Use of Marine Macroalgae for the Treatment of Municipal Wastewaters and Biomass Production for Animal Feed

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Abstract
Similarly to microalgal treatment systems, macroalgae are considered another promising approach for wastewater treatment, and could also ultimately provide an alternative animal food source in addition to a biofuel feedstock. Their large size and/or tendency to grow as dense floating mats or substrate-attached turfs lead to lower separation and drying costs than microalgae. In this study, the common macroalgae species of *Ulva lactuca* was used to investigate the capacity of macroalgae to treat different types of municipal wastewaters, and the feasibility of using the harvested biomass as a feed for the fruitfly *Drosophila melanogaster*, which is often used as an animal model for biological research. Real municipal wastewaters, including primary (PW), secondary (SW) and centrate wastewaters (CW), were employed. Results indicated that *U. lactuca* could successfully grow on all wastewaters studied with biomass productivities of 8.12-64.3 g DW(dry weight)/(m^2·d), which varied with wastewater type. SW was demonstrated as the most effective wastewater medium for *U. lactuca*. However, both relatively high nitrogen (92.5-98.9%) and phosphorus (64.5-88.6%) removal efficiencies were observed in all wastewaters, particularly in PW and SW, with the highest removal rates (N 24.7±0.97 and P 0.69±0.01 mg/(g DW·d)) observed in CW. The biomass composition varied with the wastewater type used for macroalgal growth. It was also found that, compared with the control, the addition of 20% washed *U. lactuca* into 80% standard fly food (w/w) led to an extended lifespan and stable body weights in flies, while the addition of 20% unwashed *U. lactuca* (w/w) led to reduced survival and body weights. This fundamental study demonstrates an effective approach for the macroalgae-based treatment of municipal wastewater and the simultaneous production of biomass for animal feed.

Keywords
wastewater; macroalgae; nutrient recovery; *Ulva lactuca*; animal feed, flies

INTRODUCTION

Algae (micro- and macroalgae) have great capacities for carbon dioxide (CO_2) and nutrient uptake, while simultaneously producing biomass for biofuel, bioenergy and bioproducts. Microalgae have been widely studied because of their high growth and nutrient uptake rates, as well as their potential as a biomass resource for value-added products such as biodiesels, animal feeds, and fertilizers (Skorupskaite et al. 2015). However, the high cost and environmental concerns (e.g. flocculants or coagulants required) associated with the microalgal harvesting stage pose challenges that could hinder the full-scale application of microalgae-based technologies, due to their small size (2-20 μm), relatively low growth densities (<1 g DW (dry weight)/ L), and electrostatic stability in dispersions (Ge et al. 2015). With comparatively similar biomass compositions and capacities to microalgae (Yun et al. 2015), macroalgae are larger and grow as dense floating mats or substrate-attached turfs (Yun et al. 2015), thereby potentially offering significant reductions in harvesting and dewatering costs relative to microalgae.

Macroalgae have been primarily employed in two fields related to wastewater bioremediation: nutrient and pollutant removal from municipal wastewaters (Ge and Champagne 2016) and removal of toxic metals from industrial wastewater (Wilde and Benemann 1993). However, most studies to date that have examined nutrient removal by macroalgae have focused on wastewaters with low nutrient concentrations (Yun et al. 2015, de Paula Silva et al. 2013), such as fish farm wastewater

with low nutrient concentrations (nitrogen 1.93-2.75 mg/L and phosphate 0.16-0.53 mg/L) (Cole et al. 2014), aquaculture wastewater (N 4.73-11.34 μmol/L, P 1.2-1.85 μmol/L) (Marinho-Soriano et al. 2009), and other surface water bodies contaminated by agricultural and stormwater runoff. Studies concerning nutrient-rich wastewater treatment using microalgae (Ge and Champagne 2016) have indicated that macroalgae could also have the potential to treat wastewaters with high nutrient concentrations; an approach still in its infancy in both the academic and industrial areas. In addition, the composition of the macroalgal biomass produced could be affected by the wastewater medium, which could influence subsequent biomass applications.

Macroalgae could be used as feedstocks for a variety of biomass applications, such as fertilizers and soil conditioners (Baghel et al. 2016), biofuels (Ross et al. 2008), and human and animal food (Garcia-Vaquero and Hayes 2016). With high levels of minerals, vitamins, proteins, carbohydrates and polyunsaturated fatty acid but low lipid content, macroalgae have been used as ingredients in food preparations across the world (Kumar et al. 2008). Macroalgae are a food source not only for marine animals such as the shore crab, sea bass, snakehead and shrimp (Hechinger et al. 2011, Valente et al. 2006), but have also been used as a provider of antibacterial agents for poultry and swine (Kulshreshtha et al. 2014, Walsh et al. 2013). However, some researchers also reported that incorporating macroalgae into the diets of chickens and ducks might have detrimental effects on their growth (Ventura et al. 1994, El-Deekx and Brikaa 2009). Hence, the effects of macroalgal biomass on animals should be studied on a case-by-case basis. When using macroalgae as an alternative to traditional animal food, several parameters must be considered (e.g., dose, pretreatment, temperature, etc.). In modern biological sciences, fruitflies are widely used as an attractive animal model because of their effectiveness as genetic tools, however, food consumption and food waste present concerns in the fly research community. Macroalgae could be investigated as a substitute for standard fly food to improve its economic and environmental viabilities, however, to the authors' knowledge, no such studies have been reported to date.

The purpose of this study is to investigate the possibility of using macroalgae (*Ulva lactuca*) for phosphorus and nitrogen recovery from municipal wastewater, and to provide a proof of principle that macroalgal cultivation could be considered as a technology for wastewater treatment and downstream biomass production for animal food. The mortality and weight of flies with *U. lactuca* in their diets will be examined to investigate whether the macroalgal biomass could be used as a partial alternative to standard fly food.

MATERIAL AND METHODS

Macroalgae and wastewater

U. lactuca was used as the model macroalgal species and was obtained from a local aquarium store. It was inoculated on Walne's medium with a salinity content of 32‰ (Zhu and Lee 1997), in a flat-plate aquarium (35×40×50 cm) in order to allow for acclimatization to laboratory conditions. The aquarium was equipped with an Orphek Atlantik Aquarium LED lighting platform, which can provide appropriate light spectra ranging from 380-440 nm and 650-670 nm for macroalgal growth. Two air pumps (Tetra Whisper, Canada) equipped with membrane filters provided aeration and mixing condition at a rate of around 200 mL/min. *U. lactuca* from the aquariums were used as the inoculum for the following experiments.

The wastewater used for the *U. lactuca* growth was collected from the Ravensview wastewater treatment plant (WWTP), with an average treatment capacity of 95,000 m^3/ d, located in Kingston, Canada. Three types of wastewaters were used for the macroalgal growth, including primary wastewater (PW), secondary wastewater (SW) and centrate wastewater (CW) collected from different sampling sites in the WWTP. The wastewater was stored in the laboratory refrigerator at 4℃

until use. The composition of the wastewater was as follows (mg/L): PW: NH_4^+-N 17.5±3.5 mg/L; NO_3^--N 0.43±0.02 mg/L; NO_2^--N 0.02±0.002 mg/L; TP 1.59±0.38 mg/L; Chemical oxygen demand (COD) 154±41 mg/L; SW: NH_4^+-N 0.35±0.05 mg/L; NO_3^--N 21.3±1.6 mg/L; NO_2^--N 0.01±0.001 mg/L; TP 0.11±0.02 mg/L; COD 24±3 mg/L; and CW: NH_4^+-N 648±57 mg/L; NO_3^--N 0.04±0.003 mg/L; NO_2^--N 0.02±0.001 mg/L; TP 24.8±2.22 mg/L; COD 477±32 mg/L.

Experimental setup of macroalage growth on wastewater

Macroalgae growth on wastewater. Jar test experiments using 250 mL Erlenmeyer flasks were performed in the laboratory. *U. lactuca* was exposed to one of three wastewaters with salinity maintained at around 32‰, respectively: (1) PW, (2) SW and (3) a series of CW (3% and 4%) diluted with deionized (DI) water. DI water alone (salinity 32‰) was used as a control treatment. All wastewaters were sterilized in an Autoclave at 120℃ for 20 min. Each treatment had 6 replicates and each flask contained a working volume of 200 mL and an initial total biomass of approximately 0.30±0.03 g fresh weight (FW) of *U. lactuca*. Aquarium air pumps (Tetra Whisper, Canada) connected to in-line filters and air diffusers were used to provide mixing to the cultures. An Orphek Atlantik Aquarium LED lighting platform was used to illuminate all flasks with a 24 h light cycle at temperatures between 24.0℃ and 27.5℃. The flask positions were changed daily to provide similar light intensity exposure to each flask. The volumes of the flasks were kept constant over the experimental period with the addition of DI water every day.

The biomass FW was centrifuged at 10,000 g for 5 min and weighed every day. Water samples were collected and then filtered through a 0.45 μm vacuum filter for NH_4^+-N, NO_3^--N, NO_2^--N and TP analyses at the beginning and end of each treatment. Three indicators were used to evaluate the wastewater treatment performance as per Equations (1-3), including nutrient removal efficiency (RE, %), treatment efficiencies (TE, %/d) and removal rates (RR, mg/(g DW/d)). The biomass productivity (BP, g DW/(m^2·d)) was calculated using Equation (4).

$$\text{RE}\ (\%) = \frac{C_0 - C_t}{C_0} \times 100\% \quad (1)$$

$$\text{TE}\ (\%\cdot d^{-1}) = \frac{C_0 - C_t}{C_0 \cdot d} \times 100\% \quad (2)$$

$$\text{RR}\ (\text{mg}\cdot \text{g}^{-1}\text{DW}\cdot d^{-1}) = \frac{C_0 - C_t}{m_t \cdot d} \quad (3)$$

$$\text{BP}\ (g\cdot DW\cdot m^{-2}\cdot d^{-1}) = \frac{m_0 - m_t}{(FW/DW)\cdot A\cdot d} \quad (4)$$

where C_0 and C_t are the nutrient concentrations on the first and final day (mg/L), *d* is the experimental time (day) , m_0 and m_t are the biomass weights on Day 0 and t (g), FW/DW is the fresh to dry weight ratio, and A is the area of the flask (m^2).

Biomass production for fly study. Following macroalgae growth experiment, SW was selected to cultivate *U. lactuca* in the aquarium under similar growth conditions to those noted previously. When the biomass increased to more than triple its initial mass, samples were taken and divided into two parts. One part was thoroughly rinsed with DI water to reduce the salt, sand and gravel until a salinity of less than 0.5‰ was reached in the rinse water (defined as "washed *U. lactuca*"). The other part was roughly rinsed with DI to remove sand and gravel (defined as "unwashed *U. lactuca*"). After cleaning, both biomass samples were dried at 55°C to a constant DW, powdered manually with a pestle and mortar, sifted through a piece of muslin with a pore size about 10 μm and stored for the fly study.

Experimental setup of fly feeding study

Flies. Parental *Drosophila melanogaster* of wildtype Canton-S strain (Bloomington *Drosophila* stock center at Indiana University, USA) were raised in 10 ml plastic vials and allowed to lay eggs on standard medium (0.01% molasses, 8.2% cornmeal, 3.4% killed yeast, 0.94% agar, 0.18% benzoic acid, 0.66% propionic acid) at room temperature 21-23°C, 60-70% humidity. A 12h/12 h light/dark cycle was provided using three light bulbs (Philips 13 W compact fluorescent energy saver) with lights on at 7 am and off at 7 pm every day. Male flies were collected within 2 days following eclosion (defined as Day 1) for the following experiments.

Preparation of different fly food sources. The effects of food on the flies were investigated starting on Day 1, in which two different food sources were provided. They involved the mixture of 80% standard fly standard medium (same component as mentioned above) and 20% washed or un-washed *U. lactuca* (w/w). The dose of 20% was selected according to the optimized dose determined for livestock and swine in previous studies (Kulshreshtha et al. 2014, Walsh et al. 2013) as well as our unpublished results. For each type of food source, at least 250 flies were raised in 10 vials (around 25 flies per vial) under the same conditions noted above. Flies were transferred into fresh food vials with the same food composition every 4 days. A control with 250 flies was included in 10 vials with standard fly food alone. Among the 10 vials with each food treatment (including control), 5 were used to monitor the lifespan and the other 5 were used to perform the body weight experiment.

Lifespan experiment. Flies from 5 replicate vials (25 flies per vial) of each food treatment (including the control) were maintained as long as feasible and the deaths of flies were recorded on Day 10, 20, 30, 40 and 50. Flies were considered to be dead when neither voluntary movement nor responses to external stimulation could be observed. The survival percentage on each recorded day was the average of the survival percentages from 5 vials with the same treatment.

Body weight experiment. The remaining 5 vials for each food treatment (including control) were used to monitor changes in body weight. The average fly body weight from 5 vials was recorded on Day 1, 10, 20, 30 and 40 using a Denver Instrument SI-234 balance (accuracy 0.0001 g).

Chemical analysis

Temperature, pH and dissolved oxygen (DO) were monitored using a microprocessor meter with corresponding probes (Fisher Scientific™ accumet™ Excel XL60). NH_4^+-N and NO_3^--N were analyzed using a Hach spectrophotometer (Method No. 8171). COD was analysed with an Hach Model DR/2010 spectrophotometer according to Standard Methods (APHA 2005). TP was measured using the Hach PhoVer 3 Method No. 8190 with acid-persulfate digestion. TN was measured using the Hach TNT Persulfate Digestion Method No. 10072. Salinity was measured using HACH Pocket Pro$^+$ Multi 1.

Statistical analysis

For all quantifications and graphs, means and standard deviations are given. For the biomass composition study, one-way ANOVA with post-hoc Turkey's test was performed to compare the difference between different wastewater cultures. For the fly study, the sample size was 5 in all experiments. Comparison of the survival percentage or body weights of washed and unwashed *U. lactuca* treated flies against control flies were performed as One-way ANOVA with post-hoc Dunnett's test. All *p* values presented were two-tailed. Statistical tests were performed with Prism version 5.0 (GraphPad Software, San Diego, CA).

RESULTS AND DISCUSSION

Bioremediation of wastewater and biomass production of *U. lactuca*

Biomass production. **Figure 1** shows the growth performance of *U. lactuca* grown on different wastewaters indicated by the FW and biomass productivities, as well as the other water quality parameters. The highest growth rate during the 6-day growth cycle was obtained in the SW treatment, followed by PW, 4-CW and 3-CW (**Figure 1(a)**) indicating that *U. lactuca* could grow well on all types of wastewaters employed although SW appeared to be the most effective growth medium with nitrate as the dominant nitrogen form. Growing on SW and PW, *U. lactuca* could achieve biomass productivities as high as 64.3±3.38 and 21.4±0.86 g DW/(m^2·d), respectively (**Figure 1(b)**), which was comparable to those (22-55 g TS DW/(m^2·d) and 37.6 ± 8.6 g DW/(m^2·d)) reported in studies about *U. lactuca* cultivated on natural seawater (Bruhn et al. 2011, Msuya and Neori 2008). The lower biomass productivities (7.75-10.4 g DW/(m^2·d)) observed in the CW treatments were still comparable with those of *Chaetomorpha linum* grown on PW, SW and a series of CW in our parallel studies (data not shown).

The pH was noted increased due to the significant macroalgal biomass production in both SW and PW treatments, whereas in 3- and 4-CW treatments pH was relatively stable compared to initial values, but was found to be higher with values above 8.0 (**Figure 1(c)**). Over the experimental period, salinities were relatively constant around 23.5-26.3‰ in all treatments and the temperatures were maintained at 23.9-25.7 ºC (**Figure 1(d) and 1(e)**).

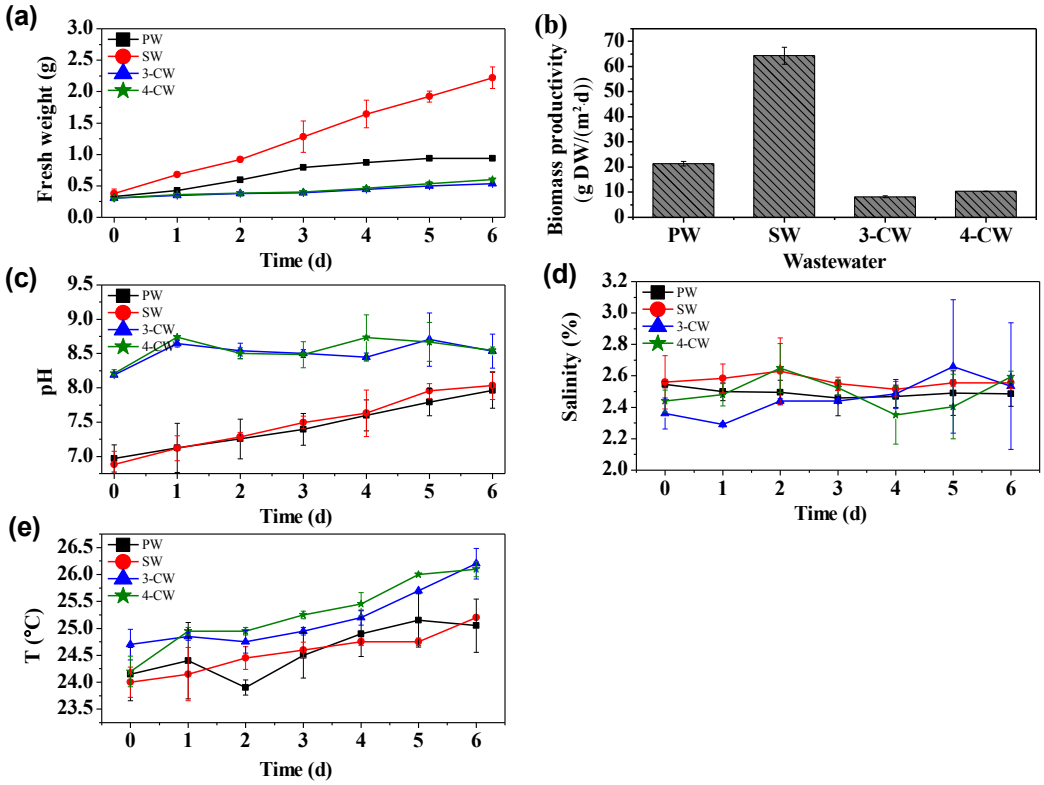

Figure 1. Comparisons of (a) biomass production (g) by fresh weight, (b) biomass productivity (g DW/(m^2·d)), (c) pH, (d) salinity (%) and (e) temperature (ºC) in flasks where *U. lactuca* were cultivated on PW, SW 3-CW and 4-CW, respectively.

These observations suggest that municipal wastewater could be used as a marine macroalgal growth medium to reduce water and nutrient requirements. However, it should be noted that the raw CW contains high concentrations of nutrient and other constituents such as heavy metals and/or free ammonia that is toxic, lipid soluble, and can traverse biological membranes in its uncharged form

under pH 8.5 (Körner et al. 2001), which could potentially inhibit macroalgal growth. As such, direct or full strength use of raw CW should be avoided as a macroalgal growth medium; corresponding pre-treatments or strategies should be established to alleviate the adverse effects and facilitate macroalgal growth on a case-by-case basis, such as the integration of CW and SW, and supplementation of CO_2 to lower pH during the macroalgae growth process.

Nutrient removal. Satisfactory nitrogen (ammonia or nitrate) removal capacities of *U. lactuca* were observed, with REs between 92.5±1.71% and 98.9±0.23% and TEs at 11.6-12.4%/d in the three types of wastewaters studied, respectively (**Table 1**), even though different growth dynamics and biomass productions were obtained as noted above. However, the nutrient removal rates used for evaluating the nutrient removal capacities per gram of biomass per day varied significantly with wastewater type. The RR of nitrogen in 3-CW was almost 24-fold greater than that observed in SW, which warrants further investigation into the nitrogen removal mechanism in macroalgae-based wastewater treatment systems. It is worth noting that the nutrient removal indicators (RE, TE and RR) calculated in **Table 1** included all nutrient losses between the influent and effluent concentrations. For example, denitrification and Anammox, as well as volatilization of ammonia would also likely contribute to nitrogen removal in addition to macroalgal uptake, although wastewater sterilization was performed prior to the experiments. Specific mechanism of alternative pathways for nitrogen removal should be further investigated.

Compared to nitrogen removal, *U. lactuca* showed lower phosphorus removal capacities particularly in CW. REs and TEs ranged between 64.5-88.6% and 8.07-11.1%/d, respectively (**Table 1**). However, the RRs of phosphorus exhibited a similar trend to that observed for nitrogen, where higher RRs were observed in CW than in PW and SW. Apart from the macroalgal metabolic assimilation process, the phosphorus in wastewaters could also be removed through struvite precipitation in the presence of phosphorus, ammonium and magnesium, under appropriate pH conditions (Huang et al. 2015, Mijangos et al. 2004). Therefore, these results suggested that the bioremediation of the municipal wastewater using *U. lactuca* could be possible and allow for simultaneous biomass production.

Table 1. Nutrient removal and treatment efficiencies and removal rates in *U.lactuca* after exposure to different types of wastewaters for 12 days. The ratio of FW to DW is 3.8.

	RE (%)		TE (%/d)		RR (mg/(g DW·d))	
Wastewater	N	P	N	P	N	P
PW	98.7±0.62	88.6±1.24	12.3±0.08	11.1±0.16	4.51±0.05	0.44±0.01
SW	98.9±0.23	77.7±14.1	12.4±0.03	9.72±1.76	1.09±0.10	0.04±0.01
3-CW	92.5±1.71	64.5±3.92	11.6±0.21	8.07±0.49	24.7±0.97	0.69±0.01
4-CW	98.8±0.34	66.8±4.47	12.4±0.05	8.34±0.56	16.8±0.34	0.38±0.07

Biomass composition. The compositions of carbon, nitrogen and phosphorus in the macroalgal biomass varied between wastewater cultures (**Figure 2**). Specifically, significant differences (p<0.05) in carbon content were observed between PW and SW, between PW and 3-CW, as well as between SW and 4-CW cultures. Similarly, nitrogen percentages in biomass were different between PW and SW, between SW and 3-CW, and between SW and 4-CW cultures. The phosphorus in the biomass cultured on 4-CW was significantly different from the biomass cultured on the other three types of wastewaters. However, the C/Ns were relatively similar ranging between 4.87±0.27 and 5.24±0.11 regardless of the wastewater cultures.

Biomass application: food supply for flies used for biological research

A number of physiological parameters could be used to examine the health of common laboratory

animal species, such as body weight, organ weight, organ volumes, blood flow speed, respiratory rate and lifespan (Davies and Morris 1993). In this study, lifespan and body weights were the two parameters selected to investigate the fly health and to determine whether the inclusion of macroalgae could be considered as an alternative to standard fly food. 20% of *U. lactuca* was chosen based on our unpublished results and the optimized dose added for livestock and swine as reported in previous studies (Kulshreshtha et al. 2014, Walsh et al. 2013).

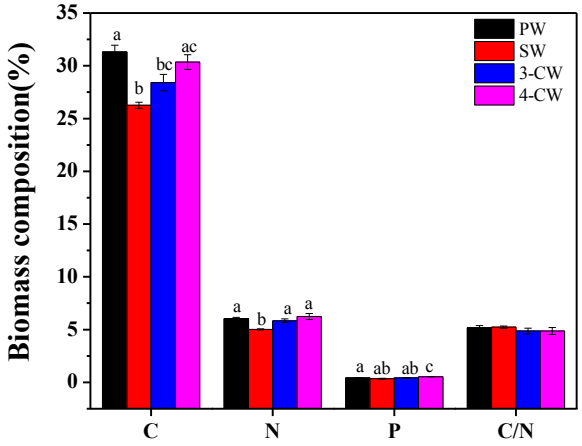

Figure 2. Biomass composition of carbon (C, %), nitrogen (N, %), phosphorus (P, %) and C/N in biomass after cultured in different wastewaters for 4 continuous growth cycles (24 days). Different small letters on the bars indicate significant difference (p<0.05).

To test the effect of food source treatment with washed and unwashed *U. lactuca*, the survival percentages and body weights were measured on specific days. Survival percentages were noted to vary with food treatment (**Figure 3**). The survival percentages of control flies and flies treated with washed *U. lactuca* did not decrease until Day 30, and significantly higher survival percentages were observed on Day 30, 40 and 50 in washed *U .lactuca* treated flies (Day 30, 40 and 50: post-hoc Dunnett's test: p<0.001). However, the survival percentage of unwashed *U. lactuca* treated flies started to decrease significantly on Day 20 and was reduced to less than half of the survival percentage of the control flies on Day 50 (Day 20: post-hoc Dunnett's test: p<0.05; Day 30, 40 and 50: post-hoc Dunnett's test: p<0.001).

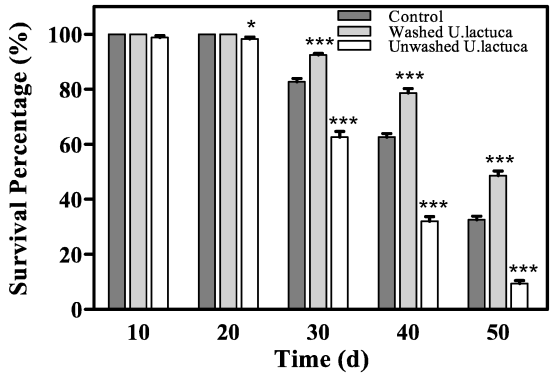

Figure 3. Survival percentage (%) of flies treated with washed or unwashed *U. lactuca* and control flies on Day 10, 20, 30, 40 and 50. Asterisks (* or ***) indicate p<0.05 or 0.001, respectively, by post-hoc Dunnett's test against control flies.

The *U. lactuca* treatment also affected the fly body weights. Because several flies had died by Day 40 and the results would be less accurate with fewer flies, the body weights were measured only until Day 40. The initial body weights of the food source treated flies were indistinguishable from the control flies on Day 1 (post-hoc Dunnett's test: p>0.05 for washed *U. lactuca*; post-hoc

Dunnett's test: p>0.05 for unwashed *U.lactuca*) (**Figure 4**). The body weights of control flies increased until Day 20 before starting to decrease. The body weights of washed *U. lactuca* treated flies, however, remained at a similar level on all tested dates, and they were significantly larger than control flies on Day 30 and 40 (Day 30 and 40: post-hoc Dunnett's test: p<0.001). On the contrary, the body weights of unwashed *U. lactuca* treated flies decreased significantly starting on Day 20 compared with control flies (Day 20 and 40: post-hoc Dunnett's test: p<0.001; Day 30: post-hoc Dunnett's test: p<0.05)

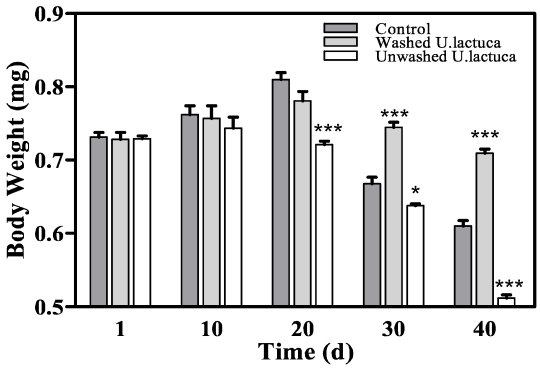

Figure 4. Body weight (mg) of flies treated with washed or unwashed *U.lactuca* and control flies on Day 1, 10, 20, 30 and 40. Asterisks (* or ***) indicate p< 0.05 or 0.001, respectively, by post-hoc Dunnett's test against control flies.

It has been reported that exclusive consumption of *U. lactuca* could have detrimental effects on blue crab (*Callinectes sapidus*) due to the production of toxic exudates (Johnson and Welsh 1985) or the insufficient nutrition provided (Belgrad and Griffen 2016). Also, less than 10% of the food consumed by blue crabs is *U. lactuca*. The current study demonstrated that 20% washed *U. lactuca* could have positive effects on flies resulting in extended lifespans and stable body weights. These findings have implications for animal laboratories, as fly food with the inclusion of 20% washed *U. lactuca* could become an alternative to the standard food. Similarly, the methanolic extracts of *Chondrus crispus*, a red macroalgae species, have been demonstrated to attenuate oxidative stress and increase the lifespan in *Caenorhabditis elegans*, probably due to the high amount of bioactive compounds in macroalgae (Sangha et al. 2013). Although the effect of macroalgae consumption on animal body weight has not been reported to date, to the authors′ knowledge, the influence of microalgae has been demonstrated, where it was indicated that the riboflavin and vitamin A in *Chlorella sp.* might be responsible for the improved growth in chicks (Combs 1952). In vertebrates including humans, dietary salt is suggested as a major contributing factor to hypertension and some other cardiovascular diseases. The results presented in this study would also suggest that the amount of salt in unwashed *U. lactuca* could also have a substantial impact on survival and body weights, which is consistent with previous studies showing the negative consequence of excess salt intake (Ollivett and McGuirk 2013, Finnie et al. 2010). The concentrations of the heavy metals in *U. lactuca*, such as arsenic, lead, mercury and cadmium, were all below the maximum recommended dietary levels (Nielsen et al. 2012), suggesting that it should be relatively safe to include *U. lactuca* in animal food. In addition, the other algae types, such as red and brown algae, have been shown to improve growth and survival better than *U. lactuca* in sea urchin (*Strongylocentrotus Drobachiensis*) (Devin et al. 2004), indicating that selection of algal type might also have an effect on fly lifespan and body weight. Although the survival percentage and body weights were improved by washing of *U. lactuca,* based on the current study, it is still unclear whether it would affect other body functions, such as motor and sensory functions, energy consumption, and nutrition metabolism. Thus the central and peripheral nervous systems and metabolic pathways of algae-treated animals, including flies, should be examined in future studies.

CONCLUSION

Effective nitrogen and phosphorus removal was observed for macroalgae cultivation in all three types of wastewaters employed in this study. The findings indicated that the growth of macroalgae may be an effective wastewater remediation technique with the added benefit of being a strong candidate for animal feed. The survival percentages and body weights of macroalgae-treated flies indicated that washed rather than unwashed *U. lactuca* could be applied as a partial substitution of traditional fly food.

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REFERENCES

Skorupskaite, V., Makareviciene, V. and Levisauskas, D. (2015) Optimization of mixotrophic cultivation of microalgae Chlorella sp. for biofuel production using response surface methodology. Algal Research 7, 45-50.

Ge, S., Agbakpe, M., Wu, Z., Kuang, L., Zhang, W. and Wang, X. (2015) Influences of Surface Coating, UV Irradiation and Magnetic Field on the Algae Removal Using Magnetite Nanoparticles. Environmental Science & Technology 49(2), 1190-1196.

Yun, J.-H., Smith, V.H. and Pate, R.C. (2015) Managing nutrients and system operations for biofuel production from freshwater macroalgae. Algal Research 11, 13-21.

Ge, S. and Champagne, P. (2016) Nutrient removal, microalgal biomass growth, harvesting and lipid yield in response to centrate wastewater loadings. Water Research 88, 604-612.

Wilde, E.W. and Benemann, J.R. (1993) Bioremoval of heavy metals by the use of microalgae. Biotechnology Advances 11(4), 781-812.

de Paula Silva, P.H., Paul, N.A., de Nys, R. and Mata, L. (2013) Enhanced production of green tide algal biomass through additional carbon supply. PloS one 8(12), e81164.

Cole, A., de Nys, R. and Paul, N. (2014) Removing constraints on the biomass production of freshwater macroalgae by manipulating water exchange to manage nutrient flux. PloS one 9(7), e101284.

Marinho-Soriano, E., Nunes, S.O., Carneiro, M.A.A. and Pereira, D.C. (2009) Nutrients' removal from aquaculture wastewater using the macroalgae Gracilaria birdiae. Biomass and Bioenergy 33(2), 327-331.

Baghel, R.S., Trivedi, N. and Reddy, C. (2016) A simple process for recovery of a stream of products from marine macroalgal biomass. Bioresource Technology 203, 160-165.

Ross, A.B., Jones, J.M., Kubacki, M.L. and Bridgeman, T. (2008) Classification of macroalgae as fuel and its thermochemical behaviour. Bioresource Technology 99(14), 6494-6504.

Garcia-Vaquero, M. and Hayes, M. (2016) Red and green macroalgae for fish and animal feed and human functional food development. Food Reviews International 32(1), 15-45.

Kumar, C.S., Ganesan, P., Suresh, P. and Bhaskar, N. (2008) Seaweeds as a source of nutritionally beneficial compounds-a review. Journal of Food Science and Technology 45(1), 1-13.

Hechinger, R.F., Lafferty, K.D., McLaughlin, J.P., Fredensborg, B.L., Huspeni, T.C., Lorda, J., Sandhu, P., Shaw, J., Torchin, M. and Whitney, K. (2011) Food webs including parasites, biomass, body sizes, and life stages for three California/Baja California estuaries. Ecology 92(3), 791-791.

Valente, L.M.P., Gouveia, A., Rema, P., Matos, J., Gomes, E.F. and Pinto, I.S. (2006) Evaluation of three seaweeds *Gracilaria bursa-pastoris*, *Ulva rigida* and *Gracilaria cornea* as dietary ingredients in European sea bass (*Dicentrarchus labrax*) juveniles. Aquaculture 252(1), 85-91.

Kulshreshtha, G., Rathgeber, B., Stratton, G., Thomas, N., Evans, F., Critchley, A., Hafting, J. and

Prithiviraj, B. (2014) Feed supplementation with red seaweeds, *Chondrus crispus* and *Sarcodiotheca gaudichaudii*, affects performance, egg quality, and gut microbiota of layer hens. Poult Sci 93(12), 2991-3001.
Walsh, A.M., Sweeney, T., O'Shea, C.J., Doyle, D.N. and O'Doherty, J.V. (2013) Effect of dietary laminarin and fucoidan on selected microbiota, intestinal morphology and immune status of the newly weaned pig. Br J Nutr 110(9), 1630-1638.
Ventura, M.R., Castañon, J.I.R. and McNab, J.M. (1994) Nutritional value of seaweed (*Ulva rigida*) for poultry. Animal Feed Science And Technology 49(1), 87-92.
El-Deekx, A.A. and Brikaa, A.M. (2009) Effect of different levels of seaweed in starter and finisher diets in pellet and mash form on performance and carcass quality of ducks. International Journal of Poultry Science 8, 1014-1021.
Zhu, C.J. and Lee, Y.K. (1997) Determination of biomass dry weight of marine microalgae. Journal Of Applied Phycology 9(2), 189-194.
APHA (2005) Standard methods for the examination of water and wastewater. American Public Health Association (APHA): Washington, DC, USA.
Bruhn, A., Dahl, J., Nielsen, H.B., Nikolaisen, L., Rasmussen, M.B., Markager, S., Olesen, B., Arias, C. and Jensen, P.D. (2011) Bioenergy potential of Ulva lactuca: Biomass yield, methane production and combustion. Bioresource Technology 102(3), 2595-2604.
Msuya, F.E. and Neori, A. (2008) Effect of water aeration and nutrient load level on biomass yield, N uptake and protein content of the seaweed Ulva lactuca cultured in seawater tanks. Journal Of Applied Phycology 20(6), 1021-1031.
Körner, S., Das, S.K., Veenstra, S. and Vermaat, J.E. (2001) The effect of pH variation at the ammonium/ammonia equilibrium in wastewater and its toxicity to Lemna gibba. Aquatic Botany 71(1), 71-78.
Huang, H., Huang, L., Zhang, Q., Jiang, Y. and Ding, L. (2015) Chlorination decomposition of struvite and recycling of its product for the removal of ammonium-nitrogen from landfill leachate. Chemosphere 136(0), 289-296.
Mijangos, F., Kamel, M., Lesmes, G. and Muraviev, D. (2004) Synthesis of struvite by ion exchange isothermal supersaturation technique. Reactive and Functional Polymers 60, 151-161.
Davies, B. and Morris, T. (1993) Physiological Parameters in Laboratory Animals and Humans. Pharmaceutical Research 10(7), 1093-1095.
Johnson, D. and Welsh, B. (1985) Detrimental effects of *Ulva lactuca* (L.) exudates and low oxygen on estuarine crab larvae. Journal Of Experimental Marine Biology And Ecology 86(1), 73-83.
Belgrad, B.A. and Griffen, B.D. (2016) The Influence of Diet Composition on Fitness of the Blue Crab, *Callinectes sapidus*. PloS one 11(1), e0145481.
Sangha, J., Fan, D., Banskota, A., Stefanova, R., Khan, W., Hafting, J., Craigie, J., Critchley, A. and Prithiviraj, B. (2013) Bioactive components of the edible strain of red alga, *Chondrus crispus*, enhance oxidative stress tolerance in *Caenorhabditis elegans*. Journal of Functional Foods 5(3), 1180-1190.
Combs, G.F (1952) Algae (*Chlorella*) as a Source of Nutrients for the Chick. Science 116(3017), 453-454.
Ollivett, T. and McGuirk, S. (2013) Salt poisoning as a cause of morbidity and mortality in neonatal dairy calves. Journal Of Veterinary Internal Medicine 27(3), 592-595.
Finnie, J., Blumbergs, P. and Williamson, M. (2010) Alzheimer type II astrocytes in the brains of pigs with salt poisoning (water deprivation/intoxication). Australian Veterinary Journal 88, 405-7.
Nielsen, M.M., Bruhn, A., Rasmussen, M.B., Olesen, B., Larsen, M.M. and Møller, H.B. (2012) Cultivation of *Ulva lactuca* with manure for simultaneous bioremediation and biomass production. Journal Of Applied Phycology 24(3), 449-458.
Devin, M., Peacock, R. and Stence, H. (2004) Development of grow-out techniques for juvenile sea urchins *Strongylocentrotus droebachiensis*. Sea urchins: fisheries and ecology, 246-254.