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# MODELING NITROGEN AND PHOSPHORUS RECOVERY POTENTIAL BY *ULVA FASCIATA*

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## ABSTRACT

**Figueira, T. A., Costa, D. S., Pollery, R. C. G., Yoneshige-Valentin, Y., Enrich-Prast, A. & Oliveira, V. P. (2023). Modeling Nitrogen and Phosporus Recovery Potential by *Ulva fasciata*. Braz. J. Aquat. Sci. Technol. 27(1). eISSN 1983-9057. DOI: 10.14210/bjast.v27n1.19100.** This study investigated the nitrogen and phosphorus uptake efficiency and recovery potential by the cosmopolitan macroalga *Ulva fasciata*. An equation to estimate biomass production, as well as nitrogen and phosphorus recovery, by *Ulva* species is further proposed. Results showed that *U. fasciata* removed 99% nitrogen ($NH_4^+$-$NO_3^-$) and 22% phosphate dissolved in water. Tissue nitrogen, phosphorus, and chlorophyll-*a* increased by 73%, 20%, and 244%, respectively, demonstrating rapid assimilation of nutrients fueling *U. fasciata* growth (5.69% day$^{-1}$). The constructed equation correlating fresh weight and area showed that 0.21 and 11.35 tons.ha$^{-1}$.year$^{-1}$ of phosphorus and nitrogen could be recovered and that 3.47 tons of dry biomass could be produced. Tissue data add to the understanding of the physiological effects of nutrient availability in *U. fasciata*. The efficient removal of dissolved nitrogen and phosphorus represents an important ecosystem service with potential for the production of value-added by-products.

**Key Words:** Environmental Impact. Eutrophication. Bioremediation. Nitrogen Uptake. Phosphate Uptake. Tissue Nutrient. Aquaculture. Sustainability.

## INTRODUCTION

The high input of nitrogen and phosphorus in marine environments is the principal cause of water quality impairment in coastal zones (Reidenbach et al., 2017). Macroalgae cultivation in sites with elevated concentrations of these elements, and subsequent extraction of value-added products, is an alternative for more reliable biomass production. It returns nutrients to the technological cycle, promotes the valorization of biomass, and improves water quality. Macroalgae belonging to the genus *Ulva* (Chlorophyta) are opportunist species with a cosmopolitan distribution, known to dominate environments with nutrient conditions unfit for other species (García-Poza et al., 2022). This genus presents high nitrogen and phosphorus absorption to sustain its rapid growth and reproductive maturation. Furthermore, *Ulva* species present broad biotechnological uses, such as food and chemicals (Figueira et al., 2020). In this study, we carried out experiments supporting a proposed equation to estimate biomass production and nitrogen and phosphorus recovery by *Ulva* species.

## MATERIAL AND METHODS

Individuals of *Ulva fasciata* were collected at Prainha Beach, Arraial do Cabo - Brazil (22º57’40"S /42º01’13"W). Specimens were rinsed with local seawater and immediately transported to the Multiuser Unit of Environmental Analysis (UMAA / UFRJ). Voucher specimens were deposited in the Institute of Bioscience Herbarium at the University of São Paulo, Brazil (SPF-57877). Individuals were acclimated to laboratory conditions for seven days prior to the experiment. Healthy thalli were maintained in sterilized seawater enriched with von Stosch culture medium (Ursi and Plastino, 2001) in a temperature-controlled room at 24.0 ± 1.0 ºC and a photoperiod of 12 hours of light. Photosynthetically active radiation (PAR) was kept at 70 $\mu mol_{photons}.m^{-2}.s^{-1}$ and was provided by 40 W daylight fluorescent tubes.

To carry out this experiment, individuals (3 g) were cultivated in 3 L Erlenmeyer flasks (1 g/L) with four replicates for each treatment (enriched water and control). The enriched water treatment consisted of sterilized seawater enriched with 200 µM ammonium ($NH_4Cl$), 8µM nitrate ($NaNO_3$), and 12 µM phosphate ($Na_2.HPO_4.12H_2O$). These concentrations were chosen with reference to the mean maximum nutrient concentration after five-year monitoring of one of the points of a eutrophic bay (Valentin et al., 2018) with potential for *Ulva* cultivation. For the control treatment, *U. fasciata* individuals were cultivated in unenriched sterilized seawater (0.05 µM $NH_4^+$, 0.41 µM $NO_3$, and 0.09 µM $PO_4^{3-}$). Erlenmeyer flasks (n = 4) filled with enriched seawater without macroalgae were used as controls. Light, photoperiod, and temperature conditions were the same as those described for the acclimatization period. The experiment lasted five days and was repeated twice.

The methodology to construct an equation describing the correlation between Fresh Mass (FM, g) and Surface Area (SA, $cm^2$) was adapted from Lubsch & Timmermans (2018). Healthy fragments (n = 34) of *U. fasciata*, ranging from 0.002 to 0.085 g (0.153 to 6.391 $cm^2$), were placed on a white surface, and covered with a Petri dish to avoid folding. A ruler placed next to the fragments was used as a reference. Photographs (Cannon PowerShot G16) were taken, and the SA was calculated using the open-source software ImageJ (ImageJ, U. S. National Institutes of Health, Bethesda, MD, USA). The colored photograph was first converted into grayscale (type 8-bit) and then into pixels using the function "binary" to highlight the pigmented (dark) area and exclude any holes. The threshold routine was set to manual and adjusted for refined analysis when necessary.

Water aliquots were collected daily, filtered through GF/F glass filters, and analyzed. Ammonium, nitrate, and phosphate concentrations were measured by methods adapted from Koroleff (1970), Grasshoff & Johannsen (1972), and Murphy & Riley (1962), respectively. All analyses were performed in triplicate. Nutrient uptake ($\mu mol.g^{-1}_{DW}.h^{-1}$) and uptake efficiency (%) were calculated according to Pedersen & Borum (1997) and Tremblay-Gratton et al. (2018), respectively. Chlorophyll-*a* contents were calculated according to Lorenzen (1967). For tissue phosphorus, nitrogen, and carbon content, samples collected at the beginning and end of the experiment were dried at 50 ºC until they reached constant mass. Tissue phosphorus content was determined according to Murphy & Riley (1962). For the analysis of total nitrogen and carbon contents, a CHNS Elemental Analyzer (Flash 2000 - Thermo Scientific) with acetanilide as a reference standard was used.

All statistical analyses were performed using Statistica 10 software, considering $p < 0.05$. SGRs were analyzed by one-way ANOVA. Dissolved nutrient concentrations, chlorophyll-*a*, and tissue nutrient contents were analyzed by two-way ANOVA. The Shapiro-Wilks and Cochran tests tested the normality and homogeneity of variance assumptions, respectively. When the data failed to achieve these assumptions, log transformation was used. A posteriori Student-Newman-Keuls test (SNK) was used to establish statistical differences in all cases.

## RESULTS AND DISCUSSION

*U. fasciata* cultivated in the enriched water treatment presented the highest growth rate (5.69 ± 0.48 $\%.d^{-1}$), while SGR in the control treatment was 2-fold lower than that in the enriched treatment ($p < 0.01$, Table 1). The SGR of *U. fasciata* cultured in enriched water was also higher than that seen in studies with *U. lactuca* cultivated in oilfield wastewater and integrated with abalone or shrimp production (de Oliveira et al., 2016; Lavania-Baloo et al., 2014; Robertson-Andersson et al., 2008). The uptake efficiency of *U. fasciata* was 22.48 ± 0.9 for phosphate, 99.95 ± 0.05 for ammonium, and 100% for nitrate.

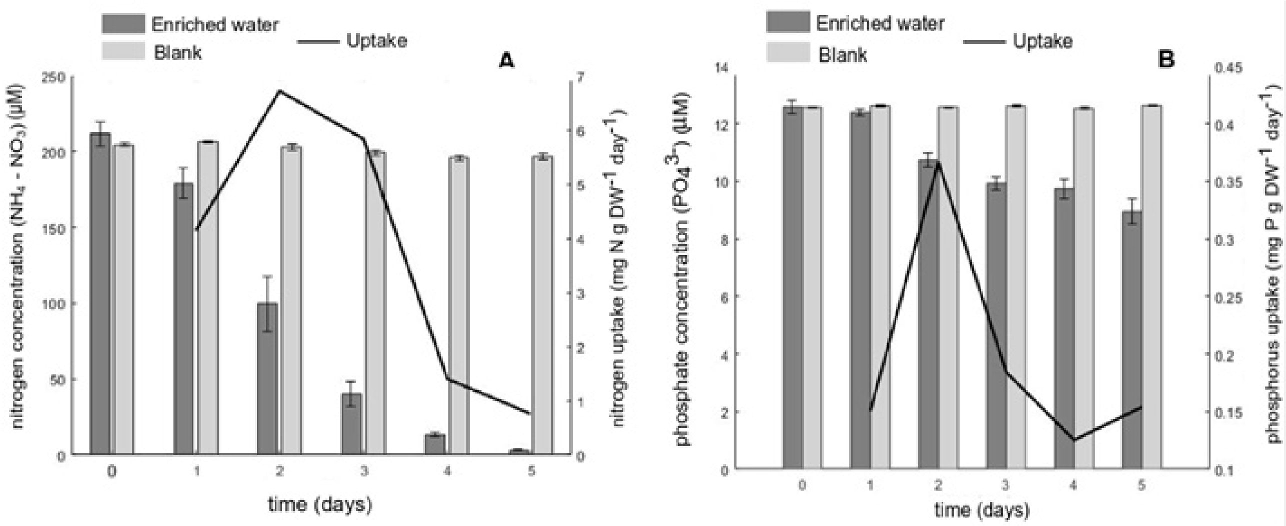
Table 1 – Chlorophyll-*a*, tissue phosphorus, nitrogen and carbon content for *U. fasciata* cultivated at two different treatments (enriched water and control). Initial (beginning of the experiment) and final state (after five days of the experiment). Data presented as mean and standard deviations. Bold values indicate significant differences according to two-way ANOVA and Newman–Keuls test (p < 0.05).

| Treatment/ Experimental period | Chl-a ($mg.g_{FW}^{-1}$) | P ($\%_{DW}^{-1}$) | N ($\%._{DW}^{-1}$) | C ($\%_{DW}^{-1}$) |
|---|---|---|---|---|
| Bioremediation | | | | |
| T = 0 day | 0.34 ± 0.07 | 0.05 ± 0.01 | 1.89 ± 0.16 | 27.07 ± 0.60 |
| T = 5 days | **1.17 ± 0.27** | **0.06 ± 0.00** | **3.27 ± 0.07** | 29.27 ± 0.71 |
| Control | | | | |
| T = 0 day | 0.34 ± 0.08 | 0.05 ± 0.01 | 2.00 ± 0.21 | 28.20 ± 1.23 |
| T = 5 days | 0.34 ± 0.03 | **0.03 ± 0.01** | 2.03 ± 0.35 | 29.73 ± 1.50 |

Nutrient uptake rates varied throughout the experiment, with higher rates on the second day for both nitrogen ($NH_4^+$) (Figure 1 (a)) and phosphorus ($PO_4^{-3}$) (Figure 1 (b)). The average uptake rates were 4.20 ± 0.43 $mg_N.g_{DW}^{-1}.d^{-1}$ and 0.25 ± 0.07 $mg_P.g_{DW}^{-1}.d^{-1}$ for nitrogen and phosphorus, respectively. In the macroalgae control treatment, nutrient concentration was below detection limits (< 0.01µM) by the first day of the experiment. The control showed no difference in nitrogen (p = 0.30) or phosphate concentrations p = 0.63) throughout the experimental period.

The nitrogen concentration used was higher than that reported in most studies. However, *U. fasciata* uptake efficiency was higher than that reported for *Ulva sp.* grown in an integrated multi-trophic system (IMTA) and cultivated in rejected water from anaerobically digested sludge (Shpigel et al., 2019). Phosphate uptake was similar to that reported for *U. lactuca* cultivated in a two-level IMTA system, either co-cultured with Pacific white shrimp or cultured in effluent discharged from an abalone tank (Nardelli et al., 2019). The uptake of nitrogen and phosphorus was highest on the second day of the experiment, followed by a sharp decrease (Figure 1 (a) and (b)). Nitrogen uptake continued to decay, while phosphorus uptake increased on the fourth day. When nitrogen pools were filled, phosphate uptake resumed. The C/N ratio (14.32) and tissue N, close to a minimum allowing for maximum growth, recorded at the start of the experiment supports the hypothesis of nitrogen as the limiting nutrient (Zollmann et al., 2021). After cultivation in enriched water, tissue N increased to a concentration similar to that found in the literature (Hernández et al., 2002).

Figure 1 – Nitrogen ($NH_4$–$NO^3$) (a) and phosphate (b) by *Ulva fasciata* over the experimental period (five days). There was no difference in the nutrient concentrations in water control treatment throughout the experiment ($p > 0.05$). Data presented as means and standard deviations ($n = 4$).

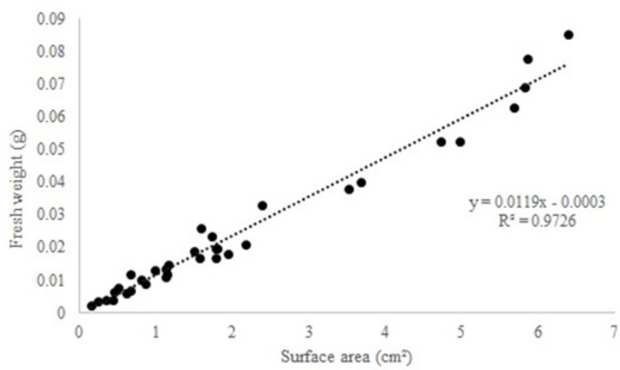



In macroalgae from the control, P content decreased by 40%, but no difference in tissue N, C, and chlorophyll-a was observed (Table 1). This decoupling between tissue phosphorus and other physiological parameters may represent a tradeoff between phosphorus reserves to sustain growth and photosynthetic capacity, as stored polyphosphates can be hydrolyzed to meet the requirements of starved macroalgae (Lee et al., 2005). Surface Area (SA) was highly correlated with Fresh Mass (FM) ($R^2 = 0.973$), and the trend line used to calculate the equivalent area versus biomass is presented in Figure 2. This correlation allowed us to construct an equation for the estimation of nutrient uptake potential over a chosen period and area (Equation 1).

$$NR = \left(1 + \frac{SGR * t}{100}\right) * \frac{AREA - 0.252}{84.03} * \frac{DM}{FM} * \frac{TN}{100} \quad \text{Equation 1}$$

Where NR is nutrient recovery (mass nutrient $area^{-1}.time^{-1}$), SGR is the specific growth rate of macroalgae, t is time, DM is the dry mass of the macroalgae, FM is fresh mass of the macroalgae, and TN is the tissue nutrient content in percentage (%). Given the results gathered and this equation, considering a year-round period and one square kilometer cultivated area, the nutrient recovery potential would be around 0.17 and 11.35 $kg.km^{-2}.year^{-1}$ of phosphorus and nitrogen, respectively. Furthermore, 347 $kg.km^{-2}.year^{-1}$ of dry biomass could be produced. In conclusion, *U. fasciata* efficiently removed dissolved nitrogen and phosphorus from the enriched water, increasing its biomass, content of tissue nutrients, and pigment. Through the constructed correlation among surface area, biomass produced, and uptake efficiency, we could estimate the nutrient recovery potential of *U. fasciata.* This is the first attempt to model nitrogen and phosphorus recovery to the technological cycle. Therefore, future studies with the cultivation of *U. fasciata* on larger scales are needed to refine the accuracy of the proposed equation.

Figure 2 – Correlation between fresh weight (g) and surface area ($cm_2$) of *U. fasciata*. Trend line: $y = 0.0119x + 0.0003$; $R^2 = 0.9726$.

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