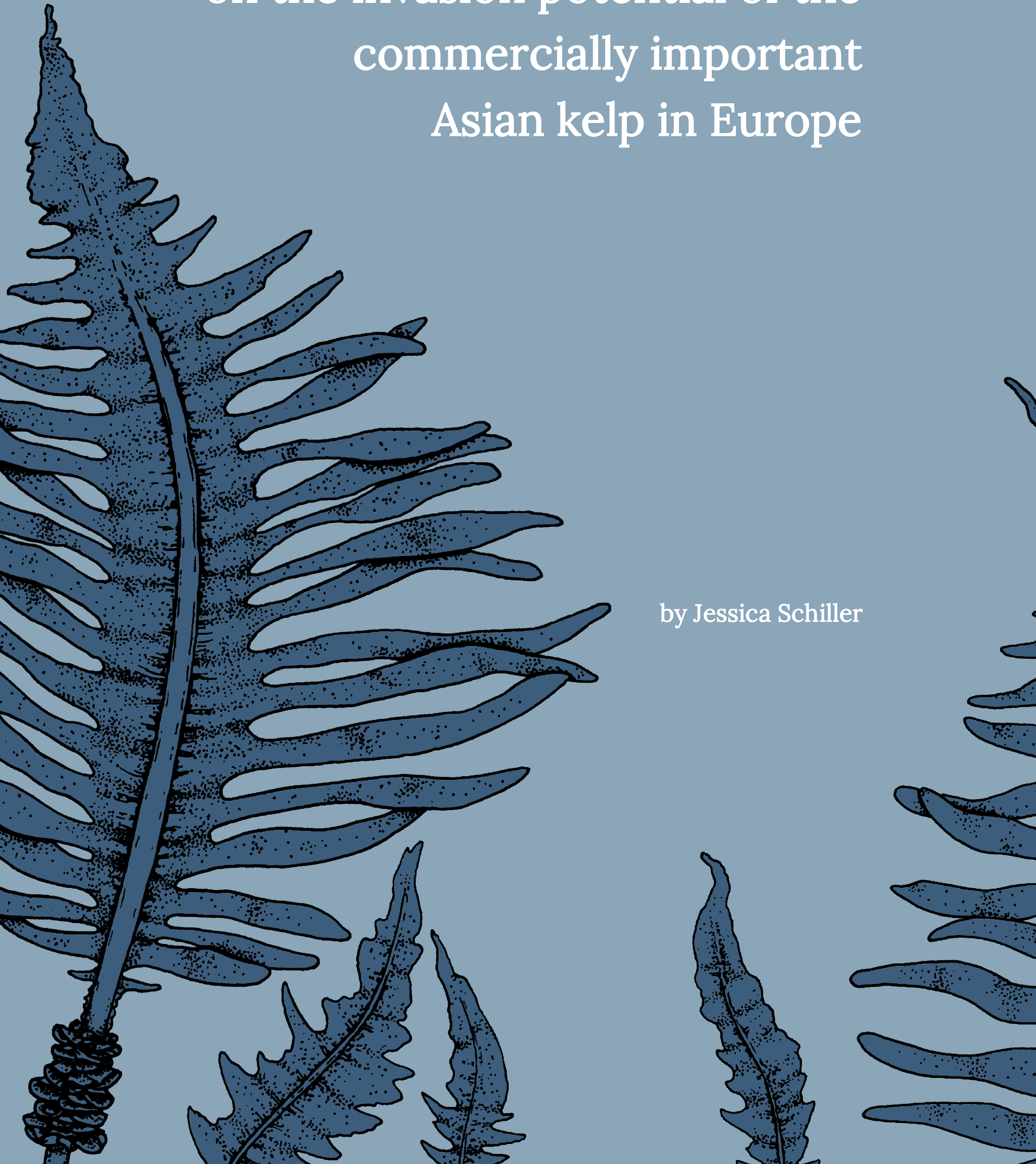
Undaria pinnatifida: on the invasion potential of the commercially important Asian kelp in Europe

by Jessica Schiller



Undaria pinnatifida: on the invasion potential of the commercially important Asian kelp in Europe

Dissertation

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by

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This research has been conducted at the Department of Marine Botany, Bremen Marine Ecology Centre for Research and Education (BreMarE), Faculty Biology/Chemistry (FB2), University of Bremen (Bremen, Germany).

Experiments were conducted at the Department of Marine Botany, University of Bremen, the CAS and Shandong Province Key Laboratory of Experimental Marine Biology, Seaweed Culture Collection Center, Institute of Oceanology, Chinese Academy of Sciences (Qingdao, China), and the Alfred Wegener Institute, Helmholtz Centre for Polar and Marine Research, Wadden Sea Station Sylt (Germany).

Analyses were performed at the Department of Marine Botany, University of Bremen, the CAS and Shandong Province Key Laboratory of Experimental Marine Biology, Seaweed Culture Collection Center, Institute of Oceanology, Chinese Academy of Sciences (Qingdao, China), and the Department of Applied Ecology & Phycology, University of Rostock, Germany.

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Photo J. Schiller

"Above all, don’t fear difficult moments. The best comes from them."

Rita Levi-Montalcini

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Summary

Non-native invasive species were the most common driver of species extinctions in the last 500 years and continue to have a dramatic impact on nearly all ecosystems worldwide. Globalization and the continuously growing world population facilitate the spread of invasive species and, at the same time, contribute to making native ecosystems more susceptible to bioinvasions.

Bioinvasions in the marine realm have historically received less attention than terrestrial ones, which led to underreporting of their occurrence and ecological- and economic impact. Particularly in light of the Climate Crisis, marine primary producers have gained increasing attention – both as a potential solution and as ecosystem engineers that the changes will impact. A marine primary producer that has gained much attention due to its near ubiquitous worldwide spread as an invasive species is the brown macroalga *Undaria pinnatifida*. It is native to East Asia and is the second most commercially important kelp species worldwide. Since its introduction to Europe in 1972, *U. pinnatifida* has spread as far north as Great Britain (1994), the Republic if Ireland (2015-2017), and the island of Terschelling in The Netherlands (2009).

In this study, a population of *U. pinnatifida* was discovered on the German Wadden Sea island of Sylt, near the Danish border. This marks the to date (2024) northernmost introduced population of the kelp. The population was self-sustaining, and a gametophyte isolate was established. Two strikingly different phenotypes were observed between the attached growing individuals and others that washed ashore from a second site. Microsatellite analysis and field observations uncovered that despite those differences, both groups were genetically highly similar, and the populations have had genetic exchange. At the same time, they were distinct from other European and native populations. Further comparison of the mitochondrial DNA and subsequent generation of haplotypes confirmed this and indicated that the population in France (Brittany) was the most likely source of the German *U. pinnatifida*. However, potential stepping-stone populations between the two locations could not be excluded.

Isolates of the German and a native Chinese population were exposed to temperatures that occur around the year at the German site. Gametogenesis at the lowest temperature occurred only in the German isolate. Sporophytes reared under standard conditions confirmed less growth limitation in the German isolate during the cold treatment, and meta-analysis including various physiological and biochemical response parameters supported this. At the same time, the German *U. pinnatifida* grew slower than the native isolate in warmer treatments, indicating a potential adaptation to the colder environment. Further studies are required to prove the latter.

While *U. pinnatifida* is currently limited to primarily artificial substrates in the Wadden Sea, its increasing proximity to the rocky shores of Scandinavia raises questions about the likelihood of an establishment there. This spread could only be enabled by human vectors. However, intact native communities have shown resilience to invasion by *U. pinnatifida* in many regions. Therefore, the best counteraction against the spread of *U. pinnatifida*, invasive species, and their unpredictable impacts is the protection and preservation of native communities that are under threat from Climate Change.

Zusammenfassung

Gebietsfremde invasive Arten waren die häufigste Ursache für Artensterben in den letzten 500 Jahren und haben weltweit dramatische Auswirkungen auf nahezu alle Ökosysteme. Globalisierung und die stetig wachsende Weltbevölkerung begünstigen die Ausbreitung invasiver Arten und tragen gleichzeitig dazu bei, dass einheimische Ökosysteme anfälliger für Bioinvasionen werden.

Bioinvasionen im marinen Bereich wurde historisch gesehen weniger Aufmerksamkeit zuteil als terrestrischen, was zu einer Unterschätzung ihrer Vorkommen, sowie ihrer ökologischen und ökonomischen Auswirkungen führte. Im Kontext der Klimakrise haben insbesondere marine Primärproduzenten zunehmend an Aufmerksamkeit gewonnen – als potenzielle Lösung und als Ökosystemingenieure, welche von den resultierenden Veränderungen betroffen sein werden. Die braune Makroalge *Undaria pinnatifida* ist ein solcher Primärproduzent und hat aufgrund ihrer nahezu weltweiten Verbreitung als invasive Art viel Interesse auf sich gezogen. Ursprünglich aus Ostasien stammend, ist sie die zweitwichtigste kommerziell kultivierte Braunalgenart weltweit. Seit ihrer Einschleppung nach Europa im Jahr 1972 hat sich *U. pinnatifida* bis nach Großbritannien (1994), Irland (2015-2017) und zur Insel Terschelling in den Niederlanden (2009) ausgebreitet.

Im Rahmen dieser Studie wurde eine selbsterhaltende *U. pinnatifida* Population auf der deutschen Wattenmeerinsel Sylt nahe der dänischen Grenze entdeckt und ein Gametophytenisolat erstellt. Sylt stellt die derzeit (2024) nördlichste Verbreitung einer eingeschleppten Population der Alge dar.

Angeschwemmte Individuen und solche, die in der Austernbank wachsend vorgefunden wurden, wiesen zwei deutlich verschiedene Phänotypen auf. Mikrosatellitenanalysen und Feldbeobachtungen zeigten, dass sich beide Gruppen trotz dieser Unterschiede genetisch sehr ähnlich waren und genetischer Austausch zwischen den Populationen stattfand. Gleichzeitig unterschieden sie sich jedoch von anderen europäischen und einheimischen asiatischen Populationen. Ein weiterer Vergleich der mitochondrialen DNA und die anschließende Erstellung von Haplotypen bestätigten dies und deuteten darauf hin, dass eine Population in Frankreich (Bretagne) die wahrscheinlichste Quelle der deutschen *U. pinnatifida* war. Potentialle intermediäre Standorte konnten jedoch nicht ausgeschlossen werden.

Isolate der deutschen und einer einheimischen chinesischen Population wurden Temperaturen ausgesetzt, die im Jahresverlauf am deutschen Standort auftreten. Nur im deutschen Isolat fand bei der niedrigsten Temperatur Gametogenese statt. Sporophyten, die unter Standardbedingungen aufgezogen und akklimatisiert wurden, bestätigten eine geringere Wachstumsbeschränkung im deutschen Isolat unter Kälteeinfluss. Eine Metaanalyse, die verschiedene physiologische und biochemische Reaktionsparameter einbezog, unterstützte dies. Gleichzeitig wuchsen deutsche *U. pinnatifida* bei höheren Temperaturen langsamer als das einheimische Isolat, was auf eine mögliche Anpassung an die kältere Umgebung hindeutet. Weitere Studien sind erforderlich, um Letzteres zu beweisen.

Während *U. pinnatifida* im Wattenmeer derzeit hauptsächlich auf künstliche Substrate beschränkt ist, wirft die zunehmende Nähe zu den felsigen Küsten Skandinaviens Fragen über die

Wahrscheinlichkeit einer dortigen Etablierung auf. Diese Ausbreitung kann nur durch menschliche Vektoren ermöglicht werden. Intakte einheimische Gemeinschaften haben jedoch in vielen Regionen eine Widerstandsfähigkeit gegen Invasionen durch *U. pinnatifida* gezeigt. Die folglich beste Maßnahme gegen die Ausbreitung von *U. pinnatifida* und anderen invasiven Arten, sowie deren unvorhersehbaren Folgen, besteht daher im Schutz und Erhalt einheimischer Gemeinschaften, die durch den Klimawandel bedroht sind.

Abbreviations

A	Antheraxanthin
Acc	Accessory pigment pool
ANOVA	Analysis of variance
C	Carbon
C:N ratio	Carbon to nitrogen ratio
Chl	Chlorophyll
CSP	Carbon sequestration potential
DNA	Deoxyribonucleic acid
DOP	Dissolved organic carbon
DPS	De-epoxidation state of the xanthophyll cycle
DPPH	2,2-diphenyl-1-picrylhydrasyl
DW	Dry weight
Fuc	Fucoxanthin
HPLC	High performance liquid chromatography
IQR	Interquartile Range
L:D	Light:dark
N	Nitrogen
n	number of samples (true replicates only)
n/a	not available, not detected, not applicable
NIS	Non-indigenous species
PCA	Principal component analysis
PCoA	Principal coordinate analysis
PERMANOVA	Permutational multivariate analysis of variances
PERMDISP	Permutational analysis of multivariate dispersion
PES	Provasoli enriched seawater
Rel. ΔL	Relative length increase (to day 0)
Rel. ΔWW	Relative wet weight increase (to day 0)
RLM	Robust linear model
ROS	Reactive oxygen species
rpm	rotations per minute
SD	Standard deviation
SST	Sea surface temperature (°C)
SW	Seawater
TE	Trolox® equivalents
V	Violaxanthin
VAZ	Xanthophyll cycle pigment pool
WW	Wet weight
β	Beta
Δ	Delta

Chapter 1

General Introduction

1.1 Invasive species and their traits

1.1.1 Non-native, introduced, and invasive species – a question of terminology

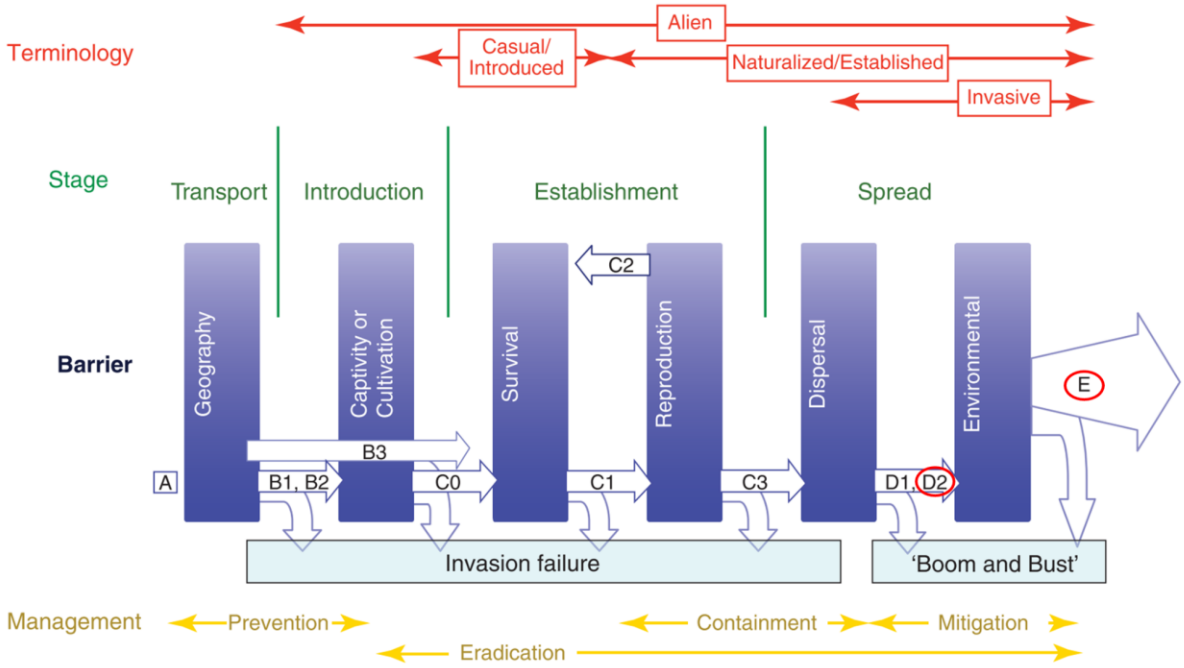
Globalization has promoted the introduction of organisms to environments they are not native to (see chapter 1.1.2). If they become established, spread, and may even have a negative impact on the receiving ecosystems, the economy, human-, animal-, or plant health, these organisms fall under most definitions of an invasive species (e.g., IUCN 2000; Colautti and MacIsaac 2004; Richardson and Pyšek 2006; Reaser et al. 2020). In the last third of the 20th century, "invasive" became one of the most used and recognized terms to refer to these species in scientific literature and public communications (Pyšek 1994). However, despite many decades of research, the terminologies are still varied and incongruent, especially between disciplines (Colautti and MacIsaac 2004), which poses difficulties for scientific understanding and policies resulting from it.

Terminology sets the tone for scientific works and discussions. Descriptions range from terms with a somewhat positive connotation, such as "exotic" or "novel," to more neutral such as "introduced," "non-native," or "non-indigenous," to potentially negatively connotated terms like "alien," "invasive," and "pest" (see Table 1.1). The choice of terminology in publications is largely up to subjective interpretation, which may be affected by conscious and subconscious decisions. Specifications may be applied to indicate a sub-group, such as using non-indigenous marine species (NIMS) instead of simply non-indigenous species (NIS) or using "invasive alien species" to highlight the non-native aspect of the species that became invasive (e.g., European Commission 2008; Cuthbert et al. 2021). While some literature aims for neutral, uniform terminologies (Colautti and MacIsaac 2004), to this date, there is still no consensus. Table 1.1 presents a non-exhaustive list of terms used in scientific literature regarding invasive species.

Different process-based approaches have been developed to overcome resistance and the seeming inability to unify terminologies within the scientific community, aiming to enable more straightforward translation between diverged disciplines and resulting policy-making. Williamson and Fitter (1996) established a statistical system to estimate the occurrence of invasions, based on the tens-rule, by which one in ten species of each stage made it to the next of their system. A later approach by Richardson et al. (2000) utilized a system that defined the invasion process by barriers that species need to overcome and associated some commonly used terms with specific crossed barriers. Similarly, Colautti and MacIsaac (2004) proposed a filter and stage-based framework incorporating determinants that positively or negatively affect a species' chance to pass through the stage. One of the latest attempts at a unified framework aims to combine previously proposed principles and further additions and specifications (Figure 1.1; Blackburn et al. 2011). The terminology used in this work will primarily be based on the framework by Blackburn et al. (2011). However, the generalized term "invasive species" or "biological invasion" may be used when no clear stage definition is required. This work mainly uses the term "invasive," as it is the most common in scientific literature regarding *U. pinnatifida*. When referencing from literature, specific terminology from the cited works may be used to preserve accuracy. When appropriate, "non-native" or "ïntroduced" will be used for their neutral nature.

Table 1.1 Non-exhaustive list of terms used in scientific literature regarding invasive species. Terms listed may occur in various forms, often as adjectives in combination with species or taxa. One exemplary source for each term is provided in the right column. (Adapted and expanded from Colautti and MacIsaac 2004.)

Term	Exemplary Source
Adventive	Hay and Villouta 1993
Alien	Pyšek et al. 2020
Casual	Williamson and Fitter 1996
Colonizing	Colautti and MacIsaac 2004
Cryptogenic	Carlton and Eldredge 2015
Escaping	Williamson and Fitter 1996
Establishing	Williamson and Fitter 1996
Exotic	Sax et al. 2007
Foreign	Young 1936
Immigrant	De Meester et al. 2007
Imported	Holzapfel and Vinebrooke 2005
Imported (≠introduced)	Williamson and Fitter 1996
Introduced	Carlton and Eldredge 2015
Invasive	Pyšek et al. 2020
Invasive alien species (IAS)	European Commission 2008
Naturalized	Sax et al. 2007
(Newly) introduced species	Simberloff 2015
Non-native	Simberloff 2015
Non-indigenous (marine) organisms/ species (NIS/NIMS)	Carlton and Geller 1993; Davidson et al. 2015
Noxious	Quinn et al. 2013
Nuisance	Zaiko et al. 2015
Pest	Hutchings et al. 2002
Spreading	Colautti and MacIsaac 2004
Temporary	Colautti and MacIsaac 2004
Transient	Colautti and MacIsaac 2004
Translocated	Colautti and MacIsaac 2004
Transplanted	Darrigran et al. 2023
Transported	Colautti and MacIsaac 2004
Waif	Colautti and MacIsaac 2004
Weed	Quinn et al. 2013



D2: Self-sustaining population in the wild, with individuals surviving and reproducing a significant distance from the original point of introduction.
E: Fully invasive species, with individuals dispersing, surviving and reproducing at multiple sites across a greater or lesser spectrum of habitats and extent of occurrence

Figure 1.1: Proposed unified framework for biological invasions (adapted from Blackburn et al. (2011)). The framework divides biological invasions into stages with barriers that need to be crossed for a species or population to move to another stage. It further associates common terminology, as well as applicable management strategies. The unfilled arrows containing alphanumerical codes (A-E) link to more detailed population categorizations in table 1 of Blackburn et al. (2011). The red circles indicate stages of the invasion in Europe.

1.1.2 Human impact on species range expansion

Species introductions and range shifts are an essential aspect of ecosystem development and evolution (e.g., Reise et al. 2023). However, human activities have disrupted this equilibrium and amplified it so that, alongside other stressors, it can lead to ecosystems that can no longer cope (e.g., Hutchings et al. 2002; Hulme et al. 2009; Seebens et al. 2017). Before human civilization, species introductions and invasions occurred naturally, such as animals or plants being transported by storms, moving across newly formed land bridges, or populating areas that became accessible due to sea level changes (e.g., Ricciardi 2007; Nathan et al. 2008).

However, as with most natural versus human-mediated phenomena, the scale sets them apart, spatially and temporally. Since the onset of human civilization, moving and introducing species has been one of its essential aspects: after the domestication of animals, they were brought with humans wherever they moved, and countless species of plants have been transplanted across continents for horticultural and food production purposes, journeys they could never have completed without human intervention (e.g., Ricciardi 2007; Sax et al. 2007). Human modification of geographical barriers has also opened up new corridors for the movement of species, such as the construction of the Panama and Suez Canals (Katsanevakis et al. 2013; Ruiz et al. 2018). Modern technological development promotes the movement of species worldwide at an unprecedented rate that is expected to increase further, as well as synergistic effects with other human-mediated drivers such as Climate Change (Ricciardi 2007). The growing world economy, progressively more effortless and cheaper international transport, and regulations that develop slower than all of these processes have promoted the introduction of species to new habitats (e.g., Ricciardi 2007; Seebens et al. 2021). Future predictions based on models that assume a "business as usual" approach regarding biological invasions estimated an increase of established alien species by 38% per continent from 2005 to 2050 (Seebens et al. 2021). Next to indirect facilitation, human activities also directly affect species introductions by a range of vectors (e.g., shipping, mariculture and fisheries, marine litter, trade, and recreational activities; e.g., Büttger et al. (2022); Gittenberger et al. (2023)). These are elaborated on in publication V (see appendix). Many marine species introductions are and have been unintentional, such as when fouling species attached to ship and boat hulls or aquaculture and fishing equipment are brought along with desired introductions as associated species, transported in ballast water, or released accidentally with aquarium water (Gittenberger et al. 2023). Intentional introductions typically are and have been for aquaculture-sometimes restoration purposes. An example is the Pacific Oyster, *Magallana gigas*, which was brought to several locations in Europe for its larger size and ultimately also the advantageous economic opportunity its cultivation provided over the native species, which was at the time in decline due to depletion (e.g., Wolff and Reise 2002; Gittenberger et al. 2023).

Another example is the introduction of the brown macroalga *Undaria pinnatifida* from the French Mediterranean coast to Brittany on its Atlantic side (Floc'h et al. 1991). In these examples and many others, it is either assumed that the cultivated species will not spread or is unlikely to spread due to environmental conditions that hinder reproduction (Floc'h et al. 1991). Another common reason for introduction is when the risk of the species' spread is accepted as a trade-off for greater socio-economic benefit (Copp et al. 2016). Both *M. gigas* and *U. pinnatifida* have spread outside of aquaculture confines despite assumptions they would not and became prominent species on many European shores.

Unlike intentional introductions, the sources and vectors of unintentional introductions are often challenging to identify (Minchin 2007; Büttger et al. 2022; Gittenberger et al. 2023). Occasionally, clear indications point to intentional introductions of an associated species, or proximity to these introductions, and other logical links are present (i.e., "direct evidence" *sensu* Minchin (2007). Fifty years after the letter by Druehl (1973) in which he predicted the spread of the brown alga *Sargassum muticum* in the northeast Atlantic, several of his and his colleague's predictions have come to pass: *S. muticum* was introduced to Europe alongside oyster spat from British Columbia (a secondary introduction of the material brought there from Japan), and became established (e.g., (Wolff and Reise 2002). Unbeknownst at the time, another brown macroalga, *U. pinnatifida*, was introduced to the French Mediterranean coast at the same time with the same vector, aquaculture, from Japan (Floc'h et al. 1991) and has since progressed northward along the European coast, facilitated by human activities as already described briefly above.

Tracing new arrivals or the further spread of bioinvasions in a region without clearly associated vectors is often impossible due to the multitude of human-facilitated vectors that overlap in many developed coastal regions, such as aquaculture, fisheries, cargo vessels, and recreational boating and watersports (Minchin 2007; Gittenberger et al. 2023). In those cases, vectors can only be identified as “likely” (in case of strong indications) or “possible” (deduction based on proximity alone; both *sensu* Minchin (2007)).

1.1.3 What makes a species invasive?

Various factors and traits play into whether or not a species has the potential to become invasive. They concern the origin, evolutionary history, and other life-history traits. Typically, newly introduced or non-native species become invasive, although population explosions or “invasions” have been documented for native species as well (Blackburn et al. 2019). Several studies argue that distinctions between native and non-native species are inconsequential for conservation concerns, both on a practical and theoretical level (e.g., Davis et al. 2011; Valéry et al. 2013). However, a more recent study provides evidence that biogeographic origin is indeed relevant to a species' potential ecological impact (Blackburn et al. 2019). This is supported by other studies highlighting that most species do not establish populations when introduced to a new habitat. An extensive analysis of recent extinction events showed that few were associated with native species. In those instances that they were, other factors could be identified as the original trigger of the invasion (Seebens et al. 2018). One prominent example is the explosive population increase of *Strongylocentrotus purpuratus*, the purple sea urchin, which led to the extinction of Steller's sea cow (*Hydrodamalis gigas*) by overgrazing its food source. The original trigger, however, was the decimation of sea otter populations due to hunting, thereby removing the natural predator of the sea urchin (Blackburn et al. 2019).

While no characterization can currently predict which particular species will become invasive, there are traits many invaders have in common that may be used for risk assessments (Alpert et al. 2000; Whitney and Gabler 2008). Invasive species often present r-selected life histories, with rapid growth, short generation times, large number of propagules or offspring, and high dispersal ability (Whitney and Gabler 2008; Cardeccia et al. 2015). Additionally, generalist traits, biotic resistances (Ricciardi et al. 2013), and high phenotypic plasticity may be associated with invasiveness (Davidson et al. 2011). In terms of their origin and evolutionary history, they often originate from regions with high species abundance, competition, predation, and other diverse stressors, which may give them competitive advantages in invaded regions with less pressure (e.g., Ricciardi et al. 2013).

1.1.4 Ecological and economic impact of invasive species

Invasive alien species in terrestrial ecosystems are the most frequent driver of recent animal and plant extinctions based on extinction data from the International Union for Conservation of Nature (IUCN) (Blackburn et al. 2019; Pyšek et al. 2020).

Coastal aquatic ecosystems, which are home to most macroalgae, are impacted by various threats. Marine bioinvasions are among these, alongside other human-mediated stressors such as Climate Change, destruction or alteration of habitats, overfishing, and pollution (Carlton and Geller 1993; Vitousek et al. 1997; Halpern et al. 2008; Davidson et al. 2015; Pyšek et al. 2020).

Invasive species can have far-reaching effects on an ecological and economic scale, the latter in many cases being affected by the prior. Their impact on community composition, biodiversity, ecosystem services, and human and animal well-being has been abundantly documented and discussed in scientific literature (Schaffelke and Hewitt 2007; Williams and Smith 2007; Davidson et al. 2015; Pyšek et al. 2020). Invasive species effectively alter the competitive relationships and functioning in the recipient habitat (Schaffelke and Hewitt 2007). Depending on their trophic level, the initial alteration varies. They may be predatory species decimating a prey population, filter feeders removing plankton (Carlton 1996; Davidson et al. 2015), or competition for resources like light, space, and nutrients, as well as structural changes to the habitat (Schaffelke and Hewitt 2007; Davidson et al. 2015). The spread of an invasive ecosystem engineer, the Pacific oyster *M. gigas*, along the Wadden Sea coast, has transformed mussel beds into oyster reefs, such as documented on the island of Sylt, Germany (Wolff and Reise 2002, K. Reise & D. Lackschewitz pers. comm.). With the complexity and uniqueness of each ecosystem, predicting the exact effect an introduced species may have is impossible, which is one of the reasons many intentional introductions lead to unintentional and unexpected consequences. The negative ecological impacts of marine and terrestrial invasive species are well documented. Some are directly observable, such as the introduction of cats and rats to islands with no prior predators that decimated local prey populations (Blackburn et al. 2019) or the invasion of the poisonous cane toad in Australia, which caused the decline of predators that died from consuming the toads, upsetting a whole ecosystems' balance (Shine 2010). In the marine realm, striking invasion examples include the predatory, poisonous lionfish invasions to the Caribbean, West Atlantic, and Mediterranean, which caused the decline of local fish abundance (Bariche et al. 2017).

Another example is the bivalve *Potamocorbula amurensis*, which led to the essential disappearance of the spring phytoplankton bloom in north San Francisco Bay over four years after its discovery (Carlton 1996). While animals often seem to have a more direct effect on their ecosystems, invasions of plants, or in the marine realm, seaweeds, can have as much impact. In the Mediterranean, the introduction and invasive spread of the green alga *Caulerpa taxifolia* has had devastating implications for species richness and abundance of the native ecosystems, made especially impactful by the alga's ability for vegetative reproduction via fragmentation and defenses against grazing (Boudouresque et al. 1995).

Economic and societal impacts of invasive species can be divided into direct and indirect impacts. Direct impacts are, e.g., costs due to the loss of ecosystem functions, damage to resources, impact on environmental amenities, or value and impacts on human health. Indirect costs include management costs, research, control and eradication measures, and education. Economic costs incurred by aquatic invasive species have been estimated to be drastically underreported compared to terrestrial species, as well as relative to their ecological impact (Davidson et al. 2015; Cuthbert et al. 2021). The conservatively summed up global costs of aquatic invasive species reached US$ 345 billion, most of which was from damage to resources by invertebrates, followed by vertebrates. In relation to the number of known invasive plant species, the associated economic costs seem likewise underreported. Future predictions indicated an increase to US$ 23 billion per annum globally in 2020 (Cuthbert et al. 2021).

One of the challenges in dealing with invasions is that they typically start inconspicuously, with few individuals and can come from all trophic levels and have far-reaching impact (Carlton 1996; Wiedenmann et al. 2001; Bariche et al. 2017). While the majority of scientific publications take a cautionary stance, advocating for better regulations, more preventive measures, and the recognition of the potential for far-reaching ecological and economic consequences of non-native species introductions (Simberloff 2005; Simberloff et al. 2013; Cuthbert et al. 2021), others argue these views are too subjective, regulations unreasonable to upkeep, and implications as likely positive as negative (Sagoff 2005). The most recent, data-based meta-analyses and modeling studies agree that bioinvasions and all types of associated impacts are only expected to increase. In contrast, our understanding of their functioning and impact still needs to be improved (Gallardo et al. 2016; Seebens et al. 2017, 2021; Pyšek et al. 2020).

1.2 Seaweeds in ecology and economy

1.2.1 Ecosystem engineers of the temperate and cold seas

In tropical seas, corals are well known to shape ecosystems and provide three-dimensional habitat structure. In cold and temperate seas, this role is carried by the large, often canopy-forming kelps of the order Laminariales (Steneck et al. 2008; Teagle et al. 2017). The possible impact of invasive species, particularly kelps, becomes abundantly clear when considering the functions large macroalgae provide as ecosystem engineers and key primary producers. Ecosystems like the Wadden Sea, where natural hard substrate is scarce, form a barrier preventing the migration of sessile species across their expanse. Invasive, habitat-forming species have an even more significant impact, changing the basis of the ecosystem and enabling new communities to develop. On natural rocky substrates, native kelp forest ecosystems thrive and form the basis of the communities. In the North Sea, these are present on the German high seas island Helgoland, the rocky shores of Great Britain and Ireland, and the Norwegian coast. The native, canopy-forming kelp species in these regions are multi-annuals, such as *Laminaria* spp., *Saccorhiza polyschides*, and *Saccharina latissima* (Bartsch et al. 2008). The multi-annual nature of these kelp forests is essential for the stability of the ecosystems they form. Kelp forests support biodiversity by being home to diverse assemblages of marine species, such as smaller seaweeds like the red alga *Palmaria palmata*, which frequently grows on the stipes of *Laminaria spp*, as well as a multitude of invertebrates, fish, marine mammals, and seabirds (Steneck et al. 2008). The kelp canopies provide many other ecosystem services such as wave and erosion protection for the coastline, nutrient cycling, and energy capture, e.g., via supplying nutrients as a grazing source or organic detritus (Duggins et al. 1989; Teagle et al. 2017; Weigel et al. 2022). Hence, they provide tremendous ecological and economic value (Costanza et al. 1997).

Kelp forests maintain a natural equilibrium of growth, decay, and predation. The primary grazers, globally, are echinoids, sometimes fish, and gastropods, all of which are, in turn, preyed on by higher trophic organisms (Steneck et al. 2008). Seaweeds play a crucial role in oxygen production and carbon fixation from the environment and are thus increasingly seen as a potential solution in addressing the world's Climate Crisis (Duarte et al. 2017; Li et al. 2022).

1.2.2 Kelp as a crop

Kelp and other seaweeds have been traditionally harvested from wild populations in many parts of the world. The history of kelp utilization is particularly prominent in Asia, where it still plays an important cultural role today (Kim et al. 2017). The development and drastic expansion of commercial cultivation of high-value species have led to an increase in harvested kelp over the latter part of the 20th and early 21st centuries. Today, seaweed cultivation is the fastest-growing sector in western aquaculture (Kim et al. 2017), as interest in utilization for areas other than direct human consumption increased. In addition to direct food consumption, seaweed components are now widely used as food additives, animal feed, and sources for pharmaceuticals-, cosmetics- and other industries (Lüning and Pang 2003; Camus et al. 2018). Global seaweed production has more than tripled in the last 20 years, to 35.8 million tons in 2019, more than 97 % of which came from aquaculture, while wild harvesting of natural populations has marginally reduced (FAO 2021). The vast majority of cultivated seaweed (>97 %) was produced in Asia, mainly China (50.8 % of global). Over the last decades, modernizing farming and breeding techniques have gained focus to meet rising market demands (see Figure 1.2; Kim et al. 2017; Hu et al. 2021; Shan and Pang 2021).

Kelp's capabilities to sequester carbon and its use in bioremediation have made kelp cultivation an attractive blue economy opportunity (Lüning and Pang 2003; Filbee-Dexter et al. 2022b). Seaweed cultivation (except land-based) does not require freshwater or arable land, minimizing competition with other food crops (Stanley and Day 2014). During biomass production, kelp is fixing carbon and releasing dissolved organic carbon (DOC) (Li et al. 2022). The main principles by which carbon sequestration via kelp cultivation is proposed are the sinking and depositing of biomass into the deep sea sediment (Ortega et al. 2019), and storage as recalcitrant DOC in the seawater (Li et al. 2022). While the general scientific consensus is that seaweeds can be used to sequester carbon, the scale is under debate. Farming trials have shown that significant amounts of biomass may be lost before harvest (Dolliver and O'Connor 2022), and recent studies highlight the negative impact ocean warming may have on the carbon sequestration potential (CSP) of seaweed ecosystems (Filbee-Dexter et al. 2022a; Wright et al. 2022).

U. pinnatifida, the commercially second most important kelp species worldwide, has historically been wild-harvested and cultivated (Shan and Pang 2021). Early farming started in the 1940s in China, based on discoveries by Youshiro Ohtsuki, followed by more extensive cultivation in 1955 and, shortly after, commercial cultivation in other areas of China, Korea, and Japan (Yamanaka and Akiyama 1993; Pang et al. 2024). Commonly, *U. pinnatifida* is mainly referred to as 'wakame' the traditional Japanese name, while other names are the Chinese 'qun dai cai' or 'sea mustard,' or the Korean 'miyeok' (Yamanaka and Akiyama 1993; Choi et al. 2008). Most cultivated wakame (2.6 million tonnes in 2019) comes from China, Japan, and The Republic of Korea, with minor commercial cultivation in France, Spain, and The Netherlands (Peteiro et al. 2016; FAO 2021; Pang et al. 2024, own observations). Most cultivated wakame has historically been exported to Japan, which is still the biggest market today (Yamanaka and Akiyama 1993; Pang et al. 2024).

Figure 1.2: Historical and modern techniques coexist in present-day kelp farming in Asia. Top left: traditional manual harvesting method by small boat in Rongcheng, China; top right: traditional kelp nursery used for spore-seeded frames with manual shading control; bottom left: improved mechanized harvesting vessel in Wando, South Korea; bottom right: mechanical-digital quality control system used for sorting dried seaweed in China. Photos by J. Schiller.

1.2.3 The kelp *Undaria pinnatifida*

A kelp that gathered attention not just as a commercially important species in Asia but in recent decades foremost as an invasive species is *Undaria pinnatifida* (Harvey) Suringar (South et al. 2017). It has even been listed as one of the world's "100 worst invasive alien species" (Lowe et al. 2000).
As a member of the order Laminariales of the class Phaeophyceae (brown algae), it shares its typical haplodiplontic heteromorphic life cycle. It consists of the microscopic haploid phase (1n; dioecious male and female spores, gametophytes, and gametes) and the diploid phase (2n), growing from microscopic to macroscopic sporophytes (Figure 1.3; Hurd et al. 2014).

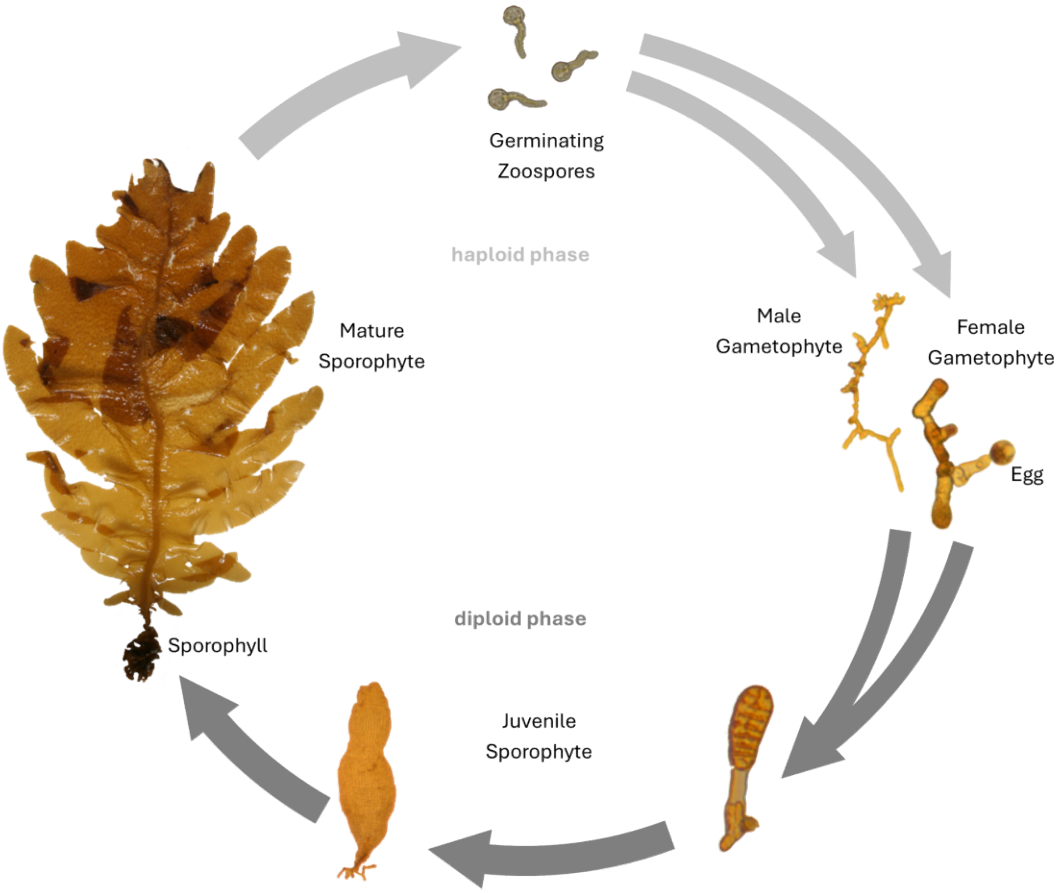


Figure 1.3: Representation of the heteromorphic life cycle of kelp (order Laminariales, Phaeophyceae) on the example of the kelp *Undaria pinnatifida* (Harvey) Suringar. The cycle is composed of the microscopic, haploid (1n) male and female gametophytes and the macroscopic, diploid (2n) sporophyte. All stages pictured are of the German wild population and the resulting gametophyte isolate. The components of the life cycle are not to scale. Photos and graphic by J. Schiller.

Mature sporophytes develop large quantities of zoospores via meiosis, contained in a reproductive tissue called sorus, which may be located on the blade or in specialized structures called sporophylls (Hurd et al. 2014). The latter is the case for *U. pinnatifida*, which forms these as frills at the base of the blade, along the edges of the stipe (Figure 1.4 and Figure 1.5, Van den Hoek et al. 1995).
Release of the zoospores into the water may occur due to ripeness but can also be triggered by osmotic or temperature stress (Van den Hoek et al. 1995; Alsuwaiyan et al. 2019). Kelp zoospores

are heterokont, containing two flagellae, and can move actively, even against slow currents (Pang and Shan 2008). They have been shown to prefer certain substrates (Petrone et al. 2011). The natural, unaided dispersal of kelp spores is limited to some hundred meters (e.g., Fredriksen et al. 1995; Gaylord et al. 2004). Attachment of released spores occurs within a short period. It is followed by the germination into male and female gametophytes, usually within 24 h for *U. pinnatifida*, while other kelps may require longer (e.g., Nielsen et al. 2016; J. Schiller pers. obs.).

Figure 1.4: Sporophylls of the invasive kelp *Undaria pinnatifida*. Each individual may release several hundred million spores (Schaffelke et al. 2005; Pang et al. 2024). Photos by J. Schiller.

The filamentous gametophytes exhibit sexual dimorphism, the extent of which seems to be species-dependent (e.g., dimorphism in *S. latissima* and *Macrocystis pyrifera* is much more pronounced than in *U. pinnatifida*; J. Schiller, pers. obs.). While male gametophytes tend to have smaller cells, those of females are larger (e.g., Destombe and Oppliger 2011). Kelp gametophytes may persist in a vegetative growing state for at least a year, likely much longer. This is regarded as an adaptation to suboptimal environments (tom Dieck (Bartsch) 1993; Carney 2011; Ebbing et al. 2021; J. Schiller pers. obs.). When environmental conditions meet the requirements, the gametophytes develop reproductive structures. Male gametophytes form antheridia, which release flagellated sperm, while female gametophytes form oogonia, producing eggs that are significantly larger than the sperm and immobile. Upon fertilization, the egg forms a diploid zygote, which develops into a young kelp sporophyte, often called ‘seedling’ in kelp cultivation (Destombe and Oppliger 2011; Luthringer et al. 2014; Peteiro et al. 2016).

The sporophyte of *U. pinnatifida* consists of a claw-like, dichotomously branched holdfast (also ‘rhizoid’), a thick, flattened stipe (also ‘cauloid’) which continues to run as a midrib through the length of the blade or lamina (also ‘phylloid’; see Figure 1.5; (Van den Hoek et al. 1995). The sporophyte’s characteristic thallus morphology gives the species its name – ‘pinnatifid,’ meaning having a cleft or lobed leaf, as it is easily recognizable by finger-like protrusions along the sides of the lamina. (Figure 1.5; Guiry (2024)). Sporophytes reach sizes of 45 to 130 cm in native habitats (e.g., Ohno et al. 1999; Shibneva and Skriptsova 2012), while cultivated ones have been reported to reach up to 3 m in length (Pérez and Kaas 1984). As proper breeding of *U. pinnatifida* was only conducted much later (Hu et al. 2021; Shan and Pang 2021), this difference is likely the effect of optimized cultivation periods and site selection, and possibly some selection for size. *U. pinnatifida* is native to South Korea, parts of Japan, and China’s Zhoushan archipelago (Hay and Villouta 1993, Morelissen et al. 2013). Its natural habitats include the lower intertidal and subtidal zones of rocky shores (Hay and Villouta 1993; Morelissen et al. 2013), while it can also be found growing on virtually any natural and artificial hard substratum (Floc'h et al. 1991; Wotton et al. 2004). In its native range, *U. pinnatifida* is a winter annual species, as the sporophyte appears in autumn, grows over the winter months, and matures in spring, releasing spores before decaying and being absent in summer during which only gametophytes persevere (e.g., Morita et al. 2003). The colder temperatures in some non-native habitats of *U. pinnatifida* allow for a second or even third annual generation to appear (James et al. 2015 and references therein). As an invasive species, *U. pinnatifida* has been demonstrated to have several advantageous traits. Gametophytes of *U. pinnatifida* undergo gametogenesis faster in the presence of other kelp species' gametophytes, and sporophylls tolerate significant desiccation, e.g., during land transport of aquaculture equipment (Bollen et al. 2017). Its ability to become fertile within short periods and its wide tolerance to various stressors compared to native species provide additional means to outcompete other kelps (e.g., Bollen et al. 2016). Since its accidental introduction to Europe, *U. pinnatifida* has spread along the European coastline, developing populations progressively further north. Further invasions worldwide include almost every continent (see Publication I).

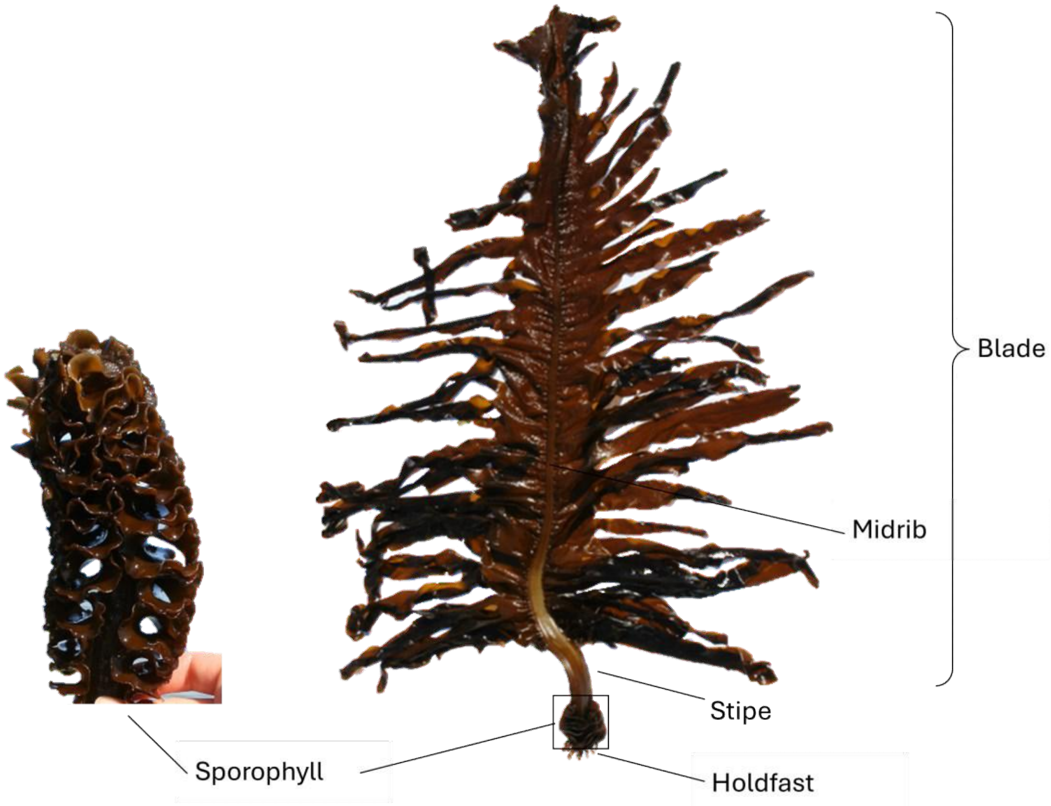


Figure 1.5: Morphology of the kelp habitus (order Laminariales, Phaeophyceae) on the example of the *Undaria pinnatifida* (Harvey) Suringar. Mature sporophyte of a Chinese farmed cultivar on the right and a close-up of a ripe sporophyll (Sylt, Germany) on the left. Photos and graphic by J. Schiller.

1.3 Acclimation, phenotypic plasticity, and adaptation in kelps

Species responses to environmental pressures can be described as four basic categories: (1) tolerance (i.e., persistence without acclimatization or adaptation), (2) persistence with acclimatization or adaptation, (3) migration (i.e., remaining within the climatic niche via range shift), and (4) extinction (Harley et al. 2012).

Kelps, being sessile organisms, cannot easily migrate away from unfavorable or changing environmental conditions as motile organisms might be able to do. They are sensitive to changes in local temperature, light, nutrient regime, and other factors (Yarish et al. 1990; Steneck et al. 2002; Hurd et al. 2014), as well as oceanographic climate (Dayton et al. 1992) and as such, need to be able to adjust. Depending on the time scale, changes occur in the genotype, the phenotype, or both. The genotype, an organism's whole genetic sequence, is relatively stable over its lifetime, while the phenotype, its observable expression, is dynamic and an interaction between the genotype and the environment (Holderegger et al. 2006; Wojczynski and Tiwari 2008). Among the mechanisms available to cope with changing circumstances, acclimation is fast, occurring within a lifetime. The reversible short-term physiological adjustment allows individual-level changes to maintain functionality while conditions change. Acclimation responses are limited to a manageable range defined by the phenotypic plasticity of a genotype (Morgan-Kiss et al. 2006). Genetic change accumulating over many generations in response to an environmental constant is long-term and called adaptation (Morgan-Kiss et al. 2006). Phenotypic plasticity is the range of different phenotypes a single genotype can express as a function of its environment (Nicotra et al. 2010). It is considered an epigenetic phenomenon (Schlichting 1986). In seaweeds, phenotypic plasticity regarding morphology and physiology in response to temperature (Reusch 2014; King et al. 2018), light (Monro and Poore 2005), waves, and currents (Fowler-Walker et al. 2006) is well documented, while little is known of their potential for evolutionary responses (Mabin et al. 2019). Adaptation in the traditional sense (i.e., genomic modifications) can occur within 20 generations or less (Prentis et al. 2008). However, in recent years, the newly developing field of epigenetics has broadened that definition to include genome-associated modifications independent of changes to the DNA sequence. These epigenetic adaptations can occur significantly faster, e.g., within single generations (Anastasiadi et al. 2021 and references therein).

U. pinnatifida in Europe could have reached the stage where adaptation is expected (see Figure 1.6). In our current era, the Anthropocene, human influence on climate and earth rivals or exceeds natural processes (Karl and Trenberth 2003), which makes successful adjustment of organisms more complex and may provide an advantage to those with higher capacity for change or faster responses (= more opportunistic species).

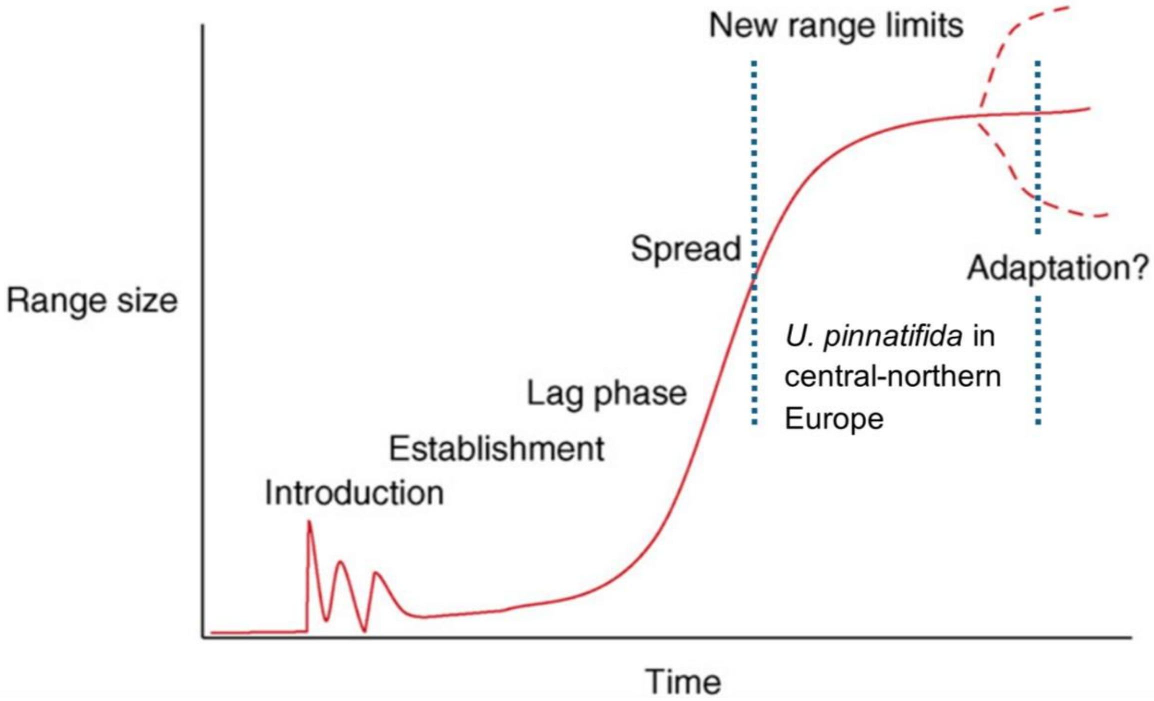


Figure 1.6: Adaptive evolution during the stages of invasion with regard to range size (adapted from Prentis et al. 2008). Considering the significant range expansion of *U. pinnatifida* in Europe, the region marked by blue dashed lines likely represents the state of the invasion, indicating that adaptations could occur.

1.5 Aim of the Study

The spread of *U. pinnatifida* in Europe from the Mediterranean over French Brittany to The Netherlands has been continuous over the last half century. All previous introduction sites were within the optimum temperature range for the species and within the temperature limits of its native distribution range. With increasing proximity to Scandinavia, invasive populations of *U. pinnatifida* are, for the first time, approaching waters with lower minimum and mean temperatures.

This study will conduct an extensive survey of likely introduction spots along the Dutch-German coastline to identify possible novel populations. Genetic characterization is crucial to establish the origin and connectivity between invasive *U. pinnatifida* populations in the vicinity of novel ones. Given that the populations under investigation in this study are distinct, an experimental approach will be used to identify potential temperature acclimations or adaptations. Research has shown that Climate Change can significantly affect the potential distribution ranges of invasive seaweeds, particularly by opening up higher latitudes (Serebryakova et al. 2017). Therefore, this study will explore the likelyhood of the northward migration of *U. pinnatifida* in Europe.

Most studies on *U. pinnatifida* have focused on its performance under elevated temperatures. Therefore, it is essential to understand its responses to conditions at its northern European distribution limit. This study offers valuable insight into the ecophysiology of an ongoing marine bioinvasion at the edge of the organism's distribution limit. In light of Climate Change and ocean warming, it provides the basis to predict a potential spread further northward in Europe and offers important data for informed species distribution modeling.

1.6 Research questions and hypotheses

Hypothesis I

***Undaria pinnatifida* will establish self-sustaining populations further North and Eastward along the European coast, predominantly in sites with a strong association with its main proposed invasion vectors in Europe (i.e., recreational boating).**

Research question 1a:

Can sporophytes of *U. pinnatifida* be found along the coast in the Dutch-German border region?

Based on the spread of *U. pinnatifida* up until the start of the study (2016), new populations may appear in the east of The Netherlands or the German Western Wadden Sea. Monitoring excursions to sites where the species is likely to appear (marinas and jetties) provide the highest chance of discovering them. Should novel populations of *U. pinnatifida* be located during monitoring, additional focus will be placed on potential stepping stone locations between the last published site and the newly established population.

Research question 1b:

Can newly established *U. pinnatifida* populations be self-sustaining, or do they rely on continuous re-supply from larger, established populations?
Species at their distributional edge may exist in the intermediate zone where they can grow vegetatively in their more robust state(s) (i.e., gametophytes or mature sporophytes in kelps), but face conditions unfavorable for reproduction. When *U. pinnatifida* sporophytes are discovered, the presence of sporophylls will be recorded and a representative number selected to undergo spore extraction. Spore release and, when successful, spore germination rate will be quantified. Germinated spores will further be used to establish a gametophyte culture and undergo gametogenesis experiments.

Hypothesis II

The smaller sporophytes found growing attached in tidal pools off Sylt, Germany, and the larger ones found floating belong to the same population - differences are due to phenotypic plasticity.

Research question 2:

Are the larger, floating (i.e. detached) sporophytes found off the coast of Sylt the source of the tidal pool population?
Kelps are known to exhibit highly plastic phenotypes in response to different environmental conditions. Other species, such as *Saccharina latissima* are known to produce smoother, elongated thalli under higher current speeds and more undulated, ribbed ones in calm waters. Previous trials with cultivating *U. pinnatifida* have shown capacity for phenotypic plasticity and as such, despite their phenotypic differences, the two populations might be genetically identical or similar. Microsatellite analysis and *in situ* observations will be used to to identify the relationship of the two populations.

Hypothesis III

The source of the newly established *U. pinnatifida* off Sylt, Germany are other, high-proximity European populations.

Research question 3:

What is the most likely source population of the newly established one off Sylt, Germany?
Given the relatively isolated location of Sylt in regard to the other populations in Europe, several of them are plausible sources. At the same time, a secondary introduction via long distance vectors is also possible. Genetic comparison of several continental European populations, as well as one from the British Isles, and native ones will enable identifying the source.

Hypothesis IV

***U. pinnatifida,* found on Sylt, Germany, has adapted to the colder sea surface temperatures and exhibits higher cold tolerance than the native, center range population from Qingdao, China, which has a lower cold tolerance but wider overall temperature tolerance.**

Research question 4a:

Does the German isolate show better performance at colder temperatures than the Chinese isolate?

One of the key indicators for stress tolerance is growth. Temperatures below the optimum are known to stagnate growth in seaweeds, and given other parameters such as light and nutrients are kept stable, to trigger other specific stress responses such as photoinhibition, change of the photosynthetic apparatus, or in the case of increased mitigation capacity, higher levels of photoprotective pigments and antioxidants. Growth is assessed via size and biomass quantification, while biochemical measurements provide insights into other internal processes.

Research question 4b:

Does the Chinese isolate have a wider temperature tolerance range than the German isolate?

Native populations are known to have higher genetic diversity than those newly established in novel habitats which often experience founder effects. A possible result of the higher diversity is a higher capacity for acclimation or adaptation via phenotypic plasticity. Should this be the case in the Chinese isolate, then sporophytes reared from it should be able to tolerate a wider range of temperatures than the German isolate.

Research question 4c:

Can gametophytes of the German isolate reproduce successfully at colder temperatures than the Chinese isolate?

Next to growth, reproduction is an essential indicator for successful adaptation to environmental conditions. In many cases, tolerance alone is not enough to enable reproduction and which makes it the true threshold for assessing whether a species can broaden its range into previously unfavorable conditions. Reproduction is assessed via formation of oogonia and antheridia and finally, viable sporophytes.

1.7 Thesis outline

The negative impact of invasive species has been recognized as one of the key drivers of biodiversity loss worldwide. Invasive species such as *U. pinnatifida* have encountered limitations at the leading edge (northward) of the habitat they invaded, e.g., in the form of suboptimal temperatures or dense, intact native kelp forests which prohibit the establishment of invader communities. However, Climate Change with all its associated impacts will cause sea surface temperatures to rise, and is generally predicted to increase the susceptibility of native coastal ecosystems. Other impacts of Climate Change are likely to improve conditions for species with invasive traits such as *U. pinnatifida,* and aid facilitate its establishment further north.
Following the framework proposed by Blackburn et al. (2011, see chapter 1.1.1), the invasion of *U. pinnatifida* in Europe has currently halted between central Europe and Scandinavia in the establishment or introduction phase (depending on location). Prior to conducting this study, the populations closest to this region were located in the Netherlands, where they are fully established, spreading and considered ‘fully invasive’ by Blackburn’s framework.

Publication I in chapter 2 of this thesis focuses on **hypothesis I** and **research questions (RQs) 1a and 1b**. It describes the results of an extensive surveying approach along the Dutch-German border region, focusing on sites *U. pinnatifida* was suspected to occur first (marinas, harbors) between summer 2016 and winter 2017, which answers RQ 1a. Upon discovery of the population at Sylt, Germany its extent as well as associated species were recorded and mapped. Temperature loggers were deployed in the tidal pools the attached population was found in, however, this data could not be retrieved due to technical issues. RQ 1b was addressed by quantifying the prevalence of reproductive tissue (sporophylls) on the attached growing sporophytes. Release of spores and their viability, occurence of gametogenesis, and development of new sporophytes were assessed in the established isolate.

Publication II in chapter 3 addresses **hypothesis II** with **RQ 2**. Tissue samples of the attached and the floating populations of *U. pinnatifida* on Sylt, recorded in publication I, were collected during the sampling decribed in chapter I, as well as during additional visits to the site. Other European populations, as well as three native Chinese ones, were sampled as a reference for assessing the similarity between the two target groups. 10 newly developed microsatellite markers were used for the amplification and the number of alleles. Observed and expected heterozygosity, inbreeding coefficient and Nei’s standard genetic distance were analyzed.

As the microsatellite markers applied in publication II did not provide enough insight into the connectivity of the European populations to identify a likely founder of the German one, **hypothesis III** was further explored via **RQ 3** in **publication III**, chapter 4. The samples used for this study originated from the same populations as those selected in publication II. Mitochondrial DNA sequences, including the partial coding region of *cox3* and intergenic noncoding loci *tatC-tLeu*, *atp8-trnS* and *trnW-trnI* were obtained, and the populations were analyzed for their haplotype composition.

Publication IV focuses on hypothesis IV with **RQs 4a**, **b**, and **c**, presented in chapter 5. It aims to investigate the phenotypical, physiological, and biochemical side of the genetic questions addressed in the previous two chapters. Isolates of the German and a native Chinese population of *U. pinnatifida* were stabilized under identical conditions before gametogenesis was induced. The obtained sporophytes were exposed to a range of low temperatures and assessed for growth rate, biomass, content of nitrogen, carbon, antioxidants, mannitol, and pigment composition. Mannitol data was later excluded from the analysis due to a lack of sample biomass. Gametogenesis induction trials were also carried out at those same temperatures.

Publication V in the appendix is a review written to address and summarize some of the factors influencing species' invasions in the form of distribution vectors, as well as outline aims and challenges of data-based prediction approaches (species distribution modeling).

1.8 List of publications and declaration of contributions

Publication I

Title: **Heading northward to Scandinavia: *Undaria pinnatifida* in the northern Wadden Sea**

Authors: Jessica Schiller, Dagmar Lackschewitz, Christian Buschbaum, Karsten Reise, Shaojun Pang, Kai Bischof

Journal: Botanica Marina, doi.org/10.1515/bot-2017-0128

Contribution of the candidate in % of the total workload:
Experimental concept and design: 70%
Experimental work and acquisition of the data: 80%
Data analysis and interpretation: 80%
Preparation of figures and tables: 100%
Drafting of the manuscript: 75%

Publication II

Title: **Genetic analysis of a recently established *Undaria pinnatifida* (Laminariales: Alariaceae) population in the northern Wadden Sea reveals close proximity between drifting thalli and the attached population**

Authors: Tifeng Shan, Shaojun Pang, Xuemei Wang, Jing Li, Li Su, Jessica Schiller, Dagmar Lackschewitz, Jason M. Hall-Spencer, Kai Bischof

Journal: European Journal of Phycology, doi.org/10.1080/09670262.2018.1532116

Contribution of the candidate in % of the total workload:
Experimental concept and design: 15%
Experimental work and acquisition of the data: 30%
Data analysis and interpretation: 20%
Preparation of figures and tables: 20%
Drafting of the manuscript: 10%

Publication III

Title: **Comparison of mitochondrial DNA sequences points to the European populations as the direct origin of *Undaria pinnatifida* that has spread to the northern Wadden Sea**

Authors: Tifeng Shan, Shaojun Pang, Yuqian Li, Jessica Schiller, Dagmar Lackschewitz, Kai Bischof

Journal: Aquatic Botany, doi.org/10.1016/j.aquabot.2023.103671

Contribution of the candidate in % of the total workload:

Experimental concept and design: 10%
Experimental work and acquisition of the data
(field sample collection and processing) : 30%
Data analysis and interpretation: 10%
Preparation of figures and tables: 0%
Drafting of the manuscript: 10%

Publication IV

Title: **Cold tolerance in the invasive brown alga *Undaria pinnatifida*: A comparative study of physiological responses in a northern European population and its native counterpart**

Authors: Jessica Schiller, Tifeng Shan, Nora Diehl, Shaojun Pang, Ulf Karsten, Kai Bischof

Journal: in preparation

Contribution of the candidate in % of the total workload:

Experimental concept and design: 90%
Experimental work and acquisition of the data: 95%
Data analysis and interpretation: 95%
Preparation of figures and tables: 100%
Drafting of the manuscript: 80%

Publication V (Appendix)

Title:	**Theories, Vectors, and Computer Models: Marine Invasion Science in the Anthropocene**
Authors:	Philipp Laeseke, Jessica Schiller, Jonas Letschert, Sara Doolittle Llanos (All authors contributed equally)
Book:	YOUMARES 9 – The Oceans: Our Research, Our Future, doi.org/10.1007/978-3-030-20389-4_10

Contribution of the candidate in % of the total workload:

Concept and design: 30%
Literature screening: 25%
Preparation of figures and tables: 25%
Drafting of the manuscript: 25%

Chapter 2 Publication I:

Heading northward to Scandinavia: *Undaria pinnatifida* in the northern Wadden Sea

Jessica Schiller, Dagmar Lackschewitz, Christian Buschbaum, Karsten Reise,
Shaojun Pang, Kai Bischof

published in
Botanica Marina
July 2018

doi.org/10.1515/bot-2017-0128

DE GRUYTER Botanica Marina 2018; 61(4): 365–371

Jessica Schiller*, Dagmar Lackschewitz, Christian Buschbaum, Karsten Reise, Shaojun Pang and Kai Bischof

Heading northward to Scandinavia: *Undaria pinnatifida* in the northern Wadden Sea

https://doi.org/10.1515/bot-2017-0128
Received 21 December, 2017; accepted 19 June, 2018; online first 11 July, 2018

Abstract: The kelp *Undaria pinnatifida*, native to East Asian shores, was unintentionally introduced with Pacific oysters into the Mediterranean in 1971. Intentional introduction from there to the French Atlantic coast 12 years later led to a gradual spread to the British Isles and the North Sea. Here, we report on the northernmost established population in continental Europe, and suggest a further spread into Scandinavian waters to be almost inevitable. In 2016, several thalli were found washed ashore at the eastern side of the island of Sylt in the northern Wadden Sea (German Bight, Eastern North Sea). Most specimens bore fertile sporophylls and thallus lengths of >1 m were common. In June 2017, 91 sporophytes were found attached to a mixed bed of Pacific oysters and native blue mussels, located just below low tide level in a moderately sheltered position. Mean thallus length was 0.2 m and the longest 0.7 m. Most had distinctive sporophylls and released spores in the laboratory. From sporophylls collected in the previous year, we successfully reared a new generation, demonstrating the kelp's potential for further spread by natural means or human vectors.

Keywords: introduced non-native species; neobiota; Pacific oysters; range expansion; *Undaria pinnatifida*.

Introduction

The brown macroalga *Undaria pinnatifida* is native to South Korea, parts of Japan and China's Zhoushan archipelago (Hay and Villouta 1993, Morelissen et al. 2013). It originally inhabits the lower intertidal and subtidal zones of rocky shores (Hay and Villouta 1993, Morelissen et al. 2013), but can also be found growing on virtually any natural and artificial hard substratum (Floc'h et al. 1991, Wotton et al. 2004). This kelp species has been a well-known food source in Asia and has recently gained increasing attention as an introduced species in many coastal areas worldwide (Hay and Luckens 1987, Nisizawa et al. 1987, Yamanaka and Akiyama 1993, Lowe et al. 2000). The first accidental introduction of *U. pinnatifida* to Europe occurred in 1971 in the Thau lagoon (French Mediterranean). Its introduction is thought to be associated with movement of Pacific oysters [*Magallana* (*Crassostrea*) *gigas*] brought from Japan for farming (Floc'h et al. 1991). In 1983, specimens were brought for cultivation experiments from the Mediterranean to Brittany (France), the first free living specimens were later detected in 1987 (Figure 1A; Minchin and Nunn 2014). These were most likely facilitated by anthropogenic vectors, and *U. pinnatifida* continued to spread along European coasts, first observed in southern England in 1994 and on the shores of Belgium and the Netherlands in 1999. Until now, the most northern occurrence of *U. pinnatifida* was in Belfast Lough (Northern Ireland) in 2015 and on the Dutch island of Terschelling in continental Europe in 2009 (Figure 1B, Gittenberger et al. 2015, Minchin et al. 2017).

In addition to dispersal by aquaculture activities and shipping, overland transport of *Undaria pinnatifida* with fishing gear has been reported (Bollen et al. 2017). Its highly plastic physiology and morphology allow *U. pinnatifida* to adapt well to many new environments, which may explain its success as a worldwide invader (Dean and Hurd 2007). Previous studies suggested that natural dispersal mechanisms, namely spore production and release, as well as severed floating thalli or sporophylls, result in only a limited range expansion of around 100 m per generation (Forrest et al. 2000). Still, the number of offspring potentially produced by individual sporophytes is high. Millions of zoospores are released from each sporophyll and, therefore, populations may grow very rapidly (Shan et al. 2016). The impact of *U. pinnatifida* on native communities may differ depending on the coastal system

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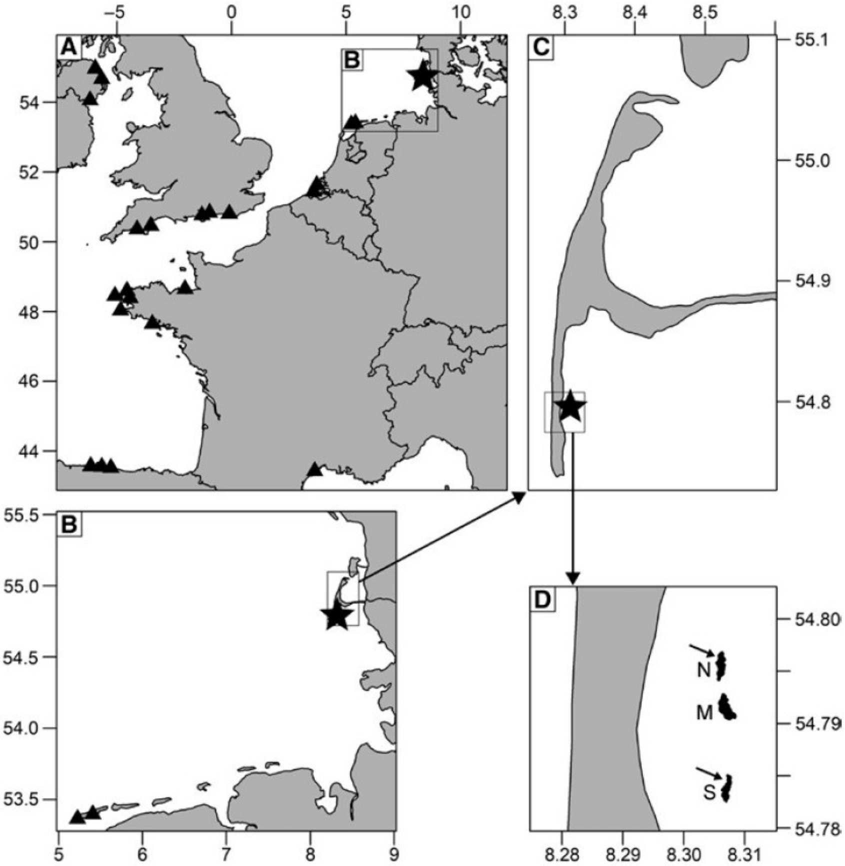

Figure 1: Distribution of *Undaria pinnatifida* populations previously reported (triangles) and the newly established one on Sylt (stars). (A) Examples of populations in Europe (Minchin and Nunn 2014, Gittenberger et al. 2015). (B) Easternmost documented European population in 2014 (Gittenberger et al. 2015) and 2017. (C) Island of Sylt with box marking the major *U. pinnatifida* wash-up zone and location of the attached *U. pinnatifida* on the sampled oyster reef (54°47′44.4″N 8°18′24.7″E). (D) Oyster reefs (north, middle, south) off the eastern shore of Sylt with arrows marking the growth sites. Numbers on axes are longitude and latitude in degrees N and E.

investigated. Native species richness may be reduced but, in some circumstances, the alga could have a positive effect where it provides an extra substratum for native species to grow (e.g. Casas et al. 2004; Irigoyen et al. 2011).

Here we report the first detection of *Undaria pinnatifida* in the northern Wadden Sea (southeastern North Sea) and provide a baseline for further research on the population development of this non-native kelp and its potential effects on native communities in the Wadden Sea ecosystem.

Materials and methods

Individuals of *Undaria pinnatifida* (Harvey) Suringar were first found washed ashore on intertidal sandflats on the eastern side of the island of Sylt in the northern Wadden Sea in August 2016 during a routine non-native species monitoring survey (for detailed information on the monitoring program see Buschbaum et al. 2012). On several occasions between August 2016 and June 2017, a total of more than 100 sporophytes were found washed ashore (hereafter “floating sporophytes”) between Rantum and Hörnum in southern Sylt (Figure 1C).

No specimens of *Undaria pinnatifida* were observed during dredge sampling of mussel reefs in the area east of the wash-up zone in March 2017. In June 2017, three oyster reefs, located in the shallow subtidal near the wash-up zone were investigated during low tide (Figure 1D). Contours and profiles of the reefs were recorded using a GPS-based application (MapMyWalk© 2017, Under Armour® Inc., Baltimore, MD, USA) and all *U. pinnatifida* sporophytes found attached to the oyster reefs were collected.

The substratum as well as major components of the associated flora and fauna were identified. *Undaria pinnatifida* sporophytes were transported to the laboratory (Alfred Wegener Institute, Helmholtz Centre for Polar and Marine Research, Wadden Sea Station Sylt, Germany), where all individuals were photographed, the presence of sporophylls was recorded and thallus lengths and midrib widths were measured. For damaged thalli, the full length without damage was estimated based on the midrib width and length. Previous studies (Castric-Fey et al. 1999) and our own experience showed that the midrib width can serve as a proxy for full sporophyte size (Schiller et al. unpublished). However, as morphology and size ratios of *U. pinnatifida* vary with growth site and conditions (Castric-Fey et al. 1999, Skriptsova et al. 2004), the midrib width in this study only serves as an independent measurement for sporophyte size distribution.

Floating sporophytes were measured in the field or digitally from photos using Image J (Schneider et al. 2012).

To test for zoospore release *in vitro*, 27 of the attached mature sporophytes were haphazardly selected and a method adapted from Shan and Pang (2009) was used. Sporophylls were cut from the plants, cleaned of epiphytes and dried in closed petri dishes for approximately 24 h at 12°C without light. Pieces of each sporophyll were immersed in petri dishes filled with fresh seawater at room temperature and natural light. They were observed under the microscope after 24 and 48 h.

For germination experiments, a washed up specimen collected in October 2016 was used to release viable spores in the laboratory (Marine Botany, BreMarE, University of Bremen, Germany). Pieces of the sporophyll were cleaned thoroughly as described in Redmond et al. (2014) and desiccated in petri dishes for 24 h at 12°C, before being immersed in Provasoli enriched sterilized natural seawater (PES). After 24–48 h at 20–30 μmol photons $m^{-2} s^{-1}$, 12:12 h L:D and 18°C, spores germinated into gametophytes. In several subsequent experiments, sporophytes were successfully obtained from this culture by adapting the method of Shan et al. (2013). Gametophyte filaments were broken up into fragments and irradiance reduced to 5 μmol photons $m^{-2} s^{-1}$ for 24 h, then increased to 50 μmol photons $m^{-2} s^{-1}$ with medium changes every 3 days.

Statistical analysis was performed in R using a generalized linear model with Gaussian dispersion parameters and $p < 0.05$ (R Core Team 2015).

Results and discussion

Sea surface temperature in late June ranged between 16 and 17°C (own measurements). However, intense irradiance and air temperatures can increase the temperature range significantly. In total, we found 91 attached specimens of *Undaria pinnatifida* (Figure 2), which were detected only on the western fringe of the northern oyster reefs, except for a single strongly damaged individual on the southern reef.

The community on the reefs was dominated by the filamentous brown alga *Ectocarpus* sp., stretching as a dense floating layer across the tide pools, with *U. pinnatifida* and other species (Table 1) growing underneath and between the *Ectocarpus* filaments. Thalli of *U. pinnatifida* grew in clusters of one to seven individuals as epibionts (attached to living surfaces) of other species (basibionts), with 34 clusters in total, spread over a system of connected tide pools no deeper than 30 cm during low tide.

Figure 2: *Undaria pinnatifida* sporophytes collected at Sylt, Germany.
(Left) Specimens washed up in the intertidal between Rantum and Hörnum during low tide. (Middle) Large fertile sporophyte found floating in tide pools of the sampled oyster reef. (Right) Individuals collected growing on oysters in the tide pools.

Table 1: *Undaria pinnatifida* associated flora and fauna identified at the northern oyster reef (54°47′44.4″N 8°18′24.7″E), Sylt, Germany.

Plantae		**Animalia**	
Chlorophyta	*Ulva* sp. (narrow)	Mollusca	*Magallana gigas*
	Ulva sp. (wide)		*Mytilus edulis*
Rhodophyta	*Gracilaria vermiculophylla*		*Littorina littorea*
	Chondrus crispus		*Crepidula fornicata*
	Dasya baillouviana	Crustacea	*Carcinus maenas*
	Ceramium rubrum		*Semibalanus balanoides*
Ochrophyta	*Ectocarpus* sp.	Polychaeta	*Lanice conchilega*
	Sargassum muticum		*Arenicola marina*
	Fucus vesiculosus	Bryozoa	*Electra pilosa*
	Chorda filum	Echinodermata	*Asterias rubens*
	Dictyota dichotoma	Cnidaria	*Metridium senile*

The majority of the thalli (91%) were attached to Pacific oysters *Magallana [Crassostrea] gigas*, while 7.7% were growing on *Mytilus edulis* and a single thallus grew on a sponge.

Nearly all of the sporophytes were damaged at the tip of the blade, likely due to wave exposure and the onset of senescence due to high temperature or age.

The mean actual length ± standard deviation (SD) of the 91 attached sporophytes was 21.5 ± 8.5 cm, while the mean full length ± SD was estimated to be 30.6 ± 10.7 cm (Figure 3). The longest thallus was estimated to be about 70 cm before damage.

Additionally, 41 floating *Undaria pinnatifida* sporophytes were photographed in January 2017 (Figure 2) of which 13 could be reliably measured from the photos and 36 and 16 were measured in late March and June 2017, respectively. Of these, the mean actual length was 68.5 ± 32.2 cm and the mean estimated length 104.7 ± 32.3 cm (Figure 3) with the longest sporophyte estimated to be 149 cm before damage. The significantly longer thalli and wider midribs compared to those found in attached thalli suggest another source habitat. Within the oyster reefs current speed is reduced with stagnant water in low tide pools. Peteiro and Freire (2011) found that *U. pinnatifida* growing at a site moderately exposed to water currents had significantly larger sporophytes, compared to a sheltered site. The average total length reported for a moderately exposed site in Northwest Spain was 122 cm, compared to 86 cm at a sheltered site (Peteiro and Freire 2011). This suggests that our floating sporophytes originate from tidal channels with swift currents rather than from another oyster reef with low tide pools.

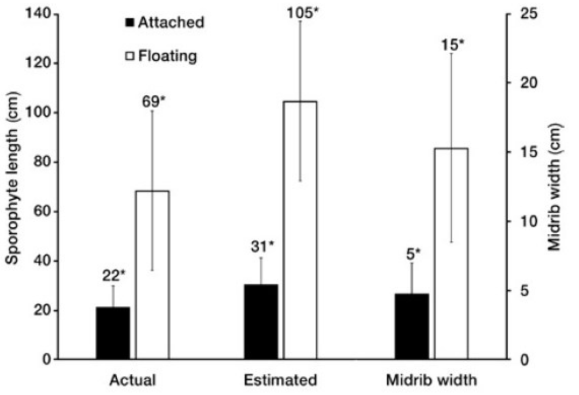

Figure 3: *Undaria pinnatifida*: actual and estimated length (left) and midrib width (right) of 91 attached (black) and 73 floating (white) sporophytes.
Values above bars are means; bars indicate standard deviations. Significant differences ($p \leq 0.05$) between the attached and floating specimens of each parameter are indicated by asterisks.

Floating sporophytes were collected over a period of more than 6 months, and they always had sporophylls, suggesting that different states of the population and several generations were documented. This agrees with the observation that *Undaria pinnatifida* sporophytes occurred year round with several recruitment pulses where maximum sea surface temperatures are ≤19.4°C (James et al. 2015 and references therein).

We identified 69 (76%) of the attached individuals that had formed sporophylls. Out of 27 tested for spore release, 85% had released spores after 4 h. After 24 h, 89% had released spores of which 82% had formed germination tubes. However, it has been noted by Forrest et al. (2000) that germination into gametophytes could occur up to 14 days after the release of spores. The high spore release and germination rates, together with the successful growth of sporophytes from this material, provide strong evidence that reproduction and potential range expansion are possible in the *Undaria pinnatifida* population from Sylt.

From field observations, it was concluded that *Undaria pinnatifida* arrived at the oyster reefs by natural dispersal through floating mature thalli. The fact that attached individuals were found only on the landward side of the reef and in tide pools, suggests that floating individuals were trapped in these pools during low tide. Spores released there are likely to remain in these pools, allowing them to settle in the observed patches. Pang and Shan (2008) argued that water velocity was the most important factor for spore attachment. *Undaria pinnatifida* spores were able to attach best under low water velocities, but attached permanently to the substratum if allowed to settle for an hour before exposure to higher velocities (Pang and Shan 2008). The tide pools sampled near Sylt provide the necessary time for settlement during low tide.

Undaria pinnatifida was not found in any harbour on Sylt, nor on the neighbouring island of Föhr or in any other oyster reef around Sylt that was surveyed. Additionally, thalli were only washed up in a rather narrow beach area on Sylt. Therefore, it is likely that only one main population exists in subtidal waters and that this is the source of the large floating sporophytes. Further surveys in a wider area will be conducted to locate this population.

Compared to other invasions in Europe and worldwide, the presence exclusively on natural substratum is rather unusual, as *Undaria pinnatifida* has shown a preference for artificial substrates (Minchin and Nunn 2014). In cases where it was found in natural habitats, it was usually after a population had been observed first on man-made structures (i.e. James and Shears 2016). Prior to the findings on Sylt, several other German marinas closer to the known Dutch populations (Emden, Bensersiel, Wilhelmshaven, Cuxhaven, Brunsbüttel, Büsum, Langeoog, Borkum, Helgoland) had been screened but *U. pinnatifida* was found in none of them (Schiller/Lackschewitz, own observations). During assessments of non-native species in the Dutch Wadden Sea by Gittenberger et al. (2015), no populations were found east of Terschelling, even though areas of hard substrate were sampled all the way to the German border.

Forrest et al. (2000) illustrated that the natural spread of *Undaria pinnatifida* is generally restricted to approximately 100 m per year even when considering floating sporophylls. The direct distance between Terschelling and Sylt is 240 km and along the coast it is 300 km (Figure 1B), so that it is unlikely that this present record of range expansion is due to natural dispersal. Coastal shipping has been shown to play a significant role in the dispersal of *U. pinnatifida* (Hay 1990), and during summer many recreational boats visit Sylt. Extensive mussel cultures on ropes and the sea floor are located in the proximity of the reported *U. pinnatifida* localities. These relied on seed mussel translocations from other North Sea regions in the past and farming vessels still frequently transfer between Sylt and other farms.

Conclusion

Our study documents the successful establishment of a *Undaria pinnatifida* population much further north on the European continental coast than previously known. Laboratory experiments proved that the majority of the sporophytes had reached maturity and released spores that were capable of germinating and forming a new generation of sporophytes *in vitro*. Thus, we conclude that a self-sustaining population now exists in the Northern Wadden Sea and may disperse northwards from there. By continuous monitoring, ecophysiological characterization, interaction studies, genetic analyses and species distribution modelling, we will aim to understand and predict its further spread.

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Bionotes

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Chapter 3 Publication II:

Genetic analysis of a recently established *Undaria pinnatifida* (Laminariales: Alariaceae) population in the northern Wadden Sea reveals close proximity between drifting thalli and the attached population

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Genetic analysis of a recently established *Undaria pinnatifida* (Laminariales: Alariaceae) population in the northern Wadden Sea reveals close proximity between drifting thalli and the attached population

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ABSTRACT
Undaria pinnatifida, a kelp species native to East Asia, has become cosmopolitan and drawn increasing attention due to its worldwide spread in recent decades. Floating fragments of this alga were found washed ashore on Sylt in 2016, the first record of this species in Germany. Thalli attached to local oyster reefs were detected in 2017. The genetic relationship between the floating and attached thalli on Sylt, as well as their relevance to the populations from northern Europe and native regions, was hitherto unknown. Here, 10 microsatellite markers were used to assess relationships between the recently established population on Sylt and five other northern European populations in France (Brittany, West English Channel), the Netherlands and England (Plymouth, West English Channel) plus three natural populations in China. Almost no genetic differentiation was detected between the floating and attached populations on Sylt, but they were genetically distinct from all the other studied northern European populations. The very low genetic diversity revealed in the new founder populations of Sylt suggests that they came from genetically similar parents. The marked reduction in both the number of alleles and heterozygosity in the northern European populations, as compared with the Chinese ones, is typical of founder effects in recently populated regions. Prominent genetic divergence was found between most of the northern European populations except those within Brittany and Sylt. Further studies will focus on identifying the putative source populations that might be found on shellfish farms, in local marinas or the benthic habitats around Sylt Island.

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KEYWORDS Genetic diversity; invasive seaweed; kelp; microsatellite; North Sea; Wakame

Introduction

The brown alga *Undaria pinnatifida* (Harvey) Suringar, an edible kelp indigenous to the North-west Pacific, has become cosmopolitan and drawn increasing attention because of its worldwide spread in recent decades (Epstein & Smale, 2017; South *et al.*, 2017). The first record of its spread to Europe was an accidental introduction to Thau lagoon (French Mediterranean) in 1971, probably accompanying the transport of Pacific oysters (*Magallana* (*Crassostrea*) *gigas*) from Japan for mariculture (Floc'h *et al.*, 1991). In 1983, this alga was intentionally introduced to Brittany on the French Atlantic coast for commercial cultivation and soon spread into the wild (Grulois *et al.*, 2011 and references therein). Its later spread in Spain is also mainly attributed to aquaculture activity (Peteiro, 2008). Probably promoted by maritime traffic, it spread to Italy, the UK, Portugal, Belgium and the Netherlands (Heiser *et al.*, 2014; Minchin *et al.*, 2017; Epstein & Smale, 2017, fig. 2). The most northerly distribution of *U. pinnatifida* in Europe is in Scotland according to fig. 2 of Epstein & Smale (2017).

Along the German coastline, *U. pinnatifida* was first found washed ashore on intertidal sandflats on the east side of the island of Sylt in the northern Wadden Sea in August 2016, during a routine monitoring survey (Schiller *et al.*, 2018). At that time, more than 100 stranded sporophytes were found ashore over a stretch of less than 100 m. Since then, mature floating sporophytes have repeatedly been found on sandflats and in the oyster reefs between Rantum and Hörnum. Subsequently, a total of 91 attached *U. pinnatifida* individuals were found growing in tide pools on the eastern (coastward) side of the island in June 2017. The origin of the floating and attached populations was unknown (Schiller *et al.*, 2018). Population genetics tools can provide clues for identifying the source of non-native species, and have been used to trace the introduction of the Asian

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seaweed *Gracilaria vermiculophylla* through very extensive sampling from the introduced and native ranges (Krueger-Hadfield *et al.*, 2017). It was also found that the introduction of this seaweed was associated with a shift in reproduction due to floating thalli (Krueger-Hadfield *et al.*, 2016). Therefore, population genetics analysis is necessary in order to provide insights into the likelihood that the attached *U. pinnatifida* individuals originated from the drifting ones, by contrasting their genetic composition with those found in other populations.

Microsatellites have been the marker of choice for genetic structure analysis due to their advantageous characteristics such as co-dominance, high polymorphism and even genomic distribution (Liu & Cordes, 2004). There were 20 microsatellites available for *U. pinnatifida* and they were used to analyse the genetic polymorphism and structure on both intercontinental and regional scales, in which French populations were most often included (Daguin *et al.*, 2005; Grulois *et al.*, 2011; Guzinski *et al.*, 2018). However, genetic structure of *U. pinnatifida* populations from different European countries has never been investigated using microsatellite markers. In order to provide more alternatives for population genetic analysis, 30 trinucleotide microsatellites have been developed *de novo* by next-generation sequencing, which proved to be highly informative (Shan *et al.*, 2018).

In this study the genetic structure of seven populations of *U. pinnatifida* from northern Europe was investigated, using microsatellite markers to infer the genetic relationship between specimens on the island of Sylt and populations in other northern European countries. Three populations from China were included in order to compare the genetic diversity between the introduced and native regions.

Materials and methods

Sampling and DNA isolation

Drift and attached populations (DEU1 and DEU2) of *U. pinnatifida* were collected on the island of Sylt in the northern Wadden Sea in 2016 and 2017, respectively (Fig. 1, Supplementary table S1). Two sporophytes from DEU1 were deposited in the herbarium at the Alfred Wegener Institute, Sylt. Five sites from three countries near the newly detected populations on Sylt were sampled, including: two (NLD1 and NLD2) from marinas in Vlissingen and Terschelling, the Netherlands; two (FRA1 and FRA2) from the Marina of the Moulin Blanc and Castle Marina (West English Channel) in Brest, Brittany, France; and one (GBR) from Plymouth (West English Channel), UK. All marina samples were taken over the widest area possible, at least several metres of floating pontoon or across the whole marina if present there. The attached and floating sporophytes on Sylt were collected from ~90 m^2 of oyster reef and 1 km of sandflat, respectively. Populations were sampled in Dalian (DL), Qingdao (QD) and Gouqi Island (GQ) in China in 2016 and used for comparison with the specimens from Europe. Thirty individuals (except GQ, with 29 individuals) were randomly chosen from each population regardless of their size and developmental stage. One piece of thallus from each individual was cleaned with sterilized seawater, dried and preserved in silica gel for DNA extraction. Genomic DNA was extracted by using DNeasy Plant Mini Kit (Qiagen, Hilden, Germany) according to the instructions and eluted by two repeated steps with 60 µl AE buffer.

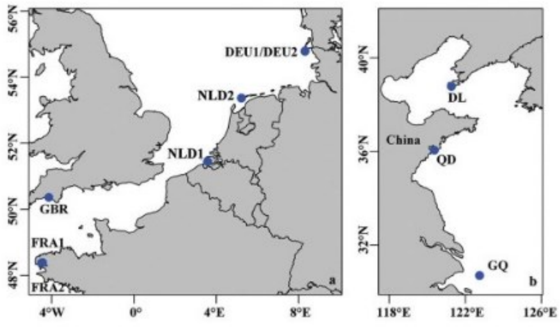

Fig. 1. Sampling map of *Undaria pinnatifida* in (a) Europe and (b) China.

Microsatellite genotyping

Microsatellite genotyping in the present study was started during the development process of the 30 new trinucleotide microsatellites and before extensive evaluation of the polymorphism at each locus conducted in Shan *et al.* (2018), so the markers used herein were selected randomly and are different from those in Shan *et al.* (2018). The 10 microsatellites employed for genetic analysis were UPN130, 161, 1143, 1528, 3177, 3197, 3205, 3530, 6327 and 9919. PCR was conducted in 20 µl volume containing AmpliTaq Gold 360 Master Mix (Applied Biosystems, USA), 0.5 µM fluorescent-labelled (forward) and unlabelled (reverse) primers and 5 ng of genomic DNA by using a T-gradient thermocycler (Biometra, Göttingen, Germany). The PCR procedure consisted of an initial denaturation at 95°C for 4 min, followed by 30 cycles of 94°C for 30 s, annealing at 55°C for 30 s, 72°C for 45 s, and a final extension at 72°C for 7 min. Microsatellite genotyping was carried out on an ABI 3730XL automated sequencer (Applied Biosystems, Carlsbad, California, USA) and allele sizes were determined with GeneMapper version 4.0.

Data analysis

Number of alleles (N_a), observed and expected heterozygosity (H_o and H_e), inbreeding coefficient F_{is}

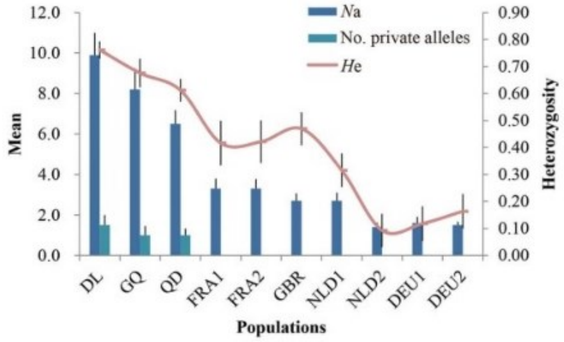

Fig. 2. The number of alleles (*Na*), private alleles and expected heterozygosity (*He*) of *Undaria pinnatifida* from northern Europe and China.

and Nei's standard genetic distance (Nei, 1972) were calculated using GenAlEx 6.5 (Peakall & Smouse, 2006, 2012). Population selfing rate was estimated by the index g_2 using the *inbreedR* package (Stoffel *et al.*, 2016) according to equation 9 in David *et al.* (2007). The probability for Hardy–Weinberg equilibrium (HWE) for each locus was tested by using ARLEQUIN version 3.11 (Excoffier *et al.*, 2005). Genetic distance between populations was used to construct a dendrogram with POPTREE software (Takezaki *et al.*, 2010), using the neighbour-joining (NJ) clustering approach. During bootstrapping, 1000 permutations were performed to evaluate the robustness of the clusters. Pairwise population genetic differentiation (F_{st} value) was assessed by using ARLEQUIN version 3.11 with 1000 permutations. To adjust for multiple comparisons, the false discovery rate (FDR) was controlled by using 'BH' method (Benjamini & Hochberg, 1995) in the *p.adjust* function in R. The adjusted P-value < 0.05 was considered to be significant.

Given that the evaluation of genetic distance and differentiation was conducted on *a priori* population level, a Bayesian model-based clustering analysis was conducted by using STRUCTURE 2.3.4 software to estimate the most likely number of genetic clusters (Pritchard *et al.*, 2000). This clustering approach was used to identify genetically distinct subpopulations based on allele frequencies. The admixture model was applied and the number of clusters (K value) was set from 2 to 10, with 10 independent runs for each fixed number K. Each run involved a burn-in length of 100 000 followed by 1 000 000 MCMC (Monte Carlo Markov Chain) repetitions. The most probable value of K was determined according to the method described in Evanno *et al.* (2005) by submitting all results files of $K = 2$ to 10 to STRUCTURE HARVESTER (Earl & VonHoldt, 2011). Among the 10 independent runs, the one with the highest Ln Pr ($X|K$) value (log probability) was chosen and visualized as bar plots by using the software DISTRUCT 1.1 (Rosenberg, 2004). Discriminant analysis of principal components (DAPC) which is implemented in *adegenet* package in R was also performed in order to analyse the genetic relationships across populations (Jombart, 2008; Jombart *et al.*, 2010).

Results

One locus (UPN161) was found to be monomorphic across all the studied northern European populations (Supplementary table S2). The number of monomorphic loci was 6, 5, 2, 7, 1, 1 and 2 in DEU1, DEU2, NLD1, NLD2, GBR, FRA1 and FRA2, respectively (Table 1 and Supplementary table S2). The average N_a, H_o and H_e were all minimum in NLD2, being 1.4, 0.110 and 0.093, respectively. Within northern European populations, the maximum N_a (3.3) was found in the two French populations, while the maximum H_e (0.469) was found in GBR. The average N_a, H_o and H_e of DEU1 and DEU2 were a little higher than those of NLD2 but lower than those of other populations. The average N_a, H_o and H_e in all three Chinese populations were much higher than those in the northern European ones (Fig. 2). Private alleles were detected at 7, 6 and 5 loci in DL, QD and GQ with an average number of 1.5, 1.0 and 1.0 at each locus, respectively. Among the 100 population-loci cases (10 populations × 10 loci), 7 cases were estimated to be significantly deviated from HWE ($P < 0.05$) after sequential Bonferroni's correction for multiple tests (Rice, 1989). Populations GBR, FRA1, FRA2, DL and QD were found to be significantly deviated from HWE ($P < 0.05$) across loci. F_{is} ranged from −0.021 in NLD2 to 0.286 in GBR (Table 1). The selfing rates, estimated from g_2, ranged from −0.171 in NLD2 to 0.146 in FRA2, with FRA1 and FRA2 showing significant departure from g_2=0 ($P < 0.05$).

With very few exceptions, all pairwise F_{st} values were high and the genetic differentiation was found to be significant (Table 2). The genetic distance between DEU1 and DEU2 was lowest (0.005) and no significant differentiation ($F_{st} = 0.016$, $P = 0.064$) was detected between them. The genetic distance (0.075) and F_{st} (0.076) between the two French populations were relatively low, although the genetic differentiation among them was estimated to be significant ($P = 0.000$). The genetic distance-based dendrogram grouped all populations into three major clusters (Supplementary fig. S1). Chinese populations and all northern European populations except NLD1 were grouped as two distinct major clusters. NLD1 was revealed to be different from the others. Although NLD2 was found to be most closely related to DEU1 and DEU2 according to the values of genetic distance, the F_{st} values between them were very high (0.519 and 0.470).

Table 1. Genetic diversity and selfing rates of the populations of *Undaria pinnatifida* from northern Europe and China.

Population	N_a	Polymorphic loci (%)	H_e	F_{is}	Selfing rate (g_2)	P (g_2=0)
DEU1	1.6	40	0.118	0.109	0.018	0.650
DEU2	1.5	50	0.164	0.043	0.143	0.064
NLD1	2.7	80	0.315	0.087	0.116	0.057
NLD2	1.4	30	0.093	−0.021	−0.171	0.988
GBR	2.7	90	0.469	0.286*	0.051	0.188
FRA1	3.3	90	0.416	0.176*	0.106	0.021
FRA2	3.3	80	0.422	0.213*	0.146	0.004
DL	9.9	100	0.761	0.087*	0.012	0.145
QD	6.5	100	0.612	0.120*	0.006	0.412
GQ	8.2	100	0.676	0.039	−0.011	0.829

N_a, number of alleles; H_e, expected heterozygosity; F_{is}, inbreeding coefficient. Selfing rate (g_2) indicates selfing rate estimated from g_2 in *inbreedR* package; P (g_2=0) the probability value for g_2=0; *significant departure from Hardy–Weinberg equilibrium ($P < 0.05$) across loci.

The most likely number of *K* was determined to be 8 by using STRUCTURE HARVESTER based on the Δ*K* value (Fig. 3, Supplementary fig. S2). Individuals from German (DEU1 and DEU2) and French populations (FRA1 and FRA2) were assigned to two individual clusters (green and red) with high proportions of membership (Q > 0.95 and Q > 0.90 on average), respectively. Individuals from other populations were grouped to six different individual clusters (brown, white, yellow, pink, orange and purple), with the proportion of membership all being higher than 0.90. No prominent admixture was found in any population. The genetic structure and genetic relationships across populations revealed by DAPC were similar to those revealed by STRUCTURE and the genetic distance-based dendrogram (Fig. 4).

Discussion

Both genetic distance and Bayesian model-based analyses showed that there was almost no genetic differentiation between the two founding populations (DEU1 and DEU2) recently detected on Sylt Island. It could be confidently established that these two populations were genetically distinct from all the other populations tested. The fact that the attached individuals were detected only one year after fertile floating ones were found at a site nearby suggests that the former is the offspring, rather than the parents, of the latter. In addition, the growth pattern on the oyster reef strongly supports this, as has previously been discussed (Schiller *et al.*, 2018). Drifting thalli are thought to play a role in long-distance dispersal (Forrest *et al.*, 2000; Valentine & Johnson, 2003; Sliwa *et al.*, 2006). It has been highlighted that this means of dispersal could explain rapid colonization on a medium scale as well as the chaotic genetic structure observed at a regional scale (Guzinski *et al.*, 2018). The fact that there is such a close genetic relationship between the drifting population and the subsequently established attached one on Sylt is a strong argument in favour of the important role that drifting thalli have played in the expansion of *U. pinnatifida* in this area.

Aquaculture and maritime traffic are regarded as the two major vectors contributing to the worldwide spread of *U. pinnatifida* (Voisin *et al.*, 2005; Thomsen *et al.*, 2016). Aquaculture has a twofold role in the introduction of *U. pinnatifida* in Europe. One is the accidental introduction with imports of cultivated oysters, which is the presumed primary introduction vector in Thau Lagoon. The other is deliberate introduction of *U. pinnatifida* itself for cultivation purposes, as is the case in Brittany. Given the fact that no individuals of *U. pinnatifida* were found in any marina on Sylt or the neighbouring island of Föhr, shipping (via hull fouling or ballast water) is probably not the vector of introduction. There is widespread commercial farming of mussels and oysters around Sylt, therefore accidental introduction with transport of aquaculture animals is likely to be the vector leading to the introduction of this alga to Sylt. It is also possible that there is an undetected benthic population near Sylt serving as the source of the floating fertile thalli, which have been estimated to disperse, in Tasmania and New Zealand, at a rate of 1–10 km per year (Sliwa *et al.*, 2006). However, in March 2017, after the floating thalli were first found on Sylt, dredge sampling of mussel reefs was performed in

Table 2. Pairwise genetic distance (below diagonal) and F_{st} values (above diagonal) in 10 populations of *Undaria pinnatifida* from northern Europe and China.

Population	DEU1	DEU2	NLD1	NLD2	GBR	FAR1	FAR2	DL	QD	GQ
DEU1		0.016	0.602*	0.519*	0.513*	0.450*	0.457*	0.457*	0.567*	0.489*
DEU2	0.005		0.566*	0.470*	0.483*	0.420*	0.422*	0.428*	0.537*	0.459*
NLD1	0.553	0.546		0.644*	0.445*	0.500*	0.487*	0.333*	0.366*	0.348*
NLD2	0.140	0.144	0.638		0.450*	0.541*	0.517*	0.477*	0.590*	0.530*
GBR	0.571	0.569	0.762	0.367		0.313*	0.259*	0.272*	0.338*	0.284*
FRA1	0.348	0.351	0.902	0.512	0.481		0.076*	0.294*	0.403*	0.296*
FRA2	0.367	0.360	0.847	0.457	0.354	0.075		0.302*	0.370*	0.277*
DL	0.956	0.925	0.812	1.005	0.928	0.898	0.986		0.183*	0.125*
QD	1.417	1.353	0.700	1.542	0.985	1.349	1.049	0.737		0.161*
GQ	0.911	0.885	0.722	1.133	0.793	0.717	0.636	0.522	0.481	

*Indicates significance at $P < 0.05$ level for F_{st} values.

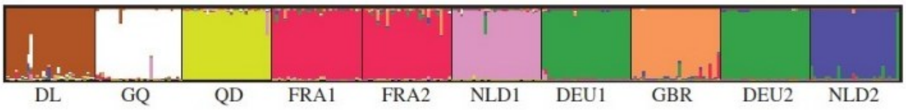

Fig. 3. Estimated genetic structure resulting from the Bayesian model-based analysis by using STRUCTURE 2.3.4 for populations of *Undaria pinnatifida* from northern Europe and China. Each individual is indicated by a vertical coloured bar, and the proportion of the colour in each bar represents the probability of membership in the relevant cluster.

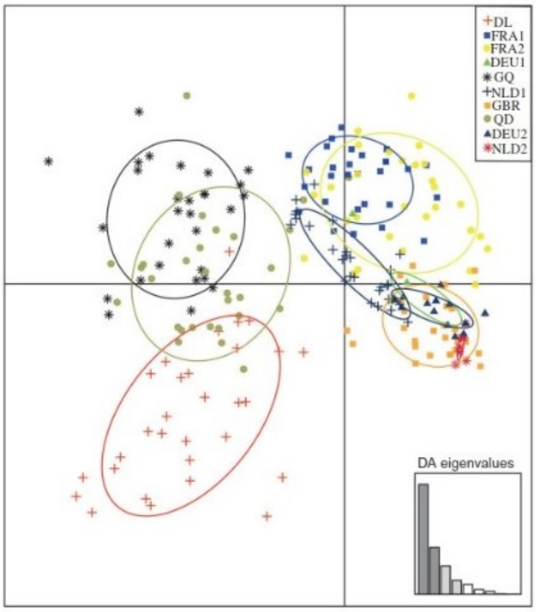

Fig. 4. Discriminant analyses of principal components for populations of *Undaria pinnatifida* from northern Europe and China.

the area east of the wash zone, but no *U. pinnatifida* were observed (Schiller *et al.*, 2018).

The founding populations of Sylt had very low genetic variation, with a high percentage of monomorphic loci (60% and 50%) and very few alleles averaged at each locus (1.6 and 1.5). This suggests that the new founders on Sylt were most probably derived from a limited number of genetically close parents from a single source. However, it is also possible that they originated from numerous individuals from one genetically depauperate source, or from individuals from different sources that were genetically similar. There exists a genetic paradox in invasive species – that introduced populations with low genetic diversity successfully become invasive. However, this paradox has not been found commonly in aquatic species (Roman & Darling, 2007). Rius *et al.* (2015) specifically showed little or no decrease in genetic diversity in marine introduced populations in comparison with the native ones. The newly founded populations of *U. pinnatifida* on Sylt are in contrast to these previous reports. Possible solutions to the genetic paradox include: being asexual or self-fertilizing; having high reproductive rates; or having high migration rates, where multiple introductions occur to ameliorate founder effects and inbreeding (Frankham, 2005). *Undaria pinnatifida* has versatile reproduction, including out- and self-fertilization and parthenogenesis, as well as the recently identified reproduction by way of monoecious gametophytes (Nakahara, 1984; Shan *et al.*, 2013; Li *et al.*, 2017). The attached DEU2 had a much higher selfing rate than the drifting DEU1, supporting the assumption that self-fertilization might play an important role in successful establishment of the new founder. Reproductive capacity of *U. pinnatifida* is also high, with hundreds of millions of zoospores released from one mature sporophyte. Sporophytes are often able to persist all year round, with multiple generations coexisting at the same time (James *et al.*, 2015). In addition, multiple introductions associated with an increase in genetic diversity might explain the success of *U. pinnatifida* in some regions, for instance in Australasia (Voisin *et al.*, 2005). All these characteristics contribute to the invasive success of this kelp (South *et al.*, 2017). The new founders on Sylt presented a valuable opportunity to sample a newly-reported population from the beginning of its establishment. We therefore also have a rare opportunity to monitor the dynamics of genetic diversity and the possible future waves of introductions from genetically differentiated sources.

Contrary to the previous results revealed by mitochondrial intergenic sequences (Voisin *et al.*, 2005), much lower genetic diversity, demonstrated by fewer N_a and private alleles as well as lower H_e, was found within the introduced populations in northern Europe as compared with those from Chinese waters. This differs from the results obtained by Daguin *et al.* (2005), in which the native Japanese population (Nagasaki) displayed the lowest genetic diversity indices when compared with introduced populations in New Zealand and France. These results suggest either that the Nagasaki population was less genetically diversified than the Chinese ones in this study, or that the origin of the population used to develop the microsatellites has an effect. The two French populations, which were from the primary introduction region in Europe, showed the highest genetic diversity within the northern European populations. They were also the only two populations with significant selfing rates, consistent with the results for the marina Moulin Blanc in Guzinski *et al.* (2018) although the selfing rates of the latter were higher (0.385–0.471). The values of N_a and H_e were very similar to those in the Bay of St-Malo (Grulois *et al*., 2011). The Terschelling population, which was first detected in 2009, had the lowest genetic diversity. Reduction in both allelic richness and heterozygosity in these introduced populations is most probably caused by founder effects (Dlugosch & Parker, 2008). Genetic diversity decreased from the earlier to the later founding populations (Fig. 2). This may be explained by more introduction events in the earlier founding populations, which mitigated the founder effects and increased genetic diversity. An alternative explanation is that the donor regions and the introduction processes could be different across European regions and seas. To determine which explanation is correct would require more extensive sampling and investigations across Europe, using populations first reported at different dates. Almost all the investigated European populations were genetically distinct from each other, except those within France and Germany. This significant divergence may be for two reasons. Firstly, populations from different countries were introduced from different sources, which were genetically distinct and the migration (gene flow) among them was low. Secondly, even though the French populations served as the primary source to spread satellite populations in other countries, genetic drift might occur in these secondary populations due to the limited number of founding individuals.

In summary, the most important finding in the present study is the close genetic proximity between the drifting thalli and the attached population established on Sylt. The source of these founders remains unknown. Tracking the origin of introduced species with a worldwide distribution is very difficult because sampling efforts to include all the putative sources are required (Rius *et al*., 2015). Further studies will focus on identifying the respective donor population, expansion routes and transportation vectors. As the ddRAD-sequencing technique has recently been successfully developed to genotype individuals of *U. pinnatifida* (Guzinski *et al*., 2018), employing the more powerful SNP markers in genetic analysis is also our goal in the future.

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Disclosure statement

No potential conflict of interest was reported by the authors.

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Supplementary information

The following supplementary material is accessible via the Supplementary Content tab on the article's online page at http://doi.org/10.1080/09670262.2018.1532116

Supplementary table S1. Sampling information for the wild populations of *Undaria pinnatifida* from northern Europe and China.

Supplementary table S2. Genetic diversity of the populations of *Undaria pinnatifida* from northern Europe and China at each microsatellite locus.

Supplementary fig. S1. Neighbour-joining dendrogram based on genetic distance among the populations of *Undaria pinnatifida* from northern Europe and China. The bar indicates the genetic distance.

Supplementary fig. S2. Delta-K graph obtained by submitting all results files of K = 2 to 10 to STRUCTURE HARVESTER.

Author contributions

S. Pang, K. Bischof and T. Shan conceived the study; T. Shan, X. Wang, J. Li, L. Su and J. Schiller performed lab work; T. Shan and X. Wang performed genetic analysis; J. Schiller, D. Lackschewitz and J. M. Hall-Spencer collected specimens from Germany and the UK. Everyone contributed to specimen collection and manuscript writing.

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Chapter 4 Publication III:

Comparison of mitochondrial DNA sequences points to the European populations as the direct origin of *Undaria pinnatifida* that has spread to the northern Wadden Sea

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Short communication

Comparison of mitochondrial DNA sequences points to the European populations as the direct origin of *Undaria pinnatifida* that has spread to the northern Wadden Sea

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ABSTRACT

The increasing spread of *Undaria pinnatifida* has caused concern in many parts of the world in recent decades, and this alga has been listed as invasive in the introduced countries. Its most recent spread in Europe was reported on Sylt island in the northern Wadden sea (German Bight, Eastern North Sea) between 2016 and 2017. Its direct origin remained unknown. In the present study, we obtained the mitochondrial DNA sequences including the partial coding region of *cox3* and intergenic noncoding loci *tatC-tLeu*, *atp8-trnS* and *trnW-trnI* from one drifting population and one attached population of *U. pinnatifida* on Sylt and compared them with the available sequences published in previous studies. For the concatenated sequences of *atp8-trnS* and *trnW-trnI*, two haplotypes (Up01 and Up03) were detected in Sylt populations with Up01 being the dominant haplotype, which was most similar to the haplotype composition identified previously in European populations. For the concatenated sequences of *cox3* and *tatC-tLeu*, two haplotypes (H1 and H9) were found in Sylt populations and they were the same as those identified previously in Brittany, France. These results suggest that European populations were most likely the direct origin of the newly established *U. pinnatifida* population on Sylt. The combined use of these sequences will be a robust tool to infer the origins of newly established populations of this seaweed in the future.

1. Introduction

Undaria pinnatifida (Harv.) Suringar is a kelp species that has been gaining growing attention due to its global spread and has been listed as invasive in many countries outside its native range (Epstein and Smale, 2017). It was initially spread to Europe by accident in the French Mediterranean in 1971, with the originally introduced stock hitchhiking the transportation of oysters from Japan for farming purposes (Floc'h et al., 1991). Then it was deliberately introduced to Brittany (French Atlantic) for cultivation in 1983, and soon cultivated individuals escaped, establishing natural populations in the wild (Floc'h et al., 1991; Floc'h et al., 1996). It later gradually spread to Spain, Italy, the UK, Belgium, the Netherlands, Portugal and Ireland (Fletcher and Manfredi, 1995; Curiel et al., 1998; Stegenga, 1999; Báez et al., 2010; Minchin and Nunn, 2014; Kraan, 2017). The most recent introduction has been reported on the island of Sylt (Germany) in the northern Wadden Sea by Schiller et al. (2018), in which drifting individuals were found to be stranded on intertidal sandflats between August 2016 and June 2017, and attached individuals were detected in June 2017.

In order to trace the origin of the individuals of *U. pinnatifida* newly found on Sylt island, microsatellite markers were used to analyze their genetic relationship with other populations from northern European

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countries, including the Netherlands, France, the UK, and three native populations from China (Shan et al., 2019). The drifting and attached populations from Sylt were revealed to be genetically close to each other, but significantly differentiated from all the other studied northern European populations. Hence, the direct origin of *U. pinnatifida* populations on Sylt remained unknown. Several mitochondrial DNA sequences are available for a large number of native and introduced *Undaria* populations worldwide. Mitochondrial coding region of *cox3* and intergenic noncoding loci *tatC-tLeu*, *atp8-trnS* and *trnW-trnI* have been used to compare the genetic diversity and sequence divergence among native and introduced populations (Voisin et al., 2005; Uwai et al., 2006). These studies showed that the mitochondrial sequences were informative to infer the origin of the introduced populations. The results suggested that aquaculture was a major vector of introduction and spread of *U. pinnatifida* in Europe and maritime traffic was involved in accelerating recurrent introduction from the native range to Australasia. Recently, genetic diversity of Chinese populations and their genetic relationship with those from Japan and Korea were clearly elucidated using *cox3* and the *tatC-tLeu* sequences (Shan et al., 2022). These studies and the associated sequence databases provide a baseline for inferring the origin of the newly established populations of *U. pinnatifida*.

In the present study we obtained the sequences of the mitochondrial coding region of *cox3* and intergenic noncoding loci *tatC-tLeu*, *atp8-trnS* and *trnW-trnI* of *U. pinnatifida* populations from Sylt and compared them with the available haplotypes of worldwide native and introduced populations published in previous studies, aiming to provide new clues for tracing the direct origin of the newly established population from Sylt.

2. Materials and methods

Twenty-four genomic DNA samples from each of the drifting and attached *U. pinnatifida* populations (DEU1 and DEU2) from the German island of Sylt in the northern Wadden Sea in 2016 and 2017 (Shan et al., 2019) were used for PCR amplification. The amplification of *atp8-trnS* and *trnW-trnI* regions were conducted according to Voisin et al. (2005). The primers were atp8-trnS-F (5′-TGTACGTTTCATATTACCTTCTTTAGC-3′) and atp8-trnS-R (5′-TAGCAAACCAAGGCTTTCAAC-3) for the *atp8-trnS* region, and trnW-trnI-F (5′-GGGGTTCAAATCCCTCTCTT-3′) and trnW-trnI-R (5′-CCTACATTGTTAGCTTCATGAGAA-3′) for the *trnW-trnI* region. The partial *cox3* gene and the *tatC–tLeu* region were amplified using the primers and PCR programs according to Uwai et al. (2006). The primers were CAF4A (5′-ATGTTTACTTGGTGRAGRGA-3′) and CAR4A (5′-CCCCACCARTAWATNGTNAG-3′) for the *cox3* gene, and tatCEF (5′-AAATAATATATTGAGATTTTAAGTCTATTCAT-3′) and tLeuR (5′-AACCTAAACACCGCGTGTATACC-3′) for the *tatC-tLeu* region. The PCR amplification was conducted using a *Taq* Master Mix (Accurate Biology, China) in a T-gradient thermocycler (Biometra, Germany). PCR products were sequenced in both directions by using the amplification primers with an ABI 3730XL automated sequencer (Applied Biosystems, USA) by the Beijing Tsingke Biotechnology Co., Ltd.

The haplotypes of *atp8-trnS* and *trnW-trnI* regions published in Voisin et al. (2005) and the *cox3* gene and the *tatC–tLeu* region in Uwai et al. (2006) and Shan et al. (2022) were downloaded from GenBank (https://www.ncbi.nlm.nih.gov/). The sequences generated in the present study were aligned to those haplotypes for comparison analysis. We first aligned each of the four sequences that we obtained in DEU1 and DEU2 to the publicly available sequences with MUSCLE in MEGA X (Kumar et al., 2018). Then we combined the *cox3* gene and the *tatC–tLeu* region to form a concatenated alignment, and combined the *atp8-trnS* and *trnW-trnI* regions to form another concatenated alignment using MEGA X. DnaSP 5.10 (Rozas et al., 2003) was used to identify different haplotypes in the two sets of concatenated alignment and compare the haplotypes of DEU1 and DEU2 to those of native and other introduced countries published previously (Voisin et al., 2005; Uwai et al., 2006; Shan et al., 2022). The number of haplotypes (N_h), haplotype diversity (h), and nucleotide diversity (π) were analyzed with ARLEQUIN 3.11 (Excoffier et al., 2005).

3. Results and discussion

The aligned sequences of *atp8-trnS* comprised 157 bp. Only one haplotype of *atp8-trnS* was detected in DEU1 and DEU2, and it is identical to the haplotype with the accession number of AY821890 (Voisin et al., 2005). The aligned sequences of *trnW-trnI* comprised 155 bp. One haplotype was detected in DEU1, being identical to the haplotype with the accession number of AY821898 (Voisin et al., 2005). In addition to this haplotype, DEU2 population had a second haplotype which was the same as the haplotype with the accession number of AY821901 (Voisin et al., 2005). The values of h and π were 0.344 ± 0.099 and 0.0022 ± 0.0006 for the *trnW-trnI* region in DEU2, respectively. The alignment of the concatenated sequences of *atp8-trnS* and *trnW-trnI* comprised 333 bp. Two haplotypes were detected for the concatenated sequences from Sylt island, identical to Up01 and Up03 of Voisin et al. (2005). DEU1 only possessed haplotype Up01, and DEU2 possessed both Up01 and Up03 (Fig. 1). The values of h and π for the concatenated sequence of *atp8-trnS* and *trnW-trnI* in DEU2 were 0.344 ± 0.099 and 0.0011 ± 0.0003, respectively (Table 1). The haplotype Up01 was dominant in the Sylt population, accounting for 90 % (43 of 48 individuals) of all the samples (Fig. 1). This haplotype was found to be ubiquitous throughout Europe and dominant in most European populations, accounting for 63 % of all observed haplotypes in Voisin et al. (2005). It was the only haplotype detected in Thau Lagoon and the French crop population. It was not found in native natural populations but was present in crop samples from Korea and Japan and hence might be the origin of primary introduction (Voisin et al., 2005). The haplotype Up03 was also found in many European natural populations, including Zeebrugge (Belgium), Hamble (Great Britain), St. Malo and Brest (France), and Cudillero (Spain). It was dominant in St. Malo and Cudillero, almost reaching a frequency of unity in the latter (Voisin et al., 2005). Interestingly, all these sites in which the haplotype Up03 was detected had the haplotype

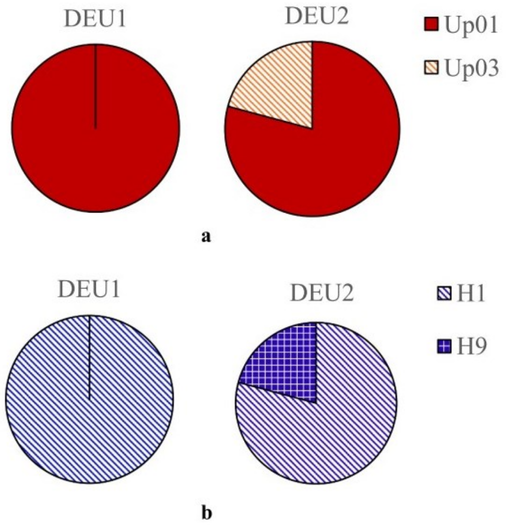

Fig. 1. Haplotypes composition of the combined *atp8-trnS* and *trnW-trnI* sequences (a) and the combined *cox3* and *tatC–tLeu* sequences (b) in the *Undaria pinnatifida* populations from Sylt island. The color areas in the pie charts are proportional to the haplotype frequency. Refer to Voisin et al. (2005) and Uwai et al. (2006) for the haplotype names. The color patterns of the haplotypes were set similarly to those two studies for the convenience of comparison.

Table 1
Genetic diversity of the Sylt populations of *Undaria pinnatifida* estimated by using the combined *atp8-trnS* and *trnW-trnI* and the combined *cox3* and *tatC-tLeu* sequences, respectively.

Population	Combined *atp8-trnS* and *trnW-trnI* sequences				Combined *cox3* and *tatC-tLeu* sequences			
	N_p	N_h	h	π ($\times 10^{-2}$)	N_p	N_h	h	π ($\times 10^{-2}$)
DEU1	0	1	0	0	0	1	0	0
DEU2	1	2	0.344 ± 0.099	0.110 ± 0.032	1	2	0.344 ± 0.099	0.042 ± 0.012

N_p, the number of polymorphic site; N_h, the number of haplotypes; h, haplotype diversity; π, nucleotide diversity

Up01 simultaneously. Hence, all of them could be the potential single source of the Sylt populations. The haplotype Up03 was not detected in native populations, at least in the samples contained in the study of Voisin et al. (2005). The haplotype composition of Sylt populations was different than that of populations from Australasia and Americas. Based on comparison of the combined sequences of *atp8-trnS* and *trnW-trnI*, the Sylt populations appear most likely to be the secondary derivative of European populations.

The aligned sequences of *cox3* comprised 431 bp. Only one haplotype was detected in DEU1 and DEU2, and it was the same as the haplotype with the accession number of AB213030 (Uwai et al., 2006). The aligned sequences of *tatC-tLeu* comprised 410 bp. One haplotype was detected in DEU1, being identical to the haplotype with the accession number of AB240644 (Uwai et al., 2006). In addition to this haplotype, DEU2 population possessed a second haplotype which was identical to the haplotype with the accession number of AB240651 (Uwai et al., 2006). The values of h and π of *tatC-tLeu* sequences in DEU2 were 0.344 ± 0.099 and 0.0009 ± 0.0003, respectively. The alignment of the concatenated sequences of *cox3* and *tatC–tLeu* was 841 bp. Two haplotypes were detected for the concatenated sequences from Sylt island, and they were identical to H1 and H9 of Uwai et al. (2006). DEU1 only possessed the haplotype H1, and DEU2 possessed both H1 and H9. The values of h and π for the concatenated sequences of *cox3* and *tatC–tLeu* in DEU2 were 0.344 ± 0.099 and 0.0004 ± 0.0001, respectively (Table 1). All individuals with the H1 haplotype from Sylt island were found to have the Up01 haplotype, and all individuals with the H9 haplotype were found to have the Up03 haplotype. Thus, when we concatenated all four sequence fragments, we still obtained two haplotypes. The haplotype H1 was dominant in the Sylt population, accounting for 90 % (43 of 48 individuals) of all the samples (Fig. 1). This haplotype was detected in specimens from Brittany, France (Uwai et al., 2006). It was a common haplotype in northern Japan, and was also found in Korean specimens and in the natural population from Gouqi Island and cultivated populations from Dalian of China (Uwai et al., 2006; Shan et al., 2022). The haplotype H9 was also detected in specimens from Brittany (Uwai et al., 2006). In fact, Brittany was found to be the only site in the study of Uwai et al. (2006) that shared the same haplotype composition with Sylt island. The haplotype H9 was not detected in the native samples, probably due to the limited number of specimens especially for those from northern Japan. Comparative results of the concatenated sequences of *cox3* and *tatC–tLeu* point to Brittany as the most probable origin of the populations from Sylt island.

Microsatellites can often reveal the subtle genetic structure even on small geographical scales, especially for kelp species such as *U. pinnatifida* which is thought to have a limited natural migration range (Grulois et al., 2011; Guzinski et al., 2018). Moreover, the founder effect is usually significant for the newly founded population due to the limited number of founding individuals, leading to significant genetic differentiation between the donor and recipient populations. Consequently, it is likely difficult to trace the origin of the newly established population according to genetic relationship analysis based on the allele frequency of microsatellites. This is the reason that we were not able to determine the origin of the *U. pinnafida* on Sylt by using microsatellites (Shan et al., 2019). In this case, the haploid mitochondrial DNA markers are more suitable to trace the general origin of a founding population as the genetic relationship can be inferred by comparing the haplotypes of these markers. In the present study, both the concatenated sequences of *atp8-trnS* and *trnW-trnI* and the concatenated sequences of *cox3* and *tatC–tLeu* showed that the haplotype composition from Sylt island was most similar to that detected in European populations, but different than that from other regions. Therefore, European populations are suggested to be the direct origin of the populations of *U. pinnatifida* established on Sylt. The combined use of these mitochondrial sequences was demonstrated to be informative in the present study and hence will be a robust tool to infer the origins of newly established populations of this seaweed in the future.

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CRediT authorship contribution statement

Tifeng Shan: Conceptualization, Methodology, Writing- Original draft preparation. **Shaojun Pang:** Supervision, Writing- Reviewing. **Yuqian Li:** Methodology. **Jessica Schiller:** Resources. **Dagmar Lackschewitz:** Resources. **Kai Bischof:** Resources, Writing- Reviewing and Editing. All authors read and approved the final manuscript.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Chapter 5 Publication IV:

Cold tolerance in the invasive brown alga *Undaria pinnatifida*: A comparative study of physiological responses in a northern European population and its native counterpart

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in preparation

Title: Cold Tolerance in the Invasive Brown Alga *Undaria pinnatifida*: A Comparative Study of Physiological Responses in a Northern European Population and Its Native Counterpart

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Abstract

Invasive species, particularly primary producers such as seaweeds, can significantly impact the ecosystems they invade, changing community structures and species composition, affecting ecosystem services and linked economies. Climate Change may alter species' range limits, highlighting the need to understand how such organisms, especially invasive ones, perform under altered environmental conditions. This study compares physiological and biochemical responses of *Undaria pinnatifida* isolates (sporophytes) from a native (Chinese) and an introduced (German) population to temperatures below the published optimum to evaluate its capacity to invade colder regions. Antioxidant content and C:N ratio did not show treatment-related patterns. Differences became visible in growth performance as an integrative measure, pigments, and during meta-analysis. The German isolate was less growth inhibited in the coldest treatment and less viable at the warmer temperatures compared to the Chinese isolate. This response pattern correlates with biogeographic origins of both isolates. Further research is required to understand the underlying mechanisms and to identify whether these differences are due to acclimation and phenotypic plasticity or adaptation. This study adds to our understanding of *U. pinnatifida*'s competitive success and ability for future spread into new biogeographic regions as an invasive species, and its response to potential temperature stress in new habitats.

Keywords: cold tolerance, temperature stress, invasive kelp, *Undaria pinnatifida*, range expansion

Introduction

Marine introductions of nonindigenous species (NIS) and bioinvasions have gained significant attention over the past decade compared to years before, with larger amounts of scientific publications and increased public interest. The capacity of invasive species to cause conspicuous large-scale changes over short periods has raised concern (Kovalenko et al. 2021 and references therein). Primary producers such as seaweeds (alongside predators) are the most disruptive group of NIS, with examples being seaweeds such as *Caulerpa* spp., *Codium fragile,* and *Sargassum muticum* (Anton et al. 2019).

The brown macroalga *Undaria pinnatifida* (Harvey) Suringar, a representative of the order Laminariales, commonly known as Wakame, has gained much scientific interest due to its near ubiquitous appearance as an introduced and even invasive species with varying levels of impact on the invaded ecosystems and connected economies (e.g., Pimentel et al., 2005). Native to the temperate waters of China, Japan and Korea (Hay and Villouta 1993, Oh and Koh 1996, Morelissen et al. 2013), this kelp has successfully established novel populations worldwide (Hay and Luckens 1987, Nisizawa et al. 1987, Yamanaka and Akiyama 1993, Lowe et al. 2000). Impacts of introductions and invasions by *U. pinnatifida* vary drastically from no reported ecological impacts, often via lack of following reports or mentions (e.g., Schiller et al. 2018), spreading populations of little or positive impacts (e.g., Irigoyen et al. 2011), to strong (negative) impacts on the native ecosystem and economy (Curiel et al. 2002, Casas et al. 2004, Forrest and Blakemore 2006). Since the initial introduction to Europe in southern France in 1971 (Floc'h et al. 1991), populations have been reported progressively further north - northern France (1987), southern England (1994), The Netherlands (1999-2009), and Northern Ireland (2015) (Minchin and Nunn 2014, Gittenberger et al. 2015, Minchin et al. 2017), including its current northern continental range limit in the German Wadden Sea in 2016 (Schiller et al. 2018). Since 2016, this population has persisted (J. Schiller pers. obs.), but no new locations along the German coastline or further north have been documented in scientific literature. Temperature is an essential driver for the formation of biogeographic patterns in macroalgae (Adey and Steneck 2001). All except two native populations of *U. pinnatifida* (Peter the Great Bay, RU; Hokkaido, JP) and all to date recorded introduced populations are located further south at sites with higher minimum sea surface temperature (SST) and, for the most part, higher maximum SST (James et al. 2015) than the population in Sylt, Germany (see Figure 1). With a reported temperature optimum of 18-21 °C for young *U. pinnatifida* sporophytes of most populations in the native range (Morita et al. 2003a, Gao et al. 2013a, Watanabe et al. 2014), this northward expansion poses questions about the underlying physiological acclimation mechanisms, particularly in response to temperature stress. Lethal temperature limits for the survival of a population have been reported as 0-30 °C based on occurrence data, while reproduction is more limited, reportedly requiring temperatures of 10-20 °C during several months of the year (James et al. 2015b and references therein). Research strongly suggests the capacity for genetic adaptation to colder temperatures in more northern Japanese populations (Gao et al. 2013a). The population at Sylt was characterized as one of low genetic diversity and clear distinction from other European populations based on microsatellite analysis (Shan et al. 2019). With the addition of mitochondrial DNA analysis, it was possible to establish other European populations, such as Brittany, France as the likely origin of the German population on Sylt (Shan et al. 2023). These data indicate a continuous northward migration of *U. pinnatifida* along the European coast from a region with warmer mean and winter temperatures (Murphy et al. 2017, Charria et al. 2020) to the colder waters (mean and minimum temperatures) of Sylt, Germany, with ample time for adaptation.

While the ecological implications of NIS spreading, establishing populations, and the potential associated challenges they pose to native ecosystems and economies are essential to consider and discuss (Pimentel et al. 2005, Robinson and Culhane 2020), these cases are also a unique opportunity to study mechanisms of range expansion and establishment (Hudson et al. 2021, Kovalenko et al. 2021). Biological invasions have been referred to as one of the most profitable avenues to test forecasts for the distribution of species and diversity (Higgins and Richardson 2014). Physiological tolerances and the capacity and presence of local adaptations are among the key factors that determine a species' potential for establishing and maintaining new populations that ultimately lead to an increase in range (Hudson et al. 2021 and references therein). Several studies have highlighted the need for more differentiation in research performed on macroalgal species with a wide latitudinal distribution, which have largely been considered as a single homogenous unit (e.g., Reed et al. 2011). Local adaptation and phenotypic plasticity are considered critical factors in deciding whether a species can overcome changing conditions and maintain populations or establish new ones (King et al. 2018). A need for experiments that examine the influence of individually manipulated factors, including temperature, on the growth of *U. pinnatifida* has been expressed in previous works (Dean and Hurd 2007). Therefore, a comparative investigation of physiological and biochemical responses of *U. pinnatifida* isolates from native and introduced populations exposed to varying temperature treatments provides a unique opportunity to help understand its capacity to invade colder regions.

We hypothesized that the German isolate, originating from a location with lower minimum and average temperatures, exhibits less stress to low temperatures, while the Chinese isolate, originating from a region of higher average and maximum temperatures, responds more favorably to warmer growth conditions.

As an acclimation to life in the intertidal and specifically tide pools that experience more sudden cold events during the growth period in winter (e.g., Scrosati et al. 2020), the German *U. pinnatifida* isolate may have a more pronounced mitigation response when exposed to the coldest treatment.

The German isolate, originating from an introduced population with lower genetic diversity (Shan et al. 2019), may have a narrower range of tolerance that leans towards the conditions present in the introduced location. In comparison, the native Chinese isolate has larger genetic diversity and, hence, a wider tolerance range.

This study aims to enhance the understanding of *U. pinnatifida*'s invasive success, as well as explore the underlying physiological intricacies of how it, as a representative of seaweeds in new habitats, responds to temperature stress by comparing an isolate from its native, warmer range with one from the introduced, colder habitat.

Materials and Methods

Media used

All cultures were grown in Provasoli Enriched Seawater (PES, 50 %, modified from Starr and Zeikus 1993) based on natural sand-filtered, ozone-treated, and pasteurized seawater from the Yellow Sea in Qingdao, China. For a short period after extraction, the German isolate was cultivated in autoclaved aged PES from the North Sea before being transferred to Qingdao, China.

Origin of gametophyte cultures

The cultures were obtained from wild populations in Sylt, Germany (G) and Qingdao, China (C) by releasing zoospores from mature sporophylls according to the method of Shan and Pang (2009). Differences in the responses of newly established and older vegetative gametophyte isolates of kelps have been observed (J. Schiller pers. obs.) and were later confirmed in other studies (Ebbing et al. 2021). Therefore, this study acquired both isolates at similar times and cultivated them under identical conditions for approximately one year before conducting the experiments to limit the latter. The resulting gametophyte cultures were kept at the Seaweed Culture Collection Centre (SCCC, Institute of Oceanology, Chinese Academy of Sciences, Qingdao, China; www.caslivealgae.com) at 14 ± 2°C and dim fluorescent white light of approximately 5 µmol photons m^{-2} s^{-1} in a 12:12 h L:D cycle. Cultures were refreshed monthly using 50 % PES (adapted from Starr and Zeikus 1993).

Obtaining of sporophytes and pre-experimental culture

Sporophytes were produced by inducing gametogenesis in vegetative gametophyte cultures of *Undaria pinnatifida* from Germany and China, respectively, following the method of Shan and Pang (2009) with minor modifications. Small amounts of filamentous gametophytes were blended with 18 °C 50 % PES in an electric blender (20000 rpm, JYL-C012, Jiuyang, China) until the female gametophyte fragments were at most 10 cells long. Some of the suspension was added to Petri dishes and filled with 50 % PES to a culture density of 20-30 fragments per microscopic field at 100× magnification (Li et al. 2014). The dishes were placed at 18 ± 0.5 °C, shaded for the first 24h, and at 60 µmol photons m^{-2} s^{-1} full spectrum LED light (GXZ-380C LED, Ningbo Jiangnan Instrument, China) with a 12:12 h L:D cycle afterward. The medium was refreshed every three days. At about 1 mm length (approximately two weeks), sporophytes were transferred to sealed 1 l beakers sealed with plastic wrap and added aeration at 14 ± 1 °C with daily media refreshments for approximately three weeks until they reached a size of about 1 cm.

Experimental setup

Sporophyte clusters (i.e., several juvenile thalli linked at the base by their holdfasts, see Figure 2) of approximately the same size were selected from the pre-experimental cultures and added to 1l beakers filled with 14 °C 50 % PES (3 beakers per origin (C, G) and temperature, 10 clusters per beaker). The beakers with added aeration were sealed with plastic wrap and placed into four temperature and light-controlled photo-incubators at 60 µmol photons m^{-2} s^{-1} full spectrum LED light (GXZ-380C LED, Ningbo Jiangnan Instrument, China), 12:12 h L:D cycle at 4, 8, 12 and 16 ± 0.5 °C, respectively. They received fresh, 50 % PES on every assessment day.

Sporophyte length assessment

On days 0, 4, 8, and 12, all sporophyte clusters of each beaker were spread flat in shallow water and photographed. Due to tissue degradation, the sporophytes incubated at 16 °C were only measured until day 8, and the tissue was not further analyzed (data L_{t0}, L_{t4}, L_{t8}, L_{t12}). The images were processed using ImageJ to measure the length of all visible, intact sporophytes (56-111 measurements per replicate). The average sporophyte length per replicate for each sampling day was determined, and the resulting absolute length increase ($\Delta L = L_{t12} - L_{t0}$) of each replicate was calculated and expressed in percent of the starting value relative ΔL (rel. ΔL).

Biomass quantification and preparation

Sporophytes were lightly patted dry, and the wet weight (WW) per replicate was determined on days 0 and 12 (WW_{t0}, WW_{t12}). The absolute biomass increase was calculated as the increase of WW between day 0 and day 12 ($\Delta WW = WW_{t12} - WW_{t0}$) and expressed in percent of WW_{t0} as relative rel. ΔWW (rel. ΔWW). To explore the relationship between biomass and plant size, the ratio of ΔWW to ΔL was calculated (ΔWW:ΔL [g cm^{-1}]). The correlation between final wet and dry weight was calculated to verify the wet weight data, which can be more prone to fluctuations. At the end of the experiment (12 days), all sporophytes of a replicate were combined and frozen at -80 °C. The samples were lyophilized, and dry weight (DW_{t12}) was quantified before they were finely ground using a benchtop homogenizer and ceramic beads (FastPrep ®-24; MP Biomedicals, Solon, OH, USA). The powder was divided into batches and weighed for the following biochemical analyses.

HPLC pigment analysis

Pigments were extracted in darkness at 4 °C for 24 h using cold 90 % acetone, following Koch et al. (2015). The extracts were vortexed, centrifuged (16000 × g, 4 °C, 5 min), and the supernatant vacuum filtered over a 45 µm cellulose acetate membrane. The analysis was performed via high-performance liquid chromatography (HPLC; LaChromElite® system with a chilled autosampler L-2200 and DAD detector L-2450; VWR-Hitachi International GmbH, Darmstadt, Germany) on a reversed-phase Spherisorb® ODS-2 column (5 µm, 25 cm × 4.6 mm ID; Waters, Milford, MA) following a modified protocol by Wright et al. (1991). Pigment peaks were measured at 440 nm and identified as chlorophyll *a* (Chl *a*), chlorophyll *c*2 (Chl *c*2), fucoxanthin (Fuc), violaxanthin (V), antheraxanthin (A), zeaxanthin (Z), and β-carotene by retention time and calibration against pigment standards (software: EZChrom Elite, Agilent Technologies, Santa Clara, CA, USA, Version 3.1.3., 2004; standards: DHI Inc., Hørsholm, Denmark). The size of the xanthophyll pool was calculated as the sum of *V*, *A*, and *Z* (*VAZ*). The de-epoxidation state of the xanthophyll cycle (DPS) was calculated as DPS = $(Z + 0.5\,A) / (V + A + Z)$ (Colombo-Pallotta et al. 2006). Ratios for Acc:Chl *a* and VAZ:Chl *a* were calculated to identify possible changes in the photosynthetic apparatus.

C:N analysis

Following Graiff et al. (2016), the total content of carbon (C), nitrogen (N), and the resulting C:N ratio were analyzed and calculated. Approximately 2 mg of the lyophilized pulverized samples were prepared in tin cartridges (6 × 6 × 12 mm) and combusted at 950 °C. The absolute content of C and N were quantified in an elemental analyzer (Vario EL III, Elementar, Langenselbold, Germany) with acetanilide (C_8H_9NO) as standard (Verardo et al. 1990).

Antioxidant capacity

To determine the radical scavenging capacity of DPPH (2,2-diphenyl-1-picrylhydrasyl), modified protocols by Brand-Williams et al. (1995) and Cruces et al. (2012) were applied. Approximately 15 mg pulverized sporophytes of each treatment replicate were homogenized in darkness at 4 °C for 24 h on a shaker plate using 1 mL of 70% acetone. The extracts were centrifuged (500 × g, 4 °C, 5 min), and in a 96-well microtiter plate, 22 µL of the supernatant were mixed with 200 µL DPPH* stock (150 µM in 100 % ethanol, triplicate measurement). After 45 min incubation in darkness, the absorbance at 520 nm was measured in a microplate spectrophotometer (FLUOstar OPTIMA, BMG Labtech, Ortenberg, Germany). To determine the antioxidant activity of the antioxidant pool, Trolox® was used as a standard (Cruces et al. 2012), and µg Trolox® equivalents (TE) were calculated per dry weight (µg TE mg^{-1} DW).

Data analysis

All statistical analyses were conducted using R (R Core Team 2023). For all datasets, the normality of the data was assessed using visual inspection of Quantile-Quantile (QQ) plots and the Shapiro-Wilk normality test, followed by Levene's test for homogeneity of variances. Outliers were removed based on the IQR (Interquartile Range) Method (k = 1.5) when required. Statistical comparisons were assessed within and between the origin groups only for the same temperature treatment. Parametric data was assessed using two-way analysis of variance (ANOVA) and Tukey's post hoc test (equal variances) or Welch's ANOVA (unequal variances). A residuals vs. fitted values plot was generated to assess the linearity, and a robust linear regression model (RLM) was applied if suitable. The Kruskal-Wallis test and either Dunn-Bonferroni (equal variances) or Games-Howell (unequal variances) post hoc tests were applied for non-parametric data. Additionally, data were analyzed by two-factor permutational multivariate analysis of variances (PERMANOVA), based on Canberra distances on the factors origin (two levels; C, G) and temperature (three levels; 4, 8, 12) and relevant response variables (TE, C:N, Chl *a*:C, rel. ΔL, rel. ΔWW, DPS, Acc:Chl *a*, VAZ:Chl *a*) with a maximum of 9999 permutations. A permutational analysis of multivariate dispersion (PERMDISP) was performed for each factor to confirm homogeneous multivariate dispersions between groups since PERMANOVA is sensitive to within-factor differences. Pairwise permutational t-tests with p-value correction for multiple testing were used for post hoc testing of the PERMANOVA results (pairwise.adonis function; Martinez Arbizu 2017). Higher-level patterns in the data were visualized and evaluated via principal coordinate analysis (PCoA) after the data was standardized using mean centering. The data did not meet the principal component analysis (PCA) requirements to assess the response variables' contribution to the distribution.

SST data acquisition

Data for sea surface temperature (SST) for the locations where the isolates were collected was accessed via NASA Ocean Biology Processing Group (2023). Monthly nighttime SST for both sites was downloaded for the years 2012-2017 (the time frame prior to collecting the isolates) and plotted in R (R Core Team 2023). The satellite data retrieved for this study shows that in the introduced habitat, Sylt, Germany, annual SST ranged from 2.4 ± 0.8 °C in February to 18.1 ± 0.4 °C in August (median with SD of satellite-derived monthly SST for the years 2012 to 2017). Near the sampling location in Qingdao, SST ranged from 5.7 ± 0.9 °C in February to 26.6 ± 1.2 °C in August. The SST range in the two locations from 2012 to 2017 was 15.7 ± 0.9 °C and 20.9 ± 1.5°C for Sylt and Qingdao, respectively (see Figure 1).

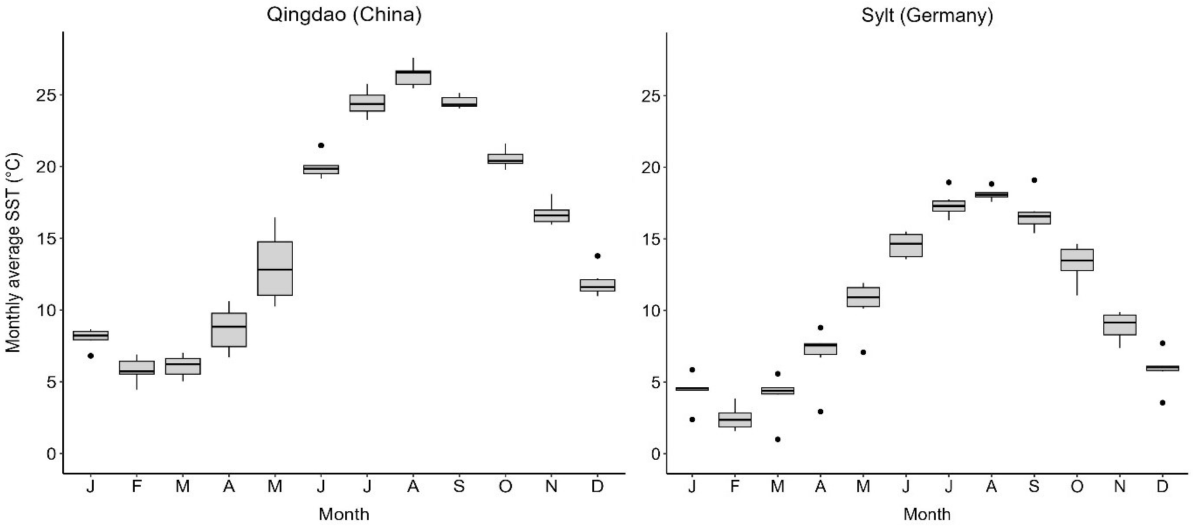

Figure 1: Monthly average sea surface temperature (SST) for the collection sites of the two isolates used in this study. Left: Qingdao, China; right: Sylt, Germany. Data accessed via NASA Ocean Biology Processing Group (2023). Each box represents the average monthly temperatures from 2012 to 2017 (n = 6), and dots represent outliers.

Results

Growth of sporophytes (morphology, length, and biomass)

Sporophytes of both isolates showed only minor morphological differences during the experiment. The most notable were slightly (not statistically confirmed) narrower blades in the German isolate and slightly wider blades with a pronounced, slim tip in the Chinese isolate (see Figure 2). When sporophytes of both isolates were grown for several months under ambient conditions in tanks, pronounced differences in morphology became visible. The German isolate produced wider thalli of light brown color, with shallower lobing and large holdfasts, while the Chinese isolate produced dark brown, longer, and deeply lobed thalli with small holdfasts (see Figure 7, suppl. material).

Sporophytes in all treatments grew healthy until day 8, when the first signs of tissue decay became visible in all 16 °C replicates (see Figure 2). This thallus decay progressed fast, and the 16 °C sporophytes could not be measured after this period, as most had broken apart from expanding circular decay points, while the remaining tissue still seemed healthy. Early signs of decay were visible also in the 12 °C replicates on day 12 when the experiment was finished. Two replicates of the German isolate had also broken apart at this temperature and could not be measured on day 12. Therefore, the statistical analysis of the length data was focused on day 8, when all treatments were still present. The treatments may hereafter be referred to by their origin (China = C, Germany = G) and experimental temperature (4, 8, 12, 16 °C).

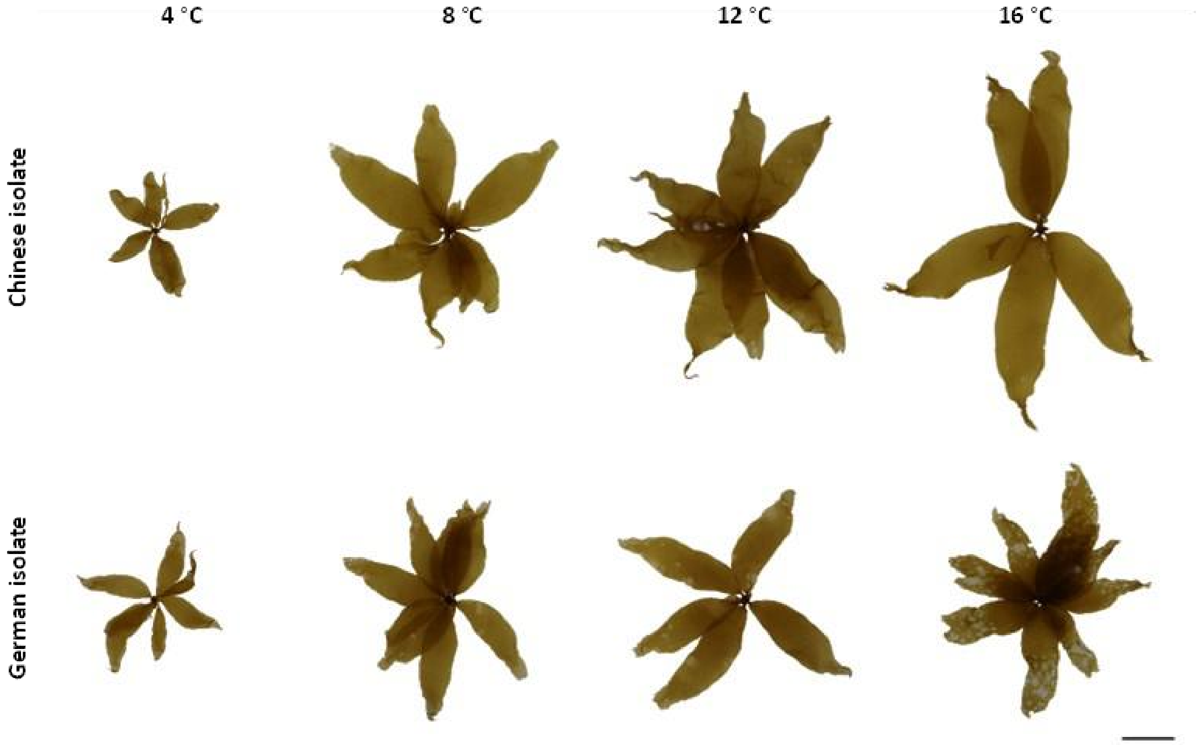

Figure 2: Representative sporophytes photographed after 8 days of treatment. Chinese (Qingdao) isolate (top) and German (Sylt) isolate (bottom) incubated at different temperatures (from left to right: 4, 8, 12, 16 °C). The scale bar represents 1 cm.

At 4 °C, sporophytes of both isolates grew significantly less than at all other temperatures ($p < 0.05$) except German ones at 12 °C ($p = 0.08$) and showed no indication of tissue decay during the experiment. They were, therefore, also statistically compared at day 12, when their growth visibly diverged (see Figure 3). Sporophytes of the German isolate (rel. ΔL 54.04 ± 5.89 %) showed more than double the length increase of Chinese ones (23.22 ± 3.44 %; $p = 0.017$). At 8 °C, the isolates did not differ significantly on day 8, and while on day 12, Chinese sporophytes had a higher length increase (239.2 ± 23.2 %) than the German ones (158.6 ± 4.3 %); this difference was, however, not significant ($p = 0.09$). The length increase of both isolates was most similar at 12 °C with no significant differences between each other but a somewhat steeper growth curve in the Chinese isolate (see Figure 3). Although it could not be statistically confirmed, 16 °C produced the highest length increase in the Chinese isolate, while in the German one, it was not distinguishable from 8 and 12 °C on day 8 (see Figure 3). The German isolate at 16 °C also showed the earliest signs of tissue decay, followed by the Chinese at 16 °C and the German at 12 °C.

To compare the length-based growth rates of the present study with literature data, they were approximately translated into area-based rates. The resulting area-based growth rates of the German isolate were 9, 28, and 29 %, and those of the Chinese isolate 4, 44, and 36 % at 4, 8, and 12 °C, respectively. The difference between the highest and lowest growth rates for each isolate was striking. It was about 3-fold in the German isolate, yet more than 10-fold in the Chinese one.

The ratio of wet weight to dry weight at day 12 (WW_{t12}:DW_{t12}) showed a linear correlation ($R = 0.99$, $p < 0.001$, data not shown), validating the WW for further analyses while confirming no difference in water content. The sporophytes of the German isolate increased significantly more in biomass than the Chinese at 4 °C (rel. ΔWW 220 ± 24 % and 95 ± 24 % respectively), while the opposite was true at 8 °C (C8 = 1928 ± 125 %, G8 = 998 ± 54 %, see Figure 4). Both cultures had the highest biomass increase at 8 °C ($p < 0.05$), although this was only significant for the Chinese culture as G8 and G12 did not vary significantly ($p = 0.6$). While the biomass increase differed only by a factor of 4.5 between G4 and G8, it was 20-fold between C4 and C8. Biomass data for G12 needs to be considered with some reservation since replicates G12b and G12c showed some breakage. Thus, small amounts of biomass may have stayed behind in the sieve, leading to a discrepancy.

As length data was only available for one replicate of G12 on the final day, the ΔWW:ΔL ratio for G12 should not be considered statistically relevant. For all other treatments, the ΔWW:ΔL ratio showcases a change in growth pattern between the different temperatures but not the origins (see Table 1). Both cultures had the lowest ΔWW:ΔL ratio at 4 °C (C4 = 0.63 ± 0.08, G4 = 0.66 ± 0.01) and the highest at 8 °C (C8 = 1.2 ± 0.04, G8 = 1.07 ± 0.13).

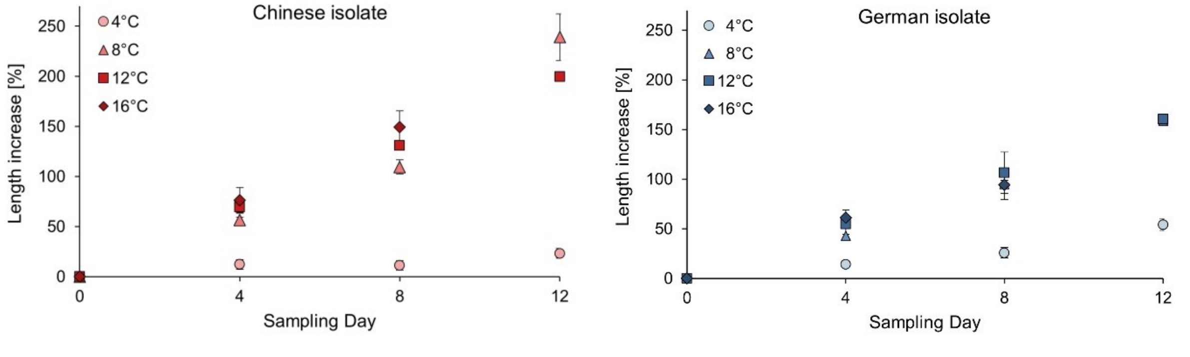

Figure 3: Relative length increase (ΔL) per temperature and origin in percent of the mean sporophyte length per replicate on day 0. Left: Chinese isolate (C), right: German isolate (G). Different shapes represent different temperature treatments (4, 8, 12, 16 °C). Values are means ± SD (n = 3 except DE, 12 °C, day 12 where n = 1). Length data for 16 °C is only shown until day 8, after which all treatments led to tissue decay. Statistically significant on day 8: C-4 °C from all other temperatures, and G-4 °C from all other temperatures; on day 12 (only 4 °C and 8 °C were compared): C-4 °C from C-8 °C and G-4 °C, and G-4 °C from G-8 °C.

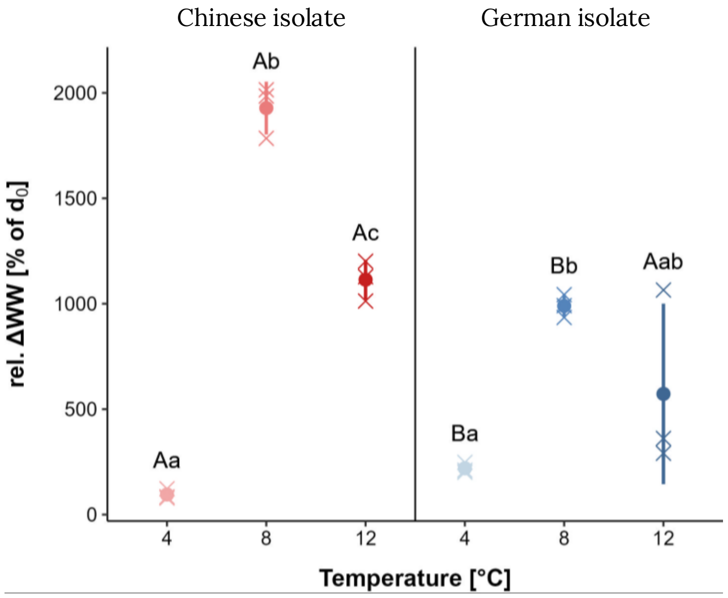

Figure 4: Relative wet weight increase (rel. ΔWW) of sporophytes of the Chinese (red, left) and the German isolate (blue, right) between day 0 and day 12, after different temperature treatments (4, 8, and 12 °C). Values are given in percent of the original WW of each replicate on day 0 of the experiment. Treatment means (dots) ± SD (whiskers) and individual replicate values (crosses); (n = 3). Significant differences between origins at the same temperature are indicated by different capital letters and between temperatures of the same origin by different lowercase letters.

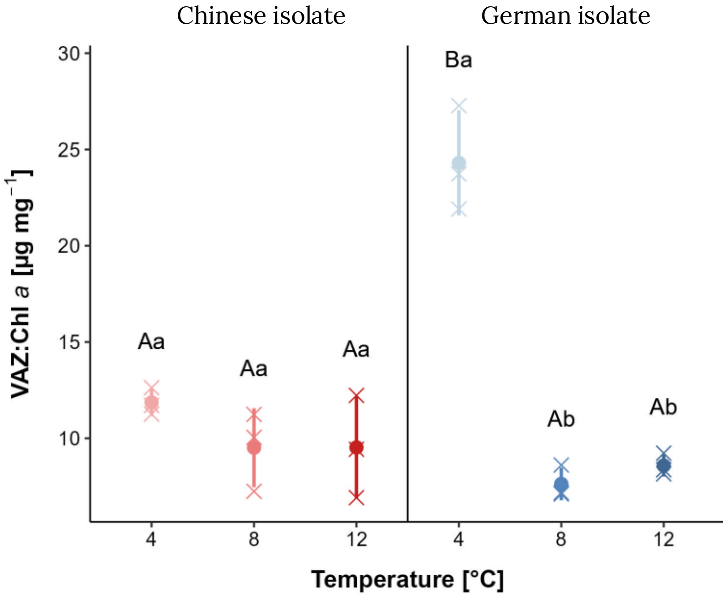

Figure 5: Ratio of the xanthophyll pool pigments (VAZ) to chlorophyll *a* in µg mg^{-1} of sporophytes of the Chinese (red, left) and the German isolate (blue, right) on day 12 of the experiment, after different temperature treatments (4, 8, and 12 °C). Treatment means (triangle) ± SD (whiskers) and individual replicate values (cross); (n = 3). Significant differences between origins at the same temperature are indicated by different capital letters and between temperatures of the same origin by different lowercase letters.

HPLC pigment analysis

The temperature treatments and origin of the isolates had a visible effect on the pigment concentrations and ratios. Most of the significant differences between the isolates occurred in the 4 °C treatment, fewer at 8 °C, and least in the 12 °C treatment (of 8 assessed concentrations, pools, and ratios 5, 3, and 0 significant differences respectively).

The size of the xanthophyll pool (VAZ) showed a pronounced difference between origins at 4 °C, with that of the Chinese isolate being the smallest of all treatments (10.1 ± 1.3 µg g^{-1} DW) and significantly smaller than that of the German isolate (23.8 ± 1.9 µg g^{-1} DW), which was the largest of all treatments it was statistically compared to C4, G8, G12 (see Table 1). At 8 °C, the Chinese isolate had its highest VAZ (24.31 ± 13.7 µg g^{-1} DW), while the German one did not differ significantly between 8 and 12 °C. However, there was a negative correlation between temperature and VAZ in the German samples (means ± SD 4, 8 12 °C: 23.8 ± 1.9, 21.6 ± 13.1, 16.3 ± 3.3 µg g^{-1} DW, respectively, see Table 1). Both isolates revealed high SDs at 8 °C, and outliers were identified and excluded from statistical analysis while being shown in the graph; this could indicate a local deviation in one of the replicates and needs to be considered during evaluation.

The only treatment with a significantly different VAZ:Chl *a* ratio was the German isolate at 4 °C ($p < 0.05$). The Chinese isolate had a ratio less than half as high (C = 11.86 ± 0.70, G = 24.30 ± 2.73), and the German 8 and 12 °C treatments were approximately three times lower (7.63 ± 0.85 and 8.58 ± 0.57). This drastic difference originates in the larger xanthophyll pool of the German isolate at 4 °C, while all other VAZ values match the general pattern of Chl *a* (see Figure 5 and Table 1). The overall concentration of accessory pigments (Acc) is less affected by origin and temperature, with only minor differences between treatments. C8 has a lower Acc concentration than C12, while the rest do not differ significantly (see Table 1). However, due to the comparatively higher concentrations of Chl *a* at 8 and 12 °C for both isolates, the Acc:Chl *a* ratio is higher at 4 °C for both isolates than the higher temperatures ($p < 0.05$). Both isolates exhibited similar ratios at each temperature and did not differ significantly from each other ($p > 0.05$).

A marked difference in the response of both isolates was observed in the DPS, which was not affected by the temperature treatments in the German isolate but rose to three times higher levels at 4 °C in the Chinese isolate (see Table 1). The differences to the other temperatures and the German isolate at the same temperature were significant ($p < 0.05$).

Overall, the German isolate revealed a higher total pigment concentration at all temperatures than the Chinese one; however, this was only significant at 4 and 8 °C ($p < 0.05$). Both isolates had the significantly highest total concentration at 8 °C (C = 3.9 ± 1.4 and G = 4.7 ± 1.8 mg g^{-1} DW, $p < 0.05$) and lowest concentration at 4 °C (C = 2.2 ± 0.1 and G = 2.5 ± 0.03 mg g^{-1} DW, $p < 0.05$). (see Table 1)

C:N analysis

All measured C:N ratios ranged between 6.2 and 7.8, indicating no nitrogen limitation. The C:N ratio in the C4 treatment (7.41 ± 0.44) was significantly higher than in C8, C12 and G4 samples (6.62 ± 0.03, 7.14 ± 0.84, and 6.70 ± 0.20 respectively, $p < 0.5$, see Table 1). In the German isolate, there were no significant differences between the treatments ($p < 0.5$). However, there was a slight trend for increased C:N ratio with increased temperature (see Table 1).

In the Chinese isolate, the Chl *a*:C ratio was significantly higher at 4 °C (0.009 ± 0.010) compared to 8 and 12 °C (both 0.007 ± 0.001, $p < 0.05$) which did not differ from each other ($p > 0.5$), while in the German isolate, 4 and 8 °C revealed significantly lower ratios (0.003 ± 0.001 and 0.004 ± 0.000) than 12 °C (0.009 ± 0.004, $p < 0.05$, see Table 1)

The carbon and nitrogen analysis produced an erroneous result for one replicate of both origins' 4 °C samples (0 reading). For the German isolate, the remaining samples produced results in line with expectations, while for the Chinese isolate, the remaining Chl *a*:C ratios deviated strongly (0.16 and 0.002) and should be considered with caution (see Table 1).

Antioxidant capacity

None of the groups differed significantly from the others when analyzed with the chosen statistical methods ($p < 0.05$). The Chinese culture tended towards higher levels of TE than the German culture. However, this was just not significant due to overall high data variation and missing values for the 4 °C treatment ($p = 0.08$; see Table 1)

Table 1: Growth and biochemical data of Chinese and German sporophytes after cultivation at different temperatures. Relative length and biomass increase [%], chlorophyll a (Chl *a*), chlorophyll c2 (Chl c2) and fucoxanthin in mg g^{-1} DW, β-carotene and xanthophyll pool (VAZ) in µg g^{-1} DW, VAZ:Chl a ratio [µg mg^{-1}], de-epoxidation state of the xanthophyll pool (DPS), ratios of Acc:Chl *a* and Chl *a* to Carbon (Chl *a*:C) in mg mg^{-1}, antioxidative potential in Trollox equivalents (TE), total Nitrogen and total Carbon in mg g^{-1} DW, as well as their ratio (C:N). Values are means ± SD (n = 3, except * n = 2 and ** n = 1). Significant differences between origins at the same temperature are indicated by different capital letters and between temperatures of the same origin by different lowercase letters.

	Qingdao, China						Sylt, Germany					
	4 °C		8 °C		12 °C		4 °C		8 °C		12 °C	
Rel. ΔL d12 [% of d0]	23.22 ± 3.44	Aa	239.16 ± 23.20	Ab	199.73 ± 4.09	Ab	54.04 ± 5.89	Ba	158.62 ± 4.30	Bb	160.21 ± 0.00**	n/a
Rel. ΔWW [% of d0]	95.25 ± 23.62	Aa	1927.91 ± 125.14	Ab	1114.05 ± 95.06	Ac	219.73 ± 24.49	Ba	989.62 ± 54.06	Bb	572.33 ± 428.01	Aab
ΔWW:ΔL	0.63 ± 0.08	Aa	1.20 ± 0.04	Ab	0.89 ± 0.11	Ac	0.66 ± 0.01	Aa	1.07 ± 0.13	Ab	1.18 ± 0.00**	n/a
Chl *a* [mg g^{-1} DW]	0.85 ± 0.06	Aa	2.41 ± 1.04	Aa	1.70 ± 0.25	Aa	0.98 ± 0.05	Ba	2.73 ± 1.33	Bb	1.89 ± 0.27	Ab
Chl *c2* [mg g^{-1} DW]	0.37 ± 0.01	Aa	0.44 ± 0.07	Aa	0.43 ± 0.05	Aa	0.37 ± 0.03	Aa	0.60 ± 0.11	Aa	0.43 ± 0.06	Aab
Fucoxanthin [mg g^{-1} DW]	0.90 ± 0.02	Aa	0.96 ± 0.25	Aa	0.87 ± 0.09	Aa	1.01 ± 0.04	Aa	1.24 ± 0.33	Aa	0.87 ± 0.16	Aa
ß-carotene [µg g^{-1} DW]	105.46 ± 3.17	Aa	62.91 ± 7.08	Ab	75.75 ± 22.76	Aa	109.37 ± 5.00	Aa	92.18 ± 10.93	Aab	61.45 ± 12.10	Ab
VAZ [µg g^{-1} DW]	10.11 ± 1.30	Aa	24.31 ± 13.69	Ab	16.55 ± 6.14	Aa	23.80 ± 1.90	Ba	21.57 ± 13.07	Bb	16.29 ± 3.29	Ab
VAZ : Chl *a* [µg mg^{-1}]	11.86 ± 0.70	Aa	9.51 ± 2.05	Aa	9.53 ± 2.66	Aa	24.30 ± 2.73	Ba	7.63 ± 0.85	Ab	8.58 ± 0.57	Ab
DPS of VAZ	0.53 ± 0.06	Aa	0.20 ± 0.08	Ab	0.25 ± 0.06	Ab	0.25 ± 0.08	Ba	0.27 ± 0.08	Aa	0.21 ± 0.06	Aa
Acc : Chl *a* [mg mg^{-1}]	1.63 ± 0.08	Aa	0.68 ± 0.20	Ab	0.83 ± 0.21	Ab	1.56 ± 0.15	Aa	0.77 ± 0.17	Ab	0.73 ± 0.04	Ab
Chl *a* :C [mg mg^{-1}]	0.009 ± 0.010	Aa	0.007 ± 0.001	Ab	0.007 ± 0.001	Ab	0.003 ± 0.001	Aa	0.004 ± 0.000	Ba	0.009 ± 0.004	Bb
Antox. pot. [TE]	45.42 ± 4.28	Aa	40.33 ± 2.63	Aa	45.03 ± 4.27	Aa	39.16 ± 5.64	Aa	35.71 ± 7.93	Aa	41.11 ± 6.60	Aa
Total N [mg g^{-1} DW]	50.45 ± 9.74*	Aa	43.21 ± 0.40	Aa	43.65 ± 5.04	Aa	68.50 ± 26.34*	Aa	43.54 ± 1.55	Aa	45.42 ± 2.05	Aa
Total C [mg g^{-1} DW]	322.46 ± 81.13*	Aa	245.15 ± 3.37	Aa	264.88 ± 2.68	Aa	395.85 ± 162.98*	Aa	249.03 ± 4.03	Aa	276.01 ± 20.77	Aa
C:N	7.41 ± 0.44*	Aa	6.62 ± 0.03	Ab	7.14 ± 0.84	Ab	6.70 ± 0.20*	Ba	6.68 ± 0.34	Aa	7.09 ± 0.42	Aa

PERMANOVA and PCoA

The response variables rel. ΔL, rel. ΔWW, TE, C:N, Chl *a*:C, Acc:Chl *a*, VAZ:Chl *a,* and DPS were included in the PERMANOVA and PCoA. The PERMANOVA indicated significant differences among the variables origin (p = 0.0006), temperature (p = 0.0001), and their interaction (p = 0.0074). Post hoc testing was only performed on the temperature group as origin had two levels (post hoc not needed). The interaction of both variables resulted in output messages that indicated unreliable results and was therefore excluded. Significant differences were identified between 4 and 8 °C (p = 0.003) and 4 and 12 °C (p = 0.006), but not 8 and 12 °C. The PCoA plot highlighted a clear separation of the 4 °C treatments from the rest and, within this group, a clear distinction between the German and Chinese isolates. While oriented closer together, there was still a good separation between both isolates within the 8 °C and 12 °C treatment, as well as both origins irrespective of temperature (see Figure 6). The two first axes of the PCoA plot explain almost 70 % of the data.

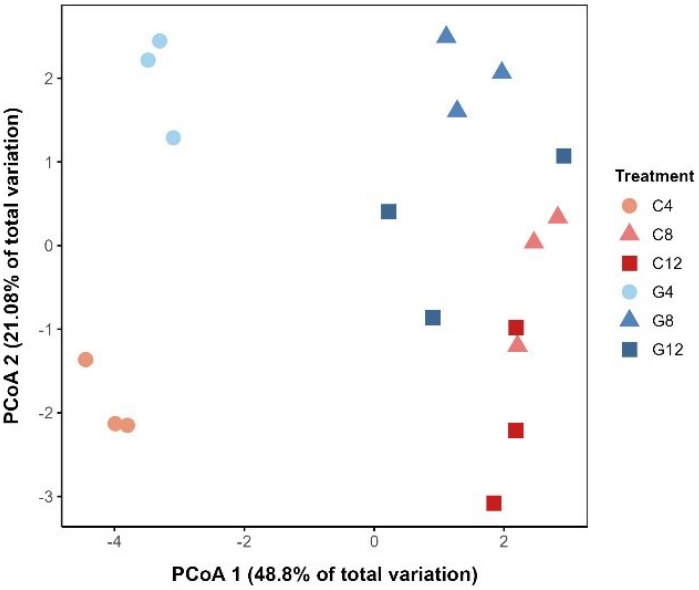

Figure 6: Principal coordinate analysis (PCoA) plot for sporophytes of *Undaria pinnatifida* sporophytes of the German (G, blue) and Chinese (C, red) isolates after cultivation under three different temperature treatments (4, 8 and 12 °C) for 12 days. The PCoA was based on Canberra distances and conducted on the scaled response variables relative length and wet weight increase (rel. ΔL, rel. ΔWW), Trolox equivalents (TE), carbon to nitrogen ratio (C:N), ratio of chlorophyll a to total carbon (Chl *a*:C), ratios of accessory pigments and xanthophyll cycle pigments to Chl *a* (Acc:Chl *a*, VAZ:Chl *a*) and the de-epoxidation state of the xanthophyll cycle (DPS). Dots, triangles, square represent 4, 8, and 12 °C, respectively.

Discussion

Physiological responses to temperature treatments

This study aimed to identify differences in morphological, physiological, and biochemical traits in response to different temperatures of sporophytes reared from an *U. pinnatifida* isolate originating from an invasive population in a region with lower average and minimum annual temperatures (Sylt, Germany) compared to a native one from a warmer region (Qingdao, China). Despite some confounding factors, such as the tissue decay of sporophytes at the highest temperature, which limited part of the data gathering, the results remained valid.

Both isolates showed distinct responses to the temperature treatments in the otherwise common garden approach. Growth (length and biomass) and reproduction are considered to be the key indicators of physiological stress (e.g., Dethier et al. 2005; Zinn et al. 2010; Harley et al. 2012; King et al. 2018). In the present study, both traits were less inhibited in the German isolate during cold exposure. While reproduction data was not included in this study, in a sub-experiment, only the German isolate became fertile at 4 °C, and the Chinese isolate reached fertility faster at 12-16 °C (Schiller et al., unpublished data). Morita et al. (2003) observed slightly larger (2-3 cm compared to 1 cm in this study) *U. pinnatifida* sporophytes from its southern distribution limit in Japan, grown for eight days, and assessed the area-based growth rate of the blades. They found the highest daily growth rate at 20 °C (25.8 %) and approximately 23, 16 and 8 % at 15, 10 and 5 °C, respectively. The approximate area-based growth rates of the German isolate in the present study were highly similar to those of Morita et al. (2003), although they used a lower nutrient concentration (20 % PES-iodine medium). The lower maximum growth rate in the German isolate compared to the Chinese one could be a result of limited genetic diversity brought on by the founder effect in the invasive population (Shan et al. 2019, 2023). The German and Japanese populations were located at the species' northern and southern distribution limits, where temperature stress occurs more frequently, while the Chinese isolate came from the species center of distribution and might be less adapted to tolerate changing conditions (Morita et al. 2003a, 2003b, Valladares et al. 2014). Gao et al. (2013b) found similar growth rates as the other Japanese study but included nutrients as a factor, identifying significantly lowered growth at the same temperature if nutrients were limited. We could not confirm this in the present study, as nutrients were abundant (fresh 50 % PES every four days, which previously did not lead to nutrient depletion, Schiller et al., unpublished data). The stable and low C:N ratio further supports that the isolates were not nitrogen limited (>10-15 N limitation; Hurd et al. 1996; Sjøtun et al. 1996). The cold temperature may have triggered nutrient accumulation in preparation for lower ambient nutrient levels in spring (Young et al. 2007). At 4 °C, total C and total N contents aligned with the data published in Gao et al. (2013b) for sporophytes grown in 25 % PES-iodine medium (i.e., the nutrient abundant treatment of the study).

In general, the biochemical analyses of both isolates supported the growth data and aligned with our hypothesis that the German isolate is better adapted or acclimated to low temperatures. The most striking example of this in the present study was the significantly higher VAZ:Chl *a* ratio, and higher content of pigments in general of the German isolate at cold temperatures (see Table 1 and Figure 5). A larger VAZ pool and its DPS are known acclimation responses to excess light, including excess light facilitated by a cold-induced lowered photosynthetic capacity (Li et al. 2009). It is likely that in the German isolate, no rise in DPS was observed due to the much larger pool size, which indicates better acclimation to the cold (Demmig-Adams and Adams 1996, Li et al. 2009). It seems likely that the coldest treatment caused such limitations in the Chinese isolate as only little biochemical protection could be built up (such as β-carotene). Mostly, patterns between the isolates were identical, with certain changes that can be expected in seaweeds exposed to low temperatures and relatively high irradiance (e.g., Li et al. 2009; Jahns and Holzwarth 2012). While irradiance remained unchanged in the present study, the cold temperature negatively affected the capacity to utilize energy in the temperature-dependent secondary photosynthetic reactions, which correlated to the lower concentration of Chl *a,* the pigment that captures light energy (Hanelt and Nultsch 2003) in relation to accessory pigments. The latter aid in photoprotection, helping to dissipate excess light energy and prevent photodamage, a process frequently described after high light exposure (e.g., Bruhn and Gerard 1996; Li et al. 2009).

The antioxidant capacities measured in this study align with levels in unstressed sporophytes assessed in another study (Bollen et al. 2016) and their observations that *U. pinnatifida* did not have to deplete its antioxidant pool to combat reactive oxygen species (ROS). They further did not find any changes in VAZ or Chl *a* content at 5 °C, and Diehl et al. (2021) did not find any changes in the pigment profile in response to temperature treatments for several kelp species. However, it is important to consider that they worked with adult thalli. The present study focused on young sporophytes, which are known to be more susceptible to environmental stress (e.g., Hanelt et al. 1997), which makes their limitations a key identifier for range limitations. Additionally, young sporophytes reared under laboratory conditions have been shown to give a valid representation of young wild material (Heinrich et al. 2016).

Despite the lack of biochemical data for the highest temperature treatment (16 °C), the earlier onset of tissue decay and much smaller appearance of the German isolate sporophytes suggest that they experienced more stress than the Chinese isolate at this temperature. This correlates with the observation that no sporophytes were found on Sylt from early to late summer, despite temperatures within the published upper tolerance limit of *U. pinnatifida* (<25 °C) and even within the optimum (20 °C, Morita et al. 2003a). These temperature requirements suggest that the German population behaves more like the cold-adapted one identified in northern Japan (Gao et al. 2013a) which had an upper tolerance limit of 22-24 °C.

The meta-analysis supported the previously discussed data. At the coldest temperature, both isolates showed clear visual separation and differed from the other temperatures.

Acclimation or adaptation?

The underlying cause for the distinct responses to the temperature treatments remains to be identified. Epigenetic mechanisms are described in seaweeds and these could significantly impact the next generation's response to stressors such as temperature (Gauci et al. 2022, Scheschonk et al. 2023). Genetic adaptation has been identified in Japanese *U. pinnatifida* populations, with northern ones exhibiting a significantly lowered temperature optimum (14-16 °C) than southern ones (18 °C) (Gao et al. 2013a). The former indicates that the temperature shift observed in the German isolate could indeed be due to adaptive mechanisms, which is undoubtedly possible for this kelp. While adaptation to novel environments may be a slow process for many invasive species, it may happen as fast as 20 generations or less and as little as 10 generations if it arises from existing alleles (i.e., standing genetic variation, Prentis et al. (2008). *U. pinnatifida* has persisted in the North Sea for more than 20 years already, having moved eastward toward German coasts more than 10 years ago (Minchin and Nunn 2014, Gittenberger et al. 2015). It is unclear when this kelp established itself in the colder waters near the German-Danish border. However, it is expected to be the latest in 2015 (D. Lackschewitz, pers. comm.). *U. pinnatifida* can produce two or more generations per year in Dutch and German waters (J. Schiller pers. obs.). Thus, genomic adaptations could have occurred within 5-10 years (Prentis et al. 2008), and epigenetic modifications could have occurred even faster (Anastasiadi et al. 2021). Both processes are, therefore, likely to produce additional adaptive traits in the coming years.

Plasticity, epigenetic modification, and genomic adaptation cannot be distinguished further without additional analyses, such as repeated generational crossing or genetic assessments (Gao et al. 2013a, King et al. 2018). Therefore, any observed differences between both isolates in the present study may be attributed to either process, which does not prevent the conclusion that a fundamental difference that gives the German *U. pinnatifida* an advantage under cold conditions exists. Previous cultivation conditions were shown to affect the performance of sporophytes due to acclimation (Heinrich et al. 2016). However, this was minimized as much as possible via the pre-cultivation in this study.

Future spread and impact

Distributional boundaries of seaweeds may not be stable and undergo shifts, reaching their distributional limits only after some time (e.g., Breeman et al. 1988), particularly if global warming promotes the disappearance of native species and leaves behind unoccupied hard substrata. Invasive species, such as *U. pinnatifida*, often have broader physiological niches and higher phenotypic plasticity (Davidson et al. 2011, Higgins and Richardson 2014), as well as shorter periods to reach fertility compared to native seaweeds, which tends to be highly advantageous when faced with changing abiotic pressures (Prentis et al. 2008, Lagos et al. 2017). It has been argued that one of the reasons *U. pinnatifida* has such success as an invasive species is its highly plastic physiology and life history traits that more closely resemble ephemeral seaweeds such as *Ulva* spp. than other kelps (Dean and Hurd 2007). Non-invasive species may have an advantage under conditions with more competition (Davidson et al. 2011), such as low nutrient environments or those with environmental patterns they are highly adapted to (e.g., winter growth of *Saccharina latissima* during the Arctic winter; Scheschonk et al. 2019). For *U. pinnatifida* as a NIS in the North Sea, eutrophication and progressive warming of the region (van Beusekom 2005 and references therein; Wiltshire et al. 2010; IPCC 2023), might be a significant advantage over native species.

Important factors that will decide if *U. pinnatifida* can establish populations further north will be the time and energy required to reach maturity, considering the drastically lowered growth rates at lower temperatures, but also its reported poorer ability to take up nutrients at low concentrations (Dean and Hurd 2007). However, considering previous shifts in its growth strategy from a winter annual to multi-annual in colder regions, it seems highly plausible that *U. pinnatifida* would appear as a spring and fall or even summer species at coasts much further north, especially if local ecosystems are impaired as a consequence of Climate Change. Impacts of invasions by *U. pinnatifida* worldwide have varied drastically, however, the current understanding points to the state of the recipient ecosystem as a key factor (South et al. 2017). Intact ecosystems are resilient, while open substrates in damaged ecosystems enable and promote the kelp's spread and negative impact (e.g., Epstein and Smale 2017; South et al. 2017). Whether *U. pinnatifida* will spread further north and establish in natural communities to a point of impact may, therefore, largely depend on the protection of intact native communities.

The distribution and abundance of marine species, including invasive ones like *U. pinnatifida,* are expected to be influenced by Climate Change in various ways. Ocean warming, observed and predicted to be especially prevalent in higher latitudes, may enable invasive species to colonize previously too-cold habitats to establish self-sustaining populations (Harley et al. 2006, James et al. 2015).

Most studies, including this one, focus foremost on the direct temperature effects of Climate Change; however, its impact is much greater. Patterns of ocean circulation are expected to change and affect upwelling areas and, thus, nutrient availability in various regions (Harley et al. 2006), including the northern coastal habitats under discussion here. Additionally, nutrient concentrations in northern coastal areas, currently disadvantageously low for *U. pinnatifida*, may increase due to higher precipitation and run-off (Harley et al. 2012). Higher frequency of extreme weather events and ocean acidification could make native communities more susceptible while at the same time providing an advantage to invasive species with higher phenotypic plasticity and tolerance to extreme conditions. Ultimately, this may create opportunities for NIS such as *U. pinnatifida* to settle and establish populations in higher latitudes (Harley et al. 2006, Davidson et al. 2011, Miller et al. 2011, Higgins and Richardson 2014).

While gaining knowledge via physiological research is important, as has been done for many decades, Harley et al. (2012) highlighted the importance of building strong mechanistic linkages to predicted future conditions and, thereby, a solid ecophysiological basis for predictions, which is still lacking. Therefore, further research on physiological responses to combined parameters and the performance of populations becomes essential. The case of *Undaria pinnatifida* in Europe provides a unique opportunity to follow these developments as there are no confounding effects of multiple populations or ecotypes mixing, but only a possible northward expansion and resulting changes and adaptations. Particularly, further comparisons of the German population with those preceding it further south in Europe, and native ones from various backgrounds may provide valuable insights. Considering this, further monitoring of its spread will be required to make these observations. Further research into *U. pinnatifida's* physiological adaptability will be essential to predict its future distribution and add to our understanding of kelp ecophysiology in the face of Climate Change.

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Chapter 6

Synoptic Discussion

6.1 Scientific contributions of this thesis

This study gathered new knowledge on the invasion status of *U. pinnatifida* in Europe, its expansion into a colder habitat, and the consequential changes in the kelp's physiological responses.

The to-date (2024) northernmost population was discovered on the island of Sylt (Germany), where it grew in association with invasive Pacific oysters. At least one more population grew likely deeper and caused sporophytes to wash ashore on the island. Both were characterized by low genetic diversity, and genetically nearly identical to each other, but distinct from other European populations. The *U. pinnatifida* population discovered in Germany likely originated from French populations in Brittany. On Sylt, it is self-sustaining and has since produced a sub-population in the island's harbor, which poses a possible risk for its further northward spread, facilitated by human vectors. Compared to sporophytes reared from a native isolate from China, the German *U. pinnatifida* sporophytes were less limited by the coldest treatment, and gametophytes even underwent gametogenesis. Strong phenotypic plasticity regarding morphology was observed in both isolates in response to being grown under laboratory conditions compared to in the wild and for wild German *U. pinnatifida* between those individuals growing in the oyster reef and those presumed to grow in the subtidal.

These new insights were individually explored in the publications presented in the previous chapters. They serve as the foundation for the synoptic discussion in the chapters hereafter.

6.2 From plasticity and acclimation to adaptation

Kelps and other seaweeds exhibit high levels of phenotypic plasticity in response to environmental conditions (see chapter 1.3). **Publications I and II** discuss the drastically different morphology of "floating" and "attached" sporophytes found on Sylt, which were proven to be of the same genetic origin in **publication II**. In **publication IV,** the juvenile sporophytes of both isolates displayed highly similar morphologies when grown under identical conditions. Figure 6.1 shows representative sporophytes of German and Chinese origin sampled in the ocean versus grown in tanks in the laboratory (see also Figure S1, appendix of publication IV for further lab-grown sporophytes). The morphologies of sporophytes of different genetic origins (Chinese native and German invasive) were more similar when grown under the same or similar conditions than sporophytes of the same origin grown under different conditions. Observations in cultivated populations in northern Spain support this (Peteiro and Freire 2014). All indications suggest that growth conditions seem to influence the morphology at least as much as the genotype, and no heritable differentiation (i.e., adaptation) occurred in the German population regarding morphology.

Figure 6.1: *U. pinnatifida* sporophytes collected from the wild, farm, and raised from isolates. From left to right: Floating sporophyte collected on Sylt (Germany), sporophyte from a farm near Qingdao (China; no wild sporophyte image available), lab-grown sporophyte of the German isolate (obtained from floating sporophytes), lab-grown sporophyte of the (wild) Chinese isolate. Images are not to scale. Photos by J. Schiller.

Sporophytes of both isolates were exposed to temperatures occurring naturally throughout the year at Sylt, Germany (see **publication IV,** Figure 1 (temperature graph)). These temperatures are lower than most native *U. pinnatifida* populations experience (James et al. 2015). At the northern limit of its native range in south-eastern Russia, *U. pinnatifida* sporophytes may grow from as low as 0 °C (Skriptsova et al. 2004). However, this northern population has not been used for farming, and *U. pinnatifida* in Europe is considered to be descended from a much more southern, farmed population (Sendai, Japan; see **publication III** and Voisin et al. 2005). The SST in Sendai ranges between 6-25 °C (2002-2008; Matsuoka et al. 2011), nearly identical to Qingdao, China, which is the population examined in this study (**see publication IV**, Figure 1 (temperature graph)). In its native range, including Sendai, *U. pinnatifida* occurs with one generation per year, and sporophytes appear in fall to winter (James et al. 2015). Therefore, gametogenesis in the native populations has likely adapted to warmer, pre-winter temperatures, which aligns with published data of gametogenesis occurring at an optimum of 10-20 °C, depending on the population (e.g., Floc'h et al. 1991; Morita et al. 2003; James et al. 2015; Sato et al. 2020). Southern range populations of *U. pinnatifida* have been shown to be adapted to warmer local conditions (Gao et al. 2013), and the results of **publication IV** confirm that the Chinese population does not tolerate cold temperatures to the same extent as the German one. Differences between the haplotype composition of the initial European population in Thau, France, compared to others along the English Channel have already been confirmed in a previous work, which hypothesized that additional introductions, or "rapid evolution" could have been possible explanations. No cold-adapted (i.e., northern native range limit) *U. pinnatifida* population or strain has been deemed a likely source of the European ones (Voisin et al. 2005). Considering the above, it is plausible that the capacity for cold tolerance was gained anew by the European *U. pinnatifida* during its northward spread.

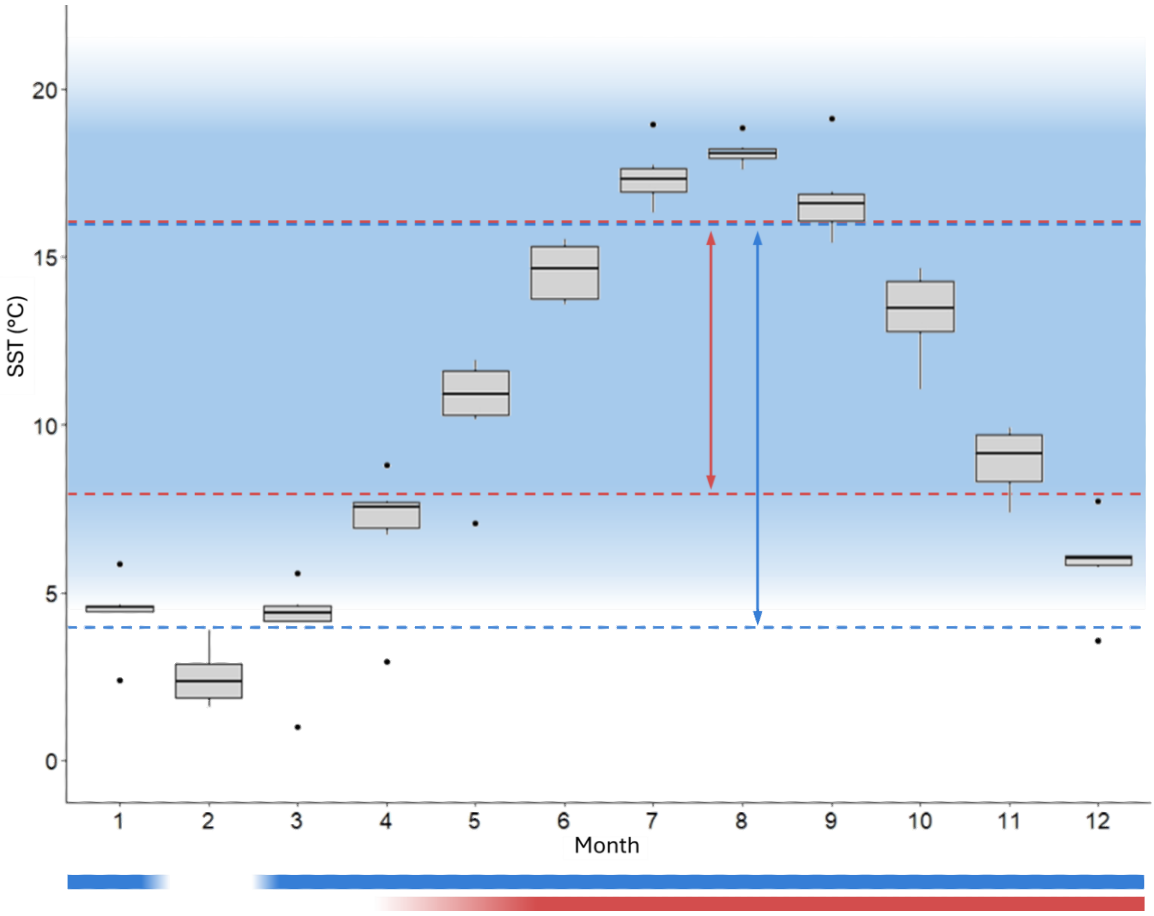

Figure 6.2: Reproductive windows for both isolates of *U. pinnatifida* confirmed in this study in the context of sea surface temperature (SST) at Sylt, Germany. Red color indicates the Chinese and blue the German isolate. Horizontal dashed lines indicate upper and lower gametogenesis temperatures tested in this study, and vertical arrows represent the range. The bars at the bottom indicate months in which reproduction and significant sporophyte growth could occur at Sylt. Blue shading indicates gametogenesis range indicated by literature (e.g., Morita et al. 2003). Each box represents the average monthly SST for the years 2012 to 2017 (n = 6), dots represent outliers. Figure by J. Schiller based on Data accessed via NASA Ocean Biology Processing Group (2023) presented in **publication IV**.

Publications II and III established that the German *U. pinnatifida* population is low in genetic diversity, which could lower its acclimation capacity and, thereby, its phenotypic plasticity. This aligns with observations of **publication IV**, which indicated lower performance at higher temperatures than the native isolate. Such a shift has likewise been documented at Japan's southern range limit of *U. pinnatifida*. Sporophytes from populations of three different latitudes all grew similarly below 18 °C. However, northern ones were limited above that temperature, which was confirmed to be an inheritable adaptation response (Gao et al. 2013). In the present case, pronounced differentiation was observed at 4 °C. At the same time, the two isolates were less clearly separated at 8, 12 (and 16) °C (see **publication IV**, Figure 3 (growth rates) and Figure 6 (PCoA plot)). While the present study lacks the multi-generational testing utilized in the example from Japan, the results still indicate that a heritable adaptation is likely in this case. Both isolates were kept under identical conditions for a year, yet performed differently afterward. In the case of phenotypic plasticity, one would expect that the isolate with larger genetic diversity (Chinese; **publication II and III**) would also exhibit acclimation. Furthermore, no evidence of gamete production in *U. pinnatifida* below 5 °C was found in scientific literature. However, it occurred in the German isolate (see **publication IV** and Figure 6.2 in this chapter). Concluding from the above, whether the tolerance range of the native isolate is truly wider than that of the introduced one would depend on their performance at temperatures high enough to cause inhibition of growth or reproduction in at least one of them. This threshold was not reached in the present study. It is possible that the cultivation in a limited volume amplified the decay at higher temperatures, either due to faster physiological processes in the sporophytes, or external factors such as pathogen activity (see **publication IV**). The reproductive temperature range observed in the present study compared to the literature is shown in Figure 6.2. It also depicts the theoretical reproductive windows based on temperature both isolates would have at Sylt, Germany, based on the experimental results of **publication IV**. The isolate obtained at Sylt would theoretically be able to reproduce throughout the year, with the exception of February, as temperatures this low were not tested in the study.

Identification of, and differentiation between adaptive mechanisms were outside of the power of the methods applied in this study. Therefore, they could not prove that an adaptation occurred, either in the form of epigenetic eco-evolutionary dynamics (i.e., genome-associated; see chapter 1.3), or genomic (i.e., adaptation in the traditional sense), but the results suggest it. Likewise, determining at what point a potential acclimation may have occurred requires further studies. The German isolate likely originated from Brittany, France, a population with higher minimum and mean annual SST (Murphy et al. 2017), which, in turn, originated from Japan or Korea (see **publication III**). While the water temperature in the most likely region of origin is near identical to Qingdao, China, other factors such as cultivation practices and genetic diversity may have affected the tolerance range. Only analyses of isolates from Brittany, and Japan, alongside the German one could definitively answer this question.

Given the documented adaptation potential of *U. pinnatifida* in literature, and the strong indications derived during the course of this study a spread further north in Europe is theoretically possible, enabled by (presumed) further adaptations to the colder environment.

6.3 Past, present, and future of *U. pinnatifida* in Europe

The history of species introductions is often impossible to reconstruct unless enabled by clear links to events, vectors, or via modern genetic technologies (see chapter 1.1.2). In the case of *U. pinnatifida* in Europe, the first two phases of the introduction (primary to the French Mediterranean, and secondary to French Brittany) have been documented, and directly linked to vectors (see Figure 6.3; Floc'h et al. 1991; Minchin 2007; Minchin and Nunn 2014). For its subsequent spread, the only conclusions can be drawn from proximity to or association with vectors, which has led to the assumption that recreational boating is one of the likely main vectors in Europe (Minchin 2007; Minchin and Nunn 2014). Other studies have identified cultivated populations from northern Japan or Korea as the likely origin for the primary introduction to Europe via haplotypes (Voisin et al. 2005). The findings presented and discussed in **publications II and III** align with this hypothesis, and further point to populations in French Brittany as the most likely source of the German *U. pinnatifida* at Sylt. Of the populations sampled for this study and those published in other works, it was the only match in haplotypes (see **publication III**).

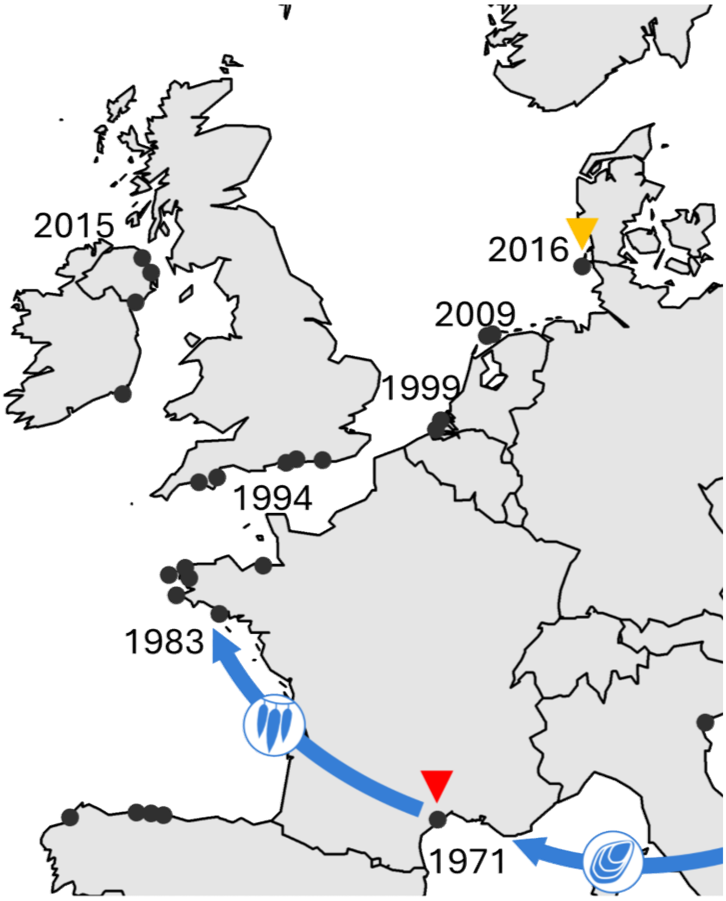

Figure 6.3: Progression of *U. pinnatifida* spread along the European coast. Dots represent exemplary populations mentioned in scientific literature, years indicate first records for the region (Minchin and Nunn 2014; Gittenberger et al. 2015, **publication I**). The red triangle indicates the first, primary introduction to Europe, the orange triangle the latest range expansion at Sylt, Germany (**publication I**). The blue arrows with symbols schematically highlight the known vectors and progression of spread (oysters, accidental from Japan, and kelp cultivation, intentional from the French Mediterranean to Brittany).

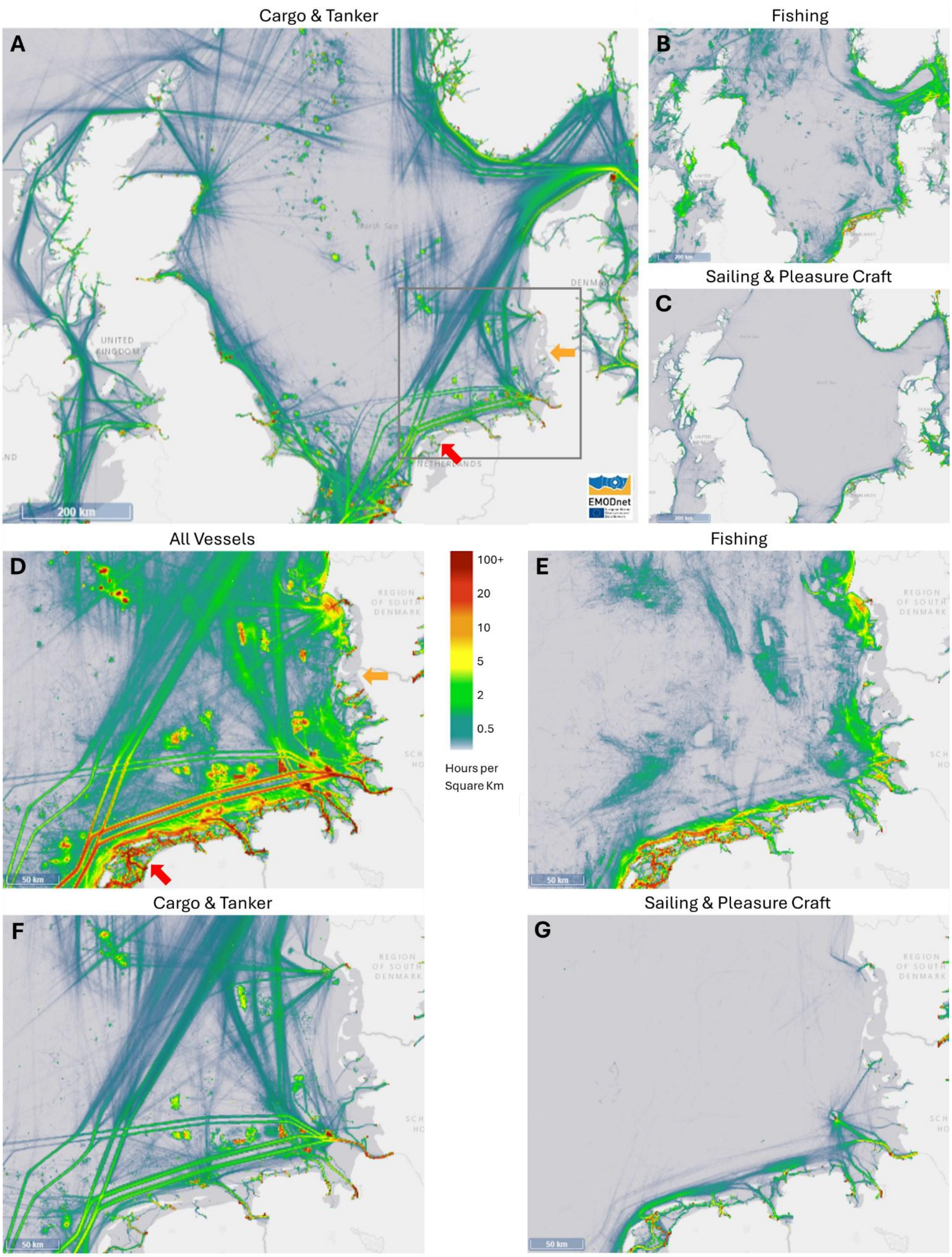

Figure 6.4: Vessel density in the Wadden Sea (2017-2022), shown as annual average hours per square Km per month. Vessel types are listed above each map. “All Vessels” includes categories not pictured here, the individual maps were sources from EMODnet.ec.europa.eu/geoviewer, see for metadata and details). The gray box in map A indicates the area depicted in map D-F. The red and orange arrows indicate the last known population prior to this study and the newly discovered (**publication 1**), north-easternmost population of *U. pinnatifida*.

Based on the genetic background, potential vectors of the spread can be inferred. In Figure 6.4, the vessel density in the North Sea (A-C) and Wadden Sea (D-G) is depicted by vessel type. Sylt was an unlikely site for the first German population, only accessed by a few vessel types, and much less frequented than other harbors of the Wadden Sea. When excluding those that move predominantly locally (e.g., ferries), only fishing vessels and recreational boating (sailing and pleasure craft) remain as possible vectors for long-distance transfer. Larger vessels do not access the harbor of Hörnum (Sylt). Considering the findings of **publication III**, fishing vessels are likewise implausible, as they do not transfer between French Brittany and Sylt. Recreational boating does, and therefore could be a possible vector. However, the first attached *U. pinnatifida* individuals were not found in the harbor, but further north in an oyster reef located near shellfish farms (D. Lackschewitz pers. comm., J. Schiller pers. obs.). While it is possible that a fertile individual detached from the hull of a boat and settled further north instead of in the harbor, accidental transfer alongside oyster- or mussel spat is also plausible. Such rare transfers would not be visible in the vessel density maps (Figure 6.4) or route density maps (not shown). Intense transfers of oysters and oyster spat have been documented since the 1980s or earlier (Drinkwaard 1998). Oyster spat cultivated at Sylt, Germany, has reportedly been imported from southern Ireland from the 1990s until the 2020s (Drinkwaard 1998; Sylter Royal 2020). However, all *M. gigas* initially originated in Asia, and many invasive species have been documented alongside its path of cultivation in Europe and elsewhere (e.g., Drinkwaard 1998, Wolff and Reise 2002). A recent analysis of direct and indirect connections in the Pacific Oyster cultivation network in Scotland showed intense interconnectivity and exchange with various other countries (Murray et al. 2020). Non-native species could easily hitchhike along those transfers. In conclusion, the vector that has brought *U. pinnatifida* to Sylt cannot unequivocally be identified, but both recreational boating and aquaculture vessels are highly likely. As the genetic analyses showed no differentiation between the floating and attached thalli, and both were of low genetic diversity, it was impossible to identify the initial point of introduction (see **publications II** and **III**). However, field observations over two years indicate that either the subtidal population was the primary one, or that it became a replenishing source after both populations were established. The floating thalli washed ashore in and around the oyster reefs with high frequency throughout the year, and fertile sporophylls often got caught in the tidal pools created by the oysters during low tide. The conditions in the pools, and retention time during low tides were ideal for triggering spore release and subsequent settlement. Additionally, the attached thalli only grew inside tidal pools, or on the landward side of the oyster reefs in small bays (see **publication I**; D. Lakschewitz pers. comm.; J. Schiller, pers. obs.).

The establishment of a new, northernmost continental European population on Sylt could potentially pave the way for further northward migration of *U. pinnatifida*. In April 2019, three years after the first thalli were discovered washed ashore on the island's east coast, a significant number of fertile individuals were found (and removed) in the boating marina at Hörnum, Sylt, about 5 km further south of the initial attached population (D. Lakschewitz pers. comm.). This discovery, along with the subsequent identification of a new generation of fertile specimens in June 2019, suggests a continuous source in the harbor itself, or in the surrounding area. With its establishment at the harbor, *U. pinnatifida* has crossed the barrier from a remote natural population from where it was unlikely to spread by natural means (see chapter 6.4; Schourup-Kristensen et al. 2023), to a location with access to a vector. The harbor at Hörnum, being well connected to neighboring islands and the mainland via recreational boating, ferries, fishing vessels, and other vessel types (see Figure 6.4 D, E, and G), poses a significant risk for further spread to these locations. Considering its spread northward, the research presented in **publications I and IV** indicates that only the barriers of time and means of transport are currently preventing it. Furthermore, in **publication II**, the attached and floating populations were shown to be genetically highly similar, indicating that a subtidal source population of fertile individuals exists near Sylt, which could promote further spread.

An indication for the possible northward progression of the *U. pinnatifida* invasion comes from the case of another invasive species, *Dasysiphonia japonica*, which shows striking similarities. The red alga was introduced from the North Pacific to French Brittany in 1984, possibly alongside oysters, and has since spread to Spain, Great Britain and Ireland, The Netherlands, Denmark, and finally Norway (Sjøtun et al. 2008). In Norway specifically, its spread has been linked to shipping and fishing activities due to its proximity to marinas and harbors. It has since become a dominant species in these habitats (Husa et al. 2004; Sjøtun et al. 2008). While *D. japonica* spread significantly faster than *U. pinnatifida*, the “stations” and likely vectors along the European coast are highly similar. Therefore, a transfer from The Netherlands, Britain, or Germany to Denmark and Norway might also be feasible for *U. pinnatifida*. A previous study deemed the range expansion of *U. pinnatifida* along large parts of the Norwegian coastline possible, given that temperature maxima were above 13 °C and minima above 0 °C (James et al. 2015). These findings were based on current (i.e., before 2015) *U. pinnatifida* populations and temperature data. They did not consider potential adaptations, or ocean warming. Both might extend the potential range even further north than initially predicted.

In its natural state, the Wadden Sea is an ecosystem of mud- and sand flats (e.g., Reise et al. 2023) that does not offer many sites suitable for the settlement of *U. pinnatifida* or other hard substrate dwellers. However, ongoing human activities such as the creation of wind farms may inadvertently create stepping stones by which sessile, invasive species can spread (Adams et al. 2014). Moreover, there seems to be a facilitation of the spread of *U. pinnatifida* by another invader, the Pacific Oyster, which established permanent hard substrate reefs in the Wadden Sea, and is a preferred settling ground for the kelp (**publication I**). Rocky shores, such as those found along the coast of Great Britain, provide much more substrate, especially when facilitated by artificial structures as stepping stones. In those cases, the propagule pressure from such sites is a significant factor affecting spillover into natural habitats (Epstein and Smale 2018; Schourup-Kristensen et al. 2023). Hull fouling and boat traffic between these hotspots are the most likely vectors by which migration northward and significant spread may occur. Thus far, *U. pinnatifida* and other invasive kelps, such as *Sargassum muticum*, have had no detectable negative effects on ecosystem functioning and biodiversity in the Wadden Sea. Despite this, the need for monitoring and regulations for vectors of invasion, in particular hull fouling, remains crucial (see **publication V**, appendix; Reise et al. 2023; Schourup-Kristensen et al. 2023).

6.4 Ecological implications and economic trade-offs

Reports on the effect of *U. pinnatifida* invasions in ecosystems worldwide vary drastically from one another (Epstein and Smale 2017). In some places, like Australasia, it primarily invaded open spaces and despite high biomass, had either none or a positive impact on native species and ecosystem productivity (South et al. 2017). However, in other regions the effects were more severe and unpredictable. For instance, different studies reported opposite effects for the same region in Argentina: one reporting higher native species diversity and abundance (Irigoyen et al. 2011), another drastically lower diversity (Casas et al. 2004). High economic costs and negative ecological impact have been associated with *U. pinnatifida* overgrowing harbors and commercial structures in Venice, and along the French coast (Sfriso and Facca 2013; Sfriso et al. 2020). In northern Portugal and Ireland, *U. pinnatifida* grows in marinas. However, no negative impact has been associated, and the kelp could not outcompete native species outside of the marinas (e.g., Veiga et al. 2014; Kraan 2017).

Despite its conspicuousness and highly focused research on the matter, *U. pinnatifida* does not seem to harm intact, natural ecosystems, but is rather a “passenger” than a “driver” of change (South and Thomsen 2016; South et al. 2017). This aligns with the highly diverging reports on the impact of the kelp in regions it invades. Those already under pressure from direct anthropogenic impact or Climate Change are more likely to experience a boom of *U. pinnatifida* once it is established. In contrast, with only minor unoccupied spaces others see only gradual spread. In particular, artificial substrates are well-documented for hosting large populations of the kelp due to the required unoccupied spaces and their typical proximity to vectors of invasion (Miller et al. 2011; Minchin and Nunn 2014; Epstein and Smale 2018).

The screening in search of such populations for **publication I** focused on such areas, and the French and Dutch reference populations were sampled in highly colonized boating marinas. The marina of Vlissingen, The Netherlands, was nearly exclusively covered by *U. pinnatifida* at the time of sampling in 2016 (J. Schiller, pers. obs.).

Responses to invasions by *U. pinnatifida* are also highly variable in scope and methodology. In a 2017 review, dedicated management strategies targeting *U. pinnatifida* were only found in New Zealand and Australia (Epstein and Smale 2017). No specific measures have been taken in many countries, possibly due to high associated costs, efforts, and often questionable success (Epstein and Smale 2017).

While *U. pinnatifida* outside of its native range is usually considered undesired, its fast growth rates compared to other kelp species make it an attractive option for biomass generation and carbon sequestration in the blue economy sector (e.g., Sato et al. 2021). Unlike Australia and New Zealand, Spain, The Netherlands, and France have opted for acceptance and inclusion of the kelp. Farming of *U. pinnatifida* has been carried out in France since 1984 (see **publication I**), in Spain since 2003 (Peteiro 2008), and in The Netherlands at least since 2021 (Dutch Seaweed Group 2022). While the farming practices in France are considered the vector by which the kelp was first brought to the East Atlantic coast, those in The Netherlands and Spain began long after its spread into the region (Peteiro 2008; Báez et al. 2010; Gittenberger et al. 2015). The farming approaches in Europe may be a risk for further spread, while they may also provide a possibility for diversification in aquaculture. In northern Spain, at the southern distribution limit of the native kelp *S. latissima*, both species have been cultivated (Peteiro et al. 2016). However, populations of native kelps are disappearing under the pressure of rising SSTs (Voerman et al. 2013), posing a risk to the region's blue economy. Unlike the native species, *U. pinnatifida* tolerates these changed conditions easily and allows for continued farming.

In The Netherlands, cultivation occurs in the Oosterschelde (Dutch Seaweed Group 2022). *U pinnatifida* occurs there in abundance (J. Schiller pers. obs.). The region is characterized by agri- and aquaculture, and the native ecosystem has been dramatically altered by coastal construction (Wetsteyn and Kromkamp 1994). Populations of the native kelp *Saccharina latissima* are scarce today, possibly due to elevated summer water temperatures and other factors (e.g., Jiang et al. 2022; J. Schiller pers. obs.). This poses a risk to proposed kelp farming approaches (e.g., Jiang et al. 2022), as native isolates are required for sustainable practices, which become more and more difficult to obtain and run the risk of low genetic diversity and unintentional inbreeding (J. Schiller, pers. obs.). As discussed in **publication IV**, the local (German) *U. pinnatifida* population still possesses the ability to grow at higher temperatures while also being able to tolerate the lower ones occurring in more shallow areas over winter. In any case, given the previous history of *U. pinnatifida*, cultivation should only be considered after careful risk assessemnt and based on scientific knowledge, rather than driven by economic prospects alone.

In other regions that rely on the ecosystem services provided by the kelp forests, their decline or damage due to Climate Change could be compensated by invaders that fill unoccupied niches or fill spaces freed up by other canopy-forming kelps. Reise et al. (2023) have warned that while preventing invasions remains essential, novel species could help the Wadden Sea ecosystem, characterized by low biodiversity, to cope with Climate Change, and have a beneficial impact. This aligns with *in situ* observations thus far. In the eight years since the discovery in Germany, no explosive spread of *U. pinnatifida* has occurred in the region despite the population stretching across at least three nearby locations (**publications II** and **III**; pers. obs.). Similar observations were made in the Republic of Ireland (Kraan 2017). This is unlike reports from Plymouth, UK, where it became highly abundant and omnipresent, especially in marinas, within a decade (Heiser et al. 2014). Recent hydrodynamic studies of the Northern Wadden Sea could provide an explanation. While the Wadden Sea shows strong intra-basin connectivity, the natural migration from one basin to the next is unlikely or slow. However, if it occurs, it does so in a northward direction, which may facilitate the northward spread of *U. pinnatifida* discussed in **publication I** (Schourup-Kristensen et al. 2023).

Regarding its spread further north, to Scandinavia along the Danish and later Norwegian coast, only speculations are possible about its impact. As discussed in previous chapters (see 1.1.3 and 1.1.4 and **publication IV**), to date, it is not considered possible to predict an invasive species' impact. However, some generalizations allow for pointing out risk factors that could lead to a more intense invasion. In the case of *U. pinnatifida,* the predominant factors facilitating spread in natural communities are unoccupied substrate, proximity to vectors (e.g., harbors), and propagule pressure from them (Epstein and Smale 2017). While proximity cannot be affected, propagule pressure can be lowered by regulations, and removal of large individuals (Schaffelke et al. 2005;

Clarke Murray et al. 2011). Open substrates freed up by the disappearance of native species are a risk factor that is only suspected to increase with Climate Change (Filbee-Dexter et al. 2020) and direct human activities such as dredge harvesting of native kelps or other organisms (Werner and Kraan 2004). Dredging impacts the local ecosystems and removes significant parts of native *Laminaria* spp. forest, which will take years to recover (Werner and Kraan 2004; Epstein and Smale 2017; Fraser et al. 2017). Rising SSTs may cause lowered fitness and patchier communities of native kelps due to loss of native species (Smale 2020). Both leave open substrate, and once occupied by invasive species such as *U. pinnatifida*, regrowth of slow-growing, multi-annual native species becomes less likely (Epstein and Smale 2017). As invasive populations of *U. pinnatifida* may occur nearly year-round, they would likely have an even higher impact (James et al. 2015). Therefore, the prevention of direct and indirect damage to native ecosystems, and the limitation of invasive species' means of spreading have to be considered the best options.

6.5 The invasion potential of *U. pinnatifida* in Europe: Research questions and hypotheses revisited

Answer to research question 1a:

Can sporophytes of *U. pinnatifida* be found along the coast in the Dutch-German border region?

Surveying of marinas and other structures often linked to *U. pinnatifida* invasions led to the discovery of a population growing in an oyster reef (*M. gigas*) on Sylt, Germany, in the Northern Wadden Sea (**publication I**). Located more than 240 km of direct distance from the nearest documented population in The Netherlands, this by far exceeded the expected range, and brought more focus to exploring associated vectors (**publication V**) and source populations (**publications II** and **III**). Furthermore, in 2019, another sub-population was discovered in the biggest harbor on Sylt. Despite repeated searches in this work's context and large species monitoring campaigns, no populations were documented in the Dutch-German border region.

Answer to research question 1b:

Can newly established *U. pinnatifida* populations be self-sustaining, or do they rely on continuous re-supply from larger, established populations?

Sporulation trials on *U. pinnatifida* sporophytes from all sub-populations found on Sylt were successful in releasing viable spores. After establishing an isolate, sporophytes were obtained, and grown out to maturity (**publications I** and **IV**). Therefore, by 2019, three self-sustaining sub-populations existed on Sylt, and field observations suggest they can replenish each other. Thus, the populations or Sylt are fully self-sustaining at this point.

Answer to research question 2:

Are the larger, floating (i.e., detached) sporophytes found off the coast of Sylt the source of the tidal pool population?

In **publication II**, the floating and attached populations' genetic connectivity was explored via microsatellite analysis. The two Sylt populations were found to be highly similar, but with a clear distinction from other European, as well as native reference populations. Likewise, a marked reduction of alleles and heterozygosity aligned with the patterns expected in a newly established population experiencing the founder effect. The genetic analyses in **publications II** and **III** could not identify which Sylt sub-population might be the source. The hydrodynamics of the region, and the frequent observation of fertile floating thalli being caught in the same tidal pools the attached ones were found in strongly suggest that either the subtidal population is the primary source, or that it became a replenishing source after both populations were established.

Answer to research question 3:

What is the most likely source population of the newly established one at Sylt, Germany?

The comparison of European *U. pinnatifida* populations based on mitochondrial DNA sequences revealed identical haplotypes in the populations from Germany as in others sampled for **publications II** and **III**, and already published ones from Brittany in France. The identified haplotypes were only found in farmed populations from Japan and Korea (Voisin et al. 2005), and introduced European ones. They were distinct from invasive populations analyzed, e.g. in the US and other parts of the world. The German population's most likely source was France, and the initial primary source population was likely Japanese or Korean (Voisin et al. 2005).

At the same time, it is essential to consider that the analyzed populations are only snapshots, and many more exist in other non-sampled sites. It is, therefore, also possible that between Brittany and Germany, further intermediary populations exist.

Answer to research question 4a:

Does the German isolate perform better at colder temperatures than the Chinese isolate?

The growth of lab-grown German sporophytes was significantly less inhibited than that of the Chinese ones at the coldest temperature (4 °C), and a significant peak of VAZ:Chl *a* indicated a higher capacity to mitigate light stress in the German isolate. In the PCoA analysis of all assessed parameters (length, biomass, antioxidants, C:N ratio, antioxidants, and pigments), the German and Chinese isolates were distinct from each other, as well as from the other temperature treatments at 4 °C. Therefore, it can be concluded that the German isolate was more viable than the Chinese at cold temperatures.

Answer to research question 4b:

Does the Chinese isolate have a wider temperature tolerance range than the German isolate?

Center-range populations often have a wider tolerance range than edge populations. The Chinese *U. pinnatifida* isolate in **publication IV** represented the former population type. While it underperformed compared to the German one at the coldest temperature both were exposed to, it showed a higher growth rate, faster induction of gametogenesis, and later onset of thallus decay in the highest temperature treatment (16 °C) in the study. Several possible explanations remain. The Chinese isolate may have

(i) a wider range, shifted towards warmer temperatures,
(ii) a similar range, shifted towards warmer temperatures,
(iii) a narrower range with similar or slightly higher lethal limits than the German isolate.

With the data obtained in **publication IV**, a wider tolerance range could not be confirmed; however, the literature suggests that reproduction is possible until 20 °C, and the lethal limit for native populations is at 27 °C.

A wider range of temperatures would need to be applied to answer this question for the isolates in question definitively.

Answer to research question 4c:

Can gametophytes of the German isolate reproduce successfully at colder temperatures than the Chinese isolate?

In **publication IV**, gametogenesis could only be triggered in the German isolate, not the Chinese one in the coldest laboratory treatment (4 °C). At 8 °C, both isolates reproduced successfully. While no temperatures between 4 and 8 °C were applied in the presented experiments, the performance at 4 °C allows the conclusion that the German isolate can reproduce at lower temperatures than the Chinese isolate.

Hypothesis I

***U. pinnatifida* will establish self-sustaining populations further North and Eastward along the European coast, predominantly in sites with a strong association with its main proposed invasion vectors in Europe (i.e., recreational boating).**

A self-sustaining population of *U. pinnatifida* was found at Sylt, Germany, in association with another invasive species, *M. gigas*, as its primary substrate. Oyster transfer has been considered the second most important vector for invasive species transfer in Europe (Wolff & Reise 2002). Since the growth site of the "floating" *U. pinnatifida* population at Sylt could not be identified prior to its subsequent spread into the nearby harbor, hull fouling and aquaculture activities are possible vectors *sensu* Michin (2007).

Hypothesis II

The smaller sporophytes found growing attached in tidal pools off Sylt, Germany, and the larger ones found floating belong to the same population – differences are due to phenotypic plasticity.

Results presented in **publications II** and **III** showed that the two populations are genetically highly similar, with only minor differences, which can likely be attributed to the founder effect at such an early stage of the invasion at Sylt. When grown out to maturity under space-restricted (i.e., tank) conditions, sporophytes reared from an isolate obtained from the floating population showed a morphology highly similar to the attached growing population at Sylt, which was likewise space-restricted in the tidal pool.

Hypothesis III

The source of the newly established *U. pinnatifida* off Sylt, Germany, is other, high-proximity European populations.

The analysis of mitochondrial DNA sequences and subsequent identification of two haplotypes present in the German populations linked them to other European populations analyzed in **publication III**, and those published in scientific literature. However, the closest match was not found in the sample from the highest proximity population (The Netherlands), but in samples from Brittany in France. Whether another population served as a stepping stone between the two sites could not be elucidated.

Hypothesis IV

U. pinnatifida, **found on Sylt, Germany, has adapted to the colder sea surface temperatures and exhibits higher cold tolerance than the native, center range population from Qingdao, China, which has a lower cold tolerance but wider overall temperature tolerance.**

In the coldest treatment presented in **publication IV**, only the German isolate underwent gametogenesis. It also grew faster, and was distinct from the Chinese one in the meta-analysis (PCoA). At the warmest applied treatment, the Chinese isolate produced sporophytes faster, grew faster, and decayed later than the German isolate. While the German isolate displayed higher cold tolerance, it is not conclusive whether this is due to acclimation or adaptation, despite the latter being feasible based on the characteristics *U. pinnatifida* displayed in other studies. The wider temperature tolerance of the Chinese isolate could not be confirmed, as no maximum or optimum temperature could be identified during the experiments presented in **publication IV**.

Hypothesis conclusion

Hypotheses I, **II**, and **III** could be confirmed via the findings of **publications I**, **II**, and **III** and the associated research questions. **Hypothesis IV** could only partially be confirmed via **publication IV**. **Research questions 4a** and **4c** support the hypothesis, while a conclusive answer to **4b** was not found. Therefore, no confirmation of whether the Chinese isolate has a wider temperature tolerance was possible.

6.6 The discrepancy between theory and practice in invasion biology

With human-mediated changes affecting the world, as discussed in the previous chapters it is clear that the field of invasion biology is essential for understanding, mitigating, and preventing invasive species' spread and negative impact. Biological invasions increase alongside human activities, and other environmental changes attenuate and amplify their effects. Within the field of invasion biology many challenges make addressing these issues more difficult. One which will not be discussed further in this study, but is continuously being addressed by others (Colautti and MacIsaac 2004; Ricciardi et al. 2013), is that of clearly lacking uniformity in terminology and categorization. Another widely criticized aspect is the lack of connectivity in research (e.g., Blackburn et al. 2011). Many studies are literature reviews focused on theoretical or hypothetical scenarios, but lacking direct observations (Blackburn et al. 2011). Studies that connect ecological theories with real-world observations of species range shifts, especially facilitated by environmental changes, are much needed. Meaningful theoretical frameworks are still not universally accepted as preferences diverge between the disciplines (e.g., terrestrial and aquatic invasion science; Blackburn et al. 2011). They are either seen as too broad, or too specific, and most of all as lacking a basis in studies that connect the non-native species and the invaded ecosystem via functional ecology (Ricciardi et al. 2013). Moreover, today's rapidly changing environmental conditions may not be adequately reflected in research that often focuses on interactions and dynamics under stable conditions (Simberloff et al. 2013). Many studies view invasive species and recipient ecosystems as static systems, disregarding evolutionary dynamics (Whitney and Gabler 2008). Much research on marine species' responses to Climate Change, including invasive ones, does not consider the potential for adaptation, thus hampering the capacity to predict how they will adjust to Climate Change (Munday et al. 2013; Reusch 2014).

Even though a tremendous amount of research has been conducted on the topic it is still not fully clear what causes a species to become invasive (see chapter 1.1.3; (Alpert et al. 2000; Whitney and Gabler 2008), and predicting exact bioinvasion events is as impossible as complete eradication in most cases (see previous chapters). Another potential pitfall in invasion studies is the diversity of comparisons that are made between different groups of species, such as invasive species in their native vs. invaded habitat, or an invasive species vs. a native one in the invaded habitat. This may answer inherently different questions that are not always differentiated (Van Kleunen et al. 2010). Some recent developments seem to move away from trying to agree on a single detailed, all-encompassing framework that defines invasions, but rather one that guides research, collaboration, and actions agains invasive species (Blackburn et al. 2020).

6.7 Conclusion

This work aimed to add to the understanding of marine bioinvasions by examining the ongoing spread of *U. pinnatifida* in Europe, which proves particularly interesting in regard to the interconnection of the invasion process and biological changes in the invading organism. The invasion of *U. pinnatifida* at the edge of its distributional range was examined in detail, and from several angles: locate its northernmost population, characterize it, uncover its source and connectivity to other populations as well as potential vectors, identify potential changes in its physiological responses to the colder environment and finally, discuss the implications for future distributions.

U. pinnatifida was found much further northwest along the European coast than expected during the conceptualization of this study. The new population was equivocal, as it offered a unique research opportunity, but also posed the risk of increased proliferation. Two populations were initially discovered at Sylt, Germany – one associated with a reef assemblage dominated by the invasive Pacific oyster *M. gigas*, the other being washed ashore with no known source. A third location in the island's harbor was discovered later. Specimens of the attached population grew much smaller than the floating ones, yet both were fertile, and the life cycle could be closed in the laboratory. All discovered sub-populations were self-sustaining.

Both sampled German *U. pinnatifida* populations were shown to be highly similar, and the former was continuously resupplied by the latter, while both were also self-sustaining. Their genetic heritage could be linked to populations from French Brittany: However, as neither all populations along the English Channel could be sampled, nor all hard substrate areas be assessed during this study, a stepping stone between the locations could not be excluded. *U. pinnatifida* likely arrived to Sylt by recreational boating, or aquaculture, and is only likely to spread from there by the former, given the isolated nature of the island with only limited boating and isolating hydrodynamics. Regardless, it is expected that the kelp will spread further north via one of the many harbor populations in central Europe. In laboratory experiments presented via this study, the first reported case of gametogenesis in the species below 5 °C was reached in the German isolate, while the native Chinese isolate remained fully vegetative. Likewise, sporophytes of the German isolate exhibited significantly less growth-limitation at the coldest treatment than its Chinese counterpart and both were clearly distinguishable during meta-analysis. Data was found insufficient to conclusively identify the underlying mechanism. However, the genetic history and connectivity of the German population, performance of the two isolates under different temperatures, and analogies to literature allow the hypothesis that adaptation, rather than acclimation might be the cause. The latest findings showcasing epigenetic temperature adaptation in kelps further support that such modifications are to be expected in a population of a historically highly adaptive species. Results obtained during this study provide valuable insight into an ongoing evolutionary process, and link it to human-facilitated bioinvasion mechanisms.

U. pinnatifida has an ambiguous future in Europe, especially in light of the Climate Crisis. It might increase the resilience in low-diversity ecosystems such as the Wadden Sea, and could provide a valuable farming resource in mono-culture areas, while also threatening the kelp forests of the rocky Norwegian shores if native kelps become less abundant due to Climate Change.

6.8 Future perspectives

The present study was able to answer many questions regarding the expansion of *U. pinnatifida* to its current northern distributional limit. At the same time, it raised new ones to be examined further in future studies. The underlying mechanisms for the cold tolerance of the German isolate remained unclear. Further research, including multi-generational cultivation and comparisons with predecessor populations (e.g., from France and Japan), would be required to prove that a heritable adaptation occurred. In addition to these more traditional approaches, which may differentiate between acclimation via phenotypic plasticity or adaptation, the novel field of epigenetics could shed light on the underlying mechanism of adaptations. Many previous studies mention "rapid adaptation or -evolution", or "micro-evolution" to indicate heritable changes that happen faster than expected via genetic adaptations (Prentis et al. 2008; Jones and Gomulkiewicz 2012). A possible explanation for these unusually fast evolutionary processes is epigenetics. Marine epigenetics is only recently being considered, and the field is very new (Eirin-Lopez and Putnam 2021). Recent findings have proven that epigenetic modifications that do not alter the genetic sequence do occur in seaweed (e.g. (Gauci et al. 2022; Scheschonk et al. 2023), and they are considered valuable tools for exploring bioinvasions (Eirin-Lopez and Putnam 2021).

While the lower tolerance limit of the isolates was explored in this work, it did not cover the entire range of temperature performance, especially at higher temperatures. Examining the entire life cycle of different *U. pinnatifida* isolates at a wider range of temperatures and with finer scaling at the suspected cold threshold could give an answer to the question of variable tolerance ranges between the isolates. Likewise, this would shed light on the possibility of spreading further north along the Norwegian coast, and into the subarctic. While it has been suggested that *U. pinnatifida* might be able to grow and reproduce anywhere temperatures align with its current distributional range (James et al. 2015; Murphy et al. 2017), this does not take into account the potential for adaptation at the distributional edge. Further abiotic factors such as light and nutrients may aid or limit a further spread. To evaluate the potential northern distribution limit of *U. pinnatifida*, excluding possible further adaptations, interactions, or possible emerging properties between temperature, light, and nutrients would require investigation as well. Specifically, life-cycle transitions (gametogenesis and sporogenesis) and maturation need to be completed within the limitations of higher latitudes for a species to persist there.

In principle, its fast growth and valuable biomass make *U. pinnatifida* an interesting cultivation opportunity for the blue economy – regarding bioremediation, carbon sequestration, and resource production. Particularly in regions where native cultivated kelp species are being driven north by rising SST, *U. pinnatifida* might offer a solution. However, its background as an invasive species makes *U. pinnatifida* an unpredictable option that requires careful, science-based considerations. In regard to potential consequences of further spread of *U. pinnatifida*, literature agrees on the impossibility of accurate prediction. However, previous occurrences of the kelp worldwide permit the assumption that intact ecosystems are relatively resilient against its invasion. The best strategy to limit or prevent the unknown consequences of this bioinvasion is therefore the protection and restoration of healthy, native ecosystems. This includes limiting direct human impact, as well as indirect impact via ocean warming in the light of our current Climate Crisis.

The invasion of *U. pinnatifida* in Europe, while highly undesired from an ecological standpoint, provides a very unique opportunity to further the understanding of species range expansions and associated evolutionary changes under the effect of Climate Change. The spread of this invasive kelp across diverse ecosystems from genetically distinct source populations, combined with its fast generation time, could make it an ideal model organism to investigate questions of eco-evolutionary dynamics such as acclimation, adaptation, and specifically epigenetics.

Chapter 7 References

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Chapter 8 Appendix

8.1 Appendix of Publication II

Table S1: Sampling information of the wild populations of *Undaria pinnatifida* from Europe and China

Code	Location	Collection time	Coordinate	No. individuals	Growing substrate
DEU1	Sylt, Germany	Winter 2016/17	54°47'N 8°18'E	30	Drifting (washed ashore)
DEU2	Sylt, Germany	June 2017	54°47'N 8°18'E	30	Oyster reef
NLD1	Marina Vlissingen, Netherland	June 2016	51°26'N 3°34'E	30	Floating pontoons, buoys
NLD2	Marina Terschelling, Netherland	July 2017	53°21'N 5°13'E	30	Floating pontoons, buoys
FRA1	Marina of the Moulin Blanc, Brest, France	June 2016	48°23'N 4°25'W	30	Floating pontoons
FRA2	Castle Marina, Brest, France	June 2016	48°22'N 4°29'W	30	Floating pontoons
GBR	Plymouth, Great Britain	July 2017	50°21'N 4°07'W	30	Floating pontoons
DL	Dalian, China	April 2016	38°47'N, 121°16'E	30	Cultivation rafts
QD	Qingdao, China	April 2016	36°03'N, 120°22'E	30	Rocky reef
GQ	Gouqi Island, China	April 2016	30°42'N, 122°45'E	29	Plastic Buoys

Table S2: Genetic diversity of the populations of *Undaria pinnatifida* from northern Europe and China at each microsatellite locus

Locus	Parameter	DEU1	DEU2	NLD1	NLD2	GBR	FRA1	FRA2	DL	QD	GQ
UPN130	N_a	1	1	2	2	3	3	3	12	6	6
	H_o	0	0	0.267	0.133	0.500	0.267	0.200	0.767	0.300	0.448
	H_e	0	0	0.231	0.320	0.389	0.289	0.309	0.732	0.424	0.660
	F_{is}	N/A	N/A	-0.154	0.583	-0.284	0.079	0.354	-0.047	0.293*	0.321
UPN161	N_a	1	1	1	1	1	1	1	7	6	6
	H_o	0	0	0	0	0	0	0	0.567	0.700	0.517
	H_e	0	0	0	0	0	0	0	0.599	0.672	0.497
	F_{is}	N/A	N/A	N/A	N/A	N/A	N/A	N/A	0.054	-0.042	-0.041
UPN1143	N_a	2	2	1	1	2	3	3	9	7	6
	H_o	0.633	0.233	0	0	0.167	0.467	0.433	0.900	0.767	0.483
	H_e	0.486	0.495	0	0	0.375	0.531	0.516	0.819	0.782	0.590
	F_{is}	-0.303	0.529	N/A	N/A	0.556	0.121	0.160	-0.098	0.019	0.181
UPN1528	N_a	1	2	3	2	4	5	4	17	7	12
	H_o	0	0.100	0.233	0.033	0.500	0.633	0.433	0.867	0.567	0.931
	H_e	0	0.255	0.292	0.033	0.653	0.746	0.583	0.918	0.686	0.867
	F_{is}	N/A	0.608	0.200	-0.017	0.234	0.151	0.256	0.056	0.173	-0.073
UPN3177	N_a	1	1	2	1	3	6	4	6	3	4
	H_o	0	0	0.333	0	0.633	0.567	0.500	0.433	0.400	0.483
	H_e	0	0	0.391	0	0.609	0.671	0.653	0.613	0.399	0.435
	F_{is}	N/A	N/A	0.148	N/A	-0.039	0.156	0.234	0.293	-0.001	-0.111
UPN3197	N_a	4	1	5	1	3	3	3	8	8	12
	H_o	0.033	0	0.200	0	0.133	0.267	0.167	0.767	0.733	0.931
	H_e	0.127	0	0.461	0	0.646	0.531	0.383	0.812	0.704	0.892
	F_{is}	0.738*	N/A	0.566	N/A	0.794*	0.498	0.565	0.056	-0.042	-0.044
UPN3205	N_a	1	2	3	1	2	2	1	6	5	5
	H_o	0	0.200	0.567	0	0.300	0.033	0	0.467	0.500	0.655
	H_e	0	0.180	0.429	0	0.473	0.033	0	0.736	0.599	0.637
	F_{is}	N/A	-0.111	-0.320	N/A	0.365	-0.017	N/A	0.366*	0.165	-0.029
UPN3530	N_a	2	2	3	3	5	2	6	12	5	8
	H_o	1.000	1.000	0.433	0.933	0.433	0.133	0.533	0.933	0.333	0.448
	H_e	0.500	0.500	0.389	0.573	0.574	0.231	0.511	0.826	0.601	0.523
	F_{is}	-1.000*	-1.000*	-0.113	-0.629	0.245	0.423	-0.045	-0.130	0.445	0.142
UPN6327	N_a	1	1	3	1	2	5	4	10	7	10
	H_o	0	0	0.333	0	0.300	0.567	0.533	0.700	0.400	0.793
	H_e	0	0	0.335	0	0.495	0.582	0.687	0.729	0.503	0.815
	F_{is}	N/A	N/A	0.005	N/A	0.394	0.027	0.223	0.040	0.205	0.026
UPN9919	N_a	2	2	4	1	2	3	4	12	11	13
	H_o	0	0.167	0.400	0	0.333	0.467	0.600	0.600	0.767	0.828
	H_e	0.064	0.206	0.625	0	0.480	0.549	0.574	0.829	0.753	0.843
	F_{is}	1.000	0.191	0.360	N/A	0.306	0.151	-0.045	0.277*	-0.018	0.018

N_a number of alleles, H_o observed heterozygosity, H_e expected heterozygosity, F_{is} inbreeding coefficient, *significant departure from Hardy-Weinberg equilibrium ($P < 0.05$)

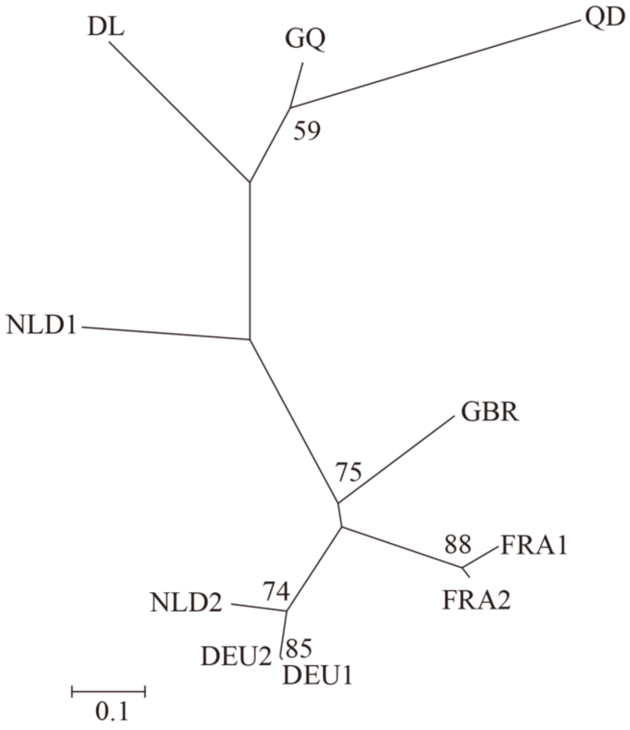

Figure S1: Genetic distance-based dendrogram that groups all populations into three major clusters.

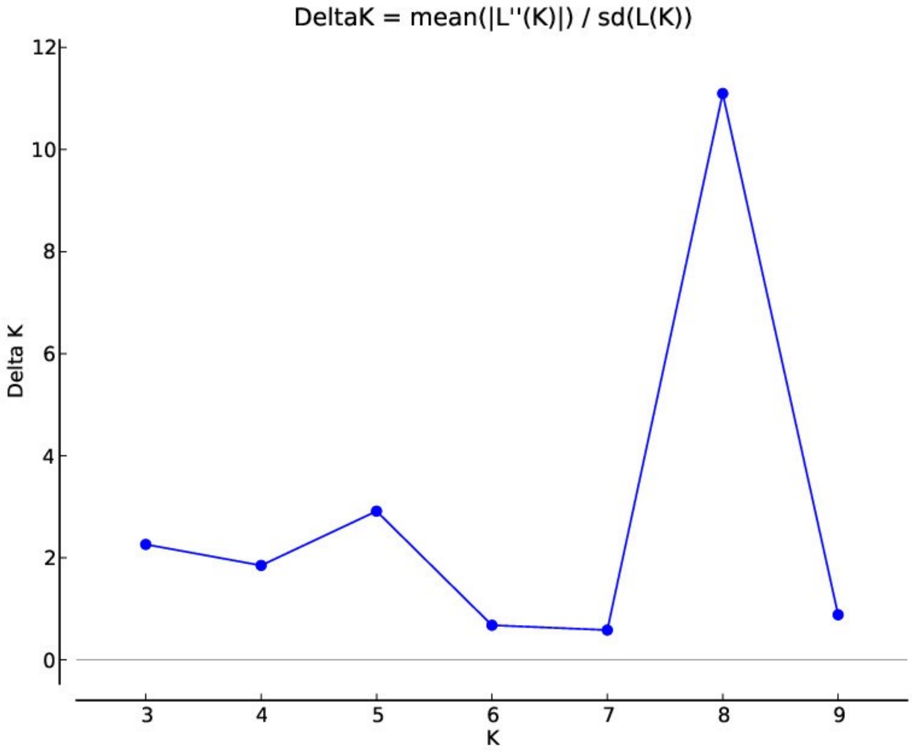

Figure S2: Most likely number of K based on the ΔK value determined using STRUCTURE HARVESTER.

8.2 Appendix of Publication IV

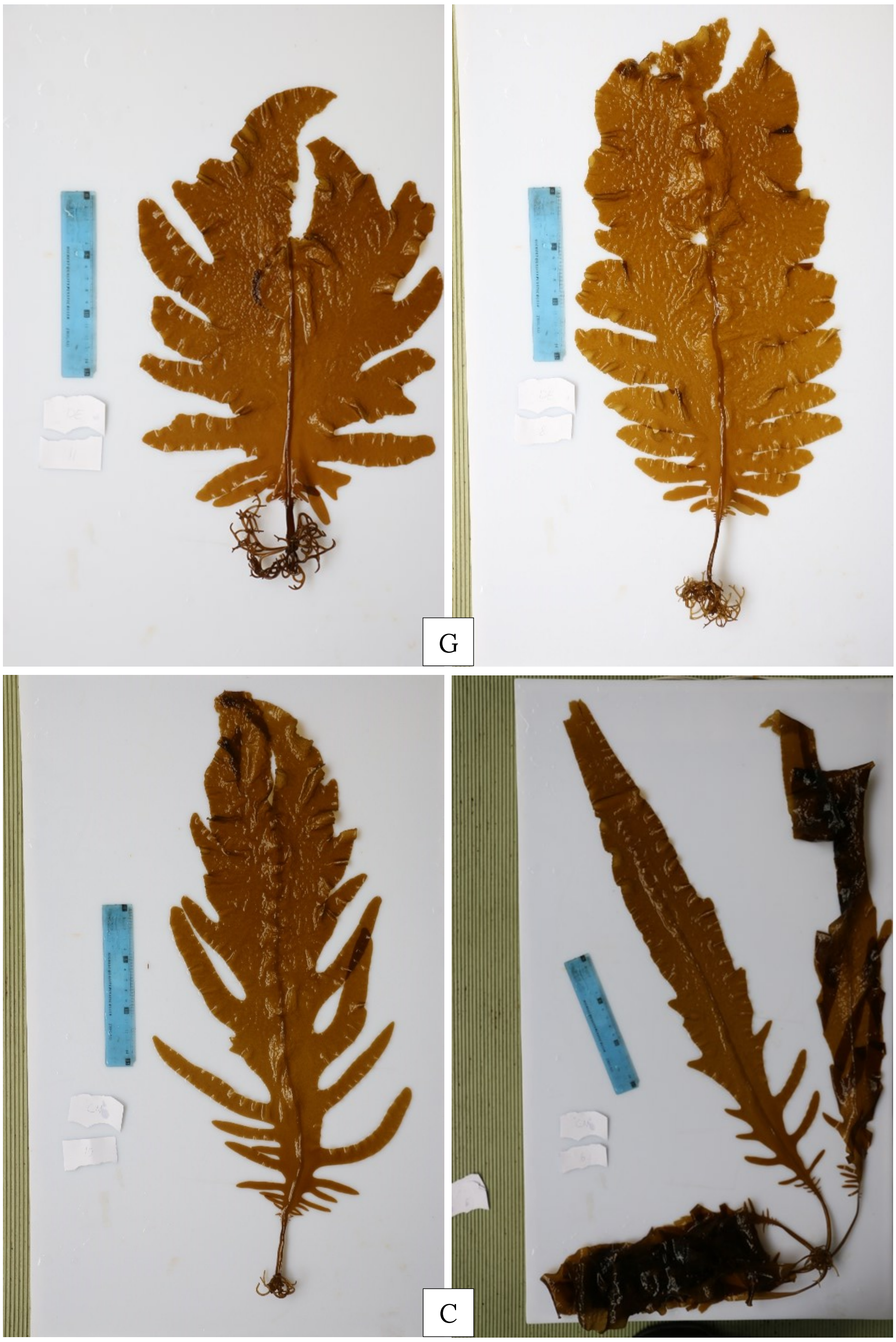

Figure S1: Sporophytes from the outgrowth experiment (unpublished data) after approx. 2 months of cultivation in large aquaria. Top: sporophytes obtained from the German isolate (G); bottom: sporophytes obtained from the Chinese isolate (C). Length of the ruler is 15 cm.

8.3 Publication V:

Theories, Vectors, and Computer Models: Marine Invasion Science in the Anthropocene

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Theories, Vectors, and Computer Models: Marine Invasion Science in the Anthropocene

10

Philipp Laeseke, Jessica Schiller, Jonas Letschert, and Sara Doolittle Llanos

Abstract
Marine invasions are well-recognized as a worldwide threat to biodiversity and cause for tremendous economic damage. Fundamental aspects in invasion ecology are not yet fully understood, as there is neither a clear definition of invasive species nor their characteristics. Likewise, regulations to tackle marine invasions are fragmentary and either restricted to specific regions or certain aspects of the invasion process. Nonetheless, marine anthropogenic vectors (e.g., vessel fouling, ballast water, aquaculture, marine static structures, floating debris, and human-mediated climate change) are well described. The most important distribution vector for marine non-indigenous species is the shipping sector, composed by vessel fouling and ballast water discharge. Ship traffic is a constantly growing sector, as not only ship sizes are increasing, but also remote environments such as the polar regions are becoming accessible for commercial use. To mitigate invasions, it is necessary to evaluate species' capability to invade a certain habitat, as well as the risk of a region of becoming invaded. On an ecological level, this may be achieved by Ecological Niche Modelling based on environmental data. In combination with quantitative vector data, sophisticated species distribution models may be developed. Especially the ever-increasing amount of available data allows for comprehensive modelling approaches to predict marine invasions and provide valuable information for policy makers. For this article, we reviewed available literature to provide brief insights into the backgrounds and regulations of major marine vectors, as well as species distribution modelling. Finally, we present some state-of-the-art modelling approaches based on ecological and vector data, beneficial for realistic risk assessments.

Keywords
Non-indigenous species · Marine vectors · Species distribution modelling · Regulations · Anthropogenic debris

10.1 Non-indigenous and Invasive Species

Non-indigenous species (NIS) can have negative effects on receiving ecosystems and are considered one of the major global threats to biodiversity (Ruiz et al. 1997; Casas et al. 2004; Raffo et al. 2009). Apart from ecological consequences, substantial economic damage can be caused by overly abundant introduced species or harmful species such as pathogens (e.g., Pimentel et al. 2000, 2001). The effects of introductions and establishments of new species in a community are unpredictable, as a multitude of biotic and abiotic factors determine the onset and further development of an invasion. Depending on the receiving habitat and the observed parameter, the same species can have negative but also positive effects (McLaughlan et al. 2014). Because of the variety of factors of each invasion, understanding them on the species-, pathway-, and ecosystem level is essential for adequate evaluation and possible management.

Despite their ecological and economic relevance, not even the basic terminology of introduced or invasive species is clearly determined among scientists and regulations. Over time, several definitions have been proposed for biological invasions. The most basic one is being a non-indigenous

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species (NIS), namely, a species introduced after the discovery of America and the onset of large-scale transatlantic ship traffic (Leppäkoski et al. 2013; Ricciardi et al. 2013). More specifically, Richardson and Pyšek (2006) defined invasion ecology as the study of human-mediated introductions of species to areas beyond their native range without considering the impact on the invaded habitat. Alpert et al. (2000) included effects of NIS and described an invasive species as "one that both spreads in space and has negative effects on species already in the space that it enters." According to Boudouresque and Verlaque (2002) introduced and invasive species can be differentiated by the conspicuous role the latter play in the recipient ecosystems, which is characterized by becoming dominant and potentially taking the place of keystone species. The previous examples show how much definitions can vary in only a few studies – with more being considered, they even begin to contradict each other, both in terminology and procedure (e.g., Blackburn et al. 2011; Guy-Haim et al. 2018).

Although not clearly defined, bioinvasions are a topic of public interest (García-Llorente et al. 2008) and there are several national eradication programs and policies established (see New Zealand, USA; Myers et al. 2000, Wotton et al. 2004). However, on a global scale, overarching regulations to mitigate marine invasions are missing. This is reflected in the EU legislative 1143/2014, which only deals with anthropogenically introduced species, but does not consider naturally introduced species. Moreover, international conventions for marine traffic are not binding across the globe or only concern certain aspects of dispersal mechanisms (see Sect. 10.2). One reason for this fragmentation among marine NIS regulations might be the influence of economic interests, which dilute scientific expertise (Margolis et al. 2005).

To develop efficient regulations, it is essential to gain an in-depth understanding of human-mediated vectors and factors influencing invasion success. Ecological Niche Models (ENM) can be powerful in evaluating invasion potential and are currently implemented at the frontier of invasion science (see Sect. 10.3). Figure 10.1 sets the framework for this article, in which we summarize knowledge on anthropogenic vectors and give insights into methods and developments of ENM as a potential forecasting tool. We intend to contribute to the understanding of bioinvasions at a broader scale and shine a light on necessary future efforts to develop efficient regulations.

10.2 Anthropogenic Vectors

Defining which vector has the highest impact in terms of the number of introductions, establishment rate, and effects on the new habitat is challenging because their effectiveness and frequency vary with time and geographical region (Williams et al. 2013). In general, failed introductions and invasions pose a problem in cross-vector analysis, because they mostly remain hidden, leading to strong biases in introduction rates per vector (Zenni and Nuñez 2013). About four decades ago, ship traffic and aquaculture were identified as the major vectors for marine human-mediated introductions (Carlton 1979). Recent studies suggest that this assumption has not changed much and efforts have been undertaken to rank vectors regarding their potential of dispersing NIS. On a global level, a positive correlation between cargo ship traffic and marine introductions reveals the vast contribution of marine traffic to create connectivity across distant geographic regions (Seebens et al. 2016). Ship traffic can be divided into two NIS pathways: the colonization of vessel hulls with sessile or small motile species (hereafter fouling species), and the transportation of organisms and their early-life stages (eggs, larvae) in ballast water tanks (Ruiz et al. 1997; Cohen and Carlton 1998; Godwin 2003). On a regional level, a cross-vector comparison in California revealed vessel fouling as the most important vector followed by ballast water and aquaculture (Williams et al. 2013). However, the authors claim that results cannot be extrapolated and are case-specific with respect to area, time, and vector composition.

This review examines the following marine vectors: vessel fouling, ballast water, mariculture, marine static structures, floating anthropogenic litter, and human-mediated climate change. This selection encompasses the major vectors, affecting most marine ecosystems worldwide. Live species trade with ornamental (Weigle et al. 2005) and bait species (Weigle et al. 2005; Fowler et al. 2016) represent minor vectors and therefore will not be elaborated in this article. Canals play an important role in the distribution of marine species on regional scales (see Gollasch 2006 for the influence of the Suez Canal on Mediterranean species composition). However, they represent the removal of physical barriers between adjacent regions and allow migration in a variety of ways (e.g., shipping related or natural dispersal), which are covered in the sections mentioned above. Therefore, we do not include an individual chapter on this vector.

10.2.1 Vessel Fouling

The importance of hull fouling for marine invasions is unquestionable. A convenient parameter to quantify the marine invasion risk through hull fouling is the wetted surface area (WSA) of ships (Miller et al. 2018) and an approach of calculating the WSA of the world fleet of commercial vessels resulted in 325×10^6 m^2(Moser et al. 2016). Marine traffic is continuously increasing and even remote areas, such as

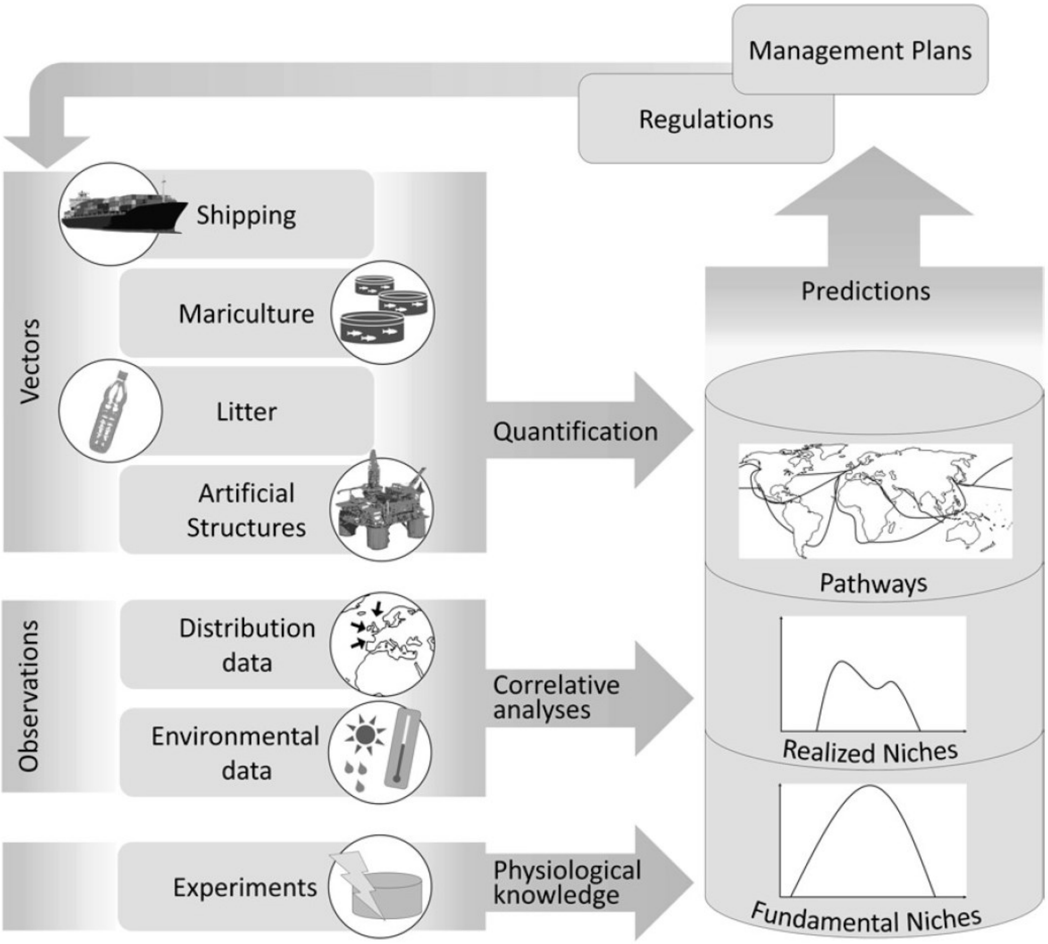


Fig. 10.1 Marine Bioinvasions in the Anthropocene: the most important vectors for alien invasive species across geographic regions are anthropogenic transportation means, such as shipping- and mariculture-related transfers. Also passively drifting litter and stable structures contribute to the transport and introduction of species. Quantification of introductions along these vectors allows for identification of major pathways across the globe. Ecological Niche Modelling can help to identify suitable environmental conditions for species in question. While correlative approaches are well established for the investigation of realized niches, laboratory studies can yield important additional information about the species' fundamental niche and hence contribute to the understanding of ecological mechanisms which influence a species' distribution potential. Transportation data and Ecological Niche Models can be combined to evaluate invasion risk. Identification of areas with high introduction pressure and understanding of the species being transported along are an important step prior to the development of regulations, management plans, and mitigation strategies. However, to date, only few international regulations are effective which successfully control the spread of species

the Arctic, become available for commercial shipping due to melting sea ice (Miller and Ruiz 2014).

Antifouling coatings are applied to vessel hulls and repel many fouling species that would normally settle on submerged vessel areas (Williams et al. 2013). Yet, there are certain organisms that are immune to antifouling components such as the bryozoan *Watersipora subtorquata*, which may serve as a foundation species providing settlement space for subsequent epibionts (Floerl et al. 2004). Small disruptions of 1–2 cm in antifouling coatings may enable the settlement of a wide range of sessile marine species, which can easily be overseen in cryptic spots like keels or propeller shafts (Piola and Johnston 2008). Godwin (2003) observed weaknesses of antifouling coatings at weld seams and spots where smaller boats were placed on wooden blocks while painted. He also assumed that slow velocities and long port stays increase the potential of sessile species to settle and survive on vessel hulls (Godwin 2003). Kauano et al. (2017) tested persistence of fouling species after being dragged with 5, 15, and 20 knots for 20 min. Although the overall trend shows a negative correlation between velocity and persistence, 90% of the species were present with at least 20% of their original abundance after being dragged with 20 knots. Some limitation to vessel fouling is provided by desiccation. Kauano et al. (2017) found that most soft-bodied sessile species died after being outside of the water for 24 hours, whereas barnacles survived 120 hours. Another example for desiccation resistance are sporophytes of the invasive kelp *Undaria pinnatifida* that released viable spores even after 3 days outside of the water (Bollen et al. 2017).

Large cargo vessels such as bulk carriers, tankers, and container ships are usually equipped with slow-speed engines (Endresen et al. 2003), meaning that they rarely travel faster than 20 knots, and are only put on dry dock every 5 years when their hulls are cleaned and repainted. Yet, these vessels represent 79% of the WSA of the commercial world fleet and substantially contribute to geographical connectivity (Moser et al. 2016; Seebens et al. 2016). In combination with the knowledge mentioned above, this may explain why vessel fouling is still a major pathway for NIS on a global scale.

Trends in the marine traffic industry favor larger container ships and hub-ports (Shenkar and Rosen 2018), from which smaller transport vessels carry goods to smaller ports, representing one example of secondary spread. Small-scale boating may contribute to secondary spread of NIS, especially in areas with intense tourism or recreational activities (Anderson et al. 2015). Many marine invertebrates, such as ascidians and bryozoans, have very short natural dispersal ranges and hence marine traffic or rafting debris is likely to enable their long-range dispersal (Petersen and Svane 1995). This is underlined by a case study in the great barrier reef where sessile NIS were found about 80 km offshore at an isolated coral reef that is frequently visited by boats (Piola and Johnston 2008).

In 2011, the International Marine Organization (IMO) published a resolution for the responsible management of vessel fouling to reduce the risk of NIS introduction (IMO 2011). However, these are mere voluntary guidelines and despite the global significance of vessel fouling for NIS dispersal, there is no enforced regulation on an international level yet. There are some examples for implemented hull fouling standards on a national and regional level represented by New Zealand (Ministry for Primary Industries 2014), and the National Park of Galapagos, Ecuador (Campbell et al. 2015). Both regulations require clean vessel hulls and antifouling coatings prior to the arrival of vessels.

10.2.2 Ballast Water

Ballast water discharge is the vector with the most management rules among the important anthropogenic dispersal mechanisms. The International Convention for the Control and Management of Ships' Ballast Water and Sediments (hereafter the BW Convention) was adopted by the IMO in 2004 and came into force in September 2017 (IMO 2004). According to requirements regulating the behavior of ballast water discharge, the BW Convention can be split into two major parts.

The first part obliges incoming vessels to exchange their ballast water at least 200 nautical miles offshore in a minimum depth of 200 m. The USA, not a signatory to the BW Convention, implemented a similar requirement. A study assessing ballast water exchanges in the USA from 2005 to 2007 found that most vessels abide with this rule, however, especially vessels that journeyed along the South and North American coasts still exchange their ballast water in coastal areas frequently (Miller et al. 2011). Similar results were obtained by a study targeting the Taiwanese maritime cargo sector showing that up to 30% of the surveyed ships exchange ballast water closer to shore than 200 nautical miles (Liu et al. 2014).

The second part of the BW Convention restricts the total amount of viable organisms in discharged ballast water to up to ten with a size of >50 μm m^{-3} plus up to ten with a size of <50 μm ml^{-1} (IMO 2004). To meet these restrictions, vessels are obliged to install ballast water treatment plants (e.g., electro-chlorination, UV treatment, and filtration). Given those conditions, Reusser et al. (2013) developed a model to predict the invasion rate per year through foreign ballast water discharge in the US Pacific Coast. Based on existing invasive species records and assuming a linear relationship between discharged organisms and successful invasions, they calculated that a new invasion would only occur every 10–100 years.

Shipping routes and source regions of ballast water affect the survivability of organisms at the ship's destination (Verling et al. 2005). For example, do transport routes through the Panama Canal expose attached specimens to tropical and partially freshwater conditions leading to temperature and osmotic stress (Miller and Ruiz 2014). The BW Convention requires ships to keep records of ballast water activities, so that uptake areas can be compared to discharge areas on demand and high risks of introductions can be avoided. Additionally, port states are empowered to conduct ballast water controls on incoming foreign ships and, if necessary, impose sanctions.

Still, a minimum risk of biointroduction remains and is positively correlated to the amount of ballast water discharged in an area (Reusser et al. 2013). This is important to consider in major ports serving as hubs for international maritime trade such as Shanghai, Singapore, or Rotterdam. Moreover, a study of the Chinese ballast water capacity confirmed the rising amount of ballast water in line with the growing maritime transport sector (Zhang et al. 2017).

10.2.3 Mariculture

Many marine species have been intentionally transported across broad geographical distances to be husbanded in aquacultures. The largest contributors to the global mariculture industry are Asian countries with China being by far the most important among them (FAO 2016). Other countries, such as Norway, Chile, and Indonesia have fast-growing mariculture industries as well (Buschmann et al. 2009). The most important cultured organisms worldwide are finfish, mollusks, crustaceans, and seaweed species. In 2014, 580 aquatic species have been registered with the FAO as husbanded species (FAO 2016). These species are often non-indigenous in the place where they are kept, meaning an escape would directly lead to an introduction into the new habitat. Examples for intentionally introduced species are the Pacific oyster *Crassostrea gigas*, domesticated salmon, and many seaweed species (Naylor et al. 2001). In contrast, accidental introductions may occur due to associated hitchhikers such as parasites, algae (e.g., *Codium fragile*), and various fouling species that live on or in aquaculture gear and husbanded species (Naylor et al. 2001).

Focusing on introductions to urban areas, Padayachee et al. (2017) investigated the taxa composition introduced by several marine vectors and found a significant difference between the categories Mariculture and Fisheries. Vertebrates were almost exclusively introduced for cultivation, while plants dominated, and were exclusive to, the equipment-facilitated arrivals. The continuous transfer of equipment and seed stock between maricultures has an especially high potential of species introduction (Forrest and Blakemore 2006). One striking example for this is the kelp *Undaria pinnatifida*, used for mariculture. It arrived to Europe alongside the Pacific oyster and has since been spread independently of oyster cultivation for farming or as a fouling species and recently reached German waters (Schiller et al. 2018). This was largely enabled by its tolerance to various conditions, including surviving overland transport on boat hulls or ropes (Bollen et al. 2017).

After vessel fouling, shellfish farming is considered the second most important vector for the 277 registered non-indigenous seaweeds worldwide. Especially red corticated algae, but also a variety of other taxa, live in association with farmed shellfish (Williams and Smith 2007). Seaweed mariculture itself is only a minor but efficient way of seaweed introductions, because farmed algae are specifically chosen for their competitiveness (Williams and Smith 2007). Interestingly, seaweed mariculture is the fastest-growing sector of aquaculture posing one-quarter of the global volume produced by aquaculture (FAO 2016). This growth is mainly due to seaweed farms in Indonesia and China that were established during the last 20 years. Between 2004 and 2014, the global aquaculture industry has grown rapidly and the percentage share of total worldwide fish harvest increased from 31.1% to 44.1% (wild catches and aquaculture products including non-food uses; FAO 2016). While regulations on an international level are missing, there are some examples for guidelines of the treatment of aquaculture organisms and gear, proposing sterilization prior to moving it to a new location. An example is the Australian National Biofouling Management Guidelines for the Aquaculture Industry that proposes different treatment methods such as exposures to air, fresh water, heat, or chemicals (NSPMMPI 2013).

10.2.4 Static Maritime Structures

There is a growing number of various static maritime structures (SMS), which are occasionally relocated and thus pose a risk to transport marine NIS or serve as stepping stones (i.e., oil and gas platforms, offshore wind farms, navigational buoys, non-cargo barges, and dry docks; Iacarella et al. 2018). Most SMS are characterized by their large and complex wetted surface area (WSA), providing space for fouling organisms, which, in turn, may attract predators (Friedlander et al. 2014; Todd et al. 2018). These artificial communities often differ from surrounding species assemblages (Stachowicz et al. 2002). Oil and gas platforms represent a major part of SMS and will therefore be the main focus of this section.

After being stationary for years, oil and gas platforms may be moved to a new service location, for repair, or decommission. To be able to navigate, they are either equipped with engines, towed by tug vessels (wet-tow), or carried on heavy lifting ships (dry-tow; Robertson et al. 2018). The former two options pose a risk for NIS dispersal, because platforms stay in the water during transport and are transported at very low speed (<8 knots), allowing associated organisms to travel along. In contrast to vessel fouling, translocated oil and gas platforms may introduce entire ecosystems to new geographical areas, including large sessile and mobile species across all trophic levels from algae to vertebrates (Ferreira et al. 2006; Yeo et al. 2009). Incidences of stranded or intentionally moved oil and gas platforms prove the introduction of a range of invertebrate species (Foster and Willan 1979; Ferreira et al. 2006; Page et al. 2006; Yeo et al. 2009), as well as fish species (Yeo et al. 2009; Wanless et al. 2010; Pajuelo et al. 2016).

Abandoned oil and gas platforms are frequently transformed into artificial reefs instead of being decommissioned ("rigs to reefs"; reviewed by Bull and Love 2019), because they foster entire marine ecosystems and due to high demolishment costs. This practice is largely unregulated with respect to its biological implications, an issue in need of addressing, considering that a large number of the roughly

7000 oil platforms worldwide were already reaching the end of their service time in 2003 (Hamzah 2003).

Iacarella et al. (2018) emphasized that regulations concerning marine NIS introductions through SMS are still missing. This is especially worrying considering that the Arctic might become more available for commercial use, including drilling operations, with decreasing sea ice.

10.2.5 Marine Litter

We have long known about how ocean currents can transport a wide variety of structures, which may then serve as a raft for fouling species (Guppy 1917; Thiel and Gutow 2005; Wichmann et al. 2012). The presence of floating plastic debris in the oceans has increased tremendously in recent decades and continues to grow (PlasticsEurope 2013). Due to this increment of potential vectors, we very well might be on the brink of a new era for marine invasions.

The exact sources of anthropogenic debris are often unknown, since trajectories of floating objects are hard to track, being influenced by seasonal variations in wind and current conditions (Kiessling et al. 2015). The United Nations Joint Group of Experts on the Scientific Aspects of Marine Pollution (GESAMP) have estimated that land-based sources account for up to 80% of the world's marine litter, 60–95% of the waste being plastic debris (Sheavly 2005). However, shipping activities have also been a major source of marine litter (Scott 1972). Despite agreements to forbid ship waste dumping (London Dumping Convention, promulgated in 1972; Lentz 1987), compliance and enforcement still pose significant challenges (Carpenter and Macgill 2005). In fact, in some regions up to 95% of all litter items are shipping-related (Van Franeker et al. 2011), and debris composition in the Baltic Sea and North Pacific Ocean leaves little doubt that ocean-based sources are major contributors to marine debris (Moore and Allen 2000; Fleet et al. 2009; Keller et al. 2010; Watters et al. 2010; Schlining et al. 2013).

The predominance of plastic as floating litter and as accumulated debris on shorelines is not due to the amounts in which it is produced relative to other types of waste, but to its remarkable persistence and durability (Andrady 2015). The long life expectancy of a piece of plastic contrasts to the natural processes of consumption and decomposition that organic flotsam eventually undergoes (Vandendriessche et al. 2007). It is because of this persistence that today we are facing the possibility of human litter more than doubling rafting opportunities, particularly at high latitudes (Barnes 2002), and potentially propagating fauna outside of their native ranges (Barnes et al. 2009; Gregory 2009) and up to the most remote polar marine environments (Barnes et al. 2010; Lusher et al. 2015). Because of its overall high numbers, plastic debris offers rafting opportunities that quantitatively surpass other floating substrata in the oceans. As Goldstein et al. (2012) suggest, many species may no longer be limited by the availability of suitable substrata to adhere to. On top of enhancing transport of rafting communities, the availability of plastic may favor the transport of certain species over others. This is because rafting communities on litter and, e.g., macroalgae are described as similar, but less species rich in the former (Winston et al. 1997; Gregory 2009).

Over 1200 taxa have been associated with natural and anthropogenic flotsam (Thiel and Gutow 2005), and many organisms and potential invaders were first described on marine litter (Jara and Jaramillo 1979; Stevens et al. 1996; Winston et al. 1997; Cadée 2003). One most notable event was the record of a 188-ton piece of a former dock, dislodged during a tsunami in Japan in 2011, stranded in Oregon and accounting for the first record of over 100 species non-native to the west coast of the USA (Choong and Calder 2013). While samples taken from beach litter collections show a bias towards sessile organisms with hard calcified structures (Winston et al. 1997; Gregory 2009), debris collected afloat include a higher diversity of soft-bodied and/or motile species (Astudillo et al. 2009; Goldstein et al. 2014). Overall, cnidarians, bryozoans, mollusks, and crustaceans seem to be the most abundant taxa registered. Today, we know plastic can host a variety of pathogens: the ciliate *Halofolliculina* sp., which targets coral skeletal structures (Goldstein et al. 2014), potential human and animal pathogens of the genus *Vibrio* (Zettler et al. 2013), and dinoflagellates known to cause harmful algal blooms (Masó et al. 2003).

What ensures colonization and survival during transport on a plastic raft? Kiessling et al. (2015) reviewed 82 publications with the aim of characterizing marine debris rafters, their biological traits, and identifying the specific conditions rafters face in order to survive their voyages. Their results suggest that a majority of species act as facultative rafters (77%), as fully sessile (59%), and as suspension feeders (72%). This can easily be compared to communities of algae rafts, which are more complex at the structural level, and more capable of hosting mobile species with different feeding patterns (Thiel and Gutow 2005).

Colonization might influence certain characteristics of a plastic raft. Floating behavior might be altered, as the added weight of rafters may stabilize an otherwise highly buoyant and unbalanced object. This would increase colonization probability (Bravo et al. 2011) and the succession of the rafting community, but heavy fouling on a plastic item may increase the raft's weight and cause it to sink (Ye and Andrady 1991; Barnes et al. 2009). If this causes death and loss of rafters, it may result in decolonization and resurfacing of the item (Ye and Andrady 1991), extending the life of plastic as a vector. The size of a particular piece of debris can also play a part in influencing the species richness and density of organisms rafting on it. Studies have shown a positive correlation between higher taxonomic richness and a larger surface area of plastic debris (Carson et al. 2013; Goldstein et al. 2014). However, this may be due to stochastic effects, biased sampling efforts (smaller items sink faster when colonized by fewer organisms) or other characteristics of the raft such as stability (Goldstein et al. 2014).

Although it is not expected that marine litter opens up novel pathways that are not available for other rafting materials (Lewis et al. 2005), it is more durable, more pervasive, and travels slower in comparison with vessel hulls, factors that might favor the survival of rafters (Barnes 2002). Therefore, the presence of plastic debris in the ocean might be adding another dimension to rafting and dispersal opportunities.

Today, we are familiar with calls to consider plastic as hazardous materials (Rochman et al. 2013), investing in better controls for waste management (European Commission 2018), and seeing strong lobbying in certain sectors of social media. As Rech et al. (2016) state, our main research priorities should center around estimating the impact of marine litter on NIS dispersal, and identifying sources and sinks by better understanding behavior of debris in ocean currents. Future research should consider unifying sampling methods to obtain comparable results and including base knowledge of local communities to better monitor arrivals of NIS while continuing our advance in taxonomic and genetic identification methods to be able to better identify species that might be cryptic or yet unknown to us (Carlton and Fowler 2018).

Finally, recognizing that the plastic problem is theoretically an avoidable one, research should be accompanied by management that aims in the direction of education and public awareness, the surveillance and protection of sink zones, and the reduction of production through taxation and banning.

10.2.6 Climate Change

Hellmann et al. (2008) identified possible ways in which climate change may affect NIS either directly or by influencing their competitors or dispersal: Firstly, climate change alters traits of habitats such as temperatures and CO_2 concentrations, which may reduce environmental constraints for marine invaders and diminish native species' competitiveness. Ultimately, this would increase the establishment rate of NIS in a new habitat. Secondly, climate change alters human-induced propagule pressure by affecting maritime tourism, cargo, and recreational activities. Finally, Hellmann et al. (2008) argued that climate change may lead to range-shifts of species, a trend that has been documented multiple times in the scientific literature, and which does not only affect NIS, but also native species (Sorte et al. 2010b; Carlton 2011; Wernberg et al. 2011; Canning-Clode and Carlton 2017; Martínez et al. 2018).

Although marine range-shifts occurs at a slower rate than marine introductions through anthropogenic vectors, the impacts on ecological communities in both scenarios can be very similar (Sorte et al. 2010a) and thus range-shifts due to human-induced climate change may be considered a type of anthropogenic introduction.

Climate change predictions include not only a change in the overall temperature but also the increasing climate variability (Rhein et al. 2013). Aperiodic cold snaps have been observed to reduce the number of invasive species (Canning-Clode et al. 2011). In this particular example, a cold snap in January 2010 in Florida, USA caused high mortalities of many marine organisms, among them the invasive porcelain crab *Petrolisthes armatus* (Firth et al. 2011; Kemp et al. 2011). Testing the survivability of *P. armatus* in cold water treatments, Canning-Clode et al. (2011) found that abnormal cold temperatures decrease the population of the invasive crab. Cold snaps limiting NIS might be relevant worldwide, but do not balance out climate change-induced range-shifts of NIS (Canning-Clode and Carlton 2017). In fact, individual examples show that NIS may expand to a broader distribution range after its population got reduced by a cold snap (Crickenberger and Moran 2013). Canning-Clode and Carlton (2017) assumed that NIS surges will eventually outnumber NIS setbacks along with predicted warming climate. This is underlined by several studies showing the beneficial impact of warmer water on NIS (Stachowicz et al. 2002; Sorte et al. 2010b; Kersting et al. 2015).

Stachowicz et al. (2002) found several benefits for non-native fouling species in warmer water temperatures. During a 10-year monitoring campaign, starting in 1991, they found a positive correlation between mean temperature and total recruitment of NIS, whereas the opposite trend was observed for native species. Additionally, non-native fouling species started their recruitment earlier in warmer waters, a remarkable advantage over native species. Stachowicz et al. (2002) also tested the growth of two non-native and one native ascidian species under different water temperatures resulting in faster growth of the former in warm water conditions. Sorte et al. (2010b) conducted mortality experiments with four native and seven non-native sessile species (bryozoans, colonial and solitary tunicates, and hydroids) in increased temperature treatments. They observed that the temperature at which only 50% of the species were alive is 3 °C higher for NIS than for native species, suggesting that NIS are more resistant to abnormally high temperatures.

Overall, there seems to be a trend of species shifting their ranges polewards along the continental coasts with proceeding climate change (Müller et al. 2009; Sorte et al. 2010a; Wernberg et al. 2011; Morley et al. 2018).

10.3 Forecasting

Of all transferred species, only a small number become truly invasive (see the "tens rule"; Williamson and Fitter 1996). Identifying the potential of an introduced species for dispersal and establishment can be useful in risk assessment. In this cause, Ecological Niche Modelling (ENM) and Species Distribution Models (SDM) can be of great help when predictions of species' potential distributions are needed. Conservation biology can, besides other applications (Guisan and Thuiller 2005; Gavin et al. 2014), profit from SDMs for risk assessment of invasions (Peterson 2003; Thuiller et al. 2005; Seebens et al. 2016). An ecological niche represents an *n*-dimensional (e.g., food-availability and temperature gradient) space in which a species can thrive (Hutchinson 1957). For distribution modelling, a model is usually calibrated on a species' niche and then projected onto the geographic space of interest. Here, the calibration process is conducted on available information of a species' known distribution and/or biological traits and the projection area is compared with the needs of a species. Like that, the suitability of an area can be evaluated and visualized. Calibration and projection can be done on historical and present-day data and allow predictions for simulated environmental conditions as, e.g., for future or past climate scenarios. The importance of invasion-risk assessments is underlined by Leung et al. (2002) who developed a bio-economic model as a framework to assess costs and benefits of invasions and their prevention efforts. Leung et al. (2002) demonstrated that investment in prevention over damage repair is to be preferred for society. For risk assessments, the recognition of suitable habitat of a species is of central interest. Hence, ENM is an important tool for policy makers to evaluate and to react to possible invasions before they can get economically or ecologically out of hand. Although ENM/SDM-related publications have become more and more abundant and yield valuable information for a diverse array of interests, there is a huge gap in the number of available publications between the terrestrial and the aquatic realm and between organizational organism levels. While a lot of studies are accessible for especially terrestrial higher plants, mammals, and birds, aquatic (small) taxa are still underrepresented (Soininen and Luoto 2014). Hence, methodological aspects in the following section are partly explained based on terrestrial studies. To understand the underlying concept of ecological niche modelling, Soberon and Peterson (2005) elaborated the work of Pulliam (2000) and presented the BAM-diagram. The BAM diagram consists of a set of suitable <u>b</u>iotic, <u>a</u>biotic and accessible (<u>m</u>ovement) spaces. Thus, A represents the fundamental niche and the intersection of B and A represents the realized niche of a species. The fundamental niche is the space which can theoretically be inhabited by a species. Contrary, the realized niche represents the fundamental niche which is actually inhabited but truncated due to abiotic or biotic factors. M can contain naturally accessible regions as well as regions which are reachable through anthropogenic influence. Restrictions of M can be inherent (dispersal capacity of a species) or external and either of natural (e.g., land bridges) or artificial character (e.g., dams; Watters 1996, Ovidio and Philippart 2002). In the context of this paper, M (with respect to dispersal vectors) and A (with respect to changing climate) play major roles. Implementation of B (as biotic interactions) into models is still an area of investigation and rather case-specific than following established concepts.

10.3.1 Limitations of Models Through Knowledge Gaps

Distribution modelling is the projection of an identified niche from one geographic range to another under the presumption that species occupy the same conditions in both regions (Peterson and Vieglais 2001). Therefore, environmental data (predictor variables) in a species' distributional range is correlated with occurrence data (response variable). Nowadays, more and more databases are becoming available to provide researchers with valuable data for predictors (e.g., bio-oracle, worldclim, MerraClim) as well as distributional data (e.g., gbif, iobis) in addition to available primary sources (e.g., herbaria, museum collections, scientific reports, field guides, citizen science projects). Although correlative models have great predictive power, they can only identify the realized niche based on available distributional and environmental data. However, species are not necessarily in equilibrium with their environment and not all suitable environmental combinations might be represented in the distributional training range (Jackson and Overpeck 2000). Hence, these models may underestimate the fundamental niche of a species, leading to narrowed projections of suitable habitat (Kearney and Porter 2009; Martínez et al. 2015). Additionally, even in native distributional ranges parts of populations are in fact sink populations (Soberon and Peterson 2005) and might, therefore, reflect unsuitable environmental conditions for reproduction but suitable for survival. There are many other possible cases, in which the observed distribution of a species does not cover all suitable environmental conditions (e.g., sampling bias, seasonality, anthropogenic influences, and recent introduction). In any case, models based on unfilled niches could lead to erroneous assumptions on suitability of habitat for a given species (Peterson 2005). Likewise, projections into niche space beyond the identified realized niche can only be speculative. Therefore, models based on physiological knowledge are an important addition to classic correlative models (Kearney and Porter 2009). These mechanistic models make use of physiological knowledge (e.g., survival thresholds or performance over environmental gradients) to identify abiotically suitable spaces (Kearney and Porter 2009; Buckley et al. 2010; Diamond et al. 2012; Martínez et al. 2015). Identification of physiological limits is more laborious than correlative modelling, but these models are not subject to incomplete distribution data. In 2017, the GlobTherm database has been launched (Bennett et al. 2018), which includes experimentally determined thermal tolerances for more than 2000 aquatic and terrestrial species, providing a promising tool for future more holistic modelling approaches. While correlative models might assume too narrow niches, mechanistic models might, in ignorance of biological influences, assume too wide niches. The resulting discrepancies might bear the potential to investigate factors influencing the prevalence of a species and be useful in invasion risk assessment.

10.3.2 Invasions and Niche Shifts

It is a central assumption of SDM that species do occupy the same niche in their novel range as in their native range and across time periods. However, this has been subject of debates and evaluation studies in the past under the term "niche shift." This term implies changes in the realized niche of a species with respect to the centroid of the niche, the margins, and/or frequency of occupied environmental conditions (Guisan et al. 2014). Although studies have been published, which suggest niche shifts in invaded territories (e.g., Maron et al. 2004; Fitzpatrick et al. 2006; Broennimann et al. 2007), ecological niches have also been shown to be a rather conservative feature of species' and can be transferred to other than the native regions (Prinzing et al. 2001; Broennimann et al. 2007; Tingley et al. 2009; Petitpierre et al. 2012). Petitpierre et al. (2012) investigated 50 holarctic terrestrial plant species from herbs to trees and found niche expansion of more than 10% in the invaded range for only 14% of the studied species. Furthermore, they stated that genetic admixing (repeated introductions or hybridization) or reduced competition in the novel range do not automatically lead to substantial niche expansions. Ecological niches are even conserved over evolutionary time scales (i.e., several million years), as has been shown by Peterson et al. (1999). The authors built ecological niche models for 37 sister taxa of birds, mammals, and butterflies, and were able to reciprocally predict the geographical distribution of the respective sister taxon with high accuracy. Larger niche dissimilarity was found only on the higher taxonomic family level. Naturally, due to shared ancestry, niches of sister taxa tend to be highly similar. This was demonstrated by comparison to more distant taxa and to what can be expected from their environmental background alone (Warren et al. 2008). However, comparison of ecological niches of sister taxa and respective outlier groups does not necessarily indicate close phylogenetic relationship (Warren et al. 2008). Hence, not only phylogenetic relationship but also the environmental framework defines the species' niches. This is in line with Ackerly (2003): Species' niches are maintained throughout space and time and adaptation in specific traits seems to have a more stabilizing function in maintaining this niche. Adaptive evolution still may occur under the following scenarios: When a species colonizes islands in environmental space (not necessarily equivalent with geographical space), in trailing edge populations during migrations, or adaptation within the occupied niche space (due to environmental changes) (Ackerly 2003). To identify true niche shifts, Guisan et al. (2014) propose to build ENMs with gradually trimmed environmental data from the native and novel range and to investigate the effects of rare climatic conditions on resulting niche overlap metrics.

Large niche shifts can erroneously be assumed when species niches are derived from unfilled niches. For example, if a species occupies environmental conditions in a new geographic region (e.g., an invaded site) which are not found in its native range. In fact, populations rather persist at the edges than in the center of their historical distributional range (Lomolino and Channell 1995), and building a niche model on occurrence data from a certain time span can only yield a snapshot of the actual niche and might result in biased projections (Faurby and Araújo 2018). Including historical distributional and environmental data may be important to prevent modelling of biased niches. Also the findings of Peterson et al. (1999) might allow to considerably enhance the available information on tolerated environmental conditions by carefully including the realized niches of sister taxa. Distributional ranges and concomitant realized niches are massively narrowed through anthropogenic influences (e.g., extinctions or displacement; Lomolino and Channell 1995) but, in contrast, can become enormously enhanced through dispersal events (anthropogenically through increasing global trade and aquaculture, Ruiz et al. 1997, or naturally through drift, Waters 2008). An example of how unfilled niches may pose problems for accurate ecological niche modelling is given by Peterson (2005): He explained how non-equilibrium distributional data may lead to biased niche assumptions and hence underestimate a species' niche as in the case in Ganeshaiah et al. (2003). Ganeshaiah et al. (2003) modelled the ecological niche of the terrestrial sugarcane woolly aphid (*Ceratovacuna lanigera*) to predict its invasion potential across India. However, they used distributional records which were collected during the process of migration, and therefore could not cover the whole range of suitable environmental conditions. Thus, the suitable range was underestimated and Peterson (2005) suggested using native distributional data to train an ENM in order to capture the whole ecological niche for more realistic information on potential habitat.

While biotic interactions play a central role in ecology, they are only recently being integrated into niche modelling. Their former omission could be due to either the difficulties of implementing highly complex and dynamic biotic interactions, population and dispersal dynamics or because of the general assumption that rather abiotic (climatic) factors are the main drivers of species distributions (Woodward and Williams 1987). Models based on climatic variables alone have good predictive power, but biotic interactions can be of major importance (Araújo and Luoto 2007; Soininen et al. 2013). However, including biotic interactions does not necessarily lead to an increase in predictive power (Raath et al. 2018), but, in contrast, might even lead to a decrease (Silva et al. 2014). Nevertheless, they can be of great importance when it comes to predicting invasion success, as has been shown by Silva et al. (2014) for the crown-of-thorns sea star.

10.3.3 Assessing Invasions

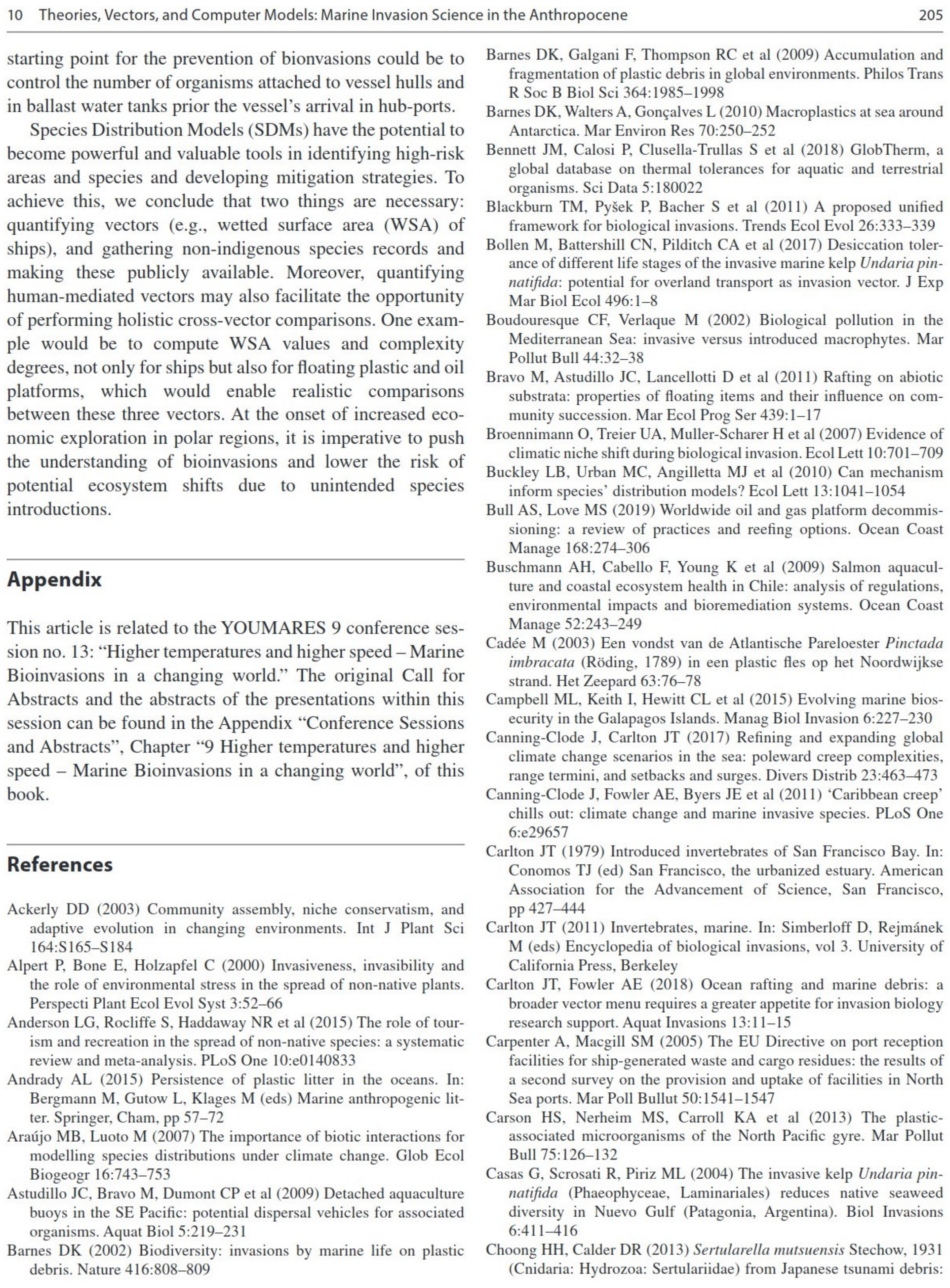
Predicted high suitability of a certain range does not imply a risk of invasion. Species distribution models give information on how well a certain area matches the requirements of a certain species. Intact environments and species communities as well as geographic obstacles may prevent the invasion of a species. In fact, successful invasions often follow several failed introductions (Sax and Brown 2000) and although a potentially suitable habitat might be available, intrinsic and extrinsic factors are able to prevent a successful invasion. Firn et al. (2011) did not find evidence for a general pattern of higher abundance in non-native ranges of introduced species ("invasion paradox," Sax and Brown 2000). Only a small fraction of the 26 investigated species did show a higher abundance in the new range. The other species were as abundant as in their native ranges or less abundant.

Simple ecological niche models can give good estimations of the extent of range shifts under projected climate conditions. However, at finer scales, models can be improved by including high-detail data for dispersal capacity and land-use data when it comes to accurate local predictions (Fordham et al. 2018). Even if data on dispersal capacity cannot be included in a model, general predictions of invasion risk are possible. Thuiller et al. (2005) showed that invasion prediction based on climatic variables in combination with economic data such as tourism and trade intensity is a usable and important tool in identifying invasion risk between regions at a global scale. Tourism and trade were used as proxies for propagule pressure from source regions (namely, South Africa) to target regions. For risk assessment of marine bioinvasions via ports and main shipping routes, an intermediate distance between origin and recipient port of 8000 to 10,000 km seems to be significant for high-risk assignment (Seebens et al. 2013). Generally, main traffic highways across the oceans exist, of which some have higher invasion probabilities than others (e.g., between Asia & Europe and Asia & North-America). Seebens et al. (2016) successfully developed a model to predict migrations of marine algae-based only on occupied environmental conditions and marine traffic data. Furthermore, they used historical invasion data to identify the invasion risk for ecoregions around the world and were able to identify the respective invasion probability.

Predictive models are not equally good among taxa. Soininen et al. (2013) found that predictive power of species distribution models decreases with decreasing body size of the organism under investigation to exceptionally low values when compared to taxonomic groups of larger body size. This might be due to the fact that especially small planktonic taxa might be drifted to unsuitable habitats and therefore exhibit source and sink populations alike within their distributional range. Soininen and Luoto (2014) further investigated how species-specific traits can influence the predictive power of distribution models. They investigated 4911 AUC values ("Area under curve," an indicator of predictive power of a model) of 50 publications on taxonomically widespread organisms. One conclusion was that predictability increases with body size, which might be due to the fact that smaller organisms are more prone to colonization-extinction dynamics, fine-scale environmental fluctuations, and have less niche plasticity than larger taxa. Interestingly, they did not find a trend in predictability over dispersal mode (i.e., passive, non-flying active, and flying) and thereby underlined the findings of Kharouba et al. (2013). Furthermore, niches of organisms from lower trophic levels might be more reliably predicted by abiotic predictors alone than from higher trophic levels (Soininen and Luoto 2014). However, this notion could not be verified in an earlier comparative study by Huntley et al. (2004) on 306 higher plant, insect, and bird taxa.

10.4 Conclusions

Under increasing globalization and blue growth (i.e., marine cargo shipping, mariculture, oil and gas drilling, and deep-sea mining), human-mediated vectors will continuously homogenize marine species assemblages across biogeographical regions. Although ballast water is the only vector with a worldwide binding regulation, guidelines to face the threat of invasions are constantly improved and official frameworks are beginning to be implemented on a broader scale. For example, the European Union is now considering plastic pollution as a threat and carrying out mitigation strategies such as banning certain one-use products. An efficient



starting point for the prevention of bionvasions could be to control the number of organisms attached to vessel hulls and in ballast water tanks prior the vessel's arrival in hub-ports.

Species Distribution Models (SDMs) have the potential to become powerful and valuable tools in identifying high-risk areas and species and developing mitigation strategies. To achieve this, we conclude that two things are necessary: quantifying vectors (e.g., wetted surface area (WSA) of ships), and gathering non-indigenous species records and making these publicly available. Moreover, quantifying human-mediated vectors may also facilitate the opportunity of performing holistic cross-vector comparisons. One example would be to compute WSA values and complexity degrees, not only for ships but also for floating plastic and oil platforms, which would enable realistic comparisons between these three vectors. At the onset of increased economic exploration in polar regions, it is imperative to push the understanding of bioinvasions and lower the risk of potential ecosystem shifts due to unintended species introductions.

Appendix

This article is related to the YOUMARES 9 conference session no. 13: "Higher temperatures and higher speed – Marine Bioinvasions in a changing world." The original Call for Abstracts and the abstracts of the presentations within this session can be found in the Appendix "Conference Sessions and Abstracts", Chapter "9 Higher temperatures and higher speed – Marine Bioinvasions in a changing world", of this book.

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