# The relevance of food availability for the tolerance to environmental stress in Asian green mussels, *Perna viridis*, from coastal habitats in Indonesia

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**Summary**

Coastal ecosystems worldwide are experiencing increasing anthropogenic pressure, mainly caused by growing human populations in near-shore urban areas and by the rising number of megacities. One of the consequences of this process is the eutrophication of marine habitats that lie in the vicinity of rivers carrying high loads of nutrients that come from agriculture and human sewage. The capital of the Republic of Indonesia, Jakarta, is an example of a megacity impacting the adjacent marine ecosystems: In Jakarta Bay excessive loads of nutrients cause frequent phytoplankton blooms and the resulting microbial activity causes hypoxia events. One of the few species that copes well with these conditions is the Asian green mussel *Perna viridis*. It forms dense aggregations on bamboo settlement stakes in the bay located within the native distributional range of the mussel that is also a well-known invader of coastal habitats. Non-native populations of this species exist in southern Japan, at some Pacific islands and in the West Atlantic. In Indonesia, *P. viridis* is native to the western parts of the archipelago but non-native to the eastern parts and was found in the non-native range as fouling on ships that cross the Indonesian archipelago from west to east. One of the reasons for its invasion success is the ability of *P. viridis* to tolerate large fluctuations in abiotic environmental conditions. Therefore, understanding the factors influencing the mussel's tolerance to environmental stress, should help to understand their invasion success. To address this question, I conducted three studies in which I exposed mussels to hypoxia in the laboratory under different scenarios. In the first study, I compared the hypoxia tolerance and nutritional status of mussels collected from a ship hull in the non-native range to those of mussels from Jakarta Bay in the native range. I found that the mussels collected from the ship hull were in a very poor nutritional status and tolerated hypoxia in the laboratory only half as long as mussels from the eutrophic Jakarta Bay. The finding suggests that transport on a ship hull may reduce the invasion potential of the species if the journey leads through areas of low food supply. The other two studies that comprise this thesis aim at assessing the potential roles of local adaptations (i.e. an irreversible modification that is manifested in the gene pool of a population), acclimation to stress (i.e. a reversible modification that is not genetically manifested) and a good nutritional status (caused by ample planktonic food supply in a eutrophic habitat) in determining the degree of tolerance to environmental stress in mussels. The idea of investigating this closer had arisen from a previous study, which found that individuals from Jakarta Bay are more tolerant to environmental stress (i.e. salinity, thermal and oxygen stress) than conspecifics from a more natural habitat in Indonesia. However, it remained unknown which mechanisms led to this difference. I approached this question by

conducting a reciprocal transplantation experiment and subsequent hypoxia tests in the laboratory with *P. viridis* from the eutrophic Jakarta Bay and an oligotrophic habitat in West Java. The experiment showed that tolerance to hypoxia was rather determined by the conditions in the habitat where the mussels had lived for two months after transplantation before exposure to stress and not by the characteristics of the habitat where they originated from. This suggests that local adaptations to stress did not occur in Jakarta Bay mussels - although they have a long history of experiencing adverse conditions – or that they have been overwritten by other determinants of tolerance to hypoxia. The main determinant of stress tolerance again was the nutritional status. In the third study of this thesis, I conducted experiments that allowed establishing a causal relationship between a high nutritional status and hypoxia tolerance. Jakarta Bay mussels that had obtained more food supply in the laboratory had a better hypoxia tolerance than Jakarta Bay mussels that had obtained less food and were in a poor nutritional status. Furthermore, acclimation to low, non-lethal concentrations of dissolved oxygen enhanced hypoxia tolerance in mussels with low nutritional states. Taken together, these results show that a good nutritional status is the most relevant determinant of tolerance to environmental stress in *P. viridis*, which implies that the mussel can benefit from eutrophication caused by anthropogenic impact. *Perna viridis* may, therefore, be a species that can extend its distributional range if anthropogenic pressure in urban, near-shore areas is increasing and contributing to eutrophication. However, it may not succeed and establish in more non-native areas if conservation efforts apply that keep tropical and subtropical coastal ecosystems in an oligotrophic state and maintain high levels of biodiversity.

## Zusammenfassung

Die Zunahme der Weltbevölkerung in städtischen Gebieten und die wachsende Anzahl an Metropolen üben einen immer stärker werdenden Druck auf küstennahe Ökosysteme aus. Diese leiden vor allem unter Eutrophierung durch landwirtschaftliche und menschliche Abwässer, welche über Flüsse in nahe an Mündungsgebieten gelegene marine Habitate gelangen. Jakarta, die Hauptstadt der Republik Indonesien, ist ein gutes Beispiel einer solchen Metropole, die die nahe gelegenen marinen Ökosysteme erheblich beeinflusst: In der Bucht von Jakarta treten häufig eutrophierungs-bedingte Planktonblüten auf, die aufgrund von erhöhter mikrobieller Aktivität das Auftreten von Hypoxie-Ereignissen verursachen. Eine der wenigen Tierarten, die gut unter diesen Bedingungen leben kann, ist die Grünlippmuschel, *Perna viridis*, die dichte Aufwuchsgemeinschaften auf Besiedelungsstrukturen in der Bucht bildet. Die Bucht von Jakarta liegt im natürlichen Verbreitungsgebiet der Muschelart, die in anderen Küstenhabitaten der Welt als invasive Art bekannt ist. Nicht-indigene Populationen der Art sind aus dem südlichen Japan, von einigen pazifischen Inseln und aus dem westlichen Atlantik bekannt. In Indonesien gehört der westliche Teil des Archipels zum indigenen-, während der östliche Teil zum nicht-indigenen Verbreitungsgebiet von *P. viridis* zählt. Dort wurden Muscheln als Aufwuchs auf Schiffen entdeckt, die den indonesischen Archipel von West nach Ost durchkreuzen. Als einer der Gründe für den Invasionserfolg der Art wird deren gute Anpassungsfähigkeit an fluktuierende Umweltbedingungen diskutiert. Daher ist es wichtig zu verstehen, welche Faktoren die Toleranz der Muscheln gegenüber Umweltstress bedingen und wie dies den Invasionserfolg beeinflusst. Um dies zu beleuchten, führte ich drei Studien durch, in denen ich die Toleranz von *P. viridis* gegenüber Hypoxie im Labor unter verschiedenen Scenarios untersuchte. In der ersten Studie verglich ich die Hypoxie- Toleranz und den Ernährungszustand von Muscheln, die von einem Schiffsrumpf im nicht-indigenen Verbreitungsgebiet stammten, mit deren von Muscheln aus der Bucht von Jakarta im indigenen Verbreitungsgebiet. Die Untersuchung zeigte, dass die Muscheln, die vom Schiffsrumpf stammten, sich in einem sehr schlechten Ernährungszustand befanden und außerdem Hypoxie nur halb so lang tolerieren konnten wie Muscheln aus der eutrophen Bucht von Jakarta, die wiederum in einem sehr guten Ernährungszustand waren. Dieses Ergebnis zeigt, dass der Transport als Rumpfaufwuchs das Invasionspotential der Art mindern kann, falls die Schiffsroute durch nährstoffarme Gebiete führt. In den beiden anderen Studien wurden die potentiellen Mechanismen, die zu einer erhöhten Toleranz gegenüber Umweltstress führen können, genauer untersucht. Diese Mechanismen sind a) lokale Adaptation (eine irreversible Modifikation im Genpool der Population), b) Akklimatisierung

an Umweltstress (eine reversible Modifikation, die nicht genetisch verankert ist) und c) ein guter Ernährungszustand, verursacht durch eine ausgeprägte Planktonverfügbarkeit im eutrophen Habitat. Eine vorhergegangenen Studie, in der festgestellt wurde, dass Muscheln aus der Bucht von Jakarta über eine höhere Toleranz gegenüber Salinitäts-, Temperatur- und Sauerstoffstress verfügen als Muscheln aus einem weniger belasteten Habitat, veranlasste mich genauer zu untersuchen, welche Mechanismen für diese Unterschiede verantwortlich sind. Ich untersuchte diese Frage mithilfe eines Transplantations- und anschließendem Hypoxie-Experiment mit *P. viridis* aus der eutrophen Bucht von Jakarta und einem oligotrophen Habitat in Westjava. Das Experiment zeigte, dass die Hypoxie-Toleranz der Muscheln in erster Linie von den Bedingungen im Habitat abhing, in dem die Tiere zuletzt, also zwei Monate lang nach der Transplantation bis direkt vor den Hypoxie-Tests, gelebt hatten. Die Herkunft der Muscheln spielte dabei keine Rolle, was dafür spricht, dass in der *P. viridis*- Population aus der Bucht von Jakarta trotz des häufigen Vorkommens von Hypoxie-Ereignissen keine lokale Adaptation stattgefunden hat, oder deren Auswirkungen von anderen Faktoren überlagert wurden. Erneut spielte hier der Ernährungszustand die größte Rolle. In der dritten Studie dieser Arbeit führte ich ein Experiment durch, in dem ich eine kausale Beziehung zwischen Ernährungszustand und Hypoxie-Toleranz herstellen konnte. Muscheln aus der Bucht von Jakarta, die im Labor mehr Nahrung zur Verfügung gestellt bekamen, hatten eine höhere Hypoxie-Toleranz als Muscheln aus der Bucht von Jakarta, die weniger Nahrung bekamen und in einem schlechten Ernährungszustand waren. Betrachtet man alle Ergebnisse im Zusammenhang, wird deutlich, dass ein guter Ernährungszustand bei *P. viridis* die größte Rolle dabei spielt Umweltstress zu tolerieren. Dies bedeutet, dass die Art von Eutrophierung profitiert, was dafür spricht, dass ihr Verbreitungsgebiet im Falle der Zunahme von anthropogenem Druck auf Küstenhabitate noch vergrößert werden kann. Andererseits kann der Etablierungserfolg der Art in nicht-indigenen Gebieten eingedämmt werden, wenn Naturschutzmaßnahmen in subtropischen und tropischen Küstenökosystemen dazu beitragen, dass eine hohe biologische Vielfalt und ein oligotropher Zustand erhalten bleiben.

## General Introduction

*An example of anthropogenic impacts in tropical coastal ecosystems: Jakarta Bay*

The global human population is growing rapidly, reaching an estimated number of 10 billion people by the year 2050 (Buhaug and Urdal 2013). As 37% of the world's population and 70% of the megacities are located within 100 km of the coastlines, marine coastal ecosystems are experiencing an increasing anthropogenic pressure (Duarte et al. 2008). Anthropogenic impact has caused a progressing loss in coastal ecosystems for the last 5 decades and, if it continues at the same rate, only 15% of the original area will remain until 2050 (Duarte et al. 2008). The ecosystems experiencing the highest loss rates are seagrass beds and coral reefs, which are both found in tropical areas, most of them in the Indo-Pacific (Duarte et al. 2008). Threats for coral reef and seagrass ecosystems are rising sea temperatures and ocean acidification caused by climate change, sedimentation and siltation due to dredging or erosion, domestic and industrial pollution and wastewater runoff causing eutrophication and desalination. All these are either linked to direct human activities or to the increasing demand for natural and fossil resources (Chadwick-Furman 1996; van der Meij et al. 2010).

An example of tropical coastal habitats experiencing all of the above mentioned anthropogenic pressures are the Jakarta Bay ecosystems. Jakarta Bay is a shallow bay (average depth 15 m) that covers an area of 514 km² and is located in the Java Sea (Nur 2001). The Bay lies in the catchment area of the Indonesian capital and megacity Jakarta with its more than 25 million inhabitants (Dsikowitzky et al. 2016). Thirteen rivers discharge into the bay, bringing along domestic sewage, but also pollutants from small-scale industries located upstream in the JABODETABEK area (the name used to describe the agglomeration of the cities Jakarta, Bogor, Depok, Tangerang and Bekasi) (Nur 2001). It is estimated that 11% of Jakarta's sewage does not get treated but goes directly into the rivers and consequently into the bay (Dsikowitzky et al. 2016). This leads to an overload of nutrients (nitrogen and phosphorous compounds) causing eutrophic to hypertrophic conditions in the inner bay area (Damar 2003). The high nutrient availability frequently leads to phytoplankton blooms, enhancing microbial decomposition and causing hypoxia events that have led to several cases of mass mortality in fish (Thoha et al. 2007). In addition to sewage, other industrial and domestic pollutants are discharged into the bay. Past studies found elevated concentrations of heavy metals, e.g. mercury, lead and cadmium (Hutagalung 1989; Williams 2000), organic micro-pollutants, e.g. polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs) and linear alkylbenzenes (LABs) (Rinawati et al. 2012) or

even the harmful component of insect repellents N,N-diethyl-m-toluamide (DEET) (Dsikowitzky et al. 2014) in water and sediment samples from Jakarta Bay. Apart from the input of nutrients and pollutants, the Jakarta Bay ecosystems have also suffered from dredging, near-shore constructions and deforestation further inland. Resulting sedimentation and siltation have caused a large change in community and habitat structure, which caused a loss of 45% of all hard coral species in the area from 1920 to 2005 (van der Meij et al. 2010) and a loss of 55% of mollusc species from 1937 to 2005 (van Der Meij et al. 2009). The reefs of Nyamuk and Onrust Island in the inner Jakarta Bay even lost 84% and 87%, respectively, of all their coral species between 1920 and 2005 (van der Meij et al. 2010). The remaining coral communities in the inner bay have a mean living hard coral cover of < 5% and are composed of only the more robust submassive and encrusting coral growth forms (Baum et al. 2015). Apart from the loss in coral reef area and diversity, most mangrove forests were lost due to a conversion into aquaculture or agriculture areas and due to land reclamation projects. The only remaining mangrove forests are located near the Angke and Bekasi estuaries (Jury et al. 2011).

The examples mentioned above illustrate the extreme environmental changes that occurred in Jakarta Bay because of industrialization and human population growth. To date, the macrozoobenthic communities in the eutrophic and hypertrophic zones of Jakarta Bay have changed into a mainly muddy bottom composition and are now dominated by deposit feeding polychaetes and two suspension feeding bivalves, i.e. *Mactra sp.* and *Chione sp*. (Taurusman 2010; van der Meij et al 2009). In the western part of the inner bay (Fig. 1), the large mud flats serve as a culture area for Asian green mussels, *Perna viridis*, which are abundant on bamboo settlement structures and constitute the livelihood for about 2000 mussel farmers (Jury et al. 2011). These mussels are opportunistic filter feeders that can cope with highly turbid water and adverse environmental conditions (Rajagopal et al. 2006). There is no information available on the abundance of *P. viridis* before culture efforts were increased in the 1970ies by the construction of spat collectors built from bamboo stakes and ropes. It is likely that mussels settled in mangrove roots and later benefitted from the increasing eutrophication of the bay. As increasing eutrophication occurred at about the same time with the intensification of mussel culture efforts, it is difficult to conclude which one of these two factors contributed more to the success of *P. viridis* in Jakarta Bay.

Jakarta Bay is only one example of a megacity causing a drastic change in coastal ecosystem composition, functioning and resilience. Rivers and megacities with a similarly severe impact on coastal environments are the Mississippi that affects the Gulf of Mexico

(USA), Kingston (Jamaica) that has an influence on the Kingston Harbour, Rio de Janeiro (Brazil) that affects the Bay of Guanabara, Chennai (India) on the Bay of Bengal, the Pearl River in Hong Kong (China) on the South China Sea and Bangkok (Thailand) on the upper Gulf of Thailand (Boonyatumanond et al. 2007; Cheung et al. 2003; Perin et al. 1997; Rajendran et al. 2005; Santschi et al. 2001). Unless management strategies for pollution prevention will be extremely improved, growing human populations in these areas will pose an increasing threat to the adjacent coastal ecosystems. This should be of growing concern as the number of urban areas is expected to increase and as existing metropolitan areas will experience further population growth worldwide, but especially in Southeast Asia, Latin America and Sub-Saharan Africa (Cohen 2004).

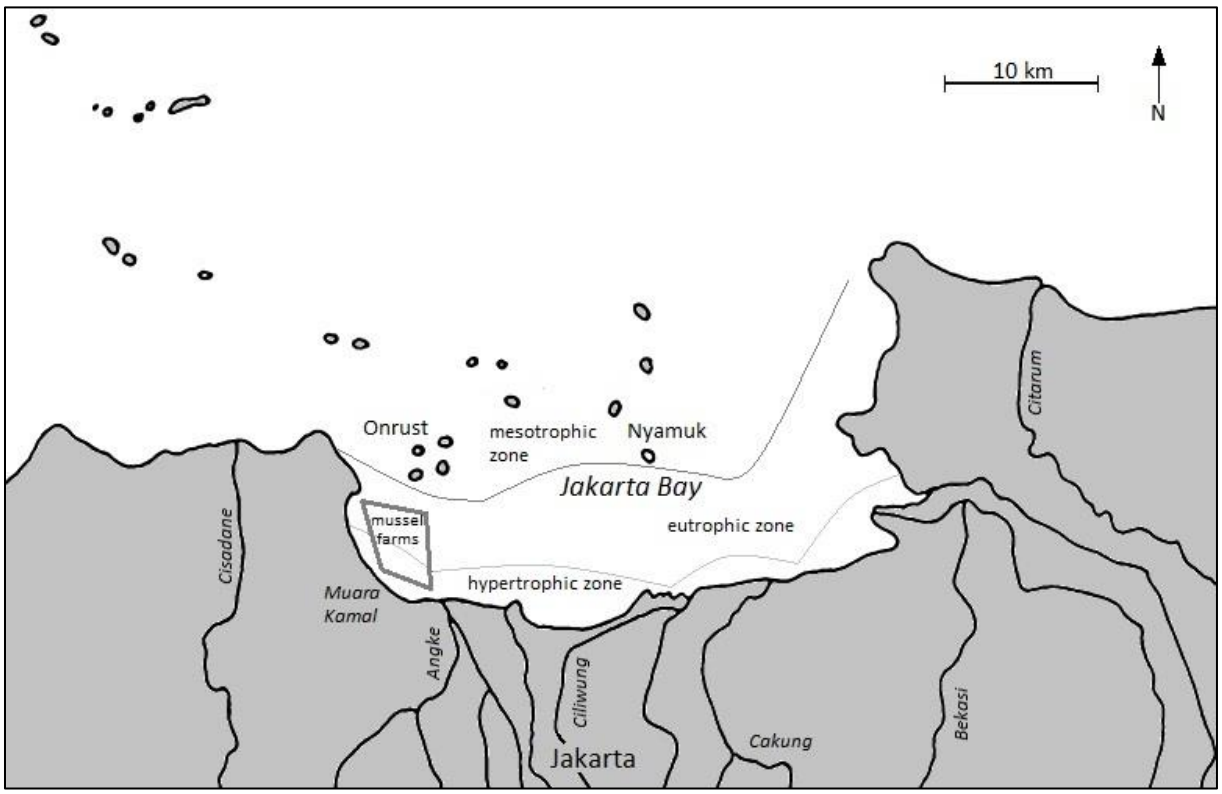


**Figure 1**: Location and trophic zonation of Jakarta Bay and adjacent islands. A total of 13 rivers discharge nutrients and pollutants into the bay. Modified according to Damar 2003.

*Global distribution, invasion history and life history traits of Perna viridis*

The Asian green mussel, *Perna viridis*, is widely distributed in subtropical and tropical coastal habitats worldwide (Rajagopal et al. 2006) and reaches the highest settling densities in environmentally impacted ecosystems, such as in Tolo Harbour, Hong Kong (Cheung 1993). *Perna viridis* native distributional range lies in the Indo-Pacific, where long established populations are known from India, Sri Lanka, Malaysia, Philippines, Thailand, Singapore and western Indonesia (Baker et al. 2007; Siddall 1980). Controversial is the status of the mussels in Hong Kong, China, which was included in the mussels' native range by some authors (Gilg et al. 2013; Huang et al. 1983) but excluded by others (Baker et al. 2007; Siddall 1980). Non-native populations of the mussel are known from southern Japan, the pacific islands Fiji, Samoa, Tonga and Tahiti and from the West Atlantic in Venezuela, Trinidad, Jamaica, Cuba and Florida, USA (Baker et al. 2007; Buddo et al. 2003; Lopeztegui-castillo et al. 2014). In Australian waters, several *P. viridis* incursions have been reported from Queensland and Western Australia but the mussels are not known to have established so far (Dias et al. 2013; McDonald 2012; McDonald 2016; Piola and McDonald 2012). In Indonesia, the suggested native range of *P. viridis* is restricted to the western part of the archipelago with the most eastern native populations in the Makassar Strait, Sulawesi. A more detailed description of the invasion status in the Indonesian archipelago is presented in chapter 1 a (Huhn et al. 2015). In this thesis, the terms "invasion" and "invasive" refer to the establishment and spread of a species in its non-native range. The terms "incursion" and "introduction" are used for the occurrence of a species in its non-native range, which does not result in establishment and spread.

The invasion success of *P. viridis* in southern Japan, named pacific islands and in the West Atlantic goes presumably back to the species' life history traits, which are a) a high reproductive output as broadcast spawner with planktonic larvae, b) the ability to firmly attach to hard substrates with byssus threads, which contributes to the ability of being transported on ship hulls or ocean rafts, c) fast growth and a short time to reach maturity, d) the opportunistic feeding habit as filter feeder with a broad planktonic diet and e) its ability to tolerate large fluctuations in environmental conditions, i.e. a high stress tolerance (Rajagopal et al. 2006). *P. viridis*, for example, tolerates salinities as low as 20‰, temperatures up to 39°C for 3.5 hours and up to 34°C for more than 10 days, high concentrations of suspended particulate matter (1200 mg/l) for more than 4 days and hypoxic conditions as low as 0.5 mg/l DO for up to 2 weeks (Huhn et al. 2016; Rajagopal et al. 2006; Shin et al. 2002; Wendling et al. 2013).

*The relevance of stress tolerance for invasion success*

Marine biological invasions often occur in anthropogenically impacted and polluted habitats (Lopeztegui-castillo et al. 2014; Piola and Johnston 2006; Stachowicz et al. 2002). Therefore, beside the fact that impacted habitats may be more vulnerable towards biological invasions (Stachowicz et al. 1999), there seems to be a link between the invasiveness of a species and its ability to tolerate environmental stress (Zerebecki and Sorte 2011). Environmental stressors that can facilitate the establishment of new species can be, for example, elevated water temperatures caused by climate change or locally by cooling water systems of power plants (Lopeztegui-castillo et al. 2014; Sorte et al. 2010), chemical pollution (Piola and Johnston 2006) or eutrophication with subsequent hypoxia events (Jewett et al. 2005). Stress tolerance is often a characteristic of r-selected species, i.e. species that show early maturity, high fecundity, rapid rates of development, high metabolic rates and short longevities (Parsons 1994; Wu 2002). These characteristics are all typically found in marine invertebrate species known to be successful invaders or species with a large distributional range (Zerebecki and Sorte 2011). This raises the question whether a high tolerance to environmental stress is a key determent for invasiveness or whether it is part of a package of characteristics that usually occur together and that all – more or less equally - contribute to the invasion success of the species. Several studies have examined the role of environmental stress tolerance in biological invasions by comparing stress responses between invasive and native species that inhabit the same environment. Jewett et al. (2005), for example, found that periodic low concentrations of dissolved oxygen caused epifaunal communities in Chesapeake Bay to be dominated by successful worldwide invaders (two tunicates, an anemone and a polychaete) rather than by the otherwise dominant but native barnacles. Among mytilids, the invasive *Mytilus galloprovincialis* was found to be more heat resistant than the native *M. trossulus* in Washington State (Schneider 2008) and more tolerant to high temperatures during aerial exposure than the native *Perna canaliculus* in New Zealand (Petes 2007). Furthermore, invasive *M. galloprovincialis* in South Africa were more tolerant to high turbidity (sand action) than native *P. perna* (Zardi et al. 2008). A comparison of the performance under hyposalinity, low oxygen and elevated temperatures between native and invasive species of mussels (3 pairs), ascidians (1 pair) and gammarids (1 pair) showed that the invasive species were generally more tolerant (Lenz et al. 2011).

A wide environmental tolerance is especially important during transport from the native into the introduced range and during the time of establishment after introduction. Habitats, in which marine non-native species get released from vectors such as ships, are

typically harbours that usually coincide with centers of anthropogenic activity. Therefore, they are often prone to pollution and/or high levels of eutrophication. Here, a high tolerance to extreme environmental conditions will certainly facilitate a species' establishment after introduction. This means that a species will more likely spread and establish self-sustaining populations if growth and reproduction can be maintained even during periods of environmental stress. Good examples are the success of *P. viridis* in Cienfuegos Bay, Cuba (Lopeztegui-castillo et al. 2014), in Kingston Harbour, Jamaica (Buddo et al. 2003) and in Tolo Harbour, Hong Kong (Cheung 1993) and of the bryozoan *Bugula neritina* in Kembla Habour, Australia (Piola and Johnston 2006). Whether a high stress tolerance is a general trait of the respective species, which evolved in correspondence with a stressful environment in the home range, or whether high tolerance levels are acquired during the transport process - e.g. by the activation of protective cellular functions such as the up-regulation of stress proteins or by a pre-selection of stress-tolerant genotypes (genetic bottle neck) - still remains mainly undetermined. To elucidate this, comparisons between native and invasive populations of the same species are necessary to test whether systematic differences in stress tolerance can be found. In the following section, different mechanisms that act on the phenotypic and the genotypic level, which may lead to an increase in performance under stress, are discussed.

*Intra-specific and inter-population differences in stress tolerance: the role of adaptation, phenotypic plasticity and energy reserves*

Tolerance to environmental stress, i.e. the ability to survive fluctuating environmental conditions that reduce growth, reproduction and/or the lifespan of an individual, differs between species as well as between populations and individuals of the same species in marine benthic invertebrates. There are various factors that contribute to the differences. Between species, different adaptations are primarily responsible for different tolerance levels: For example, temperate species are generally considered more tolerant to large fluctuations in water temperatures, whereas tropical species tolerate only temperatures in a narrow range and generally live closer to their upper thermal limits (Compton et al. 2007). Differences in stress tolerance can also exist between populations or individuals of the same species and it is interesting to understand the reasons for it, since, in addition to local adaptations, non-genetic factors may contribute to differences in environmental tolerance. Non-genetic differences between populations of the same species can go back to phenotypic plasticity, i.e. the ability of a genotype to express different phenotypes, depending on the environmental conditions

(Ghalambor et al. 2007). A form of phenotypic plasticity is acclimation, a reversible condition that develops over days to weeks upon exposure to stress (Sørensen et al. 2003) and a good example of an acclimation effect on the physiological level is the heat shock response. Heat shock proteins (hsp) are chaperones that prevent the denaturation of proteins and protect cells not only from the effects of heat stress but also from those of other stressors such as hypoxia (Lindquist and Craig 1988). Pacific oysters, *Crassostrea gigas*, for example, express higher amounts of hsp during summer than during winter and, therefore, increase their thermal limits during summer (Hamdoun et al. 2003). Another example of phenotypic plasticity in bivalves is shell formation in mytilids, which can be modified by exposure to predatory stress (Cheung et al. 2004; Reimer and Tedengren 1996), exposure to high wave action (Steffani and Branch 2003) or by the acidification of seawater (Gazeau et al. 2010). Another factor that influences stress tolerance on the phenotypic level is the nutritional condition of an individual. Most stress responses, e.g. the production of hsp, consume energy. In blue mussels, *M. edulis*, 20 to 25% of the available energy is, during heat stress, consumed by the synthesis of heat shock proteins (Anestis et al. 2007). Most bivalves react to environmental stress by closing their valves and by reducing their metabolic activity. In this state, they are not taking up food and, therefore, rely on energy reserves, e.g. glycogen that is stored in the mantle tissue (Fearman et al. 2009). A high energy budget should, therefore, also promote tolerance to environmental stress. Both, phenotypic plasticity and the ability to maintain a high energy budget can also be inheritable and are not only influenced by environmental conditions. This makes it difficult to distinguish between the effects of phenotypic plasticity, e.g. stress acclimation, good nutritional status and local adaptation when studying which factors are primarily responsible for population-specific differences in the ability to tolerate environmental stress in marine invertebrates. In fact, in some studies where local adaptation was first expected to have caused population-specific differences, it turned out that phenotypic plasticity caused by stress hardening, i.e. a short-time acclimation, or differential gene expression was indeed responsible for the observed differences, which was revealed by transplantation experiments with *M. edulis* (Altieri 2006) and by studying gene regulation of *Arctica islandica* populations from sites with different histories of environmental hypoxia (Philipp et al. 2012). In addition, the occurrence of transgenerational epigenetic effects can further complicate the identification of the responsible mechanism. When transgenerational epigenetic effects occur, information is passed from the parent to the offspring without being encoded on the DNA and can play a key role in stress tolerance. The effects can persist over generations, even after the condition causing the stress has faded (Reusch 2013). Examples among marine invertebrates, in which

local adaptation as the cause for enhanced stress tolerance has successfully been identified, are rare. Lacroix et al. (2015), for example, suggest that metabolic adaptation occurred in a *M. edulis* population chronically exposed to polycyclic aromatic hydrocarbon (PAH) in Brest harbor and in Chesapeake Bay, while behavioral adaptation in the form of swimming away from water layers of low oxygen concentration may have occurred in copepods in order to avoid hypoxia (Decker et al. 2003). Adaptation to permanent heavy metal exposure is reported from the bryozoan *Bugula neritina* (Piola and Johnston 2006) and from the annelid worm *Limnodrilus hoffmeister* (Klerks and Levinton 1989). However, it cannot be excluded that these findings go back - fully or partly - to transgenerational epigenetic effects as the authors found out that the resistance in metal tolerance in *L. hoffmeister* can be lost again within 9 generations (Klerks and Levinton 1989).

Identifying local adaptation as the mechanism that is mainly responsible for population-specific tolerance to stress would therefore require laboratory experiments that cover generations of the test organisms. Excluding local adaptations as a potential cause can, however, easier be achieved by reciprocal transplantation experiments in the field between an impacted site with a history of environmental stress and a natural site where stress regimes have not been encountered by the test organisms. If tolerance is lost from the population transplanted to the natural site or gained in the population transplanted to the impacted site within the same generation, phenotypic effects, e.g. stress acclimation or acquiring additional energy reserves, can clearly be made responsible for potential differences in stress tolerance.

*Thesis outline*

This thesis aims at contributing to the understanding of the mechanisms that are responsible for the acquisition of stress tolerance in *P. viridis*. The idea developed because substantial differences in salinity-, temperature- and oxygen stress were found between populations of *P. viridis* that came from the impacted Jakarta Bay (Java Sea) and the more natural Lada Bay (Sunda Strait), both at West Java, Indonesia (Wendling et al. 2013). In this study, higher tolerance levels were found in mussels from the impacted habitat (Wendling et al. 2013). Since, as in the case of Jakarta Bay, impacted ecosystems often coincide with harbours, i.e. potential donor sites for taking up *P. viridis* on ship hulls or in ballast water and transporting it to the non-native range, identifying the mechanisms that make the species more robust will also cast light on understanding the species' invasive potential and success.

To identify whether only one or all of named mechanisms (local adaptations, stress acclimation and good nutritional states) are responsible for the acquisition of environmental tolerance in *P. viridis*, I conducted sets of laboratory experiments, in which I exposed *P. viridis* to hypoxia under different scenarios. I chose hypoxia as the main stressor, because it, different from temperature or salinity stress, is only relevant for populations that come from the impacted Jakarta Bay and not for the populations that stem from the more natural habitats I chose as control sites. Therefore, the baseline of the experiments were three populations of *P. viridis* (two from natural and oligotrophic environments, Lada Bay in the Sunda Strait and Pelabuhan Ratu in the South Java Sea, and one from an impacted and eutrophic environment, Jakarta Bay in the Java Sea, Fig 2), which differed in terms of their stress history: the populations from the natural sites should not have experienced hypoxia in their life and in their parent generations, whereas the population from the impacted site had experienced a history of hypoxia. Establishing that the stress history differed between the populations is an indispensable pre-requisite when the role of local adaptation as a potential reason for differences in stress tolerance is under investigation. Accordingly, in this study, hypoxia was applied as a stressor for all but one experiment, in which hyposalinity was used additionally as a comparative stressor.

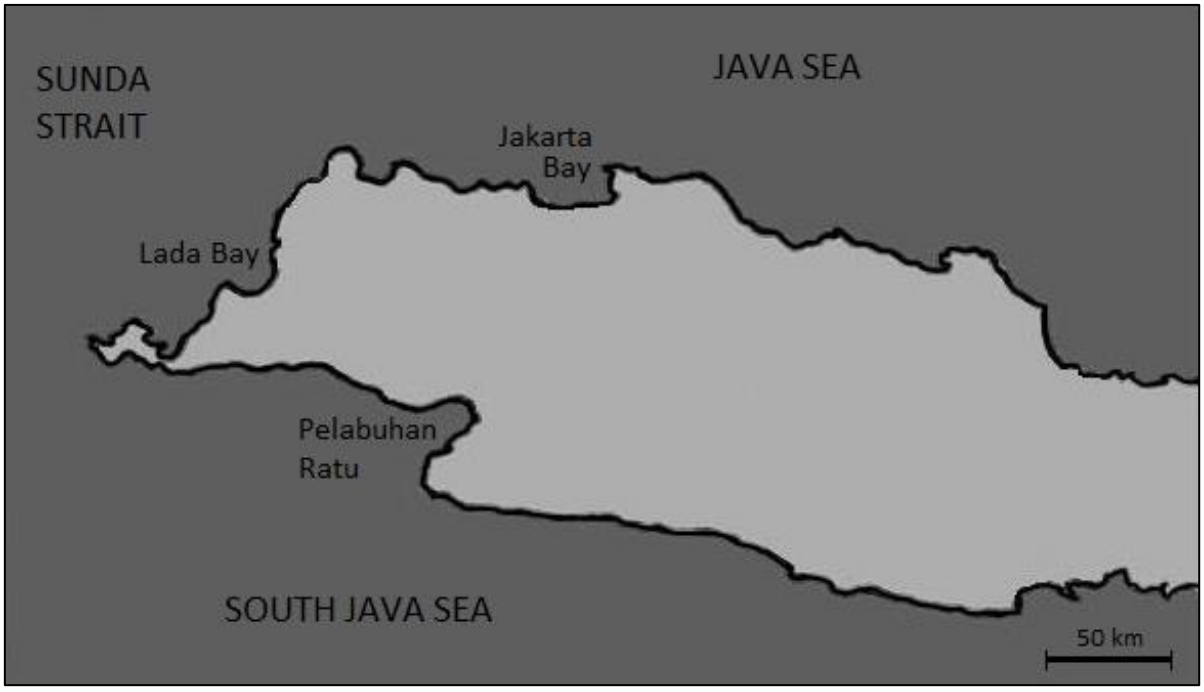


**Figure 2:** Study sites (impacted: Jakarta Bay and natural: Lada Bay and Pelabuhan Ratu) at Java Island, Indonesia. Modified from http://jafarkareem.tripod.com/westjafar.jpg.

In addition to the hypoxia experiments that aimed at identifying under which conditions the mussels are specifically tolerant, the determination of body condition indices (BCIs) played a central role in my work. Prior to each hypoxia experiment, I determined the BCI, which is a measure of the nutritional status and physical condition of a mussel, in samples which were representative of the groups of mussels I tested. BCIs of mussels can easily be determined by drying the soft tissue and dividing the dry soft tissue mass by the shell weight or the shell volume. Monitoring the BCIs of mussels from the impacted Jakarta Bay and from the more natural Lada Bay bimonthly for two years and relating this to the abundance of phytoplankton and nutrient concentrations at the respective sites gave valuable information on the connection between nutrient input, food availability and mussel BCIs. Brought in relation with the hypoxia experiments it also informed about the role of the nutritional status in stress tolerance.

The thesis is divided into three main chapters. Chapter 1a describes the distributional range of *P. viridis* in Indonesia and explains the fact that the species is considered native to the western and non-native to the eastern regions of the Indonesian archipelago. It shows data that illustrate that the nutritional status of mussels from the non-native range and from those found as fouling on a ferry that is operated in the non-native range was much lower than the nutritional status of mussels from habitats in the native range in western Indonesia. Furthermore, suggestions for possible invasion pathways from west- to east Indonesia are given and discussed. In chapter 1b, the results from hypoxia tests with mussels from the native range and mussels found as fouling on the hull of a ferry in the non-native range are presented. They suggest that a high stress tolerance is linked to a good nutritional status. This chapter, therefore, indicates that the mussels' invasion success depends on its nutritional status and, thus, can be limited by the food availability in the mussels' environment.

In chapter 2, a positive correlation between mussel food supply (plankton abundance), nutritional status and hypoxia tolerance is described. Results from reciprocal transplantation experiments with mussels between the natural Lada Bay and the impacted Jakarta Bay show that hypoxia tolerance in *P. viridis* from these sites depends more on the nutritional status and the prevailing conditions in a mussel habitat than on adaptations to specific environmental stress, i.e. hypoxia. Interestingly, in this study, mussels were, for the first time, found more tolerant when coming from the natural habitat. Also, this was the only time when mussels from the natural habitat had higher BCIs, which most likely goes back to the extreme changes that occurred in Jakarta Bay due to a beginning land reclamation project in the study year. The results hint that a good nutritional status and consequently a high stress tolerance can only be

maintained if the degree of impact does not exceed a certain level. Chapter 2, therefore, suggests that as long as anthropogenic impact is primarily expressed as eutrophication, *P. viridis* can benefit because eutrophication leads to plankton blooms that are positive for the mussels' nutritional states and stress tolerance whereas other anthropogenic influences, such as pollution by heavy metals, organochlorines or high sediment loads act negatively on the mussels' nutritional states and stress tolerance.

Chapter 3 deals with the complex responses that two environmental stressors (hypoxia and hyposalinity) can trigger in *P. viridis* populations that stem from differently impacted ecosystems. Again, the significance of the nutritional status for stress tolerance is highlighted, which was revealed in experiments with groups of mussels that were fed different amounts of food prior to exposure to hypoxia in the laboratory. Furthermore, it is suggested that acclimation to a moderate level of low oxygen can enhance mussel tolerance towards subsequent exposure to extreme hypoxia.

Taken together, the findings of the three chapters add valuable new information about how population-specific differences in stress tolerance can develop in a mytilid. They may also contribute to understanding why populations of *P. viridis* may flourish at some sites but fail at others, which can be helpful for the management of introduced populations of this species and for the effective utilization of *P. viridis* as aquaculture organism.

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Chapter 1 a

# A ferry line facilitates dispersal: Asian green mussels *Perna viridis* (Linnaeus, 1758) detected in eastern Indonesia

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## Abstract

While part of a single country, the Indonesian archipelago covers several biogeographic regions, and the high levels of national shipping likely facilitate transfer of non-native organisms between the different regions. Two vessels of a domestic shipping line appear to have served as a transport vector for the Asian green mussel *Perna viridis* (Linnaeus, 1758) between regions. This species is indigenous in the western but not in the eastern part of the archipelago, separated historically by the Sunda Shelf. The green mussels collected from the hulls of the ferries when in eastern Indonesia showed a significantly lower body condition index than similar-sized individuals from three different western-Indonesian mussel populations. This was presumably due to reduced food supply during the ships' voyages. Although this transport- induced food shortage may initially limit the invasive potential (through reduced reproductive rates) of the translocated individuals, the risk that the species will extend its distributional range further into eastern Indonesia is high. If the species becomes widely established in eastern Indonesia, there will then be an increased risk of incursions to Australia, where the mussel is listed as a high-priority pest species.

**Keywords:** invasion vector, hull-fouling, bivalve, body condition index, Indonesian archipelago

## Introduction

The Indonesian archipelago (4°N to 10°S and 95° to 141°E) lies at the confluence of the Eurasian, Indo-Australian, and Pacific tectonic plates and is framed by the Indian Ocean in the west and south and the Pacific Ocean in the north and east. Indonesia's large longitudinal extension (5200 km), its position between the two ocean basins, the current systems of the Indonesian Through Flow, and the isolation of sea basins during the last few glacial cycles, when parts of the Java and the Sunda Shelf were dry land, have caused a genetic break between marine species from the western (Sunda Shelf, Makassar Strait and Lombok Strait) and the eastern parts of the archipelago (Flores Sea, Banda Sea and Suhul Shelf) (Nuryanto and Kochzius 2009). The separation of the sea basins has also resulted in a high degree of endemism in the Indonesian archipelago, with a large number of species restricted to certain biogeographic regions (Briggs 2005; Allen 2008; Nuryanto and Kochzius 2009; Lord et al. 2012). The natural borders between the various biogeographic regions of the archipelago occur within the borders of a single country, Indonesia. The many domestic shipping lines, essential for passenger and cargo transport within Indonesia, constantly cross these biogeographic borders (Abdullah et al. 2005) and thereby represent a potential vector (as fouling on their hulls or as larvae in their ballast water tanks) for transfer of species between biogeographic regions. For example, the national ferry company (PT. Pelni) operates 24 steel passenger ships that connect all parts of Indonesia from Sumatra in the west to Papua in the east. As well, many large fishing vessels transport their catches from the major fishing grounds in the east to the markets in the west (Williams 2009). However, there have been almost no studies to evaluate whether domestic ships serve as a significant vector for transfer of marine species between the different biogeographic regions of the Indonesian archipelago.

The Asian green mussel *Perna viridis* (Linnaeus, 1758) is a well-known hull-fouling organism with a wide indigenous and non-indigenous distribution. Moreover, in Southeast Asia, it is an important aquaculture organism that constitutes a fast-growing and cheap protein source (Rajagopal et al. 2006). Because of its moderately long planktonic-larval stage (3 weeks) and its ability to attach to surfaces by means of strong byssal threads, *P. viridis* can reach areas outside its natural distributional range when transported in ballast water or as fouling on ship hulls (Baker et al. 2007; Rajagopal et al. 2006). The mussel's native range extends from the Persian Gulf to the Malaysian peninsula and includes the Indonesian islands Sumatra, Java, and Sulawesi, as well as

the Philippines (Siddall 1980). In the 1970s, the species was introduced to a number of South Pacific islands for aquaculture purposes and subsequently established populations on New Caledonia, Fiji, Tahiti, Tonga, and Samoa (Baker et al. 2007). In the West Atlantic, established populations of green mussels were detected in Trinidad, Venezuela, and Jamaica in the 1990s, and it subsequently was discovered along the southeastern coast of the United States (Buddo et al. 2003; Baker et al. 2007). In Indonesia, *P. viridis'* native range is limited to the western part of the archipelago (Siddall 1980). Several well-studied populations of *P. viridis* occur in the Java Sea, while fewer are known from the Indian Ocean and the Strait of Malacca (Table 1). In eutrophic and anthropogenically-influenced habitats in the western archipelago, such as in Jakarta Bay, Asian green mussels often occur at high densities. Here they are harvested from bamboo structures, on which they settle naturally, by mussel farmers and sold locally (Yaqin 2010). Green mussel farming in Indonesia began in the late 1970s with first attempts at yield optimization conducted in Jakarta and Banten Bay (Davy and Graham 1982). To date, there is not any evidence of green mussel populations east of the Makassar Strait (Figure 1). Here, we report the detection of *P. viridis* in the eastern part of the Indonesian archipelago (in Ambon Bay), which is outside the mussels' native distributional range, and the finding of live specimens on the hulls of two passenger ferries that regularly cross the country from west to east. The latter hints at the particular role of national Indonesian ferries as transport vectors for marine species within the archipelago. We discuss the influence of transport conditions on the mussels' invasiveness and highlight the particular risk that the Asian green mussel poses to the ecosystems of the Banda and Arafura Sea.

## Materials and Methods

We obtained information about the possible presence of *Perna viridis* in the eastern region of Indonesia from interviews with bivalve specialists of the Indonesian Institute of Science (LIPI) and the Ministry of Marine Affairs and Fisheries (KKP) in Ambon, Moluccas. These workers have occasionally sighted *P. viridis* in Ambon Bay since 1994. We confirmed this information by collecting live specimens from two locations within Ambon Bay in July and November 2012 and in March 2013. The species was identified according to morphological characteristics based on Siddall (1980). Within Ambon Bay, the mussels had settled on Mangrove roots and on mariculture platforms. Additional work will be

needed to determine whether the populations are reproducing and, if so, the extent of the populations. The focus of this study, however, was on identifying potential vectors for the transfer of the green mussel to the eastern part of Indonesia.

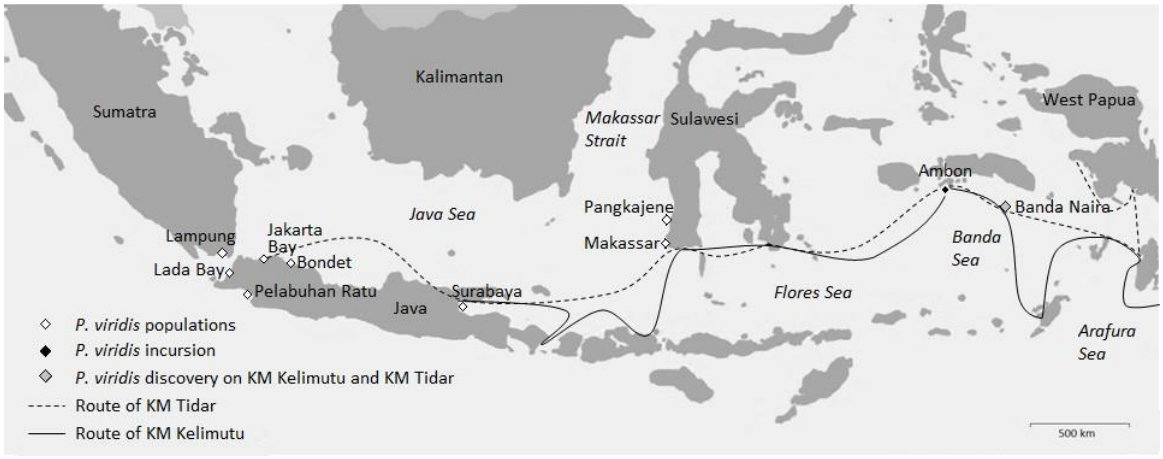


**Figure 1.** Routes of the KM Tidar and the KM Kelimutu in Indonesia and locations along the route where *Perna virdis* occurs and where it was found in this study. Ambon Bay and Banda Naira are outside the natural distribution of the species.

*Discovery of mussels on the hull of two ferries*

On 26 August 2013, live specimens of *P. viridis* were found in the front propeller slot of the ferry *KM Kelimutu*. The mussels were discovered in Banda Naira, Banda Islands, Moluccas, (Figure 1) by local divers who had been asked to free the propeller from an entangled rope. Ten specimens of the approximately 100 individuals on the ship were collected, the soft tissue separated from the shell, and the shells sent to us at the Marine Centre, Agricultural Institute Bogor (IPB), Indonesia, for identification. Here the shell length (longest anterior-posterior dimension) was measured with calipers to the nearest 0.1 mm. Two months later, on 28 October 2013, divers in Banda Naira discovered another group of live *P. viridis* on the ferry *KM Tidar*. As on the *KM Kelimutu*, the mussels had settled inside the front propeller slot, where they covered an area of about 4 m² (Figure 2). Twenty-six specimens of different sizes were collected and the shell length determined as described above.

On *KM Tidar's* next return trip to Banda on 15 November 2013, another 58 live mussels with a mean (± SD) shell length of 30.7 ± 2.1 mm were collected in order to

compare their body condition indices (BCI) to those of green mussels gathered in Ambon (mean 43.0 ± 8.1 mm, $n$ = 22) and at three locations along the coast of West Java (Figure 1) between April 2012 and May 2013: Jakarta Bay (mean 40.1 ± 5.0 mm, $n$ = 60), Lada Bay (mean 36.9 ± 6.9 mm, $n$ = 60), Pelabuhan Ratu (mean 49.6 ± 7.0 mm, $n$ = 25). The BCI is a measure of the physical condition of mussels and was calculated as the dry weight of the soft tissue (dried at 60°C until the weight remained constant, which was the case for all samples within 24 hours) divided by the dry weight of the shell. The higher the BCI, the better the mussel's nutritional status and its resistance to environmental stress (Wang et al. 2011). We used ANOVA followed by a Tukey's all-pairwise-comparison test using the free software R (R Core Team 2013) to test whether the BCIs of mussels differed significantly between the various origins. A reciprocal data transformation was conducted prior to the analysis to achieve normality.

**Figure 2.** Asian green mussel fouling on the KM Tidar, Banda Naira, 28.10.2013. Photograph by Guido Weissenfeld.

*Route and layover times of KM Tidar and KM Kelimutu*

Both ferries that had *P. viridis* on their hulls are important connections between the island of Java in the western part and the Moluccas and West Papua in the eastern part of the Indonesian archipelago. The *KM Tidar* travels between Jakarta and West Papua biweekly. On its way, it passes by Surabaya, Makassar, Bau Bau, Ambon, Banda, Tual and Dobo (Figure 1). The longest stopover is in Jakarta with a layover time of 18 hours. Layover times at the other harbours are shorter, ranging between two and four hours. The *KM*

*Kelimutu* follows a similar route, but starts its monthly journey in Surabaya (Java), passes by Benoa (Bali), Bima (Sumbawa), Makassar, Bau Bau and Wanci (Sulawesi), Ambon, Banda, Saumlaki, Tual and Dobo (Moluccas), and ends its trip after a few additional stopovers in the Arafura Sea in Merauke (West Papua). Layover times in Surabaya usually are 18 hours but can be extended to several days. The longest layover known to us was 18 days in April/May 2013. The *KM Kelimutu* visits all other harbours for two to four hours on its regular trips. In January 2013, the *KM Kelimutu* spent one month in Tanjung Priok, Jakarta Bay, which is where all Pelni ferries go for a dry dock inspection annually.

**Table 1**. Known populations of *Perna viridis* in Indonesia. References referring to a mussel population are marked by * and those referring to habitat quality by †. All but Ambon Bay are within the native distribution range of the species.

| Location | Habitat quality | Reference |
|---|---|---|
| Belawan, North Sumatra, Malacca Strait | medium anthropogenic influence | Sudharyanto et al. (2005)[*†], Hayati (2009)[*†] |
| Lampung, Sumatra, Sunda Strait | medium-high anthropogenic influence, evidence of heavy metals | Damar (2003)[†], Sudharyanto et al. (2005)[*†], Tugiyono (2007)[*†] |
| Lada Bay, Java, Sunda Strait | medium anthropogenic influence | Sudharyanto et al. (2005)[*†], Jalius et al. (2008)[*†], present study[*†] |
| Jakarta Bay, Java, Java Sea | high anthropogenic influence, heavy metals, evidence of organochlorines | Sudharyanto et al. (2005)[*†], Jalius et al. (2008)[*†], present study[*†] |
| Bondet, Java, Java Sea | high anthropogenic influence, evidence of organochlorines | Sudharyanto et al. (2005)[*†] |
| Pelabuhan Ratu, Java, Indian Ocean | medium anthropogenic influence | present study[*†], Arfin et al. (2012)[†] |
| Surabaya, East Java, Java Sea | high anthropogenic influence, evidence of organochlorines | Sudharyanto et al. (2005)*[†] |
| Pangkajene, South Sulawesi, Makassar Strait | low anthropogenic influence | Sudharyanto et al. (2005)[*†], Yaqin et al. (2011)[*†] |
| Makassar, South Sulawesi, Makassar Strait | high anthropogenic influence, evidence of heavy metals | Fachruddin & Musbir ( 2011)[*†], Lestari (2002)[*†] |
| Ambon Bay, Banda Sea | medium anthropogenic influence, evidence of TBT | present study ( possibly not established)[*†], Evans et al. (1995)[†] |

**Results**

The mean (± SD) shell lengths of *Perna viridis* specimens collected from the *KM Kelimutu* on 26 August 2013 was 28.04 ± 8.02 mm (range 18.7 to 41.7 mm, n = 10). The mean shell length of specimens collected from the *KM Tidar* on 28 October 2013 was 28.07 ± 12.55 mm (range 7.7 to 49.7 mm, n = 26). The mean BCIs of the mussels collected from the *KM Tidar* on 15 November 2013 and the BCIs of the mussels collected from Ambon and from places along the coast of West Java in 2012 and 2013 differed significantly (ANOVA, $F_{4, 220} = 121.3$; $P < 0.0001$). The BCIs of the *KM Tidar* mussels were the lowest, being, on average, 62% lower than those of mussels from Jakarta Bay, 34% lower than those of Lada Bay mussels, 13% lower than in mussels from Pelabuhan Ratu and 10% lower than in mussels from Ambon (Figure 3). With two exceptions (Ambon Bay vs. *KM Tidar* and Ambon Bay vs. Pelabuhan Ratu), all pairwise comparisons revealed significant differences (Tukey's test, $P < 0.01$, Figure 3).

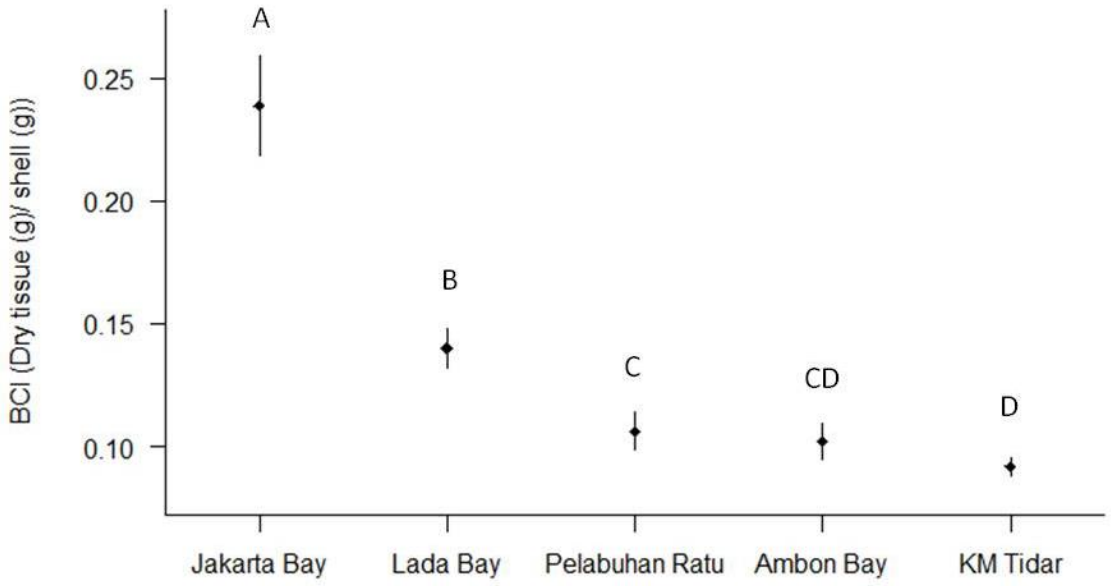


**Figure 3.** Body Condition Indices (means and 95% confidence intervals) of *Perna viridis* (shell length = 24.2 – 60.0 mm) from different locations in Indonesia. Means sharing the same letter were not significantly different (Tukey's test, $P > 0.05$)

**Discussion**

The discovery of *Perna viridis* on two ferries demonstrates the potential role of domestic ship traffic as a vector for species transfer within the Indonesian archipelago. Moreover, the current case of *P. viridis* illustrates how this anthropogenic influence can break open the faunal discontinuity between the western and eastern Indonesian archipelago, since *P. viridis* is considered to be non-indigenous in the eastern archipelago. Passenger and military naval ship traffic in the archipelago have been frequent since the mid 9th century (Rutz and Coull 1996), and, therefore, incursions of *P. viridis* to Ambon Bay or to other parts of the eastern archipelago are probably not new, but have never been reported. If a history of mussel incursions exists, the question arises, why the species did not establish in the eastern areas at an earlier date, and whether growing anthropogenic pressure would increase the potential for the mussels to establish permanent populations. If earlier incursions of *P. viridis* to the eastern Indonesian archipelago have indeed been overlooked in the past, the cryptic number of other species transported between the different biogeographic regions is presumably high as well.

The mussels collected from the *KM Kelimutu* ranged from 18.7 to 41.7 mm in shell length and those from *KM Tidar* from 7.7 to 49.7 mm. Therefore, mussels from both ferries likely stem from multiple recruitment events. Growth rates in *P. viridis* can be very variable and dependent on the habitat quality. Therefore, it is difficult to infer the ages of the mussels found on the ferries. The lowest growth rate reported for *P. viridis* in a size range of 30–50 mm is 2.29 mm per month (Cheung 1993). Using this as a reference, and taking into account that the *KM Kelimutu* had left Tanjung Priok, Jakarta Bay, 7 months before our sampling, and did not return there until the mussels were discovered in Banda Naira, it is possible that some of the larger individuals had colonized the ship in Jakarta Bay. Because of the large variation in the sizes of mussels found on *KM Kelimutu*, additional recruitment must have occurred in other locations along the route. The most likely donor harbour for the mussels on *KM Kelimutu* is Surabaya because of the long layover times known from there. In all other harbours, the 1–3 hours stops would only be sufficient for a substantial colonization of surfaces by *P. viridis* if propagule pressure was high and if the pediveligers were competent to settle.

The mussels found on the *KM Tidar* covered a nearly continuous size range. This means that all harbours on its route with known *P. viridis* populations are possible sources of colonists but, due to the above mentioned reasons, unlikely origins of mussels. If the number of recruits is a positive function of layover-time, the most likely source

population is Jakarta Bay where the ferry spends 18 hours between two trips every 14 days. There, according to local mussel fishermen, reproduction in *P. viridis* occurs throughout the year. Therefore, mussels may have settled on the *KM Tidar* during several stopovers in Jakarta in 2013. Stopovers in Surabaya are much shorter (3–6 hours), which makes this place a less likely origin. However, we cannot exclude that some mussels recruited in Jakarta Bay, while others stem from Surabaya or Makassar. Regardless of where along the ferry routes the mussels originate from, the facts that mussels A) had settled on two different ferries and B) very likely stem from several recruitment events demonstrate that the domestic ferries serve as a vector that may facilitate the west-to-east dispersal of *P. viridis* (and likely other species) in Indonesia. So far, the possible ecological consequences of the establishment of Asian green mussels in eastern Indonesia have not been investigated. Since 2012, we regularly found *P. viridis* in Ambon. However, it is unclear whether the mussels have already established a permanent population or whether the findings from there result from repeated introductions by ferries or other domestic vessels. These are not mutually exclusive mechanisms.

Not only is ferry traffic from Java and Sulawesi to Ambon frequent occurrence but the Banda and Arafura Sea are very important fishing grounds (Abdullah et al. 2005; Stacey et al. 2011). Thus, there is a lot of fishing-vessel traffic that brings with it another set of problems, in particular for adjacent jurisdictions. The control of fishing activities in the remote areas of the Arafura Sea is difficult (Stacey et al. 2011) as there is a problem with illegal crossings of fishing vessels between this region and northern Australia (Fox and Sen 2002). These fishing boats, in addition to the large cargo vessels that connect Australia to the western part of the Indonesian archipelago, may represent a pathway for the introduction of *P. viridis* to Australia. The green mussel is listed as a high priority pest species in Australia (Australian Government National System for the Prevention and Management of Marine Pest Incursions 2011) and comprehensive management efforts have been taken to prevent it from becoming established (McDonald 2012; Dias et al. 2013). The establishment of permanent populations of *P. viridis* in the Banda and Arafura Seas increases the risk of further introductions of the bivalve to Australia.

The establishment of *P. viridis* in the Banda and Arafura Seas seems likely because most of the individuals we found on the *KM Kelimutu* and the *KM Tidar* had reached reproductive size. Under favourable conditions, gonad development in *P. viridis* usually starts at a shell length of 12 mm and, when mussels are growing slowly, they

mature even at shorter lengths (Rajagopal et al. 2006). In addition, spawning events can be triggered by fluctuating environmental conditions such as sudden changes in temperature or salinity (Vakily 1989; Rajagopal et al. 2006). This may increase the likelihood of spawning during one of the layovers because many harbours are located near estuaries and therefore show pulses of low salinity. However, the mussels from *KM Tidar*, which we dissected to measure BCIs, had pale yellow to orange gonads, which indicates that they were not in a mature but in a resting or post-spawning phase within the reproductive cycle. The fact that the mussels we collected from the *KM Tidar* had significantly lower BCIs than those from coastal populations is plausible since the animals presumably experienced food shortage during transport. While the one sample of mussels examined in this study was not in spawning condition, should any of these mussels become detached from the hull, they may become reproductively active. In general, the environmental conditions in the eastern regions of the Indonesian archipelago are suitable for *P. viridis,* which is able to tolerate a wide range of environmental conditions (Rajagopal et al. 2006). On a local scale, environmental stressors such as sedimentation, fresh water runoff, or hypoxia may even favour *P. viridis* over other native hardbottom species, because of its broad environmental tolerance (Seed 1999; Wang et al. 2011).

To minimize the risk of establishment of *P. viridis* in eastern Indonesia, regular inspections of ship hulls and their cleaning from fouling organisms should be mandatory. This, in particular, applies to ferries that connect biogeographic regions within the Indonesian archipelago and to vessels on trans-border routes between Indonesia and its neighboring countries. The establishment of the Asian green mussel in the Banda and Arafura Sea, which are part of one of the world's biodiversity hotspots and support a large number of marine endemic species (Allen 2008), represents a major risk to the integrity of these unique marine ecosystems. For their future protection, comprehensive monitoring activities are needed to be able to detect and then stop invasions by species like the Asian green mussel at an early stage.

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We thank the German Academic Exchange Service (DAAD) for supporting the project with a student scholarship and the Bogor Agricultural Institute (IPB) and the Indonesian Institute of Science (LIPI), especially D. Tala and Dr. A. Syahailatua, for the support in the research activities and in obtaining all necessary permits. We would also like to thank Pak Heru (KKP Ambon), Pak Eri (KKP Pandeglang), Pak Baso and Pak Asep for their support during sampling. We also thank the anonymous reviewers for their contributions to this paper.

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Chapter 1 b:

**Tolerance to hypoxia in Asian green mussels, *Perna viridis*, collected from a ship hull in the non-native range in eastern Indonesia**

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## Abstract

Tolerance to fluctuating environmental conditions is regarded as a key trait of successful marine invasive species as it presumably promotes survival in recipient habitats, which are often anthropogenically impacted systems such as harbours. Little is known, however, about how transport of fouling organisms on ship hulls influences the condition of the transported individuals and how this is related to their tolerance to environmental stress. We investigated the influence of transport on a ship hull on the ability of Asian green mussels, *Perna viridis*, to survive low concentrations of dissolved oxygen (0.5 and 1 mg/l DO). This was done by comparing the performance under stress in mussels from a eutrophic habitat in Jakarta Bay to that of mussels that had spent their lifetime on a passenger ferry crossing the Indonesian Archipelago from Jakarta in the west to West Papua in the east. We found that the mussels that came from the eutrophic habitat survived twice as long as mussels from the ferry when exposed to low oxygen concentrations. Mussels collected from the ferry, however, had a generally higher byssus production under experimental conditions, which can be attributed to their live on a moving object where they are more exposed to currents. We suggest that Jakarta Bay mussels survived oxygen stress longer because they had higher Body Condition

Indices than their conspecifics from the ship hull and thus had more energy available for stress compensation. These results show that transport on ship hulls can weaken the robustness of *P. viridis*, if the journey leads the ship through areas of low food supply for mussels, if the stopovers in eutrophic coastal ecosystems are short and if the sailing times are long (several weeks). This finding might explain the lack of establishment ability of *P. viridis* in tropical areas of Australia, from where repeated incursions have been reported.

## Introduction

The introduction of non-native species into an area should be regarded with concern as introduced species may compete with native biota and alter ecosystem structure and function (Kimbro et al. 2013; Mack et al. 2000). However, not every introduced species also establishes and spreads, i.e. becomes invasive. Invasion success depends on the condition of the receiving habitat, the timing of the introduction, propagule pressure, i.e. the number of reproductive units introduced, and on the species' life history traits (Van Kleunen et al. 2010).

Life history traits often found in widespread invasive marine invertebrates are: a good ability to disperse as adults (e.g. by fouling on hard substrates) or during long phases as pelagic larvae, high fecundity, fast growth and a high tolerance towards changing environmental conditions (Sakai et al. 2001). Some of these adaptations may, however, also differ between individuals and populations of the same species, for instance if local adaptations have caused differences between populations from different origins or if momentary health conditions differ between individuals. Several studies compared the performance of native and non-native species co-occurring in the same habitat and found that the non-native species often had a higher tolerance to fluctuating environmental conditions (environmental stress) than the native species (Bielen et al. 2016; Jewett et al. 2005; Lenz et al. 2011; Schneider 2008). In the present study, we investigated the ability of the successful invader *Perna viridis* to survive extreme environmental conditions (hypoxia) as a function of the habitat where the respective individuals spent their life: in a eutrophic coastal ecosystem in the western, native range of the species in Indonesia, or as hull fouling on a ferry in the eastern, non-native range of the species in Indonesia. Testing the tolerance to environmental stress of individuals of marine invertebrates that came into an area as hull fouling and may compose a founder population in the non-native range, can help understand why not all incursions of non-native species result in biological invasions. Similar work has been done that investigated the copper (Cu) tolerance of invasive bryozoans, *Bugula neritina*, fouling

ship hulls coated with Cu- containing antifouling paint (Piola and Johnston 2006), however, to our knowledge, no study has ever compared the tolerance to environmental stress of a population of a marine invertebrate sampled in the native range to a population of the same species sampled from a ship hull in the non-native range. This approach can shed light on the question whether individuals do undergo any changes that might reduce or enhance the potential of establishment and spread during the transport process.

## Materials and Methods

### *Mussel collection and acclimatization*

In order to compare tolerance to environmental stress between mussels from a native population in the eutrophic Jakarta Bay (JB), West Java, Indonesia, and mussels that were collected from hull of the ferry KM Tidar (TI) at Banda Naira, Moluccas, in the non-native range in Indonesia on 15$^{th}$ November 2013, two separate laboratory experiments were conducted. The mussels collected from Muara Kamal in JB on 31$^{st}$ May 2014 (shell length (SL): 42.5 – 50.0 mm, mean SL = 45.7 mm) were tested in the marine habitat laboratory at Bogor Agricultural University (IPB), which is a 3-4 hours drive from the collection site. During transport, the mussels were kept dry in an insulation box. The experiment with the mussels from the ferry (SL: 30.2 – 45.7 mm, mean SL = 34.6 mm) was conducted in a laboratory at Banda Naira, where the mussels were collected from the sheltered leeward side inside the front propeller slot (Huhn et al. 2015). Before the experiments, mussels were cleaned from epifouling and were acclimatized to laboratory conditions for 5 days (JB mussels) and 14 days (TI mussels). During this time, they were kept in tanks with 80 l of aerated seawater at a density of 1 mussel per liter and were fed with 0.01 ml of the highly concentrated filter feeder food Sera Marin Coralliquid® (SMC) per individual and day. A 50% water exchange was conducted daily.

### *BCI determination*

Immediately after the acclimatization phase, before the beginning of the hypoxia experiment, subsamples of 20 mussels per population were removed from the tanks and immediately frozen. Individuals from Banda Naira were later transported to IPB by plane in an insulation box on ice and the samples from Jakarta Bay were frozen at IPB until further processing.

BCIs were determined as described in Huhn et al. (2015). BCIs were tested for statistically significant differences between populations with the non-parametric Wilcoxon rank sum test because the data could not be transformed in a way that normality would be achieved.

*Exposure to different concentrations of dissolved oxygen*

For the experiment in which mussels were exposed to different concentrations of dissolved oxygen (DO), 45 adult individuals per population (mean SL ± standard deviation (sd): JB = 45.4 ± 1.8 mm and TI = 34.5 ± 2.1 mm) were singled out and placed in PVC containers filled with 600 ml of seawater so that one mussel in one container represented one experimental unit. Water in the units of the control group (DO > 6 mg/l, n = 15 per population) was aerated with compressed air and aeration stones commonly used in fish tanks. For the experimental groups with lowered oxygen concentrations, water with 0.5 mg/l DO and with 1.0 mg/l DO was prepared in a header tank by nitrogen flow from a gas cylinder. When the target oxygen concentration was reached, which was validated by measuring the oxygen concentration with a WTW Oxical 3205 oxygen meter and CellOx 325 sensor, the experimental containers were filled with the respective de-oxygenized water and covered with o-ring sealed lids so that no air space remained in the container. The replication was again 15 mussel individuals per population and oxygen concentration. The same technique of decreasing the oxygen concentration was used for the daily water exchange. Experimental conditions were maintained for 14 days, during which mussels were daily fed with 0.01 ml SMC per individual. Every day, mortality was recorded in all containers by carefully touching gaping mussels with a thin stick. Mussels that did not respond to this stimulation were considered dead. Survival over 14 days was statistically compared between groups with a Coxph survival analysis in the package “Survival” in R (R Development Core Team 2013; Therneau 2013; see Huhn et al. (2016) for a detailed description of the procedure).

*Byssus thread production*

To measure the byssus production of the mussels during exposure to hypoxia, the number of byssus threads produced by each mussel individual was counted after 48 hours under the experimental conditions (0.5, 1.0 and > 6 mg/l DO). For this, every byssus terminal disc was marked with a permanent marker on the outside of the transparent PVC container while

counting to avoid double counts. Count data were analysed statistically with a generalized linear model (glm) with Poissson distribution in R (R Development Core Team 2013), which included two factors, “Population” (2 levels: Jakarta Bay and KM Tidar) and “Treatment” (3 levels: 0.5, 1.0 and > 6 mg /l DO).

## Results

### *BCIs at Experimental Start*

Before the start of the experiment, BCIs differed significantly between mussels from Jakarta Bay and those collected from the KM Tidar ferry. They were 2.5 times higher among the mussels from Jakarta Bay and this difference was statistically significant (mean $BCI_{JB}$ = 0.143, mean $BCI_{TI}$ = 0.059, Wilcoxon rank sum test: W = 400, $p < 0.001$, $n_{JB} = 20$, $n_{TI} = 20$, Figure 1).

### *Survival during exposure to hypoxia*

During 14 days of exposure to two concentrations of hypoxia (0.5 mg/l and 1.0 mg/l DO), mussels from Jakarta Bay and from KM Tidar showed progressing mortality, whereas the control groups that were maintained under 100% oxygen saturation survived by 87% (JB) and 100% (TI). There was no significant interaction between the factors “Population” and “Oxygen concentration”. Therefore, the interaction term was removed from the Coxph model. In the simplified model, the factor “Population” was highly significant ($p < 0.0001$, $\exp(coef)_{TI} = 3.02$, n = 60), while the factor “Oxygen concentration” was marginally significant ($p = 0.08$, $\exp(coef)_{1mg/l} = 0.62$, n = 60). Thus, the probability to survive under both hypoxic concentrations was higher (202%) in JB mussels than in TI mussels and the chance to survive, averaged across both populations, was slightly higher at 1 mg/l DO (38%) than at 0.5 mg/l DO (Figure 2).

### *Byssus threads*

Even though the mussels from the ferry KM Tidar were in average 10 mm smaller than the mussels from Jakarta Bay, they produced more byssus threads. This was true under all oxygen concentrations (Tab. 1, Fig. 3). The largest difference in mean byssus production between the

populations was found under hypoxia of 1 mg/l DO (3.7 times more byssus in TI than in JB mussels), followed by hypoxia (3.2 times more byssus in TI mussels) and normoxia (1.7 times more byssus in TI mussels).

**Table 1**: Byssus production in *Perna viridis* from the populations Jakarta Bay (JB) and Tidar (TI) after 48 hours of exposure to different oxygen concentrations (Treatment 0.5, 1.0 and > 6 mg/l DO). Results from a generalized linear model with Poisson distribution.

| | Estimate | SE | z value | P |
|---|---|---|---|---|
| Intercept | 1.969 | 0.061 | 32.241 | < **0.001** |
| Population (TI) | 0.742 | 0.040 | 18.635 | < **0.001** |
| Treatment (1.0 mg/l DO) | 0.614 | 0.068 | 8.988 | < **0.001** |
| Treatment (> 6 mg/l DO) | 1.777 | 0.059 | 29.994 | < **0.001** |
| Null deviance | 3348.6 | on 88 df | | |
| Residual deviance | 1487.0 | on 85 df | | |

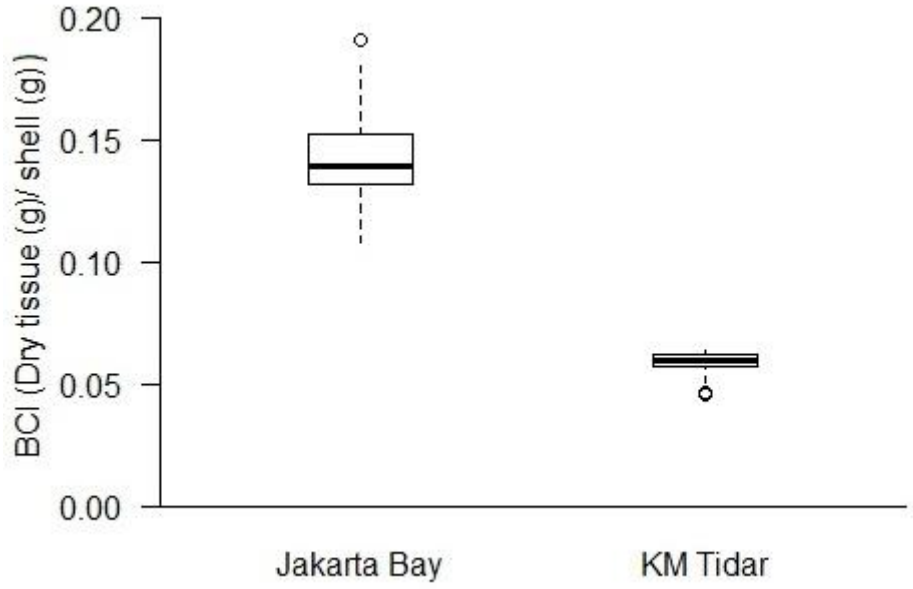


**Figure 1:** Body Condition Indices (median, interquartile range (IQR), IQR*1.5 and outliers) of *Perna viridis* from Jakarta Bay and from the ferry KM Tidar before the beginning of the hypoxia experiment.

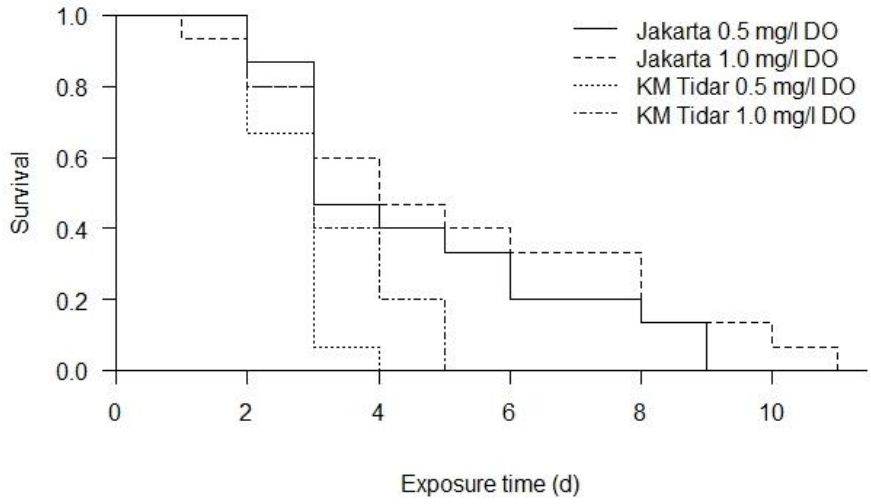


**Figure 2:** Survival of *Perna viridis* from Jakarta Bay and from the ferry KM Tidar during laboratory experiments with two different concentrations of hypoxia (0.5 and 1.0 mg/l DO).

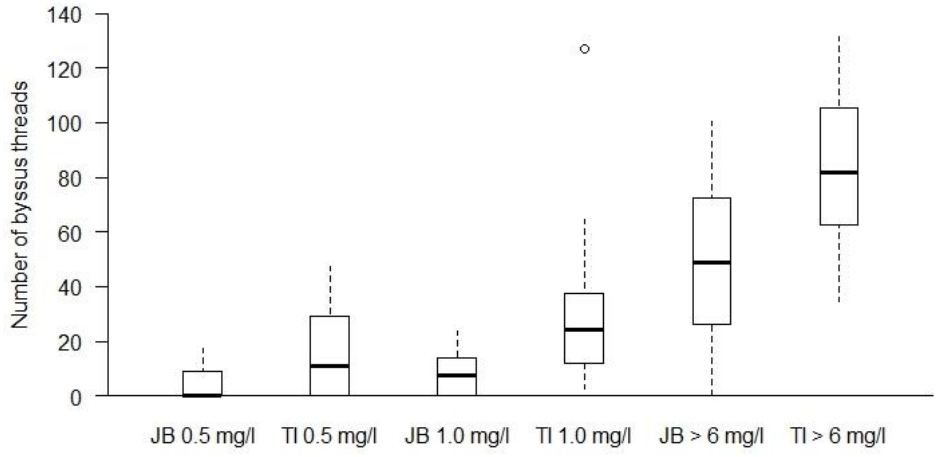


**Figure 3:** Byssus production (median, interquartile range (IQR), IQR*1.5 and outliers) in *Perna viridis* from Jakarta Bay (JB) and the ferry KM Tidar (TI) after 48 hours of exposure to different oxygen concentrations (0.5, 1.0 and > 6 mg/l DO).

## Discussion

The experiment presented here shows that Asian green mussels, *Perna viridis*, that had spent their lifetime on the hull of a ferry with short stopover times, had a lower tolerance towards two concentrations of hypoxia than mussels that came from a eutrophic coastal habitat. In contrast to the survival times, byssus production during the first 48 hours, regardless of

oxygen concentration, was higher among mussels that were collected from the ship hull. These results suggest that the environmental conditions experienced by an individual during the lifetime can play an important role in byssus formation: The mussels that came from the ferry had presumably acclimated to the prevailing drag and shear stress by forming more byssus threads in order to attach firmly to the hull when the ship is moving and maintained this acclimation over the cause of the experiment. Furthermore, as all mussels from the ferry had settled inside the front propeller slot, they were additionally exposed to strong currents produced by the propeller whenever the ship was manoeuvring sideways (during docking and departing). As the mussels that were collected from the ship hull presumably stemmed from different recruitment events, which most likely occurred in different harbours (Huhn et al. 2015), it is unlikely that the higher byssus production resulted from genetic adaptation, but rather was a consequence of acclimation to life on a moving object.

A striking difference between the mussels from JB and TI was the BCI, which was 2.5 times higher at the start of the experiment in mussels from JB - the group with the higher tolerance to hypoxia. In a eutrophic habitat, such as Jakarta Bay, there is ample food supply for filter feeders, such as mussels (Huhn et al. 2016), whereas during an open ocean journey on a ship hull, food availability is scarce, as *P. viridis* mainly feeds on phytoplankton. A proxy for phytoplankton abundance that is applicable at large spatial scales is the surface Chlorophyll *a* (Chl *a*) concentration. In Jakarta Bay, Chl *a* concentrations range from 8 to 93 µg/l (Damar 2003), whereas along the route of the ferry KM Tidar, in the open Java Sea, Flores Sea and Banda Sea, Chl *a* concentrations are much lower, ranging from 0.1 to 2.4 µg/l, depending on the season (Kinkade et al. 1997). From other bivalve species, including *Mytilus edulis*, it is known that Chl *a* concentrations are positively related to mussel growth (Philippart et al. 2014). This means that the food availability for *P. viridis* should be by magnitudes higher if mussels come from a eutrophic habitat such as Jakarta Bay than if they live on a ferry that is spending most of the time at sea travelling through oligotrophic areas. It is also not known, whether mussels on a ship hull take up oxygen and food particles while the ship is moving. If the gaping ability is hindered during ship movements, the time the mussels can spend to feed and respire would be restricted to the times that the ship is docking. At the time when the mussels were collected from KM Tidar at Banda Naira, almost all of them were found gaping and filtering (Huhn, personal observation), but we do not know whether they only resumed this activity after the ship had stopped or whether they were gaping throughout the passage.

No matter whether low phytoplankton availability during the voyage or limited gaping periods accounted for the much lower BCIs in the mussels from KM Tidar, this study shows that the mussels from the ferry had less energy reserves available than their conspecifics from the eutrophic habitat. In mytilids, energy for metabolic processes is stored in the form of glycogen, which is deposited in the mantle tissue, whereas dietary lipids, if not metabolized instantly, are mainly allocated directly to gamete formation (Fearman et al. 2009). Consequently, an insufficient diet, under which all available energy is needed for metabolic processes, would slow down gamete formation and would limit glycogen storage (Fearman et al. 2009). During extended periods of environmental stress, such as hypoxia, stored glycogen can be metabolized by anaerobic respiration while the valves stay closed to isolate the soft body from the environment. Consequently, insufficient glycogen storage would certainly shorten survival time during stress exposure. The mussels that came from KM Tidar had low BCIs, which is a valid proxy for energy reserves (Norkko et al. 2005), did not have any well-developed gonads (Huhn et al. 2015) and showed low survival during hypoxia. These results, therefore, hint at a relatively low risk of establishment and spread of *P. viridis* when arriving after a voyage as hull fouling on ships, especially if the mussels were lacking well-developed gonads because of insufficient food supply to invest energy in reproduction and not because they were collected outside the reproductive season. The risk of establishment would increase, however, if ships carrying *P. viridis* on their hulls would spend days to weeks in a eutrophic harbour or coastal area where the animals can refill their energy resources.

The present experiment highlights the relevance of energy availability for stress compensation and survival in *P. viridis*. Other effects, such as acclimation to low oxygen concentrations in mussels from Jakarta Bay, where hypoxic periods occur regularly, may additionally have contributed to the longer survival time among JB mussels. We cannot disentangle potential acclimation effects on survival time from the effect of energy availability based on this experiment but the extreme differences in BCIs (2.5 times higher in JB mussels) speak for the significant role of energy availability in survival during hypoxia (2 times higher in JB mussels).

To better understand the influence transport on ship hulls has on *P. viridis*, further studies are needed that monitor different aspects during the ships' journeys, which may influence the mussels' nutritional status and robustness. For example, parameters, such as temperature, oxygen concentration, salinity and phytoplankton abundance could be measured along the shipping routes as all these are known to influence mussel BCIs (Huhn et al. 2016; Wang et al. 2011). With small submersible cameras attached to the ship hull, the activity

patterns of mussels could be recorded to find out whether the animals are gaping while the ship is moving. Together with data on phytoplankton abundance, the gaping times would give valuable information on the potential utilization of food during an ocean voyage. Additionally, it is necessary to sample more mussels from ship hulls to find out whether the low BCIs and poorly developed gonads found in mussels from the ferry at Banda Naira in this study are a common picture or an exception. Studies that directly looked at specimens from ship hulls are rare but would add valuable information to the understanding of the potential of establishment and spread of *P. viridis* and other hull-fouling invertebrates in the non-native rang.

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Chapter 2:

# Food availability in an anthropogenically impacted habitat determines tolerance to hypoxia in the Asian green mussel *Perna viridis*

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## Abstract

The Asian green mussel *Perna viridis* is tolerant to environmental stress but its robustness varies between populations from habitats that differ in quality. So far, it is unclear whether local adaptations through stress-induced selection or phenotypic plasticity are responsible for these inter-population differences. We tested for the relevance of both mechanisms by comparing survival under hypoxia in mussels that were transplanted from an anthropogenic impacted (Jakarta Bay, Indonesia) to a natural habitat (Lada Bay, Indonesia) and vice versa. Mussels were retrieved eight weeks after transplantation and exposed to hypoxia in the laboratory. Additional hypoxia tests were conducted with juvenile mussels collected directly from both sites. To elucidate possible relationships between habitat quality and mussel tolerance, we monitored concentrations of inorganic nutrients, temperature, dissolved oxygen, salinity, phytoplankton density and the mussels' body condition index (BCI) for 20 months before, during and after the experiments. Survival under hypoxia depended mainly on the quality of the habitat where the mussels lived before the hypoxia tests and only to a small degree on their site of origin. Furthermore, stress tolerance was only higher in Jakarta than in

Lada Bay mussels when the BCIs were substantially higher, which in turn correlated with the phytoplankton densities. We explain why phenotypic plasticity and high BCIs are more likely the causes of population-specific differences in hypoxia tolerance in *P. viridis* than stress-induced selection for robust genotypes. This is relevant for understanding the role of *P. viridis* as mariculture organism in eutrophic ecosystems and invasive species in the (sub)tropical world.

## Introduction

Low oxygen concentrations are a common environmental stress for benthic animals in coastal areas worldwide (Hoffmann & Hercus, 2000) and the occurrence of hypoxic zones has doubled in frequency each decade since the 1960s (Diaz & Rosenberg, 2008). As exposure to low oxygen impairs their fitness, many bottom-dwelling invertebrates have evolved strategies to react to changes in oxygen concentrations to attenuate their detrimental consequences. These strategies comprise behavioral responses such as habitat shifts or physiological acclimation as a result of phenotypic plasticity. Furthermore, tolerance to hypoxia can be based on adaptations through natural selection (Parsons, 1994; Decker et al., 2003). In species with limited mobility, acclimation and adaptation are more important and in laboratory experiments that do not allow the escape from stress, low oxygen concentrations are therefore better tolerated by bivalves than by mobile taxa such as crustaceans (Vaquer-Sunyer & Duarte, 2008).

An example of a bivalve with a high tolerance to hypoxia is the Asian green mussel *Perna viridis* (Linnaeus 1758). Within its subtropical/tropical distributional range, it frequently occurs in eutrophic and polluted habitats such as harbors and estuaries of urbanized areas, e.g. Tolo harbor in Hong Kong, China, or Jakarta Bay, Java island, Indonesia (Cheung, 1993; Yaqin, 2010). Like other Mytilid bivalves, *P. viridis* responds to hypoxia by closing its valves and depressing its metabolism, which also results in a reduced filtering activity and food uptake (Wang et al., 2011). In addition, hypoxia damages the mussels' lysosomes, e.g. of digestive diverticula, and leads to a loss of lysosomal integrity (Fang et al., 2008). The consequence is digestive dysfunction, which – enhanced by metabolic depression – diminishes the energy budget of the mussel and leads to a low nutritional status (Nicholson, 1999). Hence, hypoxia impairs the nutritional status of mussels while, at the same time, a good nutritional status should help them tolerate hypoxic conditions better. Therefore,

individuals with rich energy reserves should perform better in environments that frequently experience low oxygen concentrations than conspecifics that are in a poor nutritional state.

Frequent exposure to hypoxia may lead to genetic adaptation in mussel populations by the selection of genes that are positively associated with the maintenance of homeostasis during environmental stress. These genes encode *heat shock proteins* (HSPs), *inhibitor of apoptosis* proteins (IAPs) or other proteins expressed during the stress response, such as *growth arrest and DNA damage proteins* (GADD45) (Zhang et al., 2012; Leung et al., 2014). Furthermore, high mutation rates, caused by enhanced transposon activity under environmental stress, may contribute to the accumulation of random mutations. This provides genetic material for the subsequent selection of stress tolerant mutants. In general, adaptations are manifested in populations if the selective force persists over generations (Piacentini et al., 2014) and chronic environmental stress can therefore alter the genetic structure of populations. This possibly leads to intra-specific genetic diversity and pronounced differences in robustness between conspecific populations, i.e. local adaptation. Local adaptation has been shown, for example, for several copepod species in response to hypoxia, salinity, thermal stress and phytoplankton toxins (Dam 2013). However, population-specific differences in stress tolerance may not always be genetically fixed. They may also go back to differential acclimation capacities. This has been described for the blue mussel *Mytilus edulis.* In intertidal habitats, *M. edulis* shows physiological responses that help tolerate hypoxia when it is exposed to the air during low tide and these reversible reactions can also be expressed by individuals that stem from subtidal habitats if they are moved to the intertidal (Altieri, 2006). Hence, in these animals, tolerance to hypoxia clearly goes back to phenotypic plasticity and not to a difference in the genetic makeup of subtidal and intertidal mussel populations (Altieri, 2006). Stress tolerance in mussels is therefore determined by the amount of energy available for their metabolism, by genetic adaptation, by phenotypic plasticity, or by a combination of all these mechanisms.

In laboratory experiments conducted by Wendling et al. (2013), individuals of *P. viridis* from the eutrophic, impacted Jakarta Bay exhibited a higher tolerance to hypoxia and other stressors than individuals from the oligotrophic, mostly natural Lada Bay. Both sites are located at the western coasts of the island of Java, Indonesia. The authors suggest that the good nutritional status of the mussels from Jakarta Bay was the main reason for the enhanced stress tolerance in these animals, but were not able to exclude a possible influence of local adaptations on the observed difference. To test whether a high stress tolerance is a permanent characteristic of mussels from Jakarta Bay and to disentangle the influences of previous

adaptations to stressful conditions and phenotypic plasticity supported by a rich food supply, we performed two experiments: First, we conducted hypoxia tolerance tests similar to those reported by Wendling et al. (2013), but used juvenile instead of adult mussels to test whether population-specific differences in stress tolerance already occur at an early life stage. Furthermore, we conducted transplantation experiments in which we reciprocally translocated adult individuals between the same two locations. We then assessed their tolerance to hypoxia after they had spent 8 weeks in the new environment. If genetic adaptations to hypoxia occurred in the mussel population from Jakarta Bay and if these were of utmost importance in determining hypoxia tolerance, then Jakarta Bay mussels should consistently perform better under hypoxia than conspecifics from Lada Bay. A different result is to be expected if no adaptations occurred or if their influence was overwritten by environmental characteristics, such as food availability, which prevailed in the habitat where the mussels resided immediately before the hypoxia event. In order to assess the influence of energy availability and energy reserves on mussel stress tolerance, we also collected data about phytoplankton densities and about the nutritional status of resident *P. viridis* individuals in both habitats over a period of 16 months prior to and 4 months after the translocation.

## Methods

*Site selection and monitoring*

Two coastal areas in West Java, Indonesia, in which *Perna viridis* is common, were selected for the experiments. The two habitats differ strongly with regard to the level of human impact: Jakarta Bay (JB), Java Sea, is influenced by anthropogenic activities and by the influx of nutrient rich river water (Arifin, 2006). The experimental site in JB (06°04’S, 106°44’E) is located near the estuary of Muara Kamal in the inner bay area that has been classified as eu- to hypertrophic by Damar (2003). From this area, toxic plankton blooms and the occurrence of Paralytic Shellfish Poisoning (PSP) caused by saxitoxin producing dinoflagellates have been reported repeatedly (Thoha et al., 2007; Andayani & Sumartono, 2012). Furthermore, it is impacted by heavy metals (Williams, 2000; Takarina & Adiwibowo, 2011) and organic pollutants such as polychlorinated biphenyl (PCB), dichlorodiphenyltrichloroethane (DDT) and polycyclic aromatic hydrocarbon (PAH) (Sudharyanto et al., 2005; Rinawati et al., 2012). In contrast to this, the second site in Lada Bay (LB) (06°30’S, 105°41’E), which is located in the Sunda Strait, is more natural, clean and oligotrophic (Jalius, 2008; Radiarta et al., 2011).

At both sites, *P.viridis* spat settles naturally on bamboo structures built by local mussel farmers.

Between April 2012 and November 2013, JB and LB were visited monthly to bimonthly to monitor a) water temperature, b) salinity, c) dissolved oxygen, d) inorganic nutrients (nitrate, nitrite, ammonia and ortho-phosphate), e) phytoplankton density, and f) body condition indices (BCIs) of resident mussels.

*Abiotic habitat characteristics*

For measuring inorganic nutrient concentrations, surface water samples were taken by filling up 1 L PVC bottles. The bottles were then transported to the Bogor Agricultural University (IPB) in an insulated box filled with ice within 4-6 hours. They were stored in the lab at -20°C until the analyses. Nitrate, nitrite, ammonia and ortho-phosphate concentrations were determined using spectrophotometry after APHA (2012) at the analytic laboratory for assessment of productivity and water quality (ProLing) at IPB. A total of 31 water samples were taken from 8 different locations at the impacted and 5 locations at the natural site at 10 and 7 sampling days, respectively, between April 2012 and November 2013. At the same occasions, sea surface temperature (SST) and oxygen concentrations (DO) were measured with an oxygen meter (WTW Oxical 3205 + CellOx 325), while salinity was determined with a handheld refractometer.

*Phytoplankton*

Phytoplankton samples were taken with a handheld net (20 µm mesh width and a diameter of 26 cm) that was vertically lowered to a water depth of 2.5 m, kept there for 60 sec and then slowly howled up. The net was then rinsed 3 times from the outside, using a wash bottle containing seawater. Each sample was transferred to an 80 ml- PVC bottle, fixed with 0.3 ml Lugol's solution and stored cool during transport. In the laboratory, samples were kept at 4°C until analysis with a light microscope using a Sedgwick rafter. Natural phytoplankton units, i.e. potential units of food for *P. viridis* such as single dinoflagellate cells, single pennate diatoms or single diatom chains, were counted in 10 cells of the Sedgwick rafter and in 3 replicates per sample. For species identification, photos of the samples were taken in a Sedgwick rafter, using the software Stream Start for Olympus CX41. Identification on the

genus or species level followed standard plankton identification literature (Yamaji, 1979; Tomas, 1997).

*Mussel BCIs*

For assessing the mussels' body condition indices (BCIs), adult mussels (23 mm – 88 mm shell length, the size spectrum of the collected individuals was similar in the two populations) were frozen after collection. Per site and sampling day, 15 - 40 individuals were collected. BCIs were determined by separating the soft body from the shell and drying both at 60°C for 24 hours. To calculate the BCI the dry weight of the soft tissue was divided by the dry weight of the shell. The use of this condition index compared to the use of other common condition indices was recommended as most suitable for adult bivalves as it is easy to measure and standardize and has a high physiological validity (Lucas & Beninger, 1985).

*Hypoxia tolerance of juvenile Perna viridis*

Juvenile *P. viridis* with a shell length of 10 – 18 mm were collected from JB and LB on July 14$^{th}$ and 15$^{th}$ 2012, respectively, and were transported to the Marine Habitat Laboratory at the IPB by car: 4 hours from JB and 6 hours from LB in cool boxes with 100 individuals in 10 l seawater. Mussels were acclimatized to laboratory conditions for 20 (JB) and 19 days (LB), during which they were kept in two glass aquaria containing 100 mussels in 120 l of aerated seawater. The water was exchanged daily and the mussels were fed with 1.25 x $10^4$ cells Coralsands living phytoplankton (DT's Premium Blend Live Marine Phytoplankton) per mussel per day. On August 2$^{nd}$, 45 of the acclimatized mussels from each population were singled out into plastic containers containing 100 ml seawater with different oxygen concentrations. Per population, 15 mussels each were exposed to 0.5 mg/l DO (hypoxia), 1 mg/l DO (low oxygen) and > 5 mg/l DO (normoxia), respectively. Hypoxic and low oxygen concentrations were achieved by bubbling nitrogen gas into a storage tank filled with seawater until the target oxygen concentration was reached. This de-oxygenized seawater was then used for the daily water exchange, in which the content of each plastic container was completely replaced by water from the storage tank. In this way, the oxygen concentration was reset to the same level in each replicate (0.5 mg/l or 1 mg/l DO) every 24 h. Directly after the daily water exchange, individuals were fed with 1.25 x $10^4$ cells Coralsands living phytoplankton (DT's Premium Blend Live Marine Phytoplankton) each. The containers were closed with a tight plastic lid and remained closed until the next day's water exchange. We

tested for vitality by carefully stimulating the siphons of gaping mussels with a thin stick. Individuals that did not respond with shell closure were considered dead. These conditions were maintained for 14 days. To obtain information on the nutritional status of the juvenile mussels before the start of the experiment, mussels were dried from the outside with a paper towel, the total wet weight was determined to the nearest 1 mg with a digital scale and the shell length was measured to the nearest 0.1 mm with a caliper. Whenever an individual died and at the last day of the hypoxia experiment the mussels were frozen at -20°C. Later, the soft tissue of the frozen mussels was removed from the shell and the shell weight was determined to the nearest 1 mg. The wet soft tissue weight before the experiment (day 0) was then calculated as the total wet weight at day 0 subtracted by the dry shell weight at the day of death and the BCI determined as wet soft tissue weight in g divided by the cubic shell length ($L^3$) in $mm^3$. When tissue wet weight is measured in living mussels, it can fluctuate with the water content of the mantel cavity. We minimized this methodological error by standardizing the weighing procedure. For this mussels were always handled in the same way: They were taken out of the water, were dried from the outside with a paper towel and were then immediately placed on a scale. This assured that water loss from the mantel cavity during the weighing process was at minimum and, hence, the random error that could be introduced by this was as small as possible. However, we could not prevent the introduction of a systematic error (i.e. overestimating tissue wet weight due to the water content of the mantel cavity), but we assume that this error was constant across mussel individuals and did therefore not bias the comparison of BCIs across the experimental groups. Furthermore, assessing the wet weight is the only non-destructive method that allows BCI determination prior to the start of the experiment.

*Mussel transplantation and retrieval*

On July 7$^{th}$ 2013, 400 mussels (20 - 30 mm shell length) were collected from JB and equal shares of them were placed in eight elastic nylon nets such as in the technique of ‘mussel binding’ commonly used in aqua farming (Jenkins, 1979). A funnel connected to a PVC pipe (2.5 cm Ø) was used to fill the mussels into the nets, while a nylon rope (0.8 cm Ø) was attached to the inside bottom of each net. Pulling the rope through the pipe while mussels were slowly fed into the funnel resulted in an even distribution of mussels around the rope. Later on, mussels attached to the rope with their byssus threads and then broke through the elastic meshes while growing. This method guaranteed that the animals experienced a food

and oxygen supply equivalent to the prevailing habitat conditions at the respective site. We tested for an effect of the net on food supply by comparing the BCIs of specimens that were transplanted within sites (see below) and grew in a net for two months with the BCIs of mussels, which were collected directly from the settlement structures where the nets were located. As the BCIs did not differ between the two groups, we assume that the net technique did not affect food or oxygen uptake in the mussels (data not shown). Four of the nets filled with mussels from JB were deployed not far from their site of origin (within-site transplantation), while another four were transported to LB (between-site transplantation: 7 h transport by car and boat, dry and cool). The procedure was repeated with animals originating from LB and four nets with mussels from there were deployed in LB and in JB, respectively. Mussels that went from LB to JB were transported by car for 7 h, while they were kept dry and cool. They were stored in aerated seawater for 6 h in an air-conditioned room near the Jakarta Bay site until the next morning and were then taken to the transplantation site by car and boat (1 h transport, dry and cool). For logistic reasons, the transportation times could not be made totally identical between groups and transportation could not be mimicked for the within-site transplanted mussels. We assume that these differences did not influence our results, since the differences in transportation times were kept at a minimum and the effect of transport on mussel condition should have faded after 2 months of residence time at the respective sites. All these activities resulted in four experimental groups: 1) mussels transplanted within Jakarta Bay (group: JB-JB), 2) mussels transplanted from Jakarta to Lada Bay (group: JB-LB), 3) mussels transplanted within Lada Bay (group: LB-LB) and 4) mussels transplanted from Lada to Jakarta Bay (group: LB-JB). After transplantation, the mussels were left in the field for a residence time of 2 months and were then retrieved and taken to the Marine Habitat Laboratory at the IPB (transported dry and cool, 6 hours from LB and 4 hours from JB). Here, the mussels were acclimatized to laboratory conditions for 10 days. For this, they were placed in four separate aquaria containing 50 mussels in 120 l of aerated seawater each. Water was exchanged daily and the mussels were fed with $1 \times 10^3$ cells/ml aquarium water ($= 2.4 \times 10^6$ cells per mussel) of living *Isochrysis galbana* per day. A sub-sample of 5 individuals from each of the 4 groups was used to assess mussel BCIs immediately after retrieval and the change in BCI was calculated as the BCI in each subtracted by the mean BCI of the subsample of mussels collected for BCI determination from the respective locations on the day when the mussels had been translocated (Figure 3, July 2013).

*Hypoxia tolerance tests with resident and translocated mussels*

After acclimatization, 30 individuals from each of the 4 experimental groups were used for the comparison of mussel hypoxia tolerance. For this, 20 of the 30 individuals were exposed to 0.5 mg/l dissolved oxygen (DO) and 10 individuals to normoxic conditions (> 5 mg/l DO), respectively. The normoxia group was smaller because survival under these conditions was expected to be 100%. Furthermore, these data were not included in the statistical comparison between mussel groups and only served to monitor potential background mortality. In our experimental design, one replicate consisted of one mussel that was placed in an air-tightly sealed plastic container with 600 ml seawater. For the normoxia treatment, punctuated lids allowed to supply the containers with air through hoses connected to an air pump. Hypoxia was achieved as described above for the juvenile mussels and the oxygen concentration was reset to the target level in each replicate every 24 hours. Mussels were fed with $2.5 \times 10^6$ cells of *I. galbana* per individual per day and mortality among them was recorded daily for 14 days. We tested for vitality by stimulation as described above. Animals that did not respond upon this stimulation with shell closure were considered dead.

*Statistical analysis: Comparison of BCIs between sites*

We tested for differences in mussel BCIs between sites using data that were collected between April 2012 and November 2013 by conducting a 2-factorial ANOVA including the factors *'Population'* (with two levels: 'JB' and 'LB') and '*Sampling time'* (with 11 levels from 'Apr 2012' until 'Nov 2013'). Data were tested for normality by plotting the residuals and were log-transformed to encounter the problem of inhomogeneous variances. For all analyses in this study, the free statistical computing software R was used (R Core Team 2013).

*Correlation between plankton abundance and BCIs of JB mussels*

To test whether mussel BCIs correlated with the changing phytoplankton abundance in Jakarta Bay, we conducted a simple linear regression with the BCIs of mussels at the sampling times in 2012 and 2013 as the dependent and phytoplankton abundance as the independent variable.

*Comparison of BCIs of juvenile mussels*

A Shapiro-test in R was used to test for normality of the BCI data of juvenile mussels (JB mussels: W = 0.979, p = 0.581; LB mussels: W = 0.989, p = 0.942). We then applied a T-test to test for significant differences between BCIs of mussels from JB and LB.

**Table 1**: Results from ANOVA. A) Influence of population and sampling time on the body condition index (BCI) of *Perna viridis* from Jakarta Bay and Lada Bay between April 2012 and November 2013. Data were log-transformed. B) Influence of habitat and origin on the BCI and C) on the change in BCI of *P. viridis* 2 months after reciprocal transplantations between Jakarta Bay and Lada Bay.

| | | **df** | **SS** | **MS** | **F** | **p** |
|---|---|---|---|---|---|---|
| A) | Sampling time | 1 | 16.36 | 16.36 | 270.1 | < 0.001 |
| | Population | 1 | 40.22 | 40.22 | 663.9 | < 0.001 |
| | Sampling time : Population | 1 | 3.32 | 3.32 | 54.8 | < 0.001 |
| | Residuals | 729 | 44.16 | 0.06 | | |
| B) | Origin | 1 | 0.0016 | 0.0016 | 3.13 | 0.1 |
| | Habitat | 1 | 0.0024 | 0.0024 | 4.78 | < 0.05 |
| | Origin : Habitat | 1 | 0.0045 | 0.0045 | 8.92 | < 0.01 |
| | Residuals | 16 | 0.008 | 0.0005 | | |
| C) | Origin | 1 | 0.0117 | 0.0117 | 23.27 | < 0.01 |
| | Habitat | 1 | 0.0024 | 0.0024 | 4.78 | < 0.05 |
| | Origin : Habitat | 1 | 0.0044 | 0.0044 | 8.92 | < 0.01 |
| | Residuals | 16 | 0.008 | 0.0005 | | |

*BCIs after re-collection of transplanted adult mussels*

BCI data of the mussels that were re-collected from the nets two months after transplantation were tested for a normality of errors using a residual plot, while a Fligner-Killeen test was used to verify whether the variances were homogenous. A two-factorial ANOVA with the factors *'Origin'* (with 2 levels: 'JB' and 'LB') and *'Habitat'* (with 2 levels: 'Impacted' and

'Natural') was used to test for the differences in BCIs between groups. A subsequent Tukey's HSD posthoc test identified which group means differed. The same tests with the same factor combinations were applied to the data of the change in BCI from the day of transplantation to the day of re-collection.

*Survival during the hypoxia tests*

Mussel survival rates during the 14 days of the hypoxia test were compared between groups with Cox proportional hazard (Coxph) survival analysis in the "survival" package (Therneau 2013). This method is based on the comparison of hazard functions, i.e. the instantaneous risk of death of an individual that belongs to a certain group, and allows applying multi-factorial designs to survival data. Assuming proportional hazards (ph) between groups, the ratio of hazard rates $hr_{group1}/\ hr_{group2}$ = *exponentiated coefficient* (exp. coef.) of two groups allows a straightforward interpretation of the risk of death. If exp. coef. is greater than 1, it indicates an increased risk of death for individuals that belong to the group represented in the numerator of the ratio, whereas a ratio smaller than 1 means a lower risk for this group (Mills, 2011). For the hypoxia experiment with juvenile mussels, a fully crossed, two-factorial design with the factors '*Origin'* (with the levels 'JB' and 'LB') and '*Treatment*' (with the levels 'Hypoxia', 'Low oxygen' and 'Normoxia') was applied to the survival data, whereas in the hypoxia test after the reciprocal transplantations the factors were '*Origin'* (with the levels 'JB' and 'LB') and '*Habitat'* (with the levels 'Impacted' and 'Natural'). We tested whether the models fulfilled the assumption of proportional hazards by using the cox.zph function in R (Mills, 2011).

## Results

*Habitat characteristics: Nutrients, temperature, dissolved oxygen and salinity*

Nitrite, ammonia and ortho-phosphate concentrations were generally higher and more variable at the impacted site in Jakarta Bay (JB) than at the natural site in Lada Bay (LB) (Figure 1). The highest concentrations of the three nutrients were found in JB during the dry seasons of 2012 and 2013, while at both sites the lowest were observed during the transition season, i.e. the period between the end of the rainy (February) and the beginning of the dry season (June) (Siregar & Koropitan, 2013), in March 2013. Nitrate concentrations did not change with

season or site. Similarly, average water temperatures also did not differ between JB and LB (Table 2). Oxygen concentrations were generally lower in JB than in LB: At 10 occasions, oxygen saturation in JB was even below 50% (= 3.25 mg/l DO at Sal = 32 and T =28°C) and the conditions were hypoxic (DO < 2 mg/l) in 5 of these cases. The lowest oxygen concentrations (0.31 mg/l and 0.88 mg/l DO) were measured on two consecutive days in July 2013 in JB. In LB, oxygen saturation was always near 100%, while the lowest value (5.18 mg/l DO = 80%, Sal = 28, T = 29.5°C) was measured in June 2013 (Table 2). Salinity was similar at both sites at most sampling days but differed with regard to the minimum value, which was 24 in JB in July 2013 and 15 in LB in December 2012.

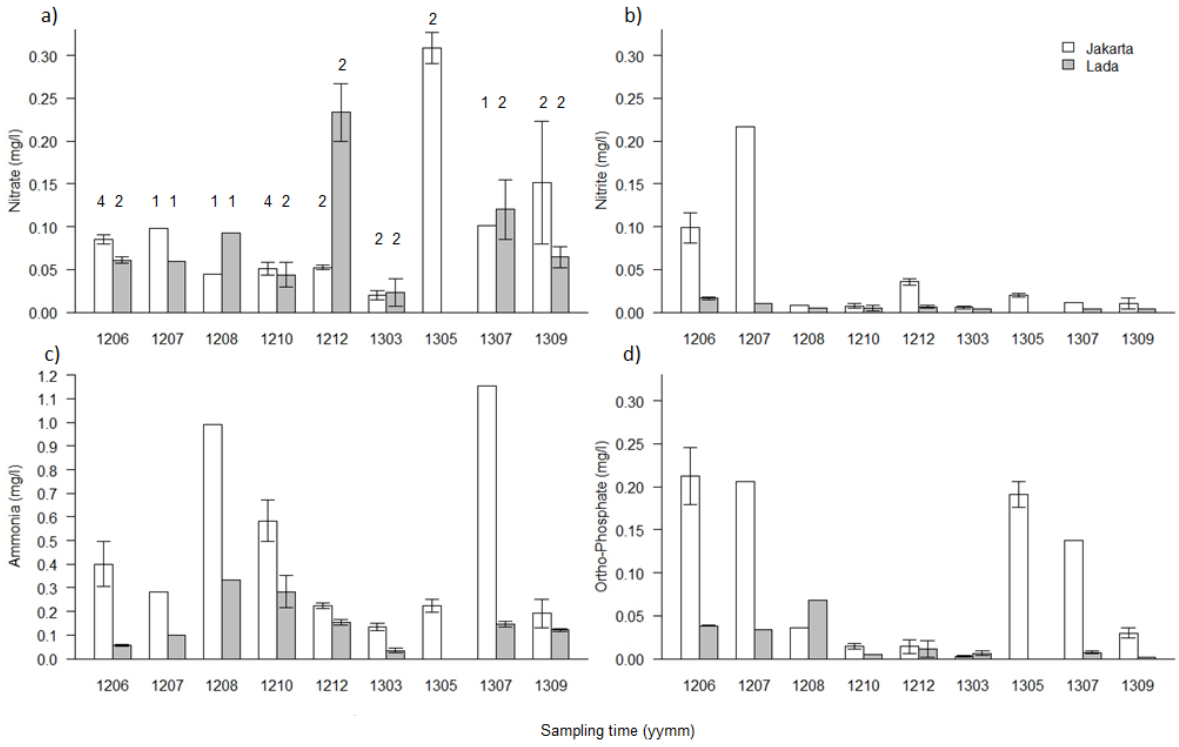


**Fig. 1** Nutrient concentrations in surface waters of Jakarta Bay and Lada Bay between June 2012 and September 2013. a) Nitrate, b) Nitrite, c) Ammonia, d) Ortho-phosphate. The number of replicates per site and sampling event is indicated above the respective bars in A. Bars show means and 95% CIs in case n > 1

*Habitat characteristics: Phytoplankton abundance and composition*

Phytoplankton abundances largely varied between sampling dates as well as between sites (Figure 2). In 7 out of 9 between-site comparisons, phytoplankton was 2- 21 times more abundant in the impacted JB than in the natural LB. The number of plankton units was on average 7 times higher in JB than in LB. However, on 2 sampling occasions, in March and in May 2013, the plankton abundance was 52 and 2 times higher in LB, respectively. Samples from the impacted JB (n = 27) contained mostly diatoms (78%, 12 genera) and were generally dominated by *Skeletonema costatum* (on average 86% of all diatoms). In contrast to this, the diatom communities in LB (n = 19) were dominated by several species belonging to 5 out of 15 identified genera: *Pleurosigma*, *Nitzschia*, *Chaetoceros*, *Rhizosolenia* and *Thalassiotrix*. On average, *Skeletonema* species represented only 16% of all diatoms in this system. However, in March 2013, the phytoplankton communities in LB were also dominated by *S. costatum*. On average, 22% of the plankton cells sampled in JB were dinoflagellates, mostly represented by *Ceratium furca* and *Peridinium spp*. Furthermore, *Prorocentrum micans* and *Dinophysis caudata* were common as well. After July 2013, an increase in *Peridinium* numbers was observed in JB that culminated in a bloom in November 2013, while *Skeletonema*, for the first time, was absent from all samples. Another dinoflagellate bloom occurred in the impacted JB in October 2012 and on this occasion up to 55% of the dinoflagellates belonged to the genus *Gymnodinium*, which contains potentially toxic species. In the natural LB, the proportion of dinoflagellates was lower: the samples consisted on average of 87% diatoms and 13% dinoflagellates (n = 19). The dinoflagellates found here belonged to the genera *Dinophysis*, *Peridinium*, *Ceratium* and *Prorocentrum*. In LB, no dinoflagellate blooms were observed during the study period. In general, phytoplankton abundances in the impacted JB were higher in 2012 than in 2013, whereas in LB no such trend was observed.

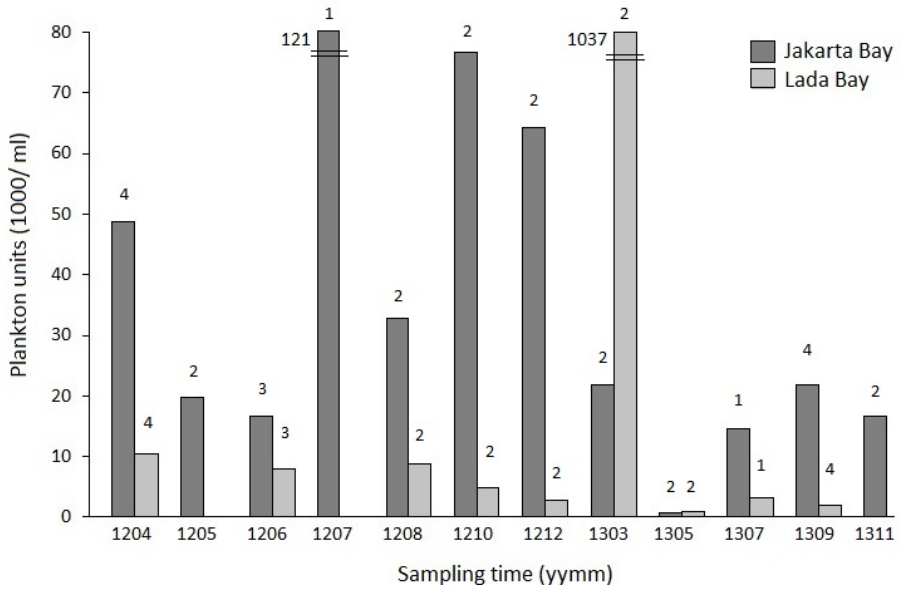


**Fig. 2** Number of plankton units in samples collected from Jakarta Bay and Lada Bay between April 2012 and November 2013. Plankton units are shown in 1000 per ml sample. The number of samples per sample site and time is indicated above the respective bars

*Habitat characteristics: Nutritional status of mussels*

BCIs of mussels that were sampled bimonthly between April 2012 and November 2013 followed an annual cycle with maximum values in August. This pattern emerged at both sites but mussel biomass was consistently higher in JB than in LB (Table 1, Figure 3). Only in September 2013, BCIs of mussels from both populations were identical. On all other occasions, mussels from the impacted JB showed a 1.3 to 2 times higher BCI than those from the natural LB. In general, the difference in BCIs between the two populations was larger in 2012 than in 2013. This was due to the fact that, although BCIs declined at both sites in the first half of 2013, the loss in biomass was stronger in mussels from JB. Their BCI in 2012 was twice the value as in 2013 (Figure 3). In JB mussels, BCIs were a positive linear function of phytoplankton abundance in the respective sampling months (simple linear regression: $F = 5.4$, r-squared = 0.38, $p < 0.05$).

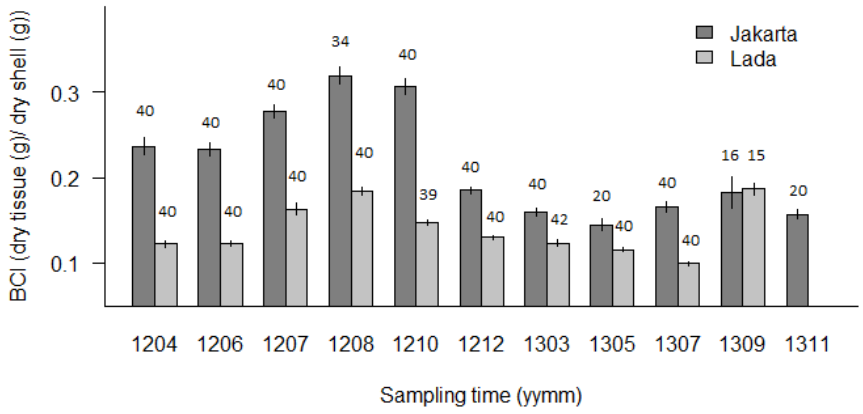


**Fig. 3** Body condition indices (means and 95% CIs, n= 746) of *Perna viridis* individuals collected from Jakarta Bay and Lada Bay between April 2012 and November 2013. The number of samples per population and time is indicated above the respective bars

*Hypoxia tolerance in juvenile mussels*

In August 2012, juvenile *P. viridis* from JB and LB differed significantly in their tolerance to low oxygen concentrations and hypoxia. Mussels from JB survived on average 1.6 days longer under low oxygen conditions (mean survival time: JB = 12.6 d; LB = 11.0 d) and 3.1 days longer under hypoxia (mean survival time: JB = 12.1 d; LB= 8.9 d) than LB mussels (coxph survival analysis, exp. coef.$_{\text{Lada,}}$ = 2.34, $p < 0.01$, n = 90). Under normoxia, mean survival times were 13.7 (JB) and 13.3 (LB) days and hence did not differ significantly between the populations (Figure 5). Not surprisingly, oxygen availability had a significant effect on survival. The risk of death was highest in the hypoxia groups and still higher under low oxygen than under normoxic conditions (coxph survival analysis, exp.coef.$_{\text{low oxygen}}$ = 3.11, p = 0.02; exp. coef.$_{\text{hypoxia}}$ = 6.35, $p < 0.001$; n = 90). The overall Cox model was significant (Likelihood ratio test = 24.37, df = 3, $p < 0.001$). BCIs of juvenile mussels differed significantly between the populations at the start of the experiment (T = 7.6, $p < 0.001$, n = 90). Mussels from JB had 16% higher BCIs than mussels from LB (Figure 4).

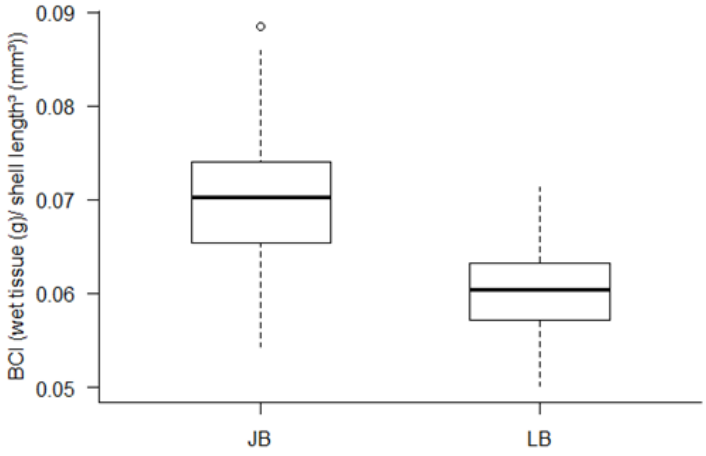


**Fig. 4** Body condition indices (median, inter-quartile range (IQR), 1.5* IQR) of juvenile mussels *Perna viridis* from Jakarta Bay (JB) and Lada Bay (LB) before the hypoxia experiment in August 2012 (n = 90)

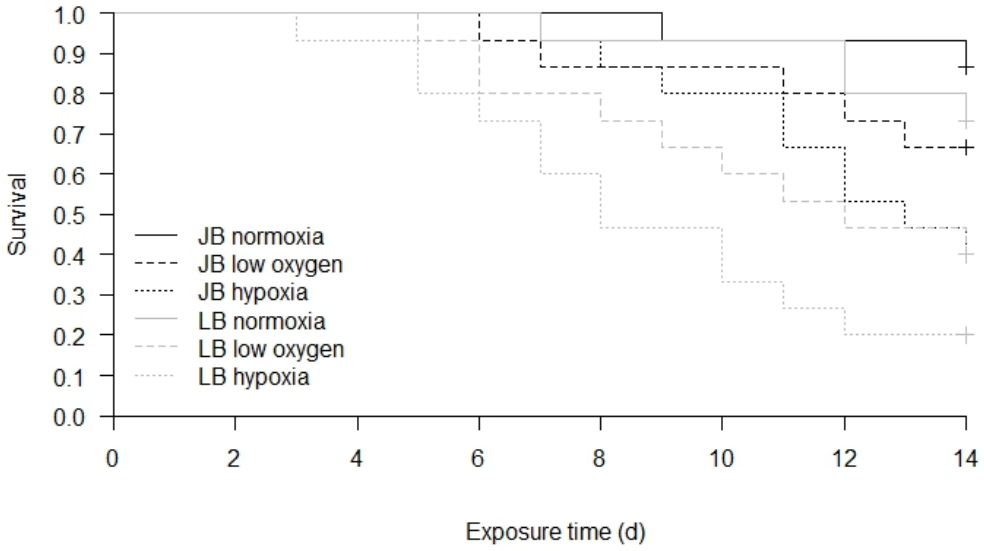


**Fig. 5** Survival of juvenile *Perna viridis* from Jakarta Bay (JB, black lines, n = 45) and Lada Bay (LB, grey lines, n = 45) under normoxia (> 5 mg/l DO), low oxygen (1 mg/l DO) and hypoxia (0.5 mg/l DO)

*Nutritional status of adult mussels 2 months after reciprocal transplantation*

Two months after transplantation, mean BCIs of adult mussels that, irrespective of their origin, had spent the time since translocation in LB (i.e. factor 'Habitat', table 1B) were 13% higher (pooled groups LB-LB and JBLB, mean BCI = 0.173) than those of mussels that were in JB (pooled groups LB-JB and JBJB, mean BCI = 0.151), while BCIs of mussels that,

irrespective of the habitat where they spent the time since transplantation, originated from JB (i.e. factor 'Origin', table 1B) were 10% higher (pooled groups JB-JB and JB-LB, mean BCI = 0.171) than of conspecifics that originated from LB (pooled groups LB-JB and LB-LB mean BCI = 0.154) . However, only the main effect of 'Habitat' and the interaction 'Habitat' x 'Origin' were significant (Table 1B). The group of mussels that had been translocated from LB to JB had a lower BCI than all other groups (Tukey's HSD $p \leq 0.05$, Figure 6a). This presumably explains the significant interaction between 'Habitat' and 'Origin' (Table 1B). Mean BCIs were 0.175 for the group JB-JB (n = 5), 0.128 for LB-JB (n = 5), 0.179 for LB-LB (n = 5) and 0.167 for JB-LB (n = 5). The change in BCI differed significantly with regard to where the mussels originated from, where they were transplanted to and with regard to the combination of both (Table 1C). During the residence time, both groups that originated from LB experienced an increase in BCI, whereas the BCI of mussels originating from JB did not change. The only group that significantly differed from all others was LB-LB (Tukey's HSD $p \leq 0.05$, Figure 6b).

*Tolerance to hypoxia 2 months after reciprocal transplantation*

Survival under hypoxia in transplanted mussels was to a small degree influenced by their origin (exp. $coef._{Lada} = 0.38$, $p < 0.01$, n = 80) and to a much larger degree by the habitat they last lived in (exp. $coef._{Lada} = 0.11$, $p < 0.001$, n = 80) (Figure 7). Eleven percent of the variance in mortality rates was explained by the factor 'Origin' and mean time to death was 0.6 days shorter among mussels originating from JB than in those from LB. At the same time, 44% of the variance was explained by the factor 'Habitat'. Here, mean time to death was 2.8 days shorter in mussels that had last resided in JB than in those that were in LB. So, both, originating from the impacted site or having resided there before the stress event made mussels more susceptible to hypoxia. This is confirmed by the fact that LB mussels (LB-JB) had higher survival rates at the impacted site than mussels that originated from there (JB-JB). Surprisingly, mussels that were transported to LB (JB-LB) had higher survival rates than those that came from there (LB-LB). This resulted in a significant interaction between the factors 'Habitat' and 'Origin' ($exp.coef._{Lada:Lada} = 2.8$, $p < 0.05$, n = 80). The overall Cox model was also significant (Likelihood ratio test = 43.02, df = 3, $p < 0.001$).

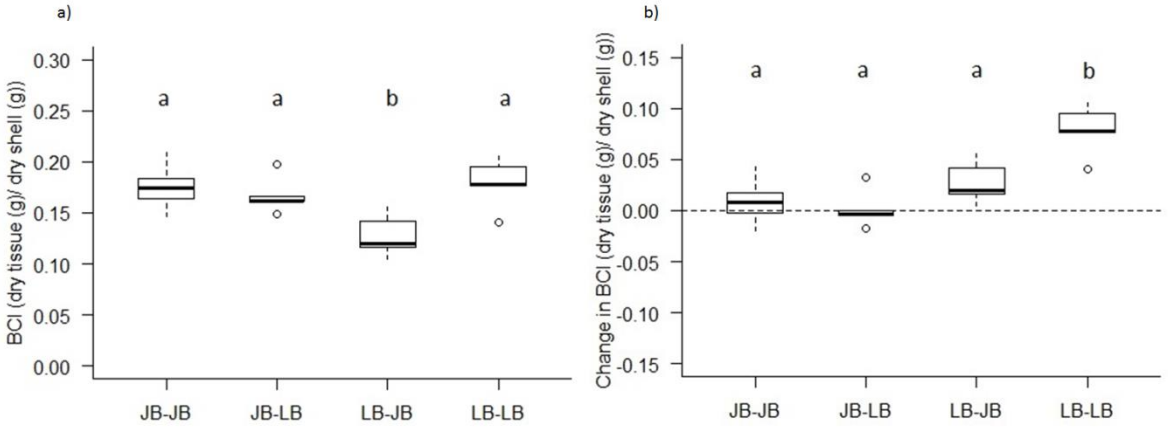


**Fig. 6** a) Body condition indices *BCIs* and b) Change in BCIs (median, inter-quartile range (IQR), 1.5* IQR) of *Perna viridis* 2 months after transplantation in September 2013. JB-JB = mussels transplanted within Jakarta Bay (n = 5), LB-JB = mussels transplanted from Lada Bay to Jakarta Bay (n = 5), LB-LB = mussels transplanted within Lada Bay (n = 5), JB-LB = mussels transplanted from Jakarta Bay to Lada Bay (n= 5). Different lowercase letters indicate significant differences between groups (Tukey's HSD posthoc test, $p \leq 0.05$)

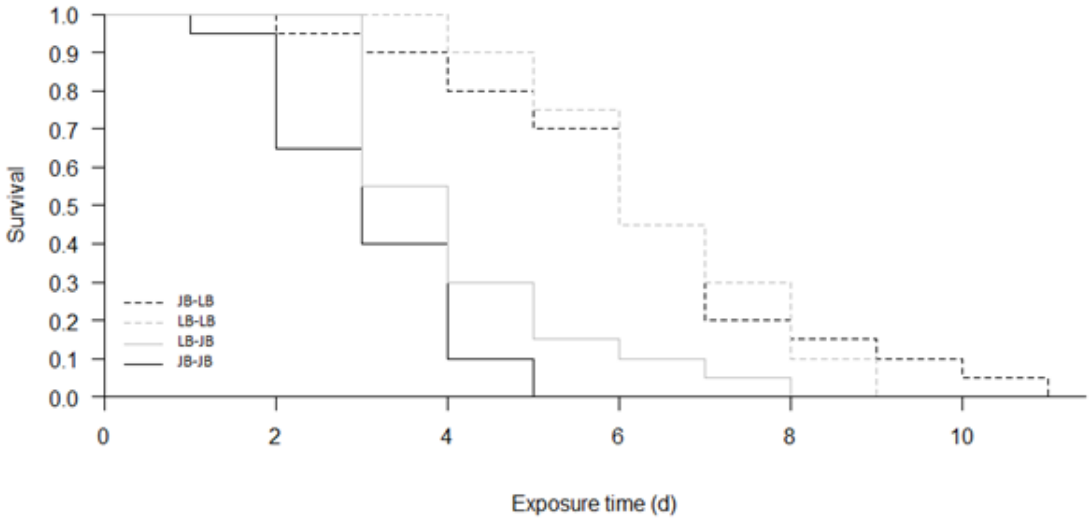


**Fig. 7** Survival of *Perna viridis* under hypoxia (0.5 mg/l DO) in the laboratory. Mussels had been transplanted within (JB-JB, n = 20; LB-LB, n = 20) and between (JB-LB, n = 20; LB-JB, n = 20) Jakarta Bay (JB) and Lada Bay (LB) 2 months prior to the hypoxia test

**Table 2**: Water temperature, dissolved oxygen and salinity between April 2012 and November 2013 at 13 different locations in Jakarta Bay (JB) and 8 different locations in Lada Bay (LB). Lada Bay values are highlighted in grey, oxygen concentrations below 50% saturation are in bold print. Measurements were conducted within 0 and 2 m below the sea surface. NA = no available data.

| Date | Location | Coordinates (Lat/ Long) | | T (°C) | DO (mg/l) | Sal |
|---|---|---|---|---|---|---|
| 19.04.2012 | JB1 | S06°04.628' | E106°43.758' | 30.0 | **1.66** | 32 |
| 19.04.2012 | JB2 | S06°04.044' | E106°44.344' | 30.5 | 4.16 | 32 |
| 19.04.2012 | JB3 | S06°04.230' | E106°44.226' | 30.9 | **2.69** | 34 |
| 19.04.2012 | JB4 | S06°04.028' | E106°43.924' | 31.4 | **2.41** | 33 |
| 30.04.2012 | LB1 | S06°29.674' | E105°46.780' | 29.3 | 5.61 | 34 |
| 30.04.2012 | LB2 | S06°29.643' | E105°46.711' | 29.3 | 5.72 | 35 |
| 30.04.2012 | LB3 | S06°29.749' | E105°46.834' | 28.7 | 5.90 | 35 |
| 26.06.2012 | LB6 | S06°29.681' | E105°46.785' | 29.5 | NA | 33 |
| 26.06.2012 | LB7 | S06°29.717' | E105°46.918' | 30.0 | NA | 33 |
| 06.07.2012 | JB9 | S06°04.509' | E106°44.027' | 29.5 | NA | 32 |
| 14.07.2012 | LB6 | S06°29.681' | E105°46.785' | 28.0 | NA | 34 |
| 14.07.2012 | JB11 | S06°04.492' | E106°43.834' | 29.5 | NA | 32 |
| 14.08.2012 | JB11 | S06°04.492' | E106°43.834' | 30.0 | NA | 30 |
| 15.08.2012 | LB6 | S06°29.681' | E105°46.785' | 26.5 | NA | 35 |
| 27.09.2012 | JB10 | S06°03.646' | E106°44.696' | 29.1 | 3.44 | 34 |
| 27.09.2012 | JB11 | S06°04.492' | E106°43.834' | 29.4 | **3.11** | 34 |
| 06.10.2012 | LB8 | S06°29.814' | E105°46.765' | 28.0 | 6.24 | 35 |
| 30.10.2012 | JB11 | S06°04.492' | E106°43.834' | 31.1 | 3.73 | 34 |
| 08.12.2012 | JB12 | S06°04.451' | E106°44.186' | 27.9 | **2.73** | 33 |
| 08.12.2012 | JB11 | S06°04.492' | E106°43.834' | 28.8 | 3.60 | NA |
| 09.12.2012 | LB8 | S06°29.814' | E105°46.765' | 29.6 | 5.40 | 15 |
| 09.12.2012 | LB7 | S06°29.717' | E105°46.918' | 30.7 | 5.43 | 20 |
| 02.03.2013 | JB10 | S06°03.646' | E106°44.696' | 28.9 | 5.11 | 33 |
| 02.03.2013 | JB13 | S06°04.626' | E106°43.814' | 29.2 | 5.83 | 33 |
| 14.03.2013 | LB10 | S06°29.811' | E105°47.100' | NA | 7.89 | 31 |
| 29.05.2013 | JB14 | S06°04.663' | E106°44.022' | 30.6 | **1.90** | 31 |
| 29.05.2013 | JB15 | S06°04.567' | E106°43.814' | 30.6 | **1.60** | 31 |
| 07.06.2013 | LB11 | S06°30.276' | E105°40.846' | 29.5 | 5.18 | 28 |
| 07.07.2013 | JB13 | S06°04.617 | E106°43.798' | 27.4 | **0.31** | 24 |
| 07.07.2013 | LB11 | S06°30.276' | E105°40.846' | 30.2 | 7.95 | NA |
| 08.07.2013 | JB15 | S06°04.567' | E106°43.814' | 28.3 | **0.88** | 25 |
| 09.09.2013 | LB11 | S06°30.276' | E105°40.846' | 27.9 | 7.20 | 32 |
| 10.09.2013 | JB17 | S06°04.966' | E106°43.770' | 31.1 | 7.20 | 29 |
| 13.11.2013 | JB18 | S06°04.371' | E106°44.204' | 29.6 | **3.13** | 31 |

## Discussion

The results from our reciprocal transplantation experiments suggest that hypoxia tolerance in the mussel populations we tested is only to a small degree determined by local adaptations to previously experienced stress, but rather depends on the environmental conditions in the habitats where the animals resided before the hypoxia event. Furthermore, we found strong correlations between the abundance of phytoplankton and the nutritional status of the Asian green mussel *Perna viridis* as well as between the mussels' nutritional status and their ability to survive hypoxia in the laboratory.

The period during which we monitored environmental conditions at the impacted site in Jakarta Bay (JB) can be divided into two phases that were distinctively different with regard to the composition and abundance of phytoplankton. This pattern is important for the interpretation of our findings. During the first phase, from April to December 2012, phytoplankton was on average 3.6 times more abundant than during the second phase that lasted from March to November 2013. In 2012, especially small chain forming diatoms, such as *Skeletonema costatum*, were very common, whereas in 2013 diatoms were rare and dinoflagellates were abundant. *P. viridis* has a higher retention efficiency for small (< 12 µm) than for large food particles (Hawkins et al., 1998), which should make the uptake of small diatoms, such as *S. costatum*, more worthwhile than ingesting dinoflagellates or pennate diatoms (Holmes et al., 1999). Since the latter were more common in 2013, food supply for *P. viridis* was presumably better in 2012. This notion is supported by the fact that the change in phytoplankton abundance and composition from 2012 to 2013 was paralleled by a change in the body condition index (BCI) of JB mussels, which was twice as high in 2012 as in 2013. Again, this observation was in accordance with hypoxia tolerance in juvenile mussels, which in 2012 was higher in JB than in LB individuals. Interestingly, in 2013, tolerance was higher in adult individuals that had resided in LB before the stress trials, which is in contrast to the 2012 experiments and to the study by Wendling et al. (2013) that was conducted in 2009/2010. In both, higher BCIs and a higher tolerance to hypoxia were found in JB mussels. Nevertheless, the new results are in line with the finding that a good nutritional status is relevant for stress tolerance as, in our transplantation experiment, the BCIs of the mussels that last resided in LB and were more robust, were higher than those of mussels from JB. Taking these findings together indicates that only if the nutritional status is higher in JB mussels, they also have a higher hypoxia tolerance than conspecifics from LB. This suggests that in the JB population, selection for hypoxia-tolerant genotypes did either not occur or that the effect was rather small and was overruled by the effect of food supply.

*The influence of environmental conditions on the nutritional status of Perna viridis*

We found substantial differences in environmental conditions between the two sites we chose for this study. These were in accordance with previously collected data from the same region. We confirmed reports about low oxygen concentrations (Arifin, 2006; Thoha et al., 2007; Jalius et al., 2008), high concentrations of inorganic nutrients (Damar, 2003; Thoha et al., 2007) and the occurrence of eutrophication-induced phytoplankton blooms (Wouthuyzen et al., 2008) from Jakarta Bay. This indicates that these conditions prevail permanently and can be considered characteristic for the bay. Moreover, in accordance with findings of Jalius et al. (2008), we observed that the concentrations of ammonia, nitrite and phosphate were generally higher and more variable in the impacted JB than in the natural LB.

The nutritional status of the mussels we collected at the two sites correlated strongly with the prevailing environmental conditions - most of all with phytoplankton abundance. It was distinctively higher in JB than in LB, especially from July to December 2012. This is in accordance with the observed difference in the nutritional status between JB and LB mussel populations: In 9 out of 10 comparisons BCIs were higher in individuals from JB than in animals from LB. Only in September 2013, no difference between the two populations was found. This presumably reflected the coincidence of a general decrease in the nutritional status of JB mussels from October 2012 to July 2013 and a sudden increase in the BCIs of LB individuals in September 2013. The decline in BCIs in JB mussels in 2013 matches perfectly with the change in phytoplankton densities at this site, which decreased after October 2012 and 30% of the variation in BCIs over time was explained by the phytoplankton abundance. In contrast to this, the abrupt change in mussel BCI in LB is not at all plausible, because it was not paralleled by a phytoplankton bloom. We can therefore not offer an explanation for this pattern.

In addition to food availability, site-specific stressors, which can act separately or in concert, could have influenced the energy budget and hence the nutritional status of the mussels: fluctuations in $pCO_2$ and pH, desalination, hypoxia, thermal stress or harmful algae blooms (HAB). Growth and shell integrity in the blue mussel *Mytilus edulis*, for example, are negatively influenced by high $pCO_2$ (Melzner et al., 2011), while the same stressor reduces calcium deposition and weight in *M. chilensis* (Duarte et al., 2014). Low oxygen concentrations (1.5 mg/l and 3 mg/l DO) and low salinities (15 and 20) lead to a reduced shell and tissue growth in *P. viridis* (Wang et al., 2011), while thermal stress is energetically costly for *M. galloprovincialis* since it increases the production of heat shock proteins (HSP) and causes metabolic depression (Anestis et al., 2007). Furthermore, the scope for growth in *P.*

*viridis* is limited when the mussel is exposed to dinoflagellates, such as *Alexandrium tamarense*, that produce toxins responsible for paralytic shellfish poisoning (PSP) (Li et al., 2002). Therefore, the fact that the mussels from JB showed significantly lower BCIs in 2013 than in 2012 must not exclusively be the consequence of lower phytoplankton abundances, but could also be due to more intense abiotic stress in 2013. The latter is supported by the observation that the lowest oxygen concentrations during the study period were measured in May and July 2013 (Table 2). Since *P. viridis* responds to hypoxia with valve closure and metabolic depression - a process that consumes the mussel's resources - (Wang et al., 2011), the low oxygen concentrations in JB may have further impaired the mussels' performance in 2013.

A third factor that could have influenced both, the decrease in phytoplankton densities and the abiotic environmental conditions in JB in 2013 possibly was the beginning of a land reclamation project. For the development of the residential area PT. Kapuk Niaga Indah (KNI) on reclaimed land (Jury et al., 2011), half of the bamboo settlement structures in the mussel culture area of Muara Kamal, where we collected the JB specimens for this study, were removed in early 2013. The project involved dredging activities near the remaining culture sites, which probably resulted in a release of toxic substances such as Cu, Cr, Pb and Zn (Takarina & Adiwibowo, 2011) and other pollutants from the sediment, as well as in a strong increase in turbidity and sedimentation. High turbidity and sedimentation have a negative influence on the primary production in JB (Siregar & Koropitan, 2013) and a high ratio of total particulate matter to particulate organic matter has a negative effect on growth in bivalves (Galimany et al., 2011). With a human population growth of 6.5% from 2010 to 2014 (http://worldpopulationreview.com, accessed 02.07.2015), Jakarta Bay is experiencing an increasing anthropogenic pressure that affects the amount of pollutants discharged into the sea. Apart from industrial pollution, domestic pollutants are becoming more significant: For example, compounds of insect repellents, such as *N*,*N*-diethyl-*m*-toluamide (DEET) and linear alkylbenzenes (LABs), another indicator of domestic pollution, are found in high concentrations in Jakarta Bay (Rinawati et al., 2012; Dsikowitzky et al., 2014).

*Adaptation versus food supply: What drives hypoxia tolerance in mussels?*

Two months after the transplantation, mussel tolerance to oxygen deprivation depended more on the characteristics of the environment where they had last lived in (44% of the variation in mortality rates) and less on the quality of their original habitat (11%). Higher tolerance to

hypoxia was found among mussels that last resided in LB, no matter whether they originally came from JB or LB. This finding suggests that local adaptations to specific prevailing stressors are of rather little relevance for hypoxia tolerance in *P. viridis*. Possibly, phenotypic plasticity in the tested mussels was more relevant. Phenotypic responses to stress can be the activation of HSPs upon exposure to thermal stress, hypoxia and low salinities (Lockwood et al. 2010; Monari et al., 2011) or behavioral responses, such as valve closure and reduction in filtration rates (Vakily, 1992; Li et al., 2002). All these mechanisms are energetically costly and compromise energy resources. The heat shock response, for example, can consume between 20 and 25% of the energy budget of blue mussels, *M. edulis* (Anestis et al., 2007) and the hypoxia- or heavy metal- induced damage of lysosomal membranes in digestive diverticula impairs the efficiency of energy uptake in *P. viridis* (Krishnakumar et al., 1990; Nicholson, 2003). Thus, individuals with a high nutritional status should survive environmental stress longer than individuals that are in a poor nutritional condition. The correlation between a good nutritional status and survival under hypoxia that we found in 2012 and the 2013 decline in hypoxia tolerance, which was paralleled by a decrease in BCIs among JB mussels, support this assumption. However, food availability alone does not determine the mussel BCIs and their hypoxia tolerance. As described above, the presence of environmental stress can diminish the energy reserves of mussels. The change in BCI in the different mussel groups during the residence period presumably means that the mussels that resided in JB were facing environmental stress, which could have been hypoxia, low pH, the presence of toxic plankton species, pollutants such as Cu, Cr, Pb, Zn and organochlorines and sedimentation. From Hong Kong, for example, it is known that growth in *P. viridis* is site-dependent and is highest in a clean habitat (Cheung, 1991). In the present study, BCIs of both mussel groups that resided in JB (LB-JB and JB-JB) did not increase from July to September 2013 (Figure 6b) even though the plankton abundance increased by the factor 1.5, whereas the mussels that came from LB and resided in LB doubled their BCIs even though the plankton abundance did not increase. Although we only measured snapshots of phytoplankton abundance, they correspond well with the overall trend that we observed in the bimonthly plankton counts from April 2012 until November 2013. This is further supported by the fact that the BCIs of the JB-LB group animals did not increase after their arrival in LB. Before they were transplanted there, the nutritional status of these mussels was twice as high as in their conspecifics from LB. However, plankton abundances in LB did obviously not suffice to maintain this difference. This implies that LB mussels are better acclimated to low food supply and possibly utilize limited energy resources better than mussels from JB. A better

utilization of food among LB mussels and the negative effects of additional stressors, e.g. dinoflagellate toxins or heavy metals acting additively or even synergistically (Crain et al., 2008) on the mussels residing in JB, can explain the better hypoxia tolerance in the JB-LB and LB-LB groups, which we observed in the lab.

Taking all our findings together an interesting image is revealed: If the nutritional status of mussels from the impacted habitat was higher than in conspecifics from the natural environment, these animals performed better under stress. However, if there was only a small or no difference in the nutritional status between populations, mussels from the natural habitat were more robust. The reason may be that adverse influences which prevailed in the impacted habitat compromised the energy reserves of resident mussels and reduced their capacity to tolerate hypoxia. Hence, only if eutrophication goes along with a high availability of energy (i.e. food) it allows filter feeders to compensate for adverse effects such as hypoxia.

The question remains why a long history of environmental stress did not lead to local adaptations in *P. viridis* from JB or, if it did, why no such effects were detectable. One reason could be the complexity of the physiological response to hypoxia in Mytilids. In *M. galloprovincialis*, for example, 107 genes are differentially expressed in response to low oxygen concentrations and they are involved in a plethora of pathways and functions, such as protein regulation, heat shock response, ribosome biogenesis, carbohydrate transport and -metabolism, and ATP synthesis (Woo et al., 2013). Most of these genes are involved in the regulation of basic cellular functions and therefore are highly conserved. Thus, a directional selection by a single environmental driver, such as hypoxia, seems unlikely (Walsh & Blows, 2009). Another reason may be the variability of environmental conditions in JB. This should favor a high standing genetic variance, i.e. a high number of alleles and high heterozygosity, over directional selection. Walsh and Blows (2009), e.g., review cases where an abundant genetic variance in a population could be maintained despite of the existence of strong selective forces, and explain this with the fact that pleiotropy, the influence of one gene on multiple traits, is generally much more common than independent traits are. So far, the genetic diversity of populations of the Asian green mussel inhabiting sites in Indonesia, which differ largely in habitat quality, has not been investigated. From the Malaysian peninsular, however, high heterozygosity in *P. viridis* from contaminated sites (Yap et al., 2002) and an overall high genetic similarity between mussel populations inhabiting different habitats are known (Ong et al., 2009). This suggests that in these populations phenotypic plasticity rather than local adaptation helps to overcome environmental stress.

*Mussel fitness and potential of spread in human-influenced habitats*

Our results suggest that the effects of human-induced environmental change on *P. viridis* do not produce highly robust mussel populations via stress-driven selection and local adaptations but can be very ambivalent: Eutrophication, when leading to blooms of target food species, can, on the one hand, enhance energy reserves and by this increase mussel tolerance to stress and hence their fitness. However, on the other hand, if microbial degradation of blooms leads to hypoxia, the surplus in energy that was gained through increased food supply may get consumed by the compensation of stress effects. Furthermore, the hypoxia-induced decline in energy reserves can be intensified by the simultaneous occurrence of other stressors such as the bloom of harmful algae, exposure to heavy metals, high ammonia concentrations and organic pollutants. Therefore, the survival of the generally robust and widely-distributed *P. viridis* in anthropogenic impacted habitats should depend on the interplay between a) the availability of energy, b) the intensity of environmental stress and c) mussel capacity to tolerate adverse conditions. The latter seems to be mainly determined by phenotypic plasticity, which, in turn, needs to be facilitated by ample energy reserves, but only to a very limited extend by local adaptation to environmental stress. This assumption, if true, would have two important consequences: First, if the competitiveness of *P. viridis* is primarily driven by immediate energy availability, the evolution of highly resistant mussel populations, with a high potential for invasions, in eutrophic and highly impacted areas is unlikely. Furthermore, population sizes of the mussel that has become an important food source in anthropogenic influenced coastal areas should decline if habitat quality changes from eutrophic and hypoxic to eutrophic, hypoxic and polluted as it is increasingly the case in worldwide coastal metropolitan areas. This is especially relevant for Asian coastal megacities where a rapidly growing human population causes an increasing input of domestic pollutants into the sea. With this study, we demonstrate how a model mariculture organism that, so far, seemed to benefit from human-made eutrophication can also become a victim of anthropogenic impact. The increasing industrial and domestic pollution that adds to the already prevailing eutrophication in coastal waters near megacities may therefore harm even very robust mariculture organisms and reduce the production of animal biomass for human consumption.

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Chapter 3

# Tolerance to stress differs between Asian green mussels *Perna viridis* from the impacted Jakarta Bay and from natural habitats along the coast of West Java

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## Abstract

It is an open question whether adverse habitat conditions, characteristic for many anthropogenically impacted coastal habitats, can determine resistance to abiotic stress in populations of residing invertebrates. We tested experimentally for differences in stress tolerance between individuals of the Asian green mussel *Perna viridis* stemming from the heavily impacted Jakarta Bay and from two natural sites, Lada Bay and Pelabuhan Ratu, West Java. Mussel performance under hyposalinity and hypoxia was assessed in laboratory assays by measuring fitness-related response variables, e.g. body condition index, relative shell weight, byssus production, respiration rates and survival. We found stress-specific and population-specific differences in mussel resistance to adverse conditions: Individuals from the impacted Jakarta Bay performed better under hypoxia than their conspecifics from the natural sites, whereas the latter were more resistant to hyposalinity. We explain these differences by differential acclimation to environmental conditions in the respective habitats and by diverging degrees of food supply.

**Keywords:** Phenotypic plasticity, acclimation, hypoxia, hyposalinity, environmental stress, benthic invertebrates

## Introduction

Coastal ecosystems in growing metropolitan areas are facing an enormous pressure due to anthropogenic activities. One important impact is the influx of nutrients and pollutants from industrial and domestic sewage as well as from agriculture. In closed and semi-closed coastal habitats such as bays, fjords and lagoons, which have a limited water exchange with the open ocean, waste water discharges can have multiple negative effects on habitat quality. They can dilute the sea water and lead to brackish conditions, while inorganic and organic pollutants accumulate and may in the long term exceed critical concentrations (Siregar & Koropitan, 2013). Furthermore, nutrients cause eutrophication (Boyd & Hutchins, 2012) that goes along with an increase in the abundance of phytoplankton. Sinking algal blooms, in turn, and their decomposition by bacteria increase the frequency of hypoxia events as well as the extent of hypoxic zones (Diaz & Rosenberg, 2008). Furthermore, they may lead to changes in the pH of the sea water (Flynn et al., 2015). Finally, toxic algal blooms can exert a further severe stress. As an overall consequence, marine organisms in closed or semi-closed habitats that are prone to waste water discharge often experience multiple environmental stressors, which can severely reduce their fitness and survival (Crain, Kroeker, & Halpern, 2008).

The maintenance of fitness and survival in a changing and stressful environment is mediated by adaptation, i.e. an irreversible change in the genetic makeup of populations that results from the selection of certain genotypes, or through acclimation, i.e. a reversible, short-term change in the performance of an individual that results from phenotypic plasticity (Boyd & Hutchins 2012). In many invertebrate groups, acclimation to environmental stress, such as elevated temperatures or low dissolved oxygen, is to a large extent achieved through the production of heat shock proteins (hsps), which are chaperones that stabilise partially unfolded and reactivate damaged proteins (Anestis, Lazou, Pörtner, & Michaelidis, 2007; Chapple, 1998; Dutta, Mustafi, Raha, & Chakraborty, 2014, Tomanek 2002). Furthermore, in bivalves that have the ability to isolate themselves from a hostile environment, acclimatory plasticity is also evident in the adjustment of behaviours such as valve closure, which often goes along with a switch from aerobic to anaerobic respiration or a change in filtration rate (Braby & Somero, 2006; Vakily, 1992). However, all of the mechanisms that are associated

with acclimation limit the amount of metabolic energy that is available for life processes such as growth and defence. Exposure to environmental stress, e.g. acidification, hyposalinity and hypoxia, can therefore also impair morphological traits like shell formation in Mytilid mussels (Gazeau et al., 2010; Melzner et al., 2011) .

Despite these negative consequences, eutrophic conditions are also likely to have positive effects on marine organisms, especially on benthic filter feeders. Due to nutrient-promoted phytoplankton blooms, mussels from eutrophic habitats should have more food available and consequently have a better nutritional status than mussels from oligotrophic habitats. In Hong Kong, for example, food consumption and growth in *P. viridis* is highly site-dependent, differing with regard to the concentration of total particulate matter (Wong & Cheung, 2003). Similar to this, Wendling et al. (2013) found substantial differences in the nutritional status (body condition index, BCI) and in the ability to tolerate three environmental stressors, i.e. hyposalinity, hypoxia and elevated water temperatures, between *P. viridis* from the natural Lada Bay and the eutrophic and impacted Jakarta Bay. Mussels from Jakarta Bay showed higher BCIs and were consistently more robust. The authors suggested that the latter trait goes back to the fact that a good nutritional status presumably enhances the mussels' ability to compensate for the negative effects of environmental stress, but were, however, not able to verify this mechanism. If it is real, it would be an example for a positive feedback loop by which a human-induced habitat change such as eutrophication enables residing species to tolerate enhanced levels of environmental stress.

However, while some highly tolerant species may even benefit from the positive aspects of nutrient load, less tolerant organisms get removed from the affected habitats. In Jakarta Bay, 95 mollusc species got locally extinct between 1937 and 2005 (van Der Meij, Moolenbeek, & Hoeksema, 2009). So it is obvious that the environmental conditions in Jakarta Bay select for tolerant organisms and/or individuals and the area is therefore an interesting system to study the effects of anthropogenic pressures on benthic communities as well as on the populations of residing species such as the Asian green mussel *P. viridis*. For instance, by comparing the characteristics of *P. viridis* individuals from Jakarta Bay to those of conspecifics from more natural habitats, we may learn which mechanisms and traits allow mussels to thrive under adverse conditions. A further interesting question in this context is, whether the selection of robust genotypes and/or an enhanced acclimation capacity fosters only tolerance to the most prevailing stressor or whether it also mediates robustness towards other abiotic pressures. To answer the latter question, we tested whether individuals of *P. viridis* from the impacted Jakarta Bay are generally more tolerant towards hypoxia than

conspecifics from more natural environments and whether their tolerance is restricted to this site-specific stressor. For this, we compared six physiological and behavioural health indicators (BCI, relative shell weight, respiration rates, byssus production, shell closing behaviour, and acclimation capacity to low oxygen) between mussels from different populations (impacted versus natural sites) under hyposalinity, which is a common stressor in estuaries, and hypoxia that is associated with eutrophication and is typical for Jakarta Bay (Friedrich et al. 2014).

## Materials & Methods

### *Study sites*

Individuals of *Perna viridis* for this study were collected at three sites along the coasts of West Java, Indonesia. The first site, Jakarta Bay, Java Sea (06°04'S, 106°44'E), is characterized by a high anthropogenic impact and a high level of eutrophication (Siregar & Koropitan, 2013). The substantial input of organic pollutants such as polychlorinated biphenyls (PCBs) or polycyclic aromatic hydrocarbons (PAHs) (Rinawati et al., 2012) led to an elevated concentration of these pollutants in the bay, while the influx of phosphorus and nitrogen compounds, mainly through human sewage, frequently causes phytoplankton blooms followed by hypoxia events (Arifin, 2006). Salinity regimes in Jakarta Bay vary with distance to the various river mouths that enter the bay, but are generally comparable to other tropical estuarine ecosystems (Arifin, 2006). The other two collection sites were located in more natural environments at the Sunda Strait (Lada Bay, 06°29'S, 105°47'E), and in the South Java Sea (Pelabuhan Ratu, 06°59'S, 106°32'E). Waters at these sites are oligotrophic and hypoxic events are unknown (Radiarta, Albasri, & Sudradjat, 2011; Wati, 2003; Wendling et al., 2013). Salinity here can vary with season and with distance from shore (Huhn et al. accepted; Wati, 2003; Wendling et al., 2013). The main difference between the impacted and the two natural sites is the larger human population size in the catchment area of rivers that enter Jakarta Bay. The agglomeration in the Jakarta metropolitan area comprised 28 million inhabitants in 2010 (Central Statistical Bureau Jakarta, 2010), whereas the Pelabuhan Ratu district had a population of 96,000 and the Panimbang district, Lada Bay, of 49,000 in 2010 (http://www.citypopulation.de/php/indonesia, accessed 20.08.2015). Additionally, the catchment area of Jakarta Bay comprises the biggest industrial area in Indonesia, whereas in Lada Bay and Pelabuhan Ratu the only industries are coal power plants for local electricity

supply. They are located 12 km from the collection site in Lada Bay and 6 km from the collection site in Pelabuhan Ratu, respectively.

*Stress experiments*

In three different laboratory experiments, we tested for population-specific differences in tolerance to abiotic stress in individuals of *Perna viridis* from the impacted and from the two more natural sites. During the first experiment (in the following referred to as Experiment A) we choose hyposalinity as a stressor and measured byssus production, shell closing behaviour (the proportion of closed-valves-observations per individual) and the nutritional status of the mussels under ocean salinity (32) as well as hyposalinity (15) and compared these values between mussels from Jakarta Bay (impacted habitat) and Lada Bay (natural habitat). In the second experiment (Experiment B), the same populations were tested for differences in survival, byssus production, shell closing behaviour and respiration rates during and after exposure to hypoxia (0.5 mg $l^{-1}$ dissolved oxygen). Finally, in the third experiment (Experiment C), the impact of different feeding regimes (i.e. low and high food supply) and different oxygen acclimation regimes (2 mg $l^{-1}$ DO versus normoxia for 4 days) on survival and byssus production under hypoxia were assessed in mussels from Jakarta Bay (impacted habitat) and Pelabuhan Ratu (natural habitat). This two-factorial experiment had a fully crossed design, which for the analysis, was applied to mussels from the two populations separately (see "statistical analysis" for the details).

*Mussel collection*

Mussels were collected from bamboo settlement structures in the above mentioned habitats. In Jakarta Bay and Lada Bay, bamboo structures are deployed for mussel farming, whereas in Pelabuhan Ratu, settlement of mussels occurs on bamboo floats that serve as fishing platforms or lobster rearing facilities. Collection dates were 22$^{th}$ January 2012 for Jakarta Bay and 24$^{th}$ February 2012 for Lada Bay (Experiment A), 2$^{nd}$ March 2013 for Jakarta Bay and 14$^{th}$ March 2013 for Lada Bay (Experiment B), and 18$^{th}$ June and 17$^{th}$ July 2014 for Jakarta Bay and 18$^{th}$ August 2014 for Pelabuhan Ratu (Experiment C). Mussels were transported to the Bogor Agricultural University by car in 4 h from Jakarta Bay, in 5 h from Pelabuhan Ratu and in 6 h from Lada Bay. In 2012, mussels were transported in an insulation box filled with seawater,

which we previously cooled down to 20°C. The water was exchanged every 30 min. In 2013 and 2014, mussels were transported dry in a cooled insulation box. At the collection dates, we measured water surface temperatures (using a laboratory glass thermometer in 2012 and a WTW Oxical 3205 + CellOx 325 sensor in 2013 and 2014), salinity (using a handheld refractometer) and in 2013 and 2014 also oxygen concentrations (using a WTW Oxical 3205 + CellOx 325 sensor). To find out whether mussels that stem from different habitat types also differ with regard to their nutritional states and shell stability, 20 mussels per population and sampling event were collected and frozen immediately for later determination of BCIs and relative shell weight.

*Acclimatization to laboratory conditions*

Before the start of the Experiments A and B, mussels were acclimatized to laboratory conditions for 10 - 12 days. In case of Experiment C, however, the acclimation phase for Jakarta Bay mussels was 62 (low food supply group) and 32 days (high food supply group), respectively. This was done to obtain mussels that differ in their nutritional state. Since we wanted to avoid starving the Jakarta Bay mussels, which usually have a high nutritional status due to the eutrophic conditions in this environment, we kept them in the laboratory for a longer time span but did not reduce food supply during this period (62 days) so that the amount of food provided allowed the mussels to display normal filtration behaviour and metabolic activity, but was less than what is available in the eutrophic habitat of Jakarta Bay. Therefore, Jakarta Bay mussels could be kept alive under these conditions for several weeks but during this period their BCIs were slowly decreasing to a state that was then comparable to the BCIs of mussels from the natural, oligotrophic habitats. Furthermore, the long acclimatization phase before the start of Experiment C presumably excluded potentially existing acclimations to hypoxia among mussels from Jakarta Bay where low oxygen concentrations are common. During laboratory acclimatization, mussels were always kept in aerated seawater at a maximum density of 1 mussel per litre (Experiment A: 4 x 30 l aquaria à 30 mussels, Experiment B and C: 4 x 100 l aquaria à 70 mussels), at a temperature of 25 - 27°C and a salinity of 32 – 33. In 2012, mussels were fed with 0.007 ml and in 2013 with 0.0142 ml of an artificial filter feeder food, Sera Marin Coralliquid® (SMC) per individual and day, while in 2014 2 x $10^6$ cells of living phytoplankton, *Isochrysis galbana*, per mussel were given twice per day and additionally 0.011 ml SMC per mussel once per day. In all experiments, half of the water in the aquaria was replaced by seawater from a 1000 l storage

tank each day. The water in the storage tank was recycled constantly using biological filtration.

*Experimental design and setup*

*Experiment A*

In Experiment A, 30 mussels from each of both habitat types were exposed to two salinity regimes: ambient (32) and hyposalinity (15). During exposure mussels were fed with the same amount of SMC as during acclimatization to laboratory conditions and were placed separately in 1 l PVC containers with aerated seawater so that a single mussel in one container represented one experimental unit. The whole water volume per container was replaced daily by filtered seawater from the storage tank that we pre-adjusted to the respective salinity by adding non-chlorinated freshwater of drinking water quality. Fluctuations in the water temperature were kept to a minimum by placing the experimental containers in a water bath. This experiment lasted for 10 days, during which survival and the number of closed mussels were recorded daily. At the end of the experiment, byssus production within 24 h and the BCI of each mussel individual were determined.

*Experiment B*

In Experiment B, 40 mussel individuals from the impacted and 32 individuals from the natural habitat were singly assigned to experimental units that were 600 ml PVC containers. Again, one mussel in one container represented one experimental unit. Half of the mussels from each habitat were then kept under normoxic conditions by providing pressured air, whereas the other half was exposed to hypoxia (0.5 mg $l^{-1}$ DO). Seawater with the respective target oxygen concentration was prepared in a header tank by adding nitrogen gas that reduced the amount of dissolved oxygen. Whenever the target concentration of < 0.5 mg $l^{-1}$ (measured with an oxygen meter WTW Oxical 3205 + CellOx 325 sensor) was reached in the header tank, the containers were slowly filled with the deoxygenated water and the oxygen concentration in the containers was checked to assure that it was between 0.47 and 0.55 mg $l^{-1}$. The containers were then closed with a lid equipped with an integrated rubber sealant and were not opened until the water exchange on the next day - when the entire water volume was exchanged using the same procedure. The mussels were fed with 3.2 x $10^5$ cells of living phytoplankton (Chlorella) per individual and day. This was done directly after the water

exchange and just before the containers were closed. Throughout a 14 days experimental period, survival and number of closed mussels were recorded daily. Furthermore, once after 48 h the number of byssus threads produced per mussel was assessed, while respiration rates and BCIs were determined at the end of the experiment.

*Experiment C*

In Experiment C, mussels from both habitat types were organized in two experimental groups of equal size. Animals in one of them received the same amount of food as during the laboratory acclimatization period (i.e. 2 x $10^6$ cells of *I. galbana* twice per day + 0.011 ml SMC once per day: 'high food supply'), whereas the others received 20% of this amount (i.e. 4 x $10^5$ cells of *I. galbana* twice per day + 0.0022 ml SMC once per day: 'low food supply'). After 12 days of differential feeding, a subsample of mussels from each combination of habitat type and feeding regime (impacted site, low food: n = 9; impacted site, high food and natural site high and low food n = 10) was drawn to determine BCIs. This revealed that at this time mean BCIs of Jakarta Bay mussels were 50% higher in the 'high food' than in the 'low food' group, while BCIs in mussels from the natural site did not differ between feeding regimes. Thus, the differential feeding was considered successful for Jakarta Bay mussels. However, since no difference in BCIs could be induced in mussels from the natural site, the factor "Food Supply" was later excluded from the analysis.

After this, 30 mussels from each two-way combination (habitat type and feeding regime) were transferred to two 15 l aquaria in equal numbers. One aquarium per group was then kept under low oxygen conditions (2 mg $l^{-1}$ DO) for 4 days, whereas the other was supplied with pressurised air to maintain normoxic conditions (6 mg $l^{-1}$ DO). The oxygen concentration was lowered in the same way as described for Experiment B. Half of the water was exchanged every day and mussels were fed with the same amount of *I. galbana* and SMC as during the 12 days prior to this phase. After 4 days, 10 individuals from each three-way factor combination (impacted habitat + low food supply + 2 mg $l^{-1}$ DO, impacted habitat + low food supply + 6 mg $l^{-1}$ DO etc., Table 1) were singled out into airtight 600 ml PVC containers filled with hypoxic seawater (0.5 mg $l^{-1}$ DO) so that one mussel in one container represented one experimental unit. The seawater in the containers was replaced daily by hypoxic water that had the target oxygen concentration as described for Experiment B and the mussels were fed with 2 x $10^6$ cells *I. galbana* per individual and day. A further group of mussels (n = 27) was kept in the same type of containers, but were supplied with pressured air

to keep the oxygen concentration normoxic (6 mg $l^{-1}$ DO). For 14 days, survival was recorded daily and after the first 48 h, the number of byssus threads produced per mussel was counted.

**Table 1:** Experimental groups in Experiment C.

| **Population** | Impacted | | | | Natural | | | |
|---|---|---|---|---|---|---|---|---|
| **Food supply** | High | | Low | | High* | | Low* | |
| **Acclimation** | 2 mg/ l DO | Ambient | 2 mg/ l DO | Ambient | 2 mg/ l DO | Ambient | 2 mg/ l DO | Ambient |
| **Treatment** | Hypoxia | Hypoxia | Hypoxia | Hypoxia | hypoxia | Hypoxia | Hypoxia | Hypoxia |
| **Replication**** | 10 (3) | 10 (3) | 10 (1) | 10 (0) | 10 (5) | 10 (5) | 10 (5) | 10 (5) |

* Groups were pooled later because BCIs did not differ

** Replication within the normoxia group in brackets

*Response variables and statistical analysis*

*BCIs and relative shell weight*

For BCI and relative shell weight calculations, the shell length *L* (longest anterior-posterior extension) was measured with a calliper and the soft body was separated from the shell using a teaspoon. Soft tissue and shell were then dried in an oven at 60°C for 24 h and the dry tissue weight *dt* as well as the dry weight of the shell *ds* was determined to the nearest 0.01 g using a digital scale. The BCI was calculated as *dt* (g)/ $L^3$ ($cm^3$) *100 and the relative shell weight as *ds* (g)/ *L* (cm).

*Byssus production*

At the end of Experiment A, mussels were transferred to clean containers and their byssus production was determined by counting the terminal discs of the byssus threads produced 24 h later. This was facilitated by marking the discs on the outside of the transparent PVC containers with a permanent marker. In Experiments B and C, the same procedure was used but in these cases, byssus production was assessed over the first 48 h after the beginning of the hypoxia phase to ensure that all replicates were still alive for the byssus counts. The number of byssus threads was standardized by the shell length of the respective mussel (in cm) to facilitate comparisons between individuals.

*Shell closing behaviour*

During the first 10 days of Experiment A and B, we recorded whether a replicate had closed valves or was gaping every day. For every mussel, the number of incidents when valves were closed was summed up over the 10 days and the sum was then divided by 10. This resulted in values between 0 and 1 (e.g. 0 if a replicate was gaping at all times, 0.5 if it was closed half of the times, 1 if the valves were closed at all times) and was referred to as "shell closing behaviour".

*Respiration rates*

In Experiment B, respirations rates after 14 days of exposure to hypoxia were determined in subsamples of replicates taken from each combination of habitat type and oxygen concentration. For this, mussels were transferred one by one to a small plastic grid which was placed in a glass container filled with 250 ml of normoxic seawater (T = 27.6 ± 0.2°C). The container was sealed with a conical rubber plug into which the oxygen sensor was inserted so that no air could enter. The sealed glass containing the mussel, the plastic grid and a magnet below the grid, was then placed on a magnetic stirrer. We recorded the oxygen concentration in the moment a mussel started gaping ($c_0$) and again after 15 min ($c_1$). Every 8 measurements, a blank water sample without mussel was measured to assess bacterial background respiration. The mean background respiration ($c_{blank}$) was then subtracted from the mussels' oxygen consumption ($c_0 - c_1$). After the measurements, mussels were frozen and the dry tissue weight was determined by separating the soft tissue from the shell and drying it at 60°C for 24 h. Mussel respiration rate (r) was calculated as the corrected decrease in dissolved oxygen per hour and gram dry soft tissue (ST):

$r = (c_0 - c_1 - c_{blank}) * 4 * ST^{-1}$ with the unit ($mg\ l^{-1}\ h^{-1}\ g^{-1}$).

*Survival*

Mussel survival was determined by carefully touching the siphons of gaping mussels with a thin stick. Mussels that responded with valve movement or mussels that were closed were considered alive.

*Statistical analysis*

For all response variables, apart from survival, effect ratios were calculated by dividing the value recorded for a single replicate belonging to a stress treatment group (hyposalinity in Experiment A and hypoxia in Experiment B and C) by the mean value of all replicates of the corresponding ambient treatment group (salinity 32 and 6 mg $l^{-1}$ DO, respectively). For example: The number of produced byssus threads by one replicate that came from a natural habitat and was exposed to hyposalinity was 36, while the mean number of byssus threads produced by all replicates from the same habitat that were kept at a salinity of 32 was 42. The resulting ratio was then 36/42 = 0.86. This approach allowed quantifying the effect of stress on the mussels as the deviation from their performance under ambient conditions. Response ratios whose 95% confidence intervals did not include 1 therefore indicate a significant deviation from mussel performance under ambient conditions. All statistical testing was done using the free statistical computing software R (R Development Core Team 2013). For all pairwise comparisons (BCIs, byssus production, shell closing behaviour and respiration rates in Experiment A & B), T-tests or, alternatively, non-parametric Wilcoxon-tests were used.

Multi-factorial analyses of variance (ANOVA) were applied to tests for differences in BCIs and in relative shell weight immediately after having collected the mussels in the field as well as to compare byssus production rates between treatment groups in Experiment C. For the first analysis, the factors 'Habitat type' with the levels '*impacted*' and '*natural*' and 'Collection Time' with the levels '*Experiment A*', '*Experiment B*' and '*Experiment C*' were included in the model. In the second analysis (Experiment C), the factors used for the modelling were 'Habitat type' with the levels '*impacted*' and '*natural*', "Food supply" with the levels *"high"* and "*low"* and "acclimation" with the levels "*yes*" and "*no*". Tukey's HSD post hoc tests were used to identify significant differences between single treatment combinations. Model residuals were tested for normal distribution using the Shapiro-Wilks-W test and for heterogeneity of variances using the Fligner-Killeen test. Data were log-transformed in case the assumptions were not met.

Mussel survival rates were compared with Cox proportional hazard (Coxph) regressions using the "survival" package for R (Therneau & Lumley, 2013). This method is based on the comparison of hazard functions, i.e. the instantaneous risk of death of an individual that belongs to a certain group, and allows applying multi-factorial designs to survival data. Assuming proportional hazards (ph) between groups, the ratio of hazard rates $hr_{group1}/\ hr_{group2}$ = *exponentiated coefficient* (exp. coef.) of two groups allows a straightforward interpretation of the risk of death. If exp. coef. is greater than 1, it indicates an

increased risk of death for individuals that belong to the group that is the numerator of the ratio, whereas a ratio smaller than 1 means a lower risk in this group (Mills, 2011). Before analysis, we tested whether the data fulfil the assumption of proportional hazards by using the cox.zph function in R (Mills, 2011). In Experiment B, we tested for the effect of 'Habitat type' with the levels 'impacted' and 'natural', while when analysing Experiment C, 'Habitat type' was not considered as a factor and both populations were analysed separately because the large difference in mean survival times between the mussels from different habitats would have led to unproportional hazards. Among the groups of mussels from the natural habitat, we tested for the influence of the factor 'Acclimation to hypoxia' with the levels 'yes' and 'no' and in the groups of mussels from the impacted habitat additionally for the factor 'Food supply' with the levels ''high' and 'low'. In Experiment A, all mussels survived the experimental phase of 10 days, so survival was excluded as a response variable. In Experiments B and C, there was 100% survival among normoxia-treated mussels, which is the reason why only hypoxia-treated mussels were included in the statistical analysis of the survival data.

## Results

*Water quality at the sampling sites*

Whenever we measured water temperature and salinity during our sampling events, they were similar between the impacted and the natural habitats. This was not the case for oxygen concentrations, which were consistently lower in the impacted habitat (Table 2).

**Table 2:** Water quality in Jakarta Bay, Lada Bay and Pelabuhan Ratu at the sampling events in 2012, 2013 and 2014.

| | **January/ February 2012** | | **March 2013** | | **June - August 2014** | |
|---|---|---|---|---|---|---|
| Habitat | Jakarta Bay (impacted) | Lada Bay (natural) | Jakarta Bay (impacted) | Lada Bay (natural) | Jakarta Bay (impacted) | Pelabuhan Ratu (natural) |
| Temperature (°C) | 26.5 | 28.5 | 28.9 | na | 29.8 | 28.9 |
| Salinity | 33 | 29 | 33 | 32 | 32 | 34 |
| Dissolved oxygen (mg $l^{-1}$) | na | na | 5.11 | 7.89 | 4.15 | 6.15 |

*Mussel body condition indices after collection*

Body Condition Indices (BCIs) of field collected mussels differed significantly between habitat types and between sampling events (Table 3). Furthermore, in 2012 and 2014 BCIs of mussels from the impacted habitat were by 39% (2012) and by 43% (2014) higher than those measured in mussels from the respective natural habitat. This led to a significant interaction. In 2013 no significant differences in BCIs were observed between sites (Figure 1).

**Table 3:** Effects of 'Habitat Type' and 'Collection Time' on the BCIs of *Perna viridis*. Results from ANOVA. Data were log-transformed to meet the assumption of normally distributed residuals. Significant comparisons ($p < 0.05$) are shown in bold.

| | **df** | **SS** | **MS** | **F** | **p** |
|---|---|---|---|---|---|
| Habitat Type | 1 | 3.652 | 3.652 | 85.17 | < **0.001** |
| Collection Time | 2 | 0.349 | 0.175 | 4.07 | < **0.05** |
| Habitat : Collection | 2 | 1.662 | 0.831 | 19.38 | < **0.001** |
| Residuals | 120 | 5.146 | 0.043 | | |

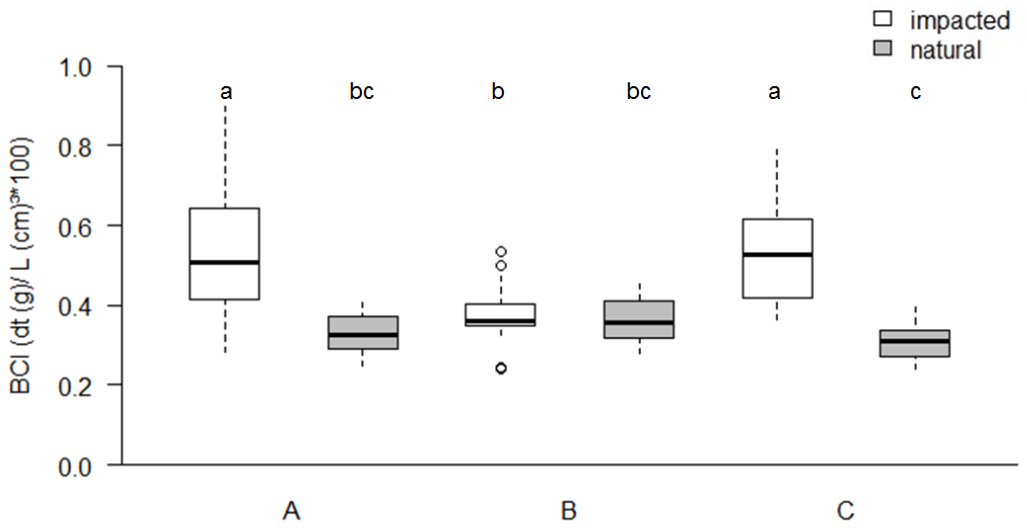


**Figure 3:** Body condition indices (median, inter-quartile range (IQR), 1.5* IQR and outliers) of *Perna viridis* immediately after collection in the field. Collection dates were A) 22.1.2012 (impacted habitat) and 24.2.2012 (natural habitat), B) 2.3.2013 (impacted) and 14.3.2013 (natural), C) 18.6.2014 and 17.7.2014 (impacted) and 12.8.2014 (natural). Sample size: n = 21 per habitat and time of collection. Groups that do not share letters are significantly different (Tukey's HSD, $p \leq 0.05$). dt = dry tissue (g), L = shell length (cm).

*Relative shell weight after collection*

There was no significant difference in relative shell weight between mussels coming from the impacted and from the natural habitats in 2012. In the other years, however, mean relative shell weights were by 23% (2013) and 68% (2014) higher in mussels from the natural habitats (Figure 2, Table 4).

**Table 4:** Effects of 'Habitat Type' and 'Collection Time' on the relative shell weight of *Perna viridis*. Results from ANOVA. Data were log-transformed to meet the assumption of normally distributed residuals. Bold figures show a significant effect ($p < 0.05$).

| | **df** | **SS** | **MS** | **F** | **p** |
|---|---|---|---|---|---|
| Habitat Type | 1 | 7.870 | 7.870 | 365.73 | < **0.001** |
| Collection Time | 2 | 0.979 | 0.490 | 22.76 | < **0.001** |
| Habitat : Collection | 2 | 6.709 | 3.354 | 155.88 | < **0.001** |
| Residuals | 120 | 2.582 | 0.022 | | |

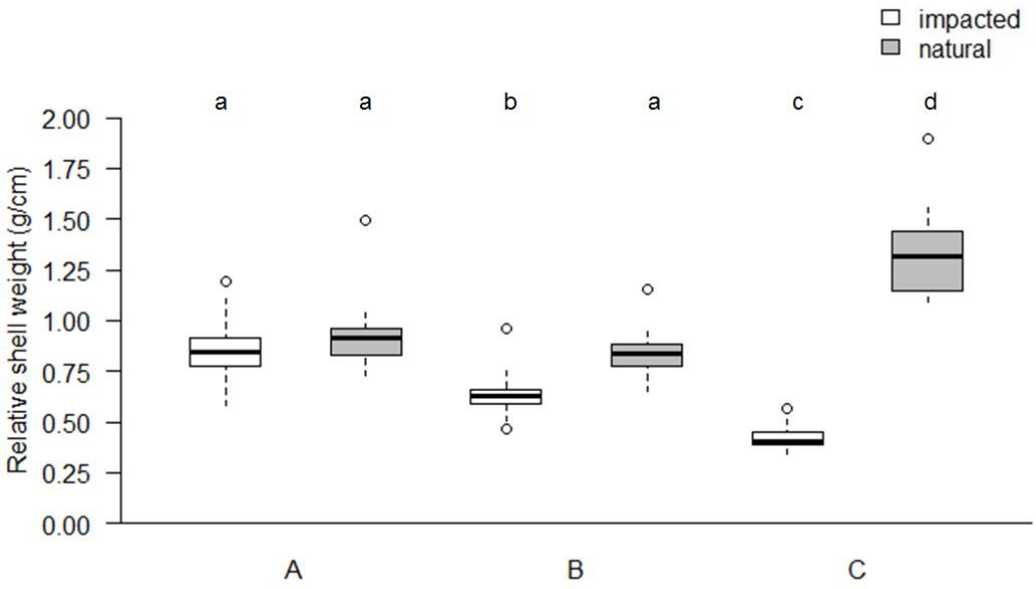


**Figure 4:** Relative shell weight (median, inter-quartile range (IQR), 1.5* IQR and outliers) of *Perna viridis* after collection from the field. Collection dates were A) 22.1.2012 (impacted habitat) and 24.2.2012 (natural habitat), B) 2.3.2013 (impacted) and 14.3.2013 (natural), C) 18.6.2014 and 17.7.2014 (impacted) and 12.8.2014 (natural). Sample size: n = 21 per habitat and collection time. Groups that do not share letters are significantly different (Tukey's HSD, $p \leq 0.05$).

*Body Condition Indices after exposure to hyposalinity (Experiment A) and hypoxia (Experiment B)*

After 10 days of exposure to a hyposalinity of 15 (Experiment A), BCI ratios did not differ between mussels from the impacted and the natural habitat (T-test, t = - 1.4, n = 60, p > 0.05, Fig 3a), but the mean ratios were < 1 in mussels from both habitat types. This shows that hyposalinity generally had a negative effect on the animals. In Experiment B, BCI ratios did not differ between mussels from different habitat types (Wilcoxon test, W = 58, n = 19, p > 0.05, Figure 3b).

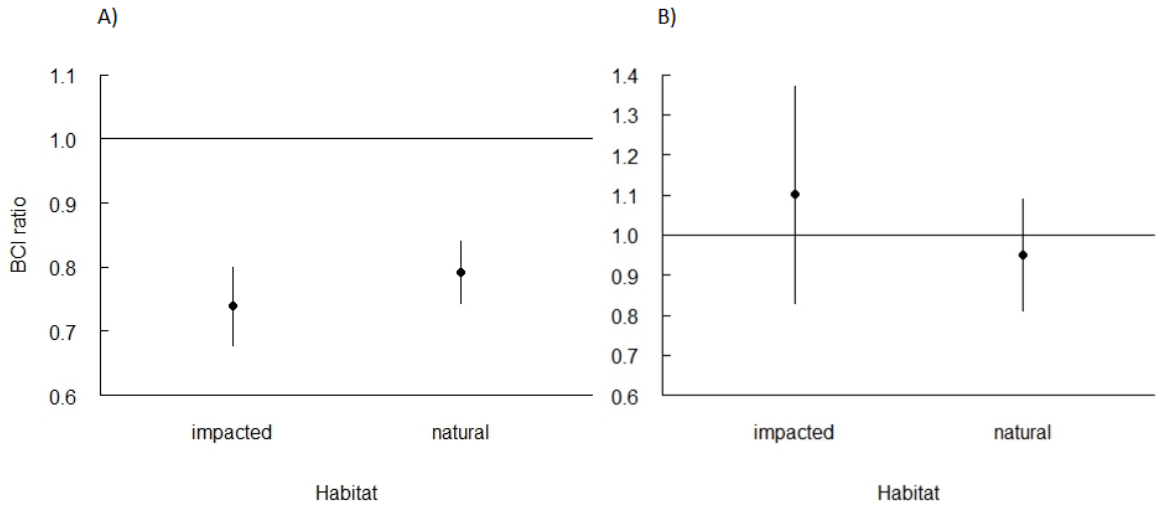


**Figure 5:** BCI ratios (BCI of stressed individual/ mean BCI in respective unstressed group) of *Perna viridis* from Lada Bay (natural habitat) and Jakarta Bay (impacted habitat) after a) 10 days of exposure to a salinity of 15 (Exp. A) and b) 14 days of exposure to hypoxia (0.5 mg $l^{-1}$ DO, Exp. B). Sample size: A) impacted = 30, natural = 30; B) impacted = 9, natural = 10. Errors bars show means and 95% CIs.

*Byssus production during exposure to hyposalinity (Experiment A) and hypoxia (Experiments B & C)*

Byssus production was reduced in the face of both stressors: Mussels under ambient conditions (salinity =32, DO = 6 mg $l^{-1}$) produced more byssus threads than conspecifics under a hyposalinity of 15 (Experiment A, Figure 4A) and under a hypoxia concentration of 0.5 mg $l^{-1}$ DO (Experiment B, Figure 4B). However, in both experiments, byssus production ratios did not differ between mussels from the different habitat types (Experiment A: T-test, T = -1.27, n = 59, $p > 0.05$; Experiment B, W = 320, n = 34, $p > 0.05$). In Experiment C, the ratio of produced byssus was significantly higher in mussels from the impacted habitat but did not differ with regard to the factors ‘Acclimation’ or ’Food supply’ and also no interaction emerged (Table 5, Figure 4C). Mussels from the natural habitat generally had a lower byssus production rate under hypoxia than under normoxia, whereas among mussels from the impacted habitat, byssus production was only reduced by hypoxia if mussels received low food supply and had been pre-acclimated to low oxygen (Figure 4C).

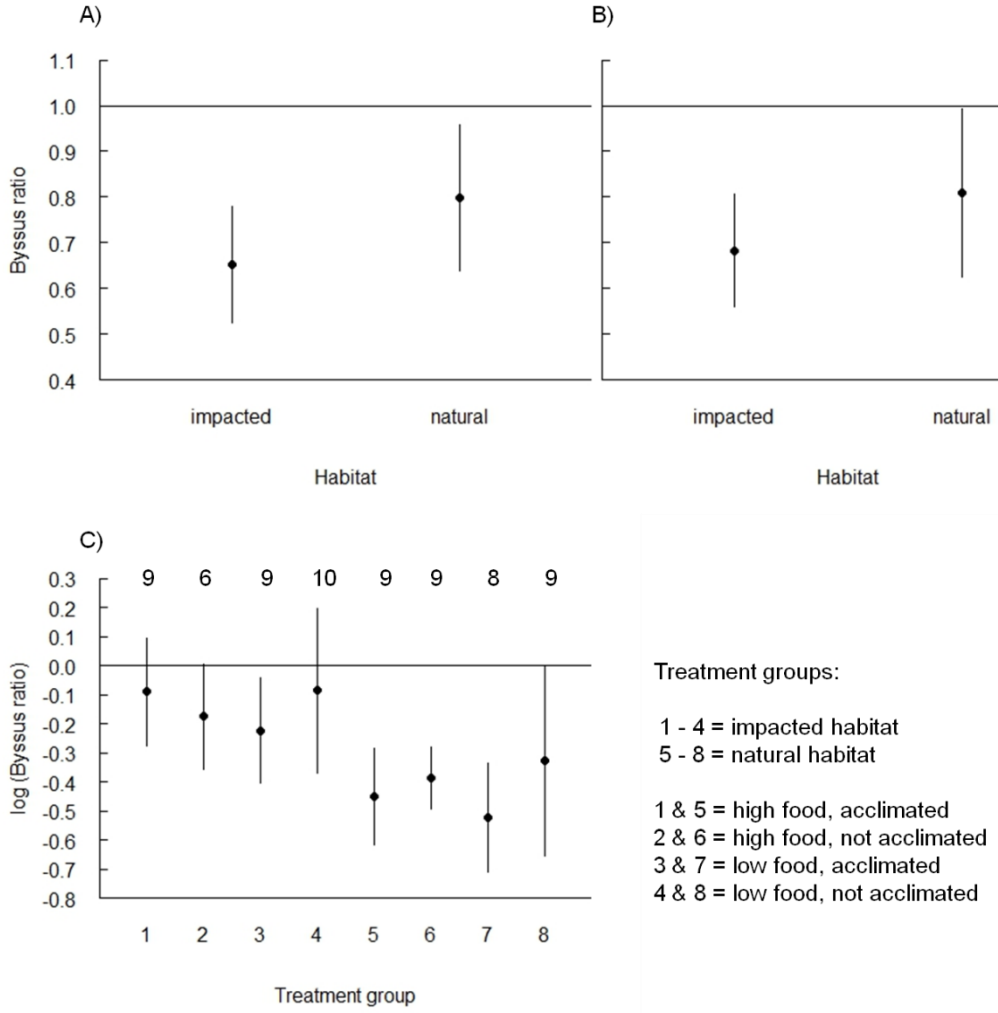


**Figure 6**: Ratios of byssus production (stressed individual/ mean of unstressed individuals) within 24 h (Experiment A) and within 48 h (Experiments B & C) of *Perna viridis* from natural habitats (Lada Bay in Exp. A & B, Pelabuhan Ratu in Exp. C and from an impacted habitat (Jakarta Bay in Exp. A, B & C) after 10 days of exposure to different a salinity of 15 and 2 days of exposure to hypoxia (0.5 mg $l^{-1}$ DO). Sample size: Exp. A: impacted = 30, natural '15' = 30; Exp. B: impacted = 19, natural = 15; Exp. C: impacted = 10, natural = 10. Sample sizes in Exp. C are indicated above error bars. Errors bars show means and 95% CIs.

*Shell closing behaviour during exposure to hyposalinity (Experiment A) and hypoxia (Experiments B)*

Shell closing behaviour under stress differed significantly between mussels from the natural and the impacted habitat during the 10 days of exposure to hyposalinity (Experiment A) and

hypoxia (Experiment B) (Wilcoxon test. Exp. A: W = 820, n = 60, p < 0.01; Exp. B: W = 3, n = 25, p < 0.01, Figure 5A & 5B). However, only in mussels from the impacted habitat the behaviour under hyposalinity deviated from the performance under ambient conditions (Figure 5A), while under hypoxia this was the case for mussels from both habitats. Here, valve closing was shown less frequently under hypoxia than under normoxia (Figure 5B).

**Table 5:** Effects of 'Habitat Type', 'Acclimation to low oxygen' and 'Food supply'on the byssus production ratios in *Perna viridis* from Pelabuhan Ratu (natural habitat) and Jakarta Bay (impacted habitat) after 10 days of exposure to hypoxia in Experiment C. Results from ANOVA. Data from log-transformed to meet the assumption of normally distributed residuals. Bold figures show a significant effect (p < 0.05).

| | **df** | **SS** | **MS** | **F** | **p** |
|---|---|---|---|---|---|
| Habitat Type | 1 | 1.35 | 1.35 | 14.45 | **< 0.01** |
| Acclimation | 1 | 0.12 | 0.12 | 1.25 | 0.27 |
| Food supply | 1 | 0.01 | 0.01 | 0.07 | 0.79 |
| Residuals | 65 | 6.06 | 0.09 | | |

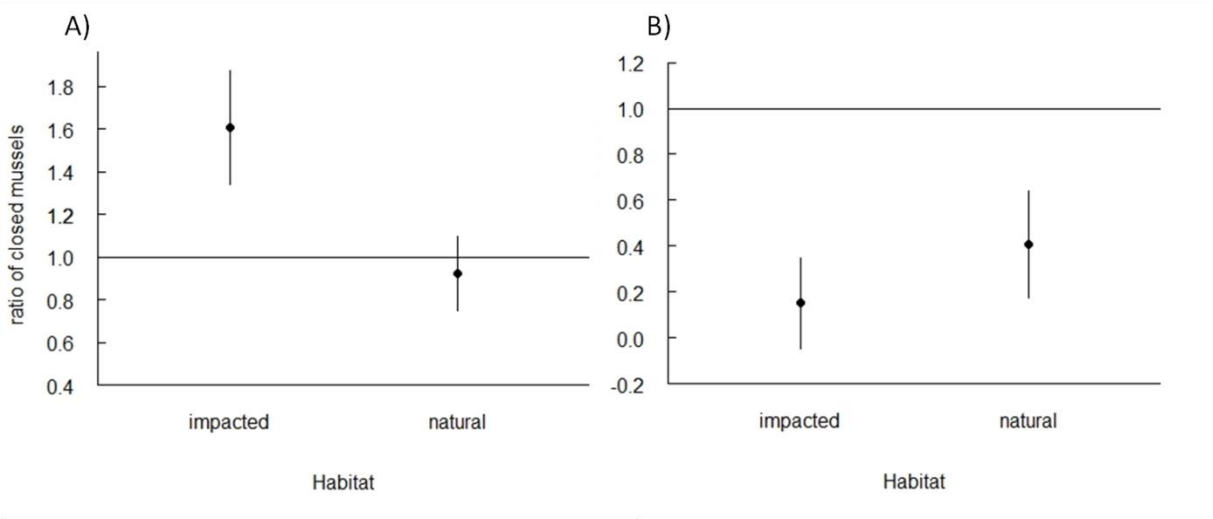


**Figure 7:** Shell closing behaviour (the proportion of closed-valves-observations in a stressed individual/ the mean proportion of closed-valves-observations in the respective unstressed group) in *Perna viridis* from Jakarta Bay (impacted habitat) and Lada Bay (natural habitat) during 10 days of exposure to hyposalinity (salinity 15, Exp. A) and hypoxia (0.5 mg $l^{-1}$ DO, Exp. B). Sample sizes: A) impacted = 30, natural = 30, B) impacted = 12, natural = 13. Errors bars show means and 95% CIs.

*Respiration rates after exposure to hypoxia (Experiment B)*

In 2013, the ratio of respiration rates was significantly higher in mussels from the natural habitat (T-test, T = -4.66, n = 18, $p < 0.01$). Mussels from the impacted habitat showed similar respiration rates after normoxia and hypoxia (Figure 6), whereas mussels from the natural habitat had higher respiration rates after they had been exposed to hypoxia (Figure 6).

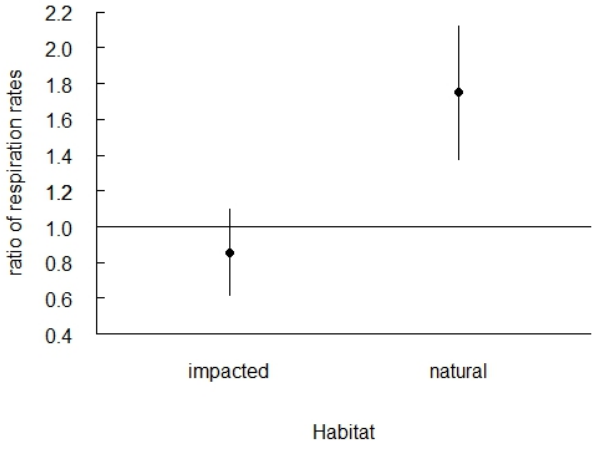


**Figure 8:** Ratio (stressed individual/ mean in unstressed group) of respiration rates of *Perna viridis* from Jakarta Bay (impacted habitat) and Lada Bay (natural habitat) after 14 days exposure to hypoxia (0.5 mg $l^{-1}$ DO = stressed) and normoxia (> 6 mg $l^{-1}$ DO = unstressed, Exp. B). Sample sizes were: impacted = 9 and natural = 9. Errors bars show means and 95% CIs.

*Survival during hyposalinity (Experiment A) and hypoxia (Experiments B & C)*

Mussel survival during exposure to a hyposalinity of 15 was 100% in both populations. During the 14 days of exposure to hypoxia in 2013, the difference in hazard ratios between the impacted and the natural population was marginally significant (coxph survival analysis, exp.coef.(natural ) = 0.3, n = 36, p = 0.07), while mean time to death was 10 days for mussels from the impacted and 12 days for mussels from the natural habitat. In 2014, however, mussel survival under hypoxia was more than twice as long in the group that was kept with high food supply, was not acclimated to low oxygen and consisted of mussels from the impacted habitat (mean time to death = 9.5 days) than among the group of mussels from the natural habitat that was kept under high food supply and was not acclimated to low oxygen (mean time to death =

4 days). This difference was significant (coxph survival analysis, exp.coef. (natural) = 11.93, n = 20, $p < 0.001$, Figure 7a).

**Table 6:** Survival of *Perna viridis* from Jakarta Bay (impacted habitat) and Pelabuhan Ratu (natural habitat) during 14 days of exposure to hypoxia (0.5 mg $l^{-1}$). Prior to the hypoxia treatment, mussels had been kept under low and high food supply for 12 days and were acclimated/ not acclimated to low oxygen concentrations (2 mg $l^{-1}$ DO) for another 5 days (experiment C). Results of the Coxph Regression Analysis. Brackets behind the respective factors indicate to which group the expected coefficient (Exp.coef.) refers. Exp.coef. < 1 indicates a lower risk of death in the respective groups whereas Exp.coef. > 1 indicates a higher risk of death. Bold figures show a significant effect ($p < 0.05$).

| Origin | Factor | df | exp.coef. | p |
|---|---|---|---|---|
| Impacted | Food (low) | 1 | 0.69 | 0.48 |
| | Acclimation (no) | 1 | 0.93 | 0.89 |
| | Food (low) : Acclimation (no) | 3 | 4.20 | < **0.05** |
| | Residuals | 40 | | |
| Natural | Acclimation (no) | 1 | 1.10 | 0.77 |
| | Residuals | 40 | | |

*Acclimation capacity to low oxygen concentrations (Experiment C)*

When analysing survival under hypoxic conditions in *P. viridis* from the impacted habitat, we observed a significant interaction between the factors 'Food Supply' and 'Acclimation to Hypoxia' (Table 6, Figure 7b). Mussels that were kept with low food supply showed higher survival under hypoxia, if they had previously been acclimated to low oxygen conditions, while there was no effect of the acclimation in case the mussels were kept with high food supply. Among the groups of mussels that were not acclimated to hypoxia, mussels with high food supply performed better (Figure 7b). Mean time to death under hypoxia in mussels originating from the impacted habitat were 9.0 (acclimated) and 9.5 days (not acclimated) after high food supply, while after low food supply mean time to death was 10.0 (acclimated) and 5.1 days (not acclimated). In mussels from the natural habitat, there was no significant effect of oxygen acclimation on survival under hypoxia (Table 6, Figure 7c) and mussels survived on average for 4.2 (acclimated) and 3.9 days (not acclimated).

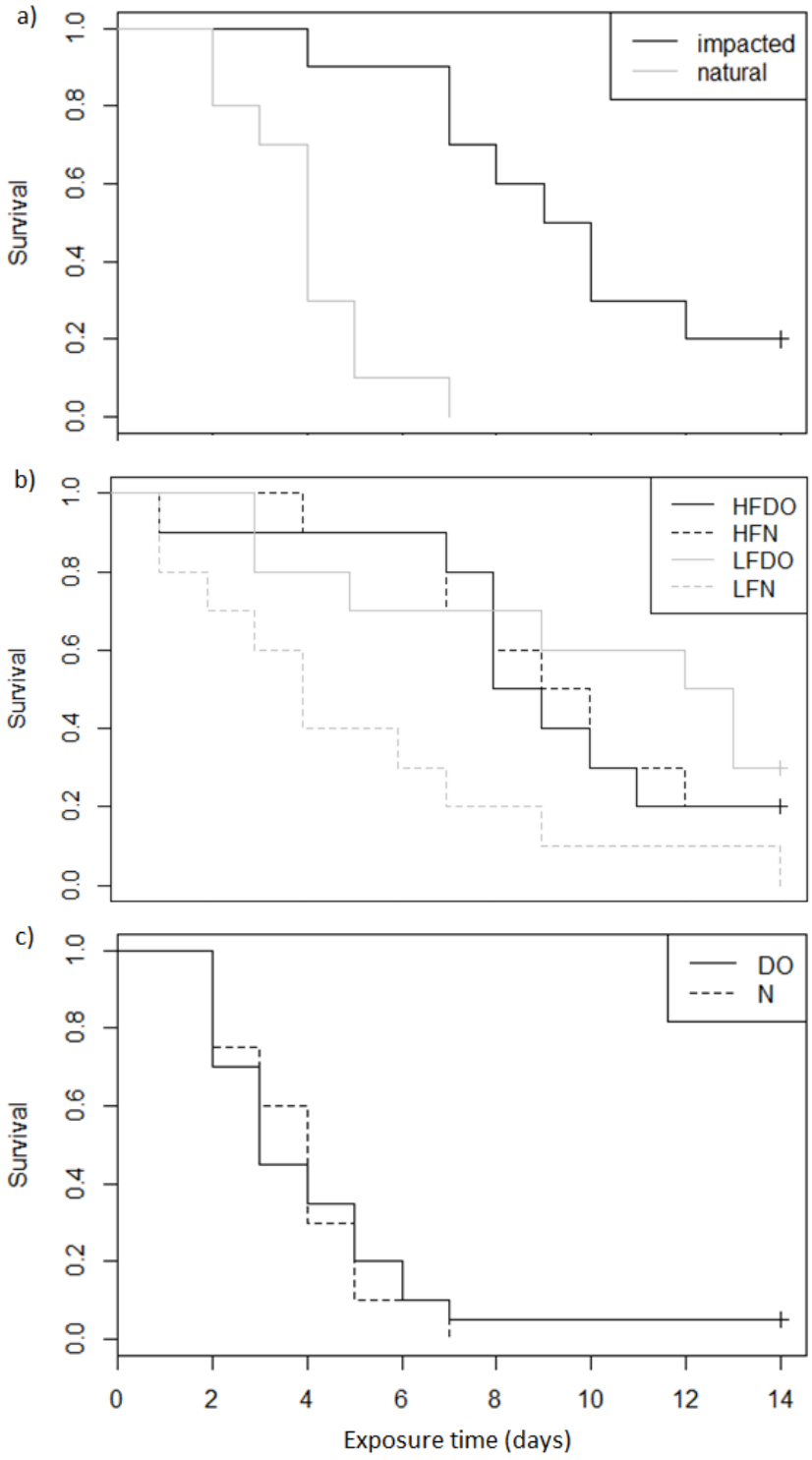


**Figure 9:** Survival during 14 days of exposure to hypoxia (0.5 mg $l^{-1}$ DO, Exp. C) in *Perna viridis* from Jakarta Bay (impacted habitat) and Pelabuhan Ratu (natural habitat). a) High food supply groups that were not acclimated to low oxygen concentrations, b) mussels from the impacted habitat, c) mussels from the natural habitat. Prior to the hypoxia exposure, mussels had been fed with high (HF) and low (LF) amounts of *Isochrysis galbana* for 12 days and were acclimated (DO) to low oxygen concentrations (2 mg $l^{-1}$ DO) or were kept under ambient conditions (N) for 5 days, respectively (b and c).

## Discussion

We assessed the effects of hyposalinity and hypoxia on individuals of *P. viridis* from habitats that differ in environmental quality by monitoring a set of physiological and behavioural response variables during laboratory stress assays. These comparisons revealed stress-specific as well as population-specific differences in mussel performance under adverse conditions. When exposed to hypoxia, which is a common stressor in impacted and eutrophic coastal areas, mussels from the impacted habitat a) had higher survival rates (Experiment C), b) had a better acclimation capacity to low oxygen (Experiment C), c) showed no deviation in respiration rates from the normoxia treatment (Experiment B), d) were more active (Experiment B) and e) produced more byssus (Experiment C) than conspecifics from the two natural habitats. This suggests that mussels from Jakarta Bay are pre-adapted to this kind of stressor or have the capacity to acclimate quickly. However, when mussels were exposed to hyposalinity only one difference between populations emerged: The proportion of inactive individuals was higher among mussels from the impacted habitat, which, in turn, suggests that mussels from natural habitats have a better capacity to tolerate hyposalinity than those from Jakarta Bay. Thus, performance under stress differed between the mussel populations we tested, but the outcome of the comparison was determined by the choice of response variables and by the type of stressor applied.

*The influence of habitat quality on shell stability and the nutritional status*

When we measured mussel BCIs and relative shell weights directly after having collected the animals in the field, these values differed significantly between mussels from the different habitats. Mussels from the impacted Jakarta Bay had on average 27% higher BCIs than conspecifics from the natural habitats, while the latter had relative shell weights that were on average 30% higher than in Jakarta Bay individuals. These differences presumably reflect differences in the habitat conditions prevailing at the respective collection sites and may also explain the diverging capacities to tolerate the two stressors we applied.

Various factors could have led to the fact that mussels from the impacted habitat had lower shell weights than their conspecifics from the natural sites. This could, for instance, be due to high settling densities and the prevalence of environmental stressors, such as hypoxia, desalination and ocean acidification, in Jakarta Bay. Furthermore, there could be a difference in predation pressure between habitat types. In Jakarta Bay, eutrophication and pollution has resulted in a decline in biodiversity during the last decades (van Der Meij et al., 2009; van der Meij et al., 2010). It is unknown, however, whether this decline also resulted in lower

predator abundances in Jakarta Bay compared to the more natural sites. It can therefore only be speculated whether a lower predation pressure, which is known to go along with thinner shells in *P. viridis* (Cheung et al., 2004), could explain the observed differences between the populations. It was further shown that shell parameters can vary between wild and cultivated populations in *Mytilus chilensis* (Valladares, Manríquez, & Suárez-Isla, 2010), with wild populations having thicker shells, which is supposedly caused by the lower settling densities compared to cultivated mussels (Valladares et al., 2010). In the impacted habitat in our study, *P. viridis* settles densely on bamboo structures provided by the mussel farmers, whereas in the natural habitats mussel densities are lower because the animals colonize the ropes, bamboo poles (Lada Bay) or floating bodies of platforms (Pelabuhan Ratu) more sparsely, which is supposedly due to the lower larvae supply in these habitats. We, for example, observed a rapid colonization by *P. viridis* spat on nets that we had deployed in Jakarta Bay in July 2012. After three months in the field, nets deployed in Jakarta Bay were densely overgrown by mussel spat whereas in Lada Bay no mussels were found (M. Huhn, pers. obs.). In addition to settlement density, environmental stressors such as hypoxia and desalination are known to have negative effects on the shell stability of *P. viridis* (Wang et al., 2012), while ocean acidification impairs calcification rates in the closely related blue mussel *Mytilus edulis* (Gazeau et al., 2010; Melzner et al., 2011). In Jakarta Bay, microbial degradation caused by phytoplankton blooms commonly reduces oxygen concentrations to hypoxic levels of less than 2 mg $l^{-1}$ DO (Arifin, 2006; Damar, 2003; Wendling et al., 2013) and salinity varies with the season and with the distance to river mouths. A previous study that covered the same sampling locations, documented that salinity in Jakarta Bay fluctuated only minimally between 30 and 34 in 2012 (Wendling et al., 2013), but values as low as 10 have been reported during the northwest monsoon in 2003 (Arifin, 2006). Water pH can vary widely in Jakarta Bay, ranging from 6.14 to 8.14 (Arifin, 2006; Thoha, Adnan, & Sidabutar, 2007). In Lada Bay and Pelabuhan Ratu, salinity can also vary with season (Wendling et al., 2013), but oxygen concentrations are generally higher than in Jakarta Bay, ranging from 5.6 mg $l^{-1}$ DO to > 7 mg $l^{-1}$ DO, respectively (Sanusi, 1994; Wendling et al., 2013). Thus, high mussel settling densities, reduced predatory pressure and the severe environmental stress in Jakarta Bay are possible reasons for the lower shell weight that we found in Jakarta Bay mussels but we have not data that would allow us to identify which or which combination of them were responsible.

Not only shell weights but also mussel BCIs differed significantly between Jakarta Bay individuals and conspecifics from the natural sites. This is presumably due to the

eutrophic conditions of Jakarta Bay, which frequently cause phytoplankton blooms that contain target food species for *P. viridis*, such as diatoms of the genera *Skletonema* and *Chaetoceros* or dinoflagellates of the genera *Dinophysis*, *Prorocentrum* or *Protoperidinium* (Damar, 2003; Holmes, Teol, Lee, & Khoo, 1999; W Wong, 1999). Food availability is therefore generally higher in the impacted than in the two natural habitats and as a consequence Jakarta Bay mussels are generally in a better nutritional status than conspecifics from natural environments (Wendling et al., 2013, Huhn et al. submitted). This positive influence can be counteracted by environmental stress that goes along with eutrophication, such as hypoxia, and which may reduce BCIs when energy is needed to mitigate its negative effects, e.g. through the synthesis of heat shock proteins (HSP) (Anestis et al., 2007). Eutrophication can therefore have ambivalent consequences for mussel performance: While ample food availability promotes a good nutritional state, severe environmental stress has the potential to inhibit mussel growth. These influences may even compensate each other and this concurrence could have caused the similarity in BCIs between the mussel populations, found in March 2013.

*Mussel activity during environmental stress*

A good nutritional status was supposedly also the reason for the better performance that Jakarta Bay mussels - in comparison to their conspecifics - exhibited under hypoxia: It is very likely that their extensive energy reserves allowed them to maintain a higher activity under low oxygen concentrations than their conspecifics from oligotrophic sites. This was indicated by the higher byssus production and the less frequent shell closing Jakarta Bay mussels showed in comparison to individuals from Lada Bay and Pelabuhan Ratu during hypoxia. Wang et al. (2012) found that exposure to hypoxia and hyposalinity affects the production of byssus threads negatively in *P. viridis*. This finding is in line with our results, but we further revealed that the extent to which byssus production is impaired can depend on mussel life history and/or population-specific adaptations to stress since the deviation in byssus production between ambient and stressful conditions differed between habitats. Valve closure during hypoxia was more pronounced in mussels from the natural than in individuals from the impacted site. This suggests that mussels from Jakarta Bay maintained a close-to-normal metabolic activity, whereas animals from the natural habitats experienced metabolic depression. Consequently, the first were probably better in using the remaining oxygen as well as the available food, whereas the second presumably more readily switched to anaerobic respiration. The anaerobic pathway, on one hand, allows a mussel to save energy because

there is no need to maintain the cilial activity that transports water to the gills (Doeller, Kraus, Shick, & Gnaiger, 1993), but, on the other hand, it further reduces its access to energy as closing of the valves limits food intake. Furthermore, anaerobic respiration is also less efficient in converting energy resources into ATP than the aerobic pathway (Riisgård & Larsen, 2014).

In addition to the observed differences in mussel behaviour during hypoxia, the moderate rates of oxygen uptake shortly after this stress also suggest adaptation or acclimation to hypoxic conditions in mussels from the impacted habitat. In these individuals, respiration rates did not differ between the group that experienced normoxic and the one that experienced hypoxic conditions, whereas in mussels from the natural habitat oxygen consumption was greatly enhanced after exposure to hypoxia. This indicates the necessity to pay back an oxygen debt (Bayne & Livingstone, 1977), which resulted from reduced oxygen uptake prior to the measurements. This again corresponds well with the fact that mussels from the natural habitat were more often found closed during hypoxia than their conspecifics from Jakarta Bay. Seemingly, groups of green mussels that come from habitats that differ with regard to the frequency of oxygen depletion have different capacities to deal with hypoxia.

*The influence of mussel nutritional status on survival during hypoxia*

Apart from different activity patterns, a difference in hypoxia tolerance between mussels from different populations was also expressed in the survival rates that we observed in Experiment C. Here, mussels from the impacted habitat survived hypoxia in the laboratory on average 2.4 times longer than mussels from the natural habitat. This corresponds well with the findings of Wendling et al. (2013), who observed higher survival during hypoxia in *P. viridis* from the Jakarta Bay compared to individuals from Lada Bay. However, surprisingly, in Experiment B of the present study, we did not find such a difference. The explanation for this is most likely the nutritional status of the mussels: In the experiments reported by Wendling et al. (2013) as well as in Experiment C of this study, individuals from the impacted habitat had substantially higher BCIs, whereas in experiment B, BCIs of both populations were identical. The relevance of the nutritional status for hypoxia tolerance in *P. viridis* is further underlined by the results of Experiment C. Here, mussels that were kept with a high food supply prior to the hypoxia test and had high BCIs survived on average twice as long as mussels that were reared with low food supply and had low BCIs. Whereas, so far, the assumption that high BCIs promote stress tolerance in *P. viridis* was based on correlations, this experiment now establishes a causal relationship between nutritional status and hypoxia tolerance.

*Influence of acclimation capacity on hypoxia tolerance*

The higher tolerance to hypoxia that we observed in mussels from the impacted habitat can, at least partly, be attributed to their high BCIs. However, a good nutritional state is presumably not the sole driver of stress tolerance in mussels. If that would be the case, the high BCIs of Jakarta Bay mussels should also have led to robustness towards hyposalinity. But this was not the case, since for none of the response variables measured it was found that mussels from the impacted habitat performed better under hyposalinity than their conspecifics from the natural sites. There was either no difference or mussels from the natural habitat performed better. Even though exposure to hyposalinity consumes energy resources - what was shown by the fact that BCIs were reduced by exposure to hyposalinity - mussels with a better nutritional status did not perform better under this stressor than individuals that had less energy available. This implies that a further factor influenced mussel performance under low ion concentrations. A likely mechanism is pre-acclimation on the individual level, which could have occurred in mussels that experienced hyposalinity already before the experiment. While in our study system, hyposalinity can occur in both, the impacted and the natural habitats, hypoxia only prevails in Jakarta Bay. Therefore, mussels from the natural habitats may have been pre-acclimated to hyposalinity but not to hypoxia, whereas mussels from the impacted habitat may have been pre-acclimated to both stressors. We did not explicitly test for mussel ability to acclimate to low salinities, but Experiment C showed that Jakarta Bay mussels had the ability to acclimate to low oxygen conditions: Mussels, which were exposed to 2 mg $l^{-1}$ DO for 4 days prior to the hypoxia stress, had a mean survival time that was twice as long as in mussels that were not acclimated to low oxygen. However, such an acclimation effect was only detected in Jakarta Bay mussels and among these only in individuals that previously received a low food supply. This suggests that a good nutritional status overrules other determinants of hypoxia tolerance, such as acclimation. Mussels from the natural habitat, however, did not acclimate to low oxygen conditions at all. A reason could be that the time of oxygen acclimation (4 days) was not long enough to induce the up-regulation of heat shock proteins (hsp) among mussels that never experienced low oxygen stress before. For the Mediterranean mussel *Mytilus galloprovincialis*, 5 days suffice to induce hsp production in response to heat stress (Anestis et al., 2007) and the temperate blue mussel *M. edulis* acclimates to low oxygen within 5 days (Bayne & Livingstone, 1977). On the base of this, one should assume that 4 days of hypoxia acclimation should suffice to induce an hsp response in the tropical *P. viridis* as metabolic processes are faster under high temperatures. Regardless of what caused the absence of acclimation effects in mussels from the natural habitat, our results

clearly show that individuals from the impacted habitat acclimated faster to hypoxia, which is presumably promoted by the frequent occurrence of hypoxic conditions in Jakarta Bay. Genetic adaptation to the prevailing environmental stressors in Jakarta Bay may also have contributed to the observed differences between mussel populations. However, reciprocal transplantation experiments between Lada Bay and Jakarta Bay showed that local adaptation to hypoxia in Jakarta Bay mussels are unlikely (Huhn et al. accepted). Taking our findings and the findings from Huhn et al. (accepted) together, we conclude that the nutritional status and the capacity to acclimate to stress are the two most important determinants of tolerance to adverse environmental conditions in mussel populations from West Java. A good nutritional status seems to be a prerequisite for hypoxia tolerance and both come along with residing in eutrophic habitats. This means that the same mechanism that promotes high BCIs in mussels, i.e. the blooming of phytoplankton, also causes hypoxia events during which high BCIs are then advantageous for mussel survival.

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# Synthesis

## *General Discussion*

Together with habitat conversion, overexploitation, pollution (nutrient loading) and climate change, biological invasions are among the five greatest causes of biodiversity loss worldwide. In coastal ecosystems the impact of all five drivers is increasing (Millennium Ecosystem Assessment, 2005). Biological invasions do not only threaten biodiversity, they also bring along severe economic losses and human health risks as they interfere with natural foodwebs and ecosystem services, impact local fisheries and aquacultures and may carry alien pathogens (Katsanevakis et al. 2014; Perrings 2002). As biological invasions are inter-connected with other main drivers of biodiversity loss, their impacts should not be regarded separately but it is important to understand how one driver can influence the impact of another. Habitat conversion and eutrophication, for example, have the potential to contribute to the success of non-native species in areas to which they have been introduced (Byers 2000; Jewett et al. 2005). Since successful marine invaders are often species with phenotypically plastic responses and a high tolerance to environmental stress (Parsons 1994), they should perform better in eutrophic, polluted and disturbed ecosystems than less tolerant native species. Support for this assumption comes from studies on impacted coastal habitats. For example, copper pollution reduced the diversity and abundance of native but not of non-native species in marine hard-substrate communities in San Francisco Bay, USA, and Port Kembla Harbour, Australia (Crooks et al. 2010; Piola and Johnston 2008). In the Mediterranean Sea, the non-native algae species *Caulerpa taxifolia* and *C. racemosa* are currently benefiting from wastewater discharge that causes an overload of the nutrients $NH_4^+$ and Phosphate, and are outcompeting an endemic seagrass (Occhipinti-Ambrogi and Savini 2003). Furthermore, in hypoxic areas of Californian salt marshes, the invasive mud snail *Batillaria attramentaria* is more competitive than the native *Cerithidea californica* (Byers 2000). Anthropogenic disturbance is also believed to be one of the reasons why San Francisco Bay became the probably most invaded estuary in the world (Cohen and Carlton 1998).

The Asian green mussel, *Perna viridis*, is a good example of a marine invader that flourishes in anthropogenic disturbed coastal ecosystems. In both, its native and non-native range, it reaches the highest population densities in highly eutrophic and often polluted systems (Buddo et al 2003; Cheung 1993; Rajagopal, Venugopalan et al. 2006). Dense *P. viridis* populations, for example, occur in Kingston Harbour (Jamaica), in the Bay of Bengal near Chennai (India), in Tolo Harbour, Hong Kong (China), in the upper Gulf of Thailand

near Bangkok (Thailand) and in Jakarta Bay (Indonesia) - all of which are anthropogenic impacted coastal habitats near megacities (Buddo et al. 2003; Huang et al. 1983; Prakoon et al. 2010; Vijayavel et al. 2007; Wendling et al. 2013). In this thesis, I addressed the question how individuals of *P. viridis* that stem from an impacted habitat acquire tolerance to the prevailing environmental stress. A previous study already showed that *P. viridis* populations from an impacted and a natural habitat in Indonesia differ in their tolerance to environmental stress (Wendling et al. 2013). Three explanations (or combinations of them) were considered by the authors as the possible reasons for this: i) mussels from the impacted habitat had undergone local adaptation due to stress as a selective force (an irreversible state that is manifested in the gene pool of a population, ii) mussels from the impacted habitat had acclimated to stress in the habitat (a reversible state that is not genetically manifested), iii) mussels from the impacted (and therefore eutrophic) habitat had a higher nutritional status which allowed them to allocate more energy to stress compensation. A fourth explanation should also be considered - the presence of transgenerational epigenetic effects that are passed from parents to the offspring without being genetically inherited (Salinas et al. 2013). These effects go back to phenotypic plasticity, i.e. the ability of a genotype to express different ecotypes in response to environmental changes (Whitman and Agrawal 2009). In the case of transgenerational epigenetic plasticity, the information to express a specific ecotype is passed from the parent to the offspring even before the offspring is confronted with the respective environmental condition. This effect is induced maternally, e.g. via DNA methylation, and can be observed over generations (Salinas et al. 2013).

To elucidate the relevance of the different explanations of population-specific differences in stress tolerance for a study system in Indonesia, I compared stress tolerance and the nutritional status between a mussel population from Jakarta Bay (JB) and two populations from more natural habitats (Lada Bay (LB), Sunda Strait and Pelabuhan Ratu (PR), South Java Sea) under different experimental scenarios (chapters 2 & 3). In the selected habitats, environmental conditions (surface temperature, salinity, dissolved oxygen, nutrient concentrations and phytoplankton abundance) were monitored during sampling. Additionally, mussels sampled from a ship hull were investigated with regard to both variables - stress tolerance and nutritional status - to test whether transport on ship hulls influences robustness and, therefore, invasion potential in *P. viridis* (chapter 1).

Reciprocal transplantations of mussels between JB and LB with subsequent laboratory stress experiments after a residential time of two months in the respective habitats suggest that local adaptation does not play a significant role in determining hypoxia tolerance of mussels

from the impacted JB (chapter 2). JB mussels were not consistently more tolerant and individuals from the natural habitat were even more tolerant to hypoxia in the laboratory when their nutritional status was good. Adaptations to local stress regimes in marine invertebrates have been reported for *Mytilus edulis*, which was exposed to PAH (Lacroix et al. 2015), and for a bryozoan as well as an annelid which were exposed to copper (Klerks and Levinton 1989; Piola and Johnston 2006). To my knowledge, however, none of these studies considered epigenetic transgenerational effects as a possible reason for the observed differences in tolerance and it is questionable whether these could have played a role. To be able to disentangle epigenetic transgenerational effects and local adaptations in an experiment, test organisms would need to be reared under controlled conditions for multiple generations (Salinas et al. 2013). As neither local adaptations, nor epigenetic transgenerational effects seemed to play a role for the population-specific differences in stress tolerance I observed in my study (chapter 2), I did not investigate this further. The leading driver for hypoxia tolerance in *P. viridis* was very likely the nutritional status of the test animals. In all hypoxia experiments that I conducted, the mussels that had the higher BCIs were also the ones with the better hypoxia tolerance. These experiments were run with i) juvenile *P. viridis* from JB and LB in August 2012 (chapter 2), ii) adult *P. viridis* from JB and LB, reciprocally transplanted, in October 2013 (chapter 2), iii) adult *P. viridis* from JB and PR, in September 2014 (chapter 3). The results from these experiments are also in correspondence with experiments conducted in 2009 and 2010 with the same mussel populations (Wendling et al. 2013). In one of the experiments of the present study (adult *P. viridis* from JB and LB, March 2013, chapter 3), no difference in hypoxia tolerance was found and, at the same time, there was also no difference in mussel BCIs between the populations. Furthermore, the only time when mussels from a natural habitat were more tolerant to hypoxia than those from an impacted site was also when they had higher BCIs (chapter 2). These two observations support the assumption that the nutritional status is the leading driver for tolerance. In all these comparisons, the nutritional status was a characteristic of the respective groups, which resulted from their life histories, and was not manipulated in the laboratory. Therefore, this interpretation relies on correlations between BCIs and hypoxia tolerance. In the last experiment (chapter 3), however, BCIs were modified under controlled conditions and thus allowed to infer about causal relationships. Therefore, in this experiment, BCI was treated as an independent factor in design. Again, a higher tolerance was found in those mussels with the higher BCI. Since the experiment allowed keeping other influential factors

constant, this finding strongly supports the assumptions that the nutritional status is the key driver for hypoxia tolerance.

The nutritional status was not the only factor that I observed to influence mussel tolerance in my experiments. An acclimation to moderately low oxygen concentrations (2 mg/l DO) over 5 days improved the performance of mussels during subsequent exposure to hypoxia (0.5 mg/l DO) (chapter 3). However, this was only the case when the nutritional status of the mussels was poor – mussels with good nutritional states did not benefit from oxygen acclimation – which again strongly suggests that the nutritional status is the most important driver of stress tolerance and might outweigh the effects of other factors. A similar concept was found for *M. edulis,* for example, in which negative effects of ocean acidification on calcification and shell formation are outweighed if the food supply is high (Thomsen et al. 2013). The idea that a good nutritional status contributes to hypoxia tolerance is plausible: when exposed to hypoxia, *P. viridis* closes its valves and down-regulates its metabolism (Wang et al. 2011). In this state, the mussel uses energy that is stored as glycogen in the mantle tissue, to maintain metabolic functions (Fearman et al. 2009). Therefore, the more energy is stored, the longer the mussels should survive during stress exposure. In this context, it is an interesting question whether mussels that have spent several generations in an impacted habitat, where they are often exposed to, e.g. low-oxygen stress, tend to store more energy as glycogen for future periods of environmental stress. If this was the case, it would be difficult to disentangle the influences of local adaptations, epigenetic transgenerational plasticity and the nutritional status in an experimental approach since at least two of the explanations would be closely interrelated.

Furthermore, it is important to notice that the individual capacities for energy storage and acclimation (both are aspects of phenotypic plasticity) are heritable. I, therefore, can, of course, not exclude local adaptation, epigenetic transgenerational effects and phenotypic plasticity as drivers of environmental tolerance. However, what I can conclude from my results is that the most important driver for tolerance to hypoxia – and probably also for tolerance to thermal stress and hyposalinity – in *P. viridis* from West Java is the momentary nutritional status that a specimen is in. This is an important finding to understand differences in stress tolerance between populations from different habitats. It also sheds light on the question why *P. viridis* is a successful circumtropical invader and why the mussels' potential to establish in new habitats is so large. In this context it is surprising that the mussel did not establish stable populations in the non-native range in the eastern Indonesian archipelago and in the subtropical and tropical Australia, although environmental conditions are suitable and

settlement substrates are present. So far, in these areas, mussels were only found on ship hulls or artificial substrates in harbours but did not spread to any natural hard substrates (Dias et al. 2013; Huhn et al. 2015; McDonald 2012; McDonald et al. 2016). If this is related to the nutritional status of the arriving mussels, it can have two reasons: a) mussels brought in as hull fouling had low BCIs due to the journey on the open oligotrophic ocean (chapter 1) or b) the habitats where the mussels were released in eastern Indonesia (e.g. Ambon Bay) and Australia (e.g. Queensland and Western Australia) are not eutrophic enough to provide sufficient amounts of phytoplankton. I measured phytoplankton abundance in Ambon Bay and found it to be even lower than in the oligotrophic habitat LB in the native range of the mussels in western Indonesia. With, in average, 2119 ± 1603 phytoplankton units per millilitre sample (mean ± sd, n = 5, Appendix I), it was comparable to the lowest phytoplankton abundances found in LB (chapter 2). I do not have comparable plankton data from Australia, however. A positive correlation between phytoplankton abundance and the nutritional status of the mussels was shown in this study (chapter 2). Furthermore, McDonald et al. (2016) relate the absence of *P. viridis* from a specific site in Singapore to prevailing low Chlorophyll *a* concentrations. Also, most well known cases of *P. viridis* establishments in the non-native range are from habitats that are highly anthropogenically impacted and eutrophic, e.g. Kingston Harbour (Jamaica), Chennai (India) and Tolo Harbour, Hong Kong (China) (Buddo et al. 2003; Huang et al. 1983; Vijayavel et al. 2007). In this study, I found the BCIs of mussels fouling on a ferry to be very poor (chapter 1). This was only one case and I did not sample further ferries. It, therefore, remains to be investigated whether this picture is typical for mussels spending their adult life on an open ocean journey. I also did not find mature gonads in any of the mussels that were collected from the ferry and, therefore, would assume that their potential to establish in new habitats is low. Again, this may not be the case for all mussels that grow as hull fouling on ferries.

In this study, hypoxia tolerance was used as a measure of robustness in mussels and a low hypoxia tolerance was interpreted to indicate low invasiveness. Of course, hypoxia tolerance is not the only determent of invasiveness, but, together with the nutritional status, it gives reliable information about the health status of a mussel. If transport on ship hulls impairs the mussels' nutritional status and mussel robustness as shown in this study, this concept also implies that transported *P. viridis* do not have a high potential to establish in new habitats. When reviewing the invasion history of *P. viridis*, it is striking that in most cases in which the mussels established and spread outside their native range, the invasion vector was either aquaculture or ballast water (Baker et al. 2007; Buddo et al. 2003). To my knowledge,

there is no known case of *P. viridis* establishment and spread after introduction via hull fouling.

Understanding the invasion pathways and, identifying donor populations responsible for *P. viridis* incursions in the non-native range, can be achieved by studying the population-genetics of the species. Gilg et al. (2013) have compared relationships between two native, one cryptogenic and five invasive populations of the mussels using Cytochrome-c-oxidase subunit I (COI) and suggested that all invasive populations originate from the introduction of a single population that subsequently spread. However, the authors were not able to identify the source population because including more populations from the native range would be necessary. I contributed to the extension of this study by sampling five mussel populations in Indonesia and the project is currently ongoing. Furthermore, I am collaborating in a project by the Department of Fisheries, Government of Western Australia, in Perth, using microsatellite DNA to identify the native and introduced populations of *P. viridis*. So far, we have developed 16 new markers of polymorphic microsatellite loci (Lukehurst et al. submitted) and screened native and non-native populations from India, Hong Kong, Taiwan, Thailand, Singapore, Indonesia and Tampa Bay, USA. The results show that only the Tampa Bay and India populations are genetically different from most of the Southeast Asian populations. Within Southeast Asia, there seems to be high connectivity and genetic exchange between Hong Kong, Taiwan, Singapore and Indonesia (McDonald et al. 2016). The high genetic similarity found between - and the high diversity found within - the different Indonesian populations tested (Jakarta Bay, Lada Bay, Pelabuhan Ratu, Makassar, Surabaya and Ambon) (McDonald et al. 2016) supports the assumption that there is a high genetic exchange between the populations. This would again make local adaptations to specific environmental conditions unlikely to be manifested in the gene pool of a single population of *P. viridis* in Indonesia.

*Conclusions*

The results of my studies emphasize the significant role that food supply plays for the tolerance of the Asian green mussel *P. viridis* to environmental stress. The motivation to test which mechanisms determine stress tolerance in *P. viridis* arose from the observation of substantial differences in stress tolerance between mussel populations. They were found between mussels from the impacted Jakarta Bay, Java Sea, and from the pristine Lada Bay, Sunda Strait, both located at the island of Java, Indonesia. The differences concerned

tolerance to desalination, thermal stress and hypoxia of which all three were higher in Jakarta Bay mussels (Wendling et al. 2013). Three main mechanisms were initially considered that could make mussels from Jakarta more robust: a) genetically fixed adaptations to environmental stress, b) acclimation or stress hardening, i.e. reversible changes in tolerance due to previously experienced stress, and c) higher food supply in the eutrophic habitat that equipped mussels with rich energy resources for stress compensation.

In four experiments, in which adult mussels from 4 populations were exposed to hypoxia and in one experiment with juvenile mussels from 2 populations, which were also exposed to hypoxia, the mussels with higher survival rates were consistently also the ones with higher body condition indices (BCIs). The only exception was one group with low BCIs, which showed higher survival rates than a group with high BCIs after it had been acclimated to low concentrations of dissolved oxygen (chapter 3). This latter finding suggests that both, a good nutritional status and acclimation to non-lethal stress levels, can enhance stress tolerance in *P. viridis*. In contrast to this, in experiments with reciprocal transplantations of mussels between an impacted and a natural site, local adaptations could be excluded as a significant driver of robustness (chapter 2). This could be concluded from the fact that mussel performance under hypoxia was not determined by the habitat where the mussels originated from, but by the habitat where the mussels had spent the last two months before the hypoxia experiment.

Both of my approaches, the correlative one presented in chapter 1 & 2 and the analysis of a causal relationship (chapter 3), suggest that a good nutritional status is vitally important for compensating environmental stress. This may also affect the invasion success of *P. viridis*: the mussels are most successful in establishing in habitats outside the native range if the receiving environment is on the one hand anthropogenically impacted, such as harbours, and on the other hand located near estuaries with high loads of nutrients (Acosta et al. 2010; Buddo et al. 2003). Jakarta Bay, even though it is located in the native range of the species, is a good example for this scenario. Here eutrophication and frequent phytoplankton blooms led to high population densities and to an overall good nutritional status of the mussels (chapter 2). Similar examples are Tolo Harbour in Hong Kong (cryptogenic) or Kingston Harbour (non-native range) in Jamaica (Buddo et al. 2003; Cheung 1993). The results from the studies presented here suggest that life in a eutrophic and impacted system can even enhance mussel resistance to adverse conditions, as long as stress levels are not too extreme and as long as the impact is associated with high food availability (chapter 2). For the invasion ecology of *P. viridis* this could mean that the species can only successfully establish in habitats outside the

native range if, after transport, it is released into a eutrophic environment with high phytoplankton abundances. At the same time, transport to the non-native range as fouling on a ship hull should minimize the invasion success of *P. viridis*, if the journey goes through oligotrophic waters and results in a poor nutritional status of the mussels (Figure 1). This may explain the lack of establishment success that *P. viridis* shows in the subtropical and tropical Australia, even though the estimated number of ships arriving in Western Australia with *P. viridis* on their hulls is 2644 over the past 5 years (McDonald et al. 2016). Therefore, *P. viridis* may not be an uncontrollable pest species that colonizes new habitats under any conditions, but is limited in its success by a) the planktonic primary production of the receiving environment and b) the conditions during transport. Conclusively, the best way to prevent invasions by *P. viridis* is presumably to maintain natural and oligotrophic conditions in tropical coastal ecosystems.

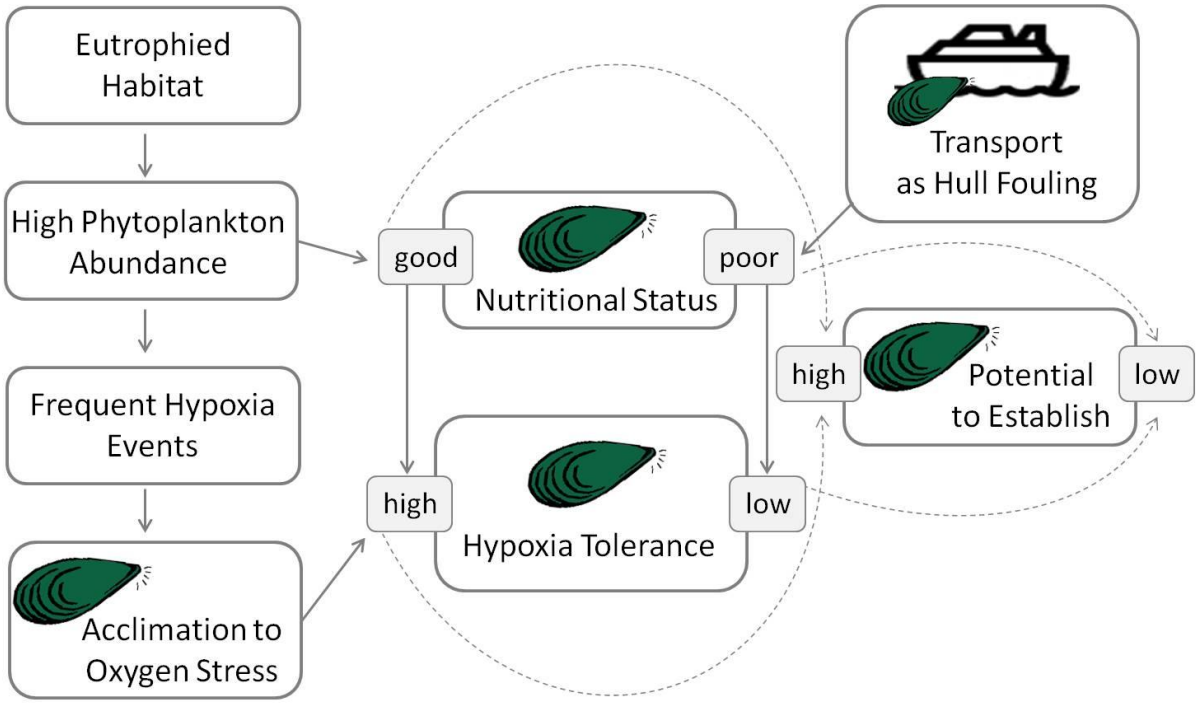


**Figure 1**: Schematic illustration of the influences of eutrophic conditions in a coastal habitat and of ship transport on the nutritional status, on hypoxia tolerance and on the potential to establish in a new habitat in *Perna viridis*. Eutrophication enhances phytoplankton abundance, which, in turn, has a positive effect on the nutritional status. The latter promotes tolerance to hypoxia. However, as microbial activity is increased, eutrophication, at the same time, can cause hypoxia events in the impacted habitat. This again can enhance tolerance to low oxygen concentrations, because exposure to short phases of hypoxia can lead to acclimation to oxygen stress. If a mussel arrives in new habitat after ship transport, however, its nutritional status is likely to be reduced because phytoplankton availability can be low during the voyage. Mussel individuals that get released into a new habitat after such a journey, e.g. due to the removal of hull fouling, are in a poor nutritional state and have a lower hypoxia tolerance. This should

reduce their potential to establish and spread. If they are, however, released into a eutrophic habitat, a good nutritional status should be regained quickly and the potential to establish should increase again. Solid lines indicate conclusions drawn from the results presented in this thesis, whereas dashed lines indicate hypotheses that need to be tested in future studies.

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## Appendix

**Appendix I:** Phytoplankton abundance in Ambon Bay. Plankton sampling and evaluation was conducted as described in chapter 2 (Huhn Zamani, von Juterzenka and Lenz 2016). “Total units” are the units of dinoflagellates and diatoms, which are potential food sources for *Perna viridis*. The concentration of dinoflagellates, diatoms and total units is shown in a millilitre of the sample and not of the water column at the sampling site (see chapter 2).

| Site | GPS location | Sampling date | Dinoflagellates per ml | Diatoms per ml | Total units per ml |
|---|---|---|---|---|---|
| AMB 1, Ambon Bay | S 03°38.217'<br>E 128°12.917' | 06.02.2013 | 920.00 | 760.00 | 1680.00 |
| AMB 2, Ambon Bay | S 03°39.883'<br>E 128°11.467' | 06.02.2013 | 777.78 | 166.67 | 944.44 |
| Lateri, Ambon Bay | S 03°38.692'<br>E 128°13.967' | 10.05.2013 | 400.00 | 1266.67 | 1666.67 |
| Hunut, Ambon Bay | S 03°38.251'<br>E 128°12.850' | 10.05.2013 | 250.00 | 4687.50 | 4937.50 |
| Hunut, Ambon Bay | S 03°38.251'<br>E 128°12.850' | 24.03.2014 | 366.67 | 1000.00 | 1366.67 |
| **mean of all sites** | | | **542.89** | **1576.17** | **2119.06** |
| **standard deviation of all sites** | | | **289.24** | **1786.13** | **1603.66** |

## Author contributions

### Chapter 1 a: A ferry line facilitates dispersal: Asian green mussels *Perna viridis* (Linnaeus, 1758) detected in eastern Indonesia

Mareike Huhn, Neviaty P. Zamani and Mark Lenz

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MH and ML developed the study concept. MH conducted the sampling, sample processing and analysis. The study was co-supervised by ML and NPZ. MH wrote and ML and NPZ edited the manuscript.

### Chapter 1 b: Tolerance to hypoxia in Asian green mussels, *Perna viridis*, collected from a ship hull in the non-native range in eastern Indonesia

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MH and ML developed the study concept. MH conducted the sampling, experiments and analysis. The study was co-supervised by ML and NPZ. MH wrote and ML and NPZ edited the manuscript.

### Chapter 2: Food availability in an anthropogenically impacted habitat determines tolerance to hypoxia in the Asian green mussel *Perna viridis*

Authors: Mareike Huhn, Neviaty P Zamani, Karen von Juterzenka and Mark Lenz

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The study was designed by MH and ML. All sampling, experimental work, data processing and analysis was conducted by MH. ML helped with the statistical analysis and KvJ with the practical application of the experimental work. NPZ supervised the work in Indonesia. MH wrote and ML, NPZ and KvJ edited the manuscript.

**Chapter 3: Tolerance to stress differs between Asian green mussels *Perna viridis* from the impacted Jakarta Bay and from natural habitats along the coast of West Java**

Mareike Huhn, Giannina S I Hattich, Neviaty P Zamani, Karen von Juterzenka and Mark Lenz

Published in Marine Pollution Bulletin (2016, in press)

MH, GSIH, KvJ and ML designed the study. MH conducted the hypoxia experiments and the field sampling in 2013 and 2014, whereas GSIH conducted the hyposalinity experiment and the field sampling in 2012. MH conducted the data analysis and ML assisted with the statistical analysis. NPZ supervised the work in Indonesia. MH wrote the manuscript, which was edited by GSIH, NPZ, KV and ML.

**Curriculum vitae**

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|---|---|
| 2016 | Price for the best oral presentation at the International Conference on Marine Bioinvasions, Sydney, Australia |
| 2016 | DAAD Travel grant for international congresses |
| 2012-2014 | DAAD (German Academic Exchange Service) scholarship for PhD students within international projects |
| 2011 | Price for one of the four best Diploma theses of the year 2011, rewarded by the faculty of Biology, University of Würzburg |
| 2010 | DAAD scholarship for international Master theses (5 months) |
| 2009 | DAAD scholarship for international internships (3 months) |

Publications

*Original research papers:*

**Huhn M**, Hattich GSI, NP, von Juterzenka K, Lenz M (2016). Tolerance to stress differs between Asian green mussels *Perna viridis* from the impacted Jakarta Bay and from natural habitats along the coast of West Java. Marine Pollution Bulletin, in press, http://dx.doi.org/10.1016/j.marpolbul.2016.02.020

**Huhn M**, Zamani NP, von Juterzenka K, Lenz M (2016). Food availability in an anthropogenically impacted habitat determines tolerance to hypoxia in the Asian green mussel *Perna viridis.* Marine Biology (2016) 163:15, DOI 10.1007/s00227-015-2786-6

**Huhn M**, Zamani NP, Lenz M (2015). A ferry line facilitates dispersal: Asian green mussels *Perna viridis* (Linnaeus, 1758) detected in eastern Indonesia. BioInvasion Records, Volume 4, Issue 1: 23–29

Wendling C, **Huhn M**, Ayu N, Bachtiar R, von Juterzenka K & Lenz M (2013). Habitat degradation correlates with tolerance to climate-change related stressors in the green mussel *Perna viridis* from West Java, Indonesia. Marine Pollution Bulletin, 71, 222–229.

*Conference contributions:*

**Huhn M**, Zamani NP, Lenz M (2016). Does transport on ferries mitigate the risk of establishment in Asian green mussels *Perna viridis*? Oral presentation, International Conference on Marine Bioinvasions, Sydney, Australia, 19$^{th}$ -21$^{st}$ Jan 2016

Maddupa H, Dias J, **Huhn M**, McDonald J (2016). Trial of an early warning system for marine pests in the Banda Islands, Moluccas, Indonesia. Oral presentation, International Conference on Marine Bioinvasions, Sydney, Australia, 19$^{th}$ -21$^{st}$ Jan 2016

**Huhn M**, Zamani NP, Lenz M (2015). Bioinvasions between Indonesian islands: Asian green mussels *Perna viridis* as hitchhikers on ferries. Oral presentation, Small Island Research in Tropical Regions (SIRTRE), Makassar, Indonesia, 15$^{th}$ – 16$^{th}$ Sep 2015

**Huhn M** (2014). Bioinvasions within a Single Country: Crossing Biogeographic Borders in the Indonesian Archipelago. Keynote, Mini-Workshop on Enhancing Marine Biodiversity Research in Indonesia, Agricultural University Bogor, Indonesia, 14$^{th}$-15$^{th}$ Nov 2014

**Huhn M**, Lenz M., von Juterzenka K, Wahl M (2013). Habitat quality influences the tolerance of the Asian green mussel *Perna viridis* towards hypoxia. Oral presentation, World Congress of Malacology, Ponta Delgada, Azores, Portugal

**Huhn M** (2013). Is fatter better? Site-related differences in body condition index and shell morphology in the Asian green mussel (*Perna viridis*) with regard to habitat quality. Oral presentation, International Conference on Marine Sciences, Agricultural University Bogor, Indonesia

**Eidesstattliche Erklärung**

Hiermit bestätige ich, dass die vorliegende Arbeit mit dem Titel „The relevance of food availability for the tolerance to environmental stress in Asian green mussels, *Perna viridis*, from coastal habitats in Indonesia“, abgesehen von der Beratung durch meine akademischen Betreuer, von mir selbstständig verfasst worden ist und keine weiteren Quellen und Hilfsmittel als die angegebenen verwendet wurden. Die vorliegende Arbeit ist unter Einhaltung der Regeln guter wissenschaftlicher Praxis der Deutschen Forschungsgemeinschaft entstanden und wurde keiner anderen Stelle im Rahmen eines Prüfungsverfahrens vorgelegt.

Folgende Teile der Arbeit wurden bereits in wissenschaftlichen Fachzeitschriften veröffentlicht oder eingereicht:

Mareike Huhn, Neviaty P. Zamani and Mark Lenz (2015) A ferry line facilitates dispersal: Asian green mussels *Perna viridis* (Linnaeus, 1758) detected in eastern Indonesia. *BioInvasion Records* 4, 1: 23–29.

Mareike Huhn, Neviaty P Zamani, Karen von Juterzenka, Mark Lenz (2016) Food availability in an anthropogenically impacted habitat determines tolerance to hypoxia in the Asian green mussel *Perna viridis*. *Marine Biology* 163, 1, 15pp.

Mareike Huhn, Giannina S I Hattich, Neviaty P Zamani, Karen von Juterzenka, Mark Lenz (2016) Tolerance to stress differs between Asian green mussels *Perna viridis* from the impacted Jakarta Bay and from natural habitats along the coast of West Java. *Marine Pollution Bulletin* (in press).

Mareike Huhn, Neviaty P. Zamani, Mark Lenz (submitted) Tolerance to hypoxia in Asian green mussels, *Perna viridis*, collected from a ship hull in the non-native range in eastern Indonesia. Submitted as short communication to the journal *Management of Biological Invasions*, Special Issue *International Conference on Marine Bioinvasions 2016*

Kiel, den 23.6.2016

Mareike Huhn