Influence of Mexican waterlily (*Nymphaea mexicana*) on ecology and water quality in the lower Vasse River, Busselton

Prepared for

the Department of Water and Environmental Regulation

February 2018

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Cover photo: Mexican waterlily (*Nymphaea mexicana*) growing in the Vasse River, March 2017.

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Summary

The Mexican waterlily, *Nymphaea mexicana* has been present as an introduced plant in the lower Vasse River for many years. Following a substantial expansion during 2013-2014 and subsequent gradual spread, it now covers a considerable area of the lower Vasse River (1.1ha). While the ongoing expansion over large areas of the river is of concern to the community and management authorities, the presence of waterlily has had a visible beneficial effect on water quality, with an absence of summer algal blooms within and adjacent to patches of waterlily. To improve understanding of the effects of the waterlilies on water quality and ecology, the Department of Water and Environmental Regulation commissioned a study in Autumn 2017. This document presents the outcomes of this study, includes a comprehensive review of relevant literature and provides recommendations for future management of Mexican waterlily in the lower Vasse River.

Study aims

The study aimed to understand the influence of waterlily in the lower Vasse River on water quality and ecology to inform future management of its growth. Specific objectives of the sampling program were to:

1. determine the influence of waterlily on water quality (physical parameters, nutrient concentrations and algal blooms) including determining if the lilies provide a sink for nutrients;
2. evaluate the potential impacts of waterlily on the ecology of the system
3. investigate characteristics associated with presence and absence of waterlily to inform future control methods and predict risk of further expansion.
4. assess the potential value of *P. decipiens* in management through competition with waterlily; and
5. evaluate the likely effects of harvesting, removal or in-situ control;

Effect of waterlilies on water quality

Waters within the dense stands of plants had very low oxygen concentrations, owing to a lack of photosynthetic activity within the water, limited replenishment from the air and high levels of decomposition. There is greater potential for oxygen replenishment of water within the lilies under windy conditions.

The lilies and the open water areas between lily patches had higher phosphorus concentrations, but lower phytoplankton growth, than the section of river downstream. A dense bloom of blue-green algae (cyanobacteria) was present downstream, but not among the patches of waterlily. This is an example of contrasting regimes: the downstream reach exhibited a turbid, phytoplankton-dominated regime; while the reach with large stands of *N. mexicana* exhibited a clear-water regime. Phosphorus concentrations, trophic interactions and physical conditions did not explain lower phytoplankton growth in the vicinity of waterlilies. Nitrogen limitation may prevent phytoplankton growth but does not explain the lack of nitrogen-fixing cyanobacteria. Additional processes such light limitation due to short water residence time between stands of waterlily and chemical inhibition of phytoplankton by waterlilies may have limited phytoplankton growth, however these processes could not be examined through this study.

Additional research to investigate the processes limiting phytoplankton growth between stands of *N. mexicana* in the lower Vasse River is recommended so these can be considered in future control of this plant.

It is difficult to determine whether the lilies act as a net nutrient sink. Nutrient uptake and storage within the plants is likely to be temporary due to nutrient release during decomposition. However, if sediments from external sources and from decomposing plants accumulate and are stored within the plant beds, they may act as an important nutrient sink. Nutrient content of sediments was lower beneath the waterlilies, which could reflect nutrient uptake by the plants. However accumulation of decomposing plant tissue, which has lower nutrient content than river sediments, may also act to lower the sediment-nutrient content.

Ecological impacts of waterlilies

Despite low oxygen conditions, the waterlily stands supported higher abundance of aquatic invertebrates than other areas of the river through providing structural habitat. Much of this abundance was due to large numbers of taxa tolerant of poor water quality. However, species richness was also higher, particularly at the edges of the lily stands where oxygen levels were higher, suggesting the lilies do enhance the aquatic invertebrate community. Notwithstanding the effect on aquatic invertebrates, there may be negative consequences of dense infestation of waterlilies on birds and fish through loss of open water habitat, which need to be considered in future management.

Potential for further expansion

Nymphaea are highly successful in nutrient-rich, slow-flowing waters such as the lower Vasse River. Their floating leaves provide a competitive advantage over submerged plants for light, they can colonise areas which are too deep for other emergent macrophytes, and they tolerate soft organic-rich substrates. There did not appear to be any characteristics of open water areas that would prevent future colonisation. Comparison of depth and sediment characteristics, river width and amount of riparian shading between areas with and without lilies upstream of the main point of infestation did not suggest any limiting factors to the further expansion of waterlilies into these areas. Shading at the Strelly Street bridge has prevented the spread of lilies downstream from this point, but the infestation present further downstream will likely spread to this point eventually.

Slower expansion of lilies from the main point of infestation downstream may be owing to deeper waters and shading by the severe algal bloom, but over time ongoing expansion will continue. If not controlled, the waterlily population is likely to continue to expand throughout the lower Vasse River through vegetative stoloniferous reproduction. It will cover increasing areas of open water and act as a source of material for dispersal to new sites.

Competition potential of Persicaria decipiens

The native semi-aquatic plant *Persicaria decipiens* (slender knotweed) occurs in several locations on the banks of the lower Vasse River and in some shallow areas grows out from the bank amongst the waterlilies. Growth of *P. decipiens* within the river channel is restricted to areas where waterlily is also growing, suggesting that *P. decipiens* may be dependent on the substrate provided by the waterlilies for growth. Lower *N. mexicana* biomass in the presence of *P. decipiens* may be owing to

shallow conditions, rather than competition, with seasonal senescence of the waterlilies observed in other shallow areas in the absence of *P. decipiens* (e. g. northern bank near site WL 2). Further planting of *P. decipiens* with the aim of replacing *N. mexicana* is not considered worthwhile because it will not have a competitive advantage in deeper waters of the river channel, it may not offer the same water quality benefits that are currently provided by *N. mexicana,* would likely maintain low oxygen conditions, and would continue to restrict open water habitat for birds.

Future management

Active management of the lilies is required to prevent additional expansion in the lower Vasse River and loss of open hater habitat, and to reduce the risk of spread to nearby wetlands, and new populations observed in wetland areas should be controlled as a priority. However, the role of waterlilies in limiting algal blooms in the lower Vasse River also need to be considered in deciding the future extent of control. Eradication of *N. mexicana* may see a return to the situation of blue-green algal blooms throughout this reach of river. Control should aim to contain and reduce the extent of waterlilies extent, rather than eradicate them.

Shading is a potential control option to preventing further spread of waterlilies. Creating barriers across the river at the edges of waterlilies may be feasible and could incorporate floating islands as bridges with wetland vegetation. More extensive control can be most effectively achieved by applying herbicide in autumn, using glyphosate- or diquat-based products approves for use in waterways. A staged, mosaic approach to control will reduce the risks of oxygen depletion associated with plant decay.

Although there is a large biomass of waterlilies present, the current autumn standing crop represents only a small proportion of nutrient load to the river. They may provide an ongoing sink for nutrients, but this is unlikely to contribute much to overall reduction of available nutrient in the river. Harvesting of the current biomass would be equivalent to about 0.5% of the annual nutrient load of the lower Vasse River.

Control of waterlilies is recommended to reduce the current extent and prevent further spread in the lower Vasse River and eradicate new infestations. The waterlilies clearly improve water clarity in adjacent areas, and a mosaic approach to control would help maintain this beneficial role.

Introduction

The introduced Mexican waterlily, *Nymphaea mexicana* is native to Mexico and the south-eastern United States, and has been present as an introduced plant in the lower Vasse River and adjacent New River Wetland for many years. During the 2013-2014 summer, *N. mexicana* underwent notably prolific growth to cover large areas of open water (Figure 1, Figure 2). Very dense stands with substantial root mats and thick mats of broad floating leaves have become well-established, with peak biomass during summer and persistence throughout the year but with lower biomass during winter. The infestation has expanded gradually from those areas colonised during period of rapid growth, and in February 2017 covered 23%, and approximately 1.15ha of the river between the stop boards at the Butter Factory Museum and the Busselton Bypass. There are several areas in which waterlily has remained notably absent. Understanding different conditions in infested areas and those where growth is restricted is important in future management of infested areas and in assessment of risk of further expansion into other areas of the Vasse River and adjacent wetlands.

Water quality problems in the lower Vasse River are well-known, with blue-green algal blooms (cyanobacteria) a regular feature from December to April each summer. This has initiated substantial management effort over the last twenty years, including restoration, remediation and catchment management, however algal blooms have continued. Given this history, it is of considerable interest that the presence of waterlily has had a visible effect on water quality. Downstream of the waterlily infestation green discolouration from the algal bloom is evident, while within and adjacent to patches of waterlily this discoloration is absent (Figure 2). The potential limitation of phytoplankton growth by the waterlilies is of great interest, although the mechanisms responsible are not understood.

The effects of the waterlily on ecological values of the river are unknown. Although the lower Vasse River is severely degraded, it nonetheless provides habitat for waterbirds and native fish. Clearly the presence of large areas of water lily will have contracted open water habitat for aquatic fauna. However, the physical structure provided by aquatic plants often increases diversity and abundance of aquatic invertebrates, providing additional food resources for birds and fish, and this has previously been shown for the lower Vasse River (Paice et al. 2016).

The Slender Knotweed, *Persicaria decipiens*, is a native semi-aquatic plant common along the banks of the lower Vasse River. This is not an endemic species – it is a cosmopolitan species found throughout Australia, New Zealand, Europe, America and Malesia. It colonises moist degraded areas and is uncommon in well-vegetated riparian zones in the South West region. This species is extending its growth out from the banks into the river channel amongst the waterlily, taking advantage of the considerable substrate provided by the waterlily. There is some interest as to whether *P. decipiens* may be useful in the control of waterlily through competition.

Document Set ID: 3250243
Version: 1, Version Date: 04/04/2018

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4. evaluate the likely effects of harvesting, removal or in-situ control; and
5. assess the potential value of *P. decipiens* in management through competition with waterlily.

To meet these objective, extensive sampling of water and sediment quality, plant nutrients and biomass and aquatic invertebrates was undertaken upstream, downstream and within the areas colonised by *N. mexicana* in the lower Vasse River during March 2017. This report presents the results of this study and a comprehensive review of relevant literature.
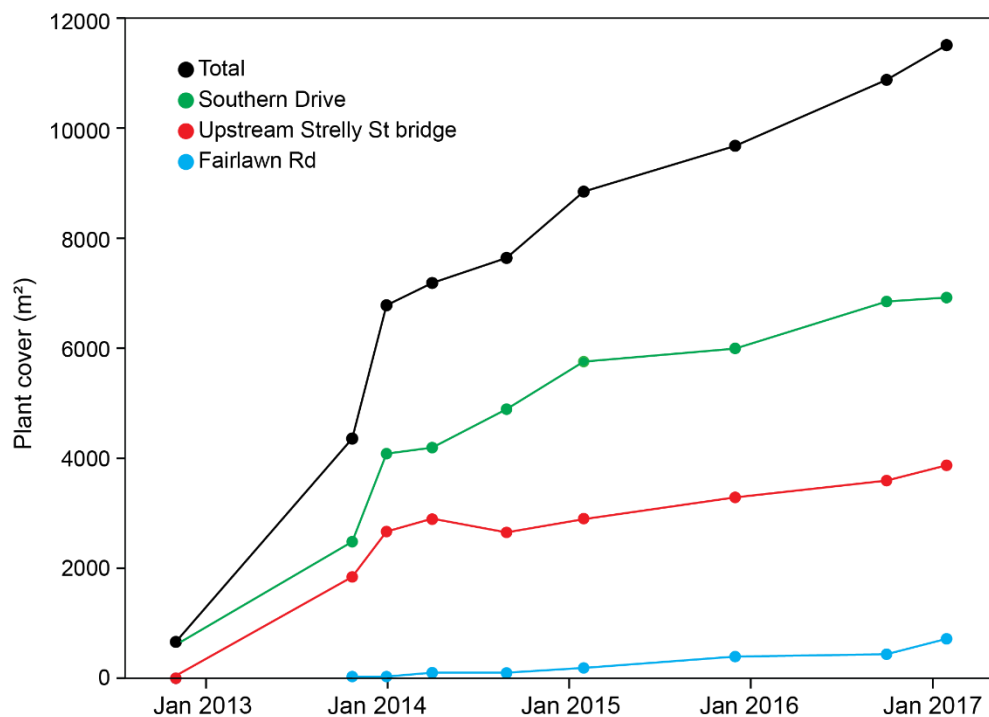
Figure 1. Plant cover over time calculated from air photos for the entire reach of the lower Vasse River (black) and main areas of growth indicated by nearby roads. Winter data was not available and cover may have been lower during winter. Data courtesy Department of Water and Environmental Regulation.

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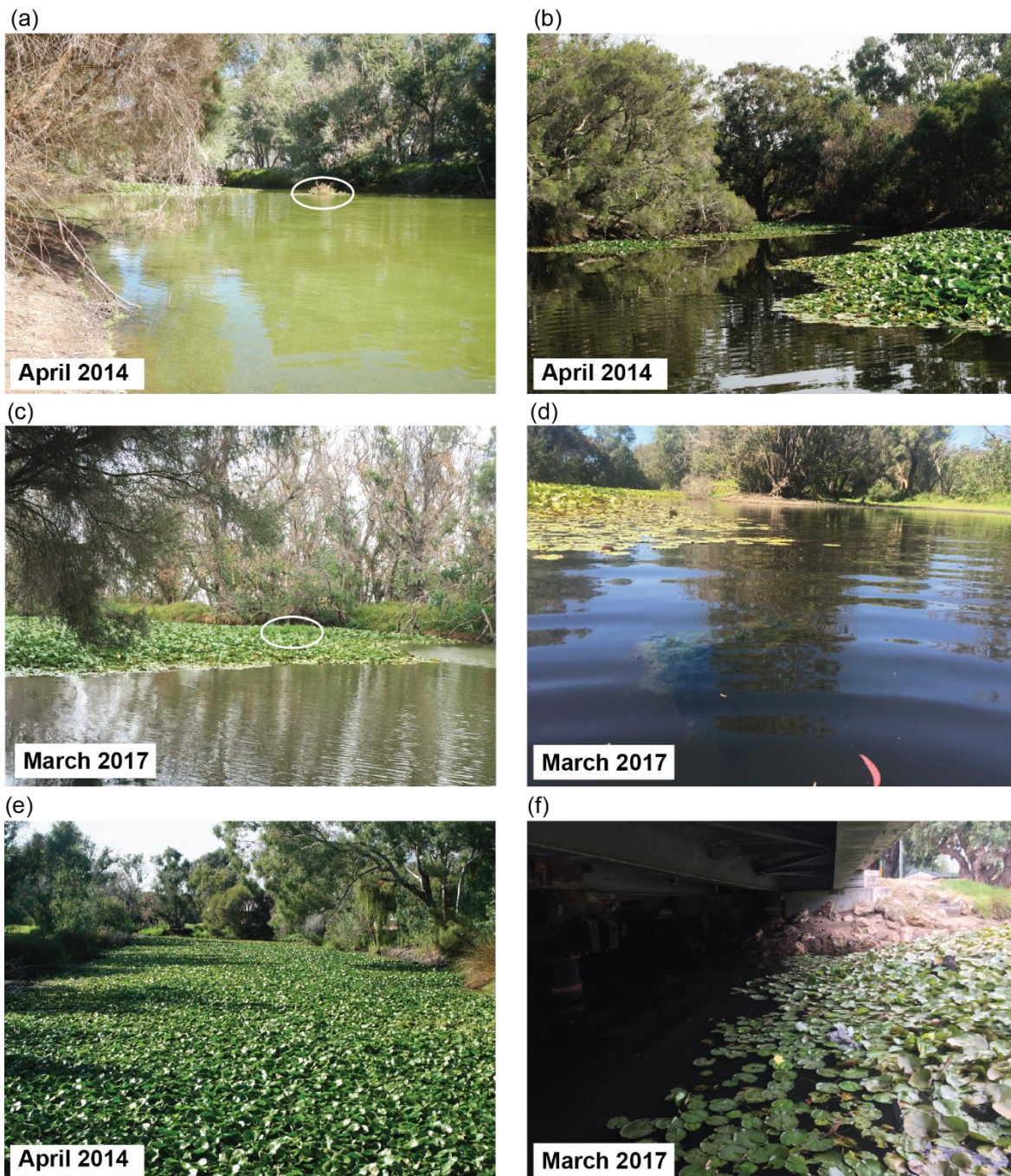
Figure 2. Photos from the lower Vasse River showing: contrasting water quality between the area downstream of *N. mexicana* and waters upstream in April 2014 (a and b) and March 2017 (c and d); limited expansion of the lily infestation over three years following period of rapid growth (a and c, highlighting a floating island as a point of reference); a pool site with clear water and a floating benthic algal mat (d); and the infestation upstream of Strelly Street bridge in April 2014 (e), which has remained similar over three years, and has not spread downstream of the bridge due to shading (f).

Characteristics of *Nymphaea mexicana*

Description and distribution

Nymphaea mexicana, also known as Mexican waterlily, Yellow waterlily and Banana lily, is a perennial floating-leaved freshwater aquatic plant with large round (orbiculate) leaves exceeding 20cm, bright yellow flowers and robust stems which may hold flowers and leaves above the water surface when plants are crowded (Sainty and Jacobs 2003) (Figure 3). As with all waterlilies, it occurs in slow-flowing waters over substrates rich in organic material (Wiersema 1988). In the lower Vasse River, extensive open areas with this type of substrate provide ideal conditions for its growth.

Nymphaea is one of five Genera within the Nymphaeaceae family, of which there are some forty species, in the water lily order Nymphaeales. Within *Nymphaea* are three sub-genera: *Nymphaea, Brachyceras and Hydrocallis. Nymphaea mexicana* is considered the most primitive species within the sub-genus Nymphaea (Capperino and Schneider 1985). Its native distribution is Mexico and south-east USA, however cultivation for ornamental use has seen it introduced extensively outside of this range.

It is recorded from 99 locations in Australia including three in Western Australia (Perth, Busselton and Manjimup) and others in south-east Queensland and scattered locations in New South Wales, Victoria and south-east South Australia (Atlas of Living Australia 2017).

Reproduction and dispersal

Nymphaea mexicana reproduces both sexually through flowering and asexually via stolons, which give rise to 'brood-bodies' and subsequent formation of clones (Wiersema, 1998). The resemblance of these perennating bodies to a bunch of bananas is the reason it is sometimes referred to as the Banana lily. Flowering commences seasonally from spring during the main growing season, and continues until dormancy in winter. Flowers are protogynous: female flower parts mature before male parts; and also allogamous: requiring different parent plants for successful pollination. Flowers open from late morning to late afternoon for two days (Caperino and Scheider 1985). Pollinators include bees, shore flies and soldier beetles, which are all present in the Vasse River. In the lower Vasse River, flowering density was highest at the outer edges of each stand of lilies and few flowers were observed in large expanses. Only one fruiting body was seen during harvesting of lilies in this study, and it is not known if this was viable.

Distinction in flower-opening times maintains reproductive isolation of many different species, but northern extension in the range of *N. mexicana* has resulted in formation of natural hybrids with *Nymphaea odorata* (Wiersema 1988). The cultivation of many ornamental hybrid varieties of waterlily is widespread. While these hybrid ornamentals are usually sterile, fertile hybrids of *N. mexicana* and *N. odorata*, exhibiting intermediate characters, do occur where they have overlapping distribution in south-east USA (Woods et al. 2005). Hybrids of *N. mexicana* which exhibit its stolon-bearing habit form extensive clones and are commonly the cause of problematic invasions (FNAA 2008). It is interesting to note that the population of *N. odorata* in the Margaret River, about 50 kilometres south of the Vasse River, has not grown as excessively. Asexual reproduction in this species is by rhizomes, rather than the more extensive stolons formed by *N. mexicana.*

While stolon formation is a common mode of asexual reproduction in *Nymphaea*, the stolons formed by *N. mexicana* are the largest of all waterlily species, and unique in the development of perennating bodies (Wiersema 1988). In the lower Vasse River, stolons were commonly 2cm in diameter and formed an extensive network among the root mass (Figure 3). This is doubtless the dominant form of reproduction in this population.

It is possible the Vasse River population is actually a hybrid of *N. mexicana*: expansion from nodes may indicate each stand has arisen from a single plant rather than by seed dispersal, and the extensive stoloniferous growth is typical of invasions elsewhere thought to be from hybrids. If not a hybrid, successful sexual reproduction may have seen broader dispersal of this plant in the region, rather than the in-situ expansion observed. This would depend on the presence of dispersal agents, namely waterbirds, which are abundant in the area. It is unknown whether the dispersal to date has occurred via movement of seeds or vegetative material – both are possible. Purple Swamphens (*Porphyrio porphyrio*) is resident in *N. mexicana* in the Vasse River, and was observed eating parts of stolons during the present study.

Growth and competition

Waterlilies are known to inhabit still or slow-moving waters up to 2m deep (DiTomaso and Healy 2003 [cited in Hoftra et al. 2013]) with sufficient direct sunlight and organic substrates, though there are no published studies of growth limitations for *N. mexicana* or related species. *Nymphaea* are highly successful within their habitat mainly because they can colonise areas which are too deep for other emergent macrophytes, and have a competitive advantage over submerged plants for light (Capperino and Schneider 1985). Nutrient-rich, slow-flowing waters provide optimal growing conditions for *Nymphaea* species, and they have a competitive advantage in these waters owing to associated conditions of high turbidity and soft substrates which are not tolerated by submerged plants (Bornette and Puijalon 2011). They obtain their nutrients from the sediments, which under these conditions are generally not limiting (King et al. 2009).

Other mechanisms for competing with other emergent plants include competition for root space and nutrients and allelopathic effects. Allelopathy is the biochemical inhibition of the growth of one species by another (van Donk and van de Bund 2002). Allelopathically active chemicals include a range of compounds, which may be exuded from growing plants into the water column (Gross 2003), or produced during decomposition (Rice 1987), and act through inhibition of physiological processes such as photosynthesis. Allelopathic interactions through bioassays have been found for various species of submerged, floating and emergent species (Quayyum et al. 1999, Szczepanska 1987, Gross 2003). Allelopathic effects on phytoplankton have only been investigated for submerged macrophyte species, perhaps because emergent species are not considered to compete with phytoplankton for either light or nutrients (Gross 2003). Associations of floating leaved macrophytes with low chlorophyll *a* concentrations have been hypothesized to arise from allelopathic effects (Nemoto and Fukuhara 2012), but have not been proven. Allelopathic studies for Nymphaeaceae have found inhibition of lettuce seedlings by *Nuphar lutea* (Elakovich and Wooten 1991), and no inhibition of wild rice by *Nymphaea odorata* (Quayyum et al. 1999).

Colonisation of new areas is dependent on successful dispersal and establishment. Seed dispersal is considered the most likely mechanism for broader distribution, but dispersal of vegetative material may also be successful (Wiersema 1988). Successful establishment following dispersal is dependent

on favourable environmental factors for development of propagules and ongoing success of reproduction. In the Vasse River there has clearly been successful dispersal, and the conditions in this system present few limitations to growth. Substantial organic sediments are present throughout the river and there is little competition by other plants. Few emergent rushes extend below the low-water mark and in many parts the banks are sufficiently steep that there is available habitat completely across the river. Submerged plants grow in some years (author observations): during this study there was a large patch of *Potamogeton crispus* directly upstream of one large patch of lilies. Competition for root space between this species and *N. mexicana* is possible, however over time the shading caused by lilies is likely to outcompete *P. crispus.*

Shade is apparently a limiting factor, evidenced by the barrier to growth presented by the Strelly St bridge. While *N. mexicana* has spread substantially in other parts of the river, it has not spread downstream of this point since becoming widely established upstream during the 2013-14 summer (Figure 2). It does grow in partial shade in some parts of the river, and also in a relatively well-shaded stormwater basin further downstream, however the complete shading presented by the bridge appears to have been a successful barrier. Expansion of the population downstream has been relatively slow (less than 30m extension in 3 years), and dense phytoplankton growth during summer months may create sufficient shade to reduce waterlily growth here.

Water depth appears to influence growth mainly through the effects of drying. This is evident in seasonally dry and very shallow parts of the river where establishment is less successful. In some of these areas, seasonal growth of the native semi-aquatic plant *Persicara decipiens* occurs as the lilies begin to die back in response to low water levels. It appears that *P. decipiens* is also able to colonise new areas due to the root mass of *N. Mexicana* providing suitable substrate and reduced depth*.* Reduced flood scouring and sediment accumulation, as has occurred in the lower Vasse River, can lead to terrestrialisation. In shallow, slow flowing waters, diverse submerged macrophyte communities are progressively replaced with Nymphaeales, which are typical of clogged habitats, and finally succeeded by emergent marsh species (Bornette and Puijalon 2011). The growth of *P. decipiens* in the shallower areas colonised by waterlily may be indicative of this successional process in the Vasse River.

As with all water lilies (order Nymphaeales), *N. mexicana* is a freshwater plant (Borsch et al. 2008), and thus is not expected to tolerate high salinity. Freshwater angiosperms are considered unable to persist at consistent salinity of over 5ppt (Nielsen et al. 2008). Information available on the salinity tolerance of *N. mexicana,* and water lilies generally, is limited. It has been described as having a salinity range of 0-3.5ppt (Stutzenbaker 1999, cited in USACE 2011) but there are no published studies on its tolerance. Dalziell (2016) investigated the effects of salinity on germination and seedling growth of four native tropical northern Australian species of *Nymphaea.* Germination was reduced above 3ppt, but some species were able to germinate in salinity of up to 17ppt, and all showed some germination in freshwater after exposure to high salinity. Seedling growth was reduced by 50% above 10ppt.
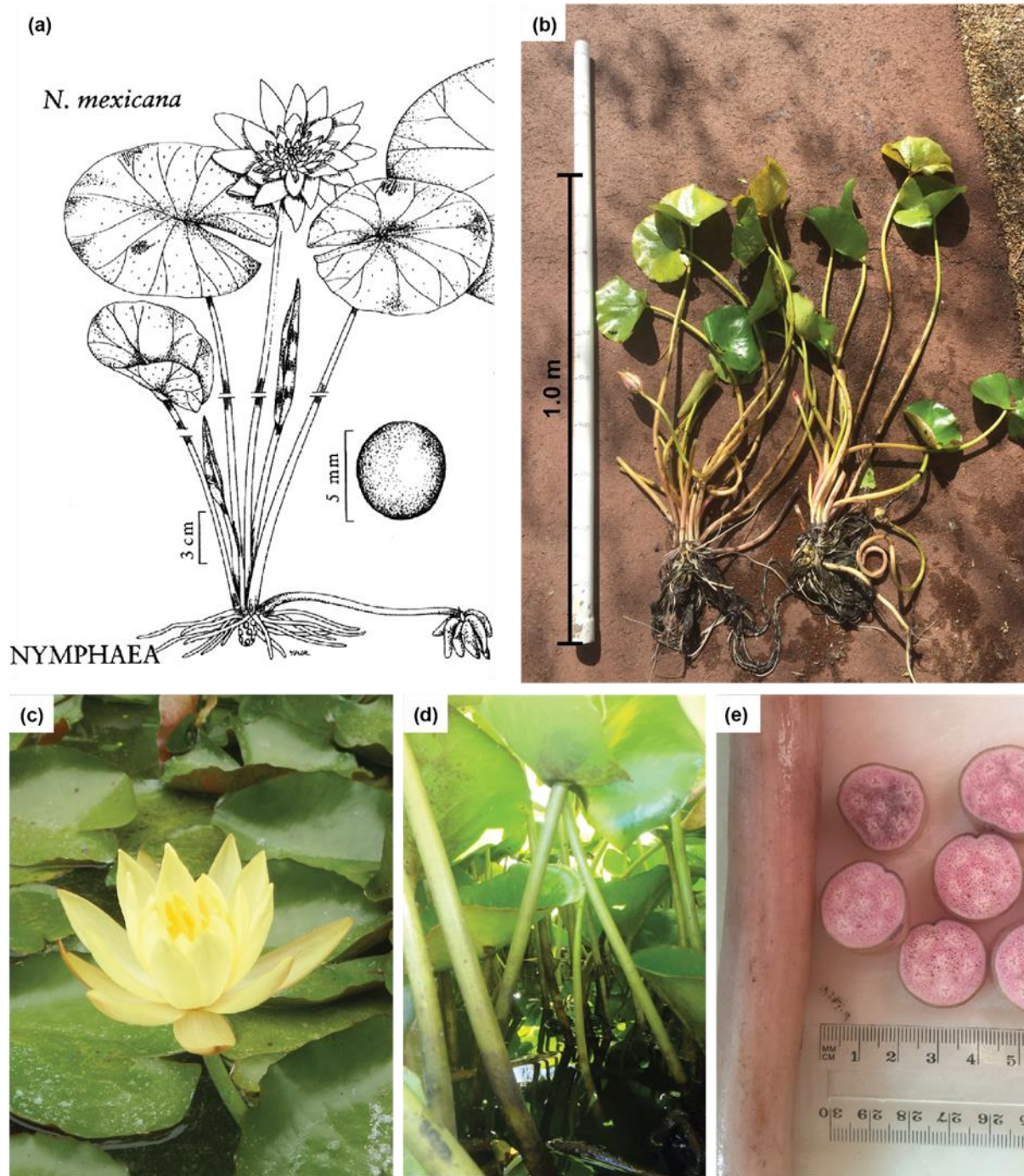
Figure 3. Morphological characteristics of Mexican waterlily *Nymphaea mexicana*: (a) diagram and (b) photo of whole plants, (c) flower, (d) dense leaves standing erect above water surface and (e) cross section of stolon showing and aerenchyma tissue. Diagram (a) from eFloras (2017).

Methods

Study area and sample sites

This study was undertaken in the lower Vasse River, between the "old railway bridge" and the Busselton Bypass bridge in Busselton, approximately 250km south of Perth in south-western Western Australia. The section of the Vasse River commonly referred to as the lower Vasse River is a 1.75km reach downstream of the interception with the Vasse Diversion through the township of Around 90% of the Vasse River catchment area is diverted directly to Geographe Bay via the Vasse Diversion to provide flood protection for the township of Busselton. The lower Vasse River has substantially reduced flow inputs and is impounded, resulting in a system resembling an elongated wetland during low rainfall months from spring to autumn. The study was undertaken from March to May 2017: water quality, plant and invertebrate sampling was completed from 2–17 March and sediment sampling was done from 2-4 May. Throughout the sampling period, a severe algal bloom was present in the area downstream of the waterlilies (cyanobacteria, blue-green algae).

The study area can be considered in three areas: the reach downstream of the waterlily infestation; the reach with extensive waterlily growth and waters between these stands; and the reach upstream. Four replicate sample sites of different site types were established within these areas Additional sites were located at the downstream and upstream edges of the reach with waterlilies and within an area where *P. decipiens* co-occurs with *N. mexicana.* Thus, there were eight different site types included in the study (Table 1, Figure 4).

Table 1. Description of different reaches and sample types in the study area.

River reach	Site type	Description	Number of sites
Downstream	Downstream	Area downstream of all waterlily growth	4
	Downstream edge	Interface of waterlilies and downstream area	1
Waterlily stands	Lilies	Within dense waterlily growth	4
	Open water	Large patches of water between waterlily stands (200-400m reaches)	4
	Pool	Small patches of water within and between waterlily stands (5-50m wide areas)	4
	Edge	Interface of open water and dense lily growth	4
	Persicaria decipiens	Area with both *P. decipiens* and *N. mexicana* (shown on results plots as *Pers. decip.*)	1
Upstream	Upstream	Area upstream of all waterlily growth	4
	Upstream edge	Interface of waterlilies and upstream area	1

Sampling Methods

Water Quality

Water quality sampling included in-situ measurement of physico-chemical profiles and secchi depth, and collection of depth-integrated samples for laboratory analysis of turbidity, nutrients (total and dissolved nitrogen and phosphorus), and chlorophyll (*a*, *b*, *c* and phaeophytin). A limited number of samples were also analysed for phytoplankton species composition and cell density by the Department of Water's Phytoplankton Ecology Unit: one sample from each site types downstream, lilies, open water, pools and upstream (site 1 for each type, Figure 4). These samples were preserved in Lugol's solution prior to analysis.

Depth-integrated water samples were collected at each site using a 45mm internal diameter clear plastic tube. Samples were submitted to the National Measurements Institute for laboratory analysis using standard analytical methods (NATA Accreditation No. 2474). Water quality sampling was completed between 2nd and 8th March 2017.

Temperature, dissolved oxygen, pH and conductivity at each site were measured at 0.2m intervals through the water column with a YSI ProDSS multiparameter sonde. Sampling was conducted at all sites over a one-week period between 10am and 2pm to reduce variation related to diurnal fluctuations of dissolved oxygen. Additional in-situ measurement of physico-chemical variables was done at each site in the early morning, between 5am and 7am during the same period (i.e. when photosynthesis, and dissolved oxygen is likely to be low). Further sampling of physico-chemical parameters was undertaken at 5m intervals along a transect through the stand of waterlilies located most upstream (vicinity of site WL4). Secchi depth was recorded at sites without plants.

Sediments

Sediment samples were taken from three of each of the following site types: downstream waterlilies (sites 3, 4 and part-way between), pools (1, 2, 4), open waters (1, 2, 4) and upstream (2, 3, 4). A core-sampler was used to obtain samples of the top 5cm for analysis of total nitrogen and total phosphorus, and the top 1cm for analysis of chlorophyll a and phaeophytin. Samples were submitted to ALS Water for analysis of sediment nutrients using standard methods (NATA Accreditation No. 825). Samples were sent to the Marine and Freshwater Research Laboratory for analysis of sediment chlorophyll a and phaeophytin (NATA 10603). Water depth and depth of flocculent sediments at sites without waterlilies was also measured with a measuring pole by measuring depth to the top of sediments and then pushing the pole through the soft sediments to the hard river bed.

Aquatic invertebrates

Aquatic invertebrate samples were collected by using a D-frame net (250μm mesh) to sweep for a 2-minute period in the vicinity of the main site types. Sweep sampling was not practical at the *P. decipiens* site because it was too shallow (<0.2m). Invertebrate samples were preserved in 70% ethanol prior to processing in the laboratory, where organisms were identified mostly to species level and counted using a dissecting microscope. Zooplankton were counted at the level of Copepoda and Cladocera; copepods were identified as Calanoida and Cyclopoida and Cladocera were identified to family level. Terrestrial beetle nymphs were present in some samples and not identified further, but were included in abundance results. Entire samples were analysed, with taxa present in very large numbers counted using volumetric subsampling. Subsampling involved

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suspension of the sample in 1L of water, thorough mixing and pouring off 25% or 50% of the sample; with repeated subsampling depending on magnitude of abundance. A minimum of 100 individuals was counted in subsamples.

Plant biomass and nutrient content

Biomass and plant nutrient samples were taken at each waterlily site and the *P. decipiens* site. At each of these sites, a plastic $0.25m^2$ quadrat (0.5m x 0.5m) was placed within the plants and all leaf and stem material cut at the base and removed. At one waterlily site below-ground material (roots, rhizomes, stolons) was also removed by digging into the sediment within the quadrat. Samples were placed in separate clean hessian bags and hung to air-dry for one week. Following air-drying, subsamples from each of these samples were taken by weight, further dried at 70°C for 48 hours, weighed and submitted for laboratory analysis of total nitrogen and total phosphorus. Plant nutrient analyses were completed by the Marine and Freshwater Research Laboratory (NATA 10603).

Data analysis

Comparison of water and sediment quality variables and invertebrate abundance and richness were made using one-way analysis of variance (ANOVA) with the single factor of site type (fixed, six levels: downstream, waterlilies, pools, open waters, edges and upstream), and four replicate sites within each site type. For physical variables, these comparisons included measurements at 0.2 and 0.4m. Tukey's and Games-Howell post-hoc tests were used to follow up significant ANOVAs to determine specific differences between each site type. All variables were checked for assumptions of normality and homogeneity of variance and when these were not met data were transformed ($Log_{10} x + 1$). Transformations were required for water and sediment nutrients, and invertebrate abundance. SPSS software was used to perform these analyses.

Aquatic invertebrate data was additionally analysed for differences in community assemblage using multivariate procedures in Primer-E: Analysis of similarity (ANOSIM) to test significance of between-site variation; similarity percentages (SIMPER) to explain dissimilarity; and multidimensional scaling to illustrate differences graphically.

Biomass and nutrient loading estimates were calculated using results from 0.25m quadrats and plant cover. Plant cover estimates from aerial photographs and GIS were provided by the Department of Water and Environmental Regulation.
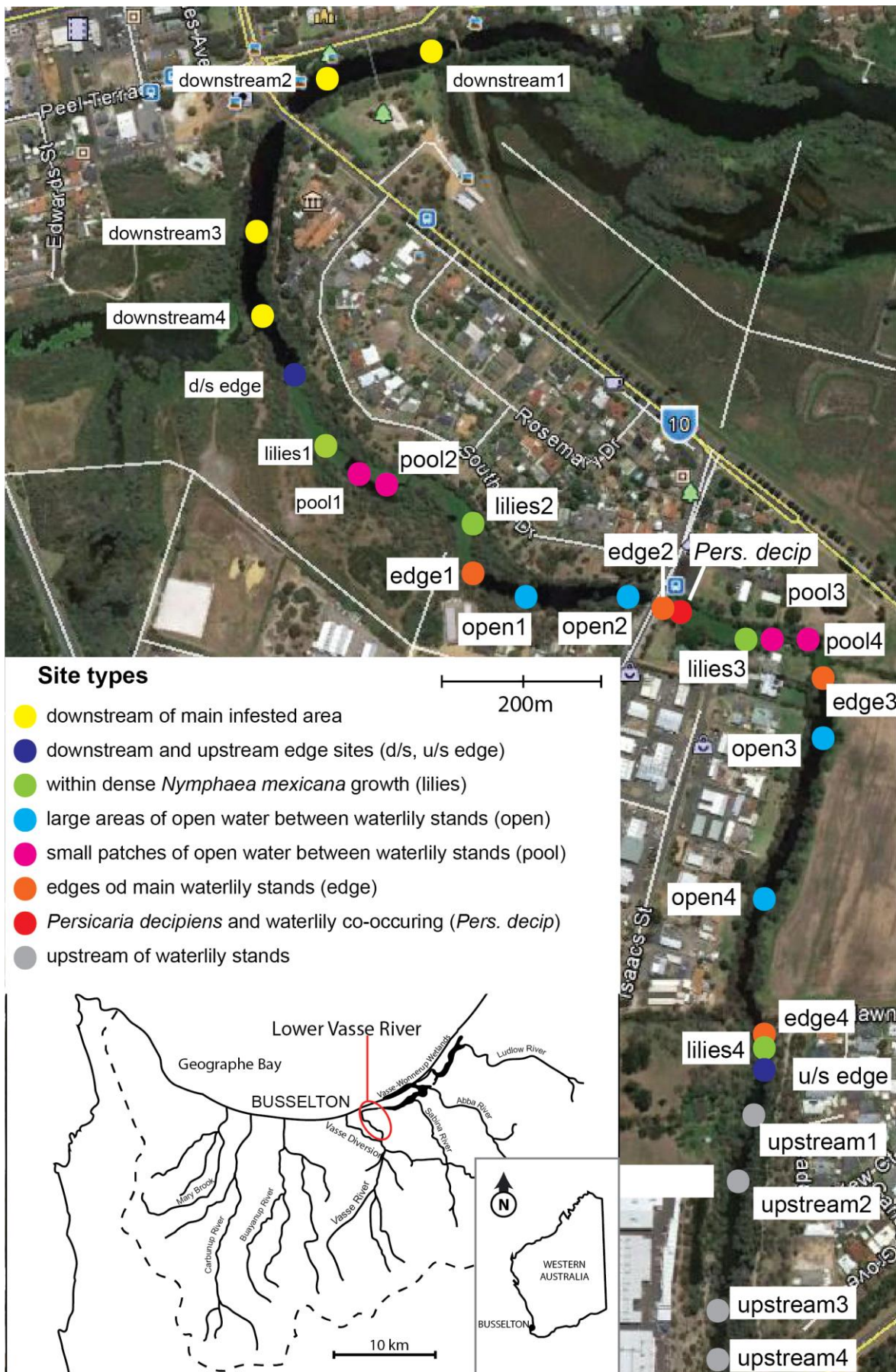
Figure 4. Study area and site locations for the project, 2017. GPS locations are provided in Appendix 2.

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Results

Water quality

Analysis of variance (ANOVA) found significant variation across sites for all water quality parameters (Table 2). Post hoc tests revealed the nature of between-site variation, and the significance of these tests are reported in the sections below.

Table 2. Results of ANOVA showing the overall effect of site type (downstream, waterlily, open water, pool, edge and upstream) on environmental variable measured in the study.

Variables		F statistic (df: 5, 18)	P value
Physico-chemical	Dissolved oxygen (0.2-0.4m)	10.25	<0.001
	Temperature	5.25	0.004
	Conductivity	46.4	<0.001
	pH	5.08	0.004
Nutrients	Total nitrogen	3.282	0.033
	Total phosphorus	23.44	<0.001
	Filterable reactive phosphorus	9.06	<0.001
	Total oxidised nitrogen	2.90	0.043
	Ammonium	22.68	<0.001
Water clarity	Turbidity	19.03	<0.001
	Chlorophyll a	9.34	<0.001
	Phaeophytin	11.97	<0.001

Oxygen

Extremely low concentrations occurred within waterlily stands, with measurements consistently below critical levels for aquatic fauna (2.0mg/L) throughout the water column during mid-day and dawn sampling (Figure 5). Post hoc testing revealed that oxygen levels for these sites were significantly lower than all other site types (P < 0.003). Water within the *Persicara decipiens* site was very shallow (<0.2m) and was anoxic during both mid-day and dawn sampling (Figure 5). Beneath the densely growing plants, aerobic respiration would deplete available oxygen, while photosynthesis would occur in emergent leaves and thus not replenish oxygen in the water.

In contrast, oxygen levels in the downstream reach were very high during mid-day sampling, particularly in the top 0.6m of water (Figure 5a), and significantly higher than other site types (P < 0.001) with the exception of open water sites. Concentrations in this area declined steadily with

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increasing depth but supersaturated conditions (>100%) persisted to 0.8m at three of these sites. These high oxygen values were doubtless a result of photosynthesis within the dense algal bloom in this reach. Dawn downstream oxygen levels were considerably lower than mid-day owing to overnight consumption via respiration, but remained above 5mg/L throughout most of the water column although bottom waters were anoxic (Figure 5b).

Mean dissolved oxygen was also very high in open water areas between lily patches: significantly higher than edge, lily and pool sites ($P < 0.001$) and upstream sites ($P = 0.016$). The highest oxygen concentrations in the study area occurred at one open water site close to an extensive meadow of the submerged plant *Potamogeton crispus* (19mg/L; >200%), which also contributes oxygen to the water column through photosynthesis. Upstream dissolved oxygen values were more 'normal'[1] (Figure 5a). Pool and edge sites showed the greatest stratification over depth, with mid-day oxygen levels at these sites moderate at the surface and very low from 0.4m to the bottom. Low oxygen is likely caused by oxygen consumption within the lilies and sediments. This was also evident at the upstream edge site. Dissolved oxygen during dawn sampling was consistently lower for all these site types, particularly in the pools (Figure 5b).

Water quality sampling for the study was undertaken during calm, sunny conditions, perhaps exacerbating the effects of deoxygenation within the lilies. An additional transect of dissolved oxygen was completed through the most upstream stand of *N. mexicana*. This stand was growing from bank to bank for 45m along the river (lilies site 4). The sampling was undertaken during windy conditions. Surface dissolved oxygen in the lilies was higher on this day (mean 6.7mg/L at 0.2m depth) compared with point sampling (2.4mg/L at 0.2m), particularly from the upstream edge to 10m inside the lily plants (Figure 6). Very low dissolved oxygen levels persisted in bottom waters and extended for at least 10m into the downstream open water area. These results suggest potential for replenishment of oxygen in the lily stand through mixing and under windier condition.

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[1] The saturation concentration of oxygen in freshwater at 20°C is around 9mg/L (Boulton *et al.* 2014)

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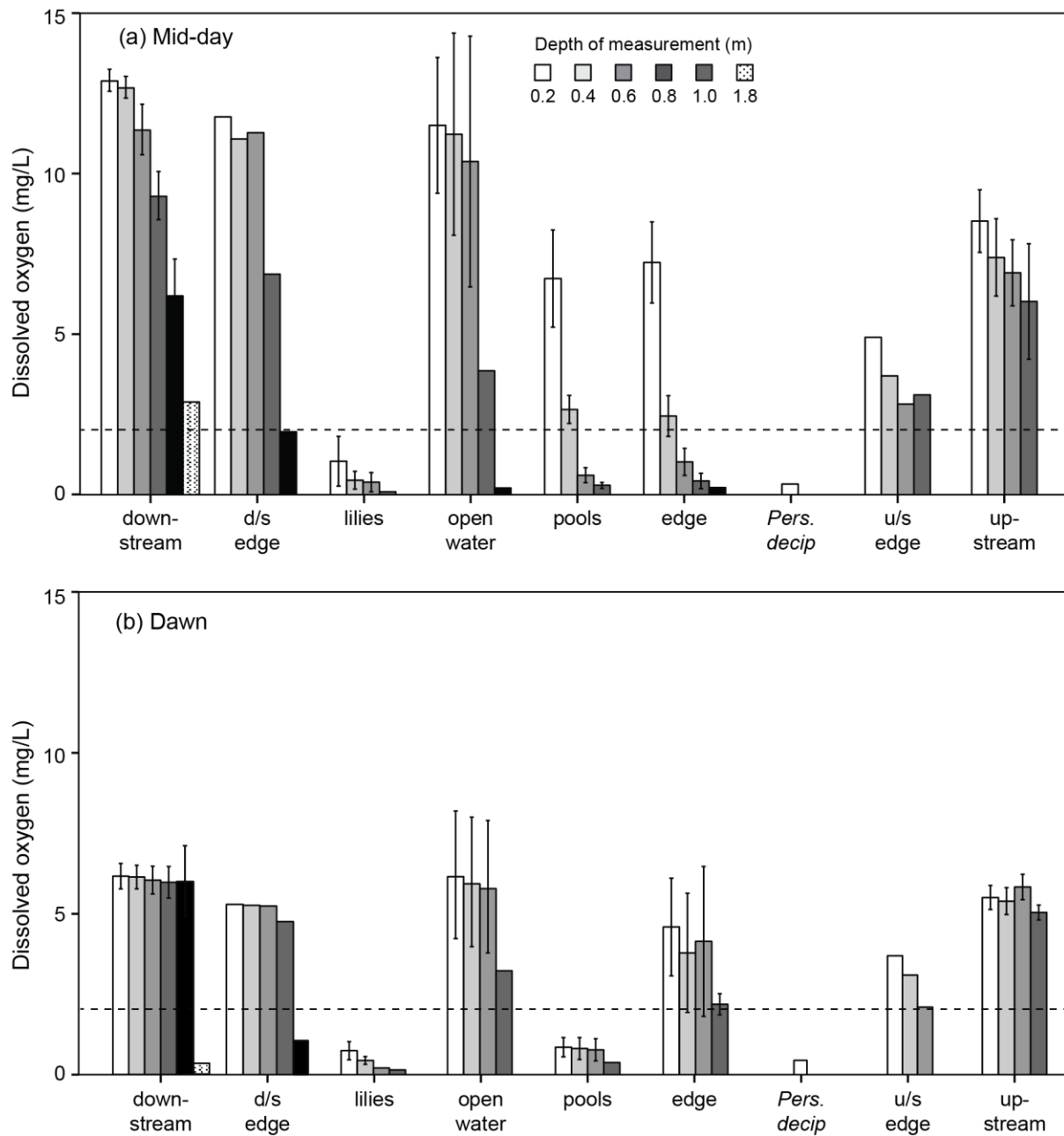
Figure 5. Mean concentrations of dissolved oxygen at different site types in the study area during (a) mid-day sampling and (b) dawn sampling, showing varying values with depth. Dashed lines indicate the critical level for aquatic fauna (2.0mg/L: Boulton et al. 2014). Error bars are +/- one standard error (n=4).
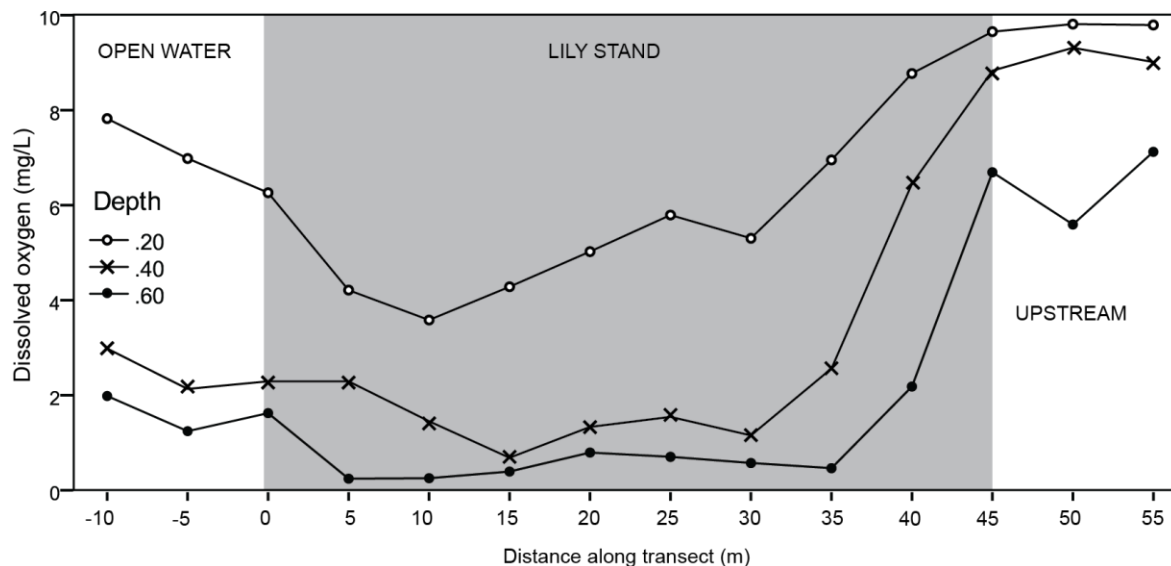
Figure 6. Dissolved oxygen concentrations in the water column across a transect through a 45m stand of *N. mexicana*. Shaded area shows the lily stand (0-45m). Transect location at waterlily site 4 (lilies4, Figure 4).

Other physico-chemical variables

Surface water temperatures ranged from 21 – 28°C in the study area, with the warmest conditions found in the shallowest open water sites and the coolest conditions beneath the lilies and at the *P. decipiens* site (Figure 7a). Significant variation in temperature between site types ($F_{5,18}$ = 5.25, P = 0.004) was mainly owing to cooler temperatures within the lilies ($P \leq 0.03$), where shading would have an effect. Minor stratification was apparent in the reduction of temperature with increasing depth at most sites. Lower temperatures of bottom waters in edge and pool sites may be an effect of shading (edges) and mixing with cooler waters within the lily stands (pools). Downstream sites were warmer than upstream sites, which have more riparian shading because the river is narrower in the upstream reach.

Conductivity was lower in the downstream reach compared with other sites (Figure 7b), with an increase in mean surface conductivity from 1284 μS/cm at downstream sites to 1541μS/cm at lily site WL1. This was despite cooler temperatures suggesting lower potential for evapoconcentration of salts. From this point of notable change, conductivity generally increased with increasing distance upstream (Figure 8). There was a significant effect of site type on conductivity ($F_{5,18}$ = 46.4, P < 0.001), and post hoc tests did show downstream sites to have lower conductivity than all other site types (P <0.001).

Results for pH ranged from 6.4 to 9.5, with higher values for downstream sites (P < 0.001) and in surface waters generally, with stratification evident in most sites (Figure 7c). This is likely associated with higher levels of photosynthesis in the algal bloom downstream and in surface waters at some sites, which reduces the concentration of dissolved CO_2. The waterlily sites had the lowest pH (6.78 – 7.23, Figure 7), with low potential for photosynthesis by algae due to a lack of sunlight reaching the water.

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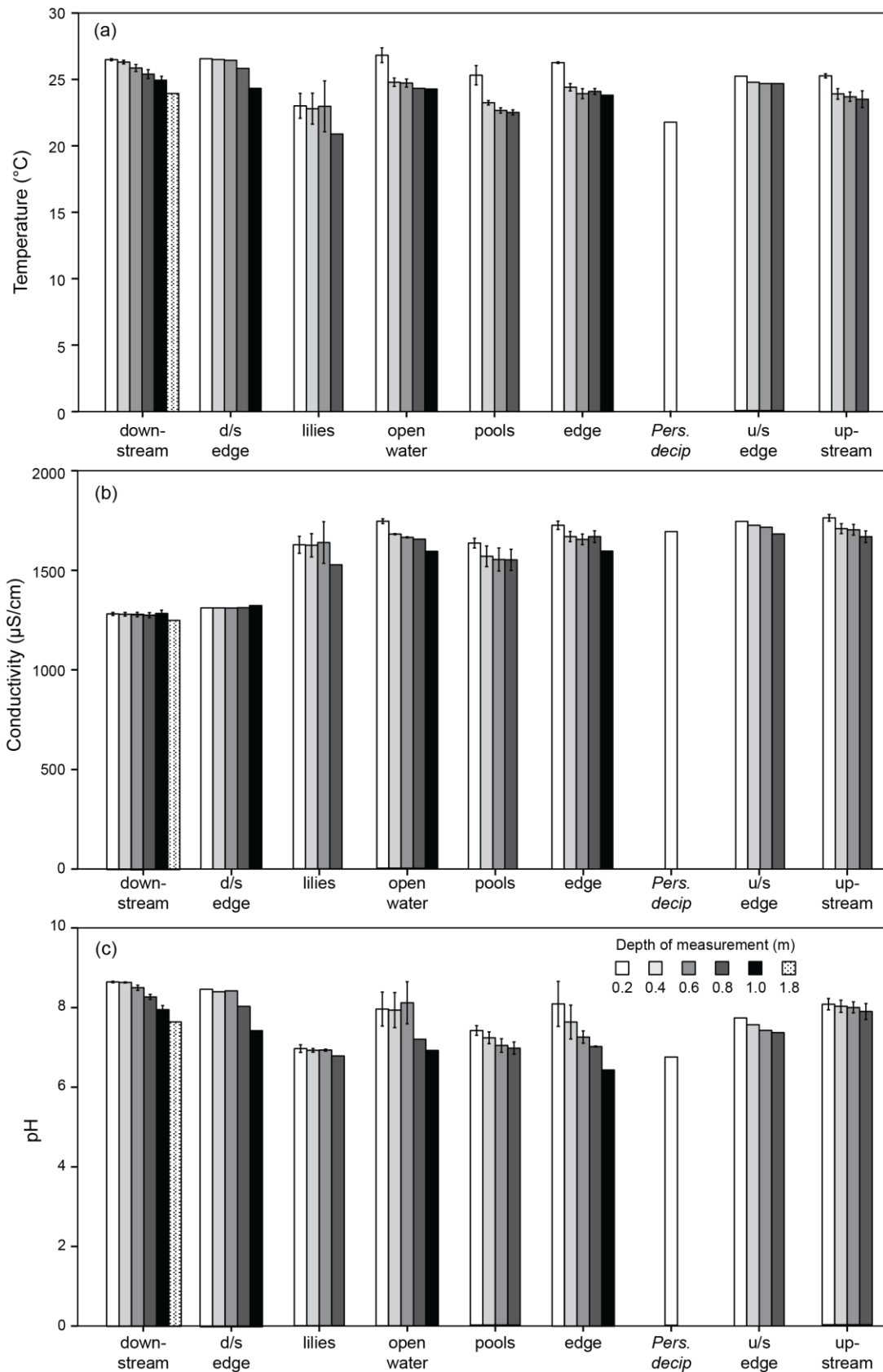
Figure 7. Mean values for in-situ water column profile measurements of (a) temperature, (b) conductivity and (c) pH for different site types in the study areas. Error bars show +/- one standard error (n=4).
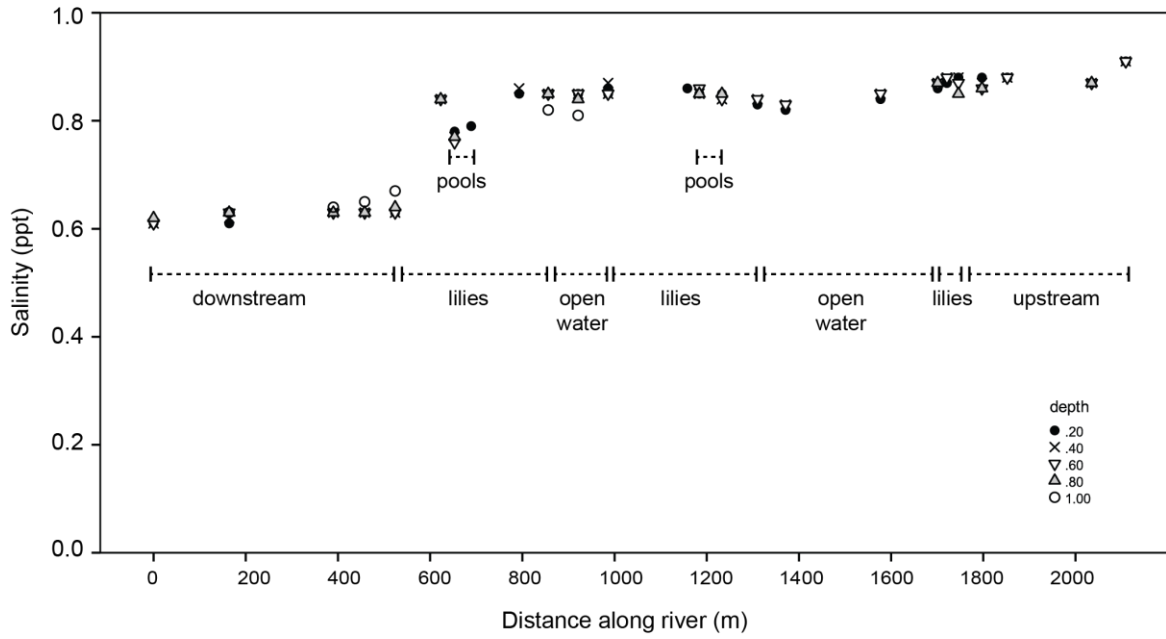
Figure 8. Salinity measurements at each site in order of increasing distance upstream. Measurements over 6 days under stable weather conditions from 1/03/2017 – 7/03/2017.

Water column nutrients

Total phosphorus (TP) concentrations were very high throughout the study area, with all samples greatly exceeding the guideline for ecosystem protection (0.06mg/L)[2], but were much higher at all sites upstream of the main infestation (Figure 9a). Sites downstream of the infestation had a mean TP concentration of 0.17mg/L, which was significantly lower than all other site types ($P \leq 0.033$). Sites among the lily populations (within lilies, open water, pools and edges) had extremely high TP, with mean values greater than 0.6mg/L. Small pools within the lily patches had significantly higher TP than downstream and upstream sites ($P \leq 0.001$), with a mean concentration of 0.88mg/L.

Filterable reactive phosphorus (FRP) concentrations were also much higher for sites among and upstream of the lilies (0.13-0.47 mg/L) compared with downstream sites (≤ 0.014mg/L) (Figure 9a) ($P \leq 0.044$). FRP in downstream sites can be attributed to all available phosphorus being held in phytoplankton cells within the dense algal bloom in this section of the river, and thus measurable as TP. However, this was not the case for other sites, where FRP concentrations were consistently much higher than the ecosystem protection guideline of 0.03 mg/L[3]. Indeed, FRP concentrations for sites among the lilies exceeded TP concentrations downstream (Figure 9a). The proportion of available phosphorus (FRP component of TP) was also considerably lower in downstream sites (mean 4.0%) compared with other sites. Sites within the lilies and at the edges of the lilies had the highest proportion of FRP (means of 47% and 45% respectively). Mean proportions of FRP in open water, pool and upstream sites were 38%, 38% and 33% respectively. So, while almost all available phosphorus in the downstream reach was being taken up by phytoplankton, this was not the case for other sites.

In contrast to phosphorus, total nitrogen (TN) was slightly lower (but not significantly) for sites among the lilies compared with downstream, with the exception of pool sites (Figure 9b). Only edge and pool sites differed significantly in TN ($P = 0.049$), having the highest and lowest TN concentrations respectively. Dissolved inorganic nitrogen - ammonium and nitrate/nitrite - were very low, consistently below guidelines for ecosystem protection[3] and often below detection limits (Figure 9c). Interestingly, oxidised nitrogen (nitrates and nitrites) were only detected at the downstream edge of the lily infestation and at the edges of lily patches, although in small amounts only. Ammonium was present at low levels for most of the study area, but not in the downstream area or within lily sites. Ammonium and nitrate/nitrite do not necessarily represent all available nitrogen, because dissolved organic nitrogen (particularly urea) is also available to phytoplankton. Because TN values were of a similar magnitude throughout the study area but TP values were much higher in sites among and upstream of waterlily patches, the TN:TP ratios for these areas differed substantially. Downstream sites had a mean TN:TP of 28.2 while values for other site types ranged from only 4.5 - 8.9 (molar mass ratio).

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[2] Ecosystem protection guideline for wetlands in south-western Australia (ANZECC and ARMCANZ 2000)
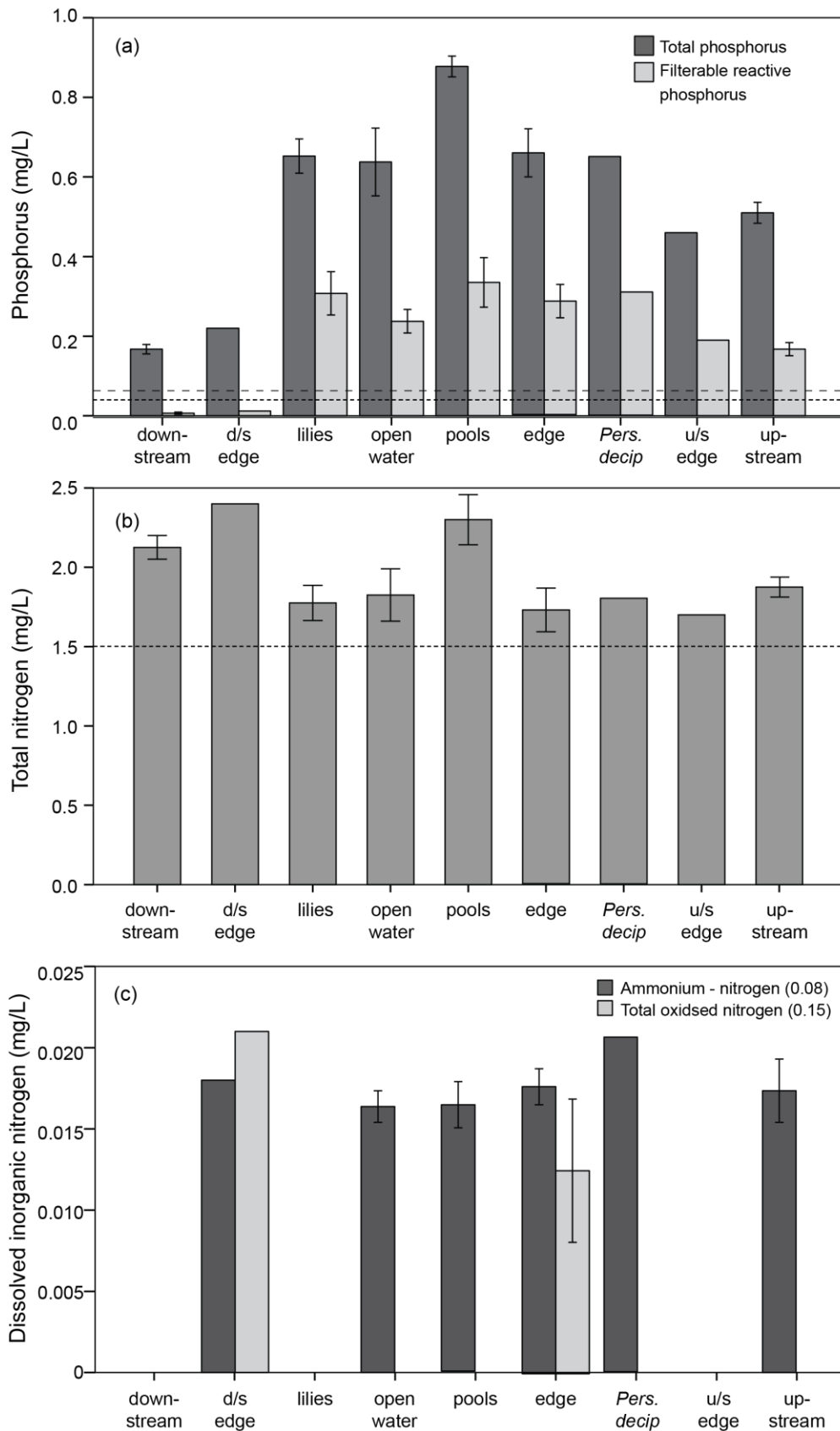
Figure 9. Mean concentrations of (a) phosphorus, (b) total nitrogen and (c) dissolved nitrogen at different site types in the study area. Dashed lines indicate ecosystem protection guidelines1: in (a) larger dashes show TP guideline, smaller dashes show FRP guideline; in (c) guidelines are shown in brackets as these values exceed the scale values. Error bars are +/- one standard error (n=4).

Water clarity

Water clarity was lower in the reach downstream of waterlily growth, with higher turbidity ($P \leq$ 0.042) and lower secchi depth than all other sites (Figure 10a, b). Higher turbidity in the downstream area was likely owing to the presence of a dense algal bloom, obvious by green discolouration of the water, which was not observed at other sites. The combined conditions of lower turbidity and shallower water depth in areas among and upstream of the lilies (Figure 10b) means light penetration through a much greater proportion of the water column, and in several cases the secchi disc was visible on the bottom. This has implications for growth of benthic algae, presented with the results of sediment sampling.
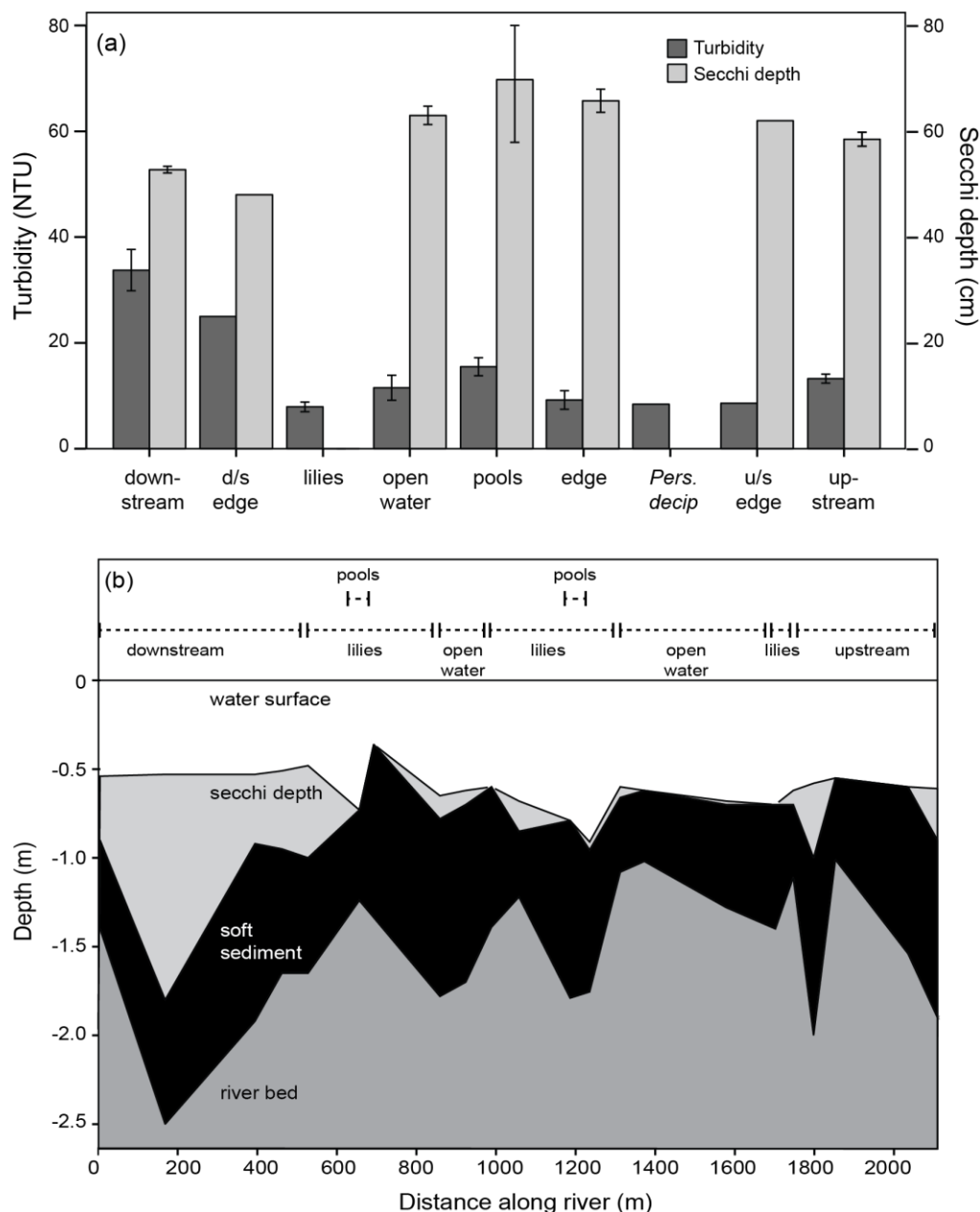
Figure 10. Water clarity for different site types in the study area indicated by turbidity and secchi depth (a); and water clarity in relation to depth and sediment layers (b). Error bars in (a) are +/- one standard error (n=4).

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Phytoplankton

Chlorophyll *a* concentrations were substantially higher in the downstream reach (0.045-0.085 mg/L) than sites within and between waterlily stands (0.005-0.036 mg/L) ($P \leq 0.002$, Figure 11a). Upstream of the waterlilies, chlorophyll *a* concentrations were somewhat higher than for site types among waterlilies (Figure 11a), although significant only compared with pools ($P = 0.005$). The very high result for chlorophyll a at the downstream edge of the lilies is attributed to an obvious accumulation of algal scum at this site, and concentrations at individual sites decreased downstream of this point. Such accumulation of chlorophyll *a* was not observed at the upstream edge site, although accumulation of debris was notable. Surprisingly, chlorophyll *a* was lower in open water and pools among lily patches than in waters within the lilies (though not significantly), which would have less available light for phytoplankton growth owing to shading by leaves.

Concentrations of chlorophyll *b* and *c* were also higher upstream and downstream of the lily stands. Phaeophytin was often higher in sites other than downstream, and represented a greater proportion of total pigments (Figure 10a). Phaeophytin is produced by digestion of chlorophyll (eg. by herbivores) and can lead to an overestimate of chlorophyll pigments in samples (Fuchs et al. 2002). So, active photosynthetic activity for sites within and upstream of waterlilies may be lower than chlorophyll results suggest, because higher phaeophytin may be included in the chlorophyll a estimate.

However, it is important to note that cell density does not equate to biomass owing to substantial variation in cell size between species, and within species at different stages of growth (Bellinger and Sigee 2010).

Phytoplankton species and cell density analysis indicated cyanobacteria (blue-green algae) were a main component of the phytoplankton community in the downstream reach, within waterlilies and in open waters (Figure 11b). The species of cyanobacteria present differed: *Anabaena spiroides* and *Oscillatoria sp.* were dominant in the downstream sample; *Oscillatoria sp.* and *Pseudoanabaena limnetica* were dominant in the waterlily sample; and *Aphanocapsa sp.* (non-nitrogen fixing) was dominant in the open water sample (Figure 11b). Reported cell densities for these three sites were below the recreational health guidelines of 15,000-20,000 cells per mL. For the downstream site, the total cell density of 14,579 (54% cyanobacteria) was not consistent with the dense algal bloom visible at this site, and did not reflect the very high chlorophyll concentrations in the downstream area. This is difficult to explain, and suggests a problem with this sample. The Department of Water and Environmental Regulation routinely samples phytoplankton near this downstream site (at the old rail bridge), and this monitoring has shown persistence of a severe cyanobacterial bloom from January to April 2017 (Figure 12a). It also shows a short-lived bloom of the non-nitrogen-fixing cyanobacteria *Aphanocapsa* at Strelly St bridge (Figure 12b), which is consistent with the presence of this species in the nearby open water site in this study.

Pool and Upstream samples also contained cyanobacteria, though at much lower densities than for other site types (Figure 11b). The pool sample had the lowest cell density and was dominated by Chlorophyta (green algae, seven species), Cryptophyta (*Cryptomonas*) and Euglenophyta (*Euglena*). The open water sample was dominated by green algae, predominantly very small *Crucigenia*.

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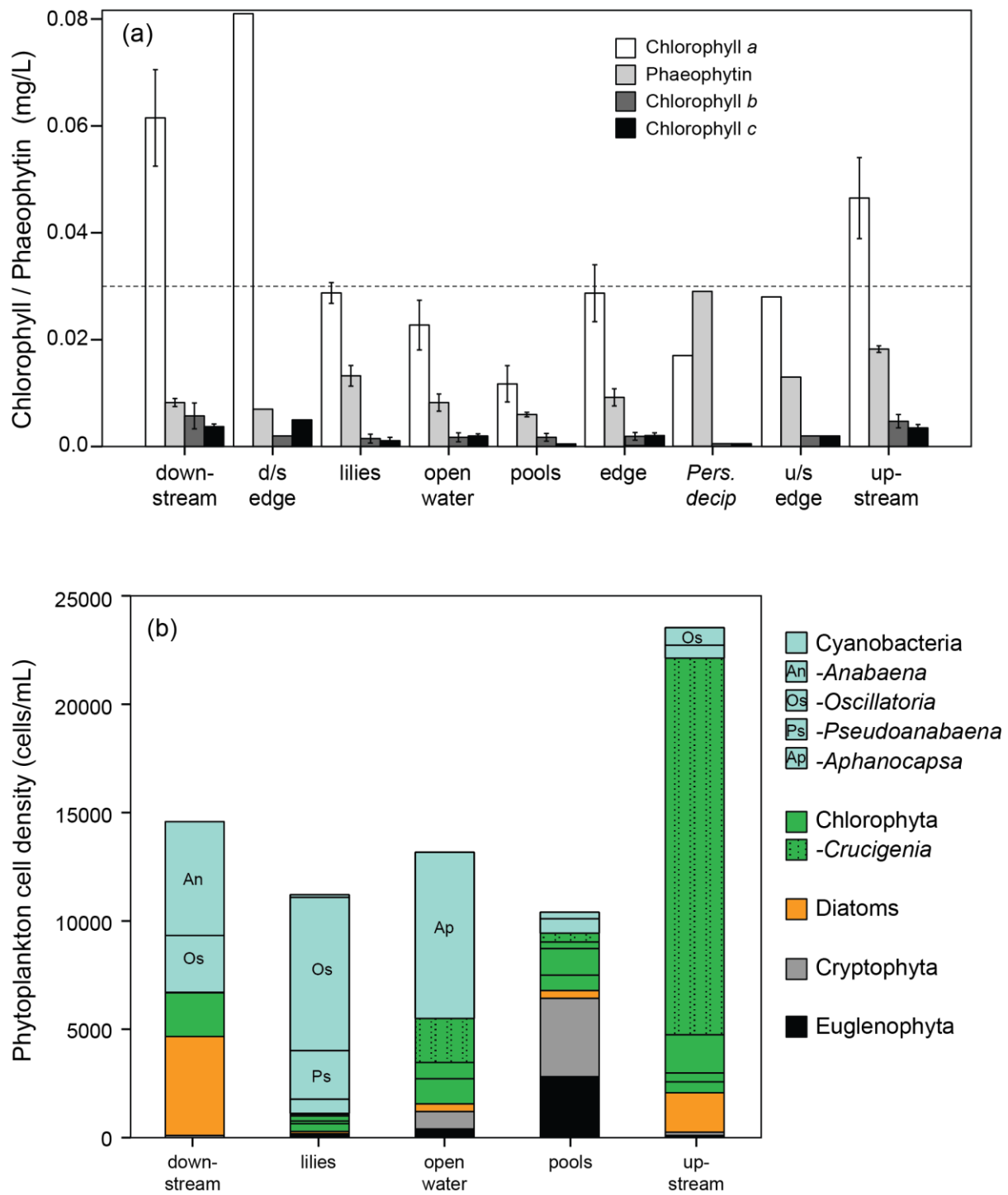
Figure 11. Phytoplankton levels in the study area indicated by chlorophyll concentration (a) and cell counts (b). Error bars in (a) are +/- one standard error (n=4).
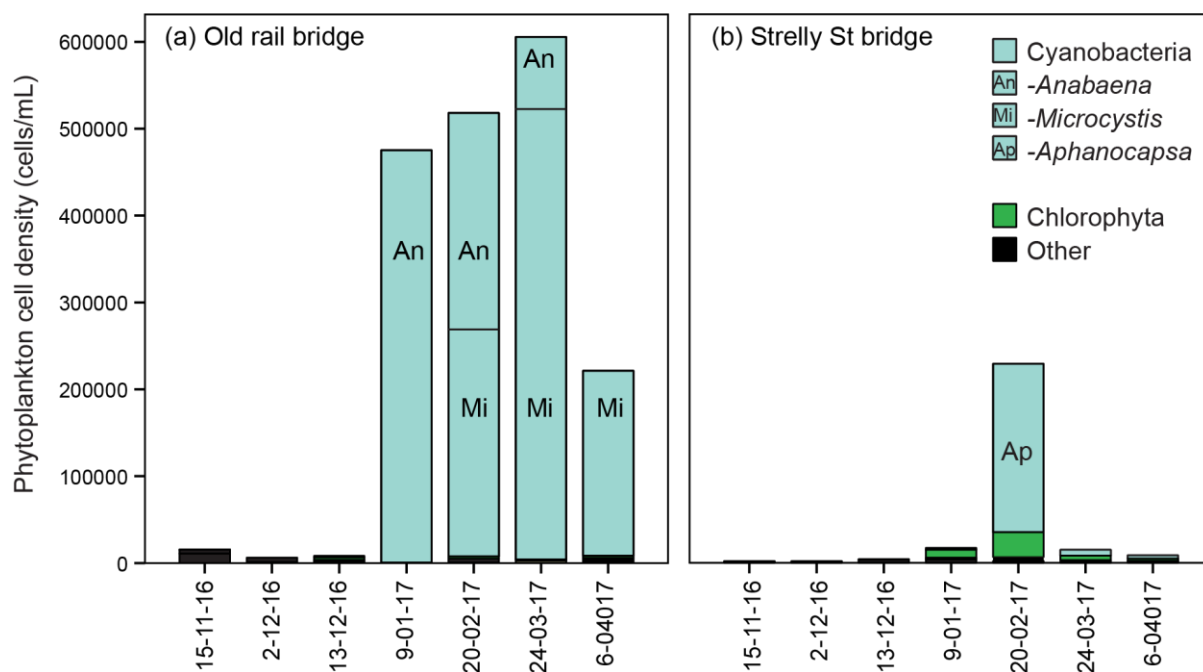
Figure 12. Phytoplankton cell density and community structure from Department of Water and Environmental Regulation routine water quality monitoring November 2016 to April 2017. The old rail bridge site (a) is close to downstream site 1 in the waterlily study; the Strelly St bridge site (b) is the same as waterlily edge site 2.

Sediments

Sediment nutrient content varied considerably between sites, with lower concentrations of phosphorus and nitrogen in sediments beneath lilies and in pools within lilies compared with the downstream reach and open water areas between patches of lilies (Figure 13a). While post hoc tests did not show lily or pool sites to be significantly lower than others, the data for lily sites was skewed by very high TN and TP at one site 5 metres from the downstream edge of the lilies (due to access difficulties). For the other two lily sites, mean TN and TP were 2.8 g/kg and 0.26g/kg respectively; substantially lower than the combined means for other sites of 10.5 g/kg TN and 1.4 g/kg TP. This may be the result of nutrient uptake by the plants, but accumulation of decomposing plant material within the sediments would also affect sediment-nutrient levels. Sediment phosphorus may also be influenced by release of phosphorus into the overlying water column from anoxic sediments. During sampling, a strong smell of hydrogen sulphide at lily sites when sediment was disturbed indicated sulphate reduction under anoxic conditions. Upstream sediment phosphorus was high and variable.

Although sites were somewhat shallower upstream from the point of lily infestation, depth of the flocculent layer of sediments was similar (Figure 10c). Depth of soft sediments could not be measured accurately within patches of lilies, however these sites had the shallowest average depth owing to the formation of substantial root mass beneath the plants.

Sediment chlorophyll and phaeophytin could not be sampled within the lilies as it was not possible to extract a surface layer of sediment at these sites. Concentrations of chlorophyll *a* were higher in surface sediments of open water sites, pools and upstream sites compared with downstream sites (Figure 13b), which is likely due to increased light reaching the bottom at these shallower, clearer sites. This was particularly evident in pool sites, where several samples had a dense benthic algal

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mat present (Figure 14a), and these sites had significantly higher chlorophyll *a* compared with other site types ($P \leq 0.034$). Phaeophytin was also high in sediment samples, which may reflect build-up of phytoplankton waste material over time. Filamentous algae was observed in open water and upstream samples (Figure 14b), resulting in elevated pigments in samples for these sites. In downstream sites, pigment levels can be attributed to blue-green algal colonies on surface of sediment samples. As the sediment was pushed up through the corer to obtain the sample, some of these colonies from the water column settled on the sediment.
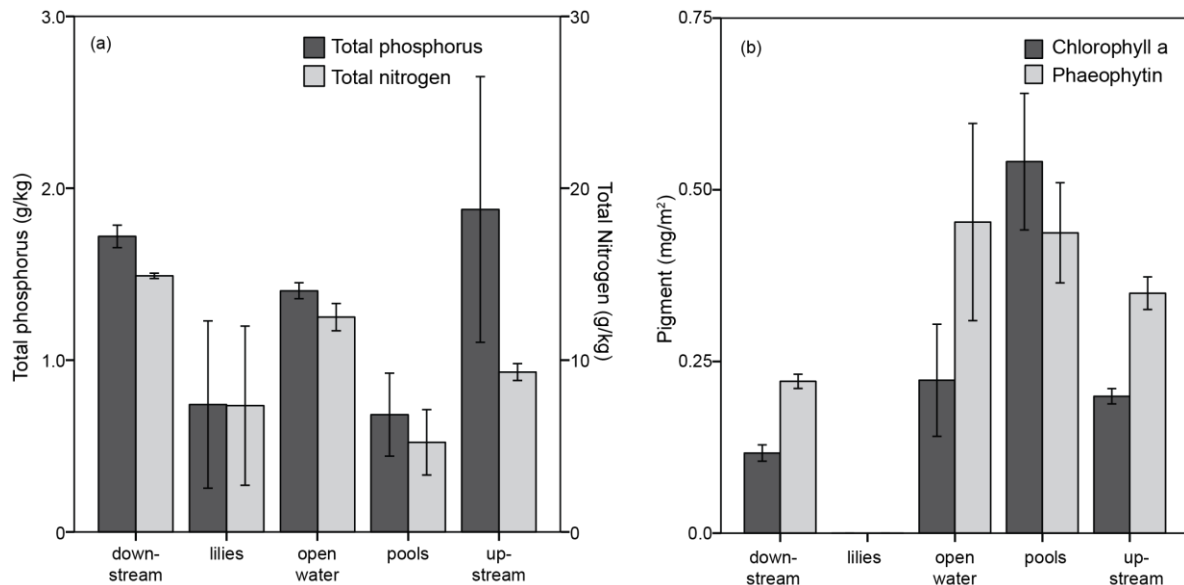
Figure 13. Sediment properties of different site types in the study area: (a) mean total phosphorus (y-axis) and total nitrogen (z-axis) in top 5cm of sediment; and (b) mean chlorophyll *a* and phaeophytin concentrations in top 1cm of sediment. Error bars are +/- one standard error (n=3). Note that photosynthetic pigments were not sampled for lily sites, because it was not possible to obtain a surface layer sample.

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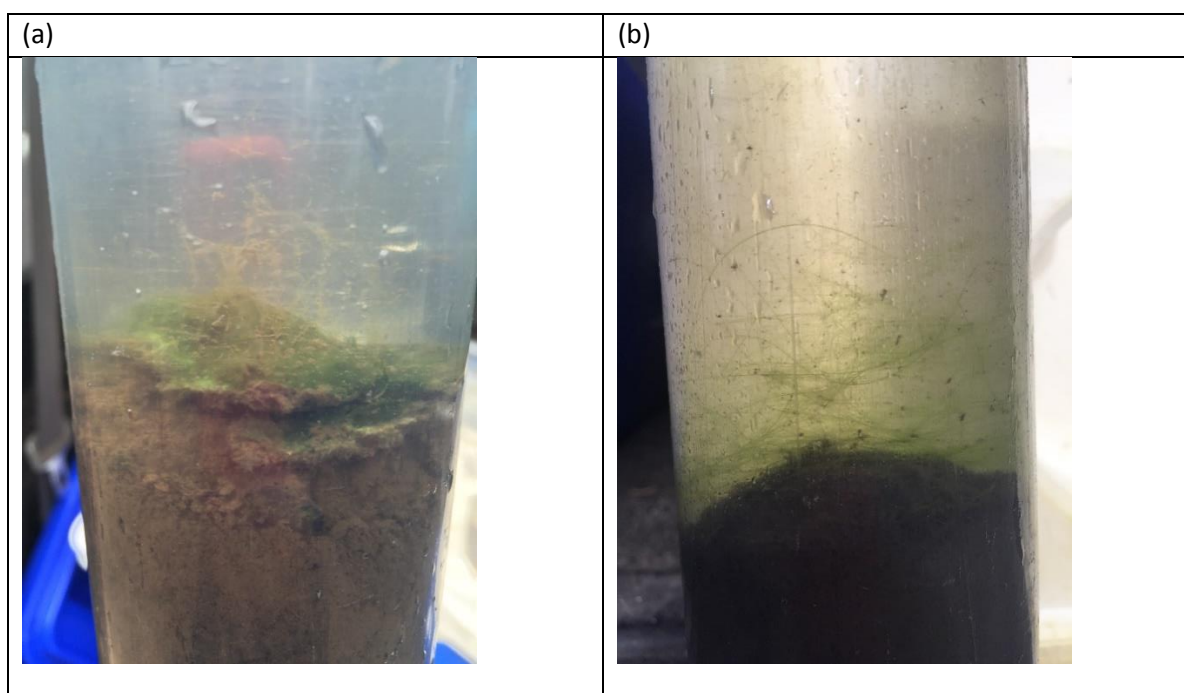
Figure 14. Benthic algae on the surface of sediment core samples from pool sites (a) and filamentous algae observed in open water and upstream sites (b).

Plant biomass and nutrient content

Aerial photography from February 2017 showed a total cover of *N. mexicana* of 11,409m². Plant biomass and nutrient data are provided in Appendix 3. Biomass results ranged from 461g/m² (dry weight) to 992g/m² for leaf material and stem (mean 732 g/m²), giving an estimated total above ground standing crop at the time of sampling of 8.4 tonnes (dry weight). The single root sample had a biomass of 1400 g/m². Although limited by a lack of replication, this suggests greater biomass in the root material than in above ground material. At the site with *Persicaria decipiens* growing amongst the lilies, above ground biomass of *P. decipiens* was at a similar level to the average for lily material in other sites (688.4g/m²), while lily biomass was much lower (72g/m²). This site was very shallow (0.2m) and low (senescing) biomass of *N. mexicana* was also observed in shallow waters without *P. decipiens.* So lower lily biomass at this site may be due to shallow conditions rather than competition with *P. decipiens*; or a combination of both.

Mean leaf/stem nutrient content of the lilies was 3.9mg/g phosphorus and 22.75mg/g nitrogen, with little variation between samples. Only one root sample was analysed, which contained 3.8mg/g phosphorus and 10.0mg/g nitrogen. At the *P. decipiens* site, leaf/stem nutrient content of the lilies was slightly higher: 5mg/g phosphorus ad 35mg/g nitrogen. Nutrient content of *P. decipiens* (above ground) was 3.7mg/g phosphorus and 14.0mg/g nitrogen. Notwithstanding the limitations of having only one root sample, estimates based on the results from this study indicate approximately 93kg of phosphorus and 350kg of nitrogen in the standing crop of waterlily (Table 3). If this were completely removed, it would equate to about 2% of the annual phosphorus load and 1% of the annual nitrogen load in waters of the Lower Vasse River.

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Table 3. Nutrient content estimates of *Nymphaea mexicana* in the lower Vasse River, based on sampling in March and Cover in February 2017 (11,409m^2), with comparisons to annual nutrient loads of the lower Vasse River (LVR).

	Biomass (dry)		Phosphorus			Nitrogen		
	g/m2	total T	mg/g	g/m2	total kg	mg/g	g/m2	total kg
Leaf and stem	732	8.35	3.9	2.9	32.6	22.8	16.7	190.0
Root	1400	15.97	3.8	5.3	60.7	10.0	14	159.7
Total	2132	24.32			93.3			349.7
LVR annual load*					4,720			33,800

*Department of Water (2010)

Invertebrates

Zooplankton[3] abundance varied greatly within and between site types (Figure 15a), with substantially more Copepods than Cladocerans. These groups are primary consumers, and are often most abundant when algae is available as a food source and there is little habitat to support predators. Cladocerans (water fleas) have greater potential to exert grazing pressure on phytoplankton, and abundance often cycles with phytoplankton density. This group was present in samples from two downstream sites with high abundance (Daphniidae; mean 3750 individuals per sample), and at low abundance at four other sites. Abundance was highest in downstream sites than other site types, while the lowest abundance was found within and at the edges of waterlilies (Figure 15a). Significant variation across site types ($F_{5,18}$ = 5.2, *P* = 0.004) was a result of significantly higher abundance in the downstream area compared with water lily, edge and pool sites (*P* = 0.008, 0.009, 0.027, respectively).

A total of 41 species of aquatic invertebrate species were present in samples from the study area, with a maximum of 19 per sample. Chironomidae midge larvae was the most common and diverse group with 9 species found. Chironomids are often the dominant taxa in nutrient-enriched wetlands (Davis and Christidis 1997). There was significant variation in species richness across site types ($F_{5,18}$ = 4.6, *P* = 0.007), owing to higher numbers of taxa found in waterlily stands and at the edges compared with all other site types (*P* = 0.009 and *P* = 0.002 respectively) (Figure 15b). Other site types did not differ significantly in species richness. This was surprising given the low levels of dissolved oxygen within the waterlilies, nonetheless the structural habitat provided by these plants appears to support greater diversity.

Samples from within waterlilies also had significantly higher mean macroinvertebrate abundance than all other site types (Figure 15c, *P. = 0.002*). Four main groups accounted for 96% of abundance in these sites: Ostracoda (38%), Oligochaete worms (29%), unidentified terrestrial nymphs (18%) and Chironomidae (11%) (Figure 15c). These groups also accounted for 67% of species richness. Ostracods are often associated with aquatic plants and are tolerant of low oxygen conditions, and Oligochaetes are mainly detritivores inhabiting sediments. Also notable during sampling were terrestrial spiders and frogs among emergent leaves of waterlilies. Chironomidae and Ostracoda

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[3] Zooplankton for this study included microcrustaceans Copepoda and Cladocera.

were dominant taxa in all site types and Corixidae (water boatmen) were also common, but abundance of these open water predators was notably lower in waterlily sites (Figure 15c). High numbers of Coenagrionidae damselfly larvae (Order Zygoptera) at one edge site is likely associated with the presence of a *P. crispus* meadow, as this group is often associated with submerged vegetation. The site types all differed substantially in taxa present at low abundance. One Cladocera family (Chyoridae) and one ostracod species (*Alboa worooa*) were found only in waterlily sites, and Leptoceridae caddisfly larvae were present at one lily site and two edge sites. Taxa restricted to waterlily and edge sites, though in very low numbers, included several that are indicative of improved structural habitat: Aeshnidae dragonfly larvae, Ceratopogonidae biting midge larvae, and Hydroptilidae caddisfly larvae (Chessman 2003).

Multivariate analyses of invertebrate abundance data further showed significant difference in invertebrate community assemblage between waterlily sites and other site types. Analysis of similarity (ANOSIM) found an overall significant effect of site type (R = 0.424, *P* < 0.001) and pairwise tests indicated that this was primarily owing to differences between waterlily and each other site type (Table 4). Assemblages of edge and upstream sites also differed significantly, but other site comparisons found no difference (Table 4). The distinct invertebrate assemblage in lily sites was additionally evident in multidimensional scaling, which also indicated separation of edge sites and some similarity between open water and upstream communities (Figure 15d). Analysis of similarity percentages (SIMPER) showed that higher abundance of oligochaetes, terrestrial nymphs, and ostracods, and lower abundance of Copepoda and Corixidae accounted for 41.4 - 50.5% of dissimilarity between invertebrate assemblage in lily sites and other sites.
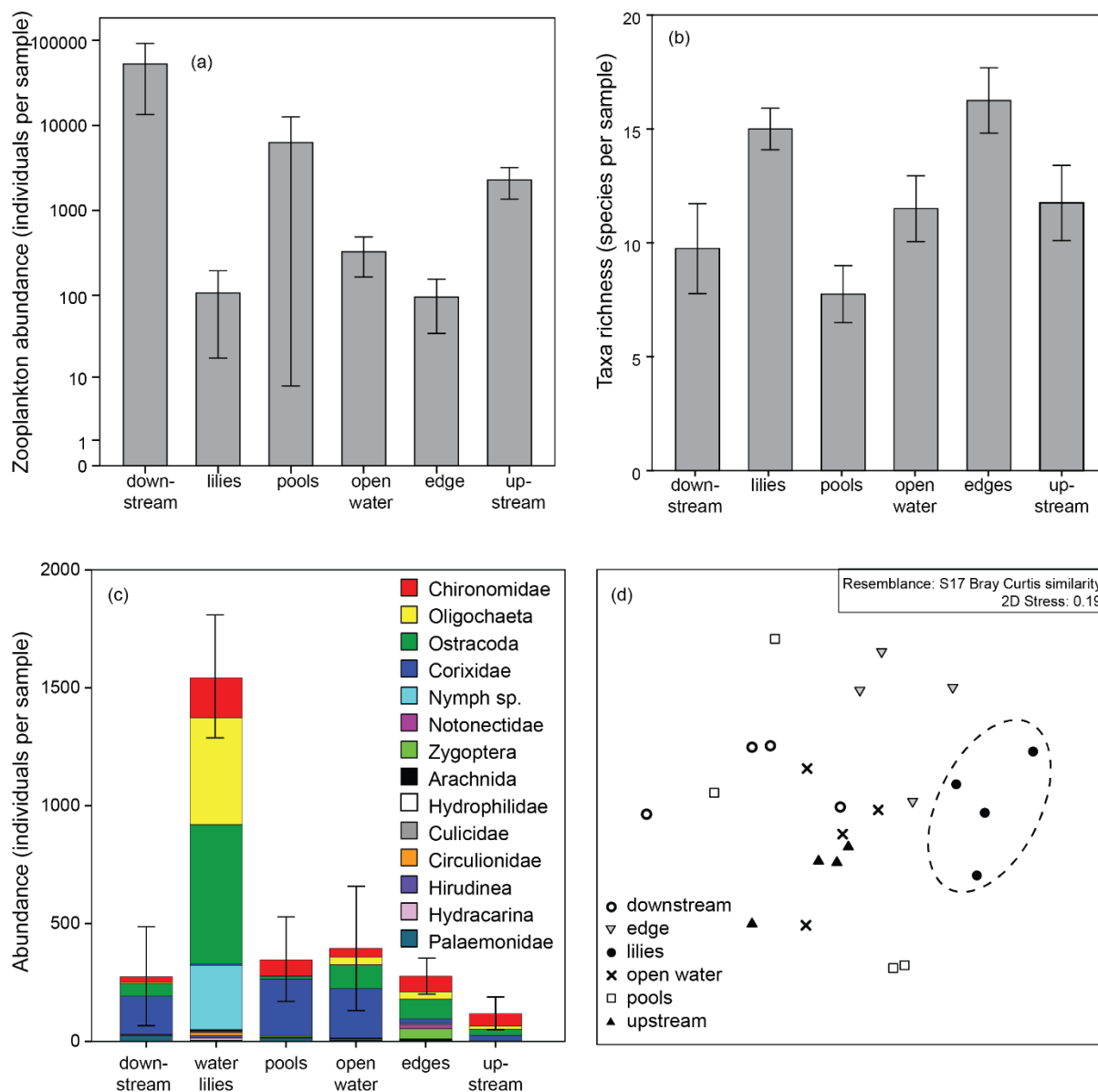
Figure 15. Comparison of invertebrate communities in different site types in the study area: (a) mean species richness; (b) mean zooplankton abundance (Copepoda plus Cladocera); (c) mean abundance of macroinvertebrate groups; and (d) Multi-dimensional scaling plot of invertebrate community assemblages, showing distinct grouping for waterlily sites (dashed line). Error bars are +/- one standard error (of total abundance in (c)). Note log10 scale in (b).

Table 4. Resemblance matrix comparing aquatic invertebrate community assemblages in different site types in the study are. Values are R statistics from ANOSIM, which are on a scale of 0 (no difference) to 1 or -1 (large difference). Asterisks indicate significant results (P < 0.05).

Site Types	Lilies	Edges	Pools	Open Water	Downstream
Edges	0.51[*]				
Pools	0.57[*]	0.17			
Open water	0.75[*]	0.19	0.16		
Downstream	0.92[*]	0.40	0.27	0.28	
Upstream	0.92[*]	0.64[*]	0.22	0.09	0.36

Discussion

The infestation of *N. mexicana* has influenced water quality and ecology in the lower Vasse River. The most important effects indicated by this study are: substantially lower oxygen levels within stands of waterlily; higher phosphorus concentrations throughout the river upstream of the point of infestation; reduced phytoplankton growth in waters between stands of waterlily; and provision of structural habitat for aquatic invertebrates. In addition, the large areas of river now covered by *N. mexicana* has substantially reduced open water habitat for birds.

Effects on oxygen

The very low oxygen levels within dense patches of *N. mexicana* in the lower Vasse River were expected. Floating-leaved aquatic plants are commonly associated with low dissolved oxygen levels (Scheffer et al. 2003; Goodwin et al. 2008). The two key processes affecting oxygen concentrations in standing freshwaters are photosynthesis and respiration (Boulton et al. 2014). Photosynthesis by the waterlilies occurs primarily in the emergent leaves with gas exchange in the air (Ritchie 2012), so there is little potential for oxygen replenishment in the water through this process. Conversely, respiration in the waters beneath the lilies continues to consume oxygen, resulting in very low oxygen conditions. The rich organic sediments in the Vasse River confound this problem due to high microbial decomposition of this existing material and additional decaying plant material (Carpenter and Lodge 1986, Boulton et al. 2014). This contrasts with phytoplankton and submerged aquatic plants, for which photosynthetic activity within the water column replenishes oxygen, with the potential to create conditions of supersaturation. This was observed in this study in the downstream reach, associated with a phytoplankton bloom, and in open waters between waterlily stands in the vicinity of the submerged macrophyte *Potamogeton crispus.*

Low oxygen levels extended throughout the water column during the sampling period when weather conditions were calm and extended beyond the extent of the plants in some cases, particularly in the small pools among plants. Average wind speed during the sampling period (16 km^{-1}) were similar to long-term average wind conditions (17km^{-1}, BoM 2017). Higher surface water oxygen concentrations were observed during transect measurements under windy conditions (26kmh^{-1}). This indicates potential for oxygen replenishment via mixing by wind, creating fluctuations in oxygen concentrations and providing more tolerable conditions for aquatic fauna at times.

Oxygenation of the sediments by aquatic plants is recognised as an important mechanism for reducing phosphorus availability in shallow aquatic systems (Carpenter and Lodge 1986, Barko and James 1998), although it varies with plant morphology and trophic status (Wigand et a. 1997). Although waterlilies have aerenchyma (tissue with air spaces) in their stems and roots (Figure 3e) which move gases throughout the plant (Richards et al. 2012), sediment oxygenation has not been reported for *Nymphaea*. High respiratory demands of the organic sediments limits the effectiveness of this process (Carpenter and Lodge 1986), which is doubtless the case in the lower Vasse River, and indeed in most habitats where waterlilies grow extensively.

Nutrients

Given observations of reduced phytoplankton growth in the vicinity of the waterlilies, it was expected that nutrient availability would be lower, however this was not found: phosphorus concentrations were substantially higher than, and nitrogen concentrations were similar to, concentrations observed downstream of the waterlilies. Lower oxygen levels associated with the lilies may increase potential release of inorganic phosphate from sediments (Mortimer 1942, Boulton et al. 2014), contributing to water-column phosphorus. Although anoxic conditions did not occur in waters between stands of waterlilies (pools and open waters), high phosphorus concentrations in these areas may be influenced by conditions within the lilies. Plants can also increase water column phosphorus through sediment uptake and subsequent release via decomposition (Carpenter and Lodge 1986, Clarke 2002).

While anoxia may have contributed phosphorus to the water column, higher phosphorus concentrations found in the upstream area, which was well-oxygenated, suggest other processes. Lower phosphorus uptake by phytoplankton within and upstream of the waterlilies, owing to much lower phytoplankton density, would also contribute to higher phosphorus concentrations. Similarity of nitrogen levels throughout the study area may be due to the dominance of cyanobacteria in the downstream all bloom, which can obtain nitrogen through fixation of atmospheric nitrogen. This naturally leads to the question of why phytoplankton growth was low within and upstream of the waterlilies, discussed further in the next section.

The most interesting outcome of plant biomass and nutrient analyses is to estimate how much nutrients are stored within waterlily biomass, to consider how much could be harvested if plants were removed; how much would be released if plants were killed with herbicide; and to what extent the lilies are a nutrient sink in the system. Removal of the standing crop of waterlilies present at the time of sampling would be the equivalent of about 2% of the annual phosphorus load and 1% of the annual nitrogen load in waters of the Lower Vasse River.

The estimated nutrient content of the plants appears to be low relative to external nutrient loading, and even less significant considering the large amount of nutrients stored in the sediments. It is difficult to know with certainty whether the lilies act as a net nutrient sink in the lower Vasse River. Nutrient uptake and storage within the plants is likely to be temporary, as nutrients are released via turnover and decay of plant material (Cronk and Fennessy 2001, Vymazal 2010). Lower sediment-nutrient content among the waterlilies compared to other sites may reflect nutrient uptake by the plants, which would be primarily from the sediments rather than the water column (King et al. 2009, Bornette and Puijalon 2011). However, accumulation of sediments from external sources and from decomposing plant material may also affect sediment-nutrient content. Plant tissue had lower nutrient content than sediments, so accumulation of this material may act to lower the sediment-nutrient content. Aside from plant uptake, permanent removal of nutrients may occur via denitrification (for nitrogen); and via sorption and burial due to accumulation of soil particles (Cronk and Fennessy 2001). If nutrients and organic material from decomposing plants are stored within the plant beds, net accumulation of nutrients would be higher than that present within the living plant biomass.

Phytoplankton

In accordance with the observed algal bloom in the downstream area, and its absence upstream of the point of waterlily infestation, significantly lower levels of phytoplankton growth, as indicated by chlorophyll a, were found within and between waterlily stands. Although cyanobacteria density results for lilies and open water sites were similar those for the downstream area, it appears that the downstream sample was an underestimate of the phytoplankton density. There was obvious discolouration at the time of sampling, and concurrent routine monitoring further indicated a persistent, severe cyanobacterial bloom in this area. One-off phytoplankton sampling without replications does limit the inferences made from this data, but it did show that cyanobacteria were present in the vicinity of the waterlilies and that the species differed from those in the downstream area. The absence of *Anabaena* and *Microcystis* within and upstream of the waterlilies, which are the common bloom-forming genera in the downstream area, is a positive sign that problematic phytoplankton growth is being influenced by the presence of the waterlilies. Further sampling of the phytoplankton community at additional sites and over time would help further understand this influence.

Low levels of phytoplankton growth (indicated by chlorophyll) within the waterlily stands can be attributed to light extinction beneath the dense emergent leaves. Shading is an obvious physical effect and has been found to be a limiting factor to phytoplankton growth in recent experimental trials by Ji et al. (2016). However, this does not adequately explain the very low levels of phytoplankton in the well-lit open water and pool areas between waterlily stands. Two of the pools (one site in each) are very small and perhaps influenced by shading from adjacent lilies, however the other two pool sites located within a larger pool are unlikely to be shaded enough to restrict phytoplankton growth. Furthermore, dense benthic algal growth was evident at these sites, suggesting sufficient available light. The large open water areas between main waterlily infestations are very unlikely to be influenced by shading within adjacent plants, and lower chlorophyll *a* extended 199m and 391m between waterlily stands. Though mean values were higher upstream, lower chlorophyll *a* concentrations (0.037 mg/L) extended to the third upstream site, 289m from the upstream edge.

Potential mechanisms limiting phytoplankton growth

Limitation of phytoplankton growth in open waters upstream of the main waterlily infestation, suggests that the algal bloom forms only in downstream waters and the waterlilies form a physical barrier to upstream dispersal, with conditions upstream from this point less conducive to phytoplankton growth. The significant increase in conductivity at the interface with the waterlilies does suggest that there is little water mixing beyond this point, and a build-up of algal scum at this downstream edge also provides evidence of a barrier effect. This leads to the question of why phytoplankton blooms do not form independently in the upstream areas, despite very high nutrient concentrations. The characteristics of this reach of river do not provide an explanation: high light availability (evidenced by the extensive waterlily growth), high nutrient availability, warm temperatures and still conditions are suitable for phytoplankton to thrive. Upstream sites were slightly (though not significantly) cooler, possibly due to greater riparian shading because the river is narrower in the upstream reach. However, this section of river has previously experienced severe

phytoplankton blooms similar to those observed in the downstream area during this study (Paice et al. 2016), suggesting abiotic conditions do not limit growth.

Although nitrogen:phosphorus ratios in the water column were indicative of nitrogen limitation within lilies, pools and open waters, this was due to very high phosphorus concentrations rather than low nitrogen, with TN concentrations similar to those downstream. TN:TP ratios are often low in hypereutrophic systems owing to the excessive availability of phosphorus, with a decline over summer as nitrogen is decreased through denitrification while phosphorus is increased through release from sediments (Moss et al. 2013). Dissolved inorganic nitrogen concentrations were very low throughout the study area, indicating very little nitrogen available for phytoplankton growth. Low nitrogen availability favours cyanobacteria which are able to fix atmospheric nitrogen (N_2), as was observed in the downstream area. But populations of cyanobacteria may not establish if there are other factors that prevent development of a substantial population of nitrogen-fixing organisms (Vrede et al. 2009). The waterlilies appear to create such a situation, but it is not possible from this study to determine the mechanisms preventing the establishment of a cyanobacterial bloom. Potential mechanisms are light limitation due to reduced water residence time in pools and open water areas, and allelopathic inhibition of phytoplankton.

Water residence time

A reduction in water (hydraulic) residence time can prevent development of cyanobacterial blooms. In a restoration context, this is generally approached through flushing, vertical mixing, or circulation through a compartmentalised system (Stroom and Kardinaal 2016). In the lower Vasse River, water movement and mixing by wind may create conditions of low residence time in the open water areas that have sufficient sunlight for phytoplankton growth. Shading within the waterlily stands prevents phytoplankton growth, and movement between stands and open waters may not allow sufficient residence time for dense populations of cyanobacteria to establish.

Allelopathy

Allelopathy in this context describes the biochemical inhibition of phytoplankton by chemicals exuded from the waterlilies into the water column. Allelopathic suppression of phytoplankton by submerged macrophytes and charophytes is recognised as a potentially important mechanism preventing phytoplankton growth and stabilising clear-water regimes in shallow lakes (van Donk and van de Bund 2002, Hilt and Gross 2008). Some aquatic angiosperms known to have allelopathic properties are the free-floating *Eichornia crassipes* (water hyacinth), the non-rooting submerged *Ceratophyllum demersum*, and the rooted submerged species *Myriophyllum spicatum*, *Elodea canadensis* and *Egeria densa* (Gross 2003, Hilt and Gross 2008, Vanderstukken et al. 2011). Rooted floating-leaved plants have received little attention in regard to allelopathic effects on phytoplankton, as they do not need to compete with phytoplankton (Gross 2003). However, *Trapa* spp. (water chestnut) and *Nelumbo nucifera* (lotus), which have similar morphology to *Nymphaea*, have also been associated with low chlorophyll a concentrations (Nemoto and Fukuhara 2012). This research concluded that allelopathic inhibition of cyanobacteria was the most likely explanation for this relationship, although there was no supporting experimental evidence.

Proving allelopathy is complex (Hilt 2015), requiring detection of allelopathic compounds in the plants and in surrounding waters; and experimental proof of inhibitory activity through laboratory

bioassays and in-situ experiments (eg. Hilt et al 2006). Allelopathic chemicals are difficult to detect in the field owing to their volatility, and determining which compounds should be analysed would be problematic, since it has not been done for this species. There are many types of compounds that may have allelopathic effects, including acetic and butyric acids, fatty acids, simple phenols and phenolic acids, flavonoids and alkaloids (Rice 1987). Phenolic compounds appear the most common for submerged plants, while alkaloids have been indicated for the floating-leaved *Nuphar lutea* (Gross 2003). Some preliminary sampling (semi-volatile organic compounds) was undertaken for this study when it became apparent that allelopathy may be occurring, but all potential indicators were below detection. However, future sampling could incorporate acidification and extraction to concentrate these compounds (Hilt et al. 2006).

Alternative regimes

This study is an example of contrasting regimes: the downstream reach exhibited a turbid, phytoplankton-dominated regime; while the reach with large stands of *N. mexicana* exhibited a clear-water regime. These alternate regimes are often described for shallow lakes with submerged macrophytes (aquatic vascular plants) dominating the clear-water regime (Scheffer et al. 1993, Davis et al. 2010). Davis et al. (2010) also defined a clear-water, benthic microbial community regime, however this is found only in hypersaline or highly acidic lakes. The key mechanisms by which submerged macrophytes maintain the clear-water regime are nutrient uptake by plants and associated epiphytes, reduced sediment resuspension and increased sedimentation (Scheffer and van Ness 2007), provision of refuge habitat for herbivorous zooplankton (Timms and Moss, 1984) and allelopathy (van Donk and van de Bund 2002). Floating plants have also been considered in the context of alternative regimes, however rooted floating-leaved macrophyte such as *Nymphaea* have not. This is presumably because these plants have no need to limit phytoplankton for successful establishment: they can outcompete phytoplankton for light due to shading emergent leaves, and do not compete for nutrients owing to their reliance on sediment for nutrition (Ji et al. 2016). This study did not determine the mechanisms causing the clear-water regime, but further investigations based on the findings here may help with long-term management of problem algal blooms in the lower Vasse River.

Aquatic fauna

Assessment of the aquatic invertebrate community was undertaken to determine both the general impact on ecology in the Vasse River, and the effect on potential for zooplankton control on phytoplankton through grazing. Although zooplankton abundance was highest in the downstream reach, there was no limitation of phytoplankton growth. The zooplankton community was dominated by small copepods, as is common for eutrophic waters in Australia (Boon and Bunn 1994), and these do not exert grazing pressure on phytoplankton (Brooks and Dodson, 1965). Cladoceran populations are more important for control of phytoplankton, but were rare throughout the study area, and thus unlikely to be a factor in lower phytoplankton densities in pools and open water adjacent to waterlilies.

The dark, anoxic conditions beneath densely-growing rooted and free-floating plants impact aquatic fauna and invasions of these plant communities are generally considered a serious threat to freshwater ecosystems (Scheffer et al. 2003). However in this study, higher abundance and richness of invertebrates was found within the lilies, despite much lower oxygen conditions. The main

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differences between the invertebrate community of lilies and other sites were higher abundance of Oligochaeta, Ostracoda and unidentified terrestrial nymphs; and fewer Corixidae and Copepods. Oligochaetes are detritivores and were common throughout the study area. Ostracods are mostly plant-associated and tolerant of low oxygen conditions, thus well-adapted to conditions in the waterlily stands (Kato et al 2016). The nymphs are indicative of the terrestrial habitat component provided by the emergent eaves, and many spiders were also observed above the water. The highest diversity, although not abundance, was found at the edges of waterlilies. Although present at very low density, some taxa that were restricted to edge sites were indicative of improved structural habitat: Aeshnidae (dragonfly larvae), Ceratopogonidae (biting midge larvae), Hydroptilidae and Leptoceridae (caddisfly larvae).

High diversity and abundance among the waterlilies was surprising given the low oxygen levels present, however as shown during transect sampling, wind-mixing may oxygenate surface waters. Higher abundance may relate to greater decomposition of dead plant material in the lilies and greater surface area for colonisation provided by the structural habitat of the plants (Diehl and Kornijów 1998). Aquatic plants are known to enhance abundance and diversity of invertebrates, owing to provision of structural habitat with greater niche diversity additional food resources (Heck and Crowder 1991, Diehl and Kornijów 1998). This has been found previously in the study area when submerged aquatic plants were grown from transplants (Paice et al. 2016). However, in the waterlily habitat, most of the additional abundance and richness was owing to taxa that are tolerant of poor water quality (Chironomidae, Oligochaeta, Ostracoda). Improved conditions were indicated for the edges of water lilies where the structural habitat of the lilies and improved oxygen conditions are present.

Birds

During sampling for this study the Purple Swamphen (*Porphyrio porphyrio*) was often observed in and around the waterlilies, walking among and on top of the plants and climbing and roosting in riparian vegetation. These birds are likely to be nesting within the emergent leaves and feeding both directly on the lilies and on small animals living amongst them. Notably, one bird was seen in the shallows, grasping and eating waterlily root material. As vegetative reproduction of *N. mexicana* is very successful, Purple Swamphens may aid further dispersal.

Although providing habitat for Purple Swamphens, no other bird species were observed using the waterlily patches. The lower Vasse River provides habitat for many other species of waterbirds. Open water habitat is vital for swimming, diving and wading, and birds are often seen nesting in branches overhanging the water. The increasing coverage of the waterlilies is potentially a direct threat to this. While there is greater diversity and abundance of aquatic invertebrates in the vicinity of the waterlilies, this does not extend into adjacent open waters where most birds feed.

Competition with *Persicara decipiens*

The native semi-aquatic plant *Persicaria decipiens* (slender knotweed) occurs in several locations on the banks of the lower Vasse River, and at some points grows out from the bank amongst the waterlilies. One of the objectives of this study was to assess the potential for *P. decipiens* to compete with *N. mexicana* and thus to be used as a management tool for control of waterlilies through gradual replacement with this native species. Field observations of the habit of the two species

revealed that while *P. decipiens* is present at a number of points along the river bank, its growth only expands into the river where waterlily is also growing. This suggests that *P. decipiens* may be dependent on the substrate provided by the waterlilies for growth. The author has observed recurring annual growth of Persicaria among the waterlilies at the site sampled in this study during the past three seasons and noted its expansion over the season as water levels drop. A similar area is colonised each season, commencing at the bank. The declining water levels are also associated with seasonal senescence of the waterlilies, as observed elsewhere in the study area in the absence of *P. decipiens* (e. g. northern bank near site WL 2). Where *P. decipiens* grows densely on the river bank but does not extend into the water, it is likely constrained by depth and/or lack of substrate. Lower *N. mexicana* biomass in the presence of *P. decipiens* is probably owing to shallow conditions at this site, which are more favourable for the semi-aquatic *P. decipiens* as water levels drop over summer.

Further planting of *P. decipiens* with the aim of replacing *N. mexicana* is not considered worthwhile because it will not have a competitive advantage in deeper waters throughout the river channel. It is common along the margins of wetlands and rivers and in winter-wet depressions, but is not fully aquatic (Sainty and Jacobs 2003, DCBA 2017). Its expansion into the Vasse River among the lilies may be an example of terrestrialisation in these shallower areas (Bornette and Puijalon 2011). In addition, it may not offer the same water quality benefits that are currently provided by *N. mexicana,* would likely maintain low oxygen conditions, and would continue to restrict open water habitat for birds.

Future expansion of waterlilies

Without intervention *N. mexicana* is likely to continue to spread throughout the lower Vasse River. There did not appear to be any characteristics of open water areas which would prevent future colonisation. Comparison of depth and sediment characteristics, river width and amount of riparian shading between areas with and without lilies upstream of the main point of infestation did not suggest any limiting factors to the further expansion of waterlilies into these areas.

Shading is likely to be a key factor limiting growth. This is evident at the Strelly Street bridge, where a large dense infestation has been established for four years without spreading downstream beyond the bridge. But riparian shading in the lower Vasse River did not appear limiting and the plants will continue to spread to the bridge from another infestation area downstream. At the downstream limit of waterlily growth in the Vasse River expansion over the last four years has been slow, with an extension in summer extent of approximately 27m downstream. This may have been restricted in summer by shading from dense phytoplankton growth. This area is also deeper than upstream. however *N. mexicana* has capacity to grow to a depth of 2m (DiTomaso and Healy 2003 [cited in Hoftra et al. 2013]) and creates shallower conditions by establishment of the root mass, and so the infestation can be expected continue to gradually expand downstream. As occurred over the 2013-2014 growing season, future spread of *N. mexicana* may involve sudden large expansions in cover.

Of particular importance is the potential for invasion of the Vasse-Wonnerup wetlands system downstream. *Nymphaea mexicana* is a freshwater plant and thus is not expected to tolerate persistent salinity over 3.5ppt (Stutzenbaker 1999 [cited in USACE 2011], Borsch et al. 2008). There are no published studies on its tolerance and its growth in seasonally saline systems such as the Vasse-Wonnerup is not known, however it is not naturally distributed in estuarine systems. Spring salinity in the most upstream region of the Vasse Estuary ranges from 0.8-26ppt, while summer

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salinities can be in excess of seawater and large areas are dry. Potential for growth in estuarine areas is probably limited by salinity, however wetland areas between the lower Vasse River and the estuary are at higher risk – indeed a new population of lilies was recently observed downstream of the stop boards.

Future management

Future control requires consideration of both the risks and the benefits of waterlilies: mainly their function in limiting algal blooms and maintaining water clarity in some areas, as opposed to potential for spread and ongoing loss of open water habitat and associated amenity. Continued occurrence of large areas of lilies also increases the risk of spread to other waterways in the region, which is a particular concern for downstream wetlands. Active management of the lilies is therefore recommended, at least to prevent further spread.

Options for control are reviewed in Appendix 1. The most effective control method would be use of herbicide in autumn, but risks of oxygen depletion associated with plant decay need to be managed. The effectiveness of shading in preventing expansion does suggest this could be a valuable control option, at least for preventing further spread. Creating barriers across the river at the edges of waterlilies may be feasible, and could incorporate floating bridges with wetland vegetation, building on the idea of floating islands which have previously been placed in the river.

Conclusions and recommendations

If it is not managed, *N. mexicana* is likely to spread throughout the river. Future management is necessary to address loss of open water habitat and prevent the spread of this invasive plants to important wetland areas, however managers also need to consider current water quality benefits provided by the lilies. Extensive control would require the use of herbicide in autumn. This will need to be staggered to minimise potential impacts from deoxygenation resulting from decomposing plants. Smaller areas of control and prevention of spread may be achievable through artificial shading, using floating barriers or floating vegetated bridges.

The presence of the waterlilies is clearly associated with reduced phytoplankton in waters between stands of plants. This is not a result of nutrient reduction, with extremely high phosphorus concentrations found in these areas, and may be explained by allelopathic effects or light limitation in patches between lilies. Notwithstanding high phosphorus levels, the apparent prevention of algal blooms provides direct benefits to water clarity, visual amenity and public health in waters between stands of waterlilies.

Although there is a large biomass of waterlilies present, the current autumn standing crop represents only a small proportion of nutrients in the river. They may provide an ongoing sink for nutrients, but this is unlikely to contribute much to an overall reduction of available nutrients in the river. Harvesting of the current biomass would be equivalent to about 0.5% of the annual nutrient load of the lower Vasse River.

Despite low oxygen conditions within the waterlilies, the structural habitat provided supports greater abundance and diversity of aquatic invertebrates than unvegetated areas. High abundance in the lilies was mainly owing to greater numbers of organisms tolerant of poor water quality, and

diversity was highest at the edges of waterlily stands, where oxygen levels were higher. Notwithstanding apparent benefits for aquatic invertebrate fauna, the loss of open water habitat for birds and fish is probably a negative ecological consequence of the large areas occupied by the waterlilies, although this was not assessed in this study.

The results of this study give rise to two key recommendations:

1) Undertake control of waterlily with consideration of the following principles:

    i. Effective herbicide control of *N. mexicana* can be achieved with glyphosate- or diquat-based products approved for use in waterways, but will likely require repeated applications. Autumn is the most effective time for control.

    ii. Eradicate new populations as a priority (eg. downstream of Butter Factory stop boards).

    iii. Aim to contain and reduce, not eradicate, the extent of waterlily in the lower Vasse River.

    iv. Control waterlilies in a mosaic pattern to reduce risk of deoxygenation resulting from decomposing plants and to maintain the role of waterlilies in improving water clarity. Control 50m sections of river between stands of waterlily to commence, and adapt management according to results.

    v. Target control effort to sections of river that provide important bird habitat, consulting with the Department of Biodiversity, Conservations and Attractions to determine these areas. Suggested examples based on the author's observations are:

- Night herons nesting near the confluence with New River Wetland;
- Spoonbills nesting in section parallel to Isaac St;
- lost open water habitat near Bunbury Street stormwater drain.

    vi. Use artificial shading to prevent further spread of waterlilies by creating barriers across the river, such as floating bridges of wetland vegetation.

    vii. Ongoing monitoring of control activities and outcomes is important to track efficacy, adapt management and share knowledge.

2) Support further research to investigate the processes limiting phytoplankton growth between stands of *N. mexicana* to inform control and contribute to management of problem algal blooms in the lower Vasse River.

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Appendix 1. Options for control of *Nymphaea mexicana*

Removal and harvesting

Complete manual removal of *N. mexicana* from the lower Vasse River is probably not logistically feasible owing to the very large biomass of plants present and the need to remove all root material to prevent regrowth. This approach would not contribute greatly to removal of internally stored nutrients from the river, given the extremely large volume of organic-rich sediments that would remain throughout the entire reach. It would also be difficult for this option to eradicate this species, and remaining vegetative material would regrow. Mechanical removal has been trialled in Goulburn Weir, and was effective in removing the above ground material, however there was 100% regrowth within just 8 months (Goulburn-Murray Water 2009).

Periodic harvesting of leaf and stem growth with the aim of ongoing nutrient removal would not contribute greatly to overall annual load reductions, as nutrient content of plants represents a small proportion of total nutrient loads for the river. In the context of average daily nutrient loads for the lower Vasse River (DoW 2010), removal of the lilies at the current level of growth would be the equivalent of about 0.5% of phosphorus and nitrogen. Nonetheless, ongoing harvesting would ensure that any nutrient removal via plant uptake is permanent and result in net removal of nutrients from the system.

Shading

The effectiveness of the Strelly Street bridge in preventing downstream expansion of waterlilies suggests that artificial shading may be an effective control option. Extensive control is probably not logistically feasible for very large areas covered by waterlily, but may be useful for control in discrete areas. Shading could be most effective to (i) contain spread of existing plants, and (ii) contain spread of smaller areas of plants following other control practices.

Shade structures could include floating barriers such as black plastic, or floating vegetated 'bridges' similar to the floating islands that have been placed previously in the lower Vasse River (Figure 15). The width of artificial shading would need to ensure shading throughout the day, and the existing road bridge provides an example of what may be effective. Vegetated structures would provide habitat above and below the water and may benefit water quality through nutrient uptake (Bu and Xu 2013, Fang et al. 2016).

Figure 16. An example of a large floating vegetated island, which could be extended to a 'bridge' across the river to prevent expansion of the waterlilies by shading, and provide additional benefits for habitat and water quality. (Image source www.aquabiofilter.com).

In situ control with herbicides

The waterlilies can be controlled using herbicide, however there is a risk of deoxygenation associated with plant decomposition. Although low oxygen conditions already occur within the lilies, widespread dieback resulting from herbicide use to control the entire infestation would likely cause more widespread deoxygenation, posing a risk to aquatic fauna and creating conditions conducive to phosphorus release from sediments. Control of *N. mexicana* with Glyphosate herbicide was suspected to have caused a downstream fish kill in Victoria in 2004 (Hofstra et al. 2013). Timing, extent and rates of herbicide use are key factors in efficacy and managing deoxygenation. While the greatest possible impact on the waterlilies is desirable, more rapid decay and control over larger areas carries greater risk of deoxygenation.

When considering control of waterlilies, it is very important to recognise the role they appear to have in limiting phytoplankton growth. Complete control of these plants would likely see a return to turbid, phytoplankton-dominated conditions that existed previously, and that continue to occur in the downstream area. It is unknown what extent of plant growth is needed to maintain benefits to water quality. There may be scope for gradual control and replacement of *N. mexicana* with native submerged species (Scheffer et al. 2003), for example *Potamogeton crispus*, *Cycnogeton* sp., and *Stuckenia pectinata*.

Glyphosate- and diquat-containing herbicides are both recommended for the control of waterlily[4]. Glyphosate has been trialled in Victoria and diquat (as Reglone®) has been trialled locally in the nearby New River Wetland, and also to control Nardoo in the Collie River. These trials are described below. Both products are likely to require repeated applications. Monitoring and reporting of effectiveness would contribute to long term management of this species locally and elsewhere.

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[4] e.g. Department of Primary Industries, NSW: http://weeds.dpi.nsw.gov.au/Weeds/Details/259

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The most effective control of *N. mexicana* in the lower Vasse River would probably be achieved using herbicide applied in autumn - prior to widespread senescence of the plants in winter. Previous trials have shown this the most effective time for control (Hofstra et al. 2013) and at this time, cooler temperatures and onset of winter flows may mitigate deoxygenation. Potential impacts at this time may also be considered relatively low in the context of prevailing poor conditions in the lower Vasse River. Water quality is very poor during this time and aquatic invertebrate communities are fairly nondescript. This study has shown that anoxic conditions already exist within waterlily infestations at this time of year, while supersaturated conditions occur downstream and may buffer the effects of further reductions in oxygen levels associated with plant decay. Natural senescence and plant decay would occur in winter regardless of herbicide use, and the onset of cooler temperatures and rainfall provides for oxygen replenishment. However potential risks associated with deoxygenation cannot be ignored and, with due consideration to potential water quality benefits provided by the waterlilies, herbicide control should be staged over time.

Glyphosate

There has been some experience in controlling *N. mexicana* with glyphosate (as Roundup Biactive®, approved for use in waterways) in Goulburn Weir in Victoria, Australia, and in the Waitakere Wetland in New Zealand. This experience suggests ongoing annual control is required. Much of the information from this experience is in grey literature and not readily available, but is reviewed by Hofstra and others (2013) in their account of trials in Goulburn Weir. Glyphosate was used effectively to manage (but not eradicate) *N. mexicana*. Prior to the downstream fish deaths in 2004, glyphosate application reduced the area covered by waterlilies from 200ha to 30ha. Subsequent trials found low and medium application rates of glyphosate (1.08 and 2.16kg/ha) were equally effective and that the most effective time for application was late summer to early autumn, but the best control was found with additional application in spring to early summer (Hofstra et al. 2013). These application rates resulted in concentrations in the water column within ecosystem protection guidelines (370 µg/L: ANZECC and ARMCANZ 2000). Applying glyphosate at very high rates (6 kg/ha) provides better control because it has a greater effect on rhizomes and stolons, reducing regrowth (Champion 2003, cited in Hofstra et al. 2013). This approach has potential to reduce plant density and extent over a shorter time frame, but would still require multiple annual treatments and may result in glyphosate concentrations above ecosystem protection guidelines.

Diquat

An alternative to glyphosate are diquat based products such as Reglone®, a non-residual herbicide frequently used to control various aquatic weeds globally (Ritter et al 2000). This herbicide is registered for control of aquatic weeds by the Australian Pesticides and Veterinary Medicines Authority (APVMA 2017). It is inactivated on contact with soil and suspended particles and a literature search found no studies indicating negative impacts on aquatic fauna. The main disadvantage of using Reglone® is its very high toxicity to humans through inhalation, skin contact and ingestion. The label lists waterlilies as a target species and although there are no published examples of its efficacy in control of *N. mexicana* or related species, it was used in the adjacent New River wetland in 2008 (L. Massey, GeoCatch, pers. comm.; Figure 17). This trial found complete control of waterlily in the treated area with no regrowth for at least two years. There was no follow-up control and the area is now re-infested.

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Reglone® has also been used successfully in the Collie River to control excessive growth of Nardoo (*Marsilea mutica*) through its ongoing Collie River Revitalisation Plan (Shire of Collie 2014). This program uses an inert bonding agent (Hydrogel) to apply the herbicide to floating-leaved plants, as it is more targeted and reduces spray drift. Experience in the Collie River indicates staged treatment reduces risks associated with decaying plants on deoxygenation.
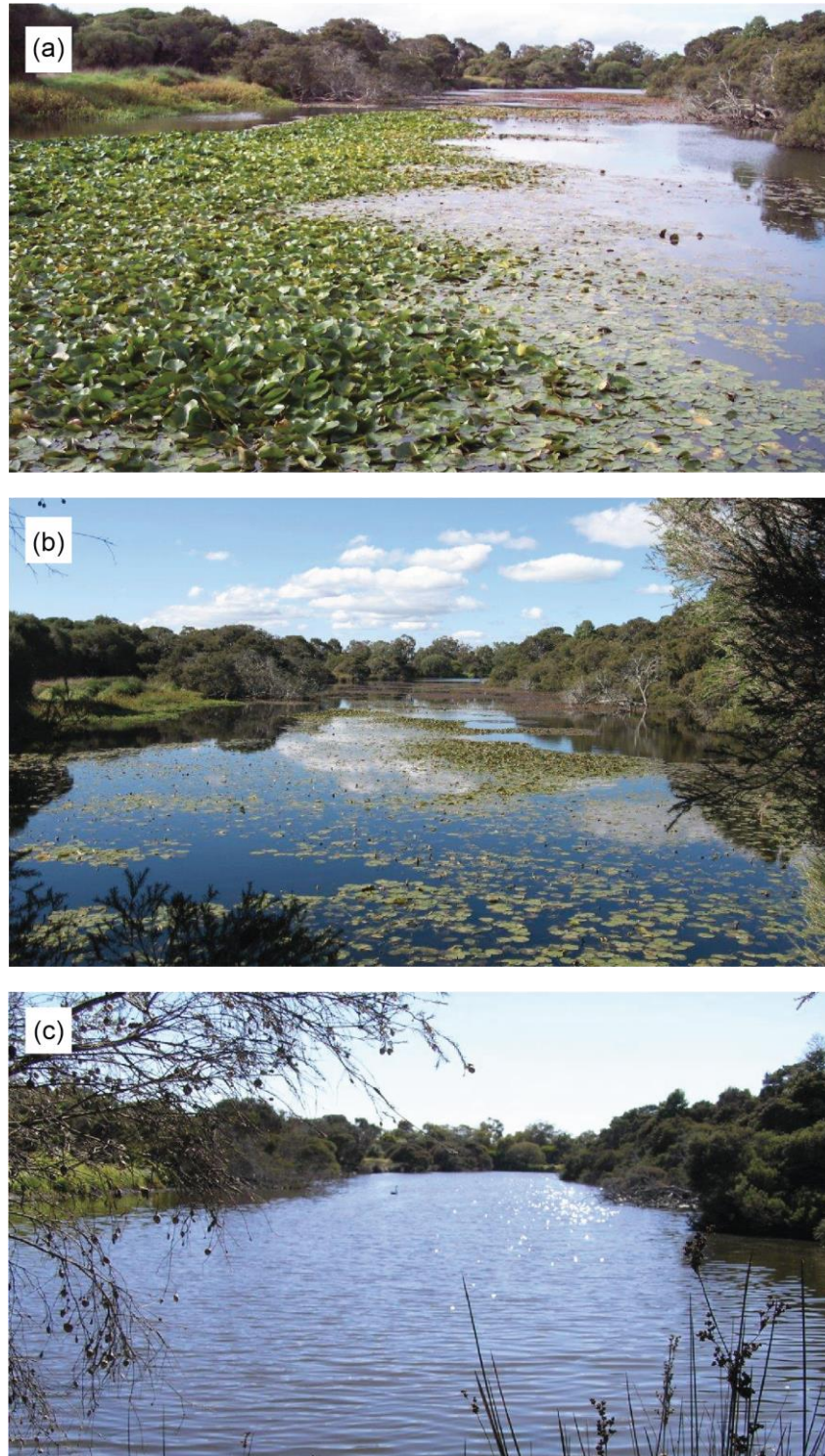
Figure 17. An area of the New River Wetland, adjacent to the lower Vasse River treated with Reglone® in May 2008: (a) before treatment in May, and after the treatment in (b) August and (c) December. Photos courtesy Lisa Massey, GeoCatch.

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Appendix 2. Study site locations.

WIR SITE CODE	Site Name	Latitude	Longitude
WLA1	lilies1	33°39.481'	115°20.896'
WLA2	lilies2	33°39.460'	115°20.865'
WLA3	lilies3	33°39.559'	115°21.101'
WLA5	lilies4	33°39.804'	115°21.128'
WLB1	downstream1	33°39.180'	115°20.872'
WLB2	downstream2	33°39.208'	115°20.768'
WLB3	downstream3	33°39.336'	115°20.731'
WLB4	downstream4	33°39.367'	115°20.748'
WLC1	open3	33°39.633'	115°21.161'
WLC2	open4	33°39.767'	115°21.111'
WLC3	upstream1	33°39.889'	115°21.108'
WLC4	upstream2	33°39.988'	115°21.094'
WLC5	upstream3	33°39.394'	115°20.765'
WLC6	upstream4	33°40.016'	115°21.104'
WLD1	d/s edge	33°39.394'	115°20.764'
WLD2	edge1	33°39.514'	115°20.924'
WLD3	edge2	33°39.542'	115°21.062'
WLD4	edge3	33°39.585'	115°21.171'
WLD5	edge4	33°39.799'	115°21.126'
WLD6	u/s edge	33°39.825'	115°21.129'
WLE1	open1	33°39.526'	115°20.922'
WLE2	open2	33°39.532'	115°20.972'
WLE3	pool3	33°39.554'	115°21.129'
WLE4	pool4	33°39.557'	115°21.155'
WLE5	pool5	33°39.463'	115°20.836'
WLE6	pool6	33°39.452'	115°20.836'
WLF1	*Pers. decip*	33°39.543'	115°21.057'

Appendix 3. Plant nutrient and biomass data

Site	Species	Sample type	TKN (mg.N/g)	TP (mg.P/g)	Biomass (dry weight/m²)
A1	*N. mexicana*	leaf + stem	24	3.7	992.0
A2	*N. mexicana*	leaf + stem	25	4.2	461.5
A3	*N. mexicana*	leaf + stem	22	3.6	866.7
A4	*N. mexicana*	leaf + stem	20	4.1	608.0
A1	*N. mexicana*	roots	10	3.8	1400.0
F1	*P. decipiens*	leaf + stem	14	3.7	688.4
F1	*N. mexicana*	leaf + stem	35	5.0	72.0