

Effects of hypoxia-induced habitat compression on growth of juvenile fish in the Neuse River Estuary, North Carolina, USA

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ABSTRACT: Hypoxia is thought to have negative effects on fish in coastal ecosystems, but quantifying those effects can be difficult. Direct exposure to hypoxia can reduce fish growth or survival, but fish can also rapidly detect and avoid low dissolved oxygen levels. However, avoidance behavior may result in indirect effects that reduce fish growth. For example, when hypoxic conditions expand, fish densities may increase in nearshore oxygenated refuges, potentially causing density-dependent reductions in growth. We evaluated this hypothesis for juvenile demersal fish species (primarily spot *Leiostomus xanthurus* and Atlantic croaker *Micropogonias undulatus*). By monitoring water quality and fish density across the Neuse River Estuary, North Carolina, USA, under varying water quality conditions during summer 2007, we showed that fish effectively avoided hypoxia despite rapidly changing conditions (minutes to hours), moving away from incursions of hypoxic water and then rapidly redistributing into affected areas after these events passed. Fish densities in nearshore oxygenated refuges increased nearly 2-fold when habitat was compressed by hypoxic waters. Spot in compressed refuges also had significantly less food in their stomachs during June. Based on published estimates of density-dependent spot growth, we estimated that average spot growth rate was reduced 17% during habitat compression events, which occurred 21.5% of the time, translating into an average reduction in growth rate of 4% over the summer. This likely is a conservative estimate of indirect hypoxia effects on growth, as hypoxia was relatively mild in 2007, and density dependence is only one indirect mechanism by which hypoxia may potentially reduce growth.

KEY WORDS: Hypoxia · *Leiostomus xanthurus* · Density-dependent growth · Habitat compression · Neuse River Estuary

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INTRODUCTION

In coastal regions globally, there is serious concern that water quality problems caused by anthropogenic eutrophication are having negative effects on ecologically and economically important living resources (Caddy 1993, Schmittner 1999, Beck et al. 2001, Craig et al. 2001, Breitburg 2002). Increased nutrient loading to rivers and estuaries from sources such as expanding urbanization and agricultural runoff change

nutrient balances and alter the oxygen budget of estuaries, which can increase the extent and magnitude of hypoxia (dissolved oxygen [DO] <2.0 mg l⁻¹) (Pinckney 2001). Increased frequency and duration of hypoxic events are commonly caused by high productivity from excess nutrients added to warm water coupled with reduced mixing rates from salinity stratification. Patterns of increased hypoxia due to anthropogenic causes have been observed in Chesapeake Bay, USA (Cooper 1995), the Gulf of Mexico (Rabal-

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ais et al. 2002), and elsewhere (Breitburg et al. 2009), including North Carolina, USA (Luettich et al. 2000). Diaz & Rosenberg (2008) found that hypoxia can alter the state of the ecosystem in an estuary (increased nutrients and organic matter, diversion of nutrients to microbial pathways instead of higher trophic levels, decreased benthic fauna), and if hypoxia is a persistent, recurring event, recovery of that ecosystem may lag several years behind a reduction in nutrient loading.

Estuaries are important not only as nurseries and essential habitat for juvenile fish but also to the economy, culture, and quality of life of coastal residents. Most of the ecologically and economically important fish and shellfish species underpinning tourism and fishing industries in North Carolina and elsewhere depend on estuarine systems for their essential nursery habitat (Weinstein 1979, Ross & Epperly 1985, Able 1999). In North Carolina, nearly 90% of commercially important fishes and 60% of recreationally important fishes are dependent on estuarine nursery areas at some point in their life cycle (Deaton et al. 2010). One of North Carolina's larger estuaries, the Neuse River Estuary (NRE), began receiving increased attention regarding eutrophication in the late 1970s (Deamer 2009), was placed on the North Carolina list of impaired waters in 1994 (based on the Clean Water Act 303(d) Criteria) (Deamer 2009), and a Neuse Nutrient Strategy was adopted by the North Carolina Environmental Management Commission in 1998 to reduce total nitrogen entering the estuary by 30%. Despite this, the Neuse River Basinwide Assessment (Deamer 2009) showed that there was no distinct reduction in overall nitrogen loading by 2006. An increasing number of fish kills in the NRE in the mid-1990s prompted detailed data collection on species and numbers of fish killed, water quality parameters, and pathology reports starting in 1997 that continues today. These data have shown peaks in fish kills in 2003 and 2008 and record highs in 2009 (North Carolina Division of Water Quality Environmental Sciences Section; annual reports available at <http://portal.ncdenr.org/web/wq/ess/fishkills>).

Fish kills are a highly visible indicator of water quality problems (e.g. Paerl et al. 1998) but generally have negligible population-level impacts, as even large fish kills typically involve only a small fraction of the population and are relatively rare. Conversely, sublethal hypoxic conditions are common and widespread and may affect a much larger portion of the fish population. Fish growth can be reduced by exposure to sublethal low or fluctuating hypoxia, as shown in experimental trials with a variety of species

(e.g. southern flounder *Paralichthys lethostigma*, Taylor & Miller 2001; spot *Leiostomus xanthurus* and menhaden *Brevoortia tyrannus*, McNatt & Rice 2004; summer flounder *Paralichthys dentatus* and winter flounder *Pseudopleuronectes americanus*, Stierhoff et al. 2006). However, fish can typically detect and avoid hypoxia, often at DO levels higher than those that would typically cause a reduction in growth (Wannamaker & Rice 2000, Eby & Crowder 2002, Bell & Eggleston 2005, Stierhoff et al. 2006).

Because fish can often avoid direct exposure to hypoxia, indirect effects of hypoxia on growth of individual fish may have greater consequences than direct effects. When hypoxic conditions expand, avoidance behavior by fish may increase densities in oxygenated refuges, typically near shore. Crowding in these oxygenated refuges may cause density-dependent effects on growth (Pihl et al. 1991, Breitburg 1992). Small-scale cage experiments in the field and larger-scale pond experiments have documented reductions in growth of juvenile estuary-dependent fish as densities increased, even in the absence of predation (Eby et al. 2005, Craig et al. 2007). In the field, reduced growth can prolong the period of vulnerability to gape-limited predators, increasing predation mortality (Sogard 1997). High densities of fish confined to a compressed oxygenated refuge may also attract predators, or predators may be confined to the oxygenated refuge as well, resulting in higher encounter rates and increased mortality of juvenile fish (Hixon et al. 2002).

Hypoxia can also indirectly affect fish growth through its impact on prey resources. As hypoxic waters expand and contract repeatedly over a season, the abundance of benthic invertebrate prey can be reduced because these sessile invertebrates cannot avoid lethal DO levels (Peterson et al. 2000, Eby et al. 2005, Powers et al. 2005). Eby et al. (2005) concluded from field caging experiments with Atlantic croaker *Micropogonias undulatus* that reductions in growth caused by reduced prey resources may surpass reductions in growth from direct exposure to short-term hypoxic events. Thus, repeated episodic hypoxic disturbances affect the interactions of juvenile fish with both their predators and their prey as well as intraspecific competitive interactions (Breitburg 1992, Breitburg et al. 1997).

While substantial effort has been devoted to water quality monitoring and modeling, rarely are linkages between poor water quality and estuarine fish population responses specifically addressed (e.g. Borsuk et al. 2003, Bowen et al. 2003, Wool et al. 2003). Scientists have hypothesized that poor water quality

conditions reduce habitat quality and create a cascade of negative effects from benthic invertebrates to higher predators (Peterson et al. 2000, Powers et al. 2005), but little empirical evidence from the field has been produced; negative effects are generally assumed but not tested. Understanding these linkages and the magnitude of their potential sublethal and lethal effects on aquatic living resources would provide much-needed information to guide water quality and natural resource management. This understanding is particularly important for juvenile fish and estuarine habitats because anthropogenic changes to estuarine habitats are increasing (Diaz & Rosenberg 2008), and the value of an estuarine habitat is often measured by its ability to serve as a nursery for juvenile fishes (Beck et al. 2001).

Because fish are highly mobile and exhibit strong hypoxia avoidance behavior, indirect effects from habitat compression and density-dependent processes in oxygenated refuges may be important mechanisms by which hypoxia impacts juvenile cohorts. However, studying these processes is complicated because unlike most other habitat features, water quality characteristics are spatially and temporally dynamic, and the size and duration of refuges vary with wind and oxygen conditions in the system. In this study, we evaluated changes in the spatial distribution and density of juvenile fishes in response to seasonal and episodic hypoxia dynamics and estimated the extent to which these changes may result in indirect effects of hypoxia on growth. We focused on juvenile spot and Atlantic croaker, as these fish were abundant, occupy the full spectrum of impacted habitats, are benthically oriented (where hypoxia is most severe), and are representative estuary-dependent fishes that support significant fisheries. We conducted our study on a cross section of the NRE that experiences frequent hypoxia and used a combination of water quality monitoring and fish sampling to address the following questions: How does the spatial distribution of hypoxia within a season and on an event basis influence fish densities? Does habitat compression caused by hypoxic events result in significant increases in densities in oxygenated water refuges? Does feeding success change in response to fish densities or water quality condition? Does relative predator abundance change in oxygenated refuges as a response to habitat compression? What potential changes in fish growth can be attributed to these indirect effects? Quantifying these effects could provide insight on the cumulative consequences of these indirect effects on fish growth and production.

MATERIALS AND METHODS

Study area

The NRE is the end point of a watershed that stretches over 400 km from the Piedmont region of North Carolina through the coastal plain to Pamlico Sound and is composed of ~5472 km of tributaries. More than 2 million people live in the Neuse River basin, and the population is expected to increase 44% from 2000 to 2020 (Deamer 2009). The watershed encompasses many urbanized and industrial areas as well as extensive farmlands and numerous confined animal feedlot operations. The NRE is a relatively shallow estuary with circulation driven primarily by wind rather than tides and with slow flushing rates in the summer (63 d, Deamer 2009). Hypoxia develops on the river bottom as the processes decomposing organic materials use up oxygen, and the water column becomes stratified because of low wind-driven mixing, particularly as water warms up during summer months. Hypoxia can quickly spread upward in the water column when no wind mixing occurs to bring oxygenated waters to the bottom. Strong, sustained winds can force downwelling of oxygenated waters on the downwind side of the river, displacing the hypoxic layer into the shallow areas on the upwind side of the basin and creating a seiche event; when the winds relax, the hypoxic layer can quickly flow back to the other side of the basin (Reynolds-Flemming & Luettich 2004). For example, winds from the southwest cause hypoxic bottom water to upwell along the southwest shoreline, but after winds relax or turn from the northeast, hypoxic water upwells on the northeast side of the river (Reynolds-Flemming & Luettich 2004). For our study transect, we selected a cross section of the NRE known for hypoxia dynamics in other studies (Luettich et al. 2000, Bell et al. 2003), with areas that range from typically oxygenated to chronically hypoxic. While several studies have documented water quality along transects down the middle of the NRE (e.g. Luettich et al. 2000), no study has continuously monitored across the width of the river for an extended period. Our transect was located 19 river km downstream of the Highway 70 bridge in New Bern, North Carolina, and extended from just upriver of the mouth of Slocum Creek to just downriver of the mouth of Beard Creek (Fig. 1). At this location, the river is oriented from northwest (upstream) to southeast (downstream), so prevailing winds generally blow across the river, creating seiche events.

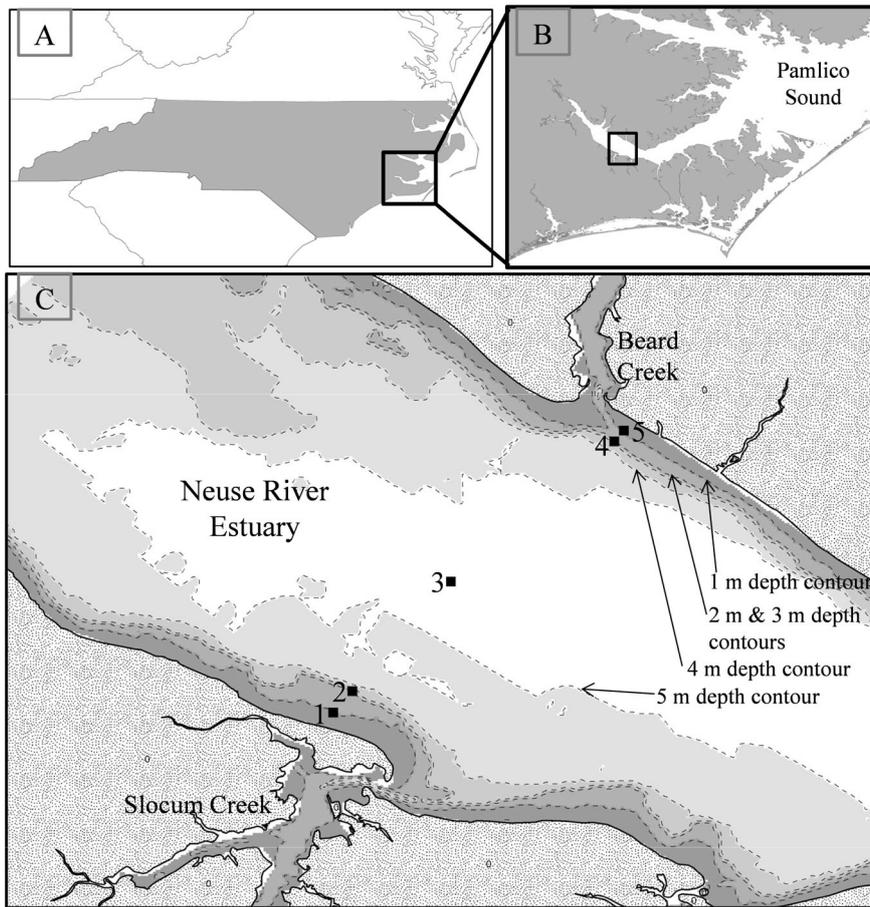


Fig. 1. (A) Study location in relation to North Carolina, (B) section of state showing Pamlico Sound and portion of the Neuse River Estuary (NRE) where study sites were located, (C) location of study sites in the NRE with depth contours. Site 1: nearshore, typically oxygenated site on the southeast side of the estuary, ~1 m depth, ~173 m from shore. Site 2: intermediate hypoxic site on the southeast side of the estuary, ~2 to 3 m depth, ~621 m from shore. Site 3: chronically hypoxic site in the middle of the estuary, ~4 m depth. Site 4: intermediate hypoxic site on the northwest side of the estuary, ~2 to 3 m depth, ~366 m from shore. Site 5: typically oxygenated site on the northwest side of the estuary, ~1 m depth, ~185 m from shore

Water quality monitoring

During the summer of 2007, 5 YSI 600XLM data sondes were deployed at sites along the transect across the NRE (Fig. 1) ~30 cm off the bottom and recorded temperature, salinity, DO, and depth every 15 min from early summer (May 25, 2007) to late summer (final retrieval September 24, 2007). Both nearshore stations (173 to 185 m from shore, ~1 m depth, south shore = Site 1, north shore = Site 5) were chosen to represent typically oxygenated areas with minimal severity of hypoxic conditions. The center station (midway between the 2 shores, ~4 m depth, Site 3) was chosen to represent areas characterized by substantial, prolonged hypoxic events. Stations located between the mid-river site and nearshore sites (621 m from south shore, Site 2, and 366 m from north shore, Site 4, ~2 to 3 m depth) represented areas that we expected would be more intermittently affected by hypoxia. Sondes were serviced every 5 to 8 d (typically 5 d, but once 10 d between cleanings) to clean off any accumulated debris and download data.

Fish sampling

We conducted approximately weekly nighttime sampling trips (total 14, May to September) to estimate the density of spot and other fish species at 5 sites corresponding to the data sonde locations. We conducted nighttime sampling to reduce visual detection and gear avoidance in shallow waters, to target fish after potentially increased feeding activity at dusk, and to sample when occurrences of hypoxic events were more likely. Each site was sampled with an otter trawl (6 m head rope and foot rope with tickler chain attached, 20 mm bar mesh wings, 5 mm bar mesh cod end) pulled parallel to shore at constant speed for 2 min (average track length ~200 m). Sites were trawled once in order from south to north, and then the same sequence was repeated 2 more times (for a total of 3 trawls at each site per sampling night). If the mid-river location (Site 3) data sonde records showed that DO levels had been continuously hypoxic over the previous week, the water column was hypoxic to a depth beyond the height range of the trawl net (at least 1 m above the bottom), and no fish were

caught during the first trawl set, it was not re-trawled during the other 2 sets. At the beginning and end of each trawl we recorded water quality readings (temperature, salinity, DO) ~10 cm below the surface and ~30 cm off the bottom using a hand-held YSI 600QS sonde. In addition, water quality data from stationary data sondes near trawl sites were collected; data sondes had been serviced within the prior 2 d. For samples with fewer than ~200 fish, all individuals were identified to species and counted. For each species, we measured a subset of 20 individuals or all individuals if fewer than 20 were collected. For larger samples, all individuals of rare species were identified, counted, and measured. The remaining sample was divided in half or quarters to obtain a subsample of ~200 fish, and the remaining fish were identified and counted. Total number of fish for a subsampled trawl was estimated by multiplying the number of fish in the subsample by the subsample's proportion of the total catch. Fish density was calculated by dividing the total number of fish caught by the area trawled, then multiplying by 3.125 to account for an estimated trawl efficiency of 32%, based on a study by Kjelson & Johnson (1978) using the same net construction, similar-sized spot, and similar depths and conducted in a nearby estuary. Trawled area was calculated by multiplying track length (determined from GPS points taken at the beginning and end of each trawl) by the width of the net mouth while fishing (estimated as 5 m, based on Kjelson & Johnson 1978). Samples were assigned to 3 DO categories based on the average of bottom DO readings at the beginning and end of the trawl: DO < 2 mg l⁻¹, DO between 2 and 4 mg l⁻¹, and DO > 4 mg l⁻¹. If DO readings at the beginning and end of a trawl indicated that the trawl track crossed the hypoxic boundary (one reading < 2 mg l⁻¹, one reading > 2 mg l⁻¹), the sample was not included in any of the above categories for analyses.

A subset of spot from each trawl (20 ind., when available) was sealed in a plastic bag and flash frozen in dry ice-ethanol slurry; later, fish were thawed, weighed (nearest 0.01 g), and measured (nearest mm standard length, SL), and stomachs were removed and placed in 10% formalin. Individual stomachs were later weighed to the nearest 0.001 g. An Index of Feeding was calculated by dividing the whole stomach wet weight by the wet weight of the fish.

To characterize the relative density of potential predators in relation to water quality, we conducted weekly nighttime gillnetting at each site. One experimental gillnet of 2.44 m height with 50 m sections of 31.8, 50.8, 63.5, and 76.2 mm bar mesh was set on the bottom parallel to shore at each station starting at

sunset, with a minimum soak time of 2.5 h. Water quality readings (temperature, salinity, DO) were recorded ~10 cm below the surface and ~30 cm off the bottom when nets were set and picked up. All fish caught were identified to species and counted, and potential predators were measured to the nearest mm SL. Potential predators were determined as carnivorous fish with a gape that would allow ingestion of fish > 60 mm SL. Catch per unit effort (CPUE) was calculated as the number of fish caught per hour soak time of the gillnet; a separate CPUE for potential predators only was calculated similarly.

Sites 1 and 5 were characterized as areas of oxygenated refuge from hypoxia, which we then assigned to 2 different habitat compression states: refuge not compressed or refuge compressed. The refuge was defined as not compressed if both its DO reading and that of its adjacent site (Site 2 or Site 4) were above 2 mg l⁻¹; the refuge was compressed if its DO reading was above 2 mg l⁻¹, but DO at its adjacent site was below 2 mg l⁻¹. A special case was also considered when there was effectively no refuge because both DO readings were below 2 mg l⁻¹, so while not a true refuge, this case is considered in our analysis (labeled by 'hypoxic') to give a representation of all conditions experienced.

Data analysis

Records of temperature, salinity, and DO from the remotely deployed data sondes were processed using Aquarius 2.0 software (Aquatic Informatics). When necessary, DO data from the 48 h preceding data sonde servicing were corrected to adjust for reduction in readings because of biofouling using a function within the Aquarius software that generated a prorated linear drift correction over time. To determine if a correction was needed, we compared the last 4 to 6 DO readings prior to servicing with the first 4 to 6 DO readings after sonde cleaning and calibration and with readings taken near the sonde with a calibrated hand-held YSI 600QS unit just before or after the sonde was serviced; if these data indicated that readings just prior to servicing were depressed > 0.75 mg l⁻¹, then the post-calibration values were used in conjunction with the Aquarius function to correct for drift. Less than 8% of our water quality records required any correction, and the only analysis in which these corrected data were used was to determine the number and duration of hypoxic events over the summer. We defined hypoxic events as follows: an event started when a DO reading was ≤ 2.0 mg l⁻¹ and

ended when 2 consecutive readings were $>2.5 \text{ mg l}^{-1}$. Then, we characterized frequency and duration of events at each site by month. Rate of onshore movement of the hypoxic front was estimated by dividing the time between the first hypoxic reading at Site 2 (or 4) and the first hypoxic reading at Site 1 (or 5) by the distance between the 2 data sondes. For ease of viewing in figures, water quality data are presented as a running average, such that for a particular time point, readings from a specified interval (45 min or 3 h) both before and after that point are averaged, and the resulting value is plotted on the figure. We examined patterns of DO and salinity in relation to wind speed and direction over short-term (1 wk) and long-term (3 mo) time periods. Wind data were obtained from the State Climate Office of North Carolina for measurements taken at Cherry Point Marine Corps Air Station in the form of hourly average wind speed and direction. Finally, we compared water quality readings from opposite sides of the NRE to evaluate if any relationship between the 2 could be detected.

The calculated value for fish density (fish m^{-2}) from each trawl sample was transformed ($\log_{10}(\text{density} + 1)$) to account for zeros and to meet the assumptions of normality and equality of variance for statistical analysis, but we present untransformed data in the 'Results' section for ease of interpretation. Gillnet CPUE values were also transformed ($\log_{10}(\text{CPUE})$) to meet the assumptions of normality and equality of variance, but we present untransformed data in the 'Results' section. The difference in densities and CPUE among water quality conditions was tested by ANOVA. Comparisons were also tested among the different habitat compression categories for both densities of fish from trawl samples and gillnet CPUE data. Index of Feeding values from the subsample of spot from each trawl were averaged to give a single Index of Feeding value for each trawl. When testing for fish length effects on Index of Feeding, individual values were used. The Index of Feeding proportions were arcsine-square root transformed and tested for differences in both water quality and habitat compression categories; untransformed values are presented in the 'Results' section. All analyses were performed in JMP 9 (SAS Institute) with an alpha level of 0.05.

RESULTS

Dissolved oxygen

DO concentrations varied substantially over the short term (hours to days) and long term (weeks to months) on both temporal and spatial scales. Hypoxic events were dynamic, with changes in DO at a site often dropping from $>6 \text{ mg l}^{-1}$ to $<2 \text{ mg l}^{-1}$ in $<1 \text{ h}$ and recovering from hypoxia just as quickly. We often recorded many hypoxic events within a week at an individual site (Fig. 2) and as many as 4 in a single day. During a typical 5 h sampling period, all 3 DO categories could be recorded. Site 3 was hypoxic 75% of total recordings for the summer, with fewer individual events but with each event longer (average 48.5 h) and a single continuous event lasting 498 h (Table 1). Intermediate sites (Sites 2 and 4) had higher percentages of time spent hypoxic (16 and 27%, respectively) than their corresponding near-shore counterparts. August exhibited the highest occurrence of total individual hypoxic events. Site 4 had the highest number of events for the season, 83, with an average duration of 7.74 h.

Consistent with observations reported by Bell et al. (2003), Reynolds-Flemming & Luettich (2004), and

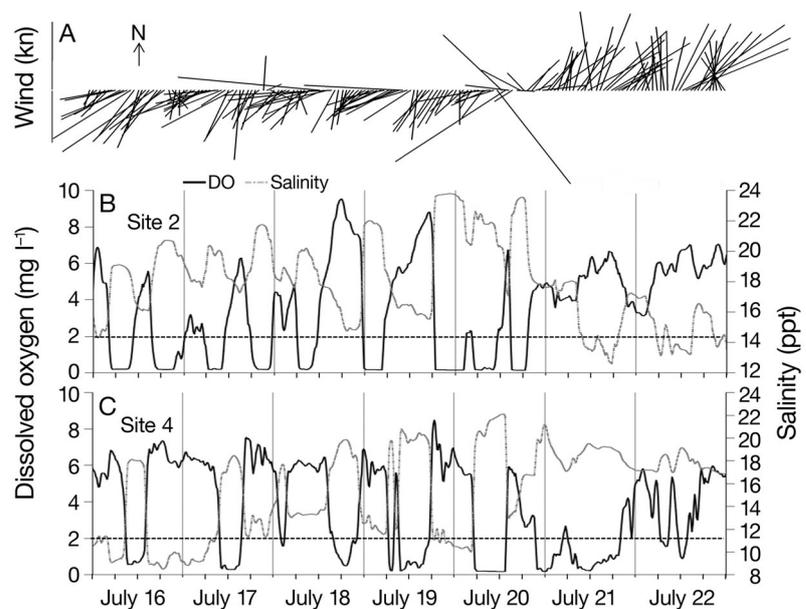


Fig. 2. (A) Feather plot of wind speed (longer lines indicate increased speed) and direction (vertical line extending upwards indicates winds from the north) at hourly intervals from July 16 to July 22, 2007. Average wind speed 8 knots (kn) and maximum wind speed 21 kn over the time period shown. Dissolved oxygen (DO) and salinity values (45 min running averages) for (B) Site 2 and (C) Site 4 over the same time period. x-axis starts at 00:00 h on July 16, 2007; vertical grey lines denote 00:00 h each day, and small tick marks are at 6 h intervals. Dashed horizontal lines indicate hypoxia (2 mg l^{-1} DO)

Table 1. Number of hypoxic events, average duration, and maximum duration in hours by month and site

Site	June			July			August			Overall		
	No. of events	Avg. duration (h)	Max. duration (h)	No. of events	Avg. duration (h)	Max. duration (h)	No. of events	Avg. duration (h)	Max. duration (h)	No. of events	Avg. duration (h)	Time <2 mg l ⁻¹ (%)
1	9	5.3	14.25	11	5	17.5	19	5.1	16	39	5.1	11
2	17	5.2	15	22	5.6	17.75	26	6.7	23.8	65	5.9	16
3	10	52.2	304.3	8	77.1	498	12	27	136.5	30	48.5	75
4	27	5.7	29.5	33	4.4	14.5	23	14.9	93.3	83	7.7	27
5	2	2.3	3.3	8	4.3	13.75	21	10.3	85.8	31	8.2	22

Eggleston et al. (2005), the hypoxia dynamics we observed were driven by wind, with winds from the southwest associated with hypoxic upwelling on the south side of the estuary and winds from the northeast associated with hypoxic upwelling on the north side of the estuary (Fig. 2). The relationship between wind direction and speed and the inverse patterns of hypoxic events on opposite sides of the estuary held

throughout the whole June–August period (Fig. 3). Because of the role wind plays in hypoxia dynamics, we observed an approximate inverse relationship between DO concentrations on opposite sides of the estuary over the season (Fig. 4). Generally, hypoxic conditions tended to occur between 17:00 and 07:00 h on the southwest side of the estuary and between 03:00 and 16:00 h on the northeast side of

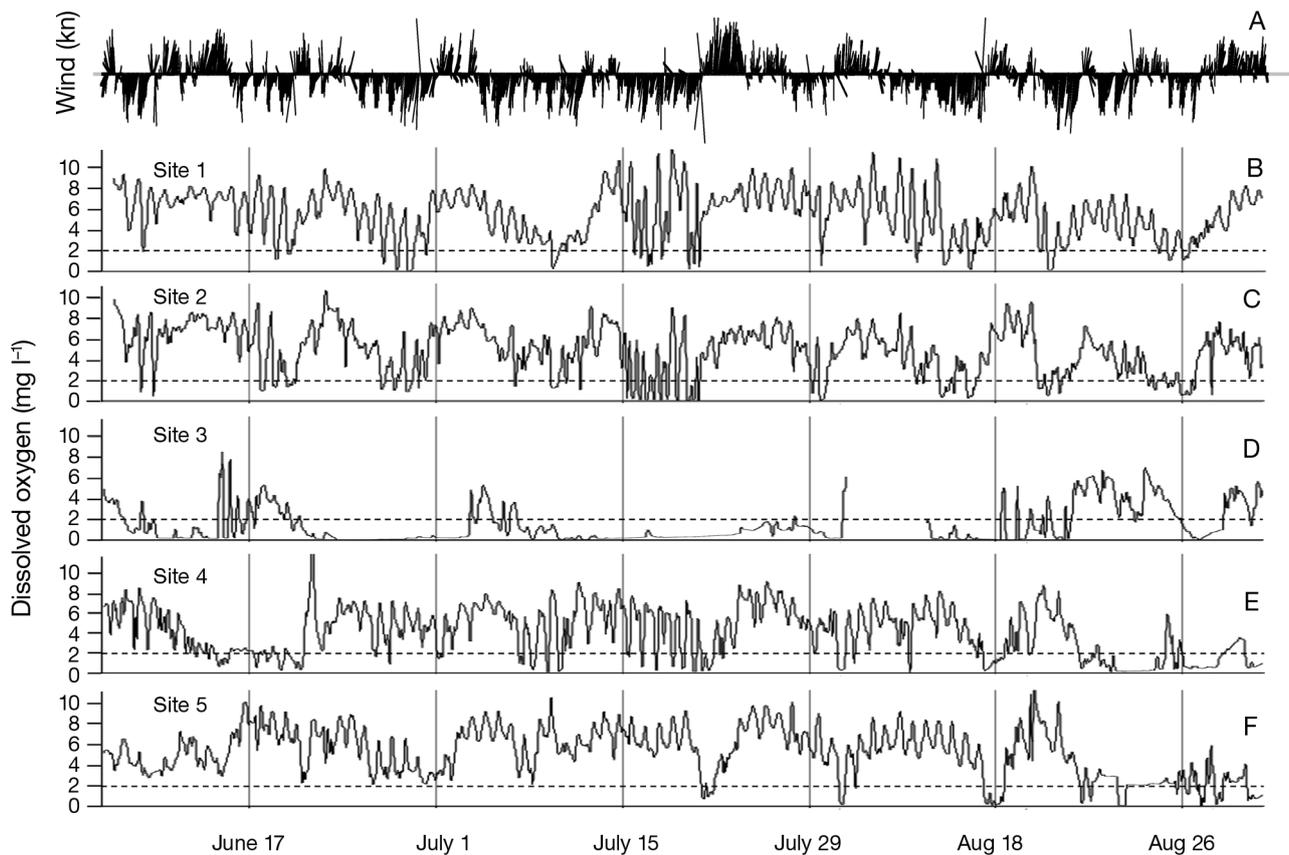


Fig. 3. (A) Feather plot of wind speed (longer lines indicate increased speed) and direction (vertical line extending upwards indicates winds from the north; much of the easterly and westerly slant is compressed because of the long time period shown as an artifact from plotting program) from June 6 to August 31, 2007. Average wind speed 6 knots (kn) and maximum wind speed 21 kn over time period shown. Dissolved oxygen (DO) concentration (plotted as a 3 h running average for clarity) for Sites (B) 1, (C) 2, (D) 3, (E) 4, and (F) 5. Sites are ordered from south shore (top) to north shore (bottom). Dashed horizontal lines indicate hypoxia (2 mg l^{-1} DO)

the estuary (Fig. 4). This pattern reflects the tendency for winds to increase in strength from the southwest during the afternoon and then die down after sunset. The relaxation of wind strength reduces upwelling, allowing seiche events to take place, where previously upwelled hypoxic water would move to the opposite side of the estuary before settling to the bottom of the basin, much like a pendulum swing. The average speed (\pm SE) of the onshore movement of the hypoxic front on the southwest side of estuary was $6.97 \pm 1.74 \text{ cm s}^{-1}$ with a maximum speed of 52 cm s^{-1} . On the northeast side of the estuary, the average speed of the hypoxic front was $4.8 \pm 1.6 \text{ cm s}^{-1}$ with a maximum speed of 24 cm s^{-1} .

In general, lower DO is associated with higher salinities because of stratification in the river. Thus, a decline in DO at a site caused by wind-driven movement of bottom waters is also typically accompanied by an increase in salinity. Over the short term, salinity values can be coupled with DO, with a stronger relationship in some periods (e.g. Site 2: June 17–23, $R^2 = 0.51$, $p < 0.0001$; July 16–22, $R^2 = 0.73$, $p < 0.0001$) but a weaker relationship in others (e.g. Site 2: July 1–7, $R^2 = 0.11$, $p < 0.0001$; August 26–31, $R^2 = 0.20$, $p < 0.0001$) (Fig. 2). However, on a longer time scale, the relationship between DO and actual salinity values breaks down because bottom-water salinity can change seasonally. For example, in 2007 average bottom salinity at Sites 2 to 4 changed from 9–13 ppt in June to 14–19 ppt in July and 15–17 ppt in August. As a result, although DO was significantly related to salinity, only a small portion of the variability in DO was explained by salinity at Sites 1 to 5 when tested over June through August (linear regression, all $p < 0.0001$, R^2 values: Site 1 = 0.14, Site 2 = 0.28, Site 3 = 0.26, Site 4 = 0.13, and Site 5 = 0.05). Even when regressions were run for data separated

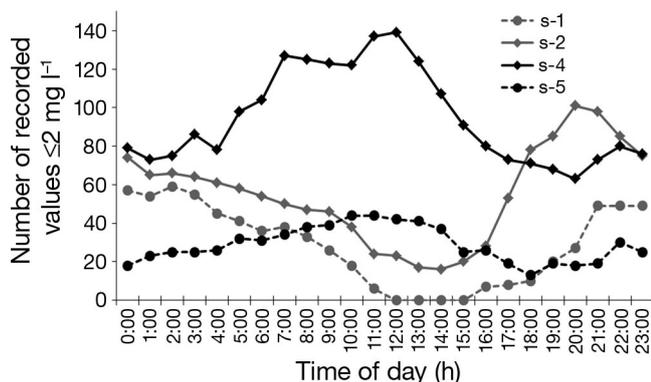


Fig. 4. Number of dissolved oxygen (DO) measurements $\leq 2 \text{ mg l}^{-1}$ by time of day (sum of readings taken during the hour) for Sites 1, 2, 4, and 5, June through August 2007

by month, the highest R^2 was 0.61 ($p < 0.0001$) for Site 3 in June, but most R^2 values were less than 0.30, with linear regression of DO on salinity not significant at Site 5 in June ($p = 0.83$) or July ($p = 0.07$).

Fish sampling

We conducted 149 trawls from May 29 to September 22, 2007. Our catch was comprised of 96% demersal species and included (in descending order of abundance) Atlantic croaker, spot, blue crab *Callinectes sapidus*, southern flounder, brown shrimp *Farfantepenaeus aztecus*, hogchoker *Trinectes maculatus*, summer flounder, bighead searobin *Prionotus tribulus*, and blackcheek tonguefish *Symphurus plagiusa*; of those, spot made up 42% and Atlantic croaker made up 56%. Non-demersal species included (in descending order of abundance) bay anchovy *Anchoa mitchilli*, pinfish *Lagodon rhomboides*, menhaden, harvestfish *Pepilus paru*, bluefish *Pomatomus saltatrix*, white mullet *Mugil curema*, silver perch *Bairdiella chrysoura*, and American eel *Anguilla rostrata*. Average size of spot increased from $70 \pm 0.5 \text{ mm SL}$ (mean \pm SE) in June to $93 \pm 1.3 \text{ mm SL}$ in August; croaker were slightly larger, increasing from $82 \pm 0.6 \text{ mm SL}$ in June to $110 \pm 1.2 \text{ mm SL}$ in August.

Throughout the season, density of demersal fish in trawl samples was strongly related to oxygen concentration. Since 96% of our trawls were made up of demersal species, the densities we report are those of demersal species only. As expected, overall fish densities declined over the season (1-way ANOVA, $df = 156$, $F = 12.07$, $p < 0.0001$; Tukey's HSD, $p < 0.05$; fish m^{-2} : June 0.45 ± 0.07 = July 0.28 ± 0.05 > Aug 0.09 ± 0.05 = Sept 0.03 ± 0.01). Mean density of fish from normoxic waters ($\text{DO} > 4 \text{ mg l}^{-1}$) was almost twice that from waters with DO between 2 and 4 mg l^{-1} and 15 times greater than densities where DO was less than 2.0 mg l^{-1} (fish m^{-2} : 1.05, 0.62, 0.06, respectively; 1-way ANOVA, $df = 138$, $F = 12.99$, $p < 0.0001$) (Fig. 5). All samples from waters where DO was greater than 2.0 mg l^{-1} contained fish; in contrast, 38% of samples from water with less than 2.0 mg l^{-1} DO had zero fish.

Densities of fish in oxygenated nearshore refuges were strongly affected by the extent to which hypoxia compacted these areas. When the nearshore oxygenated refuge was compressed (i.e. the adjacent site was hypoxic), mean density of fish (0.98 fish m^{-2}) was almost twice the mean density (0.55 fish m^{-2}) of samples in the same areas when the refuge was not compressed (i.e. the adjacent site was normoxic) and

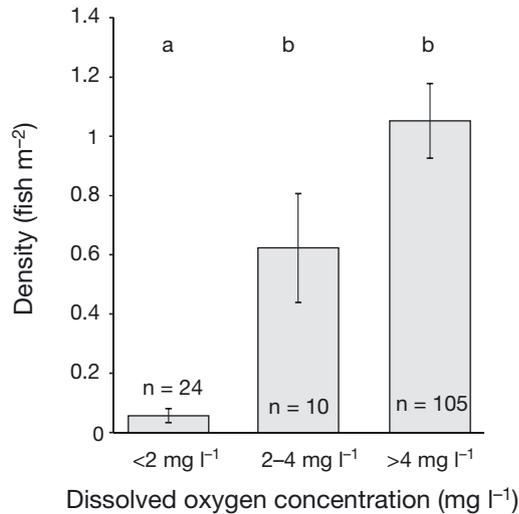


Fig. 5. Mean density \pm SE of fish collected in trawls conducted in different water quality conditions: dissolved oxygen (DO) <2 mg l⁻¹, DO 2 to 4 mg l⁻¹, and DO >4 mg l⁻¹; different letters above error bars denote statistically significant differences determined by Tukey's HSD

greater still than in the few cases when the whole area was hypoxic so there was no oxygenated refuge (0.13 fish m⁻²) (1-way ANOVA, df = 65, $p = 0.0247$) (Fig. 6).

Fish in compressed refuges had not been eating as much recently as fish in non-compressed areas during early summer. Index of Feeding in refuge areas did not vary significantly with time of day sampled (1-way ANOVA, df = 61, $F = 0.46$, $p = 0.19$) but did vary by Month (1-way ANOVA, df = 3, $F = 4.43$, $p = 0.0071$). Index of Feeding in the compressed refuge in June was about half (0.0063) of that when the refuge was not compressed (0.0108) (t -test, df = 18, $p = 0.04$); there was no difference in Index of Feeding in other months (t -tests: July, df = 25, $p = 0.97$; August, df = 2, $p = 0.56$; September, df = 4, $p = 0.39$) (Fig. 7). Index of Feeding declined slightly as the season progressed (Fig. 7). To investigate whether or not fish size had an allometric effect on Index of Feeding leading to a decrease over the season and to evaluate whether the Index of Feeding changed over the season (e.g. because of a seasonal change in food availability), we used values from individuals collected at Sites 1 and 5 when the refuge was not compressed (so as to not confound with refuge size) and ran a 2-way ANOVA to test for effects of Length (SL, mm), Month, and their interaction. Only Month was a significant factor (2-way ANOVA, df = 377, $F = 12.34$, $p < 0.0001$; effects tests: SL, df = 1, $p = 0.87$; Month, df = 3, $p < 0.0001$; Month \times SL, df = 3, $p = 0.93$). Index of Feeding from spot collected during a prolonged hypoxic event from August 8 to 10, 2007, when no

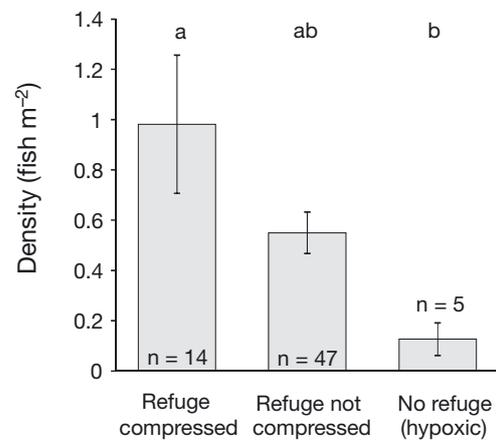


Fig. 6. Mean densities \pm SE of fish in trawls conducted at nearshore sites when the refuge was compressed, not compressed, or no refuge (hypoxic); different letters above error bars denote statistically significant differences determined by Tukey's HSD

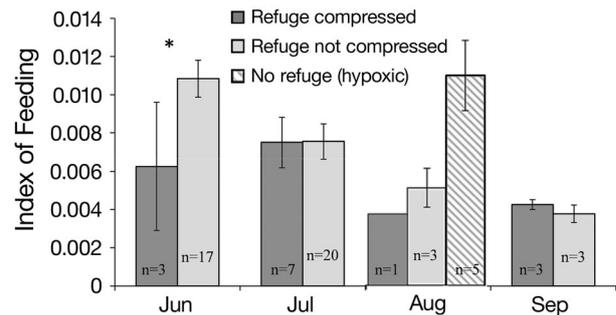


Fig. 7. Mean Index of Feeding \pm SE in refuges that were compressed and not compressed. Asterisk indicates significant difference in June. The special case of no refuge (hypoxic) is shown for August (the only month such cases occurred) but was not tested against other values. Values of n in each bar indicate the number of trawls from which fish were collected

refuge was available, was higher (1.4 ± 0.14) than average Index of Feeding during any other water condition (Fig. 7).

We gillnetted on 8 different nights from May 30 to September 23, 2007. Catches were comprised of 18% predators (bluefish, blue crab, longnose gar *Lepisosteus osseus*, Spanish mackerel *Scomberomorus maculatus*, southern flounder, red drum *Sciaenops ocellatus*, and spotted seatrout *Cynoscion nebulosus*) and 82% non-predators (menhaden, cownose ray *Rhinoptera bonasus*, Atlantic croaker, harvest fish, silver perch, spot, Atlantic stingray *Dasyatis sabina*, pinfish, and pompano *Trachinotus carolinus*), which either did not eat demersal fish or were not of the size range able to eat demersal fish. Bluefish were the most common predator caught in our gillnets (n =

527), with a trend of increasing numbers as the season progressed. There was no difference in gillnet total fish CPUE (fish h^{-1}) whether conditions were hypoxic during the net set (12.03 ± 5.19) or not (44.18 ± 7.34) (t -test, $df = 42$, $p = 0.054$) or whether the refuge was compressed (34.87 ± 23.3) or not (49.83 ± 9.84) (t -test, $df = 21$, $p = 0.52$). Predator CPUE in gillnets was not different whether conditions were hypoxic (5.76 ± 1.04) or not (6.16 ± 1.82) (t -test, $df = 41$, $p = 0.93$) or whether the refuge was compressed (5.48 ± 1.18) or not (6.32 ± 1.45) (t -test, $df = 20$, $p = 0.66$). There was no difference in predator CPUE between nearshore sites (6.13 ± 1.14) and intermediate sites (4.06 ± 0.79) (t -test, $df = 41$, $p = 0.33$).

DISCUSSION

Our study shows that fish detect and avoid hypoxia in the field and can do so in response to rapidly changing conditions (minutes to hours). Throughout the season, fish were able to move away from incursions of hypoxic water and then rapidly redistribute into affected areas after these events passed; fish responded this way repeatedly over the summer instead of permanently emigrating from areas that frequently experienced hypoxic events. Eby et al. (2005) found that early in the season, benthic prey were more abundant in deeper sites (similar to our intermediate sites), which may explain why fish return to these areas despite frequent hypoxic events. Our results supplement findings by others that fish move away from hypoxia (e.g. Pihl et al. 1991, Tyler & Targett 2007, Keller et al. 2010) but are unique in that we documented this behavior in response to hypoxic events occurring on the scale of hours and recurring repeatedly over several months.

The added movement and interactions associated with repeatedly avoiding hypoxic waters may have greater cumulative energetic and mortality consequences than evading only a few stable, long-term events over the course of a season. Energetic costs of swimming increase exponentially with swimming speed (Fry 1971). The average onshore movement rate of hypoxic waters we observed (5 to 7 $cm\ s^{-1}$) was similar to previous calculations by Luettich et al. (2000) of 6 $cm\ s^{-1}$; however, several hypoxic events greatly exceeded the average onshore speed (26 to 52 $cm\ s^{-1}$), illustrating how quickly hypoxic waters can move and fish must promptly evade. Telemetry tracking of juvenile spot in relation to oxygen conditions documented spot swimming at speeds up to 3.5 body lengths s^{-1} when avoiding hypoxia (J. K.

Craig pers. comm.). Based on the average sizes of fish in our samples, spot could swim up to 28 $cm\ s^{-1}$ and croaker 33 $cm\ s^{-1}$ to avoid hypoxic waters, making it possible for them to outswim the average onshore movement of hypoxia but unlikely to outswim our fastest calculated hypoxic movement of 52 $cm\ s^{-1}$. Some fish will likely be caught in hypoxic waters during their escape and will be subject to greater direct hypoxia consequences during this time. Many benthic species can move not only horizontally away from hypoxia but also vertically out of the more dense hypoxic waters into overlaying normoxic waters. Moving vertically a few meters or less would require negligible energy expenditure compared to prolonged rapid lateral movement. However, this behavior would separate demersal fish like spot and croaker from their normal benthic food sources, and their vulnerability to predators may be elevated while they remain in this less familiar pelagic environment. Even if spot and croaker remain near the bottom during avoidance movement, their ability to feed on benthic prey would likely also be reduced when swimming at elevated speeds. Thus, successful avoidance behavior may still result in negative indirect effects because of increased activity costs, decreased feeding, and potentially increased predation mortality.

If the hypoxic front is moving rapidly, there could be a 'snow plow' effect of fish accumulating on the hypoxic edge while moving to get out of the way of advancing hypoxia, increasing localized densities on the hypoxic front. Craig (2012) found evidence of fish aggregating on the edge of the hypoxic zone in the Gulf of Mexico. Two of our trawls that crossed the hypoxic boundary (i.e. at one end of the trawl DO was $>2\ mg\ l^{-1}$, at the other end DO was $<2\ mg\ l^{-1}$) had very high densities of fish, in the top 10 highest of the study. One trawl was conducted at Site 2 when the end of this trawl went into slightly deeper waters, encountering the hypoxic edge. The second trawl that crossed the boundary occurred at Site 1 at the start of a prolonged hypoxic event as the hypoxic front advanced toward shore. The data sonde at this site showed a rapid decline in DO and then several hours of hypoxia. We visibly observed fish aggregating near shore and then migrating laterally toward the mouth of a nearby tributary. These incidents show fish aggregating on the edge of the hypoxic zone similar to the behavior observed by Craig (2012) but on a much smaller scale. If fish are staying in these edge areas with increased density, then even more inter- and intra-species interactions could take place than we can accurately describe from the samples we collected.

Because 38% of trawls under hypoxic conditions collected no fish and the remainder collected very low numbers of fish, it is worth considering why fish were located in hypoxic waters at all. The presence of at least some of the fish in our trawls under hypoxic conditions might be an artifact of the mouth of the net extending into normoxic waters at times, catching fish that were not truly in hypoxic water. Although we did not get many fish in our trawls when sampling at intermediate sites when the bottom was hypoxic, we noted that the depth finder sonar image showed a layer of fish just above the upper boundary of the hypoxic layer. Pihl et al. (1992) suggested that fish take advantage of invertebrate prey temporarily stunned by hypoxia, and others have noted that benthic prey migrate vertically to the sediment surface during hypoxia exposure (Diaz & Rosenberg 1995, Taylor & Eggleston 2000), so the fish in these areas could be exploiting this resource (as reported by Roberts et al. 2012). We observed one instance in our study when spot may have been employing this strategy, as the Index of Feeding (0.013 ± 0.002) of spot collected in low DO waters during a prolonged hypoxic event on August 8 to 10 was 2 times higher than that of spot collected during normoxia (0.0058 ± 0.001). However, prolonged events such as this did not occur frequently at our nearshore and intermediate sites. Some researchers suggest that short hypoxic events (less than 6 h), such as those observed in our study, might not last long enough for infauna to migrate to the sediment surface, reducing the potential profitability of foraging as a behavioral response to hypoxia (Bell et al. 2003). Fish may also choose to stay in hypoxic waters to avoid predators; Froeschke & Stunz (2012) found through tank experiments that pinfish and Atlantic croaker would not go into hypoxic waters for food but would do so to avoid predators. We did not find evidence of highly increased predator density in oxygenated refuges that would drive fish into hypoxic waters. Despite the myriad reasons that could drive fish to occupy hypoxic waters, fish rarely did so in our study.

Our results showed that hypoxia has the potential to indirectly reduce growth of juvenile fish through increased densities and reduced feeding success in oxygenated refuges during periods of habitat compression. Although we only detected a decrease in Index of Feeding in June, that we were able to detect a decrease in Index of Feeding at all was surprising considering that, on average, fish were collected after only 3 h of habitat compression. Therefore, more prolonged periods of habitat compression may result in an even greater reduction in feeding suc-

cess. Since prey densities are reported to be higher earlier in the summer (Buzzelli et al. 2002, Eby et al. 2005), a lack of differences between the 2 groups later in the summer may simply be because insufficient resources were available for a difference to manifest in such a short period. Reductions in feeding or a shift in diet to less nutritional prey items could result in reduced growth (Eby et al. 2005, Powers et al. 2005). Intermittent hypoxia altered prey availability to croaker in Eby et al.'s (2005) study; they cited a decrease in food as the main cause for decreased croaker growth rates. Reduced food resources and increased competition not only for food but also for space leads some species, such as Atlantic croaker, to escalate aggressive behaviors toward conspecifics (Gibbard et al. 1979).

The nearly 2-fold increase in demersal fish densities in nearshore oxygenated refuges during periods of habitat compression has the potential to cause density-dependent effects on mortality and growth. Given that 98% of demersal fish caught were a combination of spot and croaker, it is not unreasonable to use total demersal fish densities when considering density-dependent effects on either species. Spot and Atlantic croaker distributions overlap strongly in the NRE (Eby & Crowder 2002; this study), and these species share similar diets (Stickney et al. 1975). Thus, a spot would likely experience the same density-dependent effect from both other spot and croaker. Density-dependent effects are common and can occur over multiple spatial and temporal scales (Hixon & Jones 2005). Several studies have concluded that density dependence acts most intensely on growth at low to moderate abundance levels, such as those seen in our study (Jenkins et al. 1999, Grant & Imre 2005, Martino & Houde 2012). Craig et al. (2007) measured spot growth at varying densities in experimental ponds and found a non-linear relationship between density and growth ($\text{growth} = \alpha e^{(\beta \times \text{density})}$, where $\alpha = 0.719 \pm 0.042$, $\beta = -0.426 \pm 0.056$). When we apply this equation to the fish densities we observed, the estimated growth rate of fish in compressed refuge areas (0.47 mm d^{-1}) is 17% lower than the estimated growth of fish in refuge areas that were not compressed (0.57 mm d^{-1}). These growth rates fall into the range of growth observed in wild spot (Weinstein 1983, Weinstein et al. 1984). Because prey densities and other conditions in the ponds may have been different than those in the field, the relative difference between the 2 estimates is more reliable than the actual growth rates estimates for fish in the field. In a field cage study, Eby et al. (2005) found that croaker growth rates at low densities (0.67 fish m^{-2})

were twice those at high densities (2.67 fish m^{-2}); hence, increased densities in compressed refuges would similarly affect croaker growth.

However, over the course of the summer, nearshore oxygenated refuge areas were only compressed an average of 21.5% of the time. Thus, the cumulative effect over the season would be much smaller: only a 4% reduction in growth ($0.785 \times 0.57 \text{ mm d}^{-1} + 0.215 \times 0.47 \text{ mm d}^{-1} = 0.55 \text{ mm d}^{-1}$). Such a modest decrease in growth may seem to indicate that these effects are not worth considering, but 2007 was a mild year of hypoxia; during years with more severe hypoxia, growth reduction could be more substantial. For example, if refuges were compressed 50% of the time, then estimated reductions in growth would be 9%. In all likelihood, wild fish will have lower growth than fish in ponds or cages at similar densities, as the costs of hypoxia exposure can be exacerbated in the field, where fish increase swimming speeds and distances during exposure (Craig & Rice unpubl. data), food availability can be limited, fish must actively forage and avoid predators, and hypoxia avoidance activity may reduce foraging time. We consider the degree of indirect effects on growth calculated above to be conservative. To draw more accurate comparisons, we recommend measures of actual fish growth in the field.

Levin & Stunz (2005) found that even small changes in the quantity or quality of essential fish habitat will have large effects on population dynamics, and Myers & Cadigan (1993) concluded after a study of density-dependent mortality that the juvenile stage is very important for population regulation of most species. Reduced growth rates can prolong the period that fish remain vulnerable to size-dependent predation, increasing overall mortality of the population (Houde 1987, Sogard 1997). While our methods were unable to conclusively detect an increase in predators in compressed refuges (although the trend of increased predator density was observed), others have shown that increased density of spot increased mortality from predators (Wright et al. 1993) and speculated that species compaction enhances predation risk from blue crabs via harassment and agonism (Eby & Crowder 2002, Bell & Eggleston 2005). Craig et al. (2007) also found that even in the absence of predators, mortality of spot increased with increasing densities.

Decline in the Index of Feeding over the summer is consistent with evidence produced by other researchers that benthic prey composition is altered and reduced over the summer by repeated hypoxic events in the NRE (Bell et al. 2003, Eby et al. 2005, Powers et al. 2005). Allometric effects could possibly

contribute to a decline in Index of Feeding as spot sizes increased over the summer, but we found no evidence of that over the time period and size range of fish we sampled. Decline in available food over the summer would hamper potential compensatory growth of fewer late-summer and fall survivors as pressure from hypoxia decreases (Sogard 1997). Reduced growth can result in smaller size at the beginning of winter, which can lead to reduced survival over the winter (Sogard 1997). Martino & Houde (2012) found that slower growth of age 0 striped bass was linked to a smaller age 1 year class the following spring. Using population modeling, Eby et al. (2005) estimated that reduced growth of Atlantic croaker because of hypoxia in the NRE caused a 4% decline in their population. All of the cascading effects from reduced growth because of hypoxia dynamics could lead to smaller population size and disruption of the prey base for larger predators, among many other possible disturbances of vital ecosystem and trophic links.

Hypoxic events in our study proved to be more spatially and temporally dynamic than we anticipated, especially at our intermediate sites. Other studies of water quality in the NRE (Luettich et al. 2000, Eby & Crowder 2002, Eby et al. 2005, Powers et al. 2005) have typically sampled areas once a day, during daylight hours, and generally at the same time of day (but see Bell et al. 2003 as another example of using fixed water quality instruments). As our data indicate, this sampling approach can detect some hypoxic events but miss the intervening dynamic behavior of hypoxic waters generally driven by prevailing wind patterns. Buzzelli et al. (2002) developed a model to estimate the cross-river extent of hypoxia based on water quality profiles taken down the middle of the NRE. While this model provides useful predictions for some applications, it was developed assuming a level oxycline. Our more detailed cross-river data show that the oxycline is rarely level, as evidenced by the out-of-phase pattern of DO and salinity readings on opposite sides of the NRE. Water quality readings taken at locations deeper than our intermediate sites (more toward the middle of the NRE or middle river, e.g. Reynolds-Flemming & Luettich 2004, Eby et al. 2005) show more stable and longer hypoxic events consistent with our mid-river observations, as they are not on the dynamic edge of the hypoxic water lens. While deeper sites might have less variability in hypoxic events, they are also less likely to be frequented by demersal fish (as demonstrated by small and zero catches at our site 3 in the middle of the estuary; see also Bell & Eggleston 2005); consequently, examining and extrapolating behavior, movement, and con-

sequences of hypoxia from these locations would not reflect the highly variable conditions experienced by the majority of the fish population that inhabits shallower habitats. Water quality projections based on fewer measurements can be useful for large-scale applications and when predicting generalities for a system (e.g. Buzzelli et al. 2002, Bowen et al. 2003), but scientists need to be aware that these models do not accurately depict conditions at the small spatial and temporal scale fish must respond to.

Defining and quantifying the consequences of hypoxia for fish and other living resources via either direct or indirect effects is increasingly important, as fisheries managers must employ ecosystem-based approaches to manage changing environments (Myers et al. 2000). Many studies on hypoxia focus on the direct effects of fish caught in hypoxia, but as our study and others have demonstrated, fish are usually successful in avoiding hypoxia, so more attention needs to be focused on the potential indirect effects of hypoxia and how these effects on the individual level aggregate to the population level. Our study helps us to understand sub-daily movement patterns in relation to hypoxia, which is crucial to predict ecological consequences of hypoxia (Roberts et al. 2012). The highly dynamic aspect of hypoxia in the NRE can lead to many indirect effects that cannot be fully measured and taken into account by conventional means; despite this, we were able to see some coarse effects of hypoxia on fish densities and potential density-dependent effects. When trying to quantify the impact of hypoxia on fish, we must first comprehend the response to fine-scale spatiotemporal changes from dynamic hypoxia before we can determine the consequences of environmental quality changes for fish (Rose 2000). Measuring recent growth of fish responding to hypoxic environments will more accurately depict the toll of direct and indirect costs of hypoxia as a cumulative measure of all the stressors on fish that effect growth.

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