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Exposure to Deepwater Horizon weathered crude oil increases routine metabolic demand in chub mackerel, *Scomber japonicus*

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ABSTRACT

During the 2010 Deepwater Horizon incident, the continuous release of crude oil from the damaged Macondo 252 wellhead on the ocean floor contaminated surface water habitats for pelagic fish for more than 12 weeks. The spill occurred across pelagic, neritic and benthic waters, impacting a variety of ecosystems. Chemical components of crude oil are known to disrupt cardiac function in juvenile fish, and here we investigate the effects of oil on the routine metabolic rate of chub mackerel, *Scomber japonicus*. Mackerel were exposed to artificially weathered Macondo 252 crude oil, prepared as a Water Accommodated Fraction (WAF), for 72 or 96 h. Routine metabolic rates were determined pre- and post-exposure using an intermittent-flow, swim tunnel respirometer. Routine energetic demand increased in all mackerels in response to crude oil and reached statistical significance relative to unexposed controls at 96 h. Chemical analyses of bile from exposed fish revealed elevated levels of fluorescent metabolites, confirming the bioavailability of polycyclic aromatic hydrocarbons (PAHs) in the exposure WAF. The observed increase in metabolic demand is likely attributable to the bioenergetic costs of contaminant detoxification. These results indicate that short-term exposure (i.e. days) to oil has sub-lethal toxicity to mackerel and results in physiological stress during the active spill phase of the incident.

1. Introduction

The 2010 Deepwater Horizon (DWH) disaster released an estimated 780 million liters of crude oil into the northern Gulf of Mexico over 87 days (McNutt et al., 2012), making it the largest accidental oil spill in history (Eckle et al., 2012). During the DWH event, crude oil released from the seafloor rose up through the slope waters and spread throughout mesoscale eddies in the eastern Gulf of Mexico, creating a relatively prolonged exposure window in the pelagic zone, where many fish species utilize habitat in the mixed layer. The Gulf of Mexico has a large and diverse assemblage of pelagic fishes, with representatives from nearly all of the major taxonomic families including mackerels, tunas, marlins, swordfish, sunfish, and carangids (Hoese and Moore, 1998). Thus, economic losses due to closures of recreational and commercial fisheries and negative impacts on fish populations are estimated to be in the billions of dollars over the next decade (Sumaila et al., 2012; Upton, 2011). Understanding how oil impacts the physiology of juvenile and adult pelagic fish is critical for discerning population impacts.

In addition to the more easily observed histopathological impacts on aquatic organisms (e.g. integumental lesions; Hargis et al., 1984), crude oil contains numerous chemical components that are toxic when dissolved in the water column. Of these, polycyclic aromatic hydrocarbons (PAHs) are the most prevalent and extensively studied, primarily because they are relatively water soluble and thus available for uptake by oil-exposed organisms (NRC, 2003). Metabolism of PAHs varies by compound, concentration, exposure duration, organism, life-stage, and tissue type, but many metabolites of PAHs are rapidly excreted into the urine and secreted into the bile of juvenile and adult fish (Collier et al., 2014; Tierney et al., 2013).

Decades of studies have shown that PAHs cause a range of sub-lethal effects in fish, including neoplasia, immunotoxicity, reduced growth and condition, and reduced reproductive success (as reviewed in Collier et al., 2014). Crude oil-derived PAHs are also

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directly toxic to the fish heart, as documented for the developing embryos of zebrafish (Carls et al., 2008; de Soysa et al., 2012; Hicken et al., 2011; Incardona et al., 2013), herring (Hose et al., 1996; Incardona et al., 2009), flounder (Collier et al., 2014), and tunas (Incardona et al., 2014), as well as in cardiomyocytes isolated from the hearts of juvenile bluefina and yellowfin tunas (Brette et al., 2014). In the case of growth, sublethal toxicity at the scale of individual fish can reduce the intrinsic growth and abundance of wild populations (Spromberg and Meador, 2005, 2006).

Whole-organism bioenergetics, typically measured using respirometry, provides an integrative physiological measure of sub-lethal toxicity (McKenzie et al., 2007; Whitehead, 2013). Previous respirometry studies on PAH-exposed teleosts have yielded varying results, with reported increases, decreases, and no change in metabolic rates (Table 1). However, direct comparisons across respiration studies are confounded by differences in focal species, PAH exposure protocols, water temperatures, and respirometry protocols. Previous studies have generally focused on fish from freshwater, estuarine, and benthic marine habitats, where oil spills are common. Much less is known about the sub-lethal impacts of PAHs on pelagic fish.

In this study we examine the impacts of oil on chub mackerel (*Scomber japonicus*) as a representative of the scombrid fishes (tunas, mackerels, and bonitos). Scombridae are pelagic predators with unique physiological specializations and life history traits that potentially increase their oil exposure risk, including relatively high rates of activity, mobility, and endurance. They swim continuously and with their mouths open, allowing the forward motion of the body to create a continuous flow of water across the gills (obligate ram ventilation; Magnuson, 1979). To meet a high oxygen demand, scombrids require a robust respiratory system and a well-developed circulatory system with specializations for rapid oxygen delivery. These include gills with extraordinarily large surface areas and thin membranes, a high mitochondrial content in the muscle, visceral, and gill tissues, and increased cardiac function relative to many other teleost fish (Block and Stevens, 2001; Galli et al., 2011). Large gill surface area could increase the risk of oil impairing ventilation and associated metabolic processes.

Among scombrids, chub mackerel is a favorable model species for assessing metabolic rate because they are relatively easy to acquire and maintain in captivity (Mendiola et al., 2008). Accordingly, they have been used successfully in prior flume and respiration studies (e.g. Beamish, 1984; Dickson et al., 2002; Donley and Dickson, 2000; Sepulveda and Dickson, 2000). They are also commercially important and made up 15% of global *Scrombidae* landings in 2013 (FAO, 2015). Chub mackerel live in subtropical waters (10–27 °C) of the Pacific and Indian Oceans (Fishbase, 2014b). Evolutionarily, they are closely related to Atlantic chub mackerel (*Scomber colias*) from the pelagic–neritic waters of the Gulf of Mexico and the Mediterranean Sea (Catanese et al., 2010; Cheng et al., 2011; Collette et al., 2001; Fishbase, 2014a). Here, we exposed chub mackerel to weathered MC252 crude oil at a concentration of 0.125:2500 oil:seawater to evaluate the impact of PAH exposure on pelagic predator bioenergetics.

2. Materials and methods

2.1. Experimental organisms

Chub mackerel (mean ± standard deviation: fork length = 28.4 ± 1.3 cm, weight = 273.8 ± 45.7 g) were collected in 18–21 °C surface waters off the southern coastline of California, in the California Current Large Marine Ecosystem. At this length and weight, fish were sub-adult or mature (Hernandez and Ortega, 2000). Mackerel were transported by truck to the Tuna Research

Table 1
Respiration studies on the effects of exposure to oil on the metabolic rates of teleost fishes. AMR = active metabolic rate, DO = dissolved oxygen, DWH = Deepwater Horizon, n/r = not reported, MR = metabolic rate, SMR = standard metabolic rate, WAF = water accommodated fraction, WSF = water soluble fraction.

Species	Common name	Mean fish mass (g)	Exposure duration	Additive	Concentration	Mixing method	PAH high ($\mu g\ L^{-1}$)	PAH low ($\mu g\ L^{-1}$)	Metabolic rate change	References
Pagothenia borchgrevinki	Bald notothen	50	2–72 h	Diesel fuel oil	100% WSF of 1 oil: 9 water	Stirring – WSF	n/r	n/r	Increased MR	Davison et al. (1992)
Trachinotus carolinus	Florida pompano	1.15, 1.77	24 h, 12 days	Naphthalene	n/r	n/r	300	150	Increased MR	dos Santos et al. (2006)
Solea solea	Common sole	146	5 days	Heavy fuel No. 2	1 fuel: 200 water	None	0.039	0.039	No change SMR, Decreased AMR	Davoodi and Claireaux (2007)
Solea solea	Common sole	1020	5 days	Heavy fuel No. 2	1 fuel: 200 water	None	0.039	0.039	Decreased MR	Claireaux and Davoodi (2010)
Boreogadus saida	Polar cod	30	60 min, 4 weeks	North Sea petroleum	n/r	WSF	40	4.3	Decreased MR	Christiansen et al. (2010)
Pentius sophore	Minor carp	2.68	10–168 h	Crude oil	0.2–2.5 oil: 1000 water	Shaking	n/r	n/r	Decreased MR	Prasad (1987)
Pagothenia borchgrevinki	Bald notothen	75.9	7 days	Diesel fuel oil	33% of a WSF of 1 oil: 2 water	Stirring – WSF	n/r	n/r	No change in MR at normal DO, decreased MR at low DO	Davison et al. (1993)
Macquaria novemaculeata	Australian bass	6.5	3 days	Bass Strait crude oil	1 oil: 9 water	Stirring – WAF	384	68	No change in MR	Cohen et al. (2001)
Liza aurata	Golden gray mullet	34.4	48 h	Arabian crude oil	20 g oil: 300 L water	Multiple	3.3	0.5	No change in MR	Milinkovitch et al. (2012a)
Coryphaena hippurus	Mahi-mahi	0.404–0.812	24 h	DWH slick oil	0.4%, 1.2%, and 2% WAF dilutions	WAF	30	4.2	No change in MR	Mager et al. (2014)
Scomber japonicus	Pacific chub mackerel	274	3 and 4 days	DWH weathered oil	0.125 oil: 2500 water	WAF	47.5	3.5	No change in MR (3 days), Increased MR (4 days)	This Study

and Conservation Center (Hopkins Marine Station, Stanford University, Pacific Grove, CA). The fish were acclimated for at least 1 month, at 20 °C, in a 22,300 L tank with a turn-over rate of one volume per hour.

Fish which were successfully trained to swim in a respirometer (see below for respirometer protocol) were moved to a 3780 L holding tank with a turnover rate of one volume per hour. Fish were held under natural photoperiod, with a dim moon-light over the tanks which gave enough light for mackerel to see the tank walls. Mackerel were fed a mixture of euphausiids and chopped market squid (*Loligo opalescens*) and sardines (*Sardinops sagax*) three times per week. Temperature was maintained at 20 °C in all tanks throughout the experiment.

2.2. Preparation of Water Accommodated Fraction (WAF)

WAFs were prepared in a 57 L stainless steel mixing cask with an electric motor (GE 3583, 1/4 hp, 115 VAC, Fairfield, CT) run at low speed for four hours (design specifics were modified from Barron et al., 2003). An 18 inch stainless steel drywall paddle was attached in series to the motor through a ½ in. drill chuck (Jacobs Multi Craft®, ½ × 20 in. thread, Sparks, MD) and ½ in. motor arbor (1/2 × 20 in.). The motorized mixing apparatus was affixed to the stainless steel lid by a wooden stand, with the shaft of the drywall paddle running through a hole drilled into the lid. Prior to each preparation, the stainless steel cask and drywall paddle were cleaned with three rinses each of acetone and then dichloromethane and thoroughly wiped down with paper towels.

Exposures were run at a nominal concentration of 25 ppb total PAHs in water. For each WAF preparation, 40 L of filtered seawater at 20 °C was siphoned from the holding tank directly into the mixing cask. 125 ml of DWH-MC252 artificially weathered oil was measured in a dedicated 150 ml glass graduated cylinder and then combined with the seawater in the mixing cask. Artificial weathering included gently mixing the sample while heating it to 90–105 °C to reduce the mass by 33–38% (Incardona et al., 2014). Upon completion of mixing in the cask, the WAF preparation was poured into a recirculating exposure tank, which was identical in design and volume to the holding tank.

2.3. Water sampling and water quality analysis

Water samples were taken from the exposure tank starting 20 min after introduction of the WAF preparation. Subsequent water samples were taken every 24 h until completion of the exposure protocol. Subsurface water samples (550 ml) were collected by submerging a closed separatory funnel into the exposure tank and opening the stopcock. The water sample was divided into two equal aliquots of 250 ml. One aliquot was filtered through 2.7 and 0.7 μm glass microfiber filters (GF/D and GF/F, respectively, Whatman/GE Healthcare, Piscataway, NJ) held in a glass filter holder apparatus (VWR International, Radnor, PA). The filtered and unfiltered water samples were then stored at 4 °C for water chemistry analysis. Water quality in the exposure tank was also monitored during oil exposures. Parameters included temperature and dissolved oxygen (YSI ProODO™ probe, Yellow Springs, OH), pH (Litmus paper strips, Cole Parmer, Vernon Hills, IL), salinity (refractometer, Grainger Industrial, Salinas, CA) and ammonia (Hach® Ammonia test kit, model NI-SA, Loveland, CO). Temperature, dissolved oxygen and pH were all measured on a daily basis, while salinity and ammonia were measured at the beginning and end of each exposure.

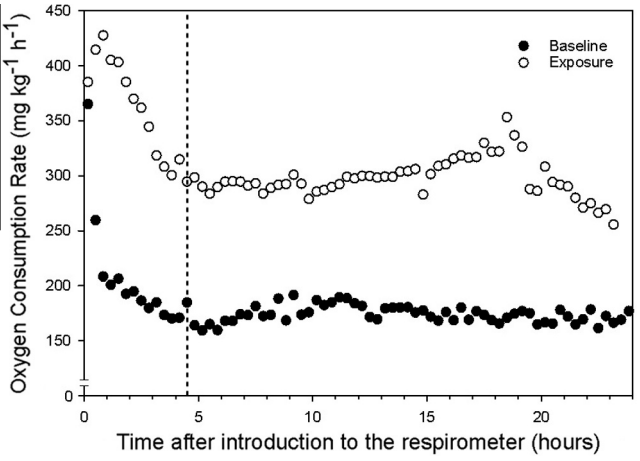

Fig. 1. Example of baseline routine and post-exposure respiration trials for an individual mackerel. This individual was exposed to oil for 96 h. The dashed black line marks the end 4.5 h period upon entry to the respirometer when the fish was allowed to recover from the stress of handling. Data during this period was not included in the analysis.

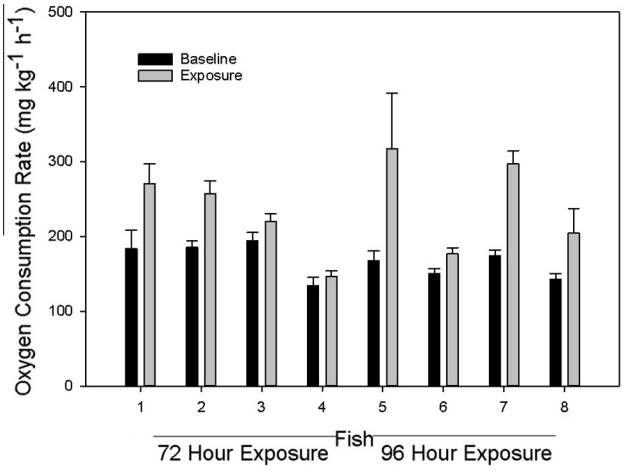

Fig. 2. Mean oxygen consumption rate, ± standard deviation, of individual Pacific mackerel exposed to oil for 72 and 96 h.

2.4. Exposure protocol

Prior to each oil exposure, an initial seawater rinse of 1000 L was run through the exposure tank's recirculating system to flush out any standing water. Following removal of the rinse water, 2500 L of seawater was pumped into the exposure tank, which was maintained at a temperature of 20 ± 0.2 °C with a dual heater (Titanium I10 heater, GLO-QUARTZ, Mentor, OH) and chiller system (Cyclone chiller, Aqua-Logic, San Diego, CA).

The WAF was added to the exposure tank and allowed to mix for 20 min. Fish were then collected from the holding tank and placed in the exposure tank. For each experiment, two flume-trained fish (see respirometer protocol) were exposed simultaneously in the experimental regime, one fish for 72 h and the other for 96 h. The tank was covered with a clear, plastic sheet for the duration of the exposures. After each exposure duration, one fish was removed from the exposure tank and transferred to the respirometer.

2.5. Respirometer protocol

For conducting the respiration trials, a 10 L intermittent flow, swim tunnel respirometer (Loligo, Denmark) was used to measure metabolic rates both before and after exposure to oil. The respirometer was supplied with seawater from a 1000 L reservoir. Dissolved oxygen concentration and temperature was measured

with a fiberoptic oxygen dipping probe and temperature probe, respectively (Presens, Germany), and recorded with Oxyview data acquisition software (Presens, Germany).

Measured metabolic rates are often impacted by a fish's lack of familiarity with swimming in a flume, requiring acclimation and training prior to experimental trials (Blank et al., 2007a,b). An extensive training regime was conducted with all mackerel prior to exposure trials to establish the routine metabolic rates within the flume environment for each individual. Training included an initial phase where each individual mackerel was introduced into the respirometer and allowed to swim at 1 body length per second for at least 4 h. During this initial training period, fish were manually assisted as necessary to ensure that they did not repeatedly hit or rest on the back grate of the respirometer, possibly resulting in injury to their caudal fins. This is a common problem when mackerel are first introduced to the respirometer, and many individual fish had to be trained several times before they were able to swim consistently for over 24 h.

Once fish successfully passed the training exercises, they were returned to the holding tank for at least 72 h. For pre-exposure routine metabolic rate (baseline) trials, fish were fasted for at least 48 h and then introduced into the respirometer for 24 h at a speed of 1 body length per second. Before oxygen respiration measurements began, fish were allowed to acclimate to the respirometer for 4.5 h to allow the effects of stress of handling and excitation from introduction to the flume to subside. After the pre-exposure trial, fish were again returned to the holding tank for at least 72 h. Fish were then randomly assigned exposure durations and the respiration protocol was repeated post-exposure (see Fig. 1). Differences in pre- and post-exposure routine metabolic rates at each experimental duration were tested using paired *t*-tests.

2.6. Bile protocol

To assess the internal dose of oil compounds in exposed fish, PAH metabolites were measured in the bile to provide a relative measure of PAH uptake in addition to the nominal exposure concentration in the surrounding water (Beyer et al., 2010). Bile was collected from oil-exposed fish post-experiment and after euthanization. As a control, bile was also collected from 3 trained mackerel after swimming in the exposure tank without oil and then being subjected to the respirometer protocol.

To collect bile, fish were euthanized by pithing and then the body cavity was opened with a clean scalpel. Using a new scalpel blade, the internal organs were severed at the esophagus and the entire internal organ mass was removed and placed on aluminum foil. The gall bladder was identified as the elongated green sac-like organ, and if there was blood on the outside of the gall bladder it was rinsed with distilled water from a squirt bottle. The gall bladder was gripped at the bile duct and then gently separated from the liver with a scalpel.

To collect bile from the gall bladder the blind end was positioned over the mouth of an amber vial and punctured with a fine scalpel. Bile was allowed to drip into the vial, and if more was needed than dripped out, the gall bladder was dipped into the vial and the blunt side of the scalpel blade was placed against it and the vial mouth and the gall bladder was pulled up so as to force the remaining liquid into the vial. The vial was placed immediately into a −20 °C freezer. All tools were rinsed with deionized water and then with isopropanol, and scalpel blades were discarded. The bile samples were sent to the NOAA laboratory in Seattle, WA on ice, and metabolites of biologically pertinent oil compounds (phenanthrene, benzo(α)pyrene, naphthalene and pyrene) were analyzed along with total protein concentration, following protocols described in (Yanagida et al., 2012).

3. Results

Fish were introduced into the oil exposed seawater tank environment that was visibly brown in color and turbid. The volatile fumes around the tank were noxious to humans without respiratory masks, and once fish were inside the tank they were difficult to locate. When visually observed, paired fish swam together within the tank.

The fish were exposed in the tank environment to a total dissolved (filtered) PAH concentration that declined over the course of the exposure trials from a high concentration of 47.5 ± 13.7 μg l^{-1} (mean ± standard deviation) at the start of the experimental procedure to a low of 3.5 ± 1.3 μg l^{-1} at 72 h, and 3.7 ± 1.1 μg l^{-1} at 96 h (Fig. 4). The PAH concentration decline in the tank was greatest within the first 24 h and concentrations remained relatively stable for the remainder of the exposure. The total unfiltered PAH concentration went from 114.3 ± 41.4 μg l^{-1} at the start of exposure trials to 91.6 ± 31.2 μg l^{-1} after 72 h and 71.0 ± 26.9 μg l^{-1} after 96 h (Fig. 5). The unfiltered PAH concentrations remained relatively stable for 48 h and then began to decline. The variation in unfiltered concentrations between experiments was greater than for the filtered concentrations. Salinity, pH, and ammonia remained constant and within biologically optimal ranges throughout all experiments.

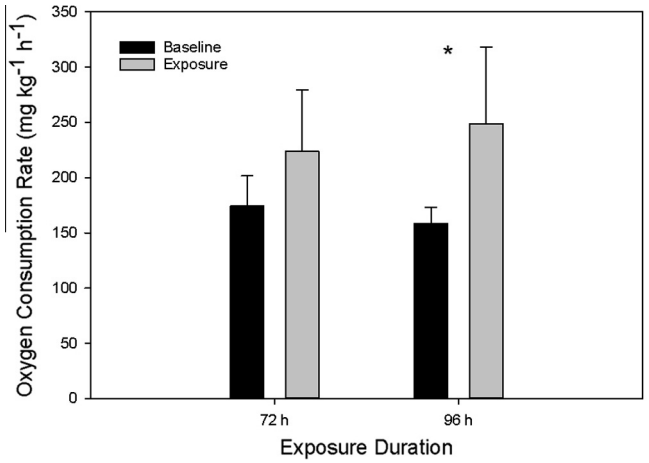

Fig. 3. Mean oxygen consumption rate, ± standard deviation, for pre- and post-oil exposures. Exposure durations were 72 and 96 h. An asterisk (*) indicates a significant difference between pre- and post-exposure metabolic rates.

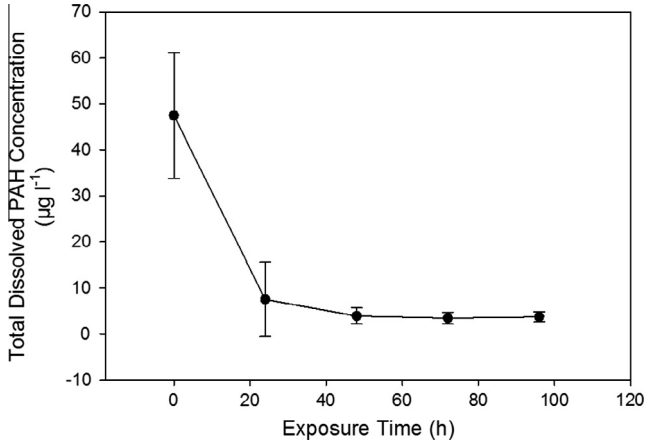

Fig. 4. Mean ± standard deviation of total dissolved (filtered) PAH concentrations.

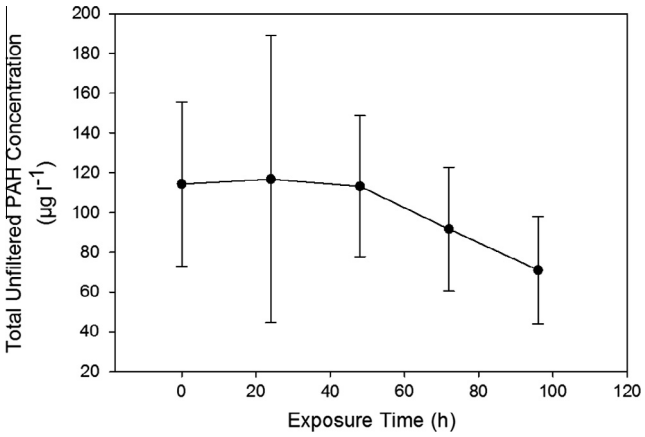

Fig. 5. Mean ± standard deviation of unfiltered PAH concentrations.

Pairs of trained mackerel were exposed to oil in four trials. Mean pre-exposure routine metabolic rate for mackerels was 167 ± 22 mg O_2 kg^{-1} h^{-1} (N = 8 fish) when swimming at 1 body length per second at 20 °C. In all cases, there was an increase in routine metabolic rates following oil exposure (Fig. 2). Exposure to oil for 96 h resulted in a statistically significant increase in metabolic rates (mean post-exposure RMR = 249 ± 69 mg O_2 kg^{-1} h^{-1}, Paired T-test, T-value = 3.21, P = 0.049). Oxygen consumption also increased in 72 h exposures, but the increase was not statistically significant (mean post-exposure RMR = 224 ± 56 mg O_2 kg^{-1} h^{-1}, Paired t-test, T-value = 2.77, P = 0.070) (Fig. 3).

The bile of mackerel that were exposed for 72 h showed the greatest increase in each of the four PAH metabolites analyzed, compared to the control mackerel (Fig. 6). On average metabolites of phenanthrene increased from 1.73 mg/ml in the control fish to 1133 mg/ml in fish exposed to oil for 72 h, benzo(α)pyrene increased from 0.05 mg/ml to 6.9 mg/ml, naphthalene increased from 7.03 mg/ml to 3200 mg/ml, and pyrene increased from 0.12 mg/ml to 52 mg/ml. The mackerel exposed for 96 h also had increased concentrations of metabolites of phenanthrene, benzo(α)pyrene, naphthalene and pyrene at 293 mg/ml, 1.9 mg/ml, 970 mg/ml, and 14 mg/ml, respectively.

4. Discussion

This study differs from other respiration exposure studies in that it: (1) exposes sub-adults and adults of a pelagic species for multiple days, (2) employs a respirometry protocol that reduces confounding factors such as SDA, stress from handling, and stress from unfamiliarity with the respirometer, and (3) uses a modified method of fractionating oil into seawater at large volumes. Mackerel exposures were conducted to discern the whole-animal metabolic response and potential for injury upon swimming through a patch of pelagic ocean habitat contaminated with oil droplets. Notably, mackerel were capable of swimming in oil over a period of 72–96 h. During this interval, fish schooled with conspecifics and were successful in negotiating the 2.5 m diameter tank despite turbid conditions. Thus, the basic swimming behavior of these obligate ram ventilators was not evidently impaired at the concentrations of oil tested. When examined over 72 h, there was a trend toward higher metabolic rates, but this was not significantly different from controls. For the longer 96 h exposure interval, however, the animals exhibited an increase in oxygen consumption that was significantly elevated from routine metabolic rate.

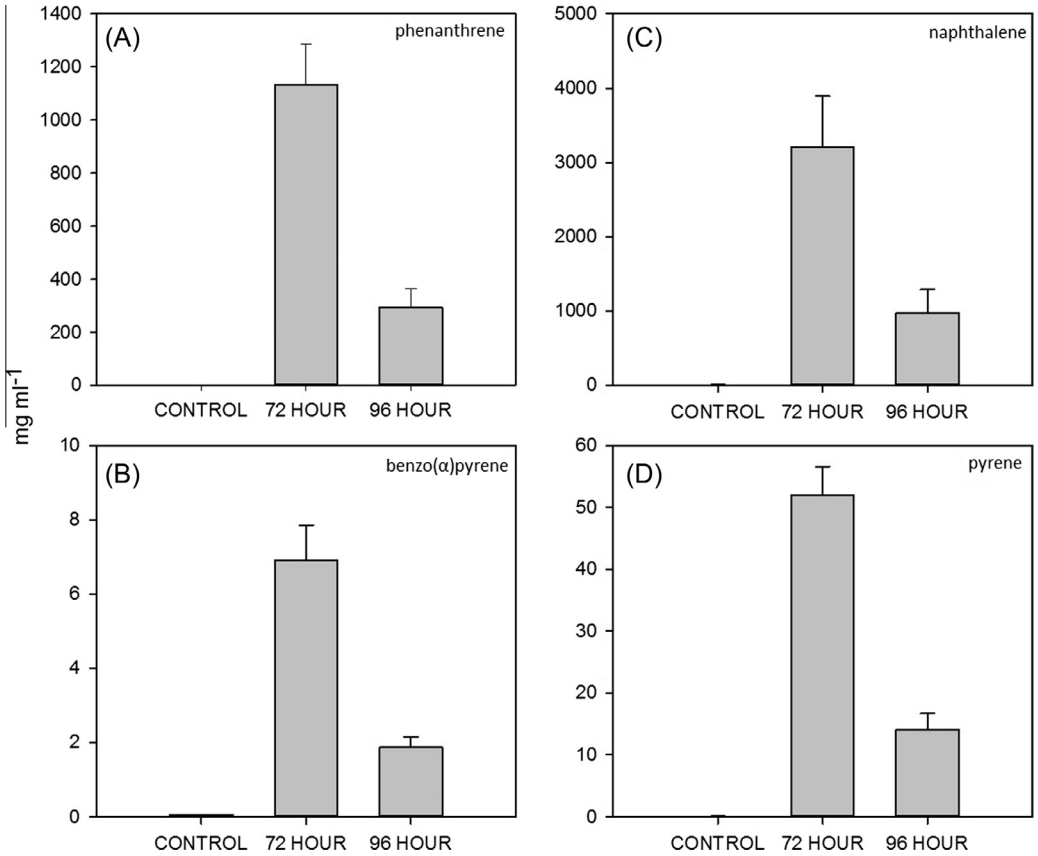

Fig. 6. Mean ± standard deviation of biliary concentrations of metabolites of four PAHs, (A) phenanthrene, (B) benzo(α)pyrene, (C) naphthalene, and (D) pyrene, in control mackerel and mackerel exposed to oil for 72 and 96 h.

The increase in metabolism at 96 h was most likely due to a physiological stress response as well as an induction of xenobiotic metabolizing enzymes. The stress response is associated with increases in circulating catecholamine and cortisol, which in turn increase energy production via increases in plasma glucose and lactate (George et al., 2013). Catecholamines bind to β-adrenoreceptors, amplifying oxygen uptake pathways and rate of delivery (Randall and Perry, 1992). The aryl hydrocarbon receptor (AHR) signaling pathway is responsible for induction of xenobiotic metabolizing enzymes such as CYP1a (Kim et al., 2013). Upregulation of these metabolizing enzymes and associated ATP demands for detoxification would also result in the increased metabolic rates observed in our study. Given that stress may have increased with the longer duration exposures, future experiments should consider monitoring cortisol in tandem with metabolism. The 24 h period in which some fish were alone in the exposure tank, following removal of the 72 h exposure fish, could have also contributed to elevated stress.

4.1. Comparison with previous studies

The routine metabolic rates measured for unexposed mackerels in this study (mean = 167 ± 22 mg O_2 kg^{-1} h^{-1}) are similar to those reported for mackerel in previous studies conducted at similar temperatures (Dickson et al., 2002; Shadwick and Steffensen, 2000). Fish behavior and swimming in both the exposure tank and the respirometer did not appear abnormal or labored.

Prior studies show increases, decreases, and no change in metabolic rates as a result of exposure to oil and PAHs (Table 1). As observed here for mackerel, PAH exposures have previously been shown to increase metabolic rates in the polar Antarctic bald notothen (*Pagothenia borchgrevinki*) as well as the tropical Florida pompano (*Trachinotus carolinus*). Bald notothen were exposed to much higher concentrations of oil than used in the current study (277:2500 relative to 0.125:2500 oil:seawater here) although exposure durations were similar (Davison et al., 1992). Florida pompano were exposed to only one PAH, naphthalene, and at much higher concentrations (300–150 ppb naphthalene relative to 3–47 ppb total PAHs here) (dos Santos et al., 2006).

By contrast, several prior studies reported a decrease in metabolic rates after exposure to oil. For example, polar cod (*Boreogadus saida*) exposed to similar concentrations of PAHs but for shorter and longer durations (1 h and 4 weeks, respectively) showed decreased metabolic rates. However, control and exposed fish were both fed prior to respirometry, and thus the measured metabolic rates included the energetic cost of specific dynamic action (SDA) (Christiansen et al., 2010). Exposure to PAHs may have impeded digestion or assimilation, resulting in decreased SDA and associated metabolic rates relative to the control, which included unimpeded digestion and SDA. In our study, fish were fasted for both their baseline and exposure runs, eliminating the confounding effects of SDA. In one study where oil was simply added to the surface of the water at much higher concentrations (12.5:2500 oil:seawater relative to 0.125:2500 in our study), adult common sole (*Solea solea*) exposed in this way for 5 days showed a decline or no change in metabolic rates (Claireaux and Davoodi, 2010; Davoodi and Claireaux, 2007). The lack of mixing and higher concentration of oil could have reduced oxygen diffusion across the gills, thereby limiting metabolic rates (Milinkovitch et al., 2012a). Similarly, minor carp (*Pentius sophore*) exposed to crude oil without thorough mixing exhibited decreased metabolic rates (Prasad, 1987).

Other studies report no change in metabolic rates following exposure to oil. Bald notothen exposed to oil for 7 days showed no change in metabolic rate, but rates did decrease under low dissolved oxygen conditions (Davison et al., 1993). Golden gray mullet (*Liza aurata*) exposed to lower PAH concentrations (0.5–3.3 μg L^{-1} relative to 3.5–47.5 μg L^{-1} here) for a shorter period of time (48 h relative to 72 and 96 h) showed no significant change in metabolic rates (Milinkovitch et al., 2012a). The higher concentration of PAHs and longer exposure time in our study could explain the differing results. Australian bass (*Macquaria novemaculeata*) exposed to higher concentrations of PAHs (68–384 μg L^{-1} relative to 3.5–47.5 μg L^{-1} here) showed no change in metabolic rate, but fish were only allowed to acclimate in the respirometer for 15 min, increasing the likelihood that stress from handling affected the results (Cohen et al., 2001). Mackerel in our study were trained in multiple trials and then acclimated to the respirometer for 4.5 h before final routine measurement, thereby minimizing any influence of handling stress. Young of the year mahi-mahi (*Coryphaena hippurus*) exposed to oil and similar concentrations of PAHs showed no change in standard or active metabolic rates (Mager et al., 2014), but mahi were only exposed to oil for 24 h (relative to 72 or 96 h here), meaning the shorter exposure time could explain the difference in results.

4.2. Bile

Each of the four PAH metabolites measured in the bile of mackerel increased in the oil exposure fish, but concentrations were 70% lower in fish exposed for 96 h relative to fish exposed for 72 h. This is consistent with the decline in dissolved waterborne PAH concentrations during the exposure, and indicates that PAHs taken up during the initial exposure period induced a metabolic response. Biliary PAH concentrations also increased in golden gray mullet exposed to oil (Milinkovitch et al., 2012b).

4.3. Dissociation of oil in seawater using the Water Accommodated Fraction (WAF) method

Oil is a complex chemical mixture containing hundreds or thousands of compounds. In order to expose aquatic organisms to the toxicological compounds contained within oil in an environmentally realistic exposure profile – one that mimicked, to the extent possible, conditions during the DWH oil spill – it was necessary to thoroughly mix oil into water. The most established and consistent method for mixing oil into water is the creation of a WAF of oil (Incardona et al., 2013; Singer et al., 2000). This method blends oil and water together at high speeds for a defined amount of time, producing a thoroughly mixed sample of oil dissolved in a small amount of water (typically less than 4 L). The WAF can then be used to either expose an organism directly or be filtered or diluted to a desired concentration.

We modified an existing method for mixing larger volumes of water (40 L) and oil together (from Barron et al., 2003), so that the WAF contained oil droplets in the solution, similar to conditions that fish might have encountered in pelagic habitats of the Gulf of Mexico during the DWH oil spill (Adcroft et al., 2010; Diercks et al., 2010). This method also increases the scale of the WAF, making it possible to expose larger juvenile and adult pelagic fish in greater volumes of seawater.

When measuring PAH levels in unfiltered and filtered seawater samples, the concentrations are consistently higher in unfiltered samples because they contain the dissolved as well as the particulate fraction of PAHs. On a total mass basis, however, the filtered fraction is proportionally more toxic to fish because dissolved PAHs absorb through the gills more easily (McKim and Erickson, 1991). The particulate (droplet) PAHs are more likely to have a physical effect – i.e., oiling the gills, thereby interfering with oxygen uptake (Claireaux and Davoodi, 2010). Both toxicological and physical effects of oil may have an impact on metabolism and thus both must be taken into consideration.

The unfiltered PAH concentrations in the exposure tank varied slightly between each experiment (Fig. 5). This variability is likely due to differences in the amount of oil that dissolves sufficiently in the seawater. After the WAF mixed for the allotted time (4 h) there were often droplets of oil on the sides of the stainless steel cask and top of the mixing vessel that did not go into solution. Droplets also appeared on the walls of the exposure tank during exposure trials. These globular droplets varied in size and shape and indicate that certain volumes of oil did not go into solution.

The filtered (dissolved) PAH concentrations were highly consistent across each of the experiments. This can be attributed to the fact that the mechanical action of the stirring paddle is consistent in dissociating similar amounts of dissolved PAHs in each experiment. While a certain amount of oil globules will stick to the sides of the mixing vessel, the globules that remain in the water are more consistently dissociated during each WAF. The concentrations of unfiltered and filtered toxic PAHs in the WAF mixture were fairly consistent between trials, indicating that the modified WAF method is suitable for conducting replicate experiments.

5. Conclusions

Experiments examining the effects of crude oil on mackerel indicate oil exposure has a significant impact on metabolic rates after four days. As an active, pelagic species, mackerel have increased aerobic potential and tissues enriched in mitochondrial capacity. Their high movement and endurance ability provides a mechanism through which they may be able to actively move away from contaminated regions. This study indicates short-term (i.e. days) exposure to oil has sub-lethal toxicity to chub mackerel, increasing energy expenditures and consuming energy that could otherwise be used for growth or reproduction. However, we cannot yet evaluate the long-term consequences of oil exposure on lifetime fitness. Longer post-exposure observation might yield more information about the potential effects of oil on survival and fitness. Oil exposure may have substantial effects on growth, fecundity or gamete production which would not be evident in this study. Additionally, aerobic scope (the metabolic range between resting and maximal metabolic rates) may have been impacted, limiting aerobic capacity for challenging behaviors such as predator avoidance, prey capture, and reproduction.

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