

INVASIVE PLANKTON

Implications of and for ballast water management

Dissertation

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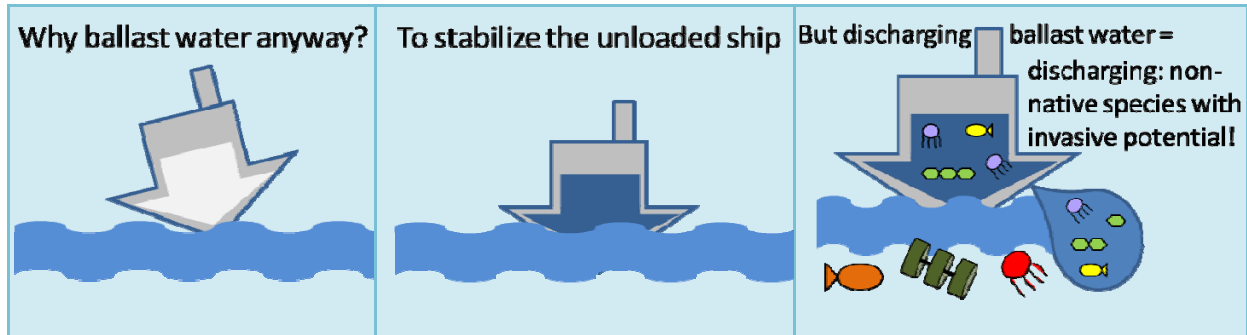


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Title: **INVASIVE PLANKTON - implications of and for ballast water management**

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Chapter 1. Introduction: Invasive species and ballast water

Invasive species

Invasive species are considered one of the biggest threats to our world's oceans (in addition to marine pollution and overexploitation). The man-aided introduction of non-native organisms via a vector (transport element) into new areas and their successful establishment as invasive species pose risks to native biodiversity and habitats (Zaiko et al. 2011). Commonly recognized is the phenomenon of competitive exclusion of native populations by invasive species (Huxel 1999), with increasing probability due to climate change (Philippart et al. 2011).

In case negative impacts are affecting ecosystem services from which humans benefit economically, for instance in terms of fisheries or aquaculture resources, then invasions are also a financial threat. Invasions might directly be harmful if pathogens are transported via ballast water, for example the bacteria *Vibrio cholera* (Ruiz et al. 2000). Mitigation strategies are difficult and most often extremely expensive. The management of the environmental and economic risks requires comprehensive knowledge about the invasion process – which is still not understood in detail.

Invasions are, however, no new phenomenon. Elton concluded already in 1958 in his leading book that biological invasions 'are so frequent nowadays in every continent and island, and even in the oceans, that we need to understand what is causing them and try to arrive at some general viewpoint about the whole business' (p.18) (Figure 1). The second following milestone in literature was an article by Carlton (1985)

'Transoceanic and interoceanic dispersal of coastal marine organisms: the biology of ballast water' because 'it helped launch a sub-discipline of bioinvasion ecology that spans academia, policy, and industry' and 'presented foundational insight for an international approach to vector management' (Davidson and Simkanin 2012).

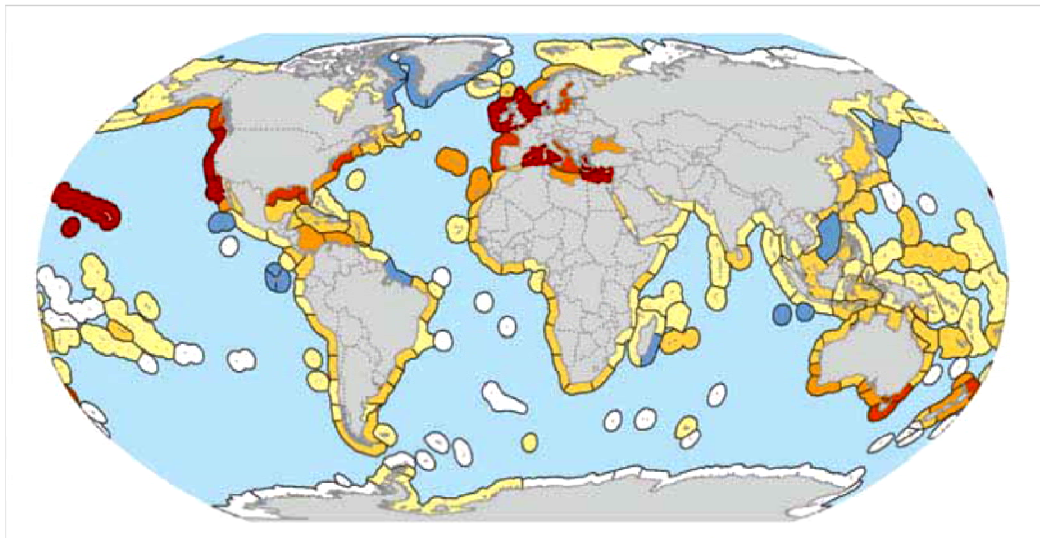


Figure 1. World map of invasions with dark red shades indicating the highest number of invasive species with harmful effects (Molnar et al. 2008).

Generally, a number of vectors play an important role for the dispersal of non-native species in the marine environment (Minchin 2007). For aquaculture projects are molluscs and fishes, for example, frequently cultured species. The Pacific Oyster *Crassostrea gigas* in the Wadden Sea is a famous example for a highly successful invader after being intentionally introduced via aquaculture (Troost 2010). Hull fouling of ship's vessels introduces unintentionally a high diversity of non-native species (Gollasch 2002).

However, **in marine environments, the main vector for the dispersal of invasive species is ballast water.** Cargo ships use ballast water to balance differences in weight during their journeys and it is estimated to account for a total volume of 3500 million tons annually on global scale (Endresen et al. 2004). Ballast water is mostly pumped up in harbors and coastal areas and thus ballast water is loaded with a high diversity of organisms (Veldhuis et al. 2006). More than 1000 species are transported in ballast water of ships including various plankton taxa, but also invertebrates and

small fishes (Gollasch et al. 2007). Ballast water tanks constitute hostile conditions for the entrained organisms. However, several studies prove that a variety of species is able to survive for several days in the dark tanks (Cordell et al. 2009). Discharging viable organisms means also giving them a chance to become established, invasive and maybe harmful. In European waters, the North Sea Region is experiencing the most severe consequences of harmful invasions (Vilà et al. 2009).

Invasive (phyto)plankton

Plankton, consisting of drifting organisms, is most likely to be caught by ballast water uptake. Among some well-studied planktonic invaders are zooplankton species belonging to copepod taxa which have been introduced into estuaries and continental fresh waters throughout the world and might outcompete native populations (Bollens et al. 2012). Devices such as the Continuous Plankton Recorder show a rapid increase of non-native species plankton species in the North Sea. One example is the invasive phytoplankton species *Coscinodiscus wailesii*, a centric diatom with a diameter of up to 500 μm and a dominant member of the North Sea phytoplankton community (Brander et al. 2003). Most recently, the phytoplankton community of the North Sea coastal area has also been invaded by *Mediopyxis helysia*, a large and chain-forming diatom (Kühn et al. 2006) (Figure 2).

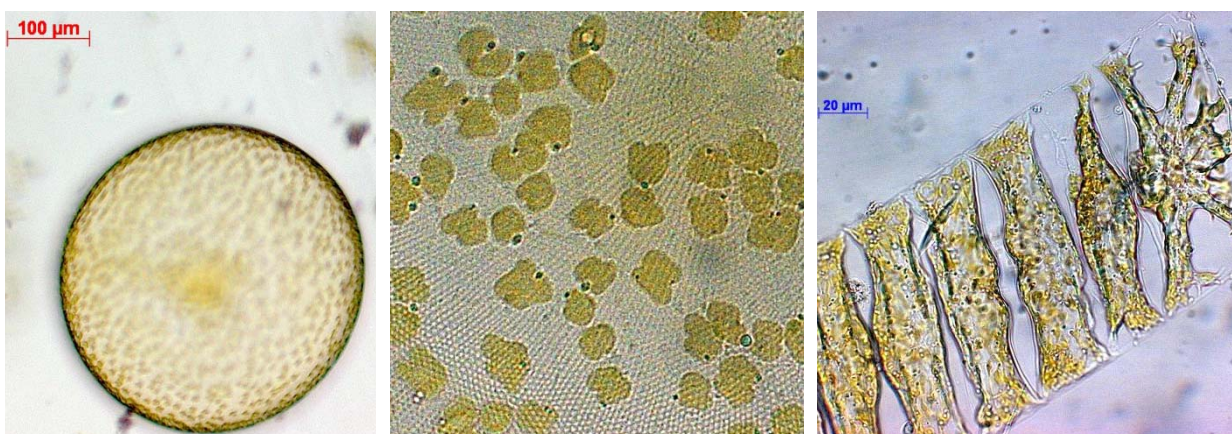


Figure 2. Left: the diatom *Coscinodiscus* sp. (middle: with higher magnification to show silica based cell walls and chloroplasts), right: *Mediopyxis helysia*, both from samples at the harbor of NIOZ, Texel, The Netherlands (Liebich V 2010).

Phytoplankton comprises hundreds of species which are usually unicellular but in some cases can form cell aggregates. Phytoplankton mainly includes photosynthetic active diatoms, which therefore form as primary producers the base of the marine food web. The second major group of phytoplankton consists of dinoflagellates, including autotrophic and heterotrophic taxa. All of which interact with cyanobacteria, viruses, bacteria, and zooplankton (Fogg 1991).

Phytoplankton is influenced by a number of factors, such as temperature, salinity, light, and nutrient availability (Loebl et al. 2009). Under optimal growth conditions many phytoplankton species can develop blooms, enhanced by eutrophication (Colijn and Beusekom 2005). These blooming species can have negative consequences by their mass occurrence (clogging of fish gills and suspension feeding organs) and decay (oxygen depletion), and also in some cases by producing toxins (Hense and Beckmann 2006). Global warming can affect timing of phytoplankton blooms, which can result in long-term changes of the phytoplankton community (Schlüter et al. 2012). Changes within the phytoplankton community based on newly introduced species at the base of the food web can consequently affect higher trophic levels to consumers, such as zooplankton and fishes and thus cause regime shifts (Möllmann et al. 2008).

Invasion theory and terminology

Defining the role of a recent invader in its new ecosystem is a very interesting approach of embracing different perspectives. One example from the Wadden Sea ecosystem is *Styela clava*, a North-West-Pacific sea squirt. It has recently become established in European coastal waters with a range from Denmark to Portugal. Research on the Wadden Sea ecosystem showed no harmful effects on the native benthic communities. Fouling on harbor surfaces and molluscs was in its extent by no means comparable to the situation at the Canadian east coast, where it is considered a pest species overgrowing blue mussel cultures. *S. clava* is only one example of an invader which is considered pest species in some regions but shows elsewhere little harmful effects – or even has some positive outcomes (Liebich 2007). **Research is necessary to understand the process of a successful invasion and its consequences and to develop efficient management, and it also gives very interesting insights on how ecosystems work.**

Attempts to explain the invasion process are based on a great variety of invasion theories. They include different stages, transitions, factors, and terminology explaining the failure or success of an invasion process. That variety is not only confusing, it hinders scientific community, public, managers, and policy makers to understand each other to a detailed level which is essential for developing monitoring and mitigation strategies – where necessary. It is important to unify different theories and models into a single comprehensive framework to support mechanistic understanding of the invasion process which is the goal of **Chapter 2. This new framework should be simple, applicable, and able to provide clear definitions and management perspectives.**

Some ideas regarding the factors which influence the invasion process seem to be used most often: propagule pressure, invasibility, and invasiveness. **Propagule pressure** is the introduced number of individuals of a species with invasive potential and the frequency of introduction events (Lawrence and Cordell 2010). **Invasibility** is the susceptibility of the system to having invasive species established (Lonsdale 1999). And **invasiveness** is the ability of species to establish in, spread, and become abundant in the recipient area (Colautti et al. 2006). Not all introduced species survive, establish or get invasive - which was long time expressed as the 'tens rule'. That means a statistical rule that '1 in 10 of those imported survive, 1 in 10 of those introduced become established, and that 1 in 10 of those established becomes a pest' (Williamson and Fitter 1996). Recently, that 'tens rule' is however seen more skeptically (Jarić and Cvijanović 2012). Vector management to decrease the number of introductions in the first place is a reasoning which most invasion studies seem to agree on.

Ballast water management

The International Convention for the Control and Management of Ships' Ballast Water and Sediments (the Ballast Water Convention) was adopted in 2004 at a diplomatic conference at the International Maritime Organization (IMO 2004). That is a specialized UN agency concerned with shipping issues. Of special importance are maritime safety and prevention, and control of marine pollution from ships. Two well known IMO conventions are the International Convention for the Safety of Life at Sea (SOLAS) and the International Convention for the Prevention of Pollution from Ships

(MARPOL). The Ballast Water Convention will enter into force when 30 states representing 35% of the world's gross tonnage signed (status of 29th November 2012: 36 states but only 29.07%).

The implementation schedule depends on ballast capacity and construction date of the vessel, but regulations will also enter into force retroactive. Latest by 2016, these rules apply to all vessels using ballast water. Ballast water management is defined by the convention as 'mechanical, physical, chemical, and biological processes, either singularly or in combination, to remove, render harmless, or avoid the uptake or discharge of Harmful Aquatic Organisms and Pathogens within Ballast Water and Sediments' (BWM/CONF/36 ANNEX p. 2) (IMO 2004).

Different systems exist to treat the ballast water in accordance with the Ballast Water Convention. These systems have to be tested for approval to comply with the convention's Ballast Water Performance Standard D-2 (Figure 3). That means a 100-1000 times reduction in organism numbers. To reduce the numbers of organisms several options are possible: mechanical separation, cavitation, heat treatment, UV-radiation and active substances. Active substance 'means a substance or organism, including a virus or a fungus that has a general or specific action on or against Harmful Aquatic Organisms and Pathogens' (BWM/CONF/36 ANNEX p. 15) (IMO 2004).

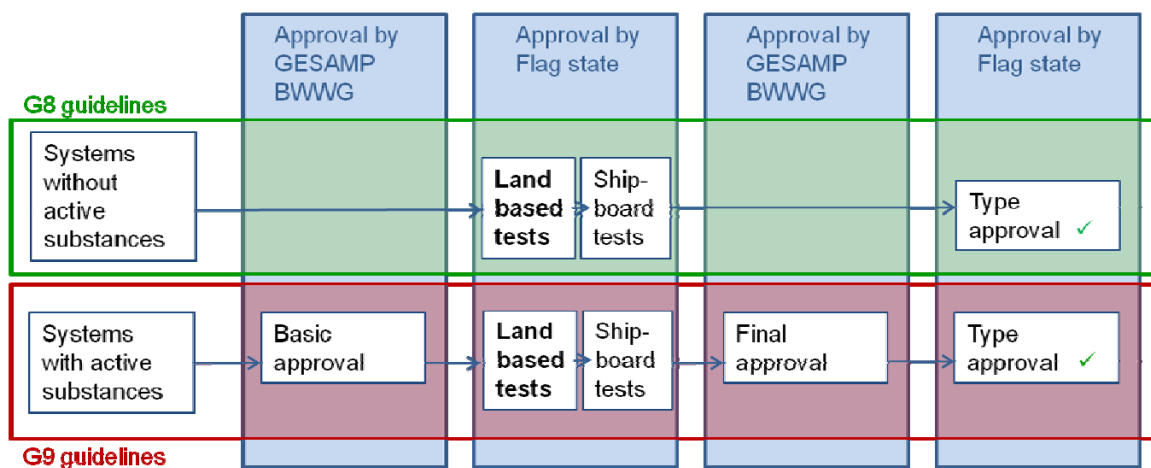


Figure 3. Overview of approval pathway for ballast water treatment systems. Flag state refers to the country where the system is registered. GESAMP is the Joint Group of Experts on the Scientific Aspects of Marine Environmental Protection, sponsored by UN organizations such as IMO. BWWG is its Ballast Water Working Group (changed after Lloyd's Register 2010).

Land-based tests of ballast water treatment systems (BWTS) are conducted at NIOZ Royal Netherlands Institute for Sea Research at the island of Texel, located at the border of the North Sea and the Wadden Sea. Despite successful type approvals, it is known from previous studies that phytoplankton has the ability to survive different ways of treatment.

When examining the efficiency of BWTS, studies about the potential re-growth of organisms should also be considered. So far, the phytoplankton which can survive disinfection and is able to recover the population (re-grow in numbers) was not identified. Their identification will, however, point out in **Chapter 3** the tough organisms which still get a chance of introduction. The question is **which phytoplankton species can most likely become invasive despite ballast water treatment?**

The Ballast Water Performance Standard D-2

Different methods to analyze phytoplankton are used, such as flow cytometry, cluster analysis, microscopy, and DNA-sequencing - also considering species smaller than 10 µm. **The comparison of these different screening methods in ballast water treatment studies** will be part of this thesis in **Chapter 4**.

Organisms smaller than 10 µm are so far not included in the D-2 standard of the Ballast Water Convention. However, marine phytoplankton is actually dominated by small cells (Teira et al. 2005). **Studies on these small phytoplankton organisms regarding invasion and ballast water management are not known so far.**

Invasions, also on lower trophic (planktonic) level, result from global movement of people and products (Figure 4). Thus, management of invasive species needs to be coordinated across national borders and is only as good as the weakest provider of control. If one of the parties does not provide adequate control, an invasive species can spread and cause damage to all (Perrings et al. 2002). However, agreement and compliance with regulations across national borders is of course challenging.

The United States Coast Guard (USCG) seemed at first not to accept IMO's D-2 standard and US states devised a standard which was 100 to 1000 times stricter, depending on size class. In 2012, the USCG decided to adopt the IMO standard

(referred to as phase-one, in Final rule: Standards for Living Organisms in Ships' Ballast Water Discharged in U.S. Waters, USCG 2012) which prevents compliance difficulties if otherwise every state would have own ballast water standards. The implementation of the stricter standard (referred to as phase-two) is postponed. For a stricter standard, techniques need also to be available and sufficient to detect compliance with this standard. Thus, **how invasive plankton and ballast water management influence each other** is discussed in **Chapter 5**.

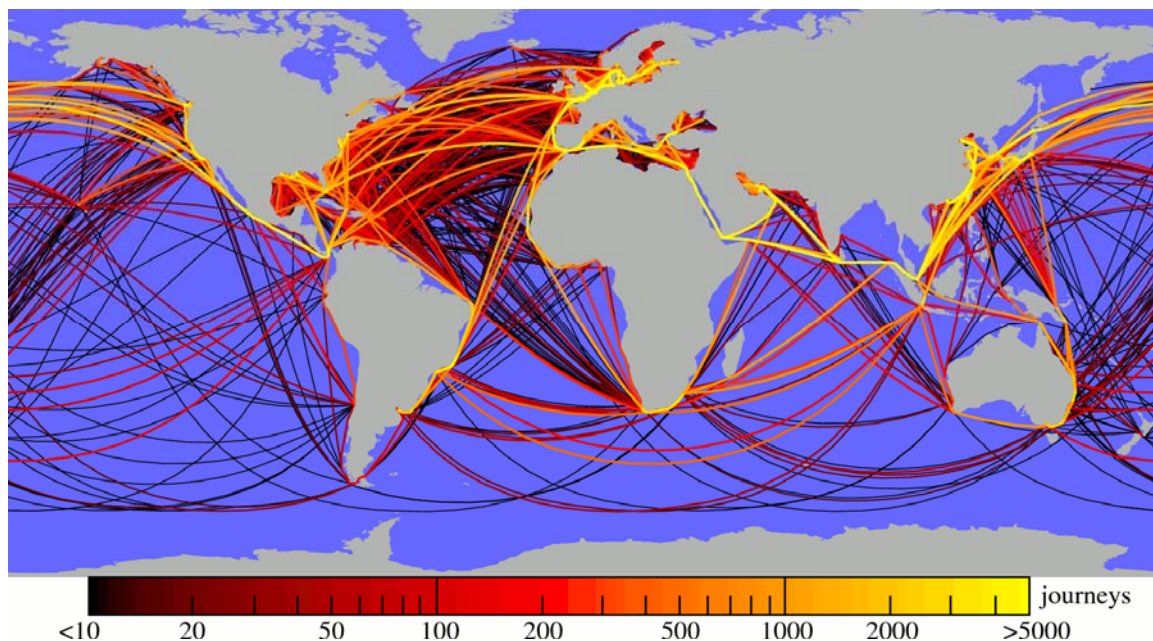


Figure 4. The complex network of global cargo ship movements (Kaluza et al. 2010) and thus ballast water movements.

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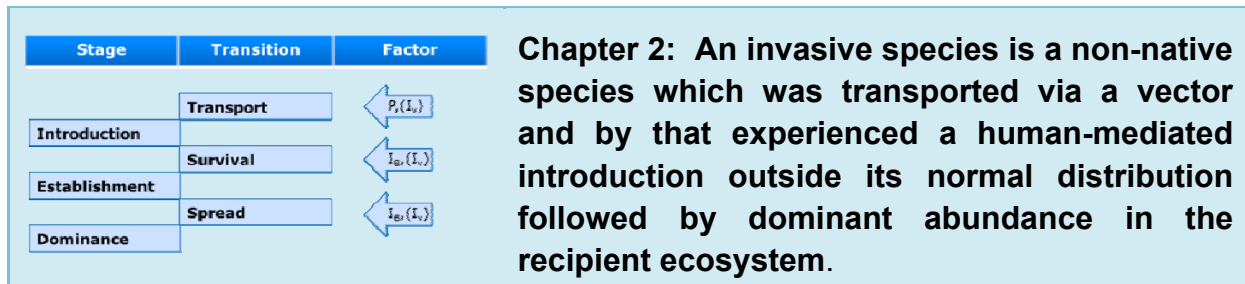
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Chapter 2. Understanding (marine) invasions through the application of a comprehensive stage-transition framework – review of invasion theory and terminology

Abstract

Non-native species can be introduced via a vector and then undergo a process which may lead towards a successful establishment and further spread as invasive species. Different invasion theories include varied stages, transitions, factors and terminology explaining the failure or success of an invasion process. This review presents a comprehensive framework consisting of three stages, each preceded by a transition. Successful invaders pass these intermediate transitions which are influenced by factors like propagule pressure, invasiveness, and invasibility. Terminology in invasion biology is still inconsistent and a new definition of ‘invasive species’ is given encompassing the major process events: an invasive species is a non-native species which was transported via a vector and by that experienced a human-mediated introduction outside its normal distribution followed by dominant abundance in the recipient ecosystem. The presented holistic stage-transition framework provides new insight into invasion theory, especially for marine environments, and valuable management options to deal with harmful marine invaders.

Keywords: invasive species, theory, success factors, ballast water management

Introduction

The introduction of non-native organisms via a human-mediated vector can lead to successful establishment and further spread of an invasive species in the recipient ecosystem. In marine environments, one of the main vectors for the introduction of invasive species is ship's ballast water (Gollasch 2006) but also hull fouling and aquaculture play an important role (Minchin 2007). Invasive species of varying taxonomic groups and regions are well-known to threaten ecosystem functioning in terrestrial, freshwater and marine environments (Vilá et al. 2009). Management actions are needed if invasive species pose a risk of harmful effects and mitigation strategies work best early in the invasion process (Byers et al. 2002).

The process of a species becoming invasive is widely discussed in literature. A conceptualization may help to understand the mechanistic nature of this process but its value depends on clear and consistent use of terminology (Richardson et al. 2000) which is still lacking. In most theories and frameworks, invasions start with one or more incidences of arrival, followed by the establishment and by a further spread to dominant occurrence in the invader's new community. For management attempts knowledge about the stage of the process at the time of assessment is crucial (Reise et al. 2006).

Development of a stage-transition framework and definition of invasive species

Terminology

Terminology in the field of invasion biology remains still inconsistent and is often biased by management perspectives. The new definition excludes the invader's possible (positive or negative) impact for a more ecological approach: **an invasive species is a non-native species which was transported via a vector and by that experienced a human-mediated introduction outside its normal distribution followed by dominant abundance in the recipient ecosystem.** This new definition and the later presented stage-transition framework are developed based on an analysis of different invasion theories. The goal is to disentangle the (so far) inconsistent use of terminology to support a mechanistic understanding of the invasion process.

Nehring (2005) (Alien species - Glossary of key terms) summarized several definitions relevant to the subject of invasive species, mainly based on the Convention on Biological Diversity (CBD). Within this scope (non-native or) '**alien species**' got 'introduced outside their normal distribution'. '**Introduction**' means 'movement of a species into an area where it is not yet present'. Nehring, however, includes to the latter CBD definition the insertion of 'movement, by human agency' (Nehring 2005, p5). 'By human agency' means a human-mediated transport element is involved, also called vector. '**Vector**' is understood as 'any living or non-living carrier that transports living organisms intentionally or unintentionally' (ICES (2003): Code of Practice on the Introductions and Transfers of Marine Organisms.- ICES, Copenhagen.) The term 'invasive alien species' (based on CBD) refers to 'an alien species whose introduction and spread threaten ecosystems, habitats or species with economic or environmental harm.' CBD (2000): Global strategy on invasive alien species. - Convention on Biological Diversity, UNEP/CBD/SBSTTA/6/INF/9, whereas Nehring replaced introduction by establishment.

However, especially the term 'invasive' is biased and often linked to economic or environmental harm (Dahlstrom et al. 2011; Lovell et al.), but several studies confirm, that **invasive species do not necessarily cause harmful effects** (Reise et al. 2006; Zaiko et al. 2011). One species might also entail a range of consequences depending on the recipient area or the assessment's perspective. The Zebra mussel *Dreissena polymorpha* and its obvious expansion of distribution into inland waters of North America is often used as prominent example of invasive species and their associated ecological modifications and economic loss (Pimentel et al. 2005).

However, this mollusc provides also an example for impacts which can be discussed from different perspectives (Maclsaac 1996 and references therein). It produces extensive fouling. The most harmful effects derive from its massive settling within water pipes but also on harbor, canal, and watercraft surfaces. Consequently, the negative effects of its invasion call control and mitigation strategies into action. The invasion of the Zebra mussel shows, however, also another side. As benthic filter feeders, they increase water quality and are therefore also intentionally introduced, for example in Dutch lakes. By filtering, they decrease on the other hand phytoplankton biomass and thus adversely affect zooplankton populations and possibly higher trophic levels.

If considering potential negative effects, invasive species can potentially do anything what other native species can do as well (Zaiko et al. 2011). And it is important to emphasize that potential threats should not be treated equally for all invaders and recipient communities (Gurevitch and Padilla 2004). An ecological approach to study invasion biology covers the human-mediated introductions of organisms and further their interspecies interactions (Richardson and Pyšek 2006). Those differ for each invasive species and area as they do for native biota.

A more neutral and ecological terminology of invasive species is given by Colautti and MacIsaac based on their stage framework (2004). In this respect 'invasive species' are species which were introduced via a transport vector, established themselves, and are widespread in the recipient ecosystem. Valéry et al. (2008) attempt to give the 'real definition' of a biological invasion based on the 'phenomenon itself' and show differences in previously suggested terminology. Regarding their conclusions, a definition of 'invasion' should also not be made based on the impact criterion - suggested by several other authors as well. Thus, **a more ecological approach in terminology seems to be appropriate.**

The definition of invasion is also often made based on a geographic criterion. Valéry et al. (2008) indicate the comparison between two geographical based ideas: saltation dispersal and diffusion dispersal. Saltation dispersal means the overcoming of a geographical barrier. This is only possible in a *jumping* way via a vector. The diffusion dispersal embraces a broader idea including a range expansion into an adjacent area. They conclude with the following definition: 'a biological invasion consists of a species acquiring a competitive advantage following the disappearance of natural obstacles to its proliferation, which allows it to spread rapidly and to conquer novel areas within recipient ecosystems in which it becomes a dominant population'.

Also the definition on 'invasions' by Valéry et al. (2008) includes the idea of rapid spread or rapid increase of spatial occupation and invasive species being a dominant part as final event of the invasion process. Valéry argues that dominance should be included in terms of the invader's sufficient density. This distinguishes invaders from just ubiquitous species which might colonize to only lower spatial degrees. The invader's dominance is indeed suggested as measure of invasion success of invasive

(terrestrial plant) success. In this respect dominance is expressed as *relative* biomass - taking also the native communities into account (Lundholm and Larson 2004). For instance, 10 individuals of the potential invasive species found on one square meter mud flat are not considered dominant if native biota are present with 100 individuals on average. 10 individuals are, however, dominant in relation to only one individual each of the native biota.

The included part of a 'competitive advantage following the disappearance of natural obstacles' could mean a vector enabled by human activities. One example is the uptake of potential invaders in ballast water and the release into another water body (Williams et al. 1988). Discussed in this respect, invasions happen only through human-mediated introduction, which can be seen as overcoming of a natural barrier. Following this idea, a simple range expansion of a species' former distribution is not considered an invasion.

Invasion theory

Understanding the invasion process is essential to create a comprehensive and clear framework and definition (which is still needed). The invasion process is conceptualized by different theories and depicted with various mechanistic models. Invasive organisms undergo consecutive events starting with an initial uptake into the transport vector. Invasion theories express these events as steps (Sakai et al. 2001; Lockwood et al. 2005), phases (Reise et al. 2006; Catford et al. 2009), stages (Levine et al. 2004; Colautti and MacIsaac 2004), transitions (Pyšek et al. 2008; Kolar and Lodge 2001), barriers (Milbau and Stout 2008; Richardson et al. 2000) and filters (Colautti et al. 2004) including varied influencing factors (Milbau and Stout 2008) and terminology.

Recently, it is regarded 'most damaging that invasion biologists have pursued their research using a variety of terminologies, using synonymous terms for the same process, different definitions of the same term, and dissecting and pursuing the invasion process in different ways' (p. 1) (Blackburn et al. 2011). Therefore, **what is needed is a unified comprehensive framework to support mechanistic understanding of the invasion process; it should be simple, applicable, and able to provide clear definitions and management perspectives.**

Altogether 29 hypotheses in (terrestrial) plant invasion ecology are, for example, reviewed by Catford et al. (2009) to incorporate the underlying conceptual ideas into a single framework on the invasion process including several consecutive events. In combination, there also is a lot of research going on about the success of the invasion process (or how fast and how many of those events one species has to undergo until it is considered 'invasive') which depends on three key factors (Catford et al. 2009; Lonsdale 1999). First, **propagule pressure** as main driver - depending on the amount of individuals and the frequency of the introduction. Second, **invasibility** of the recipient environment which reflects the susceptibility of an area to the establishment of invasive species (Alpert et al. 2000). And as third factor **invasiveness** determined by the invader's traits (Milbau et al. 2003).

Catford et al. use spread *and impact* to demonstrate a successful invasion. Also studying invasion success, Williamson and Fitter (1996) present a statistical approach to assess the proportion of 'imported' species which reach the three following stages of becoming 'introduced', 'established' and here actually called 'pest'. To reach the next stage in the invasion process these species would have to pass transitions in terms of 'escaping', 'establishing' and 'becoming a pest'. Being a *pest species* refers thereby to the term of 'invasive species'. However, as mentioned before the term of '**invasive species**' **should not necessarily be linked to harmful impacts**.

'Predicting invasion success in complex ecological networks', as addressed by Romanuk et al. (2009), is an important goal. Simulating invasions with different species in different food webs showed that determining success factors vary depending on the invasion stage – either introduction or establishment. Being a generalist increases chances to become a successful invader at the time of introduction. Already established species seem to be more likely successful when they are ranked on lower trophic levels. Not only generality but also trophic position seem to be other driving factors for invasion success.

Characterizing the invasion process by two events or stages, namely introduction and establishment, is the easiest approach to study and generalize this complex course of events. These two stages have all theoretical approaches in this review in common, either as defined stage or embedded in the definition of 'invasive species' (Richardson et al. 2000). For instance, Shea and Chesson (2002) define their first

stage as ‘transport of organisms to a new location’ which is with other authors equivalent to ‘introduction’, followed by the stages of establishment and population increase (Shea and Chesson 2002). In addition to the stages introduction, establishment, and (further spread until) dominant abundance, other stages are reviewed by Catford et al. (2009). However, the number of stages and their definitions differ among authors. In some theories ‘each of these stages presents an ecological filter’ through which the invader passes (Mitchell et al. 2006). Also other authors agree on the fact, that the consecutive stages are preceded by an ecological filter or barriers (Richardson et al. 2000; Milbau and Stout 2008). **Striking is the fact, that the terms *steps, phases* or *stages* are by some authors used equivalent to the terms and meaning of *intermediate transitions, barriers* and *filters* - and by others they are strictly separated from each other** (Table 1). However, it will be shown, that *phase, stage*, and *step* belong to a **different category of terms**, considering the invasion process, than *transition, barrier*, and *filter*.

Terminology used in invasion theory : phase, stage, step, transition, barrier, filter, factors	
Williamson & Fitter '96	stage ≠ transition (between stages); factors
Richardson et al. '00	synonymously used: phase = stage; synonymously used: barrier & factors
Kolar & Lodge '01	synonymously used: stage = transition = step; barrier, factors
Sakai et al. '01	synonymously used: stage = step; factors
Shea & Chesson '02	stage; factors
Levine et al. '04	synonymously used: phase = stage; barrier, factors
Colautti & MacIsaac '04	stage ≠ transition (between stages); filter, factors
Lockwood et al. '05	synonymously used: stage = transition = step; factors
Mitchell et al. '06	stage = (ecological) filter; factors
Reise et al. '06	phase, factors
Barney & Whitlow '08	stage, factors
Milbau & Stout '08	synonymously used: phase = stage, stage ≠ transition (between stages/phases); barriers, factors
Pyšek et al. '08	synonymously used: phase = stage, stage ≠ transition (between stages/phases); barriers, factors
Catford et al. '09	synonymously used: phase = stage = step; synonymously used: barrier = filters; factors
Romanuk et al. '09	synonymously used: phase = stage; factors
Blackburn et al. '11	stage, barrier

Table 1. Comparison of models and varied terminology describing the invasion process. Same words can have different meanings when compared between models: ‘stages’ are either separated by intermediate ‘transitions’ or used synonymously.

Colautti and MacIsaac (2004) synthesize their conceptual framework based on the following stages: stage zero bringing up the propagule residing in a potential donor region, stage one as the transport vector, stage two as introduction, stage three include the species' establishment, four and five describe different classes based on abundances. Based on these stages a terminology is provided in order to disentangle the inconsistent use of terms in invasion biology. They also change terminology towards a biogeographical (and/or ecological) approach by differing it from a so far rather 'taxonomic description' (p.136). That means that one species can be invasive in a certain area while in other areas not. In their framework, potential invaders pass through a series of filters which are affected by three determinants: propagule pressure, physic-chemical requirements of the potential invader (which could refer to invasibility), and community interactions (which could refer to invasiveness). Colautti and MacIsaac based their framework (Figure 5) on Carlton's ballast water transport model (1985), the 'tens rule' (Williamson and Fitter 1996) and the models by Richardson (2000) and Kolar and Lodge (2001); Kolar and Lodge present a transition model, where certain transitions need to get passed in a species' invasion process development until it is considered invasive.

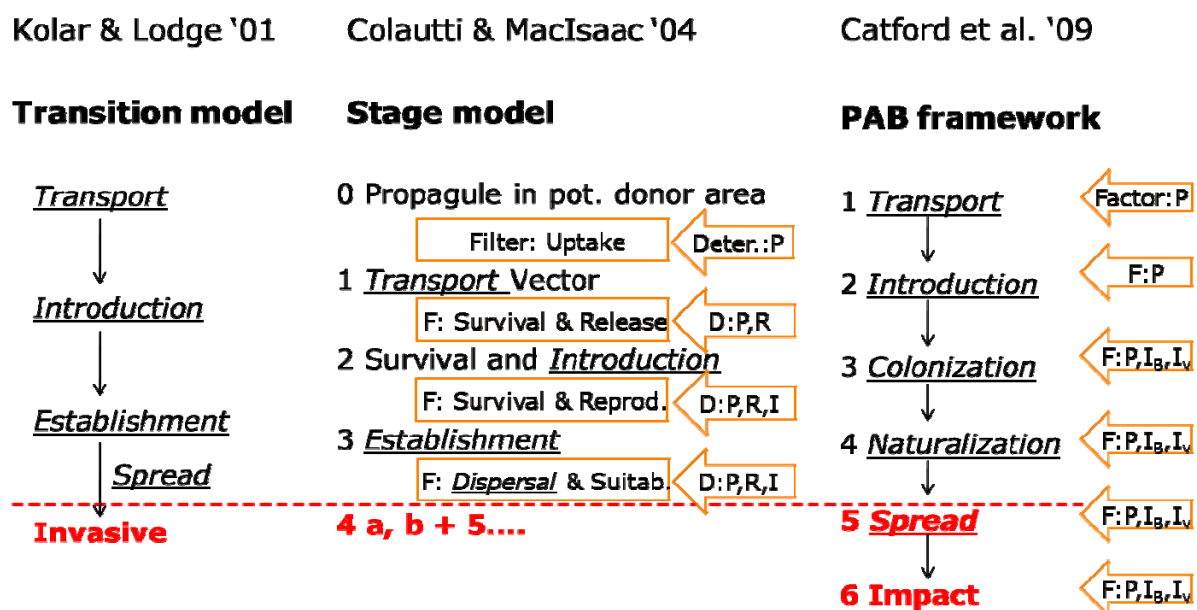


Figure 5. Comparison and conceptual analysis of models explaining the process of a species becoming invasive (red terms underneath dotted line indicate an 'invasive' species). Underlined terms are found (directly or in the same meaning) in all three models. D means determinants: propagule pressure (P), requirements of the invader (R), and community interactions (I). I_B refers to invasibility and I_V to invasiveness.

Barney and Whitlow (2008) present their state factors which can be used to study the influences on the invasion process in a more holistic manner. These factors are propagule pressure, introduced habitat (which could refer to invasibility), and invader autecology (which could refer to invasiveness). Additionally, they include source environment, and time since introduction. Also chance and timing play a role in the invasion process towards failure or success (Crawley 1989). When introduced in winter organisms from warmer areas could be more likely to fail, for example. The above mentioned factors can be again influenced by other factors. Invasibility, for instance, is influenced by certain factors which depend on spatial scale. Changing climate might for example affect invasibility on continental scale, whereas biotic interactions play a role on smaller scale (Milbau et al. 2009). Also species composition is an important factor. Higher biodiversity is positively influencing the biotic resistance to invasion (Stachowicz and Byrnes 2006), thus resulting in a lower invasibility.

A new comprehensive stage-transition framework

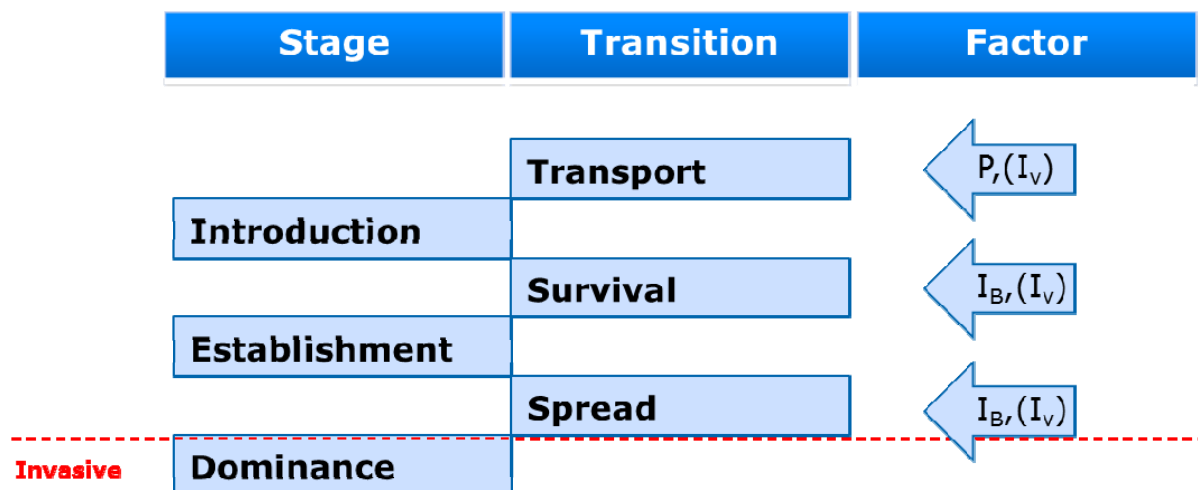


Figure 6. A new comprehensive framework explaining the invasion process (red dotted line indicates an 'invasive' species comparable to the models in Figure 5). P refers to propagule pressure, I_v to invasiveness, and I_B to invasibility.

The new comprehensive framework consists of three stages, each preceded by a transition (Figure 6). Successful invaders pass these intermediate transitions which are influenced by factors. As for the reviewed factors influencing the invasion process, propagule pressure is essential - especially in aquatic environments (Copp et al. 2007; Clark and Johnston 2009). Most theories include additionally invasibility and invasiveness as direct terms or as further meaning of them. These three factors seem more important than source environment, time since introduction, chance, and timing. Thus, the only factors included in this conceptual model are propagule pressure, invasibility, and invasiveness. These factors influence the transitions and not the stages.

Why is it important to differ between a stage and a transition?

There are several differences between a stage and a transition: implied meaning, position in the process, susceptibility to influencing factors and based on that the resulting management options. **At a stage, a step or phase can a potential invasive species stay for a while** - without further progress up to the following consecutive events. Actually, a stage is the time of a process where we can monitor (newly) introduced species. For instance, a species can stay for a while being regarded as introduced (e.g. when plankton monitoring shows it every now and then in samples) or as established (e.g. when plankton monitoring encounters it regularly in samples) but not yet present with dominant abundances.

On the other hand, the term **transition implies an intermediate and dynamic changeover**. Because of its dynamic it is not practical to monitor a transition. For instance, a species should/could not be monitored as 'is surviving after introduction' but it should be monitored as either 'introduced' or 'established'. However, because it is an intermediate changeover, it is a crucial event between stages. And by that a transition is a boundary we can interfere with. For example, we cannot prevent the introduction in itself as a stage (but we can monitor it by saying this species is or is not introduced). We can, however, prevent the transport as threshold towards introduction (Figure 7).

In case of ballast water mediated invasions the first and therefore crucial transition before the stage of introduction is the transport of propagules in ballast water

(vector). The influencing factor is propagule pressure and needs to be considered if a prevention of the changeover on to the introduction stage is aimed for. Propagule pressure simply means how many individuals of a species – and how often they are transported into the new habitat. This is the factor we can influence ourselves best by reducing numbers and transport events. In terms of ballast water a proper treatment and thus reduced propagules at discharge are the most sensible action to be undertaken.

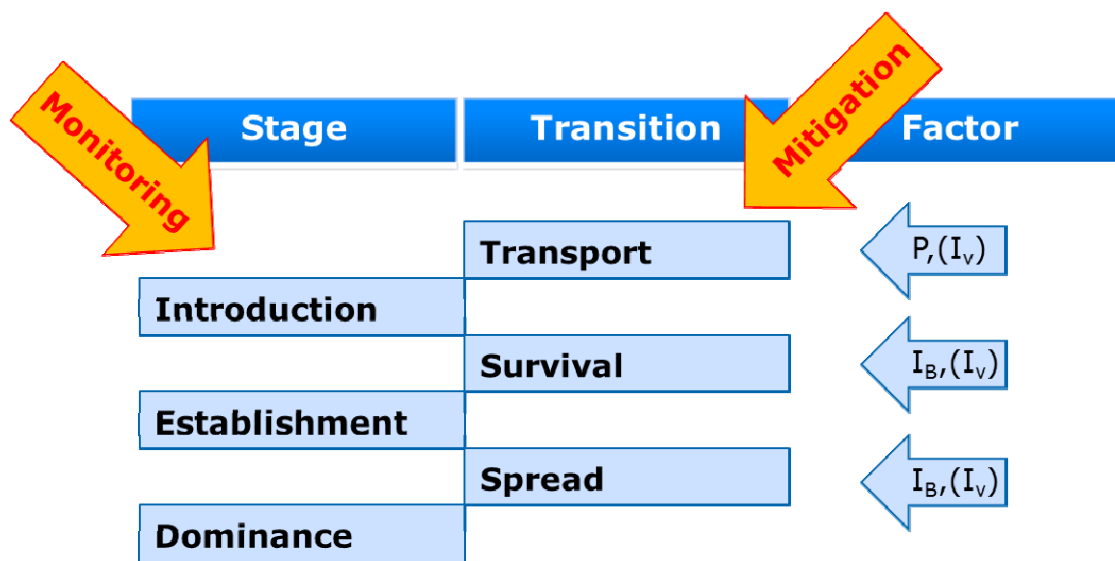


Figure 7. The new comprehensive framework explaining the invasion process. Stages can be monitored; transitions can be interfered with (target points for management/mitigation strategies). P refers to propagule pressure, I_v to invasiveness, and I_B to invasibility.

The factors which influence the intermediate transitions are again influenced by other factors. Invasibility is (as mentioned above) influenced by a number of factors. These factors can be ordered hierarchical and according to scale ranging between continental and local (site and micro) levels. Factors on local scale become significant if the invasion is supported by larger scale factors like climate. The soil type, for example, is affecting invasibility for plant invasions on local scale: between 10 kilometer and 1 meter (Milbau et al. 2009). For marine environments, the differentiation of scales with their affecting factors is a challenge since water masses

move, interact and exchange. However, benthic habitats are especially vulnerable to invasions (compared to open sea environments) (Zaiko et al. 2007). Benthic species are often found on specific substrate (Boudreau et al. 1990). Invasibility of certain substrate to benthic invaders can therefore be linked to similar factors. According to the new framework, the introduction of benthic invaders is preceded by the transport transition (human-mediated movement via vector) into new habitat. This transition and the following transitions are influenced by invasibility. That and the above mentioned theories lead to the conclusion that benthic invaders are under the right climatic conditions dependent on the substrate condition.

Management implications and application examples

Sakai et al. (2001) link the invasion process stages to certain management actions. In their model approach these stages are highly influenced by the species' population biology, which should be taken into account for the management attempt. Establishment should be prevented considering life history traits. Regarding the suggestion of eradication and control efforts, those could be addressed by environmental tolerance, dispersal mode, and genetic structure.

Management actions include, besides eradication and control attempts, also monitoring strategies. Based on the new framework the stages of the invasion process can be monitored: Is the species introduced, established, or dominantly abundant? Depending on this result the control step should be undertaken considering the next transition to prevent further invasion success. With another defined management goal can the preceded transition be tackled to prevent a stronger base of the stage and by that decrease management efforts for the transition which follows in the process framework.

For example, if a species, causing harm elsewhere, is monitored as introduced but not yet found (regularly and/or at several nearby locations) established, then the appropriate management goal is to prevent the stage of establishment. The transition to be focused on is the species' survival - which in this case can be best interfered with by removal of individuals. To prevent a stronger base of the stage 'introduction', the preceded transition of transport could be tackled (based on the factor propagule pressure) by vector management, e.g. ballast water treatment.

In case of benthic species, the underlying concept is the same, while the actions are different. A monitored and considered introduced harmful sea squirt species, for example, can also be removed to prevent its changeover to establishment. However, as mentioned before, the influencing factors play an important role as well. Invasibility is important because it is influencing each transition. In terms of benthic species this could be used for management attempts. Namely: substrate could be prepared to prevent settlement or to get rid of settled individuals.

The new definition

Summarizing and concluding above mentioned definitions ('alien', 'introduction' and 'vector') and conceptual ideas, especially the new comprehensive framework, I propose a new definition of **INVASIVE: an invasive species is a non-native species which was transported via a vector and by that experienced a human-mediated introduction outside its normal distribution followed by dominant abundance in the recipient ecosystem.** This definition includes all major events in the invasion process between initial introduction and successful dominant abundance.

Conclusion

The widely discussed factors propagule pressure, invasibility and invasiveness are used in various invasion theories. However, it should be noted that a successful invasion is always based on a match of favorable circumstances. 'Any given species can therefore become invasive at the right time and place' (p.332) (Pienimäki and Leppäkoski 2004). The factor of invasiveness is based on the species' characteristics which can be assessed in case studies. The results might help to determine species which are more likely to become invasive (Nijs et al. 2004) and thus indicate target species for monitoring. However, species' characteristics cannot be changed (therefore this factor can be neglected if the framework is used for developing management strategies, not on the other hand for research purposes).

The invasion of harmful species calls managers into action, as well as policy makers, lawyers, stakeholders and last but not least scientists. Thus, there is the urgent need

for a comprehensive framework which provides consistent reasonable terminology, which enables us to develop a mechanistic understanding, and which is applicable for management strategies. For management or mitigation strategies a great amount of data is needed. This presented stage-transition framework offers a simple base to decide where and what is to be monitored and where in the process the mitigation strategy should best be targeted.

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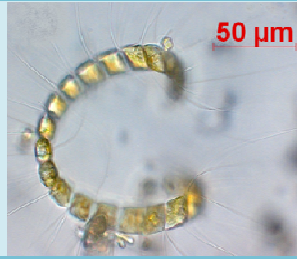
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State of the manuscript and own contribution: I initiated the study and conducted it on my own. The manuscript is submitted to *Management of Biological Invasions*. Dr. Stephan Gollasch and Dr. Justus van Beusekom provided comments which helped to improve the manuscript.



Chapter 3: Ballast water treatment with UV reduces numbers of viable organisms; but specific phytoplankton taxa such as *Thalassiosira*, *Skeletonema*, *Chaetoceros* & *Pseudo-nitzschia* can survive and re-grow and thus still become (harmful) invasive species.

Chapter 3. Re-growth of potential invasive phytoplankton following UV-based ballast water treatment

Abstract

Ballast water contains organisms which can survive the ship's journey and become established in the recipient water body when discharged. Phytoplankton species can become invasive and might be harmful by producing toxins or anoxic conditions following their blooms. Different technologies exist to treat ballast water in order to reduce the spread of invasive species. The effectiveness of a UV-based ballast water treatment system was tested in an incubation experiment over 20 days. After an initial decline in cell numbers, re-growth could be observed of certain phytoplankton taxa, namely the diatoms *Thalassiosira*, *Skeletonema*, *Chaetoceros*, *Pseudo-nitzschia*, and *Nitzschia* (order represents rank of abundance). The conclusion of this study is that a variety of taxa are able to survive UV-treatment. These may include harmful and potential invasive phytoplankton species. Long-term incubation experiments should be considered when testing the effectiveness of UV-based treatment systems. The dominant re-growing phytoplankton group was *Thalassiosira* which could be a suitable indicator organism for testing the efficiency of UV-units.

Keywords: UV-treatment, bioinvasion, *Thalassiosira*, *Skeletonema*, *Chaetoceros*, HAB

Introduction

Organisms are transported via the ballast water of ships (Carlton and Geller 1993; Williams et al. 1988). When non-indigenous species are released at the port of destination, they may become established in the recipient ecosystem and spread (Kolar and Lodge 2001). These invasive species can pose a risk to biodiversity (McGeoch et al. 2010) and, in some cases, also to human health (Ruiz et al. 2000). Presently, different methods exist to treat ballast water (Tsolaki and Diamadopoulos 2010) to reduce numbers of contained organisms in accordance with the Ballast Water Convention adopted by the International Maritime Organization (IMO) (IMO 2004). The convention includes requirements (D-2 standard) which refer to the discharge of certain concentrations and size classes of organisms. To reduce numbers of viable organisms in ballast water, one option is the use of certain wavelengths of ultraviolet light (UV-C). UV-radiation penetrates through cell membranes of organisms and damages deoxyribonucleic acids (Quek and Hu 2008). For this reason, UV-treatment is commonly used for disinfection of drinking water (Choi and Choi 2010). The lethal UV-dose is an important issue of research as phytoplankton and bacteria are able to recover. The marine diatom *Cyclotella* sp. for instance was able to repair the DNA damage caused by UV-B radiation within hours (Gieskes and Buma 1997). Even when UV-treatment (UV-C) reduced the viable count of microorganisms, remaining bacteria were able to grow again (Waite et al. 2003).

The effectiveness of UV-dosages depends largely on the organism, its size and pigments (Gregg et al. 2009). Potential survival and re-growth of (harmful) organisms after treatment should be considered when examining the effectiveness and efficiency of ballast water treatment systems (BWTS), although this is not a standard requirement of IMO's guidelines for approval of Ballast Water Management Systems G8 (Anonymous 2008). However, only a few re-growth studies have been conducted so far. For example, Stehouwer et al. (2010) showed that after using different dosages of UV-radiation, several unidentified phytoplankton groups did survive UV-treatment and re-grew in long-term incubation experiments. However, no further taxa specification of re-growers was given.

The present study aimed at examining survival and re-growth of phytoplankton after UV-treatment in long-term incubation experiments over 20 days. Flow cytometry was applied to examine timing of re-growth and to indicate numbers and size of cells. Specifically, it was the aim to identify phytoplankton genera and species by using light microscopy. Special focus was drawn on diatoms due to their high ecological relevance as a major group of the phytoplankton, the presence of some invasive and harmful species (Nehring 1998), their ability to survive several weeks in the dark (Peters 1996), and the formation of resting stages (Sugie and Kuma 2008). Several studies confirm that diatoms are commonly found in ballast water (Olenin et al. 2000; McCarthy and Crowder 2000).

Re-growth after UV-treatment may occur related to quantitative or qualitative causes. Quantitative causes include a better chance of re-growth based on more surviving individuals of species with initial high numbers. Qualitative causes include physiological cell properties which support survival and re-growth. A comparison between species that survive and re-grow and those that do not may reveal especially UV-resistant species. These species could then be considered as indicator organisms for testing the effectiveness of UV-treatment. So far, a large diversity of phytoplankton organisms has been used (Tsolaki and Diamadopoulos 2010). Using different phytoplankton species makes comparison and compliance control complicated as differences in sensitivity to UV-dosage might affect test results. A standard phytoplankton species would therefore simplify the testing of UV-based BWTS.

Phytoplankton species which are more resistant to UV-treatment and are faster to recover (repair potential damage) could re-grow and become invasive in their new environment after discharge. It is of special interest to examine the re-growth potential of harmful or invasive microalgae. To specify these re-growers and their functional aspects is essential for risk assessment and mitigation strategies. The identification of the re-growing phytoplankton groups is also crucial to determine effectiveness and efficiency of UV-treatment. For UV-units it might be more efficient to reduce the intensity if the required reduction of organism concentration is already achieved with lower dosages.

Methods

Ballast water treatment tests were conducted at the harbor of the Royal Netherlands Institute for Sea Research (NIOZ, Texel, The Netherlands). For further information on this land-based test facility for BWTS see Veldhuis et al. (2006). The treatment system in the present study used a 20 μm mesh-size filter and low-pressure UV-radiation (fixed wavelength of 254 nm). Water from the Wadden Sea (a turbid estuary) was filtered and processed with UV-radiation at intake (ballasting) and discharge (deballasting). In between, the water was stored in holding tanks for five days simulating conditions during a ship journey. Tanks had a size of 300 m^3 and were either located underground or at the surface. The temperature difference between the tanks was negligible (unpublished data). Experiments were conducted based on normal scheduled test runs according to the G8 guidelines (Anonymous 2008). They were carried out in duplicate resulting in two tanks (I & II). After filling tank I with treated water, the system was shut down and pipes were emptied. Then a control tank was filled and after another temporary shutdown, water was treated and pumped into tank II. For both replicate tanks, the water was newly treated. The first incubation experiment started 1st of April 2010 and the second one 13th of May 2010, latter with two bottles for each tank. For the control, harbor water was pumped (200 m^3/h) into a holding tank without passing through the treatment system. At day zero of the intake series water was pumped up, filtered by the system and processed with UV-radiation. The water was treated a second time after five days which is day zero of the discharge series. Each series was incubated for 20 days. Samples were collected from the control C, I Intake (filter+UV), II Intake (filter+UV), I Discharge (filter+UV+UV), and II Discharge (filter+UV+UV).

The samples were incubated in clean 10 Liter Nalgene (Rochester, USA) bottles and were kept in a climate-controlled room with a temperature of 15 °C (+/- 2°C) and a 16:8 hour light/dark period, similar to local, natural growth conditions. The bottles were placed on magnetic stirrers, which maintained gentle water movement to prevent the phytoplankton from settling. Nutrients were added at concentrations, which are typical for the Wadden Sea in early spring (PO_4 1,6 $\mu\text{mol/L}$, NO_3 20 $\mu\text{mol/L}$, SiO_3 20 $\mu\text{mol/L}$). Samples were taken daily for analyzing phytoplankton concentration and composition. Phytoplankton was quantified by flow cytometry (Coulter Epics XL-MCL with a 488 nm argon laser, Miami, USA). The flow cytometer

measures various properties of individual cells including size and chlorophyll fluorescence (Veldhuis and Kraay 2004). Samples of one milliliter were measured in triplicate, using the red autofluorescence of the chlorophyll signal to differentiate between phytoplankton and other particles. Samples for species identification (Hoppenrath et al. 2009) were examined using an inverted light microscope (Zeiss Axiovert, 400x, Oberkochen, Germany). These samples had a volume of five milliliters, they were well-mixed, and not preserved. All cells and particles in these samples were allowed to settle for at least 30 minutes.

Results

Flow cytometry:

UV-treatment decreased phytoplankton cell numbers (Figure 8). The decline in total cell numbers occurred during the first week of the treated intake and discharge samples of both replicate tanks in April as well as in May. Re-growth, indicated by an increase of cell numbers, occurred comparably in all incubation bottles after day seven. The numerical trend over the first two weeks is comparable for all replicates in both experiments. In May's discharge samples, numbers in different bottles range in extreme cases from 17200 cells per milliliter after three weeks in tank I bottle one to 300 cells per milliliter after three weeks in tank II bottle two, but in the series themselves the overall trend (first decline and re-growth after seven days) was again comparable. In both experiments, phytoplankton cell numbers in the control samples were considerably different from the treated samples.

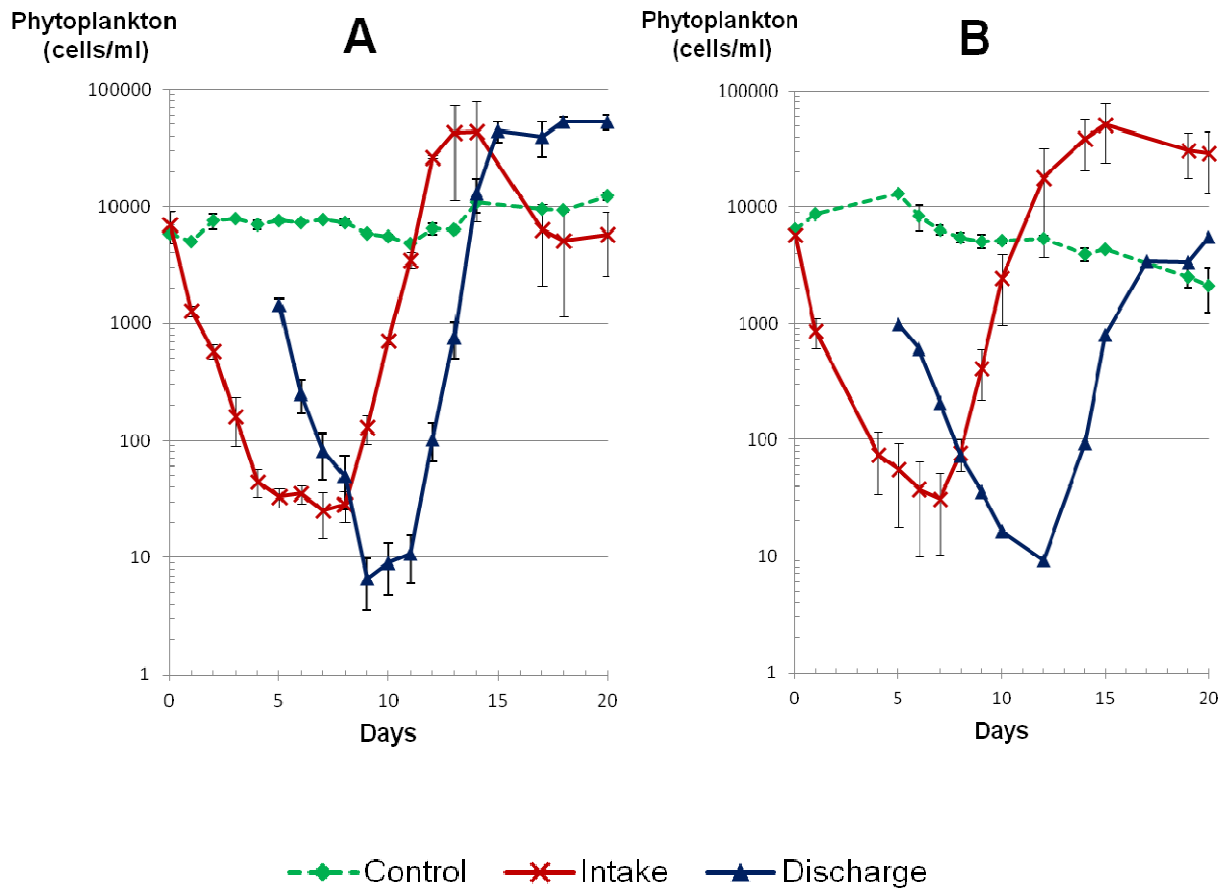


Figure 8. Phytoplankton cell concentrations after UV-treatment at intake (day 0) and discharge (day 5), analyzed by flow cytometry. Incubation experiment one was performed in April (A) and experiment two in May (B). Data points show mean of incubation samples, error bars indicate standard deviation, no error bars are given for May's discharge samples due to distinct numerical differences (see text).

Light microscopy:

In April, *Thalassiosira* was the most abundant phytoplankton group in the control sample; additional phytoplankton included the diatoms: *Asterionellopsis*, *Chaetoceros*, *Coscinodiscus*, *Ditylum*, *Guinardia*, *Nitzschia*, *Pseudo-nitzschia*, and *Skeletonema* (Figure 9). The control sample of May contained the above mentioned taxa as well as *Mediopyxis*, *Odontella*, and *Phaeocystis*. In May's control sample, *Mediopyxis* was the most abundant species.

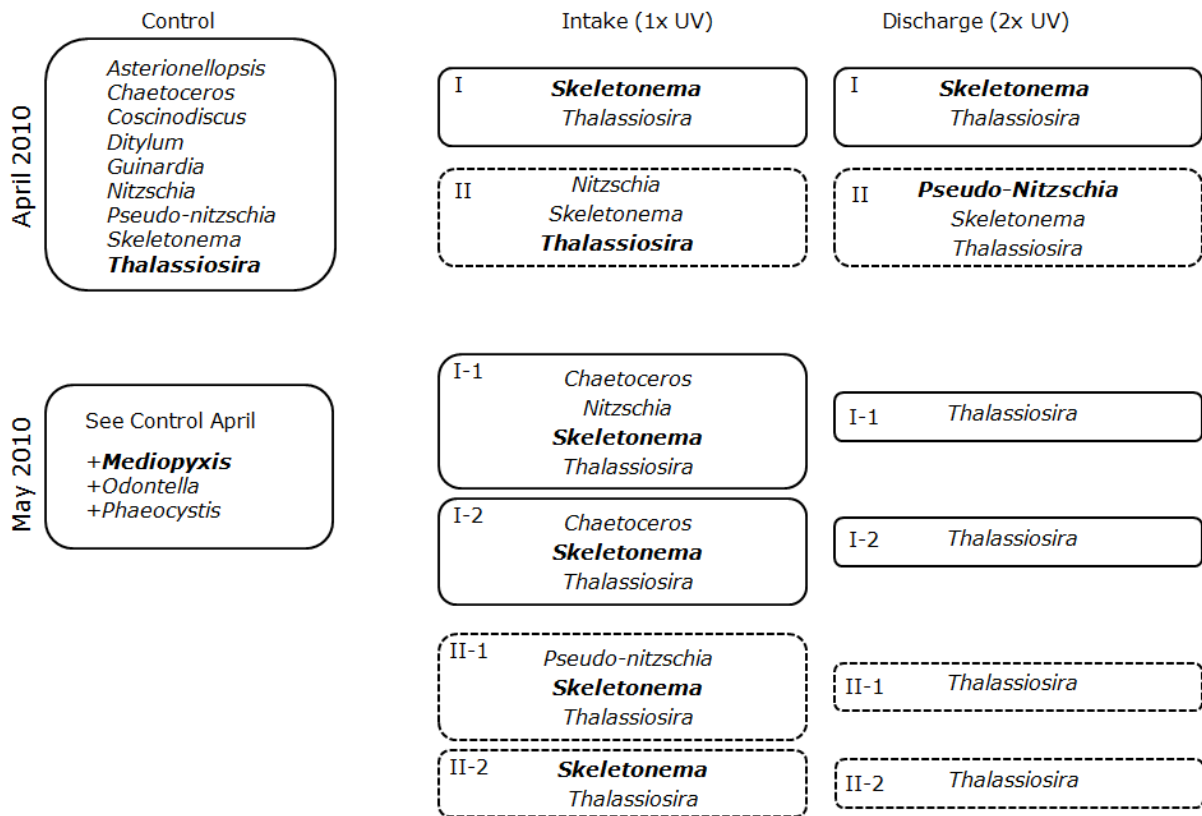


Figure 9. Overview of identified phytoplankton groups in re-growth experiments after UV-treatment. Control = untreated water, Intake = filtered and once UV-treated in replicate tanks I and II, Discharge = Intake with second UV-treatment after five days and two bottles for each tank in May. Taxa in bold letters mark the dominant group of this sample.

In the incubation experiments, the following five taxa re-grew after UV-treatment: *Thalassiosira*, *Skeletonema*, *Chaetoceros*, *Pseudo-nitzschia*, and *Nitzschia* (this order represents rank of abundance estimated from all light microscopy samples).

Thalassiosira cells were re-growing in every series of the first and second experiment. In all four discharge samples of the May series, *Thalassiosira* was the only phytoplankton group coming back. *Skeletonema* was the most abundant re-growing phytoplankton group in the intake and discharge samples of April and in all four intake samples of May. *Pseudo-nitzschia* was the most abundant group in the

April's discharge sample of the second tank. *Nitzschia* cells were re-growing in two intake samples, one from each experiment. In May, *Chaetoceros* re-grew in both bottles of tank I after being treated once with UV-radiation.

All intake samples contained, at day zero a few hours after UV-treatment, some intact *Thalassiosira* cells but rarely other phytoplankton. At day eight, all intake samples from April's and May's replicates looked comparably empty, containing single diatom cell walls without cell content. At day two or four, samples appeared in a similar way empty like samples at day eight. Ten and twelve days after UV-treatment, the April intake samples of tank I contained few *Thalassiosira* cells but more *Skeletonema*. Tank II samples at that time contained mostly *Thalassiosira* cells. In all of May's intake samples, *Skeletonema* was the most abundant phytoplankton but only occurred after day ten. In intake samples of tank I in May, *Chaetoceros* cells were nearly as abundant as *Skeletonema* cells.

Discharge samples out of tanks I and II, a few hours after the second treatment, showed no intact cells. Samples of the April series at day ten contained more *Skeletonema* than *Thalassiosira* cells (tank I) which was still the case at day 20. *Pseudo-nitzschia* was more abundant than *Skeletonema* (tank II), and by day 20 this incubation sample additionally contained some *Thalassiosira*. Discharge samples in May contained nearly no cells at days one and ten, but several *Thalassiosira* cells by day 15 and even more at day 21.

Discussion

Ballast water is the main vector for invasions in marine environments (Gollasch 2006). Phytoplankton is known to be transported via ballast water, to become invasive, and in some cases to pose a threat to ecosystem function of the recipient environment. The objectives of this study were (1) to identify if and which phytoplankton groups are re-growing after UV-treatment; (2) to find possible success factors for the survivorship of phytoplankton groups regarding usability as indicator organisms for treatment effectiveness; and (3) to evaluate if there is a risk through invasive (harmful) microalgae even though the ballast water is treated.

Re-growth of identified phytoplankton groups

Data of the flow cytometer indicate cell size and numbers but the various clusters could not refer to species level. A size range from 10 μm up to 50 μm is accurately detected by the flow cytometer. However, there is a chance that bigger and less common cells, chains or colonies are not in the measured volume which is only a part of the entire sample. This could explain that cell numbers in the treated samples outnumber cell counts of the control after approximately ten days. Control water was unfiltered, thus contained larger organisms like *Ditylum* cells, *Asterionellopsis*, and *Mediopyxis* chains. These were seen using the light microscope, but were not measured by the flow cytometer.

The main re-growing phytoplankton groups were: *Thalassiosira*, *Skeletonema*, and *Chaetoceros*. For *Thalassiosira* and *Skeletonema* it was not possible to identify at the species level (with only a light microscope). *Chaetoceros* could be identified as *C. socialis* due to its characteristic colony formation. *Skeletonema costatum* is a species mentioned in several ballast water (treatment) studies (e.g. Sutherland et al. 2001; Kang et al. 2010). There is however evidence that 'within the species complex once perceived as '*Skeletonema costatum*,' there are cases of very clear distinction among species for morphological, phylogenetic, and ecological traits.' (Sarno et al. 2005 p. 174). For the exact species of *Skeletonema*, as well as for the other mentioned diatoms in our study, additional genetical studies or identification with an electron microscope would be needed.

In April, *Thalassiosira* was the dominant phytoplankton group in the control sample. It was also re-growing in every incubation sample. These results could lead to the assumption that this re-growth is only occurring as a matter of chance, resulting from high initial numbers. *Skeletonema* was found in the control sample in numbers comparable to species which did not re-grow. However, if it was present as a re-grower it was most often (six out of eight times) also dominant. These results could indicate certain advantages of *Skeletonema* over the other phytoplankton groups. *Pseudo-nitzschia* was present in only one discharge sample as most abundant taxa but was not found before the second treatment; maybe it was present as resting cells (Orlova and Morozova 2009). In May's control sample, *Mediopyxis helysia* is the most abundant species but it did not show re-growth at all. It was the largest species

in April and May, with single cells having length measurements of 44-125 µm (apical axis or width of chain) and 27-78 µm (perivalvar axis) (Hoppenrath et al. 2009). It is therefore unlikely that *Mediopyxis helysia* was able to pass the 20 µm mesh sized filter lined in front of the UV-unit.

Success factors for the survivorship and usability as indicator organisms

The identified re-growers in the present study were all diatoms, which are ideal candidates for successful ballast water transport (McCarthy and Crowder 2000). This is because they are small, robust as vegetative cells or resting stages, and able to survive dark and unfavorable conditions in the tank. Most diatoms also have a broad temperature range; species of the genus *Chaetoceros*, *Skeletonema*, and *Thalassiosira* grew from -1,5°C up to at least 20°C (Baars 1979). Viable cultures of *Pseudo-nitzschia* were collected from ballast water tanks underlining the ability to survive darkness for days (Hallegraeff 1998). *Chaetoceros* and *Thalassiosira* species were not only found as vegetative cells in ballast water but also as resting stages (Klein et al. 2009). *Skeletonema* resting forms are also known (Durbin 1978). The formation of resting stages could facilitate survival of UV-treatment.

Re-growth of potential invasive organisms might be supported by optimal light and nutrient conditions and does not necessarily mean that re-growth occurs in dark ballast water tanks. Most invasive organisms fail also to establish after introduction (Williamson and Fitter 1996). For a successful establishment habitat invasibility and propagule pressure play an important role as well as invasiveness (Lonsdale 1999). Invasiveness is the ability to be successful in new environments and depends on species traits (Colautti et al. 2006). A high growth rate is considered to be a functional trait of a successful plant invader (van Kleunen et al. 2010). In general, smaller cells show higher growth rates than large ones (Kagami and Urabe 2001). *Chaetoceros*, *Skeletonema*, and *Thalassiosira* are small sized taxa and by their high growth rates could have an advantage when recovering and re-growing.

Species of the three re-growing genera have a broad temperature tolerance, resting forms, and high growth rates. Therefore, they appear to have greater potential to survive treatment and become invasive than the other identified microalgae. Some non-native *Thalassiosira* species are known to be already established in the North Sea (Reise et al. 1998). *Thalassiosira* cells were dominant as re-growers, are easy to

culture (unpublished data), and commonly found in the marine environment. Therefore we consider them as suitable indicator organisms for testing the effectiveness and efficiency of UV-units.

Risk evaluation for (harmful) algae invasions - despite UV-treatment

Harmful diatoms like toxic *Pseudo-nitzschia* species causing Amnesic Shellfish Poisoning can be transported via ballast water (Zhang and Dickman 1999). However, harmful diatoms are not only those producing toxins. Species of the genus *Chaetoceros* have spines which are thought to cause mechanical damage to fish gills (Bell 1961). Ecological implications of phytoplankton invasions may include changes in the biodiversity of the food-web after successful establishment. Species of *Chaetoceros*, *Skeletonema*, and *Thalassiosira* are known to form blooms (Tiselius and Kuylenstierna 1996), thus may increase local blooming events leading to anoxic conditions following their decay. Species of the identified re-growing genera might not only get invasive but also cause negative effects on the recipient ecosystem.

Conclusion

It should be noted that the tested UV-treatment system in the present study caused a decline of phytoplankton numbers in compliance with the D-2 standard. Incubation experiments are not required for the G8 guidelines but help to evaluate effectiveness and efficiency of treatment systems. Other studies also examined plankton composition in incubation experiments after UV-treatment. Waite et al. (2003) showed the decline of phytoplankton after 18 hours. The present study proves however, that possible re-growth could only be seen after seven days. Sutherland et al. (2001) conducted incubation studies lasting for 16 days. They focused on the three dominant phytoplankton taxa *Chaetoceros gracile*, *Skeletonema costatum* and *Thalassiosira sp.*; our results validate the choice of the tested genera. If incubation experiments show that there is a chance of introducing invasive (harmful) species despite treatment, additional tests should be considered.

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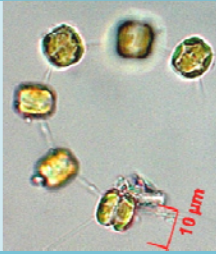
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State of the manuscript and own contribution: I initiated the study and analysed microscopic samples; ballast water treatment testing including flow cytometry was conducted by the co-authors. The manuscript has been published:

Liebich V, Stehouwer PP, Veldhuis M (2012) Re-growth of potential invasive phytoplankton following UV-based ballast water treatment. *Aquatic Invasions* 7: 29-36.



Chapter 4: After showing that specific phytoplankton taxa can survive UV-treatment, the(ir) size fraction of smaller than 10 μm is focused on regarding the D-2 standard. Treatment with electrolytic chlorination is compared and effects of UV-treatment explained.

Chapter 4. Phytoplankton screening methods and light/dark incubation after UV- ballast water treatment and electrolytic chlorination: focus on the forgotten fraction of organisms smaller than 10 μm

Abstract

Ballast water of vessels facilitates the spread of marine invasive species. Due to invasions with harmful effects, the International Maritime Organization (IMO) has adopted the Ballast Water Convention. This convention requires ballast water management, aiming to reduce the risk of further spread of organisms. The included D-2 standard sets the guidelines for testing ballast water treatment systems including the organism density in the discharged water. Organisms are divided into two size classes: 10 - 50 μm and larger than 50 μm . Apart from three pathogenic indicator microbes, organisms smaller than 10 μm are not included when testing ballast water treatment systems following the D-2 standard of the IMO. Therefore, the focus is drawn on the forgotten fraction of organisms smaller than 10 μm and their relevance for ballast water management.

A number of ballast water treatment systems based on filtration, UV-radiation and chemical disinfection were tested between 2009 and 2011 in a land-based set-up at NIOZ Royal Netherlands Institute for Sea Research (Texel). Harbor samples showed that over 90% of all natural phytoplankton was smaller than 10 μm in minimum dimension. And especially these small organisms were able to survive treatment (UV-radiation and electrolytic chlorination) and re-grow in numbers (three days later with electrolytic chlorination) after an initial decline. For comparison, a range of techniques was applied to these incubation samples: flow cytometry with cluster analysis,

microscopy, DNA-sequencing, and Pulse Amplitude Modulated fluorometry. Light and dark experiments gave additional results helping to explain the observed phenomena based on UV-induced damages and repair mechanisms. A phytoplankton cell division could be followed via the microscope. Results of microscopy identified even at species level re-growing phytoplankton – including species smaller than 10 µm in minimum dimension.

Because of the high numerical abundance of small organisms in the natural water, their ability to survive treatment and re-grow, the known harmful effects of species in this size range, and their relative easy possibility for detection, should organisms smaller than 10 µm in minimum dimension get included into the D-2 standard. Only then, the spread of marine invasive species via ballast water has a chance to be reduced effectively.

Introduction

Vessels use ballast water to maintain stability if they are not fully loaded. This ballast water contains basically all organisms which get pumped up. It acts therefore as transfer mechanism or vector for invasive species' introduction (Davidson, Simkanin 2012). Recognizing the problem, the International Maritime Organization (IMO) has adopted the International Convention for the Control and Management of Ships' Ballast Water and Sediments in 2004 (IMO 2004). Based on that Ballast Water Convention (BWC), ballast water management is required. The option of ballast water exchange will be, latest by 2016, completely replaced by on-board ballast water treatment. Several options are available to reduce numbers of viable organisms: mechanical separation, cavitation, heat treatment, **UV-radiation and active substances** (Gregg et al. 2009). However, some phytoplankton organisms are not only able to survive several weeks in the dark (tank) and recover when exposed back to light (Carney et al. 2011) but also to re-grow after ballast water treatment (Stehouwer et al. 2010). The effects of UV-treatment (C) and (natural A & B) UV-light are based on **complex physiological mechanisms protecting the cell against UV-caused damage.**

Phytoplankton organisms, as vegetative cells or as cysts, were found to survive the transport in the dark ballast water tanks, including toxic dinoflagellate species (e.g. *Gymnodinium catenatum*) (Hallegraeff 1998). Diatoms also are contained in ballast water tanks, even more than dinoflagellates (McCarthy and Crowder 2000). Among diatoms, there are species of some taxa which are found alive in ballast water and may be harmful by producing toxins, e.g. *Pseudo-nitzschia* (Sekula-Wood et al. 2011; Fire et al. 2010), by causing physical impairment to fish gills, e.g. *Chaetoceros* (Harrison et al. 1993), or by forming blooms, e.g. *Skeletonema* (Hobson and McQuoid 1997). Apart from phytoplankton with possible harmful effects (Zhang and Dickman 1999), ballast water may also contain human pathogens like the bacteria *Vibrio cholerae* (Ruiz et al. 2000).

To reduce the spread of marine invasive species, the BWC requires on-board installations to treat (disinfect) ballast water. The D-2 Ballast Water Performance Standard, which is included in the convention, sets the guidelines for testing ballast water treatment systems. The BWC specifies the amount of viable organisms allowed in ballast water upon discharge and divides them into two size classes: 10 - 50 μm and larger than 50 μm . Thus, organisms smaller than 10 μm are not considered when testing BWTS following the D-2 standard of the IMO. Only three indicator microbes are taken into account (Figure 10).

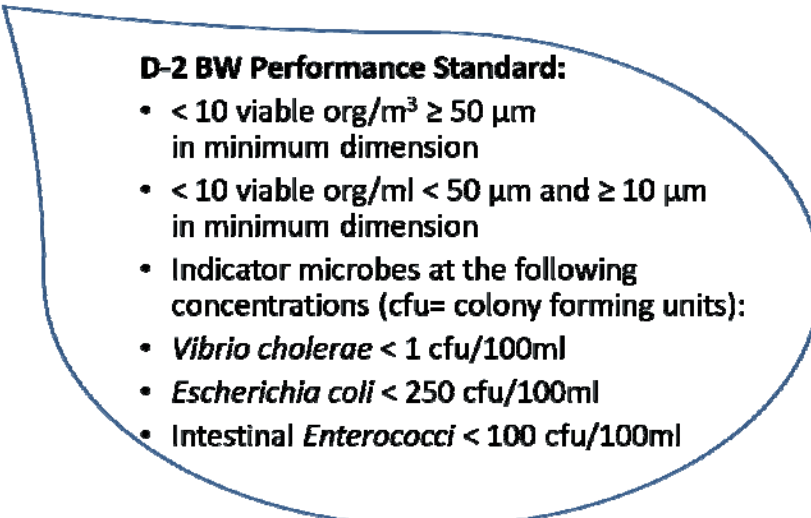
- 
- D-2 BW Performance Standard:**
- < 10 viable org/ m^3 \geq 50 μm in minimum dimension
 - < 10 viable org/ml < 50 μm and \geq 10 μm in minimum dimension
 - Indicator microbes at the following concentrations (cfu= colony forming units):
 - *Vibrio cholerae* < 1 cfu/100ml
 - *Escherichia coli* < 250 cfu/100ml
 - Intestinal *Enterococci* < 100 cfu/100ml

Figure 10. Regulation D-2 Ballast Water Performance Standard, BWM/CONF/36 ANNEX Page 22.

The size class of smaller than 10 μm in minimum dimension contains a great diversity of phytoplankton, micro-zooplankton, bacteria, and viruses. Minimum dimension in this context means the greatest dimension of the smallest visible axis of the body excluding appendages (Gollasch et al. 2007). This measuring guideline creates the very particular situation that in elongated or needle shaped diatom taxa like *Pseudo-nitzschia* (Figure 11) and *Nitzschia*, the minimum dimension can be below 5 μm (transapical axis) while cell length can be more than 30 μm (apical axis). Thus, toxic *Pseudo-nitzschia* species would fall under the lowest size class range of 10 μm in minimum dimension and therefore would not be included when testing a treatment system – according to the guidelines of the convention.

Additionally, a number of species which do not have the shape of a needle are found to be smaller than 10 μm in minimum dimension. Examples are diatoms of the group *Skeletonema* sp. and *Chaetoceros* sp. *Thalassiosira* species have a more round shape and a size of about 10 μm (Figure 11). The smaller individuals would also not be included. Many marine plankton organisms also have complex life cycles with stages of more than one size class.

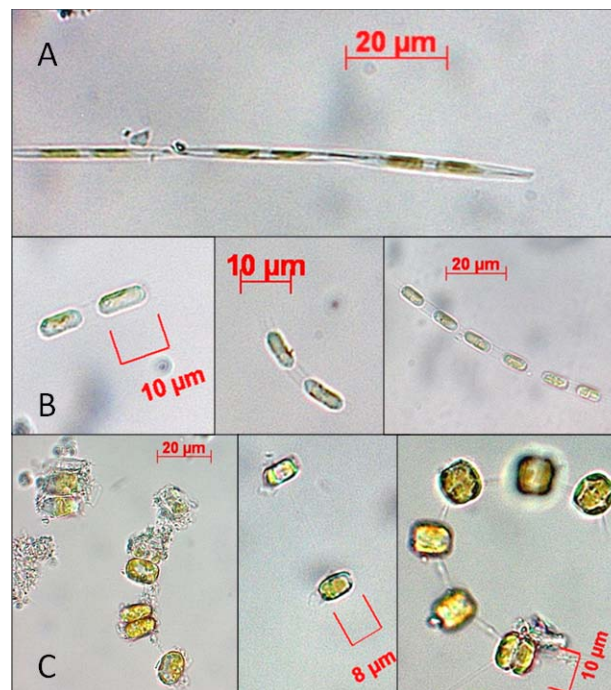


Figure 11. Phytoplankton: A *Pseudo-nitzschia* sp., B (left, middle, right) *Skeletonema* sp., C (left, middle, right) *Thalassiosira* sp.

Methods

At the land-based test facility of NIOZ Royal Netherlands Institute for Sea Research (Texel) ballast water treatment systems were tested based on IMO guidelines in spring and summer between 2009 and 2011. In 2010, incubation experiments were conducted with water of a treatment system using filtration followed by UV-radiation at intake and discharge. Phytoplankton data were collected from samples taken when testing but before treatment, serving as control. Experimental set-up and measurement schedule are described in Chapter 3 (and Liebich et al. 2012).

In 2010, incubation experiments were in addition to the before mentioned UV-system carried out in comparison with a system using filtration, hydrocyclones and electrolytic chlorination. Total Residual Oxidants (possible harmful by-products) got neutralized before discharge. A range of techniques was applied to samples of the UV-based incubation experiments in order to check for and possibly identify re-growing phytoplankton after treatment: flow cytometry with cluster analysis, microscopy and DNA-sequencing.

A flow cytometer Beckman Coulter Epics XL MCL (488 nm laser) was used to count phytoplankton cells in control and incubation samples based on their chlorophyll fluorescence. Cell size was also measured. Flow cytometric analysis was performed in triplicates. Data were displayed in two-dimensional graphs in which cluster indicated cells with similar properties. A program called Easyclus (Thomas Rutten Projects, NL) was used to analyze and compare clusters based on overall six dimensions (factors of this flow cytometer). Species identification was attempted by using settling samples for an inverted light microscope (Zeiss Axiovert). DNA was extracted for DNA-sequencing using 0.2 µm filter (GTTP, Millipore) and the UltraClean Soil DNA Isolation Kit. Primers specific for cyanobacteria and plastids were used and results compared with sequences in a Genbank using BLAST.

For the incubation samples a light: dark regime was set-up in a climate-controlled room with a temperature of 15°C and 16 hours of light and 8 hours dark. Bottles for the dark experiment were wrapped densely with aluminium foil and - apart from that difference - treated in the same way as the light (: dark) experiment.

Pulse Amplitude Modulated (PAM) fluorometry (Water-PAM, Walz GmbH) was used to give an indication of phytoplankton viability, in terms of photosynthetic efficiency.

Triplicate measurements of 3 milliliters each were taken after dark adaptation for at least 15 minutes. The sample's value was allowed to get stabilised for one minute in the PAM fluorometer. Phytoplankton viability is generally expressed as optimum quantum yield (Fv/Fm), expressed as value between 0 and 1:

> 0.5: healthy phytoplankton population

Between 0.3 and 0.5: population which is not under optimal conditions

< 0.3: unhealthy/dying population

This number gives an indication of viability while the response graph and its peak (or no peak) should also be considered.

Results

Phytoplankton cells after UV-treatment vs. electrolytic chlorination

Results of total phytoplankton **cell counts and size measurements found in control samples in spring and summer months between 2009 and 2011 indicated that phytoplankton smaller than 10 µm comprised 92% of all phytoplankton** (SD= 6%, 85 samples, 255 measurements) (Table 2).

Year	Number of control samples	Number of analysed FC samples	Average of cells <10 µm in % of total cells	Standard deviation in %
2009	21	63	92	6
2010	40	120	92	5
2011	24	72	92	6
Total	85	255	92	6

Table 2. Percentage of phytoplankton with a cell size <10 µm, from total phytoplankton in control harbor samples analyzed in triplicates by flow cytometry (FC).

Treatment of the two compared systems in 2010 resulted in a decline of organism numbers according to the D-2 Ballast Water Performance Standard. After UV-treatment and a first decline in numbers, phytoplankton showed re-growth in terms of numbers when monitored for 20 days under laboratory conditions. (Details on the re-growth of specific phytoplankton groups after the UV-system are given in Chapter 3.)

When **comparing** re-growth after using UV-radiation or **electrolytic chlorination**, latter **samples showed less increase in cell numbers starting on average three days later** at day ten. Especially in UV-samples, but also in samples after electrolytic chlorination, **re-growing cells belong mainly to the size class of smaller than 10 μm** (Figure 12).

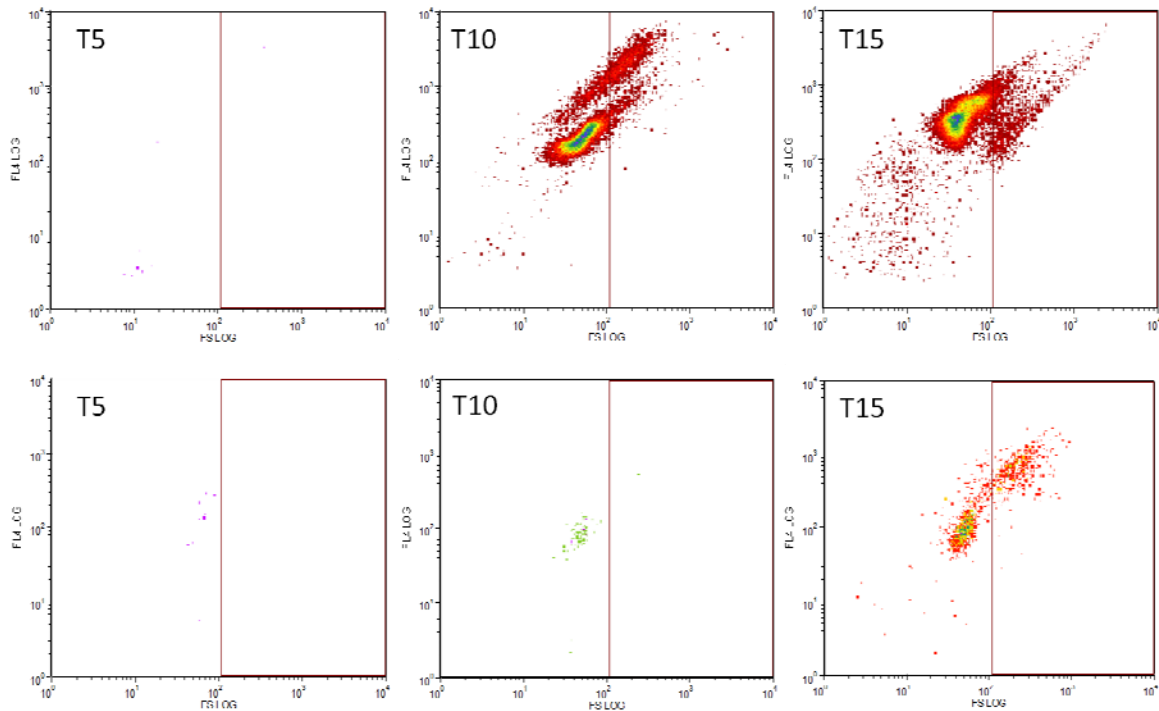


Figure 12. Flow cytometer graphs showing cluster smaller and bigger than 10 μm (borderline) of representative samples from incubation experiments with UV-system (first row) and with electrolytic chlorination (second row) at day 5, 10 and 15 after discharge.

Comparison of phytoplankton screening methods

For samples of the UV-based incubation experiment, different techniques were compared: the **number of distinguishable clusters** based on flow cytometry indicated 50 clusters in 14 samples (Table 3). That number was **comparable to results based on microscopy** with 47 clusters in the same 14 samples. The greatest diversity of 9, 12, or 13 clusters was shown in the control samples, compared to treated incubation samples. The greatest diversity indicated by molecular analysis comprehended only two clusters. In six cases, molecular results

indicated exactly the same phytoplankton group like in microscopic samples. In May's control sample (B), however, molecular analysis only identified one phytoplankton species while microscopy and flow cytometry indicated 12 different ones.

	Flow Cytometer	Microscopy	Molecular Analysis	Microscopy vs. Molecular Analysis
	Number of clusters	Number of species	Number of species	Matching species
A Control	13	9	1	0
A Intake I	1	2	2	1
A Intake II	2	3	1	1
A Discharge I	2	2	2	1
A Discharge II	4	3	1	1
B Control	12	12	1	1
B Intake I-1	1	4	n.d.	n.d.
B Intake I-2	3	3	n.d.	n.d.
B Intake II-1	3	3	1	1
B Intake II-2	1	2	n.d.	n.d.
B Discharge I-1	2	1	n.d.	n.d.
B Discharge I-2	2	1	n.d.	n.d.
B Discharge II-1	2	1	n.d.	n.d.
B Discharge II-2	2	1	n.d.	n.d.

Table 3. Number of phytoplankton species/groups analysed by flow cytometry, microscopy, and molecular analysis. Samples derived from incubation experiments in April (A) and May (B) in 2010, n.d. means no data can be shown because of the lack of molecular data.

A cell division of *Chaetoceros* sp. could be observed by microscopy 10 days after UV-treatment (Figure 13).

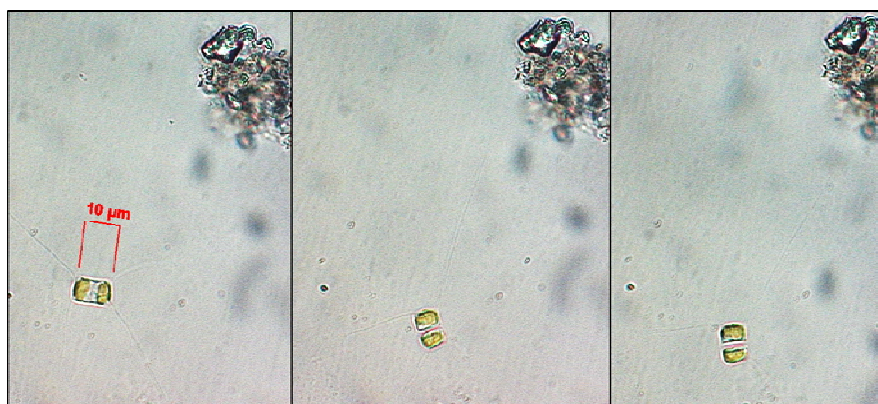


Figure 13. *Chaetoceros* cell division 10 days after first UV-treatment in incubation samples, (left: 0:00, middle: 0:03, right: 0:05) inverted microscope 400x.

Light vs. dark set-up after UV-treatment

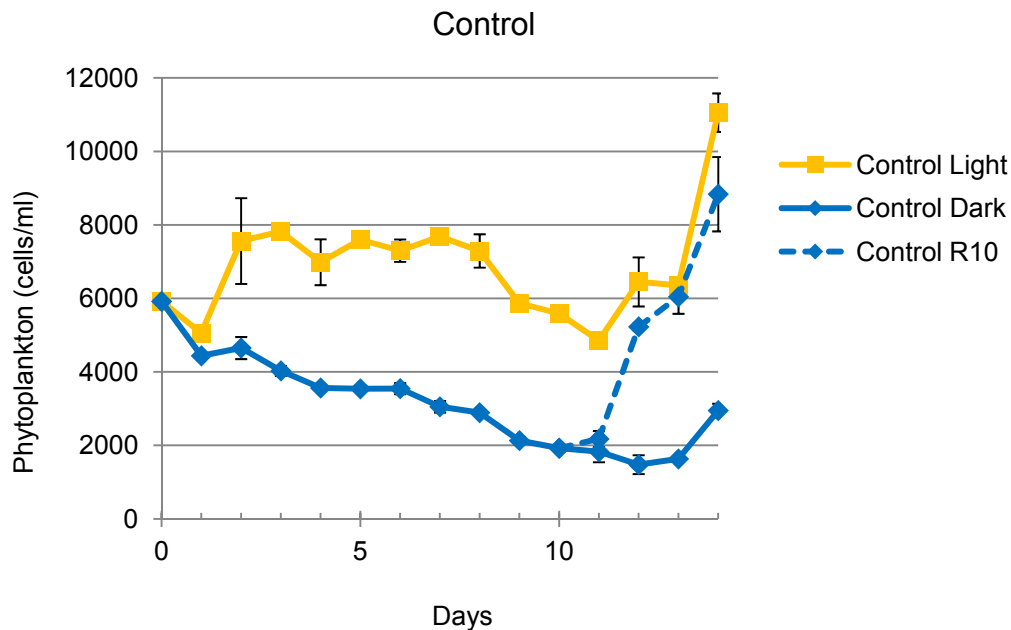


Figure 14. Total phytoplankton cells in the control under light (: dark regime), in the dark, and the dark sample re-exposed to light at day 10, measured as triplicates by flow cytometry. If error bars (standard deviation) are smaller than the symbol indicating the average, they are not displayed.

Reducing phytoplankton numbers were found in the dark control sample compared to the light: dark regime. However, when the dark sample was re-exposed to light after 10 days, a strong re-growth occurred within the next four days (Figure 14).

When the water was treated with UV-radiation at intake, thus on the first day, numbers dropped over the first eight days and started to increase afterwards indicating strong re-growth (Figure 15). Keeping the UV-treated harbor water in the dark resulted in a less steep decline over the first four days. Numbers stayed around 30 times higher than compared to the light: dark regime. Re-growth also started in the dark at day ten. When re-exposed to light at day ten, the numbers dropped over the next seven days and started to increase then with a similar slope to the re-growth in the light experiment.

The dark experiment went with its re-growing phytoplankton numbers of 4483 cells per milliliter at day 18 nearly up to starting point of 5034 cells. No measurement exists for day 20. However, the sample bottle was then re-exposed to light. That resulted into a similar drop for seven days and a following re-growth like when re-exposed at day ten.

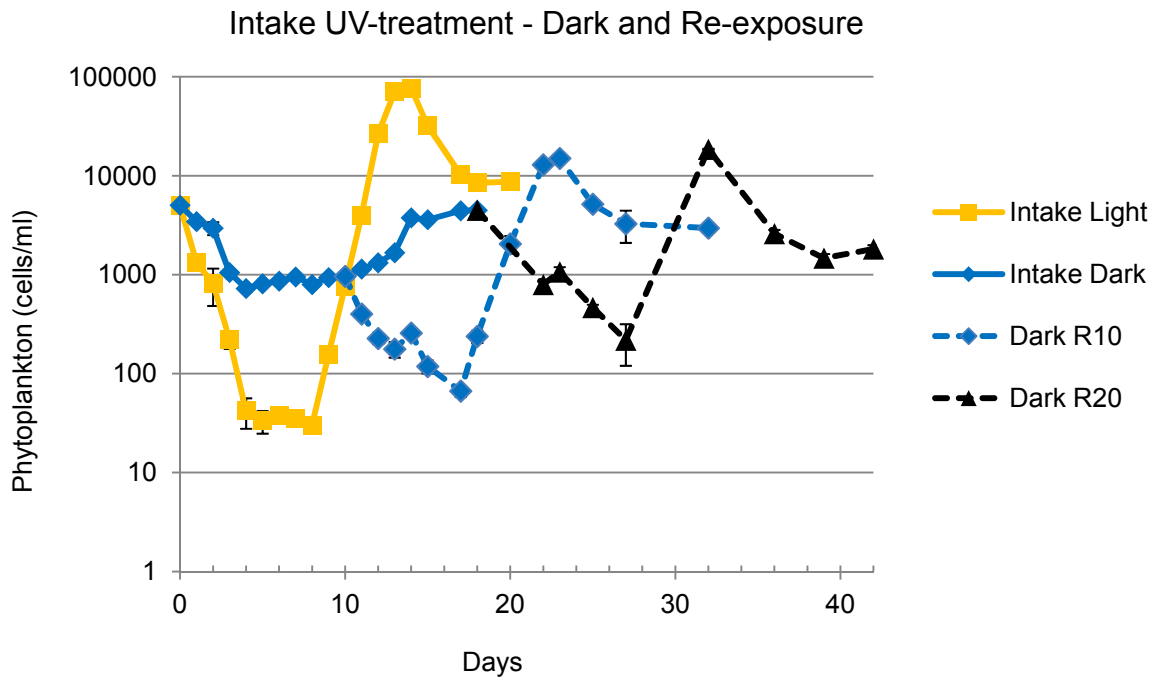


Figure 15. Total phytoplankton cells after UV-treatment at intake measured as triplicates by flow cytometry: dark, re-exposure to light at day 10 and 20. If error bars (standard deviation) are smaller than the symbol indicating the average, they are not displayed.

PAM measurements of the UV-treated samples light and dark stayed below 0.3 indicating an unhealthy or dying population (Figure 16). After eight days there was re-growth indicated in the light experiment and the PAM values increased up to a healthy value of 0.68 in the same sample.

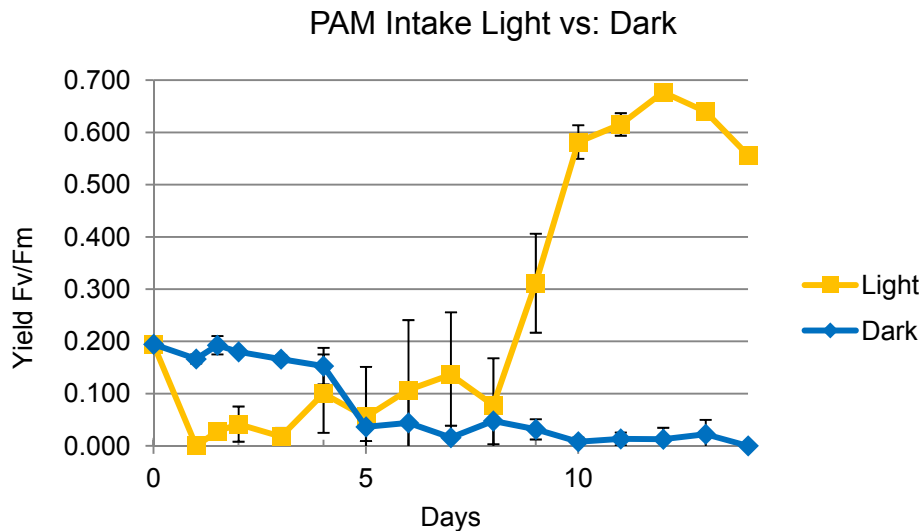


Figure 16. PAM yield after UV-treatment at intake followed over 2 weeks under light (: dark) regime and in the dark. If error bars (standard deviation) are smaller than the symbol indicating the average of three measurements, they are not displayed.

Discussion

UV-treatment vs. electrolytic chlorination and the re-growth of small phytoplankton

Incubation experiments were found to be suitable as additional tests to the IMO testing guidelines (Stehouwer et al. 2010). Both installations, using UV-radiation and electrolytic chlorination, fulfilled the requirements of the reduction of organism numbers according to the D-2 standard in 10 successful land-based test each (Veldhuis et al. 2011a; Veldhuis et al. 2011b). Hence, both systems were approvable under land-based conditions like stated in the conventions guidelines. Microscopy and flow cytometry showed best the reduction in phytoplankton group diversity between untreated control (harbor) and UV-treated samples. Although not required by the IMO, monitoring samples after treatment for at least 10 days allow organisms to show their re-growth potential.

The ability of phytoplankton to survive treatment by UV-radiation or chemical disinfection is not unexpected as it was shown in earlier studies (Stehouwer et al. 2010; Waite et al. 2003; Sutherland et al. 2001). A comparison of UV-treatment and electrolytic chlorination under favorable growth conditions indicates that latter seems

to 'inactivate' the phytoplankton for some days longer, as re-growth occurs later. Phytoplankton proved again the ability to survive disinfection.

Phytoplankton screening methods and their limits

Flow cytometry proved to be a faster tool for counting microorganisms than microscopy. This is the case especially for the smaller phytoplankton species, where more than 100 can be found per liter (Veldhuis and Kraay 2000). Phytoplankton cells smaller than 10 μm in minimum dimension can also be easily detected by flow cytometry. It was shown that over 90% of all phytoplankton was smaller than 10 μm and that especially these small organisms are able to survive treatment (UV and electrolytic chlorination) and re-grow in numbers. Also, smaller cells show higher growth rates than large ones (Kagami and Urabe 2001).

Survivorship and re-growth is not shown randomly but it is specific for certain phytoplankton groups. That conclusion was already drawn by the 2-dimensional scatter plots of the flow cytometer. However, these scatter plots cannot be used to differ between certain phytoplankton groups of similar size and chlorophyll content. The software Easyclus uses all factors of this flow cytometer to compare cell properties. In pre-tests the software was calibrated with cells of individual laboratory cell cultures. Those could be recognized in a mixed sample by the software. As further step was the software calibrated with flow cytometer runs of samples in which microscopic results found just one visible species. However, it was unreliable to detect the same phytoplankton 'species' in those samples in which microscopy clearly showed them as well. Difficulties and unreliable results may arise from the fact that the used flow cytometer generates only six parameters per measured particle, while the software is designed for flow cytometers with 20 or more variables.

To determine re-growing phytoplankton groups, microscopy proved to be a successful tool. However, the identified main re-growers *Thalassiosira*, *Skeletonema*, and *Pseudo-nitzschia* are diatoms with cryptic species complexes (Park and Lee 2010; Kooistra et al. 2008; Amato et al. 2007), making identification at the species level impossible using only light microscopy. Therefore, molecular techniques were tested additionally. However, molecular results were not accurate enough to

determine species, indicating that the chosen 16s rRNA gene is not suitable for species identification.

In addition to the discussed phytoplankton smaller than 10 µm, this size class includes also other groups of organisms like: micro-zooplankton, bacteria, and viruses. It is possible to detect viruses and bacteria by flow cytometry (Marie et al. 1999). Viruses are of course a special case since they need a host cell, thus are not useful to take into account. Bacteria, on the other hand, occur in high numbers in surface water, but also in drinking water numbers between 5.56×10^2 and 3.94×10^4 per milliliter can be found (Hoefel et al. 2003). Thus, apart from indicator microbes, high bacteria standards seem impractical and unnecessary for ballast water testing. However, to detect and count plankton smaller than 10 µm, such as diatoms, dinoflagellates, and micro-zooplankton, is not only doable but also important.

Light vs. dark set-up after UV-treatment to study survival and recovery

Since solar UV-radiation can negatively affect marine organisms, many photoprotection mechanisms have been developed (Hader et al. 2007). On the other hand, solar UV-(A) radiation is, of course, also the energy source for the photosynthesis. Quite obviously, phytoplankton populations decline when kept in the dark compared to a light: dark regime. This study, however, shows also that phytoplankton is able to use the light energy again when re-exposed. They not only survive ten days in the dark (ballast water tank) but also still show strong recovery potential resulting in even higher numbers than directly in the intake water.

Physiological changes caused by excessive UV-(A & B) radiation can include the inhibition of cell division, reduction of growth and photosynthesis - for example resulting from damage to key components in the photosystem II (Holzinger and Lutz 2006). Consequently, if the key protein D1 in photosystem II gets damaged (Aro et al. 1993) by photodegradation, the essential electron transport chain is disturbed.

Rubisco, the key enzyme of the Calvin cycle, can also be damaged by UV-radiation (Bischof et al. 2000). Rubisco is responsible for binding CO₂. If it is not functioning, among other things, NADP⁺ is not formed anymore from NADPH. (NADPH deriving from the light reaction acts otherwise as reducing agent because it is

holding/donating an extra electron). If reduced forms such as NADPH are more produced than used, that results in an over reduced electron transport chain. Then, 'electrons will leak onto O₂, which will act as an alternative electron acceptor, thereby initiating the formation of reactive oxygen species' (p.11) (Janknegt 2009). Superoxide radicals generate photooxidative stress (Foyer et al. 1994) with many harmful effects (Aguilera et al. 2002).

Re-growth after UV-treatment was discussed in Chapter 3. However, the additional dark experiments gave also valuable information. Basically, the UV-treatment resulted in a steeper decline in numbers under the light: dark regime. Thus, light seemed to increase the UV-damage as numbers dropped around 30 times lower. That might be explained by the physiological damages which result in the dysfunction of the electron transport chain. Consequently, more electrons through light induction increase the damage. The same theory can be used to explain why in both cases, when the dark sample is re-exposed to light at day 10 and 20, the first reaction is a decline in numbers. That is even happening when there was already re-growth going on in the dark sample after day 10. Interestingly, re-growth thus also occurs in the dark, but only less compared to the strong increase in numbers under light induction. Actually, the slope of re-growth is similar under the light: dark regime compared to the re-exposed (dark) samples. Re-growth started in all three cases about a week after the first decline. (Also re-growth in Chapter 3 of two incubation set-ups and at intake and discharge indicated that re-growth started after approximately one week.)

Apart from the electron related physiological damages, UV-radiation also damages DNA. However, repair mechanisms can stabilize the cell, for example by photoreactivation via the enzyme Photolyase (Sinha and Hader 2002), but photoreactivation occurs only with light induction (Buma et al. 2000). It could explain the higher re-growth under light. Actually, also other studies concluded that 'exposure of UV-disinfected water to light should be avoided to ensure that photoreactivation does not occur.' (p. 536) (Quek and Hu 2008). That is of course more realistic to achieve with drinking water in dark tanks and pipes, then with discharging ballast water. However, the real recipient water body will not only influence the organisms by different light conditions but also support less optimal growth conditions than provided in that experimental set-up.

Obviously, when it comes to re-growth after UV-treatment in the experimental set-up, it takes about a week for cells to repair their damages. Once the repair mechanism work (on physiological or DNA level) growth of the populations seems strong and probably gets stopped by natural phenomena (in the bottle) like competition and nutrient depletion. PAM results indicate healthy cells at this point.

The observed cell division of *Chaetoceros* sp. 10 days after UV-treatment in incubation samples confirms the recovery. *Chaetoceros* took five minutes for a visible cell doubling. Literature suggests under good growth conditions up to four doublings per day for *Chaetoceros muelleri* (McGinnis et al. 1997). *Chaetoceros* is not only one of the most abundant diatom taxa but with reported 18 species, as both vegetative cells and spores, also one of the most abundant and diverse taxa in ship's ballast water (Klein et al. 2009). As it was one of the main re-growers it has thus potential to become invasive, maybe with harmful effects. Therefore, it is useful to study its ecological tolerance and influencing factors, as well as its growth rate. *Chaetoceros* sp. survived treatment in more than one case and it is smaller than 10 µm in minimum dimension – since that means excluding appendages, like the spines of *Chaetoceros* species (which intertwine when cells form chains).

Because of their high numerical abundance of small organisms in natural water, their ability to survive treatment and re-grow, and the possible harmful effects (toxicity and anoxic conditions following decay of blooms) of species in this size range, should organisms smaller than 10 µm in minimum dimension get included in the D-2 standard. Only then, the spread of all marine invasive species via ballast water has a chance to be reduced effectively.

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State of the manuscript and own contribution: I initiated the microscopic analysis and the light/dark experiments after UV-treatment in order to gain understanding of the physiological phenomenon of the treatment recovery. Data analysis and microscopy were conducted by me. I am second author of the two papers which present partial data of this chapter:

Stehouwer PP, **Liebich V**, Peperzak L (2012) Flow cytometry, microscopy, and DNA analysis as complementary phytoplankton screening methods in ballast water treatment studies. *Journal of Applied Phycology*, DOI 10.1007/s10811-012-9944-8 (paper is attached as annex)

and

Van der Star I, **Liebich V**, Stehouwer PP (in press) The forgotten fraction: The importance of organisms smaller than 10 µm when evaluating ballast water treatment systems. In: *Compliance Monitoring and Enforcement - the Next R&D Challenge and Opportunity*, Proceedings of the Global R&D Forum and Exhibition on Ballast Water Management, 26–28 October 2011, Istanbul, Turkey.



Chapter 5: Not all invaders are harmful, but if they are - smart management goals are needed based on a detailed framework; e.g. a reduction in plankton numbers by UV-treatment specified as D-2 Standard. However, the D-2 Standard needs amendments based on biological results!

Chapter 5. Overall Discussion, Conclusion and Perspective

Understanding the invasion process is essential to develop smart management goals (Ch. 2)

Invasive species are often linked to adverse impacts they might cause (Simberloff 2009). This is of course the case, because harmful invaders get far more attention than those which establish new populations and seem to be mainly additions or might even have positive consequences within the native network of biota (e.g. Wonham et al. 2005). Often, invaders might not get recognized at all; especially if they are small and if they are found in the marine environment further away from the easily reachable mud flats and harbor walls. However, as a matter of fact, only harmful invaders require urgently to be 'managed'. Thus, working on management strategies means to ignore the harmless invasions.

'Management' is an elusive term and might be understood in different ways. This possibility is also the case for the term 'invasive' species. 'Managing invasive species' includes not only a great range of options but also can be approached from different perspectives. The term 'invasive species' is for a better understanding defined based on the new framework in Chapter 2: **an invasive species is a non-native species which was transported via a vector and by that experienced a human-mediated introduction outside its normal distribution followed by dominant abundance in the recipient ecosystem.** This definition is independent of the invader's possible impact. Consequently, it needs to be specified that only *harmful* invasive species are target of management strategies. Management will not be defined as term. It can be assumed that in all its different meanings goals needs to be expressed.

Management goals are not always easy to express. Maybe it is even more difficult when dealing with complex, dynamic ecosystems, where environmental consequences might be far more disastrous than when setting management goals to make a company more profitable. Nevertheless, in both cases, management goals need to be **specific, measurable, attainable, relevant, and time-based**, summarized by the acronym SMART. This helping guideline can be useful when clearly defining goals for environmental management plans (Larson et al. 2011). This is essential to develop control or mitigation strategies in case of invasive species with harmful effects.

Smart management goals are developed for **two examples** - based on the new comprehensive framework (stages: introduction, establishment, dominance; preceded by the transitions: transport, survival, spread; in turn influenced by propagule pressure, invasibility, invasiveness).

The first example is the **sea squirt *Styela clava***. Its effects on the Wadden Sea ecosystem as invader might not be evaluated as harmful (yet) but at the Canadian east coast it is considered a pest species (Ramsay et al. 2008) (Figure 17).

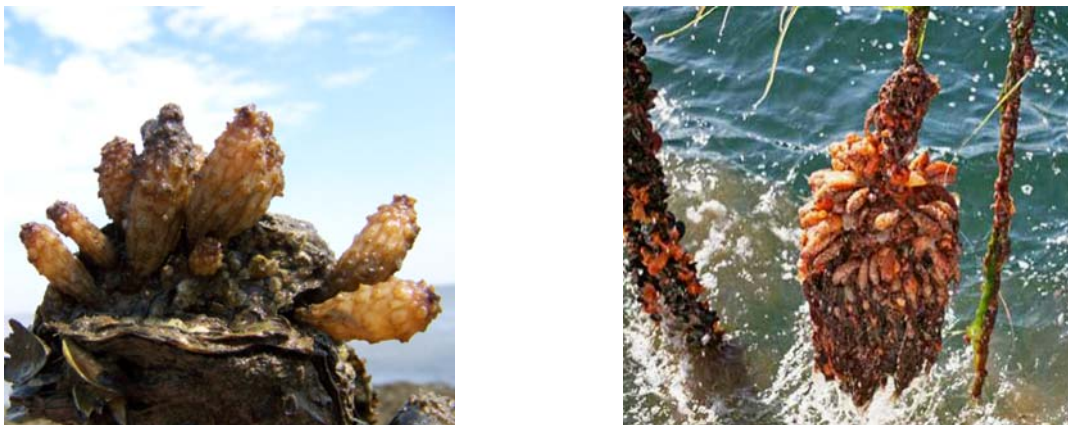


Figure 17. Left: The invasive sea squirt *Styela clava* settled on top of the Pacific Oyster, another invader in the Wadden Sea area of Sylt, Germany (Liebich V 2007), Right: *Styela clava* overgrows blue mussel cultures in Prince Edward Island, Canada (Gittenberger A 2007).

This example shows also a very interesting dispersal mechanism, especially because the short-living planktonic larvae cannot spread over great distances by themselves. The main vector is ship's hull fouling. However, *S. clava* shows a way of secondary dispersal via the formation of big bunches of up to 200 individuals (Figure 18) which float and drift (Liebich 2007).



Figure 18. Left to right: The invasive sea squirt *Styela clava* with increasing numbers of individuals having settled together and thus forming a floating bunch in the Wadden Sea area of Sylt, Germany (Liebich V 2007).

S. clava is already introduced in the Wadden Sea ecosystem. Monitoring shows that in some areas it is more abundant(-ly established) than in others. Considering this invasive ascidian to be 'established' is a safe evaluation as new generations are regularly produced. The further transition to dominance in all surrounding areas is 'spread'. As benthic invader it is dependent on the substrate. To prevent the transition towards the next stage (the survival and) further spread needs to be stopped. Targeting the substrate, eradication by hand seems most appropriate. Of course, floating bunches need to be removed to reduce the risk of a further spread.

In terms of smart management goals it could mean: monitoring indicates a population density in the harbor of List (Sylt, Germany) of 10 individuals per square meter in autumn. A specific and measurable goal would be to completely reduce that number to zero. The management action to achieve that goal would be to pull off all individuals by hand from harbor walls, pontoons but also benthic substrate such as

rocks and mussel beds. It is questionable if it is attainable to remove all *S. clava* individuals. A reduction down to one individual per 10 square meters would probably still prevent the frameworks next transition. To answer that question more precisely, more information about the population ecology needs to be considered. A reduction in numbers would be relevant to prevent further spread on to blue mussel cultures, for example. Time-wise this goal could be set until first day of spring, since its growth rate and probably reproduction ability is reduced until April.

Second example is a phytoplankton species, namely the **toxic diatom *Pseudo-nitzschia australis*** (Holtermann et al. 2010).



Figure 19. *Pseudo-nitzschia* species in (ballast) water samples at the harbor of NIOZ, Texel, The Netherlands (Liebich V 2010).

The main dispersal vector of *Pseudo-nitzschia* species is (probably) ballast water (Figure 19). Since ‘the most effective way to manage (invasive) species and their impacts is to prevent their introduction via vector regulation’ (p. 1) (Sylvester et al. 2011), the target transition is ‘transport’. To reduce the chance of an introduction, ballast water should get treated since it is the first transition towards introduction. The influencing factor is propagule pressure, thus a reduction in propagules should be achieved. In terms of management goals that could mean: in harbor X, and therefore in the intake ballast water of the ships sailing from this harbor, a concentration of 5 *P. australis* cells per milliliter is counted, thus 5000 cells per liter (that number is estimated from samples of the NIOZ harbor water.) The goal is to reduce that number in the ballast water tank to only 5 cells of *P. australis* per liter - as this would be a 1000 times reduction, like regulated in the D-2 standard. This goal is very specific and obviously measurable. It was tested to be attainable by, for example, treatment

with UV-radiation. It is relevant because *P. australis* is toxic and should be prevented from further spread and possible invasion. A time needs to be assigned, for instance half a year in which this ship leaves harbor X six times. This time is needed to evaluate the measure and to increase, for example, the UV-dosage, if incubation experiments show that *Pseudo-nitzschia* survives lower dosages.

However, discussing the D-2 standard in terms of cell numbers reveals interesting results. For a control intake to be valid, test organisms between 10 and 50 µm should be present in a total density of preferably 10.000 but not less than 1.000 individuals per milliliter, and should consist of at least five species from at least three different phyla/divisions (MEPC 58/23 ANNEX 4, p.20). Consequently: assuming 1.000 cells but then divided by five species, *P. australis* would be present with 200 individuals per milliliter in the control intake.

According to the D-2 standard, less than 10 viable organisms per milliliter – so two (10/5) or rather one (because the goal is ‘less’) cell per milliliter is allowed at discharge after treatment. That makes 1000 cells per liter times 1000 to calculate it for a cubic meter. That leaves in this example 1 million cells per cubic meter, in a common 5.000 cubic meter sized tank that sums up to 5 billion allowed viable cells in a tank! Thus, even if the required reduction of 100 to 1.000 times down to less than 10 individuals is achieved, the risk of introducing potentially invasive toxic species is only reduced but clearly not eliminated. Therefore, the D-2 standard needs to be evaluated and adjusted.

UV-treatment reveals tough plankton invaders - implications of ballast water management (Ch. 3)

Acknowledging the fact that invasive species are considered one of the biggest threats to our world’s oceans, ballast water as main dispersal vector should get managed. Ballast water management with UV-radiation is an option which is chosen comparably often. That is because many ship-owners like the idea of not using or producing any chemicals on-board (personal communication). Safety regulations for the ship’s crew would make operations more difficult and cost maybe more in terms of money and time.

However, UV-radiation was shown to allow for re-growth of phytoplankton in previous tests (Stehouwer et al. 2010). Only, those phytoplankton groups were never identified. No knowledge was available, if re-growth occurred based on coincidence, a matter of chance, or specifically for certain groups. Microscopic analysis revealed now for the first time that specific phytoplankton taxa, such as *Thalassiosira*, *Skeletonema*, *Chaetoceros*, *Nitzschia* & *Pseudo-nitzschia*, can survive and re-grow. Remarkable is *Pseudo-nitzschia* as surviving and re-growing taxa, since some of its species are toxic (Lincoln 2002).

The situation of certain organisms being able to survive disinfection of ballast water treatment systems seems similar to situations in the food industry. It is well known from literature, that for example *Escherichia coli* strains can become resistant to biocide and disinfectant use. This persistence is probably not developed based on disinfectant resistance but based on physical adaptation (Holah et al. 2002). Also an intact spore coat of *Bacillus subtilis* (commonly found in soil and the human gut) can physically protect it against artificial UV-B radiation and solar UV-B and UV-A radiation, but interestingly not against 254-nm UV-C radiation (Riesenman and Nicholson 2000). Latter is used in UV-ballast water treatment. Two major spore DNA repair pathways are, on the other hand, causing a resistance of *Bacillus subtilis* spores to artificial 254-nm UV-C radiation (Xue and Nicholson 1996). Concern is also expressed, that the application of biocides in the food sector might contribute to the development of antibiotic resistance. However, bringing food research back to aquatic science: bacteria residing in biofilms are, for example, up to 100 times more resistant to disinfectants than planktonic bacteria (White and McDermott 2001). And the marine (tank) environment needs to be taken into consideration, since factors like temperature and sediment content influence efficiency of biocides (Gregg and Hallegraeff 2007).

Not much is known about phytoplankton building up a resistance against ballast water treatment. The proof that certain taxa, such as *Thalassiosira*, *Skeletonema*, *Chaetoceros*, *Nitzschia* & *Pseudo-nitzschia*, can survive and re-grow after UV-treatment is therefore very important. *Thalassiosira*, *Skeletonema*, and *Chaetoceros*, the main re-growers by numbers, have a broad temperature tolerance, resting forms, and high growth rates. It was concluded that they have greater potential to survive treatment and become invasive than the other identified microalgae in the harbour

water. Including *Pseudo-nitzschia*, their species' invasions might have harmful effects by forming blooms, harming fish gills, producing toxins, and by 'simply' outcompeting native populations and causing regime shifts. If ballast water treatment favors certain invasive species which develop(ed) a resistance against certain treatments - is certainly a risk which should be studied further.

Implications for ballast water management: Recommendation for amendments to the D-2 standard (Ch. 4)

Members of national delegations attending the IMO MEPC BallastWaterWorking Group have participated in the development of the convention and summarize that only 'few delegations brought the biological expertise necessary for in-session discussions.' (p. 590) (Gollasch et al. 2007). A critical review of the Ballast Water Performance Standard D-2 should be considered taking into account the meanwhile achieved experience with the testing of treatment systems for compliance with D-2.

The D-2 standard can be changed, like it is the case also for other parts of the Ballast Water Convention, only after it got ratified. Official agreement by the majority of the signed parties is required. Soon the convention is expected to be ratified, probably next year (2013). Based on the results presented in this thesis, one possible recommendation could be to focus on harmful species rather than or in addition to an overall reduction of cells. However, it was shown, that species identification requires different techniques and more time. Regarding compliance testing in the harbors, that seems not applicable (yet).

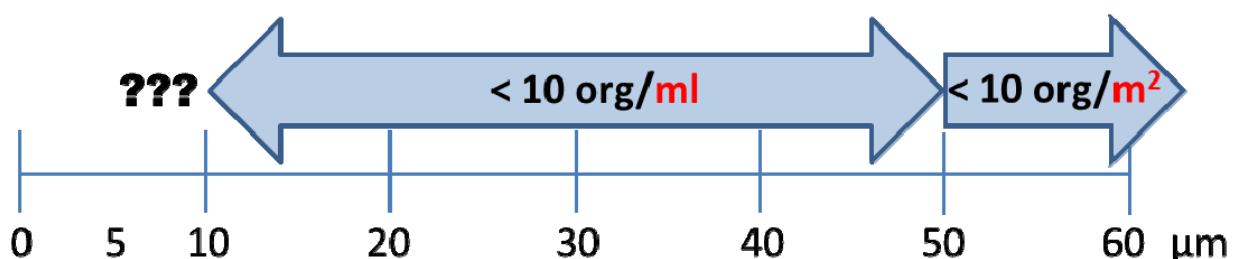


Figure 20. The D-2 standard of the Ballast Water Convention with two size classes in which less than 10 viable organisms per ml or m² respectively are allowed at discharge.

Coming back to the example of harmful *Pseudo-nitzschia* species outside of the testing size ranges of the D-2 standard (Chapter 4), one other recommendation would be to expand the D-2 standard about further 5 μm down to 5 μm in minimum dimension (Figure 20). The new size class would then include instead of 10 to 50 μm the increased range of 5 to 50 μm . However, that would consequently lead to more organisms in this size category. Still, systems would need to meet the reduction to the required 10 organisms. Only now that would mean a far greater reduction in numbers!

Another possible solution could be a third size range from 5 to 10 μm . 5 μm is reasonable because it is from my experience still visible with a normal microscope and a 400x magnification. Smaller organisms could be picked up by flow cytometry but since this is no standard method, testing and enforcement would be more difficult and thus agreement on this change less realistic.

If the size range 5 to 50 μm or 5 to 10 μm would be included into D-2, then more organisms would be included in the control. Size classes are not equally distributed in the phytoplankton community. As consequence the allowed numbers at discharge could be increased, for example, from 10 to 20 viable organisms. Or another extra 10 organisms are included in the third size class, respectively.

Final Conclusion and Perspective

Invasive plankton is the reason why the Ballast Water Convention was developed. Hence, invasive plankton has implications for ballast water management. If certain species survive, then implications might arise from it. These finding (should) then in turn influence ballast water management. Invasive plankton creates implications of and for ballast water management!

After generally discussing the findings of this thesis, it becomes clear that confusing invasion theories and terminology were disentangled in an extensive literature review. The new stage-transition framework is comprehensive based on the review's analysis. It provides consistent reasonable terminology and enables us to gain a

mechanistic understanding of the invasion process. That makes it applicable for (ballast water) management strategies and a tool for scientists and decision makers.

The framework achieved to present a simplified but comprehensive view, while invasion ecology is quite complicated. That is, because the processes are influenced by varied factors, starting by man-aided overcoming of natural barriers (and propagule pressure based on frequency). The potential invader's characteristics play an important role (invasiveness) which can be expressed by a sub-discipline of ecology, namely autecology. Synecology takes different species' interaction into consideration – thus, the invader *and* the native biota. Those interactions influence if and how fast the potential invasive species might become really invasive in the recipient area (invasibility). The stages of the invasion process are comparable to processes in population ecology: introduction, establishment and dominance (Figure 21).

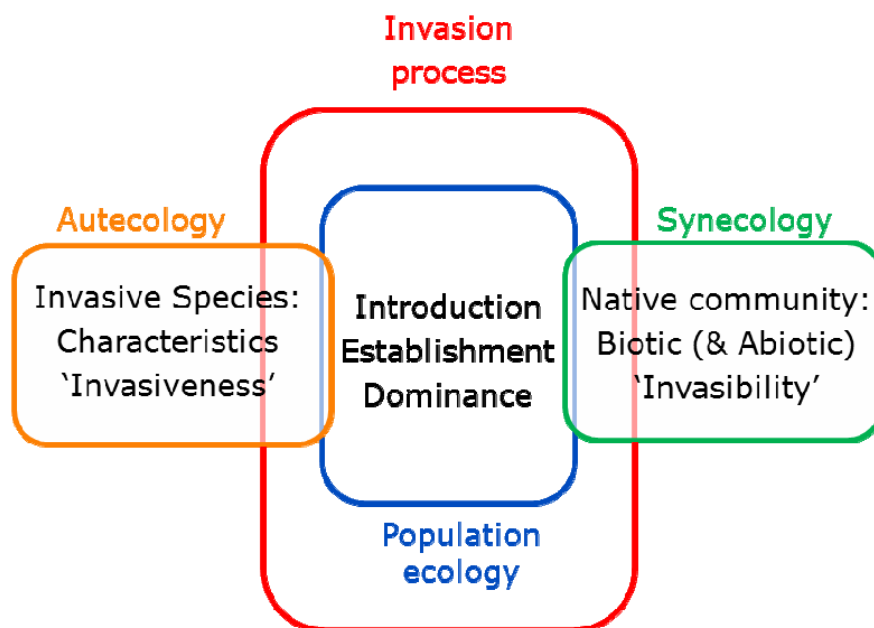


Figure 21. Overview of ecology sub-disciplines interlinking with the invasion process.

Studying the invasion process gives mechanistic insights into different sub-disciplines of ecology. For example: selection, spread, adaptation, species' interaction and globalization are developments which happen fast compared to evolutionary process. Therefore, invasion ecology offers rare chances for model systems. Predictions of

ecological and evolutionary processes can be studied in the next few years to come – in 10 years a lot can happen if you are a potential invasive species.

Therefore, invasion ecology is a very valuable discipline. It should not *only* be studied to develop mitigation strategies, for example ballast water management. Even though there might be the focus of public awareness, for instance regarding harmful plankton species with invasive potential. Globalization of species should, on the other hand, also not just be accepted. Globalization of species through invasions implies necessarily a loss of biodiversity, since resources do not allow for limitless species additions (Lennon et al. 2003). Invasion ecology should be taught to students and get funding for further research. Invasion ecology is after all 'a discipline that's too young to die' (Pyšek and Hulme 2009), it still has a lot to offer.

This thesis brought invasive plankton and ballast water management into the framework of invasion theory. As further perspective, more research is recommended to study if ballast water treatment according to the D-2 standard is sufficient, especially regarding smaller organisms. And it is very interesting to see if ballast water treatment is creating tough invaders. If this is the case, adjustments of the invasion framework might become necessary. Certainly, a broader knowledge about invasive plankton species, especially the smaller and harmful species, is needed.

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Publications derived from the dissertation

Two manuscripts have been published:

Liebich V, Stehouwer PP, Veldhuis M (2012) Re-growth of potential invasive phytoplankton following UV-based ballast water treatment. *Aquatic Invasions* 7: 29-36 (Chapter 3)

and

Stehouwer PP, **Liebich V**, Peperzak L (2012) Flow cytometry, microscopy, and DNA analysis as complementary phytoplankton screening methods in ballast water treatment studies. *Journal of Applied Phycology*, DOI 10.1007/s10811-012-9944-8 (paper is attached as annex).

One manuscript is accepted and in press:

Van der Star I, **Liebich V**, Stehouwer PP (in press) The forgotten fraction: The importance of organisms smaller than 10 µm when evaluating ballast water treatment systems. In: *Compliance Monitoring and Enforcement - the Next R&D Challenge and Opportunity*, Proceedings of the Global R&D Forum and Exhibition on Ballast Water Management, 26–28 October 2011, Istanbul, Turkey.

One manuscript is submitted:

Liebich V (submitted) Understanding (marine) invasions through the application of a comprehensive framework – review of invasion theory and terminology. Submitted to *Management of Biological Invasions*.

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Viola

*"Die Wissenschaft fängt eigentlich erst da an interessant zu werden, wo sie aufhört."
Justus von Liebig (1803-73)*

Summary

Invasive species are one of the biggest threats to our world's oceans. The man-aided introduction of non-native organisms via a vector into new areas and their successful establishment as invasive species pose risks to native biodiversity, ecosystem services, and human health. To develop effective strategies in case of negative environmental and economic impacts, detailed knowledge about the invasion process is required.

A new comprehensive stage-transition framework is presented. It unifies invasion theories which were inconsistent in the use of terminology and it provides a mechanistic understanding of the invasion process. That makes it applicable for the development of management strategies and as a tool for scientists and decision makers. The new framework consists of three stages: introduction, establishment, and dominance. Each stage is preceded by a transition: transport, survival, and spread. Successful invaders pass these intermediate transitions which are influenced by factors like propagule pressure, invasiveness, and invasibility.

'Invasive species' are defined encompassing the major process events: non-native species which were transported via a vector and by that experienced a human-mediated introduction outside their normal distribution followed by dominant abundance in the recipient ecosystem. That definition excludes, however, the invader's possible (positive or negative) impact.

The first transition in the framework is the transport via a vector and that is the best moment to mitigate introductions of species with invasive and harmful potential. Marine organisms are mainly transported via ballast water of vessels and many are able to survive in the dark tanks. In order to reduce the risk of invasions, different technologies were developed to treat ballast water according to the Ballast Water Convention adopted by the International Maritime Organization. The effectiveness of a UV-based ballast water treatment system is tested in incubation experiments over 20 days.

Long-term incubation experiments proved to be a valuable testing tool and after an initial decline in cell numbers, re-growth could be observed. Surviving phytoplankton taxa were identified for the first time: namely, the diatoms *Thalassiosira*,

Skeletonema, *Chaetoceros*, *Pseudo-nitzschia*, and *Nitzschia* (order represents rank of abundance). The conclusion is that a variety of taxa are able to survive UV-treatment. Despite approved treatment according to IMO's D-2 standard, phytoplankton species can become invasive and might become harmful by producing toxins (e.g. *Pseudo-nitzschia* species) or anoxic conditions following their blooms. *Thalassiosira* could be a suitable indicator organism for testing the efficiency of UV-units.

Methods for phytoplankton detection are used and compared: flow cytometry, cluster analysis, microscopy, and DNA-sequencing. Flow cytometry is preferable for fast organism counts. Cluster analysis and DNA-sequencing seemed unreliable to identify phytoplankton species. Results of microscopy indicate, even at species level, the re-growing phytoplankton - including species smaller than 10 µm in minimum dimension. These small organisms are so far not included in the D-2 Ballast Water Performance Standard which restricts organism counts per size class after treatment. However, they account for over 90 % of the overall phytoplankton in land-based ballast water treatment system testing, show the main re-growth after UV-treatment and electrolytic chlorination, and include harmful species. It is therefore recommended to include organisms smaller than 10 µm in minimum dimension into the D-2 standard.

This thesis showed why invasive plankton is the reason for ballast water management and in turn treatment seems to have implications for plankton invasions. Invasive plankton and ballast water management were examined based on the new framework of invasion theory. As further perspective, more research is suggested to study if ballast water treatment according to D-2 is sufficient or if amendments are needed (especially regarding smaller organisms) and if ballast water treatment is creating tough invaders.

Zusammenfassung

Invasive Arten sind eine der größten Bedrohungen für unsere Weltmeere. Durch die Einschleppung gebietsfremder Organismen und deren erfolgreiche Invasion werden die heimische Biodiversität und Ökosystemdienstleistungen gefährdet, wobei einige invasive Arten auch gesundheitsschädlich sind. Um mögliche Kosten und ökologische Schäden zu begrenzen, ist ein grundlegendes Verständnis des Invasionsprozesses notwendig.

Das neue konzeptionelle Modell erklärt nicht nur den Invasionsprozess, sondern vereinheitlicht auch ältere, zum Teil widersprüchliche, Prozessmodelle und die Terminologie. Dadurch kann es zur Entwicklung von Maßnahmen beitragen und Wissenschaftler und Entscheidungsträger bei ihrer Arbeit unterstützen. Das neue Modell besteht aus drei Stufen: Einschleppung, Ansiedlung und Dominanz. Jeder Stufe geht ein Zwischenschritt voraus: Transport, Überleben und Verbreitung. Erfolgreiche invasive Arten durchlaufen diese Prozessschritte unter Einflussnahme von Faktoren, wie Einschleppungsfrequenz und Volumen, den Eigenschaften der Art und des Zielhabitats.

Invasive Arten werden auf Basis des neuen Modells definiert als gebietsfremde Arten, die über einen Vektor außerhalb ihres normalen Lebensraumes eingeschleppt werden und sich im Zielhabitat dominant ansiedeln. Diese Definition bezieht jedoch nicht die möglichen positiven oder negativen Folgen der Invasion mit ein.

Der erste (Zwischen-)Schritt in dem Modell ist der Transport durch den Vektor. An diesem Schritt kann deshalb besonders gut interveniert werden, um die Einschleppung möglicher schädlicher invasiven Arten zu verhindern. Meeresorganismen werden hauptsächlich durch Ballastwasser von Frachtschiffen in andere Gebiete transportiert und können dabei auch mehrere Tage im dunklen Tank überleben. Um die Einschleppung gebietsfremder Arten zu reduzieren, wurden Ballastwasserbehandlungsanlagen entwickelt – nach den Richtlinien des Ballastwasser-Übereinkommens der Internationalen Seeschiffahrts-Organisation (IMO). Die Effektivität einer Behandlungsanlage, die UV-Strahlung zur Desinfizierung einsetzt, wurde im Rahmen von Inkubationsexperimenten über 20 Tage getestet.

Die Inkubationsexperimente erwiesen sich als sehr sinnvoll und zeigten nach einer anfänglichen Abnahme der Phytoplankton-Zellen erneutes Wachstum. Phytoplankton-Taxa, die überlebt haben, wurden erstmals identifiziert: *Thalassiosira*, *Skeletonema*, *Chaetoceros*, *Pseudo-nitzschia* und *Nitzschia* (wobei die Reihenfolge ihrer Abundanz entspricht). Die Schlussfolgerung ist also, dass verschiedene Phytoplankton-Taxa die UV-Behandlung überleben. Trotz Behandlung mit einem zertifizierten System nach dem D-2 Standard der IMO verbleiben Möglichkeiten, dass Phytoplankton-Arten invasiv werden und Schäden verursachen können (z.B. durch Planktonblüten oder Toxine bildende *Pseudo-nitzschia* Arten). Dabei scheint *Thalassiosira* ein guter Testorganismus für die Effektivität von Ballastwasserbehandlungsanlagen zu sein.

Methoden zur Untersuchung von Phytoplankton wurden verglichen: Durchflusszytometrie, Software zur Daten-Gruppenerkennung, Mikroskopie und DNS-Sequenzierung. Um Zellzahlen zu ermitteln, erwies sich die Durchflusszytometrie als bestes Verfahren. Die Identifizierung der Arten war durch die Software zur Daten-Gruppenerkennung und die DNS-Sequenzierung nur unzuverlässig gewährleistet. Mikroskopie war die beste Methode, um Arten zu ermitteln. Dadurch war es sogar möglich, die kleineren überlebenden Phytoplankton-Gruppen zu ermitteln (kleiner als 10 µm). Bisher sind diese kleinen Organismen nicht in dem D-2 Standard enthalten, welcher die erlaubten Zellanzahlen per Größenklasse im Abspumpwasser regelt. Allerdings machen diese kleinen Organismen den größten Teil des Phytoplanktons bei den Landtests der Behandlungsanlagen aus. Sie zeigen außerdem die höchste Überlebens- und (Wiederwachstums-)Rate nach Behandlung mit UV-Strahlung oder elektrolytischer Chlorierung und beinhalten schädliche Arten. Deshalb wird empfohlen, Phytoplankton mit einer Größe kleiner als 10 µm in den D-2 Standard zu integrieren.

Diese Dissertation hat gezeigt, warum invasives Plankton der Grund für die Entwicklung von Ballastwassermanagement ist, welches wiederum Planktoninvasionen beeinflusst. Invasives Plankton und Ballastwassermanagement wurden im Rahmen des neuen Prozessmodells analysiert. Als Folgeschritt ist zu empfehlen, weiter zu untersuchen, ob der D-2 Standard des Ballastwasser-Übereinkommens ausreicht oder Änderungen im Speziellen bezüglich kleinerer Organismen notwendig

sind und ob das heutige Ballastwassermanagement besonders resistente invasive Arten hervorbringt.

Eidesstattliche Versicherung

Hiermit erkläre ich an Eides statt, dass ich die vorliegende Dissertationsschrift selbst verfasst und keine anderen als die angegebenen Quellen und Hilfsmittel benutzt habe.

Declaration on oath

I hereby declare, on oath, that I have written the present dissertation by my own and have not used other than the acknowledged resources and aids.

Hamburg, den

Hamburg, date
