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ARTICLE

Variability in Fish Tissue Proximate Composition is Consistent with Indirect Effects of Hypoxia in Chesapeake Bay Tributaries

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Abstract

The spatial and temporal extent of summer hypoxia (dissolved oxygen [DO] concentration ≤ 2 mg/L) in Chesapeake Bay and its tributaries has been increasing for decades, consequently affecting fish distribution and abundance by shifting biomass to non-hypoxic habitats. Hypoxia in coastal waters impacts food web dynamics, thereby limiting ecosystem productivity and affecting regional fisheries. Additionally, laboratory studies of Atlantic Croakers *Micropogonias undulatus* have shown that hypoxia serves as an endocrine disruptor, reducing the production of the yolk precursor vitellogenin as well as affecting other biochemical pathways. Reproductive potential is therefore lower in hypoxia-exposed Atlantic Croakers than in fish that are taken from normoxic conditions. We examined field-caught Atlantic Croakers from three Chesapeake Bay tributaries with different DO levels to evaluate patterns in the lipid content of somatic and gonadal tissues. We found that somatic lipid content was not affected by the presence of hypoxia, whereas ovarian lipid content was significantly affected by the severity of hypoxia. Furthermore, Atlantic Croakers that were exposed indirectly to mild hypoxia (lasting hours to days) exhibited greater ovarian lipid content than fish that were captured from normoxic sites. As expected, severe hypoxia reduced the ability of Atlantic Croakers to accumulate lipids in their ovaries, likely affecting reproductive output. Stock assessment models that ignore the effects of hypoxia may yield overly optimistic production estimates for hypoxia-exposed populations, particularly if environmentally invariant fecundity and growth are assumed.

The increasing occurrence of hypoxia (dissolved oxygen [DO] concentration ≤ 2 mg/L) in coastal waters impacts food web dynamics, limits ecosystem productivity, and affects regional fisheries (Boesch et al. 2007; Diaz and Rosenberg 2008). Hypoxia also alters fish distribution and abundance by shifting biomass to non-hypoxic regions (Ludsin et al. 2009; Keller et al. 2010; Craig 2012; Buchheister et al. 2013; Craig and Bosman 2013). Depending on its timing and duration, hypoxia can generate a variety of effects on aquatic systems (Diaz and Rosenberg 2008; Seitz et al. 2009). For example, severe seasonal hypoxia in coastal systems can redirect energy from higher trophic levels (e.g., fish) to microbes, resulting in a loss of fish production and a subsequent decrease in ecosystem services (Baird et al. 2004; Diaz and Rosenberg 2008). Conversely, mild periodic hypoxia (lasting hours to days) may actually increase food availability as the fish feed opportunistically on stressed benthos (Pihl et al. 1992; Long and Seitz 2008). In between these extremes, seasonal hypoxia may lead

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to (1) alterations in the prey resources that are available to fish (Pihl et al. 1992) and (2) changes in the spatial distribution of fish (Eby and Crowder 2002; Buchheister et al. 2013; Craig and Bosman 2013).

The spatial and temporal extent of summer hypoxia in Chesapeake Bay and its tributaries has increased over the last 60 years (Kemp et al. 2005; Boesch et al. 2007). These hypoxic episodes may affect species that use estuarine habitats during summer to grow and prepare for spawning. For example, Chesapeake Bay supports a large population of Atlantic Croakers *Micropogonias undulatus* and is considered an important nursery and foraging area for the East Coast stock (ASMFC 2010). Atlantic Croakers consume primarily benthic prey in summer and use these energy sources to prepare for fall spawning (Barbieri et al. 1994; Nye et al. 2010). With the increasing occurrence and extent of summer hypoxia, critical habitat needs of Atlantic Croakers may coincide with hypoxic episodes, thereby affecting population productivity from an energetic standpoint by reducing prey availability or forcing a shift in fish spatial distribution to less-favorable habitats (e.g., suboptimal salinity or temperature ranges) that limit growth and reproduction. Furthermore, exposure to hypoxic waters for as little as 3–7 d can affect biochemical pathways and can reduce the production of important maturation hormones, potentially leading to decreased stock productivity (Rahman and Thomas 2007).

The effects of hypoxia can occur at the individual level and at the population level. At the level of the individual fish, habitat shifts in response to hypoxia may be accompanied by changes in the composition of available prey, the abundance of prey, or both, which in turn can lead to alterations in the allocation of food energy. Energy that is needed for growth or reproduction may be allocated to maintenance metabolism if hypoxia-displaced fish are unable to forage in favorable environments. This reallocation of energy resources to fish maintenance metabolism may affect population-level responses by reducing growth and reproductive potential (i.e., indirect effects). As a result, stock assessment models that ignore the effects of hypoxia may yield overly optimistic production estimates for hypoxia-exposed populations, particularly if environmentally invariant fecundity and growth are assumed.

Laboratory studies of Atlantic Croakers indicate that hypoxia serves as an endocrine disruptor, reducing the production of the yolk precursor vitellogenin as well as affecting other biochemical pathways (Wu et al. 2003; Thomas et al. 2006; Thomas and Rahman 2009, 2012). Thomas et al. (2006) corroborated their laboratory results with an examination of field-captured Atlantic Croakers and showed that the mean gonadosomatic index (GSI) and number of fully developed oocytes were significantly lower in hypoxia-exposed fish than in fish collected from normoxic environments; this finding prompted concern about hypoxia's effects on stock productivity. More recently, a reduction of at least 20% in the number of

mature eggs and sperm was observed for Atlantic Croakers collected from hypoxic sites in comparison with conspecifics that were collected from normoxic reference sites (Thomas and Rahman 2012), thus providing evidence of the sublethal effects of direct hypoxia exposure and a decrease in Atlantic Croaker reproductive potential within the Gulf of Mexico.

During summer, Chesapeake Bay tributaries exhibit a range of DO conditions that can be used to test hypotheses about the sublethal and indirect effects of hypoxia on fishes. We hypothesized that Atlantic Croakers inhabiting systems with summer hypoxia would exhibit lipid storage patterns differing from those of fish found in normoxic systems. Changes in lipid allocation among fish tissues may result either from altered energetic intake or from the disruption of biochemical pathways related to gonadal development. Altered energy intake can occur via one of three possible mechanisms: (1) energetic intake can increase due to predation on hypoxia-exposed benthos; (2) energetic intake can decrease due to competition for food resources in normoxic areas that are colonized by individuals displaced from hypoxic waters; or (3) energetic intake can decrease in hypoxic areas due to a reduction in benthic productivity or organism quality (Sturdivant et al. 2013). Through the first mechanism, fish that feed opportunistically on distressed benthic prey may either maintain or deplete their energy reserves depending on the energetic value of the consumed prey (Pihl et al. 1992). Alternatively, fish may obtain higher energy reserves if the distressed benthic prey contain sufficient protein and lipid to support fish growth and reproduction (Davis and Arnold 1997).

The second and third hypothesized mechanisms lead to changes in energy allocation between somatic and gonadal tissues because energy intake is reduced through increased competition, decreased prey availability, or decreased prey energy content. As a result, fish that experience low energy intake are hypothesized to forego the accumulation of energy reserves for spawning and may instead use stored lipids and proteins to maintain metabolism or to grow. Fish that undergo alterations in biochemical pathways as a result of hypoxia exposure may retain lipid-rich compounds in their somatic tissues because those compounds are blocked from reaching gonadal tissues (Thomas and Rahman 2009). Therefore, we expected that the ovarian lipid content in female Atlantic Croakers collected during late summer in estuaries exhibiting severe hypoxia would be lower than the ovarian lipid content in fish from estuaries with less-severe or no hypoxia. Variation in the tissue-specific lipid content of fish from hypoxic systems should be evaluated against observed patterns in fish from normoxic systems. Such comparisons are warranted because observed changes in energy storage may be ascribed to indirect effects of hypoxia as the fish undergo seasonal preparation for spawning. To test these hypotheses, we examined the tissue-specific energy content of female Atlantic Croakers and

assessed impacts on reproductive potential by comparing the responses of fish from three Chesapeake Bay tributaries (the James, York, and Rappahannock rivers) that exhibit different DO conditions during summer.

METHODS

Fish collection and processing.—Adult Atlantic Croakers were collected by using a 9.1-m bottom trawl towed from an 8.5-m research vessel; sampling protocols were from an established fish monitoring program that has been operating in Virginia tributaries consistently since 1997 (hereafter, "trawl survey"; Tuckey and Fabrizio 2013). The relative abundance and distribution of adult Atlantic Croakers in each tributary were determined from routine trawl survey observations that consisted of samples from each tributary mouth to approximately 64 km upstream near the freshwater interface. Stations that were sampled by the trawl survey included a mixture of fixed and random sites. The number of fixed stations sampled was eight in the James River, eight in the Rappahannock River, and nine in the York River. The number of random stations (stratified by depth and region) was 14 in the James River, 14 in the Rappahannock River, and 13 in the York River. Thus, a total of 22 stations were sampled in each tributary during each month. These monthly data were used to examine the relative abundance and spatial distribution of Atlantic Croakers from May to August 2011, encompassing (1) the period in which tissue samples were obtained for this study and (2) the periods prior to and during hypoxic conditions.

In each river and for each treatment period (i.e., before hypoxia [May] and during hypoxia [July]), at least 20 adult female Atlantic Croakers were retained for additional analyses. If too few Atlantic Croakers were available from the hypoxic region, which was often the case, sites immediately upstream of the hypoxic region were targeted. Sampling upstream of hypoxic regions increased the likelihood that the fish had moved through or around the hypoxic region (i.e., were not recent arrivals from nearby Chesapeake Bay). Because hypoxia does not occur in the James River, Atlantic Croakers from this system were collected from areas located at a similar distance from the river mouth as fish sampled from the York and Rappahannock rivers (Figure 1). Fish in the James River were collected on May 6 and 7 (before hypoxia was observed in the York and Rappahannock rivers) and again on July 18–20 (coinciding with the hypoxic period in the other two tributaries). In the York River, fish samples were collected on May 18, May 19, and June 3 (before hypoxia) and on July 25 and August 3 (during hypoxia). Sampling for fish in the York River continued into early June and August due to very low catches of females. Atlantic Croakers in the Rappahannock River were collected on May 13, 17, and 18 (before hypoxia) and again on July 14 (during hypoxia).
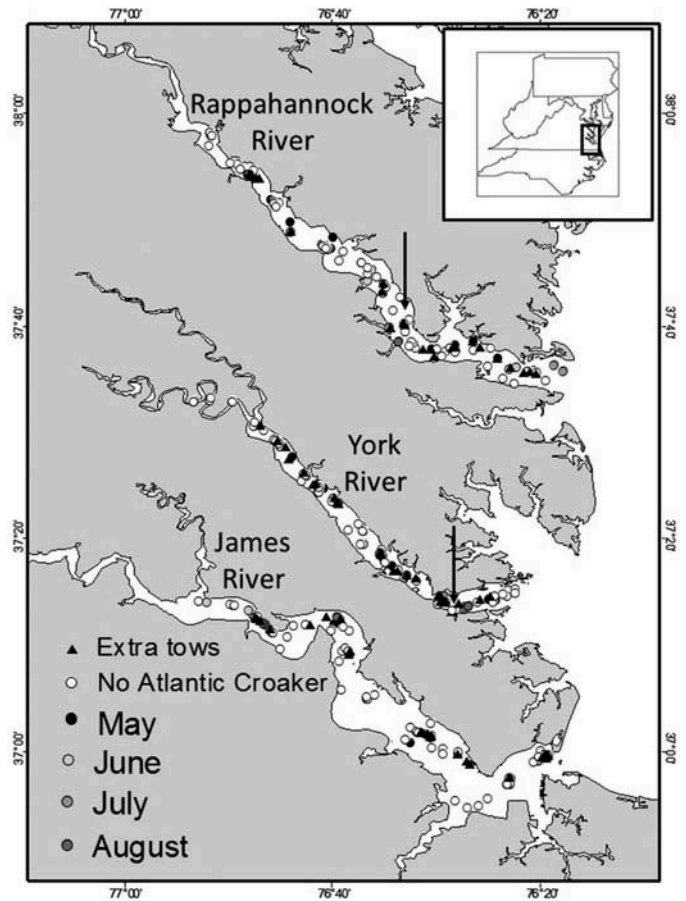

FIGURE 1. Map of the study area, showing sites that were sampled in the James, York, and Rappahannock rivers during May–August 2011. Arrows indicate the locations of water quality profiling stations (Virginia Estuarine and Coastal Observing System) in the York and Rappahannock rivers; triangles represent extra tows that were used to collect Atlantic Croakers.

Fish tissue processing.—Female Atlantic Croakers larger than 240 mm TL were retained to ensure the sampling of sexually mature fish (Barbieri et al. 1994). Upon capture, all specimens were placed on ice and returned to the laboratory for processing; TL, total weight (TW), gonad weight (GW), liver weight (LW), and somatic weight (SW; excluding stomach contents, otoliths, and ovaries) were recorded for each fish. All weights are reported as wet weights unless specified otherwise. We collected otoliths for age determination, and stomach contents were preserved and later identified to gross taxonomic groupings (e.g., crustaceans, polychaetes, and bivalves).

Because fecundity tends to increase with fish age, the age composition of Atlantic Croakers was examined to ensure that similar-aged females were compared among rivers and between treatment periods. Sagittal otoliths were sectioned and polished under transmitted light, and ages were assigned by two independent readers. When ages did not agree, a third

independent reader examined the otolith, and the final age was that agreed upon by two of the three readers.

Bottom water quality conditions (water temperature, salinity, and DO) in the James, York, and Rappahannock rivers were measured at the end of each trawl tow by using a hand-held meter (YSI Model 650 MDS). In addition, DO data for the York and Rappahannock rivers were obtained from fixed stations belonging to the Virginia Estuarine and Coastal Observing System (VECOS; www3.vims.edu/vecos/), which monitors hypoxia in these tributaries. The VECOS stations are fixed moorings that perform hourly surface-to-bottom profiles for the duration of their deployment (typically June–September) and thus provide a continuous characterization of water quality conditions throughout the summer.

Fish condition indices.—We used a combination of whole-body condition indices and tissue-specific condition metrics to evaluate the status of individual Atlantic Croakers. First, we examined fish condition using the GSI, hepatosomatic index (HSI), and relative condition factor (K_n). The GSI and HSI were calculated as

$$GSI = (GW/SW) \times 100$$

and

$$HSI = (LW/SW) \times 100.$$

Relative condition factor was calculated as

$$K_n = TW/TW',$$

where TW′ is the predicted length-specific mean weight (TW′ = $[2 \times 10^{-6}]L^{3.30}$) based on measurements from 1,666 Atlantic Croakers captured in Chesapeake Bay and its Virginia tributaries between 2010 and 2012.

Fish condition indices, which are typically calculated from the lengths and weights of individual fish, are thought to reflect nutritional state; however, such indices may be prone to length-related biases (Gerow et al. 2005). In addition, some condition indices are poorly correlated with energy density (Trudel et al. 2005; Copeland et al. 2010; Schloesser 2015). Therefore, we examined alterations in the condition and reproductive preparedness of Atlantic Croakers from each tributary by conducting proximate composition analysis of specific fish tissues (Busacker et al. 1990). Somatic tissues (whole fish minus the ovaries, otoliths, and stomach contents) were ground by using a commercial food grinder and were dried at 60°C until an asymptotic weight (i.e., dry weight) was achieved. Individual dried samples were then homogenized with a mortar and pestle and were shipped to Southern Illinois University–Carbondale for proximate composition analysis. Ovary tissue samples, which were dried first and then ground by using a mortar and pestle, could only be analyzed for lipid content due to the small amount of tissue

present, whereas somatic tissues were analyzed for full proximate composition (e.g., percentages of lipid, protein, and ash). Because carbohydrates are a minor constituent of fish tissues (typically < 0.6%; Craig et al. 1978), we followed the common practice of ignoring the carbohydrate fraction.

Statistical analyses.—Diet data were summarized by using prey frequency of occurrence and prey numeric composition in the stomach contents of Atlantic Croakers that were collected before and during hypoxia; this was done to assess potential dietary changes resulting from exposure to low DO or from hypoxia-related shifts in individual spatial distribution. Numeric composition was estimated by counting whole prey items and adding estimated proportions for partially digested prey (e.g., 0.5 for half of an organism). The use of estimated proportions likely underestimated prey consumption and is a more conservative approach than assuming that the entire organism was consumed. Stomach contents of Atlantic Croakers from each tributary were analyzed by using a cluster estimator to account for the nonindependence of fish that were captured in the same trawl tow (Buckel et al. 1999). The a priori hypothesis that diets differed between Atlantic Croakers collected before a hypoxic event and those captured during the hypoxic event was evaluated by using the adonis function in the R package "vegan" (R Development Core Team 2014; Oksanen et al. 2015). The adonis function is a nonparametric multivariate ANOVA that resembles analysis of similarity but is less sensitive to differences in dispersion (Anderson 2001).

Proximate composition results for protein, ash, and lipid were converted from percentages to grams dry weight (whole carcass minus ovaries, otoliths, and stomach contents) and are reported on a per-gram basis to account for differences in body size and the known decrease in water mass per unit protein (and ash) that occurs in larger fish (Breck 2008, 2014). Conducting analyses on a mass basis rather than a percentage basis accounts for this size-related change and produces better estimates of fish energy density (Breck 2008, 2014). Condition indices and standardized proximate composition results were analyzed with generalized linear mixed models by using Bonferroni-adjusted significance values ($\alpha = 0.05$) to account for multiple comparisons. The appropriate error structure of the model (i.e., lognormal) was determined by examining the distribution of the response variables (e.g., lipid or protein), the fit of competing models (as evaluated with Akaike's information criterion), and patterns in the residuals. Factors that were examined included the fixed effect of treatment period (i.e., before or during hypoxia exposure) nested within tributary and the random effect of tow. Tributary was treated as a fixed factor.

Additionally, to examine differences in water characteristics among tributaries, bottom water temperature and salinity were compared among tributaries and months by using general linear models that included a tributary × month interaction

term. All statistical analyses were conducted in R software or in the Statistical Analysis System version 9.3 (SAS Institute, Cary, North Carolina).

RESULTS

Water Quality in Virginia Tributaries

Virginia tributaries to Chesapeake Bay displayed monthly differences in hypoxia (Figures 2–4). The proportion of routine trawl stations (excluding supplemental stations, which were sampled to capture female Atlantic Croakers) exhibiting hypoxia during May–August 2011 was greatest in the Rappahannock River (hypoxia was observed at 28% of the trawl stations) followed by the York River (9% of trawl stations); no stations in the James River were hypoxic (Figure 2). Hypoxic episodes in the York River lasted only 1–3 d at a time and extended 3 m upward from the river bottom into the water column (mild periodic hypoxia; Figure 4). In the Rappahannock River, hypoxia persisted for most of the summer except for five 2-d periods; in this system, hypoxic waters extended 8 m from the bottom in some areas (Figure 4) and affected approximately 32 km of the river. After the passing of Hurricane Irene on August 27, 2011, the DO level in the York River remained normoxic; however, DO in the Rappahannock River fell below 2 mg/L on September 8. As expected, hypoxia was not observed in the James River during the study (lowest observed DO = 4.62 mg/L). The patterns of hypoxia observed in 2011 were consistent with annual historic patterns in these tributaries (Figure 2, inset), with (1) a greater frequency of trawl
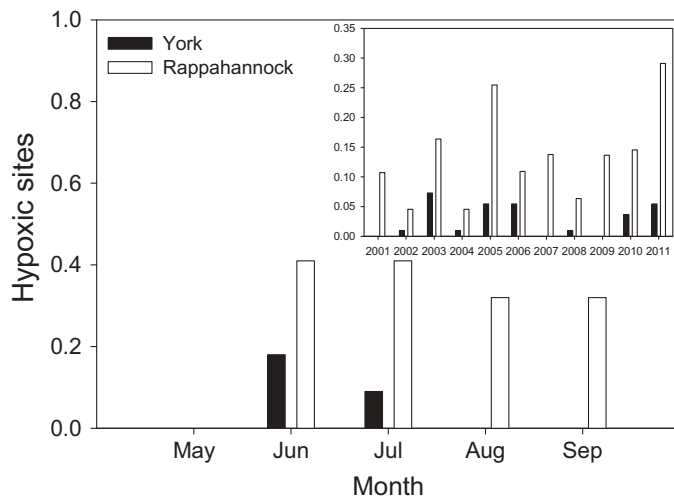

FIGURE 3. Bottom water temperature (°C), salinity (psu), and dissolved oxygen (DO; mg/L) in the James, York, and Rappahannock rivers during May–August 2011 (dots = median; boxes = quartiles; whiskers = 5th and 95th percentiles; open circles = outliers [extreme values]). The horizontal dashed line in the lower panel indicates hypoxic conditions (DO ≤ 2.0 mg/L).

stations exhibiting hypoxia in the Rappahannock River than in the York River and (2) no hypoxia in the James River during the last decade.

Bottom water temperatures averaged across all sites were significantly different among tributaries ($F_2 = 25.9$, $P < 0.001$) and among months ($F_3 = 380.4$, $P < 0.001$); the warmest mean temperatures were observed during July and August (Figure 3). Mean bottom water temperature was lowest in the James River (mean = 24.5°C, SD = 3.98), followed by the York River (mean = 24.9°C, SD = 4.17); the warmest water was observed in the Rappahannock River (mean = 25.4°C, SD = 3.60). Salinity from all sampled sites also differed significantly among tributaries ($F_2 = 13.0$, $P < 0.0001$): the York River had the highest salinity (mean = 13.5 psu, SD = 5.45), the James River displayed intermediate salinity (mean = 11.6 psu, SD = 7.27), and the Rappahannock River exhibited the lowest salinity (mean = 10.0 psu, SD = 3.83).

FIGURE 2. Proportion of trawl survey stations that exhibited hypoxia (dissolved oxygen concentration ≤ 2.0 mg/L) in the York and Rappahannock rivers during each month in 2011 and during each year (inset: 2001–2011). Data for the James River are not shown because hypoxic conditions were not observed there.
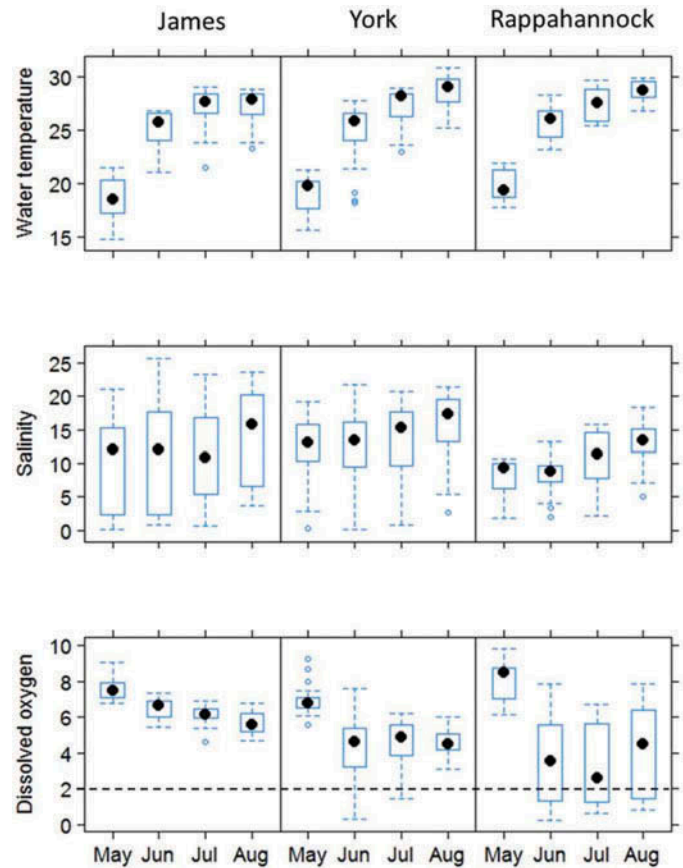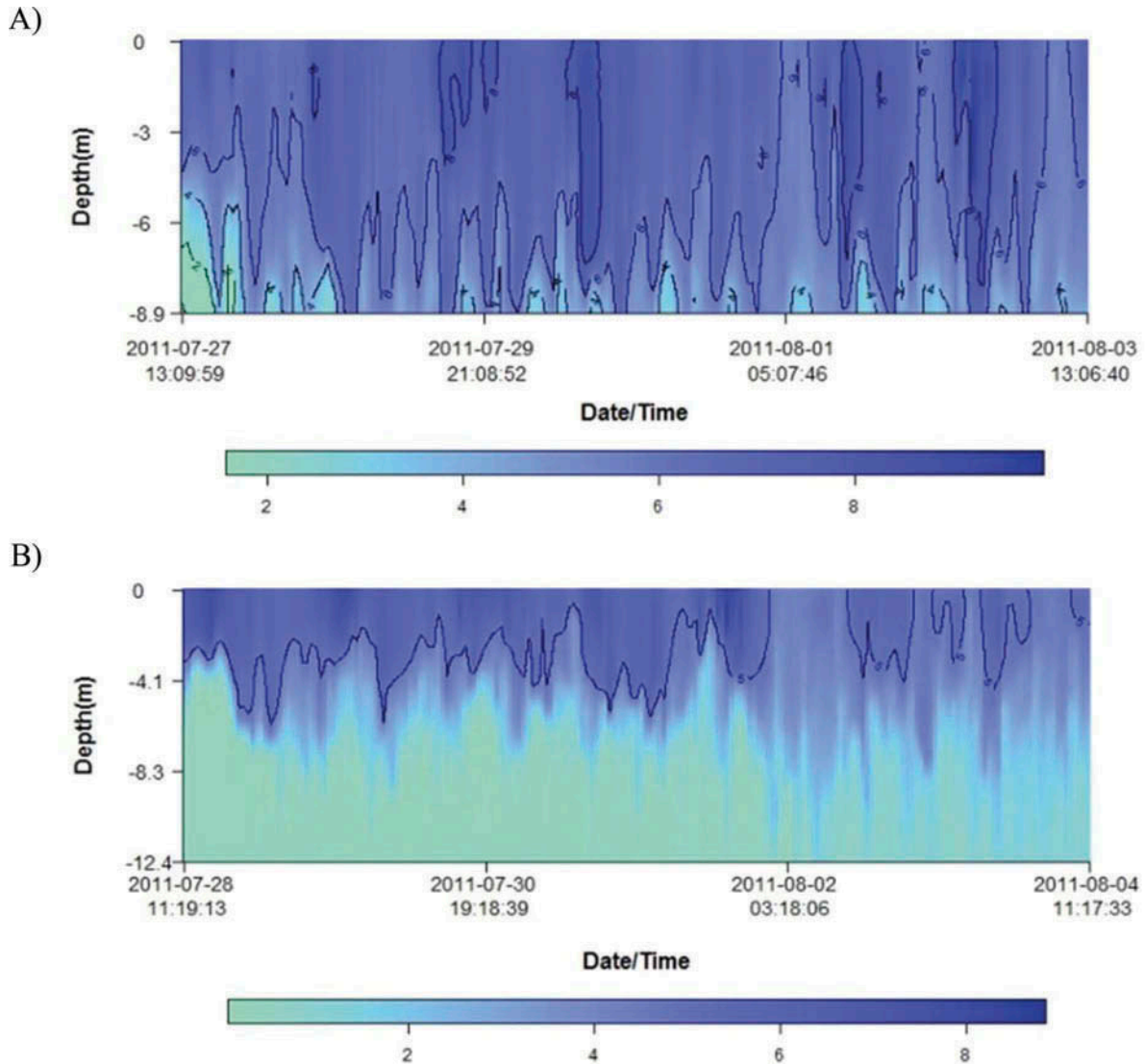
FIGURE 4. An example of weekly measurements of bottom dissolved oxygen concentration (mg/L) in (**A**) the York River (Virginia Estuarine and Coastal Observing System [VECOS] Station YRK004.26) from July 27 to August 3, 2011; and (**B**) the Rappahannock River (VECOS Station RPP021.36) from July 28 to August 4, 2011. Similar data for the York River during June 1–October 19, 2011, and for the Rappahannock River during May 26–October 10, 2011, are available from VECOS (web2.vims.edu/vecos/).

Atlantic Croaker Distribution

Most of the adult Atlantic Croakers (>240 mm TL) obtained from routine trawl survey sampling were collected in the York River (N = 216 adults), followed by the James River (N = 140 adults) and Rappahannock River (N = 110 adults). In the York River, we collected 7.5% of the Atlantic Croakers from hypoxic waters, whereas 22.7% of the fish collected in the Rappahannock River were from hypoxic waters (Figure 5). Mean water temperature at the Atlantic Croaker capture sites differed by less than 0.2°C; the average mean water temperature was 22.8°C (SD = 4.14) in the York River, 23.0°C (SD = 4.51) in the James River, and 22.9°C

(SD = 3.81) in the Rappahannock River. Average water depths were also similar, with positive catches of Atlantic Croakers occurring in water depths of 7 m (SD = 3.22) in the York River, 9.6 m (SD = 5.39) in the Rappahannock River, and 10.4 m (SD = 4.49) in the James River. Salinities where adult Atlantic Croakers were found varied among rivers and were lowest in the Rappahannock River (mean salinity = 9.5 psu, SD = 2.66), highest in the York River (15.2 psu, SD = 3.80), and intermediate in the James River (12.5 psu, SD = 7.86). Atlantic Croakers were distributed throughout the sampled portions of each river in May 2011 (Figure 6). Over the course of the study period and as hypoxia
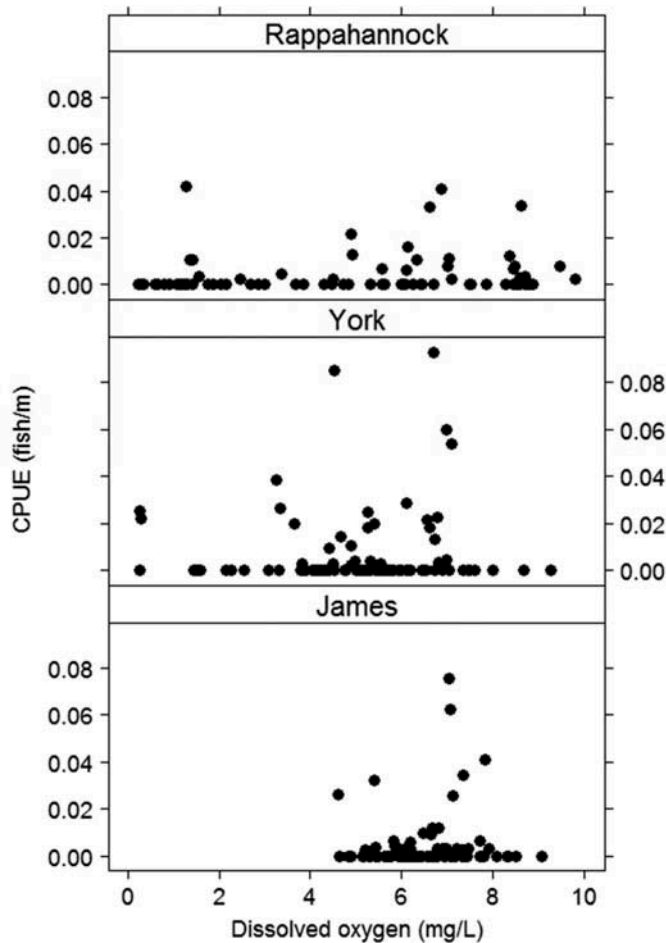
FIGURE 5. Adult Atlantic Croaker CPUE (fish/m) versus dissolved oxygen concentration (mg/L) in the Rappahannock, York, and James rivers during May–August 2011.
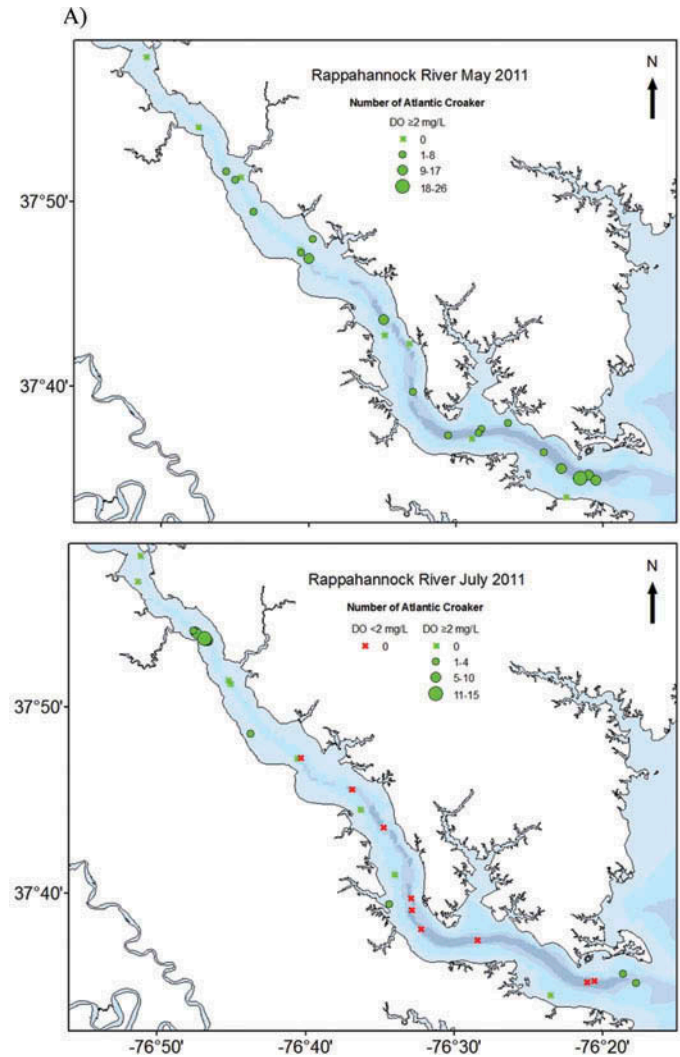

FIGURE 6. Capture locations of adult Atlantic Croakers in relation to dissolved oxygen (DO) concentration (mg/L) in the (A) Rappahannock River, (B) York River, and (C) James River during May and July 2011.

developed in the York and Rappahannock rivers, adults were found upriver in the Rappahannock River and remained in those areas through July (Figure 6). In the York River, some adults were found upriver, whereas others occupied the lower river in the vicinity of hypoxic waters (Figure 6). The distribution of Atlantic Croakers in the James River remained similar over the study period, as fish were located throughout the river (Figure 6).

Atlantic Croaker Characteristics

We retained 124 female Atlantic Croakers from 52 tows for use in proximate composition, condition, and diet analyses (Table 1). Fish collected in the James and York rivers before hypoxia were similar in size to those collected during hypoxia, whereas fish captured from the Rappahannock River during hypoxia were significantly larger than those captured before hypoxia (mean TL difference = 25 mm; $F = 5.24$, $P < 0.001$).

The mean age of Atlantic Croakers did not significantly differ among tributaries or between time periods ($F = 2.22$, $P = 0.06$), indicating that the fish were randomly mixed among rivers. Therefore, we did not consider age in any additional statistical models.

Diet

Diet indices (i.e., frequency of occurrence and numeric composition) for female Atlantic Croakers showed that prey consumption patterns before hypoxia were similar to the patterns demonstrated during hypoxia. Diets consisted of 13 prey categories and an additional category encompassing unidentifiable material (UID; Figure 7). In May, diets of fish in the James River were dominated by polychaetes, amphipods, bivalves, shrimp, and isopods; similar prey items were consumed during July
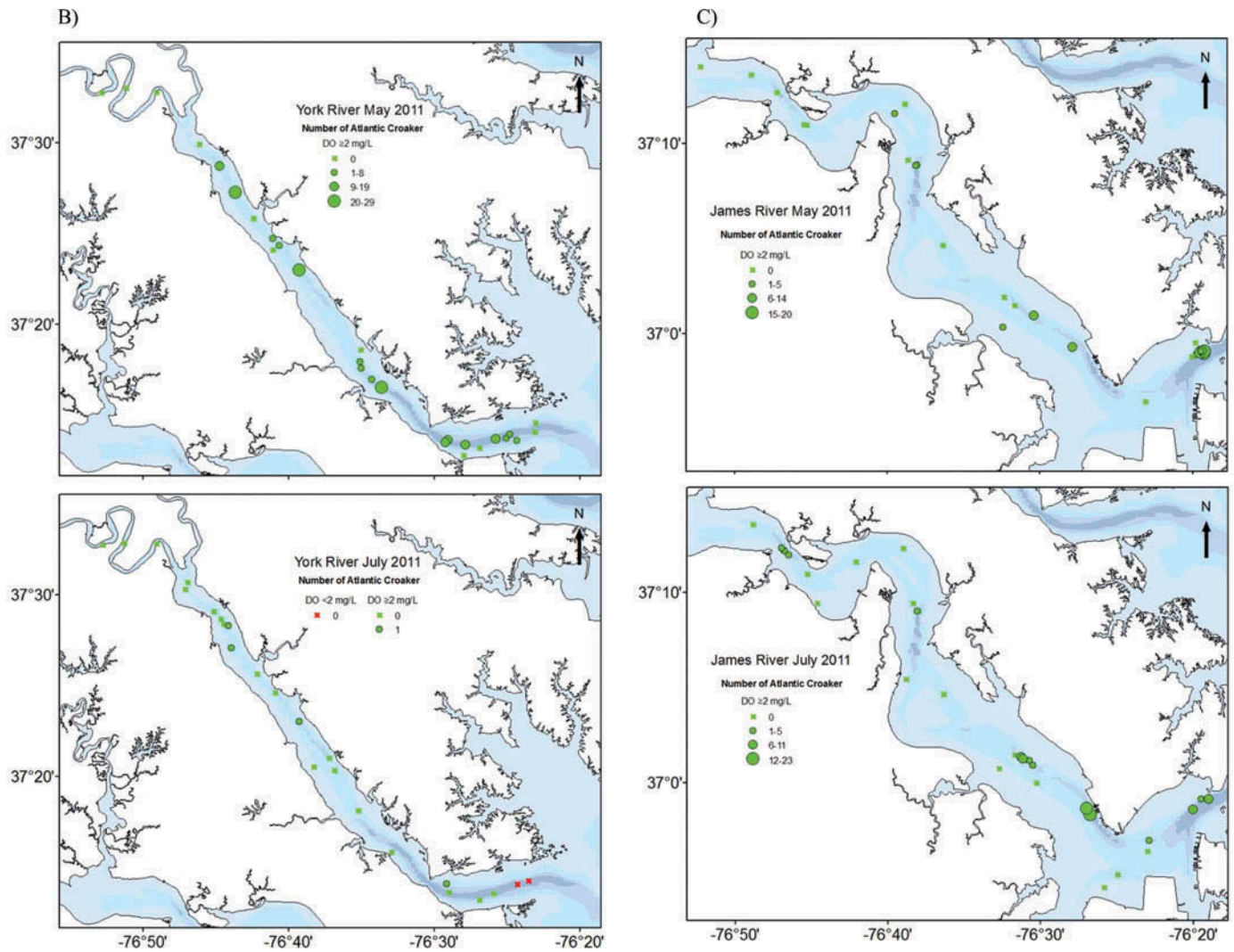
FIGURE 6. Continued.

(numeric contribution: $F = 1.685$, $P = 0.131$). Diets in the York River were also similar between normoxic and hypoxic periods (numeric contribution: $F = 0.970$, $P = 0.455$). During May in the York River, diets were dominated by polychaetes, shrimp, bivalves, amphipods, sea squirts, and crabs. During the hypoxic period (July), sea squirts increased in importance as prey items (Figure 7). The data set for the Rappahannock River failed the test for homogeneity of group dispersion ($F = 5.03$, $P = 0.039$; betadisper function in vegan); as a result, no statistical tests were conducted to compare diets between pre-hypoxic and hypoxic periods. However, qualitative inspection revealed that diets in the Rappahannock River during May consisted of bivalves, polychaetes, shrimp, and sea squirts, whereas the July diets included more amphipods and midges, along with polychaetes, shrimp, and bivalves. Comparisons of prey frequency of occurrence (data not shown) between the two periods yielded results similar to those based on numeric composition: diets did not differ between normoxic and hypoxic periods in the James and York rivers, and homogeneity of group dispersion was not met for the Rappahannock River.

Condition Metrics

Mean K_n of Atlantic Croakers was variable and tended to show a slight, nonsignificant increase between normoxic and hypoxic periods within rivers (Table 1; Figure 8).

The GSIs for fish collected in the tributaries at the start of the study averaged 0.6%; the overall mean GSI increased significantly during July ($F_{1,\ 79} = 25.73$, $P < 0.01$). The mean GSI of York River fish increased significantly ($t_{79} = 4.83$, Bonferroni-adjusted $P < 0.01$), whereas the mean GSIs of fish in the James River ($t_{79} = 2.33$, Bonferroni-adjusted $P = 0.33$) and the Rappahannock River ($t_{79} = 1.51$, $P = 1.0$) did not change.

TABLE 1. Number of female Atlantic Croakers that were collected from each Chesapeake Bay tributary during each treatment period in 2011; and mean (SE in parentheses) age, TL, weight, hepatosomatic index (HSI), gonadosomatic index (GSI), relative condition factor (K_n), and proximate composition (percent dry weight [DW]) of fish collected before and during hypoxic conditions in the York and Rappahannock rivers. Because hypoxia was not observed in the James River, data from that river are used as a reference for normoxic conditions. Lowercase letters indicate significant differences in a given factor before versus during hypoxia.

Factor	James River		York River		Rappahannock River	
	Before	During	Before	During	Before	During
N	20	23	20	18	19	24
Age (years)	5 (1.2)	4 (1.1)	4 (1.0)	4 (1.0)	4 (0.8)	4 (1.3)
TL (mm)	255 (13.6) z	255 (8.3) z	263 (27.1) z	264 (18.4) z	256 (11.9) z	280 (32.4) y
Weight (g)	208 (36.2)	215 (32.7)	239 (105.4)	229 (49.5)	222 (36.7)	325 (138.7)
HSI (%)	2.1 (0.2) z	1.6 (0.1) z	1.8 (0.1) z	1.8 (0.1) z	2.5 (0.1) y	1.8 (0.1) z
GSI (%)	0.6 (0.0) z	1 (0.2) z	0.6 (0.0) z	1.7 (0.3) y	0.5 (0.0) z	0.8 (0.1) z
K_n	97 (1.3)	101 (2.1)	97 (1.7)	96 (2.1)	103 (1.2)	106 (1.5)
Proximate composition (% DW)						
Ovarian lipid	6 (0.6) z	9 (1.3) z	6 (0.5) z	14 (1.4) y	6 (0.2) z	7 (0.9) z
Ovarian water	82 (0.2)	81 (0.9)	82 (0.2)	76 (1.6)	81 (0.3)	80 (0.7)
Somatic lipid	17 (0.8) z	31 (2.1) y	26 (1.2) z	33 (1.8) z	26 (1.2) z	35 (1.1) z
Somatic water	76 (0.3)	74 (0.6)	75 (0.4)	72 (0.6)	74 (0.6)	69 (0.7)
Somatic protein	57 (0.9) z	48 (1.9) y	56 (1.3) z	51 (2.4) y	53 (1.1) z	48 (1.3) y
Somatic ash	20 (0.7) z	20 (0.9) z	19 (0.9) z	17 (0.9) z	17 (0.8) z	13 (0.5) y

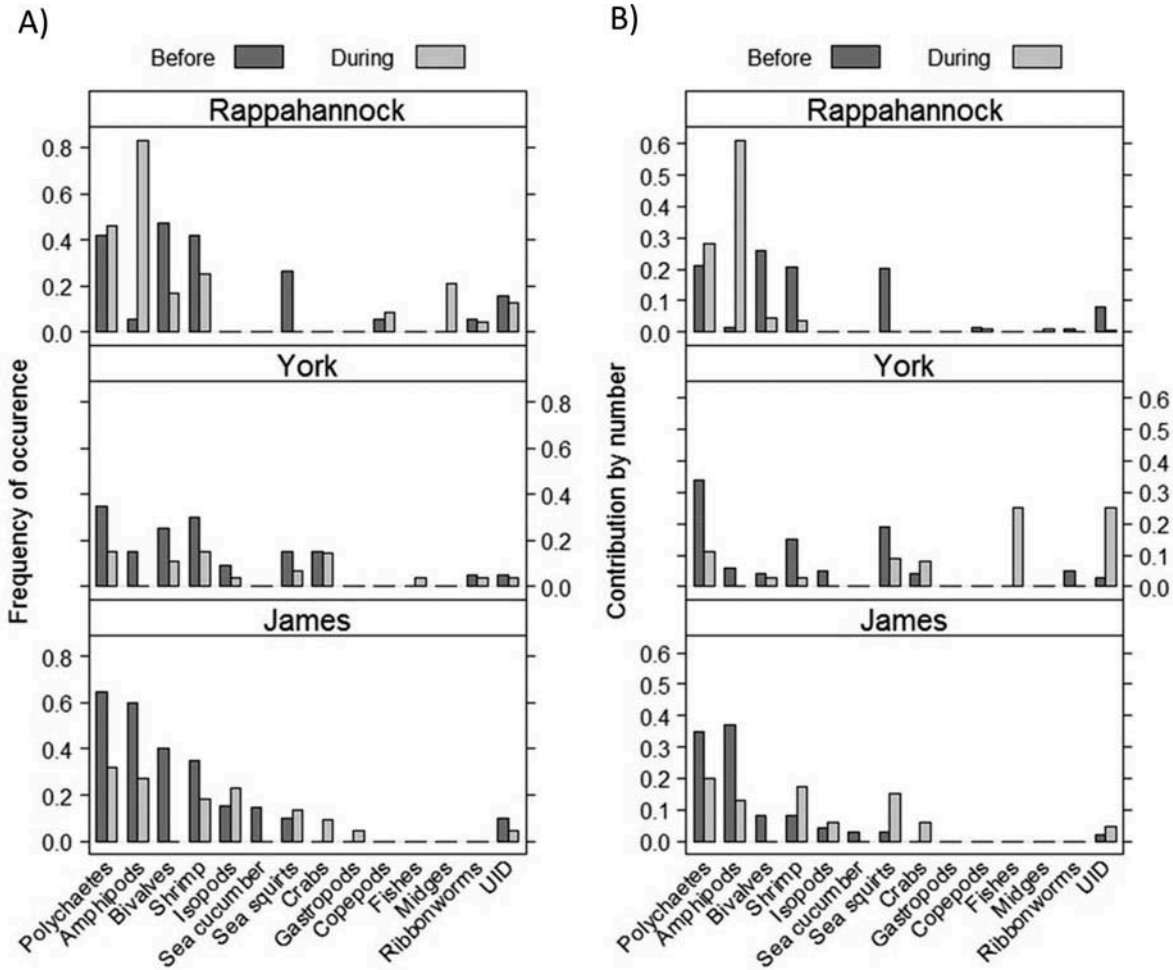FIGURE 7. Diet composition for adult female Atlantic Croakers captured in the James, York, and Rappahannock rivers before and during hypoxia in 2011: (A) prey frequency of occurrence and (B) numeric contribution of prey taxa (UID = unidentifiable material). Because hypoxia was not observed in the James River, data from that river are used as a reference for diets consumed under normoxic conditions.
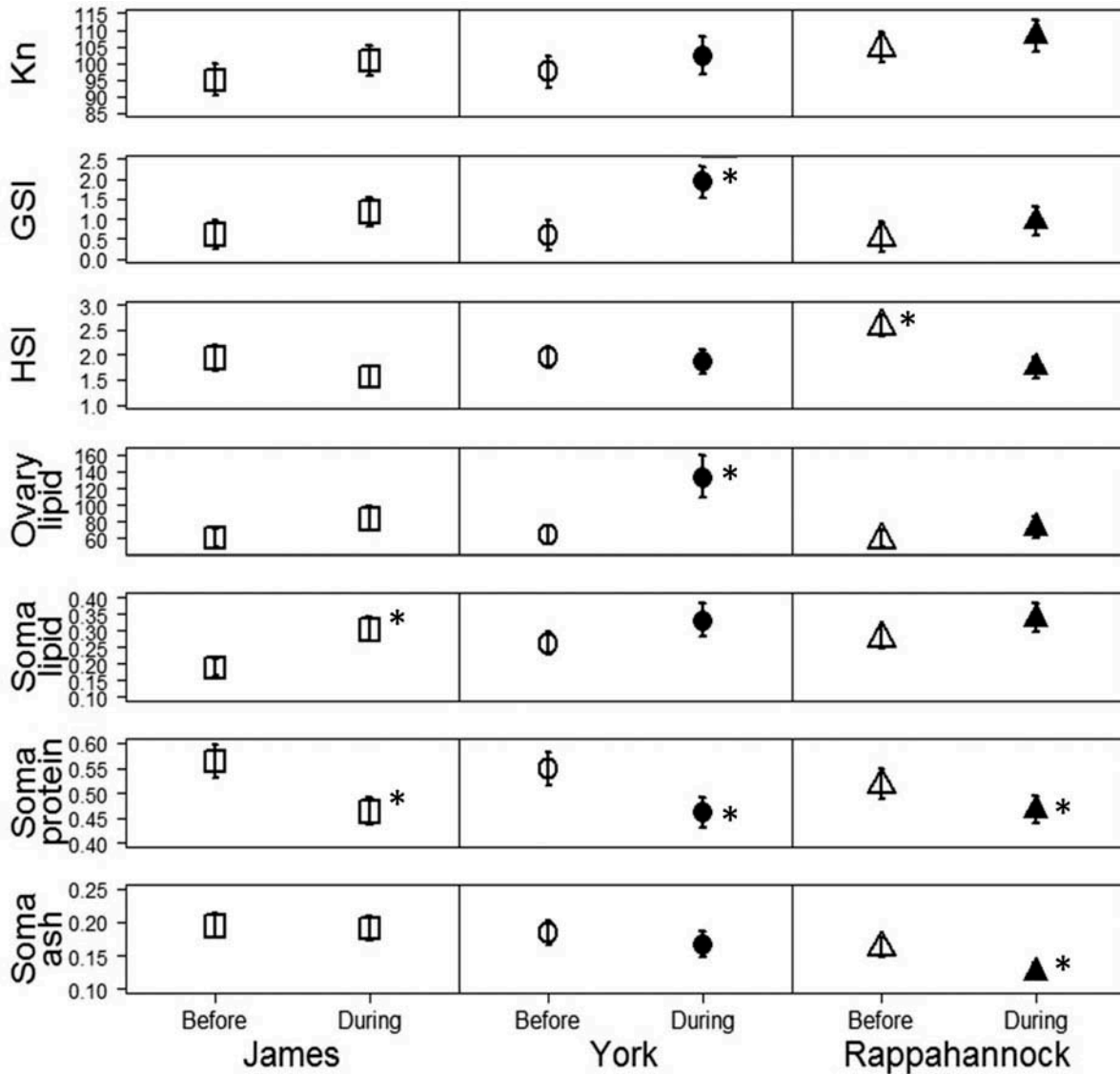
FIGURE 8. Mean (±95% confidence interval) relative condition factor (Kn), gonadosomatic index (GSI; %), hepatosomatic index (HSI; %), ovarian lipid content (mg/g), somatic lipid content (g/g), somatic protein content (g/g), and somatic ash content (g/g) of adult female Atlantic Croakers collected from the James, York, and Rappahannock rivers before (open symbols) and during (filled symbols) hypoxic conditions in 2011. Because hypoxia was not observed in the James River, data from that river are used as a reference for normoxic conditions. Asterisks indicate metrics that were significantly different between the pre-hypoxic period and the hypoxic period.

Prior to the onset of hypoxia, Atlantic Croakers collected from the Rappahannock River had significantly higher mean HSIs than fish from the James and York rivers ($F_{4, 76} = 6.85$, $P < 0.01$; Figure 8). During hypoxia, the mean HSI decreased significantly ($F_{1, 76} = 22.86$, $P < 0.01$) for fish in the Rappahannock River (28% decrease; $t_{76} = 5.54$, Bonferroni-adjusted $P < 0.01$), whereas the mean HSIs of York River and James River fish did not change significantly relative to pre-hypoxia values (Bonferroni-adjusted $P > 0.05$).

Proximate Composition

Atlantic Croaker ovaries showed significant increases in lipid accumulation from May to July ($F_{1, 79} = 33.08$, $P < 0.01$; Table 1; Figure 8). We detected a significant positive correlation between GSI and ovarian lipid content (Spearman's rho = 0.70, $N = 119$, $P < 0.004$), indicating that higher GSIs reflected increases in lipid content. The temporal increase in mean ovarian lipid content differed among tributaries ($F_{4, 79} = 6.40$, $P < 0.001$); a significant increase was observed only for the York River ($t_{79} = 5.70$, Bonferroni-

adjusted $P < 0.01$). Before the onset of hypoxic conditions in the York and Rappahannock rivers, fish had a similar mean ovarian lipid content of 60 mg/g (Table 1; Figure 8). Under normoxic conditions in the James River, mean ovarian lipid content increased by 39% between periods. The mean ovarian lipid content for York River females was 50% greater than that observed for females from the normoxic James River. The mean ovarian lipid content in Rappahannock River fish during hypoxia was 24% greater than the lipid content measured for the pre-hypoxia period but was significantly less than that observed in York River females during hypoxia ($F = 29.45$, $P < 0.01$; Table 1; Figure 8).

Mean lipid content of somatic tissues from adult Atlantic Croakers increased significantly in the James River from May to July ($t_{77} = 5.36$, Bonferroni-adjusted $P < 0.01$; Table 1; Figure 8). In May, somatic lipid content differed among the tributaries such that James River fish contained significantly less lipid than fish from the York and Rappahannock rivers ($F_{1, 77} = 32.46$, $P < 0.01$; Table 1; Figure 8). During July, the mean somatic lipid content of adults collected in all three tributaries ranged from 0.31 to 0.35 g/g.

Mean protein content of somatic tissues from Atlantic Croakers decreased significantly ($F_{1, 77} = 40.15$, $P < 0.01$) during the study, but there was no significant effect of tributary ($F_{4, 77} = 1.15$, $P = 0.34$; Table 1; Figure 8). A significant decrease ($t_{77} = 11.78$, Bonferroni-adjusted $P < 0.01$) in the ash content of tissues was observed in fish from the Rappahannock River (Figure 8).

DISCUSSION

We observed that lipid allocation to ovaries in adult female Atlantic Croakers was consistent with predictions resulting from laboratory-based studies of the sublethal and indirect effects of hypoxia. The hypothesized outcome—that female Atlantic Croakers from a system characterized by severe hypoxia (Rappahannock River) would exhibit lower mean ovarian lipid content than females from a mildly hypoxic system (York River)—was supported. We also observed that within the Rappahannock River, catches of Atlantic Croakers were greater from normoxic habitats than from hypoxic habitats, indicating that habitat shifts can occur even for a relatively hypoxia-tolerant species (Bell and Eggleston 2005; Craig and Crowder 2005).

Throughout the study period, DO in the James River remained above 4 mg/L—a critical level below which fish CPUE and fish species diversity in Chesapeake Bay decline (Buchheister et al. 2013). Therefore, DO levels in the James River can be considered normal conditions that support Atlantic Croaker feeding and preparation for spawning; as such, the James River estuary serves as a suitable system with which to make comparisons.

Development and persistence of hypoxia in Virginia tributaries of Chesapeake Bay are associated with variation in longitudinal salinity gradients (e.g., gravitational circulation; Kuo and Neilson 1987). The salinity difference between the river mouth and 30 km upriver is greatest in the James River, smallest in the Rappahannock River, and intermediate in the York River. Gravitational circulation resulting from the salinity gradient replenishes oxygen in bottom waters of the James River but is insufficient for consistent oxygen replenishment in the York River or Rappahannock River (Kuo and Neilson 1987). In the York River, hypoxia is further regulated by (1) the spring–neap tidal cycle that affects water column stratification and (2) local weather conditions that contribute to stratification or vertical mixing (Haas 1977). Kuo and Neilson (1987) found that observed differences in DO level between the York and Rappahannock rivers were attributable to the entrainment of lower-quality (i.e., hypoxic) water from main-stem Chesapeake Bay into the Rappahannock River. Thus, during years when significant hypoxia exists in Chesapeake Bay, lower DO can be expected in the Rappahannock River; indeed, we have observed this phenomenon over multiple years (our unpublished data). Similarly, results from the Chesapeake Bay Water Quality Monitoring Program (Chesapeake Bay Program 2013) corroborated this expectation: the highest summer volume of hypoxic water since 1985 was observed during 2011 (prior to the arrival of Hurricane Irene), matching the patterns of hypoxia measured within the Rappahannock River during our study. Given the unique hydrology and physical forcing that underlie the DO conditions in each of these tributary systems, impacts on demersal fish habitat within the York and Rappahannock rivers are likely to persist beyond the specific year of our study.

Data on bottom DO at our trawl survey stations and at the VECOS monitoring stations suggest that benthic productivity in the Rappahannock River was likely reduced during 2011 due to the duration and extent of hypoxia. Previous studies in the Rappahannock River have found significant reductions in benthic community biomass at the same DO levels we observed (Seitz et al. 2009; Sturdivant et al. 2013). The intensity and duration of hypoxia directly affect benthic productivity (Baird et al. 2004; Long and Seitz 2009; Sturdivant et al. 2013), and the single best predictor of benthic organism density and diversity in Chesapeake Bay is DO (by depth), which outperforms other important habitat characteristics such as sediment type and salinity (Seitz et al. 2009). Hypoxia lasting only a few hours to a few days increases the transfer of trophic energy to higher trophic levels (e.g., fish), whereas longer-lasting hypoxia results in the loss of benthic production and a shift toward microbial production (Baird et al. 2004; Powers et al. 2005; Long and Seitz 2009). The 2011 hypoxic event that persisted for more than 10 weeks in the Rappahannock River likely disrupted the system's normal benthopelagic food web. Recovery of benthic communities after hypoxic events commonly occurs due to the recruitment of invertebrates from

adjacent normoxic habitats; however, severe hypoxic events may result in only partial recovery, with subsequent long-term implications for fish production (Wu 2002; Long and Seitz 2009). Additional research is needed to evaluate the energy content of benthic prey taxa that recolonize habitats after hypoxia; this would help to determine how changes in food quality and quantity could affect fish growth and reproduction over the long term.

In our study, indirect exposure to hypoxia apparently had no effect on the relative condition of Atlantic Croakers, consistent with results from a previous study in which even direct exposure to hypoxia did not affect Atlantic Croaker condition (Thomas and Rahman 2009). Endocrine control of gonadal recrudescence in Atlantic Croakers is highly susceptible to hypoxia exposure, whereas other biochemical pathways involved in physiological processes such as metabolism and growth are regulated by additional genes (e.g., hypoxia-inducible factors) that facilitate adaptation to low-DO conditions (Thomas and Rahman 2009, 2012). These hypoxia-regulated genes maintain the overall condition of Atlantic Croakers, likely increasing survival but at a potential cost to reproductive output.

The liver, which produces vitellogenin, is important for lipid storage and processing in teleosts and is a probable site of hypoxia interference (Thomas and Rahman 2009). Changes in mean HSI observed in this study indicated a negative effect of hypoxia on HSI; Atlantic Croakers collected in the Rappahannock River showed a significant reduction in HSI between the pre-hypoxia period and the hypoxic period, whereas fish from the James and York rivers maintained similar HSIs between May and July. If vitellogenesis was disrupted in Rappahannock River fish, then energy stores may have been shifted to other processes (e.g., maintenance metabolism or growth). Other fish condition indices showed no changes that would suggest better or worse condition of Rappahannock River fish relative to James River or York River fish, but we did observe a significant increase in the size of Rappahannock River fish between May and July (no difference in mean age), suggesting that lipid stores in these fish were used for growth. However, movement of larger Atlantic Croakers into the Rappahannock River from Chesapeake Bay cannot be ruled out as a possible reason for the greater fish size observed in that river during hypoxia. An acoustic tagging study would be required to definitively evaluate Atlantic Croaker movement between Chesapeake Bay and the upper Rappahannock River areas where we collected Atlantic Croakers during July.

The observed increase in GSIs of Atlantic Croakers from the three tributaries demonstrated that preparation for fall spawning was underway. The GSIs of Atlantic Croakers collected from the normoxic James River served as a reference for the expected maturation processes in this species at this time of year, assuming that no other deleterious conditions (e.g., contaminants) affected ovary development in fish within this tributary. Patterns in mean GSI of fish from the York and Rappahannock rivers supported the hypotheses that (1) severe hypoxia has a negative effect on reproductive potential, as was previously observed during studies in the Gulf of Mexico (Thomas et al. 2006; Thomas and Rahman 2009); and (2) mild periodic hypoxia has a positive effect on reproductive preparedness. We expected that Atlantic Croakers from the James River would exhibit a higher gonadal lipid content than Rappahannock River fish, yet their lipid levels were similar. Our assumption that ovaries in James River females were not affected by other potential endocrine disruptors might not be valid, as the James River watershed is more developed than the Rappahannock River and thus may contain endocrine-disrupting compounds. However, to our knowledge, an environmental survey of potential endocrine-disrupting compounds has not been conducted for the James River or any other river in Virginia. Such a survey appears to be warranted and could provide additional insight on our observed results.

Female Atlantic Croakers from the three tributaries experienced an increase in mean lipid content of somatic and ovarian tissues, but the magnitude of the increase differed depending on the river and the tissue examined. Somatic lipid content significantly increased in fish from the James River, whereas mean ovarian lipid content significantly increased in females from the York River, more than doubling during mild periodic hypoxia. In the Rappahannock River, which exhibited severe seasonal hypoxia, lipid stores in ovarian and somatic tissues did not significantly differ between the pre-hypoxia period and the hypoxic period. We hypothesize that a reduction in ovarian lipid content will lead to a decrease in reproductive output for those females (Brooks et al. 1997). For example, hypoxia-exposed Atlantic Croakers from laboratory experiments and field-based collections have demonstrated a significantly reduced reproductive capacity (e.g., lower GSI, fecundity, and reproductive hormone levels) in comparison with fish obtained from normoxic conditions (Thomas et al. 2006; Thomas and Rahman 2009, 2012). Even exposure to hypoxia for short periods (i.e., hours) can have lasting reproductive effects due to the alteration of biochemical pathways associated with sex hormones (Cheek et al. 2009).

Habitat effects are a possible source of variability in lipid accumulation and storage in Atlantic Croakers. Although we found significant differences in bottom water temperature among tributaries, the magnitude of differences among Atlantic Croaker capture sites (0.2°C) is unlikely to be biologically meaningful for a eurythermic species (Miglarese et al. 1982). It is possible that the low-salinity habitat occupied by Atlantic Croakers in the Rappahannock River increased the metabolic demand for osmoregulation, thereby reducing the amount of energy available for reproduction. However, the greatest salinity difference between tributaries was 6 psu, which may not be large enough to affect metabolic processes in Atlantic Croakers since they

have been found in salinities ranging from 3 to 34 psu (Miglarese et al. 1982).

The low ovarian lipid levels observed in female Atlantic Croakers from the Rappahannock River may be due to direct or indirect causes. Direct exposure to hypoxia could cause endocrine disruption and result in lower lipid content of ovarian tissues. Alternatively, low lipid levels in ovaries could arise from dietary changes associated with reduced abundance of prey items, decreased energy content of prey, a change in diet composition, or a combination of such factors. Results from our diet study were inconclusive, as we observed only subtle changes in diet composition after hypoxia developed. Because Atlantic Croakers are opportunistic generalist feeders (Nye et al. 2010), they may be able to compensate for changes in habitat quality and potential shifts in prey availability, prey type, or energy content better than species with specialized diets. However, studies that have been conducted in the same Chesapeake Bay tributaries and in other systems have documented hypoxia-mediated changes in Atlantic Croaker diets (Powers et al. 2005; Long and Seitz 2008). Hypoxia-induced changes in benthic communities include a shift from longer-lived species (e.g., bivalves) to opportunistic species, such as polychaetes (Long and Seitz 2009). We did observe a decrease in the consumption of bivalves, but this occurred in all three tributaries and therefore was probably associated with predation pressure rather than hypoxia. Our study of Atlantic Croaker diets would have benefited from a larger sample size, as we used a cluster estimator to investigate diet composition; as a result, the number of fish examined ($n = 124$ individuals) was reduced to 47 cluster samples (24 clusters before hypoxia; 23 clusters during hypoxia), a sample size that may have been insufficient to characterize differences in diet composition for a generalist feeder. Increasing the frequency of sample collection for diet analysis, perhaps to a weekly basis, would also have aided in determining the potential effects of hypoxia on foraging success. This would be particularly informative for the York River, where hypoxia occurred on neap tidal cycles rather than continuously throughout the study period. Furthermore, examination of available prey densities and measurement of individual prey energy content would help to conclusively determine whether increased lipid content of Atlantic Croaker tissues was due to increased predation on energetically rich foods. It should be noted that stomach content analyses characterize the recent diet but do not account for prior feeding and energy intake that would have been assimilated into specific tissues, which is what we measured via proximate analysis. Therefore, proximate composition provides a cumulative perspective on the diet and may be a better indicator of feeding success.

Chesapeake Bay Atlantic Croakers residing in areas characterized by prolonged seasonal hypoxia may be incapable of achieving their full reproductive potential. In the Chesapeake Bay region, hypoxia is typically observed from July to September, and spawning typically occurs from August to November (Barbieri et al. 1994). Because oocyte development in Atlantic Croakers requires 10 weeks to complete (Thomas et al. 2006), recovery from sublethal or indirect effects of hypoxia may be possible only for individuals that spawn at the very end of the season. Some fish may reproduce early if sufficient energy can be allocated to egg production during May and June (prior to the onset of hypoxia); however, the early spawning fraction of the population is believed to be small (Barbieri et al. 1994). Thus, although Atlantic Croakers may feed advantageously on stressed prey, these fish may suffer reductions in reproductive output due to the inability to complete gonadal recrudescence. Instead, energy may be stored in other tissues (i.e., soma) and used for maintenance metabolism, growth, and movement. Hypoxic episodes within Chesapeake Bay are expected to increase in association with global climate change (Boesch et al. 2007; Najjar et al. 2010), resulting in (1) a greater frequency of fish exposure to hypoxia or its indirect effects and (2) subsequent potential changes in the reproductive output of Atlantic Croakers.

Ecosystem-based approaches that quantify environmental and climatic effects on fish stocks can provide managers with more realistic predictions of fish production (Keyl and Wolff 2008). In particular, stock–recruitment relationships can be improved by including environmental factors that affect stock productivity. For instance, environmental conditions that are encountered prior to spawning can affect the reproductive performance of European Anchovy *Engraulis encrasicolus* (Pecquerie et al. 2009). Comprehensive models that include variability in reproduction (e.g., weight- and age-specific fecundity; Marteinsdottir and Begg 2002; Spencer and Dorn 2013) are currently under development, and additional refinements should be considered. The clear effects of hypoxia on Atlantic Croaker reproduction should be quantified in assessment models, as bias in the number (or weight) of spawning females may lead to overestimation of the spawning stock's reproductive potential. This is particularly important given the expected increase in the number of hypoxia-affected coastal zones and the greater likelihood of increased interactions between demersal fishes and impaired habitats (Diaz and Rosenberg 2008). As a result, egg production will be closely aligned with the energy density of adult females but will not necessarily be predictable through estimates of adult female biomass (Marshall et al. 1999). However, energy density may also have limitations in predicting egg production since direct exposure to hypoxia limits vitellogenin production at the cellular level. Furthermore, our study suggests that the indirect effects of hypoxia may also impair reproductive development. To this end, investigations into biomarkers that can be used to evaluate fish exposure to hypoxia are underway (Murphy et al. 2009; Thomas and Rahman 2009). To fully characterize the effects of hypoxia (including direct and indirect exposure) on

reproduction, comprehensive studies of spawning-capable fish are needed. Given new insights, stock assessment scientists can evaluate various harvest strategies and can include environmental-change-associated uncertainty in fecundity estimates, thus providing robust estimates of recruitment under changing climate and nutrient loading regimes.

ACKNOWLEDGMENTS

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