Multiple anthropogenic stressors in Indonesia

Impacts on metabolism, spatial distribution and community composition of coral reef organisms

A dissertation by

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"In all things of nature, there is something of the marvelous"

— Artistotle

"Pollution should never be the price of prosperity"

— Al Gore

Summary

Coral reefs, especially those located near larger urban areas, are challenged by multiple anthropogenic stressors. Both local (e.g. pollution) and global stressors (e.g. ocean warming) are increasing in impact, range and frequency due to growing urbanization, industrialization and coastal development. Stressors can interact to various degrees with each other, however considerable knowledge gaps exist regarding their combined effects on species and ecosystem levels. These gaps have to be addressed if underlying mechanisms in coral reef function and degradation are to be understood. This thesis tries to contribute to a better understanding of the processes that shape coral reef communities under the influence of multiple anthropogenic stressors. Spatial impacts of stressors as well as physiological responses to pollutants and reduced water quality were determined. Coral reefs in Jakarta Bay (JB) and along the outer Thousand Islands off Jakarta in Indonesia, one of the largest megacities worldwide, are facing extreme environmental pressure by anthropogenic stressors and thus represent an ideal area to assess the combined effects of multiple anthropogenic stressors on coral reefs.

In the first part of this thesis, the spatial impact of stressors on local and regional scales of coral reefs north of Jakarta was investigated. Results indicate that the direct impact of Jakarta is mainly restricted to nearshore reefs, separating reefs in JB from reefs along the outer Thousand Islands. A spatial patchwork of differentially degraded reefs is present along the islands as a result of localized anthropogenic effects rather than regional gradients. Over 80 % of variation in benthic community composition was driven by eutrophication related factors. In addition, surfactants and diesel-borne compounds from sewage and bilge water discharges were found to be very common local pollutants, both in JB and along the outer Thousand Islands. Thus, the spatial structure of reefs in the JB/Thousand Islands reef complex is directly related to intense anthropogenic pressure from local as well as regional sources. Extremely low coral cover (2 %) and shifts to soft coral dominance, as well as 80 % lower fish abundance were found in JB compared to the outer Thousand Islands. Improved spatial management that accounts for both local and regional stressors is needed for effective marine conservation in the area.

The second part of this thesis focused on physiological responses of key coral reef organisms, the economically important fish *Siganus guttatus* and the scleractinian coral *Pocillopora verrucosa* to local chemical pollutants. Short-term exposure of the water accommodated fraction of diesel (WAF-D) and the surfactant linear alkylbenzene sulfonate

(LAS) caused metabolic stress in both species investigated. This may in the long run lead to reduced growth and reproduction rates which in turn can translate to the whole population. In *S. guttatus* WAF-D led to a decrease in metabolic rates, while LAS caused an increase, respectively. In *P. verrucosa* LAS led first to a decrease in photosynthetic efficiency and finally to severe tissue loss. Under combined exposure to both WAF-D and LAS, both pollutants interacted, resulting in metabolic depression in *S. guttatus*. Interaction of stressors became more complex when also taking temperature into account. In combination with high temperature LAS and WAF-D resulted in more severe metabolic stress for both species, however effects were not amplified (no synergism). In view of global warming, this highlights the need to account for stressor interactions in ecological studies and conservation plans. Furthermore, reduced water quality in JB affected the physiology of two dominant soft corals in the area, *Sarcophyton sp.* and *Nephthea sp. In situ* studies showed that water quality, especially inorganic nutrients and sedimentation rates, may control abundance and metabolism of both species. This may facilitate phase shifts from hard to soft coral dominance in JB. The results here demonstrate the need to better manage water quality and to reduce import of pollutants.
The ecological and physiological results of this thesis were integrated with a parallel socio-economic study. High anthropogenic pressure in JB was shown to be linked to reduced coastal livelihoods. Communities on the coastal mainland were more dependent on fisheries and thus more vulnerable because of less adaptive capacity (less livelihood assets and limited alternative income sources). The local pollution linked with overexploitation was perceived as one of the main causes to degrading fisheries. In order to improve livelihoods of people in Jakarta and along the Thousand Islands, national policy not only needs to develop adaptive capacity, but also address the initial cause of vulnerability, the environmental exposure by anthropogenic stressors.
In conclusion, this thesis demonstrates that Jakarta's local environmental impacts and the challenges it faces from global impacts such as climate change, are not separate issues, but interact closely. Reef degradation due to global and local stressors in the JB/Thousand Islands reef complex has caused loss of ecosystem services. When considering the importance of coral reefs for the livelihoods of millions of people in the area, the need for more effective coral reef management strategies is obvious.

Zusammenfassung

Korallenriffe, vor allem solche in der Nähe von größeren Städten, werden durch eine Vielzahl an anthropogenen Stressfaktoren belastet. Sowohl lokale (z.B. Verschmutzung) als auch globale Stressfaktoren (z.B. Klimaerwärmung) nehmen aufgrund wachsender Urbanisierung, Industrialisierung und Küstenentwicklung an Stärke, Verbreitung und Häufigkeit zu. Diese Stressfaktoren können auf unterschiedlichen Ebenen miteinander interagieren, jedoch sind erhebliche Wissenslücken in Bezug auf deren gemeinsame Effekte auf Art- und Ökosystemebenen vorhanden. Solche Effekte müssen untersucht werden, um zugrundeliegende Mechanismen der Korallenrifffunktion und –zerstörung zu verstehen. Diese Dissertation versucht zu einem besseren Verständnis der Prozesse, die unter dem Einfluss von multiplen anthropogenen Stressfaktoren stehen und Korallenriffgemeinschaften formen, zu kommen. Die räumlichen Auswirkungen der Stressfaktoren sowie physiologische Reaktionen auf Schadstoffe und schlechtere Wasserqualität wurden untersucht. Korallenriffe in der Bucht von Jakarta (JB) und entlang der äußeren Tausend Inseln vor Jakarta in Indonesien, eine der größten Megastädte weltweit, sind extremer Verschmutzung ausgesetzt und repräsentieren daher ein ideales Gebiet, um die gemeinsamen Auswirkungen von multiplen anthropogenen Stressfaktoren auf Korallenriffe zu untersuchen.

Im ersten Teil der Dissertation wird der räumliche Einfluss der Stressfaktoren auf lokaler und regionaler Ebene in Korallenriffen nördlich von Jakarta untersucht. Die Ergebnisse zeigen, dass der direkte Einfluss von Jakarta hauptsächlich auf küstennahe Riffe besteht und dadurch die Riffe in der Bucht von Jakarta von denen entlang der äußeren Tausend Inseln trennt. Ein räumliches Flickwerk aus unterschiedlich zerstörten Riffen ist entlang der Inseln aufgrund von eher lokalen anthropogenen Stressfaktoren als regionalen Gradienten vorhanden. Verschmutzung ist der Hauptstressfaktor, wobei über 80 % der Variation in der Zusammensetzung der benthischen Gemeinschaften durch euthrophierungsbedingte Faktoren verursacht wird. Zudem wurden Tenside und Substanzen aus Dieseltreibstoff durch Schmutzabwässer und Bilgewasserausschüttung als verbreitete Schadstoffe identifiziert, sowohl in JB als auch entlang der Tausend Inseln. Daher beruht die in Abhängigkeit von der räumlichen Entfernung zu Jakarta unterschiedliche Struktur der Riffe in dem Jakarta/Tausend Inseln-Riffkomplex direkt auf dem intensiven anthropogenen Druck durch lokale und regionale Quellen. Eine äußerst niedrige Korallenbedeckung (2 %), Verschiebungen zu einer Dominanz von Weichkorallen und 80 % niedrigere Fischabundanzen wurden im Vergleich zu den äußeren Tausend Inseln in JB gefunden. Ein

verbessertes räumliches Management, welches sowohl lokale als auch regionale Stressfaktoren berücksichtigt, ist für einen effektiveren marinen Umweltschutz in dem Gebiet notwendig.

Im zweiten Teil der Dissertation wurde der Fokus auf physiologische Reaktionen von chemischen Schadstoffen auf Schlüsselorganismen in Korallenriffen, dem ökonomisch wichtigen Korallenrifffisch *Siganus guttatus* und die Steinkoralle *Pocillopora verrucosa*, gesetzt. Eine kurzzeitige Exposition mit der wasserangereicherten Fraktion von Diesel (WAF-D) und dem Tensid linear alkyliertes Benzolsulfonat (LAS) verursachte in beiden untersuchten Arten Stoffwechselstress. Dies kann längerfristig zu verringertem Wachstum und reduzierten Reproduktionsraten führen, was sich wiederum auf die gesamte Population auswirkt. WAF-D führte zu einer reduzierten Stoffwechselrate in *S. guttatus,* wohingegen LAS einen Anstieg der Stoffwechselrate in *S. guttatus* bewirkte. In *P. verrucosa* verursachte LAS zunächst eine Verminderung der photosynthetischen Effizienz und gegen Ende einen massiven Gewebeverlust. Bei gleichzeitiger Exposition gegenüber WAF-D und LAS interagierten beide Faktoren und verursachten eine Erniedrigung der Stoffwechselrate in *S. guttatus.* Die Interaktion der beiden Schadstoffe wurde jeweils komplexer, wenn die Temperatur miteinbezogen wurde. In Kombination mit einer erhöhten Temperatur und Exposition gegenüber LAS beziehungsweise WAF-D wurde ein erhöhter Stoffwechselstress in *S. guttatus* und *P. verrucosa* gefunden, jedoch waren die Effekte nicht vervielfältigt (kein Synergismus). Im Hinblick auf den Klimawandel verdeutlichen diese Ergebnisse, dass Interaktionen von Stressfaktoren in ökologischen Studien und Umweltschutzstrategien miteinbezogen werden müssen. Die verringerte Wasserqualität in JB hat zudem die Physiologie von zwei dominanten Weichkorallen in dem Gebiet, *Sarcophyton sp.* und *Nephthea sp.*, beeinflusst. In situ-Studien zeigten, dass die reduzierte Wasserqualität vor allem durch anorganische Nährstoffe und Sedimentationsraten die Abundanz der Korallen und den Stoffwechsel beider Arten in JB bestimmen. Dies könnte in JB Verschiebungen von Hart- zu Weichkorallendominanzen erleichtern. Die Ergebnisse zeigen, dass die Wasserqualität besser geregelt sowie Schadstoffeinträge verringert werden müssen.

Die ökologischen und physiologischen Ergebnisse dieser Dissertation wurden mit denen einer parallelen sozioökonomischen Studie integriert. Es wurde gezeigt, dass der hohe anthropogene Druck in JB mit einer verringerten Lebensgrundlage der Menschen in den Dörfern in Küstennähe in Verbindung steht. Dörfer an der Küste auf dem Festland sind abhängiger von der Fischerei und daher ökonomisch weniger stabil, da sie weniger wertvolle Güter und alternative Lebensgrundlagen haben. Die lokale Verschmutzung und die übergroße Ressourcenausbeutung wurden als eine der Hauptgründe für die Verschlechterung

der Fischereiergebnisse angesehen. Um die Lebensgrundlagen der Menschen in Jakarta und entlang der Tausend Inseln zu verbessern, muss die nationale Politik nicht nur adaptive Maßnahmen entwickeln, sondern auch den eigentlichen Grund der Anfälligkeit der Lebensgrundlagen der Menschen, die Verschmutzung, angehen.

Zusammenfassend zeigt diese Dissertation, dass Jakartas lokale Umwelteinflüsse und die Herausforderungen durch globale Einflüsse wie der Klimawandel keine voneinander getrennten Probleme sind, sondern eng miteinander interagieren. Die Zerstörung der Riffe durch globale und lokale Stressfaktoren in dem Jakarta/Tausendinseln-Riffkomplex hat zu dem Verlust von Ökosystem-Funktionen geführt. In Anbetracht der Bedeutung der Korallenriffe für die Lebensgrundlagen der Millionen Menschen in dem Gebiet, sind effektivere Managementstrategien für Korallenriffe notwendig.

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General Introduction

Coral reefs in Indonesia

Coral reefs are among the most biologically diverse and productive systems in the world (Moberg and Folke 1999, Crossland et al. 1991). They occur mainly in tropical oceans within 30° of the equator (Veron 2000). Most corals live in symbiosis with unicellular dinoflagellates, the zooxanthellae, which can provide > 90 % of the energy for the coral from carbon fixed during photosynthesis (Falkowski et al. 1984). Coral reefs are primarily formed by calcification processes of scleractinian corals, which provide a habitat for benthic organisms and fish (Moberg and Folke 1999, Veron 2000). About 1/3 of all fish species worldwide occur in coral reefs (Crabbe et al. 2009). In addition, coral reefs can provide physical protection from storms, thereby protecting associated ecosystems such as seagrass beds and mangrove forests, which further provide habitats as well as hatching and fishery grounds (Hoegh-Guldberg et al. 2007).

Indonesia is the world's largest archipelago nation and one of the fastest growing countries in the world with immense urbanization, industrialization and coastal development over the last decades (Pelling and Blackburn 2014). Coral reefs in Indonesia belong to the most diverse reefs worldwide (Hoeksema and Putra 2000). They are part of the Coral Triangle, a global hotspot of biodiversity (Veron et al. 2009, Burke et al. 2012). With more than 17,000 islands and a coastline of 81,000 km (Syarif 2009), 95 % of the current population of 252 million people lives near the coast (Martinez et al. 2007). Coral reefs support fisheries and tourist sectors and millions of people in Indonesia depend directly on marine resources from coral reefs for their daily lives (Moberg and Folke 1999, Burke et al. 2012).

Anthropogenic stress

Coral reefs are increasingly under threat due to the simultaneous impact of multiple environmental stressors (Halpern et al. 2007, Hoegh-Guldberg et al. 2007, Burke et al. 2012, Ban et al. 2014 a). Growing urbanization and industrialization in coastal areas, especially in many developing countries, has led to an increase in diversity and intensity of stressors and thereby caused an enormous speed in worldwide coral reef degradation. Over 60 % of reefs worldwide are considered at immediate risk from direct human activities (Burke et al. 2012) and at least 19 % have already been permanently lost (Wilkinson 2008). Some of the most pressing stressors on coral reefs are local anthropogenic stressors such as eutrophication,

increased sedimentation, pollution with toxic chemicals and overfishing (Fabricius 2005, van Dam et al. 2011, Burke et al. 2012), as well as global stressors such as ocean warming (Pandolfi et al. 2011) (see Fig. 1).

Organisms are able to tolerate stress to a certain extent, however the exposure to multiple stressors poses an increasing threat to reefs (Wilson et al. 2010), potentially causing a higher sensitivity to further stressors (Beyer et al. 2014). 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 organisms (Ban et al. 2014 b). Effects of multiple stressors have mostly been assumed to be additive (Halpern et al. 2007). However, current literature indicates that multiple stressors tend to interact with each other. Stressors can act directly with another, or the response of an organism to one factor can be altered by the occurrence of another (Crain et al. 2008). This interaction can be a synergism (i.e. amplification), e.g. increased UV radiation greatly increases the negative effects of a toxin (reviewed by Pelletier et al. 2006), or an antagonism (i.e. reduction), e.g. when nutrient enrichment dampens the negative effect of a second stressor, such as toxic chemicals or UV (Breitburg et al. 1998). These combined effects can occur on both species levels, as well as on community or population levels (see Fig. 1).

Chemical pollution

Especially in the field of chemical stressors, the intensity and diversity of anthropogenic stressors has increased rapidly (see review van Dam et al. 2011). Terrestrial runoff from rivers and streams and urban run-off carrying large amounts of domestic wastes and industrial effluents are usually the most important routes for chemicals to enter marine waters. The array of potential contaminants is very wide and includes among others organic contaminants such as hydrocarbons, surfactants, pesticides and herbicides as well as metals and organometallic compounds from industrial waste products (see review van Dam et al. 2011).

Toxic pollutants can accumulate in marine organism such as fish or invertebrates like corals (bioaccumulation) (see reviews Logan 2007, van Dam et al. 2011) and thereby cause various physiological impairments (Sokolova and Lannig 2008, McCloskey and Chesher 1971). During stress in general, additional energy is needed to recover and maintain homeostasis. For maximum fitness, organisms have to be able to balance the energy requirements from their environment against metabolic losses through environmental stress. Over longer time periods, environmental stress such as toxic pollutants can lead to reduction in growth as well

as lowered reproductive success and offspring fitness which in turn can change population dynamics and reef community structures (Calow and Forbes 1998). The effects of pollutants vary, from sub-cellular changes such as direct effects on DNA to metabolic stress leading to physiological impairment (Logan 2007, van Dam et al. 2011). Several toxic pollutants have also been shown to alter the olfactory system in fish (Scott 2003).

Effects of toxic pollutants on marine organisms depend on dispersion, physicochemical properties and bioaccumulation and biodegradation rates of chemicals (Kookana et al. 1998). For instance, pollutants with a higher solubility in water are generally transported further offshore and pollutants that associate easily with particulate matter may be more environmentally persistent. Many pollutants are rapidly absorbed and accumulated by sediments. Therefore, the largest amounts of chemical stressors are often found in estuaries or close to large urban areas (van Dam et al. 2011). In the tropics, degradation of organic compounds is generally more rapid than in temperate regions, however higher temperatures may also increase the sorption of organic contaminants to particulate matter, which in turn can increase their persistence (Neff 1979).

Polycyclic aromatic hydrocarbons (PAHs) are the most widespread class of organic pollutants and some PAHs are considered to have mutagenic, carcinogenic, and endocrine-disrupting characteristics (Logan 2007). Sources for PAHs are combustion of organic matter at low temperatures, such as for example in biomass burning, forest fires, internal combustion engines and garbage incineration, as well as crude oil and petroleum products (Rinawati et al. 2012) Generally, mixtures containing different hydrocarbons are released to the marine environment (Capone and Bauer 1992). For instance, through the release of bilge and ballast water from boats, both from large tankers and small fishing boats alike, organic contaminants such as PAHs from diesel used to fuel these boats can enter marine waters as part of the water accumulated fraction (WAF) (chapter 3). Many coral reefs are in close proximity to shipping lanes, where contaminated bilge water is disposed of (Halpern et al. 2008). Another ubiquitous pollutant class is surfactants which are applied by households and industries in large amounts in detergents and soaps. In untreated effluents, certain classes of surfactants can occur in concentrations that may be toxic to aquatic organisms (Ankley and Burkhard 1992). Domestic detergents can contain linear alkylbenzenes (LABs) and their amount may be used as indicators for environments affected by sewage (Rinawati et al. 2012).

Chronic sub-lethal stress of certain pollutants such as PAHs may decrease the resilience of reef organisms to other environmental stressors such as increased temperature as a result of global warming (see review van Dam et al. 2011). Furthermore, short-term (pulse-like) pollution events such as oil spills are often more localized and have a direct and severe impact on marine organisms as wella as multiple trophic levels simultaneously. In contrast, recurring or chronic pollution events such as input from river floods, bilge water discharge or chronic pollution from land runoff (e.g. sewage effluent), may exert subtle effects on lower trophic levels of the system. This may have more long-term effects on species fitness (see review van Dam et al. 2011).

Eutrophication and Sedimentation

Another main stress factor for many reefs worldwide has been proposed to be eutrophication (GESAMP 2001, Fabricius 2005). Eutrophication is mainly caused by the import of high levels of nutrients (especially nitrogen and phosphorus) such as for example by land runoff, lack of sewage treatment and large-scale agri- and aquaculture (GESAMP 2001). Long term monitoring data from the Great Barrier Reef show that the overall reduction in total coral cover by 70 % is mainly due to eutrophication (Bell et al. 2014. Coral reefs generally thrive under oligotrophic conditions (Schlager 1981, Nelson et al. 2011), however nutrient enrichment can facilitate the growth of macroalgae, reduce calcification rates in corals and reduce organic enrichment of the benthos, sediment and suspended particulate organic matter (Fabricius 2005). This can lead to shifts in trophic interactions and food webs (Anderson et al. 2002, Haas et al. 2009). These shifts in community structure to new states can reduce ecological and economic values (Hughes 1994, Hoegh-Guldberg et al. 2007). Most phase shifts on coral reefs are mainly associated with shifts from hard coral-dominated to macroalgae-dominated communities (Nyström et al. 2000, Hughes et al. 2007). However, shifts to reefs dominated by other benthic organisms such as sponges, corallimorpharians, and soft corals have been reported as well (e.g. Fox et al. 2003, Ward-Paige et al. 2005, Norström et al. 2009). The processes involved in phase shifts, especially to reefs dominated by soft corals, sponges and corallimorpharians are still largely unclear (see review by Norström et al. 2009). Coral–macroalgae shifts have been linked to loss of top-down control as a result of overfishing (Hughes et al. 2007, Rasher et al. 2012). Overfishing can deprive coral reefs from essential community members (Cinner and McClanahan 2006) and often these are herbivore fish. Herbivores play a key role in reef ecosystem function by actively influencing the competition for space between corals and algae (Hughes et al. 2007, Hixon 2011). In contrast, phase shifts to sponges, corallimorpharians, and soft corals may be

driven by bottom-up control and reduction in water quality (Norström et al. 2009). For instance, short-term sedimentation and nutrient enrichment may cause replacement of corals by sea anemones on certain coral reefs (Liu et al. 2015). Increased sedimentation and turbidity often occurs simultaneously to an increase in nutrient input, (Anderson et al. 2002, Weber et al. 2006). Suspended sediments in the water column reduce light availability (higher turbidity) and thereby photosynthesis rates of corals (Philipp and Fabricius 2003). In general, corals can tolerate low levels of sedimentation (Dollar 1981), but the energy costs caused by chronic high sedimentation rates results in reduced coral growth (Rogers 1990). Sediments trapped in epilithic algal turfs have also been shown to suppress herbivory by for instance fish which can cause macroalgal phase shifts (Bellwood and Fulton 2008, Goatley and Bellwood 2012).

Temperature

Local anthropogenic stressors such as the above mentioned organic chemicals, increased nutrient concentrations or fishing pressure are often accompanied with global stressors due to climate change. Their combined exposure can result in enhanced vulnerability of ecosystems (Pörtner et al. 2014). Global stressors such as ocean acidification and global warming that are related to climate change are caused by greenhouse gas emissions (Pandolfi et al. 2011). A rise in global sea surface temperature between 0.3 - 4.8 °C has been predicted for the end of this century (IPCC 2013). Coral reefs in the tropics usually face very little variation in temperature compared to temperate regions. Hence, even small increases in water temperature can lead to a high stress in reef organisms (Maina et al. 2011, Lesser 2013). Bleaching, a phenomenon occurring worldwide during which the zooxanthellae are degenerated or expelled, is in most cases connected to rising water temperatures (Brown 1997) and can lead to severe coral mortalities if temperature conditions are prolonged (Wilson et al. 2010).

Thermal stress can enhance reaction rates and in turn increase the sensitivity of organisms to other stressors such as light and sedimentation stress and contaminants (Anthony and Connelly 2007). Temperature affects both the solubility of oxygen in the water, as well the rate of chemical reaction and thereby also the activity of enzymes. In addition, temperature affects physicochemical properties of membranes, including permeability and diffusion rates, as well as the solubility and (bio)degradation rate of chemicals. This in turn affects the toxicity of chemicals (O`Donnel et al. 1985).

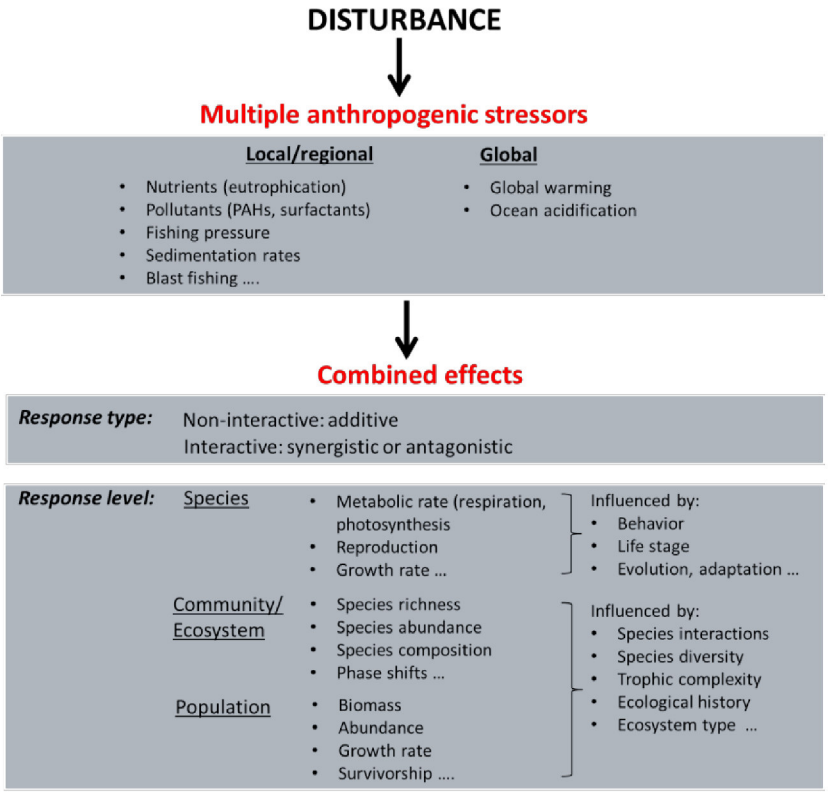

Fig. 1: General overview of multiple anthropogenic stressors (local and global) and combined effects of these stressors including response types and levels.

Determining stress in reef organisms and communities

Organisms can react to stressors in different ways and scientists have used a wide array of response indicators, from sub-cellular to metabolic indicators to determine stress responses (Niimi 1990, Logan 2007). The physiological status or health of an individual organism is often referred to as condition or fitness and reflects overall energetic requirements (McPherson et al. 2010). The metabolism of an organism combines all processes controlling the performance of an organism in terms of behavior, survival, growth and reproduction and therefore estimations of the metabolic condition can be used to reflect stress levels during changing environmental conditions (Fanslow et al. 2001, Lesser 2013). Metabolism is usually determined in terms of oxygen consumption per biomass as a measure for metabolic rate (Schmidt-Nielsen 1997). Respirometry is a well-established and acknowledged method to estimate the metabolic rate and identify stress levels caused by pollutants and temperature stress in organisms (Lesser 2013). The standard metabolic rate (SMR) refers to respiration rates for basal physiological processes in resting and unfed fish (e.g. cellular key processes such as ion and acid-base regulation, protein turnover, ventilation, circulation and excretion),

while the routine metabolic rate (RMR) reflects respiration that includes energy for locomotion, digestion etc. (McNab 1997). 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. Besides metabolic rates, the electron transport system (ETS) activity is another indirect measurement of respiration. Organisms respire through a respiratory chain of enzymes called the electron transport system. By measuring the enzyme activity of the ETS, a time averaged value of the maximum oxygen uptake rate potential can be obtained (Fanslow et al. 2001). For photosynthetic organisms such as zooxanthellate alge, the photosynthetic efficiency can be determined, either by measuring the oxygen production directly, or indirectly by measuring the quantum yield of linear electron transport (i.e. photosynthetic yield) (Philipp and Fabricius, 2003). Respiration of the coral host and photosynthesis of the symbiotic algae, are indicators of the holobiont's basal metabolic functions and can be used to determine non-lethal stress effects on corals (Porter et al. 1999, Osinga et al. 2012).

The impact of multiple stressors on coral reef communities depends on species interactions, species diversity and redundancy, trophic complexity, ecological history, and ecosystem type (Vinebrooke et al. 2004). Both temporal and spatial changes in reef community composition can be determined. Surveys are commonly used to determine factors related to biodiversity (e.g. English et al. 1994). The role of biodiversity for the stability, function, and resilience of ecosystems can provide information on how reef communities are affected by stressors (e.g. McGrady-Steed et al. 1997, Nyström et al. 2008). Biodiversity factors include for example species richness or abundances of certain benthic groups such as macroalgae, turf, corals, other macroinvertebrates as well as of fish communities. Furthermore, species can be divided into functional groups such as predators, grazers, bioeroders, primary producers and habitat builders (e.g. Bellwood et al. 2004), which can link how species diversity interacts with certain ecosystem processes (Bellwood et al. 2003). For example, quantifying the relative abundance of several key functional algae groups (crustose corallines, macroalgae and turf algae) and relating these to abundances with functional fish groups such as herbivores, provides further knowledge on coral condition and potential phase shifts (Nyström et al. 2000, Hughes et al. 2007). Besides these factors, other factors such as whole-system productivity (production, respiration, photosynthetic yield) can be measured. Commonly, several indicators are determined at the same time to measure stress in reef communities (Price et al. 2007).

Jakarta Bay/Thousand Islands and coastal livelihood vulnerability

Some of the most rapidly growing cities in the world are located in Asia. In 2015, 16 of the world's 24 mega cities (cities with more than 10 million people) will be located in Asia (Hung et al. 2006). This enormous growth however goes hand in hand with environmental destruction and increasing poverty. These mega cities are all located at the coast causing various marine and coastal environmental impacts such as decreased water quality, depletion of fishery resources, seafood contamination, land subsidence, loss of habitat, coastal littering as well as eutrophication and increased sedimentation rates. Jakarta Metropolitan Area in Indonesia is the 3rd largest urban agglomeration in the world with around 25 million inhabitants (Brinkhoff 2012.) This mega city has a unique position compared to other world mega cities: Directly to the north of Jakarta Bay (JB), and thus directly in the expected main impact area of anthropogenic stressors originating from Jakarta, the Kepulauan Seribu ("Thousand Islands") are located. This island chain extends up to 80 km off the coast and harbors many different ecosystems including coral reefs and mangroves (Arifin 2004). Large amounts of untreated sewage and industrial effluent with high pollutant levels are transported by several rivers directly into the bay (Rees et al. 1999). Especially within the bay, elevated concentrations of contaminants such as heavy metals (Rees et al. 1999), DEET (Dsikowitzky et al. 2014), surfactants (Rinawati et al. 2012, chaper 3) and oil-related pollution (Rinawati et al. 2012) are of large concern. Bradshawet al. (2010) reported that Indonesia ranked fourth among 200 countries in environmental pollution discharge.

Thousands of people in North Jakarta and along the Thousand Islands depend on the ecosystem goods and services provided by local coral reefs. However, the extreme pollution with toxic chemicals, eutrophication and sediment load in the area as well as overexploitation of marine resources are threatening coastal communities. According to quantitative data from the Indonesian Ministry of Marine Affairs and Fisheries, the decline in fish stocks in Jakarta is linear to the increasing population growth in Jakarta (KKP 2011). Yoo et al. (2014) found that North Jakarta is the most vulnerable district due to risk of flooding caused by sea level rise and degradation of water quality, has the largest slum population and a low capacity to adapt to potential negative impacts. Coastal livelihoods, especially those that rely mainly on marine resources like in the JB/Thousand Islands complex, are vulnerable to long-term changes such as increasing pollution with toxic chemicals (Ferrol-Schulte et al. 2015). Therefore, there is an increasing need to evaluate the links between the social and ecological dimensions of human vulnerability to anthropogenic stress (Cinner et al. 2013, Yoo et al. 2014, Ferrol-Schulte et al. 2015). Vulnerability is "the degree to which a system is susceptible

to and is unable to cope with adverse effects" (Adger 2006) of declining resources. The three key dimensions environmental exposure (i.e. pollution), sensitivity (i.e. the degree to which a system is affected (IPCC 2001) and adaptive capacity (i.e. economic status and infrastructure) are commonly taken to describe vulnerability (Adger et al. 2005, Hughes et al. 2005, Cinner et al. 2013).

Gaps of Knowledge

Due to the worldwide expansion of human populations around the coast, both local and global stressors are increasing in impact and range in marine environments. There are hardly any coral reefs left that are exposed to no or only single stressors (Breitburg et al. 1998, Halpern et al. 2007, Crain et al. 2008, van Dam et al. 2011). The majority of reefs, especially those located near larger urban areas, are simultaneously exposed to multiple (mostly anthropogenic) stressors. These stressors will most likely interact to various degrees with one another. However, considerable knowledge gaps exist regarding the responses on species and ecosystems levels to combined effects of multiple stressors. Exactly, these have to be addressed, if underlying mechanisms in coral reef function and degradation are to be understood and effective conservation and management plans of coral reefs are to be implemented (Ban et al. 2014 a). Especially the influence and interaction of local (i.e. single islands) vs. large-scale (i.e. regional) stressors in shaping coral reef systems is not clear. In addition, physiological background data on metabolic conditions of key reef players such as fish and corals under combined exposure to multiple anthropogenic stressors (both local and global), are still largely lacking and difficult to predict due to often highly species and stressor specific responses. Furthermore, a better understanding of effects on livelihood vulnerabilities linked to anthropogenic stressors is urgently needed.

Objectives

The overarching aim of this study is to contribute to a better understanding of the processes that shape coral reefs under the influence of multiple anthropogenic stressors close to large urban areas. JB and the Thousand Islands represent an ideal area to assess the combined effects of multiple stressors on coral reef ecosystems. Fish and reef-building corals are among the most studied groups within coral reefs. Since corals are fundamental for the existence and functioning of coral reef ecosystems, and since they require relatively stable environmental conditions, anthropogenic stress likely affects corals earlier than other organisms. Reefs have been present throughout the past 600 million years, and although they

have been affected by environmental changes in ocean chemistry, sea level and climate, show a remarkable resistance (Pandolfi 1999). As such, corals offer a unique chance to study physiological responses to stress. Similarly, fish play a key position in marine food webs and are of large economic importance to locals coastal communities. Measurements of the physiological condition of fish or corals are common tools to increase the ecological understanding of changing environments (Wilson et al. 2010).

With regard to the Jakarta Bay and Thousand Islands reef complex as a case study, this thesis has the following two main objectives:

1. To determine spatial impacts of important anthropogenic stressors on reef communities on local and regional scales and to identify potential shifts in benthic community structure.

2. To determine physiological effects of multiple anthropogenic stressors on key coral reef players with a focus on a) combined effects of two significant chemical stressors (diesel and surfactant) and temperature on the metabolic state of a fish and a scleractinian coral by means of manipulative experiments and b) effects of water quality on metabolism of dominant soft corals.

Furthermore, as an added value to the above ecological and physiological objectives of this thesis, the livelihood vulnerabilities linked to anthropogenic stressors with a focus on the use and dependence on reef fisheries resources by coastal communities were integrated based on results from a parallel socio-economic study within the Indonesian-German SPICE III Program (Science for the Protection of Indonesian Coastal Marine Ecosystems).

Approach

This thesis was conducted at the Leibniz Center for Tropical Marine Ecology (ZMT), Bremen (Germany) in collaboration with the Indonesian Research Center for Marine and Fisheries Products Processing and Biotechnology (KKP), as well as the Research Centre for Oceanography, Indonesian Institute of Sciences (LIPI). The work was carried out within the frame of the Indonesian-German SPICE III Program (Science for the Protection of Indonesian Coastal Marine Ecosystems). German Federal Ministry of Education and Research (BMBF) funded the research for this thesis. The fieldwork was carried out in Indonesia within Jakarta Bay and the Thousand Islands reef complex. Field experiments were conducted at the LIPI Pari field station in the Thousand Islands and additionally at the LIPI

Mataram Unit on Lombok. Correlative *in situ* as well as manipulative laboratory studies were performed to determine impacts of multiple anthropogenic stressors on coral reef communities close to large urban areas. In addition, studies were conducted in collaboration with other groups from the SPICE III Program to understand and evaluate livelihood vulnerabilities linked to anthropogenic stress.

Chapter and Publication Outline

This thesis is divided into this general introduction in which the overall objectives of this thesis are given, 5 chapters presenting the performed research, and a general discussion summarizing the key finding of this thesis and providing a brief outlook for future research.

In chapter 1, the reef condition along the Thousand Islands was determined. The spatial impact of anthropogenic stressors on local and regional scales on coral reefs was investigated and the main stressors identified. The first chapter is the base study on which all other chapters rely for background information.

Chapter 2 is a correlative study. Potential phase shifts of coral reefs to soft coral dominance were determined and metabolic efficiency of two dominant soft corals species, *Sarcophyton sp.* and *Nephthea sp.*, correlated with the distance to Jakarta and water quality, as well as with the benthic community composition along the island chain.

This is followed by chapter 3 and 4, in which metabolic stress responses in two key reef players, an ecologically and economically important fish (*Siganus guttatus*) and a scleractinian coral (*Pocillopora verrucosa*), were determined under exposure to two common organic pollutants (surfactants and diesel) in the JB/Thousand Islands reef complex with or without a global warming scenario. Manipulative experiments were conducted at the Thousand Islands and on Lombok.

The final chapter 5 focuses on social aspects regarding the impacts of anthropogenic stressors such as pollutants and eutrophication on coastal communities. The anthropogenic pressures and associated vulnerabilities of reef fisheries, including its dependencies and livelihoods, of the Jakarta Bay Ecosystem, are assessed.

List of publications

This thesis is based on the scientific publications listed below, with authors contribution indicated.

Publication 1)

Baum G, Januar HI, Ferse SCA, Kunzmann A. **Local and regional impacts of pollution on coral reefs along the Thousand Islands north of the megacity Jakarta, Indonesia**. *PlosOne*. In print.

The concept of this publication was developed by G. Baum, A. Kunzmann and S. Ferse. Fieldwork was performed by G. Baum with support from HI Januar and sample analyses by G. Baum. The manuscript was written by G. Baum with critical revision from all authors.

Publication 2)

Baum G, Januar HI, Wild C, Kunzmann A. **Water quality controls physiology and abundance of dominant soft corals in Jakarta Bay.** In preparation for *PlosOne*.

This study was initiated by G. Baum, C. Wild, S. Ferse and A. Kunzmann. Fieldwork was performed by G. Baum with support from HI. Januar. G. Baum analyzed the data and wrote the manuscript with revision by all authors.

Publication 3)

Baum G, Kegler P, Scholz-Böttcher BM, Abrar M, Alfiansah YR, Kunzmann A. **Metabolic stress responses of the coral reef fish *Siganus guttatus* exposed to combinations of water borne diesel, an anionic surfactant and high temperature in Indonesia.** Submitted to *Marine Pollution Bulletin*.

This study was designed by G. Baum, P. Kegler and A. Kunzmann. Fieldwork and sample analyses were performed by G. Baum and P. Kegler with support from M. Abrar and Y.R. Alfiansah. PAH-analyses was performed by B.M. Scholz-Böttcher. The manuscript was written by G. Baum with critical input by all authors.

Publication 4)

Kegler P, **Baum G**, Indriana LF, Wild C, Kunzmann A. **Physiological response of the hard coral *Pocillopora verrucosa* from Lombok, Indonesia, to two common pollutants in combination with high temperature.** In review after revisions in *PLoS ONE*.

The concept for this study was developed by P. Kegler, G. Baum and A. Kunzmann. The experiments were carried out by P. Kegler and G. Baum, aided by L. Indriana. Data analysis and interpretation was carried out by P. Kegler, G. Baum, C. Wild and A. Kunzmann. The manuscript was written by P. Kegler with critical revision from all authors.

Publication 5)

Baum G, Kusumanti I, Glaser M, Ferse SCA, van der Wulp S, Adrianto L, Kunzmann A, Schwarzbauer J, Dsikowitzky L, Dwiyitno, Breckwoldt A, Agos Heri. **Under anthropogenic stress: Linking Marine Resource Use Systems in Jakarta Bay and the Seribu Island**s. In preparation for *Marine Pollution Bulletin*.

The concept of this publication was developed by S. Ferse, with input from G. Baum, A. Breckwoldt, M. Glaser, A. Kunzmann. The vulnerability assessment work is based on the Master thesis of I. Kusumanti. Data and sample analysis were performed by all contributing authors. Baum G. wrote the manuscript together with A. Breckwoldt and input from all other authors.

Related publication not included in this thesis

Hillebrand H, **Baum G**, Donadi S, Fink D, Hamann B, Hinrichs N, Jay S, Schnetzer J. **Between ignorance and concern - interdisciplinary approaches to raising awareness on marine environments**.

This study was initiated by H. Hillebrand. The main text was written by H. Hillebrand and individual chapters were provided by all other authors.

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Chapter 1: Spatial impacts of local and regional stressors

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Local and regional impacts of pollution on coral reefs along the Thousand Islands north of the megacity Jakarta, Indonesia

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Abstract

Worldwide, coral reefs are challenged by multiple stressors due to growing urbanization, industrialization and coastal development. Coral reefs along the Thousand Islands off Jakarta, one of the largest megacities worldwide, have degraded dramatically over recent decades. The shift and decline in coral cover and composition has been extensively studied with a focus on large-scale gradients (i.e. regional drivers), however special focus on local drivers in shaping spatial community composition is still lacking. Here, the spatial impact of anthropogenic stressors on local and regional scales on coral reefs north of Jakarta was investigated. Results indicate that the direct impact of Jakarta is mainly restricted to inshore reefs, separating reefs in Jakarta Bay from reefs along the Thousand Islands further north. A spatial patchwork of differentially degraded reefs is present along the islands as a result of localized anthropogenic effects rather than regional gradients. Pollution is the main anthropogenic stressor, with over 80 % of variation in benthic community composition driven by sedimentation rate, NO_2, PO_4 and Chlorophyll a. Thus, the spatial structure of reefs is directly related to intense anthropogenic pressure from local as well as regional sources. Therefore, improved spatial management that accounts for both local and regional stressors is needed for effective marine conservation.

Introduction

Rising population numbers worldwide go hand in hand with an increase in the diversity and intensity of anthropogenic stressors. Coral reefs for instance are increasingly under pressure due to coastal development and resource use. At least 19 % of reefs worldwide have been permanently lost (Wilkinson 20008), and of those remaining, over 60 % are at immediate risk from direct human activities (Burke et al. 2012). Local anthropogenic stressors can decouple

reef communities from biophysical factors and become the principal drivers in shaping coral reef benthic community composition (Williams et al. 2015), leading to novel and unprecedented ecosystems that are composed and function in ways currently still poorly understood (Graham et al, 2014). Therefore, understanding the processes that shape coral reef communities under the influence of multiple anthropogenic stressors is one of the core challenges in coral reef ecology and conservation. The most pertinent stressors on reefs have anthropogenic origins (e.g. nutrient input, fishing pressure or turbidity gradients due to coastal development (Burke et al. 2012) and, depending on the specific location, occur at larger gradients or rather show localized impacts.

The degradation of reefs has direct impacts on coastal communities that depend on reef resources for their livelihoods. One of the main challenges of ecosystem and conservation management plans is to account for the connection between local habitats and the conflicting demands of different stakeholders on reef resources (Sale et al. 2014). Marine spatial planning, which is based on assigning different activities to specific zones and thus can account for variable location-specific factors such as increasing distance from city centres or markets, has been proposed as an alternative to current non spatially-explicit management strategies. Such approaches may be especially suitable in areas with extreme urbanisation such as megacities, where numerous different stakeholders are involved in resource uses. However, there is still a lack in knowledge in how far the influence of large urban areas extends and to which extent local and regional anthropogenic and non-anthropogenic stressors interact.

Of today´s 28 megacities (cities with more than 10 million people), sixteen are located in Asia (UN 2014), and many megacities are located at the coast, causing various human-induced marine and coastal environmental problems such as water pollution, depletion of fishery resources, seafood contamination, loss of habitat, coastal littering as well as eutrophication and increased sedimentation rates (Blackburn and Marques 2014). The Greater Jakarta Metropolitan Area, as the 3rd largest agglomeration in the world with around 25 million inhabitants (Brinkhoff 2011), and the Kepulauan Seribu ("Thousand Islands") chain, located in front of Jakarta Bay (JB), represent an ideal area to assess the relative and interactive effects of multiple stressors on coral reef ecosystems. Here, local anthropogenic impacts have caused dramatic changes in coral reef ecosystems (e.g. van der Meij et al. 2010), especially over the past decades, with a current coral cover of < 5 % for nearshore reefs within JB and < 20 % cover in offshore reefs (up to 80 km north of the coast of Jakarta) (e.g. Cleary et al. 2014). Coral reefs are of high economic and environmental importance for the

Jakarta area and island communities, for instance supporting local fisheries, aquaculture and, to a lesser extent, a growing and mostly local tourism industry (Burke et al. 2012, Fauzi and Buchary 2002). Currently, there are around 40,000 fishermen in JB and the Thousand Islands (BPS 2012).

A marked inshore-offshore gradient in heavy metal pollution, nutrient input and water transparency (Rees et al. 1999, Williams et al. 2000), coral cover (Cleary et al. 2006, 2008) and fish abundance (Madduppa et al. 2013) has been observed in the past. Cleary et al. (2006) divided the island chain into three areas based on geomorphology, oceanography and distance from Jakarta, representing a disturbance gradient with severe pollution in nearshore reefs, medium pollution in midshore reefs and relatively minor anthropogenic disturbances in offshore reefs. However, localized stressors e.g. from destructive fishing and local nutrient sources are likely to be at least equally severe as regional impacts from coastal cities. This could potentially lead to high spatial variability in reef community composition and functioning, and previous research along the Thousand Islands has not addressed local drivers sufficiently. Furthermore, pollution from large cities and localized impacts from island communities require different management approaches.

Although the Thousand Islands constitute the oldest Marine National Park in Indonesia, management of the area is still ineffective (Fauzi and Buchary 2002). Furthermore, comprehensive marine spatial management is lacking for the reefs outside the Thousand Island Marine National Park. This threatens not only local livelihoods, but also the stated goal of the Indonesian government to have 20 million hectares of marine area under effective management by 2020 (Ministry of Marine Affairs and Fisheries 2009). In order to develop successful management and conservation strategies for the coral reefs in JB and the Thousand Islands, the spatial scale at which stressors act has to be determined. Thus, the present study assessed whether spatial trends in stressors and benthic community composition reflect the distance to Jakarta, reef conditions are a reflection of localized effects, or (and to what extent) a combination of both regional and local effects is shaping local reef communities. In addition, it was examined which of the different stressors mainly shape the local structure of coral reefs along the island chain.

Material and methods

Study area

The Kepulauan Seribu (Thousand Islands) are comprised of 105 small (< 10 ha) and very low-lying (< 3 m above sea level) islands, most with lagoons and fringing reefs, reaching up

to 80 km north of the city (Arifin 2004). In 1982 Indonesia´s first Marine National Park, the Thousand Islands National Park, was established in the north of the island chain (Djohani 1994). Reef development generally is restricted to shallow depths (around 3-10 m, max. 20 m depth). Although reefs within the bay once had thriving coral communities (Sluiter 1888, Umgrove 1939), they are now dominated by sand, rubble and algae. Several islands within the bay were mined for construction purposes and have eroded away (Ongkosongo 1986, Stoddart 1986, DeVantier et al. 1998). With a total population of around 22,700 people, the island chain is densely populated. 65 % of the people live on the four main islands Panggang, Pramuka, Kelapa and Harapan (BPS 2012). Numerous stakeholders are presently involved in fishing, sand mining, tourism and aquaculture, in particular the culture of green mussels (*Perna viridis*) in JB. The latter has a daily production of around 20-25 t and involves around 3,000 fisher families (Arifin 2004). Several rivers with a combined catchment area of 2000 km^2 discharge directly into the bay and transport large amounts of untreated sewage and industrial effluent with high pollutant levels (Rees et al. 1999). Around 60 % of the bay´s shoreline has been modified due to massive urbanization and industrialization as well infrastructural development in Jakarta, and another 30 % for agricultural or aquaculture developments (Bengen et al. 2006). During the dry season, the predominantly south-easterly winds can cause polluted surface waters from the JB area to reach midshore reefs, while during the wet season, north-westerly winds blow from offshore towards JB (Cleary et al. 2008).

For this project, eight coral reef sites across the Thousand Islands chain were visited in November 2012 during the transition time between northwest and southeast monsoon. The field studies did not involve endangered or protected species. Sites within JB (nearshore zone; < 20km) as well as from the outer Thousand Islands (mid- and offshore zones; 20-45 km and > 45 km, respectively) were chosen to represent both inhabited and non-inhabited islands for each of the three zones. Following the same methodology as previous studies (Cleary et al. 2006, Moll and Suharsono 1986), reefs from the northern side of each island were visited to ensure consistent wave exposure and current regimes. In case strong waves did not allow anchoring on the northern side of islands, transects were placed slightly to the north-east (Kayu Angin Bira (B) and Gosong Conkak (C)). An exceptional case was Pari: here, the south side of the island was included to account for previously observed strong differences in coral cover between the northern and southern side of the island (Abrar and Zamani 2011, Madduppa et al. 2012) (Table 1.1, Fig. 1.1).

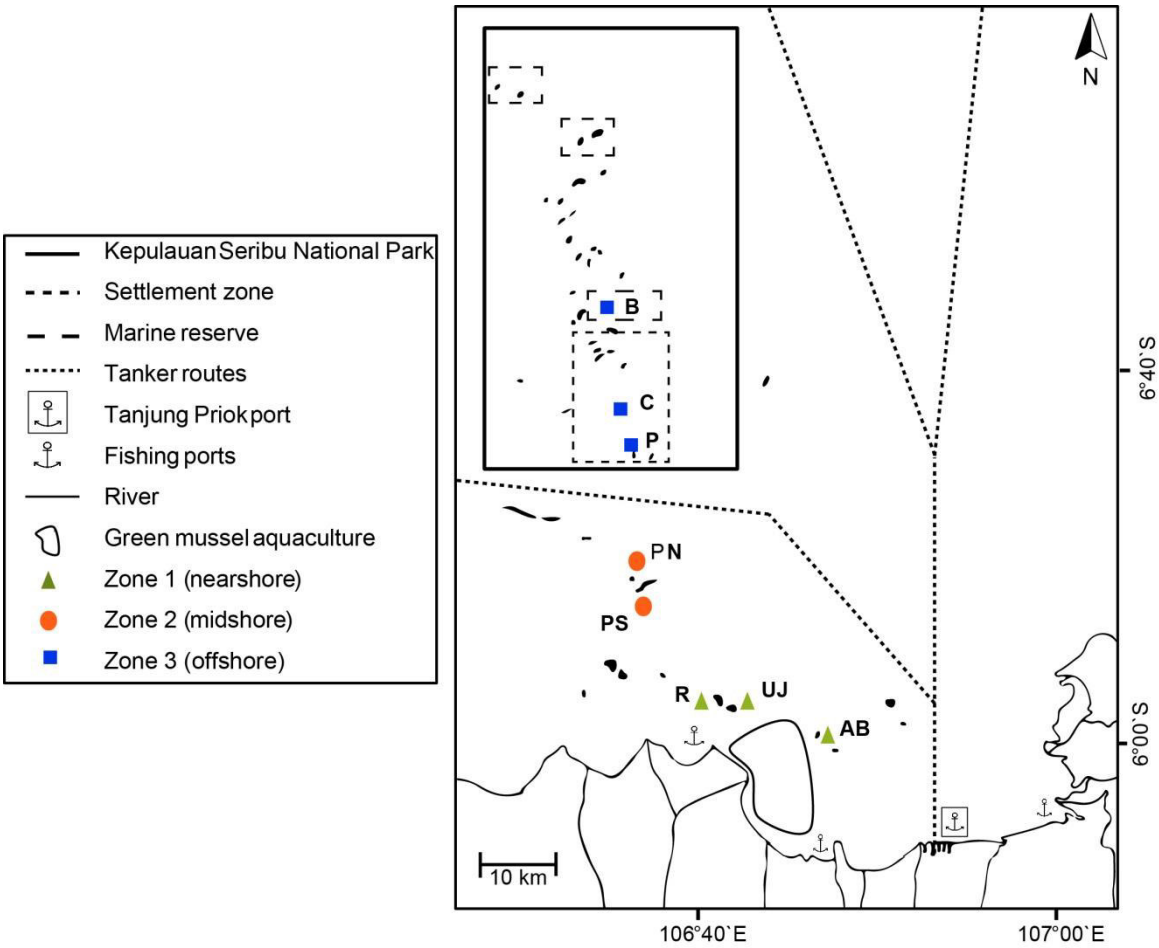

Fig. 1.1: Study area. Map includes boundaries of the Thousand Islands Marine National Park, ports and study sites from nearshore reefs (within Jakarta Bay) as well as from the outer Thousand Islands (mid- and offshore): AB = Ayer Besar, UJ = Untung Jawa, R = Rambut, PS = Pari South, PN = Pari North, P = Panggang, C = Congkak, B = Bira.

Benthic and reef fish community

Benthic habitat structure was assessed at each location using three replicate 50 m line-intercept transects at 5 +/- 0.5 m water depth (English et al. 1994). Preliminary visits to the Thousand Islands had shown that highest coral cover can be commonly found at shallower depth and that at nearshore sites turbidity was too high at greater depth to conduct accurate surveys. Therefore a depth of 5 m was chosen to allow for adequate comparison across the sites which would not be possible at greater depths. High-resolution underwater photographs were taken using a digital camera (Canon G12 in a WP-DC 28 housing) every two meters on both sides of the transect line with a 1x1 m gridded quadrat frame for reference. All three replicate transects at each site were conducted on the same day between 8:00 h and 13:00 h. For the assessment of benthic community composition, photographs were analysed using CPCE software (Kohler and Gill 2006). 50 random points were placed on each photo and

each point was assigned to one the following benthic categories: hard and soft corals, acroporid and non-acroporid corals, recently dead corals (coral structure largely intact and attached to sediment), sand, pavement and rubble (bare or overgrown with turf or crustose coralline algae; rubble objects can move during storms/currents) and macroalgae. Overall total live coral cover was calculated. In addition, corals were further separated into morphological categories (English and Wilkinson 1994, Edinger and Risk 2000) to assess coral morphology composition. The relative abundance of these standardized coral morphology categories was used to classify coral reefs from JB and the Thousand Islands using a r-K-S- ternary (triangular) plot (based on (Edinger and Risk 2000), see S1.3 Table and S1.1 Fig.).

Table 1.1: Description of sampling sites (linear distance refers to distance from each site to the port Muara Angke in Jakarta). Zonation is based on Tomascik et al. (1994) and DeVantier et al. (1998): zone 1 (nearshore; within Jakarta Bay): < 20 km; zone 2 (midshore): 20 – 45 km; zone 3 (offshore): > 45 km. Number of residents is given per `administrative village` (Indonesian: *kelurahan*) that contain several islands within the island complex Untung Jawa, Pari and Panggang.

Site	Site abbrev.	Zone	Coordinate [E]	Coordinate [S]	Linear distance to Jakarta [km]	Permanent residents	Characteristics
Ayer Besar	AB	1	106°42.242	05°58.399	11.3	-	Private, for tourism
Untung Jawa	UJ	1	106°46.911	05°58.399	16.4	1726	Small settlement
Rambut	R	1	106°41.597	05°58.202	17.3	-	No permanent settlement
Pari South	PS	2	106°36.963	05°52.094	31.4	2458	Large fringing reef, tourism, research station
Pari North	PN	2	106°37.440	05°51.001	32.6		Large fringing reef, tourism, research station
Gosong Panggang	P	3	106°35.355	05°44.664	45.7	5123	Coral key, close to very densely populated islands (Pramuka and Panggang)
Gosong Conkak	C	3	106 35.274	05 42.303	49.5	-	Coral key
Kayu Angin Bira	B	3	106°34.162	05°36.405	59.8	-	Within conservation area of the NP, i.e. no fishing activities

Fish community composition was assessed along the same transects as benthic habitat structure using underwater visual census (English et al. 1994). Number of fish species and number of individuals per species was recorded within 2.5 m of each side of the transect line

(yielding a total area of 250 m^2 per transect) by slowly swimming along the line at a constant speed. Fish surveys were always conducted by the same person at all sites to minimize observer bias. Each fish species was further assigned to one of five different feeding guilds to gain information on feeding guild composition (based on information from FishBase (Froehse and Pauly 2014): herbivores (HV), carnivores (CV), planktivores (PV), omnivores/invertivores (OVI) and obligate corallivores (OCV).

Water quality

At each sampling site, temperature (°C), dissolved oxygen (DO; mg/L), pH, salinity (PSU), turbidity (NTU) and Chl a (µg/L) concentration of the water were measured as common water quality indicators (e.g. De`ath and Fabricius 2008, Fabricius et al. 2012) at 1 and 3 m water depth using a Eureka 2 Manta Multiprobe (Eureka Environmental Engineering, Texas, USA). Measuring interval was set to 1 min. Measurements of 3-4 min duration were taken twice a day, once in the morning around 09:00 h and once in the afternoon around 13:00 h. Water samples for inorganic nutrient analysis (nitrite (NO_2), nitrate (NO_3), phosphate (PO_4), ammonia (NH_3)) were taken at each sampling site at 1 and 4.5 m water depth. Samples were stored in an ice cooler and analysed the same day using a field photometer. Dissolved inorganic nitrogen (DIN) is given as the sum of NO_2, NO_3 and NH_3.

Sediment traps made from a PVC tube with a height-to-width ratio of 7.2 (as recommended by Storlazzi et al. (2011)) were deployed at 5 +/- 0.5 m depth for 22 +/- 1 h at each location (n = 4 per site). Traps were sealed underwater prior to retrieval. All water and sediment in the tubes was transferred to plastic bottles (5 L) and samples were stored in the dark until further processing. Water was filtered through Whatman GF/C glass microfiber filters (diameter 110 mm; 1.2 µm porosity) that had been precombusted at 500 °C for 6 h and weighed. After filtering, filters were dried at 65 °C for 24 h and re-weighed. Sedimentation rate is given as total particulate mass flux (TPMF) [g m^{-2} d^{-1}] according to UNESCO (1994):

$$TPMF = DW / A_r \times T, \quad (1)$$

where DW is dry weight of trapped sediment samples [g], T is trapping duration [d] and A_r is the area of the sediment trap tube opening [m^2] with $\pi = 3.14$ and d = aperture size [cm]:

$$A_r = \pi \times (0.5\ d)^2 \times 10^{-4} \quad (2)$$

Statistical analysis

Spatial trends for each individual water and biological factor along the islands in JB and the Thousand Islands were analyzed for effects of the distance to Jakarta and localized patterns among sites. To test whether the large-scale effects emanating from Jakarta act linearly or non-linearly along the distance gradient, a number of different models were used in assessing large-scale trends: grouping of sites into *a priori* defined zones (blocked treatments: near-, mid- and offshore), gradual in- or decreases (linear regression), exponential in- or decreases (exponential regression), and linear regression with one breakpoint (i.e. two linear segments). Differences in water and biological factors among zones and sites were analysed using one-way ANOVA. Data were checked for assumptions of normality and homogeneity of variances. In case assumptions were not fulfilled, a Kruskal Wallis test was performed instead. If significant effects were detected, pairwise comparisons with the post-hoc Tukey test were performed to assess significant differences between individual factors. Univariate statistics were performed with SigmaPlot 12.5.

Multivariate statistics of spatial trends for each of the four different biological composition groups (fish community, fish feeding guild, benthic community and coral morphology) were performed using PRIMER-E software v.6 (Clarke and Gorley 2006). In order to account for different scales and units (Clarke and Ainsworth 1993), the water factors PO_4, NH_4, NO_3, turbidity and Chl a were log+1 transformed, followed by normalization of all water factors. All biological factors were square root transformed prior to further analysis in order to reduce the influence of overly abundant species (Clarke and Green 1988). Bray-Curtis similarity matrices (Bray and Curtis 1957) were calculated for all biological composition groups and Euclidian distance was used to construct the similarity matrix for water data (Clarke and Green 1988). Permutational multivariate analysis of variance (PERMANOVA) was used to test for significant differences among *a priori*-defined groups (i.e. three zones) (Anderson 2001). Distance-based redundancy analysis (dbRDA; Anderson 2001), a constrained ordination technique, was used to visualise differences between zones. Furthermore, the role of individual stressors was assessed with the BEST routine (using the BioEnv procedure based on Spearman rank correlation; Clarke et al. 2008) to determine which of the factors best explained the composition of fish and benthic composition groups. Only proximate drivers were considered here: 1) water factors for both benthic composition groups and 2) benthic community and coral morphology factors for fish composition groups respectively. Those factors identified by BEST were then used for the LINKTREE procedure (Clarke et al. 2008) in PRIMER to construct a linkage tree, a hierarchical tree that

shows how fish and benthic compositions separate into zones. The RELATE test, a comparative (mantel-type) test on similarity matrices, was used to compare the different composition groups (Spearman rank correlation; Rho = 1 indicates a perfect match; Clarke and Warwick 2001).

Results

Spatial trends by factors

A total of 22 families and 92 species of fishes were observed overall (S1 Table). Fish abundance at each site was dominated by the family Pomacentridae, encompassing > 60 % at each site, followed by Labridae with around 15 %. Within inshore reefs, total fish abundance was extremely low (65 ± 27.8 ind. 250 m^{-2}, mean ± SD), while in midshore (354 ± 72.4 ind. 250 m^{-2}, mean ± SD) and offshore reefs (335 ± 127.3 ind. 250 m^{-2}, mean ± SD) abundances were very similar. Species richness 250 m^{-2} was lowest in inshore (11 ± 4.1, mean ± SD) and similar in mid- (27 ± 5.9, mean ± SD) and offshore reefs (21 ± 2.1, mean ± SD). The highest fish diversity (Shannon biodiversity index) was found at Pari North in the midshore zone. Sites within JB (inshore) did not differ from offshore sites with regard to fish diversity (Table 1.2). Highest total coral cover of 49 % (mean hard coral cover: 47 %) was found at Pari North in the midshore zone, while near- and offshore reefs had a total coral cover of 15 ± 9.1 % (mean ± SD; the majority of which were soft corals (mainly nephtheids, xeniids and alcyoniidids); hard coral cover was 2 ± 2.3 % (mean ± SD) and 31 ± 11.1 % (mean ± SD; hard coral cover: 22 ± 7.5 %, mean ± SD), respectively (Fig. 1.2).

Table 1.2: Diversity indices for fish community. Fish diversity (Shannon H´), evenness (Simpson 1-λ), total number of species (S) and individuals (N) for each site respectively.

Site	Total species S	Total individuals N	Shannon H´(log)	Simpson 1-λ`
AB	20	58	2.54	0.9
UJ	27	99	2.82	0.92
R	18	38	2.72	0.95
PS	38	354	2.96	0.93
PN	48	353	3.1	0.94
P	37	300	2.67	0.88
C	35	354	2.81	0.92
B	30	349	2.66	0.9

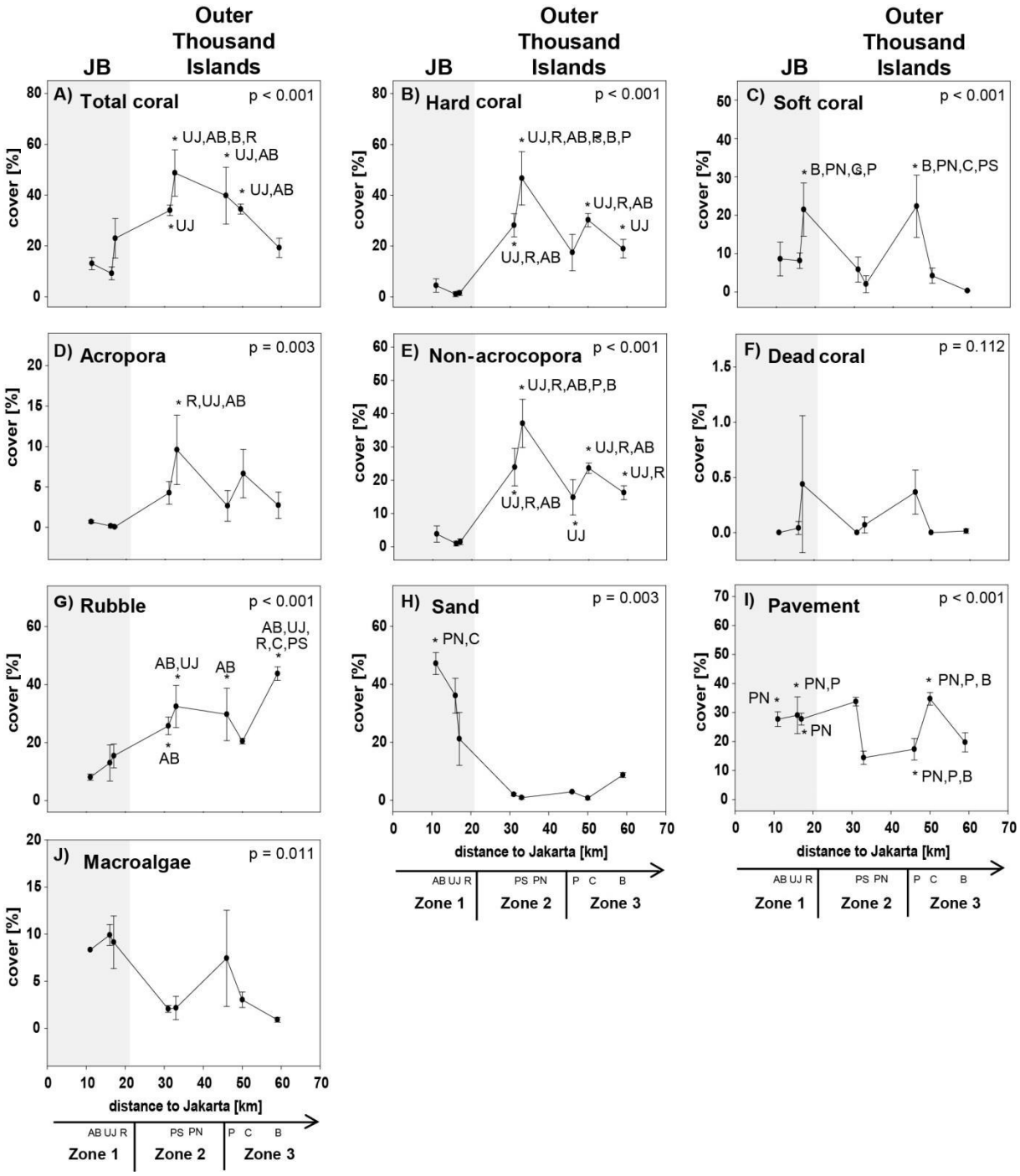

Fig. 1.2: Benthic community composition. Data for sites in Jakarta Bay (JB; grey highlighted) and outer Thousand Islands is given as mean cover (± SD) for total coral (A), hard coral (B), soft coral (C), acroporid (D), non-acroporid (E), dead coral (F), rubble (G), sand (H), pavement (I) and macroalgae (J) at each site. p-values and post hoc results for differences between sites are given for each graph. Consider different scales on y-axis. Study sites: AB = Ayer Besar, UJ = Untung Jawa, R = Rambut, PS = Pari South, PN = Pari North, P = Panggang, C = Congkak, B = Bira.

Inshore, corals were comprised almost exclusively of submassive corals which are stress-tolerant corals (S) (Veron 1985, Rogers 1990) and encrusting corals, which are competition-adapted corals (K), while foliose, digitate and branching corals were completely or almost

absent (Fig. 1.3). The two sites Pari North and Bira were dominated by competition-adapted corals (K), i.e. all non-acroporid corals that are either branching, encrusting, foliose or mushroom-shaped and are characterized as K-adapted due to their lower growth rates than acroporid corals (Moll 1983, Bak and Povel 1989). Ruderal corals however (r), i.e. all acroporid corals and tabular non-acroporid corals, which are more disturbance-adapted due to their rapid growth and mechanical fragility (Done 1982, Karlson and Hurd 1993), were not dominating any reefs (Fig. 1.3 and S1.1 Fig.).

A clear separation of all three zones was only found for the feeding guild OV and for the species richness in the subfamily Scarinae. The separation of mid- and offshores sites within the Thousand Islands was less distinct. Only some factors showed a significant separation of inshore sites and sites from the Thousand Islands (e.g. NO_2, cover of hard and non-acroporid corals, total fish abundance and species richness, abundance of Pomacentridae and Scarinae, species richness of Pomacentriade and Caesionidae; see Table 1.3 for post-hoc results; Fig. 1.4 - 1.7).

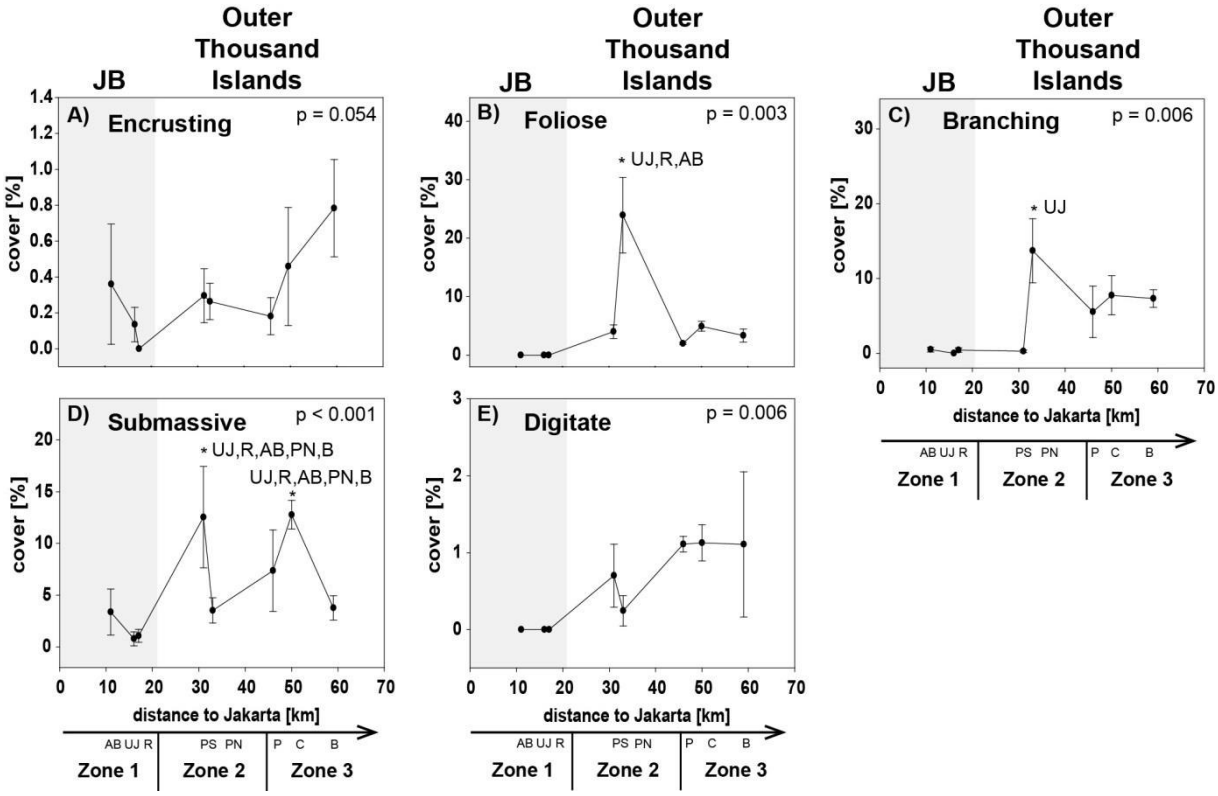

Fig. 1.3: Coral morphology composition. Data for sites in Jakarta Bay (JB; grey highlighted) and outer Thousand Islands is given as mean cover (± SD) for encrusting (A), foliose (B), branching (C), submassive (D) and digitate (E) corals at each site. p-values and post hoc results for differences between sites are given for each graph. Consider different scales on y-axis. Study sites: AB = Ayer Besar, UJ = Untung Jawa, R = Rambut, PS = Pari South, PN = Pari North, P = Panggang, C = Congkak, B = Bira.

Table 1.3: Univariate analyses to test for distance-based and localized spatial trends for 5 different fish and benthic community composition groups and environmental factors. Regression types tested were: linear or exponential de- or increase towards offshore and linear de- or increase with one breakpoint (2 segments). The regression type yielding the lowest p-value and highest R^2 is shown. Tests for zonation and localized effects were done using one-way Anova. Post-hoc results (Tukey test) for differences between zones are given in the 'zonation' column.

Factors			Distance-based effects					Localized effects
Group	Composition	Component/Factor	Regression analysis - best fit			Zonation (*a priori* groups)		p-value
			Type	p-value	R^2	p-value	Post-hoc	
Fish	Community: abundance	Caesionidiae	Linear	0.16	0.3	0.196		0.179
		Pomacentridae	Linear (2 seg.)	< 0.001	0.95	0.002	z1 vs. z2 z1 vs.z3	0.018
		Labridae	Linear	0.05	0.49	0.086		0.079
		Nemipteridae	Exp.	0.08	0.43	0.04		0.11
		Scaridae	Linear (2 seg.)	0.03	0.86	0.03	z1 vs. z2 z1 vs.z3	0.048
		Serranidae	Exp.	0.23	0.23	0.08		0.845
		Total	Linear (2 seg.)	0.01	0.93	< 0.001	z1 vs. z2 z1 vs.z3	0.014
Fish	Community: species richness	Caesionidiae	Linear	0.08	0.44	0.033	z1 vs. z2 z1 vs.z3	0.035
		Pomacentridae	Exp.	< 0.001	0.79	0.026	Z1 vs. z3	0.018
		Labridae	Linear (2 seg.)	0.02	0.9	0.002	z1 vs. z2 z1 vs.z3	0.021
		Nemipteridae	Linear	0.06	0.48	0.261		0.204
		Scaridae	Linear (2 seg.)	0.01	0.92	< 0.001	z1 vs. z2 z1 vs.z3 z2 vs.z3	0.006
		Serranidae	Linear (2 seg.)	0.58	0.36	0.148		0.774
		Total	Linear (2 seg.)	0.04	0.86	0.006	z1 vs. z2 z1 vs.z3	< 0.001
Fish	Feeding guild	CV	Linear (2 seg.)	0.25	0.61	0.445		0.41
		HV	Linear (2 seg.)	0.01	0.92	< 0.001	z1 vs. z2 z1 vs.z3	< 0.001
		OV	Linear	0.02	0.66	< 0.001	z1 vs. z2 z1 vs.z3 z2 vs.z3	< 0.001
		OVI	Linear (2 seg.)	0.21	0.65	0.382		0.005
		PV	Linear	0.07	0.45	0.159		0.08
		OCV	Exp.	0.05	0.49	0.095		0.103

Table 1.3 continued

Factors			Distance-based effects					Localized effects
Group	Composition	Component/Factor	Regression analysis - best fit			Zonation (*a priori* groups)		p-value
			Type	p-value	R^2	p-value	Post-hoc	
Benthic	Community	Dead coral	Exp.	0.81	0.01	0.798		0.112
		Hard coral	Linear	0.06	0.67	0.009	z1 vs. z2 z1 vs.z3	< 0.001
		Rubble	Exp.	0.01	0.71	0.067		< 0.001
		Pavement	Linear	0.57	0.08	0.808		< 0.001
		Sand	Linear (2 seg.)	0.01	0.94	0.061		0.003
		Macroalgae	Linear	0.47	0.09	0.543		0.011
		Soft coral	Linear	0.48	0.09	0.591		< 0.001
		Acroporid	Linear (2 seg.)	0.19	0.66	0.054		0.003
		Non-acroporid	Linear (2 seg.)	0.07	0.8	0.005	z1 vs. z2 z1 vs.z3	< 0.001
		Total coral	Linear (2 seg.)	0.03	0.87	0.062		< 0.001
	Water	Salinity	Linear	0.02	0.6	0.049	z1 vs. z3	0.007
		pH	Exp.	0.37	0.13	0.806		0.083
		DO	Exp.	0.56	0.06	0.837		0.106
		Temperature	Linear	0.44	0.1	0.357		0.114
		Turbidity	Exp.	0.15	0.32	0.011	z1 vs. z2	0.005
		Sedimentation	Linear (2 seg.)	0.01	0.92	0.244		< 0.001
		Chl a	Exp.	0.07	0.37	0.081		0.003
		PO_4	Exp.	0.7	0.03	0.421		< 0.001
		NO_3 (nitrate)	Linear	0.42	0.11	0.025		< 0.001
		NO_2 (nitrite)	Linear	0.03	0.57	0.088		< 0.001
		NH_4	Exp.	0.47	0.54	0.524		< 0.001
		DIN	Linear	0.15	0.35	0.54		< 0.001

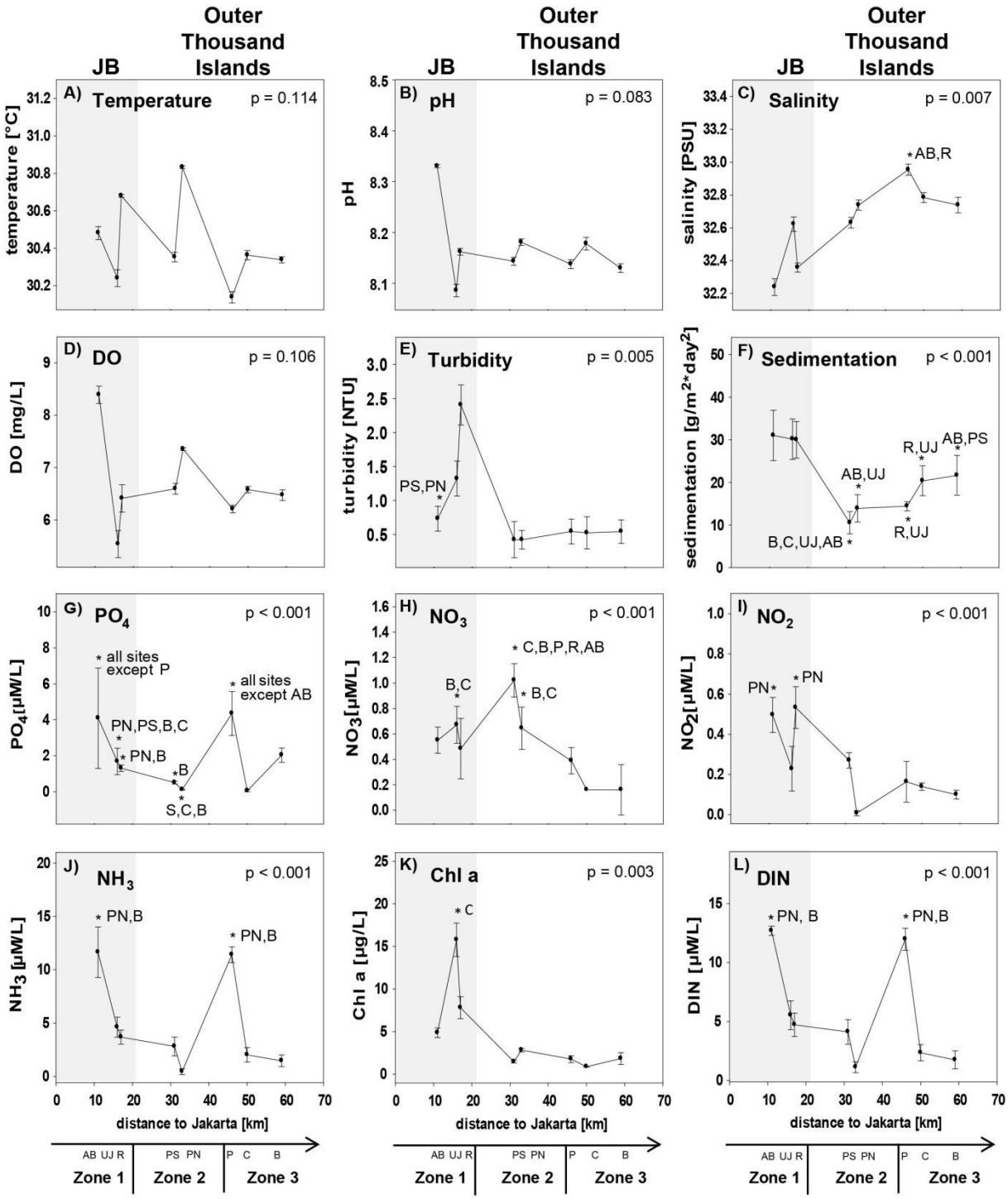

Fig. 1.4: Water quality composition. Data for sites in Jakarta Bay (JB; grey highlighted) and outer Thousand Islands: Mean values (± SD) for the factors (A) temperature [°C], (B) pH, (C) salinity [PSU], (D) DO [mg/L], (E) turbidity [NTU], (F) sedimentation, the inorganic nutrients [µM/L] PO_4 (G), NO_3 (H), NO_2 (I) as well as NH_3 (J), (K) Chl a [µg/L] and (L) dissolved inorganic nutrients (DIN; [µM/L] at each site. p-values and post hoc results for differences between sites are given for each graph. Study sites: AB = Ayer Besar, UJ = Untung Jawa, R = Rambut, PS = Pari South, PN = Pari North, P = Panggang, C = Congkak, B = Bira.

Among the different factors of each composition group, only few showed significant gradual or exponential in- or decreases with increasing distance from shore. Clear linear increases in salinity and cover of digitate corals and a decrease in NO_2 as well as exponential increases in coral rubble were found towards offshore. Other factors first decreased significantly from Ayer Besar to Rambut in JB and then either increased linearly (e.g. sand, sedimentation rate, total fish species richness) or decreased (e.g. abundance of Pomacentridae, herbivores; see Table 1.3, Fig. 1.4 - 1.7).

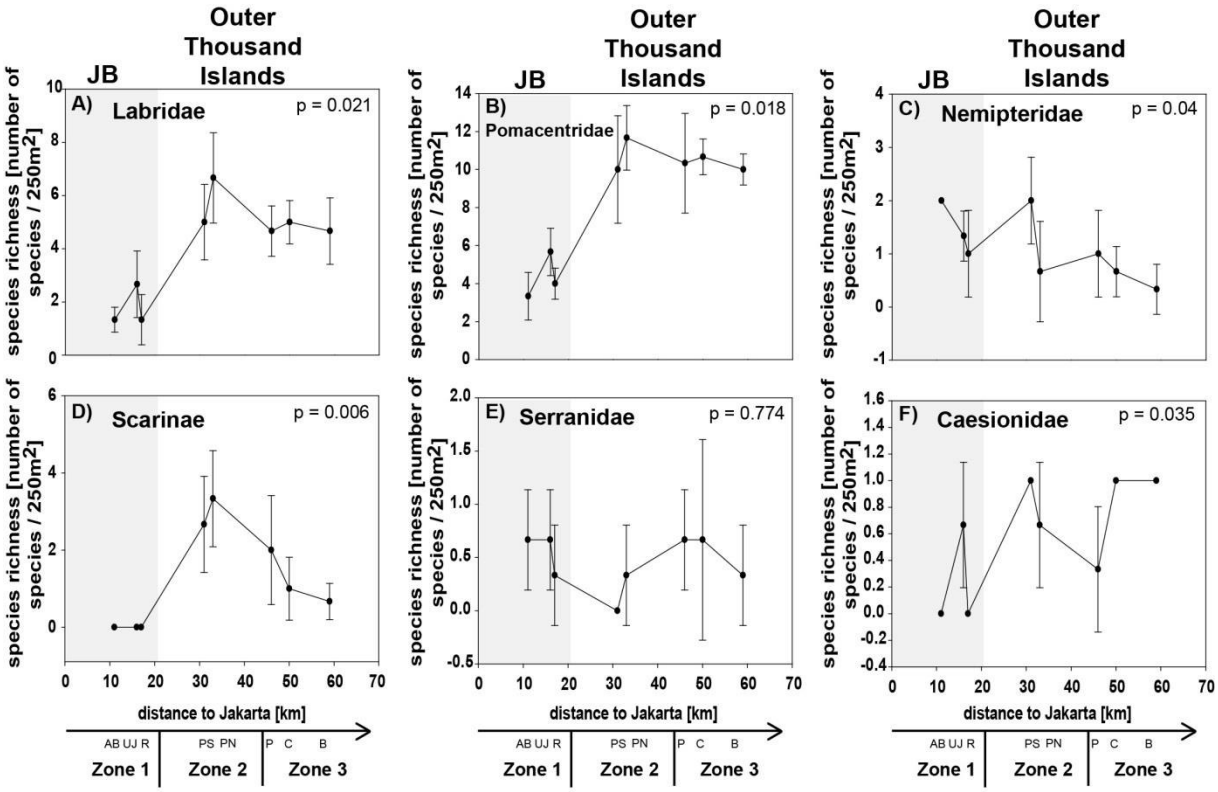

Fig. 1.5: Fish community composition: Species richness. Data for sites in Jakarta Bay (JB; grey highlighted) and outer Thousand Islands is given as mean species richness (± SD) for the families (A) Labridae (excluding Scarinae), (B) Pomacentridae, (C) Nemipteridae, (D) labrid Scarinae, (E) Serrranidae and (F) Caesionidae at each site. p-values and post hoc results for differences between sites are given for each graph. Consider different scales on y-axis. Study sites: AB = Ayer Besar, UJ = Untung Jawa, R = Rambut, PS = Pari South, PN = Pari North, P = Panggang, C = Congkak, B = Bira.

Most factors did not show a clear zonation or distance-based spatial patterns, but rather localized patterns. The concentration of PO_4 and NH_3 for example both seemed to decrease towards offshore, however both showed a significantly higher concentration at a single offshore site (Panggang) compared to all other sites. Also at Bira, a site that is furthest north and within the conservation zone of the National Park, PO_4 was higher than at midshore

sites, where concentrations were lowest overall. Similarly, such a local but significantly higher increase was found at Pari South for NO_3 concentrations and for Chl a at Untung Jawa. Also soft coral cover had two peaks at Rambut and Panggang, where cover was significantly higher than at Pari North and Bira. At Pari North coral cover (hard, acroporid, non-acroporid and foliose) was higher compared to all nearshore sites and to several offshore sites (Table 1.3, Fig. 1.4 – 1.7).

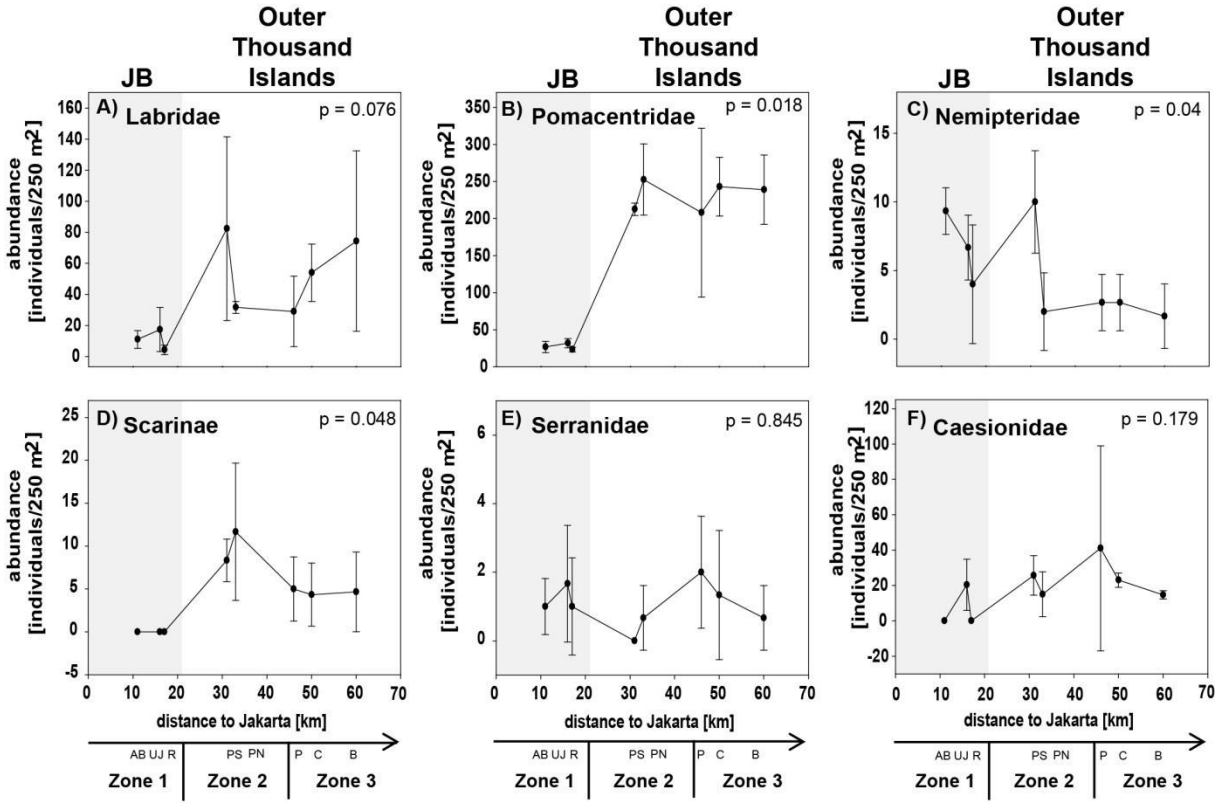

Fig. 1.6: Fish community composition: Fish abundance. Data for sites in Jakarta Bay (JB; grey highlighted) and outer Thousand Islands is given as mean values (± SD) for the families (A) Labridae (excluding Scarinae), (B) Pomacentridae, (C) Nemipteridae, (D) labrid Scarinae, (E) Serrranidae and (F) Caesionidae at each site. p-values and post hoc results for differences between sites are given for each graph. Note the different scales on y-axis. Study sites: AB = Ayer Besar, UJ = Untung Jawa, R = Rambut, PS = Pari South, PN = Pari North, P = Panggang, C = Congkak, B = Bira.

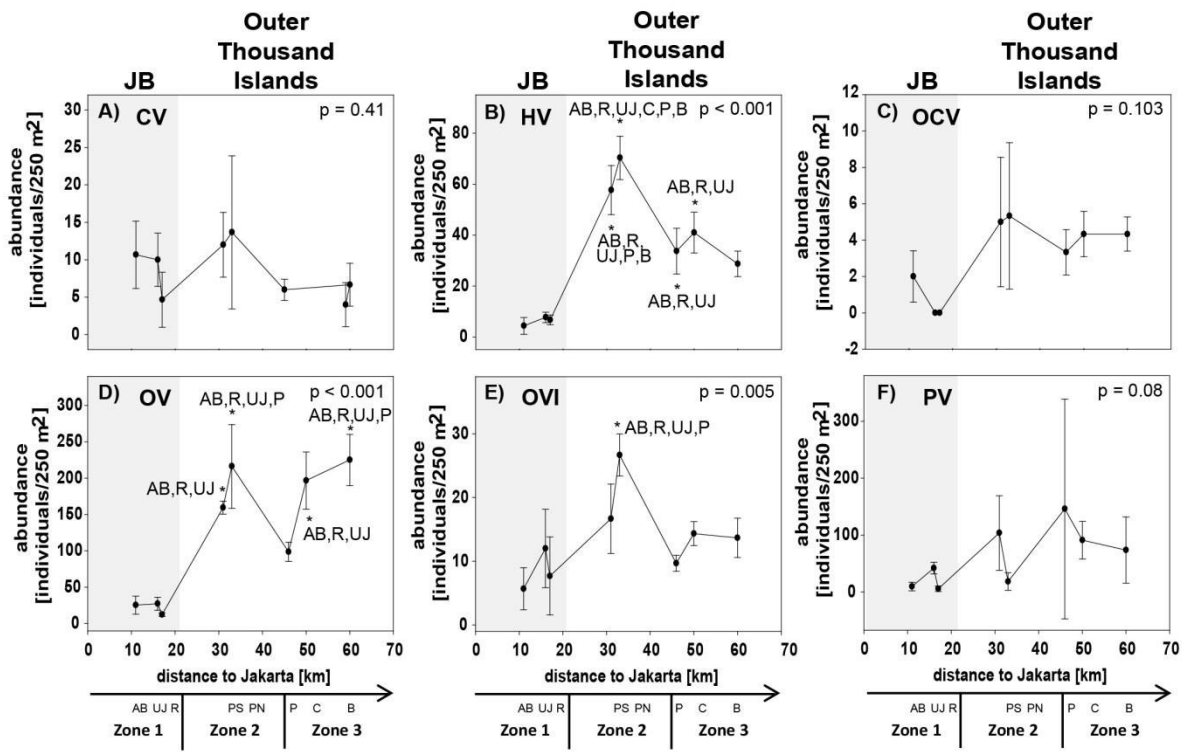

Fig. 1.7: Fish feeding guild composition. Data for sites in Jakarta Bay (JB; grey highlighted) and outer Thousand Island is given as mean abundance (± SD) for the guilds (A) corallivores (CV), (B) herbivores (HV), (C) obligate corallivores (OCV), (D) omnivores (OV), (E) omnivore/invertivores (OVI) and (F) planktivores (PV) at each site. p-values and post hoc results for differences between sites are given for each graph. Consider different scales on y-axis. Study sites: AB = Ayer Besar, UJ = Untung Jawa, R = Rambut, PS = Pari South, PN = Pari North, P = Panggang, C = Congkak, B = Bira.

Relative role of stressors and interactions between composition groups

Multivariate analysis of each composition group with a zonal distribution pattern as basis revealed an overall significant difference between zones for all composition groups (Permanova test; $p < 0.05$), although pairwise tests for effects of zones failed to detect significant differences ($p > 0.05$). The results indicate that differences were mainly between the first and one of the other two zones (S1.2 Table, S1.2 Fig.). Similar to results from the univariate analysis, the separation of nearshore sites in JB and offshore sites was distinct (Fig. 1.8). Offshore sites however clustered together and displayed no clear separation into mid- and offshore zones. For both fish composition groups and the benthic community composition, the site Panggang was less similar to the other offshore sites from the Thousand Islands (Fig. 1.8).

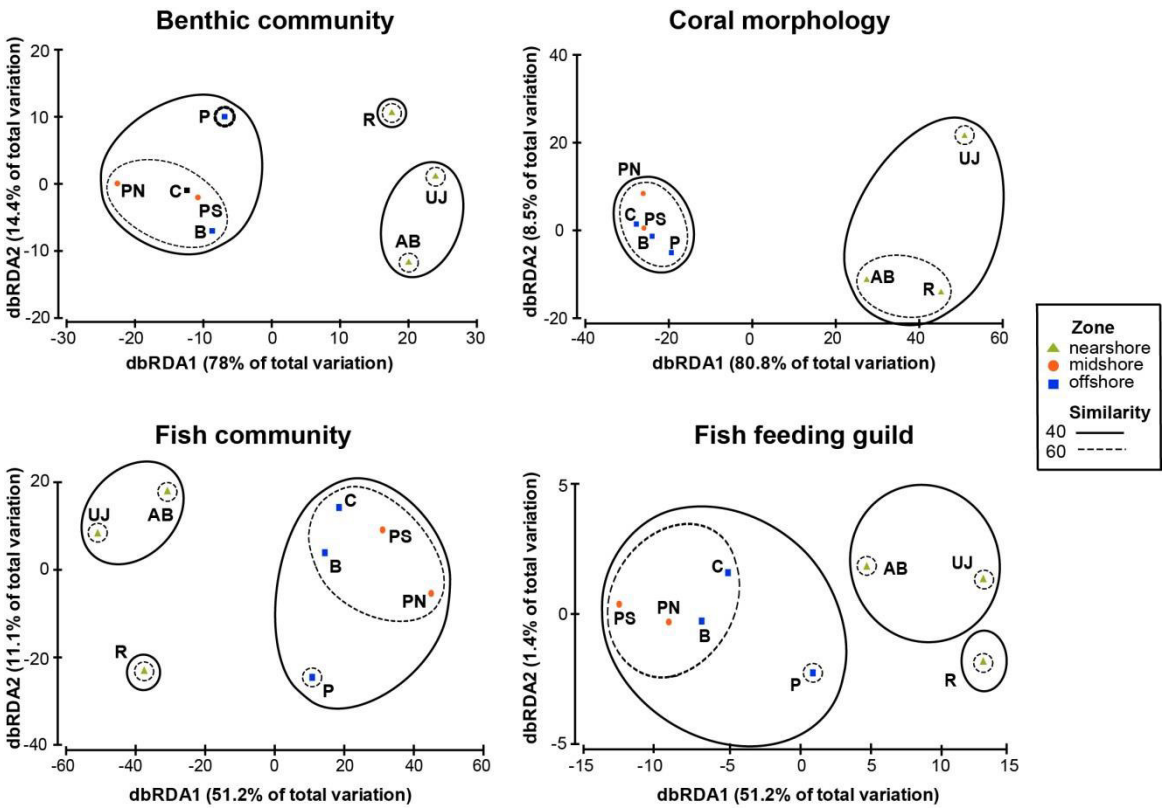

Fig. 1.8: Visualization of fish and benthic community composition based on distance-based redundancy analysis (dbRDA). Benthic community composition (A), coral morphology composition (B), fish community taxonomic composition (C) and fish feeding guild composition (D) are shown. Study sites: AB = Ayer Besar, UJ = Untung Jawa, R = Rambut, PS = Pari South, PN = Pari North, P = Panggang, C = Congkak, B = Bira.

Benthic community (p = 0.007) and coral morphology (p = 0.016) composition were significantly related to water characteristics (Table 1.4). Spatial patterns in the benthic community composition were best explained by NO_2 concentration, followed by sedimentation rate, PO_4 and Chl a, together accounting for 83 % of the observed variability. Coral morphology composition was explained by NO_2, turbidity, sedimentation and Chl a, which accounted for 88 % of the observed variability (Table 1.5).

Table 1.4: Relation (test: RELATE) between composition groups and their proximate driving composition group.

Group	Composition	Relation with		
		Composition (proximate driver)	R	p-value
Benthic	Community	Water	0.61	0.007
	Coral morphology	Water	0.59	0.016
Fish	Community	Benthic community	0.8	0.003
		coral morphology	0.88	0.002
	Feeding guild	Benthic community	0.58	0.013
		coral morphology	0.78	0.003

Table 1.5: Correlation of each composition group with their proximate driving composition group. Data is based on the test BioEnv for all sites and those sites located in the outer Thousand Islands.

Group	Composition	Correlation with				
		Composition	All Thousand Islands		Outer Thousand Islands	
			Corr	Factor	Corr	Factor
Benthic	Community	Water	0.83	Chl a NO_2 PO_4 Sed	0.69	PO_4 Sal DO Turb
	Coral morphology	Water	0.87	Tub Chl a NO_2 Sed	0.98	Temp Turb Chl a DO
Fish	Community	Benthic community	0.9	Non-acroporid Acroporid Dead coral	0.67	Non-acroporid Macroalgae Dead coral
		Coral morphology	0.88	Bottlebrush Digitate	0.89	Bottlebrush Digitate
	Feeding guild	Benthic community	0.82	Acroporid	0.81	Soft coral Pavement Hard coral Dead coral
		Coral morphology	0.86	Digitate Bottlebrush Submassive encrusting	0.94	Branching bottlebrush

Fish community taxonomic and feeding guild composition displayed high correlations with both benthic community ($p = 0.003$ and $p = 0.013$, respectively) and coral morphology composition ($p = 0.002$ and $p = 0.003$, respectively) (Table 1.5). Fish species richness was linearly correlated with total coral cover ($p = 0.03$; linear regression). Spatial patterns in both fish community (87 % of variability explained) and feeding guild (82 % of variability explained) composition were best explained by the total cover of acroporid corals, followed by total cover of non-acroporid and dead corals (Table 1.5).

The role of the main drivers for the four different composition groups is visualized in linkage tree graphs (Fig. 1.9) and in all cases shows a clear grouping of sites within JB, but a mix of sites from mid- and offshore reefs due to localized effects of anthropogenic stressors. Both for the fish community and feeding guild composition, Pari North is separated from all other reefs due to highest overall coral cover. Similarly, Panggang is separated due to it having the

lowest coral cover overall. In the two benthic composition groups, the sites Panggang and Bira together form one group due to higher PO_4 levels (benthic community composition) and higher Chl a and turbidity levels (coral morphology composition), compared to the other sites from mid- and offshore reefs.

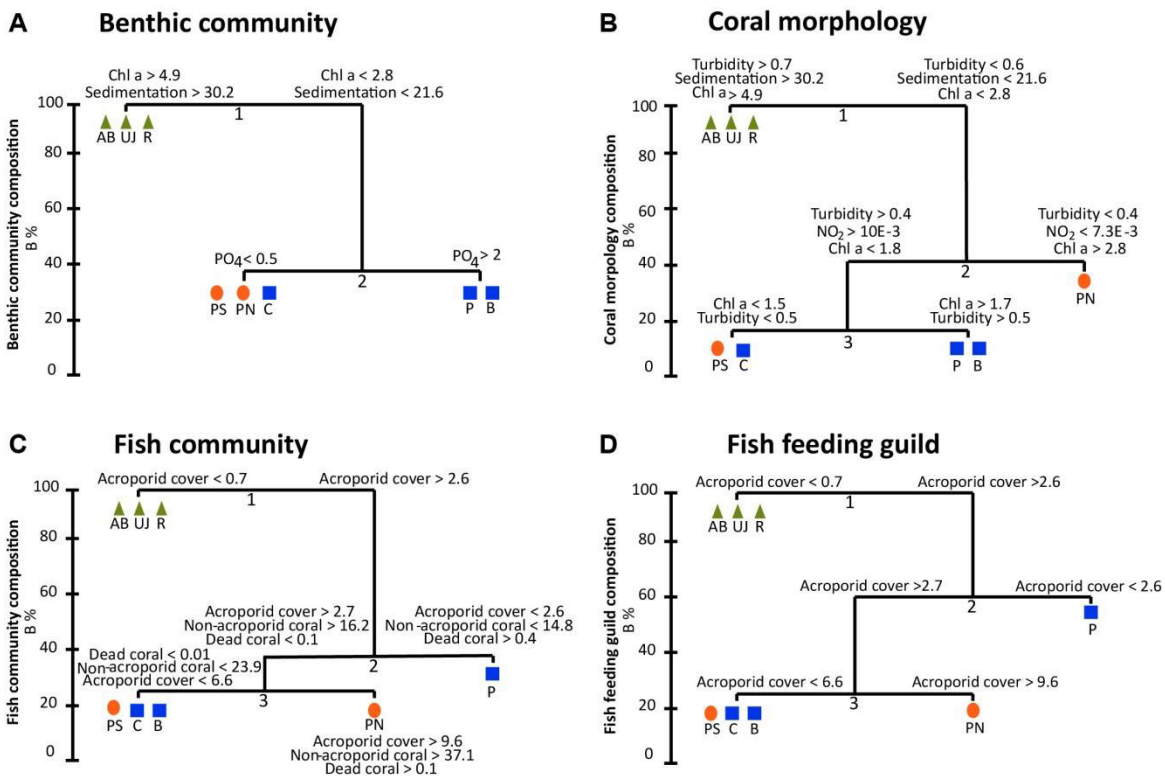

Fig. 1.9: Linkage tree and associated thresholds of proximate drivers that relate to the separation of A) benthic community, B) coral morphology, C) fish community taxonomic and D) fish feeding guild composition. Thresholds at the end of each branch indicate that a left or right path respectively should be followed through the tree. B % is the absolute measure of group differences. Study sites: AB = Ayer Besar, UJ = Untung Jawa, R = Rambut, PS = Pari South, PN = Pari North, P = Panggang, C = Congkak, B = Bira. Zones are indicated by symbols: circle (nearshore), triangle (midshore), square (offshore).

Discussion

At the sample depth of 5 m, multiple stressors affected spatial patterns in coral reef communities along the reefs of Jakarta Bay and the Thousand Islands. Using a combination of different models, we found that local and regional factors acted and interacted at different spatial scales, resulting in a mosaic of reef community configurations. Results suggest that for reefs at around 5 m water depth, the direct impact of Jakarta appears to be mainly restricted to inshore reefs within the bay, since both univariate and multivariate analysis of benthic and

fish communities showed a clear separation between sites in JB and all sites north of the bay (i.e. the northern Thousand Islands). Reefs north of the bay at this depth range, however, did not follow a gradual spatial pattern with reef condition improving towards north, but rather localized effects of anthropogenic stressors, especially those related to eutrophication, appear to shape the spatial structure of reefs. This has led to the spatial patchwork of differentially degraded reefs that was described by Rachello-Dolmen and Cleary (2007).

When the Dutch scientist Umbgrove conducted one of the first marine assessments in Indonesia around the Thousand Islands in 1929, he found a reef system with high species diversity (Umgrove 1939). However, anthropogenic influences were already documented in the early 1900s (Zaneveld and Verstappen 1952, Verstappen 1953, 1988), and especially since the 1950s, Jakarta´s rapid population growth has transformed the city into a megacity with more than 14,500 inhabitants km^{-2} in the city area (Pelling and Blackburn 2014), causing the bay to become one of the most polluted in Asia (Bengen et al. 2006). Here, results confirm that the bay is facing extreme eutrophication coupled with increased primary production and turbidity. PO_4 levels in the upper layer of JB reached 4 µM/L and DIN levels up to 13 µM/L. Damar (2003) reported an annual average of 5.1 µM/L for PO_4 and 20.1 µM/L for DIN in JB. These extremely high nutrient values are the consequence of massive land runoff, lack of sewage treatment and large-scale agri- and aquaculture. In the wider Jakarta region, about 80 % of the wastewater runs directly into the rivers through an open ditch system (Damar et al. 2012). At all sites in JB, Chl a levels were between 5 and 15 µg/L, thus far above the Eutrophication Threshold Concentration for Chl a of 0.2 - 0.3 µg/L (Bell et al. 2007), indicating high primary productivity. In addition, sites within JB had significantly higher sedimentation rates compared to offshore sites in the Thousand Islands, with up to 30 $g\ m^{-2}\ d^{-1}$. Sedimentation is considered to be a principal stress factor for coral reefs (Fabricius 2005); e.g. in Singapore, chronic exposure to high sediment loads is seen as the main stressor affecting corals (Dikou and van Woesik 2006, Huang et al. 2009). Similarly, high turbidity levels of 3–5 NTU as found in the bay can reduce light availability and thereby reduce coral photosynthesis and calcification rates (De`ath and Fabricius 2008).

These severe changes in water quality, compared to generally oligotrophic and relatively low turbid waters where coral reefs thrive, can in part explain the massive coral reef degradation. Hard coral cover is now at 2 % in the bay, and compared to sites from the northern Thousand Islands, fish abundance was reduced by around 80 %. Both uni- and multivariate statistics revealed that at the sites within the bay overall reef condition at shallow depth was far lower compared to those reefs along the Thousand Islands. Even though among all of the

measured water factors, only salinity and turbidity in JB were significantly different to offshore sites, multivariate statistics showed that when different aspects of fish and coral community composition were considered, all sites within the bay were clearly separated from the rest. This suggests that even though Jakarta is a megacity with high intensity of urbanisation, industry and shipping, the direct impact on coral reef condition at 5 m depth appears to be restricted to within the bay itself. In addition, the fact that compared to previous studies, where nearshore to offshore gradients in heavy metal pollution, nutrient input and water transparency (Rees et al. 1999, Williams et al. 2000), as well as coral cover (Cleary et al. 2006, 2008) and fish abundance (Madduppa et al. 2013), were found, hardly any overall gradual de- or increases from near- to offshore were observed, which shows that the changes in reef condition at 5 m depth and water quality are rather abrupt and not gradual. This may be a reflection of the role of those environmental factors acting at shallower water depth. Both salinity and turbidity are likely to display stronger changes in the surface waters, as plumes of runoff from Jakarta largely float on the surface, particularly beyond areas of mixing in the inner parts of the bay. While a number of factors showed linear gradients, these also displayed a breaking point at either Pari South or Pari North. For example, sedimentation rate and cover of sand gradually increased from Pari South towards offshore, while total coral cover and total fish abundance gradually decreased. Cleary et al. (2014) reported a linear increase in shallow-water (< 5 m) coral cover from in- to offshore in 1985, and in 1995 higher cover midshore than offshore. Different patterns to those observed in this study for shallow reefs along the Thousand Islands can be assumed to occur at greater depths, particularly at offshore sites with clearer water conditions, and further research is needed to determine spatial patterns at these depths.

Based on a hydrodynamic model, Koropitan et al. (2009) concluded that JB is mainly controlled by water influxes from adjacent marine waters. JB is very shallow with a mean depth of only 15 m, resulting in relatively well-mixed concentrations of for instance nutrients (Koropitan et al. 2009). Even though the south-easterly winds during northwest monsoon could potentially cause polluted water masses from JB to reach the northern Thousand Islands, Koropitan et al. (2009) estimated that bottom currents are up to 90 % slower than surface flows in JB. The relatively good reef condition at Pari Island, especially when compared to sites further north, may be seen as an indicator that water masses from Jakarta do not considerably affect offshore reefs of the Thousand Islands. Damar (2003) and Damar et al. (2012) found that the direct impact of estuarine nutrient loads coming from Jakarta is limited to nearshore areas close to river mouths. Similarly, other studies focusing on heavy metals (Rees et al. 1999, Williams et al. 2000, Hosono et al. 2011, Farhan and Lim 2012) and

organic contaminants (Rinawati et al. 2012) suggest that these pollutants affect reefs of the northern Thousand Islands far less or almost not at all. Most sources of organic pollutants along the Thousand Islands seem to be the increasing ship traffic and oil spills from oil drilling activities to the northwest of the island chain (Uneputty 1997).

Nevertheless, the overall reef condition along the Thousand Islands chain at shallow depths can be considered as being poor since total coral cover in most of the sites was < 25 % (threshold based on Gomez and Yap (year). Values of hard coral cover for near- (2 %), mid- (37 %) and offshore (22 %) reefs were similar to those reported by Cleary et al. (2014) for the Thousand Islands chain in 2011. However, the midshore reefs exceed the current average coral cover of 22.1 % reported for the Indo-Pacific area (Bruno and Selig 2007). Similarly, fish abundance and species richness in November 2012 were lower at all offshores sites compared to estimates by Madduppa et al. (2013). Fishing pressure currently is concentrated on JB where it is very high, however due to continually decreasing fish yields over the last years (KKP 2011), fishermen may be forced to move to other fishing grounds further north (Kusumanti 2013), potentially further threatening the already depleted reef fish resources of the Thousand Islands.

Even though previous studies observed gradients along the Thousand Islands in the past, this was not clearly visible by the time of the present study. Neither was there an indication that the zoning into mid- and offshore reefs as used by Cleary et al. (2006) is applicable anymore for reefs at around 5 m water depth. Hardly any factors showed significant differences between mid- and offshore reefs along the Thousand Islands at this depth. Results rather show that certain sites (especially Pari North and Panggang) differed significantly from the other sites, suggesting that the observed high spatial variability in reef condition between sites that are only separated by a few km is most likely due to highly localized effects (which will be discussed below). Such a high spatial variability on a smaller regional scale (e.g. (Selig et al. 2006) and local scales of < 20 km (e.g. Murdoch and Aronson 1999, Edmunds 2002, Berkelmans et al. 2004) have been observed in other coral reef regions. This highlights the potential role of stressors, both local and regional, in shaping the structure of benthic communities in coral reefs.

Along the Thousand Islands, the benthic community and coral morphology compositions were significantly related to anthropogenically influenced water parameters. 80 % of the variation in benthic community composition along the complete island chain could be linked to factors related to terrestrial run-off and eutrophication, especially NO_3, sedimentation, turbidity, PO_4 and Chl a, mirroring the observation from a recent ocean-wide study that local anthropogenic stressors can become the dominant factors shaping benthic reef communities

(Williams et al. 2015). Eutrophication has been proposed to be the main stress factor for many reefs worldwide. For example, long term monitoring data from the Great Barrier Reef show that the overall reduction in total coral cover by 70 % is mainly due to eutrophication (Bell et al. 2014). Eutrophication refers to the response of an ecosystem to an increase in nutrient concentrations in the water, which then leads to an increase in algae growth and turbidity (GESAMP 2001). Along the Thousand Islands, overall Chl a levels (mean: 1.7 μg/L) were above the eutrophication threshold levels of 0.2 - 0.3 μg/L (Bell et al. 2007) at all sites. While correlative in nature, the results of this study suggest that differences in benthic community and coral morphology composition between sites are caused by the presence of local sources of high nutrient values, as can be seen for the site Panggang, situated relatively in the middle of the island chain and the site Bira in the north. Mean PO_4 and NH_4 levels were increased by 60 % at Panggang compared to Pari, and at Bira PO_4 was increased by 30 %. These two islands form their own cluster in terms of community composition, with similar nutrient conditions. Benthic community composition was significantly related to increased PO_4 levels, and coral morphology composition mainly to higher turbidity and Chl a levels. At the site Panggang, the increased nutrient levels can be attributed to the proximity of nearby highly populated islands (Pramuka and Panggang), where sewage is discharged directly to the sea without any prior treatment as commonly practiced on all populated islands along the Thousand Islands chain. However, proximity to populated islands alone is not the only explanatory factor, since Bira Island is not inhabited, and the highest coral cover (both acroporid and non-acroporid cover) was found at Pari North and Pari South close to the populated Pari island complex. This island group displayed relatively low nutrient levels, suggesting that human population density does not always lead to coral decline linked to eutrophication.

However, the observed localized differences in benthic reef condition at 5 m depth along the Thousand Islands are not necessarily caused by local sources of eutrophication alone. Experimental studies are necessary to empirically establish causation, and other confounding factors may play a role as well. Many regional-scale disturbances such as predator and disease outbreaks and bleaching events have been shown to exhibit highly localized effects, leading to high spatial variability on smaller regional scales (Selig et al. 2006, Berkelmans et al. 2004). Cleary et al. (2014)] suggest that the 2010 bleaching event in the Indo-Pacific region and Indonesia has affected offshore reefs at the Thousand Islands more severely than midshore reefs. The authors reported a dramatic loss of acroporid cover offshore from 36 % in 1985 to 5 % in 1995. In the present study, cover of rubble increased significantly towards offshore, where it is now at 31 %. Although Cleary et al. (2014) suggest a tentative recovery for reefs

of the Thousand Islands chain, the present observations suggest that coral reef recovery may still be inhibited, at least in shallow areas. Large fields of rubble can shift during storms, which may hinder colonization by coral recruits (Fox and Caldwell 2006). Especially at the sites Bira and Panggang, the loss in Acropora cover is severe, with a current cover of < 3 %. Similarly, blast fishing was commonly practiced in the 1980s (Erdmann 1998).

Overall fish community composition was highly related to the benthic community composition, and almost 90 % of variability in fish community composition along the Thousand Islands could be linked to the cover of acroporid, non-acroporid and dead corals. Declines in the abundance and diversity of coral reef fish have been previously linked to an indirect effect of habitat loss (Wenger et al. 2014). Herbivores play a key role in reef ecosystem function since they actively influence the competition for space between corals and algae (Hughes et al. 2007). At Pari North and Pari South, a higher herbivore abundance was observed compared to Panggang, where average macroalgae cover was similarly high to that of sites in JB. The disappearance of herbivores as a top-down factor has been suggested to cause shifts of coral reefs towards macroalgae-dominated conditions, rather than bottom-up factors such as eutrophication (Rasher et al. 2012). Such shifts have been documented from locations around the world, but to date appear most common in the Caribbean (Norström et al. 2009).

High spatial variability in reef condition on a regional scale, as observed for the reefs of the Thousand Islands, has to be considered in future conservation and management plans. For the Thousand Islands this specifically means that the organization of the Thousand Islands National Park has to be re-evaluated, since the regulations as well as the boundaries of the park have not undergone any reformation for almost three decades (Farhan and Lim 2012), to account for demographic, economic and environmental changes. For example, there are four different government agencies involved in decision making processes regarding the National Park organization, which work independently and consequently can slow down overall progress (Farhan and Lim 2012). Localized effects have to be incorporated into management plans, and marine spatial planning that explicitly accounts for the different spatial extent of stressors as an alternative to conventional management could be a suitable approach. Marine spatial planning tries to consider the needs of all stakeholders involved in a certain system by assigning defined zones, which could be based on their distance to an urban centre to reflect differences in impacts and uses. In the present case, zoning should not only include distance from Jakarta, but consider island-specific conditions that may fall outside of larger-scale trends. For example, an approach could be to administer each populated island separately rather than grouping several populated islands into an

administrative zone, as is currently the case for the Thousand Islands. Each zone may then be assigned to certain activities, for example port and shipping closer to the city centre, followed by fishing activities, then tourism and furthest away marine reserves (Sale et al. 2014). As such a zonation roughly reflects the current spatial arrangement of activities in the Thousand Islands, incorporating them into a dedicated Marine Spatial Planning framework should be feasible. However, the specific situation in the area necessitates a few location-specific adjustments. For example, the extreme levels of pollution in the inner Jakarta Bay have caused significant heavy metal contamination in green mussels (Cordova et al. 2012), which suggests that the intense aquaculture of green mussels currently practiced there is not sustainable in terms of hazards to human health (as discussed in the news: Daily Mail Report 2013) and that further research is urgently needed. Similarly, the Thousand Islands National Park currently has conservation, residential (including fisheries) and tourism zones, however results from the present study suggest that reefs in conservation zones (Bira Island) may not necessarily have higher coral cover than reefs in areas with less protection (Congkak and Panggang) or even reefs outside of the boundaries of the National Park (Pari Island), and that this reality should be reflected in a revised zonation plan.

The current study indicated the potential for multiple stressors to interact to varying degrees along the island chain. Localized stressors appeared to shape the spatial structure of reefs rather than regional stressors. Nevertheless, it is still very difficult to study the effects of pollution on coral reefs. Cumulative effects of multiple stressors on ecological communities are barely understood, and the response of organisms and ecosystems to a suite of stressors is still not clear due to varying tolerance thresholds of the different species and complex interactions between organisms and stressors. The present study yielded correlative results, and although the observed correlations were quite strong, these should be followed by manipulative experiments to establish causal agents among the identified stressors and their interactions. In studies of interactive effects of stressors, pollution remains one of the least understood factors (Ban et al. 2014). Furthermore, Crain et al. (2008) propose that synergies may be quite common in nature, complicating the prediction of interactive effects. In addition, the effects of pollution at regional scales (compared to a single local reef) are harder to distinguish due to confounding stressors such as bleaching events, storms, fishing pressure and coral diseases. At the same time, the natural variation of reefs along water quality gradients in the absence of well-defined pollution sources has to be considered, i.e. reefs close to the main coastline are often characterized by different nutrient loads, turbidity, wave exposure and current conditions compared to reefs with oceanic conditions (Fabricius 2011).

Monitoring key biological and environmental parameters continuously over several years and across seasons is key to establish successful management and conservation plans. Any conservation and management plan, however, will only be successful if pollution in Jakarta is reduced, e.g. by implementing sewage treatment and waste disposal plans as well as reducing air pollution. Tackling these massive problems will require all stakeholders to work together, a pro-active government, and a reduction in corruption (Bengen et al. 2006, Dutton 2005). In addition, marine spatial planning that is adjusted to local conditions and takes into consideration the different spatial scales on which stressors and resource uses interact with reef communities is required to adequately address the current situation of Jakarta Bay and the Thousand Islands. While these are considerable challenges, complacency is not an option. When considering the importance of coral reefs for the livelihoods of millions of people in developing countries, including large parts of the population in large cities, the need for coral reef conservation in the vicinity of large cities such as Jakarta is obvious and repercussions of degrading habitat reef conditions are likely to be far reaching.

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Chapter 2: Responses of soft corals to reduced water quality

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Water quality controls abundance and physiology of dominant soft corals in Jakarta Bay, Indonesia

Baum G, Januar HI, Wild C, Kunzmann A

Abstract

Declining water quality is among the main reasons of coral reef degradation along the Thousand Islands off the megacity Jakarta, Indonesia. Shifts in benthic community composition, for instance to higher soft coral abundances, have been reported in degraded reefs in the Indo Pacific. However, it is not clear to what extent soft coral abundance and physiology are influenced by the water quality. Thus, in this study, benthic community composition and water quality (i.a. dissolved inorganic nutrient (DIN) concentrations, turbidity, and sedimentation rates) were assessed at three sites in Jakarta Bay (< 20 km north of Jakarta) and five sites along the outer Thousand Islands (20 – 60 km north of Jakarta). This was supplemented by measurements of photosynthetic efficiency and respiratory electron transport system (ETS) activity of the two dominant soft corals species, *Sarcophyton sp.* and *Nephthea sp.* Findings revealed extremely eutrophic water conditions within the bay compared to the outer Thousand Islands, with a 44 % higher DIN load (7.65 µM/L), 67 % higher turbidity (1.49 NTU) and a 47 % higher sedimentation rate (30.4 g m^{-2} d^{-1}). Shifts towards soft coral dominance occurred within the bay (2.4 % hard and 12.8. % soft coral cover) compared to the outer Thousand Islands (28.3 % hard and 6.9 % soft coral cover). Soft coral abundances, photosynthetic yield, and ETS activity were highly correlated with key water quality parameters, particularly inorganic nutrient concentrations and sedimentation rates. These findings suggest that water quality controls abundance and physiology of dominant soft corals in Jakarta Bay and may thus contribute to phase shifts from hard to soft coral dominance. This highlights the need to better manage water quality in order to prevent or reverse phase shifts.

Introduction

Coral reefs worldwide are characterized by a considerable loss in coral cover and species diversity (Bellwood et al. 2004, Bruno and Selig 2007). The degradation of coral reefs is often related to declining water quality linked to eutrophication and pollution as a result of urban run-off carrying large amounts of domestic wastes and industrial effluents (Fabricius 2005, van Dam et al. 2011). Eutrophication has been proposed as the main stress factor for many reefs worldwide (GESAMP 2001). For example, long term monitoring data from the Great Barrier Reef show that the overall reduction in total coral cover by 70 % is mainly due to eutrophication (Bell et al. 2014).

A growing body of literature suggests that the degradation of coral reefs is often associated with shifts in community structure to new states (e.g. Done 1992, Hughes 1994). Phase shifts on coral reefs are usually associated with shifts from hard coral-dominated to macroalgae-dominated communities (Nyström et al. 2000, Szmant 2002, Hughes et al. 2007). However, shifts to reefs dominated by other benthic organisms such as sponges, corallimorpharians, and soft corals have been reported as well (Chou and Yamazato 1990, Fox et al. 2003, Ward-Paige et al. 2005). These shifts have however received less attention, and the underlying mechanisms are still poorly understood (Norström et al. 2009). Soft corals (octocorals) represent a diverse and widespread benthic group within coral reefs in the Indo-Pacific (Dinesen 1983, Benayahu 1997, Benayahu et al. 2004) and are important for reef structure and function (Cary 1931,). Many soft coral species are successful colonizers with high fecundity and several dispersal modes (Benayahu and Loya 1985). Studies on coral–macroalgae shifts suggest that those shifts are caused by loss of top-down control as a result of overfishing (Hughes et al. 2007, Rasher et al. 2012). In contrast, phase shifts to sponges, corallimorpharians, and soft corals may be driven by bottom-up control and reduction in water quality (Holmes et al. 2000, Norström et al. 2009). However, the literature is unclear whether soft corals are more tolerant towards declining water quality compared to hard corals (Schuhmacher 1975, Dinesen 1982, De'ath and Fabricius 2010). For instance, De'ath and Fabricius (2010) found that soft coral species richness declined up to 60 % along a gradient of increasing turbidity, while other studies found a higher tolerance of soft corals towards high sedimentation rates (McClanahan and Obura 1997). In addition, there is considerably more knowledge available on hard-coral physiology than for soft corals, for instance how the metabolism of soft corals is influenced by anthropogenic stress and whether soft corals react differently than hard corals on a physiological level. Such knowledge is however crucial to understand the conditions and underlying mechanisms that

drive phase shifts to soft coral dominance, as well as needed to improve management strategies for coral reefs (Folke et al. 2004). Especially, considering that coral reefs are of huge economic and environmental importance, supporting fisheries and tourist sectors and providing habitats with high productivity and diversity, there is a growing need to understand coral reef functioning.

The photosynthetic capacity and electron transport system (ETS) activity are two indicators for the metabolism and can serve as diagnostic tools for the estimation of stress responses such as declining water quality (Jones et al. 1999, Fanslow et al. 2001, Lesser 2013, Maes et al. 2013). Photosynthetic capacity can be determined though the quantum yield of linear electron transport (i.e. photosynthetic yield = delta F/Fm´). The ETS is a multi-enzyme complex in the respiratory chain in the mitochondria during which electrons are passed along numerous enzymes and energy is generated for oxidative phosphorylation and ATP synthesis. The synthesis and degradation of these macro-enzymes depends on the respiratory requirements of the organism and therefore by measuring the ETS activity, a time averaged value of the maximum oxygen uptake rate potential is given. Since the ETS activity adjusts to changes in environmental conditions over several days and weeks, short-term fluctuations and experimental factors are less influential than for direct measurements of respiration (Bamstedt 1980, Cammen et al. 1990). Both ETS activity and photosynthetic yield can increase in organisms exposed to pollution to compensate for stress effects (i.e. produce more ATP) or decrease due to toxic effects (van Dam et al. 2011).

The Greater Jakarta Metropolitan Area, as the 3rd largest agglomeration in the world with around 25 million inhabitants (Brinkhoff 2012), and the Kepulauan Seribu ("Thousand Islands") chain, located in front of Jakarta Bay (JB), represent an ideal area to assess the effects of multiple stressors on coral reef organisms. Various human-induced marine and coastal environmental problems such as high sediment load, water pollution, depletion of fishery resources, seafood contamination, loss of habitat, coastal littering as well as eutrophication have caused severe degradation of coral reefs along the Thousand Islands. Localized effects of anthropogenic stressors appear to have led to a spatial patchwork of differentially degraded reefs (Rachello-Dolmen and Cleary 2007, Baum et al. (in review)). Although reefs within the bay once had thriving coral communities (Djohani 1994, Arifin 2004), they are now dominated by sand, rubble and algae, with a current coral cover of < 5 % for nearshore reefs within JB. Mid-and offshore reefs along the Thousand Islands have highly variable reef conditions (< 20 % cover to 50 %) (Cleary et al. 2014, Baum et al. (in review)).

In order to increase our understanding of shifts towards soft coral dominance in reefs exposed to multiple anthropogenic stressors, this study aimed to answer the following research questions: 1) How does distance to Jakarta influence key water quality parameters? 2) How does distance to Jakarta (i.e. declining water quality) influence benthic community structure in local coral reefs and are there phase shifts from hard to soft coral dominance? 3) Does water quality affect photosynthesis and ETS activity of two dominant soft corals in the area, Sarcophyton sp. (family: Alcyoniidae) and Nephthea sp. (family: Nephtheidae)? Which water quality parameters benefit metabolic condition of these soft corals? We hypothesize that closer to Jakarta a) phase shifts to soft coral dominance occur more frequently b) water quality is reduced and c) the photosynthesis and ETS activity in soft corals are negatively affected by reduced water quality. These questions were aimed to answer by a combination of benthic community survey, water quality assessments, and physiological measurements.

Material and methods

Study area

The Kepulauan Seribu (Thousand Islands) stretch up to 80 km north of Jakarta and are comprised of 105 small (< 10 ha) and very low-lying (< 3 m above sea level) islands (Arifin 2004). Indonesia´s first Marine National Park, the Thousand Islands National Park, was established in 1982 in the north of the island chain (Djohani 1994). Most islands have lagoons and fringing reefs with reef development generally restricted to shallow depths (around 3-10 m, max. 20 m depth). The island chain is densely populated (total population: 22,700 people). 65 % of the people live on the four main islands Panggang, Pramuka, Kelapa and Harapan (BPS 2012). Several rivers with a combined catchment area of 2000 km^2 discharge directly into Jakarta Bay and transport large amounts of untreated sewage and industrial effluents with high pollutant levels (Rees et al. 1999). The bay´s shoreline has been modified extensively over the last decades due to massive urbanization, industrialization and infrastructural development in Jakarta (60 % of the shoreline) as well as due to agricultural or aquaculture developments (30 % of the shoreline) (Bengen et al. 2006). During the dry season, the predominantly south-easterly winds can cause polluted surface waters from the JB area to reach midshore reefs (definition see below), while during the wet season, north-westerly winds blow from offshore towards JB (Cleary et al. 2006).

Table 2.1: Description of sampling sites (linear distance refers to distance from each site to the port Muara Angke in Jakarta).

Site	Site abbrev.	Longitude [E]	Latitude [S]	Linear distance to Jakarta [km]
Ayer Besar	AB	106°42.242	05°58.399	11.3
Untung Jawa	UJ	106°46.911	05°58.399	16.4
Rambut	R	106°41.597	05°58.202	17.3
Pari South	PS	106°36.963	05°52.094	31.4
Pari North	PN	106°37.440	05°51.001	32.6
Gosong Panggang	P	106°35.355	05°44.664	45.7
Gosong Conkak	C	106 35.274	05 42.303	49.5
Kayu Angin Bira	B	106°34.162	05°36.405	59.8

For this project, eight coral reef sites across the Thousand Islands chain were visited in November 2012 during the transition time between northwest and southeast monsoon. Sites within JB (nearshore are; < 20km) as well as from the outer Thousand Islands (mid- and offshore area; 20-45 km and > 45 km, respectively) were chosen to represent both inhabited and non-inhabited islands for each of the three zones. Following the same methodology as previous studies (Moll and Suharsono 1986, Cleary et al. 2006), reefs from the northern side or north-eastern side of each island were visited to ensure consistent wave exposure and current regimes. Reefs from the northern or north-eastern side of each island were visited to ensure consistent wave exposure and current regimes (except for Pari South: here, the south side was included to account for the observed strong differences in coral cover between the northern and southern side of the island (Abrar and Zamani 2011, Madduppa et al. 2012) (Table 2.1, Fig. 2.1).

Benthic community

Benthic habitat structure was assessed at each location, using three replicate 50 m line-intercept transects at 5 +/-0.5 m water depth (English et al. 1994). Preliminary visits to the Thousand Islands had shown that highest coral cover can be commonly found at shallower depth and that at nearshore sites turbidity was too high at greater depth to conduct accurate surveys. Therefore a depth of 5 m was chosen to allow for adequate comparison across the sites, which would not be possible at greater depth. High-resolution underwater photographs were taken, using a digital camera (Canon G12 in a WP-DC 28 housing) every two meters on both sides of the transect line with a 1x1 m gridded quadrat frame for reference. All three replicate transects at each site were conducted on the same day between 9:00 h and 13:00 h.

Photographs were analyzed using CPCE software (Kohler and Gill 2006) with 50 random points placed on each photo and each point assigned to one the following benthic categories: hard and soft corals, *Nephthea sp.*, *Sarcophyton sp.* and macroalgae. Overall total live coral cover was calculated as the sum of hard and soft coral cover.

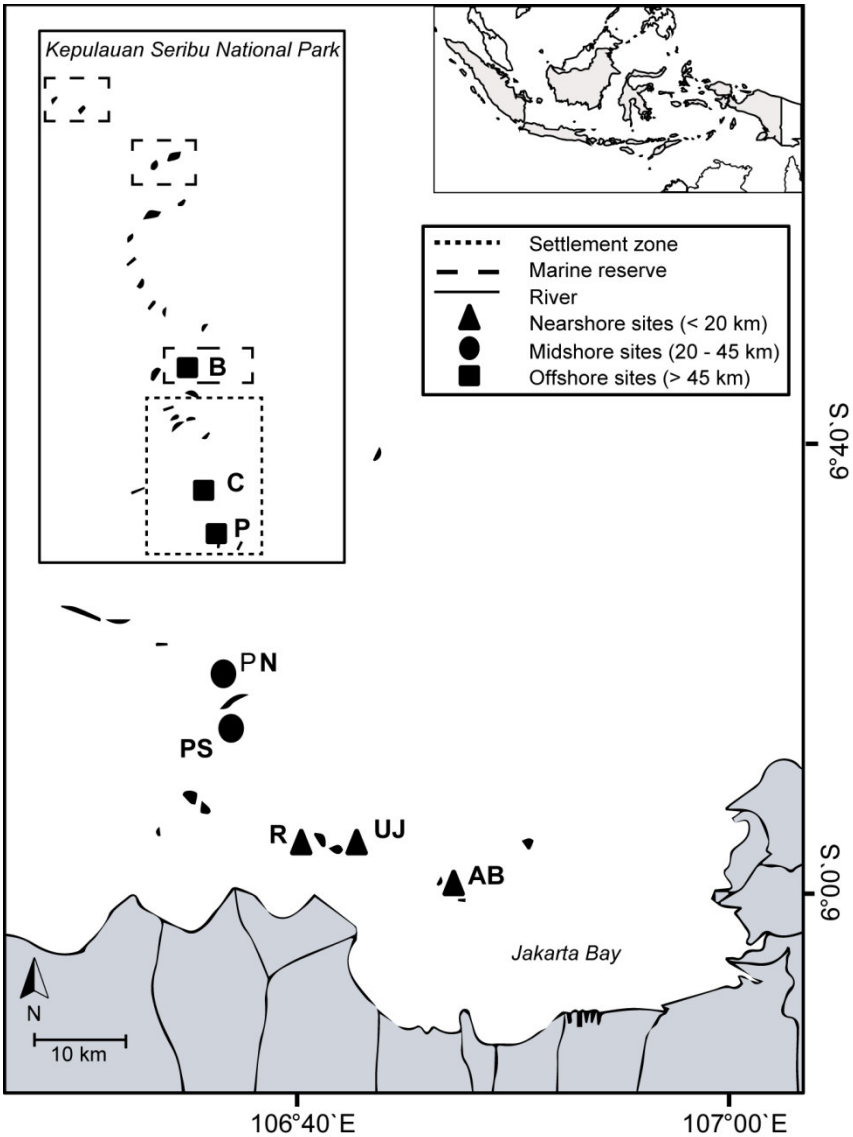

Fig. 2.1: Study area including boundaries of the Thousand Islands Marine National Park and study sites from nearshore reefs (within Jakarta Bay) as well as from the outer Thousand Islands (mid- and offshore): AB = Ayer Besar, UJ = Untung Jawa, R = Rambut, PS = Pari South, PN = Pari North, P = Panggang, C = Congkak, B = Bira.

Water quality

Anthropogenic stressors that reflect the water quality in the JB/Thousand Islands reef complex (De`ath and Fabricius 2008, Fabricius et al. 2012, Baum et al. (in review)) were

determined at each sampling site. The water parameters temperature (°C), dissolved oxygen (DO; mg/L), pH, salinity (PSU), turbidity (NTU) and Chl a (µg/L) concentration of the water were measured at 1 and 3 m water depth, using a Eureka 2 Manta Multiprobe (Eureka Environmental Engineering, Texas, USA). Measurements of 3-4 min duration (measuring interval: 1 min) were taken twice a day (~09:00 am and ~14:00 pm). Water samples for inorganic nutrient analysis (nitrite (NO_2), nitrate (NO_3), phosphate (PO_4), ammonia (NH_3)) were taken at each sampling site at 1 and 4.5 m water depth, stored in an ice cooler and analyzed the same day using a field photometer. Dissolved inorganic nitrogen (DIN) is given as the sum of NO_2, NO_3 and NH_3. Sedimentation rate was estimated by deploying sediment traps made from a PVC tube with a height-to-width ratio of 7.2 (as recommended by Storlazzi et al. (2011) at 5 +/- 0.5 m depth for 22 +/- 1 h at each site (n = 5 traps per site). Traps were sealed underwater prior to retrieval. The complete water and sediment content in the tubes was transferred to plastic bottles (5 L) and stored in the dark until further processing. Water and sediment was filtered through Whatman GF/C glass microfiber filters (diameter 110 mm; 1.2 µm porosity) that had been pre-combusted at 500 °C for 6 h and weighed. After filtering, filters were dried at 65 °C for 24 h and re-weighed. Sedimentation rate is given as total particulate mass flux (TPMF) [g m^{-2} d^{-1}] according to UNESCO (1994):

$$TPMF = DW / A_r \times T, \quad (2)$$

where DW is dry weight of trapped sediment samples [g], T is trapping duration [d] and A_r is the area of the sediment trap tube opening [m^2] with $\pi = 3.14$ and d = aperture size [cm]:

$$A_r = \pi \times (0.5\ d)^2 \times 10^{-4} \quad (3)$$

Photosynthetic yield and ETS activity of soft corals

At each site, fragments (~5 – 10 cm length) of the two soft coral species *Sarcophyton sp.* and *Nephthea sp.* were sampled (n = 5) during scuba diving at ~5 m water depth. Soft corals were chosen due their high abundances along the island chain. At nearshore sites, a sufficient number in hard coral replicates was not given. Therefore, photosynthetic yield and ETS activity in hard corals could not be measured.

Photosynthetic yield

Coral samples were placed immediately in two 100 L black plastic boxes, one box for each of the soft coral species, respectively. The boxes were filled with fresh seawater from the sampling site. The water was aerated and temperature, salinity, dissolved oxygen and pH

monitored with a WTW 340i Multiparameter system at regular intervals. 30 % of the water was exchanged every 30 min.

Corals were dark-adapted for 3 ± xx hours, by covering the boxes with a lid (mean light in the box [PAR] = 4.3 PAR; measured with Licor xyz sensor, Germany). Photosynthetic capacity was then determined by measuring the chlorophyll fluorescence of photosystem II (PS II), using a pulse-amplitude modulated fluorometer (DIVING-PAM, www.walz.com). Photosynthetic yield (also called maximum quantum yield; F_v/F_m) (Walz 1998) was measured by holding the sensor tip around 3-5 mm above the polyps (n = 7 per fragment, except for the sites Rambut (*Sarcophyton sp.* and *Nephthea sp.*) with n = 6 and Untung Jawa (*Sarcophyton sp.*) with n = 4) (Rodolpho-Metalpa et al. 2008).

Electron transport system (ETS) activity

Prior to dark-adaptation for measurement of photosynthetic yield, tissues samples were taken from each coral fragment, placed in small 2 ml glass vials and immediately stored in liquid nitrogen, until they could be placed in a -80 °C freezer. ETS activity was measured at ZMT in Bremen, Germany. Replicate number varied between the two species: n = 5 for *Nephthea* sp. (except for the sites Untung Jawa, Rambut: n = 4 and Pari North, Bira: n = 3) and n = 4 for *Sarcophyton sp.* (except for the sites Pari North, Congkak, Bira: n = 3). The soft coral tissue samples (always kept on ice between steps) were ground with a plastic mortar for 90 s in homogenization buffer (HOM; stored at -20°C) containing 1.5 mg/ml polyvinylpyrolidone (PVP), 75 µM MgSO4 x 7H2O and 0.2 % Triton X-100 in 0.1 M phosphate buffer, pH 8.5 (according to Owens and King 1975). ETS enzyme extracts were prepared in a 50-fold volume (w:v) of homogenization buffer. After 1 min of tissue lysis by ultrasonication (Bandelin, Sonopuls HD 3100) the homogenates were centrifuged for 10 min at 2°C and 1500g (Eppendorf, 5804 R). The resulting supernatant was transferred into a sterile Eppendorf cup and stored on ice until analyses. ETS activities were determined the same day, following Lannig et al. (2003) with slight modifications. The final assay volume was adjusted to 1 ml and the reaction mixture was prepared as follows in 1.5 ml single use plastic cuvettes: 500 µl assay buffer (0.1 M phosphate buffer, pH 8.5; stored at 4 °C) were mixed with 250 µl INT-solution (8 mM INT *(2-(4-Iodophenyl)-3-(4-nitrophenyl)-5-phenyl-2H-tetrazolium chloride)* in 0.1 M phosphate buffer, pH 8.5, stored at 4 °C) and 167 µl NADH-solution (7.2 mM NADH with 0.2 % Triton X-100 (v:v) in 0.1 M phosphate buffer, pH 8.5, prepared daily), stirred with a plastic stirrer and incubated for 5 min at 30 °C in a cooling-thermomixer (HLC, MKR 23) in the dark. The reaction was started by adding

150 µl of sample homogenate to the assay mixture. Immediately afterwards, the increase in absorbance of ETS activity was measured at 490 nm for 5 min with a time interval of 15 s (applying the associated measuring software UV WinLab (Perkin Elmer)) in a spectrophotometer (Perkin Elmer, Lambda 35). The resulting slope, calculated by subtracting the blank activity from sample activity, was further used to calculate enzymes activities. All samples were run in triplicate. ETS activity [µmol O_2 h^{-1} g^{-1}] was calculated according to the equation (Lannig et al. 2003):

$$\text{ETS-activity}\,[\mu\text{mol } O_2\ h^{-1}\ g^{-1}] = \frac{\Delta A\ \text{min}^{-1}}{\varepsilon \times d} \times \frac{V_{Assay}}{V_{Aliquot}} \times \frac{V_{Extract}}{m_{sample}} \times R \times 60 \qquad (1)$$

$\Delta A\ min^{-1}$: change in sample absorbance – change in blank absorbance per min

ε: molar extinction coefficient of INTP Formazan [15900 µl μmol^{-1} cm^{-1}]

d: path length of the cuvette [1 cm]

V_{Assay}: volume of the final assay mixture [1000 µl]

$V_{Aliquot}$: volume of homogenate used in the reaction mixture [150 µl]

$V_{Extract}$: volume of the original homogenate [µl]

m_{sample}: wet mass of the muscle tissue [g]

R: 0.5 (ratio of O_2 to INT of 1 : 2)

Statistical analysis

Differences between sites for any of the water quality parameters, benthic parameters or ETS activity rates and photosynthetic yields of the two different soft coral species were analyzed using one-way ANOVA. In addition, differences between JB and outer Thousand Islands for hard and soft coral cover respectively were tested for using one-way ANOVA. All data were checked for assumptions of normality and homogeneity of variances. In case assumptions were not fulfilled, a Kruskal Wallis test was performed instead. If significant effects were detected, pairwise comparisons with the post-hoc Student-Newman-Keuls test were performed to assess significant differences between individual factors. Linear regression analysis was performed to test whether gradual in- or decreases could be found in ETS activity and photosynthetic yield as well as benthic factors along the distance gradient to Jakarta. In addition, ETS activity and photosynthetic yield as well as benthic factors were

checked for linear correlation with each other and with water factors, respectively. Linear regression with one breakpoint (i.e. two linear segments) was performed in case it yielded a higher correlation. Univariate statistics were performed with SigmaPlot 12.5.

Multivariate statistics were performed using PRIMER-E software v.6 (Clarke and Gorley 2006). In order to account for different scales and units (Clarke and Ainsworth 1993), the water factors PO_4, NH_4, NO_3, turbidity and Chl a were log+1 transformed, followed by normalization of all water factors. All benthic factors were square root transformed prior to further analysis in order to reduce the influence of overly abundant species (Clarke and Green 1988). Bray-Curtis similarity matrices (Bray and Curtis 1957) were calculated for the benthic community composition and the metabolic condition (ETS activity and photosynthetic yield) of *Sarcophyton sp.* and *Nephthea sp.* A Euclidian distance was used to construct the similarity matrix for water data (Clarke and Gorley 2006). Distance-based redundancy analysis (dbRDA; Anderson 2001), a constrained ordination technique, was used to visualize differences between sites. Furthermore, the role of individual stressors was assessed with the BEST routine (using the BioEnv procedure based on Spearman rank correlation; (Clarke and Warwick 2001)) to determine which of the water and benthic factors best explained the metabolic condition and cover of *Sarcophyton sp.* and *Nephthea sp.*

Results

Benthic community

Highest hard coral cover of 47 % ± 11 % (mean ± SD) was found at Pari North in the midshore zone, while near- and offshore reefs had a hard coral cover of 2 ± 2 % and 22 ± 6 %, respectively (mean ± SD). At nearshore sites mean soft coral cover (13 ± 6 %) was significantly higher than hard coral cover ($p = 0.023$). The lowest soft coral cover was found in midshore sites (4 ± 2 %). At offshore sites mean soft coral cover was 9 ± 10 %. Total soft coral cover did not show a linear trend with decreasing cover towards offshore ($p = 0.475$), however the cover of *Nephthea sp.* significantly decreased towards north ($p = 0.02$). For *Sarcophyton sp.*, no significant relation with distance to Jakarta could be found.

Shifts towards soft coral dominance occurred at Untung Jawa ($p = 0.011$) and Rambut ($p = 0.016$). At the site Panggang (soft coral cover: 22 %) no significant difference in hard and soft coral cover was found. *Sarcophyton sp.* cover was significantly increased compared to *Nephthea sp.* cover at the two sites Rambut in JB and Panggang at the outer Thousand Islands

($p < 0.05$). Overall, soft coral cover along the Thousand Islands was highly patchy and mainly comprised of nephtheids, xeniids and alcyoniidids, of which nephtheids and alcyoniidids were dominating. Macroalgae cover was significantly different between sites and seemed higher at nearshore sites (mean 6.4 ± 4.5 %) as well as at Panggang (mean 7.4 %) compared to sites from the outer Thousand Islands, however post hoc analysis did not show significant differences between sites. Neither did macroalgae cover show a significant decrease towards offshore ($p = 0.19$) (see Fig. 2.2, Table 2.2 and 2.3).

Table 2.2: Difference in mean hard and soft coral cover at each site (n = 3 transects per site) obtained using One Way Anova analysis (p-values are given). Data for cover are given as mean ± SD. AB = Ayer Besar, UJ = Untung Jawa, R = Rambut, PS = Pari South, PN = Pari North, P = Panggang, C = Congkak, B = Bira.

Site		Cover		One-Way ANOVA				
		Mean hard coral [% cover]	**Mean soft coral [% cover]**	**DF**	**SS**	**MS**	**F**	**P**
Jakarta Bay (JB)	AB	4.5 ± 2.6	8.6 ± 5.4	1	25.05	25.05	1.27	0.323
	UJ	1.1 ± 1.0	8.2 ± 2.5	1	75.08	75.08	20.39	0.011
	R	1.5 ± 0.9	21.5 ± 8.5	1	596.57	596.57	16.28	0.016
	Mean	2.4 ± 1.5	12.8 ± 6.2	1	162.24	162.24	5.346	0.082
Outer Thousand Islands	PS	28.2 ± 4.6	5.8 ± 4.0	1	748.11	748.11	31.69	0.005
	PN	46.7 ± 10.5	2.0 ± 2.7	1	2987.84	2987.84	34.72	0.004
	P	17.5 ± 7.2	22.3 ± 10.0	1	35.34	35.34	0.4	0.56
	C	30.3 ± 2.7	4.2 ± 2.4	1	1016.93	1016.93	123.53	<0.001
	B	19.0 ± 3.7	0.3 ± 0.2	1	522.15	522.15	52.03	0.002
	Mean	28.3 ± 10.5	6.9 ± 7.9	1	1147.041	1147.041	10.684	0.011

Water quality

Most water parameters did neither show a clear separation of nearshore sites and sites from the outer Thousand Islands, nor a clear distance-based spatial pattern (i.e. with increasing distance to Jakarta), but rather localized patterns (see Baum et al. (in review) for further details). Water quality at nearshore sites in JB seemed generally worse than at sites from the outer Thousand Islands, with a 67 % higher turbidity (1.5 ± 0.7 NTU), 47 % higher sedimentation rate (30.5 ± 0.4 g m^{-2} d^{-1}), 44 % higher DIN load (7.6 ± 3.6 µM/L) and Chl a (9.5 ± 4.5 µg/L) levels in the Bay (mean ± SD), results were however not significant for all

sites from JB. For other water parameters, e.g. the concentration of PO_4 and NH_3, values seemed to decrease towards offshore, with one exception. They showed significantly higher levels at one single offshore site (Panggang), compared to all other sites ($p < 0.05$) (see Table 2.4).

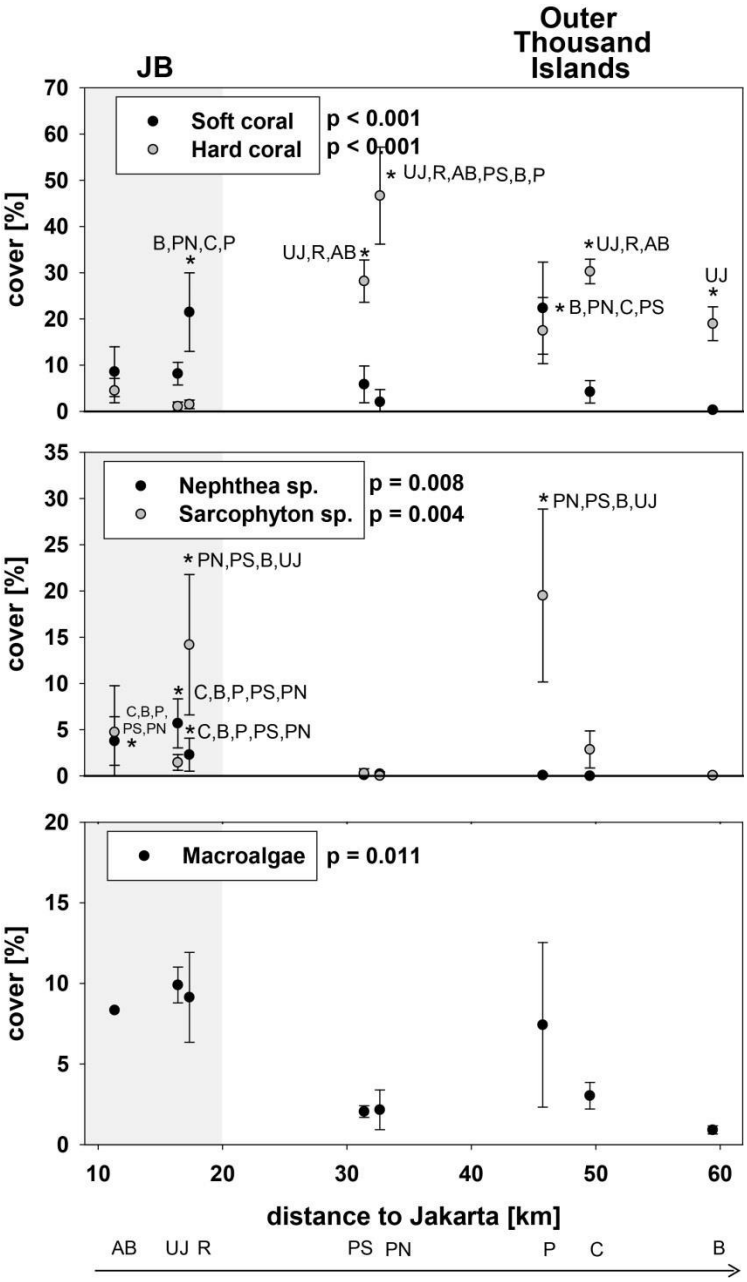

Fig. 2.2: Benthic community composition. Mean cover (± SD) for hard and soft corals, the two soft coral species *Sarcopyhton sp.* and *Nephthea sp.* as well as macroalgae for sites along the Thousand Islands (x-axis refers to distance to Jakarta). p-values and post hoc results for differences between sites are given for each graph. Consider different scales on y-axis. Study sites: AB = Ayer Besar, UJ = Untung Jawa, R = Rambut, PS = Pari South, PN = Pari North, P = Panggang, C = Congkak, B = Bira.

Table 2.4: Water quality. Data for sites in Jakarta Bay (JB) and outer Thousand Islands: Mean values for the factors temperature [°C], pH, salinity [PSU], DO [mg/L], turbidity [NTU], sedimentation, the inorganic nutrients [µM/L] PO_4, NO_3, NO_2, NH_4 and Chl a [µg/L] at each site. The % difference between JB and outer Thousand Islands as well as p-values for differences between sites along the whole island chain (one-way ANOVA) and for linear regression analysis with distance to Jakarta are given for each factor. Study sites: AB = Ayer Besar, UJ = Untung Jawa, R = Rambut, PS = Pari South, PN = Pari North, P = Panggang, C = Congkak, B = Bira.

Area	Site	Turb [NTU]	Chl a [µg/L]	PO_4 [µM/L]	NH_3 [µM/L]	NO_2 [µM/L]	NO_3 [µM/L]	Sed [g m^{-2} d^{-1}]	DIN [µM/L]	Temp [°C]	pH	Salinity [PSU]	DO
Jakarta Bay (JB)	Mean	1.49	9.48	2.36	6.65	0.42	0.57	30.39	7.64	30.47	8.19	32.41	6.78
	AB	0.73	4.86	4.09	11.64	0.5	0.55	31.02	12.69	30.48	8.33	32.24	8.39
	UJ	1.32	15.77	1.68	4.62	0.23	0.67	30.16	5.52	30.24	8.09	32.62	5.54
	R	2.4	7.81	1.31	3.7	0.53	0.48	30	4.71	30.68	8.16	32.36	6.41
Outer Thousand Islands	Mean	0.49	1.76	1.41	3.64	0.14	0.48	16.18	4.25	30.41	8.15	32.77	6.64
	PS	0.42	1.48	0.51	2.82	0.27	1.02	10.54	4.11	30.35	8.14	32.63	6.59
	PN	0.42	2.84	0.11	0.46	0.01	0.65	13.91	1.11	30.83	8.18	32.74	7.35
	P	0.54	1.78	4.35	11.41	0.16	0.39	14.41	11.96	30.14	8.14	32.95	6.21
	C	0.52	0.89	0.05	2.03	0.14	0.16	20.37	2.33	30.36	8.18	32.78	6.58
	B	0.54	1.84	2.04	1.48	0.1	0.16	21.65	1.74	30.34	8.13	32.74	6.47
% difference JB and Outer Thousand Islands		67	81.41	40.19	45.28	67.58	16.38	46.78	44.35	0.2	0.47	1.12	2.05
One-Way Anova (p-value)		0.005	0.003	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	0.114	0.083	0.007	0.106
Correlation with distance to Jakarta (p-value)		0.15	0.07	0.7	0.47	0.42	0.03	0.01 *	0.15	0.44	0.37	0.02	0.56

Photosynthetic yield

Average photosynthetic yield (F_v/F_m) of *Sarcophyton sp.* (0.65 ± 0.09) and *Nephthea sp.* (0.67 ± 0.06) did not differ between the two species. Significant differences in photosynthetic yield between sites were found for both soft corals respectively ($p < 0.001$). Subsequent post hoc analysis revealed for *Sarcophyton sp.* that all sites from JB were significantly different to almost all other sites from the outer Thousand Islands ($p < 0.05$). Overall, the yield increased for *Sarcophyton sp.* towards north ($p = 0.017$). Post hoc analysis for *Nephthea sp.* revealed a similar trend with the two sites furthest south in the Bay (AB, UJ), being significantly different to most sites from the outer Thousand islands ($p < 0.05$). The photosynthetic yield of *Nephthea sp.* did however not significantly increase towards north ($p = 0.202$) (Table 2.2 -2.3, Fig. 2.3).

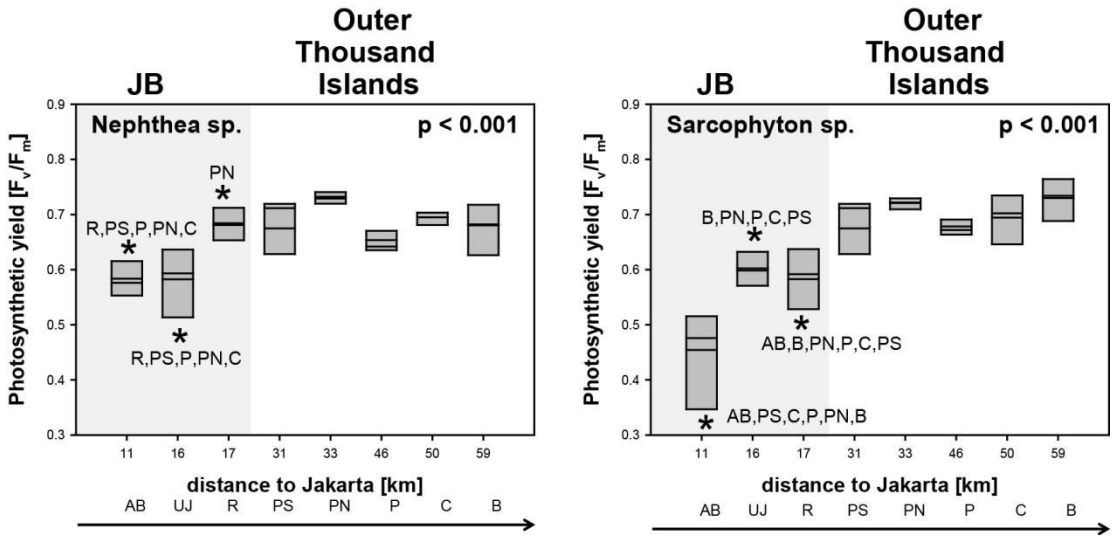

Fig. 2.3: Mean photosynthetic yield (F_v/F_m) of *Nephthea sp.* (A) and *Sarcophyton sp.* (B) for sites along the Thousand Islands (x-axis refers to distance to Jakarta). AB = Ayer Besar, UJ = Untung Jawa, R = Rambut, PS = Pari South, PN = Pari North, P = Panggang, C = Congkak, B = Bira.

ETS activity

Average ETS-activity [µmol O_2 h^{-1} g^{-1}] of *Sarcophyton sp.* (25.8 ± 8.5) and *Nephthea sp.* (24.1 ± 6.8) did not differ between the two species. Significant differences in ETS-activity between sites were found for *Nephthea sp.* ($p = 0.005$) and *Sarcophyton sp.* ($p = 0.009$). Subsequent post hoc analysis revealed for both species that the two sites AB and UJ in JB were significantly different to the midshore site PN with the highest ETS-activity suggesting that ETS might be lower in the bay compared to sites from the outer Thousand islands (Table 2.3, Fig. 2.4).

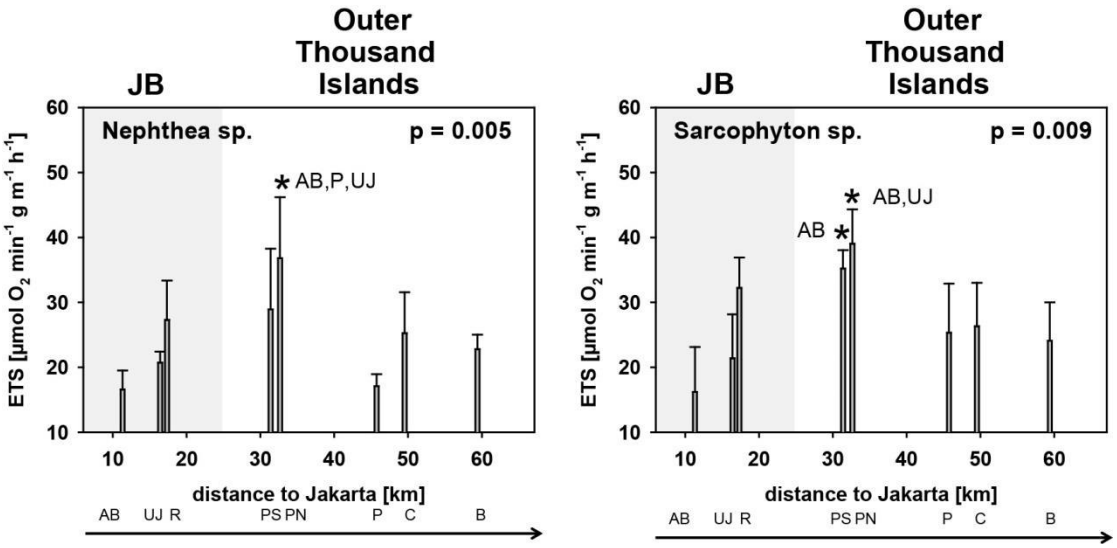

Fig. 2.4: Mean electron transport system (ETS) activity *Nephthea sp.* (A) and *Sarcophyton sp.* (B) for sites along the Thousand Islands (x-axis refers to distance to Jakarta). AB = Ayer Besar, UJ = Untung Jawa, R = Rambut, PS = Pari South, PN = Pari North, P = Panggang, C = Congkak, B = Bira.

Correlations between water quality, reef condition and ETS activity as well as photosynthetic yield

The metabolic condition (indicated by photosynthetic yield and ETS) of both *Sarcophyton sp.* and *Nephthea sp.* were well correlated with the overall water quality composition, with 79 % of the variation in *Nephthea sp.* being explained by the three water parameters PO_4, NH_3 and temperature and 67 % of the variation in *Sarcophyton sp.* being explained by the three water parameters DO, pH and temperature (table 2.2). Photosynthetic yield of *Sarcophyton sp.* was significantly lower at sites with elevated sedimentation rates ($p = 0.004$). ETS activity of *Nephthea sp.* was significantly lower at sites with elevated DIN and PO_4 levels ($p = 0.023$ and $p = 0.009$ respectively) (Table 2.5). The correlation of the metabolic condition of both soft coral species was less significant to the benthic community composition (< 15 % for both species). Similarly, only photosynthetic yield of *Nephthea sp.* is significantly correlated with the cover of *Nephthea sp.* and increases towards north ($p = 0.018$), while no relationship was found for *Sarcophyton sp.* For ETS-activity no significant correlation could be found for both species (see Table 2.5).

Along the Thousand Islands, 71 % of overall benthic community composition can be linked to the water parameters NH_3, NO_2 and turbidity. 39 % of variation in the composite cover of both *Sarcophyton sp.* and *Nephthea sp.* can be explained by the differences in sedimentation rate and NH_3 (see Table 2.5). The correlation of metabolic condition with the water composition as well as with benthic composition is visualized in Fig. 2.5 for both species and shows a

similar pattern for both species. Sites however do not separate according to their distance to Jakarta with the midshore site PN separated from the other sites and the nearshore site AB.

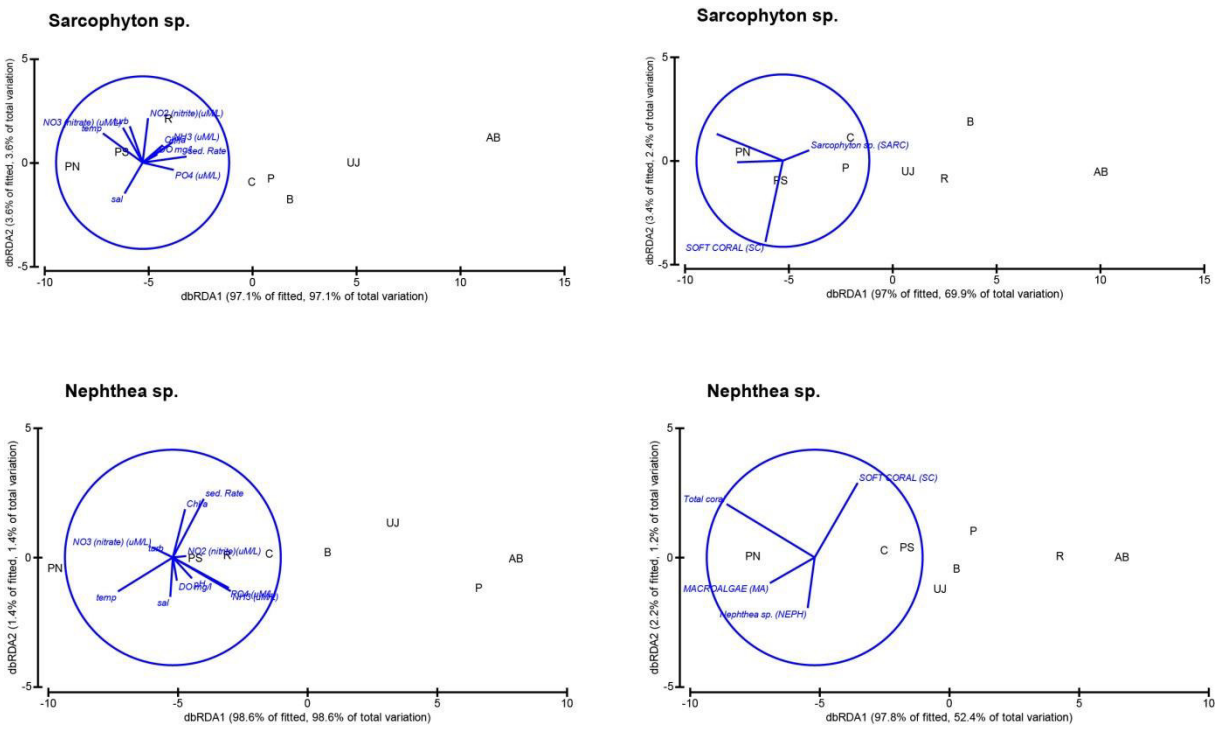

Fig. 2.5: Visualization of the metabolic condition indicated by photosynthetic yield (F_v/F_m) and electron transport system (ETS) activity of the two soft coral species *Sarcophyton sp.* and *Nephthea sp.* based on distance-based redundancy analysis (dbRDA). Water quality factors and benthic factors are overlain for both species respectively. Study sites: AB = Ayer Besar, UJ = Untung Jawa, R = Rambut, PS = Pari South, PN = Pari North, P = Panggang, C = Congkak, B = Bira.

Table 2.3: Univariate analyses (linear regression) to test for correlations between the metabolic condition indicated by photosynthetic yield (F_v/F_m) and electron transport system (ETS) activity of the two soft corals *Sarcophyton sp.* and *Nephthea sp.* as well as the benthic cover with the distance to Jakarta and water factors. p-values are given. * refers to 2 linear segments (i.e. one breaking point).

Group		Factor	Correlation with distance to Jakarta	Correlation with water parameter								
				DIN	NH3	NO2	NO3	Sed	Chl a	Turb	PO_4	Temperature
Metabolic condition	Photosynthetic yield (F_v/F_m)	*Nephthea sp.*	0.202	0.057	0.066	0.202	0.675	0.09	0.094	0.657	0.095	0.226
		Sarcophyton sp.	0.017	0.055	0.073	0.007	0.624	0.0039*	0.267	0.267	0.18	0.898
	ETS activity	*Nephthea sp.*	0.846	0.023	0.017	0.376	0.455	0.255	0.629	0.934	0.009	0.038
		Sarcophyton sp.	0.681	0.107	0.09	0.441	0.346	0.087	0.464	0.982	0.057	0.143
Benthic community (cover)		*Nephthea sp.*	0.02	0.385	0.429	0.183	0.559	0.014	0.002	0.187	0.205	0.875
		Sarcophyton sp.	0.894	0.117	0.107	0.381	0.607	0.854	0.956	0.315	0.516	0.643
		Total soft coral	0.475	0.081	0.094	0.074	0.809	0.052	0.039	0.066	0.139	0.737
		Total hard coral	0.06*	0.17	0.186	0.031	0.903	0.013	0.075	0.063	0.186	0.43
		Macroalgae	0.19*	0.118	0.125	0.179	0.994	0.649	0.684	0.129	0.205	0.715
		Total coral	0.03*	0.524	0.547	0.148	0.883	0.009	0.077	0.271	0.403	0.489

Table 2.5: Correlation between the metabolic condition indicated by photosynthetic yield (F_v/F_m) and electron transport system (ETS) activity of the two soft coral species *Sarcophyton sp.* and *Nephthea sp.*, respectively and the water quality as well as benthic community. Data are based on the test BioEnv.

Group		Correlation with water quality composition		Correlation with benthic composition	
		Corr	**Factor**	**Corr**	**Factor**
Metabolic condition	*Nephthea sp.*	0.79	PO_4 NH_3 Temperature	0.12	*Sarcophyton sp.* Macroalgae Hard coral
	Sarcophyton sp.	0.68	DO pH Temperature	0.06	Macroalgae *Nephthea sp.* Hard coral
Benthic community	Overall	0.71	NH_3 NO_2 Turbidity		
	Cover of *Nephthea sp.* + *Sarcophyton sp.*	0.39	Sedimentation rate NH_3		

Table 2.6: Univariate analyses (linear regression) to test for correlations between photosynthetic yield (F_v/F_m) and electron transport system (ETS) activity of the two soft corals *Sarcophyton sp.* and *Nephthea sp.* p-values are given.

Species	Factor	Cover *Sarcophyton sp./Nephthea sp.*
Sarcophyton sp.	Photosynthetic yield (F_v/F_m)	0.849
	ETS	0.274
Nephthea sp.	Photosynthetic yield (F_v/F_m)	0.018
	ETS	0.379

Discussion

This study suggest that water quality controls photosynthetic efficiency, ETS activity and abundance of dominant soft corals in Jakarta Bay and may thus influence phase shifts to soft coral dominance. Findings revealed extremely eutrophic water conditions and shifts towards soft coral dominance within the bay compared to the outer Thousand Islands. Both photosynthetic yield and ETS activity of the two common Indo-Pacific soft corals *Sarcophyton sp.* and *Nephthea sp.* were reduced in the bay and highly correlated with key water quality parameters, especially inorganic nutrient concentrations and sedimentation rates. A similar correlation was found for the abundances of *Sarcophyton sp.* and *Nephthea sp.*

Coral reefs along the Thousand Islands are exposed to countless different anthropogenic stressors that affect reefs both on regional and local scales (Berkelmans et al. 2004, Selig et al. 2006, Burke et al. 2012). Findings from this study revealed that the water quality is substantially decreased within the bay with extremely eutrophic conditions compared to the outer Thousand Islands. This extreme eutrophication may be the consequence of massive land runoff, lack of sewage treatment and large-scale agri- and aquaculture in the area. In JB, PO_4 levels reached 4 µM/L and DIN levels up to 13 µM/L. Along the Thousand Islands, overall Chl a levels (mean: 1.7 µg/L) were above the eutrophication threshold level of 0.2 - 0.3 µg/L (Bell et al. 2007) at all sites. Other significant stressors include increased sedimentation and turbidity rates. Sites within JB had on average a 47 % higher sedimentation rate compared to offshore sites in the Thousand Islands, with up to 30 g m^{-2} d^{-1}. There is however no clear nearshore-offshore gradient in water quality visible. Along the outer Thousand Islands, water quality between sites is variable due to locally increased concentrations of especially inorganic nutrients at specific offshore sites such as for example at Panggang where PO_4 and NH_3 peaked.

The reef condition along the Thousand Islands at shallow depths can be considered as being poor since total coral cover in most of the sites was < 25 % (threshold based on Gomez and Yap 1988). Especially, in the bay the loss in coral cover is highly dramatic with a current cover below 5 %. Currently, the highest hard coral cover can be found at midshore sites (47 %), with a subsequent significant decrease towards offshore (mean cover: 17 - 30 %). A similar pattern in hard coral cover along the distance gradient from the mainland was also observed by Cleary et al. (2014) and Baum et al. (in review) for the Thousand Islands chain. In this study, results indicate that shifts towards soft coral dominance occurred within the bay more frequently than at the outer Thousand Islands. Within the bay, 2.4 % hard and 12.8. % soft coral cover was found compared to the outer Thousand Islands where hard coral cover was 28.3 % and that of soft corals 6.9 %. Overall, the cover of *Nephthea sp.* significantly decreased towards offshore and a similar trend was found for Sarcophyton sp. Ecological studies from the 80´s already predicted that shifts to soft-coral dominance after hard coral mass mortalities (e.g. after crown-of-thorns outbreaks) can be expected (Bradbury and Mundy 1989). Even though alternative states with soft corals dominating the benthic community are not as common and widespread as coral-macroalgae phase shifts (e.g. Hughes 1994), several studies have reported coral reefs that are dominated by soft corals locally in the Indo-Pacific (Robinson 1971, Nishihira and Yamazoto 1974, Endean et al. 1988, Chou and Yamazato 1990, Fabricius 1998) and in the western Indian Ocean (Muhando and Mohammed 2002).

Along the Thousand Islands, the benthic community composition was significantly related to anthropogenically influenced water parameters. 71 % of the variation in benthic community composition along the complete island chain could be linked to factors related to terrestrial run-off and eutrophication, especially NH_3, NO_2 and turbidity. One of the main stress factors for coral reefs worldwide is eutrophication (Bell et al. 2014). Dissolved inorganic nutrients can reduce calcification rates in corals, increase macroalgae cover and reduce organic enrichment of the benthos, sediment and suspended particulate organic matter (Fabricius 2005) thereby causing a decline in coral cover.

Similar, the cover of the two soft corals *Sarcophyton sp.* and *Nephthea sp.* was linked to eutrophication related stressors. The combined cover of both Sarcophyton sp. and *Nephthea sp.* along the whole island chain was related by 40 % to the water parameters sedimentation rate and NH_3 with a generally higher cover at nearshore sites. Other studies found similar trends, e.g. McClanahan and Obura (1997) found that soft coral cover was higher at increasing levels of sediment influence. Soft corals are mainly passive suspension feeders and the more abundant genera live in symbiosis with photosynthetic endosymbionts (zooxanthellae) (Fabricius and Alderslade 2001). According to Fabricius 2011, shifts from hard to soft corals appear to be rare and restricted to productive, high-irradiance and wave-protected waters with strong currents and that, especially zooxantheallate soft corals are highly affected by turbidity (Fabricius and De'ath 2004). Neither the cover of *Sarcophyton sp.* nor of *Nephthea sp.* was however significantly affected by turbidity rates within this study. Higher sedimentation rates and Chl a levels however were positively related with higher abundances in the cover of *Nephthea sp.* and overall cover of *Nephthea sp.* was significantly reduced at sites with higher dissolved inorganic nutrient concentrations. However, in order to determine direct causal relationships between individual water stressors, long-term monitoring data is required to make definite statements explaining influences of the various water parameters.

Findings revealed that both photosynthesis and ETS activity of both soft coral species were reduced in the bay. ETS activity and photosynthetic yield values measured in this study were comparable to those measured by other authors for different marine invertebrate species (Muscatine et al. 1984, Fanslow et al. 2001, Ulstrup et al. 2011, Nahrgang et al. 2013). For both *Sarcophyton sp.* and *Nephthea sp.* a relatively high correlation between their metabolic condition (indicated by photosynthesis and ETS activity) and the overall water quality was found. 79 % of the variation in metabolic condition of *Nephthea sp.* may be explained by PO_4, NH_3 and temperature and 68 % by DO, pH and temperature for *Sarcophyton sp.* It has been

shown, that chronic exposure to dissolved inorganic nitrogen can reduce calcification rates and increase the concentrations of photopigments (Marubini and Davies 1996) and photosynthesis rates (Fabricius 2005). In contrast shading due to high turbidity and sedimentation rates of > 10 mg cm^{-2} d^{-1} (Rogers 1990), have been shown to reduce photosynthesis which than can lead to reduced calcification (Anthony and Hoegh-Guldberg 2003).

Overall, the metabolic response of soft corals is very complex, especially in areas with simultaneous exposure to different stressors such as along the Thousand Islands. The resulting final metabolic condition in soft corals under simultaneous exposure to many stressors, as was the case in this study, depends on the interactions of the various stressors. Ban et al. (2014) provide a comprehensive review of multiple stressor interactions and found that in most studies investigating effects of several stressors, photosynthesis was reduced.

For instance, in areas with high levels of particulate organic matter (POM), an important food source for soft corals (Fabricius and Dommisse 2000), some coral species can increase their heterotrophic feeding rates and thereby compensate for energy losses resulting from light reduction due to increased turbidity. This can cause an increase in gross photosynthetic and respiration rates. However, when POM is increased even further, gross photosynthesis, respiration and calcification decrease because light reduction outweighs further energy gains from POM feeding. Therefore, photosynthesis, respiration and calcification in corals are suggested to change in a modal fashion along eutrophication gradients (Tomascik and Sander 1985, Marubini and Davies 1996, Marubini 1996). This could explain why photosynthetic yield and respiration, as indicated by ETS activity in this study, were lowest at the most eutrophic and turbid sites in this study.

To our knowledge this is the first study measuring ETS activity in soft corals. We found reduced ETS levels in both species at two nearshore sites characterized by high nutrient and sedimentation levels. The ETS activity of *Nephthea sp.* was significantly lower at increasing levels of DIN and significantly linked to changes in temperature. Several studies have proposed ETS activity as a useful complementary indicator of long-term metabolic activity as they provide valuable information on the physiological status of organisms (Fanslow et al. 2001, Nahrgang et al. 2013). Here, ETS activity was clearly linked to reduced water quality and confirms that ETS is a useful stress biomarker in soft corals.

Since both photosynthesis and ETS activity as well as the cover of both soft corals was highly correlated with overall water quality, these results may suggest a higher tolerance of soft corals towards decreased water quality. Possibly, phase shifts to soft coral dominance

may therefore be driven by water quality, particularly eutrophication and sedimentation, and could be facilitated at nearshore sites in JB. Heterotrophic filter-feeders such as many soft corals have been shown to benefit more from dissolved inorganic and particulate organic nutrients than corals (Fabricius and Dommisse 2000, Fabricius 2011). In areas of high POM and nutrient levels, some soft corals may therefore outcompete hard corals that thrive more in extremely low food environments, i.e. in low nutrient environments. Metabolic condition did however not increase linearly towards offshore thus reflecting the distance to Jakarta and the improved water quality towards offshore. This may be due to a lack in a clear nearshore-offshore gradient in water quality as a result of locally increased concentrations of especially inorganic nutrients at specific offshore sites.

Nevertheless, whether soft corals as a group are more tolerant of stressful conditions such as high turbidity and sedimentation than hard corals and whether this facilitates shifts to soft coral domiance, cannot be fully answered within this study. Further knowledge on the effects of declining water quality on the physiology of soft corals such as growth rates, pigment concentrations as well as zooxanthellae densities is needed to determine whether the metabolism is more efficient under stressful conditons compared to hard corals.

Other confounding stressors that may have affected metabolic condition and shifts in benthic community composition, should be considered as well. Considering that sediments and water in JB have been reported to be contaminated with heavy metals (Rees et al. 1999, Williams et al. 2000) and other organic contaminants such as the insect repellent *N*,*N* -diethyl-*m* -toluamide (DEET) (Dsikowitzky et al. 2014), surfactants, pesticides and oil-related pollution (Rinawati et al. 2012, Baum et al. (in prep.)), a possible toxic effect with inhibition of photosystem II and the mitochondrial electron transport chain, could also explain the observed decreased rates in ETS activity and photosynthetic yield in soft corals in the bay compared to soft corals from reefs further north. A reduction in both ETS activity and photosynthetic yield rates after the exposure with chemicals has been reported by several studies (e.g. Negri et al. 2005, Biscere et al. 2015). For example, heavy metals can disturb the aerobic metabolism, e.g. Maes et al. (2013) reported reduced ETS rates in fish after copper exposure. Similarly, herbicides and antifouling agents can cause a reduction in photosynthesis in corals (see review Van Dam et al. 2011).

In addition, other factors such as the striking ability of soft corals to colonize new substrates due to their high fecundity rates (Pearson 1981, Benayahu and Loya 1977, 1985) as well as toxic and allelopathic featrures (Bakus 1981, Coll et al. 1982, Tursch and Tursch 1982, Sammarco et al. 1983, Maida et al. 1995, Fox et al. (2003) compared to hard corals may have

facilitated shifts to soft coral dominance as well and should be considered. For instance, at the offshore site Panggang, relatively high nutrient concentrations and a significantly higher cover in *Sarcophyton sp.* was found compared to other sites from the outer Thousand Islands, however the overall metabolic condition was not significantly lower as observed in JB for *Sarcophyton sp.*

Along the outer Thousand Islands, blast fishing was commonly practiced in the 1980s causing hard coral decline (Erdman 1989). Fox et al. (2003) reported locally high abundances of the soft coral *Xenia sp.* (up to 80 %) on coral rubble patches after chronic blast fishing practices in the Komodo National Park in eastern Indonesia. Many soft corals such as *Xenia sp.* are successful colonizers and have high fecundity and several dispersal modes (Benayahu and Loya 1985). These ecological traits of soft corals may have facilitated the growth of *Sarcophyton sp.* at local reefs around Panggang and given them the advantage to outcompete other hard corals. However, it is not fully understood, in what way shifts to soft coral dominance may be triggered by pulse disturbances (e.g. blast fishing) and whether a loss of resilience preceded this proximal trigger (see review Norström et al. 2009).

Results in this study suggest that water quality, particularly eutrophication, could drive phase shifts to soft coral dominance in JB. Water quality has to be improved in order to to prevent or reverse further phase shifts in the area. Even though this study is not able to determine direct causal relationships between individual stressors and changes in the ETS activity and photosynthetic yield of both *Nephthea sp.* and S*arcophyton sp.*, the current study indicates that the metabolic condition of both *Nephthea sp.* and S*arcophyton sp.* is affected by reduced water quality (and other anthropogenic stressors) and may be a useful indicator of overall metabolic condition and stress level. Currently, there is still a lack in knowledge on physiological processes and compensating mechanisms of soft corals exposed to environmental stressors, however such knowledge is essential if the processes involved in shifts of reefs dominated by hard corals to those dominated by soft corals is to be understood. Data on respiration and photosynthesis should be combined with data on energy reserves (lipids, proteins etc.) in both hard and soft corals, in order to determine cellular energy allocation during stress (Novais et al. 2013). In addition, parallel to metabolic measurements, other ecological factors, such as the ability of soft corals to colonize new substrates and their reproductive capacity, as well as growth rates and pigment concentrations should be determined to understand mechanisms involved in phase shifts. Management of coral reefs requires an understanding of the conditions under which phase shifts to different states occur. When considering the

importance of coral reefs for the livelihoods of millions of people in developing countries, the need for more effective coral reef management is obvious.

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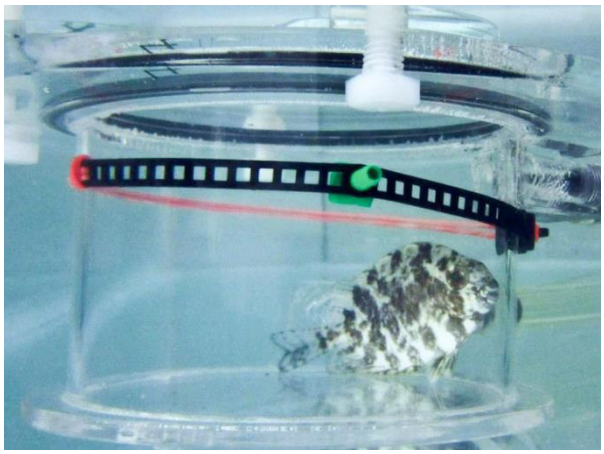
Chapter 3: Responses of fish to pollutants and temperature



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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 Σ EPA polycyclic aromatic hydrocarbons (PAH)) 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.

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 an enormous speed. 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 and many pelagic fish of high fishing value need coral reefs as breeding grounds (Crabbe et al. 2009).

Some of the most pressing stressors on coral reefs are locally, such as eutrophication due to intense 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. 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. The intensity and diversity of anthropogenic stressors has increased rapidly over the last decades, especially in the field of chemicals, such as organic pollutants. Effects of multiple stressors have mostly been assumed to be additive (Halpern et al. 2007). However, current literature indicates that multiple stressors such as chemicals interact with each other and tend to have synergistic effects on communities, i.e. the combined effect is often worse than expected (Crain et al. 2008). 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, multiple stressors have caused severe degradation of coral reef ecosystems within the bay (< 5 % coral cover) (Cleary et al. 2014, Baum et al. 2015). Jakarta Bay (JB) faces extreme eutrophication coupled with intense sedimentation (Baum et al. 2015). Several rivers with a combined catchment area of 2000 km^2 discharge directly into the bay, transport large amounts of sewage, and industrial effluents with high pollutant levels (Rees et al. 1999). Along the offshore island chain Pulau Seribu ("Thousand Islands"), a spatial patchwork of differentially degraded reefs is present along the islands as a result of localized anthropogenic effect, especially factors related to eutrophication (Rachello-Dolmen and Cleary 2007, Baum et al. 2015). 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. The major port Tanjung Priok in JB

has now become one of the leading harbors in South East Asia and tanker routes go directly through the island chain (Bengen et al. 2006). Through the release of bilge and ballast water, both from large tankers to small fishing boats, organic contaminants such as polycyclic aromatic hydrocarbons (PAHs) can enter marine waters as part of the water accommodated fraction (WAF) of fossil fuels, such as diesel used by small fishing boats. Another ubiquitous pollutant class are 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 after short-term exposure (washing of boats in reef areas) and in areas with extremely high population densities and lack of efficient sewage treatments. In JB high amounts of linear alkylbenzenes were detected recently, indicating that the bay receives very poorly treated sewage (Rinawati et al. 2012).

Local anthropogenic stressors such as the above mentioned organic chemicals are often accompanied with global stressors due to climate change. In combination, these can result in enhanced 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 enhance reaction rates 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 fish 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), as well as to LAS (Zaccone et al. 1985, Lewis 1991) at cellular levels. However very few have looked at whole-body responses (e.g. Maki 1979, Davoodi and Claireaux 2007, Christiansen et al. 2010). 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. Considering that of Indonesia´s 252 million inhabitants around 95 % live at the coast (Martinez et al. 2007), frequent use of organic pollutants all over coastal areas represents a significant pollution problem on a regional scale. 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), a common food fish in Indo-Pacific regions (Lam 1974). 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) in isolation and b) in combination, as well as c) under increased temperature reflecting a global warming scenario.

Methods

Experimental fish

Specimens of *S. guttatus,* collected along the Seribu Island chain situated north of Jakarta, were bought from the ornamental fish trader PT Dinar (http://dinardarumlestari.blogspot.de; 04.03.2015) in Jakarta. All fish were juveniles with an average wet weight of 23.4 g +/- 4.5. Two large semi-flow through cylindrical tanks (500 L) were used to acclimatize the fish for 14 days prior to experiments. 50 % 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 detect any daily fluctuations in temperature. All specimens were exposed to a constant 12 h light: 12 h dark cycle and were fed daily.

Experimental protocol

Fish were first exposed to either of three different treatments at 28 °C to resemble 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 a "global warming scenario" 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".

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 150W 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).

Respirometry

Automated intermittent-flow-through respiration runs (Fig. 3.1) 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, 106°36.716`E) in a 100 L aerated glass aquarium containing four circular acrylic glass incubation chambers

(total system volume per chamber: 885 mL) for parallel measurements of three individual fish and one blank measurement, respectively. The glass aquarium served both as a reservoir used for flushing the incubation chambers with oxygenated water and to equalize the temperature between the replicates.

Oxygen concentration within each incubation chamber was recorded continuously every 20 s using optical oxygen sensor spots and a 4-channel Firesting oxygen meter (www.pyro-science.com), calibrated prior to each experiment. A small pump (Eheim compact 300, www.eheim.com) ensured water flow within the chamber for the 10 min measurement phase, while a second pump (aquabee UP 300, www.aquabee-aquarientechnik.de) was set by a timer to flush the chambers after each measurement phase (10 min) with oxygenated water from the surrounding tank for 3 min (flush phase) (see Fig. 3.1). The experimental set-up was placed in a separate room to minimize human disturbances. The reservoir was aerated rigorously using several air stones to ensure high levels of dissolved oxygen. During respiration measurements, oxygen saturation was maintained above 85 % and water was re-oxygenated to ~95 % during the flush phase.

Mass-specific whole body oxygen consumption rates by single fish (MO_2, mg O_2 g^{-1} h^{-1}) were calculated from the temporal decline in oxygen concentration (i.e. depletion rate = slope) for each single measurement phase. The first four hours were used for the fish to acclimatize to the new surroundings before MO_2 values were used for calculations of metabolic rates. In order to test for differences between day and night measurements, calculations were performed separately for each 4 hours during the night (00:00-04:00) and during the day (09:00– 12:00). Routine metabolic rate (RMR) was determined by averaging MO_2 values of all measurement phases for day or night time measurement phases. Similarly, the 15 % quantile method was used to estimate standard metabolic rate (SMR) (Chabot and Claireaux 2008, Franklin et al. 2013).

At the end of the experiment fish were removed from the chambers and subjected to a chase protocol (after Roche et al. 2013), where they were chased for 3 min with a net in a 100 L tank containing the same water conditions of the respective treatments, followed by a 1 min air exposure. Fish were then immediately transferred back to the incubation chambers, where MO_2 determination was ensured within 30 s after the end of air exposure. MO_2 was determined for another 8.8 +/-1.4 cycles until MO_2 decreased towards normal levels again. From these MO_2 values, maximum metabolic rate (MMR) was calculated by using the 85 % quantile method. AMS was then calculated as the difference between SMR during day and

MMR for each specimen. All fish regained normal SMR values after the chase protocol within 1 to 2 hours. A postblank (3-4 cycles) was run at the end of each experiment for each fish and bacterial respiration accounted for.

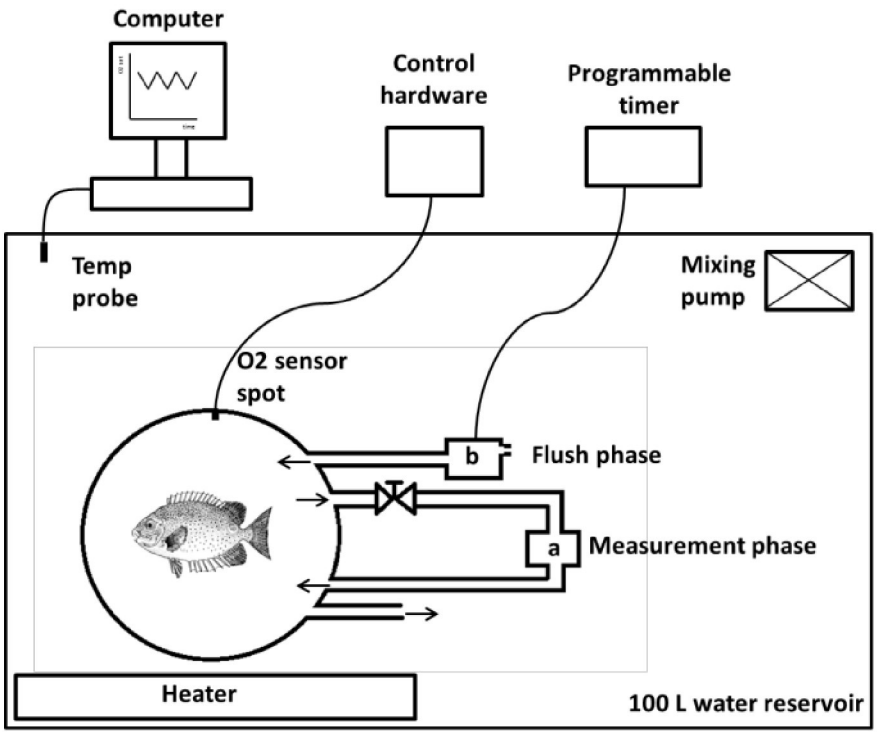

Fig. 3.1: Schematic illustration of the experimental set-up to determine whole-body oxygen consumption rates of *Siganus guttatus*. Oxygen concentration within the chamber was recorded continuously using an oxygen sensor spot. A small pump (a) ensured water flow within the chamber for the 10 min measurement phase, while a second pump (b), controlled by a timer was set to flush the chamber with surrounding oxygenated water every 10 min for 3 min (flush phase).

Analysis of pollutants

LAS:

Linear alkylbenzene sulfonate (LAS) was purchased from a local company in Jakarta (PT. Findeco Jaya, www.findeco.com) and stored at 4 °C until further usage. LAS-stock solutions (2 mg/L) were prepared daily and administered directly into the water of the experimental aquarium of each experiment respectively. 50 mL water samples for LAS analysis were taken at the start and end of each experiment (n = 3 for each). Samples were stored at 4 °C until further analysis. LAS was subsequently analyzed the same day spectrophotometrically (SQ

300 Merck Millipore Filterphotometer), using the methylene blue active substances (MBAS) method modified after George and White (1999). For the assay, 0.2 mL methylene blue - solution (preparation of stock solution: 0.062 g boric acid + 37.5 mg methylene blue + 10 mL chloroform filled to 100 mL with saltwater (35 PSU)) were added to 1 mL sample in a glass tube and vortex mixed 5 times for about 3 s. Then 6 mL chloroform were added, the solution vortex mixed for another 2 min and immediately afterwards the optical density of the chloroform layer was measured at 665 nm wavelength (n = 4 absorbance measurements per sample). Calibration curves (8-point calibration with a concentration range between 0 – 4 mg/L), using LAS as standard and seawater from each experimental tank (i.e. to ensure that exactly the same salinity is used) were prepared for each treatment. All calibration curves followed linear functions with $r^2 \geq 0.97$.

To determine LAS contamination during short-term exposure, water from 1 m depth and in 1 m distance to a small boat was sampled directly after a fisherman cleaned his boat with soap. These tasks are common practice by local fishermen, while they are anchoring at a reef (pers. observation). In addition, to reflect natural exposure conditions (sewage run-off), surface water samples were taken in 1, 10, 50 and 150 m distance to the harbor at Pari Island. All samples were analyzed for LAS the same day.

WAF-D

To reflect the local exposure with diesel-borne compounds, the water accommodated fraction of diesel (WAF-D) was prepared. The diesel used (Indonesian: "solar") was bought at a local gas station (state-owned Indonesian oil and natural gas corporation PT. Pertamina) and stored in a brown glass bottle. WAF-D- stock solutions were prepared for each experiment separately by weighing 5 g diesel on a precision scale (Sartorius ME 235 S) and adding it to 1 L of filtered sea water in a 1 L volumetric flask. The solution was capped, placed in the dark and mixed for 24 h on a magnetic stirrer, and allowed to settle for 5 min (as recommended by Singer at al. 2000). The lower phase (i.e. the water phase containing the water soluble diesel compounds including PAHs) was then used as a pollutant for the experiments. Its concentration of the EPA (US Environmental Protection Agency) PAHs was determined to reflect WAF-concentrations (as superscription) (Netherlands National Water Board 2008, Christiansen et al. 2010). For each experiment 2 L WAF-D- stock solution were added to the experimental tank containing 100 L sea water. EPA PAH-concentration was determined in the diesel itself, in WAF-D - stock solution and in experiments (one sample from the start and end of each experiment, respectively). Each

sample of 1 L was filtered (0.7 µm filter, VWR) and poisoned with 50 mL 2-propanol until further analysis. Samples were then pre-concentrated by solid phase extraction (SPE) by passing it through a preconditioned CHROMABOND® C18 PAH cartridge (6 mL, 2000 mg) by gravitation, followed by elution of PAHs from the cartridge using 5 mL dichloromethane. The dichloromethane phase was reduced to approx. 1mL and 250 µl of dimethylformamid added as a keeper. As a procedure blank, bi-destillated water samples were prepared using the same procedure. The measured Σ EPA PAH-concentration in those blanks (15.3 µg/L) reflect the local background and were subtracted from the samples analyzed. Furthermore, as a method validation procedure, the standard addition method was applied by adding 100 µl of an EPA PAH standard with known concentration (Σ EPA PAH: 1ng/L) twice to selected samples (linear functions yielded an $r^2 \geq 0.99$) (see Table S3.1 for sample list).

Additional reference surface water samples for PAH-determination were taken at sites within JB to reflect natural exposure conditions (see Fig. 3.2 for study area). Furthermore, to determine PAH-contamination during short-term exposure (bilge water discharge), water from 1 m depth and in 1 m distance to a small fisherman boat (diesel driven) was sampled directly after bilge water that had accumulated at the bottom of the boat was dumped to the surrounding water. Bilge water discharge is commonly practiced by local fishermen close to reefs (pers. observation). EPA PAH-concentration was analyzed directly in the bilge water as well.

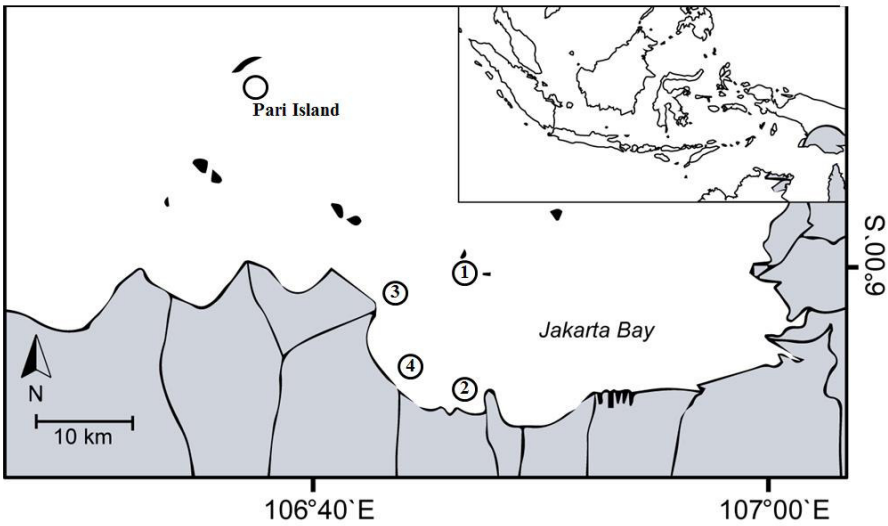

Fig 3.2: Study area. Map includes sampling stations (1-4 and Pari Island) for PAH water samples, reflecting PAH background concentration at "natural" exposure conditions in Jakarta Bay.

All samples (see Table S3.1 for full sample list) were prepared for PAH-determination the same day. Samples were transferred to Germany for further analysis at the Institute for Chemistry and Biology of the marine environment (ICBM) in Oldenburg, Germany. The analysis of 15 EPA-PAHs (acenaphthylene was not measured) was performed on a Waters ACQUITY UPLC (ultrahigh performance chromatography) system coupled to a FLD (fluorescence detector). A list of measured PAHs as well as their solubility in water (mg/L) (ATSDR 2005) is given in Table 3.1. For fluorescence detection, excitation and emission wavelengths were time programmed to receive the maximum sensitivity for each compound (c.f. Table 3.1). The PAHs were separated on a RP18 analytical UPLC column (ZORBAX Eclipse PAH Rapid Resolution HD 2.1 mm (I.D.) x 100 mm; 1.8 µm, Agilent), equipped with a C18 guard column (Vanguard 2.1 mm (I.D.) x 5 mm, Waters) at 35°C. The mobile phase consisted of water (A), and acetonitrile/water (v/v, 9/1) (B) and was run at a flow rate of 0.35 mL/min with the following gradient elution program: starting with 50 % of (A) and 50 % of (B), the mixture had 35 % of (A) and 65 % of (B) after 2.9 min and 1 % of (A) and 99 % of (B) after 4.2 min. This final proportion was held for 2.9 minutes. Subsequently it was changed to 50 % (A) and 50 % (B) reached after 1.9 min (9 min runtime) and held for 1 minute. All single steps were performed with a linear mixing gradient. Column pressure was approx. 500 bar. The injection volume was 2 µl in all cases. Samples were analyzed directly or after dilution with acetonitrile (1+1 or 1+9, respectively). The standard solutions for the 10 point calibration contained three different concentrations of distinct PAH groups each regarding different detection sensitivities, e.g. 100 ng/mL for naphthalene and acenaphthene, 20 ng/mL for fluorene, phenanthren, anthracene, fluoranthene, pyrene, benzo(a)anthracene, chrysene, benzo(b)fluoranthene, dibenzo(ah)anthracene and indeno(1,2,3cd)pyrene, and 10 ng/mL for benzo(k)fluoranthene, benzo(a)pyrene and benzo(ghi)perylene. The standard solutions used contained 100/20/10, 80/16/8, 60/12/6, 40/8/4, 20/4/2, 10/2/1, 8/1.6/0.8, 6/1.2/0.6, 4/0.8/0.4 and 2/0.4/0.2 ng/mL. All calibration curves followed linear functions with $r^2 \geq 0.98$.

Table 3.1: UPLC analysis: time program for excitation and emission wavelength of the fluorescence detector (FLD) for each detected compound: 15 different polycyclic aromatic hydrocarbons (PAH). The solubility in water (mg/L) for each PAH is given (ATSDR 2005).

Time [min]	Excitation wavelength [nm]	Emission wavelength [nm]	Detected Compound	Solubility [mg/L]
0	275	350	Naphthalene	31
			Acenaphthene	3.8
			Fluorene	1.9
			Phenanthrene	1.1
3.8	260	420	Anthracene	0.045
4.15	270	440	Fluoranthene	0.26
			Pyrene	0.132
4.7	260	420	Benzo(a)anthracene	0.011
			Chrysene	0.0015
5.2	290	430	Benzo(b)fluoranthene	0.0015
			Benzo(k)fluoranthene	0.0008
			Benzo(a)pyrene	0.0038
			Dibenzo(ah)anthracene	0.0005
			Benzo(ghi)perylene	0.00026
6.62	300	500	Indeno(1,2,3cd)pyrene	0.062

Data treatment

Univariate statistics were performed with SigmaPlot 12.5. Differences between treatments and between day- and night- measurements for SMR, RMR, MMR and AMS values, respectively, were analyzed using one-way ANOVA. To test for any single or combined effects of the stressors LAS, WAF-D and temperature, two-way ANOVAs for SMR, RMR, MMR and AMS values, respectively, were performed. Data were checked for normality and homogeneity of variances in all cases. In case assumptions were not fulfilled, a Kruskal Wallis test was performed instead of one-way ANOVA. Significant differences were then compared pairwise with the post-hoc Fisher LSD test (in case of one way ANOVAs) and Tukey test (in case of two-way ANOVAs).

Results

Experimental water conditions

Water conditions (Table 3.2) of the keeping system and during experiments were similar to ambient reef conditions with 95-100 % oxygen saturation and a salinity of 33 PSU ± 0.3. The

water temperature in the keeping system (28.2 ± 0.5 °C) was similar to the control temperature treatments (27.4 ± 0.4 °C). Elevated temperature treatments (31.2 ± 0.2 °C) were conducted 3.8 °C above control temperature treatments. No significant differences were detected for either DO, salinity or temperature for experiments within control and elevated temperature treatments, respectively.

Table 3.2: Physical water parameters in the fish keeping system and during the control and elevated temperature experiments, respectively. Dissolved oxygen (DO), salinity, pH and temperature were measured at the start and end of each experiment and once daily in the keeping system. Data are means ± SD.

Physical water parameters	Keeping	Experiments	
		Control temp	Elevated temp
DO [% sat]	98.5 ± 2.7	102 ± 2.2	99 ± 1.7
Salinity [PSU]	32.9 ± 0.4	33.1 ± 0.7	33.6 ± 0.5
pH	7.9 ± 0.4	n.a.	n.a.
Temperature [°C]	28.2 ± 0.5	27.4 ± 0.4	31.2 ± 0.2

Concentrations of pollutants

Natural exposure conditions:

Measurements within the harbor at Pari island revealed a LAS concentration between 0.6 - 0.8 mg/L within the first 50 m, while directly at the reef (150 m distance to the harbor of Pari island) values were below the detection limit and thus considered to be zero (Table 3.3). Σ EPA PAHs concentrations varied within JB and at Pari island. While they revealed 70 - 385 µg/L within the bay, water samples at Pari island contained 10.2 µg/L. All samples were on average mainly comprised of the PAHs phenanthrene (61.7 %), naphthalene (15.2 %) and fluorene (9.2 %), comprising together > 85 % of the 15 measured EPA PAHs (Table 3.3, Fig. 3.3).

Short-term exposure conditions:

Measurements taken directly after the cleaning of a boat revealed LAS concentrations of up to 5.7 ± 0.4 mg/L. Within 10 min however, dilution reduced concentration to 0.5 ± 0.3 mg/L. This shows that enhanced LAS levels occur temporarily (Table 3.3). Samples taken directly after bilge water discharge, revealed a Σ EPA PAHs concentration of up to 13374 µg/L. However, within 10 min, values fell to 294 µg/L due to dilution processes. In analogy to the LAS determinations, locally elevated PAH-levels have to be anticipated as temporary. The bilge water mainly consisted of the four PAHs naphthalene (31.7 %), acenaphtene (4.8

%) and fluorene (31.2%) and phenanthrene (32.3 %). The PAH composition between water samples taken 5 min (sample 1), as well as 10 min (sample 2) after bilge water was dumped into the water was similar to the bilge water composition, however sample 1 contained small amounts of pyrene, benzo(a)anthracene and chrysene, even though these were not detected in the bilge water itself (Table 3.3, Fig. 3.3a).

Table 3.3: PAH [Σ EPA, µg/L; n = 1 or 2] and LAS [mg/L; n = 3] concentrations during the experiments, as well as under natural and short-term exposure conditions. Start and end measurements are given separately for each experimental treatment, as well as for control and elevated temperature experiments. PAH concentrations reflecting PAH background at "natural" exposure conditions were measured at different sites in Jakarta Bay (JB) and LAS concentrations in the harbor area at Pari Island. PAH concentrations reflecting short-term exposure conditions were measured after bilge water was dumped into the water and for LAS after the washing of a fisher boat. Data are means ± SD in case of n > 2.

Group	PAH [Σ EPA, µg/L]			LAS [mg/L]		
Experiments	Control temp	start	490 ± 96	Control temp	start	2.0 ± 0.1
		end	434 ± 176		end	1.6 ± 0.1
	Elev. temp	start	1077.2	Elev. temp	start	2.1 ± 0.1
		end	603.1		end	1.6 ± 0.1
	WAF-D	start	394	LAS	start	2.1 ± 0.1
		end	57.7		end	1.7 ± 0.1
	WAF-D + LAS	start	585.7	WAF-D + LAS	start	2.0 ± 0.1
		end	810.2		end	1.5 ± 0.1
	WAF-D + temp	start	1077.2	WAF-D + temp	start	2.1 ± 0.1
		end	603.1		end	1.6 ± 0.1
Natural exposure conditions	Jakarta Bay (JB) sites	Pari Island	10.2	Pari Island: Distance to harbor	1m	0.8 ± 0.1
		1	99.2		10m	0.8 ± 0.1
		2	384.6		50m	0.6 ± 0.1
		3	226.9		150 m (reef area)	0
		4	69.7			
Short-term exposure conditions	Time after bilge water discharge	30 s	13374.7	Time after washing of boat	30 s	5.7 ± 0.4
		5 min	733.3		5 min	1.3 ± 0.1
		10 min	294.3		10 min	0.5 ± 0.3

Experiments:

LAS concentrations at the start of both control and elevated temperature treatments were around 2 mg/L ± 0.05 mg/L and decreased by 20 % to 1.6 ± 0 mg/L at the end of the experiment. No differences were detected between elevated and control temperature treatments. Neither were any substantial differences detected between the start and end concentrations among the treatments (Table 3.3). The Σ PAH concentrations in the

experiments (mean: 588 µg/L) were above the total concentrations found in JB (natural exposure; mean = 195 µg/L), but fell within the range of levels found during the bilge water discharge (294 – 733 µg/L). The WAF-D stock solution contained 10653.41 µg/L Σ PAH and was mainly composed of the PAHs phenanthrene (35.2 %), acenaphtene (30.3 %), naphthalene (18.9 %) and fluorene (9.1 %), together accounting for > 90 %. This PAH composition did not differ significantly from the composition in the bilge water ($p = 0.214$). The four main PAHs found in the WAF-D stock solution together comprised 100 % of the total composition in the bilge water: phenanthrene (32.3 %), naphthalene (31.7 %), fluorene (31.2 %) and acenaphtene (4.8 %).

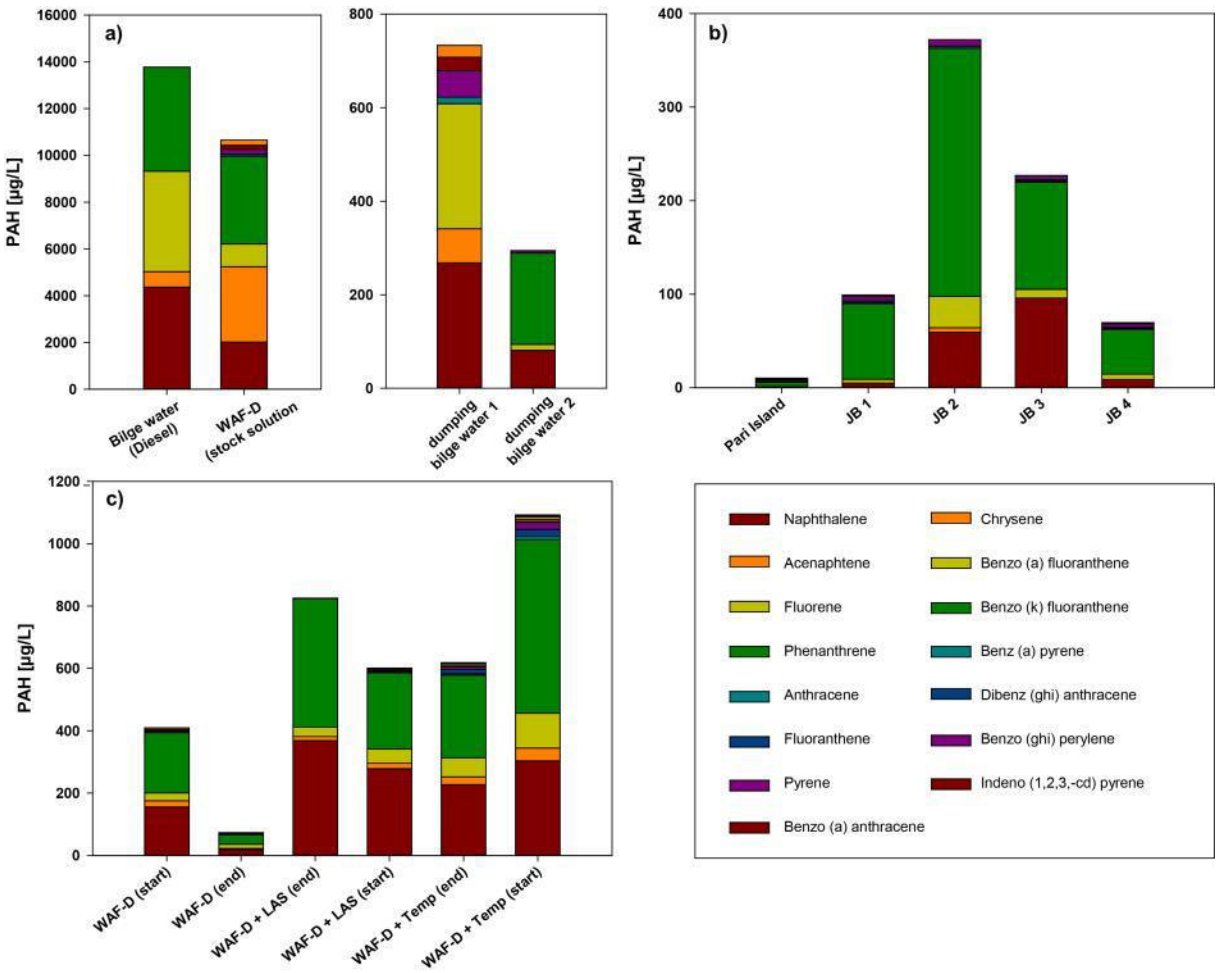

Fig. 3.3: Composition of polycyclic aromatic hydrocarbons (PAH) [µg/L] under a) short-term and b) natural exposure conditions as well as during c) the experiments. 15 EPA (US Environmental Protection Agency) PAHs were measured. PAH concentrations reflecting short-term exposure conditions were measured 5 min (dumping 1) and 10 min (dumping 2) after bilge water was discharged into the water. PAH concentrations reflecting PAH background at "natural" exposure conditions were measured at different sites in Jakarta Bay (JB 1-4) (see Fig. 3.2). Start and end measurements are given separately for each experimental treatment. The PAH composition of the WAF-D stock solution used in the experiments is shown in a) as well.

The PAH composition between the WAF-D stock solution and any of the treatment samples was not significantly different. Mean concentrations of the fours PAHs in the experiments were: phenanthrene (45.5 %), naphthalene (36.3 %), fluorene (9.8 %) and acenaphtene (3.8 %) (Table 3.3, Fig. 3.3). Σ PAH concentrations were higher in WAF-D + temp and WAF-D + LAS treatments compared to the control. In the WAF-D treatment Σ PAH concentrations decreased by 85 % to the end of the experiment, in the WAF-D + temp treatment by 44 % and in the WAF-D + LAS Σ PAH increased by 38.5 %. The overall PAH composition in samples from the different treatments (both start and end samples) differed significantly ($p = 0.028$), however the post hoc Student-Newman Keuls test revealed only a significant difference between the treatments WAF-D + Temp (start) and WAF-D + LAS (end), as well as between WAF-D + Temp (start) and WAF-D (end). In addition, the PAH composition of the four main PAHs was not significantly different between start and end samples of the treatments, however in end samples, most high-molecular PAHs of the 15 EPA-PAHs were not present any longer (Table 3.3, Fig. 3.3).

Effect of WAF-D and LAS in isolation

Mean SMR values for the control were 163.5 ± 5.45 mg O_2 kg^{-1} fish h^{-1}. There were no significant differences in metabolic rates detected between day and night measurements, in any of the treatments ($p > 0.05$) for SMR and RMR rates (see Fig. 3.4). Therefore only total (day + night) values are discussed in the following (see Table S3.2). Mean SMR values differed significantly between treatments ($p < 0.001$). SMR decreased significantly during the WAF-D treatment to 132 ± 5.3 mg O_2 kg^{-1} fish h^{-1} (p 0 0.001) and increased significantly during LAS treatment to 208.6 ± 6.8 mg O_2 kg^{-1} fish h^{-1} compared to the control ($p < 0.001$). RMR values were in all cases slightly above SMR values and followed the same significant pattern ($p = 0.006$) as for SMR values. Mean RMR values for the control were 192.3 ± 14 mg O_2 kg^{-1} fish h^{-1}. Mean MMR and AMS values also differed significantly between treatments ($p < 0.001$ and $p = 0.015$, respectively).

However, neither AMS nor MMR of the WAF-D treatment were significantly different to the control and under LAS exposure a significant increase was only found in MMR rates. Mean MMR values for the control were 334.3 ± 117.5 mg O_2 kg^{-1} fish h^{-1} and for AMS values 171.8 ± 113.6 mg O_2 kg^{-1} fish h^{-1}, respectively (Fig. 3.4 for post hoc analysis, Table S3.3).

Effect of WAF-D and LAS in combination

SMR and RMR values under combined exposure of LAS and WAF-D were significantly reduced compared to the control ($p < 0.001$ and $p = 0.001$, respectively), but not significantly different to the WAF-D treatment ($p = 0.716$ and $p = 0.532$, respectively). A significant interaction for SMR ($p = 0.001$) and RMR ($p = 0.02$) was found for the LAS + WAF-D treatment. This interaction was not significant for MMR and AMS values (Fig. 3.4, Table S3.2 and S3.3).

Effect of WAF-D and LAS in combination with temperature

Mean SMR values of 201.8 ± 4.4 mg O_2 kg^{-1} fish h^{-1} under the temperature treatment were significantly higher compared to the control ($p < 0.001$). The combination of WAF-D with elevated temperature revealed a significant interaction ($p = 0.007$). In contrast, the combination of LAS with elevated temperature revealed no significant interaction ($p = 0.146$). However, SMR values of the temp + LAS treatment (227.8 ± 13.7 mg O_2 kg^{-1} fish h^{-1}) were significantly higher compared to all other treatments ($p < 0.05$). A similar trend was observed for RMR, MMR and AMS values, with a significant increase during the temperature treatment compared to the control ($p > 0.05$). Significant interactions were found for the combination of temp and LAS ($p = 0.026$) and for temp and WAF-D ($p = 0.009$) for RMR values. There was a significant interaction detected for MMR values concerning the combined temp + LAS treatment ($p = 0.04$), however not for the temp and WAF-D treatment ($p = 0.096$). A significant interaction for AMS values between either WAF-D or LAS with elevated temperature were not observed. Mean MMR values of the treatments for temp (633.5 ± 4.4 mg O_2 kg^{-1} fish h^{-1}) and temp + LAS (554 ± 13.7 mg O_2 kg^{-1} fish h^{-1}) and LAS (533.2 ± 6.8 mg O_2 kg^{-1} fish h^{-1}) were significantly increased by 28 % compared to the control (mean: 334.3 ± 117.5 mg O_2 kg^{-1} fish h^{-1}) (Fig. 3.4, Table S3.2 and S3.3).

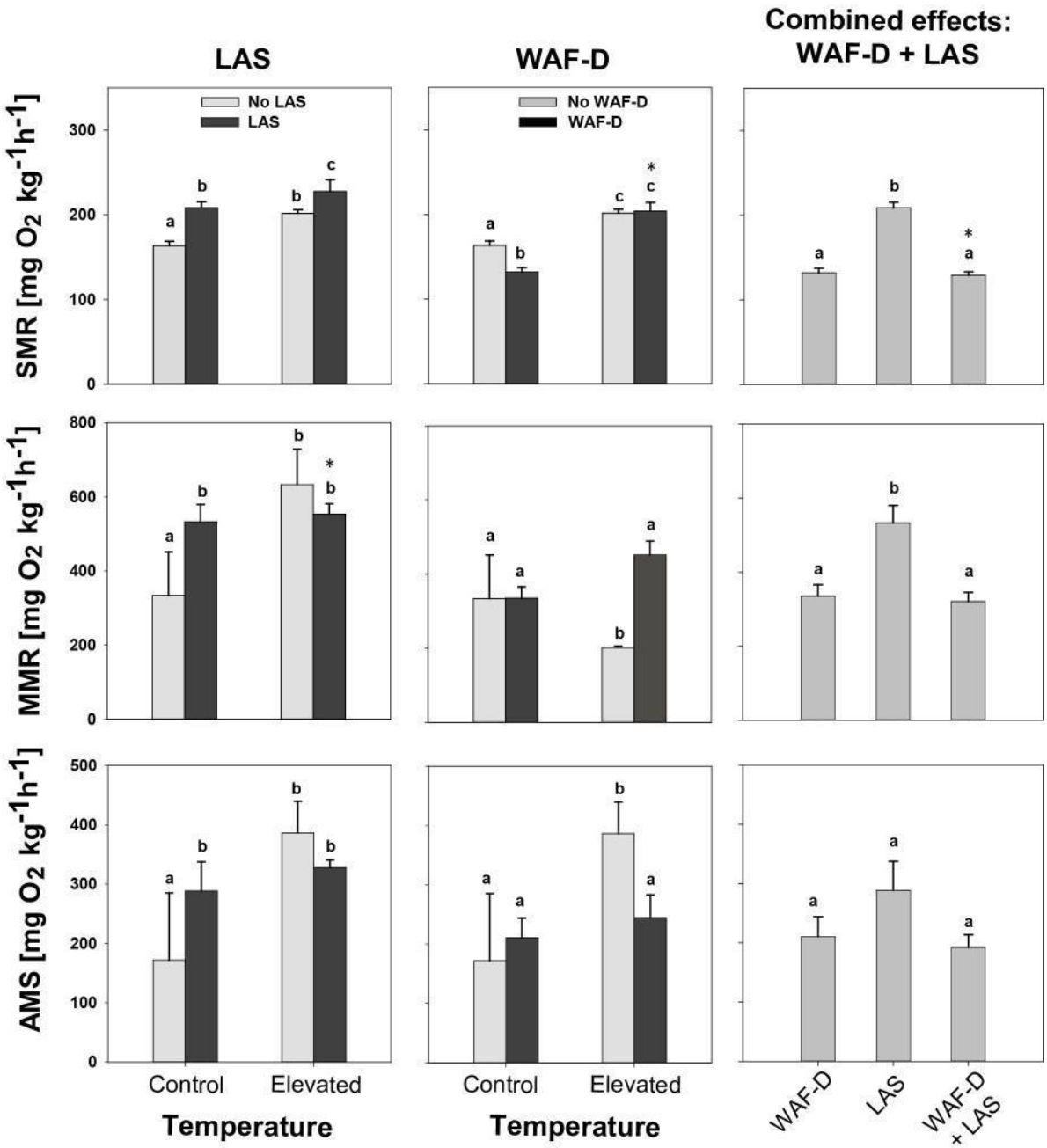

Fig. 3.4: Metabolic stress responses of *Siganus guttatus* to short-term exposure of a) LAS and b) water accommodated fraction of diesel (WAF-D) in isolation and in combination with elevated temperature respectively, as well as to c) LAS and WAF-D in combination. Metabolic rates are given as means ± SD in mg O_2 kg^{-1} h^{-1} (n = 3) at control and elevated temperature experiments: standard metabolic rates (SMR), maximum metabolic rates (MMR) and aerobic metabolic scope rates (AMS). Dissimilar letters in each of the plots represent a significant difference between treatments ($p < 0.05$, one-way ANOVA). Asterisks indicate significant interactions ($p < 0.05$; two-way ANOVA).

Discussion

Results from this study show that both LAS concentrations and diesel borne compounds such as PAHs, especially under short-term exposure conditions (i.e. bilge water discharge and

washing of boats above reef areas) can show significantly increased levels in the JB area and the Thousand Islands. Short-term exposure (16 h) to WAF-D and to sublethal concentrations of LAS significantly affect the metabolic condition of *S. guttatus*, with a decrease in SMR for WAF-D and an increase for LAS, respectively. Significant impairment in SMR can lead to trade-offs towards lower growth rates and reproduction efficiency (Calow 1991, Calow and Forbes 1998, Van Straalen and Hoffmann 2000, Logan 2007). Under combined exposure to both stressors, metabolic depression was observed as well. LAS led to a significantly higher PAH concentration in the water therefore suggesting that the effect of WAF-D (decrease in respiration) may have counteracted and neutralized the effect of LAS (increase in respiration). Results further show that a 3.8 °C increase in temperature (reflecting predicted global warming effects) may not necessarily cause further metabolic stress with regard to LAS and WAF-D toxicity. A synergistic, i.e. amplified reduced (WAF-D) or increased (LAS) change in metabolic rates was not observed. This study nevertheless highlights the need to reduce the import of these pollutants in coastal areas, if long-term effects on fish health are to be avoided.

Pollutant concentrations

Results from this study show that both LAS concentrations and PAH concentrations (Σ EPA PAH) under both natural exposure conditions in JB and the harbor area at Pari Island, as well as under short-term exposure conditions (i.e. bilge water discharge and washing of boats above reef areas) can show significantly increased levels.

Within the harbor area at Pari Island, LAS concentrations between 0.6 - 0.8 mg/L were found and after washing of boats even up to 5.7 mg/L for short time periods. These values are far above the predicted no effect concentration of 0.12 mg/L for LAS in marine fish (Tattersfield et al. 1996, van de Plassche et al. 1999). Surfactants are ubiquitous, but often regarded harmless due to their high biodegradability and speculated control concentrations in the environment (Rebello et al. 2014). However, many studies have reported highly increased LAS levels in marine environments, e.g. LAS was found in untreated sludge at high concentrations of up to 30,200 mg/kg dry weight (Berna et al. 1989), in surface waters at concentrations of up to 0.416 mg/L (Fox et al. 2000) and a few hours after massive discharges in the Gulf of Eilat of up to 5 mg/L (Shafir et al. 2014). Ivancovic and Hrenovic (2010) stated that LAS concentrations in untreated waste water close to reefs can reach 1.1 mg/L. Even though LAS is degraded relatively fast in seawater (half-life time (T50) for LAS in seawater is 6 days (Shafir et al. 2014)), pollution with LAS should not be underestimated.

LAS concentrations at the start of both the control and elevated temperature treatments were around 2 mg/L ± 0.05 mg/L, thus within the range of observed LAS concentrations in JB and at Pari Island. Similar to natural degradation of LAS in seawater over time by bacterial communities (see review Rebello et al. 2014), LAS concentrations decreased on average by 21.9 % during the course of each experiment. During the elevated temperature treatments, no significant increase in LAS degradation could be found, possibly since the 3.8 °C increase in temperatures was not enough.

The Σ PAH concentrations found in surface waters in JB (70-385 µg/L) and 10 min after bilge water discharge (294 µg/L) suggest that PAH contamination is significant in the area and could pose significant stress for fish (Logan 2007). Similar levels of the same 15 EPA PAHs were found in other parts of Indonesia in surface waters as well, e.g. Timor Sea: 54-213 µg/L (Falahudin et al. 2012) and Lampung Bay: 50-411 µg/L (Munawir 2007). PAH concentrations of up to 68.8 µg g^{-1} dry weight (PAH 15 EPA) (Falahudin et al. 2013) and 1252 ng g^{-1} dry weight (PAH 14 EPA) (Rinawati et al. 2012) have been reported for sediments in Jakarta Bay. Bilge water discharge in the JB/Thousand Islands area should also not be underestimated, especially considering the extremely high population density and boat traffic in the area. In the Netherlands, a bilge water discharge of 22,889 m^3 had been estimated for 2006 (Netherlands National Water Board 2008). A similar or even higher discharge could be assumed for the JB/Thousand Islands area.

Overall, the four PAHs phenanthrene, acenaphtene, naphthalene and fluorene dominated (> 80 %) the PAH composition in WAF-D and in the experiments, as well as from samples collected in JB and after the bilge water discharge. In addition, the Σ EPA PAH concentrations in the experiments (mean: 588 µg/L) fell within the range of levels found during the bilge water discharge. Thus it can be concluded that WAF-D is suitable to reflect the local exposure with diesel-borne compounds. However, it should be noted that WAF-D contains a number of other water soluble substances, which may have affected metabolic conditions of *S. guttatus* as well, i.e. diesel oil is commonly mixed with lubricating oil (grease) and both contain sulphur (< 1.5 %) (Netherlands National Water Board 2008). In addition, mineral oil saturated hydrocarbons (MOSH) are present (EFSA 2012). Further experimental studies are necessary to deduce individual effects of these other substances on metabolic performance of *S. guttatus*.

Similar to natural degradation of PAH in seawater over time, Σ EPA PAH concentrations decreased on average by 19.7 % during the course of the experiment. On the one hand, PAHs are naturally degraded by bacteria (see review Rebello et al. 2014), on the other hand

temperature effects, especially during the elevated temperature treatments and surface effects may have occurred. The solubility of PAHs in water is enhanced three- to four-fold by a rise in temperature from 5 to 30 °C (Neff 1979). PAHs are non-polar, hydrophobic compounds, which do not ionize. As a result, they are only slightly soluble in water (ATSDR 2005). Especially larger PAHs disappear faster since PAH solubility in water decreases as the molecular weight increases. In addition, PAHs with a higher molecular weight have a stronger surface tension and stick to surfaces more easily. Therefore, towards the end of the experiments, samples contained hardly any of the larger PAHs. Especially, obtained naphthalene concentrations in samples have to be considered with caution, due to its low solubility in water and high volatility at high temperatures.

Effects of WAF-D

Siganus sp. are herbivorous, diurnal fish (Lam 1974) and often found schooling in coastal sandy or muddy areas. Mean SMR values of 163.5 ± 5.45 mg O_2 kg^{-1} fish h^{-1} for the control fall within the range of other reported values for tropical coral reef fish, i.e. between 100 and 800 mg O_2 kg^{-1} fish h^{-1} (Nilsson and Östlund-Nilsson 2004, Nilsson et al. 2009). The present study found a 19 % decrease in SMR in fish exposed to WAF-D compared to the control. Fish are able to take up the four main PAHs found in the WAF-D either through the gills during respiration (Baussant et al. 2001), the gut via ingestion of food, sediment and detritus or directly through absorption via the skin (Logan 2007). Once taken up, these PAHs are distributed in the body via the bloodstream (Logan 2007) and accumulate in fatty tissues, such as liver and bile, where they are further oxidized, which increases their water solubility and reactivity with enzymes (Pampanin and Sydnes 2013). Generally, PAHs are rapidly eliminated in fish, either through diffusion across the gills and skins or actively through biotransformation in the liver with an elimination rate of 2 to 33 days, depending on their alkylation degree (Jonsson et al. 2004).

The observed decrease in SMR during WAF-D exposure may indicate that oxygen limitation occurs for the fish (Calow 1991). Especially under long-term exposure, this could lead to mortality and reduced digestion, as well as reduced growth rates (Christiansen and George 1995, Barron et al. 2004). Similar narcotic effects have been reported by other studies. For instance, Christiansen et al. (2010) exposed polar cod to WAF of petroleum with PAH concentrations between 0.1 and 40 µg/L (Σ 16 EPA PAHs and priority PAHs naphthalenes, phenanthrenes and dibenzothiophenes) and observed reduced RMR rates after chronic (4 weeks) and acute (60 min) exposure. The four main PAHs found in the WAF-D are known

to be toxic, especially for phenanthrene and naphthalene a wide range of studies exists on physiological effects on fish (e.g. Jee et al. 2004, Sun et al. 2006, Oliveira et al. 2008). For instance, Dos Santos et al. (2006) found a narcotic effect of the PAH naphthalene on the oxygen consumption of the fish *Trachinotus carolinus* (Florida pompano) after chronic exposure (12 days) and high concentrations (300 µg/L). However they also found that the response was time and dose dependent, since oxygen consumption increased after short-term exposures (50 min) and lower concentrations (150 µg/L).

In this study, neither AMS nor MMR were significantly reduced under WAF-D treatment compared to the control. All of the fish regained relatively normal SMR values after the chase protocol within 1 to 2 hours, suggesting that WAF-D may not cause a severe long-term dysfunction of metabolism. Other studies though have reported effects on AMS by WAF of fossil fuels or oil, e.g. in the common sole, SMR was unaffected after exposure to WAF of petroleum but MMR was significantly depressed resulting in a reduced aerobic scope (Davoodi and Claireaux 2007).

Nevertheless, the underlying physicochemical processes responsible for the reduced SMR rates in *S. guttatus* observed in this study are far from clear and require further investigation. The wide range of reported metabolic responses in fish, shows that mechanisms of the toxicity on fish by oil related components are still poorly understood. For instance, many studies have reported tendencies to increasing oxygen consumption rates in fish after exposure to hydrocarbons, either as WAF (Correa and Garcia 1990, Davison et al. 1992) or as single PAHs (Brocksen and Bayley 1973, Anderson et al. 1974, Vargas et al. 1991) and attributed this to higher energy costs due to an overall increase in stress level and detoxification processes. Fish are able to metabolize and eliminate PAHs using various enzymes, however these processes differ for each individual PAH (Hahn et al. 1994). In addition, and metabolism often varies greatly between fish species, which may lead to species dependent PAH accumulation rates (Baussant et al. 2001, Jonsson et al. 2004).

Effects of LAS

Findings revealed that metabolic rates significantly increased by 28 % during short-term exposure to LAS (1.6-2 mg/L) compared to the control. Since surfactants reduce the water surface tension, they can penetrate mitochondrial membranes, causing structural damage in the branchial respiratory epithelium (Engelhardt et al. 1981, Rosety-Rodriguez et al. 2002), which limits oxygen transfer (Agamy 2013). Aerobic processes are diminished and anaerobic oxidation takes place (Brage and Varesche 2014). Other studies have reported effects of LAS

on respiration and metabolism, e.g. increases in respiratory rate of bluegills fish (0.39 to 2.2 mg/L; Maki 1979). Overall, LAS causes lowered functional capacity of various organs, such as gills and skin (Abel 1974), oxidative stress and mucus layer damage of fishes (Susmi et al. 2010), as well as disruption of chemoreceptors (Bardach et al. 1965). Zaccone et al. (1985) reported enhanced mucus production in epidermal cells and a decrease in activity of respiratory enzymes after exposure to sublethal concentrations of the anionic surfactant sodium alkylbenezensulphonate in catfish. The observed increase in SMR rates during exposure with LAS confirms that even environmentally realistic concentrations may pose metabolic stress in *S. guttatus*. However short-term exposure with LAS did not cause a significant decrease in AMS in this study, suggesting that, similar to WAF-D, LAS may not cause a severe long-term dysfunction of metabolism. Nevertheless, under long-term exposure the increase in SMR may lead to significant impairment and trade-offs towards lower growth rates and reproduction efficiency (Calow 1991, Calow and Forbes 1998).

Combined effects of WAF-D and LAS

Under combined exposure of both LAS and WAF-D, SMR rates were significantly reduced compared to control. In general the solubility of PAHs is enhanced with increasing amount of dissolved and colloidal organic fractions such as LAS by incorporating the PAHs into micelles (Neff 1979). This means that in the presence of LAS, the concentration of hydrocarbons increases temporarily in the water phase and as result also their bioavailability (Middaugh and Whiting 1995). For instance Bajpai and Tyagi (2007) found that a laundry detergent concentration of 2 mg/L in the water can cause fish to absorb double the amount of chemicals than normally. Therefore adding for example dispersants to oil may create a more toxic solution than the non-dispersed oil (Papathanassiou et al. 1994, Ramachandran et al. 2004).

In this study, *S. guttatus* were exposed to a higher Σ PAH concentration in the WAF-D + LAS treatment compared to the WAF-D treatment. Therefore it could be postulated that the metabolic depression observed during the WAF-D treatment may have been even more severe during the WAF-D + temp treatment and neutralized/counteracted against the effect of LAS (increase in respiration). Agamy (2013) found that gills of juvenile *Siganus canaliculatus* showed the strongest histopathological response when exposed to dispersed WAF of light Arabian crude oil. Responses to only WAF or dispersant were less severe. The overall reduction in SMR during the WAF-D + LAS treatment was not stronger than during the WAF-D treatment, however a metabolic depression nevertheless occurred. In addition, all of the fish regained relatively normal SMR values after the chase protocol within 1 to 2 hours,

suggesting that the contamination of WAF-D and LAS may not cause a severe long-term dysfunction of metabolism. In the literature a vast range of reported effects of fish to WAF of fossil fuels/PAHs and LAS has been reported and understanding cumulative effects of both stressors is very difficult. Fish are a highly diverse group with various different life stages and behavioral patterns (i.e. different gill structures and respiration rates), which affect physiological responses (Neff 2002, Logan 2007). In addition, individual fitness of each fish influences responses. Furthermore, WAF-D is composed of many different water soluble substances that may affect *S. guttatus*. This may explain the observed often highly species-specific responses of fish to WAF of fossil fuels/PAHs and LAS.

Effects of WAF-D and LAS at elevated temperature

Under elevated temperature, the oxygen demand of fish increases and as result their standard and routine metabolic rate as well (Pörtner and Knust 2007). This was also observed in this study for *S. guttatus*. A reduction in aerobic scope (AMS) for *S. guttatus* under increased temperature however could not be observed, as has been reported for other coral reef fish at elevated water temperatures (31- 33 °C) compared to controls (29 °C) (Nilsson et al. 2009). At higher temperatures, the circulatory and ventilatory systems cannot keep up with the increased oxygen demand, which then leads to the reduction in AMS (Fry 1971, Pörtner and Knust 2007). *S. guttatus* may have a higher tolerance towards increased temperatures. Nilsson et al. (2009) found varying thermal tolerance levels for different reef fish species.

Higher temperature has shown to increase the uptake or detoxification rate of contaminants (Beyer et al. 2014). For instance, an elevated toxicity of metals at higher temperatures has been reported and metal uptake and accumulation increases with increasing temperature (Cairns et al. 1975, McLusky et al. 1986). Under the combined exposure of elevated temperature and LAS, SMR rates were higher than during either elevated temperature or LAS exposure alone, suggesting an increased uptake rate. However no significant interactive effect (no synergism) could be found, i.e. elevated temperature did not increase the negative effect of LAS and cause even more metabolic stress.

The combined exposure of both elevated temperature and WAF-D however had a significant interaction effect on SMR. WAF-D did not cause a decrease in SMR rates under elevated temperature. This may suggest possibly increased detoxification rates of the components of WAF-D under elevated temperature. Thus in both cases, LAS and WAF-D exposure, an increase in water temperature as expected from global warming, may not necessarily cause further metabolic stress with regard to LAS and WAF-D toxicity.

Conclusions

In this study, short-term exposure of sublethal concentrations of both WAF-D and LAS significantly caused metabolic stress in *S. guttatus*. Even though metabolic rate analysis does not give exact answers to the physiological mechanisms disrupted by the pollutants, it gives answers to the severity, since it is an indicator at the level of whole organisms with implications for populations and communities (Johns and Miller 1982). Further studies looking at trade-offs of energy allocated to detoxification processes, as well as at the underlying detoxification mechanisms using molecular indicators represent complex topics for future research (Logan 2007). In addition, further experiments are needed to determine lethal threshold values of the pollutants in *S. guttatus*, and by varying exposure times and increasing measurement intervals, additional information on the risks of low concentrations during long-term vs. high concentrations during short-term exposure can be gained. For instance, specific experiments could be designed to simulate bilge water discharge and washing of boats by e.g. exposing fish once an hour for a few minutes.

Even though both LAS and diesel-borne pollutants such as PAH are degraded naturally in marine waters in relatively short time periods (Ivancović and Hrenović 2010, Pampanin and Sydnes 2013), large-scale sewage run-off from densely populated islands and high boat traffic in the JB/Thousand Islands reef complex, suggest that these two pollutants pose a significant threat to reef organisms. For instance, assuming 10.000 boats of varying size in the area which at least once a day dump their bilge water into the water, the likelihood that fish are exposed to diesel-borne pollutants possibly even several times a day, is very high and may therefore constitute a regional problem rather than a local problem. The long-term effects on fish metabolism have to be studied. Considering that short-term exposure of WAF-D and LAS can cause significant changes to standard metabolic rates in fish, trade-offs towards lower growth rates and reproduction efficiency are highly likely, which eventually will lead to reduced fish catches. Ultimately a negative feedback to human livelihoods and food security may be the consequence, not only in local areas, but also regional areas considering the frequency and area exposed to these pollutants.

Indonesia´s continuing growth in population, especially along the coast, poses severe problems for ecosystems. The installation of effective sewage treatment plants, use of green surfactants (new class of biodegradable and biocompatible products; Patel et al. 1999, Rebello et al. 2014) and stricter guidelines for industrial waste could significantly reduce pollutant levels in the water (Clara et al. 2007). Similar, large tanker routes should not pass by close to islands with coral reefs, as is presently the case in the Thousand Islands. In addition,

marine awareness and local education campaigns could help to change the washing habits of local fishermen and reduce WAF-D and LAS pollution in the region. Without a better understanding of impacts of combined stressors on marine organisms and underlying mechanisms of WAF-D/PAH and LAS toxicity, these mitigation efforts and management strategies such as marine planning and conservation are however void.

Acknowledgements

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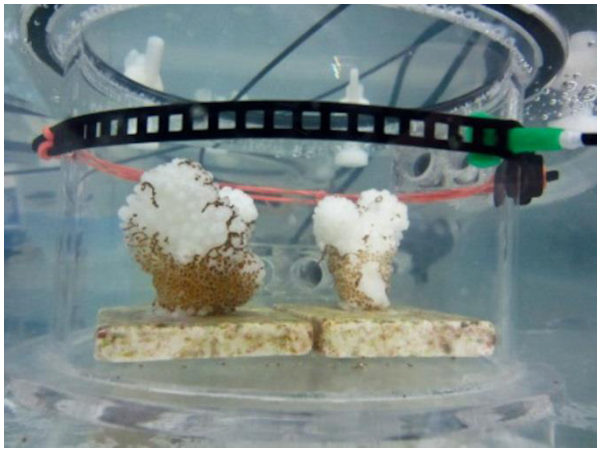
Chapter 4: Responses of corals to pollutants and temperature



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Physiological response of the hard coral *Pocillopora verrucosa* from Lombok, Indonesia, to two common pollutants in combination with high temperature

Kegler P, Baum G, Indriana LF, Wild C, Kunzmann A

Abstract

Knowledge on interactive effects of global (e.g. ocean warming) and local stressors (e.g. pollution) is needed to develop appropriate management strategies for coral reefs. Surfactants and diesel are common coastal pollutants, but knowledge of their effects on hard corals as key reef ecosystem engineers is scarce. This study thus investigated the physiological reaction of *Pocillopora verrucosa* from Lombok, Indonesia, to exposure with a) the water-soluble fraction of diesel (determined by total polycyclic aromatic hydrocarbons (PAH); 0.69 ± 0.14 mg L^{-1}), b) the surfactant linear alkylbenzene sulfonate (LAS; 0.95 ± 0.02 mg L^{-1}) and c) combinations of each pollutant with high temperature (+3 °C). To determine effects on metabolism, respiration, photosynthetic efficiency and coral tissue health were measured. Findings revealed no significant effects of diesel, while LAS resulted in severe coral tissue losses (16-95 % after 84 h). High temperature led to an increase in photosynthetic yield of corals after 48 h compared to the control treatment, but no difference was detected thereafter. In combination, diesel and high temperature significantly increased coral dark respiration, whereas LAS and high temperature caused higher tissue losses (81-100 % after 84 h) and indicated a severe decline in maximum quantum yield. These results confirm the hypothesized combined effects of both diesel and LAS with high temperatures. Our study demonstrates the importance of reducing import of these pollutants in coastal areas in future adaptive reef management, particularly in the context of ocean warming.

Introduction

With growing human populations, the anthropogenic influence on coastal ecosystems is increasing. Halpern et al. (2008) found that no marine areas are unaffected by anthropogenic

influences and 41 % are even strongly affected. About 275 million people live in close vicinity to coral reefs and most of them depend on their ecosystem services for their livelihoods (Burke et al. 2012). One third of the world's coral reefs are located in the Coral Triangle in the Indonesian/Philippines Archipelago (Burke et al. 2012), where hard coral cover declined significantly within the past decades due to a multitude of global and local stressors (i.e. factors that are diverging from the natural conditions) (Bruno & Selig 2007). About 85 % of all reefs within the coral Triangle are threatened by local stressors, up to 90 % in combination with global stressors (Burke et al. 2012). Global stress is generated by climate change, which is usually accompanied by local anthropogenic drivers, such as overfishing, pollution, sedimentation and eutrophication, which in combination result in enhanced vulnerability of the ecosystem (Knowlton & Jackson 2008, Pörtner et al. 2014, Risk et al. 2001). Global sea surface temperatures are estimated to increase up to 4.8 °C within this century (IPCC 2013). Low variances in surface temperature in tropical regions such as Southeast Asia, where organisms live already close to their upper thermal limits, leave organisms there more susceptible to climate change (Maina et al. 2011, Lesser 2013). Bleaching of corals due to a breakdown of the symbiosis between corals and their symbiotic algae is strongly associated with high sea surface temperatures, as shown in field and laboratory studies (Fournie et al. 2012, Hoegh-Guldberg et al. 1999, Wild et al. 2011). The majority of studies concerning stressors on coral reefs have focused on ocean acidification and global warming associated coral bleaching. Several studies have investigated combined effects where stressors can have additive, synergistic or antagonistic effects, and it is important that we understand these in order to develop appropriate management strategies for coral reefs (Beyer et al. 2014, Wilson et al. 2006). Synergistic effects have been found for example between temperature and light stress on photosynthesis in corals (Bhagooli & Hidaka 2004), while increased CO_2 levels and temperature had antagonistic effects Reynaud et al. 2003).

One important pollutant in the ocean is diesel, used to fuel machines and ships all over the world. Although diesel is not the only source of oil pollution (there are many others, both from anthropogenic, as well as natural sources, see Ocean Studies Board and Marine Board 2003), it is a very important one. In 2012 each day over 3.5 billion liters of motor fuels were consumed all over the world and while an effort is made to reduce this number, in growing countries like in the coral triangle region, there was a steady increase in diesel consumption over the past decade (e.g. in Indonesia from 40 million L d^{-1} to 84 million L d^{-1}) (US Energy Information Administration). Diesel is introduced to the environment via oil spills from ships and harbors, from discharge of routine tanker operations and from municipal and

urban runoff (Isobe et al. 2007, Santos et al. 2010). Among the water soluble constituents in diesel, polycyclic aromatic hydrocarbons (PAHs) pose the highest threat to the environment (Haapkylä et al. 2007, Santos et al. 2013). Several studies have discovered toxic effects of PAH on aquatic organisms, mainly fish (Logan 2007, Simonato et al. 2007, Vanzella et al. 2007, Santos et al. 2010). A range of physiological responses to oil pollution by corals, depending highly on the type of oil used, were found in previous studies, including growth impairments, mucus production and decreased reproduction (for an overview see Haapkylä et al. 2007). But most of these studies were performed before 1990 investigating effects of large oil spills and due to the many different types of oils, concentrations and durations it is hard to compare the results.

Another group of frequently used chemicals that regularly end up in the ocean are surfactants which are applied by households and industry in large quantities in detergents and soaps. In 2003 18.2 billion kg of surfactants were used all over the world (Chupa et al. 2007). Linear alkylbenzene sulfonate (LAS) is one of the most common surfactants in use (Ivanović & Hrenović 2010), the consumption of LAS alone in 2003 was 2.9 billion kg (Chupa et al. 2007). Although to some extent surfactants are eliminated from water by biodegradation within a few hours up to several days, significant proportions of surfactants attach to suspended solids and remain in the environment (Lara-Martín et al. 2010). This sorption of surfactants onto suspended solids depends on environmental factors, such as temperature, salinity or pH (Lara-Martín et al. 2010). The important role of water temperature in combination with pollution, is mainly due to enhanced reaction rates at higher temperatures, which leave organisms more sensitive to chemicals (Falahudin et al. 2012, Beyer et al. 2014).

Indonesia, the country with the largest area of coral reefs within the coral triangle, has a large and growing number of human settlements clustered along the entire coastline in close vicinity to coral reefs, and in most cases no waste water treatment is occurring (Isobe et al. 2007, Burke et al. 2012). In areas without sufficient sewage- and waste water treatments, concentrations reaching 1.1 mg L^{-1} of LAS and 0.2 mg L^{-1} of PAH from diesel can be entering the reefs (Ivanović & Hrenović 2010, Falahudin et al. 2012). Thus, there is a continuous contamination of coastal waters with these two very commonly used pollutants, turning the local stressor into a regional threat.

While several studies have investigated responses to contaminants on the cellular level, it is important to understand the effects of stressors on physiological performance on the whole-organism level (Maltby 1999). Metabolic rates are indicators of the overall energy budget of organisms and can indicate non-lethal stress responses (Porter et al. 1999). Metabolism of the coral holobiont (Rohwer et al. 2002, Bourne et al. 2009) includes host and symbiont

respiration, as well as symbiont photosynthesis and metabolic energy is needed among other processes to transport calcium to the host skeleton (Beer et al. 1998, Al-Horani et al. 2003). Kaniewska et al. (2012) showed that effects in coral physiology become apparent before any changes in calcification processes can be detected. Thus, measurements of respiration in combination with photosynthesis are a common method in coral physiological research (Lesser 2013). An increase in respiration can indicate acute stress, while a decrease can indicate either an acclimation or depression due to a stressor (Guppy & Withers 1999). To investigate photosynthetic capacity, the quantum yield of linear electron transport is a useful tool to determine coral health and serves as a diagnostic tool for the analysis of pollutants (Jones et al. 1999, Lesser 2013). Further, the ratio of photosynthesis to respiration (P:R) provides an estimate, whether a coral can live on the energy obtained from its zooxanthellae (Coles & Jokiel 1977).

To our knowledge, there are no publications describing effects of the pollutants diesel and LAS combined with high temperature on coral metabolism. This study investigates this potentially interactive effect on the physiology of a tropical reef coral *Pocillopora verrucosa* in acute exposure experiments. *P. verrucosa* is common in the study area and therefore a good representative of the scleractinian corals that are of vital importance for coral reefs. The main objective was to determine if and how the coral is affected by pollutants in isolation and combination with increased temperature. Special focus was put on whether there are combined effects between the pollutants and high temperature, because their simultaneous occurrence in the reef is likely. Oxygen consumption and photosynthetic activity were chosen as response parameters, to determine the response of the coral holobiont metabolism. The hypothesis is that the metabolism of *P. verrucosa* will be negatively affected by both pollutants and that there will be combined effects with temperature.

Material and Methods

Coral sampling and rearing

Pocillopora verrucosa fragments of approx. 5 cm height (average surface area ± SD: 112 cm^2 ± 29 cm^2) were sampled using Scuba diving at two sites (S 08°20.259', E 116°02.260' and S 08°21.768', E 116°01.897') during 4 days in July 2013 on Gili Trawangan north of Lombok, Indonesia. Both sites were similar in reef habitat, environmental conditions and measured physical water parameters. The research permit for

the study area was approved by the Indonesian ministry for research and technology (RISTEK, permit no. 176/SIP/FRP/SM/V/2013). Two fragments each from a total of 60 colonies were sampled from the two sites combined. All fragments were glued onto 5x5 cm ceramic tiles directly after sampling and brought to a rearing station located in the reef in front of the sampling island (S 08°20.750', E 116°02.608'). They were left in the reef to recover from sampling for two weeks. Water parameters (salinity, temperature, pH and dissolved oxygen) at all sites were measured before and after each dive using a Eureka Manta 2 multiprobe (Eureka Water probes, Austin, USA) and water samples were taken for environmental LAS and PAH determination. After two weeks all healthy coral fragments (two fragments each from 42 colonies) were taken to a laboratory of the Indonesian Institute for Science (LIPI) at Pemenang, Lombok. Fragments were placed in a 600 L semi-flow-through outside tank, located in a larger pool to buffer temperature fluctuations during the day. Water flow through the tank was adjusted that the entire water volume was exchanged once a day with fresh water from the reef, supplied by a pump ca. 200 m away from the shore. Water circulation within the rearing tank was created by using two circulation pumps (Hydor korallia, Hydor Ind., Sacramento, USA). Light conditions at the sampling sites were measured using a LI-1400 data logger with a Li-192 underwater quantum light sensor (LI-COR Biosciences, Lincoln, USA). They ranged from 90 to 500 µmol quanta m^{-2} s^{-1} and averaged around 200 µmol quanta m^{-2} s^{-1} during midday, therefore were adjusted to these intensities in the rearing system by shading the tank from direct sunlight. Corals received additional heterotrophic feeding with designated coral food (Coral V Power by Preis Aquaristik, Bayerfeld, Germany) once a day. Temperature, salinity, pH and dissolved oxygen were monitored daily, always at the same time, using a WTW 340i Multiparameter system (WTW GmbH, Weilheim, Germany).

Experimental protocol

Four main experiments were performed with four replicates for each treatment (see Table 4.1). Corals from the two sampling sites were randomly chosen for the different experiments. Treatments were control, increased water temperature, diesel and combination of diesel with increased temperature. For the control treatment the same protocol as for the other treatments was applied, but neither pollutant nor high temperature were applied. Two additional experiments were performed with the surfactant LAS (linear alkylbenzene sulfonate) alone and in combination with increased temperature. A reduced experimental protocol was used for these two treatments, where no respiration, but only photochemical yield was measured. Temperature was increased using Eheim Jäger 150W aquarium heaters

(Eheim GmbH & Co. KG, Deizisau, Germany). The control temperature was adjusted to 28 °C, to resemble the temperature measured in the reef. Increased temperature was 31 °C, three degrees above the control temperature. Diesel was bought at a local gas station and a water accumulated fraction (WAF) (Singer et al. 2000, Simonato et al. 2008) was produced from 5 g diesel in 1 L of filtered seawater. This solution was stirred for 24 h, then left to settle for 20 min before the lower phase with the waterborne diesel constituents was retrieved. 490 mL of this 0.5 % WAF were immediately administered to each tank at the start of the experiments. LAS was purchased from a local supplier in Indonesia (PT. Findeco Jaya, www.findeco.com) and stored at 4 °C. For each experiment 190 µL LAS were administered to each tank, resulting in a final concentration of 0.00019 %.

Table 4.1: List of all treatments administered to *Pocillopora verrucosa* during the study.

Treatment	Description
Control	control reef temperature ~28 °C, without pollutant addition
High Temperature	+3 °C, without pollutant addition
Diesel	490 mL of 0.5 % water accumulated fraction (WAF) of diesel
LAS	190 µL Linear alkylbenzene sulfonate
Diesel and Temperature	490 mL of 0.5 % WAF, +3 °C
LAS and Temperature	190 µL, +3 °C

All treatments were applied for a total of 84 h; first corals were subjected to 48 h of pre-treatment without measurements to increase the exposure time of the corals to the stressors. This was followed by 36 h of continuous oxygen measurement, during which also analysis of photosynthetic yield took place. During the pre-treatments the tanks were subjected to natural daylight adjusted to the same intensities as in the rearing tank, while the measurement tanks were artificially illuminated from 7:00 to 19:00, using a 2x20 W aquarium light (MW1-Y20X2 from Guangdong Zhenhua Electric Appliance Co. Ltd, Zhonhshan, China). The light intensities were 60 µmol quanta m^{-2} s^{-1} and a 12 h light: 12 h dark cycle was adjusted to simulate natural conditions. This difference in light intensity compared to the rearing and pre-treatment period occurred due to practical reasons of the laboratory set up, but were the same for all experiments. Pre-treatments always started in the evening and lasted for 48 h during which no heterotrophic feeding of the corals took place. Prior to the measurement phase all epiphytes and debris were removed from the coral fragments and the

tiles. Then the fragments were moved to the respiration set-up (see Fig. 4.1), which was prior to the start of measurements filled with filtered seawater and the same stressors as in the pre-treatment tanks.

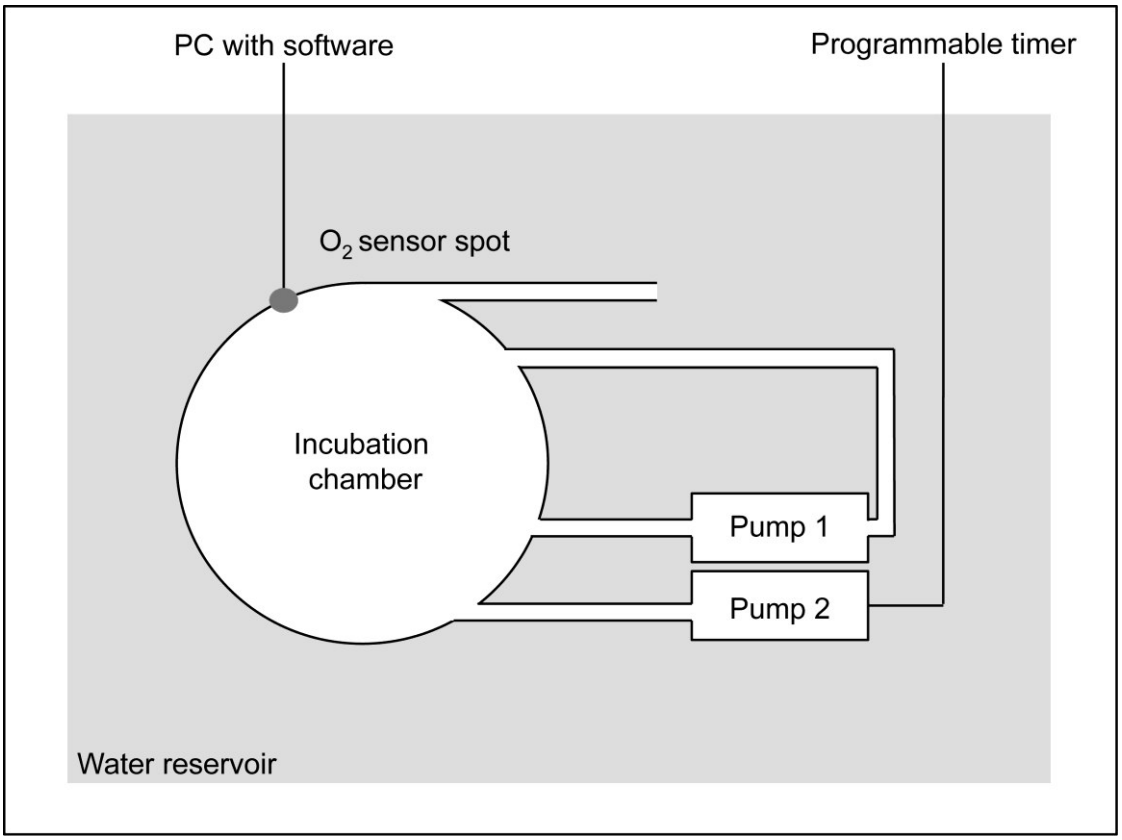

Fig. 4.1: Schematic drawing of the experimental setting. Oxygen concentrations within the incubation chambers were continuously monitored using a sensor spot and optic fiber cable. A recirculation pump (pump 1) was used to ensure continuous water flow within the chamber, while Pump 2 was turned on at programmed intervals to flush the chamber with oxygenated water from the surrounding water reservoir. Four incubation chambers were placed in the reservoir simultaneously, which served also as a temperature control.

Physical water parameters were measured every day for each pre-treatment and measurement phase, using a WTW 340i Multiparameter system (WTW GmbH, Weilheim, Germany). Water samples from pre-treatment and measurement tanks were analyzed for pollutants as described below. After each experiment the entire experimental set-up was cleaned with 70 % Ethanol and rinsed with distilled water to remove bacterial contamination. Surface area of the coral fragments was determined using the aluminum foil method described by Marsh (1970). In this method pieces of aluminum foil are fitted closely to the coral skeleton and the weight of these pieces is compared to a calibration curve with aluminum foil pieces of known size. After each experiment all fragments were photographed from two sides and the pictures

analyzed using Image J software (v1.47, National Institutes of Health, Bethesda, USA) to determine the amount of tissue loss during experiments.

Respirometry and PAM fluorometry

Measurements of oxygen fluxes took place in acrylic incubation chambers. To provide stronger oxygen fluxes and reduce the error due to individual variance, two fragments from one colony were measured in the same incubation chamber. Four of these chambers were situated within a 100 L tank, which served both as a reservoir used for flushing the incubation chambers with oxygenated water and to equalize the temperature between the replicates. All chambers were connected to a pump ensuring water circulation within the chamber and to a flush-pump, supplying oxygenated water from the surrounding water bath at programmed time intervals using a custom build timer (see Fig. 4.1 for details). 30 min measurement periods were followed by 3 min flush periods to allow for continuously high oxygen levels (>90 % saturation) within the chambers. During the entire measurement phase oxygen content within the incubation chambers was recorded using optical oxygen sensor spots and a 4-channel Firesting oxygen meter with the associated software (Oxygen Logger v.3.12.4, Pyro Science GmbH, Aachen, Germany). The system was calibrated prior to each experiment. After the coral fragments were removed from the incubation chambers blank respiration in the system was measured for another 1.5 h to determine bacterial respiration at the end of the experiment. Oxygen fluxes from respiration and photosynthesis were calculated as described below. Photosynthetic capacity was determined by measuring the chlorophyll fluorescence of photosystem II (PS II), using a pulse-amplitude modulated fluorometer (DIVING-PAM, Heinz Walz GmbH, Effeltrich, Germany). Maximum quantum yield (F_v/F_m) (Walz 1998) was measured in the beginning and end of the measurement phase in corals that were dark-adapted (1 h at the beginning of the experiment, approx. 10 h at the end of the experiment as it took place following the dark measurements). All fragments were measured three times with approx. 5 min between each measurement.

LAS and PAH determination

Water samples from each experiment were taken at the beginning and end of each pre-treatment and measurement phase. To quantify the diesel WAF within the samples total polycyclic aromatic hydrocarbons (PAHs) were determined, which are among the major soluble toxic constituents in diesel and a practical measure for the toxicity of a PAH mixture (Logan 2008). Samples for PAH determination were filtered (0.7 µm filters, VWR International, Radnor, USA) and stored with 2-propanol (50 mL) before pre-concentrating

them using solid-phase extraction (SPE) by passing it through a CHROMABOND © C18 PAH cartridge (6 ml, 2000 ng; Macherey-Nagel GmbH & Co. KG, Düren, Germany) and elution of the PAHs with 5 mL dichlormethane from the cartridge. The dichloromethane was evaporated to 1 ml and 250 µl of dimethylformamid was added as keeper. For analysis of total EPA-PAH concentrations Ultra performance liquid chromatography (UPLC) was performed at the Institute for Chemistry and Biology of the Marine Environment (ICBM) in Oldenburg, Germany. A methodological quality control was performed, using a deuterated internal standard and the standard addition method. For LAS analysis triplicate samples of 50 mL were taken and stored at 4 °C until further analysis. All LAS determination took place <24 h after sampling. Spectrophotometric analysis (SQ300 from Merck Millipore, Billerica, USA) was performed applying a modified version of the methylene blue assay for anionic surfactants (MBAS) standard method (George & White 1999). Prior to each LAS determination, a calibration curve was obtained using sodium-dodecyl sulfonate as a standard and filtered seawater from each experimental tank to ensure the same salinity.

Data analysis

Oxygen fluxes were calculated using Microsoft Excel 2010. For each 30 min measurement period the decline in oxygen concentration within the incubation chamber was calculated and standardized to the consumption in 1 h. Fluxes were determined for each 12 h dark and light period (day and night) separately, taking averages from 18-25 measurement periods. All values were further standardized to surface area of the coral and values for bacterial respiration were accounted for by including the oxygen consumption values from the blanks, measured after the experiments. Gross photosynthetic rate was estimated as the difference between dark and light respiration, assuming the same respiration rates during light and darkness (see discussion). To avoid any confounding effects due to handling stress after the pre-treatment, the first 12 h in each experiment were excluded from the analysis. Statistical analysis was performed in R (R v.3.0.2 using R Studio v.0.98.1056). All data were checked for normal distribution using the Shapiro Wilk test and for heterogeneity of variance with Levene's test. Two-way ANOVA was carried out with diesel and temperature as fixed factors for dark and light periods separately to determine significant effects of the stressors and their interaction (see Table 4.4 for the results). To determine differences between the individual treatments, a post-hoc Tukey HSD test was applied (see S4.1 table in the supporting information). In case of the photosynthetic yield in the LAS treatments, data were not normal distributed and multiple Wilcox rank sum tests were applied to detect differences between treatments.

Results

Water parameters

Physical water parameters measured within the rearing tank and during the experiments resembled those determined at the sampling sites (Table 4.2). LAS and PAH concentrations at the sampling sites were below detection limit, thus considered to reach zero. During the experiments there was no significant difference in the LAS or PAH concentrations between pre-treatment and measurement phase or between control and high temperature ($p>0.05$). The pollutant concentrations for both diesel (as measured by total PAH analysis) and LAS decreased significantly from the beginning to the end of each pre-treatment and measurement period. The values decreased from 0.69 ± 0.14 mg L^{-1} to 0.25 ± 0.05 mg L^{-1} and from 0.95 ± 0.02 mg L^{-1} to 0.87 ± 0.05 mg L^{-1} for PAH and LAS, respectively.

Table 4.2: Physical water parameters. Salinity, dissolved oxygen (DO), pH and temperature at the sampling stations (averages of both stations are shown), the rearing system and the experiments (average of the daily measured parameters at midday). Temperature for the experiments is given for the control and high treatments separately.

	Sampling stations	Coral rearing	Pre-treatments	Measurement phase
Salinity [PSU]	34.0 ± 0.2	33.7 ± 0.1	34.0 ± 0.2	34.1 ± 0.2
DO [% sat]	102.2 ± 3.0	102.8 ± 5.0	102.0 ± 1.1	96.3 ± 2.8
pH	8.1 ± 0.0	8.3 ± 0.0	8.3 ± 0.0	8.1 ± 0.1
Temp [°C]	28.3 ± 0.2	28.3 ± 0.5	28.9 ± 0.3	27.9 ± 0.1
			31.1 ± 0.2	30.9 ± 0.1

Control

As a control corals were subjected to the same experimental protocol (including pre-treatment and measurement period) like the other treatments. Without any stressor present dark respiration rates were 0.019 ± 0.005 mgO_2 h^{-1} $cm^{-2}$and light respiration rates 0.082 ± 0.003 mgO_2 h^{-1} cm^{-2}. The photosynthesis rate calculated from the difference between dark and light respiration was 0.011 ± 0.003 mgO_2 h^{-1} cm^{-2}, concluding in a P:R ratio of 0.58 ± 0.12. Maximum quantum yield did not differ between the start and end of the experiment, in both cases being 0.71 ± 0.02.

Diesel

When corals were exposed to the water accumulated fraction of diesel, the dark and light respiration rates were 0.015 ± 0.001 mgO_2 h^{-1} $cm^{-2}$and 0.006 ± 0.003 mgO_2 h^{-1} cm^{-2}, respectively (Fig. 4.2). Gross photosynthesis was 0.010 ± 0.003 mgO_2 h^{-1} cm^{-2} with a P:R ratio of 0.61 ± 0.17. The maximum quantum yield gave exactly the same results as in the control treatment with 0.71 ± 0.02 (Fig. 4.3). No significant effects of diesel exposure were detected on either of these parameters (see Table 4.3 for ANOVA results).

Temperature

Similar to diesel, temperature on its own had no effect on the oxygen fluxes or P:R ratio of the coral. Dark respiration was 0.0121 ± 0.0034 mgO_2 h^{-1} cm^{-2}, light respiration 0.003 ± 0.001 mgO_2 h^{-1} cm^{-2} (Fig. 4.2), concluding in a gross photosynthesis of 0.009 ± 0.003 mgO_2 h^{-1} cm^{-2} and a P:R ratio of 0.76 ± 0.11. At the start of the measurement phase, after corals were exposed to high temperature for 48 h, maximum quantum yield was significantly increased compared to all other treatments (p=0.0112, confirmed by Tukey HSD, supplementary information table S4.1). At this time the yield was 0.74 ± 0.01, but subsequently decreased to control levels of 0.72 ± 0.01 within 24 h until the end of the experiment (Fig. 4.3).

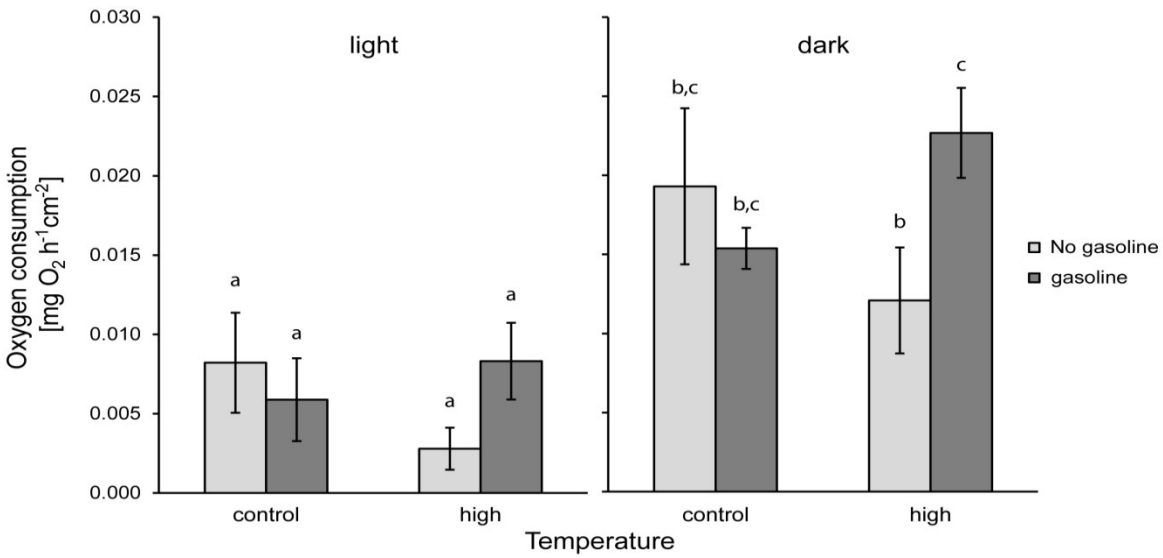

Fig. 4.2: Oxygen consumption. Consumption of oxygen by *Pocillopora verrucosa* in diesel and high temperature treatments for the light and dark period. Differences between the two periods (60-80 % lower oxygen consumption during the light period) are due to the occurring photosynthesis in light. Given are averages for each treatment (n=4) with standard deviations. Different letters indicate significant difference as found in Tukey HSD post hoc test (Supplementary information table S4.1).

Table 4.4: ANOVA results for diesel and temperature experiments. Two way analysis of variance (ANOVA) for maximum quantum yield (F_v/F_m) and respiration values. Effects of temperature (control or high) and pollutant (no pollutant or with diesel) were analyzed in isolation and in combination with each other. Significant values ($p<0.05$) are indicated by an asterisk.

	Df	(SS)	(MS)	F value	Pr (>F)
F_v/F_m (Beginning of measurement period)					
Temperature	1	3.03E-03	3.03E-03	8.963	0.011 *
Pollutant	1	0.00E+00	0.00E+00	0	1
Temperature: Pollutant	1	2.50E-05	2.50E-05	0.074	0.79
F_v/F_m (End of measurement period)					
Temperature	1	2.50E-05	2.50E-05	0.09	0.77
Pollutant	1	1.00E-04	1.00E-04	0.358	0.561
Temperature: Pollutant	1	1.00E-04	1.00E-04	0.358	0.561
Light respiration					
Temperature	1	8.93E-06	8.93E-06	1.1	0.315
Pollutant	1	1.02E-05	1.02E-05	1.252	0.285
Temperature: Pollutant	1	6.17E-05	6.17E-05	7.601	0.017 *
Dark respiration					
Temperature	1	1.00E-08	1.00E-08	0.001	0.982
Pollutant	1	4.46E-05	4.46E-05	2.947	0.112
Temperature: Pollutant	1	2.11E-04	2.11E-04	13.939	0.003 *

LAS

Exposure (>24 h) to LAS caused tissue ablations in the coral fragments (see Fig. 4.4). This ranged from 16 % tissue loss in some fragments up to 95 % in others at the end of the experiment (84 h). On average the tissue loss in the isolated LAS treatment was 53 ± 30 % (n=8). The amount of tissue ablation was always similar in both fragments originating from the same coral colony. The tissue loss hampered the measurements of photosynthetic activity. During the first measurements all coral tissues were still intact, while values from the end of the experiment can only serve as a rough prediction of actual values. Although the PAM fiber optic was always placed at positions where tissue was still visibly unimpaired, the results obtained are not reliable due to the high tissue loss. Maximum quantum yield in the treatments with LAS was 0.73 ± 0.01 at the beginning of the measurement period and 0.58 ± 0.22 at the end of the experiment (Fig. 4.3).

Table 4.3: Summary of *Pocillopora verrucosa* responses. Physiological responses of the coral holobiont for all treatments. Given are holobiont respiration measured during dark and light periods, photosynthesis rate estimated from the difference between the respiration values and maximum quantum yield measured after 48 h and 84 h. The 84 h results for treatments containing LAS are given in brackets as they are not reliable due to high tissue loss (see LAS results and discussion section). Tissue loss as seen in treatments containing LAS is given as determined at the end of the experiment.

	Dark Respiration [mgO_2 h^{-1} cm^{-2}]	**Light respiration [mgO_2 h^{-1} cm^{-2}]**	**Photosynthesis [mgO_2 h^{-1} cm^{-2}]**	**Maximum quantum yield 48 h [F_v/F_m]**	**Maximum quantum yield 84 h [F_v/F_m]**	**Tissue loss after 84 h [% loss]**
Control	0.019 ± 0.005	0.082 ± 0.003	0.011 ± 0.003	0.71 ± 0.02	0.71 ± 0.02	-
High temperature	0.012 ± 0.003	0.003 ± 0.001	0.009 ± 0.003	0.74 ± 0.01	0.72 ± 0.01	-
Diesel	0.015 ± 0.001	0.006 ± 0.003	0.010 ± 0.003	0.71 ± 0.02	0.71 ± 0.02	-
LAS	-	-	-	0.73 ± 0.01	(0.58 ± 0.22)	52.5 ± 30.15
Diesel + high temperature	0.023 ± 0.003	0.008 ± 0.002	0.014 ± 0.005	0.74 ± 0.01	0.71 ± 0.01	-
LAS + high temperature	-	-	-	0.63 ± 0.13	(0.14 ± 0.08)	92.25 ± 7.26

Combined effects

Even though neither diesel nor temperature alone had a significant effect on respiration, significant effects were found in the combined treatment. A significant interaction between diesel and temperature was detected ($p=0.0174$ in light and $p=0.0029$ in darkness, see Table 4.4). The Tukey test validated this effect only for the dark period (dark period $p=0.0107$, light $p>0.07$, see supplementary information table S4.1). The light respiration rate was 0.008 ± 0.002 mgO_2 h^{-1} cm^{-2}, dark respiration was 0.023 ± 0.003 mgO_2 h^{-1} $cm^{-2}$and thus higher than in the treatment with increased temperature alone (Fig. 4.2). But there was no significant difference between the combined and the control treatment. Despite the difference in oxygen consumption values, there was no difference in gross photosynthesis (0.014 ± 0.005 mgO_2 h^{-1} cm^{-2}), P:R ratio (0.61 ± 0.15) or maximum quantum yield (0.72 ± 0.02).The combination of high temperature and LAS led to even higher tissue ablations than LAS in isolation (on average 92 ± 7 %, with n=8, in a range from 81 to 100 %), thus results of maximum quantum yield are highly unreliable and are merely given to show the decreasing trend in the values. At the start of the measurement phase, when no

ablations of coral tissue were yet visible, maximum quantum yield in the combined LAS and high temperature treatment was 0.63 ± 0.13 and thus significantly lower than the other treatments (p=0.0265, Wilcox rank sum test). At the end of the experiment the values measured for maximum quantum yield decreased to 0.14 ± 0.08 (p=0.0294, Wilcox rank sum test), but at this point tissue loss was already at its maximum.

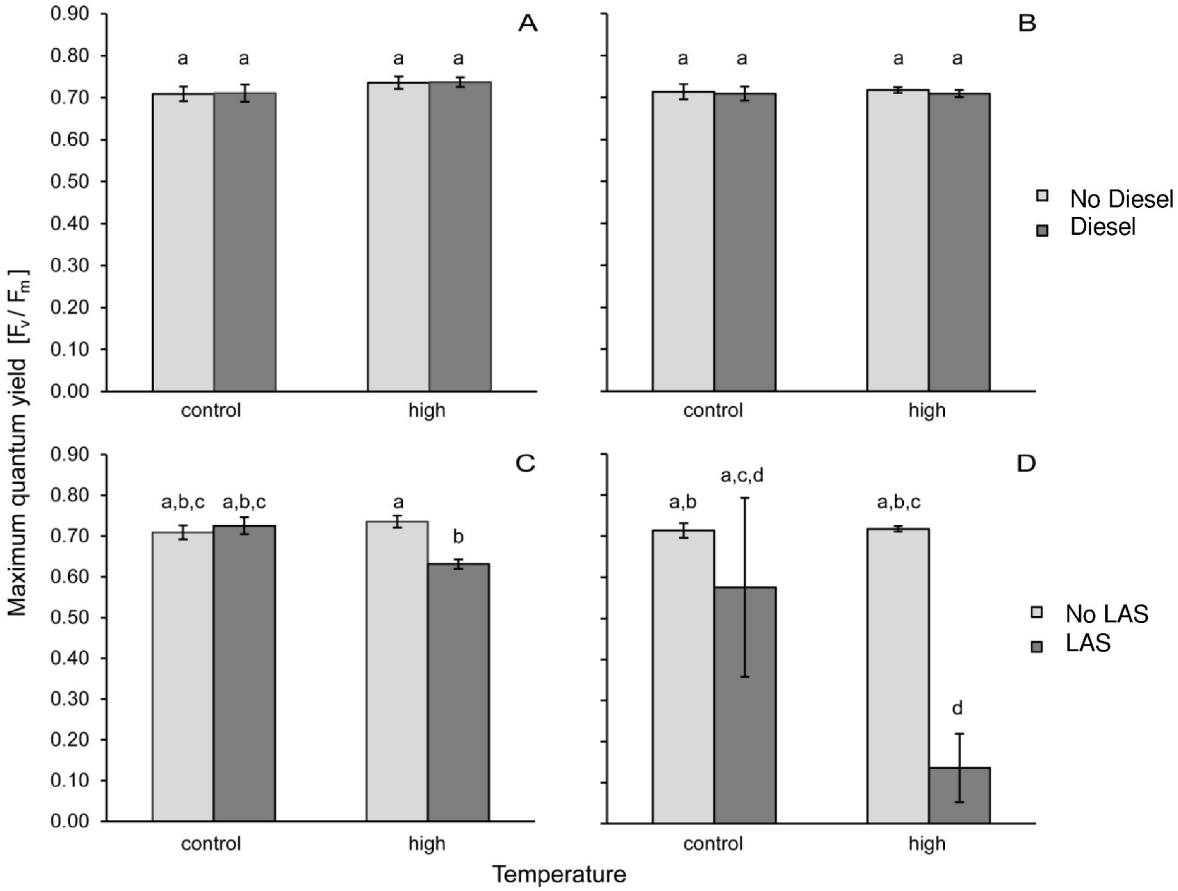

Fig. 4.3: Maximum quantum yield (F_v/F_m) of *Pocillopora verrucosa* subjected to pollutants and high temperature. WAF of diesel (A+B) and linear alkylbenzene sulfonate, LAS (C+D) were administered either individually or in combination with high temperature. Measurements took place after 48 h and 84 h (A+C and B+D, respectively). Given are averages for each treatment (n=4) with standard deviation. Different letters indicate significant differences as determined by Wilcox rank sum tests.

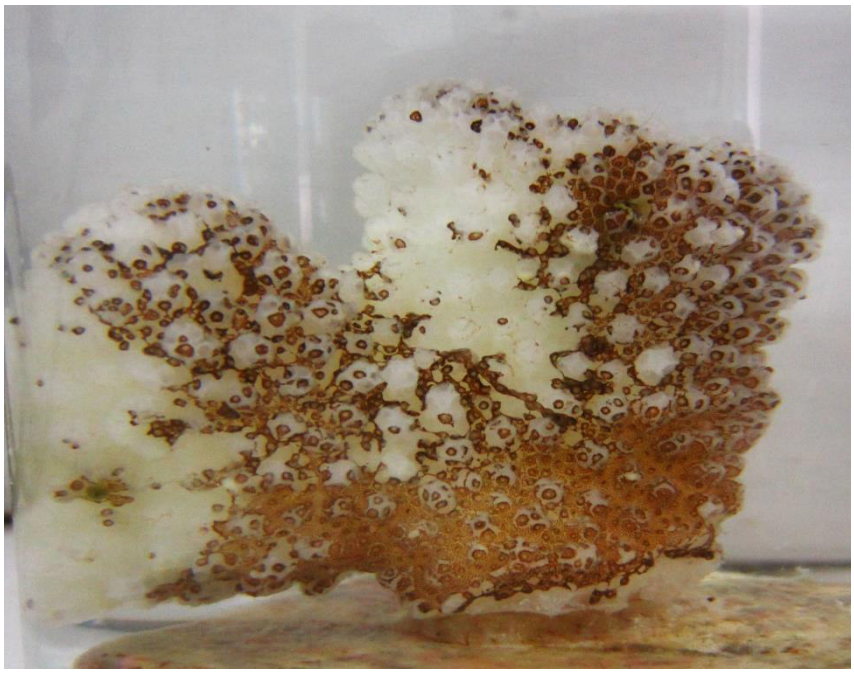

Fig. 4.4: Documentation of tissue loss due to LAS. *Pocillopora verrucosa*, subjected to LAS treatment, showing severe tissue loss after 84 h exposure.

Discussion

This study showed that the physiology of *Pocillopora verrucosa* was influenced by the three stressors temperature, diesel and LAS, but resulted in different responses. While diesel and temperature had a significant interactive effect on dark respiration, LAS on its own and in combination with temperature resulted in a severe tissue loss of coral fragments. Temperature on its own led to a significant decrease in maximum quantum yield only in the beginning of the measurement period, while diesel had no significant effect at all. Thus our working hypothesis is only partly supported by the findings, but proved right in the context of combined effects with high temperature. This confirms other studies, which have predicted a negative effect of global warming on organisms' sensitivity to chemical stressors (Beyer et al. 2014). In addition, our study highlights the importance of measuring several response parameters, as different stressors can result in diverse responses.

The analysis of ambient LAS and diesel concentrations around Gili Trawangan showed no measureable amounts of these pollutants in the water. This can be explained by the fact that the island as a tourism hotspot is kept clean and strong currents would quickly dilute any pollutants entering the water. A contrasting situation occurs in more densely populated areas, such as the Thousand Island chain off the Indonesian capital Jakarta, where also the boat

traffic is much higher, including large commercial vessels passing through the island chain. At the Thousand Islands, PAH values up to 0.23 mg L^{-1} and LAS of up to 0.9 mg L^{-1} were detected (Baum et al. in prep.). Other studies in the Indo- Pacific measured total PAH concentrations between 0.05 to 0.21 mg L^{-1} (Falahudin et al. 2012), and LAS concentrations in the Red Sea ranging from 0.001 to 0.03 mg L^{-1} (Shafir et al. 2014). During short periods, the concentrations can reach higher values close to pollutant sources (Braga & Varesche 2014, Shafir et al. 2014). One source of diesel and PAH is the regular evacuation of water from the bilge in ships. Total PAH concentrations next to a boat after discharging bilge water were still higher than the PAH concentrations in our experiments (0.9 mg L^{-1} 10 min after discharging), but would further dissolve after a longer time period. Similarly, LAS concentrations next to a boat after cleaning were still 1.3 mg L^{-1} (G. Baum et al., in prep). This shows that the pollutant concentrations used in this study are relevant in the environmental context. The decreasing PAH and LAS concentrations during the course of our experiments resemble the natural exposure conditions of corals in the reef, with initially higher concentrations that are decreasing over time. Fast degradation has been described before for LAS (Ivancović & Hrenović 2010) and to some extent also for diesel (Pampanin & Sydnes 2013). Half-life time of LAS in seawater is ca. 6 days due to biodegradation and adsorption to suspended particles (Kemp et al. 2011).

The respiration and photosynthetic yield values measured in the experiment were comparable to those measured by other authors for different coral species (Muscatine et al. 1984, Porter et al. 1999, Kemp et al. 2011, Ulstrup et al. 2011). The values given in our paper for photosynthesis are estimations for the actual rates of oxygen produced, calculated from the difference between dark and light oxygen consumption. This assumes that holobiont respiration during dark and light are the same, which is not always the case as mentioned by Lesser (2013). Therefore gross photosynthesis might be slightly underestimated by this method. Compared to other studies, the calculated P:R ratio of 0.63 was very low. Generally, a P:R ratio below 1.0 indicates lack of photosynthetically fixed carbon (Davies 1984). In our study, this is due to the low light intensities from the artificial aquarium light. The light intensities were only one third of light intensities measured in the reef, explaining why photosynthesis rates during the experiments were quite low. Still 60 µmol quanta m^{-2} s^{-1} were enough to result in photosynthesis in *P. verrucosa*.

Diesel exposure had no effect on the coral physiology. Other authors have reported negative effects of different sources of oil on corals, but the literature on the effects of diesel, other oil sources and PAH on corals is contradictory. While several studies report decreases in

maximum quantum yield (Mercurio et al. 2004), tissue alterations (Harrison et al. 1990) and damages to reproductive systems (Rinkevich & Loya 1979, Negri & Heyward 2000), there are also other studies where no effect on corals was found (Braga & Varesche 2014). In a recent study on cold water corals, DeLeo et al. (2015) also could not detect effects of crude oil treatments on coral health and proposed that initial negative effects could be mitigated, when the coral holobiont uses the hydrocarbon components as a nutrition source, as indicated before by Al-Dahash and Mahmoud (2013). In coral tissues, total PAH concentrations of 0.004 - 0.1 $\mu g\ g^{-1}$ dry mass were determined, higher than in the surrounding sediments, indicating a bioaccumulation (Ko et al. 2013, Whitall et al. 2014). Most studies on threshold values of PAH were performed on fish and crustaceans. In general LC50 concentrations for various PAH types and exposure times range from 0.0005 to 32.5 mg L^{-1} (Ministry of environment BC). In sole morphological and physiological effects occurred at PAH concentrations in sediments ranging from 0.054 to 4 $\mu g\ g^{-1}$ dry mass (Johnson et al 2002). No threshold values for corals are described in the literature. PAH metabolic products can bind to the organisms DNA and cause severe carcinogenic damage, as well as lead to alterations of the immune system (Logan 2007), change blood composition and tissue (Simonato et al. 2008).

The significantly higher photosynthetic yield due to increased temperature alone was only detected in the beginning of the experiment, indicating a high stress during the first hours that the coral could mitigate over time. Exposure time has a strong influence on the effect of temperature. While longer exposure reduces the respiration rates in corals, short term exposures can increase both respiration and photosynthetic rates (Coles & Jokiel 1977). In *Montastrea annularis* elevated temperature reduced both photosynthesis and respiration after 6 h of exposure (Porter et al. 1999). Caribbean corals showed minor decreasing effects on photochemical efficiency due to higher temperature in experiments lasting for 10 days (Fournie et al. 2012). Other studies detected negative effects on photosynthesis and respiration due to temperature stress as well (Kemp et al. 2011), although this could not statistically be replicated in the current study, where only negative trends of high temperature were measured.

Even though no significant differences to either diesel or temperature alone could be detected, when diesel was combined with temperature, dark respiration significantly increased compared to the temperature as a single stressor and was similar to the control. This combined effect can be explained by altered membrane properties due to the higher temperature, which affect fluidity and diffusion rates and thus chemical toxicity (van Dam et

al. 2011), In hard corals from the Florida Keys, Porter et al. (1999) found that increases in both temperature and salinity reduced respiration rates and if administered at the same time, the effect was mitigated during the first 36 h, but still led to death of all corals after longer exposure. Synergistic effects on maximum quantum yield were also demonstrated before between temperature and light intensities in Japanese corals, where yield decreased even stronger at higher temperatures (Bhagooli & Hidaka 2004). Reynaud et al. (2003) found an antagonistic effect of temperature in combination with increased pCO2 on calcification and photosynthesis of *Stylophora pistillata*, but not on respiration (Reynaud et al. 2003).

LAS resulted in a significant reduction of the photosynthetic yield. Even though maximum quantum yield values obtained at the end of the experiment were not reliable, in combination with high temperature decreases in yield were already seen in corals without tissue loss, showing the importance of yield measurements as early warning indicator. Declines of photosynthetic yield in corals were also measured after cyanide exposure and sedimentation, where yield values down to 0.1 during stress were recorded (Jones et al. 1999, Philipp & Fabricius 2003). The effect LAS had on the coral tissue was surprisingly severe. First tissue ablations were already visible after only 24 h exposure to 0.9 mg L^{-1} and after 84 h large parts of the coral tissue were detached from the skeleton. Trials with higher concentrations of LAS (results not shown) even lead to tissue losses up to 100 % within the first 24 h. These tissue ablations pose a severe threat to coral health, as the regeneration of tissue needs a lot of energy and time. Generally, marine species tend to be more sensitive to LAS than freshwater species. Surfactants reduce the water surface tension that aquatic organisms depend on (Braga & Varesche 2014), explaining the severe effect on coral tissue as the membrane properties are disrupted (Abel 1974, DeLeo et al. 2015). Anionic surfactants such as LAS can bind to proteins and peptides, resulting in an alteration of their structure and function (Braga & Varesche 2014). Thereby, these pollutants can alter enzymatic activities within the metabolic pathways and affect the DNA (Cserháti et al. 2002, Ivancović & Hrenović 2010). Temara et al. (2001) determined average LC50 values for marine species to be 4.3 mg L^{-1} with no observed effect concentrations at 0.3 mg L^{-1}, which is 2 mg L^{-1} lower than for freshwater species. There are only very few studies on the effects of LAS on corals, most experimental and monitoring work focused rather on fish and other invertebrates than corals. Shafir et al. (2014) performed the first study on the toxicology of detergents on hard corals and found them to be much more sensitive than many other marine organisms. LC50 for *Pocillopora damicornis* was determined to be 2.2 mg L^{-1}, for *Stylophora pistillata* even 1.0 mg L^{-1} in 24 h exposures. In their experiments genotype-specific

mortality and adaptation in *P. damicornis* after several exposures was observed. Such a genotype-specific response could explain the finding that in our experiments always both fragments from the same coral colony showed similar ablation rates.

Further experiments with different concentrations of pollutants, especially of LAS, would increase our knowledge on their effects on coral physiology and the impacts on coral reefs. It will be essential to determine threshold values to those common pollutants also for corals. Experiments with multiple pollutants with and without the increase of temperature could reveal more on interactions between them. Varying exposure times and increasing measurement intervals in the experiments would give additional information about the risks of low concentrations during long term vs. high concentrations during very short term exposure. In future research with multiple response parameters should regularly be considered, because while diesel had an effect on respiration and no effect on photosynthetic yield, significant effects on yield were detected during LAS and temperature exposure. Different stressors can evidently modify the coral physiology in several ways, and some parameters such as respiration may react faster and stronger to pollutants than others. The physiological parameters proved to react fast to environmental changes, which is supported by Kaniewska et al. (2012), who also reported faster effects of physiological responses compared to biomineralisation processes. In order to find appropriate parameters, it is further necessary to understand the underlying mechanisms of PAH and LAS toxicity in corals. Due to high variations in all response parameters, not all trends that were visible could be validated with statistical significance. Kaniewska et al. (2012) also had n=4, but proposed higher replicate numbers in order to get more robust results. Future experiments with higher replication could strengthen our findings.

This study gives further confirmation about the need for better local management in the face of global warming. Even if CO_2 emissions will be reduced, global warming will continue in future (IPCC 2013). For coral reef organisms in tropical areas, a seemingly minor increase of a few degrees can result in severe stress, particularly if other local stressors are present as shown in this study. While removing thermal stress is not achievable in the near future, coral reefs are able to recover when other stressors are removed, thus it will be critical to support resilience of reefs by changing human destructive activities (Hughes et al. 2002). Indonesia's population is strongly reef-associated, and most of the country's coastline is populated without effective sewage treatments in place (Burke et al. 2012). However, every family in each of the villages along the coast is using diesel and soap. Although at the moment no obvious effects of diesel or surfactants are visible on coral reefs, this could potentially

become a problem in near future, taking growing populations and climate change into account, and expressing the need for effective local management (Baskett et al. 2009, Burke et al. 2012). Understanding the effect of different stressors and their combinations on key organisms can strengthen the decision support needed for coral reef management (Maina et al. 2011). One way to reduce the outflow of pollutants from human settlements is the implementation of sewage treatments. Experiments on the effectiveness of sewage treatments have shown that from influent waters with LAS concentrations up to 6.7 mg L^{-1}, only max. 0.005 mg L^{-1} remained in the effluent water (Clara et al. 2007). In areas like the Netherlands, where 83 % of all waste water is treated, LAS concentrations of only 1-9 µg L^{-1} were measured in nearshore estuaries (Temara et al. 2001). Further controls on the direct discharge of diesel at least for commercial ships are necessary. At the same time, effective reef management can be achieved by communication of findings, education, training and outreach of populations living close to reefs to make them aware of the risks and the positive outcome when protecting the reefs (Burke et al. 2012).

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Chapter 5: Implications for coastal livelihoods

This chapter is in preparation as:

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Under anthropogenic stress: Linking marine resource use systems in Jakarta Bay and the Seribu Islands

Baum G, Kusumanti I, Glaser M, Ferse SCA, van der Wulp S, Adrianto L, Kunzmann A, Schwarzbauer J, Dsikowitzky L, Dwiyitno, Breckwoldt A, Agos Heri

Abstract

The Greater Jakarta Metropolitan Area is the 3rd largest agglomeration in the world with around 25 million inhabitants. Jakarta Bay and its offshore "Thousand Islands" chain, are facing extreme pollution as a result of urban (domestic and industrial) run-off. These anthropogenic impacts have caused severe degradation of local coral reefs. Furthermore, marine resources are heavily exploited for subsistence consumption and sale at local markets, providing livelihoods and food for millions of people. This study investigates anthropogenic pressures and associated vulnerabilities of the marine resource use system (i.e. fishery), including its dependencies and related livelihoods, by comparing communities from Jakarta Bay (coastal mainland) and the Thousand Islands. Data was collected from 15 sites (five island and 10 mainland sites) during household questionnaire surveys and individual interviews on issues such as resource use patterns, economic relevance of species, consumption patterns and fisheries-dependent livelihoods. Complementary data from two ship-based research excursions on consumption patterns (based on Food Frequency and Food Recall questionnaires), numerous field visits, two experimental studies, as well as data from visual underwater surveys helped to triangulate and situate the data generated during the 'village' questionnaires and interviews. Findings show that especially in the bay anthropogenic pressure was severe, with extreme pollution with toxic chemicals and eutrophication. Communities on the mainland, were more dependent on fisheries compared to communities along the islands, and thus more vulnerable because of less livelihood assets with limited alternative livelihoods. On the islands, more people can rely on alternative livelihoods. Around 85 % of fishermen in both areas regard the current state of marine natural resources as declining. Pollution (26 %), especially closer to the mainland, and overexploitation (21 %) due to rising numbers of fishermen are perceived as the main causes.

Although fish stocks are suffering, both fishermen from the mainland and the islands still depend (mainly and partially) on fisheries resources for their livelihoods. The fish C*aesio cuning* (Redbelly yellowtail fusilier) was perceived to be the most economically valuable species on the islands, because of its high marketability and easy catch. On the mainland *Rastrelliger kanagurta* (Indian mackerel) was mentioned as the most economically important species due to its easy processing. *Siganus guttatus* (Goldlined spinefoot), also mentioned from mainland and island fishermen as economically important, was investigated as a model species for its susceptibility to pollution. Metabolic stress during exposure to pollutants was observed, which may lead to trade-offs to lower growth and reproduction rates and translate to declines in fish populations. Considering its economic importance, this may affect food security. The article closes with a discussion of the three aspects of vulnerability (exposure, sensitivity and adaptive capacity) within the larger Jakarta Bay Ecosystem. In order to improve livelihoods of people, national policy should not only develop adaptive capacity, but also address the initial cause of vulnerability, i.e. environmental exposure by anthropogenic stressors.

General Discussion

Key findings and significance

Reefs are not exposed to single stressors only, but increasingly to multiple stressors simultaneously, especially close to large urban areas. Therefore, this thesis is aimed at contributing to a better understanding of the processes that shape coral reef communities (i.e. reef composition, physiology of selected key reef organisms) and thus subsequently affect coastal livelihoods under the influence of multiple anthropogenic stressors with the Jakarta Bay/Thousand Islands reef complex in Indonesia as a case study.

The key results of this thesis and their significance are summarized in the following chapter, referring back to the original research objectives from the general introduction.

Spatial impacts of important anthropogenic stressors on reef communities on local and regional scales and identification of potential shifts in benthic community structure

The spatial structure of reefs in the JB/Thousand Islands reef complex is directly related to intense anthropogenic pressure from local as well as regional sources. A high spatial variability in reef condition on a smaller regional scale was observed, highlighting the potential role of especially local stressors in shaping the structure of benthic communities in coral reefs (Fig. 1). The reef condition along the Thousand Islands has dramatically declined since the first scientists conducted investigations in the area in the beginning of the 20th century and described reef systems with high species diversity (Umgrove 1939, Tomascik et al. 1939). Especially since the 1950s, the rapid growth of Jakarta has transformed the former "Batavia" into a megacity with more than 14,500 inhabitants km^{-2} in the central city area (Pelling and Blackburn 2014). This has caused the bay to become one of the most polluted in Asia (Bengen et al. 2006). Here, results confirm that the bay is facing extreme eutrophication coupled with increased turbidity and sedimentation (**chapter 1**). This decline in water quality went hand in hand with severe changes in reef communities. Hard coral cover is around 2 % within the bay. Along the outer Thousand Islands, overall reef conditions can also be considered poor since total coral cover in most sites is < 25 % (threshold based on Gomez and Yap 1988, **chapter 1**). In addition, shifts to soft coral dominance were found in the bay (**chapter 2**). Even though shifts to soft coral dominance are far less common than those to macroalgae dominance, such shifts have been reported in other degraded reefs in the Indo Pacific (Chou and Yamazato 1990, Fox et al. 2003, Ward-Paige et al. 2005). Similarly, severe changes in fish communities were found, with currently 80 % lower fish abundance in the bay compared to sites from the outer Thousand Islands (**chapter 1**).

The results from this thesis showed a clear difference in benthic and fish communities between sites in JB and from the outer Thousand Islands. No clear nearshore to offshore gradients in water quality were found. This suggests that even though Jakarta is a megacity with high intensity of urbanization, industry and shipping, the direct impact on shallow coral reefs may be restricted to within the bay itself (**chapter 1**). In the outer Thousand Islands, localized effects of anthropogenic stressors rather than regional gradients appear to shape the spatial structure of reefs. Over 80 % of variation in benthic community composition was linked to factors related to terrestrial run-off and eutrophication, especially NO_3, sedimentation, turbidity, PO_4 and Chl a (**chapter 1**). This supports results from a recent

ocean-wide study that local anthropogenic stressors can become the dominant factors shaping benthic reef communities (Williams et al. 2015).

Physiological effects of multiple anthropogenic stressors on key coral reef players: combined effects of two significant chemical stressors (diesel and surfactant) and temperature on the metabolic state of a fish and a scleractinian coral by means of manipulative experiments

Organic toxic pollutants are of growing concern to marine ecosystems (Logan 2007, van Dam et al. 2011). Results from this thesis show that both the surfactant LAS from sewage run-off and diesel borne compounds such as PAHs from bilge water discharges are two very common local pollutants (**chapter 3**), not only within JB but also along the outer Thousand Islands due to lack of sewage treatment and high boat traffic. Short-term exposure of sublethal concentrations of WAF-D and LAS caused metabolic stress to various degrees in both coral reef key players investigated (Fig. 1). Exposure to WAF-D led to a reduction in metabolic rates in the commercially important coral reef fish *S. guttatus* (**chapter 3**), while no visible effect on hard coral metabolism of *P. verrucosa* (**chapter 4**) could be found. LAS exposure led to a significant increase in SMR in *S. guttatus*, a clear indicator of increased energy demand as a result of high stress (Caulton 1977, Sloman et al. 2000). In *P. verrucosa*, a decreased photosynthetic efficiency as well as severe tissue loss could be observed during LAS exposure (**chapter 4**). The experiments further detected interactive effects between both pollutants highlighting the need to account for stressor interactions in future management and conservation plans. Under combined exposure to both stressors, metabolic depression was observed in *S. guttatus*. LAS led to a significantly higher PAH concentration in the water, therefore suggesting that the effect of WAF-D (decrease in respiration) may have counteracted and neutralized the effect of LAS (increase in respiration) (**chapter 3**). Overall, the literature on the effects of both diesel or other oil products, as well as of surfactants on both corals and fish is ambiguous (Maki 1979, Rinkevich and Loya 1979, Zaccone et al. 1985, Harrison et al. 1990, Negri and Heyward 2000, Mercurio et al. 2004, Susmi et al. 2010, Braga and Varesche 2014, DeLeo et al. 2015), showing that underlying physiochemical processes in the toxicity of these pollutants are still barely understood. Nonetheless, even though metabolic rate analysis does not give exact answers with regards to the physiological mechanisms disrupted by the pollutants, it indicates the severity, since it reflects effects at the level of whole organisms with implications for populations and communities (Johns and Miller 1982).

The local chemical stressors investigated in this thesis are very likely to occur in combination with global warming. Results show that a 3-4 °C increase in temperature, reflecting predicted global warming effects for the end of this century (IPCC 2013), caused more severe metabolic stress with regard to LAS and WAF-D toxicity for *S. guttatus* (**chapter 3**) and *P. verrucosa* (**chapter 4**). However, in *S. guttatus* a synergistic, i.e. amplified reduced (WAF-D) or increased (LAS) change in metabolic rates, was not observed. Effects were nonetheless additive during combined exposure to high temperature and LAS, thus further decreasing the metabolic condition of *S. guttatus*. In the coral *P. verrucosa* the combination of WAF-D and high temperature led to an increase in dark respiration, and the combination of LAS and high temperature to severe tissue loss and subsequent high mortality (**chapter 4**).

While studies in the past focused primarily on other pollutants, such as heavy metals (Howard and Brown 1984, Guzmán and Jiménez 1992), single PAHs (Jee et al. 2004, Dos Santos et al. 2006, Oliviera et al. 2008) and pesticides (Richmond 1993), future studies should concentrate as well on lesser studied pollutants, such as surfactants and WAF-D. Considering the frequency and amount of bilge water discharge and untreated sewage run-off in the JB/Thousand Islands complex (**chapter 3**), these pollutants may constitute a regional problem rather than a local problem for marine organisms. This study highlights the need to reduce the import of these pollutants in coastal areas if long-term effects on fish and coral health are to be avoided.

Physiological effects of multiple anthropogenic stressors on key coral reef players: effects of water quality on metabolism of dominant soft corals

As seen in **chapter 1**, both local and regional factors interact and have caused severe reef degradation in the JB/Thousand Islands reef complex including shifts to soft coral dominance in the bay. The processes involved in shifts to soft coral dominance are still unclear, especially to what extent soft coral abundance and physiology are influenced by declining water quality (Dinesen 1982, De'ath and Fabricius 2010, Norström et al. 2009). This study contributes to the knowledge on soft coral responses to environmental stress. Findings suggest that water quality may control abundance and physiology of dominant soft corals in Jakarta Bay and may thus facilitate phase shifts from hard to soft coral dominance (Fig. 1). Water quality, particularly inorganic nutrient concentrations and sedimentation rates, were found to affect photosynthetic yield and ETS activity of two dominant soft corals species, *Sarcophyton sp.* and *Nephthea sp.* In addition, the abundances of both species were directly linked to declining water quality (**chapter 2**). Although the present study is not able

to determine direct causal relationships between individual stressors and changes in the ETS activity and photosynthetic yield of both *Nephthea sp.* and S*arcophyton sp.*, results indicate that metabolic condition in both species is affected by reduced water quality. This further highlights the need to improve management of water quality in order to prevent or reverse phase shifts.

Implications: Livelihood vulnerabilities linked to marine anthropogenic pressures

Coastal communities, especially those that rely mainly on marine resources, are highly vulnerable to marine anthropogenic pressures (Cinner et al. 2013, Yoo et al. 2014, Ferrol-Schulte et al. 2015). In the previous **chapters 1-4**, both local and global stressors were found to have severe impacts on coral reefs in JB and the Thousand Islands. Especially, local factors such as eutrophication (**chapter 1**) and chemical pollutants (**chapter 3+4**) were found to be highly important in shaping reef communities. Therefore, there is an increasing need to evaluate the links between livelihood vulnerabilities of coastal communities in this area and local stressors such as pollution. Integrating results from a parallel socio-economic study (**chapter 5**) allowed to derive implications of the thesis results for livelihoods of coastal communities in JB/Thousand Islands. For JB, where lower water quality (i.e. higher environmental exposure) was found compared to the outer Thousand Islands (**chapter 1**), results from this socio-economic study suggest that communities on the coastal mainland may be more dependent on fisheries and thus more vulnerable because of less adaptive capacity (less livelihood assets and limited alternative income sources such as tourism). Thus, the high anthropogenic pressure in JB is linked to reduced coastal livelihoods (Fig. 1). In comparison, the socio-economic study found that along the islands, more fishermen could rely on alternative income sources such as tourism. Nevertheless, although fish stocks in the JB/Thousand Islands are heavily exploited (BPS 2011, KKP 2011), results from the parallel socio-economic study found that fishermen from the mainland in Jakarta and from the islands still depend largely on fisheries resources for their livelihoods. Fishermen in general perceived overexploitation and pollution, especially closer to the mainland, as the main causes for the declining fisheries.

Physiological stress in the rabbitfish S. guttatus (**chapter 3**), which was found to be an economically important species (in fishermen perceptions; socio-economic part of **chapter 5**), as well as in *P. verrucosa* (**chapter 4**), could lead to trade-offs to lower growth and

reproduction rates which may cause declines in populations numbers in the area. Such stress could also be assumed for other fish and coral species and clearly more research into the responses of economically important fish species and corals to the most common pollutants is needed in order to find out how this may affect food security and livelihoods of coastal communities. The integrated analysis of the collected ecological and socio-economic information (**chapter 5**) highlights that Indonesian national policies should address the initial cause of vulnerability, i.e. in the case of JB/Thousand Islands exposure by anthropogenic stressors such as pollutants and eutrophication, as well as developing adaptive capacities (see Ferrol-Schulte et al. 2015).

Fig. 1: General overview of the effect of multiple anthropogenic stressors on reef communities, physiology of key reef players and coastal communities in Jakarta Bay and along the outer Thousand Islands. The numbers refer to results of the chapters of this thesis.

Anthropogenic pressure	**Jakarta Bay (nearshore: < 20 km)**	**Outer Thousand Islands (offshore: 20 – 60 km)**
Reef communities **→ Local and regional stressors interact on community level**	Extreme reef degradation: • Low coral cover (2 %) (1) • Shifts to soft coral dominance (2) • 80 % lower fish abundance Reduced water quality: • Severe eutrophication, sedimentation (1) • High chemical pollution (chronic) (3) → Clear separation of reef composition to outer islands (1)	Patchwork of differentially degraded reefs: • Mean coral cover < 20 % (1) • Trends of shifts to soft coral dominance No near- offshore gradient in water quality • local sources for nutrients and chemical (e.g. sewage run-off, bilge water discharge) (3) → Localized rather than regional stressors shape reefs (1)
Physiology of key reef players **→ Local chemical and global (high temp) stressors interact on species level**	Severe metabolic stress due to • Local (chronic and regular recurring) sources of WAF-D and LAS (3+4) • Global warming (3+4) Physiology + abundance of soft corals linked to reduced water quality (2) • possible link to phase shifts	Metabolic stress due: • Local regular recurring sources of WAF-D and LAS (3+4) • Global warming (3+4) Possible link between physiology /abundance of soft corals and water quality (2)
Coastal communities **→ Local stressors affect livelihood vulnerability**	High anthropogenic pressure → higher livelihood vulnerability: Limited alternative livelihoods and lower livelihood assets (socio-economic part in 5)	→ Lower livelihood vulnerability: More alternative livelihoods (e.g. tourism) and higher livelihood assets (socio-economic part in 5)

Conclusions and future perspectives

This thesis shows that the major threat to coral reefs in JB and the Thousand Islands that has caused the observed reef and resource decline is a result of the combination of multiple stressors acting simultaneously. This is also thought to be the cause of the worldwide reef degradation (Edinger et al. 1998, Sale 2008). While organisms may be able to mitigate some of the adverse effects of single stressors, the exposure to multiple stressors causes even more severe consequences in most cases. Chemical pollutants can influence each others toxicity as seen in this study for WAF-D and LAS, and combined exposures become more complex, when also taking non-chemical stressors such as temperature into account (Ban et al. 2014 a, Beyer et al. 2014). The anthropogenic pressure on coral reefs needs to be reduced in order to "buy time" for reefs to adapt to inevitable global warming (Knowlton and Jackson 2008, IPCC 2013).

However, given the diversity of stressors present, coupled with the great complexity of marine ecosystems and their high variability, it remains difficult to establish simple causal relationships between stressors and observed effects in organisms and to draw general conclusions (Billick and Case 1994, Adams 2005). Furthermore, the impact of multiple stressors on marine systems will depend not only on species-level responses, but additionally on species interactions, species diversity and redundancy, trophic complexity and ecological history (Vinebrooke et al. 2004). Research on community and population levels should receive more focus in the future (Breitburg et al. 1998). In addition, most studies cannot investigate potential adaptations of organisms towards environmental stress, especially if this adaptation covers multiple generations (Vinebrooke et al. 2004). For instance, reef fish responded very sensitive to temperature increases, however after several generations adaptation had occurred (Donelson and Munday 2011).

Understanding variation in stressor interactions could aid management strategies such as ocean zoning (Crowder et al. 2006) and change expected outcomes of conservation and management efforts. In case of additive effects between stressors, reducing the magnitude of any stressor should lead to a reduction of the overall stress. Antagonistic stressors may create management challenges, since all or most stressors would need to be eliminated to improve the condition of species or communities, except in cases where the antagonism is driven by a dominant stressor (e.g. Folt et al. 1999). In contrast, synergisms may respond quite favorably to the removal of a single stressor as long as the system has not passed a threshold into an alternative state (see review Crain et al. 2008).

In the light of increasing anthropogenic pressure on coral reefs, it is crucial to find indicators that are able to rapidly assess the condition of an organism and a population (Sokolova et al. 2012). Further studies looking at trade-offs of energy allocated to detoxification processes, as well as at the underlying detoxification mechanisms, using molecular indicators, represent complex topics for future research (Logan 2007). Most marine stressor pairs have not been experimentally studied in controlled factorial experiments, leaving large gaps in our understanding of the interactive effects of multiple stressors in marine ecosystems (Crain et al. 2008, Ban et al. 2014 b). This thesis focused on this gap in knowledge and answered the response to combinations of chemical pollutants and higher temperature.

Increased stress such as metabolic impairment on the organism level, as seen in the short-term exposure experiments in **chapter 3 and 4**, may shift responses to populations and ecosystem levels and reduce reef resilience and the potential for recovery (Hoegh-Guldberg et al. 2007). Trade-offs in terms of reduced survival, fitness and growth of individuals are very likely (Calow 1991, Calow and Forbes 1998, Van Straalen and Hoffmann 2000). Lower reproductive output is one of the severe consequences of chronic stress (Knowlton 2001, Baird and Marshall 2002, Logan 2007, Dixson et al. 2014). In addition, negative feedbacks to human livelihoods and food security may be the consequence (Cinner 2011), as seen in **chapter 5.**

Ecological consequences however also depend on the exposure type, including factors such as intensity, timing, frequency and duration (see review van Dam et al. 2011). For instance, regularly recurring pollution events from land runoff (e.g. sewage treatment effluent or bilge water discharge in areas with high boat traffic, see **chapter 3**) cause chronic pollution and although they may have lower pollutant concentrations than accidental pollution events such as oil spills, are more likely to affect larger areas and exert more subtle effects (incl. genetic adaptation). Such chronic pollution may interfere with the resilience of lower trophic levels, because the stress conditions persist longer, thereby affecting species fitness in the long run. This may in turn cause increased vulnerability to other stressors such as global warming (Pörtner et al. 2014). In contrast, accidental pollution events may affect all trophic levels but are often localized and may not harm the overall structure and function of large ecosystems (see review van Dam et al. 2011). Overall, not only the amount of stressors a system is exposed to simultaneously, but also the magnitude (i.e. concentrations of stressors) and frequency of exposure (e.g. number of times of bilge water release or sewage run-off) of each individual stressor will determine the stress condition of the system and lower its resilience. In the long run, the threshold for tipping points to alternate states that are often far less

economically and ecologically valuable, are lowered and recovery is hampered (Hoegh-Guldberg et al. 2007).

Since a high spatial variability in reef condition on a regional scale was observed for the reefs of the Thousand Islands due to more localized rather than regional stressors, this has to be considered in future conservation and management plans. Marine spatial planning that is adjusted to local conditions and takes into consideration the different spatial scales on which stressors and resource uses interact with reef communities (Sale et al. 2014) is an alternative to current management strategies (i.e. marine protected- and no-take areas (MPA's and NTA's; see Hoegh-Guldberg et al. 2007, Douvere 2008, Wilson et al. 2010). In addition, monitoring key biological and environmental parameters continuously over several years and across seasons is crucial for the establishment of successful management and conservation plans. Besides these reef management strategies, the involvement of local communities into reef protection is needed (Inglehart 1995, Ferse et al. 2010). Marine awareness and local education campaigns could aid the enforcement of protection areas and, for instance, help to change the washing habits of local fishermen and reduce WAF-D and LAS pollution in the region. Any conservation and management plan, however, will only be successful if pollution in Jakarta is reduced, e.g. by implementing sewage treatment and waste disposal plans (Clara et al. 2007, Rebello et al. 2014). In Indonesia, as in most adjacent countries in South-East Asia, treatment of sewage is still largely missing (Burke et al. 2012). Reducing environmental exposure by anthropogenic stressors, i.e. the initial cause of vulnerability of costal communities, will improve livelihoods of people (Ferrol-Schulte et al. 2015). Nevertheless, without a better understanding of impacts of combined stressors on marine organisms and underlying mechanisms (Knowlton and Jackson 2008), these mitigation efforts and management strategies such as marine planning and conservation are however void in the end.

Indonesia´s continuing growth in population, especially along the coast, poses severe problems for ecosystems such as coral reefs. Reef degradation due to global and local stressors will eventually cause a loss of ecosystem services that sustain millions of people (Hoegh-Guldberg et al. 2007, Burke et al. 2012). Any relief from stressors would help coral reefs and give them the chance to recover and provide livelihoods also for future generations. However, ocean management can no longer focus on individual stressors (Halpern et al. 2007), but must incorporate combined stressor effects. Combined effects of multiple stressors are still barely understood (Ban et al. 2014 a) and studies such as this thesis are needed to determine responses of organisms and ecosystems to these stressors. While

these are considerable challenges, complacency is not an option. Tackling these massive problems will require all stakeholders to work together, a pro-active government, and a reduction in corruption (Dutton 2005, Bengen et al. 2006). When considering the importance of coral reefs for the livelihoods of millions of people in developing countries, the need for more effective coral reef management is obvious.

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Abstract of the additional manuscript

Between ignorance and concern - interdisciplinary approaches to raising awareness on marine environments

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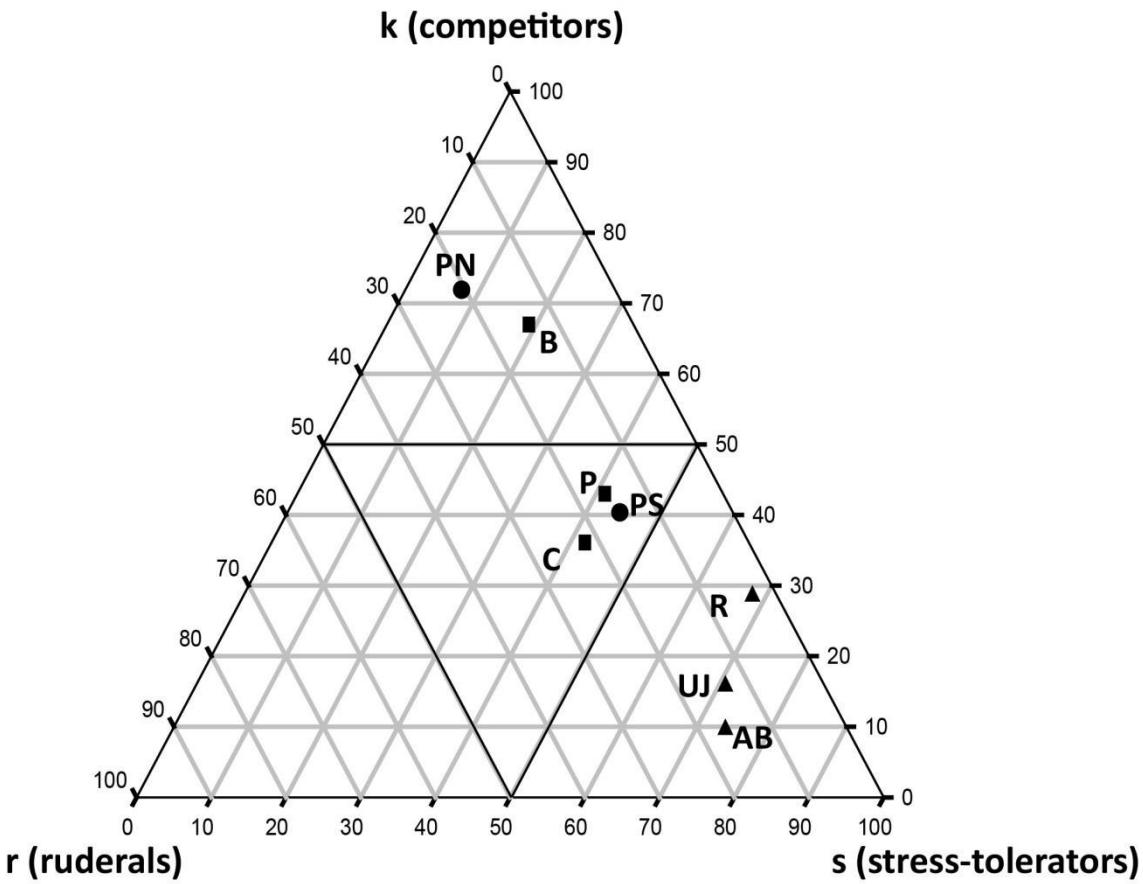
Abstract

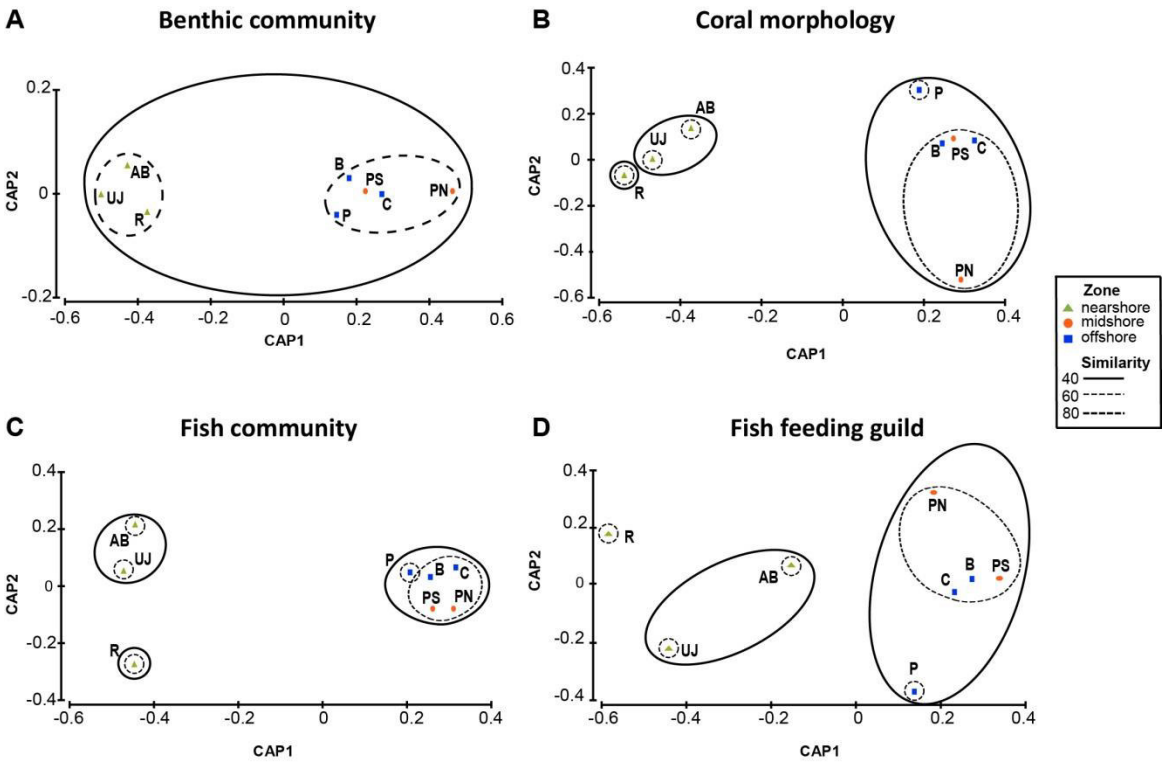
Human domination of Earth's ecosystem results in major environmental changes in marine, freshwater and terrestrial systems alike. Using the Wadden Sea as a regional example, the topics addressed in scientific articles have changed during the last decades, with environmental issues being a major part of these. However, the literacy on the status of marine environments lags behind those of other systems, both in the public awareness and in the scientific community. Here, we present exemplary analyses which might help to understand why this lack of ocean literacy exists and suggest future endeavors of how awareness can be raised. First, we show that images visualizing marine environmental change in arts and science are lacking. Artists have transported a rather ambiguous image of the Sea between rather hostile environment for humans and an idyllic place rather untouched by humans. Within the scientific literature, terrestrial ecosystems are more often illustrated by artwork visualizing the system than are articles on marine ecosystems. Second, we show that spatial planning, which becomes more important in the Sea, deals mainly with land-derived concepts. This might hinder implementation of environmental knowledge into ecosystem management. Third, we consider future actions that can enhance ocean literacy and lead to a more realistic acknowledgements of the status of and trends in marine ecosystems. Here, we focus on involving people in gaining scientific data (Citizen Science) and engaging people through effective communication science.

Appendices

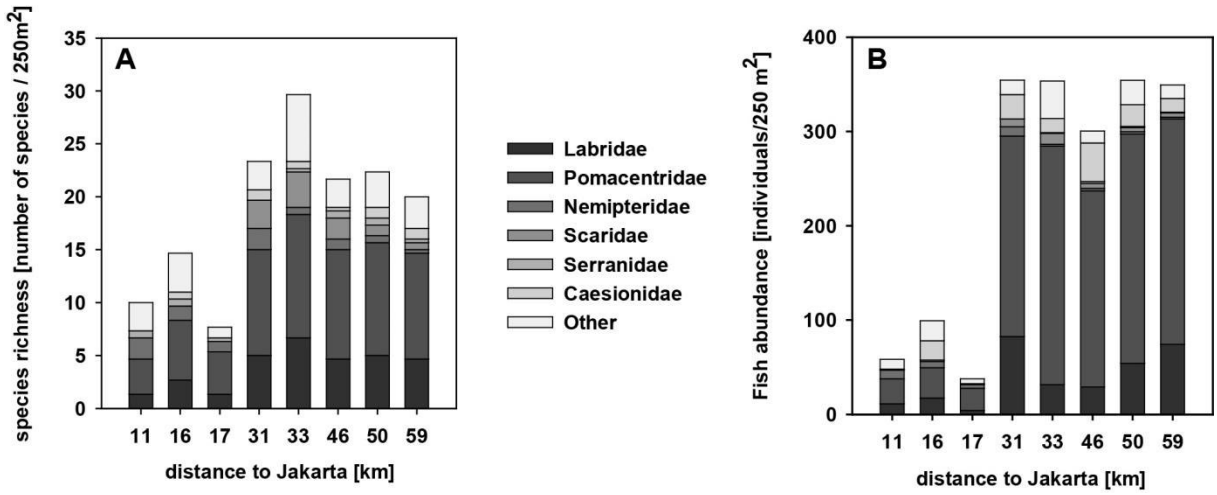
Supplementary information Chapter 1



S1.1 Fig: r-K-S ternary plot for benthic community composition along the Thousand Islands. R: disturbance-adapted ruderal corals (acroporid corals); K: competition-adapted corals (branching non-acroporid corals and foliose corals); S: stress-tolerating corals (massive and submassive corals).



S1.2 Fig: Visualization of composition groups based on a priori defined groups, i.e. zones. Canonical analysis of principal coordinates (CAP) was used for (A) benthic community, (B) coral morphology, (C) fish community and (C) fish feeding guild composition.



S1.3 Fig: Total fish abundance (A) and species richness (B) for sites in Jakarta Bay (JB) and Thousand Islands. Mean values given for the families Labridae (excluding Scarinae), Pomacentridae, Nemipteridae, labrid Scarinae, Serrranidae and Caesionidae, and Others.

S1.1 Table: Fish survey 2012. Average abundance of observed fish species per zone and assigned feeding guild for each species after FishBase (Froese and Pauly 2000): OV = omnivore, OCV = obligate corallivore, HV = herbivore, PV = planktivore, CV = carnivore, OVI = omnivore/invertivore.

No.	Family	Species	Feeding guild	Abundance per zone			Total abundance
				1	2	3	
1	Acanthuridae	*Acanthurus tristis*	PV		2		2
2		*Ctenochaetus striatus*	OV		2		2
3	Apogonidae	*Ostorhinchus compressus*	OV	9	38	23	70
4		*Cheilodipterus intermedius*	OV			12	12
5		*Cheilodipterus quinquelineatus*	OVI	6			6
6		*Fibramia thermalis*	OV	4			4
7	Aulostomidae	*Aulostomus chinensis*	CV		1	1	2
8	Caesionidae	*Caesio cuning*	PV	31	24	34	89
9	Centriscidae	*Aeoliscus strigatus*	PV	24			24
10	Chaetodontidae	*Chaetodon octofasciatus*	OCV	3	5	4	12
11		*Chelmon rostratus*	OCV	1		2	3
12		*Heniochus pleurotaenia*	OCV		3	2	5
13	Diodontidae	*Diodon hystrix*	OVI		1		1
14	Gramistidae	*Diploprion bifasciatum*	CV	3			3
15	Haemulidae	*Plectorhinchus lineatus*		1			1
16	Holocentridae	*Sargocentron rubrum*	CV	5			5
17	Labridae	*Bodianus mesothorax*			3		3
18		*Cheilinus fasciatus*	OVI		4	5	9
19		*Cheilinus undulatus*	CV			2	2
20		*Choerodon anchorago*	OVI	5	3	3	11
21		*Cirrhilabrus cyanopleura*	PV	20	96	57	173
22		*Halichoeres binotopsis*	CV	4			4
23		*Halichoeres chloropterus*	CV	3	5	4	12
24		*Halichoeres melanurus*	CV	3	5	3	11
25		*Halichoeres hortulanus*	OVI		5	2	7
26		*Halichoeres melanurus*	OV	2			2
27		*Hemigymnus fasciatus*	OVI			3	3
28		*Hemigymnus melapterus*	OVI		4	3	7
30		*Labroides bicolor*	OVI		1		1
31		*Labroides dimidiatus*	OVI	2	3	3	8
32		*Stethojulis interrupta*				1	1
33		*Stethojulis trilineata*	OVI	3	2		5
34		*Thalassoma lunare*	OVI		10	6	16
35	Lethrinidae	*Lethrinus harak*	CV	2			2
36	Lutjanidae	*Lutjanus carponotatus*	CV			2	2
37		*Lutjanus decussatus*	CV	1	3		4
38	Mullidae	*Upeneus tragula*		2			2

S1.1 Table: continued

No.	Family	Species	Feeding guild	Abundance per zone			Total abundance
				1	2	3	
39	Nemipteridae	*Pentapodus setosus*	OVI	6			6
40		*Scolopsis aurata*	OVI			3	3
41		*Scolopsis bilineata*	CV		6	5	11
42		*Scolopsis ciliata*	CV	6			6
43		*Scolopsis margaritifera*	CV		4	3	7
44		*Scolopsis monogramma*	OVI	5	4		9
45		*Scolopsis vosmeri*	OV	2	3		5
46	Ostraciidae	*Ostracion cubicus*	OV		1	1	2
47	Phemperidae	*Pempheris vanicolensis*	PV	8	22		30
48	Pomacanthidae	*Chaetodontoplus mesoleucus*	OV		3	2	5
49		*Pomacanthus sexstriatus*	HV		2	2	4
50	Pomacentridae	*Abudefduf bengalensis*	OV	4	7		11
51		*Abudefduf sexfasciatus*	OV	3	32	12	47
52		*Abudefduf vaigiensis*	OV		22	11	33
53		*Amblyglyphidodon curacao*	OV	3	33	37	73
54		*Amblyglyphidodon leucogaster*	OV		18	9	27
55		*Amphiprion ocellaris*	OV	4			4
56		*Chromis viridis*	OV		47	30	77
57		*Chrysiptera hemicyanea*	OV	10			10
58		*Dischistodus prosopotaenia*	HV		5	5	10
59		*Neoglyphidodon crossi*	OV		10		10
60		*Neoglyphidodon melas*	OV		7	7	14
61		*Neoglyphidodon nigroris*	OV		7	11	18
62		*Neopomacentrus anabatoides*	PV	12	21		33
63		*Neopomacentrus azysron*	OV		8	21	29
64		*Neopomacentrus cyanomos*	CV	5	20		25
65		*Pomacentrus alexanderae*	OV	5	33.5	48	86.5
66		*Pomacentrus amboinensis*	HV	7	12	9	28
67		*Pomacentrus armillatus*	HV	8	12	8	28
68		*Pomacentrus bankanensis*	OV		8	33	41
69		*Pomacentrus burroughi*	HV			6	6
70		*Pomacentrus chrysurus*	HV	5			5
71		*Pomacentrus cuneatus*	OV	8			8
72		*Pomacentrus javanicus*	OV	11			11
73		*Pomacentrus lepidogenys*	PV			70	70
74		*Pomacentrus littoralis*	OV	7			7
75		*Pomacentrus moluccensis*	HV		30	19	49
76		*Pomacentrus nigromarginatus*	PV		8		8
77		*Pomacentrus philippinus*	OV			18	18
78		*Pomacentrus saksonoi*				8	8
79		*Pomacentrus smithi*	OV		38	57	95
80		*Premnas biaculeatus*	OV	3		2	5

S1.1 Table: continued

No.	Family	Species	Feeding guild	Abundance per zone			Total abundance
				1	2	3	
81	Scaridae	*Cetoscarus bicolor*	HV		2		2
82		*Chlorurus bleekeri*	HV		4		4
83		*Chlorurus bowersi*	OV			2	2
84		*Chlorurus sordidus*	OV		4	6	10
85		*Scarus ghobban*	HV		2	2	4
86		*Scarus niger*	HV		4	4	8
87		*Scarus quoyi*	HV		1		1
88	Serranidae	*Cephalopholis boenak*	CV	2	2	3	7
89		*Cephalopholis microprion*	CV			3	3
90		*Epinephelus fasciatus*	CV			1	1
91	Siganidae	*Siganus virgatus*	HV		3		3
92		*Siganus rivulatus*	HV		3	4	7

S1.2 Table: Multivariate analysis of composition groups based on zonation. The Permanova test and subsequent pairwise testing of zones (nearshore: zone 1; midshore: zone 2; offshore: zone3) was used.

Group	Composition	Test pairs: zones	p-value
Fish	community	global	0.002
		1 vs. 2	0.101
		1 vs. 3	0.079
		2 v.s 3	0.105
	Feeding guild	global	0.03
		1 vs. 2	0.089
		1 vs. 3	0.116
		2 v.s 3	0.477
Benthic	community	global	0.017
		1 vs. 2	0.104
		1 vs. 3	0.091
		2 v.s 3	0.704
	Coral morphology	global	0.008
		1 vs. 2	0.084
		1 vs. 3	0.11
		2 v.s 3	0.194
Water		global	0.008
		1 vs. 2	0.092
		1 vs. 3	0.105
		2 v.s 3	0.101

S1.3 Table: Coral morphology categories for Indonesian coral reefs used to classify rks-groups (Edinger and Risk 2000).

Morphology	**Description**	**rKS group**
Acropora, branching	Staghorn corals, long thin branches	r
Acropora, bottlebrush	Mainly *A. echinata* group	r
Acropora, corymbose	Stout branches, low bushy shape	r
Acropora, digitate	Digitate, stubby, mainly *A. humilis* group	r
Acropora, tabular	Tables, mainly *A. hyacinthus* group	r
Acropora, submassive	Columns + blades, very stout, mainly *A. palifera and A. cuneata*	r
Branching coral	Branching non-Acropora corals; especially *Porites cylindrica*, some other spp.	K
Encrusting coral	Low relief, often small colonies	K
Massive-platy coralLobophyllia spp. S	Plate-like corals forming large massive colonies, especially Euphyllia,	S
Massive coral	Massive or dome-like corals of all sizes.	S
Foliose coral	Foliose, either horizontal or vertical, non-Acropora, especially Montipora, Echinopora	K
Tabular coral (non-Acropora)	Tabular non-Acropora, esp. Montipora	r
Submassive coral columnar,	Multilobate or ``lumpy'' corals, sometimes columnar or mixed massive-columnar, especially Goniopora, Galaxea	S
Mushroom coral	Free-living fungiid corals	K
Millepora	Various species of Millepora. (hydrocoral)	r
Heliopora	Blue coral (a hydrocoral)	r

Supplementary information Chapter 2

Table S2.1: Comparison of electron transport system (ETS) activity, photosynthetic yield (F_v/F_m) and benthic cover between sites (One-Way Anova and post hoc Student Newman-Kueuls Method) Replicate number varied between the two species for the ETS-activity: n = 5 for *Nephthea* sp. (except for the sites UJ, R: n = 4 and PN, B: n = 3) and n = 4 for *Sarcophyton sp.* (except for the sites PN,C, B: n = 3). For photosynthetic yield n = 7 per fragment was used (except for the sites R (*Sarcophyton sp.* and *Nephthea* sp.) with n = 6 and UJ (*Sarcophyton sp.*) with n = 4)

Factor	species	Test	DF	SS	MS	F	P	Post-hoc (Student-Newman-Keuls Method)
ETS	*Nephthea sp.*	One-Way-Anova	7	1209.8	172.8	3.97	0.005	PN vs. AB, P, UJ
	Sarcophyton sp.	One-Way-Anova	7	1309.4	187.1	3.71	0.009	PN vs. AB, UJ, AB PS vs. AB
Photo-synthetic yield	*Sarcophyton sp.*	One-Way-Anova	7	142577.3	20368.2	10.88	<0.001	R vs. AB B vs. AB,R,UJ PN vs. AB, R,UJ P vs. AB, R,UJ C vs. AB, R,UJ PS vs. AB, R,UJ UJ vs. AB
	Nephthea sp.	Kruskal-Wallis Test					<0.001	R vs. UJ,AB PS vs. UJ,AB P vs. UJ,AB PN vs. UJ,AB,R C vs. UJ,AB
Benthic cover	*Sarcophyton sp.*	Kruskal-Wallis Test					0.004	P vs PN,PS,B,UJ R vs PN, PS,B,UJ
	Nephthea sp.	Kruskal-Wallis Test					0.008	UJ vs C, B,P,PS,PN AB vs C,B,P,PS,PN R vs C,B,P,PS,PN
	Total soft coral	One-Way-Anova	7	1472	210.3	7.16	<0.001	P vs. B,PN,C,PS,UJ,AB R vs. B,PN,C,PS,UJ,AB
	total hard coral	One-Way-Anova					<0.001	
	Macroalage	One-Way-Anova					0.011	-

Supplementary information Chapter 3

Table S3.1: List of samples taken for analysis of EPA PAH (polycyclic aromatic hydrocarbons) concentration under natural exposure conditions in Jakarta Bay (JB) and under short-term exposure conditions (bilge water discharge) and during the experiments (start and end samples). Concentrations for total EPA PAH and for each of separate PAH are given in µg/L. The letters A) and B) refer to samples taken twice. As a procedure calibration, the standard addition method was used by adding an EPA PAH standard with known concentration twice to selected samples. These samples are labeled "1x" and 2x".

Group	Sample	Total PAH [µg/L]	Naphthalene	Acenaphthene	Fluorene	Phenanthrene	Anthracene	Fluoranthene	Pyrene	Benzo(a)anthracene	Chrysene	Benzo(b)fluoranthene	Benzo(k)fluoranthene	Benzo(a)pyrene	Dibenzo(ah)anthracene	Benzo(ghi)perylene	Indeno(1,2,3cd)pyrene
Natural conditions	Pari South	10	0	0	2.1	3.8	0.3	1.5	1.1	0.5	0	0.2	0	0.3	0.2	0.3	0
	JB 1	99	5.1	0	3.9	81	0.9	1.8	4.7	1.8	0	0	0	0	0	0	0
	JB 2 (B)	360	59	5.6	36	250	2.3	0	6.7	0	0	0	0	0	0	0	0
	JB 2 (A)	385	59	4.1	32	280	2.2	0	7.1	0	0	0	0	0	0	0	0
	JB 3	227	96	0	9	115	1.1	1.6	4	0	0	0	0	0.2	0	0.3	0
	JB 4	70	9	0	5.6	48	0.6	1.6	4	1.2	0	0	0	0	0	0	0
Short-term exposure	Bilge water	13775	4364	665	4297	4448	0	0	0	0	0	0	0	0	0	0	0
	Time after bilge water discharge: 10 min	733	268	73	267	0	14	0	57	28	25	0	0	0	0	0	0
	Time after bilge water discharge: 5 min	294	80	1	13	195	0	0	5	0	0	0	0	0	0	0	0
	Blank 1 (JB)	112	85	0	4.2	21	0.3	0.7	1.2	0	0	0	0	0	0	0	0
	Blank 2 (JB)	89	77	0	3.1	8.9	0	0	0	0	0	0	0	0	0	0	0

Table S3.1 continued

Group	Sample	Total PAH [µg/L]	Naphthalene	Acenaphthene	Fluorene	Phenanthrene	Anthracene	Fluoranthene	Pyrene	Benzo(a)anthracene	Chrysene	Benzo(b)fluoranthene	Benzo(k)fluoranthene	Benzo(a)pyrene	Dibenzo(ah)anthracene	Benzo(ghi)perylene	Indeno(1,2,3cd)pyrene
Exp.	WAF-D (start, A)	440	160	21	51	190	1.1	0.9	1.5	4.9	8.1	0.1	0	0.4	0.5	0	0.2
	WAF-D (start, B)	378	150	19	0	197	1.1	0	5.3	2.5	3.7	0	0	0	0	0	0
	WAF-D (end, A)	84	19	5	17	37	0.6	0.3	0.8	1.8	3.2	0	0	0	0	0	0
	WAF-D (end, B)	62	17	3.6	11	24	0.4	0.4	0.5	1.7	3.5	0	0	0	0	0	0
	WAF-D (start, 1x)	1771	445	314	110	284	57	83	86	62	63	60	30	26	61	30	60
	WAF-D (start, 2x)	2900	668	573	163	339	117	135	142	119	121	120	59	49	120	58	117
	WAF-D + LAS (end)	826	368	13	31	412	0	0	1.8	0	0	0	0	0	0	0	0
	WAF-D + LAS (start)	601	278	17	46	244	0.7	4	0.9	2.8	4.2	2.5	0.5	0	0	0	0
	WAF-D + temp (end)	618	227	25	62	265	4.7	14	8.4	1.3	4.7	5.7	1.2	0.1	0	0.3	0
	WAF-D + temp (start)	1093	304	41	112	557	10	23	23	2.9	6.8	8	1.8	1.1	1.1	0.4	1.5
	WAF-D + temp (start, 1x)	1819	597	303	112	231	56	59	58	62	63	65	31	26	63	31	62
	WAF-D + temp (start, 2x)	3051	828	581	177	293	120	135	146	118	121	122	60	51	123	60	118
	Blank 3	17	1	0.9	5.3	7.6	0.3	0.1	0.1	0.5	1.1	0	0	0.1	0	0	0
	Blank 1	13	5.3	0.5	3.3	3.4	0	0	0	0.2	0.4	0	0	0	0	0	0
	Blank 2	16	6.6	0.7	4.3	3.2	0	0	0	0.3	0.5	0	0	0	0	0	0
	WAF-D (stock solution)	10653	2014	3226	973	3754	0	92	201	171	221	0	0	0	0	0	0
	Diesel	149786	47081	269	25802	76166	0	0	0	467	0	0	0	0	0	0	0

Table 3.2: Metabolic stress responses of *Siganus guttatus* during short-term (16 h) exposure to the treatments control, WAF-D (water accommodated fraction of diesel), LAS (linear alkyl benzene sulfonate), temperature, temp +WAF-D, temp + LAS. Metabolic rates are given as means ± SD in mg O_2 kg^{-1} h^{-1} (n = 3): standard metabolic rates (SMR), routine metabolic rates (RMR), maximum metabolic rates (MMR) and aerobic metabolic scope rates (AMS). p-values are given for differences between day and night measurements of SMR and RMR (one-way ANOVA).

	Metabolic rate	**Control**	**WAF-D**	**LAS**	**WAF-D + LAS**	**Temp**	**Temp + WAF-D**	**Temp + LAS**
SMR [mg/h/g]	Total	163.5 ± 5.3	132.0 ± 5.3	208.6 ± 6.8	129.1 ± 4.0	201.8 ± 4.4	204.2 ± 10.1	227.8 ± 13.7
	Day	162.5 ± 3.8	124.7 ± 2.7	244.3 ± 14.2	128.9 ± 3.2	246.9 ± 46.8	207.9 ± 5.3	226.0 ± 17.9
	Night	181.6 ± 31.8	143.4 ± 1.5	218.6 ± 13.8	138.9 ± 20.4	229.4 ± 36.5	217.5 ± 21.1	246.6 ± 14.8
	Day/Night comparison (p-value)	0.651	0.163	0.477	0.384	0.057	0.544	0.191
RMR [mg/h/g]	Total	192.3 ± 14.0	155.7 ± 10.4	246.9 ± 8.5	144.3 ± 7.7	282.0 ± 38.0	231.5 ± 9.1	277.3 ± 5.6
	Day	181.4 ± 10.2	147.7 ± 113.5	274.6 ± 13.6	138.9 ± 4.9	300.0 ± 47.6	231.6 ± 4.8	259.2 ± 7.8
	Night	190.0 ± 27.6	160.5 ± 7.6	246.0 ± 17.3	152.6 ± 19.7	285.0 ± 75.2	235.4 ± 24.3	266.4 ± 20.0
	Day/Night comparison (p-value)	0.258	0.089	0.164	0.4	0.069	0.497	0.257
	AMS [mg/h/g]	171.8 ± 113.6	210.6 ± 33.3	288.8 ± 48.8	192.2 ± 21.6	386.6 ± 53.4	244.5 ± 38.4	328.1 ± 12.7
	MMR [mg/h/g]	334.3 ± 117.5	335.4 ± 30.7	533.2 ± 46.7	321.0 ± 24.4	633.5 ± 95.3	452.4 ± 37.2	554.1 ± 27.6

Table S3.3: Two-Way Analysis of variance for standard metabolic rates (SMR), routine metabolic rates (RMR), maximum metabolic rates (MMR) and aerobic metabolic scope (AMS) to test for significant effects of the stressors LAS, WAF-D and temperature as well as for interactions between the stressors. Post-hoc test was done with Tukey (95 % confidence interval).

Factor	Source of Variation	DF	SS	MS	F	P
	LAS	1	1335	1335	29.9	<0.001
	WAF-D	1	9236	9236	207	<0.001
	LAS x WAF-D	1	1727	1727	38.7	<0.001
	Temp	1	2478	2478	23.6	0.001
SMR	LAS	1	3797	3796	36.2	<0.001
	Temp x LAS	1	271	271	2.6	0.146
	Temp	1	9140	9149	137	<0.001
	WAF-D	1	635	635	9.5	0.015
	Temp x WAF-D	1	861	861	12.9	0.007
	LAS	1	25585	25585	10.8	0.011
	WAF-D	1	101120	101120	42.7	<0.001
	LAS x WAF-D	1	19665	19665	8.3	0.02
	Temp	1	8727	8727	3.1	0.119
RMR	LAS	1	23768	23768	8.3	0.02
	Temp x LAS	1	21320	21320	7.5	0.026
	Temp	1	2921	2921	1	0.344
	WAF-D	1	74439	74439	25.7	<0.001
	Temp x PAH	1	34369	34369	11.9	0.009
	LAS	1	25550	25550	3.9	0.084
	PAH	1	33412	33412	5.1	0.054
	LAS x WAF-D	1	34085	34085	5.2	0.052
	Temp	1	76868	76868	7.9	0.023
MMR	LAS	1	10700	10700	1.1	0.324
	Temp x LAS	1	58093	58093	6	0.04
	Temp	1	129933	129933	13.7	0.006
	WAF-D	1	24330	24330	2.6	0.147
	Temp x WAF-D	1	24905	24905	2.6	0.143
	LAS	1	7294	7294	1.2	0.314
	WAF-D	1	2510	2510	0.4	0.546
	LAS x WAF-D	1	13763	13763	2.2	0.178
	Temp	1	48425	48425	7.1	0.029
AMS	LAS	1	2568	2568	0.4	0.558
	Temp x LAS	1	23116	23116	3.4	0.104
	Temp	1	46384	46384	6.7	0.032
	WAF-D	1	8012	8012	1.2	0.312
	Temp x WAF-D	1	24563	24563	3.6	0.096

Supplementary information Chapter 4

Table S4.1: Results from post-hoc tests for diesel and temperature treatments. Tukey HSD tests performed for the control, high temperature and diesel treatments. Temperature was either "norm" (28 °C) or "high" (31 °C) and pollutant was either "none" or "diesel" (490 mL of 0.5 % WAF). Asterisks indicate significant effects (p<0.05).

		diff	lwr	upr	p adj
PAM Beginning of measurement period					
$Temperature	high-norm	0.0275	7.49E-03	0.047514	0.0112 *
$Pollutant	none-diesel	0	-2.00E-02	0.020014	1
$`Temperature:Pollutant`	norm:diesel-norm:none	0.0025	-3.61E-02	0.041067	0.9973
	high:diesel-norm:none	0.0275	-1.11E-02	0.066067	0.2026
	high:none-norm:none	0.03	-8.57E-03	0.068567	0.1503
	high:diesel-norm:diesel	0.025	-1.36E-02	0.063567	0.2689
	high:none-norm:diesel	0.0275	-1.11E-02	0.066067	0.2026
	high:none-high:diesel	0.0025	-3.61E-02	0.041067	0.9973
PAM End of measurement period					
$Temperature	high-norm	0.0025	-0.015702	0.020702	0.7699
$Pollutant	none-diesel	0.005	-1.32E-02	0.023202	0.5606
$`Temperature:Pollutant`	norm:none-high:diesel	0.0025	-3.26E-02	0.037576	0.9965
	norm:diesel-high:diesel	0.0025	-3.26E-02	0.037576	0.9965
	high:none-high:diesel	0.01	-2.51E-02	0.045076	0.8315
	norm:diesel-norm:none	0	-3.51E-02	0.035076	1
	high:none-norm:none	0.0075	-2.76E-02	0.042576	0.9188
	high:none-norm:diesel	0.0075	-2.76E-02	0.042576	0.9188
Light respiration					
$Temperature	norm-high	0.0015	-1.61E-03	0.004597	0.3149
$Pollutant	diesel-none	0.0016	-1.51E-03	0.004697	0.285
$`Temperature:Pollutant`	norm:diesel-high:none	0.0031	-2.89E-03	0.009067	0.4494
	norm:none-high:none	0.0054	-5.59E-04	0.011399	0.0803
	high:diesel-high:none	0.0055	-4.59E-04	0.011499	0.0739
	norm:none-norm:diesel	0.0023	-3.65E-03	0.008312	0.6627
	high:diesel-norm:diesel	0.0024	-3.55E-03	0.008412	0.6338
	high:diesel-norm:none	0.0001	-5.88E-03	0.006079	1
Dark respiration					
$Temperature	high-norm	0	-4.19E-03	0.004281	0.9824
$Pollutant	diesel-none	0.0033	-8.99E-04	0.007576	0.1117
$`Temperature:Pollutant`	norm:diesel-high:none	0.0033	-4.87E-03	0.011461	0.6395
	norm:none-high:none	0.0072	-9.49E-04	0.015384	0.0899
	high:diesel-high:none	0.0106	2.43E-03	0.018766	0.0107 *
	norm:none-norm:diesel	0.0039	-4.24E-03	0.012089	0.5079
	high:diesel-norm:diesel	0.0073	-8.61E-04	0.015471	0.0853
	high:diesel-norm:none	0.0034	-4.78E-03	0.011549	0.621

Erklärung

Gemäß § 6 der Promotionsordnung der Universität Bremen für die mathematischen, natur- und ingenieurwissenschaftlichen Fachbereiche vom 14.3.2007 versichere ich, dass die vorliegende Arbeit mit dem Titel

"Understanding coral reefs in an impacted world - Physiological responses of coral reef organisms to coastal pollution and global warming"

ohne unerlaubte fremde Hilfe selbstständig verfasst und geschrieben wurde.

keine anderen als die angegebenen Quellen und Hilfsmittel genutzt wurden.

die den benutzen Werken wörtlich oder inhaltlich entnommenen Stellen als solche kenntlich gemacht wurden.

es sich bei den von mir abgegebenen Arbeiten um drei identische Exemplare handelt.

Bremen, 21. September 2015

Gunilla Baum