

EFFECTS OF CRAYFISH ON THE ESTABLISHMENT OF MACROPHYTES IN A SHALLOW PEAT LAKE

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ABSTRACT

Macrophytes are the most important components of wetland ecosystems, because they provide food and habitat for animals, increase nutrient retention and reduce resuspension of the water column. Therefore, the decline of submerged macrophytes has profound consequences for ecosystem processes in shallow lakes and ponds. Restoration measures to reduce phosphorus loading have been implemented in the Loosdrecht peat lake system; however a direct return of submerged macrophytes was not apparent. Invasive herbivores intruding aquatic systems may amplify negative effects for macrophyte abundance. The red swamp crayfish *Procambarus clarkii* has invaded many water bodies throughout the world, giving rise to breeding populations that now threaten freshwater ecosystems. In this case study we tested the strength of top-down effects, including indirect effects by an omnivorous crayfish on establishment of macrophytes in two shallow ponds. One pond was characterized by a high iron concentration due to application, while the second pond had the original phosphate concentrations from the main lake. We hypothesize that *P. clarkii* exerts a strong negative impact on submerged macrophyte abundance and that macrophytes are important in crayfish diet. By means of enclosure and exclosure experiments we tested the effect of crayfish on 3 submerged macrophyte species; *Chara globularis*, *Elodea nuttallii* and *Myriophyllum spicatum*. A food web study based on stable isotope analysis was done to evaluate the contribution of macrophytes to the general diet of crayfish. Final biomass of all 3 macrophyte species was significantly higher in the full exclosures compared to all other treatments, which had roughly equally low biomasses. We found final biomass of *Chara globularis* and *Myriophyllum spicatum* to be significantly higher in the iron-rich pond. The food web study indicated that crayfish mainly feed on macro invertebrates. We conclude that crayfish have an evident negative effect on submerged macrophyte abundance, although mainly by their sloppy feeding behaviour and the consequential increase in water turbidity.

KEYWORDS: invasive species, crayfish, *Procambarus clarkii*, macrophytes, omnivory, diet selection, freshwater communities

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INTRODUCTION

Macrophytes play an essential role in structuring communities in aquatic environments. Their abundance is positively related to good water quality and therefore essential for the stabilization of the clear water state in shallow lakes (Scheffer 1998). Macrophytes increase nutrient retention, provide food and habitat for macrofauna, improve light climate for phytoplankton and reduce resuspension in the water column (Timms and Moss 1984, Carpenter and Lodge 1986, Lammens 1989, Paterson 1993, Weisner *et al.* 1994, Lauridsen *et al.* 1996). In shallow freshwater systems, increased nutrient loading has resulted in a loss of macrophytes (Jeppesen 1998). In the last century, many shallow lakes have turned eutrophic due to the high nitrogen and phosphorus loading from agricultural, industrial and domestic pollution (e.g. O'Sullivan 1992; De Haan *et al.* 1993). Nutrient pollution evokes a tip in the ecosystem balance, transforming lakes from a clear state with predatory fish and macrophytes, to a species-poor state with breams that induce sediment resuspension followed by phytoplankton dominance e.g. *Microcystis* spp. impoverishing growth conditions for submerged macrophytes (Scheffer *et al.* 1997). Because of their role as protective cover, substratum or breeding sites for many organisms, the loss of macrophytes will in turn result in an overall decrease in species diversity and abundance of fish and macrofauna (Olsen *et al.* 1991; Nyström *et al.* 1999; Dorn and Wodjak 2004). In general, a decrease in macrophyte biomass has consequences for both the diversity and ecosystem functioning of shallow freshwater wetlands, such as rivers, lakes and ponds – that deliver a wide range of important ecosystem services that are of infinite value to human well-being.

Another important reason for declining macrophyte abundance is the presence of large numbers of planktivorous and benthivorous fish. By minimizing zooplankton by consumption, planktivorous fish promote algal growth, possibly leading to blooms. Benthivorous fish stir up sediment during feeding activity and thus induce resuspending of sediment (Ter Heerdt and Hootsmans 2007). Crayfish, accounting for the largest proportion of invertebrate biomass and production in inland waters, have also been associated with reductions in macrophyte biomass. They have an indiscriminate omnivorous diet including a wide variety of benthic invertebrates, macrophytes, detritus, fish eggs and algae (Momot 1995; Stenroth and Nyström 2003). Because of their capability to integrate into the food web at many levels, once added to a system crayfish can have a large ecological impact (Lodge *et al.* 1994; Nyström *et al.* 1999; Anastácio *et al.* 2005; Roth *et al.* 2006; Gherardi 2007). The red swamp crayfish (*Procambarus clarkii*) is the most dominant cosmopolitan crayfish today, having spread from South-Central America and North-East Mexico to natural habitats in all continents excepting Australia (Huner 2002; Gherardi 2006). The rapid expansion of *P. clarkii* is mainly explained by its ecological plasticity (Gutiérrez-Yurrita *et al.* 1999). Despite their prevalence throughout Europe, the effects that invasive crayfish exert on the invaded ecosystem and the effects of such interactive outcomes on their further invasion are poorly understood (Hudina *et al.* 2011). Gut content analyses showed that *P. clarkii* feeds on diverse items in proportion to their availability and that its diet may change with habitats (Smart *et al.* 2002; Gherardi and Barbaresi 2007; Usio *et al.* 2009). Although omnivorous, crayfish are described to have diet preferences that switch according to availability of food items. This feeding selectively may exert a strong negative impact on species diversity in freshwater habitats and biota. On the short term, introduced crayfish

may reduce the standing stock of macrophytes and density of benthic invertebrates (Lodge and Lorman 1987; Cronin *et al.* 2002; Gherardi *et al.* 2011) and on the long term the invaders may alter habitat heterogeneity. Additionally, not only can crayfish exert direct consumption effects; they may also induce sediment resuspension, causing water turbidity (Rodríguez *et al.* 2003). *P. clarkii* also destroys macrophytes by nonconsumptive cutting of stems (Cronin *et al.* 2002). All in all, *P. clarkii* is believed to negatively affect macrophyte growth and survival, and are thought to stimulate a transition to and maintenance of a turbid eutrophic state (Gherardi 2006). They may inhibit establishment of macrophytes in lake restoration projects (Matsuzaki *et al.* 2009).

In recent years water managers have and will thoroughly invest in restoration projects to revive shallow lakes in the Netherlands to their original clear state with the species-rich macrophyte stands that once covered lake bottoms (van de Haterd and Ter Heerdt 2007). Lake bio-manipulation experiments in the past, by means of catching away benthic fish, have not always resulted in a regime shift as nutrient concentrations in the lake were not low enough (Projectvoorstel waternet KRW08079). Ter Heerdt and Hootsmans (2007) state that most restoration measures that have been employed in peat lakes in the Netherlands have generally failed so far due to the fact that it is impossible to catch away all fish, fast recruitment of young fish, low density of large-bodied cladocerans and wind-induced resuspension of loose bottom sediments. Moreover, the high phosphorus concentrations due to internal loading from the sediment are a problem (Boers *et al.* 1994). External application of iron into wetlands has been applied as a recovery strategy, and decreasing turbidity in the following season observed (Quaak *et al.* 1993, Boers *et al.* 1994; Smolders *et al.* 1995). Under O₂-rich conditions, iron binds with phosphate one on one and herewith forms a layer on top of the sediment that inhibits release of phosphorus from the lake-bed sediments. Lower phosphate concentrations stimulate macrophyte growth, and thus increase water clarity. Since the supply of groundwater from National Park the Utrechtse Heuvelrug was halted, the natural amount of iron in peat lakes has been very low. Addition of iron to water is believed to be an efficient measure to treat eutrophication (Boers *et al.* 1994). This method is relatively new and long-term effects are thus far unknown. The effects of iron supplementation in subsequent seasons were monitored in Lake Terra Nova in the summer of 2009 (Projectvoorstel waternet KRW08079). In an experimental pond separated from the rest of the lake, a ferric iron chloride suspension was gradually added to an estimated concentration of 100 g Fe · m⁻². The clarity of the lake significantly increased within several weeks. However, the return of submerged macrophytes held off in the succeeding season. In August 2009, a pH of 3.77 was recorded, a condition clearly not beneficial for aquatic life. This might have inhibited macrophyte growth. Interestingly, a study by Van der Welle *et al.* (2007) showed iron to have no unfavourable effect on test species *Potamogeton acutifolius*, *Elodea nuttallii* and *Stratiotes aloides*. We hypothesize that high densities of invasive crayfish may inhibit macrophyte abundance (Gherardi 2007; Sala *et al.* 2000; Gherardi 2010).

The aim of this study is therefore to evaluate the effects of *P. clarkii* on the regeneration ability of submerged macrophytes in a clear, shallow body of water. We conducted an *in situ* enclosure and exclosure experiments to study direct and indirect effect of crayfish on 3 different macrophyte species. In order to investigate direct herbivorous effect, stable isotope analysis was used to quantify the importance of macrophytes in the crayfish diet. We also determined whether changing environmental conditions, particularly the availability

of phosphorus, changed the relationships in the food web. Understanding this plant-herbivore interaction will help in designing efficient control measures to restore degraded systems.

METHODS

Study area

Terra Nova is a 85 ha shallow peat lake in The Netherlands that is part of the Loosdrecht lake system, originating from peat excavation in the 19th century (Hofstra and van Liere 1992). From 1986 onwards the macrophyte population collapsed and phosphorus content in summer increased to 0.08mg/l (Bontes *et al.* 2006) resulting in a turbid, species-poor body of water ever since (van de Haterd and Ter Heerdt 2007). The lake has a mean depth of 1.43 m and the bottom is covered with a 0.9 m organic mud layer. In the Western part of Lake Terra Nova 2 experimental ponds of approximately 4000m² each were constructed and separated from the rest of the lake by dams between the parallel banks. Water exchange and fish migration was herewith minimized. One pond is characterized by a high iron concentration due to application, while the second pond did not receive iron, and therefore had the original phosphate concentrations from the main lake. From now on we refer to the ponds as being iron-rich and iron-poor. In the iron-rich pond the amount of iron was 1.3 mg/l in April 2010 and 1.6 mg/l in September 2010. In the iron-poor pond these values were respectively 0.09 mg/l and 0.21mg/l on the same dates. *Nuphar lutea* (L.) Sm., *Nymphaea alba* L. and *Phragmites australis* (Cav.) Trin. ex Steud. are the floating-leaved macrophytes present in both ponds. Abiotic characteristics were measured in both ponds on the 30th of May 2011. Light intensity was measured *in situ* with a LI-COR Lightmeter type LI-189. The pH and conductivity were measured *in situ* with a portable probe (340i SET, 2E30-101B02, WTW Wissenschaftlich Technische Werkstätten GmbH, Weilheim, Germany).

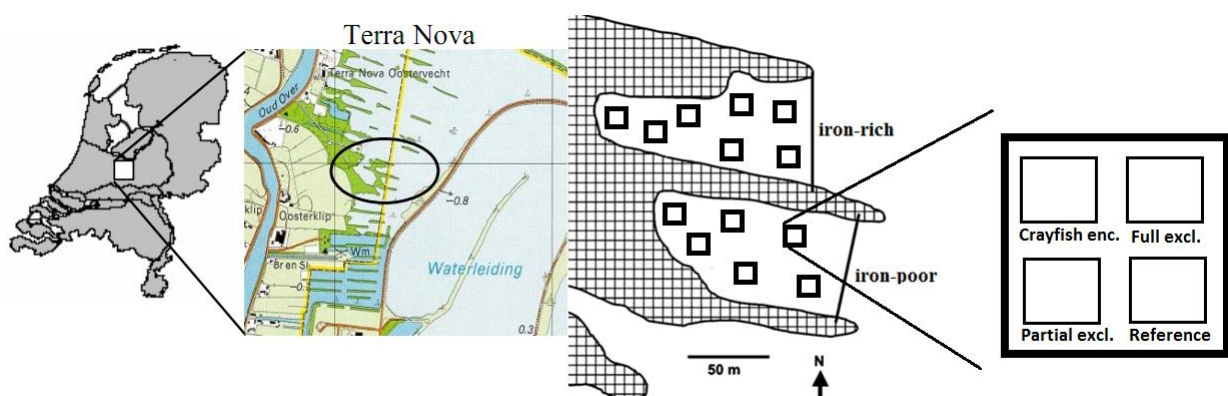


Fig. 1 Location of Terra Nova and design of the biomanipulation experiment in the field.

Density of potential herbivores

We conducted a mark and recapture method to estimate crayfish abundance. 12 Traps with cat food bait were placed in the ponds, and checked every 3 days during 5 weeks. To investigate non-crayfish herbivorous pressure, an estimation of the amount of herbivorous

birds present in the water in and around the ponds, an area encompassing 0.07km², was made. Weekly observations with binoculars were made and the absolute number and species of all water birds was measured. Fish density in the experimental ponds is limited due to biomanipulation experiments in 2002, in which electro-fishery removed all fish (Bontes *et al.* 2006).

Herbivore exclusion experiments

During April 2011, an *in situ* cage experiment was installed, consisting of 7 replicates of 4 treatments in a randomized block design; crayfish enclosures (mesh size 1 x 1 cm), full enclosures (mesh size 1 x 1 cm), partial enclosures (mesh size 5 x 10 cm) and reference areas (no cage). The cages all measured 1 x 1 x 1 m, and all sides were covered with iron mesh wire. The experimental blocks were separated at least 15-20m from other blocks. The water depth in the cages varied between 70 – 100 cm. Bamboo poles were fixed on the corners of the cages to secure a firm position in the sediment. 6 Plastic plant pots sized 11 x 11 cm - mounted in a 40x60 metal frame - were placed on the bottom of every enclosure and enclosure in which a mix of 3 macrophyte species - all commonly occurring in Lake Terra Nova - was planted in pots filled with peat from the ponds; *Chara globularis* (mean Dry weight 0.1 g; Fresh weight 0.9 g), *Elodea nuttallii* (mean DWT 0.5 g; FWT 2.6 g) and *Myriophyllum spicatum* (av. DWT 0.1 g; FWT 1.5 g). The macrophytes were collected from nearby ponds. 2 Cuttings of every species were planted in a random order into the frame. Crayfish for stocking were collected in Lake Terra Nova with the same traps described above. A mix of female and male crayfish was added to the enclosures at the density of 4 individuals·m⁻².

Macrophyte biomass was sampled 6 weeks later, when the canopy-forming species had reached the water surface. The redundant sediment was gently rinsed off from the root complex *in situ*, and the plants were hereafter thoroughly cleaned in the laboratory. Roots and shoots were separated, washed and dried at 60°C for 48 hours. The total dry weight of *Chara* plants was measured, and a distinction was made between *Elodea* and *Myriophyllum* root and shoot dry weights.

Stable isotope analysis

Sample collection

Specimens of *P. clarkii* were collected in April 2011 on the North side of Terra Nova with traps (60-mm mesh, 30-cm × 36-cm opening, 120-cm length) set overnight. We randomly selected 20 crayfish to take back to the laboratory. Food sources from dominant taxa, representing all trophic levels and potential carbon sources were sampled from Lake Terra Nova in spring (Jones and Waldron 2003). When possible, 5 replicate samples were collected for each food source. We collected invertebrate samples for stable isotope analysis by scooping through benthic sediment and the water column, and beating through aquatic vegetation. We used a zooplankton net (mesh size 330 μm) was used to collect *Daphnia*. Roots, stem and leaves were collected separately when appropriate, and thoroughly cleaned in water. All samples were frozen within 12 hours. The data points belonging to 3 fish species, *Perca fluviatilis*, *Gymnosephalus cernuus* and *Esox lucius*, are extracted from a previous food web study done in Terra Nova (data not published).

Isotopic methods and trophic model formulation

On return to the laboratory, we dried samples at 60 °C for 48 hours, ground them into a fine powder, and packed them into acid-washed tin capsules for carbon and nitrogen isotope analysis (Gilling *et al.* 2009). All samples weighed between 0.300 mg – 1.000 mg. Samples of crayfish tail muscle tissue were dissected and dried as above (Stenroth *et al.* 2006). Prior to drying, snail body tissues were dissected from their shells and shells discarded.

Stable isotopes are used to assess the transfer of energy between trophic levels of aquatic food webs (Rybczynski *et al.* 2008). It provides an indication of what a consumer has assimilated over a longer time period. We assumed a trophic enrichment of + 1 ‰ $\delta^{13}\text{C}$ (DeNiro and Epstein 1978) and +3.4 ‰ $\delta^{15}\text{N}$ (Minagawa and Wada 1984; Post 2002) for each trophic transfer. $\delta^{13}\text{C}/^{12}\text{C}$ ratios (parts per thousand, ‰, difference from the $\delta^{13}\text{C}/^{12}\text{C}$ ratio in Vienna PeeDee limestone; further referred to as ^{13}C) and $\delta^{15}\text{N}/^{14}\text{N}$ ratios (‰ difference from the $^{15}\text{N}/^{14}\text{N}$ ratio in atmospheric N_2 ; further referred to as ^{15}N) were determined in a EuroAE3000 elemental analyzer coupled online to a Finnigan Delta S isotope-ratio mass spectrometer via a Finnigan continuous – flow interface. Stable isotopes were determined at the laboratory of the Netherlands Institute of Ecology, Wageningen, the Netherlands.

Data analysis

All statistical analyses were carried out using SPSS for Windows (version 18.0, SPSS, Chicago, IL, USA). We tested the effect of treatment type and pond (expressed by crayfish density - as determined by the capture-mark and recapture method - on final macrophyte biomass, with a one-way ANOVA, with crayfish density and treatment type as fixed factors and biomass as dependent variable. The biomass data were double-root transformed to obtain normality and meet homoscedasticity assumptions. To test whether relative plant growth rate, calculated by dividing the absolute increase in biomass by the start biomass, was related to plant species, treatment type and crayfish density, a one-way ANOVA with nested design was used. A t-test was used to test for differences in abiotic factors between the ponds. The level of significance at which the null hypothesis was rejected was $\alpha = 0.05$. Bonferroni *post hoc* comparisons were applied to compare differences among means when a significant treatment effect was found.

RESULTS

Water quality

Soon after the start of the experiment, the iron-poor pond got very turbid and filamentous algae covered the surface. Compared to the iron-rich pond, very little floating-leaved macrophytes emerged. Pond types (iron-rich vs. iron-poor) differed in pH ($F_{1,2}=1.438$, $t=-4.126$, $p=0.001$), conductivity ($F_{1,2}=1.817$, $t=15.648$, $p<0.001$), the light intensity in air ($F_{1,2}=0.772$, $t=3.674$, $p=0.003$) and light intensity at 35 cm ($F_{1,2}=0.540$, $t=-9.157$, $p<0.001$) and 60 cm ($F_{1,2}=3.352$, $t=-11.19$, $p<0.001$) under the water surface (for characteristics see table 1), whereas temperature did not significantly differ between ponds. Differences in pH and conductivity were minimal.

Table 1 Abiotic characteristics of the 2 experimental ponds. Values are mean \pm SE (n=7 for both ponds)

	Iron-rich pond	Iron-poor pond
Electric Conductivity $\mu\text{S}\cdot\text{m}^{-1}$	435.6 \pm 0.53	426 \pm 0.31
pH	7.34 \pm 0.04	7.55 \pm 0.029
Light intensity at water surface ($\mu\text{mol}\cdot\text{s}^{-1}\cdot\text{m}^{-2}$ PAR ⁺) *	68.65 %	68.22 %
Light intensity at 35 cm depth ($\mu\text{mol}\cdot\text{s}^{-1}\cdot\text{m}^{-2}$ PAR ⁺) *	23.77 %	43.61 %
Light intensity at 60 cm depth ($\mu\text{mol}\cdot\text{s}^{-1}\cdot\text{m}^{-2}$ PAR ⁺) *	10.51 %	30.27 %
Temperature ($^{\circ}\text{C}$)	16.92 \pm 0.13	16.63 \pm 0.11

* relative to light intensity in air

⁺ PAR: Photosynthetic Active Radiation

Density of potential herbivores

The total number of crayfish caught in the iron-rich pond was 66 (62% male), of which 2 crayfish were caught back. In the iron-poor pond we caught 178 crayfish (54% male), including 1 recapture. No migration between ponds was observed. We used this absolute crayfish density (66 vs. 178) as a fixed factor distinguishing between ponds.

Mean densities of waterbirds were: Mallards (*Anas platyrhynchos*), Mute swans (*Cygnus olor*), Egyptian geese (*Alopochen aegyptiacus*), Gadwalls (*Anas strepera*) and Common Pochards (*Aythya ferina*); 71 km^{-2} ; Eurasian Coots (*Fulica atra*) and Greylag Geese (*Anser anser*): 142 km^{-2} ; and the Tufted Duck (*Aythya fuligula*): 285 km^{-2} .

Herbivore exclusion experiments

Initially, we introduced 4 crayfish in every enclosure. At the end of the experiment, mortality reduced crayfish in the enclosures to a final mean density of 3.14 ± 0.34 (mean \pm SE) in the iron-rich pond, and 2.71 ± 0.70 (mean \pm SE) in the iron-poor pond. Mean crayfish biomass did not significantly differ between replicates or ponds ($p > 0.05$). After a double-root transformation (Sokal and Rohlf 1995) the *Elodea* biomass was normally distributed for mean shoot weight ($F_{3,52} = 2.305$, $p = 0.088$), mean root weight ($F_{3,52} = 1.044$, $p = 0.381$) and for mean weight of *Myriophyllum* shoot biomass ($F_{3,52} = 0.199$, $p = 0.897$) and root biomass ($F_{3,52} = 1.391$, $p = 0.256$). Although the distribution of the mean weight of *Chara*'s total biomass proved not to be normal ($F_{3,52} = 3.370$, $p = 0.025$), a parametric test was performed anyway as the deviance was not so big and ANOVA's are rather robust concerning their assumptions.

Treatment type had a significant effect on mean *Chara* biomass ($F_3 = 11.113$, $p < 0.001$). *Chara* biomass was also significantly related to crayfish density ($F_3 = 4.820$, $p = 0.031$). *Chara* biomass was at least 4 times higher in the full enclosures compared to all other treatments, which had equally low *Chara* biomasses. Treatment type significantly affected mean *Elodea* shoot biomass ($F_3 = 25.046$, $p < 0.001$) and *Elodea* root biomass ($F_3 = 19.140$, $p < 0.001$). Crayfish density had no significant effect on *Elodea* shoot biomass ($F_1 = 0.191$, $p = 0.664$) or on *Elodea* root biomass ($F_1 = 0.017$, $p = 0.896$). *Myriophyllum* shoot biomass was found to be significantly affected by treatment type ($F_3 = 23.595$, $p < 0.001$) and crayfish density ($F_1 = 4.969$, $p = 0.031$). *Myriophyllum* root biomass was also significantly affected by treatment type ($F_3 = 19.404$, $p < 0.001$) and crayfish density ($F_1 = 5.335$, $p = 0.025$). *Elodea* and *Myriophyllum* biomasses of both shoots and roots were clearly highest in the full enclosures, and lowest in the references (see figure 2). Supplement 1 and 2 show the overall total biomass of *Chara* and *Myriophyllum* to be higher in the iron-rich pond.

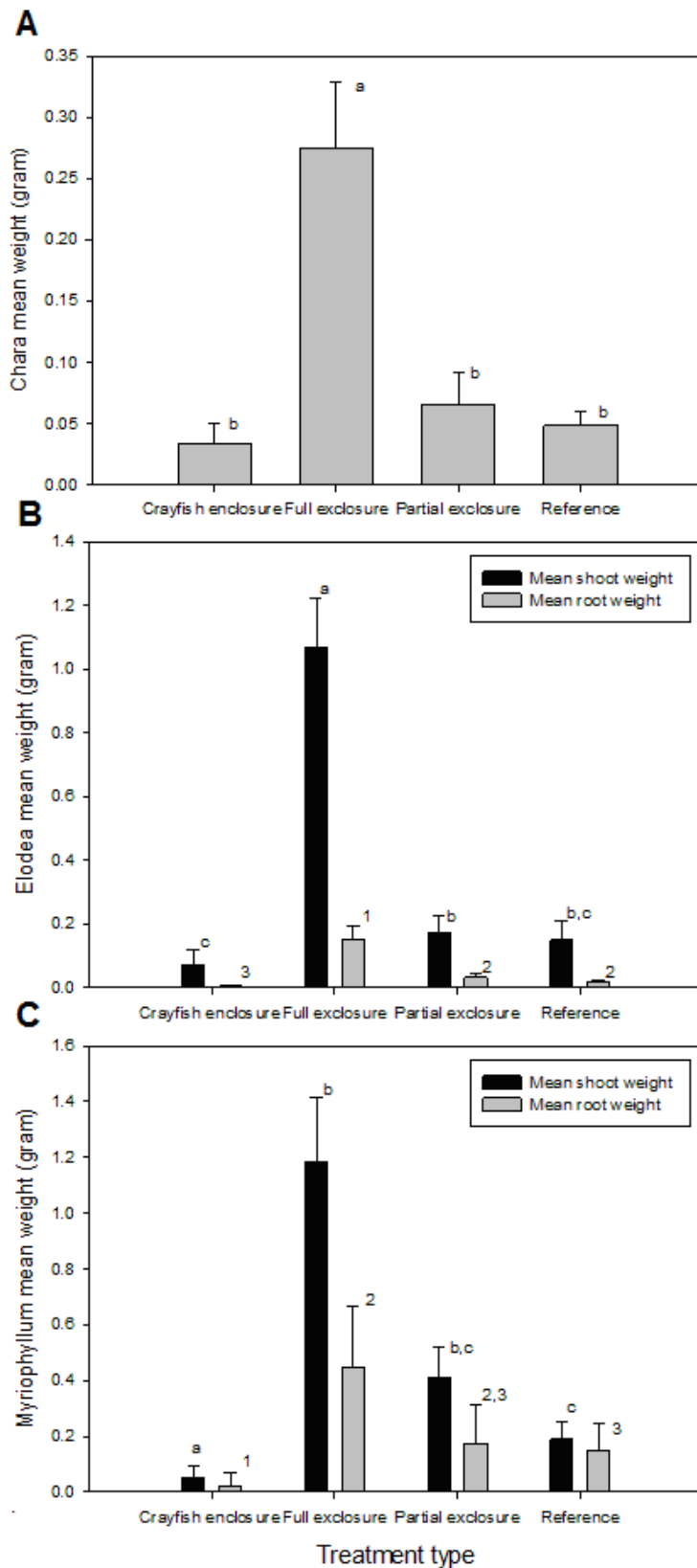


Fig. 2 Mean dry weight (\pm S.E. in grams) of final biomass for (A) Chara, (B) Elodea, (C) Myriophyllum in the 4 treatment types. In (B) and (C) a separation is made between roots and shoots. Different letters show significant differences for shoot weight, while different numbers show significant differences for root weight.

The relative growth rate (after double-root transformation) was significantly affected by plant species ($F=13.77$, $df=2$, $p<0.001$, ANOVA with nested design) and treatment type ($F_9=18.63$, $p<0.001$, ANOVA with nested design) (see figure 3). Compared to *Chara* and *Myriophyllum*, *Elodea* had the highest relative growth rate in the full enclosure. In the crayfish enclosure, the consumption rate is lowest for *Elodea*, while *Myriophyllum* is relatively the most preferred plant species by crayfish. In the partial enclosures *Chara* biomass declined, while *Elodea* and *Myriophyllum* biomass slightly increased compared to the initial biomass. In the reference, both *Chara* and *Myriophyllum* declined in biomass, while *Elodea* biomass increased.

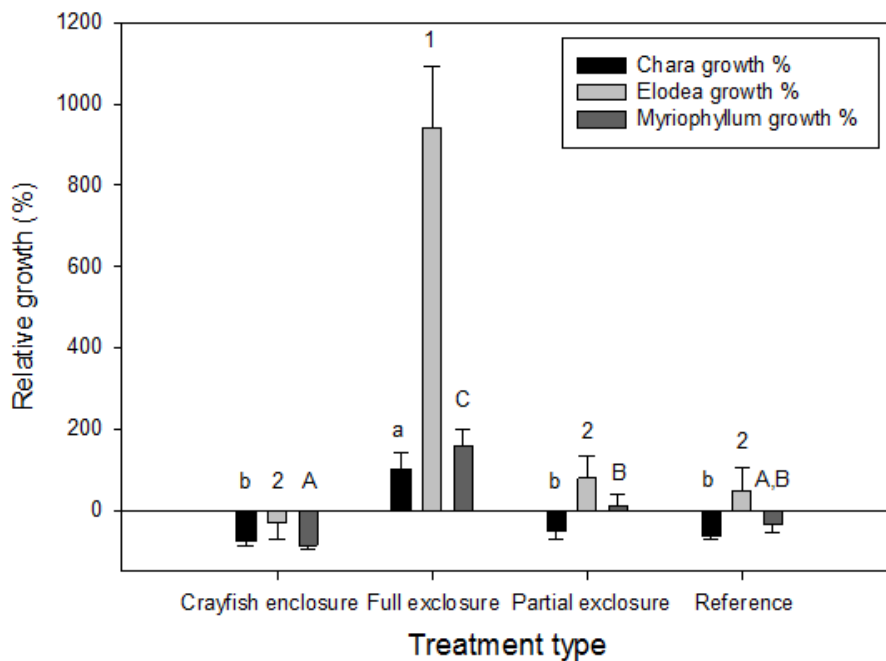


Fig. 3 Relative growth rate (%) of *Chara*, *Elodea* and *Myriophyllum* in the 4 treatment types. Treatment is nested within plant species. Different lower-case letters show sig. differences for relative *Chara* growth rate, different digits show sig. differences for relative *Elodea* growth rate and different capital letters show sig. differences for relative *Myriophyllum* growth rate.

Stable isotope analysis

We assumed that invertebrate consumers fed at trophic level 2 and crayfish at trophic level 3. The standard deviation of both nitrogen and carbon isotope values within the crayfish population (muscle samples) from Lake Terra Nova varied respectively from 6.22 to 8.46 with a mean of 7.17 for nitrogen, and from -29.70 to -22.72 with a mean of -25.86 for carbon isotope values. Sex and total length did not significantly influence isotope signature, thus the 20 samples were grouped ($p>0.05$).

Figure 4 illustrates a $\delta^{13}\text{C}$ - $\delta^{15}\text{N}$ bi-plot showing mean values and standard errors for crayfish and their potential food sources in Lake Terra Nova. Different plant parts were pooled to jointly represent the concerning plant species, and the different gastropods were grouped into 'small snails' and 'large snails'. The data points within the dotted rectangle are most important in crayfish diet. *Phragmites australis* seems to be the most important macrophyte

in *P. clarkii* diet, since this data point is relatively close to crayfish in the bi-plot. Macro invertebrates seem to be important nitrogen sources for crayfish as most food items slightly lighter than crayfish are of animal origin. Especially waterbugs, *Zygoptera* and *Trichoptera* larvae seem to be important diet components. Although not strictly within the boundaries of trophic enrichment relative to crayfish tissue, filamentous algae also seem to be a relative important food source for crayfish, as are snails and snail eggs. *Hydrarachna* and *Daphnia* have $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ signatures similar to *P. clarkii*, indicating they are in the same trophic level. Fish tissues are clearly more enriched than crayfish tissue. Detritus is characterized by a very low nitrogen signature, and does not seem to be an important direct food source for crayfish.

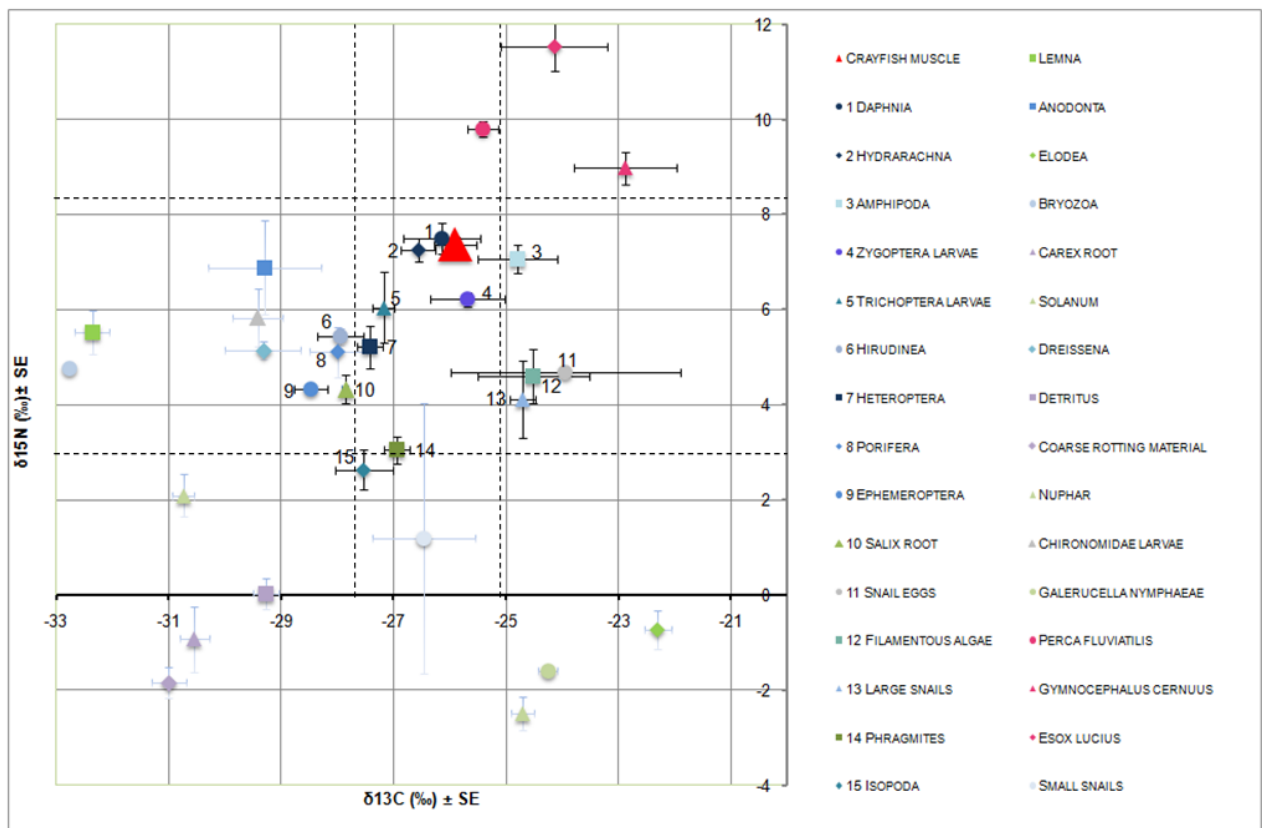


Fig. 4 Natural abundance of mean \pm SE $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of crayfish muscle and potential food items for crayfish collected from Lake Terra Nova. Note the different scales on the nitrogen and carbon axes. We assumed a trophic enrichment of 0.4 ‰ (\pm 1.2 SE) $\delta^{13}\text{C}$ (DeNiro and Epstein 1978) and 2.3 (\pm 1.6 SE) $\delta^{15}\text{N}$ (Minagawa and Wada 1984; Post 2002) for each trophic transfer, indicated by dotted lines. The data point belonging to *Perca fluviatilis*, *Gymnocephalus cernuus* and *Esox lucius* have been extracted from a similar food web study done in 2010 (data not published).

DISCUSSION

We found that, in the presence of crayfish, submerged macrophyte growth and biomass is clearly inhibited in both iron-rich and iron-poor conditions. Within ponds, biomass significantly differed between treatments. Very little plant biomass was left in the crayfish enclosures, while in the total enclosures the biomass of all species was large compared to the other treatments. The fact that in the reference treatment plant biomass was very low

indicates that overall herbivorous pressure from water birds and crayfish is high. We found little more plant biomass in the partial exclusions, indicating that crayfish have an evident overall effect on submerged macrophytes.

Crayfish were much more abundant in the iron-poor pond. When taking the crayfish density as the factor distinguishing between the 2 ponds, we found biomass of *Chara* and *Myriophyllum* to be generally higher in the iron-rich pond. This suggests that at higher densities, crayfish have more disastrous effects on these 2 submerged macrophyte species or that iron may have direct or indirect positive effects on the growth of these species. Other studies also found macrophyte biomass to be correlated with crayfish density (e.g. Chambers *et al.* 1990). Based on combined $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ signatures, the invasive crayfish seem to have conquered a high trophic position in the food web of Lake Terra Nova. Macro-invertebrates appear to be the most important group in the direct transfer of nitrogen to crayfish, indicating crayfish to be mainly carnivorous. The food web study implies that crayfish do not get their nitrogen solely by eating macrophytes, but by chiefly consuming animal tissue. These results agree with previous studies that showed crayfish to predate on macro-invertebrates with reduced escape reactions like snails, snail eggs, *Odonata* larvae, *Ephemeroptera* larvae (Rickett 1974; Hofkin *et al.* 1992; Ilhéu & Bernardo 1993; Matthews *et al.* 1993).

In literature, crayfish diet is reported to be diverse; consisting of fresh plants, animals, and algae and detritus to lesser extent. The preferences appear to switch according to availability of food items. Schoener (1971) and Ilhéu and Bernardo (1993) found that *P. clarkii* prefer animal food but will eat algae and macrophytes when animals are less abundant. Crayfish feeding preferences of fresh macrophytes are based on plant traits such as morphology, structure, chemical defences, digestibility and nutritional value (Chambers *et al.* 1990). Often, they feed upon finely branched or filamentous plants as they are easy to handle and consume (Bolser *et al.* 1998, Gherardi and Acquistapace 2007). Probable consequences of food item selectivity are an increased homogenisation of the recipient ecosystems and progressive reduction of biodiversity in shallow freshwater systems and (Lodge and Lorman 1987; Nyström *et al.* 1996, Rodríguez *et al.* 2003). This may also influence the distribution of *P. clarkii* (Cirujano *et al.* 2004). We found, however, that while all 3 submerged macrophyte species were differently affected by crayfish, none of the 3 species were consistently preferred or ignored to exclusion of the others. Crayfish thus apparently did not show any preference in appearance or growth form of the macrophytes in our case. More case studies of *P. clarkii* will contribute to a better understanding of feeding decisions and herewith provide general insights into the ecology and evolution of plant-herbivore interactions (Lodge *et al.* 1998).

Overall, light conditions proved to be better in the iron-poor pond. Reduced light may be caused by iron precipitates that are in suspension within the iron-rich pond. A possible explanation for the faster growth of the macrophytes in the full exclusions in the iron-rich pond is that these plants have grown faster towards the water surface in an attempt to capture more light. Whereas light availability at the bottom of the iron-rich pond was 3 times less than in the iron-poor pond, it was still 10% of the ambient light level and in absolute terms still plenty for macrophyte growth, which stops at approximately $6.9 \mu\text{mol} \cdot \text{m}^{-2} \cdot \text{sec}^{-1}$ (Kaj Sand-Jensen and Madsen 1991). Because iron concentration is negatively

correlated with the pH, it was unexpected to find the current pH to be significantly higher in the iron-rich pond. However, the difference is so small, that it may be a transient observation. The total amount of solids dissolved in water, measured as electric conductivity, was found to be higher in the iron-rich pond, which is in accordance to the higher iron concentration. In contrast to Chambers *et al.* (1990), we found impact of crayfish on submerged macrophyte biomass not to be related to crayfish sex. Crayfish total length was also not significantly correlated with macrophyte biomass. However, other studies have found that adults tend towards herbivory while juvenile crayfish are carnivorous or omnivorous (Mason, 1975; Olsen *et al.*, 1991; Ilhéu & Bernardo, 1993a). In this study we used only large individuals of total length > 9.0 cm size in the enclosures.

The number of crayfish in the crayfish enclosures (4 individuals · m⁻²) is probably higher than the natural density of crayfish in the ponds. Thus the results found do not mimic a situation of what submerged macrophytes realistically endure. However, in light of the growing number of non-indigenous crayfish introduced worldwide, our study indicates possible harm done when crayfish are present in large numbers (Gherardi and Acquistapace 2007).

Assuming an isolated population, the crayfish in the iron-rich pond might be on its way of recovery from the acid conditions reported in August 2009, when the pH was recorded to be equivalent to 3.77 (update pilot iron suppletion – non-published data). Although the possible toxic effects of iron on *P. clarkii* have not yet been investigated, Roldan and Shivers (1987) proved iron concentration to be toxic to another crayfish species, *Orconectes propinquus*.

Our study implies that invasive crayfish can reduce or completely eliminate multi-species macrophyte beds in a clear body of water. We state that it may very well be that crayfish interfere with and will continue to inhibit macrophyte establishment in lake-recovery projects. Due to their omnivorous diet, *P. clarkii* play complex roles in aquatic ecosystems. We proved this crayfish to have a clear negative effect on the biomass of 3 submerged macrophyte species: *Chara globularis*, *Elodea nuttallii* and *Myriophyllum spicatum*. Although stable isotope analysis showed that crayfish primarily feed on slow-moving animal prey, this does not rule out that macrophytes are not important in crayfish diet. Crayfish activity seems to affect submersed vegetation mostly indirectly, through bioturbation and shredding behavior in the process of feeding. Thus, while macrophyte attack may be indiscriminate, crayfish feeding appears selective. Indications for further research include additional case studies measuring *P. clarkii* effects throughout different aquatic ecosystems and investigating the trophic role invasive crayfish have gained in food webs. Also, quantifying the long-term effects of *P. clarkii* occurrence will highly contribute to planning science-based actions for their containment and mitigation (Gherardi and Barbaresi 2007).

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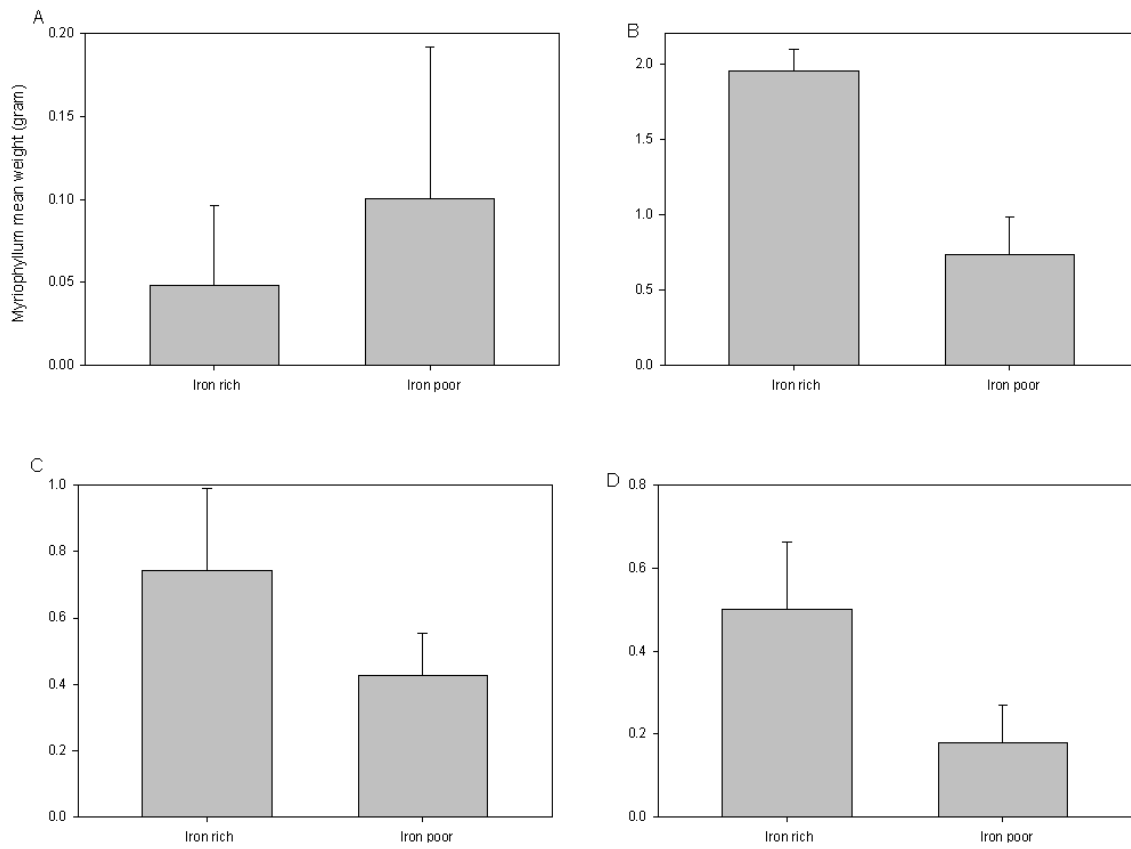
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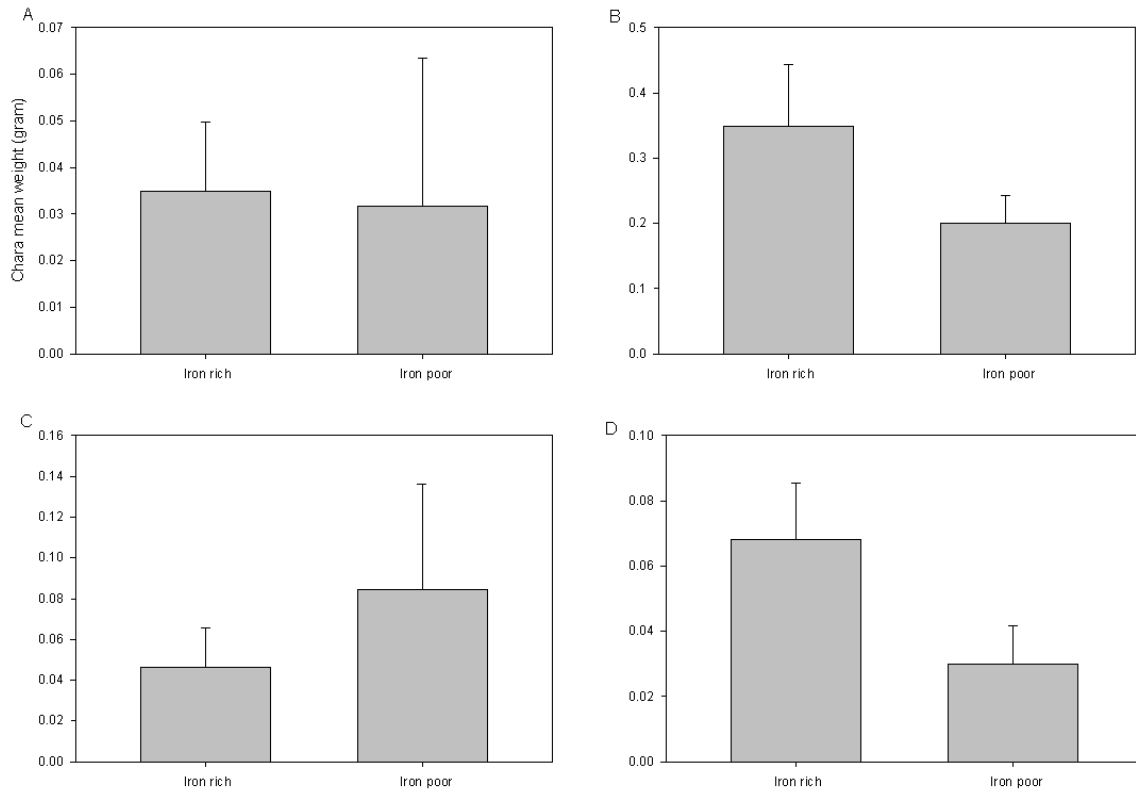
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SUPPLEMENTARY INFORMATION



Supplement 1 Mean weight (\pm S.E. in grams) of *Chara* in iron-rich and iron-poor ponds for the 4 treatments (A) Crayfish enclosure (B) Total enclosure (C) Partial enclosure and (D) Reference.



Supplement 2 Mean weight (\pm S.E. in grams) of *Myriophyllum* in iron-rich and iron-poor ponds for the 4 treatments (A) Crayfish enclosure (B) Total enclosure (C) Partial enclosure and (D) Reference.