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Metabolic performance of the coral reef fish *Siganus guttatus* exposed to combinations of water borne diesel, an anionic surfactant and elevated temperature in Indonesia

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ABSTRACT

Jakarta Bay in Indonesia and its offshore island chain, the Thousand Islands, are facing extreme pollution. Surfactants and diesel-borne compounds from sewage and bilge water discharges are common pollutants. However, knowledge of their effects on reef fish physiology is scarce. This study investigated combined and single effects of a) the water accommodated fraction of diesel (WAF-D, determined by ΣEPA polycyclic aromatic hydrocarbons (PAHs)) and b) the surfactant linear alkylbenzene sulfonate (LAS) on metabolic performance of the coral reef fish *Siganus guttatus*. Responses to combinations of each pollutant with elevated temperature (+3 °C) were determined. Short-term exposure to WAF-D led to a significant decrease in standard metabolic rates, while LAS led to an increase. During combined exposure, metabolic depression was observed. Effects of pollutants were not amplified by elevated temperature. This study highlights the need to reduce import of these pollutants and to avoid negative long-term effects on fish health.

1. Introduction

Coral reefs are increasingly under pressure due to the simultaneous impact of multiple environmental stressors. As a result of growing urbanization and industrialization in coastal areas, especially in many developing countries, coral reefs are degrading at a fast pace. At least 19% of reefs worldwide have been irreversibly destroyed (Wilkinson, 2008) and over 60% are considered at immediate risk from direct human activities (Burke et al., 2012). Coral reefs are of huge economic and environmental importance in many developing countries, supporting fisheries and tourist sectors and providing many different habitats with high productivity and diversity. About one-third of all fish species worldwide occur in coral reefs (Crabbe et al., 2009).

Some of the most pressing stressors on coral reefs are from local sources, such as eutrophication due to sewage and terrestrial run-off, increased sedimentation, pollution with toxic chemicals, and overfishing, as well as global stressors such as ocean warming. These stressors influence overall species abundances, as well as composition and diversity of coral reef communities. The intensity and diversity of anthropogenic stressors has increased rapidly over the last decades, especially regarding toxic chemicals, such as organic pollutants. Research into cumulative and interactive impacts of multiple stressors is still not very frequent (Crain et al., 2008) and even less so on coral reef fish. Effects of multiple stressors have mostly been assumed to be additive (Halpern et al., 2007). However, current literature indicates that some chemicals occurring in mixtures can interact, resulting in multiplicative effects (Crain et al., 2008; Laetz et al., 2009). This interaction can be synergistic (i.e. amplification) or antagonistic (i.e. reduction) (Dunne, 2010).

In Jakarta, a megacity with around 25 million inhabitants in the Greater Jakarta Metropolitan Area, and along its offshore island chain Pulau Seribu ("Thousand Islands"), multiple stressors have caused severe degradation of coral reef ecosystems (Cleary et al., 2014; Baum et al., 2015). Jakarta Bay (JB) faces severe eutrophication coupled with sedimentation (Baum et al., 2015). Several rivers with a combined catchment area of 2000 km^2 discharge directly into the bay, transporting large amounts of sewage and industrial effluents with high pollutant levels (Rees et al., 1999).

With a total population of around 22,700 people, the island chain is densely populated (BPS, 2012).

Along the islands and in Jakarta Bay, numerous stakeholders are presently involved in fishing (around 40,000 fishermen, BPS, 2012), sand mining, tourism, aquaculture and transport. This has led to intensive boat traffic, both from smaller boats such as fishermen boats, ferries and tourist boats as well as from larger vessels and tankers (Bengen et al., 2006). Through the release of bilge and ballast water, both from large tankers to small fishing boats, organic contaminants such as

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polycyclic aromatic hydrocarbons (PAHs) can enter marine waters as part of the water accommodated fraction (WAF) of fossil fuels, including diesel fuel used by small fishing boats. Another ubiquitous pollutant class is surfactants. In untreated effluents, certain classes of surfactants can occur in concentrations that are toxic to aquatic organisms (Ankley and Burkhard, 1992). Anionic surfactants such as linear alkyl benzene sulfonates (LAS) are widely used as domestic detergents. LAS are quickly degraded in water and often found below detection limits, however high amounts of linear alkylbenzenes can be found associated with washing of boats in reef areas as well as in areas with extremely high population densities and poor sewage treatment. In JB high amounts of linear alkylbenzenes were detected in water recently, indicating that the bay receives very poorly treated sewage (Koike et al., 2012).

Local anthropogenic stressors such as the above mentioned organic chemicals may be exacerbated by geographically broader stressors such as temperature changes related to climate change. In combination, these can result in increased vulnerability of the ecosystem (Risk et al., 2001; Knowlton and Jackson, 2008; Pörtner et al., 2014). A global rise in sea surface temperature of up to 4.8 °C within this century has been predicted (IPCC, 2013). Higher water temperature can increase reaction rates as well as metabolism and in turn increase the sensitivity of organisms to contaminants (Falahudin et al., 2012; Beyer et al., 2014).

Because of the key position of fish in many marine food webs and their economic importance, fish are suitable indicator species. Fish can be exposed to diesel-borne compounds and LAS in the water column (Logan, 2007). Numerous studies have addressed biochemical responses of adult fish and embryonic development to hydrocarbons, either as WAF of fossil fuels (e.g. Agamy, 2012, 2013), or as single PAHs (Baussant et al., 2001; Dos Santos et al., 2006; Carls and Meador, 2009), as well as to LAS (Zaccone et al., 1985; Lewis, 1991) at cellular levels. However, very few have looked at whole-body responses in adult fish (e.g. Maki, 1979; Davoodi and Claireaux, 2007; Christiansen et al., 2010). In comparison to many cellular indicators such as the activity of specific enzymes, metabolic rates reflect the overall energetic requirements of an individual fish and thus detect overall stress levels, even when organisms are exposed to sublethal concentrations of contaminants. An increase in respiration can indicate acute stress and higher oxygen demand, while a decrease can either occur due to acclimation or depression due to the toxic effects of a stressor (Guppy and Withers, 1999). Respirometry is a well-established and acknowledged method to estimate the metabolic rate and indentify stress levels caused by pollutants and temperature stress in fishes (Schreck, 1990). The standard metabolic rate (SMR) refers to respiration rates for basal physiological processes in resting and unfed fish, while the routine metabolic rate (RMR) reflects respiration that includes energy for locomotion, digestion, etc. (Sokolova et al., 2012). By measuring the maximal metabolic rate (MMR) under high stress, the aerobic metabolic scope (AMS), i.e. the energy that is available for fitness-related functions (Fry, 1971), can be estimated as the difference to the SMR.

Organic pollutants are of growing concern to marine ecosystems due to their increasing presence close to large urban areas, their high number of different individual compounds and the high likelihood of interactive effects. Hence, future research should focus more on detecting interactive effects in order to predict changes in ecosystems such as coral reefs more accurately.

To our knowledge, there are no publications describing effects of diesel-borne compounds and LAS in combination with increased water temperature on fish metabolism. This study investigates in acute exposure experiments the potentially interactive effects of the two stressors WAF-D (water accommodated fraction of diesel) and LAS combined with elevated temperature, on whole animal oxygen consumption rates in juvenile *Siganus guttatus* (Siganidae, Rabbitfishes), an important herbivore in coral reefs (Fox et al., 2009) and economically important fish species in the JB/Thousand Islands area (Kusumanti, 2013). With regard to the short-term exposure of diesel-borne compounds and surfactants close to coral reefs characterized by high boat traffic (bilge water discharge) and sewage run-off, this study aimed to determine how respiration rate of *S. guttatus* is affected by WAF-D and LAS a) separately and b) in combination, as well as c) under increased water temperature.

2. Methods

2.1. Experimental fish

Specimens of *S. guttatus*, collected along the Thousand Island chain situated north of Jakarta, were bought from the ornamental fish trader PT Dinar (http://dinardarumlestari.blogspot.de; 04.03.2015) in Jakarta. Two large semi-flow through cylindrical tanks (500 L) were used to acclimatize the fish for 14 days prior to experiments. Fifty percent of the water in the tank was exchanged daily with filtered sea water (0.2 μm). Water circulation within the keeping tank was created by using two aquaria pumps (Hydor korallia, www.hydor.com). The water used for treatment tanks and for the experiments was obtained directly from a nearby reef and UV- sterilized, as well as treated with calcium hypochlorite solution before storage. The water parameters salinity, temperature, pH and dissolved oxygen (DO) were monitored daily in the morning using a WTW 340i Multiparameter system. Additionally, temperature data loggers (HOBO Pendants from www.onsetcomp.com) were deployed in all tanks to measure fluctuations in temperature. All specimens were exposed to a constant 12 h light:12 h dark cycle and were fed daily using commercial dry fish feeds ad libitum.

2.2. Experimental protocol

Fish were first exposed to one of three different treatments at 28 °C, to resemble natural temperature conditions found in the reef: "control" or exposure to one of the two different pollutants linear alkylbenzene sulfonate "LAS" or the water accommodated fraction of diesel "WAF-D". These treatments were then repeated under increased water temperatures with three degrees above control temperature (31 °C): elevated water temperature "temp" (31 °C) and a combination of either LAS or WAF-D with elevated temperature; "LAS + temp" and "WAF-D + temp". During the exposure to each treatment, metabolic rates were measured continuously (see below for details). All fish were juveniles with an average wet weight of 23.4 g ± 4.5. No difference in weight between treatments was present ($p = 0.393$) (see Table S6 for wet weight measurements of each fish).

Salinity, pH and dissolved oxygen were measured at the start and end of each experiment, using a WTW 340i Multiparameter system. Additionally a temperature data logger (HOBO Pendant from www.onsetcomp.com) was deployed in the glass aquarium. Temperature in treatments with elevated temperature was maintained using Eheim Jäger 150 W aquarium heaters (www.eheim.com). A 12 h light:12 h dark cycle was adjusted to simulate the natural conditions. Each incubation chamber was shielded at the sides with a black plastic bag to prevent visual contact between fish. After each experiment, the entire experimental set-up was cleaned thoroughly with a mild hypochlorite solution followed by rinsing with fresh- and distilled water. All specimens were starved for 24 h prior to respirometry to remove any confounding effects of feeding on metabolic rate (Ross et al., 1992; Jordan and Steffensen, 2007).

2.3. Respirometry

Automated intermittent-flow-through respiration runs (Fig. 1) were conducted with always three fish running in parallel replicates for each treatment. Experiments started at around 6:30 pm and ended at around 4:30 pm the following day (total duration: 16.7 h ± 0.5). Experiments were conducted at the Pulau Pari Research Station (5°51.756′S,