Assessing the Effects of Thermal Stress, Hypoxia, and Hydrogen Sulfide Exposure on the Survival of the Gulf Toadfish (Opsanus beta)

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By

Charles Groppe

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Assessing the Effects of Thermal Stress, Hypoxia, And Hydrogen Sulfide Exposure on the Survival of the Gulf Toadfish (*Opsanus beta*) (December 2022)

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Northern Biscayne Bay experienced recurring mass mortality fish kill events in 2020 and 2021, largely composed of gulf toadfish (*Opsanus beta*). Low dissolved oxygen values were recorded during these periods of mortality, however, the duration and severity of hypoxia recorded in the Bay has not been confirmed as lethal to *O. beta*. Hydrogen sulfide (H_2S), a toxic gas formed naturally in aquatic sediments under hypoxic or anoxic conditions, can further inhibit respiration and decrease survival time in aerobic organisms. This study investigated how thermal and hypoxic conditions recorded in the bay affect *O. beta* survival, and whether exposure to H_2S under these same conditions altered rates of mortality. Juvenile toadfish were exposed to different combinations of thermal stress (32°C), varying levels of hypoxia (≤ 0.5 or 1 mg/L O_2), and H_2S (7.5 uM) in a non-flow-through seawater (32-35 ppt salinity) aquarium system to assess survivorship over time. Extreme hypoxia at 0.5 mg/L O_2 yielded a median time till death (LT50) of 1.49 ± 0.32 hours that was significantly shorter than the survival times for hypoxia at 1.0 mg/L O2 alone (8.64 ± 1.64 hours) and hypoxia at 1 mg/L and 7.5 ppm H2S (7.62 ± 1.29 hours). Linear regression analysis on fish survival under the 1 mg/L O_2 treatment suggested larger fish by weight may survive longer under hypoxic conditions, but no significant regressions were found under the other treatments. Results from this

study confirm the lethality of hypoxia durations recorded during the 2020 fish kill, and further experimentation coupled with field collection within Northern Biscayne Bay will be useful to understand the potential effects and critical thresholds of hydrogen sulfide exposure and hypoxia in regard to *Opsanus beta* survivorship.

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Chapter 1: Introduction

Biscayne Bay is a shallow inner-shelf embayment adjacent to the Atlantic coastline of South Florida which links upland freshwater sources to oceanic saltwater flows creating a coastal lagoon (Kruczynski & Fletcher, 2012). Northern Biscayne Bay (NBB) is a segment of the bay situated north of the Rickenbacker Causeway in a heavily urbanized environment bordered by the cities of Miami along the western shoreline and Miami Beach along the eastern shoreline (Fig. 1).

Figure 1. Map of Northern Biscayne Bay using satellite imagery taken from Google Maps TerraMetrics 2022 © data.

As an estuarine system, NBB is strongly influenced by the mixing of converging freshwater and saltwater flows. Anthropogenic alterations have caused massive shifts to

historical freshwater flow regimes (Cantillo *et al.*, 2000), changing the timing and duration of flows which led to higher average salinities and greater variability in water quality (Score & Jacoby, 2006; Langevin, 2003). Fresh water is delivered to the bay through multiple inputs including surface runoff, canal flow, and groundwater discharge (Stalker *et al.,* 2009; Browder *et al.,* 2005), forming spatial salinity gradients and altering nutrient loading patterns (Wang *et al.,* 2003; Caccia & Boyer, 2005). Mainland shoreline sites in NBB with more variable salinity patterns have been found to have lower overall fish species richness, with such sites being dominated by species capable of withstanding the wide range of salinity fluctuation (Serafy *et al.*, 1997). While the limiting nutrient in NBB is still in question (Chin, 2020), canals were found to be the largest contributor of nitrogen to the bay and provide the bulk of phosphate loading in NBB specifically (Caccia & Boyer, 2007). Shallow estuaries and lagoons are already vulnerable to eutrophication effects brought on by increased nutrient loading (Valiela *et al.*, 1997; Whitall *et al.*, 2007), and NBB has been shown to be the segment of Biscayne Bay at highest risk of eutrophication from nutrient pollution with the highest measurements of chlorophyll-a concentration, total phosphorus, and dissolved inorganic nitrogen (Briceño *et al.*, 2011). NBB is also experiencing significantly higher rates of increasing chlorophyll-a and phosphate concentrations in comparison to other segments within the bay, indicating that its proximity to multiple sources of nutrient input coupled with reduced circulation and flushing are inducing greater rates of decline over time (Millette *et al.*, 2019).

The fluctuation in nutrient availability and other water properties such as salinity and temperature drives variability in the composition of benthic vegetation (Lirman &

Cropper, 2003; Biber & Irlandi, 2006; Wingard *et al.*, 2003) and in turn alters the health and ecological functioning of these structurally important communities (Deegan *et al.*, 2002). NBB has experienced drastic declines recently in seagrass beds (Lirman *et al.*, 2016) and historically in mangrove ecosystems (Peters *et al.,* 2015), both of which are functionally vital to many species within the bay (Gilby *et al.,* 2018; Serafy *et al.*, 2003). Seagrass ecosystems and their associated benthic macroalgal communities serve as valuable locations of nitrogen cycling and primary production (Yarbro & Carlson, 2008; Terrados & Borrum, 2004). Shifts in these communities to greater concentrations of macrophytic algae have been linked to lower rates of photosynthesis due to higher levels of light attenuation, restricted vertical mixing of the water column, and lower levels of oxygen production and availability (Sand-Jensen, 1989; Miranda & Hodges, 2000). Additionally, blooms of macrophytic drift algae can deteriorate seagrass ecosystems and hamper their recovery and resilience, further lowering ecosystem functioning within the bay (Santos *et al.*, 2020). Coastal zones have experienced increases in eutrophication-driven hypoxic events globally (Howarth *et al.* 2011, Diaz & Rosenburg, 2008), but nutrient pollution must be investigated in tandem with physical factors such as stratification and salinity to better understand the formation of hypoxia in estuarine habitats (Lowery, 1998). Temperature and freshwater discharge can exacerbate stratification effects on hypoxia along the thermocline and halocline in coastal areas (Fennel & Testa, 2019), and globally, oceans are experiencing a decline in oxygen driven by the compounding effects of rising temperatures on oxygen solubility and depth zonation (Keeling *et al.*, 2010). If coastal hypoxia occurs for long enough durations or in

tandem with other physical stressors that exacerbate respiration, such as thermal stress, severe consequences including large scale fish die offs can occur (Breitburg, 2002).

In August 2020, a fish kill occurred in NBB resulting in an estimated mortality of at least 27,000 fish over six days (Silverstein *et al.*, 2021). Hypoxia (≤ 2 mg/L dissolved oxygen) was determined to be the most probable cause of mortality, and extreme hypoxic (≤ 1 mg/L) and anoxic conditions (0 mg/L) were also detected by continuous 15-minute resolution monitoring instruments during the event. The Biscayne Bay Scientific Coordination (BBSC) group, composed of researchers and stakeholders from government, academia, and NGOs, generated a consensus statement suggesting that hypoxia as a contributing factor and that several key factors synergistically worsened these conditions. High water temperatures during the month of August may have potentially lowered the solubility of oxygen and further stratified the water column along the thermocline. Additionally, low wind movement during the fish kill may have limited surface turnover in the upper water column., a physical factor that has been shown to enhance dissolved oxygen (DO) conditions through forced re-aeration in coastal estuaries (Hull *et al.*, 2008). Anomalously high canal flow was reported during the year of 2020, which further stratified the water column along the halocline at canal mouths and could have contributed heavily to the decline in seagrass ecosystems and their protection against extreme hypoxia in NBB. The BBSC found that an algal bloom occurred in the month of August in 2020, however, it occurred post-fish kill and was most likely a result of the die-off and subsequent nutrient release rather than a cause. Oxygen fluctuated along a diel pattern within NBB similar to patterns previously detected in nearby Florida Bay (Borum *et al.*, 2005), indicating that remaining seagrass and algal communities were

able to offset some oxygen depletion through photosynthesis during the day. Finally, although the BBSC group suspected canal flow played a deleterious role in affecting NBB water quality, early findings suggested that hypoxic waters most likely originated within the bay itself.

In September of 2021, a fish kill event occurred once again, but at a much lesser magnitude than the previous year , with an estimated mortality of hundreds of fish across a three-day span (Irela Bagué, personal communication). Hypoxia was recorded in the bay, but to lesser extremes, as both oxygen concentrations and hypoxia episode duration lengths were not as severe as in 2020. Like in 2021, a synergistic concurrence of high temperatures, little wind action, and high canal flow occurred before and during the fish kill and may have contributed to the formation of hypoxia in the water column (Fig. 2). The Biscayne Bay Aquatic Preserve (BBAP) BB14 buoy sensor within the bay itself experienced technical difficulties during the 2021 fish kill period and was unable to provide water quality data within the basin, however, an alternative sensor from the BBAP monitoring system within the Little River canal confirmed similar physical parameters of elevated water temperature within the bay. Utilizing data collected at the LR03 sensor within the canal, it appeared yet again that hypoxic waters originated within the bay (Fig. 3). Dissolved oxygen originally showed cyclical increases with increasing salinity indicating bay waters in the canal were well oxygenated, however during the fish kill periods the synchrony between the two parameters snapped and oxygen values decreased with increasing salinity. Canal flow in 2021 was once again higher than historical averages, however, not as anomalous as in 2020 (Fig. 4). Although algae blooms were suggested to play a role in creating hypoxic conditions within the bay,

chlorophyll concentrations in both years show spikes following each fish kill event, rather than preceding them, indicating they were results and not causes of each kill (Fig. 5). Hypoxia was once again implicated to be the cause of fish mortality in the bay, however, to date, no definitive causes have been identified for either fish kill.

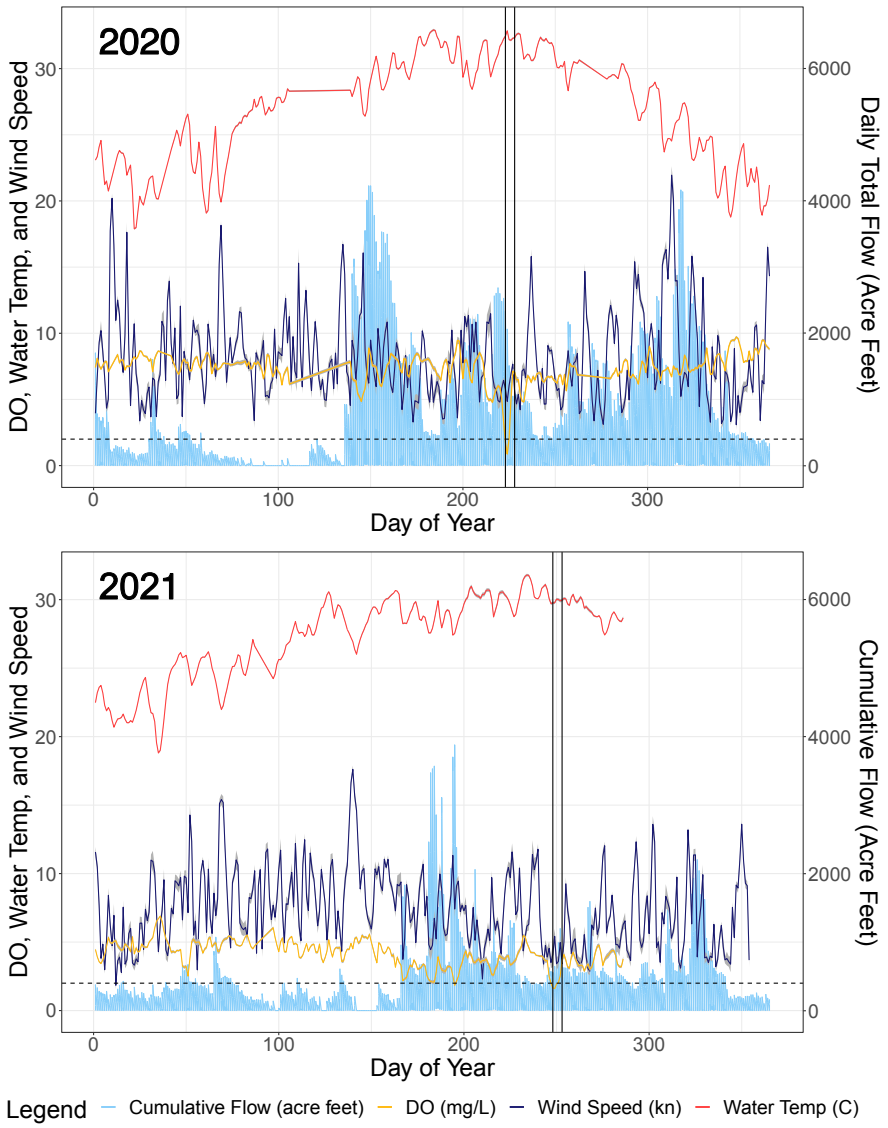

Figure 2. Timelines of DO, canal flow, water temperature, and wind speed for 2020 (top) and 2021 (bottom). DO and water temperature daily averages were created using data collected at the BBAP's LR03 sensor, while daily averages for windspeed were calculated using data from NOAA's Virginia Key Station sensor. Little River daily flows were calculated using data from SFWMD's DBHYDRO database. Vertical solid lines outline each year's respective fish kills, horizontal dashed lines represent the hypoxia threshold at 1 mg/L, and gray ribbons around each line represent ± 1 se. Incomplete DO and temperature readings are due to limited sensor availability. The LR03 sensor did not begin taking readings until March 17, 2020, and data obtained in 2021 only had readings up to October 13.

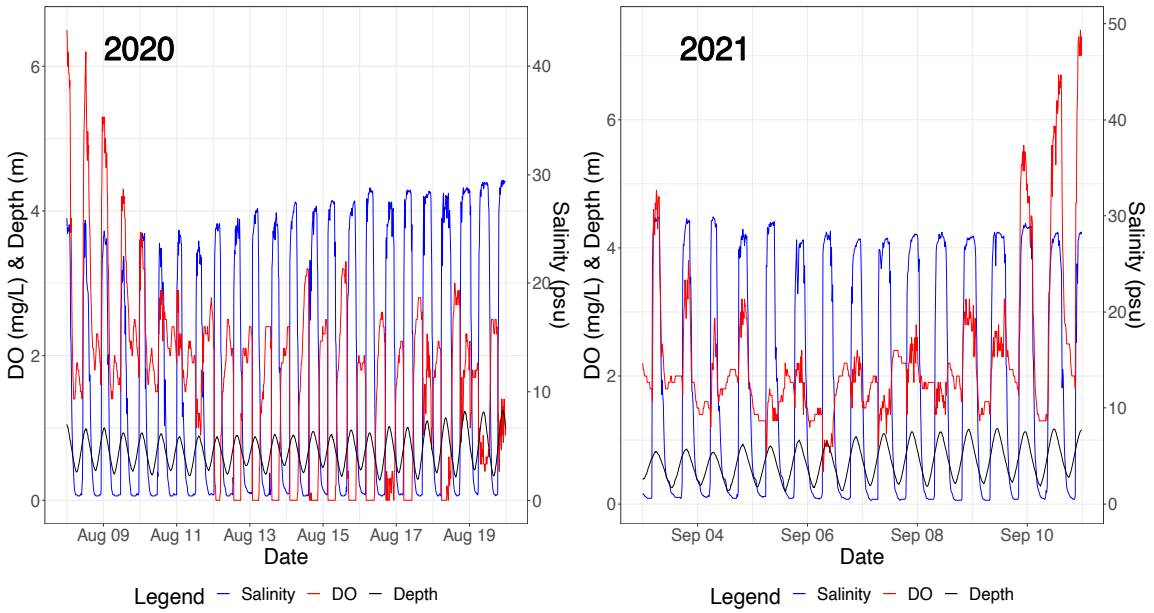

Figure 3. DO, depth, and salinity curves before, during, and after the fish kill events in 2020 and 2021. All readings taken from recordings at the BBAP's LR03 sensor. Oxygen originally cycles in synchrony with salinity but breaks the cycle during the fish kill to show lower oxygen values during higher salinity periods, indicating hypoxic conditions flushed into the canal from the bay.

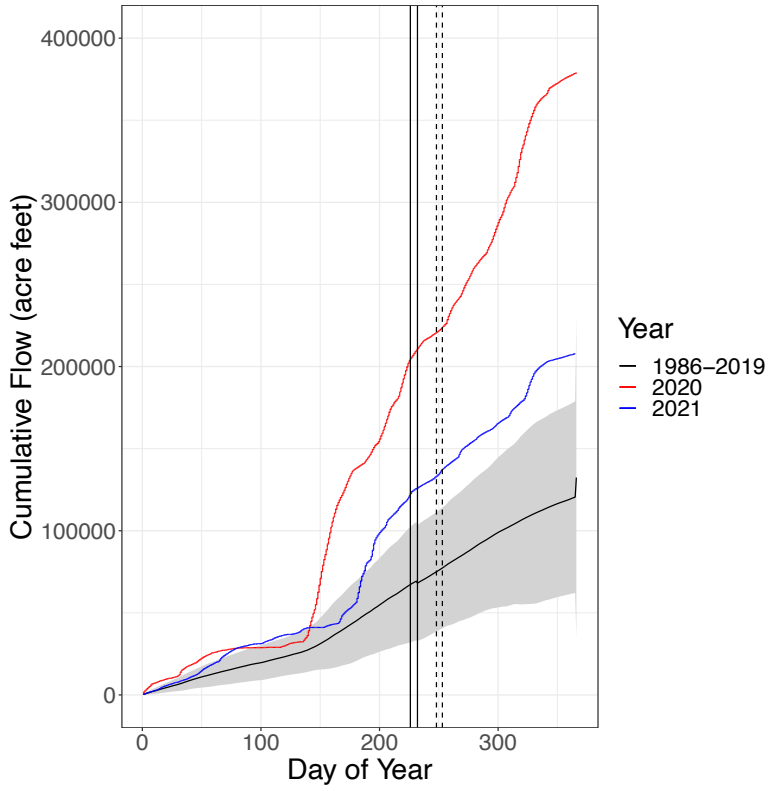

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Figure 4. Cumulative yearly canal flow in the Little River Canal measured in acre-feet. Historical data shown in the graph is an average cumulative flow value for each day of the year across all years from 1986-2019, with the gray ribbon representing ± 1 SD. Vertical black lines outline the fish kill events in 2020 (solid) and 2021 (dashed). Data taken from SFWMD's DBHYDRO database.

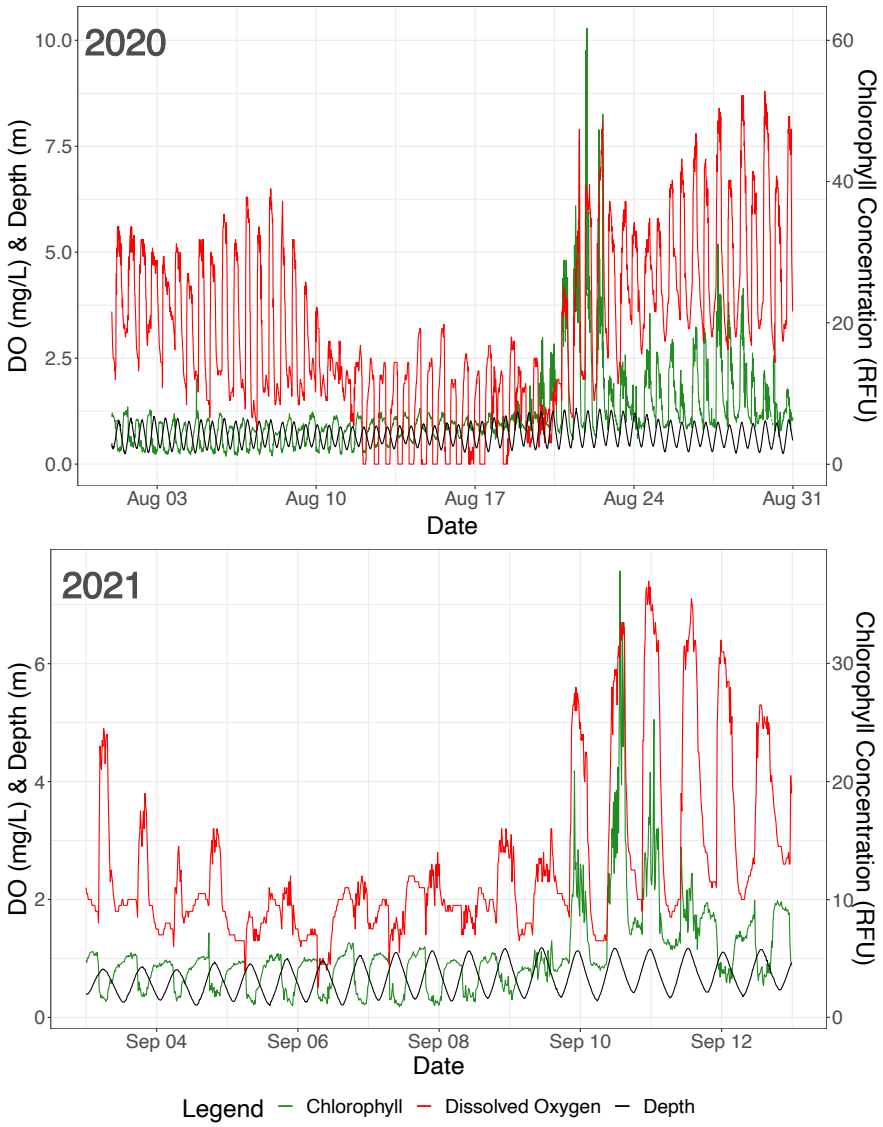


Figure 5. Chlorophyll fluorescence, DO, and depth readings collected by the LR03 sensor before, during, and after fish kill episodes in 2020 and 2021. Chlorophyll fluorescence units are not specified by BBAP, used as relative fluorescence levels for analysis in this report.

While hypoxia has previously been reported as a factor in fish kills in multiple coastal ecosystems, the causes and stressors associated with each die-off can be highly variable. Along deeper coastal ecosystems, bays can experience hypoxia through upwelling and crowding of surface waters by affected fish populations, resulting in exacerbated respiration rates in oxygen-limited water and potential mass mortality events (Stauffer *et al.*, 2012). In such cases, hypoxia not only creates a stressful environment for

aerobic organisms, but also forces populations into altering their behavior to further decrease the potential for respiratory relief. While fish altered their behavior to surface swimming and heavily crowded upper portions of the water column in NBB, the hydrology, location, and depth of NBB essentially negate the likelihood that upwelling can be a factor. In coastal areas of Texas, the largest fish mortalities occurred in warmer months as warm temperatures and poor water circulation further stratified water columns and led to deadlier levels of hypoxia (Thronson & Quigg, 2008). While the authors noted these physical stressors are causative factors in many hypoxic fish kills, they stressed concern that further urbanization of coastal areas could influence nutrient loading patterns and further exacerbate physical conditions through eutrophication. In a tropical estuary in India, nutrient loading from freshwater inputs and lack of flushing and circulation were found to be the direct causes of hypoxia and anoxia which in turn caused mass fish mortality events (Ram *et al.*, 2014). Nutrient inputs formed a perpetual oxygen-deficient environment within the Indian estuary, but the worst conditions of anoxia and the periods of greatest mortality occurred in between monsoon seasons when circulation was lowest. Organic matter (OM) is a byproduct of algal blooms and can form lethal levels of hypoxia in estuaries, however, OM can also be directly loaded into bays via freshwater discharge from polluted watersheds resulting in hypoxia episodes of similar severity (Paerl *et al.*, 1998). Organic matter can also be transported from offshore locations via currents and wind, as is the case with pelagic drift algae like *Sargassum. Sargassum* can accumulate along nearshore habitats and induce hypoxia as well as degrade water quality, resulting in mass mortality across multiple faunal species (Rodriguez-Martinez *et al.*, 2019). In comparison to these other ecosystems' struggles

with hypoxia, NBB exhibits similar deficiencies in circulation and has experienced its worst hypoxia and mortality episodes in the warmer months. NBB has also experienced increases in urbanization and anthropogenic influences along its watershed and South Florida has seen recent influxes in *Sargassum* along its shores (Collado-Vides *et al*., 2020), suggesting an increase of organic matter loading to the bay. While NBB has shown higher levels of eutrophication in comparison to other segments of the bay (Millette *et al.*, 2019), it did not experience algal blooms prior to either fish kill and does not experience continuous durations of oxygen deficiency like in the Tapi Estuary (Ram *et al.*, 2014). Without evidence of an algal bloom driving hypoxia during these fish kills, physical stressors should be treated as potential causative factors of hypoxia acting synergistically to induce mass mortality. However, broader investigations on the parameters of the bay raises questions on the capabilities of these stressors alone being responsible for the fish kills.

Hypoxic events have been recorded at the BB14 and LR03 stations multiple times since 2019, yet only a portion of these events have occurred immediately preceding or during the fish kills. Utilizing continuous DO logging data from the 2020 fish kill, the longest duration of any hypoxic event at a 2 mg/L threshold was 18.5 hours, and the longest duration at a 1 mg/L threshold level was 10 hours. While no study has examined the lethal time threshold for toadfish at these oxygen concentrations, a previous study analyzing the effects of fluoxetine on hypoxia tolerance held the gulf toadfish (*Opsanus beta*) at 2.45 mg/L O_2 for 22 hours without inducing mortality, showing their capacity to handle low oxygen concentrations for long periods of time (Amador *et al.*, 2018). There appears to be a relationship between temperature and hypoxia duration for the events

which occurred in the basin during the 2020 fish kill (Fig. 6), which might suggest that synergistic impacts of temperature and low oxygen concentrations surpassed the threshold levels of fish survival in the bay.

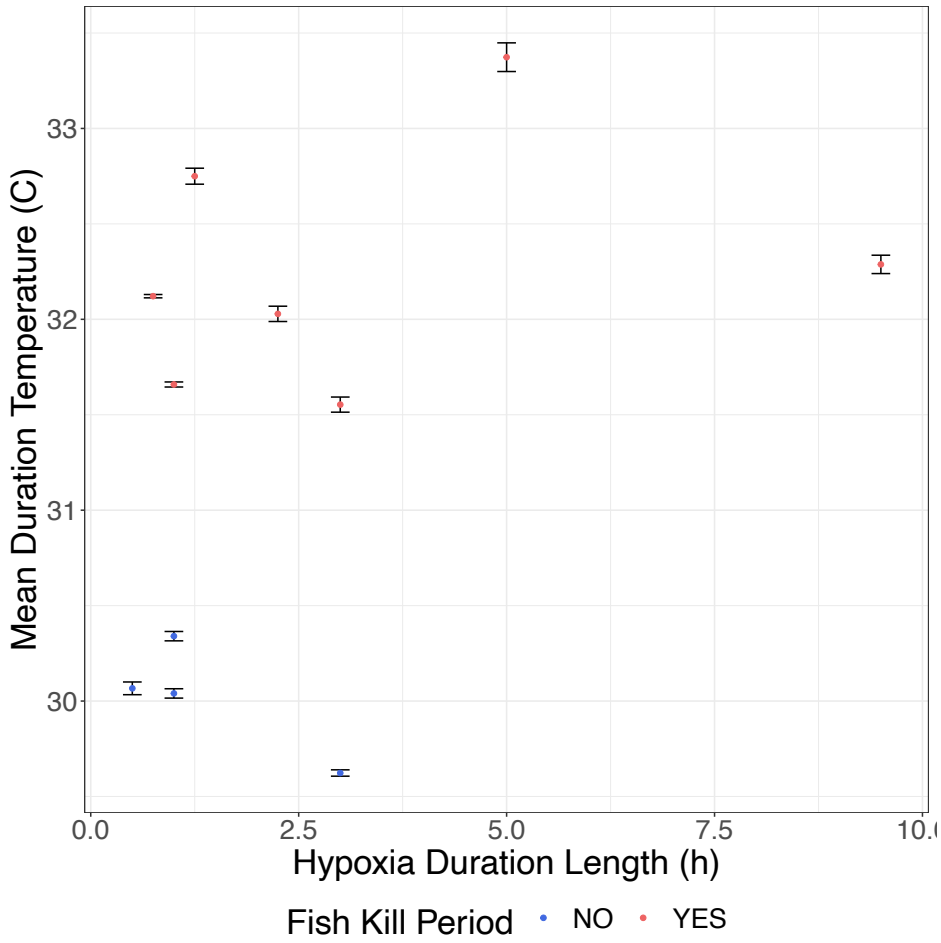


Figure 6. Hypoxia (≤ 1 mg/L) duration length vs. mean temperature for each episode recorded at the BB14 sensor from 2019 - 2020. Error bars depict ± 1 S.E., means are computed by averaging all temperature readings within each duration.

Recent experiments by the Grosell laboratory at the Rosenstiel School of Marine & Atmospheric Science (RSMAS) have found evidence counter to this assumption however, showing that juvenile and larval *O. beta* show innate signs of hypoxia tolerance in metabolic responses to hypoxia at a variety of temperatures (Frank *et al.*, 2022). This finding is particularly intriguing given that *O. beta* was not only present in both fish kills, but was the predominant species killed in the 2020 event, making up an estimated 44% of

all fish mortality (Silverstein *et al.*, 2021). Therefore, while hypoxia may have been a contributing stressor in the fish kills, it may not have been the primary cause of death, opening the investigation further for additional factors responsible for mortality.

Given the stratification of oxygen concentrations with depth, benthic substrates can have lower rates of oxygenation from a lack of mixing with surface waters. These substrates are home to a vast variety of marine bacteria, and these key bacterial and prokaryotic communities are largely responsible for the decomposition and remineralization of nutrients in coastal estuaries (Nixon, 1981). Microbial communities in the benthos vary in rates of nutrient cycling and biogeochemical capabilities, and their composition and abundance has been shown to fluctuate with elevated temperatures, organic matter loading, and oxygen concentrations (Hicks *et al.*, 2018; Albert *et al.*, 2021; Rousi *et al.*, 2019). In extremely hypoxic (< 1 mg/L O_2) or anoxic conditions, benthos diversity is highly reduced and dominated by specialized genera capable of tolerating low DO conditions (Levin *et al.*, 2009), many of which utilize efficient anaerobic respiration mechanisms to survive (Coskun *et al.*, 2019). Sulfate reducing bacteria (SRB) make up a large portion of this group, utilizing sulfate as a terminal electron acceptor for growth and produce various forms of sulfide as byproducts (Muyzer & Stams, 2008). The biodegradation of organic matter by SRBs results in production of hydrogen sulfide (H_2S), a toxic gas with a complex relationship with many water quality parameters. The solubility of H_2S holds inverse relationships with both temperature and salinity, and it is rapidly oxidized to sulfate in oxygenated water (Morse *et al.*, 1987). The ionization and oxidation rates of H_2S are heavily affected by pH. Both rates increase with increasing pH, thus lower percentages of total dissolved sulfide in the water column are

in the form of un-ionized H_2S in waters with higher alkalinity (Morse *et al.*, 1987; Baird *et al.*, 2017). Under healthy conditions in tropical estuaries like NBB, warm, normoxic waters with a pH of 7 - 8 will greatly limit the concentrations and toxicity of H_2S. However, if OM loading were to exceed the rate of consumption by bacteria, benthic waters and substrates could become hypoxic or anoxic (Levin *et al.*, 2009). The excess OM present would then be utilized by SRBs capable of tolerating the oxygen deficient conditions, resulting in the production of H_2S in deoxygenated waters. Maximum H_2S concentrations from decomposing organic material under saline conditions are significantly higher than in freshwater environments due in part to greater availability of sulfate (Letelier-Gordo *et al.*, 2020), and H_2S toxicity greatly increases in lower oxygen concentrations (Baird *et al.*, 2017). Thus, the capacity for high H_2S concentrations in hypoxic waters could potentially indicate a new source of mortality during the fish kills in 2020 and 2021.

H_2S can induce mortality through apnea and respiratory arrest in a manner similar to hypoxia. H_2S inhibits cytochrome-c-oxidase and reduces its activity in the electron transport chain in many mammals including humans, thus disrupting oxidative phosphorylation in cells and limiting ATP production and cellular respiration as a whole (Guidotti, 1996). This same reduction in cytochrome-c-oxidase activity has been found in multiple fish species (Torrans & Clemens, 1982; Bagarinao & Vetter, 1989; Forgan & Forster, 2010), and further studies into H_2S toxicity have revealed its capability to produce detrimental reactive oxygen species which can also cause cellular damage (Truong *et al.*, 2006). Sulfide exposure has also been shown to convert hemoglobin to sulfhemoglobin (SHb) which is incapable of carrying O_2 (Shen, 2015), however, while

sulfide exposure can produce SHb in fish, SHb production does not confer the same loss in O_2 transport in fish (Affonso *et al.*, 2002; Bagarinao & Vetter, 1992). Generally, tidal marsh fishes and other fish genera accustomed to areas of higher environmental sulfide concentrations show greater tolerance to the toxic gas in comparison to more open coast and pelagic marine species (Bagarinao & Vetter, 1989). Well adapted fish can oxidize small concentrations of H_2S to thiosulfate within the blood, and additional studies suggest that symbiotic relationships with sulfide-oxidizing bacteria or enhanced oxidation via mitochondrial processes increase tolerance in these species (Bagarinao & Vetter, 1989, Bagarinao, 1992). These natural capabilities are not abnormal given H_2S is naturally produced in most animals as it has some uniquely beneficial uses including the inhibition of apopotosis (Sen *et al.*, 2012), vasorelaxation to regulate blood pressure (Yang *et al.*, 2008), and as an O_2 sensor in tissues of cardiovascular and respiratory systems (Olson, 2015). Advanced investigation in the medical field continues to find more cellular systems reliant on the functionality of H_2S, however, these physiological processes utilize extremely small concentrations of hydrogen sulfide. As concentration grows, physiological damage increases and eventually thresholds of mortality are reached.

Sublethal concentrations of H_2S caused damage to the gills and liver of pelagic Atlantic salmon smolts over both gradual long-term experiments as well as short term acute exposures, indicating the toxicity of the compound can damage multiple essential organs even in small doses (Kiemer *et al.*, 1995). This physical damage can lead to indirect mortality even in the cases of organisms well adapted to sulfide environments. A recent study on H_2S-tolerant fish found the molecule impaired functionality within the eyes and drastically limited vision, which could inhibit predator avoidance and ultimately

lower fitness in prey species (Allore *et al.*, 2021). The effect of H_2S on respiration can also alter fish behavior as they experience lower tolerances to physical parameters such as thermal stress and hypoxia, as fish have been shown to seek cooler waters, swim along the surface where water is more oxygenated, and fully emerge from the water column to breathe atmospheric air (Skandalis *et al.*, 2020; Bagarinao & Vetter, 1989; Rossi *et al.*, 2019). In instances where behavioral adjustments are ineffective and tolerance thresholds are exceeded, mass mortality events may occur. H_2S has been ruled as the primary cause of death in a number of fish kills across a variety of marine settings including tropical fish farms and natural waters (Bagarinao & Lantin-Olaguer, 1998), shallow seas (Debol'skava *et al.*, 2005), and coastal estuaries (Lamberth *et al.*, 2010). Hypoxic conditions were recorded in all of these fish kills, but hypoxia could be either a facultative stressor enabling higher concentrations of H_2S or a result of H_2S reducing O_2 availability and redox potential within the sediments and water column (Maeda & Kawai, 1988). When coupled with hypoxia, H_2S acts synergistically as a catalyst for death, resulting in shorter survival times and higher rates of mortality (Bagarinao & Lantin-Olaguer, 1998; Vaquer-Sunyer & Duarte, 2010). Thus, shorter hypoxic durations that are incapable of killing fish from hypoxia alone may become much more lethal in the presence of H_2S, putting hypoxia-tolerant species at risk of succumbing to mortality. With this theory in mind, the present study aimed to test the factors of thermal stress, hypoxia, and H_2S exposure on *O. beta* to examine if this hypoxia-tolerant fish species shows significantly different rates of mortality in response to added H_2S exposure.

Chapter 2. Methods

2.1 *Experimental Animals*

Juvenile gulf toadfish (*Opsanus beta*) bred, fed, and maintained by the Grosell laboratory at the University of Miami's Rosenstiel School of Marine and Atmospheric Science (RSMAS) were utilized in this study. Gulf toadfish were selected for this experiment because they made up large proportions of the fishes killed in the 2020 fish kill (Miami Waterkeeper, 2020). For preliminary experiments, 20 individuals of similar size at two years of age were collected and moved to two separate tanks filled with 25 L of seawater. For all follow-up experiments ranging from the dates of April 25th to June 8th, one-year-old toadfish were selected for use due to their greater availability. Toadfish were fed every three days, with two-year-old fish receiving thawed squid and one-year-old fish receiving thawed artemia. Fish would not be fed within three days of their designated experimental trial, and all individuals were treated with malachite green immediately upon transfer to their respective aquaria to reduce risk of infection and once again every seven days. All handling and manipulation of fish were approved by the University of Miami's Institutional Animal Care and Use Committee (IACUC) on protocol 21-193.

2.2 *Experimental Design*

Controls for all experimental runs were carried out during the acclimation process. The control experimental trial involved holding fish at 32 °C without any additional stress treatments for a period of 24 hours, after which the same sample of fish would be exposed to their designated treatment level. Fish were slowly acclimated to the

temperature of 32 °C by raising the temperature of their aquarium 1 °C per day, and feeding regiments were continued during the acclimation period.

For preliminary studies, oxygen was lowered gradually from 6 mg/L to 2 mg/L over 4 hours after 24 hours of 32 °C exposure. This lowering rate matched drops recorded in the natural environment by the BB14 DO sensor during the 2020 fish kill. For sulfide-infusion trials, sodium sulfide solution was gradually added upon reaching the 2 mg/L DO threshold and the 24-hour experimental window commenced. Sulfide solution was continuously pumped in over 24 hours to keep sulfide concentrations stable, and water samples were taken throughout the experiment to measure total sulfide and hydrogen sulfide concentrations. Oxygen was kept stable via controlled nitrogen and air bubbling for 24 hours and fish mortality was assessed continuously for the first eight hours. Cameras were then set up adjacent to the tank to capture images every minute for the remainder of the 24-hour window, which allowed for mortality times to be estimated overnight. After 24 hours, a water sample was taken from H_2S-infused tanks to confirm final sulfide concentrations, and any surviving toadfish from the hypoxia-only trials were remediated through gradual oxygenation and cooling of the aquarium. Surviving toadfish from hydrogen sulfide experiments were euthanized via MS-222 immersion. Preliminary trials aimed to hold a target concentration of 4.4 uM H_2S, and sulfide measurements as well as mortality rates were reevaluated after the preliminary experimental run.

Subsequent trials investigated the effects of lower oxygen concentrations and higher H_2S concentrations on *O. beta* survival utilizing the same experimental design and apparatus as preliminary studies. Experimental hypoxia and hydrogen sulfide trials were carried out at a dissolved oxygen concentration of 1 mg/L, while extreme hypoxia trials

held a target concentration of 0.5 mg/L O_2. The oxygen lowering rate was extended so that O_2 concentration fell from 6 mg/L to 1 mg/L over the course of five hours or 6 to 0.5 mg/L over 5.5 hours, again matching observed rates of decline. Follow-up hydrogen sulfide trials were conducted at a target concentration of 8 uM H_2S, which was established using the same apparatus as in preliminary trials. While preliminary trials utilized sample sizes of ten fish per experimental run, sample sizes varied in follow-up experiments due to limitations in fish availability and mortality during the acclimation process (Table 1).

Experimental Trial Date	Treatment	Sample Size	Fish Mortality Size Range (g)
April 25th	Hypoxia	10	NA
May 23rd	Hypoxia	9	0.21 - 0.55
May 27th	Hypoxia	10	0.20 - 0.36
May 31st	Extreme Hypoxia	8	0.26 - 0.78
June 6th	Hydrogen Sulfide	7	0.19 - 0.79
June 8th	Hydrogen Sulfide	6	0.39 - 1.11

Table 1. Sample sizes as well as size ranges of the fish that succumbed to mortality during each respective experimental run following preliminary trials.

2.3 *Experimental Apparatus*

Experimental runs were conducted in 37 L glass aquaria filled with 25 L of seawater. Seawater was sourced directly from Bear Cut, a channel connecting Biscayne Bay to the Atlantic Ocean between Virginia Key and Key Biscayne (Fig. 1), then filtered and aerated through a filtration pump system at RSMAS prior to entering the aquarium. Each aquarium utilized a non-flow-through design to facilitate maintenance of sulfide concentration, oxygen saturation, and temperature. Temperature was controlled via a Finnex digital heater temperature control system, which utilized a submersible heater and thermometer sensor to actively monitor and stabilize water temperature. Water was

circulated via a submersible pump stationed alongside the heater to promote temperature uniformity throughout the tank. Oxygen concentration was controlled via an O2-stat system consisting of a Vernier LabView 3 controller, Vernier optical dissolved oxygen sensor, LabQuest digital control unit (DCU) and two solenoid valves. One solenoid valve controlled the flow of N2 gas to an airstone and the other solenoid valve controlled the flow of air to a second airstone. The LabView 3 actuated the N2 solenoid valve when O2 rose above the setpoint and actuated the air solenoid valve whenever O2 fell below the setpoint. The Vernier probe was able to measure both temperature and oxygen levels throughout the experimental run and log it in the LabQuest file system for verification after each experimental run. The surface of the water was covered as much as possible with plastic-aluminum sheeting to reduce atmospheric gas exchange and increase the efficiency of the aquarium in holding minimal oxygen levels. Air was bubbled in through an air stone via the same digital control system connected to the Vernier probe to allow for re-aeration of the water and a return to predetermined oxygen concentrations. Preliminary studies found oxygen concentrations decreased rapidly over time with the infusion of sodium sulfide solution to less than 1 mg/L O_2, thus re-aeration was necessary to control for confounding factors. Hydrogen sulfide infusion was carried out via an automated pump system connected to IV bags with minimal atmospheric headspace, and pump rates were determined by sulfide concentration and IV bag volume.

2.4 *Sulfide Infusion and Measurement*

Sulfide concentrations were created by dissolving sodium sulfide nonahydrate ($Na_2S \bullet 9\ H_2O$) crystals in degassed water at oxygen concentrations ≤ 1 mg/L. Sodium sulfide

crystals were rinsed in degassed water and blotted dry prior to weighing as outlined in the APHA methods for creating standard sulfide solutions (Baird *et al.*, 2017). Sodium sulfide crystal weight for solutions were calculated using APHA calculations dependent on pH, temperature, and salinity. After weighing, crystals were dissolved in nitrogen-bubbled water to avoid oxidation of hydrogen sulfide concentrations within the solution. Sulfide solutions were then stored in separate 1 L IV bags for continuous infusion via automated pump, and an initial high concentration sulfide solution was added directly to the tank in small increments to gradually achieve the target sulfide concentration within the aquarium. Solutions were stored in IV bags to minimize headspace and oxidation throughout the experiment, and multiple IV bags were used in each experimental run due to volume constraints on solution saturation. Supersaturation in IV bags caused sodium sulfide to precipitate and settle out, resulting in a release of unequal levels of hydrogen sulfide over time. This unequal release had the potential to lower the realized concentration of sulfide within the tank, thus multiple bags of lower concentration solutions were utilized to keep realized concentrations as consistent as possible over time.

Sulfide concentrations were measured via two methods of spectrophotometry, both of which were calibrated through experimental trials without animals. Both methods required the formation of five standard sulfide concentrations ranging from 1 to 8 mg/L S^{2-}. To prepare sulfide standards, sodium sulfide crystals were rinsed and dried prior to weighing as described previously. First, 0.75 g $Na_2S \bullet 9\ H_2O$ were weighed out and dissolved and mixed in 100 mL of degassed seawater to create a header solution of 100 mg/L. From this solution, 1 mL was taken and added to 99 mL of degassed seawater to create a solution of 1 mg/L S^{2-}. To keep the header solution from oxidizing, a nitrogen

bubbler was continuously running within the header solution while standards were being prepared. Thus, as soon as the first 1 mg/L standard was created, it was run through both spectrophotometry methods immediately. Upon finishing analysis, the next standard was created by pulling 2 mL from the header tank and adding 98 mL, and this procedure was repeated until standards of 1, 2, 4, 6, and 8 mg/L S^{2-} had been analyzed to create calibration curves. During experimentation, samples were taken after the first 15 minutes to measure initial sulfide concentration, then once every half-hour for at least the first nine hours of experimentation. While samples were not taken overnight, sulfide concentrations were measured once again in the morning as soon as possible and sampling continued every half-hour until the 24-hour experimental window closed.

The first method of spectrophotometry was an adapted version of the methylene blue method from the APHA standard methods of sulfide measurement (Baird *et al.,* 2017). The method involved treating a water sample with amine-sulfuric acid reagent, ferric chloride solution, and diammonium hydrogen phosphate solution, then using colorimetric analysis to compare the resulting solution to the calibration curves formed from the standards mentioned previously. The protocol used in this study largely follow the APHA procedure but deviated slightly by using a sample of filtered seawater as a blank rather than treating a second aquarium sample with blanking reagents. Preliminary experimental runs without animals found that utilizing a seawater blank alternative was more consistent and efficient in measuring total dissolved sulfide concentrations, thus no blanking reagents were used during actual experimental trials. The secondary method was adapted from an online study by Applied Analytics in which they showcased differences in hydrogen sulfide composition across varying pH levels (Applied Analytics, n.d.). In

this method, sulfide concentration and hydrogen sulfide concentration were measured directly from tank samples using a wavelength of 229 nm and quartz cuvettes. The spectrophotometer was once again blanked with filtered seawater, however no reagents were added to tank samples prior to spectrophotometric assessment. As with the blue methylene method, calibration curves were created from the standards mentioned previously and used to calculate sulfide concentrations from tank samples. Due to higher efficiency and precision (Fig. 7), only this direct measurement method was used to measure sulfide concentrations during the June 6th and June 8th experimental trials.

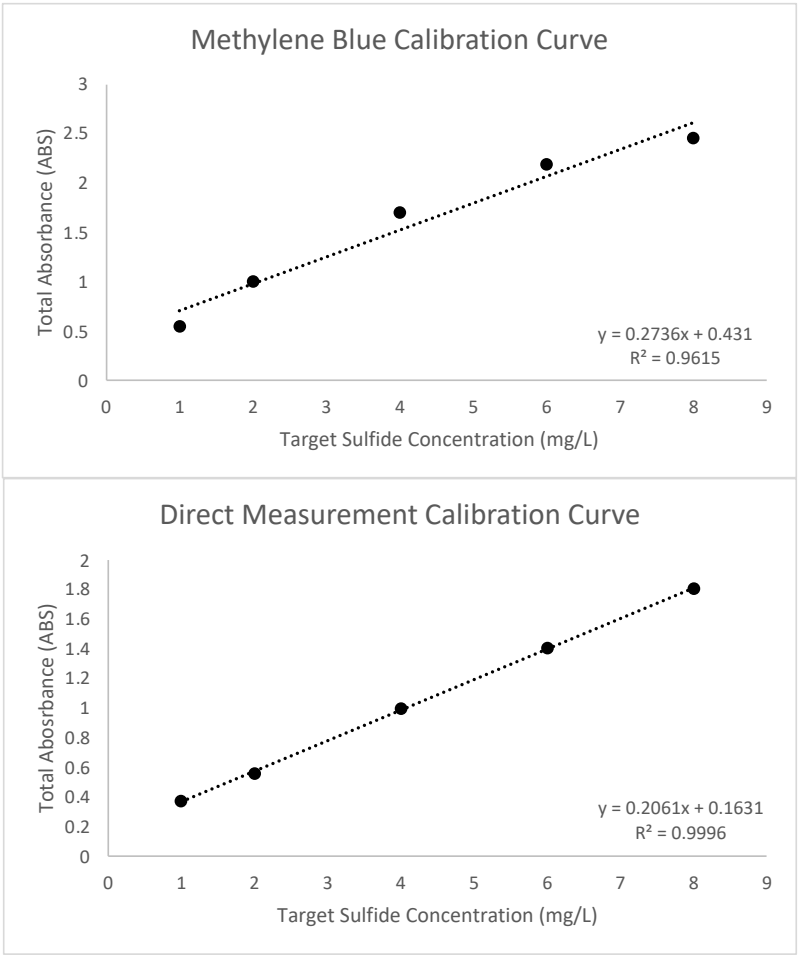


Figure 7. Comparison of calibration curves between the two spectrophotometric methods, with linear regression equations and R-squared values given for each set of standards.

2.5 *Data Analysis*

Temperature and oxygen concentration were continuously logged by the Vernier digital system at a rate of one sample every ten seconds. These data logs were then extracted from the logger and loaded into Microsoft Excel and RStudio for statistical analysis. Means and standard errors for temperature and DO were calculated for each trial run for comparison, and oxygen durations under specified concentration thresholds were calculated for each experimental run to assess possible differences in hypoxia severity. Sulfide concentration measurements were recorded in absorbance units and converted to total sulfide concentration in mg/L using calculations derived from standard calibration curves. The total sulfide concentrations were then converted to H_2S concentrations in uM utilizing calculations from the APHA *Standard Methods of the Examination of Water and Wastewater* (Baird *et al.*, 2017). H_2S concentrations were plotted over time.

Fish survival was observed continuously throughout the 24-hour period, and time elapsed until mortality was recorded for each fish in hours. Survival was observed through a combination of constant in-person observation and continuous timelapse photography at a 1-minute resolution. Elapsed time was then compared to percentage of sample mortality and plotted to show rate of mortality over time. In addition to survival time, dry weight and fork length was recorded for each fish that died during experimentation. LT50 curves, which estimate the amount of time needed to induce mortality in 50% of test individuals, were calculated for each experimental run by fitting a three-parameter Weibull type 2 model with an upper limit of 1 to binomial data using the ‘drm’ function in the *drc* package in R (https://cran.r-project.org/package=drc, Ritz & Strebig 2016). LT50s were originally calculated using a three-parameter log-logistic regression curve

fitted to binomial data with an upper limit of 1, however, AIC model selection determined the Weibull type 2 model to have the best fit. The slopes and estimated LT50 values of each curve were then tested for significance by carrying out a t-test on each estimated LT50 parameter using the 'compParm' function in *drc*. Additionally, average mortality time was compared between treatment types as well as among experimental runs using ANCOVA with fish weight as a covariate. Linear regressions were also calculated between fish weight and survival time for each treatment group.

Chapter 3. Results

3.1 *Water Quality*

The preliminary hypoxia trial held an average dissolved oxygen concentration of 1.89 ± 0.001 mg/L O_2 with an average temperature of 31.7 ± 0.003 °C, while the preliminary hydrogen sulfide trial averaged 1.15 ± 0.006 mg/L O_2 and 31.9 ± 0.004 °C. Dissolved oxygen concentration ranged from 1.54 – 2.21 mg/L O_2 and 0.51 – 3.32 mg/L O_2 in the preliminary hypoxia and hydrogen sulfide trials, respectively. The preliminary H_2S trial held an average concentration of 3.47 ± 0.15 uM H_2S over the experimental trial, however, H_2S fell from a starting concentration of 3.85 uM to 2.55 uM H_2S over the 16-hour experimental period.

Following the preliminary trials, DO levels were dropped and maintained below target O_2 concentration thresholds of 1 mg/L and 0.5 mg/L across all experimental runs dating from April 25th to June 8th (Fig. 8). Initial hypoxia trials at 1 mg/L ranged in DO concentration from 0.44 to 1.44 mg/L O_2 over the 24-hour experimental period, while replicate trials maintained a tighter scope of oxygen with ranges of 0.76-1.06 mg/L O_2 and 0.69-1.16 mg/L O_2 on May 23rd and May 27th, respectively. H_2S replicate trials held lower DO concentrations on average than all 1mg/L O_2 hypoxia trials, and DO values fell as low as 0.48 and 0.32 mg/L O_2 during the June 6th and June 8th trials, respectively. Experimental runs for all treatments held average temperatures within 0.2 °C of the target 32 °C threshold (Supplemental Fig. 1). H_2S concentrations averaged 7.51 ± 0.05 uM H_2S and 7.64 ± 0.05 uM H_2S on June 6th and June 8th respectively (Supplemental Fig. 2). Hydrogen sulfide concentration ranged from 6.96 – 8.07 uM H_2S in the first sulfide infusion experiment compared to a range of 7.18 – 8.26 uM H_2S in the replicate sulfide

trial. Aquaria pH was measured at 8.14 and 8.16 prior to both hydrogen sulfide experiments, thus H_2S composed roughly 2% of all sulfide present in the aquaria.

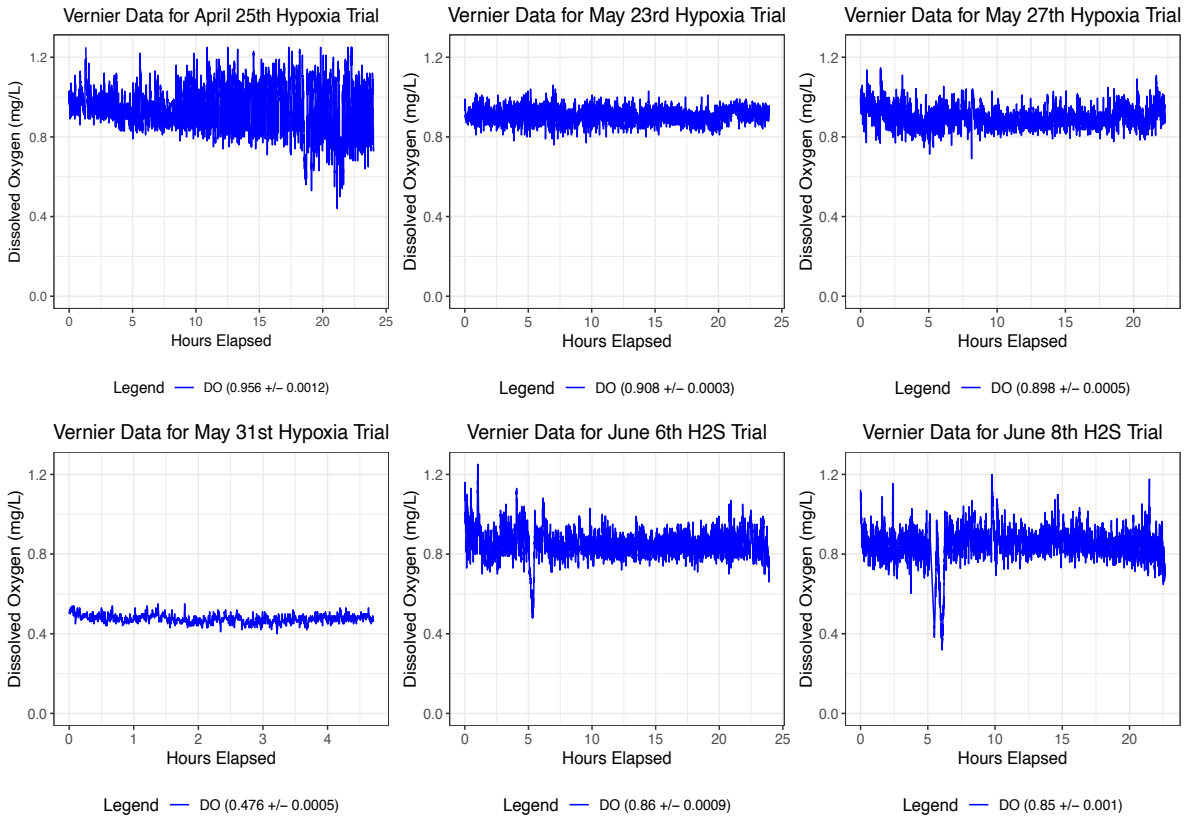


Figure 8. Dissolved oxygen data recorded by the Vernier optical probe in each experimental trial following preliminary studies. Oxygen was recorded continuously at a ten second sampling rate, and legend captions denote the average DO ± the standard error for each experimental run.

3.2 *Mortality by Treatment*

Preliminary hypoxia exposure at a target concentration of 2 mg/L O_2 resulted in no toadfish mortality over a 24-hour period, while preliminary hydrogen sulfide infusion at the same target oxygen threshold yielded complete mortality in 13.6 hours. A three-parameter log-logistic regression calculated the LT50 of the hydrogen sulfide preliminary trial to be 9.83 ± 0.39 hours, and survival time averaged 10.3 ± 0.82 hours for the sample population.

In follow-up studies, ANCOVA analysis found extreme hypoxia resulted in a significantly lower average time until mortality than the hypoxia and hydrogen sulfide treatments when including fish weight as a covariate and a Bonferroni correction (Fig. 9, $F(2, 33) = 9.89$, $p < 0.001$). Hypoxia and H_2S treatments did not yield significantly different times until death, however, using fish weight as a covariate did alter the relationship between average survival time under the two experiments (Supplemental Fig. 3).

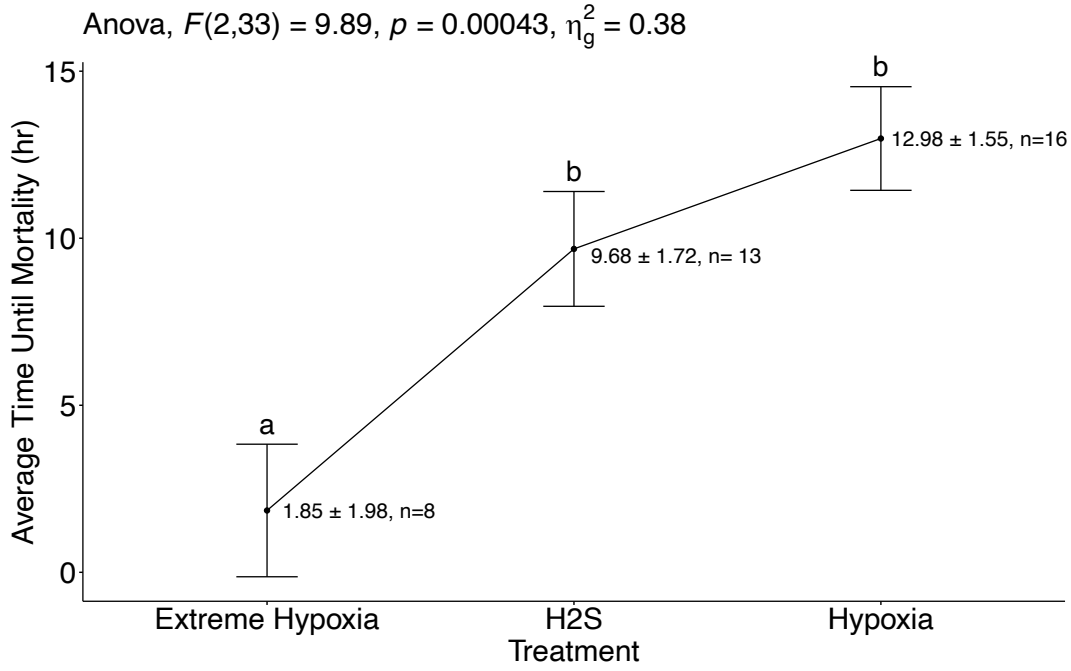


Figure 9. Average time until mortality under each treatment. Error bars depict ± 1 SE using ANCOVA analysis with weight as a covariate, labels denote the average survival time ± 1 SE in hours as well as total sample size for each treatment. Survival time under the extreme hypoxia treatment was significantly lower than under the H_sS and hypoxia treatments; no other significant differences by treatment were found.

ANCOVA analysis with the fish weight covariate and Bonferroni correction also found significantly different average times until mortality between individual trial runs of each treatment (Fig. 10, $F(4, 31) = 8.94$, $p < 0.001$). The hypoxia replicate from April 25th

was excluded from ANCOVA analysis due to no mortality throughout the trial. The covariate weight was not statistically independent of the variables treatment ($F(2,34) = 7.802$, $p=0.002$) and experimental trial ($F(4,32) = 5.48$, $p = 0.002$) were not statistically significant from one another, however, nonparametric covariate analysis was not possible in R. Kruskal-Wallis rank sum tests with Bonferroni correction found significantly lower survival times under the extreme hypoxia treatment in comparison to hydrogen sulfide and hypoxia ($X^2 = 15.033$, d.f. = 2, $p = 0.001$), as well as significant differences in survival time between individual experimental runs ($X^2 = 18.147$, d.f.=4, $p = 0.001$).

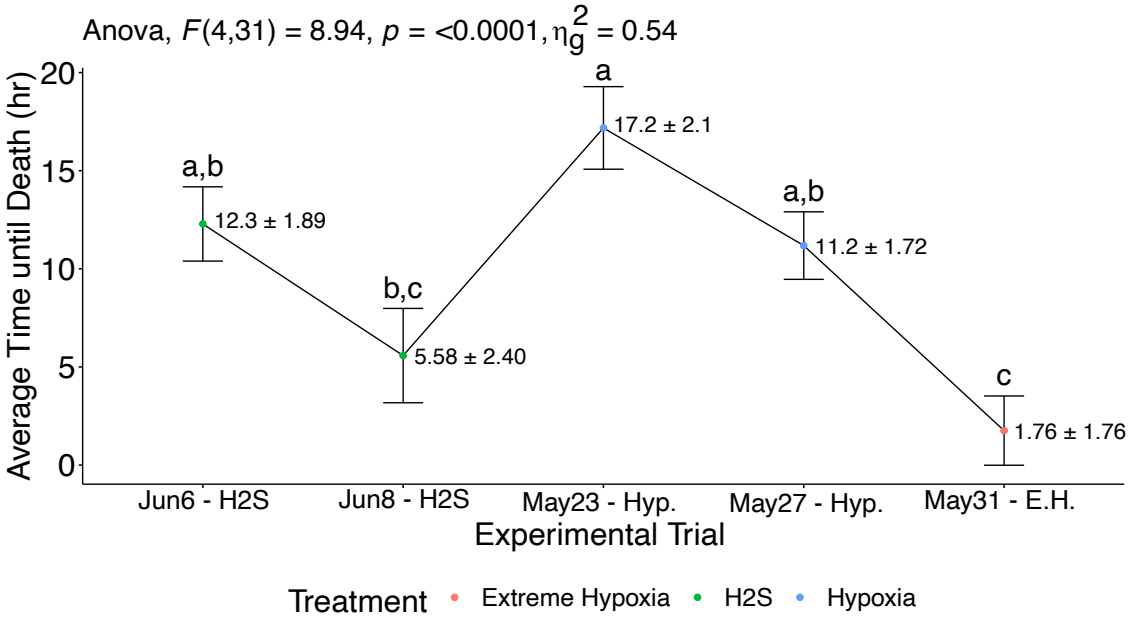


Figure 10. Average time until mortality for each replicate run. Error bars depict ± 1 SE using ANCOVA analysis with weight as a covariate, labels denote average survival time ± 1 SE in hours, letters denote significance between replicate trials, and dot colors indicate the treatment of each experimental trial.

Mortality was also compared across treatments using constructed LT50 curves to incorporate surviving fish from each trial in total mortality assessment. Extreme hypoxia yielded a significantly lower estimated LT50 value of 1.49 ± 0.32 hours than the H_2S (7.62 ± 1.29 hours) and hypoxia (8.64 ± 1.64 hours) treatments (Supplemental Table 1).

Individual LT50 curves were calculated for each replicate run and yielded significantly different LT50 values between and within treatments (Fig. 11, Supplemental Table 2). The April 25th hypoxia trial was removed from LT50 analysis as zero mortality within the 24-hour study window cannot be used to compute an estimated lethal time until death. Slopes of LT50 curves were compared both by treatment and by replicate run, and no significant differences were found. Individual LT50 curves were graphed to portray significant differences in LT50 by replicate trial (Supplemental Fig. 4).

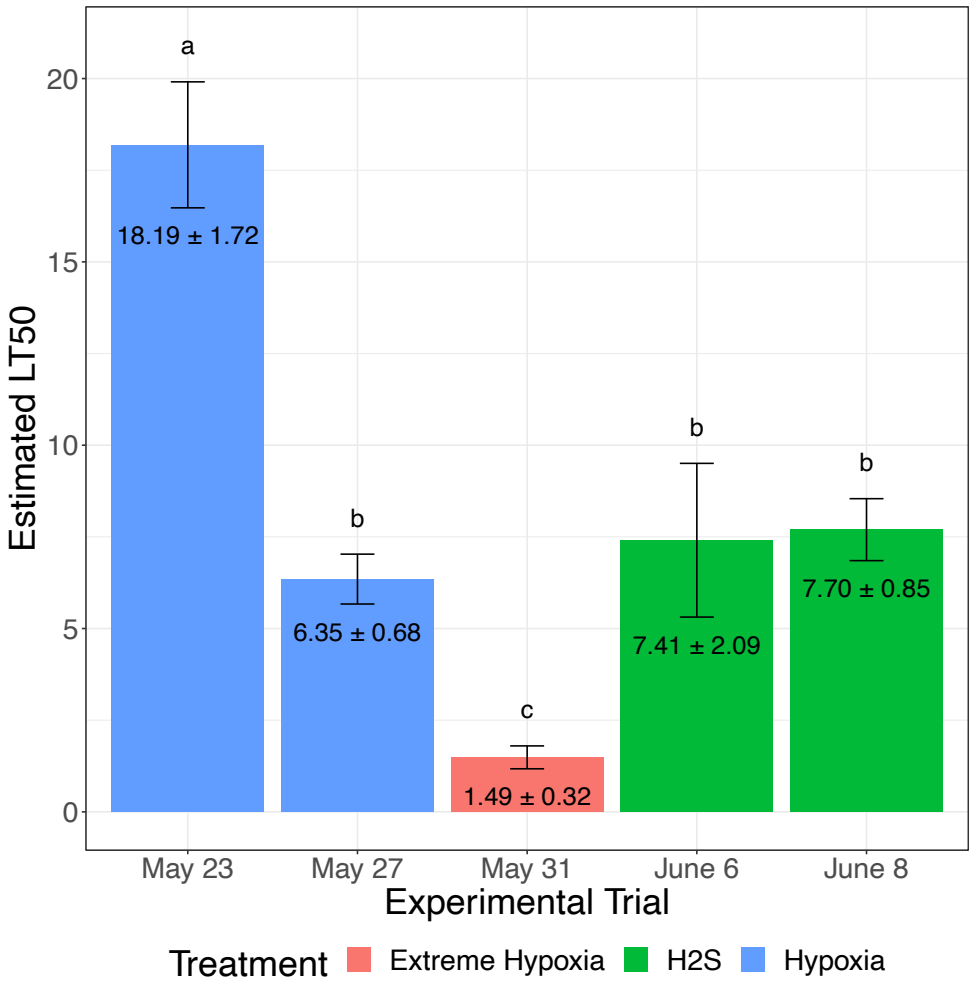


Figure 11. Comparison of estimated LT50 values by individual experimental trial. Models were fitted to proportion of mortality data over time, and error bars depict 1 SE around the estimated LT50 value. Letters denote significance from pairwise t-tests on estimated LT50 parameters, labels list LT50 ± 1 SE.

3.3 *Effect of Weight on Mortality*

Fish weights and lengths were not collected following preliminary trials but were recorded for every fish that died in follow-up experiments. Fish weight data violated Bartlett tests for homogeneity of variance among trials ($p < 0.001$) and among days ($p = 0.001$). Kruskal Wallis tests found fish weight was significantly lower in hypoxia trials ($X^2 = 10.75$, d.f. = 2, $p = 0.005$) and significantly different among replicate trial days ($X^2 = 13.803$, d.f. = 4, $p = 0.008$). For all trials at 1 mg/L O_2 regardless of treatment, fish survival time increased with increasing fish weight (Supplemental Fig. 5, $F(1,27) = 5.194$, $p = 0.03$). Likewise, survival time increased with fish weight within the hypoxia treatment at 1 mg/L O_2 ($F(1,14) = 5.286$, $p = 0.04$). However, no such relationships emerged in the other two treatments (Fig. 12).

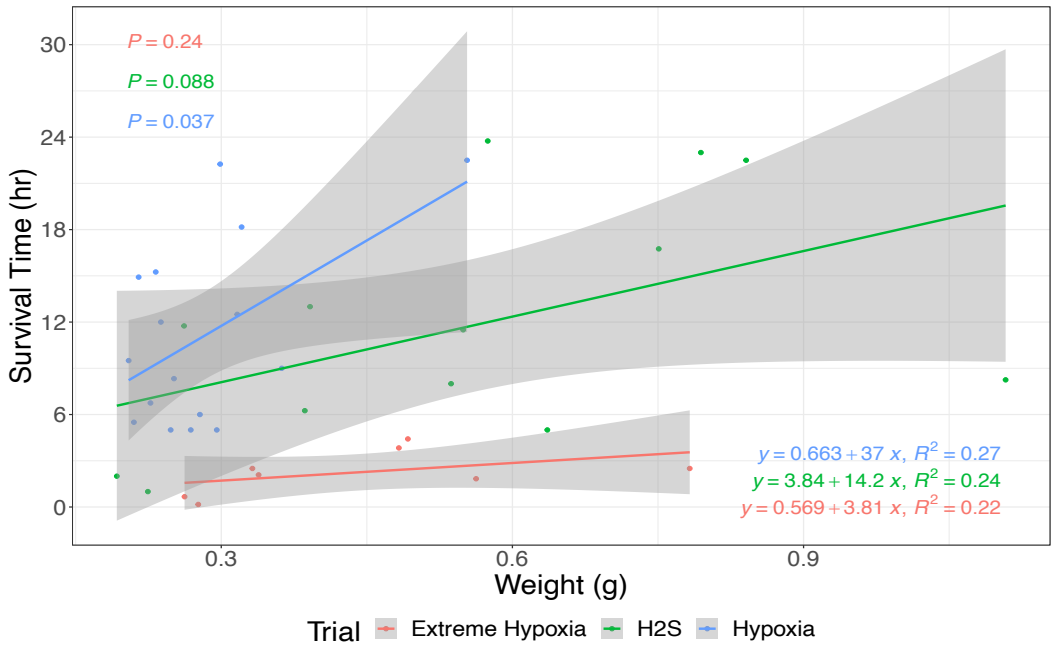


Fig. 12. Effect of fish weight (g) on survival time in each respective treatment group. Data only reflect fish that died within each experiment; surviving fish were not weighed. Lines reflect linear regressions for each treatment type, ribbons illustrate 95% confidence bands. Equations and r-squared values are shown in the top left for each line, while P-values are shown in the bottom right corner.

Chapter 4. Discussion

Comparison of mortality between the three treatments found that extreme hypoxia at 0.5 mg/L O_2 yielded significantly faster rates of mortality in juvenile toadfish than hypoxia at 1 mg/L O_2 as well as hypoxia with hydrogen sulfide infusion with an average concentration of around 7.5 uM H_2S. While previous studies have shown that *Opsanus beta* exhibits similar cardiovascular and respiratory responses to acute hypoxia as other teleost species (McDonald *et al.*, 2010), toadfish are generally viewed as a hypoxia-tolerant species (Frank *et al.*, 2022; Ultsch *et al.*, 1981; Amador *et al.,* 2018). Total survival of juveniles during the preliminary hypoxia trial at 2 mg/L O_2 and initial follow-up experiment at 1 mg/L O_2 supports the notion that gulf toadfish have high tolerances to hypoxia under the generally accepted dissolved oxygen threshold of 2 mg/L. However, the rapid decline in survival time following minimal decreases in oxygen concentration past 1 mg/L supports studies contending such universal levels of hypoxia thresholds are not applicable to all species (Vaquer-Sunyer & Duarte, 2008). The fourfold difference in survival time under extreme hypoxia conditions compared to hypoxia illustrates a stark threshold in survivorship over a range of just 0.4 mg/L O_2. Comparatively, *Leistostomus xanthurus* and *Brevoortia tyrannus*, which occupy similar occasionally hypoxic estuarine habitats as *Opsanus beta* during juvenile stages, showed similar exponential drops in LT50 across a narrower range of dissolved oxygen values at overall higher levels of oxygen (Shimps *et al.*, 2005). Thus, while *Opsanus beta* may show greater hypoxia tolerance at oxygen levels that are harmful for co-inhabitant estuarine fishes, lethality still increases at an exponential rate once a threshold is reached. During the 2020 fish kill in North Biscayne Bay, durations of oxygen concentrations ≤ 0.5 mg/L were recorded on

consecutive days from August 10 – 12, with multiple durations surpassing the estimated LT50 time derived from this experiment (Supplemental Table 3). Environmental levels of hypoxia should not be examined at all-encompassing baseline concentrations, but rather at species-specific critical thresholds derived from research on the physiological processes, survival and behavioral capabilities of focal organisms (Farrell & Richards, 2009).

The lack of significant differences between pooled hypoxia and hydrogen sulfide treatments was surprising, especially given the differences in oxygen concentration between the two treatments. Not only did the sulfide infusion treatment introduce concentrations of toxic H_2S, but both trials had significantly lower levels of dissolved oxygen than either hypoxia trial. The preliminary hydrogen sulfide trial experienced a drastic drop in oxygen over the experimental period due to the rapid oxidation rates of sulfide which have been shown to reduce oxygen concentrations along sulfidic redox boundary layers (Morse *et al.*, 1987; Maeda *et al.*, 1988). Follow-up trials used the adjusted Vernier system with a minimal oxygen threshold to bubble in oxygen when DO concentrations dropped too low, and this remediation of oxygen directly to the tank may have presented a confounding variable on fish survival. Behaviorally, fish exhibited similar responses of surface swimming and loss of equilibrium across both treatments prior to death, and some fish were also observed to detect areas of higher oxygen concentration through their movement to the oxygen bubbling air stone over time. It is difficult to discern whether apneic responses and mortality were due to the toxicity of H_2S or the effect of the chemical on oxygen concentrations, so future studies utilizing a flow-through system for oxygen maintenance might help to address these difficulties.

Additionally, while this pilot study was unable to utilize power analyses to estimate adequate sample size and had a limitation of fish availability, further replication of experimental trials with greater sample sizes may result in clearer statistical distinctions among treatments.

Another potential reason for lack of differences between hydrogen sulfide and hypoxia trials could be the concentration of H_2S was too minimal to detect significant toxicity effects. Previous studies on species along the San Diego coast show tidal marsh species such as killifish could survive longer than 20 hours at concentrations up to 50 uM H_2S; however, these studies were not done in tandem with hypoxia (Bagarinao & Vetter, 1989). Environmental data reports from fish kills in the canal tributaries of a bay in Delaware showed H2S concentrations reached millimolar levels at depths of 4.5m within the canals (Luther *et al.*, 2004), indicating canal systems can foster extremely high levels of H2S production. In the *Thalassia* die-offs in Florida Bay in 1990, porewater total sulfide levels were millimolar which indicates H_2S porewater concentrations could have been as high as 45 uM, assuming a pH of 8.14 and temperature of 32 °C (Carlson *et al*., 1994*)*. A 2017 Florida Power and Light (FPL) assessment report found porewater sulfide concentrations ranging from 0 - 400 mg/L S^2 across sample sites in southern Biscayne Bay, however, no specific site locations or other available water quality data was provided in this report (FPL, 2017). There are currently no available data on hydrogen sulfide concentrations in northern Biscayne Bay during these recurring fish kill events, however, pH levels during the August 2020 event ranged from 7.5 to 7.7 and thus almost 10% of available sulfide was available as unionized H_2S within the porewater compared to the 2.5% proportion of sulfide used in laboratory experiments (pH 8.1-8.15). Bottom

water surveys collected by the Florida Department of Environmental Protection did not record quantifiable concentrations of sulfide, however, these collections were taken several days after the fish kill as part of a pilot field survey (Aliza Karim, personal communication). Field surveys projected for the summer and fall months of 2022 plan to take more accurate porewater and bottom water samples throughout the bay on a regular basis, providing potentially useful spatiotemporal datasets on realized sulfide concentrations in Northern Biscayne Bay. Additionally, further experiments investigating the release of porewater sulfide concentrations into the bottom layer of the water column may offer more insight on the potential sulfide concentration maxima in demersal fish habitats. Utilizing these field data, future studies could use natural sulfide concentrations in tandem with pH levels during fish kill events to produce a more accurate environment for comparison of hypoxia and hydrogen sulfide on toadfish survival.

Survival time did not vary with fish weight under extreme hypoxia conditions, but fish weight held a significantly positive linear relationship with survival time across all mortalities during 1 mg/L O_2 trials, including those with hydrogen sulfide infusion. Additionally, accounting for weight in covariate analysis shifted the comparison of survival times between hypoxia and hydrogen sulfide trials, indicating weight may have played a role in survivorship under both treatments. Size as a factor of fish hypoxia tolerance has long been debated, and a metanalysis study found that while body size had no impact on oxygen uptake ability under hypoxia, survival time under severe hypoxia does increase exponentially with body mass due to scaling of anaerobic metabolism and glycolysis (Nilsson & Ostlund-Nilsson, 2008). In another demersal Batrichoidid species, *Poricthhys notatus*, males exhibited greater tolerances to hypoxia stress than their female

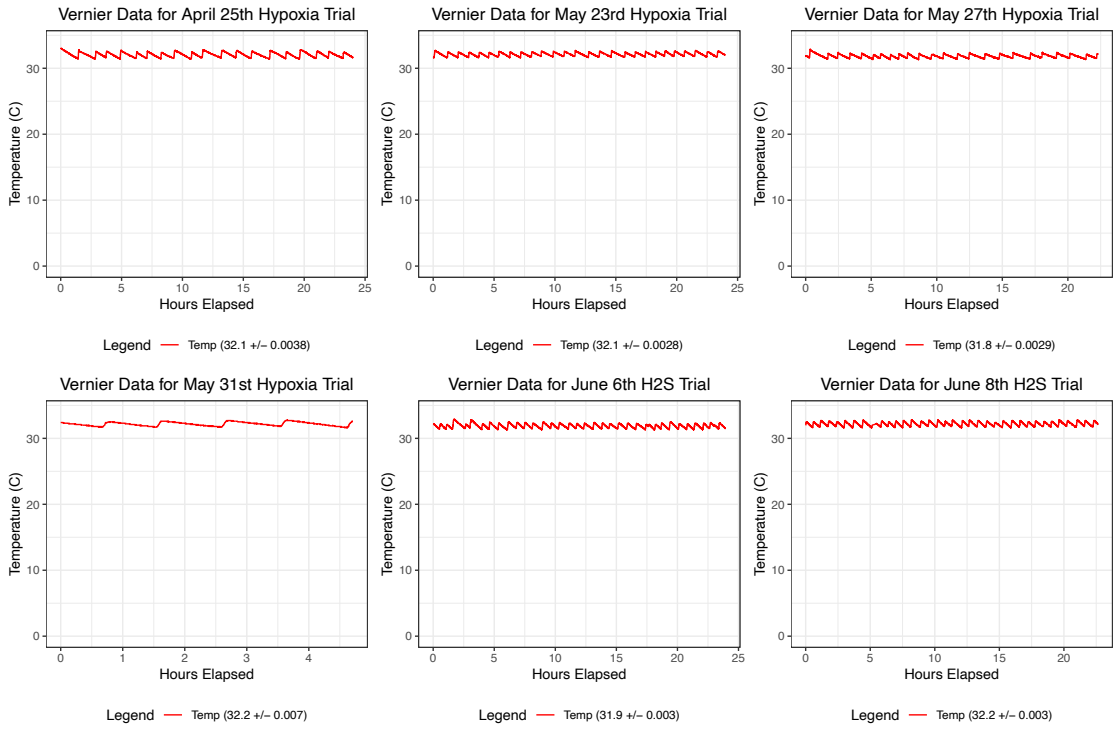
counterparts due to higher glycolytic potential with size (LeMoine *et al.*, 2014), suggesting the idea of larger size advantages could apply to the related *Opsanus beta* as well. However, studies on juvenile estuarine fish found species-specific differences in relationships between size and hypoxia survival times (Shimps *et al.*, 2005), and larval and juvenile gulf toadfish have been found to exhibit similar capacities for hypoxia tolerance despite differences in size by utilizing differing metabolic strategies (Frank *et al.*, 2022). Thus, assessing a wider range of body sizes is necessary to fully understand how fish weight correlates with hypoxia tolerance in *Opsanus beta*. It is important to note that no juvenile fish floated to the surface after succumbing to mortality, even those that died overnight and had multiple hours within the tank to float to the surface. If larger toadfish are more resilient to hypoxia but were still observed floating along the surface following the recent Biscayne Bay fish kills, it can be assumed that a large quantity of juvenile toadfish also died during these fish kills and remained unaccounted for in carcass surveys.

While this study was unable to significantly differentiate the effects of hydrogen sulfide and hypoxia on mortality in toadfish, it did verify that *Opsanus beta* can succumb to mortality in durations of low oxygen concentrations recorded in northern Biscayne Bay. Seasonal recurrence is a common trait detected in many of the world's hypoxic zones (Rabotyagov *et al.*, 2014), with many aquatic ecosystems forming hypoxic bottom waters during summer months when stratification within the water column naturally intensifies (Klump *et al.*, 2018; Bishop *et al.*, 2006; Fennel & Testa 2019). Recurring hypoxia can impact demersal fish species at sublethal levels through the reduction of habitat availability and the increase in density-dependent interactions including an

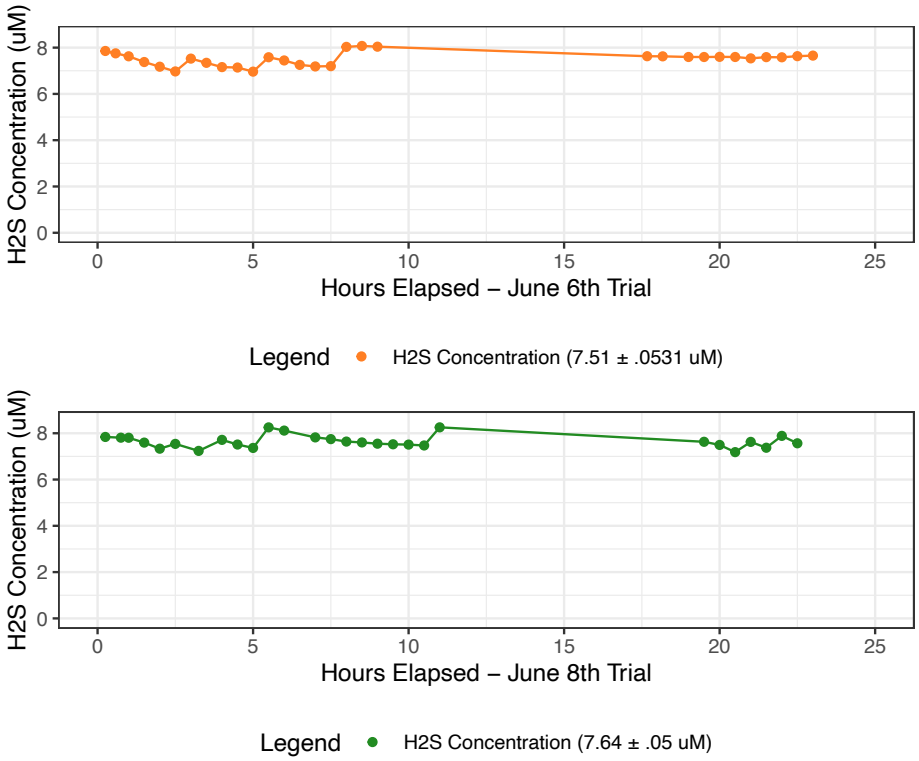
increase in predation risk on smaller, younger organisms (Orio *et al.*, 2019; Eby *et al.*, 2005). Population studies on *Opsanus beta* in the 1990s found abundance to correlate positively with nocturnal dissolved oxygen and seagrass cover (Serafy *et al.*, 1997), indicating gulf toadfish exhibit similar traits of preferential avoidance of lower oxygenated environments. Juvenile estuarine species have been found to behaviorally prioritize predator avoidance over hypoxia avoidance; however (Froeschke & Stunz, 2012), and it is unclear how juvenile toadfish behave during these intense hypoxia episodes. Multiple fish species show greater hypoxia tolerances and altered behavioral reactions and physiological processes with increased preconditioning and acclimatization (Nilsson & Renshaw, 2004; Rees *et al.*, 2001), thus local *Opsanus beta* populations may start to exhibit phenotypical differences with recurring fish kill events in northern Biscayne Bay. Ultimately, ecological assessments in NBB should investigate the spatial and temporal distributions of *Opsanus beta* and other demersal species to determine whether recurring hypoxia and previous fish kills have significantly altered local populations.

SUPPLEMENTAL MATERIAL

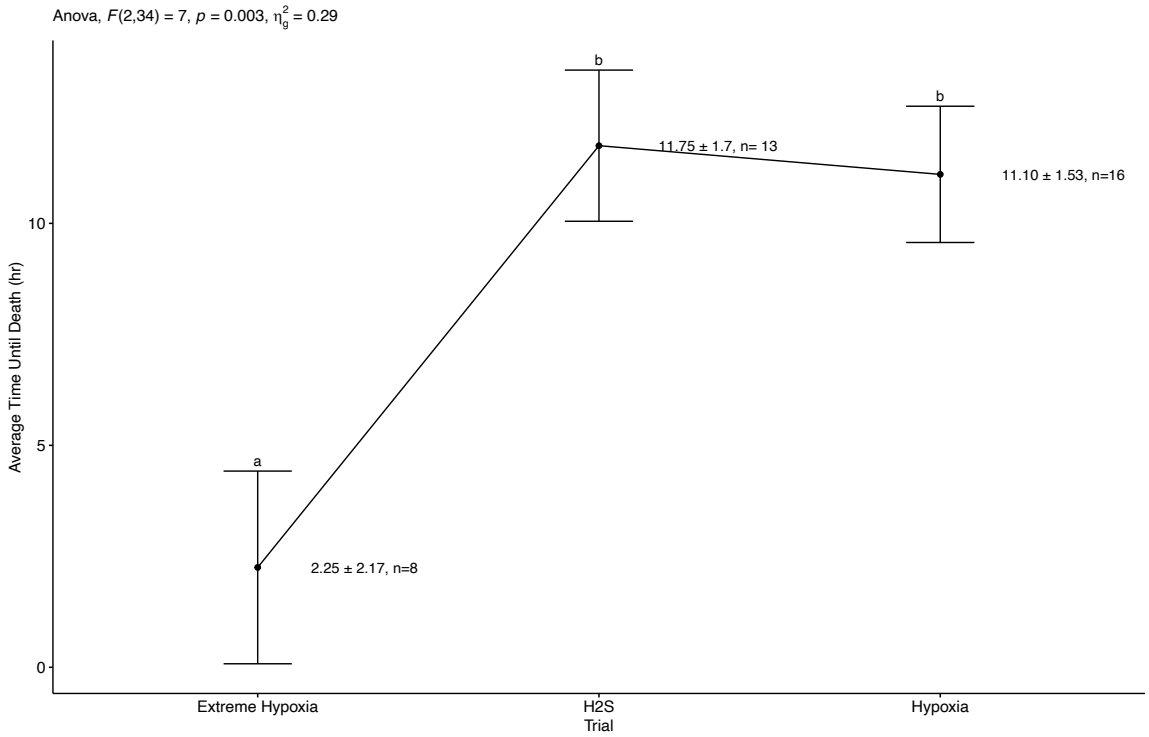
FIGURES



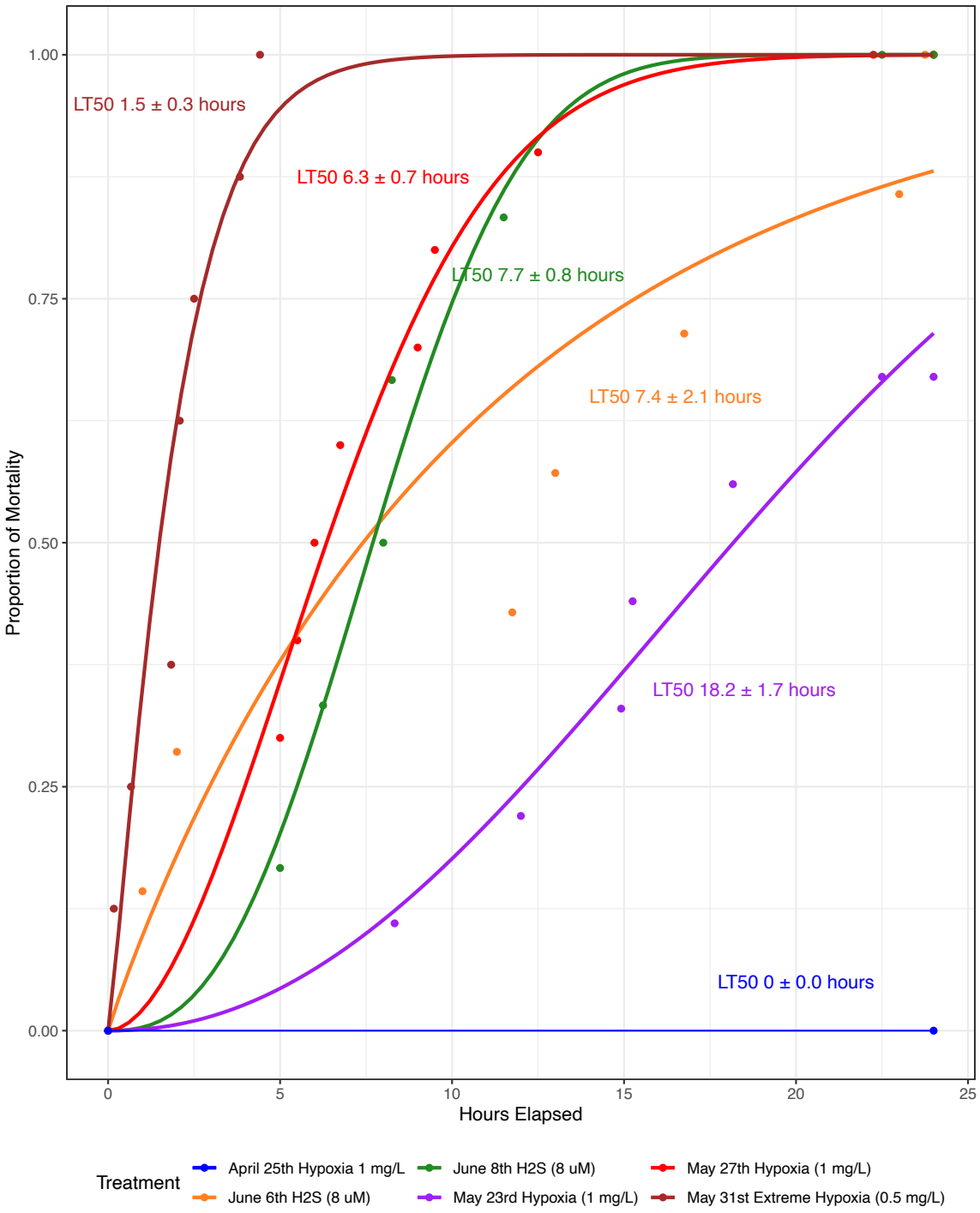
Supplemental Figure 1. Temperature data recorded by the Vernier optical probe in each experimental trial following preliminary studies. Temperature was recorded continuously at a ten second sampling rate, and legend captions denote the average temperature in Celsius ± the standard error for each experimental run.



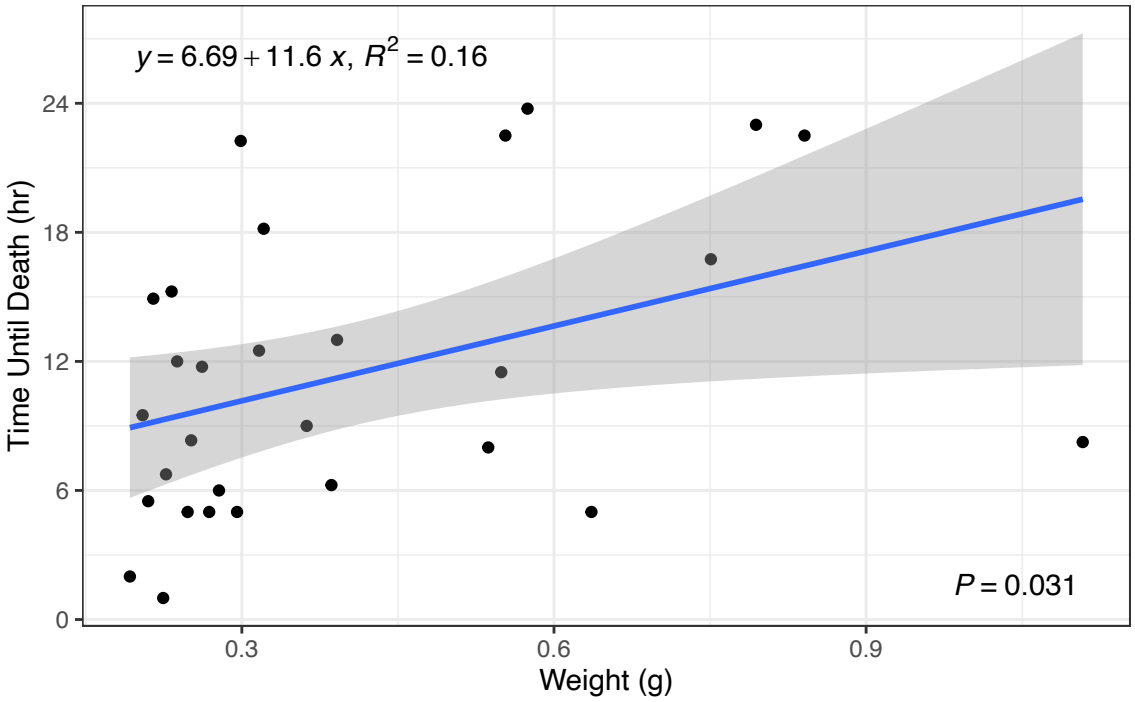
Supplemental Figure 2. Measured hydrogen sulfide concentrations over time from each replicate experimental run. Target concentration was 8 uM H2S; average concentration ± standard error for each trial are listed under their respective graphs.



Supplemental Figure 3. Average time until mortality under each treatment using ANCOVA analysis without weight as a covariate. Error bars depict ± 1 SE; labels denote the average survival time ± 1 SE in hours as well as total sample size for each treatment.



Supplemental Figure 4. Proportion of mortality over time calculated by fitting three-parameter Weibull type 2 models with an upper limit of 1 to binomial data using the drc package in R. An additional line is included to show proportion of mortality for the April 25th Hypoxia trial in which no fish died over the span of 24 hours. Labels denote the estimated LT50 for each curve ± standard error.



Supplemental Figure 5. Linear regression of time until death by fish weight for all fish that died during hypoxia and hydrogen sulfide treatments under a target oxygen threshold of 1 mg/L. Gray shading depicts the 95% confidence bands around the regression; R^2 and p-value are shown in the upper left and lower right corners respectively.

TABLES

Comparison of LT50 Parameter Estimates by Treatment					
Treatment Comparison	Estimate	Std. Error	t-value	p-value	Significance
Hypoxia-Extreme Hypoxia	12.1697	2.2137	5.4975	3.85E-08	***
Hypoxia-H2S	3.7681	2.5944	1.4524	0.1464	
Extreme Hypoxia-H2S	-8.4016	1.4401	-5.8342	5.40E-09	***

Supplemental Table 1. Output table of t-test comparisons on estimated LT50 parameters for each treratment generated by the compParm function in R.

Comparison of LT50 Parameter Estimates by Day					
Experimental Trial Comparison	Estimate	Std. Error	t-value	p-value	Significance
M23-M27	13.93901	2.53837	5.4913	3.99E-08	***
M23-M31	19.62798	2.46885	7.9503	1.82E-15	***
M23-J6	10.77903	3.39885	3.1714	0.0015172	**
M23-J8	12.79582	2.62663	4.8716	1.11E-06	***
M27-M31	5.68897	0.76904	7.3975	1.39E-13	***
M27-J6	-3.15998	2.45935	-1.2849	0.1988324	
M27-J8	-1.14319	1.18128	-0.9678	0.3331653	
M31-J6	-8.84895	2.38752	-3.7063	0.0002103	***
M31-J8	-6.83216	1.02337	-6.6761	2.45E-11	***
J6-J8	2.01679	2.55034	0.7908	0.4290666	

Supplemental Table 2: Output table of t-test comparisons on estimated LT50 parameters for each experimental trial generated by the compParm function in R.

Extreme Hypoxia Durations (≤ 0.5 mg/L) Recorded at BB14				
Date	Start Time	Duration (hr)	Average DO (mg/L)	Average Temperature (C)
8/10/20	6:15:00	3	0.16538462	31.55315
8/10/20	19:30:00	1.25	0.27333333	32.74983
8/11/20	2:15:00	9.5	0.06051282	32.28777
8/11/20	18:45:00	5	0.0952381	33.37333
8/12/20	4:45:00	0.75	0.3	32.12125
8/12/20	6:00:00	2.25	0.066	32.0286
8/12/20	8:45:00	1	0.254	31.6586

Supplemental Table 3. Extreme hypoxia durations under a threshold level of 0.5 mg/L O_2 recorded at the BB14 sensor. Average dissolved oxygen concentration and temperature were calculated for each duration.

REFERENCES

Affonso, E. G., Polez, V. L. P., Corrêa, C. F., Mazon, A. D. F., Araujo, M. R. R., Moraes, G., & Rantin, F. T. (2002). Blood parameters and metabolites in the teleost fish Colossoma macropomum exposed to sulfide or hypoxia. *Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology*, *133*(3), 375-382.

Albert, S., Bonaglia, S., Stjärnkvist, N., Winder, M., Thamdrup, B., & Nascimento, F. J. (2021). Influence of settling organic matter quantity and quality on benthic nitrogen cycling. *Limnology and Oceanography*, *66*(5), 1882-1895.

Allore, C. A., Rossi, G. S., & Wright, P. A. (2021). Seeing in the swamp: hydrogen sulfide inhibits eye metabolism and visual acuity in a sulfide-tolerant fish. *Biology Letters*, *17*(9), 20210329.

Amador, M. H., Schauer, K. L., & McDonald, M. D. (2018). Does fluoxetine exposure affect hypoxia tolerance in the Gulf toadfish, Opsanus beta?. *Aquatic Toxicology*, *199*, 55-64.

Applied Analytics. (n.d.). *Ph-independent measurement of hydrogen sulfide in liquids*. Retrieved February 24, 2022, from https://aai.solutions/news/ph-independent-measurement-of-hydrogen-sulfide-in-liquids

Bagarinao, T. (1992). Sulfide as an environmental factor and toxicant: tolerance and adaptations in aquatic organisms. *Aquatic Toxicology*, *24*(1-2), 21-62.

Bagarinao, T., & Lantin-Olaguer, I. (1998). The sulfide tolerance of milkfish and tilapia in relation to fish kills in farms and natural waters in the Philippines. *Hydrobiologia*, *382*(1), 137-150.

Bagarinao, T., & Vetter, R. D. (1989). Sulfide tolerance and detoxification in shallow-water marine fishes. *Marine Biology*, *103*(3), 291-302.

Bagarinao, T., & Vetter, R. D. (1992). Sulfide-hemoglobin interactions in the sulfide-tolerant salt marsh resident, the California killifish Fundulus parvipinnis. *Journal of Comparative Physiology B*, *162*(7), 614-624.

Baird, R. B., Bridgewater, L. L., Eaton, A. D., & Rice, E. W. (2017). *Standard methods for the examination of water and wastewater*. American Public Health Association.

Biber, P. D., & Irlandi, E. A. (2006). Temporal and spatial dynamics of macroalgal communities along an anthropogenic salinity gradient in Biscayne Bay (Florida, USA). *Aquatic Botany*, *85*(1), 65-77.

Bishop, M. J., Powers, S. P., Porter, H. J., & Peterson, C. H. (2006). Benthic biological effects of seasonal hypoxia in a eutrophic estuary predate rapid coastal development. *Estuarine, Coastal and Shelf Science*, *70*(3), 415-422.

Borum, J., Pedersen, O., Greve, T. M., Frankovich, T. A., Zieman, J. C., Fourqurean, J. W., & Madden, C. J. (2005). The potential role of plant oxygen and sulphide dynamics in die-off events of the tropical seagrass, Thalassia testudinum. *Journal of Ecology*, *93*(1), 148-158.

Breitburg, D. (2002). Effects of hypoxia, and the balance between hypoxia and enrichment, on coastal fishes and fisheries. *Estuaries*, *25*(4), 767-781.

Briceño, Henry O., Joseph N. Boyer and Peter W. Harlem. 2011. Ecological Impacts on Biscayne Bay and Biscayne National Park from Proposed South Miami-Dade County Development, and Derivation of Numeric Nutrient Criteria for South Florida Estuaries and Coastal Waters. NPS TA# J5297-08- 0085, Florida International University, Southeast Environmental Research Center Contribution # T- 530, 145 p

Browder, J. A., Alleman, R., Markley, S., Ortner, P., & Pitts, P. A. (2005). Biscayne Bay conceptual ecological model. *Wetlands*, *25*(4), 854-869.

Caccia, V. G., & Boyer, J. N. (2005). Spatial patterning of water quality in Biscayne Bay, Florida as a function of land use and water management. *Marine Pollution Bulletin*, *50*(11), 1416-1429.

Caccia, V. G., & Boyer, J. N. (2007). A nutrient loading budget for Biscayne Bay, Florida. *Marine Pollution Bulletin*, *54*(7), 994-1008.

Cantillo, A. Y., Pikula, L., Hale, K. K., Collins, E. V., & Caballero, R. (2000). Biscayne Bay environmental history and annotated bibliography.

Carlson Jr, P. R., Yarbro, L. A., & Barber, T. R. (1994). Relationship of sediment sulfide to mortality of Thalassia testudinum in Florida Bay. *Bulletin of marine Science*, *54*(3), 733-746.

Chin, D. A. (2020). Source Identification of Nutrient Impairment in North Biscayne Bay, Florida, USA. *Journal of Environmental Engineering*, *146*(9), 04020101.

Collado-Vides, L., Cifuentes, P., Bally, N., Iporac, L. A. R., & Olszak, S. (2020). Variability of nutrients and trace metals tissue content in two pelagic sargassum (Ochrophyta, Phaeophyceae) species from South Florida compared with global data. *72nd Gulf and Caribbean Fisheries Institute* (pp. 263-267).

Coskun, Ö. K., Özen, V., Wankel, S. D., & Orsi, W. D. (2019). Quantifying population-specific growth in benthic bacterial communities under low oxygen using H218O. *The ISME journal*, *13*(6), 1546-1559.

Debol'skaya, E., Yakushev, E., & Sukhinov, A. (2005). Formation of fish kills and anaerobic conditions in the Sea of Azov. *Water resources*, *32*(2).

Deegan, L. A., Wright, A., Ayvazian, S. G., Finn, J. T., Golden, H., Merson, R. R., & Harrison, J. (2002). Nitrogen loading alters seagrass ecosystem structure and support of higher trophic levels. *Aquatic Conservation: Marine and Freshwater Ecosystems*, *12*(2), 193-212.

Diaz, R. J., & Rosenberg, R. (2008). Spreading dead zones and consequences for marine ecosystems. *science*, *321*(5891), 926-929.

Eby, L. A., Crowder, L. B., McClellan, C. M., Peterson, C. H., & Powers, M. J. (2005). Habitat degradation from intermittent hypoxia: impacts on demersal fishes. *Marine Ecology Progress Series*, *291*, 249-262.

Farrell, A. P., & Richards, J. G. (2009). Defining hypoxia: an integrative synthesis of the responses of fish to hypoxia. In *Fish physiology* (Vol. 27, pp. 487-503). Academic Press.

Fennel, K., & Testa, J. M. (2019). Biogeochemical controls on coastal hypoxia. *Annual Review of Marine Science*, *11*, 105-130.

Florida Power and Light. (2017). *Site Assessment Report; Ammonia in Surface Waters, Turkey Point Facility: March 17* (ECRC-17-014963). http://www.psc.state.fl.us/library/filings/2017/09365-2017/Support/EXH%2062%2020170007%20-%20Staff%27s%203rd%20POD%20No.%2025%20-%20Attachment%20No.%201.pdf

Forgan, L. G., & Forster, M. E. (2010). Oxygen consumption, ventilation frequency and cytochrome c oxidase activity in blue cod (Parapercis colias) exposed to hydrogen sulphide or isoeugenol. *Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology*, *151*(1), 57-65.

Frank, L., Serafy, J., Grossel, M. (2022). *The metabolic response of the larval gulf toadfish, Opsanus beta, during combined thermal and hypoxia stress and how it develops with age*. [Manuscript submitted for publication]. *Department of Marine Biology & Ecology, University of Miami.*

Froeschke, J. T., & Stunz, G. W. (2012). Hierarchical and interactive habitat selection in response to abiotic and biotic factors: the effect of hypoxia on habitat selection of juvenile estuarine fishes. *Environmental Biology of Fishes*, *93*(1), 31-41.

Gilby, B. L., Olds, A. D., Connolly, R. M., Maxwell, P. S., Henderson, C. J., & Schlacher, T. A. (2018). Seagrass meadows shape fish assemblages across estuarine seascapes. *Marine Ecology Progress Series*, *588*, 179-189.

Guidotti, T. L. (1996). Hydrogen sulphide. *Occupational Medicine*, *46*(5), 367-371.

Hicks, N., Liu, X., Gregory, R., Kenny, J., Lucaci, A., Lenzi, L., ... & Duncan, K. R. (2018). Temperature driven changes in benthic bacterial diversity influences biogeochemical cycling in coastal sediments. *Frontiers in Microbiology*, *9*, 1730.

Howarth, R., Chan, F., Conley, D. J., Garnier, J., Doney, S. C., Marino, R., & Billen, G. (2011). Coupled biogeochemical cycles: eutrophication and hypoxia in temperate estuaries and coastal marine ecosystems. *Frontiers in Ecology and the Environment*, *9*(1), 18-26.

Hrycik, A. R., Almeida, L. Z., & Höök, T. O. (2017). Sub-lethal effects on fish provide insight into a biologically-relevant threshold of hypoxia. *Oikos*, *126*(3), 307-317.

Hull, V., Parrella, L., & Falcucci, M. (2008). Modelling dissolved oxygen dynamics in coastal lagoons. *Ecological Modelling*, *211*(3-4), 468-480.

Keeling, R. F., Körtzinger, A., & Gruber, N. (2010). Ocean deoxygenation in a warming world. *Annual review of marine science*, *2*, 199-229.

Kiemer, M. C., Black, K. D., Lussot, D., Bullock, A. M., & Ezzi, I. (1995). The effects of chronic and acute exposure to hydrogen sulphide on Atlantic salmon (Salmo salar L.). *Aquaculture*, *135*(4), 311-327.

Klump, J. V., Brunner, S. L., Grunert, B. K., Kaster, J. L., Weckerly, K., Houghton, E. M., ... & Valenta, T. J. (2018). Evidence of persistent, recurring summertime hypoxia in Green Bay, Lake Michigan. *Journal of Great Lakes Research*, *44*(5), 841-850.

Kruczynski, W.L. and P.J. Fletcher (eds.). 2012. *Tropical Connections: South Florida's marine environment*. IAN Press, University of Maryland Center for Environmental Science, Cambridge, Maryland. 492 pp.

Lamberth, S. J., Branch, G. M., & Clark, B. M. (2010). Estuarine refugia and fish responses to a large anoxic, hydrogen sulphide,"black tide" event in the adjacent marine environment. *Estuarine, Coastal and Shelf Science*, *86*(2), 203-215.

Langevin, C. D. (2003). Simulation of submarine ground water discharge to a marine estuary: Biscayne Bay, Florida. *Ground Water*, *41*(6), 758-771.

Letelier-Gordo, C. O., Aalto, S. L., Suurnäkki, S., & Pedersen, P. B. (2020). Increased sulfate availability in saline water promotes hydrogen sulfide production in fish organic waste. *Aquacultural Engineering*, *89*, 102062.

Levin, L. A., Ekau, W., Gooday, A. J., Jorissen, F., Middelburg, J. J., Naqvi, S. W. A., ... & Zhang, J. (2009). Effects of natural and human-induced hypoxia on coastal benthos. *Biogeosciences*, *6*(10), 2063-2098.

Lirman, D., & Cropper, W. P. (2003). The influence of salinity on seagrass growth, survivorship, and distribution within Biscayne Bay, Florida: field, experimental, and modeling studies. *Estuaries*, *26*(1), 131-141.

Lirman, D., J. Monty, C. Avila, E. Buck, M. O. Hall, S. Bellmund, P. R. Carlson, and L. Collado- Vides. (2016). Summary report for Biscayne Bay, pp. 234-245, *in* L. Yarbro and P. R. Carlson, eds. Seagrass Integrated Mapping and Monitoring Report No. 2. Fish and Wildlife Research Institute Technical Report TR-17 version 2, St. Petersburg, Florida, 281 p.

Lowery, T. A. (1998). Modelling estuarine eutrophication in the context of hypoxia, nitrogen loadings, stratification and nutrient ratios. *Journal of Environmental Management*, *52*(3), 289-305.

Luther, G. W., Ma, S., Trouwborst, R., Glazer, B., Blickley, M., Scarborough, R. W., & Mensinger, M. G. (2004). The roles of anoxia, H2S, and storm events in fish kills of dead-end canals of Delaware inland bays. *Estuaries*, *27*(3), 551-560.

Maeda, H., Kawai, A. (1988). Hydrogen sulfide production in bottom sediments in the northern and southern Lake Biwa. *日本水産学会誌*, *54*(9), 1623-1634.

McDonald, M. D., Gilmour, K. M., Walsh, P. J., & Perry, S. F. (2010). Cardiovascular and respiratory reflexes of the gulf toadfish (Opsanus beta) during acute hypoxia. *Respiratory Physiology & Neurobiology*, *170*(1), 59-66.

Millette, N. C., Kelble, C., Linhoss, A., Ashby, S., & Visser, L. (2019). Using spatial variability in the rate of change of chlorophyll a to improve water quality management in a subtropical oligotrophic estuary. *Estuaries and Coasts*, *42*(7), 1792-1803.

Miranda, L. E., & Hodges, K. B. (2000). Role of aquatic vegetation coverage on hypoxia and sunfish abundance in bays of a eutrophic reservoir. *Hydrobiologia*, *427*(1), 51-57.

Morse, J. W., Millero, F. J., Cornwell, J. C., & Rickard, D. (1987). The chemistry of the hydrogen sulfide and iron sulfide systems in natural waters. *Earth-Science Reviews*, *24*(1), 1-42.

Muyzer, G., & Stams, A. J. (2008). The ecology and biotechnology of sulphate-reducing bacteria. *Nature Reviews Microbiology*, *6*(6), 441-454.

Nilsson, G. E., & Östlund-Nilsson, S. (2008). Does size matter for hypoxia tolerance in fish?. *Biological Reviews*, *83*(2), 173-189.

Nilsson, G. E., & Renshaw, G. M. (2004). Hypoxic survival strategies in two fishes: extreme anoxia tolerance in the North European crucian carp and natural hypoxic preconditioning in a coral-reef shark. *Journal of Experimental Biology*, *207*(18), 3131-3139.

Nixon, S. W. (1981). Remineralization and nutrient cycling in coastal marine ecosystems. In *Estuaries and Nutrients* (pp. 111-138). Humana Press.

Olson, K. R. (2015). Hydrogen sulfide as an oxygen sensor. *Antioxidants & Redox Signaling*, *22*(5), 377-397.

Orio, A., Bergström, U., Florin, A. B., Lehmann, A., Šics, I., & Casini, M. (2019). Spatial contraction of demersal fish populations in a large marine ecosystem. *Journal of Biogeography*, *46*(3), 633-645.

Paerl, H. W., Pinckney, J. L., Fear, J. M., & Peierls, B. L. (1998). Ecosystem responses to internal and watershed organic matter loading: consequences for hypoxia in the eutrophying Neuse River Estuary, North Carolina, USA. *Marine Ecology Progress Series*, *166*, 17-25.

Peters, J. R., Yeager, L. A., & Layman, C. A. (2015). Comparison of fish assemblages in restored and natural mangrove habitats along an urban shoreline. *Bulletin of Marine Science*, *91*(2), 125-139.

Rabalais, N. N., & Turner, R. E. (2019). Gulf of Mexico hypoxia: Past, present, and future. *Limnology and Oceanography Bulletin*, *28*(4), 117-124.

Rabotyagov, S. S., Kling, C. L., Gassman, P. W., Rabalais, N. N., & Turner, R. (2014). The Economics Of Dead Zones: Causes, Impacts, Policy Challenges, And A Model Of The Gulf Of Mexico Hypoxic Zone. *Review of Environmental Economics and Policy*, *8*(1), 58-79.

Ram, A., Jaiswar, J. R. M., Rokade, M. A., Bharti, S., Vishwasrao, C., & Majithiya, D. (2014). Nutrients, hypoxia and mass fishkill events in Tapi Estuary, India. *Estuarine, Coastal and Shelf Science*, *148*, 48-58.

Rees, B. B., Sudradjat, F. A., & Love, J. W. (2001). Acclimation to hypoxia increases survival time of zebrafish, Danio rerio, during lethal hypoxia. *Journal of Experimental Zoology*, *289*(4), 266-272.

Rodríguez-Martínez, R. E., Medina-Valmaseda, A. E., Blanchon, P., Monroy-Velázquez, L. V., Almazán-Becerril, A., Delgado-Pech, B., ... & García-Rivas, M. C. (2019). Faunal mortality associated with massive beaching and decomposition of pelagic Sargassum. *Marine Pollution Bulletin*, *146*, 201-205.

Rossi, G. S., Tunnah, L., Martin, K. E., Turko, A. J., Taylor, D. S., Currie, S., & Wright, P. A. (2019). Mangrove fishes rely on emersion behavior and physiological tolerance to persist in sulfidic environments. *Physiological and Biochemical Zoology*, *92*(3), 316-325.

Rousi, H., Korpinen, S., & Bonsdorff, E. (2019). Brackish-water benthic fauna under fluctuating environmental conditions: the role of eutrophication, hypoxia, and global change. *Frontiers in Marine Science*, *6*, 464.

Sand-Jensen, K. A. J. (1989). Environmental variables and their effect on photosynthesis of aquatic plant communities. *Aquatic Botany*, *34*(1-3), 5-25.

Santos, R. O., Varona, G., Avila, C. L., Lirman, D., & Collado-Vides, L. (2020). Implications of macroalgae blooms to the spatial structure of seagrass seascapes: The case of the Anadyomene spp.(Chlorophyta) bloom in Biscayne Bay, Florida. *Marine Pollution Bulletin*, *150*, 110742.

Score, A., & Jacoby, C. (2006). Historical and planned changes in the south Florida ecosystem. *EDIS*, *2006*(33). Retrieved from https://journals.flvc.org/edis/article/view/116253

Sen, N., Paul, B. D., Gadalla, M. M., Mustafa, A. K., Sen, T., Xu, R., ... & Snyder, S. H. (2012). Hydrogen sulfide-linked sulfhydration of NF-κB mediates its antiapoptotic actions. *Molecular Cell*, *45*(1), 13-24.

Serafy, J. E., Hopkins, T. E., & Walsh, P. J. (1997). Field studies on the ureogenic gulf toadfish in a subtropical bay. I. Patterns of abundance, size composition and growth. *Journal of Fish Biology*, *50*(6), 1258-1270.

Serafy, J. E., Lindeman, K. C., Hopkins, T. E., & Ault, J. S. (1997). Effects of freshwater canal discharge on fish assemblages in a subtropical bay: field and laboratory observations. *Marine Ecology Progress Series*, *160*, 161-172.

Serafy, J. E., Faunce, C. H., & Lorenz, J. J. (2003). Mangrove shoreline fishes of Biscayne Bay, Florida. *Bulletin of Marine Science*, *72*(1), 161-180.

Shen, X., Kolluru, G. K., Yuan, S., & Kevil, C. G. (2015). Measurement of H2S in vivo and in vitro by the monobromobimane method. In *Methods in Enzymology* (Vol. 554, pp. 31-45). Academic Press.

Shimps, E. L., Rice, J. A., & Osborne, J. A. (2005). Hypoxia tolerance in two juvenile estuary-dependent fishes. *Journal of Experimental Marine Biology and Ecology*, *325*(2), 146-162.

Silverstein, R. (2020, August 31). Consensus statement on fish kill and algae bloom in Biscayne Bay [Web log message]. Retrieved from https://www.miamiwaterkeeper.org/consensus_statement_on_fish_kill_and_algae_bloom_in_biscayne_bay

Skandalis, D. A., Dobell, C. D., Shaw, J. C., & Tattersall, G. J. (2020). Hydrogen sulfide exposure reduces thermal set point in zebrafish. *Royal Society Open Science*, *7*(11), 200416.

Stalker, J. C., Price, R. M., & Swart, P. K. (2009). Determining spatial and temporal inputs of freshwater, including submarine groundwater discharge, to a subtropical estuary using geochemical tracers, Biscayne Bay, South Florida. *Estuaries and Coasts*, *32*(4), 694-708.

Stauffer, B. A., Gellene, A. G., Schnetzer, A., Seubert, E. L., Oberg, C., Sukhatme, G. S., & Caron, D. A. (2012). An oceanographic, meteorological, and biological 'perfect storm'yields a massive fish kill. *Marine Ecology Progress Series*, *468*, 231-243.

Terrados, J., & Borum, J. (2004). European seagrasses: an introduction to monitoring and management. *EU Project M and MS*, 8-11.

Thronson, A., & Quigg, A. (2008). Fifty-five years of fish kills in coastal Texas. *Estuaries and coasts*, *31*(4), 802-813.

Torrans, E. L., & Clemens, H. P. (1982). Physiological and biochemical effects of acute exposure of fish to hydrogen sulfide. *Comparative Biochemistry and Physiology. C: Comparative Pharmacology*, *71*(2), 183-190.

Truong, D. H., Eghbal, M. A., Hindmarsh, W., Roth, S. H., & O'Brien, P. J. (2006). Molecular mechanisms of hydrogen sulfide toxicity. *Drug Metabolism reviews*, *38*(4), 733-744.

Ultsch, G. R., Jackson, D. C., & Moalli, R. (1981). Metabolic oxygen conformity among lower vertebrates: the toadfish revisited. *Journal of Comparative Physiology*, *142*(4), 439-443.

Urbina, M. A., & Glover, C. N. (2013). Relationship between fish size and metabolic rate in the oxyconforming inanga Galaxias maculatus reveals size-dependent strategies to withstand hypoxia. *Physiological and Biochemical Zoology*, *86*(6), 740-749.

Valiela, I., McClelland, J., Hauxwell, J., Behr, P. J., Hersh, D., & Foreman, K. (1997). Macroalgal blooms in shallow estuaries: controls and ecophysiological and ecosystem consequences. *Limnology and Oceanography*, *42*(5part2), 1105-1118.

Vaquer-Sunyer, R., & Duarte, C. M. (2008). Thresholds of hypoxia for marine biodiversity. *Proceedings of the National Academy of Sciences*, *105*(40), 15452-15457.

Vaquer-Sunyer, R., & Duarte, C. M. (2010). Sulfide exposure accelerates hypoxia-driven mortalit. *Limnology and Oceanography*, *55*(3), 1075-1082.

Wang, J. D., Luo, J., & Ault, J. S. (2003). Flows, salinity, and some implications for larval transport in south Biscayne Bay, Florida. *Bulletin of Marine Science*, *72*(3), 695-723.

Whitall, D., Bricker, S., Ferreira, J., Nobre, A. M., Simas, T., & Silva, M. (2007). Assessment of eutrophication in estuaries: pressure–state–response and nitrogen source apportionment. *Environmental Management*, *40*(4), 678-690.

Wingard, G. L., Cronin, T. M., Dwyer, G. S., Ishman, S. E., Willard, D. A., Holmes, C. W., ... & Budet, C. (2003). *Ecosystem history of southern and central Biscayne Bay: Summary report on sediment core analyses* (No. 2003-375). US Geological Survey.

Yang, G., Wu, L., Jiang, B., Yang, W., Qi, J., Cao, K., ... & Wang, R. (2008). H_2S as a physiologic vasorelaxant: hypertension in mice with deletion of cystathionine γ-lyase. *Science*, *322*(5901), 587-590.

Yarbro, L. A., & Carlson, P. R. (2008). Community oxygen and nutrient fluxes in seagrass beds of Florida Bay, USA. *Estuaries and Coasts*, *31*(5), 877-897.