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The effects of oil induced respiratory impairment on two indices of hypoxia tolerance in Atlantic croaker (*Micropogonias undulatus*)

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HIGHLIGHTS

- Oil exposure results in impaired maximum metabolic rate and aerobic scope.
- The oil exposures do not cause an increase in critical oxygen threshold.
- The oil exposures do not cause a decrease in time to loss of equilibrium.
- Repeatability of critical oxygen threshold is affected by oil exposure.
- Wide scale effects of oil exposure on hypoxia tolerance are not apparent.

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ABSTRACT

The Gulf of Mexico was home to the *Deepwater Horizon* oil spill, and is also known to exhibit seasonal declines in oxygen availability. Oil exposure in fish is known to impact oxygen uptake through cardiac impairment, which raises questions about the additive effects of these two stressors. Here we explore this question on the Atlantic croaker using two measures of hypoxia tolerance: critical oxygen threshold (P_{crit}), and time to loss of equilibrium (LOE). We first demonstrated that 24 h exposure to 10.1 and 23.2 $\mu g\ l^{-1}$ ΣPAH_{50} significantly impaired oxygen uptake. There was no effect of exposure on P_{crit} or LOE. Exposure did result in significantly different repeatability between pre- and post-exposure P_{crit}, suggesting that hypoxia tolerant individual may see greater impacts following exposure. These results suggest oil exposure does not have wide scale detrimental outcomes for hypoxia tolerance in fish, yet there may be fine scale impairments of ecological significance.

1. Introduction

The Northern Gulf of Mexico was home to the largest oil spill in United States history – the 2010 *Deepwater Horizon* oil spill – which released more than 700 million liters of crude oil (Crone and Tolstoy, 2010; Camilli et al., 2012). The time period of the spill coincided with the spawning season of many commercially important fish species, which spurred intense interest in the detrimental effects of oil on these species (see review Buskey et al., 2016). As expected, the effects of oil were most pronounced in early life stages, where sublethal pericardial edema, craniofacial and spinal deformities, significantly reduced cardiac output and a slew of transcriptional changes were observed (Incardona et al., 2014; Edmunds et al., 2015; Esbaugh et al., 2016; Xu et al., 2016, 2017; Khursigara et al., 2017). This presumably contributed to the significantly reduced survival of larval fishes observed at parts per billion concentrations of polycyclic aromatic hydrocarbons (PAHs) (Esbaugh et al., 2016; Stieglitz et al., 2016a; Khursigara et al., 2017). Interestingly, the *Deepwater Horizon* oil spill also coincided with a secondary environmental issue common to the Northern Gulf of Mexico – oxygen minimum zones.

The nutrient-rich freshwater discharge from the Mississippi River delta stimulates algal production, which in combination with a suite of physical factors can lead to large seasonal hypoxic zones in the Northern Gulf of Mexico (Rabalais et al., 2001; Bianchi et al., 2010). Importantly, hypoxia and oil exposure are both known to reduce aerobic scope by constraining maximum metabolic rate (MMR) (Claireaux et al., 2004; Davoodi and Claireaux, 2007; Mager et al., 2014; Ern et al., 2016; Stieglitz et al., 2016b). Presumably the

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effects of oil are due to perturbations in cardiac function that interfere with excitation-contraction coupling, alter adrenergic responses and limit cardiac output (Milinkovitch et al., 2013; Brette et al., 2014; Nelson et al., 2016, 2017; Khursigara et al., 2017), while the effects of hypoxia relate to perfusion and diffusion limitations that impair oxygen supply capacity. The independent underlying causes for the effects of hypoxia and oil exposure on aerobic scope suggest that these two environmental stressors may act additively to cause further physiological impairment. The available evidence generally supports this hypothesis; however, the effects are not always straightforward.

Hypoxia has been shown to enhance toxicity (Dasgupta et al., 2015) and exacerbate oil induced DNA damage and reduce egg production in sheepshead minnow (*Cyprinodon variegatus*) (Dasgupta et al., 2016; Hedgpeth and Griffitt, 2016), and a series of studies on common sole (*Solea solea*) demonstrated that oil exposure increased critical oxygen threshold (P_{crit}) (Claireaux et al., 2004; Davoodi and Claireaux, 2007). This is defined as the environmental PO_2 whereby an animal can no longer maintain standard metabolic rate (SMR) through aerobic metabolism, which is a crucial fitness metric as anaerobic metabolism is inherently unsustainable. This finding is presumably the direct result of additive effects of oil and hypoxia on MMR (Fig. 1a); however, energetic allocation toward detoxification pathways that raise SMR may also contribute. Similar findings have also been observed in the anaerobic metric, loss of equilibrium (LOE) (Mauduit et al., 2016). Hypoxia has also been demonstrated to exacerbate oil induced cardiac impairment in embryonic zebrafish (*Danio* rerio) (Cypher et al., 2017). This would suggest that oil exposure may reduce the ability of fish to make the cardiorespiratory adjustments that compensate for environmental hypoxia, such as the characteristic bradycardia and elevated stroke volume (reviewed by Gamperl and Driedzic, 2009). But perhaps the most intriguing recent findings relate to the differential effects of oil on hypoxia-tolerant and hypoxia-sensitive individuals of the European seabass (*Dicentrarchus labrax*). By incorporating the concepts of repeatability and individual variation (see review Killen et al., 2016), the work by Zhang et al. (2017) demonstrated that hypoxia-tolerant individuals – as defined by LOE – showed an increased P_{crit} relative to unexposed controls; yet hypoxia-sensitive individuals were unaffected by oil exposure. More simply, oil only impacted the most hypoxia tolerant individuals.

On this background, the current study sought to test the hypothesis that oil induced respiratory impairment will significantly reduce the hypoxia tolerance of marine fish native to the northern Gulf of Mexico. This hypothesis was tested on the Atlantic croaker (*Micropogonias undulatus*), which is an established model organism for exploring the effects of hypoxia on fish in the northern Gulf region (e.g. Thomas et al., 2007, 2015; Thomas and Rahman, 2010). We first explored the effects of non-weathered oil on respiratory performance through the examination of aerobic scope. A second series of paired experiments tested the effects of acute oil exposure on P_{crit}, both with respect to the tested population as a whole, and with respect to the effect on specific individuals. A final series of unpaired experiments examined the effects of acute oil exposure on time to LOE at 11 mmHg PO_2, which is an indicator of the combined aerobic and anaerobic capacity of the organism to withstand hypoxia.

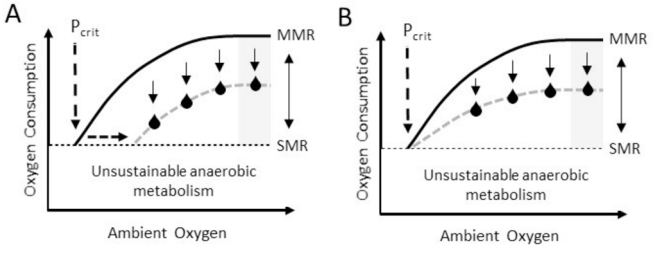

Fig. 1. Two scenarios depicting the effects of oil exposure on hypoxia tolerance in fish. Panel A: The oil induced reduction in maximum metabolic rate (MMR) observed under normoxia (grey shading) is retained across all oxygen partial pressures. This would result in a significantly elevated critical oxygen tension (P_{crit}), and a reduced time to loss of equilibrium (LOE) when exposed to oxygen levels below P_{crit}. Panel B: The oil induced reduction in MMR observed under normoxia is mitigated as environmental oxygen decreases. This would result in unaffected P_{crit} and time to LOE despite the observed reduction in MMR under normoxia.

2. Materials and methods

2.1. Experimental animals

All experimental procedures were performed under the auspices of the University of Texas at Austin Institutional Animal Care and Use Committee (AUP-2015-00147; AUP-2014-00375). All *M. undulatus* (25.6 ± 0.6 g; mean ± S.E.M; $N = 77$) were obtained from local fishermen and acclimated for at least two weeks in recirculating in-door 150 l tanks supplied with filtered seawater originating from the Corpus Christi ship channel, and maintained at 24 °C. Following acclimation, fish were tagged using 8 mm PIT tags (Biomark, Idaho, USA) following the user's guide for identification purposes, and allowed to recover for at least one week prior to experimentation. Fish were fed daily with commercial fish pellets (Aquafeed, Cargill, USA) and tanks were siphoned periodically to remove debris.

2.2. Oil exposure protocol

This study utilized a non-weathered crude oil (MASS), which originated from a Massachusetts pipeline and has been designated as an approved surrogate for the MC252 *Deepwater Horizon* source oil. MASS oil was delivered to the University of Texas Marine Science Institute through proper chain of custody and stored at 4 °C with headspace filled with N_2 to preserve volatile composition. MASS oil was used to prepare high energy water accommodated fractions (HEWAFs) at a loading rate of 1 g of oil per liter of seawater, as previously described (Esbaugh et al., 2016; Khursigara et al., 2017). All HEWAFs were prepared the day of use. Fish were individually exposed to 0, 1, or 2% nominal HEWAF concentrations (3 L solution volume) in seamless glass tanks immediately after introduction into the tanks for 24 h with light aeration. Temperature, salinity, oxygen and pH were measured before and after the exposure, and water samples were taken for PAH analysis at the beginning and end of each exposure. PAH analysis was performed commercially by ALS environmental under extraction protocol EPA 3510C and measurement protocol 8270D SIM. Samples were spiked with fluorine-d10, fluoranthene-d10 and terphenyl-d14 to assess extraction efficiency, with general recovery of >80%, >90% and >90%, respectively. Detection limits ranged from 4.5 to 20.5 ng l^{-1} depending on the specific PAH. All samples were stored at 4 °C and delivered within one week of collection. Ammonia samples were taken at the end of each exposure and assessed using a colorimetric assay (Verdouw et al., 1978). Fish were not fed during exposures.

2.3. Aerobic scope determination

Oxygen uptake (MO_2) was measured using computerized intermittent-flow respirometry (Loligo Systems, Denmark) (Lefevre et al., 2011; Chabot et al., 2016) at 24 °C immediately following the oil exposure ($N = 4–6$ per treatment). A standard chase protocol was employed to elicit maximum metabolic rate (MMR), whereby