**Evaluating Nutrient Reduction, Grazing and Barley Straw as Measures Against Algal Growth**

# Evaluating grazing, barley straw and nutrient reduction as measures against filamentous algal growth

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## ABSTRACT

*Abstract* The aim of our study is to investigate whether it is possible to reverse the effects of cultural eutrophication, as nuisance filamentous algae blooms in freshwaters ecosystems. Our experimental set up mimic shallow eutrophic moat of Krapperup castle (Southern Sweden), which is an example of extremely impaired ecosystem, subject to filamentous algae blooms (*Cladophora* spp.) We conducted an indoor mesocosm experiment to test three ecological sustainable treatments I) reduced nutrient concentration, II) invertebrate grazers and III) barley straw treatment, which may constitute measures against filamentous algal growth and thereby improve the quality of the ecosystem services provided by water bodies. We tested treatments effect on surface *Cladophora* coverage, on *Cladophora* growth on artificial macrophyte, on the phytoplankton community (diatoms and cyanobacteria) and we measured *Cladophora* and macrophyte biomass at the end of the experiment. We also did a ratio between final macrophyte and final *Cladophora* biomasses. Our results show a decrease in cyanobacteria and diatom concentrations in all mesocosms as *Cladophora* biomass increased, suggesting that the microalgae suffered from nutrient competition with *Cladophora*. In the present study, a tendency for lower *Cladophora* final biomass, as well as coverage, was observed in the treatment where the concentration of nutrients was reduced. Barley straw treatment was the only treatment promoting macrophyte growth, but this occurred without any notable effect on *Cladophora* biomass. Hence, our study adds to previous knowledge by identifying that the primary effect of barley straw is an enhancement of macrophyte growth, whereas the direct effects on filamentous algae is negligible. In a broader context, barley straw treatments of eutrophic waters may therefore promote a shift from dominance by filamentous algae to macrophytes as main primary producers. Hence, in accordance with the alternative stable state theory (Scheffer et al., 1993), barley straw treatment may be a tool to shift a system from algal to macrophyte dominance, and thereby lead to clearer water. Moreover, our experiment show that barley straw may be effective to inhibit cyanobacterial growth. Altogether, our experimental results have important implications for the challenge of reversing nuisance algal blooms in highly eutrophicated systems.

## INTRODUCTION

Clean water is a crucial resource for drinking, irrigation, industry, transportation, recreation, fishing, hunting, support of biodiversity, and also for aesthetic reasons (Carpenter, 1998). Scientists and policy-makers have widely come to accept that natural resources need to be protected from the destructive actions of human activities, since people inevitably harm natural resources as they use them. Moreover, the human population size is globally increasing, which is translated into more pronounced impact on water, land and atmospheric resources (Pretty et al., 2003). In fact, in the past decades, tremendous volumes of urban, agricultural and industrial wastewater have been produced, which have greatly increased the input of nutrients, such as. nitrogen and phosphorus, as well as pollutants into natural water bodies (Abdel-Raouf et al., 2012; Boelee et al., 2011; Xin et al., 2010). Eutrophication is a leading cause of impairment of many freshwater and coastal marine ecosystems in the world (Chislock et al., 2013). This phenomenon is characterized by excessive plant and algal growth due to the increased availability of one or more limiting growth factors needed for photosynthesis, such as phosphorus or nitrogen (Schindler,2006). A relevant question is therefore if there are possibilities to reverse such effects by making ecologically sustainable changes. For example, many waters exposed to eutrophication are overgrown with filamentous macroalgae, e.g. *Cladophora* spp., covering macrophytes and leading to reduced quality of ecosystem services (Phillips et al. 1978; 2016). In order to address the question if such nuisance growth can be counteracted, we conducted an experiment to test three ecologically sustainable solutions to reduce filamentous *Cladophora* sp. growth in an extremely degraded ecosystem. We experimentally tested the effects on *Cladophora* growth from reduced nutrient concentration, invertebrate grazers and barley straw treatment, which may constitute measures against filamentous algal growth and thereby improve the quality of the ecosystem services provided by water bodies.

## MATERIAL AND METHODS

### Site description

Krapperup is a castle in Höganäs municipality, Scania, in southern Sweden (56° 15' 26.01" N; 12° 31' 28.83" E), representing a well-connected environment with castle, park and modern farming. The total area of 2750 hectares includes farmhouses, pastures and forests (http://krapperup.se) The moat surrounding the castle is characterized by shallow waters with an average depth of 0.46 m. We performed an exploratory survey of the Krapperup moat in September 2016. The average turbidity was 7.3 NTU, i.e. the water was clear and light easily penetrated to the bottom. The average pH in the moat was 7.8. Total nutrient concentrations measured in the waters of the moat were very high with an average total phosphorous concentration of 213 µg/L and an average total nitrogen concentration of 1450 µg/L. Macrophytes in the moat belong to the genus *Potamogeton* but its coverage in the moat was low, only 6% PVI (Percent Volume Infested). The average PVI of the filamentous algae *Cladophora* was 32.5%, with highest values in the East- and South-facing sides of the moat, where it reached a PVI of 90%.

In November 2016, we collected material for the indoor mesocosm experiment from the Krapperup moat: 150L of unfiltered water, 100L of sediment and macrophytes (*Potamogeton pectinatus*). The water was collected in plastic jerry cans. The sediment was collected by using hand nets and placed in dark plastic boxes. Macrophytes were harvested manually from the moat and placed in a dark bucket with moat water. All the material collected was transported to the experimental facilities within four hours of sampling.

**Experimental set-up**

Several factors may affect the distribution of the nuisance filamentous algae, among others inhibition by barley straw , grazing pressure and reduced nutrient supply can be simulated in laboratory experiments. The three treatments tested in our experiment were therefore:

I) **Barley treatment:** Fresh barley straw + barley straw extract, to induce chemical inhibition Aerobical decomposition of barley straw has been shown to inhibit growth of algae under both laboratory and field conditions (Caffrey, 1999; Martin et al., 1999; Ridge

et al., 1996; Geiger et al., 2005; Ball et al., 2011; Houman et al. 2011). A primary requirement for the successful use of barley straw is the maintenance of aerobic conditions (Ridge et al., 1996). Unstable, short-lived algal inhibitors are released during the aerobic decomposition of the straw. These are highly selective against planktonic and filamentous algae (Newman et al., 1993; Barrett et al., 1999) and are algistatic rather than algicidal. There is strong evidence that these algal inhibitors are derived from oxidised polyphenolics released from solubilised lignin (Ridge & Pillinger, 1996), although the precise nature of the inhibitors or their mode of action remain unknown. The effects of more than 100 barley straw treatments in the U.K. and Ireland were documented by Newman & Barrett (1993) and results revealed that algal control was achieved to at least some extent, in all types of water bodies, but was most pronounced in smaller ponds (< 5 ha). Barley straw is now in widespread use as a method for controlling algal growth, since it is inexpensive and show no adverse ecological effects (Geiger et al., 2005). However, some studies have shown a weak or even absent inhibitory effect on algae (Boylan & Morris, 2003; Prygiel et al., 2014). Furthermore, some studies suggest that barley straw should be placed in the body of water several months before bloom conditions are expected (Geiger, 2005). Therefore, to prevent that fresh barley straw masked the effect from straw-derived algistatic components, we decided to test both fresh straw and straw extract in our study. This approach has been previously used in several studies (Ball et al., 2001; Houman, 2011). We applied this treatment to test the effectiveness of barley straw derived components in inhibiting *Cladophora* growth in the moat;

II) II) **Grazers**: snails are an important component of the benthic community in many freshwater systems, and several experimental studies have shown that snail grazing has strong effects on epiphytic algal biomass, species composition, architecture, and productivity (Brönmark, 1989; Stevenson et al., 1996). Freshwater pulmonate snails are commonly found in association with macrophytic vegetation and their epiphyton

(Underwood, 1992). These macrophytes provide sites for snail oviposition, access to the air-water interface and shelter, and provide a surface for periphytic algal development, which constitutes a major source of the food of freshwater snails (Underwood, 1992). Filamentous green algae, such as *Cladophora*, are the most noted nuisance periphyton group (Biggs & Price, 1987; Welch, Horner & Patmont, 1989; Welch, Quinn & Hickey, 1992). The removal of periphyton by grazing invertebrates is well documented (Lamberti & Moore, 1984; Colletti et al., 1987; Jacoby, 1987; Steinman et al., 1987; Power, Steward & Matthews, 1988; Feminella & Hawkins, 1995), even if earlier studies (Gregory, 1983) emphasized grazer preference for diatoms and avoidance of the larger filamentous green algae. *Lymnaea stagnalis* occurs naturally in the study site (Krapperup Castle) and is known to be a grazer on *Cladophora* (Brönmark et al., 1991), although other studies have shown that snails prefer diatoms (Calow and Calow 1975; Skoog 1978). In any case, growth and reproduction of *L. stagnalis* can be sustained on a diet of *Cladophora*, but growth rate is higher when fed more high-ranked food items (Skoog 1978). *Lymnaea stagnalis* is predicted to have high population densities in small ponds without predators (Brönmark, 1989), such as the Krapperup moat. We applied this treatment to test if a high presence of grazers can limit the growth of *Cladophora* on macrophytes and thus improve the health status of macrophytes, triggering a shift to a state with macrophytes as dominant primary producers;

III) III) **Reduced nutrient concentration**: the main cause of algal accumulations is an over-enrichment of nutrients, particularly phosphorus and nitrogen in the water (Søndergaard, 2007). We applied this treatment to test if it was sufficient to decrease the concentration of nutrients in the water column to inhibit *Cladophora* growth.

**Experimental set up and maintenance**

The experiment was run for two and a half months, from November 2016 to January 2017, in the greenhouse of Lund University (55° 42’ N 13° 12’ E). The experimental mesocosms consisted of 28

transparent polypropylene aquaria (39x28x28 cm) with a capacity of 22L. To initiate the experiment, we placed 3 cm of previously homogenized moat sediment at the bottom of each aquarium and then filled each of them with 10 L of unfiltered water collected from the moat and tap water in different proportions depending on the treatment. We did not filter neither the sediment nor the water, ensuring a certain number of grazers in each treatment, thereby mimicking natural ecosystems. Each experimental treatment had 7 replicates and each aquarium was filled with 6L of unfiltered water from the Krapperup moat and 4 L of tap water. In the barley straw treatment we put 10g of fresh barley straw (packed in a plastic net) in each aquarium and also added 35 mL of barley straw extract. The extract was prepared by cutting 10g of fresh barley straw into uniform lengths (2 cm) which were then boiled in 250 ml tap water according to Ball et al. (2001). After cooling, the solution was filtered through a glass fibre filter and the filtered volume was adjusted to 250 ml. To each aquarium we put 6L of unfiltered water from the moat and 4L of tap water. In the grazers treatment we added 25 *Lymnea stagnalis* snails to each aquarium. Snails were taken both from the moat and from an aquaria culture at Lund University. In the treatment with reduced nutrient concentrations in the water by adding only 2L, instead of 6L, of unfiltered water from the moat to each aquaria and filling up the remaining 8L with tap water. The control aquaria were identical to all other treatments except that they did not receive any experimental treatment.

In each aquarium, we planted 2.5 g (wet weight) *of Potamogeton pectinatus* collected from the moat and 2.5 g of *Myriophyllum spicatum* (fresh weight) collected in a stream near the greenhouse. We added *Myriophyllum spicatum,* even if it is not a species naturally present in the moat, because *Potamogeton* is known to be rapidly out-competed by other species (Birkinshaw et al., 2013). In the experiment, we also aimed at testing the effect of *Cladophora* on macrophytes and *vice versa*. For this reason, we needed a macrophyte species that was more likely to survive for the entire experimental period. *Myriophyllum spicatum* occurs in various sediment types and can tolerate low-light environments, such as highly eutrophic waters (Smith & Barko 1990), and is therefore widely used for eutrophic lake restoration (Gao et al. 2007). We also added artificial plastic macrophytes (9

branches) to each aquarium in order to estimate *Cladophora* growth on standardized surface areas with macrophyte-like morphology. At the beginning of the experiment the weight and coverage of *Cladophora* on artificial macrophytes was zero. The aquaria were kept at 14-10h light-dark cycle and the water temperature in the aquaria ranged between 14.5 and 16.5°C during the experiment. Distilled water was added weekly to compensate for evaporation losses.

**Sample collection and analysis**

Temperature, dissolved oxygen (using OxyGuard Handy Oxygen Meter) and pH (using a pH meter) were measured every 10 days, and every 20 days (in total four sampling sessions) *Cladophora* coverage on the surface of the water was visually estimated for each aquarium and photos were taken. Similarly, *Cladophora* growth on artificial macrophytes was measured every 20 days (in total four sampling sessions), cutting one branch of the artificial macrophyte in each aquarium and then scrubbing off attached *Cladophora*. Finally, we weighed the branch and the filamentous algae growing on it (dry weight). For each sampling, a growth rate was obtained using the formula:

$$x = \frac{Cladophora\ weight}{artificial\ macrophyte\ branch\ weight + Cladophora\ weight} * 100$$

Samples for assessment of chlorophyll-a concentration (µg chl-a/l) were taken from each aquarium every 20 days (in total four sampling occasions), inside a submerged plastic bottle with a filter (50 µm mesh size) to avoid contamination by *Cladophora* filaments. Chlorophyll was analysed with a fluorometer (AlgaeLabAnalyser, ALA, bbe moldaenke, Germany), within one hour of sampling. Chlorophyll-a analysis were performed to assess the effect of different treatments on the phytoplankton community, particularly on cyanobacteria in view of their possible toxicity, and diatoms, since they are nutritious food for many grazers. At the end of the experiment all macrophytes and *Cladophora* were taken out of each aquarium, dried and weighted. The above and belowground part of the macrophytes were weighted separately.

**Data analyses**

Two-way repeated measures Analysis of Variance (RM-ANOVA), with sampling session and treatment as factors, was used to analyse the treatment effect and its duration on *Cladophora* coverage on the surface, *Cladophora* growth on artificial macrophytes, as well as diatom and cyanobacteria biomass expressed as μg chl-a/l. Data were log-transformed to achieve a normal distribution. Pairwise differences between treatments were checked with the Tukey's post-hoc test. Kruskal-Wallis test was used to evaluate the treatment effect on total *Cladophora* and macrophyte biomasses in each aquarium at the end of the experiment, as well as on the ratio between the final biomass of *Cladophora* and that of macrophytes. RM-ANOVA, Tukey's post-hoc test and graphs were performed with R (R Development Core Team, 2015) while the Kruskal-Wallis test was performed in StatXact v5 (© Cytel Software, 2001).

## RESULTS

The oxygen saturation trend was similar for all the treatments and ranged between 100% and 145%.

Similarly, the pH trend was similar for all the treatments and ranged between pH 8.3 and pH 9.6.

### Cladophora growth in the treatments

*Cladophora* coverage was significantly affected by the treatment and the sampling session (Table 1). It increased significantly in all treatments between sampling sessions 1, 2 and 3 (Tukey's post-hoc: $p < 0.001$), but then reached its peak biomass. *Cladophora* coverage was significantly lower in the reduced nutrients treatment compared to the control (Tukey's post-hoc: $p = 0.032$). There were no significant differences in (?) the other treatments compared to the control, although there were tendencies for a lower *Cladophora* coverage in the snail treatment (Fig. 1).

*Cladophora* growth on artificial macrophytes was significantly affected by the sampling session but not by the treatment (Table 1). In particular, it increased in all treatments, but it increased slower than *Cladophora* coverage on the surface, since it did not show a significant difference between sampling

sessions 1 and 2. The growth significantly increased between sampling sessions 2 and 3 (Tukey's post-hoc: $P < 0.001$). There were not significant differences between treatments, although there were tendencies for a lower growth in the nutrient treatment. The barley straw treatment initially inhibited *Cladophora* growth on artificial macrophytes, but the effect disappeared after about a month. The snail treatment showed no initial effect on *Cladophora* growth on artificial macrophytes, although there was a tendency for reduced *Cladophora* biomass at the end of the experiment. The total *Cladophora* biomass increased in all treatments over time, but did not differ significantly among treatments, although there was a tendency for lower biomass in the reduced nutrient treatment (Table 2). Hence, none of the treatments applied had any significant effect on the growth of *Cladophora*.

**Phytoplankton community responses**

Cyanobacteria concentration (µg chl-a/l) was significantly affected by both sampling session and treatment (Table 1), and showed an inverse hump-shaped response since it initially significantly decreased in all treatments between sampling sessions 1 and 2 (Tukey's post-hoc: $p < 0.001$). However, the concentration of cyanobacteria (µg chl-a/l) showed a significant increase in all treatments towards the end of the experiment (Tukey's post-hoc: $p = 0.001$). Considering the different treatments, the cyanobacteria concentration (µg chl-a/l) was significantly lower in the barley straw treatment compared to all the other treatments and the control (Tukey's post-hoc: $p = 0.014$). Barley straw inhibited cyanobacterial growth, but this seemed to be a short-term effect, since cyanobacteria concentration increased again towards the end of the experiment.

The diatom concentration (µg chl-a/l) was significantly affected by both time and treatment (Table 1). It initially decreased in all treatments (Tukey's post-hoc: $p < 0.001$), whereas the following sampling sessions did not show any significant differences. However, the diatom concentrations were significantly higher in the barley straw treatment compared to all other treatments and the control (Tukey's post-hoc: $p < 0.031$).

**Macrophytes response**

Macrophyte final biomass showed significant differences between treatments and the control (Table 2). In particular, total macrophyte biomass was significantly higher in the straw treatment compared to the other treatments and the control (Kruskal-Wallis: $p$ = 0.0021). These differences were significant with regard to the aboveground part of macrophytes, whose biomass was significantly higher in the straw treatment (Kruskal-Wallis: $p$ = 0.0015). There were no significant differences in the belowground macrophyte biomass, although there was a tendency for higher biomasses in the straw treatment. Significant differences among treatments were observed for the ratio between the final weight of cladophora and the final weight of macrophytes (Kruskal-Wallis: $p$ = 0.002), with lower values in the straw treatment compared to the others.

## DISCUSSION

Our experimental results show that the *Cladophora* coverage started to increase substantially in all treatments from the beginning of the experiment and then declined after reaching its peak. None of the treatments used in the experiment resulted in significant declines in *Cladophora* growth. Instead the total *Cladophora* biomass increased in all treatments during the study. However, there was a decrease in cyanobacteria and diatom concentrations in all aquaria as *Cladophora* biomass increased, suggesting that the microalgae suffered from nutrient competition with *Cladophora* (Cheney and Hough 1983).

Barley straw have been shown to inhibit a wide range of algae, comprising both filamentous types, as *Cladophora* (Weltch et al., 1990), and phytoplankton (Gibson et al., 1990) including cyanobacteria (Newman & Barrett, 1993). However, based on the finding that diatoms may occur in tanks of rotting barley straw, it was suggested that these algae are resistant to straw inhibitors (Ridge et al., 1996). The anti-algal activity of barley straw has yet to be elucidated, although several authors suggest that phenolic compounds derived from lignin play a role in the inhibition of algae (Ridge and Pillinger, 1996). Our results confirmed these previous studies, showing that *Cladophora* coverage on

the surface and *Cladophora* growth on artificial macrophytes seemed to be limited in the barley straw treatment, although this effect disappeared after about a month. However, our results show that barley straw may be effective to inhibit cyanobacterial growth, but even this turned out to be a short-term effect, since cyanobacteria concentration increased again towards the end of the experiment. Hence, barley straw treatment had, for all the components examined, a short-term effect. Our results show that barley straw promoted diatom growth, since the diatom concentration was significantly higher in the barley straw treatment. As suggested by Ridge et al. (1996), diatoms seem to be resistant to barley straw effects and in our study they seem to be further favoured by the decline in cyanobacteria in the barley straw treatment. Barley straw have been suggested to produce conditions favouring establishment of a diverse plant and faunal community (Welch et al., 1990; Barrett & Newman, 1993; Everall & Lees, 1996; Caffrey et al., 1999). Moreover, Caffrey (1999) demonstrated that after barley straw treatment, water transparency improved and *Myriophyllum spicatum* and *Elodea canadensis* showed increased growth. Our results are in line with those, showing that barley straw treatment was the only treatment promoting macrophyte growth. Previous studies (Welch et al., 1990; Barrett& Newman, 1993; Caffrey, 1999; Everall & Lees, 1996), suggested macrophyte growth to be enhanced where barley straw was used, following a reduction in algal biomass. Our results show barley straw to promote macrophytes growth, but this occurred without any notable effect on *Cladophora* biomass. Hence, our study adds to previous knowledge by identifying that the primary effect of barley straw is an enhancement of macrophyte growth, whereas the direct effects on filamentous algae is negligible. A likely explanation to findings from previous studies may therefore be that macrophytes are stimulated by barley straw and that a larger macrophyte biomasses compete with algae for nutrients, thereby leading to a secondary effect on algae. In a broader context, barley straw treatments of eutrophic waters may therefore promote a shift from dominance by filamentous algae to macrophytes as main primary producers. Hence, in accordance with the alternative stable state theory (Scheffer et al., 1993), barley straw treatment may be a tool to shift a system from algal to macrophyte dominance, and thereby lead to clearer water.

Although removal of periphytic algae by grazing invertebrates is well documented (Lamberti & Moore, 1984; Jacoby, 1987; Steinman et al., 1987; Power, Steward & Matthews, 1988; Steinman 1991; Feminella & Hawkins, 1995), earlier studies (Gregory, 1983) have emphasized a grazer preference for diatoms and avoidance of the larger filamentous algae, such as *Cladophora*. In the present study, a tendency for lower *Cladophora* final biomass, as well as coverage, was observed in the treatment where the concentration of nutrients was reduced.

The nuisance growth of *Cladophora* in Krapperup castle moat is likely a result of an excessive nutrient (phosphorous and nitrogen) loading to the water. Therefore, none of the treatments applied in the experiment (even the nutrients reduction one) had low enough nutrient concentrations to reach the desired effect of reducing *Cladophora* biomass. However, our study clearly identifies a strong positive response of submerged macrophytes to barley straw treatment, suggesting that the compounds released by barley may not primarily be affecting nuisance algal growth, such as *Cladophora* and cyanobacteria, but rather stimulate their macrophyte competitors. A deeper understanding of barley straw bio-stimulation effect on macrophytes would be useful to assess its potential as a converter from a situation where filamentous algae is the dominant primary producers to one in which they are outcompeted by macrophytes.

Altogether, our experimental results have important implications for the challenge of reversing nuisance algal blooms in highly eutrophicated systems. Although conclusions derived from experimental studies have to be interpreted with caution, they provide a useful intermediate scale between short-term laboratory studies and whole lake manipulations, especially by mimicking water bodies structurally similar and by enabling replication (Urrutia-Cordero, 2017).

In conclusion, we show that nutrient reduction is the only treatment that manifested any tendency to reduce the biomass of filamentous algae. However, barley straw treatment resulted in a considerable increase in macrophyte growth, therefore the amount of filamentous algae per macrophyte was lower. Hence, although we found no direct effect of barley straw on filamentous algal growth, barley straw

may alter the dominance pattern between filamentous algae and macrophytes, indirectly leading to clearer water.

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**Table 1** Results of the RM-ANOVA. For both factors tested (Treatment and Session) the F and *p* values are reported

| | Treatment | Session |
|---|---|---|
| Cladophora coverage | $F_{3,101} = 2.76$; $P = 0.046$ | $F_{3,101} = 86.3$; $p < 0.001$ |
| Cladophora weight | $F_{3,101} = 1.64$; $P = 0.186$ | $F_{3,101} = 25.8$; $p < 0.001$ |
| Diatom chl-a | $F_{3,101} = 10.3$; $P < 0.001$ | $F_{3,101} = 30.8$; $p < 0.001$ |
| Cyanobacteria | $F_{3,101} = 6.62$; $P < 0.001$ | $F_{3,101} = 29.0$; $p < 0.001$ |

**Table 2** Results of the Kruskal-Wallis test on the final biomass of filamentous Cladophora, as well as toale, above- and below-ground macrophyte biomasses. For each variable, the W and p values are reported

| | |
|---|---|
| Total *Cladophora* biomass | $W = 6.090$; $p = 0.1007$ |
| Total macrophytes biomass | $W = 12.72$; $p = 0.0021$ |
| Below-ground macrophytes biomass | $W = 7.031$; $p = 0.0584$ |
| Above-ground macrophytes biomass | $W = 14.08$; $p = 0.0015$ |

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**Figure legends**

**Figure 1**. Box Plots showing *Cladophora* surface coverage in response to the different treatments at the four sampling sessions.

**Figure 2**. Box Plots of *Cladophora* biomass on artificial macrophytes in the different treatments at four sampling occassions.

**Figure 3**. Box Plots showing Cyanobacteria concentrations (µg chl-a/l) in response to the different treatments in the four sampling occassions.

**Figure 4**. Box Plots showing Diatom concentrations (µg chl-a/l) in response to the different treatments at the four sampling occassions.

**Figure 5**. Box Plots showing *Cladophora* final biomassesin response to the different treatments. Stars represent significant differences.

**Figure 6**. Box Plots showing macrophyte final biomasses in response to the different treatments.

**Figure 7.** Box Plots showing final macrophyte above-ground biomasses in response to the different treatments.

**Figure 8.** Box Plots showing macrophyte final below-ground biomasses in response to the different treatments.

**Figure 9.** Box Plots showing final Cladophora and macrophyte weight ratio in response to the different treatments.

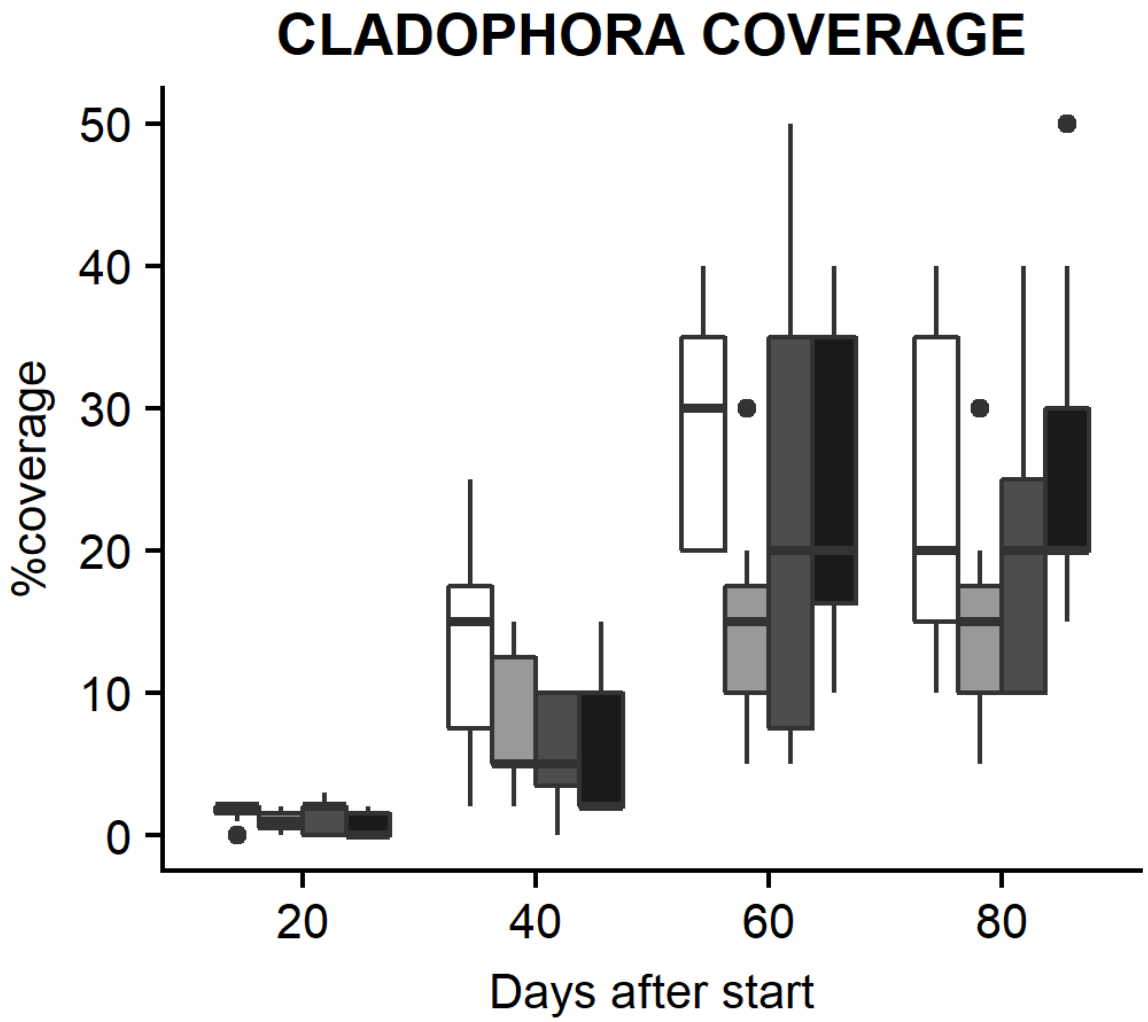
CLADOPHORA COVERAGE
%coverage
50
40
30
20
10
0
20
40
60
80
Days after start

Figure 1.

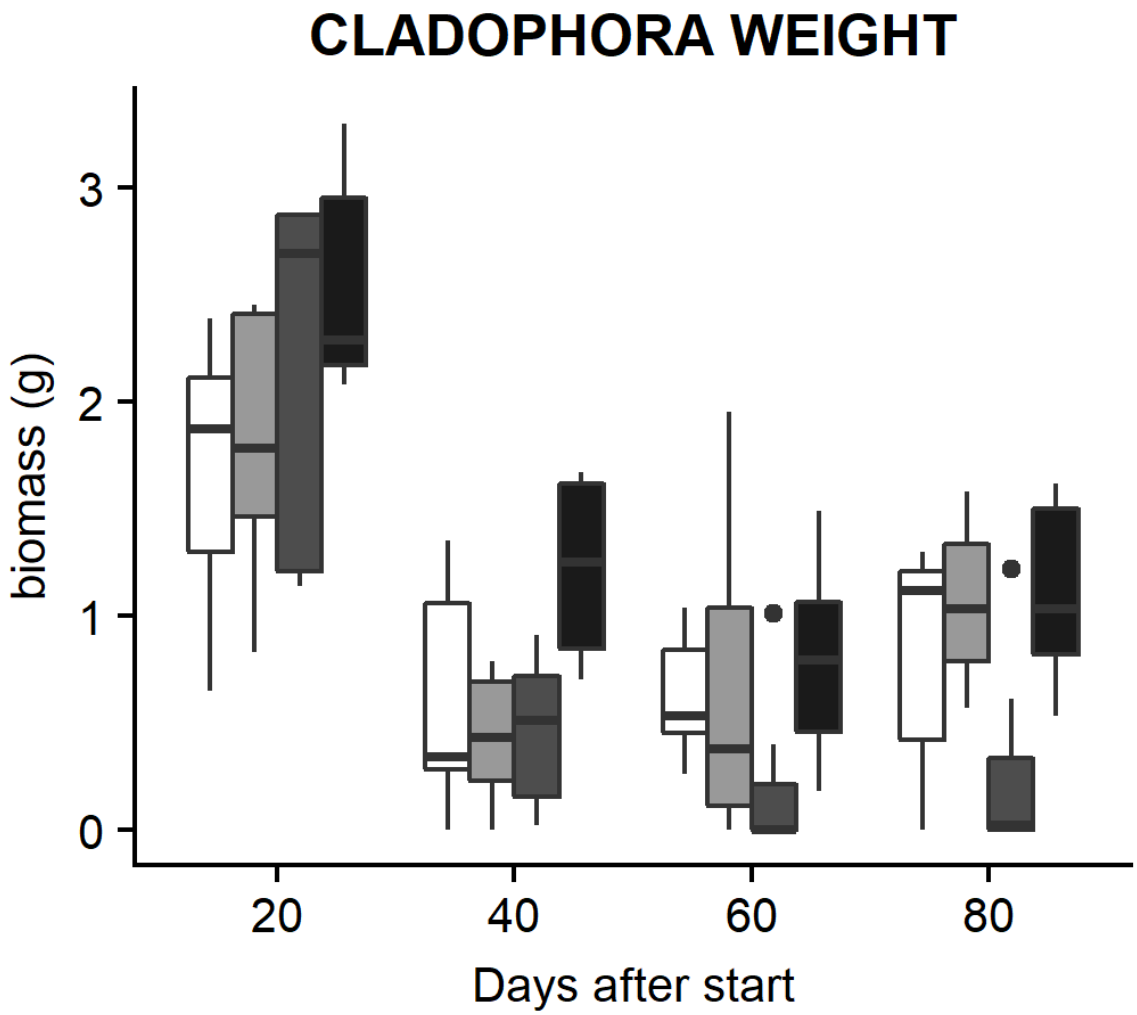
CLADOPHORA WEIGHT
biomass (g)
0
1
2
3
20
40
60
80
Days after start

Figure 2.

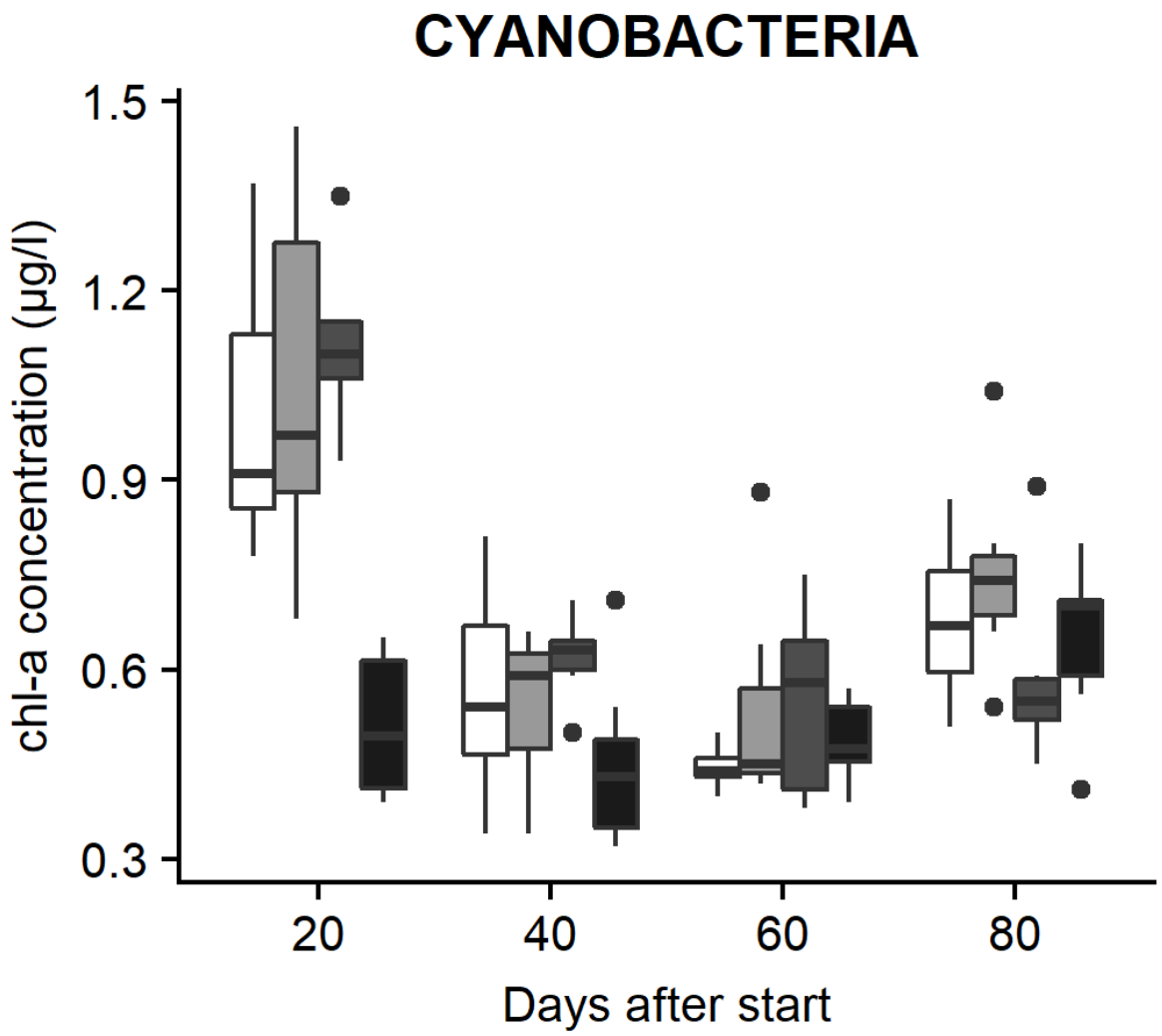
CYANOBACTERIA
chl-a concentration (µg/l)
1.5
1.2
0.9
0.6
0.3
20
40
60
80
Days after start

Figure 3.

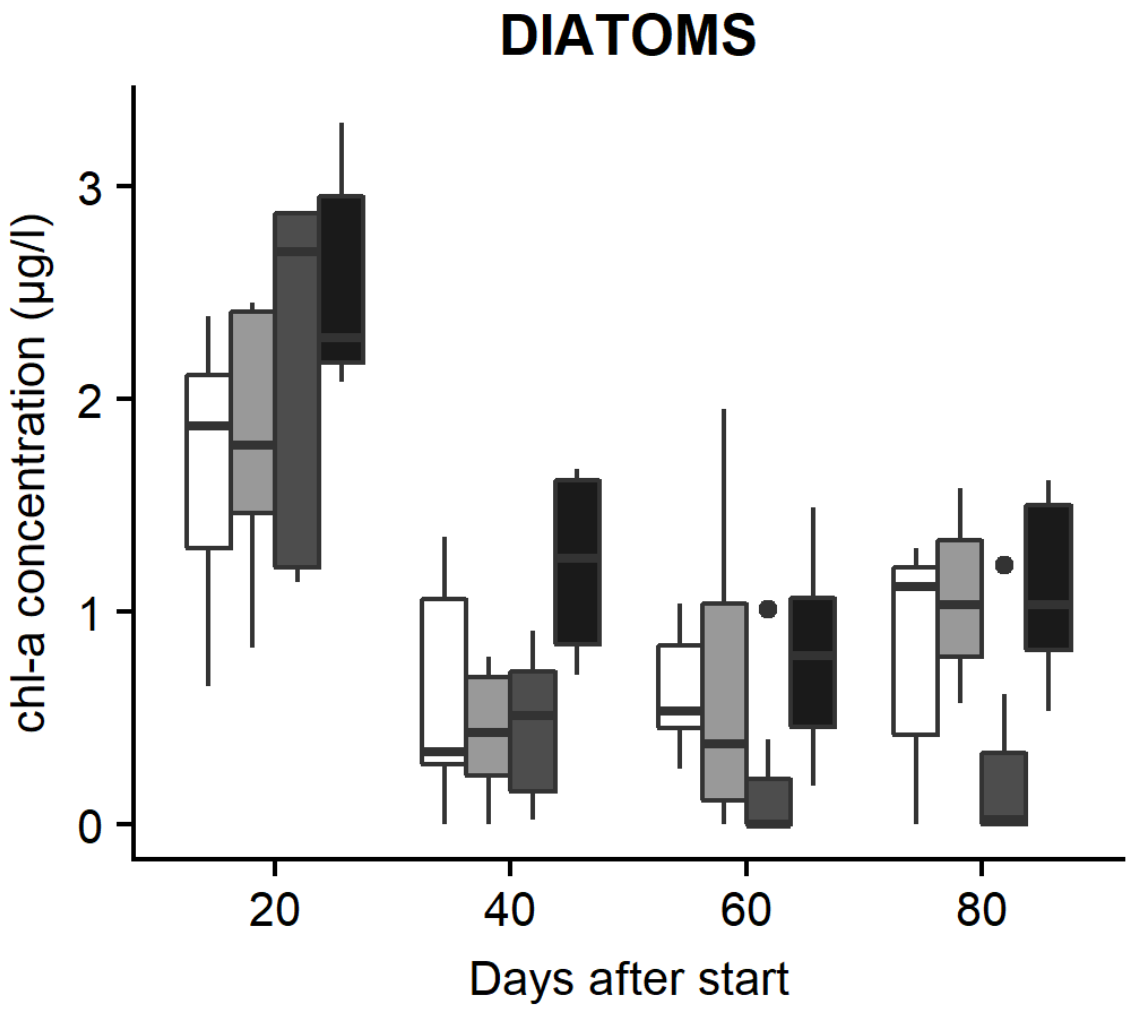
DIATOMS
chl-a concentration (µg/l)
0
1
2
3
20
40
60
80
Days after start

Figure4.

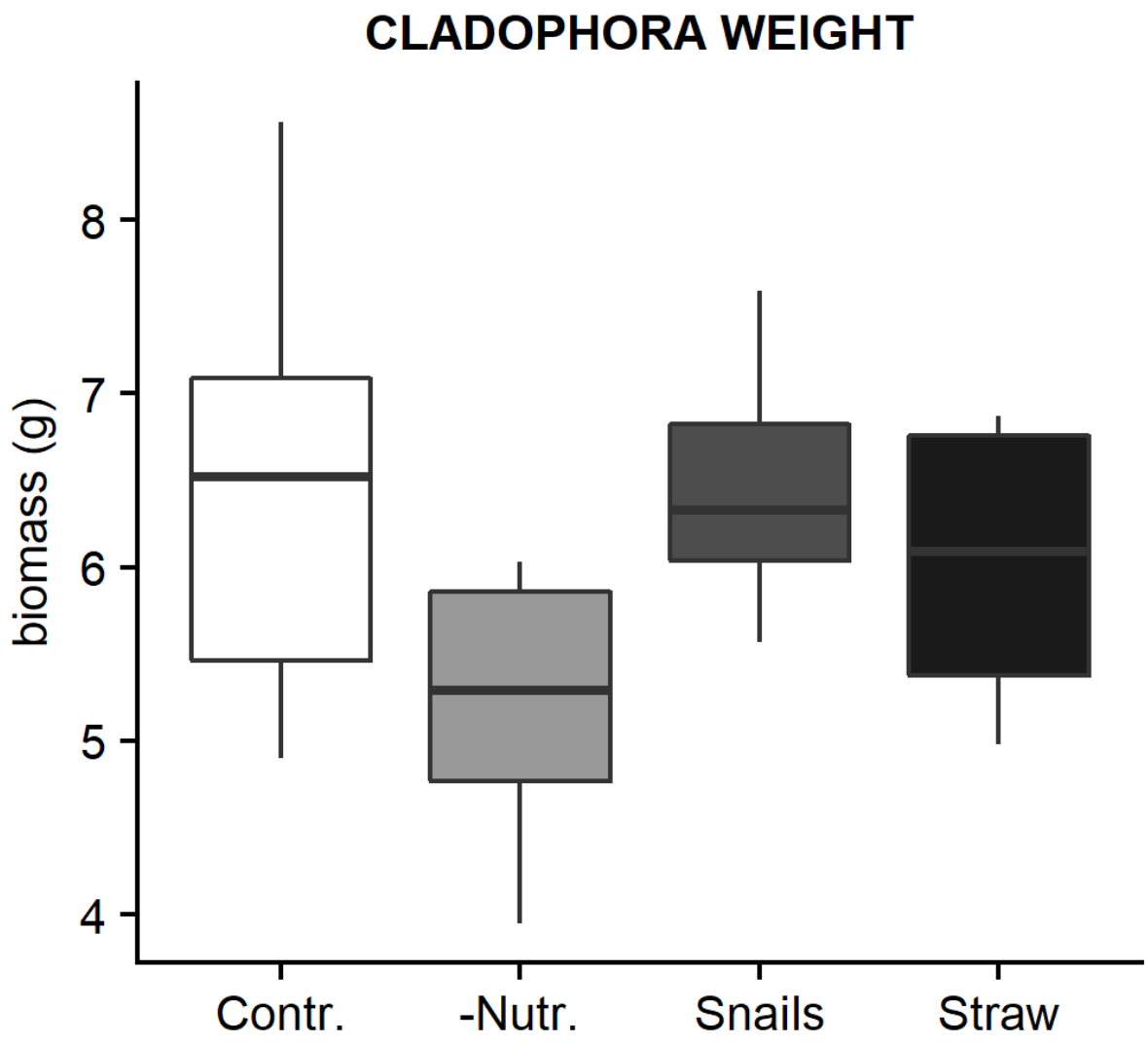


Figure5.

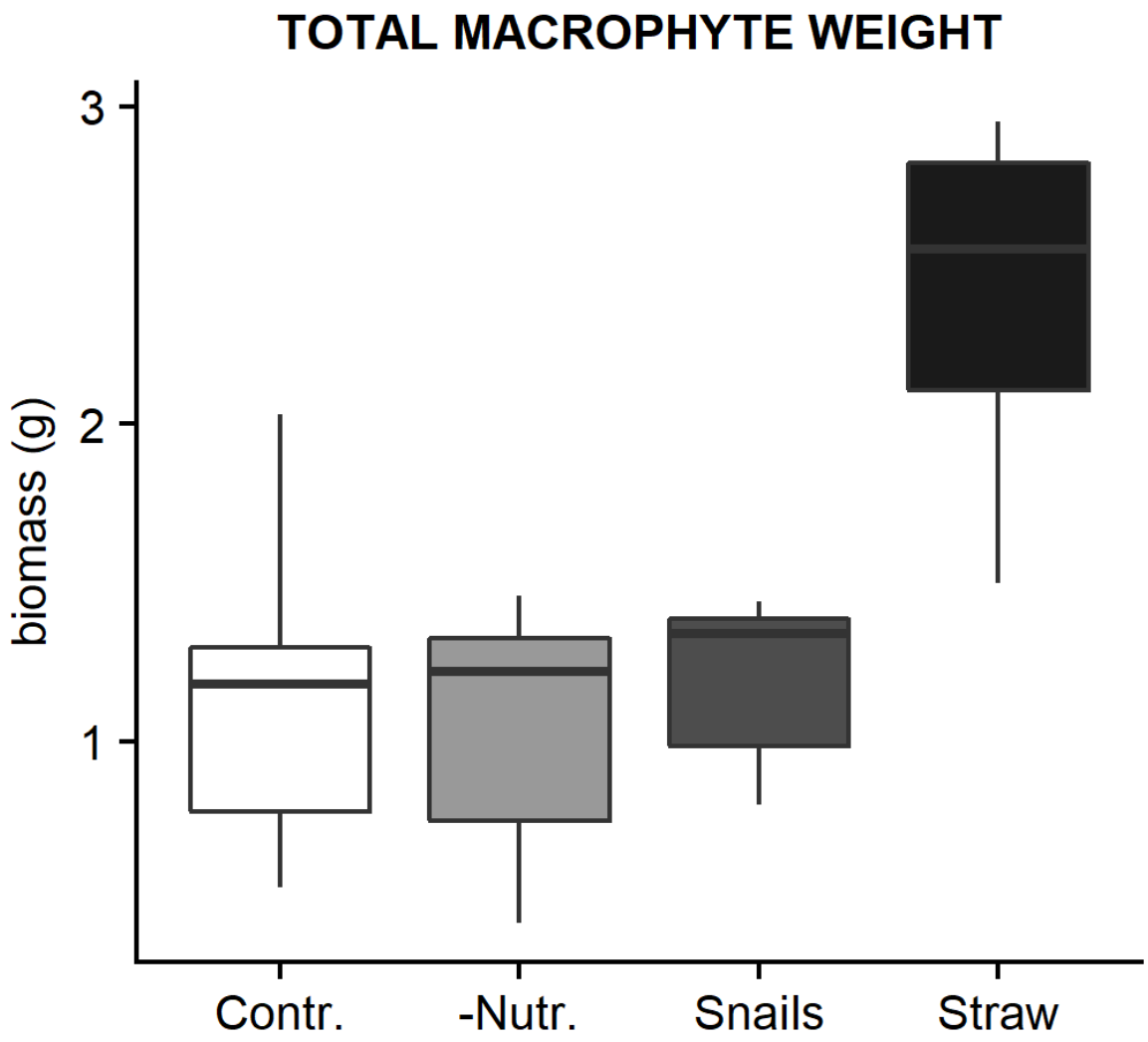
TOTAL MACROPHYTE WEIGHT
biomass (g)
3
2
1
Contr.
-Nutr.
Snails
Straw

Figure 6.

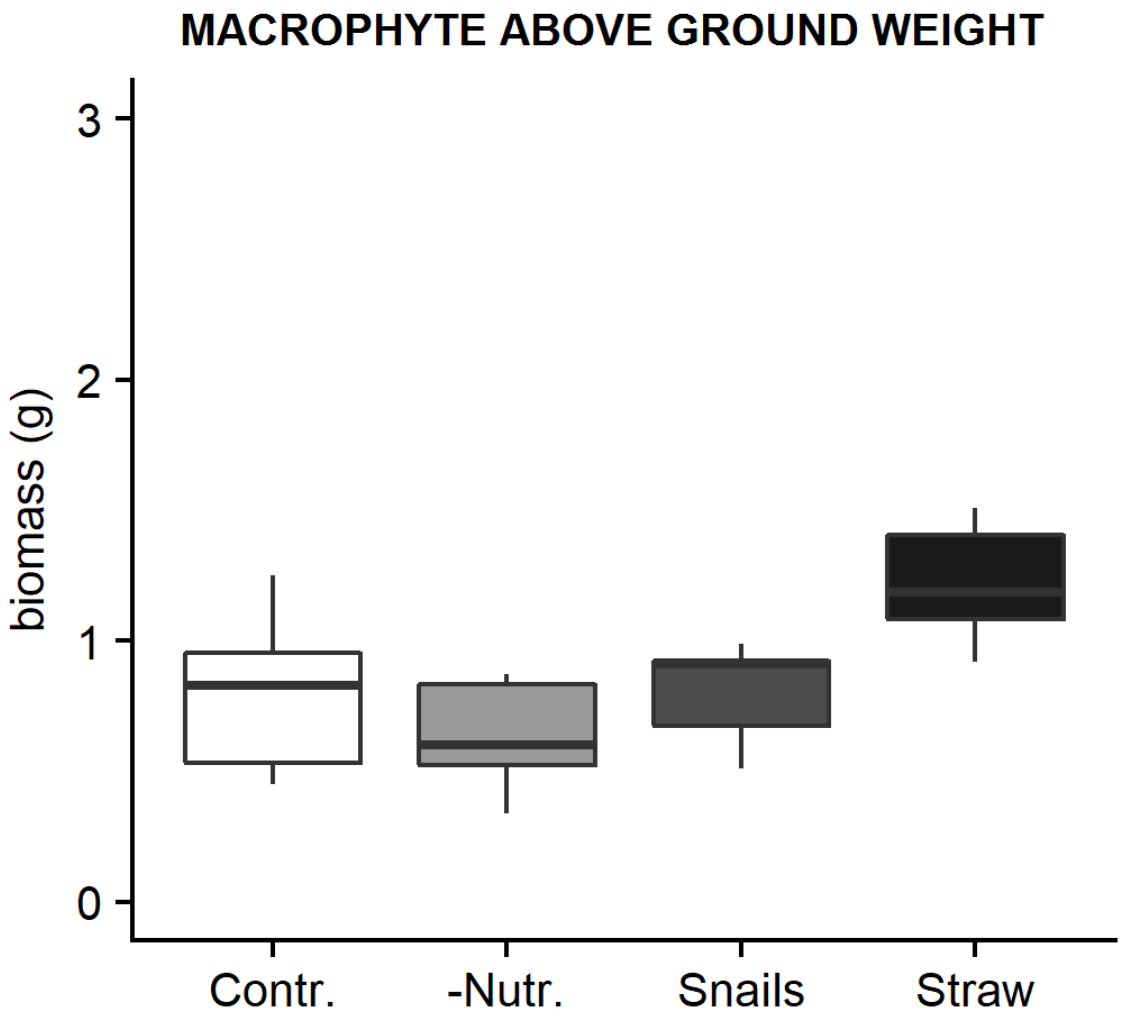
MACROPHYTE ABOVE GROUND WEIGHT
biomass (g)
3
2
1
0
Contr.
-Nutr.
Snails
Straw

Figure7.

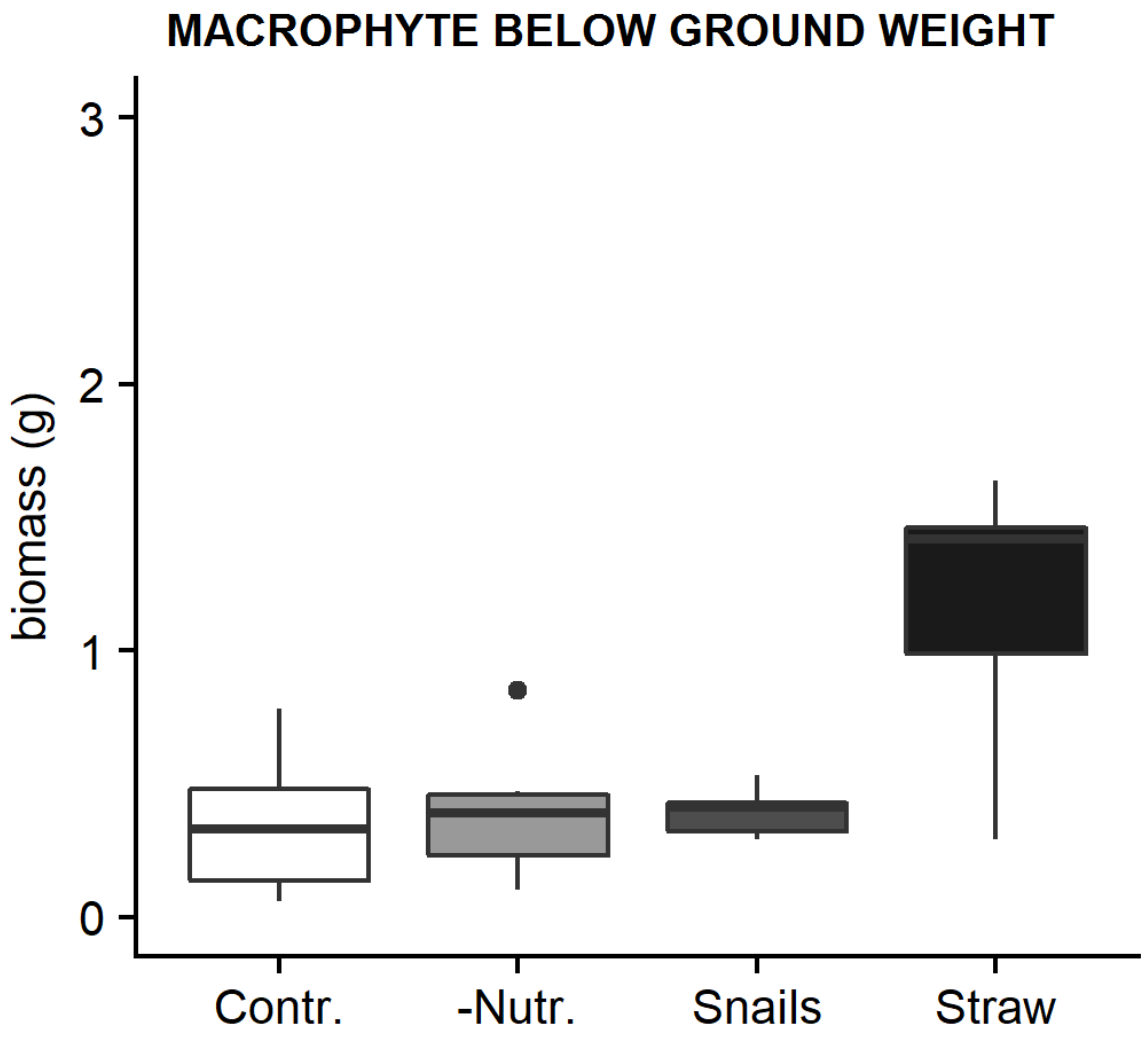
MACROPHYTE BELOW GROUND WEIGHT
biomass (g)
3
2
1
0
Contr.
-Nutr.
Snails
Straw

Figure 8.

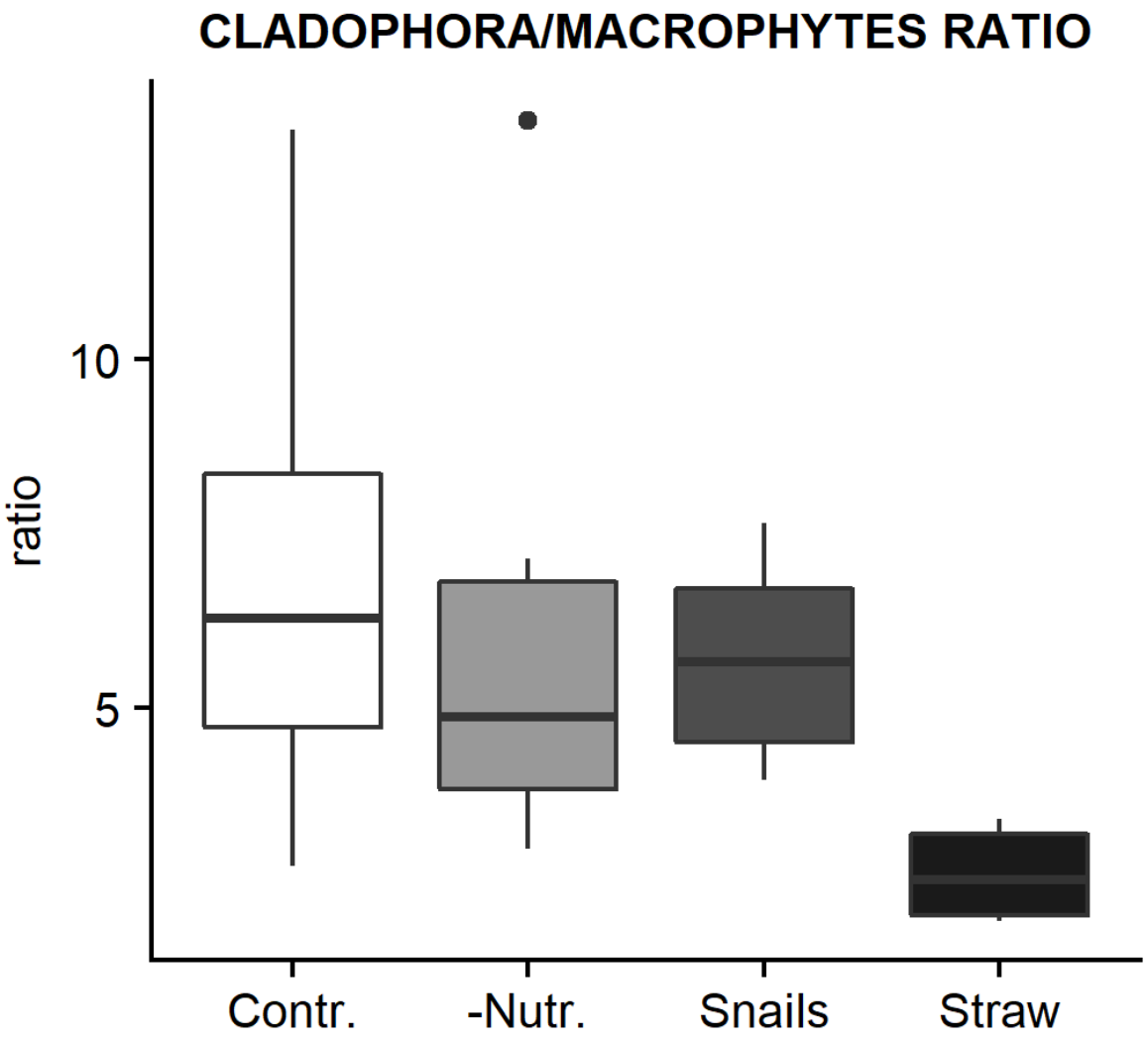
CLADOPHORA/MACROPHYTES RATIO
ratio
10
5
Contr.
-Nutr.
Snails
Straw

Figure 9.