Fragment dispersal and its role for the successful spread of native and invasive alien aquatic plants

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Nonagon Infinity opens the door …

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Summary

Aquatic plant invasions pose a major threat to the biodiversity and functionality of freshwater ecosystems and harm human well-being and the economy. Most invasive alien aquatic plants predominantly reproduce through vegetative means in their introduced range, with unspecialized plant fragments being considered as the most important propagules. However, there is still a lack of knowledge about the species-specific dispersal capacity by plant fragments and the underlying dynamics in streams. According to the new EU Regulation 1143/2014 on the prevention and management of the introduction and spread of invasive alien species, information on a species' reproduction and its spread patterns is mandatory. Thus, in order to comply with the EU Regulation, laboratory and field studies in lowland streams were conducted to assess the fragment dispersal capacity of native and invasive alien aquatic plants based on four key traits, comprising (i) fragmentation rate, (ii) drift distance, (iii) desiccation resistance relevant for overland dispersal to isolated waters and (iv) the regeneration and colonization potential of fragments. The findings of this thesis emphasize that fragment dispersal capacity is a major driving force behind the successful and rapid spread of many aquatic plant invaders worldwide. While the dispersal and invasion success of submerged species such as *Myriophyllum spicatum*, *Potamogeton crispus*, *Elodea canadensis*, *Elodea nuttallii* and *Hydrilla verticillata* can largely be attributed to a high fragment dispersal capacity, fragment dispersal seems to play only a minor role for the invasiveness of *Lagarosiphon major* and *Myriophyllum heterophyllum*. However, it was documented that fragment dispersal is strongly controlled by the hydrological and hydraulic stream properties and generally enhanced in streams characterized by high discharge and turbulent flow conditions. The spread of invasive alien aquatic plants therefore deserves particular attention in larger streams, as long as the degree of flow disturbance does not prevent the establishment and persistence of aquatic plants.

Zusammenfassung

Invasionen durch gebietsfremde Wasserpflanzen stellen eine Hauptbedrohung für die biologische Artenvielfalt und Funktionalität von Süßwasserökosystemen dar und schaden dem Wohlbefinden des Menschen sowie der Wirtschaft. Die meisten invasiven gebietsfremden Wasserpflanzen vermehren sich in ihren nicht-heimischen Verbreitungsgebieten überwiegend vegetativ, wobei unspezialisierte Pflanzenfragmente als die wichtigsten Verbreitungseinheiten angesehen werden. Es mangelt jedoch immer noch an Wissen über die artspezifische Ausbreitungskapazität durch Pflanzenfragmente und die zugrunde liegende Dynamik in Fließgewässern. Gemäß der neuen EU-Verordnung 1143/2014 zur Prävention und dem Management der Einführung und Ausbreitung invasiver gebietsfremder Arten sind Informationen zur Fortpflanzung und den Ausbreitungsmustern einer Art obligatorisch. Um der EU-Verordnung zu entsprechen, wurden Labor- und Feldstudien in Fließgewässern des Tieflandes zur Bewertung der Fragmentverbreitungskapazität einheimischer und invasiver gebietsfremder Wasserpflanzen anhand von vier Schlüsseleigenschaften durchgeführt: (i) Fragmentierungsrate, (ii) Driftdistanz, (iii) Austrocknungsresistenz, die für die Ausbreitung über Land in isolierten Gewässern relevant ist, und (iv) Regenerations- und Kolonisierungspotential von Fragmenten. Die Ergebnisse dieser Arbeit verdeutlichen, dass die Fragmentverbreitungskapazität ein maßgeblicher Faktor für die erfolgreiche und schnelle Ausbreitung vieler invasiver Wasserpflanzen weltweit ist. Während der Ausbreitungs- und Invasionserfolg submerser Arten wie *Myriophyllum spicatum*, *Potamogeton crispus*, *Elodea canadensis*, *Elodea nuttallii* und *Hydrilla verticillata* größtenteils auf eine hohe Fragmentverbreitungskapazität zurückgeführt werden kann, scheint die Fragmentverbreitung nur eine untergeordnete Rolle für die Invasivität von *Lagarosiphon major* und *Myriophyllum heterophyllum* zu spielen. Es konnte jedoch gezeigt werden, dass die Fragmentverbreitung stark von den hydrologischen und hydraulischen Fließgewässereigenschaften abhängt und in Gewässern, die sich durch hohe Abfluss- und turbulente Strömungsbedingungen auszeichnen, generell erhöht ist. Folglich erfordert die Ausbreitung invasiver gebietsfremder Wasserpflanzen in größeren Fließgewässern besondere Aufmerksamkeit, solange die Etablierung und Beständigkeit von Wasserpflanzen nicht durch die Strömungsbedingungen verhindert werden.

Contents

CHAPTER 1

General introduction

Biological invasions

Since the beginning of what is known as the ‘Great Acceleration’ in 1950, human activities have altered ecosystems more extensively than ever before in the history of mankind (Steffen *et al.*, 2007). Human-induced environmental changes cause global loss of biodiversity even beyond a postulated safe operating space for humanity (planetary boundary framework; Rockström *et al.*, 2009; Steffen *et al.*, 2015b) and have led to the onset of a new era: The Anthropocene (Crutzen, 2002; Steffen *et al.*, 2007, 2015a).

Biological invasions are among the five major drivers of biodiversity loss and degradation of ecosystems, together with habitat change, climate change, overexploitation and environmental pollution, i.e. increased nutrient loadings (Millennium Ecosystem Assessment, 2005). Besides habitat loss, biological invasions were recognized to pose the greatest threat to biodiversity in freshwater ecosystems (Allan & Flecker, 1993; Sala *et al.*, 2000). Freshwater ecosystems are considered as the most vulnerable ecosystems worldwide, and despite only covering 0.8% of Earth’s surface, they are hotspots of biodiversity that harbor a much higher relative species richness than its terrestrial and marine counterparts (Dudgeon *et al.*, 2006; Strayer & Dudgeon, 2010; Moorhouse & Macdonald, 2015). Apart from the ecological damage by reducing native species richness and abundance and by altering ecosystem functionality, invasive alien species impair ecosystem services, human well-being and economy (Hulme *et al.*, 2009; Pyšek & Richardson, 2010; Vilà *et al.*, 2010, 2011). Previously, the global economic costs associated with invasive alien species including both environmental damages and management efforts were broadly estimated to be in the range of USD 1.4 trillion per year (corresp. >3% of the world economy in 2000), though this value is now expected to be much higher (Pimentel *et al.*, 2001). Other estimates further indicate that invasive alien species cause annual costs of USD 120 billion in the United States (Pimentel *et al.*, 2005) and up to EUR 20 billion in Europe (Kettunen *et al.*, 2008).

The deliberate and inadvertent introduction of alien species beyond biogeographical barriers due to human activity is a well-known phenomenon (e.g. Lodge, 1993; Mills *et al.*, 1993; Vitousek *et al.*, 1996, 1997; Mack *et al.*, 2000) that is largely driven by trade globalization (Gaston *et al.*, 2003; Hulme, 2007; Hulme *et al.*, 2008; Westphal *et al.*, 2008). Once introduced, alien species must overcome abiotic and biotic filters, which hinder establishment, persistence and expansion (Levine *et al.*, 2004; Divíšek *et al.*, 2018). The dispersal of species, however, is generally limited by major climatic disjunctions (e.g. temperate to tropical regions), with species tolerant to a wide range of climates being

Terminology

Native species[1]: Species that have originated in a given area without human involvement or that have arrived there without intentional or unintentional intervention of humans from an area in which they are native.
(*Synonyms*: Indigenous)

Alien species[1,2]: Species in a given area whose presence there is due to intentional or unintentional introduction as a result of human activity, or which have arrived there without human involvement from an area in which they are alien.
(*Synonyms*: Exotic, introduced, non-native, non-indigenous)

Casual alien species[1,2]: Alien species that may flourish and even reproduce occasionally outside cultivation in an area, but that eventually die out because they do not form self-replacing populations, and rely on repeated introductions for their persistence.

Naturalized species[1,2]: Alien species that sustain self-replacing populations for at least ten years without direct human intervention (or in spite of human intervention) by recruitment from offspring capable of independent growth.
(*Synonyms*: Established)

Invasive alien species[1,2,3]: Naturalized species that produce reproductive offspring, often in very large numbers, at considerable distances from the parents, and thus have the potential to spread over a large area. Invasive alien species threaten human health, economy and/or native biological diversity.

> *Note*: The term 'invasive species' is still ambiguous and often used to refer solely to alien species. However, human-induced habitat change can also confer a competitive advantage on native species, which may then spread rapidly and cause significant environmental damage, thus be considered as invasive (Valéry *et al.*, 2008, 2009). Changes in the environment may additionally explain lag times observed between the introduction of an alien species and the onset of its invasive behavior (Crooks, 2005).
>
> **IAAP** = **I**nvasive **A**lien **A**quatic **P**lant

Propagule pressure[4]: The frequency with which a species is introduced to a site, combined with the number of individuals in each introduction event.

[1] Pyšek *et al.* (2004), [2] Richardson *et al.* (2000), [3] Hulme (2007), [4] Simberloff (2009)

considered to naturalize and invade new regions more frequently (Pyšek *et al.*, 2009; Murphy *et al.*, 2019). Likewise, a high phenotypic plasticity, that is the capacity of a genotype to express different phenotypes under variable environmental conditions, is regarded as an important property of successful invaders as it may confer adaptive benefits and allows a species to occupy broader ecological niches (Richards *et al.*, 2006; Davidson *et al.*, 2011).

In Europe alone there are more than 14,000 alien species that have established self-sustaining populations, with terrestrial plants accounting for the largest share of alien species (European Alien Species Information Network – EASIN, Katsanevakis *et al.*, 2015). However, only a small proportion of naturalized species becomes invasive and causes significant ecological and/or economic damage (Mack *et al.*, 2000; Vilà *et al.*, 2010, 2011). According to the widely used 'tens rule', ~10% of introduced species (i.e. imported species found in the wild outside cultivation) become naturalized and, in turn, ~10% of naturalized species become a pest, suggesting that ~1% of introduced species are successful invaders (Williamson, 1993; Williamson & Fitter, 1996). While the tens rule may be appropriate for terrestrial plant species, it does not apply to freshwater aquatic plants, as far more than 1% of introduced alien aquatic plant species become invasive in their introduced range (see Hussner, 2012). In Europe, the absolute number of alien species is clearly much lower for aquatic (~96 species) than for terrestrial plants (~6,200 species) but the proportion of alien aquatic plants considered as invasive within the European Union is about 32 times greater (Hussner, 2012; EASIN, Katsanevakis *et al.*, 2015). Not surprisingly, aquatic plant species tend to show broader distributions than terrestrial plants (Santamaría, 2002; but see Murphy *et al.* (2019) for an overall narrow global distribution of aquatic plants on species level). Reasons for this primarily include the large-scale uniformity of the aquatic environment (Cook, 1985; Barrett *et al.*, 1993) and the high phenotypic and physiological plasticity of aquatic plants in general (Riis *et al.*, 2010; Pedersen *et al.*, 2013). Up to a certain degree, however, it still remains unresolved why so many aquatic plant species are successful invaders worldwide (Fleming & Dibble, 2015).

Aquatic plant invasions in freshwater ecosystems

Aquatic plants generally play a crucial role for the structure and functionality of freshwater ecosystems (Jeppesen *et al.*, 1998). They fulfill a wide range of functions, e.g. supporting macroinvertebrate and fish diversity by providing habitat and food (Pelicice *et al.*, 2005; Rennie & Jackson, 2005; Bakker *et al.*, 2016), reducing sediment resuspension and retaining nutrients (Horppila & Nurminen, 2005; Cotton *et al.*, 2006) and inhibiting phytoplankton blooms (Gross, 2003). The rampant and dense growth of invasive alien aquatic plants (IAAPs), however, causes enormous ecological and economic damages (Strayer, 2010; Gallardo *et al.*, 2016).

From an ecological perspective, IAAPs can displace native macrophyte species (Santos *et al.*, 2011), alter community composition and reduce diversity of zooplankton, macro-invertebrates and fish (Stiers *et al.*, 2011a; Coetzee *et al.*, 2014; Kuehne *et al.*, 2016; Stiers & Triest, 2017) and consequently impair food web structure (Villamagna & Murphy, 2010). Moreover, they can impede water flow and lead to increased sedimentation, influence nutrient cycling, induce hypoxia or even anoxia within the water column and may ultimately cause the collapse of ecosystem functioning (Carpenter & Lodge, 1986; Dandelot *et al.*, 2005; Urban *et al.*, 2006). These environmental impacts, in turn, have severe repercussions on human well-being and the economy (Vilà *et al.*, 2010; Verhofstad & Bakker, 2019). Dense stands of IAAPs are reported to foster mosquito breeding sites which promote transmission of human pathogens, cause large losses in commercial fishing, increase the flood risk by obstructing water flow, block navigation and irrigation channels and interfere with hydroelectric power generation by clogging water intakes of hydropower plants (Holm *et al.*, 1969; Clayton & Champion, 2006; Thouvenot *et al.*, 2013). It was further documented that IAAPs can depreciate lakefront property value by 20-40% (Halstead *et al.*, 2003) and impede or even completely shut down recreational use of water bodies, causing substantial monetary losses to tourism (Holm *et al.*, 1969; Aiken *et al.*, 1979). In the United States, the mere costs associated with management of IAAPs are about USD 100 million per year (Pimentel *et al.*, 2005), with high annual costs of 14.5 million for control of a single submerged species, *Hydrilla verticillata* (L.f.) Royle, in Florida lakes (Langeland, 1996). Incurred expenses for IAAP management in Europe are also expected to be high (Brundu, 2015), as e.g. more than EUR 14 million were spent for removal of the free floating invader *Eichhornia crassipes* (Mart.) Solms from a 75 km stretch of the Guadiana River in Spain (EPPO, 2008).

A new directive: EU Regulation 1143/2014

To counteract the serious threat of invasive alien species to the European Union (EU), the European Parliament and the Council adopted the EU Regulation 1143/2014 (EU, 2014), which entered into force on the 1st of January 2015. The EU Regulation sets out rules on three major subjects to mitigate the adverse impacts of invasive alien species, including (i) prevention of introduction and further spread, (ii) early warning and rapid eradication and

Table 1 Invasive alien aquatic plants (IAAPs) of Union concern (based on last update EU 2019/1262): Native range and distribution within the EU

Species	Native range	Distribution in EU
Alternanthera philoxeroides (Mart.) Griseb.	South America	ES, FR, IT
Cabomba caroliniana A. Gray	South America	AT, BE, DE, DK, FR, (*GB*), HU, NL, PL, SE
Eichhornia crassipes (Mart.) Solms	South America	BE, CZ, DE, ES, FR, (*GB*), HU, IT, NL, PT, RO
Elodea nuttallii (Planch.) H. St. John	North America	AT, BE, BG, CZ, DE, DK, (*GB*), FI, FR, HR, HU, IE, IT, LU, NL, PL, RO, SE, SK
Gymnocoronis spilanthoides (D. Don ex Hook. & Arn.) DC.	South America	HU, IT
Hydrocotyle ranunculoides L.f.	North-. Central-, South America	BE, DE, (*GB*), FR, HU, IE, IT, NL
Lagarosiphon major (Ridley) Moss	South Africa	BE, DE, ES, FR, (*GB*), HU, IE, IT, NL, PT
Ludwigia grandiflora (Michx.) Greuter & Burdet	South America	BE, DE, ES, FR, (*GB*), HU, IE, IT, NL
Ludwigia peploides (Kunth) P. H. Raven	South America	BE, DE, ES, FR, (*GB*), GR, HR, IT, NL, PT
Myriophyllum aquaticum (Vell.) Verdcourt	South America	AT, BE, DE, ES, FR, (*GB*), HU, IE, IT, LU, NL, PT, RO
Myriophyllum heterophyllum Michx.	North America	AT, BE, DE, ES, FR, HR, NL
Salvinia molesta D. S. Mitch.	South America	AT, BE, DE, ES, FR, IT, NL, PT

Data on IAAP distribution based on Hussner (2012), Tsiamis *et al.* (2017) and European and Mediterranean Plant Protection Organization (EPPO) Global Database (2019). IAAP distribution includes established populations as well as casual occurrences.
ISO country codes of the 27 EU member states and the United Kingdom: [AT] Austria, [BE] Belgium, [BG] Bulgaria, [CY] Cyprus, [CZ] Czech Republic, [DE] Germany, [DK] Denmark, [EE] Estonia, [ES] Spain, [FI] Finland, [FR] France, [*GB*] *United Kingdom* (withdrawel from EU, 31.01.2020), [GR] Greece, [HR] Croatia, [HU] Hungary, [IE] Ireland, [IT] Italy, [LT] Lithuania, [LU] Luxembourg, [LV] Latvia, [MT] Malta, [NL] Netherlands, [PL] Poland, [PT] Portugal, [RO] Romania, [SE] Sweden, [SI] Slovenia, [SK] Slovakia

(iii) management. It prioritizes target species at Union level by implementing a list of invasive alien species of Union concern (Article 4, EU Regulation 1143/2014) that are subjected to stringent enforcements (Genovesi *et al.*, 2015). Hitherto, the EU Regulation is the most important environmental policy measure of Europe towards meeting the Aichi Target 9 of the Convention on Biological Diversity (CBD), which foresees that ‘by 2020, invasive alien species and pathways are identified and prioritized, priority species are controlled or eradicated, and measures are in place to manage pathways to prevent their introduction and establishment.’

At the present time the list of invasive alien species of Union concern comprises a total number of 66 species, including 30 animal and 36 plant species (latest update: 25 July 2019, EU 2019/1262). Among the plant species, 12 aquatic plants are listed whose current distri-

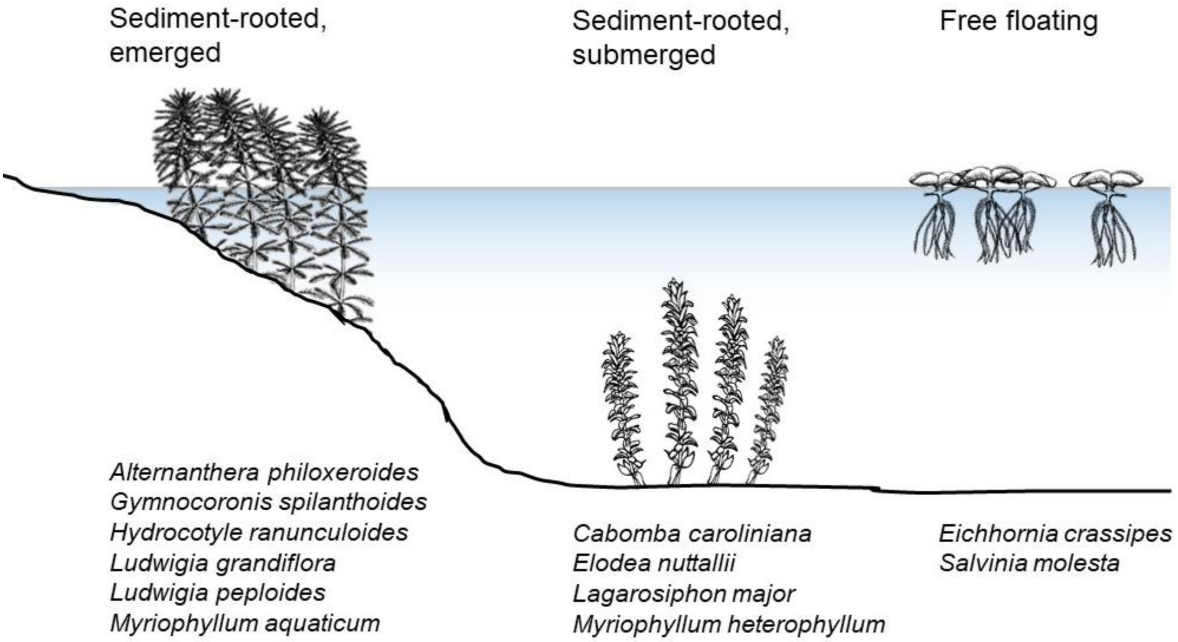

Figure 1 Growth forms of invasive alien aquatic plants (IAAPs) of Union concern (modified after Hussner *et al.*, 2017)

bution within the EU varies widely (Table 1). While *Elodea nuttallii* is the most widespread IAAP, others, such as *Alternanthera philoxeroides* and *Gymnocoronis spilanthoides*, still show narrow distributions. It must, however, be taken into account that some species are limited to few freshwater systems and only reported as casual for several member states, e.g. *E. crassipes* (Brundu *et al.*, 2012; Hussner, 2014). Following a simple classification of their growth forms (acc. Hussner *et al.*, 2017), the 12 IAAPs of Union concern can be subdivided in (i) six sediment-rooted emerged growing species, (ii) four sediment-rooted submerged growing species and (iii) two free floating species (Figure 1).

Invasive alien aquatic plants in the light of the EU Regulation

In the past, there has already been tremendous research interest in understanding which traits enable a species to become invasive (invasiveness of species), which factors determine the susceptibility of a community or habitat to invasive species (invasibility of recipient community) and how invasiveness is linked with invasibility (Lodge, 1993; Rejmanek & Richardson, 1996; Alpert *et al.*, 2000; Davis *et al.*, 2000; Levine, 2000; Kolar & Lodge, 2001; Shea & Chesson, 2002; Levine *et al.*, 2004; van Kleunen & Richardson, 2007; Pyšek & Richardson, 2008; Levine & D'Antonio, 2010; Pyšek *et al.*, 2012). Despite their high proportion of invasive species, however, aquatic plants were mostly neglected in these studies. More recently, efforts have been made to unravel the invasion mechanisms of aquatic plants in freshwater ecosystems (e.g. Fleming & Dibble, 2015; Thomaz *et al.*, 2015). There is evidence that competitive strength, thus invasiveness, rather than invasibility determines the invasion success of IAAPs (Capers *et al.*, 2007; Alofs & Jackson, 2014; Muthukrishnan *et al.*, 2018; Louback-Franco *et al.*, 2019), though biotic resistance by native vegetation can certainly play an important role on a smaller spatial scale (Petruzzella *et al.*, 2018, in press; Pulzatto *et al.*, 2019). It must nevertheless be considered that invasions are context-specific and that invasiveness of aquatic plants only takes effect when specific environmental requirements are met (Richardson & Pyšek, 2006). Invasions are complex and often associated with spatio-temporal dynamics of multiple factors. Still, the successful spread of IAAPs must be to some extent attributed to highly efficient reproductive strategies and propagule dispersal mechanisms (Barrett *et al.*, 1993; Santamaría, 2002; Wang *et al.*, 2017).

A widely recognized key determinant of invasion success is the quantity of propagules arriving at a receiving site and their frequency of release, i.e. propagule pressure (Lockwood *et al.*, 2005; Colautti *et al.*, 2006; Simberloff, 2009). In other words, the more propagules introduced into a habitat, the higher the likelihood of successful establishment of IAAPs. Propagule pressure was found to contribute to both species invasiveness and community invasibility (Von Holle & Simberloff, 2005; Colautti *et al.*, 2006). Even moderately high propagule pressure can overwhelm biotic resistance of the resident plant community, particularly in highly disturbed ecosystems such as streams (Von Holle & Simberloff, 2005; Colautti *et al.*, 2006; Carr *et al.*, 2019).

According to the EU Regulation, information on the reproduction, spread patterns and dynamics of invasive alien species of Union concern as well as on potential pathways of introduction and spread is mandatory (Article 5(1), EU Regulation 1143/2014). Consequently, assessment of the dispersal capacity, pathways and dynamics of IAAPs is required to derive prevention and management measures, and ultimately to comply with the EU Regulation by achieving containment of IAAPs.

Introduction pathways

For alien aquatic plants, ornamental (or horticultural) trade has been recognized as the major general pathway of introduction worldwide (Maki & Galatowitsch, 2004; Brunel, 2009; Champion *et al.*, 2010; Hussner *et al.*, 2014b; Azan *et al.*, 2015). Especially in recent times, the trading and import of alien plants is strongly facilitated by easily accessible and poorly regulated online commerce (Padilla & Williams, 2004; Mazza *et al.*, 2015). A total number of at least 247 alien aquatic plant species was found to be imported into the EU, of which the vast majority (88%) was associated with aquarium use (Brunel, 2009). Almost all IAAP species of Union concern have been actively traded within the EU for indoor aquarium and outdoor purposes in spite of their invasiveness (Brunel, 2009; Hussner *et al.*, 2014b), whereby intentional release (e.g. *Ludwigia* spp.; EPPO, 2011) and unintentional escape from confinement by disposal of aquarium contents (e.g. *Hydrocotyle ranunculoides*; EPPO, 2006) are regarded as the most important introduction pathways of IAAPs into member states of the EU (pathways acc. Hulme *et al.*, 2008). Some others may be unintentionally introduced as contaminants of commodities, e.g. *A. philoxeroides*, which was identified as a contaminant of bonsai growing media and bird seeds (van Denderen *et al.*, 2010; EPPO, 2016a). Moreover, the extra-range dispersal of IAAPs may also be linked with inadvertent transport of plants attached to boats (Wilson *et al.*, 2009; Rothlisberger *et al.*, 2010), though boating likely plays a minor role for the introduction of IAAPs into EU member states.

Apart from human-mediated introduction pathways, IAAPs might also be introduced via natural vectors such as waterfowl (Green, 2016). Zoochorous dispersal through waterfowl, however, is often rather limited in dispersal distance and efficiency and most likely restricted to aquatic plant seeds and fronds of small pleustophytes like *Lemna* spp. (Coughlan *et al.*, 2015, 2017b; Lovas-Kiss *et al.*, 2018, 2019).

Spread pathways and mechanisms

Once established, the production and subsequent dispersal of propagules mainly drives the further spread on a regional scale. Aquatic plants are often limited in their sexual reproduction and dispersal via seeds, due to the lack of floral induction, ineffective pollination, low seed viability, low germination rates and failure of seedlings to establish (Titus & Hoover, 1991). Moreover, the absence of a sex frequently restricts alien aquatic plant species solely to vegetative spread mechanisms within their introduced range, e.g., *Elodea canadensis* Michx., *Egeria densa* Planch., and *Myriophyllum aquaticum* (Orchard, 1981; Cook & Urmi-König, 1985). Indeed, half of the IAAPs of Union concern, including all sediment-rooted submerged growing species (Figure 1), do not produce seeds within the EU (Table 2). Others such as *Ludwigia* spp. that are known to produce significant amount of seeds, appear to have only limited germination success, while *Salvinia molesta* is sterile (Table 2). Even though *A. philoxeroides*, *E. crassipes* and *G. spilanthoides* were found to be capable of seed formation within the EU, and despite the lack of quantitative data, they

Table 2 Invasive alien aquatic plants (IAAPs) of Union concern (based on last update EU 2019/1262): Sexual reproduction within the EU

Species	Sexual reproduction in EU			
	Number of seeds/spores produced	Viability	Germination in the field	References
Alternanthera philoxeroides	NA	NA	NA	
Cabomba caroliniana	None (no seeds)	-	-	Matthews *et al.* (2013), Scheers *et al.* (2019)
Eichhornia crassipes	NA	NA	NA	
Elodea nuttallii	None (no seeds)	-	-	Cook & Urmi-König (1985), Josefsson (2011)
Gymnocoronis spilanthoides	NA	High	NA	Ardenghi *et al.* (2016)
Hydrocotyle ranunculoides	Medium	Low	Low	EPPO (2006), Birch *et al.* (2015)
Lagarosiphon major	None (no seeds)	-	-	Cook (1982), Caffrey *et al.* (2011)
Ludwigia grandiflora	High	Medium	None/low	Dandelot *et al.* (2005), Ruaux *et al.* (2009), Hussner *et al.* (2016b)
Ludwigia peploides	High	High	NA	Dandelot *et al.* (2005), Ruaux *et al.* (2009)
Myriophyllum aquaticum	None (no seeds)	-	-	Hussner (2008)
Myriophyllum heterophyllum	None (no seeds)	-	-	Hussner (2008)
Salvinia molesta	None/low	None	-	EPPO (2017)

mainly disperse asexually (EPPO, 2008, 2016a, 2017b). Consequently, the successful spread of IAAPs of Union concern must be based on effective vegetative reproduction.

The dispersal of vegetative propagules and local expansion via clonal growth are widely viewed as the primary spread mechanisms of most aquatic plant species (Sculthorpe, 1967; Grace, 1993; Santamaría, 2002). Aquatic plants may disperse through different types of vegetative propagules, including turions, tubers, rhizomes, stolons, shoot fragments, ramets and even entire uprooted plants (Barrat-Segretain, 1996). Specialized propagules such as turions or subterranean tubers serve as important storage organs for many species and can seasonally contribute in large part to local recruitment, particularly following unfavorable conditions e.g. during winter (Sastroutomo, 1981; van Wijk, 1989; Thullen, 1990; Suzuki & Stuefer, 1999). Unlike specialized propagules, unspecialized plant fragments are readily available and pose the most important means for intermediate and large distance dispersal (Barrat-Segretain, 1996; Sand-Jensen *et al.*, 1999; Boedeltje *et al.*, 2003, 2004). It is therefore suggested that efficient dispersal through plant fragments decisively contributes to the invasiveness of IAAPs (Jacobs & MacIsaac, 2009). The fragment dispersal capacity of a species principally depends on a combination of (i) the specific fragmentation rate, (ii) the fragment dispersal distance and (iii) the potential for regeneration (i.e. initiation of new growth and development of new propagules) and colonization (i.e. anchorage within the sediment) of fragments.

Plant fragments can be formed either by self-induced abscission (autofragmentation) or by external disturbances (allofragmentation), such as water movement (Sand-Jensen, 2008) foraging of macroinvertebrates, fish or waterfowl (Figuerola & Green, 2002; Carreira *et al.*, 2014; Bakker *et al.*, 2016; Boedeltje *et al.*, 2019) and human activities (Anderson, 1998; Skaer Thomason *et al.*, 2018). Besides the degree of environmental disturbance, the species-specific biomechanical properties largely determine the number of plant fragments produced, and thus influence propagule pressure (Puijalon *et al.*, 2011; Miler *et al.*, 2012, 2014). Fragments of aquatic plants generally preserve a high viability, though differences in the regeneration and colonization abilities among species and different fragment types are well documented (e.g. Barrat-Segretain *et al.*, 1998, 1999; Barrat-Segretain & Bornette, 2000; Barrat-Segretain *et al.*, 2002; Riis *et al.*, 2009; Umetsu *et al.*, 2012a; Vári, 2013). For most widespread submerged species, very small stem fragments consisting only of a single node are sufficient to develop new roots and/or shoots (Langeland & Sutton, 1980; Bickel, 2012; Kuntz *et al.*, 2014). Some few others such as *M. aquaticum* and *Ludwigia grandiflora* are even able to regenerate from single detached leaves (Hussner, 2009). Still, the likelihood of regeneration is generally expected to increase with larger fragment size (Dong *et al.*, 2010a; Redekop *et al.*, 2016; Bickel, 2017). As root formation determines the colonization potential of fragments, species characterized by a preference for fast root formation are likely to colonize and establish more rapidly. Regeneration and colonization abilities might, however, be subject to seasonal fluctuations, as was shown for *E. canadensis* (Barrat-Segretain & Bornette, 2000). Abiotic factors such as temperature as well as nutrient, inorganic carbon and light availability influence fragment regeneration and growth performance of aquatic plants (e.g. Riis *et al.*, 2012; Pedersen *et al.*, 2013; Kuntz *et al.*, 2014; Dülger *et al.*, 2017). Therefore, the environmental conditions of the receiving habitat strongly control the successful establishment of dispersed propagules (Bornette & Puijalon, 2009, 2011).

The spread of IAAPs within interconnected water bodies does largely differ from dispersal to hydrologically isolated habitats, and is mainly driven by water movement in both lentic and lotic systems (Nilsson *et al.*, 2010; Kim *et al.*, 2019). In stream ecosystems, fragment dispersal is of greatest relevance due to the high degree of disturbance by flow (Sand-Jensen *et al.*, 1999). The presence of flow naturally promotes propagule pressure as it facilitates allofragmentation and downstream transport of fragments (Riis & Biggs, 2003; Heidbüchel *et al.*, 2016). Not surprisingly, streams are pathways of rapid spread and regarded as significant invasion corridors for IAAPs (e.g. Hussner, 2014; Scheers *et al.*, 2019). First comprehensive field studies in lowland streams already reported high fragment numbers of up to >6,000 drifting plant fragments per hour for a single species (*Stuckenia pectinata* L.; Heidbüchel *et al.*, 2016) and drift distances ranging from only few meters to several kilometers (Johansson & Nilsson, 1993; Riis & Sand-Jensen, 2006). However, the fragment dispersal dynamics in streams are still not well understood (but see Riis, 2008). Contrary to the drift dispersal within connected waters, the spread of IAAPs to hydrologically isolated sites requires specific vectors for overland transport. While overland transport of hitchhiking fragments by natural vectors such as waterfowl is presumed to be rare (Coughlan *et al.*, 2017a), human-mediated dispersal via trailered boating and contaminated water sport equipment of e.g. anglers and canoeists were recognized as the major spread pathways of IAAPs to isolated waters (Johnstone *et al.*, 1985; Johnson *et al.*, 2001; Rothlisberger *et al.*, 2010; Anderson *et al.*, 2014). During overland transport, plants are exposed to drying conditions and suffer from evaporative water loss. Thus, the successful spread to hydrologically isolated sites is mainly hinged on the ability of fragments to retain viability and withstand desiccation, i.e. desiccation resistance (Barnes *et al.*, 2013; Bickel, 2015; Bruckerhoff *et al.*, 2015).

Although it has long been recognized that spread through vegetative propagules is the primary dispersal mode for most aquatic plants (Sculthorpe, 1967; Nichols & Shaw, 1986; Sand-Jensen *et al.*, 1999), comprehensive studies on the role of fragment dispersal, particularly in streams, are largely absent (but see Riis, 2008; Heidbüchel *et al.*, 2016). There is still a great lack of precise information about the species-specific fragment dispersal capacity required for the risk assessment of alien aquatic plants, including those already listed as IAAPs of Union concern (e.g. Fleming & Dibble, 2015).

Prevention & management

According to the EU Regulation, effective and efficient prevention and management strategies against invasive alien species must be implemented, including containment, control and eradication when feasible (EU, 2014). Prevention from introduction, further spread and re-infestation following management is in general more desirable than eradication and ongoing management measures (EU, 2014).

Preventing IAAPs from future introduction e.g. by applying trading bans is widely acknowledged as the most cost-efficient way to reduce the risk of aquatic plant invasions (e.g. Mack *et al.*, 2000; Hussner *et al.*, 2017). Still, misidentification of IAAPs cannot be fully excluded, highlighting the importance of early detection measures that allow for rapid eradication (Hussner *et al.*, 2016b, 2017). Once established, measures preventing IAAPs from further spread constitute a promising and feasible approach for successful containment (Panetta & Cacho, 2014). This requires information on potential pathways of spread in order to prioritize interventions most cost-effectively (Brunel, 2009). Additionally, different control measures may be applied to reduce IAAP biomass or, if possible, even eradicate infestations. The management opportunities against IAAPs comprise mechanical/physical, biological, chemical and indirect control measures and have been recently detailed in a comprehensive review (Hussner *et al.*, 2017). In Europe, mechanical control methods are the most commonly applied measures against IAAPs. The feasibility and efficacy of these methods, however, strongly depend on the growth form of the IAAP species (emerged/submerged/free floating), the physical properties of the habitat and the seasonal timing of application (e.g. Newman, 2010). Most importantly, mechanical harvesting frequently generates vast numbers of plant fragments, and thus may unintentionally promote the further spread of a target IAAP (Anderson, 1998, 2003; Riis, 2008). Consequently, knowledge about the fragment dispersal pathways and dynamics is of utmost importance for the development and implementation of efficient measures preventing IAAPs from further spread and the adaptation of existing management options (Brundu, 2015; Hussner *et al.*, 2017).

Thesis outline & objectives

In this thesis, I elucidate the vegetative dispersal of aquatic plants via plant fragments and its role for the invasion success of IAAPs. Therefore, comparative laboratory and field studies in different lowland stream systems were conducted, considering native and (invasive) alien aquatic plant species. The studies herein are generally centered around three major objectives:

I. Assessment of the species-specific fragment dispersal capacity and its relevance for the invasiveness of IAAPs

II. Identification and evaluation of fragment dispersal pathways and dynamics

III. Derivation of prevention and management measures against IAAPs

Throughout the thesis, I will address different key traits of fragment dispersal capacity within the context of the invasion continuum, i.e. from introduction to nuisance growth (Figure 2). These traits comprise (i) fragmentation rate, (ii) drift distance, (iii) desiccation resistance and (iv) the regeneration and colonization potential of aquatic plant fragments. The studies give particular attention to the spread within interconnected and between isolated water bodies following initial establishment of aquatic plants (proliferation cycle in Figure 2). Thus, I largely focus on post-introduction fragment dispersal of established IAAPs and measures for containment of their further spread.

I. & II. Fragment dispersal capacity, pathways & dynamics

Stream ecosystems are highly susceptible to IAAP invasions, as flow strongly facilitates fragmentation and downstream dispersal, and thus naturally enhances propagule pressure. Due to the high relevance of fragment dispersal in running waters, field investigations were performed in small to medium-sized streams located within the Lower Rhine region in West Germany.

In **Chapter 2**, a field study was conducted to quantify the number of fragments and estimate fragmentation rates in different streams systems, combined with assessment of the regeneration/colonization potential indicated by root formation of the fragments under standardized conditions. Subsequently, in **Chapter 3**, more precise information on the species-specific fragmentation rates and the influence of the flow regime were gathered.

In **Chapter 4**, drift distances of fragments from morphologically distinct species and the underlying fragment retention patterns in streams were investigated, while in **Chapter 5**, a laboratory approach was applied to examine the species- and fragment type-specific desiccation resistance and its relevance for overland dispersal to isolated waters.

In **Chapter 6**, the fragment type-specific regeneration and colonization abilities and the influence of water depth were assessed under standardized conditions.

Lastly, all findings are merged in the synthesis, **Chapter 7**, to detail the fragment dispersal capacity of aquatic plant species in consideration with existing literature and to resolve the role of fragment dispersal for the invasiveness of IAAPs. The relevance of different spread pathways and the fragment dispersal dynamics within different stream systems are further addressed and discussed.

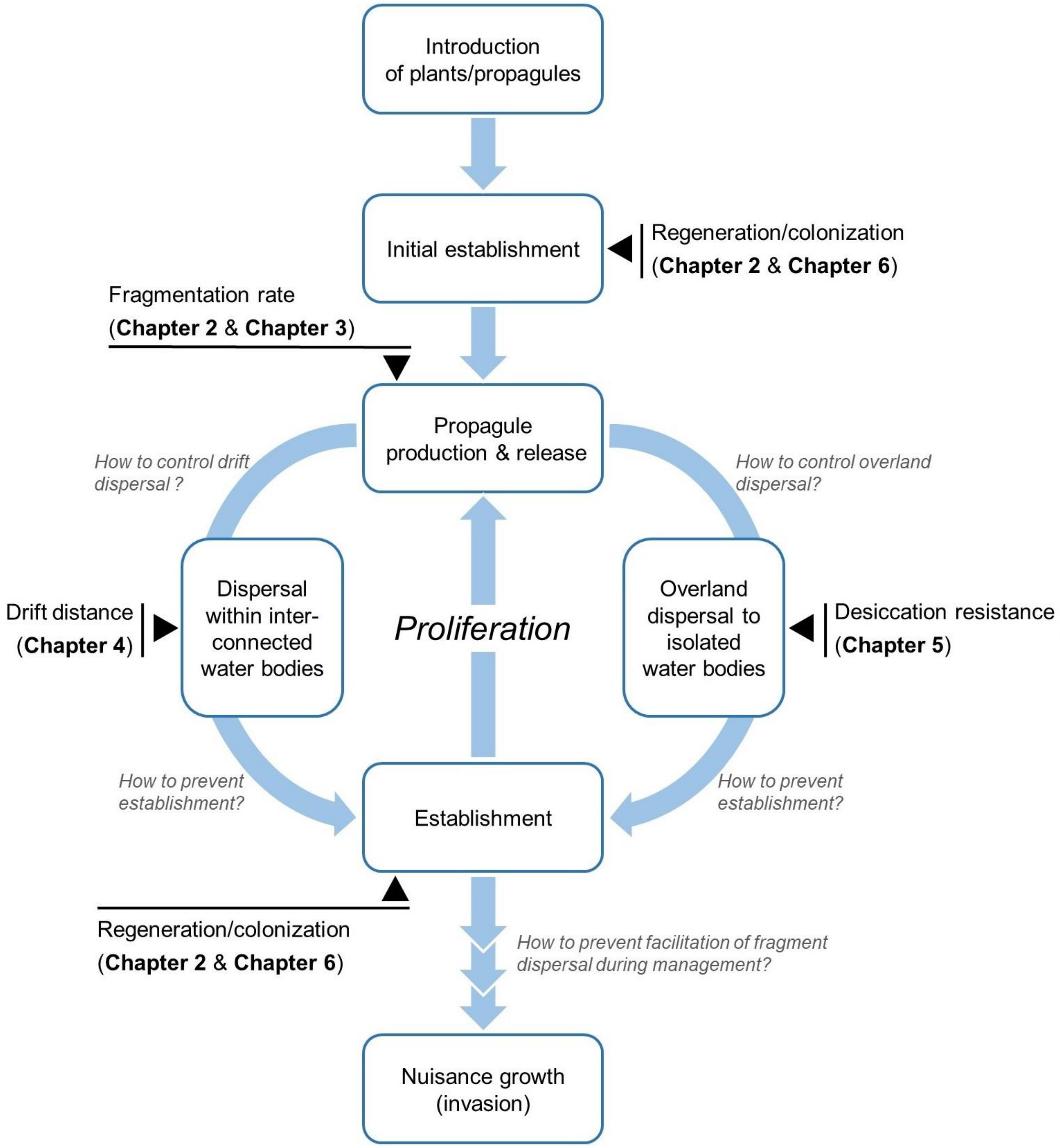

Figure 2 Overview of fragment dispersal within the invasion continuum. Traits of fragment dispersal capacity and its relevance in the dispersal process are given with reference to the chapters of this thesis, respectively. Intervention points of interest related with major objective III of this thesis are indicated by grey text

III. Implications for prevention & management of IAAPs

In the synthesis (**Chapter 7**), implications for the prevention of further spread of IAAPs and management options are derived and discussed. More specifically, I particularly point out measures of containment, preventing IAAPs (i) from further spread within interconnected running water systems and (ii) from new introduction to isolated water bodies by intervening overland dispersal. Finally, potential tools that help to assess the vegetative spread potential of IAAPs are considered.

CHAPTER 2 |

Species-specific fragmentation rate and colonization potential partly explain the successful spread of aquatic plants in lowland streams

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Abstract

The vegetative spread potential of aquatic plant species is largely based on the quantity of dispersed plant fragments (propagule pressure) and their potential for regrowth and establishment, i.e. fragment regeneration and colonization. In streams, fragment dispersal is of particular significance as the exposure of plants to flow facilitates fragmentation and downstream drift of fragments. We conducted field investigations to quantify the relevance of fragment dispersal and the species-specific propagule pressure due to fragmentation in five small to medium-sized German streams. These field surveys were combined with determination of the potential for regeneration/colonization of fragments collected in the field indicated by relative root formation under standardized conditions. In general, the number of drifting fragments tended to increase with larger stream size. We documented species-specific differences in fragmentation rate, which contributed to weak correlations between the number of drift units and specific plant cover within four streams. The overall likelihood for root formation increased significantly with increasing fragment size and was highest for the invasive *Elodea nuttallii* (70% of fragments). We conclude that the fragment dispersal capacity in streams is highly species-specific and that propagule pressure alone cannot explain the successful spread of invasive species like *Myriophyllum heterophyllum*.

Keywords aquatic macrophytes, fragment dispersal, hydrochory, invasive species, propagule pressure, regeneration

Introduction

The wide distribution of aquatic plants in general and the rapid spread of invasive alien aquatic plant species in particular are a global phenomenon that is largely due to a combination of the large scale uniformity of aquatic environments (Cook, 1985) and the high phenotypic and physiological plasticity of many aquatic plants (Sculthorpe, 1967; Riis *et al.*, 2010; Pedersen *et al.*, 2013; Hussner *et al.*, 2016). Nevertheless, the wide distribution of aquatic plant species requires highly effective spread mechanisms (Santamaría, 2002; Fleming & Dibble, 2015).

The dispersal by seeds is largely limited in numerous submerged aquatic plant species, particularly in their introduced range, due to the lack of floral induction, ineffective pollination, low seed viability, failure of seedlings to establish (Titus & Hoover, 1991) or simply the presence of only one sex (e.g. *Elodea canadensis* Michx.*,* *Egeria densa* Planch. and *Myriophyllum aquaticum* (Vell.) Verdcourt; Orchard, 1981; Cook & Urmi-König, 1985). And even for species that are known to produce significant amounts of seeds, the spread within and between water bodies appears to be almost exclusively asexual, as it was documented for e.g. invasive alien *Ludwigia* species in Europe (Okada *et al.*, 2009; Thouvenot *et al.*, 2013). Consequently, it must be concluded that aquatic plant species predominantly spread through vegetative propagules rather than seeds (Boedeltje *et al.*, 2003, 2004).

For some aquatic plant species, specialized vegetative organs such as tubers and turions significantly contribute to propagule banks and can seasonally play a substantial role for short distance dispersal (e.g. *Hydrilla verticillata* (L.f.) Royle, *Potamogeton crispus* L., *Stuckenia pectinata* L.; Bowes *et al.*, 1979; Sastroutomo, 1981; van Wijk, 1989). By contrast, unspecialized shoot fragments are readily available and regarded as the most important dispersal units among the different types of vegetative propagules, particularly for the intermediate range extension of submerged plant species (Grace, 1993; Barrat-Segretain, 1996). These plant fragments are either formed self-induced by autofragmentation or through external disturbances such as water movement (Sand-Jensen, 2008), foraging of invertebrates, fish or waterfowl (Figuerola & Green, 2002; Bakker *et al.*, 2016) and human activity (e.g. during mechanical weed control; Anderson, 1998), which is termed as allofragmentation. Hence, fragment dispersal is largely determined by the degree of environmental disturbance and the biomechanical fragmentation properties of a species (Schutten *et al.*, 2005; Bociag *et al.*, 2009; Liffen *et al.*, 2011; Miler *et al.*, 2012, 2014;

Łoboda *et al.*, 2019). The number of plant fragments produced is further correlated with the specific plant biomass and is the major determinant of the propagule pressure of aquatic plants, which is considered as the key factor for the spread potential and invasion success of a species (Lockwood *et al.*, 2005, 2009; Jacobs & MacIsaac, 2009; Simberloff, 2009).

While the dispersal of vegetative propagules to hydrologically isolated habitats relies on overland transport through specific vectors (e.g. human or waterfowl; Rothlisberger *et al.*, 2010; Green, 2016), the vegetative spread of aquatic plants within interconnected water bodies is mainly controlled by water movement (Boedeltje *et al.*, 2003). Particularly in running waters, the formation and downstream dispersal of allofragments is highly facilitated by the ubiquitous presence of flow, which contributes to the rapid spread of aquatic plants in streams (Riis & Biggs, 2003). Indeed, high numbers up to >6,000 drifting plant fragments h^{-1} were documented for a single submerged species (*Stuckenia pectinata*) within a medium-sized, fast flowing river (River Erft, Germany; Heidbüchel *et al.*, 2016). Moreover, the capacity of drifting propagules depends on the stream characteristics (Riis & Sand-Jensen, 2006; Riis, 2008) and generally increases at higher levels of discharge, especially during flood events (Boedeltje *et al.*, 2004; Heidbüchel *et al.*, 2016). As further fragmentation during downstream transport is likely and as most species have the potential to initiate new growth from small stem fragments consisting only of a single node (Langeland & Sutton, 1980; Kuntz *et al.*, 2014; Bickel, 2017), the propagule pressure may substantially increase with increasing drift duration. Propagule pressure is therefore assumed to be additionally influenced by fragment size (see also Heidbüchel *et al.*, 2016). Previously, it was shown that the number of drifting fragments does not accumulate in the course of a stream but declines exponentially when released at a given point (Riis & Sand-Jensen, 2006; Heidbüchel *et al.*, 2016). The few existing field studies report drift distances ranging from only a few meters to several kilometers, whereby the maximum drift distance of plant fragments decreased with smaller stream size (Johansson & Nilsson, 1993; Boedeltje *et al.*, 2003; Riis & Sand-Jensen, 2006).

Apart from the major role of fragment number, the likelihood of successful spread also depends on the regeneration potential of the dispersed fragments, i.e. the initiation of secondary shoots and roots (Barrat-Segretain, 1996). Most aquatic plants produce highly viable fragments, though the minimum fragment size required for regeneration strongly differs among the species (Hussner, 2009; Riis *et al.*, 2009). Additionally, the regeneration capacity is species- and fragment-specific (Barrat-Segretain *et al.*, 1998; Umetsu *et al.*, 2012a; Kuntz *et al.*, 2014; Heidbüchel & Hussner, 2019) and generally increases with increasing fragment size (Redekop *et al.*, 2016; Bickel, 2017). Besides the species- and fragment-specific properties, fragment regeneration is to a large extent influenced by abiotic factors such as temperature, light, carbon and nutrient availability, emphasizing that the environmental conditions of the receiving habitat control the establishment success of plant fragments (Xie *et al.*, 2010, 2018; Riis *et al.*, 2012; Kuntz *et al.*, 2014; Hussner *et al.*, 2015). Consequently, plant fragments must settle at suitable habitats in order to establish new macrophyte stands.

Drifting fragments can be trapped by a variety of retention agents, such as lentic zones, obstacles (e.g. stones) and, most importantly, pre-existing vegetation (Johansson & Nilsson, 1993; Riis & Sand-Jensen, 2006; Riis, 2008). Once a fragment is retained, it must anchor within the sediment for colonization. In streams, initial colonization of fragments was recognized as the main bottleneck for the successful establishment of aquatic plants (Riis, 2008). Thus, a high potential for root formation indicates an increased likelihood of colonization through root anchorage and contributes to the rapid spread of invasive species (Riis *et al.*, 2009; Vári, 2013; Heidbüchel & Hussner, 2019).

In conclusion, even though propagule pressure is considered to play a key role for the spread potential of invasive aquatic plants in running waters, comprehensive quantitative field studies on fragment dispersal are scarce (Riis, 2008; Heidbüchel *et al.*, 2016) and detailed information on the species-specific fragmentation rates in the field are still lacking. Moreover, while the regeneration and colonization abilities of artificial plant fragments (simulated fragmentation by cutting/breaking) have been intensively studied for many species (Barrat-Segretain *et al.*, 1998, 1999; Barrat-Segretain & Bornette, 2000; Barrat-Segretain *et al.*, 2002; Riis *et al.*, 2009; Umetsu *et al.*, 2012a; Vári, 2013; Kuntz *et al.*, 2014; Heidbüchel & Hussner, 2019), surprisingly little is known about the regeneration and colonization potential of naturally formed and dispersed fragments collected in the field.

Here, we quantified the vegetative spread potential of aquatic plants through drifting plant fragments by conducting field surveys in lowland streams under normal discharge conditions. We aim (i) to evaluate the overall relevance of fragment dispersal in different streams, (ii) to determine the species-specific fragmentation rate in the field, (iii) to analyze the potential for regeneration and colonization according to fragment size of fragments collected in the field and (iv) to show differences in fragment dispersal among the species.

Materials and methods

Study area and sampling procedure

The study was conducted in five small to medium-sized lowland streams within the Lower Rhine region in Germany during summer (June-Aug) and autumn (Oct) in 2017. All streams studied are largely straightened and differ in stream size, ranging from a small ditch (Gustorfer Graben) to a medium-sized river (Niers). For each stream, two to four sampling locations were selected based on the presence and community composition of aquatic vegetation in the upstream reach, respectively (Figure 1). The minimum distance between two consecutive sampling locations of a stream differed strongly and ranged from 0.3 (GG1-GG2, Gustorfer Graben) up to 16.9 km (GB2-GB3, Gillbach).

At each sampling location, water depth as well as flow velocity (MiniWater20 Mini, Schiltknecht Messtechnik AG, Gossau, Switzerland) were determined mid-stream and drifting plant fragments were collected once a month (Table 1). The collection of plant fragments was always performed by a single person using a hand-held fishing net (mesh size: 6 mm) either from the river banks or from within the stream. During the samplings, all visible drifting plant material was collected over a duration of 2 to a maximum of 20 min, which was repeated four times (n = 4 per sampling date and sampling location). The sampling duration varied according to the amount of drifting plant material on a respective sampling day to ensure sufficiently large sampling sizes (i.e. to avoid samples without fragments at sampling locations of smaller streams). All samplings were generally performed under normal runoff conditions (as indicated by measurements of water depth and flow velocity; Table 1) to avoid effects of strongly altered discharge on fragmentation during floods and droughts. The collection of plant fragments at locations of the same stream was always carried out on the same day, with samplings conducted from downstream to upstream locations.

Fresh plant material of each sample was carefully packed in sealed plastic bags filled with some water from a respective sampling location and subsequently transported in a polystyrene box. In most cases, samples were immediately evaluated in the laboratory and further used in the regeneration/colonization trials. Some samples, however, were stored in a fridge at 4 °C for less than 24 h until further processing. The storage did not influence fragment regeneration of the species investigated.

Evaluation of plant fragments

Overall, only plant fragments potentially able to regenerate were considered, i.e. fragments consisting of at least the known minimum size required for regeneration of a species (defined based on existing information and observations during the regeneration/colonization trials of this study). We determined the number of drifting plant fragments (hereafter referred to as drift units) and the number of regenerative subunits of each fragment (hereafter referred to as potential propagation units; Table 2). We further determined the drift unit (fragment) length and root length of each individual fragment. During the evaluation, all plant material was handled with great care to prevent the fragments from suffering mechanical damage or damage through desiccation.

Due to the 6 mm mesh size of the hand-held fishing net used, small free-floating species such as *Lemna* spp. were excluded from our study. However, the mesh size was suitable to trap single leaves (e.g. of *Myriophyllum aquaticum)* and small fragments consisting only of a single node that is the minimum fragment size required for regeneration of many species investigated within this study (Table 2). Plant material of *Callitriche* collected at the different sampling locations was not determined to species level and thus summarized (hereafter referred to as *Callitriche* spp).

Determination of fragmentation rate

In the midst of the investigation period, in August, vegetation mappings were performed to quantify the specific plant cover and to subsequently calculate the specific fragmentation rates within the five streams. It was previously documented that the drift distance of the majority of plant fragments in smaller streams is very low (<300 m; Riis & Sand-Jensen, 2006). Thus, vegetation mappings were only conducted along a reach of up to 600 m upstream of a respective sampling location to obtain an adequate estimate of the plant cover responsible for the release of the fragments collected. The species-specific plant coverage (in percent of stream surface area) was thereby estimated for 10 m stream sections and combined with data on the stream surface area of each 10 m section (*A*), which was determined by using polygons in Google Earth (Google Inc., CA, USA). The specific plant cover of a species across the upstream reach of a sampling location was then calculated as the sum of the specific cover of each 10 m section:

$$\text{Specific plant cover (m}^2\text{)} = \sum_{i=1}^{n} \frac{\text{specific coverage}_i}{100} \times A_i \qquad (1)$$

By combining the data on the mean number of drift units per day at each sampling site with the data on specific plant cover, species-specific fragmentation rates were calculated for each stream and sampling date as follows:

$$\text{Fragmentation rate }(\mathrm{m}^{-2}\mathrm{d}^{-1}) = \frac{\sum_{i=1}^{n} \bar{x}_{\text{No. of drift units}_i}}{\sum_{j=1}^{k} \text{specific plant cover}_j} \qquad (2)$$

Due to the inaccuracy of estimating coverage for very small populations, fragmentation rates were only calculated for a species when the sum of the specific plant cover in the upstream reaches of all sampling locations of a stream was >5 m² to avoid unrealistically high fragmentation rates.

Determination of regeneration/colonization potential

Following the evaluation of the plant fragments collected in the field, the potential for regeneration/colonization indicated by root formation was examined in a laboratory experiment. Therefore, the relative root formation of fragments without pre-existing roots was documented per species and sampling location according to five size classes (fragment length: <5, 5-10, 10-15, 15-20, 20-30 cm). Larger plant fragments >30 cm in length were not further included in the regeneration/colonization trials, because the likelihood of regeneration is generally expected to increase with increasing fragment size and most fragments of this size already possessed roots. The trials were only conducted with fragments collected during July, August and October. Randomly selected plant fragments of the four replicates obtained from a given sampling location were pooled according to species and size class and grown under standardized conditions in 0.25, 0.7 or 5 L plastic containers. The container size differed according to the absolute number of fragments collected for a given fragment size class of a species to provide approximately similar ratios of plant biovolume to water medium. For example, a single large fragment of *Myriophyllum spicatum* (size class: 20-30 cm) was placed in a 0.7 L container, while 42 small fragments of *Callitriche* spp. (size class: <5 cm) were placed in a 5 L container. Overall, 416 containers were prepared, each including 1 to 42 plant fragments (1,840 fragments in total; for detailed information see supplementary Table S1). All plastic containers were filled with a modified general purpose medium for aquatic plant cultivation (acc. Smart & Barko, 1985) containing 2 mg NO_3^--N L^{-1} and 0.1 mg PO_4^{3-}-P L^{-1}. The fragments were grown at room temperature under a lighting installation with fluorescent lamps (Lumilux cool daylight, OSRAM Licht AG, Munich, Germany) and exposed to a L:D 16:8 (h light:dark) simulated photoperiod at a light intensity of 96.5 ± 19.4 µmol photons m^{-2} s^{-1} (± *SD*; measured above the containers) for 28 days.

The regeneration/colonization trials lasted over 28 days as most fragments initiate growth of new roots and shoots within this period (e.g. Kuntz *et al.*, 2014; Heidbüchel & Hussner, 2019). The onset of root formation was assessed on a weekly basis by checking for roots and measuring root length. Concomitantly, the medium was exchanged and containers were cleaned weekly to restore nutrients and to mitigate algal growth. Once a fragment developed new roots >1 cm in length, it was considered as successfully regenerated through root formation, while completely degraded fragments were deemed as died off. Fragments classified as successfully regenerated were subsequently excluded from the experiment.

Statistical analysis

Statistical analysis of the data was undertaken in R version 3.5.1 (R Core Team). In order to test the combined effects of sampling location and sampling date (month) on number of drift units, data on number of drift units was transformed (+1) to obtain positive values prior to applying a generalized linear model (GLM) with gamma distribution. Due to strong violation of homoscedasticity and normality, differences among the species studied within all streams in number of drift units, number of potential propagation units and drift unit size (i.e. (1) potential propagation units per drift unit and (2) drift unit length) were assessed by Kruskal-Wallis tests, followed by pairwise Mann-Whitney *U* tests (FDR-corrected) for multiple comparison. Pearson product-moment correlations were conducted to check the linear relationship between the natural log (ln) of number of drift units and the ln of specific plant cover in the upstream reaches, respectively. Furthermore, the species-specific fragmentation rates in the different streams were analyzed by one-way ANOVA, followed by Tukey's HSD post-hoc test for multiple comparison after data was ln-transformed to achieve homoscedasticity.

For six species, the likelihood for root formation of fragments collected in the field depending on the previously defined size classes was assessed by mixed effects Cox proportional hazards models (CoxPHme) with sampling location and sampling date (month) specified as random effects. These analyses were only carried out when the data set (number of fragments without pre-existing roots used in the regeneration/colonization trials) for a given species was sufficiently comprehensive ($n \geq 20$; *Callitriche* spp., *Myriophyllum spicatum*, *Myriophyllum heterophyllum*, *Stuckenia pectinata*, *Elodea nuttallii*, *Elodea canadensis*). Within the CoxPHme models, sampling location and sampling date were not included as explanatory variables, as the likelihood of root formation was analyzed under standardized conditions in the lab. Instead, random intercepts were specified for sampling location and sampling date, respectively, to compensate for potential differences in fragment vitality at the initial of the regeneration/colonization trials. The assumption of proportional hazards was checked for each model by statistical tests and visual inspection of the scaled Schoenfeld residuals. Hazard ratios (HR) >1 and <1 indicate an increased or decreased likelihood of root formation at each point in time with increasing size class of a species, respectively, while a HR of 1 indicates no difference between different size classes.

Results

Fragment drift and fragmentation rate

Overall, the number of drift units differed significantly among the sampling locations and sampling dates (sampling location x month: $\chi^2_{(39,\, n = 224)} = 333.75$, $P < 0.0001$) and generally tended to increase with larger stream size (Figure 2a). The lowest number of drift units on overall average was documented for the smallest stream (Gustorfer Graben: 5 ± 1 h^{-1}), followed by the streams Düssel (34 ± 8 h^{-1}), Brückerbach (222 ± 87 h^{-}1) and Gillbach (243 ± 75 h^{-1}), while the highest number of drift units was found for the largest stream investigated (Niers: 1,134 ± 306 h^{-1}; ± 1 *SE*). In total, fragments of 13 aquatic plant taxa were collected at the sampling locations, whereby the species composition of the fragment drift at a respective sampling location was relatively consistent throughout the study (Figure 2b). Among the species, particularly *Callitriche* spp., *Myriophyllum spicatum* and *Stuckenia pectinata* contributed to the number of drift units collected in the streams.

A maximum number of 5,160 drift units h^{-1} was documented for *Callitriche* spp. (mean of 514 h^{-1}), which showed significantly higher numbers of drift units than the other species, except for *Myriophyllum heterophyllum* (Figure 3a). Additionally, high means of 104 and 44 drift units h^{-1} were documented for *S. pectinata* and *M. spicatum*, respectively, whereas the majority of the remaining species showed mean values not exceeding 10 drift units h^{-1}. As with number of drift units, differences among the species were almost identical for the number of potential propagation units, with a maximum of 102,672 potential propagation units h^{-1} observed for *Callitriche* spp. (Figure 3b). The highest mean numbers of potential propagation units were documented for *Callitriche* spp. (7,026 h^{-1}), followed by *S. pectinata* (1,556 h^{-1}), *M. spicatum* (1,128 h^{-1}) and *M. heterophyllum* (1,072 h^{-1}), while the means for the majority of the other species were lower than 100 potential propagation units h^{-1}. For many species, fragments were frequently absent in samples collected at the sampling locations where a respective species occurred, which is why medians for numbers of drift units and potential propagation units are often zero (Figure 3a,b).

Among the species, strong differences existed in drift unit size expressed as number of potential propagation units per drift unit ($\chi^2_{(12,\, n = 6124)} = 551.79$, $P < 0.0001$) and drift unit length ($\chi^2_{(12,\, n = 6124)} = 753.16$, $P < 0.0001$), respectively (Figure 3c,d). Drift unit size ranged from a minimum of 1 potential propagation unit per drift unit and corresponding drift unit length of 0.1 cm (*Callitriche* spp., *Elodea nuttallii*, *Ceratophyllum demersum*) up to a maximum of 15,391 potential propagation units per drift unit and corresponding drift unit length of 10,698.7 cm (patch of *Callitriche* spp.), with overall medians of 7 potential propagation units per drift unit and 5.6 cm drift unit length. The mean number of potential propagation units per drift unit was lowest for *Vallisneria spiralis* (1 drift unit^{-1}) and highest for *M. heterophyllum* (68 drift unit^{-1}), whereas the mean drift unit length was lowest for *Myriophyllum aquaticum* (2.9 cm) and highest for *Sparganium emersum* (48.5 cm). Particularly *V. spiralis* and *S. emersum* were characterized by a low number of potential propagation units per drift unit that differed significantly from most other species, but, by contrast, showed relatively high drift unit lengths.

When taking into account the species-specific plant cover in upstream reaches, there was a poor linear relationship between the natural log of number of drift units and the natural log of specific plant cover for the streams Gustorfer Graben, Düssel, Brückerbach and Niers, which was reflected by low r^2 values ≤ 0.30 (Figure 4a-c,e). For the stream Gillbach, however, the number of drift units was well and significantly correlated with specific plant cover (Figure 4d).

The estimated fragmentation rates differed significantly among the species and tended to show stream-specific differences ($F_{(18,\, 57)} = 4.01$, $P < 0.0001$; Figure 5). Nevertheless, the differences observed between fragmentation rates of the same species in different streams were not significant. The highest fragmentation rate of 51 ± 10 drift units m^{-2} specific plant cover d^{-1} was documented for *Callitriche* spp. in the river Niers, which was significantly higher than the fragmentation rates of the other species, except for *S. pectinata* and *M. spicatum* in the Gillbach (24 ± 6 and 21 ± 13 drift units m^{-2} d^{-1}, respectively) and *E. nuttallii* in the river Niers (17 ± 9 drift units m^{-2} d^{-1}; ± 1 *SE*). Conversely, fragmentation rates of *M. heterophyllum*, *Egeria densa*, *C. demersum*, *V. spiralis* and *S. emersum* were very low, with mean values <1 drift unit m^{-2} specific plant cover d^{-1}.

Regeneration/colonization potential of fragments

Within the study, the presence of roots was used as indicator for fragment viability and the potential for anchorage in the sediment. The proportion of drift units with pre-existing roots was distinctly different among the species, though differences in the absolute number of drift units must be considered (Table 3). For *M. heterophyllum*, *V. spiralis* and *S. emersum*, high relative numbers of collected drift units with pre-existing roots were documented (61-80% of drift units), while the other species showed notably lower proportions of drift units possessing roots.

When exposed to standardized laboratory conditions, the relative root formation and mortality of plant fragments collected in the field varied strongly among the species and fragment size classes (Table 4, Figure 6). Overall, fragments of *E. nuttallii* showed the highest root formation capacity (70% of fragments), followed by *M. spicatum* (64%), *E. canadensis* (57%), *S. pectinata* (52%) and *M. heterophyllum* (45%), while only 23% of *Callitriche* fragments developed new roots. The vast majority of plant fragments developed new roots within 14 days (overall, 84% of fragments that successfully regenerated through root formation). For all species, the likelihood of root formation increased significantly with fragment size (Figure 6). Each increase in fragment size class enhanced the likelihood of root formation by a factor of 1.2 (*M. spicatum*, HR = 1.21, 95% CI = 1.09-1.33, *P* <0.001) to 2.8 (*E. canadensis*, HR = 2.78, 95% CI = 1.53-5.04, *P* <0.001). Likewise, fragment mortality tended to decrease with increasing fragment size class in all species and was lowest for the *Elodea* species (*E. canadensis*, 0% of all fragments; *E. nuttallii*, 10%), whereas fragments of *Callitriche* spp. showed the highest mortality (57%; Table 4). However, it must be noted that the absolute numbers of fragments used in the regeneration/colonization trials differed considerably among the species and size classes.

Discussion

The dispersal of aquatic plants through plant fragments is of particular relevance in running waters as the water current facilitates fragment formation, and thus naturally enhances propagule pressure (Riis & Sand-Jensen, 2006; Heidbüchel *et al.*, 2016). We found that the number of drifting plant fragments generally tends to increase with increasing stream size. Yet it must be noted that the quantity of fragments produced may vary considerably along a stream, mainly due to differences in biomass and species composition of the aquatic vegetation, species-specific biomechanical properties and the strong habitat heterogeneity of streams. When it comes to analyzing the effect of stream size in more detail, it is further recommended to normalize for discharge by calculating the number of drift units per m^3 water. Moreover, local differences in flow can affect plant growth and fragmentation (Riis & Biggs, 2003; Redekop *et al.*, 2016). Hence, the fragmentation rate of a species may be higher when inhabiting more lotic sites, even though the phenotypic plasticity of aquatic plants must be considered (Puijalon *et al.*, 2008; Riis *et al.*, 2010; Miler *et al.*, 2014). Within our study, in situ fragmentation rates were species- rather than stream-specific, supporting the view that aquatic plants are characterized by a high phenotypic plasticity and adapt to the local hydraulic conditions. Strong differences in species-specific fragmentation rate among co-occurring species further contribute to the weak correlations between the number of drift units and the respective plant coverage of a species observed for almost all streams. For the stream Gillbach, by contrast, the number of drifting fragments was well correlated with specific plant coverage, which is mainly due to similar fragmentation rates of the most dominant species *Myriophyllum spicatum* and *Stuckenia pectinata*. Nevertheless, it must be noted that the local flow conditions experienced by the individual plant stands were not quantified. Earlier studies already showed that increased discharge, especially during flood events, can promote the number of drifting propagules and may often lead to uprooting of large macrophyte patches (Boedeltje *et al.*, 2004; Heidbüchel *et al.*, 2016). As we conducted sampling of the fragment drift under normal runoff conditions, the documented numbers of drifting propagules and fragmentation rates are expected to increase at higher levels of discharge. Still, our results demonstrate that, even at normal discharge, the extent of the fragment drift in smaller streams can be high, and may be vastly increased in larger rivers (cf. Heidbüchel *et al.*, 2016). Most notably, mechanical weed control results in the formation of numerous plant fragments (Owens *et al.*, 2001; Riis, 2008), but is a common measure against invasive alien aquatic plant species (Hussner *et al.*, 2017). Thus, mechanical weed control can substantially enhance the propagule pressure and rapid spread of a target invasive aquatic plant species (Anderson, 1998).

Uprooting of larger plant stands may be initiated by strong local disturbances, e.g. foraging by herbivores (Bakker *et al.*, 2016), and facilitated by the ongoing drag forces imposed by flow (Sand-Jensen, 2003, 2008). This seems reasonable as we collected large drift units up to >100 m in total length (patch of *Callitriche* spp.). Yet, sediment erosion during floods is regarded as the main cause for uprooting of aquatic vegetation (Riis & Biggs, 2003), emphasizing that the resistance against uprooting not only depends on the species-specific biomass allocation to roots and root architecture but also on sediment characteristics

(Schutten *et al.*, 2005; Sand-Jensen & Møller, 2014). As all field surveys were generally conducted at normal discharge, uprooting is expected to have played only a minor role compared to fragmentation in our study. The firm root anchorage within the sediment, short stems and belowground or ground-based formation of ramets in species such as the rhizome-forming *Sparganium emersum* and the stoloniferous *Vallisneria spiralis* cause great resistance against stem breakage and uprooting (Liffen *et al.*, 2011; Pollen-Bankhead *et al.*, 2011) and thus explain the low fragment dispersal capacities observed for these species. The benefits of clonal integration, however, facilitate effective dispersal in the direct vicinity and largely determine the spread potential and invasion success of many species, including *Myriophyllum aquaticum*, *Alternanthera philoxeroides* (Mart.) Griseb. and the free-floating *Eichhornia crassipes* (Mart.) Solms which are listed as invasive alien species within the European Union (EU regulation No. 1143/2014; Xiao *et al.*, 2007, 2011; You *et al.*, 2013a; Wang *et al.*, 2016b, 2017; Yu *et al.*, 2019). Conversely, species like *M. spicatum*, *S. pectinata*, *Elodea* spp. and *Callitriche* spp. produce numerous fragments as the force at which stem breakage (but also uprooting) occurs is relatively low (Schutten *et al.*, 2005; Miler *et al.*, 2014). This is also reflected by the fragmentation rates documented in our study and highlights the high propagule pressure of these species within running waters. Particularly in the case of the fragile *Callitriche* spp., the low specific breaking strength (Miler *et al.*, 2014) largely explains the high fragmentation rates observed. Interestingly, fragmentation rates of the invasive alien *Myriophyllum heterophyllum* seem to be very low. It must however be considered that this species grew at rather low flow conditions in the stream Düssel and is in general sturdy in structure.

As many drifting plant fragments are rather large in size but most aquatic plant species are able to regenerate even from small fragments consisting of a single node (Langeland & Sutton, 1980; Kuntz *et al.*, 2014; Bickel, 2017), the number of potential propagation units of a fragment may be high. Indeed, we show that the number of dispersed potential propagation units often differs from the number of drift units by a magnitude of 10. Hence, further fragmentation over the duration of downstream drift may strongly increase the propagule pressure of a species, depending on the specific biomechanical properties and the degree of external disturbances. This is of particular relevance for species with a high likelihood of regeneration even from small fragments, such as *M. spicatum* and the invasive *Elodea nuttallii*.

In order to establish new populations, drifting propagules must be retained and settle at suitable habitats. The drift distance of plant fragments is limited by all kind of physical obstacles such as stones or aquatic and riparian vegetation (Johansson & Nilsson, 1993; Riis & Sand-Jensen, 2006; Riis, 2008). Thus, a higher coverage of vegetation and number of obstacles in upstream reaches have likely limited the amount of drifting plant fragments collected at a sampling location of the streams studied. Patch dynamics, species-specific flexibility and reconfiguration potential of the present vegetation as well as the floating characteristics of the drifting fragments, however, differently affect fragment retention (Sand-Jensen, 2003; Riis & Sand-Jensen, 2006; Sarneel, 2013; Cornacchia *et al.*, 2019). The degree of fragment buoyancy varies among the species, and while fragments of e.g. *Myriophyllum* spp. float at the water surface, buoyancy of *Elodea canadensis* and the invasive species *E. nuttallii* and *Lagarosiphon major* (Ridley) Moss is rather low as fragments are neutrally buoyant (Riis & Sand-Jensen, 2006; Cornacchia *et al.*, 2019; Heidbüchel & Hussner, 2019).

Even though the amount of drifting plant fragments can be enormous, only a small proportion will successfully establish. In streams, initial colonization (i.e. first anchorage within the sediment) was recognized as the main bottleneck limiting the establishment success of aquatic plant fragments (Riis, 2008). Our results indicate that fragmentation rates of *Callitriche* spp. are very high, whereas the colonization potential (as indicated by relative root formation) of *Callitriche* fragments seems to be very low, at least under our experimental conditions. Consequently, despite the high propagule pressure, the vegetative spread potential of *Callitriche* spp. is to a large extent limited by its low regeneration capacity and failure of initial colonization. Although the number of released drift units of *S. emersum*, *V. spiralis* and the invasive *M. heterophyllum* seems to be low, the large fragment size and the fact that most drift units already possessed roots are assumed to benefit colonization success. Smaller fragments of *M. heterophyllum* are further characterized by a weak potential for regeneration (see also Heidbüchel & Hussner, 2019; Heidbüchel *et al.*, 2019a), indicating that the low fragment dispersal capacity in general and the low propagule pressure in particular are not explaining the invasiveness of this species.

We show that the likelihood for regeneration and colonization of drifting fragments collected in the field increases significantly with increasing fragment size (see also Redekop *et al.*, 2016; Bickel, 2017). In our study, particularly in the case of *E. nuttallii*, the combination of relatively high fragmentation rates and high root formation capacities highlights the strong vegetative spread potential of this species and its role as successful invader. Similarly, a recent mesocosm study documented higher fragmentation and regeneration rates of *E. canadensis* compared to *Egeria densa* and *L. major*, two species also known for their invasiveness (Redekop *et al.*, 2016). This illustrates that fragmentation and regeneration rates can only partly explain the invasion success of some species. However, it is well known that the regeneration and colonization success of plant fragments is controlled by the environmental conditions of the receiving habitat (Franklin *et al.*, 2008; Bornette & Puijalon, 2011) and may show seasonal variation (Barrat-Segretain & Bornette, 2000). As abiotic factors such as temperature, light, carbon and nutrient availability influence plant growth and fragment regeneration (Riis *et al.*, 2012; Kuntz *et al.*, 2014; Hussner *et al.*, 2015), the documented root formation capacities may strongly rely on our experimental conditions and can be substantially different under field conditions. Species-specific optima in abiotic factors such as nutrient availability are likely to occur, which is why some species may have been favored, while others may have been adversely affected by the experienced conditions in our experiment

In conclusion, the fragment dispersal capacity and thus the vegetative spread potential through plant fragments is highly species-specific, but may vary significantly between different aquatic systems. Propagule pressure must be considered as one of the major drivers for the successful spread and invasiveness of species characterized by high fragmentation rates as well as high regeneration and colonization capacities, e.g. *M. spicatum*, *S. pectinata* and *E. nuttallii* (Riis *et al.*, 2009; Umetsu *et al.*, 2012a; Vári, 2013; Redekop *et al.*, 2016). It must be considered, however, that the successful establishment of plant fragments is strongly influenced by the environmental conditions of the receiving habitat and can vary throughout the season (Barrat-Segretain & Bornette, 2000; Riis & Biggs, 2003; Riis *et al.*, 2012; Kuntz *et al.*, 2014). Therefore, further studies on initial fragment colonization and establishment under field conditions are highly required (but see Riis, 2008). Finally, as phenotypic plasticity is known to affect the biomechanical properties of a plant species (Miler *et al.*, 2014), there is a great need for detailed information on the species-specific fragmentation rate in the field to extend our understanding of the relevance of propagule pressure for the vegetative spread potential and invasion success of aquatic plant species.

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Table 1 Sampling schedule and stream properties at the sampling locations of the five streams studied during the investigation period

					Sampling date			
Stream	Sampling location	Stream width (cm)	Water depth[1] (cm)	Flow velocity[1] (m s^{-1})	June	July	August	October
Gustorfer Graben	GG1	210	23.5-38.0	0.08-0.19	03/06/2017	06/07/2017	02/08/2017	02/10/2017
	GG2	170	14.0-21.0	0.23-0.30	03/06/2017	06/07/2017	02/08/2017	02/10/2017
Düssel	D1	370	28.0-37.0	0.23-0.33	26/06/2017	19/07/2017	04/08/2017	13/10/2017
	D2	670	53.5-77.0	0.06-0.08	26/06/2017	19/07/2017	04/08/2017	13/10/2017
Brückerbach	BB1	570	52.0-82.5	0.09-0.29	22/06/2017	21/07/2017	07/08/2017	13/10/2017
	BB2	785	68.5-87.5	0.06-0.14	22/06/2017	21/07/2017	07/08/2017	13/10/2017
	BB3	650	41.0-51.5	0.11-0.40	22/06/2017	21/07/2017	07/08/2017	13/10/2017
Gillbach	GB1	480	45.0-58.0	0.34-0.42	03/06/2017	14/07/2017	14/08/2017	02/10/2017
	GB2	460	30.0-36.0	0.58-0.64	03/06/2017	14/07/2017	14/08/2017	02/10/2017
	GB3	400	55.0-58.5	0.30-0.30	03/06/2017	14/07/2017	14/08/2017	02/10/2017
Niers	N1	550	148.0-151.0	0.05-0.06	10/06/2017	22/07/2017	04/08/2017	11/10/2017
	N2	880	82.0-86.0	0.10-0.10	10/06/2017	22/07/2017	04/08/2017	11/10/2017
	N3	560	67.0-79.0	0.10-0.15	10/06/2017	22/07/2017	04/08/2017	11/10/2017
	N4	600	31.0-37.0	0.30-0.38	10/06/2017	22/07/2017	04/08/2017	11/10/2017

[1] For water depth and flow velocity, minimum/maximum values are shown. Values were determined mid-stream

Table 2 Regenerative subunits, i.e. potential propagation units, of drift units considered for the species collected within this study and corresponding references

Species	Propagation units considered for regeneration	References
Callitriche spp.	Single nodes of stem fragment	This study
Myriophyllum spicatum L.	Single nodes of stem fragment	Kuntz *et al.* (2014)
Myriophyllum heterophyllum Michx.	Single nodes of stem fragment	Kuntz *et al.* (2014)
Myriophyllum aquaticum (Vell.) Verdc.	Single leaves and single nodes of stem fragment	Hussner (2009); Kuntz *et al.* (2014)
Stuckenia pectinata L.	Single nodes of stem fragment	This study
Potamogeton crispus L.	Single nodes of stem fragment	Fritschler *et al.* (2008)
Potamogeton pusillus agg.	Single nodes of stem fragment	This study
Elodea nuttallii Planch. St. John	Single nodes of stem fragment	This study
Elodea canadensis Michx.	Single nodes of stem fragment	This study
Egeria densa Planch.	Double nodes of stem fragment	Riede (1920); Fritschler *et al.* (2008)
Ceratophyllum demersum L.	Single nodes of stem fragment	Kuntz *et al.* (2014); Fritschler *et al.* (2008)
Vallisneria spiralis L.	Whole shoots	Sculthorpe (1967)
Sparganium emersum Rehmann	Whole shoots	Sculthorpe (1967)

Table 3 Number of drift units with pre-existing roots (w/ roots) for the species collected within this study

Species	Absolute No. of drift units	Absolute No. of drift units w/ roots	Relative No. of drift units w/ roots (%)
Callitriche spp.	3,751	767	20
Myriophyllum spicatum	800	106	13
Myriophyllum heterophyllum	57	35	61
Myriophyllum aquaticum	20	2	10
Stuckenia pectinata	1,180	105	9
Potamogeton crispus	23	0	0
Potamogeton pusillus agg.	8	3	38
Elodea nuttallii	197	27	14
Elodea canadensis	11	3	27
Egeria densa	7	2	29
Ceratophyllum demersum	4	0	0
Vallisneria spiralis	59	43	73
Sparganium emersum	10	8	80

Table 4 Fragment survival, relative root formation (as indicator for regeneration-/colonization potential) and fragment mortality of selected species depending on fragment size class after 28 days of growth under standardized conditions

Species	Fragment size class	n	Fragment survival		Fragment mortality
			Rel. root formation	No root formation	
	(cm)		(%)	(%)	(%)
Callitriche spp.	<5	486	23	19	58
	5-10	232	23	21	56
	10-15	81	19	22	59
	15-20	46	24	17	59
	20-30	16	38	31	31
Myriophyllum spicatum	<5	99	49	1	49
	5-10	117	62	2	37
	10-15	68	68	0	32
	15-20	55	69	4	27
	20-30	43	88	2	9
Myriophyllum heterophyllum	<5	3	0	67	33
	5-10	8	25	38	38
	10-15	5	60	20	20
	15-20	0	NA	NA	NA
	20-30	4	100	0	0
Stuckenia pectinata	<5	135	32	30	38
	5-10	105	61	7	32
	10-15	72	56	15	29
	15-20	45	73	4	22
	20-30	27	78	7	15
Elodea nuttallii	<5	69	54	29	17
	5-10	38	82	13	5
	10-15	24	92	8	0
	15-20	5	100	0	0
	20-30	6	83	17	0
Elodea canadensis	<5	12	25	75	0
	5-10	7	100	0	0
	10-15	1	100	0	0
	15-20	1	100	0	0
	20-30	0	NA	NA	NA

Shown are data for fragments collected in July, August and October and corresponding n values. NA indicates no fragments collected (n = 0)

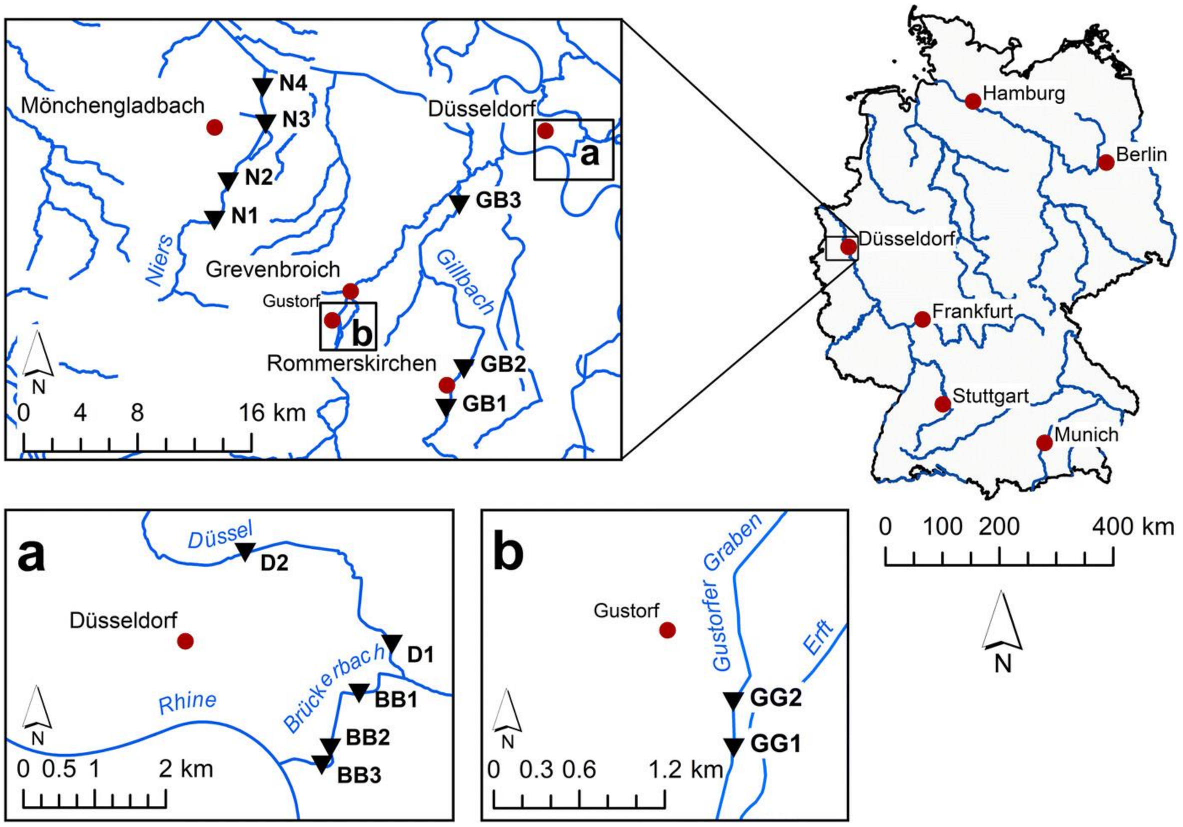


Figure 1 Locations of the sampling sites along the five streams Gustorfer Graben (GG), Düssel (D), Brückerbach (BB), Gillbach (GB) and Niers (N) within the Lower Rhine region, Germany

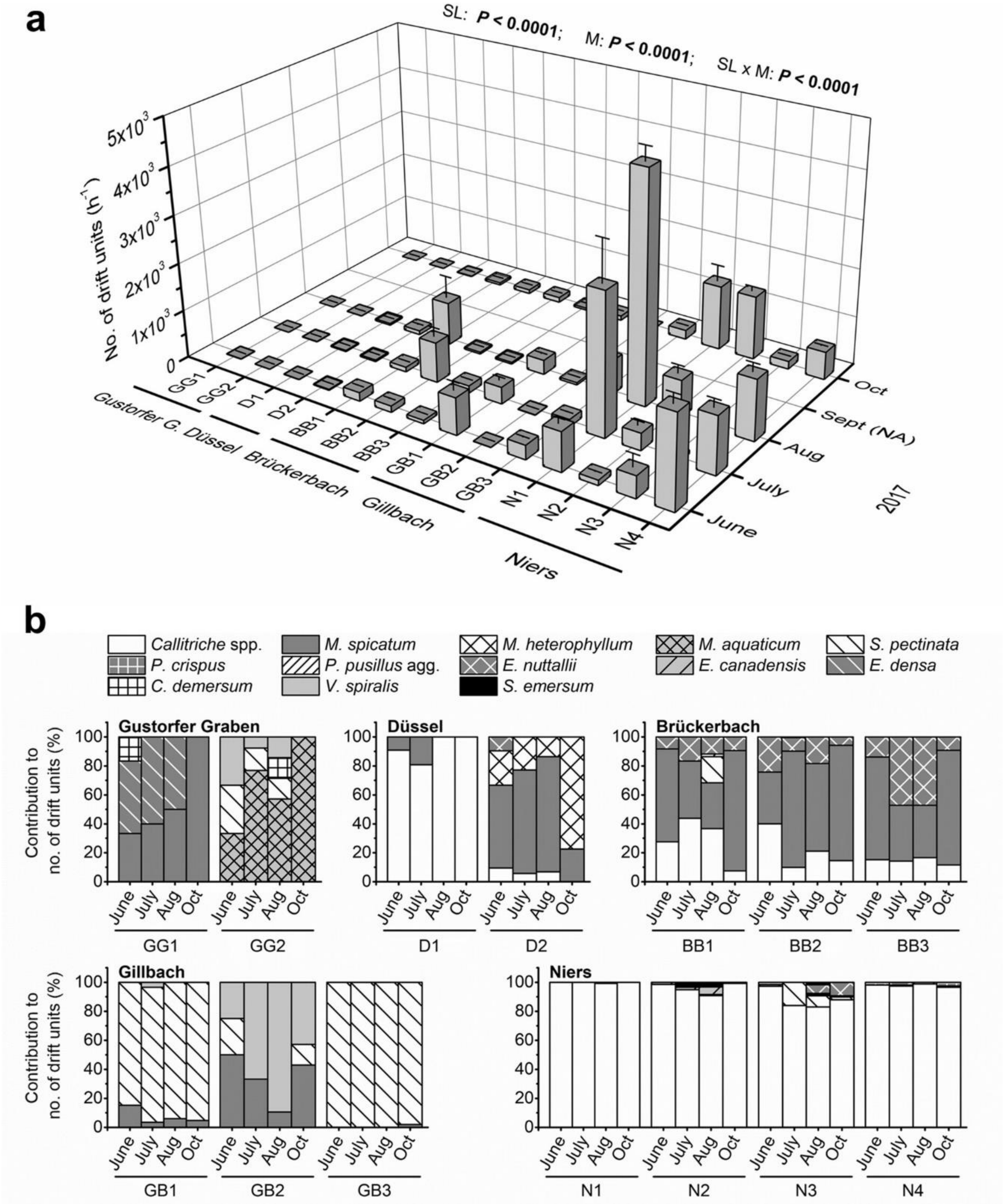
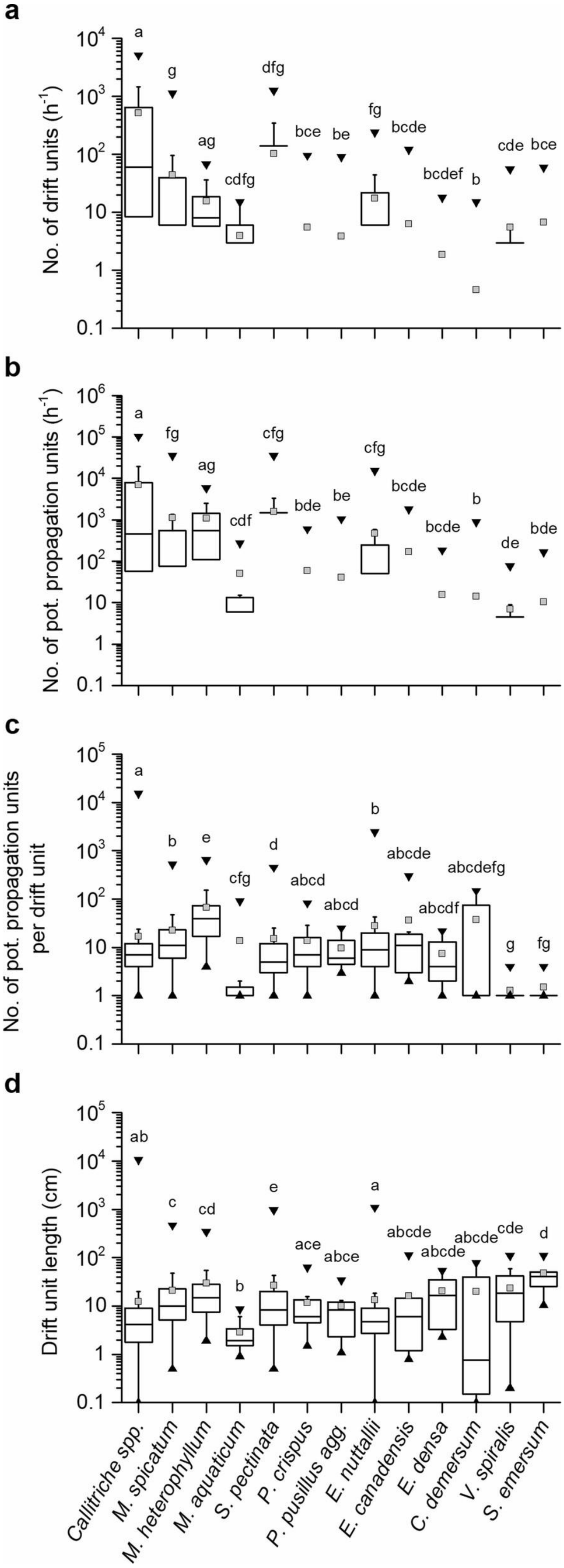


Figure 2 a Number of drift units at the sampling locations of the five streams over the investigation period (per month) and **b** corresponding species compositions of collected plant fragments. Shown are mean values (± 1 *SE* in **a**). Significant effects of sampling location (SL) and month (M) on No. of drift units in **a** and of the interaction term (SL x M) are indicated by *P* values written in bold (*P* <0.05; GLM). Exact sampling dates within a respective month for each sampling location are shown in Table 1



◄ **Figure 3 a** Number of drift units, **b** number of potential propagation units and drift unit size (**c** number of potential propagation units per drift unit and **d** drift unit length) for each species sampled at the sampling locations where a respective species was present. Shown are 25th and 75th percentiles (bottom and top of boxes, respectively), medians (horizontal lines in boxes), means (grey squares), 1.5 × interquartile ranges (whiskers) and the minimum/maximum values (▲ and ▼, respectively). Different *letters* indicate significant differences ($P < 0.05$; pairwise Mann-Whitney *U* tests)

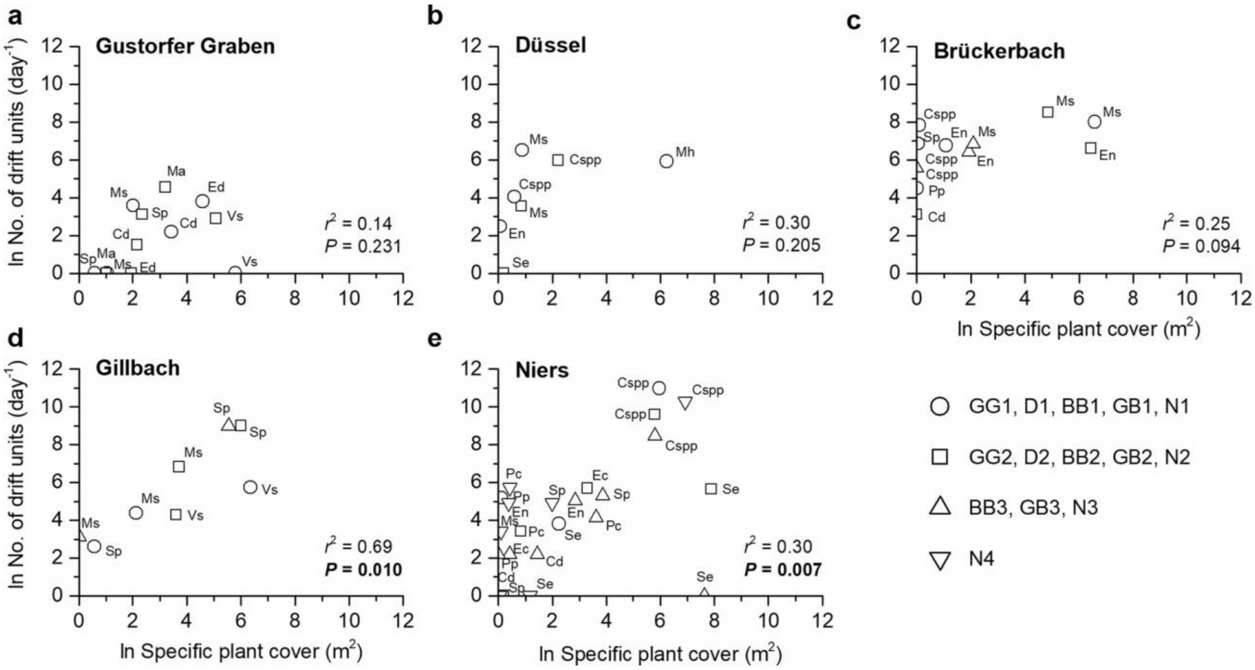


Figure 4 Correlations between the natural log (ln) of number of drift units and the ln of specific plant cover in reaches located upstream of the sampling sites for each species and sampling location of the five streams **a** Gustorfer Graben, **b** Düssel, **c** Brückerbach, **d** Gillbach and **e** Niers. Shown are mean values of replicates of the whole investigation period for each species and sampling location as well as corresponding coefficients of determination. Significant correlations are indicated by P values written in bold (P <0.05; Pearson correlation). Cspp, *Callitriche* spp.; Ms, *Myriophyllum spicatum*; Mh, *Myriophyllum heterophyllum*; Ma, *Myriophyllum aquaticum*; Sp, *Stuckenia pectinata*; Pc, *Potamogeton crispus*; Pp, *Potamogeton pusillus* agg.; En, *Elodea nuttallii*; Ec, *Elodea canadensis*; Ed, *Egeria densa*; Cd, *Ceratophyllum demersum*; Vs, *Vallisneria spiralis;* Se, *Sparganium emersum*)

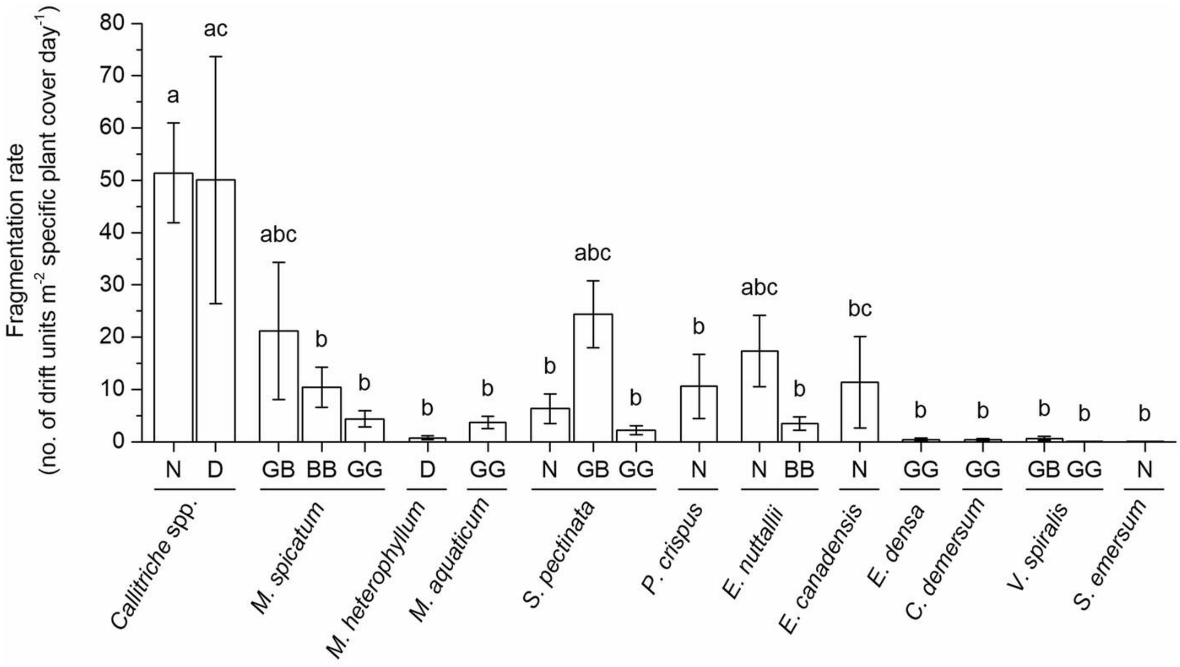


Figure 5 Species-specific fragmentation rates within the five streams Gustorfer Graben (GG), Düssel (D), Brückerbach (BB), Gillbach (GB) and Niers (N) based on the sampling locations studied. Shown are mean values ± 1 *SE*. Different *letters* indicate significant differences (P <0.05; Tukey's HSD test)

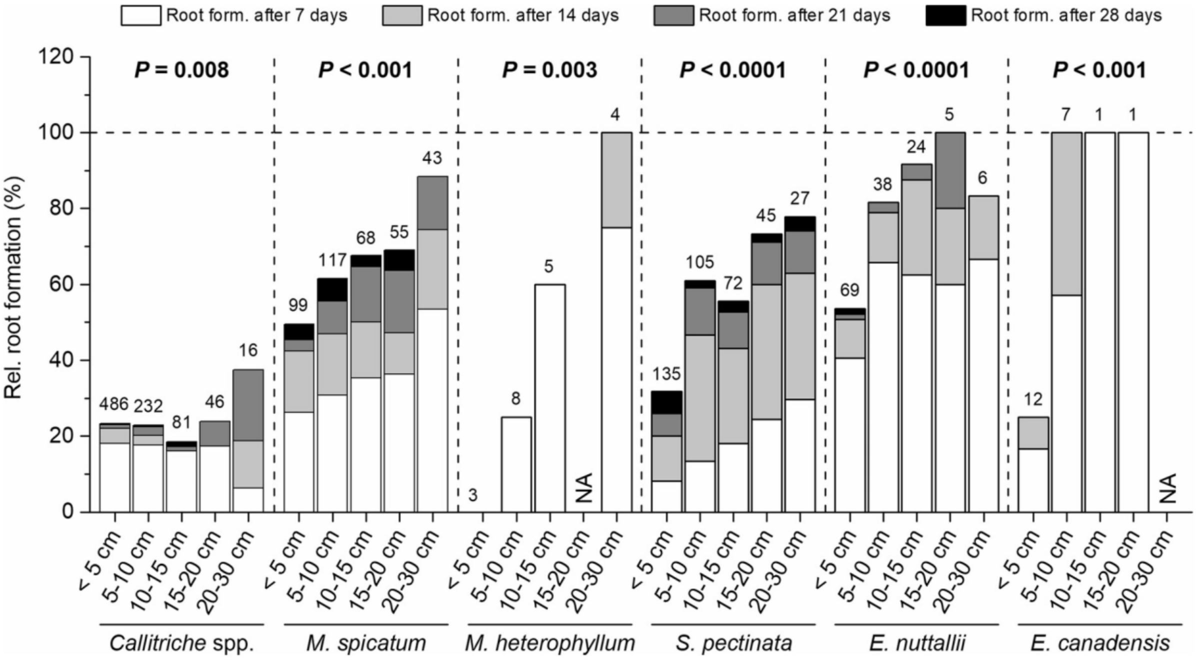


Figure 6 Relative root formation (as indicator for regeneration/colonization potential) of selected species depending on fragment size class. Shown are data for fragments collected in July, August and October and corresponding n values above the columns. NA indicates no fragments collected (n = 0). Significant effects among increasing size classes of a species are indicated by *P* values written in bold (*P* <0.05; CoxPHme)

Supporting information

Table S1 Total number of plastic containers used for growing the plant fragments, total number of fragments without pre-existing roots and the number of fragments without pre-existing roots per container (minimum/maximum values) for the species and fragment size classes included in the regeneration/colonization trials within this study

Species	Fragment size class (cm)	*n* containers	*n* fragments	*n* fragments per container
Callitriche spp.	<5	46	486	1-42
	5-10	37	232	1-26
	10-15	22	81	1-10
	15-20	19	46	1-5
	20-30	10	16	1-3
Myriophyllum spicatum	<5	29	99	1-15
	5-10	27	117	1-20
	10-15	20	68	1-13
	15-20	18	55	1-17
	20-30	20	43	1-10
Myriophyllum heterophyllum	<5	3	3	1
	5-10	4	8	1-5
	10-15	3	5	1-3
	20-30	2	4	2
Myriophyllum aquaticum	<5	1	5	5
	5-10	2	4	1-3
Stuckenia pectinata	<5	16	135	1-34
	5-10	16	105	1-17
	10-15	15	72	1-11
	15-20	12	45	1-7
	20-30	13	27	1-4
Potamogeton crispus	<5	2	2	1
	5-10	1	2	2
	20-30	1	1	1
Potamogeton pusillus agg.	<5	2	2	1
	5-10	2	2	1
Elodea nuttallii	<5	18	69	1-9
	5-10	17	38	1-6
	10-15	14	24	1-4
	15-20	3	5	1-3
	20-30	3	6	1-3
Elodea canadensis	<5	4	12	1-6
	5-10	4	7	1-3
	10-15	1	1	1
	15-20	1	1	1
Egeria densa	<5	1	1	1
	10-15	1	1	1
	15-20	1	1	1
Ceratophyllum demersum	<5	2	2	1
Vallisneria spiralis	<5	1	2	2
	5-10	1	1	1
	20-30	1	4	4

CHAPTER 3 |

Falling into pieces: In situ fragmentation rates of submerged aquatic plants and the influence of discharge in lowland streams

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Abstract

The formation and dispersal of viable plant fragments are considered as key determinants for the vegetative spread potential and competitive strength of submerged aquatic macrophytes. Although it is known that the disturbance by flow facilitates fragment dispersal in streams, detailed information on in situ fragmentation rates and the influence of discharge are still lacking. We determined the fragmentation rates (i.e. number of fragments per biovolume of a given species) of the four widespread aquatic plant species *Myriophyllum spicatum*, *Potamogeton crispus*, *Elodea canadensis* and *Elodea nuttallii* in defined sections of small to medium-sized German streams with different flow regimes. We further measured chlorophyll *a* fluorescence (F_v/F_m: maximum quantum yield of photosystem II) to denote the viability of plant fragments collected in the field. We documented stream- and species-specific differences in fragmentation rate, ranging from 74 ± 20 (*P. crispus*) up to 1,260 ± 376 fragments m^{-3} specific biovolume d^{-1} (*E. canadensis*; ± *SE*). Fragmentation rates generally increased in streams characterized by rather high discharge conditions but were negligibly associated with minor discharge fluctuations at a given stream section. This effect was significant for *M. spicatum* and *E. nuttallii*, but not for *P. crispus* and *E. canadensis*. Overall, a high portion of fragments was viable, as indicated by F_v/F_m values >0.58 in 95% of all fragments. Our results demonstrate that fragmentation rates of submerged aquatic plants are to a certain extent controlled by the discharge conditions of a stream and highlight the strong vegetative spread potential of the four species studied.

Keywords aquatic macrophytes, chlorophyll fluorescence, hydrochory, invasive species, propagule pressure, regeneration, spread potential

Introduction

The number of aquatic plant propagules introduced into a new habitat (i.e. propagule pressure) is considered as the key factor for successful establishment, subsequent spread and the invasiveness of a species (Lockwood *et al.*, 2005, 2009; Simberloff, 2009). Among the identified introduction and spread pathways of aquatic plants, it seems reasonable that differences exist in the number of propagules moving along a pathway. Human-mediated spread through ornamental and horticultural trade (Maki & Galatowitsch, 2004) as well as zoochorous dispersal, especially via waterbirds (Green, 2016), are regarded as the most relevant pathways for long distance dispersal and the introduction of alien species in regions beyond their native range. Conversely, intermediate and short distance dispersal most likely depend in large part on environmental vectors such as water movement and on the local population size of established aquatic plants that are documented to produce high numbers of sexual and asexual propagules (Boedeltje *et al.*, 2003; Heidbüchel *et al.*, 2016, 2019b).

Submerged aquatic macrophytes predominantly disperse asexually as the majority of propagules produced is vegetative (Barrat-Segretain, 1996; Boedeltje *et al.*, 2003). Reasons for this are, in particular, the uniformity of aquatic environments on a larger scale (Cook, 1985), the high phenotypic and physiological plasticity of aquatic plants (Riis *et al.*, 2010; Pedersen *et al.*, 2013; Hussner *et al.*, 2016a) and the ineffective formation of viable seeds (Titus & Hoover, 1991). Moreover, many widespread species are even lacking seed production in their introduced range due to the presence of only one sex, and thus are often exclusively limited to vegetative reproduction (e.g. *Elodea canadensis* Michx., *Elodea nuttallii* (Planch.) H. St. John; Cook and Urmi-König, 1985).

Among the vegetative propagules of submerged plants, unspecialized plant fragments, particularly shoot fragments, pose the most important dispersal units (Grace, 1993; Barrat-Segretain, 1996). Consequently, the number of viable plant fragments produced largely determines the propagule pressure of submerged aquatic plants (Jacobs & MacIsaac, 2009). Plant fragments are either formed self-induced by autofragmentation or by allofragmentation, that is fragmentation mediated by external disturbances such as water flow (Sand-Jensen, 2008), foraging of invertebrates or waterfowl (Figuerola & Green, 2002; Bakker *et al.*, 2016) or human activity, e.g. during mechanical weed control (Anderson, 1998). Differences in the biomechanical properties among the species additionally determine the specific fragmentation rate (Bociag *et al.*, 2009; Miler *et al.*, 2012, 2014). Hence, the number of plant fragments produced, and thus propagule pressure, is mainly dependent on a combination of the species-specific biomechanical properties and the experienced degree of environmental disturbances.

In streams, fragment dispersal is of particular relevance as shear stress mediated by flow promotes allofragmentation and downstream drift of plant fragments (Riis & Biggs, 2003). High numbers of drifting plant fragments were already documented within differently sized streams, albeit extensive field studies are scarce (Boedeltje *et al.*, 2003; Heidbüchel *et al.*, 2016, 2019b). Thereby, the quantity of vegetative propagules generally depends on the upstream located population size and abundance of aquatic plant species, the stream characteristics and the environmental conditions, though seasonal differences were found to be of minor relevance (Riis, 2008; Heidbüchel *et al.*, 2016). The number of drifting plant fragments within the same stream was further documented to increase with increasing discharge and can be substantially enhanced during floods (Boedeltje *et al.*, 2004; Heidbüchel *et al.*, 2016). It is thus reasonable to assume that the fragmentation rates of submerged species differ between streams, with higher fragmentation rates expected in streams characterized by high discharge and turbulent flow conditions (i.e. increased hydraulic stress). By contrast, submerged aquatic plants may adapt to the mechanical stress imposed by the water current as they are characterized by a high phenotypic plasticity (Puijalon *et al.*, 2008; Riis *et al.*, 2010; Miler *et al.*, 2014).

Apart from the number of released plant fragments, the likelihood of fragment regeneration (i.e. development of secondary roots and shoots) is regarded as a crucial indicator for the colonization potential and establishment success of vegetative aquatic plant propagules (Riis, 2008; Heidbüchel & Hussner, 2019). Regeneration generally increases with larger fragment size (Redekop *et al.*, 2016; Bickel, 2017; Heidbüchel *et al.*, 2019b), but differs among species and fragment types (Barrat-Segretain *et al.*, 1998; Riis *et al.*, 2009; Umetsu *et al.*, 2012a; Heidbüchel & Hussner, 2019). During downstream dispersal, further fragmentation of larger drifting plant fragments is highly likely. As most submerged aquatic plants are able to regenerate from very small fragments consisting only of a single node (Langeland & Sutton, 1980; Kuntz *et al.*, 2014), the propagule pressure may consequently be enhanced with increasing drift duration (Heidbüchel *et al.*, 2016). Recently, it was demonstrated that measurements of chlorophyll *a* fluorescence can precisely denote fragment viability of submerged aquatic plants (Heidbüchel *et al.*, 2019a). The vast majority of plant fragments regenerate above a critical F_v/F_m, i.e. maximum quantum yield of photosystem II, of 0.4 (Heidbüchel *et al.*, 2019a). It must be noted, however, that regeneration and colonization of aquatic plants is strongly influenced by abiotic factors such as temperature, light, nutrient and carbon availability (Riis *et al.*, 2012; Kuntz *et al.*, 2014; Hussner *et al.*, 2015). Consequently, the successful establishment of dispersed plant fragments is to a large extent determined by the environmental conditions of the receiving habitat (Bornette & Puijalon, 2011).

In conclusion, despite the numerous studies on the biomechanical resistance of aquatic plants to stem breakage (Schutten *et al.*, 2005; Bociag *et al.*, 2009; Liffen *et al.*, 2011; Miler *et al.*, 2012, 2014; Łoboda *et al.*, 2019) and the few existing quantitative studies on fragmentation rates of aquatic plants (Heidbüchel *et al.*, 2016, 2019b; Redekop *et al.*, 2016), detailed information on species-specific fragmentation rates in the field particularly considering the influence of flow are hitherto absent.

Here, we investigated the fragment dispersal capacity of submerged aquatic plants in small to medium-sized German lowland streams with different flow regimes. We hypothesize (i) that submerged species show stream- and species-specific differences in fragmentation rate, (ii) that fragmentation rates increase at higher levels of discharge and (iii) that most plant fragments produced are viable.

Material and methods

Study area and species

The four submerged aquatic plant species M*yriophyllum spicatum* L., *Potamogeton crispus* L., *Elodea canadensis* Michx. and *Elodea nuttallii* (Planch.) H. St. John are successful invaders that belong to the most troublesome species around the globe (Bolduan *et al.*, 1994; Hussner *et al.*, 2017). Field surveys were conducted in 15 to 33 m long (82-211 m^2) stream sections of five small to medium-sized lowland streams within the Lower Rhine region in Germany (Table 1). For each of the species, fragmentation was investigated in sections of two different streams with contrasting flow regimes. Sections of the streams Brückerbach (BB), Gillbach (GB), and Jüchener Bach (JB) were characterized by monospecific macrophyte populations, whereas multiple species were abundant within the stream sections of the Cloer (C1, C2) and the river Niers (N). For sections of the streams Brückerbach, Gillbach and Cloer, in-stream vegetation was composed of small isolated patches of submerged plants, whereas stream sections of the Jüchener Bach and Niers were more heavily vegetated and composed of larger interconnected macrophyte patches. The coverage of the sampled species strongly differed among the stream sections, ranging from <1 (*P. crispus*, N) up to 58% (*E. nuttallii*, JB).

Experimental procedure

Field surveys at a given sampling location were performed on three to four sampling days with different discharge conditions in late summer and autumn (Aug-Oct 2018; Table 1). Therefore, the stream sections of interest were cordoned off to quantify the plant fragments formed within a given section by using constructed drift barriers consisting of polyvinylchloride fences with a mesh size of 10 mm (Figure 1). While the upstream drift barrier served the purpose of retaining drift material from outside the stream sections, fragments of the species studied were collected at the downstream drift barrier. After setting up the drift barriers, a latency time of at least 10 min was included before sampling commenced to avoid collection of inadvertently caused drifting plant fragments. On each sampling day, drifting plant material was collected over a period of 15 to 85 min, which differed according to the absolute number of plant fragments collected. All samplings were replicated three times under the environmental conditions on each sampling day ($n = 3$). Samplings conducted on a respective sampling day were treated as replicates, because environmental fluctuations, particularly in flow, were expected to influence fragmentation rate. Collected plant material was packed in plastic bags and stored in the fridge at 8 °C for less than 48 h until further evaluation.

Furthermore, water depth and flow velocity (measured ~5 cm below the water surface; MiniWater20 Mini, Schiltknecht Messtechnik AG, Gossau, Switzerland) were determined at intervals of 20 or 40 cm (depending on stream width) in front of upstream drift barriers on each sampling day. Based on the measurements, discharge was then calculated as the sum of discharge for each water column (20 or 40 cm column width):

$$\text{Discharge } [\mathrm{m}^3\ \mathrm{s}^{-1}] = \sum_{i=1}^{n} w_i \times d_i \times v_i \tag{1}$$

with w_i being the water column width [m], d_i being the water column depth [m] and v_i being the flow velocity [m s^{-1}] at a respective water column.

On the first sampling day and when distinct changes in aquatic vegetation were observed (see for example vegetation coverage for the Niers; Table 1), the length, width and height of individual plant stands of each species were measured. In the following, specific plant cover (length [m] x width [m]) and biovolume (length [m] x width [m] x height [m]) of the species within the stream sections were calculated. Data for plant biovolume was further used to calculate fragmentation rate (see Eq. 2 below; for fragmentation rates based on specific plant cover see supplementary Table S1).

Evaluation of plant fragments

Only drifting plant material potentially able to regenerate was considered according to the minimum known fragment size required for regeneration. For all species, the minimum known size for regeneration consists of a stem fragment with a single node (Heidbüchel *et al.*, 2019b and references therein). Within the study, a regenerative drifting plant fragment was termed as drift unit. All drift units collected were exclusively composed of shoot fragments. Based on the number of drift units collected over a given sampling period, we estimated the number of drift units per day. By combining data on the number of drift units [d^{-1}] and specific biovolume within a stream section [m^3], the species-specific fragmentation rates were subsequently calculated as follows:

$$\text{Fragmentation rate } [\mathrm{m}^{-3}\mathrm{d}^{-1}] = \frac{\text{drift units}}{\text{specific biovolume}} \tag{2}$$

Moreover, drift unit size was determined by measuring fragment length and by counting the number of regenerative subunits, i.e. nodes, of a fragment (hereafter referred to as potential propagation units; acc. Heidbüchel et al., 2016).

In order to denote viability of the drift units collected, measurements of the maximum quantum yield of PSII in a dark adapted state (F_v/F_m) were performed on entire plant fragments using an Imaging-PAM fluorometer (IMAG-MAX, Heinz Walz GmbH, Effeltrich, Germany). Therefore, all plant material was evaluated under dark conditions to achieve dark adaptation (i.e. maximum F_v/F_m), which is often reached after a short incubation time of 5 min in the dark (see Heidbüchel *et al.*, 2019a).

Statistical analysis

All statistical analyses were carried out in R version 3.5.1 (R Core Team). Due to violation of parametric assumptions, differences between the fragmentation rates of the four species in different streams were examined by Kruskal-Wallis test with pairwise Mann-Whitney *U* tests (FDR-corrected) as post-hoc. Likewise, differences among the species in drift unit size (expressed as drift unit length and number of potential propagation units per drift unit, respectively) and in fragment F_v/F_m, were assessed by Kruskal-Wallis tests, followed by pairwise Mann-Whitney *U* tests for multiple comparison. A principle component analysis (PCA) was performed to examine differences between the species growing under different flow conditions within sections of different streams.

Results

Fragmentation rate

Overall, the specific fragmentation rates differed significantly among the species and stream sections ($\chi^2_{(7,\ n\ =\ 93)}$ = 43.28, *P* <0.0001) and ranged from a minimum of 74 ± 20 (*Potamogeton crispus*, C1) to a maximum of 1,260 ± 376 drift units m^{-3} specific biovolume d^{-1} (± *SE*) documented for *Elodea canadensis* in the river Niers (Figure 2, Table S1). Fragmentation rates were stream- rather than species-specific and generally higher in streams characterized by increased discharge conditions and flow velocities (GB, N; see Table 1), respectively. This effect was significant for *Myriophyllum spicatum* (*P* = 0.008) and *Elodea nuttallii* (*P* = 0.021), but not for *P. crispus* and *E. canadensis*. Correspondingly, fragmentation rates of *M. spicatum* and *E. nuttallii* were significantly correlated with discharge (*P* = 0.016 and *P* = 0.020, respectively; Pearson correlation), while rather weak correlations were documented for *P. crispus* and *E. canadensis* (Figure S1).

According to the principle component analysis (PCA), both principle component one (PC1) and two (PC2) clearly separated the sampling sites of different streams rather than the species from each other (Figure 3). PC1 and PC2 together explained 79.0% of the total variance in the dataset, with discharge, flow velocity, specific biovolume and coverage showing similarly high loadings (each contributing 22.5-23.9% to the cumulative share of PC1 and PC2). Fragmentation rate contributed least to PC1 and PC2 (7.4%) and was to some extent correlated with discharge but almost entirely uncorrelated with specific plant biovolume and coverage.

Fragment size and viability

Drift unit size in terms of length and potential propagation units per drift unit ranged from a minimum of 0.2 cm and a corresponding number of 1 potential propagation unit per drift unit (*P. crispus*) to a maximum of 243.8 cm (*M. spicatum*) and 340 potential propagation units per drift unit (*E. nuttallii*), with overall medians of 7 cm and 12 potential propagation units per drift unit, respectively (Figure 4). Even though the drift unit size ranges were similar among the species, significant differences were documented in drift unit length ($\chi^2_{(3,\ n\ =\ 840)}$ = 30.16, *P* <0.0001) and potential propagation units per drift unit ($\chi^2_{(3,\ n\ =\ 840)}$ = 11.81, *P* = 0.008). Drift units of *M. spicatum* were significantly longer compared to those of *E. canadensis* and *E. nuttallii*, and *E. nuttallii* showed significantly higher numbers of potential propagation units per drift unit than *E. canadensis*.

For all species, F_v/F_m ratios of drift units were very high, with medians ranging from 0.70 (*M. spicatum*, *P. crispus*, *E. nuttallii*) to 0.72 (*E. canadensis*) and a corresponding overall median of 0.71 (Figure 5). In total, 95% of all drift units possessed F_v/F_m values >0.58. Only a single fragment of *E. nuttallii* (0.1% of all drift units) showed a F_v/F_m below the recently postulated critical F_v/F_m value for regeneration of 0.40 (Heidbüchel *et al.*, 2019a).

Discussion

The specific fragment dispersal capacity largely determines the vegetative spread potential of submerged aquatic plants and is naturally enhanced in running waters due to increased hydraulic stress by flow (Boedeltje *et al.*, 2003; Riis, 2008; Heidbüchel *et al.*, 2016). A high fragmentation rate and high fragment viability increase the propagule pressure and likelihood of colonization and therefore promote the successful spread of aquatic plants (Jacobs & MacIsaac, 2009).

We found that the four submerged aquatic plant species, *Myriophyllum spicatum*, *Potamogeton crispus* and in particular *Elodea canadensis* and *Elodea nuttallii*, show high in situ fragmentation rates. This is to a large extent based on their biomechanical properties, as low forces (1.6-6.9 N) cause stem breakage of the species (Schutten *et al.*, 2005; Puijalon *et al.*, 2011; Miler *et al.*, 2014; Łoboda *et al.*, 2019). For *P. crispus*, even very low breaking strengths were reported when growing in rivers (Miler *et al.*, 2014; Łoboda *et al.*, 2019) and the estimated fragmentation rate in a medium-sized river was high (Heidbüchel *et al.*, 2016). Surprisingly, however, we documented low fragmentation rates for *P. crispus* compared to the other species within the stream sections studied. This might be due to a low degree of local disturbance and hydraulic stress experienced by plants of *P. crispus* on a smaller scale. Unfortunately, the flow velocity and drag force experienced by each individual plant stand of the species were not quantified within our study and should be considered in future field studies to allow for more precise information on the relationship between flow and fragmentation rate. It must further be noted that effects resulting from the conditions associated with the stream sections chosen cannot be ruled out and likely have contributed to the fragmentation rates observed within our study. Nevertheless, our results particularly highlight the strong fragmentation of *E. canadensis* (cf. Redekop *et al.*, 2016) and high propagule pressure exerted by the four species studied.

As the presence of flow facilitates the natural spread via plant fragments in streams, general differences in discharge among different streams and fluctuations in discharge within the same stream are expected to influence the fragmentation rates of submerged species (Riis & Biggs, 2003; Boedeltje *et al.*, 2004; Heidbüchel *et al.*, 2016). We provide evidence that fragmentation rates are generally promoted in streams with higher levels of discharge, though minor fluctuations in discharge seem to be negligible. Hence, the extent of fragment dispersal capacity is assumed to increase with increasing hydraulic stress and stream size (see also Heidbüchel *et al.*, 2019b). Fragmentation rates of a single species can still be differently affected by flow, illustrating that species-specific acclimatization in growth characteristics (e.g. plant size, stem thickness, degree of lignification or other structural features) in response to flow shape the propagule pressure and thus vegetative spread potential of submerged species (Puijalon *et al.*, 2005, 2008; Miler *et al.*, 2014; Schoelynck *et al.*, 2015). Strong fluctuations in discharge such as floods enhance the number of drift units and can even lead to uprooting of a large proportion of the aquatic vegetation (Boedeltje *et al.*, 2004). Particularly during mechanical weed control, which is commonly applied to counteract the impacts of invasive aquatic plant species (Hussner *et al.*, 2017), enormous amounts of fragments are released (Anderson, 1998; Owens *et al.*, 2001). Consequently, the fragmentation rates documented for the species within our study are expected to be substantially higher when plants are experiencing strong disturbances.

It must, however, be noted that the large differences in coverage, biomass and structure of the in-stream vegetation among the sampling sites and differences in fragment buoyancy of the species may have influenced the number of collected plant fragments. A higher coverage of aquatic vegetation and a low specific fragment buoyancy (e.g. for *Elodea* spp.) increase fragment retention, though patch dynamics must be taken into account (Riis & Sand-Jensen, 2006; Cornacchia *et al.*, 2019; Heidbüchel & Hussner, 2019). Additionally, in-stream vegetation characterized by large interconnected rather than small isolated macrophyte patches could have reduced fragmentation as a consequence of higher mutual shelter from drag forces imposed by flow, i.e. reduced border effect (Sand-Jensen, 2003, 2008). Thus, fragmentation rates of species growing in the stream sections of the Jüchener Bach and river Niers, particularly *E. nuttallii,* may be higher when growing in sections composed of small patches isolated from one another. It must be further considered that our results only cover a short investigation period and indicate fragmentation of the species in late summer/autumn. Fragmentation rates may differ at different plant life stages and with changing environmental conditions, although seasonality was found to have a minor impact on the quantity of dispersed fragments (Riis, 2008; Heidbüchel *et al.*, 2016).

Due to the fact that most submerged species are able to initiate new growth from very small fragments consisting only of a single node (Langeland & Sutton, 1980; Kuntz *et al.*, 2014; Bickel, 2017), further fragmentation by mechanical stress during downstream drift likely increases propagule pressure. We show that the number of potential propagation units, and thus potential propagule pressure of submerged aquatic plants, is often more than ten times greater than the number of drift units.

However, even though the propagule pressure based on fragmentation can be high (Heidbüchel *et al.*, 2016, 2019b), only a small proportion of drift units will successfully establish. Initial colonization, that is the initial anchorage within the substrate, was postulated to be the main bottleneck limiting the establishment of aquatic plants in streams (Riis, 2008) and depends on the potential to regenerate new roots (Riis *et al.*, 2009; Heidbüchel & Hussner, 2019). According to chlorophyll fluorescence measurements, the viability and regeneration potential of fragments collected in the field is very high for all species studied. Furthermore, the regeneration capacity is expected to be high as fragment size distinctly exceeded the minimum size required for regeneration and as the likelihood of regeneration increases with increasing fragment length and number of potential propagation units (Redekop *et al.*, 2016; Bickel, 2017; Heidbüchel *et al.*, 2019b). Nevertheless, the regeneration and colonization abilities differ among aquatic plant species (Barrat-Segretain *et al.*, 1998; Riis *et al.*, 2009) and are strongly controlled by the environmental conditions of the receiving habitats (Barrat-Segretain *et al.*, 1999; Riis *et al.*, 2012; Kuntz *et al.*, 2014; Hussner *et al.*, 2015). Our results thus imply that plant fragments of *M. spicatum*, *P. crispus*, *E. canadensis* and *E. nuttallii* have a high potential for successful establishment when settled at suitable habitats, explaining their role as successful invaders worldwide. Further studies on the regeneration, colonization and performance of plant fragments in the field are still needed to gain a comprehensive view of the species-specific vegetative spread potential (but see Riis, 2008).

Lastly, with respect to the initial hypotheses, we conclude (i) that fragmentation rates of submerged aquatic plants in lowland streams are stream- and species-specific, though stream-specific differences were more pronounced in our study. We further conclude (ii) that fragmentation rates are generally increased in streams with higher levels of discharge but are not affected by minor fluctuations in discharge to a large extent. For the species studied, we demonstrated (iii) that the vast majority of plant fragments collected in the field is highly viable. Finally, our results emphasize the high fragment dispersal capacity and thus high vegetative spread potential and invasion success of the four submerged species studied (Bolduan *et al.*, 1994; Hussner *et al.*, 2017).

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Table 1 Locations and properties of the stream sections selected for quantification of the fragmentation rates of *Myriophyllum spicatum*, *Potamogeton crispus*, *Elodea canadensis* and *Elodea nuttallii*

Stream	Stream section	Coordinates	Sampling days	Mean width	Length	Surface area	Mean flow velocity[1]	Discharge[1]	In-stream vegetation pattern[2]	Sampled species	Coverage[3]
		[°]		[m]	[m]	[m^2]	[$m\ s^{-1}$]	[$m^3\ s^{-1}$]			[%]
Brückerbach	BB	N51.191443, E6.813255	4	5.5	25.9	143.1	0.09 (0.07-0.11)	0.21 (0.18-0.23)	Isolated patches	*M. spicatum*	15.3
Gillbach	GB	N51.037307, E6.691762	3	5.6	15.2	85.3	0.62 (0.60-0.65)	0.83 (0.75-0.88)	Isolated patches	*M. spicatum*	4.9
Cloer	C1	N51.246025, E6.478586	4	3.7	22.5	82.4	0.04 (0.02-0.07)	0.03 (0.02-0.04)	Isolated patches	*P. crispus*	5.3
	C2	N51.247223, E6.464753	4	4.7	32.5	151.2	0.03 (0.02-0.04)	0.05 (0.03-0.08)	Isolated patches	*E. canadensis*	2
Jüchener Bach	JB	N51.215720, E6.554536	4	2.7	33	87.5	0.09 (0.07-0.11)	0.06 (0.05-0.09)	Interconnected patches	*E. nuttallii*	57.5
Niers	N	N51.171460, E6.473345	4	8.1	26	210.6	0.14 (0.12-0.17)	0.28 (0.22-0.31)	Interconnected patches	*P. crispus*,	0.6-1.4
										E. canadensis,	3.4-4.3
										E. nuttallii	6.0-7.6

[1] Flow velocity and discharge conditions during the surveys are indicated by mean values and minimum/maximum values in parentheses. Values were determined in front of upstream drift barriers, respectively

[2] Vegetation patterns refer to all species present at a given sampling location (single or multiple species)

[3] Values for coverage refer to the vegetation cover of the sampled species, respectively

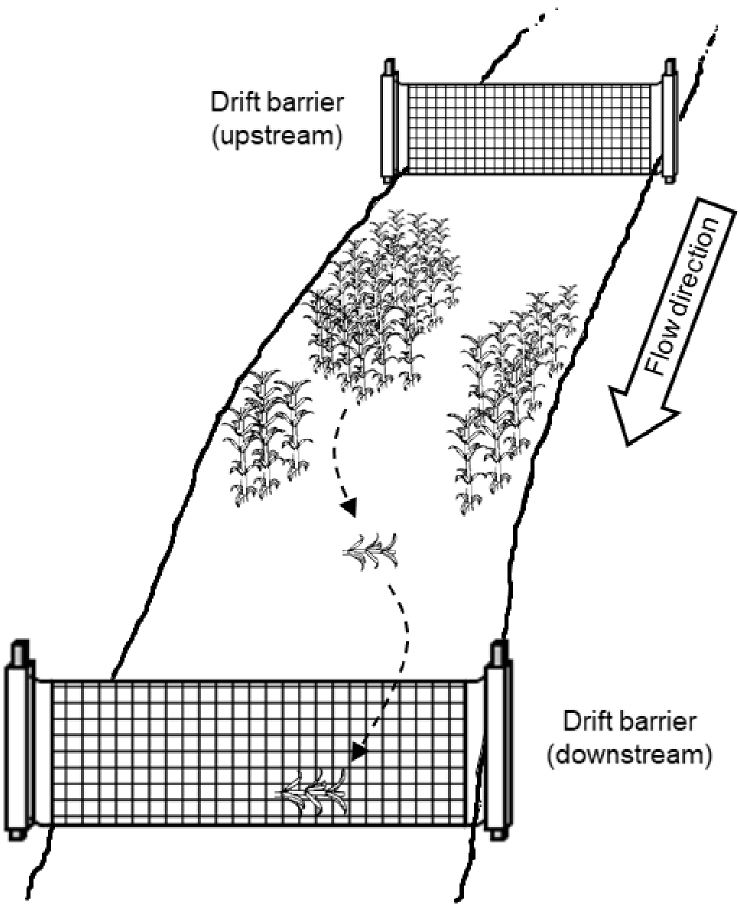


Figure 1 Schematic overview of the experimental setup in the field

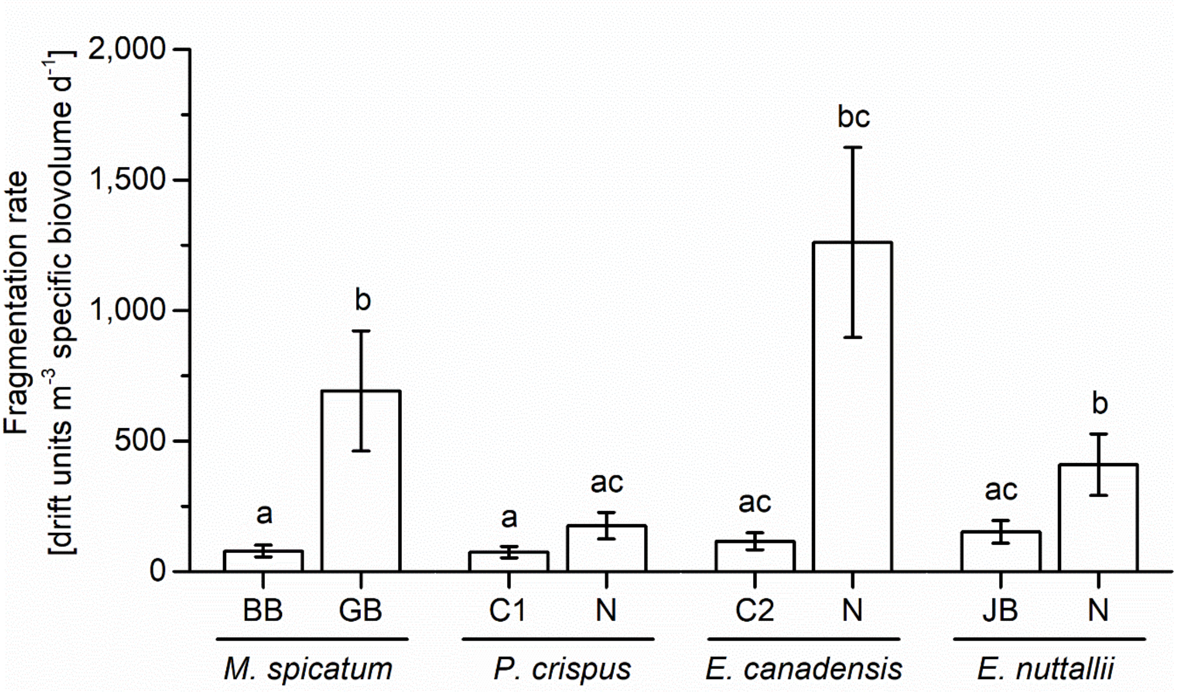


Figure 2 In situ fragmentation rates of *Myriophyllum spicatum*, *Potamogeton crispus*, *Elodea canadensis* and *Elodea nuttallii* at sampling locations of streams with different runoff regimes (see Table 1). Shown are mean values ± 1 *SE* (n = 12 and n = 9 for *M. spicatum*, GB; based on 3 repetitive measurements per sampling day and stream section). Different letters indicate significant differences (*P* <0.05; pairwise Mann-Whitney *U* tests)

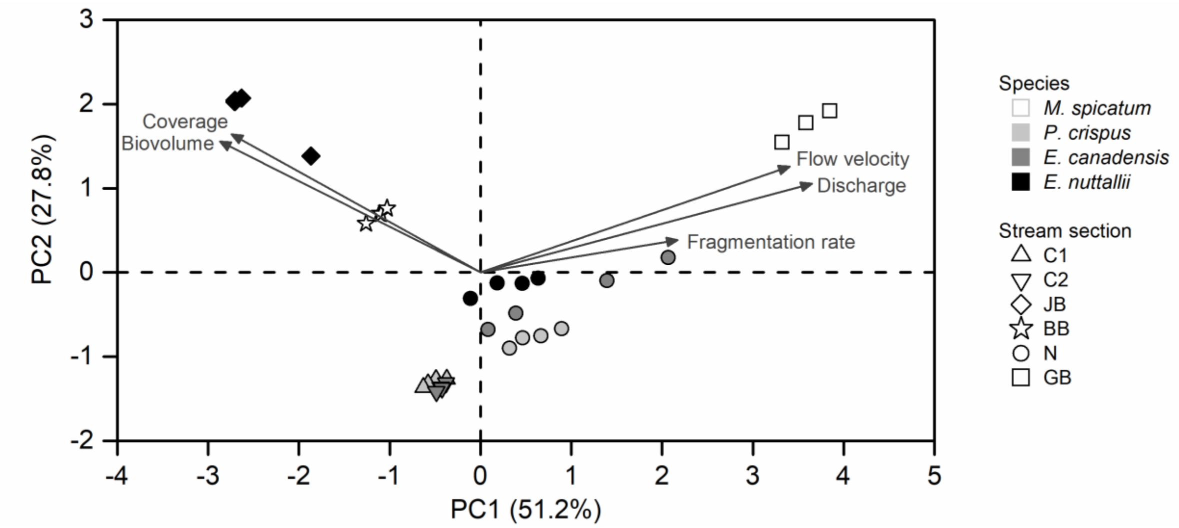


Figure 3 Principal component analysis (PCA) biplot of the four species *Myriophyllum spicatum*, *Potamogeton crispus*, *Elodea canadensis* and *Elodea nuttallii* at sampling locations of streams with different runoff regimes (see Table 1). Data used for PCA consisted of plant (coverage, biovolume and fragmentation rate) and flow parameters (discharge, flow velocity). Loadings of these parameters on PC1 and PC2 are indicated by dark grey arrows. Percent variance explained by each PC is indicated in parentheses

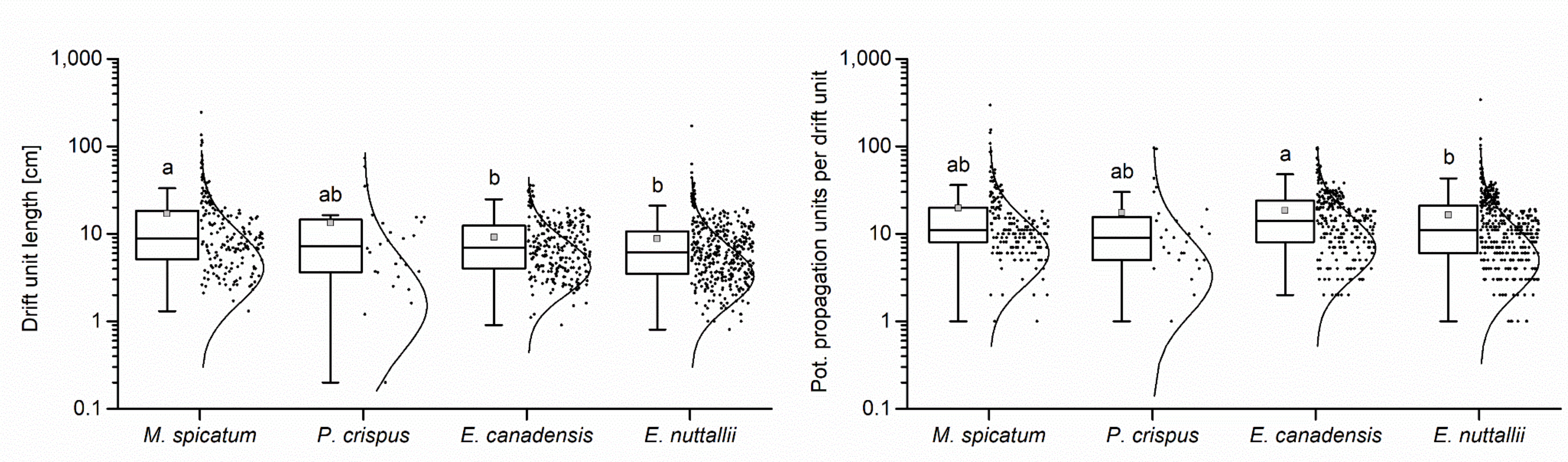


Figure 4 Drift unit size according to length and potential propagation units per drift unit of collected plant fragments for *Myriophyllum spicatum*, *Potamogeton crispus*, *Elodea canadensis* and *Elodea nuttallii*. Shown are 25th and 75th percentiles (bottom and top of boxes, respectively), medians (horizontal lines in boxes), means (grey squares) and 1.5 × interquartile ranges (whiskers). Raw data points and log-normal distribution curves are presented next to boxplots. Different letters indicate significant differences (P <0.05; pairwise Mann-Whitney U tests)

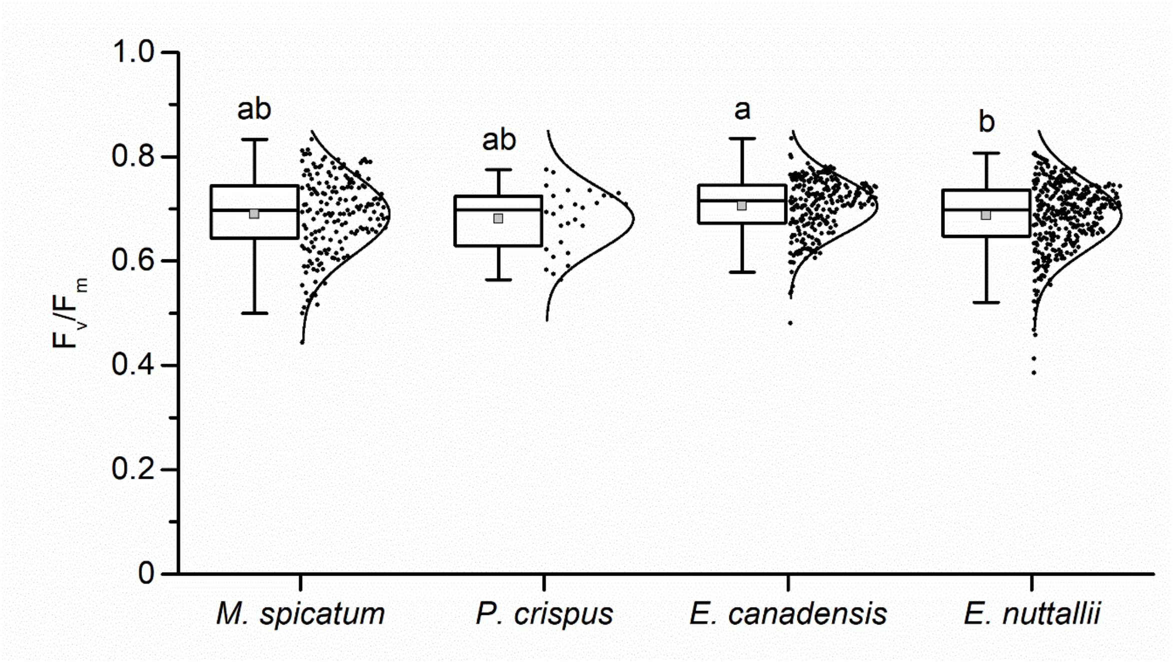
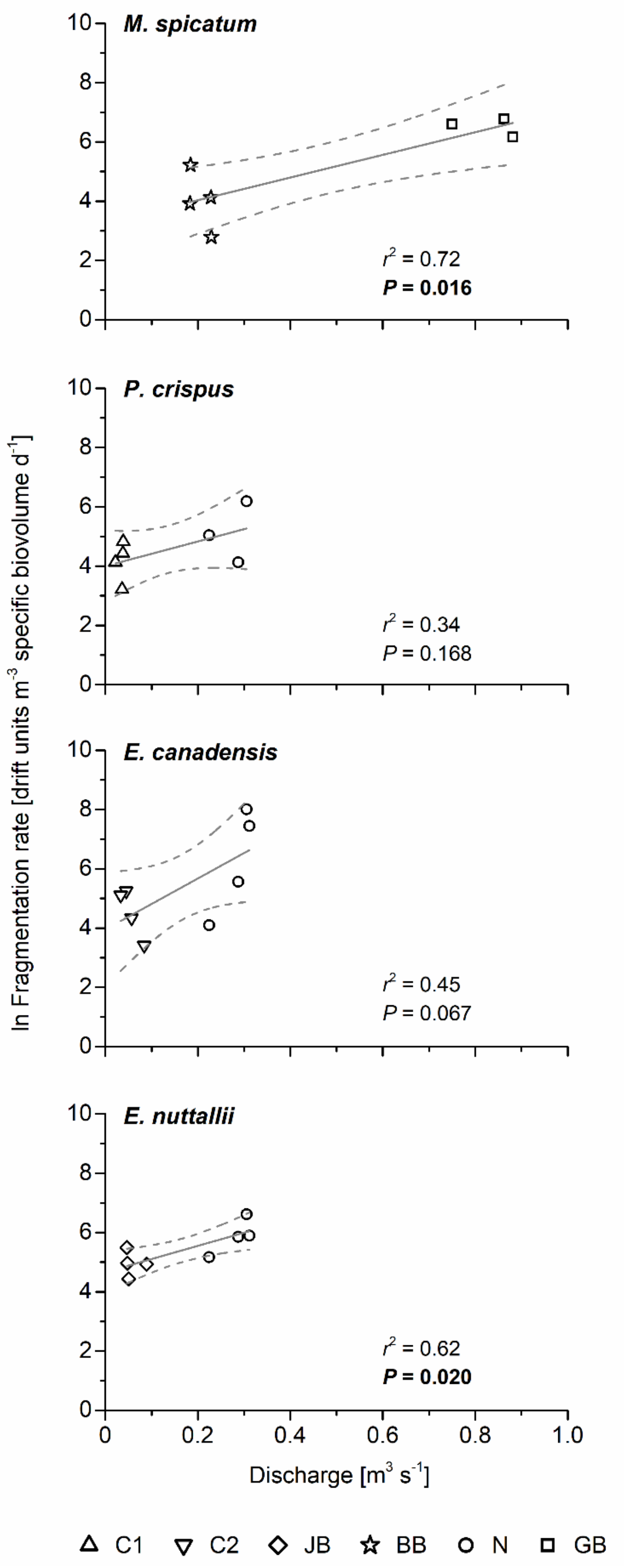


Figure 5 F_v/F_m ratio (as indicator for fragment viability) of collected plant fragments for *Myriophyllum spicatum*, *Potamogeton crispus*, *Elodea canadensis* and *Elodea nuttallii*. Shown are 25th and 75th percentiles (bottom and top of boxes, respectively), medians (horizontal lines in boxes), means (grey squares) and 1.5 × interquartile ranges (whiskers). Raw data points and normal distribution curves are presented next to boxplots. Different letters indicate significant differences (P <0.05; pairwise Mann-Whitney U tests)

Supporting information

Table S1 In situ fragmentation rates based on specific plant cover and specific biovolume, respectively, for *Myriophyllum spicatum*, *Potamogeton crispus*, *Elodea canadensis* and *Elodea nuttallii* at sampling locations of streams with different runoff regimes (see Table 1)

Species	Stream section	Fragmentation rate [drift units m^{-2} specific plant cover d^{-1}]	Fragmentation rate [drift units m^{-3} specific biovolume d^{-1}]
M. spicatum	BB	25 ± 6	78 ± 20
	GB	63 ± 13	691 ± 147
P. crispus	C1	8 ± 2	74 ± 20
	N	12 ± 5	176 ± 118
E. canadensis	C2	24 ± 7	115 ± 32
	N	168 ± 50	1,260 ± 376
E. nuttallii	JB	24 ± 4	152 ± 22
	N	62 ± 10	409 ± 67



◀ **Figure S1** Correlation between the natural log (ln) of fragmentation rate and discharge for *Myriophyllum spicatum*, *Potamogeton crispus*, *Elodea canadensis* and *Elodea nuttallii* at sampling locations of streams with different runoff regimes (see Table 1). Shown are mean values for each sampling day, linear regression lines (solid lines) and corresponding 95% confidence intervals (dashed lines). Coefficients of determination (r^2) are displayed for each species. Significant correlations are indicated by *P* values written in bold (*P* <0.05; Pearson correlation)

CHAPTER 4

Go with the flow: Fragment retention patterns shape the vegetative dispersal of aquatic plants in lowland streams

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Abstract

1. The dispersal of aquatic plant propagules is highly facilitated in streams due to flow. As many aquatic plants predominantly spread through vegetative propagules, the specific retention and thus drift distance of dispersed plant fragments largely contribute to the rapid spread along the course of a stream.
2. We determined fragment retention for four aquatic plant species (*Elodea canadensis*, *Myriophyllum spicatum*, *Ceratophyllum demersum*, *Salvinia natans*; representing four different common morpho-structural groups) in sections of small to medium-sized German streams with different levels of stream sinuosity.
3. The number of fragments showed a logistic decline over drift distance. In small streams, 90% of drifting fragments were retained at distances (D_{90}) of only 5-9 m and 19-70 m, while higher D_{90} values of 116-903 m and 153-2,367 m were determined for a medium-sized stream. The likelihood of retention thereby decreased significantly with increasing stream size and was reduced in straightened stream sections.
4. Differences in retention were more strongly related to fragment buoyancy rather than fragment size and morphology. Increasing buoyancy significantly lowered the likelihood of fragment retention over drift distance by a factor of 3 to 8, whereas contrasting effects were documented for size and morphology of fragments.
5. The relevance of different obstacles was highly stream section-specific and depended on obstacle abundance, distribution and the degree of submergence/emergence.
6. Our findings elucidate the dynamic retention patterns of plant fragments and highlight the strong interplay between extrinsic (stream) and intrinsic (fragment) properties. We conclude that straightened lowland streams of intermediate size promote the rapid dispersal of invasive aquatic plants and are particularly prone to invaders producing large amounts of small and highly buoyant plant fragments. Still, information on the species-specific fragment colonization dynamics in the field is highly required to improve our understanding of the vegetative dispersal capacity of invasive aquatic plants in stream ecosystems.

Keywords aquatic macrophytes, hydrochory, invasive species, propagule pressure, vegetative reproduction

Introduction

Aquatic plants generally tend to show broader distributions across the globe than terrestrial plant species. This is in great part due to a combination of the large-scale uniformity of aquatic environments (Cook, 1985), the overall high phenotypic and physiological plasticity of aquatic plants (e.g. Riis *et al.*, 2010; Hussner *et al.*, 2016) and highly efficient spread mechanisms (Santamaría, 2002).

While the reproduction and dispersal via seeds is often limited (Titus & Hoover, 1991), most widespread aquatic plant species successfully disperse through vegetative means (Grace, 1993; Barrat-Segretain, 1996). Some of the most troublesome aquatic plants are even restricted to vegetative dispersal in their introduced range as seed production is not possible due to the absence of either female or male plants, e.g. *Elodea canadensis* Michx., *Egeria densa* Planch. and *Myriophyllum aquaticum* (Vell.) Verdcourt in Europe (Orchard, 1981; Cook & Urmi-König, 1985). Moreover, some others such as the invasive *Ludwigia* spp. apparently spread almost exclusively through vegetative propagules although they are known to produce numerous seeds in both their native and introduced range (Okada *et al.*, 2009; Thouvenot *et al.*, 2013). Consequently, it seems evident that not only successful spread but also the invasiveness of aquatic plant species must be to a certain extent attributed to a high vegetative dispersal capacity (Fleming & Dibble, 2015; Hussner *et al.*, 2017).

It is therefore not surprising that the majority of aquatic plant propagules collected in streams were found to be vegetative (Boedeltje *et al.*, 2003, 2004; Heidbüchel *et al.*, 2016). Among the different types of vegetative propagules, unspecialized plant fragments may contribute to plant dispersal throughout the whole year and pose the most important dispersal units, particularly for submerged species (Barrat-Segretain, 1996; Heidbüchel *et al.*, 2016). These plant fragments are predominantly formed through external disturbances and generally preserve a high viability as most aquatic plants can regenerate (i.e. initiate new growth) even from small stem fragments consisting of only a single node (e.g. Langeland & Sutton, 1980; Kuntz *et al.*, 2014; Heidbüchel *et al.*, 2019b). Indeed, fragments of many aquatic plants collected in the field were highly viable (Boedeltje *et al.*, 2003, 2004; Heidbüchel *et al.*, 2019b; Heidbüchel & Hussner, 2020). Additionally, the likelihood of regeneration increases with larger fragment size (Redekop *et al.*, 2016; Bickel, 2017; Heidbüchel *et al.*, 2019b), though species and fragment type-specific differences occur (Barrat-Segretain *et al.*, 1999; Barrat-Segretain & Bornette, 2000; Riis *et al.*, 2009; Umetsu *et al.*, 2012a).

While the dispersal of aquatic plant propagules to distant hydrologically isolated habitats requires specific vectors for overland transport (Johnstone *et al.*, 1985; Johnson *et al.*, 2001; Green, 2016), the spread within interconnected and temporarily connected waterbodies in floodplains is mainly driven by water movement, i.e. hydrochory (Johansson & Nilsson, 1993; Johansson *et al.*, 1996; Nilsson *et al.*, 2010). Particularly in streams, flow not only promotes fragmentation but also strongly facilitates unidirectional dispersal of drifting fragments (Johansson & Nilsson, 1993; Riis & Sand-Jensen, 2006; Heidbüchel *et al.*, 2016, 2019b). Thus, the number and frequency of propagules dispersed to downstream located habitats, i.e. propagule pressure (e.g. Simberloff, 2009), is naturally enhanced. As propagule pressure is widely viewed as the key factor for both the invasiveness of species and invasibility of the recipient community (Von Holle & Simberloff, 2005; Colautti *et al.*, 2006), the perturbed characteristics imposed by flow make running water ecosystems particularly susceptible to invasive aquatic plant species (Jacobs & MacIsaac, 2009). Besides propagule pressure, a high dispersal distance is considered as a major trait of invasive species (Pyšek *et al.*, 2004) and likely explains the rapid spread of aquatic plants along the course of a stream.

However, while hydrochory via seeds has been extensively examined, there is only a low number of available studies on hydrochory via vegetative propagules as reviewed in Nilsson *et al.*, (2010), Catford & Jansson (2014) and Jones *et al.* (in press). Studies considering the flow-mediated dispersal of plant fragments mostly focused on riparian and emerged aquatic plants (Johansson & Nilsson, 1993; Boedeltje *et al.*, 2003, 2004, 2008; Sarneel, 2013) but less on submerged and free floating species (Riis & Sand-Jensen, 2006; Riis, 2008; Heidbüchel *et al.*, 2016). Some of the few existing field investigations on fragment retention of aquatic plants in running waters already documented drift distances ranging from few meters up to several kilometers (<5 km) for the majority of plant fragments in small and medium-sized streams (Johansson & Nilsson, 1993; Riis & Sand-Jensen, 2006). In general, propagule retention depends on a combination of extrinsic (stream properties) and intrinsic (propagule properties) factors. Plant fragments may be trapped by all kind of physical barriers, including stones, debris, deadwood, riparian vegetation and aquatic vegetation. The latter was recognized as the most important retention agent (Riis & Sand-Jensen, 2006; Riis, 2008), though biotic interactions with the resident vegetation can impede or facilitate establishment of propagules (Chadwell & Engelhardt, 2008; Thiébaut & Martinez, 2015). Apart from obstacles, flow dynamics such as lentic zones and eddies, which are often associated with hydromorphological characteristics (e.g. pools and riffles in meanders), may also act as important drift barriers (Johansson & Nilsson, 1993). It therefore seems reasonable to assume that drift distance depends on the level of stream sinuosity and channelization results in reduced fragment retention. Moreover, increasing discharge was found to enhance drift distance and alter propagule retention dynamics due to flooding of protruding obstacles, as was documented for floating wooden cubes (Engström *et al.*, 2009). Due to general differences among growth forms, e.g. submerged, free floating and emerged growing plants, and specific differences in the extent of aerenchymatic tissue, buoyancy of fragments varies among species (Sculthorpe, 1967). A high fragment buoyancy that is preserved over a long period was found to increase drift distance, whereas fragment size was of minor importance (Riis & Sand-Jensen, 2006; Sarneel, 2013; Cornacchia *et al.*, 2019). However, the fragment sizes employed in these studies only covered stem fragments >7.5 cm in length (Johansson & Nilsson, 1993; Riis & Sand-Jensen, 2006; Riis, 2008; Cornacchia *et al.*, 2019), albeit the majority of fragments found in small to medium-sized streams is often smaller (Heidbüchel *et al.*, 2019b; Heidbüchel & Hussner, 2020). It is further likely that morphological and structural differences such as leaf shape and flexibility influence the drift distance and retention dynamics. In an earlier study, drifting fragments exponentially declined over distance (Riis & Sand-Jensen, 2006). This may not always be the case, as the complex interplay between different extrinsic and intrinsic factors suggests a more dynamic relationship.

Although it is well known that vegetative propagules rather than seeds determine recruitment and establishment of aquatic plants (Sand-Jensen *et al.*, 1999; Riis, 2008), there is a lack of comprehensive field studies on fragment retention patterns combining intrinsic factors of submerged and clonal free floating species and extrinsic factors of different streams. In order to improve assessment of the vegetative dispersal capacity for widespread aquatic plant species, we investigated the drift dispersal and underlying fragment retention patterns in small to medium-sized streams with different levels of stream sinuosity for plant fragments of submerged and a floating species. We hypothesize (i) that fragment retention is generally mitigated in straightened streams characterized by higher discharge, (ii) that differences in fragment buoyancy, size and morphology drive the retention patterns in streams, with drift distance increasing with higher buoyancy, smaller size and more compact morphology of fragments, and lastly, (iii) that the specific relevance of retention agents varies within and between stream sections.

Methods

Study area and species

Fragments of three submerged species *Elodea canadensis* Michx., *Myriophyllum spicatum* L., and *Ceratophyllum demersum* L. and the floating *Salvinia natans* L. (All.) were released in German lowland streams (Figure 1a). The species are either native (*M. spicatum*, *C. demersum*, *S. natans*) or naturalized (*E. canadensis*) to Europe but are well-known for their invasion success elsewhere. All species represent morpho-structurally distinct groups of aquatic plants that differ in their overall fragment buoyancy (*E. canadensis*: rigid, entire submerged leaves, low buoyancy; *M. spicatum*: flexible, dissected submerged leaves, high buoyancy; *C. demersum*: rigid, dissected submerged leaves, very high buoyancy; *S. natans*: entire floating leaves, very high buoyancy).

Four stream sections representing different combinations of either meandering or straightened (indicated by sinuosity index; SI = channel length/straight line distance) and small or medium-sized streams (indicated by discharge) were selected for the field surveys (Figure 1b, Table 1). All streams investigated were located within the Lower Rhine region in Western Germany. Channel length of the stream sections ranged from a minimum 40 m (KB) to a maximum of 480 m (GB1), according to stream size, potential drift distance of fragments and practical feasibility. Aquatic vegetation that is considered to be one of the most important retention agents was present in all stream sections, even though there were strong differences in coverage of submerged and emerged aquatic vegetation. The total coverage of aquatic vegetation was distinctly higher in the straightened sections (JB: 61.0%, GB2: 55.3%) than in the meandering sections (KB: 8.8%, GB1: 7.2%; for determination of plant coverage see chapter 2.3 below).

Fragment preparation

Unbranched apical fragments of the submerged species *E. canadensis*, *M. spicatum* and *C. demersum* with fragment lengths of 3, 6 and 12 cm were used in the field experiments. These fragment sizes were chosen as they represent the first quartile (Q1 = 3 cm), median (Q2 = 6 cm) and third quartile (Q3 = 12 cm) of the fragment length documented for aquatic plant species in previous field studies on fragment dispersal in small to medium-sized streams (Heidbüchel *et al.*, 2019b; Heidbüchel & Hussner, 2020). For *S. natans*, a single fragment size class consisting of fragments with two floating fronds and a diameter of 3-4 cm was used.

All fragments applied in a respective field survey were prepared the preceding day by cutting plant material according to size classes (*n* = 30 per species, fragment size and field survey). Plant material of *E. canadensis* and *C. demersum* originated from a pond system at the University of Düsseldorf (Germany), while plants of *M. spicatum* and *S. natans* were obtained from laboratory cultures of the University of Düsseldorf. Prepared plant fragments were color-coded with acrylic spray paint (DUPLI-COLOR platinum, MOTIP DUPLI GmbH, Haßmersheim, Germany) according to species and fragment size to facilitate fragment retrieval. For each consecutive field survey at the same stream section, fragments of a given species and fragment size were spray-painted in a different color to avoid biased re-collection and to mitigate effects of color on retrieval rate. Spray-painted fragments were no longer viable, allowing for fragment release in streams where the species did not occur in the resident plant community.

Moreover, fragment buoyancy was evaluated for each set of fragments in one to six 5 L plastic containers (28 × 19 × 14 cm, LWH) filled with tap water. Therefore, a buoyancy score was determined before ($\mathrm{BS}_{\mathrm{control}}$) and after spray-painting (BS) of fragments for each species and fragment size:

$$\text{Buoyancy score} = \frac{n_{\mathrm{s}} \times 0 + n_{\mathrm{sf}} \times 0.5 + n_{\mathrm{f}} \times 1}{n_{\mathrm{tot}}} \tag{1}$$

Where n_{s} is the number of fragments that had sunk to the bottom, n_{sf} is the number of fragments that floated below the surface or stood upright in the water column, n_{f} is the number of fragments that floated at the surface and n_{tot} is the total number of fragments (= 30 per species, size and field survey). Spray-painting of the plant fragments only slightly reduced fragment buoyancy by 2.3 ± 2.4% (mean ± *SD*). The lowest overall BS was documented for *E. canadensis* (0.49), followed by *M. spicatum* (0.84), while the maximum possible BS of 1 was documented for fragments of *C. demersum* and the floating *S. natans*. Fragment buoyancy of *E. canadensis* and *M. spicatum* further tended to increase with increasing fragment length, whereas differently sized fragments of *C. demersum* showed consistently high BS (see Table S1).

All spray-painted plant fragments were packed in sealed plastic bags filled with some tap water and stored in a fridge at 4°C over night until further use in the field surveys. Plastic bags containing the fragments were transported in a polystyrene box.

Fragment drift surveys

For each stream section, four fragment drift surveys were conducted on four different days during summer (June-August) in 2019 to provide a sufficiently large sampling size of fragments retrieved per species and fragment size. Field surveys at a respective stream section were carried out under normal discharge conditions (see Table 1) within 14 days to avoid effects of fluctuations in environmental conditions on fragment retention. Overall, 120 fragments per species and fragment size were released during the field surveys at the stream sections, respectively.

During each field survey, all 300 fragments were randomized and released one by one within 10 min in the middle of a stream. Drift barriers consisting of either temporarily installed polyvinylchloride fences (mesh size: 10 mm) or a person using a hand-held fishing net (mesh size: 6 mm) prevented fragments from drifting outside the defined reaches. Following the release of fragments, a lag time of 1 h was included before re-collection of fragments commenced. This lag time proved necessary as most of the drifting fragments that were not retained in the stream sections reached the drift barriers within this time, particularly in sections of the stream Gillbach. Retrieval of fragments was always conducted by the same person wading carefully upstream from the end of a stream section to fragment release point. Due to stream section- and fragment-related differences, fragment retrieval differed between the stream sections, species and fragment sizes (Table S2). While 97.3% (KB) and 84.6% (JB) of all fragments could be retrieved in sections of the small streams, lower proportions of 51.8% (GB1), and 66.7% (GB2) were found in the medium-sized Gillbach. Laser distance measurements (GLM 120 C Professional, Robert Bosch Power Tools GmbH, Stuttgart, Germany) were performed to accurately determine the drift distance from release point. Therefore, distance markers were deployed along the banks of the stream sections. For every single fragment retrieved, drift distance, water depth at the point where retention occurred, i.e. retention depth (bottom/middle/surface), and type of retention agent were recorded.

Water depth and flow velocity (measured ~5 cm below the water surface; MiniWater20 Mini, Schiltknecht Messtechnik AG, Gossau, Switzerland) were determined on each sampling day across the streams at intervals of 20 (KB, JB) or 40 cm (GB1, GB2), depending on stream width, for calculation of discharge. Discharge was calculated as the sum of discharge for each 20 or 40 cm water column:

$$\text{Discharge } (\text{m}^3\ \text{s}^{-1}) = \sum_{i=1}^{n} w_i \times d_i \times v_i \quad (2)$$

Where w_i is water column width (m), d_i is water column depth (m) and v_i is the corresponding flow velocity of a water column (m s^{-1}).

Moreover, coverage of the aquatic vegetation (% of stream surface area) was estimated visually for smaller subsections during the first field survey at a respective stream section. The total coverage for submerged and emerged aquatic vegetation in each section was then calculated as the sum of coverages for the subsections. Stream section area (m^2) was determined by using polygons in Google Earth (Google LLC, Mountain View, CA, USA) or calculated based on stream width measurements along a section.

Determination of fragment retention parameters

Within our study, the decline in the relative number of drifting fragments over distance was most accurately described by a five-parameter logistic regression with two slopes (based on Ricketts & Head, 1999):

$$\text{Drifting fragments (\%)} = A + \frac{100-A}{1+f_x\times(\frac{\text{distance}}{EC_{50}})^{-k_1}+(1-f_x)\times(\frac{\text{distance}}{EC_{50}})^{-k_2}} \tag{3}$$

With

$$f_x = \frac{1}{1+(\frac{\text{distance}}{EC_{50}})^{\bar{C}_f}} \tag{4}$$

$$\bar{C}_f = \frac{2\times|k_1|\times k_c}{1+k_c} \tag{5}$$

And

$$k_2 = k_1 \times k_c \tag{6}$$

The retention coefficients k_1 and k_2 (i.e. rate constants) thereby describe the two different curvatures of a regression curve and were used to explain differences in the decline of fragments over distance between the species and different fragment sizes. Additional parameters (D_{50}, D_{90} and D_{95}) were derived from the fitted regression curves, indicating the distances (m) at which 50%, 90% and 95% of fragments were retained, respectively. All regressions were performed in SigmaPlot 14 (Systat Software Inc., San Jose, CA, USA).

In order to compare the relevance of different retention agents along the stream course, retention strength (i.e. the relative fragment retention by a certain retention agent type within a subsection) was calculated for main groups of retention agent types in either 5 (KB), 25 (JB) or 50 m (GB1, GB2) subsections of each investigated stream section as follows:

$$\text{Retention strength} = \frac{n_{\text{rtot}}}{n_{\text{s}}} \times \frac{n_{\text{r}}}{n_{\text{rtot}}} \qquad (7)$$

Where n_{s} is the number of fragments (retrieved) that entered a subsection, n_{rtot} is the total number of fragments retained within a subsection and n_{r} is the number of fragments retained within a subsection by a specific retention agent type, respectively. Hence, a retention strength of 1 would imply that a given retention agent type was responsible for retaining all fragments that entered a respective subsection. Retention strength was only calculated for subsections until 95% of all fragments retrieved in a stream section were retained.

Statistical analysis

In order to examine differences in fragment decline among the species and stream sections and to identify the most relevant retention parameters, a principal component analysis (PCA) was performed on data for fragment retention parameters derived from the logistic regressions (k_1, k_2, D_{50}, D_{90} and D_{95}). Generalized linear mixed models (GLMM) were applied to test the influence of species (i.e. fragment morphology), buoyancy score (BS) and fragment size on drift distance for each stream section, and included field survey date as random effect. In addition to the GLMMs, mixed-effects Cox proportional hazard regression (CoxPHme) was used to examine the influence of BS on the likelihood of fragment retention over drift distance for each stream section and to test the influence of fragment size on retention probability in the stream sections separately for *E. canadensis*, *M. spicatum* and *C. demersum*, respectively. Within these CoxPHme models, random intercepts were specified for field survey date. To examine differences in the likelihood of retention between fragments of a floating and fragments of a submerged species that are equal in BS and similar in size, *S. natans* and 3-cm *C. demersum* fragments were compared (*S. natans* vs 3-cm *C. demersum*). Therefore, CoxPHme models were conducted for each stream section, including field survey date as random effect. CoxPHme models were further used to analyze the effect of discharge as indicator of stream size and the effect of stream sinuosity (meandering vs straightened) on the likelihood of fragment retention over drift distance among the four stream sections, with stream section included as random effect. Statistical tests and visual inspection of the scaled Schoenfeld residuals were conducted to check the assumption of proportional hazards for each CoxPHme model. In case that assumption of proportional hazards was violated, stratification was integrated in the models to achieve proportionality. A hazard ratio (HR) <1 or >1 indicates a decreased or increased likelihood of retention over drift distance with each increase in factor level (or between two single levels), respectively, while an HR of 1 indicates no difference in retention probability. For each HR, 95%

confidence intervals (CI) are given. All statistical analyses were carried out in R version 3.6.1 (R Core Team).

Results

Decline of drifting fragments

Overall, the relative number of drifting fragments logistically declined with increasing distance from release point. Based on the principal component analysis (PCA) on fragment retention parameters derived from the regressions, PC1 and PC2 together accounted for 76.2% of the total variance in the dataset (Figure 2). Data points for fragments were generally well discriminated by PC1 according to stream section rather than species, except for three data points of GB1 (12-cm fragments of the submerged species). The highest loading, i.e. the influence of a variable on the principal components, was documented for distance at which 90% of fragments were retained (D_{90}), followed by D_{95} and D_{50}, contributing 25.6%, 21.6% and 20.7%, respectively, to the cumulative share of PC1 and PC2. Thus, differences in the decline of drifting fragments were strongest among the stream sections and best explained by D_{90}. Small streams showed low D_{90} of only 5-9 m (KB) and 19-70 m (JB), while higher D_{90} values of 116-903 m and 153-2,367 m were determined for GB1 and GB2, respectively. The likelihood of retention over drift distance significantly decreased with increasing discharge conditions of a stream by a factor of 75 (HR = 0.01, 95% CI = <0.01-0.04, $p < 0.0001$), indicating that fragment retention was significantly enhanced in larger streams with higher discharge. Moreover, retention probability within the straightened stream sections was about 19 times lower than for the meandering sections (HR = 0.05, 95% CI = 0.04-0.08, $p < 0.0001$).

Within the stream sections, the species showed strong variation in the decline of drifting fragments and differed significantly in their drift distances (Figure 3, Table 2). According to the logistic regressions, the lowest overall D_{90} of 6 m was found for fragments of *E. canadensis* in KB, while the highest D_{90} of 1,216 m was documented for *C. demersum* in the straightened section GB2 (retention parameters of the regressions are summarized in Table S3). In GB2, 3-cm fragments of *C. demersum* even showed a maximum D_{90} and corresponding D_{95} of 2,367 and 4,255 m, respectively. Differences in the decline of drifting fragments were particularly pronounced between fragments of *E. canadensis* and *M. spicatum* and the highly buoyant fragments of *C. demersum* and *S. natans* (Figure 3).

Fragment buoyancy strongly contributed to the observed differences in the decline of drifting fragments and significantly influenced drift distance in all stream sections (Table 2). An increase in buoyancy score (BS) corresponded to a lower retention depth (Figure S1a) and significantly decreased the likelihood of fragment retention over drift distance by a factor of 3 (GB1: HR = 0.33, 95% CI = 0.24-0.45, $p < 0.0001$) to 8 (GB2: HR = 0.13, 95% CI = 0.09-0.18, $p < 0.0001$).

For fragment size, a significant effect on overall drift distance of the submerged species was documented in GB1, but not in KB, JB and GB2 (Table 2). In stream sections of the medium-sized Gillbach, increasing fragment size significantly enhanced the likelihood of retention over distance for *E. canadensis* (GB2: HR = 1.66, 95% CI = 1.14-2.41, $p = 0.008$) and *C. demersum* (GB1: HR = 1.15, 95% CI = 1.11-1.20, $p < 0.0001$, GB2: HR = 1.04, 95% CI = 1.00-1.08, $p = 0.039$), even though the increase in retention probability was low. For *M. spicatum*, by contrast, an increase in fragment size was coupled with a significant reduction of the likelihood of fragment retention by a factor of 1.1 in all stream sections, except for GB1 (KB: HR = 0.94, 95% CI = 0.90-0.97, $p < 0.0001$, JB: HR = 0.88, 95% CI = 0.85-0.91, $p < 0.0001$, GB2: HR = 0.93, 95% CI = 0.89-0.97, $p < 0.0001$). Despite the marginally lowered retention probability, this effect corresponded well to the increase in buoyancy associated with an increasing fragment length of *M. spicatum*.

Contrasting effects were documented for surface-associated floating type, comparing *S. natans* and 3-cm *C. demersum* fragments. The likelihood of retention of floating *S. natans* relative to *C. demersum* fragments was significantly reduced in JB (HR = 0.69, 95% CI = 0.49-0.97, $p = 0.032$) but enhanced in GB2 (HR = 1.88, 95% CI = 1.32-2.69, $p < 0.001$). No significant differences were observed in KB and GB1.

Dispersal speed of fragments differed between the stream sections according to flow conditions. Fragments not retained within the stream sections GB1 ($n = 58$) and GB2 ($n = 99$), i.e. collected at the end of a section, drifted 12.0 ± 2.7 and 14.8 ± 4.2 m min^{-1} (means ± *SD*), respectively. In JB, fragments approximately drifted 4.5 m min^{-1} (only based on a single fragment), while no fragments were retrieved at the end of KB.

Relevance of retention agents

Corresponding to the overall decline in the number of drifting fragments, retention strength of retention agents showed stream section-specific fluctuations along the stretches and differed among retention agent types in the individual subsections (Figure 4). Overall, retention strength within the stream sections was highest for deadwood in GB1 (0.70), for riparian vegetation in KB (0.59) and for submerged vegetation in the straightened sections JB (0.59) and GB2 (0.40).

Among all fragment types, a lower buoyancy was associated with enhanced fragment retention by stream bed and submerged structures (Figure 5, Figure S1b). Conversely, emerged obstacles were increasingly important for the retention of highly buoyant fragments, particularly of *C. demersum* and *S. natans* (Figure 5). In KB, the largest proportions of all fragments were retained either by the stream bed (63.4-80.3% of *E. canadensis* and 3-cm *M. spicatum* fragments) or terrestrial vegetation, i.e. roots of riparian trees within the water forming a shallow riffle (48.7-88.2% of *C. demersum*, *S. natans*, 6-cm and 12-cm *M. spicatum* fragments). Likewise, fragments of *E. canadensis* and *M. spicatum* in the straightened section JB were mostly retained by submerged vegetation (55.0-91.1%), whereas the majority of *C. demersum* and *S. natans* fragments were retained by emerged aquatic vegetation (61.0-96.1%). In GB1, most drifting fragments of the species, except for 3-cm fragments of *E. canadensis* and *M. spicatum* that were mainly retained by stream bed, were intercepted by large deadwood blockades (44.8-93.2%), even though deadwood covered only 3.3% of the stream section. In accordance with the high coverage of 55.3%, submerged vegetation consistently contributed most (38.0-57.8%) to fragment retention in GB2.

Discussion

Control of fragment retention by extrinsic and intrinsic factors

Vegetative drift dispersal of unspecialized plant fragments is the primary dispersal mode for many aquatic plant species inhabiting streams as it allows them to cope with frequent disturbance by flow (Sand-Jensen *et al.*, 1999; Riis & Biggs, 2003). Large dispersal distances thereby contribute to the specific fragment dispersal capacity and may explain the rapid spread of invasive aquatic plant species along the stream course (Fleming & Dibble, 2015; Hussner *et al.*, 2017).

We found that fragment retention characteristics, and thus drift distances, are highly dynamic but influenced by the general stream properties in the first place. Stream size as well as the abundance, distribution and the degree of submergence/emergence of retention agents strongly impair fragment retention. As shown for small to medium-sized streams, drift distance of plant propagules generally increases with larger stream size. The range of drift distances in our study fits well with those documented for plant fragments in other small and medium-sized streams (Johansson & Nilsson, 1993; Riis & Sand-Jensen, 2006). Hence, the vast majority of plant fragments may be retained between a few meters and ~5 km, though it must be considered that fluctuations in discharge (Engström *et al.*, 2009) and seasonal effects (e.g. growth of submerged vegetation; Riis & Sand-Jensen, 2006) strongly alter retention patterns. In larger streams, drift distances of fragments can even be considerably greater due to the low interaction with potential retention agents when transported in the main flow (e.g. Andersson *et al.*, 2000). However, fragment dispersal is then expected to be restricted by very long drift durations and loss of fragment viability. Hydromorphological differences due to channelization additionally influence fragment retention (cf. Johansson & Nilsson, 1993). We documented greater drift distances in straightened relative to meandering stretches, albeit aquatic vegetation coverage was six to eight times higher in the straightened sections. This may be at least partly due to the complex interplay between flow and patch dynamics of the resident aquatic vegetation (Cornacchia *et al.*, 2019). At high flow velocities, propagules more likely drift unimpeded over submerged plants that are bent towards the stream bed rather than being trapped within patches. There are, however, species-specific differences in plant flexibility and the potential for reconfiguration (Sand-Jensen, 2003, 2008), suggesting different effects on retention depending on resident species identity. Interestingly, our results particularly pronounce the strong retention capacity of large deadwood (which retained the majority of fragments in GB1 despite low in coverage and number). Deadwood may consequently be deployed in stream restoration to effectively facilitate potential (re-)establishment of aquatic plants (Engström *et al.*, 2009) but may also serve as a useful measure to limit the dispersal of invasive aquatic plant species. In general, however, obstacle identity is less important for fragment retention than the degree of submergence/emergence, as the relevance of protruding obstacles increases with increasing fragment buoyancy (see also Riis & Sand-Jensen, 2006).

Among the intrinsic factors, fragment buoyancy is most likely the strongest determinant of retention probability. We found that increasing buoyancy is coupled with a lower retention depth and an increase in drift distance, while effects of fragment size and morphology seem to be of secondary importance. Floating aquatic plants, including invasive species like *Salvinia molesta* D. S. Mitch., *Pistia stratiotes* L. and *Eichhornia crassipes* (Mart.) Solms, are thus expected to have the highest potential for long-distance dispersal and rapid range extension in streams (Hussner *et al.*, 2014a; Brundu, 2015; Heidbüchel *et al.*, 2016), but do not necessarily show higher drift distances than highly buoyant fragments of submerged species. Conversely, the dispersal speed of species with a low fragment buoyancy such as *E. canadensis* and *Elodea nuttallii* Planch. St. John (Cornacchia *et al.*, 2019) may to a large extent be limited by their low drift distances. In the case of both *Elodea* species, however, high fragmentation rates and regeneration abilities likely compensate for a low drift distance and ensure a high fragment dispersal capacity (Heidbüchel *et al.*, 2019b; Heidbüchel & Hussner, 2020). Even though several studies already acknowledged the importance of fragment buoyancy for vegetative dispersal (Riis & Sand-Jensen, 2006; Sarneel, 2013; Stafford-Bell *et al.*, 2015; Cornacchia *et al.*, 2019), little is known about the temporal fluctuations in buoyancy characteristics and the influence of abiotic factors such as light and temperature, particularly for submerged aquatic plants. Additionally, fouling of aquatic plants by e.g. epiphytic algae (Cattaneo & Kalff, 1980) and deposition of seston may significantly reduce buoyancy of plant fragments formed in the field. Nevertheless, buoyancy alone cannot explain the retention dynamics within streams. Our findings demonstrate that fragment size can have contrasting effects on retention and generally becomes more important in larger streams. Within medium-sized streams, smaller fragments are inclined to have a lowered likelihood of retention, at least if similar to fragments of *E. canadensis* and *C. demersum*. Observations in the field further suggest that branching of aquatic plant fragments affects the impact of fragment size on retention. Highly branched fragments are more compact and might be less likely retained compared to unbranched fragments of equal total fragment length (pers. obs.). Due to the strong influence of buoyancy on fragment retention, it was difficult to single out effects associated with plant morphology and structure. However, the high flexibility of *M. spicatum* (Miler *et al.*, 2014) likely facilitated tangling of the fragments around obstacles, and thus contribute to its low drift distances despite having a relatively high buoyancy. Flow-mediated effects may have additionally influenced buoyancy of the delicate *M. spicatum* fragments in the field, as we determined buoyancy under controlled conditions excluding water movement. Other fragment morphologies than those of the species studied, e.g. rosette growth with strap-like leaves of species like the invasive *Vallisneria spiralis* L., can still behave differently and deserve further attention.

In an earlier study, fragments of *E. canadensis* and *Ranunculus peltatus* Schrank were documented to decline exponentially over drift distance in vegetated small to medium-sized streams (Riis & Sand-Jensen, 2006). We demonstrate that the retention dynamics associated with the stream and propagule-specific properties do not always result in an exponential decline of propagules. Here, we propose a logistic relationship for the decline in the number of drifting fragments over distance that was suitable to describe the different retention dynamics observed in our study.

Successful establishment following retention

Once retained, plant fragments must colonize, i.e. anchor within the sediment, to avoid recurrent dislodgement. Hydraulic fluctuations can impede colonization and increase drift distance and duration. Not surprisingly, initial colonization was found to be the major bottleneck for the successful establishment of aquatic plants in streams (only 3.4% of retained fragments), emphasizing the importance of retention at suitable habitats for a sufficiently long period to anchor in the sediment (Riis, 2008).

This aspect is of particular importance, because most species are in general not limited by their regeneration and colonization capacities, even though differences among species and fragment types exist (Barrat-Segretain *et al.*, 1999; Barrat-Segretain & Bornette, 2000; Riis *et al.*, 2009; Umetsu *et al.*, 2012a). As the regeneration probability increases with increasing fragment size (e.g. Heidbüchel *et al.*, 2019b), larger fragments are expected to remain viable for a longer period and may become more important when regeneration is constrained by less optimal environmental conditions of the receiving habitat. Thus, despite the lower drift distances documented for 12-cm fragments of *E. canadensis* and *C. demersum* in a medium-sized stream, new plant stands might often result from these larger fragments. The likelihood of colonization is particularly high for aquatic plants characterized by fast regeneration, such as *M. spicatum* and the widespread hydrocharitaceans *E. canadensis*, *E. nuttallii*, *Lagarosiphon major* (Ridley) Moss and *Hydrilla verticillata* (L.F.) Royle (Heidbüchel & Hussner, 2019; Heidbüchel *et al.*, 2019b). However, even under controlled conditions, plant fragments of highly regenerative species do not initiate new growth before seven days (Kuntz *et al.*, 2014; Heidbüchel & Hussner, 2019; Heidbüchel *et al.*, 2019a). It is therefore likely that retention documented here represents transient stopping places during early downstream dispersal.

Moreover, the timing of retention is viewed as an important factor. While propagules of emerged and riparian species were found to reach suitable habitats during high flow (Engström *et al.*, 2009; Catford & Jansson, 2014), colonization of submerged species is increased at low water levels (Riis, 2008; Heidbüchel & Hussner, 2019). As we only conducted field surveys under normal runoff conditions, the proportion of fragments retained at suitable sites might be lower than at high/low water levels. Nevertheless, the environmental conditions of the receiving habitat, e.g. flow conditions, sediment type, nutrient, carbon and light availability, strongly control the successful colonization and establishment of aquatic plants (Bornette & Puijalon, 2011; Sand-Jensen & Møller, 2014; Ellawala *et al.*, 2019). While submerged species may settle at many sites, particularly when associated with the stream bed or submerged vegetation (Riis & Sand-Jensen, 2006; Riis, 2008), floating species are largely restricted to lentic to slow-flowing sites such as sheltered littoral zones or emerged aquatic vegetation patches (e.g. Neuenschwander *et al.*, 2009). Indeed, *S. natans* fragments were readily trapped by riparian vegetation providing suitable habitats. But although resident aquatic vegetation is viewed as a crucial retention agent, competitive effects in dense plant beds can limit resource availability and impede the establishment success of arriving fragments (Capers *et al.*, 2007; Chadwell & Engelhardt, 2008; Petruzzella *et al.*, 2018). For future studies, it is reasonable to integrate the likelihood of retention at sites suitable for colonization rather than sole retention probability.

Albeit initial colonization poses the limiting factor for the dispersal success of aquatic plants, there is a major gap in knowledge about the underlying mechanisms (i.e. rooting strategies) and the likelihood of fragment colonization in the field. Some species can rapidly allocate biomass towards the development of long, fine roots to anchor in the sediment while still floating (e.g. *M. spicatum*; Heidbüchel & Hussner, 2019), whereas fragments of others might have to sink before being able to colonize. Tracking of fragments over the whole dispersal process, i.e. from release to successful establishment, is highly needed to elucidate these species-specific limitations for initial colonization. However, a high number of released fragments, thus also propagule pressure, as well as high regeneration and colonization abilities generally enhance the overall likelihood for successful establishment of a species (Heidbüchel *et al.*, 2019b).

Implications for assessment of fragment dispersal capacity

Besides fragmentation rate and the regeneration/colonization abilities, drift distance and fragment retention characteristics are considered as major determinants of the species-specific fragment dispersal capacity in streams and therefore contribute to the assessment of the invasion potential of a species (Fleming & Dibble, 2015; Heidbüchel *et al.*, 2016, 2019b). Based on our results, fragment buoyancy is an overall reliable predictor of drift distance and can be easily determined for all kind of fragments. Determination of a buoyancy score for a set of plant fragments constitutes an example of an efficient straightforward approach to assess buoyancy, though more sophisticated methods may also be implemented (e.g. by force transducer measurements; Cornacchia *et al.*, 2019). As fragments can drift for a long period, the specific buoyancy dynamics over time should be further addressed (cf. Sarneel, 2013). We emphasize that information on fragment buoyancy help to evaluate the fragment dispersal capacity of invasive species in order to comply with legislations and regulations tackling the management of invasive species and the prevention of their further spread, such as the European Union regulation on invasive alien species (EU regulation 1143/2014).

Conclusion

Finally, with regard to the hypotheses, we conclude (i) that stream size and channelization influence the likelihood of fragment retention, which is lowered in larger straightened streams. (ii) Buoyancy rather than fragment size and morphology drives the fragment retention patterns among the fragment types covered in this study, with highly buoyant fragments showing a strongly attenuated likelihood of retention over drift distance. Fragment size becomes a more relevant determinant in larger streams, while the growth form and morphology of plants can have contrasting effects on retention. Lastly, (iii) fragment retention is defined by the specific distribution of retention agents along the stream course and the degree of obstacle submergence/emergence rather than retention agent identity.

We stress the strong interplay between extrinsic and intrinsic properties for fragment retention of submerged and floating aquatic plants. Straightened medium-sized streams pose rapid dispersal corridors and are highly susceptible to invasive aquatic plant species that produce large amounts of small fragments with a high buoyancy. Thus, measures reducing the propagule pressure of established invasive species, e.g. targeted trapping by applying artificial retention agents, may be particularly useful within such stream systems. Information on fragment buoyancy further helps to evaluate the species-specific fragment dispersal capacity and its relevance for the invasion success of a species. However, there is a great need for holistic approaches unraveling the fragment colonization patterns and the specific likelihood of initial colonization in the field.

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Table 1 Locations and properties of the stream sections of small to medium-sized German streams selected for the fragment drift field surveys

Stream	Stream section	Coordinates	SI	Surface area	Length	Width	Flow velocity[a]	Discharge	Coverage	
									Submerged aquatic veg.	Emerged aquatic veg.
		(°)		(m^2)	(m)	(m)	($m\ s^{-1}$)	($m^3\ s^{-1}$)	(%)	(%)
Krumbach	KB	N51.274462, E6.917729	1.25	69	40	1.74 ± 0.20	0.16 ± 0.03	0.05 ± 0.01	1.2	7.6
Jüchener Bach	JB	N51.214566, E6.554294	1.00	423	225	1.89 ± 0.21	0.23 ± 0.06	0.07 ± 0.02	44.2	16.8
Gillbach	GB1	N51.130066, E6.664686	1.22	936	480	2.80 ± 0.81	0.26 ± 0.04	0.30 ± 0.02	7.0	0.2
	GB2	N51.04293, E6.698442	1.01	2,006	450	4.47 ± 0.51	0.49 ± 0.07	0.52 ± 0.12	55.3	<0.1

Stream sinuosity for the stream sections is indicated by sinuosity index (SI). For stream width, flow velocity and discharge, mean values ± *SD* are shown. Flow velocity and discharge measurements are based on four sampling days per stream section ($n = 4$).

[a] Values determined mid-stream (~5 cm below water surface) at one single site.

Table 2 Summary of the generalized linear mixed models (GLMM) for the influence of species (i.e. fragment morphology), buoyancy score (BS) and fragment size on drift distance in the stream sections KB (Krumbach), JB (Jüchener Bach), GB1 and GB2 (Gillbach)

	Species		BS		Fragment size	
Stream section	χ^2 (*df*, *n*)	*p* value	χ^2 (*df*, *n*)	*p* value	χ^2 (*df*, *n*)	*p* value
KB	34.15 (3, 1167)	**<0.0001**	47.80 (1, 1167)	**<0.0001**	3.48 (1, 1167)	0.062
JB	30.22 (3, 1015)	**<0.0001**	135.49 (1, 1015)	**<0.0001**	2.83 (1, 1015)	0.093
GB1	11.50 (3, 622)	**0.009**	32.05 (1, 622)	**<0.0001**	54.31 (1, 622)	**<0.0001**
GB2	14.08 (3, 800)	**0.003**	11.47 (1, 800)	**<0.001**	1.28 (1, 800)	0.259

Shown are χ^2 values of the test statistic and corresponding *p* values. Significant *p* values are written in bold ($p < 0.05$).

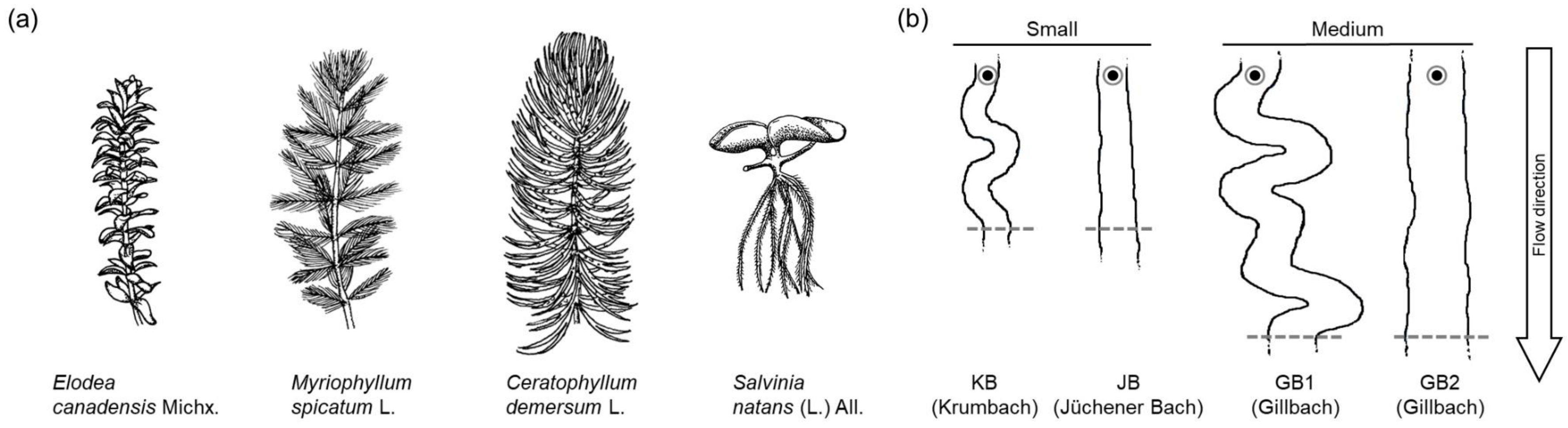


Figure 1 (a) Fragment morphology of the four aquatic plant species studied and (b) simplified overview of the meandering (KB, GB1) and straightened sections (JB, GB2) of small to medium-sized German streams at which field surveys were carried out (see Table 1). Fragment release points (dots) and drift barriers limiting the maximum possible drift distance (dashed grey lines) are indicated. Scientific drawings by M. Sachs (© 2019 Maria Sachs)

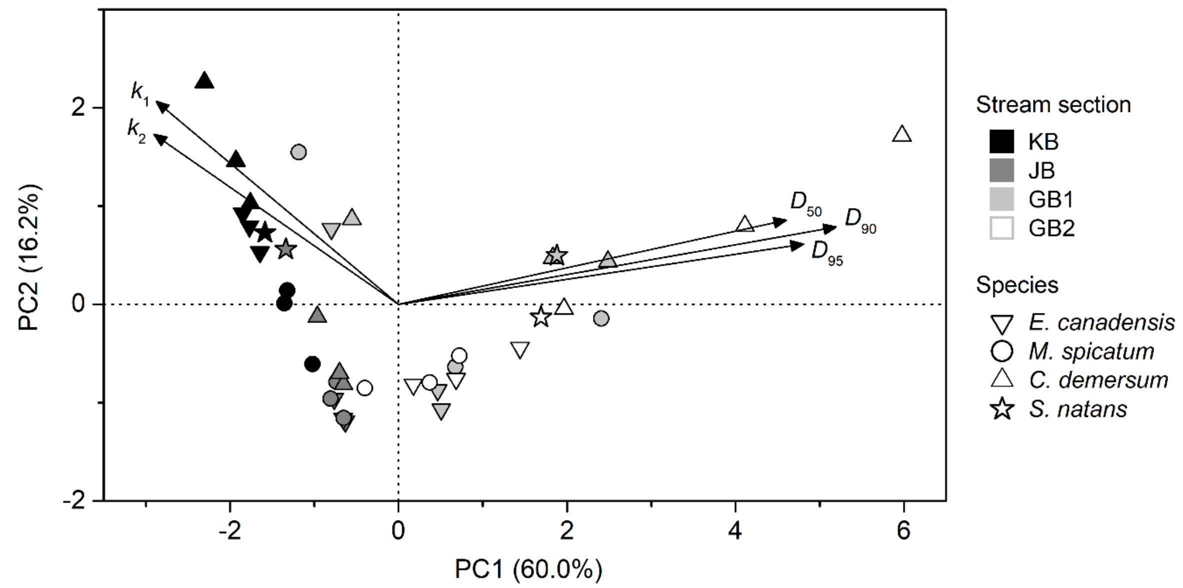


Figure 2 Principal component analysis (PCA) biplot of fragment retention parameters derived from the logistic regressions (Eq. 3) for *Elodea canadensis*, *Myriophyllum spicatum*, *Ceratophyllum demersum* and *Salvinia natans* in the stream sections KB, JB, GB1 and GB2. Species are indicated by symbols, while shading of symbols gives stream sections. Loadings on PC1 and PC2 for the retention coefficients (k_1, k_2) and distances at which 50%, 90% and 95% of fragments were retained (D_{50}, D_{90}, D_{95}) are indicated by black arrows. Percent variance explained by each PC is given in parentheses

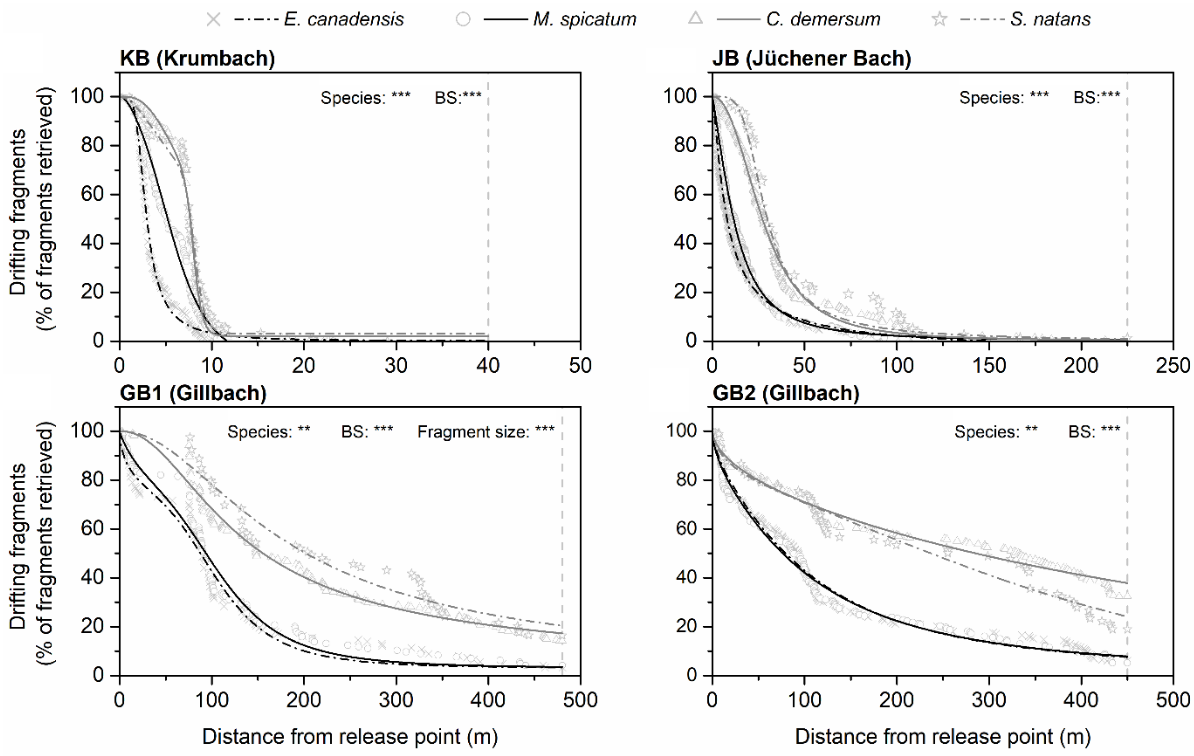


Figure 3 Decline in relative number of drifting fragments of *Elodea canadensis*, *Myriophyllum spicatum*, *Ceratophyllum demersum* and *Salvinia natans* with increasing distance from release point for the stream sections KB, JB, GB1 and GB2. Shown are data points, fitted logistic regression curves (acc. Eq. 3) and maximum possible drift distances, i.e. stream section lengths (vertical dashed grey lines). Corresponding retention parameters for each logistic fit are given in Table S3. Significant effects of species, buoyancy score (BS) and fragment size on drift distance (see Table 2) are asterisked according to significance level (**p* <0.05, ***p* <0.01, ****p* <0.001; GLMM)

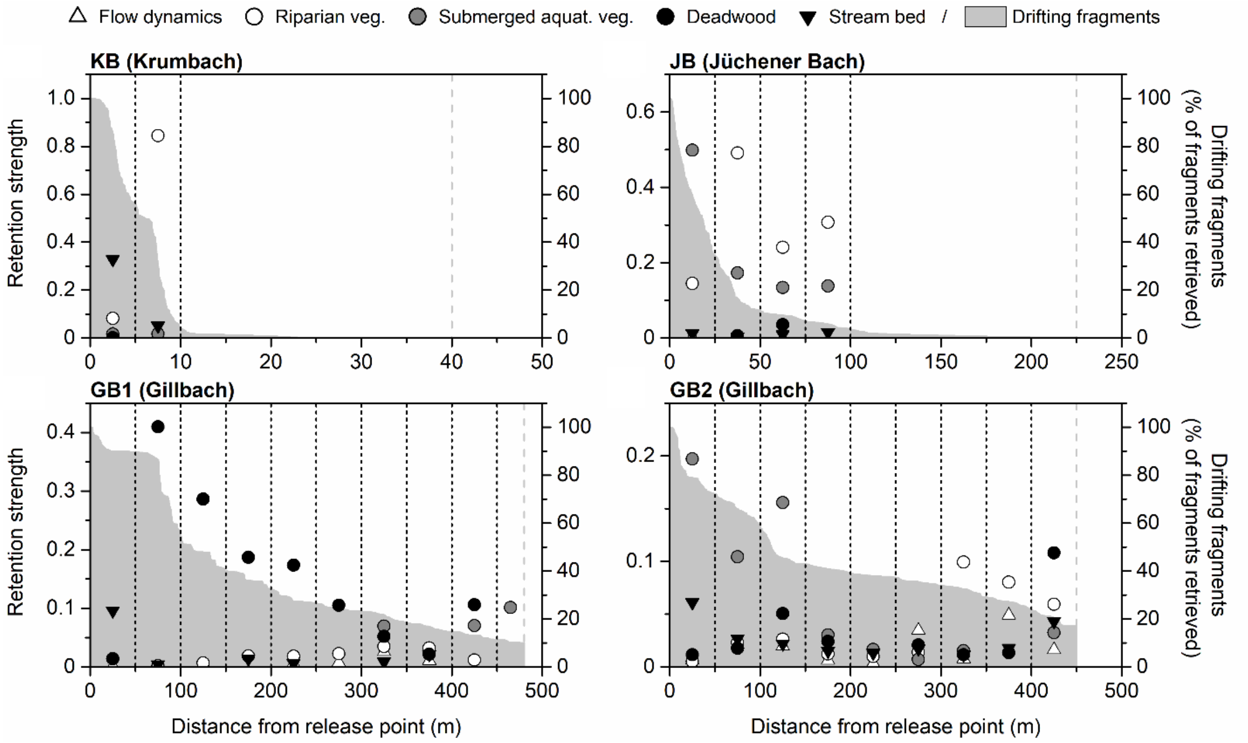
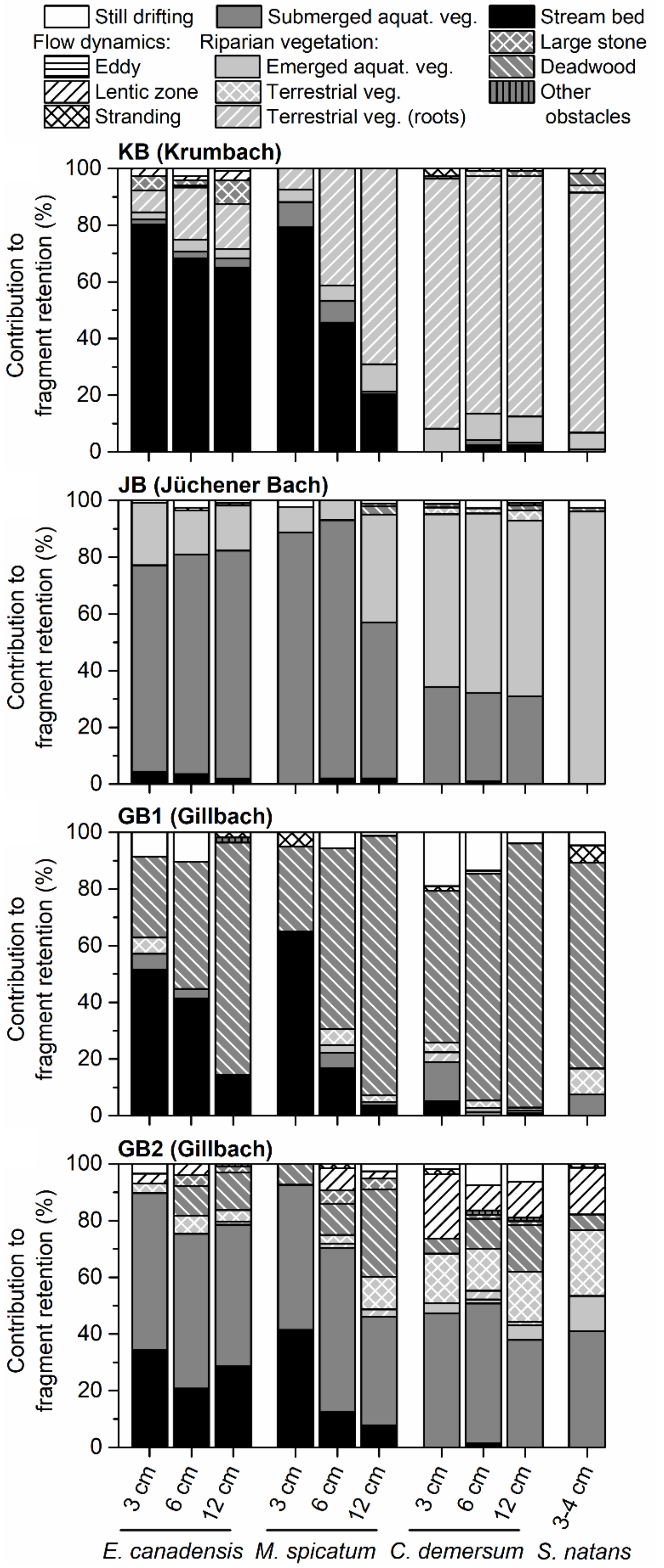


Figure 4 Retention strength of the most relevant retention agent types for subsections of the stream sections KB, JB, GB1 and GB2. Corresponding overall declines in the relative number of drifting plant fragments of all species (grey-shaded areas) are shown on secondary y-axes. Borders of the subsections (vertical dotted black lines) and maximum possible drift distances, i.e. stream section lengths (vertical dashed grey lines) are indicated. Retention strength is only shown for subsections until 95% of all fragments retrieved in a stream section were retained and if retention strength of a retention agent type was >0



◄ **Figure 5** Contribution of retention agents to fragment retention of *Elodea canadensis*, *Myriophyllum spicatum*, *Ceratophyllum demersum* and *Salvinia natans* depending on fragment size for the stream sections (a) KB, (b) JB, (c) GB1 and (d) GB2

Supporting information

Table S1 Fragment buoyancy score of *Elodea canadensis*, *Myriophyllum spicatum*, *Ceratophyllum demersum* and *Salvinia natans* depending on fragment size before ($BS_{control}$) and after spray-painting (BS) of plant fragments

		Fragment buoyancy		
Species	Fragment size (cm)	$BS_{control}$	BS	Discrepancy
E. canadensis	Total	0.51	0.49	-0.02
	3	0.46	0.44	-0.02
	6	0.53	0.48	-0.05
	12	0.56	0.54	-0.02
M. spicatum	Total	0.89	0.84	-0.05
	3	0.81	0.75	-0.06
	6	0.88	0.82	-0.06
	12	0.96	0.94	-0.02
C. demersum	Total	1	1	0
	3	1	1	0
	6	1	1	0
	12	0.99	0.99	0
S. natans	Ø 3-4	1	1	0

Table S2 Number of fragments released and fragments retrieved of *Elodea canadensis*, *Myriophyllum spicatum*, *Ceratophyllum demersum* and *Salvinia natans* depending on fragment size for the stream sections KB (Krumbach), JB (Jüchener Bach), GB1 and GB2 (Gillbach)

			KB		JB		GB1		GB2	
Species	Fragment size (cm)	n_{released}	$n_{\mathrm{retrieved}}$	Retrieval (%)	$n_{\mathrm{retrieved}}$	Retrieval (%)	$n_{\mathrm{retrieved}}$	Retrieval (%)	$n_{\mathrm{retrieved}}$	Retrieval (%)
E. canadensis	Total	360	357	99.2	342	95.0	123	34.2	217	60.3
	3	120	117	97.5	114	95.0	37	30.8	33	27.5
	6	120	120	100	115	95.8	31	25.8	84	70.0
	12	120	120	100	113	94.2	55	45.8	100	83.3
M. spicatum	Total	360	344	95.6	290	80.6	145	40.3	193	53.6
	3	120	112	93.3	89	74.2	22	18.3	41	34.2
	6	120	115	95.8	101	84.2	39	32.5	65	54.2
	12	120	117	97.5	100	83.3	84	70.0	87	72.5
C. demersum	Total	360	348	96.7	305	84.7	275	76.4	300	83.3
	3	120	110	91.7	83	69.2	77	64.2	95	79.2
	6	120	118	98.3	109	90.8	93	77.5	100	83.3
	12	120	120	100	113	94.2	105	87.5	105	87.5
S. natans	Ø 3-4	120	118	98.3	78	65.0	79	65.8	90	75.0
Grand total		1,200	1,167	97.3	1,015	84.6	622	51.8	800	66.7

n_{released} refers to the overall number of fragments released in each stream section on four different sampling days

Table S3 Fragment retention parameters derived from logistic regressions (Eq. 3) of *Elodea canadensis*, *Myriophyllum spicatum*, *Ceratophyllum demersum* and *Salvinia natans* depending on fragment size for the stream sections KB (Krumbach), JB (Jüchener Bach), GB1 and GB2 (Gillbach)

Species	Fragment size (cm)	k_1	k_2	D_{50} (m)	D_{90} (m)	D_{95} (m)	RMSE	r^2
KB								
E. canadensis	Total	5.16 ± 0.93	2.50 ± 0.85	3	6	8	2.94	0.99
	3	6.80 ± 0.19	2.31 ± 0.22	3	5	8	2.46	0.99
	6	4.73 ± 1.93	2.29 ± 1.79	3	7	10	4.16	0.98
	12	5.54 ± 1.87	2.66 ± 1.75	3	6	8	3.85	0.99
M. spicatum	Total	4.65 ± 0.15	0.72 ± 0.06	5	9	10	4.57	0.98
	3	5.06 ± 0.11	1.57 ± 0.12	4	8	11	2.73	0.99
	6	4.52 ± 0.16	0.86 ± 0.07	5	9	10	4.20	0.98
	12	12.73 ± 0.59	0.55 ± 0.03	7	9	10	3.46	0.99
C. demersum	Total	19.03 ± 0.85	1.11 ± 0.06	8	9	10	3.71	0.99
	3	25.91 ± 1.87	1.24 ± 0.14	8	9	10	4.52	0.98
	6	21.27 ± 1.16	0.93 ± 0.07	8	9	9	3.76	0.99
	12	15.60 ± 0.86	1.24 ± 0.11	7	9	10	4.38	0.98
S. natans	Ø 3-4	16.65 ± 1.31	0.70 ± 0.09	8	9	10	5.01	0.98
JB								
E. canadensis	Total	1.84 ± 0.02	0.55 ± 0.02	8	44	70	1.93	1.00
	3	1.77 ± 0.04	0.49 ± 0.03	8	46	73	3.01	0.99
	6	1.66 ± 0.43	0.80 ± 0.42	7	43	75	2.14	0.99
	12	2.25 ± 0.05	0.46 ± 0.02	9	36	49	2.68	0.99
M. spicatum	Total	2.58 ± 0.03	0.45 ± 0.01	11	42	63	2.07	0.99
	3	3.31 ± 0.08	0.58 ± 0.03	7	19	26	2.69	0.99
	6	1.83 ± 0.04	0.52 ± 0.03	9	42	59	2.44	0.99
	12	4.64 ± 0.10	0.51 ± 0.02	21	43	61	2.18	0.99
C. demersum	Total	4.02 ± 0.07	0.84 ± 0.04	27	64	85	3.10	0.99
	3	4.36 ± 0.20	0.49 ± 0.04	26	58	84	3.59	0.99
	6	4.97 ± 0.15	0.54 ± 0.03	27	56	87	2.97	0.99
	12	4.65 ± 0.08	1.29 ± 0.06	28	70	117	2.02	1.00
S. natans	Ø 3-4	4.62 ± 3.45	2.17 ± 3.30	30	67	96	5.55	0.97
GB1								
E. canadensis	Total	3.95 ± 0.25	0.22 ± 0.02	88	201	289	5.06	0.97
	3	1.01 ± 0.07	0.18 ± 0.03	45	464	661	4.32	0.98
	6	2.30 ± 0.20	0.17 ± 0.02	91	342	498	4.74	0.97
	12	15.51 ± 1.64	0.48 ± 0.07	94	116	131	4.89	0.98
M. spicatum	Total	3.95 ± 0.20	0.34 ± 0.03	94	220	323	5.26	0.97
	3	1.01 ± 0.15	0.34 ± 0.22	20	862	3,874	6.42	0.95

Table S3 (continued)

	6	2.30 ± 0.24	0.31 ± 0.08	107	433	659	7.97	0.93
	12	5.97 ± 3.76	2.87 ± 3.60	93	174	227	6.19	0.96
C. demersum	Total	2.09 ± 0.28	1.17 ± 0.25	156	828	1,630	3.08	0.98
	3	2.89 ± 0.22	0.35 ± 0.04	302	903	1,248	3.70	0.97
	6	2.53 ± 0.18	0.83 ± 0.14	191	862	1,561	3.60	0.97
	12	4.30 ± 2.20	2.12 ± 1.99	106	251	362	6.59	0.94
S. natans	Ø 3-4	2.59 ± 0.27	0.82 ± 0.25	202	868	1,522	5.10	0.95
GB2								
E. canadensis	Total	2.08 ± 0.05	0.28 ± 0.01	79	372	591	3.86	0.98
	3	1.33 ± 0.07	0.37 ± 0.05	46	744	2,048	3.06	0.99
	6	1.88 ± 0.08	0.26 ± 0.02	83	463	776	4.18	0.97
	12	2.83 ± 0.12	0.29 ± 0.02	84	253	349	4.00	0.98
M. spicatum	Total	2.00 ± 0.05	0.26 ± 0.01	76	379	611	3.60	0.98
	3	1.56 ± 1.60	0.76 ± 1.47	14	153	423	7.01	0.94
	6	2.42 ± 0.11	0.28 ± 0.03	88	326	479	4.42	0.98
	12	2.18 ± 0.05	0.43 ± 0.02	101	482	796	2.45	0.99
C. demersum	Total	1.62 ± 0.11	0.24 ± 0.01	285	1,216	1,500	2.91	0.97
	3	1.68 ± 0.14	0.24 ± 0.02	341	2,367	4,255	2.54	0.98
	6	1.19 ± 0.16	0.26 ± 0.03	262	1,848	2,529	3.53	0.96
	12	2.20 ± 0.17	0.23 ± 0.01	256	748	864	3.38	0.97
S. natans	Ø 3-4	2.53 ± 0.20	0.23 ± 0.02	237	654	775	4.56	0.96

For retention coefficients k_1 and k_2, mean values ± 1 *SE* are shown. D_{50}, D_{90} and D_{95} indicate the distances at which 50%, 90% and 95% of fragments were retained, respectively. Goodness of model fits is indicated by root mean square errors (RMSE) and r^2 values

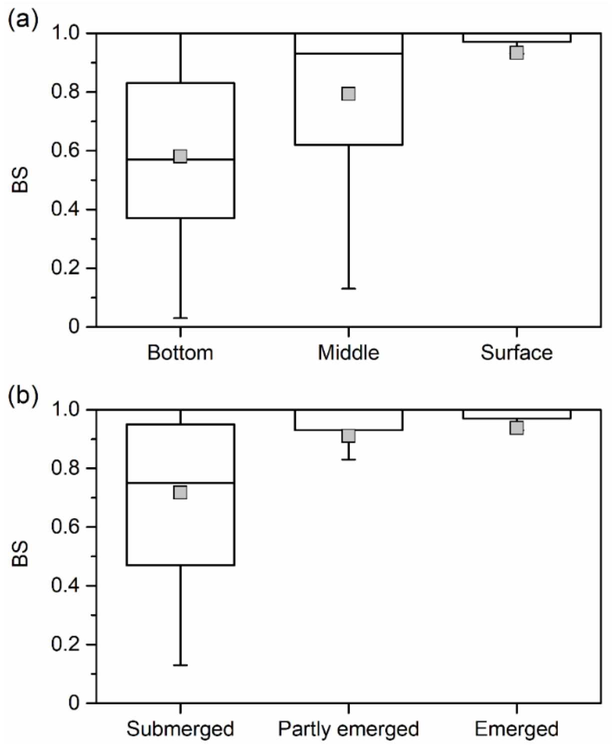


Figure S1 The effect of buoyancy score (BS) on (a) retention depth and (b) obstacle position (degree of submergence/emergence) associated with retention of fragments released in small to medium-sized streams. Shown are 25th and 75th percentiles (bottom and top of boxes, respectively), medians (horizontal lines in boxes), means (grey squares) and 1.5 × interquartile ranges (whiskers)

CHAPTER 5

Chlorophyll fluorometry sheds light on the role of desiccation resistance for vegetative overland dispersal of aquatic plants

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Abstract

1. Submerged aquatic plant species predominantly spread through vegetative propagules, particularly shoot fragments. While it is known that the successful establishment of fragments in isolated water bodies is largely determined by retention of fragment viability during overland transport (i.e. desiccation resistance), detailed information on species-specific desiccation resistance is still scarce and the underlying mechanisms remain uncharted.
2. We combined measurements of chlorophyll *a* fluorescence (F_v/F_m: maximum quantum yield of photosystem II) with determination of water loss and post-desiccation survival and regeneration to examine the desiccation resistance of shoot fragments without and with apical tips for six submerged aquatic plant species (*Myriophyllum spicatum*, *Myriophyllum heterophyllum*, *Ceratophyllum demersum*, *Lagarosiphon major*, *Elodea canadensis*, *Hydrilla verticillata*).
3. Overall, the relationship between F_v/F_m and relative water loss was nonlinear, and a decrease in F_v/F_m was significantly related to reduced fragment survival and regeneration. We determined an overall critical minimum of 0.40 in F_v/F_m and a critical maximum of 84% in water loss for regeneration. Differences in the relationships between water loss and desiccation time and between F_v/F_m and water loss were species-specific rather than fragment type-specific. Plant fragments of *M. spicatum* (fragments without apices excluded), *M. heterophyllum* and *C. demersum* maintained a high F_v/F_m even after losing a large proportion of the initial water content, while the F_v/F_m for *L. major*, *E. canadensis* and *H. verticillata* decreased more rapidly with advancing water loss.
4. Maintaining the function of the photosynthetic apparatus at high water loss can prolong fragment viability during overland transport. Our results suggest that aquatic plants own species-specific mechanisms to cope with water deficit. Measurements of chlorophyll fluorescence can precisely predict the likelihood of fragment regeneration and constitute a useful tool to assess the spread potential of aquatic plants.

Keywords aquatic macrophytes, imaging PAM, invasive species, plant fragments, water deficit

Introduction

Aquatic plant species generally show broader ranges than terrestrial plants, even though their habitats are more isolated (Santamaría, 2002). This is caused by various factors, such as the uniformity of the aquatic environment on a larger scale (Cook, 1985) and the high phenotypic and physiological plasticity of many aquatic plants (Puijalon *et al.*, 2008; Riis *et al.*, 2010; Eusebio Malheiro *et al.*, 2013; Pedersen *et al.*, 2013; Hussner *et al.*, 2016a). Beyond that, the wide geographical distribution of aquatic plant species must be based on highly effective spread mechanisms (Santamaría, 2002).

Submerged aquatic plants predominantly disperse via vegetative means, with simple shoot fragments being the most important propagules (Barrat-Segretain, 1996; Boedeltje *et al.*, 2003). Several alien species are limited exclusively to vegetative dispersal in their introduced range, due to the presence of only one sex (e.g. *Elodea canadensis* Michx. and *Egeria densa* Planch. in Europe; Cook & Urmi-König, 1985). In general, plant fragments of most aquatic plant species are highly viable and may regenerate through the formation of secondary shoots and roots, while the presence of apical tips additionally allows for regeneration through fragment elongation (Barrat-Segretain, 1996). The minimum fragment size required for regeneration (i.e. initiation of new growth), however, differs among species (Hussner, 2009; Riis *et al.*, 2009), with submerged plants being commonly able to regenerate from small shoot fragments consisting only of a single node (Langeland & Sutton, 1980; Kuntz *et al.*, 2014).

The formation and dispersal of fragments depend on species-specific fragmentation properties (Redekop *et al.*, 2016) as well as on disturbances through environmental factors like water movement and flow (Sand-Jensen, 2008), foraging by invertebrates, fish and waterbirds (Bakker *et al.*, 2016; Figuerola & Green, 2002) and human-mediated effects e.g. through recreation and mechanical weed control (Anderson, 1998; Owens *et al.*, 2001). Some of the few existing field studies on the vegetative dispersal of aquatic plants already documented high numbers of fragments in the drift of running waters (Boedeltje *et al.*, 2003; Riis & Sand-Jensen, 2006; Heidbüchel *et al.*, 2016), which explains the rapid and successful spread of submerged aquatic macrophytes within interconnected water bodies. Conversely, the dispersal between isolated waters, such as lakes, depends on vectors transporting the fragments to the receiving water bodies (Rothlisberger *et al.*, 2010; Green, 2016). It was recently shown that birds, particularly mallard ducks, can act as vectors for vegetative propagules of floating *Lemna* spp. (Coughlan *et al.*, 2017b). However, it is still unclear how frequent animal-mediated dispersal of submerged plant fragments occurs and whether it significantly contributes to the spread between hydrologically unconnected sites (Johnstone *et al.*, 1985; Coughlan *et al.*, 2017a). By contrast, human-mediated overland transport, such as trailered boating, was recognized as the major driving force for the vegetative dispersal of aquatic plants to isolated water bodies (Johnstone *et al.*, 1985; Johnson *et al.*, 2001; Rothlisberger *et al.*, 2010) and the extra-range dispersal of invasive alien aquatic species in general (Wilson *et al.*, 2009; Anderson *et al.*, 2014). Plant fragments can be attached to water sport equipment of e.g. anglers and canoeists, but are more frequently found attached to trailers or wrapped around boat propellers (Johnson *et al.*, 2001; Rothlisberger *et al.*, 2010). Once introduced into new water bodies, the likelihood for establishment of the transported fragments largely depends on the viability at the time of introduction and subsequent potential for regeneration (Johnstone *et al.*, 1985).

The relative water content (RWC) of aquatic plants (~86-95% of fresh mass) is comparable to the water content of many herbaceous terrestrial plants (Sculthorpe, 1967). However, submerged species show adaptations to the aquatic environment, such as less lignified vascular tissue, thin leaves with a high surface area to volume ratio and an extremely reduced cuticle, which facilitate transpiration once a plant is exposed to drying conditions (Sculthorpe, 1967). As a consequence, submerged plant fragments rapidly suffer from water deficit when out of water, though the rate of water loss differs among species (Barnes *et al.*, 2013). The water content of plants generally consists of bulk cytoplasmic water and hydration water that is forming the hydration shells of macromolecules. While effects of bulk cytoplasmic water loss may be reversed upon rehydration, the loss of hydration water results in protein denaturation and irreversible damage to membranes, thus limiting plant viability (Hoekstra *et al.*, 2001). Additionally, ongoing water loss promotes the generation of reactive oxygen species (ROS; especially in chloroplasts when exposed to light), causing oxidative stress and damage to the photosynthetic apparatus (Smirnoff, 1993; Dinakar *et al.*, 2012). The negative effects of ROS are in turn mitigated through antioxidant metabolites and enzymes that scavenge free radicals (e.g. Kranner & Birtić, 2005).

During overland transport, the water loss rate and viability of plant fragments strongly depend on the environmental conditions (e.g. Bruckerhoff *et al.*, 2015). A low vapor pressure deficit (VPD; as a function of temperature and relative humidity), stagnant air and clumping of fragments mitigate evaporative loss of water and might cause prolonged viability of fragments (Jerde *et al.*, 2012; Bickel, 2015; Bruckerhoff *et al.*, 2015; Coughlan *et al.*, 2018). Even though water deficit generally reduces the survival and regeneration abilities of plant fragments, the resistance to desiccation can differ among species, determining the viability of fragments after overland transport (Barnes *et al.*, 2013). Moreover, the regeneration capacity of fragments is influenced by additional factors such as fragment type (e.g. single leaves, stem sections with leaves, presence of an apical tip; Langeland & Sutton, 1980; Hussner, 2009; Umetsu *et al.*, 2012a; Kuntz *et al.*, 2014) or fragment length (Riis *et al.*, 2009; Redekop *et al.*, 2016; Bickel, 2017). Besides the specific regeneration abilities and environmental conditions during overland transport, the properties of the receiving habitat may also limit the successful establishment of plant fragments. Abiotic factors such as temperature, light, nutrient and carbon availability are known to influence plant growth and fragment regeneration (Riis *et al.*, 2012; Kuntz *et al.*, 2014; Hussner *et al.*, 2015).

Invasive alien aquatic plants (IAAPs) pose a serious threat to aquatic ecosystems. Currently, the European Union has listed 10 IAAPs, for which strategies and measures are required to stop their further introduction into the EU and limit their spread within the European countries (EU, 2014). While the dispersal into isolated water bodies is largely based on the overland transport of plant fragments, information about the species- and fragment type-specific resistance to desiccation is substantive (Hussner *et al.*, 2017). In previous studies, the resistance of aquatic plant fragments to desiccation has so far been examined by determining the regeneration potential of plant fragments following designated periods of desiccation and by using water or mass loss as the only indicators for the viability of fragments (Johnstone *et al.*, 1985; Silveira *et al.*, 2009; Evans *et al.*, 2011; Jerde *et al.*, 2012; McAlarnen *et al.*, 2012; Barnes *et al.*, 2013; Bickel, 2015; Bruckerhoff *et al.*, 2015; Coughlan *et al.*, 2018). However, water and mass loss may be less accurate indicators for fragment viability than physiological parameters (specifically chlorophyll (Chl) fluorescence parameters), as the degree damage to the photosynthetic apparatus through oxidative stress at given water contents and the proportions of bulk cytoplasmic and hydration water may differ among species.

Here we analyzed the species- and fragment type-specific resistance to desiccation for two different fragment types of six submerged species by combining pulse amplitude modulated (PAM) Chl fluorometry with measurements of water loss and determination of fragment viability following desiccation. The goals of this approach were (1) to obtain more detailed information about the resistance of plant fragments to desiccation in general and to identify differences among species and fragment types, (2) to elucidate the specific link between photosytem II (PSII) quantum yield, water loss and post-desiccation viability and (3) to establish non-invasive PAM measurements as a useful tool to denote fragment viability, contributing to the determination of species-specific spread potentials.

Methods

Plant material and cultivation

The six species (M*yriophyllum spicatum* L., *Myriophyllum heterophyllum* Michx., *Ceratophyllum demersum* L., *Lagarosiphon major* (Ridley) Moss, *Elodea canadensis* Michx., and *Hydrilla verticillata* (L.f.) Royle) are widely distributed, known for their rapid spread and among the most troublesome aquatic weeds worldwide (Hussner *et al.*, 2017). Recently, *M. heterophyllum* and *L. major* have been listed as invasive species within the European Union (EU, 2014).

Among the species, morphological differences occur. *M. heterophyllum*, *C. demersum* and *L. major* were generally sturdier in leaf and stem structure than *M. spicatum*, *E. canadensis* and *H. verticillata*. Moreover, the aquatic plant species were characterized by either dissected (*M. spicatum*, *M. heterophyllum*, *C. demersum*) or entire submerged leaves (*L. major*, *E. canadensis*, *H. verticillata*), which are very thin with a leaf thickness typically <300 µm (Sand-Jensen & Frost-Christensen, 1999).

Plants of *M. spicatum* were sampled from the River Erft (Germany), while *M. heterophyllum*, *C. demersum*, *E. canadensis* and *L. major* were collected from a pond system at the University of Düsseldorf (Germany). *H. verticillata* originated from a laboratory stock of the National Institute of Water and Atmospheric Research (NIWA, New Zealand).

All plant species were cultivated separately in 65 L plastic tanks (57 x 39 x 42 cm, LWH) filled with a 5 cm layer of washed sand (obtained from the River Rhine, Germany) and a general purpose medium for aquatic plant cultivation (acc. Smart & Barko, 1985). In every culture, plants grew anchored in the sediment at room temperature and were exposed to a 16:8 h (light:dark) simulated photoperiod with a photosynthetic photon flux density (PPFD) of 124.2 ± 33.7 µmol photons m^{-2} s^{-1} (± *SD*; measured 1 cm above the water surface) for at least two weeks. During cultivation, evaporated water was compensated by regularly adding deionized water.

Preparation

In order to examine the desiccation resistance of aquatic plant fragments, a two-step approach was employed, combining (i) measurements of water loss and Chl fluorescence (F_v/F_m) followed by (ii) the determination of post-desiccation fragment survival and regeneration (Figure 1).

Randomly selected unbranched shoot fragments without (w/o) and with (w/) apical tips were obtained from the plant cultures and cut to an initial length of 6 cm shortly before use in the experiment. These shoots differed in their corresponding number of nodes among the species and fragment types (Table 1). Plant fragments already possessing buds were avoided to prevent biased regeneration. For each species and fragment type, a total of 25 fragments were used in both (i) desiccation and (ii) post-desiccation viability trials.

Five additional plant fragments were further taken to assess the initial relative water content (RWC) per species and fragment type (Table 1). Fresh mass (FM) was determined after adherent water was removed by using highly absorbent tissue papers. Plants were then dried to a constant weight at 85 °C (for at least 24 h) and dry mass (DM) was determined. The RWC was calculated according to the following equation:

$$\text{RWC (\%)} = 100 \times \frac{\text{FM}-\text{DM}}{\text{FM}} \qquad (1)$$

(i) Water loss and Chl fluorescence measurements

Measurements of water loss were conducted by determining the loss in fragment mass, whereas the quantum yield of PSII photochemistry in a dark adapted state (F_v/F_m) was used as the Chl fluorescence parameter. The measurements were performed in a series of desiccation trials according to species and fragment type. Collected plant fragments were kept in general purpose culture medium (acc. Smart & Barko, 1985) and placed in the dark for at least 5 min to achieve dark adaptation before desiccation trials were performed. Previous test measurements showed that a short dark adaptation time of 5 min is sufficient for the aquatic plant species to obtain maximum F_v/F_m ratios. All F_v/F_m measurements were performed on 6 cm long fragments utilizing an Imaging-PAM fluorometer (IMAG-MAX, Heinz Walz GmbH, Effeltrich, Germany).

After dark incubation, the initial F_v/F_m ratio was assessed first, followed by removal of adherent water with absorbent tissue papers and determination of FM (i.e. initial fragment mass). Plant fragments were subsequently placed onto a plastic tray in a dark chamber without air circulation/movement. Within the dark chamber, fragments were exposed to ambient conditions of 40.5 ± 9.5% relative humidity, 23.5 ± 0.7 °C and a corresponding vapor pressure deficit (VPD) of 1.72 ± 0.23 kPa (± *SD*). Measurements of relative humidity and temperature were performed at the beginning and at the end of each desiccation trial. During the desiccation trials, measurements of F_v/F_m and loss in fragment mass of each individual fragment were repeatedly performed in varying intervals until a target F_v/F_m (i.e. final specific F_v/F_m value) was observed. A minimum time difference between two consecutive measurements on a respective fragment of at least 5 min was included to ensure dark recovery of PSII. For each species and fragment type, plant fragments (n = 25) were desiccated until final specific F_v/F_m values were equally distributed between the initial maximum F_v/F_m (ca. 0.70) and a minimum F_v/F_m of about 0.30 (threshold value indicating serious damage to PSII). Thus, desiccation periods differed among species and fragment types according to specific decrease rates in F_v/F_m. This approach further entailed more frequent measurements of F_v/F_m and loss in fragment mass of a given fragment, the lower its final F_v/F_m. Fragments that reached specific final F_v/F_m values were subsequently used for the determination of fragment survival and regeneration.

Based on the measurements of fragment mass, the relative water loss (%) was finally calculated as follows:

$$\text{Water loss (\%)} = 100 \times \frac{(\text{initial } M_{\text{frag}} - \text{initial DM}) - (M_{\text{frag}} - \text{initial DM})}{\text{initial } M_{\text{frag}} - \text{initial DM}} \quad (2)$$

Whereby initial M_{frag} (g) is the initial fragment mass (equal to FM) and M_{frag} (g) is the fragment mass after a given desiccation period. The initial DM (g) was calculated as FM x DM/FM (note that DM/FM was assessed for additional plant fragments (n = 5), which were not used in the experiment).

(ii) Determination of fragment survival and regeneration

Once the fragments reached their specific final F_v/F_m values, they were directly moved to 260 mL plastic jars (7 cm in diameter and height) filled with a modified general purpose culture medium (acc. Smart & Barko, 1985), additionally containing 2 mg NO_3^--N L^{-1} and 0.1 mg PO_4^{3-}-P L^{-1}. As plant fragments of each treatment covered final F_v/F_m values from 0.70 to 0.30, fragment survival and regeneration were assessed in response to various degrees of damage through desiccation.

All fragments were subsequently grown at a 16:8 h (light:dark) simulated photoperiod and a PPFD of 100.4 ± 17.0 µmol photons m^{-2} s^{-1} (± *SD*; measured 1 cm above the water surface) for 28 days. These post-desiccation viability trials lasted for 28 days as previous investigations demonstrated that the majority of plant fragments initiate new growth within this period (see also Kuntz *et al.*, 2014). The medium was exchanged and plastic jars were cleaned as well as randomly repositioned on a weekly basis to maintain nutrient concentrations, to mitigate algal growth and to avoid position effects. Likewise, fragment survival and regeneration were assessed by weekly determination of fragment length and length of secondary shoots and/or roots if present.

In case of complete degradation of plant material, fragments were deemed as died off, while fragments that were not degraded after 28 days or successfully regenerated were classified as survived. To evaluate the onset of fragment growth, plant fragments were considered as regenerated if either fragment length increased by ≥2 cm, total secondary shoot length increased by ≥1.5 cm or total secondary root length increased by ≥3 cm (acc. Heidbüchel & Hussner, 2019). These low thresholds in fragment length and length of new shoots and roots were suitable to detect differences in post-desiccation viability for the 6 cm fragments of all species studied. Successfully regenerated fragments were removed from the experiment and not further examined.

Following the post-desiccation viability trials, overall survival and regeneration were calculated as the number of fragments which did not die off after 28 days or did not successfully regenerate relative to the total number of fragments per species (n = 50), respectively. Critical F_v/F_m (F_{crit}) and water loss (W_{crit}) values were calculated to indicate general thresholds for survival and regeneration of the species, respectively. F_{crit} represents the F_v/F_m above which 95% of data points for regenerated or survived fragments of all species may be found (equal to 5th percentile), while W_{crit} represents the water loss below which 95% of data points for either regenerated or survived fragments of all species may be found (equal to 95th percentile).

Statistical analysis

In order to describe the relationships between relative water loss and desiccation time and between F_v/F_m and water loss, respectively, nonlinear functions were fit to the data of each species and fragment type using MATLAB R2017a (Mathworks, Massachusetts, USA). The applied models were chosen based on functional plausibility and the evaluation of goodness of fit parameters (RMSE, SSE and adjusted r^2).

$$\text{Water loss (\%)} = 100 - A_1 \times e^{(-k_1 \times \text{desic. time})} \tag{3}$$

$$\text{Water loss (\%)} = 100 - (A_1 \times e^{(-k_1 \times \text{desic. time})} + A_2 \times e^{(-k_2 \times \text{desic. time})}) \quad (4)$$

Data on water loss depending on desiccation time were either described by asymptotic mono-exponential (Eq. 3) or bi-exponential (Eq. 4) functions with asymptotes set at 100%.

$$\mathrm{F_v/F_m} = a \times (1 - e^{(-k \times \text{water loss})}) + b \quad (5)$$

The model fits of F_v/F_m depending on water loss exclusively followed a mono-exponential function (Eq. 5). Differences in a respective coefficient of the fitted models for the two fragment types (w/o and w/ apex) of a species were tested by t-tests. The fitted curves of both fragment types were considered significantly different if at least one coefficient differed at the alpha level of 0.05. To denote differences in the species- and fragment type-specific relationships between relative water loss and desiccation time and between F_v/F_m and water loss, values for desiccation time at 50% water loss (W_{50}) and for water loss at a F_v/F_m of 0.55 ($F_{0.55}$) were calculated based on the fitted regression curves, respectively. The F_v/F_m of 0.55 was chosen because it represents the overall mean F_v/F_m value of all successfully regenerated plant fragments within the study.

Statistical analysis of the survival and regeneration of plant fragments following desiccation was carried out in R version 3.5.0 (R Core Team). Cox proportional hazards regression was used to test for differences in the overall survival and regeneration among the six species and to assess the combined influence of fragment type and F_v/F_m on survival and regeneration of each species separately. Water loss was not included in the models, as it was a poor indicator of both fragment survival and regeneration. Cox proportional hazards regression is commonly used in time to event analysis and addresses the probability of an event (survival/regeneration) at each point in time. All Cox regressions were subsequently followed by computing analysis of variance (ANOVA) tables. Differences in the overall survival and regeneration among the species were further analyzed by applying Tukey contrasts as post-hoc test. For each Cox regression, the assumption of proportional hazards was checked by statistical tests and visual inspection of the scaled Schoenfeld residuals.

Results

Desiccation of fragments

Desiccation of plant fragments generally followed species-specific patterns, as there were strong differences in the relationships between relative water loss and desiccation time as well as between F_v/F_m and water loss. For all species, imaging of F_v/F_m clearly demonstrated that F_v/F_m of plant fragments decreased with increasing water loss and that leaves desiccated first, while stems maintained a higher F_v/F_m for a longer period of time (exemplarily shown for *M. spicatum* and *L. major* in supplementary Figure S1). Consequently, the difference in F_v/F_m between leaves and stems increased at higher levels of water loss (Figure S1).

According to the nonlinear regression curves, relative water loss of plant fragments over desiccation time at a VPD of 1.72 ± 0.23 kPa (± *SD*) was more similar among *M. spicatum*, *E. canadensis* and *H. verticillata* on the one hand and *L. major*, *M. heterophyllum* and *C. demersum* on the other hand (Figure 2). The species *M. spicatum*, *E. canadensis* and *H. verticillata* were characterized by rapid initial water loss, whereas *L. major*, *M. heterophyllum* and *C. demersum* showed a comparably slower water loss during the initial phase before loss of water slowly leveled off (Figure 2). This is reflected by the high rate constants (k_1) of the former and rather low rate constants of the latter group of species (Table 2). Plant fragments of *M. spicatum*, *E. canadensis* and *H. verticillata* already lost 50% of the initial water content within a desiccation period of 0.3-0.4 h (Figure 2a,d,f, Table 2). In the case of *H. verticillata* fragments, however, water loss followed a mono-exponential function and loss of the vast majority of the water content (exceeding the W_{crit} of 84% for regeneration) even occurred within a very short period <1 h, which was reflected by a short late phase of decelerated water loss (Figure 2f). Conversely, fragments of *L. major*, *M. heterophyllum* and *C. demersum* lost 50% of the initial water content after considerably longer desiccation periods, with W_{50} values ranging from 0.9 (*L. major*, w/ apex) to 1.8 h (*M. heterophyllum*, w/ apex; Figure 2b,c,e, Table 2). Based on differences in the regression coefficients, plant fragments w/o and w/ apical tips indicated distinct relationships between water loss and desiccation time for all species (Table 2). Particularly, fragments w/o apical tips of *M. heterophyllum* showed a stronger increase in relative water loss over time and thus lower W_{50} when compared to fragments w/ apical tips (Figure 2c, Table 2). Conversely, the regression curves for fragments of *L. major* indicated that water loss during the early phase was slower for fragments w/o than for fragments w/ apices, which was, however, reversed at higher levels of water loss (Figure 2b, Table 2).

The F_v/F_m ratio decreased with increasing water loss in a curvilinear manner for all species, though regression curves were more similar among fragments of *M. spicatum*, *M. heterophyllum* and *C. demersum* and for the three hydrocharitacean species *L. major* (at least w/ apex), *E. canadensis* and *H. verticillata*, respectively (Figure 3). Shoot fragments of *M. spicatum*, *M. heterophyllum* and *C. demersum* were able to maintain relatively high F_v/F_m values even after losing a high percentage of the initial water content, followed by a rapid decline in F_v/F_m with any further water loss (Figure 3a,c,e, Table 3). According to the regression curves, a F_v/F_m ratio of 0.55 was still documented after a water loss between 72 (*M. spicatum*, w/o apex) and 83% (*C. demersum*, w/ apex) of initial water content, excluding *M. spicatum* w/o apex which showed a lower $F_{0.55}$ value of 63% (Figure 3a,c,e, Table 3). By contrast, fragments of *L. major*, *E. canadensis* and *H. verticillata* were characterized by a rather continuous decrease in F_v/F_m with increasing water loss along with considerably lower $F_{0.55}$ values ranging from 52 (*H. verticillata*, w/o apex) to 64% (*H. verticillata*, w/ apex), respectively (Figure 3b,d,f, Table 3). The relationship of F_v/F_m to relative water loss differed between fragments w/o and w/ apices for *M. spicatum*, *M. heterophyllum*, *C. demersum* and, particularly, *L. major*, as indicated by differences in the regression coefficients (Figure 3a-c,e, Table 3). Fragments w/ apical tips of *M. spicatum*, *M. heterophyllum* and *C. demersum* tended to maintain a high F_v/F_m over a wider range of water loss than fragments w/o apices (Figure 3a,c,e, Table 3). The relationship between F_v/F_m and water loss for fragments w/o apical tips of *L. major* resembled the pattern observed in *M. spicatum*, *M. heterophyllum* and *C. demersum*. It must be noted, however, that initial F_v/F_m values of *L. major* fragments w/o apices were fairly low (0.66 ± 0.08; ± *SD*) and scatter in the data was high (Figure 3b).

Survival and regeneration following desiccation

Subsequent to the desiccation trials, post-desiccation survival and regeneration (i.e. initiation of new growth) of fragments were determined depending on water loss and F_v/F_m. Although fragment survival and regeneration of the species and fragment types partly corresponded to water loss (Figure 4), it was a less suitable indicator than F_v/F_m and excluded from the statistical analysis.

For all species, 95% of fragments that survived showed F_v/F_m values above a F_{crit} of 0.36 and corresponding values of water loss below a W_{crit} of 91%. Fragment survival decreased significantly with decreasing F_v/F_m for *M. spicatum*, *L. major*, *H. verticillata* and *C. demersum* (Figure 4a,b,e,f, Table 4). Conversely, the mortality of fragments of *M. heterophyllum* and *E. canadensis* was not associated with F_v/F_m (Figure 4c,d, Table 4). Single plant fragments of the species were even able to survive at minimum F_v/F_m values of 0.30-0.43 (Figure 4, Table 5). The overall likelihood of fragment survival following the desiccation trials was distinctly lower for *E. canadensis* (54% of fragments) and *M. heterophyllum* (62%) compared to the other species investigated (84-90%; Figure 4). The only significant difference in fragment survival, however, was documented between *E. canadensis* and *L. major* (Table 4). In the case of *M. spicatum* and *M. heterophyllum*, fragments w/o apical tips showed a significantly lower survival than fragments w/ apical tips (Figure 4a,c, Table 4).

Based on post-desiccation regeneration of all species, 95% of fragments that successfully regenerated were characterized by F_v/F_m values above a F_{crit} of 0.40 and by water loss values below a W_{crit} of 84%. As with fragment survival, regeneration strongly corresponded to F_v/F_m. The likelihood of regeneration per unit time decreased significantly with decreasing F_v/F_m in case of all species, except for *M. heterophyllum* (Figure 4, Table 4). Still, single fragments of *M. spicatum* (w/ apex), *H. verticillata* and *L. major* (w/ apex) were even able to regenerate below the determined F_{crit} of 0.40, whereas fragments of *M. heterophyllum* and *E. canadensis* did not regenerate below a threshold of 0.52 in F_v/F_m and water losses exceeding 79 and 56%, respectively (Figure 4a-d,f, Table 5). Particularly the regeneration of fragments of *M. spicatum* and *H. verticillata* (at least w/ apical tips) pointed out that not only the overall likelihood but also the time required for regeneration was strongly related to F_v/F_m (Figure 4a,f). The overall likelihood of regeneration following desiccation was significantly different among the species, with *H. verticillata* (74% of fragments) and *M. spicatum* (72%) showing the highest regeneration capacities (Figure 4, Table 4). By contrast, *M. heterophyllum* possessed the lowest regeneration capacity of only 8% (Figure 4b). Plant fragments w/o apical tips of *M. heterophyllum*, *H. verticillata* and particularly of *L. major* had a significantly lower likelihood of regeneration per unit time than fragments w/ apical tips (Figure 4b,c,f, Table 4).

Discussion

The successful establishment of aquatic plant fragments following overland dispersal into hydrologically unconnected waters primarily depends on the combination of the experienced desiccation conditions, the desiccation period and the specific resistance of fragments to desiccation, particularly the potential to retain viability after introduction into a new water body (Johnstone *et al.*, 1985). Our results provide detailed information about the species-specific desiccation resistance of fragments without and with apical tips and demonstrate that Chl fluorescence (F_v/F_m) is an overall reliable indicator for the survival and regeneration potential of plant fragments.

The strong species-specific rather than fragment type-specific differences in the rate of water loss are most likely caused by morphological and anatomical differences. All of the investigated species are characterized either by dissected (*M. spicatum, M. heterophyllum,* *C. demersum*) or entire leaves (*L. major, E. canadensis, H. verticillata*). However, a rapid water loss is not assigned to a particular leaf morphology (see also Barnes *et al.*, 2013). Due to the generally very thin leaves of submerged aquatic plants (<300 µm; Sand-Jensen & Frost-Christensen, 1999), leaf thickness can be expected to play only a minor role in explaining the differences in desiccation rate. Species showing a rather slow initial water loss (*M. heterophyllum, C. demersum, L. major*) were sturdier in structure (more pronounced strengthening tissue; Sculthorpe, 1967) and had a higher shoot diameter compared to the more delicate *M. spicatum, E. canadensis* and *H. verticillata*. Moreover, particularly the invasive *H. verticillata* is characterized by a rapid decrease of water content compared to other submerged plant species (cf. Barnes *et al.*, 2013 and our data). The densely packed leaves at apical tips of species may retain water for a longer time (McAlarnen *et al.*, 2012), as documented for *M. heterophyllum* fragments, but it may also be reasonable that the fine structure of juvenile leaves promote evaporative loss.

Nevertheless, a slow water loss can generally prolong fragment viability when transported to hydrologically unconnected sites (Johnstone *et al.*, 1985). We document regeneration of e.g. single *M. spicatum* fragments after a maximum desiccation period of 5.7 h, while others reported no regeneration after only 3 h (Jerde *et al.*, 2012; Barnes *et al.*, 2013). This is most likely a consequence of differences in the experimental setup, as the rate of evaporative water loss and viability of plant fragments will differ due to various factors, like vapor pressure deficit (VPD; Coughlan *et al.*, 2018), wind speed (Bickel, 2015), precipitation (Bruckerhoff *et al.*, 2015) and clumping of plants (Jerde *et al.*, 2012; Bickel, 2015). Thus, the viability of fragments exposed to air might be notably prolonged under field conditions (up to 18 and 48 h for single and coiled fragments of *M. spicatum*, respectively; Bruckerhoff *et al.*, 2015). Potential vectors for fragment dispersal, such as water sport equipment, boats and waterbirds, additionally provide different microclimatic envelopes that influence desiccation and viability of fragments (Coughlan *et al.*, 2018).

A high evaporative water loss may involve loss of hydration water and limits the likelihood of fragment survival and regeneration. The hydration water content was postulated to be ~0.3 g H_2O per g DM (Hoekstra *et al.*, 2001), though there is a lack of information on the relative proportions of bulk cytoplasmic water and hydration water for aquatic plants. By estimating the hydration WC using 0.3 g H_2O per g DM, fragments of some species, like *E. canadensis*, hardly regenerate (and survive) after losing relatively low amounts of bulk cytoplasmic water (see also Johnstone *et al.*, 1985; Barnes *et al.*, 2013), while others are able to regenerate even after almost complete loss of bulk cytoplasmic water, e.g. 94% water loss of *M. spicatum* (2.7% estimated hydration WC; this study) or ~97% water loss of *Cabomba caroliniana* A. Gray (2.3% estimated hydration WC; Bickel, 2015). Previously, efforts have been made to elucidate the relationship between water loss (or mass loss) and viability, though it is likely that water and mass loss are not precisely indicating physiological stress (Johnstone *et al.*, 1985; Silveira *et al.*, 2009; Evans *et al.*, 2011; Jerde *et al.*, 2012; McAlarnen *et al.*, 2012; Barnes *et al.*, 2013; Bickel, 2015; Bruckerhoff *et al.*, 2015; Coughlan *et al.*, 2018). We show that the F_v/F_m ratio accurately predicts fragment viability and is nonlinear to relative water loss. Still, there are species-specific differences in the relationship between F_v/F_m and water loss, which may be linked to differences in the ability of the species to cope with oxidative stress caused by water deficit. A high content of non-enzymatic antioxidants (e.g. ascorbate and glutathione) and antioxidant enzymes (e.g. superoxide dismutase, catalase, ascorbate peroxidase and glutathione reductase) prevent the photosynthetic apparatus from damage through ROS (Kranner & Birtić, 2005; Nayyar & Gupta, 2006). Thus, the species that maintained a relatively high F_v/F_m even when losing a large fraction of water content (*M. spicatum, M. heterophyllum, C. demersum*) could presumably show higher contents of antioxidants than the hydrocharitacean species (*L. major, E. canadensis, H. verticillata*). Despite the fact that regeneration following desiccation generally corresponded well to F_v/F_m, single fragments of *M. spicatum, H. verticillata* and *L. major* were even able to regenerate at very low F_v/F_m values (<0.40). As we determined the overall F_v/F_m of shoot fragments and because stems desiccated much slower than leaves, it is reasonable that meristematic tissue at the nodes was sporadically viable even though the overall F_v/F_m was low. Nevertheless, our results demonstrate that the viability of leaves strongly determines the likelihood of regeneration at the nodes, emphasizing the low regeneration potential of stem fragments that are lacking leaves.

Excessive transpiration during overland transport clearly reduces F_v/F_m and the regeneration potential of plant fragments, but the general regeneration abilities strongly depend on the species and fragment type in the first place (Barrat-Segretain *et al.*, 1998; Riis *et al.*, 2009; Kuntz *et al.*, 2014). The low regeneration capacities documented for fragments of the invasive species *M. heterophyllum* and *L. major* (w/o apices) are most likely assigned to an overall low likelihood of regeneration rather than a consequence of water deficit (cf. Heidbüchel & Hussner, 2019). Hence, F_v/F_m measurements can accurately predict the potential for fragment regeneration only if a fragment is generally able to regenerate and shows at least moderately high regeneration abilities under unstressed conditions. Many aquatic plant species were found to own high regeneration rates even for very small fragments (e.g. fragments consisting of one node in *M. spicatum* or single leaves in *Myriophyllum aquaticum* (Vell.) Verdc.; Langeland & Sutton, 1980; Hussner, 2009; Kuntz *et al.*, 2014). Here, we used shoot fragments with a length of 6 cm, but the size of fragments found attached to boats and trailers and in the drift of running waters can be distinctly higher (Rothlisberger *et al.*, 2010; Heidbüchel *et al.*, 2016). As the regeneration abilities and resistance to desiccation increase with increasing fragment length and number of nodes (McAlarnen *et al.*, 2012; Redekop *et al.*, 2016; Bickel, 2017), fragments found in the field are expected to have an increased likelihood of regeneration and establishment in new water bodies. Besides fragment length, the likelihood of regeneration and the resistance of plant fragments to desiccation further depend on the type of fragment. Our results generally reaffirm that fragments possessing apical tips show higher regeneration abilities than fragments without apices, particularly in the case of the invasive *L. major* (cf. Riis *et al.*, 2009; Umetsu *et al.*, 2012a). Yet, there are rather minor differences in the response to desiccation between fragments without and with apices.

Specialized propagules, such as turions of *Potamogeton crispus* L. (Bruckerhoff *et al.*, 2015), fragments of emerged plant parts, e.g. of the invasive and highly regenerative *M. aquaticum* (Barnes *et al.*, 2013), or even whole floating plants (Coughlan *et al.*, 2018) are able to withstand desiccation and remain viable for a much longer period than submerged plant fragments. It must be noted, however, that the resistance of a species to desiccation may differ distinctly between populations due to the high phenotypic plasticity of aquatic plants (Riis *et al.*, 2010; Eusebio Malheiro *et al.*, 2013), explaining the contrasting findings in desiccation resistance for *C. demersum* (cf. Barnes *et al.*, 2013 and our data).

Once introduced into an isolated water body, the likelihood of regeneration and the establishment success of plant fragments are strongly influenced by the environmental conditions at the receiving site. Factors such as temperature, light, nutrient and carbon availability are known to influence the photosynthetic acclimation, growth and regeneration of aquatic plants (Hussner, 2009; Lambert & Davy, 2011; Riis *et al.*, 2012; Kuntz *et al.*, 2014; Hussner *et al.*, 2015). Furthermore, the successful establishment of fragments is controlled by the specific potential for initial colonization (i.e. root anchorage within the sediment), which is in turn dependent on e.g. water depth (Heidbüchel & Hussner, 2019) and locally present vegetation (Chadwell & Engelhardt, 2008).

To conclude, high regeneration abilities of submerged aquatic plants and the ability to maintain the potential for regeneration when exposed to drying conditions are key factors that determine the establishment success of plant fragments transported into isolated water bodies. Desiccation may be tolerated for a certain time by reducing evaporative water loss (due to morphological and anatomical features; e.g. *L. major*), by maintaining functioning of the photosynthetic apparatus even at high water loss (due to physiological features; e.g. *M. spicatum*) or a combination of both (e.g. *M. heterophyllum* and *C. demersum*). Still, the experienced environmental conditions during overland transport strongly control the desiccation resistance and viability of aquatic plant fragments (Bruckerhoff *et al.*, 2015). The viability of a fragment can be precisely predicted by F_v/F_m measurements, provided that the fragment is generally able to regenerate. Where information on minimum fragment size required for regeneration and on the regeneration capacity of a species is available, measurements of F_v/F_m may be further used as a tool to evaluate the viability following overland transport or to optimize management of IAAPs by evaluating applications of e.g. herbicides (Hussner *et al.*, 2017) and disinfectants (Cuthbert *et al.*, 2018). Thus, Chl fluorescence measurements constitute a promising tool in biosecurity to help preventing IAAPs from further spread in their introduced range. It must, however, be noted that the application in the field can be complicated and Chl fluorescence measurements in the laboratory may be inevitable.

Acknowledgements

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Table 1 Initial number of nodes and initial relative water content (RWC) of 6 cm long plant fragments of different fragment types (FT; without (w/o) and with (w/) apex) for *Myriophyllum spicatum*, *Myriophyllum heterophyllum*, *Ceratophyllum demersum*, *Lagarosiphon major*, *Elodea canadensis* and *Hydrilla verticillata*. Shown are mean values ± *SD*

Species	FT	No. of nodes ($6\ cm^{-1}$)	RWC[1] (%)
M. spicatum	w/o apex	5 ± 1	92.1 ± 1.0
	w/ apex	10 ± 2	91.1 ± 1.1
M. heterophyllum	w/o apex	10 ± 4	93.8 ± 1.0
	w/ apex	20 ± 5	94.2 ± 0.6
C. demersum	w/o apex	14 ± 2	91.8 ± 1.6
	w/ apex	17 ± 1	87.3 ± 1.4
L. major	w/o apex	26 ± 6	92.5 ± 1.8
	w/ apex	38 ± 6	87.1 ± 3.2
E. canadensis	w/o apex	26 ± 6	90.5 ± 1.6
	w/ apex	41 ± 3	90.0 ± 2.7
H. verticillata	w/o apex	7 ± 2	85.8 ± 2.1
	w/ apex	17 ± 3	86.8 ± 2.2

[1] Determined for additional plant fragments not used in the experiment (n = 5)

Table 2 Summary of nonlinear regression models on water loss of plant fragments over time and W_{50} values (i.e. desiccation time at 50% water loss) for different fragment types (FT; without (w/o) and with (w/) apex) of *Myriophyllum spicatum*, *Myriophyllum heterophyllum*, *Ceratophyllum demersum*, *Lagarosiphon major*, *Elodea canadensis* and *Hydrilla verticillata*

			Coefficients (95% CI)									
Species	FT	Equation	A_1		k_1		A_2		k_2		Significant difference	W_{50} (h desic. time)
M. spicatum	w/o apex	Eq. 4	62.78 (59.01 – 66.56)	**p <0.0001**	4.69 (4.11 – 5.28)	**p <0.0001**	37.61 (34.14 – 41.08)	**p <0.0001**	0.21 (0.17 – 0.25)	p = 0.440	**Yes**	0.3
	w/ apex	Eq. 4	79.10 (73.62 – 84.59)		3.03 (2.64 – 3.42)		20.10 (14.68 – 25.52)		0.17 (0.06 – 0.27)			0.3
M. heterophyllum	w/o apex	Eq. 3	96.94 (95.29 – 98.58)	p = 0.307	0.55 (0.53 – 0.57)	**p <0.0001**					**Yes**	1.2
	w/ apex	Eq. 3	98.38 (96.10 – 100.66)		0.37 (0.36 – 0.39)							1.8
C. demersum	w/o apex	Eq. 3	97.80 (95.72 – 99.88)	p = 0.584	0.62 (0.60 – 0.65)	**p <0.0001**					**Yes**	1.1
	w/ apex	Eq. 3	98.66 (96.42 – 100.91)		0.54 (0.51 – 0.56)							1.3
L. major	w/o apex	Eq. 4	30.25 (6.44 – 54.06)	**p = 0.002**	1.12 (0.47 – 1.77)	p = 0.689	68.28 (44.05 – 92.52)	**p = 0.004**	0.29 (0.21 – 0.37)	**p = 0.007**	**Yes**	1.5
	w/ apex	Eq. 4	65.91 (57.45 – 74.36)		1.24 (1.06 – 1.43)		33.84 (25.03 – 42.65)		0.15 (0.09 – 0.21)			0.9
E. canadensis	w/o apex	Eq. 4	55.26 (51.51 – 59.01)	**p = 0.002**	5.09 (4.42 – 5.76)	**p = 0.027**	44.54 (40.94 – 48.14)	**p = 0.002**	0.23 (0.19 – 0.28)	p = 0.108	**Yes**	0.4
	w/ apex	Eq. 4	63.37 (60.02 – 66.73)		4.24 (3.84 – 4.64)		36.60 (33.25 – 39.95)		0.17 (0.11 – 0.23)			0.3
H. verticillata	w/o apex	Eq. 3	97.90 (95.89 – 99.91)	p = 0.853	2.27 (2.16 – 2.38)	**p = 0.011**					**Yes**	0.3
	w/ apex	Eq. 3	98.13 (96.72 – 99.53)		2.44 (2.37 – 2.52)							0.3

Shown are estimated values and 95% confidence intervals (CI) for each coefficient of the respective equation. Significant differences between coefficients for the two fragment types of a species are indicated by p values written in bold (p <0.05; t-test). Regression models for the fragment types of a species were deemed as significantly different if at least one coefficient differed significantly. All coefficients differed significantly from zero (p <0.05)

Table 3 Summary of nonlinear regression models on F_v/F_m depending on water loss of plant fragments and $F_{0.55}$ values (i.e. relative water loss at F_v/F_m of 0.55) for different fragment types (FT; without (w/o) and with (w/) apex) of *Myriophyllum spicatum*, *Myriophyllum heterophyllum*, *Ceratophyllum demersum*, *Lagarosiphon major*, *Elodea canadensis* and *Hydrilla verticillata*

Species	FT	Coefficients (95% CI)						Significant difference	F0.55 (% water loss)
		a		*k*		*b*			
M. spicatum	w/o apex	0.017 (0.002 – 0.033)	*p* = 0.081	-0.031 (-0.041 – -0.022)	***p* = 0.006**	0.656 (0.636 – 0.676)	*p* = 0.148	**Yes**	63
	w/ apex	0.004 (7 x 10^{-4} – 0.007)		-0.049 (-0.058 – -0.040)		0.674 (0.659 – 0.689)			72
M. heterophyllum	w/o apex	0.001 (4 x 10^{-4} – 0.002)	***p* = 0.013**	-0.060 (-0.067 – -0.052)	***p* < 0.0001**	0.694 (0.685 – 0.702)	***p* = 0.037**	**Yes**	79
	w/ apex	1 x 10^{-4} (2 x 10^{-4} – 2 x 10^{-4})		-0.085 (-0.095 – -0.075)		0.673 (0.636 – 0.690)			81
C. demersum	w/o apex	0.010 (0.004 – 0.016)	***p* = 0.010**	-0.041 (-0.048 – -0.034)	***p* = 0.002**	0.732 (0.720 – 0.745)	***p* = 0.012**	**Yes**	73
	w/ apex	0.002 (0.001 – 0.003)		-0.054 (-0.059 – -0.049)		0.752 (0.743 – 0.760)			83
L. major	w/o apex	0.003 (-8 x 10^{-4} – 0.007)	***p* = 0.001**	-0.051 (-0.065 – -0.036)	***p* < 0.0001**	0.611 (0.596 – 0.626)	***p* < 0.0001**	**Yes**	60
	w/ apex	0.109 (0.054 – 0.164)		-0.017 (-0.021 – -0.012)		0.720 (0.705 – 0.734)			56
E. canadensis	w/o apex	0.054 (0.024 – 0.085)	*p* = 0.217	-0.025 (-0.031 – -0.019)	*p* = 0.064	0.714 (0.695 – 0.733)	*p* = 0.262	No	56
	w/ apex	0.035 (0.022 – 0.048)		-0.032 (-0.036 – -0.027)		0.728 (0.713 – 0.742)			57
H. verticillata	w/o apex	0.055 (0.022 – 0.087)	*p* = 0.150	-0.024 (-0.030 – -0.018)	*p* = 0.266	0.683 (0.665 – 0.701)	*P* = 0.079	No	52
	w/ apex	0.030 (0.015 – 0.045)		-0.028 (-0.033 – -0.023)		0.704 (0.689 – 0.718)			64

Shown are estimated values and 95% confidence intervals (CI) for each coefficient of Eq. 5. Significant differences between coefficients for the two fragment types of a species are indicated by *p* values written in bold ($p < 0.05$; t-test). Regression models for the fragment types of a species were deemed as significantly different if at least one coefficient differed significantly. All coefficients differed significantly from zero ($p < 0.05$)

Table 4 ANOVA results for the effect of species and the combined effect of fragment type (FT; without (w/o) and with (w/) apex) and F_v/F_m on survival and regeneration of *Myriophyllum spicatum*, *Myriophyllum heterophyllum*, *Ceratophyllum demersum*, *Lagarosiphon major*, *Elodea canadensis* and *Hydrilla verticillata*

	FT		F_v/F_m		FT x F_v/F_m	
Species	χ^2 (df, n)	*p* value	χ^2 (df, n)	*p* value	χ^2 (df, n)	*p* value
Survival[1]						
M. spicatum[ab]	12.43 (1, 139)	**<0.001**	27.93 (1, 139)	**<0.0001**	1.47 (1, 139)	0.225
M. heterophyllum[ab]	15.01 (1, 169)	**<0.001**	0.06 (1, 169)	0.800	0.10 (1, 169)	0.757
C. demersum[ab]	3.65 (1, 162)	0.056	5.62 (1, 162)	**0.018**	0.66 (1, 162)	0.418
L. major[a]	1.54 (1, 144)	0.215	20.62 (1, 144)	**<0.0001**	0.01 (1, 144)	0.931
E. canadensis[b]	0.52 (1, 157)	0.473	2.34 (1, 157)	0.126	0.12 (1, 157)	0.734
H. verticillata[ab]	0.27 (1, 141)	0.605	23.20 (1, 141)	**<0.0001**	<0.01 (1, 141)	0.990
Regeneration[2]						
M. spicatum[a]	2.83 (1, 139)	0.092	34.30 (1, 139)	**<0.0001**	1.37 (1, 139)	0.242
M. heterophyllum[d]	5.14 (1, 169)	**0.023**	2.05 (1, 169)	0.152	<0.01 (1, 169)	1
C. demersum[bc]	1.34 (1, 162)	0.246	8.66 (1, 162)	**0.003**	0.79 (1, 162)	0.375
L. major[ab]	19.75 (1, 144)	**<0.0001**	11.74 (1, 144)	**<0.001**	0.65 (1, 144)	0.422
E. canadensis[cd]	2.87 (1, 157)	0.090	9.86 (1, 157)	**0.002**	2.45 (1, 157)	0.117
H. verticillata[a]	6.19 (1, 141)	**0.013**	5.81 (1, 141)	**0.005**	5.81 (1, 141)	**0.007**

Shown are χ^2-values of the likelihood ratio test statistic and corresponding *p* values. Significant *p* values are written in bold ($p < 0.05$). Significant differences between species are indicated by different letters ($p < 0.05$; Tukey contrasts)

[1] Overall effect of species on survival: χ^2 (5, 912) = 19.95, ***p* = 0.001**

[2] Overall effect of species on regeneration: χ^2 (5, 912) = 81.94, ***p* <0.0001**

Table 5 Minimum F_v/F_m and maximum water loss values documented in our study for fragment survival and regeneration, respectively, according to different fragment types (FT; without (w/o) and with (w/) apex) of *Myriophyllum spicatum*, *Myriophyllum heterophyllum*, *Ceratophyllum demersum*, *Lagarosiphon major*, *Elodea canadensis* and *Hydrilla verticillata*. NA indicates no observation of fragment survival or regeneration

		Survival		Regeneration	
Species	FT	Min. F_v/F_m	Max. water loss (% of initial WC)	Min. F_v/F_m	Max. water loss (% of initial WC)
M. spicatum	w/o apex	0.41	78	0.41	78
	w/ apex	0.32	94	0.32	94
M. heterophyllum	w/o apex	0.31	91	NA	NA
	w/ apex	0.30	91	0.52	79
C. demersum	w/o apex	0.36	89	0.43	84
	w/ apex	0.43	96	0.43	91
L. major	w/o apex	0.39	43	0.46	31
	w/ apex	0.38	82	0.38	82
E. canadensis	w/o apex	0.33	79	0.52	56
	w/ apex	0.34	70	0.65	47
H. verticillata	w/o apex	0.32	80	0.32	80
	w/ apex	0.36	80	0.39	80

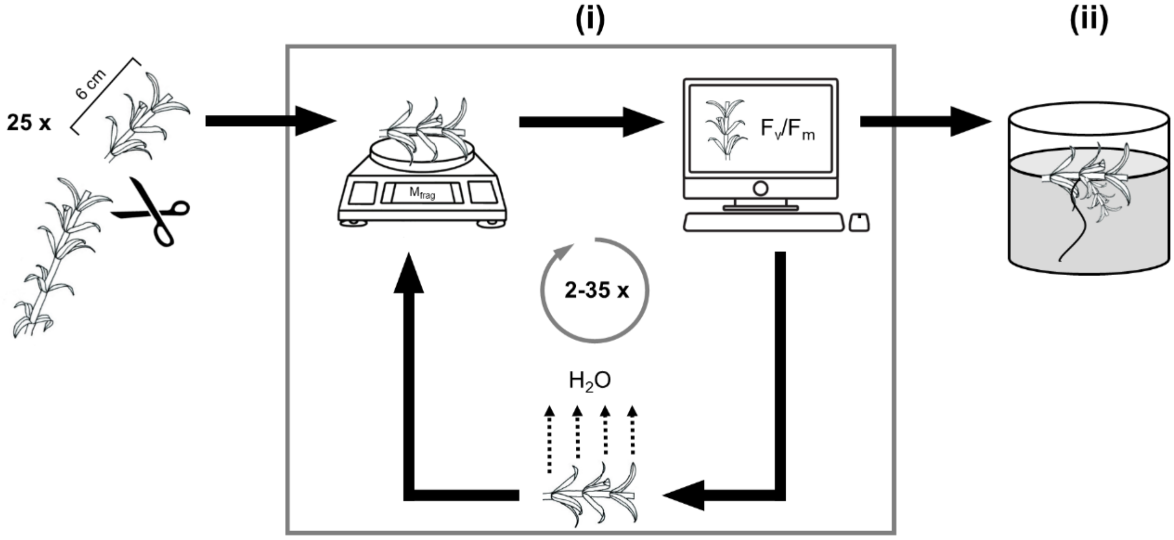

Figure 1 Schematic overview of the experimental procedure illustrating (i) desiccation trials combining measurements of water loss and Chl fluorescence (F_v/F_m) and (ii) post-desiccation trials for determination of fragment survival and regeneration

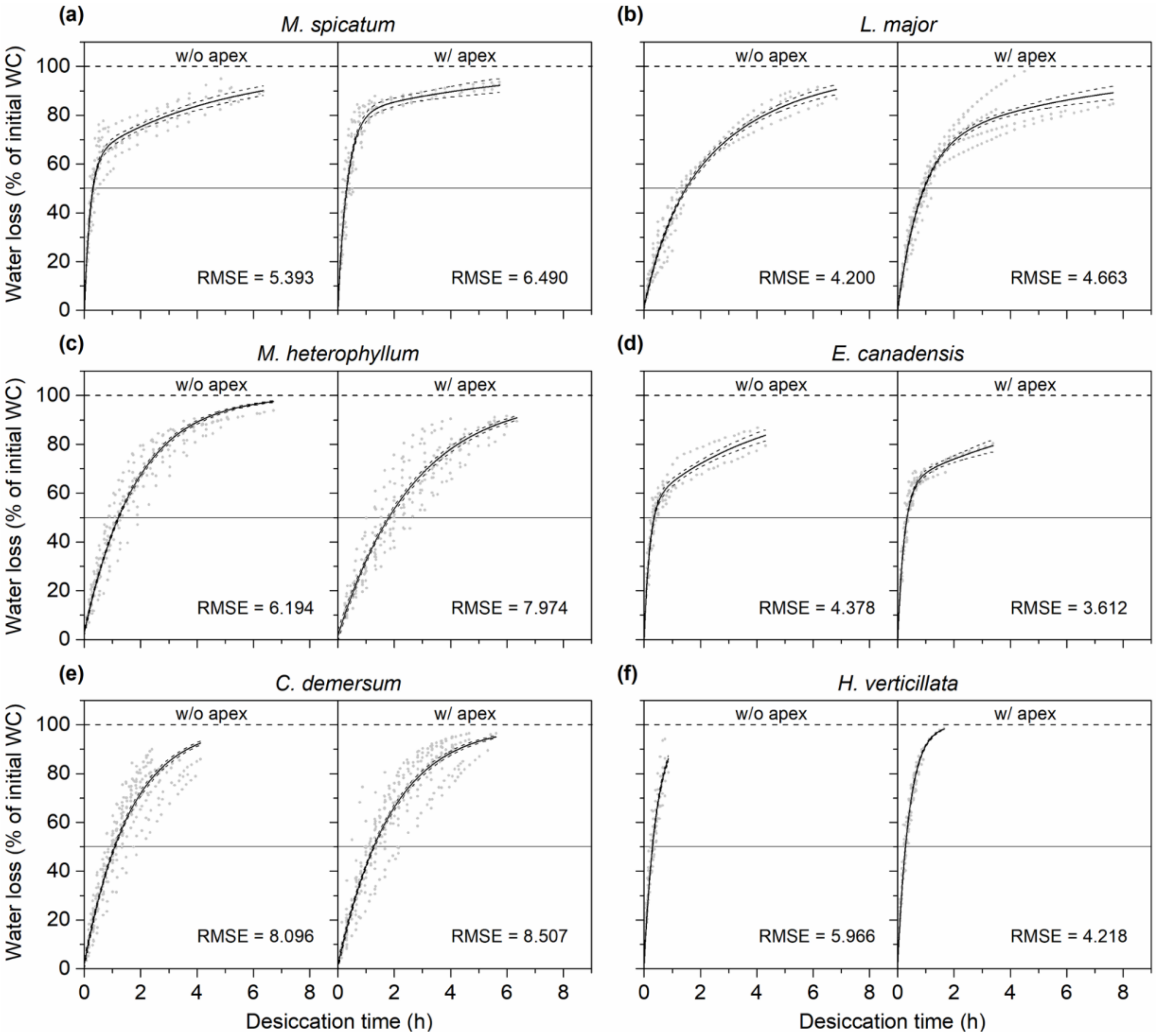

Figure 2 Relative water loss of plant fragments without (w/o) and with (w/) apical tips over time during desiccation trials for (a) *Myriophyllum spicatum*, (b) *Lagarosiphon major*, (c) *Myriophyllum heterophyllum*, (d) *Elodea canadensis*, (e) *Ceratophyllum demersum* and (f) *Hydrilla verticillata*. Shown are raw data points of repeated measures, fitted regression curves (solid lines) and corresponding 95% confidence intervals (dashed lines). Intersections of solid grey lines with regression curves indicate desiccation time at 50% water loss (W_{50}). Goodness of model fits is indicated by root mean square errors (RMSE). Slopes of all fitted curves were significantly different from zero ($p < 0.0001$)

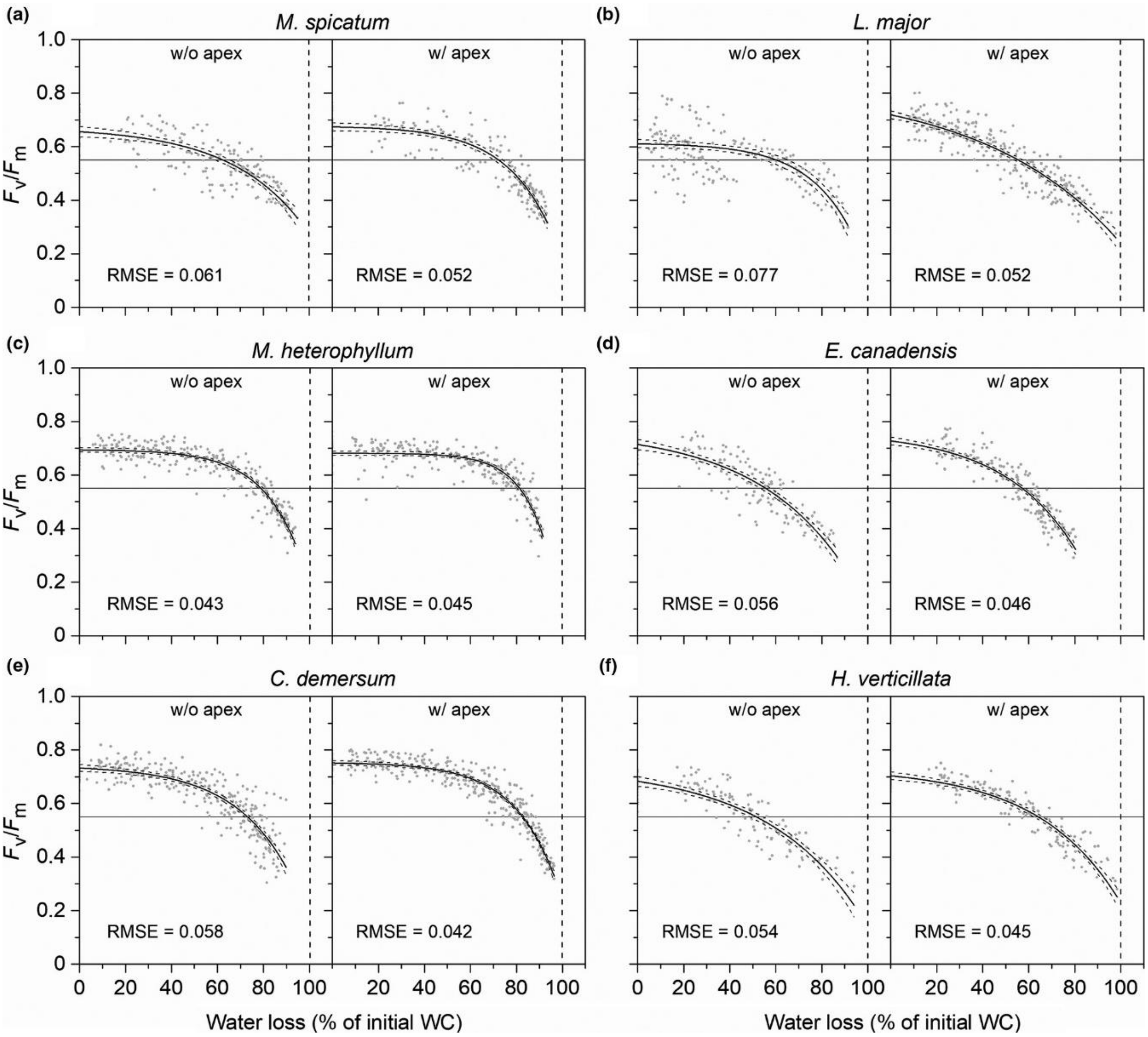

Figure 3 Relationship between F_v/F_m ratio and relative water loss of plant fragments without (w/o) and with (w/) apical tips during desiccation trials for (a) *Myriophyllum spicatum*, (b) *Lagarosiphon major*, (c) *Myriophyllum heterophyllum*, (d) *Elodea canadensis*, (e) *Ceratophyllum demersum* and (f) *Hydrilla verticillata*. Shown are raw data points of repeated measures, fitted regression curves (solid lines) and corresponding 95% confidence intervals (dashed lines). Intersections of solid grey lines with regression curves indicate relative water loss at F_v/F_m of 0.55 ($F_{0.55}$). Goodness of model fits is indicated by root mean square errors (RMSE). Slopes of all fitted curves were significantly different from zero (p <0.0001)

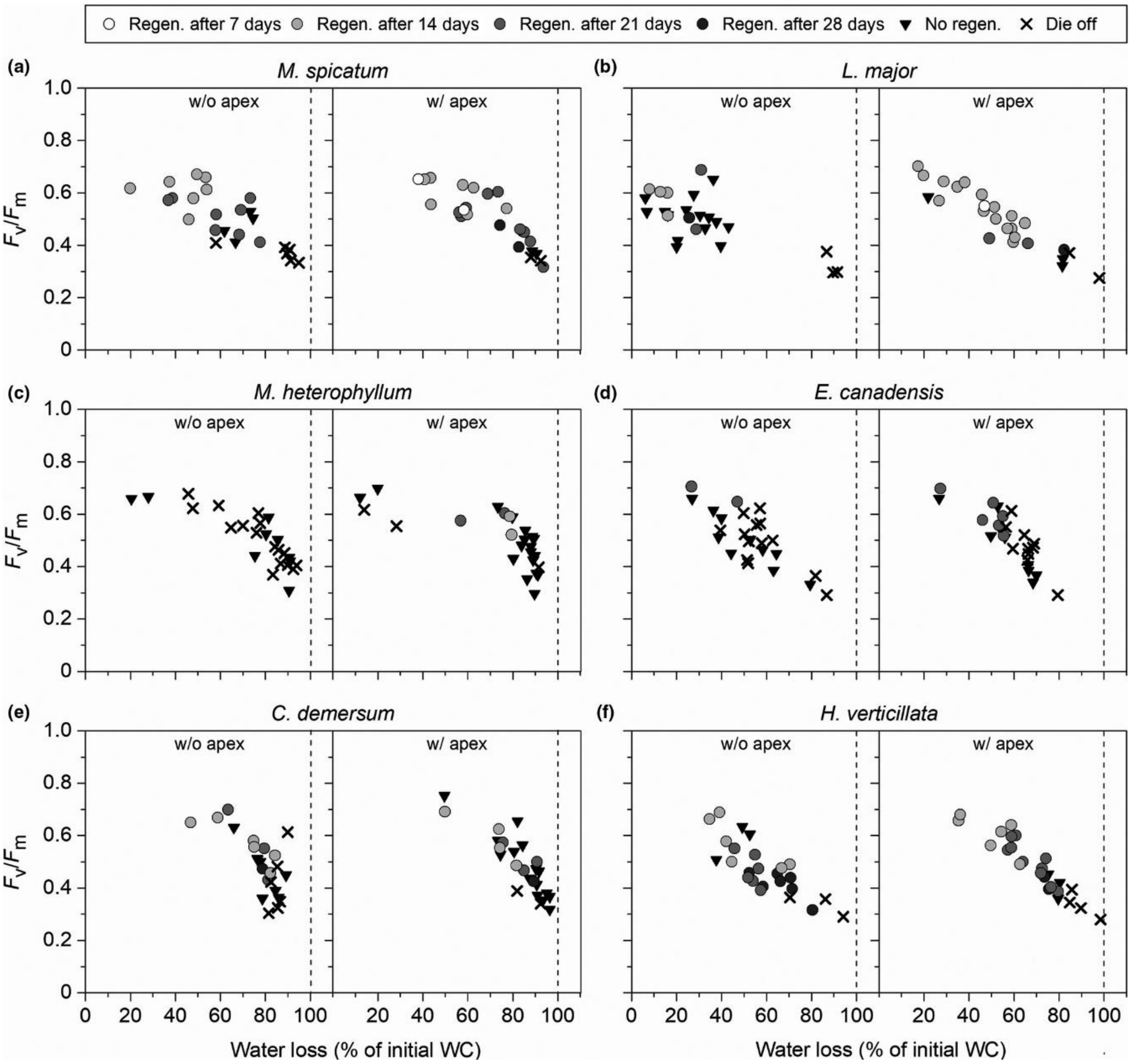

Figure 4 Survival and regeneration of plant fragments without (w/o) and with (w/) apical tips following desiccation depending on specific final F_v/F_m values and relative water loss for (a) *Myriophyllum spicatum*, (b) *Lagarosiphon major*, (c) *Myriophyllum heterophyllum*, (d) *Elodea canadensis*, (e) *Ceratophyllum demersum* and (f) *Hydrilla verticillata*. The experiment lasted for 28 days. Fragments that have not survived are indicated by **X**, while fragments considered as survived but not regenerated are indicated by **▼**. Data points depicted per species and fragment type (n = 25) represent single data points also shown in Figure 3

Supporting information

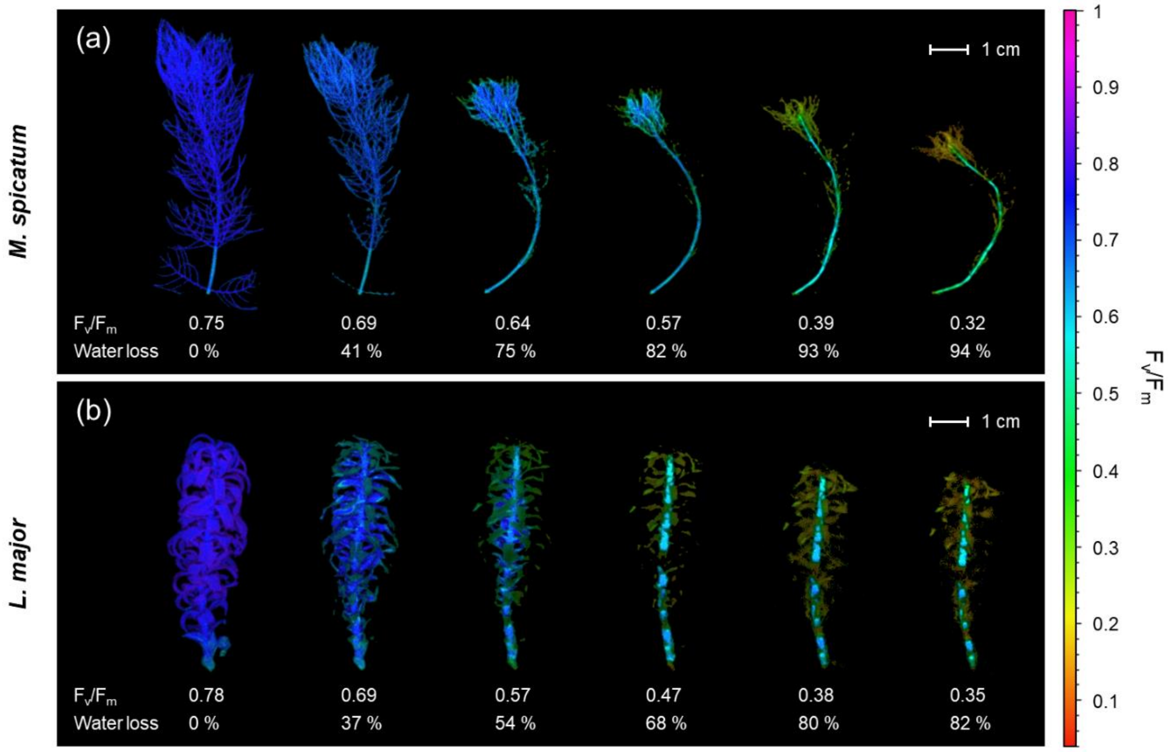

Figure S1 Illustration of F_v/F_m measurements on single plant fragments with (w/) apical tips of (a) *Myriophyllum spicatum* and (b) *Lagarosiphon major* during desiccation trials recorded by Imaging-PAM. Image series correspond to desiccation periods of 5.7 and 6.0 h for fragments of *M. spicatum* and *L. major*, respectively

CHAPTER 6

Fragment type and water depth determine the regeneration and colonization success of submerged aquatic macrophytes

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Abstract

Submerged aquatic plants predominantly disperse via vegetative means, with shoot fragments being the most important propagules. The establishment of new macrophyte stands largely depends on two parameters, (i) the regeneration of plant fragments and (ii) the successful anchorage of these fragments in the sediment (colonization). Here we studied both the regeneration and colonization abilities of six submerged aquatic plant species (*Myriophyllum spicatum*, *Myriophyllum heterophyllum*, *Ceratophyllum demersum*, *Lagarosiphon major*, *Elodea canadensis* and *Hydrilla verticillata*) with two fragment types (with and without apical tips, 6 cm fragment length) at two water depths (12 and 3 cm). Overall, *M. spicatum*, *L. major*, *E. canadensis* and *H. verticillata* showed stronger regeneration and colonization abilities than *C. demersum* and *M. heterophyllum*. In *M. spicatum*, *L. major* and *H. verticillata*, fragments with apical tips had a significantly higher likelihood of regeneration and colonization than fragments without apices. The presence of apical tips increased regeneration by a factor of up to 5 (*L. major*), and colonization by a factor of up to 6.5 (*H. verticillata*). Water depth had an even stronger effect on colonization of *M. spicatum*, *L. major*, *E. canadensis* and *H. verticillata*, but did not affect regeneration of all species. A low water depth significantly increased the likelihood of colonization by a factor of 3.5 (*L. major*) to 31 (*M. spicatum*). Our findings demonstrate the differences in the likelihood for initial colonization of fragments among aquatic plant species, thereby allowing conclusions to be drawn on species-specific spread potentials through plant fragments.

Keywords dispersal, establishment, fragmentation, invasive alien aquatic plants (IAAPs), spread, vegetative reproduction

Introduction

Aquatic plants reproduce either from seeds or from vegetative means (Sculthorpe, 1967). The production of seeds differs largely between aquatic plants, and while some species produce large amounts of seeds (in both native and introduced range, e.g. *Ludwigia grandiflora* (Michx.) Greuter & Burdet; Thouvenot *et al.*, 2013), some others hardly develop viable seeds even in their native range (e.g. *Myriophyllum aquaticum* (Vell.) Verdcourt; Orchard, 1981). Conversely, the production of viable vegetative propagules is reported as a common trait of aquatic plants (Barrat-Segretain, 1996; Boedeltje *et al.*, 2003). These include tubers, rhizomes, stolons, turions, shoot fragments or even entire plants, whereby shoot fragments are considered the most important propagules (Boedeltje *et al.*, 2003; Riis & Sand-Jensen, 2006; Heidbüchel *et al.*, 2016).

Aquatic plant fragments are produced either by autofragmentation (the self-induced production of fragments) or allofragmentation (fragmentation caused by disturbances; Riis *et al.*, 2009). The number of fragments produced is thereby determined by species-specific fragmentation rates (Redekop *et al.*, 2016) and the level of disturbance (e.g. flow in streams or mechanical damage during weed control; Anderson, 1998; Riis, 2008; Sand-Jensen, 2008). In running waters, the number of plant fragments produced further differs between species due to the specific biomass located upstream and may range from single fragments (*Hydrocotyle ranunculoides* L. F.) to >6000 fragments per hour (*Stuckenia pectinata* (L.) Boerner), as documented for a medium sized river (River Erft, Germany; Heidbüchel *et al.*, 2016). Additionally, great variety was reported for propagule size, ranging from small fragments consisting of single nodes to >400 interconnected shoots of *Vallisneria spiralis* L. (Heidbüchel *et al.*, 2016).

Apart from the large differences in fragment number and propagule size found in the field, contrasting findings are documented in the potential for regeneration (the production of new roots, shoot apices and/or shoot elongation) and colonization (anchorage within the sediment) of vegetative propagules. The regeneration and colonization abilities may not only differ between species but also between different types of vegetative propagules. While, for example, rhizomes and stolons are hardly able to regenerate (Barrat-Segretain *et al.*, 1998), most shoot fragments may elongate and develop new roots and/or shoots (e.g. Umetsu *et al.*, 2012a; Vári, 2013; Bickel, 2017). The minimum size of a fragment needed for regeneration, however, differs between aquatic plant species (Langeland & Sutton, 1980; Barrat-Segretain *et al.*, 1998; Hussner, 2009). New roots and shoots are formed at the nodes of stems in almost all cases (Kuntz *et al.*, 2014; but see Hussner (2009) for the regeneration from detached leaves in the invasive *M. aquaticum*), illustrating that the likelihood of regeneration increases with increasing number of nodes per fragment (Bickel, 2017). Concomitantly, the regeneration capacity increases whilst the time until regeneration decreases with increasing fragment length (Redekop *et al.*, 2016). Furthermore, fragments with apical tips usually show better and faster regeneration than fragments without apices (Riis *et al.*, 2009; Umetsu *et al.*, 2012a). The regeneration type (formation of adventitious roots or shoots or concurrent growth of both) during early regeneration of fragments differs among the species (Kuntz *et al.*, 2014). A preference for early formation of new shoots emphasizes a higher potential for the production of new propagules that in turn may be further dispersed (i.e. increases propagule pressure), while fast root formation indicates an increased likelihood of rapid colonization.

Within water bodies, aquatic plant fragments are mainly dispersed by water movement, though the spread distance is limited by habitat factors like the size of the water body and the flow conditions (Riis & Sand-Jensen, 2006; Heidbüchel *et al.*, 2016). Additionally, the fragments must settle at suitable sites for successful colonization and subsequent establishment (Barrat-Segretain *et al.*, 1998; Riis, 2008). The colonization and establishment of fragments depend on various factors, including seasonality, nutrient availability and sediment type (Barrat-Segretain & Bornette, 2000; Wang *et al.*, 2009; Kuntz *et al.*, 2014; Li *et al.*, 2015b, 2016a). As for the natural spread and establishment of aquatic plants through fragments, initial colonization, i.e. first anchorage in the sediment, was found to be the main bottleneck in streams (Riis, 2008). Besides propagule pressure and fragment dispersal range, colonization success additionally determines the invasiveness of aquatic plant species (Lockwood *et al.*, 2005, 2009) and may further explain lag times between the introduction of an alien species and the onset of its invasive behavior (Crooks, 2005).

Due to the buoyancy of viable plant material, fragments capable of regeneration are generally floating at or close to the water surface, though the degree of buoyancy can be species- and fragment-specific (Santamaría, 2002). It therefore seems evident that the likelihood of rooting during initial colonization increases with decreasing water depth. Surprisingly, however, earlier studies on the colonization of floating fragments of submerged plants either did not consider different water depths (Barrat-Segretain *et al.*, 1998, 1999, 2002; Barrat-Segretain & Bornette, 2000; Riis *et al.*, 2009; Vári, 2013) or did not single out the effect of water depth as competition effects between species were included (Thiébaut & Martinez, 2015). Some others investigated the effect of different water depths on the establishment and performance of shoot fragments planted into the sediment, so that fragments were already anchored before root formation occurred (Li *et al.*, 2015a, 2016b).

We hypothesize that the colonization rates of plant fragments will decrease with increasing water depth in a given range, as long as the distance between fragment and sediment surface does not prevent the anchorage of fragments within the sediment. By contrast, regeneration rates of fragments are not expected to be affected by the distance between fragment and sediment surface. We further expect higher regeneration and colonization rates for shoot fragments with apical tips than for fragments without apical tips, as the presence of apices allows for regeneration through shoot elongation. To test our hypotheses, we studied the regeneration and colonization abilities of six submerged aquatic weed species by combining fragments with (w/) and without (w/o) apical tips and two water depths, i.e. distances between the floating fragments and the sediment surface.

Material and methods

Plant material and cultivation

All six submerged plant species (*Myriophyllum spicatum* L., *Myriophyllum heterophyllum* Michx., *Ceratophyllum demersum* L., *Lagarosiphon major* (Ridley) Moss, *Elodea canadensis* Michx. and *Hydrilla verticillata* (L.f.) Royle) belong to the most troublesome aquatic weeds worldwide (Hussner *et al.*, 2017). In Europe, *M. heterophyllum* and *L. major* have currently been listed as invasive species (EU regulation No. 1143/2014) and their spread within the European countries must be controlled.

Plants of *M. heterophyllum*, *C. demersum*, *L. major* and *E. canadensis* were sampled from a pond system near the University of Düsseldorf (Germany), *M. spicatum* was sampled from the River Erft (Germany) and *H. verticillata* originated from laboratory stocks (NIWA, New Zealand).

Prior to the use in the experiment, plants were cultivated separately in 65 L plastic tanks (57 x 39 x 42 cm) for at least two weeks. The plastic tanks were filled with a 5 cm sediment layer of washed sand from the River Rhine (Germany), and a general purpose medium for aquatic plant cultivation was added (Smart & Barko, 1985). Water loss due to evaporation was compensated by adding deionized water whenever required. The plants grew anchored in the sediment and were exposed to a 16/8 h light/dark cycle at a light intensity of 124.2 ± 33.7 µmol photons m^{-2} s^{-1} (± *SD*; measured 1 cm above the water surface).

Experimental setup

A two factorial experiment was conducted to combine the effects of two different fragment types (w/ and w/o apex) and water depths (high and low) on regeneration and colonization of six plant species over time. Unbranched shoot fragments were taken from plant cultures with an initial length of 6 cm and corresponding number of nodes of 10 ± 2 and 5 ± 1 (*M. spicatum*), 20 ± 5 and 10 ± 4 (*M. heterophyllum*), 17 ± 1 and 14 ± 2 (*C. demersum*), 38 ± 6 and 26 ± 6 (*L. major*), 41 ± 3 and 26 ± 6 (*E. canadensis*) and 17 ± 3 and 7 ± 2 (*H. verticillata*; ± *SD*) for fragments w/ and w/o apical tips, respectively. Shoot fragments w/ apical tips were taken from the top while fragments w/o apices were cut from the middle part of either main stems or secondary branches. If buds were present, they were removed prior to the experiment to avoid premature regeneration. Plant fragments were placed individually into either high or low plastic jars, which were 7 cm in diameter with a height of 19 cm (high treatment) and 7 cm (low treatment). The plastic jars were either filled with 4 cm (high treatment) or 1 cm (low treatment) thoroughly washed sand obtained from the River Rhine. Subsequently, modified Smart & Barko (1985) medium (containing 2 mg NO_3^--N L^{-1} and 0.1 mg PO_4^{3-}-P L^{-1}) was added, resulting in a final water depth of 12 cm (high treatment) and 3 cm (low treatment) above the sand (Figure 1). Thus, both treatments had the same water layer to sand layer ratio of 3:1 to compensate for effects through nutrient release from the substrate (even though the sand was already washed). As viable aquatic plant material is generally buoyant, the different water depths concomitantly resulted in different distances between fragments and the sediment surface. Both water depths represent different shallow water conditions that allow for anchorage within the sediment in a realistic way and are commonly experienced by fragments in the field (e.g. if fragments are dispersed to riparian zones or retained by obstacles in shallow streams).

All plant fragments were exposed to a simulated photoperiod of 16/8 h light/dark at a light intensity of 94.2 ± 20.5 µmol photons m^{-2} s^{-1} (± *SD*), representing summer day lengths and an average light availability for submerged plants, for 28 days. Own preliminary investigations showed that most fragments initiate adventitious growth within 28 days (see Kuntz *et al.*, 2014) and slowly degrade when no formation of adventitious roots and shoots occurred within this period. The treatments with low water depths were additionally placed 12 cm higher than the high water depth treatments by using pedestals to provide similar light conditions (see Figure 1). All plastic jars were randomly repositioned, cleaned and water was exchanged twice a week to avoid position effects and enhanced algal growth as well as to maintain nutrient concentrations. For each treatment, 10 plant fragments were investigated at the same time. The experiment was repeated, resulting in a total of 20 replicates per fragment type, water depth and species (overall n = 480).

Determination of regeneration and colonization parameters

Fragment growth, formation of new roots and shoots and colonization of plant fragments were examined twice a week by measuring fragment length as well as adventitious root and shoot lengths and by checking for rooting of fragments in the sediment. During the measurements, care was taken to prevent fragments from suffering mechanical damage and desiccation. Plant fragments were considered as regenerated if either (i) total adventitious root length was ≥3 cm, (ii) total adventitious shoot length was ≥1.5 cm or (iii) fragment length increased by ≥2 cm. As the number of nodes per fragment differed between the species and with fragment type, we calculated the relative number of nodes that developed adventitious roots and/or shoots, i.e. the number of nodes with roots and/or shoots per number of total fragment nodes, at the time of regeneration. If fragments were visibly anchored in the sediment (mainly by its roots), they were considered as colonized. It must be noted that the colonization abilities of *C. demersum* are expected to differ distinctly from the other species investigated, as this species generally does not develop roots (Sculthorpe, 1967).

During the study period, self-induced detachment of new shoots was observed for *M. spicatum* fragments in the high water depth treatments. This detachment of adventitious shoots was termed as shoot propagation. Detached shoots represent autonomous propagules which in turn are able to regenerate and colonize.

Once successful colonization or shoot propagation was documented, the respective fragment was excluded from the experiment, and thus not further examined. If degradation of a plant fragment occurred, it was considered as died off.

To evaluate the trade-off in the formation of new roots and shoots, a root-shoot index (RSI) based on lengths of adventitious roots and shoots was calculated as follows:

$$\mathrm{RSI} = l_r \text{ or } l_s \text{ [cm]} / l_{total} \text{ [cm]} \tag{1}$$

whereby l_r or l_s is either total adventitious root length (l_r, expressed as negative value) or the total adventitious shoot length (l_s, expressed as positive value) and l_{total} is the sum of total adventitious root and total adventitious shoot length. Consequently, the RSI represents the proportion between root and shoot formation, varying from -1 (indicating maximum preference for root formation) to 1 (indicating maximum preference for shoot formation), with values of either -0.5 or 0.5 indicating no preference for roots or shoots at the time of regeneration or colonization.

Statistical analysis

Statistical analysis was carried out using the software R version 3.4.1. Differences in regeneration and colonization rates between species and the combined effects of fragment type and water depth on regeneration and colonization rates of each species (time-to-event data) were tested by applying Cox proportional hazards regression models with mixed effects (CoxPHme). In case of *M. spicatum*, CoxPHme was also used to assess differences in shoot propagation rate depending on fragment type. As repeated measures were nested in the split design of the experiment (experiment was repeated), iteration and fragment ID were specified as nested random effect. The assumption of proportional hazards was checked by Schoenfeld tests and graphical inspection of the scaled Schoenfeld residuals. Hazard ratios (HR), i.e. the ratios of the hazard rates of two levels, reported for fragment type give fragments w/ apices in relation to fragments w/o apices (reference level), whereas HRs for water depth compare low relative to high treatments (reference level). A HR <1 or >1 indicates a lower or higher event probability per unit time relative to the reference level, respectively, while a HR of 1 indicates no difference. Interactions of fragment type and water depth were tested by subsequently conducting analyses of variances (ANOVA). In case no events were documented for a given treatment (e.g. low, w/ apex treatment for regeneration rate of *M. heterophyllum*), the respective data was excluded from the statistics.

Due to strong violation of normality (assessed by Q-Q plots of residuals), data on root-shoot indexes (RSI) of all species were analysed by permutational analysis of variances (pANOVA) with random effects in order to assess the combined effects of fragment type, water depth and differences between time of regeneration and colonization. Permutational ANOVAs for the effect of water depth and time of regeneration/colonization on RSI were followed by Tukey's HSD tests for multiple comparisons.

Results

During the experiment, buoyancy of plant fragments in the high water depth treatments differed between the species. Fragments of *Myriophyllum spicatum* and *Myriophyllum heterophyllum* remained floating at the water surface, whereas *Ceratophyllum demersum*, *Elodea canadensis*, *Hydrilla verticillata* and *Lagarosiphon major* were characterized by a low degree of buoyancy, as several fragments gradually sunk. The highest percentage of fragments that remained floating at the surface for more than 50% of the time until successful colonization or termination of the experiment was documented for *M. spicatum* and *M. heterophyllum* (both 100% of fragments), followed by *L. major* (53%), *H. verticillata* (43%), *C. demersum* (33%) and was lowest for *E. canadensis* (23%).

Mortality of fragments was only documented in *M. heterophyllum*, with die-off rates of 27.5 and 25% for fragments w/ and w/o apical tips after 28 days, respectively. Further fragmentation of plant fragments, i.e. breakage without strong mechanical influence, was observed for *C. demersum* (w/o apex: 2.5% of fragments), *L. major* (w/ apex: 2.5%) and particularly for *H. verticillata* (w/ apex: 25%, w/o apex: 2.5%).

Regeneration of fragments

Overall, the regeneration rates for plant fragments of *M. spicatum* (99%) and *H. verticillata* (98%) were highest, followed by *L. major* (76%), *E. canadensis* (83%) and *C. demersum* (56%), while the lowest regeneration rate of 11% was documented for *M. heterophyllum*. The time required until no further regeneration of fragments was observed (i.e. to reach maximum regeneration capacity) was distinctly shorter for *M. spicatum* (11 days; except for the w/o apex, high water depth treatment), *H. verticillata* (11 days; w/ apex, high) and *L. major* (14 days; w/ apex, high) than for *M. heterophyllum* (21 days; w/o apex, high and low), *E. canadensis* (21 days; w/ and w/o apex, low) and *C. demersum*, which most likely not reached maximum regeneration capacity at the end of the experiment (Figure 2). In *M. spicatum*, *H. verticillata* and *L. major*, fragments possessing apical tips had a significantly increased likelihood of regeneration that was more than two-fold higher in both *M. spicatum* and *H. verticillata*, and more than five-fold higher in *L. major* compared to fragments w/o apices (Table 1; Figure 2a,d,f). Conversely, regeneration was not significantly affected by water depth in case of all species (Table 1; Figure 2).

Among the species, differences in the relative number of nodes that developed adventitious roots and/or shoots of fragments considered as regenerated were documented. The relative number of nodes at which formation of new roots and/or shoots occurred was highest for *M. spicatum* (47.1 ± 3.1%), followed by *C. demersum* (23 ± 1.5%), *M. heterophyllum* (19.4 ± 3.3%) and *H. verticillata* (14.3 ± 0.7%), and was distinctly lower for *L. major* (7.2 ± 0.5%) and *E. canadensis* (5.3 ± 0.4%; ± 1 *SE*).

Colonization of fragments

The highest colonization rates were documented for *M. spicatum* (66%), *L. major* (63%) and *H. verticillata* (58%), followed by *E. canadensis* (40%), with both *M. spicatum* (w/o apex, high) and *L. major* (w/ apex, low) already reaching maximum colonization capacity when no further colonization of fragments was observed after 14 days (Figure 3a,d-f). Fragments of *C. demersum* and *M. heterophyllum*, by contrast, were not able to colonize – with the exception of one single fragment of *M. heterophyllum* (Figure 3b,c). As in the case of regeneration, fragments with apical tips showed a significantly higher likelihood of colonization by a factor of 3.7 (*L. major*), 3.9 (*M. spicatum*) and 6.5 (*H. verticillata*) relative to fragments w/o apices (Table 1; Figure 3a,d,f). Though colonization rate of *L. major* fragments w/ apical tips in the high water depth treatment was very similar to the colonization rate observed for fragments w/o apices at low water depth, no significant interaction of fragment type and water depth was found (Table 1; Figure 3d). The effect of water depth on colonization was stronger than the effect of fragment type and significant for all species that successfully anchored in the sediment. A low water depth vastly increased the likelihood of colonization that was 3.5 (*L. major*), 13.1 (*E. canadensis*), 26 (*H. verticillata*) and even 30.7 times higher (*M. spicatum*) than for a larger depth (Table 1; Figure 3a,d-f). This enhanced likelihood of colonization when exposed to a low water depth was documented for all three hydrocharitacean species despite the overall low buoyancy of plant fragments in the high water depth treatments (Table 1; Figure 3d-f).

Interestingly, plant fragments of *M. spicatum* in the high water depth treatments that did not colonize after 17 and 14 days (w/ and w/o apical tips, respectively) started to detach new shoots (Figure 4). This shoot propagation rate (i.e. the relative number of fragments that detached adventitious shoots) of *M. spicatum* increased up to 35 (w/ apex) and 65% (w/o apex) at the end of the experiment. When including the portion of colonized fragments, similar values of 85 and 80% of fragments that either detached new shoots or colonized were documented for fragments w/ and w/o apices, respectively. The likelihood of shoot propagation per unit time did not differ significantly between the two fragment types (HR = 0.91, 95% CI = 0.36-2.30, $P = 0.85$).

Trade-off between shoot and root formation

Among the species, strong differences were observed in the trade-off between the formation of adventitious roots and shoots expressed as root-shoot index (RSI). While *C. demersum* lacks roots, thus exclusively developed new shoots (RSI = 1, overall), and *M. heterophyllum* tended to show no particular preference for roots or shoots (RSI = 0.54 ± 0.12), *H. verticillata* (RSI = -0.68 ± -0.02), *M. spicatum* (RSI = -0.70 ± -0.02), *E. canadensis* (RSI = -0.77 ± -0.02) and *L. major* (RSI = -0.90 ± -0.02; ± 1 *SE*) indicated a stronger preference towards root formation. The influence of fragment type on RSI was generally less pronounced than the effects of water depth and time of regeneration/colonization (Table 2). Furthermore, the RSI was not significantly affected by water depth at time of regeneration or colonization (Figure 5), except for the RSI of *M. spicatum* at time of regeneration (Tukey's HSD, $P < 0.001$). No strong differences between the RSI at time of regeneration and the RSI at time of colonization (in combination with water depth) were documented (Figure 5).

Discussion

Besides the number of plant fragments produced and fragment release rate, the regeneration and colonization success of fragments largely contribute to the species-specific spread potential and may explain the invasiveness of aquatic plants (Santamaría, 2002; Lockwood *et al.*, 2009). We documented strong differences in the regeneration and colonization rates of fragments between the species that indicate general differences in regeneration and colonization abilities, though the abiotic parameters within our study presumably had an additional effect.

It has been documented that temperature as well as nutrient, light and carbon availability influence growth and regeneration of plant fragments (Kuntz *et al.*, 2014; Hussner *et al.*, 2015). Although an overall increase of regeneration rate with e.g. increasing nutrient availability was found (Kuntz *et al.*, 2014), species-specific optima are likely to occur. The regeneration abilities found in this study are therefore likely to differ with varying environmental conditions, which must be taken into account when comparing our findings with other studies. More recently, a strong effect of carbon availability on root production was reported (Hussner *et al.*, 2015, 2016a), illustrating that CO_2 depletion may not only attenuate the likelihood of regeneration but also reduce the likelihood of colonization. As the medium of Smart & Barko (1985) used in our experiment shows a relatively high water pH of 7.9, and thus low CO_2 availability, when in equilibrium with ambient air, the low performance of *Myriophyllum heterophyllum* fragments might be due to its slow growth under CO_2 depletion compared to the other species we investigated (Hussner & Jahns, 2015; Hussner *et al.*, 2016a).

Apart from *M. heterophyllum*, our findings emphasize the high regeneration capacity of aquatic plant fragments, particularly of those possessing apical tips (Riis *et al.*, 2009; Umetsu *et al.*, 2012a). Many aquatic plant species, however, are even capable of regeneration from small fragments only consisting of single nodes, including all species investigated in our study (Langeland & Sutton, 1980; Fritschler *et al.*, 2008; Kuntz *et al.*, 2014). Due to the larger fragment size found in the field (Heidbüchel *et al.*, 2016) relative to the fragment size we used and the generally increasing regeneration capacity with increasing fragment length (Redekop *et al.*, 2016; Bickel, 2017), it could be expected that the vast majority of plant fragments produced under field conditions are able to regenerate and initialize new macrophyte stands. But in the field, many plant fragments do not establish even though the number of fragments produced and the potential for regeneration are high. It seems reasonable that this is caused by the low overall success of fragments to colonize, as initial colonization is considered the main bottleneck for successful establishment of plant fragments (initial colonization of drifting shoot fragments was only 0.034% in the River Aarhus, Denmark; Riis, 2008). The presented results indicate that fragments of *Myriophyllum spicatum*, *Lagarosiphon major* (particularly w/ apices), *Hydrilla verticillata* and to a somewhat lesser extent *Elodea canadensis* are less limited by initial colonization than *M. heterophyllum* and *Ceratophyllum demersum*, highlighting the potential of these species to rapidly colonize new sites and spread.

Most plant fragments are dispersed at or close to the water surface, making it evident that water depth influences colonization of fragments as long as the distance between floating fragment and substrate does not prevent fragments from anchorage. Indeed, we found that even relatively small differences in shallow water depth have a strong impact on initial colonization of plant fragments. A low water depth drastically increases the likelihood of colonization, indicating that very shallow sites, e.g. in riparian zones, are more likely to become colonized by aquatic plants and experience the highest pressure for colonization. The degree of fragment buoyancy can further differ among species and fragment types (Riis & Sand-Jensen, 2006; Sarneel, 2013). However, successful colonization still seems to be facilitated at lower water depths even if buoyancy of fragments is low, as indicated by our findings for the hydrocharitacean species (*L. major*, *E. canadensis* and *H. verticillata*). Differences in the floating behavior and morphology of plant fragments are still likely to result in different patterns of retention by obstacles (Johansson & Nilsson, 1993; Riis & Sand-Jensen, 2006). This indicates that less buoyant fragments have a higher likelihood of being retained by submerged structures and may colonize deeper waters more rapidly than fragments floating at the water surface, such as those of *M. spicatum* and *M. heterophyllum*.

The retention of plant fragments prior to anchorage in the sediment increases in shallow sections that are free of vegetation, which was demonstrated for small to medium sized streams (Riis & Sand-Jensen, 2006). Drifting fragments may be retained by lentic zones or obstacles, such as stones, debris and, most importantly, present vegetation (Johansson & Nilsson, 1993; Riis & Sand-Jensen, 2006; Riis, 2008). It must nevertheless be considered that species-specific interactions between introduced fragments and the vegetation present in the receiving habitat strongly influence the colonization success of a species (Chadwell & Engelhardt, 2008; Thiébaut & Martinez, 2015). As we neglected effects of species interactions, the colonization abilities of the species investigated may be strongly altered once a fragment is retained by patches of vegetation.

In running waters, the natural spread via plant fragments is particularly relevant, as allofragmentation is promoted by flow in general and can strongly increase due to fluctuations in discharge (Truscott *et al.*, 2006; Heidbüchel *et al.*, 2016; Redekop *et al.*, 2016). High levels of disturbance, however, might in turn facilitate uprooting of plants and increase the risk of failure during colonization at suitable sites (Riis, 2008). The resistance to those disturbances depends on the extent of root anchorage, which is influenced by the species-specific preference for root formation and root architecture in general and by the type of sediment at the colonized location (Wang *et al.*, 2009; Sand-Jensen & Møller, 2014; Li *et al.*, 2016a). According to our findings, *L. major*, *E. canadensis*, *M. spicatum* and *H. verticillata* have a higher preference for root formation and thus seem to have a greater colonization potential and higher resistance to uprooting than *M. heterophyllum* and the rootless *C. demersum*. These features most likely contribute to the rapid spread via fragments and great invasion success of the former species.

Besides colonization through root anchorage within the sediment, other colonization mechanisms not based on rooting may also be involved in the establishment of plant stands, but were neglected in our study. Once fragments are retained by obstacles, subsequent deposition of sediment is likely to play an important role in facilitating initial colonization. This passive colonization may represent the major mechanism for initial anchorage of *C. demersum*, which commonly invades running waters and inhabits lotic sites (e.g. Hussner & Lösch, 2005). Additionally, *C. demersum* is known to exhibit specialized branches with finely divided leaves that support anchorage within the sediment (Sculthorpe, 1967). The development of these so-called rhizoid shoots, however, was not observed in our experiment, and it remains unclear how frequent rhizoid shoots are formed in the field.

When not able to colonize within a given time, the preference of fragments for the development and subsequent release of secondary propagules constitutes an efficient way to increase propagule pressure and the likelihood for successful colonization at favorable sites. This detachment of even smaller propagules was only documented for *M. spicatum*, which also showed the highest relative number of nodes at which the formation of new shoots and/or roots occurred among the species investigated. It is likely that the trade-off between colonization of fragments and further propagation by detachment of adventitious shoots can be found in other submerged species, especially in those with a very high regeneration capacity even from small fragments similar to *M. spicatum*. Nevertheless, this trade-off and the overall high regeneration and colonization abilities of *M. spicatum* (see also Riis *et al.*, 2009) might largely explain its strong spread potential through plant fragments and role as successful invader worldwide (e.g. in its introduced range in North America; Aiken *et al.*, 1979).

To conclude, the strong effect of water depth on colonization rate implies that very shallow water habitats, such as riparian zones, are highly susceptible to colonization and recolonization of both native and invasive alien submerged aquatic plants. The high regeneration and colonization rates found for plant fragments of *M. spicatum*, *L. major*, *H. verticillata* and, to some lesser extent, *E. canadensis* reflect their strong potential for successful colonization at suitable sites. Thus, these species are most likely less limited by the bottleneck of initial colonization, emphasizing their high vegetative spread potential and invasiveness. Plant growth and fragmentation rate, however, differ among species and with habitat conditions, which must be considered with respect to the species-specific propagule pressure and spread potential in different water bodies (Riis *et al.*, 2009; Redekop *et al.*, 2016).

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Table 1 Summary of mixed effects cox regressions (CoxPHme) for the combined effects of fragment type (FT) and water depth (WD) on regeneration and colonization rates of the six species

	FT (w/ apex vs w/o apex)			WD (low vs high)			FT x WD	
	HR	95% CI	*P* value	HR	95% CI	*P* value	X^2 (df, n)	*P* value
Regeneration rate								
M. spicatum	2.12	1.26-3.59	**0.005**	1.66	0.99-2.78	0.053	9.21 (1, 349)	**0.002**
M. heterophyllum	0.31	0.03-3.03	0.320	2.24	0.53-9.43	0.270	NA	NA
C. demersum	0.66	0.30-1.48	0.320	0.89	0.41-1.92	0.760	0.83 (1, 797)	0.362
L. major	5.34	2.22-12.84	**<0.001**	1.03	0.43-2.48	0.950	0.19 (1, 521)	0.665
E. canadensis	1.77	0.86-3.63	0.120	1.33	0.64-2.76	0.450	0.63 (1, 581)	0.427
H. verticillata	2.34	1.23-4.45	**0.009**	1.07	0.57-2.04	0.830	0.39 (1, 456)	0.534
Colonization rate								
M. spicatum	3.93	1.02-15.10	**0.046**	30.74	8.24-114.70	**<0.0001**	1.72 (1, 594)	0.190
M. heterophyllum	NA	NA	NA	NA	NA	NA	NA	NA
C. demersum	NA	NA	NA	NA	NA	NA	NA	NA
L. major	3.65	1.18-11.25	**0.024**	3.45	1.11-10.74	**0.032**	0.44 (1, 684)	0.506
E. canadensis	1.04	0.14-7.62	0.970	13.13	2.82-61.13	**<0.001**	0.03 (1, 815)	0.867
H. verticillata	6.50	1.26-33.66	**0.026**	26.00	5.36-126.10	**<0.0001**	1.48 (1, 805)	0.224

Hazard ratios (HR) indicate the ratio of hazard rates of treatment (w/ apex; low WD) and reference levels (w/o apex; high WD). NA indicates missing statistical analysis due to a lack of observations. Significant *P* values are written in bold (P <0.05)

Table 2 Summary of permutational ANOVAs (pANOVA) for the combined effects of time of regeneration/colonization (RC), fragment type (FT) and water depth (WD) on root-shoot indices (RSI) of the six species

			P value						
	dfn	dfd	RC	FT	WD	RC x FT	RC x WD	FT x WD	RC x FT x WD
RSI									
M. spicatum	1	122	**<0.001**	0.137	0.452	0.625	**0.007**	0.386	0.768
M. heterophyllum	NA	NA	NA	NA	NA	NA	NA	NA	NA
C. demersum	NA	NA	NA	NA	NA	NA	NA	NA	NA
L. major	1	101	**0.022**	**0.004**	**0.040**	0.384	0.164	**0.034**	0.178
E. canadensis	1	88	0.247	0.216	**0.020**	1.000	0.788	0.865	1.000
H. verticillata	1	114	0.264	0.941	**0.014**	0.742	**0.041**	0.105	0.502

Degrees of freedom (dfn, dfd) apply to all main effects and interactions of a given species. NA indicates missing statistical analysis due to a lack of observations (*M. heterophyllum*) or uniform outcome (*C. demersum*). Significant *P* values are written in bold (P <0.05)

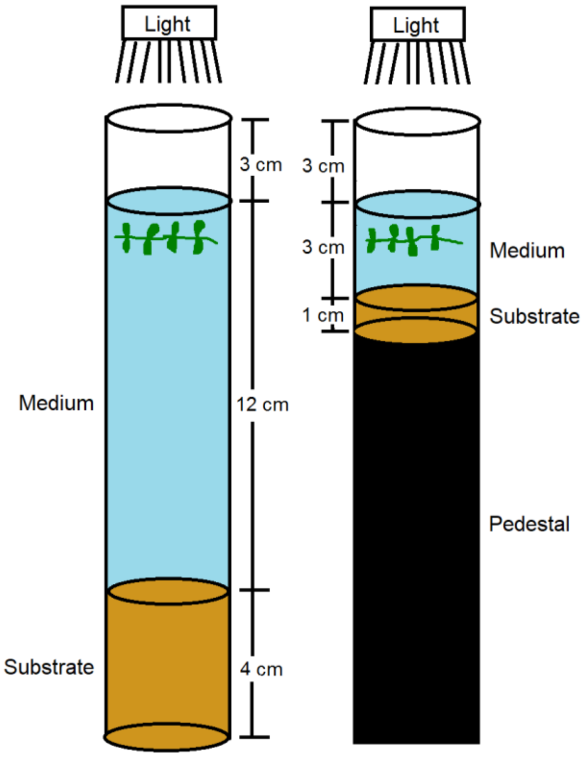

Figure 1 Experimental setup with high (left) and low (right) water depth treatments (indicating different distances between floating plant fragments and substrate surface)

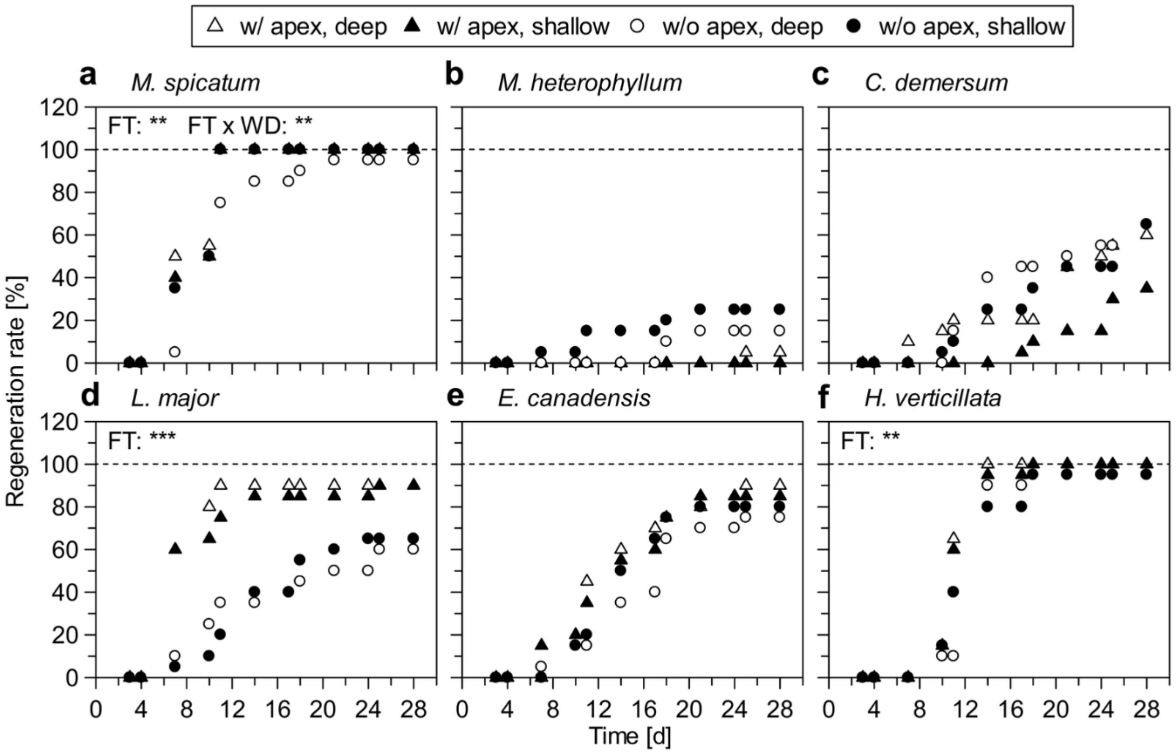

Figure 2 Regeneration rates of plant fragments for **a** *Myriophyllum spicatum*, **b** *Myriophyllum heterophyllum*, **c** *Ceratophyllum demersum*, **d** *Lagarosiphon major*, **e** *Elodea canadensis* and **f** *Hydrilla verticillata* depending on fragment type (FT; w/, w/o apex) and water depth (WD; high, low). Shown are relative numbers of fragments that developed adventitious roots and/or shoots and/or increased in length over 28 days. Significant effects are asterisked according to significance level (* *P* <0.05, ** *P* <0.01, *** *P* <0.001; CoxPHme)

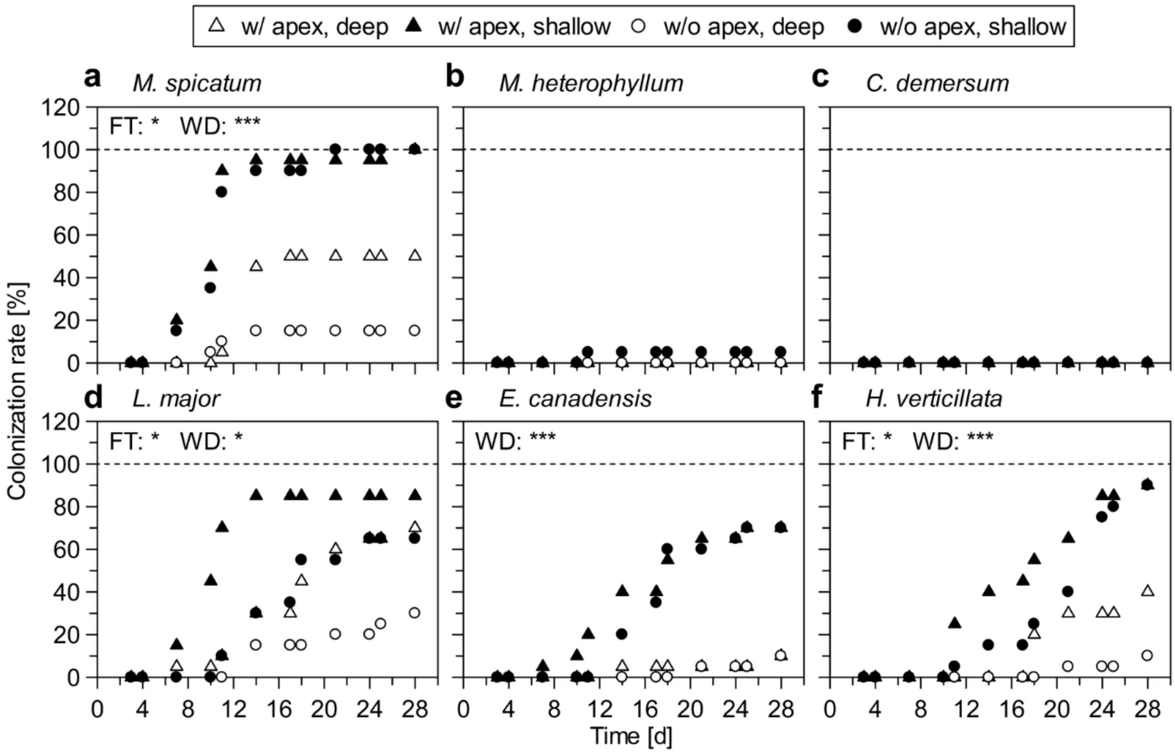

Figure 3 Colonization rates of plant fragments for **a** *Myriophyllum spicatum*, **b** *Myriophyllum heterophyllum*, **c** *Ceratophyllum demersum*, **d** *Lagarosiphon major*, **e** *Elodea canadensis* and **f** *Hydrilla verticillata* depending on fragment type (FT; w/, w/o apex) and water depth (WD; high, low). Shown are relative numbers of fragments that anchored within the sediment over 28 days. Significant effects are asterisked according to significance level (* *P* <0.05, ** *P* <0.01, *** *P* <0.001; CoxPHme)

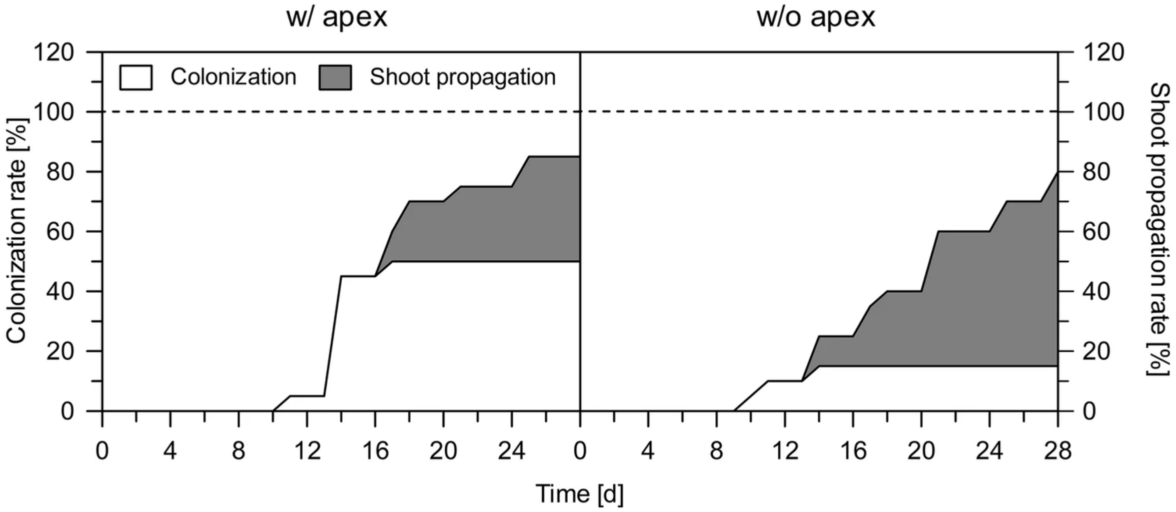

Figure 4 Proportion of colonization and shoot propagation rates of *Myriophyllum spicatum* plant fragments with (left) and without apical tips (right) for high water depth treatments over 28 days

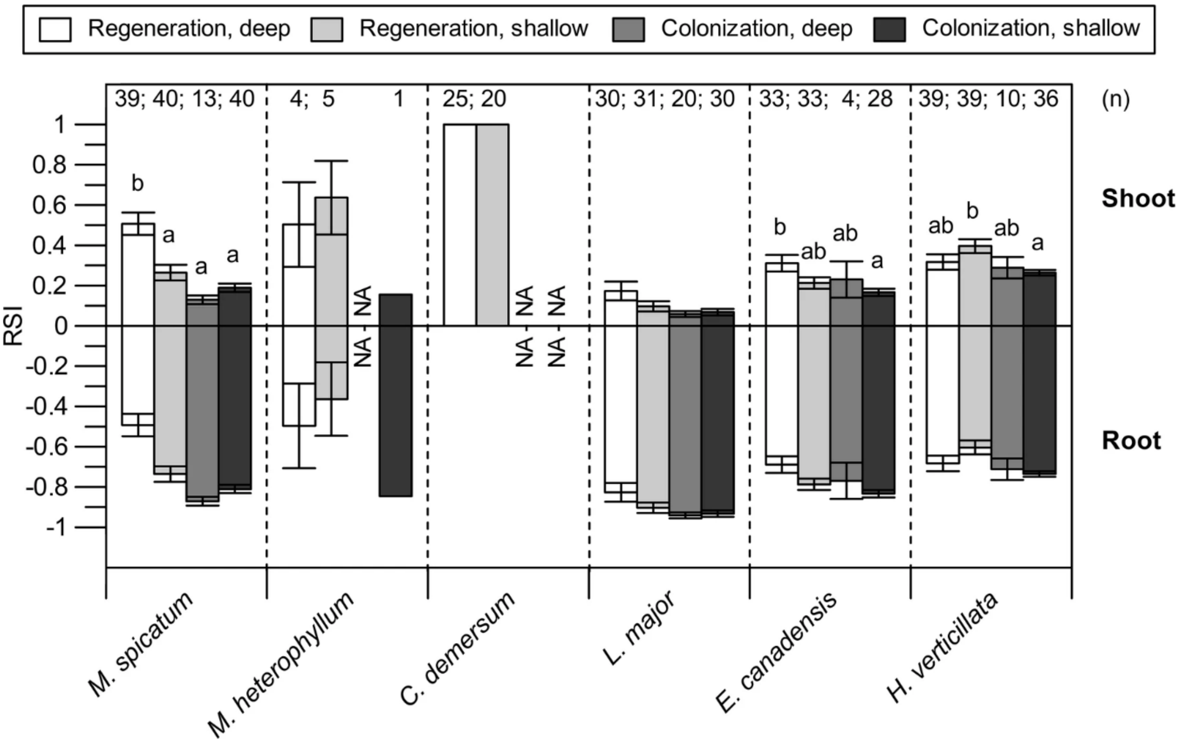

Figure 5 Root-shoot indices (RSI) of plant fragments depending on water depth (high, low) and time of regeneration/colonization. Shown are mean values (columns) ± 1 *SE* (error bars) and corresponding n-values above the columns. NA indicates no formation of adventitious shoots and roots within the respective treatment. Different letters indicate significant differences for the respective species (P <0.05; Tukey's HSD test)

CHAPTER 7

Synthesis & outlook

Invasive alien aquatic plant (IAAP) species pose a global threat to biodiversity and functionality of freshwater ecosystems (Strayer, 2010; Brundu, 2015; Gallardo *et al.*, 2016). Most IAAPs show a low degree of genetic variability and predominantly reproduce through vegetative means in their introduced range (Grace, 1993; Okada *et al.*, 2009; Riis *et al.*, 2010). Consequently, the rapid spread and invasion success of IAAPs must be assigned to a certain extent to efficient vegetative spread mechanisms (Santamaría, 2002; Fleming & Dibble, 2015). Unspecialized plant fragments constitute the most important propagules as they are readily available in large quantities and generally preserve a high viability (Barrat-Segretain, 1996). Surprisingly, detailed information on the species-specific fragment dispersal capacity are to date scarce and the underlying dynamics have not been thoroughly addressed. Understanding the relevance of spread pathways and fragment dispersal dynamics of IAAPs, however, is crucial for the development of adequate prevention and management measures and the adaptation of existing methods in order to comply with directives and legislations such as the EU regulation 1143/2014 (EU, 2014; Hussner *et al.*, 2017)

The three main objectives of this thesis were (I) to assess the species-specific fragment dispersal capacity and its relevance for the invasiveness of IAAPs, (II) to identify and evaluate fragment dispersal pathways and the underlying dynamics and (III) to derive prevention and management measures against IAAPs from the findings. Throughout the **Chapters 2-6**, comparative laboratory and field studies were conducted, particularly focusing on fragment dispersal of native and (invasive) alien aquatic plant species in temperate lowland stream systems. Moreover, the relevance of different dispersal pathways was considered by investigating traits that determine the spread potential to hydrologically connected and to hydrologically isolated sites.

Relevance of fragment dispersal capacity for invasiveness

Running water ecosystems are particularly susceptible to aquatic plant invasions. The hydrologic connectivity and physically perturbed characteristics imposed by the flow regime strongly facilitate the rapid spread of IAAPs and make streams significant invasion corridors (e.g. Scheers *et al.*, 2019). As opposed to lentic systems, the central role of recruitment in response to flow disturbance for maintaining plant populations, the facilitation of downstream dispersal and the low relevance of biotic resistance especially emphasize the strong competitive advantage of species with high fragment dispersal capacities in stream ecosystems (Barrat-Segretain & Amoros, 1996a,b; Sand-Jensen *et al.*, 1999; Riis & Biggs, 2003; Alofs & Jackson, 2014).

In **Chapter 2**, high numbers of up to >1,000 drifting fragment per hour were reported within small to medium-sized streams (see also Boedeltje *et al.*, 2003, 2004; Riis, 2008; Heidbüchel *et al.*, 2016). These findings generally illustrate the high potential of fragment dispersal as efficient spread mechanism for aquatic plant species even in small streams (Sand-Jensen *et al.*, 1999). The fragment dispersal capacity of a species depends on a combination of the specific fragmentation rate, the fragment dispersal distance within and between water bodies and the potential of fragments to regenerate and colonize, i.e. to initiate new growth and anchor within the sediment, respectively. The specific fragmentation rate further indicates the propagule pressure of a species, which is regarded as a central determinant of invasion success (Von Holle & Simberloff, 2005; Colautti *et al.*, 2006; Simberloff, 2009; Carr *et al.*, 2019).

Based on the findings within the **Chapters 2-6** and in consideration with existing literature, data on different key traits including fragmentation rate, drift distance, desiccation resistance and regeneration/colonization potential were compiled to detail the species-specific fragment dispersal capacity for IAAPs of Union concern as well as for other alien and native species addressed within this thesis (Table 1). The table gives particular attention to the role of fragment dispersal in stream ecosystems, even though the summarized information is derived from many different field, mesocosm or laboratory approaches comprising variable experimental conditions.

Overall, the capacity for fragment dispersal is expected to be high for the majority of the aquatic plant species considered herein (Table 1). There are, however, species-specific differences which are at least partly related with plant growth form. Emerged growing species, e.g. *H. ranunculoides* and *Ludwigia* spp., tend to show lower fragment dispersal capacities than free floating and submerged species and are mainly limited by their low fragmentation rates. Reasons for this include, in particular, the sturdy structure and robust growth of emerged species as well as their general preference for rather less lotic and perturbed habitats, such as riparian zones (e.g. Sculthorpe, 1967; Hussner & Lösch, 2005). In the case of *S. emersum*, very low fragmentation rates were documented despite growing in its submerged form exposed to the main flow in the medium-sized river Niers (**Chapter 2**). Hence, even though there is a lack of information on the relevance of fragment dispersal for species such as *G. spilanthoides*, it is anticipated that the degree of disturbance induced fragmentation is generally attenuated in species capable of emergent growth. For the free floating IAAPs *E. crassipes* and *S. molesta* as well as for *S. natans*, fragment dispersal capacity is assumed to be high, though information on fragmentation rate is largely absent. Albeit floating species such as *Salvinia* spp. are known to be very fragile and readily detach fronds, fragment dispersal likely plays a less prominent role in streams as these species favor habitats sheltered from high flow disturbance, especially standing waters such as lakes (Room, 1983). Free floating IAAPs can still settle at lentic or slow flowing sites along streams, where they may grow in dense mats and limit the local light availability for native submerged vegetation (Strayer, 2010; Stiers *et al.*, 2011a; Gałka & Szmeja, 2013). Nevertheless, it must be concluded that the fragment downstream flux and propagule pressure for both emerged and free floating IAAPs is generally lowered in stream ecosystems due to their growth characteristics and ecological niche preferences.

Conversely, submerged species are frequently inhabiting lotic sites and can cope with high levels of hydraulic stress, as long as flow disturbance does not prevent them from growth and persistence (Riis & Biggs, 2003; Sand-Jensen, 2003, 2008; Puijalon *et al.*, 2011). Hence, it seems reasonable that the overall fragment dispersal capacity is strongly increased for submerged IAAPs growing exposed to the main flow. There are, however, profound differences in the capacity for fragment dispersal among the submerged species addressed within this thesis, particularly among the submerged IAAPs of Union concern (Table 1). Most notably, the high fragment dispersal capacity of *E. nuttallii*, which is the most widespread IAAP within the EU, emphasizes its rapid spread in streams and likely explains in large part the global invasion success of this species (e.g. Zehnsdorf *et al.*, 2015; Steen *et al.*, 2019). High propagule pressure and a strong potential for rapid colonization and re-establishment following disturbance combined with its well performance under variable environmental conditions due to its high adaptive plasticity make *E. nuttallii* a resilient nuisance plant and superior competitor even to other species known for their efficient vegetative dispersal, e.g. *E. canadensis* (Barrat-Segretain *et al.*, 2002; Barrat-Segretain, 2005; Szabó *et al.*, 2019). Similarly, the competitive strength of *C. caroliniana* is also expected to rely on its efficient fragment dispersal, even though fragmentation rate has not yet been adequately assessed. Recent modelling of its spread revealed that a model integrating both vector-based (i.e. fragment dispersal mediated by flow and human activities) and environmental niche models was much more similar to the mere vector-based model than the predictions by the environmental niche model (Jacobs & MacIsaac, 2009).

Table 1 Fragment dispersal capacity of aquatic plant species addressed in this thesis

Species	Status in EU	Growth form	Fragmentation rate	Drift distance	Desiccation resistance
Alternanthera philoxeroides	Invasive alien	Emerged	NA	High [81]	Very high [28,74]
Cabomba caroliniana	Invasive alien	Submerged	NA	NA	Medium [54,62]
Eichhornia crassipes	Invasive alien	Free floating	NA	NA	Very high [83]
Elodea nuttallii	Invasive alien	Submerged	**High** [**C2**,**C3**,18,46]	Low [80]	Medium [25,76]
Gymnocoronis spilanthoides	Invasive alien	Emerged	NA	High [81]	NA
Hydrocotyle ranunculoides	Invasive alien	Emerged	Low [68]	NA	Very high [32]
Lagarosiphon major	Invasive alien	Submerged	Low [71]	NA	**Medium/high** [**C5**,76]
Ludwigia grandiflora	Invasive alien	Emerged	Low [77]	NA	NA
Ludwigia peploides	Invasive alien	Emerged	NA	NA	NA
Myriophyllum aquaticum	Invasive alien	Emerged	**Medium** [**C2**,68]	NA	Very high [54]
Myriophyllum heterophyllum	Invasive alien	Submerged	**Low** [**C2**]	NA	**High** [**C5**,54]
Salvinia molesta	Invasive alien	Free floating	NA	NA	High [20]
Egeria densa	Alien (invasive elsewhere)	Submerged	**Medium** [**C2**,64,68,71]	NA	Medium [36,54]
Elodea canadensis	Alien (invasive elsewhere)	Submerged	**High** [**C2**,**C3**,23,46,71]	**Low** [**C4**,24]	**Low** [**C5**,8,25,54,75]
Hydrilla verticillata	Alien (invasive elsewhere)	Submerged	Very high [16,53]	NA	**Medium** [**C5**,36,54,67]
Vallisneria spiralis	Alien (invasive elsewhere)	Submerged	**Low** [**C2**,68]	NA	NA
Callitriche spp.	Native	Submerged	**Very high** [**C2**,46,60]	NA	NA
Ceratophyllum demersum	Native (invasive elsewhere)	Submerged	**Medium/high** [**C2**,18,23,68,79]	**High** [**C4**]	**Medium** [**C5**,54]
Myriophyllum spicatum	Native (invasive elsewhere)	Submerged	**High** [**C2**,**C3**,23,46,60,61,79]	**Low/medium** [**C4**]	**Medium/high** [**C5**,43,49,50,54,63]
Potamogeton crispus	Native (invasive elsewhere)	Submerged	**High** [**C2**,**C3**,60,68,82]	NA	Low/medium [54,63]
Salvinia natans	Native (invasive elsewhere)	Free floating	NA	**High** [**C4**]	NA
Sparganium emersum	Native (invasive elsewhere)	Emerged	**Low** [**C2**,44,45,55]	NA	NA
Stuckenia pectinata	Native (invasive elsewhere)	Submerged	**High** [**C2**,31,46,60,68,79,82]	NA	Medium [11]

Chapter contributions to specific traits are written in bold and references are given in

[a]Selection of relevant studies referring to variable fragment sizes (not only to minimum size

[b]Only regeneration considered. Colonization is 'none/low' as *C. demersum* lacks roots

[c]Only regeneration considered. Colonization is generally negligible for free floating species

based on the findings within the chapters and existing literature

Regeneration/colonization		Fragment dispersal capacity
Min. fragment size required for regeneration	Regeneration/colonization rate[a]	
Small [37]	High [37,38,42,48,64]	High
Small [47]	High [47,75]	Medium/high
Small/medium [3]	High[c] [58,73,83]	High
Small [**C2**,29]	**Medium/high** [**C2**,17,21,29,39,41,59]	High
Very small [40]	NA	NA
Small [33]	High [33]	Medium
Small [85]	**Medium** [**C6**,71]	Low/medium
Very small [33]	High [33,66]	Medium
Small [19]	High [19,56]	Medium
Very small [33]	Medium [29,33,41,57,59,66,78]	Medium
Small [27,59]	**Low/medium** [**C2**,**C6**,27,29,59]	Low
Small [12,20]	High[c] [7,20]	High
Small/medium [2,29]	Medium [29,51,71,72]	Medium
Small [**C2**,29]	**Medium/high** [**C2**,**C6**,13-15,17,21,22,29,35,39,59,71]	Medium/high
Small [6]	**Medium/high** [**C6**,6,41,51,52,59,67]	High
Medium [4]	High [4,85]	Medium
Small [**C2**,1]	**Medium** [**C2**]	High
Small [29,59]	**High**[b] [**C6**,14,29,59]	High
Small [26,59]	**High** [**C2**,**C6**,14,29,35,59,69,70]	High
Small [29,30]	High [29,34]	High
Small [5]	High[c] [84]	High
Medium [9,13]	High [13-15]	Medium
Small [**C2**,10]	**High** [**C2**,85]	High

parentheses (see next page for reference list). IAAPs of Union concern are written in red required for regeneration)

[**C2**] **Chapter 2**, [**C3**] **Chapter 3**, [**C4**] **Chapter 4**, [**C5**] **Chapter 5**, [**C6**] **Chapter 6**

[**1**] Arber (1920), [**2**] Riede (1920), [**3**] Gay (1960), [**4**] Sculthorpe (1967), [**5**] Zutshi & Vass (1971), [**6**] Langeland & Sutton (1980), [**7**] Room (1983), [**8**] Johnstone *et al.* (1985), [**9**] Sand-Jensen *et al.* (1989), [**10**] van Wijk (1989), [**11**] van Wijck & de Groot (1993), [**12**] Lemon & Posluszny (1997), [**13**] Barrat-Segretain *et al.* (1998), [**14**] Barrat-Segretain *et al.* (1999), [**15**] Barrat-Segretain & Bornette (2000), [**16**] Rybicki *et al.* (2001), [**17**] Barrat-Segretain *et al.* (2002), [**18**] Boedeltje *et al.* (2003), [**19**] Dandelot (2004), [**20**] Owens *et al.* (2004), [**21**] Barrat-Segretain (2005), [**22**] Mielecki & Pieczyńska (2005), [**23**] Schutten *et al.* (2005), [**24**] Riis & Sand-Jensen (2006), [**25**] Barrat-Segretain & Cellot (2007), [**26**] Gao *et al.* (2007), [**27**] Hussner & Krause (2007), [**28**] van Oosterhout (2007), [**29**] Fritschler *et al.* (2008), [**30**] Ganie *et al.* (2008), [**31**] Bociag *et al.* (2009), [**32**] EPPO (2009), [**33**] Hussner (2009), [**34**] Jiang *et al.* (2009), [**35**] Riis *et al.* (2009), [**36**] Silveira *et al.* (2009), [**37**] Dong *et al.* (2010a), [**38**] Dong *et al.* (2010b), [**39**] JianMin *et al.* (2010), [**40**] Panetta (2010), [**41**] Xie *et al.* (2010), [**42**] Dong *et al.* (2011), [**43**] Evans *et al.* (2011), [**44**] Liffen *et al.* (2011), [**45**] Pollen-Bankhead *et al.* (2011), [**46**] Puijalon *et al.* (2011), [**47**] Bickel (2012), [**48**] Dong *et al.* (2012), [**49**] Jerde *et al.* (2012), [**50**] McAlarnen *et al.* (2012), [**51**] Umetsu *et al.* (2012a), [**52**] Umetsu *et al.* (2012b), [**53**] Zhu *et al.* (2012), [**54**] Barnes *et al.* (2013), [**55**] Hamann & Puijalon (2013), [**56**] Robert *et al.* (2013), [**57**] You *et al.* (2013a), [**58**] You *et al.* (2013b), [**59**] Kuntz *et al.* (2014), [**60**] Miler *et al.* (2014), [**61**] Zhu *et al.* (2014), [**62**] Bickel (2015), [**63**] Bruckerhoff *et al.* (2015), [**64**] Fan *et al.* (2015), [**65**] Schoelynck *et al.* (2015), [**66**] Thiébaut & Martinez (2015), [**67**] Baniszewski *et al.* (2016), [**68**] Heidbüchel *et al.* (2016), [**69**] Li *et al.* (2016a), [**70**] Li *et al.* (2016b), [**71**] Redekop *et al.* (2016), [**72**] Thiébaut *et al.* (2016), [**73**] Wang *et al.* (2016), [**74**] You *et al.* (2016), [**75**] Bickel (2017), [**76**] Coughlan *et al.* (2018), [**77**] Skaer Thomason *et al.* (2018), [**78**] Xie *et al.* (2018), [**79**] Zhu *et al.* (2018), [**80**] Cornacchia *et al.* (2019), [**81**] Kim *et al.* (2019), [**82**] Łoboda *et al.* (2019), [**83**] Yu *et al.* (2019), [**84**] Zhang *et al.* (2019) [**85**] own preliminary studies & observations

Due to the high accuracy of this modelling approach, fragment dispersal capacity must be considered as the major driving force for the invasive spread of *C. caroliniana*. Moreover, a high fragment dispersal capacity clearly constitutes a key component for the invasiveness of other submerged aquatic plant species such as the native *M. spicatum*, *P. crispus* and Asian *H. verticillata*. These species are well known for their competitive strength and role as successful invaders throughout their introduced range, particularly in North America (Aiken *et al.*, 1979; Bolduan *et al.*, 1994; Langeland, 1996). In addition to their efficient fragment dispersal, all three species are characterized by high growth rates and adaptive physiological performance, e.g. a high efficiency to use bicarbonate as additional inorganic carbon source when invading vegetated habitats and growing in dense plant stands (Madsen & Sand-Jensen, 1991; Hussner *et al.*, 2016a; Yin *et al.*, 2017; Fasoli *et al.*, 2018). Interestingly, whilst *H. verticillata* is amongst the species with the highest fragment dispersal capacities and the climatic conditions of at least Southern Europe match those in its invasive range, this species has not, to date, become a permanent nuisance within the EU (e.g. Hussner, 2012).

As opposed to the majority of the submerged species addressed, *L. major* and *M. heterophyllum*, both listed as IAAPs of Union concern, possess rather weak capacities for fragment dispersal. It is therefore not surprising that these species are reported to expand only slowly within the EU and often do not seem to spread in an invasive way (EPPO, 2016b). Established populations, however, are very resilient and can cause great damage on a local scale, as was reported for lakes in Germany and Ireland (Hussner & Krause, 2007; Caffrey *et al.*, 2010, 2011). Consequently, their competitive strength and invasion potential must rely on other traits. A high bicarbonate use capacity and thus the ability to maintain high growth rates when CO_2 availability becomes limited at high pH is regarded as the key trait for the strong competitive advantage of *L. major* over other macrophytes and allows for its dense growth (James *et al.*, 1999; Stiers *et al.*, 2011b; Cavalli *et al.*, 2012). *M. heterophyllum*, by contrast, is not characterized by high growth rates and shows only a low efficiency in HCO_3^- utilization (Hussner & Jahns, 2015; Dülger *et al.*, 2017). The invasion success of *M. heterophyllum* is most likely attributed to its evergreenness and high resilience. Maintaining biomass during winter and pre-seasonal growth can compensate for lowered growth rates in the main season and allow *M. heterophyllum* to overgrow and outcompete seasonal native species in the long term (cf. Greulich & Bornette, 2003). Increased water temperature in winter is further expected to promote the competitive strength and dominance of evergreen species (Netten *et al.*, 2011; Hussner, 2014). Previously, it has been postulated that evergreen species are usually more efficient in their propagation through plant fragments than seasonal (or summergreen) species (Bornette & Puijalon, 2009). The findings in Table 1, however, indicate that evergreen aquatic plant species such as *M. heterophyllum* and *V. spiralis* are characterized by considerably lower fragment dispersal capacities than many seasonal species (e.g. *Elodea* spp. and *M. spicatum*), which produce high numbers of viable fragments over the growing season (**Chapter 2**).

Albeit limited propagule supply often seems to constrain fragment dispersal capacity, i.e. low fragmentation rates due to high structural resistance to stem breakage and/or avoidance of disturbance (preference for less lotic habitats), the aquatic plant species addressed herein were principally not limited by their regeneration and colonization abilities (Table 1). Most species require only small fragments consisting of a single node for regeneration and show high regeneration and/or colonization rates (but see *M. heterophyllum*). Even for species with rosette growth forms, e.g. *S. emersum* and *V. spiralis*, regenerating fragments can be relatively small, as long as the basal/apical meristem is present.

To conclude, efficient fragment dispersal must be considered as a key feature of many IAAPs, particularly for submerged species in stream ecosystems. Knowledge gaps on the traits of many species could be filled by the findings within the chapters of this thesis. Still, while the regeneration and colonization abilities of fragments have been intensively studied, knowledge on the fragmentation rates of free floating and emerged growing species and on drift distance remains scarce and deserves further attention (Table 1). It must nevertheless be noted that extrinsic factors influence the spatial and temporal dispersal patterns (Chambers *et al.*, 1991; Sand-Jensen *et al.*, 1999; Riis & Biggs, 2003; Franklin *et al.*, 2008). The fragment dispersal capacity of a species is therefore subjected to fluctuations in the experienced environmental conditions.

Fragment dispersal pathways & dynamics

Throughout **Chapter 2**-**6**, the specific relevance of different spread pathways and the underlying dynamics of fragment dispersal, particularly the influence of hydraulic and hydromorphological stream properties, were further elucidated. Seasonal effects during summer and autumn seem to play only a minor role for fragment dispersal capacity in the different stream systems investigated (**Chapter 2**, **Chapter 3**; see also Heidbüchel *et al.*, 2016). Submerged species maintained high fragmentation rates and fragments remained highly viable over the whole investigation periods. For seasonal species, fragment dispersal is still largely restricted in winter when environmental conditions become unfavorable for growth and plants senescence (e.g. Bornette & Puijalon, 2011). However, seasonal species often produce specialized storage organs for overwintering that enable fast regrowth once the environmental conditions become more favorable, e.g. turions in *P. crispus* or tubers in *H. verticillata* and *S. pectinata* (Bowes *et al.*, 1979; Sastroutomo, 1981; van Wijk, 1989). These specialized propagules are generally less buoyant but may commonly be dispersed over longer distances when attached to floating fragments (own observations). The formation of specialized vegetative propagules combined with high release rates of unspecialized plant fragments represents an adaptive dispersal strategy for some species that clearly fosters the resilience and invasive spread in their introduced range, e.g. *P. crispus* (Bolduan *et al.*, 1994).

Aquatic plants show plastic responses in morphology and structure to abiotic factors, such as nutrient conditions in the sediments (Zhu *et al.*, 2018) and hydrodynamic forces (Sand-Jensen, 2008; Miler *et al.*, 2012). Due to the overall high phenotypic plasticity of IAAPs (Puijalon *et al.*, 2008; Riis *et al.*, 2010), it seems reasonable that adaptation to hydraulic stress allows species to operate similar fragmentation rates under different flow conditions, and thus to avoid excessive biomass loss to the current while maintaining a certain propagule pressure. However, I found that fragmentation rate is strongly controlled by the flow regime in the first place, with higher fragmentation rates in streams characterized by increased levels of discharge (**Chapter 3**). This indicates that the successful spread of IAAPs growing under perturbed flow conditions in larger streams is most likely limited by the ability to cope with hydrodynamic forces, i.e. maintenance of growth, rather than propagule flux. Even though minor fluctuations in flow of a stream have only little effect on fragmentation rate (**Chapter 3**), strongly increased discharge especially during flood events still enhances fragmentation and may even cause uprooting of large plant stands through sediment erosion (Riis & Biggs, 2003; Heidbüchel *et al.*, 2016). A previous study, however, showed that macrophyte patches grown at a flow velocity of 0.2 m s^{-1} lost only <1% of plant biomass after a short exposure to 1.5 m s^{-1} (Riis & Biggs, 2003). These findings may at least partly be related to the mutual shelter provided in plant patches, which strongly alters the resistance to flow and fragment formation on the individual level (Sand-Jensen, 2003, 2008). As the size of fragments found in small to medium-sized streams often exceeds the minimum size required for regeneration by a factor of >10 (**Chapter 2**, **Chapter 3**), further fragmentation during downstream transport can considerably enhance propagule pressure, emphasizing that rapid invasion may be strongly facilitated when IAAPs establish in turbulent streams. Larger plant fragments may still establish more frequently than smaller fragments as the likelihood for regeneration increases with larger fragment size (**Chapter 2**). Nevertheless, despite the vast number of dispersed fragments, only few will successfully establish as primary colonization was found to be the main bottleneck for establishment (Rybicki *et al.*, 2001; Riis, 2008; Bickel, 2017). In Danish lowland streams, only 3.4% of fragments retained by obstacles were able to successfully colonize, while, in turn, only 19% of successfully colonized fragments failed to establish (Riis, 2008). As most IAAPs are generally not limited in their regeneration abilities, this aspect is of particular importance and requires further research to unravel the species-specific restrictions through initial colonization in the field (but see **Chapter 6**). It thus seems reasonable to assume that specific initial colonization dynamics rather than propagule pressure could explain the invasion success of some IAAPs (e.g. efficient rooting strategies that enhance retention and colonization).

The range expansion of IAAPs on a regional scale is strongly influenced by the potential of a species to spread within interconnected water bodies (e.g. along streams) and between hydrologically isolated sites. Based on the compiled information, strong differences in the potential for propagule movement along these different pathways exist among the species addressed (Table 1). Within interconnected water bodies, the rapid dispersal of IAAPs is largely associated with high drift distances. Although field studies on drift distances of plant fragments are very scarce, the results in **Chapter 4** highlight that fragment buoyancy is an overall reliable indicator of drift distance (thus also fragment retention) and can be easily assessed. Consequently, species with a high buoyancy, including all free floating and emerged species herein, are expected to show larger drift distances than those characterized by less buoyant fragments such as the submerged hydrocharitaceans *Elodea* spp., *H. verticillata* and *L. major* (see also **Chapter 6**). Drift distances and fragment retention patterns, however, are strongly dependent on the flow regime and hydromorphological properties of a stream as well as on the abundance of retention agents (**Chapter 4**). In larger straightened stream systems characterized by high discharge, fragments may generally drift more unimpeded over considerably larger distances than in smaller, meandering streams, particularly when fragments are highly buoyant (see also Riis & Sand-Jensen, 2006). Still, a large drift distance alone is not directly linked with increased establishment success as retention sites must allow for colonization. Fragments with a low buoyancy (including specialized organs such as turions of e.g. *P. crispus*) might be more frequently lodged at suitable habitats for colonization despite being limited in dispersal distance. For submerged species, colonization of fragments is more likely and much faster at low water depths (**Chapter 6**), indicating that shallow sites, such as riparian zones, are most frequently invaded and that colonization particularly takes place during periods of low discharge (see also Riis, 2008). Likewise, fragments of floating and emerged species increasingly colonize at low water depths (Thiébaut & Martinez, 2015) but, by contrasts, might predominantly reach suitable habitats following higher levels of discharge when the probability for deposition of propagules in the riparian zones is enhanced (Engström *et al.*, 2009). Besides water depth and flow, other abiotic factors, e.g. temperature, light, nutrient and carbon availability (Riis *et al.*, 2012; Kuntz *et al.*, 2014; Hussner *et al.*, 2015), as well as biotic interactions control the establishment and growth of IAAPs at the receiving site (Franklin *et al.*, 2008; Thomaz *et al.*, 2015; Pulzatto *et al.*, 2019). Shading by riparian vegetation can generally reduce growth of aquatic plants but may not, however, prevent invasive species with high fragment dispersal capacities like *E. nuttallii* and *M. spicatum* from establishment, even if incident light is reduced by 94% (Zefferman, 2014; Ellawala *et al.*, 2019). Moreover, contrasting effects are documented for the influence of pre-existing aquatic vegetation on the establishment of arriving fragments. Macrophyte beds can serve as important retention agents (see also **Chapter 4**) that facilitate initial colonization of IAAPs, though competitive effects, by contrast, can limit resource availability for arriving propagules (Chadwell & Engelhardt, 2008; Thiébaut & Martinez, 2015; Petruzzella *et al.*, 2018). High levels of propagule pressure may still allow IAAPs to overwhelm the biotic resistance by native vegetation (Chadwell & Engelhardt, 2008; Li *et al.*, 2015a; You *et al.*, 2016a; Louback-Franco *et al.*, 2019). It is important to stress that there remains great need for field studies integrating the retention and establishment rate at suitable habitats and, at best, tracking fragments over the whole dispersal process from release to establishment.

Unlike drift dispersal within connected water bodies, the spread of IAAPs to hydrologically isolated habitats requires specific vectors for overland transport and largely depends on the ability of aquatic plant fragments to withstand drying conditions (**Chapter 5**). The desiccation resistance of free floating and emerged aquatic plants is generally considered to be much higher compared to submerged species (Table 1), as their growth form involves different adaptations to air exposure, e.g. thicker leaves with pronounced cuticle (Sculthorpe, 1967). Among the submerged aquatic plants addressed, desiccation resistance of fragments shows strong variation. While *E. canadensis* is particularly prone to desiccation, species that are more robust in structure such as the IAAPs *L. major* and *M. heterophyllum* show high desiccation resistances. Thus, albeit both species are rather limited in their overall fragment dispersal capacities, plant fragments may have an increased probability to remain viable when moved to distant isolated waters. The movement of fragments along this pathway, however, depends on the abundance of potential vectors, especially the frequency of human activities, and the likelihood of fragments to attach to a certain vector in the first place (Jacobs & MacIsaac, 2009; Rothlisberger *et al.*, 2010). Plants growing near haul-out areas in water bodies extensively used for trailered boating are assumed to be more frequently dispersed to hydrologically isolated sites. It must be further considered that the environmental conditions during overland transport strongly control the desiccation resistance and dispersal success of IAAPs (e.g. Bruckerhoff *et al.*, 2015). Several factors such as wind speed, precipitation, sun light, clumping of fragments and sediment covering plant material along with the experienced vapor pressure deficit (VPD) influence water loss and fragment viability over the duration of overland transport (Bickel, 2015; Bruckerhoff *et al.*, 2015; Coughlan *et al.*, 2018). In **Chapter 5**, I found that aquatic plant species possess different mechanisms to cope with desiccation that are either related with structural features, physiological features or a combination of both. These mechanisms, particularly differences in the maintenance of the photosynthetic apparatus in response to water loss, are still not fully understood and require further research.

Implications for prevention & management of IAAPs

Taking into account the findings of this thesis, implications for the prevention and management of IAAPs can be derived to counteract the nuisance growth of IAAPs with respect to the EU regulation (EU, 2014). Potential measures that intervene in the spread of IAAPs at different stages of the invasion process are proposed (Figure 1). As ongoing management incurs high costs (Oreska & Aldridge, 2011; Hussner *et al.*, 2017) and prevention is generally more desirable (Vander Zanden *et al.*, 2010; Caplat & Coutts, 2011), special emphasis is placed on preventing IAAPs from further spread within stream ecosystems and overland dispersal to isolated water bodies. The containment of detected infestations of IAAPs characterized by high fragment dispersal capacities (e.g. *E. nuttallii* and *C. caroliniana*) thereby deserve particular attention as these species are expected to expand with great rapidity once they become established in streams (Scheers *et al.*, 2019).

Prevention of introduction & further spread

Reducing propagule pressure is generally regarded as a promising approach to limit the successful spread of IAAPs (Chadwell & Engelhardt, 2008; Meyerson & Pyšek, 2013). By intercepting fragments during the early growing season, drift dispersal and establishment of IAAPs can be interfered and competition with native species attenuated as consequence of

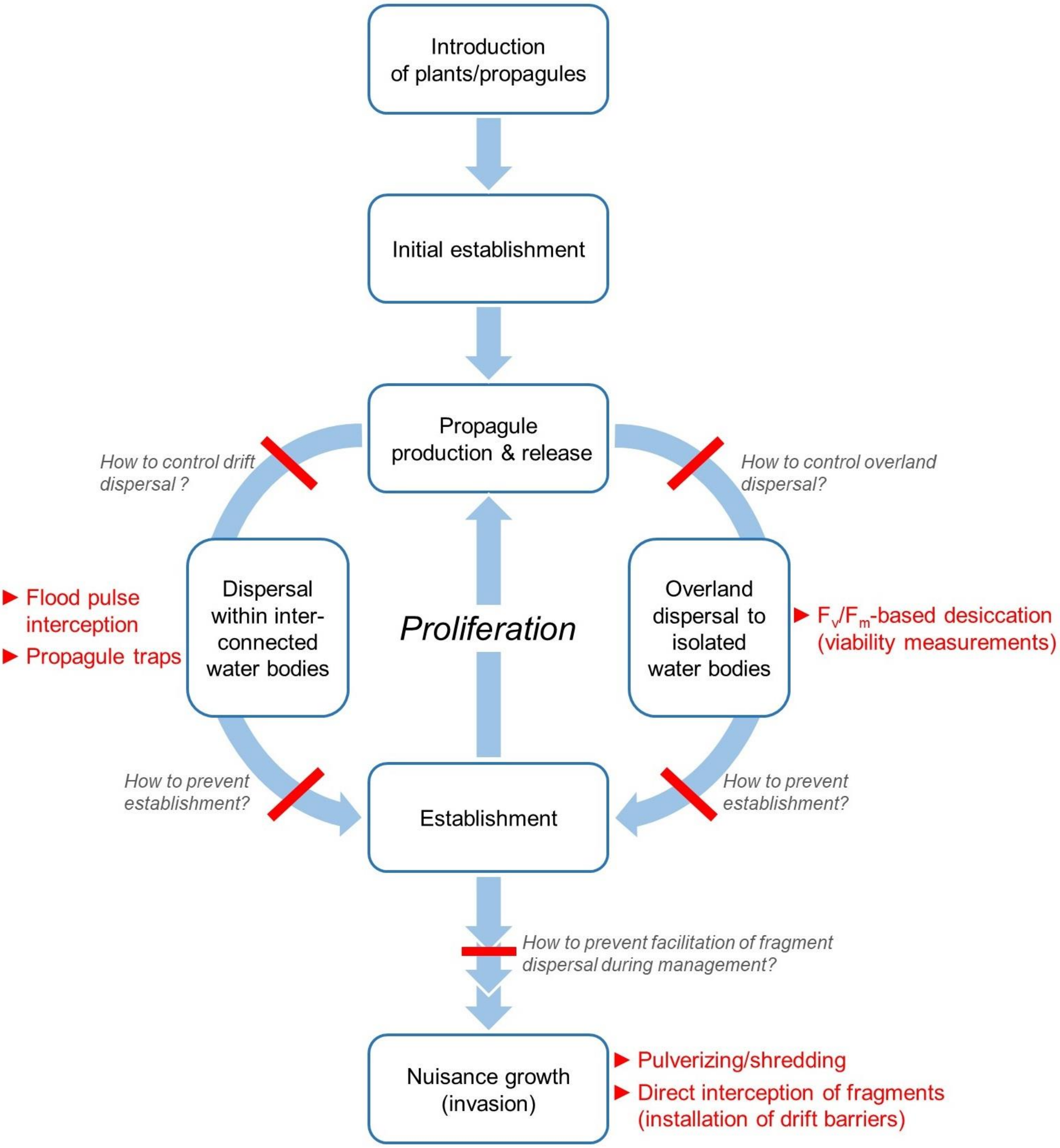

Figure 1 Overview of potential measures derived from the findings to intervene in fragment dispersal of IAAPs within the invasion continuum. Intervention points are indicated by red bars. See text for further explanation of the proposed prevention/management measures

reduced downstream propagule pressure. As significant quantities of fragments are formed and released during high discharge (see **Chapter 3**, Heidbüchel *et al.*, 2016), recurrent interception of plant material in response to heavy rainfall events and elevated discharge is probably most important for limiting the downstream flux of IAAPs. For this purpose, fragment collection should take place at relevant sites (i.e. before stream junctions) and may be supported by stationary installations for fragment interception. In impounded semi-natural and artificial streams, flood pulses may be induced to initiate interception of produced plant fragments. Moreover, targeted trapping by establishing obstacles at selected retention sites may pose an alternative (or additional) measure to reduce propagule pressure. These 'propagule traps' must therefore be regularly maintained and freed from fragments during receding water levels following high discharge events. The efficient use of propagule traps, however, is likely restricted to rather shallow streams that allow for collection of fragments. It must nevertheless be noted that the proposed measures may be costly as they must be carried out repeatedly and should be preferentially combined with management options to reduce propagule production and the further spread of established IAAPs in the long term (see Hussner *et al.*, 2017).

Fragment dispersal of IAAPs to isolated water bodies is largely driven by the intensity of human activities as plant material often adheres to boats or leisure equipment (Johnstone *et al.*, 1985; Rothlisberger *et al.*, 2010; Anderson *et al.*, 2014). Biosecurity campaigns promoted by stakeholders, such as the 'Check, Clean, Dry' campaign in the UK, aim to raise public awareness of invasive alien species and endorse best-practice for recreational users to prevent IAAPs from further spread (Anderson *et al.*, 2014). For biosecurity protocols incorporating desiccation as prevention measure, knowledge on the species-specific desiccation resistance and drying times required to kill off IAAP fragments as well as on the adequate disposal of plant material is substantial (see **Chapter 5**). To support the efficiency of such biosecurity protocols, measurements of fragment viability may be integrated in controlled desiccation procedures (e.g. active drying by using fans). As the F_v/F_m ratio precisely indicates fragment viability (**Chapter 5**), drying of plant material can be supported by F_v/F_m measurements in an efficient way. This could be realized by implementing control stations for screening of plant material before entering a water system. Likewise, chlorophyll *a* measurements could also prove a useful tool in other promising biosecurity measures, such as application of disinfectants (Cuthbert *et al.*, 2018), decontamination in hot water (Anderson *et al.*, 2015) or exposure to steam (Crane *et al.*, 2019; Coughlan *et al.*, 2020). Knowledge on the species-specific desiccation resistance of aquatic plants can be further transferred to evaluate the repercussions of water drawdowns on aquatic plant communities, and thus help to assess the efficacy of commonly applied winter drawdowns in controlling IAAPs (Langeland, 1996; Barrat-Segretain & Cellot, 2007; Wersal & Madsen, 2011; Dugdale *et al.*, 2013).

Mechanical control

Mechanical control represents the most commonly applied type of management measure against IAAPs within the EU and is generally very versatile, as e.g. mowing boats can be utilized to unselectively remove vast amounts of plant biomass while hand-weeding/pulling allows for selective control of a target species (Thiébaut & Dutartre, 2009; Brundu, 2015; Hussner *et al.*, 2017). Management methods incorporating mowing and cutting often cause the formation of numerous viable allofragments (Anderson, 1998, 2003; van Valkenburg & Rotteveel, 2010), which in turn may be dispersed and establish new populations. Consequently, mechanical measures can unintentionally promote the further spread of a target IAAP species, particularly when applied in stream systems. To avoid the excessive production of viable fragments, plants could be pulverized or shredded to a size smaller than the minimum fragment size required for regeneration (Figure 1; see Table 1 for min. size required for regeneration). If the produced plant material is not removed, however, decomposition of large amounts of plant matter likely results in significant nutrient increase and may cause oxygen depletion (Greenfield *et al.*, 2007). Combining mowing or cutting with direct interception of produced plant fragments constitutes a more promising approach to manage IAAPs (as long as infestations are not overly large) without risking inadvertent facilitation of spread. Cutting and immediate collection of plant material e.g. by using landing nets can be operated simultaneously. Particularly in smaller streams, temporarily installed drift barriers may prove useful for eradication measures against IAAPs, which are usually recommended to be conducted from upstream to downstream infestations (see **Chapter 2** & **3**). It must still be noted that the frequency and timing of measures is important for successful containment and eradication (Newman, 2010). Control methods are most efficient if conducted in advance of the main season when growth receded and fragment dispersal capacity is limited by unfavorable environmental conditions. Nevertheless, mechanical management and eradication options are often labor-intensive, costly and might not always show high efficacy as IAAPs may rapidly regrow following cutting (Howard-Williams *et al.*, 1996; Sabbatini & Murphy, 1996). Hence, sound execution is of crucial importance and careful measures such as hand-pulling are probably most efficient, at least for smaller infestations (Thiébaut & Dutartre, 2009).

Concluding remarks

Although aquatic plant invasions have been widely recognized as a major global threat to the biodiversity and functionality of freshwater ecosystems, it is still not fully understood why such a large proportion of aquatic plant species becomes invasive and, more specifically, what traits confer their invasion success (Fleming & Dibble, 2015; Hussner *et al.*, 2017). Based on the findings within this thesis, the vegetative dispersal capacity via plant fragments must be considered as a major driving force behind the successful and rapid spread of IAAPs worldwide. However, fragment dispersal capacity does not necessarily explain the invasion success of all IAAPs of Union concern over native species within the EU. While the invasiveness of submerged IAAPs such as *E. nuttallii* and *C. caroliniana* can be in large part assigned to high fragment dispersal capacities, fragment dispersal seems to be less relevant in explaining the invasive behavior of *L. major*, the evergreen *M. heterophyllum* and emerged growing invaders like *Ludwigia* spp. and *H. ranunculoides*. It must nevertheless be considered that the findings do not point out an overall low vegetative spread potential for the latter IAAPs but rather demonstrate the substantial role of fragment dispersal for the competitive strength and invasion success of submerged native species such as *M. spicatum* and *P. crispus* in their introduced range.

While invasions are context-specific and often complex, there is great need for holistic approaches that help to predict and counteract aquatic plant invasions. Modelling the spread of IAAPs is considered as a powerful tool in decision making regarding efficient intervention on the further expansion of IAAPs (e.g. Jacobs & MacIsaac, 2009). Reliable modelling approaches, however, require a sound data basis. Information on the species-specific fragment dispersal capacity presented within the thesis allows to draw conclusions on the propagule flux and likelihood for establishment of IAAPs. In stream ecosystems, fragment dispersal is strongly controlled by the hydrological properties and flow dynamics and may be of greatest relevance in medium-sized lowland streams (see also Riis & Sand-Jensen, 2006). To improve modelling of the spread of IAAPs in the future, information on the species-specific fragment dispersal capacity must be integrated in vector-based models and combined with habitat suitability models considering assumptions on the spatial and temporal dynamics of the hydrological regime.

For successful containment of IAAPs characterized by high fragment dispersal capacities (e.g. *E. nuttallii* and *C. caroliniana*), measures interfering with propagule flux are highly needed. The feasibility and efficacy of potential measures, however, strongly depend on the extent of infestation and the characteristics of the invaded water bodies such as the degree of hydrological connectivity.

References

A

Aiken SG, Newroth R, Wile I. **1979**. The biology of Canadian weeds: 34. *Myriophyllum spicatum* L. *Canadian Journal of Plant Sciences* **59**: 201–215.

Allan JD, Flecker AS. **1993**. Biodiversity conservation in running waters. *BioScience* **43**: 32–43.

Alofs KM, Jackson DA. **2014**. Meta-analysis suggests biotic resistance in freshwater environments is driven by consumption rather than competition. *Ecology* **95**: 3259–3270.

Alpert P, Bone E, Holzapfel C. **2000**. Invasiveness, invasibility and the role of environmental stress in the spread of non-native plants. *Perspectives in Plant Ecology, Evolution and Systematics* **3**: 52–66.

Anderson LWJ. **1998**. Dissipation and movement of Sonar and Komeen following typical applications for control of *Egeria densa* in the Sacramento/San Joaquin Delta, and production and viability of *E. Densa* fragments following mechanical harvesting (1997/1998). *Environmental Impact Report*. California Department of Boating and Waterways, 79 pp.

Anderson LWJ. **2003**. A review of aquatic weed biology and management research conducted by the United States Department of Agriculture - Agricultural Research Service. *Pest Management Science* **59**: 801–813.

Anderson LG, Dunn AM, Rosewarne PJ, Stebbing PD. **2015**. Invaders in hot water: A simple decontamination method to prevent the accidental spread of aquatic invasive non-native species. *Biological Invasions* **17**: 2287–2297.

Anderson LG, White PCL, Stebbing PD, Stentiford GD, Dunn AM. **2014**. Biosecurity and vector behaviour: evaluating the potential threat posed by anglers and canoeists as pathways for the spread of invasive non-native species and pathogens. *PLoS ONE* **9**: 1–10.

Andersson E, Nilsson C, Johansson ME. **2000**. Plant dispersal in boreal rivers and its relation to the diversity of riparian flora. *Journal of Biogeography* **27**: 1095–1106.

Arber A. **1920**. *Water plants: A study of aquatic angiosperms*. Cambridge University Press.

Ardenghi NMG, Barcheri G, Ballerini C, Cauzzi P, Filippo G. **2016**. *Gymnocoronis spilanthoides* (Asteraceae, Eupatorieae), a new naturalized and potentially invasive aquatic alien in S Europe. *Willdenowia* **46**: 265–273.

Azan S, Bardecki M, Laursen AE. **2015**. Invasive aquatic plants in the aquarium and ornamental pond industries: A risk assessment for southern Ontario (Canada). *Weed Research* **55**: 249–259.

B

Bakker ES, Wood KA, Pagès JF, Veen GF (Ciska), Christianen MJA, Santamaría L, Nolet BA, Hilt S. **2016**. Herbivory on freshwater and marine macrophytes: A review and perspective. *Aquatic Botany* **135**: 18–36.

Baniszewski J, Cuda JP, Gezan SA, Sharma S, Weeks ENI. **2016**. Stem fragment regrowth of *Hydrilla verticillata* following desiccation. *Journal of Aquatic Plant Management* **54**: 53–60.

Barnes MA, Jerde CL, Keller D, Chadderton WL, Howeth JG, Lodge DM. **2013**. Viability of aquatic plant fragments following desiccation. *Invasive Plant Science and Management* **6**: 320–325.

Barrat-Segretain MH. **1996**. Strategies of reproduction, dispersion, and competition in river plants: A review. *Vegetatio* **123**: 13–37.

Barrat-Segretain MH. **2005**. Competition between invasive and indigenous species: Impact of spatial pattern and developmental stage. *Plant Ecology* **180**: 153–160.

Barrat-Segretain MH, Amoros C. **1996a**. Recovery of riverine vegetation after experimental disturbance: A field test of the patch dynamics concept. *Hydrobiologia* **321**: 53–68.

Barrat-Segretain MH, Amoros C. **1996b**. Recolonization of cleared riverine macrophyte patches: Importance of the border effect. *Journal of Vegetation Science* **7**: 769–776.

Barrat-Segretain MH, Bornette G. **2000**. Regeneration and colonization abilities of aquatic plant fragments: Effect of disturbance seasonality. *Hydrobiologia* **421**: 31–39.

Barrat-Segretain MH, Bornette G, Hering-Vilas-Bôas A. **1998**. Comparative abilities of vegetative regeneration among aquatic plants growing in disturbed habitats. *Aquatic Botany* **60**: 201–211.

Barrat-Segretain MH, Cellot B. **2007**. Response of invasive macrophyte species to drawdown: The case of *Elodea* sp. *Aquatic Botany* **87**: 255–261.

Barrat-Segretain MH, Elger A, Sagnes P, Puijalon S. **2002**. Comparison of three life-history traits of invasive *Elodea canadensis* Michx. and *Elodea nuttallii* (Planch.) H. St. John. *Aquatic Botany* **74**: 299–313.

Barrat-Segretain MH, Henry CP, Bornette G. **1999**. Regeneration and colonization of aquatic plant fragments in relation to the disturbance frequency of their habitats. *Archiv für Hydrobiologie* **145**: 111–127.

Barrett SCH, Eckert CG, Husband BC. **1993**. Evolutionary processes in aquatic plant populations. *Aquatic Botany* **44**: 105–145.

Bickel TO. **2012**. Ecology of the submersed aquatic weed *Cabomba caroliniana* in Australia. In: Eldershaw V, ed. 18th Australasian Weeds Conference, Melbourne, 8–11 October. Weed Society of Victoria: 21–24.

Bickel TO. **2015**. A boat hitchhiker's guide to survival: *Cabomba caroliniana* desiccation resistance and survival ability. *Hydrobiologia* **746**: 123–134.

Bickel TO. **2017**. Processes and factors that affect regeneration and establishment of the invasive aquatic plant *Cabomba caroliniana*. *Hydrobiologia* **788**: 157–168.

Birch J, Joyce CB, Bilotta GS, van Valkenburg JLCH. **2015**. Impact of the invasive non-native species *Hydrocotyle ranunculoides* L.f. (Floating Pennywort) on native macrophyte communities. In: 14th International Symposium on Aquatic Plants, Edinburgh, 14–18 September. Presented poster.

Bociag K, Gałka A, Łazarewicz T, Szmeja J. **2009**. Mechanical strength of stems in aquatic macrophytes. *Acta Societatis Botanicorum Poloniae* **78**: 181–187.

Boedeltje G, Klutman B, Schaap M, Sollman P, de Vos M, Lenssen JPM, Verberk WCEP. **2019**. Plant dispersal in a temperate stream by fish species with contrasting feeding habits: The role of plant traits, fish diet, season, and propagule availability. *Frontiers in Ecology and Evolution* **7**: 1–15.

Boedeltje G, Bakker JP, Bekker RM, van Groenendael JM, Soesbergen M. **2003**. Plant dispersal in a lowland stream in relation to occurence and three specific life-history traits of the species in the species pool. *Journal of Ecology* **91**: 855–866.

Boedeltje G, Bakker JP, Brinke A Ten, van Groenendael JM, Soesbergen M. **2004**. Dispersal phenology of hydrochorous plants in relation to discharge, seed release time and buoyancy of seeds: The flood pulse concept supported. *Journal of Ecology* **92**: 786–796.

Boedeltje G, Ozinga WA, Prinzing A. **2008**. The trade-off between vegetative and generative reproduction among angiosperms influences regional hydrochorous propagule pressure. *Global Ecology and Biogeography* **17**: 50–58.

Bolduan BR, Van Eeckhout GC, Quade HW, Gannon JE. **1994**. *Potamogeton crispus* – the other invader. *Lake and Reservoir Management* **10**: 113–125.

Bornette G, Puijalon S. **2009**. Macrophytes: Ecology of aquatic plants. *Encyclopedia of Life Sciences*: 1–9.

Bornette G, Puijalon S. **2011**. Response of aquatic plants to abiotic factors: A review. *Aquatic Sciences* **73**: 1–14.

Bowes G, Holaday AS, Haller WT. **1979**. Seasonal variation in the biomass, tuber density, and photosynthetic metabolism of *Hydrilla* in three Florida lakes. *Journal of Aquatic Plant Management* **17**: 61–65.

Bruckerhoff L, Havel J, Knight S. **2015**. Survival of invasive aquatic plants after air exposure and implications for dispersal by recreational boats. *Hydrobiologia* **746**: 113–121.

Brundu G. **2015**. Plant invaders in European and Mediterranean inland waters: Profiles, distribution, and threats. *Hydrobiologia* **746**: 61–79.

Brundu G, Stinca A, Angius L, Bonanomi G, Celesti-Grapow L, D'Auria G, Griffo R, Migliozzi A, Motti R, Spigno P. **2012**. *Pistia stratiotes* L. and *Eichhornia crassipes* (Mart.) Solms.: Emerging invasive alien hydrophytes in Campania and Sardinia (Italy). *EPPO Bulletin* **42**: 568–579.

Brunel S. **2009**. Pathway analysis: Aquatic plants imported in 10 EPPO countries. *EPPO Bulletin* **39**: 201–213.

C

Caffrey J, Millane M, Evers SL, Moran H. **2011**. Management of *Lagarosiphon major* (Ridley) Moss in Lough Corrib–a review. *Biology and Environment: Proceedings of the Royal Irish Academy* **111B**: 205–212.

Caffrey JM, Millane M, Evers S, Moran H, Butler M. **2010**. A novel approach to aquatic weed control and habitat restoration using biodegradable jute matting. *Aquatic Invasions* **5**: 123–129.

Capers RS, Selsky R, Bugbee GJ, White JC. **2007**. Aquatic plant community invasibility and scale-dependent patterns in native and invasive species richness. *Ecology* **88**: 3135–3143.

Caplat P, Coutts SR. **2011**. Integrating ecological knowledge, public perception and urgency of action into invasive species management. *Environmental Management* **48**: 878.

Carpenter SR, Lodge DM. **1986**. Effects of submersed macrophytes on ecosystem processes. *Aquatic Botany* **26**: 341–370.

Carr AN, Hooper DU, Dukes JS. **2019**. Long-term propagule pressure overwhelms initial community determination of invader success. *Ecosphere* **10**: e02826.

Carreira BM, Dias MP, Rebelo R. **2014**. How consumption and fragmentation of macrophytes by the invasive crayfish *Procambarus clarkii* shape the macrophyte communities of temporary ponds. *Hydrobiologia* **721**: 89–98.

Catford JA, Jansson R. 2014. Drowned, buried and carried away: Effects of plant traits on the distribution of native and alien species in riparian ecosystems. *New Phytologist* **204**: 19–36.

Cattaneo A, Kalff J. 1980. The relative contribution of aquatic macrophytes and their epiphytes to the production of macrophyte beds. *Limnology and Oceanography* **25**: 280–289.

Cavalli G, Riis T, Baattrup-Pedersen A. **2012**. Bicarbonate use in three aquatic plants. *Aquatic Botany* **98**: 57–60.

Chadwell TB, Engelhardt KAM. **2008**. Effects of pre-existing submersed vegetation and propagule pressure on the invasion success of *Hydrilla verticillata*. *Journal of Applied Ecology* **45**: 515–523.

Chambers PA, Prepas EE, Hamilton HR, Bothwell ML. **1991**. Current velocity and its effect on aquatic macrophytes in flowing waters. *Ecological Applications* **1**: 249–257.

Champion PD, Clayton JS, Hofstra DE. **2010**. Nipping aquatic plant invasions in the bud: Weed risk assessment and the trade. *Hydrobiologia* **656**: 167–172.

Clayton J, Champion P. **2006**. Risk assessment method for submerged weeds in New Zealand hydroelectric lakes. *Hydrobiologia* **570**: 183–188.

Coetzee JA, Jones RW, Hill MP. **2014**. Water hyacinth, *Eichhornia crassipes* (Pontederiaceae), reduces benthic macroinvertebrate diversity in a protected subtropical lake in South Africa. *Biodiversity and Conservation* **23**: 1319–1330.

Colautti RI, Grigorovich IA, MacIsaac HJ. **2006**. Propagule pressure: A null model for biological invasions. *Biological Invasions* **8**: 1023–1037.

Cook CDK. **1982**. Pollination mechanisms in the Hydrocharitaceae. In: Symoens JJ, Hooper SS, Compere P, eds. Studies on aquatic vascular plants. Brussels: Royal Botanical Society of Belgium, 1–15.

Cook CDK. **1985**. Range extensions of aquatic vascular plant species. *Journal of Aquatic Plant Management* **23**: 1–6.

Cook CDK, Urmi-König K. **1985**. A revision of the genus *Elodea* (Hydrocharitaceae). *Aquatic Botany* **21**: 111–156.

Cornacchia L, van der Wal D, van de Koppel J, Puijalon S, Wharton G, Bouma TJ. **2019**. Flow-divergence feedbacks control propagule retention by in-stream vegetation: The importance of spatial patterns for facilitation. *Aquatic Sciences* **81**: 17.

Cotton JA, Wharton G, Bass JAB, Heppell CM, Wotton RS. **2006**. The effects of seasonal changes to in-stream vegetation cover on patterns of flow and accumulation of sediment. *Geomorphology* **77**: 320–334.

Coughlan NE, Armstrong F, Cuthbert RN, Eagling LE, Kregting L, Dick JTA, MacIsaac HJ, Crane K. **2020**. Dead and gone: Steam exposure kills layered clumps of invasive curly waterweed *Lagarosiphon major*. *Aquatic Botany* **162**: 103204.

Coughlan NE, Cuthbert RN, Kelly TC, Jansen MAK. **2018**. Parched plants: Survival and viability of invasive aquatic macrophytes following exposure to various desiccation regimes. *Aquatic Botany* **150**: 9–15.

Coughlan NE, Kelly TC, Davenport J, Jansen MAK. **2017a**. Up, up and away: Bird-mediated ectozoochorous dispersal between aquatic environments. *Freshwater Biology* **62**: 631–648.

Coughlan NE, Kelly TC, Jansen MAK. **2015**. Mallard duck (*Anas platyrhynchos*)-mediated dispersal of Lemnaceae: A contributing factor in the spread of invasive *Lemna minuta*? *Plant Biology* **17**: 108–114.

Coughlan NE, Kelly TC, Jansen MAK. **2017b**. "Step by step": High frequency short-distance epizoochorous dispersal of aquatic macrophytes. *Biological Invasions* **19**: 625–634.

Crane K, Cuthbert RN, Dick JTA, Kregting L, MacIsaac HJ, Coughlan NE. **2019**. Full steam ahead: Direct steam exposure to inhibit spread of invasive aquatic macrophytes. *Biological Invasions* **21**: 1311–1321.

Crooks JA. **2005**. Lag times and exotic species: The ecology and management of biological invasions in slow-motion. *Ecoscience* **12**: 316–329.

Crutzen PJ. **2002**. Geology of mankind. *Nature* **415**: 23.

Cuthbert RN, Coughlan NE, Crane K, Caffrey JM, Macisaac HJ, Dick JTA. **2018**. A dip or a dab: Assessing the efficacy of virasure® aquatic disinfectant to reduce secondary spread of the invasive curly waterweed *Lagarosiphon major*. *Management of Biological Invasions* **9**: 259–265.

D

Dandelot S. **2004**. *Les Ludwigia spp. invasives du sud de la France: Historique, biosystématique, biologie et écologie*. PhD thesis, Paul Cézanne University Aix-Marseille III, Marseille, France.

Dandelot S, Verlaque R, Dutartre A, Cazaubon A. **2005**. Ecological, dynamic and taxonomic problems due to *Ludwigia* (Onagraceae) in France. *Hydrobiologia* **551**: 131–136.

Davidson AM, Jennions M, Nicotra AB. **2011**. Do invasive species show higher phenotypic plasticity than native species and, if so, is it adaptive? A meta-analysis. *Ecology Letters* **14**: 419–431.

Davis MA, Grime JP, Thompson K. **2000**. Fluctuating resources in plant communities: A general theory of invasibility. *Journal of Ecology* **88**: 528–534.

van Denderen D, Tamis W, van Valkenburg J. **2010**. Risks of introduction of alien plant species, particularly from the genus *Ambrosia*, via seeds imported for fodder and birdseed. *Gorteria: Tijdschrift voor Onderzoek aan de Wilde Flora* **34**: 65–85.

Dinakar C, Djilianov D, Bartels D. **2012**. Photosynthesis in desiccation tolerant plants: Energy metabolism and antioxidative stress defense. *Plant Science* **182**: 29–41.

Divíšek J, Chytrý M, Beckage B, Gotelli NJ, Lososová Z, Pyšek P, Richardson DM, Molofsky J. **2018**. Similarity of introduced plant species to native ones facilitates naturalization, but differences enhance invasion success. *Nature Communications* **9**: 4631.

Dong BC, Alpert P, Guo W, Yu FH. **2012**. Effects of fragmentation on the survival and growth of the invasive, clonal plant *Alternanthera philoxeroides*. *Biological Invasions* **14**: 1101–1110.

Dong BC, Liu RH, Zhang Q, Li HL, Zhang MX, Lei GC, Yu FH. **2011**. Burial depth and stolon internode length independently affect survival of small clonal fragments. *PLoS ONE* **6**: 1–6.

Dong BC, Yu GL, Guo W, Zhang MX, Dong M, Yu FH. **2010a**. How internode length, position and presence of leaves affect survival and growth of *Alternanthera philoxeroides* after fragmentation? *Evolutionary Ecology* **24**: 1447–1461.

Dong BC, Zhang MX, Alpert P, Lei GC, Yu FH. **2010b**. Effects of orientation on survival and growth of small fragments of the invasive, clonal plant *Alternanthera philoxeroides*. *PLoS ONE* **5**: 1–6.

Dudgeon D, Arthington AH, Gessner MO, Kawabata ZI, Knowler DJ, Lévêque C, Naiman RJ, Prieur-Richard AH, Soto D, Stiassny MLJ, *et al.* **2006**. Freshwater biodiversity: Importance, threats, status and conservation challenges. *Biological Reviews of the Cambridge Philosophical Society* **81**: 163–182.

Dugdale TM, Butler KL, Clements D, Hunt TD. **2013**. Survival of cabomba (*Cabomba caroliniana*) during lake drawdown within mounds of stranded vegetation. *Lake and Reservoir Management* **29**: 61–67.

Dülger E, Heidbüchel P, Schumann T, Mettler-Altmann T, Hussner A. **2017**. Interactive effects of nitrate concentrations and carbon dioxide on the stoichiometry, biomass allocation and growth rate of submerged aquatic plants. *Freshwater Biology* **62**: 1094–1104.

E

Ellawala C, Matheson FE, Riis T. **2019**. Shading constrains the growth of invasive submerged macrophytes in streams. *Aquatic Botany* **158**: 103125.

Engström J, Nilsson C, Jansson R. **2009**. Effects of stream restoration on dispersal of plant propagules. *Journal of Applied Ecology* **46**: 397–405.

EPPO. **2006**. *Hydrocotyle ranunculoides*. *EPPO Bulletin* **36**: 3–6.

EPPO. **2008**. *Eichhornia crassipes*. *EPPO Bulletin* **38**: 441–449.

EPPO. **2009**. *Report of a Pest Risk Analysis for Hydrocotyle ranunculoides*. Paris.

EPPO. **2011**. *Ludwigia grandiflora* and *L. peploides* Onagraceae - water primroses. *EPPO Bulletin* **41**: 414–418.

EPPO. **2016a**. *Alternanthera philoxeroides* (Mart.) Griseb. *EPPO Bulletin* **46**: 8–13.

EPPO. **2016b**. *Myriophyllum heterophyllum* Michaux. *EPPO Bulletin* **46**: 20–24.

EPPO. **2017a**. *Gymnocoronis spilanthoides*. *EPPO Bulletin* **47**: 544–548.

EPPO. **2017b**. *Salvinia molesta* D.S. Mitch. *EPPO Bulletin* **47**: 531–536.

EPPO. **2019**. EPPO Global Database (available online). https://gd.eppo.int [accessed 02.01.2020]

European Union (EU). **2014**. Regulation (EU) No 1143/2014 of the European Parliament and the Council of 22 October 2014 on the prevention and management of the introduction and spread of invasive alien species. *Official Journal of the European Union* **317**: 35–55.

Eusebio Malheiro AC, Jahns P, Hussner A. **2013**. CO_2 availability rather than light and temperature determines growth and phenotypical responses in submerged *Myriophyllum aquaticum*. *Aquatic Botany* **110**: 31–37.

Evans CA, Kelting DL, Forrest KM, Steblen LE. **2011**. Fragment viability and rootlet formation in Eurasian watermilfoil after desiccation. *Journal of Aquatic Plant Management* **49**: 57–62.

F

Fan S, Yu H, Liu C, Yu D, Han Y, Wang L. **2015**. The effects of complete submergence on the morphological and biomass allocation response of the invasive plant *Alternanthera philoxeroides*. *Hydrobiologia* **746**: 159–169.

Fasoli JVB, Mormul RP, Cunha ER, Thomaz SM. **2018**. Plasticity responses of an invasive macrophyte species to inorganic carbon availability and to the interaction with a native species. *Hydrobiologia* **817**: 227–237.

Figuerola J, Green AJ. **2002**. Dispersal of aquatic organisms by waterbirds: A review of past research and priorities for future studies. *Freshwater Biology* **47**: 483–494.

Fleming JP, Dibble ED. **2015**. Ecological mechanisms of invasion success in aquatic macrophytes. *Hydrobiologia* **746**: 23–37.

Franklin P, Dunbar M, Whitehead P. **2008**. Flow controls on lowland river macrophytes: A review. *Science of the Total Environment* **400**: 369–378.

Fritschler N, Hussner A, Busch J. **2008**. Regenerationsfähigkeit von indigenen und neophytischen Wasserpflanzen. In: Deutsche Gesellschaft für Limnologie (DGL)-Tagungsbericht 2007. 199–203.

G

Gałka A, Szmeja J. **2013**. Phenology of the aquatic fern *Salvinia natans* (L.) All. in the Vistula Delta in the context of climate warming. *Limnologica* **43**: 100–105.

Gallardo B, Clavero M, Sánchez MI, Vilà M. **2016**. Global ecological impacts of invasive species in aquatic ecosystems. *Global Change Biology* **22**: 151–163.

Ganie AH, Reshi Z, Wafai BA. **2008**. Multiple reproductive strategies contribute to invasiveness of *Potamogeton crispus* L. (Potamogetonaceae) in freshwater ecosystems of Kashmir Himalaya, India. In: Sengupta M, Dalwani R, eds. Proceedings of Taal 2007: The 12th World Lake Conference. 1067–1073.

Gao Y, Yu X, Liu J, Yang W, Li D, Liu Y. **2007**. Production of adventitious roots and buds on fragments of *Myriophyllum spicatum* L. *Acta Hydrobiologica Sinica* **31**: 730.

Gaston KJ, Jones AG, Hänel C, Chown SL. **2003**. Rates of species introduction to a remote oceanic island. *Proceedings of the Royal Society B: Biological Sciences* **270**: 1091–1098.

Gay PA. **1960**. Ecological studies of *Eichhornia crassipes* Solms. in the Sudan: I. Analysis of spread in the Nile. *Journal of Ecology* **48**: 183–191.

Genovesi P, Carboneras C, Vilà M, Walton P. **2015**. EU adopts innovative legislation on invasive species: A step towards a global response to biological invasions? *Biological Invasions* **17**: 1307–1311.

Grace JB. **1993**. The adaptive significance of clonal reproduction in angiosperms: An aquatic perspective. *Aquatic Botany* **44**: 159–180.

Green AJ. **2016**. The importance of waterbirds as an overlooked pathway of invasion for alien species. *Diversity and Distributions* **22**: 239–247.

Greenfield BK, Siemering GS, Andrews JC, Rajan M, Andrews Jr. SP, Spencer DF. **2007**. Mechanical shredding of water hyacinth (*Eichhornia crassipes*): Effects on water quality in the Sacramento-San Joaquin River Delta, California. *Estuaries and Coasts* **30**: 627–640.

Greulich S, Bornette G. **2003**. Being evergreen in an aquatic habitat with attenuated seasonal contrasts - a major competitive advantage? *Plant Ecology* **167**: 9–18.

Gross EM. **2003**. Allelopathy of aquatic autotrophs. *Critical Reviews in Plant Sciences* **22**: 313–339.

H

Halstead JM, Michaud J, Hallas-Burt S, Gibbs JP. **2003**. Hedonic analysis of effects of a nonnative invader (*Myriophyllum heterophyllum*) on New Hampshire (USA) lakefront properties. *Environmental Management* **32**: 391–398.

Hamann E, Puijalon S. **2013**. Biomechanical responses of aquatic plants to aerial conditions. *Annals of Botany* **112**: 1869–1878.

Heidbüchel P, Hussner A. **2019**. Fragment type and water depth determine the regeneration and colonization success of submerged aquatic macrophytes. *Aquatic Sciences* **81**: 6.

Heidbüchel P, Hussner A. **2020**. Falling into pieces: In situ fragmentation rates of submerged aquatic plants and the influence of discharge in lowland streams. *Aquatic Botany* **160**, 103164.

Heidbüchel P, Jahns P, Hussner A. **2019a**. Chlorophyll fluorometry sheds light on the role of desiccation resistance for vegetative overland dispersal of aquatic plants. *Freshwater Biology* **64**: 1401–1415.

Heidbüchel P, Kuntz K, Hussner A. **2016**. Alien aquatic plants do not have higher fragmentation rates than native species: A field study from the River Erft. *Aquatic Sciences* **78**: 767–777.

Heidbüchel P, Sachs M, Stanik N, Hussner A. **2019b**. Species-specific fragmentation rate and colonization potential partly explain the successful spread of aquatic plants in lowland streams. *Hydrobiologia* **843**: 107–123.

Hoekstra FA, Golovina EA, Buitink J. **2001**. Mechanisms of plant desiccation tolerance. *TRENDS in Plant Science* **6**: 431–438.

Von Holle B, Simberloff D. **2005**. Ecological resistance to biological invasion overwhelmed by propagule pressure. *Ecology* **86**: 3212–3218.

Holm LG, Weldon LW, Blackburn RD. **1969**. Aquatic weeds. *Science* **166**: 699–709.

Horppila J, Nurminen L. **2005**. Effects of different macrophyte growth forms on sediment and P resuspension in a shallow lake. *Hydrobiologia* **545**: 167–175.

Howard-Williams C, Schwarz AM, Reid V. **1996**. Patterns of aquatic weed regrowth following mechanical harvesting in New Zealand hydro-lakes. *Hydrobiologia* **340**: 229–234.

Hulme PE. **2007**. Biological invasions in Europe: Drivers, pressures, states, impacts and responses. *Issues in Environmental Science and Technology* **25**: 56–80.

Hulme PE, Bacher S, Kenis M, Klotz S, Kühn I, Minchin D, Nentwig W, Olenin S, Panov V, Pergl J, *et al.* **2008**. Grasping at the routes of biological invasions: A framework for integrating pathways into policy. *Journal of Applied Ecology* **45**: 403–414.

Hulme PE, Pyšek P, Nentwig W, Vilà M. **2009**. Will threat of biological invasions unite the European Union? *Science* **324**: 40–41.

Hussner A. **2008**. *Ökologische und ökophysiologische Charakteristika aquatischer Neophyten in Nordrhein-Westfalen*. PhD thesis, Heinrich-Heine-University, Düsseldorf, Germany.

Hussner A. **2009**. Growth and photosynthesis of four invasive aquatic plant species in Europe. *Weed Research* **49**: 506–515.

Hussner A. **2012**. Alien aquatic plant species in European countries. *Weed Research* **52**: 297–306.

Hussner A. **2014**. Long-term macrophyte mapping documents a continuously shift from native to non-native aquatic plant dominance in the thermally abnormal River Erft (North Rhine-Westphalia, Germany). *Limnologica* **48**: 39–45.

Hussner A, Heidbüchel P, Heiligtag S. **2014a**. Vegetative overwintering and viable seed production explain the establishment of invasive *Pistia stratiotes* in the thermally abnormal Erft River (North Rhine-Westphalia, Germany). *Aquatic Botany* **119**: 28–32.

Hussner A, Hofstra D, Jahns P, Clayton J. **2015**. Response capacity to CO_2 depletion rather than temperature and light effects explain the growth success of three alien Hydrocharitaceae compared with native *Myriophyllum triphyllum* in New Zealand. *Aquatic Botany* **120**: 205–211.

Hussner A, Jahns P. **2015**. European native *Myriophyllum spicatum* showed a higher HCO_3^- use capacity than alien invasive *Myriophyllum heterophyllum*. *Hydrobiologia* **746**: 171–182.

Hussner A, Krause T. **2007**. Biology of the alien aquatic plant *Myriophyllum heterophyllum* Michaux in urban city lakes of Düsseldorf. *Acta Biologica Benrodis* **14**: 67–75.

Hussner A, Lösch R. **2005**. Alien aquatic plants in a thermally abnormal river and their assembly to neophyte-dominated macrophyte stands (River Erft, Northrhine-Westphalia). *Limnologica* **35**: 18–30.

Hussner A, Mettler-Altmann T, Weber APM, Sand-Jensen K. **2016a**. Acclimation of photosynthesis to supersaturated CO_2 in aquatic plant bicarbonate users. *Freshwater Biology* **61**: 1720–1732.

Hussner A, Nehring S, Hilt S. **2014b**. From first reports to successful control: A plea for improved management of alien aquatic plant species in Germany. *Hydrobiologia* **737**: 321–331.

Hussner A, Stiers I, Verhofstad MJJM, Bakker ES, Grutters BMC, Haury J, van Valkenburg JLCH, Brundu G, Newman J, Clayton JS, *et al.* 2017. Management and control methods of invasive alien freshwater aquatic plants: A review. *Aquatic Botany* **136**: 112–137.

Hussner A, Windhaus M, Starfinger U. **2016b**. From weed biology to successful control: An example of successful management of *Ludwigia grandiflora* in Germany. *Weed Research* **56**: 434–441.

I

–

J

Jacobs MJ, MacIsaac HJ. **2009**. Modelling spread of the invasive macrophyte *Cabomba caroliniana*. *Freshwater Biology* **54**: 296–305.

James CS, Eaton JW, Hardwick K. **1999**. Competition between three submerged macrophytes, *Elodea canadensis* Michx, *Elodea nuttallii* (Planch.) St John and *Lagarosiphon major* (Ridl.) Moss. *Hydrobiologia* **415**: 35–40.

Jeppesen E, Søndergaard M, Søndergaard M, Christoffersen K (Eds). **1998**. *The structuring role of submerged macrophytes in lakes*. New York: Springer.

Jerde CL, Barnes MA, DeBuysser EK, Noveroske A, Chadderton WL, Lodge DM. **2012**. Eurasian watermilfoil fitness loss and invasion potential following desiccation during simulated overland transport. *Aquatic Invasions* **7**: 135–142.

Jiang J, An S, Zhou C, Guan B, Sun Z, Cai Y, Liu F. **2009**. Fragment propagation and colonization ability enhanced and varied at node level after escaping from apical dominance in submerged macrophytes. *Journal of Integrative Plant Biology* **51**: 308–315.

JianMin M, LingWei H, QianRu H, YongJing Z, Ping J, TongXia J, HuiYing M. **2010**. Regeneration of fragments of *Elodea nuttallii* and *Hydrilla verticillata*. *Acta Hydrobiologica Sinica* **34**: 525–532.

Johansson ME, Nilsson C. **1993**. Hydrochory, population dynamics and distribution of the clonal aquatic plant *Ranunculus lingua*. *Journal of Ecology* **81**: 81–91.

Johansson ME, Nilsson C, Nilsson E. **1996**. Do rivers function as corridors for plant dispersal? *Journal of Vegetation Science* **7**: 593–598.

Johnson LE, Ricciardi A, Carlton JT. **2001**. Overland dispersal of aquatic invasive species: A risk assessment of transient recreational boating. *Ecological Applications* **11**: 1789–1799.

Johnstone IM, Coffey BT, Howard-Williams C. **1985**. The role of recreational boat traffic in interlake dispersal of macrophytes: A New Zealand case study. *Journal of Environmental Management* **20**: 263–279.

Jones PE, Consuegra S, Börger L, Jones J, Garcia de Leaniz C. Impacts of artificial barriers on the connectivity and dispersal of vascular macrophytes in rivers: A critical review. *Freshwater Biology*, in press. DOI: 10.1111/fwb.13493

Josefsson M. **2011**. NOBANIS - invasive alien species fact sheet – *Elodea canadensis*, *Elodea nuttallii* and *Elodea callitrichoides*. From: Online database of the European Network on Invasive Alien Species - NOBANIS. www.nobanis.org [accessed 10.01.2020]

K

Katsanevakis S, Deriu I, D'Amico F, Nunes AL, Sanchez SP, Crocetta F, Arianoutsou M, Bazos I, Christopoulou A, Curto G, *et al.* 2015. European alien species information network (EASIN): Supporting European policies and scientific research. *Management of Biological Invasions* **6**: 147–157.

Kettunen M, Genovesi P, Gollasch S, Pagad S, Starfinger U, ten Brink P, Shine C. **2008**. *Technical support to EU strategy on invasive species (IAS) - Assessment of the impacts of IAS in Europe and the EU (final module report for the European Commission)*. Brussels.

Kim JY, Yano T, Nakanishi R, Tagami H, Nishihiro J. **2019**. Artificial wave breakers promote the establishment of alien aquatic plants in a shallow lake. *Biological Invasions* **21**: 1545–1556.

van Kleunen M, Richardson DM. **2007**. Invasion biology and conservation biology: Time to join forces to explore the links between species traits and extinction risk and invasiveness. *Progress in Physical Geography* **31**: 447–450.

Kolar CS, Lodge DM. **2001**. Progress in invasion biology: Predicting invaders. *Trends in Ecology & Evolution* **16**: 199–-204.

Kranner I, Birtić S. **2005**. A modulating role for antioxidants in desiccation tolerance. *Integrative and Comparative Biology* **45**: 734–740.

Kuehne LM, Olden JD, Rubenson ES. **2016**. Multi-trophic impacts of an invasive aquatic plant. *Freshwater Biology* **61**: 1846–1861.

Kuntz K, Heidbüchel P, Hussner A. **2014**. Effects of water nutrients on regeneration capacity of submerged aquatic plant fragments. *Annales de Limnologie - International Journal of Limnology* **50**: 155–162.

L

Lambert SJ, Davy AJ. **2011**. Water quality as a threat to aquatic plants: Discriminating between the effects of nitrate, phosphate, boron and heavy metals on charophytes. *New Phytologist* **189**: 1051–1059.

Langeland KA. **1996**. *Hydrilla verticillata* (L.F.) Royle (Hydrocharitaceae), "the perfect aquatic weed". *Castanea* **61**: 293–304.

Langeland KA, Sutton DL. **1980**. Regrowth of *Hydrilla* from axillary buds. *Journal of Aquatic Plant Management* **18**: 27–29.

Lemon GD, Posluszny U. **1997**. Shoot morphology and organogenesis of the aquatic floating fern *Salvinia molesta* D.S. Mitchell, examined with the aid of laser scanning confocal microscopy. *International Journal of Plant Sciences* **158**: 693–703.

Levine JM. **2000**. Species diversity and biological invasions: Relating local process to community pattern. *Science* **288**: 852–854.

Levine JM, Adler PB, Yelenik SG. **2004**. A meta-analysis of biotic resistance to exotic plant invasions. *Ecology Letters* **7**: 975–989.

Levine JM, D'Antonio CM. **2010**. Elton revisited: A review of evidence linking diversity and invasibility. *Oikos* **87**: 15–26.

Li F, Qin Y, Zhu L, Xie Y, Liang S, Hu C, Chen X, Deng Z. **2016a**. Effects of fragment size and sediment heterogeneity on the colonization and growth of *Myriophyllum spicatum*. *Ecological Engineering* **95**: 457–462.

Li HL, Wang YY, Zhang Q, Wang P, Zhang MX, Yu FH. **2015a**. Vegetative propagule pressure and water depth affect biomass and evenness of submerged macrophyte communities. *PLoS ONE* **10**: 1–12.

Li F, Zhu L, Xie Y, Jiang L, Chen X, Deng Z, Pan B. **2015b**. Colonization by fragments of the submerged macrophyte *Myriophyllum spicatum* under different sediment type and density conditions. *Scientific Reports* **5**: 1–9.

Li F, Zhu L, Xie Y, Liang S, Hu C, Chen X, Deng Z. **2016b**. Fragment growth performance of the invasive submerged macrophyte *Myriophyllum spicatum* under conditions of different water depths and sediment types. *Aquatic Ecology* **50**: 727–734.

Liffen T, Gurnell AM, O'Hare MT, Pollen-Bankhead N, Simon A. **2011**. Biomechanical properties of the emergent aquatic macrophyte *Sparganium erectum*: Implications for fine sediment retention in low energy rivers. *Ecological Engineering* **37**: 1925–1931.

Łoboda AM, Bialik RJ, Karpiński M, Przyborowski Ł. **2019**. Two simultaneously occurring *Potamogeton* species: Similarities and differences in seasonal changes of biomechanical properties. *Polish Journal of Environmental Studies* **28**: 1–16.

Lockwood JL, Cassey P, Blackburn T. **2005**. The role of propagule pressure in explaining species invasions. *Trends in Ecology & Evolution* **20**: 223–228.

Lockwood JL, Cassey P, Blackburn TM. **2009**. The more you introduce the more you get: The role of colonization pressure and propagule pressure in invasion ecology. *Diversity and Distributions* **15**: 904–910.

Lodge DM. **1993**. Biological invasions: Lessons for ecology. *Trends in Ecology & Evolution* **8**: 133–137.

Lonsdale WM. **1999**. Global patterns of plant invasions and the concept of invasibility. *Ecology* **80**: 1522–1536.

Louback-Franco N, Dainez-Filho MS, Souza DC, Thomaz SM. **2019**. A native species does not prevent the colonization success of an introduced submerged macrophyte, even at low propagule pressure. *Hydrobiologia*, DOI: 10.1007/s10750-019-04116-w.

Lovas-Kiss Á, Sánchez MI, Wilkinson DM, Coughlan NE, Alves JA, Green AJ. **2019**. Shorebirds as important vectors for plant dispersal in Europe. *Ecography* **42**: 956–967.

Lovas-Kiss Á, Vizi B, Vincze O, Molnár V. A, Green AJ. **2018**. Endozoochory of aquatic ferns and angiosperms by mallards in Central Europe. *Journal of Ecology* **106**: 1714–1723.

M

Mack RN, Simberloff D, Lonsdale WM, Evans H, Clout M, Bazzaz FA. **2000**. Biotic invasions: Causes, epidemiology, global consequences, and control. *Ecological Applications* **10**: 689–710.

Madsen TV, Sand-Jensen K. **1991**. Photosynthetic carbon assimilation in aquatic macrophytes. *Aquatic Botany* **41**: 5–40.

Madsen JD, Smith DH. **1997**. Vegetative spread of eurasian watermilfoil colonies. *Journal of Aquatic Plant Management* **35**: 63–68.

Maki K, Galatowitsch S. **2004**. Movement of invasive aquatic plants into Minnesota (USA) through horticultural trade. *Biological Conservation* **118**: 389–396.

Matthews J, Beringen R, Lamers LPM, Odé B, Pot R, Van Der Velde G, Van Valkenburg JLCH, Verbrugge LNH, Leuven RSEW. **2013**. Risk analysis of the non-native fanwort (*Cabomba caroliniana*) in the Netherlands. *Reports Environmental Science*: 442.

Mazza G, Aquiloni L, Inghilesi AF, Giuliani C, Lazzaro L, Ferretti G, Lastrucci L, Foggi B, Tricarico E. **2015**. Aliens just a click away: The online aquarium trade in Italy. *Management of Biological Invasions* **6**: 253–261.

McAlarnen LA, Barnes MA, Jerde CL, Lodge DM. **2012**. Simulated overland transport of Eurasian watermilfoil: Survival of desiccated plant fragments. *Journal of Aquatic Plant Management* **50**: 147–149.

Meyerson LA, Pyšek P. **2013**. Manipulating alien plant species propagule pressure as a prevention strategy for protected areas. In: Foxcroft LC, Pyšek P, Richardson DM, Genovesi P, eds. Plant invasions in protected areas: Patterns, problems and challenges. Dordrecht: Springer, 473–486.

Mielecki M, Pieczyńska E. **2005**. The influence of fragmentation on the growth of *Elodea canadensis* Michx. in different light conditions. *Polish Journal of Ecology* **53**: 155–164.

Miler O, Albayrak I, Nikora V, O'Hare M. **2012**. Biomechanical properties of aquatic plants and their effects on plant – flow interactions in streams and rivers. *Aquatic Sciences* **74**: 31–44.

Miler O, Albayrak I, Nikora V, O'Hare M. **2014**. Biomechanical properties and morphological characteristics of lake and river plants: Implications for adaptations to flow conditions. *Aquatic Sciences* **76**: 465–481.

Millennium Ecosystem Assessment. **2005**. *Ecosystems and human well-being*. Washington, D.C.: Island Press.

Mills EL, Leach JH, Carlton JT, Secor CL. **1993**. Exotic species in the Great Lakes: A history of biotic crises and anthropogenic introductions. *Journal of Great Lakes Research* **19**: 1–54.

Moorhouse TP, Macdonald DW. **2015**. Are invasives worse in freshwater than terrestrial ecosystems? *Wiley Interdisciplinary Reviews: Water* **2**: 1–8.

Murphy K, Efremov A, Davidson TA, Molina-Navarro E, Fidanza K, Crivelari Betiol TC, Chambers P, Tapia Grimaldo J, Varandas Martins S, Springuel I, *et al.* 2019. World distribution, diversity and endemism of aquatic macrophytes. *Aquatic Botany* **158**: 103127.

Muthukrishnan R, Hansel-Welch N, Larkin DJ. **2018**. Environmental filtering and competitive exclusion drive biodiversity-invasibility relationships in shallow lake plant communities. *Journal of Ecology* **106**: 2058–2070.

N

Nayyar H, Gupta D. **2006**. Differential sensitivity of C3 and C4 plants to water deficit stress: Association with oxidative stress and antioxidants. *Environmental and Experimental Botany* **58**: 106–113.

Netten JJC, van Zuidam J, Kosten S, Peeters ETHM. **2011**. Differential response to climatic variation of free-floating and submerged macrophytes in ditches. *Freshwater Biology* **56**: 1761–1768.

Neuenschwander P, Julien MH, Center TD, Hill MP. **2009**. *Pistia stratiotes* L. (Araceae). In: Muniappan R, Reddy GVP, Raman A, eds. Biological control of tropical weeds using arthropods. Cambridge University Press, 825–872.

Newman JR. **2010**. EUPHRESCO-DeCLAIM – a decision support system for management of invasive aquatic macrophytes. *Aspects of Applied Biology* **104**: 19–20.

Nichols SA, Shaw BH. **1986**. Ecological life histories of the three aquatic nuisance plants, *Myriophyllum spicatum*, *Potamogeton crispus* and *Elodea canadensis*. *Hydrobiologia* **131**: 3–21.

Nilsson C, Brown RL, Jansson R, Merritt DM. **2010**. The role of hydrochory in structuring riparian and wetland vegetation. *Biological Reviews* **85**: 837–858.

O

Okada M, Grewell BJ, Jasieniuk M. **2009**. Clonal spread of invasive *Ludwigia hexapetala* and *L. grandiflora* in freshwater wetlands of California. *Aquatic Botany* **91**: 123–129.

van Oosterhout E. **2007**. Alligator weed control manual. *New South Wales Department of Primary Industries*: 90.

Orchard A. **1981**. A revision of South American *Myriophyllum* (Haloragaceae) and its repercussions on some Australian and North American species. *Brunonia* **4**: 27–65.

Oreska MPJ, Aldridge DC. **2011**. Estimating the financial costs of freshwater invasive species in Great Britain: A standardized approach to invasive species costing. *Biological Invasions* **13**: 305–319.

Owens CS, Madsen JD, Smart RM, Steward RM. **2001**. Dispersal of native and nonnative aquatic plant species in the San Marcos River, Texas. *Journal of Aquatic Plant Management* **39**: 75–79.

Owens CS, Smart RM, Dick GO. **2004**. Regeneration of giant salvinia from apical and axillary buds following desiccation or physical damage. *Journal of Aquatic Plant Management* **42**: 117–119.

P

Padilla DK, Williams SL. **2004**. Beyond ballast water: Aquarium and ornamental trades as sources of invasive species in aquatic ecosystems. *Frontiers in Ecology and the Environment* **2**: 131–138.

Panetta FD. **2010**. Seed persistence of the invasive aquatic plant, *Gymnocoronis spilanthoides* (Asteraceae). *Australian Journal of Botany* **57**: 670–674.

Panetta FD, Cacho OJ. **2014**. Designing weed containment strategies: An approach based on feasibilities of eradication and containment. *Diversity and Distributions* **20**: 555–566.

Pedersen O, Colmer TD, Sand-Jensen K. **2013**. Underwater photosynthesis of submerged plants – recent advances and methods. *Frontiers in Plant Science* **4**: 1–19.

Pelicice FM, Agostinho AA, Thomaz SM. **2005**. Fish assemblages associated with *Egeria* in a tropical reservoir: Investigating the effects of plant biomass and diel period. *Acta Oecologica* **27**: 9–16.

Petruzzella A, van Leeuwen CHA, van Donk E, Bakker ES. Direct and indirect effects of native plants and herbivores on biotic resistance to alien aquatic plant invasions. *Journal of Ecology*, in press. DOI: 10.1111/1365-2745.13380

Petruzzella A, Manschot J, Van Leeuwen CHA, Grutters BMC, Bakker ES. **2018**. Mechanisms of invasion resistance of aquatic plant communities. *Frontiers in Plant Science* **9**: 134.

Pimentel D, McNair S, Janecka J, Wightman J, Simmonds C, O'Connell C, Wong E, Russel L, Zern J, Aquino T, *et al.* 2001. Economic and environmental threats of alien plant, animal, and microbe invasions. *Agriculture, Ecosystems and Environment* **84**: 1–20.

Pimentel D, Zuniga R, Morrison D. **2005**. Update on the environmental and economic costs associated with alien-invasive species in the United States. *Ecological Economics* **52**: 273–288.

Pollen-Bankhead N, Thomas RE, Gurnell AM, Liffen T, Simon A, O'Hare MT. **2011**. Quantifying the potential for flow to remove the emergent aquatic macrophyte *Sparganium erectum* from the margins of low-energy rivers. *Ecological Engineering* **37**: 1779–1788.

Puijalon S, Bornette G, Sagnes P, I CBL. **2005**. Adaptations to increasing hydraulic stress: Morphology, hydrodynamics and fitness of two higher aquatic plant species. *Journal of Experimental Botany* **56**: 777–786.

Puijalon S, Bouma TJ, Douady CJ, van Groenendael J, Anten NPR, Martel E, Bornette G. **2011**. Plant resistance to mechanical stress: Evidence of an avoidance-tolerance trade-off. *New Phytologist* **191**: 1141–1149.

Puijalon S, Léna J-P, Rivière N, Champagne J-Y, Rostan J-C, Bornette G. **2008**. Phenotypic plasticity in response to mechanical stress: Hydrodynamic performance and fitness of four aquatic plant species. *New Phytologist* **177**: 907–917.

Pulzatto MM, Cunha ER, Dainez-Filho MS, Thomaz SM. **2019**. Association between the success of an invasive macrophyte, environmental variables and abundance of a competing native macrophyte. *Frontiers in Plant Science* **10**: 1–11.

Pyšek P, Jarošík V, Hulme PE, Pergl J, Hejda M, Schaffner U, Vilà M. **2012**. A global assessment of invasive plant impacts on resident species, communities and ecosystems:

The interaction of impact measures, invading species' traits and environment. *Global Change Biology* **18**: 1725–1737.

Pyšek P, Jarošík V, Pergl J, Randall R, Chytrý M, Kühn I, Tichý L, Danihelka J, Chrtek Jun J, Sádlo J. **2009**. The global invasion success of Central European plants is related to distribution characteristics in their native range and species traits. *Diversity and Distributions* **15**: 891–903.

Pyšek P, Richardson DM. **2008**. Traits associated with invasiveness in alien plants: Where do we stand? In: Nentwig W, ed. Biological invasions. Ecological studies (analysis and synthesis). Berlin, Heidelberg: Springer, 97–125.

Pyšek P, Richardson DM. **2010**. Invasive species, environmental change and management, and health. *Annual Review of Environment and Resources* **35**: 25–55.

Pyšek P, Richardson DM, Rejmánek M, Webster GL, Williamson M, Kirschner J. **2004**. Alien plants in checklists and floras: Towards better communication between taxonomists and ecologists. *Taxon* **53**: 131–143.

Q

–

R

Redekop P, Hofstra D, Hussner A. **2016**. *Elodea canadensis* shows a higher dispersal capacity via fragmentation than *Egeria densa* and *Lagarosiphon major*. *Aquatic Botany* **130**: 45–49.

Rejmanek M, Richardson DM. 1996. What attributes make some plant species more invasive? *Ecology* **77**: 1655–1661.

Rennie MD, Jackson LJ. **2005**. The influence of habitat complexity on littoral invertebrate distributions: Patterns differ in shallow prairie lakes with and without fish. *Canadian Journal of Fisheries and Aquatic Sciences* **62**: 2088–2099.

Richards CL, Bossdorf O, Muth NZ, Gurevitch J, Pigliucci M. **2006**. Jack of all trades, master of some? On the role of phenotypic plasticity in plant invasions. *Ecology Letters* **9**: 981–993.

Richardson DM, Pyšek P. **2006**. Plant invasions: Merging the concepts of species invasiveness and community invasibility. *Progress in Physical Geography* **30**: 409–431.

Ricketts JH, Head GA. **1999**. A five-parameter logistic equation for investigating asymmetry of curvature in baroreflex studies. *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology* **277**: 441–454.

Riede W. **1920**. Untersuchungen über Wasserpflanzen. *Flora oder Allgemeine Botanische Zeitung* **114**: 1–118.

Riis T. **2008**. Dispersal and colonisation of plants in lowland streams: Success rates and bottlenecks. *Hydrobiologia* **596**: 341–351.

Riis T, Biggs BJF. **2003**. Hydrologic and hydraulic control of macrophyte establishment and performance in streams. *Limnology and Oceanography* **48**: 1488–1497.

Riis T, Lambertini C, Olesen B, Clayton JS, Brix H, Sorrell BK. **2010**. Invasion strategies in clonal aquatic plants: Are phenotypic differences caused by phenotypic plasticity or local adaptation? *Annals of Botany* **106**: 813–822.

Riis T, Madsen T V., Sennels RSH. **2009**. Regeneration, colonisation and growth rates of allofragments in four common stream plants. *Aquatic Botany* **90**: 209–212.

Riis T, Olesen B, Clayton JS, Lambertini C, Brix H, Sorrell BK. **2012**. Growth and morphology in relation to temperature and light availability during the establishment of three invasive aquatic plant species. *Aquatic Botany* **102**: 56–64.

Riis T, Sand-Jensen K. **2006**. Dispersal of plant fragments in small streams. *Freshwater Biology* **51**: 274–286.

Robert H, Lafontaine R-M, Beudels-Jamar RC, Delsinne T. **2013**. Risk analysis of the water primrose *Ludwigia peploides* (Kunth) P.H. Raven. - risk analysis report of non-native organisms in Belgium. *Royal Belgian Institute of Natural Sciences for the Federal Public Service Health, Food chain safety and Environment*: 35.

Rockström J, Steffen W, Noone K, Persson Å, Chapin FS, Lambin EF, Lenton TM, Scheffer M, Folke C, Schellnhuber HJ, *et al.* **2009**. A safe operation space for humanity. *Nature* **461**: 472–475.

Room PM. **1983**. ‘Falling apart’ as a lifestyle: The rhizome architecture and population growth of *Salvinia molesta*. *The Journal of Ecology* **71**: 349.

Rothlisberger JD, Chadderton WL, McNulty J, Lodge DM. **2010**. Aquatic invasive species transport via trailered boats: What is being moved, who is moving it, and what can be done. *Fisheries* **35**: 121–132.

Ruaux B, Greulich S, Haury J, Berton JP. **2009**. Sexual reproduction of two alien invasive *Ludwigia* (Onagraceae) on the middle Loire River, France. *Aquatic Botany* **90**: 143–148.

Rybicki NB, McFarland DG, Ruhl HA, Reel JT, Barko JW. **2001**. Investigations of the availability and survival of submersed aquatic vegetation propagules in the tidal Potomac River. *Estuaries* **24**: 407–424.

S

Sabbatini MR, Murphy KJ. **1996**. Submerged plant survival strategies in relation to management and environmental pressures in drainage channel habitats. *Hydrobiologia* **340**: 191–195.

Sala OE, Chapin FS, Armesto JJ, Berlow E, Bloomfield J, Dirzo R, Huber-Sanwald E, Huenneke LF, Jackson RB, Kinzig A, *et al.* **2000**. Global biodiversity scenarios for the year 2100. *Science* **287**: 1770–1774.

Sand-Jensen K. **2003**. Drag and reconfiguration of freshwater macrophytes. *Freshwater Biology* **48**: 271–283.

Sand-Jensen K. **2008**. Drag forces on common plant species in temperate streams: Consequences of morphology, velocity and biomass. *Hydrobiologia* **610**: 307–319.

Sand-Jensen K, Andersen K, Andersen T. **1999**. Dynamic properties of recruitment, expansion and mortality of macrophyte patches in streams. *International Review of Hydrobiology* **84**: 497–508.

Sand-Jensen K, Frost-Christensen H. **1999**. Plant growth and photosynthesis in the transition zone between land and stream. *Aquatic Botany* **63**: 23–35.

Sand-Jensen K, Møller CL. **2014**. Reduced root anchorage of freshwater plants in sandy sediments enriched with fine organic matter. *Freshwater Biology* **59**: 427–437.

Sand-Jensen K, Jeppesen E, Nielsen K, van der Bijl L, Hjermind L, Nielsen LW, Iversen TM. **1989**. Growth of macrophytes and ecosystem consequences in a lowland Danish stream. *Freshwater Biology* **22**: 15–32.

Santamaría L. **2002**. Why are most aquatic plants widely distributed? Dispersal, clonal growth and small-scale heterogeneity in a stressful environment. *Acta Oecologica* **23**: 137–154.

Santos MJ, Anderson LW, Ustin SL. **2011**. Effects of invasive species on plant communities: An example using submersed aquatic plants at the regional scale. *Biological Invasions* **13**: 443–457.

Sarneel JM. **2013**. The dispersal capacity of vegetative propagules of riparian fen species. *Hydrobiologia* **710**: 219–225.

Sastroutomo SS. **1981**. Turion formation, dormancy and germination of curly pondweed, *Potamogeton crispus* L. *Aquatic Botany* **10**: 161–173.

Scheers K, Denys L, Jacobs I, Packet J, Smeekens V, Adriaens T. **2019**. *Cabomba caroliniana* Gray (Cabombaceae) invades major waterways in Belgium. *Knowledge & Management of Aquatic Ecosystems* **420**: 22.

Schoelynck J, Puijalon S, Meire P, Struyf E. **2015**. Thigmomorphogenetic responses of an aquatic macrophyte to hydrodynamic stress. *Frontiers in Plant Science* **6**: 43.

Schutten J, Dainty J, Davy AJ. **2005**. Root anchorage and its significance for submerged plants in shallow lakes. *Journal of Ecology* **93**: 556–571.

Sculthorpe CD. **1967**. *The biology of aquatic vascular plants.* London: Arnold.

Shea K, Chesson P. **2002**. Community ecology theory as a framework for biological invasions. *Trends in Ecology & Evolution* **17**: 170–176.

Silveira MJ, Thomaz SM, Mormul RP, Camacho FP. **2009**. Effects of desiccation and sediment type on early regeneration of plant fragments of three species of aquatic macrophytes. *International Review of Hydrobiology* **94**: 169–178.

Simberloff D. **2009**. The role of propagule pressure in biological invasions. *Annual Review of Ecology, Evolution, and Systematics* **40**: 81–102.

Skaer Thomason MJ, McCort CD, Netherland MD, Grewell BJ. **2018**. Temporal and nonlinear dispersal patterns of *Ludwigia hexapetala* in a regulated river. *Wetlands Ecology and Management*: 1–12.

Smart RM, Barko JW. **1985**. Laboratory culture of submersed freshwater macrophytes on natural sediments. *Aquatic Botany* **21**: 251–263.

Smirnoff N. **1993**. The role of active oxygen in the response of plants to water deficit and desiccation. *New Phytologist* **125**: 27–58.

Stafford-Bell RE, Chariton AA, Robinson RW. 2015. Prolonged buoyancy and viability of *Zostera muelleri* Irmisch ex Asch. vegetative fragments indicate a strong dispersal potential. *Journal of Experimental Marine Biology and Ecology* **464**: 52–57.

Steen B, Cardoso AC, Tsiamis K, Nieto K, Engel J, Gervasini E. **2019**. Modelling hot spot areas for the invasive alien plant *Elodea nuttallii* in the EU. *Management of Biological Invasions* **10**: 151–170.

Steffen W, Broadgate W, Deutsch L, Gaffney O, Ludwig C. **2015a**. The trajectory of the Anthropocene: The Great Acceleration. *Anthropocene Review* **2**: 81–98.

Steffen W, Crutzen PJ, McNeill JR. **2007**. The Anthropocene: Are humans now overwhelming the great forces of nature? *Ambio* **36**: 614–621.

Steffen W, Richardson K, Rockström J, Cornell SE, Fetzer I, Bennett EM, Biggs R, Carpenter SR, De Vries W, De Wit CA, *et al.* 2015b. Planetary boundaries: Guiding human development on a changing planet. *Science* **347**.

Stiers I, Crohain N, Josens G, Triest L. 2011a. Impact of three aquatic invasive species on native plants and macroinvertebrates in temperate ponds. *Biological Invasions* **13**: 2715–2726.

Stiers I, Njambuya J, Triest L. 2011b. Competitive abilities of invasive *Lagarosiphon major* and native *Ceratophyllum demersum* in monocultures and mixed cultures in relation to experimental sediment dredging. *Aquatic Botany* **95**: 161–166.

Stiers I, Triest L. 2017. Impact of non-native invasive plant species cover on phytoplankton and zooplankton communities in temperate ponds. *Aquatic Invasions* **12**: 385–395.

Strayer DL. 2010. Alien species in fresh waters: Ecological effects, interactions with other stressors, and prospects for the future. *Freshwater Biology* **55**: 152–174.

Strayer DL, Dudgeon D. 2010. Freshwater biodiversity conservation: Recent progress and future challenges. *Journal of the North American Benthological Society* **29**: 344–358.

Suzuki JI, Stuefer JF. 1999. On the ecological and evolutionary significance of storage in clonal plants. *Plant Species Biology* **14**: 11–17.

Szabó S, Peeters ETHM, Várbíró G, Borics G, Lukács BA. 2019. Phenotypic plasticity as a clue for invasion success of the submerged aquatic plant *Elodea nuttallii*. *Plant Biology* **21**: 54–63.

T

Thiébaut G, Dutartre A. 2009. Management of invasive aquatic plants in France. *Aquatic Ecosystem Research Trends*: 25–46.

Thiébaut G, Gillard M, Deleu C. 2016. Growth, regeneration and colonisation of *Egeria densa* fragments: The effect of autumn temperature increases. *Aquatic Ecology* **50**: 175–185.

Thiébaut G, Martinez L. 2015. An exotic macrophyte bed may facilitate the anchorage of exotic propagules during the first stage of invasion. *Hydrobiologia* **746**: 183–196.

Thomaz SM, Mormul RP, Michelan TS. 2015. Propagule pressure, invasibility of freshwater ecosystems by macrophytes and their ecological impacts: A review of tropical freshwater ecosystems. *Hydrobiologia* **746**: 39–59.

Thouvenot L, Haury J, Thiébaut G. 2013. A success story: Water primroses, aquatic plant pests. *Aquatic Conservation: Marine and Freshwater Ecosystems* **803**: 790–803.

Thullen JS. **1990**. Production of axillary turions by the dioecious *Hydrilla verticillata*. *Journal of Aquatic Plant Management* **28**: 11–14.

Titus JE, Hoover DT. **1991**. Toward predicting reproductive success in submerged freshwater angiosperms. *Aquatic Botany* **41**: 111–136.

Truscott AM, Soulsby C, Palmer SCF, Newell L, Hulme PE. **2006**. The dispersal characteristics of the invasive plant *Mimulus guttatus* and the ecological significance of increased occurrence of high-flow events. *Journal of Ecology* **94**: 1080–1091.

Tsiamis K, Gervasini E, Deriu I, D'Amico F, Nunes A, Addamo A, Cardoso A. **2017**. *Baseline distribution of invasive alien species of Union concern*. Ispra: Publications Office of the European Union.

U

Umetsu CA, Evangelista HBA, Thomaz SM. **2012a**. The colonization, regeneration, and growth rates of macrophytes from fragments: A comparison between exotic and native submerged aquatic species. *Aquatic Ecology* **46**: 443–449.

Umetsu CA, Evangelista HBA, Thomaz SM. **2012b**. Colonization, regeneration potential and growth rates of fragments of the exotic aquatic macrophyte *Hydrilla verticillata*. *Aquatic Biology* **16**: 197–202.

Urban RA, Titus JE, Zhu WX. **2006**. An invasive macrophyte alters sediment chemistry due to suppression of a native isoetid. *Oecologia* **148**: 455–463.

V

Valéry L, Fritz H, Lefeuvre JC, Simberloff D. **2008**. In search of a real definition of the biological invasion phenomenon itself. *Biological Invasions* **10**: 1345–1351.

Valéry L, Fritz H, Lefeuvre JC, Simberloff D. **2009**. Invasive species can also be native... *Trends in Ecology & Evolution* **24**: 585.

van Valkenburg J, Rotteveel T. **2010**. *Cabomba caroliniana* Gray, a subtropical surprise at Loosdrecht. *Gorteria* **34**: 106–118.

Vári Á. **2013**. Colonisation by fragments in six common aquatic macrophyte species. *Fundamental and Applied Limnology* **183**: 15–26.

Verhofstad MJJM, Bakker ES. **2019**. Classifying nuisance submerged vegetation depending on ecosystem services. *Limnology* **20**: 55–68.

Vilà M, Basnou C, Pyšek P, Josefsson M, Genovesi P, Gollasch S, Nentwig W, Olenin S, Roques A, Roy D, *et al.* **2010**. How well do we understand the impacts of alien species on ecosystem services? A pan-European, cross-taxa assessment. *Frontiers in Ecology and the Environment* **8**: 135–144.

Vilà M, Espinar JL, Hejda M, Hulme PE, Jarošík V, Maron JL, Pergl J, Schaffner U, Sun Y, Pyšek P. **2011**. Ecological impacts of invasive alien plants: A meta-analysis of their effects on species, communities and ecosystems. *Ecology Letters* **14**: 702–708.

Villamagna AM, Murphy BR. **2010**. Ecological and socio-economic impacts of invasive water hyacinth (*Eichhornia crassipes*): A review. *Freshwater Biology* **55**: 282–298.

Vitousek PM, D'Antonio CM, Loope LL, Rejmánek M, Westbrooks R. **1997**. Introduced species: A significant component of human-caused global change. *New Zealand Journal of Ecology* **21**: 1–16.

Vitousek PM, D'Antonio CM, Loope LL, Westbrooks R. **1996**. Biological invasions as global environmental change. *American Scientist* **84**: 468–478.

W

Wang P, Alpert P, Yu FH. **2016a**. Clonal integration increases relative competitive ability in an invasive aquatic plant. *American Journal of Botany* **103**: 2079–2086.

Wang S, Jin X, Jiao L, Wu F. **2009**. Response in root morphology and nutrient contents of *Myriophyllum spicatum* to sediment type. *Ecological Engineering* **35**: 1264–1270.

Wang M-Z, Liu Z-Y, Luo F-L, Lei G-C, Li H-L. **2016b**. Do amplitudes of water level fluctuations affect the growth and community structure of submerged macrophytes? *Plos One* **11**: e0146528.

Wang Y-J, Müller-Schärer H, van Kleunen M, Cai A-M, Zhang P, Yan R, Dong B-C, Yu F-H. **2017**. Invasive alien plants benefit more from clonal integration in heterogeneous environments than natives. *New Phytologist* **216**: 1072–1078.

Wersal RM, Madsen JD. **2011**. Comparative effects of water level variations on growth characteristics of *Myriophyllum aquaticum*. *Weed Research* **51**: 386–393.

Westphal MI, Browne M, MacKinnon K, Noble I. **2008**. The link between international trade and the global distribution of invasive alien species. *Biological Invasions* **10**: 391–398.

van Wijck C, de Groot CJ. **1993**. The impact of desiccation of a freshwater marsh (Garcines Nord, Camargue, France) on sediment-water-vegetation interactions - Part 2: The submerged macrophyte vegetation. *Hydrobiologia* **252**: 95–103.

van Wijk RJ. **1989**. Ecological studies on *Potamogeton pectinatus* L. III. Reproductive strategies and germination ecology. *Aquatic Botany* **33**: 271–299.

Williamson M. **1993**. Invaders, weeds and the risk from genetically manipulated organisms. *Experientia* **49**: 219–224.

Williamson M, Fitter A. **1996**. The varying success of invaders. *Ecology* **77**: 1661–1666.

Wilson JRU, Dormontt EE, Prentis PJ, Lowe AJ, Richardson DM. **2009**. Something in the way you move: Dispersal pathways affect invasion success. *Trends in Ecology & Evolution* **24**: 136–144.

X

Xiao K, Yu D, Wang L, Han Y. **2011**. Physiological integration helps a clonal macrophyte spread into competitive environments and coexist with other species. *Aquatic Botany* **95**: 249–253.

Xiao K, Yu D, Xu X, Xiong W. **2007**. Benefits of clonal integration between interconnected ramets of *Vallisneria spiralis* in heterogeneous light environments. *Aquatic Botany* **86**: 76–82.

Xie D, Hu Y, Mormul RP, Ruan H, Feng Y, Zhang M. **2018**. Fragment type and water nutrient interact and affect the survival and establishment of *Myriophyllum aquaticum*. *Hydrobiologia* **815**: 205–213.

Xie D, Yu D, Yu LF, Liu CH. **2010**. Asexual propagations of introduced exotic macrophytes *Elodea nuttallii*, *Myriophyllum aquaticum*, and *M. propinquum* are improved by nutrient-rich sediments in China. *Hydrobiologia* **655**: 37–47.

Y

Yin L, Li W, Madsen T V., Maberly SC, Bowes G. **2017**. Photosynthetic inorganic carbon acquisition in 30 freshwater macrophytes. *Aquatic Botany* **140**: 48–54.

You WH, Han CM, Fang LX, Du DL. **2016a**. Propagule pressure, habitat conditions and clonal integration influence the establishment and growth of an invasive clonal plant, *Alternanthera philoxeroides*. *Frontiers in Plant Science* **7**: 1–11.

You WH, Han CM, Liu CH, Yu D. **2016b**. Effects of clonal integration on the invasive clonal plant *Alternanthera philoxeroides* under heterogeneous and homogeneous water availability. *Scientific Reports* **6**: 1–8.

You W, Yu D, Liu C, Xie D, Xiong W. **2013a**. Clonal integration facilitates invasiveness of the alien aquatic plant *Myriophyllum aquaticum* L. under heterogeneous water availability. *Hydrobiologia* **718**: 27–39.

You W, Yu D, Xie D, Yu L. **2013b**. Overwintering survival and regrowth of the invasive plant *Eichhornia crassipes* are enhanced by experimental warming in winter. *Aquatic Biology* **19**: 45–53.

Yu H, Shen N, Yu D, Liu C. **2019**. Clonal integration increases growth performance and expansion of *Eichhornia crassipes* in littoral zones: A simulation study. *Environmental and Experimental Botany* **159**: 13–22.

Z

Vander Zanden MJ, Hansen GJA, Higgins SN, Kornis MS. **2010**. A pound of prevention, plus a pound of cure: Early detection and eradication of invasive species in the Laurentian Great Lakes. *Journal of Great Lakes Research* **36**: 199–205.

Zefferman E. **2014**. Increasing canopy shading reduces growth but not establishment of *Elodea nuttallii* and *Myriophyllum spicatum* in stream channels. *Hydrobiologia* **734**: 159–170.

Zehnsdorf A, Hussner A, Eismann F, Rönicke H, Melzer A. **2015**. Management options of invasive *Elodea nuttallii* and *Elodea canadensis*. *Limnologica* **51**: 110–117.

Zhang LM, Alpert P, Si C, Yu FH. **2019**. Interactive effects of fragment size, nutrients, and interspecific competition on growth of the floating, clonal plant *Salvinia natans*. *Aquatic Botany* **153**: 81–87.

Zhu G, Cao T, Zhang M, Ni L, Zhang X. **2014**. Fertile sediment and ammonium enrichment decrease the growth and biomechanical strength of submersed macrophyte *Myriophyllum spicatum* in an experiment. *Hydrobiologia* **727**: 109–120.

Zhu G, Yuan C, Di G, Zhang M, Ni L, Cao T, Fang R, Wu G. **2018**. Morphological and biomechanical response to eutrophication and hydrodynamic stresses. *Science of the Total Environment* **622–623**: 421–435.

Zhu G, Zhang M, Cao T, Ni L, Zhong A, Fu H. **2012**. Effects of sediment type on stem mechanical properties of the submerged macrophyte *Hydrilla verticillata* (L.f.) Royle. *Fresenius Environmental Bulletin* **21**: 468–474.

Zutshi DP, Vass KK. **1971**. Ecology and production of *Salvinia natans* Hoffim; in Kashmir. *Hydrobiologia* **38**: 303–320.

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Selbstständigkeitserklärung

Ich versichere an Eides Statt, dass die vorliegende Dissertation von mir selbständig und ohne unzulässige fremde Hilfe unter Beachtung der “Grundsätze zur Sicherung guter wissenschaftlicher Praxis an der Heinrich-Heine-Universität Düsseldorf” erstellt worden ist. Die Dissertation wurde in gleicher oder ähnlicher Form noch keiner anderen Fakultät vorgelegt und ich habe bisher keinerlei erfolglose Promotionsversuche unternommen. Darüber hinaus ist mir bekannt, dass jedweder Betrugsversuch zum Nichtbestehen oder zur Aberkennung der Prüfungsleistung führen kann.

Düsseldorf, den 19.05.2020

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(Patrick Heidbüchel)

Research articles and contributions

Here, bibliographical information of the published and unpublished articles included in this thesis are listed according to chapters. For each article, a short description of my contribution is given.

Published articles

Chapter 2 |

Heidbüchel P, Sachs M, Stanik N, Hussner A. **2019**. Species-specific fragmentation rate and colonization potential partly explain the successful spread of aquatic plants in lowland streams. *Hydrobiologia* **843**: 107–123.

Journal: Hydrobiologia

Role: First author & author for correspondence

Contribution: Contributed to conceptualization and design of the study, performed experiments and analyzed the data, wrote the manuscript (first draft & revisions) in consultation with co-author(s)

Chapter 3 |

Heidbüchel P, Hussner A. **2020**. Falling into pieces: In situ fragmentation rates of submerged aquatic plants and the influence of discharge in lowland streams. *Aquatic Botany* **160**, 103164.

Journal: Aquatic Botany

Role: First author & author for correspondence

Contribution: Contributed to conceptualization and design of the study, performed experiments and analyzed the data, wrote the manuscript (first draft & revisions) in consultation with co-author(s)

Chapter 5 |

Heidbüchel P, Jahns P, Hussner A. **2019**. Chlorophyll fluorometry sheds light on the role of desiccation resistance for vegetative overland dispersal of aquatic plants. *Freshwater Biology* **64**: 1401–1415.

Journal:	Freshwater Biology
Role:	First author & author for correspondence
Contribution:	Contributed to conceptualization and design of the study, performed experiments and analyzed the data, wrote the manuscript (first draft & revisions) in consultation with co-author(s)

Chapter 6 |

Heidbüchel P, Hussner A. **2019**. Fragment type and water depth determine the regeneration and colonization success of submerged aquatic macrophytes. *Aquatic Sciences* **81**: 6.

Journal:	Aquatic Sciences
Role:	First author & author for correspondence
Contribution:	Contributed to conceptualization and design of the study, performed experiments and analyzed the data, wrote the manuscript (first draft & revisions) in consultation with co-author(s)

Articles submitted in revised form

Chapter 4 |

Heidbüchel P, Sachs M, Hamzehian N, Hussner A. Go with the flow: Fragment retention patterns shape the vegetative dispersal of aquatic plants in lowland streams.

Role:	First author & author for correspondence
Contribution:	Contributed to conceptualization and design of the study, performed experiments and analyzed the data, wrote the manuscript (first draft & revisions) in consultation with co-author(s)