ALLELOPATHIC EFFECTS OF NATIVE AND INVASIVE FRESHWATER MACROPHYTES ON PHYTOPLANKTON AND AQUATIC INVERTEBRATES

Lake Nagambie. Photo credit: Fariba Moslih Pakdel

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Abstract

Submerged macrophytes are important components of many freshwater ecosystems. They provide habitats and food resources for a wide range of organisms and can control algal blooms via indirect and direct interactions such as shading, competition and allelopathy. Allelopathy is the production of biochemicals by primary producers that negatively affect the growth of potential competitors. Allelopathic submerged macrophytes can play a central role in the restoration of eutrophic lakes and maintaining a clear water state. However, invasive macrophytes have displayed negative impacts on fish, macroinvertebrates and native macrophytes. Invasive macrophytes usually displace native plants and form mono-specific stands, which are a less suitable habitat and food source for many biota. The 'Novel Weapons Hypothesis' (NWH) posits that exotic species will have the potential to become invasive if they produce novel biochemistry to which native species are not adapted. These chemicals may also exhibit deterrent activity against invertebrates. Hence, the allelopathic potential of introduced species is an influential strategy in invasion biology. On the other hand, the 'Enemy Release Hypothesis' (ERH) proposes that invasive species are successful because they escape their natural enemies in their new ranges. *Cabomba caroliniana*, *Egeria densa* and *Elodea canadensis* are invasive macrophyte species in Australia and many other countries. I performed 'screening of live material' assays to investigate the allelopathic effects of these invasive species and to compare their bioactivity to that of a range of native macrophytes using native microalgae (cyanobacteria and green algae) as the target organisms. A mesocosm study was conducted to further test the potential allelopathic effect of *C. caroliniana* on *Microcystis aeruginosa,* and to compare its effect to *Potamogeton ochreatus* (native). The density of free-living microalgae was also measured (OD_{750}) in the control ponds (without macrophytes) and in ponds where either native or invasive macrophytes were grown. I also examined the effects of *C. caroliniana* on invertebrates and compared it to *P. ochreatus* stands using mono-culture (single species of either native or invasive plant), co-culture (native and invasive plants grown together) and control (no plant) settings. Finally, I tested the ERH and the NWH by investigating the biochemical activity of *C. caroliniana* on a native moth larva and a native vascular plant.

Cabomba caroliniana exhibited a potent species-specific algicidal activity whereas the algicidal activity of *E. canadensis* and *E. densa* was weaker. *Potamogeton ochreatus* and *Potamogeton crispus* (native macrophytes) also showed species-specific allelopathic effects on the target organisms. *Cabomba caroliniana* inhibited the growth of *M. aeruginosa* in the mesocosm study; however, the effect was similar to that of *P. ochreatus*. The microalgal populations were significantly lower in the ponds where *C. caroliniana* was present compared to the ponds with *P. ochreatus* and the controls. Furthermore, the % cover of *P. ochreatus* was significantly lower when grown with *C. caroliniana* compared to the controls. Community dynamics of the invertebrates were similar in native and invasive macrophyte stands in the single species and in the co-culture experiments, but displayed a seasonal pattern in most comparisons. The moth larvae caused a high level of feeding damage on *C. caroliniana* and *P. ochreatus*; nevertheless, the damage was greater on *C. caroliniana*. My findings suggest that *C. caroliniana*, *E. canadensis* and *E. densa* may use their novel biochemistry to enhance their invasiveness, although their effect is dependent on the counterpart organisms in the recipient community, and they may use a combination of different strategies to intensify their invasive potential. For instance, *C. caroliniana* can use its novel biochemistry against competitors such as phytoplankton and plants, but may use a herbivory tolerance strategy instead of chemical defence when exposed to herbivory. In addition, *C. caroliniana* may have 'positive' impacts on associated fauna (e.g. providing refuge) and some generalist herbivores (food source) in specific freshwater systems. However, these positive effects do not outweigh the overall adverse impacts of *C. caroliniana* imposed on a system. Allelopathic native macrophytes can also regulate the growth of invasive species and therefore may have the potential to be used as biological control agents in future management plans. As a consequence, systems that contain a high diversity of native plants and generalist herbivores (a wide range of native 'enemies') may perhaps be less prone to invasion by exotic macrophytes.

Thesis declaration

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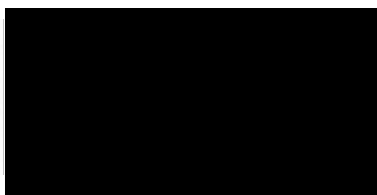
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The ideas, development and write-up of all chapters in this thesis were the principal responsibility of myself, the PhD candidate working within the Monash University School of Biological Sciences under the supervision of Professor Jenny Davis of Charles Darwin University, Professor John Beardall of Monash University, Dr Tricia Wevill of Deakin University and Dr Lien Sim. The inclusion of co-authors reflects the collaborative-based research conducted during the course of this PhD program. My contribution to each data chapter involved the following:

Thesis chapter	Publication title	Publication status	Extent of candidate's contribution
2	Novel chemical weapons: growth inhibition of cyanobacteria and green algae by invasive and native macrophytes	In review (*Ecology and Evolution*)	Conception, design, data collection, data analysis, manuscript preparation
3	The influence of native and invasive macrophytes on aquatic invertebrate communities: a mesocosm study	In review (*Ecology and Evolution*)	Conception, design, data collection, data analysis, manuscript preparation
4	Bioactivity of an invasive macrophyte species on a potential native enemy and a native competitor	In review (*Aquatic Botany*)	Conception, design, data collection, data analysis, manuscript preparation
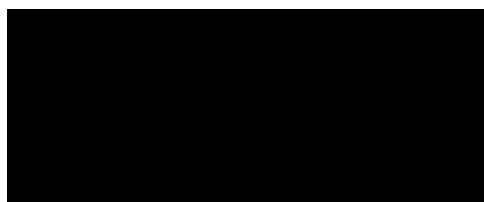
Candidate's Signature   Date: 17/02/2017

The undersigned hereby certify that the above declaration correctly reflects the nature and extent of the candidate and co-authors' contributions to this work.

Main Supervisor's Signature   Date: 17/02/2017

Acknowledgements

The role of allelopathic native and invasive macrophytes in freshwater systems

Lake Nagambie infested by *Cabomba caroliniana*. Photo credit: Fariba Moslih Pakdel

Introduction

Wetlands preserve wildlife and support biodiversity by providing habitat and food sources for a wide range of organisms (Gopal, 1999; Zhao and Song, 2004). Furthermore, wetlands may capture and filter nutrients and pollution from surrounding areas, supply water, abate floods and are used for recreational purposes (Mitch and Gosselink, 1986; Kadlec and Brix, 1995; Costanza et al., 1997; Nuttall et al., 1997; Ellis et al., 2003; Fisher and Acreman, 2004; Sheoran and Sheoran, 2006). Natural or human-induced eutrophication and associated algal blooms are major threats to these systems (Ansari et al., 2010).

Phytoplankton species generally have a faster growth rate than higher plants. Nutrient enrichment and consequent eutrophication and turbidity can accelerate phytoplankton growth. This may ultimately lead to the disappearance of 'macrophytes' (submerged plants and macroalgae) and the system can shift to a phytoplankton-dominated state (Scheffer et al., 2001; Ansari et al., 2010). Submerged macrophytes play pivotal roles in stabilising the alternative clear-water macrophyte-dominated state recognised by Scheffer et al. (2001). They can halt the increase of phytoplankton biomass directly or indirectly via several interrelated mechanisms and, contribute to water quality and water transparency (Fig. 1) (Scheffer, 1998; Mulderij et al., 2007a; Scheffer and van Nes, 2007). Submerged macrophytes have a greater competitive ability for nutrient uptake and hence can limit the growth of phytoplankton and epiphytes (Brammer, 1979; Gopal and Goel, 1993; van Donk and van de Bund, 2002; Lürling et al., 2006). Furthermore, macrophytes can increase grazing pressure indirectly by providing refuge for algal-grazing zooplankton against predators (Jeppesen et al., 1997) and therefore have positive physical effects on invertebrate communities (Watkins el., 1983; Timms and Moss, 1984). Reduction of sediment re-suspension (Horppila and Nurminen, 2003), shading effects (Mulderij et al., 2007a) and allelopathy against phytoplankton (van Donk and van de Bund, 2002) are other macrophyte-mediated mechanisms that can improve water quality.
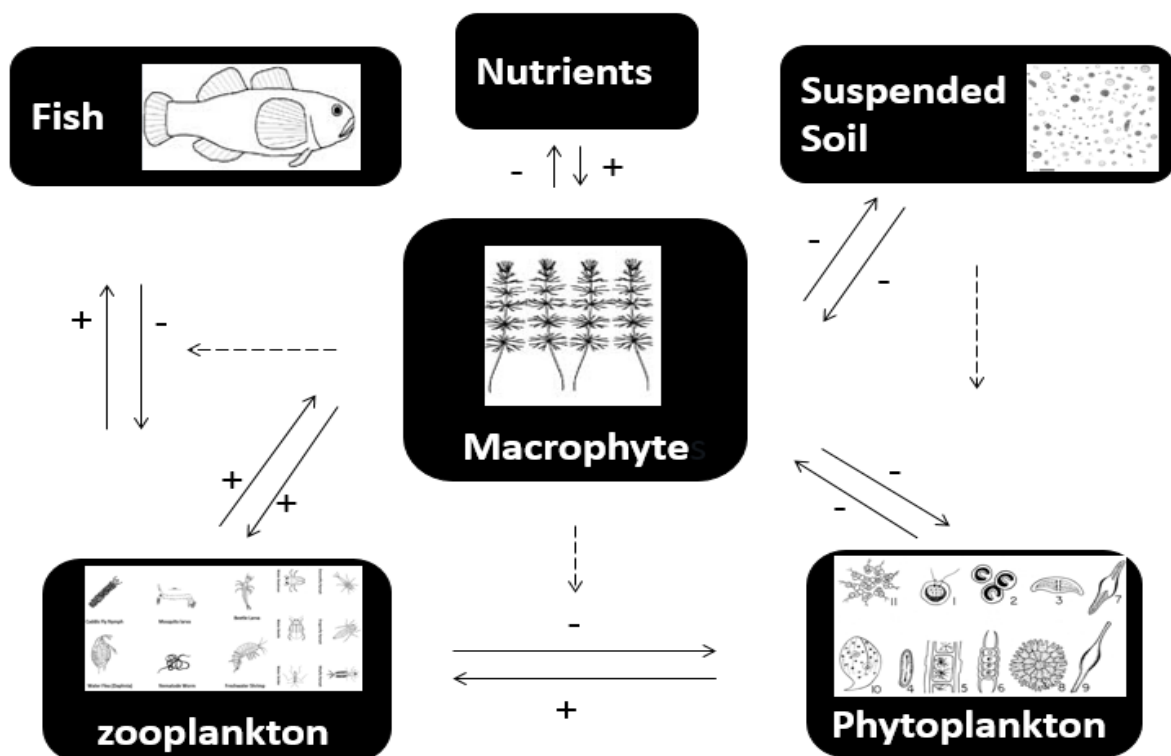
Fig. 1. A schematic diagram showing direct (solid lines) and indirect (dashed lines) interactions between macrophytes and phytoplankton/zooplankton.

Allelopathy is a very effective strategy to overcome competition and refers to any harmful (or beneficial) effect of one plant imposed on another plant, herbivores and/or microorganisms through chemical intervention, which involves the production and release of biochemical substances into the surrounding environment (Willis, 1985; 2008). These chemicals, termed allelochemicals, can be continuously released by a donor plant (via leaves and stems) into the environment (in the air or water), released as a product of microbial degradation of plant residues, or can be exuded from the root into the soil (Zeng et al., 2008). Allelopathic species may limit the growth and survival of co-occurring species as well as those that have the capacity to colonise (Willis, 1985). For example, the occurrence of extensive meadows of *Chara australis* and *Potamogeton crispus* has coincided with a decline in microalgal blooms regularly forming in a eutrophic lake in south-eastern Australia (pers. comm. Peter Symes, 2012). Pakdel et al. (2013) found antagonistic effects of these macrophytes against the cyanobacterium, *Anabaena variabilis*. They suggested that the allelopathic potential of these macrophytes might be responsible for the prevention of blooms formed by cyanobacteria. Allelopathic interactions also exist between macrophytes (Gopal and Goel, 1993; Gross, 2003a) and some phytoplankton species exhibit bioactivity against macrophytes (Keating, 1977; 1978; Gross et al., 1991; Gross, 1999; Gross, 2003a).

Invasion is another key threat to freshwater systems (Miller et al., 1989; Lassuy, 1995; Burlakova and Karatayev, 2007) and is considered one of the major causes of biodiversity decline in lakes and streams (Sala et al., 2000). Exotic freshwater plants are introduced either intentionally or accidentally through aquaculture, aquarium trade and hitchhiking by ships, boats and yachts (Kolar and Lodge, 2000; Kay and Hoyle, 2001; Martin and Coetzee, 2011). Some of these exotic species can become invasive and cause significant negative environmental and economic impacts (Pimentel et al., 2005; Lockwood et al., 2013; Gallardo et al., 2016).

Allelopathy in freshwater systems

The concept of allelopathy has long been applied to terrestrial environments (Molisch, 1937 cited in Willis, 1985; Muller, 1966; 1969; Gibbs, 1974) and has been expanded to aquatic systems over the last few decades (Hutchinson, 1975; Anthoni et al., 1980; Blindow, 1987; Gross, 2003a; Ferrier et al., 2005; Hu and Hong 2008; Svensson et al., 2013; Vanderstukken et al., 2014). Observations of low densities of epiphytes and phytoplankton in the proximity of some submerged and floating macrophytes led to primary investigations of allelopathy between macrophytes and phytoplankton in the late 1970s and early 1980s (Brammer, 1979; Wium-Andersen et al., 1982; 1983).

Nevertheless, demonstrating the existence of allelopathy in a natural setting is challenging. A range of biotic and abiotic factors such as nutrient levels, light and temperature conditions, competition, physiology and the structure of counterpart species can interfere with the production and action of macrophyte allelochemicals on phytoplankton and other macrophytes (Gross, 2003b; Hilt and Gross, 2008). Consequently, it is difficult to separate direct and indirect impacts of macrophytes such as shading, competition and existence of zooplankton in macrophyte beds from the effects of allelopathy on phytoplankton and other counterpart macrophyte species.

The allelopathic potential of macrophytes differ and the allelopathic outcome appears to be regulated by both the macrophyte and the phytoplankton counterpart species that are present (Mulderij et al., 2003; Erhard, 2006). Of the submerged macrophytes, *Myriophyllum* and *Ceratophyllum* are the most active genera that have been investigated to date, both exhibiting strong algicidal activity against a wide range of microalgal species (Hilt and Gross, 2008). For example, *M. spicatum*, *M. verticillatum* and *C. demersum* inhibit the growth of a variety of planktonic and epiphytic species of cyanobacteria, chlorophytes (green algae) and diatoms (Gross et al., 1996; Gross et al., 2003a; 2003b; Hilt et al., 2006). The inhibitory effects of other macrophytes such as *Chara*, *Potamogeton* and *Elodea* are more moderate (Hilt and Gross, 2008).

The responses of phytoplankton species to allelopathy also vary and they exhibit different susceptibility to allelochemicals (Mulderij et al., 2003; Mohamed and Al Shehri, 2010). Growth of *Microcystis*, *Anabaena*, *Scenedesmus* and diatoms such as *Nitzschia* has been shown to be inhibited by a wide range of macrophyte species, although the level of inhibition was different in each case (Gross et al., 1996; Nakai et al., 1999; Gross et al., 2003a; Erhard and Gross, 2006; Hilt et al., 2006; Lürling et al., 2006; Mulderij et al., 2007b; Wu et al., 2007a; 2007b; He et al., 2008; Zhang et al., 2009a; 2009b; Takeda et al., 2011) and it is uncertain whether these differences are due to species dependent effects or differences in experimental conditions which affect the responses of the microalgae and cyanobacteria to allelochemicals. Allelopathic macrophytes are known to constrain the growth of cyanobacteria to a greater extent than they constrain growth in diatoms and chlorophytes (Körner and Nicklisch, 2002; Hilt and Gross, 2008). Moreover, planktonic cyanobacteria and green algae are more vulnerable to allelochemicals compared to epiphytic species (Erhard and Gross, 2006).

Allelopathic potential of invasive macrophytes

Although submerged macrophytes play important positive roles in freshwater systems, invasive macrophyte species are known to negatively affect many native species of fish, macroinvertebrates and macrophytes in the recipient community (Thiebaut et al., 1997; Dextrase and Mandrak, 2006; Bryant and Papas, 2007; Svensson et al., 2013). A study, conducted by Bryant and Papas (2007), of nine wetlands in south-eastern Australia found that submerged native macrophytes have greater positive impacts on macroinvertebrate community composition by supporting a higher richness of macroinvertebrates compared to invasive macrophyte species. Adverse impacts of invasive macrophytes were also found on the abundance of several fish species, including some that are threatened (Lassuy, 1995; Dextrase and Mandrak, 2006; Schultz and Dibble, 2012). Furthermore, invasive species can outcompete and eventually eliminate native macrophytes (Thiebaut et al., 1997; Svensson et al., 2013). This may lead to the formation of dense, mono-culture, macrophyte meadows, which is a less suitable habitat and food source for zooplankton, benthic macroinvertebrates, fish and waterfowl, and can lead to seasonal water quality issues (Thiebaut et al., 1997; Barrat-Segretain, 2001; Erhard and Gross, 2006; Herb and Stefan, 2006).

Different interconnected mechanisms drive biological invasions and together can determine the success of invasive species (Mitchell et al., 2006; Fleming and Dibble, 2015). Many invasive species are superior competitors in their new ranges and can utilise nutrients more efficiently than native species (Burns, 2004; Funk and Vitousek, 2007). Furthermore, they have a rapid growth rate and produce a large amount of plant biomass compared to their native counterparts (Grotkopp and Rejmánek, 2007). Exotic plant species escape natural enemies in their native ranges and hence can become highly abundant in the new environment (the 'Enemy Release Hypothesis' (ERH), Crawley, 1997; Elton, 2000; Liu and Stiling, 2006). Therefore, they are less prone to herbivory and can gain a significant advantage over native species (Maron and Vilà, 2001; Liu and Stiling, 2006).

Allelopathy has long been proposed as another important mechanism responsible for the success of invasive plant species (Steenhagen and Zimdahl, 1979). As predicted by the 'Novel Weapons Hypothesis' (NWH), non-indigenous plant species have the potential to outcompete native competitors by producing chemicals that are novel to the native species (Callaway and Aschehoug, 2000; Callaway and Ridenour, 2004). This will allow the invaders to establish and spread in the new environment and displace native species through allelopathic interaction. Furthermore, some of the chemicals produced by macrophytes can be toxic to zooplankton and can limit their growth, reproduction and survival (Alvarez et al., 2016). Invasive macrophytes with allelopathic activity may have adverse impacts on the fitness of freshwater herbivores, consequently, some herbivores prefer natives over invasive species (Morrison and Hay, 2011). The NWH has been supported by studies of terrestrial and, to a smaller extent, marine communities (Carpenter and Cappuccino, 2005; Cappuccino and Arnason, 2006; Inderjit et al., 2006; Svensson et al., 2013). However, adequate empirical evidence to support the NWH and the role of allelopathy in freshwater macrophyte invasion is lacking. Of many studies testing the allelopathic potential of invasive species in freshwater systems, Marko et al. (2008) explicitly related allelopathy to invasiveness of *M.*

spicatum and found that this invasive species has a higher concentration of carbon, polyphenols and lignin that could contribute to the invasiveness of the species and provide it with a competitive advantage. Understanding the mechanisms that are responsible for the invasiveness of macrophytes is important for decision-making strategies and controlling invasive species (Sax et al., 2007; Davis, 2009).

Allelochemicals produced by some invasive macrophytes can persist in the soil/sediments and can have a legacy effect on future species. It could be inferred that allelopathic legacy effects of some invasive species could be more remarkable than direct effects because allelochemicals can persist after death or removal of the macrophyte and hence have a long-term impact on future community structure within the system (Inderjit et al., 2011). This suggests that the legacy effects of allelochemicals produced by some invasive macrophytes can make the mechanical control of these species less effective. Legacy effects can be absent in some invasive macrophytes but overall, both immediate allelopathic and allelopathic legacy effects of invasive macrophytes can be influenced by competition in natural conditions (Del Fabbro and Prati, 2015). Therefore, the effects of other factors should also be considered in controlling invasive macrophytes.

Allelochemicals

Allelopathic macrophytes produce myriad chemical compounds including polyphenols and phenolic acids, lipophilic constituents, hydrolysable tannins, alkaloid substances, and many other unidentified allelochemicals (Table 1). Some of these chemical substances have stronger algicidal properties than others (Gopal and Goel, 1993; Gross et al., 1996). Phenolic acids and polyphenolic compounds are the most common allelochemicals found in numerous species of macrophytes (Jones, 1995; Gross et al., 1996). Many macrophytes with similar growth forms produce similar groups of allelochemicals despite their great taxonomic variation (Gopal and Goel, 1993). Oxygenated fatty acids and alkaloids are groups of allelochemicals found in most *Potamogeton* and *Najas* species, *Vallisneria americana*, *M. spicatum*, *C. demersum* and *E. canadensis* (Ostrofsky and Zettler, 1986; Gopal and Goel, 1993). *Potamogeton* species and other monocotyledons produce a relatively high level of alkaloids compared to dicotyledons (Ostrofsky and Zettler, 1986). The strong, pungent smell of many charophytes indicates the production of allomones such as trithiane and dithiolane (Gopal and Goel, 1993). *Myriophyllum spicatum* produces the polyphenol tellimagrandin II whereas *M. verticillatum* and *M. alterniflorum* lack tellimagrandin II. Nevertheless, algicidal other, hydrolysable, polyphenols are found in *M. verticillatum* and *M. alterniflorum* (Hilt et al., 2006) and they exhibit the same degree of allelopathy as *M. spicatum* (Gross et al., 1996).

Since allelochemical compounds of macrophytes are released into the water column, they need to be sufficiently hydrophilic and adequately concentrated in order to reach and provoke the target organisms effectively (Gross, 2003a). Some of these compounds are labile and are readily metabolized, hence constant exudation is required to prolong their deleterious effects (Gopal and Goel, 1993; Jasser, 1995; Gross, 1999; Nakai et al., 1999; Gross, 2003a). Allelochemicals such as lipophilic compounds, elemental sulphur and oxygenated fatty acids have a potent algicidal property. These chemicals are not water-soluble and may act mainly through direct cell-cell contact. It has been suggested that hydrophilic compounds primarily act on planktonic species, whereas lipophilic compounds mainly target epiphytic species (Wium-Andersen et al., 1983; Gopal and Goel, 1993; Gross, 2003a). The production of lipophilic constituents may have evolved in macrophytes to prevent epiphytic algal growth that are in direct contact with macrophytes. Low epiphytic growth on *C. demersum* was proposed to be caused by the release of elemental sulphur that has a strong algicidal property and is ten times more toxic to algae than the trithiane compounds produced by *Chara* species (Gopal and Goel, 1993). The stronger toxicity of these compounds may be an evolutionary advantage to compensate for their low solubility in water. The difference in the nature of allelochemicals excreted by macrophytes is an important measure in determining macrophyte success. Macrophytes such as *Chara*, *Ceratophyllum* and *Myriophyllum* species that produce hydrophilic allelochemicals are likely to be more effective at supressing the growth of other species existing further away, because hydrophilic compounds have the ability to diffuse through the waterbody and reach the target species.

Lipophilic substances produced by species such as *Potamogeton* also have very strong growth inhibitory effects. Furthermore, most of these species (i.e. *Chara* and *Potamogeton*) excrete multiple chemical substances (Table 1) (Gopal and Goel, 1993).

Some invasive macrophytes produce different chemical compounds to the native congener. Nevertheless, the quantity of the chemicals produced can be similar (Jarchow and Cook, 2009). In contrast, other invasive species are capable of producing a higher concentration of chemical substances in comparison to the closely related native species (Marko et al., 2008). Furthermore, the novelty of these chemicals in a recipient environment is an important factor determining their effects on the target species.

Allelochemicals have different modes of action on phytoplankton, often targeting two main physiological processes: enzyme activity and photosynthesis (many allelochemicals interfere with Photosystem II (PS II) activities) (Gross, 2003b). They may also destroy the cell membrane (Leflaive and Ten-Hage, 2007) (Table 1). Some chemical substances affect several processes/components of the afflicted species depending on other environmental conditions. Most macrophytes produce multiple allelochemicals. (Gopal and Goel, 1993; Xian et al., 2006; Jarchow and Cook, 2009; Zhang et al., 2010). For instance, Marko et al. (2008) found a high diversity of chemical substances produced by both native and invasive macrophytes. This strategy may be an advantage to ensure the effectiveness of allelochemicals on several target species and increases the probability of inhibition of the afflicted species.

Table 1. Summary of studies on the allelochemicals of macrophytes and their mode of actions on target species (phytoplankton), (?: target species and/or the mode of action not known).

References	Allelochemicals	Macrophyte(s) (donor)	Phytoplankton (target)	Mode of action
1	Alkaloids	*Potamogeton* * *V. americana* *M. spicatum* *C. demersum* *E. canadensis*	?	?
2	Flavonoids	Lemnaceae* (free floating plants)	?	?
3, 4, 5	Phenolic compounds	*Elodea* * *C. demersum* *Najas marina* ssp. *intermedia* *Stratiotes* *	Cyanobacteria, chlorophytes, epiphytic algae & diatom (*Nitzschia*)	Destruction of photosynthesis
2,16, 17	Caffeic acids	*Stratiotes* * *E. canadensis* *Lemna minor*	Cyanobacteria & algae	Destruction of photosynthesis
2	Oxygenated fatty acids	*Ruppia** *Potamogeton** *Thalassia sp.* *Najas**	Phytoplankton & epiphytic diatom *Nitzschia palea*	?
2	4-methylthio-1,2-dithiolane and 5-methylthio-1,2,3-trithiane	*Chara globularis* *Chara**	Phytoplankton & epiphytic diatom *N. palea*	Destruction of Photosynthesis
6	Elemental sulphur	*C. demersum*	Epiphytes	?
2	Dithiolane substances	*Nitella**	Epiphytes	Destruction of Photosynthesis

2, 7, 8, 9	Tellimagrandin II & other hydrolysable polyphenols (e.g. gallic and vanillic acids), labile sulphur compounds	*M. spicatum* *Myriophyllum ** *C. demersum* *V. americana*	Algae & cyanobacteria	Destruction of extracellular enzyme activity, destruction of photosynthesis
10	Cyclic sulphur compounds	*Chara* sp.	Diatoms & phytoplankton	Destruction of photosynthesis
11, 18	Ethyl 2-methylacetoacetate	*Phragmites**	*Scenedesmus obliquus Selenastrum capricornutum Microcystis aeruginosa*	Cell deformation & destruction of cell membrane
2, 12, 13	P-hydroxybenzoic acid, ρ-coumaric acid and vanillic acid	*V. americana*	*M. aeruginosa*	Destruction of cell membrane
14	Cyanogenic compounds	*Myriophyllum**		?
15	2-Ethyl-3-methylmaleimide, & β-carotene derivatives	*Vallisneria spiralis* L.	*M. aeruginosa*	Destruction of photosynthesis ?

* More than one species within the genus contain the compound(s).

1- Ostrofsky and Zettler (1986), 2- Gopal and Goel (1993), 3- Gross et al. (2003b), 5- Mulderij et al. (2007b), 6-Wium-Andersen et al. (1983), 7- Gross et al. (1996), 8- Körner and Nicklisch (2002), 9- Gross (2003b), 10- Wium-Andersen et al. (1982), 11- Men et al. (2007), 12- Zhang et al. (2008) cited by Zhang et al. (2010), 13- Zhang et al. (2010), 14- Gibbs (1974), 15- Xian et al. (2006). 16- Mulderij et al. (2005a, b), 17- Zhu et al. (2010), 18- Li and Hu (2005).

Allelopathy versus other factors

The allelopathic potential of macrophytes affecting microalgae is influenced by biotic and abiotic factors such as competition, season, and nutrient and light availability (Gross, 2003a; Mulderij et al., 2007a; 2007b; Bauer et al., 2009). Competition between microalgal and macrophyte species is believed to be a more significant factor affecting microalgal growth than allelopathy (Lürling et al., 2006), so in allelopathic investigations it is important to also acknowledge the effect of resource limitation and competition.

Light is the main limiting resource in many wetland systems, particularly for submerged species (Erhard, 2006). Rapid growth of epiphytic algae and phytoplankton may enforce additional shading on macrophytes (Gross, 2003a; Kirk, 2010) therefore limiting their growth. In addition, synergy between shading caused by excessive growth of periphyton and herbivory can diminish resilience of macrophytes, leading to an imminent macrophyte collapse followed by a sudden shift of the system to a turbid phytoplankton-dominated state (Hidding et al., 2016). Jupp and Spence (1977) observed a decline in *Potamogeton filiformis* biomass, which was primarily attributed to the shading effects of algal blooms consisting of floating cyanobacteria such as *Anabaena* species. Conversely, shading can limit the growth of microalgae in systems where macrophytes are well established. This may explain the low densities of phytoplankton in vegetated areas; in particular areas dominated by floating species (van Donk and van de Bund, 2002). High light availability per se can enhance the production and exudation of some macrophyte allelochemicals (Gross, 2003b) thus reinforcing macrophyte dominance.

Nutrient availability is another major factor regulating the structure and dynamics of macrophyte communities and the abundance of phytoplankton species in aquatic systems (Gopal and Goel, 1993; Barko and James, 1998), and may interact directly with allelopathy (Gross et al., 2007). Macrophytes can suppress microalgal growth directly through nutrient competition (Fitzgerald, 1969). Furthermore, nutrient availability may alter the allelopathic effect of macrophytes on microalgae. Some macrophytes have reduced allelopathic potential when nutrients are limited (Mulderij et al., 2007b). In contrast, the inhibitory effect of some macrophytes on microalgae is greater under low nutrient conditions (Fitzgerald, 1969; Lürling et al., 2006), suggesting that nutrient limitation exerts additional stress on some microalgae. Hence, these nutrient-exhausted microalgae exhibit greater susceptibility to allelochemicals (Mulderij et al., 2007b).

The physiology and physical structure of macrophytes can influence their allelopathic potential against microalgae. For instance, submerged macrophytes can suppress microalgal growth more effectively than emergent species because more of the plant biomass is under water where active production of allelochemicals occurs (Gopal and Goel, 1993; Hilt and Gross, 2008). The effective secretion of allelochemicals may be influenced by leaf anatomy. Submerged macrophytes have very thin leaves, which lack stomata and have fewer tight cell connections compared with emergent species, an anatomy that can facilitate the excretion of allelopathic substances (Hutchinson, 1975).

Some macrophytes are known to produce chemical substances in particularly high levels during the primary growth stages of their life cycle (Planas et al., 1981; Gross, 2000; Mulderij et al., 2003). This indicates that these macrophytes invest more in chemical defences at an earlier life stage, which is one strategy to allow the organism to survive during this vulnerable period. For instance, Mulderij et al. (2003; 2005a) found that young macrophytes had stronger bioactivity and excreted a greater amount of allelochemicals compared with older individuals. Inhibition of the growth of *Scenedesmus obliquus* also occurred only when exposed to younger *Stratiotes* species (Mulderij et al., 2005a). Younger leaves of *P. illinoensis* and *Scirpus subterminalis* produced allelochemicals more effectively than senescent leaves of these species (Burkholder et al., 1990). Season is another factor determining the level of the chemical constituents produced by macrophytes. For instance, *M. spicatum* produces tellimagrandin II in high concentrations in spring when phytoplankton growth is intense and competition for light is strong (Gross, 2003b; Hilt and Gross, 2008).

Competition and allelopathic interactions between macrophytes

Interactions such as resource competition can determine the ultimate pattern of macrophyte species dominance (McLay, 1974; Gross, 2003a; Spencer and Rejmánek, 2010). Emergent macrophyte species rely solely on sediments as the source of nutrients, while free-floating macrophytes acquire their nutrients from the water column. Submerged species are capable of obtaining nutrients from both sediments and the surrounding water, hence lowering the nutrient level in the system (Wetzel, 1988; Gopal and Goel, 1993). Furthermore, the ability to utilize alternative sources of nutrients enables submerged macrophytes to survive even when the nutrient level of the water column is low. This provides them with a competitive advantage (Barko and James, 1998). Competition is expected to take place predominantly among species with comparable resource requirements (Gopal and Goel, 1993; Spencer and Rejmánek, 2010). For example, Spencer and Rejmánek (2010) detected greater interspecific competition than intraspecific competition between *P. pectinatus* and *P. gramineus* under high light availability.

Competition for space can also have an impact on the distribution and abundance of macrophytes (Gopal and Goel, 1993). Two major types of growth forms are exhibited by submerged macrophyte species. Stoloniferous species form rosettes and do not develop long leaves or shoots (i.e. *Chara*) whereas rooted or non-rooted canopy forming species (i.e. *P. pectinatus* and *C. demersum* respectively) have long shoots and their leaves extend near the water surface (Spence, 1982).

Competition may be minimal among species with distinctly different structures and resource requirements. As a result, they are expected to co-exist in a particular system due to limited competition for space, (i.e. stoloniferous versus canopy forming species), or limited nutrient competition, (i.e. free-floating macrophytes versus rooted submerged species) (Gopal and Goel, 1993). Nevertheless, McLay (1974) has reported the exclusion of the submerged macrophyte, *P. pectinatus*, by the emergent species, *S. californicus*. The free-floating species *Lemna perpusilla* was also negatively affected by *S. californicus*. Furthermore, McLay (1974) detected an antagonistic interaction between *L. perpusilla* and *P. pectinatus*. As mentioned earlier, allelopathic interactions occur among macrophyte species (Elakovich, 1989; Gopal and Goel, 1993; Gross, 2003a; Svensson et al., 2013) hence, this interference between the free-floating, emergent and submerged species could be due to chemical intervention rather than competition. *Eleocharis coloradoensis* (dwarf spike- rush) is known to have allelopathic effects on *E. canadensis, E. nutallii, N. guadalupensis*, and several *Potamogeton* species (Frank and Dechoretz, 1980; Gopal and Goel, 1993). It can be deduced then that an allelopathic interaction may be more critical than competition in determining the dominance of macrophyte species with distinct resource requirements and structures.

Invasive species have specific physiological traits such as fast growth rate and a greater photosynthetic ability under a broad range of environmental and nutrient conditions (Jahnke et al., 1991; Dendène et al., 1993; Ozimek et al., 1993). They are more successful in invading disturbed habitats such as eutrophic systems; therefore nutrient reduction can have a negative impact on some invasive species (Baltz and Moyle, 1993; Quinn et al., 2011; Gérard et al., 2014). Some invasive macrophytes are capable of storing nutrients in their tissues when nutrient concentration in the system is high or, utilise nutrients more efficiently in poor-nutrient conditions compared to native macrophytes (Mony et al., 2007; Yarrow et al., 2009; Wersal and Madsen, 2011; Gérard et al., 2014). Although nutrient availability is important in regulating the growth and, ultimately, the success of plant species, there are other factors that affect competition between invasive and native macrophytes (Vanderstukken et al., 2011; Tabassum and Leishman, 2016). Thus, mechanisms such as allelopathy can be responsible for the success of invasive species and can provide them with a competitive advantage in response to nutrient availability.

Native macrophytes with allelopathic potential may be used as biological control agents to manage algal blooms or invasive macrophyte species. Charophytes are important allelopathic species in aquatic ecosystems as they are

rapid colonizers and are pioneer species after restoration by biomanipulation (Mulderij et al., 2003). Charophytes impede sediment re-suspension, deliver oxygen to the sediments and more importantly, are known as 'nutrient sinks' in shallow lakes (Kufel and Kufel, 2002). Hence, *Chara* species would be one potential candidate in restoration plans. *Potamogeton* is another genus known to have allelopathic potential and its rapid vegetative propagation would be an advantage in restoration processes (Nichols and Byron, 1986; Berger and Schagerl, 2004; Zhang et al., 2009b; Pakdel et al., 2013).

Methodological issues in allelopathic investigations in freshwater systems

Despite a recent increase in research focus on the role of allelopathic effects of macrophytes in freshwater systems (Mulderij et al., 2007a; Qin et al., 2010; Takeda et al., 2011; Vanderstukken et al., 2011), this area is still a subject of debate, because the confirmation of allelopathic interactions *in situ* is challenging (Gross et al., 2007). There is a list of requirements that should be addressed to demonstrate the existence of allelopathic interactions between species (Willis, 1985). In order to fulfil these requirements, any study of allelopathy must be designed to reveal the production and release of allelochemicals by donor species and transportation of these chemicals through the surrounding environment. In addition, the uptake and consequent pattern of inhibition of the target species must be confirmed. Finally, the pattern of inhibition must be primarily explained by the allelopathic interaction between donor-target species rather than other biotic and abiotic factors such as competition, disease, light or temperature. (Willis, 1985; Gross et al., 2007).

A range of methodologies has been employed to investigate allelopathic interactions between macrophytes and microalgae. The most common laboratory experiments include the use of macrophyte extracts (plants' constituents extracted in a solvent), exudates (liquids in which the macrophytes have been incubated) or live material (Nakai et al., 1999; Mulderij et al., 2005b; Wu et al., 2007a; 2007b; He et al., 2008; Mohamed and Al Shehri, 2010; Vanderstukken et al., 2011). Extract experiments give no substantial ecological evidence about the natural release of allelochemicals (Gross et al., 2007) and when conducted in laboratories, may underestimate the effect of allelochemicals if the chemical substances are labile, or if bacterial metabolism occurs during the exudation period. Constant exudation of allelochemicals occurs in nature whereas this cannot be replicated in many laboratory experiments (Gross, 2003a; Gross et al., 2007). Co-existence experiments (using live material) are approaches more comparable to natural conditions. However, on their own, these still fail to give ecological proof of the presence of allelopathy. Co-existence experiments only reflect the inter-specific interactions between donor and target species, which could be related to mechanisms other than allelopathy (e.g. competition for light, nutrients or other resources). Another inherent problem with co-existence studies is the existence of other associated organisms such as bacteria, epiphytes and zooplankton, which may alter the interaction between macrophytes and phytoplankton (Gross et al., 2007). Utilising sterile dialysis cassettes is one method that can be used to demonstrate the allelopathic potential of macrophytes on microalgae by eliminating the effects of other organisms. A combination of laboratory and coexistence experiments undertaken in outdoor conditions within the one study could perhaps provide the most defensible outcomes. Furthermore, the effects of other confounding factors such as competition should be eliminated. Studies of allelopathic interactions between different macrophyte species can also provide more reliable conclusions if the effects of factors such as competition, light and shading is minimized.

Research question

The present understanding of allelopathic interactions in aquatic systems is incomplete and fragmentary. Despite extensive research and literature, there is much that remains to be explored (Gross et al., 2007; Mulderij et al,. 2007a; Hilt and Gross, 2008; Hu and Hong 2008; Zhang et al., 2009a; 2009b; Zhu et al., 2010; Takeda et al., 2011). However, information accumulated to date strongly suggests that allelopathic interactions between submerged macrophyte species and other freshwater organisms do exist. There is a hope that in the long term, different

pieces of evidence, combined with recent advances in technology and methodologies in this area, will ultimately lead us to the answers with regard to the effects of allelopathy in freshwater systems (Gross et al., 2007).

Mesocosm studies and field investigations have seldom been undertaken to study the relationships between freshwater macrophytes, microalgae and invertebrates in the context of allelopathy to test the NWH and the ERH. Furthermore, an explicit test of the NWH has not been conducted in freshwater systems. However, some aquatic invasive species are known to produce chemical substances and have allelopathic effects on native species (in freshwater systems, Erhard and Gross, 2006; Marko et al., 2008; in marine systems, Svensson et al., 3013). There are also insufficient studies testing the NWH using invasive macrophytes as the donor species and freshwater native vascular plants as the target organism. Furthermore, most studies investigating the effects of invasive macrophytes on other biota such as invertebrates are descriptive. The indirect effects of the macrophyte community on microalgal blooms have been investigated using outdoor ponds (Schriver et al., 1995; Bakker et al., 2010). However, to date, not many outdoor experiments have been carried out to test for variation between the allelopathic effects of native and invasive freshwater macrophytes.

The main objective of this study was to address the knowledge gap by following a line of investigations—from laboratory trials to a mesocosm study—to explore the role of native and invasive allelopathic macrophytes in freshwater systems and their interaction with other organisms. The allelopathic potential of several invasive macrophyte species was investigated on green algal and cyanobacterial species and was compared to the allelopathic potential of native macrophytes to determine whether allelopathy is the key trait responsible for the success of these invaders. This included a set of assays to screen live material in controlled laboratory conditions. An outdoor experiment was carried out to further explore the mechanisms responsible for the invasion success of invasive species by investigating the allelopathic effects of native and invasive macrophytes on a cyanobacterial species that causes noxious blooms around the globe and in Australia. The density of free-living microalgae was also measured in ponds where native or invasive macrophytes were present (Chapter 2). The deterrent activity of native and invasive macrophytes on invertebrate communities was investigated by examining the community dynamics of macro and microinvertebrates in native and invasive macrophyte stands. Macrophytes were grown either as mono-culture or co-culture stands in order to determine if invertebrates prefer native versus invasive species. This experiment was conducted to determine if the mono-specific stands of invasive macrophytes were a less suitable habitat for invertebrates and if they deter invertebrates via allelopathy. (Chapter 3). Furthermore, the bioactivity of an invasive macrophyte was investigated on two potential native 'enemies' to test the ERH and the NWH (Chapter 4). This research may provide further insights into understanding the allelopathic effects of native and invasive macrophytes on microalgae and aquatic invertebrates. The current study also sought to determine whether the role of allelopathic native versus invasive macrophytes differs in some wetland systems. A conceptual model has been created to summarise the structure of the study to investigate the major research question (Fig. 2).

Thesis Structure Outline:

This thesis comprises five chapters: a general introduction, three data chapters and a general discussion. Each data chapter is self-contained and is a manuscript under revision a peer-reviewed scientific journal. For this reason, some aspects are presented in more than one chapter and there might be some overlap, particularly in the introduction of different chapters. All data chapters included in this thesis have been written in the style of the journal to which they have been or will be submitted. However, section headings, format, numbering and referencing have been amended to be consistent across the thesis. The terms 'exotic', and 'non-indigenous', 'allelopathic activity' and 'bioactivity', 'mono-specific' and 'mono-culture', 'macrophyte stands', 'macrophyte meadows' and 'macrophyte beds' and, 'microalga' and 'phytoplankton' have been used interchangeably throughout the thesis.

Chapter 1:   **General introduction: The role of allelopathic native and invasive macrophytes in freshwater systems**

The introductory chapter included background information on the allelopathic effects of native and invasive macrophytes on phytoplankton, macroinvertebrates and zooplankton. This chapter reviewed allelopathic interactions between some of the most studied native and invasive macrophyte taxa and other organisms and considered the effects of various biotic and abiotic factors on allelopathy of macrophytes. The negative effects of invasive macrophyte species on freshwater biota were briefly described. The majority of examples provided in this chapter were about allelopathic effects of macrophytes on phytoplankton because studies of allelopathic interactions among macrophyte species, especially native and invasive macrophytes in freshwater systems are scarce.

Chapter 2:   **Novel chemical weapons: growth inhibition of cyanobacteria and green algae by invasive and native macrophytes**

Allelopathic effects of selected native and invasive macrophytes were investigated on the most dominant species of microalgae that form blooms in many wetlands around the globe, including Australia. This was performed as a series of laboratory experiments, including a test of the species-specificity of macrophyte allelochemicals on selected microalgae. This chapter included an investigation of the allelopathic potential of three invasive macrophyte species present within wetlands in several states of Australia. A mesocosm study was carried out to ascertain the effectiveness of allelochemicals in suppressing microalgal growth in natural systems using dialysis cassettes. Moreover, the effect of native versus invasive macrophyte species on the growth of naturally occurring microalgae was investigated (Fig. 2).

Chapter 3:   **The influence of native and invasive macrophytes on aquatic invertebrate communities: a mesocosm study**

The taxon richness, abundance, community composition and distribution of functional feeding groups (FFG) of invertebrates in native and invasive macrophyte meadows were investigated. Two different mesocosm studies were conducted to 1) determine the effect of native and invasive macrophytes on the invertebrate communities and 2) investigate invertebrates' preference for native vs. invasive macrophyte stands as refuge (Fig. 2).

Chapter 4:   **Bioactivity of an invasive macrophyte species on a potential native enemy and a native competitor**

In this study, the ERH and the NWH were scrutinised simultaneously by investigating the bioactivity of an invasive macrophyte in order to determine whether the invasive species uses its novel chemicals against natural 'enemies' such as competitors and herbivores. The allelopathic effect of *C. caroliniana* was examined on a common native

macrophyte in the wetlands in Australia. The native moth larva, *Parapoynx rugosalis*, is known to feed on *Potamogeton* species. The damage caused via herbivory by the moth larvae on *C. caroliniana* and its natural food source was compared to determine whether the invasive macrophytes had anti-herbivore activity (Fig. 2).

Chapter 5:   General discussion

The findings from all data chapters (2-4) were integrated and implications and future research were discussed in this part of the thesis. The outcomes from the three data chapters were placed in the context of broader ecological applications and some suggestions were made to explain the importance of investigating the allelopathic potential of freshwater macrophytes, specifically invasive species. These investigations may provide valuable insight into understanding the impacts of invasive macrophytes on freshwater communities and hence inform better management strategies.

Central Research Question: What are the roles of allelopathic native and invasive macrophytes in freshwater systems?

Study Aim: To compare the allelopathic effects of native and invasive macrophytes on cyanobacteria, green algae and invertebrate communities in freshwater systems, and to test the NWH and ERH by investigating the bioactivity of an invasive macrophyte.

Main chapter objectives

Objective 1. Assessing the allelopathic potential of native and invasive macrophytes on microalgae

Objective 2. Assessing the invertebrate communities in native and invasive macrophyte beds in a mono-culture and co-culture settings

Objective 3. Assessing the bioactivity of an invasive macrophyte on two native 'enemies'

Main chapter aims

Chapter 2.

a) Investigate allelopathic effects of several native and invasive macrophytes on the growth of microalgae

b) Examining the allelopathic potential of *P. ochreatus* (native) and *C. caroliniana* (invasive) on *Microcystis aeruginosa*

c) Measure microalgal density in the presence of *P. ochreatus* and *C. caroliniana*

Chapter 3.

a) Investigate deterrent activity of a native and an invasive macrophyte on invertebrate communities

b) Investigate invertebrate communities in native vs. invasive macrophyte stands in a co-culture setting

Chapter 4.

Investigate bioactivity of *C. caroliniana* on two potential native 'enemies' using a native macrophyte and a native herbivorous moth larva
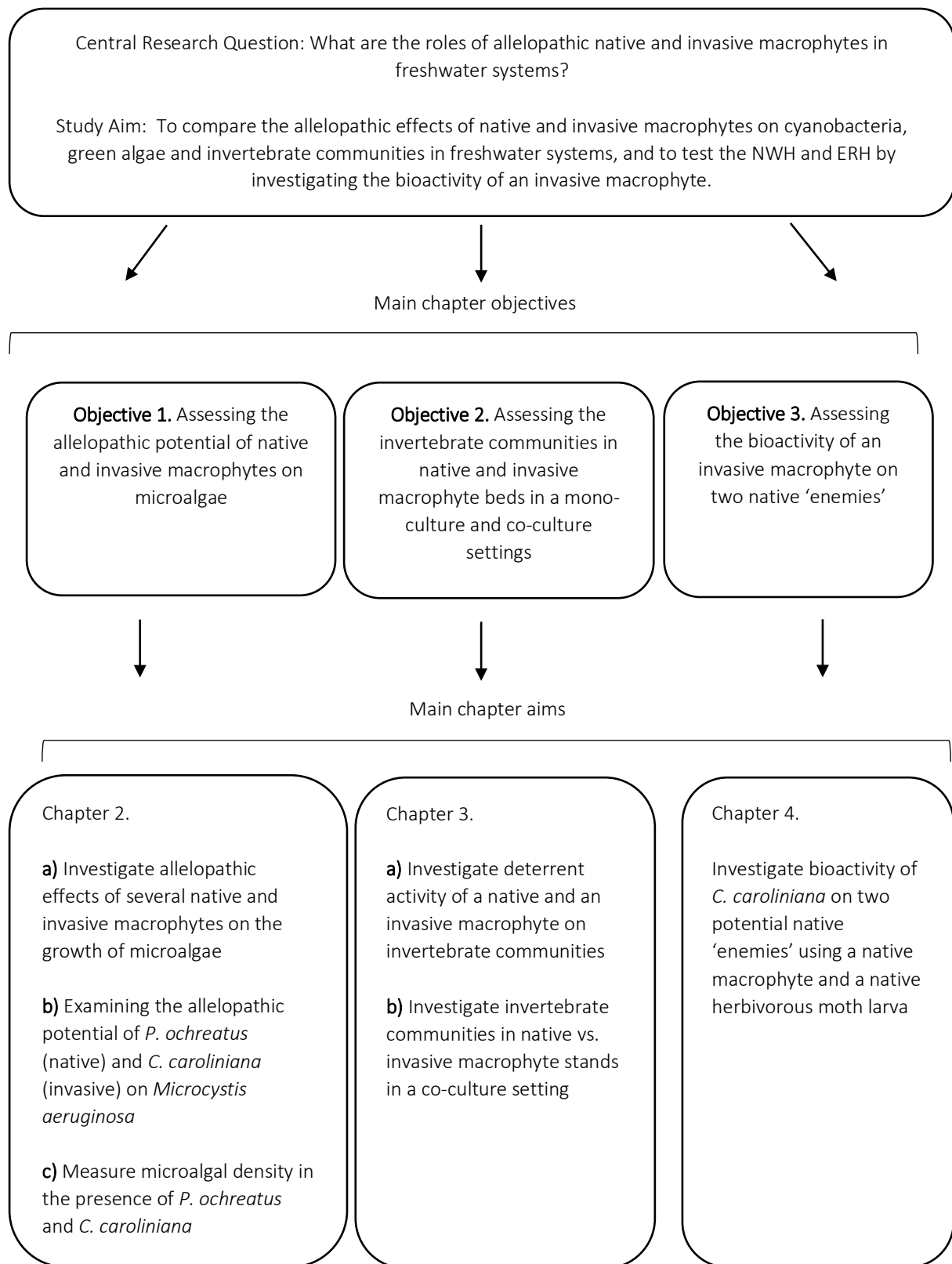
Fig. 2. A conceptual framework of the thesis summarising the scope and the aims of each chapter

References

Alvarez, M. F., Benítez, H. H., Villegas Cortés, J. C., Gabellone, N. A. & Claps, M. C. (2016) Effect of tannins on survival and development of *Daphnia menucoensis* and the abundances of bacteria. *Chemistry and Ecology*, **32,** 742-755.

Ansari, A. A., Gill, S. S., Lanza, G. R. & Rast, W. (2010) *Eutrophication: Causes, Consequences and Control.* Springer Verlag, Netherlands.

Anthoni, U., Christophersen, C., Madsen, J. O., Wium-Andersen, S. & Jacobsen, N. (1980) Biologically active sulfur compounds from the green alga *Chara globularis*. *Phytochemistry,* **19,** 1228-1229.

Bakker, E. S., Van Donk, E., Declerck, S. A. J., Helmsing, N. R., Hidding, B. & Nolet, B. A. (2010) Effect of macrophyte community composition and nutrient enrichment on plant biomass and algal blooms. *Basic and Applied Ecology,* **11,** 432-439.

Baltz, D. M. & Moyle, P. B. (1993) Invasion resistance to introduced species by a native assemblage of California stream fishes. *Ecological Applications,* **3,** 246-255.

Barko, J. W. & James, W. F. (1998) Effects of submerged aquatic macrophytes on nutrient dynamics, sedimentation and resuspension. *The Structuring Role of Submerged Macrophytes in Lakes* (eds E. Jeppesen, M. Søndergaard & K. Christoffersen), pp. 197–217. Springer, New York.

Barrat-Segretain, M. H. (2001) Invasive species in the Rhône River floodplain (France): replacement of *Elodea canadensis* Michaux by *E. nuttallii* St. John in two former river channels. *Archiv für Hydrobiologie,* **152,** 237-251.

Bauer, N., Blaschke, U., Beutler, E., Gross, E. M., Jenett-Siems, K., Siems, K. & Hilt, S. (2009) Seasonal and interannual dynamics of polyphenols in *Myriophyllum verticillatum* and their allelopathic activity on *Anabaena variabilis*. *Aquatic Botany,* **91,** 110-116.

Berger, J. & Schagerl, M. (2004) Allelopathic activity of Characeae. *Biologia,* **59,** 9-15.

Blindow, I. (1987) The composition and density of epiphyton on several species of submerged macrophytes - the neutral substrate hypothesis tested. *Aquatic Botany,* **29,** 157-168.

Brammer, E. S. (1979) Exclusion of phytoplankton in the proximity of dominant water-soldier (*Stratiotes aloides*). *Freshwater Biology,* **9,** 233-249.

Bryant, D. & Papas, P. (2007) Macroinvertebrate communities in artificial wetlands - the influence of macrophytes. Arthur Rylah Institute for Environmental Research Technical Report Series No. 168. Arthur Rylah Institute for Environmental Research.

Burkholder, J. M. & Wetzel, R. G. (1990) Epiphytic alkaline phosphatase on natural and artificial plants in an oligotrophic lake: re-evaluation of the role of macrophytes as a phosphorus source for epiphytes. *Limnology and Oceanography*, **35,** 736-747.

Burlakova, L. E. & Karatayev, A. Y. (2007) The effect of invasive macrophytes and water level fluctuations on unionids in Texas impoundments. *Hydrobiologia,* **586,** 291-302.

Burns, J. H. (2004) A comparison of invasive and non-invasive dayflowers (Commelinaceae) across experimental nutrient and water gradients. *Diversity and Distributions*, **10,** 387-397.

Callaway, R. M. & Aschehoug, E. T. (2000) Invasive plants versus their new and old neighbors: a mechanism for exotic invasion. *Science,* **290,** 521-523.

Callaway, R. M. & Ridenour, W. M. (2004) Novel weapons: invasive success and the evolution of increased competitive ability. *Frontiers in Ecology and the Environment,* **2,** 436-443.

Cappuccino, N. & Arnason, J. T. (2006) Novel chemistry of invasive exotic plants. *Biology Letters,* **2,** 189–193.

Carpenter, D. & Cappuccino, N. (2005) Herbivory, time since introduction and the invasiveness of exotic plants. *Journal of Ecology,* **93,** 315-321.

Costanza, R., d'Arge, R., deGroot, R., Farber, S., Grasso, M., Hannon, B., Limburg, K., Naeem, S., O'Neill, R. V., Paruelo, J., Raskin, R. G., Sutton, P. & Belt, M. V. D. (1997) The value of the world's ecosystem services and natural capital. *Nature,* **387,** 253-260.

Crawley, M. J. (1997) *Plant Ecology*. Blackwell Science, Oxford.

Davis, M. A. (2009) *Invasion Biology*. Oxford University Press, New York, USA.

Del Fabbro, C. & Prati, D. (2015) The relative importance of immediate allelopathy and allelopathic legacy in invasive plant species. *Basic and Applied Ecology,* **16,** 28-35.

Dendène, M. A., Rolland, T., Trémolieres, M. & Carbiener, R. (1993) Effect of ammonium ions on the net photosynthesis of three species of *Elodea. Aquatic Botany,* **46,** 301-315.

Dextrase, A. & Mandrak, N. (2006) Impacts of alien invasive species on freshwater fauna at risk in Canada. *Biological Invasions,* **8,** 13-24.

Elakovich, S. D. & Wooten, J. W. (1989) Allelopathic aquatic plants for aquatic weed management. *Biologia Plantarum,* **31,** 479-486.

Ellis, J. B., Shutes, R. B. E. & Revitt, D. M. (2003) *Guidance Manual for Constructed Wetlands* (ed R&D Technical Report P2-159/TR2), pp. 90. Environment Agency, UK.

Elton, C. S. (2000) *The Ecology of Invasions by Animals and Plants*. University of Chicago Press, Chicago.

Erhard, D. & Gross, E. M. (2006) Allelopathic activity of *Elodea canadensis* and *Elodea nuttallii* against epiphytes and phytoplankton. *Aquatic Botany,* **85,** 203-211.

Erhard, D. (2006) Allelopathy in aquatic environments. *Allelopathy: A Physiological Process with Ecological Implications* (eds M. J. Reigosa, N. Pedrol & L. Gonzalez), pp. 433-450. Springer, Dordrecht, Netherlands.

Ferrier, M. D., Butler, B. R. & Terlizzi, E. (2005) The effects of barley straw (*Hordeum vulgare*) on the growth of freshwater algae. *Bioresource Technology,* **96,** 1788-1795.

Fisher, J. & Acreman, M. C. (2004) Wetland nutrient removal: a review of the evidence. *Hydrology and Earth System Science,* **8,** 673-685.

Fitzgerald, G. P. (1969) Some factors in the competition or antagonism among bacteria, algae and aquatic weeds. *Journal of Phycology,* **5,** 351-9.

Fleming, J. P. & Dibble, E. D. (2015) Ecological mechanisms of invasion success in aquatic macrophytes. *Hydrobiologia*, **746,** 23-37.

Frank, P. A. & Dechoretz, N. (1980) Allelopathy in dwarf spikerush (*Eleocharis coloradoensis*). *Weed Science,* **28,** 499-505.

Funk, J. L. & Vitousek, P. M. (2007) Resource-use efficiency and plant invasion in low-resource systems. *Nature*, **446,** 1079-1081.

Gallardo, B., Clavero, M., Sánchez, M. I. & Vilà, M. (2016) Global ecological impacts of invasive species in aquatic ecosystems*. Global Change Biology*, **22,** 151-163.

Gérard, J., Brion, N. & Triest, L. (2014) Effect of water column phosphorus reduction on competitive outcome and traits of *Ludwigia grandiflora* and *L. peploides*, invasive species in Europe. *Aquatic Invasions*, **9,** 157-166.

Gibbs, R. D. (1974) *Chemotaxonomy of Flowering Plants*. McGill & Queens University Press, Montreal.

Gopal, B. & Goel, U. (1993) Competition and allelopathy in aquatic plant-communities. *Botanical Review,* **59,** 155-210.

Gopal, B. (1999) Natural and constructed wetlands for wastewater treatment: potentials and problems. *Water Science and Technology,* **40,** 27-35.

Gross, E. (2000) Seasonal and spatial dynamics of allelochemicals in the submersed macrophyte *Myriophyllum spicatum* L. *Verhandlungen der Internationalen Vereinigung für Limnologie,* **27,** 2116-2119.

Gross, E. M. (1999) Allelopathy in benthic and littoral areas: Case studies on allelochemicals from benthic cyanobacteria and submerged macrophytes. *Principles and Practices in Plant Ecology: Allelochemical Interactions* (eds K. M. M. Inderjit, Dakshini & C. L. Foy), pp. 179-199. CRC Press, Boca Raton, FL.

Gross, E. M. (2003a) Allelopathy of aquatic autotrophs. *Critical Reviews in Plant Sciences,* **22,** 313-340.

Gross, E. M. (2003b) Differential response of tellimagrandin II and total bioactive hydrolysable tannins in an aquatic angiosperm to changes in light and nitrogen. *Oikos,* **103,** 497-504.

Gross, E. M., Erhard, D. & Ivanyi, E. (2003a) Allelopathic activity of *Ceratophyllum demersum* L. and *Najas marina* ssp. *intermedia* (Wolfgang) Casper. *Hydrobiologia,* **506,** 583-589.

Gross, E. M., Feldbaum, C. & Graf, A. (2003b) Epiphyte biomass and elemental composition on submersed macrophytes in shallow eutrophic lakes. *Hydrobiologia*, **506-509,** 559-565.

Gross, E. M., Hilt, S., Lombardo, P. & Mulderij, G. (2007) Searching for allelopathic effects of submerged macrophytes on phytoplankton—State of the art and open questions. *Hydrobiologia,* **584,** 77-88.

Gross, E. M., Meyer, H. & Schilling, G. (1996) Release and ecological impact of algicidal hydrolysable polyphenols in *Myriophyllum spicatum*. *Phytochemistry,* **41,** 133-138.

Gross, E. M., Wolk, C. P. & Juttner, F. (1991) Fischerellin, a new allelochemical from the freshwater cyanobacterium *Fischerella muscicola. Journal of Phycology,* **27,** 686-692.

Grotkopp, E. & Rejmánek, M. (2007) High seedling relative growth rate and specific leaf area are traits of invasive species: phylogenetically independent contrasts of woody angiosperms. *American Journal of Botany*, **94,** 526-532.

He, F., Deng, P., Wu, X. H., Cheng, S. P., Gao, Y. N. & Wu, Z. B. (2008) Allelopathic effects on *Scenedesmus obliquus* by two submerged macrophytes *Najas minor* and *Potamogeton malaianus*. *Fresenius Environmental Bulletin,* **17,** 92-97.

Herb, W. R. & Stefan, H. G. (2006) Seasonal growth of submersed macrophytes in lakes: the effects of biomass density and light competition. *Ecological Modelling,* **193,** 560-574.

Hidding, B., Bakker, E. S., Hootsmans, M. J. M. & Hilt, S. (2016) Synergy between shading and herbivory triggers macrophyte loss and regime shifts in aquatic systems. *Oikos,* **125,** 1489-1495.

Hilt, S. & Gross, E. M. (2008) Can allelopathically active submerged macrophytes stabilise clear-water states in shallow lakes? *Basic and Applied Ecology,* **9,** 422-432.

Hilt, S., Ghobrial M. G. N. & Gross, E. M. (2006) In situ allelopathic potential of *Myriophyllum verticillatum* (Haloragaceae) against selected phytoplankton species. *Journal of Phycology,* **42,** 1189-1198.

Horppila, J. & Nurminen, L. (2003) Effects of submerged macrophytes on sediment resuspension and internal phosphorus loading in Lake Hiidenvesi (southern Finland). *Water Research,* **37,** 4468-4474.

Hu, H. & Hong, Y. (2008) Algal-bloom control by allelopathy of aquatic macrophytes-a review. *Frontiers of Environmental Science & Engineering in China* **2,** 421-438.

Hutchinson, G. E. (1975) *A Treatise on Limnological Botany.* Wiley, New York.

Inderjit, Callaway, R. M. & Vivanco, J. M. (2006) Can plant biochemistry contribute to understanding of invasion ecology? *Trends in Plant Science,* **11,** 574–580.

Inderjit, Evans, H., Crocoll, C., Bajpai, D., Kaur, R., Feng, Y.-L., Silva, C., Carreón, J. T., Valiente-Banuet, A., Gershenzon, J. & Callaway, R. M. (2011) Volatile chemicals from leaf litter are associated with invasiveness of a neotropical weed in Asia. *Ecology*, **92,** 316-324.

Jahnke, L. S., Eighmy, T. T. & Fagerberg, W. R. (1991) Studies of *Elodea nuttallii* grown under photorespiratory conditions. I. Photosynthetic characteristics. *Plant, Cell and Environment*, **14,** 147-156.

Jarchow, M. E. & Cook, B. J. (2009) Allelopathy as a mechanism for the invasion of *Typha angustifolia*. *Plant Ecology*, **204,** 113-124.

Jasser, I. (1995) The influence of macrophytes on a phytoplankton community in experimental conditions. *Hydrobiologia,* **306,** 21-32.

Jeppesen, E., Lauridsen, T. L., Kairesalo, T. & Perrow, M. R. (1997) Impact of submerged macrophytes on fish-zooplankton interactions in shallow lakes. *The Structuring Role of Submerged Macrophytes in Lakes* (eds E. Jeppesen, M. Søndergaard, M. Søndergaard & K. Christoffersen), pp. 91-114. Springer-Verlag, New York, NY.

Jones, H. L. (1995) Allelopathic ability of various aquatic plants to inhibit the growth of *Hydrilla verticillata* (L.f.) Royle and *Myriophyllum spicatum* L. *Aquatic Plant Control Research Program*. U.S. Army Corps of Engineers, Waterways Experiment Station.

Jupp, B. & Spence, D. (1977) Limitations on macrophytes in a eutrophic lake, Loch Leven: I. Effects of phytoplankton. *The Journal of Ecology*, **65,** 175-186.

Kadlec, R. & Brix, H. (1995) *Wetland Systems for Water Pollution Control, 1994.* Elsevier Science Publishing Co.

Kay, S. H. & Hoyle, S. T. (2001) Mail order, the Internet, and invasive aquatic weeds. *Journal of Aquatic Plant Management*, **39,** 88-91.

Keating, K. I. (1977) Allelopathic influence on blue-green bloom sequence in a eutrophic lake. *Science,* **196**, 885-887.

Keating, K. I. (1978) Blue-green algal inhibition of diatom growth: transition from mesotrophic to eutrophic community structure. *Science,* **199,** 971-973.

Kirk, J. T. O. (2010) *Light and Photosynthesis in Aquatic Ecosystems.* Cambridge University Press, Cambridge.

Kolar, C. S. & Lodge, D. M. (2000) Freshwater nonindigenous species: interactions with other global changes. *Invasive Species in a Changing World*, 3-30.

Körner, S. & Nicklisch, A. (2002) Allelopathic growth inhibition of selected phytoplankton species by submerged macrophytes. *Journal of Phycology,* **38,** 862-871.

Kufel, L. & Kufel, I. (2002) *Chara* beds acting as nutrient sinks in shallow lakes-a review. *Aquatic Botany,* **72,** 249-260.

Lassuy, D. R. (1995) Introduced species as a factor in extinction and endangerment of native fish species. *Uses and Effects of Cultured Fishes in Aquatic Ecosystems* (eds H. L. Schramm & R. G. Piper), pp. 391-396. American Fisheries Society, Bethesda.

Leflaive, J. & Ten-Hage, L. (2007) Algal and cyanobacterial secondary metabolites in freshwaters: a comparison of allelopathic compounds and toxins. *Freshwater Biology,* **52,** 199-214.

Li, F. M. & Hu, H. Y. (2005) Isolation and characterization of a novel antialgal allelochemical from *Phragmites communis*. *Applied and Environmental Microbiology,* **71,** 6545–6553.

Liu, H. & Stiling, P. (2006) Testing the enemy release hypothesis: a review and meta-analysis. *Biological Invasions*, **8,** 1535-1545.

Lockwood, J. L., Hoopes, M. F. & Marchetti, M. P. (2013) *Invasion Ecology*. John Wiley & Sons, Oxford.

Lürling, M., van Geest, G. & Scheffer, M. (2006) Importance of nutrient competition and allelopathic effects in suppression of the green alga *Scenedesmus obliquus* by the macrophytes *Chara*, *Elodea* and *Myriophyllum*. *Hydrobiologia,* **556,** 209-220.

Marko, M. D., Gross, E., Newman, R. & Gleason, F. K. (2008) Chemical profile of the North American native *Myriophyllum sibiricum* compared to the invasive *M. spicatum*. *Aquatic Botany,* **88,** 57-65.

Maron, J. L. & Vilà, M. (2001) When do herbivores affect plant invasion? Evidence for the natural enemies and biotic resistance hypotheses. *Oikos*, **95**, 361-373.

Martin, G. D. & Coetzee, J. A. (2011) Pet stores, aquarists and the internet trade as modes of introduction and spread of invasive macrophytes in South Africa. *Water SA*, **37,** 371-380.

McLay, C. (1974) The distribution of duckweed *Lemna perpusilla* in a small southern California lake: an experimental approach. *Ecology,* **55,** 262-276.

Men, Y. J, Hu, H. Y & M, L. F. (2007) Effects of the novel allelochemical ethyl 2-methylacetoacetate from the reed (*Phragmitis australis* Trin) on the growth of several common species of green algae. *Journal of Applied Phycology,* **19,** 521-527.

Miller, R. R., Williams, J. D. & Williams, J. E. (1989) Extinctions of North American fishes during the past century. *Fisheries*, **14,** 22-38.

Mitch, W. J. & Gosselink, G. J. (1986) *Wetlands.* Van Nostrand Reinhold, New York, USA.

Mitchell, C. E., Agrawal, A. A., Bever, J. D., Gilbert, G. S., Hufbauer, R. A., Klironomos, J. N., Maron, J. L., Morris, W. F., Parker, I. M. & Power, A. G. (2006) Biotic interactions and plant invasions. *Ecology Letters*, **9,** 726-740.

Mohamed, Z. A. & Al Shehri, A. M. (2010) Differential responses of epiphytic and planktonic toxic cyanobacteria to allelopathic substances of the submerged macrophyte *Stratiotes aloides*. *International Review of Hydrobiology,* **95,** 224-234.

Molisch, H. (1937) *Der Einfluss einer Pflanze auf die Andere-Allelopathie.* Fisher, Jena.

Mony, C., Koschnick, T. J., W. T., H. & S., M. (2007) Competition between two invasive Hydrocharitaceae (*Hydrilla verticillata* (L.f.) (Royle) and *Egeria densa* (Planch)) as influenced by sediment fertility and season. *Aquatic Botany,* **86,** 236-242.

Morrison, W. E. & Hay, M. E. (2011) Herbivore preference for native vs. exotic plants: generalist herbivores from multiple continents prefer exotic plants that are evolutionarily naïve. *PLoS One,* **6,** e17227.

Mulderij, G., Mau, B., van Donk, E. & Gross, E. M. (2007b) Allelopathic activity of *Stratiotes aloides* on phytoplankton—towards identification of allelopathic substances. *Hydrobiologia*, **584,** 89-100.

Mulderij, G., Mooij, W. M. & van Donk, E. (2005a) Allelopathic growth inhibition and colony formation of the green alga *Scenedesmus obliquus* by the aquatic macrophyte *Stratiotes aloides*. *Aquatic Ecology,* **39,** 11-21.

Mulderij, G., Mooij, W. M., Smolders, A. J. P. & van Donk, E. (2005b) Allelopathic inhibition of phytoplankton by exudates from *Stratiotes aloides*. *Aquatic Botany,* **82,** 284-296.

Mulderij, G., van Donk, E. & Roelofs, J. G. M. (2003) Differential sensitivity of green algae to allelopathic substances from *Chara*. *Hydrobiologia,* **491,** 261-271.

Mulderij, G., Van Nesc, E. H. & Van Donka, E. (2007a) Macrophyte-phytoplankton interactions: the relative importance of allelopathy versus other factors. *Ecological Modelling,* **204,** 85-92.

Muller, C. H. (1966) The role of chemical inhibition (allelopathy) in vegetational composition. *Bulletin of the Torrey Botanical Club*, **93,** 332-351.

Muller, C. H. (1969) Allelopathy as a factor in ecological process. *Plant Ecology,* **18,** 348-357.

Nakai, S., Inoue, Y., Hosomi, M. & Murakami, A. (1999) Growth inhibition of blue-green algae by allelopathic effects of macrophyte. *Water Science and Technology,* **39,** 47-53.

Nichols, S. A. & Byron, H. S. (1986) Ecological life histories of the three aquatic nuisance plants, *Myriophyllum spicatum*, *Potamogeton crispus* and *Elodea canadensis*. *Hydrobiologia,* **131,** 3-21.

Nuttall, P. M., Boon A. G. & Rowell, M. R. (1997) Review of the design and management of constructed wetlands, Report 180. Construction Industry Research and Information Association, Westminster, London, UK.

Ostrofsky, M. L. & Zettler, E. R. (1986) Chemical defences in aquatic plants. *Journal of Ecology,* **74,** 279-287.

Ozimek, T., Van Donk, E. & Gulati, R. D. (1993) Growth and nutrient uptake by two species of *Elodea* in experimental conditions and their role in nutrient accumulation in a macrophyte-dominated lake. *Hydrobiologia,* **251,** 13-18.

Pakdel, F. M., Sim, L., Beardall, J. & Davis, J. (2013) Allelopathic inhibition of microalgae by the freshwater stonewort, *Chara australis*, and a submerged angiosperm, *Potamogeton crispus*. *Aquatic Botany*, **110,** 24-30.

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.

Planas, D., Sarhan, F., Dube, L., Godmaire, H. & Cadieux, C. (1981) Ecological significance of phenolic compounds of *Myriophyllum spicatum*. *Verhandlungen des Internationalen Verein Limnologie*, **21,** 1492-1496.

Qin, S., Zhou, J., Yang, T., Duan, Y., Huang, P. & Shang, W. (2010) Growth Inhibition of Algae from Landscape Water by Submerged Macrophytes. *Bioinformatics and Biomedical Engineering (ICBBE), 2010 4th International Conference on Bioinformatics and Biomedical Engineering*, pp. 1-3.

Quinn, L. D., Schooler, S. S. & Van Klinken, R. D. (2011) Effects of land use and environment on alien and native macrophytes: lessons from a large-scale survey of Australian rivers. *Diversity and Distributions*, **17,** 132-143.

Sala, O. E., Chapin, F. S., Armesto, J. J., Berlow, E., Bloomfield, J., Dirzo, R., Huber-Sanwald, E., Huenneke, L. F., Jackson, R. B. & Kinzig, A. (2000) Global biodiversity scenarios for the year 2100. *Science*, **287,** 1770-1774.

Sax, D. F., Stachowicz, J. J., Brown, J. H., Bruno, J. F., Dawson, M. N., Gaines, S. D., Grosberg, R. K., Hastings, A., Holt, R. D. & Mayfield, M. M. (2007) Ecological and evolutionary insights from species invasions. *Trends in Ecology & Evolution*, **22,** 465-471.

Scheffer, M. & van Nes, E. H. (2007) Shallow lakes theory revisited: various alternative regimes driven by climate, nutrients, depth and lake size. *Hydrobiologia*, **584,** 455-466.

Scheffer, M. (1998) *Ecology of Shallow Lakes.* Chapman & Hall, London, New York.

Scheffer, M., Carpenter, S., Foley, J. A., Folke, C. & Walkerk, B. (2001) Catastrophic shifts in ecosystems. *Nature,* **413,** 591-596.

Schriver, P., Bogestrand, J., Jeppesen, E. & Sondergaard, M. (1995) Impact of submerged macrophytes on fish-zooplankton-phytoplankton interactions - large-scale enclosure experiments in a shallow eutrophic lake. *Freshwater Biology,* **33,** 255-270.

Schultz, R. & Dibble, E. (2012) Effects of invasive macrophytes on freshwater fish and macroinvertebrate communities: the role of invasive plant traits. *Hydrobiologia,* **684,** 1-14.

Sheoran, A. S. & Sheoran, V. (2006) Heavy metal removal mechanism of acid mine drainage in wetlands: a critical review. *Minerals Engineering,* **19,** 105-116.

Spence, D. H. N. (1982) The zonation of plants in freshwater lakes. *Advances in Ecological Research,* **12,** 37-125.

Spencer, D. F. & Rejmánek, M. (2010) Competition between two submersed aquatic macrophytes, *Potamogeton pectinatus* and *Potamogeton gramineus*, across a light gradient. *Aquatic Botany,* **92,** 239-244.

Steenhagen, D. A. & Zimdahl, R. L. (1979) Allelopathy of leafy spurge (*Euphorbia esula*). *Weed Science,* **27,** 1-3.

Svensson, J. R., Nylund, G. M., Cervin, G., Toth, G. B. & Pavia, H. (2013) Novel chemical weapon of an exotic macroalga inhibits recruitment of native competitors in the invaded range. *Journal of Ecology,* **101,** 140-148.

Tabassum, S. & Leishman, M. R. (2016) Trait values and not invasive status determine competitive outcomes between native and invasive species under varying soil nutrient availability. *Austral Ecology*, **41,** 875-885.

Takeda, F., Nakano, K., Nishimura, O., Shimada, Y., Fukuro, S., Tanaka, H., Hayashi, N. & Inamori, Y. (2011) Allelopathic potential of *Potamogeton pusillus* community against *Microcystis aeruginosa*. *Journal of Water and Environment Technology,* **9,** 21-28.

Thiebaut, G., Rolland, T., Roach, F., Tremolieres, M., Muller, S. & Bergot, F. (1997) Some consequences of the introduction of two macrophyte species, *Elodea canadensis* Michaux and *Elodea nuttallii* St. John, in continental aquatic ecosystems: example of two areas in the North-East of France: Alsace Plain and Northern Vosges. *Bulletin Francais de la Peche et de la Pisciculture*, **344-345,** 441-452.

Timms, R. M. & Moss, B. (1984) Prevention of growth of potentially dense phytoplankton populations by zooplankton grazing, in the presence of zooplanktivorous fish, in a shallow wetland ecosystem. *Limnology and Oceanography*, **29,** 472-486.

van Donk, E. & van de Bund, W. J. (2002) Impact of submerged macrophytes including charophytes on phyto- and zooplankton communities: allelopathy versus other mechanisms. *Aquatic Botany,* **72,** 261-274.

Vanderstukken, M., Declerck, S. A., Decaestecker, E. & Muylaert, K. (2014) Long-term allelopathic control of phytoplankton by the submerged macrophyte *Elodea nuttallii*. *Freshwater Biology*, **59,** 930-941.

Vanderstukken, M., Mazzeo, N. & Van Colen, W. (2011) Biological control of phytoplankton by the subtropical submerged macrophytes *Egeria densa* and *Potamogeton illinoensis*: a mesocosm study. *Freshwater Biology*, **56,** 1837-1849.

Watkins, C., Shireman, J. V. & Haller, W. T. (1983) The influence of aquatic vegetation upon zooplankton and benthic macroinvertebrates in Orange Lake, Florida. *Journal of Aquatic Plant Management*, **21,** 78-83.

Wersal, R. M. & Madsen, J. D. (2011) Influences of water column nutrient loading on growth characteristics of the invasive aquatic macrophyte *Myriophyllum aquaticum* (Vell.) Verdc. *Hydrobiologia,* **665,** 93-105.

Wetzel, R. G. (1988) Water as an environment for plant life. *Vegetation of Inland Waters: Handbook of Vegetation Science* (ed J. J. Symoens), pp. 113-154. Kluwer Academic Publishers, Dordrecht.

Willis, R. J. (1985) The historical bases of the concept of allelopathy. *Journal of the History of Biology,* **18,** 71-102.

Willis, R. J. (2008) *The History of Allelopathy.* Springer, Dordrecht.

Wium-Andersen, S., Anthoni, U. & Houen, G. (1983) Elemental sulphur, a possible allelopathic compound from *Ceratophyllum demersum*. *Phytochemistry,* **22,** 2613-2613.

Wium-Andersen, S., Anthoni, U., Christophersen, C. & Houen, G. (1982) Allelopathic effects on phytoplankton by substances isolated from aquatic macrophytes (Charales). *Oikos,* **39,** 187-190.

Wolf, V. C., Berger, U., Gassmann, A. & Müller, C. (2011) High chemical diversity of a plant species is accompanied by increased chemical defence in invasive populations. *Biological Invasions*, **13,** 2091-2102.

Wu, Z. B., Deng, P., Wu, X. H., Luo, S. & Gao, Y. N. (2007b) Allelopathic effects of the submerged macrophyte *Potamogeton malaianus* on *Scenedesmus obliquus*. *Hydrobiologia,* **592,** 465-474.

Wu, Z. B., Zhang, S. H., Wu, X. H., Cheng, S. P. & He, F. (2007a) Allelopathic interactions between *Potamogeton maackianus* and *Microcystis aeruginosa*. *Allelopathy Journal,* **20,** 327-338.

Xian, Q., Chen, H., Liu, H., Zou, H. & Yin, D. (2006) Isolation and identification of antialgal compounds from the leaves of *Vallisneria spiralis* L. by activity-guided fractionation. *Environmental Science and Pollution Research,* **13,** 233-237.

Yarrow, M., Marin, V. H., Finlayson, M., Tironi, A., Delgado, L. E. & Fischer, F. (2009) The ecology of *Egeria densa* Planchon (Liliopsida: Alismatales): a wetland ecosystem engineer? *Revista Chilena de Historia Natural,* **82,** 299-313.

Zeng, R. S., Mallik, A. U. & Luo, S. M. (2008) *Allelopathy in Sustainable Agriculture and Forestry.* Springer-Verlag, New York, London.

Zhang, S. H., Cheng, S. P., Wang, H. Q., He, F. & Wu, Z. (2009b) Allelopathic interactions between the *Potamogeton* spp. and toxic cyanobacteria (*Microcystis aeruginosa*). *Allelopathy Journal,* **23,** 379-390.

Zhang, T. T., He, M., Wu, A. P. & Nie, L. W. (2009a) Allelopathic effects of submerged macrophyte *Chara vulgaris* on toxic *Microcystis aeruginosa*. *Allelopathy Journal,* **23,** 391-401.

Zhang, T. T., Zheng, C. Y., Hu, W., Xu, W. W. & Wang, H. F. (2010) The allelopathy and allelopathic mechanism of phenolic acids on toxic *Microcystis aeruginosa*. *Journal of Applied Phycology*, **22,** 71-77.

Zhang, T., He, M., Wu, A. & Nie, L. (2008) Allelopathic inhibition of ρ-hydroxybenzoic acid on *Microcystis aeruginosa* Kuetz with no toxicological effects on *Cyprinus carpio* Linnaeus. *Acta Scientiae Circumstantiae,* **28,** 1887-1893.

Zhao, Q. G. & Song, J. (2004) *Wetland Utilization and Protection in China.* Elsevier, Amsterdam.

Zhu, J., Liu, B., Wang, J., Gao, Y. & Wu, Z. (2010) Study on the mechanism of allelopathic influence on cyanobacteria and chlorophytes by submerged macrophyte (*Myriophyllum spicatum*) and its secretion. *Aquatic Toxicology,* **98,** 196-203.

Chapter 2.

Novel chemical weapons: growth inhibition of cyanobacteria and green algae by invasive and native macrophytes

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Declaration for thesis chapter two

Declaration by candidate
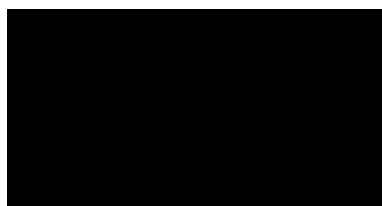
In the case of Chapter 2, the nature and extent of my contribution to the work was the following:

Nature of contribution	Extent of contribution
I mostly conceived and designed the experiments, performed the outdoor and lab work, analysed all of the data and was the primary author of the manuscript	80%

The following co-authors contributed to the work. If co-authors are students at Monash University, the extent of their contribution in percentage terms must be stated:

Name	Nature of contribution	Extent of contribution (%) for student co-authors only
John Beardall	Conception of ideas, supervision and editing	5%
Jenny Davis	Conception of ideas, supervision and editing	5%
Lien Sim	Conception of ideas, supervision and editing	5%
Tricia Wevill	Conception of ideas, supervision and editing	5%

Candidate's Signature   Date: 17/02/2017

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Abstract

1. Some invasive plant species are allelopathic, producing biochemicals that reduce the growth, reproduction and survival of competitors and other organisms. This can confer a competitive advantage, particularly where the invasive species possess novel biochemistry to which the native species are not adapted ('Novel Weapons Hypothesis', NWH). Relatively little is known about the direct phytotoxic effects of invasive plants on microalgae in aquatic systems.

2. We performed assays to test the NWH in freshwater systems by investigating the allelopathic potential of live material from three invasive and four native macrophytes, using several native microalgal species as the target organisms. We furthermore investigated the allelopathic effects of *Cabomba caroliniana* (invasive) and *Potamogeton ochreatus* (native) on cyanobacteria using dialysis cassettes and on free living microalgae with and without nutrient addition.

3. All three invasive macrophytes exhibited the potential for allelopathic impacts on microalgae, with the strongest effect exerted by *C. caroliniana*. In most cases, the effect of *C. caroliniana* was stronger than that of native species. We also found a strong inhibitory effect of *C. caroliniana* and *P. ochreatus* on the growth of *M. aeruginosa* using dialysis cassettes in mesocosms. However, the density of free microalgae in ponds containing *C. caroliniana* was significantly lower than in the ponds with *P. ochreatus* and the controls. The effects were similar in nutrient addition ponds and after nutrient addition to the ponds ceased.

4. These outcomes suggest that these invasive species are allelopathically active. However, the success of allelopathy as an invasion strategy may vary depending on the composition of native microalgal communities already present within a system.

5. *Synthesis*. This study shows that these invasive macrophytes have strong potential allelopathic effects towards microalgae; however, such effects were species-specific in two of the invasive species tested. Our findings also provide evidence that *C. caroliniana* is allelopathically active towards *M. aeruginosa* and other microalgal species in non-laboratory conditions and this mechanism may have an important role in facilitating the success of exotic macrophytes in the invaded areas.

Key-words: allelopathy, biological invasion, cyanobacteria, freshwater macrophytes.

Introduction

The invasion and displacement of native plants by invasive species are major threats that can lead to declines in biodiversity and local extinctions in many systems (Mooney and Cleland, 2001). The impact of invasion is considerably greater in aquatic systems than in terrestrial environments. For example, invasive species are predicted to be the major cause of biodiversity decline in lake systems and are classified as the third major driver of biodiversity loss in streams (after land-use and climate change) in less than a century (Sala et al., 2000).

There are various biotic (e.g. biodiversity of native macrophyte species (angiosperms and macroalgae)) and abiotic (disturbance, water chemistry, water regime, sediment conditions, light availability and shading by riparian vegetation) factors that affect macrophyte invasions in freshwater systems (Thomaz et al., 2015). Furthermore, invasive macrophyte species have specific attributes that enable them to establish and proliferate from their point of introduction. It is important to understand the mechanisms that make some non-indigenous plant species successful invaders, because of the significant impacts of invasive plants on native communities. Understanding these mechanisms helps us to predict the potential spread of the invasive species and to manage and control their future distribution (Jacobs and MacIsaac, 2009; Fleming and Dibble, 2015). Multiple studies have demonstrated a decline in plant biodiversity due to either the direct or the indirect effects of invasive species (Wilcove et al., 1998; Gurevitch and Padilla, 2004; Svensson et al., 2011). Competition is a direct interaction between native and invasive species that can determine individual plant success and ultimately, plant diversity (Fleming and Dibble, 2015). Invasive species in general have a greater capacity to competitively restrain the growth of natives in the new ranges than at home (Callaway et al., 2011; Inderjit et al., 2011a). Allelopathy is another direct interaction that can determine community structure and can alter competitive outcomes (Molisch, 1937; Willis, 1985; 2008). Allelopathy is the production and release of chemical substances by plants that reduces the growth, reproduction and survival of competitors and other organisms (Willis, 2008). This mechanism has been proposed to be used by invasive plants (Steenhagen and Zimdahl, 1979), but has taken longer to be recognised as an effective strategy in invasion biology (Inderjit et al., 2011b).

Exploitative competition for nutrients and allelopathic interactions are two major biological processes that occur between freshwater macrophytes and phytoplankton (Gopal and Goel, 1993; Vanderstukken et al., 2011). These can be major elements that drive the occurrence of alternative states, namely the 'macrophyte-dominated state' and the 'phytoplankton-dominated state' (Scheffer, 1998), in shallow freshwater wetlands. Phytoplankton have a greater ability than freshwater macrophytes to grow under high nutrient conditions because they have a higher rate of biomass development. This can lead to the formation of blooms that cover the surface of the waterbody; limiting light penetration into the deeper parts of the waterbody and shading out submerged macrophytes (Scheffer, 1998; Ansari et al., 2010). Submerged macrophytes on the other hand, can restrain phytoplankton biomass via competition for nutrients and light and chemical interference (van Donk and van de Bund, 2002; Mulderij et al., 2007; Pakdel et al., 2013).

The 'Novel Weapons Hypothesis' (NWH) predicts that allelopathic plant species have the potential to become invasive when introduced into a new environment because the native species are not adapted to the novel biochemistry of the non-indigenous species (Callaway and Aschehoug, 2000). Hence, invasive aquatic plants with allelopathic activity may be capable of controlling the development of phytoplankton or native macrophytes and become competitively superior through chemical interference. This strategy may allow the invasive species to expand their distribution or invade new areas.

The effect of plant biochemistry on biological invasion has been frequently tested among plant species in terrestrial environments. Many studies have also investigated the allelopathic potential of freshwater plants on phytoplankton in their native ranges (Hutchinson, 1975; Anthoni et al., 1980; Blindow, 1987; Gross, 2003; Ferrier et al., 2005; Hu and Hong, 2008) and found that macrophyte species with allelopathic potential can control the

growth of phytoplankton even in nutrient rich environments (Pakdel et al., 2013). However, studies investigating the role of allelopathy in aquatic invasions are fairly recent and scarce, but have shown that some invasive macrophyte species produce allelopathic chemical substances (Jarchow and Cook, 2009; Svensson et al., 2013). Erhard and Gross (2006) found a negative impact of invasive freshwater macrophytes on epiphytic algae and cyanobacteria via chemical intervention. The inhibitory effect of another invasive plant, *Eichhornia crassipes*, was also observed on *Microcystis aeruginosa* (Wu et al., 2012). Moreover, Svensson et al. (2013) found that the marine invasive macroalga, *Bonnemaisonia hamifera* can allelopathically exclude native competitors and hence form mono-specific stands. To date, however, there have been no studies that use laboratory techniques, mesocosm studies or field surveys to explicitly test allelopathy and the direct phytotoxic effects of invasive plants on their competitors in freshwater systems.

Cabomba caroliniana, *Egeria densa* and *Elodea canadensis* are submerged perennial plants that have been introduced, either deliberately or accidentally, to many parts of the world, and are considered noxious weeds in various countries including Australia (Mackey and Swarbrick, 1997; Parsons and Cuthbertson, 2001; Sainty and Jacobs, 2003; ISSG, 2005; Brundu, 2015). There are many problems associated with the introduction and spread of these species including impediment of water flow and interference with the recreational use of waterways. They usually displace native macrophytes and form mono-specific stands, which are a less suitable habitat and food source for zooplankton and other biota. Consequently, they reduce biodiversity and the overall quality of the wetland (Mackey and Swarbrick, 1997; Thiebaut et al., 1997; Parsons and Cuthbertson, 2001; Sainty and Jacobs, 2003).

Our aim was to examine the role of allelopathy in the antagonistic interaction between these three invasive macrophytes and their competitors. We specifically sought to investigate the potential allelopathic effects of these three invasive species on the growth of microalgae (green algae and cyanobacteria) and to test whether the effect of their biochemistry differs from that of co-occurring native macrophytes.

Materials and methods

EXPERIMENT 1-SCREENING OF LIVE MATERIAL ASSAY

We performed 'screening of live material' assays to examine the potential allelopathic activity of the invasive macrophytes on microalgae and compare their activity to those of Australian natives (Table 1). We carried out a plate diffusion assay following the procedures described in Berger and Schagerl (2004) and Pakdel et al. (2013). The experiment was conducted in a 23±1 °C constant temperature room (CT room) with a 14:10 light: dark period and 50 µmol photons PAR m^{-2} s^{-1} during the light phase, these conditions being optimal for growth of the green algae and cyanobacteria used.

Study organisms

We conducted the screening assays using several native and invasive macrophytes as the donor species and native microalgae as the target organism (Table 1). *Cabomba caroliniana* is native to South America. This species has become a highly invasive plant in the United States, Canada and several European, Asian and Pacific countries as well as Australia (Ørgaard, 1991; ISSG, 2005; Brundu, 2015). *Cabomba caroliniana* is currently a weed of national significance in some of these countries. It reproduces sexually by producing seeds and vegetatively via fragmentation (Mackey and Swarbrick, 1997). *Elodea canadensis* and *E. densa* spread via fragmentation and are native to North America and parts of South America respectively (Parsons and Cuthbertson, 2001; Sainty and Jacobs, 2003). All three species grow rooted in sediments, inhabit both standing and flowing waters, and thrive in nutrient-rich environments.

Fresh plant material of both native and invasive macrophytes were collected from different systems during spring and summer from 2013 to 2016. We collected *C. caroliniana* from Lake Nagambie (36°47′0″S 145°8′30″E), an artificial reservoir located in the Goldfields area in northern Victoria. *Potamogeton crispus* and *P. ochreatus* were collected from the Hampton Park Wetland in Hampton Park, Victoria (38°02′14.1″S 145°16′24.9″E). All other macrophyte species were obtained from the Department of Environment and Primary Industries located in Frankston, Victoria. The macrophytes were gently washed under tap water to remove debris and aquatic invertebrates. In order to control for variability in anatomy and physiology of the donor organisms, only young leaves and stems of the macrophytes containing the apical meristem were used.

Microcystis aeruginosa and *A. circinalis* were purchased from the CSIRO Australian National Algae Culture Collection, Hobart, Tasmania. All other microalgal species were obtained from cultures held in the Algal Physiology Laboratory at Monash University, which had previously been isolated from water samples collected on campus. The target organisms were cultured separately in 250 mL sterilised Erlenmeyer flasks with 100 mL BG-11 (*A. variabilis* and *Synechococcus* sp.) and MLA (*A. circinalis M. aeruginosa*, *Chlorella* sp. and *S. quadricauda*) medium modified after Mohamed and Al Shehri (2010) and Bolch and Blackburn (1996) respectively. Flasks were incubated in a CT room at Monash University, Clayton under the same laboratory conditions described above.

Table 1. List of selected macrophytes and microalgal species used in the experiments.

	Invasive	Native
Macrophyte	*Cabomba caroliniana* Gray (water fanwort)	*Ceratophyllum demersum* L. (hornwort)
	Egeria densa Planch (Brazilian elodea)	*Nitella* sp. A. Braun
	Elodea canadensis Michaux (Canadian elodea)	*Potamogeton crispus* L. (curly pondweed)
		Potamogeton ochreatus Raoul (blunt pondweed)
	Cyanobacteria	**Green algae**
Microalgae	*Anabaena circinalis*-CS534, Rabenhorst	*Chlorella* sp.-Chl1
	Source, Tullaroop Reservoir, Victoria, Australia	Source, Monash University, Victoria, Australia
	Anabaena variabilis-Avar1, Kützing	*Scenedesmus quadricauda*-Scenq1 (Turpin)
	Source, Monash University, Victoria, Australia	Brébisson
	Microcystis aeruginosa- CS338, (Kützing)	Source, Monash University, Victoria, Australia
	Lemmermann	
	Source, Gippsland Lakes, Victoria, Australia	
	Synechococcus sp.-Syn1	
	Source, Monash University, Victoria, Australia	

Experimental procedure

Fresh plant material was used in these assays shortly after collection. The effect of potential exudation of allelochemicals by macrophyte shoots on the growth of microalgae was investigated in this experiment using agar-solidified medium in Petri dishes. Macrophytes were considered allelopathically active if the growth of the cyanobacterial or algal species was significantly lower in treatments containing live macrophyte material than controls (without macrophytes). Other factors such as nutrient and light levels and temperature were standardised in the laboratory in order to separate the effect of these factors from those of allelopathy. Thirty mL of 1.0% (distilled water) agar (Merck 1614) formed the basic layer of four replicate sterile Petri dishes. Culture medium (0.2% agar) enriched with the target organism at a cell density of 5×10^5 cells mL^{-1} was added after 2 h. The Petri dishes were then incubated for 24 h. Following the incubation period, 0.5 g per dish (approximately equivalent to 10 g/L in a natural environment) of either macrophyte was placed in the centre of each Petri dish, partially covered by the second layer of culture medium. Controls were made identically, but without the macrophyte material. The density of epiphytic algae was checked using microscopy to ensure that it was negligible. The plates were incubated for 8 days and the areas of microalgal growth inhibition (clear areas) were measured at day 8. Clear areas were quantified by placing a gridded square drawn on a clear plastic (1cm × 1cm)

on top of each Petri dish and measuring the areas that appeared clear to the naked eye. The size of the areas of inhibited growth was used as an indication of the allelopathic potential of the macrophytes.

EXPERIMENT 2-DIALYSIS CASSETTES

We also conducted a co-culture experiment to examine the allelopathic potential of *C. caroliniana* and the native macrophyte, *P. ochreatus* on *M. aeruginosa* in conditions outside the laboratory. When investigating allelopathic interactions taking place outside controlled laboratory conditions, one inherent problem is the presence of organisms associated with the macrophytes such as bacteria, epiphytes and zooplankton. These associated organisms may alter the interaction between macrophytes and microalgae in co-existence studies (Gross et al., 2007). Therefore, in this experiment, we used sterile dialysis cassettes to exclude contamination and grazing by other organisms. Water and dissolved chemicals were able to pass through these membranes without the microalgae escaping.

Study organisms

Potamogeton ochreatus is a native species that occurs in both standing and flowing waters. We chose this perennial plant for further investigation because it grows well in nutrient-rich environments and is very common across a range of wetland types in Victoria (Jessop et al., 1986; Sainty and Jacobs, 2003). *Microcystis aeruginosa* was chosen as the target organism in this experiment because it is one of the main toxin-producing cyanobacterial species that forms blooms in many parts of the world including Australian wetlands (Croome et al., 2012; Falconer, 2012; Williams and Cole, 2013). We collected *C. caroliniana* and *P. ochreatus* from Lake Nagambie and Hampton Park Wetland respectively (details above). *Microcystis aeruginosa* was cultured in 250 mL sterilised Erlenmeyer flasks with 100 mL MLA solution. Flasks were incubated under the same conditions used for experiment 1.

Experimental procedure

The experiment was conducted outdoors in the Jock Marshall Reserve (JMR) at Monash University, Victoria, during summer 2014-2015. Outdoor fibreglass ponds of 1.65 m radius, 0.5 m height and approximately 1000 L capacity were used. We added 15 cm of soil covered with 3 cm washed white sand to each pond to provide a benthic substrate. Rainwater from the tanks near the plant facility centre was used to fill the ponds. Plant material was gently washed free of debris and aquatic organisms and was introduced into the ponds in the first week of December 2014. Filamentous algae were removed from the shoots by hand. In three of the ponds, 15 cm shoots of *C. caroliniana* were planted with 2-3 cm of the stem embedded in the sediment and the remaining length, containing the apical meristem, above the sediment. No secondary branches were present on the shoots. *Potamogeton ochreatus* (whole plant) was planted by embedding the rhizomes into the sediment. Macrophytes were planted in the ponds (three replicate mesocosms for each plant species) to cover 50% of the total area of the ponds. The same procedure was applied to the controls, but no macrophytes were added. The ponds were covered by bird netting (1 mm^2 mesh size, 10-15% shading) to prevent wind or animal dispersal of invasive species into the nearby JMR wetland. Three slow-release fertiliser tablets (Osmocote, Plus Trace Elements - Water Gardens & Aquatic Plants, Scotts, Australia) were added to each pond every two weeks to mitigate potential nutrient limitation and competition on cyanobacterial growth.

Introduction of dialysis cassettes containing *M. aeruginosa* into the ponds took place after one year at the end of January 2015. We measured cell density of the cyanobacteria prior to the experiment using a haemocytometer and a compound microscope. We also measured optical density (OD) of the same sample at 750 nm in a UV visible spectrophotometer (Cary, 50 Bio). Our preliminary results showed a high positive correlation between cell density and OD$_{750}$; therefore, we used OD$_{750}$ as a parameter to determine the growth of the cyanobacteria. Sterile dialysis cassettes (Slide-A-Lyzer dialysis cassette, 10000MWCO, 0.5-3 mL, Thermo Fisher Scientific) were soaked in

distilled water for one minute prior to use. Unialgal cultures of *M. aeruginosa* (3 mL) with an initial concentration of $OD_{750} = 0.61$ were added to the cassettes and were placed in the mesocosms. We suspended three cassettes in each pond, just below the surface of the water near the plants, by attaching them to floats. The OD_{750} of the cyanobacteria grown in the control and the treatment ponds was then measured every day for five days. The OD_{750} reading of each replicate at each sampling date was recorded to determine the growth rate of *M. aeruginosa* in the presence of macrophytes over time.

EXPERIMENT 3-CO-CULTURE ASSAY

Furthermore, we tested the allelopathic potential of invasive and native macrophytes by measuring natural populations of microalgae growing in ponds (used in experiment 2) containing *C. caroliniana* and comparing it to the ponds with *P. ochreatus* and the controls with and without nutrient addition. Two water samples (5 mL) were collected from the surface water no more than 2 cm deep from within each pond in mid-February 2015 (total replicates of six pooled water samples). The OD_{750} of the water samples from the control and the treatment ponds were measured using the same procedures described in experiment 2. We then discontinued nutrient addition to the ponds in order to investigate the effects of potential nutrient limitation on the bioactivity of the two macrophytes on microalgae. The OD_{750} measurements were repeated after one year in mid-Feb 2016. The OD_{750} of the samples from the macrophyte treatment was compared to the controls and between the two years. Given the complex structure of macrophytes such as *C. caroliniana* and *P. ochreatus*, measuring surface area of the plants is difficult. As a consequence, we used % cover as a proxy for plant biomass.The % cover of the macrophytes in the ponds was measured and was compared between the two sampling occasions to ensure that plant density was not affected by possible nutrient limitation.

STATISTICAL ANALYSIS (ALL EXPERIMENTS)

We used single factor ANOVAs to compare the final percentage of cleared areas of each microalgal species caused by the macrophytes between the different treatments and controls at the end of experiment 1. A Linear Mixed Effects Model (LMEM) was undertaken to compare the cyanobacterial OD_{750} in the treatments with the controls in the experiments 2. Treatment (macrophytes or control) and time (day) were included as fixed effects in the analysis. Replicates were used as a random effect to avoid pseudo-replication (Logan, 2010). Two-factor ANOVAs were run to compare the microalgal OD_{750} in the treatment (containing plants) and the control ponds in different nutrient conditions in the experiment 3. All data were checked for normality and homoscedasticity. We transformed data when necessary to improve normality. Data recorded as percentages (% of microalgae cleared in petri dish) were arcsine square root-transformed. Tukey's *post hoc* tests were then performed to assess significant differences between different treatments. We used the R statistical programme (RStudio, ver. 3.3.1 (2016-06-21)) with a criterion of $\alpha = 0.05$ for statistical significance.

Results

EXPERIMENT 1-SCREENING OF LIVE MATERIAL ASSAY

We found significant effects of macrophyte presence on the clearing area of all target species (Table 2). The effect of macrophytes on microalgal growth was species-specific (Fig. 1, Table 3). Both native and invasive macrophytes (except *E. densa* and *P. ochreatus*) inhibited the growth of all cyanobacteria (Fig. 1, Table 3). Furthermore, green algae exhibited less sensitivity than cyanobacteria when exposed to both native and invasive macrophytes. The effect of *C. caroliniana* was stronger than most native species (Fig. 1, Table 3).

Table 2. Single factor ANOVAs results: the effect of macrophyte treatment on the growth of green algal and cyanobacterial species (cleared areas caused by the presence of macrophytes by the end of experiment 1, *** = $p < 0.001$).

	df	F-value	p-value
Cyanobacteria			
Anabaena circinalis	7	23.76	***
Anabaena variabilis	7	40.79	***
Microcystis aeruginosa	7	50.17	***
Synechococcus sp.	7	19.74	***
Chlorophytes			
Chlorella sp.	7	5.084	***
Scenedesmus quadricauda	7	21.81	***
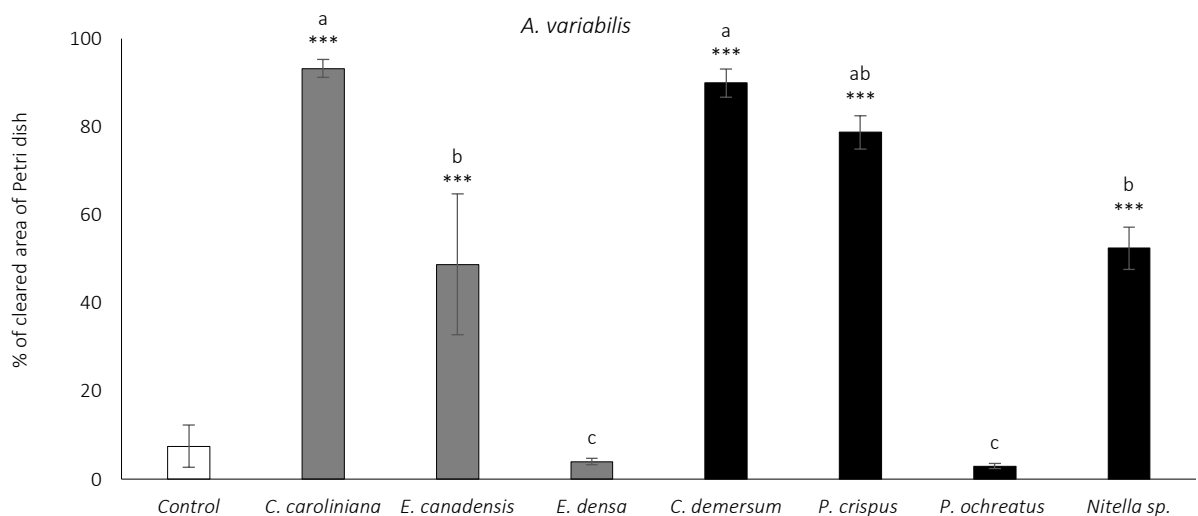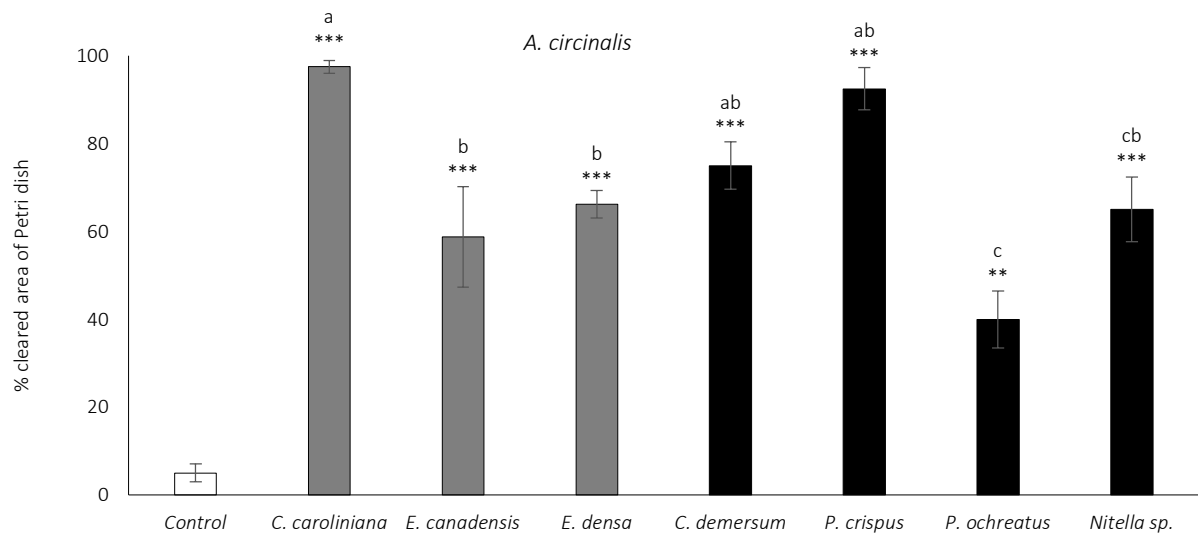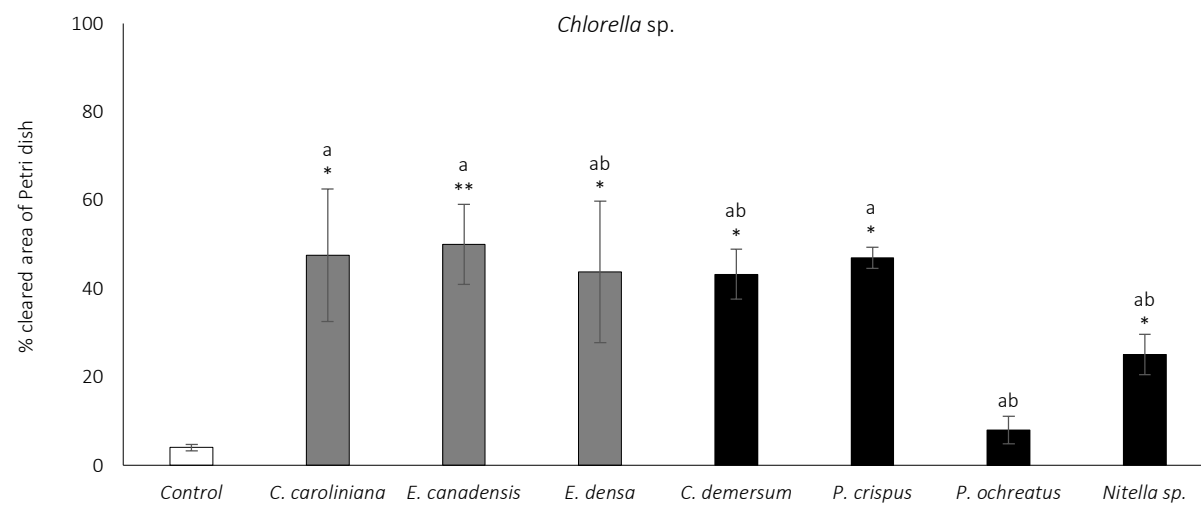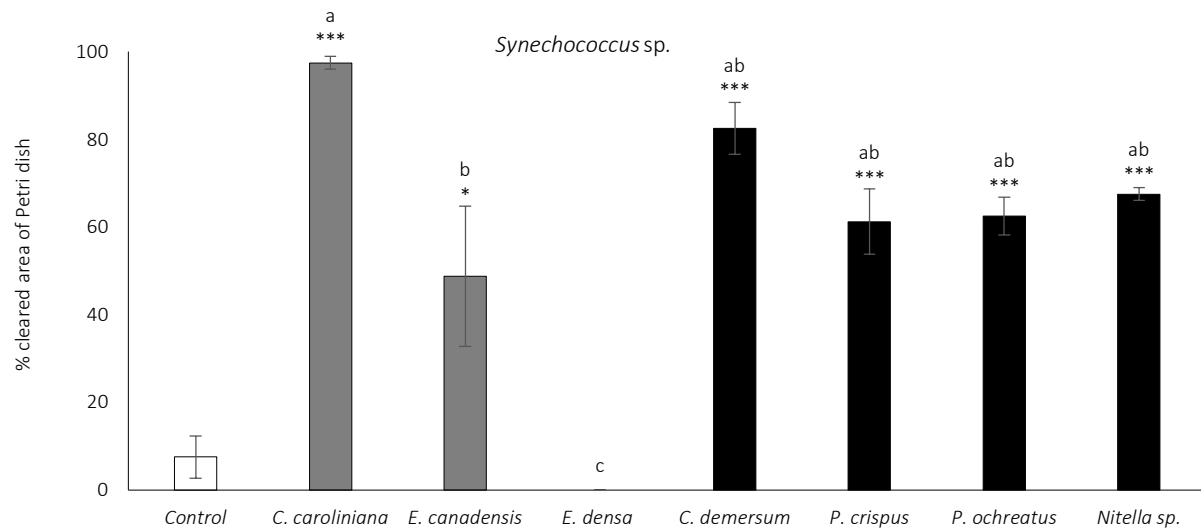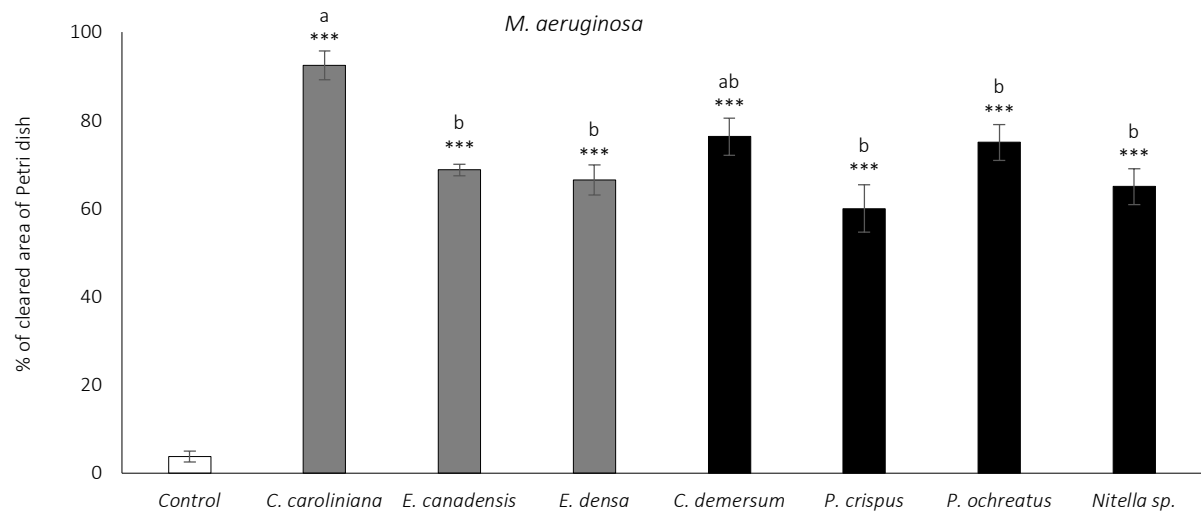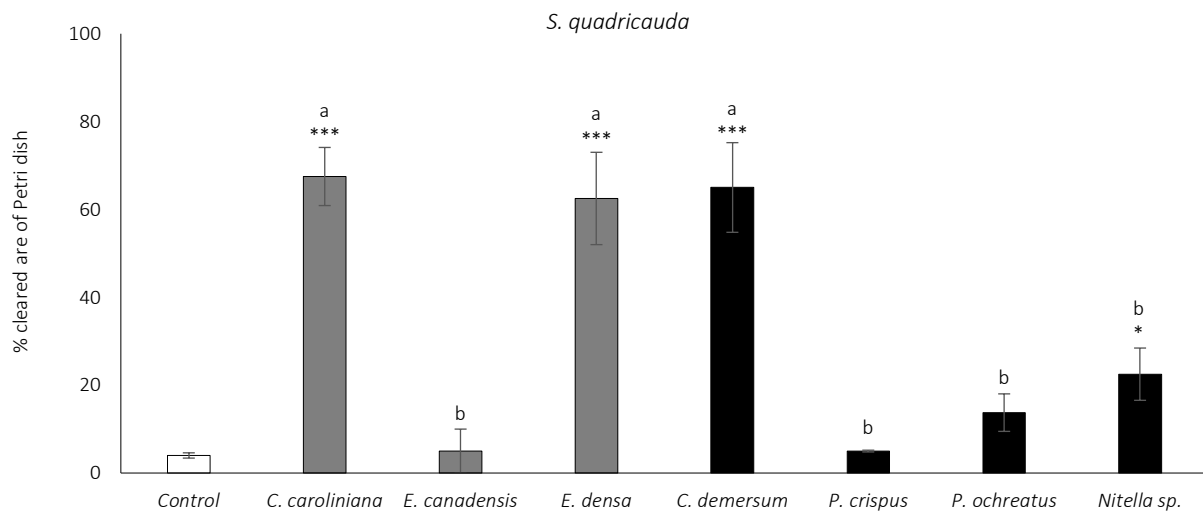
Fig. 1. Mean (±SE) percentage of Petri dish area cleared of the microalgal species in the presence of native (black bars) and invasive (grey bars) macrophytes live material and controls (white bars) at day 8 after initial inoculation (n = 4). Asterisks indicate significant differences compared to controls (* = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$) and letters represent significant differences comparing the growth inhibitory effects of native and invasive macrophytes on the target species ($\alpha = 0.05$).

Note: the clearing area of *Synechococcus* sp. = 0 in the presence of *E. densa*.

Table 3. Outcome of Tukey's *post hoc* tests comparing the effects of live material of different native and invasive macrophytes and controls on the growth of cyanobacteria and green algae. (* = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$).

Macrophytes	A. circinalis	A. variabilis	M. aeruginosa	Synechococcus sp.	Chlorella sp.	S. quadricauda
C. caroliniana - E. densa	*	***	***	***	0.99	0.99
C. caroliniana - C. demersum	0.19	0.99	0.07	0.99	0.99	0.99
C. caroliniana - Nitella sp.	*	**	***	0.99	0.60	**
C. caroliniana - E. canadensis	**	**	**	*	0.99	***
C. caroliniana - P. crispus	0.99	0.75	*	0.90	1.00	***
C. caroliniana - P. ochreatus	***	***	***	0.98	*	***
C. caroliniana - Control	***	***	***	***	*	***
E. densa - C. demersum	0.96	***	0.55	***	1.00	0.99
E. densa - Nitella sp.	0.99	***	0.99	***	0.78	**
E. densa - E. canadensis	0.98	***	0.99	**	0.99	***
E. densa - P. crispus	0.08	***	0.69	***	0.99	***
E. densa - P. ochreatus	*	1.00	0.92	***	0.11	***
E. densa - Control	***	0.98	***	0.99	*	***
E. canadensis - C. demersum	0.56	***	0.82	0.08	0.99	***
E. canadensis - Nitella sp.	0.99	0.99	0.99	0.08	0.48	0.55
E. canadensis - P. crispus	0.39	0.052	0.92	0.23	0.99	0.97
E. canadensis - P. ochreatus	*	***	0.69	0.10	*	0.99
E. canadensis - Control	***	**	***	*	**	0.99
C. demersum - Nitella sp.	0.93	***	0.40	1.00	0.80	**
C. demersum - P. crispus	0.47	0.37	0.99	0.99	0.99	***
C. demersum - P. ochreatus	**	***	0.07	1.00	0.12	***
C. demersum - Control	***	***	***	***	*	***
Nitella sp. - *P. crispus*	0.11	0.12	0.55	0.99	0.85	0.26
Nitella sp. - *P. ochreatus*	0.06	***	0.97	1.00	0.63	0.97
Nitella sp. - Control	***	**	***	***	0.48	0.26
P. crispus - P. ochreatus	***	***	0.12	0.99	0.06	0.80
P. crispus - Control	***	***	***	***	*	0.80
P. ochreatus - Control	**	0.98	***	***	0.99	1.00

EXPERIMENT 2-DIALYSIS CASSETTES

The growth of *M. aeruginosa* was reduced when exposed to *C. caroliniana* or *P. ochreatus* compared to the controls (Fig. 2). We found significant effects of treatment (F = 32.34, *df* = 37, *p* < 0.001) and time (F = 9.39, *df* = 37, *p* < 0.01), however, the effects of their interaction was non-significant. Tukey's *post hoc* tests revealed significant differences between the OD_{750} of the cyanobacteria where *C. caroliniana* or *P. ochreatus* were present compared to the controls (*p* < 0.001, Fig. 2). Nonetheless, no significant differences were found in the OD_{750} of the cyanobacteria in the presence of *C. caroliniana* compared to *P. ochreatus* (Fig. 2).
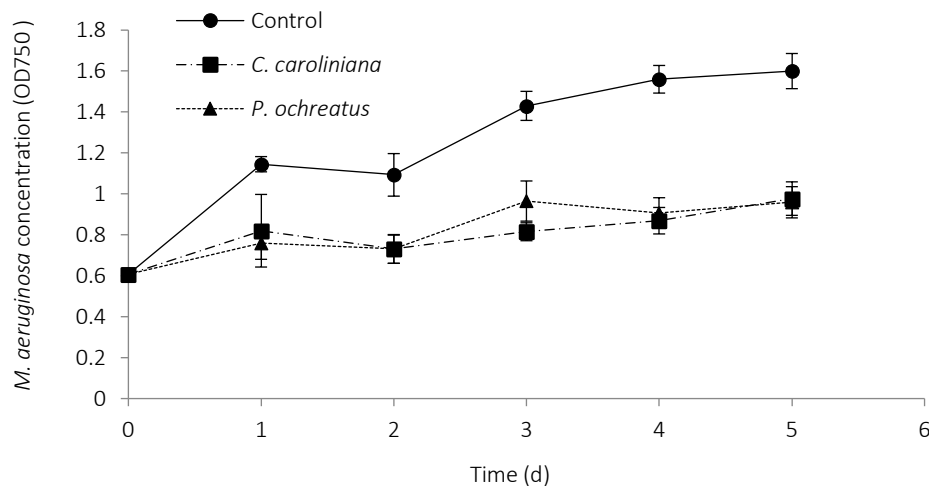
Fig. 2. Mean (±SE) concentration (OD_{750}) of *Microcystis aeruginosa* in the presence of macrophytes and control at different days (n = 9).

EXPERIMENT 3-CO-CULTURE ASSAY

We found a lower algal density in the ponds where *C. caroliniana* was present compared to *P. ochreatus* and controls. There was a significant effect of macrophyte treatment (F = 20.11, *df* = 2, *p* < 0.001), although, the effects of time (year) and their interaction were not significant. We found a significant difference between the OD_{750} of water samples taken from the ponds containing *C. caroliniana* compared to the controls (*p* < 0.001, Fig. 3). The OD_{750} of water samples obtained from the ponds with *P. ochreatus* was significantly higher than the ponds with *C. caroliniana* (*p* < 0.001, Fig. 3). However, the OD_{750} of water samples form *P. ochreatus* and the control ponds were similar (Fig. 3). Furthermore, there was no significant difference in the % cover of the two macrophytes with and without nutrient addition (Fig. 4).
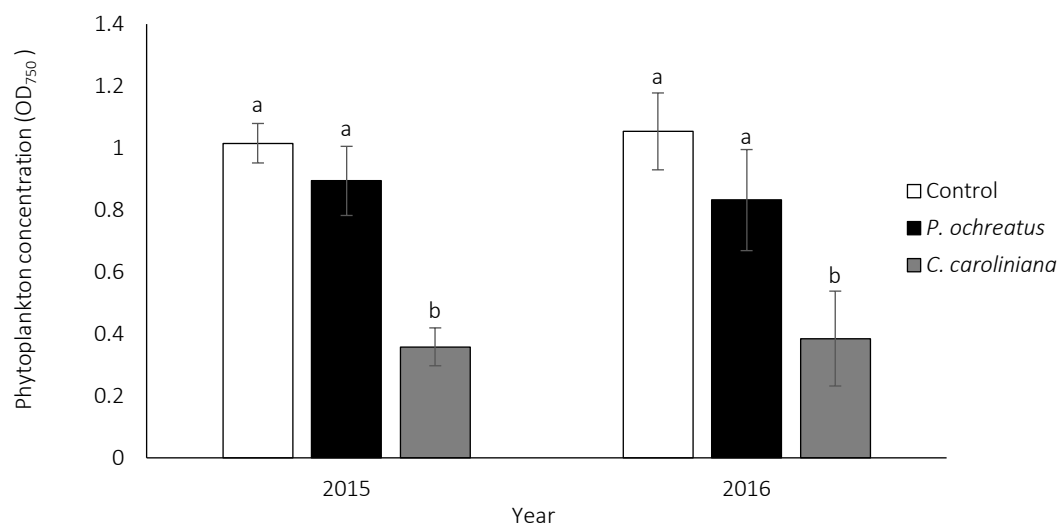
Fig. 3. Mean (±SE) concentration (OD_{750}) of phytoplankton in the ponds containing macrophytes (either native or invasive) and the controls (no macrophytes) with (2015) and without (2016) nutrient addition (n = 6).
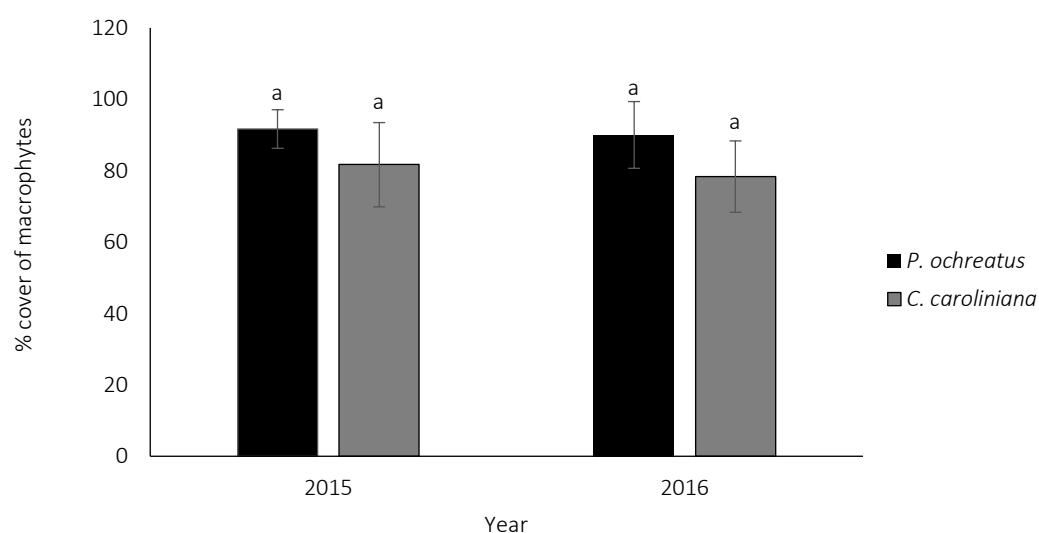
Fig. 4. Mean (±SE) % cover of *Potamogeton ochreatus* (native) and *Cabomba caroliniana* (invasive) in the nutrient addition condition (2015) and after nutrient addition stopped (2016) (n = 3).

Discussion

The presence of both native and invasive macrophytes had significant deleterious effects on the growth of the target microalgal species, although *C. caroliniana* had a stronger bioactivity than most native species. In order to focus on the potential effects of naturally-released chemical metabolites on target species, we minimised the effects of nutrient limitation in all three experiments (throughout experiments 1 and 2 and in the first part of experiment 3) and controlled the effects of light and temperature on the interactions between the species in the live material assays. The lack of an effect using highly competitive species such as *E. densa* and *E. canadensis* may also indicate that microalgae were not nutrient limited and there might be some other strategies such as potential allelopathy that affected the target organisms (which was weak on some target species and stronger on others).

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Moreover, we conducted the mesocosm study with and without nutrient addition and found that nutrient level did not impact the bioactivity of the macrophytes. In addition, the % cover of the macrophyte stands was not affected after nutrient addition ceased. The natural community of algae also showed a consistent density despite nutrient addition/no addition in all macrophyte treatments and controls, which indicates that nutrient limitation had a minor effect on the density of phytoplankton. We also excluded bacterial contamination by using sterile dialysis cassettes. Although zooplankton grazing had no substantial impact on phytoplankton biomass in a previous mesocosm study (Vanderstukken et al., 2011), we excluded the effects of grazing by conducting live material assays in experiment 1 and using dialysis cassettes in experiment 2. Studies using purified macrophyte compounds or plant culture filtrates can demonstrate the algicidal activity of submerged macrophyte tissues, but they provide no substantial ecological evidence about the natural release of allelochemicals in *situ* (Gross et al., 2007). Glomski et al. (2002) for instance, identified secondary metabolites in the tissues of two invasive macrophytes, *Hydrilla verticillata* and *Myriophyllum spicatum*; however, these compounds could not be detected in the water column. Hence, we endeavoured to employ a set of methodologies that considered all these factors, which has enabled us to conclude with a greater certainty that the growth inhibition of the target species was largely due to the allelopathic potential of the macrophytes.

We found strong allelopathic effects of the three invasive species on the microalgae in most donor-target species combinations in experiment 1, although the effects were species-specific in the presence of *E. canadensis* and *E. densa*. Species-specific allelopathic potential was also evident in most native macrophytes, which could be due to adaptation of microalgae to allelopathy of the particular donor plant (Reigosa et al., 1999; Müller-Schärer et al., 2004). *Elodea canadensis* inhibited the growth of all cyanobacterial and one green algal species. Cyanobacteria are known to have a higher susceptibility to allelopathy and have been inhibited more strongly than green algae and diatoms by allelopathic activity of a broad range of macrophytes (Körner and Nicklisch, 2002; Hilt et al., 2006; Hilt and Gross, 2008; Bauer et al., 2009; Mohamed and Al Shehri, 2010; Pakdel et al., 2013). However, *E. densa* did not exert any effects on *A. variabilis* and furthermore, it stimulated the growth of *Synechococcus* sp. Growth stimulation of *Scenedesmus brevispina* was also evident in the presence of crude extracts of *E. canadensis* (Erhard and Gross, 2006), which indicates that some allelopathic compounds may benefit the target organisms more than they harm. Our findings suggest that species-specific allelopathic effects of macrophytes can lead to a decline in the abundance of certain species, mainly cyanobacteria, in natural systems, which may in turn change the structure of microalgal communities exposed to these chemicals. *Egeria densa* for instance, was shown to reduce the relative abundance of *Scenedesmus* species and that led to changes in overall community structure of phytoplankton (Vanderstukken et al., 2011).

Several studies have demonstrated that *E. canadensis* and *E. densa* have the capacity to diminish the growth of different phytoplankton species by releasing chemical substances under non-laboratory conditions (Erhard and Gross, 2006; Vanderstukken et al., 2011), although *Elodea* species have revealed only a moderate bioactivity compared with other macrophytes (Hilt and Gross, 2008). To our knowledge, however, no previous study has tested the allelopathic activity of *C. caroliniana* under experimental field conditions.

Some plants have the potential to suppress other organisms via chemical intervention outside their native ranges, despite the absence of an effect in their natural environment (Callaway et al., 2008). These species, therefore, can become more abundant in the invaded areas than in their native ranges because they either face weaker recipient(s) or have stronger effects outside their home range. Nevertheless*, C. caroliniana* and *E. canadensis* have shown allelopathic effects inside and/or outside their natural distribution under controlled conditions (Nakai et al., 1996; 1999; Erhard and Gross, 2006). *Cabomba caroliniana* is more aggressive than *E. canadensis* and *E. densa,* and has displayed a greater level of invasiveness around the globe. In our study, *C. caroliniana* exhibited a potent allelopathic activity greater than *E. canadensis, E. densa* and most of the native species tested. Furthermore, it showed the ability to control algal blooms in outdoor conditions with and without nutrient addition while the native species showed no significant detrimental effects (experiment 3). This may provide

evidence for the existence of novel biochemistry in *C. caroliniana*, which would enable this invasive species to overcome competitors from a different group and could enhance its invasiveness.

Cabomba caroliniana had similar bioactivity to *C. demersum* and, to a lesser degree, *P. crispus* in experiment 1. The physical structure of macrophytes can impact the extent of their bioactivity on microalgae (Gopal and Goel, 1993; Hilt and Gross, 2008). *Cabomba caroliniana* and *C. demersum* have a similar structure and numerous finely dissected leaves; these provide a greater surface area for the active release of allelochemicals into the surrounding environment.

Although some invasive species are known to be chemically defended, both native and invasive plants can have similar levels of chemical defences (Huang et al., 2010; Lind and Parker, 2010). For example, Jarchow and Cook (2009) found that the invasive emergent macrophyte, *Typha angustifolia* produces different soluble phenolic compounds to the native congener, nevertheless, the quantity of the chemicals produced is similar. Wolf et al. (2011) on the other hand, found a higher concentration of volatile secondary metabolites produced by the invasive population of *Tanacetum vulgare* compared to a native population of the same species. Furthermore, they found a great level of chemical diversity in invasive and native populations of *T. vulgare*. Marko et al. (2008) also found higher concentrations of secondary compounds such as lignin and polyphenols in the tissues of the invasive *Myriophyllum spicatum* compared to the closely related native macrophyte, *M. sibiricum*. They attributed the invasion success of *M. spicatum* and the displacement of its native competitors to these differences. Our results indicate there is no general pattern signifying that the use of allelopathy is exclusive to invasive species but rather native species with strong allelopathic potential may possess invasive traits and can use similar processes to advance their ecological success (Lind and Parker, 2010). Therefore, native plants with allelopathic potential similar to that of the invasive macrophytes may have the capacity to control the growth of invasive species. Nevertheless, while both native and invasive species have the potential to produce allelopathic substances, the use of allelochemicals combined with other strategies may provide invasive macrophytes with a competitive advantage over myriad native species in *situ*.

Cabomba caroliniana demonstrated similar allelopathic potential to *P. ochreatus* against *M. aeruginosa* in experiment 2. The growth of *M. aeruginosa* has been impeded by allelopathic potential of several *Potamogeton* species in previous studies (Wu et al., 2007; Zhang et al., 2009; Takeda et al., 2011). Notionally, many allelochemical compounds are labile and can be rapidly metabolised by bacteria when released into the water column (Gross, 1999; Gross, 2003). Therefore, these chemicals should be constantly excreted in order to affect the target organisms. Since these compounds are released into the water column, they need to be sufficiently hydrophilic and adequately concentrated in order to reach and influence the target organisms effectively (Gross, 2003). We have not identified the allelochemicals produced by *C. caroliniana* and *P. ochreatus* in our study; nonetheless, we believe that the allelochemicals of these species are most likely hydrophilic, highly concentrated and frequently exuded. However, the allelochemicals released by *C. caroliniana* clearly had stronger effects compared to *P. ochreatus* in experiment 3 and perhaps could supress the growth of a wider range of target organisms. This suggests that the strength of allelochemicals produced by *C. caroliniana* was greater than *P. ochreatus* and could affect a wider range of microalgae. Further studies to test the composition of the chemicals released by these macrophytes, in particular by *C. caroliniana*, are warranted.

In contrast to Müller-Schärer et al. (2004), Vanderstukken et al., (2014) found that phytoplankton species were not capable of building resistance to allelochemicals released by *Elodea*. Thus, the effects of the novel biochemistry of species such as *C. caroliniana* may also persist if the target species do not adapt to the allelochemicals produced. Accordingly, *C. caroliniana* may have a prolonged negative impact on a native community of microalgae via allelopathy. The decomposing material of some macrophyte species is also capable of producing allelochemicals (Hu and Hong, 2008; Zeng et al., 2008), therefore, the allelopathic activity of some species may persist even after the plant is manually removed from a system.

Although the immediate allelopathic effects of *E. canadensis* and *E. densa* may only partially account for the success of these invasive species, traits such as fast growth rate and enhanced photosynthetic ability under a wide range of environmental conditions allow macrophytes such as *Elodea* and *Egeria* to become invasive (Jahnke et al., 1991; Dendène et al., 1993; Ozimek et al., 1993). For instance, *E. densa* has the capacity to significantly reduce nutrient concentrations in the water column and has a greater competitive ability for nutrient uptake (Mony et al., 2007; Yarrow et al., 2009). This shows that invasion success is driven by a much more complex interaction of different factors, rather than a single factor. Potentially several interrelated mechanisms may work together (Mitchell et al., 2006; Fleming and Dibble, 2015). Two principal elements that govern invasive potential are species-specific traits and environmental characteristics (Jacobs and MacIsaac, 2009; Wersal and Madsen, 2011; Tabassum and Leishman, 2016). Invasion is more likely to occur in disturbed systems (Pimm and Hyman, 1987; Baltz and Moyle, 1993; Quinn et al., 2011). Eutrophication for instance, can positively affect invasive species. Moreover, some invaders have the ability to alter the invaded habitats and increase eutrophication (Gallardo et al., 2016). Since most invasive species thrive in nutrient-rich environments, their reliance on high levels of nutrients can be a key predictor of their future spread as well as the severity of their invasion in a particular system. Nevertheless, the competitive interactions between invasive and native macrophytes are not always mediated by nutrient availability (Vanderstukken et al., 2011; Tabassum and Leishman, 2016). Accordingly, other traits such as allelopathic potential can play a central role in the success of invasive species and provide a competitive advantage in response to nutrient availability. Additionally, invasive species can supress the growth of native macrophytes under a broad range of environmental conditions (Herb and Stefan, 2006). *Cabomba caroliniana,* for example, demonstrated a potent bioactivity against microalgae with and without nutrient addition. This suggests that the allelopathic potential of this species may override nutrient competition or at minimum, be advantageous, enabling *C. caroliniana* to invade eutrophic as well as oligotrophic systems. Although we conducted our mesocosm experiment with and without nutrient additions, the effects should be tested under a wider range of nutrient concentrations.

Most studies of invasion by macrophytes in aquatic systems have concentrated on the impacts of invasive species (consequences) rather than the mechanisms behind invasion success (causes). This has resulted in a large literature on management strategies. However, understanding the mechanisms that are responsible for the success of invasive macrophytes is needed to identify species that are likely to invade a new region or eradicate those that are already present. Furthermore, it would assist us to better predict the future spread of invaders. *Cabomba caroliniana* can be active in eutrophic systems and its effects may persist even if the nutrient loading of the system is removed or minimised. Kuhar et al. (2010) found that *E. canadensis* had a non-uniform distribution in invaded areas and it did not display its invasiveness in heterogeneous systems where a rich macrophyte community containing *Potamogeton* was present. We chose *P. ochreatus* as the native study species in the latter experiments because it is very common in wetlands in south-eastern Australia. We found antagonistic interactions between *C. caroliniana* and *P. ochreatus* in a separate study, which was mediated by the allelopathic potential of the two species (Pakdel et al., submitted). The two macrophytes are rooted and hence use the same source of nutrients from sediments and from the water column. Since the establishment and development of invasive species could be limited if they occupy a similar habitat to the local species (Abram, 1983; Fargione et al., 2003; Von Holle and Simberloff, 2004), *P. ochreatus* may possibly be used to halt the growth of *C. caroliniana* in infested wetlands and limit its further distribution. *Potamogeton crispus* also displayed allelopathic activity similar to *C. caroliniana*. Therefore, *Potamogeton* species can be candidates for further investigation of the interactions between native and invasive macrophytes. *Ceratophyllum demersum* exhibited strong allelopathic potential towards microalgae in our study, with an effect of similar strength to that of *C. caroliniana*. Hence *C. demersum* could also be used in future investigations.

Conclusion

Our study indicates that the novel biochemistry of three invasive species could be used against competitors from different groups of aquatic autotrophs. Furthermore, allelopathy is an effective adaptive trait facilitating the success of these species outside their natural environment. All three invasive macrophytes exhibited allelopathic potential but the effect of *C. caroliniana* was strongest in most situations. *Elodea canadensis* and *E. densa* showed species-specific effects, which may be due to the longer history of their introduction in Australia. This may have provided the native microalgal species with an opportunity to build resistance to the novel biochemistry of these species. Some native species, such as *C. demersum* and *P. crispus,* exhibited a similar allelopathic potential to *C. caroliniana*. This study has taken the first step towards identifying some of the mechanisms that can lead to the successful establishment of the invasive macrophytes in particular, *C. caroliniana.* Further studies of how native and invasive macrophytes interact will help identify potential macrophyte candidates that could regulate the growth and spread of *C. caroliniana* and perhaps other invasive macrophyte species.

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References

Abrams, P. (1983) The theory of limiting similarity. *Annual Review of Ecology and Systematics*, **14,** 359-376.

Ansari, A. A., Gill, S.S., Lanza G.R. & Rast W. (2010) *Eutrophication: Causes, Consequences and Control*. Springer Verlag.

Anthoni, U., C. Christophersen, Madsen, J.O., Wium-Andersen S. & Jacobsen, N. (1980) Biologically active sulfur compounds from the green alga *Chara globularis*. *Phytochemistry*, **19,** 1228-1229.

Baltz, D. M. & Moyle, P. B. (1993) Invasion resistance to introduced species by a native assemblage of California stream fishes. *Ecological Applications*, **3,** 246-255.

Bauer, N., Blaschke, U., Beutler, E., Gross, E. M., Jenett-Siems, K., Siems, K. & Hilt, S. (2009) Seasonal and interannual dynamics of polyphenols in *Myriophyllum verticillatum* and their allelopathic activity on *Anabaena variabilis*. *Aquatic Botany,* **91,** 110-116.

Berger, J. & Schagerl, M. (2004) Allelopathic activity of Characeae. *Biologia*, **59,** 9-15.

Blindow, I. (1987) The composition and density of epiphyton on several species of submerged macrophytes - the neutral substrate hypothesis tested. *Aquatic Botony*, **29,** 157-168.

Bolch, C. & Blackburn, S. (1996) Isolation and purification of Australian isolates of the toxic cyanobacterium *Microcystis aeroginosa* Kutz. *Journal of Applied Phycology*, **8,** 5-13.

Brundu, G. (2015) Plant invaders in European and Mediterranean inland waters: profiles, distribution, and threats. *Hydrobiologia*, **746,** 61-79.

Callaway, R. M. & Aschehoug, E. T. (2000) Invasive plants versus their new and old neighbors: a mechanism for exotic invasion. *Science*, **290,** 521-523.

Callaway, R. M., Cipollini, D., Barto, K., Thelen, G. C., Hallett, S. G., Prati, D., Stinson, K. & Klironomos, J. (2008) Novel weapons: invasive plant suppresses fungal mutualists in America but not in its native Europe. *Ecology*, **89,** 1043-1055.

Callaway, R. M., Waller, L. P., Diaconu, A., Pal, R., Collins, A. R., Mueller-Schaerer, H. & Maron, J. L. (2011) Escape from competition: neighbors reduce *Centaurea stoebe* performance at home but not away. *Ecology,* **92,** 2208-2213.

Croome, R., Wheaton, L., Henderson, B., Oliver, R., Vilizzi, L., Paul, W. & McInerney, P. (2012) Examining 30 years of phytoplankton data for the Murray River, with particular emphasis on the factors determining the presence and abundance of cyanobacteria. *Recent Advances in Cyanobacterial Research, 3th National*

Cyanobacteria Conference. Murray-Darling Freshwater Research Centre, CSIRO Land and Water, Canberra, Australia.

Dendène, M. A., Rolland, T., Trémolières, M. & Carbiener, R. (1993) Effect of ammonium ions on the net photosynthesis of three species of *Elodea*. *Aquatic Botany,* **46,** 301-315.

Erhard, D. & Gross, E. M. (2006) Allelopathic activity of *Elodea canadensis* and *Elodea nuttallii* against epiphytes and phytoplankton. *Aquatic Botany,* **85,** 203-211.

Falconer, I. R. (2012) Cyanobacteria and recreational lake management. *Recent Advances in Cyanobacterial Research, 3th National Cyanobacteria Conference*. Canberra, Australia.

Fargione, J. E., Brown, C. S. & Tilman, D. (2003) Community assembly and invasion: an experimental test of neutral versus niche processes. *Proceedings of the National Academy of Sciences*, **100,** 8916–8920.

Ferrier, M. D., Butler B. R. & Terlizzi, E. (2005) The effects of barley straw (*Hordeum vulgare*) on the growth of freshwater algae. *Bioresource Technology*, **96,** 1788-1795.

Fleming, J. P. & Dibble, E. D. (2015) Ecological mechanisms of invasion success in aquatic macrophytes. *Hydrobiologia*, **746**, 23-37.

Gallardo, B., Clavero, M., Sánchez, M. I. & Vilà, M. (2016) Global ecological impacts of invasive species in aquatic ecosystems. *Global Change Biology,* **22,** 151-163.

Glomski, L. A. M., Wood, K., Nicholson, R. & Lembi, C. (2002) The search for exudates from Eurasian watermilfoil and hydrilla. *Journal of Aquatic Plant Management,* **40,** 17-22.

Gopal, B. & Goel, U. (1993) Competition and allelopathy in aquatic plant-communities. *Botanical Review*, **59,** 155-210.

Gross, E. M. (1999) Allelopathy in benthic and littoral areas: case studies on allelochemicals from benthic cyanobacteria and submerged macrophytes. *Principles and Practices in Plant Ecology: Allelochemical Interactions* (eds K. M. M. Inderjit, Dakshini & C. L. Foy), pp. 179-199. CRC Press, Boca Raton, FL.

Gross, E. M., 2003. Allelopathy of aquatic autotrophs. *Critical Reviews in Plant Sciences*, **22,** 313-340.

Gross, E. M., Hilt, S., Lombardo, P. & Mulderij, G. (2007) Searching for allelopathic effects of submerged macrophytes on phytoplankton—State of the art and open questions. *Hydrobiologia*, **584,** 77-88.

Gurevitch, J. & Padilla, D. K. (2004) Are invasive species a major cause of extinctions? *Trends in Ecology & Evolution*, **19,** 470-474.

Herb, W. R. & Stefan, H. G. (2006) Seasonal growth of submersed macrophytes in lakes: the effects of biomass density and light competition. *Ecological Modelling,* **193,** 560-574.

Hilt, S. & Gross, E. M. (2008) Can allelopathically active submerged macrophytes stabilise clear-water states in shallow lakes? *Basic and Applied Ecology,* **9,** 422-432.

Hilt, S., Ghobrial M. G. N. & Gross, E. M. (2006) In situ allelopathic potential of *Myriophyllum verticillatum* (Haloragaceae) against selected phytoplankton species. *Journal of Phycology,* **42,** 1189-1198.

Hu, H. & Hong, Y. (2008). Algal-bloom control by allelopathy of aquatic macrophytes-a review. *Frontiers of Environmental Science & Engineering in China*, **2,** 421-438.

Huang, W., Siemann, E., Wheeler, G. S., Zou, J., Carrillo, J. & Ding, J. (2010) Resource allocation to defence and growth are driven by different responses to generalist and specialist herbivory in an invasive plant. *Journal of Ecology*, **98,** 1157-1167.

Hutchinson, G. E. (1975) *A Treatise on Limnological Botany*. Wiley, New York.

Inderjit, Evans, H., Crocoll, C., Bajpai, D., Kaur, R., Feng, Y.-L., Silva, C., Carreón, J. T., Valiente-Banuet, A., Gershenzon, J. & Callaway, R. M. (2011a) Volatile chemicals from leaf litter are associated with invasiveness of a neotropical weed in Asia. *Ecology,* **92,** 316-324.

Inderjit, Wardle, D. A., Karban, R. & Callaway, R. M. (2011b) The ecosystem and evolutionary contexts of allelopathy. *Trends in Ecology & Evolution*, **26,** 655-662.

ISSG, (2005) *Global Invasive Species Database (GISD)*. Auckland, New Zealand: University of Auckland. http://www.issg.org/database

Jacobs, M. J. & MacIsaac, H. J. (2009) Modelling spread of the invasive macrophyte *Cabomba caroliniana*. *Freshwater Biology* **54,** 296–305.

Jahnke, L. S., Eighmy, T. T. & Fagerberg, W. R. (1991) Studies of *Elodea nuttallii* grown under photorespiratory conditions. I. Photosynthetic characteristics. *Plant, Cell and Environment* **14,** 147-156.

Jarchow, M. E. & Cook, B. J. (2009) Allelopathy as a mechanism for the invasion of *Typha angustifolia*. *Plant Ecology*, **204**, 113-124.

Jessop, J., Black, J. M. & Toelken, H. R. (1986) *Flora of South Australia: Lycopodiaceae-Rosaceae*. South Australian Government Printing Division, Australia.

Körner, S. & Nicklisch, A. (2002) Allelopathic growth inhibition of selected phytoplankton species by submerged macrophytes. *Journal of Phycology,* **38,** 862-871.

Kuhar, U., Germ, M. & Gaberščik, A. (2010) Habitat characteristics of an alien species *Elodea canadensis* in Slovenian watercourses. *Hydrobiologia,* **656,** 205-212.

Lind, E. M. & Parker, J. D. (2010) Novel weapons testing: are invasive plants more chemically defended than native plants? *Plos One*, **5**, e10429.

Logan, M. (2010) *Biostatistical Design and Analysis Using R: A Practical Guide*. Wiley-Blackwell, Hoboken, NJ.

Mackey, A. & Swarbrick, J. (1997) The biology of Australian weeds. 32. *Cabomba caroliniana* Gray. *Plant Protection Quarterly*, **12**, 154-165.

Marko, M. D., Gross, E., Newman, R. & Gleason, F. K. (2008) Chemical profile of the North American native *Myriophyllum sibiricum* compared to the invasive *M. spicatum*. *Aquatic Botany,* **88,** 57-65.

Mitchell, C. E., Agrawal, A. A., Bever, J. D., Gilbert, G. S., Hufbauer, R. A., Klironomos, J. N., Maron, J. L., Morris, W. F., Parker, I. M. & Power, A. G. (2006) Biotic interactions and plant invasions. *Ecology Letters,* **9,** 726-740.

Mohamed, Z. A. & A. M. Al Shehri, 2010. Differential responses of epiphytic and planktonic toxic cyanobacteria to allelopathic substances of the submerged macrophyte *Stratiotes aloides*. *International Review of Hydrobiology*, **95,** 224-234.

Molisch, H. (1937) *Der Einfluss einer Pflanze auf die Andere-Allelopathie*. Fisher, Jena.

Mony, C., Koschnick, T. J., W.T., H. & S., M. (2007) Competition between two invasive Hydrocharitaceae (*Hydrilla verticillata* (L.f.) (Royle) and *Egeria densa* (Planch)) as influenced by sediment fertility and season. *Aquatic Botany,* **86,** 236-242.

Mooney, H. A. & Cleland, E. E. (2001) The evolutionary impact of invasive species. *Proceedings of the National Academy of Sciences of the United States of America*, **98,** 5446–5451.

Mulderij, G., Van Nesc, E. H. & Van Donka, E. (2007) Macrophyte-phytoplankton interactions: the relative importance of allelopathy versus other factors. *Ecological Modelling,* **204,** 85-92.

Müller-Schärer, H., Schaffner, U. & Steinger, T. (2004) Evolution in invasive plants: implications for biological control. *Trends in Ecology & Evolution,* **19,** 417-422.

Nakai, S., Hosomi, M., Okada, M. & Murakami, A. (1996) Control of algal growth by macrophytes and macrophyte-extracted bioactive compounds. *Water Science and Technology*, **34,** 227-235.

Nakai, S., Inoue, Y., Hosomi, M. & Murakami, A. (1999) Growth inhibition of blue-green algae by allelopathic effects of macrophyte. *Water Science and Technology,* **39,** 47-53.

Ørgaard, M. (1991) The genus *Cabomba* (Cabombaceae)—a taxonomic study. *Nordic Journal of Botany*, **11**, 179-203.

Ozimek, T., Van Donk, E. & Gulati, R. D. (1993) Growth and nutrient uptake by two species of *Elodea* in experimental conditions and their role in nutrient accumulation in a macrophyte-dominated lake. *Hydrobiologia*, **251,** 13-18.

Pakdel, F. M., Sim, L. Beardall J. & Davis, J. (2013) Allelopathic inhibition of microalgae by the freshwater stonewort, *Chara australis*, and a submerged angiosperm, *Potamogeton crispus*. *Aquatic Botany*, **110,** 24-30.

Pakdel, M. F., Wevill, T., Sim, L., Beardall, John. & Davis, J. (submitted). Bioactivity of an invasive macrophyte species on two potential native 'enemies'. *Journal of Freshwater Biology.*

Parsons, W. T. & Cuthbertson, E. G. (2001) *Noxious Weeds of Australia*. 2nd Edition. CSIRO Publishing, Collingwood.

Pimm, S. L. & Hyman, J. B. (1987) Ecological stability in the context of multispecies fisheries. *Canadian Journal of Fisheries and Aquatic Sciences,* **44,** s84-s94.

Quinn, L. D., Schooler, S. S. & Van Klinken, R. D. (2011) Effects of land use and environment on alien and native macrophytes: lessons from a large-scale survey of Australian rivers. *Diversity and Distributions,* **17,** 132-143.

Reigosa, M. J., Sanchez-Moreiras, A. & Gonzalez, L. (1999) Ecophysiological approach in allelopathy. *Critical Reviews in Plant Sciences.,* **18,** 577-608.

Sainty, G. & Jacobs, S. (2003) *Waterplants in Australia: A Field Guide*, 4 edn. Sainty & Associates, Australia.

Sala, O. E., Chapin, F. S., Armesto, J. J., Berlow, E., Bloomfield, J., Dirzo, R., Huber-Sanwald, E., Huenneke, L. F., Jackson, R. B. & Kinzig, A. (2000) Global biodiversity scenarios for the year 2100. *Science*, **287,** 1770-1774.

Scheffer, M., 1998. *Ecology of Shallow Lakes.* Chapman & Hall, London , New York.

Steenhagen, D. A. & Zimdahl, R. L. (1979) Allelopathy of leafy spurge (*Euphorbia esula*). *Weed Science,* **27,** 1-3.

Svensson, J. R., Nylund, G. M., Cervin, G., Toth, G. B. & Pavia, H. (2013) Novel chemical weapon of an exotic macroalga inhibits recruitment of native competitors in the invaded range. *Journal of Ecology,* **101**, 140-148.

Tabassum, S. & Leishman, M. R. (2016) Trait values and not invasive status determine competitive outcomes between native and invasive species under varying soil nutrient availability. *Austral Ecology*, **41,** 875-885.

Takeda, F., Nakano, K., Nishimura, O., Shimada, Y., Fukuro, S., Tanaka, H., Hayashi N. & Inamori, Y. (2011) Allelopathic potential of *Potamogeton pusillus* community against *Microcystis aeruginosa*. *Journal of Water and Environment Technology,* **9**, 21-28.

Thiebaut, G., Rolland, T., Robach, F., Tremolieres, M., Muller, S. & Bergot, F. (1997) Some consequences of the introduction of two macrophyte species, *Elodea canadensis* Michaux and *Elodea nuttallii* St. John, in continental aquatic ecosystems: Example of two areas in the North-East of France: Alsace Plain and Northern Vosges. *Bulletin Francais de la Peche et de la Pisciculture*, **344-345,** 441-452.

Thomaz, S. M., Kovalenko, K. E., Havel, J. E. & Kats, L. B. (2015) Aquatic invasive species: general trends in the literature and introduction to the special issue. *Hydrobiologia,* **746,** 1-12.

van Donk, E. & van de Bund, W. J. (2002) Impact of submerged macrophytes including charophytes on phyto- and zooplankton communities: allelopathy versus other mechanisms. *Aquatic Botany,* **72,** 261-274.

Vanderstukken, M., Declerck, S. A., Decaestecker, E. & Muylaert, K. (2014) Long-term allelopathic control of phytoplankton by the submerged macrophyte *Elodea nuttallii*. *Freshwater Biology,* **59,** 930-941.

Vanderstukken, M., Mazzeo N. & Van Colen, W. (2011) Biological control of phytoplankton by the subtropical submerged macrophytes *Egeria densa* and *Potamogeton illinoensis*: a mesocosm study. *Freshwater Biology*, **56,** 1837-1849.

Von Holle, B. & Simberloff, D. (2004) Testing Fox's assembly rule: does plant invasion depend on recipient community structure? *Oikos*, **105,** 551–563.

Wersal, R. M. & Madsen, J. D. (2011) Influences of water column nutrient loading on growth characteristics of the invasive aquatic macrophyte *Myriophyllum aquaticum* (Vell.) Verdc. *Hydrobiologia,* **665,** 93-105.

Wilcove, D. S., Rothstein, D., Dubow, J., Phillips, A. & Losos, E. (1998) Quantifying threats to imperiled species in the United States. *BioScience*, **48,** 607-615.

Williams, B. & Cole, B. (2013) Mining monitored data for decision-making with a Bayesian network model. *Ecological Modelling*, **249,** 26-36.

Willis, R. J. (1985) The historical bases of the concept of allelopathy. *Journal of the History of Biology*, **18,** 71-102.

Willis, R. J. (2008) *The History of Allelopathy*. Springer, Dordrecht.

Wolf, V. C., Berger, U., Gassmann, A. & Müller, C. (2011) High chemical diversity of a plant species is accompanied by increased chemical defence in invasive populations. *Biological Invasions*, **13,** 2091-2102.

Wu, X., Zhang, Z., Chen, D., Zhang, J., Yang, W. & Jin, Y. (2012) Allelopathic effects of *Eichhornia crassipes* on the growth of *Microcystis aeruginosa*. *Journal of Agricultural Science and Technology*. A, **2,** 1400-1406.

Wu, Z. B., Zhang, S. H., Wu, X. H., Cheng, S. P. & He, F. (2007) Allelopathic interactions between *Potamogeton maackianus* and *Microcystis aeruginosa*. *Allelopathy Journal,* **20,** 327-338.

Yarrow, M., Marin, V. H., Finlayson, M., Tironi, A., Delgado, L. E. & F., F. (2009) The ecology of *Egeria densa* Planchon (Liliopsida: Alismatales): a wetland ecosystem engineer? *Revista Chilena de Historia Natural,* **82,** 299-313.

Zeng, R. S., Mallik, A. U. & Luo, S. M. (2008) *Allelopathy in Sustainable Agriculture and Forestry.* Springer-Verlag, New York, London.

Zhang, S. H., Cheng, S. P., Wang, H. Q., He, F. & Wu, Z. (2009) Allelopathic interactions between the *Potamogeton* spp. and toxic cyanobacteria (*Microcystis aeruginosa*). *Allelopathy Journal,* **23,** 379-390.

Chapter 3.

The influence of native and invasive macrophytes on aquatic invertebrate communities: a mesocosm study

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Declaration for thesis chapter three

Declaration by candidate
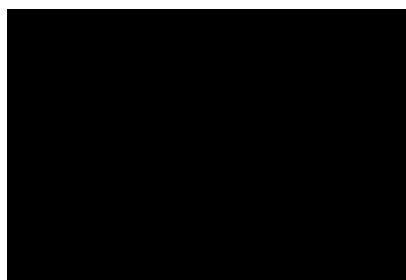
In the case of Chapter 3, the nature and extent of my contribution to the work was the following:

Nature of contribution	Extent of contribution
I mostly conceived and designed the experiments, performed the outdoor and lab work, analysed all of the data and was the primary author of the manuscript	80%

The following co-authors contributed to the work. If co-authors are students at Monash University, the extent of their contribution in percentage terms must be stated:

Name	Nature of contribution	Extent of contribution (%) for student co-authors only
John Beardall	Conception of ideas, supervision and editing	5%
Jenny Davis	Conception of ideas, supervision and editing	5%
Lien Sim	Conception of ideas, supervision and editing	5%
Tricia Wevill	Conception of ideas, supervision and editing	5%

Candidate's Signature

Date: 17/02/2017

Main Supervisor's Signature

Date: 17/02/2017

Abstract

1. Macrophytes support biodiversity by providing a food source and habitat for many freshwater organisms. However, invasive macrophytes can have extensive negative effects on different biota (e.g. zooplankton and macroinvertebrates). Invasive macrophytes usually form mono-specific stands that provide a habitat that is less suitable than mixed stands. They may also produce novel chemicals that can have deleterious and deterrent effects for native aquatic species (the 'Novel Weapons Hypothesis', NWH).

2. We conducted two mesocosm experiments to investigate the impacts of an invasive macrophyte on the community dynamics of aquatic invertebrates. The taxon richness, abundance, community composition and distribution patterns of functional feeding groups (FFG) of invertebrates were determined in mono-culture (single plant species) and co-culture (native and invasive plants grown together) stands of *Cabomba caroliniana* and *Potamogeton ochreatus*, and were compared to the controls (no macrophytes).

3. Four major FFG were found in the native and invasive macrophyte stands and in the controls. Community dynamics of the invertebrates were similar in native and invasive macrophyte stands in both single species and co-culture settings. Furthermore, the community dynamics of invertebrates were similar in the mono-culture and in co-culture stands of macrophytes where invertebrates were given a choice of selecting either native or invasive plant as refuge.

4. Our findings suggest that mono-culture stands of invasive macrophytes can support invertebrates at similar levels to those found in mono-culture stands of native plants. The complex structure of *C. caroliniana* may have 'positive' impacts on associated fauna in some systems by providing refuge and ample substrate for epiphyte attachment, which can offset the chemical effects of *C. caroliniana*. Furthermore, invertebrates may not discriminate between native and invasive plants with similar levels of habitat complexity.

5. *Synthesis*. This study shows that habitat complexity and food availability are important criteria for invertebrates when selecting macrophytes as habitat. Invasive macrophytes with complex physical structures may be able to support equivalent invertebrate community dynamics as native plants. The physical complexity of invasive plants can override their chemical attributes, although this cannot mask the adverse effects of invasive macrophytes imposed on other biota and on overall ecosystem function.

Key-words: allelopathy, enemy release hypothesis, freshwater invasions, invasion ecology, novel weapons hypothesis, zooplankton.

Introduction

Invasion by non-indigenous species is one of the primary causes of biodiversity loss in lakes and streams (Sala et al., 2000). Globalisation and human mediated impacts in particular, have been effective in facilitating and accelerating the introduction of exotic aquatic plants worldwide (Kolar and Lodge, 2000; Kowarik, 2003; Meyerson and Mooney, 2007). Exotic plants are introduced either intentionally via aquaculture and the aquarium trade or accidentally through hitchhiking long-distances (e.g. accidentally transported via ships, boats, yachts and animal vectors) (Kolar and Lodge, 2000; Kay and Hoyle, 2001; Martin and Coetzee, 2011). While the majority of exotic species fail to establish successfully and increase their range (Williamson, 1996), some have the capacity to become invasive and cause extensive environmental and economic impacts (Pimentel et al., 2005; Lockwood et al., 2013; Gallardo et al., 2016).

Understanding the principal mechanisms that drive biological invasions can help mitigate the impacts of invasive species (Sax et al., 2007; Davis, 2009). Several hypotheses have been proposed to describe the invasion success of exotic species. There is substantial empirical evidence to support the 'Novel Weapons Hypothesis' (NWH) and the 'Enemy Release Hypothesis' (ERH) (Jeschke et al., 2012). The NWH postulates that exotic plant species have the capacity to become invaders if they contain novel biochemistry such as unique anti-herbivore and allelochemical compounds (chemical substances produced by autotrophs that can limit the growth and survival of competitors, herbivores and pathogens (Willis, 2008)) (Callaway and Aschehoug, 2000; Callaway and Ridenour, 2004; Cappuccino and Arnason, 2006; Svensson et al., 2013). The native species in the recipient community are not adapted to these unique chemicals and consequently will be negatively affected and/or outcompeted via chemical intervention (Callaway and Aschehoug, 2000). This trait has long been recognised as an important mechanism responsible for the success of invasive plant species (Steenhagen and Zimdahl, 1979). On the other hand, the ERH proposes that exotic species can become highly abundant in the invaded areas because they are liberated from their natural enemies in their native homes (Crawley, 1997; Elton, 2000; Maron and Vilà, 2001; Liu and Stiling, 2006). Exotic plant species are therefore expected to be less prone to attacks by primary consumers and subsequent herbivory damage, and can gain a significant advantage over natives (Morrison and Hay, 2011a; Enge et al., 2012). Invasive species can also have deterrent activity against zooplankton and macroinvertebrates (Jarchow and Cook, 2009; Wolf et al., 2011) by producing chemicals that are toxic to them and can have a major impact on their fitness (Morrison and Hay, 2011b). For example, polyphenols such as tannins were found to affect the physiology and behaviour of *Daphnia menucoensis* and reduced its mobility, survival and development (Alvarez et al., 2016).

Native macrophytes (aquatic angiosperms and macroalgae) are important components of freshwater systems because they provide refuge and a food source for a wide range of biota, and enhance water quality by nutrient cycling and reducing phytoplankton growth (Jeppesen et al., 1997; Scheffer, 1998; Lürling et al., 2006; Scheffer and van Nes, 2007; Dhote and Dixit, 2009; Ansari et al., 2010; Muylaert et al., 2010). Pioneer investigations into the positive physical effects of macrophytes on invertebrate communities were conducted by Watkins et al. (1983) and Timms and Moss (1984) who found a greater richness and abundance of zooplankton and benthic macroinvertebrates in vegetated areas compared to open water. Invertebrates used the plants as a shelter during daytime but were out in the open water grazing upon phytoplankton at night time. These studies highlighted the importance of vegetation as a spatial and temporal refuge for zooplankton and benthic macroinvertebrates against predatory fish.

Despite the important positive role of submerged macrophytes, invasive species are known to negatively affect freshwater native fauna as well as native macrophytes (Lassuy, 1995; Thiebaut et al., 1997; Dextrase and Mandrak, 2006; Schultz and Dibble, 2012; Gallardo et al., 2016). Overall macroinvertebrate richness was found to be lower in wetlands where invasive species were the prevalent macrophytes than wetlands that were dominated by natives (Bryant and Papas, 2007). Furthermore, invasive species can outcompete and eventually eliminate native

macrophytes (Thiebaut et al., 1997; Svensson et al., 2013). This may lead to the formation of dense, mono-culture, macrophyte meadows, which is a less suitable habitat and food source for zooplankton, macroinvertebrates, fish and waterfowl, and can cause seasonal water quality issues (Thiebaut et al., 1997; Barrat-Segretain, 2001; Brönmark and Hansson, 2005; Erhard and Gross, 2006; Herb and Stefan, 2006).

A considerable amount of research on the interactions between invasive macrophytes and associated fauna has focused on the effects of invasive plants (e.g. structural complexity, habitat heterogeneity, allelopathy, etc.) on zooplankton and macroinvertebrate communities rather than the cause of their success (see Schultz and Dibble, 2012 and references therein). There are gaps in our understanding of the interactions between invasive macrophytes and invertebrates with many aspects that need to be explored. The majority of studies conducted in this area have been descriptive and confined to counting or measuring invertebrates (e.g. richness and abundance) in naturally-occurring populations. Furthermore, the major hypotheses of invasion biology have been tested in terrestrial environments more rigorously than freshwater systems (Jeschke et al., 2012). For instance, the explicit investigation of the concept of NWH in freshwater systems has been neglected. Moreover, many studies investigating the allelopathic effects of invasive macrophytes have concentrated on using phytoplankton and epiphytic algae as the target species (Erhard and Gross, 2006; Hilt and Gross, 2008), rather than native macrophytes or aquatic invertebrates. Studies comparing faunal biodiversity in mono-specific (either native or invasive) and multiple species (mixed native and invasive) stands are scarce and not many studies have investigated habitat preference by aquatic fauna when both native and invasive plants are present in the system. There are multiple interrelated factors that affect the habitat preferences of aquatic fauna. Invasive plants with novel biochemistry can reduce the growth of phytoplankton and epiphytic algae, which are a major food source for many zooplankton and macroinvertebrates, via allelopathy (Erhard and Gross, 2006; Willis, 2008).

It is difficult to generalise the patterns of invasion by reviewing related literature because there is a geographical bias towards studies of temperate regions, particularly in the Northern Hemisphere. There are fewer studies from tropical areas and warmer inland waters to examine the relationships between native and invasive species (Fridley et al., 2007). Invasion success can be different in tropical and sub-tropical freshwaters compared with temperate areas, due to differences in abiotic filters such as disturbance, water chemistry, light, sediment type and hydrology (Thomaz et al., 2015). In spite of numerous adverse impacts on freshwater systems caused by invasive macrophytes, some species exhibited a 'positive' or neutral effects on other biota such as invertebrates in tropical and sub-tropical regions (see Thomaz et al., 2015 and references therein).

Although some areas of the Australian continent are classified as temperate, inland waters in Australia have different characteristics (e.g. physical and chemical properties) compared to similar waterbodies in temperate regions in the Northern Hemisphere (Boulton et al., 2014). In view of this, the current study seeks to answer the questions 'Do invasive macrophytes have a negative, positive or neutral impact on the community structure of invertebrates in the temperate regions of Australia, where the abiotic filters of freshwater systems are different to other parts of the world? Do the ERH and NWH apply to freshwater invasion in these systems via the same mechanisms as freshwater systems elsewhere?'

We conducted a set of quantitative experiments to test the ERH and the NWH using the invasive macrophyte, *Cabomba caroliniana* A. Gray (green cabomba or cabomba) as the study species. Native to eastern North and South America, *C. caroliniana* is a rooted freshwater perennial plant, which lives in stagnant or slow-moving waters (Godfrey and Wooten, 1979). It has been introduced to myriad freshwater systems in the United States, Canada and several European and Asian-Pacific countries including Australia. *Cabomba caroliniana* is regarded as a weed of national significance in many of these countries due to its high level of invasiveness and its profound economic and environmental impacts (Ørgaard, 1991; Mackey and Swarbrick, 1997; Parsons and Cuthbertson, 2001; Sainty and Jacobs, 2003; ISSG, 2005; Brundu, 2015). It thrives in eutrophic systems, has a fast growth rate, and produces a large amount of biomass. *Cabomba caroliniana* reproduces via fragmentation and seed

production. It is an extremely persistent plant that can exclude native vegetation and can have a great adverse impact on freshwater native animals (Mackey and Swarbrick, 1997; Thiebaut et al., 1997; Parsons and Cuthbertson, 2001; Sainty and Jacobs, 2003). Infestations have been recorded in several states in Australia including two wetland systems in Victoria, Goulburn Weir, north of Nagambie and Casey's Weir, North of Benalla.

The specific aim of this study was to investigate whether the allelopathic activity of *C. caroliniana* has a negative impact on the establishment of aquatic macro and microinvertebrates (referred to as 'invertebrates' herein) by comparing the community structure and composition of invertebrates in beds of *C. caroliniana* and in beds of the native macrophyte, *Potamogeton ochreatus* Raoul, commonly known as blunt pondweed. *Potamogeton ochreatus* is a submerged rhizomatous native plant inhabiting slow-moving and stationary freshwater systems. This perennial plant can grow well in nutrient rich environments and is very common in different wetlands around Australia. Furthermore, we sought to examine the community structure and composition of invertebrates in systems where both native and invasive macrophytes persist and to determine whether they prefer the native species as refuge when both habitat types are available. We also tested the ERH by comparing the presence of invertebrates from different functional feeding groups (FFG) in native and invasive macrophyte beds.

Materials and methods

EXPERIMENT 1

We investigated and compared the community dynamics of invertebrates in mono-culture stands of native and invasive macrophytes and the controls. We collected *C. caroliniana* from Lake Nagambie (36°47′0″S 145°8′30″E) and *P. ochreatus* from the Hampton Park wetlands (38°02'14.1"S 145°16'24.9"E) in Victoria during February 2014. Prior to the planting of macrophytes, we added 15 cm of top soil to the bottom of the ponds (capacity, 1200 L; height, 0.6 m; diameter, 1.2 m) overlaid by 5cm washed white sand. The ponds were filled with rainwater held in tanks at the plant facility centre within the Jock Marshall Reserve (JMR) at Monash University, Clayton campus, Victoria. Macrophyte material was washed under tap water to remove debris and any conspicuous macroinvertebrates present. The top 15 cm of *C. caroliniana*, bearing the apical meristem, and whole *P. ochreatus* were planted. Each species was separately planted in different ponds to cover 50% of the total pond area. Controls were set up with no macrophytes present. Three replicates for each treatment and for the control were established. The ponds were covered using bird netting (1 mm^2 mesh size, 10-15% shading) during the course of the experiments to avoid the spread of these invasive species to the nearby wetland in the JMR. To be consistent, the controls containing no macrophytes were also covered. Slow-release nutrient tablets (Osmocote, Plus Trace Elements - Water Gardens & Aquatic Plants, Scotts, Australia) were added to each pond every two weeks to enhance the growth of the macrophytes and to mitigate potential nutrient limitation. Sampling of invertebrates was performed in spring, summer and autumn of 2014-2015. We collected samples twice in spring and summer (at the start and in the middle of the season) starting the first week of September 2014 until mid-April 2015, but sampling was undertaken only once in autumn. At each sampling occasion, 10 L water was collected from across each pond, through the macrophyte beds and close to the bottom of the ponds. The water sample was then filtered through a 60 μm sieve. All samples were preserved in 70% ethanol for further analysis of the invertebrates.

EXPERIMENT 2

We examined the community structure and composition of invertebrates in systems where both native (*P. ochreatus*) and invasive (*C. caroliniana*) macrophytes persist. We also investigated whether invertebrates prefer the native species as refuge when both habitat types are available. Top soil (5 cm) was added to the bottom of small plastic ponds (capacity, 120 L; height, 0.45 m; diameter, 0.6 m) and was covered by 2 cm white washed sand. Rainwater held in the tanks at the plant facility centre within the JMR was used to fill the ponds. Plastic partitions (2 cm^2 mesh size) were placed in the middle of each pond. Soft pine timber was used as a frame to hold

the partitions. Partitions were soaked in rainwater for one week prior to installation to eliminate any contamination. Any debris and conspicuous invertebrates were removed from the macrophyte material by gently washing with tap water. The top 15 cm of *C. caroliniana*, bearing the apical meristem and the whole *P. ochreatus* were then planted. We planted both *C. caroliniana* and *P. ochreatus* in three ponds with each plant covering 30% of the pond (60% of each pond in total). Each macrophyte was planted on one side of the ponds separated from the other plant on the other side by the partitions. This allowed the movement of invertebrates and the transfer of water and nutrients from one side to the other while creating a physical barrier between the two plants, and eliminating the effects of shading and competition for space. In addition, the invertebrates were not restricted in selecting refuge. To set up the controls, we added each plant to three ponds separately to cover 30% of one side in each pond (6 ponds in total); however, the other half of the ponds had no macrophytes. Therefore, we had three replicates for the treatment (two macrophytes each covering 30% of half of the ponds at each end) and controls (3 × each macrophyte covering 30% of half of the ponds and no plant on the other side) totalling nine. All ponds were covered and nutrients were added using the same protocol as in experiment 1. At each sampling occasion (details in experiment 1), 5 L water was collected from each side of the ponds through the macrophyte beds and close to the sediment. Each water sample was then filtered through a 60 µm sieve. All samples were preserved in 70% ethanol for further analysis of the invertebrates.

SAMPLING PROCESS (EXPERIMENTS 1 & 2)

Observational assessment revealed no feeding damage on *C. caroliniana*, but some degree of herbivory was evident on the leaves of *P. ochreatus*. Invertebrates were counted and were identified to the lowest taxonomic level possible with the aid of dissecting and compound microscopes. Taxa were assigned to different FFG to assess the abundance of each group and the number of taxa present in each group in different treatments during the three seasons. Invertebrate identifications and functional classifications were undertaken with reference to Davis and Christidis (1997), Gooderham and Tsyrlin (2002) and the Australian freshwater invertebrate identification webpage (Bug Guide) created by the Murray Darling Freshwater Research Centre (2013).

STATISTICAL ANALYSIS (EXPERIMENTS 1 & 2)

We performed a series of two-factor repeated measures ANOVAs to compare the differences in invertebrate taxon richness, taxon abundance, FFG richness and FFG abundance between macrophyte treatments and seasons for experiment 1. The same type of analyses were also used to compare taxon richness, taxon abundance, FFG richness and FFG abundance between macrophyte treatments and seasons for experiment 2. Tukey's *post hoc* tests were performed on both factors (treatment and season) when the outputs of ANOVAs were significant, in order to identify where the differences lay between levels of each factor. Prior to analyses, data were checked for normality and homogeneity of variances. We conducted a Levene test to check if there was heteroscedasticity in the data since we had unequal number of samples. The output indicated no heterogeneity of variances (F value = 0.214, *df* = 2, *p* = 0.81), therefore, no transformation was required (Levene, 1960). All data analyses were performed in the R Statistical Program (RStudio, ver. 3.3.1 (2016-06-21)) using the aov function with an α-value of 0.05 as a criterion for statistical significance.

Multivariate analyses were performed to explore relationships between season, macrophyte treatment (native, invasive, single species, multiple species and control) and invertebrate community composition in experiments 1 and 2. Bray-Curtis non-metric multidimensional scaling (NMDS) was used to visually depict similarities in the assemblages of invertebrates present in native and invasive macrophyte stands in the mono-culture and co-culture settings in experiment 1 and 2 respectively (Bray and Curtis, 1957). We conducted a Permutational Multivariate Analysis of Variance (PERMANOVA) to assess the statistical variations of our grouping based on the rank similarities from the Bray-Curtis similarity matrices with 999 permutations (Oksanen, 2009). In order to test for homogeneity of multivariate group dispersions (variances), we perfumed Euclidean PERMANOVA tests.

Furthermore, we ran pairwise permutation tests to test the statistically significant differences between treatments and between seasons. All multivariate analyses were carried out using package vegan (Oksanen et al., 2007) in the R Statistical Program (RStudio, ver. 3.3.1 (2016-06-21)). An α-value of 0.05 was used as a criterion for statistical significance.

Results

EXPERIMENT 1

Taxon richness

There was a statistically significant seasonal difference in the invertebrate taxon richness (F =30.528, *df* = 2, *p* < 0.001). The overall taxon richness was found to be significantly lower in autumn compared with spring and summer (*p* < 0.001, Fig. 1). However, we found no significant differences in the taxon richness present in the stands of *C. caroliniana* compared to *P. ochreatus* and the controls (Fig. 1). Furthermore, the interaction between season and macrophyte treatment was non-significant.
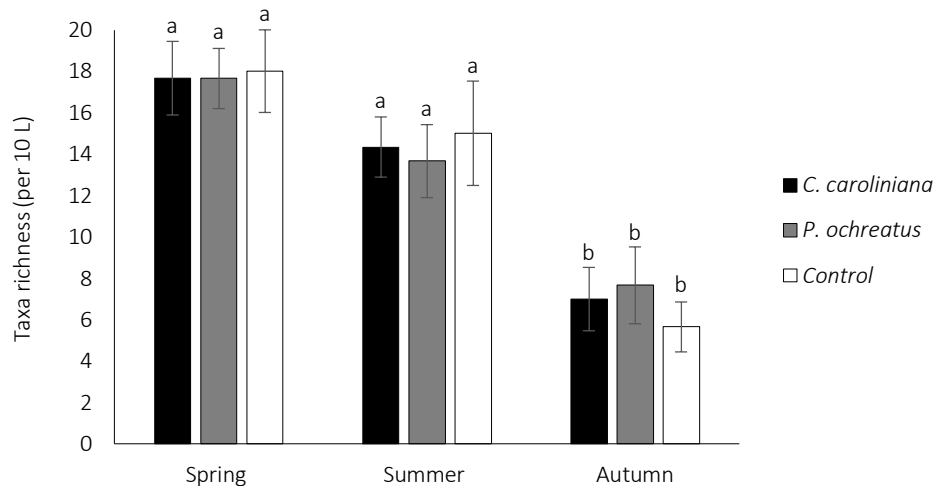
Fig. 1. Mean (±SE) number of invertebrate taxa present in the native (*P. ochreatus*) and invasive (*C. caroliniana*) macrophyte stands and in the controls (without macrophytes). Letters represent significant differences comparing the three seasons (α = 0.05).

Abundance of taxa

Season had a significant effect on the abundance of taxa (F = 10.95, *df* = 2, *p* < 0.001), however, there were no statistically significant differences in the abundance of taxa found in *C. caroliniana* and *P. ochreatus* stands and in the controls (Fig. 2). Furthermore, there was no statistically significant interaction between season and treatment. Invertebrate taxon abundance was considerably lower during autumn compared to summer and spring (*p* < 0.001 and *p* < 0.01 respectively, Fig 2).
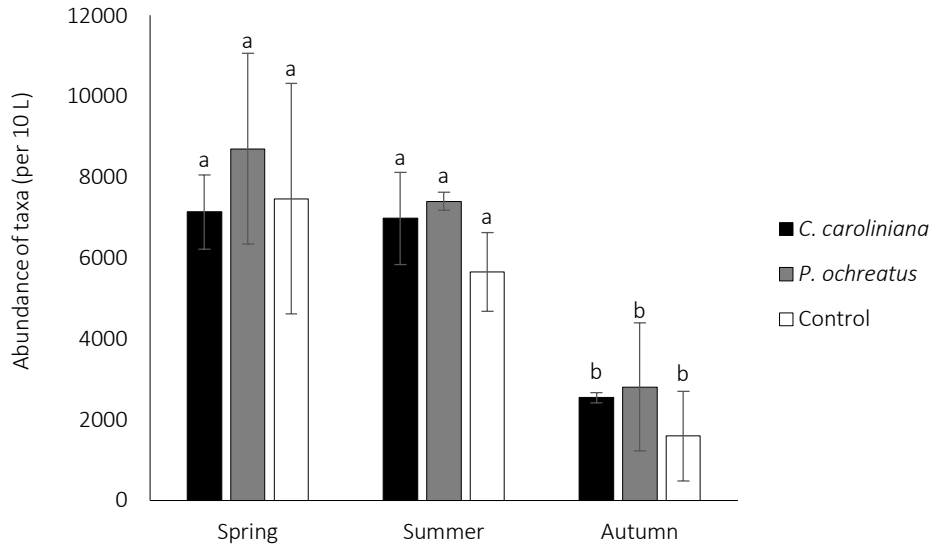
Fig. 2. Mean (±SE) abundance of invertebrate taxa present in the native (*P. ochreatus*) and invasive (*C. caroliniana*) macrophyte stands and in the controls (without macrophytes). Letters represent significant differences comparing the three seasons (α = 0.05).

Richness of taxa in each functional feeding groups

Four major FFG were identified in both native and invasive macrophyte stands and in the controls; filtering collectors, scrapers, gathering collectors and predators. The number of filtering collector taxa was similar in native and invasive macrophyte stands and in the controls, and it was consistent throughout all three seasons (Fig. 3). The richness of scrapers and gathering collectors was affected by season (F = 3.95, *df* = 2, *p* < 0.05 and F = 24.11, *df* = 2, *p* < 0.001 respectively), although, it did not differ in the stands of *P. ochreatus* and *C. caroliniana* and in the controls (Fig. 3). There was a significantly lower number of taxa classified as scrapers (*p* < 0.05) and gathering collectors (*p* < 0.001) found in autumn than spring (Fig. 3). The number of taxa present in the latter group also declined during summer compared to spring (*p* < 0.01). Furthermore, there was a higher number of gathering collectors found during summer than autumn (*p* < 0.05). We found a consistent number of predatory taxa in all treatments and during all three seasons (Fig. 3).
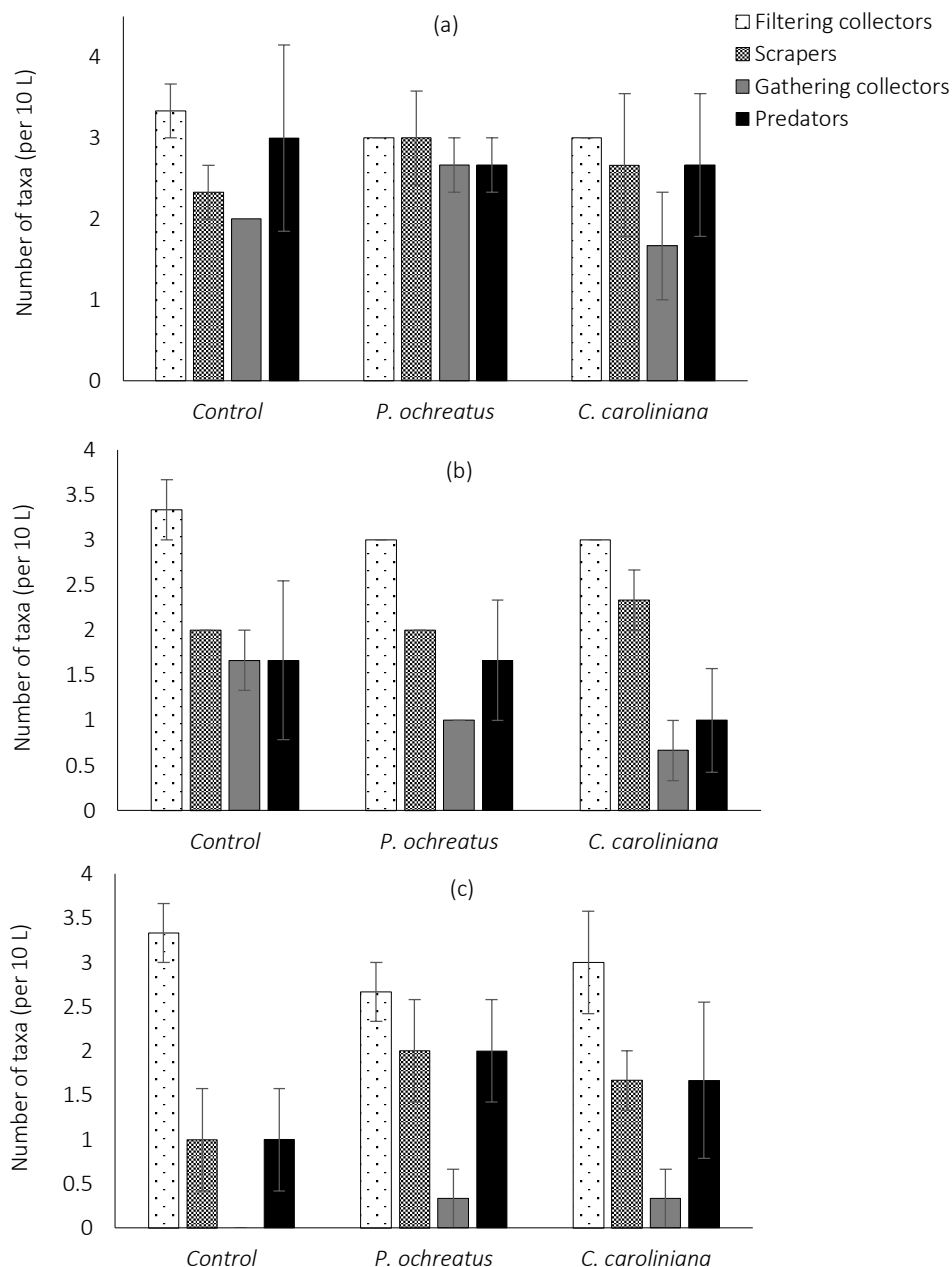
Fig. 3. Mean (±SE) number of invertebrate taxa present in different functional feeding groups in the ponds with the native (*P. ochreatus*) and invasive (*C. caroliniana*) macrophytes and the controls during spring (a), summer (b) and autumn (c) ($\alpha = 0.05$). In some cases error bars were too small to be visible in the figure.

Abundance of taxa in each functional feeding group

There were no significant interactions between season and macrophyte treatment in all comparisons, although, we found a statistically significant seasonal pattern in the abundance of filtering collectors (F = 6.45, *df* = 2, *p* < 0.01) and gathering collectors (F = 3.63, *df* = 2, *p* < 0.05, Fig. 4). The abundance of filtering collectors was significantly higher in spring than in autumn (*p* < 0.05, Fig. 4). We also found a higher abundance of filtering collectors during summer compared to autumn (*p* < 0.01, Fig. 4). Moreover, there was a significantly higher number of gathering collectors in spring in comparison to autumn (*p* < 0.05, Fig. 4). However, the abundance of scrapers and predatory taxa was similar throughout all three seasons (Fig. 4). The differences in the abundance of all FFG present in *P. ochreatus* and *C. caroliniana* stands and in the control ponds were non-significant (Fig. 4).
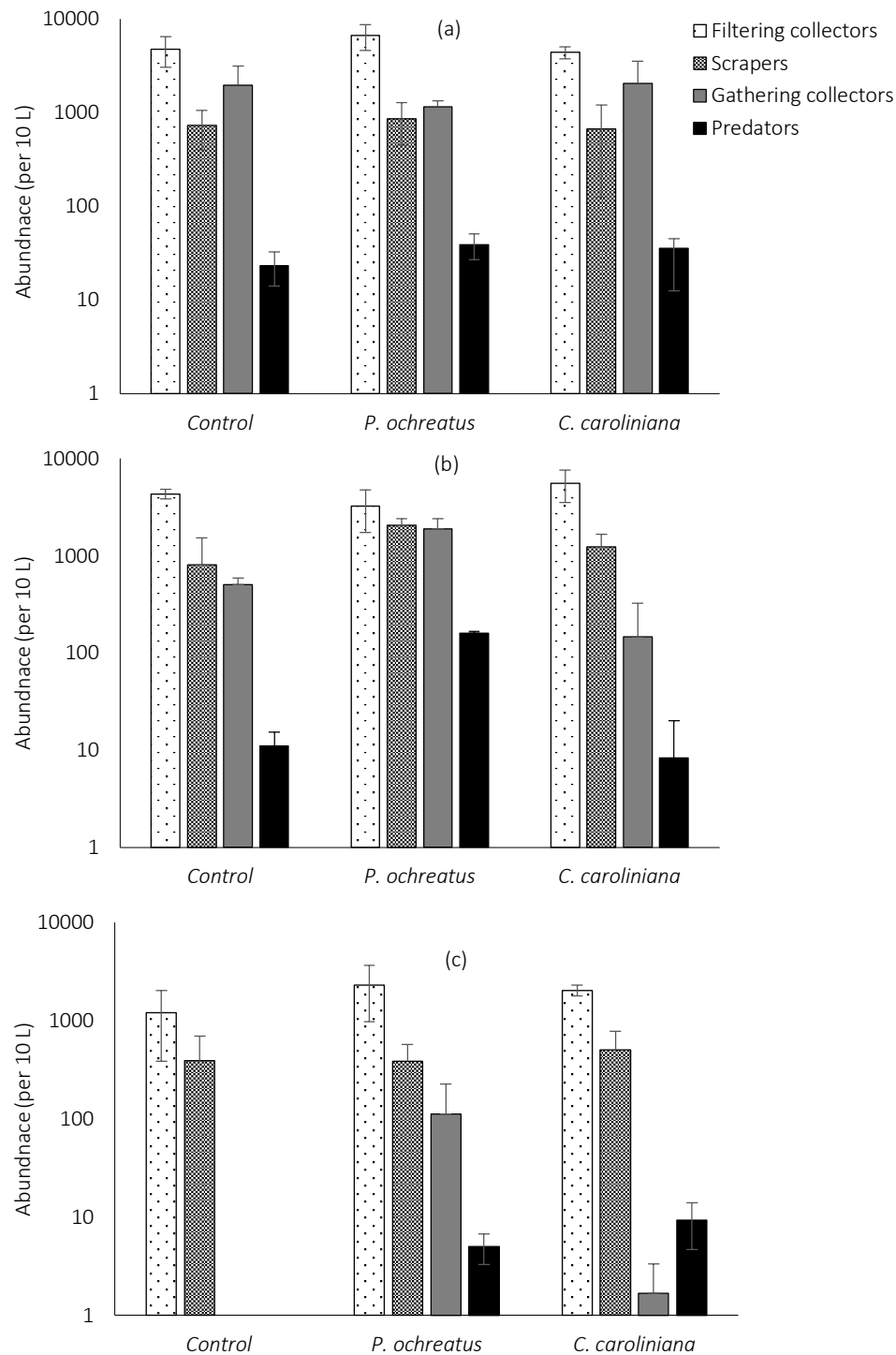
Fig. 4. Mean (±SE) abundance of invertebrates allocated to different functional feeding groups in the ponds with the native (*P. ochreatus*) and invasive (*C. caroliniana*) macrophytes and the controls during spring (a), summer (b) and autumn (c) (α = 0.05). The y-axis was (log10) transformed to illustrate the graphs clearly.

Community composition

Ordination of invertebrate community composition did not show distinct clustering by macrophyte treatment (native or invasive) and control (Fig. 5). We found significant dissimilarity in invertebrate composition between seasons, despite the overlap in seasonal groupings (R = 0.11, *p* < 0.001, Fig. 5). Results from the Euclidean

PERMANOVA tests showed no significant heterogeneity of dispersion within groups. Furthermore, the interaction between season and macrophyte treatment did not show any significant effects on the invertebrate community compositions.
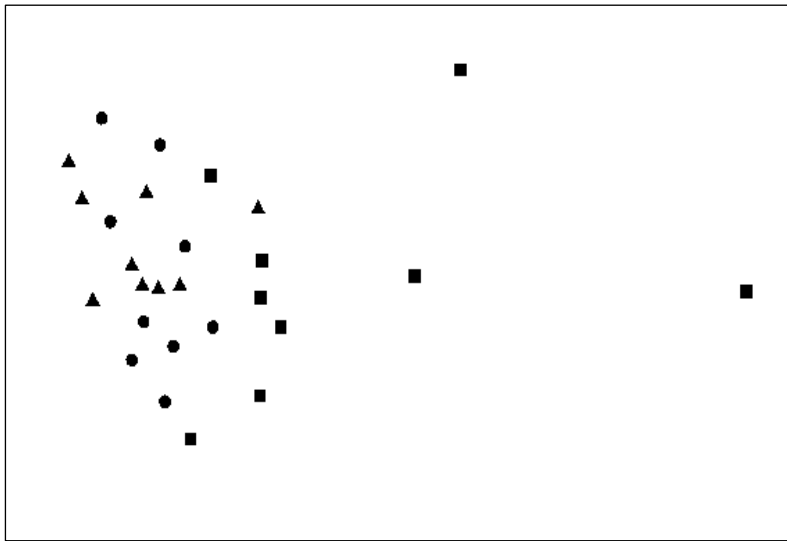
Fig. 5. Non-metric multidimensional scaling (NMDS) of Bray-Curtis similarities illustrating invertebrate community composition grouped by season; spring (triangles), summer (circles) and autumn (squares) in experiment 1 (α = 0.05).

EXPERIMENT 2

Taxon richness

Taxon richness was similar throughout all three seasons (Fig. 6). We found no significant differences in invertebrate taxon richness present in *C. caroliniana* when grown separately than when grown in the mixed species ponds (Fig. 6). Taxon richness was also similar in *P. ochreatus* stands in the single species ponds compared to *P. ochreatus* stands in the co-culture setting (Fig. 6). Moreover, the richness of invertebrate taxa was similar when comparing the native (*P. ochreatus*) and invasive (*C. caroliniana*) macrophyte stands in both the single species and the co-culture settings (Fig. 6).
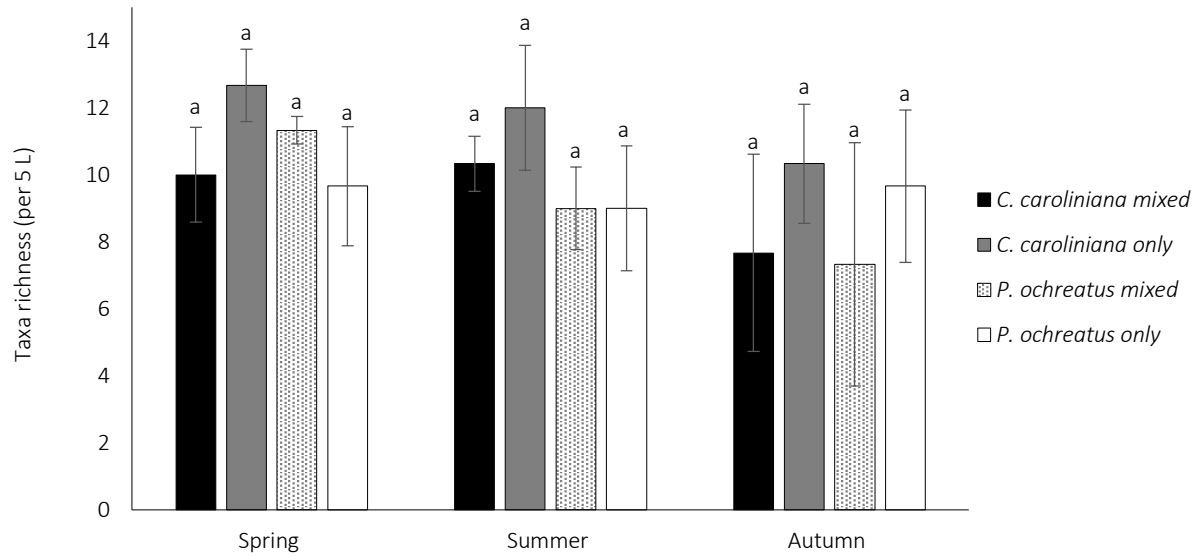
Fig. 6. Mean (±SE) number of invertebrate taxa present in native (*P. ochreatus*) and invasive (*C. caroliniana*) macrophyte stands when grown separately and when grown together in a mixed species setting. Letters represent significant differences comparing the three seasons ($\alpha = 0.05$).

Abundance of taxa

There were no statistically significant differences in the abundance of taxa across all three seasons (Fig. 7). The number of invertebrates found in *P. ochreatus* grown separately was similar to *P. ochreatus* stands in the mixed species ponds (Fig. 7). We also found no significant differences in the abundance of taxa in *C. caroliniana* stands in single species planting compared to the co-culture setting (Fig. 7). Furthermore, the abundance of invertebrates was similar in native and invasive macrophyte stands in single species and in the mixed species planting (Fig. 7).
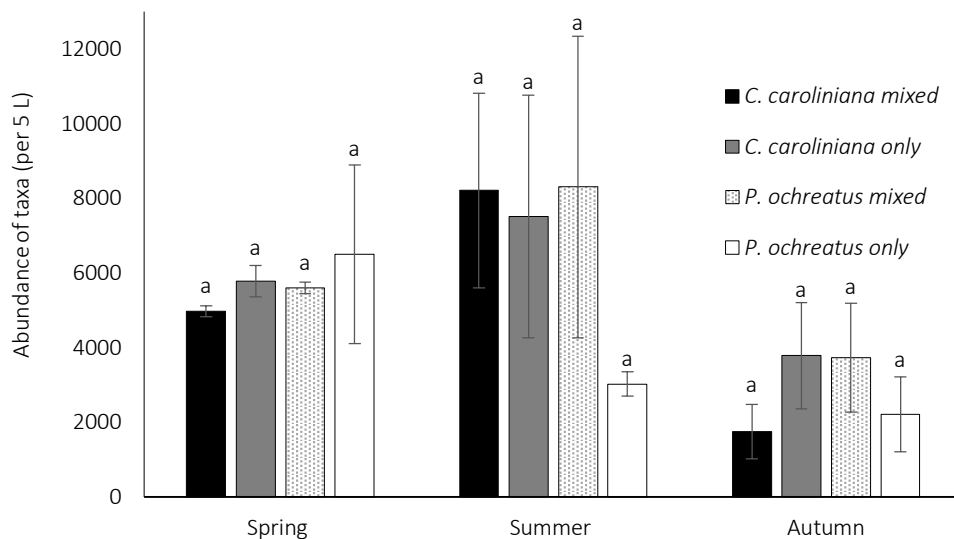
Fig. 7. Mean (±SE) abundance of invertebrate taxa present in native (*P. ochreatus*) and invasive (*C. caroliniana*) macrophyte stands when grown separately and when grown together in a mixed species setting. Letters represent significant differences comparing the three seasons ($\alpha = 0.05$).

Richness of taxa in each functional feeding group

The FFG found in this experiment were the same as those present in the experiment 1. There were no interactions between season and macrophyte treatment when examining the four groups of invertebrates. However, we found statistically significant seasonal patterns in the taxon richness of filtering collectors (F = 3.50, df = 2, p < 0.05) and scrapers (F = 12.41, df = 2, p < 0.001). There was a lower number of filtering collector taxa present in autumn compared to summer (p < 0.05, Fig. 8). Furthermore, taxon richness of scrapers was significantly higher in spring and summer than autumn (both p < 0.001, Fig. 8). Taxon richness of gathering collectors and predators was similar in all three seasons (Fig. 8). We found no significant differences in the taxon richness of all FFG found in the mono-culture and co-culture settings (Fig. 8).
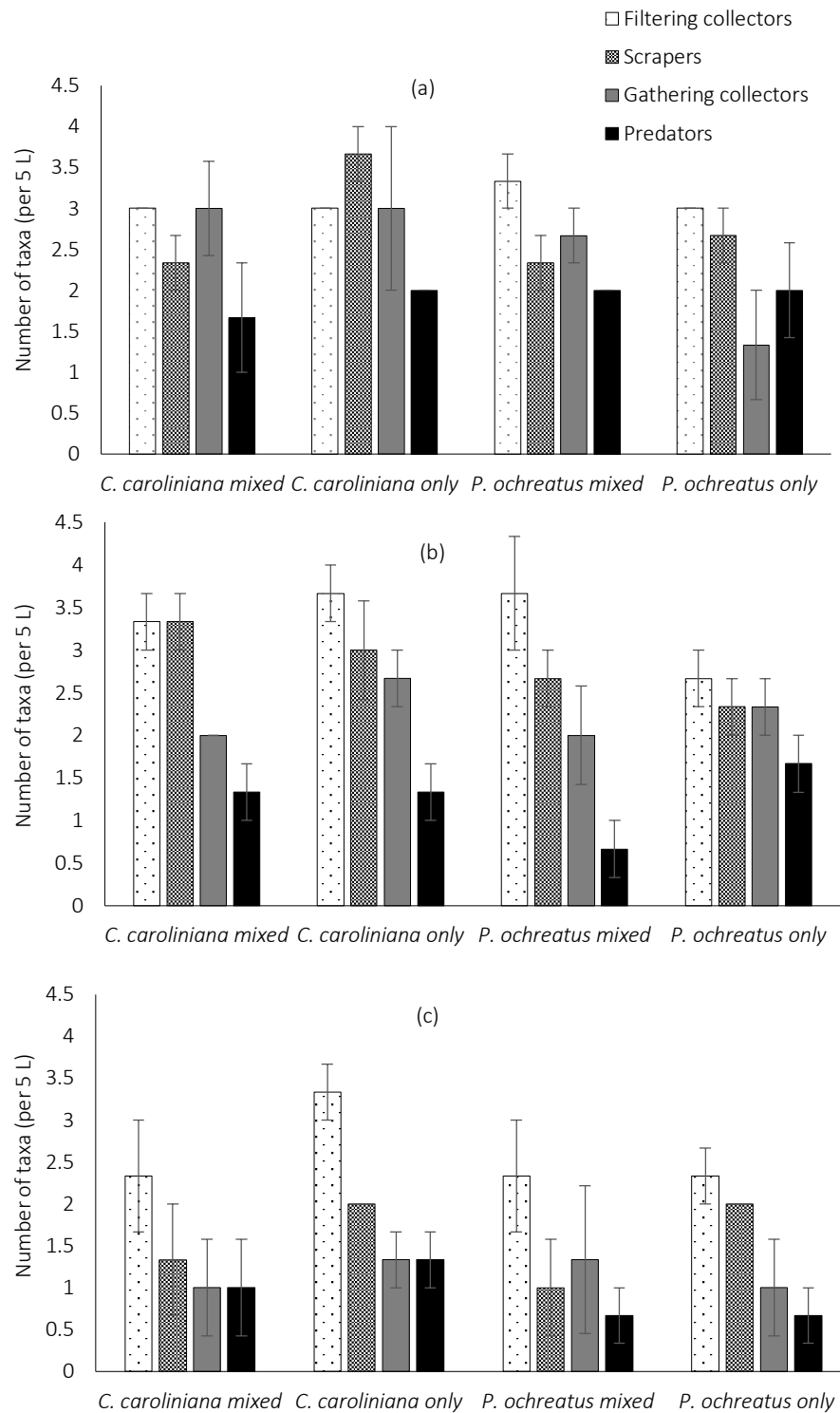
Fig. 8. Mean (±SE) number of taxa found in each functional feeding group in the ponds with native (*P. ochreatus*) and invasive (*C. caroliniana*) macrophyte stands during spring (a), summer (b) and autumn (c) in mono-culture and co-culture settings (α = 0.05).

Abundance of taxa in each functional feeding group

The abundance of filtering collector, scraper and predatory taxa was similar in native and invasive macrophyte stands in both mono-culture and co-culture settings (Fig. 9). Furthermore, there was no seasonal variation in the

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abundance of taxa in these three FFG. However, the abundance of gathering collectors was affected by season (F = 3.64, df = 2, p < 0.05, Fig. 9). There was a higher abundance of gathering collectors in spring compared to autumn (p < 0.05).The interactions between the main factors were non-significant in all comparisons.
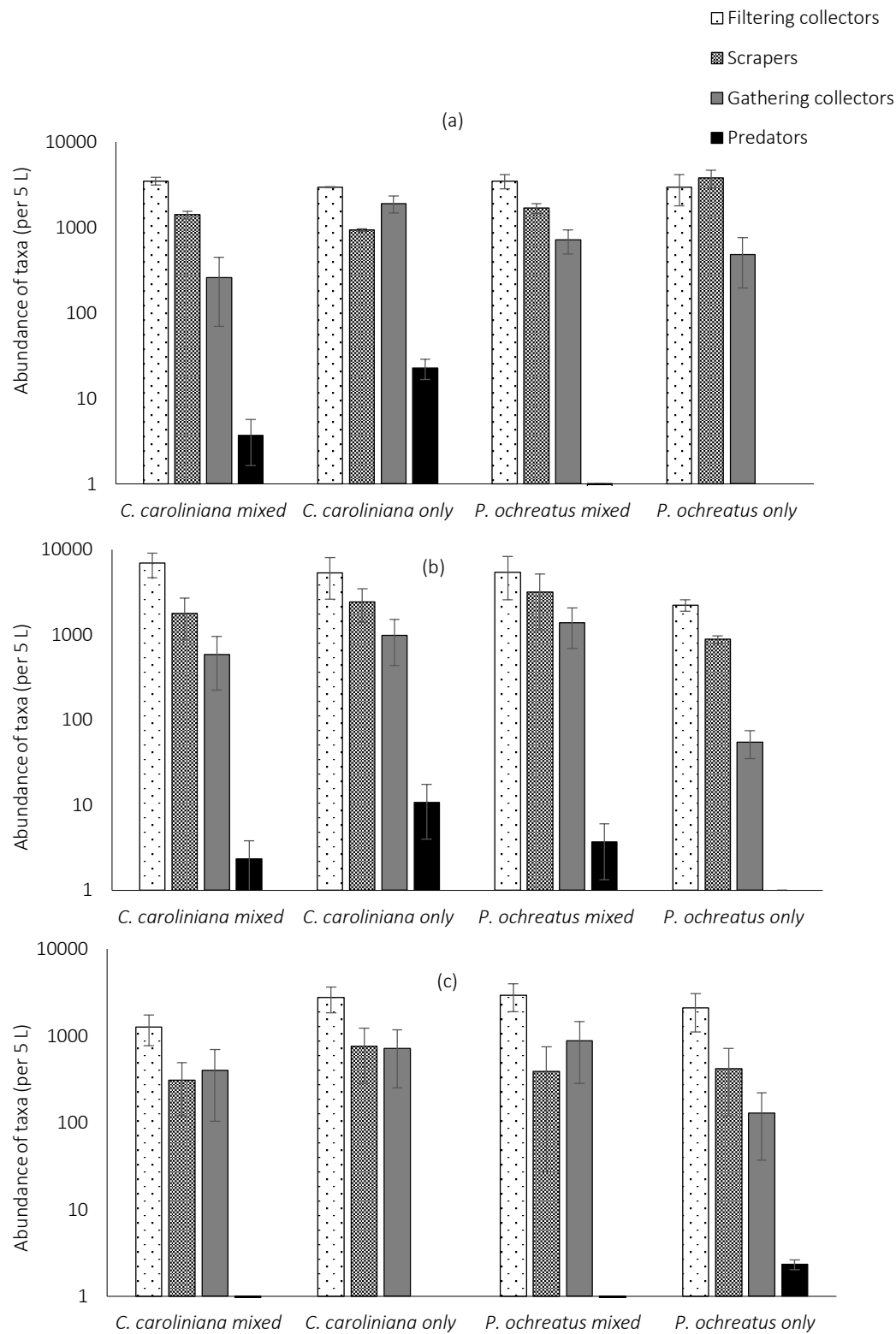
Fig. 9. Mean (±SE) abundance of invertebrates found in different functional feeding groups in the ponds with the native (*P. ochreatus*) and invasive (*C. caroliniana*) macrophyte stands during spring (a), summer (b) and autumn

(c) in mono-culture and co-culture settings (α = 0.05). The y-axis was (log10) transformed to illustrate the graphs clearly.

Community composition

We found no distinctive clustering of the invertebrate communities by macrophyte treatment when native and invasive species were grown separately or in the co-culture setting in experiment 2. Season had a significant effect on ordination of the invertebrate community compositions, although no distinctive clustering by season was shown (R = 0.13, *p* < 0.01, Fig. 10). Euclidean PERMANOVA tests revealed no significant heterogeneity of dispersion within groups. In addition, we found no statistically significant effect of the interaction between season and macrophyte treatment on invertebrate community composition.
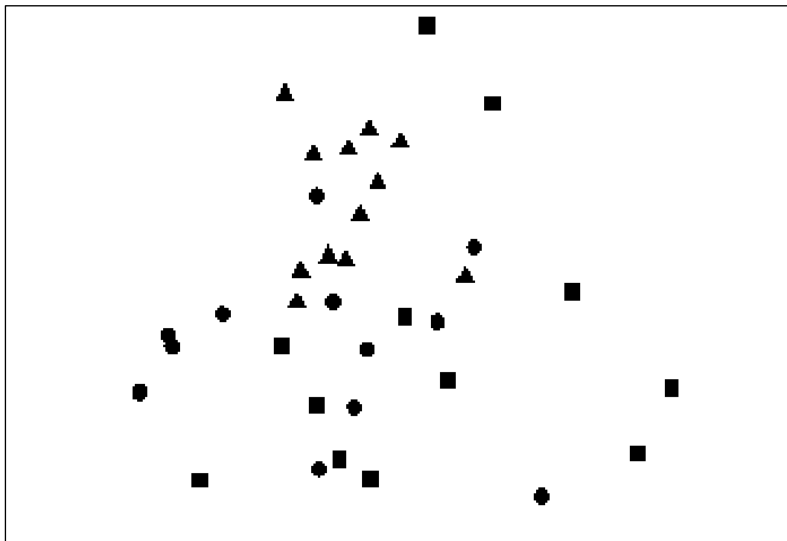
Fig. 10. Non-metric multidimensional scaling (NMDS) of Bray-Curtis similarities illustrating invertebrate community compositions grouped by season; spring (triangles), summer (circles) and autumn (squares) in experiment 2 (α = 0.05).

Discussion

Our findings demonstrated that the richness, abundance and composition of aquatic invertebrate communities in mono-culture stands of *C. caroliniana* were similar to those found in mono-culture stands of *P. ochreatus*. Overall, the invasive species had positive effects on the invertebrates similar to that of the native macrophyte. *Cabomba caroliniana* exhibited a strong induced defence against herbivorous invertebrates such as crayfish and snails in the United States (Morrison and Hay, 2011b). Moreover, it showed deleterious effects on the growth of phytoplankton species by producing anti-algal bioactive compounds (Nakai et al., 1996; 1999) and restrained germination of terrestrial vascular plant species by exuding chemical substances (El-Ghazal and Riemer, 1986). However, in our study, *C. caroliniana* did not have a deterrent effect on the invertebrate communities. The deterrent activity of macrophytes is largely associated with the counterpart invertebrate species involved; hence macrophytes are able to deter some invertebrate species while others may not be affected. Zooplankton can select habitat according to the specific information they receive from the odour emitted by a particular plant, which can be an indicator of the risk or benefit the plant may pose on them (Gutierrez and Mayora, 2016). This indicates that bioactivity of *C. caroliniana* may have deleterious effects on some species, but positive or no impact on others.

In addition to the chemical aspects, the physical structure of a plant plays an important role in a natural system. Richness and abundance of macroinvertebrates tend to increase when habitat complexity increases. Therefore, stands of invasive species with highly dissected leaves and roots can support a highly diverse community of invertebrates. For example, an increase in macroinvertebrate density was found in mono-culture stands of several invasive macrophytes with high habitat complexity compared to the native macrophytes tested (Strayer et al., 2003; Kelly and Hawes, 2005; Hogsden et al., 2007). Conversely, taxon richness and abundance of invertebrates can decline in stands of invasive plants with a simple structural architecture (Houston and Duivenvoorden, 2003). *Hymenachne amplexicaulis* is a riparian plant with a low habitat complexity. In a study conducted by Houston and Duivenvoorden (2003), *H. amplexicaulis* replaced the native submerged macrophytes with highly dissected leaves. Subsequently, the abundance of macroinvertebrates was reduced in *H. amplexicaulis* compared with the native plants. These examples suggest that the physical attributes (habitat complexity) that *C. caroliniana* provides is a key criterion for invertebrates when selecting a plant as refuge, which may supersede other factors such as the effect of bioactive compounds. Thereby, the structure and composition of invertebrate communities associated with native and invasive macrophytes is likely to be similar if the structure of the plants is similar. Douglas and O'Connor (2003) demonstrated that invasive species can have very little or no impact on macroinvertebrate communities if they have a physical structure similar to that of natives. Unlike *C. caroliniana*, *P. ochreatus* does not possess dissected leaves, but it has numerous creeping rhizomes (and/or turions) and forms an intertwined structure as it grows, which together increase the complexity of the habitat it provides for different fauna. Thus, the structural complexity of the two species appears to be comparable.

Structural complexity of plants can also enhance the surface area for attachment of epiphytic algae. *Cabomba caroliniana* contained a higher density of epiphytic algae on its leaves compared with the native macrophytes in a previous study (Hogsden et al., 2007). A study conducted by Sultana et al. (2004) also revealed that 18 different epiphytic algae were able to colonise and grow on the broad leaves of *Potamogeton perfoliatus*. Although some herbivorous invertebrates feed directly on macrophytes, the majority graze upon planktonic and epiphytic algae. This shows that in addition to offering shelter, *C. caroliniana* and *P. ochreatus* can have positive impacts on invertebrates indirectly by providing substrates for epiphytic algae, hence increasing food availability (Hogsden et al., 2007). Therefore, the abundance of certain taxa that use epiphyton as a major food source can increase in invasive plants that support a higher algal biomass compared to native plants, and that can lead to changes in the structure and composition of macroinvertebrate communities (Kelly and Hawes, 2005; Hogsden et al., 2007). This can explain the high richness and abundance of scrapers within *C. caroliniana* and *P. ochreatus* stands in our experiments. Observational assessment of the macrophytes tested in our study revealed no significant herbivory damage on *C. caroliniana*, but numerous *P. ochreatus* leaves were damaged. Moreover, we found a higher number of hygrophilous snails in *P. ochreatus* stands when it was grown next to *C. caroliniana*, which indicates that the snails avoided the invasive macrophyte and selected *P. ochreatus* stands as a feeding site. Low consumption of highly successful invasive macrophytes can directly be attributed to the use of novel chemical defence against native herbivores that are evolutionarily naïve; a strategy that can explain the success of plant invasion (Enge et al., 2012). This suggests that *C. caroliniana* may produce bioactive compounds to deter enemies such as herbivores that feed on macrophytes; nevertheless, the algal-grazing species use the chemically defended *C. caroliniana* as refuge to avoid predation. Aumack et al. (2010) found that meso-grazer amphipods of the western Antarctic Peninsula grazed on the palatable epiphytic algae and filamentous endophytes growing on plants, but used the chemically defended red algae as refuge.

Community dynamics of the invertebrates were comparable in mono-culture stands of the two macrophytes and in non-vegetated ponds. This similar structure and composition of invertebrates in the vegetated ponds and in non-vegetated settings also suggests that the availability of epiphytes as a food source was more important than shelter for the invertebrates, particularly in synthetic systems such as the one used in our study, where predatory fish were absent. We observed multiple dense patches of algae in the control ponds that formed, presumably due to high nutrient concentration. These patches of algae supported similar invertebrate communities to those found

in the vegetated ponds. In addition, habitat preference for vegetated and non-vegetated areas varies for different species. In a previous study conducted by Watkins et al. (1983), crustacean zooplankton and benthic macroinvertebrates were shown to prefer vegetated areas, whereas rotifers selected non-vegetated limnetic areas of the lake. This signifies that macrophytes appear to have a greater impact on benthic macroinvertebrates than on zooplankton. However, in our study, the composition of invertebrates was similar in all macrophyte and non-macrophyte settings.

Overall, *C. caroliniana* in our study exhibited impacts that could be interpreted as ecologically desirable, by providing viable habitat for a range of invertebrates. In a separate study, we have shown that *C. caroliniana* may be able to provide some benefits to water quality by controlling the growth of several cyanobacterial and green algal species via chemical intervention (Pakdel et al., submitted). Environmentally positive impacts have also been identified in several invasive macrophytes in previous studies conducted in tropical and sub-tropical areas (see Thomaz et al., 2015 and references therein). For example, *Eichhornia crassipes* enhanced water quality and increased the density and diversity of invertebrates, fish and waterbirds, which was attributed to an increased habitat heterogeneity and complexity provided by the macrophyte (Brendonck et al., 2003; Villamagna and Murphy, 2010; Villamagna et al., 2010).

However, the general negative impacts of invasive macrophytes should not be overlooked and caution must be taken when interpreting their neutral or positive influences on species diversity or overall ecosystem function. The density of invasive macrophytes can also determine the impacts they pose on other biota. Invasive species in general, have a high growth rate and regeneration capacity (Hussner, 2009). Examples mentioned earlier indicate that high complexity of some invasive macrophytes can support a higher diversity and density of macroinvertebrates. Nonetheless, some invasive macrophytes can have neutral or positive impacts on other organisms at low or moderate densities (Villamagna and Murphy, 2010; Carniatto et al., 2013) and their impacts can switch to negative if the extent of the invasion changes and plant biomass increases (Carniatto et al., 2013). Horizontal distribution and density of two freshwater unionids was found to be limited in dense stands of *M. spicatum* (invasive) and *Nelumbo lutea* (noxious native) (Burlakova and Karatayev, 2007). Stiers et al. (2011) also found a negative relationship between three invasive species, *Hydrocotyle ranunculoides*, *Ludwigia grandiflora* and *M. aquaticum* cover and invertebrate abundance. The decomposing plant material mixed with sediments formed a dense mat, which created unsuitable conditions for invertebrate colonisation and hence led to a lower abundance of invertebrates (Stiers et al., 2011). Dense suspended mats formed by interlocking individuals of invasive plants can decrease the concentration of dissolved oxygen in the water column and form a barrier between water and the atmosphere, which restricts oxygen exchange (Perna and Burrows, 2005; Villamagna and Murphy, 2010). There are a limited number of taxa that can tolerate low dissolved oxygen concentrations (hypoxia). Thus, macroinvertebrate communities under dense mats of invasive macrophytes are expected to be less diverse and predominantly consist of species that are more tolerant to hypoxic and anoxic conditions (Strayer et al., 2003; Kornijów et al., 2010; Villamagna and Murphy, 2010; Stiers et al., 2011). Hypoxia in dense mono-specific stands of invasive species can also impact the physiology of macroinvertebrates. *Sida crystalline* (water flea) collected from invasive macrophyte stands possessed more haemoglobin than individuals that were found within native macrophyte beds (Strayer et al., 2003). Therefore, the effects of invasive species on community structure of invertebrates may appear positive, but the impacts might be different at a molecular level. Moreover, an invasive plant may positively affect certain species, communities or even an ecosystem while the effects might be adverse on others, therefore, there is no universal ecological measure of impacts designate to invasive plant species (Pyšek et al., 2008; Thomaz and Michelan, 2011; Pyšek et al., 2012).

In a separate study, we demonstrated that *C. caroliniana* and *P. ochreatus* could produce similar % cover under our experimental conditions (Pakdel et al., submitted). This can in part explain the similar structure and composition of invertebrate communities in the mono-culture stands of *C. caroliniana* and *P. ochreatus*. Furthermore, it can describe why the invertebrates did not differentiate between the native and invasive

macrophytes in the co-culture setting where they were given a choice. However, the degree of *C. caroliniana* biomass production can differ in nature. *Cabomba caroliniana* has a very fast growth rate and can produce a great amount of plant material in invaded areas (Schooler et al., 2009). This trait is accompanied by other mechanisms used by *C. caroliniana*, which together can shift the positive impact of the plant to negative over time. Nevertheless, the positive impacts of habitat complexity provided for invertebrates by *C. caroliniana* should be considered in management plans. Habitat diversity should be maximised after removal of *C. caroliniana* or any other invasive species and should be done in a timely fashion to mitigate habitat loss (Kovalenko et al., 2010).

In summary, our synthesis showed a positive influence of the invasive species on the community dynamics of aquatic invertebrates similar to that of the native macrophyte. Irrespective of the novel biochemical effects on certain herbivores, the complex physical structure of *C. caroliniana* can benefit a range of aquatic invertebrates directly by providing refuge and indirectly by increasing substrate for attachment of epiphytes. Although *C. caroliniana* and *P. ochreatus* do not have similar structure, they possess comparable complexity. Essentially, herbivorous invertebrates (e.g. snails) that feed directly on macrophytes avoided *C. caroliniana* and fed on *P. ochreatus* where the plants were grown together, but algal-grazing species used the two plants equally as a shelter. Our study demonstrated that unlike freshwater systems of temperate regions in the Northern Hemisphere, the effects of invasive species such as *C. caroliniana* could be positive on certain biota in Australia. Furthermore, *C. caroliniana* may selectively use its novel biochemistry to enhance its invasion potential and deter enemies such as herbivores. Despite the positive effects of *C. caroliniana* on invertebrates, the plant can have detrimental impacts on the overall ecosystem, hence, the overall negative and positive impacts of *C. caroliniana* should be considered in management plans. We recommend that the NWH and ERH should be tested on additional invasive macrophyte species and on species with a range of physical forms. Furthermore, the deterrent activity of invasive plants on native enemies should be tested using a number of generalist and specialist herbivores that feed on macrophytes.

Acknowledgements

References

Alvarez, M. F., Benítez, H. H., Villegas Cortés, J. C., Gabellone, N. A. & Claps, M. C. (2016) Effect of tannins on survival and development of *Daphnia menucoensis* and the abundances of bacteria. *Chemistry and Ecology*, **32,** 742-755.

Ansari, A. A., Gill, S. S., Lanza, G. R. & Rast, W. (2010) *Eutrophication: Causes, Consequences and Control*. Springer-Verlag, Netherlands.

Aumack, C. F., Amsler, C. D., McClintock, J. B. & Baker, B. J. (2010) Chemically mediated resistance to mesoherbivory in finely branched macroalgae along the western Antarctic Peninsula. *European Journal of Phycology,* **45,** 19-26.

Barrat-Segretain, M. H. (2001) Invasive species in the Rhône River floodplain (France): replacement of *Elodea canadensis* Michaux by *E. nuttallii* St. John in two former river channels. *Archiv für Hydrobiologie,* **152,** 237-251.

Boulton, A., Brock, M., Robson, B., Ryder, D., Chambers, J. & Davis, J. (2014) *Australian Freshwater Ecology: Processes and Management*. John Wiley & Sons, Chichester, West Sussex.

Bray, J. R. & Curtis, J. T. (1957) An ordination of the upland forest communities of southern Wisconsin. *Ecological Monographs,* **27,** 325-349.

Brendonck, L., Maes, J., Rommens, W., Dekeza, N., Nhiwatiwa, T., Barson, M., Callebaut, V., Phiri, C., Moreau, K. & Gratwicke, B. (2003) The impact of water hyacinth (*Eichhornia crassipes*) in a eutrophic subtropical impoundment (Lake Chivero, Zimbabwe). II. Species diversity. *Archiv für Hydrobiologie,* **158,** 389-405.

Brönmark, C. & Hansson, L. A. (2005) *The Biology of Lakes and Ponds*. Oxford University Press, Oxford.

Brundu, G. (2015) Plant invaders in European and Mediterranean inland waters: profiles, distribution, and threats. *Hydrobiologia*, **746,** 61-79.

Bryant, D. & Papas, P. (2007) Macroinvertebrate communities in artificial wetlands - the influence of macrophytes. Arthur Rylah Institute for Environmental Research Technical Report Series No. 168. Arthur Rylah Institute for Environmental Research.

Burlakova, L. E. & Karatayev, A. Y. (2007) The effect of invasive macrophytes and water level fluctuations on unionids in Texas impoundments. *Hydrobiologia*, **586,** 291-302.

Callaway, R. M. & Aschehoug, E. T. (2000) Invasive plants versus their new and old neighbors: a mechanism for exotic invasion. *Science*, **290,** 521-523.

Callaway, R. M. & Ridenour, W. M. (2004) Novel weapons: invasive success and the evolution of increased competitive ability. *Frontiers in Ecology and the Environment*, **2,** 436-443.

Cappuccino, N. & Arnason, J. T. (2006) Novel chemistry of invasive exotic plants. *Biology Letters*, **2,** 189-193.

Carniatto, N., Thomaz, S. M., Cunha, E. R., Fugi, R. & Ota, R. R. (2013) Effects of an invasive alien Poaceae on aquatic macrophytes and fish communities in a Neotropical reservoir. *Biotropica*, **45,** 747-754.

Crawley, M. J. (1997) *Plant Ecology*. Blackwell Science, Oxford, Cambridge.

Davis, J. & Christidis, F. (1997) *A Guide to Wetland Invertebrates of South Western Australia.* Frank Daniels Pty, Perth, Australia.

Davis, M. A. (2009) *Invasion Biology.* Oxford University Press, New York, USA.

Dextrase, A. & Mandrak, N. (2006) Impacts of alien invasive species on freshwater fauna at risk in Canada. *Biological Invasions,* **8,** 13-24.

Dhote, S. & Dixit, S. (2009) Water quality improvement through macrophytes-a review. *Environmental Monitoring and Assessment*, **152,** 149-153.

Douglas, M. M. & O'Connor, R. A. (2003) Effects of the exotic macrophyte, para grass (*Urochloa mutica*), on benthic and epiphytic macroinvertebrates of a tropical floodplain. *Freshwater Biology,* **48,** 962-971.

El-Ghazal, R. A. K. & Riemer, D. N. (1986) Germination suppression by extracts of aquatic plants. *Journal of Aquatic Plant Management,* **24,** 76-79.

Elton, C. S. (2000) *The Ecology of Invasions by Animals and Plants.* University of Chicago Press, Chicago.

Enge, S., Nylund, G. M., Harder, T. & Pavia, H. (2012) An exotic chemical weapon explains low herbivore damage in an invasive alga. *Ecology*, **93,** 2736-2745.

Erhard, D. & Gross, E. M. (2006) Allelopathic activity of *Elodea canadensis* and *Elodea nuttallii* against epiphytes and phytoplankton. *Aquatic Botany,* **85,** 203-211.

Fridley, J., Stachowicz, J., Naeem, S., Sax, D., Seabloom, E., Smith, M., Stohlgren, T., Tilman, D. & Holle, B. V. (2007) The invasion paradox: reconciling pattern and process in species invasions. *Ecology,* **88,** 3-17.

Gallardo, B., Clavero, M., Sánchez, M. I. & Vilà, M. (2016) Global ecological impacts of invasive species in aquatic ecosystems. *Global Change Biology*, **22,** 151-163.

Godfrey, R. K. & Wooten, J. W. (1979) *Aquatic and Wetland Plants of Southeastern United States: Monocotyledons.* University of Georgia Press, US.

Gooderham, J. & Tsyrlin, E. (2002) *The Waterbug Book: A Guide to the Freshwater Macroinvertebrates of Temperate Australia.* CSIRO Publishing, Australia.

Gutierrez, M. F. & Mayora, G. (2016) Influence of macrophyte integrity on zooplankton habitat preference, emphasizing the released phenolic compounds and chromophoric dissolved organic matter. *Aquatic Ecology,* **50,** 137-151.

Herb, W. R. & Stefan, H. G. (2006) Seasonal growth of submersed macrophytes in lakes: the effects of biomass density and light competition. *Ecological Modelling,* **193,** 560-574.

Hilt, S. & Gross, E. M. (2008) Can allelopathically active submerged macrophytes stabilise clear-water states in shallow lakes? *Basic and Applied Ecology,* **9,** 422-432.

Hogsden, K. L., Sager, E. P. & Hutchinson, T. C. (2007) The impacts of the non-native macrophyte *Cabomba caroliniana* on littoral biota of Kasshabog Lake, Ontario. *Journal of Great Lakes Research,* **33,** 497-504.

Houston, W. A. & Duivenvoorden, L. J. (2003) Replacement of littoral native vegetation with the ponded pasture grass *Hymenachne amplexicaulis*: effects on plant, macroinvertebrate and fish biodiversity of backwaters in the Fitzroy River, Central Queensland, Australia. *Marine and Freshwater Research,* **53,** 1235-1244.

Hussner, A. (2009) Growth and photosynthesis of four invasive aquatic plant species in Europe. *Weed Research,* **49,** 506-515.

ISSG, (2005) *Global Invasive Species Database (GISD)*. Auckland, New Zealand: University of Auckland. http://www.issg.org/database

Jarchow, M. E. & Cook, B. J. (2009) Allelopathy as a mechanism for the invasion of *Typha angustifolia*. *Plant Ecology*, **204**, 113-124.

Jeppesen, E., Søndergaard, M., Søndergaard, M. & Christoffersen, K. (1998) *The Structuring Role of Submerged Macrophytes in Lakes*. Springer-Verlag, New York.

Jeschke, J., Aparicio, L. G., Haider, S., Heger, T., Lortie, C., Pyšek, P. & Strayer, D. (2012) Support for major hypotheses in invasion biology is uneven and declining. *NeoBiota,* **14,** 1-20.

Kay, S. H. & Hoyle, S. T. (2001) Mail order, the Internet, and invasive aquatic weeds. *Journal of Aquatic Plant Management*, **39,** 88-91.

Kelly, D. J. & Hawes, I. (2005) Effects of invasive macrophytes on littoral-zone productivity and food web dynamics in a New Zealand high-country lake. *Journal of the North American Benthological Society,* **24,** 300-320.

Kolar, C. S. & Lodge, D. M. (2000) Freshwater nonindigenous species: interactions with other global changes. *Invasive Species in a Changing World*, 3-30.

Kornijów, R., Strayer, D. L. & Caraco, N. F. (2010) Macroinvertebrate communities of hypoxic habitats created by an invasive plant (*Trapa natans*) in the freshwater tidal Hudson River. *Fundamental and Applied Limnology/Archiv für Hydrobiologie,* **176,** 199-207.

Kovalenko, K. E., Dibble, E. D. & Slade, J. G. (2010) Community effects of invasive macrophyte control: role of invasive plant abundance and habitat complexity. *Journal of Applied Ecology,* **47,** 318-328.

Kowarik, I. (2003) Human agency in biological invasions: secondary releases foster naturalisation and population expansion of alien plant species. *Biological Invasions*, **5,** 293-312.

Lassuy, D. R. (1995) Introduced species as a factor in extinction and endangerment of native fish species. *Uses and Effects of Cultured Fishes in Aquatic Ecosystems* (eds H. L. Schramm & R. G. Piper), pp. 391-396. American Fisheries Society, Bethesda.

Levene, H. (1960) Robust tests for equality of variances1. Contributions to probability and statistics. *Essays in Honor of Harold Hotelling,* **2,** 278-292.

Liu, H. & Stiling, P. (2006) Testing the enemy release hypothesis: a review and meta-analysis. *Biological Invasions,* **8,** 1535-1545.

Lockwood, J. L., Hoopes, M. F. & Marchetti, M. P. (2013) *Invasion Ecology*. John Wiley & Sons, Oxford.

Lürling, M., van Geest, G. & Scheffer, M. (2006) Importance of nutrient competition and allelopathic effects in suppression of the green alga *Scenedesmus obliquus* by the macrophytes *Chara*, *Elodea* and *Myriophyllum*. *Hydrobiologia,* **556,** 209-220.

Mackey, A. & Swarbrick, J. (1997) The biology of Australian weeds. 32. *Cabomba caroliniana* Gray. *Plant Protection Quarterly*, **12,** 154-165.

Maron, J. L. & Vilà, M. (2001) When do herbivores affect plant invasion? Evidence for the natural enemies and biotic resistance hypotheses. *Oikos,* **95,** 361-373.

Martin, G. D. & Coetzee, J. A. (2011) Pet stores, aquarists and the internet trade as modes of introduction and spread of invasive macrophytes in South Africa. *Water SA*, **37,** 371-380.

Meyerson, L. A. & Mooney, H. A. (2007) Invasive alien species in an era of globalization. *Frontiers in Ecology and the Environment*, **5,** 199-208.

Morrison, W. E. & Hay, M. E. (2011a) Herbivore preference for native vs. exotic plants: generalist herbivores from multiple continents prefer exotic plants that are evolutionarily naïve. *PLoS One,* **6,** e17227.

Morrison, W. E. & Hay, M. E. (2011b) Induced chemical defences in a freshwater macrophyte suppress herbivore fitness and the growth of associated microbes. *Oecologia,* **165,** 427-436.

Muylaert, K., Pérez-Martínez, C., Sánchez-Castillo, P., Lauridsen, T.L., Vanderstukken, M., Declerck, S.A.J., Van der Gucht, K., Conde-Porcuna, J.M., Jeppesen, E., De Meester, L. & Vyverma, W. (2010) Influence of nutrients, submerged macrophytes and zooplankton grazing on phytoplankton biomass and diversity along a latitudinal gradient in Europe. *Hydrobiologia,* **653,** 79-90.

Nakai, S., Hosomi, M., Okada, M. & Murakami, A. (1996) Control of algal growth by macrophytes and macrophyte-extracted bioactive compounds. *Water Science and Technology,* **34,** 227-235.

Nakai, S., Inoue, Y., Hosomi, M. & Murakami, A. (1999) Growth inhibition of blue-green algae by allelopathic effects of macrophyte. *Water Science and Technology,* **39,** 47-53.

Oksanen, J. (2009) Multivariate analysis of ecological communities in R: vegan tutorial. *URL:[http://cc. oulu. fi/, jarioksa/opetus/metodi/vegantutor. pdf].*

Oksanen, J., Kindt, R., Legendre, P., O'Hara, B., Stevens, M. H. H., Oksanen, M. J. & Suggests, M. (2007) The vegan package. *Community Ecology Package,* **10,** 631-637.

Ørgaard, M. (1991) The genus *Cabomba* (Cabombaceae)—a taxonomic study. *Nordic Journal of Botany*, **11,** 179-203.

Pakdel, M. F., Wevill, T., Sim, L., Beardall, John. & Davis, J. (submitted). Bioactivity of an invasive macrophyte species on two potential native 'enemies'. *Journal of Freshwater Biology.*

Pakdel, M. F., Wevill, T., Sim, L., Beardall, John. & Davis, J. (submitted). Novel chemical weapons: growth inhibition of cyanobacteria and green algae by invasive and native macrophytes. *Ecology and Evolution*.

Parsons, W. T. & Cuthbertson, E. G. (2001) *Noxious Weeds of Australia*. 2nd Edition. CSIRO Publishing, Collingwood.

Perna, C. & Burrows, D. (2005) Improved dissolved oxygen status following removal of exotic weed mats in important fish habitat lagoons of the tropical Burdekin River floodplain, Australia. *Marine Pollution Bulletin,* **51,** 138-148.

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.

Pyšek, P., Jarošík, V., Hulme, P. E., 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., Richardson, D. M., Pergl, J., Jarošík, V., Sixtová, Z. & Weber, E. (2008) Geographical and taxonomic biases in invasion ecology. *Trends in Ecology & Evolution,* **23,** 237-244.

Sainty, G. & Jacobs, S. (2003) *Waterplants in Australia: A Field Guide, 4 ed*. Sainty & Associates, Australia.

Sala, O. E., Chapin, F. S., Armesto, J. J., Berlow, E., Bloomfield, J., Dirzo, R., Huber-Sanwald, E., Huenneke, L. F., Jackson, R. B. & Kinzig, A. (2000) Global biodiversity scenarios for the year 2100. *Science*, **287,** 1770-1774.

Sax, D. F., Stachowicz, J. J., Brown, J. H., Bruno, J. F., Dawson, M. N., Gaines, S. D., Grosberg, R. K., Hastings, A., Holt, R. D. & Mayfield, M. M. (2007) Ecological and evolutionary insights from species invasions. *Trends in Ecology & Evolution,* **22,** 465-471.

Scheffer, M. & van Nes, E. H. (2007) Shallow lakes theory revisited: various alternative regimes driven by climate, nutrients, depth and lake size. *Hydrobiologia*, **584,** 455-466.

Scheffer, M. (1998) *Ecology of Shallow Lakes.* Chapman & Hall, London, New York.

Schooler, S., Cabrera-Walsh, W. & Julien, M. (2009) *Cabomba caroliniana* gray (Cabombaceae). *Biological control of tropical weeds using arthropods.* Cambridge University Press, Cambridge, 88-107.

Schultz, R. & Dibble, E. (2012) Effects of invasive macrophytes on freshwater fish and macroinvertebrate communities: the role of invasive plant traits. *Hydrobiologia,* **684,** 1-14.

Steenhagen, D. A. & Zimdahl, R. L. (1979) Allelopathy of leafy spurge (*Euphorbia esula*). *Weed Science,* **27,** 1-3.

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

Strayer, D. L., Lutz, C., Malcom, H. M., Munger, K. & Shaw, W. H. (2003) Invertebrate communities associated with a native (*Vallisneria americana*) and an alien (*Trapa natans*) macrophyte in a large river. *Freshwater Biology,* **48,** 1938-1949.

Sultana, M., Asaeda, T., Manatunge, J. & Ablimit, A. (2004) Colonisation and growth of epiphytic algal communities on *Potamogeton perfoliatus* under two different light regimes. *New Zealand Journal of Marine and Freshwater Research,* **38,** 585-594.

Svensson, J. R., Nylund, G. M., Cervin, G., Toth, G. B. & Pavia, H. (2013) Novel chemical weapon of an exotic macroalga inhibits recruitment of native competitors in the invaded range. *Journal of Ecology,* **101,** 140-148.

The Murray Darling Freshwater Research Centre (TMDFRC), (2013) *Identification and Ecology of Australian Freshwater Invertebrates*, TMDRC, Australia, http://www.mdfrc.org.au/bugguide/ (accessed 28 January 2015).

Thiebaut, G., Rolland, T., Robach, F., Tremolieres, M., Muller, S. & Bergot, F. (1997) Some consequences of the introduction of two macrophyte species, *Elodea canadensis* Michaux and *Elodea nuttallii* St. John, in continental aquatic ecosystems: Example of two areas in the North-East of France: Alsace Plain and Northern Vosges. *Bulletin Francais de la Peche et de la Pisciculture*, **344-345,** 441-452.

Thomaz, S. M. & Michelan, T. S. (2011) Associations between a highly invasive species and native macrophytes differ across spatial scales. *Biological Invasions,* **13,** 1881-1891.

Thomaz, S. M., Kovalenko, K. E., Havel, J. E. & Kats, L. B. (2015) Aquatic invasive species: general trends in the literature and introduction to the special issue. *Hydrobiologia,* **746**, 1-12.

Timms, R. M. & Moss, B. (1984) Prevention of growth of potentially dense phytoplankton populations by zooplankton grazing, in the presence of zooplanktivorous fish, in a shallow wetland ecosystem. *Limnology and Oceanography*, **29,** 472-486.

Villamagna, A. M. & Murphy, B. R. (2010) Ecological and socio-economic impacts of invasive water hyacinth (*Eichhornia crassipes*): a review. *Freshwater Biology,* **55,** 282-298.

Villamagna, A. M., Murphy, B. R. & Trauger, D. L. (2010) Behavioral response of American coots (*Fulica americana*) to water hyacinth (*Eichhornia crassipes*) in Lake Chapala, Mexico. *Waterbirds,* **33,** 550-555.

Watkins, C., Shireman, J. V. & Haller, W. T. (1983) The influence of aquatic vegetation upon zooplankton and benthic macroinvertebrates in Orange Lake, Florida. *Journal of Aquatic Plant Management,* **21,** 78-83.

Williamson, M. (1996) *Biological Invasions*. Chapman & Hall, London.

Willis, R. J. (2008) *The History of Allelopathy*. Springer, Dordrecht

Wolf, V. C., Berger, U., Gassmann, A. & Müller, C. (2011) High chemical diversity of a plant species is accompanied by increased chemical defence in invasive populations. *Biological Invasions,* **13,** 2091-2102.

Chapter 4.

Bioactivity of an invasive macrophyte species on a potential native enemy and a native competitor

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Declaration for thesis chapter four

Declaration by candidate
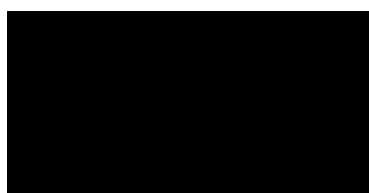
In the case of Chapter 4, the nature and extent of my contribution to the work was the following:

Nature of contribution	Extent of contribution
I mostly conceived and designed the experiments, performed the outdoor and lab work, analysed all of the data and was the primary author of the manuscript	80%

The following co-authors contributed to the work. If co-authors are students at Monash University, the extent of their contribution in percentage terms must be stated:

Name	Nature of contribution	Extent of contribution (%) for student co-authors only
John Beardall	Conception of ideas, supervision and editing	5%
Jenny Davis	Conception of ideas, supervision and editing	5%
Lien Sim	Conception of ideas, supervision and editing	5%
Tricia Wevill	Conception of ideas, supervision and editing	5%

Candidate's Signature

Date: 17/02/2017

Main Supervisor's Signature

Date: 17/02/2017

Abstract

1. Many invasive plants have important interactions with other biota in their introduced ranges. They are free from their co-evolved enemies, hence, are less prone to attacks by herbivores ('Enemy Release Hypothesis', ERH) or, may produce chemicals that are novel to the native community ('Novel Weapons Hypothesis', NWH). These mechanisms together may deliver a competitive advantage to invasive species over natives. *Cabomba caroliniana* is a freshwater plant that has become a noxious weed worldwide. We tested the ERH and NWH by investigating the interactions between *C. caroliniana* and two potential native 'enemies'; a competitor and a herbivore.

2. We tested the allelopathic effects of *C. caroliniana* on *Potamogeton ochreatus* in a co-culture outdoor experiment. We also examined the anti-herbivore activity of *C. caroliniana* by comparing the herbivory damage on *C. caroliniana* and *P. ochreatus* caused by the larvae of *Parapoynx rugosalis* under controlled conditions.

3. We found a significantly lower % cover of *P. ochreatus* and *C. caroliniana* when grown together in the co-culture ponds compared to the controls (single species planting) in the allelopathy experiment. A high level of feeding damage was inflicted on the invasive species in the herbivore release experiment. Although the native plant also had a high level of herbivory damage, there was a significantly higher amount of damage to the invasive versus the native macrophyte.

4. Our findings suggest that *C. caroliniana* could have a potent allelopathic potential towards native competitors and vice versa. However, no deterrent activity of *C. caroliniana* was detected against *P. rugosalis*.

5. *Synthesis. Cabomba caroliniana* can use its novel biochemistry to overcome competition and may be able to thrive in systems where other potential competitors are absent. Nevertheless, the presence of allelopathic native plants such as *P. ochreatus* can regulate its growth. In addition, generalist herbivores such as *P. rugosalis* appear to be resistant to the biochemicals of *C. caroliniana* and *C. caroliniana* is tolerant to herbivory by some aquatic invertebrates. *Cabomba caroliniana* has a high biomass turnover even from small fragments, which is an important strategy that can compensate for biomass loss due to herbivory.

Key-words: allelopathy, freshwater invasions, herbivory damage, macrophytes, novel weapons.

Introduction

The introduction and spread of invasive species are exacerbated as a result of globalisation and human activities (Meyerson and Mooney, 2007; Mayer-Pinto et al., 2015). Invasive species can reduce biodiversity and can have various adverse environmental, economic and social impacts (Sala et al., 2000; Lodge et al., 2006; Pimentel, 2011; Lockwood et al., 2013; Gallardo et al., 2016). Many exotic plant species can be competitively superior in their non-native ranges even though these species may not be strong competitors in their natural locales (Callaway and Aschehoug, 2000). Moreover, the effects of neighbouring plants can be less detrimental on invasive species in the invaded region than at home (Callaway et al., 2011). There are several hypotheses that can explain the success of invasive species. Research has endorsed some of these hypotheses, including the 'Novel Weapons Hypothesis' (NWH) (Callaway and Aschehoug, 2000) and the 'Enemy Release Hypothesis' (ERH) (Crawley, 1997), whereas support for other hypotheses is declining (see Jeschke et al., 2012 and references therein).

Some plants, known as allelopathic plants, can reduce the growth and survival of immediate competitors and herbivores through chemical interference (Molisch, 1937; Willis, 1985; 2008). Allelopathic activity has long been proposed as an important trait of invasive plant species (Steenhagen and Zimdahl, 1979). The mechanism has increasingly become recognised as one of the key drivers that intensify invasive potential of plants (Inderjit et al., 2011). This has led to the formation of the NWH, which poses that exotic plant species with allelopathic potential can become invasive by producing novel biochemicals to which the recipient native communities are not resistant (Callaway and Aschehoug, 2000). The NWH was initially formulated for chemically mediated interactions among plant species in terrestrial environments (Callaway and Ridenour, 2004), but was later extended to include the anti-herbivore, antimicrobial and antifungal activity of invasive plants (Cappuccino and Arnason, 2006; Verhoeven, 2009). In addition, the investigation of the NWH has recently been expanded from terrestrial environments to other systems such as marine areas (Svensson et al., 2013). Some invasive freshwater macrophyte species (aquatic plants and macroalgae) are known to produce allelopathic chemical substances (Erhard and Gross, 2006; Dandelot et al., 2008). The chemical diversity and the quantity of different chemical substances were found to be greater in some freshwater invasive macrophytes compared with native species (Marko et al., 2008; Wolf et al., 2011). However, studies investigating allelopathic interactions between native and invasive vascular aquatic plant species are scarce and to date, no studies have been conducted to test the NWH in freshwater systems (Jeschke et al., 2012).

The fitness of plant species can be regulated by other organisms such as herbivores, competitors, parasites and pathogens. According to the ERH, invaders escape these natural enemies in their exotic ranges, which enables them to enhance their competitive ability compared with their native neighbours (Williamson, 1996; Crawley, 1997; Torchin et al., 2003). Plant-herbivore interactions in aquatic systems are predominantly dependent on the abundance and feeding preferences of herbivores for certain host plant species. Some herbivores prefer invasive species over natives (Parker and Hay, 2005; Morrison and Hay, 2011a), others feed on native macrophytes and avoid invasive plants (Erhard et al., 2007; Xiong et al., 2008), whereas some have no preference for either invasive or native macrophytes (Grutters et al., 2016).

A range of invasive macrophytes exhibit induced chemical defences as a response to herbivory, which can negatively affect the fitness and growth of herbivorous invertebrates and can lower plants' palatability (Morrison and Hay, 2011b; Rothhaupt et al., 2015). Erhard et al. (2007) found that the generalist herbivorous larvae of *Acentria ephemerella* avoid feeding on the invasive species, *Elodea nuttallii*. In their study, *A. ephemerella* fed on the native macrophyte, *Potamogeton perfoliatus*, however, the larvae also avoided the leaf disks of *P. perfoliatus* when they were coated with partially purified flavonoids and crude extracts of *E. nuttallii*. Some aquatic plants can induce defence and re-allocate nutrients to different tissues thus lowering the palatability of these tissues when they are attacked by herbivores. For instance, when exposed to herbivory by insects, *M. spicatum* can

reduce the nutritional value of its tissues by allocating less nitrogen to parts that are vulnerable to grazing and re-allocate it to more secure tissues (Rothhaupt et al., 2015).

Invasive species possess a number of additional traits that can govern their successful establishment and spread in the new areas. Many invasive species for instance, have a fast growth rate and produce a large amount of plant material via seed production and/or fragmentation (Grotkopp and Rejmánek, 2007; Silveira et al., 2009). A meta-analysis conducted by van Kleunen et al. (2010) demonstrated that functional traits of plant species including overall fitness, physiology, shoot allocation, leaf area allocation, growth rate and size were significantly enhanced in invasive species compared to native and non-invasive exotic species. These findings suggest that the potential invasiveness of plants can be determined and predicted by their performance related traits. Invasive species also have an increased competitive ability and are capable of utilising available nutrients more efficiently than native species (Burns, 2004; Funk and Vitousek, 2007; van Kleunen et al., 2010; Fan et al., 2013; Fleming and Dibble, 2015).

Cabomba caroliniana Gray, commonly known as green cabomba or cabomba, is a freshwater perennial macrophyte native to North and South America, which lives in slow-moving and standing waters. It has been disseminated around the world as an aquarium plant and is currently a noxious weed in the United States (outside its natural range), Canada and several European and Asian-Pacific countries including Australia. *Cabomba caroliniana* is a Weed of National Significance in many of these countries and is on the list of invasive species of union concern in the EU (Ørgaard, 1991; Mackey and Swarbrick, 1997; Parsons and Cuthbertson, 2001; Sainty and Jacobs, 2003; ISSG, 2005; Schooler et al., 2009; Brundu, 2015). It has a high rate of biomass production and grows well in eutrophic as well as oligotrophic environments. It is highly invasive and has detrimental impacts on waterways. It can spread both vegetatively via fragmentation and sexually by producing seeds, although production of fertile seeds (and fruits) has not been documented in Australia. *Cabomba caroliniana* has been recorded in several waterways along the east coast of Australia (Mackey and Swarbrick, 1997; Sainty and Jacobs, 2003).

The aim of this study was to investigate the ERH and the NWH in freshwater systems by examining the potential allelopathic and anti-herbivore activity of *C. caroliniana* using two different potential native 'enemies' as the target organisms; a freshwater vascular plant as a competitor and a generalist herbivore.

Materials and methods

ALLELOPATHY EXPERIMENT

Study species

Potamogeton ochreatus Raoul (blunt pondweed), a native pondweed common in many wetlands located in south-eastern Australia was used as our target species. This perennial plant inhabits freshwater systems and grows well in nutrient rich environments. *Potamogeton* species are known to have allelopathic potential (He et al., 2008; Zhang et al., 2009; Takeda et al., 2011; Vanderstukken et al., 2011; Pakdel et al., 2013), therefore, *P. ochreatus* was used to test the allelopathic antagonistic interactions between the two macrophytes and determine which species have stronger allelopathic effects.

We collected *C. caroliniana* from Lake Nagambie (36°47′0″S 145°8′30″E) and *P. ochreatus* from Hampton Park Lake (38°02'14.1"S 145°16'24.9"E) in Victoria during summer 2014. An outdoor experiment was carried out using hard-plastic ponds (capacity, 120 L; height, 0.45 m; diameter, 0.6 m) in the Jock Marshall Reserve (JMR) at Monash University, Clayton, Victoria, during the period February 2014—May 2016. The bottom of each pond was covered with 5 cm top-soil overlaid by 1 cm of washed sand one day prior to the addition of macrophytes. The ponds were

then filled with rainwater held in header tanks within the JMR. Plastic mesh partitions (2 cm^2 diameter) were placed across the middle of each pond. The partitions were soaked in rainwater for one week prior to use to eliminate any contamination.

Macrophyte material was washed free of debris and zooplankton prior to planting. The top 15 cm of *C. caroliniana*, bearing the apical meristem, was cut and planted in the pond. For *P. ochreatus*, the whole plant (including rhizomes, shoots and leaves) was used. Both *C. caroliniana* and *P. ochreatus* were introduced into three ponds with each species planted to cover 30% of the sediment surface (Fig. 1). The two plant species were separated by partitions. This allowed the transfer of water, nutrients and potential allelochemicals from one side to the other while eliminating the effects of shading and competition for space between the two species. To set up the controls, we added each species to three separate ponds, at the same planting % cover (30%) on one side of the pond but leaving the other half of the pond unplanted (Fig. 1). Consequently, we had three replicates of the treatment and three replicates for each of the controls (totalling nine ponds). Two slow-release fertiliser tablets (Osmocote, Plus Trace Elements - Water Gardens & Aquatic Plants, Scotts, Australia) were added to each pond every two weeks for 20 weeks to avoid potential nutrient limitation. The ponds were covered by bird netting (1 mm^2 diameter mesh, 10-15% shading) throughout the experiment to avoid the spread of the invasive species by animal vectors and the excessive growth of filamentous and planktonic algae. The growth of *C. caroliniana* and *P. ochreatus* was measured as the % cover filling the entire area at each side of the ponds after 20 weeks and was compared to the controls containing either *C. caroliniana* or *P. ochreatus*.
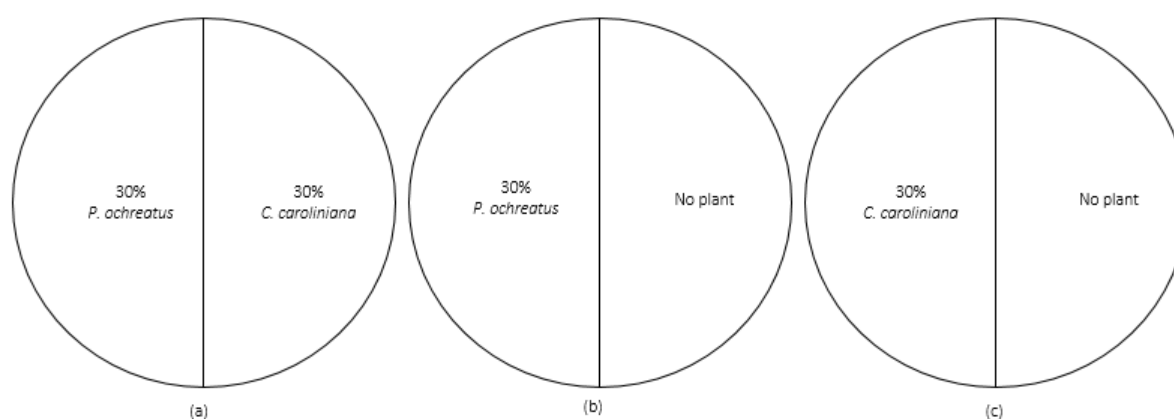
Fig. 1. Diagram showing the experimental set up in the allopathy investigation. a) mixed species plantings containing *Cabomba caroliniana* and *Potamogeton ochreatus*, b) single species planting of *P. ochreatus* (control) and c) single species planting of *C. caroliniana* (control) all separated by a partition.

HERBIVORY EXPERIMENT

Study species

Most direct herbivory on living macrophytes is by invertebrate herbivores from the families and orders of primarily terrestrial insect species including aquatic lepidopterans, Curculionidae, Chrysomelidae and specialist dipterans (Newman, 1991). We used a moth larva, *Parapoynx rugosalis* Möschler as a potential enemy to examine the deterrent activity of *C. caroliniana* on a generalist aquatic invertebrate herbivore. *Parapoynx rugosalis* is a native moth species in the family Crambidae. We selected this species because generalist herbivores are more likely to feed on and hence impact invasive macrophytes compared with specialist herbivores. The aquatic larvae of *Parapoynx* species feed on *Potamogeton* species, although, they are also known to feed on several other macrophyte species from different genera including *Vallisneria*, *Hydrilla*, *Nymphaea*, *Ludwigia* and *Cabomba* (Habeck, 1974; Buckingham and Bennett, 1989; Mueller and Dearing, 1994; Schooler et al., 2006; Hutchinson et

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al., 2015). The larvae of *P. rugosalis* use leaf fragments of the host plant to make stationary or portable cases to cover themselves (Habeck, 1974). We further tested the effect of herbivory by *P. rugosalis* on the % cover of its common food source, *P. ochreatus* and compared the herbivory damage on *C. caroliniana* and *P. ochreatus* caused by the larvae.

Parapoynx rugosalis larvae were collected from the outdoor ponds in the JMR. The experiment was carried out in a controlled temperature (CT) room (23 ± 1 °C, 14:10 light:dark period and 70 µmol photons PAR m^{-2} s^{-1}) at the School of Biological Sciences, Monash University, Victoria during summer 2015. We added 5cm top soil covered with 1-2cm white washed sand to small glass aquaria. The aquaria were then filled with distilled water. *Cabomba caroliniana* (shoots with apical meristem) and *P. ochreatus* (whole plant) were planted separately in each aquarium to cover 50% of the total area. Six replicates were established for each plant. The aquaria were then incubated in the CT room for one week to allow the plants to establish. Five *P. rugosalis* larvae (approximately 5mm) were added to three aquaria (treatments) and the other three (controls) remained free of herbivores. The herbivory damage of plants (% cover loss) caused by the larvae was recorded after 3 days and was compared to the controls.

STATISTICAL ANALYSIS

We used two-factor ANOVAs tests to compared the % cover of *C. caroliniana* and *P. ochreatus* when they were grown together to when they were grown separately in the control ponds in the allelopathy experiment. Furthermore, the % cover of *P. ochreatus* was compared to *C. caroliniana* in the mixed species ponds by conducting the same analysis. We also performed two-factor ANOVAs (in which the factor levels were species and treatment combined) to compare the effect of larval feeding on the % cover of *C. caroliniana* and *P. ochreatus* in the herbivory experiment. ANOVAs were followed by Tukey's *post hoc* tests when significant results were found. All data were tested for normality and homogeneity of variances. Data analysis was carried out using the R Statistical Program (RStudio, ver. 3.3.1 (2016-06-21)) with a criterion of $\alpha = 0.05$ as the significance level.

Results

ALLELOPATHY EXPERIMENT

We found significant effects of macrophyte treatment (single plant versus mixed species) and macrophyte species (native and invasive) (F = 60.31, *df* = 1, *p* < 0.001 and F = 19.7, *df* = 1, *p* < 0.001 respectively). The interaction between treatment and macrophyte species was non-significant. The % cover of *P. ochreatus* grown separately was significantly higher than its % cover when grown in the same ponds as *C. caroliniana* (*p* < 0.01, Fig. 2). There was also a higher % cover of *C. caroliniana* when grown separately than when grown in the mixed species ponds (*p* < 0.01, Fig. 2). In addition, our results revealed a significant difference between the % cover of *C. caroliniana* and *P. ochreatus* when grown next to each other (*p* < 0.05, Fig. 2). However, *C. caroliniana* had a similar % cover to *P. ochreatus* when the two plants were grown separately.
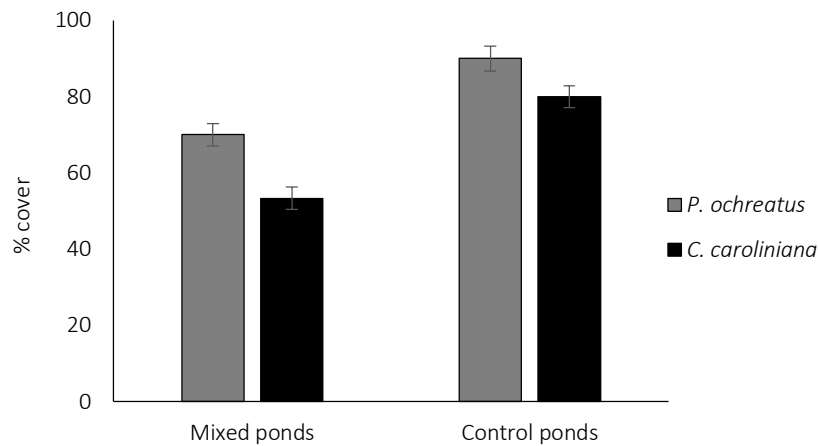
Fig. 2. Mean (±SE) % cover of *Potamogeton ochreatus* (grey bars) and *Cabomba caroliniana* (black bars) in the mixed ponds (grown together) and the controls (grown alone).

HERBIVORY EXPERIMENT

We found a statistically significant effect of the interaction between herbivory treatment and macrophyte species (F = 28.04, *df* = 1, *p* < 0.001). Cover by both *C. caroliniana* and *P. ochreatus* was significantly lower in the presence of the larvae compared to the controls (*p* < 0.001 and *p* < 0.001 respectively, Fig. 3). Furthermore, *C. caroliniana* had significantly lower % cover compared to *P. ochreatus* following exposure to the larvae (*p* < 0.001, Fig. 3) with the larvae causing a 95 ± 3 % cover decrease for *C. caroliniana* but only a 48.3 ± 7.3 % cover drop for *P. ochreatus*. The % cover of *C. caroliniana* was similar to *P. ochreatus* in the control aquaria where the larvae were absent.
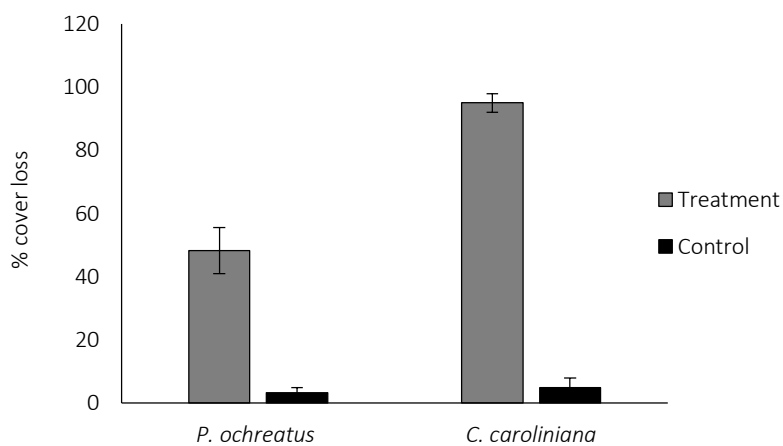
Fig. 3. Mean (±SE) herbivory damage (shown as % cover loss) on macrophytes in the presence of the moth larva, *Parapoynx rugosalis* (treatment) and when the larva was absent (control).

Discussion

ALLELOPATHY EXPERIMENT

Our findings suggest that growth of the native macrophyte, *P. ochreatus* (measured as % cover) can be constrained by allelopathic effects of the invasive species and that *C. caroliniana* uses its novel biochemistry to enhance its invasiveness. Moreover, the presence of *P. ochreatus* can have a negative effect on the % cover of *C.*

caroliniana. By the end of our experiment, the cover by both macrophytes had entirely filled the total available areas (half of each pond) in most ponds in control plantings (single species plantings). In mixed species plantings, the extent of cover by both macrophytes was lower. This suggests that there is an antagonistic relationship between *C. caroliniana* and *P. ochreatus* and further, that allelopathy may be a two-way interaction between these species. Thereby, the NWH may not be specific to invasive macrophytes, and invasive species may be similarly affected by the novel biochemicals produced by some native counterparts. *Cabomba caroliniana* is known to have an allelopathic effect on phytoplankton (Nakai et al., 1996; 1999) and has been shown to supress germination of terrestrial vascular plant species in its native habitat (El-Ghazal and Riemer, 1986). A range of *Potamogeton* species are also allelopathically active towards phytoplankton (He et al., 2008; Zhang et al., 2009; Takeda et al., 2011; Pakdel et al., 2013).

Although nutrient availability is a major factor regulating the structure and dynamics of macrophyte communities in aquatic systems (Gopal and Goel, 1993; Barko and James, 1998; Gross, 2003), competition for nutrients is rarely a limiting factor to growth for *C. caroliniana* and *P. ochreatus* because both macrophytes are submerged species that are capable of acquiring nutrients from sediments and the water column (Wetzel, 1988; Gopal and Goel, 1993; Barko and James, 1998). Hence, even when the nutrient level of surrounding water is low, there is an alternative source of nutrients in the sediment. However, invasive species are more successful in colonizing eutrophic habitats (high nutrient concentrations) and can be negatively affected by nutrient reduction (Gérard et al., 2014). Moreover, they can store nutrients such as phosphorus in their tissues when nutrient levels are high or, acquire nutrients from the water column in adverse conditions, thereby possibly outcompeting native species when the nutrient level of the system declines (Wersal and Madsen, 2011; Gérard et al., 2014). Consequently, we minimised the potential for nutrient limitation to influence % cover of the macrophyte species by adding nutrients to all ponds.

Competition for resources such as space is also inevitable between macrophytes that occupy similar niches (Schallenberg and Waite, 2004; Casanova and Zhang, 2007; Stiers et al., 2011). Both *C. caroliniana* and *P. ochreatus* are canopy forming submerged species that inhabit shallow as well as deep water. In our study, we separated the species in mixed ponds with the use of a partition so that allelopathic interactions would not be confounded with competition for space. Therefore, we can conclude that the antagonistic interaction between the two species is more likely due to chemical interference rather than competition for resources such as nutrients and space. To our knowledge, this study is the first to determine a negative effect of *C. caroliniana* on an aquatic vascular plant species outside its natural distribution and to show possible allelopathic potential of a native plant on an invasive species in freshwater system.

Cabomba caroliniana forms monospecific stands in its introduced ranges but co-exists with several submerged macrophytes including *Potamogeton* species in its native habitat (Schooler et al., 2009). In our study, *P. ochreatus* appeared to supress the growth of the invasive macrophyte. Our findings suggest that *C. caroliniana* should also be able to co-exist with *P. ochreatus* in its introduced areas. Moreover, the presence of native competitors, in particular those with allelopathic potential may limit the invasive potential of *C. caroliniana* and perhaps other exotic macrophytes. A previous study conducted by Levine et al. (2004) also showed that native competitors may not be able to prevent invasion, but they can limit the invasion success of some macrophyte species.

We conducted our experiment using mature plant material. However, the effect of invasive plant biochemistry may differ on native species at different ages and native macrophytes may be affected greatly during their more vulnerable life stages. For example, water containing the chemical released by two invasive *Ludwigia* species was found to significantly decrease germination and increase seedling mortality of two native plants (Dandelot et al., 2008). Additionally, macrophytes exhibit different levels of allelopathy at different life stages (Mulderij et al., 2003). In general, younger more active macrophytes are more chemically defended than older individuals and can have a greater allelopathic impact on other species (Planas et al., 1981; Gross, 2000; Mulderij et al., 2003;

Mulderij et al., 2005). Consequently, the outcome of the allelopathic interactions between *C. caroliniana* and *P. ochreatus* may be affected by the age of the two plants. Invasion success of *C. caroliniana* may be markedly reduced and it may not be able to establish in systems where native species, in particular those with allelopathic potential, are already present. On the other hand, immature native plants may be incapable of tolerating the novel biochemistry of *C. caroliniana* and may be outcompeted once *C. caroliniana* is established. This suggests that systems that are healthy and contain native macrophytes have a greater and prolonged ability to resist infestation by *C. caroliniana* and are less likely to be invaded. Nonetheless, even with a minor change in the system, conditions may favour one species more than the other, depending on their growth form and life strategy. In due course, one species may become dominant and overcome the other (Stiers et al., 2011). Disturbed systems are more prone to invasion (Pimm and Hyman, 1987; Baltz and Moyle, 1993) and invasive species respond more positively to disturbance than natives (Quinn et al., 2011). *Cabomba caroliniana* proliferates through fragmentation. This growth strategy combined with the use of other mechanisms such as allelopathy can therefore increase the potential of *C. caroliniana* to establish more rapidly than native species in a disturbed system.

Cabomba caroliniana can grow well in eutrophic systems (Schooler et al., 2009). Although resource availability plays an important role in the success of invasive species, in freshwater systems where native competitors and herbivores abound, the role of resource availability diminishes (Levine, 2000; Kennedy et al., 2002; Davis, 2009; Michelan et al., 2013). Native macrophytes with allelopathic potential may be used as biocontrol agents. However, it should be considered that some biocontrols might be very successful in one geographic area but fail to establish or control invasive species in other regions (Kamath, 1979; Room et al., 1981). Additionally, some invasive species can be managed using biocontrols but have the capacity to build resistance due to rapid adaptive evolution (Müller-Schärer, 2004), hence, prolonged monitoring is warranted. Further research investigating the antagonistic interactions between *C. caroliniana* and a range of native macrophytes at different life stages and under different environmental conditions should be performed. Moreover, combined studies looking into the interactions between the native and invasive macrophytes *in situ* and in the laboratory would be beneficial.

HERBIVORY EXPERIMENT

We found some degree of herbivory damage on both native and invasive macrophytes when the herbivore, *P. rugosalis* was released into the aquaria. The results clearly show that the invasive species had a lower resistance to herbivory by the moth larvae compared to the native macrophyte. *Cabomba caroliniana* contains allelochemical compounds that are active towards competing species (El-Ghazal and Riemer, 1986; Nakai et al., 1996; 1999). However, the compounds did not exhibit deterrent activity against the larvae in our study. This may be explained by several factors including the nutrient content and palatability of the plant, the tolerance of *C. caroliniana* to herbivory, the positive response of the larvae to allelochemicals produced by *C. caroliniana*, the biotic resistance of herbivores to invasive species and/or the physical characteristic of leaf and stem tissues (e.g. toughness) of the plant.

In some cases, the feeding preference and growth of herbivores can be related to nutrient composition and stoichiometry of the invasive plant species rather than their specific invasive traits such as allelopathy (Grutters et al., 2016). Overall, nitrogen content of a plant is the foremost nutrient that defines its food quality (White, 2012). Phosphorus is another important chemical to aquatic herbivorous invertebrates and may limit their growth (Perkins et al., 2004). Riemer and Toth (1970) found that *C. caroliniana* had a much higher nitrogen and phosphorus concentration in its tissues than other macrophyte species tested in the same family. In addition, the water extract of *C. caroliniana* contained more nitrogen than other macrophytes from different families (El-Ghazal and Riemer, 1986). The availability of resources such as nitrogen (or light) has a proportional relationship with the concentration of defensive compounds (Bryant et al., 1983). A study conducted by Duarte (1992) demonstrated that C:N and C:P ratios decline when nitrogen and phosphorus concentration in the tissues of freshwater plants

increases. Tissue concentrations of tellimagrandin II, the main polyphenol found in *Myriophyllum spicatum* also increased at low nitrogen and light availability (Gross, 2003). *Cabomba caroliniana* was grown in ample nutrient concentration, which could have had an effect on the C:N and C:P ratios in the plant tissues. Invasive species growing in a system with high resource availability in general, have a high nitrogen concentration in their tissues and invest less in chemical defence (e.g. production of phenolic compounds), thus, are more susceptible to enemies (Blumenthal, 2006). Many aquatic invertebrates such as Lepidopterans and snails prefer feeding on nutritious plants that contain the highest nitrogen level than those that are more chemically defended (Newman et al., 1996; Burlakova et al., 2009). For example, the larvae of *A. ephemerella* have the ability to grow on the invasive allelopathic macrophyte, *M. spicatum*, nevertheless, hydrolysable polyphenols present in *M. spicatum* can affect the larval growth. The larvae grow better on *Potamogeton perfoliatus*, which is less defended and is more nutritious (Choi et al., 2002; Walenciak et al., 2002). This was also shown by the feeding preferences of fish species. Fish avoided feeding on the chemically defended *M. spicatum*, while, consumption of *M. spicatum* increased when the C:N ratio of the plant was lowered (Dorenbosch and Bakker, 2011). This indicates that nutrient content of plants can mask the deterrent activity of allelochemical substances.

In contrast, larvae of another aquatic insect, *Parapoynx stratiotata* avoided feeding on *C. caroliniana* in its introduced range even though the nutrient composition of *C. caroliniana* was similar to all other native and invasive plant species that were investigated (Grutters et al., 2016). *Parapoynx obscuralis* also avoided feeding on and defoliating the leaves or stems of *C. caroliniana* in Central Texas (Hutchinson et al., 2015). *Cabomba caroliniana* exhibited an induced defence against herbivory by crayfish and a snail species (Morrison and Hay, 2011b). However, in our study, there was prominent herbivory damage on *C. caroliniana* while the effect was reduced on the native plant tested. This suggests that the response of invasive species to herbivory and hence the plant-herbivore interactions is unique and may be variable. Contrary to what the ERH predicts, some invasive plants can experience greater, rather than lesser, feeding damage by naturally recruited native herbivores compared to native plants, which can be explained by biotic resistance of native invertebrates (Agrawal and Kotanen, 2003; Parker and Hay, 2005; Morrison and Hay, 2011a). As a result, invasive macrophytes can be prone to a higher herbivore load and therefore a greater level of herbivory damage than natives (Liu and Stiling, 2006).

On the other hand, some plants tolerate herbivory via rapidly regrowing, reproducing and replacing the damaged tissues after herbivory (Strauss and Agrawal, 1999). A study conducted by Wang et al. (2011) demonstrated that Chinese tallow trees from introduced populations achieved a larger size compared to tallow trees from native populations. Herbivores had a higher density and a larger pupal size when feeding on introduced tallow trees than on natives. Despite higher herbivore loads, the introduced populations exhibited a greater herbivore tolerance; thereby, the overall impact of the herbivores on plant performance was lower on invasive plants compared to the native populations (Wang et al., 2011). Certain aquatic plant species can propagate quickly by producing new branches or can enhance fragmentation, thus increase reproduction, in response to herbivory (Lemoine et al., 2009). *Cabomba caroliniana* may use chemical defence against some herbivores but tolerate herbivory by others. The plant has a fast growth rate and can grow from small fragments even after it is heavily damaged (Schooler et al., 2009). This strategy may compensate for the biomass loss due to herbivory. As opposed to herbivory avoidance and tolerance, some invasive species produce chemical substances that attract aquatic herbivores. For example, the milfoil weevil, *Euhrychiopsis lecontei* is attracted to the chemicals exuded by *M. spicatum* (Marko et al., 2005). The chemical composition of *C. caroliniana* has not been studied in detail; therefore, we have no evidence to support this assumption. Nevertheless, the plant-herbivore interactions between other invasive macrophytes such as *M. spicatum* and different herbivores show that one plant can have deterrent activity on certain herbivores but attract others.

Physical attributes (e.g. toughness) of a plant can also determine its palatability. Plant toughness can be more important than chemical defence in defining feeding preferences of some herbivores (Pennings et al., 1998). Herbivory damage on *C. caroliniana* caused by the larvae in our study ranged from intense exploitation of the

leaves (food source) to defoliation of the main stems (protective cases). *Cabomba caroliniana* has finely dissected leaves and soft fragile stems, hence, the larvae preferentially used the stem of *C. caroliniana* to build its protective cases. This resulted in a higher damage of *C. caroliniana* compared to *P. ochreatus* whereas *P. ochreatus* only experienced damage to sections of its leaves. Mueller and Dearing (1994) noticed that *P. rugosalis* larvae endure intense predation when they construct their protective cases using adult leaves of the water lily, *Nymphaea ampla* compared to young leaves. This indicates that higher toughness of the leaf can limit the ability of the larvae to form their cases and consequently increases the level of predation they face. Accordingly, these larvae might prefer *C. caroliniana* over *P. ochreatus* if the two plants co-exist in a system, due to a higher risk of predation if they are exposed for longer when cutting the tough leaves of *P. ochreatus*.

The results from our study indicate that allelopathy may not increase the invasiveness of *C. caroliniana per se*, but it can confer an advantage to enhance the invasion success of the plant. Invasion success is not driven by a single factor, rather multiple interconnected strategies are used together, which can determine the degree of species invasiveness (Mitchell et al., 2006; Fleming and Dibble, 2015). *Cabomba caroliniana* may be prone to attacks by some aquatic herbivores, nevertheless, other traits such as reproduction via vegetative propagules (Ørgaard, 1991), high resilience to desiccation (Bickel, 2015), capacity to grow under a range of nutrient conditions (Bickel, 2012) and a high regeneration potential (Bickel, 2012) together with allelopathic activity against a range of competitors (El-Ghazal and Riemer, 1986; Nakai et al., 1996) enable this species to become a highly invasive weed.

Conclusion

There is insufficient research on freshwater invasions; hence, many applied ecological studies lack adequate empirical information, which can assist us in decision-making strategies to control invasive species in freshwater systems. Understanding the specific traits that are responsible for the success of invasive macrophytes would be beneficial in predicting the future spread of these species. Our study demonstrated an antagonistic interaction between *C. caroliniana* and *P. ochreatus*, which is possibly due to chemical intervention. Combined with its other competitive attributes, *C. caroliniana* can use its novel biochemistry to enhance its invasion success. In addition, allelopathic native competitors may have the potential to be used as biological control agents to control invasive macrophytes such as *C. caroliniana*. We found that broad generalist herbivores such as *P. rugosalis* can be tolerant to the biochemicals of *C. caroliniana*, however, the deterrent activity of *C. caroliniana* should be tested on a wider range of aquatic invertebrates including specialist herbivores or those that have a narrower feeding range. The allelopathy and the herbivory treatments were conducted in separate experiments in our study; consequently, we were not able to delve into the effect of factors in combination, or with more realistic conditions in which these factors interact. The herbivores in our experiment were essentially offered 'no choice' between the two macrophyte species, therefore, it is difficult to determine how the combination of allelopathy and natural herbivory would play out *in situ*. Further research needs to include simultaneous testing of a wider range of plant competitors with allelopathic potential and the effect of herbivory by moth larvae on a wider range of native macrophytes.

Acknowledgements

References

Agrawal, A. A. & Kotanen, P. M. (2003) Herbivores and the success of exotic plants: a phylogenetically controlled experiment. *Ecology Letters,* **6,** 712-715.

Baltz, D. M. & Moyle, P. B. (1993) Invasion resistance to introduced species by a native assemblage of California stream fishes. *Ecological Applications,* **3,** 246-255.

Barko, J. W. & James, W. F. (1998) Effects of submerged aquatic macrophytes on nutrient dynamics, sedimentation and resuspension. *The Structuring Role of Submerged Macrophytes in Lakes* (eds E. Jeppesen, M. Søndergaard & K. Christoffersen), pp. 197–217. Springer, New York.

Bickel, T. O. (2012) Ecology of the submersed aquatic weed *Cabomba caroliniana* in Australia. *Eighteenth Australasian Weeds Conference, Melbourne*, pp. 8-11.

Bickel, T. O. (2015) A boat hitchhiker's guide to survival: *Cabomba caroliniana* desiccation resistance and survival ability. *Hydrobiologia,* **746,** 123-134.

Blumenthal, D. M. (2006) Interactions between resource availability and enemy release in plant invasion. *Ecology Letters,* **9,** 887-895.

Brundu, G. (2015) Plant invaders in European and Mediterranean inland waters: profiles, distribution, and threats. *Hydrobiologia,* **746,** 61-79.

Bryant, J. P., Chapin III, F. S. & Klein, D. R. (1983) Carbon/nutrient balance of boreal plants in relation to vertebrate herbivory. *Oikos*, **40,** 357-368.

Buckingham, G. & Bennett, C. (1989) Laboratory host range of *Parapoynx diminutalis* (Lepidoptera: Pyralidae), an Asian aquatic moth adventive in Florida and Panama on *Hydrilla verticillata* (Hydrocharitaceae). *Environmental Entomology,* **18,** 526–530.

Burlakova, L. E., Karatayev, A. Y., Padilla, D. K., Cartwright, L. D. & Hollas, D. N. (2009) Wetland restoration and invasive species: apple snail (*Pomacea insularum*) feeding on native and invasive aquatic plants. *Restoration Ecology,* **17,** 433-440.

Burns, J. H. (2004) A comparison of invasive and non-invasive dayflowers (Commelinaceae) across experimental nutrient and water gradients. *Diversity and Distributions,* **10,** 387-397.

Callaway, R. M. & Aschehoug, E. T. (2000) Invasive plants versus their new and old neighbors: a mechanism for exotic invasion. *Science,* **290,** 521-523.

Callaway, R. M. & Ridenour, W. M. (2004) Novel weapons: invasive success and the evolution of increased competitive ability. *Frontiers in Ecology and the Environment,* **2,** 436-443.

Callaway, R. M., Waller, L. P., Diaconu, A., Pal, R., Collins, A. R., Mueller-Schaerer, H. & Maron, J. L. (2011) Escape from competition: neighbors reduce *Centaurea stoebe* performance at home but not away. *Ecology,* **92,** 2208-2213

Cappuccino, N. & Arnason, J. T. (2006) Novel chemistry of invasive exotic plants. *Biology Letters,* **2,** 189–193.

Casanova, M. & Zhang, L. (2007) Fleurieu Peninsula swamp ecology, swamp hydrology and hydrological buffers. *Unpublished report to the Steering Committee for the Independent Assessment and Recommendation of Buffers to Apply in Relation to Fleurieu Swamps. January*.

Choi, C., Bareiss, C., Walenciak, O. & Gross, E. M. (2002) Impact of polyphenols on growth of the aquatic herbivore *Acentria ephemerella*. *Journal of Chemical Ecology,* **28,** 2245-2256.

Crawley, M. J. (1997) *Plant Ecology*. Blackwell Science, Oxford.

Dandelot, S., Robles, C., Pech, N., Cazaubon, A. & Verlaque, R. (2008) Allelopathic potential of two invasive alien *Ludwigia* spp. *Aquatic Botany,* **88,** 311-316.

Davis, M. A. (2009) *Invasion Biology.* Oxford University Press, Oxford.

Dorenbosch, M. & Bakker, E. S. (2011) Herbivory in omnivorous fishes: effect of plant secondary metabolites and prey stoichiometry. *Freshwater Biology*, **56,** 1783-1797.

Duarte, C. M. (1992) Nutrient concentration of aquatic plants: patterns across species. *Limnology and Oceanography,* **37,** 882-889.

El-Ghazal, R. A. K. & Riemer, D. N. (1986) Germination suppression by extracts of aquatic plants. *Journal of Aquatic Plant Management,* **24,** 76-79.

Erhard, D. & Gross, E. M. (2006) Allelopathic activity of *Elodea canadensis* and *Elodea nuttallii* against epiphytes and phytoplankton. *Aquatic Botany,* **85,** 203-211.

Erhard, D., Pohnert, G. & Gross, E. M. (2007) Chemical defence in *Elodea nuttallii* reduces feeding and growth of aquatic herbivorous Lepidoptera. *Journal of chemical ecology,* **33,** 1646-1661.

Fan, S., Liu, C., Yu, D. & Xie, D. (2013) Differences in leaf nitrogen content, photosynthesis, and resource-use efficiency between *Eichhornia crassipes* and a native plant *Monochoria vaginalis* in response to altered sediment nutrient levels. *Hydrobiologia,* **711,** 129-137.

Fleming, J. P. & Dibble, E. D. (2015) Ecological mechanisms of invasion success in aquatic macrophytes. *Hydrobiologia,* **746,** 23-37.

Funk, J. L. & Vitousek, P. M. (2007) Resource-use efficiency and plant invasion in low-resource systems. *Nature,* **446,** 1079-1081.

Gallardo, B., Clavero, M., Sánchez, M. I. & Vilà, M. (2016) Global ecological impacts of invasive species in aquatic ecosystems. *Global Change Biology,* **22,** 151-163.

Gérard, J., Brion, N. & Triest, L. (2014) Effect of water column phosphorus reduction on competitive outcome and traits of *Ludwigia grandiflora* and *L. peploides*, invasive species in Europe. *Aquatic Invasions,* **9,** 157-166.

Gopal, B. & Goel, U. (1993) Competition and allelopathy in aquatic plant-communities. *Botanical Review,* **59,** 155-210.

Gross, E. (2000) Seasonal and spatial dynamics of allelochemicals in the submersed macrophyte *Myriophyllum spicatum* L. *Verhandlungen der Internationalen Vereinigung für Limnologie,* **27,** 2116-2119.

Gross, E. M. (2003) Differential response of tellimagrandin II and total bioactive hydrolysable tannins in an aquatic angiosperm to changes in light and nitrogen. *Oikos,* **103,** 497-504.

Grotkopp, E. & Rejmánek, M. (2007) High seedling relative growth rate and specific leaf area are traits of invasive species: phylogenetically independent contrasts of woody angiosperms. *American Journal of Botany,* **94,** 526-532.

Grutters, B. M. C., Gross, E. M. & Bakker, E. S. (2016) Insect herbivory on native and exotic aquatic plants: phosphorus and nitrogen drive insect growth and nutrient release. *Hydrobiologia,* **778,** 209-220.

Habeck, D. H. (1974) Caterpillars of *Parapoynx* in relation to aquatic plants in Florida. *Florida Agricultural Experiment Station,* **12,** 15-18.

He, F., Deng, P., Wu, X. H., Cheng, S. P., Gao, Y. N. & Wu, Z. B. (2008) Allelopathic effects on *Scenedesmus obliquus* by two submerged macrophytes *Najas minor* and *Potamogeton malaianus*. *Fresenius Environmental Bulletin,* **17,** 92-97.

Hutchinson, J. T., Huston, D. C. & Gibson, J. R. (2015) Defoliation of cultured creeping primrose willow (*Ludwigia repens*) and other aquatic plants by *Parapoynx obscuralis* (Lepidoptera: Crambidae). *Southwestern Entomologist,* **40,** 227-232.

Inderjit, Wardle, D. A., Karban, R. & Callaway, R. M. (2011) The ecosystem and evolutionary contexts of allelopathy. *Trends in Ecology & Evolution*, **26,** 655-662.

ISSG, (2005) Global Invasive Species Database (GISD). Auckland, New Zealand: University of Auckland. http://www.issg.org/database

Jeschke, J., Aparicio, L. G., Haider, S., Heger, T., Lortie, C., Pyšek, P. & Strayer, D. (2012) Support for major hypotheses in invasion biology is uneven and declining. *NeoBiota,* **14,** 1-20.

Kamath, M. (1979) A review of biological control of insect pests and noxious weeds in Fiji (1969-1978). *Fiji Agricultural Journal,* **41,** 55-72.

Kennedy, T. A., Naeem, S., Howe, K. M., Knops, J. M., Tilman, D. & Reich, P. (2002) Biodiversity as a barrier to ecological invasion. *Nature,* **417,** 636-638.

Lemoine, D. G., Barrat-Segretain, M.-H. & Roy, A. (2009) Morphological and chemical changes induced by herbivory in three common aquatic macrophytes. *International Review of Hydrobiology,* **94,** 282-289.

Levine, J. M. (2000) Species diversity and biological invasions: relating local process to community pattern. *Science,* **288,** 852-854.

Levine, J. M., Adler, P. B. & Yelenik, S. G. (2004) A meta-analysis of biotic resistance to exotic plant invasions. *Ecology Letters,* **7,** 975-989.

Liu, H. & Stiling, P. (2006) Testing the enemy release hypothesis: a review and meta-analysis. *Biological Invasions,* **8,** 1535-1545.

Lockwood, J. L., Hoopes, M. F. & Marchetti, M. P. (2013) *Invasion Ecology.* John Wiley & Sons, Oxford.

Lodge, D. M., Williams, S., MacIsaac, H. J., Hayes, K. R., Leung, B., Reichard, S., Mack, R. N., Moyle, P. B., Smith, M. & Andow, D. A. (2006) Biological invasions: recommendations for US policy and management. *Ecological Applications,* **16,** 2035-2054.

Mackey, A. & Swarbrick, J. (1997) The biology of Australian weeds. 32. *Cabomba caroliniana* Gray. *Plant Protection Quarterly,* **12,** 154-165.

Marko, M. D., Gross, E., Newman, R. & Gleason, F. K. (2008) Chemical profile of the North American native *Myriophyllum sibiricum* compared to the invasive *M. spicatum*. *Aquatic Botany,* **88,** 57-65.

Marko, M. D., Newman, R. M. & Gleason, F. K. (2005) Chemically mediated host-plant selection by the milfoil weevil: a freshwater insect–plant interaction. *Journal of Chemical Ecology,* **31,** 2857-2876.

Mayer-Pinto, M., Johnston, E., Hutchings, P., Marzinelli, E., Ahyong, S., Birch, G., Booth, D., Creese, R., Doblin, M. & Figueira, W. (2015) Sydney Harbour: a review of anthropogenic impacts on the biodiversity and ecosystem function of one of the world. *Marine and Freshwater Research,* **66,** 1088-1105.

Meyerson, L. A. & Mooney, H. A. (2007) Invasive alien species in an era of globalization. *Frontiers in Ecology and the Environment,* **5,** 199-208.

Michelan, T. S., Thomaz, S. M. & Bini, L. M. (2013) Native macrophyte density and richness affect the invasiveness of a tropical Poaceae species. *PloS One,* **8,** e60004.

Mitchell, C. E., Agrawal, A. A., Bever, J. D., Gilbert, G. S., Hufbauer, R. A., Klironomos, J. N., Maron, J. L., Morris, W. F., Parker, I. M. & Power, A. G. (2006) Biotic interactions and plant invasions. *Ecology Letters,* **9,** 726-740.

Molisch, H. (1937) *Der Einfluss einer Pflanze auf die Andere-Allelopathie.* Fisher, Jena.

Morrison, W. E. & Hay, M. E. (2011a) Herbivore preference for native vs. exotic plants: generalist herbivores from multiple continents prefer exotic plants that are evolutionarily naïve. *PLoS One,* **6,** e17227.

Morrison, W. E. & Hay, M. E. (2011b) Induced chemical defences in a freshwater macrophyte suppress herbivore fitness and the growth of associated microbes. *Oecologia,* **165,** 427-436.

Mueller, U. G. & Dearing, M. D. (1994) Predation and avoidance of tough leaves by aquatic larvae of the moth *Parapoynx rugosalis* (Lepidoptera: Pyralidae). *Ecological Entomology,* **19,** 155-158.

Mulderij, G., Mooij, W. M. & van Donk, E. (2005) Allelopathic growth inhibition and colony formation of the green alga *Scenedesmus obliquus* by the aquatic macrophyte *Stratiotes aloides*. *Aquatic Ecology,* **39,** 11-21.

Mulderij, G., van Donk, E. & Roelofs, J. G. M. (2003) Differential sensitivity of green algae to allelopathic substances from *Chara*. *Hydrobiologia,* **491,** 261-271.

Müller-Schärer, H., Schaffner, U. & Steinger, T. (2004) Evolution in invasive plants: implications for biological control. *Trends in Ecology & Evolution,* **19,** 417-422.

Nakai, S., Hosomi, M., Okada, M. & Murakami, A. (1996) Control of algal growth by macrophytes and macrophyte-extracted bioactive compounds. *Water Science and Technology,* **34,** 227-235.

Nakai, S., Inoue, Y., Hosomi, M. & Murakami, A. (1999) Growth inhibition of blue-green algae by allelopathic effects of macrophyte. *Water Science and Technology,* **39,** 47-53.

Newman, R. M. (1991) Herbivory and detritivory on freshwater macrophytes by invertebrates: a review. *Journal of the North American Benthological Society,* **10,** 89-114.

Newman, R. M., Kerfoot, W. C. & Hanscom, Z. (1996) Watercress allelochemical defends high-nitrogen foliage against consumption: effects on freshwater invertebrate herbivores. *Ecology,* **77,** 2312-2323.

Ørgaard, M. (1991) The genus *Cabomba* (Cabombaceae)–a taxonomic study. *Nordic Journal of Botany,* **11,** 179-203.

Pakdel, F. M., Sim, L., Beardall, J. & Davis, J. (2013) Allelopathic inhibition of microalgae by the freshwater stonewort, *Chara australis*, and a submerged angiosperm, *Potamogeton crispus*. *Aquatic Botany,* **110,** 24-30.

Parker, J. D. & Hay, M. E. (2005) Biotic resistance to plant invasions? Native herbivores prefer non-native plants. *Ecology Letters,* **8,** 959-967.

Parsons, W. T. & Cuthbertson, E. G. (2001) *Noxious Weeds of Australia*. 2nd Edition. CSIRO Publishing, Collingwood.

Pennings, S. C., Carefoot, T. H., Siska, E. L., Chase, M. E. & Page, T. A. (1998) feeding preferences of a generalist salt-marsh crab: relative importance of multiple plant traits. *Ecology,* **79,** 1968-1979.

Perkins, M. C., Woods, H. A., Harrison, J. F. & Elser, J. J. (2004) Dietary phosphorus affects the growth of larval *Manduca sexta*. *Archives of Insect Biochemistry and Physiology,* **55,** 153-168.

Pimentel, D. (2011) Environmental and economic costs associated with alien invasive species in the United States. *Biological Invasions: Economic and Environmental Costs of Alien Plant, Animal, and Microbe Species,* (2nd edn). CRC Press, Boca Raton, FL**,** 411-430.

Pimm, S. L. & Hyman, J. B. (1987) Ecological stability in the context of multispecies fisheries. *Canadian Journal of Fisheries and Aquatic Sciences,* **44,** s84-s94.

Planas, D., Sarhan, F., Dube, L., Godmaire, H. & Cadieux, C. (1981) Ecological significance of phenolic compounds of *Myriophyllum spicatum*. *Verhandlungen des Internationalen Verein Limnologie,* **21,** 1492-1496.

Quinn, L. D., Schooler, S. S. & Van Klinken, R. D. (2011) Effects of land use and environment on alien and native macrophytes: lessons from a large-scale survey of Australian rivers. *Diversity and Distributions,* **17,** 132-143.

Riemer, D. & Toth, S. (1970) Chemical composition of five species of Nymphaeaceae. *Weed Science***,** 4-6.

Room, P. M., Harley, K. L. S., Forno, I. W. & Sands, D. P. A. (1981) Successful biological control of the floating weed *Salvinia*. *Nature,* **294,** 78-80.

Rothhaupt, K. O., Fornoff, F. & Yohannes, E. (2015) Induced responses to grazing by an insect herbivore (*Acentria ephemerella*) in an immature macrophyte (*Myriophyllum spicatum*): an isotopic study. *Ecology and Evolution,* **5,** 3657-3665.

Sainty, G. & Jacobs, S. (2003) *Waterplants in Australia: A Field Guide*, 4 edn. Sainty & Associates, Australia.

Sala, O. E., Chapin, F. S., Armesto, J. J., Berlow, E., Bloomfield, J., Dirzo, R., Huber-Sanwald, E., Huenneke, L. F., Jackson, R. B. & Kinzig, A. (2000) Global biodiversity scenarios for the year 2100. *Science,* **287,** 1770-1774.

Schallenberg, M. & Waite, E. (2004) Survey of aquatic macrophytes in Lake Waihola, summer 2002-2003. *Limnology Report,* New Zealand.

Schooler, S., Cabrera-Walsh, W. & Julien, M. (2009) *Cabomba caroliniana* gray (Cabombaceae). *Biological control of tropical weeds using arthropods.* Cambridge University Press, Cambridge**,** 88-107.

Schooler, S., Julien, M. & Walsh, G. C. (2006) Predicting the response of *Cabomba caroliniana* populations to biological control agent damage. *Australian Journal of Entomology,* **45,** 327-330.

Silveira, M. J., Thomaz, S. M., Mormul, R. P. & Camacho, F. P. (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.

Steenhagen, D. A. & Zimdahl, R. L. (1979) Allelopathy of leafy spurge (*Euphorbia esula*). *Weed Science,* **27,** 1-3.

Stiers, I., Njambuya, J. & Triest, L. (2011) 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.

Strauss, S. Y. & Agrawal, A. A. (1999) The ecology and evolution of plant tolerance to herbivory. *Trends in Ecology & Evolution,* **14,** 179-185.

Svensson, J. R., Nylund, G. M., Cervin, G., Toth, G. B. & Pavia, H. (2013) Novel chemical weapon of an exotic macroalga inhibits recruitment of native competitors in the invaded range. *Journal of Ecology,* **101,** 140-148.

Takeda, F., Nakano, K., Nishimura, O., Shimada, Y., Fukuro, S., Tanaka, H., Hayashi, N. & Inamori, Y. (2011) Allelopathic potential of *Potamogeton pusillus* community against *Microcystis aeruginosa*. *Journal of Water and Environment Technology,* **9,** 21-28.

Torchin, M. E., Lafferty, K. D., Dobson, A. P., McKenzie, V. J. & Kuris, A. M. (2003) Introduced species and their missing parasites. *Nature,* **421,** 628-630.

van Kleunen, M., Weber, E. & Fischer, M. (2010) A meta-analysis of trait differences between invasive and non-invasive plant species. *Ecology Letters,* **13,** 235-245.

Vanderstukken, M., Mazzeo, N. & Van Colen, W. (2011) Biological control of phytoplankton by the subtropical submerged macrophytes *Egeria densa* and *Potamogeton illinoensis*: a mesocosm study. *Freshwater Biology,* **56,** 1837-1849.

Verhoeven, K. J., Biere, A., Harvey, J. A. & Van Der Putten, W. H. (2009) Plant invaders and their novel natural enemies: who is naive? *Ecology Letters,* **12,** 107-117.

Walenciak, O., Zwisler, W. & Gross, E. M. (2002) Influence of *Myriophyllum spicatum*-derived tannins on gut microbiota of its herbivore *Acentria ephemerella*. *Journal of Chemical Ecology,* **28,** 2045-2056.

Wang, Y., Huang, W., Siemann, E., Zou, J., Wheeler, G. S., Carrillo, J. & Ding, J. (2011) Lower resistance and higher tolerance of invasive host plants: biocontrol agents reach high densities but exert weak control. *Ecological Applications,* **21,** 729-738.

Wersal, R. M. & Madsen, J. D. (2011) Influences of water column nutrient loading on growth characteristics of the invasive aquatic macrophyte *Myriophyllum aquaticum* (Vell.) Verdc. *Hydrobiologia,* **665,** 93-105.

Wetzel, R. G. (1988) Water as an environment for plant life. *Vegetation of Inland Waters: Handbook of Vegetation Science* (ed J. J. Symoens), pp. 113-154. Kluwer Academic Publishers, Dordrecht.

White, T. C. (2012) *The Inadequate Environment: Nitrogen and the Abundance of Animals.* Springer Science & Business Media, Berlin, New York.

Williamson, M. (1996) *Biological Invasions.* Chapman & Hall, London.

Willis, R. J. (1985) The historical bases of the concept of allelopathy. *Journal of the History of Biology,* **18,** 71-102.

Willis, R. J. (2008) *The History of Allelopathy.* Springer, Dordrecht.

Wolf, V. C., Berger, U., Gassmann, A. & Müller, C. (2011) High chemical diversity of a plant species is accompanied by increased chemical defence in invasive populations. *Biological Invasions,* **13,** 2091-2102.

Xiong, W., Yu, D., Wang, Q., Liu, C. & Wang, L. (2008) A snail prefers native over exotic freshwater plants: implications for the enemy release hypotheses. *Freshwater Biology,* **53,** 2256-2263.

Zhang, S. H., Cheng, S. P., Wang, H. Q., He, F. & Wu, Z. (2009) Allelopathic interactions between the *Potamogeton* spp. and toxic cyanobacteria (*Microcystis aeruginosa*). *Allelopathy Journal,* **23,** 379-390.

General Discussion
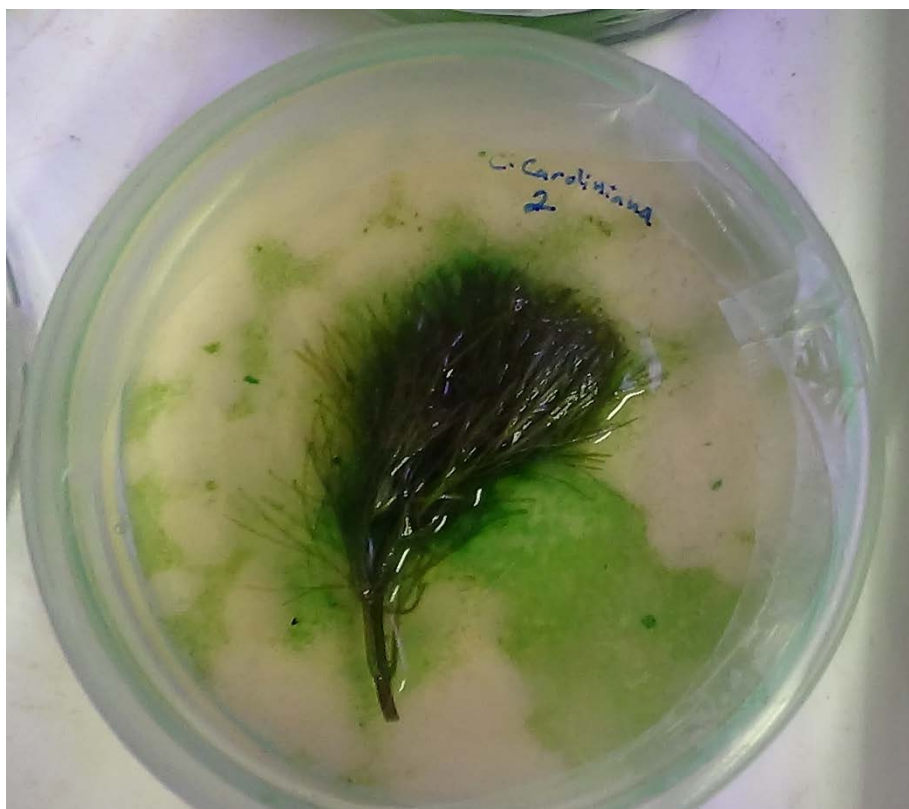
Area of *Anabaena variabilis* growth inhibition caused by live material of *Cabomba caroliniana*.
Photo credit: Fariba Moslih Pakdel

Overview

This study sought to address some of the knowledge gaps in freshwater invasion by investigating the potential allelopathic effects of invasive submerged macrophytes on different organisms inhabiting freshwater systems. I used three invasive freshwater plants, *Egeria densa*, *Elodea canadensis* and *Cabomba caroliniana* to test the potential allelopathic effects of these species on competitors such as microalgae (cyanobacteria and green algae), and compared their effects to a range of native macrophytes (refer to Table 1, Chapter 2 for the full list of donor and target species). A further objective of this study was to test two of the major hypotheses in invasion biology, the NWH and the ERH in order to explain and predict the invasion potential of exotic invaders and to seek evidence supporting these hypotheses in freshwater systems (Chapters 2—4). I tested the bioactivity of *C. caroliniana* on different competitors (microalgae and a macrophyte), a herbivorous moth larva and on community dynamics of aquatic invertebrates (macroinvertebrates and zooplankton), and compared its bioactivity to a native macrophyte, *Potamogeton ochreatus*. Information acquired via this study can deliver an empirical basis for understanding the different traits that are responsible for the success of invasive macrophyte species in freshwater systems. This information can be used in effective management strategies to mitigate the impacts and the spread of invasive species. A summary of the main objectives, primary aims and major findings of the study is illustrated in Fig. 1.

Objective 1: Assessing the allelopathic potential of native and invasive macrophytes on microalgae (Chapter 2)

My study found strong species-specific deleterious effects of the three invasive macrophytes towards the microalgal species tested. Species-specific inhibitory effects were also seen in some native macrophytes against the microalgae. Many macrophytes were shown to exhibit species-specific allelopathic activity on planktonic and epiphytic cyanobacteria, green algae and diatoms (Körner and Nicklisch, 2002; Hilt et al., 2006; Hilt and Gross, 2008; Bauer et al., 2009; Mohamed and Al Shehri, 2010; Pakdel et al., 2013). In my study, the effect of *C. caroliniana* was stronger than *E. densa* and *E. canadensis.* Previous research demonstrated that *E. canadensis* and *E. densa* have the ability to constrain the growth of different phytoplankton species via allelopathy under non-laboratory conditions (Erhard and Gross, 2006; Vanderstukken et al., 2011). However, allelopathic effects of *Elodea* species were found to be relatively moderate compared with other macrophytes (Hilt and Gross, 2008). *Cabomba caroliniana* has exhibited a higher level of invasiveness than *E. canadensis* and *E. densa* around the globe (Mackey and Swarbrick, 1997; Parsons and Cuthbertson, 2001; Sainty and Jacobs, 2003; ISSG, 2005; Brundu, 2015). The strong potential allelopathic activity of *C. caroliniana* against competitors such as microalgae can explain its greater invasion success compared with the other two invasive species.

I also found stronger inhibitory effects of *C. caroliniana* against microalgae compared to most native macrophytes, but it had similar bioactivity to *C. demersum* and to a lesser degree, *P. crispus*. In addition, a strong inhibitory effect of *C. caroliniana* and *P. ochreatus* was found on the growth of *M. aeruginosa* using dialysis cassettes in the mesocosm experiment. However, the density of naturally-occurring microalgae was significantly lower in the mesocosms where *C. caroliniana* was present than in mesocosms with *P. ochreatus* and the controls (no plants present) in the nutrient addition experiment and after nutrient addition to the mesocosms ceased. The allelopathic potential of some invasive macrophytes can be similar to that of natives (Jarchow and Cook, 2009; Huang et al., 2010; Lind and Parker, 2010), yet some invasive macrophytes can produce a higher concentration and greater diversity of chemical compounds that can enhance their invasiveness (Marko et al., 2008; Wolf et al., 2011). *Cabomba caroliniana* may be able to produce stronger and/or more diverse allelochemical compounds, which can supress a wider range of microalgal species compared to native macrophytes such as *P. ochreatus*. In summary, the success of invasive species can vary depending on the composition of native microalgal communities already present within a system. *Cabomba caroliniana* may use its allelopathic potential to overcome competition with microalgae. This mechanism may have an important role in facilitating the success of *C. caroliniana* and other invasive macrophytes in their introduced range, but the use of this mechanism is not

restricted to invasive species. Further study of the potential ecosystem dynamics of macrophytes, in native, exotic or mixed stands is warranted.

Objective 2: Assessing the invertebrate communities in native and invasive macrophyte beds in a mono-culture and co-culture settings (Chapter 3)

I found four major functional feeding groups (FFG) in the native and invasive macrophyte beds and in the controls. Community dynamics of the invertebrates (taxa richness, abundance, richness and abundance of FFG and community compositions) were similar in native and invasive macrophyte stands in both mono-culture (either native or invasive plant) and co-culture (native and invasive plants grown together) settings. Furthermore, invertebrates did not discriminate between the native and invasive macrophytes when they were given a choice of selecting either plant as a refuge. These results suggest that mono-specific stands formed by invasive macrophytes can support equivalent invertebrate community dynamics to mono-specific stands of native species. *Cabomba caroliniana* has a complex structure, which may have 'positive' impacts on associated invertebrates in some systems by providing shelter and by enhancing substrate for epiphyte attachment. Thereby, the positive physical aspects of *C. caroliniana* can offset its adverse effects on some invertebrates. Mono-specific stands of invasive species that offer a greater level of habitat complexity compared to native macrophytes have been shown to support a higher density of macroinvertebrates (Strayer et al., 2003; Kelly and Hawes, 2005; Hogsden et al., 2007). This indicates that invertebrates may not differentiate between native and invasive plants with a similar physical architecture when selecting habitat, although this may not mitigate the negative impacts of invasive macrophytes such as *C. caroliniana* enforced on other biota and on overall ecosystem function. Furthermore, the community dynamics of invertebrates present in native and invasive macrophytes were similar to those found in open water (controls). This suggests that despite previous findings, some invertebrate communities may not differentiate between macrophyte habitats and open water in certain systems.

Objective 3: Assessing the bioactivity of an invasive macrophyte on two native 'enemies' (Chapter 4)

I tested the bioactivity of *C. caroliniana* on a native plant (allelopathy experiment) and on a herbivorous invertebrate (herbivory experiment). A significantly lower % cover of *P. ochreatus* and *C. caroliniana* was found when the two plants were grown together in the same ponds compared to the controls (single species planting) in the allelopathy experiment. In addition, the larvae of *Parapoynx rugosalis* inflicted a high level of feeding damage on *C. caroliniana* in the herbivory experiment. The larvae also caused great herbivory damage on the native plant, *P. ochreatus*, although the amount of damage was significantly lower on the native versus invasive plant. These outcomes indicate that *C. caroliniana* may use its novel chemical weapons against competitors, but use a herbivory tolerance strategy when exposed to herbivores. Furthermore, *C. caroliniana* may have the capacity to establish and spread in systems where other potential native 'enemies' such as competitors and herbivores are absent. Nevertheless, the presence of native enemies can regulate its growth. Past research also revealed that although native enemies may not be able to prevent invasion, they could limit the invasion success of some macrophyte species (Levine et al., 2004). *Cabomba caroliniana* has a fast biomass turnover and can grow from small fragments (Schooler et al., 2009). This strategy can counteract the biomass loss of *C. caroliniana* and other invasive macrophytes due to herbivory (Lemoine et al., 2009).

Central Research Question: What are the roles of allelopathic native and invasive macrophytes in freshwater systems?

Study Aim: To compare the allelopathic effects of native and invasive macrophytes on cyanobacteria, green algae and invertebrate communities in freshwater systems, and to test the NWH and ERH by investigating the bioactivity of an invasive macrophyte

Main chapter objectives

Objective 1. Assessing the allelopathic potential of native and invasive macrophytes on microalgae

Objective 2. Assessing the invertebrate communities in native and invasive macrophyte beds in a mono-culture and co-culture settings

Objective 3. Assessing the bioactivity of an invasive macrophyte on two native 'enemies'

Main chapter aims

Chapter 2.

a) Investigate allelopathic effects of several native and invasive macrophytes on the growth of microalgae

b) Examining the allelopathic potential of *P. ochreatus* (native) and *C. caroliniana* (invasive) on *Microcystis aeruginosa*

c) Measure microalgal density in the presence of *P. ochreatus* and *C. caroliniana*

Chapter 3.

a) Investigate deterrent activity of a native and an invasive macrophyte on invertebrate communities

b) Investigate invertebrate communities in native vs. invasive macrophyte stands in a co-culture setting

Chapter 4.

Investigate bioactivity of *C. caroliniana* on two potential native enemies using a native macrophyte and a native herbivorous moth larva

Main chapter outcomes

a) The native and invasive macrophytes exhibited species-specific allelopathic effects on microalgae.

b & c) *Cabomba caroliniana* could negatively affect the growth of *M. aeruginosa* and other microalgal species possibly via using its novel chemical weapons.

a) The native and invasive macrophytes did not show any deterrent activity towards the invertebrates.

b) The invertebrates did not discriminate between native and invasive macrophyte beds (when they were given a choice to select refuge) perhaps due to the similar habitat complexity provided by both macrophytes.

Cabomba caroliniana exhibited a strong allelopathic potential against the native competitor, although no deterrent activity of *C. caroliniana* was detected towards the moth larvae.
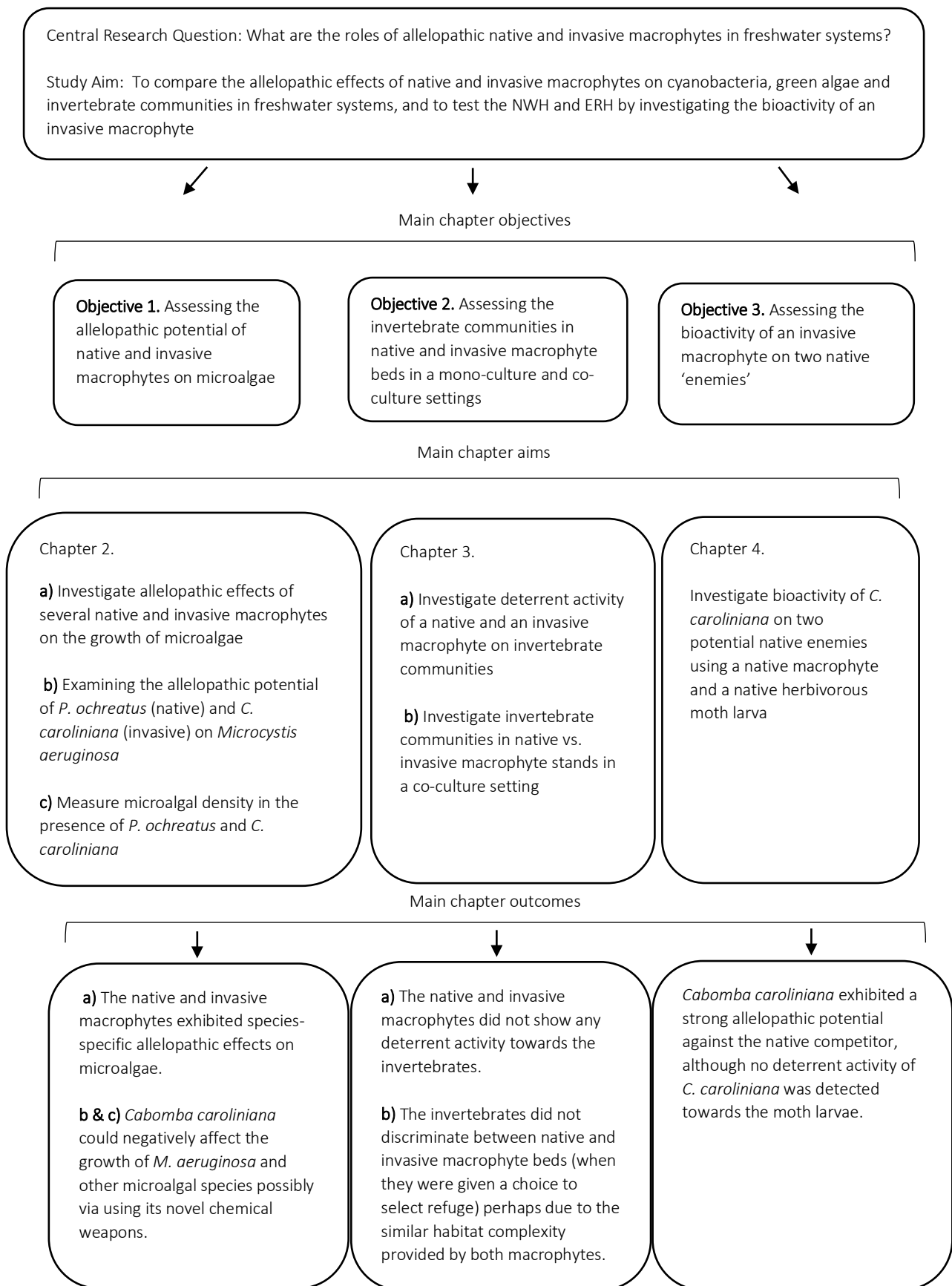
Fig. 1. A conceptual framework of the thesis summarising the scope, aims and final outcomes of each chapter

Discussion and management implications

Many studies have been conducted to recognise and evaluate the impacts of invasive freshwater macrophytes on different biota (Lassuy, 1995; Dextrase and Mandrak, 2006; Bryant and Papas, 2007; Hogsden et al., 2007; Schultz and Dibble, 2012). However, the steering strategies used by invasive macrophytes that cause these impacts are poorly understood. Understanding the mechanisms behind the success of invasive species would help in the making of management decisions that are more reliable. Invasive aquatic macrophytes use several important strategies including prolific growth and an ability to grow from fragments (Hussner, 2009; Schooler et al., 2009), high plasticity under stressful conditions (Stiers et al., 2011 Jahnke et al., 1991; Dendène et al., 1993; Ozimek et al., 1993), increased competitive ability (Fan et al., 2013; Fleming and Dibble, 2015), induced chemical defence against herbivory (Rothhaupt et al., 2015) and production of allelochemicals (Erhard and Gross, 2006) that can be novel to the native macrophytes (in marine systems, Svensson et al., 2013).

My study suggests that *E. densa*, *E. canadensis* and *C. caroliniana* can use their novel biochemistry to inhibit the growth of specific microalgae (experiment 1, Chapter 2). However, the effects of *E. densa* and *E. canadensis* were strong on some of the target species, but weak or absent on others. This suggests that the allelopathic potential of *E. densa* and *E. canadensis* may be relatively moderate compared to other macrophyte species (Hilt and Gross, 2008). Furthermore, the longer introduction history of *E. densa* and *E. canadensis* in Australia may have provided some of the native microalgal species with an opportunity to become resistant towards the novel chemical weapons of these species over time while others remained susceptible (Müller-Schärer et al., 2004). The species-specific allelopathic effects of invasive macrophytes can lead to changes in community composition of microalgae by reducing the abundance of certain species. For example, Vanderstukken et al. (2011) demonstrated that reduction in the relative abundance of *Scenedesmus* species caused by *E. densa* could alter the overall community structure of phytoplankton. Cyanobacteria are more sensitive to allelochemicals produced by macrophytes (Körner and Nicklisch, 2002; Mulderij et al., 2003; Hilt and Gross, 2008; Mohamed and Al Shehri, 2010). As a result, invasive macrophytes are expected to impact cyanobacteria more significantly compared with green algae and diatoms.

Unlike *E. densa* and *E. canadensis*, *C. caroliniana* displayed strong inhibitory effects against all microalgae in the laboratory and in outdoor conditions (experiments 1-3, Chapter 2). The plant could also suppress the growth of the native macrophyte (allelopathy experiment, Chapter 4). Furthermore, the hygrophilous snails avoided feeding on *C. caroliniana* and preferred the native plant (experiment 2, Chapter 3). Nevertheless, *C. caroliniana* did not show any deterrent activity towards the herbivorous moth larvae and other invertebrates (herbivory experiment, Chapter 4 and experiment 1-2, Chapter 3 respectively). These outcomes suggest that *C. caroliniana* may also selectively utilise its novel biochemicals, particularly against competing species, but can benefit using alternative strategies towards others. Hence, it appears that invasion success of freshwater macrophytes is not driven by a single mechanism. In fact, a combinbation of different strategies are employed by invasive macrophytes, which can strengthen their invasiveness in an introduced range and can intensifiy their chance to overcome myriad counterpart organisms (Mitchell et al., 2006; Fleming and Dibble, 2015).

Indeed, explicit reference to the mechanisms used by invasive species alone cannot define the invasion potential of macrophytes. Fundamentally, other associated factors such as environmental and ecological characteristics, including biotic and abiotic filters, within a system also play a pivotal role in the success of these species (Jacobs and MacIsaac, 2009; Wersal and Madsen, 2011; Fleming and Dibble, 2015; Tabassum and Leishman, 2016). Invasion is more likely to occur in disturbed systems (Pimm and Hyman, 1987; Baltz and Moyle, 1993; Quinn et al., 2011) and many invasive plants thrive in nutrient-rich environments (Gallardo et al., 2016). Thereby, the reliance of most invasive macrophyte species on high levels of nutrients can be a key predictor of their future spread as well as the degree of their invasiveness in a particular system. Nutrient availability can also alter the

chemical properties and hence the chemical defence of invasive plants (Bryant et al., 1983). For example, an increased nitrogen concentration in the system can lead to a decline in C:N ratio in plant tissues (Duarte, 1992). Alternatively, tissue concentrations of certain allelochemical substances can increase at low nitrogen and light availability (Gross, 2003a). Many aquatic invertebrates such as Lepidopterans and snails prefer feeding on nutritious plants that contain the highest nitrogen level than those that are more chemically defended (Newman et al., 1996; Choi et al., 2002; Walenciak et al., 2002; Burlakova et al., 2009). Therefore, high nutrient availability in a system can sometimes have an adverse impact on invasive species because the plant may invest less in chemical defence (e.g. production of phenolic compounds) and become more palatable (e.g. more nitrogen content in the tissues), thus it can be more susceptible to enemies (Newman et al., 1996; Blumenthal, 2006; Burlakova et al., 2009).

Exploitative competition with native competitors and the activity of enemies are two main biotic filters that can influence the invasion success of macrophytes. A higher diversity and density of native competitors can reduce the likelihood that a system will be invaded (Xu et al., 2004; Klironomos et al., 2006; Fleming and Dibble, 2015; Thomaz et al., 2015). Competition for resources will be intense if a greater number of species occupy a site, which means less resources will be available for the new arrivals (Elton, 2000). Furthermore, a community of native species with high richness and greater functional diversity has a greater chance of containing a few species that possess biotic resistance towards invasive species (Levine, 2000; Fargione and Tilman, 2005). The susceptibility of a system to invasion can also decline when native enemies (herbivores and pathogens) are abundant (Levine et al., 2004; Xiong et al., 2008; Davis, 2009; Fleming and Dibble, 2015; Thomaz et al., 2015). However, a lower diversity of native enemies is not always regarded as leading to less damage because even one or two enemies can have a marked impact on invasive species (Maron and Vilà, 2001; Parker and Hay, 2005; Schooler et al., 2006; Morrison and Hay, 2011). Despite the importance of resource availability in determining the success of invasive species, high biodiversity of native competitors and herbivores in freshwater systems can reduce its importance (Levine, 2000; Kennedy et al., 2002; Davis, 2009; Michelan et al., 2013).

In a previous study, *E. canadensis* showed a non-uniform distribution in invaded areas and it did not display its invasiveness in heterogeneous systems where a rich macrophyte community containing *Potamogeton* was present (Kuhar et al., 2010). This indicates that healthy systems with a greater diversity and/or density of native macrophytes have a higher capacity to resist infestation by *C. caroliniana* and other exotic macrophytes. Nonetheless, *C. caroliniana* might have a greater potential to invade systems that are facing disturbance because the plant can grow from heavily damaged fragments in eutrophic as well as oligotrophic systems (Schooler et al., 2009). In contrast, since the successful establishment and spread of invasive species would diminish if they occupy a similar niche to the resident species (Abram, 1983; Fargione et al., 2003; Von Holle and Simberloff, 2004), native allelopathic macrophytes with a similar niche may possibly be capable of regulating the growth of invasive macrophytes in infested wetlands and limit their further distribution. In my study, *P. crispus* displayed allelopathic activity similar to that of *C. caroliniana* towards microalgae, and it could limit the growth of *C. caroliniana*.

The moth larvae could also cause a great deal of feeding damage on *C. caroliniana*, although snails avoided feeding on this macrophyte. Aquatic Lepidoptera have been extensively studied as potential candidates to control non-indigenous invasive and nuisance aquatic macrophytes (Habeck, 1974; Schooler et al., 2006). Schooler et al. (2006) predicted that moth larvae from the Crambidae family may be able to control *C. caroliniana* in Australia. However, many aquatic crambids are highly polyphagous (Herlong, 1979; Stoops et al., 1998). In the classical biocontrol of weeds, the host specificity of the herbivore should be ensured prior to release in order to prevent negative impacts on endangered or ecologically important plants.

Overall, allelopathy alone is not responsible for the success of invasive species such as the one used in my study, but it can provide an advantage to increase the invasiveness of macrophytes. Invasive macrophyte species employ a set of several mechanisms to enhance their success in an introduced area (Mitchell et al., 2006; Fleming and

Dibble, 2015). *Cabomba caroliniana* may be prone to attacks by some aquatic herbivores. Nevertheless, other traits such as reproduction via vegetative propagules (Ørgaard, 1991), high resilience to desiccation (Bickel, 2015), capacity to grow under a range of nutrient conditions (Bickel, 2012) and a high regeneration potential (Bickel, 2012), together with allelopathic activity against a range of competitors (El-Ghazal and Riemer, 1986; Nakai et al., 1996), enable this species to become a highly invasive weed. For example, *C. caroliniana* may use chemical defence against competitors and certain enemies but tolerate herbivory by others. The fast growth rate of the plant from small damaged fragments may compensate for the biomass loss due to herbivory (Schooler et al., 2009). *Egeria densa* and *E. canadensis* also possess a high competitive ability for nutrient uptake, and have a fast growth rate and an enhanced photosynthetic ability under a wide range of environmental conditions (Jahnke et al., 1991; Dendène et al., 1993; Ozimek et al., 1993; Mony et al., 2007; Yarrow et al., 2009). These traits combined with their allelopathic potential deliver an advantage for *E. densa* and *E. canadensis* over native species.

In my study, the three invasive macrophytes demonstrated impacts that can be considered ecologically desirable by reducing the growth of nuisance microalgae. Furthermore, *C. caroliniana* could benefit invertebrates directly as a food source and offering refuge, and indirectly by providing substrate for epiphyte attachment. Several invasive macrophytes exhibit positive impacts on other freshwater biota (Brendonck et al., 2003; Villamagna and Murphy, 2010; Villamagna et al., 2010; see Thomaz et al., 2015 and references therein), although these cannot override the adverse impacts that invasive macrophytes pose on other biota and on overall ecosystem function. For example, the formation of monospecific stands, which is a common phenomenon after invasion by non-indigenous macrophytes, reduces overall plant diversity and consequently alters aquatic invertebrate community composition over time. Furthermore, the high biomass production of invasive macrophytes can restrict open water habitat for waterbirds. In spite of these effects, the positive impacts of habitat complexity provided for invertebrates by invasive species such as *C. caroliniana* should be considered in management plans. Habitat diversity should be maximised after removal of *C. caroliniana* or any other invasive species. In addition, the effects of invasive plants on microalgae and water quality should be considered. Hence, the growth of native residents should be encouraged in a timely fashion to mitigate habitat loss and formation of algal blooms after the removal of invasive macrophytes (Kovalenko et al., 2010).

Recommendations for future research

One of the major objectives of my study was to identify some of the key mechanisms used by invasive freshwater plants that can enhance their successful establishment and spread in introduced areas. I performed the screening of live material assays under laboratory conditions using non-axenic plant material to investigate the allelopathic activity of native and invasive macrophytes on microalgae (experiment 1, Chapter 2). Biotic and abiotic factors such as competition, season, and nutrient and light availability can affect the allelopathic potential of macrophytes on microalgae (Gross, 2003b; Mulderij et al., 2007; Bauer et al., 2009). Minimising the effects of these factors in a natural setting is unlikely. Therefore, in order to obtain results that are more reliable, I conducted a separate mesocosm study using sterile dialysis cassettes to account for some of these factors influencing interactions between the donor-target species (experiment 2, Chapter 2). Bacterial contamination can change the effects of allelochemicals that are labile and hence, can alter the interactions between macrophytes and microalgal species (Gross et al., 2007). Although I excluded bacteria from the dialysis cassettes in the mesocosm experiment (experiment 2, Chapter 2), bacteria co-exist with macrophytes in nature. Finally, I ran the mesocosm experiment investigating the allelopathic effects of *C. caroliniana* and *P. ochreatus* on naturally-occurring microalgae without bacterial exclusion (experiment 3, Chapter 2). Allelopathic experiments using a range of different methodologies can enable us to conclude with a greater certainty that the interactions between counterpart species are due to chemical intervention rather than other factors. Hence, future studies should employ a set of methods considering all factors that impact allelopathic potential of macrophytes against microalgae.

I minimised the effects of nutrient limitation on the allelopathic potential of the macrophytes and on the interactions between donor-target species (all chapters). Invasive species can regulate the growth of native competitors under a wide range of environmental conditions (Herb and Stefan, 2006; Mony et al., 2007; Schooler et al., 2009; Yarrow et al., 2009). *Cabomba caroliniana*, for example, is capable of invading eutrophic as well as oligotrophic systems. Moreover, nutrient availability in the system can affect the concentration of defensive chemicals produced by invasive macrophytes (Blumenthal, 2006). Accordingly, I recommend further investigation of the bioactivity of invasive macrophytes towards other species under a wider range of nutrient concentrations.

I used *Parapoynx rugosalis* as a native herbivore to test the deterrent activity of the native and invasive macrophytes and found that broad generalist herbivores such as *P. rugosalis* could be tolerant to the chemical compounds of *C. caroliniana* (herbivory experiment, Chapter 4). *Parapoynx* species are known to feed on many macrophyte species within different genera (Habeck, 1974; Buckingham and Bennett, 1989; Mueller and Dearing, 1994; Schooler et al., 2006; Hutchinson et al., 2015). Insects such as the stem boring weevil, *Hydrotimetes natans*, and larvae of the moth species, *Parapoynx diminutalis* and *Paracles* sp. demonstrated the potential for effective control of *C. caroliniana* (Buckingham and Bennett, 1989; Schooler et al., 2006). My study indicates that some aquatic herbivores can be used as biocontrol agents to manage the growth of invasive macrophytes including *C. caroliniana*; nevertheless, the larvae may also negatively affect non-target native organisms. As a result, the effects of herbivory by both specialist and generalist aquatic herbivores should be tested on various native and invasive macrophytes.

I conducted the allelopathy and the herbivory treatments in separate experiments (Chapter 4). These factors (allelopathic interactions between macrophytes and herbivory) interact in natural conditions, hence the effect of these factors should be tested simultaneously. Moreover, studies offering the invertebrates a 'choice' to select either native or invasive macrophyte as a food source would be more realistic. Further research needs to include concurrent testing of a wider range of plant competitors with allelopathic potential and the effect of herbivory by different aquatic herbivores on a range of native and invasive macrophytes.

Explicit investigations of the NWH and ERH are scarce in freshwater systems. Thus, the NWH and ERH should be tested using additional invasive macrophyte species, including free-floating and emergent species. In addition, investigations of the NWH and ERH on species with a range of physical forms would be beneficial. Further investigation into the interactions between a greater number of native and invasive macrophytes, and between invasive macrophytes and native herbivores can assist us to identify and select species that have the potential to be used as candidates to control invasive species such as *C. caroliniana*. In my study, *P. ochreatus* exhibited bioactivity similar to that of *C. caroliniana*. Furthermore, *P. ochreatus* could suppress the growth of *C. caroliniana*. *Ceratophyllum demersum* has a physical structure comparable to *C. caroliniana* and showed a strong allelopathic potential similar to that of *C. caroliniana* towards microalgae. Therefore, *C. demersum* would be able to provide a habitat that is similar to *C. caroliniana*. The two native macrophytes, *P. ochreatus* and *C. demersum* could be candidates for further investigation of the interactions between native and invasive macrophytes, and between macrophytes and other associated fauna.

Conclusion remarks

In summary, my study suggested that the three invasive macrophytes use their novel chemical weapons against competitors. The effects of *E. densa* and *E. canadensis* may be weak or absent on certain microalgae, especially on cyanobacteria. In contrast, *C. caroliniana* showed the ability to constrain the growth of all competing species; however, it had a specie-specific deterrent activity against the invertebrates. The moth larva of *P. rugosalis* inflicted considerable herbivory damage on *C. caroliniana*, but the hygrophilous snails avoided the plant. Furthermore, *C. caroliniana* displayed a positive impact on other invertebrates, which could be attributed to the physical structure of the plant. This indicates that the complex physical architecture of invasive species such as *C.*

caroliniana can provide shelter and feeding sites for associated fauna, and is an important criterion for invertebrates when selecting a plant as a refuge. The bioactivities of the invasive macrophytes were comparable to those of the native species in some circumstances. Both native and invasive macrophytes exhibited species-specific allelopathic potentials against the microalgae. Furthermore, the native macrophyte, *P. ochreatus* had an antagonistic interaction with *C. caroliniana*. While both native and invasive species have the potential to produce allelopathic substances, the novelty of these chemicals may provide invasive macrophytes with a competitive advantage over many native species. Invasive and native macrophytes that possess a physical structure with comparable complexity can support equivalent invertebrate communities. Nonetheless, the marked negative impacts of invasive freshwater macrophytes on the overall ecosystem should not be underestimated in management strategies. For example, fast biomass production of invasive macrophytes can cause a loss of open water habitat and that will have marked negative impact on other biota such as waterbirds. In conclusion, invasive macrophytes such as *C. caroliniana* can use their novel biochemistry in combination with other strategies to enhance their invasion success. However, the presence of native competitors and herbivores can regulate their growth to minimise their invasiveness.

References

Abrams, P. (1983) The theory of limiting similarity. *Annual Review of Ecology and Systematics*, **14,** 359-376.

Baltz, D. M. & Moyle, P. B. (1993) Invasion resistance to introduced species by a native assemblage of California stream fishes. *Ecological Applications,* **3,** 246-255.

Bauer, N., Blaschke, U., Beutler, E., Gross, E. M., Jenett-Siems, K., Siems, K. & Hilt, S. (2009) Seasonal and interannual dynamics of polyphenols in *Myriophyllum verticillatum* and their allelopathic activity on *Anabaena variabilis. Aquatic Botany,* **91,** 110-116.

Bickel, T. O. (2012) Ecology of the submersed aquatic weed *Cabomba caroliniana* in Australia. *Eighteenth Australasian Weeds Conference.* Melbourne, pp. 8-11.

Bickel, T. O. (2015) A boat hitchhiker's guide to survival: *Cabomba caroliniana* desiccation resistance and survival ability. *Hydrobiologia,* **746,** 123-134.

Blumenthal, D. M. (2006) Interactions between resource availability and enemy release in plant invasion. *Ecology Letters,* **9,** 887-895.

Brendonck, L., Maes, J., Rommens, W., Dekeza, N., Nhiwatiwa, T., Barson, M., Callebaut, V., Phiri, C., Moreau, K. & Gratwicke, B. (2003) The impact of water hyacinth (*Eichhornia crassipes*) in a eutrophic subtropical impoundment (Lake Chivero, Zimbabwe). II. Species diversity. *Archiv für Hydrobiologie,* **158,** 389-405.

Brundu, G. (2015) Plant invaders in European and Mediterranean inland waters: profiles, distribution, and threats. *Hydrobiologia*, **746,** 61-79.

Bryant, D. & Papas, P. (2007) Macroinvertebrate communities in artificial wetlands - the influence of macrophytes. Arthur Rylah Institute for Environmental Research Technical Report Series No. 168. Arthur Rylah Institute for Environmental Research.

Bryant, J. P., Chapin III, F. S. & Klein, D. R. (1983) Carbon/nutrient balance of boreal plants in relation to vertebrate herbivory. *Oikos*, **40,** 357-368.

Buckingham, G. & Bennett, C. (1989) Laboratory host range of *Parapoynx diminutalis* (Lepidoptera: Pyralidae), an Asian aquatic moth adventive in Florida and Panama on *Hydrilla verticillata* (Hydrocharitaceae). *Environmental Entomology,* **18,** 526–530.

Burlakova, L. E., Karatayev, A. Y., Padilla, D. K., Cartwright, L. D. & Hollas, D. N. (2009) Wetland restoration and invasive species: apple snail (*Pomacea insularum*) feeding on native and invasive aquatic plants. *Restoration Ecology,* **17,** 433-440.

Callaway, R. M. & Aschehoug, E. T. (2000) Invasive plants versus their new and old neighbors: a mechanism for exotic invasion. *Science,* **290,** 521-523.

Choi, C., Bareiss, C., Walenciak, O. & Gross, E. M. (2002) Impact of polyphenols on growth of the aquatic herbivore *Acentria ephemerella*. *Journal of Chemical Ecology,* **28,** 2245-2256.

Crawley, M. J. (1997) *Plant Ecology*. Blackwell Science, Oxford.

Davis, M. A. (2009) *Invasion Biology.* Oxford University Press, Oxford.

Dendène, M. A., Rolland, T., Trémolieres, M. & Carbiener, R. (1993) Effect of ammonium ions on the net photosynthesis of three species of *Elodea*. *Aquatic Botany,* **46,** 301-315.

Dextrase, A. & Mandrak, N. (2006) Impacts of alien invasive species on freshwater fauna at risk in Canada. *Biological Invasions,* **8,** 13-24.

Duarte, C. M. (1992) Nutrient concentration of aquatic plants: patterns across species. *Limnology and Oceanography,* **37,** 882-889.

El-Ghazal, R. A. K. & Riemer, D. N. (1986) Germination suppression by extracts of aquatic plants. *Journal of Aquatic Plant Management,* **24,** 76-79.

Elton, C. S. (2000) *The Ecology of Invasions by Animals and Plants.* University of Chicago Press, Chicago.

Erhard, D. & Gross, E. M. (2006) Allelopathic activity of *Elodea canadensis* and *Elodea nuttallii* against epiphytes and phytoplankton. *Aquatic Botany*, **85,** 203-211.

Fan, S., Liu, C., Yu, D. & Xie, D. (2013) Differences in leaf nitrogen content, photosynthesis, and resource-use efficiency between *Eichhornia crassipes* and a native plant *Monochoria vaginalis* in response to altered sediment nutrient levels. *Hydrobiologia,* **711,** 129-137.

Fargione, J. E. & Tilman, D. (2005) Diversity decreases invasion via both sampling and complementarity effects. *Ecology Letters,* **8,** 604-611.

Fargione, J. E., Brown, C. S. & Tilman, D. (2003) Community assembly and invasion: an experimental test of neutral versus niche processes. *Proceedings of the National Academy of Sciences*, **100,** 8916–8920.

Fleming, J. P. & Dibble, E. D. (2015) Ecological mechanisms of invasion success in aquatic macrophytes. *Hydrobiologia,* **746,** 23-37.

Gallardo, B., Clavero, M., Sánchez, M. I. & Vilà, M. (2016) Global ecological impacts of invasive species in aquatic ecosystems. *Global Change Biology,* **22,** 151-163.

Gérard, J., Brion, N. & Triest, L. (2014) Effect of water column phosphorus reduction on competitive outcome and traits of *Ludwigia grandiflora* and *L. pelploides*, invasive species in Europe. *Aquatic Invasions,* **9,** 157-166.

Gross, E. M. (2003a) Differential response of tellimagrandin II and total bioactive hydrolysable tannins in an aquatic angiosperm to changes in light and nitrogen. *Oikos,* **103,** 497-504.

Gross, E. M. (2003b) Allelopathy of aquatic autotrophs. *Critical Reviews in Plant Sciences,* **22,** 313-340.

Gross, E. M., Hilt, S., Lombardo, P. & Mulderij, G. (2007) Searching for allelopathic effects of submerged macrophytes on phytoplankton—State of the art and open questions. *Hydrobiologia,* **584,** 77-88.

Habeck, D. H. (1974) Caterpillars of *Parapoynx* in relation to aquatic plants in Florida. *Florida Agricultural Experiment Station,* **12,** 15-18.

Herb, W. R. & Stefan, H. G. (2006) Seasonal growth of submersed macrophytes in lakes: The effects of biomass density and light competition. *Ecological Modelling,* **193,** 560-574.

Hilt, S. & Gross, E. M. (2008) Can allelopathically active submerged macrophytes stabilise clear-water states in shallow lakes? *Basic and Applied Ecology,* **9,** 422-432.

Hilt, S., Ghobrial M. G. N. & Gross, E. M. (2006) In situ allelopathic potential of *Myriophyllum verticillatum* (Haloragaceae) against selected phytoplankton species. *Journal of Phycology,* **42,** 1189-1198.

Hogsden, K. L., Sager, E. P. & Hutchinson, T. C. (2007) The impacts of the non-native macrophyte *Cabomba caroliniana* on littoral biota of Kasshabog Lake, Ontario. *Journal of Great Lakes Research,* **33,** 497-504.

Huang, W., Siemann, E., Wheeler, G. S., Zou, J., Carrillo, J. & Ding, J. (2010) Resource allocation to defence and growth are driven by different responses to generalist and specialist herbivory in an invasive plant. *Journal of Ecology*, **98,** 1157-1167.

Hussner, A. (2009) Growth and photosynthesis of four invasive aquatic plant species in Europe. *Weed Research,* **49,** 506-515.

Hutchinson, J. T., Huston, D. C. & Gibson, J. R. (2015) Defoliation of cultured creeping primrose willow (*Ludwigia repens*) and other aquatic plants by *Parapoynx obscuralis* (Lepidoptera: Crambidae). *Southwestern Entomologist,* **40,** 227-232.

ISSG, (2005) *Global Invasive Species Database (GISD)*. Auckland, New Zealand: University of Auckland. http://www.issg.org/database

Jacobs, M. J. & MacIsaac, H. J. (2009) Modelling spread of the invasive macrophyte *Cabomba caroliniana*. *Freshwater Biology* **54,** 296–305.

Jahnke, L. S., Eighmy, T. T. & Fagerberg, W. R. (1991) Studies of *Elodea nuttallii* grown under photorespiratory conditions. I. Photosynthetic characteristics. *Plant, Cell and Environment* **14,** 147-156.

Jarchow, M. E. & Cook, B. J. (2009) Allelopathy as a mechanism for the invasion of *Typha angustifolia*. *Plant Ecology*, **204,** 113-124.

Kelly, D. J. & Hawes, I. (2005) Effects of invasive macrophytes on littoral-zone productivity and food web dynamics in a New Zealand high-country lake. *Journal of the North American Benthological Society,* **24,** 300-320.

Kennedy, T. A., Naeem, S., Howe, K. M., Knops, J. M., Tilman, D. & Reich, P. (2002) Biodiversity as a barrier to ecological invasion. *Nature,* **417,** 636-638.

Klironomos, J., Maron, J., Morris, W., Parker, I., Power, A., Seabloom, E., Torchin, M. & Vzquez, D. (2006) Biotic interactions and plant invasions. *Ecology Letter,* **9,** 1461-0248.

Kolar, C. S. & Lodge, D. M. (2000) Freshwater nonindigenous species: interactions with other global changes. *Invasive Species in a Changing World*, 3-30.

Körner, S. & Nicklisch, A. (2002) Allelopathic growth inhibition of selected phytoplankton species by submerged macrophytes. *Journal of Phycology,* **38,** 862-871.

Kovalenko, K. E., Dibble, E. D. & Slade, J. G. (2010) Community effects of invasive macrophyte control: role of invasive plant abundance and habitat complexity. *Journal of Applied Ecology,* **47,** 318-328.

Kowarik, I. (2003) Human agency in biological invasions: secondary releases foster naturalisation and population expansion of alien plant species. *Biological Invasions*, **5,** 293-312.

Kuhar, U., Germ, M. & Gaberščik, A. (2010) Habitat characteristics of an alien species *Elodea canadensis* in Slovenian watercourses. *Hydrobiologia,* **656,** 205-212.

Lassuy, D. R. (1995) Introduced species as a factor in extinction and endangerment of native fish species. *Uses and Effects of Cultured Fishes in Aquatic Ecosystems* (eds H. L. Schramm & R. G. Piper), pp. 391-396. American Fisheries Society, Bethesda.

Lemoine, D. G., Barrat-Segretain, M. H. & Roy, A. (2009) Morphological and chemical changes induced by herbivory in three common aquatic macrophytes. *International Review of Hydrobiology,* **94,** 282-289.

Levine, J. M. (2000) Species diversity and biological invasions: relating local process to community pattern. *Science,* **288,** 852-854.

Levine, J. M., Adler, P. B. & Yelenik, S. G. (2004) A meta-analysis of biotic resistance to exotic plant invasions. *Ecology Letters,* **7,** 975-989.

Lind, E. M. & Parker, J. D. (2010) Novel weapons testing: are invasive plants more chemically defended than native plants? *Plos One*, **5,** e10429.

Lockwood, J. L., Hoopes, M. F. & Marchetti, M. P. (2013) *Invasion Ecology*. John Wiley & Sons, Oxford.

Lodge, D. M., Williams, S., MacIsaac, H. J., Hayes, K. R., Leung, B., Reichard, S., Mack, R. N., Moyle, P. B., Smith, M. & Andow, D. A. (2006) Biological invasions: recommendations for US policy and management. *Ecological Applications,* **16,** 2035-2054.

Mackey, A. & Swarbrick, J. (1997) The biology of Australian weeds. 32. *Cabomba caroliniana* Gray. *Plant Protection Quarterly*, **12,** 154-165.

Marko, M. D., Gross, E., Newman, R. & Gleason, F. K. (2008) Chemical profile of the North American native *Myriophyllum sibiricum* compared to the invasive *M. spicatum*. *Aquatic Botany,* **88,** 57-65.

Maron, J. L. & Vilà, M. (2001) When do herbivores affect plant invasion? Evidence for the natural enemies and biotic resistance hypotheses. *Oikos,* **95,** 361-373.

Mayer-Pinto, M., Johnston, E., Hutchings, P., Marzinelli, E., Ahyong, S., Birch, G., Booth, D., Creese, R., Doblin, M. & Figueira, W. (2015) Sydney Harbour: a review of anthropogenic impacts on the biodiversity and ecosystem function of one of the world. *Marine and Freshwater Research,* **66,** 1088-1105.

Meyerson, L. A. & Mooney, H. A. (2007) Invasive alien species in an era of globalization. *Frontiers in Ecology and the Environment*, **5,** 199-208.

Michelan, T. S., Thomaz, S. M. & Bini, L. M. (2013) Native macrophyte density and richness affect the invasiveness of a tropical Poaceae species. *PloS One,* **8,** e60004.

Mitchell, C. E., Agrawal, A. A., Bever, J. D., Gilbert, G. S., Hufbauer, R. A., Klironomos, J. N., Maron, J. L., Morris, W. F., Parker, I. M. & Power, A. G. (2006) Biotic interactions and plant invasions. *Ecology Letters,* **9,** 726-740.

Mohamed, Z. A. & A. M. Al Shehri, 2010. Differential responses of epiphytic and planktonic toxic cyanobacteria to allelopathic substances of the submerged macrophyte *Stratiotes aloides*. *International Review of Hydrobiology*, **95,** 224-234.

Molisch, H. (1937) *Der Einfluss einer Pflanze auf die Andere-Allelopathie.* Fisher, Jena.

Mony, C., Koschnick, T. J., W. T., H. & S., M. (2007) Competition between two invasive Hydrocharitaceae (*Hydrilla verticillata* (L.f.) (Royle) and *Egeria densa* (Planch)) as influenced by sediment fertility and season. *Aquatic Botany,* **86,** 236-242.

Morrison, W. E. & Hay, M. E. (2011) Herbivore preference for native vs. exotic plants: generalist herbivores from multiple continents prefer exotic plants that are evolutionarily naïve. *PLoS One,* **6,** e17227.

Mueller, U. G. & Dearing, M. D. (1994) Predation and avoidance of tough leaves by aquatic larvae of the moth *Parapoynx rugosalis* (Lepidoptera: Pyralidae). *Ecological Entomology,* **19,** 155-158.

Mulderij, G., van Donk, E. & Roelofs, J. G. M. (2003) Differential sensitivity of green algae to allelopathic substances from *Chara*. *Hydrobiologia,* **491,** 261-271.

Mulderij, G., Van Nesc, E. H. & Van Donka, E. (2007) Macrophyte-phytoplankton interactions: the relative importance of allelopathy versus other factors. *Ecological Modelling,* **204,** 85-92.

Müller-Schärer, H., Schaffner, U. & Steinger, T. (2004) Evolution in invasive plants: implications for biological control. *Trends in Ecology & Evolution,* **19,** 417-422.

Nakai, S., Hosomi, M., Okada, M. & Murakami, A. (1996) Control of algal growth by macrophytes and macrophyte-extracted bioactive compounds. *Water Science and Technology*, **34,** 227-235.

Newman, R. M., Kerfoot, W. C. & Hanscom, Z. (1996) Watercress allelochemical defends high-nitrogen foliage against consumption: effects on freshwater invertebrate herbivores. *Ecology,* **77,** 2312-2323.

Ørgaard, M. (1991) The genus *Cabomba* (Cabombaceae)—a taxonomic study. *Nordic Journal of Botany*, **11**, 179-203.

Ozimek, T., Van Donk, E. & Gulati, R. D. (1993) Growth and nutrient uptake by two species of *Elodea* in experimental conditions and their role in nutrient accumulation in a macrophyte-dominated lake. *Hydrobiologia*, **251,** 13-18.

Pakdel, F. M., Sim, L., Beardall, J. & Davis, J. (2013) Allelopathic inhibition of microalgae by the freshwater stonewort, *Chara australis*, and a submerged angiosperm, *Potamogeton crispus*. *Aquatic Botany*, **110,** 24-30.

Parker, J. D. & Hay, M. E. (2005) Biotic resistance to plant invasions? Native herbivores prefer non-native plants. *Ecology Letters,* **8,** 959-967.

Parsons, W. T. & Cuthbertson, E. G. (2001) *Noxious Weeds of Australia*. 2nd Edition. CSIRO Publishing, Collingwood.

Pimentel, D. (2011) Environmental and economic costs associated with alien invasive species in the United States. *Biological Invasions: Economic and Environmental Costs of Alien Plant, Animal, and Microbe Species,* (2nd edn). CRC Press, Boca Raton, FL**,** 411-430.

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.

Pimm, S. L. & Hyman, J. B. (1987) Ecological stability in the context of multispecies fisheries. *Canadian Journal of Fisheries and Aquatic Sciences,* **44,** s84-s94.

Pyšek, P., Danihelka, J., Sádlo, J., Chrtek Jr, J., Chytrý, M., Jarošík, V., Kaplan, Z. & Krahulec, F. (2012) Catalogue of alien plants of the Czech Republic: checklist update, taxonomic diversity and invasion patterns. *Preslia,* **84,** 155-255.

Quinn, L. D., Schooler, S. S. & Van Klinken, R. D. (2011) Effects of land use and environment on alien and native macrophytes: lessons from a large-scale survey of Australian rivers. *Diversity and Distributions,* **17,** 132-143.

Rothhaupt, K. O., Fornoff, F. & Yohannes, E. (2015) Induced responses to grazing by an insect herbivore (*Acentria ephemerella*) in an immature macrophyte (*Myriophyllum spicatum*): an isotopic study. *Ecology and Evolution,* **5,** 3657-3665.

Sainty, G. & Jacobs, S. (2003) *Waterplants in Australia: A Field Guide*, 4 edn. Sainty & Associates, Australia.

Sala, O. E., Chapin, F. S., Armesto, J. J., Berlow, E., Bloomfield, J., Dirzo, R., Huber-Sanwald, E., Huenneke, L. F., Jackson, R. B. & Kinzig, A. (2000) Global biodiversity scenarios for the year 2100. *Science,* **287,** 1770-1774.

Sax, D. F., Stachowicz, J. J., Brown, J. H., Bruno, J. F., Dawson, M. N., Gaines, S. D., Grosberg, R. K., Hastings, A., Holt, R. D. & Mayfield, M. M. (2007) Ecological and evolutionary insights from species invasions. *Trends in Ecology & Evolution*, **22,** 465-471.

Schooler, S., Cabrera-Walsh, W. & Julien, M. (2009) *Cabomba caroliniana* gray (Cabombaceae). *Biological control of tropical weeds using arthropods.* Cambridge University Press, Cambridge**,** 88-107.

Schooler, S., Julien, M. & Walsh, G. C. (2006) Predicting the response of *Cabomba caroliniana* populations to biological control agent damage. *Australian Journal of Entomology,* **45,** 327-330.

Schultz, R. & Dibble, E. (2012) Effects of invasive macrophytes on freshwater fish and macroinvertebrate communities: the role of invasive plant traits. *Hydrobiologia,* **684,** 1-14.

Stiers, I., Njambuya, J. & Triest, L. (2011) 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.

Stoops, C. A., Adler, P. H. & McCreadie, J. W. (1998) Ecology of aquatic Lepidoptera (Crambidae: Nymphulinae) in South Carolina, USA. *Hydrobiologia,* **379,** 33-40.

Strayer, D. L., Lutz, C., Malcom, H. M., Munger, K. & Shaw, W. H. (2003) Invertebrate communities associated with a native (*Vallisneria americana*) and an alien (*Trapa natans*) macrophyte in a large river. *Freshwater Biology,* **48,** 1938-1949.

Svensson, J. R., Nylund, G. M., Cervin, G., Toth, G. B. & Pavia, H. (2013) Novel chemical weapon of an exotic macroalga inhibits recruitment of native competitors in the invaded range. *Journal of Ecology,* **101,** 140-148.

Tabassum, S. & Leishman, M. R. (2016) Trait values and not invasive status determine competitive outcomes between native and invasive species under varying soil nutrient availability. *Austral Ecology*, **41,** 875-885.

Thomaz, S. M., Kovalenko, K. E., Havel, J. E. & Kats, L. B. (2015) Aquatic invasive species: general trends in the literature and introduction to the special issue. *Hydrobiologia,* **746**, 1-12.

Vanderstukken, M., Mazzeo, N. & Van Colen, W. (2011) Biological control of phytoplankton by the subtropical submerged macrophytes *Egeria densa* and *Potamogeton illinoensis*: a mesocosm study. *Freshwater Biology,* **56,** 1837-1849.

Villamagna, A. M. & Murphy, B. R. (2010) Ecological and socio-economic impacts of invasive water hyacinth (*Eichhornia crassipes*): a review. *Freshwater Biology,* **55,** 282-298.

Villamagna, A. M., Murphy, B. R. & Trauger, D. L. (2010) Behavioral response of American coots (*Fulica americana*) to water hyacinth (*Eichhornia crassipes*) in Lake Chapala, Mexico. *Waterbirds,* **33,** 550-555.

Von Holle, B. & Simberloff, D. (2004) Testing Fox's assembly rule: does plant invasion depend on recipient community structure? *Oikos*, **105,** 551–563.

Walenciak, O., Zwisler, W. & Gross, E. M. (2002) Influence of *Myriophyllum spicatum*-derived tannins on gut microbiota of its herbivore *Acentria ephemerella*. *Journal of Chemical Ecology,* **28,** 2045-2056.

Wersal, R. M. & Madsen, J. D. (2011) Influences of water column nutrient loading on growth characteristics of the invasive aquatic macrophyte *Myriophyllum aquaticum* (Vell.) Verdc. *Hydrobiologia,* **665,** 93-105.

Willis, R. J. (1985) The historical bases of the concept of allelopathy. *Journal of the History of Biology,* **18,** 71-102.

Willis, R. J. (2008) *The History of Allelopathy.* Springer, Dordrecht.

Wolf, V. C., Berger, U., Gassmann, A. & Müller, C. (2011) High chemical diversity of a plant species is accompanied by increased chemical defence in invasive populations. *Biological Invasions*, **13,** 2091-2102.

Wu, X., Zhang, Z., Chen, D., Zhang, J., Yang, W. & Jin, Y. (2012) Allelopathic effects of *Eichhornia crassipes* on the growth of *Microcystis aeruginosa*. *Journal of Agricultural Science and Technology*. A, **2,** 1400-1406.

Xiong, W., Yu, D., Wang, Q., Liu, C. & Wang, L. (2008) A snail prefers native over exotic freshwater plants: implications for the enemy release hypotheses. *Freshwater Biology,* **53,** 2256-2263.

Xu, K., Ye, W., Cao, H., Deng, X., Yang, Q. & Zhang, Y. (2004) The role of diversity and functional traits of species in community invasibility. *Botanical Bulletin of Academia Sinica,* **45,** 149-157.

Yarrow, M., Marin, V. H., Finlayson, M., Tironi, A., Delgado, L. E. & Fischer, F. (2009) The ecology of *Egeria densa* Planchon (Liliopsida: Alismatales): a wetland ecosystem engineer? *Revista Chilena de Historia Natural,* **82,** 299-313.

Appendices

Appendix 1. List of invertebrate taxa (zooplankton and macroinvertebrates) found in the presence of *Potamogeton ochreatus* (native), *Cabomba caroliniana* (invasive) and controls (no plants) during three different seasons. (Experiment 1, chapter 3).

	Spring			Summer			Autumn		
	Invasive	Native	Control	Invasive	Native	Control	Invasive	Native	Control
Anisoptera	X	X	X	X	X	X	X	X	✓
Calanoida	✓	✓	✓	✓	✓	✓	✓	✓	✓
Ceratopogonidae	X	X	✓	X	X	X	X	X	X
Chironomidae	✓	✓	✓	X	X	✓	✓	X	X
Chydoridae	✓	✓	✓	✓	✓	✓	✓	✓	✓
Collembola	✓	✓	✓	X	X	X	X	X	X
Corixidae	X	X	X	X	X	X	✓	X	X
Culicidae	X	X	✓	X	X	✓	✓	X	✓
Cyclopoida	✓	✓	✓	✓	✓	✓	✓	✓	✓
Daphniidae	✓	✓	✓	✓	✓	✓	✓	✓	✓
Dytiscidae	X	✓	X	✓	✓	X	X	X	X
Hydrachnidiae	✓	✓	✓	✓	✓	✓	✓	X	✓
Hydridae	✓	✓	✓	✓	✓	✓	✓	✓	✓
Hygrophila	✓	✓	✓	✓	X	X	X	✓	X
Micronecta	✓	X	X	X	✓	X	✓	✓	X
Nematoda	X	X	✓	X	X	X	X	X	X
Notonectidae	X	X	X	X	✓	X	X	✓	X
Oribatida	✓	✓	X	✓	X	✓	X	X	X
Ostrocoda	✓	✓	✓	✓	✓	✓	✓	✓	✓
Rhynchobdellida	X	X	X	X	X	X	X	✓	X
Rotifera	✓	✓	✓	✓	✓	✓	X	✓	X
Veliidae	✓	X	✓	X	X	✓	✓	✓	✓

Appendix 2. List of invertebrate taxa (zooplankton and macroinvertebrates) found in the presence of *Potamogeton ochreatus* (native), *Cabomba caroliniana* (invasive) and controls (no plants) in mono-culture and co-culture settings during three different seasons. (Experiment 2, chapter 3).

	Spring		Summer		Autumn	
	Invasive	Native	Invasive	Native	Invasive	Native
Mono-culture setting						
Calanoida	✓	✓	✓	✓	✓	✓
Chironomidae	✓	✓	✓	✓	✓	✓
Chydoridae	✓	✓	✓	✓	✓	✓
Collembola	X	✓	X	X	X	X
Corixidae	✓	X	X	X	X	X
Culicidae	X	X	✓	X	X	✓
Cyclopoida	✓	✓	✓	✓	✓	✓
Daphniidae	✓	✓	✓	✓	✓	✓
Hydrachnidiae	✓	✓	✓	✓	✓	X
Hydridae	X	✓	✓	✓	✓	✓
Hygrophila	✓	X	✓	X	X	X
Micronecta	✓	X	✓	X	X	X
Oribatida	✓	✓	✓	✓	✓	X
Ostrocoda	✓	✓	✓	✓	✓	✓
Rhynchobdellida	✓	X	X	X	X	X
Rotifera	✓	✓	✓	✓	✓	✓
Veliidae	X	X	✓	✓	✓	X
Co-culture settings						
Bivalvia	X	X	✓	✓	✓	✓
Calanoida	✓	✓	✓	✓	✓	✓
Chironomidae	✓	✓	✓	X	✓	✓
Collembola	✓	✓	X	X	X	X
Corixidae	✓	X	X	X	X	X
Culicidae	✓	X	X	✓	X	X
Cyclopoida	✓	✓	✓	✓	✓	✓
Daphniidae	✓	✓	✓	✓	✓	✓
Hydrachnidiae	✓	✓	✓	✓	X	✓
Hydridae	X	✓	✓	✓	✓	✓
Hygrophila	✓	✓	✓	X	X	✓
Oligochaeta	X	X	✓	X	X	X
Oribatida	X	X	✓	✓	X	X
Ostrocoda	✓	✓	✓	✓	✓	✓
Rotifera	✓	✓	✓	✓	✓	✓
Veliidae	✓	✓	✓	✓	✓	✓