Biol Invasions (2018) 20:3107–3122
https://doi.org/10.1007/s10530-018-1762-8

ORIGINAL PAPER

Heat challenges can enhance population tolerance to thermal stress in mussels: a potential mechanism by which ship transport can increase species invasiveness

Mark Lenz · Yasser Ahmed · João Canning-Clode · Eliecer Díaz · Sandra Eichhorn · Armin G. Fabritzek · Bernardo A. P. da Gama · Marie Garcia · Karen von Juterzenka · Patrik Kraufvelin · Susanne Machura · Lisa Oberschelp · Filipa Paiva · Miguel A. Penna · Felipe V. Ribeiro · Martin Thiel · Daniel Wohlgemuth · Neviaty P. Zamani · Martin Wahl

Received: 11 August 2017 / Accepted: 21 May 2018 / Published online: 24 May 2018

Abstract It is unclear whether transport by human vectors can increase the robustness of translocated populations and thereby enhance their invasiveness. To test this concept, we investigated the effect of heat stress on the tolerance of mussel populations towards a second stress event of the same kind. The heat challenges we mimicked can be faced by marine invertebrates that are transported through regions with high sea surface temperatures on ship hulls or in ballast water tanks. The study included 5 mussel species that were collected at sites in Brazil, Chile, Finland, Germany (Baltic Sea) and Portugal. In parallel laboratory experiments, monospecific groups of individuals were exposed to heat challenges that caused 60–83% mortality in the experimental groups within 15–28 days. The surviving individuals were exposed to a second stress event of the same kind, while their survival was then compared to the robustness of conspecifics that had not been exposed to elevated temperatures before. We observed that thermal tolerance was significantly enhanced by previous heat stress experience in case of *Semimytilus algosus* from Chile and in case of *Mytilus edulis* from

---

Eliecer Díaz, Patrik Kraufvelin, Daniel Wohlgemuth:
Tvärminne Zoological Station, J.A. Palménin tie 260, 10900
Hanko, Finland (address during field work).

M. Lenz (✉) · S. Machura · F. Paiva · M. Wahl
Marine Ecology Department, GEOMAR Helmholtz
Centre for Ocean Research Kiel, Düsternbrooker Weg 20,
24105 Kiel, Germany
e-mail: mlenz@geomar.de

Y. Ahmed · K. von Juterzenka · N. P. Zamani
Department of Marine Science and Technology, Bogor
Agricultural University, Jalan Lingkar Akademi, Kampus
IPB Darmaga, Bogor 16680, West Java, Indonesia

J. Canning-Clode · F. Paiva
MARE – Marine and Environmental Sciences Centre,
Quinta do Lorde Marina, Sítio da Piedade, 9200 –
044 Caniçal, Madeira Island, Portugal

J. Canning-Clode
Cais do Carvão, 9000-107 Funchal, Madeira, Portugal

J. Canning-Clode
Department of Oceanography and Fisheries, Centre of
IMAR of the University of the Azores, Rua Prof. Dr.
Frederico Machado, 4, PT-9901-862 Horta, Azores,
Portugal

J. Canning-Clode
Smithsonian Environmental Research Center, 647
Contees Wharf Road, Edgewater, MD 21037, USA

E. Díaz
Department of Environmental Sciences, University of
Helsinki, PO Box 65 (Viikinkaari 1), 0014 Helsinki,
Finland

S. Eichhorn
Department Marine Biology, University of Rostock,
Albert-Einstein-Str. 3, 18059 Rostock, Germany

Germany. Our results suggest that heat challenges, which marine invertebrates experience during transport, can enhance stress tolerance in founder populations of these species in their non-native range by potentially increasing the frequency of genetically adapted genotypes. This points at the necessity to learn more about selection acting on organisms during human-mediated transport—in the aquatic but also in the terrestrial environment.

Keywords Heat · Mytilids · Selection · Stress tolerance · Survival · Transport

Introduction

Biological invasions are a common phenomenon in many aquatic and terrestrial ecosystems worldwide and their frequency has been increasing for decades (Simberloff et al. 2013; Turbelin et al. 2017). The establishment and spread of non-native species can cause substantial economic damage and has the potential to alter the structure and functioning of the affected ecosystems (Carlton 2002; Bax et al. 2003; Simberloff 2011). The development of methods and legislative regulations that allow the successful control and management of bioinvasions therefore constitutes a pressing task for scientists, managers and politicians who are concerned with environmental policies and conservation ecology (Dahlstrom et al. 2011; Ojaveer et al. 2014). For this, however, it is indispensable to understand which abiotic and biotic factors determine invasion success in species that are introduced into new habitats. Among several species-specific characteristics, such as mobility, reproductive output and competitiveness, which could play a key role for invasion success, tolerance to stressful environmental conditions has been identified as a core quality (Lockwood et al. 2007; Catford et al. 2009; Van Kleunen et al. 2010).

Previous studies have reported that some non-native species are more stress tolerant than taxonomically related native species with corresponding ecological roles (Schneider and Helmuth 2007; Zardi et al. 2007; Schneider 2008; Lenz et al. 2011; Zerebecki and Sorte 2011; Bates et al. 2013; Lejeusne et al. 2014). Whether robustness, i.e. the capacity to survive adverse environmental conditions caused by heat, oxygen limitations or low salinities, in these species is an inherent trait that evolved in response to a harsh environment—long before they become invasive in other regions of the world—or whether stress tolerance was acquired during the invasion process remains unclear. The latter comprises the transport

A. G. Fabritzek
Department of Ecology, Institute of Zoology, Johannes Gutenberg-University of Mainz, Johann-Joachim-Becher-Weg 13, 55099 Mainz, Germany

B. A. P. da Gama · F. V. Ribeiro
Departamento de Biologia Marinha, Universidade Federal Fluminense (UFF), Caixa Postal 100644, Niterói,
Rio de Janeiro CEP 24001-970, Brazil

M. Garcia
Department of Evolutionary Biology and Animal Ecology, Faculty of Biology, University of Freiburg,
Hauptstrasse 1, 79104 Freiburg, Germany

P. Kraufvelin
Department of Aquatic Resources, Institute of Coastal Research, Swedish University of Agricultural Sciences,
Skolgatan 6, 74242 Öregrund, Sweden

L. Oberschelp
Mathematisch-Naturwissenschaftliche Fakultät,
Fachbereich Geowissenschaften, Eberhard Karls
Universität Tübingen, Geschwister-Scholl-Platz,
72074 Tübingen, Germany

M. A. Penna · M. Thiel
Facultad de Ciencias del Mar, Universidad Católica del Norte, Larrondo 1281, Coquimbo, Chile

M. Thiel
Millennium Nucleus Ecology and Sustainable Management of Oceanic Island (ESMOI), Coquimbo, Chile

M. Thiel
Centro de Estudios Avanzados en Zonas Áridas (CEAZA), Coquimbo, Chile

Present Address:
Y. Ahmed
Department Utilization of Fisheries Resources, Satya Negara Indonesia University, Jl. Arteri Pondok Indah No 11, Kebayoran Lama 12240, Jakarta, Indonesia

from the donor region into the recipient region, the release into the new environment after arrival as well as the establishment and spread in habitats from which the species was previously absent (Lockwood et al. 2007). The first mechanism would rather constitute a prerequisite for invasion success, while the second mechanism could take place during the invasion process and could influence its outcome. If the first is true, this would mean that some but not all species can become invasive, while in the second case any organism with a wide ecological niche and/or a broad tolerance range could become a potential invader—given that its robustness is selected for during the invasion process.

The assumption that robustness can be acquired during the invasion leads to the question of which mechanisms have the potential to enhance stress tolerance in individuals or populations of plants or animals and at which stage of the invasion process they occur? Worldwide biological invasions in the marine environment are facilitated by anthropogenic vectors that can transport species over long distances in short time intervals (Carlton and Ruiz 2005). Hence, the majority of successful range expansions of aquatic and terrestrial species that have been documented during the last decades was presumably initiated by human-facilitated, intentional or unintentional transport of organisms (Levine and D'Antonio 2003; Floerl et al. 2009; Hulme 2009; Anderson et al. 2015). This causality is well documented for marine systems in which shipping traffic, mainly by container vessels, plays a key role for the dispersal of marine life forms beyond their natural distribution limits (Seebens et al. 2013; Williams et al. 2013; Bailey 2015).

Vessels mainly offer two different options to transport algae or invertebrates from one sea area to another: their hulls and their ballast water tanks (Ruiz et al. 2000). Ship hulls, although most often coated with antifouling paints, represent a large solid settlement substratum for algae and sessile invertebrate species such as mussels (Drake and Lodge 2007; Davidson et al. 2009; Chapman et al. 2013; Chan et al. 2015). The latter colonize these surfaces, especially when the antifouling coating is old, damaged, or masked by other foulers, as larvae or post-larvae and this can occur in any environment in which these life stages are abundant. During their adult life, the animals remain on the ship hulls until they die or fall-off. During this time they often reach sexual maturity and can then release gametes into the water column (e.g. Coutts and Dodgshun 2007).

The second mode of vessel-based species transport is the uptake of marine life forms, from bacteria to fish, with the ballast water that regulates the buoyancy of ships (Endresen et al. 2004; Bailey 2015). Ballast water tanks are commonly emptied or filled when cargo is loaded or released—unless the loaded freight equals the released one—and, although the conditions inside these tanks are stressful, many organisms survive transport and will then be released during the next water exchange (Gollasch et al. 2000; Klein et al. 2010).

Both ways of transport, on the hull and inside the ballast water tanks, expose organisms to stressful abiotic conditions, but the prevailing stressors differ between the two environments (Wonham et al. 2001; Minchin and Gollasch 2003). During the voyage from one destination to another, the temperature inside ballast water tanks follows the sea surface temperature with a delay of 1–2 days (Gollasch et al. 2000). Furthermore, oxygen concentration in the tanks can decrease due to consumption by the organisms present, but can be re-increased due to mixing caused by movements of the ship (Gollasch et al. 2000), while salinity may change abruptly in case the ballast water is exchanged. Ballast water exchange at sea, i.e. > 200 nautical miles from shore, is now mandatory in many countries worldwide to reduce the amount of viable stages of marine life forms inside ballast water tanks before an over-sea vessel reaches its destination (Carney et al. 2017). In contrast to ballast water tanks, oxygen limitations are unlikely at the hulls, but the ambient salinity may change as well when the ship passes through estuaries, rivers or artificial waterways (Cohen 2006; Davidson et al. 2008). Heat stress can affect both environments, for instance, when the shipping route leads from temperate to tropical waters. Hence, during a ship voyage that starts and ends in a temperate environment but leads

---

Present Address:
K. von Juterzenka
Leibniz Centre for Tropical Marine Research (ZMT), Fahrenheitstraße 6, 28359 Bremen, Germany

Present Address:
D. Wohlgemuth
Ocean and Earth Science, University of Southampton, Waterfront Campus, European Way, Southampton SO14 3ZH, UK

through tropical waters (see Kaluza et al. (2010) for examples), all organisms that are present on or inside the vessel and which originate from the temperate zone can experience temperature fluctuations of more than 20 °C (Hua and Hwang 2012). Consequently, during the passage, temperatures can be reached that constitute a severe heat stress for temperate organisms and this stress can persist for days to weeks (Gollasch et al. 2000). The stress may lead to partial mortality among the transported organisms that could select for those genotypes that exhibit the largest tolerance to elevated temperature. If this is the case, the frequency of tolerant genotypes in the transported population should increase during the voyage (Sakai et al. 2001). Hence, population robustness towards this stressor should be higher at the end of the journey than at its beginning.

However, so far, there are no empirical data that document such effect and therefore we designed a global study consisting of parallel experiments that mimicked heat stress during a transoceanic voyage in laboratory environments. As test organisms we used different bivalves of the family Mytilidae. These animals, due to their capacity to attach to solid surfaces, are frequently transported as hull fouling but also in ballast water tanks (e.g. Wonham 2004; Murray et al. 2011; Casoli et al. 2016; Huhn et al. 2016a). Our test organisms came from different latitudes and were therefore adapted to different thermal environments, ranging from cold-temperate regions (Finland) to the tropics (Brazil). We specifically tested the hypothesis that stress-induced mortality enhances population resistance, measured as the survival rate during a second stress event of the same kind. At the same time, we checked whether the origin of the species influences the outcome of this test.

Methods

Collection sites and test organisms

Parallel experiments were conducted at five different locations worldwide using the laboratory facilities of marine research institutes in Niterói (Brazil), Coquimbo (Chile), Tvärminne (Finland), Kiel (Germany) and Cascais (Portugal) from April to September 2012. At these sites, specimens from one locally abundant mytilid species were collected for experiments that tested whether the average robustness of a group of individuals can be enhanced by partial mortality that is induced by a preceding stress event of the same kind.

The species chosen for this study have a wide distributional range and often occur in large abundances. Furthermore, they have a life trait that makes them likely candidates for transport by human vectors: All mytilids form byssus threads which allow them to attach to solid surfaces such as ship hulls (Aguilera et al. 2017). Therefore, they are likely bioinvaders. The following paragraphs describe the sampling sites, sampling procedures and the way of transport to laboratories. More detailed information about these aspects is provided in Table 1.

South-Western Atlantic, Brazil

The test species in Brazil was the intertidal brown mussel *Perna perna*. This species is considered native in West Africa and in India (Hicks et al. (2001), while its status on the coasts of South America is under discussion. While many authors consider it native in these regions, Souza et al. (2003), after the analysis of archeological records, suggested that the species was introduced from West Africa during the 16th century. More recently, *P. perna* invaded the Gulf of Mexico and by this showed its invasive potential (Hicks et al. 2001). For our study, individuals of the mussel were collected at the beach of Boa Viagem in the city of Niterói in Guanabara Bay (22°54′S, 43°07′W). The coastline here is mostly rocky with interspersed sand beaches and has a tidal range of 1.3 m. Sea surface temperature usually ranges between 15 and 27 °C and is lowest in July and highest in January. The highest temperature that was recorded in Guanabara Bay during the last 15 years was 33.4 °C (source for all maximum temperature information: http://orca.science.oregonstate.edu/2160.by.4320.8day.hdf.sst.modis.php). Salinity is typically between 32 and 35. In May 2012, individuals of *P. perna,* which were 30-50 mm in size (umbo to the posterior end of the shell), were collected from rocks by cutting their byssus with a scalpel. Mussels were transported to the laboratory in seawater filled containers within 20 min.

Table 1 Information about test species collection, acclimation to laboratory and experimental conditions and experimental phases

Experimental group	Species	Water temperature when collected	Transport to laboratory	Acclimation to laboratory	Water temperature during acclimation	Acclimation to stress	Stress event 1 (Group A only)				Recovery (Group A only)		Stress event 2 (Group A + B)			Mortality (%)
		(°C)	(min)	(d)	(°C)	(°C/d)	(d)	(°C)	N	Mortality (%)	(d)	(°C)	(d)	(°C)	N	
A	*Mytilus edulis*	14.5	30	7	15	2	28	28	102	80	14	15	28	28	18	50
	M. galloprovincialis	18	30	7	19–20	1–2	21	29	160	83	14	20	16	29	12	100
	M. trossulus	3	30	21	3–13	3	17	27.5	160	77	14	15	19	27.5	12	66
	Perna perna	23	20	6	19	2	20	32	124	60	14	19	20	32	15	100
	Semimytilus algosus	14	10	7	12.5–15	3	15	28	222	71	18	14	18	28	31	87
B	*M. edulis*	14.5	30	7	15	2	28	15			14	15	28	28	36	86
	M. galloprovincialis	18	30	7	19–20	1–2	21	20			14	20	16	29	13	100
	M. trossulus	3	30	21	3–13	3	17	15			14	15	19	27.5	19	74
	P. perna	23	20	6	19	2	20	19			14	19	20	32	15	100
	S. algosus	14	10	7	12.5–15	3	15	14			18	14	18	28	27	92
C	*M. edulis*	14.5	30	7	15		28	15			14	15	28	15	21	0
	M. galloprovincialis	18	30	7	19–20		21	20			14	20	16	20	25	0
	M. trossulus	3	30	21	3–13		17	15			14	15	19	15	20	0
	P. perna	23	20	6	19		20	19			14	19	20	19	30	17
	S. algosus	14	10	7	12.5–15		15	14			18	14	18	14	26	0

The length of stress events does not include the time needed to acclimatize the animals to the target temperature

Table 2 Husbandry conditions during laboratory acclimation and the experiment

Species	Volume of maintenance tank (l)	Volume of experimental units (l)	Type of food	Amount of food
Mytilus edulis	60	0.30	DT's Premium Blend Marine Live Phytoplankton	0.05×10^6 cells per individual per d
M. galloprovincialis	375	0.33	DT's Premium Blend Marine Live Phytoplankton	25×10^6 cells per individual per d
M. trossulus	20	0.60	DT's Premium Blend Marine Live Phytoplankton	12×10^6 cells per individual per d
Perna perna	600	0.30	DT's Premium Blend Marine Live Phytoplankton	6.5×10^6 cells per individual per d
Semimytilus algosus	2000	0.68	*Isochrysis* sp. from a lab monoculture	$30–60 \times 10^5$ cells per individual per d

South-Eastern Pacific, Chile

The mytilid species in Chile was *Semimytilus algosus*, which is common along the shores of Northern-Central Chile where it colonizes rocks in inter- and subtidal habitats. It is native in these regions, but is also known as a successful marine invader from the coasts of Southern Africa (de Greef et al. 2013). We collected specimens in a size range of 20–30 mm in the Bay of Guayacan near Coquimbo (29°58′S, 71°21′W) by carefully detaching the animals from buoys by cutting their byssus. This was done in May and June 2012. Animals were transported to the nearby laboratory in dry conditions within 10 min. The Bay of Guayacan is fully marine with a salinity of 34, while the sea surface temperature in this area, which is influenced by coastal upwelling, takes minimum values around 13 °C in July and maximum values of about 20 °C in January (Table 1). The maximum temperature that was recorded in the Bay of Guayacan during the last 15 years was 21.4 °C.

Northern Baltic Sea proper, Finland

In Finland, the test species was the bay mussel *Mytilus trossulus*, which originates from the North Pacific, but can also be found at the east coast of North America (north of Gulf of Maine) and in parts of Northern Europe (McDonald et al. 1991, Väinölä and Strelkov 2011). However, it is not clear at what time it reached the regions which are located outside its native range (Väinölä and Strelkov 2011). Very recently, it has been found as an invasive species in the Strait of Magellan (Oyarzún et al. 2016). Specimens of this species were collected from subtidal rocks near Käringarna ca. 80 km east of Helsinki (Tvärminne, 59°50′N, 23°17′O) by the use of a bottom sleigh in April 2012. Sea surface temperature in the south-eastern Baltic Sea ranges from 0 °C in January/February to 22° in July/August. The highest temperature recorded during the last 15 years was 23.8 °C. The Baltic Sea is a semi-enclosed sea area with a mostly brackish water body that exhibits no tides. Salinity in Tvärminne is between 5.5 and 7.0. From the catch, we selected intact individuals in a size range of 7.5–12.5 mm and transported them to the laboratory submersed in seawater within 30 min.

Western Baltic Sea, Germany

In Germany, individuals of the blue mussel *Mytilus edulis* were collected in the Kiel Fjord (54°20′N, 10°09′E), Western Baltic, in September 2012. This mytilid species is native to the northern parts of the Northwest as well as the Northeast Atlantic (Seed 1992), but has been introduced to Argentina and Chile (Seed 1992) and the Mediterranean Sea (Casoli et al. 2016). The Kiel Fjord has sandy to muddy benthos habitats with boulders here and there that constitute the only natural hard substrata in the region. Sea surface temperatures in this south-western part of the Baltic Sea cover the same range as in Tvärminne, Finland (0–22 °C), while the maximum temperature recorded during the last 15 years was 23.2 °C. Salinity fluctuates between 15 and 20. The mussels (40–50 mm) were carefully detached from pilings in the shallow subtidal by cutting their byssus with a knife. After this they were transported to the laboratory in dry conditions within 30 min.

North-Eastern Atlantic, Portugal

In Portugal, the Mediterranean mussel *Mytilus galloprovincialis* was used as a test species. It is native to the Northeast Atlantic and the Mediterranean, but has invaded coastlines outside this range for instance in Australia, Chile, New Zealand, North America and South Africa (Toro et al. 2005; Elliott et al. 2008; Westfall and Gardner 2010). Individuals of *M. galloprovincialis* were collected in a rocky intertidal environment near Guia-Cascais (38°41′N, 9°27′W) in May and June 2012. In this region, the sea surface temperature of the Atlantic ranges from 13 °C in January/February to 21 °C in July/August and the highest temperature that was recorded here during the last 15 years was 21.5 °C. The water is fully marine with a salinity of 36, while the tidal amplitude along the rocky coastline is semidiurnal with tides that can range up to 4 m. After detaching the mussels (30–40 mm) from rocks in the intertidal by cutting their byssus threads, they were placed in buckets filled with seawater for transport. Transportation time to the laboratory did not exceed 30 min.

Husbandry conditions

After arrival in the respective laboratories, all mussels were cleaned from large-sized epibionts, such as hydrozoans, bryozoans, barnacles and macroalgae, by carefully brushing or scraping off the foulers. Furthermore, we documented the size of all individuals using calipers to measure the distance from the umbo to the posterior end of the shell. Prior to the start of the heat stress experiments, animals were acclimatized to laboratory conditions for time spans varying between 1 and 3 weeks (Table 1). During this period, mortality among individuals, which, after collection, ranged between 0 and 5% per day, dropped below 1% per day in all test species. At all sites, a group of individuals (i.e. Group C with n = 20–30) was kept under these conditions throughout the entire experimental phase without any further manipulation (Fig. 1). For acclimating and keeping the animals as well as for the heat stress experiments, we used indoor or outdoor tanks of different sizes (Table 2). These tanks received seawater either from a re-circulating water cycle that consisted of a reservoir and a filter unit (in Brazil and Portugal) or from a flow-through system supplied with water from the nearby sea (in Chile, Finland and Germany). The water inside the tanks was aerated by bubbling it with pressured air.

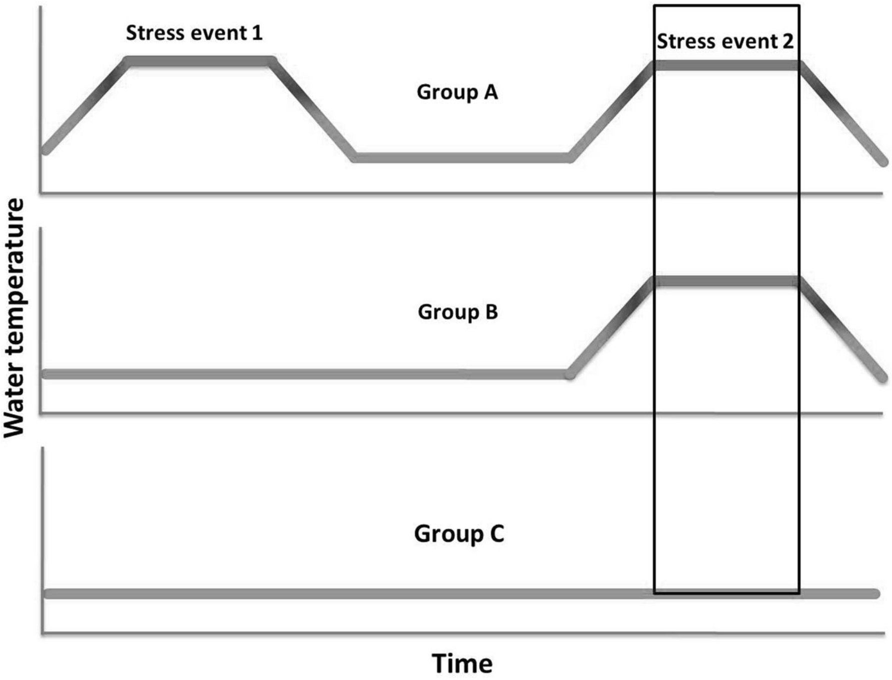


Fig. 1 Schematic illustration of the experimental design that was adopted at all study sites. One group of mytilid test individuals experienced two heat stress events (Group A), while a second group only experienced one (Group B). A further group of animals was kept under the same conditions as Group A and B, but was not stressed at any time (Group C). The box indicates the time span during which mortality rates were recorded in all three experimental groups

During all phases of the studies, animals were continuously fed, while the type of food and the amounts fed differed between species. Most of the mytilids were fed with DT's Premium Blend Live Marine Phytoplankton once a day (Table 2). This is a mixture of living microalgae that contains *Nanochloropsis oculata*, *Phaeodactylum tricornutum* and *Chlorella* sp. with a cell size of 2–20 μm at a concentration of approximately 2.5×10^6 cells ml^{-1} (M. Huhn, pers. comm.) (Table 2). Furthermore, we frequently monitored the water quality in the tanks by measuring nitrite, nitrate and ammonium (with chemical test kits or photometric procedures), oxygen concentrations (with hand-held oxymeters, e.g. VWR DO 200) and salinity (with refractometers, e.g. S/Mill-E by ATAGO). Faeces that accumulated in the tanks and dead animals were removed daily.

Experimental design

At all sites we had three experimental groups: The first group, Group A, was exposed to heat stress two times, Group B was stressed only once and Group C was not exposed to heat stress at any time (Fig. 1). For the stress events, the water temperature inside the experimental units (see 'Experimental set-up' section) was increased in a stepwise manner until a previously defined target temperature was reached (Fig. 1). After the target temperature had been maintained for a site-specific time span (Table 1), heat stress was relaxed by lowering the temperature back to ambient conditions at the same rate as it had been increased. During the first stress event, which was applied only to Group A, we kept several individuals of the test animals together in one experimental unit due to space constraints. This first event was, at all sites, followed by a 14–18 days-long recovery phase (Table 1), during which the water temperature was brought back to the ambient level. The length of the first stress event varied between 17 and 28 days (Table 1) and this discrepancy in lengths was because the duration of stress events was adjusted to the robustness of the test animals (see 'Pilot studies and heat stress regimes'). The following stress event, which was applied to both Group A and Group B, was in most cases of the same length as the first stress event (Table 1). Water temperature was increased in the same manner and to the same target temperature as for the first stress event, but we placed only one individual of the test species in each of the experimental units to ensure independency between replicates.

Our response variables were the mortality rates that occurred in Group A and Group B during the second stress event. To test whether the selection of individuals that were tolerant of heat stress increased the robustness of Group A towards a second stress event of the same kind, we compared mortality rates between Group A and Group B. At all sites, the individuals in Group B stem from the pool of animals that was collected at the beginning of the study, but they were only stressed once. Furthermore, they had spent the same time in the laboratory as the individuals that were stressed twice and were kept under exactly the same conditions. During the application of heat stress, all experimental containers were inspected daily to detect dead animals and to remove faeces. At each site, a further group of test individuals was kept under exactly the same conditions as the other experimental groups throughout the course of the study, but were not stressed at all (Group C, Fig. 1). This group served to assess the background mortality among the test animals.

Experimental set-up

During the heat stress events, animals were placed in small-sized experimental units, which were made of glass or plastic and had a volume of 0.3–0.7 l (Table 2). To manipulate the temperature inside the units, a part of the larger tanks, in which we acclimated the test animals, were now used as thermobaths. For this, they were filled with water, which we warmed with common electric aquaria heaters (e.g. Aquarium Heizer 300 Watt, EHEIM Jäger). The side walls of the tanks were insulated with Styrofoam panels to reduce heat loss. Submersed pumps ensured homogenous mixing of the water body inside the baths to avoid the formation of temperature gradients. Furthermore, we monitored the thermal regimes inside of the thermobaths every hour throughout the experiments using temperature loggers (HOBO Pendant® Temperature Data Logger). The smaller sized, single experimental units were placed in these thermobaths, but their interior was completely isolated from the surrounding water body to avoid any water exchange. The units were supplied with pressurized air that was led through a diffusor stone to keep oxygen concentrations inside the containers above stressful levels. The water inside the experimental units, which were not connected to any flow-through or re-circulating system, was renewed manually every day or every other day to maintain a good water quality. During the heat stress events, the water was replaced by seawater that was previously heated to the temperature that prevailed in the containers at the moment of exchange, while the units were re-connected to the seawater flow-through or the re-circulating system during the recovery phase (see below).

Pilot studies and heat stress regimes

The temperature regimes for the induction of heat stress and the duration of the stress events differed between species (Table 1). Both settings were adjusted in a way that allowed inducing a maximum mortality of 90% in Group A within a time span not longer than 4 weeks. It was our objective to reach a mortality rate that was substantial enough to select a group of robust individuals, but which would not reach 100% to leave replicates in Group A for the second stress event during which we compared tolerance towards heat stress between Group A and B. At all sites, we conducted pilot studies with various temperature regimes to identify the most suitable stress level for the induction of mortality during the first stress event (data not shown). At the same time, we took care that the chosen temperature levels did not exceed values that can be experienced by ship hull fouling organisms during a trans-equatorial passage, e.g. in the Pacific (Brown et al. 2015).

Statistical analyses

Survival data were visualized by Kaplan–Meier curves, while in case of proportional hazards Log-Rank tests or, in case of non-proportional hazards, Peto–Wilcoxon procedures allowed to test the null hypothesis that survival rates do not differ between groups of individuals that were previously exposed to heat stress (Group A) and those that were not (Group B). These tests were done for all 5 test species investigated in this study. All graphs and analyses were produced using the free statistical computing software R (R Development Core Team 2014) with the packages ‘survival’ and ‘ggplot2’.

Results

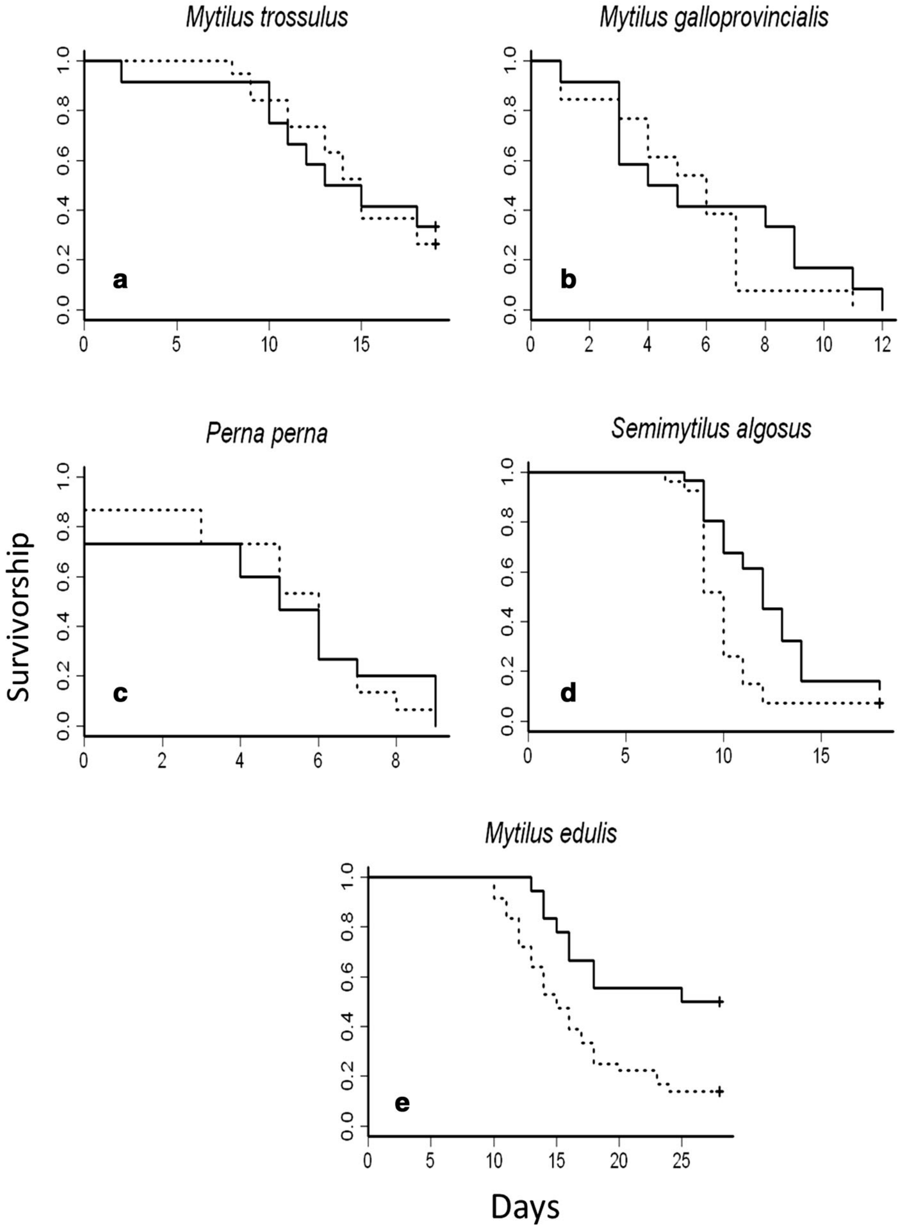
In three of the mytilid test species, no difference in robustness towards heat stress emerged between groups of individuals that survived a preceding heat challenge (Group A) and those that were not previously stressed (Group B). This was the case for the bay mussel *Mytilus trossulus* from Finland (Log-Rank Test: $\chi^2 = 0$, $p = 0.93$), the Mediterranean mussel *M. galloprovincialis* from Portugal (Peto-Wilcoxon Test: $\chi^2 = 0$, $p = 0.83$) and the brown mussel *Perna perna* from Brazil (Log-Rank Test: $\chi^2 = 0.1$, $p = 0.81$, Fig. 2a–c). In contrast to this, mussels of the species *Semimytilus algosus*, which was tested in Chile, showed an increased robustness towards heat when they survived a previous exposure to thermal stress. Mortality rates in this species were significantly lower among survivors of a preceding stress event than among non-stressed conspecifics (Log-Rank Test: $\chi^2 = 8.6$, $p = 0.003$, Fig. 2d), while median survival time was 10 days in the first and 12 days in the second group. The same picture was observed for individuals of the blue mussel *M. edulis* from the Western Baltic. In this species, survival under heat stress was again significantly higher among individuals that already lived through a preceding stress event than in the non-stressed reference group (Log-Rank Test: $\chi^2 = 8.6$, $p = 0.003$) (Fig. 2e). Median survival time was 15 days in the first and 25 days in the second group.

The mortality among individuals that were not stressed at all (i.e. Group C) during the time stress event 2 was applied to Group A and Group B is shown in Table 1.

Discussion

In a global multi-sited experimental approach with five species of mytilids, we tested whether selection of tolerant individuals by stress-induced mortality enhances population robustness towards thermal stress. We observed this effect in two of the mussel species: *Semimytilus algosus* from Chile and *Mytilus edulis* from the Southwestern Baltic Sea, i.e. in 40% of the cases. The extent to which previously experienced stress enhanced population robustness, however, varied strongly between these two species. In *S. algosus*, the difference in the median time to death between experimental groups was 2 days and therefore

Fig. 2 **a–e** Survival under elevated temperatures in groups of mytilids that previously experienced partial mortality due to heat stress (Group A, solid lines) and in groups that did not experience stress-induced mortality (Group B, hatched lines)



relatively small compared to the effect size that was observed for *M. edulis* (i.e. 10 days). It is impossible to assess whether such a small effect is relevant for invasion success after transport or not. So far, no empirical data exist that would allow to tell how big an increase in robustness in a given group of individuals of a given species has to be to enhance invasiveness significantly. However, depending on the status of the gonads in the transported animals, the release of gametes can occur at any time after the arrival in the new habitat. The longer the arrivers survive in the new environment the more likely it is that they reproduce—given that other fertile conspecifics are present.

If our findings are representative for the whole taxonomic group of mussels and the type of stressor we investigated, they indicate that an increase in population robustness through the influence of adverse environmental conditions, for instance during ship transport, could be frequently expected (ca. 40% of all cases). Furthermore, the extent to which robustness is enhanced by this process can be substantial such as in the case of *M. edulis*. Although our results provide a strong hint about a potential increase in robustness in adult individuals during the first weeks after their arrival in a new habitat, they do not allow to make assumptions about the further fate of these individuals after the initial phase. Furthermore, they can, of course, not give information about whether their potential offspring will survive under the conditions at the site of arrival.

Every day, numerous marine species are transported on the hulls or in the ballast water tanks of oceangoing vessels (Carlton 1999). During transoceanic voyages that cover different climate zones, these organisms can experience temperature regimes that can deviate substantially from the thermal environment that they are adapted to. Especially in cases when voyages start in temperate zones and lead through equatorial regions (Kaluza et al. 2010), transported individuals can experience severe heat stress for days to weeks. With regard to both, the magnitude of increase in water temperature and the length of the stress phase (i.e. resident time in tropical and subtropical waters), many of the worldwide ship transport scenarios are comparable to the conditions we realized in our study (e.g. Gollasch et al. 2000; Briski et al. 2011; Hua and Hwang 2012; Sutherland and Levings 2013). Hence, the mechanism that we observed in two of our five test species, i.e. an increase in population robustness as a consequence of previously experienced environmental stress, may also be relevant for marine invertebrates that are transported by oceangoing vessels. This would mean that human-mediated transport of species has the potential to modify stress tolerance and therefore invasiveness in founder populations of the translocated organisms. Ship traffic is therefore not only an important vector that allows numerous marine organisms to reach areas from which they were previously absent; it can also increase their potential to successfully invade these habitats by enhancing their tolerance to adverse environmental conditions.

An important question in this context is which of the numerous abiotic pressures occurring during oceanic voyages can mediate a relevant increase in robustness, which, at a later stage of the invasion process, enables species to establish and spread in the new habitat? The most common stressors that marine invertebrates face in subtidal coastal ecosystems, naturally and through human activities, are hypoxia (Jewett et al. 2005; Diaz and Rosenberg 2008; Rabalais et al. 2010), heat stress (Ganning 1971; Smale and Wernberg 2013) and hyposalinity (Goodbody 1961; Braby and Somero 2006). So, any selection for tolerance towards these stressors should have the potential to increase species invasiveness. Here, it is important to notice that selection for heat tolerance can, at the same time, also increase the capacity to tolerate hypoxia and vice versa (Kültz 2005). This is because many of the physiological responses to abiotic stress are universal and they can therefore mediate tolerance to several different stressors, what is known as cross tolerance (Bayley et al. 2001; Kültz 2005; Sinclair et al. 2013). Hence, even if there is a discrepancy between the kind of stress experienced during transport and the adverse environmental conditions in the new habitat, a selection that occurred during the voyage may enhance the invasiveness in founder populations of introduced species.

Our experimental approach does not allow the unambiguous identification of the mechanism that was responsible for the change in stress tolerance that we observed in two of the five species we tested. Possibly, it was a change in the frequency of stress tolerant genotypes, which followed from partial mortality among the individuals of the test populations. Alternatively, it could have been a consequence of stress hardening. The latter is part of the phenotypic response of an organism to environmental stress, which, for instance, includes the synthesis of heat shock proteins that safeguard the three-dimensional structure of enzymes under elevated temperatures (Kültz 2005). Reversible phenotypic responses of this kind mediate stress tolerance that can prevail even after the stress has ceased. This is because heat stress proteins persist for short time spans. Furthermore, their synthesis is not stopped immediately after the stress ceased but may continue for some hours or days (Malmendal et al. 2006; Brun et al. 2008; Bahrndorff et al. 2009). However, we assume that the length of the recovery phase that we inserted in between the two stress events was sufficiently long to exclude hardening as a possible explanation for enhanced robustness in pre-stressed animals. Unfortunately, we were not able to test this by quantifying the abundance of heat-shock proteins in the animals. This would have been the most direct evidence to confirm that there was no carry-over of phenotypic acclimation to previously experienced stress. Another option would have been to let the recovery phase last for several months, but this was not possible due to time constraints.

Although we clearly showed that previously experienced stress accompanied by partial mortality among the test animals can influence the resistance of survivors to a new stress event of the same kind, we observed substantial differences in the response within the group of bivalves, which are difficult to understand. The majority of mytilids did not reveal any influence of stress-induced mortality on population resistance, while none of them showed the opposite effect, i.e. pre-stressed individuals were less robust than non-stressed conspecifics. However, the reasons for the absence of an observable effect may vary from species to species. In the case of the tropical *P. perna*, it could be that these animals are already very close to their upper temperature tolerance limit so that the differences between genotypes with regard to heat tolerance within the population are too small to produce any measurable effect under the experimental conditions we realized (Compton et al. 2007). However, this certainly does not apply to *M. trossulus* from Finland and to *M. galloprovincialis* from Portugal. These two species inhabit marine environments in which summer maximum water temperatures usually do not exceed 22 °C and they should therefore exhibit a higher flexibility to adapt to elevated temperatures. Furthermore, the two species that exhibited an effect, *S. algosus* from Chile and *M. edulis* from Germany, both come from thermal environments that are not very different from the ones in the Gulf of Finland or at the coast of Portugal. Also with regard to other variables, which may have been important for the outcome of this study, such as the level of mortality that was induced during the first stress event, there was no pronounced difference between *M. trossulus* and *M. galloprovincials* on the one hand and *S. algosus* and *M. edulis* on the other. We can therefore not offer a plausible explanation for the absence of a measurable effect in the first two species. This also means that the latitude or thermal environment from which a population of mussels stems is not a reliable predictor of whether stress-induced mortality can enhance its robustness towards heat stress.

The size of the effect that we observed was smaller in *S. algosus* than in *M. edulis*, while the stress that was applied in these two systems was very similar. In Chile, the ambient temperature was increased by 14 °C from 14 to 28 °C, while in Germany the change was about 12 °C when we increased the water temperature from 16 to 28 °C. However, *S. algosus* showed a sudden increase in mortality after 10 days and both groups of test animals, pre-stressed and non-stressed, reached $> 80\%$ mortality after 14 days. In case of *M. edulis,* the pre-stressed group stabilized at 40–50% mortality after 15 days of exposure to elevated temperatures, while in the group of non-stressed animals, the number of survivors continuously declined until the end of the experiment after 28 days. A possible reason for the larger effect size could be that the mussels that were kept in Germany received less food than the mytilids at the other sites and therefore had less energy available for stress compensation. In this case, the influence of the pre-stress may have been more dominant.

An enhanced tolerance towards stress is presumably of particular relevance during the early phases of biological invasions, when a relatively small number of transported specimens is released into an environment that may confront them with harsh abiotic conditions. Many important harbours worldwide are located in estuaries where ambient salinity is low and arriving marine species are immediately exposed to osmotic stress that may limit their survival, establishment and further dispersal (Miller et al. 2007; Ruiz et al. 2013). The more individuals that survive this filter, the more likely it is that the introduced species can establish a permanent population. Therefore, tolerance to stress has repeatedly been suggested as a key trait that determines the invasive success of aquatic but also terrestrial species (Lockwood et al. 2007; Catford et al. 2009; Van Kleunen et al. 2010). In this context, several studies have shown that marine invasive invertebrates are generally more tolerant to abiotic stressors, e.g. hypoxia, hyposalinity and heat stress, than taxonomically related and ecologically similar native species or species that have so far not been recognized as invaders (Schneider and Helmuth 2007; Zardi et al. 2007; Schneider 2008; Gröner et al. 2011; Lenz et al. 2011; Sareyka et al. 2011; Zerebecki and Sorte 2011; Bates et al. 2013; Lejeusne et al. 2014). It is therefore vitally important for our understanding of biological invasions to identify all mechanisms that have the potential to enhance the capacity of species to tolerate adverse conditions during transport or after arrival in the new habitat. These comprise (a) the release from other environmental pressures, such as predation, competition or parasitism (Torchin et al. 2001; Keane 2002; Clay 2003; Colautti et al. 2004), because this should increase the amount of metabolic energy that is available for the compensation of stress effects; (b) the ample availability of energy, e.g. in eutrophic environments that can help to mitigate stress effects by providing energy for, e.g., the synthesis of heat shock proteins (Labarta et al. 1997; Wendling et al. 2013; Jeno and Brokordt 2014; Huhn et al. 2016b), and (c) stress-induced mortality during transport that increases the mean tolerance to stress in a group of introduced animals or plants by increasing the frequency of robust genotypes (Sakai et al. 2001). Our study, although conducted in a laboratory environment and not in situ, hints at the relevance of the last process and points at the necessity to learn more about selection acting on organisms that are transported on ship hulls or in ballast water tanks. If vessels on trans-oceanic routes are not only vectors but also a selective barrier or training field for stress tolerance in invertebrates, this is a further strong point indicating that there is an urgent need for the prevention of hull fouling as well as ballast water contamination and the establishment of worldwide management regulations.

Acknowledgements The study was realized in the framework of the international research and student training programme GAME (Global Approach by Modular Experiments) that is coordinated by GEOMAR Helmholtz Centre for Ocean Research Kiel, Germany. It was generously funded by Lighthouse Foundation, mare Zeitschrift und Buch, HydroTechnik Lübeck, Okeanos Stiftung für das Meer, Hatlapa Marine Equipment, Terra Water, CONTROS Systems & Solutions GmbH, Oktopus GmbH, Hydro-Bios, KUM, Brunsbüttel Ports. J. Canning-Clode was supported by a starting grant in the framework of the 2014 FCT Investigator Programme (IF/01606/2014/CP1230/CT0001). We thank two anonymous reviewers for their valuable suggestions, which led to a substantial improvement of the manuscript. Furthermore, we thank Rainer Kiko for providing us the maximum water temperature data for the various study sites.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

References

Aguilera MA, Thiel M, Ullrich N, Luna-Jorquera G, Buschbaum C (2017) Selective byssus attachment behavior of mytilid mussels from hard- and soft-bottom coastal systems. J Exp Mar Biol Ecol 497:61–70

Anderson LG, Rocliffe S, Haddaway NR, Dunn AM (2015) The role of tourism and recreation in the spread of non-native species: a systematic review and meta-analysis. PLoS ONE 10:3–15

Bahrndorff S, Marien J, Loeschke V, Ellers J (2009) Dynamics of heat-induced thermal stress resistance and Hsp70 expression in the springtail, *Orchesella cincta*. Funct Ecol 23:233–239

Bailey SA (2015) An overview of thirty years of research on ballast water as a vector for aquatic invasive species to freshwater and marine environments. Aquat Ecosyst Health Manage 18:261–268

Bates AE, McKelvie CM, Sorte CJB, Morley SA, Jones NAR, Mondon JA, Bird TJ, Quinn G (2013) Geographical range, heat tolerance and invasion success in aquatic species. Proc R Soc B 280:20131958

Bax N, Williamson A, Aguero M, Gonzalez E, Geeves W (2003) Marine invasive alien species: a threat to global biodiversity. Mar Pol 27:313–323

Bayley M, Petersen SO, Knigge T, Köhler HR, Holmstrup M (2001) Drought acclimation confers cold tolerance in the soil collembolan *Folsomia candida*. J Insect Physiol 47:1197–1204

Braby C, Somero G (2006) Following the heart: temperature and salinity effects on heart rate in native and invasive species of blue mussels (genus *Mytilus*). J Exp Biol 209:2554–2566

Briski E, Bailey SA, MacIsaac HJ (2011) Invertebrates and their dormant eggs transported in ballast sediments of ships arriving to the Canadian coasts and the Laurentian Great Lakes. Limnol Oceanogr 56:1929–1939

Brown JN, Langlais C, Gupta AS (2015) Projected sea surface temperature changes in the equatorial Pacific relative to the Warm Pool edge. Deep Sea Res II 113:47–58

Brun NT, Bricelj VM, MacRae TH, Ross NW (2008) Heat shock protein responses in thermally stressed bay scallops, *Argopecten irradians*, and sea scallops, *Placopecten magellanicus*. J Exp Mar Biol Ecol 358:151–162

Carlton JT (1999) The scale and ecological consequences of bioinvasions in the world's oceans. In: Sandlund OT, Schei PJ, Viken A (eds) Invasive species and biodiversity management. Kluwer Academic Publishers, Dordrecht, pp 195–212

Carlton JT (2002) Bioinvasion ecology: assessing invasion impact and scale. In: Leppäkoski E et al (eds) Invasive aquatic species of Europe: distribution, impacts and management. Kluwer Academic Publishers, Dordrecht, pp 7–19

Carlton JT, Ruiz GM (2005) Vector science and integrated vector management in bioinvasion ecology: conceptual frameworks. In: Mooney HA et al (eds) Invasive alien species: a new synthesis. Island Press, Covelo, pp 36–58

Carney KJ, Minton MS, Holzer KK, Miller AW, McCann LD, Ruiz GM (2017) Evaluating the combined effects of ballast water management and trade dynamics on transfers of marine organisms by ships. PLoS ONE 12:e0172468. https://doi.org/10.1371/journal.pone.0172468

Casoli E, Ventura D, Modica MV, Belluscio A, Capello M, Oliverio M, Ardizzone GD (2016) A massive ingression of the alien species *Mytilus edulis* L. (Bivalvia: Mollusca) into the Mediterranean Sea following the Costa Concordia cruise-ship disaster. Mediterr Mar Sci 17:404–416

Catford JA, Jansson R, Nilsson C (2009) Reducing redundancy in invasion ecology by integrating hypotheses into a single theoretical framework. Divers Distrib 15:22–40

Chan FT, MacIsaac HJ, Bailey SA (2015) Relative importance of vessel hull fouling and ballast water as transport vectors of nonindigenous species to the Canadian Arctic. Can J Fish Aquat Sci 72:1230–1242

Chapman JW, Breitenstein RA, Carlton JT (2013) Port-by-port accumulations and dispersal of hull fouling invertebrates between the Mediterranean Sea, the Atlantic Ocean and the Pacific Ocean. Aquat Invasions 8:249–260

Clay K (2003) Conservation biology: parasites lost. Nature 421:585–586

Cohen A (2006) Species introductions and the Panama Canal. In: Gollasch S, Galil BS, Cohen A (eds) Bridging divides: maritime canals as invasion corridors. Springer, Dordrecht, pp 127–206

Colautti RI, Ricciardi A, Grigorovich IA, MacIsaac HJ (2004) Is invasion success explained by the enemy release hypothesis? Ecol Lett 7:721–733

Compton TJ, Rijkenberg MJA, Drent J, Piersma T (2007) Thermal tolerance ranges and climate variability: a comparison between bivalves from differing climates. J Exp Mar Biol Ecol 352:200–211

Coutts ADM, Dodgshun TJ (2007) The nature and extent of organisms in vessel sea-chests: a protected mechanism for marine bioinvasions. Mar Pollut Bull 54:876–886

Dahlstrom A, Hewitt CL, Campbell ML (2011) A review of international, regional and national biosecurity risk assessment frameworks. Mar Pol 35:208–217

Davidson IC, McCann LD, Fofonoff PW, Sytsma MD, Ruiz GM (2008) The potential for hull-mediated species transfer by obsolete ships on their final voyages. Divers Distrib 14:518–529

Davidson IC, Brown CW, Sytsma MD, Ruiz GM (2009) The role of containerships as transfer mechanisms of marine biofouling species. Biofouling 25:645–655

De Greef K, Griffiths CL, Zeeman Z (2013) Deja vu? A second mytilid mussel, *Semimytilus algosus*, invades South Africa's west coast. Afr J Mar Sci 35:37–41

Diaz RJ, Rosenberg R (2008) Spreading dead zones and consequences for marine ecosystems. Science 321:926–929

Drake JM, Lodge DM (2007) Hull fouling is a risk factor for intercontinental species exchange in aquatic ecosystems. Aquat Invasions 2:121–131

Elliott J, Holmes K, Chambers R, Leon K, Wimberger P (2008) Differences in morphology and habitat use among the native mussel *Mytilus trossulus*, the non-native *M. galloprovincialis*, and their hybrids in Puget Sound, Washington. Mar Biol 156:39–53

Endresen O, Behrens HL, Brynestad S, Andersen AB, Skjong R (2004) Challenges in global ballast water management. Mar Pollut Bull 48:615–623

Floerl O, Inglis GJ, Dey K, Smith A (2009) The importance of transport hubs in stepping-stone invasions. J Appl Ecol 46:37–45

Ganning B (1971) On the ecology of *Heterocypris salinus*, *H. incongruens* and *Cypridopsis aculeata* (Crustacea: Ostracoda) from Baltic brackish-water rockpools. Mar Biol 8:271–279

Gollasch S, Lenz J, Dammer M, Andres HG (2000) Survival of tropical ballast water organisms during a cruise from the Indian Ocean to the North Sea. J Plankton Res 22:923

Goodbody I (1961) Mass mortality of a marine fauna following tropical rains. Ecology 42:150–155

Gröner F, Lenz M, Wahl M, Jenkins SR (2011) Stress resistance in two colonial ascidians from the Irish Sea: the recent invader *Didemnum vexillum* is more tolerant to low salinity than the cosmopolitan *Diplosoma listerianum*. J Exp Mar Biol Ecol 409:48–52

Hicks D, Tunnell J, McMahon R (2001) Population dynamics of the nonindigenous brown mussel *Perna perna* in the Gulf of Mexico compared to other worldwide populations. Mar Ecol Prog Ser 211:181–192

Hua J, Hwang WH (2012) Effects of voyage routing on the survival of microbes in ballast water. Ocean Eng 42:165–175

Huhn M, Zamani NP, Lenz M (2016a) A ferry line facilitates dispersal: Asian green mussels *Perna viridis* (Linnaeus, 1758) detected in eastern Indonesia. BioInvasion Rec 4:23–29

Huhn M, Zamani NP, von Juterzenka K, Lenz M (2016b) Food availability in an anthropogenically impacted habitat determines tolerance to hypoxia in the Asian green mussel *Perna viridis*. Mar Biol 163:15

Hulme PE (2009) Trade, transport and trouble: managing invasive species pathways in an era of globalization. J Appl Ecol 46:10–18

Jeno K, Brokordt K (2014) Nutritional status affects the capacity of the snail *Concholepas concholepas* to synthesize Hsp70 when exposed to stressors associated with tidal regimes in the intertidal zone. Mar Biol 161:1039–1049

Jewett EB, Hines AH, Ruiz GM (2005) Epifaunal disturbance by periodic low levels of dissolved oxygen: native vs. invasive species response. Mar Ecol Prog Ser 304:31–44

Kaluza P, Kölzsch A, Gastner MT, Blasius B (2010) The complex network of global cargo ship movements. J R Soc Interface 7:1093–1103

Keane R (2002) Exotic plant invasions and the enemy release hypothesis. Trends Ecol Evol 17:164–170

Klein G, MacIntosh K, Kaczmarska I, Ehrman JM (2010) Diatom survivorship in ballast water during trans-Pacific crossings. Biol Invasions 12:1031–1044

Kültz D (2005) Molecular and evolutionary basis of the cellular stress response. Annu Rev Physiol 67:225–257

Labarta U, Fernandez-Reiriz MJ, Babarro JMF (1997) Differences in physiological energetics between intertidal and raft cultivated mussels *Mytilus galloprovincialis*. Mar Ecol Prog Ser 152:167–173

Lejeusne C, Latchere O, Petit N, Rico C, Green AJ (2014) Do invaders always perform better? Comparing the response of native and invasive shrimps to temperature and salinity gradients in south-west Spain. Estuar Coast Shelf Sci 136:102–111

Lenz M, da Gama BAP, Gerner NV, Gobin J, Gröner F, Anil H, Jenkins SR, Kraufvelin P, Mummelthei C, Sareyka J, Xavier EA, Wahl M (2011) Non-native marine invertebrates are more tolerant towards environmental stress than taxonomically related native species: results from a globally replicated study. Environ Res 111:943–952

Levine JM, D'Antonio CM (2003) Forecasting biological invasions with increasing international trade. Conserv Biol 17:322–326

Lockwood JL, Hoopes MF, Marchetti MP (2007) Invasion Ecology, 1st edn. Blackwell, Oxford

Malmendal A, Overgaard J, Bundy JG, Sørensen JG, Nielsen NC, Loeschcke V, Holmstrup M (2006) Metabolomic profiling of heat stress: hardening and recovery of homeostasis in Drosophila. Am J Physiol Reg I 291:205–212

McDonald JH, Seed R, Koehn RK (1991) Allozymes and morphometric characters of three species of *Mytilus* in the Northern and Southern Hemispheres. Mar Biol 111(3):323–333

Miller AW, Ruiz GM, Minton MS, Ambrose RF (2007) Differentiating successful and failed molluscan invaders in estuarine ecosystems. Mar Ecol Prog Ser 322:41–51

Minchin D, Gollasch S (2003) Fouling and ships' hulls: how changing circumstances and spawning events may result in the spread of exotic species. Biofouling 19:111–122

Murray CC, Pakhomov EA, Therriault TW (2011) Recreational boating: a large unregulated vector transporting marine invasive species. Divers Distrib 17:1161–1172

Ojaveer H, Galil BS, Minchin D, Olenin S, Amorim A, Canning-Clode J, Chainho P, Copp GH, Gollasch S, Jelmert A, Lehtiniemi M, McKenzie C, Mikus J, Miossec L, Occhipinti-Ambrogi A, Pecarevic M, Pederson J, Quilez-Badia G, Wijsman JWM, Zenetos A (2014) Ten recommendations for advancing the assessment and management of non-indigenous species in marine ecosystems. Mar Pol 44:160–165

Oyarzún PA, Toro JE, Canete JI, Gardner JPA (2016) Bioinvasion threatens the genetic integrity of native diversity and a natural hybrid zone: smooth-shelled blue mussels (*Mytilus* spp.) in the Strait of Magellan. Biol J Linn Soc Lond 117:574–585

R Core Team (2014) R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. http://www.R-project.org/

Rabalais NN, Diaz RJ, Levin LA, Turner RE, Gilbert D et al (2010) Dynamics and distribution of natural and human-caused hypoxia. Biogeosciences 7:585–619

Ruiz GM, Fofonoff PW, Carlton JT, Wonham MJ, Hines AH (2000) Invasion of coastal marine communities in North America: apparent patterns, processes, and biases. Annu Rev Ecol Syst 31:481–531

Ruiz GM, Fofonoff PW, Galil A, Minton MS, Miller WA (2013) Geographic variation in marine invasions among large estuaries: effects of ship and time. Ecol Appl 23:311–320

Sakai AK, Allendorf FW, Holt JS, Lodge DM, Molofsky J, With KA, Baughman S, Cabin RJ, Cohen JE, Ellstrand NC, McCauley DE, O'Neil P, Parker IM, Thompson JN, Weller SG (2001) The population biology of invasive species. Annu Rev Ecol Syst 32:305–332

Sareyka J, Kraufvelin P, Lenz M, Lindström M, Tollrian R, Wahl M (2011) Differences in stress tolerance and brood size between a non-indigenous and an indigenous gammarid in the northern Baltic Sea. Mar Biol 158:2001–2008

Schneider KR (2008) Heat stress in the intertidal: comparing survival and growth of an invasive and native mussel under a variety of thermal conditions. Biol Bull 215:253–264

Schneider KR, Helmuth BST (2007) Spatial variability in habitat temperature may drive patterns of selection between an invasive and native mussel species. Mar Ecol Prog Ser 339:157–167

Seebens H, Gastner MT, Blasius B (2013) The risk of marine bioinvasion caused by global shipping. Ecol Lett 16:782–790

Seed RS (1992) Systematics, evolution and distribution of mussels belonging to the genus *Mytilus*: an overview. Am Malacol Bull 9:123–137

Simberloff D (2011) How common are invasion-induced ecosystem impacts? Biol Invasions 13:1255–1268

Simberloff D, Martin JL, Genovesi P, Maris V, Wardle DA, Aronson J, Courchamp F, Galil B, Garcia- Berthou E, Pascal M, Pysek P, Sousa R, Tabacchi E, Vila M (2013) Impacts of biological invasions: what's what and the way forward. Trends Ecol Evol 28:58–66

Sinclair BJ, Ferguson LV, Salehipour-Shirazi G, MacMillan HA (2013) Cross-tolerance and cross-talk in the cold: relating low temperatures to desiccation and immune stress in insects. Integr Comp Biol 53:545–556

Smale DA, Wernberg T (2013) Extreme climatic event drives range contraction of a habitat-forming species. Proc R Soc B 280:20122829

Souza RCCL, Fernandes FC, Silva EP (2003) A study on the occurrence of the brown mussel Perna perna on the sambaquis of the Brazilian coast. Rev Mus Arqueol Etnol 13:3–24

Sutherland TF, Levings CD (2013) Quantifying non-indigenous species in accumulated ballast slurry residuals (swish) arriving at Vancouver, British Columbia. Prog Oceanogr 115:211–218

Torchin ME, Lafferty KD, Kuris AM (2001) Release from parasites as natural enemies: increased performance of a globally introduced marine crab. Biol Invasions 3:333–345

Toro JE, Ojeda JA, Vergara AM, Castro GC, Alcapán AC (2005) Molecular characterization of the Chilean blue mussel (*Mytilus chilensis* Hupé 1854) demonstrates evidence for the occurrence of *Mytilus galloprovincialis* in southern Chile. J Shellfish Res 24:1117–1121

Turbelin AJ, Malamud BD, Francis RA (2017) Mapping the global state of invasive alien species: patterns of invasion and policy responses. Global Ecol Biogeogr 26:78–92

Väinölä R, Strelkov P (2011) *Mytilus trossulus* in Northern Europe. Mar Biol 158(4):817–833

Van Kleunen M, Dawson W, Schlaepfer D, Jeschke JM, Fischer M (2010) Are invaders different? A conceptual framework of comparative approaches for assessing determinants of invasiveness. Ecol Lett 13:947–958

Wendling CC, 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. Mar Pollut Bull 71:222–229

Westfall KM, Gardner JPA (2010) Genetic diversity of Southern hemisphere blue mussels of the genus *Mytilus* (Mytilidae; Bivalvia) and the identification of nonindigenous taxa. Biol J Linn Soc Lond 101:898–909

Williams SL, Davidson IC, Pasari JR, Ashton GV, Carlton JT, Crafton RE, Fontana RE, Grosholz ED, Miller AW, Ruiz GM, Zabin CJ (2013) Managing multiple vectors for marine invasions in an increasingly connected world. Bioscience 63:952–966

Wonham MJ (2004) Mini-review: distribution of the Mediterranean mussel *Mytilus galloprovincialis* (Bivalvia: Mytilidae) and hybrids in the Northeast Pacific. J Shellfish Res 23:535–543

Wonham MJ, Walton WC, Ruiz GM, Frese AM, Galil BS (2001) Going to the source: role of the invasion pathway in determining potential invaders. Mar Ecol Prog Ser 215:1–12

Zardi GI, Nicastro KR, McQuaid CD (2007) Sand and wave induced mortality in invasive (*Mytilus galloprovincialis*) and indigenous (*Perna perna*) mussels. Mar Biol 153:853–858

Zerebecki RA, Sorte CJB (2011) Temperature tolerance and stress proteins as mechanisms of invasive species success. PLoS ONE 6:e14806