatment	3	0.4	0.75
MCHC (g/dL)	Site	**3**	**5.7**	**0.02**
	Treatment	1	2.0	0.16
	Site × Treatment	3	2.3	0.09
Plasma cortisol (ng/mL)	Site	3	4.5	0.06
	Treatment	**1**	**4.3**	**0.04**
	Site × Treatment	**3**	**3.1**	**0.03**
Plasma glucose (mmol/L)	Site	3	1.5	0.23
	Treatment	**1**	**8.9**	**0.004**
	Site × Treatment	3	1.9	0.13
Plasma lactate (mmol/L)	Site	**3**	**3.0**	**0.04**
	Treatment	**1**	**144.1**	**<0.0001**
	Site × Treatment	3	2.6	0.06
Plasma Na^+ (mEq/L)	Site	3	0.7	0.53
	Treatment	1	0.5	0.50
	Site × Treatment	3	1.5	0.24
Plasma K^+ (mEq/L)	Site	3	0.5	0.66
	Treatment	1	1.7	0.20
	Site × Treatment	3	1.1	0.34
Plasma Cl^- (mEq/L)	Site	**3**	**5.8**	**0.002**
	Treatment	1	2.3	0.13
	Site × Treatment	**3**	**5.2**	**0.003**
Muscle water content (%)	Site	**3**	**9.0**	**<0.0001**
	Treatment	1	0.08	0.78
	Site × Treatment	3	1.6	0.21

DISCUSSION

During this study, we documented nine hypoxic events (defined as DO concentration remaining at or below 2.0 mg/L

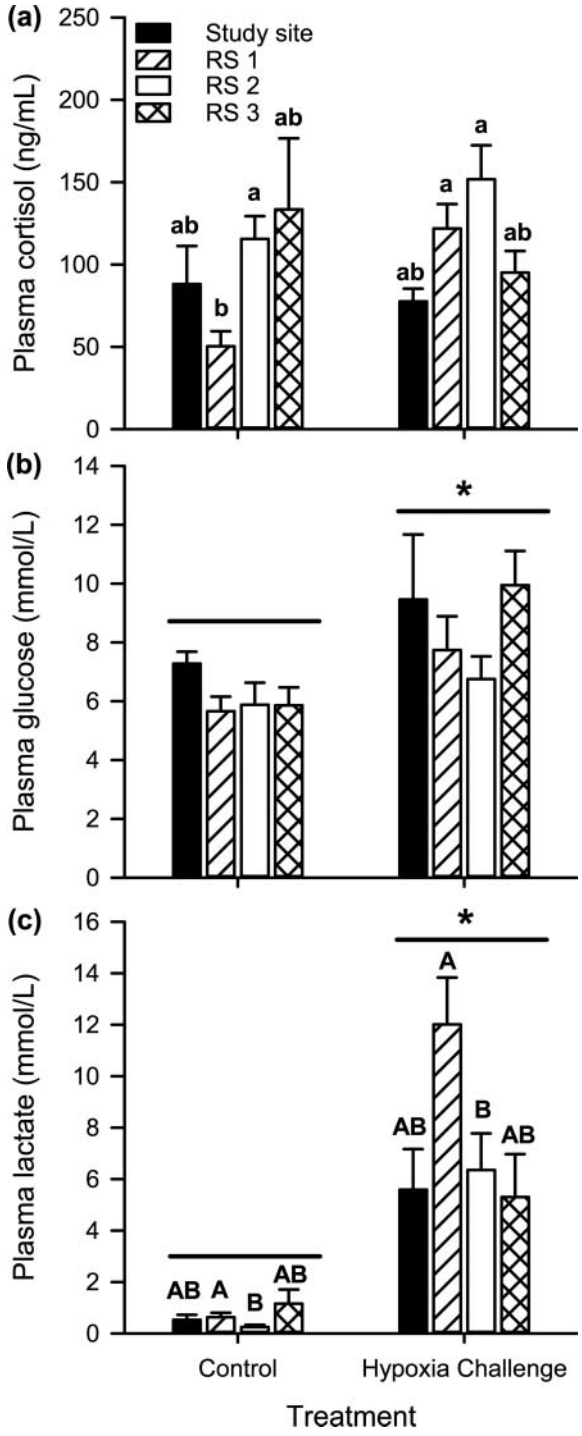

FIGURE 4. Concentrations of (**a**) plasma cortisol, (**b**) plasma glucose, and (**c**) plasma lactate for Largemouth Bass from the study site in the Chicago Area Waterway System (CAWS) and three reference sites (RS; North Shore Channel of the CAWS, Busse Lake, Des Plaines River) subjected to low DO challenge of 2.0 mg/L for 6 h. Control treatment fish were held for 6 h at 6.5 mg/L. Error bars show ±1 SE. Lowercase letters represent statistical differences between individual bars, uppercase letters represent significant differences across sites only, and an asterisk represent a significant treatment effect; bars not sharing the same letter are significantly different from others in that group ($\alpha = 0.05$). Sample sizes ranged from $n = 7$ to 11.

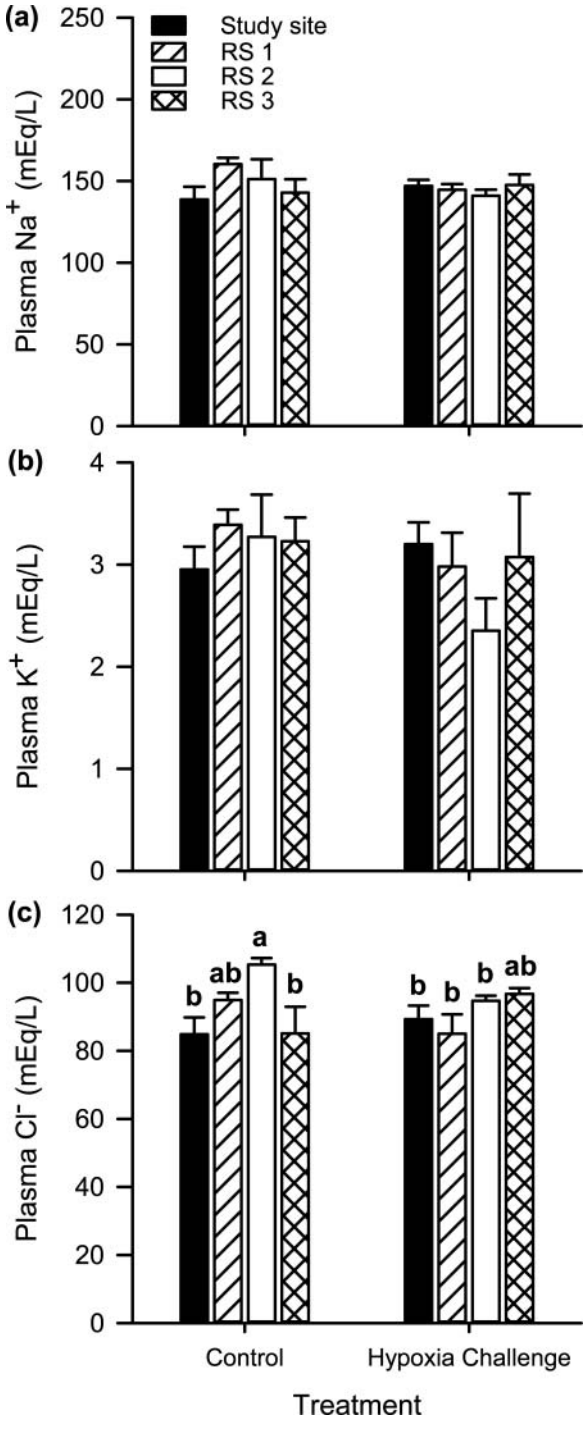

FIGURE 5. Concentrations of (**a**) plasma sodium (Na^+), (**b**) plasma potassium (K^+), and (**c**) plasma chloride (Cl^-) for Largemouth Bass from the study site in the Chicago Area Waterway System (CAWS) and three reference sites (RS; North Shore Channel of the CAWS, Busse Lake, Des Plaines River) subjected to low DO challenge of 2.0 mg/L for 6 h. Control treatment fish were held for 6 h at 6.5 mg/L. Error bars show ±1 SE, and lowercase letters represent statistical differences between individual bars; bars not sharing the same letter are significantly different from others in that group ($\alpha = 0.05$) and lack of letters indicates no significant differences. Sample sizes ranged from $n = 7$ to 11.

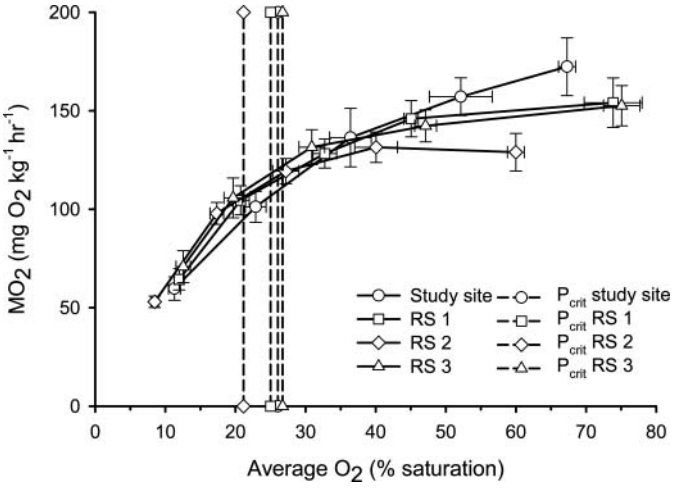

FIGURE 6. The relationship between oxygen consumption (MO_2) and percent saturation of the water for seven Largemouth Bass collected from the study site in the Chicago Area Waterway System (CAWS) and three reference sites (RS; North Shore Channel of the CAWS, Busse Lake, Des Plaines River). Mean P_{crit} values (i.e., critical oxygen tension) for each population are indicated by dashed vertical lines with symbols matching consumption line symbols. Error bars show ±1 SE for both MO_2 (vertical) and % saturation (horizontal).

for a minimum of four consecutive days), which varied in duration from 4 to 52 d; eight of these nine events were associated with CSO discharges and/or rainfall events. Reductions in DO in urban systems can be caused by the uptake of oxygen from the water column through both biological and chemical processes, particularly during periods of high flow rates generated by pumped sewage, coupled with suspension of highly organic, nutrient-rich, bottom sediments (Waterman et al. 2011).

Rain-induced reductions of DO within the CAWS did not appear to exert a strong influence on the movement patterns of Largemouth Bass. More specifically, reductions in DO to 2.0 mg/L or lower did not produce a consistent reduction in numbers of Largemouth Bass residing in locations within the study area (Table 1), and the probability of Largemouth Bass completely leaving the study area never reached 100%, even when DO dropped to anoxic levels (0 mg/L) (Figure 2). There are at least three potential explanations for why Largemouth Bass did not appear to exhibit pronounced shifts in behavior or position following exposure to low DO. First, water bodies can exhibit large variability in DO concentration across small areas, (Wetzel 1983). It is possible Largemouth Bass were spatially seeking and inhabiting waters with DO concentration higher than what was reported by the stationary sondes (e.g., shifts in swimming depth), eliminating the need for them to move out of an area entirely despite apparent low oxygen conditions. These potential shifts, however, could not be detected by the telemetry gear used for this study, which did not generate position solutions in three dimensions. Second, habitat selection by animals typically involves choices and trade-offs (i.e., food versus shelter versus predation versus competition: Mittlebach 2008; Hasler et al. 2009). Largemouth Bass within the study area may have chosen to remain in hypoxic conditions due to the presence of prey items, refuge from flow, or other factors (Pyke et al. 1977; Mittlebach 2008; Bartumeus and Catalan 2009), which may be less costly to the fish than movements away from a hypoxic area. Third, Largemouth Bass are both relatively tolerant to hypoxia and can induce plastic changes in their physiology that can improve oxygen transport, potentially allowing them to persist in low oxygen environments. VanLandeghem et al. (2010), for example, showed that, when exposed to hypoxia (2 mg/L) for up to 6 h, Largemouth Bass exhibited only minor physiological disturbances, while Furimsky et al. (2003) showed that, when subjected to graded hypoxia (90, 60, and 45 torr), Largemouth Bass did not exhibit increased hematocrit levels or a decrease in total blood O_2 content compared with Largemouth Bass held at normoxic conditions, indicating a tolerance to hypoxia as well as a high affinity for binding O_2 at lower oxygen tensions. Thus, Largemouth Bass likely make habitat choices that include factors other than just oxygen, suggesting that the choice to remain in hypoxic waters outweighs the costs associated with low DO exposure, particularly in light of physiological tolerances to low oxygen (Jeffres et al. 2006; Cocherell et al. 2010).

Interestingly, free-swimming Largemouth Bass collected from the CAWS showed signs of an improved ability to transport oxygen (increased Hct, Hb, and MCHC levels) relative to fish from some of the reference sites, possibly as a result of repeated exposures to low DO observed within the study site. More specifically, Largemouth Bass sampled from the study site showed elevated Hct compared with reference site 1 and elevated Hb compared with all three reference sites (but results for Hb were marginally nonsignificant), and MCHC values for fish sampled from the study site were almost one-third higher than in fish from the reference sites (Table 2). Hematocrit is the percentage of packed red cells in a known amount of whole blood, Hb is the total concentration of the oxygen-binding protein within the whole blood, while MCHC is the amount of Hb within a known volume of red cells (i.e., the amount of Hb per cell: Houston 1990). Increased values of Hct and Hb have both been observed in numerous species as a result of extended exposure to low DO (Tun and Houston 1986; Petersen and Petersen 1990; Timmerman and Chapman 2004). For example, Atlantic Cod *Gadus morhua* exposed to 6 weeks of hypoxia (~40% air saturation) resulted in increases of 11% and 14% for Hct and Hb, respectively, compared with cod held at normoxic conditions (Petersen and Gamperl 2011). More recently, Gaulke et al. (2014) showed that prolonged exposure of Largemouth Bass to DO levels of 3.0 mg/L resulted in significant increases to both Hb and Hct levels, indicating there is a potential for improvements in oxygen transport mechanisms for Largemouth Bass. An increase in red cells is often caused by the release of erythropoietin from the renal glands (Jensen et al. 1993), and increases in this

hormone can stimulate splenic releases of stored red cells in Rainbow Trout *Oncorhynchus mykiss* (Lai et al. 2006). Increases in Hct allow for a larger surface area to bind oxygen within the blood, likely conferring a beneficial advantage (either a plastic acclimation or an adaptation) to the fish to survive in a low DO environment with seemingly little physiological or behavioral cost. Together, results from this study indicate that free-swimming Largemouth Bass from the CAWS likely had an improved ability to transport oxygen relative to fish from control sites due to increases in both Hct and Hb.

Following a low DO exposure of 2.0 mg/L for 6 h in the laboratory, differences in physiological responses of Largemouth Bass across sites were similar to patterns seen in the field. More specifically, Largemouth Bass collected from both the CAWS and reference sites experienced an increase in both plasma glucose and plasma lactate, indicating an upregulation of the stress response (Figure 4) (Wendelaar Bonga 1997), but differences across sites were minor (only reference site 1 showed a significantly elevated production of lactate compared with other sites). Lactate is produced when oxygen delivery to tissues is not sufficient to sustain aerobic respiration forcing fish to respire anaerobically to meet energetic demands, and glucose is produced as part of the stress response and is released to fuel aerobic tissues such as heart or gills (Barton et al. 1986). The increase in both plasma glucose and plasma lactate was similar in magnitude to past studies involving Largemouth Bass and hypoxia (VanLandeghem et al. 2010) and indicated that the concentration and duration of DO exposure was successful at creating a physiological disturbance for Largemouth Bass. Cortisol is a hormone that is part of the primary stress response for fish and is produced following the onset of a stressor to help maintain homeostasis (Barton et al. 1986). Plasma cortisol values following hypoxia exposure increased significantly between two different reference sites; however, Largemouth Bass at only one site showed a significant increase in cortisol, suggesting that this intensity and/or duration of hypoxia did not necessitate production of stress hormones to assist in maintaining homeostasis. Interestingly, fish at two of the three control sites displayed an increase in Hct after low DO exposure, but fish from the study site did not have increased Hct concentrations following the hypoxia challenge, suggesting the study-site fish had a tolerance to hypoxia (Figure 3). Similarly, Largemouth Bass from the study site showed MCHC levels that were significantly higher than in those from two of the control sites, again suggesting the study-site fish have an an improved ability to transport oxygen. Fish exposed to hypoxia often experience a loss of ions from plasma as a result of greater blood perfusion of the gill, which works to improve oxygen uptake (Gonzalez and McDonald 1992). Largemouth Bass did not experience a loss of cations from plasma, and only one site displayed a reduction in Cl^-, providing additional evidence that a 6-h exposure to 2.0 mg/L DO did not disturb ionic balances. Together, results from the current study demonstrated that DO exposure of 2.0 mg/L for 6 h in the laboratory resulted in anaerobic metabolism for these Largemouth Bass, but fish residing in the study area that regularly experienced low DO displayed a reduced physiological response compared with fish from some control sites, likely due to their improved ability to transport oxygen.

Critical oxygen tension (P_{crit}) is the point at which an animal can no longer maintain aerobic respiration and begins to respire anaerobically as a result of a lack of oxygen (Richards 2009). Hypoxia-tolerant fish have lower P_{crit} values compared with fish that are less hypoxia tolerant (Richards 2009; Iversen et al. 2010), as fish that are hypoxia tolerant can change to anaerobic respiration (oxyconformation) at a lower oxygen saturation than can fish that are less hypoxia tolerant. In the current study, P_{crit} values did not vary between Largemouth Bass collected from the study site compared with Largemouth Bass collected from the other reference sites (Figure 6). Reductions in P_{crit} can occur through modifications in ventilation rate and amplitude, enhanced oxygen transport, or modifications in cardiovascular function (Randall 1982; Mandic et al. 2009; Richards 2009). Largemouth Bass from the study site were not regulating metabolic rates or oxygen-carrying capacity differently than fish from the reference site, despite residing in an environment where reductions in DO are frequent and occur for extended periods of time.

Free-swimming Largemouth Bass collected in the wild did not appear to be experiencing chronic stress or impaired nutrition compared with free-swimming Largemouth Bass sampled from the three reference sites (Table 2). For example, resting (control) values of glucose for Largemouth Bass (2–5 mmol/L) and cortisol (10–30 ng/mL: VanLandeghem et al. 2010) were similar to concentrations displayed in the current study. Fish that have experienced extended periods of time with little or no feeding can display reductions in concentrations of triglycerides or plasma proteins (Wagner and Congleton 2004; Congleton and Wagner 2006; Hanson and Cooke 2009). Over extended periods of time, this can result in a loss of weight for fish (Gingerich et al. 2010). All Largemouth Bass sampled in the field had W_r values that were above a weight of 100, which is considered normal for healthy fish, and fish residing in the study site did not have W_r values that were significantly lower than at any of the reference sites. Largemouth Bass in the study site that resided in areas exposed to frequent and extended periods of rain-induced reductions in DO were therefore not in poor nutritional condition or suffering from chronic stress compared with fish collected from the three reference sites.

While the results from this study did not indicate a strong negative effect of reductions in DO on the condition or behavior of Largemouth Bass, there are four caveats to this conclusion to address. First, Largemouth Bass residing in these areas could experience negative consequences due to their environment that were not quantified in our study, such as reduced growth, impaired reproduction, reduced immune

function, increased parasite loads, endocrine disruption, or truncated life expectancy relative to fish from less disturbed locations (Wendelaar Bonga 1997). Increased barge traffic and artificial embankments, for example, can reduce reproductive potential in the Oder-Havel Kanal, Germany, for young of year Roach *Rutilus rutilus* and Eurasian Perch *Perca fluviatilis* (Arlinghaus et al. 2002). Similarly, a reduced growth rate was observed in Atlantic Cod exposed to chronic hypoxia (45% and 56% O_2 saturation) due to decreased food consumption and decreased activity (Chabot and Dutil 1999). Future studies of hypoxia on wild free-swimming fishes should examine metrics such as these that could change due to exposure to hypoxia and may influence fish. Second, it is often difficult to separate the true effects of hypoxia alone from other ancillary stressors that often occur in concert with hypoxia, (particularly in disturbed habitats) such as elevated carbon dioxide, high hydrogen sulfide, toxic organics, and metals (Kramer 1987). For example, larval fish exposed to varying concentrations of mercury (Hg) and lead (Pb) suffered lethal acute effects (Hall 1991). Third, we intentionally used fish from a narrow size range to avoid potential effects of body size on oxygen tolerance. Large fish may have a metabolic advantage over smaller fish during periods of low oxygen (Nilsson and Östlund-Nilsson 2008), although laboratory studies with Largemouth Bass showed that smaller fish (23–500 g) can inhabit water with lower DO concentration than can larger fish (1,000–3,000 g: Burleson et al. 2001). Lack of movement away from hypoxic areas may therefore have been partially driven by our use of relatively small fish. Finally, while sample sizes of bass used in our physiological analyses are robust and consistent with previous work on the topic of hypoxia (Wagner and Congleton 2004; VanLandeghem et al. 2010), because of restraints from fish movement and challenges from working within such a highly modified environment, sample sizes for movement analyses were limited to fewer than four fish per hypoxic event. Future studies on this topic should increase the numbers of tagged individuals in additional hypoxic areas to supplement the findings reported here.

Despite Largemouth Bass displaying limited behavioral and physiological responses to repeated exposure to rain-induced reductions in low DO (e.g., no strong pattern of avoidance areas of hypoxia, no loss of ions from plasma during extended hypoxia exposure), this does not mean that CSO events and rain-induced hypoxia in urban environments should be disregarded or ignored. While absolute fish diversity in the CAWS study site was high (over 30 species recorded; data available at http://www.mwrd.org), the species present at the site consist mainly of generalists and/or hypoxia-tolerant species (e.g., sunfishes *Lepomis* spp., Common Carp *Cyprinus carpio*, catfishes *Ictalurus* spp.) rather than habitat specialists or hypoxia-intolerant species that may exist where oxygen levels are higher (e.g., salmonids Salmonidae, Walleye *Sander vitreus*) (Nilsson and Östlund-Nilsson 2008). Largemouth Bass may not be representative of these other fishes because of their physiological tolerance to hypoxia (Furimsky et al. 2003; Gaulke et al. 2014) and previous tendency to inhabit water with low DO (Hasler et al. 2009). While the infrastructure for treatment of the additional burden of rain within the CAWS is unique to Chicago and its metropolitan area, combined sewer systems that use pumping stations and experience CSO discharges are common in urban systems, both nationally and internationally. Furthermore, stormwater discharges and nonpoint source runoff can also cause low DO problems in many waterways and the episodic low-DO events that occur in the CAWS are not unique to Chicago (Gray 1997; Brombach et al. 2005; Casadio et al. 2010). Current water quality standards presume that DO must remain above specific thresholds to be protective of resident fish communities, and CSOs are regulated primarily on the basis of effects on DO and other contaminants (Andrés-Doménech et al. 2010; Casadio et al. 2010; Gasperi et al. 2010). Results from this study, when combined with previous research on pollutant loads associated with CSO discharges, can enhance regulation decisions that serve to protect aquatic biota contained within these systems. In addition, this study serves as a model for applying telemetry to free-swimming, wild fish populations to identify changes in movement and activity patterns in urban systems caused by anthropogenic stressors.

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