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Effects of the *Deepwater Horizon* Oil Spill on Pelagic Fish Species of the Gulf of Mexico

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UNIVERSITY OF MIAMI

EFFECTS OF THE *DEEPWATER HORIZON* OIL SPILL ON PELAGIC FISH SPECIES OF THE GULF OF MEXICO

By

John D. Stieglitz

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The *Deepwater Horizon* oil spill of 2010 is the largest unintended marine oil spill in history. The point-source location of the spill, below the pelagic zone of the northern Gulf of Mexico (GOM), resulted in distribution of crude oil throughout the water column in the open ocean pelagic environment as well as along near-shore coastal habitats. Additionally, unprecedented use of chemical dispersants at both the surface and at depth resulted in increased dissolution of the crude oil into the aqueous phase. Given the importance of regional finfish and shellfish fisheries in the GOM, much attention has been directed to quantifying the damage to these natural resources. Polycyclic aromatic hydrocarbons (PAHs) comprise some of the most toxic chemicals within crude oil. While a considerable amount of information exists on the effects of PAHs on aquatic invertebrate and vertebrate species, especially those in areas of previous oil spills such as Alaska following the *Exxon Valdez* oil spill, little is known regarding the effects of such toxins on tropical pelagic species. Pelagic fish species such as mahi-mahi (*Coryphaena hippurus*), tuna (*Thunnus spp.*), and cobia (*Rachycentron canadum*) can present unique challenges in captivity and laboratory settings, which has limited their inclusion in previous assessments of damage from marine oil spills. Results from this dissertation provide novel techniques and technology to successfully maintain and volitionally spawn cobia and mahi-mahi throughout the year, allowing for toxicity testing to be conducted on

such species on a continuous basis. Additionally, development of an innovative bioassay system for quantifying the effects of *DWH* crude oil on the early life-stages (ELSs) of these species allowed for testing of whether UV-radiation results in increased toxicity of *DWH* crude oil on mahi-mahi ELSs. Given the finding that photo-induced toxicity of *DWH* crude oil is over nine times that of the same crude oil under limited UV-radiation exposure, it is likely that many of the laboratory (i.e. limited UV-radiation) experiments quantifying toxicity of the spilled oil represent conservative estimates. In the same respect that UV-radiation is often left out of acute toxicity quantification efforts, the sub-lethal effects of crude oil exposure on adult life stage animals are also difficult to quantify and therefore poorly represented in damage assessment efforts. This dissertation provides evidence of *DWH* crude oil induced sub-lethal impacts to the swimming capacity and efficiency of young adult mahi-mahi at environmentally-relevant exposure levels. Overall, this dissertation provides novel insights into the effects of the *DWH* oil spill on economically and ecologically valuable pelagic fish species allowing for accurate quantification of the damages incurred by these important natural resources.

To my family, without your love and support this would not have been possible
...and especially to my wife and children.

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LEGAL DISCLAIMER

Data presented here are a subset of a larger toxicological database that is being generated as part of the *Deepwater Horizon* Natural Resource Damage Assessment, therefore, these data will be subject to additional analysis and interpretation which may include interpretation in the context of additional data not presented here.

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    - Chapter 4: *Submitted*
        - Stieglitz, J.D., Mager, E.M., Hoenig, R.H., Benetti, D.D., & Grosell, M. (2014) Impacts of *Deepwater Horizon* crude oil exposure on adult mahi-mahi (*Coryphaena hippurus*) swimming performance.
- RELATED RESEARCH (significant contribution during Ph.D. research):
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CHAPTER 1:

INTRODUCTION

OVERVIEW

The search for novel sources of oil and gas continues throughout the world, as energy providers face the challenge of delivering power to a growing human population. Current estimates indicate up to 85% of the world's population relies on energy derived from hydrocarbon resources (oil, coal, and natural gas)(Mills 2012). Given the long timescales necessary to generate these resources through natural processes, this hydrocarbon-based energy reliance has led to dwindling global supplies and higher hydrocarbon costs (Sorrell et al. 2010). In order to keep up with global demand, energy companies have targeted resource deposits deemed previously inaccessible, whether technologically or economically. As with any extractive method used to obtain natural resources, there are environmental impacts associated with hydrocarbon extraction, with certain methods having greater potential impacts than others. Of the most common forms of extraction, oil and gas drilling in oceanic habitats represents a method with the potential for large-scale environmental impacts due primarily to the hydrodynamic connectivity of the marine ecosystem (Cowen et al. 2007, Cowen and Sponaugle 2009), the difficulty in containing accidental hydrocarbon releases, and the challenging nature of drilling into the seafloor at great depths. Environmental impacts of offshore oil and gas drilling are not restricted to those associated with crude oil spills, as impacts may also occur from byproducts such as drilling muds, natural gas, and other compounds used or produced in the offshore drilling process (Boesch and Rabalais 1987, Holdway 2002). In order to understand the potential impacts of such compounds on the local flora and fauna

of the drilling location, toxicity tests may be conducted to quantify effect levels for different drilling compounds, notably the extracted crude oil. In terms of aquatic species, toxicity tests have traditionally used "model species" in acute and chronic toxicity bioassays (USEPA 2002a, 2002b). However, effect levels for such "model species" have been shown to vary significantly between organisms. Such variation amongst test species illustrates the importance of including local flora and fauna in toxicity testing to provide a more accurate assessment of the potential impacts of oil and gas extraction on specific habitats. While it is technically impossible to test every potential environmental stressor resulting from oil drilling on every species within the immediate drilling area, in order to understand the potential damage from releases of oil drilling byproducts in the offshore environment toxicity testing should likely include relevant species and life stages for the habitats that would encounter such pollution.

Amongst these byproducts, one of the most toxic groups of hydrocarbons, known as aromatics, is found in crude oil. Aromatics are compounds which contain a six-carbon atom benzene ring structure and they are distinguished from one another by the number of aromatic rings in their chemical structure, ranging from one to five (Rand 1995). Aromatics with two or more linked benzene rings are known as polycyclic, or polynuclear, aromatic hydrocarbons (PAHs), and there are more than 100 different PAHs, with crude oil typically comprised of complex mixtures of PAHs as opposed to a single PAH compound. These lipophilic petrogenic compounds are frequently divided into two different classifications: low molecular weight PAHs and high molecular weight PAHs, depending on the number of aromatic rings (Tuvikene 1995). PAHs having three or fewer rings represent the low-weight compounds and PAHs having more than three

aromatic rings represent the high-weight group. With an increase in molecular weight (i.e. rings) comes an increase in the lipophilicity, the potential for bioaccumulation, and an increase in the octanol/water partition coefficient (log K_{ow}). Traditionally an increased log K_{ow} has been linked to increased toxicity or carcinogenesis (Tuvikene 1995), though recent studies examining PAH effects on teleost embryos reveal that cardiotoxicity can be driven by both low and relatively high weight PAHs (Incardona et al. 2011, 2014). Of additional concern in the environment is the natural process of PAH weathering (i.e. a shift in PAH composition from high to low molecular weight compounds), as these higher molecular weight PAHs are also more ecologically persistent compared to the lower-weight PAHs (Rice et al. 2001).

Based on previous research resulting from oil spills throughout the world, notably the *Exxon Valdez* spill in Prince William Sound in 1989, it has been found that PAHs exhibit different levels and mechanisms of toxicity depending on a number of factors, most notably the type of oil and species. Specifically for the oil component: the weathering stage, chemical composition (i.e. PAH ratio or mixture), duration, and ambient environmental conditions of the oil exposure can all significantly impact the toxicity of the oil (Tuvikene 1995). As for the organism component: the species, life stage, trophic level, and life history traits of the organism will have important effects on the overall toxicity of the oil exposure. Given the numerous factors and costs involved in determining species-specific toxicity of specific types of crude oil, it generally takes an oil spill event to highlight the gaps in baseline eco-toxicological knowledge of a particular hydrocarbon extraction region such as the GOM.

OBJECTIVES

The lack of baseline knowledge on the effects of PAHs on subtropical pelagic fish found in the GOM came to light following the *Deepwater Horizon* (*DWH*) oil spill, lasting from April 20th to July 15th 2010. This is the largest marine oil spill ever to occur in the United States and second largest marine spill in the world, resulting in the release of over 4 million barrels of crude oil into the GOM (Camilli et al. 2010, Crone and Tolstoy 2010, McNutt et al. 2012). In addition, significant quantities of chemical dispersant (primarily Corexit 9500 and Corexit 9527) were used as part of the clean-up effort, with ~771,000 gallons of dispersants applied at depth near the wellhead while additional amounts were applied at the sea surface (Kujawinski et al. 2011). As previously mentioned, much of the toxicological information used to develop predictions of short and long-term physiological effects of this event come from eco-toxicology research using "classic" test organisms that are potentially unrepresentative of the economically important finfish species of the GOM. The aim of this dissertation research was to determine some of the physiological impacts of oil and dispersant exposure on different life stages of species representative of economically valuable GOM finfish. To date, it has been virtually impossible to conduct rigorous toxicity testing on such species as a result of the difficulty in obtaining suitable amounts of homogenous biological material (i.e. fish) at varying life stages for statistically sound toxicity tests to be conducted. One of the primary objectives of this research was to utilize advancements in marine finfish aquaculture to allow for site-specific and fishery-relevant species to be included in hydrocarbon toxicological bioassays. Specifically, the following questions were addressed:

A) How can GOM pelagic fish be captured and environmentally-induced to spawn volitionally year-round in captive conditions?

B) Will current LC50 bioassay methods and technology allow for effective crude oil toxicity testing of the early life-stages (ELSs) of GOM pelagic teleosts?

C) Does solar UV-radiation increase the toxicity of *DWH* crude oil, and what are the potential implications of this relationship?

D) What are the sub-lethal effects of *DWH* crude oil on young adult mahi-mahi (*Coryphaena hippurus*), a species representative of economically important GOM pelagic fish?

E) Over 4 years after the oil spill incident, what have we learned about the effects of the *DWH* incident on pelagic teleosts?

BACKGROUND AND SIGNIFICANCE

The oil and natural gas reserves of the GOM represent one of the nation's largest sources of hydrocarbon energy and account for approximately 30% of domestic hydrocarbon production (U.S. Energy Information Administration 2012). As part of the extraction of this valuable resource, in combination with hydrocarbon releases from natural oil seeps (>16,800,000 gallons $year^{-1}$ into the GOM), petroleum transportation, refining, and consumption, there is a significant amount of oil entering the GOM every year (National Research Council 2003). However, there is little attention paid to such "routine" releases of hydrocarbons, and it is the larger spills that garner the public's

attention when they occur. These incidents stand out as a result of the sheer quantity of oil released into the ecosystem over such a short timeframe. There are two spills which stand out as a result of the quantity of oil released into the GOM: the Ixtoc 1 spill of 1979 (3.33 million barrels of oil)(Patton et al. 1981) and the *Deepwater Horizon* spill of 2010 (> 4 million barrels of oil) (Camilli et al. 2010, Crone and Tolstoy 2010, McNutt et al. 2012). Due to the complexity of oil spill events, accurately assessing the ecological impacts of such incidents becomes a challenge (Teal and Howarth 1984). Such is the case when trying to compare the effects of the *DWH* spill with the Ixtoc 1 spill. Both spills occurred approximately 50 miles offshore in the Gulf of Mexico and resulted from the blowout of an underwater oil well. However, the water depth at the site of the Ixtoc 1 spill was 170 ft. while at the *DWH* spill site the damaged wellhead was at a water depth of approximately 5,000 ft. Similar cleanup and containment strategies were used in each spill, yet in the case of the *DWH* incident, mass quantities of chemical dispersants (Corexit) were also applied at depth to the source of the spill (Kujawinski et al. 2011), representing the first time in history that chemical dispersants had been applied under such conditions. Unfortunately, there was limited ecological impact research conducted following the Ixtoc 1 spill and therefore little was learned, in terms of ecological and biological impacts of crude oil on GOM pelagic fish.

Leading up to the *DWH* event, much of the knowledge base of ecological impacts of large oil spills in marine habitats stemmed from research resulting from the *Exxon Valdez* oil spill in 1989. This spill released approximately 11 million gallons of Alaskan North Slope crude oil into Alaskan coastal waters (Incardona et al. 2013), especially in the Prince William Sound area, and at the time represented the largest domestic marine

oil spill in history. Through research conducted in the wake of the *Exxon Valdez* spill came a greater understanding of PAH-induced effects on teleosts (Incardona et al. 2004, 2010, 2013). While such findings provided clues as to what might occur in the GOM following the *DWH* spill, there were key differences between the two spills. Specifically, the sheer quantity of oil released in the *DWH* incident was over 15x that of the *Exxon Valdez* oil spill, and the types of oil differed as well: Louisiana light crude vs. North Slope crude, respectively. Aside from the obvious difference in the mechanism of oil spill occurrence, *Exxon Valdez* ship grounding vs. Macondo-252 wellhead blowout, the environmental conditions (climate, water temperature, etc.) of each spill were markedly different. Notably, the temperatures differed significantly, with the *DWH* spill occurring in a deep subtropical sea (warm) and the *Exxon Valdez* spill occurring in Prince William Sound, a shallower sub-Arctic sheltered water body (cold). Additionally, the mitigation response differed, particularly in terms of chemical dispersant use. Any use of dispersants represents a "conscious decision to increase the hydrocarbon load (resulting from a spill) on one component of the ecosystem (e.g. the water column) while reducing the load on another (e.g. coastal wetland)"(National Research Council 2005). However, in the case of the *DWH* incident, where dispersants were applied at depth and on the sea surface (Kujawinski et al. 2011), this decision resulted in effectively entraining substantial hydrocarbon loads within the water column (Diercks et al. 2010, Wade et al. 2011), including significant amounts within the proposed study species' habitat: the pelagic zone of the GOM. While toxicity comparisons between the *Exxon Valdez* and *DWH* spills have recently been conducted indicating that ELSs of fish exhibit similar crude oil induced cardiotoxic response (Incardona et al. 2013), many knowledge gaps remain

concerning the specific effects of the *DWH* incident on different life stages of predatory pelagic teleosts in the GOM.

Previous research on the impacts of oil spills on teleosts provides some basis for assessing the impacts of the *DWH* event. As mentioned earlier, part of the toxicity related to oil stems from PAHs that leach out of the oil or oil-laden sediments and into the water column (Carls et al. 2008). For the following dissertation research, oil preparations were made to allow for toxicity testing of *DWH*-specific oils. These preparations consisted of the water accommodated fractions (WAFs) of crude oil, which are solutions that can be developed in a way to represent different oils at different stages of weathering, thereby allowing for toxicity testing of ecologically relevant compounds, concentrations, and weathering stages for each experiment. Indeed, the effects of PAHs on terrestrial and aquatic organisms have been well documented, largely as a result of some of the larger oil spills over the past few decades that were well studied in subsequent years. It is known that PAH exposure, both acute and chronic, induces a number of detrimental effects in fish. As a result of their lipophilicity, PAHs can easily penetrate the biological membranes in marine organisms by passive diffusion in cells, where the compounds are specifically bound to the cytosolic aryl-hydrocarbon receptor (AhR) (Stegeman and Hahn 1994, Tuvikene 1995). This passive uptake of PAHs leads to bioaccumulation in organisms, even in waters with low concentrations of PAHs (Tuvikene 1995). However, research suggests that fish and other vertebrates are able to metabolize PAHs through oxidation, reduction, hydrolysis, and conjugation reactions catalyzed by various enzymes which are primarily located in the liver and in some extrahepatic tissues, notably the kidney (Varanasi 1989, Stegeman and Lech 1991, Tuvikene 1995, D'Adamo et al. 1997,

Whitehead et al. 2011). The toxicity of PAHs is revealed following biotransformation to toxic metabolites through metabolic activation within the organism (Cavalieri and Rogan 1985, 1995, Varanasi and Stein 1991, Tuvikene 1995). PAH exposure is known to induce the formation of mixed function oxygenase in fish, leading to the biotransformation of the PAH compound (Stegeman and Lech 1991). The biotransformation occurs through a series of monooxygenase reactions catalyzed by the cytochrome P450 family of isoenzymes, specifically those which belong to the subfamily CYP1A, which are known to be induced by various PAH compounds (Stegeman and Lech 1991, Tuvikene 1995, Willett et al. 1997). Increasingly in ecotoxicology, induction of CYP1A protein expression, which is known as a hallmark of AhR signaling pathway activation, is being used as a sensitive biomarker for exposure to select planar PAHs and other hydrocarbons (Whitehead et al. 2011). Other commonly used biomarkers for PAH exposure include induction of 7-ethoxyresorufin-*O*-deethylase (EROD) activity, which is a dependent enzymatic reaction of the P450 family of hemoproteins, specifically CYP1A, that are responsible for catalyzing the oxidative metabolism of xenobiotics in marine animals (Varanasi et al. 1989, Willett et al. 1997). While PAHs have the potential to be carcinogenic, mutagenic, tumorigenic, teratogenic, and immunotoxic in vertebrates, these effects are often compounded by environmental conditions, such as the exposure to ultraviolet light, which has been shown to increase PAH toxicity by orders of magnitude (Ankley et al. 2003). The phototoxic properties of PAHs, especially in the more weathered or higher-molecular weight compounds, has been well documented in marine and freshwater organisms (Oris and Giesy Jr. 1986, 1987, Arfsten et al. 1996, Ankley et al. 1997, Barron et al. 2003, Lampi et al. 2006, Björn and Huovinen 2008, Incardona et

al. 2012b). Additionally, while the metabolism of xenobiotics in marine organisms frequently results in detoxification of the chemical, the problem is often reversed when it comes to the metabolism of PAHs, whereby the metabolites of the enzymatically transformed PAHs are more toxic than the parent compound (Varanasi 1989). Research conducted in the wake of the *Exxon Valdez* spill suggest a common syndrome of embryolarval PAH-induced toxicity across a number of teleost species, which is characterized by yolk sac and pericardial edema, curvature of the body axis, and jaw reductions (Marty et al. 1997, Carls et al. 1999, 2008, Heintz et al. 1999, Couillard 2002, Pollino and Holdway 2002, Incardona et al. 2004, 2005, 2013). Additionally, evidence suggests that measurement of CYP1A, and associated enzymatic activity (EROD), may not provide an accurate assessment of ecological damage or organism exposure to PAHs due to the protective, as opposed to causative, role of the CYP1A pathway in petrogenic PAH toxicity, particularly in terms of embryolarval exposure to weathered crude oil (Incardona et al. 2005). Such findings suggest that better tools need to be developed to assess PAH effects in aquatic ecosystems, and the previously conducted research underscores the need to conduct toxicity testing at different teleost life stages to better understand the ecotoxicological effects of open ocean oil spills.

Sub-lethal effects of xenobiotics in aquatic ecosystems have been associated with long term effects on fish stocks throughout the world (Bue et al. 1998, Heintz et al. 2000, Robinet and Feunteun 2002). However, such effects are extremely difficult to detect and quantify, especially in the case of PAHs distributed in the pelagic region of the open ocean, as in the case of the *DWH* incident. Much of the oil associated with this spill never reached coastlines, but was instead distributed throughout the water column, due in large

part to the use of chemical dispersants on both the surface and at depth during the spill (Kujawinski et al. 2011). Given the documented range of the spill, it is safe to assume that numerous pelagic marine organisms encountered PAH-contaminated waters. Of those encounters that were not fatal, the effects of sub-lethal exposure have been virtually impossible to identify by cursory examination of animals in their routine metabolic state. Indeed, one of the most sensitive endpoints of PAH toxicity is related to the cardiac system, in which performance and development are impaired as a result of sub-lethal PAH exposure (Incardona et al. 2004, 2010). Aside from direct observation of the heart operating under routine conditions, which is only possible for early embryonic stages, the sub-lethal cardiac impairment may be revealed through examination of the aerobic scope of the organism using swim chamber respirometry. The maximum aerobic swimming speed (U_{crit}) of an organism has been shown to be a very sensitive endpoint in toxicity testing (Plaut 2001, Mager and Grosell 2011, Tierney 2011, Mager et al. 2014), and in combination with the calculation of aerobic scope, this respirometric test allows for determination of whether the metabolic impairment was a loading stress (increased routine metabolic costs) or a limiting stress (reduced maximum rate of oxygen consumption) (Brett 1958, Wilson et al. 1994, Killen et al. 2007, Mager and Grosell 2011). These swim chamber respirometer tests can be performed on a variety of teleost life stages, depending on the fish size capacity of the swim chamber, and tests can be performed to examine the effects of both chronic and acute xenobiotic exposure. Additionally, such tests allow for cross-species comparisons to determine if different teleosts are more or less sensitive to the similar PAH exposures. Results are highly ecologically relevant, particularly for migratory pelagic species such as mahi-mahi and

tuna. These teleosts are known for maintaining elevated routine metabolic rates which are necessary to support the numerous metabolic demands of these high performance predators in the pelagic environment (Brill and Bushnell 1991, Benetti et al. 1995a, Brill 1996, Korsmeyer et al. 1996, Block and Stevens 2001). Additionally, these species have unique biochemical, physiological, and morphological attributes that allow them to support such high metabolic rates (large, thin gill surface areas, robust cardiovascular performance capabilities, elevated hemoglobin levels, endothermic adaptations, and numerous other "high-performance" attributes), yet these qualities may predispose them to a potentially greater intake of PAH contamination as a result of the known biochemical pathways of PAH toxicity in teleosts. Therefore, given what is known regarding the toxicity of PAHs and the differences in induction response to PAH exposure amongst fish species and life stages (Tuvikene 1995), a paucity of knowledge remains regarding the sub-lethal effects of *DWH*-specific PAH exposure on GOM-specific fish species.

In order to test the effects of the *DWH* oil spill on relevant GOM pelagic fish, it was necessary to obtain reliable supplies of test organisms with a known life history and homogenous size/age structure. Building upon research conducted at the University of Miami Experimental Hatchery (UMEH), consistent production methods were developed for the challenging pelagic fish species utilized in this research. In recent years, significant progress has been made in developing aquaculture techniques for test organisms similar or identical to those used in this study (Benetti et al. 2008a, 2008b, 2010b, Stieglitz et al. 2012b). However, in order to maintain the ability to conduct toxicity testing for the majority of the calendar year (Objective A), specialized techniques have been developed to obtain volitional spawns from the fish on a continuous basis

(Stieglitz et al. 2012a), details of which appear in Chapter 2. Additionally, advancements in broodstock capture and handling, as well as larval rearing, weaning, and juvenile growout, have been accomplished for species used in the following research that allowed for consistent production of test organisms. Through developments in marine aquaculture, it was possible to determine the effects of the *DWH* oil spill event on GOM pelagic fish species at different life stages. Additionally, novel technology and exposure techniques were developed to accurately assess the impacts (Objectives B and C), allowing for not only the characterization of the damage but also the quantification, as described in Chapter 3. Also, the sub-lethal effects of *DWH* crude oil on larger life stage pelagic fish were examined (Objective D), revealing important insights, detailed in Chapter 4, on the physiological impacts to young adult mahi-mahi. Quantifications of effects on pelagic fish resources may help resource managers determine the overall impact of the *DWH* oil spill. Furthermore, now that it is over four years since the spill, it's important to note what we have learned about the effects of the spill on pelagic teleosts in the GOM (Objective E) and where more research might be needed, as described in Chapter 5. The following research describes the development of innovative methods and technology that has allowed for the testing and reporting of quantifiable impacts for economically and ecologically valuable pelagic fish species of the GOM following the *DWH* oil spill of 2010.

CHAPTER 2:

DEVELOPMENT OF TECHNIQUES FOR YEAR-ROUND SPAWNING OF PELAGIC FISH IN CAPTIVITY[1]

SUMMARY

In order to be able to conduct crude oil toxicity bioassays and produce fish for use in experiments year-round, it was necessary to develop techniques to spawn fish in captivity throughout the year without compromising the quality of the offspring. While the focus of this chapter is on cobia (*Rachycentron canadum*), similar methods of environmental control have been recently applied to mahi-mahi (*Coryphaena hippurus*) broodstock yielding similar results (i.e. year-round volitional spawning in captivity). The development of these novel techniques for tropical and sub-tropical pelagic species allows for unprecedented availability of experimental animals throughout the year, thereby facilitating research looking into the effects of environmental impact events such as the *Deepwater Horizon* oil spill in the Gulf of Mexico.

Year-round control of the spawning cycle of cobia has been established by using water temperature manipulation. To compare the effectiveness of using this method to induce volitional spawning in cobia, two 80 m^3 recirculating aquaculture systems (RAS) were used. Temperatures in one of the maturation tanks ("Mat 1") were maintained between 27 – 29ºC for 12 months of the 15.5 month study period. Temperatures in the

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[1] *This chapter, in similar format and content, has been published. Citation:* Stieglitz, J.D., Benetti, D.D., Hoenig, R.H., Sardenberg, B., Welch, A.W., & Miralao, S. (2012) Environmentally conditioned, year-round volitional spawning of cobia (*Rachycentron canadum*) in broodstock maturation systems. *Aquaculture Research* 43(10), 1557-1566.

second maturation tank ("Mat 2") were allowed to fluctuate naturally throughout the year and ranged from 20 to 32 ºC. A total of 101 spawning events occurred in the tanks between the spring of 2008 and the summer of 2009 (April 3, 2008 to June 17, 2009). Of the 38 total spawning events in Mat 1, 17 of them (44.7% of all Mat 1 spawning events) occurred during the off-season (fall and winter). The egg viability rates did not differ significantly (P >0.05) between on and off-season spawns in Mat 1. Conversely, cobia broodstock exposed to natural water temperatures (no environmental manipulation) in Mat 2 followed the natural pattern of warm water (>26°C) dependence, limiting egg production to spring and summer seasons. This method of water temperature manipulation allows for effective control of the cobia reproductive cycle without compromising egg viability.

BACKGROUND

Cobia (*Rachycentron canadum*) are found throughout the world's tropical and subtropical seas, with rare occurrence in the eastern Pacific (Fowler 1944, Briggs 1960, Collette 1999) and no reported presence in the Mediterranean (Golani and Ben-Tuvia 1986). A migratory pelagic species, cobia has become one of the leading candidates for aquaculture development across its global distribution as a result of rapid growth rates, a limited wild fishery and excellent palatability (Liao et al. 2004, Benetti et al. 2008a, 2008b, 2010a). This species also possesses euryhaline life history traits that allow it to be cultured successfully at reduced salinities, permitting the expansion of culture operations inland (Atwood et al. 2004, Resley et al. 2006).

As with all aquaculture species, the ability to control the reproductive cycle to allow for year-round production of cobia juveniles is critical for the commercial success of this species (Arnold et al. 2002). Research on cobia reproduction indicates that this species has a protracted reproductive season that generally runs from about April to early October in U.S. waters (Arnold 1991, Biesiot et al. 1994, Lotz et al. 1996, Arnold et al. 2002, Kaiser and Holt 2005), however in regions that experience year-round water temperatures suitable for reproductive activity there have been reports of spontaneous year-round spawning (Liao et al. 2004). They are gonochoristic multiple spawners (Schaffer and Nakamura 1989, Arnold et al. 2002) and have been induced to spawn successfully in captivity using both hormonal stimulants and environmental conditioning methods (Arnold et al. 2002, Benetti et al. 2008a). Many species, both fresh and saltwater, have been successfully conditioned to spawn during months outside of their natural spawning seasons (off-season) through the use of hormonal stimulants or photothermal environmental manipulations (Roberts et al. 1978, Macquarrie et al. 1979, Rowan and Stone 1996, Benetti 1997, Tate and Helfrich 1998, Benetti et al. 2001, Migaud et al. 2004, Watanabe et al. 2006). Some have argued, however, that off-season spawns could result in reduced egg quality (Carrillo et al. 1989, Brooks et al. 1997, Penney et al. 2006) and/or damage to the reproductive systems of the broodstock from extending the natural spawning season. The establishment of a year-round spawning stock of cobia is dependent on meeting the nutritional and environmental requirements of the broodstock to ensure that any spawns obtained are of the highest quality (Brooks et al. 1997, Izquierdo et al. 2001, Mylonas and Zohar 2007). By developing successful off-season spawning methods for cobia, commercial operators would no longer be tied to

seasonal availability of seedstock, which has proven to be a significant industry bottleneck (Holt et al. 2007). This study presents the data and results of research conducted at the University of Miami Experimental Hatchery (UMEH) aimed at developing techniques for environmentally conditioned, year-round volitional spawning of cobia broodstock.

MATERIALS AND METHODS

The cobia broodstock used in this study were maintained at UMEH in two outdoor 80 m^3 recirculating maturation systems as described in Benetti et al. (2008a), referred to individually as maturation tank number 1 ("Mat 1") and maturation tank number 2 ("Mat 2"). The sole method of environmental manipulation used to condition cobia to spawn at UMEH was water temperature manipulation, i.e., natural photoperiod was maintained in both Mat 1 and Mat 2.

Between October 28, 2008 and April 2, 2009, the water temperature in Mat 1 was maintained above 26°C (Table 2.1). This temperature regime was maintained during the off-season (fall and winter) months when cobia would normally cease spawning and enter the refractory period. Temperature in the tank was maintained using a water heater/chiller unit (118,000 BTUs, AquaCal AutoPilot Inc., St. Petersburg, Florida, USA). In April 2009, after more than twelve months of maintaining a warm water temperature regime in Mat 1, the water temperature was lowered to a mean temperature of 22°C (±1°C), thereby mimicking an off-season water temperature regime for the majority of the remaining study period. Three days before the conclusion of the trial, the cool water (off-season) temperature regime in Mat 1 was reversed again, resulting in the water temperature rising

3°C over a short timeframe and remaining at that level until the conclusion of the study. Throughout the study period, the control tank, Mat 2, was not thermally controlled and water temperature was allowed to match the ambient water temperature in the region.

Table 2.1. Water temperature regimes and associated dates for the two maturation tanks (maturation tank 1 and maturation tank 2).

	Maturation Tank 1 (Temperature Controlled)	Maturation Tank 2 (Seasonal Temperatures)
Dates of On-Season Water Temperature Regime (> 26°C)	April 3, 2008 to April 2, 2009	April 3, 2008 to October 27, 2008 and April 3, 2009 to June 17, 2009
Dates of Off-Season Water Temperature Regime (< 26°C)	April 3, 2009 to June 17, 2009	October 28, 2008 to April 2, 2009
Study Period Duration	April 3, 2008 to June 17, 2009 (15.5 months)	

The study period encompassed the 15.5 months between April 3, 2008 and June 17, 2009. For the purposes of this study, the off-season was defined as the period in which the predominant ambient water temperature in Mat 2 was below 26°C and the on-season (spring and summer) was defined as the period in which the predominating ambient water temperature in Mat 2 was above 26°C. This corresponded to the following defined seasons: “On-season” from April 3, 2008 to October 27, 2008 and from April 3, 2009 to the end of the study period (June 17, 2009). “Off-season” was defined as October 28, 2008 to April 2, 2009. Throughout the entire study period water temperature, dissolved oxygen, and ammonia (TAN) were measured daily for both tanks.

Broodstock cobia of 10.5 kg average weight were maintained in systems at an average density of 0.75 – 1.2 kg/m^3. The average sex ratio in each tank was 1:1, but there were short periods of time during the study period where the sex ratio in Mat 2 was 1.4:1 females to males. The broodstock were fed 2% – 4% of their total biomass, once a day, six days per week using a diet of squid (*Loligo spp.*) and sardines (*Sardinella spp.*), with

an occasional addition of fresh pink shrimp (*Farfantepenaeus duorarum*). A dietary supplement comprising a commercially prepared marine finfish vitamin and mineral mix (Vitamin Pre-Mix, Aquafauna Bio-Marine, Inc., Hawthorne, California, USA), astaxanthin, and lecithin was packed into 1 g gelatin capsules and fed to the broodstock twice per week at a rate of 1% of the daily food ration. All cobia used in this study were F1 generation broodstock that were the progeny of two separate groups of wild cobia collected off the southeast coast of Florida and cross-bred in the summer of 2007. The fish selected as F1 generation broodstock were chosen for superior growth rates and morphological characteristics.

The 500-L egg collectors attached to each broodstock tank were monitored twice daily (dawn and dusk) for the presence of eggs. Additional visual monitoring of the fish would usually augur the occurrence of a spawn within 24-hours as signs of spawning (abdominal swelling, chasing behavior between males and females, and decreased feeding behavior) were usually evident as females ovulated and hydrated in the hours prior to every spawning event. Eggs were collected in the attached 500-L tanks that were fitted with flow-through PVC standpipes covered in 500 μm mesh and provided with light aeration. Using soft nylon nets, eggs were transferred from the egg collectors to 20-L buckets filled with UV-treated seawater of the same temperature and salinity as the collection tank. Using methods described in Benetti et al. (2008a), non-viable eggs were allowed to settle to the bottom where they were siphoned from the base of the 20-L buckets into 2-L beakers filled with 500-mL of seawater for volumetric measurement and then discarded. The floating, viable eggs were carefully transferred in small batches to 2-L beakers filled with 500-mL of seawater, where volumetric measurements were made.

The percentage viability was calculated as the ratio of viable eggs to the total number of eggs (i.e. viable plus non-viable). Following enumeration, viable eggs were transferred to two 1000-L incubators inside the hatchery. Each incubator was fitted with a 500 µm mesh standpipe and was supplied with filtered seawater at a temperature within 0.5°C of the water in the egg collecting tank. Eggs were stocked in the incubators at a density of 300 – 500 eggs/L and aeration was provided via an air-ring at the base of the standpipe. A prophylactic treatment of formalin (37% formaldehyde solution) was applied to the eggs at a dosage of 100 ppm for 1 hour. Supplemental oxygen was also supplied at a very low rate in the incubators to maintain dissolved oxygen levels at or above saturation levels (7 – 9 mg L^{-1} at 27 – 29^{o}C) throughout the incubation and hatching periods. Regular siphoning of the incubators and surface skimmers were used to reduce the buildup of chorions, detritus, and non-viable eggs. The following morning (12 – 14 hours post hatch) a 1-L beaker was used to collect random volumetric samples of larvae from the incubators and the larvae in each sample were counted. A minimum of three random samples were taken from each incubator and the mean of the larval counts was extrapolated to obtain the number of larvae in each 1000-L incubator. Hatch rates were calculated as the number of larvae present in the tank divided by the number of viable eggs stocked, and represented as a percentage.

In order to examine whether moon phase was correlated with spawning occurrence, the percentage of lunar illumination (U.S. Naval Observatory 2010) was graphed against the spawning data for Mat 1 over a one-year lunar cycle (March 2008 – March 2009). Moon phase, delineated as quarters (25% intervals) of lunar illumination ranging from 0% to 100%, and spawning events were compared using analysis of

variance (ANOVA) with differences considered significant when $P<0.05$. The on- and off-season viability rates were also compared using ANOVA. The eggs and larvae produced over the duration of this study were used in successful larval rearing trials, transferred to other research institutions, sold to private companies, or discarded. All values reported represent mean values (± standard deviation) unless otherwise noted.

RESULTS

Between April 3, 2008 and June 17, 2009 a total of 38 and 63 volitional spawning events occurred in Mat 1 and Mat 2, respectively. Total viable egg production resulting from these spawning events over the study period was calculated to be 39,199,251 and 94,751,481 viable eggs in Mat 1 and Mat 2, respectively, resulting in a combined production of over 133 million viable eggs. Results from both tanks are summarized in Table 2.2. Of this total number of spawning events, 17 of them (44.7% of all Mat 1 spawning events) occurred in Mat 1 during the off-season between October 28, 2008 and April 2, 2009. A spawning event was considered to be any day in which any number of fish spawned in a maturation tank. The spawning temperature in both tanks, measured as the water temperature in the tank on the day of a spawning event, ranged from 21.0°C to 31.0°C. The spawn which occurred at the low temperature range (21.0°C) occurred in Mat 1 and was the one outlying spawning event (as seen in May 2009, Fig. 2.1) which occurred after two females from a warm water (ambient temperature) holding tank were added to Mat 1 in early May 2009. One of the females subsequently spawned within a week of the transfer under the Mat 1 reduced temperature regime. Data collected from this spawning event indicated that it was a poor event (164,000 viable eggs and 59%

viability rate) in comparison to average spawns. Precluding this outlying event, spawning temperature ranged from 23.8°C to 31.0°C and averaged 28.1°C. There was no significant difference in spawning temperature between tanks (P>0.05).

Table 2.2. Summary of results form the two maturation tanks (maturation tank 1 and maturation tank 2) over the 15.5 month duration of the study period (April 2008 – mid-June 2009). Values are presented as mean ± SD.

	Maturation Tank 1 (Temperature Controlled)	Maturation Tank 2 (Seasonal Temperatures)
Number of Spawning Events[a]	38	63[a]
Number of Spawning Events (On-Season)[a]	21	63[a]
Number of Spawning Events (Off-Season)	17	0
Study Period Water Temperature °C (Mean ± SD)	26.6 ± 3.1	25.7 ± 3.1
On-Season Spawning Water Temperature °C (Mean ± SD)	27.9 ± 2.3	28.6 ± 1.4
Off-Season Spawning Water Temperature °C (Mean ± SD)	28.2 ± 1.8	N/A
mL of eggs per spawning event (Mean ± SD)[b]	2,284 ± 1,033	3,003[b] ± 2,193[b]
Total mL of eggs over 15.5 month study	77,665	183,159
Number of eggs mL^{-1} (Mean ± SD)	505 ± 86	517 ± 86
Number of eggs per spawning event (Mean ± SD)[b]	1,152,919 ± 521,493	1,553,303[b] ± 1,134,490[b]
Total number of eggs over 15.5 month study period	39,199,251	94,751,481
Viability rate % (Mean ± SD)	90.5 ± 11.6	90.9 ± 12.0
Hatch rate % (Mean ± SD)	75.8 ± 22.4	86.4 ± 10.4

[a] Indicates spawning events and does not account for multiple-female spawning events, which if counted, would bring the total number of Mat 2 spawns to at least 79.
[b] Number and mL of eggs per spawning event in Mat 2 reflects the multiple-female events, serving to increase the mean number and mL of eggs per event (See Results and Discussion).

The mean number of viable eggs per mL was 505 (± 86) mL^{-1} and 517 (± 86) mL^{-1} for Mat 1 and Mat 2 respectively with no significant difference between tanks (P>0.05)(Table 2.2). Mean spawning viability rates for Mat 1 and Mat 2 were 90.5% (± 11.6%) and 90.9% (± 12.0%) respectively, and the difference between Mat 1 on-season and Mat 2 on-season viability rates was not significant (P>0.05). Off-season Mat 1 viability rates averaged 89.5% (± 11.6%), but the difference in Mat 1 on-season and off-season rates was not significant (P>0.05). Hatch rates of fertilized eggs produced from Mat 1 and Mat 2 during the 15.5 month study period were 75.8% (± 22.4%) and 86.4% (± 10.4%) respectively, though the difference between tanks was not significantly different (P>0.05).

Spawning events in both tanks followed a pattern that was strongly related to water temperature (Fig. 2.1 and Fig. 2.2). In Mat 1, fish continued to spawn as long as the water temperature was kept above 27^{o}C. However, several exceptionally strong cold fronts resulted in occasional fluctuations that decreased water temperature below the desired level. These drops in water temperature were short in duration (< 2 – 3 days) and water temperature was rapidly restored to the desired spawning temperature (≥ 27°C) at which point spawning events resumed. Following the temperature induced off-season spawning in Mat 1, at the beginning of the natural spawning season for cobia in 2009, i.e. April 2009, the temperature regime in Mat 1 was reversed, with water temperatures intentionally lowered to a mean temperature of 22.0°C (± 1.0°C) for the majority of the remaining study period (Fig. 2.1). This reduced temperature regime effectively halted spawning activity in the Mat 1 broodstock, with the exception of the one outlying event previously discussed. Three days prior to the conclusion of the study, the Mat 1 temperature regime was reversed again, resulting in a water temperature increase of 3°C over a short timeframe, which was enough of a stimulant to induce volitional spawning events (Fig. 2.1).

The spawning events that took place in Mat 2 (Fig. 2.2) followed a seasonal pattern also strongly correlated to temperature, with spawns occurring only throughout the on-season. During the off-season, no spawning activity occurred in Mat 2. The following spring, as soon as the temperature in Mat 2 reached 26°C, pre-spawning behavior was observed, and the first spawn of 2009 occurred on April 3, 2009 (Fig. 2.2).

Figure 2.1. Maturation tank #1 (Mat 1) spawning events over the 15.5 month study period. Note the steady occurrence of spawning events in the off-season months from October to April.

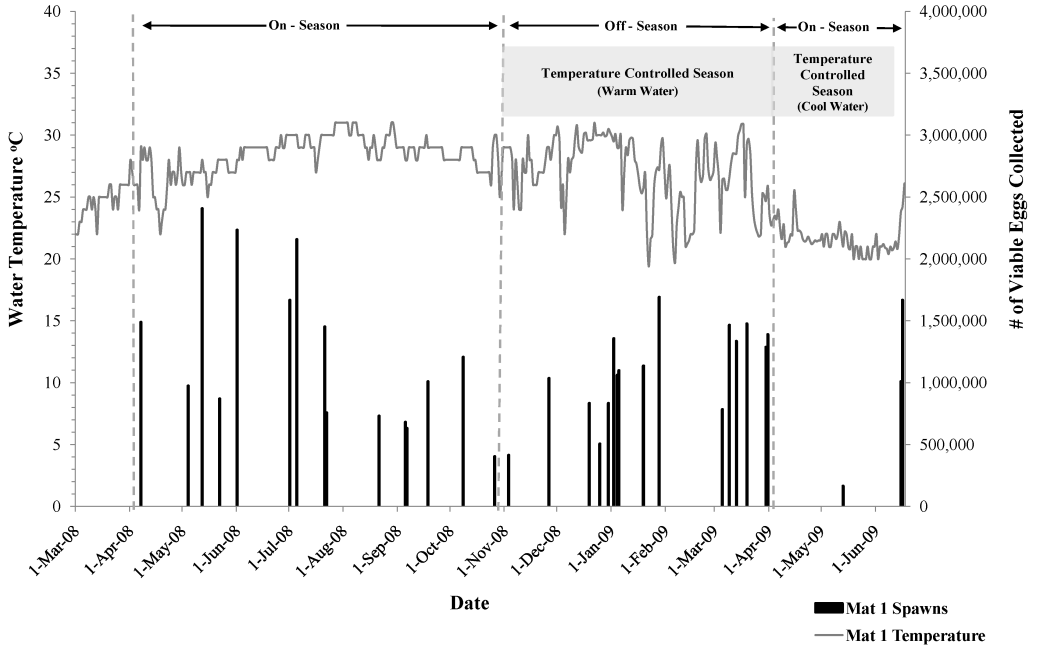
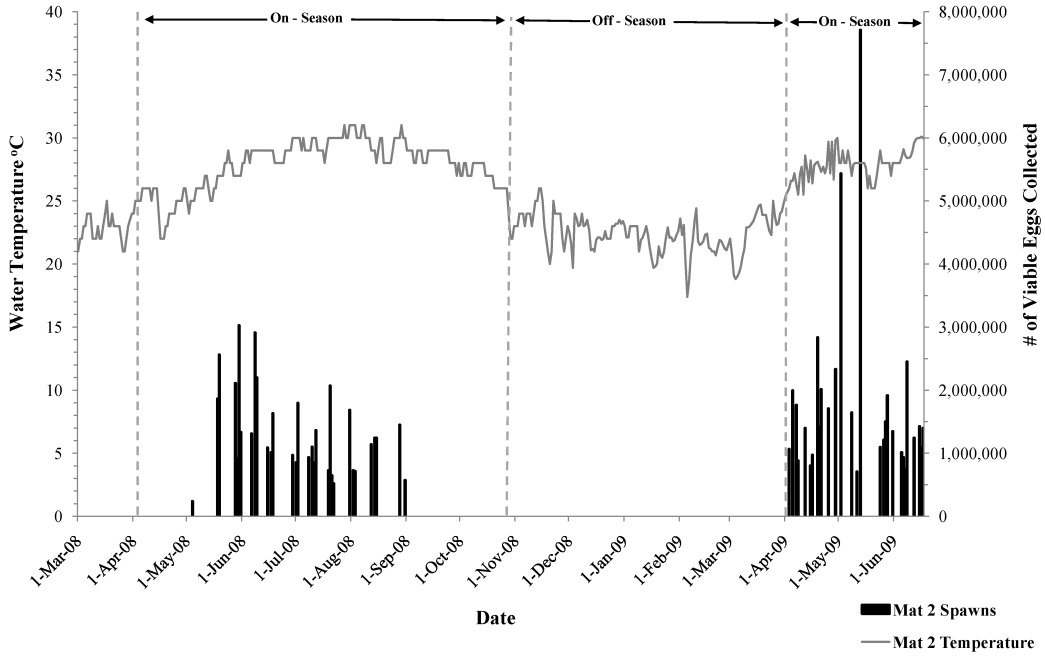


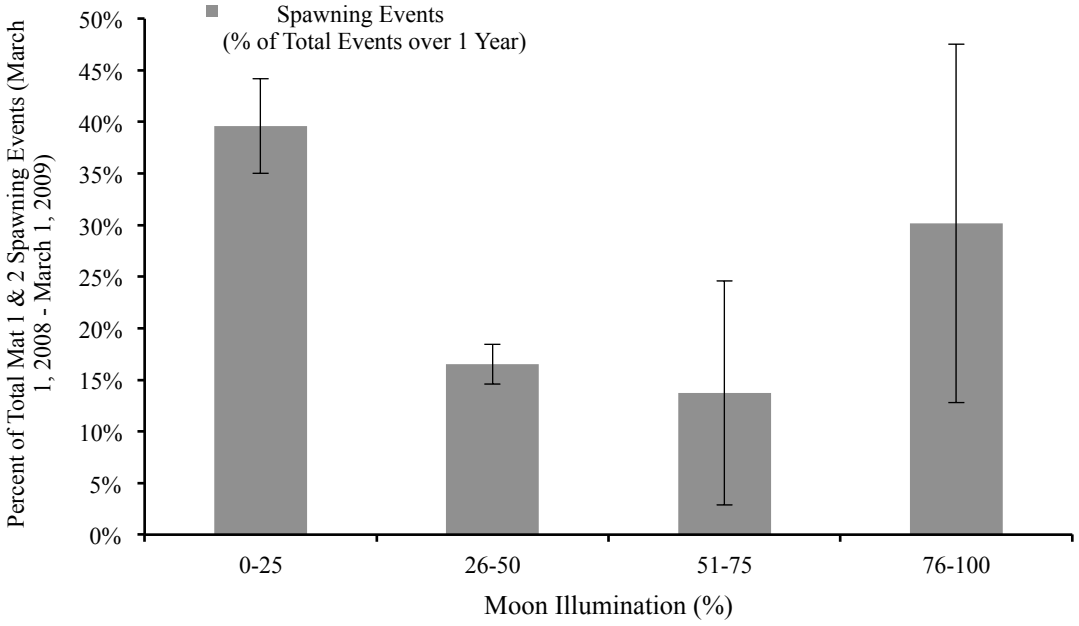
Figure 2.2. Maturation tank #2 (Mat 2) spawning events over the 15.5-month study period. Note the natural spawning cycle illustrated in the graph as opposed to the off-season pattern exhibited in Fig. 2.1, as well as the increased magnitude of spawns resulting from the multiple-female spawning events that took place in Mat 2.



As previously mentioned, courtship and pre-spawning behavior was routinely observed in tanks during the daylight hours preceding a spawning event. It was frequently possible to determine which female was undergoing ovulation and hydration, as the ventral region of their bodies would exhibit significant and clearly visible swelling prior to release of oocytes. There were at least 16 instances in which multiple female cobia in Mat 2 were observed to be exhibiting signs of spawning and resulting egg counts the following morning corroborated that multiple fish had spawned. However, it was not possible to trace oocytes to specific females in the tank, making it impossible to determine the exact number of eggs spawned per individual female cobia. This also meant that the number of times individual fish spawned in Mat 2 was higher than the 63 reported spawning events. Given the 16 confirmed instances in which multiple females spawned on a single day in Mat 2, the number of times individual fish spawned in Mat 2 was at least 79. The repeated occurrence of multiple females participating in a spawning event in Mat 2 resulted in the mean number of eggs per spawning event (1,553,303 ± 1,134,490 eggs per event) for this tank to appear greater than the number of eggs per Mat 1 spawning event (1,152,919 ± 521,493 eggs per event) (Table 2.2). Despite similar stocking densities in both Mat 1 and Mat 2, there did not appear to be any instances of multiple female spawning events in Mat 1 during the study period, though a plausible explanation is presented (Discussion section).

There appeared to be no relationship between spawning events and moon phase. The mean lunar illumination on evenings of spawning events was 46% (± 33%) illumination in Mat 1 and 55% (± 37%) illumination in Mat 2 (Fig. 2.3), with no significant difference between spawning events and moon phases ($P>0.05$).

Figure 2.3. Moon phase correlation with spawning events over a 1-year timeframe (March 2008 – March 2009). Spawning in both tanks took place during multiple phases of the moon throughout the on- and off-seasons and was not significantly correlated with any particular moon phase.



DISCUSSION

The results of this study demonstrate that off-season spawning of cobia is possible using only water temperature manipulation. Similar results have been demonstrated for other marine species, such as red drum (*Sciaenops ocellatus*) (Arnold 1988) and Nassau grouper (*Epinephelus striatus*) (Tucker et al. 1996), and the results support reports of cobia spawning spontaneously year-round in areas that experience water temperatures suitable for continuous reproductive activity (Liao et al. 2004). This study differs from previous reports of induced spawning of cobia, in that water temperature manipulation was the only method used to induce reproductive activity, as opposed to the use of

photoperiod and/or hormonal induction (Franks et al. 2001, Arnold et al. 2002, Kilduff et al. 2002).

Highlighting the strong correlation between water temperature and spawning success is the fact that the spawning event that occurred in Mat 1 at 21°C during the reduced temperature regime (April 3, 2009 – June 14, 2009) had the lowest overall egg production and lowest viability rate of all events reported in this study. The poor results were likely due to the reduced reproductive activity of the broodstock maintained under this off-season temperature regime. The outlying spawn was also most likely a result of the transfer of the two female fish that were in peak reproductive condition (i.e. previously held in a warm water temperature regime) to the maturation tank that was being maintained at lower temperatures. Given that the males were being held at temperatures below those which would trigger reproductive activity and maturation, it is likely that this could also account for the low viability rates and low numbers of viable eggs collected. Other than this singular outlying spawning event, the reduced temperature regime was successful in terminating the fish's reproductive activity, indicating that temperature manipulation can be used to effectively control spawning in this species.

Interestingly, the reversal of the cool water (off-season) temperature regime in Mat 1 (April 2009 – mid-June 2009) which resulted in warm water (on-season) temperatures induced the cobia to spawn volitionally and illustrated how effective temperature manipulation is for inducing broodstock cobia to spawn. Other pelagic marine teleost species maintained in captivity have exhibited similar spawning dependence on water temperatures, with continuous spawning occurring above a minimal

water temperature threshold (Benetti 1997, Margulies et al. 2007). As with cobia, both Pacific yellowtail (*Seriola mazatlana*) and yellowfin tuna (*Thunnus albacares*) appear to be keenly aware of surrounding water temperatures and can rapidly induce hydration and spawning when provided with the proper environmental cues (Benetti 1997, Margulies et al. 2007). The opposite effect was also illustrated in this study, as strong seasonal cold fronts cooled the water temperatures below optimal spawning temperatures (≥27°C) for short periods of time (2 – 3 days), resulting in a cessation of spawning activity during the period of cooler temperatures, followed by immediate resumption of spawning once water temperatures climbed back to optimal spawning temperatures. This illustrates how slight changes in water temperature over relatively short timeframes, on the order of days, can be effective in inducing or ceasing spawning behavior in certain marine teleost species, underscoring the importance of maintaining temperature control in marine fish hatcheries for efficient broodstock management to maintain year-round fingerling production.

Spawning activity did not appear to be dependent on photoperiod in this study. During the on-season in Mat 1, fish spawned every 14.4 days on average. During the off-season, the cobia in Mat 1 spawned every 9.2 days on average. These spawning frequencies are consistent with those reported for wild cobia in the southern United States (Brown-Peterson et al. 2001). Had photoperiod been a limiting factor the fish would not have continued their spawning activity in the off-season or the number of spawns would have been significantly less frequent in occurrence, despite the manipulation of water temperature. This is possibly due to the fact that for most tropical species water temperature is a more important exogenous trigger for maturation and gametogenesis

than photoperiod, as tropical species do not experience the significant shifts in day length that occur at higher latitudes and are therefore not as reproductively dependent on photoperiod spawning cues (Lam 1983, Harvey and Carolsfeld 1993). Similarly, although numerous pelagic teleosts possess spawning cycles that are tied to moon phases (Johannes 1978) and anecdotal evidence seems to support this trend for wild cobia (Benetti et al. 2008a), moon phase did not appear to be correlated with spawning of cobia in this study, as spawning events occurred year-round throughout the lunar cycle (Fig. 2.3). However, all broodstock used in this study were F1 generation fish, so the lack of lunar phase effect on spawning might be limited to captive-bred broodstock.

There appeared to be no adverse effects on gamete quality of the year-round cobia spawning as there were no significant decreases in egg production per spawning event or viability rates. Mean values for off-season hatch rates were formed from smaller sample sizes, as not all eggs produced in the off-season were stocked to obtain hatch rates. Previous unpublished trials conducted by the authors suggest that acute cold shock to cobia eggs has a negative impact on the survival and hatch rate of viable cobia eggs. Therefore, the eggs produced from off-season spawns were only stocked in the UMEH incubators when ambient water temperatures were above 23°C and only after the eggs had been slowly acclimated (roughly 30 minutes of acclimation for each 0.5°C decrease in temperature) to the water temperature of the flow-through incubators. When acclimated in this manner, the off-season eggs had hatch rates exceeding 85%.

Contrary to the expectation that year-round spawning in Mat 1 would have resulted in a greater number of spawns from this tank as compared to Mat 2, there were in fact more recorded spawning events in Mat 2 during the study period. It is likely that the

periods of time in Mat 2 in which the sex ratio was 1.4:1 females to males accounted for the greater number of spawns and the occurrence of multiple-female spawning events in Mat 2. Previous unpublished trials by the authors support this assumption, as experimental stocking of tanks with broodstock sex ratios of 2:1 females to males has resulted in more frequent spawning events than when stocking at sex ratios of 1:1 females to males. Furthermore, prior to the temperature induced extension of the Mat 1 spawning season, the Mat 2 broodstock were spawning more frequently than those in Mat 1, thereby supporting the idea that sex ratios of increased females to males results in greater spawning frequency amongst this species in captivity.

One of the keys to maintaining consistent spawning and egg production is the use of a diet that is sufficient to fuel continued reproductive activity beyond the traditional spawning season (Izquierdo et al. 2001). Since captive fish are not subject to the same energetic demands as wild fish (i.e. foraging, migrating, avoiding predators, etc.), sustained year-round spawning in some marine fish species is possible given the use of proper diet and environmental conditions (Arnold 1988). Our results suggest that the diet and environmental conditions provided to the cobia during this study were sufficient to allow for successful, high-quality year-round spawning of this species in captivity, ensuring continuous supply of eggs, larvae and fingerlings for research and commercial purposes.

CONCLUSION

Though there have been reports of year-round spawning success with cobia in southeast Asia (Liao et al. 2004), this study represents, to the best of our knowledge, the

first time cobia have been environmentally-induced to spawn volitionally throughout the year in the Western Hemisphere. These techniques could allow commercial cobia hatcheries to operate year-round, thereby eliminating seasonal restrictions on fingerling production. The correlation of water temperature manipulation and off-season spawning obviates the need for commercial hatcheries to invest and install specialized lighting systems for photoperiod manipulation of cobia spawning cycles, as has been used when attempting to obtain off-season spawns of other species (Carlson 1973, Roberts et al. 1978, Macquarrie et al. 1979, Arnold 1991, Tate and Helfrich 1998, Watanabe et al. 1998, 2006). Additionally, this study presents methods that can be used to cycle multiple populations of broodstock "on" and "off" throughout the year to allow reproductive resting periods for broodstock while maintaining year-round production of viable eggs. Though the physiological effects of an extended reproductive season on cobia are unknown and there did not appear to be any negative effects in this study, it is expected that utilization of a rotational spawning schedule for separate groups of broodstock fish would be beneficial over extended periods of time.

The development of year-round spawning techniques described in this study have also been successfully applied to captive mahi-mahi, both wild and F1 generation broodstock, with results similar to those reported for cobia. While mahi-mahi spawn fewer eggs per spawning event, the females are able to spawn every other day in captivity while the males can spawn daily. Due to the aggressive nature of mature male, i.e. "bull", mahi in captivity, the optimal sex ratio for this species is 2 – 4 females: 1 male in each tank. Using water temperature manipulation and optimal nutritional management, it has been possible to spawn mahi-mahi in captivity every week of the year regardless of

outside weather conditions. Such consistent spawning allows for use of this species as a pelagic teleost model organism that can be used in the damage assessment process of open ocean pollution events such as the *Deepwater Horizon* oil spill of 2010.

ACKNOWLEDGMENT

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CHAPTER 3:

A NOVEL SYSTEM FOR EMBRYO-LARVAL TOXICITY TESTING OF PELAGIC FISH: PHOTO-INDUCED TOXICITY OF *DEEPWATER HORIZON* CRUDE OIL

SUMMARY

Key differences in the developmental process of pelagic fish embryos, in comparison to embryos of standard test fish species, present challenges to obtaining sufficient control survival needed to successfully perform traditional 96-hr bioassays. A novel exposure system, the pelagic embryo-larval exposure chamber (PELEC), has been developed to conduct successful bioassays on the early life stages (ELSs; embryos/larvae) of pelagic fish. Using this system, it was possible to significantly improve control survival in pelagic fish ELS bioassays compared to commonly used static exposure methods. Results demonstrate that control performance of mahi-mahi (*Coryphaena hippurus*) embryos in the PELEC system (89.8% ± 2.12), measured as percent survival after 96-hrs, tended to outperform agitated static exposure (76.8% ± 4.49) and outperformed static exposure (67.5% ± 4.79) systems ($P<0.001$). The PELEC system was subsequently used to test the effects of photo-induced toxicity of crude oil on mahi-mahi ELSs. Results indicate a significant increase in toxicity of *Deepwater Horizon* (*DWH*) crude oil (LC50 = 0.7 μg L^{-1} ΣPAH[50]) during co-exposure to ambient sunlight compared to filtered ambient sunlight (LC50 = 6.5 μg L^{-1} ΣPAH[50]).

BACKGROUND

Recent research following the BP *Deepwater Horizon* (*DWH*) oil spill of 2010 has reported the potential for specific physiological impacts, notably cardiac impairment and reductions in swim performance, resulting from crude oil exposure (Brette et al. 2014, Incardona et al. 2014, Mager et al. 2014). One goal of the injury assessment is to quantify the effect of the spill on economically and ecologically important teleost fish species in the Gulf of Mexico (GOM) (McCrea-Strub et al. 2011, Sumaila et al. 2012). However, quantifying toxicity to resident, non-model species following such events using commonly accepted toxicological tests (96-hr bioassays) and endpoints (i.e. LC50) faces many challenges resulting primarily from the difficulty in obtaining and working with such species in a controlled environment.

A plethora of studies have documented the effects of crude oil exposure on a variety of marine biota, with research on teleost early life stages (ELSs; embryos/larvae) revealing a host of common lethal and sub-lethal effects. Many of these outcomes appear to be the result of cardiotoxic effects resulting from acute exposure to polycyclic aromatic hydrocarbons (PAHs) in crude oil during embryonic development (Carls et al. 1999, 2008, Heintz et al. 1999, Couillard 2002, Incardona et al. 2004, 2014, Hicken et al. 2011). Specifically, acute PAH exposure in teleost ELSs has been shown to induce defects in cardiac function, pericardial and yolk sac edema, neurodevelopmental abnormalities, jaw deformations, and other defects during morphogenesis (Incardona et al. 2004, 2011, 2013, 2014, Carls et al. 2008, Irie et al. 2011, de Soysa et al. 2012). While teleost ELSs are putatively the most sensitive fish life stage to crude oil exposure, the scientific literature suggests that effect thresholds vary significantly between species and

crude oil compositions (Incardona et al. 2014). Due to these differences, it is imperative to conduct toxicity tests replicating the likely exposure scenarios encountered by native, pelagic teleost ELSs in the GOM during the *DWH* spill event. To date, *DWH*-specific toxicity research has revealed numerous sub-lethal effects of crude oil exposure that occur at low- and sub- part per billion total PAH concentrations ($\mu g\ L^{-1}$ ΣPAH) in seawater, yet there are no published reports of lethal exposure thresholds for high-value pelagic teleosts, such as mahi-mahi (Brette et al. 2014, Incardona et al. 2014, Mager et al. 2014). This study aims to produce the first reported lethal effect thresholds for such species, while resolving the challenges surrounding incorporation of these animals in crude oil acute toxicity testing.

Marine natural resource impact assessment research in the United States frequently utilizes traditional toxicological testing methods, such as those published by the American Society for Testing and Materials (ASTM) and the United States Environmental Protection Agency (USEPA) (USEPA 2002a, ASTM - E47 Committee 2005). The use of these prescribed procedures facilitates comparisons among other relevant studies and allows for regulatory applications. This is particularly important for determination of the biological effects of an impact event, whereby acute toxicity tests provide an estimation of the pollutant's toxicity using a commonly accepted metric, such as the median lethal concentration (LC50). Of central importance to such tests is the ability to attain acceptably high levels of survival in control treatments as defined in the protocols used in order to provide a baseline against which treatment effects can be accurately measured. Moreover, control survival of a test is one measure of test quality that provides evidence of organism vigor, physiological quality, suitability of the

treatment chambers, and overall test conditions (ASTM - E47 Committee 2005), particularly in the absence of published ASTM guidelines for a specific species and/or lifestage (e.g., pelagic fish embryos). In combination with a necessity to maintain high control survival is a need for treatment chambers to contain sufficient numbers of test animals (*n*) to allow for high power statistical analysis of the results. An additional benefit to a higher *n* per replicated treatment chamber is the ability to harvest sufficient numbers of test animals from replicates for use in post-hoc analyses such as morphometric imaging, immunohistochemical analysis, and ecotoxicogenomics (Snape et al. 2004). Accurate determination of the effects of acute and chronic environmental impact events on GOM representative species is aided by robust experimental design incorporating such analyses.

Due to the fact that the pelagic waters of the GOM represent an important spawning site for numerous tropical and subtropical marine finfish species, it is likely that pelagic marine fish embryos, specifically those species of high economic value to the commercial and recreational fishing industries, such as mahi-mahi (*Coryphaena hippurus*) and yellowfin tuna (*Thunnus albacares*), are impacted by environmental perturbations which occur in their habitat (Diaz and Rosenberg 2008, Fabry et al. 2008, Riebesell and Tortell 2011, Muhling et al. 2012). Recent efforts to quantify such impacts employed standard toxicity test methods using stationary, covered 1 L beakers as the replicate treatment chambers (Incardona et al. 2014). Although this approach was successful, obtaining reasonably high control survival was often a challenge, sometimes necessitating repeated tests. This approach was later modified by adding agitation (~ 60 lateral oscillations per minute) using a reciprocal shaker (Eberbach Model 6000 Mid-

Range Reciprocal Shaker) that resulted in marginal improvement to control survival (Incardona et al. 2014). Thus, in order to obtain high control survival during acute toxicological bioassays, a novel exposure system, the pelagic embryo-larval exposure chamber (PELEC), was designed and assessed for control survival using mahi-mahi, or dolphin fish, a GOM-representative pelagic teleost species.

The *DWH* oil spill overlapped temporally and geographically with the spawning seasons and grounds of numerous pelagic fish species (Palko et al. 1982, Collette 2010, Muhling et al. 2012). These species have pelagic egg and larval stages that serve to position ELSs in surface waters of the GOM (Johnson 1978, Palko et al. 1982, Ditty et al. 1994). Additionally, the pelagic zone of the GOM receives significant ultraviolet (UV) radiation penetration, particularly in summer months and recent years (Whitehead et al. 2000, Tedetti and Sempéré 2006). The photo-induced toxicity of crude oil during co-exposure to UV-radiation has been shown to dramatically decrease the LC50 of PAHs to many aquatic species (Pelletier et al. 1997, Little et al. 2000, Barron et al. 2003). Therefore, it was hypothesized that the impact of the *DWH* oil spill on the ELSs of pelagic fish species, such as mahi-mahi, may be underestimated using traditional laboratory bioassays that do not incorporate exposure to levels of UV-radiation present in natural sunlight. Consequently, following control performance testing of the novel PELEC system, the system was used to assess the effects of *DWH* crude oil exposure both with and without the addition of UV-radiation on survival of ELS mahi-mahi to determine potential photo-induced toxicity of *DWH* crude oil to an economically and ecologically valuable pelagic finfish species of the GOM.

MATERIALS AND METHODS

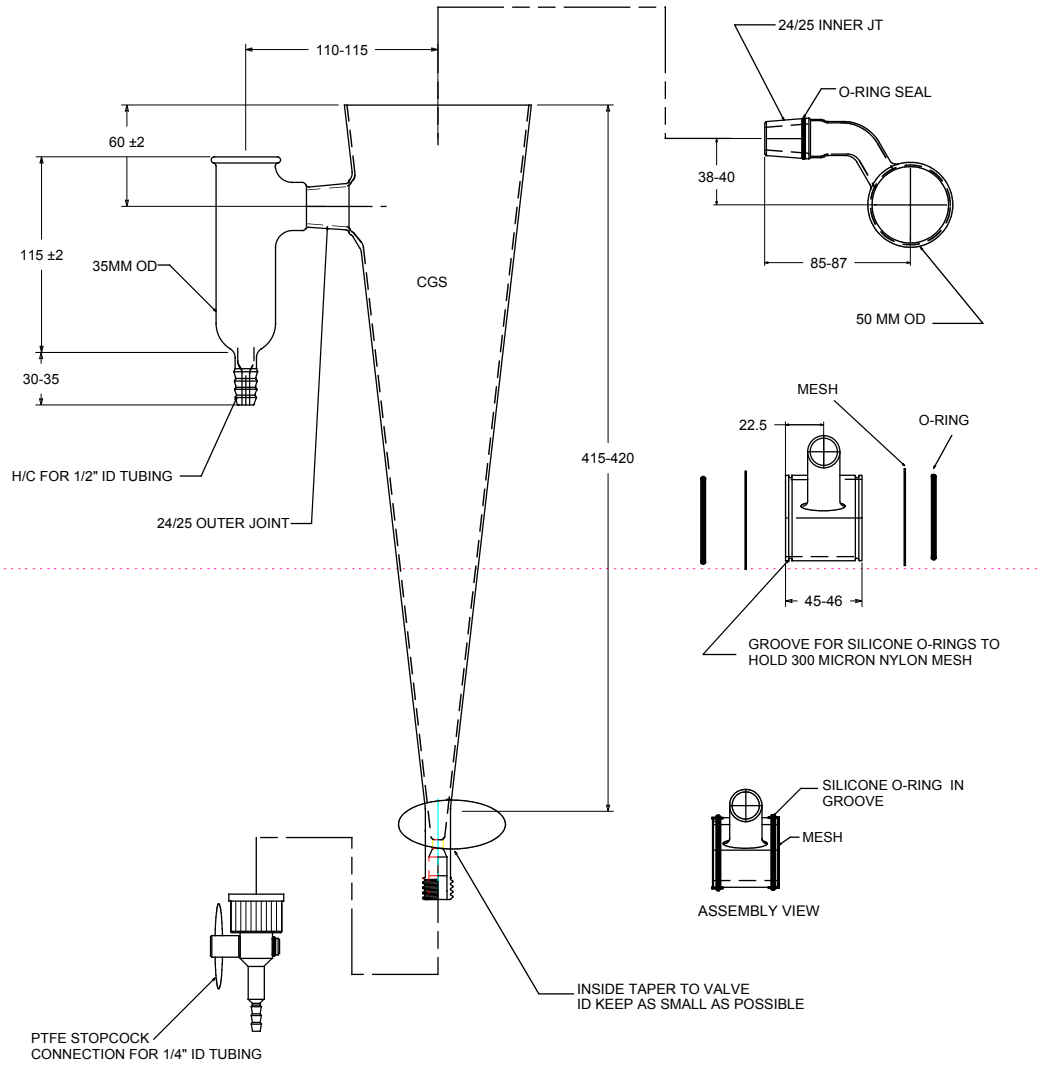
Control Testing of PELEC System. 96-hr survival tests were conducted to compare control performance using the two previously utilized methods (i.e., static and agitated beakers) and the newly developed PELEC system. Mahi-mahi embryos were obtained from captive volitionally spawning broodstock maintained in 80,000 liter seawater tanks, at the University of Miami Experimental Hatchery (UMEH) on Virginia Key, Florida, USA. Embryos were collected the morning of a spawn by removing the eggs from the egg collector attached to the broodstock tank and placing them in a bucket containing 15-L of filtered and UV-sterilized seawater at a density of 300-500 eggs per liter. Embryo collection and handling methods followed standard UMEH protocols, as described by Stieglitz et al. (2012a). Embryos were then placed in a beaker of filtered/UV-sterilized seawater and transferred to the University of Miami environmental chamber (set to 27°C) for bioassay set-up. A Leica Zoom2000 stereoscope at 45x magnification was used to assess embryo quality and for counting purposes.

Four replicates were used in each of the 96-hr control survival tests (static, agitated static, or PELEC system), and each test was carried out three separate times using a new batch of embryos for each test (total *n* of 12 per exposure system). 1 L glass beakers were used for the static and agitated static exposures. For the static agitation treatment, beakers were placed on a tray attached to a reciprocal shaker (Eberbach Model 6000 Mid Range Reciprocal Shaker) operated continuously at a low speed (~ 60 oscillations per minute) during each test. The PELEC system is comprised of a custom designed conical glass vessel (Kimble Chase, Rockwood, TN), similar to an Imhoff cone used in water quality testing (American Public Health Association et al. 1994), coupled

with a 1 L glass beaker. The total volume of each PELEC unit (cone + beaker) is 1.8 L and the dimensions of the cone are shown in Figure 3.1. Each cone was outfitted with an overflow spout for draining into the 1 L glass beaker, and each had a polytetrafluoroethylene (PTFE) stopcock on the bottom. The test solution within one PELEC unit, in this case filtered and UV-sterilized seawater, was circulated between the cone and beaker of each respective unit using a peristaltic pump (4-Channel Peri-Star Pro™, World Precision Instruments, Inc.) and silicone tubing (size #17, 0.25 inches ID). Each 4-channel peristaltic pump supplied flow to 4 PELEC units simultaneously (i.e., one channel per independent PELEC unit). Pump flow for each unit was directed such that water was drawn from the glass beaker and delivered to the cone via the bottom stopcock at a low flow rate (~100 mL/min) to keep embryos gently suspended and circulating in the cone. Embryos/larvae were retained in the cone using a glass excluder attachment extending from the overflow drain with 300 micron nylon mesh fastened on both sides with silicone o-rings (Fig. 3.1). Glass dishes were used to cover the tops of all cones and beakers within the different exposure systems to limit evaporation. All replicates were maintained within an environmental control chamber at 27°C ambient temperature with a 16:8 light dark photoperiod. Embryos were randomly distributed into each exposure system unit at a density of 20 embryos per liter in the static and agitated beakers (1 L test solution volume each), and at a density of 40 embryos in the PELEC system units (1.8 L of test solution volume each) using a large-bore Pasteur pipette. Water quality parameters including temperature, pH, salinity, and dissolved oxygen (DO_2) in each exposure chamber were monitored daily. Survival was scored at the conclusion of the 96-hr period. Statistical differences in mean survival data between exposure systems were

tested using analysis of variance (ANOVA) and a post-hoc multiple range comparison test (Tukey's HSD). Outliers were detected using Grubb's outlier test. All statistical analyses were performed using XLSTAT (version 2014.3.02, Addinsoft™, USA).

Figure 3.1. Schematic drawing of the pelagic embryo-larval exposure chamber (PELEC). Coupled with the overflow beaker (not shown), the total volume of the PELEC system is 1.8 L. Measurements in millimeters (mm).



Use of PELEC System for Determining Photo-induced Toxicity of DWH Crude Oil. In order to test the effects of *DWH* crude oil in combination with natural sunlight, the PELEC system was adapted for use in an outdoor environment. This was accomplished by combining the previously described bioassay system with a temperature controlled water bath in which the reservoir chambers (1 L beakers) for each independent PELEC unit were partially submerged in a closed loop recirculating water bath that was connected to a heat pump (1/2 hp Delta Star® DSHP-6, Aqua Logic Inc., San Diego, California, USA) to allow for accurate temperature control of all PELEC units. Fifteen independent PELEC units were set up under the cover of clear, >95% UV-transmittance plastic sheeting (KNF CleanRoom Products, Tamaqua, PA, USA) and fifteen were set up alongside under clear, UV-opaque (~10% UV transmittance) plastic sheeting (GAM Products, Los Angeles, CA, USA).

The crude oil used in this study (referred to as "Slick A") was collected at the site of the *DWH* Oil Spill on July 29, 2010 and was obtained from barge number CTC02404. This barge received oil from a number of skimmer vessels (sample ID CTC02404-02) and was subsequently transferred under custody to the University of Miami. The oil was prepared as a high-energy water-accommodated fraction (HEWAF) within 24-hrs of the start of the exposure period by mixing at a loading rate of 1 g of oil per liter of 1 μm filtered, UV-sterilized seawater in a Waring CB15 blender (Torrington, CT) at low speed for 30 seconds. Following mixing, the entire solution was transferred to a glass separatory funnel. It was allowed to settle for 1-hour in the funnel, after which 90% of the solution was drained and retained for use as 100% WAF that was diluted for test exposures. Nominal % WAF dilutions for the full UV exposed treatment group were as

follows: 0 (control), 0.03, 0.12, 0.5, 2.0, while dilutions for the limited UV exposed group were as follows: 0 (control), 0.5, 2.0, 8.0, 32.0. Each exposure dilution, including controls, was replicated in triplicate using a total of 30 independent PELEC units for the experiment. Initial water samples for PAH analysis were obtained from each of the bulk nominal % WAF dilutions prior to addition to the PELEC units and final water samples were obtained at the conclusion of the 96-hr test. The water samples for PAH analysis were collected in 250 mL amber glass bottles, with 96-hr samples for each exposure concentration being comprised of an even mix (~83 mL each) of water samples from the three replicates in each treatment group. The 250 mL water samples were shipped on ice overnight to ALS Environmental (Kelso, WA) for analysis by gas chromatography / mass spectrometry – selective ion monitoring (GC/MS-SIM; based on EPA method 8270D). The reported ΣPAH[50] values represent the sum of 50 PAH analytes (Fig. 3.4).

UV-A (λ = 380nm) was measured continuously during the daylight hours of the exposures using a BioSpherical PUV-2500 radiometer (BioSpherical Instruments, San Diego, CA, USA). Using a JAZ Radiometer (Ocean Optics, Dunedin, FL, USA), the glass of each PELEC unit was determined to be UV-transparent (λ = 380nm). Total UV exposure time, integrated dose, and mean UV intensity are reported in Table 3.1. Dissolved oxygen and water temperature parameters were obtained using a ProODO handheld optical DO_2 probe and meter (YSI, Inc., Yellow Springs, OH). Salinity was measured using a refractometer, and pH was measured using a PHM201 meter (Radiometer, Copenhagen, Denmark) fitted with a glass electrode. All of the aforementioned water chemistry measurements were taken on a daily basis, including at initial (0-hr) and final (96-hr) time points. Total ammonia was measured at the end of the

96-hr period using a colorimetric assay (Verdouw et al. 1978). Survival in each PELEC unit was scored at the conclusion of the 96-hr test.

Unless otherwise stated, all data are presented as mean ± standard error of the mean (SEM) and reported ΣPAH[50] concentrations represent geometric means of initial (0-hr) and final (96-hr) ΣPAH[50] concentrations. The LC50 values were estimated by fitting response data (mean survival at 96-hrs) and log transformed exposure concentrations (geometric mean of initial and final ΣPAH[50] measurements) to a tolerance type Gaussian model with two parameters using the Toxicity Relationship Analysis Program (TRAP; version 1.21a)(Erickson 2013) freely available from the United States Environmental Protection Agency (USEPA).

Table 3.1. Total UV exposure time, integrated dose, and mean UV intensity of the 96-hr bioassay. All UV measurements refer to UV-A (λ = 380nm).

UV Exposure	Duration (hrs)	Integrated UV (mW/cm2 s)	Mean UV (mW/cm2/nm)
Day 1	1:56	493.08	0.07
Day 2	8:12	1895.69	0.06
Day 3	8:24	1738.93	0.06
Day 4	8:33	1537.01	0.05
	Total	**Total**	**Mean**
	27:06:00	**5664.71**	**0.06**

RESULTS

Control Testing of PELEC System. Survival of mahi-mahi embryos after 96-hrs in the PELEC system (89.8% ± 2.12) was greater than in the agitated static exposure system (76.8% ± 4.49) and significantly exceeded survival in the static exposure (67.5%

± 4.79) system (Tukey's HSD test, $P<0.001$)(Fig. 3.2). One replicate with full mortality within the agitated static exposure was determined to be an outlier using Grubb's test ($P<0.01$), and therefore mean survival data for this exposure system based on an n of 11. Water quality parameters amongst all treatments were nearly identical (Table 3.2).

Figure 3.2. Results of 96-hr bioassays comparing three different exposure systems. Control performance of mahi-mahi embryos, measured as percent survival after 96-hrs, was higher in the PELEC system than in the agitated or traditional static exposure system. Different letters represent significant differences between treatment groups (Tukey's Test, $P<0.001$). Values expressed as mean ± SEM.

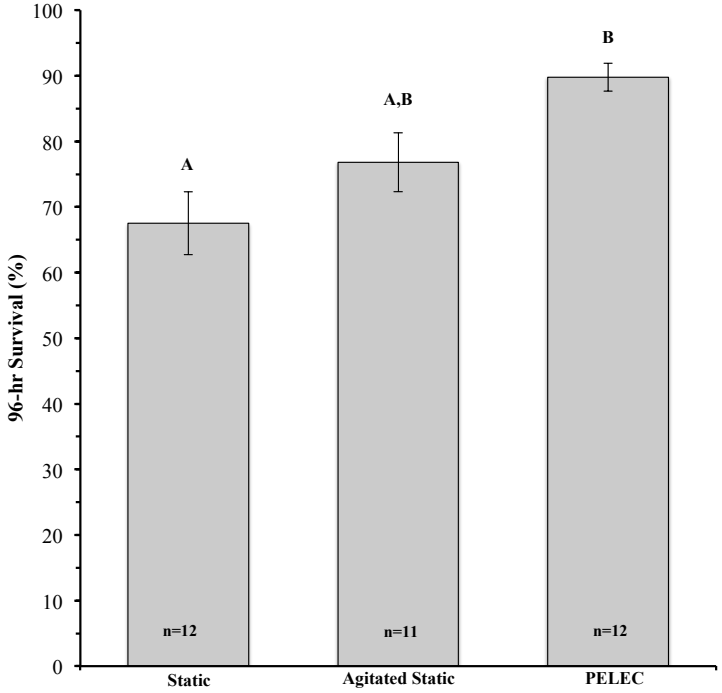


Table 3.2. Water quality parameters of the three different exposure systems (static, agitated static, and PELEC) over the course of the 96-hr bioassays. Values expressed as mean ± SEM.

Treatment	Water Temperature (°C)	Dissolved Oxygen (mg L^{-1})	pH	Salinity ($^{0}/_{00}$)
Static	25.6 ± 0.13	6.52 ± 0.04	8.10 ± 0.01	35.0 ± 0.00
Agitated Static	26.2 ± 0.21	6.62 ± 0.02	8.12 ± 0.01	35.0 ± 0.00
PELEC	25.5 ± 0.13	6.72 ± 0.01	8.14 ± 0.01	36.6 ± 0.30

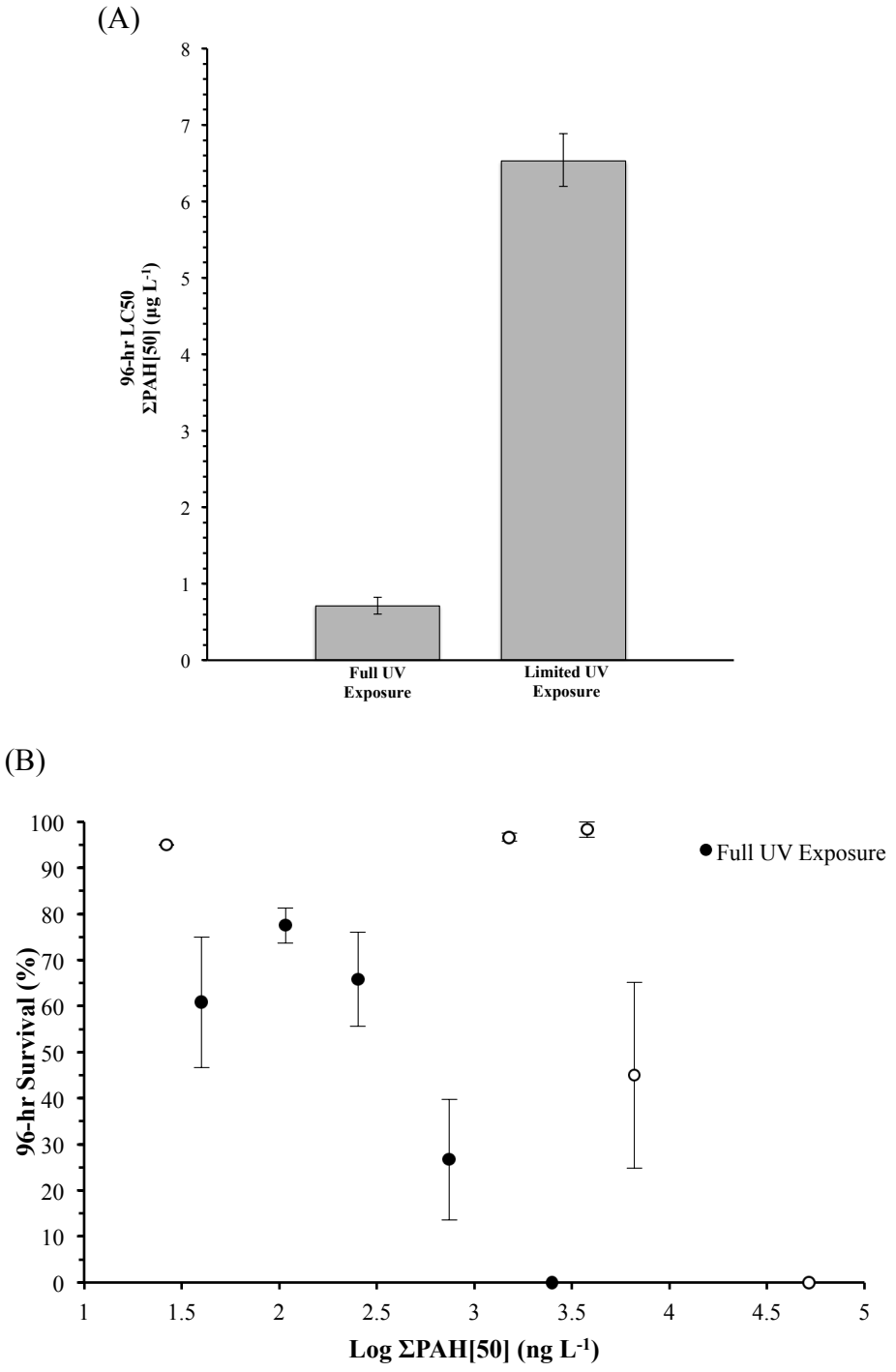
Use of PELEC System for Testing Photo-induced Toxicity of DWH Crude Oil.

There was a greater than nine fold increase in toxicity of *DWH* crude oil to organisms maintained under full spectrum sunlight ('full UV exposure') in a natural diurnal pattern over the course of the 96-hr bioassay compared to organisms kept under the filtered sunlight treatment ('limited UV exposure'). Acute toxicity, measured as 96-hr LC50, of *DWH* Slick A HEWAF in combination with UV-radiation was 0.7 μg L^{-1} ΣPAH[50] (95% CI: 0.6 – 0.8 μg L^{-1} ΣPAH[50]), whereas acute toxicity of the same *DWH* Slick A HEWAF without UV-radiation exposure was 6.5 μg L^{-1} ΣPAH[50] (95% CI: 6.2 – 6.9 μg L^{-1} ΣPAH[50]) (Fig. 3.3). There were no significant differences in water chemistry data between replicates (Table 3.3). The ΣPAH[50] concentrations in each exposure decreased over the course of the 96-hr experiment (Table 3.3). However, ΣPAH[50] concentrations within the full UV exposure treatments decreased more than in the limited UV treatment (Table 3.3), as might be expected given the likely increased photolytic breakdown of PAHs in the full UV exposure treatment.

Table 3.3. Water quality parameters (mean ± SEM) and **Σ**PAH[50] data, expressed as the geometric mean of initial (0-hr) and final (96-hr) exposure concentrations, with initial **Σ**PAH[50] data expressed in parentheses.

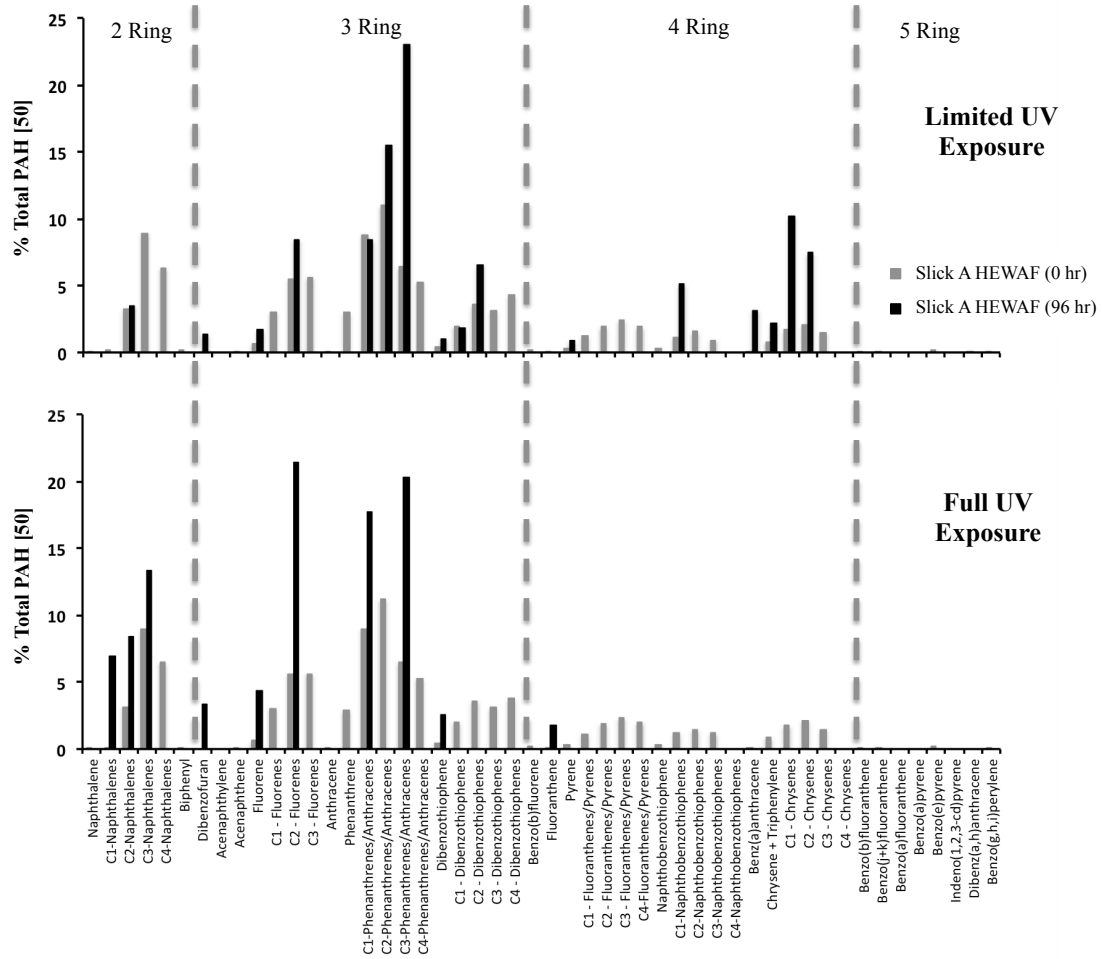
Treatment	ΣPAH (μg L^{-1})	Water Temperature (°C)	Dissolved Oxygen (mg L^{-1})	pH	Salinity ($^{0}/_{00}$)	Total Ammonia (μM)
Full UV Exposure						
Control	0.04 (0.03)	27.19 ± 0.62	6.44 ± 0.07	8.17 ± 0.02	33.33 ± 0.47	0
0.03% HEWAF	0.11 (0.53)	26.62 ± 0.22	6.45 ± 0.04	8.18 ± 0.01	33.33 ± 0.47	0
0.12% HEWAF	0.25 (2.73)	26.67 ± 0.29	6.43 ± 0.05	8.18 ± 0.01	33.33 ± 0.47	0
0.5% HEWAF	0.75 (10.38)	26.16 ± 0.22	6.48 ± 0.04	8.18 ± 0.01	33.33 ± 0.47	0
2% HEWAF	2.50 (44.28)	25.67 ± 0.26	6.50 ± 0.03	8.18 ± 0.01	33.33 ± 0.47	0
Limited UV Exposure						
Control	0.03 (0.03)	27.01 ± 0.31	6.42 ± 0.06	8.17 ± 0.01	33.44 ± 0.44	0
0.5% HEWAF	1.51 (14.80)	26.22 ± 0.29	6.48 ± 0.04	8.18 ± 0.01	33.33 ± 0.47	0
2% HEWAF	3.78 (43.58)	26.33 ± 0.24	6.48 ± 0.04	8.18 ± 0.01	33.44 ± 0.44	0
8% HEWAF	6.62 (175.91)	26.49 ± 0.37	6.41 ± 0.05	8.18 ± 0.01	33.44 ± 0.44	0
32% HEWAF	51.91 (654.98)	25.96 ± 0.43	6.45 ± 0.05	8.17 ± 0.02	33.44 ± 0.44	0

Figure 3.3. Acute toxicity, quantified as 96-hr LC50, of Slick A HEWAF exposure with limited and full UV-radiation exposure (A). Values expressed in μg L^{-1} ΣPAH[50], with upper and lower 95% confidence intervals indicated by error bars (A). Graph of mahi-mahi ELS survival (mean ± SEM) at different crude oil exposure concentrations, expressed in Log ng L^{-1} ΣPAH[50], from both the limited and full UV-radiation exposure treatments (B). Reported ΣPAH[50] concentrations represent the geometric means of initial (0-hr) and final (96-hr) ΣPAH[50] concentrations.



Additionally, the GC/MS-SIM analysis of initial and final water samples indicates a distinct difference between the final ΣPAH[50] compositions of the full UV exposure treatments compared to the limited UV exposure treatments (Fig. 3.4).

Figure 3.4. Initial (0-hr) and final (96-hr) percent composition for 50 PAH analytes as determined by GC/MS-SIM for 2% dilutions of the Slick A HEWAF exposures, both in the full and limited UV-radiation exposed treatments.



Differences in the PAH species found between the two treatments are most apparent in the overlapping 0.5% and 2% dilutions of the full UV exposed treatment compared to the limited UV exposed treatment (0.5%: 0.75 μg L^{-1} vs. 1.51 μg L^{-1} ΣPAH[50]; 2%: 2.50 μg

L^{-1} vs. 3.78 μg L^{-1} ΣPAH[50], respectively) (Table 3.3). However, the decrease was not evenly proportional between individual PAHs. There was near total elimination of 4-ring PAHs in the full UV exposed treatment after 96 hours, though specific 4-ring PAHs, notably Benz(a)anthracene, Chrysene+Triphenylene, and Chrysenes (C-1 and C-2), within the limited UV exposed treatment increased in the amount of relative contribution (% ΣPAH[50]) between initial (0-hr) and final (96-hr) sampling.

DISCUSSION

It was hypothesized that variability in control survival from static 96-hr bioassays could be attributed, in large part, to changes in embryo and larval buoyancy throughout the initial stages of development as well as a need for agitation of the micro-boundary layer of test solution proximal to the embryonic chorion. Different species of pelagic teleosts exhibit diverse life history strategies, such as changes in egg buoyancy during development, that allow for reproductive success despite numerous selective pressures in the open ocean (Ospina-Álvarez et al. 2012). Specifically, the change in buoyancy from positive to negative in the 2 – 4 hours leading up to hatch has been documented in pelagic teleosts other than mahi-mahi, such as yellowfin tuna, and likely results from the change in specific gravity that occurs as the osmotic permeability of the chorion increases and its structure softens prior to hatch (Blaxter 1969, Margulies et al. 2007). While the pre-hatch onset of negative buoyancy likely confers a benefit to these organisms in the wild, serving to position embryos and yolk-sac larvae below the neuston layer (Margulies et al. 2007), this phenomenon may reduce survival and complicate testing for treatment effects

in static beaker bioassays. Additionally, due to high metabolism and rapid embryo and larval development of many sub-tropical pelagic marine fish, such as mahi-mahi (Benetti 1992), suspension and movement throughout the treatment solution may reduce the risk of hypoxia at the boundary layer of the chorion in the embryonic stage and the cutaneous layer in the larval stage. Theoretically, both the agitated static and PELEC systems should ameliorate any such hypoxia risk, though the latter system tends to allow for higher survival over 96-hrs than the former system. Increases in relative survival in the PELEC system suggest that an upwelling water movement may be more effective at reducing mortality caused by exposure systems than the mostly superficial lateral water movement provided by agitation. Additionally, the engineering of the PELEC system allows for full replacement of exposure media without physically touching test organisms, a key benefit for short-term window of exposure studies that may be conducted within a traditional 96-hr bioassay timeframe.

Use of the PELEC system to test the effects of crude oil in combination with exposure to ultraviolet radiation on ELSs of mahi-mahi reveals one potential application of this novel exposure bioassay system. The *DHW* incident of 2010 represents the largest marine oil spill in the United States, releasing over 4 million barrels of crude oil into the GOM over the course of 87 days in the summer of 2010 (Camilli et al. 2010, Crone and Tolstoy 2010, McNutt et al. 2012). The temporal and spatial aspects of the spill, as well as the accompanying ΣPAH concentrations in the water documented during the spill (Diercks et al. 2010, Wade et al. 2011, Incardona et al. 2014), are particularly significant for this study since the pelagic zone of the GOM represents an important spawning and feeding ground for top trophic level pelagic fish species, such as mahi-mahi (Palko et al.

1982, Teo et al. 2007, Rooker et al. 2012). This economically- and ecologically-valuable gamefish species inhabits and spawns in the pelagic waters of the GOM during the time of year the *DWH* incident occurred (Palko et al. 1982). Additionally, mahi-mahi ELSs are relatively transparent, and are commonly found in the surface and near-surface layers of the ocean, potentially increasing their exposure to waters in contact with or near surface oil slicks. Furthermore, residence in surface and near-surface waters increases the likelihood of exposure to significant levels of UV-radiation. Research on predatory pelagic fish species with similar life histories to mahi-mahi, such as tuna (*Thunnus spp.*) and amberjack (*Seriola spp.*), indicates sub-lethal and lethal impacts of *DWH*-specific PAH exposure in the low (~1 – 15) μg L^{-1} ΣPAH range in laboratory settings (i.e. limited UV-radiation exposure) (Incardona et al. 2014). Numerous upper pelagic zone water samples collected from the GOM during the active spill phase had ΣPAH concentrations in excess of 1 μg L^{-1} (Incardona et al. 2014), including reported ΣPAH concentrations up to 84 μg L^{-1} in field collected water samples (Diercks et al. 2010, Wade et al. 2011). Acute toxicity of the *DWH* crude oil in this study, quantified as LC50 over 96-hrs, in the full UV exposed treatment (0.7 μg L^{-1} ΣPAH[50]) was nearly an order of magnitude greater than occurred under laboratory settings (6.5 μg L^{-1} ΣPAH[50]). This study reveals that laboratory estimates that do not include UV exposure as a variable potentially underestimate ecological damage incurred by the *DWH* incident. Additionally, this study represents the first published LC50 quantification of acute lethal impacts of the *Deepwater Horizon* oil spill on the ELSs of a resident GOM pelagic fish species. Previous studies performed under fluorescent light report effect thresholds for resident GOM pelagic fish species as low as 1.2 μg L^{-1} ΣPAH[50] (reduced swim

performance)(Mager et al. 2014) in juvenile mahi-mahi and 0.9 μg L^{-1} ΣPAH[50] (pericardial edema)(Incardona et al. 2014) in ELSs of Southern bluefin tuna (*Thunnus maccoyii*), a congeneric of the bluefin tuna species found in the GOM, the Atlantic bluefin tuna (*T. thynnus*). The low effect thresholds reported by Incardona et al. (2014) and Mager et al. (2014) may represent conservative estimates of *DWH* oil toxicity on these endpoints since they do not account for photo-induced toxicity.

Photo-induced toxicity of PAHs occurs through two distinct mechanisms: absorption of UV by a photosensitizer PAH and eventual production of reactive oxygen species (ROS) or photo-oxidation resulting in the generation of toxic, modified photoproducts (Lee 2003). In the present study, it was not possible to discern between these two mechanisms. However, previous studies examining the photo-induced toxicity of PAHs on larval teleosts following the *Exxon Valdez* (Barron et al. 2003) and *Cosco Busan* (Incardona et al. 2012a) oil spills indicate that the increased mortality observed in the full UV exposed treatment group likely resulted primarily from photosensitization, whereby absorbed PAHs in the embryonic mahi-mahi reacted with absorbed solar radiation to produce reactive oxygen species that in turn resulted in oxidative stress (Little et al. 2000, Lee 2003). Given the differences noted between the final ΣPAH[50] compositions of the full UV exposure treatments compared to the limited UV exposure treatments, as well as the uneven pattern of degradation between the treatments, it is likely that photo-oxidation increased the amount of ΣPAH[50] degradation in the full UV exposed treatment, while the increased degradation of higher-weight PAHs in this treatment likely occurred through direct photolysis (Fig. 3.4 and Table 3.3) (Lee 2003). In the full UV exposure treatments, the proportion of 2- and 3-ring PAHs increased

relative to the total. In the limited UV exposure treatment, however, 3- and 4-ring PAHs increased in proportion to the total (Fig. 3.4). The uneven pattern of proportional PAH decline provides insight on the effects of solar UV-radiation on *DWH* crude oil compounds, while the increased mortality observed in the full UV exposure treatment reveals the toxic effects of this interaction. The photo-induced crude oil toxicity study illustrates one way in which the PELEC bioassay system allows researchers to successfully quantify the effects of environmental impact events, such as the *DWH* oil spill, on challenging pelagic fish ELSs using commonly accepted toxicological tests, metrics, and endpoints.

CONCLUSION

It has been demonstrated that for pelagic fish ELSs, the novel PELEC system significantly improves test performance, and the system has been to show that co-exposure to UV-radiation increases the acute toxicity of *DWH* spill oil to a resident, GOM fish species by nearly an order of magnitude. These findings illustrate the importance of including natural sunlight in assessments of *DWH* spill oil as well as the PELEC system's potential application in ecotoxicological assessments.

ACKNOWLEDGMENT

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project, and the help of Ty Curran of the University of North Texas in conducting the UV exposure trial. MG is a Maytag Professor of Ichthyology. This work was supported by funds provided as part of the Natural Resource Damage Assessment (NRDA) for the *DWH* oil spill. The data presented here are a subset of a larger toxicological database that is being generated as part of the *DWH* NRDA, therefore, these data will be subject to additional analysis and interpretation which may include interpretation in the context of additional data not presented here.

CHAPTER 4:

IMPACTS OF *DEEPWATER HORIZON* CRUDE OIL EXPOSURE ON ADULT MAHI-MAHI (*CORYPHAENA HIPPURUS*) SWIM PERFORMANCE

SUMMARY

The temporal and geographic attributes of the *Deepwater Horizon* (*DWH*) incident in 2010 likely exposed pelagic gamefish species, such as mahi-mahi, to *DWH* crude oil. While much of the research assessing the effects of the spill has focused on early life stages of fish, studies examining whole-animal physiological responses of adult marine fish species are lacking. The present study demonstrates that acute exposure to a sub-lethal concentration of the water accommodated fraction of *DWH* crude oil results in significant swim performance impacts on young adult mahi-mahi, representing the first report of acute sub-lethal toxicity on adult pelagic fish in the Gulf of Mexico following the spill. At an exposure concentration of 8.4 ± 0.6 μg L^{-1} sum of 50 selected polycyclic aromatic hydrocarbons (ΣPAH[50]; mean of geometric means ± SEM), significant decreases in the critical (U_{crit}) and optimal (U_{opt}) swimming speeds of 14% and 10%, respectively, (P<0.05) were observed. In addition a 20% reduction in the maximum metabolic rate (MMR) and a 29% reduction in aerobic scope resulted from exposure to this level of ΣPAHs. Using environmentally relevant crude oil exposure concentrations and a commercially and ecologically valuable Gulf of Mexico fish species, the present results provide insight into the effects of the *DWH* oil spill on adult pelagic fish.

BACKGROUND

The Gulf of Mexico (GOM) supports one of the most prolific marine fisheries in the United States of America. A number of high-value and ecologically important marine fish species that utilize the variety of GOM habitats for many or all life stages (Gibbs and Collette 1959, Palko et al. 1982, Muhling et al. 2012, Rooker et al. 2012), including mahi-mahi (*Coryphaena hippurus*), or dolphin fish, are well known worldwide to commercial and recreational fishermen as well as seafood consumers. Unfortunately, based on recent studies of early life stages (ELSs) (Incardona et al. 2014, Mager et al. 2014), the *Deepwater Horizon* (*DWH*) oil spill in the summer of 2010, which released over 4 million barrels of crude oil into the GOM over the course of 87 days (Camilli et al. 2010, Crone and Tolstoy 2010, McNutt et al. 2012), likely impacted native GOM pelagic fish, including mahi-mahi. The impacts caused by exposure of ELSs to *DWH* crude oil include disruptions to cardiac form and function, as well as a host of other ontogenetic developmental defects (Incardona et al. 2014, Mager et al. 2014) that are consistent with those well documented for a number of other teleosts and oil types (Heintz et al. 1999, Incardona et al. 2009, 2012a, 2013, Hicken et al. 2011, Jung et al. 2013). Such negative impacts are of great concern for apex pelagic predatory species, such as mahi-mahi, that possess high metabolic demands to support robust feeding and migratory abilities needed for survival, growth, and reproduction in the oceanic pelagic environment (Benetti et al. 1995a, 1995b, Brill 1996, Dickson 2011). In general, it is thought that larger life stage fish may be able to withstand higher levels of environmental stressors due to increased metabolic capabilities, reduced cumulative body burdens of pollutant exposure, and the positive scaling of aerobic scope with size (Brett 1965). To test the hypothesis that

whole-animal physiological impacts of sub-lethal *DWH* crude oil exposure extend to larger life stage pelagic fish, swim performance and swimming efficiency studies were conducted on adult mahi-mahi following transient exposure to *DWH* crude oil. Swim performance studies of fish have been used to reveal the sub-lethal impacts of a number of environmental stressors (Hammer 1995, Heath 1995, Tudorache et al. 2013), including marine pollution events such as crude oil spills (Thomas and Rice 1987, Kennedy and Farrell 2006, Mager et al. 2014) that lead to exposure to toxic PAHs. When combined with respirometry analysis, swim performance studies allow for determination of aerobic scope, the energy available for sustained activity. Such analysis can reveal loading and limiting stressors on animals that may otherwise not be apparent in traditional impact quantification efforts. Additionally, given the suggestion that reductions in swim performance following transient crude oil exposure to juvenile mahi-mahi (mean mass: 0.40 – 0.81 g) (Mager et al. 2014) may result at least partially from a reduced swimming efficiency (Mager et al. 2014, Yanase et al. 2014), high-speed video analysis of swimming kinematics was incorporated to study a potential contribution of this effect. Due to the long migrations and vast areas covered by these animals in search of prey and spawning sites, sub-lethal impacts to swimming efficiency may have significant impacts on stocks of these high-value species.

MATERIALS AND METHODS

Experimental Animals:

Mahi-mahi embryos were obtained as described previously (Mager et al. 2014) from wild adult broodstock spawning volitionally in 80 m^3 seawater tanks at the

University of Miami Experimental Hatchery (UMEH) and raised to young adult stages. All mahi-mahi used in the present study were maintained in flow-through tanks and fed daily a diet of freshly thawed natural prey (squid, sardines, and silversides), along with regular additions of vitamin and mineral supplements. Fish used in the swim study were randomly selected from the holding tank and briefly examined to make sure there were no visible injuries or damage to the caudal fin. Following use in the swim study, necropsies were performed on each fish to confirm the lack of any external or internal abnormalities.

Preparation of Water Accommodated Fractions (WAFs):

The crude oil used in this study (referred to as "Slick A"; sample ID CTC02404-02) was collected at the site of the *DWH* oil spill on July 29, 2010 from a barge (#CTC02404) receiving oil from a number of skimmer vessels and was subsequently transferred under custody to the University of Miami. The high-energy WAF (HEWAF) preparations were made within 24 h of the start of each exposure period, and were prepared as described previously (Mager et al. 2014) using a loading rate of 1 g of oil per liter of 1 μm filtered, UV-sterilized seawater.

24 h Mahi-mahi exposures:

The static exposure methods used in this study are based on those described by Mager et al.(Mager et al. 2014). Exposures were conducted in 2,500-L cylindrical fiberglass tanks at UMEH with a total test solution volume of approximately 360 – 900 L, depending on exposure concentration. The test medium was natural, filtered, and UV-

sterilized seawater. On the day of each exposure, the treatment tank was filled with seawater, after which a circulation pump (Danner Supreme Mag Drive Pump, Model MD12) attached to the center drain outflow pipe was turned on to generate a directional current of approximately 1 body length per second (BL s^{-1}) within the treatment tank to facilitate ram ventilation by the fish. A short center standpipe was used in each tank to reduce the chance of fish becoming trapped in the bottom drain, and a light amount of aeration was provided at the base of the standpipe to maintain dissolved oxygen levels at or near saturation. Individual fish were exposed to control seawater or nominal HEWAF dilutions of 0.4%, and ~ 1.7% for 24 hrs. Exposures were administered by adding HEWAF to the treatment tank following initiation of the directional current. After a short period of mixing (~5 minutes), fish were randomly selected from the holding tank using a long handled net and were immediately placed into the treatment tank. Mortalities during the 24 hr exposure were minimal, and only occurred at the highest concentration (3 individuals at the 1.7% dilution). The total *n* for each of the three treatment groups is listed in Table 4.1. Due to the rapid growth of mahi-mahi, fish remained at the desired test size (~250 g) for only a matter of a week or two. Consequently, a total of 5 different cohorts of mahi-mahi were used for testing, with at least one individual from each cohort tested for control performance to minimize the potential for confounding factors due to batch variability. Fish were fed on the morning of each exposure in their holding tank, approximately 2.5 – 3 hrs prior to exposure, and were not fed during the 24 hr exposure period.

Water quality and ΣPAH analysis:

Water quality parameters measured at the beginning and end of each exposure period for each replicate were as follows: ΣPAH, temperature, dissolved oxygen (DO), pH, total ammonia and salinity. The initial (0-hr) water samples for ΣPAH analysis were obtained following the WAF addition and short mixing period in the treatment tank, and the final samples were obtained just prior to transfer of the fish to the swim chamber respirometer. Samples for ΣPAH analysis were collected in 250 mL amber glass bottles that were shipped on ice overnight to ALS Environmental (Kelso, WA) for analysis by gas chromatography / mass spectrometry – selective ion monitoring (GC/MS-SIM; according to EPA method 8270D). The reported ΣPAH[50] values represent the sum of 50 selected PAH analytes (Table 4.3). Salinity was measured using a refractometer and pH was measured using a PHM201 meter (Radiometer, Copenhagen, Denmark) fitted with a glass electrode. Water temperature and DO were measured using a ProODO handheld optical DO probe and meter (YSI, Inc., Yellow Springs, OH) and total ammonia was measured using a colorimetric assay (Verdouw et al. 1978).

Swimming Performance:

A 90-L Brett-type swim tunnel respirometer and AutoResp™ 2.1.0 software (Loligo Systems, Denmark) were used to assess swim performance. Using this system, a critical swim velocity (U_{crit}) test was performed (Brett 1964), while oxygen consumption (MO_2) was measured with a Pt100 fiber-optic probe connected to a Fibox 3 minisensor oxygen meter (PreSens Precision Sensing GmbH, Germany) using intermittent respirometry (20 minute measurement periods). Two point calibration of the oxygen

sensor was performed daily using filtered and UV-sterilized seawater that was vigorously aerated using an air-stone to obtain 100% air saturation for the high calibration point and 0% O_2 saturation, obtained by submerging the oxygen sensor in a solution of 10 g L^{-1} Na_2SO_3 (Sigma-Aldrich, St. Louis, MO), for the low calibration point. Water temperature within the swim tunnel respirometer was maintained using a 1/3 hp heat pump (Aqua Logic, Inc., Delta Star, San Diego, CA), while the risk of metabolic waste build-up within the swim chamber was mitigated by maintaining a constant flow of 1 μm filtered, UV-sterilized seawater water through the buffer tank during the swimming performance test. Water flow speed within the swim tunnel respirometer was calibrated using linear regression methods, whereby a handheld anemometer coupled with a 30 mm cylinder probe vane wheel flow sensor (Höntzsch GmbH, Germany) was used to determine the water flow speed (cm s^{-1}) at a particular motor output (Hz) setting. Accuracy of the handheld anemometer readings were confirmed using high-speed (30 fps) video analysis (GoPro Hero 2, San Mateo, CA) of liquid dye injections into the working section of the swim tunnel at various motor output (Hz) settings.

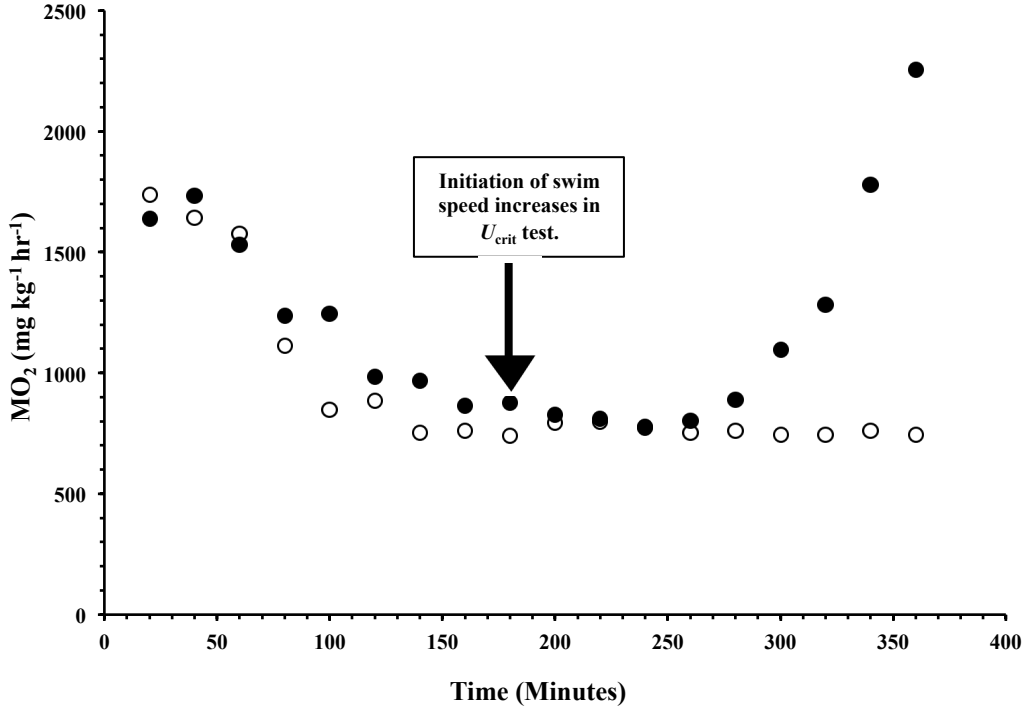
Following the 24 hr exposure period fish were gently transferred and acclimated to the swim tunnel by introducing a slow water flow to encourage swimming, while they were manually prevented from contacting the sides. Once the fish were swimming steadily in the chamber at a slow speed, the lid was bolted onto the swim tunnel and MO_2 measurements were initiated during the acclimation period. When necessary during the early portion (i.e. initial few measurement periods or "loops") of the acclimation period, supplemental O_2 was used to rapidly re-establish ~95 – 100% O_2 saturation in the water following MO_2 measurement intervals to minimize the time acclimating fish spent in

<95% O_2 saturated water. The brief use of O_2 aided in the recovery of the fish following handling, after which ambient aeration in the buffer tank was used for the remainder of the acclimation period and during the U_{crit} swim performance test. The duration of the acclimation period was determined by conducting a preliminary experiment in which young adult mahi-mahi were fed, isolated in the control treatment tank for 24 hrs, and transferred to the swim tunnel using the methods described previously. The MO_2 of the fish was monitored for 18 – 24 hrs while the fish swam at a low acclimation speed (~0.6 BL s^{-1}). Results indicate that MO_2 stabilizes after approximately 3 hrs and thereafter MO_2 readings are interpreted as routine metabolic rate (RMR) (Fig. 4.1), defined as the average energy utilization at a minimal swim speed following acclimation. Therefore, all fish used in swimming performance tests were acclimated for a minimum of 2.5 – 3 hrs, with the ramped speed portions of the U_{crit} test not commencing until acclimation MO_2 readings were within ~10% of each other over two consecutive 20 minute measurement periods. A custom-built enclosure for the entire swim tunnel respirometer allowed for stable experimental conditions within the chamber at all times, while eliminating disturbance from monitoring activities. During the U_{crit} test, fish were monitored in real-time using two different camera angles, as well as by peering into an opening at the top rear portion of the enclosure that was not visible to the fish.

Following the acclimation period, water flow velocity was increased ~0.5 BL s^{-1} (12 – 13 cm s^{-1}) every 20 minutes until the fish fatigued. Fatigue was confirmed using video analysis, and was defined as the point at which the fish began to continuously rest on the rear screen of the swim tunnel, was pushing off the rear screen in a burst and glide form of locomotion, or became pinned sideways against the rear screen and was unable to

recover to normal swimming behavior. Following completion of the swim test, the fish were euthanized by MS-222 overdose, after which the mass (g) and fork length (FL) in cm were obtained. U_{crit}, expressed in BL s^{-1}, was calculated using the equation described by Brett (Brett 1964): $U_{crit} = [U_f + (T/t)dU]$/cm FL, where U_f(cm sec^{-1}) is the highest swim velocity maintained for a full interval, T (s) is the time spent at the final velocity, t is the time interval (s) and dU is the increment in swim speed (cm s^{-1}).

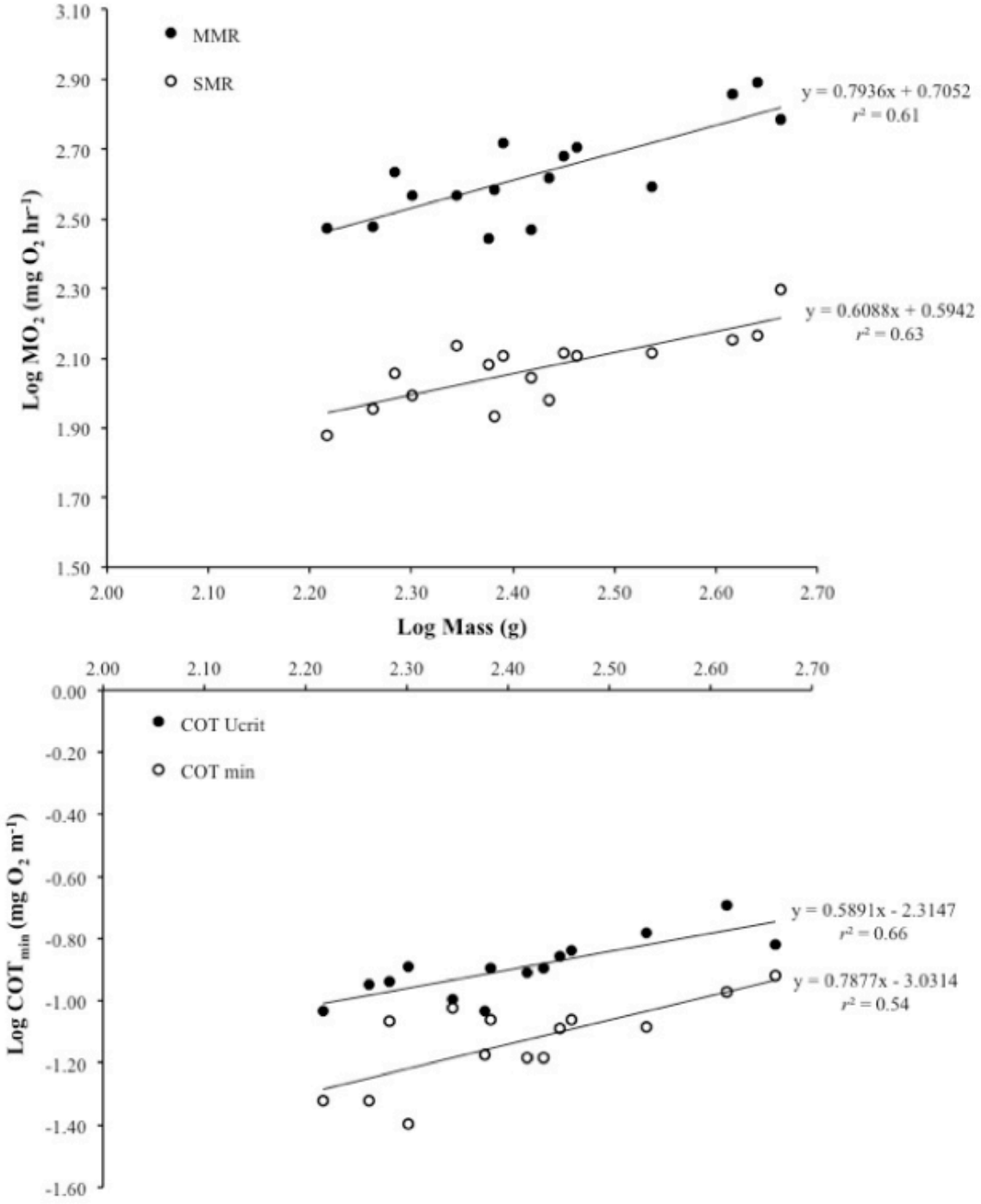
Figure 4.1. Typical acclimation pattern for young adult mahi-mahi used in this swim performance study. Initiation of speed interval increases that comprise the U_{crit} test occurred after approximately 3 hrs of acclimation at a slow steady swimming speed (~0.6 BL s^{-1}). Black dots represent a typical MO_2 pattern in a U_{crit} test, and white dots represent an individual swimming at acclimation speed for 6 hrs.



Metabolic Rates and Aerobic Scope:

The combination of MO_2 (mg O_2 kg^{-1} hr^{-1}) data at each swim speed and determination of U_{crit} allowed for calculation of aerobic scope. Aerobic scope is defined as the difference between the maximum metabolic rate (MMR) and the standard metabolic rate (SMR) (See Mager et al. 2014 for definition of these parameters). MO_2 data was log transformed and plotted vs. swim speed (BL s^{-1}) to obtain these values (Mager et al. 2014). Given that empirical data of this type have been modeled in fish swim performance studies using both least squares linear regression and exponential regression (Sepulveda and Dickson 2000), both modeling analyses were performed to determine which best fit the data (highest r^2 values). The exponential regression best modeled the data for 74% of the individuals compared to the linear regression (26%), though differences in r^2 were minor between the two methods. In order to maintain uniformity in analytical methods between individuals, the exponential regression was used and the SMR (y-intercept) and MMR (extrapolated MO_2 value at U_{crit}) were derived from the resulting equation. Only individuals with regression $r^2 \geq 0.7$ were used for the aerobic scope analysis. Since metabolic rate is known to scale with mass and there was variation in the body sizes of fish used in this study, the SMR and MMR data for each individual were normalized for the effect of mass by scaling all such values to a standard mass of 250 g before calculating aerobic scope. To eliminate the influence of treatment effects, only data from the control treatment group were used to generate the metabolic scaling coefficients (Fig. 4.2). SMR, MMR, and Aerobic Scope were estimated for each individual fish, and mean values of each parameter for each treatment group are presented in the Results section.

Figure 4.2. Metabolic rate data was scaled to a standard fish mass (250 g) using log MO_2 or COT vs. log mass plot of control treatment fish used in each analysis (n = 16 and 14, respectively) to determine metabolic scaling coefficients for SMR and MMR (top graph) as well as COT_{min} and COT_{Ucrit} (bottom graph).



Cost of Transport:

The energetic expense of movement over a distance (mg O_2 kg^{-1} m^{-1}), termed the cost of transport (COT), was quantified for each individual by dividing the MO_2 by swimming velocity at each velocity increment. The resulting parabolic shaped plot was fit to a second order (k=2) polynomial regression model and was used to determine the cost of transport at U_{crit} (COT_{Ucrit}). Additionally, the model was used to calculate the optimal swimming speed (U_{opt}), which is the speed at which swimming required the minimum cost of transport (COT_{min}) and was determined by fitting the first derivative of the polynomial model equation to zero (Palstra et al. 2008). Only individuals with regression $r^2 \geq 0.7$ were used for the COT analysis (Table 4.1). As described in the previous section, all COT data was scaled to a standard fish mass of 250 g using only data from the control group to generate the metabolic scaling coefficients (Figure 4.2).

Swimming Kinematics:

A subsample of fish from the 1.7% HEWAF and control treatments (see Table 4.1 for *n*) were analyzed for tail beat frequency (TBF) and stride length using high speed (30 fps) video recordings (GoPro Hero 2, San Mateo, CA) of individual fish at different swim speed intervals. Tail beat was defined as one complete oscillation of the tail. Frequency was determined by completing three separate analyses of 5 beat intervals, whereby the time (tenths of a second) to complete 5 tail beats was quantified for each interval. The number of tail beats (5) was then divided by the average of the three times to obtain TBF in beats per second, expressed as hertz (Hz). The distance traveled per tail beat, also known as stride length (SL), was calculated as the swim speed (cm s^{-1}) divided by the

TBF and is expressed as fractions of the body length of each individual fish. TBF and SL values for each treatment group are presented as mean ± standard error of the mean (SEM).

Statistical Analysis:

All data are presented as mean ± standard error of the mean (SEM). Statistical differences were analyzed using either one-way ANOVA or ANCOVA, with differences between treatment groups determined with appropriate post-hoc tests noted specifically in each section. Outliers within individual treatment groups were detected using Grubbs outlier test ($\alpha = 0.05$). Statistical analysis was performed using XLSTAT (version 2014.3.02, Addinsoft™, USA). Values were considered significantly different at $P<0.05$.

RESULTS

Experimental Animals, Water Quality, and PAH Exposures:

The GC-MS/SIM analyses revealed concentrations of ΣPAHs in the 0.4% and 1.7% Slick A HEWAF treatments of 2.3 ± 0.1 and 8.4 ± 0.6 μg L^{-1} ΣPAH[50], respectively (represented as means ± SEMs of the geometric means of the initial and final ΣPAH[50] concentrations from each individual exposure; Table 4.2). The primary groups used in analysis of this study are the control and 8.4 μg L^{-1} ΣPAH[50] treatment groups. At the 2.3 μg L^{-1} ΣPAH[50] exposure, the fish were significantly smaller ($P<0.05$) and water temperatures during the swim performance analyses of these fish were significantly warmer ($P<0.05$) compared to the other treatment groups (Table 4.1 and Table 4.2). Given the thermal and mass sensitivity of metabolic processes, these factors

likely explain the differences in metabolic rates (SMR & MMR) (Fig. 4.4) and COT (Fig. 4.5 and Fig. 4.6) between this treatment and controls. There were no significant differences in size (mass and length) and water temperature conditions between the control and 8.4 μg L^{-1} ΣPAH[50] treatment groups. Given the number of individual exposures used in this study, whereby each fish was exposed individually prior to each swim performance test, the results of the GC-MS/SIM water chemistry analysis for each treatment group were analyzed statistically to determine if there were any outliers in the water chemistry data. Individuals with water chemistry results deemed to be significant outliers (P<0.05, Grubb's test) were removed from further analysis, and no data for these individuals are included in any of the following analyses. Not all fish completed the swim performance test successfully, with failed tests occurring either from mortality during the exposure period (11% of fish exposed to 8.4 μg L^{-1} ΣPAH[50] and 0 mortalities in either of the other two treatments) or from a failure to acclimate to the swimming chamber, which occurred for fish in both the control and 8.4 μg L^{-1} ΣPAH[50] treatment groups (6% and 11% of fish, respectively). Within the 8.4 μg L^{-1} ΣPAH[50] treatment group, an additional 11% of exposed fish acclimated to the swim chamber, but were unable to complete the U_{crit} test given that they only completed a partial swim interval above acclimation speed. Such fish are not included in the presented data.

Table 4.1. Treatment group biometric data for mahi-mahi used in this study.

Treatment	*n*	Mass (g)	Fork Length (cm)	Age (dph)
Control	16 (16^a;14^b;6^c)	278 ± 23	29.1 ± 0.8	129 ± 10
0.4% HEWAF	7 (7^a;6^b;0^c)	196 ± 9	26.5 ± 0.5	119 ± 10
1.7% HEWAF	18 (15^a;17^b;16^c)	298 ± 15	30.7 ± 0.6	121 ± 5

Values are expressed as mean ± standard error of the mean (SEM). Only individuals with exponential (SMR,MMR) or polynomial (COT) regression r^2 values ≥ 0.7 were used for the respective analyses. The *n* numbers in parentheses indicate the number of fish form within each treatment group used for metabolic rate (SMR, MMR)^a, cost of transport (COT)^b, and kinematics (TBF, SL)^c analyses. The abbreviation "dph" refers to: days-post-hatch.

Table 4.2. Exposure concentrations and water quality parameters during the 24 h acute exposures prior to swim performance testing.

Treatment	ΣPAH (µg L^{-1})	Water Temp. (°C)	pH	D.O. (mg L^{-1})	Total Amm. (µM L^{-1})	Salinity (ppt)
Control (4)	0.09 ± 0.01 (0.05 ± 0.01)	27.9 ± 0.5	8.00 ± 0.02	6.50 ± 0.07	12.14 ± 3.6	34.1 ± 0.6
0.4% HEWAF (7)	2.30 ± 0.10 (7.33 ± 0.35)	28.9 ± 0.3	8.05 ± 0.02	6.42 ± 0.05	2.38 ± 1.2	32.1 ± 0.9
1.7% HEWAF (18)	8.40 ± 0.59 (24.19 ± 1.35)	26.6 ± 0.4	7.89 ± 0.03	6.73 ± 0.08	29.99 ± 4.9	34.2 ± 0.3

Values are expressed as mean ± standard error of the mean (SEM). The ΣPAH[50] values represent the mean (± SEM) of the geometric means of initial and final exposure concentrations, with values in parentheses indicating the mean (± SEM) of only the initial exposure concentrations from each individual exposure. Sample sizes for ΣPAH analyses for each treatment group are indicated in parentheses next to the group name.

Table 4.3. List of the 50 selected PAH analytes used for calculation of reported ΣPAHs.

Acenaphthene
Acenaphthylene
Anthracene
Benz(a)anthracene
Benzo(a)fluoranthene
Benzo(a)pyrene
Benzo(b)fluoranthene
Benzo(b)fluorene
Benzo(e)pyrene
Benzo(g,h,i)perylene
Benzo(j+k)fluoranthene
Biphenyl
C1 - Chrysenes
C1 - Dibenzothiophenes
C1 - Fluoranthenes/Pyrenes
C1 - Fluorenes
C1 - Naphthalenes
C1 - Naphthobenzothiophenes
C1 - Phenanthrenes/Anthracenes
C2 - Chrysenes
C2 - Dibenzothiophenes
C2 - Fluoranthenes/Pyrenes
C2 - Fluorenes
C2 - Naphthalenes
C2 - Naphthobenzothiophenes
C2 - Phenanthrenes/Anthracenes
C3 - Chrysenes
C3 - Dibenzothiophenes
C3 - Fluoranthenes/Pyrenes
C3 - Fluorenes
C3 - Naphthalenes
C3 - Naphthobenzothiophenes
C3 - Phenanthrenes/Anthracenes
C4 - Chrysenes
C4 - Dibenzothiophenes
C4 - Fluoranthenes/Pyrenes
C4 - Naphthalenes
C4 - Naphthobenzothiophenes
C4 - Phenanthrenes/Anthracenes
Chrysene+Triphenylene
Dibenz(a,h)anthracene
Dibenzofuran
Dibenzothiophene
Fluoranthene
Fluorene
Indeno(1,2,3-cd)pyrene
Naphthalene
Naphthobenzothiophene
Phenanthrene
Pyrene

Swimming Performance:

Swimming performance, measured as U_{crit}, of the 2.3 μg L^{-1} ΣPAH[50] treatment group (3.94 ± 0.35 BL s^{-1}) was similar to that of the control treatment group (4.08 ± 0.12 BL s^{-1}). However, the U_{crit} of the 8.4 μg L^{-1} ΣPAH[50] treatment group was significantly reduced by 14% (3.51 ± 0.14 BL s^{-1}) compared to controls (P<0.05, Tukey's HSD test) (Fig. 4.3). Similarly, there was no apparent effect of the 2.3 μg L^{-1} ΣPAH[50] treatment on U_{opt} (2.80 ± 0.18 BL s^{-1}) compared to the control group (2.80 ± 0.09 BL s^{-1}), yet at the higher concentration (8.4 μg L^{-1} ΣPAH[50]), the U_{opt} decreased significantly to 2.52 ± 0.07 BL s^{-1} (P<0.05, Dunnett's test) (Fig. 4.3 and Fig. 4.6).

Metabolic Rates and Aerobic Scope:

There was no significant difference in SMR among treatment groups (P>0.05)(Fig. 4.4). However, there was a significant decrease in MMR for the 8.4 μg L^{-1} ΣPAH[50] treatment group (1327 ± 75 mg O_2 kg^{-1} hr^{-1}) (P<0.05, Tukey's HSD test) compared to controls (1652 ± 78 mg O_2 kg^{-1} hr^{-1}) (Fig. 4.4). This reduction in MMR contributed to a significantly reduced aerobic scope (849 ± 85 mg O_2 kg^{-1} hr^{-1}) compared to controls (1194 ± 77 mg O_2 kg^{-1} hr^{-1})(P<0.05, Tukey's HSD test)(Fig. 4.4).

Cost of Transport:

No significant impact of transient crude oil exposure on COT at U_{crit} (COT_{Ucrit}) was observed (Fig. 4.5). The COT_{min} was highest in the 2.3 μg L^{-1} ΣPAH[50] treatment (0.38 ± 0.04 mg O_2 kg^{-1} m^{-1}) (P<0.05, Tukey's HSD test), while the mean COT_{min} in the

control and the 8.4 µg L^{-1} ΣPAH[50] treatment groups were identical (0.29 ± 0.02 mg O_2 kg^{-1} m^{-1} and 0.29 ± 0.01 mg O_2 kg^{-1} m^{-1}, respectively; Fig. 4.6).

Figure 4.3. Swimming performance of young adult mahi-mahi in response to *DWH* crude oil exposure. Different letters indicate significant differences between treatment groups (P<0.05) for each respective swim performance parameter. Abbreviations: body lengths per second (BL s^{-1}); critical swimming speed (U_{crit}); optimal swimming speed (U_{opt}).

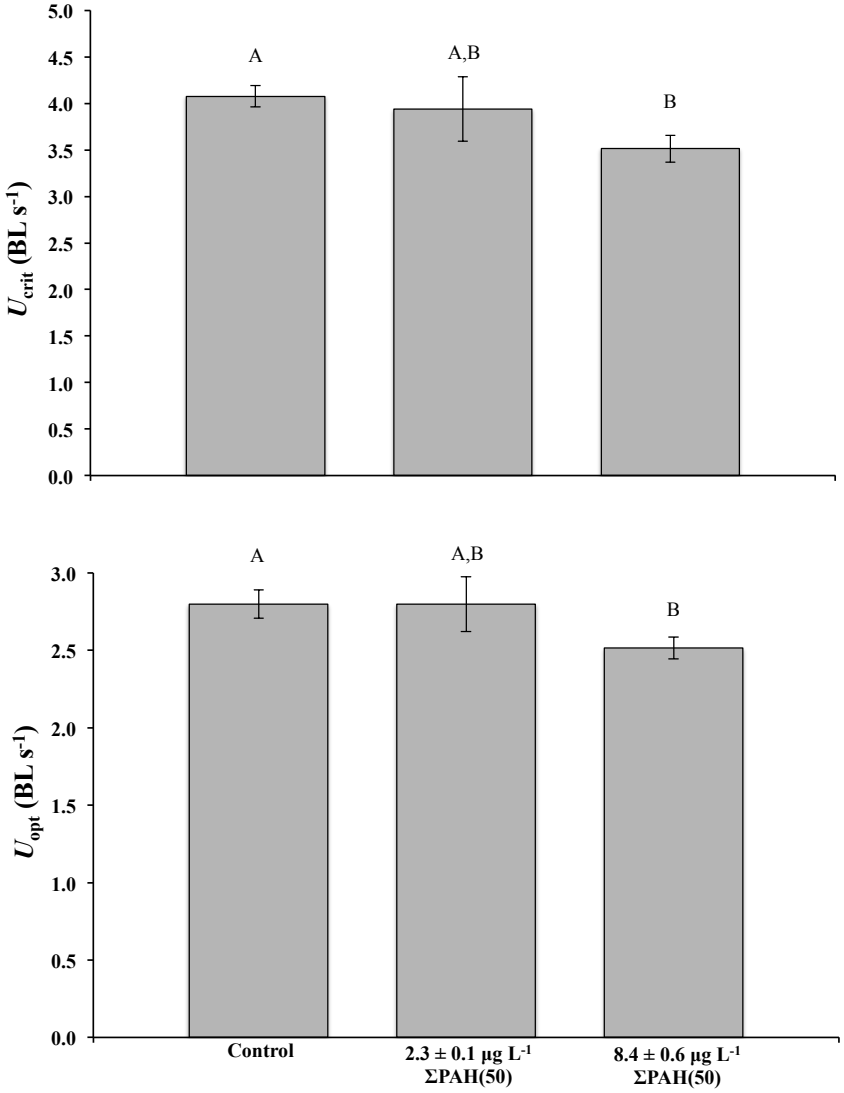


Figure 4.4. Metabolic rates of young adult mahi-mahi derived from swim performance respirometry. Standard metabolic rate (SMR), top; maximum metabolic rate (MMR), middle; and aerobic scope, bottom. Data shown has been normalized for mass, as described in 'Materials and Methods' and in 'Figure 4.2'. Different letters indicate significant differences between treatment groups (P<0.05).

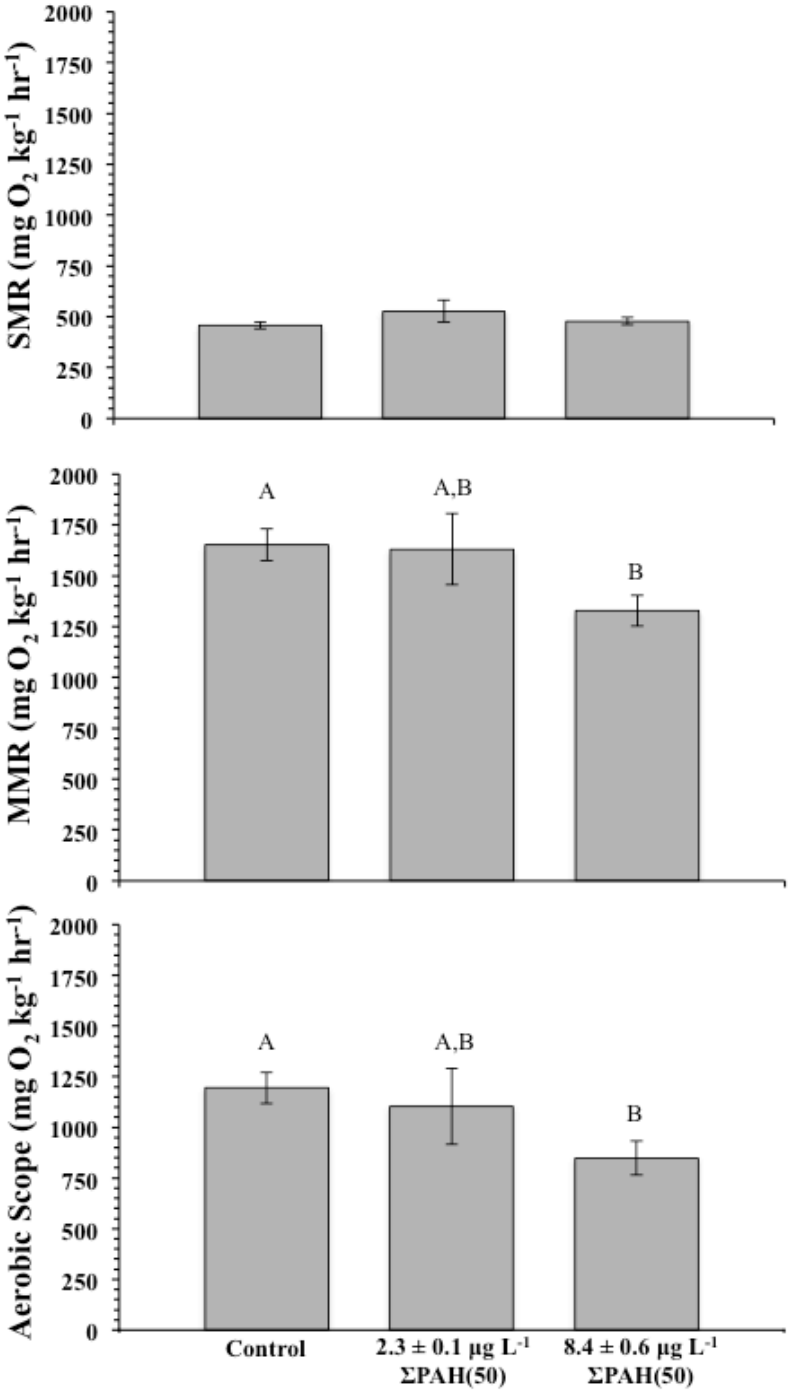


Figure 4.5. Cost of transport at both the upper critical swimming speed (COT_{Ucrit}) and at the optimal swimming speed (COT_{min}). Different letters indicate significant differences between treatment groups (P<0.05).

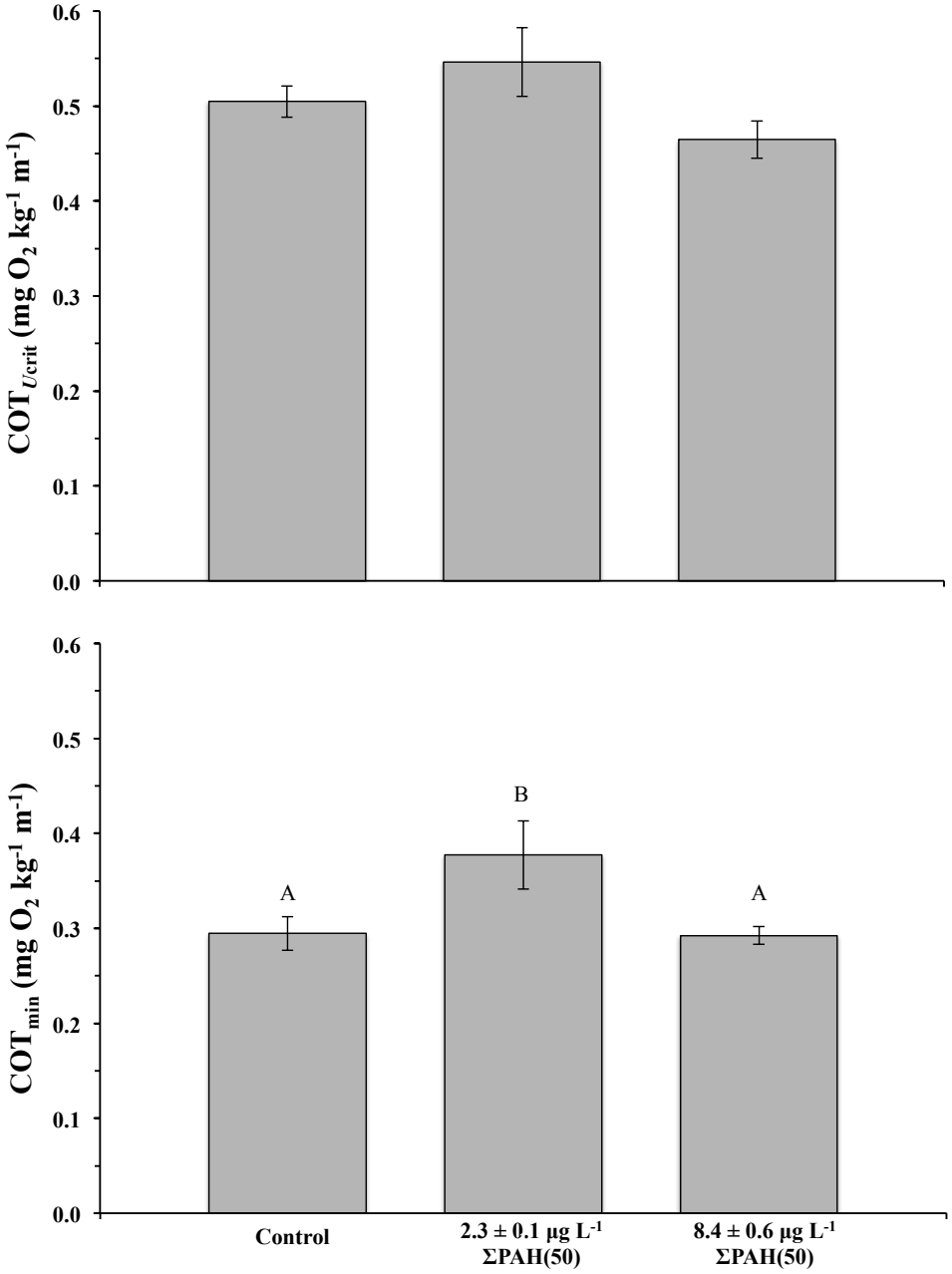
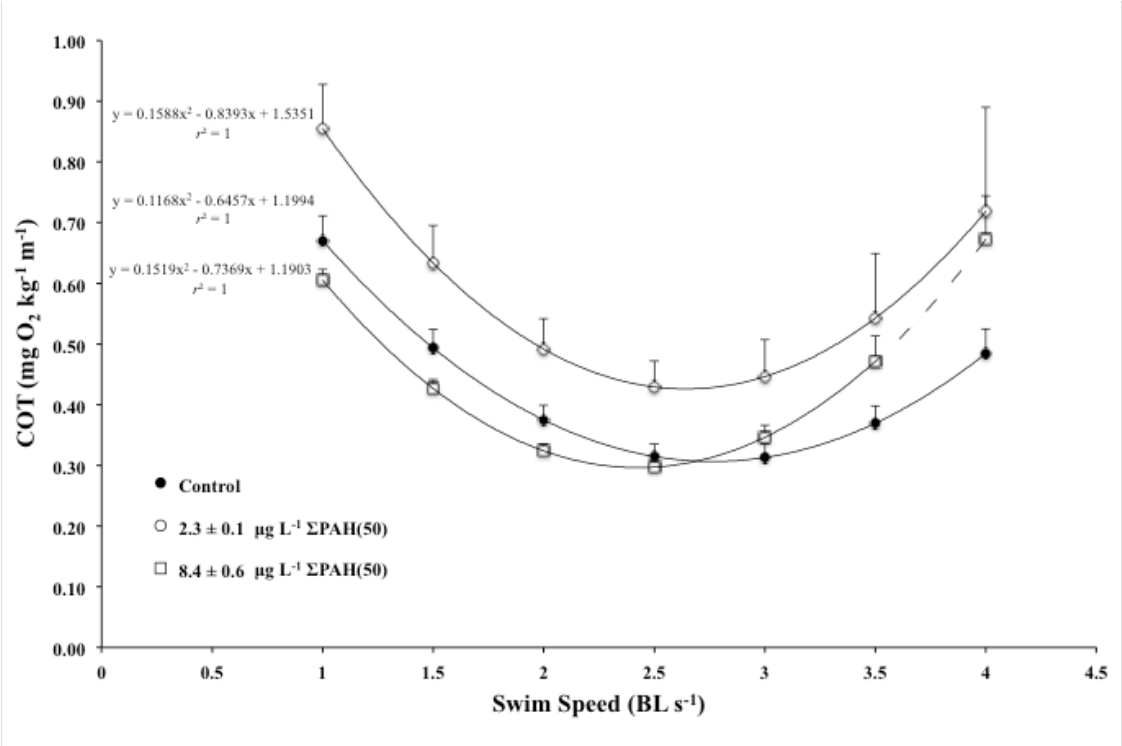


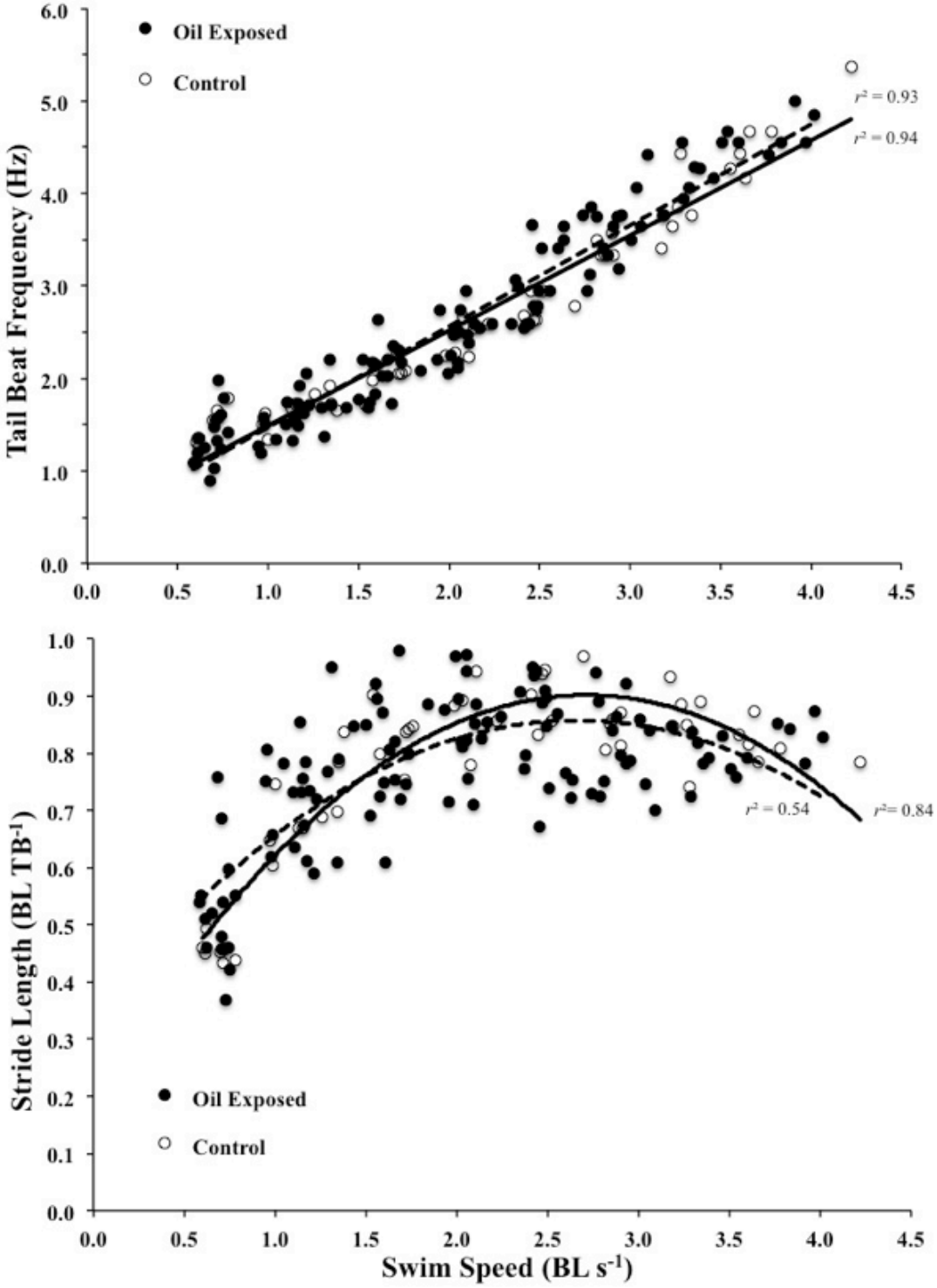
Figure 4.6. Cost of transport (COT) of young adult mahi-mahi at different swimming speeds. Each data point represents the COT (mean ± SEM) at swimming speeds from 1 – 4 BL s^{-1}. There was a significant decrease in swimming efficiency, or U_{opt}, in the 8.4 µg L^{-1} ΣPAH[50] treatment group (*open squares*) compared to control fish (*filled circles*) (*see: 'Figure 4.3'*). Dashed line indicates extrapolated data, as fish in the 8.4 µg L^{-1} ΣPAH[50] treatment group (*open squares*) had a significantly reduced U_{crit} (*see: 'Figure 4.3'*). *Note: Elevated COT of the 2.3 µg L^{-1} ΣPAH[50] treatment group (*open circles*) is likely due to significant differences (P<0.05) in mean size and swimming temperature of this group (*see: 'Results – Experimental Animals'*).



Swimming Kinematics:

Measurements of TBF and SL in a subsample of control and 8.4 µg L^{-1} ΣPAH[50] treatment group fish revealed no significant effects (P>0.05, ANCOVA) (Fig. 4.7). Both groups exhibited linear increases in TBF with increasing swimming velocity, while SL followed a parabolic pattern with the highest values, expressed as BL TB^{-1}, in the mid-range of swimming velocities.

Figure 4.7. Tail beat frequency (TBF) and stride length (SL) of young adult mahi-mahi at different swimming speeds following acute 8.4 μg L^{-1} ΣPAH[50] exposure (*dashed line*) and no oil (control) treatment (*solid line*).



DISCUSSION

Given the spatiotemporal aspects of the *DWH* oil spill and the documented contamination of the GOM pelagic environment (Crone and Tolstoy 2010, Leifer et al. 2012, McNutt et al. 2012, Ryerson et al. 2012, Sammarco et al. 2013), it is very likely that top trophic level pelagic species such as mahi-mahi encountered transient crude oil exposures well above those tested in this study (Muhling et al. 2012). Field samples of *DWH* crude oil ΣPAH concentrations in the pelagic environment have been reported as high as 84 μg L^{-1} ΣPAH (Diercks et al. 2010, Wade et al. 2011), which is 10-times greater than the high experimental dose used in this study. Furthermore, the composition of the *DWH* Slick A HEWAFs used in this study are nearly identical in composition to those of previous studies and samples obtained from the GOM during the spill (Incardona et al. 2014, Mager et al. 2014). Therefore, the current discoveries that swimming performance (U_{crit}), efficiency (U_{opt}), MMR, and aerobic scope were significantly decreased in the 8.4 ± 0.6 μg L^{-1} ΣPAH[50] treatment are relevant to the PAH exposure conditions experienced in the GOM, and these findings add to previous work suggesting juvenile pelagic fish were negatively impacted by the oil spill (Mager et al. 2014).

To date, many of the efforts aimed at investigating the effects of the *DWH* spill on high-value pelagic species have focused on the ELSs (Incardona et al. 2013, 2014, Mager et al. 2014), while impacts to later life stages have been more challenging to quantify accurately. Interestingly, when compared to juvenile mahi-mahi (0.4 – 0.8 g, 28 – 37 dph) exposed and tested under similar conditions as those employed in this study, the larger young adult mahi-mahi appear more sensitive since a significant decrease in U_{crit} occurs at 8.4 μg L^{-1} ΣPAH[50] vs. 30 μg L^{-1} ΣPAH[50] (Mager et al. 2014). However,

the magnitude of the decrease in U_{crit} is approximately 14% at the 8.4 μg L^{-1} ΣPAH[50] exposure concentration used in this study compared to a 22% decrease in U_{crit} observed at the 30 μg L^{-1} ΣPAH[50] exposure concentration (Mager et al. 2014). The lack of significant impacts to U_{crit} on juvenile mahi-mahi exposed to similar concentrations (Mager et al. 2014) may simply reflect the fact that aerobic scope positively scales with fish mass thereby allowing aerobic scope impacts to be more easily detected in the larger young adult mahi-mahi of this study. Furthermore, the absence of significant impacts in COT_{Ucrit} of the oil exposed young adult mahi-mahi in this study echo the lack of significant impacts seen in this parameter following acute 24 hr *DWH* crude oil exposure at the juvenile stage (Mager et al. 2014). However, the fact that COT_{Ucrit} is similar between the two significantly different U_{crit} speeds of the control and 8.4 μg L^{-1} ΣPAH[50] treatment group in this study indicates reduced swimming efficiency in the oil exposed treatment group with the lower U_{crit}. Given the significant differences in both U_{opt} and U_{crit} between control and 8.4 μg L^{-1} ΣPAH[50] treatment groups, this study reveals that oil-exposed young adult mahi-mahi are significantly slower, and arguably less efficient, swimmers than their non-exposed counterparts both at higher speeds and at cruising, or optimal, speeds. Additionally, given the increased rate of failed tests, largely due to mortality, at the 8.4 μg L^{-1} ΣPAH[50] exposure level, it is hypothesized that this concentration may represent a threshold above which few fish are able to survive long enough to fully complete swim performance testing. Therefore, our findings and conclusions for the 8.4 μg L^{-1} ΣPAH[50] treatment group are likely conservative.

The observed 10% decrease in U_{opt} is similar in magnitude to the 14% decrease in U_{crit}, indicating a significant impact of transient crude oil exposure on not only the high-

end of the young adult mahi-mahi swimming ability, but also on the optimal, or cruising, speed of the fish. Impacts to U_{crit} can affect the fish's ability to feed and flee effectively, as young adult mahi-mahi rely on speed and endurance to capture prey in a pelagic environment, as well as to avoid becoming prey to larger predatory pelagic species such as billfish, sharks, tuna, and larger mahi-mahi (Adams 2009). Of similar importance to life in the pelagic environment is the ability to swim efficiently at a cruising speed to facilitate ram ventilation and allow for the great distances traveled by pelagic predators in search of prey and suitable reproductive environments (Palko et al. 1982, Oxenford 1999, Block et al. 2001). Although this study documented a significant impact of *DWH* crude oil exposure to the U_{opt}, and thus swimming efficiency, of young adult mahi-mahi, there were no apparent impacts on the overall COT_{min} and COT_{Ucrit} (Fig. 4.5 and Fig. 4.6). Reduced swimming efficiency resulting from *DWH* oil exposure was recently documented for juvenile mahi-mahi (Mager et al. 2014), and the occurrence of a similar COT at two significantly different U_{crit} speeds in this study indicates a reduced swimming efficiency in the adult mahi-mahi exposed to oil. The elevated COT in the 2.3 μg L^{-1} ΣPAH[50] treatment group (Fig. 4.5 and Fig. 4.6) is likely attributable to the significantly smaller size structure of this treatment group (Table 4.1), which also supports the slightly elevated SMR seen in this treatment group (Fig. 4.5).

Research suggests that migratory animals predominantly utilize the most efficient swimming speed (i.e., U_{opt}) to travel long distances (Weihs 1973, Videler 1993, Palstra et al. 2008, Shadwick et al. 2013), and the cruising speed of unexposed young adult mahi-mahi in this study (2.9 km hr^{-1}) is similar, when size and scaling relationships are accounted for, to data reported for other highly migratory species (Gooding et al. 1981,

Videler 1993, Lutcavage et al. 2000, Sepulveda and Dickson 2000). This notable high-speed cruising ability, combined with elevated metabolic rates, specialized physiology and biochemistry, and substantial aerobic capacities, allows for these apex pelagic predators to survive in the energy depauperate oceanic pelagic environment (Stevens and Dizon 1982, Dickson 1995, Brill 1996, Korsmeyer and Dewar 2001). Therefore, the significant reduction in U_{opt} of mahi-mahi in this study indicates that crude oil exposed fish may be unable to keep up with other, non-exposed, fish in a school, potentially leaving them open to higher rates of predation and reduced foraging and spawning opportunities.

As previously mentioned, determination of aerobic scope allows for insight into whether a stressor or toxin, in this case, *DWH* crude oil, is causing a loading stress (increased SMR) or limiting stress (reduced MMR) (Mager et al. 2014). The observed reduction of aerobic scope is attributable to a significant decrease in MMR following transient crude oil exposure at exposure levels in the low parts per billion (ppb) range (8.4 ± 0.6 μg L^{-1} ΣPAH[50]), while there was no significant difference in SMR amongst treatment groups indicating that *DWH* crude oil acts as a limiting stressor (Fig. 4.5). The limiting stress indicates that crude oil exposure impairs oxygen uptake and/or oxygen transport capabilities, reducing oxygen delivery and thus limiting the overall metabolic capability of the fish. Reduced oxygen delivery in *DWH* oil exposed fish is consistent with recent reports of impaired isolated myocyte function in tuna following in vitro exposure to PAHs (Brette et al. 2014). While there are no long-term studies on the effects of reduced metabolic capacity in predatory pelagic fish species, such impacts are bound to reduce the overall fitness of the exposed animals.

To provide insight on whether decreases in swimming speed or efficiency might be due to excitation-contraction uncoupling of skeletal muscle contractions that may occur in a similar manner as described for cardiac myocytes (Brette et al. 2014), high-speed video analysis was incorporated into the analysis of a sub-set of individuals in this study. The lack of a significant relationship between oil exposure and TBF or SL at a variety of swimming speeds suggests that the reduced MMR and reduced aerobic scope are the primary drivers of the oil-induced reductions in swim performance seen at this advanced life stage. Mechanisms behind these drivers may include crude oil induced damage to gill oxygen uptake and/or cardiac output. Gill damage is a documented effect of crude oil exposure, commonly resulting in reduced oxygen uptake from damages such as filament thickening, hyperplasia, and hemorrhaging (Khan and Kiceniuk 1984, Evans 1987, Tuvikene 1995, Alkindi et al. 1996, Whitehead et al. 2011). Also, as previously mentioned, *DWH* crude oil has been shown to disrupt excitation-contraction coupling in cardiomyocytes (Brette et al. 2014). Such cardiotoxic effects are believed to cause arrhythmias that likely reduce cardiac output, a notion supported by the reduced MMR limiting aerobic scope in the current study. These effects may be more pronounced in 'high performance' pelagic teleosts, such mahi-mahi and tuna, due to the larger gill surface areas, thinner gill water-blood barrier in these species compared to other active fishes and finally the oxygen-dependent energetic requirements necessary for maintaining such specialized features (Brill et al. 2001).

Given the physiological and anatomical adaptations of apex pelagic predators, such as mahi-mahi, tuna, and billfish, which require the rapid cycling of metabolic substrates in the body to support their life processes, these species may be more sensitive

to sub-lethal crude oil exposure than other teleosts with more limited metabolic demands. This is supported by results of this study revealing significant impacts to swim performance from a relatively short exposure to *DWH* crude oil at low ΣPAH concentrations. The documented effects provide insight into the effects of sub-lethal *DWH* crude oil on whole animal physiology of these high-performance pelagic teleosts at a life stage hypothesized to be rather impervious to such damages when compared to earlier life stages of these species. Clearly, determination of the sub-lethal effects of events such as the *DWH* oil spill on all life stages of potentially impacted species is beneficial to understanding and quantifying injury to natural resources.

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interpretation which may include interpretation in the context of additional data not presented here.

CHAPTER 5:

DISCUSSION

The *DWH* oil spill occurred more than four years ago, yet scientists and resource managers are still trying to determine the cumulative effects of this unprecedented environmental event. While much of the biological damage assessment research has utilized traditional toxicity testing organisms, evidence has shown that in particular, the effects of the oil spill on high-value teleosts of the GOM could be of use in assessing the damage due to the spill. This dissertation not only provides insight into the effects of the spill on these organisms (addressing 'Chapt.1: Objective E'), but the techniques and technologies developed as part of this work also open the door for greater inclusion of open ocean pelagic fish, notably mahi-mahi and tuna, in future toxicology and damage assessment processes. Additionally, such advancements have allowed for more detailed analyses of results seen in associated studies examining the effects of the *DWH* oil spill on pelagic species.

At the center of this research lies the intersection of aquaculture and ecotoxicology, whereby the ability to capture, maintain, and spawn challenging pelagic fish in captivity opens the door for novel species to be included in ecotoxicological damage assessments. As presented in Chapter 2, it was determined that pelagic fish could be environmentally conditioned to spawn year-round using only water temperature manipulation (addressing 'Chapt. 1: Objective A'). While the chapter is focused on cobia, the reported methods were successfully applied to mahi-mahi with few, if any, modifications. Given the rapid growth rate of the tropical pelagics discussed in this dissertation, maintaining a consistent supply of experimental animals at the desired life

stage required numerous cohorts to be available virtually year-round which is only possible through the advancements in captive fish spawning reported in this dissertation.

Building upon the consistent availability of experimental animals facilitated by the research described in Chapter 2, it was necessary to develop technology and techniques that allow for conducting bioassays with the ELSs of such species. Embryos of some pelagic species, notably mahi-mahi and tuna, have a tendency to become negatively buoyant prior to hatch. This phenomenon may reduce control survival in static bioassays. The PELEC system, described in Chapter 3, details the significant improvement in performance, measured as percent control survival, of the innovative bioassay system when compared to traditional static beaker systems (addressing 'Chapt.1: Objective B'). Using this system, combined with a temperature control system, it was possible to also conduct bioassays outdoors under environmentally-relevant UV-radiation exposure. Previous research on photo-induced toxicity reveals a common trend whereby UV-radiation alters the pattern and amount of PAH degradation compared to that which occurs under low-UV exposure scenarios (i.e. under artificial laboratory lighting). The results presented in Chapter 3 yield information that suggests many of the reported effect thresholds for pelagic species in the GOM may be conservative estimates, due to the low-UV exposure conditions under which many of the bioassays were conducted (addressing 'Chapt.1: Objective C').

Clearly, the information presented in this dissertation and other associated studies have shown that the ELSs of pelagic teleosts are very sensitive to PAHs from the water accommodated fractions of *DWH* crude oil (Incardona et al. 2014, Mager et al. 2014). While cardiotoxicity occurs in ELSs at levels as low as 1.2 $\mu g\ L^{-1}$ ΣPAH[50] in mahi-

mahi (Mager et al. 2014) and 0.9 μg L^{-1} ΣPAH[50] in Southern bluefin tuna (*Thunnus maccoyii*), a closely related congeneric of the Atlantic bluefin tuna (*T. thynnus*) (Incardona et al. 2014), such effect levels have not been directly tied to acute mortality. Doing so would require bioassays to couple the LC50 data with morphological data similar to that which has been reported in recent studies on the sub-lethal effects of *DWH* crude oil on the ELSs of such species. The previously described PELEC system has been facilitating such studies due to its unique attributes suited for conducting acute toxicological bioassays on challenging ELSs of pelagic teleosts (addressing 'Chapt.1: Objective B'). Given the increasing interest in identifying and developing biomarkers for PAH-induced cardiotoxicity in fish (Incardona et al. 2009), especially for high-value species such as mahi-mahi and tuna species, the PELEC system offers key advantages to resolving this issue. The process of biomarker development requires greater numbers of individuals in each replicated treatment compared to embryo densities previously used with mahi-mahi and cobia (i.e. >20 embryos per replicate) due to the amounts of RNA that need to be harvested and the number of individuals that must be imaged at each concentration to obtain statistically significant results in cardiotoxicity studies using morphological differences and heart-rate as the quantifiable endpoints. Pursuant to this research, the PELEC system, consisting of 30 individual units allowing for proper replication and number of exposure concentrations, was transported to the Inter-American Tropical Tuna Commission (IATTC) Achotines Laboratory in the Republic of Panama to conduct toxicological bioassays on yellowfin tuna embryos. While full analysis of the study is ongoing, the LC50 and cardiotoxicity results exhibited a strong dose response and an LC50 was calculated for *DWH*-specific crude oil, representing the

first crude oil toxicological bioassay to ever be successfully completed on a tuna species. Additionally, the study yielded significant quantities of yellowfin tuna RNA that is being used to develop biomarkers for PAH-induced cardiotoxicity. Therefore, the PELEC system has allowed for novel insight on the effects of the *DWH* incident on pelagic fish species of the GOM in a number of different ways.

Aside from increasing our understanding of effects of the *DWH* incident on pelagic fish ELSs, this dissertation also provides novel insights on effects to later life-stage animals. Damages to fishery natural resources are not confined to incidences of acute mortality. In many cases, sub-lethal effects can be as damaging to a fishery, if not more so, given the typically lower effect levels required to induce sub-lethal damage. As presented in Chapter 4, young adult mahi-mahi were shown to have effect thresholds in the low part per billion (ppb) range (8.4 μg L^{-1} ΣPAH[50]), resulting in reduced upper swim speed (U_{crit}), decreased swimming efficiency, and a collapsed aerobic scope (addressing 'Chapt.1: Objective D'). This work elucidates results found in similar studies on juvenile mahi-mahi, where reduced U_{crit} and swimming efficiency were documented, yet aerobic scope impacts were not detected (Mager et al. 2014). The results presented in Chapter 4 represent the first reported whole-animal effects of the *DWH* incident on adult life-stage animals, helping to fill an important knowledge gap. Additionally, this data provides a clear link between sub-lethal *DWH* crude oil induced damage and a significant decrease in fitness. Such impacts suggest that current estimates of damage to predatory pelagic fish resources may be conservative, given the metabolic and swimming performance impacts noted in the young adult mahi-mahi and the importance of these life processes in pelagic species' survival.

CONCLUSION

The results presented in this dissertation help further our understanding of the impacts of the *DWH* oil spill on GOM pelagic fish species. Furthermore, this research contributes significantly to our knowledge of the unique physiology of the "high-performance" pelagic fish species utilized in this project. Given the ever-expanding search for hydrocarbon energy sources in the deep sea, particularly in the GOM, resource managers and those in the petroleum industry will both benefit from the results of this research, as they will have a greater understanding of the true effects of the *DWH*-incident as well as significantly greater knowledge for handling future oil spills in the region. Prior to this research, impacts of hydrocarbons in the sea have been based on "classic" test organisms, many of which are orders of magnitude more tolerant to the impacts of PAHs than the actual species and life stages of teleosts found in the pelagic region of the GOM. However, through utilization of advanced marine aquaculture techniques using high-value GOM-specific pelagic species, it is now be possible to accurately assess the effects of the *DWH* incident on such organisms. Additionally, future studies focused on embryonic stages of pelagic teleosts previously deemed virtually impossible due to the challenges of working with such delicate test organisms are now possible with the novel embryo exposure system described in Chapter 2. Impacts of this research may also be realized in the aquaculture industry, in that by demonstrating the value of advanced marine aquaculture in ecotoxicology, aquaculturists may find that there are alternative uses for their products (i.e. as bioassay test organisms) aside from the traditional end users (i.e. seafood consumers). Likewise, scientific findings on swimming energetics, metabolic rates and general physiology presented in this dissertation can be

applied to methods currently used to raise pelagic species in hatcheries and growout operations to optimize environmental parameters, enhance nutrition, decrease cannibalism, increase survival rates and improve overall aquaculture technologies. Also, portions of the GOM have been considered as candidate zones for development of offshore aquaculture. The results of this research allow farm operators to better understand the effects of future oil spills in their region and how various levels of PAH contamination may effect certain life stages of their fish crop. Finally, given the novel insight on effect thresholds and mechanisms of *DWH* crude oil toxicity in GOM pelagic teleosts, it is clear that data gleaned from other oil spills, such as the *Exxon Valdez*, and from "classic" test organisms do not always accurately represent the true effects of the *DWH* spill on the ecologically- and economically-valuable pelagic predatory fish species of the GOM. Overall, the *DWH* oil spill had a profound effect on the pelagic teleosts of the GOM, and only time will tell what the long-term effects of the spill event will be on such species.

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