HYPOXIA TOLERANCE OF THE INVERTEBRATES ASSOCIATED WITH WATER-CHESTNUT BEDS (*TRAPA NATANS* L.) IN THE HUDSON RIVER

A Final Report of the Tibor T. Polgar Fellowship Program

Mariana Carolina Teixeira

Polgar Fellow

Freshwater Ecology Post-Graduation Program
Universidade Estadual de Maringá
Maringá, PR, Brazil

Project Advisor:

David L. Strayer
Cary Institute of Ecosystem Studies
Millbrook, NY 12545

ABSTRACT

Aquatic macrophytes provide structures to be colonized and used as refuge, shelter and feeding sites by many organisms. As ecosystem engineers, they also have major effects on the physical and chemical features of water and sediment. Low oxygen concentrations are one of the recurrent impacts of dense beds of aquatic macrophytes, so the macroinvertebrates that inhabit macrophyte beds may have to cope with hypoxia. This study tested the survival of six groups of macroinvertebrates associated with different species of macrophytes (*Trapa natans* or submerged species) under hypoxia. The hypothesis tested was that the organisms that inhabit stands of the invasive *T. natans* are more resistant to low oxygen concentrations than those that inhabit submerged aquatic vegetation (SAV). Chironomids and amphipods associated with *T. natans* were more resistant than those associated with submerged species. Gastropods, ostracods and planarians from the two habitats were equally resistant to hypoxia, with high survival in both cases. Zygopterans were the most impaired group, with no survival at all. The results indicate the responses to hypoxia vary among groups, and so do the mechanisms through which they occur.

TABLE OF CONTENTS

LIST OF FIGURES AND TABLES

INTRODUCTION

Biological invasions have become one of the greatest threats to the integrity of Earth's ecosystems (Strayer 2010). Invasive species are usually exotic (but not always) to the habitat they colonize and very successful in them, often to the detriment of the former inhabitants. They bring in new traits that represent new pressures to the system, frequently culminating in undesirable impacts to native communities and the environment. In freshwater habitats, which are especially sensitive to anthropogenic impacts (Dudgeon et al. 2006), invasions are recurrent and their effects have become obvious and a cause of concern (Strayer 2010).

Aquatic macrophytes create distinctive habitats within aquatic ecosystems. They provide structures to be colonized by an array of organisms, and are used as refuge, shelter and feeding sites (Agostinho et al. 2007; Mormul et al. 2010). They also have major effects on the physical and chemical features of water and sediment (Urban et al. 2009; Bini et al. 2010; Kleeberg et al. 2010; Tall et al. 2011; Rodríguez et al. 2012), and, because of that, are considered ecosystem engineers (Bouma et al. 2005; Caraco et al. 2006). When macrophytes are invasive, their "engineering" can become a problem if it causes changes that the original inhabitants of the invaded area cannot cope with. Macrophytes have a great potential of becoming invasive because they adapt easily to a large range of habitats, have high rates of vegetative reproduction and fast growth (Santamaría 2002).

A few macrophyte traits are often related to impacts in their environment and to other organisms when they become invasive. Biomass production, photosynthesis and decomposition, for example, are three functions through which macrophytes can alter

habitat complexity, light penetration, pH and dissolved oxygen concentration (Schultz and Dibble 2012). Low oxygen concentrations have been identified as one of the recurrent impacts of dense growth of aquatic macrophytes (Caraco et al. 2006; Bunch et al. 2010; Turner et al. 2010; Desmet et al. 2011). Overnight respiration in large macrophyte beds usually creates a daily variation pattern in dissolved oxygen (DO) levels, with lower values at sunrise and higher values when light is most available for photosynthesis (Jones et al. 1996; Miranda et al. 2000; Thomaz et al. 2001; Colon-Gaud et al. 2004). Besides plants' metabolic activities, oxygen dynamics also depend on the physical structure of the stands, as they regulate light incidence and water circulation (Jones et al. 1996; Miranda and Hodges 2000; Bunch et al. 2010). In floating-leaved and emergent species, for example, the diel pattern can be overcome by the constant shading and reduced exchange at the water-air interface caused by the biomass that lies above the surface. Furthermore, the oxygen produced during photosynthesis by the aerial biomass is liberated to the atmosphere while the oxygen dissolved in the water is still consumed by respiration and decomposition. This unbalanced uptake can lead to more prolonged hypoxia or anoxia in dense beds of floating-leaved or emergent plants, which has been shown to occur in many macrophyte beds (Bunch et al. 2010; Turner et al. 2010; Desmet et al. 2011), among them water-chestnut beds in the Hudson River (Caraco and Cole 2002).

The water-chestnut *Trapa natans* L. is native to temperate and tropical Eurasia and Africa (Crow and Hellquist 2000), and was introduced in the United States in 1874 (Hummel and Kiviat 2004). In 1930, it reached the Hudson River (Hummel and Kiviat 2004), where it is now widespread, forming stands of hundreds of hectares (Nieder et al.

2004). This macrophyte is rooted in the sediment, with a submerged stem and feather

leaves, and a dense canopy of floating leaves (Hummel and Kiviat 2004). *T. natans* has

already replaced part of the native aquatic vegetation, dominated by the submerged

species *Vallisneria americana* Michx. (water-celery), in many parts of the Hudson River

(Strayer et al. 2003). The native *V. americana* is also rooted in the sediment, but its

leaves are long and flat, grow from rhizomes, and the whole plant is submersed. Such

different growth forms, shown in Figure 1, create distinct habitats for all macrophyte-

associated organisms, as much in their physical structuring as in their influence on

physical and chemical parameters of water and sediments.
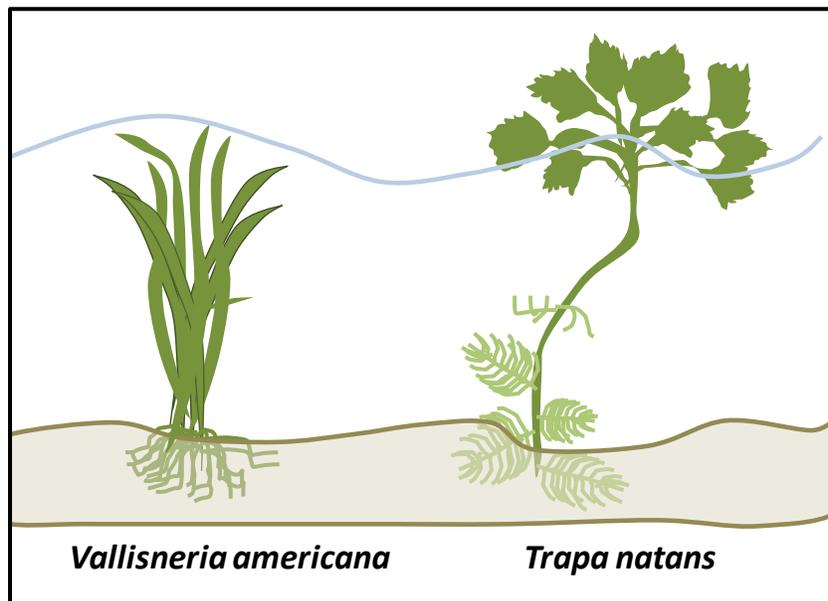
Figure 1.　　**Comparison between the submerged growth form of *Vallisneria
americana* (water-celery) and the floating-leaved form of *Trapa natans*
(water-chestnut).**

The case of *T. natans* in the Hudson River has been thoroughly investigated

(Caraco and Cole 2002; Hummel and Findlay 2006; Goodwin et al. 2008). Tidal

influence has been shown to add to its engineering on DO dynamics. Caraco and Cole (2002) showed that the DO levels in *T. natans* beds varied in a 12.5 h tidal cycle, with high peaks (ca. 7.5 mg.L^{-1}) during high tides as fresh, well-oxygenated water flows in from the open water of the river, and lows of 0 to 2.5 mg.L^{-1} during low tides. In *V. americana* stands however, DO never dropped below 5 mg.L^{-1} and was always higher than in the open water surrounding the bed (Caraco and Cole 2002). Therefore, the hypoxic to anoxic events that take place in *T. natans* beds are temporally limited by the tides, lasting approximately six hours with DO values from 0 to 2.0 mg.L^{-1} (Caraco and Cole 2002). These low DO values could be maintained for much longer periods in large beds that are not subjected to periodic flushes of oxygen-rich water.

Such different habitats created by *T. natans* in relation to the former dominant species *V. americana* could have led to changes in the macrophyte-dwelling communities. Strayer et al. (2003) compared the communities of aquatic macroinvertebrates in stands of *T. natans* and *V. americana* in the Hudson River. They found that although some community attributes, such as species richness and diversity, did not differ between the native and invasive stands, taxonomic composition differed greatly. The ability of species to deal with the distinctive dissolved oxygen dynamics with its periodic hypoxic events was thought to explain part of the differences between *T. natans-* and *V. americana*-associated macroinvertebrate communities, along with predator pressure and food availability, both which would also be influenced by *T. natans* engineering (Strayer et al. 2003). Kornijów et al. (2010) attempted to unveil the role of dissolved oxygen dynamics in determining the macroinvertebrate community in *T. natans* beds. They found that species richness, diversity and density did not differ among

microhabitats (beds' edges versus center; leaves versus sediments) with different degrees of oxygen depletion in *T. natans* beds, meaning that the macroinvertebrates inhabiting these beds can and do cope with periodic short-term hypoxia.

Dissolved oxygen concentration in aquatic habitats has diverse effects on the physiology, behavior, life cycle, growth capacity, distribution, reproductive success and immunological system of animals (Ekau et al. 2010). Morphological, physiological and behavioral adaptations to low levels of dissolved oxygen have been reported for many aquatic organisms, including auxiliary respiratory appendages (Robinson et al. 1991; Marziali et al. 2006), migration to oxic microhabitats (Apodaca and Chapman 2004; Sesterhenn et al. 2013), use of atmospheric air (Hanley and Ultsch 1999; Seuffert and Martin 2010; Penha-Lopes et al. 2010), induction of respiratory pigments (Spicer 1993; Panis et al. 1996) and anaerobic metabolism (Frank 1983; Hamburger et al. 1995; Hamburger et al. 2000).

The aim of this study was to investigate whether the organisms that inhabit stands of the invasive *T. natans* are more resistant to low oxygen concentrations than those that inhabit submerged aquatic vegetation (*V. americana* and *Myriophyllum spicatum* L. (European milfoil)) stands. This study also aims to discuss which adaptations could provide *T. natans*-dwelling macroinvertebrates with a better capacity to cope with hypoxia.

METHODS

Study area

The North and South Tivoli Bays, shown in Figure 2, encompass ca. 290 ha of freshwater tidal marshes, subtidal shallows and intertidal mudflats, along 3 km of the eastern shore of the Hudson River. The marshes are dominated by narrow-leaved cattail (*Typha angustifolia* L.), the subtidal shallows are dominated by the submerged species *V. americana* and *M. spicatum*, and the South Bay mudflats are dominated by the invasive *T. natans* (Yozzo et al. 2005). All these macrophytes have been shown to support dense and diverse macroinvertebrate communities (Strayer et al. 2003; Kornijów et al. 2010; Yozzo and Osgood 2013). These areas are also used by many fishes, such as black bass (*Micropterus sp.*), white perch (*Morone americana*) and common carp (*Cyprinus carpio*), waterfowl, many birds, and snapping turtles (Yozzo et al. 2005).
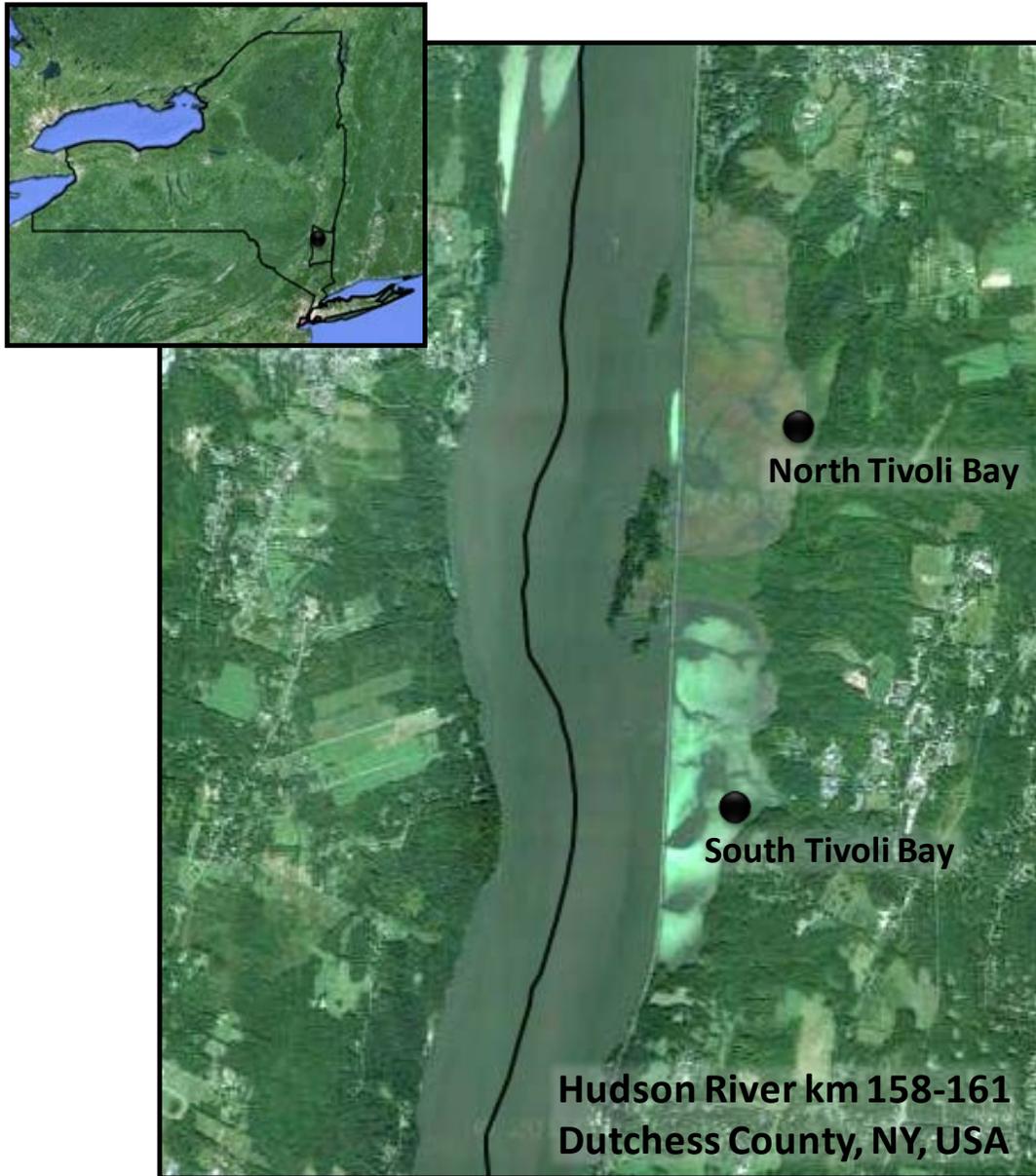
Figure 2.　　**Sampling sites in the Tivoli North and South Bays.**

Experiments

Sampling occurred from mid August until late September 2013, a period that encompassed peak biomass for invasive *T. natans* (coded "TNA" throughout), and for

submerged *V. americana* and *M. spicatum* (coded "SAV" throughout) (Caraco and Cole 2002). Animals were collected from a large TNA bed in Tivoli South Bay and from an SAV bed in Tivoli North Bay by putting whole plants into a submerged bucket, gently shaking them, pouring the water into a plankton net (118 μm mesh size) and collecting the retained material, which often included mud and debris, in jars. In SAV beds, some samples were collected by sweeping the net in the beds to avoid destroying the plants. All collected material was kept on ice and immediately taken to the lab for sorting.

Organisms were sorted under a stereomicroscope, separated by groups and kept in jars with river water. The most abundant groups in the samples were Chironomidae, Gastropoda, Amphipoda, Ostracoda, Zygoptera and Planariidae. All jars were kept in a large tray containing water and ice packs to keep temperature low. With the exception of Amphipoda, no organisms were kept longer than 24 hours before the trials, and the Amphipoda that were kept longer (up to five days) were in a large plastic tray (ca. 10 L) with an air pump providing high oxygen levels, and live chironomids were added as food.

To determine the resistance of organisms to hypoxia, a series of laboratory assays were run by manipulating dissolved oxygen levels and measuring the survival of different invertebrates. River water was sparged with N_2 gas until low concentrations of DO (0.5 to 1.0 $mgO_2.L^{-1}$) were achieved for the hypoxic treatment and aerated (when necessary) until saturation for the normoxic treatment (8 - 9 $mgO_2.L^{-1}$). These concentrations are within the range of variation found by Caraco and Cole (2002) in TNA (low DO) and SAV (high DO) beds. The assays were run in 300 ml biological oxygen demand bottles. Water temperature was not controlled and averaged 22.2±0.7°C (mean±SD). After all bottles were filled with water, and oxygen and temperature were measured with a

handheld YSI ProODO meter, the organisms were added and bottles sealed. Each trial

consisted of one group being tested in a set of five replicates for each treatment, run

according with organism's availability. In a few cases, only three replicates were run, or

the five replicates were run in blocks of two and three replicates. Five individuals were

used in each bottle with the exception of ostracods, for which ten individuals were used

per bottle.

Two different exposure times were tested: six hours, which was based on the

length of hypoxic events observed in the Hudson River (Caraco and Cole 2002), and

eighteen hours, which meant to represent an environment without tidal influence and

consequently longer hypoxic periods. At the end of each trial, DO and temperature were

measured in each bottle and the organism's survival was verified. Animals were

checked, under magnification when necessary, and considered alive when moving

spontaneously or in response to touching. The total number of organisms recovered,

which differed for some groups from the initial number probably due to predation and/or

difficulties in handling, was used to calculate the survival percentage. All organisms

were kept in 70% alcohol for later taxonomic identification.

Statistical analysis

Welch t-tests (Welch 1947) were used to test for differences between the survival

of organisms from different origins (TNA vs. SAV) within each taxonomic group

(Amphipoda, Odonata, Gastropoda, Chironomidae, Ostracoda and Planariidae) and

exposure time (six and eighteen hours). A paired Welch *t*-test was also run including

data from all taxonomic groups to test for differences in survival between origins within

each exposure time. In all analyses, the response variable was the percentage of survival in the hypoxic treatment divided by the percentage of survival in the normoxic treatment. Tests were performed with R (R Core Team 2013).

RESULTS

DO initial and final concentrations in hypoxic treatments averaged 0.69 ± 0.2 $mgO_2.L^{-1}$ and 0.25 ± 0.3 $mgO_2.L^{-1}$, while normoxic concentrations averaged 8.65 ± 0.3 $mgO_2.L^{-1}$ at the beginning of experiments and 7.29 ± 1.5 $mgO_2.L^{-1}$ at the end. The normoxic controls of all trials (SAV and TNA, 6 and 18 hours, n=117) averaged $97\pm9\%$ of survival, which indicates that handling and experimental conditions did not by themselves kill many animals; however, the survival values used in the analyses did take into account the survival in normoxic treatments, as mentioned in methods.

The results of *t*-tests can be seen in Table 1 and in Figure 3. When all taxa were considered, there were no significant differences in survival between organisms associated with TNA or SAV beds for either exposure time. When each taxonomic group was tested separately, significant differences were only found for Amphipoda in the six hour trial and for Chironomidae in the eighteen hour trial. Both groups showed higher survival of organisms associated with TNA rather than SAV, as predicted in the hypothesis. Nevertheless, none of the remaining groups (Zygoptera, Ostracoda, Gastropoda, Planariidae) differed in their survival according to organisms' origin.

Zygoptera had no survival at all in low DO at either exposure time and regardless of the origin of the organisms. On the other hand, Ostracoda had high survival in all hypoxic treatments, independent of originating from TNA or SAV.

Table 1. **Comparison (Welch *t*-test) between *Trapa natans*- and submerged aquatic vegetation-dwelling macroinvertebrates' survival to hypoxia. Significant differences (*p*<0.05) are in bold.**

Groups	Exposure	t	Df	*p*
All	6h	1.52	20	0.1442
	18h	-0.85	27	0.4048
Amphipoda	6h	5.72	5.54	**0.0016**
	18h	-	-	-
Zygoptera	6h	-1.44	4	0.2228
	18h	-	-	-
Gastropoda	6h	-1	2	0.4226
	18h	-2.25	5.74	0.0676
Chironomidae	6h	1.12	4.53	0.3181
	18h	3.64	4	**0.0220**
Ostracoda	6h	-1.05	4	0.351
	18h	1.01	7.34	0.344
Planariidae	18h	-1.04	5.68	0.342

When considering all groups, Amphipoda, Gastropoda and Chironomidae, survival was higher in the six hour trials than in the eighteen hour ones. Moreover, when survival between origins in different exposure times was compared, there seemed to be an interaction between these factors, with a smaller difference between origins in the six hour treatment than in the eighteen hour one.
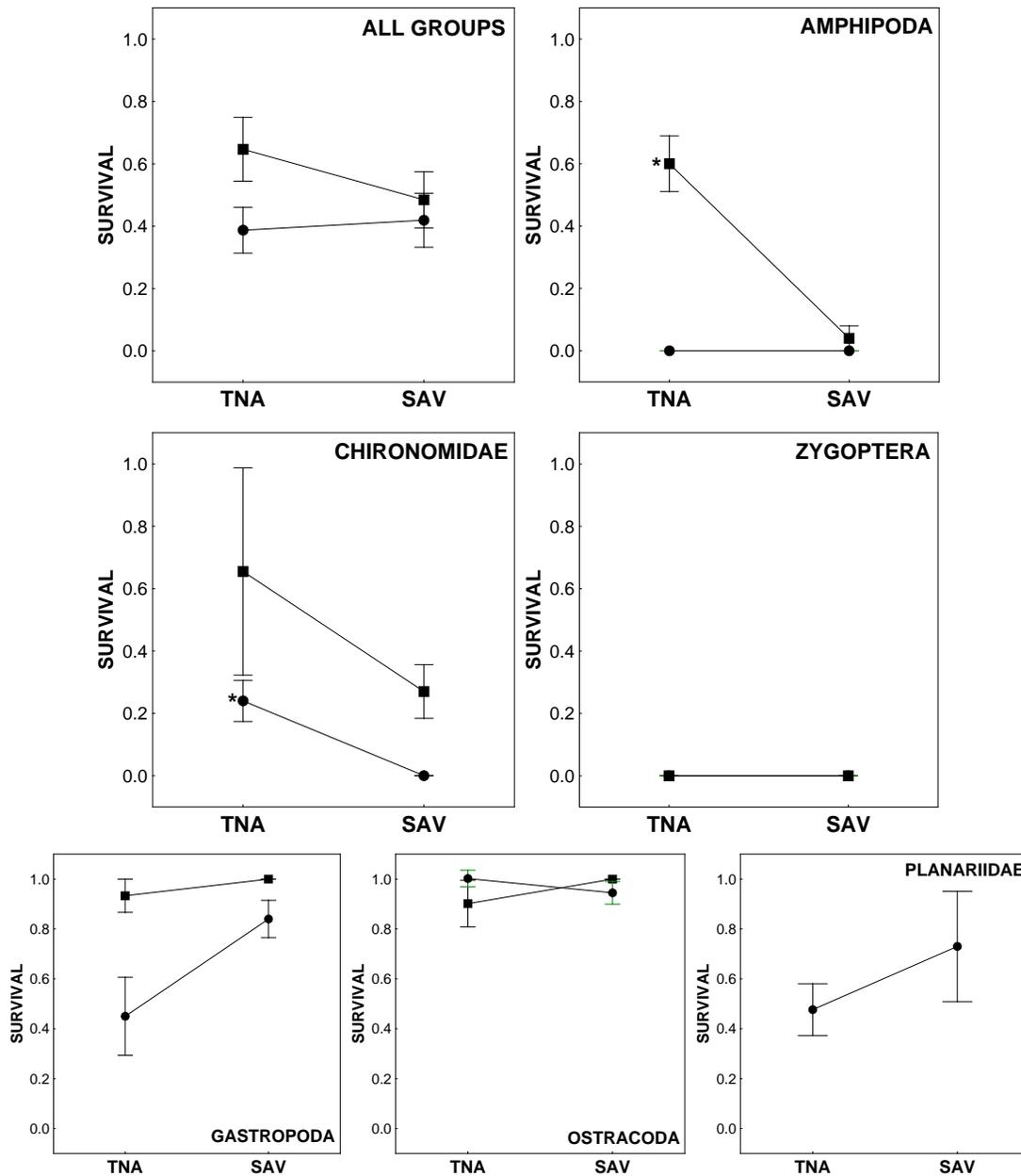
Figure 3. Survival under hypoxia (mean ± standard error) of organisms associated with *T. natans* (TNA) and submerged aquatic vegetation (SAV) in six (■) and eighteen (●) hours exposure. The * indicates significant difference between TNA and SAV (*p*<0.05).

DISCUSSION

Mixed support was found for the hypothesis that macroinvertebrates associated with TNA beds are more resistant to hypoxia than those inhabiting SAV. Data for Amphipoda and Chironomidae supported this hypothesis, while data for the other tested groups did not. Thus, the results indicate that the differences in community composition found by Strayer et al. (2003) between TNA and *Vallisneria americana* (which is one of the two species in SAV) stands can be attributed only in part to differential resistance to hypoxia.

The mechanisms involved in some groups' resistance to hypoxia can only be inferred to a certain extent. Higher survival of Chironomidae associated with TNA could be related to the presence of hemoglobin in animals living in TNA beds and its absence or lower quantity in SAV organisms, since there is great inter-specific variation in hemoglobin content among chironomids (Panis et al. 1996). However, Strayer et al. (2003) found more hemoglobin-bearing chironomid species in *V. americana* than in TNA beds, so this might not be the main mechanism here. Chironomids also possess morphological adaptations, such as thoracic horns and fringed anal lobes that tend to be more developed in species coping with low DO (Marziali et al. 2006). These structures, together with undulatory movements of the abdomen (Panis et al. 1996), can increase ventilation, but the animals would still rely on DO. It has been shown though that some chironomids can turn to an anaerobic metabolism that could give them resistance to short (a few days) and even long-term (a few months) hypoxia (Frank 1983; Hamburger et al. 1995; Hamburger et al. 2000). Species differ in their ability to perform anaerobic

metabolism (Frank 1983), and this could account for the higher survival of TNA-dwelling chironomids in relation to the SAV ones.

The other group that showed higher survival to hypoxia when collected from TNA beds was Amphipoda, in the six hour trials. In the eighteen hour trials, however, all animals died, revealing that their resistance is limited to short-term exposure to hypoxia. Previous studies (Hoback and Barnhart 1996; Spicer et al. 2002) showed that the species *Gammarus pseudolimnaeus* and *Gammarus pulex* were highly sensitive to low DO, with up to 100% mortality in 90 minutes of anoxic conditions for *G. pulex*. An attribute that could enhance survival of amphipods in low DO environments would be their ability to escape to more oxygenated areas due to their good swimming skills; however, in this experiment, this mechanism was impaired because the animals were enclosed in an arena with uniformly low DO. Moreover, in the field, this behavior might increase predation risk and thus mortality (Kolar and Rahel 1993). The presence of the respiratory pigment hemocyanin was demonstrated in many amphipods (Stucliffe 1984; Spicer 1993) and it might be an important physiological adaptation to hypoxia. On the other hand, a low DO-induced anaerobic metabolism was shown to be poor and of little importance for *G. pulex* (Spicer et al. 2002), so the survival during short-term hypoxia of Amphipoda species in this experiment is more likely to be related to the presence of respiratory pigments.

Out of the groups tested, Zygoptera was the most strongly impaired by hypoxia, with no survival at all in low DO at either exposure time and regardless of the origin of the organisms. As tracheal gill breathers, they do tend to be more sensitive to hypoxia than other groups (Chapman et al. 2004). It was also observed that these animals were much more abundant in SAV (data not shown), meaning that DO might play a part in

their distribution. They possess a morphological accessory, their caudal lamellae, that can assist oxygen uptake among other functions (Apodaca and Chapman 2004; Sesterhenn et al. 2013); however, even though most of the animals used had their lamellae intact, they did not survive even the short-term hypoxia. This suggests they must possess some kind of behavioral adaptation to escape it, since they were found in TNA beds. In fact, this group is known to migrate towards the water's surface to find higher DO concentrations (Robinson et al. 1991; Apodaca and Chapman 2004; Sesterhenn et al. 2013). This might be an important mechanism for the Zygoptera in Hudson River habitats, since Strayer et al. (2003) found them to occur on plants but not sediments, and Kornijów et al. (2010) collected them from TNA leaves but not from other parts of the plants, suggesting a dependence on more oxygenated areas of TNA beds.

At the other extreme, Ostracoda were highly resistant to hypoxia, even in the eighteen hour exposure treatment, regardless of origin, with an average survival of 80%. These results agree with former works that showed high survival of freshwater ostracods under hypoxia (Hagerman 1969; Rossi et al. 2002), although others have stated Ostracoda can be very sensitive to low DO levels (Rosseti et al. 2004; Ruiz et al. 2013). A migratory behavior towards oxygen-rich microhabitats was described by many authors as an adaptation of ostracods to hypoxia (Hagerman 1969; Corbari et al. 2004; Corbari et al. 2005), especially because they seem incapable of regulating their oxygen uptake according to DO availability (Corbari et al. 2004; Corbari et al. 2005). Although no evidence was found in the literature for anaerobic metabolism in ostracods (this study; Rossi et al. 2002), some species contain hemoglobin (Fox 1957), which may assist their survival under hypoxia.

Gastropods did not differ in survival by habitat of origin and had relatively high survival rates under hypoxia. Calow (1975) showed around 60% survival after five days of anoxia for *Ancylus fluviatilis* and up to 80% after weeklong anoxia for *Planorbis contortus* (values estimated from his figure 7). Animals in this group can be air- or water-breathers or both, and although some species might rely on their access to the atmosphere (Penha-Lopes et al. 2010; Seuffert and Martin 2010), some, even pulmonates, do not (this study; Burky and Burky 1977; Hanley and Ultsch 1999). The presence of hemoglobin was considered unimportant for *Planorbella duryi* and *Helisoma anceps*, since they showed similar responses to hypoxia as non-hemoglobin-bearing species (Hanley and Ultsch 1999). Also, pulmonate and prosobranch snails did not differ in their responses to hypoxia (Hanley and Ultsch 1999). No papers were found demonstrating the occurrence of anaerobic metabolism in Gastropoda, so their survival is more likely to be related to their great capacity of regulating their oxygen consumption under varying DO concentrations (Hanley and Ultsch 1999).

Planarians also showed high resistance to hypoxia and no effect of origin in these experiments; however, Rivera and Perich (1994) identified DO as the most critical out of six variables affecting survival and reproduction of four species of planarians (*Dugesia dorotocephala*, *D. tigrina*, *Cura foremanni* and *Dendrocelopsis vaginatus*). Their experiments consisted of a two week exposure to different levels of DO, and none of the species survived the 0 and 2 mg.L^{-1} DO levels. In this shorter-term (18 hours) hypoxia treatment though, planarians were very successful. Some flatworms contain hemoglobin (Crompton and Smith 1963), but no studies were found reporting the occurrence of anaerobic metabolism in these animals.

Macroinvertebrates inhabiting hypoxic macrophyte beds such as TNA beds in the Hudson have two alternatives to cope with the hypoxic habitat within the beds: they can escape to more oxygenated micro-habitats, or possess adaptations to survive through hypoxia. The first alternative was not available for the animals in this experiment, so with the exception of Zygoptera, which must survive hypoxia by escaping to oxygenated zones, all other groups tested must have adaptations to survive hypoxia in place. Some groups in these experiments (Gastropoda, Ostracoda, Planariidae) displayed the same ability to survive hypoxia whether their habitat requires them to do so (TNA beds) or not (SAV). On the other hand, Chironomidae and Amphipoda inhabiting TNA beds seem to have been selected by their better resistance to hypoxia than their relatives in SAV.

Because of their engineering activities on DO, macrophyte beds can impose challenges as well as benefits for the macroinvertebrates that live there. Whether through their regular dark respiration and daylight photosynthesis or growth form-related DO fluctuations, large macrophyte beds represent habitats with abiotic filters that may require specific adaptations of organisms. The results show that these challenges can be met in several different ways by macroinvertebrates: probable movement to nearby oxygenated habitats by zygopterans, short-term (6-18 hour) tolerance of hypoxia by amphipods and chironomids, and long-term (>18 hour) tolerance of hypoxia by gastropods, ostracods and planarians. The fact that different invertebrates meet the challenge of hypoxia in different ways means that different kinds of hypoxic macrophyte beds may support different assemblages of macroinvertebrates. Specifically, the spatial and temporal extent of hypoxia will be critical (whether well-oxygenated refuges are nearby, or whether the hypoxia lasts for longer than the invertebrate can tolerate). Likewise, shifts in

macrophyte species composition caused by species invasions or other reasons may have

differential effects on macroinvertebrates that are caused by different oxygen regimes.

ACKNOWLEDGEMENTS

REFERENCES

Agostinho, A.A., S.M. Thomaz, L.C. Gomes, and S.L.S.M. Baltar. 2007. Influence of the macrophyte *Eichhornia azurea* on fish assemblage of the Upper Paraná River floodplain (Brazil). Aquatic Ecology 41:611-619.

Apodaca C.K. and L.J. Chapman. 2004. Larval damselflies in extreme environments: behavioral and physiological response to hypoxic stress. Journal of Insect Physiology 50:767-775.

Bini, L.M., S.M. Thomaz, and P. Carvalho. 2010. Limnological effects of *Egeria najas* Planchon (Hydrocharitaceae) in the arms of Itaipu Reservoir (Brazil, Paraguay). Limnology 11:39-47.

Bouma, T.J., M.B. De Vries, E. Low, G. Peralta, I. C. Tánczos, J. Van De Koppel, and P.M.J. Herman. 2005. Trade-offs related to ecosystem engineering: a case study on stiffness of emerging macrophytes. Ecology 86:2187-2199.

Bunch, A.J., M.S. Allen, and D.C. Gwinn. 2010. Spatial and temporal hypoxia dynamics in dense emergent macrophytes in a Florida lake. Wetlands 30:429-435.

Burky, K.A., and A.J. Burky. 1977. Buoyancy changes as related to respiratory behavior in an amphibious snail, *Pomacea urceus* (Müller), from Venezuela. The Nautilus 91:97-104.

Calow, P. 1975. The respiratory strategies of two species of freshwater gastropods (*Ancylus fluviatilis* Müll and *Planorbis contortus* Linn) in relation to temperature, oxygen concentration, body size, and season. Physiological Zoology 48:114-129.

Caraco, N.F., and J.J. Cole. 2002. Contrasting impacts of a native and alien macrophyte on dissolved oxygen in large river. Ecological Applications 12:1496-1509.

Caraco, N.F., J.J. Cole, S. Findlay, and C. Wigand. 2006. Vascular plants as engineers of oxygen in aquatic systems. BioScience 56:219-225.

Chapman, A., K.R. Schneider, Apodaca, C. and C. A. Chapman. 2004. Respiratory ecology of macroinvertebrates in a swamp-river system of East Africa. Biotropica 36:572-585.

Colon-Gaud, J., W.E. Kelso, and D.A. Rutherford. 2004. Spatial distribution of macroinvertebrates inhabiting hydrilla and coontail beds in the Atchafalaya Basin, Louisiana. Journal of Aquatic Plant Management 42:85-91.

Corbari, L., P. Carbonel, and J. Massabuau. 2004. How a low tissue O_2 strategy could be conserved in early crustaceans: the example of the podocopid ostracods. The Journal of Experimental Biology 207:4415-4425.

Corbari, L., P. Carbonel, and J. Massabuau. 2005. The early life history of tissue oxygenation in crustaceans: the strategy of the myodocopid ostracod *Cylindroleberis mariae*. The Journal of Experimental Biology 208:661-670.

Crompton, D.W.T., and M.H. Smith. 1963. Haemoglobin in a flatworm. Nature 197:1118-1119.

Crow, G.E. and C.B. Hellquist. 2000. Aquatic and wetland plants of northeastern North America. University of Wisconsin Press, Madison.

Desmet, N.J.S., S. V. Belleghem, P. Seuntjens, T.J. Bouma, K. Buis, and P. Meire. 2011. Quantification of the impact of macrophytes on oxygen dynamics and nitrogen retention in a vegetated lowland river. Physics and Chemistry of the Earth 36:479-489.

Dudgeon, D., A.H. Arthington, M.O. Gessner, Z. Kawabata, D.J. Knowler, C. Lévêque, R.J. Naiman, A. Prieur-Richard, D. Soto, M.L.J. Stiassny, and C.A. Sullivan. 2006. Freshwater biodiversity: importance, threats, status and conservation challenges. Biological Reviews 81:163-182.

Ekau, W., H. Auel, H.O. Pörtner, and D. Gilbert. 2010. Impacts of hypoxia on the structure and processes in pelagic communities (zooplankton, macro-invertebrates and fish). Biogeosciences 7:1669-1699.

Fox, H.M. 1957. Haemoglobin in the Crustacea. Nature 164:59.

Frank, C. 1983. Ecology, production and anaerobic metabolism of *Chironomus plumosus* L. larvae in a shallow lake. II Anaerobic metabolism. Archiv für Hydrobiologie 96:354-362.

Goodwin, K., N. Caraco, and J. Cole. 2008. Temporal dynamics of dissolved oxygen in a floating-leaved macrophyte bed. Freshwater Biology 53:1632-1641.

Hagerman, L. 1969. Respiration, anaerobic survival and diel locomotory periodicity in *Hirschmannia viridis* Müller (Ostracoda). Oikos 20:384-391.

Hamburger, K., P.C. Dall and C. Lindegaard. 1995. Effects of oxygen deficiency on survival and glycogen content of *Chironomus anthracinus* (Diptera, Chironomidae) under laboratory and field conditions. Hydrobiologia 297:187-200.

Hamburger, K., P.C. Dall, C. Lindegaard, and I.B. Nilson. 2000. Survival and energy metabolism in an oxygen deficient environment: field and laboratory studies on the bottom fauna from the profundal zone of Lake Esrom, Denmark. Hydrobiologia 432:173-188.

Hanley, R.W., and G.R. Ultsch. 1999. Ambient oxygen tension, metabolic rate, and habitat selection in freshwater snails. Archiv für Hydrobiologie 144:195-214.

Hoback, W.W., and M.C. Barnhart. 1996. Lethal limits and sublethal effects of hypoxia on the amphipod *Gammarus pseudolimnaeus*. Journal of the North American Benthological Society 15:117-126.

Hummel, M., and E. Kiviat. 2004. Review of world literature on water chestnut with implications for management in North America. Journal of Aquatic Plant Management 42:17-27.

Hummel, M., and S. Findlay. 2006. Effects of water chestnut (*Trapa natans*) beds on water chemistry in the tidal freshwater Hudson River. Hydrobiologia 559:169-181.

Jones, J. I., K. Hardwick, and J.W. Eaton. 1996. Diurnal carbon restriction on the photosynthesis of dense stands of *Elodea nuttallii* (Planch) St. John. Hydrobiologia 340:11-16.

Kleeberg, A., J. Köhler, T. Sukhodolova and A. Sukhodolov. 2010. Effects of aquatic macrophytes on organic matter deposition, resuspension and phosphorus entrainment in a lowland river. Freshwater Biology 55:326-345.

Kolar, C.S., and F.J. Rahel. 1993. Interaction of a biotic factor (predator presence) and an abiotic factor (low oxygen) as an influence on benthic invertebrate communities. Oecologia 95:210-219.

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

Marziali, L., V. Lencione, and B. Rossaro. 2006. Adaptations of pupae of Chironomidae (Insecta: Diptera) to oxygen-poor habitats. Polish Journal of Ecology 54:687-693.

Miranda, L.E., and K.B. Hodges. 2000. Role of aquatic vegetation coverage on hypoxia and sunfish abundance in bays of a eutrophic reservoir. Hydrobiologia 427:51-57.

Miranda, L.E., M.P. Driscoll, and M.S. Allen. 2000. Transient physicochemical microhabitats facilitate fish survival in inhospitable aquatic plant stands. Freshwater Biology 44:617-628.

Mormul, R.P., S.M. Thomaz, M.J. Silveira, and L. Rodrigues. 2010. Epiphyton or macrophyte : Which primary producer attracts the snail *Hebetancylus moricandi*? American Malacological Bulletin 133:127-133.

Nieder, W.C., E. Barnaba, S. E. G. Findlay, S. Hoskins, N. Holochuck, and E.A. Blair. 2004. Distribution and abundance of submerged aquatic vegetation and *Trapa natans* in the Hudson River estuary. Journal of Coastal Research: Special Issue 45:150-161.

Panis, L.I., B. Goddeeris, and R. Verheyen. 1996. On the relationship between vertical microdistribution and adaptations to oxygen stress in littoral Chironomidae (Diptera). Hydrobiologia 318:61-67.

Penha-Lopes, G., F. Bartolini, S. Limbu, S. Cannicci, Y. Mgaya, E. Kristensen, and J. Paula. 2010. Ecosystem engineering potential of the gastropod *Terebralia palustris* (Linnaeus, 1767) in mangrove wastewater wetlands - A controlled mesocosm experiment. Environmental Pollution 158:258-266.

R Core Team. 2013. R: a language and environment for statistical computing. R Foundation for Statistical Computing. Vienna, Austria. http://www.R-project.org (accessed October 31, 2013).

Rivera, V.R., and M.J. Perich. 1994. Effects of water quality on survival and reproduction of four species of planaria (Turbellaria: Tricladida). Invertebrate Reproduction and Development 25:1-7.

Robinson, J.V., L.R. Shaffer, D.D. Hagemier, and N.J. Smatresk. 1991. The ecological role of caudal lamellae loss in the larval damselfly, *Ischnura posita* (Hagen) (Odonata: Zygoptera). Oecologia 87:1-7.

Rodríguez, P., H. Pizarro, and M.S. Vera. 2012. Size fractionated phytoplankton production in two humic shallow lakes with contrasting coverage of free floating plants. Hydrobiologia 691:285-298.

Rosseti, G., M. Bartoli, and K. Martens. 2004. Limnological characteristics and recent ostracods (Crustacea, Ostracoda) of freshwater wetlands in the Parco Oglio Sud (Northern Italy). Annales de Limnologie 40:329-341.

Rossi, V., E.B.A. Todeschi, A. Gandolfi, M. Invidia, and P. Menozzi. 2002. Hypoxia and starvation tolerance in individuals from a riverine and a lacustrine population of *Darwinula stevensoni* (Crustacea: Ostracoda). Archiv für Hydrobiologie 154: 151-171.

Ruiz, F., M. Abad, A.M. Bodergat, P. Carbonel, J. Rodríguez-Lázaro, M.L. Gonzalez-Regalado, A. Toscano, E.X. Garcia, and J. Prenda. 2013. Freshwater ostracods as environmental tracers. International Journal of Environmental Science and Technology 10:1115-1128.

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

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

Sesterhenn. T.M., E.E. Reardon, and L.J. Chapman, 2013. Hypoxia and lost gills: respiratory ecology of a temperate larval damselfly. Journal of Insect Physiology 59:19-25.

Seuffert, M.E., and P.R. Martin. 2010. Dependence on aerial respiration and its influence on microdistribution in the invasive freshwater snail *Pomacea canaliculata* (Caenogastropoda, Ampullariidae). Biological Invasions 12:1695-1708.

Spicer, J. 1993. Oxygen binding by amphipod (Crustacea) haemocyanins. Marine Behavior and Physiology 24:123-136.

Spicer, J., C.L. Dando, and L. Maltby. 2002. Anaerobic capacity of a crustacean sensitive to low environmental oxygen tensions, the freshwater amphipod *Gammarus pulex* (L.). Hydrobiologia 477: 189-194.

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

Strayer, D.L. 2010. Alien species in fresh waters: ecological effects, interactions with other stressors, and prospects for the future. Freshwater Biology 55:152-174.

Stucliffe, D.W. 1984. Quantitative aspects of oxygen uptake by *Gammarus* (Crustacea, Amphipoda): a critical review. Freshwater Biology 14:443-489.

Tall, L., N. Caraco, and R. Maranger. 2011. Denitrification hot spots: dominant role of invasive macrophyte *Trapa natans* in removing nitrogen from a tidal river. Ecological Applications 21:3104-3114.

Thomaz, S.M., A. Enrich-Prast, J.F. Gonçalves Jr., A.M. dos Santos, and F.A. Esteves. 2001. Metabolism and gaseous exchanges in two coastal lagoons from Rio de Janeiro with distinct limnological characteristics. Brazilian Archives of Biology and Technology 44:433-438.

Turner, A.M., E.J. Cholak, and M. Groner. 2010. Expanding American lotus and dissolved oxygen concentrations of a shallow lake. The American Midland Naturalist 164:1-8.

Urban, R. A., J.E. Titus, and W.X. Zhu. 2009. Shading by an invasive macrophyte has cascading effects on sediment chemistry. Biological Invasions 11:265-273.

Welch, B.L. 1947. The generalization of Student's' problem when several different population variances are involved. Biometrika 34: 28-35.

Yozzo, D.J., J.L. Andersen, M.M. Cianciola, W.C. Nieder, D.E. Miller, S. Ciparis, and J. McAvoy. 2005. Ecological profile of the Hudson River National Estuarine Research Reserve. New York Department of Environmental Conservation.

Yozzo, D.J., and D.T. Osgood. 2013. Invertebrate communities of low-salinity wetlands: overview and comparison between *Phragmites* and *Typha* marshes within the Hudson River estuary. Estuaries and Coasts 36:575-584.