



IMTA with *Gracilaria vermiculophylla*: Productivity and nutrient removal performance of the seaweed in a land-based pilot scale system

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ABSTRACT

The use of ecological engineering tools for the development of a more sustainable aquaculture is crucial. In this context, seaweed based Integrated Multi-Trophic Aquaculture (IMTA) systems are being designed to mitigate the environmental problems caused by several forms of fed aquaculture. Several macroalgal species, namely some from the genus *Gracilaria*, have been shown to be efficient biofilters. *Gracilaria vermiculophylla* thrives in Ria de Aveiro lagoon, Portugal (40°38'N, 8°43'W). It has been an unexploited resource for the production of agar. A seaweed cultivation system with 1200 L tanks was installed at a sole and turbot land-based aquaculture facility to evaluate the potential of this species as the biofilter component of an IMTA system. A year round, full factorial experiment was done, testing for the influence of stocking density (3, 5 and 7 kg m⁻² (fw)), water exchange rate (100 and 200 L h⁻¹) and time of the year on *G. vermiculophylla*'s relative growth rates (RGR), productivity and nutrient removal.

G. vermiculophylla was able to maintain a good overall performance; however, results indicate that the culture conditions require adaptations throughout the year in order to attain successful productivities. In general, biomass production and nutrient removal were negatively related to the cultivation densities in the system. In the tanks seeded with 3 kg fw m⁻², the production of *G. vermiculophylla* was 0.7 ± 0.05 kg dw m⁻² month⁻¹; this biomass removed 221 ± 12.82 g m⁻² month⁻¹ of carbon and 40.54 ± 2.02 g m⁻² month⁻¹ of nitrogen (± 0.03% of the monthly fish N inputs). Temperature and light were the main environmental factors conditioning the growth and nutrient removal performance of the seaweed. With the appropriate upscaling, this pilot IMTA system is ready for implementation at fish aquaculture operations. *G. vermiculophylla* has proved to be an efficient component of land-based IMTA systems with environmental and potentially economic benefits for the fish farm.

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1. Introduction

Globally, aquaculture continues to be the fastest growing animal food-producing sector. In 2006 it had already equalled wild fisheries in the world's fish supply. Nonetheless, growth rates for aquaculture production are beginning to slow down (FAO, 2009), partially due to the increasing public concerns about aquaculture practices and fish quality. Worries about genetic modified organisms, sanitary issues but also the perception that aquaculture can harm the environment has lead to a rather negative social image of the aquaculture industry (FAO, 2009). In fact, in water downstream from aquaculture farms, changes in the levels of oxygen, suspended organic matter, inorganic nutrient, heavy metals and even drugs can be measured (Buschmann et al., 2008a; De Casabianca et al., 1997; Mendiguchía et al., 2006; Mente et al., 2006; Sanderson et al., 2008). These may negatively

impact the downstream biological communities (e.g. Holmer et al., 2008).

The use of ecological engineering tools, such as Integrated Multitrophic Aquaculture (IMTA), to convert monoculture into an ecological and thus more sustainable aquaculture is crucial (Buschmann et al., 2008a; Chopin et al., 2008; Costa-Pierce, 2010; Naylor et al., 2000). In IMTA, seaweeds assimilate the fish-excreted ammonia, phosphate and CO₂, converting them into potentially valuable biomass. With this treatment, effluents can recirculate back to the fish ponds or be discharged without endangering the environment (Chopin et al., 2001; Neori et al., 2004). The advantages of using seaweeds as the biofilter component of the IMTA systems came to light more than 30 years ago (Ryther et al., 1975) but are now becoming widely accepted (Hayashi et al., 2008; Neori et al., 2007; Ridler et al., 2007).

Thus, IMTA arises as a sustainable approach, with positive environmental and socio-economic benefits for the fed aquaculture industry (FAO, 2009; Nobre et al., 2010; Ridler et al., 2007). Its application in land-based intensive fish farms can also be a tool to increase recirculation practices and establish full recirculation

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aquaculture systems (RAS) with all its known associated benefits (Piedrahita, 2003; Wik et al., 2009). Moreover, regulations and incentives on this matter are slowly coming into effect, for example, in Europe (European Regulation N°710/2009) and USA (http://seagrant.gso.uri.edu/sustainable_seafood/ecolabeling.html).

The economic value of the biomass, besides its biofiltration efficacy, is a consideration in choosing the seaweed species to work with in IMTA systems. The cultivation of seaweeds in IMTA promotes higher productivity levels and with less variability than natural seaweed beds due to higher and more constant nutrients availability (Abreu et al., 2009; Lüning and Pang, 2003). Good examples of significant revenues for the fish aquaculture when adopting the IMTA approach already exist with *Gracilaria* (Abreu et al., 2009; Buschmann et al., 2001; Neori et al., 2000), *Ulva* (Bolton et al., 2009) and kelp species (Buschmann et al., 2010; Chopin et al., 2008).

Gracilaria is one of the world's most cultivated and valuable seaweeds (Buschmann et al., 2008b; Oliveira et al., 2000; Yarish and Pereira, 2008). Traditionally, its economic importance comes from the phycocolloid industry, being the main source of agar (Armisen, 1995; Peng et al., 2009). Several studies have successfully used *Gracilaria* species as biofilters. These were mostly developed in tanks (Buschmann et al., 1994, 1996; Chow et al., 2001; Friedlander and Levy, 1995; Hanisak, 1987; Martinez-Áragon et al., 2002; Matos et al., 2006; Neori et al., 1998, 2000), offshore systems (Abreu et al., 2009; Anderson et al., 1999; Buschmann et al., 2008c; Halling et al., 2005; Troell et al., 1997) and to a lesser extent in ponds (Haglund and Pedersen, 1993; Jones et al., 2002; Marinho-Soriano et al., 2002, 2009; Nelson et al., 2001; Shpigel and Neori, 1996). The majority of the studies achieved good results on productivity and agar content, the main destiny for the biomass. Changes in the amount and quality of this phycocolloid in algae produced with distinct nutrient conditions are also well documented (Martinez and Buschmann, 1996; Troell et al., 1997; Friedlander, 2001).

Gracilaria vermiculophylla thrives in Ria de Aveiro lagoon, Portugal (40°38'N, 8°43'W). This taxonomic classification was molecularly confirmed by Saunders (2009); until then, this species was misidentified as *G. verrucosa* and *G. bursa-pastoris* (e.g. Matos et al., 2006). It is currently the dominant species at that site (Silva et al., 2004), being present throughout the year (Abreu et al., 2010). *G. vermiculophylla* may have been exploited at this lagoon since the 70s as a component of the vegetable mixture ("moliço") which was used as a soil conditioner (Silva, 1985).

G. vermiculophylla withstands a wide range of environmental parameters and is now cosmopolitan in its occurrence (Nyberg and Wallentinus, 2009). Controlled experiments with the species revealed similar growth rates at a broad range of temperature and light conditions with no differences between the different life stages. Laboratory nitrogen uptake studies revealed high N uptake values and a preference for ammonia N sources (Abreu et al., 2010). A small scale IMTA cascade system testing the biofiltration efficiency of several macroalgae species (Matos et al., 2006) provided results indicative of a good performance of *G. vermiculophylla* (misidentified in that study as *G. bursa-pastoris*). Moreover, this species produces food grade agar (Villanueva et al., 2009) and can be used as animal protein replacement in fish feed (Matos et al., 2006; Pereira et al., 2010). All these facts make *G. vermiculophylla* a potential good candidate for the biofilter component of an IMTA system.

A review by Troell et al. (2003), listed some of the main lacks in IMTA research. These include studies at relevant scales for extrapolation or commercial implementation; experimental designs to facilitate statistical analyses and comparisons; systems designed to couple nutrient removal efficiency plus high seaweed productivity and long term studies to evaluate the temporal variability of the systems.

In this study, we hypothesized that the productivity and consequent nutrient removal performance of the seaweed biofilter are affected by its cultivation density and the water flow (e.g. nutrient load) of the system. It was expected that the optimal combination of

these factors would change throughout time due to the seasonal variation of environmental parameters. The scale under which this study was conducted was expected to provide information necessary for the implementation of a commercial scale system of this kind.

2. Methodology

2.1. The cultivation system

The *G. vermiculophylla* cultivation system was established at a land-based fish commercial intensive aquaculture farm (A. Coelho & Castro, Lda) in northern Portugal. This facility produces annually around 40 t of turbot (*Scophthalmus rhombus* (Linnaeus, 1758)), 5 t of sea bass (*Dicentrarchus labrax* Linnaeus, 1758) and 500 000 Senegalese sole juveniles (*Solea senegalensis* Kaup, 1858). Recently, the company has been moving towards a full recirculation aquaculture system (RAS), thus its collaboration in this study.

Twelve 1200 L (foot print of 1.5 m² each) white polyethylene tanks (light transparency ≈ 10%) were set to receive independent flows of water (mix of fish effluent with clean seawater) and were used to grow the seaweed (Fig. 1). The mean concentration of the nutrients in that water was 7.2 mg L⁻¹ (nitrate), 2.2 mg L⁻¹ (total ammonium) and 1.8 mg L⁻¹ (ortho-phosphate), during the 9 months that lasted the experiment. The water flow was adjusted manually for each tank and the seaweeds were kept in constant movement by air diffusers placed in the bottom of the tanks. This helped to maximize the seaweeds' exposition to light and nutrients in the water. The cleaner effluent from the seaweed tanks was re-introduced into the fish water system.

2.2. Environmental parameters

Water temperature was monitored throughout the study. For each water flow condition, 2 sensors (ONSET, Tidbit, USA) were set to continuous register the average hourly temperature. The incident sunlight was also monitored. Every week, through day cycles measurements, the number of light hours and the irradiance was registered with a spherical light sensor (Spherical Quantum Scalar Sensor Mod. QSL – 2100 Biospherical Instruments – Inc, USA). These measures were always associated with photosynthesis' monitoring



Fig. 1. System for seaweed cultivation. 12 white polyethylene tanks (1200 L; 1.5 m²). These were continuously supplied with the fish effluents and the outflow was recirculated back to the fish tanks. In detail, *Gracilaria vermiculophylla* biomass exhibiting good coloration and with no epiphytes.

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