BIOFILTERING EFFICIENCY AND PRODUCTIVE PERFORMANCE OF MACROALGAE WITH POTENTIAL FOR INTEGRATED MULTI-TROPHIC AQUACULTURE (IMTA)*

Beatriz CASTELAR[1]; Marcelo Duarte PONTES[1,2]; Wanessa de Melo COSTA[1]; Luan César Fontes MOURA[1]; Giselle Eler DIAS[1,2]; Felipe Schwahoffer LANDUCI[1,3]; Renata Perpetuo REIS[4]

ABSTRACT

Seaweeds have many uses in industry and agriculture and many species have potential for integrated multi-trophic aquaculture (IMTA), since they are efficient in removing nutrients from water. The efficiency of *Ulva flexuosa, U. fasciata* and *Gracilaria birdiae* in removing nutrients from enriched water and their productive performance in outdoor tanks were quantified. These seaweeds (50 g; n = 5) were grown in tanks containing 50 L of eutrophic seawater, with a salinity of 30, a temperature of 28.5 ± 2.8 °C, an irradiance of 547 ± 458 µmol photons m^{-2} s^{-1} and aeration. The nutrients levels were recorded daily and when total nitrogen removal was detected, the biomass was measured. After five days of cultivation, more than 98% of NH_3 (H = 1.1; *P* = 0.56) and NO_3^- (H = 2.7; *P* = 0.25) and 62.1% of PO_4^{3-} (H = 0.0; *P* = 0.90) had been removed from the tanks. However, the mean daily growth rate (4.5 ± 2.5% day^{-1}) and productivity (3.5 ± 1.9 g m^{-2} day^{-1}) of *U. fasciata* and *G. birdiae* were higher than *U. flexuosa* (-13.6 ± 7.7% day^{-1}; -6.24 ± 2.8 g m^{-2} day^{-1}; *P*<0.01), demonstrating that microalga contamination by this species promoted high removal efficiency in the tanks, but a low productive performance. Based on these results, *U. fasciata* and *G. birdiae* show a greater potential for use in IMTA to improve water quality and produce biomass.

Keywords: *Gracilaria birdiae*; productivity; daily growth rate; nutrient removal; *Ulva fasciata*; *Ulva flexuosa*

EFICIÊNCIA BIOFILTRADORA E DESEMPENHO PRODUTIVO DE MACROALGAS COM POTENCIAL PARA AQUICULTURA MULTI-TRÓFICA INTEGRADA (AMTI)

RESUMO

As macroalgas são utilizadas em diversos setores industriais e agrícolas. Além disso, muitas espécies apresentam potencial para aquicultura multitrófica integrada (AMTI), pois são eficientes na remoção de nutrientes da água. A eficiência biofiltradora e desempenho produtivo das macroalgas *Ulva flexuosa*, *U. fasciata* e *Gracilaria birdiae* e foram quantificados em tanques *outdoor*. As algas (50 g; n = 5) foram cultivadas em tanques de 50 L, com água eutrofizada, salinidade 30 e temperatura média 28,3 °C. Os nutrientes foram monitorados diariamente e a biomassa foi mensurada assim que detectada a extinção do nitrogênio. Após cinco dias de cultivo, mais de 98% de NH_3 (H = 1,1; *P* = 0,56) e de NO_3^- (H = 2,7; *P* = 0,25) e 62,1% de PO_4^{3-} (H = 0,0; *P* = 0,9) foram removidos dos tanques. Contudo, a taxa de crescimento (% dia^{-1}) e a produtividade (g m^{-2} dia^{-1}) de *U. fasciata* e de *G. birdiae* (4,5 ± 2,5% dia^{-1}; 3,5 ± 1,9 g m^{-2} dia^{-1}) foram superiores às de *U. flexuosa* (13,6 ± 7,7% dia^{-1}; 6,2 ± 2,8; *P*<0,01), cuja contaminação por microalgas contribuiu para elevada eficiência de retirada nos tanques desta espécie, mas baixo desempenho produtivo. Desta forma, *U. fasciata* e *G. birdiae* foram indicadas para AMTI tanto para a manutenção da qualidade da água quanto para aproveitamento de biomassa.

Palavras chave: *Gracilaria birdiae*; produtividade; remoção de nutrientes; taxa de crescimento diário; *Ulva fasciata*; *Ulva flexuosa*

Original Article/Artigo Científico: Recebido em 30/11/2014 – Aprovado em 11/09/2015

[1] *Fundação Instituto de Pesca do Estado do Rio de Janeiro (FIPERJ). Av. das Américas, 31501 – CEP: 23032-050 – Rio de Janeiro – RJ – Brazil. e-mail: beatrixcastelar@gmail.com (corresponding author); wanessademelo@gmail.com; luan.fontes.moura@gmail.com*

[2] *Universidade Federal Rural do Rio de Janeiro (UFRRJ). Rod. BR 465 - km 7 – CEP: 23851-970 – Seropédica – RJ – Brazil. e-mail: marcelodpontes@gmail.com; gieler@bol.com.br*

[3] *Universidade Federal do Rio Grande (FURG). Rua do Hotel, 2 – CEP: 96210-030 – Rio Grande – RS – Brazil. e-mail: felipeslanduci@hotmail.com*

[4] *Instituto de Pesquisas Jardim Botânico do Rio de Janeiro (JBRJ). R. Pacheco Leão, 915 – CEP: 22460-030 – Rio de Janeiro – RJ – Brazil. e-mail: repereis@gmail.com*

* *Financial support: CNPq (303582/2014-16) - Bolsa de produtividade em pesquisa.*

INTRODUCTION

Global aquaculture development is the result of the development and constant improvement of new production technologies (FAO, 2014). However, the intensification of aquaculture increases waste production, as well as water and energy consumption (MARTINS *et al.*, 2011). In view of these problems caused by aquaculture, Recirculation Aquaculture Systems (RAS) represent an alternative type of cultivation that minimizes the impacts generated by intensive animal aquaculture (ROSENTHAL *et al.*, 1986). Recirculation Aquaculture Systems offer various advantages over conventional aquaculture, including a reduction of water consumption (VERDEGEM *et al.*, 2005; MARTINS *et al.*, 2010), and improved control over water quality and occurrence of disease and the ability to control escapes of animals to the environment (ZOHAR *et al.*, 2005).

One of the principles of RAS is the use of filters that enhance the development of nitrifying bacteria responsible for the oxidation of ammonia to nitrate, although these metabolic wastes accumulate in the system and can harm the development and health of animals (DAVIDSON *et al.*, 2009; GOOD *et al.*, 2009). The nitrate that accumulates in RAS can cause chronic toxicity to fish, particularly in systems that operate with low water renewal (DAVIDSON *et al.*, 2014). A contemporary method to deal with this issue is to use organisms of different trophic levels in RAS to those used in integrated multi-trophic aquaculture (IMTA). NEORI (2008) reports that seaweeds can filter these undesirable substances, and can take advantage of using their biomass for an economic purpose and can be turned into an ecologically-balanced farm.

Many species of macroalgae are effective in removing nutrients from water and show a high potential for IMTA in RAS (HAYASHI *et al.*, 2008; NEORI, 2008; MARINHO *et al.*, 2013). Several studies have proven the efficiency of macroalgae in retaining nitrogen compounds derived from IMTA (NEORI *et al.*, 2003; NEORI, 2008; COPERTINO *et al.*, 2009; CRUZ-SUÁREZ *et al.*, 2010; MARINHO *et al.*, 2013). In addition, IMTA uses various industrial applications for algae (NEORI, 2008; BIXLER and PORSE, 2010) and these are recognized as a high-quality food and have traditionally been used as food and feed (FLEURENCE, 1999; FAO, 2014), especially in Asian countries (KUMAR *et al.*, 2008). Many algae species have a suitable chemical composition for use as an ingredient in aquaculture with the potential for large-scale production (AL HAFEDH *et al.*, 2014), without being anti-nutritional (PEREIRA *et al.*, 2012).

Despite several applications, macroalgae production is in its infancy in Brazil and in the south and southeastern is limited to the exotic species, *Kappaphycus alvarezii*. Macroalgae of the genera *Ulva* and *Gracilaria* are notable for having a global occurrence (GUIRY and GUIRY, 2015) and for being suitable for intensive cultivation in tanks (RAPOSO *et al.*, 2014; CASTELAR *et al.*, 2014). In this context, the aim was to evaluate the biofiltering efficiency of three species of Brazilian macroalgae: *Ulva flexuosa, U. fasciata* and *Gracilaria birdiae* and to analyze their productive performance, with the goal of improving water quality and generating biomass for different uses.

MATERIAL AND METHODS

Specimens of *Ulva* were collected on rocky shores in southeastern Brazil (*U. flexuosa* – 23°03'42"S, 43°34'02"W; *U. fasciata* – 23°02'42"S, 43°32'03"W) and the seedlings of *G. birdiae* in northeastern Brazil (12°48'00"S, 38°14'00"W). These species were acclimated for 21 days in outdoor cylindrical tanks containing 50 L seawater, with constant aeration. Temperature and light were natural and the tanks were protected of rain by a translucent PVC (Polyvinyl Chloride) tile. The water was obtained from a tide channel adjacent to the mangrove of the Reserva Biológica de Guaratiba (Biological Reserve of Guaratiba), Rio de Janeiro, RJ, therefore, it was naturally enriched in macro-nutrients (ammonia, nitrite, nitrate and phosphate).

After acclimation, 50 g (wet mass) of each species was placed into an outdoor tank containing 50 L seawater, with an algal density per tank of 1 g L^{-1}. Five tanks were used in the experiment for each species (n = 5). Cultures were grown in the same abiotic conditions of acclimatization, of 15 days. Temperature and light were quantified hourly using data loggers

(ONSET/HOBO UA-002-64). Salinity was measured with a portable refractometer, to maintain it at 30 PSU. The concentrations of ammonia, nitrite, nitrate and phosphate were measured daily via colorimetry.

After five days, the filtering efficiency of each alga was evaluated by the formula of removal efficiency (RE), i.e., the capacity of the alga to remove nutrients from water: RE = (Initial nutrient – final nutrient)/initial nutrient x 100. After seven days of cultivation, the biomass production was measured by the daily growth rate, (DGR) (% day^{-1}) = $[(\text{final mass}/\text{initial mass})^{1/\text{time of cultivation}} - 1] \times 100$ and productivity (P) was assessed by the formula: P (g m^{-2} day^{-1}) = [(final mass - initial mass)/(area x cultivation time)]; both formulae were used by CASTELAR *et al.* (2014).

Biotic and abiotic data were tested for normality and homogeneity of the variance. Since the assumptions of parametric analyses were not met, the Kruskal-Wallis test was used to test differences in RE, DGR and P between the three algal species (ZAR, 1996). Differences between species were identified using the *post hoc* test of multiple comparisons of mean ranks for all groups. The data are presented as means ± standard deviation. The confidence interval for significance tests was 95% (P = 0.05) and the Bonferroni correction of significance was applied to ensure conservativity of analysis when unplanned comparisons among means were made. Statistica 7.0. software from StatSoft Inc. was used for analyses.

RESULTS

The water temperature and irradiance were similar among the tanks of different species during the experimental period. The temperature varied from 24.2 to 34.9 °C, with a mean of 28.5 ± 2.8 °C and the irradiance varied from 29 to 1,675 µmol photons m^{-2} s^{-1}, with a mean of 547 ± 458 µmol photons m^{-2} s^{-1}.

The three species were apparently efficient in removing nutrients, mainly nitrogen, from the water, but the green algae (*Ulva* spp.) removed nutrients more rapidly than the red alga (*G. birdiae*). After one day of cultivation, 81.9 ± 6.3% of the ammonia (NH_3) was removed in tanks containing *Ulva,* and by the third day, 89.1 ± 4.7% of the nitrate (NO_3^-) was removed. After five days of cultivation, 98.2 ± 3.7% NH_3 (H = 1.1; P = 0.56) and 98.4 ± 3.0% NO_3^- (H = 2.7; P = 0.25) and 62.1 ± 0.0% of phosphate (PO_4^{3-}) (H = 0.0; P = 0.90) were also removed by the three macroalgae (Figure 1). However, the growth performance among species was significantly different. The mean daily growth rate and mean productivity of *U. fasciata* and *G. birdiae* (4.5 ± 2.5% day^{-1}; 3.5 ± 1.9 g m^{-2} day^{-1}) were superior to those obtained for *U. flexuosa* (-13.6 ± 7.7% day^{-1}; -6.24 ± 2.8 g m^{-2} day^{-1}; P<0.01; Figure 2).

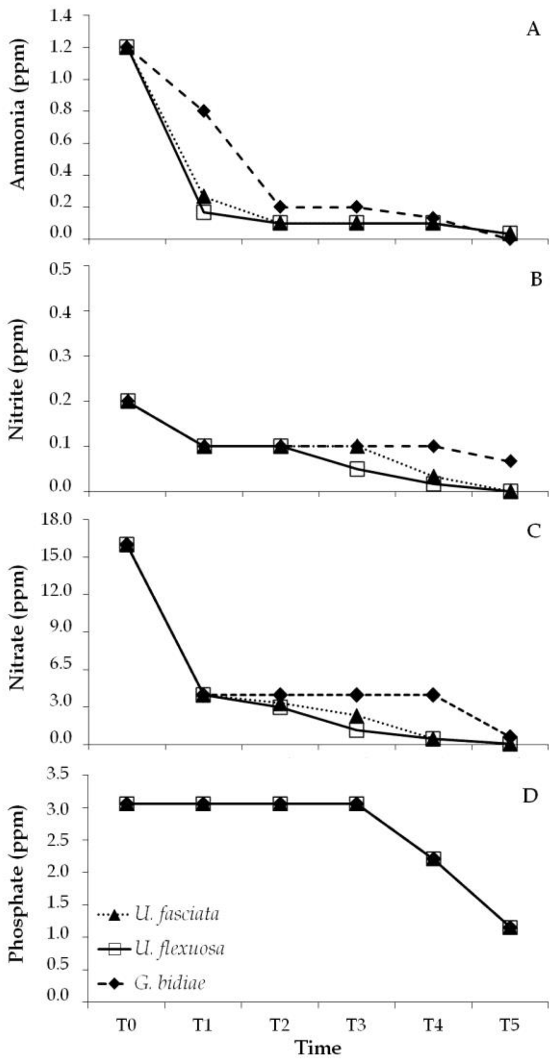

Figure 1. Nutrients concentration (ppm) throughout the experimental period (initial, T0, to five days of experiment, T5). A = ammonia (NH_3); B = Nitrite (NO_2); C = Nitrate (NO_3^-); D = Phosphate (PO_4^{3-}).

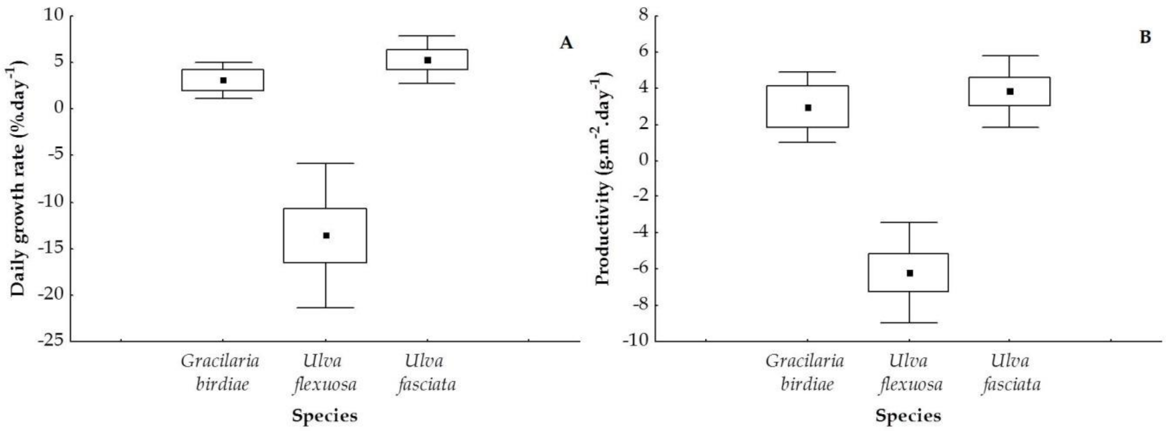

Figure 2. Mean (black squares); standard error (empty rectangles) and standard deviation (bars) of daily growth rate (A) and productivity (B) of *Gracilaria birdiae*, *Ulva flexuosa* and *Ulva fasciata* after five days of experiment.

DISCUSSION

All studied species were apparently efficient in removing nutrients from seawater, since after five days almost all the dissolved nitrogen was removed and the RE values were similar or higher than those observed in several other studies using macroalgae (Table 1). However, the RE data must be interpreted with caution. Some studies used control tanks, without macroalgae, to discriminate the amount of nutrients absorbed by macroalgae from that by other sinks (MARINHO-SORIANO *et al.*, 2011; COPERTINO *et al.*, 2009). The use of control tanks is questionable, because of differences in the dynamics between tanks with and without macroalgae. In addition to nutrient removal, macroalgae change the availability of light, affect gas exchange, and produce metabolites, which promote changes in pelagic and benthic microbiota (CRUZ-SUÁREZ *et al.*, 2010), i.e., the micro-community differs between tanks with and without macroalgae. Therefore, the nutrient removal values observed in tanks without macroalgae cannot be simply subtracted from those observed in tanks with macroalgae. Moreover, growth data complement the RE interpretation. Macroalgae are not the exclusive sink for nutrient removal in the environment, and microalgae, bacteria and the volatilization of ammonia are also efficient sinks. Despite of this, in commercial aquaculture recycling some nutrient in another product is useful.

Our results agree with the RE results for *U. fasciata* and *G. birdiae* cultivated with *Litopenaeus vannamei* (RAPOSO *et al.*, 2014) and are also similar to those of other studies that cultured *Ulva* spp. in animal effluent and in domestic effluents and found that they efficiently removed nutrients (NEORI *et al.*, 2003; YOKOYAMA and ISHIHI, 2010; OLIVEIRA *et al,*. 2012; SODE *et al.*, 2013; BALOO *et al.*, 2014). Moreover, the more rapid assimilation of ammonia than nitrate confirms the preference of *Ulva* for ammonia, as was shown to be an important function in IMTA for *U. rigida* and *U. clathrata* (= *Enteromorpha clathrata*) by ANÍBAL *et al.* (2014).

The reuse of seawater, which reduces production costs and improves water quality by macroalgae removing nutrients in IMTA, triggers a series of environmental benefits that culminate in improving the productive performance of other organisms in the system. RAPOSO *et al.* (2014) found an increase in the growth of shrimp (*Litopenaeus vannamei*) when cultured together with *U. fasciata*. In addition, seaweeds were more efficient than traditional biofilters used in bacterial RAS. When *U. lactuca* was cultured in RAS with the paua abalone (*Haliostis iris*), ammonia levels were lower than those observed using a bacterial filter (0.03 mg L^{-1} *vs.* 0.10 mg L^{-1}), and the nitrate level was undetectable and the pH was less variable, whereas the algal biomass increased to 50% (CAHILL *et al.*, 2010).

Table 1. Maximal values of nutrients (NH_3 = ammonia; NO_3^- = nitrate; PO_4^{3-} = phosphate) removal efficiency (RE; %), daily growth rate (DGR; % day^{-1}) and productivity (P; wet mass; g m^{-2} day^{-1}) of both *Ulva* and *Gracilaria* cultivated in different aquaculture systems (IA = indoor aquaria; IMTA = integrated multi-trophic aquaculture; OT = outdoor tanks; RAS = recirculation aquaculture system) by different authors.

Species	System	Nutrient	ER	DGR	P	References
Ulva fasciata	OT	NH_3 NO_3^- PO_4^{3-}	100 100 62.1	8.4	6.4	Present study
Gracilaria birdiae	OT	NH_3 NO_3^- PO_4^{3-}	100 99.2 62.1	5.2	5.1	Present study
Ulva lactuca	OT	NH_3	85.0	ND	194*	VANDERMEULEN and GORDIM, 1990
Gracilaria birdiae	IA	NH_3 NO_3^- PO_4^{3-}	34.0 100 93.5	3.6	3.7	MARINHO-SORIANO *et al.*, 2009
Ulva lactuca	IMTA + fish	NH_3 NO_3^-	93.2 24.0	10.6	267.4	AL-HAFEDH *et al.*, 2014
Gracilaria caudata	OT + *Artemia*	NH_3 NO_3^- PO_4^{3-}	30.0 70.0 NE	ND	ND	MARINHO-SORIANO *et al.*, 2011
Ulva lactuca	IA	NH_3 NO_3^- PO_4^{3-}	28.8 28.8 41.4	34.4	ND	NIELSEN *et al.*, 2012
Ulva lactuca *Gracilaria edulis*	RAS + shrimp	NH_3	40 70	4.7 4.3	ND	BALOO *et al.*, 2014
Gracilaria chilensis	IMTA + abalone	NH_3 NO_3^- PO_4^{3-}	100* 73.3* 63.1*	ND	ND	MACCHIAVELLO and BULBOA, 2014
Ulva lactuca	IMTA + abalone	NH_3 NO_3^- PO_4^{3-}	100* 80.8* 73.3*	2.6	73.6	MACCHIAVELLO and BULBOA, 2014
Ulva fasciata *Gracilaria birdiae*	IMTA + shrimp	NO_3^-	97 94	0.2 1.1	ND	RAPOSO *et al.*, 2014

**Calculated data based on cited study; ND – no data*

Although *U. fasciata* and *G. birdiae* showed lower DGR and P values than those observed in other studies, the productive performance of these species can be considered satisfactory, since they lead to an estimated mean production potential of 12 t ha^{-1} $year^{-1}$. Furthermore, considering the use of biomass in the market of high-value bioactive products (KUMAR *et al.*, 2008; YOKOYAMA and ISHIHI, 2010; ALMEIDA *et al.*, 2011), the cultivation of seaweeds in IMTA becomes even more economically interesting.

Ulva fasciata and *G. birdiae* continued to grow, even though the temperature was higher than 30 °C for 31% of the experimental period. YOKOYAMA and ISHIHI (2010) stated that biofilters adapted to high temperatures are necessary to remove the increased amount of nutrients discharged from fish farms during the warm season, when the feeding activity of fish increases, especially tropical fish (BEAMISH, 1981). Also, *Ulva* and *Gracilaria* species are widely distributed along the Brazilian coast (MOURA, 2010; PLASTINO and

OLIVEIRA, 2002) and grow in the intertidal (KOEMAN, 1985; PLASTINO and OLIVEIRA, 2002), indicating their ability to tolerate wide ranges of irradiance, temperature, salinity and even desiccation.

The low daily growth rate, low productivity and high nutrient removal observed in *U. flexuosa* tanks were attributed to microalgal contamination (Penales, Bacillariophyta), since a rapid biomass loss was observed following the covering of the thallus early during the experiment. In contrast, CASTELAR *et al.* (2014) reported high growth rates and productivity for this species in the same outdoor system, suggesting the potential of *U. flexuosa* cultivation.

CONCLUSION

Ulva fasciata and *G. birdiae* cultivated in outdoor tanks remove nutrient from seawater with a high efficiency and show satisfactory growth even in high thermal conditions, and can therefore act as a biofilter for the release of nutrients by fish. These species are suitable for RAS and IMTA to improve water quality and generate biomass. Methods to control the contamination of *U. flexuosa* cultures by microalgae must be investigated, since the feasibility of this species was previously reported.

ACKNOWLEDGMENTS

This research was supported the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), productivity grant (process nº303582/2014-16). We are grateful to Dr. Marcos Nunes, from the Bahia Federal University (UFBA) for assistance in field to collect *Gracilaria birdiae* and its identification.

REFERENCES

AL-HAFEDH, Y.S.; ALAM, A.; BUSCHMANN, A.H. 2014 Bioremediation potential, growth and biomass yield of the green seaweed, *Ulva lactuca* in an integrated marine aquaculture system at the Red Sea coast of Saudi Arabia at different stocking densities and effluent flow rates. *Reviews in Aquaculture*, *7*(3): 161-171.

ALMEIDA, C.L.F. de; FALCÃO, H. de S.; LIMA, G.R. de M.; MONTENEGRO, C. de A.; LIRA, N.S.; ATHAYDE-FILHO, P.F. de; RODRIGUES, L.C.; SOUZA, M. de F.V. de; BARBOSA-FILHO, J.M.; BATISTA, L.M. 2011 Bioactivities from Marine Algae of the Genus *Gracilaria*. *International Journal of Molecular Sciences, 12(7)*: 4550-4573.

ANÍBAL, J.; MADEIRA, H.T.; CARVALHO, L.F.; ESTEVES, E.; VEIGA-PIRES, C.; ROCHA, C. 2014 Macroalgae mitigation potential for fish aquaculture effluents: an approach coupling nitrogen uptake and metabolic pathways using *Ulva rigida* and *Enteromorpha clathrata*. *Environmental Science and Pollution Research*, *21*(23): 13324-13334.

BALOO, L.; AZMAN, S.; SAID, M.I.M.; AHMAD, F.; MOHAMAD, M. 2014 Biofiltration potential of macroalgae for ammonium removal in outdoor tank shrimp wastewater recirculation system. *Biomass and Bioenergy*, *66*(1): 103-109.

BEAMISH, F.W.H. 1981 Swimming performance and metabolic rate of three tropical fishes in relation to temperature. *Hydrobiologia, 83*(2): 245-254.

BIXLER, H.J. and PORSE, H. 2011 A decade of change in the seaweed hydrocolloids industry. *Journal of Applied Phycology*, *23*(3): 321-335.

CAHILL, P.L.; LOKMAN, M.; HURD, C.L. 2010 Keeping the water clean: seaweed biofiltration outperforms traditional bacterial biofilms in recirculating aquaculture. *Aquaculture, 306*(1-4): 153-159.

CASTELAR, B.; REIS, R.P.; CALHEIROS, A.C. dos S. 2014 *Ulva lactuca* and *U. flexuosa* (Chlorophyta, Ulvophyceae) cultivation in Brazilian tropical waters: recruitment, growth, and ulvan yield. *Journal of Applied Phycology*, *26*(5): 1989-1999.

COPERTINO, M.D.; TORMENA, T.; SEELIGER, U. 2009 Biofiltering efficiency, uptake and V assimilation rates of *Ulva clathrata* (Roth) J. Agardh (Chlorophyceae) cultivated in shrimp aquaculture waste water. *Journal of Applied Phycology*, *21*(1): 31-45.

CRUZ-SUÁREZ, E.L.; LEÓN, A.; PEÑA-RODRÍGUEZ, A.; RODRÍGUEZ-PEÑA, G.; MOLL, B.; RICQUE-MARIE, D. 2010 Shrimp/*Ulva* co-culture: A sustainable alternative to diminish the need for artificial feed and improve shrimp quality. *Aquaculture, 301*(1-4): 64–68.

DAVIDSON, J.; GOOD, C.; WELSH, C.; BRAZIL, B.; SUMMERFELT, S. 2009 Heavy metal and waste metabolite accumulation and their potential effect on rainbow trout performance in a replicated water reuse system operated at low or high system flushing rates. *Aquacultural Engineering*, *41*(2): 136-145.

DAVIDSON, J.; GOOD, C.; WELSH, C.; SUMMERFELT, S.T. 2014 Comparing the effects of high vs. low nitrate on the health, performance, and welfare of juvenile rainbow trout *Oncorhynchus mykiss* within water recirculating aquaculture systems. *Aquacultural Engineering, 59*(1): 30–40.

FAO. 2014 *The State of World Fisheries and Aquaculture*. 233 p. Available at: <http://www.fao.org/3/a-i3720e.pdf> Access on: 30 Oct. 2014.

FLEURENCE, J. 1999 Seaweed proteins: biochemical, nutritional aspects and potential uses. *Trends in Food Science & Technology, 10*(1)*:* 25-28.

GOOD, C.; DAVIDSON, J.; Welsh, C.; BRAZIL, B.; SNEKVIK, K.; SUMMERFELT, S. 2009 The impact of water exchange rate on the health and performance of rainbow trout *Oncorhynchus mykiss* in water recirculation aquaculture systems. *Aquaculture*, *294*(1): 80-85.

GUIRY, M.D. and GUIRY, G.M. 2015 AlgaeBase. Available at: <http://www.algaebase.org> Access on: 31 ago 2015.

HAYASHI, L.; YOKOYA, N. S.; OSTINI, S.; PEREIRA, R.T.L.; BRAGA, E.S.; Oliveira, E.C. 2008 Nutrients removed by *Kappaphycus alvarezii* (Rhodophyta, Solieriaceae) in integrated cultivation with fishes in recirculating water. *Aquaculture, 277*(3-4): 185-191.

KOEMAN, R.P.T. 1985 *The taxonomy of* ***Ulva*** *Linnaeus, 1753, and* ***Enteromorpha*** *Link, 1820, (Chlorophyceae) in the Netherlands*. 201p. (Doctoral Thesis. University of Groeningen). Available at: <http://dissertations.ub.rug.nl/faculties/science/1985/r.p.t.koeman/> Access on: 25 Sep. 2014.

KUMAR, C.S.; GANESAN, P.; SURESH, P.V.; BHASKAR, N. 2008 Seaweeds as a source of nutritionally beneficial compounds. A Review. *Journal of Food Science and Technology-Mysore, 45*(1): 1-13.

MACCHIAVELLO, J.; BULBOA, C. 2014 Nutrient uptake efficiency of *Gracilaria chilensis* and *Ulva lactuca* in an IMTA system with the red abalone *Haliotis rufescens*. *Latin American Journal of Aquatic Research, 42*(3): 523-533.

MARINHO-SORIANO, E.; NUNES, S.O.; CARNEIRO, M.A.A.; PEREIRA, D.C. 2009 Nutrients removal from aquaculture wastewater using the macroalgae *Gracilaria birdiae*. *Biomass and Bioenergy*, *33*(2): 327-331.

MARINHO-SORIANO, E.; AZEVEDO, C.A.A.; TRIGUEIRO, T.G.; PEREIRA, D.C.; CARNEIRO, M.A.A.; CAMARA, M.R. 2011 Bioremediation of aquaculture wastewater using macroalgae and *Artemia*. *International Biodeterioration & Biodegradation*, *65*(1): 253-257.

MARINHO, G.; NUNES C.; SOUSA-PINTO, I.; PEREIRA, R.; REMA, P.; VALENTE, L.M.P. 2013 The IMTA-cultivated Chlorophyta *Ulva* spp. as a sustainable ingredient in Nile tilapia (*Oreochromis niloticus*) diets. *Journal of Applied Phycology, 25*(5): 1359-1367.

MARTINS, C.I.M.; EDING, E.H.; VERRETH, J.A.J. 2011 Stressing fish in Recirculating Aquaculture Systems (RAS): does stress induced in one group of fish affect the feeding motivation of other fish sharing the same RAS? *Aquaculture Research*, *42*(9): 1378-1384.

MARTINS, C.I.M.; EDING, E.H.; VERDEGEM, M.C.J.; HEINSBROEK, L.T.N.; SCHNEIDER, O.; BLANCHETON, J.P.; ROQUE D'ORBCASTEL, E.; VERRETH, J.A.J. 2010 New developments in recirculating aquaculture systems in Europe: A perspective on environmental sustainability. *Aquacultural Engineering*, *43*(3): 83-93.

MOURA, C.W.N. 2010 Ulvophyceae. In: FORZZA, R.C. *Catálogo de plantas e fungos do Brasil. Vol. 1.* Rio de Janeiro: Andrea Jakobsson Estúdio, Instituto de Pesquisas Jardim Botânico do Rio de Janeiro. p.438-448.

NEORI, A. 2008 Essential role of seaweed cultivation in integrated multi-trophic aquaculture farms for global expansion of mariculture: an analysis. *Journal of Applied Phycology*, *20*(5): 567–570.

NEORI, A.; MSUYA, F.E.; SHAULI, L.; SCHUENHOFF, A.; KOPEL, F.; SHPIGEL, M.A. 2003 A novel three stage seaweed (*Ulva lactuca*) biofilter design for integrated mariculture. *Journal of Applied Phycology, 15*(6): 543–553.

NIELSEN, M.M.; BRUHN, A.; RASMUSSEN, M.B.; OLESEN, B.; LARSEN, M.M.; MOLLER, H.B.

2012 Cultivation of *Ulva lactuca* with manure for simultaneous bioremediation and biomass production. *Journal of Applied Phycology*, *24*(3): 449-458.

OLIVEIRA, V.P.; FREIRE, F.A.M.; MARINHO-SORIANO, E. 2012 Influence of depth on the growth of the seaweed *Gracilaria birdiae* (Rhodophyta) in a shrimp pond. *Brazilian Journal of Aquatic Science and Technology, 16*(1): 33-39.

PEREIRA, R.; VALENTE, L.M.P.; SOUSA-PINTO, I.; REMA, P. 2012 Apparent nutrient digestibility of seaweeds by rainbow trout (*Oncorhynchus mykiss*) and Nile tilapia (*Oreochromis niloticus*). *Algal Research*, *1*(1): 77-82.

PLASTINO, E.M. and OLIVEIRA, E.C. 2002 *Gracilaria birdiae* (Gracilariales, Rhodophyta), a new species from the tropical South American Atlantic with terete frond and deep spermatangial conceptacles. *Phycologia, 41*(4): 389-396.

RAPOSO, D.; OLIVEIRA, S.R.; AFONSO, F.; FERNANDES, F.O.; MARINHO-SORIANO, E. 2014 Performance of shrimp *Litopenaeus vannamei* and seaweeds *Gracilaria birdiae* and *Ulva fasciata* in an integrated multi-trophic aquaculture system. In: AQUACULTURE EUROPE, Trondheim, 2014. *Anais*... Trondheim: European Aquaculture Society.

ROSENTHAL, H.; CASTELL, J.D.; CHIBA, K.; FORSTER, J.R.M.; HILGE, V.; HOGENDOORN, H.; MAYO, R.D.; MUIR, J.F.; MURRAY, K.R.; PETIT, J.; WEDEMEYER, G.A.; WHEATON, F.; WICKINS, J. 1986 *Flow-through and recirculation systems*. EIFAC. 100p.

SODE, S.; BRUHN, A.; BALSBY, T.J.; LARSEN, M.M.; GOTFREDSEN,A.; RASMUSSEN, M.B. 2013 Bioremediation of reject water anaerobically digested waste water sludge with macroalgae (*Ulva lactuca*, Chlorophyta). *Bioresource Technology 146: 426-435*.

VANDERMEULEN, H. and GORDIM, H. 1990 Ammonium uptake using *Ulva* (Chlorophyta) in intensive fishpond systems: mass culture and treatment of effluent. *Journal of Applied Phycology 2*(4): 363-374.

VERDEGEM, M.C.J.; BOSMA, R.H.; VERRETH, J.A.J. 2005 Reducing water use for animal production through aquaculture. *International Journal of Water Resources Development, 22*(1): 101-113.

YOKOYAMA, H. and ISHIHI, Y. 2010 Bioindicator and biofilter function of *Ulva* spp. (Chlorophyta) for dissolved inorganic nitrogen discharged from a coastal fish farm - potential role in integrated multi-trophic aquaculture. *Aquaculture, 310*(1-2): 74-83.

ZAR, J.H. 1996 *Biostatistical analysis*. New York: Prentice Hall, Upper Saddle River. 662p.

ZOHAR, Y.; TAL, Y.; SCHREIER, H.J.; STEVEN, C.; STUBBLEFIELD, J.; PLACE, A. 2005 Commercially feasible urban recirculated aquaculture: addressing the marine sector. In: COSTA-PIERCE, B. *Urban Aquaculture*. Cambridge: CABI Publishing. p.159–171.