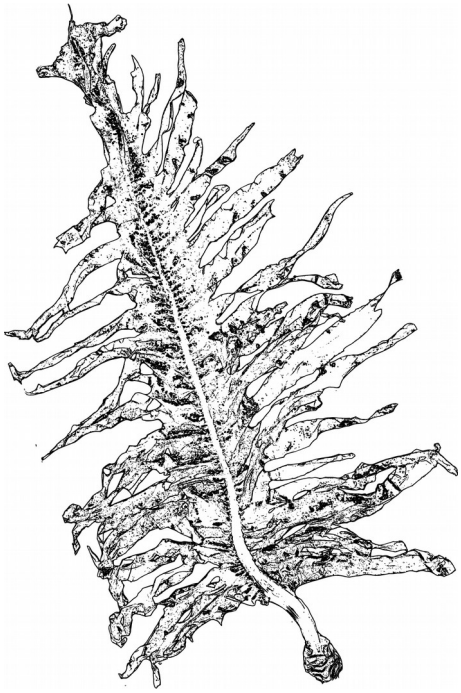
Range expansion mechanisms of the invasive kelp *Undaria pinnatifida*



Dissertation

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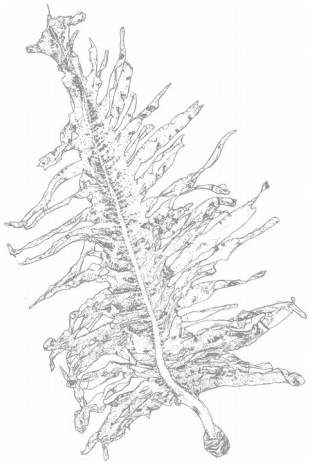

“We must make no mistake;
we are seeing one of the greatest convolutions
of the world's flora and fauna.”

Charles Elton
The Ecology of Invasions by Animals and Plants (1958)

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Summary

Species' invasions are being accelerated by anthropogenic activities to an extent that now threatens biological diversity on a global scale. Considered a driver of biotic homogenization and loss to biodiversity, the invasive kelp *Undaria pinnatifida* has recently established itself along coastlines worldwide. In addition to alterations to native communities, the canopy-forming kelp is capable of altering the environmental conditions of invaded habitats. Despite attempts to prevent its spread, *U. pinnatifida* has proven to be a highly successful invader and is expected to further expand its geographic distribution. The major aim of this thesis was to shed light on factors and characteristics that support the invasive potential of *U. pinnatifida* along various phases of its invasion process. This was achieved by focusing on traits specific to different life history stages and evaluating their role in the invasion success.

Specific desiccation tolerances were determined for different life stages of *U. pinnatifida* to test the likelihood of transport survival under emerged conditions. Photosynthetic measurements revealed that morphological features of the sporophylls protected the contained zoospores from desiccation, and as a result, accounted for a particularly high resistance to exposed conditions. Based on the capability to survive extended periods of air exposure and the kelp's distinctive trait to foul mobile maritime structures, overland transport is proposed as an effective invasion vector for *U. pinnatifida*.

A multivariate laboratory experiment, including physiological and biochemical analyses, demonstrated the exceptional tolerance of *U. pinnatifida* to various combinations of temperature and salinity, which was supported by its highly resilient antioxidant pool. This tolerance may facilitate the kelp's transport to new habitats and, thus, support the successful establishment of invasive populations. Based on its pronounced salinity tolerance, the kelp is expected to also invade low salinity environments, such as estuaries and lagoons. At the same time, the invader outperformed native New Zealand kelps, especially with regard to elevated temperatures. This suggests that *U. pinnatifida* might experience an additional competitive advantage in an increasingly warming ocean.

Summary

Detailed observations of simultaneously developing gametophytes demonstrated the occurrence of previously unobserved inter-specific interactions at microscopic stages. Interactions of *U. pinnatifida* and the giant kelp *Macrocystis pyrifera* resulted in an enhancement of the invader's gametophyte growth and oogonia formation. As events during the gametophyte stage crucially influence the subsequent sporophyte generation, this facilitation might contribute to its invasion success, particularly when newly available substrates are colonized by propagules. Such ecological aspects of kelp gametophytes are largely understudied, partly due to the difficulties of identifying kelp species at microscopic stages. In order to promote a straightforward differentiation, results of laboratory tests and earlier studies were scrutinized to discuss the benefits and shortcomings of a newly developed statistical approach and related methods.

Based on the experimental outcome of this thesis and relevant literature, most aspects of the projected climate change are expected to foster the invasion success of *U. pinnatifida*. With its physiological tolerance, the kelp is not only capable of adapting to increasing water temperatures but may also benefit if competing species are adversely affected. Moreover, the constantly increasing availability of artificial substrates and transport vectors, provided by human development and growing maritime traffic, will further promote the worldwide spread of *U. pinnatifida*.

In conclusion, this thesis assessed previously unobserved invasion pathways of *U. pinnatifida* and displayed a comprehensive set of mechanisms and diverse characteristics that facilitate the kelp's spread into previously unafflicted areas. Identified to be key factors of its invasion success, the extraordinary physiological tolerance and the complex interplay of different life history stages might enable *U. pinnatifida* to span an enormous geographic range in the coming decades. The knowledge gained from this thesis contributes to a complete understanding of the factors underpinning this invasion, a prerequisite for the development of effective management strategies and the prevention of severe ecological implications associated to the kelp's establishment in new habitats.

Zusammenfassung

Die Zunahme biologischer Invasionen durch anthropogene Einflüsse stellt eine Bedrohung für die Biodiversität der Erde dar. Unlängst trägt die invasive Makroalge *Undaria pinnatifida*, die sich an zahlreichen Küsten weltweit etabliert hat, zur biotischen Homogenisierung und zum Verlust von Artenvielfalt bei. Neben Veränderungen heimischer Artengemeinschaften, zeigt sich *U. pinnatifida* fähig, lokale Umweltbedingungen zu modifizieren. Trotz zahlreicher Versuche die Ausbreitung zu verhindern, erwies sich *U. pinnatifida* vielerorts als erfolgreicher Einwanderer, dessen geographisches Verbreitungsgebiet sich voraussichtlich auch zukünftig vergrößern wird. Das Ziel dieser Arbeit war es daher, ökologische Faktoren und physiologische Charakteristiken von *U. pinnatifida* zu ermitteln, die das große invasive Potential der Makroalge begünstigen. Das Hauptaugenmerk lag hierbei auf spezifischen Eigenschaften der unterschiedlichen Lebensstadien sowie auf der Beurteilung ihrer Rolle für den Invasionserfolg.

Um die Überlebenschancen bei Transporten außerhalb des Wassers zu ermitteln, wurden die Austrocknungstoleranzen unterschiedlicher Lebensstadien von *U. pinnatifida* bestimmt. Anhand von Photosynthese-Messungen konnte gezeigt werden, dass morphologische Besonderheiten der Sporophylle die enthaltenen Zoosporen vor der Austrocknung bewahren und infolgedessen eine hohe Austrocknungsresistenz bedingen. Aufgrund dieser Fähigkeit, die Bedingungen an der Luft für gewisse Zeiträume zu überstehen sowie der markanten Eigenschaft der Makroalge mobile, maritime Strukturen zu besiedeln, kommen Transporte über Land als effektiver Invasionsmechanismus in Frage.

In einem multivariaten Laborexperiment wurde mittels physiologischer und biochemischer Analysen die hohe Temperatur- und Salinitäts-Toleranz von *U. pinnatifida* nachgewiesen, die auf einem stabilen Antioxidantienpool beruhte. Es ist anzunehmen, dass diese physiologische Toleranz den Transport der Makroalge sowie die erfolgreiche Ansiedlung invasiver Populationen begünstigt. Darüber hinaus zeigte das Experiment, dass die Toleranz des Einwanderers diejenige einheimischer Tange Neuseelands übertraf, insbesondere hinsichtlich erhöhter Temperaturen. Bei ansteigenden Wassertemperaturen, könnte sich daher ein Konkurrenzvorteil für *U. pinnatifida* ergeben.

Zusammenfassung

Lichtmikroskopische Untersuchungen von Tang-Gametophyten zeigten bislang unbeobachtete interspezifische Interaktionen der mikroskopischen Lebensstadien. Dabei förderte die Anwesenheit des heimischen Riesentangs *Macrocystis pyrifera* das Wachstum und die Oogenese von *U. pinnatifida*. Da die Geschehnisse im Gametophyten-Stadium die nachfolgende Sporophytengeneration entscheidend beeinflussen, könnte die beobachtete Interaktion den Invasionserfolg von *U. pinnatifida* lokal unterstützen. Derartige ökologische Untersuchungen an Tang-Gametophyten sind selten, was, unter anderem, auf Probleme bei der Unterscheidung verschiedener Arten im mikroskopischen Stadium zurückzuführen ist. Um eine zweckmäßige Unterscheidung voranzutreiben, wurden Vor- und Nachteile einer neu entwickelten statistischen Methode sowie bereits etablierter Techniken erarbeitet.

Die experimentellen Ergebnisse dieser Arbeit sowie die Analyse relevanter Literatur legen nahe, dass die prognostizierten Klimaveränderungen den Invasionserfolg von *U. pinnatifida* in vielen Aspekten begünstigen werden. Ihre physiologische Toleranz wird der Makroalge die Anpassung an steigende Wassertemperaturen erleichtern und einen Konkurrenzvorteil gegenüber heimischen Arten bedingen, sollten diese nachteilig betroffen sein. Zusätzlich steigt die Verfügbarkeit von Transportwegen und künstlichen Hartsubstraten durch den wachsenden maritimen Verkehr sowie die fortschreitende Erschließung mariner Lebensräume und begünstigen so die Ausbreitung von *U. pinnatifida* in bislang unbesiedelte Gebiete.

Die vorliegende Dissertation dokumentiert ökologische Mechanismen und physiologische Charakteristiken von *U. pinnatifida*, welche die Ausbreitung der invasiven Makroalge begünstigen und offenbart bislang unerforschte Invasionswege. Als Schlüsselfaktoren dieses Invasionserfolgs schaffen die beobachtete physiologische Toleranz und das komplexe Wechselspiel unterschiedlicher Lebensstadien die Voraussetzung für die Besiedlung eines Gebietes enormer geographischer Reichweite. Die im Rahmen dieser Arbeit gewonnenen Erkenntnisse eröffnen ein umfassendes Verständnis von Faktoren, die dem Invasionserfolg von *U. pinnatifida* zugrunde liegen, und können somit zur Entwicklung effektiver Strategien gegen die weitere Ausbreitung der Makroalge in neue Lebensräume genutzt werden.

Chapter 1: General introduction

1.1 The history of species' introductions

"I do not pretend to indicate the exact lines and means of migration, or the reason why certain species and not others have migrated; (...). We cannot hope to explain such facts, until we can say why one species and not another becomes naturalised by man's agency in a foreign land."

Charles Darwin (1859)

Already recognized by naturalist Charles Darwin over 150 years ago, the introduction of species into new habitats is not a recent phenomenon. In prehuman times, shifts in species' distributions and introductions into new areas were induced by fluctuating climatic conditions, e.g. following the end of the last glacial period about 10 000 years ago (Lodge 1993). Additionally, species migrated to new areas once geographic barriers were removed by natural processes, e.g. tectonic movements or sea-level variations (Dana 1975, Burney 1995, Stigall & Lieberman 2006, Levine 2008). A well studied example of historic introductions is the 'Great American Biotic Interchange' that was triggered by the closure of the Isthmus of Panama about three million years ago. The formation of an inter-American land bridge induced a major, two-directional exchange of biota between North and South America, two ecosystems which had previously been isolated from one another (Brown & Sax 2004, Smith & Klicka 2010). As a result, many recent South American genera are descendants of their northern ancestors (Webb 2006).

Over the course of human technological development new means of species' dispersal emerged. Humans have fostered the introduction of plants and animals outside their native range by cultivation, domestication and translocation of species for food production and horticulture for centuries (Mack 1991, Mack 2000, Zeder 2008). Intentional and unintentional human-mediated, inter-oceanic transports of species via wooden ships have been occurring since the 1400s (Carlton 1999). The continuing development of infrastructure further invalidated geographic and physical barriers for species' introductions. In 1869, the opening of the Suez Canal, an artificial corridor connecting the Mediterranean and the Red Sea for watercraft, created a new route for the

interchange of marine biota (Steinitz 1968, Galil et al. 2015). It is estimated that over half of the multicellular species introduced to the Mediterranean entered through the Suez Canal (Galil et al. 2014).

With the onset of globalisation in recent decades, species' introductions have reached a new magnitude and differ substantially from ancient events with regards to introduction rate, dispersal distances and the extent of affected regions (Vitousek et al. 1997, Ricciardi 2007, Hulme 2009). Increasing global transport and trade have enhanced the connectivity of ecosystems and accelerated the spread of species. Scientists emphasize that the current, human-mediated exchange of organisms occurs on a global scale and simultaneously concerns all continents including remote ecosystems, such as oceanic islands (Burney 1995, Cassey et al. 2005, Ricciardi 2007). While historic long-distance dispersals, e.g. by rafting, have been rare events, species are now transported across the globe on a daily basis, arriving in environments they would have never reached by natural means (Burney 1995). Today's geographic and taxonomic patterns of species introductions appear to be strongly driven by human variables, such as population density, trade, transport and wealth (Perrings et al. 2005, Pyšek et al. 2005). In addition, synergistic effects with global warming might drastically accelerate the impact of human activities on species' distributions (Ricciardi 2007).

1.2 Towards a consistent definition of species' invasions

As a result of the long history of research on species introductions and its parallel development across different taxonomic groups and environments (Blackburn et al. 2011), a variety of incongruent terminologies and definitions are in use (Pyšek 1995, Richardson et al. 2000, Ruiz & Carlton 2003, Colautti & MacIsaac 2004). Darwin (1859) used the term 'naturalized' to characterise the non-native status of a species, while other scientists use terms such as 'alien', 'nonindigenous', 'pest' or 'exotic' species (Colautti & MacIsaac 2004). In ecological abstracts (1970 - 1993) on plant introductions the term 'invasive' was most frequently used, with its occurrence in publications increasing over time (Pyšek 1995). This term is widely recognized, both by the public and the scientific community (Ruiz & Carlton 2003). Despite its common application, a consensus on a precise and uniform definition has not yet been reached (Pyšek 1995, Ruiz & Carlton 2003, Blackburn et al. 2011). In recent years, a number of authors reviewed the usage of different terminologies and proposed frameworks to determine the status of species (Colautti & MacIsaac

2004, Falk-Petersen et al. 2006, Blackburn et al. 2011).

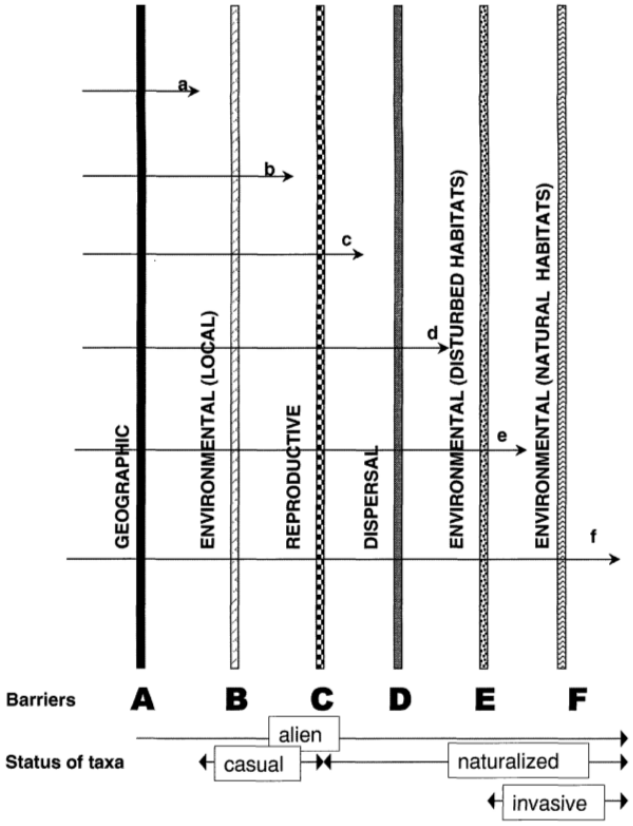

Figure 1.1 Scheme of the invasion process represented as a series of barriers a species has to overcome and the associated terminology (primarily designed for plant introductions). The barriers are (A) major geographical barriers, most often overcome by man's agency, (B) environmental conditions (biotic and abiotic) at the introduction site, (C) barriers to reproduction, (D) barriers to local or regional dispersal, away from the initial introduction site, (E) environmental barriers in human-modified habitats, and (F) environmental barriers in natural habitats. The arrows (a – f) represent the paths followed by organisms to reach the different states. Taken from Richardson et al. (2000).

For a proper definition it is helpful to develop an explicit understanding of the 'native' status. It is widely accepted that 'native' species occur in a specific habitat independent of human activities (Pyšek 1995). According to Webb (1985), a species which evolved in an area before the beginning of the Neolithic age or which has arrived since that time independent of human activity, can be termed 'native'. Although native to a specific region, a species can become invasive to another. This invasion process, as described by Richardson et al. (2000), requires a species to overcome a series of abiotic and biotic barriers (Fig. 1.1). Based on the failure and success of a species tackling these barriers, phases of the process and the associated terminologies can be defined

(Richardson et al. 2000). According to this scheme a species is considered invasive once it has spread from the site of initial introduction, is capable of reproducing and dispersing and is able to cope with the new abiotic and biotic environmental conditions (Richardson et al. 2000). The scheme of Richardson et al. (2000), although originally established with regard to plants, was used as basis for more recent frameworks on species' invasions (Colautti & MacIsaac 2004, Blackburn et al. 2011). Blackburn et al. (2011) merged schemes of Richardson et al. (2000) and Williamson & Fitter (1996) in order to design a framework applicable to all human-mediated invasions. Recognizing that some introduced species live solely in captivity and cultivation, an additional barrier ('escape from captivity and cultivation') was incorporated (Blackburn et al. 2011). Some authors proposed to use the term 'invasion' for conditions in which a new species impacts the ecology or economy of the receiving environment (IUCN 1999, Davis & Thompson 2000, Davis & Thompson 2002). Richardson et al. (2000) noticed that the presence of an invasive species may cause detectable ecological or economic consequences. In contrast, Blackburn et al. (2011) excluded impact-based aspects from the definition of invasive species, claiming that impacts may occur inconsistently throughout the invasion phases or might be difficult to determine.

1.3 The ecological and economic impact of invaders

Without a doubt, some introduced species are beneficial to humans and their economy, such as food crops and livestock which are often grown outside the native range (Vitousek et al. 1997, Sax et al. 2007). However, a major ecological concern associated to the continual introduction of species is the potential homogenisation of global diversity. Although the establishment of species in new areas may increase local diversity, the occurrence of extinctions associated with human-mediated introductions as well as the replacement of endemic species by widespread and well-established species are leading to a decrease of overall gobal species richness (Vitousek et al. 1997, Brown & Sax 2004, Cassey et al. 2005). McKinney & Lockwood (1999) pointed out that this homogenisation may be further enhanced as promoted species are not randomly distributed among taxa. Incoming invasive species may threaten native diversity by competition, predation or herbivory (Brown et al. 2002, Gurevitch & Padilla 2004). Many invasive species are capable of altering habitat structure and impact environmental conditions, e.g. water quality, nutrient cycling or vegetation (Ehrenfeld 2003, Andersen et al. 2004, Gurevitch & Padilla 2004), which suggests that native biota may also be indirectly affected by invasive species.

In addition to ecological issues, invasive species may cause significant economic losses (Pimentel et al. 2001). Pimentel et al. (2000) estimated that the costs of non-indigenous species in the US totalled $137 billion per year. Losses to the yield of different industries, e.g. due to alterations to ecosystem services and costs for prevention and control are among the most prominent expenses related to invasive species (Xu et al. 2006, Pejchar & Mooney 2009). Concerned industries comprise agriculture, forestry, stockbreeding, fishery, water transportation, tourism and human health (Xu et al. 2006). Another problem is the man-mediated introduction of livestock or human diseases (Ruiz et al. 1997, Vitousek et al. 1997).

1.4 Vectors of marine invasions

Since watercraft was the driving force of the first human-mediated long-distance introductions, it comes as little surprise that many invasions occurred in the marine environments. Mimicking speed and port-residency of the 16th century with a replica of Sir Francis Drake's famous galleon 'Golden Hinde', Carlton & Hodder (1995) described the arising fouling community along a 800 km transect. Carlton (1999) assumed that such ancient wooden vessels might have easily transported more than 150 species that bored into the hull, fouled hull and anchor systems, or thrived in sand or water ballast. Even today, shipping pathways represent the dominant vector for marine introductions (Fofonoff et al. 2003, Streftaris et al. 2005, Gollasch 2006, Molnar et al. 2008). As a result, harbours and marinas are prone to be the points of first entry for invasive species on new coasts (Reise et al. 1999, Ashton et al. 2006). A species directly arriving from its native range is considered a primary introduction (Minchin et al. 2009).

The invasion risk for a specific coastal ecosystem depends on shipping intensity and connections (Seebens et al. 2013). Increasing number, size and speed of vessels as well as greater volumes of ballast water transported have accelerated the frequency of unintentional species arrivals (Carlton 1996, Reise et al. 1999). Despite the efforts of antifouling strategies, marine organisms fouling large vessels, such as cargo and container ships, continue to be transported around the globe (Piola & Johnston 2008, Davidson et al. 2009, Piola et al. 2009). Ballast water and sediment have been recognized as the major vectors for species' introductions (Williams et al. 1988, Olenin et al. 2000, Gollasch et al. 2002, Mineur et al. 2007). Various organisms have been observed in ballast tanks, including bacteria, viruses, zoo- and phytoplankton, planktonic stages of benthic organisms, meiobenthic and vertebrate species (Hallegraeff & Bolch 1992, Gollasch et al. 2000, Ruiz et al.

2000, Wonham et al. 2000, Pertola et al. 2006, Radziejewska et al. 2006, Drake et al. 2007, Flagella et al. 2007, Klein et al. 2010). Depending on species' traits and the abiotic conditions, individuals might not only survive trans-oceanic travels, but reproduce within the ballast water tanks (Gollasch et al. 2000), thereby enhancing the invasion potential.

A second key vector of species' introductions is the transport of target aquaculture organisms and associated species, e.g. epibiota or parasites, that are unintentionally transported (Streftaris et al. 2005, Gollasch 2006, Molnar et al. 2008). This phenomenon is well displayed by the importation of oysters for aquaculture activities (Naylor et al. 2001, Ruesink et al. 2005). The Pacific oyster *Crassostrea gigas* was repeatedly imported to Europe and proved capable of reproducing in European waters, resulting in the spread and establishment of wild populations outside designated aquaculture areas (Drinkwaard 1999, Troost 2010). In association with the introduction of *C. gigas* the arrival of different alga and copepod species was observed (Peréz et al. 1984, Holmes & Minchin 1995). Species travelling with oysters might be transported fouling the oyster shell, occurring within the mantle cavity or tissue, as well as in unoccupied shells of dead oysters (Verlaque et al. 2007).

From its initial introduction site, a species may further spread via secondary transport by natural means or human-mediated transport (Minchin et al. 2009). Recreational boating is a prominent vector for secondary spread, including intra- and interoceanic travels, as well as overland transport with trailered boats (Murray et al. 2011). Other vectors for both primary and secondary spread of marine organisms comprise the release of ornamental species, fisheries and recreational water uses (Whitfield et al. 2002, Padilla & Williams 2004, Minchin et al. 2009). Research and education may also contribute to species introductions by escape or discard of experimental organisms or intentional outplantings for field experiments (Kornmann & Sahling 1994, Minchin et al. 2009).

Species may be introduced simultaneously by several mechanisms and in some cases it might not be possible to identify the transport agent of arriving species (Ruiz et al. 2000, Minchin et al. 2009). Extensive analyses of historical and observational data may shed light on vectors to specific regions (Semmens et al. 2004) and in many cases genetic methods have helped to determine the donor region of introduced marine populations (Estoup & Guillemaud 2010, Geller et al. 2010).

1.5 Concepts of invasibility and invasiveness

Ecologists have always been fascinated by the question which factors determine the success or failure of species' invasions. The observation, that the proportion of persistent invaders varies across habitat types gave rise to the idea that some ecosystems are more susceptible to incoming species than others, a concept commonly known as 'invasibility' (Williamson & Fitter 1996, Lonsdale 1999). Considered an intrinsic property of an environment, invasibility is affected by the region's climate, the level of disturbance and, for the most part, by local biological conditions (Lonsdale 1999). At the forefront of this, native species' diversity is discussed to be a major determinant of environmental invasibility (Elton 1958, Tilman 1997, Lonsdale 1999). This hypothesis is based on the assumption that more diverse communities better resist the invasion of new species than do species-poor assemblages. Even though this relationship was substantiated by experimental results on grassland communities (Tilman 1997), other authors contested a causal link between biodiversity and invasibility (Levine & D'Antonio 1999, Lonsdale 1999). Some researcher attributed the observed correlation to a third parameter that simultaneously impacts biodiversity and susceptibility to invasions, such as the availability of open space (Stachowicz et al. 1999, Stachowicz et al. 2002). Accordingly, an experiment on a subtidal marine invertebrate community demonstrated that, at constant levels of diversity, the recruitment rates of new invaders depended on the availability of free settlement space (Stachowicz et al. 2002). A more general approach by Davis et al. (2000) proposed fluctuations in resource availability to be a key factor controlling community invasibility. Thus, the often observed increase in invasibility following disturbance events might be a result of the subsequently increasing availability of resources, e.g. by the addition of nutrients (Davis et al. 2000). As a determinant of resource use, the identity of native functional groups was recognized as an influence to invasion resistance (Pokorny et al. 2005, Arenas et al. 2006). This in turn explains observations by Darwin (1859), who found an environment to be more susceptible to invasions by species from genera not represented within the native community.

Not only properties of the receiving environment determine invasion success, but also characteristics of the incoming organism, referred to as its 'invasiveness', play an important role in the process (Williamson & Fitter 1996, Blackburn et al. 2011). In order to identify common traits of invaders, observational and manipulative experiments compared properties of native and invasive species (e.g. Vilà & Weiner 2004, van Kleunen et al. 2010, Davidson et al. 2011). Propagule pressure, as a result of the organisms quality or provided by human forces, is considered to

explain much of the variation in invasion success (Williamson & Fitter 1996). Additionally, high growth rates, short generation times and broad physiological tolerance are, in many cases, attributed to successful invaders (Dukes & Mooney 1999, Fletcher & Farrell 1999, Grotkopp et al. 2002, Sorte et al. 2010, Zerebecki & Sorte 2011). The largely accepted enemy release hypothesis states, that invaders experience reduced regulation through native predators, herbivores, parasites or diseases in the new habitat (Keane & Crawley 2002, Caulotti et al. 2004, Troost 2010). By this mechanism, the invader is capable of rapidly increasing its abundance and distribution range. However, these interactions among the receiving habitat and the incoming organism might be species-specific and regionally-dependent (Radford & Cousens 2000), indicating that invasion success cannot be determined by a consistent combination of species' traits (Williamson & Fitter 1996, Radford & Cousens 2000).

1.6 The invasive kelp *Undaria pinnatifida*

A striking example of a global invasion is the spread of the laminarian kelp *Undaria pinnatifida* (Harvey) Suringar 1873 that was ranked among the '100 worst invasive alien species' (Lowe et al. 2000). One of three species of the genus *Undaria,* it is native to the shores of Japan, Korea, China and South-East Russia (Fig. 1.2; Saito 1965, Saito 1972, Saito 1975, Yamanaka & Akiyama 1993). The genus name is derived from the Latin word 'unda' for 'wave', referring to its undulating thallus shape (Silva et al. 2002). In its native range *U. pinnatifida* is both harvested from nature and commercially cultivated, supporting a vast industry for food production (Tseng 2001, Silva et al. 2002). Commonly known as 'wakame' in Japan and as 'qun dai cai' in China, products from *U. pinnatifida* are popular for their high fibre content (Yamanaka & Akiyama 1993, MacArtain et al. 2007). A huge variety of products exist, ranging from soup and seaweed salad to powder and wakame-based snacks, that are increasingly exported to Europe and America (Yamanaka & Akiyama 1993, Radmer 1996, Lee 2010).

In order to protect its natural occurrence propagation techniques have been studied in Japan (Saito 1975, Silva et al. 2002). These methods included the deposition of stones and sporophylls on the seafloor, as well as blasting of rocks to optimise water depth and surface area for zoospore settlement (Saito 1975). Mass cultivation of *U. pinnatifida* began in 1955 (Saito 1975). For this, ropes are inoculated with zoospores and transplanted to the sea (Silva et al. 2002). Mature thalli are harvested from boats or by divers (Saito 1975). First cultivation attempts in China used

specimens imported from Japan or Korea (Tseng 2001) and for cultivation purpose *U. pinnatifida* was intentionally introduced to Taiwan in 1981 (ICES 2007).

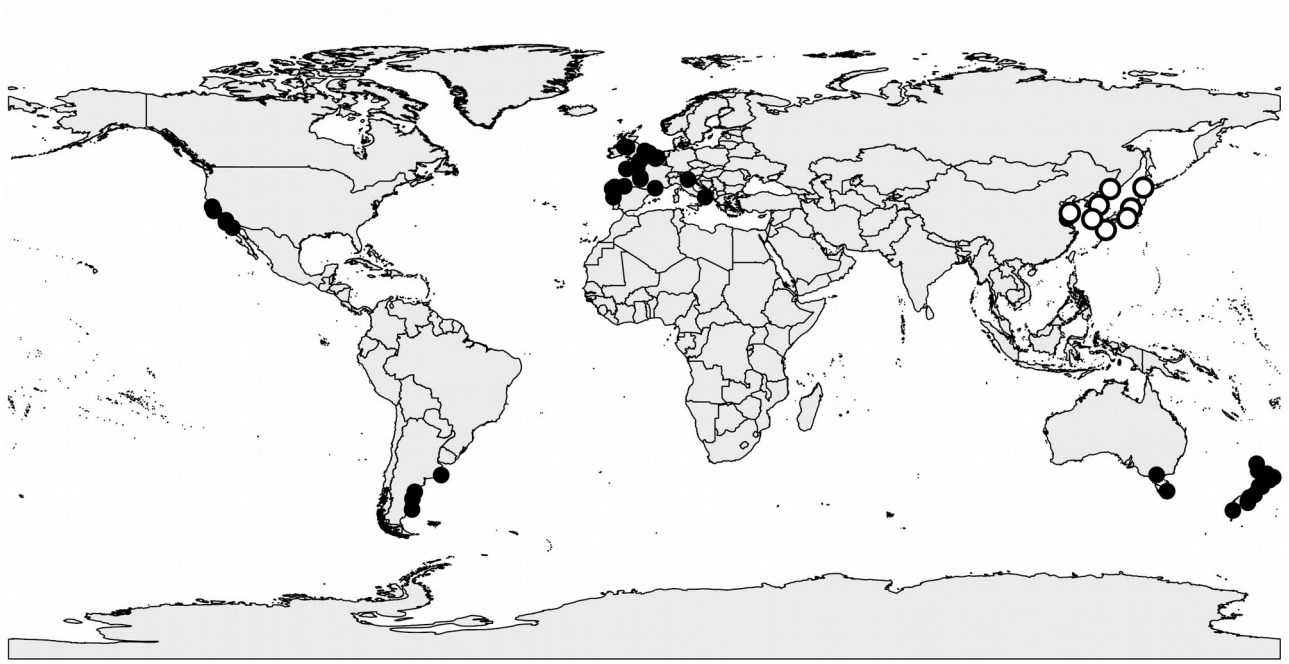

Figure 1.2 Global range of native and introduced *Undaria pinnatifida* populations. White circles indicate native distributions and black circles mark invasive populations. This map was created by Merle Bollen, compiled distribution data was adopted from James et al. (2015).

Its first unintentional introduction outside the native range was recorded in the Thau lagoon, France in 1971 (Peréz et al. 1981, Verlaque 2001, ICES 2007). The kelp arrived accidentally, most likely with oyster imports from Asia (Peréz et al. 1981, ICES 2007). A decade later, in 1983, *U. pinnatifida* was deliberately introduced to three sites in the North Atlantic (Brittany, France) for pilot farming experiments by the French Institute for the exploitation of the Sea (IFREMER; Hay & Villouta 1993). France and several other European countries including Norway, Scotland and Ireland announced interest in cultivating *U. pinnatifida* for human consumption (Floc'h et al. 1991). According to some laboratory experiments temperature regime at the designated field sites was unsuitable to complete the reproductive cycle of *U. pinnatifida* (Floc'h et al. 1991). However, in 1987 specimens were found growing outside the aquaculture site on immersed supporting structures of a mussel farm and to be capable of reproducing in the North Atlantic (Floc'h et al. 1991). Shortly thereafter, *U. pinnatifida* was first observed in New Zealand, growing on a breakwater in Wellington Harbour (Hay & Luckens 1987) and one year later it showed its first

appearance in Tasmania, Australia (Sanderson 1990). For these introduction events, shipping from the native range of *U. pinnatifida* are assumed as the vector (Hay & Luckens 1987, Sanderson 1990). In the following two decades the kelp established itself along many shorelines throughout Europe (Belgium, Italy, Portugal, Spain, the Netherlands, UK) and North and South America (Argentina, Chile, Mexico, USA; Fig. 1.2; ICES 2007 and references therein). Initial sightings of *U. pinnatifida* in the invaded range are often recorded from artificial substrates, such as breakwaters, walls, wooden and concrete wharf piles, supporting structures used in aquaculture, steel cables, ropes and buoys (Peréz et al. 1981, Hay & Luckens 1987, Floc'h et al. 1991, Parsons 1995, Curiel et al. 1998). The kelp predominantly established itself in ports and marina environments, although was found to colonise natural rocky reefs in some of the invaded regions (Stuart 2004, Heiser et al. 2014, Arnold et al. 2016).

Like all kelps, the annual *U. pinnatifida* exhibits a heteromorphic, life cycle with a macroscopic, diploid sporophyte alternating with microscopic, haploid male and female gametophytes (Fig. 1.3). The sporophyte exhibits a blade-like lamina with a stipe extending into a flattened midrib, in native habitats it grows 45 to 110 cm long (Saito 1972, Choi et al. 2007). In contrast, cultivated sporophytes with a length of up to 3 m have been recorded (Peréz et al. 1984). A dichotomously branched holdfast attaches the sporophyte to the substrate. Thallus growth is initiated in the meristematic region at the interface of stipe and blade (Saito 1965) and a folded sporophyll arises along the stipe in maturing plants (Nelson 2013). Flagellated zoospores that are released from mature sporophylls reach 9 µm in length (Saito 1965). A zoospore seems capable of swimming against minor currents (< 8 cm s^{-1}) and selectively chooses its settlement ground (Saito 1975, Petrone et al. 2011). After settlement, spores germinate and develop into thin-celled male gametophytes and females that are characterized by relatively shorter and thicker cells (Saito 1965). A more detailed description of gametophyte morphology and development in laboratory conditions is provided in chapter 5 of this thesis. The release of spermatozoids from the antheridia of male gametophytes, as well as their attraction, is triggered by pheromones emitted by the female gamete (Lüning & Müller 1978, Maier 1982). Male and female gamete fuse to form a zygote that develops into a young, multicellular sporophyte.

In native habitats the seaweed displays a winter annual life cycle with seasonal senescence of the sporophyte occurring at water temperatures exceeding 24 °C (James et al. 2015). At this temperature the microscopic gametophytes cease growing and enter a resting stage, that was described by Saito (1975) as spherical cells enclosed by thickened cell walls (Saito 1975). This

ability of its gametophytic stage to sustain unfavourable conditions may enable *U. pinnatifida* to be transported with ballast water and preserve its viability. Gametophytes resume growth in autumn (October, November) and begin maturation at temperatures below 22 °C (Saito 1965, Saito 1975). The seasonal growth pattern of *U. pinnatifida* sporophytes, however, is variable and depends on the local temperature regime (Saito 1975, James et al. 2015). Thus, in some invaded regions, e.g. France, Tasmania and New Zealand, *U. pinnatifida* sporophytes display a persistent year-round phenology (Hay & Villouta 1993, Castric-Fey et al. 1999, James et al. 2015).

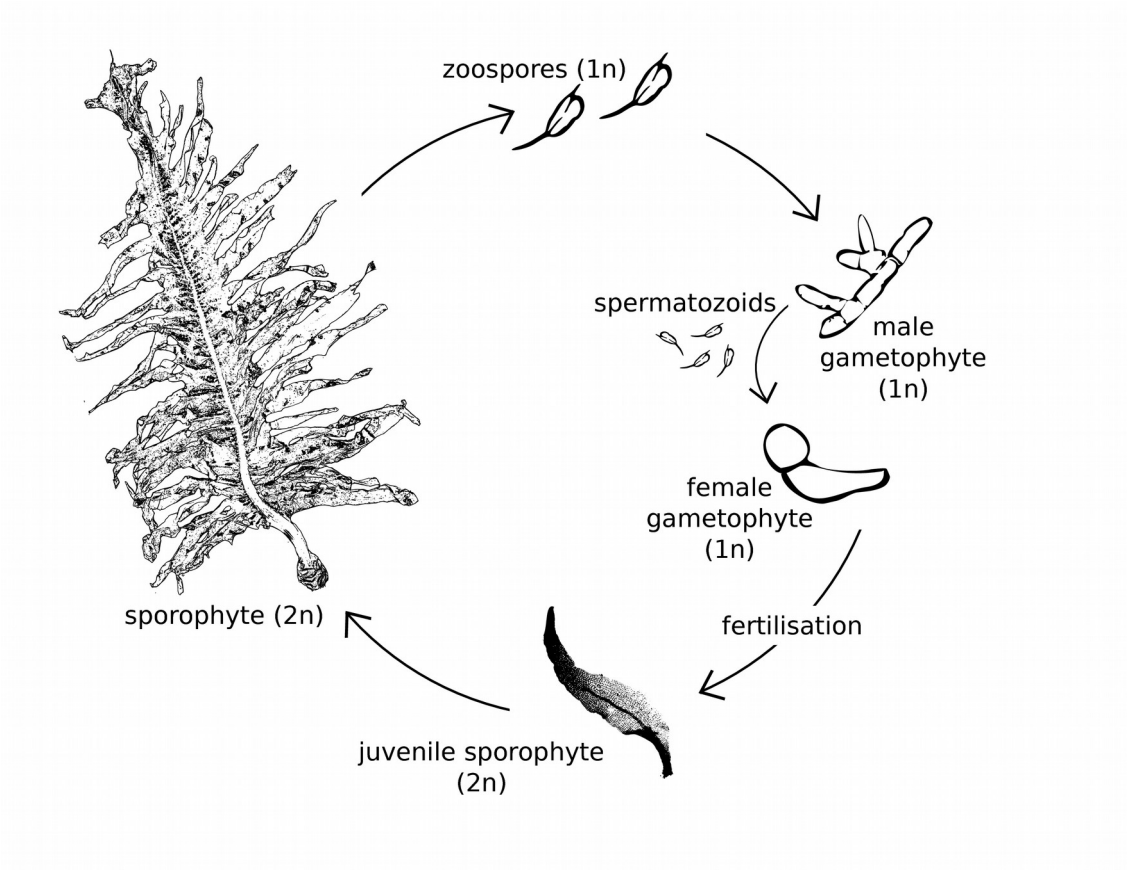

Figure 1.3 Schematic representation of the life cylce of *Undaria pinnatifida* which alternates between a diploid (2n) sporophyte and haploid (1n) gametophytes. This graphic was created by Merle Bollen.

As a canopy-forming species *U. pinnatifida* is considered to be an ecosystem engineer (*sensu* Jones et al. 1994) capable of altering environmental conditions and habitats (Crooks 2009, Irigoyen et al. 2011), e.g. by growing in previously unvegetated areas (Wallentinus & Nyberg 2007). Temporal stability of the biogenic habitat may be affected when a community shifts from perennial to annual species (Arnold et al. 2016). Consequently, ecological concerns have

accompanied the spread of *U. pinnatifida* into new habitats. The kelp is thought to compete with native seaweed species for habitat space and light (Curiel et al. 1998, Farrell & Fletcher 2006), impact native species abundance and diversity (Hay & Villouta 1993, Piriz et al. 2003, Casas et al. 2004, Irigoyen et al. 2011) and has established itself as the dominant species in some habitats (Battershill et al. 1998). Differences in the associated fauna and feeding preferences of native grazers might induce further alterations to natural community composition and impact trophic relationships (Raffo et al. 2009, Jiménez et al. 2015).

1.7 Thesis outline

The invasive kelp *U. pinnatifida* has established itself along many coastlines worldwide and based on temperature requirements its invaded range is predicted to expand (James et al. 2015). In the opening quotation, Darwin (1859) points out that a comprehensive understanding of a species' expansion mechanisms is indispensable in order to predict future distribution patterns and the ecological impacts of an invasive species. Furthermore, such knowledge is a prerequisite for potential prevention techniques. In this thesis, factors and characteristics favouring the invasive success of *U. pinnatifida* are studied along various phases of its invasion process. The traits of different life history stages were studied in order to answer the overarching research question:

“Which characteristics of the kelp *U. pinnatifida* facilitate its invasion into new habitats?”

Different sites along the New Zealand coast were chosen as study area for this thesis. Due to its geographic isolation New Zealand is renowned for the high level of endemism of its native biota, as including seaweeds (Diamond 1990, Gordon et al. 2010). As an oceanic archipelago that receives shippings and tourists from all around the world, New Zealand is vulnerable to species' introductions (Vitousek et al. 1997). Eradication efforts began soon after the introduction and spread of *U. pinnatifida* in New Zealand by way of manual sporophyte removal, chemical treatments (e.g. sodium hypochlorite and a brominated micro-biocide) and the application of localised heat treatments to sterilise gametophyte banks (Stuart 2004, Hunt et al. 2009). Therefore, its special ecological condition and its hands-on environmental policy, make New Zealand an ideal area to study species' invasions.

Before an organism may become invasive in an area, it needs to be transported through man's

agency (Sakai et al. 2001), i.e. the organism has to overcome the first barrier (A) in the scheme by Richardson et al. (2000; Fig. 1.1). While different water routes have been assessed, e.g. ballast water, hull fouling or drifting (Hay & Luckens 1987, Hay 1990, Forrest et al. 2000, Farrell & Fletcher 2004), possibilities of emerged transport have not been considered for the spread of *U. pinnatifida*. However, as a common fouling species (Hay 1990, Wotton et al. 2004), *U. pinnatifida* may be carried outside the water via fouled aquaculture equipment or trailered boats, as observed for other aquatic organisms (Buchan & Padilla 1999, Johnson et al. 2001, Shurin & Havel 2002). Therefore, in chapter 2, the potential for transport in emerged conditions is evaluated testing desiccation tolerance of various life stages of *U. pinnatifida*.

Chapter 3 discusses the barrier provided by environmental conditions (barrier B; Fig. 1.1; Richardson et al. 2000) encountered by the arriving organism. Invasion success is partly regulated by abiotic conditions (Levine 2008), such as temperature and salinity, and may be impacted by changes in abiotic parameters (Crooks et al. 2011), e.g. caused by global warming. As a result, a broad physiological tolerance is considered to be advantageous for invaders (Dukes & Mooney 1999), especially as less tolerant species in the same habitat are suggested to be disproportionally negatively affected by the impacts of climate change (Sorte et al. 2010, Zerebecki & Sorte 2011). Thus, chapter 3 compares species-specific physiological traits of *U. pinnatifida* to native kelps under combinations of various temperature and salinity regimes. The results shed light on potential future interactions among the regarded species and their expected distribution patterns.

Interactions with native biota exhibit a further barrier that needs to be overcome by an invasive species (barriers E and F; Fig. 1.1; Richardson et al. 2000). Invasive seaweeds bear the potential of altering structure and function of the invaded ecosystems (Schaffelke & Hewitt 2007), e.g. by outcompeting native habitat forming species. Consequently, chapter 4 investigates the interactions of *U. pinnatifida* with a native New Zealand kelp, concentrating on widely unnoticed interactions among their gametophytes.

Studying ecological aspects of kelp gametophytes challenges the experimenters due to the microscopic size of gametophytes. This implies difficulties in observing these life stages in the field and distinguishing between species. In chapter 5, the results of both published studies and own results from laboratory pre-experiments are combined to depict specific features of culture-grown gametophytes as well as challenges and current methodologies for studying microscopic kelp stages.

In particular, the following hypotheses were assessed:

I Various life stages of *U. pinnatifida* display specific tolerances to desiccation, and thus, display distinct potential for transport in emerged conditions. (Chapter 2)

II *U. pinnatifida* is more resistant to changing abiotic conditions than native kelps. (Chapter 3)

III Interactions between the invasive *U. pinnatifida* and native kelp species occur at gametophyte stage. (Chapter 4)

IV The projected climate change will foster the spread of *U. pinnatifida*. (Chapter 3, 4)

Synthesizing the experimental outcome of this thesis and relevant literature, chapter 6 revisits these hypotheses and reviews invasion mechanisms of *U. pinnatifida* at different phases of the invasion process. Additionally, the impact of human-induced changes on the kelp's invasion success and associated ecological implications are discussed with respect to future distribution patterns. In closing, an outlook on future research foci is provided and a synoptic conclusion highlights the most prominent results of this thesis.

1.8 Publication outline and declaration of contribution

The presented dissertation is based on the following publications:

1 **Publication:** Desiccation tolerance of different life stages of the invasive marine kelp *Undaria pinnatifida*: Potential for overland transport as invasion vector
(presented in chapter 2)
Authors: M. Bollen, C. N. Battershill, C. A. Pilditch, K. Bischof
Journal: Journal of Experimental Marine Biology and Ecology (submitted)

For the different experimental sections of this study, specific research questions and experimental designs were developed by M. Bollen, C. Battershill, C. Pilditch and K. Bischof. Laboratory set-ups and field sampling were accomplished by M. Bollen with assistance of technical staff of the University of Waikato, New Zealand, and the team of the Coastal Marine Field Station in Tauranga, New Zealand. M. Bollen carried out the experimental work, the ecological evaluation and statistical analyses. M. Bollen wrote the manuscript and prepared all figures. All co-authors discussed and revised the manuscript.

2 **Publication:** Salinity and temperature tolerance of the invasive alga *Undaria pinnatifida* and native New Zealand kelps: Implications for competition
(presented in chapter 3)
Authors: M. Bollen, C. A. Pilditch, C. N. Battershill, K. Bischof
Journal: Marine Biology (2016) 163: 194; doi: 10.1007/s00227-016-2954-3

The experimental idea and set-up were designed by M. Bollen, C. Pilditch and K. Bischof. With assistance of the technical staff of the University of Waikato, New Zealand, M. Bollen carried out the experiment and monitored physiological parameters. Biochemical analyses were performed by M. Bollen with technical assistance for the HPLC measurements at the University of Bremen, Germany. M. Bollen processed data and accomplished the statistical analyses. M. Bollen wrote the manuscript and prepared all figures. All co-authors discussed and revised the manuscript.

3 **Publication:** Interactions between microscopic gametophytes of the invasive kelp *Undaria pinnatifida* and the native *Macrocystis pyrifera* – Implications for invasive success
(presented in chapter 4)
Authors: M. Bollen, C. D. Hepburn, K. Bischof
Journal: in preparation

M. Bollen, C. Hepburn and K. Bischof developed the experimental idea and designed the laboratory set-up. Laboratory work, sampling of macroalgal specimens and gametophyte monitoring using light microscopy was accomplished by M. Bollen with assistance of the team of Portobello Marine Laboratory, New Zealand. The statistical differentiation and analysis was realized by M. Bollen with the support of the Institut für Statistik of the University of Bremen, Germany. M. Bollen wrote the manuscript and prepared all figures. All co-authors discussed and revised the manuscript.

Chapter 2: Desiccation tolerance of different life stages of the invasive marine kelp *Undaria pinnatifida* - Potential for overland transport as invasion vector

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In review: Journal of Experimental Marine Biology and Ecology

2.1 Abstract

Overland-transport in emerged conditions, including the translocation of specimens fouled on trailered boat hulls, aquaculture ropes or entangled in fishing gear, is recognized as a short-distance vector for the introduction of invasive aquatic species. Here, the desiccation tolerance for different life stages of the invasive kelp *Undaria pinnatifida* was determined to test the likelihood of emerged transport. Water content, photosynthetic quantum yield (Fv/Fm) as well as the capacity to release viable zoospores was monitored for mature sporophylls during a five-day exposure to air conditions. For newly settled spores (16 h-post-release) and developing gametophytes (30 h-post-release) survival and growth was observed after 1, 3, 6, 12 and 24 h of desiccation. Additionally, zoospore settlement and desiccation survival (after 3, 12 and 48 h) of seven-day-old gametophytes was determined growing on different rope materials (polyethylene, nylon, polypropylene, hemp) in high (99 % relative air humidity, RH) and typical (59 % RH) air humidity conditions. Viable zoospores were released from mature sporophylls after three days of desiccation. Less than 0.4 % of spores and gametophytes survived air exposure, however, single gametophytes endured 12 h of desiccation. These specimens exhibited enhanced average lengths, growing up to 70 % larger than control gametophytes. Attached to ropes, gametophytes survived 48 h of desiccation and settlement and desiccation survival did not differ between rope materials. Overall, this study proposes emerged transport, especially the translocation of mature sporophylls, to represent a potential spread mechanism for the invasive *U. pinnatifida*, that should be considered for pest management.

2.2 Introduction

Species invasions in marine habitats have dramatically increased in the last decades (Ruiz et al. 1997, Gollasch 2006, Rilov & Crooks 2009) causing severe ecological and economic concerns (Anil et al. 2002, Grosholz 2002, Perrings et al. 2002). Introduction vectors are often associated with a variety of human activities, such as shipping, aquaculture and the aquarium trade (Padilla & Williams 2004, Streftaris et al. 2005, Hellmann et al. 2008). While shipboard transport in submerged conditions, e.g. within ballast water or attached to boat hulls, represents the most common source of introduction (Ruiz et al. 1997, Molnar et al. 2008), aquatic species may travel short distances overland, potentially experiencing air exposure (Rothlisberger et al. 2010, Bacela-Spychalska et al. 2013). Via fouled aquaculture equipment or trailered boats, organisms might be carried outside the water from one port to the next (Johnson et al. 2001). Even though a number of studies have assessed this vector for freshwater invasions (Buchan & Padilla 1999, Shurin & Havel 2002, Bacela-Spychalska et al. 2013), investigations for marine organisms are scarce. For the invasive marine alga, *Caulerpa taxifolia*, emerged transport on anchors was suggested as a vector (Creese et al. 2004, West et al. 2007), with the assumption it has an ability to survive extended time periods out of the water. Such tolerance to desiccation will determine the likelihood and dispersal distance for emerged transport conditions.

Causing adverse effects on native populations (Hay & Villouta 1993, Battershill et al. 1998, Curiel et al. 1998, Piriz et al. 2003), the Asian kelp *Undaria pinnatifida* (Harvey) Suringar (Laminariales) was ranked among the '100 of the world's worst invasive alien species' (Lowe et al. 2000). Following its first unintentional introduction to the French Mediterranean coast in 1971, the invader has established on many coastlines worldwide (ICES 2007, and references therein). *U. pinnatifida* exhibits a heteromorphic life cycle involving a macroscopic, diploid sporophyte generation alternating with microscopic haploid male and female gametophytes. Both, sporophyte and gametophyte of *U. pinnatifida*, are commonly growing on artificial and potentially portable substrates, i. e. rope and boat hulls (Hay 1990, Wotton et al. 2004). Physiological tolerance and ecological function varies with life history stages, thus, successful overland transport and establishment is likely to vary also. Extensive resistance to darkness (tom Dieck 1993), potentially enables gametophytes of *U. pinnatifida* to travel long distances within ballast water tanks of international shipping, as suggested for the introduction to New Zealand in 1987 (Hay & Luckens 1987). Sporophytes, however, might reach new habitats through drifting (Forrest et al. 2000) or attached to hulls of recreational boats (Hay 1990, Farrell & Fletcher 2004). Thus, different life

stages should be considered for investigations on invasion vectors.

The aim of this study was to evaluate the potential of emerged transport for the invasive *U. pinnatifida*, testing survival times of different life stages in desiccation conditions. Varying rope materials might provide different water-holding capacities and thereby impact survival of potentially transported specimens. Thus, water-holding capacity of varying rope materials was compared and zoospore settling preference and desiccation resistance of gametophytes was tested.

2.3 Materials & Methods

2.3.1 Sporophyll desiccation experiment

Specimens of *Undaria pinnatifida* were collected from Tauranga Harbour, New Zealand (37°38'S, 176°10'E), in September 2013. Sporophylls were cut from ten kelp specimens and exposed to air in a temperature-controlled incubator (15°C, 59 % relative air humidity (RH), 70 - 80 µmol m^{-2} s^{-1} photosynthetic active radiation (PAR) irradiance, 12:12 hour light cycle) for five days. Parameters (tissue water content, photosynthetic quantum yield in terms of Fv/Fm, spore release, spore mobility, spore germination rates and gametophyte length) were recorded for each day of the experiment (d 0 – 5, where d 0 is the day of collection). For the assessment of tissue water content, two discs (diameter 2.05 cm) were cut from six haphazardly chosen sporophylls and weight determined before and after drying at 100 °C. Variable chlorophyll (Chl) *a* fluorescence of photosystem (PS) II (Fv/Fm, maximum quantum yield) was recorded for six replicate sporophylls using a pulse amplitude-modulated fluorometer (DivingPAM, Walz, Effeltrich, Germany). Photosynthetic measurements were conducted for sporophyll areas proximal to the midrib (sheltered) and distal to the midrib (exposed; Fig. 2.1), after 5 min of dark-adaption. Spores were released from two seaweed discs (2.05 cm diameter) for eight replicate sporophylls. Release of zoospores was triggered by immersion into sterilized seawater enriched with unbuffered nutrients after Provasoli (1968; Provasoli enriched seawater, PES) for 30 min. Most unaffected looking areas of the sheltered sporophyll were chosen in order to determine the maximum desiccation duration still enabling *U. pinnatifida* to release viable spores. A fourfold determination of spore density and mobility (proportion of moving spores, %) was accomplished for each replicate sporophyll using a hemocytometer. Thereafter, spore solutions were diluted to a density of 5 x 10^5 spores ml^{-1} and seeded onto glass cover slips placed in plastic petri dishes (9 cm diameter). Spores were allowed

to settle for 16 hours before the culture medium (PES) was exchanged. Spores and subsequent gametophytes were cultured in an incubator (15°C, 70 - 80 µmol m^{-2} s^{-1} PAR irradiance, 12:12 hour light cycle). Germination rates (proportion of germinated spores, %) were determined two days after spore release. A spore was considered to have germinated when a germination tube could be observed. Total length, as well as lengths of the pigmented gametophyte body and the germination tube were monitored five days after spore release. Measurements were conducted for 20 haphazardly chosen gametophytes per replicate.

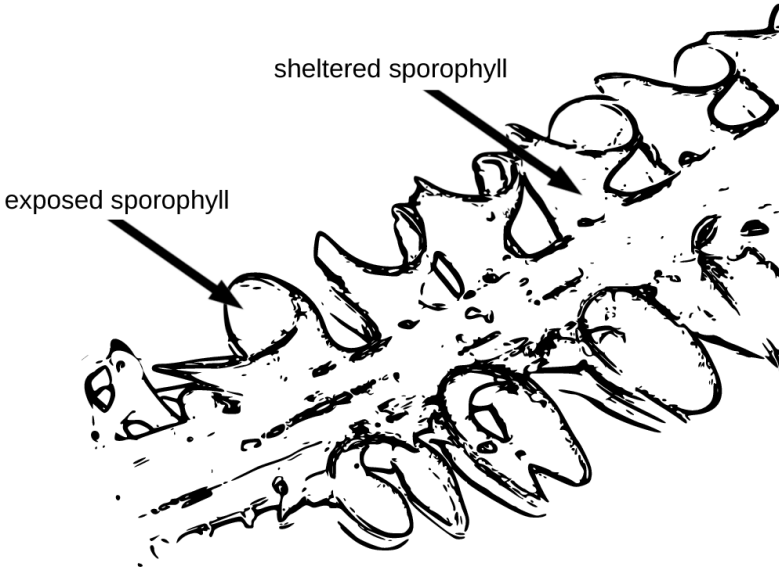

Figure 2.1 Schematic drawing of a sporophyll of *Undaria pinnatifida*, indicating sheltered (proximal to the midrib) and exposed (distal to the midrib) sporophyll parts.

2.3.2 Spore desiccation experiment

Spores were released from fertile sporophylls of *U. pinnatifida* and gametophyte cultivation was accomplished as described in section 2.3.1. Spores were exposed to different desiccation durations (treatments: 1, 3, 6, 12 and 24 h) by removing single glass cover slips, inoculated with *U. pinnatifida* spores, from the petri dishes and placing them in an incubator (15°C, 59 % RH, 70 - 80 µmol m^{-2} s^{-1} PAR irradiance, 12:12 hour light cycle). The exposure was initiated at two different development times of the spores / gametophytes: (a) 16 h post-release (group 1) when most spores were observed to have settled and initiated germination tube growth and (b) 30 h post-release (group 2) when nuclear translocation along the germination tube is considered to be completed (Pillai et al. 1992). After desiccation exposure cover slips were re-submerged in the petri

dishes. Survival and gametophyte length was assessed four days after initiation of desiccation exposure, consistently, five and six days after spore release for group 1 and 2. Gametophyte density was determined in the microscope in four fields-of-vision for each replicate on the monitoring day. In order to determine reliable survival rates, a minimum of 24 fields-of-vision was assessed if survival was extremely low including fields-of-vision that did not contain any surviving gametophytes. Survival of gametophytes in desiccation treatments is expressed in relation to the control (% of control). Since, in many cases, the germination tube was not detectable after desiccation, the length of the pigmented body was measured for 20 haphazardly chosen gametophytes per replicate.

2.3.3 Rope experiment

A closed seawater flow system (150 L) was installed and a chiller used to keep water temperature at 15°C. To avoid the entry of seaweed spores, the seawater was filtered to 1 µm. A 12:12 hour light cycle was established for kelp development. Ropes (10 cm length, 1 cm diameter) of different materials (polyethylene, PE; nylon; polypropylene, PP and hemp) were fully submerged hanging vertically from wooden racks. Ropes were randomly distributed in the tank with 1 to 2 cm distance to the next.

Fertile material of *U. pinnatifida* was collected from Tauranga Harbour in September 2014. Sporophylls were carefully cleaned with paper towel to remove epibiota and desiccated in a dark chamber at 4 °C over night. Spore release was triggered by reimmersion into filtered (1 µm) seawater. To ensure equal spore distribution throughout the tank spore solution (2 L, 4 x 10^5 spores ml^{-1}) was poured in under 15 min of random stirring. In order to monitor spore settlement four glass microscope slides were installed vertically in different areas of the tank. Settlement density on glass slides was determined 16 h after spore release and did not differ between tank areas ($F_{(3/35)}$ = 1.25, p = 0.31). Spores and arising gametophytes were cultured for seven days prior to the start of the desiccation experiment. Ropes were exposed to different desiccation durations (3, 12 and 48 h) and two humidity conditions: in air condition within the culture room (15 °C, 59 % RH, 'air') and within plastic bags (15 °C, 99 % RH, 'humid') mimicing transport in humid conditions, e.g. during shipboard transport. During desiccation ropes were hanging vertically. In order to prevent bias due to the movement of ropes, e.g. gametophyte loss, control ropes were lifted out of the water, as it was done for treatment ropes, but immediately placed back into the water. Water in the tank flow system was exchanged before ropes were placed back after exposure. Density and

length of the subsequent sporophyte generation was assessed two months after spore release.

2.3.4 Statistical analyses

All statistical analyses were conducted using the statistic programme “R” (R Core Team 2013). Multi-factorial analyses of variance (ANOVA) were applied to assess the impact of experimental factors on the respective variable. When the assumption of normally distributed data or homogeneous variances was not met, non-parametric statistical analyses according to Brunner & Munzel (2013) were applied involving ranking of the variable prior to the ANOVA.

For the sporophyll desiccation experiment one-way ANOVAs were applied to assess the impact of the factor (desiccation duration) on different experimental variables (water content, spore release, spore mobility, germination rate, gametophyte length). A two-way ANOVA investigated the impact of two factors (sporophyll area, desiccation duration) on the experimentally determined Fv/Fm. However, significant interactions between factors were encountered indicating a combined effect of factors on the experimental variable. The interpretation of main effects may be incomplete or misleading in the presence of significant interactions. Therefore, testing 'simple effects' of one factor at a fixed level of another factor is a common follow-up method in the case of significant interactions (Keppel & Wickens 2004). This was accomplished by testing differences between cell means in a one-factorial design, with combined levels of both factors (one-way ANOVA , factor: sporophyll area_desiccation duration).

The impact of desiccation duration on the variables (survival rate, gametophyte length) for the spore desiccation experiment was assessed using one-way ANOVAs (factor: desiccation duration).

In order to find out if rope material and desiccation duration impact water content of the ropes, a two-way ANOVA was applied (variable: water content, factors: rope type, desiccation duration). Significant interactions between the factors were encountered, thus, simple effects were tested using a one-way ANOVA (variable: water content, variable: rope type_desiccation duration). The impact of experimental factors (rope type, humidity, desiccation duration) on sporophyte number and length was assessed applying a three-way ANOVA. Here, significant interactions were also detected for the analysis of sporophyte length. Therefore, the variable was assessed at fixed humidity conditions (control, air, humid), employing a one-way ANOVA for control conditions (variable: sporophyte length, factor: rope type) and two-way ANOVAs for humid and air desiccation

conditions (variable: sprophyte length, factors: rope type, desiccation duration). In the case of significant interactions between factors of the two-way ANOVAs, a one-way ANOVA investigating simple effects was applied, involving combination of factor levels (factor: rope type_desiccation duration).

All post hoc analyses were accomplished applying Tukey's honest significant difference (HSD) test. Significant differences between group means are denoted by different letters.

2.4 Results

2.4.1 Sporophyll desiccation experiment

Water content accounted for 84 % of total fresh weight (FW) of *U. pinnatifida* sporophylls at the start of the experiment (d 0; Fig. 2.2). No significant reduction was observed after one day of desiccation, but after that water content continually declined ($F_{(1/34)}$ = 193.4, $p < 0.001$; groups identified by HSD test are indicated in Fig. 2.2). By day 5 discs of *U. pinnatifida* had lost 62 % of initial water content and water content accounted for 32 % of FW.

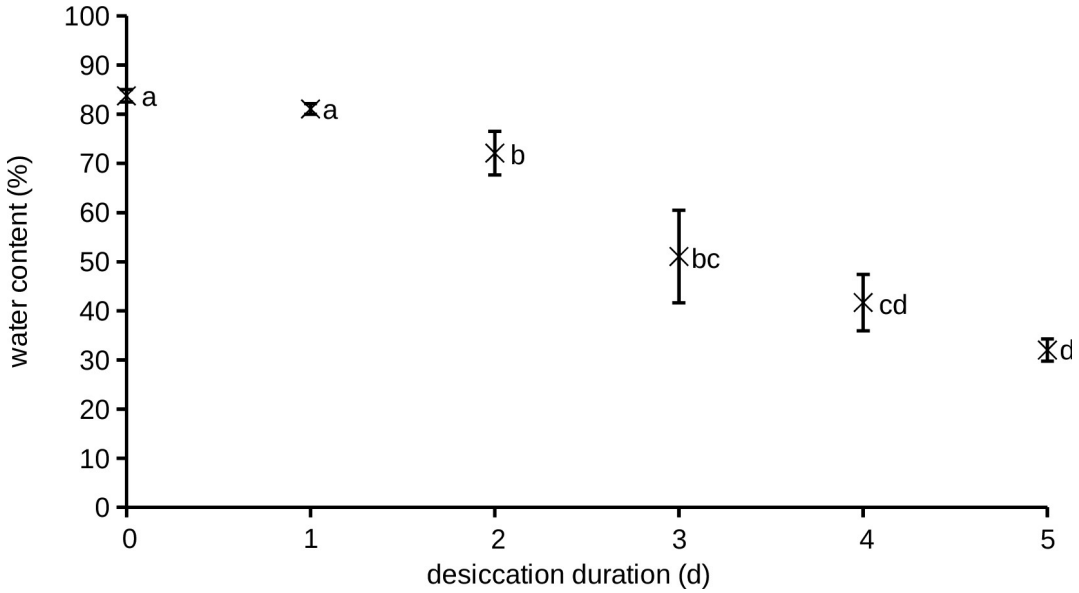

Figure 2.2 Water content (mean ± SE, n = 6) of *Undaria pinnatifida* sporophylls during exposure to desiccation (day 0 = start of the exposure) at 15°C. Different letters denote statistically significantly different groups identified by Tukey's honest significant difference (HSD) test.

Two-way ANOVA investigating the impact of sporophyll area and desiccation duration on Fv/Fm encountered significant interactions between factors ($F_{(9/32)}$ = 73.9, p < 0.001). One-way ANOVA of simple effects ($F_{(15/32)}$ = 73.9, p < 0.001) and subsequent HSD test identified higher Fv/Fm for sheltered sporophyll parts compared to exposed parts over the desiccation duration (Fig. 2.3). Drastic reductions of Fv/Fm were observed during the first two days for exposed sporophyll parts, exhibiting only 26 % of initial Fv/Fm on day 2 (Fv/Fm = 0.189). In contrast, sheltered sporophyll areas did not display significant reductions in Fv/Fm after one day of exposure, however, Fv/Fm continuously decreased during the rest of the experiment. Reductions in Fv/Fm of 11 % on d 2 (Fv/Fm = 0.653) and 33 % (Fv/Fm = 0.495) on d 3 were detected for sheltered sporophyll parts. At the end of the experimental exposure, Fv/Fm was close to zero for the entire sporophyll.

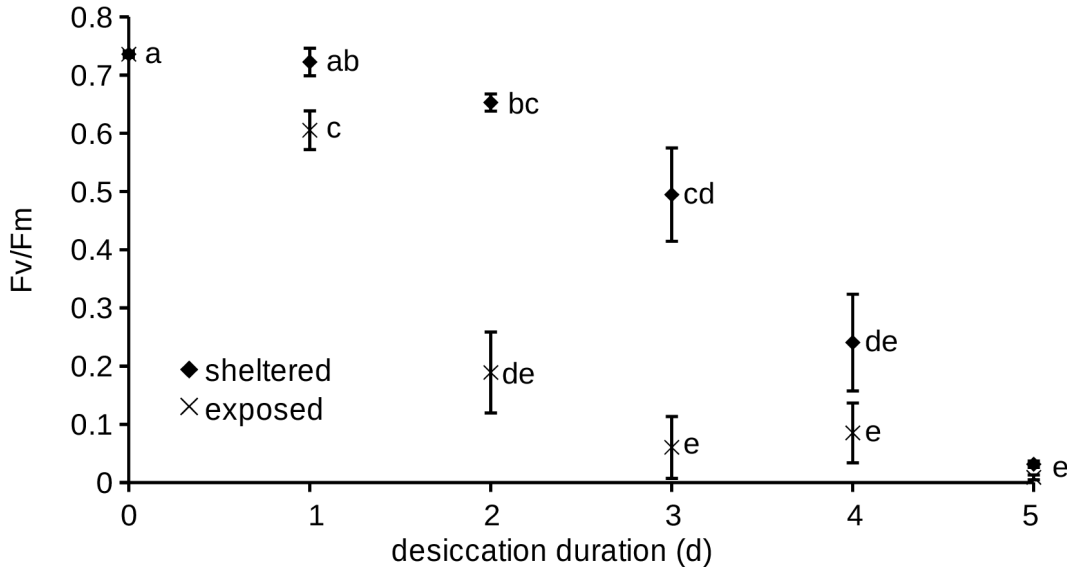

Figure 2.3 Photosynthetic quantum yield (Fv/Fm; mean ± SE, n = 6) of *Undaria pinnatifida* sporophylls exposed to different durations of desiccation, with day 0 being the day of collection. Measurements were taken proximal (sheltered) and distal (exposed) of the midrib. Different letters denote statistically significantly different groups identified by Tukey's honest significant difference (HSD) test.

Desiccation duration significantly impacted quantity ($F_{(5/38)}$ = 29.7, p < 0.001) and mobility ($F_{(5/38)}$ = 29.76, p < 0.001) of released spores. Both, highest number of released spores (20.8 mio spores cm^{-2} sporophyll) and highest proportion of mobile spores (66 %) were observed on d 1 (Fig. 2.4). Number of released spores on d 2 and 3 was comparable to spore quantity on d 0, however, the proportion of mobile spores was significantly reduced by 56 % on d 2 and by 74 % on d 3. Spore

quantities were substantially reduced by 75 % on d 4 and by almost 100 % on d 5 when no moving spores were detected.

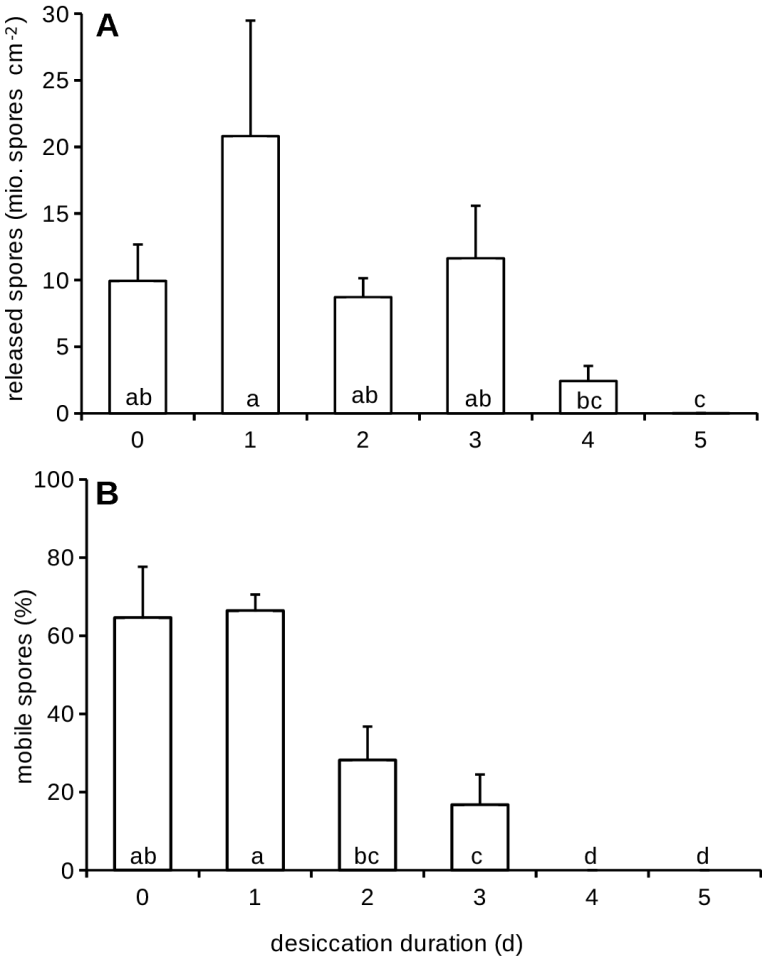

Figure 2.4 Means (± SE, n = 8) of (A) number of spores and (B) proportion of mobile spores released by *Undaria pinnatifida* after different durations of desiccation (day 0 = sporophyte collection from the field). Different letters denote statistically significantly different groups identified by Tukey's honest significant difference (HSD) test.

A high proportion (91 %) of spores released on the day of sampling (d 0) exhibited a germination tube two days after release (Fig. 2.5A). Germination success was significantly reduced with increasing desiccation duration ($F_{(4/29)}$ = 13.25, $p < 0.001$). Only half of the replicates exhibited germinated spores on d 3, resulting in an average reduction in germination success by 40 % compared to d 0. No spore germination was observed on d 4 and since no spores had been released after five days of sporophyll desiccation, germination rate could not be determined.

Total length of gametophytes determined five days after spore release ranged from 10 µm to 72.5 µm. One-way ANOVA detected a significant effect of desiccation on gametophyte total length ($F_{(3/536)}$ = 3.40, $p = 0.02$). Individuals released on d 2 were on average shorter compared to those

released on d 0 (Fig. 2.5B). However, length of gametophyte pigmented body ($F_{(3/536)}$ = 1.21, p = 0.31) and germination tube ($F_{(3/533)}$ = 2.44, p = 0.06) were not affected by exposure to desiccation.

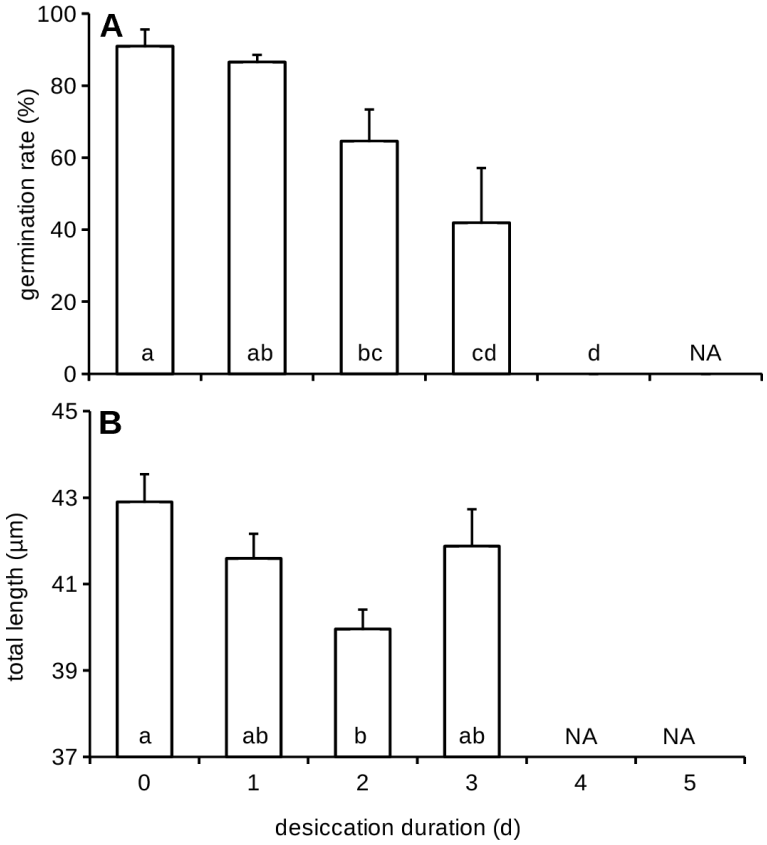

Figure 2.5 (A) Zoospore germination rate (means ± SE, n = 8) and (B) total gametophyte length (means ± SE, n = 80 - 160) of *Undaria pinnatifida* released from sporophylls exposed to desiccation for different durations (day 0 = sporophyll collection from the field). Different letters denote statistically significantly different groups identified by Tukey's honest significant difference (HSD) test. NA = not detected.

2.4.2 Spore desiccation experiment

Survival rates of gametophytes were significantly impacted by desiccation duration for group 1 (desiccation duration intitiated 16 h post-release; $F_{(5/18)}$ = 4.14, p = 0.01) and group 2 (desiccation duration intitiated 30 h post-release; $F_{(5/19)}$ = 43.81, p < 0.001; Fig. 2.6A). Survival rates of gametophytes after desiccation were generally low, with less than 0.4 % of gametophytes surviving desiccation in any treatment. No surviving individuals were detected in 3, 6, 12 and 24 h of desiccation for group 1 and after 24 h of desiccation for group 2.

Gametophytes having endured desiccation were on average 42 % and 58 % longer for group 1 ($F_{(1/117)}$ = 14.13, p < 0.001) and group 2 ($F_{(4/314)}$ = 27.84, p < 0.001) compared to the control (Fig. 2.6B). Length-frequency distributions of group 2 (Fig. 2.7) display higher minimal and maximal

length of gametophytes after all desiccation periods. Single individuals grew up to 73 % (after 1 and 6 h desiccation) larger than the longest gametophyte of the control.

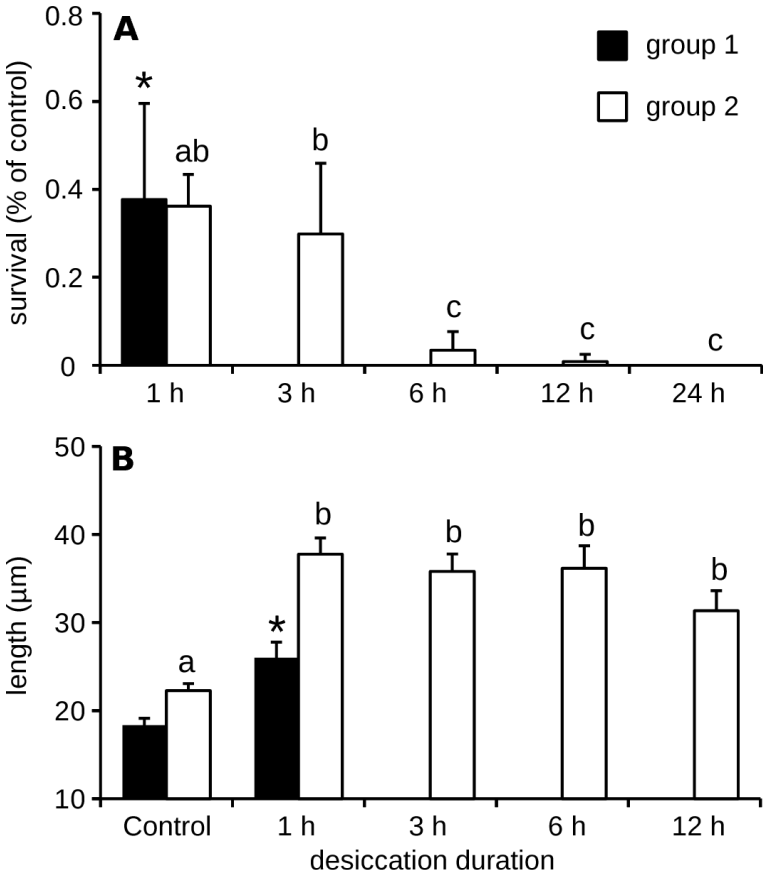

Figure 2.6 (A) survival (means ± SE) and (B) pigmented body length (means ± SE) of *Undaria pinnatifia* gametophytes exposed to varying times of desiccation. Exposure was initiated 16 h post-release (group 1) and 30 h post-release (group 2). Statistical analyses were performed separately for group 1 and group 2. The asterisk denotes a significant difference to the control by Tukey's honest significant difference (HSD) test applied to group 1. For group 2 different letters denote statistically significantly different groups identified by HSD test.

2.4.3 Desiccation on ropes

A two-way ANOVA investigating the impact of rope type and desiccation time on water content encountered significant interaction between factors ($F_{(9/32)} = 4.41$, $p < 0.001$). One-way ANOVA of simple effects ($F_{(15/32)} = 34.35$, $p < 0.001$) and subsequent HSD test (Fig. 2.8) detected significant differences in the reduction in water content during the desiccation period for different rope types.

Hemp had the highest initial water content of about 66 % of FW. Water content accounted for 48 % and 43 % of DW for nylon and PE and PP was significantly lower with only 10 % of DW. After 3 h of desiccation in air conditions water content was reduced to only 0.8 % of DW for PP, while the decrease was less rapid for all other rope materials. However, after 48 h of desiccation water content accounted for less than 4 % of DW for all rope types.

The highest number of sporophytes per rope, 614 individuals, was detected on one replicate PP rope (Table 2.1). On at least one replicate per rope type surviving sporophytes were found in all desiccation treatments. However, single replicates not exhibiting surviving sporophytes occurred after exposure of 48 h to air conditions for all rope types, except for hemp. A three-way ANOVA investigating dependence of sporophyte number on experimental factors (rope type, humidity, desiccation duration) did not detect significant interactions between factors. No effect of rope material on the number of sporophytes was identified ($F_{(3/85)} = 1.67$, $p = 0.18$). However, humidity ($F_{(2/85)} = 37.38$, $p < 0.001$) and desiccation time ($F_{(2/85)} = 12.12$, $p < 0.001$) significantly impacted the number of sporophytes. Sporophyte density was highest in the controls (no desiccation) and was reduced by 54 % and 90 % when exposed to humid and air conditions, respectively (HSD test groups: 'control' a, 'humid' b, 'air' c). Sporophyte number was reduced by 53 % after 3 h of desiccation compared to the control (HSD test groups: 'control' a, '3 h' b, '12 h' c, '48 h' c). About 16 % and 21 % of sporophytes were detected after desiccation periods of 12 h and 48 h.

Length of sporophytes ranged from 0.6 mm (hemp, 3 h, humid) to 43 mm (hemp, 48 h, humid). Investigating the impact of factors (rope type, humidity, desiccation time) on sporophyte length a three-way ANOVA encountered significant interactions between factors indicating that the impact of one factor depended on the level of another factor (humidity x desiccation time: $F_{(2/1134)} = 3.84$, $p = 0.02$). Therefore, length of sporophytes was analysed at fixed humidity conditions (control, air, humid). A one-way ANOVA (factor: rope type) compared length of sporophytes on different rope types in control conditions and detected significant differences ($F_{(3/282)} = 8.18$, $p < 0.001$). Subsequent HSD identified groups (HSD test: 'PE' a, 'nylon' ab, 'PP' bc, 'hemp' c), with PE rope hosting the longest sporophytes (5.8 mm average length) and hemp rope hosting the shortest (4.4 mm average length). Two-way ANOVA performed for air conditions detected significant impacts of both factors (rope type, desiccation time) on sporophyte length (rope type: $F_{(3/189)} = 4.95$, $p < 0.01$; desiccation time: $F_{(2/189)} = 3.33$, $p = 0.04$). HSD test identified shortest sporophytes occurring on hemp rope (HSD test groups: 'PE' a, 'nylon' a, 'PP' ab, 'hemp' b) and after 48 h of desiccation (HSD test groups: '3 h' ab, '12 h' a, '48 h' b). For humid conditions significant interactions between

factors were encountered by the two-way ANOVA. One-way ANOVA of simple effects ($F_{(11/663)}$ = 14.04, $p < 0.001$) and subsequent HSD test detected shorter sporophytes on hemp ropes compared to all combination of rope types and desiccation durations.

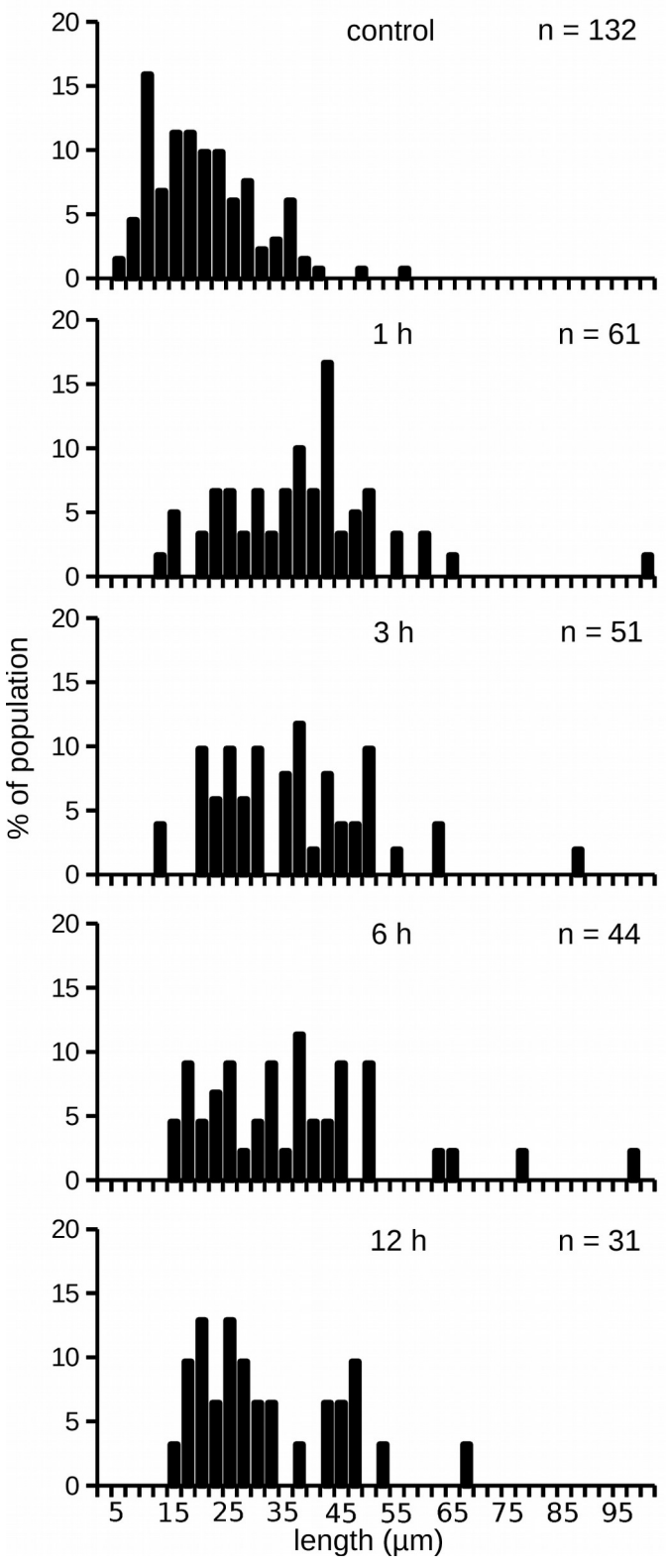

Figure 2.7 Length-frequency distributions of *Undaria pinnatifida* gametophyte populations after exposure to desiccation durations of 1, 3, 6 and 12 h, and control gametophytes that did not experience desiccation. N = number of individuals assessed.

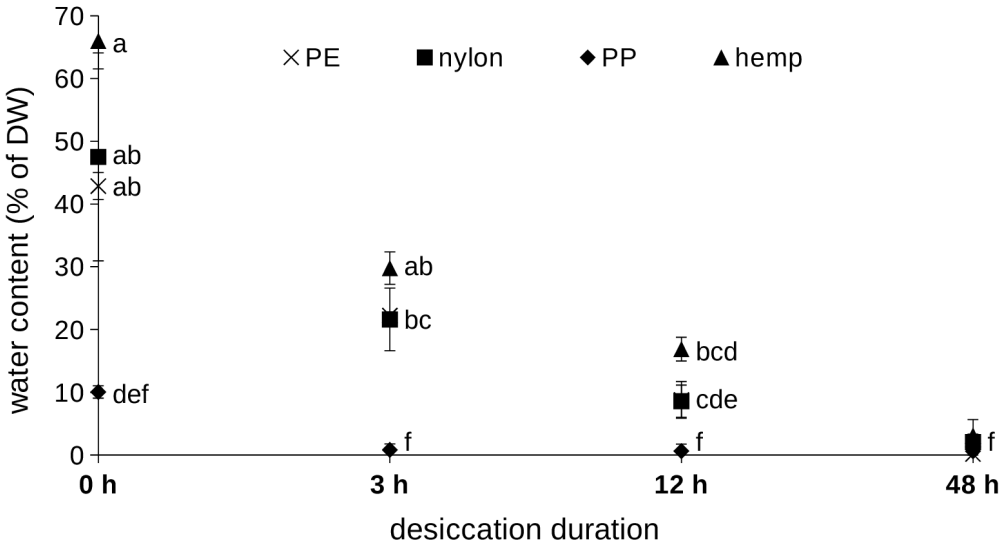

Figure 2.8 Water content (means, ± SE, n = 3) of different rope materials (polyethylene, PE; nylon; polypropylene, PP; hemp) in 15 °C and 59 % relative air humidity. Different letters denote statistically significantly different groups identified by Tukey's honest significant difference (HSD) test.

2.5 Discussion

Transport of microscopic life stages, such as zoospores and gametophytes, represents a potentially important vector for the spread of invasive algae (Flagella et al. 2007). Results of this study demonstrate that gametophytes of the invasive *U. pinnatifida* are capable of surviving short, from port to port, overland transports, e.g. attached to trailered boat hulls or fouling on aquaculture equipment.

Yet unreleased from sporophylls, mature propagules survived even longer desiccation periods. In this experiment, *U. pinnatifida* was capable of releasing viable zoospores from sporophylls after three days of desiccation in ambient air humidity. Release of zoospores is triggered by re-immersion into seawater. Thus, transport of mature sporophylls does not require recovery of the desiccated tissue for successful reproduction, potentially enhancing the pace and effectiveness of the spread (Sliwa 1999). Additionally, the simultaneous release of zoospores is advantageous for the establishment in a new environment since a minimum density of 1 spore mm^{-2} was found to be a prerequisit for successful recruitment in kelps (Reed 1990). From zoospores, when released in

favourable conditions, a new population might arise in previously uninvaded habitats. Therefore, results of this study suggest transport of fertile sporophylls in emerged conditions, e.g. entangled on boat trailers, in fishing gear or fouling on aquaculture equipment, to be an effective short-distance vector for the invasion by *U. pinnatifida*.

Table 2.1 Number of *Undaria pinnatifida* sporophytes on different rope materials (polyethylene, PE; nylon; polypropylene, PP; hemp) and after exposure to varying durations of desiccation at gametophyte stage. Each value represents the number of sporophytes on one rope replicate. Details on statistical analyses and results are given in section 2.4.3.

	Number of sporophytes per rope						
	control	**air**			**humid**		
		3 h	**12 h**	**48 h**	**3 h**	**12 h**	**48 h**
PP	150	9	1	0	341	37	74
	21	20	1	0	364	0	77
	125	0	0	1	266	78	1
	614	5	0	0	421	68	10
PE	260	279	4	3	355	180	94
	430	162	7	0	1	61	214
	29	44	2	0	74	1	35
	255	59	0	2	6	0	2
	545						
nylon	278	129	1	2	93	1	82
	28	58	0	2	120	0	197
	356	0	2	2	58	266	23
	181	154	0	0	154	197	0
hemp	18	34	12	1	413	102	58
	580	13	2	2	47	155	183
	62	74	2	5	11	234	10
	279	98	4	2	28	257	188

The long resistance time to desiccation of *U. pinnatifida* sporophylls may be a result of their specific morphology. The measurements of photosynthetic quantum yield in this study demonstrated that sporophyll areas close to the midrib were more resistant to desiccation than distant areas. This observation suggests that the folded morphology of the *U. pinnatifida* sporophyll (Nelson 2013) protects proximal sporophyll areas from water loss, enabling the enclosed zoospores to sustain extended desiccation periods. In similar temperature and humidity conditions thalli of the invasive *Codium fragile* ssp. *tomentosoides* survived for only one day (Schaffelke &

Deane 2005) and *Lessonia nigrescens* zoospores that were released from 18-hours-desiccated reproductive blades also displayed depressed germination rates (Fonck et al. 1998). High humidity has been shown to substantially prolong desiccation resistance time for *C. fragile* (Schaffelke & Deane 2005). During shipboard transport *U. pinnatifida* specimens are likely to experience high humidity, potentially preserving the ability to release viable zoospores for much longer time spans than those observed in this study.

Microscopic life stages of *U. pinnatifida* in this study became more resistant to desiccation with ongoing development. Less than one percent of zoospores that had just settled (16 h post-release) survived exposure to desiccation for one hour and all zoospores died during longer desiccation periods. In contrast, more developed gametophytes that had completed nuclear translocation along the germination tube (30 h post-release; Pillai et al. 1992), survived desiccation durations of up to 12 h. A study by Forrest & Blakemore (2006) demonstrated that cultured gametophytes of *U. pinnatifida* (2 – 4 weeks old) were capable of surviving up to three days in somewhat colder temperature (10 °C) and similar humidity conditions. Increased gametophyte size, which implies a reduction of the surface to volume ratio, might contribute to reduced loss of cellular water during desiccation, thereby reducing susceptibility to air exposure.

Interestingly, gametophytes having endured desiccation were on average longer than control gametophytes, regardless of developmental stage at initiation of desiccation and desiccation duration. Additionally, minimal gametophyte length was greater after desiccation compared to gametophytes in control treatments suggesting that the smallest individuals did not survive the exposure. This might partly explain enhanced average lengths. However maximum gametophyte length, which is drastically raised after desiccation by up to 70 %, suggests that another effect is at work. Gametophyte growth and subsequent sporophyte recruitment are known to be affected by settlement density (Reed 1990, Reed et al. 1991, Steen 2003) and reduced gametophyte lengths occurred in high settlement densities for *U. pinnatifida* and *Macrocystis pyrifera* (Bollen et al. unpublished, own observation). Thus, reduced settlement densities in the desiccation treatments, that occurred due to high mortality during exposure, may explain the observed enhanced average length of gametophytes. In kelp gametophytes, spermatozoid release is triggered by pheromones (Lüning & Müller 1978, Maier 1982). Since the effectiveness of pheromones is limited to 1 mm (Müller 1981), the extended lengths of gametophytes might facilitate recruitment by overcoming the critical distance between individuals.

Microstructure of substrates, such as surface topography, is known to impact settlement of seaweed zoospores (Callow et al. 2002, Schumacher et al. 2007). Furthermore, colour and pattern of substrate surfaces may impact algal settlement, survival and growth (Hodson et al. 2000, Swain et al. 2006, Finlay et al. 2008, Shine et al. 2010, Geng et al. 2015) and spores of *U. pinnatifida* were observed to favour positively charged substrates for settlement (Petrone et al. 2011). In this study, however, settlement density of *U. pinnatifida* sporophytes, as a result of initial zoospore density, germination and survival, did not differ between rope materials. Attached to the ropes, seven-day old *U. pinnatifida* gametophytes were capable of surviving extended periods of desiccation. Even though high mortalities occurred in response to the exposure, few gametophytes survived up to 48 hours of desiccation, successfully recruited and gave rise to young sporophytes. Refuge from desiccation provided by the microstructure of ropes might favour the survival of gametophytes. These results suggest, that gametophytes of *U. pinnatifida*, a common fouling species on floats and moorings (Hay 1990, Parsons 1995, Fletcher & Farrell 1999, James & Shears 2016), might be translocated with aquaculture rope.

Although water holding capacity varied for the different investigated rope materials, survival of gametophytes was not impacted by rope material. Arising sporophytes grew on average shorter on the only assessed natural material in this study, hemp rope. It was traditionally used by Chinese mariculturists (Tseng 1993), but had to be soaked in water prior to its deployment in order to clean it from undesirable substances (Tseng 1993). Additionally, hemp might be attacked by marine fungi (Chandrika 1974). Therefore, reduced average length of sporophytes on hemp rope might be related to specific substances or microbiological properties of natural fibres.

2.6 Conclusion

In conclusion, this study demonstrates that overland transport of different life history stages of the invasive *U. pinnatifida*, e.g. on trailered boats, is a possible short-distance invasion vector. Releasing zoospores simultaneously after re-immersion into seawater, sporophylls seem to exhibit a very high spread potential, with sporophyll areas proximal to the midrib being protected from desiccation due to the folded sporophyll morphology. Just settled zoospores were most susceptible to desiccation, however, resistance rapidly increased with ongoing gametophyte development. The ability of *U. pinnatifida* to survive short periods outside the water, at different life history stages, should be considered for pest management.

2.7 Acknowledgements

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Chapter 3: Salinity and temperature tolerance of the invasive alga *Undaria pinnatifida* and native New Zealand kelps: Implications for competition

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3.1 Abstract

Invasive species are generally believed to be more tolerant to varying abiotic conditions than native species. Here, we report the combined effect of temperature (5, 15, 20 and 25 °C) and salinity (33, 24, 18, 12 and 6 S_A) on the performance of the invasive kelp *Undaria pinnatifida* and two native kelp species (*Lessonia variegata*, *Ecklonia radiata*) from Tauranga Harbour, New Zealand (37°38'S, 176°10'E, 2014). Vegetative blade discs were exposed to temperature and salinity treatments in a 10-day laboratory experiment and the physiological response was assessed employing photosynthetic (Fv/Fm, ETR_{max}) as well as biochemical parameters (chlorophyll *a*, xanthophylls and antioxidant pool size). *U. pinnatifida* sustained a high photosynthetic quantum yield in most treatments, with a negative synergistic effect on photosynthetic yield expressed at 25 °C and low salinities (12, 6 S_A). *E. radiata* died in salinities below 18 S_A, except at 5 °C and *L. variegata* was highly susceptible to elevated temperatures (20, 25 °C). Antioxidant pool size showed species-specific responses to the experimental conditions, being most resilient in *U. pinnatifida*. Overall, *U. pinnatifida* displayed broader tolerance to the experimental salinity and temperature conditions than native kelps. The abilities to cope with a wide range in abiotic factors and to thrive in estuarine conditions might contribute to higher competitive strength compared to native kelps leading to its invasion success, especially with regard to ocean warming.

3.2 Introduction

The introduction of species into new habitats is a phenomenon of growing ecological and economical concern (Ruiz et al. 1997, Kolar & Lodge 2001, Perrings et al. 2002). As an outcome of global trade, transportation and tourism the connectivity of previously isolated ecosystems is rapidly increasing and therefore facilitating the introduction of non-indigenous species (NIS) in marine environments (Carlton & Geller 1993, Carlton 1995). In addition, world aquaculture production has dramatically increased in the past decade, including the annual harvesting of 25 million tonnes of seaweed and algae (FAO 2014). As a consequence, aquaculture is now regarded as one of the main vectors for the introduction of NIS (Welcomme 1992).

The immense ecological importance of species invasions is reflected by a growing body of literature (Kolar & Lodge 2001, Lowry et al. 2013) and seaweeds represent a considerable proportion (up to 38 %) of marine NIS (Schaffelke et al. 2007). An introduced species is not necessarily an “invasive” species unless it has an impact on the community, ecosystem or economy (Davis & Thompson 2000, 2002). Under such conditions, the invader may substantially affect structure and function of the indigenous community (Ruiz et al. 1999, Williams & Smith 2007). Predicting future invasions, as well as understanding conditions, vectors and traits that facilitate successful establishment are current challenges to invasion research (Carlton 1996, Byers et al. 2002).

Invasive seaweeds tend to exhibit both, rapid growth rates and the ability for short- and long-distance dispersal (Valentine et al. 2007, Andreakis & Schaffelke 2012). A prominent example of the detrimental impact of invasive seaweeds is the introduction of the siphonous chlorophyte *Caulerpa taxifolia* in the Mediterranean (Meinesz et al. 1993, Meinesz et al. 2001), which was associated with a marked loss in biodiversity at the infested sites (Boudouresque et al. 1995). Worldwide, 407 seaweed introduction events have been recorded so far, encompassing 277 different species (Williams & Smith 2007).

Listed among the “100 worst alien species” (Lowe et al. 2000) the invasive Asian kelp *Undaria pinnatifida* (Harvey) Suringar (Laminariales) has been introduced to many coastlines worldwide, including different countries in Europe, New Zealand, Australia, Argentina, California and Mexico (ICES 2007, and references therein). In Europe, *U. pinnatifida* was first observed in 1971 (Etang de Thau, France) after its accidental introduction with imported oysters from Japan (Peréz et al.

1981) and as a target species of aquaculture-activities was deliberately established in other areas (Floc'h et al. 1991, ICES 2007). In New Zealand, the invasive kelp most likely arrived with international shipping (Hay & Luckens 1987). The vegetative gametophytes of *U. pinnatifida* are capable of surviving extended periods of darkness (tom Dieck 1993) and exhibit a broad temperature tolerance (tom Dieck 1993, Henkel & Hofmann 2008), enabling the kelp to be transported over long distances in ballast water tanks. Furthermore, sporophytes of *U. pinnatifida* might reach new habitats as fouling organisms on hulls of vessels (Hay 1990, Farrell & Fletcher 2004). From its initial introduction sites *U. pinnatifida* most likely spreads naturally through spore dispersal or drifting sporophytes, as observed along the New Zealand coast (Forrest et al. 2000).

Globally, ecological concerns are associated with the establishment of *U. pinnatifida* in new environments as it potentially competes with native seaweed species for habitat space and light (Curiel et al. 1998, Farrell & Fletcher 2006). Consequently, *U. pinnatifida* was observed to monopolize space (Battershill et al. 1998) and cause decreases in native seaweed density and diversity (Casas et al. 2004). Differences in the associated fauna and feeding preferences of native grazers might induce further alterations to natural community composition and impact trophic relationships (Raffo et al. 2009, Jiménez 2015). In Argentina a significant decrease in fish abundance was observed in some reefs covered by *U. pinnatifida* (Irigoyen et al. 2011a), and serious economic concerns may arise if commercial species are affected (Orensanz et al. 2002). However, the extent and type of effect may be community or regionally dependent. Irigoyen et al. (2011b) suggested an increase in prey abundance due to the provision of a structurally complex habitat by *U. pinnatifida*.

Invasive species are generally believed to be more tolerant to changing abiotic factors than natives (Zerebecki & Sorte 2011). Physiological tolerance to environmental conditions, such as temperature and salinity, determine geographic distribution of many species (Crain et al. 2004, Bozinovic 2011) and regulate invasion success (Levine 2008). To adapt to the projected changing climatic conditions, i.e. rising surface temperature and ocean acidification (IPCC 2014), broad physiological tolerance might be advantageous (Dukes & Mooney 1999). Compared to invasive species within the same habitat, less tolerant natives are suggested to be disproportionally negatively affected by the impacts of climate change (Sorte et al. 2010, Zerebecki & Sorte 2011). Competitive interactions between native and invasive species may also be affected due to alterations in physiological optima and limited resource availability (Dukes & Mooney 1999, Occhipinti-Ambrogi 2007).

Relatively few studies have assessed the comparative tolerance of native and invasive seaweed species. A study by Liu & Pang (2010) demonstrated that the invasive *Grateloupia turuturu* was more tolerant to changing environmental conditions than the morphologically similar non-invasive *Palmaria palmata*. Similarly, high resistance to sedimentation, desiccation and varying nutrient conditions, enabled the non-native red alga *Gracilaria vermiculophylla* to dominate a lagoon environment (Thomsen et al. 2006).

Photosynthetic quantum yield and antioxidative potential are commonly used as proxies for comparative stress susceptibility (Maxwell & Johnson 2000, Arora et al. 2002). Based on species-specific stress tolerance predictions can be made on inter-specific competition and the potential for future establishment and range expansion.

In this study we compare species-specific physiological traits under combinations of varying temperature and salinity regimes of the invasive *U. pinnatifida* and two native New Zealand kelps in order to assess differences in stress tolerance and competitive strength. Our specific aim was to investigate how abiotic stress is reflected in photophysiological and biochemical properties of different kelps. We hypothesized that *U. pinnatifida* would be more tolerant to different experimental conditions, which might provide a competitive advantage for its future distribution.

3.3 Materials and Methods

3.3.1 Study site and kelp species

Specimens of the invasive *U. pinnatifida* and two native kelps (*Ecklonia radiata* (C. Agardh) J. Agardh, Laminariales; *Lessonia variegata* J.Agardh, Laminariales) were collected from Tauranga Harbour, New Zealand (37°38'S, 176°10'E) in October 2014. Average surface water temperatures in the harbour range from about 15 °C in August and September up to 20 - 22 °C in February (Chappell 2013, MetOcean View). Seawater salinities in some parts of the harbour might be reduced down to 2.5 - 5 S_A following a freshwater inflow event from Wairoa River (Pritchard et al. 2009) but at the collection site salinities are above 32.5 S_A.

In New Zealand, *U. pinnatifida* grows at the mean low water line (Hay & Luckens 1987, Curiel et al. 1998) as well as in intertidal rockpools (Russel et al. 2008), but is also present down to 15 m water

depth (Saito 1975). *E. radiata* and *L. variegata* might occur on a vertical range from 0 to 20 m, but show highest abundances in intermediate depths (*E. radiata:* 4 - 10 m; *L. variegata*: 3 - 15 m) (Schiel 1990, Schiel & Nelson 1990).

3.3.2 Sampling and experimental set-up

Thallus discs of five individuals per species were cut from the median part of the blade using a 2.05-cm diameter cork-borer. For *U. pinnatifida* discs were taken proximal to the midrib, at least 20 cm above the sporophyll and 10 cm below the distal thallus end. A total of at least 400 discs were cut from each species (approximately 80 discs per individual). All vegetative blade discs were carefully cleaned, removing epibiota. To allow for recovery from the cutting process and for acclimation to culture conditions seaweed discs (separated by species) were cultivated under constant bubbling in seawater (15 °C) for 24 h prior to the experimental exposure. Experimental treatments were set-up in temperature-controlled incubators at four temperatures (5, 15, 20 and 25 °C). Temperatures were chosen to replicate mean summer (20 °C) and winter (15 °C, ambient water temperature at the collection time) water temperatures in the harbour. The 5 °C and 25 °C treatments were selected in order to test temperatures close to minimal and maximal extremes from the literature. Upper critical temperatures recorded for *U. pinnatifida* sporophytes from laboratory studies range from 22 - 27 °C (Morita et al. 2003, Gao et al. 2013) suggesting that 25 °C might induce temperature stress for the specimens. *E. radiata* is considered to be a warm-temperate species, however, studies investigating thermal tolerance of its sporophytes are scarce. The optimal temperature range of *E. radiata* gametophytes lies somewhere between 12 - 20 °C (Novaczek 1984), while the upper thermal limit was detected to be 27 - 28 °C (tom Dieck 1993). Salinities (absolute salinity (S_A), 6, 12, 18, 24, 33 S_A) were obtained by dilution of 1-µm filtered seawater with distilled (reverse osmosis) water. 33 S_A refers to ambient seawater salinity at the sampling site, reduced salinities correlate with salinities that might occur inside Tauranga Harbour due to riverine input following rainfall events (Pritchard et al. 2009).

Three flasks were prepared for each temperature-salinity combination (one for each species), prior to the addition of 20 monospecific kelp discs. We consider each single disc as a replicate. Discs were maintained in 300 mL of medium continuously bubbled with air in 70 - 80 µmol m^{-2} s^{-1} photon irradiance on a 12:12-h light cycle throughout the experimental exposure. Discs for biochemical analyses (pigment and antioxidant analysis) were stored at -80 °C after 10 d of exposure for later processing. When no photosynthetic signal was detected from the kelp discs, storage for

biochemical analyses was accomplished on the specific experimental day. Levels of replication for each analysis are explained below.

3.3.3 Photosynthetic parameters

Chlorophyll (Chl) *a* fluorescence parameters (Fv/Fm, maximum quantum yield) were recorded after 1, 3, 6 and 10 d of experimental exposure using a pulse amplitude-modulated fluorometer (DivingPAM; Walz, Effeltrich, Germany). Ten randomly selected kelp discs (replicates) from each treatment were assessed after dark-adaption (5 min). For the generation of photosynthesis versus irradiance curves (PE curves) three kelp discs for each treatment were exposed to a series of gradually increasing actinic irradiances at 30-s intervals 10 d after the initial exposure. The position of the fibre optic was held constant during the measurement procedure using 'Magnet Sample Holders DIVING-MLC' (Walz). Relative electron transport rate (ETR) was calculated from PE curves as described by Schreiber et al. (1994). Subsequently, maximum electron transport rate (ETR_{max}) was defined by PE curve fitting after Jassby & Platt (1976).

3.3.4 Pigment analysis

Pigment analysis was performed in quintuplicate for each experimental treatment. Deep-frozen kelp discs were lyophilized for 24 h and pulverized at 4 m s^{-1} for 20 s in a high-speed benchtop homogenizer (FastPrep ®-24; MP Biomedicals, Solon, OH, USA). Pigments were extracted from the resulting seaweed powder in 1.5 mL of 90 % acetone for 24 h at 4 °C in darkness. Samples were vortexed and centrifuged (5 min, 4 °C, 16,000 × g) before the supernatant was filtered through a cellulose acetate (CA) membrane filter (45 µm). Pigments were analysed by high-performance liquid chromatography (HPLC) on a LaChromElite® system equipped with a chilled autosampler L-2200 and a DAD detector L-2450 (VWR-Hitachi International GmbH, Darmstadt, Germany). A reversed phase column Spherisorb ODS-2 column (25 cm × 4.6 mm, 5 µm particle size; Waters, Milford, MA) was used for the separation of pigments according to the method described by Wright & Jeffrey (1997) using the modified procedure for the Spectraphysics system. Pigments were identified by co-chromatography with pigment standards for Chl *a*, Chl *b*, lutein, antheraxanthin, zeaxanthin, violaxanthin, neoxanthin and β-carotene obtained from DHI Lab Products (Hørsholm, Denmark) using the software EZChrom Elite ver. 3.1.3. Chl *a* concentration is expressed in relation to thallus dry weight (DW; µg Chl *a* mg^{-1} DW). Pool size of xanthophyll cycle pigments (∑VAZ) was calculated from the sum of concentrations of xanthophylls involved in the

cycle (∑VAZ = violaxanthin + antheraxanthin + zeaxanthin) and is expressed in relation to Chl *a* concentration (µg µg^{-1} Chl *a*).

3.3.5 Antioxidant activity

DPPH (2,2-diphenyl-1-picrylhydrasyl) radical scavenging activity was determined using modified protocols of Brand-Williams et al. (1995) and Cruces et al. (2012) for five replicate kelp discs per treatment. Deep-frozen seaweed discs were freeze-dried and pulverized at 4 m s^{-1} for 20 s in a high-speed benchtop homogenizer (FastPrep ®-24; MP Biomedicals, Solon, OH, USA). Antioxidants were extracted from the samples (approximately 10 mg DW) in 5 mL of 70 % acetone for 24 h under constant shaking at 4 °C in darkness. After centrifugation (5 min, 500 rpm, 4 °C), 22 µL of the supernatant of each sample was mixed with 200 µL DPPH-solution (150 µM prepared in 100 % ethanol) in a 96-well microtiter plate (trifold determination for each replicate). Absorbance was measured at 520 nm after 45 min, when the reaction was finished, employing a photometer and the software FLUOstar OPTIMA (FLUOstar OPTIMA, BMG Labtech, Ortenberg, Germany). Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) was used as a standard, activity of the antioxidant pool is expressed as µg trolox equivalents (TE) per dry weight (µg TE mg^{-1} DW).

3.3.6 Statistics

All statistical analyses were conducted using the statistic programme “R” (R Development Core Team 2008). Multi-factorial analyses of variance (ANOVA) were applied to assess the impact of experimental factors on the respective variable. Since the assumption of normally distributed data was not met, non-parametric statistical analyses according to Brunner & Munzel (2013) were applied involving replacement of variable values by ranks prior to the ANOVA.

For each experimental variable the general dependence on factors was approached employing a four-way ANOVA (variable: Fv/Fm; factors: species, measurement day, temperature, salinity) and three-way ANOVAs (variable: ETRmax, Chl *a* concentration, ∑VAZ, TE; factors: species, temperature, salinity), respectively. Significant interactions between specific factors were encountered for all analyses indicating a combined effect of these factors on the experimental variable. However, as the interpretation of main effects may be incomplete or misleading in the presence of significant interactions, testing “simple effects” of one factor at a fixed level of another factor is a common follow-up method in the case of significant interactions (Keppel & Wickens

Table 3.1 Summary of the multi-factorial analyses of variance (ANOVA) for photosynthetic quantum yield. aov = ANOVA, 4f = four-factorial analysis, 3f = three-factorial analysis. Factor degree of freedom and residual degree of freedom for the F value are displayed in parentheses. Significant interactions are presented for each analysis, Sp = species, D = day, T = temperature, S = salinity.

		Factors								Interactions
		Species		Day		Temperature		Salinity		
Variable	aov	*F* value	*p* value	*F* value	*p* value	*F* value	*p* value	*F* value	*p* value	
Photosynthetic quantum yield (Fv/Fm)	4f	*(2/2444) 281.62*	*<0.001*	*(3/2444) 119.53*	*<0.001*	*(3/2444) 235.50*	*<0.001*	*(4/2444) 262.68*	*<0.001*	*Sp x D: $F_{(6/2444)} = 29.86$, $p < 0.001$* *Sp x T: $F_{(6/2444)} = 87.15$, $p < 0.001$* *D x T: $F_{(9/2444)} = 14.34$, $p < 0.001$* *Sp x S: $F_{(8/2444)} = 5.95$, $p < 0.001$* *D x S: $F_{(12/2444)} = 2.09$, $p = 0.01$* *T x S: $F_{(12/2444)} = 13.87$, $p < 0.001$* *Sp x D x T: $F_{(18/2444)} = 9.60$, $p < 0.001$* *Sp x D x S: $F_{(24/2444)} = 2.10$, $p = 0.001$* *Sp x T x S: $F_{(24/2444)} = 6.73$, $p < 0.001$* *D x T x S: $F_{(36/2444)} = 2.44$, $p < 0.001$* *Sp x D x T x S: $F_{(72/2444)} = 2.02$, $p < 0.001$*
U. pinnatifida	3f			*(3/863) 21.82*	*<0.001*	*(3/863) 70.44*	*<0.001*	*(4/863) 91.07*	*<0.001*	*D x T: $F_{(9/863)} = 9.35$, $p < 0.001$* *D x S: $F_{(12/863)} = 2.86$, $p < 0.001$* *T x S: $F_{(12/863)} = 11.66$, $p < 0.001$* *D x T x S: $F_{(36/863)} = 1.76$, $p = 0.004$*
E. radiata	3f			*(3/739) 55.77*	*<0.001*	*(3/739) 63.91*	*<0.001*	*(4/739) 99.67*	*<0.001*	*D x T: $F_{(9/739)} = 14.58$, $p < 0.001$* *D x S: $F_{(12/739)} = 1.85$, $p = 0.03$* *T x S: $F_{(12/739)} = 6.18$, $p < 0.001$*
L. variegata	3f			*(3/842) 130.89*	*<0.001*	*(3/842) 391.87*	*<0.001*	*(4/842) 94.16*	*<0.001*	*D x T: $F_{(9/842)} = 15.53$, $p < 0.001$* *D x S: $F_{(12/842)} = 2.47$, $p < 0.001$* *T x S: $F_{(12/842)} = 8.15$, $p < 0.001$* *D x T x S: $F_{(36/842)} = 4.33$, $p < 0.001$*

2004). Therefore, a three-way ANOVA (variable: Fv/Fm, factors: measurement day, temperature, salinity) and two-way ANOVAs (variables: ETRmax, Chl *a* concentration, ∑VAZ, TE, factors: temperature, salinity) were applied separately for each kelp species. In presence of significant interactions differences between cell means were tested in a one-factorial design (simple effects). Therefore levels of two factors were combined before applying a one-way ANOVA (factor: temperature_salinity). When high numbers of factor levels made this procedure inappropriate due to extremely low α-errors, a graphical comparison of cell means was accomplished.

In order to detect inter-specific differences in ambient conditions (15 °C, 33 S_A) a one-way ANOVA (variables: Fv/Fm, ETRmax, Chl *a* concentration, ∑VAZ, TE; factor: species) was applied for each experimental variable at the specific factor levels.

For post hoc analyses Tukey's honest significant difference (HSD) test was applied. Significant differences between group means are denoted by different lower cases for temperature, capital letters for salinity and italic small letters for the combined factor (temperature_salinity) in the text.

3.4 Results

3.4.1 Photosynthetic parameters

Significant interactions between factors were detected by the four-way ANOVA and the three-way ANOVAs for each species indicating that the impact of one factor on the variable depended on the level of another factor (Table 3.1). Due to the high number of factor levels a graphical comparison of cell means was accomplished. Photosynthetic quantum yield of *U. pinnatifida* was sustained on a high level after one day of experimental exposure and was only impacted by the combination of highest temperature (25 °C) and low salinities (12, 6 S_A; Fig. 3.1). Similar patterns were observed on day 3, 6 and at the end of the experimental exposure (day 10) when no photosynthetic signal could be detected for the aforementioned treatments. Contrasting this, photosynthetic quantum yield of *E. radiata* drastically decreased over the experimental duration. Specimens of *E. radiata* disintegrated or did not exhibit measurable photosynthetic signals in salinities down from 18 S_A in all temperatures except for the 5 °C treatment after 10 d of exposure. Similarly, only one day after the start of the experimental exposure, a reduction of 84 % was evident for the photosynthetic quantum yield of *L. variegata* in 25 °C and 6 S_A compared to the photosynthetic yield measured in

ambient conditions (15 °C, 33 S_A). By day 6 no photosynthetic quantum yield could be measured in any of the *L. variegata* specimens in 25 °C and the yield of individuals in 20 °C was considerably reduced by up to 70 %.

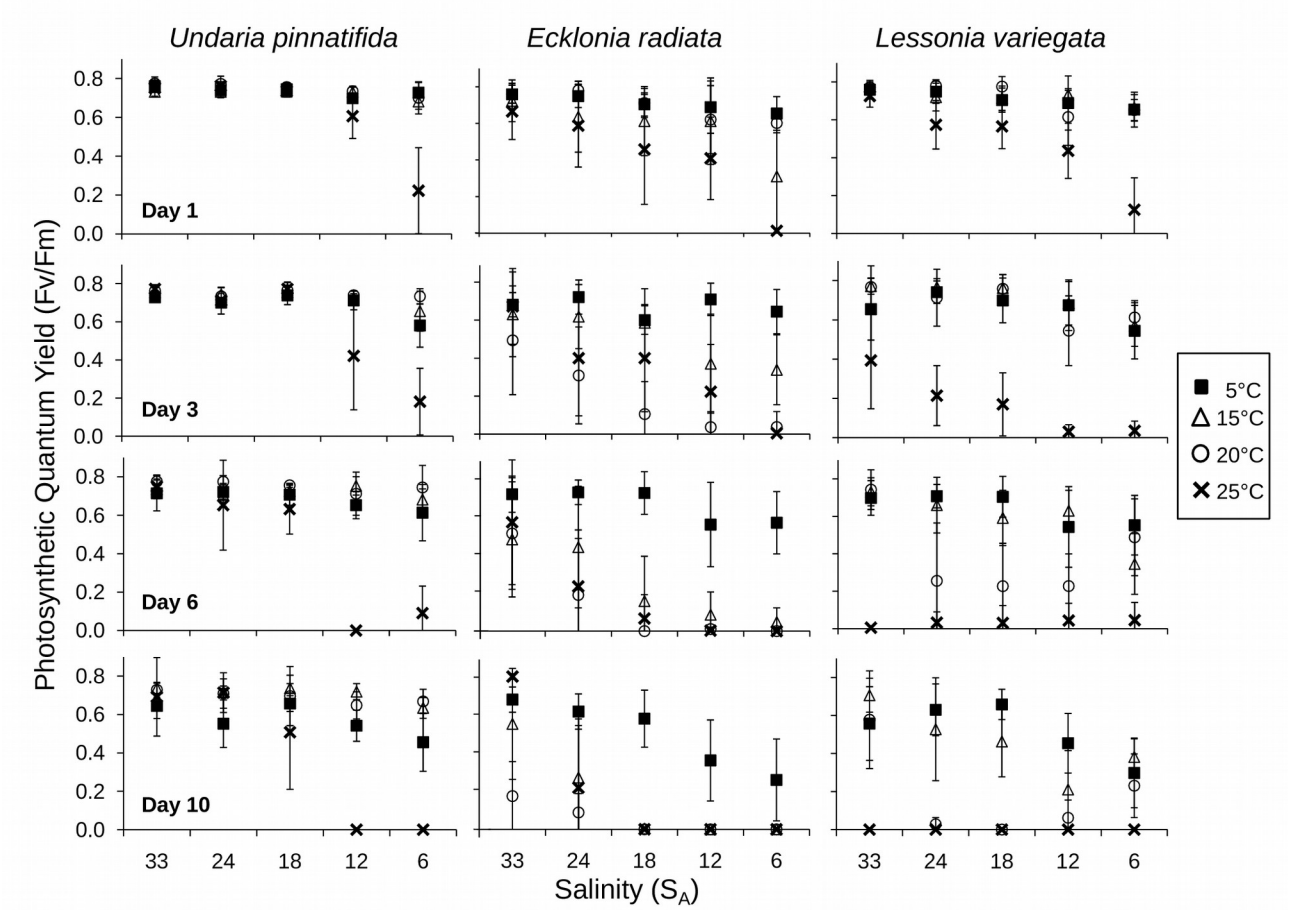

Figure 3.1 Means (± SD, n = 10) of photosynthetic quantum yield (Fv/Fm) of three kelp species (*Undaria pinnatifida*, *Ecklonia radiata*, *Lessonia variegata*) exposed to various temperatures (5, 15, 20 and 25 °C) and salinities (33, 24, 18, 12 and 6 S_A) measured after 1, 3, 6 and 10 days of experimental exposure.

Maximum electron transport rate (ETR_{max}) ranged from 23.56 (*E. radiata*, 5 °C, 12 S_A) to 109.97 µmol e^- m^{-2} s^{-1} (*E. radiata*, 5 °C, 33 S_A) (Table 3.2). Due to significant interactions between factors (species, salinity) in the three-factorial analysis (Table 3.3) separate two-way ANOVAs were accomplished for each species. Two-way ANOVA for *U. pinnatifida* detected a significant interaction between temperature and salinity. Subsequent one-way ANOVA for simple effects ($F_{(15/38)}$=3.01, $p < 0.01$) indicated differences between cell means. ETR_{max} values were observed to decrease with increasing temperatures in ambient seawater salinity (33 S_A) resulting in a reduction

of ETR_{max} of 22 % in 25 °C compared to ambient water temperature (15 °C). However, this pattern was not statistically significant (HSD test for simple effects: 5 °C *ab*, 15 °C *ab*, 20 °C no data available (NA), 25 °C *b*). ETR_{max} values of *U. pinnatifida* describe an optimum curve along the experimental salinities in 5 °C with the highest value of ETR_{max} occurring in 24 S_A (104.5 µmol $e^- m^{-2} s^{-1}$) (HSD test for simple effects: 33 S_A *ab*, 24 S_A *a*, 18 S_A *ab*, 12 S_A *ab*, 6 S_A *ab*). The same pattern, but less pronounced, was indicated for *L. variegata* in 5 °C with the highest ETR_{max} value at 24 S_A (88.48 µmol $e^- m^{-2} s^{-1}$). However, the effect of salinity on ETR_{max} was not statistically significant for *L. variegata*. Temperature impacted ETR_{max} of *L. variegata* with highest ETR_{max} occurring in 5 °C (HSD test: 5 °C a, 15 °C b, 20 °C ab, 25 °C NA). Two-way ANOVA detected no effect of temperature on ETR_{max} of *E. radiata*, however, ETR_{max} significantly varied with salinity. The lowest measured value of ETR_{max} occurred in 5 °C and 12 S_A (23.6 µmol $e^- m^{-2} s^{-1}$) but HSD test did not detect significant differences between temperature means.

Table 3.2 Means (± SD, n = 3) of maximum electron transport rate (ETR_{max}) of three kelp species (*Undaria pinnatifida*, *Ecklonia radiata*, *Lessonia variegata*) exposed to various temperatures (5, 15, 20 and 25 °C) and salinities (33, 24, 18, 12 and 6 S_A) determined after 10 days of experimental exposure. Details on statistical analyses and results are given in the text.

ETR_{max} (µmol $e^- m^{-2} s^{-1}$)		Salinity (S_A)				
		33	24	18	12	6
Undaria pinnatifida	5°C	64.3 ± *8.1*	104.5 ± *13.9*	78.0 ± *10.0*	63.3 ± *7.8*	51.2 ± *7.7*
	15°C	52.8 ± *8.7*	54.72 ± *5.9*	45.6 ± *22.9*	63.6 ± *28.9*	67.6 ± 14.6
	20°C		92.7 ± *5.3*	65.3 ± *5.2*	100.5 ± *40.8*	64.5 ± *15.3*
	25°C	41.2 ± *9.3*	46.7 ± *32.8*			
Ecklonia radiata	5°C	110.0 ± *19.1*	74.7 ± *27.4*	85.7	23.6 ± *17.7*	48.3 ± *5.8*
	15°C	53.5 ± *26.0*	56.5 ± *30.7*			
Lessonia variegata	5°C	71.8 ± *10.3*	88.5 ± *41.9*	82.0 ± *7.0*	81.5 ± *9.4*	49.4 ± *42.6*
	15°C	45.8 ± *6.5*	51.3 ± *4.3*	53.6 ± *6.1*		34.3 ± *8.0*
	20°C	65.7 ± *5.2*				

3.4.2 Pigment analysis

Three-way ANOVA detected significant interactions between factors (species, temperature, salinity) for the Chl *a* concentration per dry weight (Table 3.3). Chl *a* concentration at ambient conditions (15 °C, 33 S_A) was highest in *U. pinnatifida* (one-way ANOVA: $F_{(2/14)} = 4.53$, $p = 0.03$) exhibiting 2-fold concentrations compared to native kelps (Fig. 3.2).

The two-way ANOVA detected a significant interaction between factors for *U. pinnatifida.* Subsequent one-way analysis of simple effects ($F_{(18/65)} = 5.24$, $p < 0.001$) and HSD test detected differences in Chl *a* concentration between temperatures with highest Chl *a* concentrations occurring in 5 °C, except for the 24 S_A-treatment where no differences between temperatures were indicated. At ambient levels of seawater salinity a distinct pattern of decreasing Chl *a* concentration with increasing temperature was apparent for *U. pinnatifida* (HSD test for simple effects: 5 °C *a*, 15 °C *ab*, 20 °C *ab*, 25 °C *b*) with a reduction of 38 % in Chl *a* content in 25 °C compared to the 5 °C-temperature treatment. A high rate of disc disintegration occurred in the combination of 25 °C and low salinities (12, 6 S_A). Both factors, temperature and salinity significantly influenced Chl *a* content of native kelp species as displayed by the results of the two-way ANOVA. Chl *a* content of *L. variegata* displayed a continual decrease with decreasing salinities (HSD test: 33 S_A A, 24 S_A B 18 S_A B, 12 S_A B, 6 S_A C), exhibiting an averaged reduction of 58 % from ambient seawater salinity to the lowest experimental salinity treatment. Highest Chl *a* concentration for *L. variegata* was detected in 15 °C, lowest concentration in 25 °C (HSD test: 5 °C ab, 15 °C a, 20 °C ab, 25 °C b). No clear pattern could be identified for the Chl *a* content of *E. radiata*, the highest value being 235 µg Chl *a* mg^{-1} DW (25 °C, 18 S_A).

Significant interactions between factors (species, temperature, salinity) were detected for ∑VAZ (Table 3.3). In ambient conditions (15 °C, 33 S_A) no difference of ∑VAZ was detected between species (one-way ANOVA: $F_{(2/14)} = 2.46$, $p = 0.12$). A significant effect of temperature and salinity on ∑VAZ was identified for *U. pinnatifida* and *E. radiata* (two-way ANOVA). ∑VAZ was continuously reduced with decreasing salinities for the invasive kelp (HSD test: 33 S_A A, 24 S_A AB, 18 S_A AB, 12 S_A AB, 6 S_A B) and *E. radiata* (HSD test: 33 S_A A, 24 S_A B, 18 S_A AB, 12 S_A B, 6 S_A B), displaying an average reduction of 21 % and 42 % in 6 S_A compared to 33 S_A, respectively (Table 3.4). ∑VAZ of *E. radiata* was highest in 5 °C treatments (HSD test: 5 °C a, 15 °C b, 20 °C b, 25 °C b). For *L. variegata* the two-way ANOVA detected significant interactions between factors. One-way ANOVA ($F_{(19/75)} = 7.32$, $p < 0.001$) indicated differences between cell means and subsequent HSD test for

Table 3.3 Summary of the multi-factorial analyses of variance (ANOVA) for maximum electron transport rate (ETR_{max}), chlorophyll *a* content, xanthophyll pool size (∑VAZ) and antioxidant capacity (Trolox equivalents). av = ANOVA, 3f = three-factorial analysis, 2f = two-factorial analysis, DW = dry weight. Factor degree of freedom and residual degree of freedom for the F value are displayed in parentheses. Significant interactions are presented for each analysis, Sp = species, T = temperature, S = salinity.

		Factors						**Interactions**
		Species		Temperature		Salinity		
Variables	av	*F* value	*p* value	*F* value	*p* value	*F* value	*p* value	
ETR_{max} ($\mu mol\ e^{-}\ m^{-2}\ s^{-1}$)	3f	*(2/66) 0.172*	*0.84*	*(3/66) 9.81*	*<0.001*	*(4/66) 2.01*	*0.10*	*Sp x S: $F_{(8/66)} = 3.11$, $p = 0.004$*
U. pinnatifida	2f			*(3/38) 6.67*	*<0.001*	*(4/38) 1.64*	*0.18*	*T x S: $F_{(8/38)} = 2.32$, $p = 0.04$*
E. radiata	2f			*(1/14) 2.60*	*0.12*	*(4/14) 4.21*	*0.02*	
L. variegata	2f			*(2/14) 13.05*	*<0.001*	*(4/14) 1.88*	*0.17*	
Chlorophyll *a* content ($\mu g\ Chl\ a\ mg^{-1}\ DW$)	3f	*(2/212) 169.13*	*<0.001*	*(3/212) 4.97*	*<0.01*	*(4/212) 1.56*	*0.19*	*Sp x T: $F_{(6/212)}$=6.10, $p < 0.001$* *Sp x S: $F_{(8/212)}$=8.87, $p < 0.001$* *T x S: $F_{(12/212)}$=1.89, $p = 0.04$*
U. pinnatifida	2f			*(3/65) 19.43*	*<0.001*	*(4/65) 0.70*	*0.60*	*T x S: $F_{(11/65)}$=3.02, $p = 0.002$*
E. radiata	2f			*(3/71) 4.85*	*<0.01*	*(4/71) 5.18*	*<0.01*	
L. variegata	2f			*(3/76) 3.30*	*0.02*	*(4/76) 16.71*	*<0.001*	
∑VAZ ($\mu g\ \mu g^{-1}$ Chl *a*)	3f	*(2/206) 113.43*	*<0.001*	*(3/206) 24.98*	*<0.001*	*(4/206) 18.38*	*<0.001*	*Sp x T: $F_{(6/206)} = 8.23$, $p < 0.001$* *T x S: $F_{(12/206)} = 2.46$, p 0 0.005* *Sp x T x S: $F_{(21/206)} = 2.02$, $p = 0.006$*
U. pinnatifida	2f			*(3/62) 3.98*	*0.01*	*(4/62) 3.29*	*0.02*	
E. radiata	2f			*(3/69) 16.35*	*<0.001*	*(4/69) 8.17*	*<0.001*	
L. variegata	2f			*(3/75) 20.20*	*<0.001*	*(4/75) 7.15*	*<0.001*	*T x S: $F_{(12/75)} = 4.15$, $p < 0.001$*
Trolox equivalents ($\mu g\ TE\ mg^{-1}\ DW$)	3f	*(2/202) 347.08*	*<0.001*	*(3/202) 25.73*	*<0.001*	*(4/202) 25.83*	*<0.001*	*Sp x T: $F_{(6/202)} = 16.95$, $p < 0.001$* *Sp x S: $F_{(8/202)} = 2.66$, $p = 0.008$* *T x S: $F_{(12/202)} = 4.21$, $p < 0.001$* *Sp x T x S: $F_{(23/202)} = 3.19$, $p < 0.001$*
U. pinnatifida	2f			*(3/68) 22.23*	*<0.001*	*(4/68) 12.51*	*<0.001*	*T x S: $F_{(12/68)} = 1.96$, $p = 0.04$*
E. radiata	2f			*(3/59) 16.23*	*<0.001*	*(4/59) 7.84*	*<0.001*	*T x S: $F_{(11/59)} = 3.14$, $p = 0.002$*
L. variegata	2f			(3/75) 29.56	<0.001	*(4/75) 12.83*	<0.001	*T x S: $F_{(12/75)} = 3.67$, $p < 0.001$*

simple effects detected highest ∑VAZ for *L. variegata* in 5 and 15 °C (33S_A). In 25 °C ∑VAZ of *L. variegata* was reduced compared to the ambient water temperature.

3.4.3 Antioxidant activity

Detected antioxidant values ranged from 7 to 54 µg TE mg^{-1} DW (Fig. 3.3). Three-way ANOVA identified significant interactions between all factors (species, temperature, salinity) (Table 3.3). *L. variegata* exhibited the lowest antioxidant concentrations in relation to thallus dry weight (one-way ANOVA: $F_{(2/11)}$=17.18, $p < 0.001$), 49 % and 58 % lower compared with *U. pinnatifida* and *E. radiata*, respectively (15 °C, 33 S_A). Two-way ANOVAs for each species identified interactions between factors (temperature, salinity) and differences between cell means were indicated when testing for simple effects (*U. pinnatifida*: $F_{(19/68)} = 7.93$, $p < 0.001$, *E. radiata*: $F_{(18/59)} = 6.36$, $p < 0.001$, *L. variegata*: $F_{(19/75)} = 9.68$, $p < 0.001$). The antioxidant pool in *U. pinnatifida* revealed the least reductions over the range of experimental conditions. Average antioxidant pool size of *U. pinnatifida* displayed a distinct optimum curve for temperature at each salinity level, except for the 6 S_A treatment, with highest pool sizes occurring in 15 °C. This pattern was, however, not statistically significant (HSD test for simple effects). HSD test of simple effects indicated consistently decreasing antioxidant pool size with lower salinities for *U. pinnatifida*, exhibiting 29 % reduction in 6 S_A compared to ambient seawater salinity. However, the antioxidant pool of *U. pinnatifida* was never depleted. Antioxidant levels of *E. radiata* and *L. variegata* displayed dramatic decreases. The combination of low salinities (18 - 6 S_A) and elevated temperatures (20, 25 °C) completely exhausted antioxidant levels in *L. variegata*. Average antioxidant pool size was reduced by 72 % for *L. variegata* and 53 % for *E. radiata* in the lowest salinity treatment compared with ambient seawater salinities. For the two native kelps HSD test for simple effects indicated the most resilient antioxidant pool in 5 °C, antioxidant levels were entirely depleted in *L. variegata* in 25 °C.

3.5 Discussion

3.5.1 Species-specific physiological response

Displaying least reductions in photosynthetic quantum yield, as a proxy of plant stress, the invasive *U. pinnatifida* was more tolerant to the various experimental conditions applied in this study than the investigated native kelp species. Each single experimental factor, temperature and salinity, did

not impact photosynthetic quantum yield, however, the combined effects of highest temperature and low salinity treatments affected performance of *U. pinnatifida* specimens. The deleterious effect of several combined stress factors most often exceeds the simple additive effect of their single action, an effect known as “cross-synergism” (Alexieva et al. 2003). However, a reduction in maximum electron transport was observed as a consequence of elevated temperatures for *U. pinnatifida*. Thus, high temperatures affected photosynthetic rates but did not affect efficiency of photosystem II (PSII) or survival of *U. pinnatifida*. In contrast, specimens of the endemic *L. variegata* were strongly impacted by elevated temperatures displaying no photosynthetic signal and massive disintegration in 25 °C.

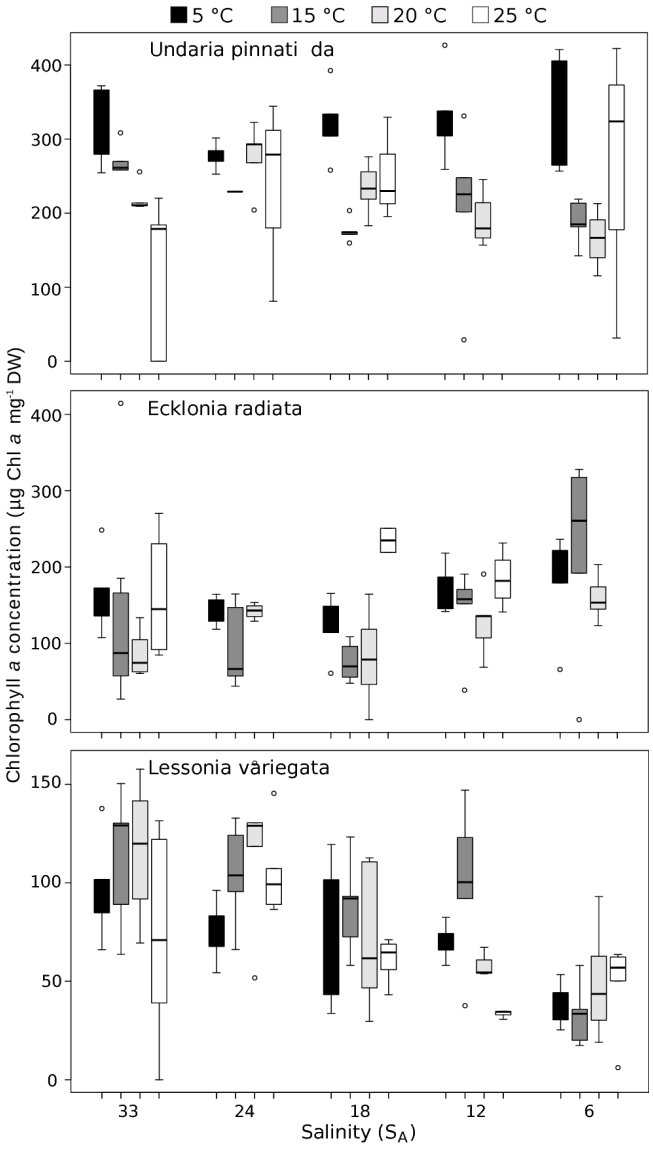

Figure 3.2 Tissue chlorophyll *a* concentrations (boxplots, n = 5) of *Undaria pinnatifida*, *Ecklonia radiata* and *Lessonia variegata* exposed to various temperatures (5, 15, 20 and 25 °C) and salinities (33, 24, 18, 12 and 6 S_A) after 10 days of experimental exposure. Chlorophyll *a* concentration is expressed in relation to thallus dry weight (DW) (µg Chl *a* mg^{-1} DW). Bottom and top of the boxes in the plot depict the first and third quartiles, respectively. The median is represented by the band inside box. The ends of the whiskers are designed to show minimum and maximum value still within 1.5 x Interquartile range (IQR) of the lower and upper quartile. Values outlying these ranges are displayed by single dots. Details on statistical analyses and results are given in the text.

Most striking differences in the physiological reaction to abiotic factors between kelp species occurred with measured antioxidant capacity in this study. While pool size of the invasive kelp was most resilient and never depleted, drastic reductions were detected for the two native kelps in response to the experimental conditions. New Zealand's endemic *L. variegata* displayed complete exhaustion of antioxidants in 25 °C as well as in the combination of low salinities (18 – 6 S_A) and various temperatures (15, 20, 25 °C). Exhausted antioxidant levels indicate the occurrence of oxidative stress and the generation of reactive oxygen species (ROS), potentially leading to inhibition and destruction of the photosynthetic apparatus, DNA, proteins and cell membranes (Kumar et al. 2014). The availability of antioxidants is a crucial defence mechanism to protect from cellular damage (Burritt et al. 2002). Therefore, high antioxidant levels are suggested to represent a physiological adaption (Kumar et al. 2014) to extreme environmental conditions, such as those occurring in elevated shore habitats. Consistently, higher antioxidant levels have been detected in intertidal macroalgae compared to subtidal species (Ross & Van Alstyne 2007). Thus, the resilient antioxidant pool of *U. pinnatifida*, observed in this study might promote its tolerance against various physico-chemical stresses, such as temperature and salinity alterations.

L. variegata displayed high susceptibility to elevated experimental temperatures (25 °C). Rapid exhaustion of its antioxidant pool in 25 °C is consistent with high sensitivity to elevated temperatures displayed by the photosynthetic quantum yield. Additionally, reduced chlorophyll *a* concentration and xanthophyll pool size in 25 °C confirm the negative impact of elevated water temperatures on the physiological condition of *L. variegata*. Data on salinity and temperature tolerance for *L. variegata* is scarce. However, Nelson (2005) tested sporophyte growth of *L. variegata* at three temperatures (10, 12 and 15 °C) and detected highest growth rates in 15 °C. Our results of photosynthetic quantum yield, chlorophyll *a* concentration and antioxidant capacity demonstrate higher susceptibility to reduced salinities in 20 °C compared to 15 °C, suggesting an optimum physiological performance of *L. variegata* in 15 °C. Our observations, however, indicate that 25 °C exceeds the tolerance limit of *L. variegata* sporophytes.

In ambient seawater salinity, the physiological condition of *E. radiata* was not impacted by experimental temperatures. However, reduced salinities impacted all investigated parameters in *E. radiata*. Drastically reduced photosynthetic quantum yield as well as impacted antioxidant and pigment concentrations indicate that low salinities down from 18 S_A induce stress in *E. radiata* sporophytes. This is consistent with Burridge et al. (1999), who observed zoospore germination and gametophyte growth of *E. radiata* to be negatively impacted by reduced salinities. In the lowest

temperature treatment *E. radiata* displayed the most resilient photosynthetic quantum yield and antioxidant concentration in reduced salinities, suggesting that it exhibits the highest acclimation potential in 5 °C.

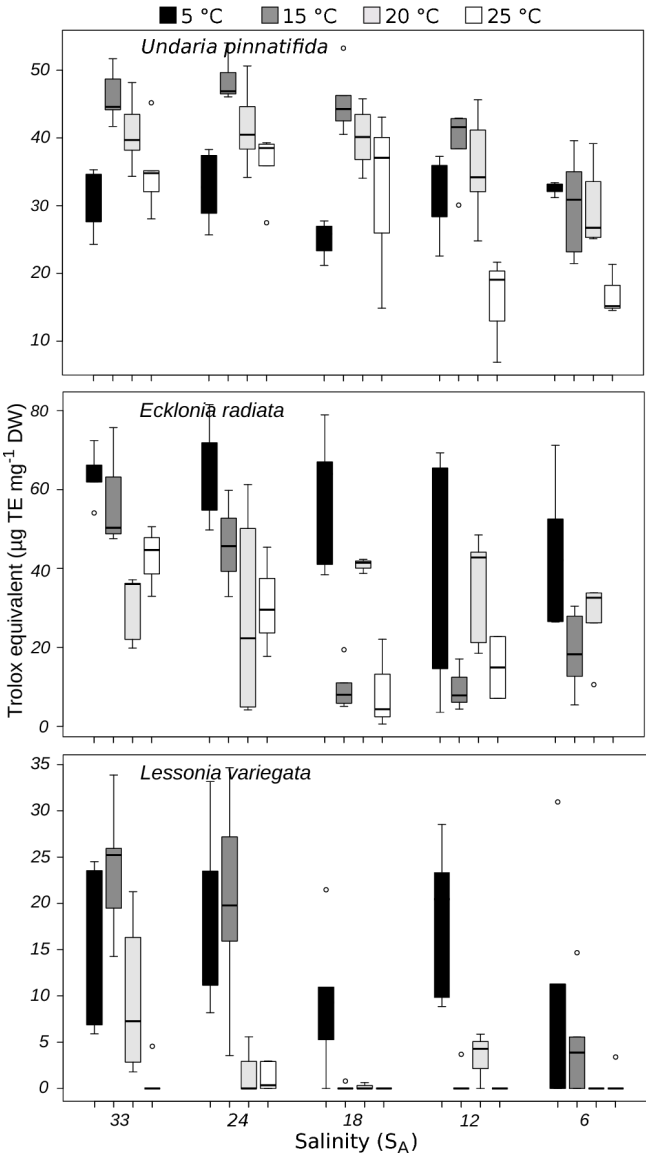

Figure 3.3 Antioxidant pool size (boxplots, n = 5) of *Undaria pinnatifida*, *Ecklonia radiata* and *Lessonia variegata* exposed to various temperatures (5, 15, 20 and 25 °C) and salinities (33, 24, 18, 12 and 6 S_A) after 10 days of experimental exposure. Pool size is expressed as Trolox equivalents per dry weight (µg TE mg^{-1} DW). Bottom and top of the boxes in the plot depict the first and third quartiles, respectively. The median is represented by the band inside box. The ends of the whiskers are designed to show minimum and maximum value still within 1.5 x Interquartile range (IQR) of the lower and upper quartile. Values outlying these ranges are displayed by single dots. Details on statistical analyses and results are given in the text.

Tolerance to salinity changes is one critical factor for vertical distribution limits of seaweed species in the intertidal zone (Kumar et al. 2014). From studies on different algal species hyposaline conditions are known to influence growth (Bjærke & Rueness 2004), water content (Luo & Liu 2011), photosynthetic activity and chlorophyll *a* concentrations (Karsten 2007), respiration rates (Ogata & Takada 1968) as well as carbon and nitrogen metabolism (Kakinuma et al 2006). In our

study, reduced salinities did not limit survival or markedly impact chlorophyll *a* concentration and photosynthetic quantum yield of *U. pinnatifida*. However, reduced antioxidant levels and xanthophyll pool sizes reflected the potential generation of ROS as consequence of low salinities. While zoospores of *U. pinnatifida* germinated in salinities down to 8 S_A (Bite 2001) and microscopic gametophytes remain viable at salinities as low as 6 S_A (Peteiro & Sánchez 2012) young sporophytes (up to 4 mm long) only survived salinities down to 16 S_A (Peteiro & Sánchez 2012). Our results suggest that adult sporophytes of *U. pinnatifida* are capable of enduring hyposaline conditions down to 6 S_A, at least for short time periods.

Table 3.4 Means (± SD, n = 5) of xanthophyll cycle pigment pool (∑VAZ) of three kelp species (*Undaria pinnatifida*, *Ecklonia radiata*, *Lessonia variegata*) exposed to various temperatures (5, 15, 20 and 25 °C) and salinities (33, 24, 18, 12 and 6 S_A) determined after 10 days of experimental exposure. ∑VAZ is expressed in relation to chlorophyll (Chl) *a* concentration (µg $µg^{-1}$ Chl *a*). Details on statistical analyses and results are given in the text.

∑VAZ (µg $µg^{-1}$ Chl *a*)		Salinity (S_A)				
		33	24	18	12	6
Undaria pinnatifida	5°C	0.078 ± *0.005*	0.073 ± *0.012*	0.086 ± *0.023*	0.070 ± *0.005*	0.069 ± *0.007*
	15°C	0.076 ± *0.002*	0.075	0.074 ± *0.006*	0.073 ± *0.007*	0.073 ± 0.006
	20°C	0.087 ± *0.010*	0.075 ± *0.009*	0.076 ± *0.015*	0.079 ± *0.011*	0.070 ± *0.015*
	25°C	0.075 ± *0.016*	0.051 ± *0.008*	0.070 ± *0.022*		0.035 ± *0.011*
Ecklonia radiata	5°C	0.073 ± *0.009*	0.062 ± *0.011*	0.067 ± *0.004*	0.055 ± *0.009*	0.057 ± *0.010*
	15°C	0.053 ± *0.018*	0.035 ± *0.011*	0.050 ± *0.018*	0.065 ± *0.040*	0.028 ± *0.017*
	20°C	0.058 ± *0.018*	0.038 ± *0.011*	0.031 ± *0.018*	0.035 ± *0.007*	0.025 ± *0.006*
	25°C	0.060 ± *0.019*		0.038 ± *0.011*	0.036 ± *0.006*	
Lessonia variegata	5°C	0.065 ± *0.008*	0.046 ± *0.007*	0.062 ± *0.007*	0.054 ± *0.006*	0.056 ± *0.011*
	15°C	0.068 ± *0.010*	0.056 ± *0.011*	0.052 ± *0.013*	0.055 ± *0.003*	0.051 ± *0.005*
	20°C	0.061 ± *0.003*	0.056 ± *0.004*	0.057 ± *0.016*	0.053 ± *0.003*	0.059 ± *0.009*
	25°C	0.027 ± *0.015*	0.025 ± *0.004*	0.090 ± *0.047*	0.032 ± *0.003*	0.037 ± *0.003*

Carbon dioxide solubility increases with decreasing salinities (Weiss 1974), potentially enhancing the availability of carbon dioxide to photosynthesis in reduced salinities. Elevated CO_2 levels are known to increase the photosynthetic performance of macroalgae (Johnson et al. 2012, Olischläger et al. 2012). This correlation might explain the observation that *U. pinnatifida* exhibited highest ETR_{max} values at lower than ambient seawater salinities (24 S_A,). When salinity is further reduced, physiological stress might exceed the positive effect of hyposaline conditions.

3.5.2 Ecological implications

Temperature is a main driver in seaweed biogeography and ocean water temperature is projected to increase continuously in the coming decades (IPCC 2014). Organisms susceptible to temperature changes or living close to their critical temperature limit might experience restrictions in physiological performance, as predicted for corals (Fitt et al. 2001). Schiel et al. (2004) reported a decrease in abundance of temperature-sensitive algae over the duration of ten years in response to artificial warming induced by the thermal outfall of a power-generating station. Our findings suggest that physiological conditions of the endemic *L. variegata* will be strongly impacted if water temperatures exceed 25 °C for multiple days, potentially leading to reduced abundances in the intertidal. In contrast, we did not detect major restrictions to the physiological performance of *U. pinnatifida* in 25 °C indicating that the kelp is capable of withstanding 25 °C water temperature. Based on satellite-derived Sea Surface Temperature (SST) data James et al. (2015) suggested that extensive areas of the world coastlines might be suitable for the invasion of *U. pinnatifida*.

Salinities in Tauranga Harbour can be significantly lower than the ocean outside the harbour (Pritchard et al. 2009). Close to the inlet of Wairoa river, the main freshwater source of Tauranga Habour, extremely low salinities (0 – 5 S_A) might be experienced for at least short periods of time (Pritchard et al. 2009). The pronounced tolerance to salinity for *U. pinnatifida* sporophytes, found in our study, potentially enables the kelp to further expand its range into less saline areas of Tauranga Harbour and elsewhere. In Venice lagoon invasive stands of *U. pinnatifida* are already established, experiencing salinities of 16 to 38 S_A (Curiel et al. 1998). However, timescale and frequency of salinity fluctuations will be important factors determining acclimation rates for the invasion success (Lee & Bell 1999) in environments of varying salinities e.g. lagoons. Experiments comparing short and long term salinity exposure may add valuable information to predict future distribution patterns.

Physiological impairment of *E. radiata* and the endemic *L. variegata* in high temperatures, as

observed in this study, might favour the spread of the less impacted *U. pinnatifida* in a competitive situation (e.g. for space), eventually resulting in local dominance of *U. pinnatifida* in the intertidal. *U. pinnatifida* has been shown to benefit from disturbance of native canopy forming kelps (Valentine & Johnson 2003, 2004). Therefore, impaired performance of native species may be a critical invasion factor.

In our study area, Tauranga Harbour (Te Awanui) located in the Bay of Plenty, New Zealand, *U. pinnatifida* was first observed in 2005 (Russel et al. 2008). The invader vigorously grows on hard substrata in close vicinity to the examined native kelp species. *U. pinnatifida* within Tauranga Harbour currently co-occurs with other macrophytes such as *Carpophyllum mascalocarpum*, *C. flexuosum* and *Sargassum spp.* as well as *E. radiata*. Together with subcanopy species such as *Ulva*, *Codium, Corallina spp.* as well as a number of small foliose red algae. It currently is not present to any great extent on the outer coast, but given the rapid spread observed in coastal regions adjacent to ports such as Wellington, Napier and Gisborne (the latter two with similar salinity and temperature profiles to Tauranga), it is expected to be interacting with coastal *E. radiata* and *L. variegata* imminently (Hay & Luckens 1987, Hay 1990, Battershill et al. 1998). Indeed, the relatively low abundance of *E. radiata* inside the harbour in areas of *U. pinnatifida* presence and on reef habitat that would normally otherwise support *E. radiata* could signal a competitive displacement already in action.

In conclusion, this study demonstrates that adult sporophytes of *U. pinnatifida* exhibit a wide physiological tolerance to synergistic effects of temperature and salinity, suggesting the potential invasion of brackish environments. Species-specific physiological reactions to abiotic stress have been observed, strikingly demonstrating the importance of antioxidant pool size for stress regulation in kelps. *U. pinnatifida* exhibited considerably higher tolerance to abiotic factors than the native kelps, supporting the generally accepted assumption that invasive species are more tolerant to abiotic stresses than natives. Especially with regard to its performance in elevated temperature conditions, *U. pinnatifida* might experience a competitive advantage in a warming ocean, and further expand its invaded range.

3.6 Acknowledgements

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3.7 References

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Chapter 4: Interactions between microscopic gametophytes of the invasive kelp *Undaria pinnatifida* and the native *Macrocystis pyrifera* – Implications for invasive success

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4.1 Abstract

Once established in new habitats, invasive seaweeds interact with the local community, potentially altering the structure and functioning of ecosystems. Interactions between microscopic kelp stages can have significant implications for subsequent macroscopic sporophyte populations and the habitat they create. This study explored interactions between gametophytes of the invasive kelp *U. pinnatifida* and the native kelp *Macrocystis pyrifera*. Gametophyte growth was monitored in a laboratory experiment in three settings: a) in a mixed culture of both species, b) in the presence of older intra- and interspecific gametophytes and c) in physically separated mono-cultures but sharing the same medium with the other species. Additionally, the effect of settling density on gametophyte growth metrics was assessed. In the presence of *M. pyrifera* pigmented bodies of *U. pinnatifida* were consistently longer and oogonia formation was enhanced by 48 %. In contrast, no response of the pigmented body was observed for *M. pyrifera* in the presence of *U. pinnatifida*. Germination tubes of both species were elongated in direct physical proximity of inter-specific gametophytes and in high settling densities. Our results suggest a facilitation of growth for *U. pinnatifida* gametophytes by the presence of *M. pyrifera* and highlight the importance of microscopic life stages for interactions among kelps.

4.2 Introduction

Interactions among species play an important role in shaping marine communities (Berlow 1999, Bruno et al. 2003, Kordas et al. 2011). Illustrating this mechanism, ecosystem engineers create complex habitats and modulate resource availability for associated biota (Jones et al. 1994). Kelps are prominent marine engineers that alter physical conditions at a local scale and provide food, nursery ground and refuge from predation for associated organisms (Limbaugh 1955, Coleman & Williams 2002, Graham et al. 2007). As a consequence, kelp forests are considered to be highly productive ecosystems in near-shore environments (Dayton 1985, Steneck et al. 2002). Yet, man's agency pressures these communities, resulting in modifications of habitat structures, shifts of species distributions and alterations to food web dynamics (Kjerfve 1994, Hoegh-Guldberg & Bruno 2010, Doney et al. 2012). Furthermore, anthropogenic activities have dramatically accelerated the number of marine invasions during the last decades (Hewitt 2003, Wonham & Carlton 2005). This addition of new species is leading to novel interactions with the local intertidal community (Bax et al. 2003). For example, the invasive green alga *Codium fragile* inhibited recruitment of native species after its establishment in kelp beds of the North Atlantic (Levin et al. 2002). Accordingly, through their interactions with natives, incoming species might modify habitat structure or other ecosystem services of concerned environments and adversely impact native biodiversity (Schaffelke & Hewitt 2007).

The annual kelp *Undaria pinnatifida* (Harvey) Suringar, native to the Northwest Pacific (Japan, China, Korea) has successfully established on many coasts outside its natural range as a result of shipping and mariculture activities (Peréz et al. 1981, Hay & Luckens 1987, Hay & Villouta 1993, Verlaque 2001, ICES 2007). The role *U. pinnatifida* exhibits in interactions with local macroalgal species in invaded areas remains unclear. Although Floc'h et al. (1991) suggested that the kelp bears a low competitive ability in native macroalga assamblages, a number of subsequent studies indicate adverse effects for invaded communities (Curiel et al. 1998, Piriz et al. 2003, Jiménez et al. 2015). *U. pinnatifida* seems capable of replacing smaller red and green algae (Hay & Villouta 1993) and was observed to monopolize space in intertidal habitats (Battershill et al. 1998). However, the mechanism of this dominance and to what extent human-induced changes, such as enhanced nutrient concentrations, favour the invasion could often not be identified. Attributes that are proposed to contribute to the invasiveness of *U. pinnatifida* are its high reproductive output, fast growth rate and the ability of specific life history stages to sustain unfavourable conditions (Fletcher & Farrell 1999, Thompson 2004). The kelp seems to be especially successful on newly

available substrates, e.g. cleared space following disturbance events, that are colonised by propagules (Valentine & Johnson 2003, 2004, Johnson et al. 2004, Carnell & Keough 2014). Thus, interactions at microscopic life stages could be a key mechanism for the spread of *U. pinnatifida* and its invasion a model for microscopic life history interaction between invasive and native species.

Like all kelps *U. pinnatifida* exhibits a heteromorphic life cycle. A macroscopic diploid sporophyte alternates with microscopic haploid male and female gametophytes, which play a crucial role for the recruitment success. Most investigations on the interaction of introduced or invasive species with the native seaweed flora employ observational or manipulative field studies on the sporophyte generation (Forrest & Taylor 2002, Casas et al. 2004, Valentine & Johnson 2005, Farrell & Fletcher 2006, Raffo et al. 2009, Thompson & Schiel 2012). In the field, microscopic stages can only be observed indirectly through arising sporophytes (Wotton et al. 2004) or by outplanting microscopic slides. As a consequence little is known about the interactions of microscopic stages of kelps.

Based on this lack of knowledge we performed a laboratory experiment to reveal whether interactions among kelp gametophytes could contribute to the success of *U. pinnatifida* as an invasive species. In Otago Harbour, New Zealand, *U. pinnatifida* eastablished in direct vicinity to the native giant kelp *Macrocystis pyrifera* (Linnaeus) C. Agardh. Since both kelps belong to the order Laminariales, development at microscopic stage is very similar, with gametogenesis in optimal conditions starting seven to ten days after spore release (North 1987, Morelissen et al. 2013). In this study, gametophyte development immediately after spore release was monitored in experimental treatments designed to study intra- as well as inter-specific interactions of *U. pinnatifida* and *M. pyrifera*. Specific objectives were (a) to investigate the effect of settlement density on gametophyte growth and (b) to assess the species-specific response to the presence of inter-specific specimens on gametophyte length and oogonia formation.

4.3 Materials and Methods

4.3.1 Spore release, set-up and response variables

Fertile material of *Macrocystis pyrifera* and *Undaria pinnatifida* was collected from Otago Harbour, New Zealand (45°47'S, 170°42'E) in November 2013. Algal material was rinsed with fresh water

and wiped with paper towels to remove sediment and epibiota. Thereafter, it was desiccated in a dark chamber at 15 °C for 4 h or overnight. Spore release was triggered by re-immersion into sterilized seawater enriched with unbuffered nutrients after Provasoli (1968; PES) and indirect sunlight.

Several glass cover slips were placed into plastic petri dishes (9 cm diameter) and inoculated with seaweed spores (specific procedures for each experiment are explained below). This set-up enabled continuous monitoring without disturbance as single cover slips could be removed from the petri dishes at different sampling times. Petri dishes were installed in a culture room and conditions were kept at 15 °C and 10 - 20 µmol m^{-2} s^{-1} photon irradiance in a 12:12 h light cycle. For all experimental designs (explained below), all treatments and controls consisted of three petri dishes, each supplied with spores from different individuals. Replicates infested with diatoms or not matching the required settling density were excluded from the analysis.

To evaluate the impact of the different treatments (explained below) total length of the gametophytes, as a measure of gametophyte growth, was determined two days after spore release (day 2). Additionally, length of the germination tube and the pigmented body (Fig. 4.1) were separately determined five days after spore release (day 5). Measurements were conducted on at least 50 haphazardly chosen gametophytes per petri dish.

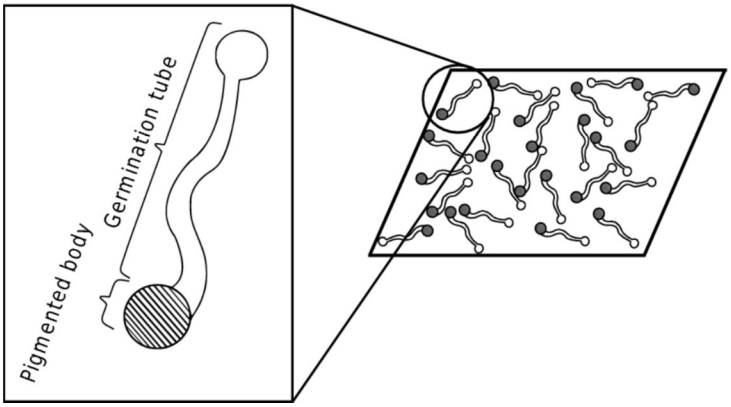

Figure 4.1 Schematic drawing of the arrangement of gametophytes on microscopic cover slips during the experimental procedures, the discrimination between pigmented body and germination tube of gametophytes is illustrated in the magnification, lengths of both add together to the total gametophyte length.

4.3.2 Density dependent growth / Intra-specific interaction experiment

In order to evaluate the effect of intra-specific interactions we investigated gametophyte growth at different settlement densities for each species separately. Therefore, petri dishes contained only

con-specifics. In accordance to Reed et al. (1991) who discovered delayed egg maturation in settlement densities >300 spores mm^{-2}, settlement densities > 300 spores mm^{-2} were defined as 'high density' in our study, settlement densities < 200 spores mm^{-2} as 'low density'. Different settlement densities were obtained by diluting the initial spore suspensions to 2 x 10^5 spores ml^{-2} (low density) and 4 x 10^5 spores ml^{-2} (high density), respectively. Actual settlement density was ascertained two days after spore release using light microscopy. Replication and monitoring of gametophyte growth was accomplished as described above.

4.3.3 Inter-specific interaction experiments

Inter-specific interactions were assessed by mixing spore solutions of *U. pinnatifida* and *M. pyrifera* and synchronous culturing of spores and gametophytes of both species within the same petri dish. Gametophytes of *U. pinnatifida* and *M. pyrifera* are morphologically very similar, hence, specimens growing close to each other pose the problem of definite species classification. Pre-experiments in the same experimental conditions (15°C, 10 - 20 µmol m^{-2} s^{-1} photon irradiance, 12:12 h light cycle) revealed that gametophytes of *U. pinnatifida* are on average longer than those of *M. pyrifera* (Table 4.1). However, visual allocation could not be reliably accomplished, thus, three experimental designs were developed in order to overcome the problem of identification (explained below).

Table 4.1 Statistical analysis of the pre-experiment comparing the mean lengths of *U. pinnatifida* and *M. pyrifera* gametophytes five days after spore release. Results of the non-parametric Kruskal-wallis test are displayed. n = number of individuals assessed.

	Mean length (µm) ± SE	n	Statistical analysis
Undaria pinnatifida	34.9 ± 1.1	30	H = 42.917 p < 0.001
Macrocystis pyrifera	17.8 ± 0.6	30	

Experimental design 1: Mixed group

We observed gametophyte growth of *M. pyrifera* and *U. pinnatifida* in a mixed culture with same-aged gametophytes of the respective other species (treatment). Thus, spore solutions of *M.*

pyrifera and *U. pinnatifida* were mixed in equal densities (4 x 10^5 spores ml^{-2}) before being disseminated into shared petri dishes. The resulting total density of the treatments was > 300 spores mm^{-2}, in which each species represents half of the total density (single species density). Thus, in order to distinguish between intra-specific and inter-specific interactions, two types of controls were established for each species: control 1 = con-specifics matching the proportion of each single species in the treatment (single species density) and control 2 = con-specifics matching the added up density of both species in the treatment (total density). For the treatment gametophyte lengths of 100 haphazardly chosen individuals were assessed in order to examine approximately 50 per species. Discrimination between the two species was accomplished statistically as explained below in the 'Statistical analyses' section. Replication and monitoring of gametophyte growth was accomplished as described above.

Experimental design 2: Presence of older gametophytes

In order to distinctly discriminate gametophytes of *M. pyrifera* and *U. pinnatifida* a difference in age between the gametophytes was established. The experimental procedure is illustrated in Fig. 4.2a. Newly released spores of one species were disseminated to five-day old gametophytes (old gametophytes) of the same (intra-specific interaction) and the respective other species (inter-specific interaction). For each seeding event spore suspensions were diluted to low densities (2 x 10^5 spores ml^{-2}), hence, total settling density adding up densities of old and newly established (young) gametophytes resulted in > 300 gametophytes mm^{-2}. Similar to experimental design 1, two types of controls were established for each species: control 1 = con-specifics matching the proportion of the young gametophytes in the treatments (young gametophyte density) and control 2 = con-specifics matching the added up density of old and young gametophytes (total density). In the treatments, 50 haphazardly chosen individuals of young gametophytes were monitored. Replication and monitoring of gametophyte growth was accomplished as described above.

Experimental design 3: Growth physically separated in the same medium

The experimental procedure is illustrated in Fig. 4.2b. Spore solutions of *M. pyrifera* and *U. pinnatifida* (4 x 10^5 spores ml^{-2}) were disseminated into separate petri dishes containing microscopic cover slips and spores were allowed to settle for 16 - 18 h. The resulting cover slips inoculated with con-specifics (monoculture cover slips) exhibited settling densities > 300 gametophytes mm^{-2}. Monoculture cover slips of each species were transferred into one common

petri dish and provided with fresh medium. Thus, gametophytes grew without physical contact, but shared the same culture medium (treatment). Gametophytes were cultured for ten days and medium was exchanged on day 5. Replication of treatment and control was accomplished as described above. Besides monitoring of gametophyte lengths, reproductive capacity, in terms of the proportion of oogonia-bearing gametophytes, was determined ten days after spore release (day 10). In order to avoid bias by mistaking males and immature females, all gametophytes were included in the measure. The percentage of oogonia-bearing gametophytes (gametophyte with oogonia / total number of gametophytes) was determined for at least 10 fields-of-view for each petri dish, resulting in a minimum of 150 assessed individuals for each treatment and control. Pigmented body length of oogonia bearing females was determined on day 10.

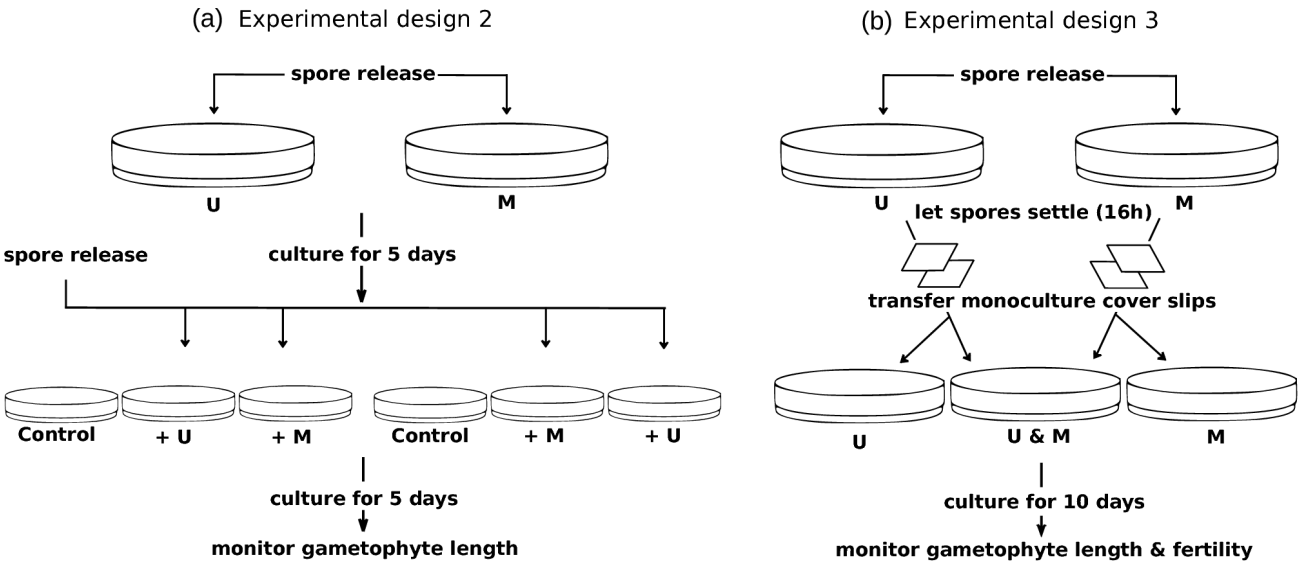

Figure 4.2 Schematic plan of the procedures for (a) experimental design 2 (“older gametophytes”) and (b) experimental design 3 (“Growth physically separated in the same medium”) applied to investigate interactions among kelp gametophytes, U = *Undaria pinnatifida*, M = *Macrocystis pyrifera*, + indicates the addition of spores to already established (five-day old) gametophytes, replication is not diagrammed, details on replication are explained in the text.

4.3.4 Statistical analyses

Data of the investigated replicates (petri dishes) was pooled for each treatment and the controls. For each species differences in mean gametophyte length between different treatments and

controls were tested using one-factorial analyses of variance (ANOVA) operated in the statistic programme “R” (R Development Core Team 2013). When ANOVA indicated significant differences post-hoc comparisons were carried out using Tukey's HSD test. When the assumption of normal distribution or homogeneous variances was not met the non-parametric Kruskal-Wallis test was applied, subsequently followed by the non-parametric Wilcoxon test for post hoc analysis. In Fig. 4.3 and Fig. 4.5 to 4.8 different letters denote differences between group means, represented in lowercases for *U. pinnatifida* and in italic lowercases for *M. pyrifera.*

Since gametophytes of *U. pinnatifida* are on average longer than those of *M. pyrifera* (Table 4.1) dissemination of mixed spore suspensions (as performed in experimental design 1) resulted in bimodal length frequency distributions. To determine mean length of each species in the mixed species group, each gametophyte had to be allocated to one of the two species. Based on the pronounced length difference between gametophytes of the two species the allocation was statistically accomplished applying a cluster analysis from the R package “pdfCluster” (Azzalini & Menardi 2014). Providing length data of the mixed culture separately for day 2 and day 5, the package performs cluster analyses via kernel density estimation (Azzalini & Torelli 2007, Menardi & Azzalini 2014), more details on the package are described in Azzalini & Menardi (2014). Thus, no data of the control distributions was used for the allocation procedure of the mixed species culture.

To assure accuracy of the cluster analysis, a quality assessment for the allocation process was performed. For this, datasets containing length data of gametophytes of both species - representing the bimodal length-frequency distribution of mixed species cultures - were assembled with prior knowledge of species identity for each single gametophyte. These datasets (n = 1000) were created by random drawing with replacement from the original mono-specific control data of each species (n = 120) and subsequent merging, resulting in a total of 240 individual gametophyte lengths in each dataset. After application of the cluster analysis to the created datasets, correct allocation of each gametophyte was tested. Two potential mistakes were considered for the analysis (mistake 1: assigning an individual of *U. pinnatifida* to *M. pyrifera*, mistake 2: assigning an individual of *M. pyrifera* to *U. pinnatifida*). In 25.6 % of cases a false number of distributions was detected (1, 3 or 4 clusters). However, when the cluster analysis identified two length-distributions only 12.4% of individuals were mismatched. Consequently, the proposed procedure seems to provide adequate accuracy. Means of the separated length-distributions were determined and differences between treatment and control were tested as described above.

4.4 Results

4.4.1 Density dependent growth / Intra-specific interaction

Settling density significantly affected gametophyte metrics in both species (Fig. 4.3). Total length of *U. pinnatifida* was reduced in the high density treatment ($F_{1/328}$ = 31.410, p < 0.001) but remained unaffected for *M. pyrifera* (H = 0.033, p = 0.856). Pigmented body length was reduced by 33 % for *U. pinnatifida* (H = 11.240, p < 0.001) and by 13 % for *M. pyrifera* (H = 25.073, p < 0.001) in the high density treatments. An elongation of the germination tube by 1.7 µm was observed in high densities for both species (*U. pinnatifida*: H = 113.081, p < 0.001; *M. pyrifera*: H = 18.001, p < 0.001).

4.4.2 Inter-specific interaction experiments

Experimental design 1: Mixed group

Gametophyte length-frequency distributions of the mixed culture and control 2 are displayed in Fig. 4.4 for each species on day 2 and day 5. Based on the controls, mean and maximum length was greater for *U. pinnatifida* compared to *M. pyrifera* on both measuring days. The length-frequency distribution of the mixed culture is bimodal due to an overlap of the single species distributions. This pattern is readily identifiable on day 2, but less clear on day 5 when male and female gametophytes start to develop morphological differences. The longest individual in the mixed culture exceeded maximum length of the *U. pinnatifida* control 2 by 5 µm (12 %) on day 2 and by 7.5 µm (16 %) on day 5. Additionally, visual observation suggests that the second peak of the bimodal distribution in the mixed culture, presumably accounting for *U. pinnatifida*, was shifted towards higher lengths compared to control 2 of *U. pinnatifida*. Accordingly, after allocation of gametophytes of the mixed culture, statistical analysis demonstrated that total length of *U. pinnatifida* five days following spore release was enhanced in the mixed culture (treatment) by 6 µm (18 %) compared to control 1 and by 10 µm (33 %) compared to control 2 ($F_{2/449}$ = 95.25, p < 0.001; Fig. 4.5). The pigmented gametophyte body of *U. pinnatifida* in the mixed culture was on average 2 µm (22 %) longer compared to control 2, however, 3 µm (18 %) shorter compared to control 1 (H = 119.327, p < 0.001). The germination tube was elongated in the mixed culture in comparison with both controls, with an increase of 10 µm (51 %) in relation to control 1 and 8 µm (38 %) relative to control 2 (H = 159.960, p < 0.001). Even though Kruskal-wallis test indicated an

effect of the three levels (control 1, control 2, treatment) on *M. pyrifera* total length (H = 7.285, p = 0.026), the post hoc Wilcoxon test did not identify significant differences between level means. Compared to control 1, the pigmented body of *M. pyrifera* was 1 µm (15 %) shorter (H = 42.603, p< 0.001) while the germination tube was elongated by 3 µm (49%; H = 37.574, p < 0.001). No significant difference was detected between pigmented body lengths of control 2 and the treatment, but the germination tube was elongated by 2 µm (20 %) in the mixed culture compared to control 2.

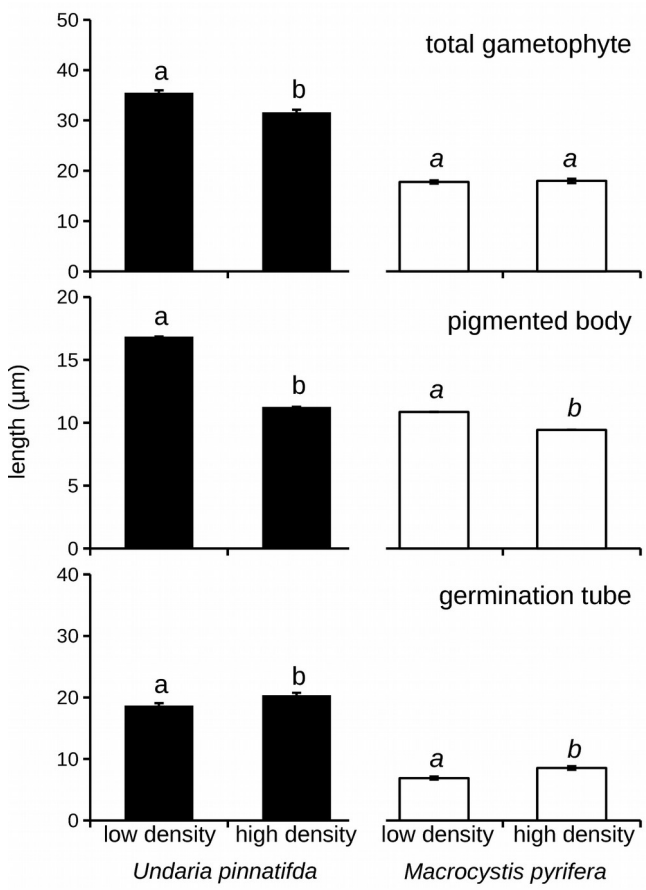

Figure 4.3 *Intra-specific interaction experiment:* Mean lengths (± SE, n = 150) of total gametopyhte, the pigmented body and the germination tube for *U. pinnatifida* and *M. pyrifera* five days after spore release in low (< 200 gametophytes mm^{-2}) and high (> 300 gametophytes mm^{-2}) densities, different letters denote differences between group means, represented in lowercases for *U. pinnatifida* and in italic lowercases for *M. pyrifera*, details of the statistical analysis are explained in the text.

Experimental design 2: Presence of older gametophytes

Total length of *U. pinnatifida* gametophytes grown in presence of intra- and inter-specific old gametophytes did not differ from lengths observed in control 1, however, were enhanced compared to control 2 five days after spore release (H = 61.496, p < 0.001; Fig. 4.6). Additionally, gametophytes of *U. pinnatifida* grown in the presence of inter-specific specimens were 3 µm (8 %)

longer than those grown in the presence of intra-specific specimens. The pigmented body of *U. pinnatifida* gametophytes was longer in the presence of inter-specific gametophytes compared to control 2 and the presence of older conspecifics, but was longest in control 1 ($H = 195.669$, $p < 0.001$). In both treatments, the presence of intra- and inter-specific gametophytes, the germination tube of specimens was elongated compared to both controls ($H = 126.209$, $p < 0.001$). For *M. pyrifera* lengths of the total gametophyte and germination tube were enhanced in presence of both, intra- and inter-specific gametophytes compared to both controls (total gametophyte length: $H = 93.295$, $p < 0.001$; germination tube: $H = 148.844$, $p < 0.001$). The pigmented body of young *M. pyrifera* gametophytes did not differ between control 2 and the two treatments, however, was longest in control 1 ($H = 36.365$, $p < 0.001$).

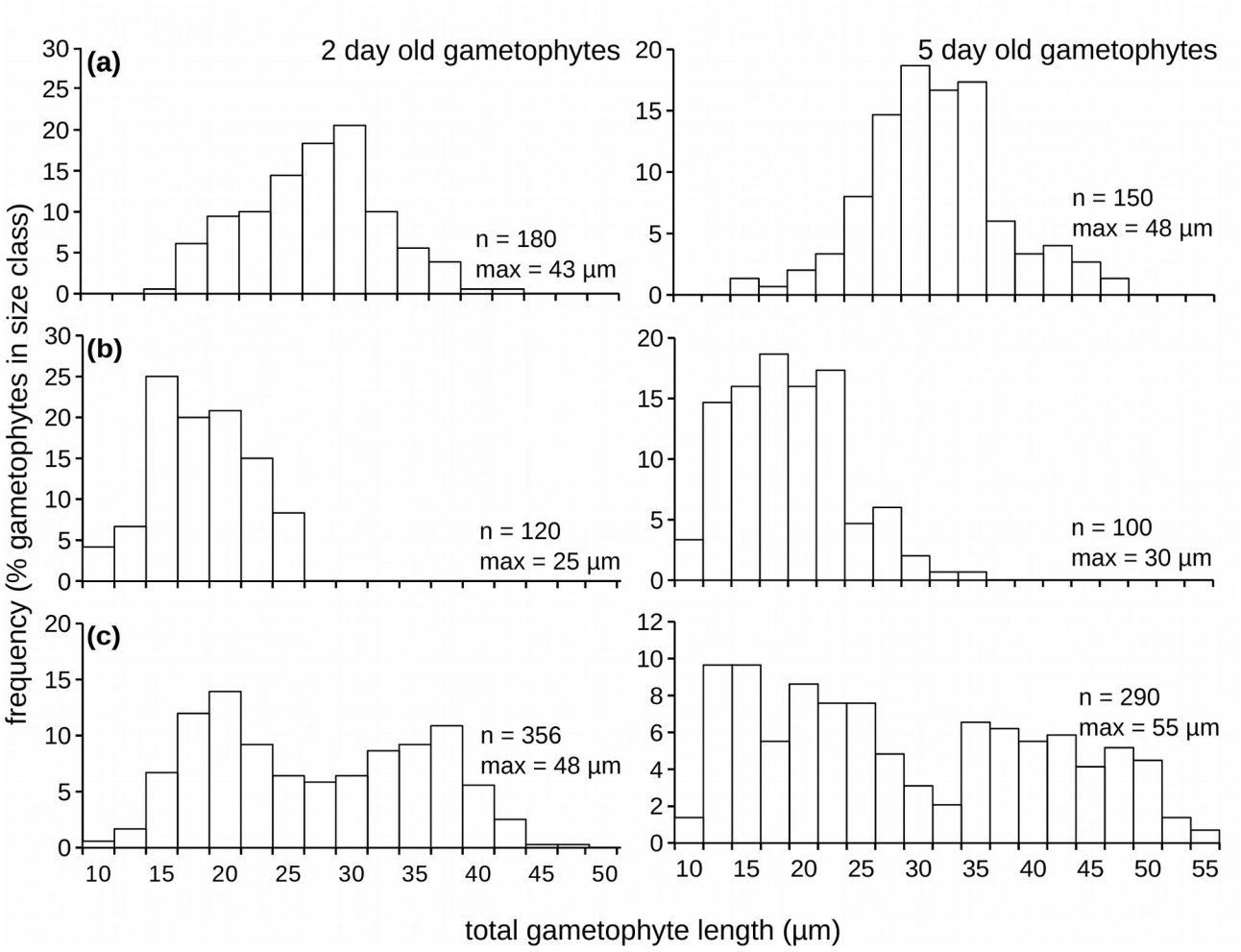

Figure 4.4 *Inter-specific interaction experiment, experimental design 1: Mixed group:* Length-frequency distribution of gametophytes two and five days after spore release for (a) *U. pinnatifida* (b) *M. pyrifera* and (c) both species grown in a mixed culture, n = number of individuals assessed, max = maximum length.

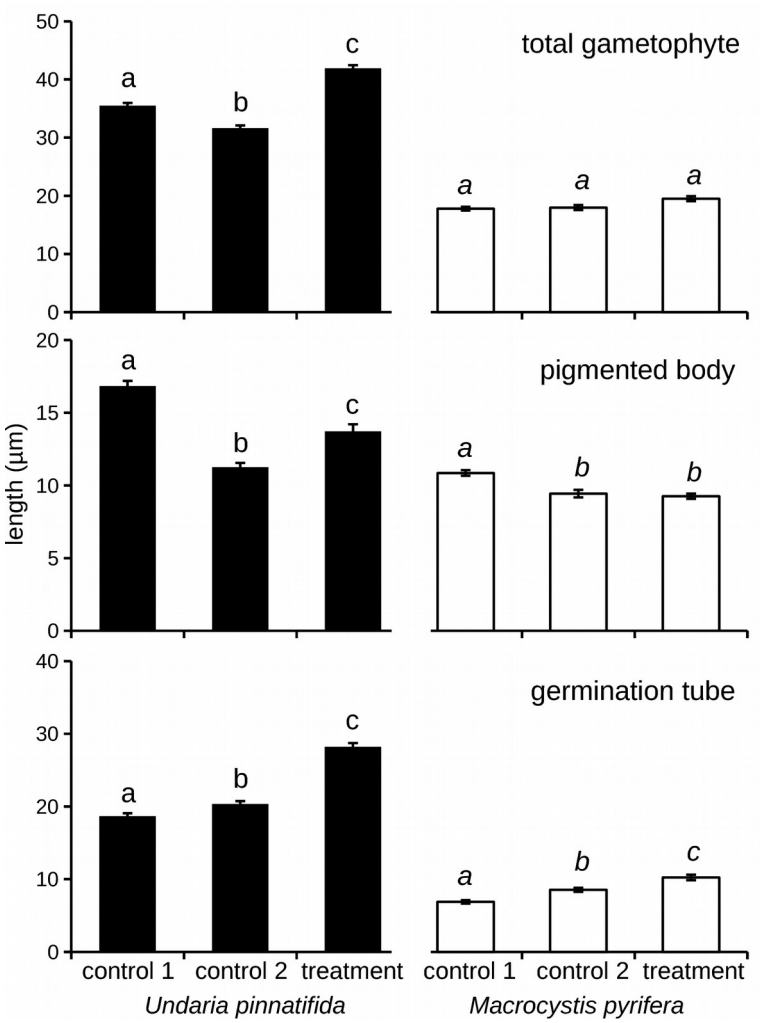

Figure 4.5 *Inter-specific interaction experiment, experimental design 1: Mixed group:* Mean lengths (± SE, n = 150) of total gametophyte, pigmented body and the germination tube of *U. pinnatifida* and *M. pyrifera* five days after spore release grown in a mixed culture with the respective other species (treatment). Control 1 = specimens in settling densities matching the density of one species of the mixed culture (= ½ of total density), control 2 = gametophytes in settling densities matching the totaled density of both species (total density), different letters denote differences between group means, represented in lowercases for *U. pinnatifida* and in italic lowercases for *M. pyrifera*, details of the statistical analysis are explained in the text.

Experimental design 3: Growth physically separated in the same medium

U. pinnatifida total length (H = 11.643, p < 0.001) and the length of the pigmented body (H = 33.742, p < 0.001) were enhanced by 2.4 µm (8 %) and 2.6 µm (22 %) on day 5 when *M. pyrifera* was present (Fig. 4.7). However, germination tube length of *U. pinnatifida* did not differ between treatment and control (H = 0.175, p = 0.676). The presence of *U. pinnatifida* gametophytes did not impact the length of total gametophyte (H = 1.706, p = 0.192), pigmented body (H = 0.008; p = 0.929) or germination tube (H = 2.383, p = 0.123) of *M. pyrifera*. Additionally, no effect of the presence of inter-specific gametophytes on the proportion of oogonia bearing *M. pyrifera* gameotphytes was observed (H = 0.087, p = 0.768; Fig. 4.8). For *U. pinnatifida* oogonia formation was enhanced by 48 % ($F_{1/43}$ = 13.09, p < 0.001) in the treatment compared to the control resulting

in 75 % more oogonia compared to *M. pyrifera*. Female gametophytes in this study never bore more than one oogonium. Pigmented body lengths of oogonia bearing females on day 10 did not differ between treatment and control for *U. pinnatifida* (H = 0.629, p = 0.428) and *M. pyrifera* ($F_{1/43}$ = 1.134, p = 0.293).

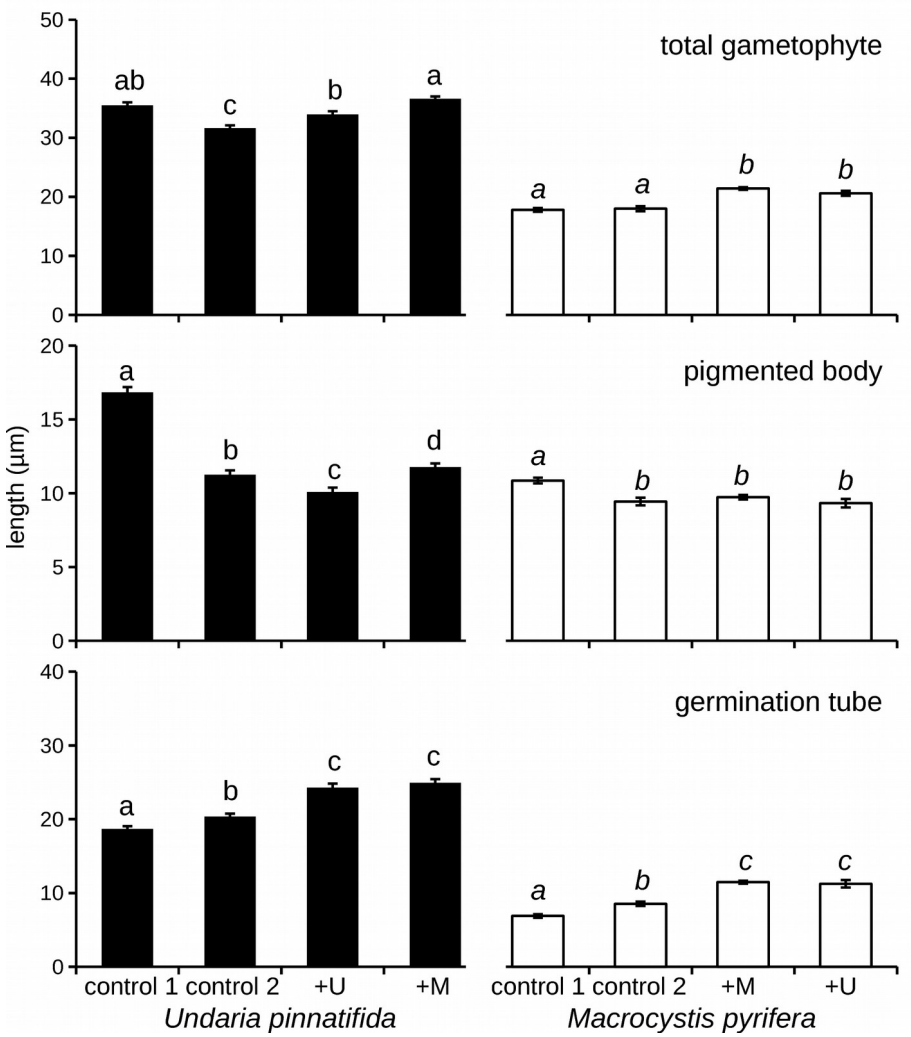

Figure 4.6 *Inter-specific interaction experiment, experimental design 2: Presence of older gametophytes:* Mean lengths (± SE, n = 150) of total gametophyte, the pigmented body and the germination tube of *U. pinnatifida* and *M. pyrifera* five days after spore release. Specimens were seeded to five-day old gametophytes of the same and the respective other species. Control 1 = specimens in settling densities matching the density of newly established gametophytes (= ½ of total density), control 2 = specimens in settling densities matching the totaled density of older and newly established gametophytes (total density), +U = gametophytes seeded to established *Undaria pinnatifida* gametophytes, +M = gametophytes seeded to established *Macrocystis pyrifera* gametophytes, different letters denote differences between group means, represented in lowercases for *U. pinnatifida* and in italic lowercases for *M. pyrifera*, details of the statistical analysis are explained in the text.

4.5 Discussion

Our results demonstrate that interactions among kelp gametophytes may impact gametophyte growth and reproductive potential. This is in accordance with earlier studies, that observed an effect of gametophyte interactions on subsequent sporophyte recruitment (Reed 1990, Chapman 2005). The experiments of Chapman (2005) revealed higher rates of sporophyte recruitment for the kelp *U. pinnatifida* in the presence of *Egregia menziesii* gametophytes (physically separated by a membrane filter). Similarly, in our study, gametophytes of *U. pinnatifida* exhibited longer pigmented bodies when *M. pyrifera* gametophytes were present. This was most evident in experimental design 3, when the density of *U. pinnatifida* gametophytes was identical in the control and in presence of *M. pyrifera* gametophytes, hence, intra-specific competition as driver of the observed effect could be rule out. The enhanced pigmented body length provides a larger surface of photosynthetically active tissue potentially promoting growth and providing energy for oogonia production and subsequent sporophyte recruitment. Consistently, we discovered a higher proportion of oogonia bearing *U. pinnatifida* gametophytes ten days after spore release in the presence of inter-specific gametophytes. Mature females did not exhibit a reduction of length or multiple oogonia indicating an enhancement, not an alteration, of gametophyte development.

The interaction between the investigated species is asymmetrical. No growth response or impact on oogonia formation was observed for *M. pyrifera* gametophytes in presence of physically distant *U. pinnatifida* gametophytes (experimental design 3). In direct physical vicinity to inter-specific gametophytes an elongation of the germination tube in excess of the elongation due to intra-specific interactions was detected (experimental design 2). Since no negative effect on *M. pyrifera* gametophytes was detected, the observed interaction is unlikely to be the result of a competitive situation. In fact, the performance of *U. pinnatifida* seems to be stimulated by the presence of *M. pyrifera*, indicating facilitation. Such positive interactions among species have received far less attention compared to the adverse impacts of competition and predation (Bertness & Leonard 1997).

4.5.1 Mode of functioning

Our study did not investigate the physiological mechanism driving the observed interaction in more detail but provides some interesting avenues for future research. The occurrence of the interaction without direct physical vicinity (experimental design 3) suggests a chemically mediated effect.

While negative allelochemical interactions are well-known for seaweeds (Kakisawa 1988, Schmitt et al. 1995, Suzuki et al. 1998, Råberg et al. 2005), reports on comparable positive interactions are scarce. A recent study demonstrated a stimulating effect of the green alga *Monostroma arctium* on *Porphyra yezoensis* (Xu et al. 2013). The authors proposed that metabolites of *M. arctium* enhanced cell membrane permeability or the activity of enzymes in *P. yezoensis*. Alkaloids, cyclic peptides, terpens, volatile organic compounds and even pheromones may be involved in such interactions (Leflaive & Ten-Hage 2007). However, in contrast to the named study, the kelps investigated in our study belong to the same order (Laminariales) and the chemical ecology is likely to be much more similar, e.g. the pheromonal system of *M. pyrifera* and *U. pinnatifida* was found to be indistinguishable (Maier & Müller 1986, Maier et al. 2001). Therefore, the potential for differences in metabolites seems to be less pronounced. To find out the exact effectiveness in the tested *Undaria*/*Macrocystis* system will require careful chemical analyses.

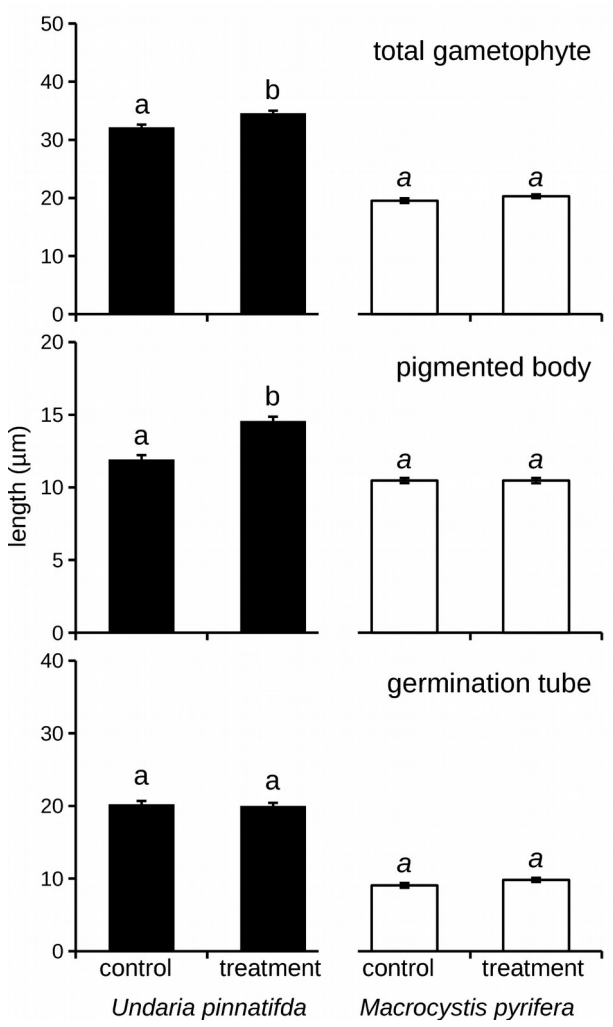

Figure 4.7 *Inter-specific interaction experiment, experimental design 3: Growth physically separated in the same medium:* Mean lengths (± SE, n = 150) of total gametophyte, the pigmented body and the germination tube of *U. pinnatifida* and *M. pyrifera* five days after spore release. Specimens were grown on monoculture cover slips sharing medium with conspecifics on further cover slips (control) or the respective other species (treatment), different letters denote differences between group means, represented in lowercases for *U. pinnatifida* and in italic lowercases for *M. pyrifera*, details of the statistical analysis are explained in the text.

4.5.2 Intra-specific effects

High settlement densities (> 300 spores mm²) reduced the size of the pigmented body for *U. pinnatifida* and *M. pyrifera*. Negative effects of elevated densities on growth and survival have been observed in a number of seaweed species (*Macrocystis pyrifera* & *Pterygophora californica*, Reed 1990; *Sargassum muticum*, Steen 2003; *Fucus spp*., Steen & Scrosati 2004; *Pyropia torta*, Conitz et al. 2013). Reed et al. (1991) showed that high densities (3000 spores mm^{-2}) could even inhibit maturation of kelp gametophytes. Intensified intra-specific competition for resources, e.g. substrate, nutrients or light (Carpenter 1990), causes such negative effects (Steen & Scrosati 2004) and is likely to be the driver of the growth reduction for gametophytes in higher settlement densities observed in our study.

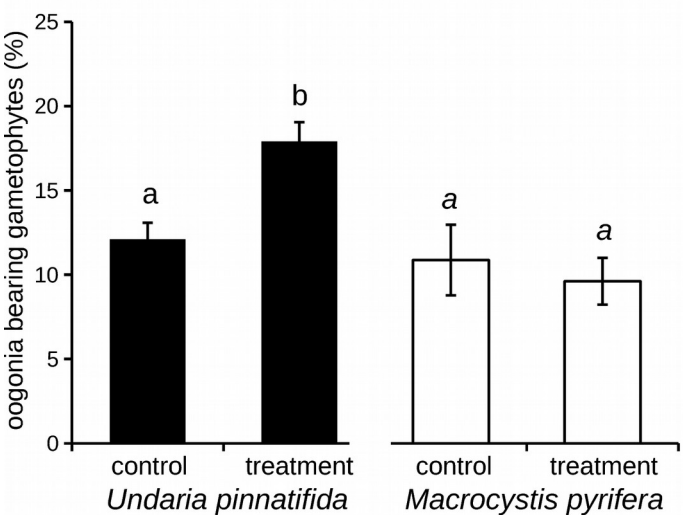

Figure 4.8 *Inter-specific interaction experiment, experimental design 3: Growth physically separated in the same medium:* Proportion of gametophytes of *U. pinnatifida* and *M. pyrifera* bearing oogonia (means ± SE) ten days after spore release. Specimens were grown on monoculture cover slips sharing medium with conspecifics on further cover slips (control) and the respective other species (treatment), different letters denote differences between group means, represented in lowercases for *U. pinnatifida* and in italic lowercases for *M. pyrifera*, details of the statistical analysis are explained in the text.

4.5.3 The role of the germination tube

The germination tube of both species investigated was elongated in direct vicinity of intra- and inter-specific gametophytes, even when the pigmented body remained unaffected. The absence of

this phenomenon in cultures where *U. pinnatifida* and *M. pyrifera* were separated suggests that the germination tube plays a role in direct physical interactions between kelp gametophytes. Furthermore, the response of the germination tube to experimental conditions was the same for *U. pinnatifida* and *M. pyrifera* in each experimental design. The physiological relevance of the germination tube is not fully resolved. After spore germination, the germination tube begins to elongate. Only when germination tube elongation is completed, nuclear division and the translocation of one daughter nucleus along the germination tube is initiated (Pillai et al. 1992). Synchronous to nuclear translocation, a bulbous structure differentiates at the distal end of the germination tube and thereafter the first gametophytic cross wall is formed (Pillai et al. 1992). Anderson & Hunt (1988) suggest a photosynthetic function based on their observations of elongated tubes when gametophytes were grown in high irradiances. Inconsistently, no effect of irradiance on germ tube length could be detected by Han et al. (2011). Germination tube elongation is used as an endpoint in toxicity studies and is considered to be less variable than spore germination (Anderson & Hunt 1988). Reductions of germination tube length were observed under heavy metal concentrations (Anderson & Hunt 1988), in contact with sediments of a sewage outfall (Tegner et al. 1995) and after UV exposure (Huovinen et al. 2007). In our study, however, enhancement of the germination tube length did not necessarily involve an enhancement of pigmented gametophyte length. Thus, in gametophyte interactions, germination tube length does not seem to indicate fitness of gametophytes. A greater adhesion capacity (Pereira et al. 2011) or pre-emption of space might be achieved due to the larger area of the germination tube.

4.5.4 Ecological implications

Scaling up results from laboratory analyses to the natural environment always poses the problem of deficient complexity, e. g. the interplay of abiotic conditions and biological interactions. However, for studies on kelp gametophytes laboratory research is almost indispensable due to the difficulties associated with observing these microscopic organisms *in situ* (Dayton 1985).

As mentioned earlier, the observed interaction between the kelp gametophytes can be interpreted as facilitation of *U. pinnatifida* gametophytes by the presence of *M. pyrifera*. Keeping in mind that density-dependent gametophyte mortality is inevitable due to the immense size difference between gametophytes and sporophytes of kelps (Reed 1990, Amsler et al. 1992), it is likely that only one individual adult sporophyte would occupy an area previously covered by a vast number of gametophytes. In this case, the observed accelerated gametophyte development of *U. pinnatifida*

in our study, as a result of the facilitative effect of the presence of *M. pyrifera* gametophytes, potentially favours earlier sporophyte recruitment and a size lead of *U. pinnatifida* sporophytes over those of *M. pyrifera*. In photosynthetic organisms larger thallus size might provide a competitive advantage by shading of smaller individuals (Schmitt et al. 1986, 1987) or overgrowth. Consequently, a competitive advantage for *U. pinnatifida* sporophytes might arise from the facilitation by *M. pyrifera* at gametophyte stage. This scenario requires synchronous release of inter-specific spores and the co-occurrence of fertile material of the investigated species supports this assumption. Thus, the relative dominance of *U. pinnatifida* sporophytes on newly available substrata, observed in some areas (Valentine & Johnson 2003, 2004, Carnell & Keough 2014) could indeed be explained by the observed interaction at microscopic stage.

In kelp gametophytes the release of spermatozoids from the antheridia as well as their attraction is triggered by pheromones released by the female gamete (Lüning & Müller 1978, Maier 1982). As mentioned before, pheromonal system of *M. pyrifera* and *U. pinnatifida* was found to be indistinguishable (Maier & Müller 1986, Maier et al. 2001) potentially allowing chemical interference between their gametophytes (Müller 1981). For a similar scenario Amsler et al. (1992) speculated that pheromones released by the early mature species might trigger spermatozoid release in both species. Thus, once the second species reaches fertility, there might be a shortage of sperm, ultimately resulting in reduced recruitment success (Amsler et al. 1992). Early maturity, as observed in our study, might set the scene for such a hypothetical interference between *U. pinnatifida* and *M. pyrifera*.

In conclusion, intra- and inter-specific interactions among kelp gametophytes might impact gametophyte development. We observed a facilitation of the invasive *U. pinnatifida* by the presence of *M. pyrifera* gametophytes, potentially contributing to its establishment success. This study underpins the importance of microscopic life stages and the need to consider the entire life cycle when determining the invasive potential of kelps.

4.6 Acknowledgements

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Chapter 5: Methodological challenges - working with kelp gametophytes

Public interest in kelp mostly refers to the macroscopic sporophyte generation, as well as it is the sporophyte that is in the focus of most scientific research. Yet, gametophytes hold a key role in the life cycle of kelp and particularly annual species, like the invasive *Undaria pinnatifida*, depend on gametophyte longevity in the seasonal absence of their sporophytes (Dayton 1985, Edwards 2000). However, the microscopic size and morphological similarity across species hinder in situ studies on kelp gametophytes (Schiel & Foster 2006) and most conclusions concerning this life history stage are indirectly drawn from surveillance of the arising sporophyte generation (Wotton et al. 2004). As a consequence, gametophytes are often considered a 'black box' in the life cycle of kelps (Johansson et al. 2013). In spite of these methodological difficulties under field conditions, different physiological traits of gametophytes have been unravelled through minute laboratory-based microscopy work (e.g. tom Dieck 1993). Beside observations of newly released spores and subsequently developing gametophytes, it is common practice for experimenters to work with cultured gametophytes, some of which have been cultivated in laboratory conditions for years or even decades. These studies are usually engaged with a single species (Devinny & Volse 1978, Lüning 1981, Novaczek 1984), in many cases with regard to economically important kelps (Westermeier et al. 2006, Zhang et al. 2008, Xu et al. 2009, Wang et al. 2011). Major research efforts focussed on factors that regulate growth and fertility in gametophytes in order to foster effective production techniques for aquaculture purposes. In contrast, ecological aspects, like interactions among kelp gametophytes, have rarely been investigated. The lack of such studies might, in part, be attributed to difficulties associated with the distinction of interacting kelp species at gametophyte stage (Chapter 4). However, over the last decades experimenters developed a number of techniques to overcome this methodological problem (Table 5.1). Interactions among kelp gametophytes might comprise physical and chemically mediated effects, as well as competition for resources (Chapter 4, Chapman 2005, Reed 1990). While chemical impacts might be effective solely by sharing the same medium with interacting species (Chapman 2005), direct physical vicinity, as occurring in nature, might be required to observe the full range of interactive effects (Chapter 4). In preparation to Chapter 4, these methods were tested for the differentiation of *U. pinnatifida* and *Macrocystis pyrifera* gametophytes. Here, this chapter provides an overview on different approaches, discusses benefits and shortcomings and includes results of pre-experiments to chapter 4.

5.1 Differentiation among kelp gametophytes

5.1.1 Arising sporophytes

A straightforward approach to the problem of allocating morphologically similar gametophytes to the respective species is the cultivation of specimens until arising sporophytes can be identified by morphological features, e.g. the appearance of a midrib (Reed 1990). This method offers high accuracy of the allocation and integrates sporophyte recruitment, an ecologically relevant measure. However, a major drawback to this technique lies in the merely indirect monitoring of events at gametophyte stage. The surveillance of sporophytes might lead to false conclusions in case multiple scenarios explain the observed condition. As an example, higher numbers of sporophyte recruits might be the result of a facilitation by an interacting species at gametophyte stage, as observed for *U. pinnatifida* and *M. pyrifera* in chapter 4. This scene is likely to be misinterpreted as competition by sole observation of sporophyte recruits. Furthermore, this experimental set-up potentially requires extended observation times until gametophytes develop and the subsequent sporophytes exhibit morphological features for identification. This process takes 10 to 15 weeks for sporophytes of *M. pyrifera* and *Pterygophora californica*, before specimens reach a size of 2 cm and can be distinguished (Reed 1990). For *U. pinnatifida*, the prominent midrib became visible in some specimens two months after the initial spore release (Chapter 2). In essence, using a simple set-up this method provides high accuracy for the identification of species', however, supports only indirect observations of gametophyte interactions.

Table 5.1 Summary of methods used to differentiate morphologically similar gametophytes of different kelps.

Differentiation method	Drawbacks	Reference
Arising sporophytes	Relatively long observation times Indirect observation of events at gametophyte stage	Reed (1990)
Physical separation	Exclusion of physical interaction effects	Chapman (2005) Chapter 4
Fluorescent labelling	Potential impact on gametophyte development Successive seeding of zoospores	Edwards (1999)
Statistical differentiation	Re-evaluation required for new combinations of interacting species' Restriction to specific developmental phases	Chapter 4

5.1.2 Physical separation

Chapman (2005) proposed an approach that allows direct observations of gametophyte development. For this, specimens of different species' are growing together in the same medium but physically separated by a membrane filter. Equivalent to this technique, zoospores of different species may be seeded to several glass slips that are labelled and placed in the same medium (Chapter 4 – experimental design 3). These set-ups assure high accuracy for the allocation of gametophytes and direct monitoring of gametophyte metrics, e.g. length and gamete formation, throughout the experiment. On the other hand, the lack of the physical component of the interaction is likely to result in alterations to the interaction effects, as illustrated in chapter 4 of this thesis. As a consequence, the full range of interactive effects might not be displayed by this method (Chapter 4).

5.1.3 Fluorescent labelling

Fluorescent labelling has been used for investigations on various macroalgal species (Table 5.2) and was introduced as an identification method for microscopic kelp stages by Edwards (1999). The employed fluorescent stain Fungi-Fluor® (Polyscience, Inc.) consists of a solution of Calcofluor white (solution A, 0.05 % Cellufluor) that stains polysaccharides in the cell walls of various organisms. The counterstain (solution B) contains the azo dye Evans Blue, applied to reduce background fluorescence. According to the protocol of Edwards (1999), one-day-old gametophytes are immersed in a solution of Fungi-Fluor® and filtered seawater for 24 h. After this procedure, a second, unlabelled species may be seeded to the same substrate for interaction. Fluorescing blue under UV fluorescence microscopy (transmittance 330 - 385 nm), labelled specimens are readily identifiable and can be distinguished from unlabelled gametophytes. This approach constitutes a powerful tool for the work with kelp gametophytes and was shown to be effective under both laboratory and field conditions (Edwards 1999). Notwithstanding, the proposed labelling procedure takes two days, causing successive seeding of the interacting gametophyte species. The resulting difference in the age of the developing inter-specific gametophytes potentially influences the interactions among species, as was demonstrated by the results of Chapman (2005) and chapter 4 of this thesis.

For the observation of interactions between inter-specific gametophytes of the exact same age, a method allowing to label zoospores of *U. pinnatifida* immediately after release from the sporophyll

Table 5.1 Summary of protocols used with the vital stain Calcofluor-white in order to label living macroalgal stages.

Study	Species investigated	Stain concentration	Staining duration	Stain impact
Cole 1964	Laminariales	0.01% Calcofluor White ST	up to 48 h	delayed gametophyte development
Waaland & Waaland 1975	*Griffithsia pacifica* Kylin *G. tenuis* C. Agardh *G. globulifera* Harvey ex Kützing *Antithamnion kylinii* N. L. Gardner *Callithamnion sp.*	0.01% Calcofluor White ST	24 h 15 - 30 min	cell abnormalities no alteration to development
Chopin & Floc'h 1987	*Chondrus crispus* Stackhouse	0.01 % Cellufluor (Calcofluor White M2R)	1 h	not mentioned
Garbary et al. 1988	*Antithamnion defectum* Kylin	0.01 % Calcofluor White M2R 0.0005 % Calcofluor White M2R 0.0001 % Calcofluor White M2R	30 min 30 min 30 min	reduced growth no reduction in growth observed no reduction in growth observed
Belliveau et al. 1990	*Antithamnion kylinii* N.L. Gardner	0.01% Cellufluor 0.0005% Cellufluor 0.0001% Cellufluor	30 min several days several days	reduced growth stunted, cell abnormalities stunted, cell abnormalities
Edwards 1999	*Macrocystis pyrifera* (Linnaeus) C. Agardh *Pterygophora californica* Ruprecht	0.05 % Fungi-Fluor® (0.01 % Cellufluor)	24 h	not mentioned
Martone 2010	*Calliarthron cheilosporioides* Manza	0.02 % Calcofluor White M2R	5 min	not mentioned

had to be developed. Therefore, the protocol of Edwards (1999) was adapted, testing different combinations of stain concentrations and staining durations, that are summarized in table 5.3. Although stain concentrations equivalent to those used by Edwards (1999) impaired germination of zoospores (Table 5.3, approach 1), lower concentrations allowed gametophyte development, still enabling identification of labelled specimens using fluorescence microscopy (Table 5.3, approach 2 and 3). Under UV, the initial spore fluoresced blue, while newly synthesized tissue, i.e. germination tube and thallus of the gametophyte, remained unstained. This is in accordance with results of Waaland & Waaland (1975) who found the stain to be immobile in the cell wall of red algae. In contrast, Edwards (1999) reported that the stain diluted throughout the gametophyte thallus during growth, but was not incorporated in the emerging sporophytes. Despite this inconsistency, the described adaptions to the protocol of Edwards (1999) allowed to stain newly released zoospores and the developing gametophytes could be identified by the adherent labelled initial spore.

Table 5.3 Summary of approaches to label newly released zoospores of *U. pinnatifida* with the fluorescent stain Fungi-Fluor®. Spore release and culture conditions were used as described in Chapter 4 (section 4.3.1) and gametophyte development was evaluated two days after spore release.

Approach	Timing of staining (after spore release)	Stain concentration (Fungi-Fluor® Solution A)	Staining duration	Impact
1	immediately	20 %	24 h	impaired zoospore germination
2	immediately	1 drop / 18 ml	4 h	impact on length
3	immediately	1 drop / 18 ml	48 h	impact on length

The physiology of experimental organisms is, in many cases, susceptible to the use of chemicals in experimental set-ups. Likewise, the fluorescent stain, in the pre-experiment, impacted the development of kelp gametophytes (Table 5.3). Gametophyte average length (Fig. 5.1) and maximum length (Fig. 5.2) of *U. pinnatifida* were significantly altered compared to control conditions. Consistently, Cole (1964) observed delayed gametophyte development in low concentrations of the brightener and adverse effects of the stain arose for various red algae, e.g. abnormalities in cell shape and reduced growth rates (Waaland & Waaland 1975, Garbary et al. 1988, Belliveau et al. 1990). The occurrence of physiological implications constitutes a major drawback to the use of fluorescent labelling with macroalgae gametophytes. In particular, effects of

experimental forces might be misinterpreted if they are superimposed by staining impacts. Extensive experimentation is required in order to develop protocols that minimize physiological impacts of the stain. Shorter staining durations might reduce the impact on physiology, as indicated by results of Waaland & Waaland (1975) and the pre-experiment (Fig. 5.1, 5.2). Encouraging the use of extremely short staining durations, Martone (2010) revealed that a staining time of 5 min is sufficient to label algae thalli for more than a year. Additionally, the physiological impact and staining success with low concentrations of Calcofluor white should be evaluated. A promising approach is the immersion of mature sporophylls to the stain prior to zoospore release. Given that the spores incorporate the stain through the sporophyll tissue this technique enables synchronous release and settlement of labelled and unlabelled zoospores.

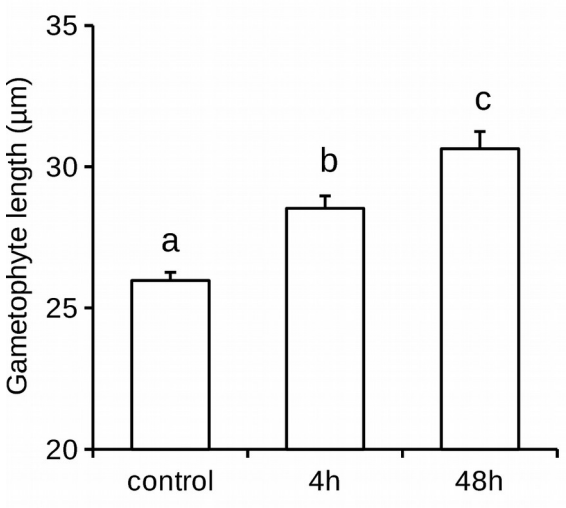

Figure 5.1 Total gametophyte length of *U. pinnatifida* (mean ± SE) after exposure to low concentrations of Fungi-Fluor® staining solution A (one drop added to 18 ml) for 4 and 48 h immediately after spore release. Spore release and culture conditions were used as described in Chapter 4 (section 4.3.1). Gametophyte length of at least 50 haphazardly chosen specimens was determined 48 h after spore release and analysed with the non-parametric Kruskal-Wallis-test ($H = 49.1081$, $p < 0.001$) and subsequent Wilcoxon-Test. Different letters denote significant differences between treatments.

5.1.4 Statistical differentiation

A statistical approach was used to distinguish gametophytes of *U. pinnatifida* and *M. pyrifera* in chapter 4 of this thesis (experimental design 1). This procedure is based on an up front detected valid difference in gametophyte length between the investigated species. By means of this length difference, a non-parametric cluster analysis via kernel density estimations allocated single

gametophytes to the respective species (Chapter 4). A strong benefit of this method is the opportunity to minutely observe events of synchronously developing and interacting inter-specific gametophytes without potential bias by chemicals. Yet, the method holds a number of drawbacks. First, a valid difference in gametophyte length between the regarded species is a prerequisite and needs to be re-evaluated for each combination of investigated species prior to the start of the experiment. Second, the suitability of this technique might be restricted to a certain phase during gametophyte development. Once morphological dissimilarities of male and female intra-specific gametophytes superimpose inter-specific dissimilarities, statistical differentiation might no longer be applicable or needs to be applied separately for each sex. Additionally, compared to earlier introduced methods, the statistical differentiation might provide less accuracy for the allocation, an error rate of 12.4 % was determined for the investigation in chapter 4. In this specific case, non-parametric methods had to be applied. However, the use of parametric methods for normally distributed population data might considerably increase the accuracy for the statistical approach.

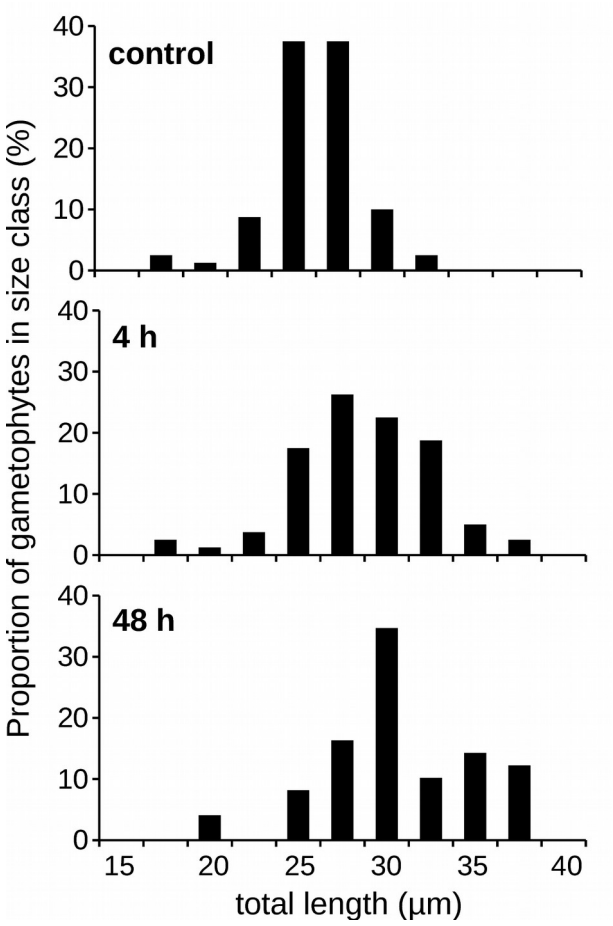

Figure 5.2 Length-frequency distribution of *U. pinnatifida* gametophytes after exposure to low concentrations of Fungi-Fluor® staining solution A (one drop added to 18 ml) for 4 and 48 h immediately following spore release. Spore release and culture conditions were used as described in Chapter 4 (section 4.3.1). Gametophyte length of at least 50 haphazardly chosen specimens was determined 48 h after spore release.

In summary, the presented differentiation methods constitute useful tools for the work with kelp gametophytes, however, each holds significant drawbacks. Therefore, careful selection of the appropriate method is required in order to comply with specific experimental goals. Employing a combination of different approaches, as accomplished in chapter 4 of this thesis, potentially allows for more detailed results. Research efforts should focus on the reduction of handling bias for the differentiation between inter-specific kelp gametophytes in order to encourage studies on ecological interactions.

5.2 Ecological relevance of laboratory studies on kelp gametophytes

Natural environments are by far more complex than laboratory settings and ecological conclusions can only to a limited extent be drawn from laboratory results. For kelp gametophytes, additional uncertainty concerns the comparability of gametophyte morphology in the field and under laboratory conditions. Characteristics of gametohytes developing under laboratory conditions have been described by various authors. In optimal culture conditions, female gametophytes typically reach maturation at a single-cell-stage (Bolton & Levitt 1985, Reed et al. 1991). In contrast, sterile filamentous and multicellular gametophytes arise in unfavourable conditions (Pang & Wu 1996, Nelson 2005) that may become fertile once conditions improve. To what extend these laboratory grown specimens resemble gametophyte in the field is largely unknown. Scare experiments indicate discrepancies concerning growth rates and sporophyte recruitment (Deysher & Dean 1986, Edwards 1999). This implies that findings from laboratory experiments might not replicate the way gametophytes respond in the field.

On the other hand, the possibility to observe isolated effects of environmental variables in controlled laboratory conditions provides valuable insights to physiological tolerances and ecological processes of investigated organisms. These investigations might also serve as a basis for the development of conservation techniques, as demonstrated by the successful eradication of *U. pinnatifida* from a sunken trawler off the Chatham Islands, New Zealand (Wotton et al. 2004). Based on the results of laboratory studies, an effective heat-treatment has been developed, dedicated to eradicate the kelp's highly resistant gametophytes (Webb & Allen 2001, Wotton et al. 2004). Thus, even though results from laboratory studies might deviate from the same investigations under field conditions, these observations confine boundaries for possible scenarios and foster our understanding of microscopic kelp stages.

5.3 Conclusion

Overall, progress in understanding the dynamics of kelp populations depends on better knowledge of microscopic stages (Schiel & Foster 2006) and their role within kelp life history. Laboratory studies on kelp gametophytes already substantially contributed to explain physiological aspects and, ultimately, to unravel the 'black box' of microscopic life stages in kelps. In order to facilitate the investigations of ecological questions, the presented studies worked towards a straightforward differentiation between microscopic stages of different kelps. However, with regard to the above-mentioned concerns, research efforts should aim for the minimization of handling bias. At the same time, validation to laboratory-drawn conclusions with adequate field data should be initiated. Especially, techniques allowing in-situ observations of microscopic gametophytes hold the potential to revolutionize kelp research. Investigations of ecological aspects of kelp gametophytes, like inter-specific interactions, provide valuable insight to a largely overlooked scene and foster a comprehensive understanding of the entire life cycle of kelp.

5.4 References

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Chapter 6: Synoptic discussion

The kelp *Undaria pinnatifida* is continuously increasing its invaded range (ICES 2007), potentially threatening native biodiversity and ecosystem functioning at concerned coastlines (Hay & Villouta 1993, Battershill et al. 1998, Curiel et al. 1998, Piriz et al. 2003, Wotton et al. 2004). The experimental outcome of this thesis displays the significant invasive potential of *U. pinnatifida*, uncovering both ecological characteristics and physiological mechanisms that may contribute to its invasion success (Table 6.1). In this chapter, results of chapter 2 to 4 as well as relevant literature are synthesized with respect to two fundamental aspects of the kelp's invasion. The first section (6.1) revisits the question raised at the beginning of this thesis: “Which characteristics of the kelp *U. pinnatifida* facilitate its invasion into new habitats?”. According to the invasion scheme of Richardson et al. (2000; see chapter 1; Fig. 1.1), potential invasion pathways of *U. pinnatifida* will be trailed, highlighting traits that facilitate its invasion to previously unconcerned habitats at different phases of the process. En route, aspects of the initially conceived hypotheses I and III (Chapter 1) will be encountered and discussed. Hypotheses II and IV will be reviewed in section 6.2 that discusses the complex interplay of human-induced changes with the invasion success of *U. pinnatifida* in the light of future distribution patterns. Considerations of ecological implications (6.3), perspectives on future research foci (6.4) and a synoptic conclusion of this thesis (6.5) are disclosed at the end of this chapter.

6.1 Resolving barriers – life cycle considerations

The likelihood of a non-native macroalga overcoming abiotic and biotic barriers, as well as its necessary physiological abilities vary throughout the invasion process (Nyberg & Wallentinus 2005). Failure to overcome any one barrier may result in a species' failure to become invasive (Blackburn et al. 2011). In kelps, sporophytes and gametophytes do not only differ in size, but also exhibit fundamental physiological differences, e.g. with respect to ploidy and thallus organisation. Consequently, physiological traits and ecological functions specific to each life history stage of *U. pinnatifida* might contribute to tackle the different invasion barriers. This emphasizes the necessity to consider the entire life cycle with regard to the kelp's invasion.

6.1.1 Transport vectors

The availability of a transport vector and the presence of a suitable life stage that is able to travel by this vector determine the spread and dispersal of macroalgal invasions (Schaffelke et al. 2006, Flagella et al. 2007), i.e. the success in overcoming barrier A and D (Fig. 1.1). For intercontinental and long-distance dispersal, the possession of microscopic stages is considered to be advantageous (Nyberg & Wallentinus 2005) and in *U. pinnatifida* seem to largely depend on its gametophyte stage (Hay & Luckens 1987, Aguilar-Rosas et al. 2004, ICES 2007). Microscopic gametophytes display extensive resistance to darkness (tom Dieck 1993) allowing *U. pinnatifida* to travel long geographic distances within ballast water tanks of international shippings. This mechanism is thought to be the vector for the kelp's introduction to New Zealand in 1987 (Hay & Luckens 1987). In unfavourable conditions, gametophytes may form thick-walled resting stages (Saito 1975), even making transport with sediment ballast possible (ICES 2007).

Many marine vectors involve mobile artificial structures (Mineur et al. 2012). The distinctive trait of *U. pinnatifida* to foul a variety of these substrates, e.g. buoys or mollusc shells, fosters the unintentional transport into new biogeographical provinces (Hay 1990, Parsons 1995, Fletcher & Farrel 1999, Wotton et al. 2004, James & Shears 2016). This pathway is most effective over short distances, allowing for potential transport of both sporophytes and gametophytes of *U. pinnatifida* (Hay 1990, Forrest et al. 2000). In particular, its arrival in previously unconcerned ports by fouling hulls of recreational boats has been acknowledged (Hay 1990, Farrell & Fletcher 2004). In a similar manner, the invader might be transported overland, e.g. attached to trailered boats or fouling aquaculture equipment (Chapter 2). The ability to travel by this vector depends on tolerance to desiccation and the ability to take refuge in emerged conditions. As an example, by means of their microscopic size gametophytes of *U. pinnatifida* survived air exposure in small humid crevices in boat hulls or anchor wells (Hay 1990). In direct air exposure, the specific folded morphology of *U. pinnatifida* sporophylls constitutes a self-protecting mechanism that shields proximal parts and the contained zoospores from desiccation (Chapter 2). Simultaneous release of zoospores is triggered by re-immersion of the sporophyll into seawater. As a result, overland transport via desiccated sporophylls provides especially high chances for suitable settling densities that enable gamete fusion and subsequent sporophyte recruitment. The possession of a winged sporophyll is unique to *U. pinnatifida* in its genus (Morita et al. 2003, Uwai et al. 2007, Hwang et al. 2012) and may, in part, contribute to its invasion success. These morphological differences between life stages of *U. pinnatifida* and life stage specific desiccation tolerances, demonstrated by the experimental results

of chapter 2, result in distinct potentials for overland transport and confirm the initially conceived hypothesis I: ***'Various life stages of* U. pinnatifida *display specific tolerances to desiccation, and thus, display distinct potential for transport in emerged conditions'*.**

Table 6.1 Summary of experimental results linked to life stage specific traits of *U. pinnatifida* that might contribute to overcoming abiotic or biotic barriers throughout the invasion process. Labelling of the invasion barriers (A – F) refers to the definitions by Richardson et al. (2000).

	Barrier	Traits of *Undaria pinnatifida*	Indication for invasion	Thesis chapter
Sporophyte	*B*	Tolerance to various temperature regimes	Potential to invade extended geographic areas	Chapter 3
	B	Tolerance to various salinity regimes	Potential to invade low salinity regions e.g. estuaries or the Baltic Sea	Chapter 3
	A/D	Desiccation tolerance of sporophylls	Potential for overland transport, e.g. fouling trailered boats	Chapter 2
	E/F	Superior tolerance to elevated temperatures compared with native kelps	Potential for higher adaptability and competitive strength in warming climatic conditions	Chapter 3
Gametophyte	*B*	Indiscriminate settling on various rope materials	Extended availability of suitable substrate for settlement	Chapter 2
	A/D	Tolerance to desiccation	Potential for overland transport, e.g. fouling trailered boats	Chapter 2
	E/F	Facilitation by inter-specific kelp gametophytes	Earlier sporophyte recruitment, potential for local dominance	Chapter 4

6.1.2 The physical environment

Following the arrival in a new habitat, survival and establishment of a non-native species depend on its tolerance to the local physical environment, i.e. barrier B (Fig. 1.1; Richardson et al. 2000, Levine 2008). Thus, similarities between native and recipient habitat are considered favourable for

invasion success (Boudouresque & Verlaque 2002). Physiological tolerance of an organism determines the potential niche, i.e. the range of suitable habitat conditions, and hence, the likelihood of invasion success (Dukes & Mooney 1999, Levine 2008). While only one life stage capable of using a vector might be sufficient for transport, all life stages of a species need, at least temporarily, to be tolerant to local abiotic conditions in order to complete its life cycle and flourish in new habitats. Gametophytes of *U. pinnatifida* are known for their exceptional tolerance to various environmental conditions, e.g. temperature, salinity, darkness and pH (tom Dieck 1993, Forrest & Blakemore 2006, Henkel & Hofmann 2008, Peteiro & Sánchez 2012, Leal et al. 2017). While the gametophyte alone might be able to inhabit a by far more extended area, the potential range of *U. pinnatifida* is restricted by the physiological abilities of its sporophyte. However, as gametophytes of *U. pinnatifida* are capable of resting in unfavourable conditions (Saito 1975), the kelp may inhabit regions that are seasonally outside the sporophyte's tolerance limits. As a result, an annual phenology is displayed in some areas, with seasonal absence of the sporophyte generation (Saito 1975, Hay & Villouta 1993, Castric-Fey et al. 1999, James et al. 2015).

Considered a major driver of biogeography, temperature was used to predict the potential global range of *U. pinnatifida* (James et al. 2015). While this investigation provides the first impressions on the potential of the invasive kelp relative to temperature, also other environmental factors will ultimately determine the actual extent of future distributions, e.g. salinity regime and wave exposure (Lee & Bell 1999, Kumar et al. 2014). Additionally, synergistic effects among these factors will influence specific tolerances (Chapter 3, Alexieva et al. 2003). With regard to a combination of temperature and salinity conditions, chapter 3 of this thesis demonstrates that sporophytes of *U. pinnatifida* exhibit considerable tolerance to single abiotic factors and low susceptibility to adverse synergistic effects, at least for short time periods. The experimental results from this study found its highly resistant antioxidant pool, that protects the kelp from cellular damage (Burritt et al. 2002), to be a promoter of the observed tolerance. While some authors contest the ability of *U. pinnatifida* to establish in low salinity regions (Minchin & Nunn 2014), results of this study and additional studies display its tolerance to various salinities (Chapter 3, Bite 2001, Peteiro & Sánchez 2012). This research suggests that the kelp might invade low salinity habitats. The occurrence of *U. pinnatifida* in lagoons (Peréz et al. 1981, Curiel et al. 1998), a habitat with known salinity fluctuations, further substantiates this claim. Consequently, *U. pinnatifida* was predicted to expand its range into less saline regimes of already concerned areas and elsewhere (Chapter 3).

A critical moment in the life history of kelps, and thus for the invasion success, is the availability of suitable substrate for settlement (Dayton 1985). Due to the strong link of invasion vectors and human activities, *U. pinnatifida* most often arrives in anthropogenically altered environments (Hay & Luckens 1987, Farrell & Fletcher 2006). These environments are increasingly dominated by artificial substrates that are considered to be 'stepping stones' or 'corridors' for marine invasions (Mineur et al. 2012). Advantageous effects of these substrates on marine invasions are attributed to the commonly sparse occupation by native species and the provision of settlement features in areas lacking natural bedrock (Floc'h et al. 1996, Ruiz et al. 2009). Suggesting a high tolerance to settlement ground for *U. pinnatifida*, the kelp occurs on a variety of artificial substrates (Parsons 1995, ICES 2007) and settled indiscriminately on different rope materials (Chapter 2) as well as other materials (e.g. concrete, steel, fibreglass, basalt; personal observation). From artificial structures, *U. pinnatifida* is thought to migrate to adjacent natural habitats, as observed along the French Atlantic coast (Floc'h et al. 1996). Therefore, its tolerance to settlement ground might foster both the establishment and persistence of *U. pinnatifida* in invaded habitats (Nyberg & Wallentinus 2005).

6.1.3 Interplay with native biota

Biological conditions, such as native biodiversity, and interactions with native species might impact the success of an invader (Schaffelke et al. 2006). Competition with native canopy-forming species is considered to impede establishment of *U. pinnatifida* in some regions (Floc'h et al. 1991). In the same way, the typical absence of potential native competitors might explain the frequent occurrence of *U. pinnatifida* on artificial substrates (Floc'h et al. 1996). Disturbance of local communities through storm or grazing events may further facilitate the spread of the kelp (Valentine & Johnson 2003, 2004, Johnson et al. 2004). By impacting native species, grazers may cause an indirect positive effect on invaders. For example, heavy grazing of sea-urchins in Tasmania accounted for the formation of barren areas and prevented native recruitment, which subsequently promoted an increase of *U. pinnatifida* densities (Johnson et al. 2004). Converse effects arise on the condition that *U. pinnatifida* is itself impacted by grazing activities. In California, grazing of the native kelp crab *Pugettia producta* on sporophytes prevented individuals of *U. pinnatifida* from reaching reproductive maturity and in turn adversely affected its establishment (Thornber et al. 2004). These examples indicate that the effect of native biota on the invasion success of *U. pinnatifida* is regionally and species-dependent.

Positive interactions among species, e.g. symbiosis or facilitation, typically receive far less attention than the adverse effects of competition and grazing (Bertness & Leonard 1997). However, these interactions might come with consequences for the success of an invader. In example, during the seasonal absence of *U. pinnatifida* sporophytes in New Zealand the persistence of the species was found to be facilitated by red turf algae harbouring its gametophytes (Thompson & Schiel 2012). While this conclusion was drawn indirectly from surveillance of arising sporophytes, chapter 4 of this thesis includes direct observations of interactions among native and invasive kelp gametophytes in a laboratory experiment. Here, a facilitation of *U. pinnatifida* gametophytes arose from the presence of native kelp gametophytes. This positive effect on the performance of *U. pinnatifida* gametophytes might ultimately result in local dominance and foster the spread of the invasive kelp. Illustrating the impact of interactions at gametophyte stage on successive sporophyte generations, the experimental results of chapter 4 emphasize the importance to consider all life stages of kelps with regard to species' interactions and confirm hypothesis III: ***'Interactions between the invasive* U. pinnatifida *and native kelp species occur at gametophyte stage'***.

6.2 Effects of human development and climate change

Earth has been substantially altered by humans (Vitousek et al. 1997). More than half of the global population inhabits areas within 100 km of a shoreline (Small & Nicholls 2003), thereby continuously increasing pressure on coastal ecosystems and associated biota (Kjerfve 1994). Anthropogenic activities transform concerned environments, alter global biogeochemistry and species communities, ultimately resulting in changing climatic conditions and loss of biological diversity (Vitousek et al. 1997). Furthermore, invasions of marine species into new habitats are directly fostered by human development, e.g. strengthening of vectors, or are accelerated by feedback-effects of human pressure on ecosystems (Fig. 6.1; Boudouresque & Verlaque 2010, Cook et al. 2013). There is an ongoing debate to what extent invasive species benefit from the observed changes (Mac Dougall & Turkington 2005).

One major component of change is the continuous growth of human infrastructure. Intensification of maritime traffic and increasing numbers of shipping routes constantly enhance the availability of transport vectors for marine invasions and the accessibility to previously remote environments (Boudouresque & Verlaque 2010). At the same time, these anthropogenic activities continuously

increase the presence of artificial substrates in the marine realm. As *U. pinnatifida* primarily travels with vessels or aquaculture species (ICES 2007), there is a high chance to arrive in harbour or marina environments that provide suitable artificial hard substrata for settlement, e.g. harbour walls and piers. The coupling of increasing vector activity and increasing availability of artificial structures accelerate species' invasions, like that of *U. pinnatifida* (Mineur et al. 2012). As long as humans continue to intensify these activities in the marine realm, the spread of *U. pinnatifida* is predicted to increase in parallel.

Changing climatic conditions are considered to impact invasions at every stage of the process (Cook et al. 2013). This feedback mechanism among two simultaneous human-induced processes was not acknowledged in the original representation of Fig. 6.1 (Vitousek et al. 1997). Yet, the scheme was updated, highlighting the acceleration of species' invasions by climate change (Fig 6.1, grey arrows) that has been discussed by subsequent studies (Hellmann et al. 2008, Rahel & Olden 2008, Boudouresque & Verlaque 2010) and in chapter 3 of this thesis with regard to the propagation of *U. pinnatifida* in New Zealand. The projected increase in sea-surface temperature (SST; IPCC 2014) might alter potential distributions, i.e. physical niches, of invasive intertidal species', especially at distribution boundaries (Sutherst 2000). This mechanism can be observed in force with short time scales for the invasion of *U. pinnatifida*. Satellite-derived SST data (http://www.esrl.noaa.gov/psd/) indicate that the range suitable for *U. pinnatifida* shifted between the period 1980 – 1990 and 2006 – 2016 (Fig. 6.2). Due to an increase in average yearly maximum SST over the last two decades its potential range in the North Atlantic is expanding northwards, reaching as far as the southern coastline of Iceland in recent years (Fig. 6.2A). Along the British coastline, an acceleration of the northward spread is expected, as zoospore germination is considered to be more effective in warmer waters (Cook et al. 2013). Consequently, assessments for the presence of the kelp are initiated at its current distribution limits (e.g. northern European countries; Minchin & Nunn 2014, Cook et al. 2015). Naturally occurring marginal spread and its enhancement by global warming are, however, often superimposed and a clear distinction between the processes might be difficult or infeasible (Boudouresque & Verlaque 2010). In contrast, Watanabe et al. (2014) suspects the southernmost native populations in Japan to be threatened by rising seawater temperatures. Fig. 6.2B shows a regression of the potential distribution border along the southern Japanese coastline due to the increase of minimum SST over the last decades. The southernmost populations in Kagoshima Bay, Japan, that were investigated by Watanabe et al. (2014) are outside the defined suitable temperature requirements for both considered periods (Fig. 6.2B), indicating that a more complex set of properties determines persistence of these edge

populations. However, provided that temperatures will continue to increase, these local populations may face extinction (Watanabe et al. 2014). Similar trends can be observed for the potential range of *U. pinnatifida* in some invaded regions, e.g. a southward shift of the potential range north of New Zealand (Fig. 6.2C). If this trend wears on, New Zealand's northernmost populations in Rangaunu Harbour, that were described by James et al. (2014), might retreat under future temperature conditions.

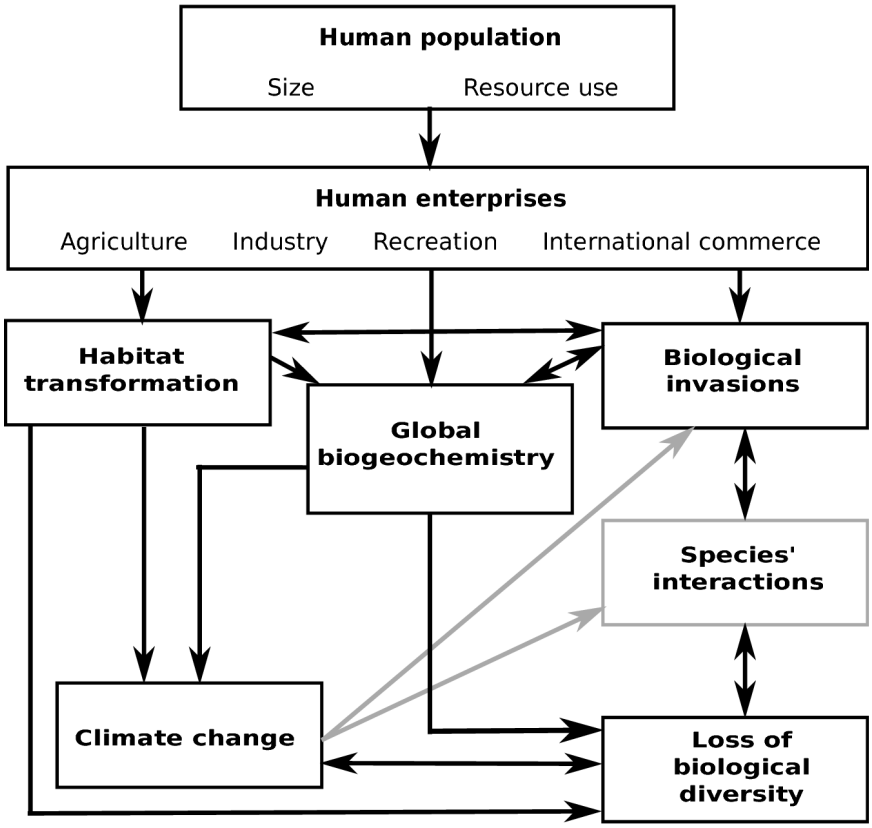

Figure 6.1 A conceptual model illustrating direct and indirect anthropogenic effects and on Earth's ecosystems, including the interplay with biological invasions (modified from Vitousek et al. 1997). In order to highlight the impact of climatic changes on biological invasions, as well as the combined effect of these components on interactions among species' in concerned communities, the original representation by Vitousek et al. (1997) was updated. Black colour indicates components adopted from Vitousek et al. (1997), added compartments and arrows are displayed in grey.

Among the projected changes to world's climate, alterations to freshwater inflow are expected in some locations, caused by increasing frequency and number of rainfall events (Fowler & Hennessy 1995). As a consequence, seawater salinity close to river inlets might experience periodical

fluctuations, especially in semi-enclosed environments like Tauranga Harbour, New Zealand. In distinct areas of Tauranga Harbour salinities as low as 0 – 5 S_A have already been reported (Pritchard et al. 2009) and are predicted to occur more frequently in future, potentially impacting local alga species (Ogata & Takada 1968, Bjærke & Rueness 2004, Kakinuma et al. 2006, Karsten 2007, Luo & Liu 2011). However, due to its salinity tolerance (Chapter 3, Bite 2001, Peteiro & Sánchez 2012), *U. pinnatifida* is unlikely to be negatively affected by these events and might benefit if native species are adversly impacted.

A shift in weather conditions to more intense storms is projected for future climate conditions (Collins et al. 2013) which may be beneficial for the expansion of *U. pinnatifida* by way of two mechanisms. First, the dislodgement of fertile *U. pinnatifida* sporophytes that carry viable zoospores could contribute to natural marginal spread along the shoreline (Minchin & Nunn 2014). Second, increased storm intensities might disturb native communities, resulting in cleared intertidal space suitable for settlement by the invader. As previously stated, *U. pinnatifida* has been shown to profit from similar disturbances (Valentine & Johnson 2003, 2004, Johnson et al. 2004). A cleared space could set the scene for interactions among propagules, like those observed in chapter 4. Given that early life history stages of a native kelp facilitate *U. pinnatifida*, the invader is likely to dominate the impacted area. Consequently, increased availability of cleared intertidal space, as a result of intensifying storm events, might contribute to local persistence of the invasive kelp.

These predicted changes to Earth's climate and ecosystems will also impact native species and their interactions with invaders (Dukes & Mooney 1999, Occhipinti-Ambrogi 2007, Boudouresque & Verlaque 2010). The isolated effect of *U. pinnatifida* on natural communities and connected interactions are not yet fully resolved (see section 6.1). Thus, evaluating the ways changing climatic conditions influence these dynamic interactions is difficult, to say the least. In general, invasive species are believed to be more tolerant to changing abiotic factors than natives (Zerebecki & Sorte 2011). Supporting this statement and hypothesis II: ***'U. pinnatifida is more resistant to changing abiotic conditions than native kelps'***, *U. pinnatifida* demonstrated superior tolerance regarding temperature and salinity regimes compared to New Zealand's indigenous kelp species *Ecklonia radiata* and *Lessonia variegata* in chapter 3 of this thesis. On this account, less tolerant natives are suggested to be disproportionally negatively affected by climatic alterations (Sorte et al. 2010, Zerebecki & Sorte 2011). In a competitive situation, impaired performance of natives might ultimately lead to local dominance of the invader (Cook et al. 2013). Potentially signalling this competitive displacement already in action, *U. pinnatifida* was present on

reef habitat in Tauranga Harbour, New Zealand, that would otherwise normally support the native kelp *E. radiata* (Chapter 3). This indicates an indirect positive effect for the invader due to locally rising temperatures. In the event that temperatures continue to increase, some native species might fail to adapt (Occhipinti-Ambrogi 2007), indirectly strengthening the advantage for more tolerant invaders.

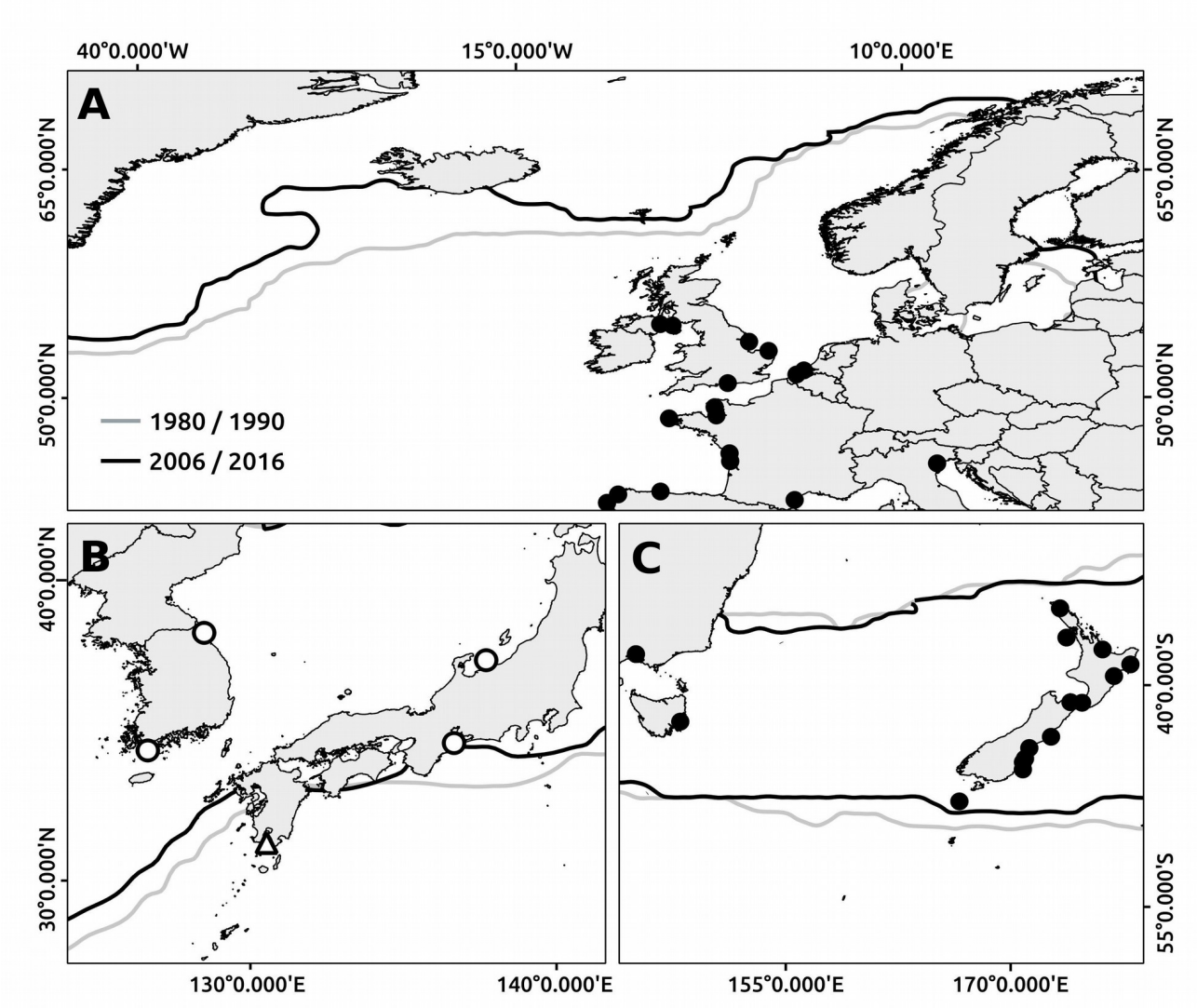

Figure 6.2 Potential distribution of *U. pinnatifida* (A) in the North Atlantic, (B) along the coast of Japan and (C) New Zealand calculated according to James et al. (2015) for two time periods (1980 – 1990 and 2006 – 2016). White circles indicate native distributions, the white triangle displays the southernmost native population and black circles mark invasive populations. Compiled distribution data was adopted from James et al. (2015), the location of the southernmost population refers to Watanabe et al. (2014). Sea-surface temperature (SST) data was derived from http://www.esrl.noaa.gov/psd//.

To conclude with the impact of human-induced changes on the invasion of *U. pinnatifida*, increasing human activities in the marine realm by way of coastal development and maritime traffic, are considered to facilitate dispersal and establishment of the kelp. Additionally, in support of the initially posed hypothesis IV: ***'The projected climate change will foster the spread of* U. pinnatifida'**, most aspects of the projected climate change are expected to foster the kelp's invasive success. *U. pinnatifida* might directly benefit from the projected changes, e.g. by an increase of its potential range, or indirectly, given that the competing native species are adversely affected.

6.3 Ecological implications

In the same way as invasion success is impacted by local biota, structure and functioning of ecosystems and performance of native species can be altered by the incoming organism (Dukes & Mooney 1999, Levin et al. 2002, Bax et al. 2003, Schaffelke & Hewitt 2007). The resulting ecological impact of an invasive macroalga is considered to be correlated with thallus size (Nyberg & Wallentinus 2005), and thus, seems to be primarily determined by the sporophyte stage in kelps. Consistently, an increase in local diversity was linked to the establishment of *U. pinnatifida* in some regions, due to the provision of structurally diverse habitat by its sporophyte (Stuart 2004, Irigoyen et al. 2011). The ability to use zones for settlement that are infrequently colonized by native habitat-forming species, e.g. artificial substrates, might increase the overall habitat available to associated organisms. This mechanism is thought to generate additional shelter and habitat in elevated intertidal habitats that are periodically exposed to air conditions (Fig. 6.3; Curiel et al. 1998).

On the other hand, numerous studies have emphasized the adverse effects of *U. pinnatifida* on invaded communities. In comparison to native canopy-forming species, the habitat provided by *U. pinnatifida* might be structurally dissimilar and, in some areas, seasonally absent. In Argentina, loss of transitory habitat for reef fish is expected to be a consequence of the introduction of *U. pinnatifida* (Irigoyen et al. 2011). As a result, the establishment of *U. pinnatifida* induced various changes to native species composition (Battershill et al. 1998, Arnold et al. 2016) reducing the density and diversity of local communities (Hay & Villouta 1993, Piriz et al. 2003, Casas et al. 2004). In particular, decreasing densities of the giant kelp *Macrocystis pyrifera* have been recognized in response to the establishment of *U. pinnatifida* (Piriz et al. 2003). This replacement

of a large, habitat-forming species by the structurally less complex *U. pinnatifida* is expected to cause massive reductions to the habitat availiable to a vast range of associated organisms.

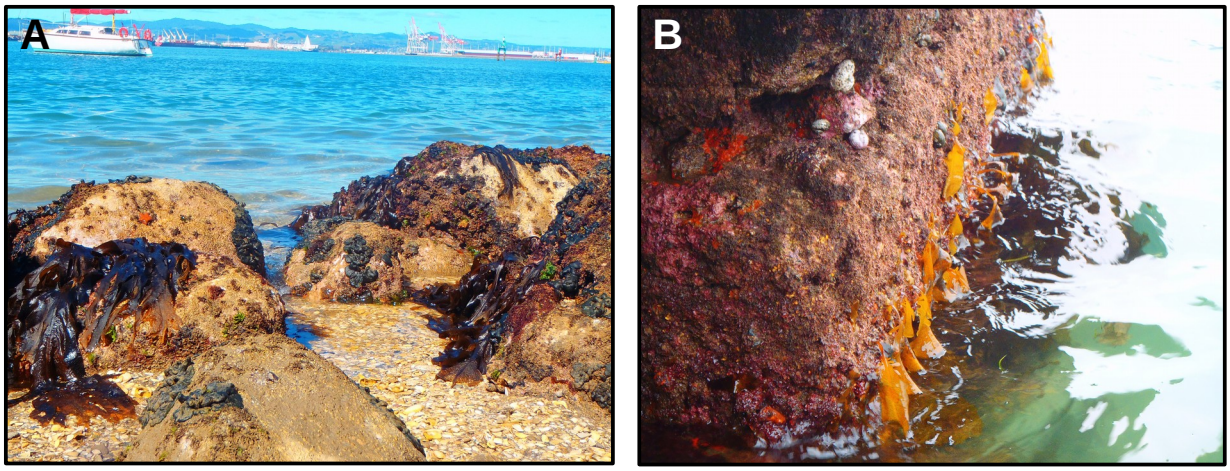

Figure 6.3 (A) Mature and (B) juvenile sporophytes of *U. pinnatifida* growing exposed to air conditions in Tauranga Harbour, New Zealand.

Species' invasions on a global scale, like the spread of *U. pinnatifida*, seriously threaten global diversity by biotic homogenization, a process of communities becoming more similar (McKinney & Lockwood 1999). Although homogenization does not necessarily go along with low local biodiversity (Davis 2003), replacement of unique endemic species with already widespread ones will ultimately reduce spatial diversity (McKinney & Lockwood 1999). With regard to its cosmopolitan distribution, flourishing on all continents except Africa and Antarctica (ICES 2007), and its continuously increasing invaded range, *U. pinnatifida* already is and will continue to be a major driver of biotic homogenization in rocky intertidal habitats (Miller 2004, Boudouresque et al. 2011).

6.4 Future perspectives

U. pinnatifida is expected to further expand its invasive range in the coming decades. The potential deleterious impact of invasive species and the acceleration of the combined effects of anthropogenic development and climate change inevitably necessitate strong management strategies. Prevention and eradication methods will only be effective at an early stage of the

invasion process (Hobbs & Humphries 1995, Bax et al. 2003, Acosta et al. 2010). Identification of high-risk areas for introductions and comprehensive knowledge of available transport vectors are powerful tools for prevention and the early detection of invasions and should be in the focus of future research activities.

Continuous monitoring along distribution edges and high-risk areas, as conducted in the UK (Cook et al. 2015), will facilitate eradication attempts in newly invaded environments. However, such monitoring activities are often limited by financial and personnel constraints (Delaney et al. 2008). At the same time, the value and quality of data collected by volunteers, so-called citizen scientists, is increasingly recognized (Silvertown 2009, Crall et al. 2011). For this, *U. pinnatifida* seems to be a suitable organism: The macroscopic sporophyte has a readily recognizable shape and its preference to foul floating structures in harbour areas allow facile observations from land. Efforts should be made to involve public resources: An active dialogue between research and citizen scientists needs to be initiated, providing means of education for volunteers via online platforms or public lectures.

Knowledge of habitat requirements helps to concentrate monitoring efforts on suitable locations. James et al. (2015) compiled available data on temperature limits of *U. pinnatifida* and detected strips of uninvaded coastline that are suitable to harbour the kelp. The potential range of *U. pinnatifida* could be further defined if additional abiotic factors such as salinity or depth requirements were incorporated. Studies investigating tolerance to single abiotic factors, like chapter 3 of this thesis, provide valuable information for these predictions of its global distribution and should be encouraged. Alterations to Earth's climate will also have implications to the future distribution of *U. pinnatifida*. Modelling approaches integrating these changes are critical in order to correctly project future distribution patterns.

In order to evaluate the ecological impact of *U. pinnatifida*, knowledge is required on both the degree and direction of interactions with local communities and alterations to habitat conditions. As the impact of *U. pinnatifida* seems to be regionally dependent, field observations from various locations will be required to integrate the full spectrum of potential interactions. Assessment of interactions at all life stages of the kelp will provide a comprehensive understanding of the complex biological relationships. In order to understand in which way climate change influences these interactions, scenarios should be tested at different temperature regimes, e.g. using mesocosm experiments.

Major research efforts should be concerned with effective prevention and eradication methods. Studies investigating possibilities to reduce fouling of *U. pinnatifida*, e.g. testing colours of aquaculture rope and studies evaluating effectiveness of different prevention procedures, like Forrest & Blakemore (2006), provide essential information for the establishment of guidelines in order to reduce the invasion risk and should be encouraged. The strong link between invasion vectors and maritime activities advocates for cooperation with the concerned industries with regard to the implementation of management measures.

6.5 Synoptic conclusion

This thesis displays the comprehensive set of mechanims and diverse physiological characteristics of *U. pinnatifida* that facilitate its spread into previously unconcerned areas and documents the minute interplay of different life stages tackling barriers encountered during the invasion process. In particular, the research in this thesis assessed and discussed potential transport vectors, physiological capabilities, interactions with native kelps, as well as the impact of changing climatic conditions with respect to the invasion of *U. pinnatifida* on the basis of four hypotheses conceived at the beginning of the work (Chapter 1).

First, in support to hypothesis I, experimental results of this thesis revealed life stage specific tolerances to desiccation for the invasive kelp. Morphological features of the sporophylls accounted for especially high resistance to emerged conditions. Based on this capability to survive extended periods of air exposure, overland transport was proposed as an effective short-distance vector.

Second, by means of a multivariate laboratory experiment involving extensive physiological and biochemical analyses, the exceptional tolerance of *U. pinnatifida* to various temperatures and salinities was illustrated. This tolerance might facilitate the kelp's transport to new habitats and support successful establishment. Confirming hypothesis II, the invader outperformed native New Zealand kelps, especially with regard to elevated temperatures.

Moreover, the employment of a statistical approach enabled minute observations of simultaneously developing inter-specific gametophytes and demonstrated, in accordance to hypothesis III, that species' interactions also occur at microscopic stages. The regarded interaction with the giant kelp *Macrocystis pyrifera* resulted in a facilitation of the performance of *U. pinnatifida* gametophytes.

In a final step, in support to hypothesis IV, most aspects of the projected climate change are expected to foster the invasive success of *U. pinnatifida*. Its physiological tolerance is expected to enable the kelp to adapt to increasing seawater temperatures in the course of global warming. The

kelp might directly benefit from the projected changes, e.g. by an increase of its potential range, or indirectly, given that competing species are adversely affected by increasing water temperatures. Particularly in regions where native kelps are concerned, *U. pinnatifida* could become locally dominant. In addition to this, the constantly increasing availability of artificial substrates and transport vectors, provided by human development and growing maritime traffic, are considered to further foster the spread of *U. pinnatifida*.

Overall, with its extraordinary physiological tolerances and effective invasion mechanisms, *U. pinnatifida* is capable of spanning an enormous geographic range in coming decades. This invasion success is essentially promoted by the complex interplay of different life history stages. Considered a driver of biotic homogenization and loss to biodiversity, the invasive kelp is expected to cause severe ecological problems. The global scale of this invasion, as well as its acceleration by man's agency make the development of effective prevention measures and eradication techniques of utmost importance.

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List of abbreviations

∑VAZ	xanthophyll cycle pigments (µg µg^{-1} Chl *a*)
1n	haploid
2n	diploid
ANOVA	Analyses of variance
CA	cellulose acetate
Chl	chlorophyll
DAD detector	Diode array detector
DFG	German Research Foundation
DNA	deoxyribonucleic acid
DPPH	2,2-diphenyl-1-picrylhydrasyl
DW	dry weight (kg)
ETR	relative electron transport rate (µmol e$^-$ m^{-2} s^{-1})
ETR_{max}	maximum electron transport rate (µmol e$^-$ m^{-2} s^{-1})
FAO	Food and Agriculture Organization
Fv/Fm	photosynthetic quantum yield
FW	fresh weight (kg)
HPLC	high-performance liquid chromatography
HSD	Tukey's honest significant difference
ICES	International Council for the Exploration of the Sea
IFREMER	French Research Institute for Exploitation of the Sea
IPCC	Intergovernmental Panel on Climate Change
IQR	interquartile range
ISSG	Invasive Species Specialist Group
IUCN	International Union for Conservation of Nature
max	maximum length
n	number of individuals / samples assessed
NA	not detected / not available
NIS	non-indigenous species
NIWA	National Institure of Water and Atmospheric Research, Ltd (New Zealand)
NOAA	National Oceanic and Atmospheric Administration
PAM	pulse amplitude modulation
PAR	photosynthetic active radiation (400-700 nm; µmol photons m^{-2} s^{-1})
PE	polyethylene

PE curve	photosynthesis versus irradiance curve
PES	Provasoli enriched seawater
PP	polypropylene
PSII	photosystem II
RH	relative air humidity (%)
ROS	reactive oxygen species
S	salinity (S_A)
S_A	absolute salinity
SD	standard deviation
SE	standard error
Sp	species
SSC	Species Survival Commission
SST	sea-surface temperature (°C)
T	temperature (°C)
TE	Trolox equivalents
UNESCO	United Nations Educational, Scientific and Cultural Organization
*x*f	*x*-factorial analysis

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