

Hypoxia-induced behaviors of the ctenophore *Mnemiopsis leidyi*

by

Lauren Ann Gentry

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Approved by

Anthony G. Moss, Chair, Associate Professor and Marine Program Coordinator, Biological
Sciences

Raymond P. Henry, Professor and Associate Dean for Research in the College of Sciences and
Mathematics, Biological Sciences

Kenneth M. Halanych, Professor and Schneller Endowed Chair, Biological Sciences

Debbie R. Folkerts, Assistant Professor, Biological Sciences

Abstract

The hypoxia-associated behaviors of the ctenophore *Mnemiopsis leidyi* are not well understood, despite the ecological significance of this species and the widespread nature of hypoxic (low oxygen) events. The interface of hypoxic and normoxic water (the oxycline) is also associated with suspended sulfur-reducing bacteria, which provide a source of food to plankton and other animals. Wild-caught specimens of *M. leidyi* were exposed to an artificially-created oxycline ($PO_2 > 140$ Torr above, < 60 Torr below) in the laboratory and the time the animals spent physically touching this oxycline was quantified. We found that these animals spent a disproportionate amount of time in the oxycline location, suggesting that they are able to sense the oxycline based only upon the oxygen gradient. *M. leidyi* specimens were also exposed to an artificial, discrete oxygen source in order to mimic the availability of oxygen from aquatic photosynthetic plants and algae. Animals associated with this oxygen source apparatus by wrapping their oral lobes around it or lingering within 2 cm. Specimens in hypoxic (< 60 Torr PO_2) conditions were 18.7 times as likely to associate with this apparatus as animals in aerated water (> 140 Torr PO_2) ($p=0.0088$), suggesting that *M. leidyi* may be able to utilize aquatic photosynthetic plants and algae as an oxygen source during hypoxic events. Given the increases in marine hypoxia worldwide, these behaviors by a well known invasive species may represent a previously unexplored ecological impact.

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Table of Contents

Abstract.....	ii
Acknowledgments.....	iii
List of Tables	vii
List of Figures.....	viii
Chapter 1. Introduction to Thesis	1
1.1 Hypoxia	1
1.2 Physiological responses to hypoxia in marine animals	2
1.3 Behavioral responses to hypoxia in marine animals	6
1.4 Introduction to ctenophores	7
1.5 Ctenophore physiological responses to hypoxia	9
1.6 Ctenophore behavioral responses to hypoxia	10
1.7 Medusae behavioral responses to oxyclines	11
1.8 Discussion	11
1.9 References	13
Chapter 2. <i>Mnemiopsis leidyi</i> behavioral responses to an artificially-created oxycline.....	24
2.1 Introduction	24
2.2 Materials and Methods	29
2.2.1 Specimen collection and husbandry	29
2.2.2 Oxycline behavior experiments	29

2.2.3 Determination of oxygen partial pressure threshold for hypoxia-associated behaviors	31
2.2.4 Aquarium oxycline particulate layer analysis	32
2.2.5 Respiratory experiments	32
2.2.6 Analyses	33
2.3 Results	36
2.3.1 χ^2 -test	36
2.3.2 Compositional Analysis	36
2.3.3 Selectivity Ratio	36
2.3.4 Determination of oxygen partial pressure threshold	37
2.3.5 Swimming times	37
2.3.6 Results of SEM/EDS	38
2.3.7 Respiratory results	39
2.4 Discussion	39
2.5 References	43
Chapter 3. Locomotory behavioral response of <i>Mnemiopsis leidyi</i> to a focal, discrete, artificial oxygen source	65
3.1 Introduction	65
3.2 Materials and Methods	68
3.2.1 Specimen collection and husbandry	68
3.2.2 Localized oxygen source experiments.....	69
3.2.3 Analyses	71
3.3 Results	72
3.4 Discussion	73

3.5 References	76
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List of Tables

Chapter 2

Table 1: Size-dependent oxycline responses of <i>M. leidy</i>	60
Table 2: Results of χ^2 analysis of location utilization.....	61
Table 3: Compositional analysis preference ranking matrix	62
Table 4: Simplified preference ranking matrix.....	62
Table 5: Results of experiments determining behavioral oxygen threshold.....	63
Table 6: EDS analysis of particles from aquarium oxycline	64

Chapter 3

Table 1: Outline of experiment and controls	88
Table 6: Results of binomial logistic regression.....	89

List of Figures

Chapter 2

Figure 1: Diagram of naturally forming aquarium oxycline.....	51
Figure 2: White particulate layer in laboratory aquarium.....	52
Figure 3: Diagram of experimental tank	53
Figure 4: Diagram of respiration cell	54
Figure 5: Graph of available and utilized tank locations	55
Figure 6: Graph of Manly selectivity ratios	56
Figure 7: Graph of percent time swimming	57
Figure 8: Scanning electron micrograph of particles from aquarium oxycline	58
Figure 9: Graph of mass-specific oxygen consumption rates	59

Chapter 3

Figure 1: <i>Mnemiopsis</i> association with algae in aquarium.....	84
Figure 2: Diagram of Leafyi localized oxygen source apparatus	85
Figure 3: Diagram of positions used in experimental trials	86
Figure 4: <i>Mnemiopsis</i> association with dialysis tubing of Leafyi	87

Chapter 1. Introduction to thesis

1.1 Hypoxia

Marine organisms live in a three-dimensional world dictated by abiotic parameters, some of which can also be affected by biotic activity. Temperature, salinity, and density all contribute to oxygen (O_2) availability and define aquatic niche boundaries (Edwards, 1987). Conversely, photosynthetic plankton produce oxygen vital to the myriad of organisms that decrease sea water O_2 levels via aerobic respiration. While oxygen concentrations can range from 0 mg L^{-1} to approximately 8 mg L^{-1} (0-100% air saturation) depending on salinity and temperature, photosynthetically active organisms can increase the oxygen concentrations in shallow estuaries to $>16 \text{ mg L}^{-1}$ ($>200\%$ air saturation). Both sessile and mobile aquatic organisms have thus evolved a host of behavioral, physiological, and anatomical adaptations to thriving in this variable environment. An abundance of research has been devoted to studying O_2 availability in aquatic systems, with impacts ranging from fisheries to public health (Breitburg, 2002; Fleming et al., 2002). Despite this significant effort, it is not well understood how many species of gelatinous invertebrates respond when confronted with low oxygen conditions.

The occurrence of both episodic and persistent hypoxia (low oxygen) is an important physical feature of many marine habitats, with natural permanent hypoxia representing at least one million km^2 of sea floor (Helly and Levin, 2004). Often the result of oceanic currents and upwelling patterns, some persistent oxygen minimum zones (OMZs) can remain stable for thousands of years (Reichart et al., 1998). Such massive OMZs can even be traced in the geological record to at least 34 million years ago (McCann, 2008). On a smaller scale, warm weather, microbial degradation of organic matter, and shallow water may create ephemeral hypoxia by stratifying the water column by density. In the absence of strong wind or waves,

bottom waters are rarely re-aerated and remain hypoxic for days to weeks (Rabalais et al., 1994). This O₂ depletion is often intensified by nutrient input from anthropogenic sources, the effects of which are already increasing worldwide (Justić et al., 2003; Legović, 1987). Shallow embayments may also become entirely hypoxic, resulting in substantial migrations of fish and crustaceans to the surface. One such event, known as ‘Jubilee’, is an annual occurrence in Mobile Bay, Alabama (USA) and may have provided an historically celebrated windfall of seafood for Native Americans and European settlers (Loesch, 1960; Outlaw and Taylor, 2012).

Both seasonal hypoxia and OMZs are well demonstrated and widely dispersed along the Atlantic coasts of North and South America (Breitburg, 1990; Diaz and Rosenberg, 2008), as well as in the Gulf of Mexico and the Black Sea (Gupta et al., 1996; Zaitsev, 1992). These areas are home to a multitude of natural and invasive gelatinous zooplankton, including the ctenophore *Mnemiopsis leidyi* (Purcell et al., 2001a). Substantial effort has been put into determining the vertical distributions and diel migrations of ctenophores and cnidarian medusae (referred to henceforth simply as jellies) in areas of low dissolved O₂ (Purcell et al., 2001a). Previous efforts have also explored the physiological effects of hypoxia on gelatinous zooplankton (Thuesen et al., 2005a), although little work has been done on these animals’ specific behavioral responses to hypoxia.

1.2 Physiological responses to hypoxia in marine animals

Previous efforts have attempted to discern general patterns of physiological adaptation to the widespread presence of hypoxia. Childress and Seibel (1998) suggest that in order to survive, animals in oxygen minimum layers may exhibit: (1) adaptation of mechanisms for increased acquisition of oxygen from water, (2) reduction in metabolic rates, and/or (3) use of anaerobic

metabolism. These methods may include hyperventilation in the form of increased frequency and volume of water intake for oxygen extraction, or cardiac responses in the form of bradycardia and hypertension. While the underlying mechanisms of cardiac responses are well understood in fishes, the physiological significance of these hypoxia-induced cardiovascular reflexes is not firmly established. It is thought that hypertension, an elevation of aortic pressure, may allow for the recruitment of previously unperfused gill lamellae for gas diffusion, while reflex bradycardia, a slowed heart rate, may allow for longer oxygen extraction from venous blood by the myocardium (McKenzie et al., 2009; Perry and Desforges, 2006). Many species of aquatic animal exhibit more than one hypoxic response simultaneously or serially. As such, it is generally accepted that animals employ the most energetically favorable strategy given the conditions of the hypoxia (severity, duration of exposure, etc.) (review by Perry et al., 2009).

While some fishes, such as the bluegill and spangled perch, may respond to mild hypoxia with significant bradycardia (Gehrke and Fielder, 1988; Marvin Jr. and Burton, 1973), others, such as the sea raven, may require extensive exposure to severe hypoxia to elicit the same response (Saunders and Sutterlin, 1971). This disparity of cardiac responses in fishes may reflect species-variable thresholds for chemoreceptor stimulation. These differences may also be the result of a disparity between the gill surface areas of these taxa.

These physiological responses in aquatic vertebrates must be initiated and mediated by oxygen chemoreceptors which inhibit the “leaky” potassium channels important to setting the resting membrane potential in neuroepithelial cells (NECs). When confronted with low O₂ conditions, K⁺ channel inhibition in oxygen-sensing NECs originally from zebrafish (*Danio rerio*) reduces membrane permeability to K⁺, resulting in membrane depolarization (Zachar and Jonz, 2012). This hypoxia stimulus is also believed to activate voltage-dependent Ca⁺ channels

resulting in a brief rise of intracellular Ca^+ which initiates neurosecretion (Jonz et al., 2015). Unexpectedly, these cells are located in the fish's gill filaments, rather than closer to the central nervous system (Perry et al., 2009). Lungbreathing fish, on the other hand, rely entirely upon oxygen-sensing NECs found internally, suggesting that external oxygen chemoreceptors may have evolved exclusively in the actinopterygians (ray-finned fishes) (reviewed by Perry et al., 2009).

Invertebrates, on the other hand, may rely on more than cardiac and ventilation adaptations to survive hypoxia. Invertebrates able to oxyregulate (maintain a constant rate of oxygen consumption during progressive hypoxia) may increase ventilation and circulation to compensate, while many other species are adapted to utilize metabolic control in the face of low ambient oxygen (Herreid II, 1980). In contrast to vertebrates' singular respiratory protein, hemoglobin, invertebrates have four respiratory pigments involved in O_2 transport. The respiratory pigment hemocyanin is temperature-sensitive and blood pH-sensitive in the deep water jumbo squid (*Dosidicus gigas*) and coastal shore crab (*Carcinus maenas*), one of many metabolic adaptations (Lallier and Truchot, 1989; Seibel, 2013). For those invertebrates functionally or temporarily unable to oxyregulate, oxyconformation (varying oxygen consumption directly with environmental partial pressure of oxygen) often requires anaerobic metabolism. While hypoxic anaerobic energetic output is generally less than that of aerobic metabolism under normoxia, its use by invertebrates is considered a widespread and effective method for surviving low ambient oxygen tensions (Mangum and van Winkle, 1973). For the purpose of clarification, oxyconformity is generally divided into a three groups representing a spectrum of metabolic strategies. Type I conformers utilize a homeometabolic response in which the animal is able to maintain its energetic expenditure despite the increased reliance on

anaerobic metabolism. Type II poikilometabolic conformers are unable to compensate for falling oxygen levels with anaerobic respiration and thus reduce energetic expenditure in proportion to a loss of oxygen. These extremes are moderated by the Type III heterometabolic conformers. Here, the animal's energetic output may fall in hypoxia, but anaerobiosis is able to partially compensate above that of a Type II organism (Herreid II, 1980). These strategies for physiologically mediated hypoxia tolerances are greatly modified by each species, and are linked with the acquisition of external insulating structures (e.g. cuticles, exoskeletons, and dermal plates). This phylogenetic trend indicates a gradient of increasing aerobic regulation (Type I) as ventilation of respiratory tissues becomes active rather than passive (Mangum and van Winkle, 1973).

For species with drastic changes during ontological development, hypoxia tolerances may change throughout an individual's life. While the adult medusae of the cnidarian *Chrysaora quinquecirrha* cannot survive more than two days at concentration of oxygen of 0.5 mg L^{-1} (approx. 6% air saturation of sea water), their benthic polyps can survive and even asexually reproduce at this level of severe hypoxia for more than five days (Condon et al., 2001). The adults are thought to survive short term hypoxic events without switching to anaerobic respiration through use of the intragel oxygen contained in their mesogleal body layer (Thuesen et al., 2005a). Mesoglea, a largely acellular structural matrix in jellies, maintains oxygen concentrations above that of the environment during exposure to hypoxia. Thuesen suggests that this gel may provide an oxygen reservoir for jellies, many of which can oxyregulate for over 2 hour in hypoxic conditions (2005b). Although the use of intragel oxygen in polyps has yet to be studied, the mesogleal presence of the enzyme phosphoenol pyruvate carboxykinase (an enzyme found in many anaerobic pathways) suggests that polyps may be able to switch between aerobic

and anaerobic metabolism to survive periods of low O₂ (Condon et al., 2001; Lin and Zubkoff, 1977). This effect has also been observed in the blue mud shrimp, *Upogebia pugettensis*, which switches from oxyregulation during intermolt stages to oxyconformation postmolt (Thompson and Pritchard, 1969).

1.3 Behavioral responses to hypoxia in marine animals

The majority of recorded hypoxia-evoked behavioral reactions involve some sort of avoidance mechanism. Within an environment, many species of fish avoid areas of anoxia (<0.5 mg O₂ L⁻¹) by simply swimming away. When confronted with inescapable aquatic hypoxia, the sculpin family of fishes (Family Cottidae) will partially or entirely emerge from the water onto land (Yoshiyama et al., 1995). Able to respire with air through skin, gills, buccal cavities, and air-breathing organs, this partial breathing may even be as efficient in air and gill respiration in water (Taylor et al., 2010; Yoshiyama and Cech, 1994). Many fish may also utilize aerial respiration without fully emerging from the water (Sloman et al., 2008). This behavior is usually reported as gasping at the surface of the water.

Mobile escape from hypoxia is not as well demonstrated in invertebrates, but does include the black sea cucumber (*Holothuria forskali*) creeping from sites of low oxygen to other aquatic sites of high oxygen (Newell and Courtney, 1965) and the emergence of crabs and amphibious snails from hypoxic water in order to breath air (Jones, 1961; Taylor et al., 1977). Other behavioral responses to hypoxia may include increasing surface area for respiration, such as in sea anemones (Sassaman and Mangum, 1972), and a reduction in activity, such as in the mud shrimp (*Callinassa californiensis*) (Torres et al., 1977). Whether such lethargy is a mechanism of hypoxia survival or a result of the physiological stress of hypoxia is still

undetermined. Perhaps the most highly derived behavioral response to hypoxia is that of the bulb-tentacle sea anemone (*Entacmaea quadricolor*), which is oxygenated throughout the night by the fanning motion of its mutualistically symbiotic two-banded anemonefish (*Amphiprion bicinctus*) (Szczebak et al., 2013).

Low dissolved oxygen may also alter other trophic interactions in pelagic food webs through behavioral modification. Breitburg (1992) found that low dissolved oxygen reduces the escape ability of fish larvae, thus making them easier prey for ctenophores and other predators. This lab has also recorded that low oxygen conditions alter attack rates and foraging time of predatory cnidarians and predatory fishes, as well as depress the prey susceptibility behaviors of zooplankton (Breitburg, 1994; Breitburg et al., 1999, 2001; Decker et al., 2004). It has also been shown that copepods will stay in hypoxic zones for extended periods, presumably to seek shelter from predators (Vanderploeg et al., 2009). Thus, hypoxia may act to concentrate susceptible prey in stratified environments, disable prey in homogeneously hypoxic areas, and provide shelter for tolerant prey species.

1.4 Introduction to ctenophores

Ctenophores are globally ubiquitous gelatinous zooplankton, characterized by eight locomotory rows of ciliated comb plates. *Mnemiopsis leidyi*, a lobate ctenophore, is a cruising predator of other zooplankton. Their populations often bloom seasonally in coastal waters, with both egg and sperm being released from each simultaneously hermaphroditic individual (Carre et al., 1990). The ecological significance of this species has been widely recognized since the introduction of North American *Mnemiopsis leidyi* in the Black Sea in the 1980s, where their presence collapsed the food web and devastated fisheries (Ivanov et al., 2000a; Shiganova,

1998). This occurred as a result of the invasive ctenophores feeding on fish eggs and larvae as well as the fish themselves, which severely exacerbated decades of overharvest of the main fisheries (Ivanov et al., 2000b). Without any natural predators in the Black Sea, *M. leidyi* not only reduced fish populations, but also inhibited recovery of damaged fish stocks for decades.

Adult *M. leidyi* ranging from 1-50mm hunt small crustaceans, the eggs and larvae of fish, protists, and other zooplankton (Rapoza et al., 2005). Their method of prey capture involves the synergistic function of two processes (Waggett and Costello, 1999). First, weakly swimming prey are entrained by the fluid flow pattern created by auricular ciliary beating. This low-velocity mechanism transports prey between the oral lobes and into contact with tentillae (oral tentacles). These tentillae entangle the prey, contract, and deposit the food in troughs leading to the mouth, often breaking off and being consumed themselves (Main, 1928). However, this ciliary flow has little impact on adult copepods and stronger-swimming prey; thus *M. leidyi* also hunts by capturing prey between its lobes. Swimming with lobes outstretched, the ctenophore will close its lobes around prey, who will subsequently collide with the sticky inner surface of the oral lobes (Colin et al., 2010). Once entrapped by colloblasts (Franc, 1978) and mucus, prey are then transported down

ciliated grooves into the mouth (Costello, 1999).

Mnemiopsis leidyi, while precise and exacting in movement with synchronized rows of comb-like ctenes, is functionally planktonic (Haddock, 2007). Unable to escape currents and tides, they are at the mercy of their environment. As such *M. leidyi* is a euryhaline osmoconformer found at depth up to 200m, salinities from <2-38‰ (Purcell et al., 2001a), and is eurythermal, tolerating temperatures of 2-32° C (Costello et al., 2006). These animals may also

be able to survive the generally hostile circumstances of $<12^{\circ}\text{C}$ and a third of food availability by delaying maturation to an adult stage by up to a year (van der Molen et al., 2015).

1.5 Ctenophore physiological responses to hypoxia

The ability of the ctenophore *Mnemiopsis leidyi* to survive low oxygen situations seems to lie in their regulation of aerobic respiration, rather than upon anaerobic adaptations. As the majority of their oxygen consumption occurs in association with the external locomotory ctenes, they have a very low metabolic oxygen demand and can oxyregulate down to very low concentrations of oxygen ($>0.5\text{mg L}^{-1}$) (Gyllenberg and Greve, 1979; Thuesen et al., 2005b). Thuesen et al. proposed that at this critical pressure there is no longer enough environmental oxygen to maintain the partial pressure of oxygen sufficient for oxygen diffusion into mitochondria associated with comb plate cilia (2005b). While the relatively small oxygen diffusion requirements of these epithelial features allow these animals to survive extreme hypoxia ($>0.5\text{ mg O}_2\text{ L}^{-1}$) better than medusae, anoxia ($<0.5\text{ mg O}_2\text{ L}^{-1}$) is still deadly moments after exposure (Thuesen et al., 2005b).

Another form of hypoxia relief may also lie in the mesogleal tissue layer unique to gelatinous organisms. Like cnidarians, this connective tissue in ctenophores is a clear, partly cellular, jelly-like structure sandwiched between an inner gastrodermal layer and an outer epithelial layer (Hernandez-Nicaise, 1991). Made up of a loose mesh-like network of microfilaments, mesogleal gel is responsible for the hydrostatic skeleton of ctenophores, providing structure and muscular support (Alexander, 1964; Weber and Schmid, 1985). As in medusea, it is thought that this fibrous gel may also be a source of short-term oxygen supply. Diffusion gradients between the seawater, mesoglea, and metabolically active tissues allow the

mesoglea of some medusae, and to a lesser extent ctenophores, to supply the musculature and tissues with oxygen during sudden or extreme hypoxia (Thuesen et al., 2005a, 2005b). Though this and other mechanisms make *M. leidy* resilient to hypoxic fatality, they are not invulnerable to the stress of low oxygen life. In experimental studies, ctenophores exposed to 1.5 and 2.5 mg L⁻¹ dissolved oxygen (approx. 20-30% air saturation) for 4 days had significantly reduced growth as compared to air-saturated control animals. These same animals also displayed significantly reduced egg production (Grove and Breitburg, 2005).

1.6 Ctenophore behavioral responses to hypoxia

In the highly oxygen variable habitat of Chesapeake Bay, studies have shown that the ctenophore *Mnemiopsis leidy* may actually use the hypoxic bottom waters for refuge from predatory non-gelatinous zooplankton (Purcell et al., 2001b). Whether this hypoxic sanctum is protected by their predators' inability to tolerate the hypoxia or due to the predators primarily associating with hypoxia-intolerant prey is currently unknown. This hypoxic refuge effect is well studied in the extremely hypoxia tolerant bearded gobies (*Sufflogobius bibarbatus*) off the coast of Namibia. Here, the gobies are protected in their low oxygen benthos from their hypoxia-intolerant predator, the Cape hake (*Merluccius capensis*).

Another benefit of low dissolved O₂ events may be the stratifying and condensing nature of bottom-layer hypoxia common to estuaries. Intolerant prey items such as crustaceans, fish larvae, and other planktonic organisms are restricted to the oxygenated upper layers, leaving them concentrated and more vulnerable to predation by ctenophores (Purcell et al., 2014). Generally more tolerant to hypoxia than their prey, *M. leidy* are actually able to feed on susceptible and thus impaired zooplankton at a higher rate in mild hypoxia than in normoxia

(Decker et al., 2004). *M. leidy*'s tolerance to low oxygen exposure and their increased feeding and escape rates in hypoxia may contribute, in part, to their disruptive effects on the pelagic food chains in frequently hypoxic environments.

1.7 Cnidarian medusae behavioral responses to oxyclines

Although generally avoiding hypoxia, studies of persistent hypoxic zones have shown an accumulation of jellies at the oxycline (normoxic-hypoxic interface) above areas of low dissolved oxygen. These animals aggregate along the oxycline where sulfur oxidizing bacteria can thrive on hydrogen sulfide produced by decay processes (Decker et al., 2004; Dugdale et al., 1977; Levin, 2003). Bayly and Hamner have both reported jellies making excursions into these interface layers presumably in pursuit of the copepods and other plankton feeding on the oxycline's suspended "bacterial plate" (Bayly, 1986; Hamner et al., 1982a). This oxycline-positive behavior has also been recorded in laboratory conditions (Thuesen et al., 2005a). When exposed to a stratified tank of normoxia and hypoxia, the cnidarian medusae *Aurelia labiata* continuously swam back and forth through the oxycline, significantly increasing their swimming distance over homogenously normoxic and anoxic controls. The behavioral responses of ctenophore jellies to oxyclines is, as of yet, unexplored.

1.8 Discussion

Hypoxia is a ubiquitous feature of marine systems worldwide. Whether the result of large scale oceanic currents or local warm weather, low O₂ conditions can exist in a habitat for a few hours or thousands of years. Marine organisms have thus developed a host of physiological and anatomical adaptations to allow them to survive such hostile conditions. Such adaptations include metabolism regulation, anaerobic respiration, specialized respiratory proteins, and

specialized respiratory structures. While these features are well studied in many fishes and invertebrates, it remains unclear how many marine organisms behave when confronted with hypoxia.

Despite their significant impact on zooplankton populations and their potential for exploitation of hypoxic zones, the behavioral interactions of *M. leidy* with hypoxia and oxyclines is yet to be elucidated. As the areas of greatest ecological impact by *M. leidy* are also areas of pervasive hypoxia, such behaviors could be an essential, yet currently underexplored, component of ecological dynamics. In this study, the ctenophore *Mnemiopsis leidy* has been examined with regard to its response to artificially-produced laboratory hypoxic environments under controlled conditions. This work focuses on the interactions of the ctenophore with oxyclines, both stationary and mobile. While previous research focused on the hypoxia-induced physiological tolerances and *in situ* distributions of *M. leidy*, this work specifically explores the ability of *M. leidy* to sense and behaviorally respond to artificially created oxyclines, and the nature of their association and preference for oxyclines, both artificially created and naturally occurring in the laboratory.

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Chapter 2. *Mnemiopsis leidyi* behavioral responses to an artificially-created oxycline

2.1 Introduction

Oxygen (O_2) is not as readily available in aquatic environments as in air. Slower diffusion, lower concentrations, and faster local depletion all lead to variability in O_2 availability for marine organisms (see review by Newell, 2013). While oxygen concentrations can range from 0 mg L^{-1} to approximately 8 mg L^{-1} (0-100% air saturation) depending on salinity and temperature, photosynthetically active organisms can increase the oxygen concentrations in shallow estuaries to $>16 \text{ mg L}^{-1}$ ($>200\%$ air saturation). Aquatic animals have thus evolved physiological and behavioral responses to what can be a variable and challenging habitat. While O_2 availability is a well studied physical parameter in many marine ecosystems, it is not well understood how many species - especially invertebrates - respond when confronted with low oxygen (hypoxic) environments. Previous efforts explored the physiological effects of hypoxia on gelatinous zooplankton, including ctenophores and cnidarians (referred to henceforth simply as jellies) (Gyllenberg and Greve, 1979; Thuesen et al., 2005a, 2005b). Others determined the vertical distribution of jellies in areas of low dissolved O_2 (Purcell et al., 2001b; Sarah E. Kolesar, 2010). However, recent publications commented on the plethora of research on hypoxia-induced physiology in marine creatures in the midst of a dearth of behavioral information about gelatinous organisms (Gray et al., 2002; Spicer, 2014; Vaquer-Sunyer and Duarte, 2008).

O_2 depletion may be intensified by excess nutrient input from anthropogenic sources, which enhances microbial activity and O_2 consumption. These effects are predicted to increase worldwide, with so-called “dead-zones” proliferating in recent years (Altieri and Gedan, 2015; Diaz and Rosenberg, 2008; Justić et al., 2003; Legović, 1987; Melzner et al., 2012). The

occurrence of both episodic and persistent hypoxia is an important physical feature of many marine habitats, with natural permanent hypoxia estimated to represent one million km² of sea floor (Gray et al., 2002; Helly and Levin, 2004). Warm weather, microbial degradation of organic matter, and shallow water may create ephemeral hypoxia by density-driven stratification of the water column, thus preventing the re-aeration of bottom waters (Rabalais et al., 1994).. Seasonal and persistent hypoxia are well demonstrated and widely dispersed along the Atlantic coasts of North and South America (Breitburg, 1990; Diaz and Rosenberg, 2008), as well as in the Gulf of Mexico and the Black Sea (Gupta et al., 1996; Zaitsev, 1992). Despite this, many cnidarians and ctenophores (henceforth referred to as jellies) are found in high densities within or immediately adjacent to areas of low dissolved O₂. These areas also represent both the natural and invasive habitats of the ctenophore *Mnemiopsis leidyi* (Purcell et al., 2001a, 2001b).

Ctenophores, like *Mnemiopsis leidyi*, are gelatinous zooplankton, characterized by eight locomotory rows of ciliated comb plates known as ctenes. *M. leidyi*, a lobate ctenophore, is a cruising predator of other zooplankton. Their populations often bloom seasonally in coastal waters. *M. leidyi*, although precise and exacting in movement, is functionally planktonic, and is thus able to adapt to many environments. Like cnidarians, ctenophores lack the ability to control the mass-transfer of oxygen and other gases through their body tissues. Diffusion across their body surface, which is only one cell thick, is their only known mechanism of respiration (Purcell et al., 2001b). They are also highly tolerant of hypoxic conditions, possibly as a result of high surface area for gas exchange, and are able to survive and function at levels lethal to many fishes and other gelatinous zooplankton (Purcell et al., 2001b).

The ability of the ctenophore *Mnemiopsis leidyi* to survive low oxygen situations seems to lie in their relatively low levels of aerobic respiration, rather than upon specifically evolved

anaerobic adaptations. As the majority of their metabolism occurs in association with the external locomotory ctenophore rows, they have a relatively low metabolic oxygen demand and can oxyregulate down to very low concentrations of oxygen ($>0.5 \text{ mg L}^{-1}$) (approx. 6% air saturation of sea water) (Gyllenberg and Greve, 1979; Thuesen et al., 2005b). At this critical partial pressure (P_{crit}), it is thought that there is no longer enough environmental O_2 to maintain the partial pressure of oxygen sufficient for diffusion into the mitochondria associated with external comb plate cilia (Thuesen et al., 2005b). While the relatively small oxygen diffusion requirements of these epithelial features allow these animals to survive extreme hypoxia ($>0.5 \text{ mg L}^{-1}$) better than medusae, anoxia ($<0.5 \text{ mg O}_2 \text{ L}^{-1}$) is still deadly moments after exposure (Thuesen et al., 2005b).

In the highly oxygen variable habitat of Chesapeake Bay, studies showed the ctenophore *Mnemiopsis leidyi* may actually use the hypoxic bottom waters for refuge from predatory non-gelatinous zooplankton (Breitburg et al., 2003; Purcell et al., 2001b). Whether this hypoxic sanctuary is provided by the predators' inability to tolerate the hypoxia or due to the predators primarily associating with prey in normoxic water is not known. Another benefit to ctenophores may be the stratifying and concentrating nature of bottom-layer hypoxia common to estuaries. Low O_2 -intolerant prey items — such as crustaceans, fish larvae and other planktonic organisms — are restricted to the oxygenated upper layers, leaving them concentrated and more vulnerable to predation by ctenophores (Purcell et al., 2014). Generally more tolerant of low dissolved oxygen than prey which may become trapped in hypoxia, *M. leidyi* in these areas are able to feed on susceptible and thus impaired zooplankton at a higher rate in mild hypoxia than in normoxia (Decker et al., 2004). *M. leidyi*'s tolerance of low oxygen exposure and their increased feeding and escape rates in hypoxia may thus contribute, in part, to their disruptive effects on the pelagic

food chains in frequently hypoxic environments.

The decay processes which promote O₂ depletion often result in the production of hydrogen sulfide (Levin, 2003; Oguz et al., 2000). The oxycline (normoxic-hypoxia interface zone) thus also becomes a chemocline (oxygen-sulfide interface zone), providing a favorable environment for sulfide-oxidizing bacteria (Matsuyama and Shirouzu, 1978). These bacteria frequently form thick, white mats which dominate hypoxic and anoxic benthic surfaces (Utne-Palm et al., 2010). Should these bacteria and other particles form a layer at the chemocline in the water column, this forms a suspended “bacterial plate”, below which hydrogen sulfide levels increase and oxygen levels may drop to anoxia (Hamner et al., 1982b). These bacterial plates are a feature of enclosed (e.g., brackish lakes, the Black and Baltic seas), semi-enclosed (e.g., estuaries) and open systems (e.g., oceanic OMZs) (Casamayor et al., 2001; Farnelid et al., 2013; Hamner et al., 1982b; Jessen et al., 2016; Sullivan et al., 2014). While hydrogen sulfide is a poison that bonds with iron in the mitochondrial cytochrome enzymes, thus inhibiting cellular respiration (Bagarinao, 1992), bacteria found in this chemocline may provide a food source in otherwise inhospitable conditions. In brackish lakes, bacterial productivity at the chemocline may come close to the primary productivity of the oxygenated conditions above (Matsuyama and Shirouzu, 1978; Sorokin and Donato, 1975). Studies of persistent hypoxic zones found medusae accumulating just above these oxyclines (Decker et al., 2004) and even making frequent excursions into these chemocline/oxycline interfaces, presumably in pursuit of the copepods which feed on the bacterial plate at the chemocline (Bayly, 1986; Hamner et al., 1982a).

Cnidarian oxycline-associated behavior was recorded in laboratory conditions by Thuesen et al. (2005a). When exposed to a stratified tank of normoxia and hypoxia, the cnidarian *Aurelia labiata* continuously swam back and forth through the oxycline, significantly increasing

their swimming distance as compared to homogeneously normoxic and anoxic controls. These results indicated the animals respond to oxyclines despite the absence of the chemical or prey cues found *in situ*. The question then remains: can *Mnemiopsis leidyi* detect oxyclines? If so, they may be able to exploit hypoxic waters as a resource, gaining a competitive advantage over non-tolerant prey and predators.

A behavior was observed in the laboratory, which further prompted this question. After a power outage turned off the pump to a laboratory tank, a white layer of particles formed a few inches from the bottom of the tank (**Fig. 1**). This layer was less than a centimeter thick, made up of brilliant white particles >1 mm in length, and appeared to be more viscous than the surrounding water. This was assumed to be an analog of the bacterial plate found at the chemocline/oxycline of hypoxic estuaries and oceanic zones. Initial sampling confirmed this layer represented an oxycline in the tank, with O₂ readings below the layer being severely hypoxic (< 1 mg L⁻¹). A number of *M. leidyi* were observed leaving the surface of the water, quickly and directly approaching the layer, then slowly entering the layer with opened lobes, taking the white particles into their oral cavity (**Fig. 2**). Soon after, the animals would return to the surface of the water. The tank also contained an established population of copepods, many of which were observed making excursions into the layer, although the ctenophores did not appear to be selectively feeding on these copepods.

As a result of these observations, the following experiments were then designed to answer three questions prompted by the laboratory behaviors: (1) Can *Mnemiopsis leidyi* sense oxyclines? (2) If so, are they associating with these oxyclines? And (3) Is the particulate layer which the animals were feeding on in the aquaria analogous to reports of the bacterial plates found at oxy/chemoclines in the wild?

2.2 Materials and Methods

2.2.1 Specimen collection and husbandry

Adult specimens of *Mnemiopsis leidyi* were collected from the Gulf of Mexico off the coast of Dauphin Island (AL, USA) by plankton net and driven to Auburn University (Alabama, USA) in sealed 5-gallon buckets. Other animals were ordered from Gulf Specimen Marine Laboratories, Inc. (Panacea, FL, USA) and shipped to Auburn overnight in water from their habitat. Animals were transferred to 50 gallon kreisel tanks with sumps and overhead reservoirs (21 °C; 34 ‰; sea water) which were cycled and aerated by pump every 12 hours. Animals used in respiratory experiments (see below) were held at a salinity of 25 ‰ until use. Animals were fed brine shrimp (*Artemia salina*) nauplii every day and allowed to feed *ad libitum*. Prior to experiments, specimens were placed in 50 mL of the experimental water and allowed to acclimatize for at least 1 hr.

2.2.2 Oxycline behavior experiments

In order to test *Mnemiopsis leidyi*'s oxycline-associated behaviors, animals (n=8) were added to the top of a tank which contained normoxic water in the top half, a thin oxycline at the middle, and a hypoxic bottom half (**Fig. 3**). The experimental tank was a 1 cm-thick acrylic cylinder (60 cm tall x 10 cm internal diameter) with inflow ports at the top and bottom and two outflow ports in the center. Two independent water sources could be pumped into the top and bottom inflow ports. A slow drip of deionized water into the top of the tank allowed for a salinity decrease of <0.5‰ in the top half, resulting in a thin (<0.5 cm) interface of the top and bottom layers between the center outflow ports. Preliminary tests confirmed this slight halocline was undetectable by the animals (see section 2.2.3). The hypoxic water source for the bottom of the

tank was first dripped through a nitrogen stripping column containing Bio-Balls (Coralife), allowing us to create a sharp horizontal oxycline at the center of the tank. It was determined by other experiments (see section 2.2.3) that *Mnemiopsis leidyi* begins exhibiting hypoxia-associated behaviors at an oxygen concentration between 60-70 Torr (2.9 – 3.3 mg O₂ L⁻¹). Thus, all hypoxic experiments were performed below 60 Torr. Anoxia (<10 Torr; <0.5 mg O₂ L⁻¹) is lethal to *M. leidyi* and thus was never used (Thuesen et al., 2005b). The top layer of normoxic water was air-saturated (>140 Torr) by a bubble stone in a reservoir before use. Controls were performed on animals placed in entirely normoxic (n=6) and entirely hypoxic tanks (n=6). Recent experiments demonstrated the possibility of an intrinsic circadian rhythm in *Mnemiopsis leidyi* (Sasson and Ryan, 2016); therefore, all experiments were conducted during daylight hours.

The force of the pumps sheared and damaged the animals, so the oxycline was established in the absence of animals, confirmed by addition of a small amount of fluorescein powder to the water surface, and the pumps and addition of DI water were then stopped. The oxycline would slowly degrade after many hours, though it was still detectable after 24 hours. After confirmation of an oxycline, animals (n=13) were then gently added at the surface and time-lapse recorded at 5 second intervals with a high definition camcorder (GZ-HM320BU; JVC) for the duration of the experiment. The locations of the animals in the tank were recorded at 5-sec intervals. As the specimens all displayed a very narrow range of locations in the tank, the categories of locations were designated as:

Top: The animal was physically touching the surface of the water

Bottom: The animal was physically touching the acrylic floor of the tank

Interface: The animal was lingering with some part of its body through the oxycline. The animal was not passing through on its way to the surface or floor.

Travel: The animal was not lingering but was actively swimming in a vertical fashion from one location to another anywhere in the tank.

Water samples were removed by 1 mL syringe from the inflow and outflow ports and PO₂ was measured by two Clark-type polarographic O₂ electrodes (E5046; Radiometer) connected to a dual chamber O₂ meter (OM200; Cameron Instruments) to determine PO₂. Two identical electrodes were used to confirm readings and were calibrated to 0 Torr (0 mg O₂ L⁻¹) with sodium sulfite and to 155 Torr (7.84 mg O₂ L⁻¹) with air-saturated sea water.

2.2.3 Determination of oxygen partial pressure threshold for hypoxia-associated behaviors

Recent literature indicates the need for a shift in the definition of “hypoxia” from rigid physical parameters to species-specific measurements (Diaz and Breitburg, 2009; Farrell and Richards, 2009; Gray et al., 2002; Hofmann et al., 2011; Melzner et al., 2012; Vaquer-Sunyer and Duarte, 2008). Essentially, what is hypoxic to one species may not be considered low oxygen by another species. In order to determine the threshold PO₂ at which *M. leidy* began displaying hypoxia-induced behaviors, animals (n=6) were introduced to the experimental tank (**Fig. 3**) with a range of hypoxia in the lower half (150, 100, 80, 70, 60 Torr). The PO₂ at which the animals would no longer cross the oxycline was taken as the behavioral hypoxic partial pressure (P_{hyp}) for *Mnemiopsis leidy*. All hypoxic experiments were performed below this P_{hyp}. This experimental design also tested the behaviors of the animals at the slight halocline in the absence of an oxycline (150 Torr on top, 150 Torr on bottom). Animals were removed from the

tank and placed in aerated sea water ($\text{PO}_2 > 140$ Torr) between each trial while the new hypoxic PO_2 in the tank was established.

2.2.4 Aquarium oxycline particulate layer analysis

We were able to reproduce conditions in the laboratory aquariums leading to the oxycline particulate layer occurrence (see section 2.1) by feeding the resident animals in the kreisel tanks to excess and turning off the pumps that cycled the seawater. Within a day, a thin white particulate layer would form near the floor of the tank. This layer formed only in the presence of multiple *M. leidy* in the tank, and was time-lapse recorded as described above. Samples of the particles were collected and filtered (0.2 μm filters), washed with deionized water, placed on carbon tape coated aluminum stubs, and sputter coated with gold for scanning electron microscopy (SEM) and energy-dispersive X-ray spectroscopy (EDS) to determine elemental composition.

2.2.5 Respiratory experiments

To obtain a mass-specific oxygen consumption rate (VO_2) of the *Mnemiopsis leidy* being used in this laboratory, animals ($n=12$) were starved for 24 hours and weighed (wet weight g) under water. To do this, animals were drained on a ladle and gently added to a known mass of water. Afterwards, animals were placed in sealed acrylic respiration cells (9 cm x 9 cm; RC400; Strathkelvin) (**Fig. 4**). The PO_2 of their chamber water was recorded continuously over several days using two Clark-type polarographic O_2 electrodes (E5046; Radiometer) connected to a dual chamber O_2 meter (OM200; Cameron Instruments). Respiration experiments were carried out in filtered sea water (0.2 μm filter, 25‰) and were continued until specimens laid down on the

bottom of the chamber and ceased to display movement of comb plates (indicating death).

Animals were allowed to swim freely in the chambers during these experiments.

Initial results (see below) indicated animals were harmed by even slight movement of a stir bar, and that the black rubber (butyl) O-rings that sealed the chambers were quite toxic to *M. leidyi*. The chambers in all subsequent experiments were sealed with plastic paraffin film (Parafilm M(R), Bemis NA). Data obtained from tests with stir bars or rubber O-rings are not presented here. Other specimens were observed engulfing the tip of the O₂ electrode in their oral cavity, which prevented accurate readings by the O₂ meter. This behavior left behind enough mucus to impair the ability of the electrode for the rest of the experiment. Data obtained from animals that engulfed the probe within 24 hours are not included here. Both the rubber toxicity and capture of the electrode by the animals have been previously reported by Thuesen et al. (Thuesen et al., 2005b).

2.2.6 Analyses

*X*²-test

Videos of oxycline behavior experiments were analyzed post-experiment and the locations of the animals in the tank were recorded at 5-sec intervals. The percentage of the tank belonging to each of the above categories was calculated using the vertical distance of the tank included in that category. Utilization of the locations was calculated as the proportion of time each location was used by the animals (n=8), and a χ^2 test was performed to detect deviation from a null hypothesis of random use of the tank. Preliminary trials indicated that animals with a volume of less than 15.75 cm³ (as measured by calipers along three planes of symmetry) did not respond to any treatments or display oxycline-responsive behaviors (**Table 1**). Rather, these small animals

never left the surface of the water, an observation previously recorded by Waduwara and Moss (unpublished data 2011). Several possibilities were suggested for this size-dependent result, such as the smaller animals representing an earlier life stage or a higher surface area to volume ratio for gas diffusion than the larger specimens. However, no obvious anatomical differences were observed. Animals below this size threshold were not used in further experiments or included in analyses.

Compositional Analysis

Preference for location in the tank was assessed using a compositional habitat analysis (CHA) approach (Aebischer et al., 1993). This approach is most often used to determine whether radio-tagged or camera-sited terrestrial animals are spending a disproportionate amount of time in a habitat type in relation to how much of that habitat type is available in the animals' home range. The percentage of the tank belonging to each of the above four categories was calculated to determine its relative availability. Utilization of the location was determined by video and calculated as the proportion of time each animal spent in that location during the experimental trial.

This data was transformed to logarithmic ratios to provide independence of linearity (Aitchison, 1986) and comparisons of the proportional use between each location and the other three was calculated using the formula $d_i = \ln(X_{ui}/X_{uj}) - \ln(X_{ai}/X_{aj})$, in which X_{ui} is the proportion of habitat used by habitat category i , X_{ai} is the proportion of habitat category i available, and d_i is the calculated matrix value. Each individual ctenophore was analyzed separately, and the averages were displayed as a matrix of comparisons. In this matrix, a positive cell value ($d_i > 0$ for location i relative to location j) indicates that location i is used more than would be expected

if there were no preference between i and j . The total number of positive cells in each row was used to rank the locations in order of increasing proportionate use. While some locations were not used by certain individuals, log ratio transformations do not allow for values of 0. Thus, all zero values were replaced with 0.01 (Aebischer et al., 1993).

Selectivity Ratio Analysis

Using the same values for availability and utilization of locations in the tank, Manly's selectivity ratio (used/available) for each location category was then calculated. This technique is used to estimate resource selection functions for a habitat (Manly et al., 2007), and calculates ratios for all locations of all individuals, rather than the pair-wise tests of the Compositional Analysis. A value greater than 1 indicates a positive resource selectivity for that category, while a value less than one indicates the animals were spending less time in that location relative to how much of that location is available in the tank.

Comparisons of swimming behavioral data were made using analysis of variance (ANOVA) followed by a Tukey's *post-hoc* analysis. All analyses were performed using statistical software R version 3.1.3, package adehabitatHS.

2.3 Results

2.3.1 χ^2 –test of tank use in oxycline behavioral experiments

Use of locations within the tank differed in relationship to each location's availability (**Table 2**), suggesting these animals were not randomly distributed in the tank throughout the experiments ($df=3, \chi^2 = 1011.17, p<0.0001$).

2.3.2 Compositional Analysis

A total of 8 *Mnemiopsis leidyi* specimens were filmed in the experimental tank in the presence of an artificial oxycline. While the Top, Bottom, and Interface locations each represented less than 4% of the tank, the animals spent approximately 41%, 27%, and 24% of their time in these locations, respectively. On the other hand, Travel represented the other 91% of the tank, and the animals spent less than 7% of their time in this area. These results indicate that *M. leidyi* associated with interfaces (e.g., the air/water interface, the tank/water interface, and the normoxia/hypoxia interface) (**Fig. 5**). This also suggests the animals are able to detect the artificial oxycline and treat it as if it were a physical, rather than chemical, interface. Further matrix analysis ranked the tank locations according to paired comparisons of utilization and availability as Top>Interface>Bottom>Travel, further suggesting *M. leidyi* can not only detect the oxycline (Interface), but is spending time in it over other locations in the tank (**Tables 3 and 4**).

2.3.3 Selectivity Ratio

Mainly selectivity ratios (used/available) for all experiments (n=8) for Interface, Top, Bottom, and Travel locations were 16, 10.8, 8.1, and 0.08 respectively. This indicates that

Mnemiopsis leidyi selected heavily to spend time in the oxycline zone (Interface), the surface of the water (Top), and the floor of the tank (Bottom). The ratios also indicate the animals selected against spending time in the other 91% of the tank (Travel) (**Fig. 6**).

2.3.4 Determination of oxygen partial pressure threshold

By introducing six *M. leidyi* specimens to artificially created oxyclines with progressively lower partial pressures of oxygen on the hypoxic side of the cline, we were able to determine that the animals began responding to the oxycline at a bottom PO₂ between 70 – 60 Torr (**Table 5**). These responses consisted of the animals approaching and lingering inside the 0.5 cm-thick oxycline but not crossing through it. When the PO₂ of the bottom half of the oxycline was ≥ 70 Torr, the specimens passed through the oxycline without hesitation. This also indicated that the animals were not responding to the halocline used to stabilize the oxycline interface in each trial.

2.3.5 Swimming times

As the animals often made quick excursions down to the oxycline before returning to the surface, their time spent in the actual oxycline area could be quite small. Thus, we also analyzed the amount of time each animal spent moving around in the tank and compared this to controls of entirely normoxic and hypoxic tanks (**Fig. 7**). The ANOVA results indicate the animals made significantly more excursions into the water column when the oxycline was present ($p < 0.01$). This further suggests the ability of *Mnemiopsis leidyi* to detect and respond positively to oxyclines.

These controls were also used as qualitative analysis to confirm the animals were not associating with physical features of the tank located near the artificial oxycline. During the

entirely normoxic and hypoxic trials, the animals did not linger in the middle of tank where the oxycline would be during experiments.

2.3.6 Results of SEM/EDS analysis of aquarium oxycline particulate matter

Time lapse video revealed the animals in the holding aquarium in the laboratory fed upon the naturally forming white oxycline layer every few minutes throughout the night. The animals approached the layer, stopped, opened their oral lobes, and submerged their bodies half-way into the layer in order to feed. However, the animals all died between 6 and 22 hours after first beginning feeding. It appeared that after many hours of feeding on the particles, they were having difficulty breaking out of the layer to return to the surface of the tank. Many returns to the layer still occurred, with each successive excursion taking longer for the animal to escape the suspended layer of particles. One by one, each of the animals eventually succumbed and was unable to escape. It appears the animals would survive only a few minutes on the severely hypoxic floor of the holding tank before all comb plate beating stopped, indicating death.

The shape and chemical compositions of the white particles harvested from the *M. leidy* holding tank were examined via scanning electron microscopy (SEM) and energy-dispersive X-ray spectroscopy (EDS). The dried white particles showed a uniform composition of spheres (**Fig. 8**), and unknown bacteria were seen dispersed throughout. The EDS qualitative chemical analysis of these spheres showed they were primarily sulfur (**Table 6**), indicating the particulate layer was roughly analogous to the sulfur-heavy bacterial plates reported at the oxy/chemoclines of naturally occurring hypoxic zones.

2.3.7 Respiratory results

All specimens of *Mnemiopsis leidyi* (n=6) for which unaffected data were obtained (see Methods) maintained their VO_2 under declining oxygen concentrations for at least 24 hours (**Fig. 9**). After this point, data for all animals were obscured due to the their ‘capture’ of the oxygen electrodes. The size range of specimens was 2.42–6.14 g, but there was no significant effect of size on respiration rates of these animals. As the experiments were not able to continue into hypoxia due to the ‘capture’ of the probe by the animals, no oxyregulation was recorded. The VO_2 range obtained from these animals were within the range of results of *Mnemiopsis leidyi* respirometry experiments done by Thuesen et al. at similar partial pressures of oxygen (2005b).

2.4 Discussion

Although a number of investigators have previously explored the physiological tolerances and effects of hypoxia on the ctenophore *Mnemiopsis leidyi*, cnidarians, and other marine invertebrates, there is a scarcity of hypoxia-induced behavioral information for these animals. Natural hypoxia, a nearly ubiquitous feature of marine environments, is often the result of oxygen consumption during microbial decomposition of detritus. Under these low oxygen conditions, sulfur-reducing bacteria utilize elemental sulfur to oxidize hydrogen or organic molecules, leading to the production of hydrogen sulfide as a waste product (Levin, 2003; Oguz et al., 2000). Sulfur-oxidizing bacteria, which oxidize hydrogen sulfide to elemental sulfur or sulfate, and these sulfur particles may form a suspended “bacterial plate” at the oxycline (normoxic-hypoxia interface zone) (Matsuyama and Shirouzu, 1978), and provide a source of productivity and food in an otherwise inhospitable environment (Hamner et al., 1982b; Sorokin and Donato, 1975).

In this study, captive *M. leidyi* were observed consuming copepods and suspended particles at a naturally forming oxycline in a laboratory tank. SEM and EDS analysis of particles from this layer revealed bacteria and sulfur, indicating the animals were feeding upon a substance analogous to oxycline bacterial plates in the ocean. This suggests *Mnemiopsis leidyi* may be able to gain a competitive advantage in hypoxic waters normally deficient in prey. In the wild, access to this layer and exposure to the respiratory poison hydrogen sulfide (Bagarinao, 1992) may be more limited, explaining the death of the laboratory animals that fed on the particulate layer throughout the night.

The behavior of the animals in a tank with an artificial oxycline further suggests that *M. leidyi* may be able to detect oxygen interfaces using only the oxygen gradient as a cue. While these animals generally spend the majority of their time stationary at the surface of the water or on the floor of the tank, the presence of an artificial oxycline clearly altered their use of the space. The analyses suggest *Mnemiopsis leidyi*'s locations in the tank are not random (**Table 1**); rather, it significantly prefers the razor-thin (<0.5 cm) oxycline over the other 91% of the tank (**Table 3**). The mechanism by which these animals sense the oxygen disparity is not clear, but may be identified by future studies. It was also observed that animals in the wild do not generally spend their time in contact with the surface of the water due to the damaging effects of wind and waves. On days when the water is stationary (as it is in a laboratory setting), *M. leidyi* can be observed hanging at the top of the water as in these experiments (pers. comm., Moss 2016).

Further analysis also revealed a strong selection for spending time in the oxycline. This resource selection was greater than that of any other location (**Fig. 5**) and was generally observed as repeated excursions away from the surface, into the oxycline, and back to the surface. Animals

were frequently seen opening their lobes in a feeding-like manner upon entry into the oxycline, but no other stereotyped behaviors could be elucidated from the interactions.

Excursions to the oxycline were frequent enough to significantly increase the time the animals spent swimming in the water column as compared to normoxic and hypoxic controls without an oxycline (**Fig. 6**). This result is in agreement with similar findings in experiments with cnidarian jellies (Thuesen et al., 2005a), and supports the observation of oxycline association by gelatinous organisms (Bayly, 1986; Decker et al., 2004; Hamner et al., 1982b). However, the hypoxic levels used in this study never approached lethality. *In situ* animals may be more reticent to spend time associating with deadly anoxia.

These data also exemplify the value of applying classic ecological methods such as compositional habitat analysis (CHA) to laboratory studies. In the case of determining preference for an oxycline, comparison to control conditions would be inappropriate due to the inability of the animals to utilize a feature that is unavailable in the controls. This analysis also provides a more nuanced understanding of selection behaviors. Although Manly's selectivity ratios indicated the selection for spending time in the oxycline was greatest in relation to its availability, CHA results revealed the animals preferred the surface of the water over the oxycline. This could be assumed from observations of the animals' excursions, but was not predicted by the χ^2 analysis or selectivity ratios. While not originally developed for laboratory application, the compositional matrix markedly improves our understanding of how *M. leidy* interacts with its environment.

These experiments demonstrate that with only simple neurological pathways, *Mnemiopsis leidy* can exhibit complex behavioral patterns adapted to a specific set of environmental features. *Mnemiopsis leidy*'s appears to exploit hypoxic waters for refuge from predators and

facilitated capture of disabled prey (Decker et al., 2004; Keister et al., 2000; Purcell et al., 2001b); these new data, on the other hand, indicate *M. leidyi* may seek hypoxic zones rather than simply benefit from their fortuitous appearance. As the areas of greatest impact by *M. leidyi* are also areas of pervasive hypoxia, advantageous use of oxyclines could be an essential component of these environments' ecological dynamics.

While much of the world's marine environments feature some degree of episodic or persistent hypoxia, these events are predicted to increase as a result of anthropogenic influences (Justić et al., 2003; May, 1973; Melzner et al., 2012). Anthropogenic nutrient input and overfishing are also both contributing to an increase in populations of jellies worldwide (Legović, 1987; Purcell, 2012). Further, *Mnemiopsis leidyi*'s behaviors in the presence of an oxycline may allow it to outcompete non-hypoxia tolerant species, use an underutilized food source (the bacterial plate) in low production environments, or influence trophic cascades at persistent oxyclines. Should *M. leidyi* take advantage of this perfect storm of conditions, their devastating dominance of the Black Sea could develop in our increasingly anthropogenically influenced oceans.

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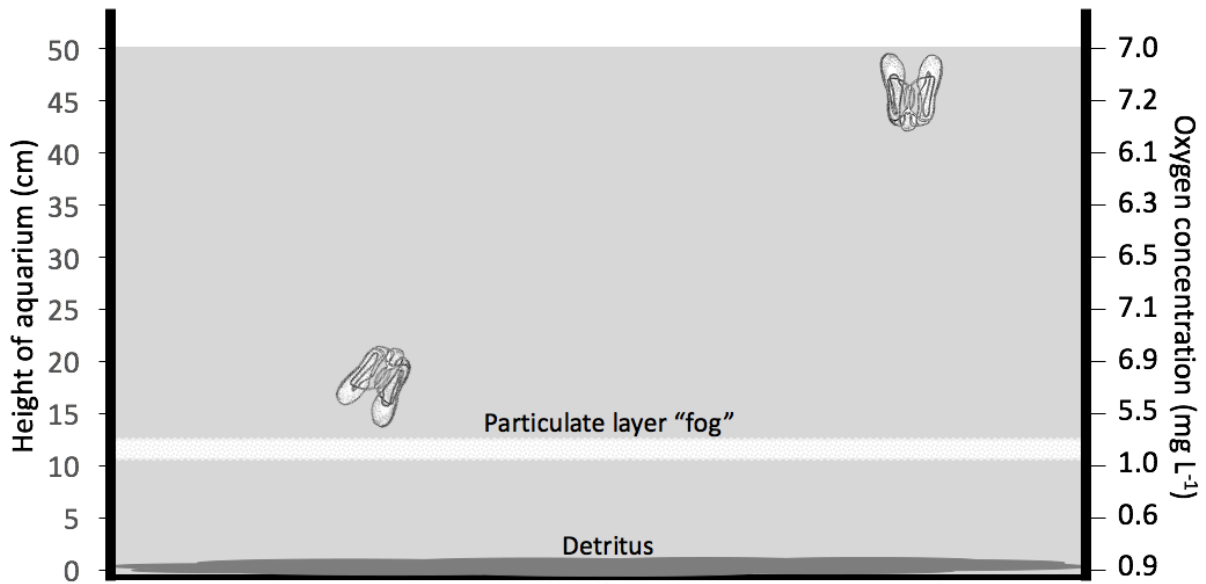


Fig. 1 Diagram of laboratory aquarium in which the naturally forming oxycline and associated particulate layer was first observed. The animals (shown to scale at 6 cm long) were observed making continuous excursions down to this layer before dying between 6 and 22 hours later (sea water; 34‰; 21 °C).



Fig. 2 Picture of the white particulate layer created at the oxycline in a *Mnemiopsis leidyi* laboratory aquarium. One of the animals can be seen feeding on this material. The water above this layer was normoxic and below was hypoxic (**Fig. 1**). Photo courtesy of J. Daniel (Moss Lab), Oxygen readings courtesy of R. Henry and T. Moss.

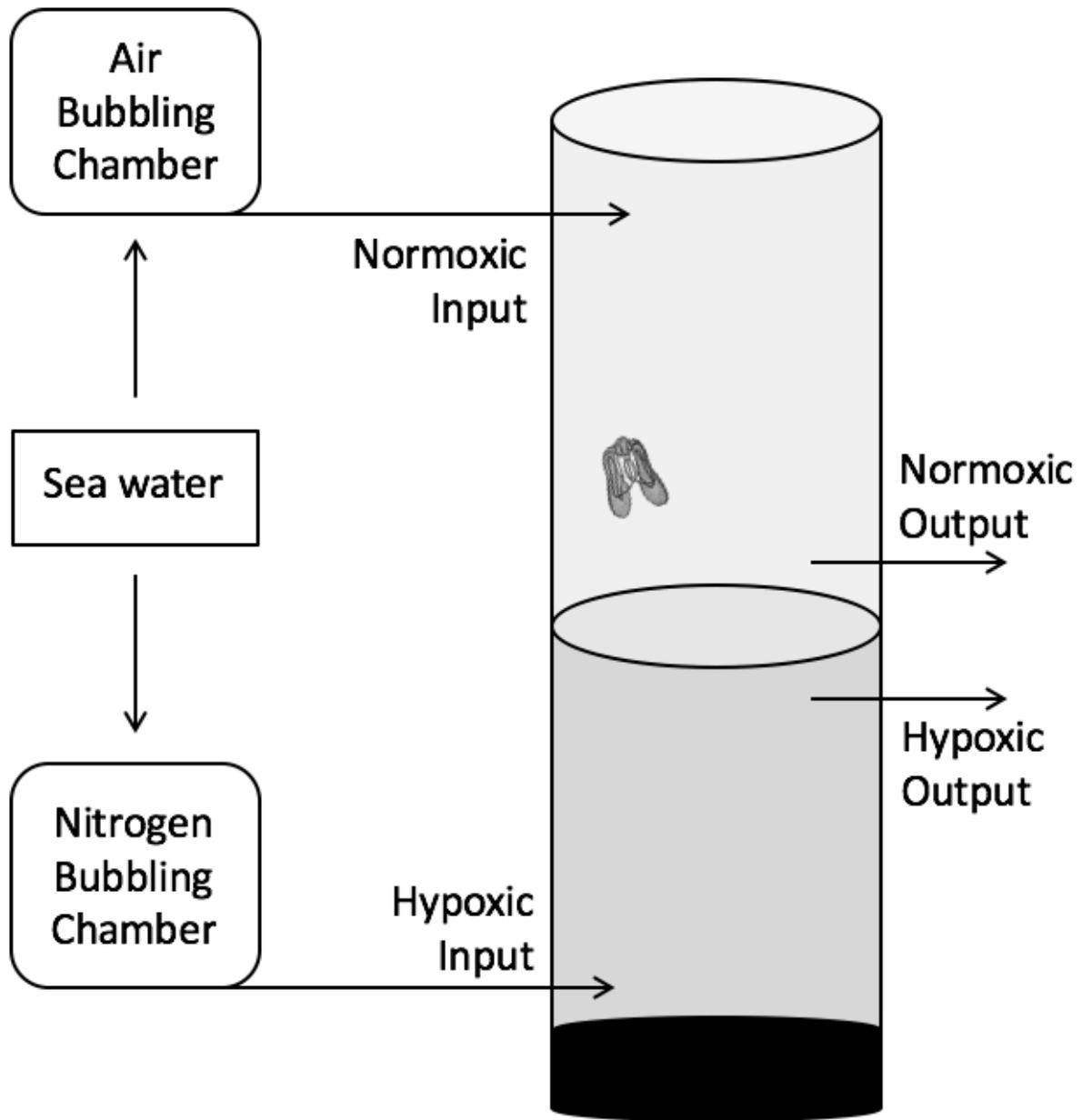


Fig. 3 Experimental cylindrical tank in which an artificially produced oxycline was formed by layering air-saturated normoxic sea water ($PO_2 > 130$ Torr; 34‰; 21 °C) over oxygen-stripped hypoxic sea water. Water flow was stopped before animals were added for trials. A 3 cm *Mnemiopsis* is shown to scale. Ctenophore image: (Wood et al. 2015)

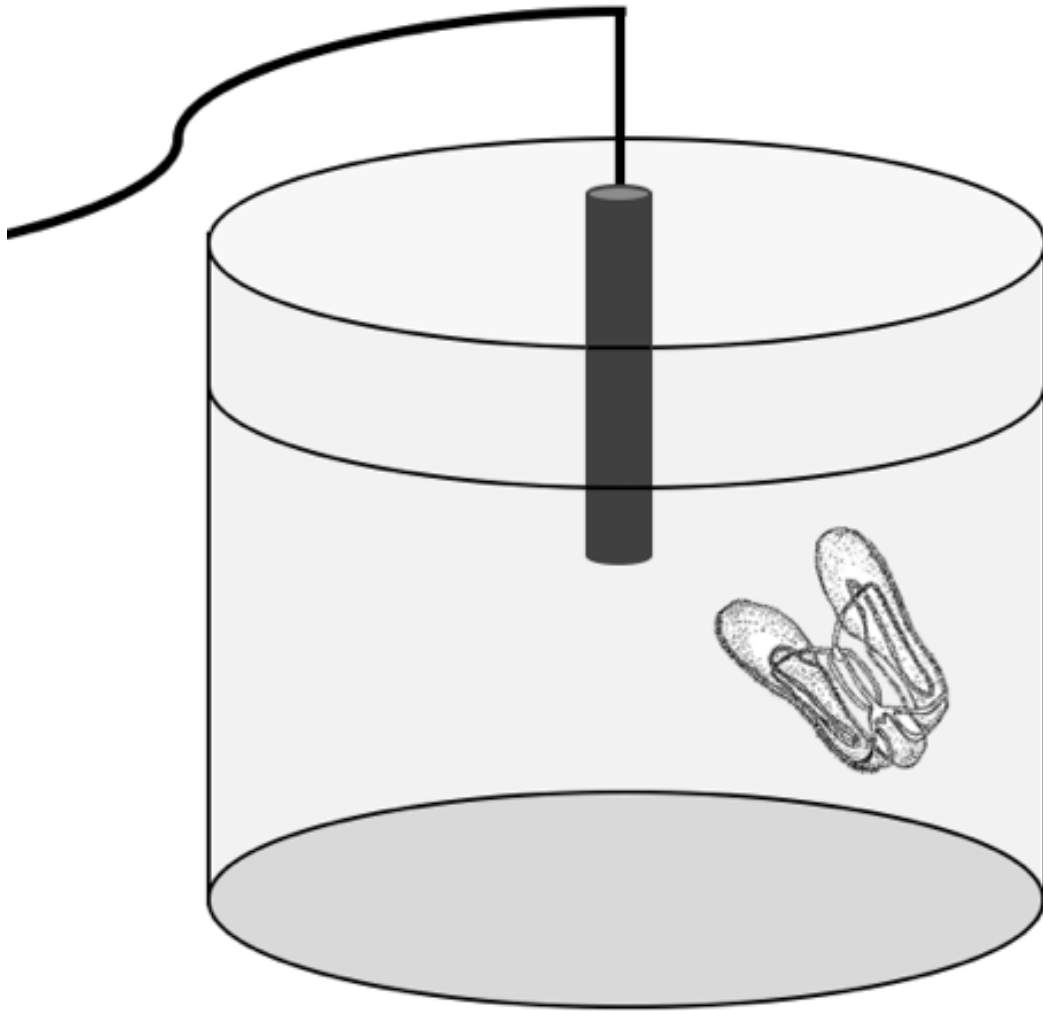


Fig. 4 Respiration cell used to determine the mass-specific oxygen consumption rates of *M. leidyi* in progressive hypoxia. Ctenophore image: (Wood et al.)

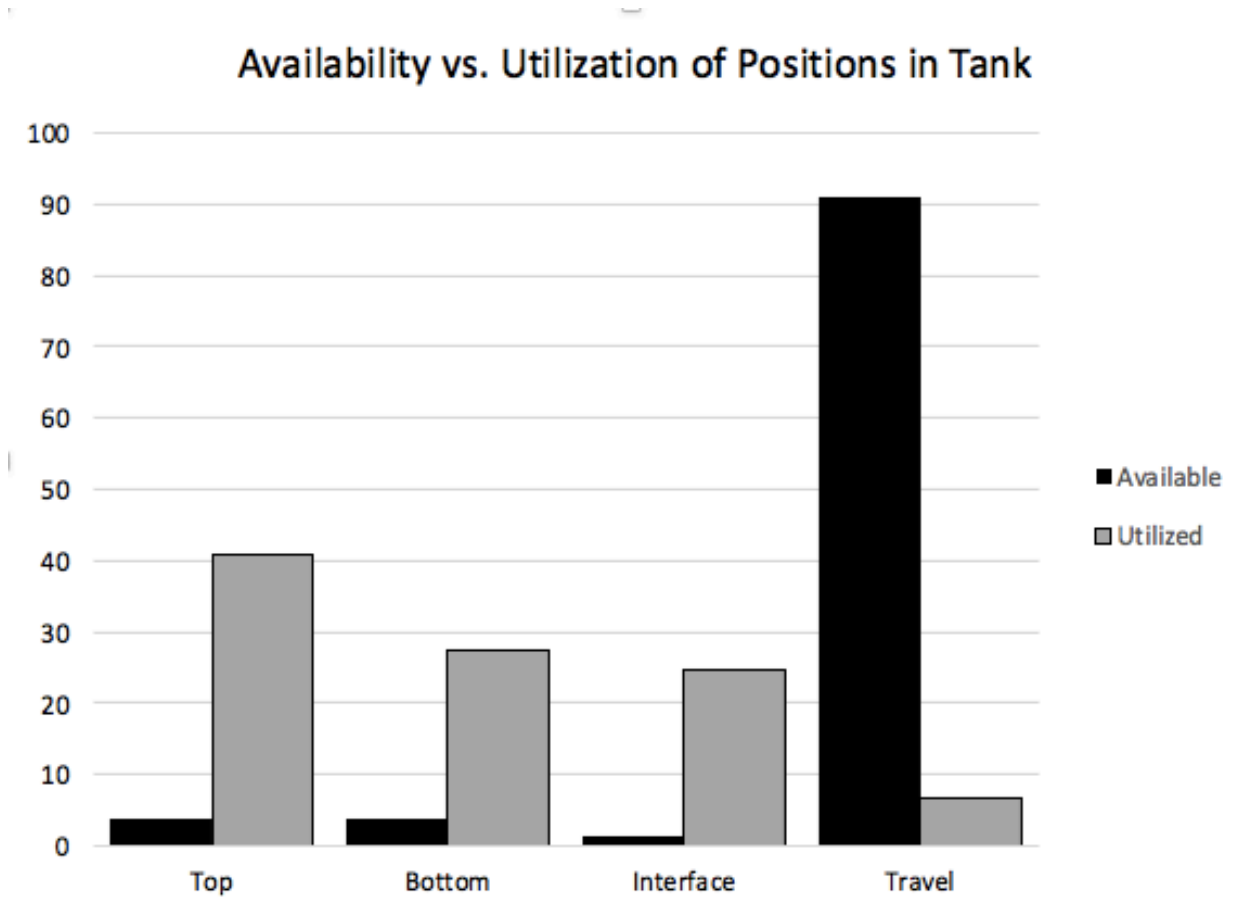


Fig. 5 Comparisons of the percent of the tank (21 °C; 34 ‰; sea water) available to *M. leidy* (n=8) in each location category vs. the percent time that the animals spent in each location during the experiments. While the Top ($PO_2 > 130$ Torr), Bottom ($PO_2 < 60$ Torr), and Interface locations each represented less than 4% of the tank, the animals spent approximately 41%, 27%, and 24% of their time in these locations, respectively. On the other hand, Travel represented the other 91% of the tank, and the animals spent less than 7% of their time in this area. This indicates that these animals spent a disproportionate amount of time in some locations and were treating the oxycline interface as a specific location.

Manly Selectivity Ratios

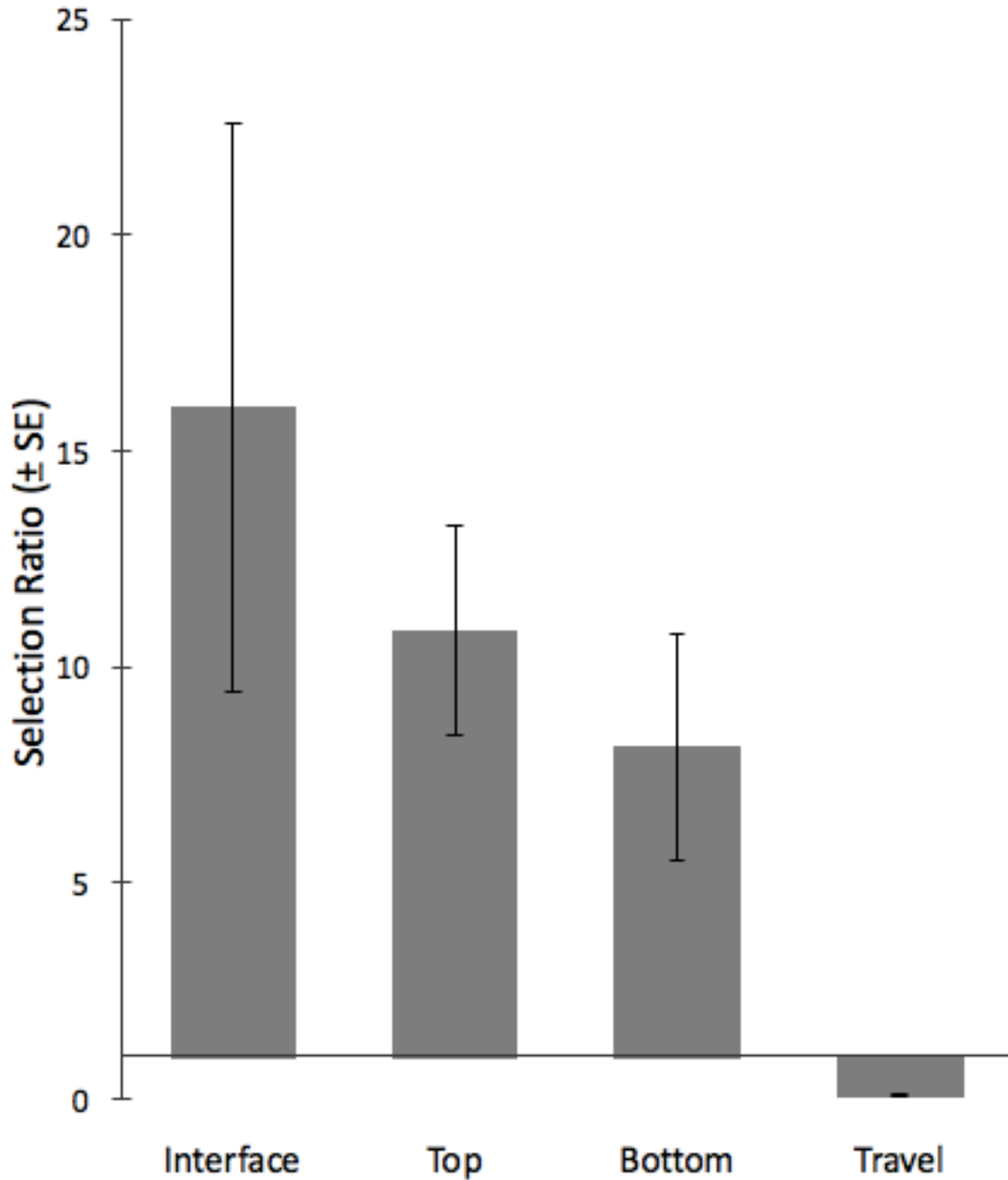


Fig. 6 Manly selectivity ratios (used/available) indicating the extent to which each location in the *Mnemiopsis leidyi* experimental tank was selected for relative to its availability. Values >1 represent selection for, while values <1 represent selection against that location.

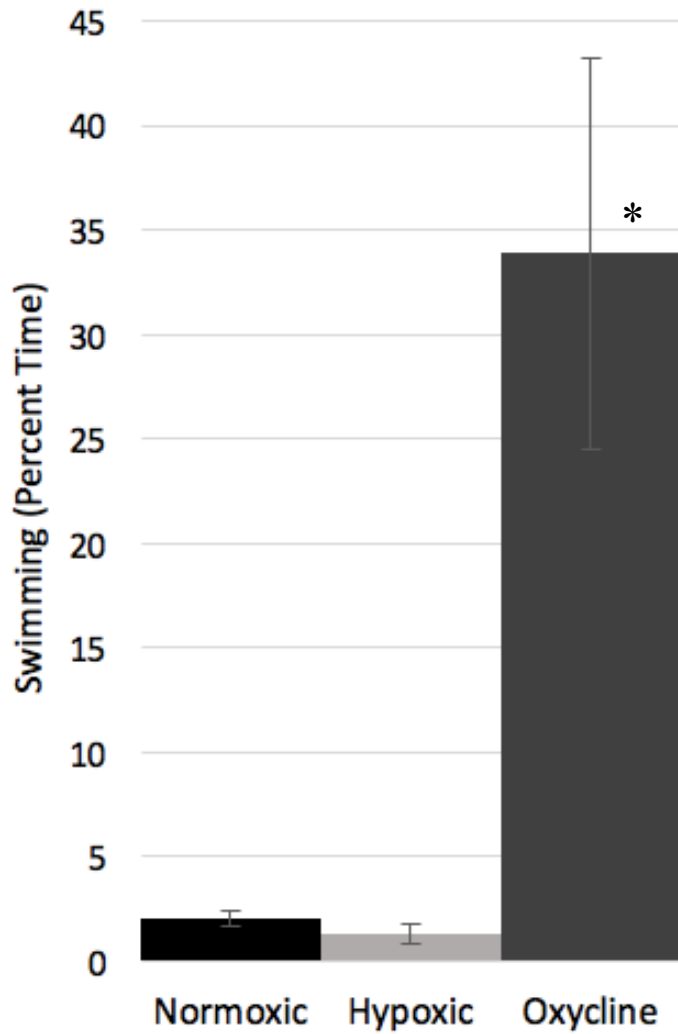


Fig. 7 Comparisons of the percent time *Mnemiopsis leidyi* spent swimming in the water column of the tank when the tank contained entirely normoxic water, entirely hypoxic water, or was split by an oxycline in the middle of the tank. This increase in swimming represents the excursions the animals made from the water's surface to the oxycline and back to the surface.

*Significance: $p < 0.05$

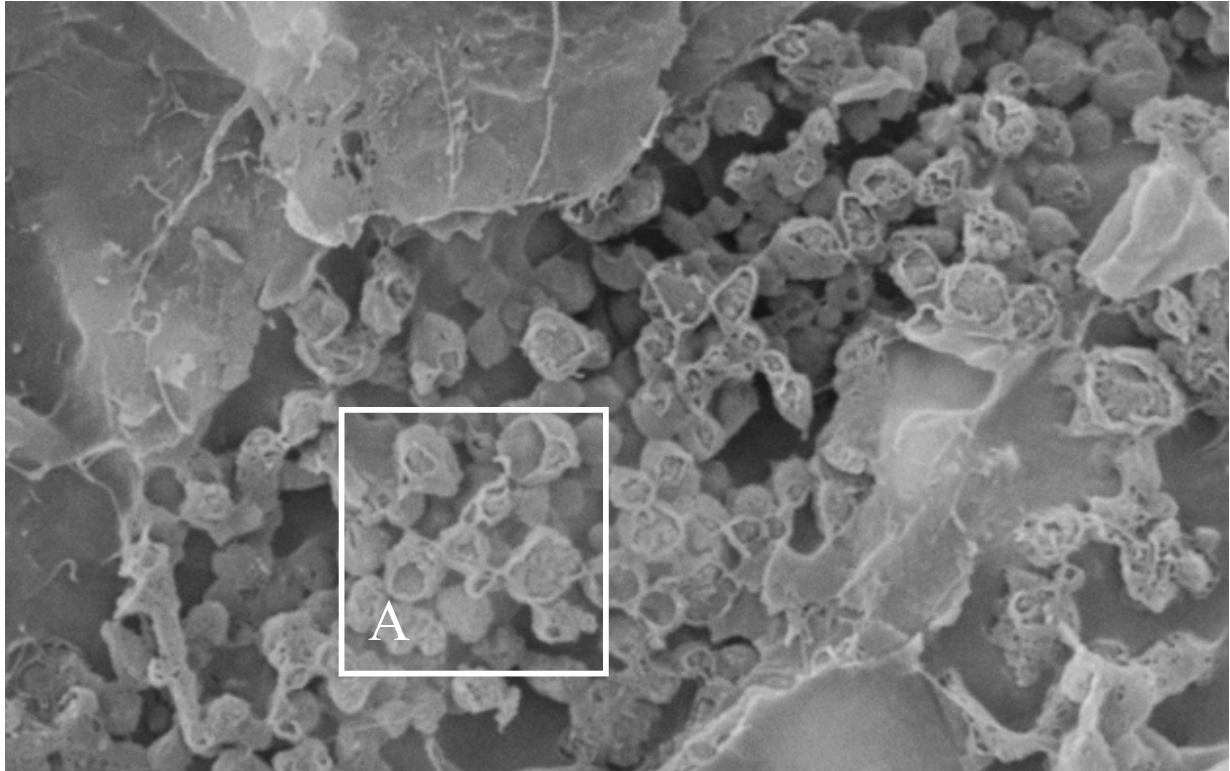


Fig. 8 Irregular spheres of the white particles collected from the oxycline in the *M. leidyi* holding tank. The particles are seen between ripped pieces of filter. Box A denotes the portion analyzed by EDS (**Table 5**).

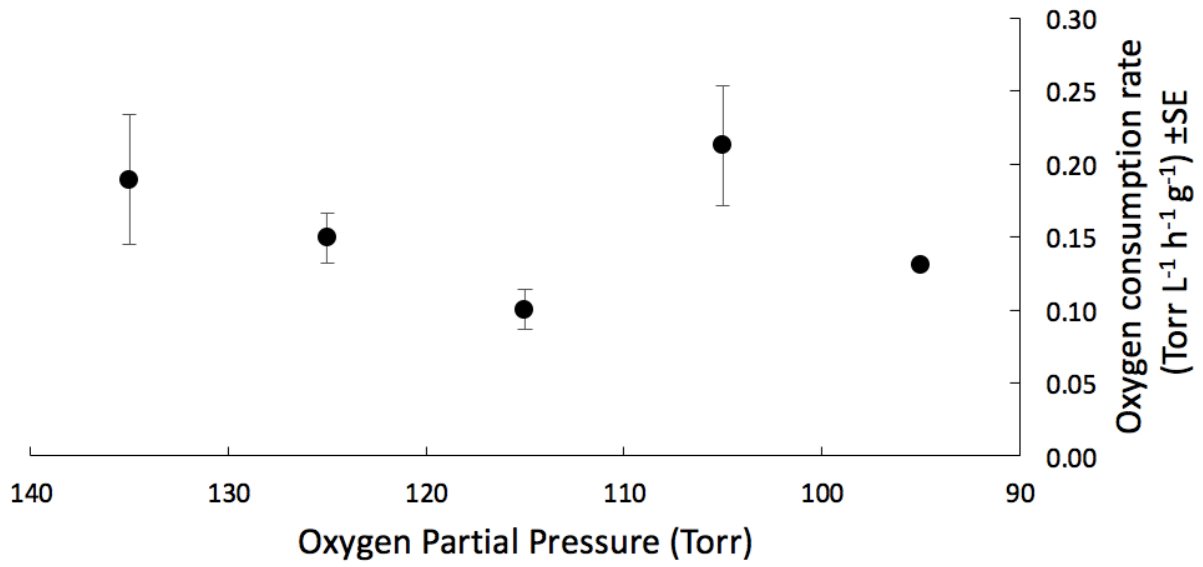


Fig. 9 Mass-specific oxygen consumption rates in progressive hypoxia for *Mnemiopsis leidyi* (n=6). These rates did not change appreciably (slope = 0.025) over the first 24 hours of oxygen consumption.

Animal size (cm ³)	n	# of responses
>15.75 cm ³	8	8
<15.75 cm ³	3	0

Table 1: Results of size-dependent *M. leidy* responses to an artificially created oxycline. After preliminary trials, specimens <15.75 cm³ were no longer used in experiments or analyses.

Location	Observed	Expected
Top	40.89	3.77
Bottom	27.39	3.77
Travel	6.87	91.13
Interface	24.86	1.32
	$\chi^2 = 1011.17$	p<0.001

Table 2 Results of the χ^2 analysis, comparing percent of time *Mnemiopsis leidyi* used each location in the tank (observed values) to the percent of the tank which represented each location (expected values).

	Top	Interface	Bottom	Travel	Rank
Top		0.06975	1.5759	11.3302	3
Interface	-0.06975		1.05735	11.8575	2
Bottom	-1.5759	-1.0575		3.2024	1
Travel	-11.3302	-11.8575	-3.2024		0

Table 3 Compositional analysis preference ranking matrix for location utilization by *Mnemiopsis leidy* in an experimental tank containing an artificial oxycline. Each cell displays the values of t , in the t-tests comparing the row and column locations. A positive value indicates that the row location is used more than would be expected if there were no preference between the row and column locations. Rank is a count of total number of positive cells and indicates increasing proportional use.

	Top	Interface	Bottom	Travel	Rank
Top		+	+	+++	3
Interface	-		+	+++	2
Bottom	-	-		+++	1
Travel	---	---	---		0

Table 4 Simplified preference ranking matrix for *Mnemiopsis leidy* location selection.

+ indicates preference for the row category to the column category, while - indicates the row location was not preferred to the column location. Tripled signs indicate significance ($p < 0.05$).

n	PO₂ in Top Half of Tank (Torr)	PO₂ in Bottom Half of Tank (Torr)	# responses to oxycline
6	150	150	0
6	150	100	0
6	150	80	0
6	150	70	0
6	150	60	6

Table 5 Results of experiments determining the PO₂ threshold at which *Mnemiopsis leidyi* begins displaying hypoxia-associated behaviors (e.g. avoidance, refusal to cross oxycline, etc). These results indicate that the animals respond behaviorally to hypoxia at a PO₂ from 70 – 60 Torr (21 °C; 34 ‰; sea water).

Element	Percent
Sulfur	19%
Carbon*	75%
Oxygen	5%
Gold*	1%

Table 6 EDS analysis the percent of each element found in Box A of **Fig. 7** of the particles from the oxycline in the *M. leidyi* holding tank. * The carbon and gold were deemed to be artifacts of the SEM stub and method of preparation.

Chapter 3. Locomotory behavioral response of *Mnemiopsis leidyi* to a focal, discrete, artificial oxygen source

3.1 Introduction

Although the availability of oxygen is often taken for granted in terrestrial environments, breathing can be a persistent struggle for marine organisms. Oxygen (O_2) is not as readily available in aquatic environments as in air. Slower diffusion, lower concentrations, and faster local consumption by aerobic organisms all lead to variability in O_2 availability for those marine creatures. Oxygen concentrations can range from 0 mg L^{-1} to approximately 8 mg L^{-1} (0-100% air saturation) depending on salinity and temperature, photosynthetically active organisms can increase the oxygen concentrations in shallow estuaries to $>16 \text{ mg L}^{-1}$ ($>200\%$ air saturation). Both sessile and mobile estuarine organisms have evolved physiological and behavioral responses to what can be a changeable, challenging environment. While O_2 availability has been heavily researched in many marine systems (Helly and Levin, 2004), it is not well understood how many species - especially invertebrates - respond when confronted with low oxygen (hypoxic) environments. Hypoxia is generally defined as oxygen concentrations below 2.0 mg L^{-1} (approximately 30% air saturation), though species-specific physiological O_2 thresholds are highly variable (Mangum and van Winkle, 1973; Steckbauer et al., 2011; Vaquer-Sunyer and Duarte, 2008). The vertical distributions and diel migrations of cnidarian medusa and ctenophores (referred to henceforth simply as jellies) in areas of low dissolved O_2 have been studied in multiple marine habitats (Ilamner and Hauri, 1981; Purcell et al., 2014, 2001b; Kolesar, 2010). However, recent publications suggest a need for more hypoxia-induced behavioral information in order to better elucidate the ecological impacts of gelatinous organisms in low oxygen conditions (Gray et al., 2002; Spicer, 2014).

The occurrence of both episodic (minutes to days) and persistent (months to years) hypoxia is an important physical feature of many habitats populated by jellies such as estuaries, bays, shallow coastlines, and marine lakes (Breitburg, 1992; Breitburg et al., 2003; Hamner et al., 1982a; Purcell et al., 2001b). Warm weather, microbial degradation of organic matter, and shallow water may create ephemeral hypoxia by stratifying the water column by density, preventing the re-aeration of bottom waters (Officer et al., 1984; Rabalais et al., 1994). O₂ depletion may also be intensified by acidification and excess nutrient input from anthropogenic sources, which enhances microbial activity and O₂ consumption (Justić et al., 2003; Melzner et al., 2012). These effects are predicted to increase worldwide, thus artificially inflating the prevalence, frequency, and intensity of temporary hypoxic events (Altieri and Gedan, 2015; Conley et al., 2011; Diaz and Rosenberg, 2008; Legović, 1987; May, 1973). Ephemeral hypoxic episodes may last from minutes to days and are common and widely dispersed along the Atlantic coasts of North and South America (Breitburg, 1990; Diaz and Rosenberg, 2008), as well as in the Gulf of Mexico and the Black Sea (Gupta et al., 1996; Zaitsev, 1992). These areas represent both the natural and invasive habitats of the ctenophore *Mnemiopsis leidyi* (Purcell et al., 2001a).

Ctenophores, like *Mnemiopsis leidyi*, are gelatinous zooplankton found in all oceans and characterized by eight locomotory rows of ciliated comb plates known as ctenes. *M. leidyi* uses two oral lobes to entrap zooplankton and other small prey items. The ecological significance of this species has been widely recognized since the introduction of the North American *Mnemiopsis leidyi* to the Black Sea in the 1980s, where they collapsed the food web and devastated fisheries. *M. leidyi*, while precise and exacting in movement, is functionally planktonic (Haddock, 2007). Unable to escape currents and tides, they are at the mercy of their environment, and have accordingly evolved mechanisms to adapt to many conditions. Like

cnidarians, ctenophores lack the ability to control the mass-transfer of oxygen and other gases through their body tissues. Diffusion across their outer epithelium, which is only one cell thick, is their only known mechanism of respiration (Purcell et al., 2001b), though ventilation via pumping of their comb plates may provide additional oxygen exposure. They are also highly tolerant of hypoxic conditions, possibly as a result of high surface area for gas exchange, and are able to survive and function at levels lethal to many fishes and other gelatinous zooplankton (Finenko et al., 2006; Purcell et al., 2001b)

For highly mobile animals the most frequently observed response to hypoxia is avoidance. Fish will generally avoid areas of low oxygen, while even sea cucumbers will creep along the benthic substrate to find sites of higher oxygenation (Farrell and Richards, 2009; Newell and Courtney, 1965). When confronted with inescapable hypoxia, many species of fishes, crabs, and amphibious snails will partially or entirely emerge from the water onto land where they are able to partially respire through their skin, gills, or modified lungs (Jones, 1961; Taylor et al., 1977; Yoshiyama and Cech, 1994; Yoshiyama et al., 1995). Other fishes may even gasp at the surface of the water to gulp air for added respiration (Sloman et al., 2008). Perhaps the most highly derived behavioral response to hypoxia is that of the bulb-tentacle sea anemone (*Entacmaea quadricolor*), which is oxygenated throughout the night by the fanning motion of its mutualistically symbiotic two-banded anemonefish (*Amphiprion bicinctus*) (Szczebak et al., 2013). All of these behaviors provide a competitive advantage over animals unable to compensate for the low availability of oxygen in their environment.

Recent observations suggest the functionally planktonic *Mnemiopsis leidyi* may be able to survive hypoxia by utilizing temporary, localized, discrete sources of oxygen (e.g., photosynthesizing aquatic plants and macroalgae). Accounts of *M. leidyi* in the West Falmouth

Harbor, (Falmouth, MA, USA) describe animals sliding down blades of benthic seagrass, presumably collecting organisms for food and possibly photosynthetically derived oxygen (Moss pers. obs. 2011). This presumption was further supported by laboratory *Mnemiopsis leidyi* in Auburn University (Auburn, AL, USA) repeatedly associating with the bubbles found underneath floating mats of green algae (**Fig. 1**). This behavior only occurred when the tanks contained excessive amount of decomposing material and were hypoxic ($<2.0 \text{ mg L}^{-1}$). This study was then performed to test (1) if *M. leidyi* is able to detect a discrete, focal source of oxygen. And if so (2) do they associate with this source in a manner indicative of utilization of the oxygen?

3.2 Materials and Methods

3.2.1 Specimen collection and husbandry

Specimens of *Mnemiopsis leidyi* were collected via plankton net from the Gulf of Mexico off the coast of Dauphin Island (AL, USA) and driven to Auburn University (Alabama, USA) in sealed 5-gallon buckets. Other specimens were ordered from Gulf Specimen Marine Laboratories, Inc. (Panacea, FL, USA) and shipped to Auburn overnight in water from their habitat. Animals were transferred to 50 gallon kreisel tanks with sumps and overhead reservoirs (21 °C; 34 ‰; sea water) and the tanks were cycled and aerated by pump every 12 hours. Animals were fed brine shrimp (*Artemia salina*) nauplii every morning and allowed to feed *ad libitum*. Prior to experiments, specimens were placed in 50mL of the experimental water and allowed to acclimatize for at least 1 hr.

3.2.2 Localized Oxygen Source Experiments

Mnemiopsis leidyi was tested for response to a discrete oxygen source by use of O₂ diffusion through dialysis tubing. We built an apparatus composed of two glass pipes connected by a 4 cm length of dialysis tubing (16mm diameter, MWCO 12-14,000, Spectra/por, Spectrum Labs) in a “U” configuration (**Fig. 2**). The tops of these glass pipes were used as the inflow and outflow connections for water pumped from reservoirs. Glass rods and silicone sealant reinforced the structural integrity of the apparatus, termed “Leafyi” after its association with aquatic leaves and *M. leidyi*.

For each hypoxic experimental trial, a 1 cm-thick acrylic cylinder (60 cm tall x 10 cm internal diameter) was filled (21 °C; 34 ‰; sea water) and bubbled with nitrogen to scavenge oxygen until the partial pressure of oxygen (PO₂) in the tank reached hypoxia (<40 Torr; 25 % air saturation). Normoxic water was air-saturated (>130 Torr; 83 % air saturation) with a bubble stone and pumped through the Leafyi apparatus. Preliminary trials showed that the water within 4 cm of the dialysis tubing increased in PO₂ by 15 Torr (approx. 10% air saturation) in 30 minutes without appreciably increasing the PO₂ of the entire cylinder (1 Torr; 0.6% air saturation). All experiments and controls were performed as such (outlined in **Table 1**):

Hypoxic Experiment: Animals were placed in the cylindrical tank of hypoxic water and normoxic water was run through the Leafyi apparatus.

Hyperoxic Experiment: Animals were placed in normoxic water in the cylinder and oxygen gas was used to create hyperoxic sea water (> 400 Torr; 289% air saturation) to test their association with an oxygen source in normoxic conditions.

Normoxic Control: To control for any animal response to the movement of water through the Leafyi, the animals were placed in a normoxic cylinder and normoxic water was run through the Leafyi.

Hypoxic Control: To control for any animal response to a negative oxygen gradient (oxygen being removed from the water), the animals were placed in a normoxic cylinder and hypoxic water was pumped through the Leafyi.

Materials Control: A materials-only control was created by wrapping a length of empty dialysis tube around a straight piece of glass pipe. The tube was secured with silicone sealant to mimic all substances found on the Leafyi. This control pipe was presented to animals in a normoxic cylinder.

Once the PO₂ of the Leafyi and cylinder water were stable, animals were gently added to the top of the tank. To prevent the animals from obtaining oxygen from the surface of the water a thin circle of plastic aquarium mesh was secured 1 cm below the surface. To account for the effect of the Leafyi's distance from the surface, the dialysis tube of the apparatus was either placed in the top half of the tank approximately 20 cm from the surface of the water (designated the "Upper" position) or in the bottom half of the tank approximately 40 cm from the surface of the water (designated the "Lower" position) (**Fig. 3**). Once an animal had maintained contact with the dialysis tubing or had stayed within 2 cm of the tubing for over a minute, the trial was considered a successful response and was ended. Each animal was exposed to the Leafyi in the Upper and Lower positions multiple times (**Table 1**). If the animal failed to associate with the Leafyi within 1 hour the trial was considered a non-response. Between each trial the animals

were removed and the cylinder was momentarily bubbled with either nitrogen gas or air to homogenize O₂ concentrations throughout.

Water samples were collected via 1 mL syringe and PO₂ measurements were made by two Clark-type polarographic O₂ electrodes (E5046; Radiometer) connected to a dual chamber O₂ meter (OM 200, Cameron Instruments). Two identical chambers were used to confirm readings, and electrodes were calibrated to 0 Torr with sodium sulfite and to 155 Torr with air-saturated sea water. Recent studies demonstrated the possibility of an intrinsic circadian rhythm in *Mnemiopsis leidyi* (Sasson and Ryan, 2016); therefore, all experiments were conducted during daylight hours. It was discovered in earlier experiments that specimens under 15.75 cm³ (as measured by calipers along three planes of symmetry) rarely leave the surface of the water, and thus only larger animals were used for these trials.

3.2.3 Analyses

A binomial logistic regression was used to detect deviation from a null hypothesis of no effect of Upper and Lower locations of the Leafyi apparatus. Further binomial logistic regressions tested the relative effects of treatments on the odds *M. leidyi* would associate with the dialysis tubing of the “Leafyi.” We also compared the control treatments to each other to test for significance within those treatments. As each individual was tested multiple times, a random statement was used in each regression to account for pseudoreplication. All analyses were performed using statistical software package R version 3.1.3.

3.3 Results

75 trials were performed on 30 individual *Mnemiopsis leidyi*. Out of the 27 experimental trials, 17 associations with the Leafyi oxygen source were observed. The majority of the interactions consisted of the animals wrapping their oral lobes around the dialysis tubing (**Fig. 4**). These individuals would maintain contact until the apparatus was gently shaken to remove them. In each of the controls, at least one animal was observed lingering within 3 cm the dialysis tubing of the Leafyi long enough to be considered an association (**Table 2**). However, none of these control interactions consisted of the animals wrapping their oral lobes around the dialysis tube. Results also indicated no significant difference between likelihood of responses between any of the control treatments ($p>0.5$).

Results of the binomial logistic regression showed that the animals in the experimental trials were (outlined in **Table 2**):

- (A) **Hypoxic Experiment** animals were 8.5 (1.49-48.54, \pm 95% C.I.) times as likely to interact with the dialysis tubing as **Hyperoxic Experiment** specimens ($p=0.0140$). This indicates that the animals' association with an oxygen source occurred only when the animal was exposed to hypoxic conditions.
- (B) **Hypoxic Experiment** animals were 18.7 (1.99-174.92, \pm 95% C.I.) times as likely to associate with the Leafyi oxygen source as **Normoxic Control** animals ($p=0.0088$), indicating that the animals only associated with Leafyi when it was providing oxygen to the tank.
- (C) **Hypoxic Experiment** animals were 18.7 (1.99-174.92, \pm 95% C.I.) times as likely to associate with the Leafyi as animals in **Hypoxic Control** trials ($p=0.0088$). This suggests

that the specimens did not associate with the oxygen gradient created by the hypoxic Leafyi removing oxygen from the cylinder.

(D) Hypoxic Experiment animals were 18.7 (1.99-174.92, \pm 95% C.I.) times as likely to respond to the apparatus as animals exposed to the **Materials Control** ($p=0.0088$), meaning the animals were not attracted to the substances used to create

(E) Hypoxic Experiment animals were 14.6 (4.25-50.33, \pm 95% C.I.) times as likely to interact with the Leafyi as animals in all other control and experimental trials combined ($p=1.43e-05$).

3.4 Discussion

Despite the frequent efforts of researchers to explore the physiological tolerances and effects of hypoxia (low oxygen) on the ctenophore *Mnemiopsis leidyi* and other marine invertebrates, hypoxia-induced behaviors of these creatures are still not well understood (Gray et al., 2002; Spicer, 2014). While highly mobile animals are often able to escape temporary hypoxia that lasts only minutes to days, physiological adaptations are generally required for the survival of creatures trapped in hypoxic events. We suggest that *M. leidyi* is able to detect and possibly utilize for oxygen an apparatus analogous to *in situ* small-scale oxygen sources (e.g., photosynthesizing aquatic leaves and macroalgae).

Specimens immersed in hypoxic water were significantly more likely to associate closely with a dialysis tube providing oxygen than specimens in normoxic water, suggesting such oxytaxis (movement towards oxygen) is likely triggered by exposure to hypoxia (**Table 2**). This is further supported by the animals' lack of response to a hyperoxic oxygen source while in normoxic water. While the "Leafyi" did not appear to be increasing the PO_2 of the entire cylinder

in these experiments, the responses of the animals suggest that the gradient was present but not detectable by our instruments. The physiological mechanism by which these animals sensed the oxygen source is not clear, but may be elucidated by future studies.

While hypoxia can lead to large reductions in abundance and diversity of fishes and marine invertebrates, this extreme form of oxytaxis may represent a *Mnemiopsis*-specific form of relief from ephemeral hypoxic events. Other studies observed *Mnemiopsis leidyi*'s hypoxia tolerance allowing for the exploitation of hypoxic waters for refuge from predators and for facilitated capture of disabled prey (Decker et al., 2004; Keister et al., 2000; Purcell et al., 2001b). However, while resilient to death via hypoxia, ctenophores are not invulnerable to the stresses of low oxygen exposure. In experimental studies, ctenophores exposed to low dissolved O₂ had significantly reduced growth and egg production as compared to air-saturated control animals (Grove and Breitburg, 2005). *M. leidyi* is also only tolerant down to about 5 Torr (3% air saturation), below which they die quickly (Thuesen et al., 2005b). While oceanic oxygen is generally derived from atmospheric and environmental input, using oxygen directly from the photosynthetic source may allow *M. leidyi* to survive and even thrive in conditions of severe or prolonged hypoxia. While these results indicate that the presence of seagrass or macroalgae may provide a microhabitat for the ctenophores, it is unlikely this method of hypoxia relief is viable for weeks or months in persistent hypoxic zones. However, future studies may prove otherwise.

Should *Mnemiopsis leidyi*'s oxytaxis provide a competitive advantage over its prey and predator species the implications could be significantly adaptive for such populations. *M. leidyi* is known to have a powerful perturbing effect on the plankton populations in Chesapeake Bay, where ephemeral hypoxia is a nearly daily occurrence in summer (Breitburg, 1990; Purcell and Decker, 2005; Purcell et al., 2001a). In the frequently hypoxic Black Sea, invasive *M. leidyi* was

responsible for decimating entire ecosystems and devastating already overharvested fisheries (Purcell et al., 2001a; Shiganova, 1998). While these hypoxic events are natural features of the world's oceanic coasts and estuaries, anthropogenic influences (e.g., nutrient input and ocean acidification) are already increasing the severity, prevalence, and frequency of such phenomena (Altieri and Gedan, 2015; Justić et al., 2003; Melzner et al., 2012). Worldwide populations of jellies are also booming as a result of overfishing and other anthropogenic stressors on marine ecosystems (Purcell, 2012). Our observation of ctenophore oxytaxis reveals expanded chemosensory capabilities in this ancient sister taxon to all of Metazoa (Whelan et al., 2015). Therefore, this new behavior carries not only interest to species-specific science, but also evidence of the vast manners by which some creatures are able to thrive over others in our increasingly disturbed oceans.

3.5 References

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Fig. 1 Three *Mnemiopsis leidyi* ctenophores associating with the bottom of a floating mat of green algae in a laboratory holding aquarium at Auburn University. Bubbles from this mat can be seen near the top of the picture.

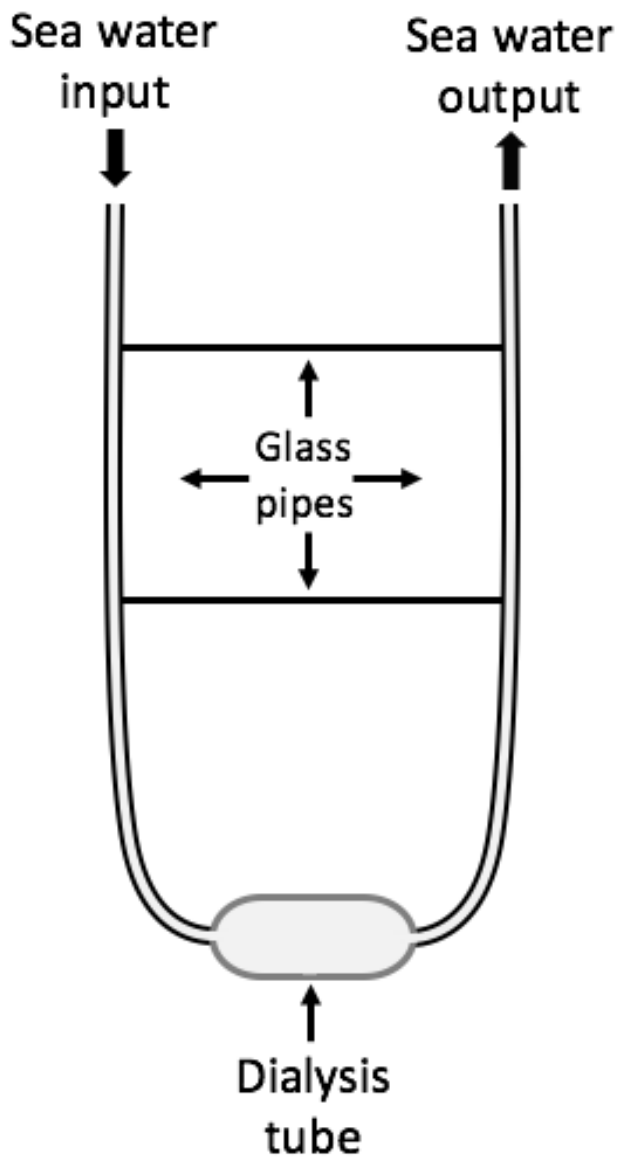


Fig. 2 Leafyi localized oxygen source apparatus

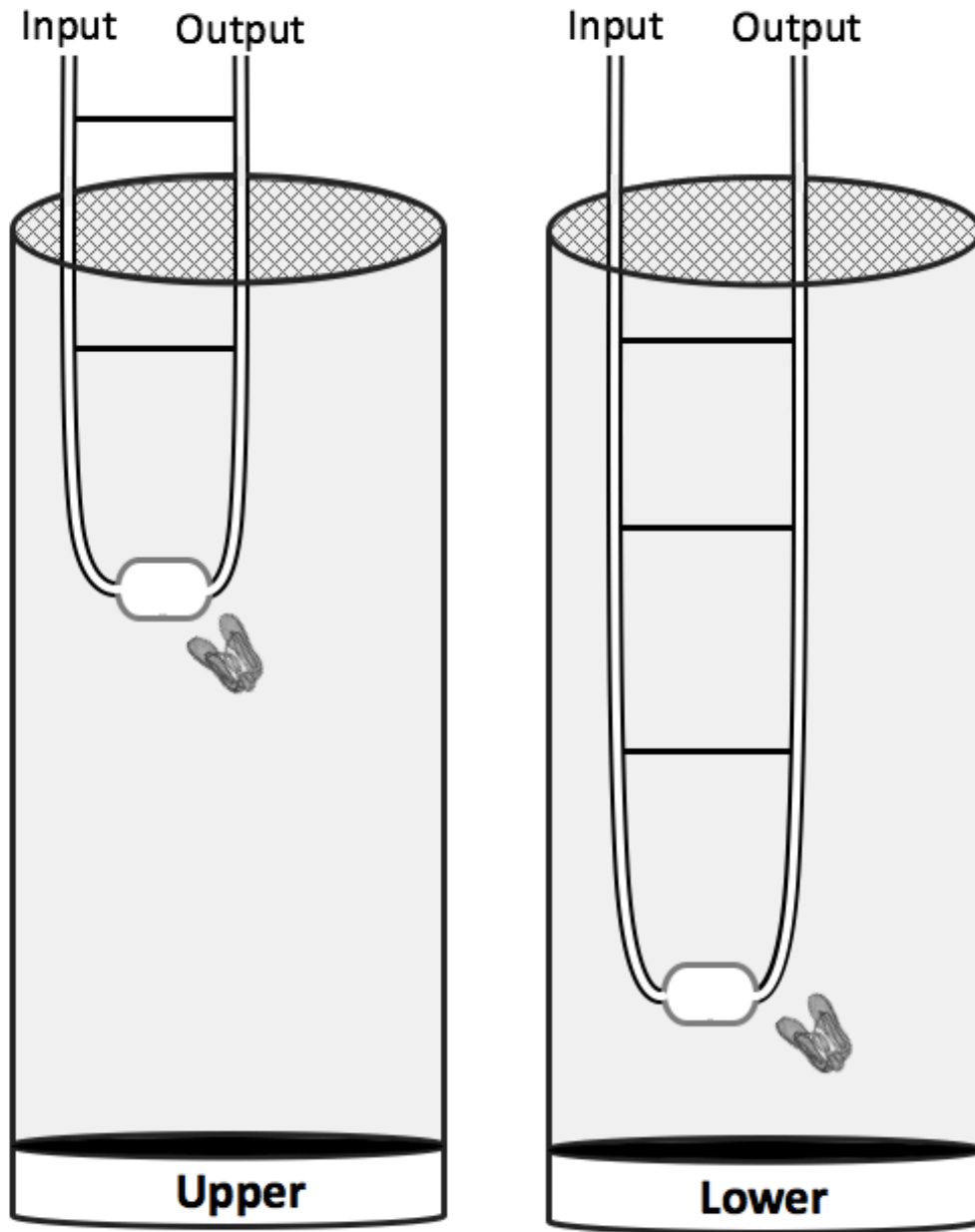


Fig. 3 Two positions (Upper, Lower) of the Leafyi apparatus during trials. Each animal was introduced to both positions at least once. Mesh was placed at the surface of the water to prevent the animals from obtaining oxygen there. One 3 cm *Mnemiopsis leidyi* is shown to scale.

Ctenophore image: (Wood et al.)



Fig. 4 Picture of *Mnemiopsis leidyi* wrapping its oral lobes around the dialysis tubing of the Leafyi apparatus during an experimental trial.

	Leafyi water	Cylinder water	n	Total # trials
Hypoxic Experiment	Normoxic	Hypoxic	6	27
Hyperoxic Experiment	Hyperoxic	Normoxic	6	12
Normoxic Control	Normoxic	Normoxic	6	12
Hypoxic Control	Hypoxic	Normoxic	6	12
Materials Control	Materials only	Normoxic	6	12

Table 1 Outline of experiments and controls for Leafyi apparatus to test localized oxygen source behaviors in *Mnemiopsis leidyi*. Normoxia was defined as being >140 Torr, while hypoxia was <40 Torr.

	Leafyi Water	Cylinder Water	n	Total # trials	Total # associations	Beta estimate (Hypoxic Experimental animals were ___ times as likely to associate)	p-value
Hypoxic Experiment	Normoxic	Hypoxic	6	27	17	NA	NA
Hyperoxic Experiment (A)	Hyperoxic	Normoxic	6	12	2	8.5	0.0140
Normoxic Control (B)	Normoxic	Normoxic	6	12	1	18.7	0.0088
Hypoxic Control (C)	Hypoxic	Normoxic	6	12	1	18.7	0.0088
Materials Control (D)	Materials only	Normoxic	6	12	1	18.7	0.0088
All Treatments except Hypoxic Experiment (E)	NA	NA	24	48	5	14.6	1.43e ⁻⁰⁵

Table 2 Results of binomial logistic regressions comparing the likelihood of *Mnemiopsis leidyi* associating with the Leafyi apparatus oxygen source under variable states of oxygenation (**A-D**). (**E**) Indicates comparison of Hypoxic experimental responses to all other treatments combined.