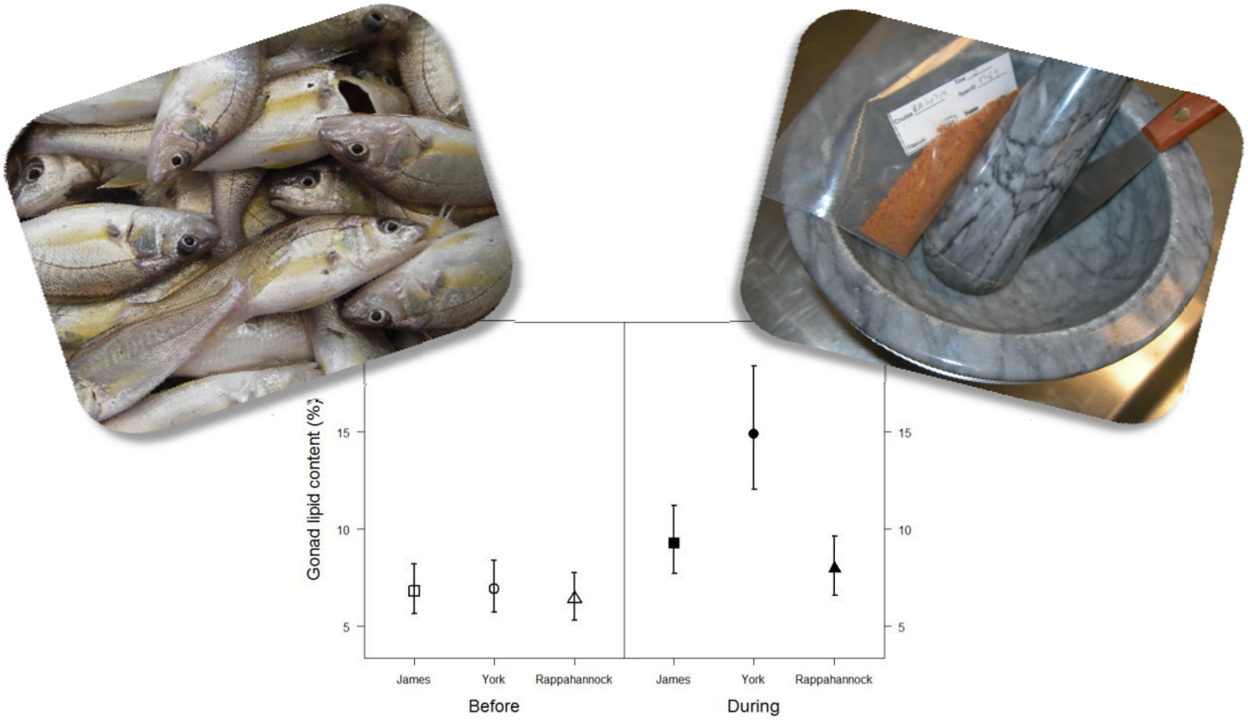
Establishing Field-based Evidence for the Effects of Hypoxia on the Reproductive Capacity of Chesapeake Bay Fishes

Final Report to:

NOAA Chesapeake Bay Office



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Executive summary

Low dissolved oxygen in Chesapeake Bay tributaries is likely to increase with climate change, affecting water quality and aquatic resources. To understand and manage aquatic resources effectively, the relationship between the potential stressor and the resource must be understood and quantified at both the individual and the population level. In this study, we examined the effects of hypoxia on fish energy content to assess impacts on lipid accumulation in different tissues by comparing the response of fish from three Chesapeake Bay tributaries that exhibit different levels of dissolved oxygen. We used adult female Atlantic Croaker collected before and during hypoxia exposure from three tributaries that exhibit a range of dissolved oxygen content in summer from no hypoxia (James River), to mild periodic hypoxia associated with the spring-neap tidal cycle (York River), to seasonal hypoxia lasting most of the summer (Rappahannock River). Significant changes in tissue lipid content were observed in all three tributaries, and the effects of hypoxia were tributary specific varying with the severity of hypoxia. Variability in dissolved oxygen conditions observed in this study shows that constant water-quality monitoring is necessary to properly characterize the degree of hypoxia and enable prediction of subsequent effects on demersal, bottom-feeding fishes. Finally, our results from examining a model species (Atlantic Croaker) may be applied to other species, such as Striped Bass or Summer Flounder, to understand and quantify the effects of hypoxia on fish condition and reproduction.

Introduction

Ecosystem-based Fisheries Management (EBFM) in Chesapeake Bay promotes a shift in management tactics requiring field-based evidence of theoretical linkages among species and between species and their environment. How do stressors identified through EBFM approaches affect fishery resources and how can we quantify those effects to incorporate them into assessment models? To answer these fundamental questions, the relationship between the potential stressor and the resource must be understood and quantified at both the individual and the population level. Whereas the effects of fishery removals and variable recruitment on population dynamics and productivity of fishery resources can be assessed and modeled, the indirect effects of environmental perturbations on the processes that govern productivity are less discernable. For example, is fish reproductive capacity altered by low dissolved oxygen in Chesapeake Bay tributaries? Laboratory investigations suggest this is the case, but inferences from laboratory studies are restricted to confined, controlled conditions, which may be mitigated by behavioral adaptations of fish in the wild (e.g., avoidance of hypoxic waters; Bell and Eggleston 2005; Ludsin et al. 2009). A field-based approach to understanding the indirect effects of environmental perturbations is required.

Evidence from studies in Chesapeake Bay and other areas show that the timing and duration of hypoxia has varying impacts on aquatic systems (Kemp et al. 2005; Diaz and Rosenberg 2008; Seitz et al. 2009). For example, severe seasonal hypoxia (Diaz and Rosenberg 2008) in coastal systems can result in a shift of energy from higher trophic levels (e.g., fish) to microbes and thus, a decrease in ecosystem services (Baird et al.

2004; Diaz and Rosenberg 2008). Conversely, mild periodic hypoxia may actually increase food availability to fish as fish feed opportunistically on stressed benthos (Pihl et al. 1992). Between these extremes (e.g., mild seasonal hypoxia), low dissolved oxygen may lead to alterations in the prey resources available to fish (Pihl et al. 1992), as well as to changes in the spatial distribution of fish (Eby and Crowder 2002; Bell and Eggleston 2005).

At the individual fish level, habitat shifts in response to hypoxia may be accompanied by changes in available prey , which may, in turn, lead to alterations in the allocation of food energy: energy that is needed for growth or reproduction may be allocated instead to maintenance metabolism if hypoxia-displaced fish are unable to forage effectively. This reallocation of energy resources to fish maintenance metabolism may affect population-level responses by reducing growth and reproductive potential. As a result, some stock assessment models (e.g., egg-per-recruit models) may yield overly optimistic recruitment estimates for populations exposed to hypoxia, if models assume environmentally-invariant fecundity. Alternatively, if prey resources are stressed and fish can feed advantageously on those stressed organisms, then energy intake may actually increase under hypoxic conditions (Pihl et al. 1992). Hypoxia-induced effects on fisheries resources, such as Striped Bass (*Morone saxatilis*), Summer Flounder (*Paralichthys dentatus*), and Atlantic Croaker (*Micropogonias undulatus*), have direct implications for resource management, particularly since these fishes forage in estuaries during summer months when hypoxia is commonly present.

In this study, we examined the effects of hypoxia on fish energy content to assess potential impacts on reproductive potential by comparing the response of fish from three Chesapeake Bay tributaries that exhibit different levels of dissolved oxygen. We used adult female Atlantic Croaker collected before and during hypoxia exposure from three tributaries that exhibit a range of dissolved oxygen content in summer from no hypoxia (James River), to mild periodic hypoxia associated with the spring-neap tidal cycle (York River), to seasonal hypoxia lasting most of the summer (Rappahannock River). We assumed that the effects of hypoxia would affect river system food web dynamics in each tributary or result in a shift in the distribution of fishes such that the lipid content of Atlantic Croaker collected from within a tributary would be representative of local conditions. We expected to observe a reduction in gonad energy content in the hypoxic Rappahannock River and an increase in gonad energy content in the mildly hypoxic York River relative to the energy content found in gonads of Atlantic Croaker collected in the normoxic James River. We also assumed that accumulation of energy in Atlantic Croaker from the James River would represent the typical seasonal changes associated with gonad development in preparation for fall spawning. This investigation serves as a proof-of-concept study, using Atlantic Croaker as a model species, to establish a direct relationship between low dissolved oxygen and effects on population productivity by evaluating hypoxia-induced changes in fish energy content (measured by proximate composition analysis), diet composition, and potential reproductive capacity.

Methods

Field collections

Female Atlantic Croaker were collected using a 9.1-m bottom trawl towed from an 8.5-m research vessel applying sampling protocols from an established fish sampling program that has been operating in Virginia tributaries consistently since 1988 (Tuckey and Fabrizio 2013). Tows were conducted in the York and Rappahannock rivers (Figure 1) in the vicinity of known areas of hypoxia to assess effects of exposure at two treatment levels: before (May) and during (July, September and October) hypoxia. In each river and for each treatment level, numerous five-minute tows were completed in the vicinity of hypoxia to capture 20 adult female Atlantic Croaker. If an insufficient number of Atlantic Croaker were present in the hypoxic region, sampling shifted upriver until the targeted sampling size was obtained. Atlantic Croaker from the James River were collected from regions with similar salinity as those found in the York and Rappahannock rivers to serve as a comparison because hypoxia is not known to occur in the James River.

Fish condition assessment

Alterations to growth or reproductive preparedness of fish resulting from exposure to hypoxia can be investigated by examining general condition, nutritional state, or energy content of fish tissues (i.e., proximate composition analysis;

Busacker et al. 1990). Fish condition indices, typically calculated from length and weight of individual fish, are thought to reflect nutritional state, but such indices are prone to length-related biases (Gerow et al. 2005). In addition, condition indices may be poorly correlated with energy density (Trudel et al. 2005; Copeland et al. 2010). Other non-invasive methods to assess fish condition, such as the use of microwave technology (Crossin and Hinch 2005) or ultrasound (Probert and Shannon 2001) remain exploratory or unproven. We chose to use proximate composition analysis to understand changes in energy allocation to growth and reproduction resulting from exposure to hypoxia. The proximate composition of fish tissue (% lipid, % protein, % water) reflects nutritional state and correlates highly with energy density (Trudel et al. 2005). On average, fish muscle tissue consists of 16-21% protein, 0.2-25% lipid, 1.2-1.5 % ash, and 66-81% water, with carbohydrates typically comprising an insignificant proportion (<0.5 %) of the total dry weight of fish (Huss 1995). The proportions of these constituents vary greatly among species, and for a given species, variations due to age, sex, season, and environmental factors have been observed (Huss 1995). In addition, the lipid content of ovaries depends on the stage of development (Biesiot et al. 1994) and may be used to gauge reproductive preparedness. Thus, proximate composition should allow us to discern differences in energy content due to summertime hypoxia as female Atlantic Croaker forage and store energy in preparation for fall spawning.

Samples processed for proximate composition analysis were from fish greater than 240 mm TL to ensure sexual maturity (Barbieri et al. 1994). Samples retained for proximate composition were processed for gonadal and somatic weights (wet weight, excluding stomach contents and gonads). We also removed otoliths for age determination and retained stomach contents for diet analysis. Stomach contents were identified to gross taxonomic groupings (e.g., crustaceans, polychaetes, bivalves). Water-quality data (water temperature, salinity, and dissolved oxygen) for the York and Rappahannock rivers were obtained from the Virginia Estuarine & Coastal Observing System (VECOS; http://www3.vims.edu/vecos/), which performs continuous water quality monitoring in the vicinity of hypoxic areas, as well as from the endpoint of each tow using a portable water quality meter.

Tissue samples (soma and gonads) of whole Atlantic Croaker were ground using a commercial food grinder and dried at 60 °C until an asymptotic weight (dry weight) was achieved. Individual dried samples were then homogenized using a mortar and pestle for shipment to Southern Illinois University, Carbondale for proximate composition analysis. Gonadal tissue samples could only be analyzed for lipid content due to the small amount of tissue present, whereas somatic tissues were analyzed for full proximate composition (i.e., % lipid, % protein, % ash).

Statistical analysis

Data from stomach contents were analyzed separately for each river using a cluster estimator to account for the non-independence of Atlantic Croaker captured in the same trawl sample (Buckel et al. 1999). Diet data were summarized using frequency of occurrence and percent occurrence by number before and during hypoxia in each river.

Relative abundance estimates of Atlantic Croaker were calculated based on a stratified-random sampling design using a delta-lognormal model to estimate stratum means. All Atlantic Croaker greater than 240 mm TL were considered adults and all Atlantic Croaker under a specific size range during specified months were considered young-of-the-year (e.g., <135 mm TL in May, <160 in June, <180 in July, and <220 in August). Estimates of abundance were calculated separately for each river and are weighted averages accounting for the different spatial extent of each system.

Proximate composition results were analyzed with general linear mixed models (SAS proc MIXED; Littell et al. 2006); the appropriate error structure of the model was determined by examining the distribution of the response variables (e.g., % lipid, % protein), as well as residuals from the linear model. Model factors included the fixed effects of treatment (i.e., before and during hypoxia exposure), river, and the treatment-river interaction with hypoxia nested within river, as well as the random effect of tow.

Results

Atlantic Croaker collections

We collected 124 female Atlantic Croaker from 62 tows for proximate composition and diet analysis in 2011 and an additional 26 female Atlantic Croaker from 17 tows in 2012 (Table 1). We targeted our sampling around hypoxic regions, but were forced to shift our sampling upriver to collect sufficient numbers of adult females. We chose to move our sampling effort upriver to ensure that the Atlantic Croaker that were collected had been residing in the river and had a greater potential of being directly exposed to hypoxia or conditions resulting from hypoxia and were not recent arrivals from Chesapeake Bay.

Hypoxia

The Rappahannock and York rivers exhibited varying intensities of hypoxia that occurred in the deeper portions of each river. Onset of hypoxia began in late May in the Rappahannock River (Figure 2) and late June in the York River during 2011 (Figure 3). As in previous years, hypoxia was not observed in the James River during the study period. In 2011, the York River exhibited numerous mild periodic hypoxic events of short duration that extended upwards into the water column approximately 3 m from the river bottom and were located primarily in the lower river (Figure 7). Hypoxia in the Rappahannock River persisted during most of the summer, rose 8 m from the bottom, and extended approximately 32 km along the river axis (Figure 6).

In 2012, different patterns of bottom hypoxia were observed in the York River with persistent hypoxia in the York River from 16 to 19 July and from 8 to 16 August 2012 with the remainder of the year more similar to that observed in 2011 (Figures 3 and 4). Hypoxia in the Rappahannock River during 2012 also differed from that observed in 2011 with several periods of normoxic conditions during summer months (e.g., 27 June–9 July, 2 August–6 August, 21 August–3 September; Figures 2 and 5). There was no evidence of hypoxia in the James River during 2012, although DO measurements of 2–3 mg/L were observed (Figure 8).

Atlantic Croaker diets

Subtle changes in diet composition were found before and during hypoxia exposure in this study, and fewer Atlantic Croaker stomachs contained prey items after the onset of hypoxia. All of the stomachs examined from the James and Rappahannock rivers and 90% of those from the York River contained food prior to hypoxia. After the onset of hypoxia in the York and Rappahannock rivers, 78% of Atlantic Croaker in the James River, 96% from the Rappahannock River, and 39% of those from the York River contained prey. Diets of female Atlantic Croaker consisted of 13 different prey categories and an additional category consisting of unidentifiable material (Figures 9 and 10).

Diet composition of prey items (by number) in the James River was dominated by polychaetes, amphipods, bivalves, and shrimp early in the sampling period (Figure 9). In the York River, diets of Atlantic Croaker were dominated by three prey categories before hypoxia: polychaetes, shrimp, and sea squirts (Figure 9). During hypoxia, Atlantic Croaker diets in the York River were dominated by five prey categories including polychaetes, sea squirts, crabs, fishes, and unidentifiable material. Diets in the Rappahannock River prior to the onset of hypoxia consisted of bivalves, polychaetes, shrimp, and sea squirts (Figure 9). During hypoxia, Rappahannock River Atlantic Croaker diets consisted primarily of amphipods and polychaetes. During the periods when hypoxia was observed in the York and Rappahannock rivers, Atlantic Croaker diets in the James River contained fewer numbers of polychaetes, amphipods, and bivalves compared with samples before hypoxia occurred.

Assessment of prey items in Atlantic Croaker diets through frequency of occurrence provides results similar to those observed through contribution by number. Polychaetes, amphipods, bivalves, shrimp, isopods, and sea squirts were among the top prey items found in stomachs of Atlantic Croaker (Figure 10). Five out of nine (56%) prey categories in the Rappahannock River and six out of ten (60%) prey categories in the James River showed higher frequency of occurrence of prey items before hypoxia, whereas eight out of ten (80%) prey categories showed higher frequencies before hypoxia in the York River.

Relative abundance and distribution

Indices of abundance for juvenile and adult Atlantic Croaker from the VIMS Juvenile Fish Trawl Survey are greater in the James River compared with the York and Rappahannock rivers between 1988 and 2012 (Figure 11). Recruitment of young-of-the-year Atlantic Croaker was low in 2011 and increased in 2012 in all three systems, whereas adult Atlantic Croaker indices remained stable during the same period (Figure 11).

The distribution of adult Atlantic Croaker in May encompassed a major portion of each river during 2011 and 2012 (Figures 6 - 8). Over the course of the study period as hypoxia developed, Atlantic Croaker moved up-river in the Rappahannock River and remained there until July (Figure 6). In the York River, Atlantic Croaker also moved up-river, though some remained in portions of the lower river in the vicinity of hypoxic waters (Figure 7). The distribution of Atlantic Croaker in the James River was split between upper-river sites and those closer to the mouth (Figure 8).

Proximate composition

Proximate composition analysis of gonadal tissues showed significant variation in lipid accumulation before and after hypoxic episodes. The lipid content of Atlantic Croaker gonads increased seasonally in all three rivers, but the proportional increase varied depending on river. Prior to the onset of hypoxia, female Atlantic Croaker from all three rivers had similar lipid content of gonadal tissues (Tables 1 and 2). Gonad percent lipid content in the James River increased significantly as summer progressed demonstrating the expected accumulation of lipids in preparation for fall spawning (F= 5.65, P=0.019; Figure 12). In the York River, gonad percent lipid increased significantly after hypoxia exposure and this increase was greater than that observed in Atlantic Croaker from the James River (Table 2; F=24.16 , P<0.0001; Figure 12). In the Rappahannock River, the percent lipid content of gonads after hypoxia exposure also increased, but was not significant (F=2.01, P=0.1591; Figure 12).

Somatic tissue percent lipid content varied at the outset of the study with James River Atlantic Croaker containing signficantly fewer lipids than those found in fish from the York and Rappahannock rivers (Table 2; Figure 13). After hypoxia exposure in the York and Rappahannock rivers, fish from all three rivers showed similar lipid content of somatic tissues; this pattern implies that somatic lipid content increased significantly in fish from the James River, but less so in fish from the other tributaries. There was a significant effect of hypoxia on lipid content of somatic tissues with lower levels observed before hypoxia (Table 2).

There was no evidence of effects of hypoxia on protein content (%) of somatic tissues. Relative to levels observed prior to hypoxia, the percent protein decreased significantly (F = 48.35, $P<0.001$) in all three rivers resulting in similar protein content, approximately 45%, at the end of the study (Table 1; Figure 14).

Discussion

Significant changes in tissue lipid content were observed in all three tributaries, and the effects of hypoxia were tributary specific varying with the severity of hypoxia. Our expected outcome that Atlantic Croaker from the York River would have more lipids in gonad tissues compared with the James and Rappahannock rivers was partially supported (2011 only). Changes in gonad lipid content observed during 2012 differed from those observed in 2011 demonstrating interannual variation at the tributary level. It should be noted that smaller sample sizes collected during 2012 limits our conclusions, but patterns in DO levels and lipid accumulation in Atlantic Croaker tissues were consistent among years. The results of this study also demonstrate that Atlantic Croaker served as a suitable model species since they are relatively abundant and can provide sufficient samples for proximate composition analysis. However, targeting only female Atlantic Croaker resulted in the collection of twice as many fish as needed for the study since Atlantic Croaker are not sexually dimorphic and cannot be identified by external inspection. Other species (e.g., Striped Bass, Summer Flounder, and Spot) that are known to feed within Chesapeake Bay in preparation for spawning may show similar effects from hypoxic conditions and warrant investigation.

Hypoxia in Chesapeake Bay tributaries

Dissolved oxygen in the James River remained above hypoxic levels throughout the study period, although there were periods of low DO (2-3 mg/L) in 2012. It has been shown that in the Chesapeake Bay, fish catch-per-unit-effort and fish species diversity decrease at DO levels below 4 mg/L (Buchheister et al. 2013). Thus, DO levels in the James River may still affect fish distribution and abundance, yet not be considered hypoxic by the strict definition we used (DO <2 mg/L).

During 2011, the York River experienced mild periodic hypoxia and the Rappahannock River experienced severe seasonal hypoxia (Diaz and Rosenberg 2008). Periods of hypoxia in the York River during 2011 were likely regulated by local weather conditions and the spring-neap tidal cycle that affects water column stratification (Haas 1977). In the Rappahannock River, bottom waters remained hypoxic for most of the summer except for five, 2-day periods and immediately following Hurricane Irene. After the passing of Hurricane Irene on 27 August 2011, DO in the York River remained normoxic from surface to bottom, however DO in the Rappahannock River remained above 2 mg/L only until 8 September when DO returned to hypoxic levels. Thus the influence of weather events on hypoxia can cause substantial changes to DO levels complicating interpretation of field-based studies (Baustian et al. 2009).

A switch in DO characteristics occurred in 2012 with the York River experiencing longer periods of hypoxia with the occurrence of a week-long period of low DO. In contrast, the Rappahannock River exhibited periods of hypoxia with shorter duration in 2012 with intermittent periods of normoxia that persisted for days to weeks. Development of hypoxia in Virginia tributaries has been investigated previously and it was determined that variability in longitudinal salinity gradients (e.g., gravitational circulation) explained the distribution of oxygen in Virginia tributaries (Kuo and Neilson 1987). The James River has the strongest salinity difference between the river mouth and 30 km upriver, the Rappahannock the weakest difference, and the York River is intermediate. Gravitational circulation resulting from the salinity gradient replenishes oxygen in bottom waters of the James River, but is insufficient to do so in the York or Rappahannock rivers (Kuo and Neilson 1987). Kuo and Neilson (1987) concluded that observed differences in DO levels between the York and Rappahannock rivers was the result of entrainment of lower quality (i.e., hypoxic) water into the Rappahannock River from Chesapeake Bay. Thus years when significant hypoxia exists in Chesapeake Bay, lower DO can be expected in the Rappahannock River. Results from Maryland Department of Natural Resources’ Chesapeake Bay Water Monitoring Program (Chesapeake Bay Program 2013) corroborate this hypothesis as the highest summer volume of hypoxic water (since 1985) was observed in 2011 (prior to the arrival of Hurricane Irene) and the second smallest volume of hypoxic water was observed in 2012, matching patterns of hypoxia measured within the Rappahannock River during this study.

The intensity of hypoxia is important because it directly affects benthic productivity (Baird et al. 2004; Long and Seitz 2009). The single best predictor for benthic organism density and diversity in Chesapeake Bay was DO, outweighing other habitat characteristics such as sediment type, salinity, and depth (Seitz et al. 2009). Hypoxia lasting only a few hours to a few days increases trophic transfer of energy to higher trophic levels (e.g., fishes), whereas hypoxia that lasts longer results in the loss of benthic production and a shift towards microbial production (Baird et al. 2004, Powers et al. 2005; Long and Seitz 2009). Recovery of benthic communities following hypoxic events commonly occurs due to high recruitment of invertebrates from nearby normoxic habitats, but severely hypoxic years can result in only partial recovery, having long-term implications for fish production (Wu 2002; Long and Seitz 2009).

Hypoxia and tissue lipid content

Atlantic Croaker from all three tributaries experienced an increase in lipid content of somatic and gonadal tissues each year, however the magnitude of the increase varied. During years of mild periodic hypoxia (i.e., York River 2011, Rappahannock River 2012), ovary lipid content of Atlantic Croaker from the York and Rappahannock rivers increased by more than 125% compared with gonad tissue samples prior to the onset of hypoxia. When mild seasonal hypoxia was present (i.e., York River 2012, Rappahannock River 2011), Atlantic Croaker from the York and Rappahannock rivers increased lipid stores in somatic and ovarian tissues, but at reduced levels. This reduction in lipid accumulation results from either a dietary shift through a change in abundance of prey items, a shift in diet composition, increases in maintenance metabolism, changes in distribution of Atlantic Croaker from preferred habitats, or a combination of these factors. Results from our diet study were inconclusive though we did note subtle changes in diet composition and stomach fullness after hypoxia versus before hypoxia. Our diet analysis would benefit from a larger sample size, examination of available prey items, and determination of energy content of individual prey items to conclusively determine if increased lipid content of Atlantic Croaker tissues is due to opportunistic predation during periods of periodic hypoxia.

Proximate composition analysis also revealed annual differences in lipid content of Atlantic Croaker tissues from the James River even though the James River remained normoxic throughout the study period. Because we observed similar abundance indices of adult Atlantic Croaker in the James River during 2011 and 2012, it is unlikely that intraspecific competition for food resources is the source of the observed differences in tissue lipid content. Other potential sources include changes in Atlantic Croaker distribution, salinity, water temperature, or variability in food-web energy content that is independent of DO levels. We found that diets of Atlantic Croaker from the James River had more (by number) polychaetes, amphipods, and bivalves at the start of the study (May 2011) compared with periods later in the summer suggestive of food limitation or prey switching. Regardless of the cause, interannual differences in lipid content (of gonads and somatic tissues) were significant. It is also possible that DO values of 2-3 mg/L are sufficiently low to stress some benthic invertebrates and allow

Atlantic Croaker to feed opportunistically, resulting in the significant increase in gonad lipid content in 2012 relative to values observed in 2011. It should be noted, however that our sample size from the James River in 2012 was small (n=11).

Implications and significance

Increasing occurrence of hypoxia in coastal waters due to eutrophication affects food web dynamics, limiting ecosystem productivity and impacting regional fisheries (Boesch et al. 2007; Diaz and Rosenberg 2008). Hypoxia has been shown to affect fish distribution and catch-per-unit-effort by shifting biomass to regions outside of those affected by hypoxia (Keller et al. 2010; Buchheister et al. 2013; Craig and Bosman 2013). Distributional shifts in fish biomass may increase competition for resources due to increased density of conspecifics or others predators, or may reduce energetic intake due to lower prey quality in newly inhabited areas. In our study of Atlantic Croaker, a species that is relatively hypoxia tolerant (Bell and Eggleston 2005), we also observed a shift in fish distribution above and below the region of hypoxia indicating that distributional shifts occur at large (e.g., northern Gulf of Mexico) and small (<32 km) spatial scales. We found that the degree of hypoxia affected the ability of Atlantic Croaker to accumulate lipids in gonadal tissues. The lipid content of fish tissue was related to the severity of hypoxia with an increase in lipid content of somatic and gonad tissues under near-hypoxic to mildly hypoxic conditions. Under more severe levels of hypoxia, somatic and gonadal tissues accumulated fewer lipids, likely reducing reproductive output that year.

The effects of hypoxia on reproductive capacity has been studied extensively in Atlantic Croaker from the Gulf of Mexico and it has been determined 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; Thomas and Rahman 2012). Atlantic Croaker exposed to hypoxia through laboratory experiments and field-based collections had significantly reduced reproductive capacity (e.g., reduced GSI's, fecundity, and reproductive hormone levels) than those obtained from normoxic conditions (Thomas and Rahman 2006). Even exposure to short periods of hypoxia can have lasting effects on reproduction because of the disruption in vitellogenin production (Cheek et al. 2009). So, although fish may feed advantageously on stressed prey, they may suffer reduced reproductive output due to the inability to complete gonadal recrudescence. That raises the question: What happens to excess lipids accumulated under mild periodic hypoxia when biochemical pathways are blocked by the presence of hypoxia? According to Thomas et al. (2006), Atlantic Croaker oocyte development requires 10 weeks to complete. Since Atlantic Croaker spawn from August to November in the Chesapeake Bay region (Barbieri et al. 1994), recovery from hypoxia exposure may only be possible for those individuals spawning at the very end of the season as hypoxia typically breaks down during September/October. This would mean that the majority of Atlantic Croaker that reside in hypoxic VA tributaries during the summer may be incapable of achieving their full reproductive potential (i.e., a portion of the population may not complete oocyte development).

Variability in DO conditions observed in this study shows that constant water-quality monitoring is necessary to properly characterize the degree of hypoxia and enable prediction of subsequent effects on demersal, bottom-feeding fishes. Conditions necessary for the development of hypoxia in riverine estuaries are basin-specific and are related to the longitudinal salinity gradient, local nutrient loads, as well as proximity to neighboring hypoxic regions (e.g., Chesapeake Bay). Local weather and episodic storm events can also have a dramatic impact on water-quality characteristics by returning systems to normoxic conditions that may last days to weeks. For our study, we used data from the VECOS water profilers that were in depths of 9.1 m (York River) and 12.2 m (Rappahannock River), whereas our water-quality readings performed at the end of each tow typically were from greater depths. For example, during June and July 2011, 12 out of 18 of the hypoxic stations in the Rappahannock River were at 15.2 m or greater. In addition, all of the York River stations that were hypoxic in those months were in depths greater than 9.1 m. Therefore, data from the VECOS water profilers may not be indicative of what is happening in the deepest portions of the Rappahannock and York rivers.

Expected increases in water temperature and sea-level rise and anticipated increases in hypoxic episodes in Chesapeake Bay due to global climate change (Boesch et al. 2007; Najjar et al. 2010) challenge our ability to predict the effects of climate change on fish reproductive condition. As the occurrence of hypoxia increases, fishes will be exposed to biochemical changes that reduce reproductive output. However, mild periodic hypoxia increases lipid storage and improves fish condition to such an extent that if a suitable period of time elapses following hypoxia exposure, full reproductive potential may still be realized. The determining factor will be the time of recovery at the cellular-level following hypoxia exposure relative to the time required to fully prepare for spawning.

Stock-recruitment relationships may be improved by including factors affecting stock productivity through EBFM approaches that quantify environmental and climatic effects and provide managers with more realistic predictions of fish production (Keyl and Wolff 2008). Comprehensive models that include variability in reproduction, such as weight-specific fecundity (Spencer and Dorn 2013), are currently under development and additional refinements should be considered. For example, it has been found that environmental conditions encountered prior to spawning can affect reproductive performance of similarly-sized individuals in European Anchovy (*Engraulis encrasicolus*; Pecquerie et al., 2009). The effects of hypoxia on Atlantic Croaker reproduction need to be quantified in assessment models, as numbers of spawning females may overestimate spawning stock reproductive potential. As a result, young-of-the-year recruitment strength may be more closely aligned with adult female energy density and not necessarily predictable through estimates of adult female biomass. However, energy density itself may have limitations since hypoxia exposure limits vitellogenin production at the cellular level. To this end, investigations into biomarkers that can be used to evaluate fish exposure to hypoxia are underway (Thomas and Rahman 2009). The development of hypoxia biomarkers, and an understanding of time to recovery from hypoxia exposure relative to spawning season may provide key answers explaining variability in juvenile recruitment of estuarine-dependent fishes.

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Table 1. The number of female Atlantic Croaker collected by river, treatment, and year, and mean (SE) age, length, weight and proximate composition results by tissue and component tested.

	2011		2012	
Site	Before	During	Before	During
James River				
N	20	23	8	3
Age (yr)	4.7 (1.2)	3.7 (1.1)	4.5 (2.3)	4.3 (2.3)
Length (mm)	254.7 (13.6)	254.5 (8.3)	288.6 (32.6)	287 (37.2)
Weight (g)	208.2 (36.2)	214.8 (32.7)	309.1 (123.0)	341.3 (123.0)
Gonad lipid	6.4 (0.56)	9.2 (1.29)	5.9 (0.74)	15.5 (5.06)
Gonad water	82.2 (0.22)	80.7 (0.91)	82.7 (0.33)	71.3 (5.54)
Soma lipid	17.0 (0.77)	31.3 (2.08)	24.0 (2.09)	36.8 (4.47)
Soma water	76.6 (0.34)	73.9 (0.62)	74.8 (1.52)	71.0 (1.15)
Soma protein	57.1 (0.91)	47.6 (1.85)	56.0 (2.67)	46.2 (0.42)
Soma ash	20.0 (0.73)	20.1 (0.89)	19.1 (1.5)	16.8 (2.61)
York River				
N	20	18	4	4
Age (yr)	4.3 (1.0)	3.7 (1.0)	5.0 (2.2)	2.5 (1.0)
Length (mm)	262.6 (27.1)	263.5 (18.4)	269.5 (32.4)	251.3 (3.5)
Weight (g)	239.3 (105.4)	228.8 (49.5)	266.7 (130.7)	258.3 (46.3)
Gonad lipid	6.1 (0.46)	13.7 (1.35)	10.1 (2.36)	16.0 (3.27)
Gonad water	82.1 (0.20)	75.7 (1.57)	81.8 (0.95)	71.0 (5.02)
Soma lipid	25.7 (1.20)	32.9 (1.75)	30.5 (5.09)	44.2 (1.59)
Soma water	74.7 (0.43)	72.1 (0.59)	73.8 (1.25)	68.3 (0.48)
Soma protein	55.8 (1.31)	51.2 (2.38)	53.3 (3.55)	38.4 (0.81)
Soma ash	19.3 (0.87)	17.2 (0.88)	16.6 (1.98)	14.5 (2.40)
Rappahannock River				
N	19	24	5	2
Age (yr)	4.0 (0.8)	4.0 (1.3)	4.2 (2.2)	3.0 (0)
Length (mm)	255.5 (11.9)	280.2 (32.4)	253.8 (5.3)	293.5 (9.2)
Weight (g)	222.3 (36.7)	324.7 (138.7)	233.2 (16.9)	395.9 (54.2)
Gonad lipid	5.6 (0.20)	7.4 (0.85)	6.9 (0.43)	18.6 (3.15)
Gonad water	81.4 (0.25)	80.4 (0.68)	81.8 (0.20)	78.0 (3.00)
Soma lipid	26.4 (1.15)	34.9 (1.11)	36.3 (2.64)	30.8 (4.71)
Soma water	74.2 (0.56)	69.4 (0.66)	71.4 (1.36)	68.0 (1.00)
Soma protein	53.4 (1.06)	47.6 (1.25)	47.6 (2.54)	45.5 (2.55)
Soma ash	17.2 (0.79)	13.2 (0.47)	14.8 (1.20)	9.5 (2.40)

Table . Summary of mixed-model parameter estimates describing the relationship between gonad and soma lipid content (%) and the effects of river, year (2011 and 2012), and factor (Before and During hypoxia) nested within river.

	Estimate	Std. error	Prob.	Adj. R^2
Gonad				0.504
Intercept	2.8587	0.1440	<0.0001	
Factor	-0.7643	0.1261	<0.0001	
York(Before)	0	.	.	
James(Before)	-0.01802	0.1186	0.8797	
Rappahannock(Before)	-0.07724	0.1202	0.5223	
York(During)	0	.	.	
James(During)	-0.4717	0.1230	0.0003	
Rappahannock(During)	-0.6257	0.1230	<0.0001	
Year 2011	-0.3173	0.1182	0.0093	
Soma				0.248
Intercept	3.7232	0.0722	<0.001	
Factor	-0.2711	0.0842	0.0019	
York(Before)	0	.	.	
James(Before)	-0.3625	0.0785	<0.0001	
Rappahannock(Before)	0.0637	0.0786	0.4200	
York(During)	0	.	.	
James(During)	-0.0956	0.0825	0.25	
Rappahannock(During)	0.0079	0.0853	0.9263	
Year 2011	-0.296	0.0530	<0.0001	

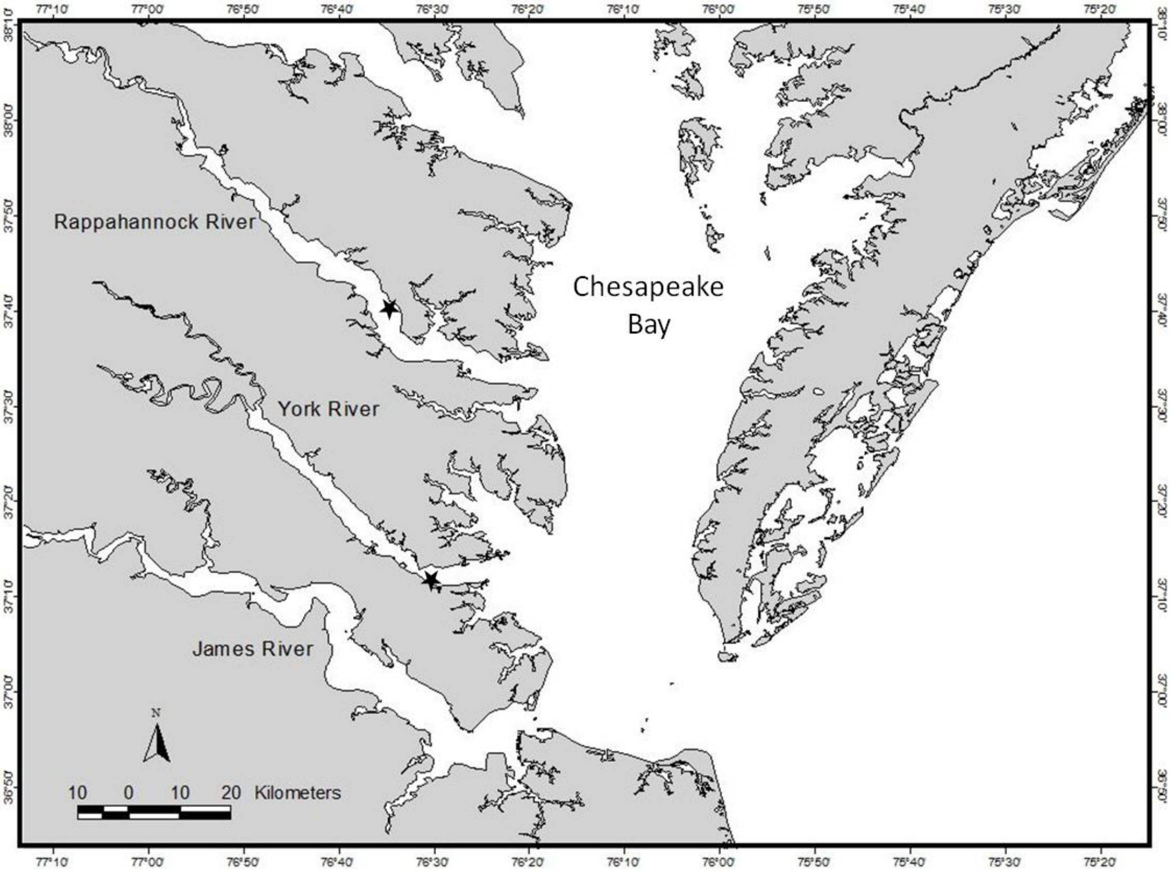

Figure 1. Map of study area showing the James, York, and Rappahannock rivers. Stars indicate the location of the VECOS water quality profiling stations.

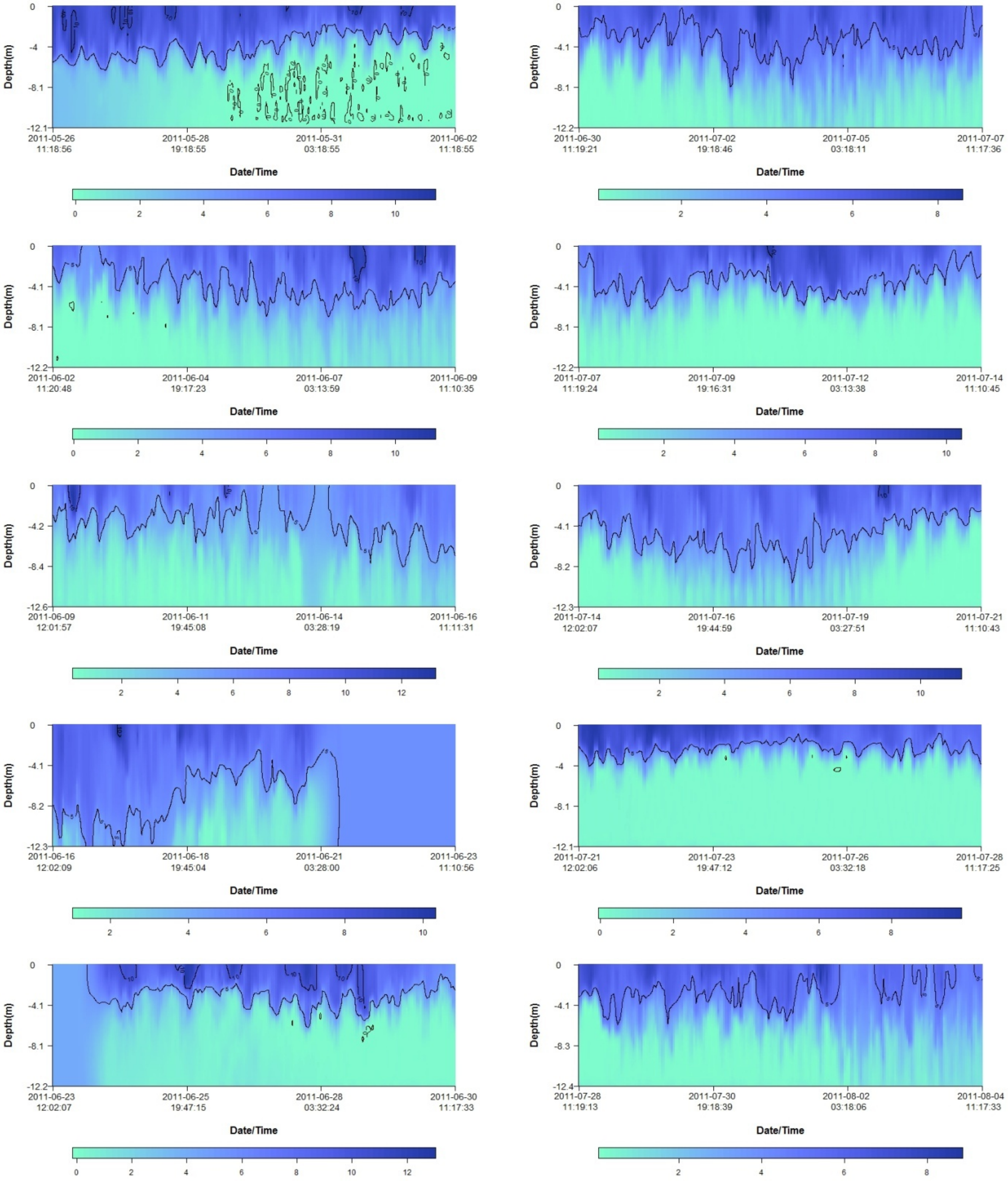

Figure 2. Weekly dissolved oxygen (mg/L) vertical profile from the VECOS Rappahannock River Depth Profiler (RPP021.36) from 26 May to 4 August, 2011.

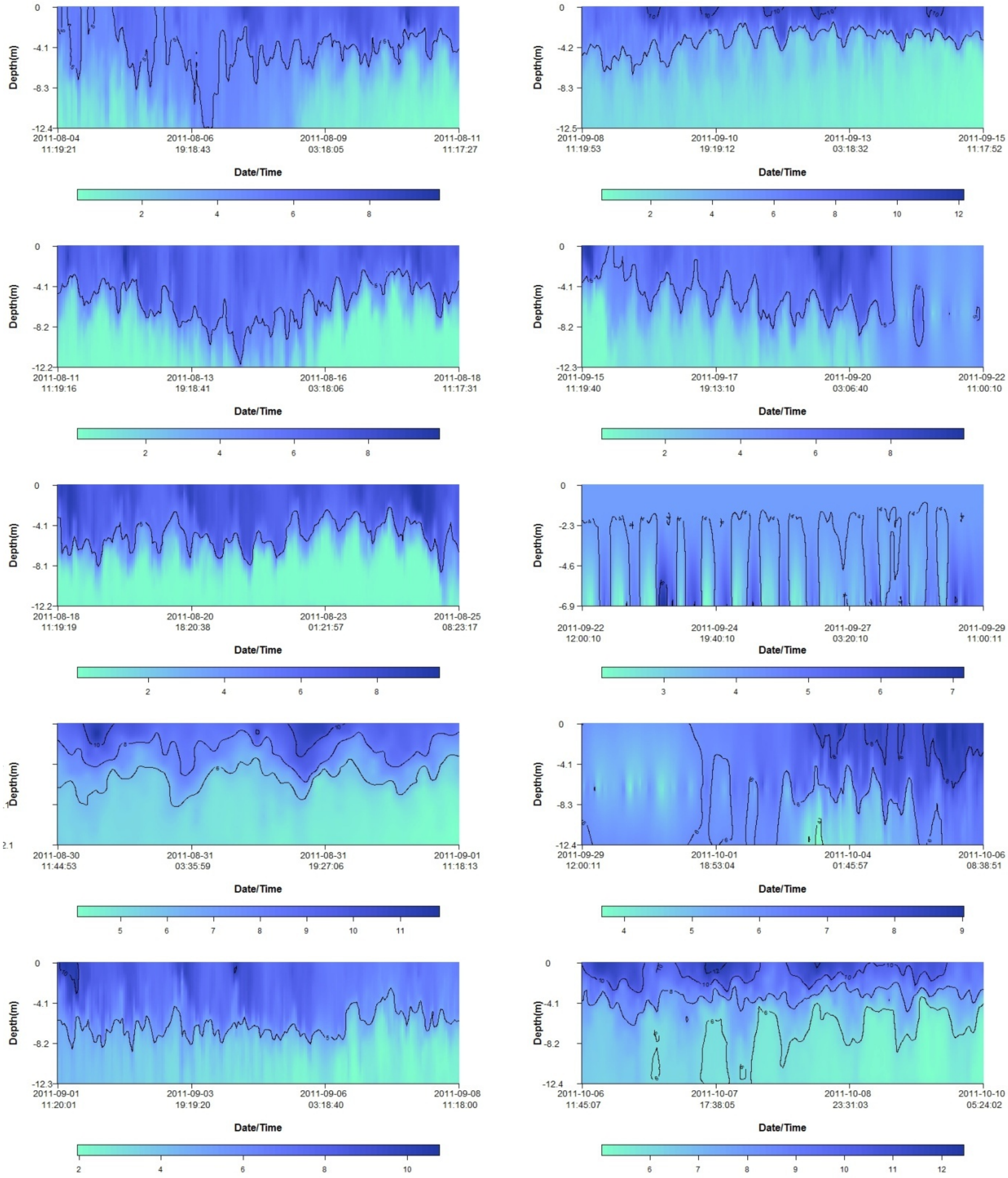

Figure 2 continued. Weekly dissolved oxygen (mg/L) vertical profile from the VECOS Rappahannock River Depth Profiler (RPP021.36) from 4 August to 10 October, 2011. The water quality monitoring gear was removed from the water on 25 August and redeployed on 30 August 2011 due to Hurricane Irene.

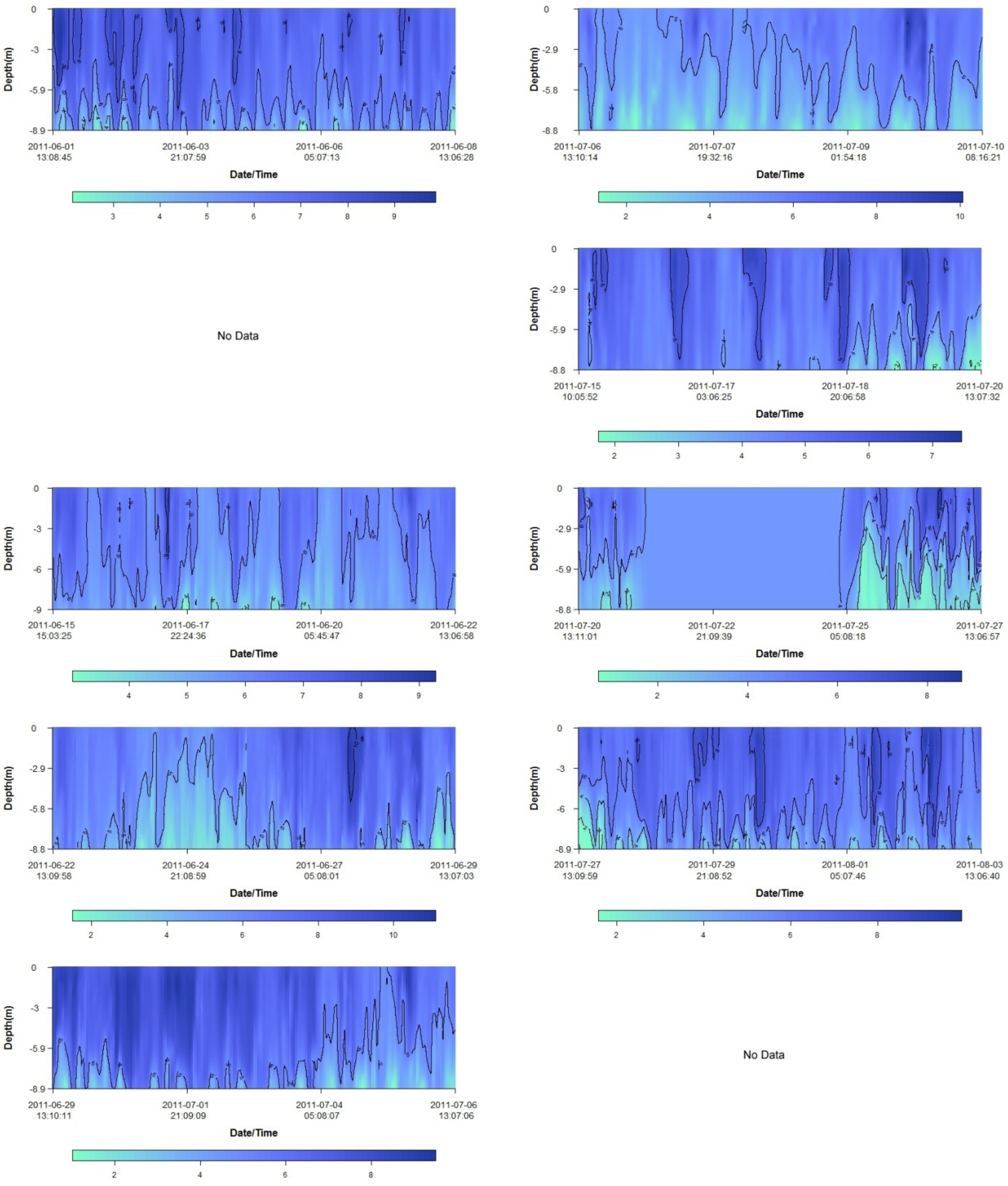

Figure 3. Weekly dissolved oxygen (mg/L) vertical profile from the VECOS York River Vertical Profiler (YRK004.26) from 1 June to 3 August, 2011.

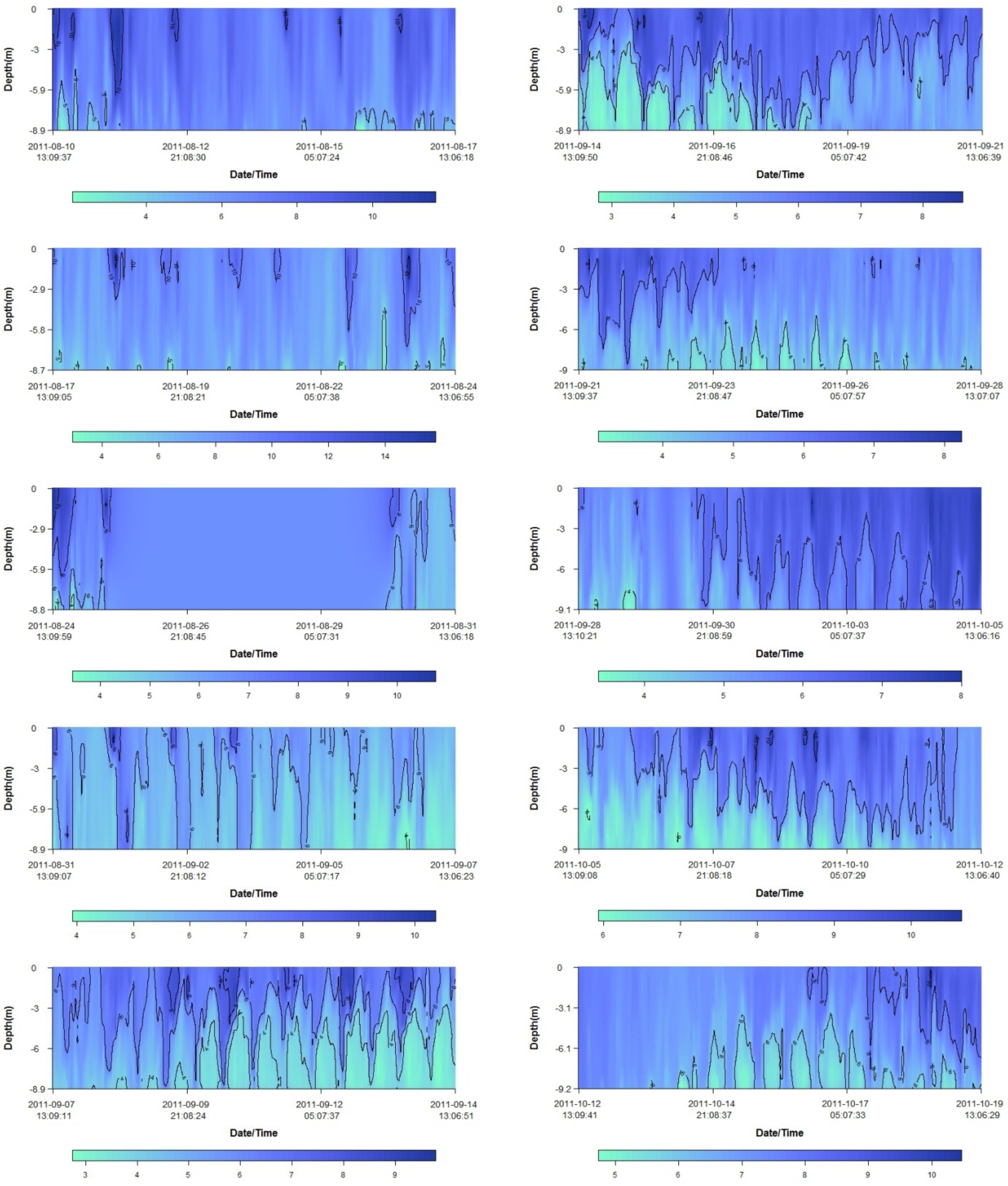

Figure 3 continued. Weekly dissolved oxygen (mg/L) vertical profile from the VECOS York River Vertical Profiler (YRK004.26) from 10 August to 19 October, 2011. The water quality monitoring gear was removed from the water on 25 August and redeployed on 30 August 2011 due to Hurricane Irene.

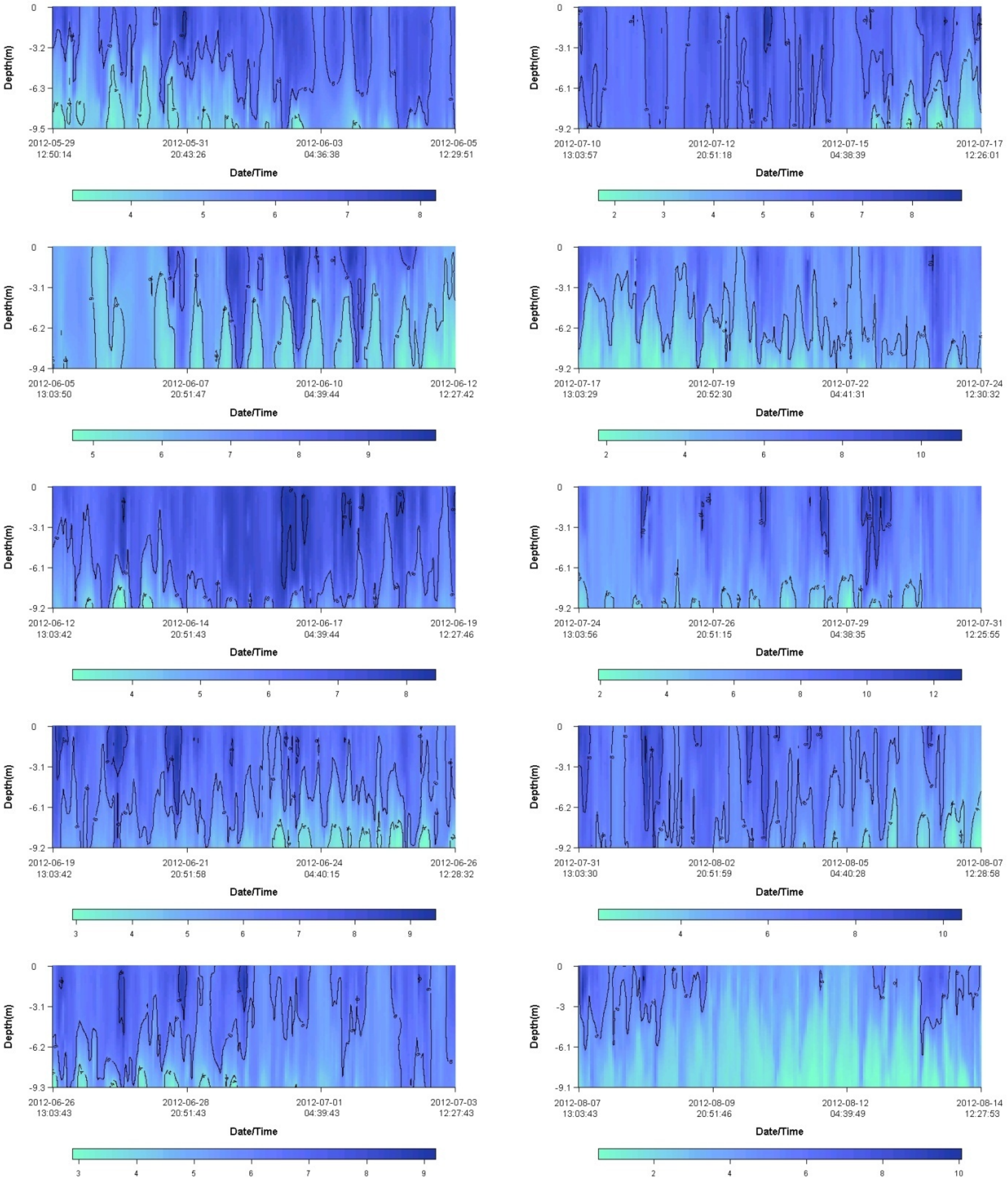

Figure 4. Weekly dissolved oxygen (mg/L) vertical profile from the VECOS York River Vertical Profiler (YRK004.26) from 29 May to 14 August, 2012.

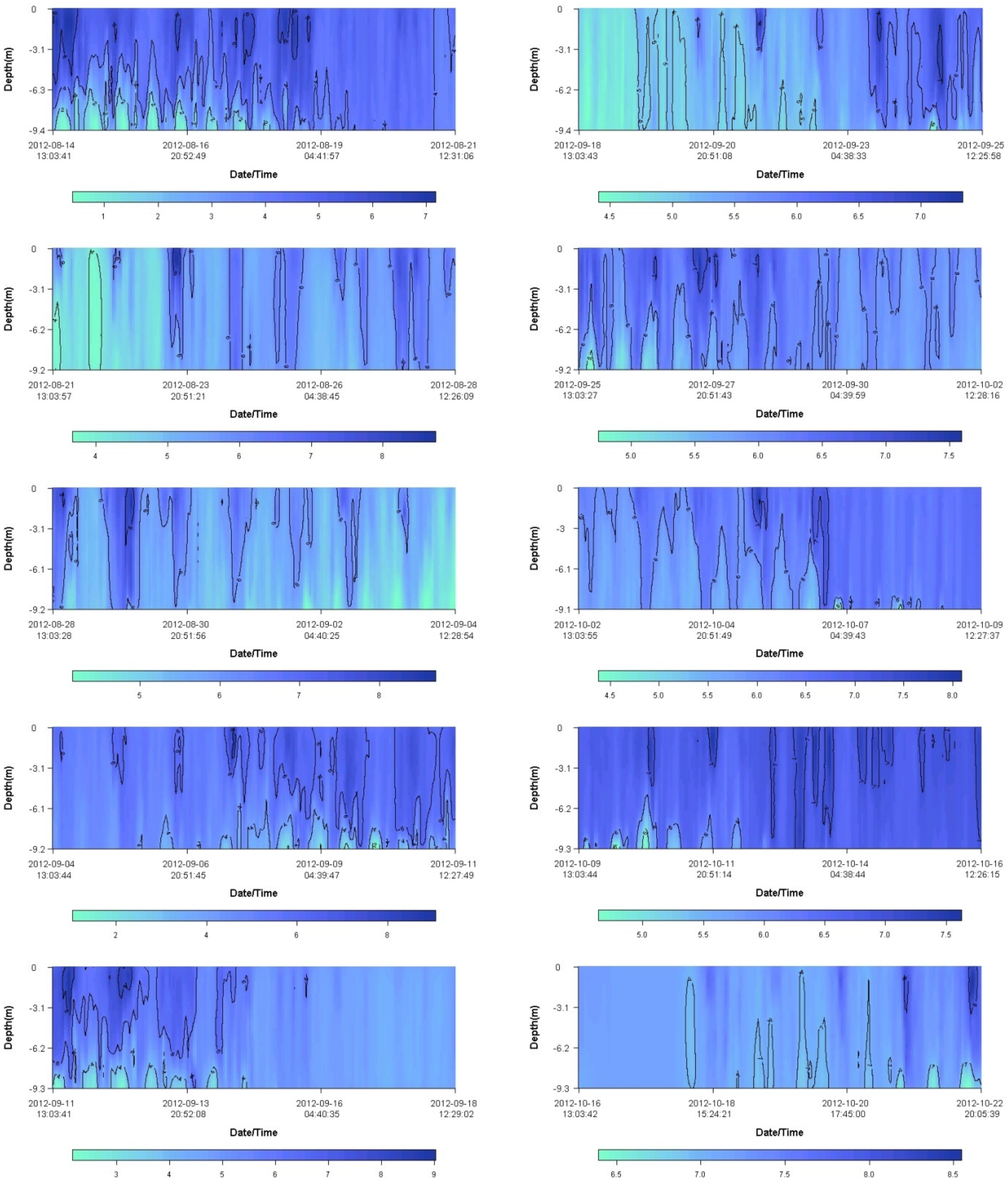

Figure 4 continued. Weekly dissolved oxygen (mg/L) vertical profile from the VECOS York River Vertical Profiler (YRK004.26) from 14 August to 22 October, 2012.

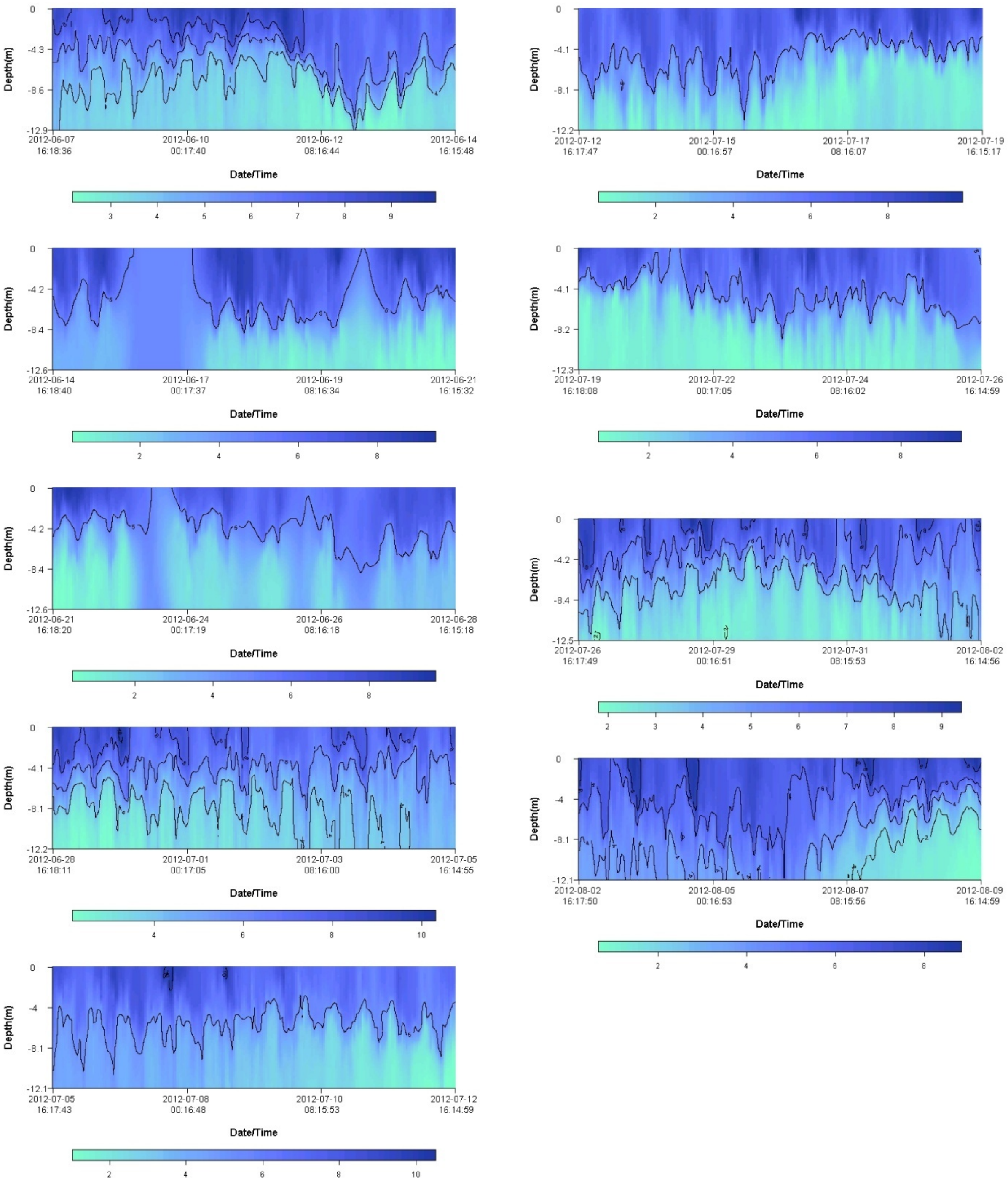

Figure 5. Weekly dissolved oxygen (mg/L) vertical profile from the VECOS Rappahannock River Depth Profiler (RPP021.36) from 7 June to 9 August, 2012.

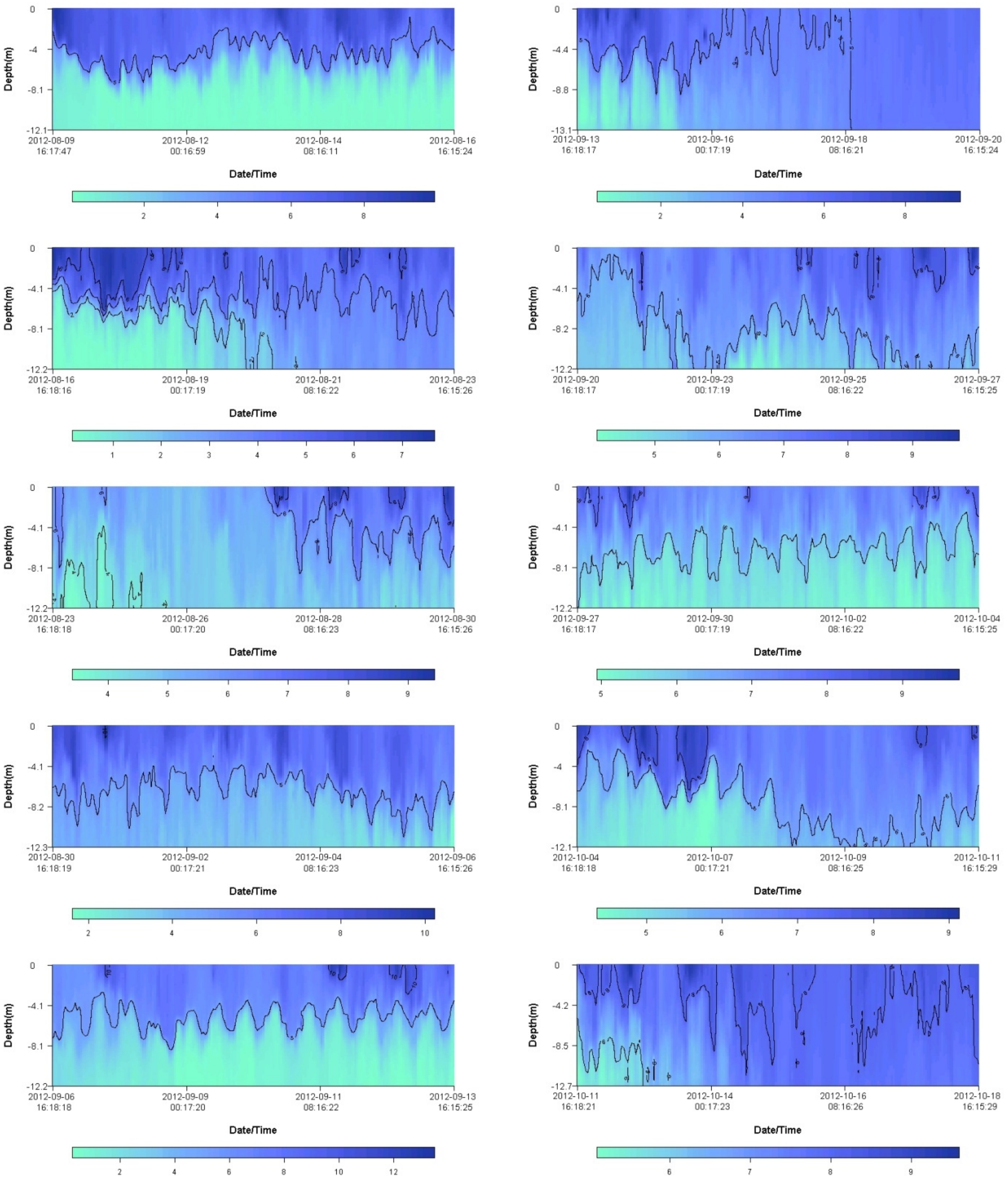

Figure 5 continued. Weekly dissolved oxygen (mg/L) vertical profile from the VECOS Rappahannock River Depth Profiler (RPP021.36) from 9 August to 18 October, 2012.

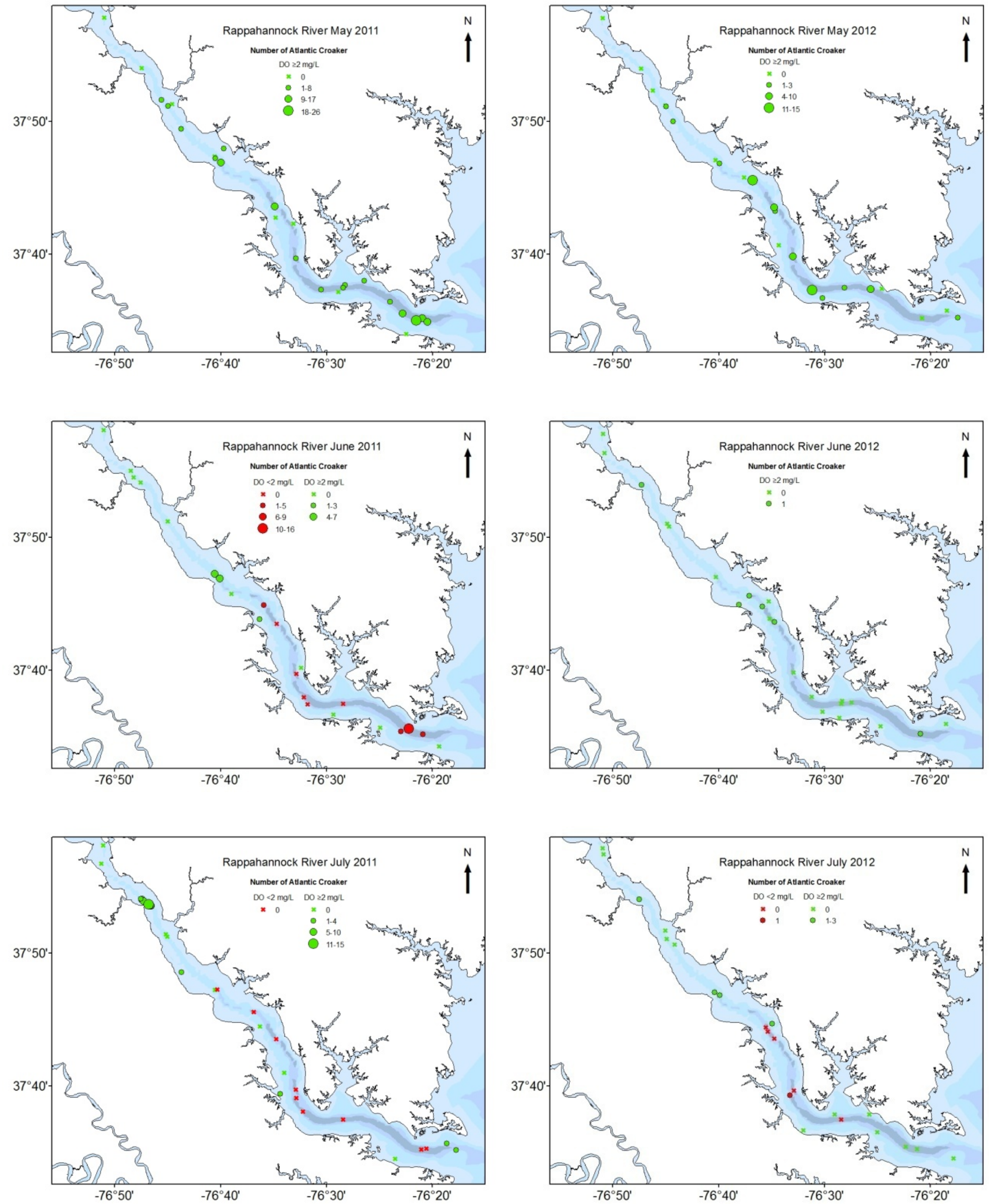

Figure 6. VIMS Trawl Survey sampling stations in the Rappahannock River from May to July, 2011 (left) and 2012 (right). The number of Atlantic Croaker captured in each tow is indicated by the size of the symbol and bottom dissolved oxygen content is indicated by color (DO<2 mg/L is shown in red, DO≥2 mg/L is shown in green).

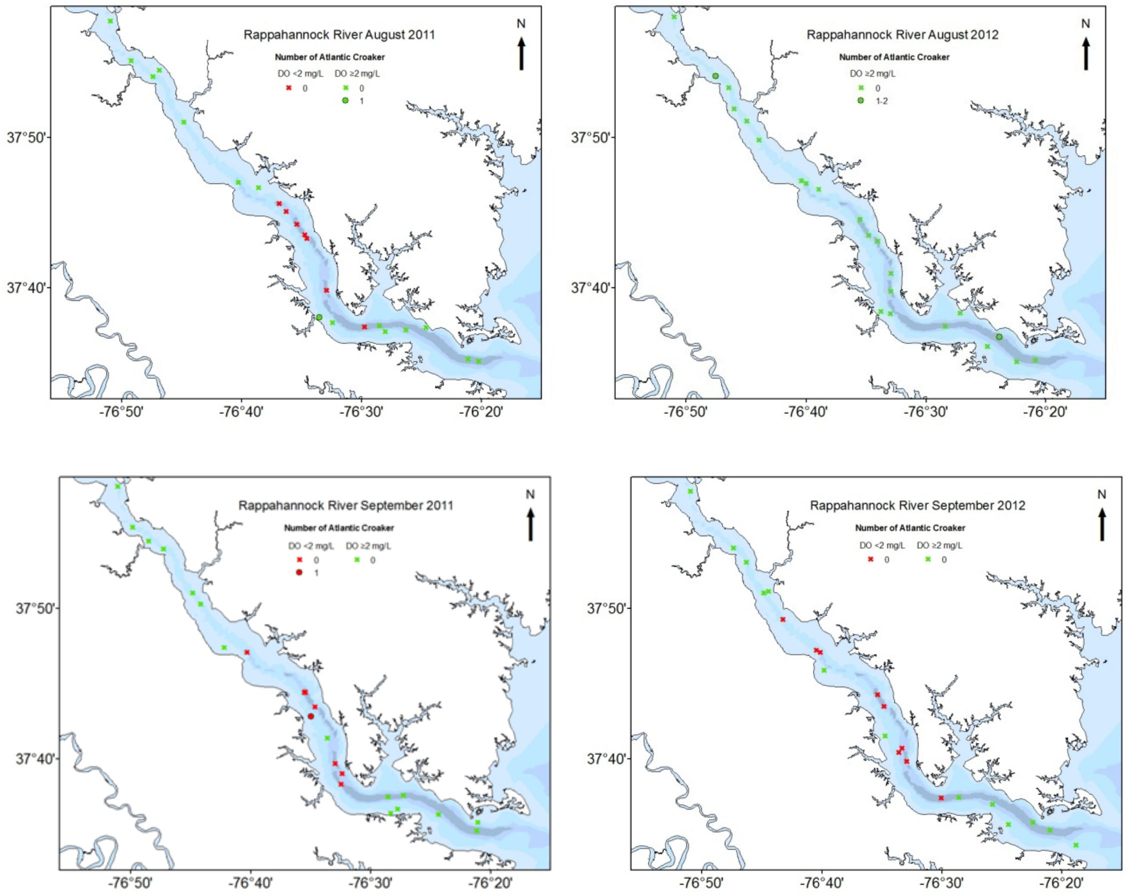

Figure 6 continued. VIMS Trawl Survey sampling stations in the Rappahannock River from August to September, 2011 (left) and 2012 (right). The number of Atlantic Croaker captured in each tow is indicated by the size of the symbol and bottom dissolved oxygen content is indicated by color (DO<2 mg/L is shown in red, DO≥2 mg/L is shown in green).

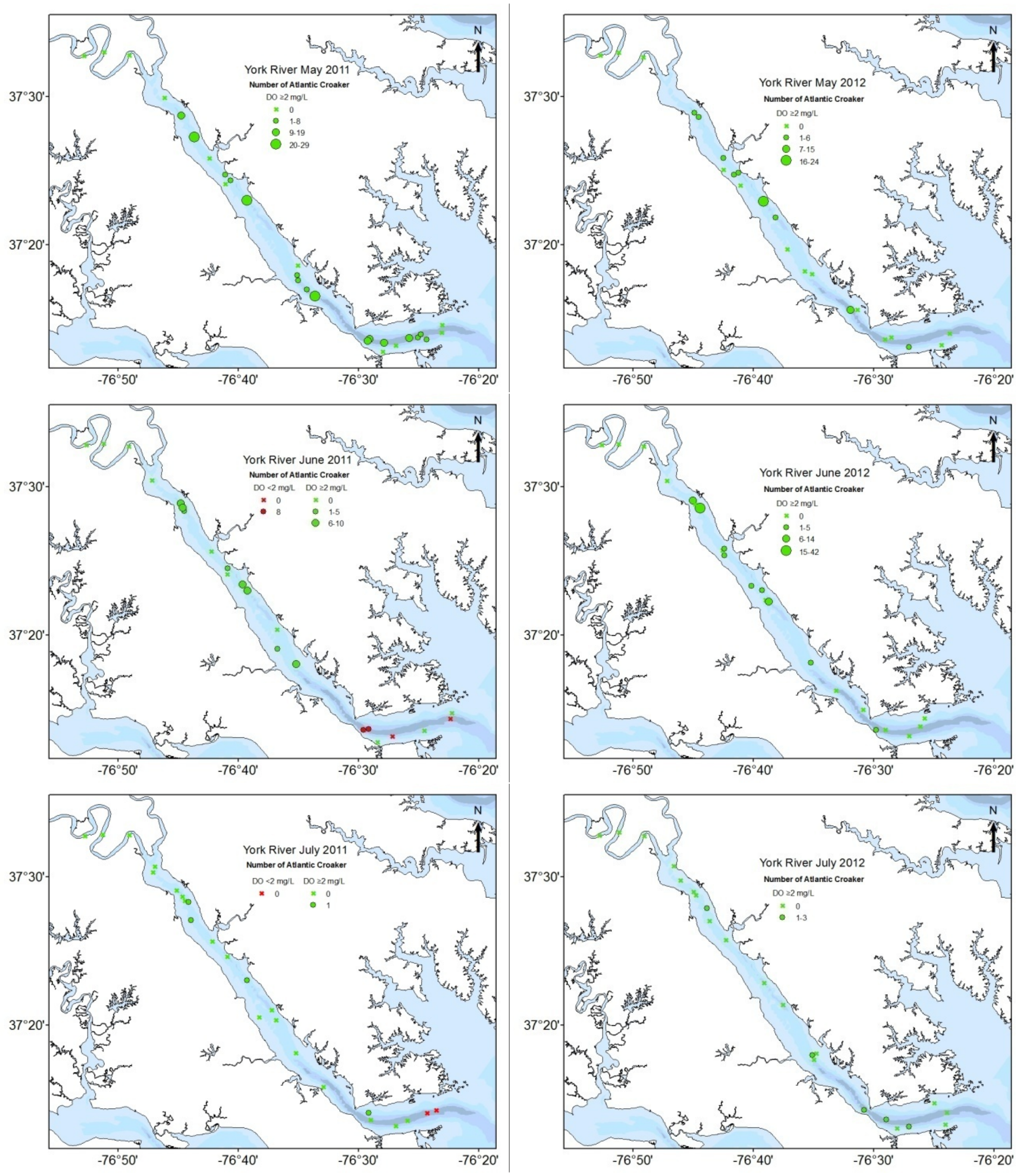

Figure 7. VIMS Trawl Survey sampling stations in the York River from May to July, 2011 (left) and 2012 (right). The number of Atlantic Croaker captured in each tow is indicated by the size of the symbol and bottom dissolved oxygen content is indicated by color (DO<2 mg/L is shown in red, DO≥2 mg/L is shown in green).

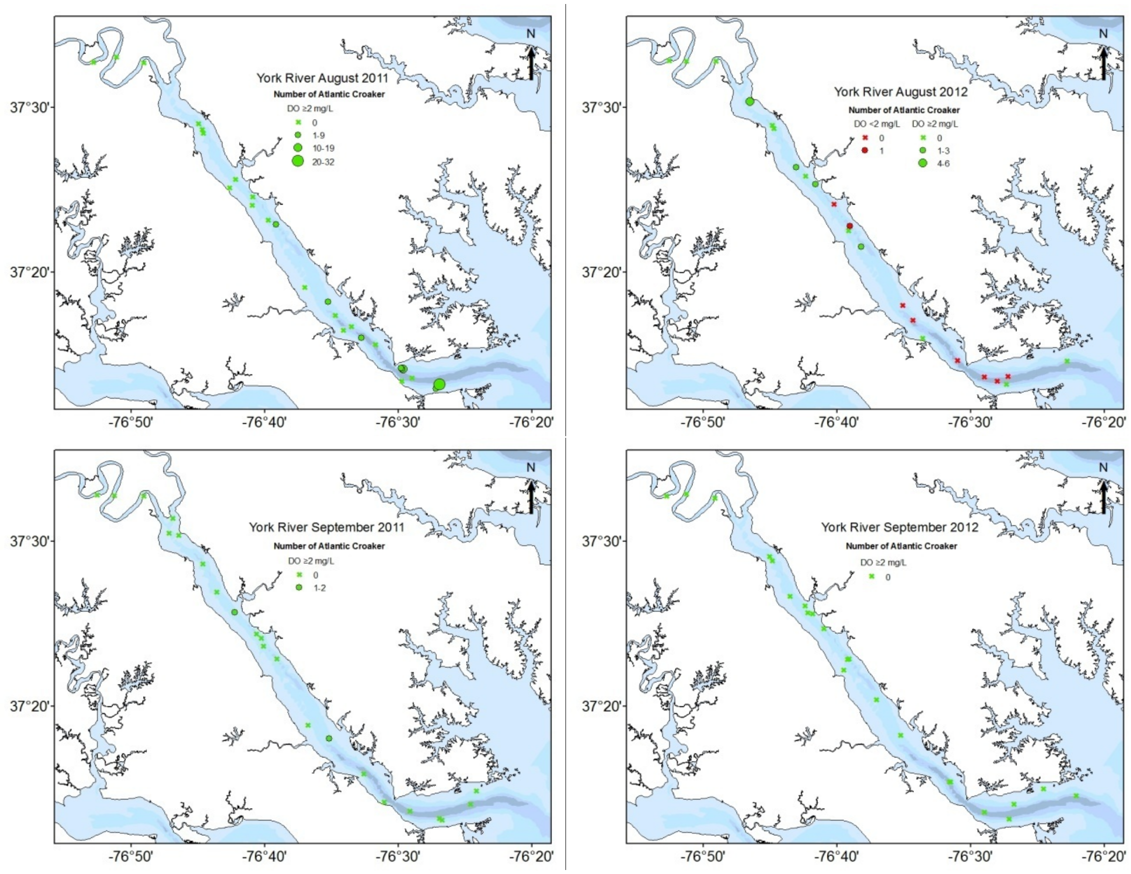

Figure 7 continued. VIMS Trawl Survey sampling stations in the York River from August to September, 2011 (left) and 2012 (right). The number of Atlantic Croaker captured in each tow is indicated by the size of the symbol and bottom dissolved oxygen content is indicated by color (DO<2 mg/L is shown in red, DO≥2 mg/L is shown in green).

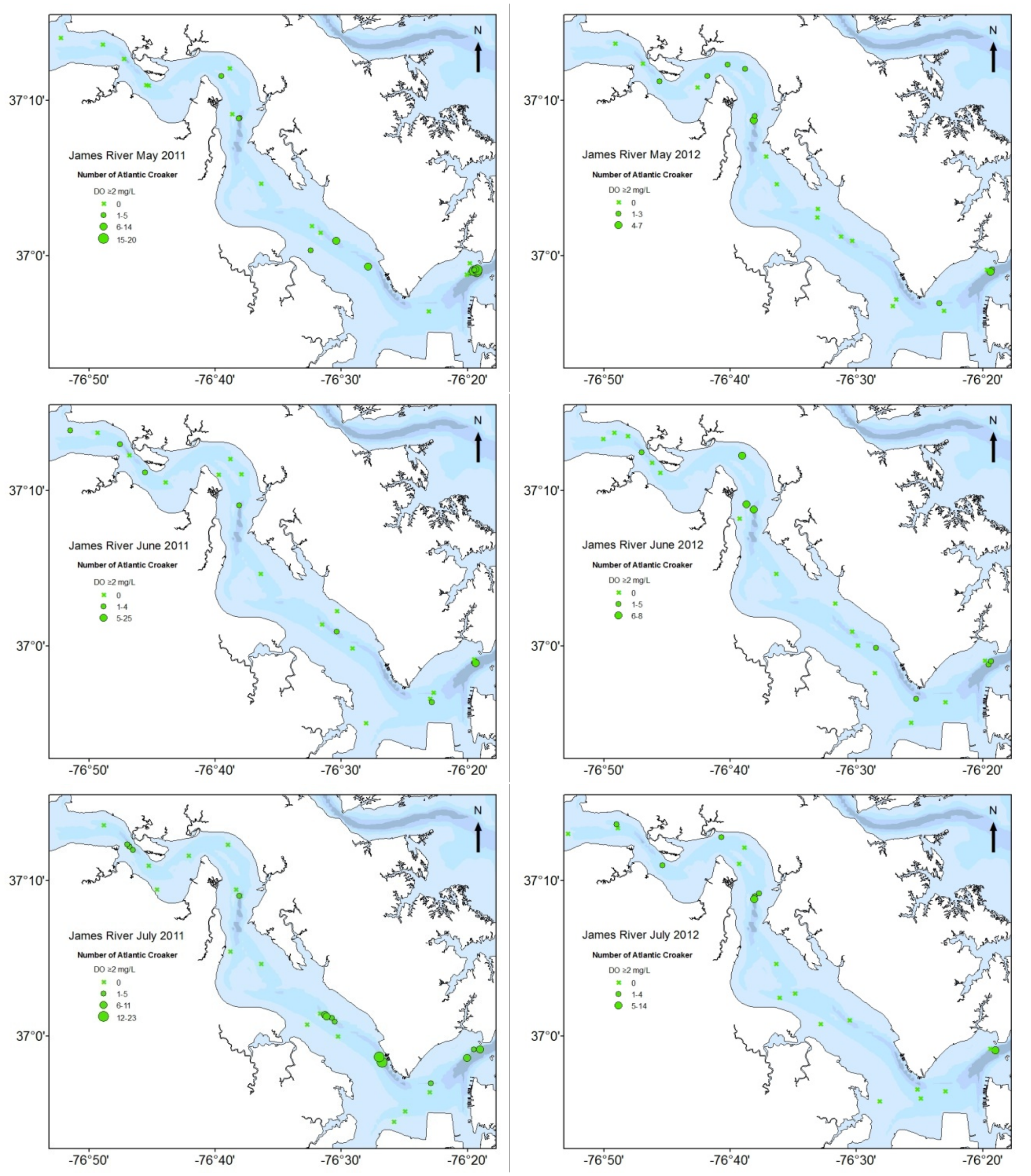

Figure 8. VIMS Trawl Survey sampling stations in the James River from May to July, 2011 (left) and 2012 (right). The number of Atlantic Croaker captured in each tow is indicated by the size of the symbol and bottom dissolved oxygen content is indicated by color (DO<2 mg/L is shown in red, DO≥2 mg/L is shown in green).

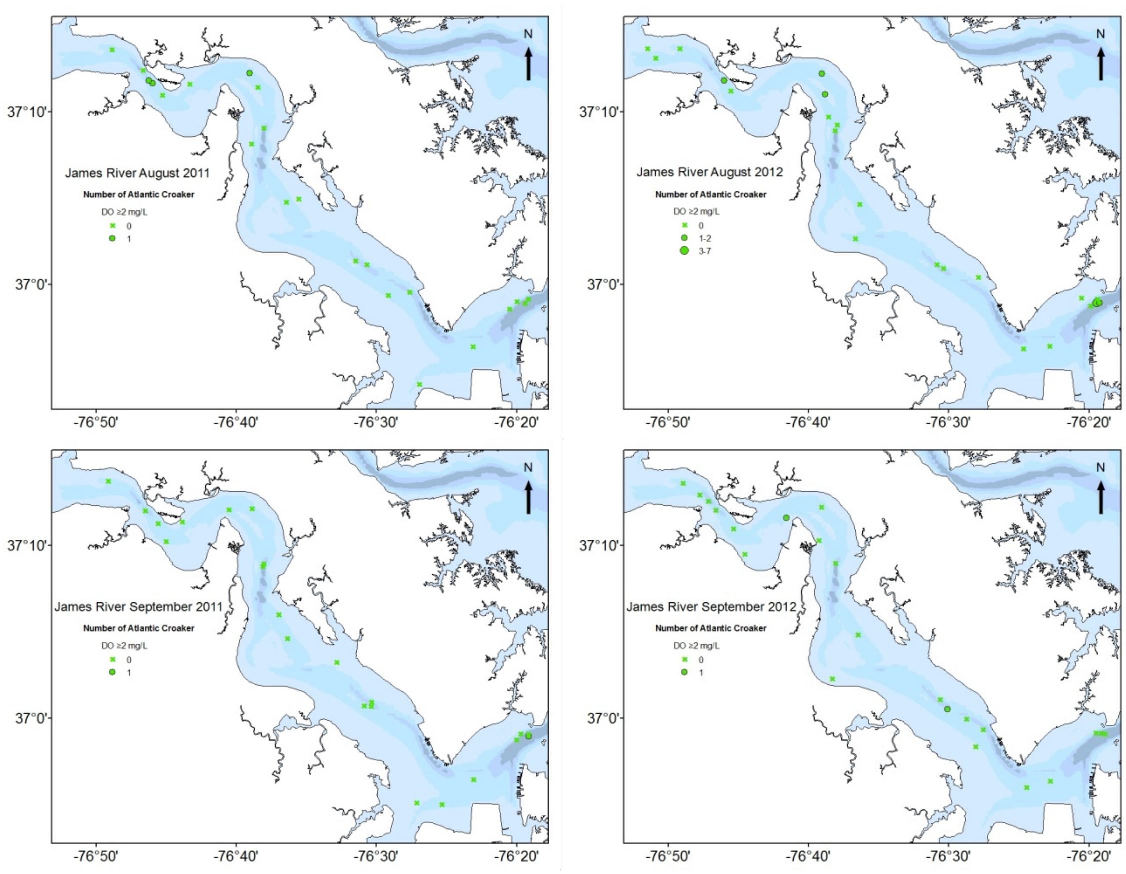

Figure 8 continued. VIMS Trawl Survey sampling stations in the James River from August to September, 2011 (left) and 2012 (right). The number of Atlantic Croaker captured in each tow is indicated by the size of the symbol and bottom dissolved oxygen content is indicated by color (DO<2 mg/L is shown in red, DO≥2 mg/L is shown in green).

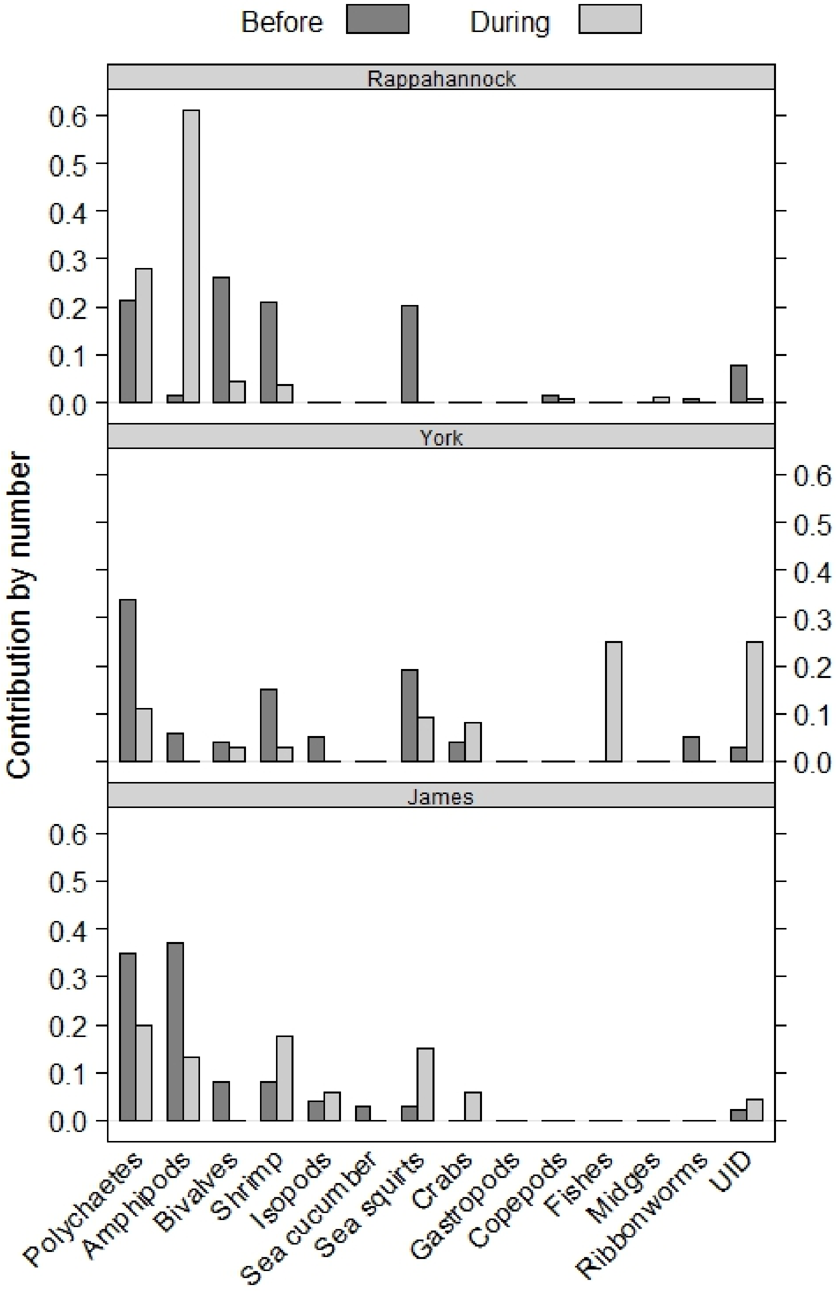

Figure 9. Prey composition by number for diets of female Atlantic Croaker collected in the Rappahannock, York, and James rivers before and during hypoxia.

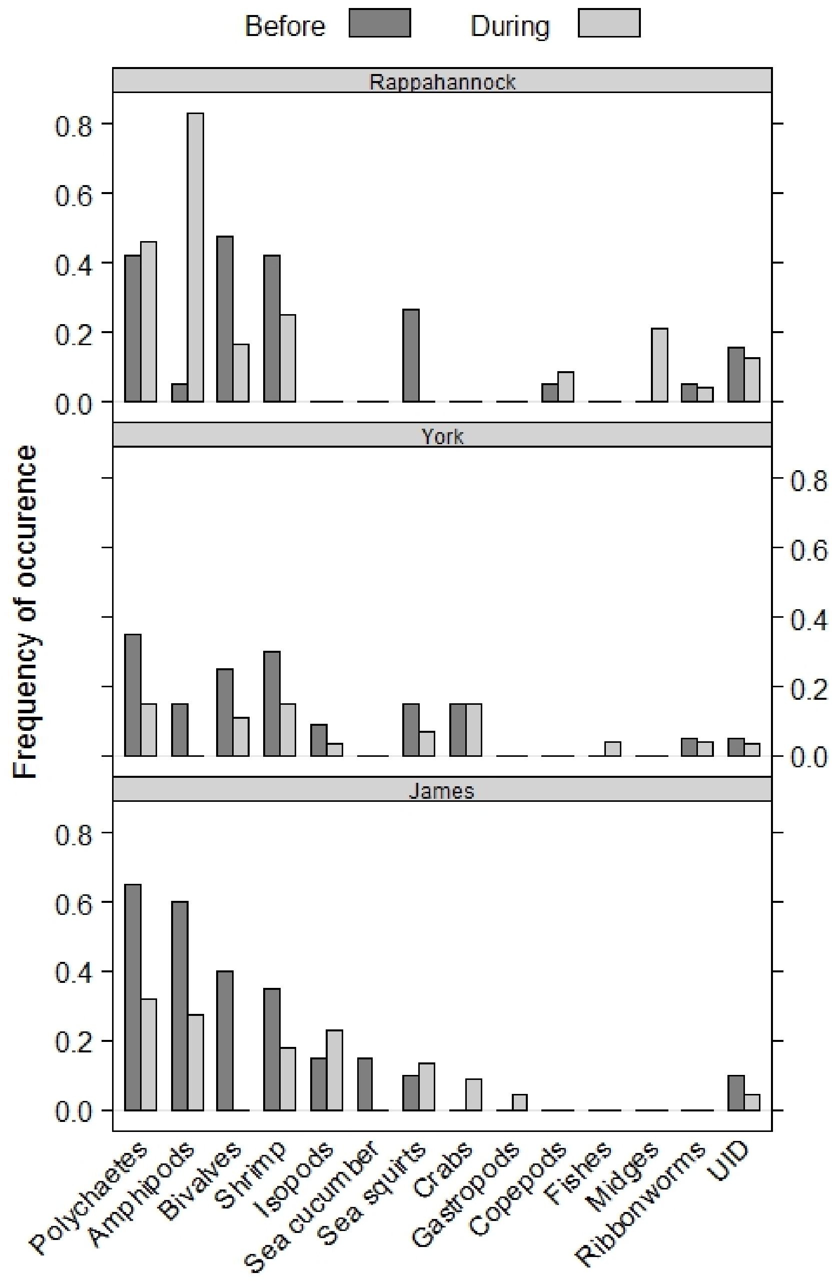

Figure 10. Prey composition by frequency of occurrence for diets of female Atlantic Croaker collected in the Rappahannock, York, and James rivers before and during hypoxia.

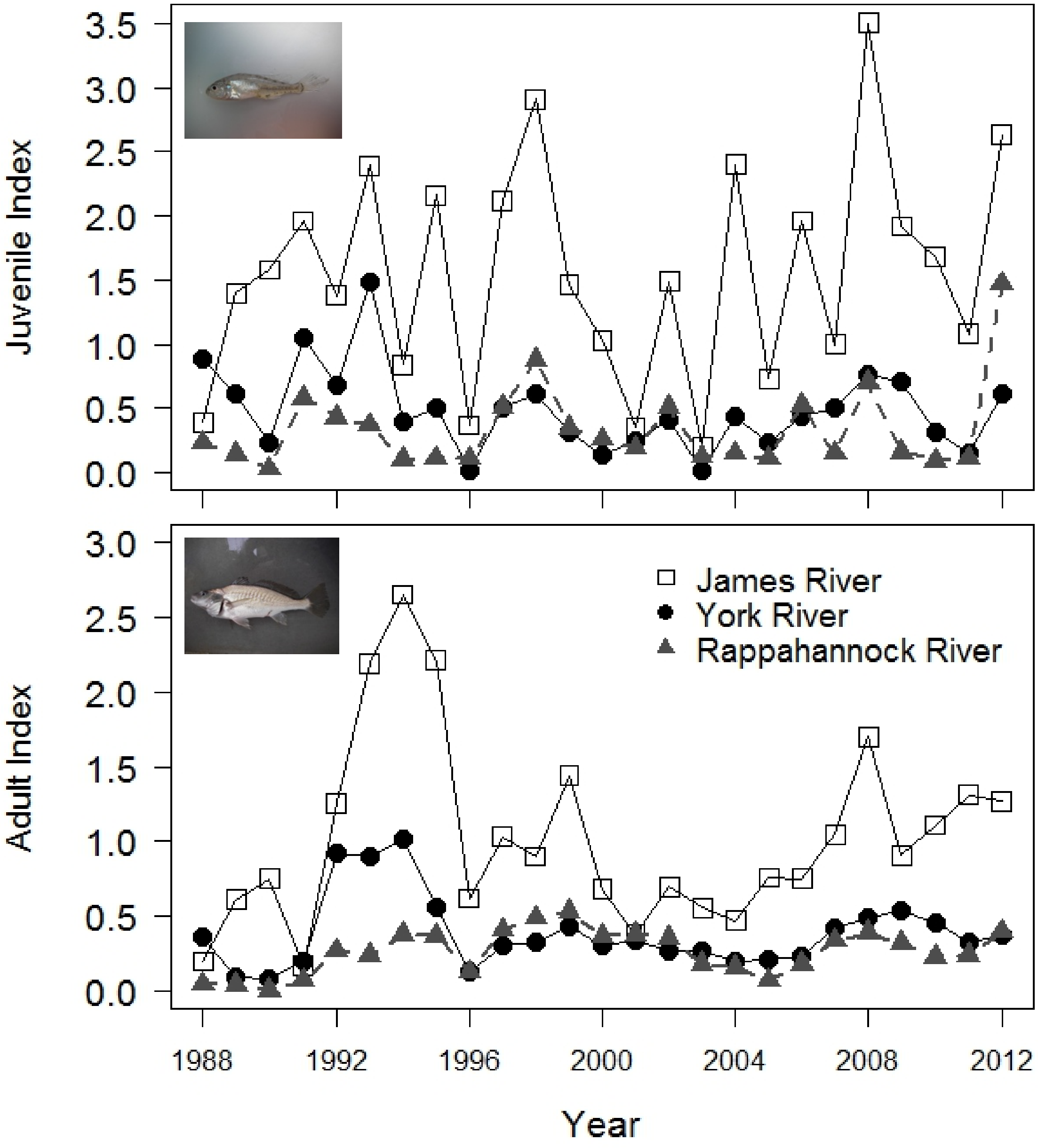

Figure 11. Juvenile (top) and adult (bottom) relative abundance indices from the Virginia Institute of Marine Science Juvenile Fish Trawl Survey, 1988–2012.

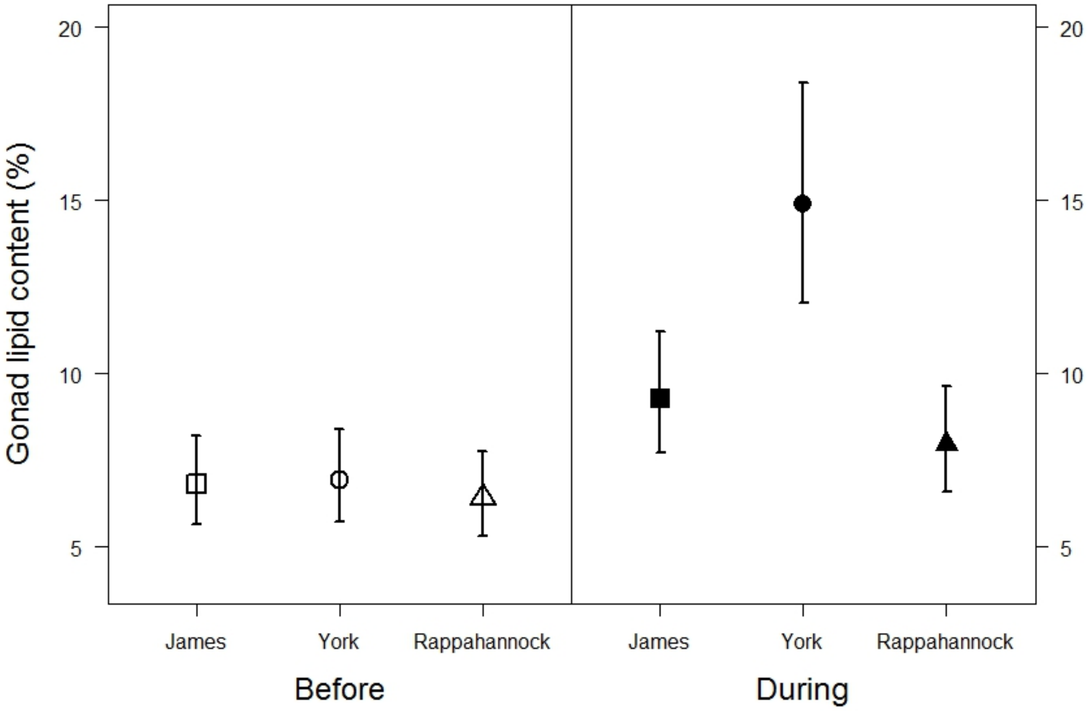

Figure 12. Model-adjusted mean lipid content (%) of Atlantic Croaker gonad tissue from the James, York, and Rappahannock rivers before and during exposure to hypoxia.

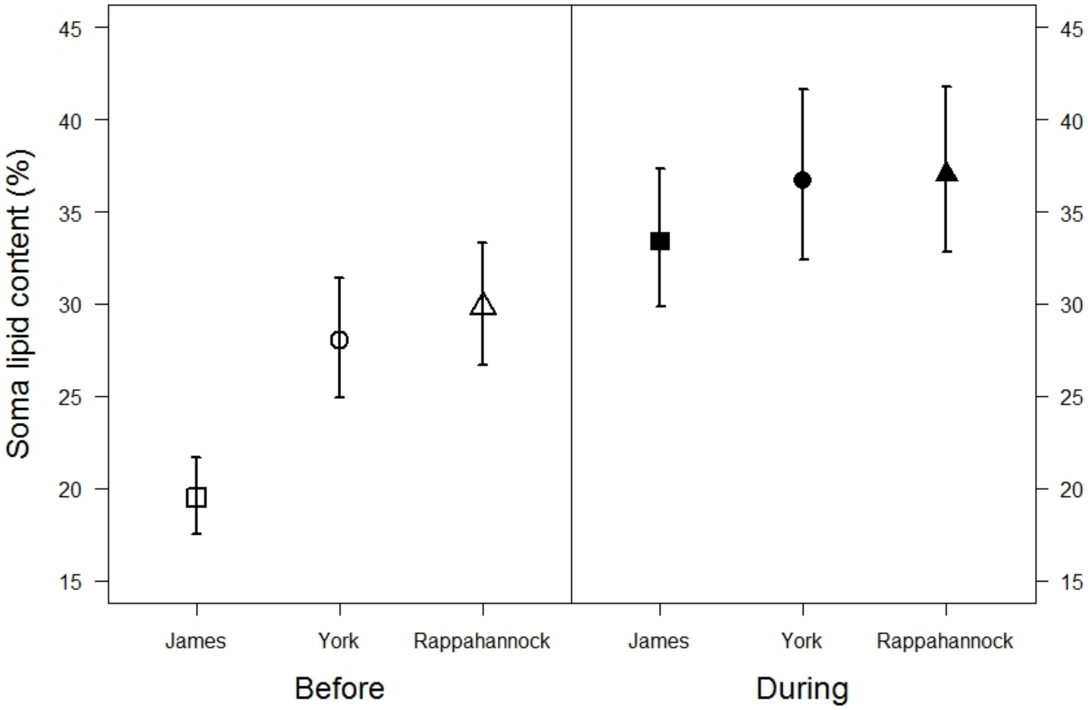

Figure 13. Model-adjusted mean lipid content (%) of Atlantic Croaker somatic tissue from the James, York, and Rappahannock rivers before and during exposure to hypoxia.

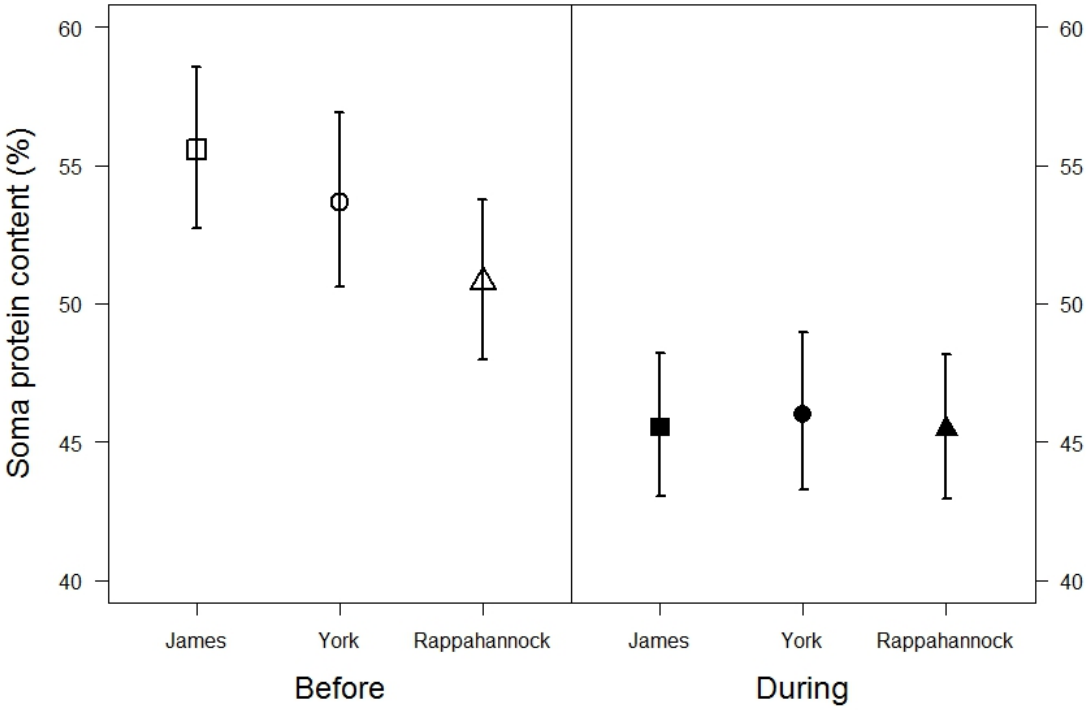

Figure 14. Model-adjusted mean protein content (%) of Atlantic Croaker somatic tissue from the James, York, and Rappahannock rivers before and during exposure to hypoxia.

Outreach and presentations

Data from this study were used in several presentations and outreach events:

Tuckey, T. D. The Juvenile Fish Trawl Survey: Current investigations and future plans. VIMS Fisheries Seminar Series, 24 October, 2012.

Tuckey, T. D. and M. C. Fabrizio. 2013. Establishing field-based evidence for the effects of hypoxia on the reproductive capacity of Chesapeake Bay fishes. 27th Annual Meeting of the Tidewater Chapter of the American Fisheries Society, Solomons, MD. March 2013.

Fabrizio, M. C. Keynote Address: Effects of Hypoxia on Chesapeake Bay Fishes. Mid-Atlantic Meeting of the Ecological Society of America. Dover, DE. April 2013.

Fabrizio, M. C. Effects of hypoxia on Chesapeake Bay Fishes. Smithsonian Environmental Research Center, Edgewater, MD. May 2013.