

Article

# Effects of Short-Duration and Diel-Cycling Hypoxia on Predation of Mussels and Oysters in Two Tributaries of the Chesapeake Bay

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**Abstract:** Although the effects of persistent hypoxia have been well established, few studies have explored the community-level effects of short-duration and diel-cycling hypoxia, for example on predator–prey interactions. Consumer stress models predict that mobile predators will flee hypoxia, while prey stress models predict that sessile species, unable to avoid hypoxic water, will be more susceptible to predation. To test these hypotheses, we studied the effects of diel-cycling hypoxia on predation of the hooked mussel, *Ischadium recurvum*, and eastern oyster, *Crassostrea virginica*, in field experiments in two Chesapeake Bay, USA tributaries. We conducted a complementary laboratory experiment that tested the impact of short-duration hypoxia on predation of the two bivalve species by the ecologically and commercially important blue crab, *Callinectes sapidus*. Although we did not observe a significant effect of diel-cycling hypoxia on predation in the field, we did observe an effect of short-duration hypoxia in the laboratory. *Callinectes sapidus* exhibited depressed feeding rates and reduced preference for *I. recurvum* in hypoxic conditions. In both field and lab results, we observed a strong preference of predators for *I. recurvum* over *C. virginica*, indicating that the relatively understudied mussel *I. recurvum* merits greater consideration as a part of estuarine food webs.

**Keywords:** water quality; food web; prey preference; *Crassostrea virginica*; *Ischadium recurvum*; *Callinectes sapidus*

## 1. Introduction

Hypoxia is a condition of reduced dissolved oxygen (DO) in aquatic systems. Hypoxic events can have profound impacts on estuarine and coastal ecosystems. In many systems, hypoxia is linked to anthropogenic eutrophication, promoting phytoplankton and microbial production and diverting energy from higher trophic levels to microbial communities [1]. Diel-cycling hypoxia is not as well studied as more persistent hypoxia, but it is also linked to eutrophication. While seasonal hypoxic events typically develop in deeper, stratified waters and can last on the order of weeks to months, diel-cycling hypoxia occurs daily in eutrophic shallow water systems. In diel-cycling hypoxia, nighttime respiration consumes DO, while daytime photosynthesis replenishes DO in the system [2]. The Chesapeake Bay, the largest estuary in the United States and one of the largest estuaries in the world, experiences an annual summer hypoxic event in the stratified main channel that has been attributed to increases in nitrogen inputs into the Bay [3,4]. The Bay also experiences diel-cycling hypoxia in its shallow-water tributaries [5,6]. The volume of the persistent hypoxia in the Chesapeake Bay is tracked annually [7], however, the volume of water exhibiting diel-cycling hypoxia is not monitored.

While persistent hypoxia can cause mass mortality of macrobenthic species [8,9], diel-cycling hypoxia is not necessarily lethal. It can instead induce behavioral [10,11], reproductive [12],

and physiological [13] changes in estuarine inhabitants. Lethal and sub-lethal thresholds to hypoxia can vary greatly by taxa and these thresholds have been linked to mobility [14]. Mobile species often have lower physiological tolerances to hypoxia but have the ability to flee unsuitable conditions [9,15]. Lacking the ability to physically escape a hypoxic area, sessile species typically have higher tolerances to hypoxia. Many utilize behavioral and physiological responses to survive periods of low dissolved oxygen [14,16–18].

It is possible that sub-lethal responses impact various community-level interactions, such as predator–prey dynamics. Several studies suggest that responses to hypoxia follow a consumer stress model [19], with less tolerant predators fleeing hypoxic areas and creating a predation refuge effect of hypoxia for non-mobile prey [20,21]. Others argue that behavioral responses induced by hypoxia support a prey stress model [22] by increasing the susceptibility of prey to predators [23,24]. For example, some clams reduce burial depth and increase siphon extension during hypoxia, which makes them more vulnerable to predation [24–26].

We conducted a two-part study in the field and the laboratory which investigated the influence of short-duration hypoxia on predation of two common Chesapeake Bay bivalve species, *Ischadium recurvum* and *Crassostrea virginica*. Primary predators of Chesapeake bivalves include the blue crab, *Callinectes sapidus*, and several species of sciaenid and flat fish (*Leiostomus xanthurus*, *Micropogonias undulatus*, and *Trinectes maculatus*). Benthic predator guild assemblage studies indicate that in Rhode River (one of our field sites) *L. xanthurus* and *C. sapidus* are very common (comprising approximately 52% and 13% of predator assemblage, respectively), while *T. maculatus* and *M. undulatus* are less common (comprising 6.3% and 2.5% of predator assemblage) [27]. *Callinectes sapidus* is known to consume bivalves as a significant proportion of its diet, however most of these fish species are more likely to feed on siphons and other benthic infauna [27,28].

In the field, we tested whether diel-cycling hypoxia promoted or deterred predation on bivalves and if the effect was enhanced by the severity of the hypoxic event. Additionally, we measured dissolved oxygen levels prior to field trials to determine if water quality had any lagged effects on predation rates. We hypothesized that, given the lower dissolved oxygen tolerances of mobile predators, hypoxia would cause predators to relocate to less hypoxic areas nearby and thus reduce foraging at our sites. *Callinectes sapidus*, a key benthic bivalve predator, is known to be able to detect and avoid hypoxic conditions [29]. We predicted that this effect would be stronger during more severe hypoxic events, and that the period of decreased foraging would last longer than the period of hypoxia, assuming that predators who fled might not immediately return.

In the laboratory, we tested the feeding response of the blue crab *Callinectes sapidus*, on two common bivalve species, *Ischadium recurvum* and *Crassostrea virginica*, in 14-h predation trials under normoxic (>5 mg/L DO), moderately hypoxic (3 mg/L DO), and severely hypoxic (2 mg/L DO) conditions. These periods of reduced DO were meant to represent the short-duration hypoxia experienced by these organisms due to diel-cycling hypoxia in their natural environment. *Callinectes sapidus* was selected as our focal predator in the laboratory, because it is a bivalve predator that is common in the tidal tributaries where the field study was conducted and known to greatly influence benthic community structure in estuarine environments [30]. Although *C. sapidus* is an opportunistic forager with a varied diet, bivalve molluscs make up a significant portion (up to 40%) of its diet [27,31]. Previous studies have shown that *C. sapidus* exhibits a preference for bivalve prey with lower handling times [32], but this preference has not been explored in the context of hypoxia. Hypoxia reduces the overall feeding rates of *C. sapidus* [33], potentially as a result of decreased prey-handling efficiency [34]. We hypothesized that blue crabs would consume fewer prey overall in lower dissolved oxygen treatments, supporting a consumer stress model [19]. We also hypothesized that a preference would be observed for prey species that were easier to handle and consume under normoxic conditions, but that preference might be less pronounced with increasing hypoxia.

## 2. Materials and Methods

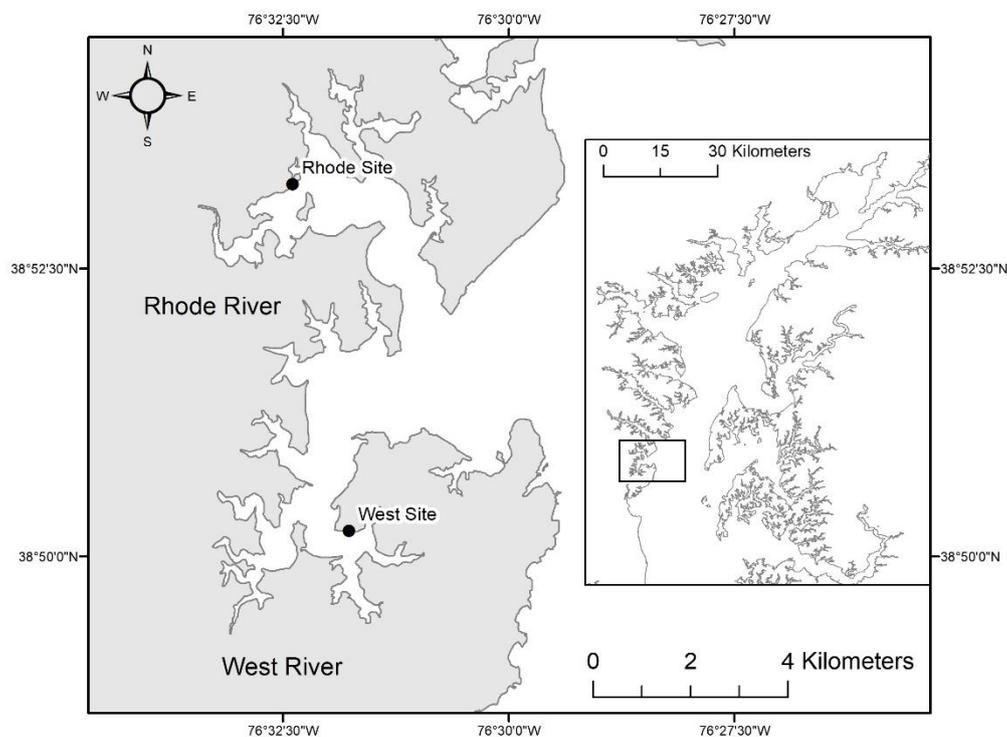
### 2.1. Study Species

Our field and laboratory studies included the eastern oyster (*Crassostrea virginica*) and the hooked (or bent) mussel (*Ischadium recurvum*), two native bivalve species commonly preyed upon by the blue crab, *Callinectes sapidus* [32,35]. *Crassostrea virginica* is a commercially and ecologically important species and the focus of numerous restoration efforts throughout the region [36–38]. *Ischadium recurvum* is an epizoic species often associated with *C. virginica* oyster beds [39–41].

*Crassostrea virginica* were purchased from Marinetics, Inc., an aquaculture facility in Cambridge, MD. *Ischadium recurvum* were manually collected from artificial oyster beds in the Rhode River at the Smithsonian Environmental Research Center and in Prospect Bay at the Chesapeake Bay Environmental Center. In field and lab trials, we used small and similarly sized individuals of both species, <35 mm shell height, to avoid introducing a potentially confounding size refuge for prey items [35,42]. Shell heights of individuals of *C. virginica* selected for trials were  $29 \pm 3.69$  mm (mean  $\pm$  SD) and of *I. recurvum* were  $27.16 \pm 3.04$  mm. Prior to trials, organisms were held in indoor tanks of flowing seawater from the Rhode River.

### 2.2. Field Sites

We conducted our field study in two brackish tributaries of the Chesapeake Bay. The first site was located at the Smithsonian Environmental Research Center (SERC) on the Rhode River, Edgewater, MD; the second site was located at the Chesapeake Yacht Club on the West River, Shady Side, MD (Figure 1). These sites were chosen for their proximity to each other and their distinctive water quality patterns. Both sites experience diel-cycling hypoxia, but hypoxia is generally less severe and less persistent in the Rhode than in the West River. At each site, salinity, pH, dissolved oxygen, chlorophyll, and water temperature were recorded in 15-min intervals by a multi-parameter YSI 6600 sonde (Table S1). Sondes were regularly maintained and data quality assured by SERC at the Rhode River site and by the Maryland Department of Natural Resources Eyes on the Bay Program [43] at the West River site.



**Figure 1.** Map of field study sites in the Rhode and West Rivers, Chesapeake Bay.

### 2.3. Field Experiment

A series of predation mortality trials were conducted at each site ( $n = 14$  at Rhode River,  $n = 11$  at West River) from mid-July to early September—the summer months when diel-cycling hypoxia is regularly observed in these tributaries. Each trial lasted 48 h. In each trial, 10 individuals of each bivalve species were deployed in replicate open mesocosms subject to predation ( $n = 2$  per species per trial) and control mesocosms in which prey were protected from predators by vexar mesh ( $n = 2$  per species per trial). Mesocosms were plastic containers,  $16 \times 16 \times 12$  cm (l  $\times$  w  $\times$  h) in size, lined with oyster shells secured to the container with marine epoxy resin to provide a realistic, structurally complex environment (Figure S1). Each mesocosm contained only one prey species. Bivalves were tethered with fishing line to the shell substrate in each mesocosm and kept in a holding tank for at least 24 h prior to deployment to allow acclimation and for mussels to attach to the shell substrate. In the field, containers were buried flush with the sediment surface in water approximately 1 m deep. Mesocosms were spaced 1 m apart.

Water quality monitoring (YSI 6600) data were available during all trial periods except during and briefly following Hurricane Irene, August 26–28, 2011. During this time, no trials could be completed at the West River site. From the water quality monitoring data, we calculated the duration of moderate hypoxia as the proportion of time under 5.0 mg/L O<sub>2</sub>, a level which can cause sub-lethal effects in mobile species [14], and the duration of severe hypoxia as the proportion of time under 2.0 mg/L O<sub>2</sub>, a threshold used in other studies involving *C. sapidus* [33,44,45]. We examined the duration of moderate and severe hypoxia within three time windows: (1) the 48 h during the trial period; (2) the 24 h preceding the trial; and (3) the combined 72 h of the trial itself and the preceding 24 h. The three time windows were selected to detect: (1) immediate effects; (2) delayed effects due to the departure of predators during an earlier period of hypoxia; and (3) cumulative effects of hypoxia occurring before and during the trial.

For each mesocosm, we calculated the percent predation mortality as the proportion of individuals predated (shell or remnants of shell remained) or entirely missing from the enclosure at the end of the trial. Replicate mesocosms were averaged to calculate the mortality of each species in each treatment during each trial. We calculated predation mortality by correcting the mortality in open mesocosms for the rate of environmental mortality that occurred in control mesocosms using Abbott's correction [46]:  $(X - Y)/X * 100$ , where  $X$  is % survival in controls and  $Y$  is % survival in open mesocosms. Corrected predation mortality data were arcsine square root transformed prior to analysis, and normality checked with a quantile-quantile plot.

We tested the effects of site, bivalve species, duration of moderate hypoxia, and duration of severe hypoxia on predation mortality in multiple linear regression models using the `lm` function in R. To test for immediate, delayed, and cumulative effects of hypoxia, we constructed three regression models corresponding to the three time windows described above. We also tested for main and interactive effects of bivalve species and site on predation mortality in two-way analysis of variance (ANOVA).

### 2.4. Laboratory Experiment

We performed a laboratory experiment to more directly study the effect of short exposure to three dissolved oxygen regimes—normoxia, moderate hypoxia, and severe hypoxia—on blue crab *C. sapidus* predation on the two bivalve species, *C. virginica* and *I. recurvum*. Individuals of *C. sapidus* were captured in crab pots and bottom trawls in the Rhode River for use in the laboratory study. Only adult male crabs over 10 cm in carapace width were used to eliminate differences in preference due to gender or life stage. Crabs were starved for 24 h prior to trials.

Dissolved oxygen regimes were established in seven-gallon aquarium tanks filled with seawater from the Rhode River. Salinity in the lab matched the river salinity level of 8.5 ppt  $\pm$  1.16 SD, and temperature was equilibrated to room temperature, 20 °C. Normoxic conditions ( $> 5.0$  mg/L O<sub>2</sub>) were maintained with aeration. Hypoxia was achieved by bubbling nitrogen gas into tanks until the target dissolved oxygen concentration was reached, and these levels were maintained by sealing the

full tank with a Plexiglas cover secured with duct tape, such that there was no head space in the tank. Plexiglas was also placed on normoxic tanks as a control measure and to prevent crab escape. The target dissolved oxygen concentration was 3.0 mg/L O<sub>2</sub> for the moderate hypoxia treatment and 2.0 mg/L O<sub>2</sub> for the severe hypoxia treatment. After DO levels reached the target level, seven individuals of each bivalve species were placed in each tank along with one blue crab. Crabs were allowed to feed overnight for 14 h, a short-duration of hypoxia similar to that which occurs in the field during diel-cycling hypoxia. In the morning, crabs were removed from tanks and the number and identity of bivalves consumed was recorded. Crabs ( $n = 8$ ) were run in each dissolved oxygen treatment, in random order, in a within-subjects experimental design. Crabs were given rest periods between trials to recover from any potential stress of prior treatments. We ran most crabs (6 of 8) through each dissolved oxygen regime twice, to reduce within-subject error.

We calculated the proportion of each prey species and total number of prey consumed by the crab in each trial. Proportion data were arcsine square root transformed prior to analysis. Prey preference was calculated using Manly's  $\alpha$ , a prey selection index that accounts for the prey consumed relative to the prey available [47], as:

$$\alpha = \frac{U_m/A_m}{U_m/A_m + 1 - U_m/1 - A_m}, \quad (1)$$

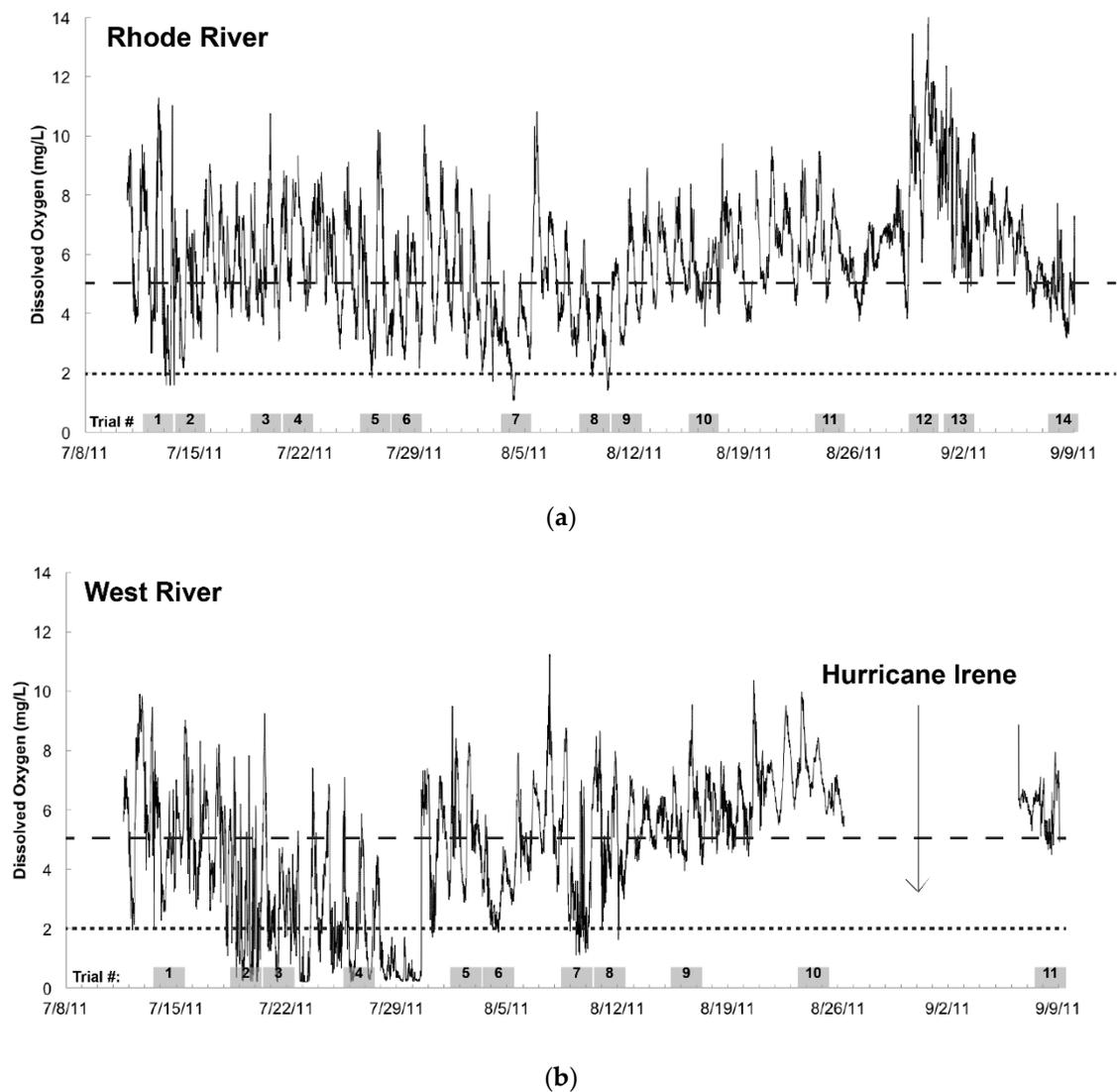
In this equation,  $U_m$  is the consumption, or use, of prey species  $m$  as a proportion of the total prey items consumed and  $A_m$  is the availability of prey species  $m$  as a proportion of the total prey items available. When a predator is selecting between two prey species, an  $\alpha > 0.5$  indicates a selection preference for the species.

We used a repeated measures ANOVA to test for differences in selection preference and total prey consumption between dissolved oxygen regimes nested within crab identity to account for individual crab behavior. Crab ("subject") identity was a random factor and dissolved oxygen regime was a fixed factor in this model. For significant effects in repeated measures ANOVA, paired t-tests were used to detect differences between group means. All analyses were conducted in R statistical software (version 3.3.3, R Core Team 2017, R Foundation for Statistical Computing, Vienna, Austria).

### 3. Results

#### 3.1. Field Experiment

Dissolved oxygen was lower and more variable in the West River than the Rhode River (Figure 2), as expected. Water temperature and salinity were similar at the two sites. During the period of field trials, water temperature averaged 29 °C ± 2 SD and 28 °C ± 2 SD and salinity averaged 8.5 ppt ± 1.16 SD and 9.3 ppt ± 1.38 SD at the Rhode and West Rivers, respectively.



**Figure 2.** (a) Dissolved oxygen during the study period in the Rhode River; (b) Dissolved oxygen during the study period in the West River. The timing of field trials is indicated by gray bars along the time axis. The dashed and dotted lines indicate dissolved oxygen thresholds of 5 mg/L and 2 mg/L, respectively. The water quality sonde was removed from the West River during Hurricane Irene.

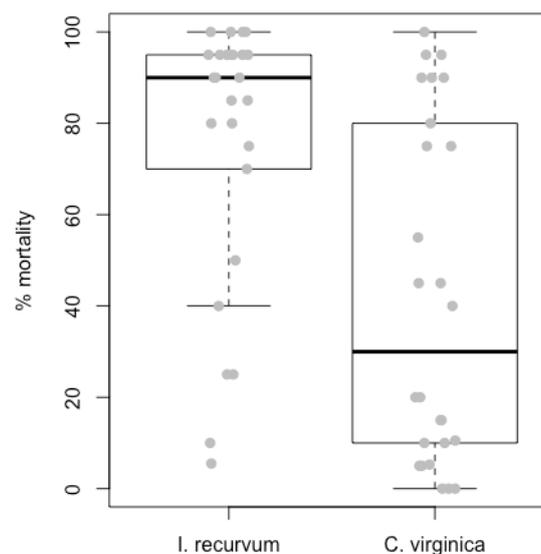
Dissolved oxygen varied greatly at the field sites throughout the duration of the study and during each trial (Figure 2, Figure S4). During trials, the duration of moderate hypoxia (from 2 to 5 mg/L) ranged from 0% to 94% (mean 39%) of trial time, and the duration of severe hypoxia (<2 mg/L) ranged from 0% to 87% (mean 10%) of trial time (Figure 2). However, hypoxia duration did not have a statistically significant effect on predation rate on either of the two species at either site (Table 1, Figures S2 and S3). Nor did site significantly affect predation mortality, even when dissolved oxygen duration was removed from the explanatory model (Table 2). Pooled across both sites, predation mortality was significantly higher for *I. recurvum* ( $74.6\% \pm 5.8$  mean  $\pm$  SE) than for *C. virginica* ( $39.7\% \pm 7.3$  mean  $\pm$  SE) (Table 1, Figure 3). Environmental mortality was low for both species during predation trials: only two individuals (0.4%) of *C. virginica* died in closed controls across all trials, and no individuals of *I. recurvum* died in closed controls.

**Table 1.** Summary of multiple linear regression models of predation mortality, testing for the effect of bivalve species, species  $\times$  site, duration of moderate hypoxia, and duration of severe hypoxia during, before, and before plus during, predation trials. Significant  $p$ -values are in bold.

Predictor	During Trial (Immediate Effect)		Pre-Trial (Delayed Effect)		Pre & During (Cumulative Effect)	
<i>Model Statistics</i>	<i>Adj R<sup>2</sup> = 0.86</i> <i>F<sub>5,46</sub> = 3.331</i>		<i>Adj R<sup>2</sup> = 0.22</i> <i>F<sub>5,44</sub> = 3.703</i>		<i>Adj R<sup>2</sup> = 0.20</i> <i>F<sub>5,44</sub> = 3.405</i>	
	<i>t</i>	<i>p</i>	<i>t</i>	<i>p</i>	<i>t</i>	<i>p</i>
bivalve species	−2.461	<b>0.0177</b>	−2.528	<b>0.0152</b>	−2.497	<b>0.0163</b>
site	−1.099	0.2776	−0.067	0.9467	−0.463	0.6457
bivalve species $\times$ site	−0.327	0.7448	−0.596	0.5545	−0.588	0.5592
moderate hypoxia	0.108	0.9142	0.234	0.8162	−0.253	0.8016
severe hypoxia	0.664	0.5101	−1.06	0.2948	−0.033	0.9734

**Table 2.** Summary statistics from a two-way analysis of variance (ANOVA) of the effects of species and site on predation mortality in the field. Significant  $p$ -values are in bold.

Predictor	Degrees of Freedom	<i>F</i>	<i>p</i>
bivalve species	1	13.82	<b>&lt;0.001</b>
site	1	2.823	0.099
bivalve species $\times$ site	1	0.111	0.741

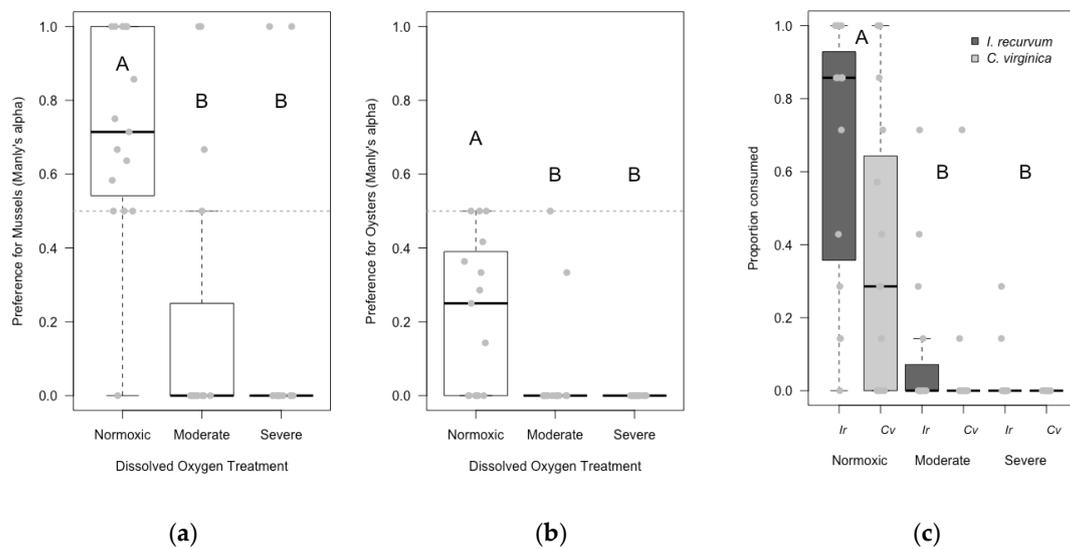


**Figure 3.** Proportion of prey consumed in field experiment trials by species. Bold black bars indicate the median value, box ends and whiskers indicate the interquartile and full ranges of the data, and gray points indicate data values.

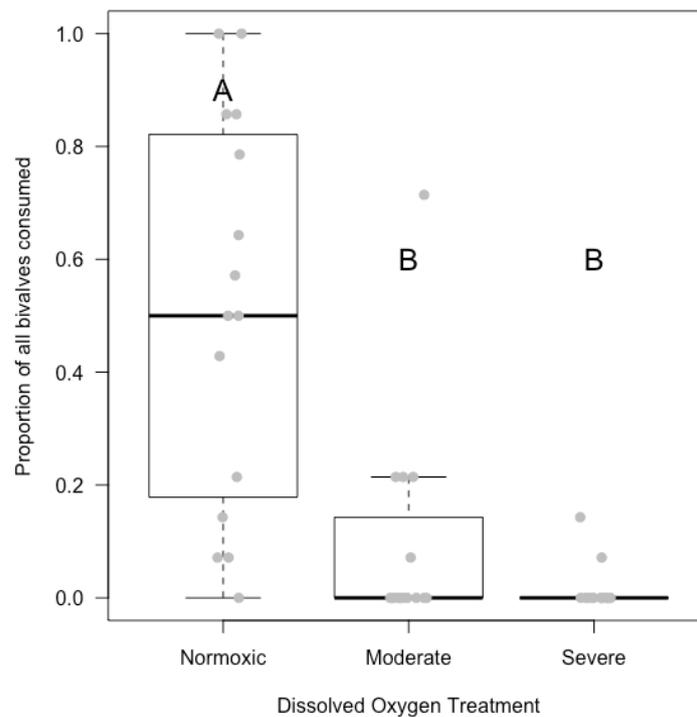
### 3.2. Laboratory Experiment

There was a significant effect of dissolved oxygen regime nested within crab identity on selection preference (ANOVA:  $F_{2,14} = 8.537$ ,  $p = 0.004$ ), with crabs showing a stronger preference for *I. recurvum* in normoxic than in moderately or severely hypoxic conditions (normoxic vs. moderate:  $t_7 = -3.279$ ,  $p = 0.013$ ; normoxic vs. severe:  $t_7 = -3.094$ ,  $p = 0.017$ ) (Figure 4). Similarly, there was a significant effect of dissolved oxygen regime nested within crab identity on the total number of prey consumed ( $F_{2,14} = 20.97$ ,  $p < 0.0001$ ), with crabs consuming more prey in normoxic conditions than in moderately or severely hypoxic conditions (normoxic vs. moderate:  $t_7 = t = -4.753$ ,  $p = 0.002$ ; normoxic vs. severe:  $t_7 = -4.822$ ,  $p = 0.002$ ) (Figure 5). On average, crabs consumed  $51\% \pm 35$  mean  $\pm$  SD of prey during

normoxic trials, compared to  $8.9\% \pm 19$  mean  $\pm$  SD of prey during moderately hypoxic trials and  $1.5\% \pm 4.1$  mean  $\pm$  SD of prey during severely hypoxic trials.



**Figure 4.** (a) *Callinectes sapidus* prey selection preference (Manly's  $\alpha$ ) for mussels (*Ischadium recurvum*) in laboratory experiments, grouped by dissolved oxygen regime; (b) *Callinectes sapidus* preference for oysters (*C. virginica*). In (a) and (b) preference values above 0.5, marked by a gray dotted line, indicate a preference for that prey species. There was a significant effect of dissolved oxygen regime in repeated measures ANOVA; letters indicate a significant difference in post-hoc Tukeys tests; (c) Proportion of mussels and oysters consumed in each dissolved oxygen regime. *Ir*: *Ischadium recurvum*, *Cv*: *Crassostrea virginica*. In box and whisker plots, black bars indicate the median value, box ends and whiskers indicate the interquartile and full ranges of the data, and gray points indicate data values.



**Figure 5.** Total proportion of prey items consumed by crabs in each dissolved oxygen regime in the laboratory experiment. Values are means  $\pm$  SE. There was a significant effect of dissolved oxygen regime in repeated measures ANOVA; letters indicate a significant difference in post-hoc t-tests.

## 4. Discussion

### 4.1. Hypoxia Lowered Predation Rates in the Laboratory; Inconclusive in the Field

We observed decreased predation on *I. recurvum* and *C. virginica* by *C. sapidus* in response to hypoxia during our laboratory experiment, however the same effect was not seen in field predation trials. The duration of moderate or severe hypoxia during field trials varied greatly but had no significant influence on predation rates. Taken together, these two experiments indicate that, while predation on bivalves by crabs is strongly affected by short-duration hypoxia in the lab, that effect does not translate predictably to real predation rates in a system experiencing diel-cycling hypoxia.

Our laboratory findings may have differed from our field study findings due to the presence of bivalve predators other than *C. sapidus* at our sites. White perch *Morone americana*, a known bivalve predator, can be quite abundant in these rivers [48,49]. Other bivalve predators, such as spot *Leiostomus xanthurus* and hogchoker *Trinectes maculatus*, have also been recorded at these sites [27,49]. These predators may respond differently than *C. sapidus* to short-duration hypoxia, accounting for the differences between the results of our field study and laboratory experiment.

Another possible explanation for the incongruence between our field and lab study results could be that our field trials included daytime hours. Predators including *C. sapidus* may have compensated for reduced nighttime feeding with increased daytime feeding, thereby negating the effect of reduced feeding during short-duration episodes of hypoxia. This type of compensatory feeding has been observed in oysters in response to diel-cycling hypoxia [50]. In addition, crabs were kept in environments with constant DO levels in the laboratory study, while DO in the field varied temporally (Figure 2) and spatially [43]. Hypoxic conditions are known to reduce digestion rates in crabs [51] and crabs have been shown to seek out areas with higher oxygen levels to digest food foraged from hypoxic areas [52]. Crabs from our field mesocosms could have been able to forage and digest food items in a more favorable environment while laboratory crabs would have had to digest in constant low DO tanks where their ability to do so is decreased. Furthermore, crabs that foraged and fed to satiation in the field could be replaced by additional hungry crabs, leading to higher observed feeding rates in normoxic conditions in the field than in the lab. Future studies should continue to examine oxygen dynamics in the field. Although we did not find a relationship that would support either a prey stress or consumer stress model in regards to short-duration hypoxia, our results did capture predation over two-day diel-cycles and indicate that predation is occurring in spite of nightly hypoxic conditions. This is in contrast to the disruption of energy flow into microbial communities that commonly characterizes systems degraded by more persistent forms of hypoxia [1]. Variability in both the timing, time scales, and spatial extent of hypoxic events merits further attention with regards to their potential impacts on estuarine communities. Based on our findings, we propose hypoxia on a short time scale may negatively impact individuals, but communities as a whole that experience diel-cycling hypoxia may be more resilient than systems affected by persistent hypoxia, with the accompanying capacity to maintain populations of higher trophic level species of ecological and economic interest.

### 4.2. Blue Crab Preference for Hooked Mussels over Eastern Oysters

In both the field and the laboratory, we observed a predator preference for *I. recurvum* over *C. virginica*, revealing interesting new details about predator–prey dynamics in the Chesapeake Bay. Although capable of consuming both species, *C. sapidus* may find *I. recurvum* to be a preferred prey item, given its shape and the ease of opening compared to *C. virginica*. In dissertation work by B. Aronhime, preference for *I. recurvum* over *C. virginica* in blue crabs was shown, however these trials used large oyster shells > 60 mm in length, with many much smaller epizoic mussels attached [53]. Our study was more consistent in terms of both the size and number of prey species present in our mesocosms, allowing a more specific test of prey preference ( $n = 7$ ,  $n = 10$  individuals of each species in the lab and field respectively; shell heights of *C. virginica* and *I. recurvum* were  $29 \text{ mm} \pm 3.69$  (mean  $\pm$  SD) and  $27.16 \text{ mm} \pm 3.04$  (mean  $\pm$  SD) respectively). With densities of *I. recurvum* in the thousands of

individuals/m<sup>2</sup> on restored oyster reefs [32,39,54], its presence may reduce predation pressure on recovering oyster populations.

Interestingly, we did observe a decrease in preference for mussels in hypoxic conditions in the lab. While clearly impacted by reduced feeding activity (Figure 5), this result also suggests a change in selectivity by crabs in stressful conditions. Crabs in moderately hypoxic conditions still consumed an average of 1.25 bivalves, but there was no observed preference for *I. recurvum* over *C. virginica* in these conditions. This finding may be a behavioral response by the crab, or alternatively, could result from prey stress reducing differences in prey handling time due to potential physiological changes in prey.

The generally high consumption rates in the field (>80% predation in some trials) indicate that hooked mussels are important components of Chesapeake Bay food webs, strongly linked to the commercially and ecologically important blue crab. However, they appear in the experimental literature only briefly thus far [32,42,55,56] and rarely as a focal organism. We still lack considerable information about their biology or life history. Our results suggest hooked mussels merit greater consideration in future studies. In particular, understanding the ecological controls on the distribution and abundance of *I. recurvum* could help us to understand oyster reef ecosystems and estuarine food webs.

## 5. Conclusions

We found a strong preference in *C. sapidus* for *I. recurvum* over *C. virginica* in the lab that mirrored predation mortality in the field. While laboratory results indicated a strong effect of dissolved oxygen on predation mortality, this did not translate into differences over two-day field trials that varied in diel-cycling hypoxia. Compensatory feeding by *C. sapidus* or a multi-species predator assemblage effect is likely at play. More research is required to fully understand the effect of diel-cycling hypoxia on predator–prey interactions in estuarine food webs.

**Supplementary Materials:** The following are available online at <http://www.mdpi.com/1424-2818/12/3/87/s1>, Figure S1: Image of field mesocosm experimental set-up. Figure S2: Predation mortality of *I. recurvum* and *C. virginica* in the field with varying durations of severe hypoxia. Figure S3: Predation mortality of *I. recurvum* and *C. virginica* in the field with varying durations of moderate hypoxia. Figure S4: Percent dissolved oxygen (% DO) in the Rhode River and West River over the time frame of the field experiment. Table S1: Water quality conditions in each field trial: mean salinity, DO concentration (mg/L), % DO, and temperature.

**Author Contributions:** Conceptualization, E.N. and K.B.G.; formal analysis, J.M. and K.B.G.; funding acquisition, K.B.G.; methodology, E.N.; writing—original draft, E.N. and K.B.G.; writing—review and editing, J.M. and K.B.G. All authors have read and agreed to the published version of the manuscript.

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**Conflicts of Interest:** The authors declare no conflict of interest.

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