

***Sagittaria* as a bioindicator of stream nutrient status: effects of nitrogen
and phosphorus on leaf size and shape**

by

Katherine M. Standen

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Supervisors: Patricia A. Chambers, Ph.D., Water Science & Technology,
Environment Canada and Biology Department, UNB
Joseph M. Culp, Ph.D., Water Science & Technology,
Environment Canada and Biology Department, UNB

Examining Board: Linley Jesson, Ph.D., Biology Department, UNB
Stephen Heard, Ph.D., Biology Department, UNB
Michelle Gray, Ph.D., Faculty of Forestry and Environmental
Management, UNB

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Dean of Graduate Studies

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Abstract

Eutrophication of freshwater is an increasing problem globally as human populations, animal husbandry and agriculture intensify, and is associated with many deleterious consequences, including algal blooms, invasion of opportunistic species and, ultimately, changes in ecological functioning. These issues within Lake Winnipeg, MB, Canada, have led to research to develop bioindicators of nutrients within streams in the Red River, the largest nutrient contributing river to the lake. The research presented herein examines the use of the aquatic plant *Sagittaria cuneata* (Sheldon) as a bioindicator of phosphorus (P) and nitrogen (N). *S. cuneata* is an abundant species in rivers and streams in Manitoba, and has plastic emergent-leaf morphology and easily identifiable features. Plasticity within the genus has been linked to several factors, such as P concentration and water depth; therefore, *S. cuneata* was proposed as a potential bioindicator. Two studies were conducted to assess the relationship of leaf morphology with N and P: a controlled experiment where nutrients were added to either sediment or water, and a field study wherein *S. cuneata* leaves were collected in streams with varying water and sediment chemistry. Experimental results showed that plants used sediment nutrients to increase biomass, and that leaf morphology varied more during the growing season in sediment-enriched compared to water-enriched plants, which showed little variability of leaf morphology. Field studies indicated leaf morphology was variable at all scales of replication, and many variables contributed to leaf size, including sediment and water chemistry. Together, these results indicated that leaf morphology was affected by nutrient availability, but also complex interactions among other environmental variables. Thus, *S. cuneata* is not currently a reliable indicator of nutrient and future studies should further pursue the variability of leaf morphology.

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Chapter 1

General Introduction

1.1 Background

1.1.1 Nutrients in Aquatic Systems

Nitrogen (N) and phosphorus (P) are essential for life of aquatic and terrestrial plants and animals, and an insufficiency of either or both nutrients often limits plant production in aquatic ecosystems (Chambers & Kalff 1985). N and P are important elements in many physiological processes in plants, the most important of these processes being the formation of proteins and ribosomal RNA in plant cells (Loladze & Elser 2011; Moss et al. 2013). Phosphorus is an important constituent of nucleic acids, phospholipid barriers and several coenzymes and, most importantly, is crucial for the energy components in adenosine triphosphate (ATP) and adenosine diphosphate (ADP) (Correll 1998). Nitrogen is a key component of amino acids, nucleotides, nucleic acids, chlorophylls and coenzymes (Raven et al. 1999). These two nutrients are important for basic cellular functions and limiting quantities of one or both will constrain plant growth. However, excessive influxes of N and P into freshwater systems (nutrient loading) can lead to large increases in plant production and biomass, including algal blooms that can be toxic to aquatic organisms and contaminate drinking water supplies.

Increased nutrient loading has implications (both positive and negative) for freshwater macrophytes, including decreased biodiversity and increased photosynthetic and biomass production. Nitrogen loading can affect macrophyte communities and individuals in several ways, including the replacement of submerged aquatic species with floating and emergent species (Feuchtmayr et al. 2009; Moss et al. 2013). In some cases, a complete loss of submerged species may occur at high N concentrations (Gonzales Sagrario et al. 2005) as a result of several conditions, such as shading by

epiphytic algae (Phillips et al. 1978). N additions may also lead to reduced biodiversity, and an increase in growth and establishment of invasive species (Blann et al. 2009). High levels of phosphorus also have negative influences on a freshwater system. Phosphorus has been linked to increased production of aquatic plants, and large concentrations can result in an increased frequency of algal blooms, with potentially toxic effects (Dolman et al. 2012; Moss et al. 2013). Though many other environmental factors interact to influence the growth of aquatic macrophytes, such as light availability (Binzer et al. 2006), water velocity (Butcher 1933) and sediment composition (Madsen et al. 2001), nutrients have been implicated as major drivers in plant growth, and significant changes to plant communities and water quality can occur as a result of nutrient loading.

1.1.2 Effects of Eutrophication

Eutrophication is a process in aquatic ecosystems whereby excessive inputs of N and P from natural or anthropogenic sources lead to undesirable and detrimental effects such as algal blooms, decreasing water clarity, and declining populations of native species. Nutrient loading and, consequently, eutrophication, can lead to depletion of oxygen in bottom habitats, thus creating “dead zones” (Rabalais et al. 2002; Diaz & Rosenberg 2008; Rao et al. 2008) with deleterious implications for benthic organisms and fishes. An ecosystem undergoing eutrophication stress may also cause ideal environmental conditions for establishment of potentially harmful invasive species, which may be better adapted to decreased water quality than local species (EC & MWS 2011) and, thus, lead to decreases of native populations. The many physicochemical alterations in aquatic ecosystems caused by eutrophication result in shifts in community

structure, leading to dramatic changes in ecological functioning and services within a freshwater ecosystem.

Eutrophication of aquatic ecosystems is an increasing global problem, with a prime example of this process and its deleterious consequences being Lake Winnipeg (Manitoba, Canada). Nutrient loading from crop agriculture, animal husbandry, and municipal wastewater effluent has been identified as the cause of an increased occurrence of potentially toxic algal blooms in Lake Winnipeg, which is the 10th largest lake in the world and has the 2nd largest watershed in Canada (EC & MWS 2011). Declining water quality, caused by an increased frequency of algal blooms, is impairing the recreation and tourism industry in Manitoba as a result of unsightly and unsafe swimming conditions, and is threatening local fish populations and, consequently, negatively influencing both commercial and recreational fisheries (EC & MWS 2011). These changes are causing significant economic losses in the Province of Manitoba and are detrimental to ecological functioning within the lake; thus, the Province of Manitoba and the Government of Canada are committed to reducing nutrient loading and eutrophication of Lake Winnipeg. The Red River Valley within the Lake Winnipeg watershed has been implicated as the major nutrient contributor to the lake (68% of P and 34% of N annually; EC & MWS 2011); thus, this watershed has been the focus of studies on sources, fate and effects of added nutrients (e.g., Yates et al. 2012).

1.1.3 Bioindicators to Assess Eutrophication

In recent years, flora and fauna have been assessed for their usefulness in diagnosing and monitoring changes in ecosystem conditions in response to added

nutrients (as well as other anthropogenic stressors). Biological indicators, or bioindicators, are species, populations or communities used to assess and monitor the ecological health and function of an ecosystem over time. Changes in morphology, behaviour or biochemical processes in certain species, presence/absence of sensitive taxa, as well as the structure of the entire biotic community can all be used as indicators of stressors, both anthropogenic (pollution, land use changes) and natural (drought, flooding, irregular freeze/thaw events) (Holt & Miller 2011). Bioindicators, as opposed to chemical analyses, provide integrated information about biological, physical and chemical states of an environment over an organism's life span and can indicate indirect effects of pollutants that chemical analyses cannot decipher (Holt & Miller 2011). Bioindicators can also be used to detect bioaccumulation of contaminants in the food web that may not be observed with chemical analyses of water or sediment samples. They also have the advantage that general properties (often biological traits such as body size) can be used universally, while specific features such as local species composition can be used to identify conditions specific to a single region.

Aquatic macrophytes have been suggested as potential bioindicators because this group has many desirable characteristics: they are generally sessile, respond to environmental variation, are easily identified to genus and often species, and are abundant in many regions. Only a few studies have examined the use of macrophytes as indicators (e.g., Carbiener et al. 1990; Tremp & Kohler 1995; Robach et al. 1996; Thiebaut & Muller 1999; Richards & Ivey 2004; Haury et al. 2006; Ceschin et al. 2010; Tyrrell 2014). For example, Ceschin et al. (2010) examined the use of the macrophyte assemblage as a bioindicator of water quality in Italy and found certain phytocoenoses

(i.e., a group of plants considered as a whole) preferentially established in specific water conditions. Specifically, the phytocoenoses *Myriophylletum spicati* and *Potametum pectinati* were indicative of hypertrophic water, whereas the two phytocoenoses *Potametum crispum* and *Nasturtietum officinalis* generally developed in meso-eutrophic waters. Notably, research within the genus *Sagittaria* showed that leaf morphology responded predictably to environmental variation (Wooten 1986; Dorken & Barrett 2004; Richards & Ivey 2004), suggesting that species of *Sagittaria* may be useful as indicators of environmental conditions.

Sagittaria cuneata (Sheldon), commonly known as arrowhead or katniss, is an abundant aquatic macrophyte found throughout southern Manitoba with a widespread distribution across North America from British Columbia to mainland Newfoundland, and south to Texas. *Sagittaria* spp. reproduce both sexually, by producing achenes (a type of fruit that contains a single seed and does not open at maturity; Harris & Harris 1994), or asexually, by producing stolons (modified stems; Harris & Harris 1994) and tubers (Marburger 1993). Tubers are clones of the parent plant that are an avoidance strategy for freezing, drought, and mechanical and herbivore damage, and often remain within 30 cm of the parent plant (Haynes & Holm-Nielsen 1994), suggesting that genetic clones will establish relatively close to the parent plant.

Sagittaria cuneata is heterophyllous, having both submerged phylloid-shaped and emergent sagittate-shaped leaves, the latter exhibiting extreme leaf plasticity (i.e., variability in leaf size and shape) (Fernald 1950; Sculthorpe 1967). This variability in emergent leaf morphology has been linked to several factors. Phosphorus concentration has previously been shown to affect width of emergent leaf blades in *S. lancifolia*, with

wider leaf blades found at higher P concentrations (Richards & Ivey 2004). Dorken & Barrett (2004) found differences in leaf size and shape of *S. latifolia* with sexual system (monoecious versus dioecious populations). In addition, they noted increases in leaf size in response to fertilization for both mon- and dioecious plants. Lastly, water depth has been shown to influence emergent leaf size (e.g., Wooten 1986; Howard & Mendelssohn 1995). Wooten (1986) found a decrease in emergent leaf size of several *Sagittaria* species with increasing water depth because energy is directed to increasing petiole length in deeper waters at the expense of leaf size. The previous research within the genus *Sagittaria* suggests that leaf plasticity may respond predictably to environmental variation, and provides evidence for the potential use of *S. cuneata* as an indicator of nutrient conditions in the Red River valley. For this reason, and because of the widespread distribution of *S. cuneata* and the high anthropogenic nutrient inputs to tributaries in southern Manitoba, *S. cuneata* is proposed as a potential bioindicator of nutrients in the Red River Valley.

1.2 Objectives & Predictions

This thesis examines the utility of the emergent aquatic plant, *Sagittaria cuneata* (Sheldon - Alismataceae) as a bioindicator of stream nutrients using two approaches: A) a controlled, nutrient manipulation experiment (Chapter 2) and B) a field study conducted in a range of agriculturally-influenced streams in southern Manitoba, Canada (Chapter 3). Also included are a detailed introduction chapter (Chapter 1) and concluding chapter (Chapter 4) that summarizes and integrates the results of the field and experimental studies, and notes future directions. Two major themes are addressed: 1) the effects of N and P in water and sediment on emergent leaf morphology of

Sagittaria cuneata and 2) the development of leaf morphology of *S. cuneata* as a bioindicator of stream nutrients.

1.2.1 Chapter 2 – Experimental Study

The objective of the experimental study was to determine the effects of high and low levels of added sediment and water nutrients (nitrogen (N) and phosphorus (P)) on leaf size and shape of the aquatic plant, *S. cuneata*, when confounding variables such as sediment composition, water depth, velocity and clarity, and incident light are standardized and monitored across all treatments.

Several studies have demonstrated that rooted macrophytes access nutrients from the sediment in which they grow (e.g. Barko & Smart 1980, 1981; Chambers & Kalff 1985), and because *S. cuneata* is a rooted, emergent macrophyte, it likely draws the majority of N and P from sediment. Thus, I predicted that *S. cuneata* grown with added sediment nutrients would be more successful (i.e., taller, larger biomass, larger leaves) than plants exposed to added water nutrients. However, because nutrients in water can bind with fine, silty sediment, (Goltermann 1995), I also predicted that the water-enriched treatment levels would be more productive than the control treatment level.

To determine whether N or P was the key nutrient stimulating plant growth and determining leaf size and shape, treatment levels were factorial in design, with high and low levels of N and P. Aquatic macrophytes have been shown to require an N to P molar ratio (N:P) of between 8:1 and 30:1 (Moss et al. 2013), although if quantities of both nutrients are excessive, then the N:P becomes irrelevant and another factor becomes limiting (Moss et al. 2013). Under low nutrient conditions, N and P have also been

shown to have a synergistic effect, meaning that additions of both N and P will have a larger effect than singular additions of either nutrient (Allgeier et al. 2011). Testing of experimental soil that had not been treated with nutrients indicated, on the basis of N:P, that sediment was N-limited, suggesting that inputs of N rather than P would increase plant growth; thus, I predicted that plants in treatments with high added N, regardless of P concentration, would have larger biomass and emergent leaves than those with low added N.

1.2.2 Chapter 3 – Field Study

The objective of the field study was to determine the association between leaf size and shape of *Sagittaria cuneata* and concentrations of N and P in water and sediment in streams draining the Red River Valley, Manitoba, Canada. Previous research demonstrates that sediment chemistry plays a larger role in plant growth than water chemistry for aquatic plants that are rooted in the sediment (e.g. Barko & Smart 1980, 1981; Chambers & Kalff 1985). Furthermore, many studies indicate that macrophytes have a larger biomass (e.g., Neill 1990; Carr & Chambers 1998) and produce larger leaves in higher nutrient conditions (e.g., Dorken & Barrett 2004; Richards & Ivey 2004). Thus, I predicted that plants growing in nutrient-rich sediment would be larger and produce larger leaves than those in nutrient-poor sediment, and that sediment chemistry would have a greater influence on plant growth than water chemistry. Stream water may still supply some nutrients to plant growth; thus, when sediment chemistry was similar, plants were expected to have higher biomass and larger leaves in those streams with greater water nutrients.

To address whether N or P was driving changes in leaf morphology, both nutrients were considered individually in empirical models. Phosphorus has previously been related to leaf morphology in the genus *Sagittaria*: Richards & Ivey (2004) found that leaf blades of *S. lancifolia* were wider in high P than in low P concentrations. Plants generally respond to increases in nutrients that are present in limited quantities (e.g., Neill 1990; Vymazal et al. 1995), suggesting that increasing leaf width in *S. lancifolia* with P was because this nutrient was in limited supply in the area of study (the Florida everglades; Richards & Ivey 2004). Because N is generally limiting in sediments and water in Manitoba, I predicted that higher N concentrations in sediments and (to a lesser extent) water would result in increased leaf size in *S. cuneata*.

1.3 References

- Allgeier J.E., Rosemond A.D. & Layman C.A. (2011) The frequency and magnitude of non additive responses to multiple nutrient enrichment. *Journal of Applied Ecology*, **48**, 96-101.
- Barko J.W. & Smart R.M. (1980) Mobilization of sediment phosphorus by submersed freshwater macrophytes. *Freshwater Biology*, **10**, 229-238.
- Barko J.W. & Smart R.M. (1981) Sediment based nutrition of submersed macrophytes. *Aquatic Botany*, **10**, 339-352.
- Binzer T, Sand-Jensen K & Middelboe A. (2006) Community photosynthesis of aquatic macrophytes. *Limnology and Oceanography*, **51**(6), 2722-2733.
- Blann K., Anderson J., Sands G. & Vondracek B. (2009) Effects of agricultural drainage on aquatic ecosystems: a review. *Critical Reviews in Environmental Science and Technology*, **39**(11), 909-1001.
- Butcher R.W. (1933) Studies on the ecology of rivers. I. On the distribution of macrophytic vegetation in the rivers of Britain. *Journal of Ecology*, **21**, 58-91.
- Carbiener R., Trémolières M., Mercier J.L. & Ortscheidt A. (1990) Aquatic macrophytes communities as bioindicators of eutrophication in calcareous oligosaprobe stream waters (Upper Rhine plaine, Alsace). *Vegetatio*, **86**, 71-88

- Carr G.M. & Chambers P.A. (1998) Macrophyte growth and sediment phosphorus and nitrogen in a Canadian prairie river. *Freshwater Biology*, **39**, 525-536.
- Ceschin S, Zuccarello V & Caneva G. (2010) Role of macrophyte communities as bioindicators of water quality: Application on the Tiber River basin (Italy). *Plant Biosystems*, **144** (3), 528-536.
- Chambers P.A. & Kalff J. (1985) The influence of sediment composition and irradiance on growth and morphology of *Myriophyllum spicatum* L. *Aquatic botany*, **22**, 253-263.
- Correll D. (1998) The role of phosphorus in the eutrophication of receiving waters: a review. *Journal of Environmental Quality*, **27**, 261-266
- Diaz R.J. & Rosenberg R. (2008) Spreading dead zones and consequences for marine ecosystems. *Science*, **321** (5891), 926-929.
- Dolman A., Rücker J., Pick F., Fastner J., Rohrlack T., Mischke U. & Wiedner C. (2012) Cyanobacteria and cyanotoxins: the influence of nitrogen vs. Phosphorus. *PloS ONE*, **7**(6), 1-14.
- Dorken M.E. & Barrett S.C.H. (2004) Phenotypic plasticity of vegetative and reproductive traits in monoecious and dioecious populations of *Sagittaria latifolia* (Alismataceae): a clonal aquatic plant. *Journal of Ecology*, **92**, 32-44.
- EC & MWS (Environment Canada & Manitoba Water Stewardship). (2011) State of Lake Winnipeg: 1999- 2007. Available at: http://www.manitoba.ca/waterstewardship/water_quality/state_lk_winnipeg_report/pdf/state_of_lake_winnipeg_rpt_technical_low_resolution.pdf.
- Fernald M.L. (1950) *Gray's Manual of Botany*. American Book Company, New York, New York, USA.
- Feuchtmayr H., Moran R., Hatton K., Connor L., Heyes T., Moss B., Harvey I. & Atkinson D. (2009) Global warming and eutrophication: effects on water chemistry and autotrophic communities in experimental, hypertrophic, shallow lake mesocosms. *Journal of Applied Ecology*, **46**, 713-723.
- Golterman H.L. (1995) The labyrinth of nutrient cycles and buffers in wetlands: results based on research in the Camargue (southern France). *Hydrobiologia*, **315**, 39-58.
- Gonzales Sagrario M., Jeppesen E., Goma J., Søndergaard M., Lauridsen T. & Landkildehus F. (2005) Does high nitrogen loading prevent clear-water conditions in shallow lakes at moderately high phosphorus concentrations? *Freshwater Biology*, **50**, 27-41.

- Harris J.G. & Harris M.W. (1994) *Plant identification terminology: an illustrated glossary*. Spring Lake Publishing, Spring Lake, Utah, USA.
- Haury J., Peltre M.C., Tremolieres M., Barbe J., Thiebaut G., Bernez I., Daniel H., Chatenet P., Haan-Archipof G., Muller S., Dutartre A., Laplace-Treyture C., Cazaubon A. & Lambert-Servien E. (2006) A new method to assess water trophy and organic pollution – The Macrophyte Biological Index for Rivers (IBMR): Its application to different types of river and pollution. *Hydrobiologia*, **570**, 153-158.
- Haynes R.R. & Holm-Nielsen L.B. (1994) The Alismataceae. *Flora Neotropica*, **64**, 1-122.
- Holt E.A. & Miller S.W. (2011) Bioindicators: using organisms to measure environmental impacts. *Nature Education Knowledge*, **3**(10), 8.
- Howard R. & Mendelssohn I. (1995) Effect of increased water depth on growth of a common perennial freshwater-intermediate marsh species in coastal Louisiana. *Wetlands*, **15**, 82-91.
- Loladze I. & Elser J. (2011) The origins of the Redfield nitrogen-to-phosphorus ratio are in a homeostatic protein-to-rRNA ratio. *Ecology Letters*, **144**, 244-250.
- Madsen J.D., Chambers P.A., James W.F., Koch E.W. & Westlake, D. F. (2001) The interaction between water movement, sediment dynamics and submersed macrophytes. *Hydrobiologia*, **444**, 71–84.
- Marburger J. (1993) Biology and management of *Sagittaria latifolia* Willd. (Broad-leaf arrowhead) for wetland restoration and creation. *Restoration Ecology*, **December 1993**, 248-255.
- Moss B., Jeppensen M., Søndergaard M., Lauridsen T. & Zhengwen L. (2013) Nitrogen, macrophytes, shallow lakes and nutrient limitation: resolution of a current controversy? *Hydrobiologia*, **710**, 3-21.
- Neill C. (1990) Effects of nutrients and water levels on emergent macrophyte biomass in a prairie marsh. *Canadian Journal of Botany*, **68**, 1007-1014.
- Phillips G.L., Eminson D.F. & Moss B. (1978) A mechanism to account for macrophyte decline in progressively eutrophicated freshwaters. *Aquatic Botany*, **4**, 103-126.
- Rabalais N.N., Turner R.E. & Wiseman W.J. (2002) Gulf of Mexico hypoxia, aka “dead zone”. *Annual Review of Ecology and Systematics*, **33**, 235-263.

- Rao Y.R., Hawley N., Charlton M.N. & Schertzer W.M. (2008) Physical processes and hypoxia in the central basin of Lake Erie. *Limnology and Oceanography*, **53**(5), 2007-2020.
- Raven P., Evert R. & Eichhorn S. (1999) Plant Nutrition and Soils. In *Biology of Plants* (6th Edition pp 726-749). New York, New York, USA: W.H Freeman and Company
- Richards J.H. & Ivey C.T. (2004) Morphological plasticity of *Sagittaria lancifolia* in response to phosphorus. *Aquatic Botany*, **80**, 53-67.
- Robach F., Thiébault G., Trémolières M. & Muller S. (1996) A reference system for continental running waters: Plant communities as bioindicators of increasing eutrophication in alkaline and acid waters in north-east France. *Hydrobiologia*, **341**, 67-76.
- Sculthorpe C.D. (1967) *The Biology of Aquatic Vascular Plants*. Edward Arnold Publishers Ltd, London, UK.
- Thiebaut G. & Muller S. (1999) A macrophyte communities sequence as an indicator of eutrophication and acidification levels in weakly mineralised streams in north-eastern France. *Hydrobiologia*, **410**, 17-24.
- Trempe H. & Kohler A. (1995) The usefulness of macrophyte monitoring-system, exemplified on eutrophication and acidification of running waters. *Acta Bot Gallica*, **142** (6), 541-550.
- Tyrrell C.D. (2014) The utility of growth form for predicting and evaluating aquatic plant nutrient relations. Ph.D. dissertation. University of New Brunswick, Fredericton, NB, Canada
- Vymazal J., Craft C.B. & Richardson C.J. (2008) Plant community response to long-term N and P fertilization. – In: Richardson C. J. (ed.), *The Everglades experiments*. Springer, New York, New York, USA, pp. 505-527.
- Wooten J. (1986) Variations in leaf characteristics of six species of *Sagittaria* (Alismataceae) caused by various water depths. *Aquatic Botany*, **23**, 321-327.
- Yates A., Culp J. & Chambers P. (2012). Estimating nutrient production from human activities in subcatchments of the Red River, Manitoba. *Journal of Great Lakes Research*, **38**, 106-114.

Chapter 2

Leaf morphology of arrowhead (*Sagittaria cuneata*) as a bioindicator of nitrogen and phosphorus for prairie streams: experimental investigations

2.1 Abstract

The emergent aquatic plant, *Sagittaria cuneata*, has been proposed as a bioindicator of nitrogen (N) and phosphorus (P) in the agriculturally dominated Red River Valley, Manitoba, Canada, because of its leaf plasticity, common occurrence and easily identifiable characteristics. To evaluate its suitability as a nutrient bioindicator, I conducted a controlled experiment where 90 *S. cuneata* plants were grown for 10-weeks in one of 9 different treatment levels where high or low levels of N and P were added to either nutrient-poor water (4 treatment levels) or nutrient-poor sediment (4 treatment levels), in addition to a control treatment level. No differences in leaf size or shape were observed among the sediment treatment levels, or water treatment levels; thus, all sediment treatment levels were pooled into one group and all water treatment levels were pooled into another. Sediment-enriched plants produced 3.8 and 9.3X more emergent leaves than water-enriched and control plants, respectively, and had significantly larger biomass and final height. Emergent leaves produced by sediment-enriched plants were variable in size and shape, with larger leaves produced as the experiment progressed, whereas water-enriched and control plants produced small emergent leaves that remained consistent in size and shape over time. By conducting this experiment in an outdoor mesocosm, thus eliminating and standardizing confounding effects of water depth, clarity, velocity, incident light and sediment composition, this study clearly showed that *S. cuneata* grown in nutrient-enriched sediments exhibited larger leaves, greater productivity, and a unique leaf shape compared to water-enriched and control groups, especially as the experiment progressed. To validate the use of this plant as a bioindicator of nutrients, future studies should test a wider nutrient gradient to

further delve into effects of high and low levels of N and P on *S. cuneata* leaf morphology.

2.2 Introduction

Bioindicators are useful tools to quickly and economically diagnose environmental stressors, and are characterized as common species, or groups of species, with easily identifiable features that exhibit plastic responses to different environmental variables (Holt & Miller 2011). Changes in morphology, behaviour and biochemical processes in certain species, presence or absence of taxa in a community, as well as the structure of the entire biotic community can be used as indicators of stressors, both anthropogenic (pollution, land use changes) and natural (drought, flooding, irregular freeze/thaw events). In streams, bioindicators can be used to detect changes in water quality, such as eutrophication from excess nutrient inputs. The benthic macroinvertebrate (BMI) community is commonly used as a bioindicator in stream habitats (Hodkinson & Jackson 2005) because it possesses many of the ideal traits of bioindicators, such as plastic responses to stressors, high abundance, and easily identifiable characteristics (Holt & Miller 2011). Aquatic macrophytes also possess many beneficial traits of bioindicators: they are usually sessile, easily sampled and identified to genus (and often species), and respond to environmental stressors (e.g., nutrients, light, substrate texture) at the individual, population and assemblage level; however, few studies have explored the use of macrophytes as bioindicators (e.g., Carbiener et al. 1990; Tremp & Kohler 1995; Robach et al. 1996; Thiebaut & Muller 1999; Richards & Ivey 2004; Haury et al. 2006; Ceschin et al. 2010).

The aquatic macrophyte genus *Sagittaria* (Alismataceae) has the potential to be a useful bioindicator of nutrient status because of its easily identifiable characteristics, known plastic leaf morphology, and previous association with environmental conditions. *Sagittaria* is a submersed or emergent genus with extremely variable leaf size and shape (Arber 1920; Fernald 1950; Sculthorpe 1967): the emergent blades range from lanceolate (lance-shaped) to sagittate (arrow-shaped) while the submerged leaves may be subulate (awl-shaped) or ribbon-like. Environmental conditions have been shown to influence leaf size and shape in this genus (e.g., Wooten 1986; Dorken & Barrett 2004; Richards & Ivey 2004). For example, Richards & Ivey (2004) found that *S. lancifolia* plants growing in a high P solution (1 mM) produced wider emergent leaves, more leaves in total and larger plants overall than plants in a low P solution (10 μ M), suggesting that higher P concentration increased plant and leaf size in *S. lancifolia*. Similarly, Dorken & Barrett (2004) noted that emergent leaf size increased with fertilization in *S. latifolia* plants of differing sexual system (i.e., mon- vs. dioecious plants). Water depth also influences leaf size, with decreasing leaf size with increasing depth (Wooten 1986). These predictable changes in leaf size with environmental conditions suggest that the genus *Sagittaria* may be a useful bioindicator of environmental stressors.

Sagittaria cuneata (Alismataceae) is a widespread species across North America (Crowe & Hellquist 2000) and is particularly common and abundant in the prairie region of Manitoba, Canada. Here, excessive nutrient inputs from agriculture, municipal waste water effluent, and animal husbandry contribute to the eutrophication of Lake Winnipeg, the 10th largest freshwater lake on Earth (EC & MWS 2011). Eutrophication is resulting

in many problems within the lake, including potentially toxic algal blooms, and invasion of opportunistic species, such as common carp (*Cyprinus carpio*) and zebra mussels (*Dreissena polymorpha*). The increasing frequency of algal blooms is negatively impacting the recreation and tourism industry and is threatening local fish populations and, consequently, negatively influencing both commercial and recreational fisheries (EC & MWS 2011). These changes are detrimental to ecological functioning in the lake and are causing important economic losses to the province. Thus, the Province of Manitoba and Government of Canada are committed to reducing eutrophication and nutrient loading of Lake Winnipeg, and are funding research to develop bioindicators of nutrients in tributaries of the Red River, which is the highest annual nutrient contributor to Lake Winnipeg (EC & MWS 2011).

The overall aim of my study was to investigate the use of the emergent aquatic macrophyte known as arrowhead or katniss (*Sagittaria cuneata* - Alismataceae), as a bioindicator of nutrient status. To complement a field investigation of *S. cuneata* distribution and abundance in relation to environmental factors (Chapter 3), I conducted a controlled experiment in which plants were grown in an outdoor mesocosm facility in standardized low-nutrient water or sediment, which were independently supplemented with high or low levels of nitrogen (N) and phosphorus (P). By eliminating the confounding effects of variability in incident light, sediment composition and water depth, clarity and velocity, the experimental design was intended to provide a controlled assessment of whether leaf morphology is influenced by N or P in water or sediment and ultimately, whether *S. cuneata* is a useful bioindicator of the nutrient status of southern Manitoba streams.

2.3 Methods

2.3.1 Experimental Design

A controlled experiment was conducted in which 90 *Sagittaria cuneata* plants were grown in 1 of 9 nutrient treatment levels: four combinations of high (H) or low (L) sediment P and N (Sediment-HP, HN; -HP, LN; -LP, HN; and -LP, LN); four combinations of high or low water P and N (Water-HP,HN; -HP, LN; -LP, HN; and -LP,LN); and a control (Tables 2.1 and 2.2). Nutrient concentrations were selected based on high and low N and P values from water and sediment samples collected in streams of the Red River Valley in Manitoba, Canada (Table 2.3, and Chapter 3).

2.3.2 Experimental details

Tubers (i.e., nutrient-filled structures produced on underground rhizomes to overwinter) were collected along the banks of two tributaries (Tobacco Creek (site TC01) and Cooks Creek (site LR01)) with known, abundant populations of monoecious *S. cuneata* in the Red River Valley, Manitoba, Canada, in October 2013 and shipped to the University of New Brunswick (UNB) in chilled coolers within 24 hours of collection. Tubers were cleaned with 5% bleach solution to remove harmful organisms (Hunter-Cario 2007), rinsed with distilled water and stored moist in Ziploc bags at 1-4°C (adapted from McNinch & Garbisch 1991) for 7 months.

Tubers were removed from cold storage, weighed, and planted 5-8 cm deep in a low-nutrient sediment composed of 1/3 loam and 2/3 sand in individual, 12.7 x 12.7 cm square pots on May 24-25th, 2014. Pots were placed in water-filled coolers in the UNB Research Greenhouse to break tuber dormancy. On June 18th, 2014, 90 plants were

selected from the stock for inclusion in the experiment based on health and submerged leaf size. Emergent leaves were removed and any plants not selected for study remained in the greenhouse for observation.

The experiment was conducted in the outdoor mesocosm facility at the Agriculture and Agri-Foods Canada facility in Fredericton, NB, Canada. Treatment levels and plants were randomly assigned to 20 L buckets that were evenly spaced on a level deck covered by a 50% shade cloth to reduce heat exposure. Plants remained in 12.7 x 12.7 cm square pots in which they were germinated, and were placed in water-filled buckets for easy maintenance and observation. Sediment and control treatment level buckets received 16 L of de-chlorinated municipal water. For the water treatment levels, stock solutions were made with municipal water and enriched with potassium phosphate (KH_2PO_4) and ammonium nitrate ($(\text{NH}_4)(\text{NO}_3)$) (Table 2.1), and 16 L was transferred into appropriate buckets. For the sediment treatment levels, nutrients were added in the form of slow release fertilizer (Table 2.2) that was wrapped in two layers of landscaping fabric to allow for permeability and ease of replacement (e.g., Figure 2.1), and buried just below the sediment surface. Nutrient-enriched water and slow-release fertilizer packs were changed biweekly to maintain a consistent nutrient supply, and all buckets were scrubbed with 5% bleach solution and subsequently rinsed to reduce algal build up. Six light and air temperature loggers (HOBO Pendant Temperature/Light Data Logger) were randomly placed on the platform to ensure that light was evenly distributed throughout the study site. Two air pumps were set up to continuously bubble through every bucket to keep CO_2 and oxygen levels consistent among treatment levels.

Plants were monitored daily and all new emergent leaves were traced and digitized using a Canon Scanner to determine if leaf size and shape changed during the growing period. Water depth was measured weekly, and pH, dissolved oxygen (DO), turbidity and specific conductivity (SPC) were measured every 2 to 3 weeks with a YSI handheld multiparameter instrument to ensure consistency among treatment levels and with natural patterns observed in Manitoba (Table 2.3). At the beginning, mid-point and end of the experiment, water samples were taken from a random selection of 27 buckets (3 per treatment level) and shipped within 24 hours to the National Laboratory for Environmental Testing (NLET) in Burlington, Ontario, to determine total nitrogen (TNw) and phosphorus (TPw) concentrations, and TNw to TPw ratios (N:Pw). Sediment was sampled from each pot at the end of the experiment, dried at 60°C for 5 days and then ground and sieved to 1 mm. Three samples per treatment level (27 total) were subsequently analyzed for bioavailable nutrients measured as Olsen-P (henceforth called Sed P; Olsen et al. 1954), and nitrate and ammonium (henceforth called Sed N). Sed P determinations were performed at the Institut de Recherche et de Développement en Agroenvironnement (IRDA) in Québec City, Québec and Sed N analyses were performed at the Agriculture and Agri-Foods Canada research station in Harrow, Ontario.

After a 10-week growing period, all plants were harvested, separated into parts (i.e., roots, stems, tubers and leaves) and individually weighed to determine aboveground (AG) and belowground (BG) biomass, and AG:BG biomass ratio. Tubers produced by each plant were counted, weighed individually, and dried at 60°C for 5

days before being reweighed. All plants and all harvested leaves were placed in a standard plant press and dried at 40°C for 7 days and reweighed.

To determine leaf size and shape, all scanned leaf images were analyzed for size and shape data (Figure 2.2) using ImageJ (Rasband 1997-2014). Leaf size was denoted by 6 measurements: leaf length, blade length, lobe length, blade width, lobe angle and distance between lobes (Figure 2.2A). Differences in leaf shape were assessed using geometric morphometrics (Bookstein 1991; Rohlf & Marcus 1993; Adams et al. 2004; Zelditch et al. 2004; Mitteroecker & Gunz 2009; Adams et al. 2012) based on 12 landmarks chosen to represent leaf shape (Figure 2.2B). Dorken & Barrett (2004) assumed bilateral leaf symmetry and used only 4 landmarks on one side of the leaf (e.g., landmarks 1, 7, 9, 11 from Figure 2.2B) for geometric morphometrics. Landmarks in Figure 2.2B were selected because not all leaves were bilaterally symmetrical, and to include the width of blades and lobes, which are important features of leaf shape. Euclidean coordinates were determined for each landmark on each leaf using ImageJ (Rasband 1997-2014) and used for statistical analyses.

2.3.3 Plant Mortality

Of the 9 treatment levels, all but one had 100% survival from the date of set-up until the end of the experiment 10 weeks later. Treatment level Sediment-LP,HN (Treatment #4 in Tables 2.1 and 2.2) had only 4 of the initial 10 plants survive. During the first 5 weeks of the experiment, plants that died were replaced with plants from the stock growing in the greenhouse. None of the replacement plants died, but slowly deteriorated over the remainder of the experiment. Values of N in sediment and water

for treatment level Sediment-LP,HN exceeded all other values in the experimental study (Tables 2.1 and 2.2) and measured values in Manitoba (Table 2.3). Because of its high mortality, poor performance of replacement plants and high sediment and water N concentrations, treatment level Sediment-LP,HN was removed from statistical analyses, although was included in tables of environmental and chemistry data for comparison.

2.3.4 Statistical Analyses

Testing Model Assumptions

All statistical analyses were completed using the statistical software, *R* (R Development Core Team 2013), except for Mixed Model Analyses of Variance (ANOVAs), where SYSTAT[®] 11.0 was used. The assumption of normality of residuals was tested using the Shapiro-Wilk test (Royston 1982) and viewed using Q-Q plots (Becker et al. 1988) for all ANOVA models. Using the *R* package car v 2.0-19 (Fox & Weisberg 2011), Brown-Forsythe tests were used to test homogeneity of variance for ANOVA (Brown & Forsythe 1974). *R* package lmtest v 0.9-33 (Zeileis & Hothorn 2002) was used to conduct Studentized Bruesch-Pagan (Koenker 1981) tests to assess heteroscedasticity in linear regression models, and diagnostic plots were viewed to visually assess normality of residuals, heteroscedasticity, and linearity for all linear regression models. When necessary, variables were transformed as indicated in tables and figures to meet assumptions.

Initial Analyses

To determine the influence of categorical variables (i.e., treatments, bucket position quantified as row number in proximity to edge of experimental site) on plant

metrics (leaf size, plant height, biomass), one-way fixed effects ANOVAs were conducted using the model: dependent variable = μ + independent variable + ϵ . To determine the association between initial tuber mass and initial plant height with plant metrics, Kendall's *tau* correlation coefficients (Hollander & Wolfe 1973) were determined. Simple linear regressions were conducted to determine the association between the date tubers germinated and the date each plant became emergent (i.e., produced the first emergent leaf) with plant metrics using the model: dependent variable = $\alpha + \beta_1(\text{independent variable}) + \epsilon$.

Environmental and Chemistry Variables

Because pH, specific conductivity, water depth, dissolved oxygen, turbidity, TNw, and TPw were measured at least 3 times during the study, mixed model ANOVAs were calculated to evaluate differences in variables among weeks and treatment levels. The model used was: variable = μ + Treatment + Week + Treatment*Week + Plant (Treatment) + Week*Plant (Treatment) + ϵ , where treatment level and week are fixed effects and plant is a random effect nested in treatment. Sediment chemistry (Sed P and Sed N) was measured once at the termination of the experiment; therefore, one-way fixed effects ANOVAs were calculated to determine differences in sediment chemistry among treatment levels using the model: sediment chemistry = μ + Treatment + ϵ .

Differences in Plant Metrics among Treatment Levels

Differences in plant metrics (final plant height and mass, and average length of all leaves produced by each plant) among treatment levels within each nutrient treatment group were not observed. Therefore, all 3 remaining sediment treatment levels

(Sediment – HP,HN; - HP,LN; - LP,LN) were pooled into one group, and all 4 water treatment levels (Water – HP,HN; -HP,LN; -LP,HN; -LP,LN) were likewise pooled into one group, giving three distinct treatment groups based on nutrient type (i.e., sediment-enriched (30 replicates), water-enriched (40 replicates), and control (10 replicates)). These 3 distinct nutrient treatment groups were considered for further analysis.

Leaf Size

A Pearson correlation matrix was calculated using leaf size and mass data to determine if a summary size variable (SSV) could be used as a proxy for leaf size in ANOVA models. SSVs were considered appropriate if there were significant ($p < 0.05$) correlation coefficients with all other variables, if mean r was large relative to other variables, and based on ease of measurement (i.e., variables with less subjective interpretation). Afterwards, differences in leaf size in nutrient treatment groups over time were assessed using a two-way fixed effects ANOVA of the model: leaf size = μ + Group + Week + Group*Week + ϵ . Tukey's HSD test was used as a *post hoc* analysis to further assess trends in data when a significant main effect or interaction occurred.

Leaf Shape

A generalized Procrustes analysis (GPA; Gower 1975; Rohlf & Slice 1990) was conducted using R package geomorph v 1.1-6 (Adams & Otárola-Castillo 2013). A GPA rotates and scales the Euclidean coordinates of all 12 landmarks (see Figure 2.2) to remove size characteristics from data, resulting in 12 sets of scaled coordinates (i.e., X and Y coordinates for landmarks 1, 2, etc.) that solely represent the shape of individual leaves (henceforth called shape data). To determine whether leaf shape grouped by week

and/or nutrient treatment groups, a principal component analysis (PCA) was conducted on the shape data using *R* package geomorph v 1.1-6 (Adams & Otárola-Castillo 2013).

Differences in leaf shape among nutrient treatment groups were compared temporally to determine whether these differences were consistent over time. A discriminant function analysis (DFA; Ripley 1996) was conducted using shape data from the sampling period when leaf size was most different among nutrient treatment groups (weeks 7 and 8) in *R* package MASS v 7.3-29 (Venables & Ripley 2002). From the DFA of shape data of weeks 7 and 8, classification functions (CFs) were determined for each treatment group (sediment, water and control). These classification functions were used to predict the treatment group to which an unknown emergent leaf would belong based on its shape data. Testing the CFs with data collected from different sampling periods examines whether similar distinctions in leaf shape among treatment groups were found throughout the experiment (i.e., weeks 3 through 6, and 9 through 10). High accuracy of CFs for shape data from each time period would suggest consistent leaf shape in treatment groups over time, whereas low accuracy suggests that leaf shape was changing as the experiment progressed. Prior to analysis, shape variables (i.e., scaled X and Y coordinates of all 12 landmarks) were examined for correlation and strongly correlated variables ($|r| > 0.6$) were removed to avoid collinearity.

2.4 Results

2.4.1 Nutrient Variables

Water chemistry measurements were variable among treatment levels and over time (Table 2.1), and both TN_w and TP_w differed significantly with an interaction

between week and treatment (Table 2.4), suggesting that water chemistry was changing throughout the experiment. The 4 water-enriched treatment levels were designed to have 1.5 and 0.5 mg/L P and 3.0 and 0.6 mg/L N in the High and Low treatment levels, respectively, though these concentrations were not consistently met. However, the efficacy of the water-enriched treatment levels was confirmed because, though concentrations were not always as intended, treatment levels with High added nutrients often had higher TPw and usually had higher TNw concentrations than those with low added nutrients (Table 2.1). TPw and TNw concentrations were more variable among the sediment treatment levels; however, values were generally less than those for the High N and P in water treatment levels. In addition, water chemistry generally decreased with elapsed time following replenishment of water: design concentrations were observed in the water treatment levels only on the occasion when water sampling was conducted 2 days after nutrient replenishment.

Sed P and Sed N were significantly different among treatments ($F_{7,16} = 57.55$, $p < 0.001$ and $F_{7,16} = 29.05$, $p < 0.001$, respectively; Appendix Table A1) and showed similar trends as TPw and TNw in water-enriched treatment levels (Table 2.2). Sediment nutrients were consistently low in control and water treatment levels, and were elevated in 3 of the 4 sediment treatment levels, although the design goal of having High nutrients about 4-5X greater than Low nutrients was not achieved. Sediment-LP, LN resembled more the control and water treatment levels in sediment chemistry, despite having 125 mg/kg N and 130 mg/kg P added biweekly over the 10-week experiment (Figure 2.3).

Ratios of N:P in water were significantly different among treatments ($F_{7,16} = 13.16$, $p < 0.001$; Appendix Table A1). Generally, N:P in water was similar in Water-LP, LN, control and sediment treatment levels (excluding Sediment-LP, HN), and Water-LP, HN had significantly higher N:P than all other treatment levels (Table 2.1). N:P ratios in water represent a large range of N-limited (e.g., Water-HP, HN; -HP, LN), P-limited (e.g., Water-LP, HN) and co-limited (e.g., Control; Water-LP, LN) conditions (Figure 2.4a). N:P in the sediment was also significantly different among treatments ($F_{7,16} = 4.62$, $p = 0.006$; Appendix Table A1); however, average N:P in sediment was less than 7:1 for all treatment levels (Table 2.2; Figure 2.4b). Sediment-LP, HN had extremely high N concentrations in sediment and water, and this fact, combined with the loss of 6 of 10 replicates, resulted in the decision to omit this treatment from further consideration.

2.4.2 Environmental Variables

Light levels and air temperature were comparable to natural ranges measured in Manitoba (Table 2.3), though were slightly variable within the experimental site. Light and air temperature were generally higher for the 4 light and temperature loggers situated near the edge of the experimental site (light range: 0 – 276 000 lux; temperature range: 7 – 46 °C, average: 22 °C) compared to the 2 loggers near the centre of the site (light range: 0 – 143 000 lux; temperature range: 7 – 40 °C, average: 22 °C) (Appendix Figure A1). Despite slight variations in light and temperature regimes across the site, randomization of the treatment levels among the 90 buckets resulted in bucket position (i.e., proximity of each bucket to edges of the experimental site: 1 being on the edge, 2 being the second row from the edge and 3 being in the centre of the site) having no

effect on average leaf size ($F_{2,51} = 3.06$, $p = 0.656$), final plant height ($F_{2,71} = 1.34$, $p = 0.277$), or final plant biomass ($F_{2,77} = 1.12$, $p = 0.333$) (Appendix Table A2).

Physicochemical factors were variable throughout the experiment (Table 2.5), but were generally consistent with measured values in Manitoba (Table 2.3). Mixed Model ANOVAs showed that water depth, turbidity, SPC, and DO differed with an interaction between week and treatment level (Table 2.6). However, pH only differed among weeks. Overall, sediment treatment levels and the control treatment level had similar SPC during the experiment, and water treatments were more variable (Table 2.5). Generally, Water-HP,HN and Water-HP,LN had lower SPC than most other treatments during a given week, and other significant differences did not follow any trends. DO exceeded 85% in all instances and generally, treatment Water-HP,HN had higher DO than other treatments, but other significant differences do not follow a pattern. Turbidity did not exceed 25 NTU in most instances, and was especially variable during weeks 1 and 9, but no consistent patterns emerge. Water depth varied, on average, by about 6.5 cm between any two buckets during a given week, and did not drop below 23 cm, nor exceed 39 cm at any point during the experiment, suggesting that, though there was a significant interaction, water depth was relatively consistent throughout the experiment.

2.4.3 Plant Survival and Growth

Results of ANOVA showed that final plant biomass and plant height were not affected by initial planting conditions: initial tuber mass ($F_{7,72} = 1.63$, $p = 0.141$) and initial plant height ($F_{7,72} = 0.75$, $p = 0.630$) did not differ among treatment levels (Appendix Table A3). Likewise, initial tuber mass and initial plant height were not

significantly correlated with final plant height ($r = 0.11$, $p = 0.165$; $r = 0.06$, $p = 0.298$, respectively) or final plant mass ($r = 0.12$, $p = 0.125$; $r = 0.04$, $p = 0.355$, respectively). Tuber collection site and date tubers were planted did not significantly influence initial plant height ($F_{1,78} = 3.05$, $p = 0.085$ and $F_{1,78} = 0.00$, $p = 0.982$, respectively), final plant height ($F_{1,78} = 1.11$, $p = 0.295$ and $F_{1,78} = 2.82$, $p = 0.097$, respectively), or final plant biomass ($F_{1,78} = 0.01$, $p = 0.936$ and $F_{1,78} = 0.41$, $p = 0.524$, respectively) of experimental plants (Appendix Table A4). Similarly, simple linear regressions showed that date of tuber germination was not associated with initial and final plant height, or final plant mass (Figure 2.5; Appendix Table A5). The date when plants produced the first emergent leaf had no relationship with final plant height and biomass, but showed a weak relationship with initial plant height (Figure 2.5).

Results of ANOVA showed that plants growing in sediment treatment levels were similar among the 3 remaining levels (i.e, Sediment-HP,HN; -HP,LN; -LP,LP). Similarly, plants growing in water treatment levels did not show differences among the 4 levels (i.e., Water-HP,HN; -HP,LN; -LP,HN; -LP,LN). Average leaf length ($F_{2,23} = 1.31$, $p = 0.288$), plant biomass ($F_{2,27} = 1.13$, $p = 0.339$), and final plant height ($F_{2,27} = 0.05$, $p = 0.950$) were not significantly different among the 3 sediment treatment levels; likewise, average leaf length ($F_{3,22} = 2.67$ $p = 0.073$), plant biomass ($F_{3,36} = 1.75$, $p = 0.173$), and final plant height ($F_{3,36} = 1.60$, $p = 0.207$) did not differ among the 4 water treatment levels (Appendix Table A6). Because differences in plant metrics among treatment levels within nutrient treatment groups were not observed, regardless of the observed differences in water and sediment chemistry, all 3 sediment treatment levels were pooled into one group, and all 4 water treatment levels were likewise pooled into

one group, giving three distinct treatment groups based on nutrient enrichment type (i.e., sediment-enriched, water-enriched, and control).

Comparison of plants among the three treatment groups (sediment-enriched, water-enriched and control) demonstrated that sediment-enriched plants were more productive than the other two treatment groups (Table 2.7). Of the plants in the sediment-enriched treatment group, 87% produced emergent leaves compared to the 65 and 20% that produced emergent leaves in water-enriched and control treatment groups, respectively. For tuber productions, 100% of sediment- and 98% of water-enriched plants produced tubers compared to only 70% of control plants. When emergent leaves and tubers were counted, sediment-enriched plants produced 3.8 and 9.3X more leaves and 6.4 and 15.2X more tubers than water-enriched and control treatment groups, respectively. Final biomass, belowground (BG) and aboveground (AG) biomass, AG:BG biomass ratio, tuber mass, final plant height, and number of leaves and tubers per plant were significantly different among nutrient treatment groups (Table 2.8) and Tukey's HSD test showed that sediment-enriched plants had higher final biomass (as well as AG, BG and tuber mass), larger AG:BG biomass ratio, were taller, and had more leaves and tubers than water-enriched and control treatment groups (Table 2.8). In addition, water-enriched plants produced 2.5X more emergent leaves and had significantly more tubers per plant than control plants (Table 2.8).

2.4.4 Leaf Size

Six determinants of leaf size were measured on all emergent leaves collected throughout the experiment, and all were found to be highly correlated ($r > 0.80$, $p <$

0.05; Table 2.9). Leaf length was chosen as a summary size variable (SSV) based on r values (all $r > 0.80$), significant ($p < 0.05$) relationships, and a high mean r (0.92) compared to all other variables (Table 2.9). Area (mean $r = 0.93$) and lobe length (mean $r = 0.94$) were also strong choices; however, length is the simplest measurement (i.e., area requires a computer program to calculate) and requires less subjective interpretation (i.e., lobe length may vary based on landmarks chosen). Other variables were dismissed based on lower mean r values (range: 0.86-0.91) relative to length, area and lobe length.

Leaf length was significantly influenced by an interaction between nutrient treatment groups and weeks (Table 2.10). Results of Tukey's HSD test showed that leaf length was not significantly different among treatment groups during the first week in which emergent leaves were present (i.e., week 3; Figure 2.6). During subsequent weeks, plants in the sediment-enriched treatment group produced emergent leaves that were significantly larger than those grown in water-enriched and control treatment groups. Tukey's HSD test identified that leaves produced in the sediment-enriched treatment group during week 3 were significantly smaller than all other weeks, weeks 4, 5, 6 and 10 were of a similar size, and leaves from weeks 7 and 8 were significantly larger than most other weeks. For plants in the water-enriched and control treatment groups, emergent leaves remained small and similar in size throughout the experiment, though these groups did not produced emergent leaves in weeks 9 and 10.

2.4.5 Leaf Shape

Principal components analysis (PCA) suggested that leaf shapes were variable among nutrient treatment groups and over time (Figure 2.7), though this variation was

relatively low based on scales of the axes for principal components (PC) 1 and PC 2. About 57% of shape variation was explained by the gradients associated with PC 1 and PC 2, which corresponded to the distance between lobes (variation between landmarks 5 and 9) and lobe length (variation between landmarks 5 and 7, or 9 and 7), respectively. Visual examination of PC 1 and PC 2 collectively (Figure 2.7) showed that for the duration of the experiment, leaves produced in the control treatment group clustered near or just left of the PC 1 origin and below PC 2 origin. This treatment group had low replication of emergence, producing only 7 emergent leaves throughout the entire experiment. Similar to control plants, the shape of emergent leaves from the water-enriched treatment group showed little variation over the 10-week experiment, clustering near or below the PC 1 and PC 2 intercept. In contrast, leaf shape of the sediment-enriched treatment group changed on a weekly basis, moving from near the PC 1 and PC 2 intercept on week 3 to extremes on the PC 2 axis (indicative of broader lobe distances) as time passed. On average, leaves produced by plants in the sediment-enriched treatment group also had longer lobes compared to those produced in water-enriched treatment group.

Discriminant functions analysis (DFA) for weeks 7 and 8 showed clear separation of leaves among nutrient treatment groups (Figure 2.8). Classification functions (CF) correctly classified leaves from weeks 7 and 8 into each of the three nutrient treatment groups with 86% accuracy (Table 2.7). Individually, CFs accurately assigned 97% of sediment-enriched leaves, 64% of water-enriched leaves and 33% of control leaves. Using the CFs determined with shape data from weeks 7 and 8, leaves produced during remaining weeks were classified into treatment groups with varying

accuracy (Table 2.11). Generally, as weeks progressed, CFs increased in accuracy, with leaves from weeks 7 and 8 classified with the most accuracy. Leaves produced by the sediment-enriched treatment group were classified with 50% accuracy during weeks 3 and 4, with the remaining 50% of leaves incorrectly classified as belonging to the water-enriched treatment group, suggesting that early leaves produced in the sediment-enriched treatment group resembled water-enriched leaves. After weeks 3 and 4, CF accuracy increased steadily with time period in the sediment-enriched group. CFs were more consistent for water-enriched than sediment-enriched treatment groups, and varied between 56 to 83% accuracy over time. The CF for the control treatment group was variably accurate, likely because of poor emergent leaf replication in this group. Leaves produced by the water-enriched treatment group were incorrectly classified as sediment-enriched most often, and were also classified as controls in some instances.

2.5 Discussion

When the emergent plant *Sagittaria cuneata* was grown under controlled conditions, extreme differences were observed among plants growing in nutrient-enriched soil, nutrient-enriched water, and controls, where no nutrients were added. Sediment-enriched plants were taller, had higher biomass, produced more leaves and tubers, and had larger and distinctly more sagittate leaves than the water-enriched and control treatment groups. Moreover, the latter two groups were indistinguishable in leaf size, plant height and biomass, differing only in the fact that water-enriched plants produced more tubers and leaves than control plants. Many rooted aquatic macrophytes preferentially utilize sediment nutrients over water nutrients (Nichols & Keeny 1976a,b; Best & Mantai 1978; Carignan & Kalff 1980; Barko & Smart 1980, 1981; Chambers &

Kalff 1985; Smith & Adams 1986; Demars & Harper 1998; Madsen et al. 2001), and plant growth is frequently limited by nutrient availability (Ågren et al. 2012). The observation that sediment-enriched plants were more productive than either water-enriched or control plants indicates that *S. cuneata* was better at accessing sediment compared to water nutrient sources. Plants growing in the water-enriched treatment group sustained marginally higher productivity than control plants, consistent with the slightly higher Sed-P concentration of the water-enriched treatment group compared to sediments of the control treatment group. Although nutrients were not added to sediments of the water-enriched treatment levels, P added to the water likely bonded to loam in the sediment (Goltermann 1995). Plants in the control treatment group grew poorly, and often remained in juvenile, submerged form, suggesting that they did not have access to sufficient nutrients to reach maturity and produce emergent leaves. Moreover, the apportionment of biomass between above- and below-ground structures also differed between the 3 nutrient treatment groups: the AG:BG ratio was greater for the plants growing in the sediment-enriched treatment group and did not differ between the water-enriched and control plants. Terrestrial and aquatic plants have been shown to preferentially allocate production to AG biomass when nutrients are not limiting and, conversely, to reduce AG biomass in nutrient limiting habitats (Neill 1990; Hossain et al. 2004; Ket et al. 2011) because of a switch from aboveground to belowground biomass production to increase root surfaces for sediment nutrient uptake in nutrient poor environments (Morris & Bradley 1999; Richards & Ivey 2004; Darby & Turner 2008). In the current study, water-enriched and control plants had limiting sediment nutrients, and were allocating biomass production to roots to increase surfaces for

sediment nutrient uptake. Sediment-enriched plants were not nutrient limited, and thus, allocated nutrients to increase photosynthetic capacity and sustain higher productivity than either of the other nutrient treatment groups. The drastic differences observed between plants in the sediment-enriched treatment group and the water-enriched and control treatment groups, and previous research showing that most rooted macrophytes uptake nutrients from the sediment, suggest that *S. cuneata* utilizes sediment nutrients more than water nutrients, and that nutrient limitation in sediment results in lower productivity and poor performance of *S. cuneata* plants.

In addition to differences in biomass in response to sediment nutrient enrichment, emergent leaf size varied among the three nutrient treatment groups. In the sediment-enriched treatment group, emergent leaves increased in size as the experiment progressed, until peaking during weeks 7 and 8, and subsequently decreasing in size until termination of the experiment. In contrast, leaves produced in water-enriched and control treatment groups were smaller than the sediment-enriched treatment group (except during week 3), and remained similarly sized through the entire experiment. Leaf size of terrestrial plants has been shown to decrease in P-limiting (Atkinson 1973; Jurik et al. 1982) and N-limiting (Medina 1970; Gulmon & Chu 1981; Jurik et al. 1982) environments. Aquatic plants have shown similar trends, and within the genus *Sagittaria*, Wooten (1970, 1986) found decreasing leaf size with increasing water depth of several species and Dorken & Barrett (2004) observed an increase in leaf size of *S. latifolia* with increased fertilization. Similarly, Richards & Ivey (2004) found an increasing width of emergent *S. lancifolia* leaves in higher P environments. In the present study, water depth varied by no more than 6.5 cm among treatment levels during

any given time period and was, on average, consistent among all treatment levels; thus, water depth was unlikely to be influencing leaf size. Rather, nutrients added to the sediment-enriched treatment group were undoubtedly responsible for the greater biomass and increased leaf size of these plants. Since larger leaves increase photosynthetic area (Niinemetsan & Kull 1994; Ribeiro et al. 1994; Ackerly & Donoghue 1998; Stenberg 1998), effectively increasing the amount of solar energy plants can harvest, sediment-enriched plants continued to produce larger leaves as the season progressed to maximize photosynthetic capacity. Near the end of the growing season (weeks 9 and 10), leaf size in the sediment-enriched treatment group began to decrease because plants were allocating nutrients to either clonal (i.e., tuber production) or sexual reproduction (i.e., flower and seed production) instead of vegetative structures (Bazzaz et al. 1987; Reekie & Bazzaz 1987). In fact, upon harvesting, sediment-enriched plants were found to have produced 6.4 and 15.2X more tubers than the water-enriched and control treatment groups, respectively. Plants in water-enriched and control treatment groups had limited sediment nutrients, and without access to high sediment nutrients, could not sustain the high productivity of emergent leaves observed in sediment-enriched plants.

Plants grown in nutrient-enriched sediments showed not only greater productivity and larger leaves, but also a divergence in leaf shape. Specifically, emergent leaves of the sediment-enriched treatment group showed increasingly greater distance between lobes each week, diverging from the water-enriched and control treatment groups as the experiment progressed. Leaf shape of aquatic macrophytes is determined by an interaction of genetic and environmental factors, the most important

environmental factors being water depth and nutrient availability, but also temperature, light quality, day length, and relative humidity (Bruni et al. 1995; Wells & Pigliucci 2000; Dorken & Barrett 2004). In the case of another *Sagittaria* species (*S. latifolia*), Dorken & Barrett (2004) found that while monoecious and dioecious populations produced leaves of differing shapes, leaf shape for plants of both sexual systems changed based on resource availability. In particular, Dorken & Barrett (2004) found that plants in high fertilizer treatments produced thinner leaves than those in low fertilizer treatments, with these differences becoming more pronounced after flowering. Similar to Dorken & Barrett (2004), the current study found differences in leaf shape in response to nutrient-enrichment and with plant maturation. Initially, *S. cuneata* leaves in the sediment-enriched treatment group, changed shape to maximize photosynthetic capacity, and reduce self-shading, as suggested by Nicotra et al. (2011), but as the growing season progressed, shape changes may result from a switch from non-reproductive to mature (reproductive) forms.

The present study identified that *S. cuneata* grown in nutrient-enriched sediments exhibited greater productivity, larger leaves and a unique leaf shape compared to water-enriched and control treatment groups, especially as the experiment progressed. By conducting the experiment in an outdoor mesocosm, the aim was to eliminate confounding effects of variability in water velocity and sediment composition, and standardize environmental factors that are known to influence the growth of macrophytes, such as pH (Titus & Stone 1982; Titus et al. 1990; Pagano & Titus 2004), water depth (Wooten 1986), DO (Goodwin et al. 2008), turbidity (Hudon et al. 2000; Madsen et al. 2001; Jones et al. 2012), incident light (Binzer et al. 2006) and water

temperature (Monneveux et al. 2003). Though these environmental factors were variable (especially DO and turbidity), the effects were negligible based on previous research (e.g., Titus & Stone 1982, Wooten 1986; Hudon et al. 2000; Monneveux et al. 2003; Binzer et al. 2006; Goodwin et al. 2008) and randomization of buckets and treatment levels within the experimental site. Low DO can lead to changes in plant respiration (Peñuelas et al. 1988; Sorrell & Dromgoole 1989), but the level at which these changes occur is uncertain. However, DO in the present study remained over 85% in all instances, and is likely sufficient to maintain plant respiration. Though leaf size is known to be influenced by water depth (e.g., Wooten 1986), the changes in depth were generally minor among treatment levels at any given time (~6.5 cm), and were unlikely influencing leaf size. Light availability is crucial for plant growth, and light decreases with increasing water turbidity (Hudon et al. 2000; Madsen et al. 2001; Jones et al. 2012), leading to a reduction in plant photosynthesis. However, water turbidity becomes irrelevant when plants are emergent, because light is readily available to emergent leaves. Extreme temperatures limit physiological processes, such as photosynthesis (Monneveux et al. 2003), and prolonged exposure may result in dormancy or death (Sculthorpe 1967; Levitt 1972; Christy & Sharitz 1980; Best & Boyd 2003). In the present study, however, temperature did not drop below 0°C and exceeded 40°C in rare, short instances. Although temperature, light, water depth and DO varied among treatments and over time during the 10-week experiment, randomization of treatment levels in the 90 buckets resulted in no significant differences in pH among treatment levels. DO exceeded 85% and turbidity, although showing an interaction between week and treatment, exhibited no consistent pattern in relation to treatment level and became

irrelevant for emergent plants. Moreover, average leaf size, and final plant height and biomass showed no relationship with location of the buckets within the experimental site, suggesting that differences in temperature and light were not influencing plant growth. These observations indicate that the primary factor explaining the greater productivity, and larger and unique shape of *S. cuneata* leaves grown in nutrient-enriched sediments compared to the control and water-enriched treatment groups was undoubtedly the presence of added nutrients in the sediment.

2.6 Conclusions

Although many factors interact to influence the size and shape of emergent *Sagittaria* leaves (e.g., Dorken & Barrett 2004), this experiment has clearly shown that sediment nutrients are crucial to the successful growth of *S. cuneata*, and that leaves produced by plants growing with additions of sediment nutrients were variable in both size and shape as the growing season progressed. While I attempted to establish a high or low, N and P design, the chemistry data, especially in the sediment, did not confirm this design. In addition, plants from high and low, N and P sediment treatments and water treatments did not respond to differences in nutrient concentrations; therefore, it is difficult to determine the singular effects of N and P, and effects of high and low levels of nutrients, on leaf morphology of arrowhead. Given the lack of a clear dose response relationship between leaf shape or size and nutrient concentrations, use of arrowhead as a bioindicator is not yet warranted; however, its leaf plasticity may still prove useful as a guide to levels of sediment nutrients within tributaries of Manitoba. More research into responses of leaf morphology to levels of N and P in the sediment is necessary to fully develop *S. cuneata* as a bioindicator. Due to the progressive changes in leaf shape as the

experiment advanced, using arrowhead as a bioindicator would entail choosing a standardized time to collect leaves, thus removing influence of timing on plants. For example, collecting leaves directly after flowering would be an appropriate time, because flowering is an obvious event, and has been shown to cause changes in leaf shape in *S. latifolia* (Dorken & Barrett 2004).

2.7 References

- Ackerly D.D. & Donoghue M.J. (1998) Leaf size, sapling allometry, and Corner's rules: phylogeny and correlated evolution in maples (*Acer*). *The American Naturalist*, **152**, 767-791.
- Adams D.C. & Otárola-Castillo E. (2013) geomorph: an R package for the collection and analysis of geometric shape data. *Methods in Ecology and Evolution*, **4**, 393-399.
- Adams D.C., Rohlf F.J. & Slice D.E. (2004). Geometric morphometrics: ten years of progress following the 'revolution'. *Italian Journal of Zoology*, **71**, 5-16.
- Adams D.C., Rohlf F.J. & Slice D.E. (2012) A field comes of age: Geometric morphometrics in the 21st century. *Hystrix*, **24**(1), 7-14.
- Ågren G.I., Wetterstedt J.Å.M. & Billberger M.F.K. (2012) Nutrient limitation on terrestrial plant growth - modeling the interaction between nitrogen and phosphorus. *New Phytologist*, **194**, 953-960.
- Arber A. (1920) *Water Plants - A Study of Aquatic Angiosperms*. Cambridge University Press, Cambridge, UK.
- Atkinson D. (1973) Some general effects of phosphorus deficiency on growth and development. *New Phytologist*, **71**, 101-111.
- Barko J.W. & Smart R.M. (1980) Mobilization of sediment phosphorus by submersed freshwater macrophytes. *Freshwater Biology*, **10**, 229-238.
- Barko J.W. & Smart R.M. (1981) Sediment based nutrition of submersed macrophytes. *Aquatic Botany*, **10**, 339-352.
- Bazzaz F., Chiariello N.R., Coley P.D. & Pitelka L.F. (1987) Allocating resources to reproduction and defense. *BioScience*, **37**(1), 58-67.

- Becker R.A., Chambers J.M. and Wilks A.R. (1988) *The New S Language: a programming environment for data analysis and graphics*. Wadsworth & Brooks/Cole, Pacific Grove, CA, USA.
- Best E.P.H. & Boyd W.A. (2003). A simulation model for growth of the submerged aquatic macrophyte sago pondweed (*Potamogeton pectinatus* L.). US Army Corps of Engineers, Washington, DC.
- Best M.D. & Mantai K.E. (1978) Growth of *Myriophyllum*: sediment or lake water as the source of nitrogen and phosphorus. *Ecology*, **59**, 1075-1080.
- Binzer T, Sand-Jensen K & Middelboe A. (2006) Community photosynthesis of aquatic macrophytes. *Limnology and Oceanography*, **51**(6), 2722-2733.
- Bookstein F.L. (1991) *Morphometric tools for landmark data: Geometry and Biology*. Cambridge University Press, Cambridge, UK.
- Brown M.B. & Forsythe A.B. (1974) Robust tests for the equality of variances. *Journal of the American Statistical Association*, **69**, 364-367.
- Bruni N.C., Young J.P. & Dengler N.G. (1995) Leaf developmental plasticity of *Ranunculus flabellaris* in response to terrestrial and submerged environments. *Canadian Journal of Botany*, **74**, 823-837.
- Carbiener R., Trémolières M., Mercier J.L. & Ortscheidt A. (1990) Aquatic macrophytes communities as bioindicators of eutrophication in calcareous oligosaprobe stream waters (Upper Rhine plaine, Alsace). *Vegetatio*, **86**, 71-88
- Carignan R. & Kalff J. (1980) Phosphorus sources for aquatic weeds: Water or sediments? *Science*, **207**, 987- 988.
- Ceschin S, Zuccarello V & Caneva G. (2010) Role of macrophyte communities as bioindicators of water quality: Application on the Tiber River basin (Italy). *Plant Biosystems*, **144** (3), 528-536.
- Chambers P.A. & Kalff J. (1985) The influence of sediment composition and irradiance on growth and morphology of *Myriophyllum spicatum* L. *Aquatic botany*, **22**, 253-263
- Christy E.J. & Sharitz R.R. (1980) Characteristics of three populations of a swamp annual under different temperature regimes. *Ecology*, **6**, 454–460.
- Crow G.E. & Hellquist C.B. (2000) *Aquatic and wetland plants of northeastern North America: volume 2. Angiosperms, monocotyledons*. University of Wisconsin Press, Madison, Wisconsin, USA.

- Darby F.A. & Turner R.E. (2008) Below- and aboveground biomass of *Spartina alterniflora*: response to nutrient addition in a Louisiana salt marsh. *Estuaries and Coasts*, **31**(2), 326-334.
- Demars B.O.L. & Harper D.M. (1998) The aquatic macrophytes of an English lowland river system: assessing response to nutrient enrichment. *Hydrobiologia*, **384**, 75-88.
- Dorken M.E. & Barrett S.C.H. (2004) Phenotypic plasticity of vegetative and reproductive traits in monoecious and dioecious populations of *Sagittaria latifolia* (Alismataceae): a clonal aquatic plant. *Journal of Ecology*, **92**, 32-44.
- EC & MWS (Environment Canada & Manitoba Water Stewardship). (2011) State of Lake Winnipeg: 1999-2007. Available at: https://www.gov.mb.ca/waterstewardship/water_quality/state_lk_winnipeg_report/pdf/state_of_lake_winnipeg_rpt_technical_high_resolution.pdf
- Fernald M.L. (1950) *Gray's Manual of Botany*. American Book Company, New York, USA.
- Fox J. & Weisberg S. (2011) *An {R} companion to applied regression*, Second Edition. Sage, Thousand Oaks, CA, USA.
<http://socserv.socsci.mcmaster.ca/jfox/Books/Companion>.
- Golterman H.L. (1995) The labyrinth of nutrient cycles and buffers in wetlands: results based on research in the Camargue (southern France). *Hydrobiologia*, **315**, 39-58.
- Goodwin K., Caraco N. & Cole, J. (2008) Temporal dynamics of dissolved oxygen in a floating-leaved macrophyte bed. *Freshwater Biology*, **53**, 1632-1641.
- Gower J.C. (1975) Generalized Procrustes analysis. *Psychometrika*, **40**, 33-51.
- Gulmon S.L. & Chu C.C. (1981) The effects of light and nitrogen on photosynthesis, leaf characteristics, and dry matter allocation in the chaparral shrub, *Diplacus aurantiacus*. *Oecologia*, **49**, 207-212.
- Haury J., Peltre M.C., Tremolieres M., Barbe J., Thiebaut G., Bernez I., Daniel H., Chatenet P., Haan-Archipof G., Muller S., Dutartre A., Laplace-Treytoure C., Cazaubon A. & Lambert-Servien E. (2006) A new method to assess water trophy and organic pollution – The Macrophyte Biological Index for Rivers (IBMR): Its application to different types of river and pollution. *Hydrobiologia*, **570**, 153–158.

- Hodkinson I & Jackson J. (2005) Terrestrial and aquatic invertebrates as bioindicators for environmental monitoring, with particular reference to mountain ecosystems. *Environmental Management*, **35** (5), 649-666.
- Hollander M. & Wolfe D.A. (1973) *Nonparametric Statistical Methods*. John Wiley & Sons. New York, New York, USA.
- Holt E.A. & Miller S.W. (2011) Bioindicators: using organisms to measure environmental impacts. *Nature Education Knowledge*, **3**(10), 8.
- Hossain M.A., Ishimine Y., Hikaru A. & Kuramochi H. (2004) Effect of nitrogen fertilizer application on growth, biomass production and N-uptake of torpedograss (*Panicum repens* L.). *Weed Biology and Management*, **4**, 86-94.
- Hudon C., Lalonde S. & Gagnon P. (2000) Ranking the effects of site exposure, plant growth form, water depth, and transparency on aquatic plant biomass. *Canadian Journal of Fisheries and Aquatic Sciences*, **57**, 31-42.
- Hunter-Cario L. (2007) Propagation protocol for broadleaf arrowhead *Sagittaria latifolia* Willd. (Alismataceae). *Native Plants Journal*, **8**(2), 80-83.
- Jones J.I., Collins A.L., Naden P.S. & Sear D.A. (2012) The relationship between fine sediment and macrophytes in rivers. *River Research and Application*, **28**, 1006-1018.
- Jurik T.W., Chabot J.F & Chabot B.F. (1982) Effects of light ad nutrients and leaf size, CO₂, exchange, and anatomy in wild strawberry (*Fragaria virginiana*). *Plant Physiology*, **70**(4), 1044-1048.
- Ket W.A, Schubauer-Berigan J.P & Craft C.B. (2011) Effects of five years of nitrogen and phosphorus additions on a *Zizaniopsis miliacea* tidal freshwater marsh. *Aquatic Botany*, **95**(1), 17-23.
- Koenker R. (1981) A note on studentizing a test for heteroscedasticity. *Journal of Econometrics*, **17**, 107-112.
- Levitt J. (1972) *Responses of plants to environmental stresses*. Academic Press, New York, New York, USA.
- Madsen J.D., Chambers P.A., James W.F., Koch E.W. & Westlake, D. F. (2001) The interaction between water movement, sediment dynamics and submersed macrophytes. *Hydrobiologia*, **444**, 71-84.
- McIninch S. & Garbisch E. (1991) Oxygen requirement of dormant wetland plants. *Wildflower*, **4**, 8-13.

- Medina E. (1970) Relationship between nitrogen level, photosynthetic capacity, and carboxydismutase activity in *Atriplex patula* leaves. *Carnegie Inst Wash Year Book*, **69**, 655-662.
- Miller R.G. (1981) *Simultaneous Statistical Inference*. Springer, New York, New York, USA.
- Mitteroecker P. & Gunz P. (2009) Advances in geometric morphometrics. *Evolutionary Biology*, **36**, 235-247.
- Monneveux P., Pastenes C. & Reynolds MP (2003) Limitations to photosynthesis under light and heat stress in three high-yielding wheat genotypes. *Journal of Plant Physiology*, **160**, 657-666.
- Morris J.T. & Bradley P.M (1999) Effects of nutrient loading on the carbon balance of coastal wetland sediments. *Limnology and Oceanography*, **44**, 699-702.
- Neill C. (1990) Effects of nutrients and water levels on emergent macrophyte biomass in a prairie marsh. *Canadian Journal of Botany*, **68**, 1007-1014.
- Nichols D.S. & Keeney D.R. (1976a) Nitrogen nutrition of *Myriophyllum spicatum*: variation of plant tissue nitrogen concentration with season and site in Lake Wingra. *Freshwater Biology*, **6**, 137-144.
- Nichols D.S. & Keeney D.R. (1976b) Nitrogen nutrition of *Myriophyllum spicatum*: uptake and translocation of N by shoots and roots. *Freshwater Biology*, **6**, 145-154.
- Nicotra, A.B., Leigh A., Boyce C.K., Jones C.S., Niklas K.J, Royer D.L. & Tsukaya H. (2011) The evolution and functional significance of leaf shape in the angiosperms. *Functional Plant Biology*, **38**, 535–552.
- Niinemets U. & Kull K. (1994) Leaf weight per area and leaf size of 85 Estonian woody species in relation to shade tolerance and light availability. *Forest Ecology and Management*, **70**, 1-10.
- Olsen S.R., Cole C.V., Watanabe F.S. & Dean L.A. (1954) Estimation of available phosphorus in soils by extraction with sodium bicarbonate. USDA circular 939. U.S. Government Printing Office, Washington, DC, USA.
- Pagano A. & Titus J. (2004) Submersed macrophyte growth at low pH: Contrasting responses of three species to dissolved inorganic carbon enrichment and sediment type. *Aquatic Botany*, **79**, 65-74.

- Peñuelas J., Murillo J. & Azcón-Bieto, J. (1988) Actual and potential dark respiration rates and different electron transport pathways in freshwater aquatic plants. *Aquatic Botany*, **30**, 353-362.
- R Development Core Team. (2013) R: A language and environment for statistical computing. *R Foundation for Statistical Computing*, Vienna, Austria. <http://www.R-project.org/>.
- Rasband W.S. (1997-2014) ImageJ, U. S. National Institutes of Health, Bethesda, Maryland, USA. <http://imagej.nih.gov/ij/>.
- Reekie E.G. & Bazzaz F. (1987) Reproductive effort in plants. 3. Effect of reproduction on vegetative activity. *The American Naturalist*, **129**(6), 907–919.
- Ribeiro S.P., Pimenta H.R. & Fernandes G.W. (1994) Herbivory by chewing and sucking insects on *Tabebuia ochracea*. *Biotropica*, **26**, 302-307.
- Richards J.H. & Ivey C.T. (2004) Morphological plasticity of *Sagittaria lancifolia* in response to phosphorus. *Aquatic Botany*, **80**, 53-67.
- Ripley B.D. (1996) *Pattern Recognition and Neural Networks*. Cambridge University Press. Cambridge, UK.
- Robach F., Thiébault G., Trémolières M. & Muller S. (1996) A reference system for continental running waters: Plant communities as bioindicators of increasing eutrophication in alkaline and acid waters in north-east France. *Hydrobiologia*, **341**, 67–76.
- Rohlf F.J. & Marcus L.F. (1993) A revolution in morphometrics. *Trends in Ecology & Evolution*, **8**, 129-132.
- Rohlf F.J. & Slice D.E. (1990) Extensions of the Procrustes method for the optimal superimposition of landmarks. *Systematic Zoology*, **39**, 40-59.
- Royston P. (1982) An extension of Shapiro and Wilk's *W* test for normality to large samples. *Applied Statistics*, **31**, 115-124.
- Sculthorpe C.D. (1967) *The Biology of Aquatic Vascular Plants*. Edward Arnold Publishers Ltd, London, UK.
- Smith C.S. & Adams M.S. (1986) Phosphorus transfer from sediment by *Myriophyllum spicatum*. *Limnology and Oceanography*, **31**, 1312-1321.
- Sorrell B.K. & Dromgoole F.I. (1989) Oxygen diffusion and dark respiration in aquatic macrophytes. *Plant, Cell & Environment*, **12**, 293-299.

- Stenberg P. (1998) Implications of shoot structure on the rate of photosynthesis at different levels in a coniferous canopy using a model incorporating grouping and penumbra. *Functional Ecology*, **12**, 82-91.
- Thiebaut G. & Muller S. (1999) A macrophyte communities sequence as an indicator of eutrophication and acidification levels in weakly mineralised streams in north-eastern France. *Hydrobiologia*, **410**, 17-24.
- Titus J, Feldman R & Gris   D. (1990) Submersed macrophyte growth at low pH. I. CO₂ enrichment effects with fertile sediment. *Oecologia*, **84**(3), 307-313.
- Titus J & Stone W. (1982) Photosynthetic response of two submersed macrophytes to dissolved inorganic carbon concentration and pH. *Limnology and Oceanography*, **27**(1), 151-160.
- Trempe H. & Kohler A. (1995) The usefulness of macrophyte monitoring-system, exemplified on eutrophication and acidification of running waters. *Acta Bot Gallica*, **142** (6), 541-550.
- Venables W.N. & Ripley B.D. (2002) *Modern Applied Statistics with S*. Fourth Edition. Springer, New York, New York, USA.
- Wells C.L. & Pigliucci M. (2000) Adaptive phenotypic plasticity: the case of heterophylly in aquatic plants. *Perspectives in Plant Ecology, Evolution and Systematics*, **3**, 1-18.
- Wooten J.W. (1970) Experimental investigations of the *Sagittaria graminea* complex: Transplant studies and genecology. *Journal of Ecology*, **58**(1), 233-242.
- Wooten J.W. (1986) Variations in leaf characteristics of six species of *Sagittaria* (Alismataceae) caused by various water levels. *Aquatic botany*, **23**, 321-327.
- Zeileis A. & Hothorn T. (2002) Diagnostic Checking in Regression Relationships. *R News*, **2**(3), 7-10. <http://CRAN.R-project.org/doc/Rnews/>.
- Zelditch M.L., Swiderski D.L., Sheets H.D. & Fink W.L. (2004) *Geometric morphometrics for biologists: a primer*. Elsevier/Academic Press, Amsterdam.

2.8 Tables and Figures

Table 2.1. Water nutrients in a 10-week nutrient enrichment experiment: experimental design and conditions used to assess the effect of nutrient enrichment in the water on *Sagittaria cuneata* characteristics. Indicated are the nutrient additions to water every two weeks, total nutrients added for the entire experiment, average ($\pm$ SE) total nitrogen (TNw) and phosphorus (TPw) in the water, sampled on 3 dates, and average N:P ratio throughout the entire experiment. The following notation is used: high (H), low (L), nitrogen (N), phosphorus (P), water (w). Each treatment level had 10 replicates, except treatment level 4 (Sediment – LP,HN), which had 4. “Days” indicates number of days elapsed between when water was replenished with nutrients and when water was sampled. Note: water nutrients were not added to sediment or control treatment levels. For manipulation of sediment nutrients in the sediment treatment levels, see Table 2.2.

#	Treatment Level	Nutrients Added to Water (mg/L)		Total Nutrients Added (mg)		TPw (mg/L)			TNw (mg/L)			Avg. N:P
		P	N	P	N	Early Aug (2 days)	Mid July (12 days)	Late Aug (20 days)	Early Aug (2 days)	Mid July (12 days)	Late Aug (20 days)	
1	Control	0	0	0	0	0.006 (0.0001)	0.008 (0.001)	0.011 (0.002)	0.089 (0.002)	0.190 (0.019)	0.175 (0.011)	14.69 (1.75)
Sediment												
2	HP,HN	0	0	0	0	0.055 (0.014)	0.021 (0.004)	0.489 (0.201)	0.167 (0.013)	0.738 (0.316)	1.990 (0.402)	16.80 (8.75)
3	HP,LN	0	0	0	0	0.042 (0.009)	0.036 (0.011)	0.137 (0.054)	0.164 (0.016)	0.325 (0.029)	2.633 (0.982)	18.29 (9.90)
4	LP,HN ¹	0	0	0	0	0.021 (0.002)	0.059 (0.044)	0.079 (0.049)	0.283 (0.088)	3.65 (1.808)	12.113 (1.047)	270.29 (122.60)
5	LP,LN	0	0	0	0	0.041 (0.085)	0.014 (0.031)	0.017 (0.0001)	0.215 (0.013)	0.721 (0.001)	0.328 (0.003)	27.43 (14.86)
Water												
6	HP,HN	1.5	3.0	120	240	1.468 (0.005)	0.135 (0.071)	0.084 (0.013)	2.750 (0.024)	0.428 (0.11)	0.492 (0.069)	4.07 (0.70)
7	HP,LN	1.5	0.6	120	48	1.520 (0.004)	0.123 (0.043)	0.134 (0.017)	0.622 (0.019)	0.585 (0.21)	1.071 (0.147)	4.51 (1.21)
8	LP,HN	0.5	3.0	40	240	0.018 (0.002)	0.023 (0.001)	0.023 (0.002)	2.89 (0.014)	0.663 (0.184)	1.15 (0.194)	87.97 (21.53)
9	LP,LN	0.5	0.6	40	48	0.019 (0.001)	0.017 (0.001)	0.023 (0.003)	0.563 (0.008)	0.271 (0.045)	0.276 (0.005)	20.11 (2.76)

¹Sediment- LP,HN included for comparison only

Table 2.2. Sediment nutrients in a 10-week nutrient enrichment experiment: experimental design and conditions used to assess the effect of nutrient enrichment in the sediment on *Sagittaria cuneata* characteristics. Indicated are the nominal amount of nutrients added to sediment every two weeks, fertilizer type and amount added to sediment every two weeks, total nominal amount of nutrients added over the entire experiment, and final concentrations of nitrogen (Sed N, measured as nitrate and ammonia) and phosphorus (Sed P, measured as Olsen P) in the sediment (mean $\pm$ SE), as well as the ratio of Sed N to Sed P (N:P). The following notation is used: high (H), low (L), nitrogen (N), phosphorus (P), sediment (Sed). Each treatment had 10 replicates, except treatment level 4 (Sediment- LP,HN), which had 4 due to plant mortality. Note: sediment nutrients were not added to control or water treatment levels. For manipulation of water nutrients in the water treatment levels, see Table 2.1.

#	Treatment Level	Nutrients Added to Sediment (mg/kg)		Fertilizer Amount & Type (N-P-K)	Total Nutrients Added (g)		Final Concentrations (mg/kg)		
		P	N		Sed P	Sed N	Sed P	Sed N	N:P
1	Control	0	0	N/A	0	0	8.02 (1.35)	17.35 (2.25)	2.26 (0.40)
	Sediment								
2	HP,HN	405	500	5 g Plant-Prod Smartcote (14-14-14)	1.53	1.92	174.67 (36.83)	50.38 (8.66)	0.30 (0.02)
3	HP,LN	405	125	1.5 g Miracle-Gro Shake 'n Feed (9-18-9) + 3 g bonemeal	1.51	0.40	37.60 (3.20)	72.98 (6.51)	1.94 (0.05)
4	LP,HN ¹	130	500	6 g Miracle-Gro Shake 'n Feed (12-4-8)	0.51	1.82	67.00 (43.64)	239.16 (93.37)	6.95 (4.75)
5	LP,LN	130	125	1.5 g Plant-Prod Smartcote (14-14-14)	0.46	0.58	10.06 (1.85)	13.41 (1.38)	1.42 (0.31)
	Water								
6	HP,HN	0	0	NA	0	0	9.32 (0.86)	15.98 (3.35)	1.81 (0.54)
7	HP,LN	0	0	NA	0	0	8.81 (1.14)	14.87 (1.49)	1.70 (0.08)
8	LP,HN	0	0	NA	0	0	8.43 (0.72)	14.81 (1.16)	1.77 (0.11)
9	LP,LN	0	0	NA	0	0	8.62 (0.67)	13.38 (0.38)	1.57 (0.13)

¹Sediment – LP,HN included for comparison only

Table 2.3. Average ($\pm$ SE), minimum and maximum values of environmental variables observed during a field study in streams in the Red River Valley, Manitoba, Canada, in July 2013 and August 2014, and during a 10-week nutrient experimental study conducted in New Brunswick, Canada from June to August 2014.

Variable	Field Study			Experimental Study		
	Avg.	Min.	Max.	Avg.	Min.	Max.
pH	8.3 (0.1)	7.9	8.7	8.7 (0.1)	8.0	10.6
DO (%)	78 (4)	20	120	105 (0.5)	85	158
Water Depth (cm)	36 (1)	5	98	27.3 (0.1)	22.6	39.0
Light (lux)	43 000 (233)	0	330 669	42 000 (307)	0	275 557
Air Temp. (°C)	20.8 (0.1)	0	51	22.9 (0.1)	6.6	45.9
SPC (S/m)	688 (54)	290	1442	252 (1.6)	161	379
TPw (mg/L)	0.360 (0.121)	0.038	2.739	0.187 (0.417)	0.006	1.530
TNw (mg/L)	1.267 (0.107)	0.528	2.510	1.416 (0.272)	0.086	14.900
Sed P (mg/kg)	30.77 (2.12)	4.76	126.00	36.95 (11.50)	5.72	228.00
Sed N (mg/kg)	60.25 (4.14)	5.84	193.43	50.26 (16.2)	10.64	370.96
N:P ratio (sediment)	2.22 (0.15)	0.38	6.40	2.19 (0.56)	0.27	16.45
N:P ratio (water)	10.42 (2.22)	0.916	37.474	32.78 (17.80)	0.378	517.86

Table 2.4. Mixed Model ANOVA results of water chemistry measured 3 times during a 10-week nutrient experiment (beginning, middle and end of the experiment). Total nitrogen (TNw) and phosphorus (TPw) were measured in the same 24 buckets (3/nutrient treatment level) each time. Buckets contained one *Sagittaria cuneata* plant that was growing in 1 of 7 nutrient treatment levels manipulating either sediment or water nutrients, or a control, and water was changed biweekly in each bucket. Significant ($p < 0.05$) and interpretable results are indicated by bold font.

Dependent Variable	Source of Variation	d.f.	Mean Square	F	p
log ₁₀ (TNw)	Treatment	7	0.75	29.84	< 0.001
	Week	2	0.25	7.26	0.003
	Treatment * Week	14	0.40	11.66	< 0.001
	Plant (Treatment)	16	0.03	.	.
	Week * Plant (Treatment)	32	0.03	.	.
	Residual	0	.	.	.
log ₁₀ (TPw)	Treatment	7	2.81	50.04	< 0.001
	Week	2	0.94	26.31	< 0.001
	Treatment * Week	14	0.50	14.08	< 0.001
	Plant (Treatment)	16	0.06	.	.
	Week * Plant (Treatment)	32	0.04	.	.
	Residual	0	.	.	.

Table 2.5. Water conditions in the nutrient enrichment experiment where 80 *Sagittaria cuneata* plants were grown in individual buckets for 10 weeks. Presented are the average ($\pm$ SE) dissolved oxygen (DO), specific conductivity (SPC), turbidity, pH and depth of the water in each of 9 treatment levels. The following notation is used: high (H) or low (L), nitrogen (N) and phosphorus (P) (see also Tables 2.1 and 2.2). Each treatment had 10 replicates, except for Sediment-LP,HN (Treatment level 4) which had 4 due to plant mortality. DO, SPC, turbidity and pH were measured every 2-3 weeks (4-5 times during the experiment) and water depth was measured weekly starting on week 2 (9 times during the experiment).

#	Treatment Level	DO (%)	SPC (μ S/cm)	Turbidity (NTU)	pH	Water Depth (cm)
1	Control	101 (1)	265 (4)	2.6 (0.5)	8.51 (0.03)	27.6 (0.3)
	Sediment					
2	HP,HN	110 (2)	247 (5)	9.3 (1.0)	8.67 (0.17)	27.5 (0.2)
3	HP,LN	104 (1)	269 (4)	8.4 (1.3)	8.68 (0.03)	27.6 (0.3)
4	LP,HN¹	105 (2)	331 (12)	8.0 (1.8)	8.50 (0.06)	27.1 (0.4)
5	LP,LN	102 (1)	264 (4)	4.9 (0.6)	8.68 (0.03)	27.7 (0.2)
	Water					
6	HP,HN	111 (2)	231 (4)	3.9 (0.5)	9.08 (0.09)	27.3 (0.3)
7	HP,LN	107 (1)	251 (4)	2.6 (0.6)	8.78 (0.06)	27.3 (0.3)
8	LP,HN	103 (1)	240 (5)	4.1 (0.6)	8.62 (0.03)	27.0 (0.2)
9	LP,LN	102.3 (1)	250 (4)	4.6 (2.0)	8.55 (0.17)	27.0 (0.2)

¹Sediment- LP,HN is included for comparison only

Table 2.6. Mixed Model ANOVA results of environmental variables measured 4-9 times during a 10-week nutrient experiment. Water depth (9 measures), turbidity (4 measures), specific conductivity (SPC, 4 measures), dissolved oxygen (DO, 5 measures) and pH (5 measures) were measured in 80 buckets (10/nutrient treatment level) during the experiment. Buckets contained one *Sagittaria cuneata* plant that was growing in 1 of 7 nutrient treatment levels manipulating either sediment or water nutrients, or a control (see tables 2.1 and 2.2). Significant ($p < 0.05$) and interpretable results indicated by bold font.

Dependent Variable	Source of Variation	d.f.	Mean Square	F	p
Water Depth (cm)	Treatment	7	5.34	1.20	0.315
	Week	8	277.54	278.30	< 0.001
	Treatment * Week	56	1.74	1.75	0.001
	Bucket (Treatment)	72	4.46	.	.
	Week * Bucket (Treatment)	576	0.99	.	.
	Residual	0	.	.	.
Turbidity (NTU)	Treatment	7	225.21	8.99	< 0.001
	Week	3	458.39	15.96	< 0.001
	Treatment * Week	21	143.21	4.99	< 0.001
	Bucket (Treatment)	72	25.03	.	.
	Week * Bucket (Treatment)	216	28.72	.	.
	Residual	0	.	.	.
SPC (S/m)	Treatment	7	7113.44	11.93	< 0.001
	Week	3	29 914.54	148.53	< 0.001
	Treatment * Week	21	1937.09	9.62	< 0.001
	Bucket (Treatment)	72	596.23	.	.
	Week * Bucket (Treatment)	216	201.40	.	.
	Residual	0	.	.	.
log ₁₀ (DO) (%)	Treatment	7	0.01	21.62	< 0.001
	Week	4	0.11	428.41	< 0.001
	Treatment * Week	28	0.00	10.301	< 0.001
	Bucket (Treatment)	72	0.00	.	.
	Week * Bucket (Treatment)	288	0.00	.	.
	Residual	0	.	.	.
log ₁₀ (pH)	Treatment	7	0.01	1.50	0.182
	Week	4	0.01	3.85	0.005
	Treatment * Week	28	0.00	0.97	0.511
	Bucket (Treatment)	72	0.00	.	.
	Week * Bucket (Treatment)	288	0.00	.	.
	Residual	0	.	.	.

Table 2.7. Plant production at the termination of the 10-week nutrient enrichment experiment growing *Sagittaria cuneata* in individual buckets. Treatment groups are the control (n = 10), the plants with nutrients added to the water (n = 40), and the plants with nutrients added to the sediment (n = 30). Indicated are percentage of plants that produced emergent leaves and tubers, and average ($\pm$ SE) plant biomass, aboveground (AG) and belowground (BG) biomass, AG:BG biomass ratio, total tuber mass, number of leaves and tubers per plant, and final height of *Sagittaria cuneata* plants. Values with different lower-case letters for a given response variable denote significance ($p < 0.05$) based on Tukey's Honestly Significant Difference (HSD) test (see Table 2.8).

Treatment Group	Produce Leaves (%)	Produce Tubers (%)	Biomass (g)	AG Biomass (g)	BG Biomass (g)	AG:BG Biomass	Tuber Mass (g)	Leaves per plant	Tubers per plant	Height (cm)
Control	20	70	2.39 a (0.37)	0.90 a (0.13)	1.40 a (0.215)	0.53 a (0.12)	0.13 a (0.05)	0.7 a (0.5)	0.9 a (0.2)	20.4 a (0.6)
Sediment	87	100	45.86 b (5.66)	22.67 b (2.89)	21.89 b (2.99)	0.77 b (0.07)	4.44 b (0.53)	7.1 b (0.7)	15.2 b (1.5)	31.7 b (1.4)
Water	65	98	3.66 a (0.27)	1.56 a (0.11)	2.04 a (0.17)	0.55 a (0.05)	0.28 a (0.05)	1.7 a (0.3)	2.1 c (0.2)	23.7 a (0.6)

Table 2.8. Results of ANOVA and *post hoc* comparisons examining differences in plant metrics of *Sagittaria cuneata* for each nutrient treatment group (sediment-enriched, water-enriched and control; see Appendix Table A6) in a 10-week nutrient experiment. The following notation is used: AG, aboveground biomass; BG, belowground biomass; AG:BG, above- to belowground biomass ratio. All measurements (biomass, tuber mass, and height) and counts (tubers and leaves) were conducted at the end of the experiment. Dependent variables were all log transformed. Significant ($p < 0.05$) and interpretable results are indicated by bold font

Dependent Variable	Source of Variation	d.f.	Mean Square	F	p	Tukey's HSD <i>p</i> -values		
						Sediment - Water	Sediment - Control	Water - Control
Biomass	Group	2	52.97	102.56	<0.001	<0.001	<0.001	0.215
	Residual	77	0.52					
AG Biomass	Group	2	59.06	101.71	<0.001	<0.001	<0.001	0.113
	Residual	77	0.58					
BG Biomass	Group	2	47.80	85.86	<0.001	<0.001	<0.001	0.359
	Residual	77	0.56					
AG:BG Biomass	Group	2	0.67	5.92	0.004	0.007	0.039	0.279
	Residual	77	0.11					
Tuber Mass	Group	2	15.54	72.30	<0.001	<0.001	<0.001	0.785
	Residual	77	0.22					
Leaves per Plant	Group	2	13.75	25.64	<0.001	<0.001	<0.001	0.135
	Residual	77	0.54					
Tubers Per Plant	Group	2	27.03	101.96	<0.001	<0.001	<0.001	0.026
	Residual	77	0.27					
Height	Group	2	0.70	5.62	0.005	0.015	0.023	0.503
	Residual	77	0.13					

Table 2.9. Pearson correlation matrix of r values of all measured leaf size and mass characteristics of *Sagittaria cuneata* leaves collected at the termination of a 10-week nutrient amendment experiment. B/W refers to the distance between the lobes of each plant (see Figure 2.2). All correlations are significant ($p < 0.05$).

Variable	Area	Width	Blade Length	Lobe Length	B/W Lobes	Length	Fresh Mass
Width	0.97						
Blade Length	0.95	0.94					
Lobe Length	0.92	0.95	0.93				
B/W Lobes	0.84	0.90	0.85	0.91			
Length	0.96	0.96	0.98	0.98	0.88		
Fresh Mass	0.91	0.90	0.87	0.83	0.79	0.86	
Dry Mass	0.93	0.93	0.84	0.84	0.82	0.85	0.89

Table 2.10. Two-way ANOVA results of differences in length of emergent *Sagittaria cuneata* leaves among 3 nutrient treatment groups (sediment, water and control; see Appendix Table A6), and weeks during a 10-week nutrient amendment experiment. Plant began producing emergent leaves in week 3 of the experiment, and leaves were not produced by all groups during weeks 5, 9 and 10. Leaf length was log transformed to meet assumptions of homogeneity of variance and normality of residuals. Tukey's HSD results for significant interaction are presented in Figure 2.6. Significant ($p < 0.05$) and interpretable results are indicated by bold font.

Dependent Variable	Source of Variation	<i>d.f.</i>	Mean Square	<i>F</i>	<i>p</i>
log₁₀ (Leaf Length)	Group	2	12.17	186.48	<0.001
	Week	4	1.41	21.60	<0.001
	Group x Week	8	0.59	9.03	<0.001
	Residual	172	0.07		

Table 2.11. Assessment of changes in leaf shape of each nutrient treatment group during the 10-week nutrient enrichment experiment with *Sagittaria cuneata*. Treatment groups are the control (n = 10), the plants with nutrients added to the water (n = 40), and the plants with nutrients added to the sediment (n = 30) (see Appendix Table A6). Indicated are the number of emergent *S. cuneata* leaves produced during each time period, and percentage of leaves from each period that were correctly classified into each nutrient treatment group using classification functions produced by a discriminant functions analysis of leaf shape data during weeks 7 and 8. In addition, percentage of emergent leaves produced by sediment-enriched plants incorrectly classified as water-enriched, and water-enriched leaves incorrectly classified as sediment-amended plants are included. NA represents the lack of emergent leaves produced during weeks 9 and 10 in the water-amended and unamended control groups.

Weeks	Leaves Produced	Total Accuracy (%)	Correctly Classified			Incorrectly Classified	
			Sediment (%)	Water (%)	Control (%)	Sediment classified as Water (%)	Water classified as Sediment (%)
3 and 4	24	54	50	83	0	50	17
5 and 6	91	69	75	56	100	25	41
7 and 8	94	86	97	64	33	3	28
9 and 10	63	81	81	NA	NA	19	NA



Figure 2.1. Example fertilizer ball used to manipulate sediment nutrients in a 10-week nutrient experiment. Fertilizer balls were made with slow-release fertilizer and wrapped with two layers of landscaping fabric to ensure easy replacement every two weeks. Also pictured are *Sagittaria cuneata* roots that grew into the ball to access the nutrients.

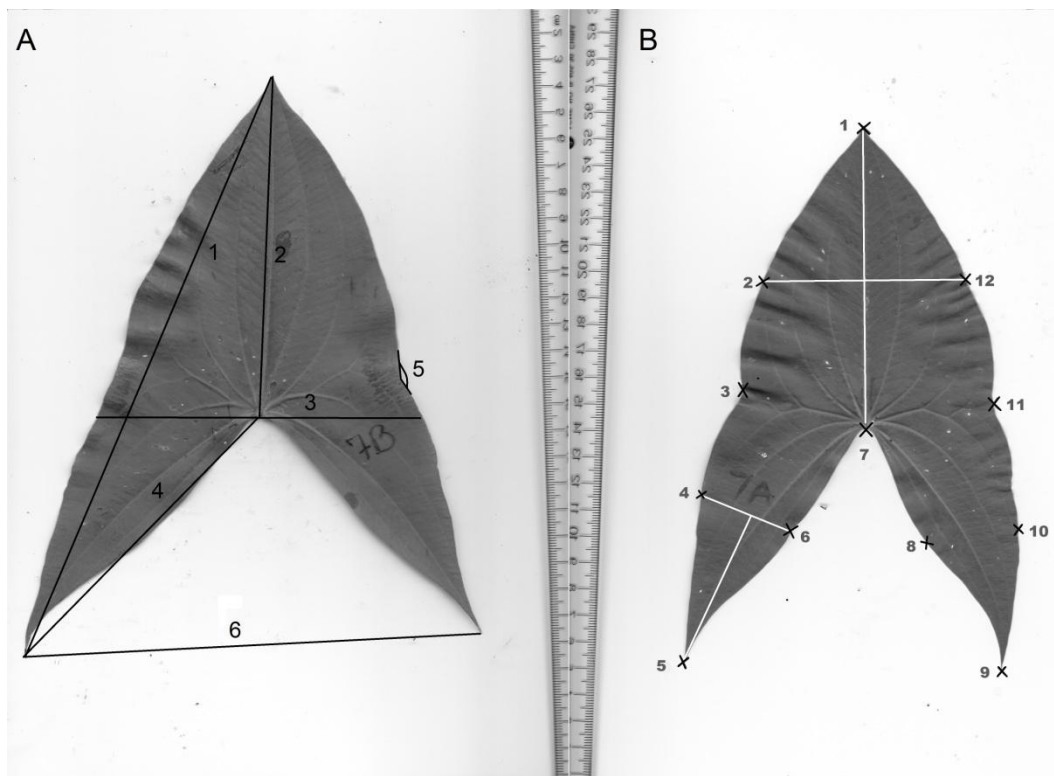


Figure 2.2. *Sagittaria cuneata* leaves depicting (A) the six size measurements taken on each leaf: length (1), blade length (2), width (3), lobe length (4), lobe angle (5), and distance between lobes (6); and (B) the 12 landmarks chosen on each leaf to represent leaf shape

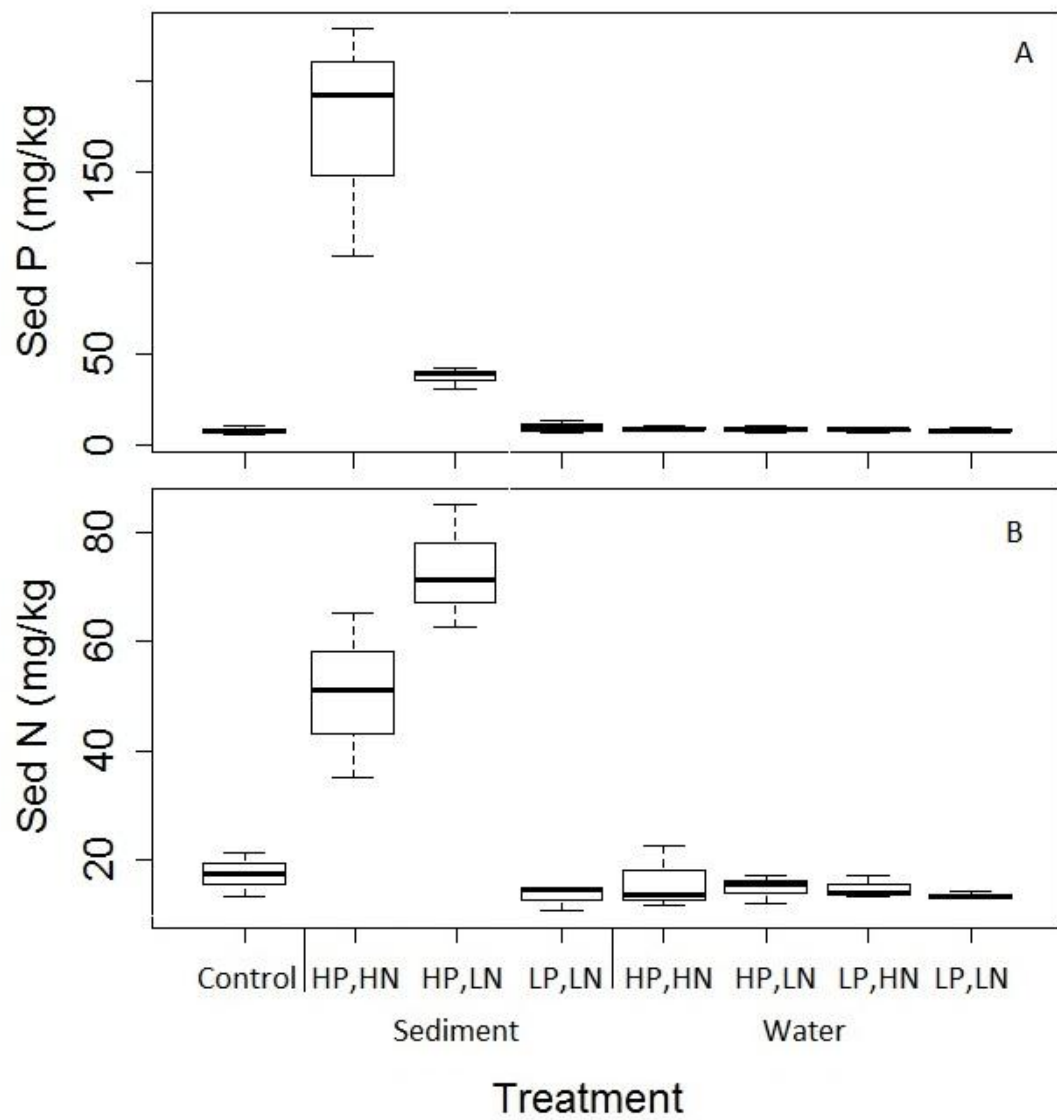


Figure 2.3. Barplots depicting variation in phosphorus in sediment (Sed P measured as Olsen P) (A) and nitrogen in sediment (Sed N measured as $\text{NO}_3 + \text{NH}_4$) (B) among 8 nutrient treatment levels during a ten-week experiment where 80 *Sagittaria cuneata* were grown in individual buckets from June to August in 2014. Sediment samples were collected from 3 buckets per treatment level at the termination of the experiment.

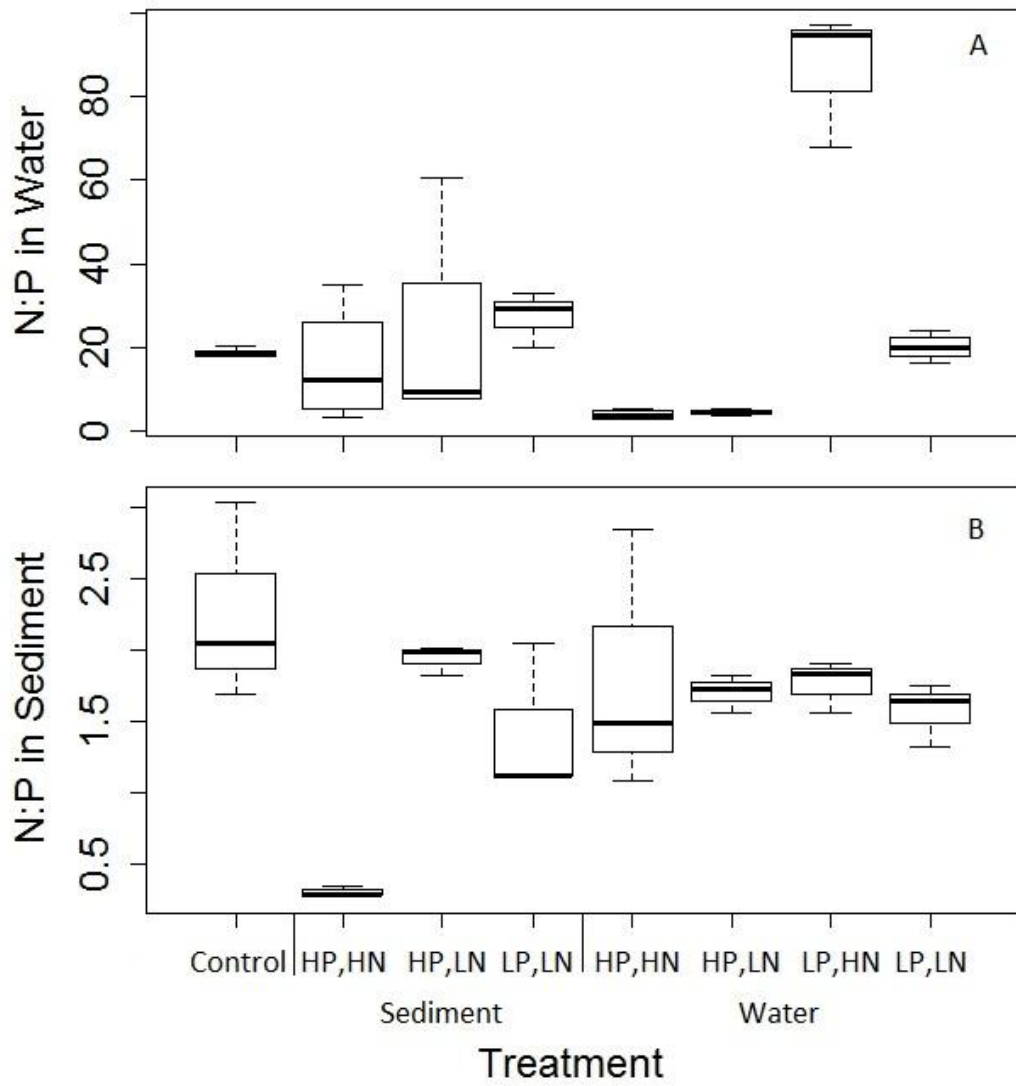


Figure 2.4. Barplots depicting variation in N:P ratios in water samples (A) and sediment samples (B) among 8 nutrient treatment levels during a ten-week experiment where 80 *Sagittaria cuneata* were grown in individual buckets from June to August in 2014. Water samples were collected from 3 buckets per treatment level 3 times during the experiment and N:P ratios were averaged across the 3 sampling periods for each bucket. Sediment samples were collected from 3 buckets per treatment level at the termination of the experiment.

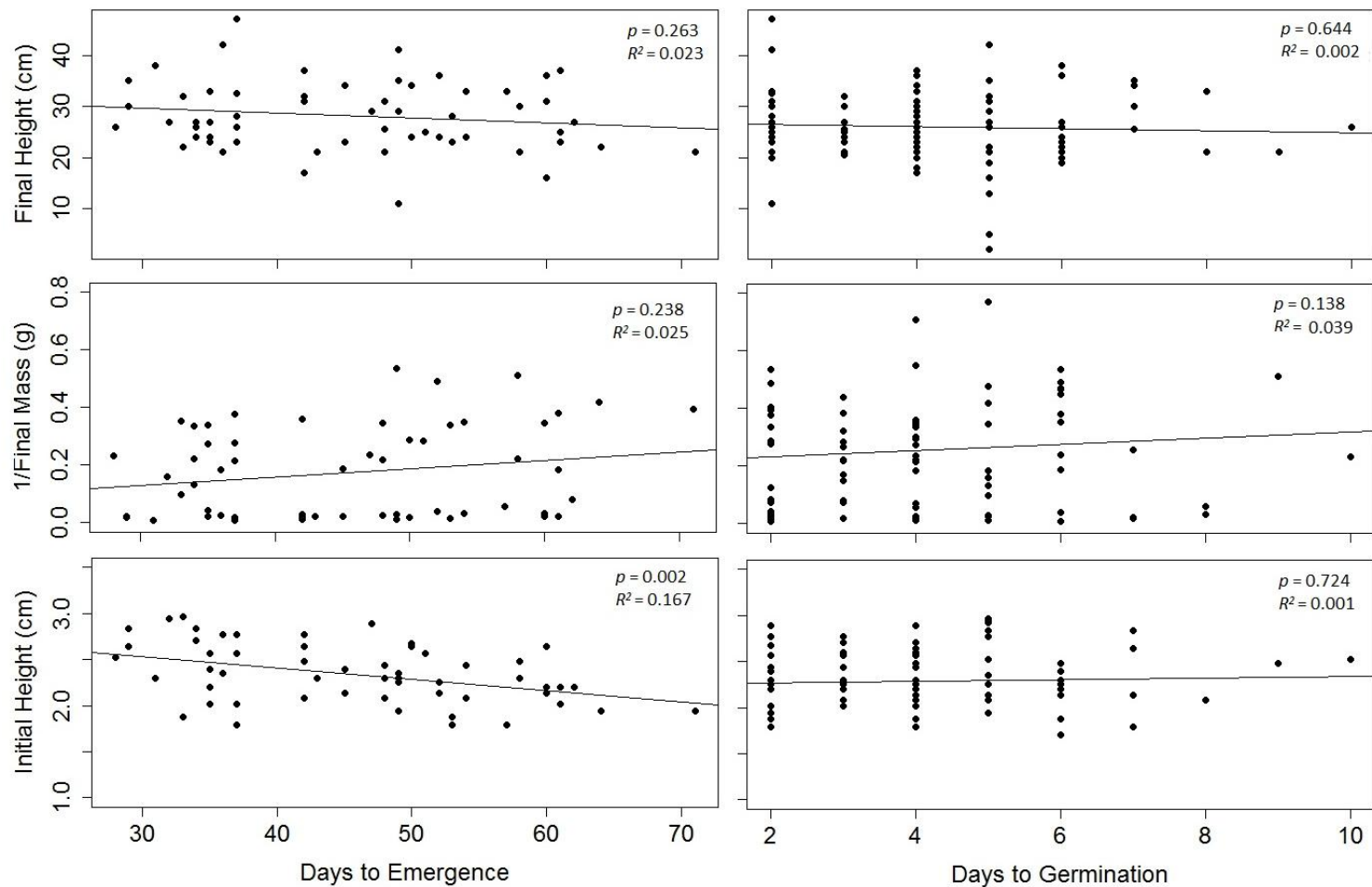


Figure 2.5. Variation of final plant height (cm) and mass (g), and initial plant height (cm) with the number of days each *Sagittaria cuneata* plant (n = 80) took to produce the first emergent leaf (a, b and c, respectively) and to germinate (d, e and f, respectively). Plants were grown in individual buckets in 1 of 7 nutrient treatment levels or a control (see table 2.1 and 2.2) for 10 weeks.

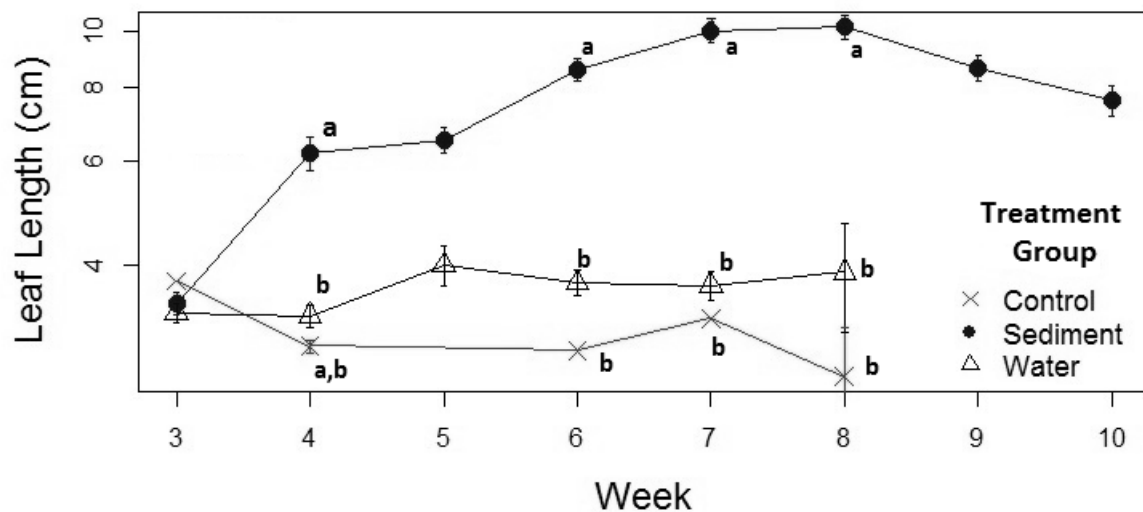


Figure 2.6. Average length ($\pm$ SE) of emergent *Sagittaria cuneata* leaves ($n = 2-45$, total = 272 leaves), on a logged axis, grown in 1 of 3 different nutrient treatment groups (sediment-enriched, water-enriched and control) during a 10-week nutrient experiment. Different letters during a given week denote significance ($p < 0.05$) based on Tukey's HSD pairwise comparison tests. Plants began producing emergent leaves in week 3, and water-enriched and/or control plants did not produced emergent leaves in weeks 5, 9 and 10.

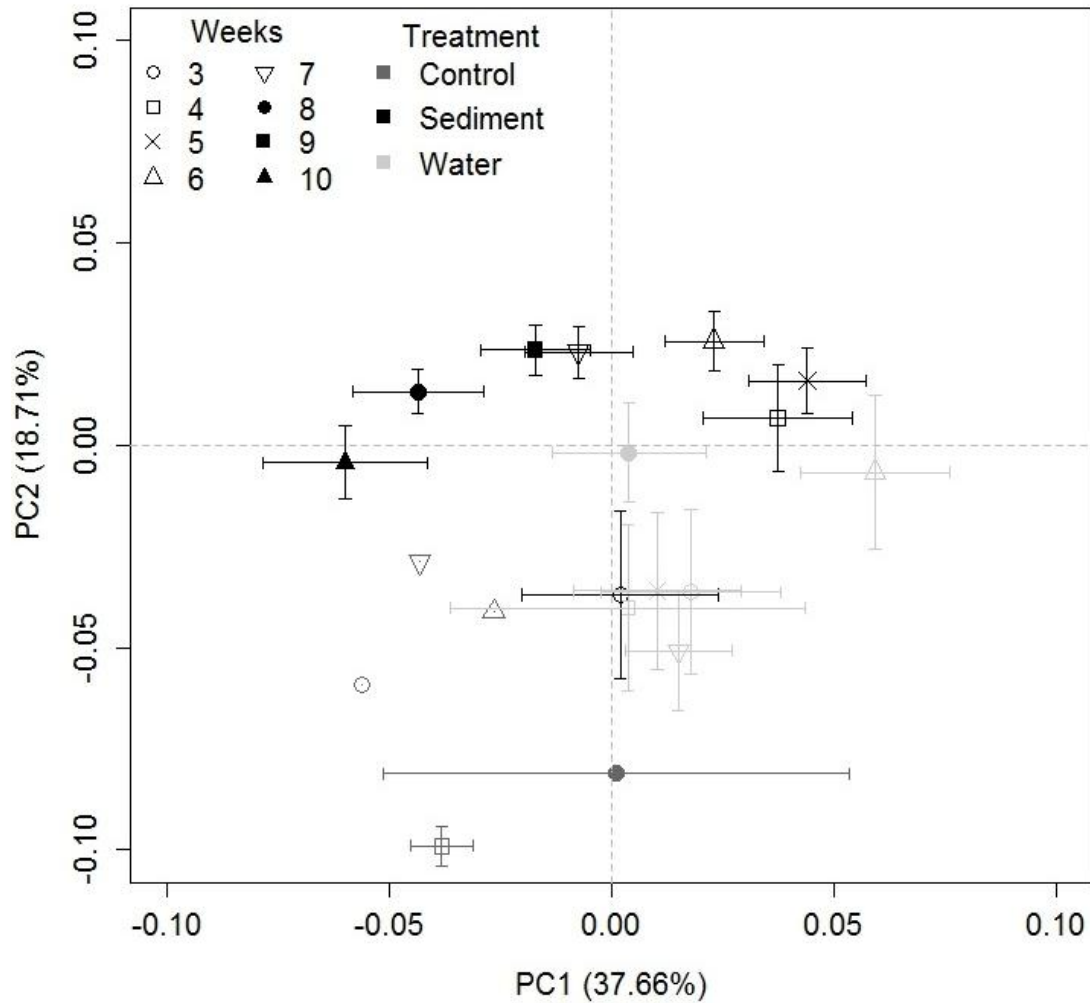


Figure 2.7. Bivariate plot of the first two principal components (PC) depicting variation in landmark coordinates (i.e., shape) of emergent *Sagittaria cuneata* leaves ($n = 2-40$, total = 272 leaves) grown in 1 of 3 nutrient treatment groups (sediment-enriched, water-enriched and control) during a 10-week experiment. Leaves began emerging in week 3 of the experiment.

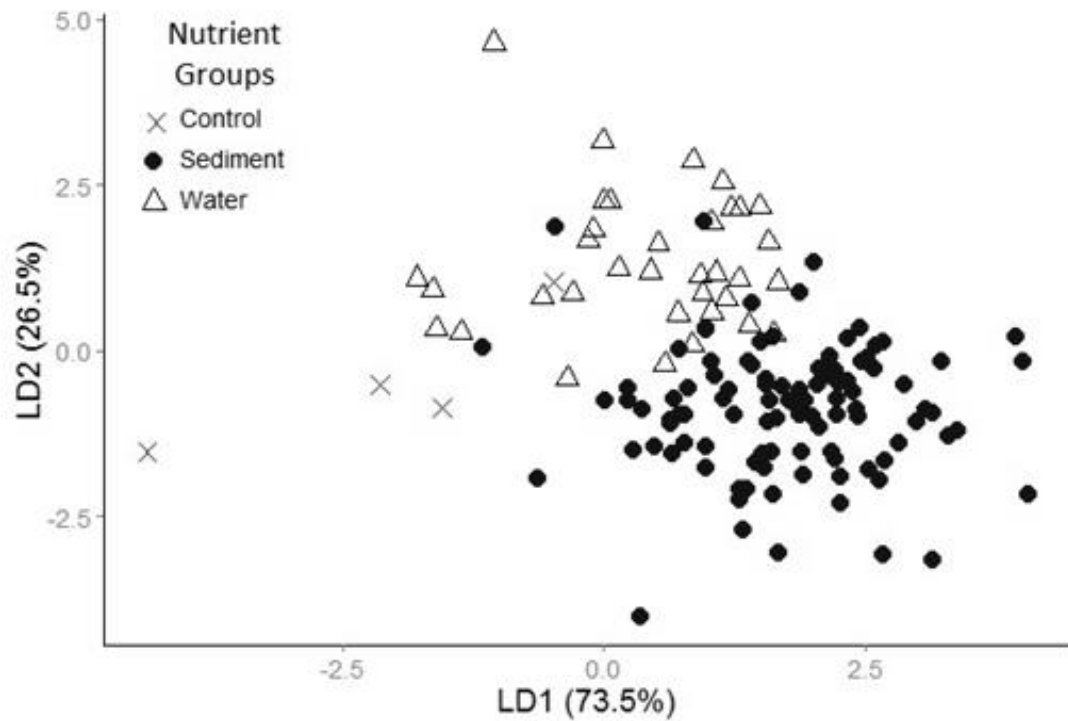


Figure 2.8. Plot of a discriminant functions analysis (DFA) of variations in shape of emergent *Sagittaria cuneata* leaves produced during weeks 7 and 8 of a 10-week nutrient amendment experiment. There is clear separation of leaf shape based on different nutrient treatment groups (sediment-enriched (66 leaves), water-enriched (25 leaves) and control (3 leaves)). Linear discriminant (LD) 1 shows the majority of separation (88%) of leaf shape among nutrient treatment groups.

Chapter 3

Leaf morphology of arrowhead (*Sagittaria cuneata*) as a bioindicator of nitrogen and phosphorus for prairie streams: *in situ* observations

3.1 Abstract

With increasing anthropogenic disturbance in terrestrial and aquatic habitats, there has been a push to develop ways to economically and accurately diagnose stressors of ecosystems, and bioindicators are becoming widely used. The emergent aquatic plant *Sagittaria cuneata* has been proposed as a bioindicator of stream nutrients, nitrogen (N) and phosphorus (P), within the agriculturally dominated Red River Valley, Manitoba, Canada, because of its easily identifiable characteristics, abundance and emergent leaf plasticity. To assess the efficacy of *S. cuneata* as a bioindicator, a field study was conducted in August 2014 during which 2 to 3 leaves were collected from 15 plants at 15 tributary sites ($n = 42\text{-}45$ leaves/site) with varying extents of nutrient inputs. Modelling showed that leaf size was influenced by several variables, including water and sediment nutrients, site location (i.e., latitude), pH and water temperature, though only 19% of variation was explained by this model. Leaf shape was related to N and P in stream water, with increasing nutrients associated with inward rotation of leaf lobes (i.e., narrower distance between lobes) and narrowing of leaf blades. Leaf size and shape variation was relatively similar at all scales of replication (leaf, plant, quadrat and site), suggesting that many complex processes are influencing leaf morphology. Although leaf size and shape variability was naturally high, their weak relationships with stream nutrients made developing leaf morphology of *S. cuneata* as an indicator of nutrients difficult. However, *S. cuneata* presents an ideal model plant to study the natural variability of leaf morphology, and future studies should further delve into leaf morphology within single tributary sites to determine potential drivers.

3.2 Introduction

Nutrient availability is a major driver in plant growth, and nitrogen (N) and phosphorus (P) are the nutrients most commonly limiting successful growth. Availability of P has been shown to have a positive relationship with aquatic macrophyte growth (Chambers et al. 1991; Carr & Chambers 1998; Heaney et al. 2001; Sagova-Mareckova et al. 2009). For example, Carr & Chambers (1998) found that macrophytes with larger biomass grew in higher P sediments, and, similarly, Neill (1990) found that *Typha* spp. exhibited greater biomass with sediment N fertilization. In streams, nutrients are present in both the water column and sediment; however, many rooted macrophytes preferentially utilize sediment as the main source of nutrients (Nichols & Keeny 1976a,b; Best & Mantai 1978; Carignan & Kalff 1980; Barko & Smart 1980, 1981; Chambers & Kalff 1985; Smith & Adams 1986; Demars & Harper 1998; Madsen et al. 2001) possibly because concentrations of bioavailable nutrients are usually lower in water than underlying sediment (Barko et al. 1986). This known link between macrophyte growth and nutrient availability presents an ideal opportunity to study and develop aquatic plants as possible reliable indicators of nutrient conditions in streams.

In recent years, concentrations of nutrients in many streams have increased due to the intensification of agriculture, animal husbandry and wastewater effluent, leading to eutrophic conditions (e.g., EC & MWS 2011). With the increase in anthropogenic disturbance, there has been a push to develop ways to economically and accurately diagnose stressors in aquatic ecosystems. Biological indicators, or bioindicators, are widely used tools to detect the impacts of environmental stressors, and are characterized as common species, or groups of species, with easily identifiable features and plastic

responses to environmental variables (Holt & Miller 2011). Aquatic plants have been little studied as potential indicators of water quality (e.g., Carbiener et al. 1990; Tremp & Kohler 1995; Robach et al. 1996; Thiebaut & Muller 1999; Richards & Ivey 2004; Haury et al. 2006; Ceschin et al. 2010), though this group possesses the appropriate traits: they are sessile, easily sampled and identified to genus (and often species), and respond to environmental stressors (e.g., nutrients, light, substrate texture, etc.) at the individual, population and assemblage level. For example, the free-floating aquatic species *Lemna minor* is currently used as a biomonitoring tool for mining effluent, and Gopalapillai et al. (2014) found that root length in *Lemna minor* was inhibited by increasing nickel concentrations in water and could be used as a sensitive and reliable indicator of nickel toxicity.

The aquatic plant genus *Sagittaria* is well known for the variability of size and shape of its emergent foliage (Arber 1920; Fernald 1950; Sculthorpe 1967) and, thus, has the potential to be a useful bioindicator. In particular, several different environmental variables have been found to affect the size and shape of emergent leaves in the genus. Richards & Ivey (2004) found that leaf blades of *S. lancifolia* were wider in high P than low P environments. Water depth has also been shown to influence emergent leaf size in several *Sagittaria* species, with smaller leaves produced in deeper waters because energy is directed to increasing petiole length in deeper water at the expense of emergent leaf size (Wooten 1970, 1986). Leaf size and shape have also been shown to vary between monoecious and dioecious populations of *S. latifolia*, with dioecious plants producing larger leaves than monoecious plants, and showing more drastic responses to addition of fertilizer (Dorken & Barrett 2004). *Sagittaria cuneata* is

an easily identifiable and distinct species of *Sagittaria* that is widely abundant and exhibits extreme plasticity in emergent foliage, with leaves varying in shape from lanceolate (lance-shaped) to sagittate (arrow-shaped). This known leaf plasticity, and the previous association of leaf morphology with nutrients in the genus, provides an ideal starting point for assessing the use of *S. cuneata* as a bioindicator of stream nutrients.

In Lake Winnipeg in Manitoba, nutrient loading from sources such as agriculture, animal husbandry and wastewater effluent has resulted in an increased occurrence of algal blooms with potentially toxic effects, and increased opportunities for establishment of invasive species (EC & MWS 2011). Declining water quality in Lake Winnipeg is impairing the recreation and tourism industry and threatening local fish populations and, consequently, negatively influencing both commercial and recreational fisheries and ecological functioning within the lake (EC & MWS 2011). Thus, the Province of Manitoba and the Government of Canada are committed to reducing eutrophication of Lake Winnipeg. This entails not only understanding nutrient dynamics within the Lake, but also the sources, processing and delivery of nutrients from the land base to the streams that dissect the watershed and ultimately discharge to the lake.

The aim of my study was to investigate *S. cuneata* as an indicator of nutrients concentrations in southern Manitoba, Canada. The study was conducted in the Red River Valley, which is the watershed responsible for the largest share of nutrient loading annually to Lake Winnipeg (68% of P, 34% of N load; EC & MWS 2011). By analyzing how N and P, and a variety of supporting physicochemical variables influence leaf size and shape in the field, I aimed to evaluate the relative role of ambient environmental

conditions in influencing *S. cuneata* phenology, and ultimately develop leaf morphology (both size and shape) as a bioindicator of N and/or P in the Red River valley.

3.3 Methods

3.3.1 Study Sites

All sampling was conducted along streams in the Red River Valley, Manitoba, Canada, in mid-August 2014. The Red River Valley spans about 13 000 km² in Manitoba (116 000 km² in total), with about 76% of that land currently used for agricultural activities (Red River Basin Board 2000). The watershed was originally covered in prairie grasslands, marshes and pothole lakes (Hanuta 2001) that were drained by ditches and streams for agricultural use. I selected fifteen tributary sites (Figure 3.1) based on previously established study sites in Manitoba selected by Yates et al. (2012) because of their variability in nutrient inputs. Yates et al. (2012) estimated the amount of N and P produced by human activities (livestock numbers, human population, cropland area) within multiple subcatchments of the Red River Valley. The activity densities and nutrient production estimates were then analyzed, and an overall Human Activity Gradient (HAG) was generated using a principal component analysis (PCA; as in Yates & Bailey 2010). Using this information, multiple study sites were selected and established based on varying inputs of nutrients from all sources. Using a combination of HAG sites and newly developed study sites which were selected to potentially fill gaps within the HAG, 18 were chosen for the present study based on presence of *Sagittaria cuneata*. However, on assessment of all sites, 3 were deemed unsuitable because of absence or low abundance of *S. cuneata* during 2014. Each of the 15 sites

selected for study were on independent tributaries within the Red River Valley, with stream orders between 2 and 4.

3.3.2 Field Methods

Given the patchy distribution of plants within a site, five 900 cm² quadrats were haphazardly placed along a 100 m reach to select plants for sampling with minimum bias (adapted from sampling design recommended by Downing & Anderson 1985). To investigate the effects of N and P in water and sediment on leaf size and shape, 2 or 3 leaves were collected from each of 3 *Sagittaria cuneata* plants in all 5 quadrats at each tributary site (n = 42 to 45 leaves/site; adapted from sampling design recommended by Cornelissen *et al.* 2003). Sampled plants were mature (i.e., flowering and/or bearing fruit), so that they could be properly identified and were present in at least 5 cm of water to ensure a potential influence of water chemistry. Only the 3 newest (or 2, if the plant did not have more than 2 emergent leaves), fully formed emergent leaves on each plant were collected, since these leaves would be most influenced by current N and P conditions. Each sample leaf was weighed (fresh mass) and scanned before being pressed and dried at 40°C for 7 days. After specimens were dried, they were reweighed to determine leaf dry matter content (LDMC; fresh mass/dry mass). Scanned images were analyzed using ImageJ to determine the size and morphometric characteristics of each leaf (Figure 3.2) and specific leaf area (SLA; area/dry mass) was calculated. In addition to leaf collection, all leaves on study plants were counted, and plant height and water depth were measured.

To quantify nutrients, one grab water sample was collected at each site for determination of total P (TPw) and total N (TNw) using 500 mL Nalgene sampling bottles. Sediment samples were collected from each quadrat (5 per site) to a depth of 10 cm using 90 mL Starplex Scientific Inc. containers. Water chemistry samples were chilled and shipped within 24 hours to the University of Alberta and analyzed following the Flow Injection Analysis method (Růžička & Hansen 1975). Sediment samples were dried, frozen and subsequently analyzed for bioavailable sediment nutrients in the forms of Olsen-P (henceforth called Sed P; Olsen et al. 1954) at the Institut de Recherche et de Développement en Agroenvironnement (IRDA) in Québec City, Québec, and nitrate and ammonium (henceforth called Sed N for the sum of nitrate and ammonium, or Sed-NO₃ when ammonium was excluded) at the Agriculture and Agri-Foods Canada research station in Harrow, Ontario. Water quality was assessed at each site using a YSI handheld multiparameter instrument. Because of equipment restraints, light and air temperature loggers (HOBO Pendant Temperature/Light Data Logger) were placed at 11 of the 15 sites to determine the average daylight LUX and air temperature (°C) over the summer (May-August 2014).

3.3.3 Statistical Analyses

Testing Model Assumptions

All statistical analyses were completed using the statistical software, *R* (R Development Core Team 2013). Using *R* package *lmtest* v 0.9-33 (Zeileis & Hothorn 2002), Studentized Bruesch-Pagan (Koenker 1981) tests were used to assess heteroscedasticity in linear regression models, and normality of residuals was tested using Shapiro-Wilk test (Royston 1982). Rainbow tests (Utts 1982) ensured linearity in

each regression model because each model had true replication (i.e., multiple leaves were collected at each site, but predictors such as water chemistry were only sampled once). *R* package *car* v 2.0-19 (Fox & Weisberg 2011) was used to conduct Brown-Forsythe tests to test homogeneity of variance for ANOVA (Brown & Forsythe 1974). Diagnostic plots were viewed to visually assess normality of residuals, linearity, and heteroscedasticity for all simple and multiple linear regression models. When 2 or more variables were strongly correlated ($|r| > 0.70$), one variable was removed from models based on relevance to the study and biology of macrophytes. For example, I omitted total dissolved phosphorus (TDP) and soluble reactive phosphorus (SRP) and retained TPw because all 3 variables were strongly correlated, and TPw includes both TDP and SRP. To meet assumptions, several variables were transformed as indicated in tables and figures. To ensure overfitting did not occur, a maximum of 7 variables were considered per linear regression model (i.e., half of the number of sites sampled).

Environmental Variation

Initially, water chemistry and quality were examined to determine whether these variables varied among sites. To test for differences in Sed N and Sed P among sites, one-way analyses of variance (ANOVAs) (Underwood 1997) were conducted using the model: chemistry = μ + Site + ε . Site was considered a random variable because 15 sites were chosen to represent nutrient conditions across the Red River valley.

Leaf Variation

A Pearson correlation matrix was calculated using all measured leaf size (see Figure 3.2A) and mass characteristics to determine if a summary size variable (SSV)

could be used in multiple linear regression and ANOVA models as a proxy for leaf size. SSVs were considered appropriate if there were significant ($p < 0.05$) correlations with all other variables, if mean r was large relative to other variables, and based on ease of measurement of the variable (i.e., variables with less subjective interpretation).

To test for significant variation in leaf size within levels of replication (leaf (2 or 3 levels) plant (3 levels), quadrat (5 levels), site (15 levels)), a three-way nested ANOVA (Underwood 1997) was used to test the following model: leaf size = μ + Site + Quadrat(Site) + Plant(Quadrat(Site)) + ϵ , where the error term refers to the variation at the leaf scale. Variance components were estimated for all random factors using R package *lme4* (Bates et al. 2014) to determine at which scale the majority of random variation of leaf size occurred.

Modelling Leaf Size

Several multiple linear regression models predicting leaf length were developed *a priori* based on known relationships of nutrients (water and sediment) and water depth with macrophyte growth (Table 3.1). After assessment of *a priori* models, *post hoc* models were developed to further explore which environmental variables influenced leaf size of *S. cuneata*. These models included water nutrients and water temperature as well as other nutrient variables (Sed-NO₃), water quality (pH) and regional (latitude, longitude) variables. Multiple linear regressions followed the general model: leaf length = $\alpha + \beta_1 (X_1) + \beta_2 (X_2) \dots + \beta_i (X_i) + \epsilon$. Leaf length data were not averaged at any replicate scale because this would result in a loss of information (as suggested by Zar

2010). Instead, all values of leaf length were included in models to assess whether variability in leaf size could be attributed to environmental conditions.

To determine which model was the “best” fit, the corrected Akaike Information Criterion (AICc; Sugiura 1978; Hurvich & Tsai 1991) was calculated for each model using *R* package AICcmodavg v 2.0-3 (Mazerolle 2015). The model with the lowest AICc value was deemed “best”, and models with a change in AICc (i.e., Δ_i) of less than 4 were considered plausible. Models with Δ_i of 4 to 7 were considered less plausible, and values over 7 were determined as implausible (as suggested by Anderson 2008). Also, if a single variable was added to a model and the Δ_i changed by 2 units or less, this variable was deemed to be “pretending” (Anderson 2008). Pretending variables occur because each variable added to the model incurs a penalty of 2 ($AIC = -2\log(\text{likelihood}) + 2K$, where K is the number of parameters in each model), therefore every model with an additional term will decrease AIC by about 2 units when the likelihood is similar (Anderson 2008). Because AICc corrects for sample size, the value of 2 units can vary. However, if an added variable decreased AICc by 2 units or less, then it was not likely contributing to a better fit model, especially when regression statistics showed that it was not influencing variation (as suggested by Anderson 2008).

Leaf Shape

Differences in leaf shape were assessed using geometric morphometrics (Bookstein 1991; Rohlf & Marcus 1993; Adams et al. 2004; Zelditch et al. 2004; Mitteroecker & Gunz 2009; Adams et al. 2012) of 12 landmarks chosen to represent leaf shape (see Figure 3.2B), and Euclidean coordinates were determined for landmarks on

each leaf using ImageJ (Rasband 1997-2014). In R package geomorph v 1.1-6 (Adams & Otárola-Castillo 2013), a generalized Procrustes analysis (Gower 1975; Rohlf & Slice 1990) was used to rotate and scale all leaves to remove size characteristics from data, resulting in 12 sets of scaled Euclidean coordinates (i.e., X and Y coordinates for landmarks 1, 2, 3, etc.) that represented the shape of individual leaves (henceforth called shape data).

A principal components analysis (PCA) was conducted on shape data using R package geomorph v 1.1-6 (Adams & Otárola-Castillo 2013) to assess and compare individual leaf shapes among and within sites. Principal components (PC) 1 and 2 were examined to ensure that each showed a meaningful shape gradient which could then be used in further statistical analyses.

To determine at which level of replication (site, quadrat, plant, leaf) leaf shape was most variable, PC 1 and PC 2 were used as summarizing shape variables in three-way nested ANOVAs (Underwood 1997) of the model: leaf shape = μ + Site + Quadrat(Site) + Plant(Quadrat(Site)) + ϵ , where the error term represents the leaf scale. Variance components were estimated for each factor using R package *lme4* (Bates et al. 2014) to determine at which scale the majority of variation in PC 1 and 2 occurred.

To evaluate whether leaf shape related to water and sediment nutrients, simple linear regressions were calculated using the model: leaf shape = $\alpha + \beta_1(X_1) + \epsilon$. Simple linear regressions were used instead of multiple linear regressions because nutrient variables violated the assumption of multicollinearity, and thus, not all variables could be included in models. PC 1 and PC 2 were used as responses to determine whether

these gradients, which represented gradients of shape, changed predictably with N or P in water or sediment. If a strong relationship existed between either PC 1 or PC 2 and a nutrient variable, then this feature of shape may be indicative of stream nutrient status.

3.4 Results

3.4.1 Environmental Variation

Comparison of pH, specific conductivity (SPC), dissolved oxygen (DO), average summer temperature and daylight level, and stream nutrients (TPw, TNw, Sed N and Sed P) showed that environmental factors were generally variable among sites (Table 3.2), providing a range of growing environments for *S. cuneata*. Average summer air temperature and pH were generally consistent among sites, varying between 19 to 22°C and 7.9 to 8.6, respectively. DO was more variable among sites, but remained above 50% in all instances. SPC and average daily light level (LUX) were quite variable, and ranged between 388 to 1290 $\mu\text{S}/\text{cm}$ and 6400 to 79 000 LUX, respectively. As expected, TPw and TNw varied among sites, ranging from 0.04 to 2.74 and 0.53 to 2.51 mg/L, respectively; Sed P ($F_{14,60} = 14.58$, $p < 0.001$) and Sed N ($F_{14,60} = 7.22$, $p < 0.001$) were significantly different among sites (Appendix Table B1). Thus, nutrient concentrations in water and sediment among sites provided a variety of nutrient conditions that will influence plant growth and, potentially, leaf morphology of *S. cuneata*.

3.4.2 Leaf Size Variation

Variability and plasticity of emergent leaves was visually evident based on field collections of *Sagittaria cuneata*. Figure 3.3 illustrates the large range in size and variations in shape of emergent arrowhead leaves collected in streams in the Red River

Valley in July 2013 and August 2014. These pictures summarize the plasticity of this species, and are consistent with observations of plasticity in other *Sagittaria* species.

To reduce the number of variables in ANOVA and multiple linear regression models, leaf length was chosen as a summary size variable (SSV) based on significant ($p < 0.05$) relationships with all leaf size and mass characteristics, and a high mean r relative to all other variables ($r = 0.68$) (Table 3.3). Area (mean $r = 0.69$) and lobe length (mean $r = 0.68$) were also strong choices; however, length is the simplest measurement (i.e., area requires a computer program to calculate, whereas length can be measured in the field) and requires less subjective interpretation during measurement (i.e., lobe length may vary based on landmarks chosen). All other variables were dismissed based on low mean r values (range -0.25 to 0.67) relative to length, area and lobe length, and because not all relationships were significant.

Results of a three-way nested ANOVA demonstrated that leaf length varied significantly at all levels of replication (Table 3.4), and variance components for all random effects suggested that variation was similar at all scales (~25%), although was marginally higher at the plant level (28%). Figure 3.4 demonstrates the large variation of leaf length within quadrats per site and among sites. Differences between minimum and maximum leaf length within a site varied between 8.5 cm at AS05A (range: 6.8 to 15.3 cm), and 20.4 cm at BR05 (range: 4.3 to 24.7 cm); the average difference in length among all sites was 13.4 cm, demonstrating the large variation in leaf size within a site.

3.4.3 Leaf Size Modelling

A priori Models

To determine the relationship of leaf length with all variables in *a priori* models, each variable was plotted (Figure 3.5, Appendix Table B2). Leaf length showed no relationship with Sed P or water depth, and weak, positive relationships with Sed N and TPw. Leaf length also showed a weak, negative relationship with TNw. Comparison of *a priori* multiple linear regression models using AIC_c indicated that three models were considered plausible (the “best” model and those with Δ_i less than 2) (Table 3.5): model 9 ($F_{5,656} = 17.78$, $p < 0.001$, $R^2 = 0.11$), which was a combination of all 5 variables (TNw, TPw, Sed N, Sed P, and water depth; Table 3.6); model 7 ($F_{2,659} = 40.78$, $p < 0.001$, $R^2 = 0.11$), which included both water chemistry variables (Table 3.7); and model 8 ($F_{4,657} = 21.20$, $p < 0.001$, $R^2 = 0.11$), which included all 4 chemistry variables (Table 3.8). Model 9 was dismissed as the most useful model because the addition of water depth resulted in a AIC_c decrease of only 1.7, suggesting that this variable was “pretending” to be important (as suggested by Anderson 2008; Arnold 2010). Similarly, the removal of sediment chemistry variables in model 7 resulted in an AIC_c decrease of 0.9 units, suggesting that Sed P and Sed N were not adding valuable information to the model. Furthermore, multiple linear regressions showed no change in R^2 values with the removal of water depth, Sed P and Sed N (all models explained about 11% of variation; Table 3.5), and these variables were non-significant (Tables 3.6, 3.7, and 3.8). Model 7, therefore, was selected as the best model. Variables in model 7 (TPw and TNw) both had significant, negative influences on leaf length, contrary to expectations.

Post Hoc Models

Post hoc model exploration suggested that there were three plausible models explaining leaf length based on AIC_c scores (Table 3.9): model 16, with the variables

Sed-NO₃, TNw, latitude, water temperature, pH and water depth ($F_{6,655}$: 27.85, $p < 0.001$, $R^2 = 0.19$; Table 3.10); model 17, which includes all variables in model 16 except water depth ($F_{5,656}$: 32.79, $p < 0.001$, $R^2 = 0.19$; Table 3.11); and model 15, which is model 17 with the addition of TPw ($F_{6,655}$: 27.31, $p < 0.001$, $R^2 = 0.19$; Table 3.12). Model 17 was selected as the most useful model because the addition of water depth to model 16 resulted in a decrease of only 0.7 AICc units, suggesting that water depth is an uninformative variable. Similarly, the addition of TPw to model 15 resulted in an increase of 1.9 AICc units, suggesting that TPw is a pretending variable. Furthermore, all models explained about 19% of leaf length variation, suggesting that the removal of 2 variables in model 17 (water depth and TPw, both of which were non-significant) did not result in lost information (Tables 3.10, 3.11 and 3.12). *A priori* models were about 64 AICc units higher than the best *post hoc* model and explained only 11% of size variation, suggesting that *post hoc* model 17, with the addition of water quality (i.e., pH, water temperature), Sed-NO₃ and site location variables, better represented leaf length variation than *a priori* models of nutrients and water depth alone. Therefore, model 17 was selected as the best model representing leaf size variation among field sites.

Variables in model 17 were all significantly influencing leaf length variation, except pH ($p = 0.055$), and represent a combination of water and sediment chemistry, site location and water quality parameters (Table 3.11). Specifically, Sed-NO₃ and pH had a positive influence on leaf length variation, whereas nitrogen in water (TNw), site location (latitude) and water temperature had a negative influence on leaf length. Based on partial R^2 for each variable, TNw had the largest influence on leaf length (partial $R^2 = 0.12$), and pH had the smallest influence on leaf size (partial $R^2 = 0.01$), whereas all

other variables had moderate influences (partial R^2 range from: 0.02 to 0.07). In combination, all variables explain about 19% of leaf length variation ($R^2 = 0.19$).

3.4.4 Leaf Shape

Principal components analysis (PCA) showed that leaf shape was variable within and among sites (Figure 3.6). Raw principal components (PC) scores suggested a large amount of variation in leaf shape within sites, and little distinct grouping. Individually, PC 1 explained about 56% of leaf shape variation and corresponded to differences in the rotation of the lobes, with lobes rotated outwards (i.e., further apart) on the negative end of the x-axis and lobes rotated inwards (i.e., closer together) on the positive side. PC 2 explained 14% of shape variation and related to width of leaf blades, with wider blades on the negative end of the y-axis and narrow leaf blades on the positive side.

To further explore variation in PC 1 and PC 2 at all scales of replication, a three-way nested ANOVA was conducted and results determined that both PC 1 and PC 2 varied at all scales (leaf, plant, quadrat, site) (Table 3.13). Variance components for PC 1 and PC 2 showed that variation in the smallest scale (i.e., leaves) explained the most random variation (41 and 36%, respectively), whereas site (30 and 21% respectively), quadrat (13 and 14%, respectively) and plant (16 and 28%, respectively) generally explained less. However, variation is relatively similar among all replication levels.

To determine the relationship of shape, summarized as PC 1 (corresponding to lobe rotation) and PC 2 (related to leaf width) scores, with N and P in sediment and water, simple linear regressions were conducted (Figure 3.7). PC 1 and PC 2 showed no relationship with Sed P ($F_{1,656}$: 1.24, $p = 0.266$, $R^2 = 0.002$ and $F_{1,656}$: 0.23, $p = 0.635$, R^2

= 0.0003, respectively) and Sed N ($F_{1,656}$: 1.19, p = 0.275, R^2 = 0.002 and $F_{1,656}$: 2.18, p = 0.140, R^2 = 0.003, respectively)(Table 3.14). In contrast, PC 1 and PC 2 showed significant, positive relationships with both TPw ($F_{1,656}$: 34.26, p < 0.001, R^2 = 0.05 and $F_{1,656}$: 80.41, p < 0.001, R^2 = 0.11, respectively) and TNw ($F_{1,656}$: 98.9, p < 0.001, R^2 = 0.13 and $F_{1,656}$: 56.95, p < 0.001, R^2 = 0.08, respectively) (Table 3.15), indicating that increasing TPw and TNw were weakly associated with inward rotation of leaf lobes (i.e., narrower distance between lobes) and narrowing of leaf blades (all R^2 < 0.15).

3.4.5 Leaf Shape - Field versus Experimental Study

To compare leaf shape in the field study to the 10-week nutrient manipulation experimental study (Chapter 2), a PCA of emergent leaf shape data from both studies was conducted (Figure 3.8). PC scores were averaged per site and per nutrient treatment group from the experiment (sediment-enriched, water-enriched, or control) to view trends within shape data. PC 1 and PC 2 explained about 68% of leaf shape variation, and corresponded to variation in the rotation of the lobes, with lobes rotated outwards (i.e., further apart) on the negative end of the x-axis and lobes rotated inwards (i.e., closer together) on the positive side, and the width of leaf blades, with wider blades on the negative end of the y-axis and narrow leaf blades on the positive side, respectively. Leaves from all 3 treatment groups of the experimental study grouped together, and were less variable and separated from the field sites.

3.5 Discussion

For most plant species, phenological traits, especially leaf morphology, express a multitude of shapes and sizes, and *Sagittaria cuneata* is no exception. This field study

demonstrated variability of leaf length and leaf shape (summarized as PC 1 and PC 2) at all levels of replication. Previous research has also shown the extreme plasticity of intra-specific plant traits (e.g., Joel et al. 1994; Bruschi et al. 2003; Dorken & Barrett 2004; Hovenden & Vander Schoor 2004; Richards & Ivey 2004). For example, Bruschi et al. (2003) found that, for populations of the oak *Quercus petraea* across Italy, leaf area significantly differed among populations, individuals within a single population and even within a single tree. Similarly, Joel et al. (1994) found that leaf area of the terrestrial species *Metrosideros polymorpha* ranged, on average, from about 3-14 cm² among populations in Hawaii. For macrophytes, height of the wetland plant *Ludwigia leptocarpa* varied, on average, from 69 to 118 cm among 3 different populations (Christy & Sharitz 1980) and leaf shape of *Sagittaria lancifolia* varied from narrow to wide leaf blades among populations in the Florida Everglades (Richards & Ivey 2004). Plasticity among plant populations does not, however, occur at random, and is often related to differences in environmental conditions (e.g., water temperature, nutrients, altitude, etc.) and/or genetics. Such plasticity is thought to be an important adaptation to constant environmental fluctuations (Wells & Pigliucci 2000): plants, unlike most animals, are unable to move from unfavourable conditions and instead, must adapt to their surroundings. Yet, whilst plants can express a multitude of phenotypes that allow them to compensate for changes in their habitat, some traits may be selected in certain environments to ensure individual (and often population) persistence over time. For example, Hovenden & Vander Schoor (2004) found that beech trees (*Nothofagus cunninghamii*) collected from various altitudes and grown in similar conditions expressed leaf thickness similar to the population of origin, suggesting a genetic control

of leaf thickness that is adaptive to altitudinal changes. Variability in plants traits, therefore, has two major drivers: a genetic component and an environmental component, and each of these factors will influence a plant's response to habitat variability.

In the present study, leaf length of *S. cuneata* was positively related to Sed-NO₃ and stream water pH, and negatively influenced by TNw, water temperature and latitude. However, though significant, this model explained only 19% of the variation in leaf length. Multiple environmental variables interact to influence physiological processes and plant growth, which, in turn, affect plant traits such as leaf morphology. Nutrient availability and water depth are often considered the most influential factors in leaf shape (e.g., Bruni et al. 1995; Wells & Pigliucci 2000) and leaf size variability (e.g., Wooten 1986; Richards & Ivey 2004). For example, Dorken & Barrett (2004) found that leaf size of *S. latifolia* increased with fertilization, and Wooten (1986) demonstrated decreasing leaf size of several *Sagittaria* species with increasing water depth. Yet, while nutrients are the primary determinant of plant size, other variables have been implicated: temperature, light quality, day length, and relative humidity (Wells & Pigliucci 2000). For example, Barko & Smart (1981) found that the macrophyte *Hydrilla verticillata* had higher biomass with increasing temperatures from 16 to 32°C. Similarly, Titus et al. (1990) reported that biomass of the macrophyte *Vallisneria americana* was higher with increasing pH due to the greater availability of dissolved inorganic carbon (DIC). The finding that only 19% of leaf length variation of *S. cuneata* was explained by the best fit model suggests that: (1) factors not examined in this study (e.g., sediment composition, light availability) may have influenced leaf size, or (2) this species is inherently variable and leaf variation can only be explained in part by environmental factors.

Similar to leaf length, the shape of emergent *S. cuneata* leaves was considerably variable, which was explained, in part, by TNw and TPw concentrations. Specifically, increasing TPw and TNw were weakly associated with inward rotation of leaf lobes (i.e., narrower distance between lobes) and narrowing of leaf blades, though only 5-13% of variation was explained in each instance. Likewise, Dorken & Barrett (2004) found that leaf blades of *S. latifolia* were narrower in high water nutrients than in low nutrients. Interestingly, sediment nutrients showed no relationship with leaf shape, though sediment nutrients are preferentially used by rooted macrophytes (e.g., Barko & Smart 1980, 1981) and resulted in leaf shape changes over time in *S. cuneata* (Chapter 2).

While *S. cuneata* showed considerable variability in leaf size and shape among sites, these traits were also variable among quadrats within site and about 25% of variation in leaf size occurred at the quadrat level, though only 12-14% of variation in leaf shape was explained at this scale. Variation at the quadrat scale is likely because tributaries are naturally heterogeneous, meaning that environmental conditions vary with location in a stream. Sediment composition may vary within a site because of differences in the quality and quantity of deposited sediment, and this will directly influence where plants take root. Schutten et al. (2005) suggested that tall, rooted macrophytes favour fine, nutrient-rich sediment; thus, plants will preferentially grow in areas with these conditions. Because aquatic plants generally reproduce vegetatively (Jones et al. 2012), dense stands often form and this, in turn, results in slower water velocity (Madsen et al. 2001; Jones et al. 2012) and increased deposition of fine organic matter (Vermaat et al. 2000; Jones et al. 2012), effectively changing sediment composition and increasing nutrients (Jones et al. 2012). Plant growth is also influenced

by light availability: Balaguer et al. (2001) found that when grown in shaded environments, the terrestrial oak *Quercus coccifera* generally had leaves that were less lobed and larger compared to those grown in high light, likely to increase surface area for light absorption. Shading from canopy cover, riparian vegetation and structures (i.e., bridges) along some sections of a tributary provides differing levels of light, directly influencing growth of macrophytes within the stream. Therefore, *S. cuneata* plants sampled from quadrats within a site will exhibit different leaf sizes and shapes.

Variation in leaf size was also approximately 25% among plants within a quadrat and among leaves within a plant, demonstrating that equal variation in size occurred at all four scales (site, quadrat, plant and leaf). Plant shape (summarized as PC 1 and PC 2) was relatively similar across all scales, but was always highest at the leaf scale (35-40%). Leaf size variation among plants within quadrats and leaves within plants likely occurs because of age differences. Miller (1987) demonstrated that later germination date in 7 terrestrial plant species lead to decreased biomass, suggesting that leaf size would be smaller in plants that establish later in the growing season. In addition, competition may cause differences between plants growing in a small area (e.g., Goldberg 1987). One plant may out-compete its neighbour and acquire more resources to allocate to vegetative growth, effectively increasing leaf size in one plant over another. For example, Goldberg 1987 found that the growth of the goldenrod, *Solidago canadensis* was reduced by 17-65% in the presence of neighbouring plants. Leaf size can also change throughout the growing season in a single plant. For example, leaf size will increase throughout a growing season to increase photosynthetic area (Niinemetsan & Kull 1994; Ribeiro et al. 1994; Ackerly & Donoghue 1998; Stenberg 1998) and thus,

increase energy uptake in the plant. In addition, reproduction can limit leaf size because nutrients are allocated to reproductive instead of vegetative structures (Bazzaz et al. 1987; Reekie & Bazzaz 1987). Leaf size and shape have also been shown to differ during the growing season in *S. cuneata* (see Chapter 2), where plants grown with added sediment nutrients had increasing leaf size until peaking, and decreasing in size, likely because of resource allocation to reproduction. Because plants within a quadrat may vary in age, the differences in leaf morphology observed among plants within a quadrat is likely because some plants established later than and/or outcompeted others, giving a plant less time to acquire resources to allocate to vegetative structures and causing leaves to be smaller. Similarly, leaf morphology differences within a plant likely occur because leaves produced after flowering (i.e., the newest leaves) would be smaller than those produced before flowering (i.e., the older leaves).

In addition to environmental heterogeneity within streams, genetics may play a part in the variability of plant traits. The observations that measured environmental variables explained only 19% of leaf length variation indicates that other factors (environmental, genetic, or both) are responsible for further leaf size variation. Though genetic influences were not explicitly examined in this study, the lack of similarity in leaf shape between plants from the field survey (present chapter) and experimental plants grown from tubers collected from two field sites (Chapter 2) suggests that genetic variation may not be an influential driver of leaf morphology. Although there are few studies of genetic and environmental factors controlling *Sagittaria* phenology (e.g., Dorken & Barrett 2004), other plants have been found to have variations in leaf morphology explained by both factors. For example, Hovenden & Vander Schoor (2004)

found that, when several genotypes of the tree *Nothofagus cunninghamii* were grown under uniform conditions, leaf thickness differed significantly among genotypes and was similar to the population of origin, suggesting a genetic influence. In contrast, leaf length and width did not differ among genotypes, suggesting that environmental conditions, and not the population of origin, were influencing leaf size (Hovenden & Vander Schoor 2004). Because leaf shape of plants grown in the experimental study (Chapter 2) differed from those collected at field sites where tubers were collected (TC01 and LR01), the large variability in measured and unmeasured (i.e., sediment composition) environmental conditions, in addition to the age structure of the *S. cuneata* population, is likely driving the plasticity of plant traits observed in this study. This plant variability, though interesting from an ecological view, proves difficult for developing a bioindicator of nutrients within individual tributaries.

3.6 Conclusions

Sagittaria cuneata is a highly variable species, expressing a multitude of sizes and shapes of emergent foliage at multiple scales of replication. Analysis of *S. cuneata* leaf size and shape with a number of physicochemical variables identified nutrients in both the water and sediment as factors that in part explained this variability. However, the full extent of this variation is likely explained by an interaction of many environmental variables (and less affected by genotypic variability). Environmental heterogeneity within a stream, and age structure of both the population of plants and individual leaves collected from each plant likely influenced the diverse leaf morphology observed in this study. Because the large variability cannot yet be fully explained, leaf morphology of *S. cuneata* is presently an unreliable bioindicator of

nutrients. It does, however, present an ideal system for studies of leaf morphology and phenotypic plasticity. To further study influences of environmental variables on leaf morphology, detailed future studies should track water quality and chemistry variables over an entire season to obtain more accurate estimates of these factors both within sites and within individual patches of plants. Also, placing light and air temperature loggers at every site to include these factors in models, sampling from more quadrats within a site to allow determination of whether environmental heterogeneity within streams is influencing leaf morphology, determining relative coverage and biomass of plants at each site, and tracking the age of each plant and new leaf would help disentangle the effects of age structure in the population and the individual plant.

3.7 References

- Ackerly D.D. & Donoghue M.J. (1998) Leaf size, sapling allometry, and Corner's rules: phylogeny and correlated evolution in maples (*Acer*). *The American Naturalist*, **152**, 767-791.
- Adams D.C. & Otárola-Castillo E. (2013) geomorph: an R package for the collection and analysis of geometric shape data. *Methods in Ecology and Evolution*, **4**, 393-399.
- Adams D.C., Rohlf F.J. & Slice D.E. (2004). Geometric morphometrics: ten years of progress following the 'revolution'. *Italian Journal of Zoology*, **71**, 5-16.
- Adams D.C., Rohlf F.J. & Slice D.E. (2012) A field comes of age: Geometric morphometrics in the 21st century. *Hystrix*, **24**(1), 7-14.
- Anderson D.R. (2008) *Model based inference in the life sciences: A primer on evidence*. Springer, New York, New York, USA.
- Arber A. (1920) *Water Plants - A Study of Aquatic Angiosperms*. Cambridge University Press, Cambridge, UK.
- Arnold T.W. (2010) Uninformative parameters and model selection using Akaike's information criterion. *Journal of Wildlife Management*, **74**, 1175–1178.

- Balaguer L., Martínez-Ferri E., Valladares F., Pérez-Corona M.E., Baquedano F.J., Castillo F.J. and Manrique E. (2001) Population divergence in the plasticity of the response of *Quercus coccifera* to the light environment. *Functional Ecology*, **15**, 124-135.
- Barko J.W., Adams M.S. & Clesrceri N.L. (1986) Environmental Factors and Their Consideration in the Management of Submersed Aquatic Vegetations: A Review. *Journal of Aquatic Plant Management*, **24**, 1-10.
- Barko J.W. & Smart R.M. (1980) Mobilization of sediment phosphorus by submersed freshwater macrophytes. *Freshwater Biology*, **10**, 229-238.
- Barko J.W. & Smart R.M. (1981) Sediment based nutrition of submersed macrophytes. *Aquatic Botany*, **10**, 339-352.
- Bates D., Maechler M., Bolker B. & Walker S. (2014) *lme4: Linear mixed-effects models using Eigen and S4*. R package version 1.1-7. <http://CRAN.R-project.org/package=lme4>
- Bazzaz F., Chiariello N.R., Coley P.D. & Pitelka L.F. (1987) Allocating Resources to Reproduction and Defense. *BioScience*, **37**(1), 58-67.
- Best M.D. & Mantai K.E. (1978) Growth of Myriophyllum: sediment or lake water as the source of nitrogen and phosphorus. *Ecology*, **59**, 1075-1080.
- Bookstein F.L. (1991) *Morphometric tools for landmark data: Geometry and Biology*. Cambridge University Press, Cambridge, UK.
- Brown M.B. & Forsythe A.B. (1974) Robust tests for the equality of variances. *Journal of the American Statistical Association*, **69**, 364-367.
- Bruni N.C., Young J.P. & Dengler N.G. (1995) Leaf developmental plasticity of *Ranunculus flabellaris* in response to terrestrial and submerged environments. *Canadian Journal of Botany*, **74**, 823-837.
- Bruschi P., Grossoni P. & Bussotti F. (2003) Within-and among-Tree Variation in leaf morphology of *Quercus petraea* (Matt .) Liebl. natural populations. *Trees*, **17**, 164-172.
- Carbiener R., Trémolières M., Mercier J.L. & Ortscheidt A. (1990) Aquatic macrophytes communities as bioindicators of eutrophication in calcareous oligosaprobe stream waters (Upper Rhine plaine, Alsace). *Vegetatio*, **86**, 71-88
- Carignan R. & Kalff J. (1980) Phosphorus sources for aquatic weeds: Water or sediments? *Science*, **207**, 987- 988.

- Carr V.E. & Chambers P.A. (1998) Macrophyte growth and sediment phosphorus and nitrogen in a Canadian prairie river. *Freshwater Biology*, **39**, 525-536.
- Ceschin S, Zuccarello V & Caneva G. (2010) Role of macrophyte communities as bioindicators of water quality: Application on the Tiber River basin (Italy). *Plant Biosystems*, **144** (3), 528-536.
- Chambers P.A. & Kalff J. (1985) The influence of sediment composition and irradiance on the growth and morphology of *Myriophyllum spicatum* L. *Aquatic Botany*, **22**, 253-263.
- Chambers P.A., Prepas E.E., Hamilton H.R. & Bothwell M.L. (1991). Current velocity and its effect on aquatic macrophytes in flowing waters. *Ecological Applications*, **1**, 249-257.
- Christy E.J. & Sharitz R.R. (1980) Characteristics of three populations of a swamp annual under different temperature regimes. *Ecology*, **6**, 454-460.
- Cornelissen J., Lavorel S., Garnier E., Díaz S., Buchmann N., Gurvich, D., Reich P., ter Steege H., Morgan H., van der Heijden M., Pausas J. & Poorter H. (2003). A handbook of protocols for standardised and easy measurement of plant functional traits worldwide. *Australian Journal of Botany*, **51**, 335-380.
- Demars B.O.L. & Harper D.M. (1998) The aquatic macrophytes of an English lowland river system: assessing response to nutrient enrichment. *Hydrobiologia*, **384**, 75-88.
- Dorken M.E. & Barrett S.C.H. (2004) Phenotypic plasticity of vegetative and reproductive traits in monoecious and dioecious populations of *Sagittaria latifolia* (Alismataceae): a clonal aquatic plant. *Journal of Ecology*, **92**, 32-44.
- Downing J.A. & Anderson M.R. (1985) Estimating standing biomass of aquatic macrophytes. *Canadian Journal of Fisheries and Aquatic Sciences*, **42**, 1860-1869.
- Environment Canada & Manitoba Water Stewardship. (2011) State of Lake Winnipeg: 1999-2007. Available at:
https://www.gov.mb.ca/waterstewardship/water_quality/state_lk_winnipeg_report/pdf/state_of_lake_winnipeg_rpt_technical_high_resolution.pdf
- Fernald M.L. (1950) *Gray's Manual of Botany*. American Book Company, New York, USA.
- Fox J. & Weisberg S. (2011) *An {R} companion to applied regression*, Second Edition. Sage, Thousand Oaks, CA, USA.
<http://socserv.socsci.mcmaster.ca/jfox/Books/Companion>.

- Goldberg D.E. (1987) Neighborhood competition in an old-field plant community. *Ecology*, **68**, 1211–1223.
- Gopalapillai Y., Vigneault B. & Hale B.A. (2014) Root length of aquatic plant, *Lemna minor* L., as an optimal toxicity endpoint for biomonitoring of mining effluents. *Integrated Environmental Assessment and Management*, **10**(4), 493–497.
- Gower J.C. (1975) Generalized Procrustes analysis. *Psychometrika*, **40**, 33–51.
- Hanuta I. (2001) A reconstruction of wetland information in pre-settlement southern Manitoba using a geographic information system. *Canadian Water Resources Journal*, **26**, 183–194.
- Haury J., Peltre M.C., Tremolieres M., Barbe J., Thiebaut G., Bernez I., Daniel H., Chatenet P., Haan-Archipof G., Muller S., Dutartre A., Laplace-Treyture C., Cazaubon A. & Lambert-Servien E. (2006) A new method to assess water trophy and organic pollution – The Macrophyte Biological Index for Rivers (IBMR): Its application to different types of river and pollution. *Hydrobiologia*, **570**, 153–158.
- Heaney S.I., Foy R.H., Kennedy G.J.A., Crozier W.W. & O'Connor W.C.K. (2001) Impacts of agriculture on aquatic systems: lessons learnt and new unknowns in Northern Ireland. *Marine and Freshwater Research*, **52**, 151–163.
- Holt E.A. & Miller S.W. (2011) Bioindicators: using organisms to measure environmental impacts. *Nature Education Knowledge*, **3**(10), 8.
- Hovenden M.J. & Vander Schoor J.K. (2004) Nature vs. nurture in the leaf morphology of Southern beech, *Nothofagus cunninghamii* (Nothofagaceae). *New Phytologist*, **161**, 585–594.
- Hurvich C.M & Tsai C-L. (1991) Bias of the corrected AIC criterion for underfitted regression and time series models. *Biometrika*, **78**, 499–509.
- Joel G., Aplet G. & Vitousek P.M. (1994) Leaf morphology along environmental gradients in Hawaiian *Metrosideros polymorpha*. *Biotropica*, **26**(1), 17–22.
- Jones J.I., Collins A.L., Naden P.S. & Sear, D.A. (2012) The relationship between fine sediment and macrophytes in rivers. *River Research and Applications*, **28**, 1006–1018
- Koenker R. (1981) A note on studentizing a test for heteroscedasticity. *Journal of Econometrics*, **17**, 107–112.
- Madsen J.D., Chambers P.A., James W.F., Koch E.W. & Westlake, D. F. (2001) The interaction between water movement, sediment dynamics and submersed macrophytes. *Hydrobiologia*, **444**, 71–84.

- Mazerolle M.J. (2015) AICcmodavg: Model selection and multimodel inference based on(Q)AIC(c). R package version 2.0-3. <http://CRAN.Rproject.org/package=AICcmodavg>.
- Miller T.E. (1987) Effects of emergence time on survival and growth in an early old-field plant community. *Oecologia*, **72**(2), 272–278.
- Mitteroecker P. & Gunz P. (2009) Advances in geometric morphometrics. *Evolutionary Biology*, **36**, 235-247.
- Neill C. (1990) Effects of nutrients and water levels on emergent macrophyte biomass in a prairie marsh. *Canadian Journal of Botany*, **68**, 1007-1014.
- Nichols D.S. & Keeney D.R. (1976a) Nitrogen nutrition of *Myriophyllum spicatum*: variation of plant tissue nitrogen concentration with season and site in Lake Wingra. *Freshwater Biology*, **6**, 137-144.
- Nichols D.S. & Keeney D.R. (1976b) Nitrogen nutrition of *Myriophyllum spicatum*: uptake and translocation of N by shoots and roots. *Freshwater Biology*, **6**, 145-154.
- Niinemets U. & Kull K. (1994) Leaf weight per area and leaf size of 85 Estonian woody species in relation to shade tolerance and light availability. *Forest Ecology and Management*, **70**, 1-10.
- Olsen S.R., Cole C.V., Watanabe F.S. & Dean L.A. (1954) Estimation of available phosphorus in soils by extraction with sodium bicarbonate. USDA circular 939. U.S. Government Printing Office, Washington, DC, USA.
- R Development Core Team. (2013) R: A language and environment for statistical computing. *R Foundation for Statistical Computing*, Vienna, Austria. <http://www.R-project.org/>.
- Rasband W.S. (1997-2014) ImageJ, U. S. National Institutes of Health, Bethesda, Maryland, USA. <http://imagej.nih.gov/ij/>.
- Red River Basin Board (2000) Inventory team report: hydrology. Available at: http://www.redriverbasincommission.org/hydrology_report.PDF
- Reekie E.G. & Bazzaz F. (1987) Reproductive effort in plants. 3. Effect of reproduction on vegetative activity. *The American Naturalist*, **129**(6), 907–919.
- Ribeiro S.P., Pimenta H.R. & Fernandes G.W. (1994) Herbivory by chewing and sucking insects on *Tabebuia ochracea*. *Biotropica*, **26**, 302-307.

- Richards J.H. & Ivey C.T. (2004) Morphological plasticity of *Sagittaria lancifolia* in response to phosphorus. *Aquatic Botany*, **80**, 53-67.
- Robach F., Thiébault G., Trémolières M. & Muller S. (1996) A reference system for continental running waters: Plant communities as bioindicators of increasing eutrophication in alkaline and acid waters in north-east France. *Hydrobiologia*, **341**, 67-76.
- Rohlf F.J. & Marcus L.F. (1993) A revolution in morphometrics. *Trends in Ecology & Evolution*, **8**, 129-132.
- Rohlf F.J. & Slice D.E. (1990) Extensions of the Procrustes method for the optimal superimposition of landmarks. *Systematic Zoology*, **39**, 40-59.
- Royston P. (1982) An extension of Shapiro and Wilk's *W* test for normality to large samples. *Applied Statistics*, **31**, 115-124.
- Růžička J. & Hansen E.H. (1975) Flow injection analyses: Part I. A new concept of fast continuous flow analysis. *Analytica Chimica Acta*, **78**(1), 145-157.
- Sagova-Mareckova M., Petrusek A. & Kvet J. (2009) Biomass production and nutrient accumulation in *Sparganium emersum* Rehm. after sediment treatment with mineral and organic fertilisers in three mesocosm experiments. *Aquatic Ecology*, **43**, 903-913.
- Schutten J., Dainty J. & Davy A.J. (2005) Root anchorage and its significance for submerged plants in shallow lakes. *Journal of Ecology*, **93**, 556-571.
- Sculthorpe C.D. (1967) *The Biology of Aquatic Vascular Plants*. Edward Arnold Publishers Ltd, London, UK.
- Smith C.S. & Adams M.S. (1986) Phosphorus transfer from sediment by *Myriophyllum spicatum*. *Limnology and Oceanography*, **31**, 1312-1321.
- Stenberg P. (1998) Implications of shoot structure on the rate of photosynthesis at different levels in a coniferous canopy using a model incorporating grouping and penumbra. *Functional Ecology*, **12**, 82-91.
- Sugiura N. (1978) Further analysis of the data by Akaike's information criterion and the finite corrections. *Communications in Statistics: Theory and Methods*, **A7**, 13-26.
- Thiebaut G. & Muller S. (1999) A macrophyte communities sequence as an indicator of eutrophication and acidification levels in weakly mineralised streams in north-eastern France. *Hydrobiologia*, **410**, 17-24.
- Titus J, Feldman R & Grisé D. (1990) Submersed macrophyte growth at low ph. I. CO₂ enrichment effects with fertile sediment. *Oecologia*, **84**(3), 307-313.

- Trempe H. & Kohler A. (1995) The usefulness of macrophyte monitoring-system, exemplified on eutrophication and acidification of running waters. *Acta Bot Gallica*, **142** (6), 541-550.
- Underwood A.J. (1997) *Experiments in Ecology: Their Logical Design and Interpretation Using Analysis of Variance*. Cambridge University Press, Cambridge, UK.
- Utts J.M. (1982) The rainbow test for lack of fit in regression. *Communications in Statistics - Theory and Methods*, **11**, 1801-1815.
- Vermaat J.E., Santamaria L. & Roos P.J. (2000) Water flow across and sediment trapping in submerged macrophyte beds of contrasting growth form. *Archiv für Hydrobiologie*, **148**, 549-562.
- Wells C.L. & Pigliucci M. (2000) Adaptive phenotypic plasticity: the case of heterophylly in aquatic plants. *Perspectives in Plant Ecology, Evolution and Systematics*, **3**, 1-18.
- Wilson P., Thompson K. & Hodgson J. (1999) Specific leaf area and leaf dry matter content as alternative predictors of plant strategies. *New Phytologist*, **143**, 155-162.
- Wooten J.W. (1970) Experimental investigations of the *Sagittaria graminea* complex: Transplant studies and genecology. *Journal of Ecology*, **58**(1), 233-242.
- Wooten J.W. (1986) Variations in leaf characteristics of six species of *Sagittaria* (Alismataceae) caused by various water levels. *Aquatic botany*, **23**, 321-327.
- Yates A. & Bailey R. (2010). Improving the description of human activities potentially affecting rural stream ecosystems. *Landscape Ecology*, **25**, 371-382.
- Yates A., Culp J. & Chambers P. (2012). Estimating nutrient production from human activities in subcatchments of the Red River, Manitoba. *Journal of Great Lakes Research*, **38**, 106-114.
- Zar J. H. (2010). *Biostatistical Analysis 5th edition*. Prentice Hall, New Jersey, USA.
- Zeileis A. & Hothorn T. (2002) Diagnostic Checking in Regression Relationships. *R News*, **2**(3), 7-10. <http://CRAN.R-project.org/doc/Rnews/>.
- Zelditch M.L., Swiderski D.L., Sheets H.D. & Fink W.L. (2004) *Geometric morphometrics for biologists: a primer*. Elsevier/Academic Press, Amsterdam.

3.8 Tables and Figures

Table 3.1. *A priori* models explaining variation in length of *Sagittaria cuneata* leaves collected from 15 streams of the Red River Valley in southern Manitoba, Canada, based on variables previously noted as influencing leaf size. Abbreviations for terms are found within the table; α refers to the value of the intercept and ε is the error term.

Model Number	Prediction	Model Structure
1	Positive Influence of total water phosphorus (TPw)	$= \alpha + \beta_1(\text{TPw}) + \varepsilon$
2	Positive Influence of total water nitrogen (TNw)	$= \alpha + \beta_1(\text{TNw}) + \varepsilon$
3	Positive Influence of Olsen P (Sed P)	$= \alpha + \beta_1(\text{Sed P}) + \varepsilon$
4	Positive Influence of nitrate and ammonium (Sed N)	$= \alpha + \beta_1(\text{Sed N}) + \varepsilon$
5	Negative influence of water depth	$= \alpha - \beta_1(\text{water depth}) + \varepsilon$
6	Positive influence of Sed P and Sed N	$= \alpha + \beta_1(\text{Sed P}) + \beta_2(\text{Sed N}) + \varepsilon$
7	Positive influence of TPw and TNw	$= \alpha + \beta_1(\text{TPw}) + \beta_2(\text{TNw}) + \varepsilon$
8	Positive influence of Sed P, Sed N, TPw, TNw	$= \alpha + \beta_1(\text{Sed P}) + \beta_2(\text{Sed N}) + \beta_3(\text{TPw}) + \beta_4(\text{TNw}) + \varepsilon$
9	Positive influence of Sed P, Sed N, TPw, TNw and negative influence of depth	$= \alpha + \beta_1(\text{Sed P}) + \beta_2(\text{Sed N}) + \beta_3(\text{TPw}) + \beta_4(\text{TNw}) - \beta_5(\text{depth}) + \varepsilon$

Table 3.2. Environmental conditions at 15 tributary sites in the Red River valley in southern Manitoba, Canada, in August 2014. Sediment phosphorus (Sed P) and nitrogen (Sed N), total phosphorus and nitrogen in water (TPw and TNw, respectively), pH, specific conductivity (SPC), dissolved oxygen (DO), water temperature and depth, air temperature and light during daylight hours are presented. Sediment chemistry (5 samples/site), depth (15 measurements/site), and air temperature (average of temperature measurements taken every 15 minutes from early-June to late-August, 2014) and light measurements (average of daylight LUX measurements taken every 15 minutes from early-June to late-August, 2014) presented as mean with SE (in parentheses). All other variables were singular measurements. Blank spaces represent data missing for these variables due to equipment failure or absent loggers.

Site	Sediment		Water							Air Temp. (°C)	Light (lux)
	Sed P (mg P/kg)	Sed N (mg N/kg)	TPw (mg/L)	TNw (mg/L)	pH	SPC (µS/cm)	DO (%)	Temp (°C)	Depth (cm)		
AS05A	50.24 (19.70)	70.71 (3.88)	1.35	2.41	8.55	703	77	20	20.80 (1.44)	22 (0.11)	71 250 (950)
BR04A	13.78 (1.42)	26.47 (0.35)	0.12	1.08	8.26	508	54	21	11.93 (1.18)	21 (0.11)	56 500 (1052)
BR05	23.04 (2.90)	36.51 (1.94)	0.12	1.21	8.46	517	89	21	12.73 (1.10)	22 (0.11)	72 500 (1006)
LA02	30.90 (1.97)	79.86 (3.93)	2.74	2.51	8.01	837		16	40.67 (1.86)		
LA03A	40.26 (7.73)	83.47 (6.65)	0.42	1.04	8.25	814	76	21	86.00 (2.33)	21 (0.12)	57 500 (998)
LR01	11.29 (1.72)	24.83 (1.77)	0.08	1.01	8.57	505	96	23	30.25 (2.65)	21 (0.11)	74 000 (1085)
LR03A	32.28 (4.75)	121.24 (5.77)	0.04	1.38	8.13	599	86	18	36.80 (1.81)	21 (0.11)	69 500 (990)
LR04	12.36 (0.94)	57.20 (1.24)	0.09	0.87	8.18	865	57	25	48.30 (1.85)	21 (0.11)	72 750 (1056)
MO04	41.72 (2.83)	66.28 (2.60)	0.12	0.53	8.33	1021	74	19	31.87 (1.17)	19 (0.06)	6 500 (111)
MO06	42.70 (2.85)	67.97 (2.30)	0.28	1.61	8.24	734	67	24	54.73 (5.13)	22 (0.11)	79 250 (1108)
MR02	52.66 (3.64)	86.70 (3.50)	0.36	1.38	8.20	473	75	22	38.20 (2.02)		
RO02	26.06 (2.74)	14.86 (2.22)	0.04	1.33	8.27	556	85	21	22.13 (2.53)	20 (0.09)	45 250 (891)
RT02A	17.26 (1.16)	14.31 (0.68)	0.25	0.91	8.42	517		21	25.33 (3.84)		
RT06	31.44 (4.38)	40.37 (2.06)	0.15	1.19	8.18	388	73	21	15.27 (1.31)	21 (0.10)	67 500 (945)
TC01	41.90 (4.80)	64.99 (5.67)	0.10	1.08	7.93	1290	71.	22	64.80 (1.01)		

Table 3.3. Pearson correlation matrix of r values of all measured leaf size (see Figure 3.2) and mass characteristics of 658 *Sagittaria cuneata* leaves collected from 15 streams of the Red River Valley, Manitoba, Canada in August 2014. B/W refers to the distance between lobes. Significant correlations ($p < 0.05$) are indicated by bold font.

Variable	Area	Width	Blade Length	Lobe Length	B/W Lobes	Length	Dry Mass	Fresh Mass	SLA
Width	0.95								
Blade Length	0.83	0.76							
Lobe Length	0.88	0.86	0.89						
B/W Lobes	0.83	0.88	0.75	0.86					
Length	0.88	0.82	0.96	0.97	0.78				
Dry Mass	0.89	0.85	0.80	0.82	0.77	0.83			
Fresh Mass	0.95	0.91	0.82	0.86	0.82	0.86	0.95		
SLA	0.28	0.28	0.16	0.23	0.17	0.21	-0.13	0.07	
LDMC	-0.24	-0.26	-0.17	-0.25	-0.24	-0.22	0.07	-0.19	-0.74

Table 3.4. Results of three-way Nested ANOVA testing the significant level of replication of emergent leaves of *Sagittaria cuneata* using the following model: $\sqrt{\text{leaf length}} = \mu + \text{site} + \text{quadrat (site)} + \text{plant (quadrat (site))} + \varepsilon$, where 2-3 leaves were collected from each of 3 plants sampled in 5 quadrats at all 15 sites (42-45 leaves per site, 658 total). All emergent leaves were collected from 15 streams of the Red River Valley, Manitoba, Canada in August 2014. Variance components were included for all random effects. Significance ($p < 0.05$) is indicated by bold font.

Source of Variation	d.f.	Mean Square	F	p	Variance Components		
						Estimate	%
Sites	14	3.83	58.50	< 0.001	σ^2_s	0.067	23
Quad (Site)	60	0.99	15.10	< 0.001	$\sigma^2_{Q(S)}$	0.076	26
Plant ((Quad) Site)	150	0.30	4.59	< 0.001	$\sigma^2_{P(Q(S))}$	0.081	28
Residual	432	0.07			σ^2_e	0.065	23

Table 3.5. Comparison of tested *a priori* models for predicting length of emergent *Sagittaria cuneata* leaves in 15 streams of the Red River Valley, Manitoba, Canada in August 2014, using multiple linear regression models and corrected Akaike Information Criterion (AIC_c). Best and competing models based on AIC_c are shown in bold. Also presented are the adjusted R squared (R^2_{adj}) for multiple linear regression models and multiple R squared (R^2) for simple linear regression models, root mean squared error (RMSE), change in AIC_c (Δ_i), relative model likelihood, and model weight (Ω_i).

Model #	Model Variables	R^2_{adj}	R^2	RMSE	AIC _c	Δ_i	Likelihood	Ω_i
9	√Sed P, √Sed N, TNw, TPw, √water depth	0.11		0.50	975.1	0.0	1.00	0.45
7	TNw, TPw	0.11		0.50	975.9	0.8	0.68	0.31
8	√Sed P, √Sed N, TNw, TPw	0.11		0.50	976.8	1.7	0.43	0.19
2	TNw	.	0.10	0.51	979.3	4.2	0.00	0.06
1	TPw	.	0.05	0.52	1018.5	43.4	0.00	0.00
4	√Sed N	.	0.01	0.53	1047.1	72.0	0.00	0.00
6	√Sed P, √Sed N	0.003	.	0.53	1048.9	73.8	0.00	0.00
3	√Sed P	.	0.001	0.53	1050.8	75.6	0.00	0.00
5	√water depth	.	0.18	0.53	1050.9	75.8	0.00	0.00

Sed P, bioavailable phosphorus in sediment in the form of Olsen-P; Sed N, nitrate and ammonia in sediment;
TNw, total nitrogen in water; TPw, total phosphorus in water

Table 3.6. Results of the multiple linear regression of *a priori* model 9, which modelled the influence of several environmental variables on the length of emergent *Sagittaria cuneata* leaves collected from 15 tributary sites of the Red River valley, Manitoba, Canada, in August 2014. Included are the standard partial coefficient regressions for each independent variable and the value for the intercept, t-values and *p*-values of the model: $\sqrt{\text{leaf length}} = \alpha + \beta_1 (\sqrt{\text{Sed P}}) + \beta_2 (\sqrt{\text{Sed N}}) + \beta_3 (\text{TPw}) + \beta_4 (\text{TNw}) + \beta_5 (\sqrt{\text{Water Depth}}) + \varepsilon$. Abbreviations include: Sed P, sediment phosphorus measured as Olsen-P; Sed N, sediment nitrogen measured as $\text{NO}_3 + \text{NH}_4$; TNw, total nitrogen in water; TPw, total phosphorus in water. Variables are transformed as indicated to meet assumptions of linear regression.

Variable	Coefficient	t-value	<i>p</i> -value
Intercept	4.27	38.6	< 0.001
$\sqrt{\text{Sed P}}$	0.03	1.9	0.064
$\sqrt{\text{Sed N}}$	0.01	0.6	0.525
TPw	-0.0002	2.9	0.004
TNw	-0.001	-7.2	< 0.001
$\sqrt{\text{Water Depth}}$	-0.02	-1.9	0.054

Table 3.7. Results of the multiple linear regression of *a priori* model 7, which modelled the influence of total nitrogen (TNw) and phosphorus (TPw) in water on the length of emergent *Sagittaria cuneata* leaves collected from 15 tributary sites of the Red River valley, Manitoba, Canada, in August 2014. Included are the standard partial coefficient regressions of each independent variable, the value of the intercept, t-values and *p*-values of the model, $\sqrt{\text{leaf length of Sagittaria cuneata}} = \alpha + \beta_1 (\text{TPw}) + \beta_2 (\text{TNw}) + \varepsilon$.

Variable	Coefficient	t-value	<i>p</i> -value
Intercept	4.27	56.34	< 0.001
TPw	-0.0001	2.33	0.020
TNw	-0.0005	-6.78	< 0.001

Table 3.8. Results of the multiple linear regression of *a priori* model 8, which modelled the influence of water and sediment chemistry on the length of emergent *Sagittaria cuneata* leaves collected from 15 tributary sites of the Red River valley, Manitoba, Canada, in August 2014. Included are the standard partial coefficient regressions of each independent variable, the value of the intercept, t-values and *p*-values of the model: $\sqrt{\text{leaf length}} = \alpha + \beta_1(\sqrt{\text{Sed P}}) + \beta_2(\sqrt{\text{Sed N}}) + \beta_3(\text{TPw}) + \beta_4(\text{TNw}) + \varepsilon$. Abbreviations include: Sed P, sediment phosphorus measured as Olsen-P; Sed N, sediment nitrogen measured as $\text{NO}_3 + \text{NH}_4$; TNw, total nitrogen in water; TPw, total phosphorus in water. Variables are transformed as indicated to meet assumption of linear regression.

Variable	Coefficient	t-value	<i>p</i> -value
Intercept	4.17	43.27	< 0.001
$\sqrt{\text{Sed P}}$	0.02	1.57	0.118
$\sqrt{\text{Sed N}}$	-0.0002	-0.02	0.984
TPw	0.0001	2.44	0.015
TNw	-0.0005	-6.89	< 0.001

Table 3.9. Comparison of *post hoc* and the best *a priori* models to predict length of emergent *Sagittaria cuneata* leaves in streams of the Red River Valley, Manitoba, Canada in August 2014 (n = 42-45 leaves per site). Variables are transformed as indicated to meet assumptions of regression. Best and competing models based on the corrected Akaike Information Criterion (AIC_c) are shown in bold. Also presented are adjusted R squared (R^2_{adj}) and multiple R squared (R^2) for multiple and simple linear regression models respectively, root mean squared error (RMSE), change in AIC_c (Δ_i), relative model likelihood, and model weight (Ω_i).

Model #	Model Variables	R^2_{adj}	R^2	RMSE	AIC_c	Δ_i	Likelihood	Ω_i
16	NO₃, TNw, latitude, Temp_w, pH, $\sqrt{\text{water depth}}$	0.20	.	0.48	910.9	0	1.00	0.48
17	NO₃, TNw, latitude, Temp_w, pH	0.19	.	0.48	911.6	0.7	0.70	0.33
15	NO₃, TNw, TPw, latitude, Temp_w, pH	0.19	.	0.48	913.5	2.6	0.27	0.13
14	NO ₃ , TNw, TPw, latitude, Temp _w	0.19	.	0.48	914.9	4.0	0.13	0.06
11	NO ₃ , TNw, TPw, latitude	0.14	.	0.49	951.7	40.8	0.00	0.00
13	NO ₃ , TNw, TPw, latitude, $\sqrt{\text{Sed P}}$	0.14	.	0.49	952.4	41.5	0.00	0.00
12	NO ₃ , TNw, TPw, latitude, longitude	0.14	.	0.49	953.7	42.8	0.00	0.00
10	NO ₃ , TNw, TPw	0.12	.	0.50	971.3	60.4	0.00	0.00
<i>A priori</i> 9	$\sqrt{\text{Sed P}}$, $\sqrt{\text{Sed N}}$, TNw, TPw, $\sqrt{\text{water depth}}$	0.11	.	0.50	975.1	64.3	0.00	0.00
9	NO ₃ , TNw	0.11	.	0.50	975.2	64.3	0.00	0.00
<i>A priori</i> 7	TPw, TNw	0.11	.	0.50	975.9	65.0	0.00	0.00
<i>A priori</i> 8	$\sqrt{\text{Sed P}}$, $\sqrt{\text{Sed N}}$, TNw, TPw	0.11	.	0.50	976.8	65.9	0.00	0.00
1	longitude, latitude	0.05	.	0.52	1015.5	104.6	0.00	0.00
5	NO ₃	.	0.02	0.53	1040.8	129.9	0.00	0.00
8	NO ₃ , Temp _w	0.01	.	0.53	1041.9	131.0	0.00	0.00
7	NO ₃ , pH	0.01	.	0.53	1042.8	131.9	0.00	0.00
4	NO ₃ , $\sqrt{\text{Sed P}}$	0.01	.	0.53	1042.8	131.9	0.00	0.00
6	NO ₃ , pH, Temp _w	0.01	.	0.53	1043.7	132.8	0.00	0.00
3	$\sqrt{\text{Sed P}}$, $\sqrt{\text{Sed N}}$	0.003	.	0.53	1048.9	138.0	0.00	0.00
2	pH, Temp _w	-0.001	.	0.53	1051.7	140.8	0.00	0.00

TNw, total nitrogen in water; TPw, total phosphorus in water; NO₃, nitrate in sediment; Temp_w, water temperature (°C);

Sed P, bioavailable phosphorus in sediment in the form of Olsen-P; Sed N, nitrate plus ammonia in sediment

Table 3.10. Results of the multiple linear regression of *post hoc* model 16, which modelled the influence of several environmental and regional variables on the length of emergent *Sagittaria cuneata* leaves collected from 15 tributary sites of the Red River valley, Manitoba, Canada, in August 2014. Included are the standard partial coefficient regressions of each independent variable, the value of the intercept, t-values and *p*-values of the model: $\sqrt{\text{leaf length}} = \alpha + \beta_1 (\text{NO}_3) + \beta_2 (\text{TNw}) + \beta_3 (\text{latitude}) + \beta_4 (\text{Temp}_w) + \beta_5 (\text{pH}) + \beta_6 (\sqrt{\text{water depth}}) + \varepsilon$. Abbreviations include: NO_3 , nitrate in sediment; TNw, total nitrogen in water; Temp_w , water temperature. Variables are transformed as indicated to meet assumption of linear regression.

Variable	Coefficient	t-value	p-value
Intercept	21.11	7.16	< 0.001
NO₃	0.081	3.74	< 0.001
TNw	-0.0004	-9.33	< 0.001
Latitude	-0.36	-6.02	< 0.001
Temp_w	-0.08	-7.26	< 0.001
pH	0.32	2.49	0.013
$\sqrt{\text{Water Depth}}$	0.02	1.65	0.099

Table 3.11. Results of the multiple linear regression of *post hoc* model 17, which modelled the influence of several environmental and regional variables on the length of emergent *Sagittaria cuneata* leaves collected from 15 tributary sites of the Red River valley, Manitoba, Canada, in August 2014. Included are the standard partial coefficient regressions of each independent variable, the value of the intercept, t-values and *p*-values of the model: $\sqrt{\text{leaf length}} = \alpha + \beta_1 (\text{NO}_3) + \beta_2 (\text{TNw}) + \beta_3 (\text{latitude}) + \beta_4 (\text{Temp}_w) + \beta_5 (\text{pH}) + \varepsilon$. Abbreviations include: NO_3 , nitrate in sediment; TNw, total nitrogen in water; Temp_w , water temperature. Variables are transformed as indicated to meet assumption of linear regression.

Variable	Coefficient	Partial R ²	t-value	p-value
Intercept	21.77	-	7.47	< 0.001
NO₃	0.07	0.017	3.40	< 0.001
TNw	-0.0004	0.116	-9.29	< 0.001
Latitude	-0.36	0.051	-5.95	< 0.001
Temp_w	-0.07	0.071	-7.08	< 0.001
pH	0.23	0.006	1.92	0.055

Table 3.12. Results of the multiple linear regression of *post hoc* model 15, which modelled the influence of several environmental and regional variables on the length of emergent *Sagittaria cuneata* leaves collected from 15 tributary sites of the Red River valley, Manitoba, Canada, in August 2014. Included are the standard partial coefficient regressions of each independent variable, the value of the intercept, t-values and *p*-values of the model: $\sqrt{\text{leaf length}} = \alpha + \beta_1 (\text{NO}_3) + \beta_2 (\text{TNw}) + \beta_3 (\text{TPw}) + \beta_4 (\text{latitude}) + \beta_5 (\text{Temp}_w) + \beta_6 (\text{pH}) + \varepsilon$. Abbreviations include: NO_3 , nitrate in sediment; TNw, total nitrogen in water; TPw, total phosphorus in water ; Temp_w , water temperature. Variables are transformed as indicated to meet assumption of linear regression.

Variable	Coefficient	t-value	<i>p</i> -value
Intercept	21.84	7.47	< 0.001
NO₃	0.07	3.35	< 0.001
TNw	-0.0004	-5.67	< 0.001
TPw	-0.00001	-0.34	0.732
Latitude	-0.36	-5.95	< 0.001
Temp_w	-0.07	-6.48	< 0.001
pH	-0.21	1.85	0.064

Table 3.13: Results of three-way nested ANOVAs testing the significant level of replication of shape of emergent leaves of *Sagittaria cuneata* using the following model: $\log_{10}(\text{leaf shape summary} + 0.5) = \mu + \text{site} + \text{quadrat}(\text{site}) + \text{plant}(\text{quadrat}(\text{site})) + \varepsilon$, where 2-3 leaves were collected from each of 3 plants sampled in 5 quadrats at all 15 sites (42-45 leaves per site, 658 total). All emergent leaves were collected from 15 streams of the Red River Valley, Manitoba, Canada in August 2014. Variance components were included for all random effects. Significance ($p < 0.05$) is indicated by bold font.

Dependent Variable	Source of Variation	d.f.	Mean Square	F	p	Variance Components		
							Estimate	%
$\log_{10}(\text{PC1} + 0.5)$	Sites	14	0.85	37.52	< 0.001	σ^2_s	0.017	30
	Quad (Site)	60	0.11	4.98	< 0.001	$\sigma^2_{Q(S)}$	0.007	13
	Plant ((Quad) Site)	150	0.05	2.21	< 0.001	$\sigma^2_{P(Q(S))}$	0.009	16
	Residual	432	0.02			σ^2_e	0.023	41
$\log_{10}(\text{PC2} + 0.5)$	Sites	14	0.16	30.63	< 0.001	σ^2_s	0.003	21
	Quad (Site)	60	0.03	6.48	< 0.001	$\sigma^2_{Q(S)}$	0.002	14
	Plant ((Quad) Site)	150	0.02	3.06	< 0.001	$\sigma^2_{P(Q(S))}$	0.004	29
	Residual	428	0.01			σ^2_e	0.005	36

Table 3.14. Results of simple linear regressions modelling the influence of sediment nutrients on the summary shape variables (PC 1 and PC 2) of emergent *Sagittaria cuneata* leaves collected from 15 tributary sites of the Red River valley, Manitoba, Canada, in August 2014. Included are the standard partial coefficient regressions of each independent variable and the value of the intercept, t-values and *p*-values of the *a priori* multiple linear regression model, $\log_{10}(\text{PC} + 0.5) = \alpha + \beta_1 (\text{sediment chemistry}) + \varepsilon$. Abbreviations include: Sed P, sediment phosphorus measured as Olsen-P; Sed N, sediment nitrogen measured as $\text{NO}_3 + \text{NH}_4$. Variables are transformed as indicated to meet assumption of linear regression.

Response	Variable	Coefficient	t-value	p-value
$-\log_{10}(\text{PC1} + 0.5)$	Intercept	-0.75	-24.32	< 0.001
	$\sqrt{\text{Sed N}}$	0.004	1.09	0.275
$\log_{10}(\text{PC1} + 0.5)$	Intercept	-0.68	-20.33	< 0.001
	$\sqrt{\text{Sed P}}$	-0.01	-1.11	0.266
$\log_{10}(\text{PC2} + 0.5)$	Intercept	-0.68	-44.37	< 0.001
	$\sqrt{\text{Sed N}}$	-0.002	-1.48	0.140
$\log_{10}(\text{PC2} + 0.5)$	Intercept	-0.71	-42.50	< 0.001
	$\sqrt{\text{Sed P}}$	0.001	0.48	0.635

Table 3.15. Results of several simple linear regressions modelling the influence of water nutrients on the summary shape variables (PC 1 and PC 2) of emergent *Sagittaria cuneata* leaves collected from 15 tributary sites of the Red River valley, Manitoba, Canada, in August 2014. Included are the standard partial coefficient regressions of each independent variable and the value of the intercept, t-values and *p*-values of the *a priori* multiple linear regression model, $\log_{10}(\text{PC} + 0.5) = \alpha + \beta_1(\text{water chemistry}) + \varepsilon$. Abbreviations include: TNw, total nitrogen in water; TPw, total phosphorus in water. Variables are transformed as indicated to meet assumption of linear regression.

Response	Variable	Coefficient	t-value	<i>p</i> -value
$\log_{10}(\text{PC1} + 0.5)$	Intercept	-2.34	-14.34	< 0.001
	$\log_{10}(\text{TNw})$	0.23	9.95	< 0.001
$\log_{10}(\text{PC1} + 0.5)$	Intercept	-0.97	-22.14	< 0.001
	$\log_{10}(\text{TPw})$	0.05	5.85	< 0.001
$\log_{10}(\text{PC2} + 0.5)$	Intercept	-2.34	-14.34	< 0.001
	$\log_{10}(\text{TNw})$	0.23	9.95	< 0.001
$\log_{10}(\text{PC2} + 0.5)$	Intercept	-0.97	-22.14	< 0.001
	$\log_{10}(\text{TPw})$	0.05	5.85	< 0.001

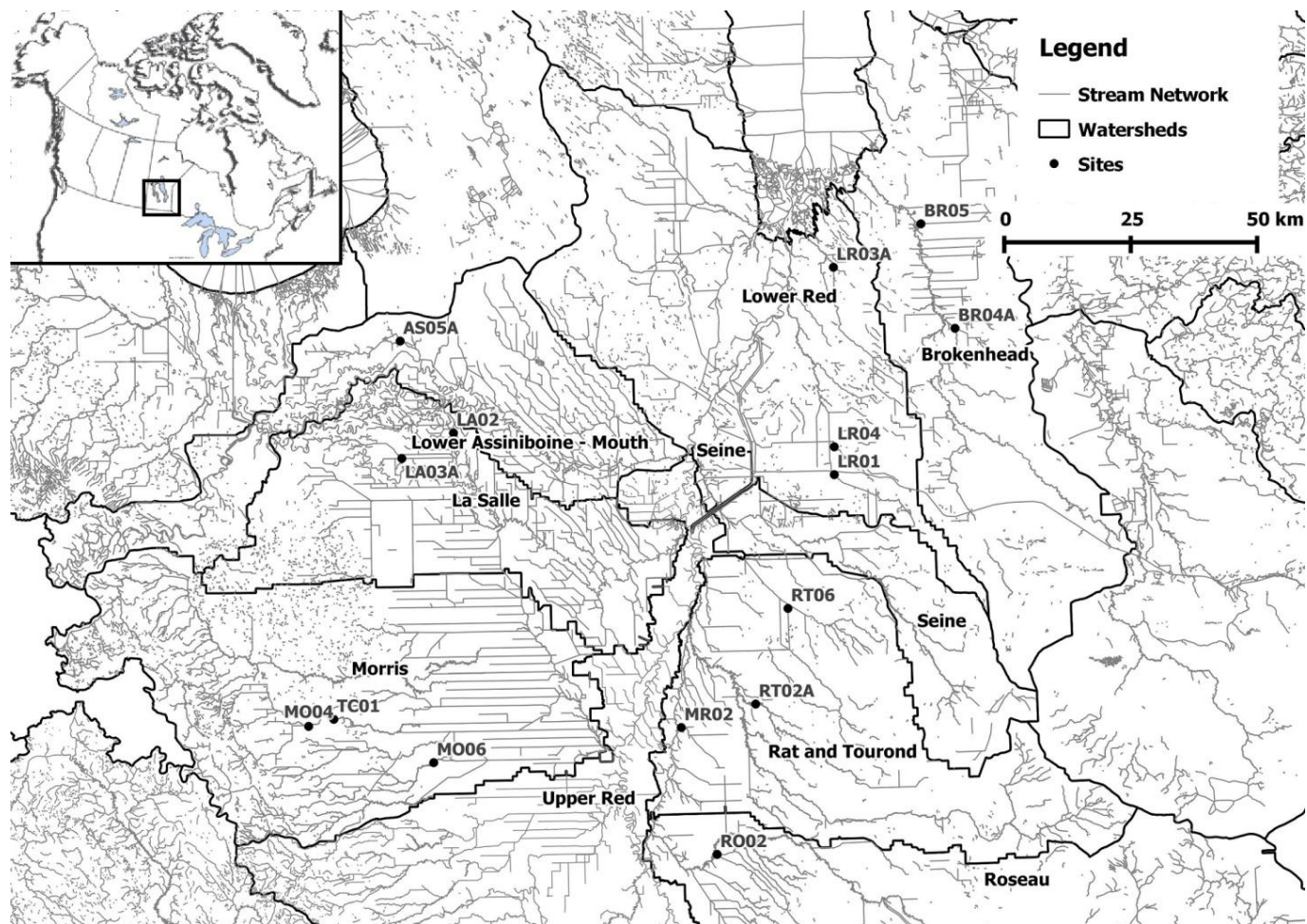


Figure 3.1. Map of the 15 sites sampled in August 2014 in independent tributaries of the Red River Valley in southern Manitoba, Canada.

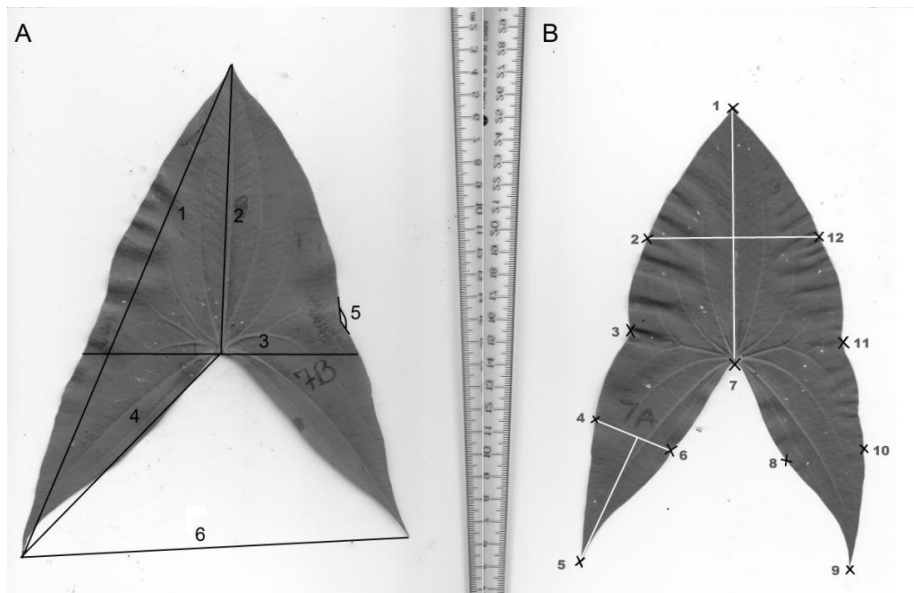


Figure 3.2. *Sagittaria cuneata* leaves showing (A) measurements taken on each leaf: (1) length, (2) blade length, (3) width, (4) lobe length, (5) lobe angle, and (6) between lobes; and (B) 12 landmarks that represent shape of each leaf.

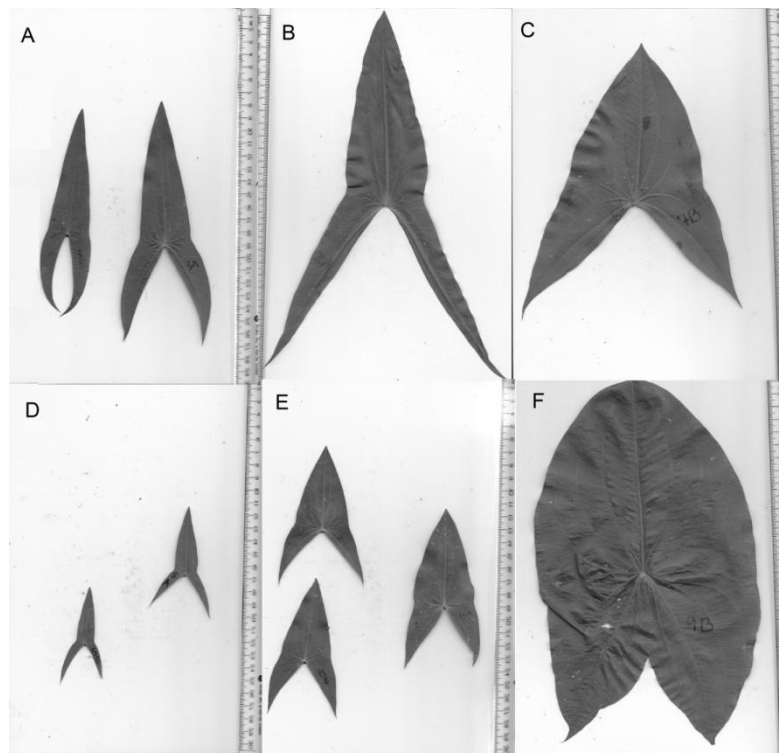


Figure 3.3. Examples of size and shape variability in *Sagittaria cuneata* leaves collected from several 15 streams of the Red River Valley, Manitoba, Canada in July 2013 and August 2014. All pictures show the same scale using a standard 30 cm ruler.

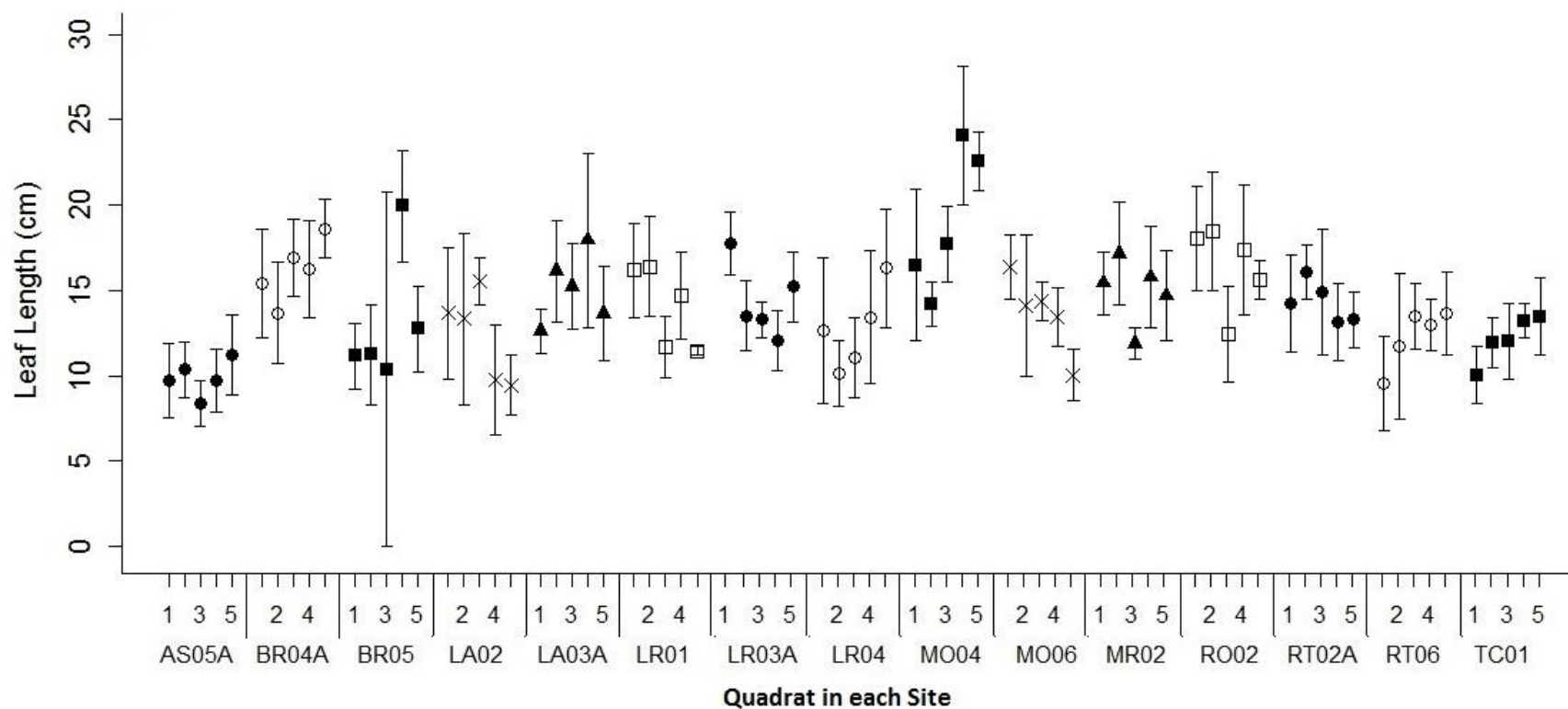


Figure 3.4. Variation in leaf length of *Sagittaria cuneata* within and among 15 different tributary sites in the Red River, Manitoba, Canada, in August 2014. Data are presented as average length (cm) ($\pm$ SD, $n = 7-9$ leaves per quadrat) of emergent leaves collected from each haphazardly placed quadrat. Different symbols and shades are used for ease of visualization of quadrats between sites.

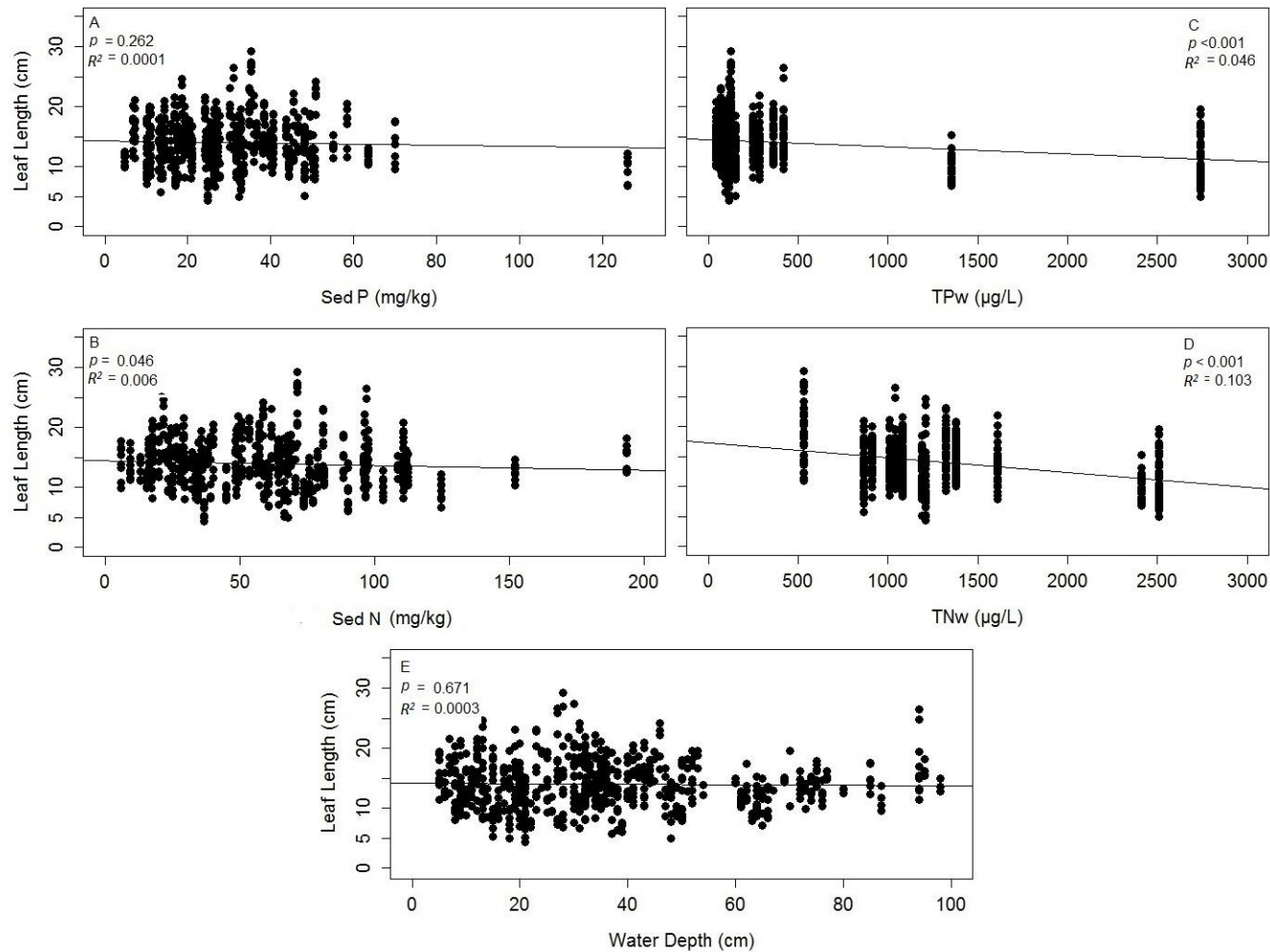


Figure 3.5. Variation in length of all 658 emergent *Sagittaria cuneata* leaves collected from 15 tributary sites ($n = 42\text{--}45/\text{site}$) of the Red River, Manitoba, Canada, in August 2014 in relation to phosphorus in sediment (A), nitrogen in sediment (B), total phosphorus in water (C), total nitrogen in water (D) and water depth (E) (see Appendix Table B2).

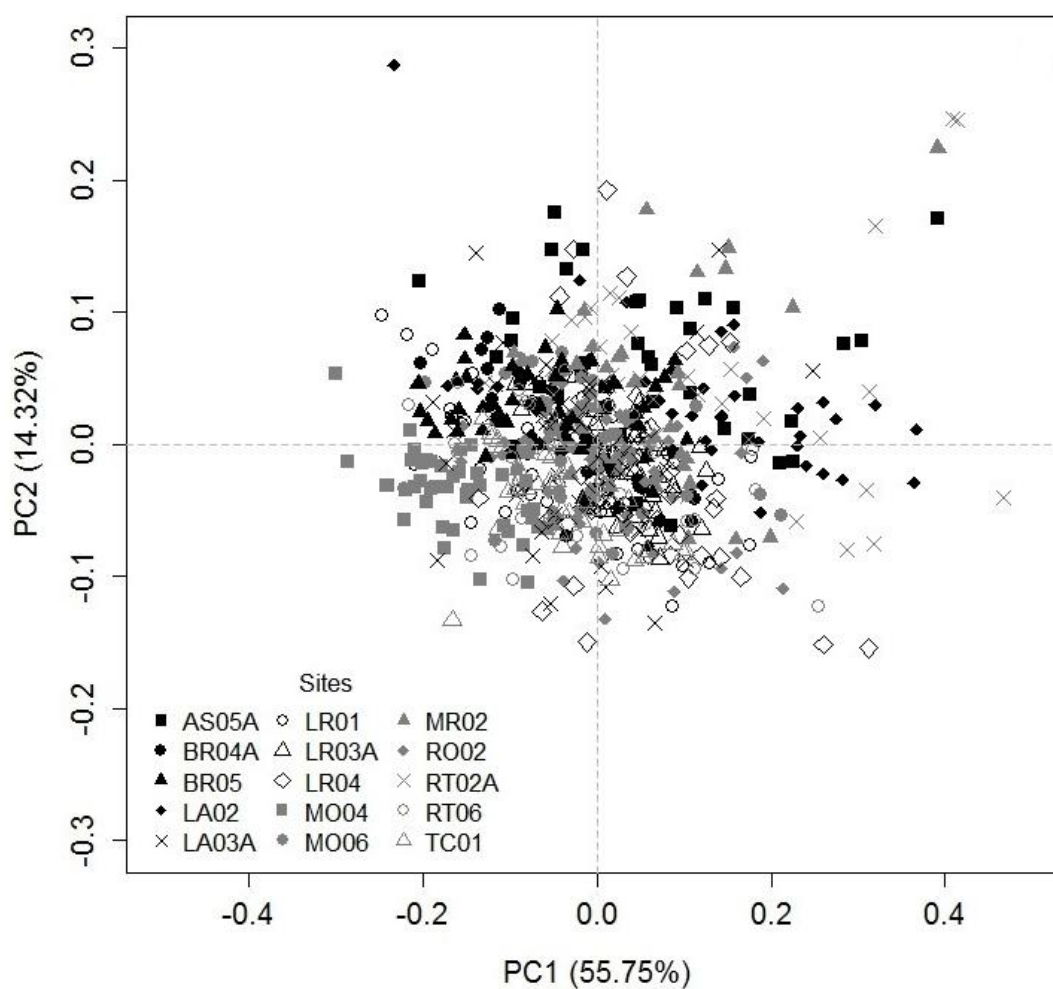


Figure 3.6. Bivariate plot of unaveraged ($n = 42 - 45$ leaves per site, 658 total leaves) principal components (PC) 1 and 2 scores summarizing variation in 12 sets of Euclidean landmark coordinates (i.e., shape, see Figure 3.2) of *Sagittaria cuneata* leaves collected from 15 tributary sites in the Red River Valley, Manitoba, Canada, in August 2014.

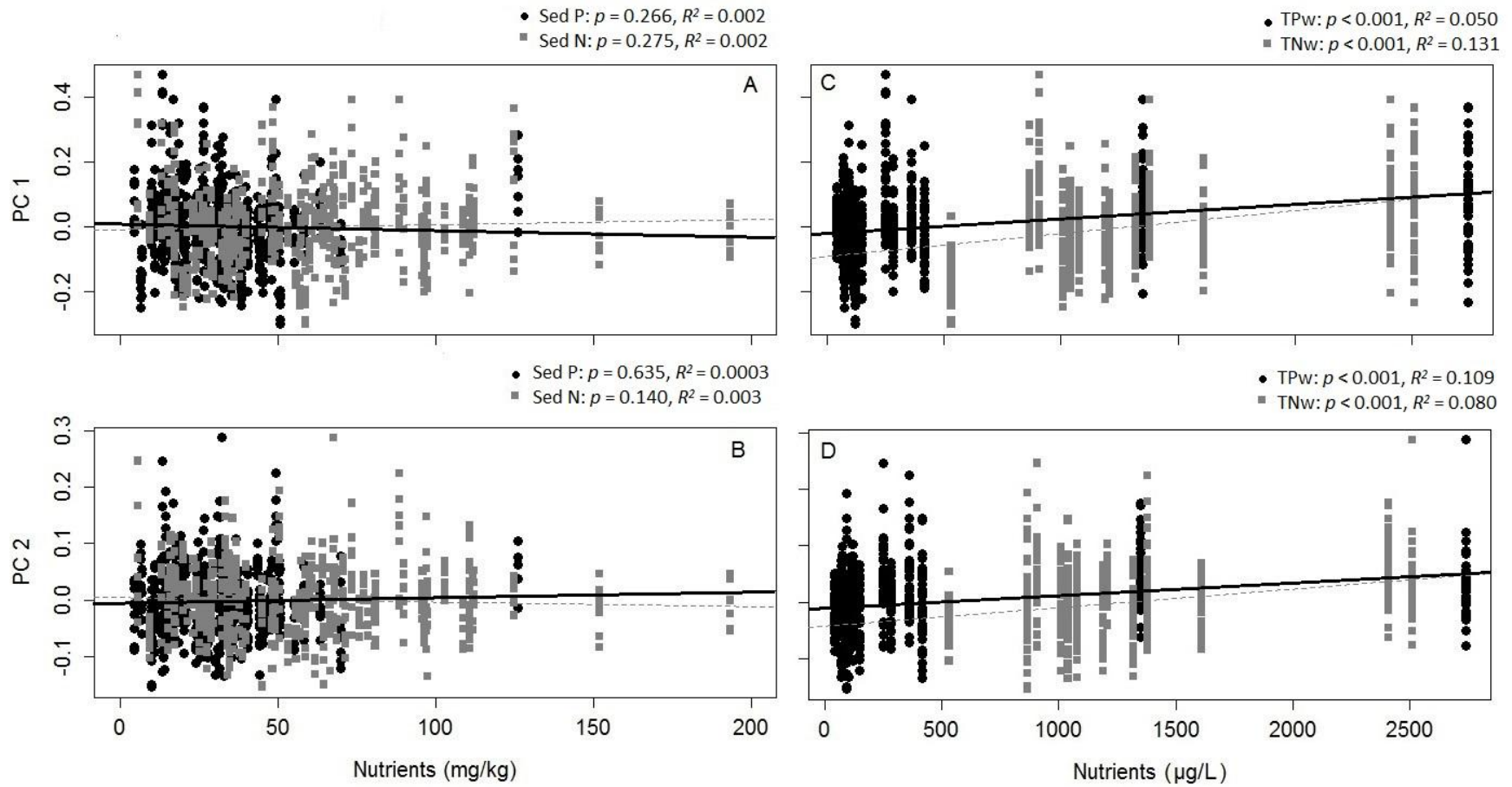


Figure 3.7. Variation of principal components (PC) 1 and 2 scores, summarizing emergent leaf shape of *Sagittaria cuneata* ($n = 42-45$ leaves per site, 658 leaves total) collected from 15 streams of the Red River Valley, Manitoba, Canada in August 2014, with sediment nutrients (A and B, respectively) and water nutrients (C and D, respectively) (see Tables 3.14 and 3.15).

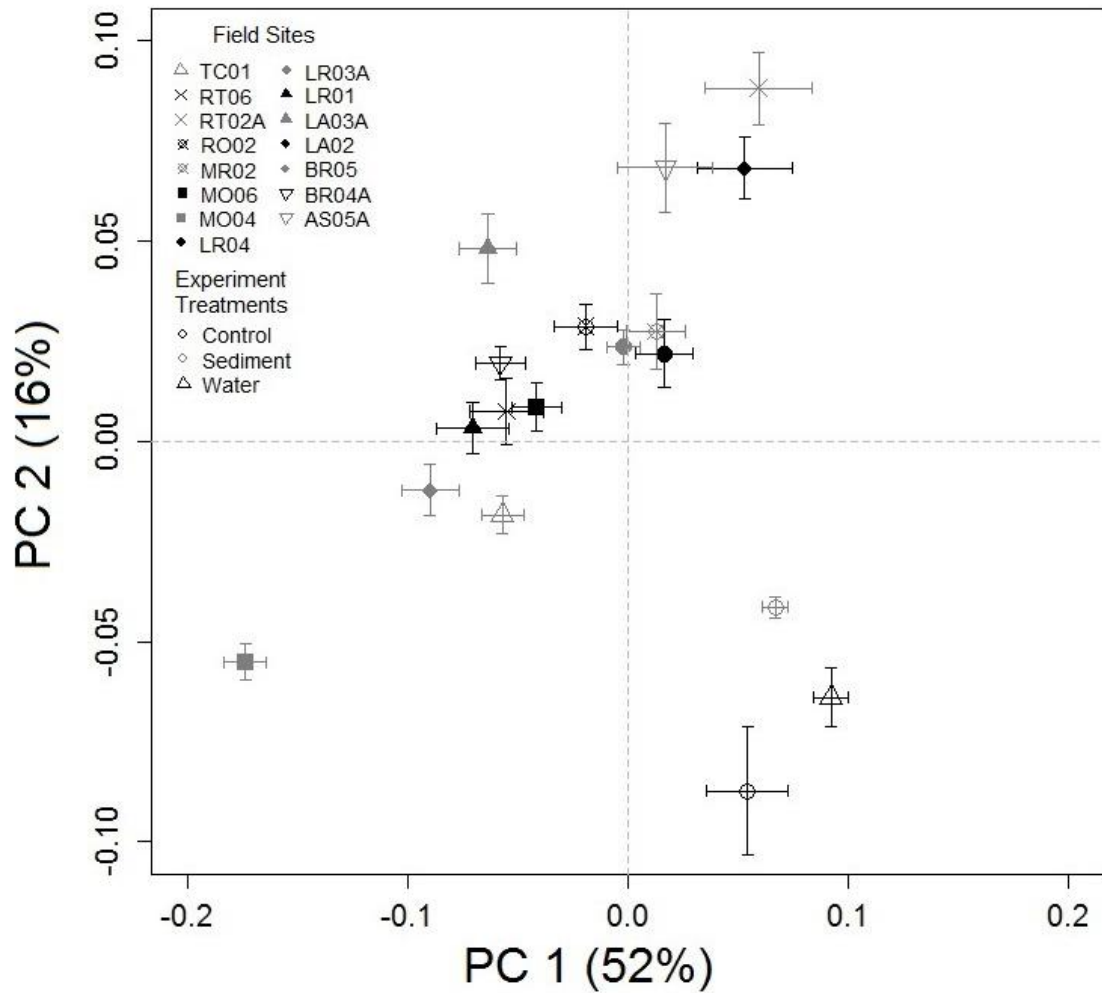


Figure 3.8. Bivariate plot of averaged ($\pm$ SE) principal components (PC) 1 and 2 summarizing variation in 12 Euclidean landmark coordinates (i.e., shape, see Figure 3.2) of *Sagittaria cuneata* leaves collected from 15 tributary sites in the Red River Valley, Manitoba, Canada, in August 2014 ($n = 42-45$ leaves per site, 658 total leaves), and leaves collected from a controlled nutrient enrichment experiment ($n = 7-200$ leaves per group, 272 total leaves) with plants growing in three different nutrient treatment groups (water-enriched, sediment-enriched and control).

Chapter 4

General Conclusions and Synthesis

This study forms part of a larger initiative investigating the effects of nutrient loading and eutrophication from anthropogenic sources on aquatic ecosystems in the Red River Valley, Manitoba, Canada. The goal of my project was to determine whether the emergent aquatic plant, arrowhead or katniss (*Sagittaria cuneata* - Sheldon) could be used as a reliable bioindicator of stream nutrient concentrations. Specifically, I investigated whether emergent leaf morphology (size and shape) could reliably predict concentrations of nitrogen (N) and/or phosphorus (P), the two nutrients implicated in eutrophication. To achieve this goal, I undertook two different studies: a controlled nutrient manipulation study designed to separate the effects of N and P added to either low-nutrient sediment or water (Chapter 2), and a field study investigating the association of multiple environmental variables on leaf morphology in streams with varying inputs of N and P from anthropogenic sources (Chapter 3).

Both studies presented interesting results on the extreme variability of the emergent foliage of *S. cuneata*. The experimental study demonstrated that when plants have access to sufficient sediment nutrients, leaf size and shape will change throughout the growing season for many reasons. Increasing leaf size during vegetative plant growth leads to increasing leaf area for solar energy capture (Niinemetsan & Kull 1994; Ribeiro et al. 1994; Ackerly & Donoghue 1998; Stenberg 1998), and thus, a greater photosynthetic rate. However, near the end of the growing seasons, plants will focus on reproductive growth at the expense of vegetative growth (Bazzaz et al. 1987; Reekie & Bazzaz 1987); thus, leaf size will decrease. This observation in the experimental study led to an explanation of why leaf size and shape varied at the leaf scale in the field study. Approximately 25% of leaf size variation occurred among leaves within a single

plant, likely because plants had recently started flowering, and thus, were allocating nutrients to reproduction rather than vegetative structures such as leaves.

Interestingly, leaf size and shape in the field study did not respond to sediment nutrients as clearly as in the experimental study. In fact, leaf shape variation (summarized as PC 1 and PC 2) was explained in part by N and P in water, but not by nutrients in the sediment. This may be because the experiment was designed to manipulate either sediment or water nutrients while standardizing the other nutrient medium, whereas nutrients in sediment and water from field sites are dependent on many sources and vary independently. Leaf size in the field study varied with multiple environmental and regional variables, including N in the water and sediment, though P was not implicated. Based on N:P ratios from the field sites, N was often limiting in both the sediment and water; therefore, N concentrations in both mediums would drive plant growth. This distinction was less clear in the experiment, where individual effects of N and P could not be separated.

The field study demonstrated that leaf morphology was variable at all scales of replication (leaves, plants, quadrats and sites). Interestingly, variability in leaf size and shape was relatively similar at all scales, suggesting that *S. cuneata* is naturally a variable species. Variation in leaf size among plants within quadrats is likely because of differences in germination dates (e.g., Miller 1987). When plants germinate later, they have less time to establish and, therefore, have decreased biomass and leaf size compared to plants that germinate earlier. In addition, competition was likely influencing leaf size in the field study, as plants often grew in close proximity (e.g., Goldberg 1987). Thus, plants that germinated later would be outcompeted for valuable

resources, such as nutrients and light, by those that germinated earlier. The experiment was designed such that each plant was independent, therefore competition did not occur. In addition, germination date was not implicated in plant productivity, likely because all experimental plants germinated within 8 days. If there were several weeks between germinations dates in the field sites, than plants would likely exhibit large differences in biomass and leaf size, especially when competition is taken into consideration.

Variability in leaf morphology at the quadrat and site scales occurred because of environmental heterogeneity (e.g., Balaguer et al. 2001). Many environmental variables will vary along a single tributary, such as sediment composition and chemistry, and light availability. Because these variables are crucial for plant growth and productivity, variations within a single site will lead to large differences in plants at the quadrat scale. Environmental variables will also differ among sites, as each site will have varying nutrient inputs, sediment composition, light availability and water quality. The tendency of aquatic plants to reproduce vegetatively (Jones et al. 2012) suggests that plants within a site will be more genetically similar than those among sites, which may lead to the differences in leaf morphology. However, comparison of emergent leaves among plants within the field and experimental studies demonstrated that leaf shapes among plants within the controlled experiment were different from those within the field study, even though tubers used in the experiment were collected from two field sites (TC01 and LR01); thus, I can conclude that genetic differences were unlikely influencing leaf shape of *S. cuneata*. Rather, differing environmental conditions, in addition to the age structure of individual leaves and plants, are the major drivers in variability of leaf morphology in both the field and experimental studies.

This thesis had two main objectives: 1. to determine the effects of N and P in water and sediment on leaf morphology of *S. cuneata*; and 2. to develop leaf morphology of *S. cuneata* as a reliable bioindicator of stream nutrients in southern Manitoba. The field study demonstrated that many environmental and regional variables influenced leaf size, including N in sediment and water, and that leaf shape was weakly related to N and P in water, but not the sediment. In addition, the experimental study demonstrated the importance of sediment chemistry, though the effects of N and P could not be separated. Though the results of each study provided interesting information on the life history of these plants and the relationship between leaf morphology of *S. cuneata* and the surrounding environment, the variability of leaf morphology, especially in the field, proves difficult for use as a bioindicator. A good bioindicator would be at the site scale, where an interested person or researcher could walk into a stream and diagnose the level of nutrients based on the leaf size and/or shape of *S. cuneata*. However, the field study demonstrated equal variability among the leaf, plant, quadrat and site scale, suggesting large natural variability within the species. In addition, conflicting results regarding the importance of sediment and water nutrients in both studies makes it incredibly difficult to develop a bioindicator. Therefore, I conclude that *Sagittaria cuneata* is not a reliable indicator of stream nutrients at this time. However, this natural variability offers interesting opportunities to study how individuals and populations of *S. cuneata* respond to the surrounding environment.

To better understand the influences of environmental variables on the emergent leaf morphology of *Sagittaria cuneata*, further research is required. To explore variability in leaf morphology at the quadrat and site scales, future research would

require careful selection of patches of *S. cuneata* plants within multiple sites that have varying environmental conditions, such as full sun, partially shaded or fully shaded areas, and collection of data at each of these points within the stream (i.e., water temperature, depth, light, sediment and water chemistry, sediment composition). A future experiment should further explore responses to sediment nutrient additions by growing plants in discrete N and P treatments spanning a range of concentrations. For example, this could be accomplished by using relatively low nutrient sediment collected from a tributary in Manitoba that sustains a population of *S. cuneata*, and applying a range of treatments. Also, the use of different slow-release fertilizers in the experimental study to manipulate nutrients caused unexpected differences in sediment chemistry among the sediment treatment levels. A new method of altering sediment N and P must be explored to attain desired sediment nutrient concentrations and better assess the influences of each nutrient, such as supplementing with agar cubes that have added nutrients. This way, roots can grow into the agar to directly access nutrients. Future research may advance our knowledge of the variability and plasticity of the emergent foliage of *Sagittaria cuneata*, and provide information on how these traits respond to environmental variation.

4.1References

- Ackerly D.D. & Donoghue M.J. (1998) Leaf size, sapling allometry, and Corner's rules: phylogeny and correlated evolution in maples (*Acer*). *The American Naturalist*, **152**, 767-791.
- Bazzaz F., Chiariello N.R., Coley P.D. & Pitelka L.F. (1987) Allocating resources to reproduction and defense. *BioScience*, **37**(1), 58-67.

- Balaguer L., Martínez-Ferri E., Valladares F., Pérez-Corona M.E., Baquedano F.J., Castillo F.J. and Manrique E. (2001) Population divergence in the plasticity of the response of *Quercus coccifera* to the light environment. *Functional Ecology*, **15**, 124-135.
- Goldberg D.E. (1987) Neighborhood competition in an old-field plant community. *Ecology*, **68**, 1211-1223.
- Jones J.I., Collins A.L., Naden P.S. & Sear. D.A. (2012) The relationship between fine sediment and macrophytes in rivers. *River Research and Applications*, **28**, 1006-1018
- Miller T.E. (1987) Effects of emergence time on survival and growth in an early old-field plant community. *Oecologia*, **72**(2), 272–278.
- Niinemets U. & Kull K. (1994) Leaf weight per area and leaf size of 85 Estonian woody species in relation to shade tolerance and light availability. *Forest Ecology and Management*, **70**, 1-10.
- Reekie E.G. & Bazzaz F. (1987) Reproductive effort in plants. 3. Effect of reproduction on vegetative activity. *The American Naturalist*, **129**(6), 907–919.
- Ribeiro S.P., Pimenta H.R. & Fernandes G.W. (1994) Herbivory by chewing and sucking insects on *Tabebuia ochracea*. *Biotropica*, **26**, 302-307.
- Stenberg P. (1998) Implications of shoot structure on the rate of photosynthesis at different levels in a coniferous canopy using a model incorporating grouping and penumbra. *Functional Ecology*, **12**, 82-91.

Appendix A – Supplementary Information for Chapter 2

Table A1. One-way ANOVA results of differences in sediment nutrient concentrations and ratios of N:P in both water and sediment among 8 treatment levels (see Tables 2.1 and 2.2) in the enrichment experiment using *Sagittaria cuneata*. N:P in water was the average N:P for each bucket over the 3 measuring periods. Note that the treatment level 4 (Sediment-LP,HN) was omitted from analysis due to high plant mortality. The following notation is used: Sed P, bioavailable sediment P in the form of Olsen-P; Sed N, bioavailable sediment nitrogen measured as nitrate and ammonia. A significant treatment effect ($p < 0.05$) is indicated by bold font. Variables were transformed as indicated.

Dependent Variable	Source of Variation	d.f.	Mean Square	F	p
log₁₀ (Sed P)	Treatment	7	3.60	57.55	<0.001
	Residuals	16	0.06		
Sed N	Treatment	7	1517.32	29.05	<0.001
	Residuals	16	52.24		
N:P in sediment	Treatment	7	1.01	4.62	0.006
	Residuals	16	0.22		
log₁₀ (N:P in water)	Treatment	7	3.90	13.16	<0.001
	Residuals	22	0.30		

Table A2. One-way ANOVA results examining differences in plant metrics of *Sagittaria cuneata* based on the proximity of each bucket to edges of the experimental site (i.e., bucket position). There were three bucket positions: the first row from the site edge, second row from site edge, and all remaining buckets in the centre of the site (27, 20 and 49 buckets, respectively). Variables were transformed as indicated.

Dependent Variable	Source of Variation	d.f.	Mean Square	F	p
Final Plant Height	Bucket Position	2	64.49	1.34	0.268
	Residual	77	48.15		
Plant Biomass⁻¹	Bucket Position	2	0.06	1.12	0.333
	Residual	77	0.05		
Average Leaf Length	Bucket Position	2	3.06	0.43	0.656
	Residual	51	7.21		

Table A3. One-way ANOVA results examining differences in initial plant condition (tuber mass in g and plant height in cm) among nutrient treatment levels (see Table 2.1 and 2.2) for 80 *Sagittaria cuneata* plants.

Dependent Variable	Source of Variation	d.f.	Mean Square	F	p
Initial Tuber Mass	Treatment	7	0.01	1.63	0.141
	Residual	72	0.01		
Initial Plant Height	Treatment	7	10.79	0.75	0.630
	Residual	72	14.37		

Table A4. One-way ANOVA results examining differences in initial and final plant height (cm), and final biomass (g) of the 80 *S. cuneata* plants used in a 10-week nutrient enrichment experiment. Collection site refers to the 2 field sites where tubers were collected (TC01 and LR01, Chapter 3) and planting date refers to when tubers were initially planted (2 dates: May 24-25, 2014). Variables are transformed as indicated to meet assumptions of ANOVA.

Dependent Variable	Source of Variation	d.f.	Mean Square	F	p
log₁₀ (Initial Plant Height)	Collection Site	1	0.48	3.05	0.085
	Residual	78	0.16		
	Planting Date	1	0.00	0.00	0.982
	Residual	78	0.16		
Final Plant Height	Collection Site	1	53.98	1.11	0.295
	Residual	78	48.49		
	Planting Date	1	134.04	2.82	0.097
	Residual	78	47.47		
1/Plant Biomass	Collection Site	1	0.00	0.01	0.936
	Residual	78	0.05		
	Planting Date	1	0.02	0.41	0.524
	Residual	78	0.05		

Table A5. Regression statistics for the influence of number of days until each plant germinated (Days to Germination) and produced emergent leaves (Days to Emergence) on the initial and final plant height (cm), and final plant biomass (g) of 80 *Sagittaria cuneata* plants grown in a 10-week nutrient experiment in 2014. Presented are the standard partial coefficient regressions, t-values and *p*-values of the simple linear regression model, dependent variable = $\alpha + \beta_1$ (Independent Variable) + ε . Variables are transformed as indicated to meet assumption of linear regression.

Dependent Variable	Independent Variable	Coefficient	t-value	<i>p</i>-value
log₁₀(Initial Height)	Intercept	2.26	20.85	<0.001
	Days to Germination	0.01	0.35	0.724
log₁₀(Initial Height)	Intercept	2.91	16.64	< 0.001
	Days to Emergence	-0.01	-3.32	0.002
Final Height	Intercept	26.82	13.43	< 0.001
	Days to Germination	-0.20	-0.46	0.644
Final Height	Intercept	32.46	8.28	<0.001
	Days to Emergence	-0.09	-1.13	0.263
Final Mass⁻¹	Intercept	0.21	3.19	0.002
	Days to Germination	0.01	0.72	0.471
Final Mass⁻¹	Intercept	0.04	0.44	0.663
	Days to Emergence	0.00	1.51	0.138

Table A6. Results of secondary one-way ANOVAs assessing differences in plant metrics of *Sagittaria cuneata* among treatment levels within either the water treatment group (Treatment level 6-9) or the sediment treatment group (Treatment levels 2, 3 and 5) (see Tables 2.1 and 2.2). Note that Sediment- LP,HN (Treatment level 4) was omitted from analysis due to high plant mortality. Variables were transformed as indicated.

Dependent Variable	Source of Variation	d.f.	Mean Square	F	p
Final Height	Sediment Treatments	2	3.43	0.05	0.950
	Residual	27	66.43		
	Water Treatments	3	19.37	1.56	0.207
	Residual	36	12.13		
1/ (Final Biomass)	Sediment Treatments	2	0.06	1.13	0.339
	Residual	27	0.05		
	Water Treatments	3	0.26	1.75	0.173
	Residual	36	0.15		
Average Leaf Length	Sediment Treatments	2	4.57	1.31	0.288
	Residual	23	3.48		
	Water Treatments	3	1.31	2.67	0.073
	Residual	22	0.49		

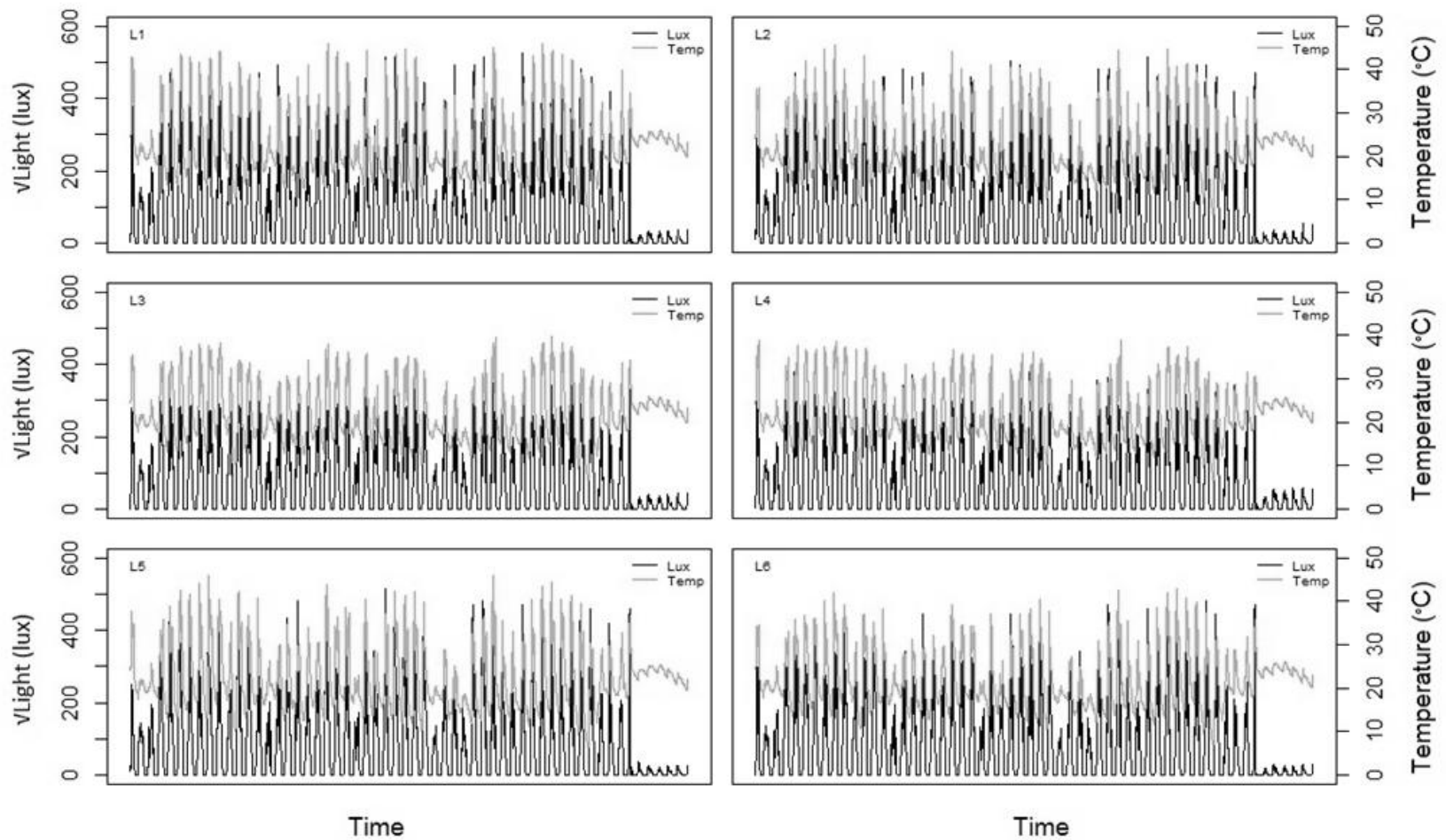


Figure A1. Square root of relative light (lux) and air temperature (°C) over time in designated buckets (L1 to L6) randomly distributed throughout the experimental site of a 10-week nutrient enrichment experiment. Loggers were deployed July 14th, 2014 and removed Aug 22nd, 2014.

Appendix B – Supplementary Information for Chapter 3

Table B1. Results of one-way ANOVAs testing the variation of sediment nutrients among 15 sampling sites ($n = 5$ per site) in the Red River valley, Manitoba, Canada, collected in August 2014. Variables are transformed as indicated to meet assumptions of ANOVA. Significant ($p < 0.05$) main effects are indicated by bold font.

Dependent Variable	Source of Variation	<i>d.f.</i>	Mean Square	<i>F</i>	<i>P</i>
$\sqrt{\text{Sed N}}$	Site	14	17.744	7.229	<0.001
	Residual	60	2.458		
$\log_{10}(\text{Sed P})$	Site	14	1.451	14.576	<0.001
	Residual	60	0.099		

Table B2. Results of simple linear regressions modelling the influence of several environmental variables on the leaf length of emergent *Sagittaria cuneata* leaves collected from 15 tributary sites of the Red River valley, Manitoba, Canada, in August 2014. Included are the standard partial coefficient regressions of each independent variable and the value of the intercept, t-values and *p*-values of the model, $\sqrt{\text{leaf length}} = \alpha + \beta_1 (\text{independent variable}) + \varepsilon$. Abbreviations include: TNw, total nitrogen in water; TPw, total phosphorus in water; Sed P, sediment phosphorus measured as Olsen-P; Sed N, sediment nitrogen measured as $\text{NO}_3 + \text{NH}_4$. Variables are transformed as indicated to meet assumption of linear regression.

Variable	Coefficient	t-value	<i>p</i> -value
Intercept	3.73	31.87	< 0.001
$\log_{10}(\text{Sed P})$	-0.01	-0.21	0.831
Intercept	3.73	54.43	< 0.001
$\sqrt{\text{Water Depth}}$	-0.01	-0.43	0.671
Intercept	3.84	54.82	< 0.001
$\sqrt{\text{Sed N}}$	-0.02	-1.99	0.047
Intercept	4.26	42.52	< 0.001
$\log_{10}(\text{TPw})$	-0.11	-5.63	< 0.001
Intercept	4.14	77.18	< 0.001
TNw	-0.0003	-8.70	< 0.001

Curriculum Vitae

Candidates Full Name: Katherine Marie Standen

Universities Attended:

University of New Brunswick, Bachelor of Science, 2008-2013

University of New Brunswick, Master of Science, 2013-2016

Conference Presentations:

Standen K.M., Chambers P.A., Culp J.M. 2015. Arrowhead (*Sagittaria cuneata*) as a bioindicator of nitrogen and phosphorus for prairie streams. Society of Freshwater Science Conference Milwaukee, WI, May 2015. Oral Presentation.

Standen K.M., Chambers P.A., Culp J.M. 2014. *Sagittaria* as a bioindicator of stream nutrient status: The effects of nitrogen and phosphorus on leaf size and shape. Canadian Rivers Institute AGM, Charlottetown, PE, Oct 2014. Poster Presentation.