

UNIVERSIDADE FEDERAL RURAL DE PERNAMBUCO
DEPARTAMENTO DE PESCA E AQUICULTURA
PROGRAMA DE PÓS-GRADUAÇÃO EM RECURSOS PESQUEIROS E AQUICULTURA

UTILIZAÇÃO DE OSTRAS E MACROALGAS COMO BIOFILTRO PARA EFLUENTES DE
CULTIVO DE CAMARÃO MARINHO

Irã Menezes Guimarães

Recife, PE
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Orientador: Dr. Alfredo Olivera Gálvez

Dissertação apresentada ao Programa de Pós-
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CULTIVO DE CAMARÃO MARINHO

Por: Irú Menezes Guimarães

Esta dissertação foi julgada para a obtenção do título de **Mestre em Recursos
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RESUMO

A eficiência de um sistema de tratamento integrado para melhorar a qualidade da água de viveiros de camarão foi avaliada utilizando-se processos sedimentação, filtração por ostras e absorção por macroalgas, assim como também foram avaliadas diferentes densidades de ostra e macroalga no tratamento de efluentes de sistemas autotrófico e heterotrófico de cultivo de camarão. Espécie nativa de ostra (*Crassostrea rhizophorae*) e macroalga (*Gracilaria* sp.) foram selecionadas devido a disponibilidade local e potencial para a aqüicultura no Nordeste do Brasil. A água do efluente decantou por 24 h sem aeração (primeira fase) e nas duas seguintes fases (ostra e macroalga) o efluente foi transferido para as unidades experimentais, permanecendo por 24 h em cada fase com aeração. Também foram avaliadas três densidades de ostra (0,2, 0,4 e 0,8 ostra.L⁻¹) e macroalga (2,0, 4,0 e 8,0 g.L⁻¹) no tratamento do efluente durante 48 h (24 h em cada fase). Variáveis químicas e físicas foram analisadas durante o período dos experimentos, 0 a 72 h no primeiro e 0 a 48 h no segundo. Variações nas concentrações de clorofila-a, feofitina, fósforo total, fosfato total, ortofosfato, amônia total, nitrato, nitrito, sólidos suspensos totais, sólidos suspensos orgânicos e sólidos suspensos inorgânicos mostraram que os dois organismos filtradores reduziram significativamente a concentração de diferentes poluentes no efluente de cultivo de camarão, entretanto as densidades de ostra e macroalga devem ser mais estudadas.

Palavras-chave: *Crassostrea rhizophorae*, *Gracilaria* sp., *Litopenaeus vannamei*, qualidade de água, sedimentação, densidade.

ABSTRACT

The efficiency of an integrated treatment system to improve the water quality from shrimp pond was assessed laboratory-scale by using sedimentation, oyster filtration and macroalgal absorption (trial 1), and the evaluation of oysters and macroalgae densities in effluent treatment of autotrophic and heterotrophic shrimp culture systems (trial 2). Native species of oyster (*Crassostrea rhizophorae*) and macroalgae (*Gracilaria sp.*) were selected due to their local availability and aquaculture potential in northeastern Brazil. In trial 1, the effluent water was left to settle for 24 h without aeration (first phase) and in the next two phases (oyster and macroalgae) the effluent water was drained into experimental units (and control) for 24h each with aeration. In trial 2, three densities of oyster (0.2, 0.4 and 0.8 oyster.L⁻¹) and macroalgae (2.0, 4.0 and 8.0 g.L⁻¹) were assessed during 48 h to treat effluent water (24 h for each phase). Chemical and physical variables were measured during experiments periods (0 to 72 h for trial 1 and 0 to 48 h for tria 2). Variations in the concentration of chlorophyll a, pheophytin, total phosphorus, total phosphate, orthophosphate, total ammonia, nitrate, nitrite, total suspended solids, organic suspended solids and inorganic suspended solids showed that the two biological filters reduced significantly the concentration of the different pollutants in the shrimp effluent, however oyster and macroalgae densities should be more studied.

Keywords: *Crassostrea rhizophorae*, *Gracilaria sp.*, *Litopenaeus vannamei*, water quality, settling, density.

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1 INTRODUÇÃO

A carcinicultura brasileira cresceu aceleradamente na última década e início desta com o cultivo da espécie exótica *Litopenaeus vannamei* principalmente na região Nordeste, e considerada uma das principais atividades em alguns estados. Em 1995, a produção era de 2.007 t, aumentando para 90.190 t em 2003 e caindo para 65.000 t em 2006 (FAO, 2008).

Entretanto, esse crescimento da carcinicultura foi desordenado e insustentável, acarretando na degradação do ambiente de cultivo, aparecimento de patologias e queda na produção. A expansão da área de produção e o aumento das densidades de cultivo acarretou na discussão sobre os impactos ambientais que a atividade pode gerar. Um de seus focos era a descarga de efluentes não tratados diretamente nos estuários e rios, o que poderia contribuir com a eutrofização do meio (ZIEMANN et al., 1992; HOPKINS et al., 1993; PAEZ-OSUNA et al., 1998). Autores como Currie (1994) e Nascimento (1998) alertavam sobre os processos de transformação dos recursos naturais e a produção de efluentes que poderiam chegar a prejudicar a própria atividade.

Os sistemas de cultivo empregados nas fazendas de camarão são considerados semi-fechados baseados no manejo da água, onde esta é bombeada para o sistema, utilizada nos viveiros e depois descartada de volta ao meio sem reaproveitá-la (WHEATON, 1982). As renovações de água que necessitam os viveiros de camarões estão relacionadas à capacidade de carga dos mesmos, devido à redução da qualidade da água provocada pelo excesso de matéria orgânica e metabólitos.

A água dos efluentes normalmente tem concentrações mais altas de nutrientes dissolvidos e partículas suspensas que a água de abastecimento (PHILLIP et al., 1993; BRIGGS e FUNGE-SMITH, 1994; MCINTOSH e FITZSIMMONS, 2003). O cultivo de animais aquáticos produz uma grande quantidade de nutrientes ao ambiente na forma de

resíduos metabólicos (BEVERIDGE, 1996). Kautsky e Folke (1989) enfatizam que apenas 25% dos nutrientes adicionados como alimento é incorporado pelos animais, enquanto que o restante permanece no ambiente, podendo causar impactos. Os referidos autores também mencionam que nos nutrientes contidos no alimento, 60% do nitrogênio (N) e 11% do fósforo (P) são lançados diretamente na água pela excreção dos camarões e pela dissolução dos *pellets*, e finalmente, só 25% do N e 23% do P são absorvidos pelos camarões.

O descarte desses efluentes, comprovadamente ricos em nutrientes, além de provocar um impacto ambiental nas áreas estuarinas, caracteriza-se como um desperdício de energia que poderia estar sendo utilizada na produção de biomassa. A aplicação de sistemas de tratamento de efluentes com organismos biorremediadores, principalmente animais filtradores e plantas é uma alternativa interessante para contribuir com a sustentabilidade da produção de camarão marinho.

A ostra do mangue, *Crassostrea rhizophorae*, e a macroalga *Gracilaria* sp. são recursos bastante explorados pelas populações locais ao longo da costa nordestina do Brasil (MARINHO-SORIANO et al. 2002; OLIVERA et al. 2006). Recentemente, o cultivo da *C. rhizophorae* vem sendo desenvolvido nos estuários e em canais de efluentes das fazendas de camarão marinho *Litopaneus vannamei* como uma alternativa à pesca artesanal e por pequenos carcinicultores (OLIVERA et al., 2006). Já houve experiências de cultivo de *Gracilaria* sp. em efluentes do cultivo de *L. vannamei* em fazendas do Nordeste (MARINHO-SORIANO et al., 2002) e mais recentemente, tem sido considerada um substituto parcial para a ração industrializada usada na alimentação do camarão (MARINHO-SORIANO et al., 2007).

2 REVISÃO DE LITERATURA

2.1 Sistemas de cultivo de camarão marinho

Os sistemas de cultivo de camarão marinho podem ser classificados de acordo com a dinâmica dos microrganismos presentes no ecossistema do viveiro, os quais são fotossintetizantes como as microalgas que dependem da radiação solar para seu crescimento, ou não fotossintetizante como determinados grupos de bactérias, ditas heterotróficas por não possuírem a capacidade de sintetizar seu próprio alimento, ou seja, que necessitam para sua nutrição da presença de matéria orgânica no ambiente (SILVA e SOUZA, 1998). Uma vez que esses organismos são a base desse ecossistema, é importante considerar os conceitos de sistemas de cultivo autotrófico e heterotrófico em fazendas de camarão.

Os cultivos convencionais baseiam-se na utilização de microalgas como um dos meios de manutenção da qualidade de água nos viveiros, sendo considerados sistemas fotoautotrófico (HOPKINS et al., 1996; AVNIMELECH et al., 1994; EBELING et al., 2006; CRAB et al., 2007). O manejo desses viveiros implica em técnicas de fertilização para induzir o crescimento da população fitoplanctônica, controle da biomassa algal e renovações constantes de água, sendo o mais empregado nas fazendas de carcinicultura no Brasil. Algumas desvantagens desse tipo de sistema são as variações diárias nos níveis de oxigênio dissolvido, pH e amônia, como também aumento excessivo da biomassa de microalgas (BURFORD et al., 2003), quando não há um manejo adequado.

O sistema de cultivo heterotrófico diferencia-se do cultivo autotrófico pela ausência de fertilização para o crescimento de microalgas e pela inoculação de bactérias através de probióticos com o intuito de diminuir a taxa de conversão alimentar e melhorar a qualidade de água do viveiro e do efluente. Esse sistema tende a ser mais estáveis que os sistemas tipicamente dominados por fitoplâncton. A produção heterotrófica não é diretamente

dependente de luz e, portanto não tem sua produtividade limitada por esta. Em viveiros de cultivo heterotrófico, componentes orgânicos servem como fonte de energia e podem estar disponíveis tanto na coluna d'água quanto no fundo do viveiro (SCHROEDER, 1978). Esse tipo de sistema vem sendo estudado e estimulado por alguns pesquisadores com o intuito de aumentar a produtividade nas fazendas (MOSS, 2002; BURFORD et al., 2003; BURFORD et al., 2004; WASIELESKY et al., 2006).

Os principais impactos gerados sobre a qualidade da água em sistemas heterotróficos descritos por Ebeling et al. (2006) são a produção muito maior de biomassa bacteriana quando comparado com a biomassa algal em cultivos autotróficos e conseqüente incremento de sólidos suspensos, aumento da produção de dióxido de carbono devido ao baixo consumo da alcalinidade como fonte de carbono, e a não produção de nitrito e nitrato.

2.2 Tratamento de efluentes

Os impactos ambientais decorrentes da expansão das áreas de cultivo de camarão e o aumento das densidades de cultivo tem sido bastante discutidos, principalmente os efeitos da liberação de efluentes não tratados na elevação da carga de nutrientes e sedimentos nos ambientes costeiros (WANG, 1990; MACINTOSH e PHILLIPS, 1992; ZIEMANN et al., 1992; HOPKINS et al., 1993; BRIGGS e FUNGE-SMITH, 1994; PAEZ-OSUNA et al., 1998; TROTT e ALONGI, 2000).

O cultivo de animais aquáticos produz e libera grandes quantidades de resíduos metabólicos ao ambiente (BEVERIDGE, 1996). A água do efluente normalmente apresenta altas concentrações de nutrientes dissolvidos e partículas suspensas em comparação com a água de abastecimento dos viveiros de cultivo de camarão (PHILLIP et al., 1993; BRIGGS e FUNGE-SMITH, 1994; MCINTOSH e FITZSIMMONS, 2003), possuindo grande quantidade de energia que poderia ser revertida em biomassa.

A utilização de organismos biorremediadores é uma forma de explorar o uso dos efluentes da carcinicultura como fonte de energia e ao mesmo tempo reduzir descargas ao ambiente, através do cultivo de ostras e macroalgas (MARINHO-SORIANO et al., 2002; JONES et al., 2002). A combinação desses organismos promove a remoção partículas suspensas e nutrientes dissolvidos na água (SHPIGEL, 1993).

Estudos demonstram que macroalgas são capazes de tratar o efluente proveniente da criação de animais de maneira eficiente (QIAN, et al., 1996; TROELL et al., 1999; NELSON et al., 2001; JONES et al., 2001). Em um sistema experimental de cultivo integrado de peixe, abalone e macroalga, esta última foi capaz de reduzir em 67% a carga de amônia (NEORI et al., 2000). Além de reduzirem os níveis de nitrogênio e fósforo, macroalgas *Gracilaria* sp. foram cultivadas com relativo sucesso em efluentes de fazendas de camarão na região Nordeste do Brasil (MARINHO-SORIANO et al., 2002).

As ostras podem melhorar a qualidade de água de efluentes de viveiros de camarão significativamente (WANG e JAKOB, 1991; HOPKINS et al., 1993; JONES e PRESTON, 1999; JONES et al., 2001). O uso de bivalves como ostras, mexilhões ou mariscos como biofiltros naturais pode ser uma maneira efetiva de remoção de pequenas partículas em suspensão dos efluentes (WANG, 1990; HOPKINS et al., 1993) e o componente orgânico dos efluentes dos viveiros de camarão provêm uma rica fonte de alimentação para os bivalves (NEWELL e JORDAN, 1983).

Quando se utiliza somente ostras no tratamento de efluentes, pode haver um aumento aparente na concentração de nutrientes na água, mas devido ao seu hábito alimentar que não depende de ração (fonte externa de alimento) em contraste com o cultivo de camarão, a liberação desses nutrientes não altera a quantidade de nutrientes preexistente. Ocorre uma redução de 25% através do plâncton consumido, enquanto que

30% se sedimentam como fezes e 45% são dissolvidos na água (KAUTSKY E FOLKE, 1989).

Um sistema de tratamento de efluentes utilizando ostras da espécie *Saccostrea commercialis* com peso úmido de 40 g, numa densidade de 1,6 ostras/L, e macroalgas da espécie *Gracilaria edulis* numa densidade de 20 g/L, foi testado obtendo uma redução substancial nos nutrientes, sólidos suspensos, fitoplâncton e carga bacteriana no efluente (JONES et al., 2001).

3 ARTIGO A SER SUBMETIDO PARA PUBLICAÇÃO

3.1 ARTIGO 1 (Experimental Integrated Treatment System to Shrimp Pond Effluent)

EXPERIMENTAL INTEGRATED TREATMENT SYSTEM TO SHRIMP POND EFFLUENT

SISTEMA EXPERIMENTAL DE TRATAMENTO INTEGRADO PARA EFLUENTES DE VIVEIROS DE CAMARÃO

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ABSTRACT

The efficiency of an integrated treatment system to improve the water quality from shrimp pond was assessed laboratory-scale by using sedimentation, oyster filtration and macroalgal absorption (trial 1), and the evaluation of oysters and macroalgae densities in effluent treatment of autotrophic and heterotrophic shrimp culture systems (trial 2). Native species of oyster (*Crassostrea rhizophorae*) and macroalgae (*Gracilaria sp.*) were selected due to their local availability and aquaculture potential in northeastern Brazil. In trial 1, the effluent water was left to settle for 24 h without aeration (first phase) and in the next two phases (oyster and macroalgae) the effluent water was drained into experimental units (and control) for 24h each with aeration. In trial 2, three densities of oyster (0.2, 0.4 and 0.8 oyster.L⁻¹) and macroalgae (2.0, 4.0 and 8.0 g.L⁻¹) were assessed during 48 h to treat effluent water (24 h for each phase). Chemical and physical variables were measured during experiments periods (0 to 72 h for trial 1 and 0 to 48 h for tria 2). Variations in the concentration of chlorophyll *a*, pheophytin, total phosphorus, total phosphate, orthophosphate, total ammonia, nitrate, nitrite, total suspended solids, organic suspended solids and inorganic suspended solids showed that the two biological filters reduced significantly the concentration of the different pollutants in the shrimp effluent, however oyster and macroalgae densities should be more studied.

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Keywords: *Crassostrea rhizophorae*, *Gracilaria* sp., *Litopenaeus vannamei*, water quality, settling, density.

RESUMO

A eficiência de um sistema de tratamento integrado para melhorar a qualidade da água de viveiros de camarão foi avaliada utilizando-se processos sedimentação, filtração por ostras e absorção por macroalgas, assim como também foram avaliadas diferentes densidades de ostra e macroalga no tratamento de efluentes de sistemas autotrófico e heterotrófico de cultivo de camarão. Espécie nativa de ostra (*Crassostrea rhizophorae*) e macroalga (*Gracilaria* sp.) foram selecionadas devido a disponibilidade local e potencial para a aquicultura no Nordeste do Brasil. A água do efluente decantou por 24 h sem aeração (primeira fase) e nas duas seguintes fases (ostra e macroalga) o efluente foi transferido para as unidades experimentais, permanecendo por 24 h em cada fase e com aeração. Também foram avaliadas três densidades de ostra (0,2, 0,4 e 0,8 ostra.L⁻¹) e macroalga (2,0, 4,0 e 8,0 g.L⁻¹) no tratamento do efluente durante 48 h (24 h em cada fase). Variáveis químicas e físicas foram analisadas durante o período dos experimentos, 0 a 72 h no primeiro e 0 a 48 h no segundo. Variações nas concentrações de clorofila-*a*, feofitina, fósforo total, fosfato total, ortofosfato, amônia total, nitrato, nitrito, sólidos suspensos totais, sólidos suspensos orgânicos e sólidos suspensos inorgânicos mostraram que os dois organismos filtradores reduziram significativamente a concentração de diferentes poluentes no efluente de cultivo de camarão, entretanto as densidades de ostra e macroalga devem ser mais estudadas.

Palavras-chave: *Crassostrea rhizophorae*, *Gracilaria* sp., *Litopenaeus vannamei*, qualidade de água, sedimentação, densidade.

INTRODUCTION

Brazilian shrimp aquaculture production had an expressive increment with the culture of the white shrimp *Litopenaeus vannamei*, which increased from 2.007 t in 1995 up to 60.190 t in 2003 (FAO, 2008). However, that raise was unplanned and not sustainable, and had caused the culture environment degradation, occurrence of diseases and consequently a decline in production.

Environmental impacts from the expansion of shrimp farms and increasing of culture densities has been hardly discussed, mainly the effects of no treated effluent to elevate sediment and nutrient loadings in coastal environments (WANG, 1990; MACINTOSH and PHILLIPS, 1992; ZIEMANN *et al.*, 1992; HOPKINS *et al.*, 1993; BRIGGS and FUNGE-SMITH, 1994; PAEZ-OSUNA *et al.*, 1998; TROTT and ALONGI, 2000). Some authors had alerted about the transformation processes of the natural resources and effluent production by shrimp industry could negatively affect itself (CURRIE, 1994; NASCIMENTO, 1998).

Aquatic animal culture produces and releases great amount of metabolic residues to the environment (BEVERIDGE, 1996). Effluent water often presents higher dissolved nutrient concentrations and suspended particles than affluent water (PHILLIP *et al.*, 1993; BRIGGS and FUNGE-SMITH, 1994; MCINTOSH and FITZSIMMONS, 2003), and its discharge is a waste of energy, that could be used to produce biomass. The culture of oysters and macroalgae is a way to make use of that energy and to reduce the discharges (JONES *et al.*, 2002; MARINHO-SORIANO *et al.*, 2002).

Mangrove oyster *Crassostrea rhizophorae* and macroalgae *Gracilaria* sp. are stocks very exploited at the Brazilian northeast coast (MARINHO-SORIANO *et al.*, 2002; OLIVERA *et al.*, 2006). More recently *C. rhizophorae* (OLIVERA *et al.*, 2006) and *Gracilaria* sp. (MARINHO-SORIANO *et al.*, 2002; MARINHO-SORIANO *et al.*, 2007) have been cultured also in shrimp farm effluent.

Studies demonstrated that macroalgae can efficiently treat effluent water from animal production systems, reducing nitrogen and phosphorus compounds (QIAN, *et al.*, 1996; TROELL *et al.*, 1999; NEORI *et al.*, 2000; NELSON *et al.*, 2001; JONES *et al.*, 2001; MARINHO-SORIANO *et al.*, 2002). Oysters, as others filter feeding mollusks, can improve water quality in shrimp ponds, as they effectively remove small suspended particles from effluents and the organic fraction provides a rich food source (WANG, 1990; WANG and JAKOB, 1991; HOPKINS *et al.*, 1993; JONES and PRESTON, 1999; JONES *et al.*, 2001; NEWELL and JORDAN, 1983).

In the present study, an integrated treatment system was assessed in laboratory-scale to improve the water quality from *L. vannamei* pond culture by using *C. rhizophorae* and *Gracilaria* sp.. These native species were selected due to their local availability and aquaculture potential in northeastern Brazil.

MATERIAL AND METHODS

Trial 1: System efficiency

Oysters *C. rhizophorae* were collected at Rio Formoso estuary, PE, (8°40'S/35°06'W) and macroalgae *Gracilaria sp.* were harvested from natural beds in Itamaracá, PE (7°45'S/34°49'W). Shrimp pond effluent water was collected after 60 days of commercial culture from a *Litopenaeus vannamei* pond (3 ha; 30 shrimp.m⁻²).

The experimental design consisted of two treatments, a biological treatment with native oyster *C. rhizophorae* and the red macroalgae *Gracilaria sp.* and other by control treatment without oysters and macroalgae. Experiment was carried out under controlled temperature (28 to 30°C) and photoperiod (12:12).

The experiment was processed in three different phases. The first phase (settling) was common for both treatments, when the effluent water was resuspended and left to settle for 24 h in the collection drum (70 L) without aeration. In the second phase (oysters) this water was drained into 10 L experimental units (three for each treatment) with aeration for 24 h. In the biological treatment of this phase, 17 oysters with a mean length of 3.20 cm and 5.88 g of mean total weight were placed in each experimental unit (1.7 oyster.L⁻¹). For the last phase (macroalgae), the water from the previous phase was drained into 5 L experimental units (3 for each treatment) with aeration for 24h. The biological treatment of this phase was arranged by including 10 g of the red macroalgae in each experimental unit (2.0 g.L⁻¹). Chemical variables were measured before (0h) and after (24h) the settling phase, and each 6 hours during the oyster (24-48h) and macroalgae (48-72h) phases.

Trial 2: Densities evaluation

Wild mangrove oysters *C. rhizophorae* were collected from estuaries in north coast of Pernambuco State, Brazil. Oysters presented 58.22 g mean total weight, 4.97 g mean dry weight and 8.27 cm mean length. The red macroalgae *Gracilaria sp.* were collected in an experimental farm at Pau Amarelo beach, Pernambuco, Brazil. The effluent water came from two experimental culture of *Litopenaeus vannamei* in laboratory, an autotrophic and an heterotrophic culture system. Shrimp were reared during four weeks in a 50 L polyethylene tank with closed flow-through system (salinity of 30). In autotrophic and heterotrophic systems, shrimps were fed with commercial food,

This experimental treatment system had two phases: first 24 h with oyster filtration and more 24 h with macroalgae filtration. The effluent from each culture system filled 12

rectangular tanks with 10 L of water. First, three densities of oyster (0.2, 0.4 and 0.8 oyster.L⁻¹) were arranged into the tanks with aeration and no additional food (3 repetitions by density). Then, oyster were removed and three stock densities of macroalgae (2.0, 4.0 and 8.0 g.L⁻¹) were added into the tanks with aeration and photoperiod of 12:12 (3 repetitions by density) (Table 1). Water quality was monitored in the beginning of the experiment and each 8 h.

Table 1 - Experimental design of trial 2.

Treatment	Oyster (oyster.L ⁻¹)	Macroalgae (g.L ⁻¹)
Control	0	0
T1	0.2	2.0
T2	0.4	4.0
T3	0.8	8.0

Water analysis

Dissolved oxygen, oxygen saturation, temperature, pH, conductivity and salinity were measured with a Multi Probe System YSI Model 556. Water samples were collected to laboratorial analyzes of total ammonia (KOROLEFF, 1976), nitrite (GOLTERMAN *et al.*, 1978), nitrate (MACKERETH *et al.*, 1978), orthophosphate, total phosphate and total phosphorus (A.P.H.A., 1995), chlorophyll-*a* and pheophytin (NUSCH, 1980), total suspended solids (TSS), organic suspended solids (OSS) and inorganic suspended solids (ISS) (A.P.H.A., 1995).

Statistical analysis

Differences between treatments were tested using analysis of variance (ANOVA) with time as a repeated measurement and Tukey's test for multiple comparisons of means at a significance level of 0.05.

RESULTS

Trial 1

Temperature, oxygen and pH

Data of temperature, dissolved oxygen concentration and pH were pooled for each treatment as they did not vary significantly within experimental phases (oyster and macroalgae) (Table 2).

Table 2 - Mean values of temperature, dissolved oxygen and pH in the control and biological treatments of shrimp pond effluent (Trial 1).

Variable	Control	Biological
Temperature (°C)	27.39±1.11 ^a	27.33±1.13 ^a
D.O. (mg.L ⁻¹)	4.47±1.40 ^a	4.52±1.34 ^a
pH	8.04±0.13 ^a	7.96±0.11 ^a

Nitrogen compounds

Nitrate concentration increased from 0.009 up to 0.024 mg.L⁻¹ during the first 24 h (settling). During oysters filtration, the nitrate concentration continued rising and reached 0.0475 mg.L⁻¹ during the third phase (macroalgae). Overall the mean values for nitrate were significantly higher during the last two phases of the biological treatment (Figure 1a).

The concentration of nitrite was almost zero during settling phase and then increased sharply up to 0.012 mg.L⁻¹ during the two phases of biological treatment (Figure 1b). However, this increase did not occur in the control treatment, which showed statistically lower values compared to the biological treatment (Figure 1b).

In the first two phases the total ammonia nitrogen concentration (0.029 mg.L⁻¹) followed a similar pattern observed for nitrite in both treatments (Figures 1b and 1c), reaching 0.021 and 0.268 mg.L⁻¹ in the control and biological treatments after 48 h, respectively. However, during the last 24 h the macroalgal absorption (biological treatment) reduced the total ammonia concentration significantly to 0.046 mg.L⁻¹ (Figure 1c).

Phosphorus compounds

Overall concentrations of phosphorus compounds showed wide variations during the experimental period, but the biological treatment was more efficient in reducing these compounds than the control treatment. Final concentrations of total phosphorus, total phosphate and orthophosphate in the effluent water were lower than the initial concentrations after the biological treatment (Figure 1d, 1e and 1f).

In the biological treatment, total phosphorus concentration after the effluent treatment dropped to only 1.41% of the initial concentration, but total phosphate and orthophosphate concentrations showed dramatic reductions of 14.71 and 10.89%, respectively.

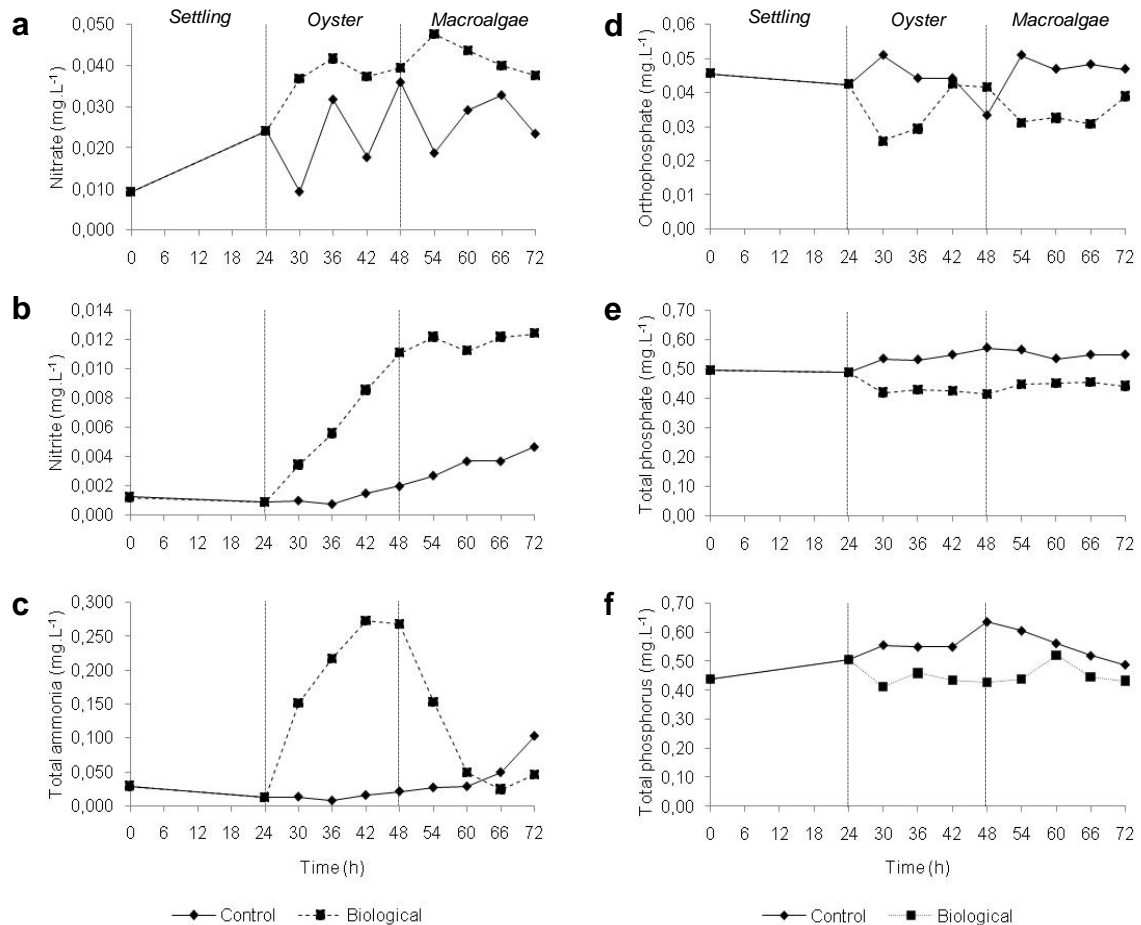


Figure 1 - Concentration of nitrate (a), nitrite (b) and total ammonia (c), orthophosphate (d), total phosphate (e) and total phosphorus (f) during sedimentation, and oyster filtration and macroalgae filtration of shrimp pond effluent in trial 1.

Suspended Solids

The total suspended solids concentration during settling phase did not differ from the initial amount (0.0720 g.L⁻¹). During the oysters and macroalgae phases at biological treatment, significantly lower values of total suspended solids were observed compared to the control. The final concentration of total suspended solids in the biological treatment (0.0653 g.L⁻¹) represented a decrease of 9.72% relative to the initial amount, whereas the control treatment this concentration increased from 0.0732 g.L⁻¹ to 0.0828 g.L⁻¹ (Figure 2a).

The organic suspended solids concentration was slightly reduced during the settling phase, but the biological treatment further reduced this concentration by 41.85% relative to the initial amount. Mean values of organic suspended solids were significantly lower in oyster and macroalgae phases in the biological treatment (Figure 2c).

During settlement, the inorganic suspended solids concentration increased from 0.0532 to 0.0595 g.L⁻¹. Consistent with the organic solids, the concentration of inorganic suspended solids were lower in the biological treatment phases, resulting in a reduction of 8.52% (Figure 2b).

Chlorophyll-a

The chlorophyll-*a* concentration dropped markedly in both treatments during the settling phase without aeration. This concentration continued decreasing during the oyster phase (biological: 0.0104 to 0.0037 µg.L⁻¹) and control treatment (0.0104 to 0.0069 µg.L⁻¹). However, there was an increase during the last 24h in the control treatment (to 0.0134 µg.L⁻¹), while the biological treatment (macroalgae) further reduced the final concentration by 90% relative to the initial concentration (Figure 2d).

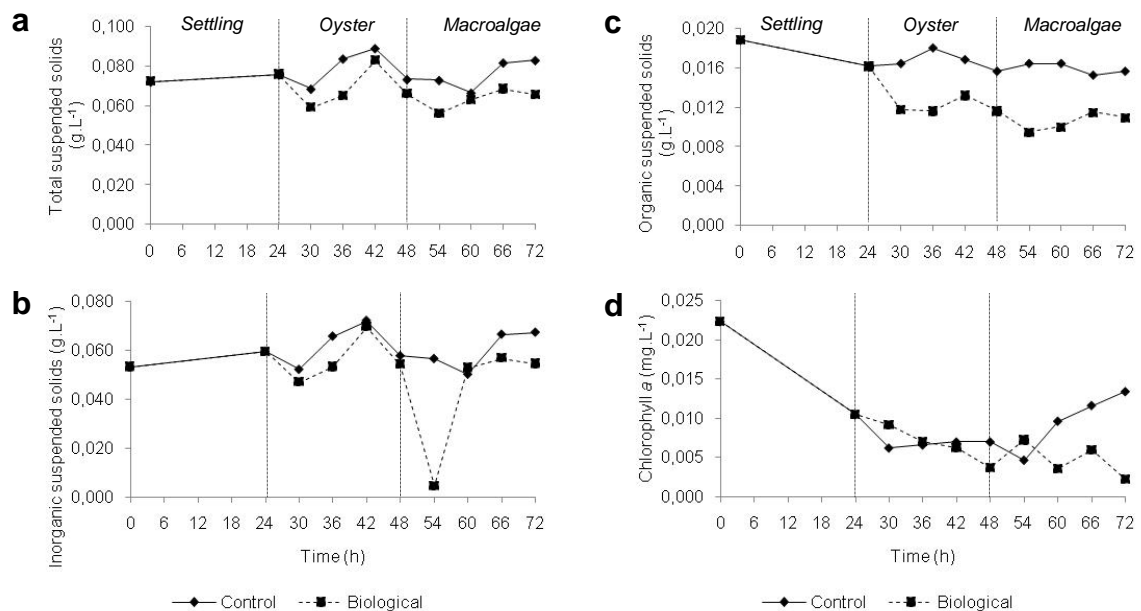


Figure 2 - Concentration of total suspended solids (a), inorganic suspended solids (b), organic suspended solids (c) and of chlorophyll-*a* (d) during sedimentation, and oyster filtration and macroalgae filtration of shrimp pond effluent in trial 1.

Trial 2

Temperature, oxygen and pH

During Trial 2, temperature and dissolved oxygen concentration did not differ among treatments in both effluent sources (autotrophic and heterotrophic culture systems) (Table 3). The pH presented the same pattern in autotrophic and heterotrophic effluent treatment, with a decreasing tendency toward higher densities.

Table 3 – Means values of temperature, dissolved oxygen (D.O.) and pH in autotrophic and heterotrophic effluent treatment (Trial 2).

Variable	Control	T2	T4	T8
<i>Autotrophic:</i>				
Temperature (°C)	27.29±1.46 ^a	27.16±1.43 ^a	27.37±1.46 ^a	27.42±1.73 ^a
D.O. (mg.L ⁻¹)	5.15±0.30 ^a	5.09±0.32 ^a	5.04±0.29 ^a	4.94±0.35 ^a
pH	7.92±0.02 ^a	7.87±0.05 ^{ab}	7.85±0.07 ^{bc}	7.81±0.12 ^c
<i>Heterotrophic:</i>				
Temperature (°C)	26.81±1.85 ^a	26.73 ±1.81 ^a	26.95±1.86 ^a	26.77 ±1.92 ^a
D.O. (mg.L ⁻¹)	5.38±0.37 ^a	5.32±0.37 ^a	5.29±0.36 ^a	5.29±0.39 ^a
pH	8.17±0.02 ^a	8.14±0.02 ^b	8.13±0.02 ^b	8.12±0.03 ^b

Nitrogen compounds

In heterotrophic effluent treatment, nitrogen compounds levels did not differ among treatments after oyster and macroalgae filtration phases. However, differences were found when the period of treatment was considered (0 to 48 h). Nitrate concentration presented differences among times, decreasing from 25.676 to 21.787 mg.L⁻¹ in 24 h and to 15.628 mg.L⁻¹ in 48 h, a reduction about 39% (Figure 3a). Nitrite concentration showed differences between time 0 h and the other sample times, with a reduction from 0.107 to 0.082 mg.L⁻¹ (Figure 3b). Total ammonia concentration had a similar statistical pattern observed for nitrite, reaching undetected levels after 24 h (Figure 3c).

In autotrophic effluent treatment, nitrate and total ammonia concentration did not present differences among Control, T1 and T2 treatments in relation to densities and over the time (Figures 3d and 3f). Nevertheless, the treatment with highest densities (T3) differed to the others after macroalgae phase (48 h), where total ammonia increased from 0.052 mg.L⁻¹ in

time 24 h to 0.218 mg.L⁻¹, just as nitrate concentration raised from 3.045 to 5.376 mg.L⁻¹. During the experiment, nitrite concentration in the Control did not vary, but in T3 it was higher than in other treatments and increased significantly over the time (Figure 3e). Nitrite level in T1 and T2 differed to Control only after macroalgae filtration phase (48 h).

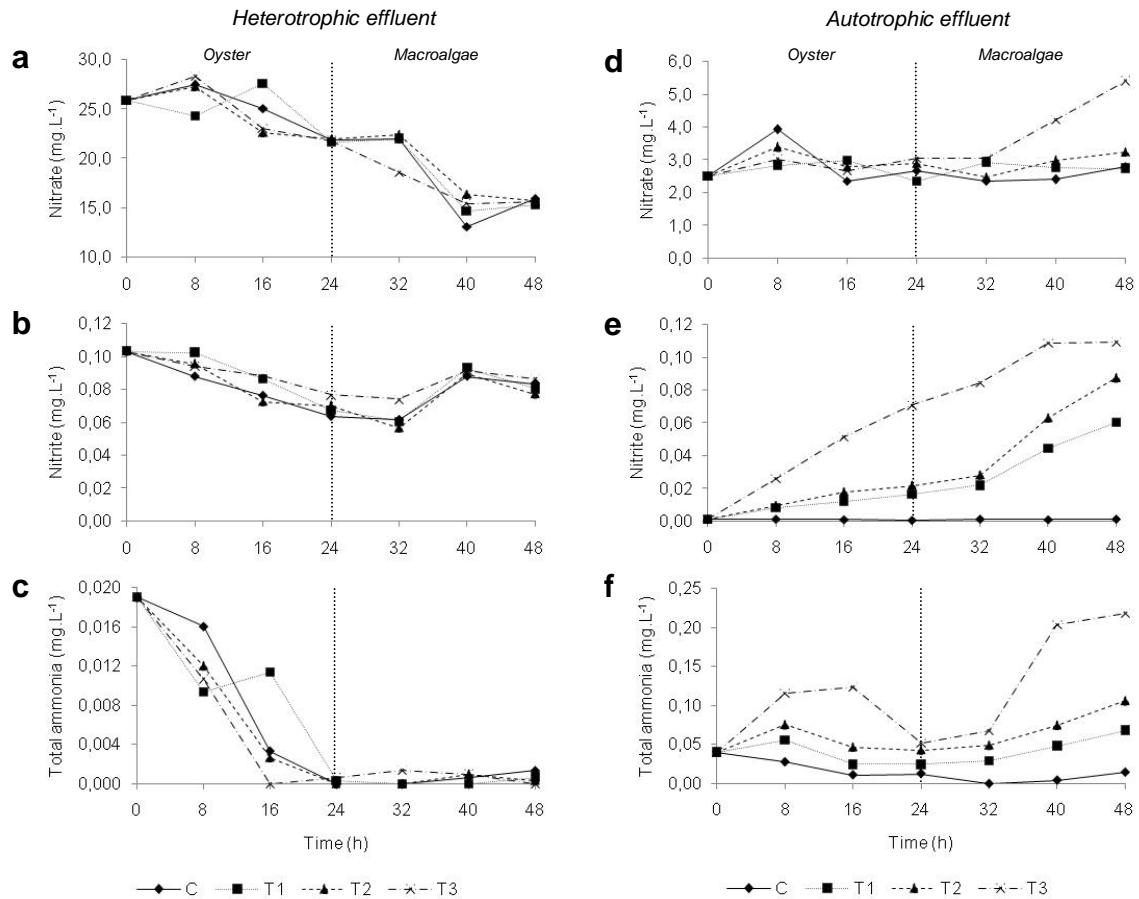


Figure 3 - Concentration of nitrate (a, d), nitrite (b, e) and total ammonia (c, f) during integrated treatment of effluent from autotrophic and heterotrophic shrimp culture system in trial 2.

Phosphorus compounds

In heterotrophic effluent treatment, phosphorus compounds presented no differences among control and the three combinations of oyster and macroalgae densities (Figures 4a and 4b). Nevertheless, orthophosphate concentration presented differences over the time, unlike total phosphorus concentration. Orthophosphate concentration was significantly lower in the end of the trial, reaching 1.929 mg.L⁻¹ with a reduction of 8.6% (Figure 4b).

In autotrophic effluent treatment, concentrations of orthophosphate in the treatments increased after oyster (24 h) and macroalgae (48 h) phases (Figure 4c). Significant differences

among the treatments were only observed after the last phase, when Control presented the lowest concentration (1.739 mg.L^{-1}) and T1 (1.913 mg.L^{-1}) was similar to T2 (2.016 mg.L^{-1}), which was similar to T3 (2.141 mg.L^{-1}). Total phosphorus concentrations had a reduction after oyster phase, but increased after macroalgae phase (Figure 4d). Total phosphorus levels did not present differences among treatments.

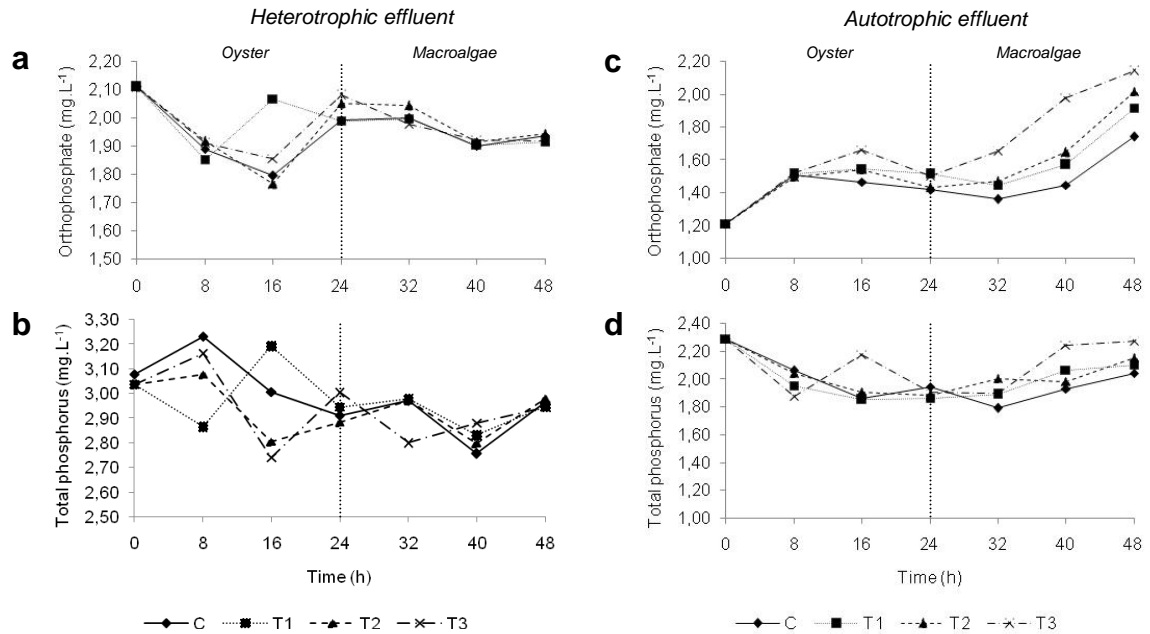


Figure 4 - Concentration of orthophosphate (a, c) and total phosphorus (b, d) during integrated treatment of effluent from autotrophic and heterotrophic shrimp culture system in trial 2.

Chlorophyll-a and Pheophytin

The concentrations of chlorophyll-*a* and pheophytin were not significant different among treatments and over the time in heterotrophic effluent (Figure 5a and 5b), as observed for chlorophyll-*a* in autotrophic effluent for T2 and T3 (Figure 5c). However, chlorophyll-*a* concentration decreased significantly in the first 16 h from 0.011 to 0.001 mg.L^{-1} for Control and T1 in autotrophic effluent. These treatments also showed a reduction in pheophytin concentration about 91% in 16 h (Control) and 78% in 24 h (T1) (Figure 4d).

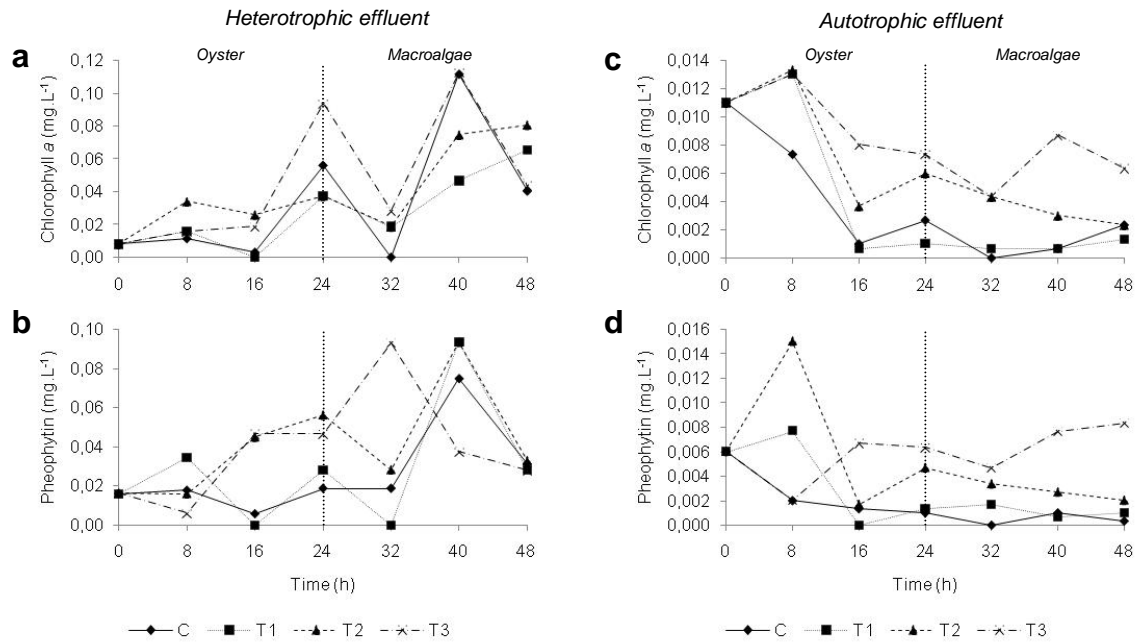


Figure 5 - Concentration of chlorophyll-*a* (a, c) and pheophytin (b, e) during integrated treatment of effluent from autotrophic and heterotrophic shrimp culture system in trial 2.

DISCUSSION

Sedimentation ponds are recommended in shrimp farms to reduce the discharge of suspended particles in coastal environments. Notwithstanding, the settlement characteristics of the effluent waters is likely to vary depending on several factors including the pond soil type, quality of receiving waters and management practices (ZIEMANN *et al.*, 1992). The lack of significant settlement of TSS in this study was probably related with its higher proportion of fine particles of inorganic solids (about 73%) than organic matter. In accordance, ZIEMANN *et al.* (1992) argued that higher proportions of inorganic matter (72%) are usually found in the effluent from earthen shrimp ponds. Previous studies showed that significant proportions of TSS is removed by settlement, but the remained suspended organic matter (e.g. phytoplankton, bacteria and detritus from shrimp feed) is more efficiently removed by the oysters (JONES and PRESTON, 1999; JONES *et al.*, 2001).

Under laboratory scale, the sedimentation of *Marsupenaeus japonicus* pond effluent effectively reduced the concentration of chlorophyll *a* (72%) (JONES *et al.*, 2001). A reduction of 53% only for chlorophyll *a* was recorded in Trial 1 during the settling phase (24 h) and 76% for chlorophyll *a* and 91% for pheophytin in Trial 2 (autotrophic) during first 16 h in the Control treatment, which operated as a settlement tank. The present study also confirmed previous findings that around 60% of the chlorophyll *a* was removed by the settlement under

still-water (non-flow) condition and the remained phytoplankton was removed by the oyster filtration (JONES and PRESTON, 1999). Nevertheless, JONES *et al.* (2002) argued that flow-through systems could improve the removal of chlorophyll *a* by oysters as the phytoplankton remains in suspension.

The use of bivalves in fish or shrimp culture has been applied to improve water quality by removing particulate organic matter and to enhance the economical value of aquaculture in land-based systems (SHPIGEL *et al.*, 1993; BODVIN *et al.*, 1996; SANDIFER and HOPKINS, 1996). Filtration by oysters effectively reduced TSS concentration in previous studies (KINNE *et al.*, 2000; JONES *et al.*, 2001; JONES *et al.*, 2002), but in the present study this reduction was probably shadowed due to the high concentration of inorganic solids remained in suspension. During the filtration, oysters showed preferential selection of organic over inorganic particles (NEWELL and JORDAN, 1983). This feeding behavior was supported in this study, as oysters removed a larger fraction of OSS (28%) compared to ISS (9%). It is suggested that the decrease in the chlorophyll *a* concentration during the same period (65%) due to phytoplankton consumption, contributed significantly to the OSS reduction. Oyster biofiltration also accounted for most of the decrease chlorophyll *a* in previous studies (KINNE *et al.*, 2000; JONES *et al.*, 2001).

Phosphorus in natural waters is usually found in the form of phosphates (PO_4^{3-}), which can be in inorganic (including orthophosphates) or organic forms (organically-bound phosphates). Organic phosphate is formed primarily by biological processes and contributed to effluent water by excretion and food residues. In the present study in Trial 1, increases of PO_4^{3-} and total phosphorus in the control treatment were probably related to the sediment release, whereas oyster filtration efficiently removed these phosphorus compounds at a greater rate than their inputs from the sediment and excretion (JONES *et al.*, 2001).

In autotrophic Trial 2, filtration by oyster and macroalgae didn't interfered on total phosphorus, the reduction in the first 24 h can be associated to settlement of organic particles, and the increase after this time could be explained by decomposition of organic matter settled. In this same trial, orthophosphate increased since the beginning of the experiment probably due mineralization of organic matter. In heterotrophic effluent treatment (Trial 2), orthophosphate concentration was reduced sharply in first 16 h probably due bacterial uptake, since aerobic heterotrophic bacteria convert nitrogen and phosphorus into bacterial biomass (EBELING *et al.*, 2006; SCHNEIDER *et al.*, 2007), and slightly raised until 48 h probably due organic matter contribution by oysters and macroalgae.

Nitrogen is often considered a limiting factor in marine ecosystems (DAY *et al.*, 1989) and its discharge from aquaculture in large amounts can create unhealthy eutrophication in natural coastal waters (COSTA-PIERCE, 1996; HOPKINS *et al.*, 1995a,b; WU, 1995). Although this problem was intensified in our integrated treatment system by the excretion of the oysters, following macroalgal biofiltration decreased significantly the concentration of total ammonia. Macroalgae can assimilate high quantities of dissolved organic and inorganic nutrients, usually with ammonia preference (D'ELIA and DeBOER, 1978; RYTHER *et al.*, 1981; SCHUENHOFF *et al.*, 2003; VERGARA *et al.*, 1993). Several *Gracilaria* species quickly assimilated ammonia from aquaculture effluent, including *Gracilaria edulis* (JONES *et al.*, 1996; JONES *et al.*, 2001), *Gracilaria parvispora* (GLENN *et al.*, 1999) and *Gracilaria conferta* (NEORI *et al.*, 1998). The potential of ammonia uptake by *Gracilaria sp.* was clearly indicated in this study after the oyster treatment, decreasing in 83% the total ammonia concentration from 0.270 mg.L⁻¹ to 0.046 mg.L⁻¹ at the end of the experiment (Trial 1).

It has been suggested that *Gracilaria* respond more rapidly to ammonia than nitrate (HANISAK, 1983; GLENN *et al.*, 1999; JONES *et al.*, 2001), consistent with our finding that ammonia rather than nitrate was significantly reduced during macroalgal absorption compared to the control treatment. Nitrifying bacteria in the aerobic sediment layers and free-living forms in the water column are probably related with the NO₂/NO₃ increase in the biological treatment in Trials 1 and 2 (autotrophic), in addition, increases since 32 h until 48 h in Trial 2 can be associated to oyster feces. Additionally, oysters could also stimulate nitrification by enhancing the movement of N to the aerobic superficial sediments and nitrifying bacteria in their digestive tract (BOUCHER and BOUCHER-RODONI, 1988). In Trial 2 (heterotrophic) total ammonia concentration was sharply decreased and nitrate was reduced slower not due oyster or macroalgae filtration, but due heterotrophic bacteria uptake. It seems that nitrate was more effectively reduced when level of total ammonia reached near zero, which can be explained by the preference of this second nitrogen source for bacteria (VRIENS *et al.*, 1989; RITTMANN and McCARTY, 2001; SCHNEIDER *et al.*, 2006).

The densities of oysters and macroalgae densities evaluated in this study, seems to be very low to interfere in the effluent treatment. Therefore, oyster densities should be higher for *C. rhizophorae*, since the same densities of Sydney rock oyster *Saccostrea commercialis* were used to treat *Penaeus japonicus* culture effluent with significant results in improving water quality (JONES and PRESTON, 1999). JONES *et al.* (2002) detected that a low density of 0.2 oyster.L⁻¹ (10 g.L⁻¹) presented best results in relation to survival in effluent treatment system.

Macroalgal absorption inefficiency seems to be related to the no occurrence of photosynthesis process during last 24 h.

CONCLUSIONS

The results of this study showed the viability to improve the water quality from shrimp pond effluent by sedimentation and biological integrated treatment system using native oyster and macroalgae species in a laboratory scale. Furthermore, these species can provide an additional source of income for shrimp farmers, converting the available energy, which would be wasted, into biomass.

Dramatic differences between autotrophic and heterotrophic effluent were recorded and different treatment strategies should be developed for each one. The impact that autotrophic or heterotrophic effluents have to the environment is significant if they weren't properly treated respecting their specificities.

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4 CONSIDERAÇÕES FINAIS

Os resultados desse estudo demonstraram que ostras *Crassostrea rhizophorae* e macroalgas *Gracilaria* sp. são organismos que podem ser utilizados no tratamento de efluentes de viveiros de camarão marinho *Litopenaeus vannamei*. Contudo, estudos mais direcionados devem ser realizados principalmente em relação às densidades de cultivo e restrições fisiológicas dos animais co-cultivados, assim como experimentos em uma escala maior.

O efluente advindo de viveiros de cultivo em sistema autotrófico possui características distintas dos provenientes de cultivos em sistema heterotrófico. O primeiro apresenta níveis mais altos de amônia, portanto os elementos do sistema de tratamento para esse efluente deverá focar a essa substância como principal alvo. Já o efluente de meio heterotrófico apresenta maior concentração de sólidos suspensos devido à grande biomassa de bactérias, logo a redução do nível de material em suspensão deverá ser priorizado no sistema de tratamento.

O impacto que os efluentes autotróficos ou heterotróficos podem acarretar ao ambiente é significativo se não forem tratados adequadamente respeitando as peculiaridades de cada um. Não há como apontar, baseando-se nos resultados desse estudo, qual causaria o menor impacto. Contudo, pode-se afirmar que um sistema de tratamento integrado, aplicando-se os conceitos da biorremediação, é eficiente na melhoria da qualidade ambiental dos viveiros e áreas circunvizinhas à fazenda, assim como na otimização do aproveitamento da energia disponível, transformando rejeito em biomassa.

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ANEXOS

ANEXO 1 - Normas para publicação no Boletim do Instituto de Pesca

INSTRUCTIONS TO AUTHORS

The *BOLETIM DO INSTITUTO DE PESCA* is a scientific journal that aims to publish original papers related to Fisheries, Aquaculture and Limnology. It is a yearly publication, with the necessary number of tomes. The articles issued by the *Boletim do Instituto de Pesca* may be categorized as follows: Research Article, Short Communication, Review Article or Case Report. They may be written in Portuguese, English or Spanish and should be composed of the following items:

TITLE: Concise and informative, **written in Portuguese and English (also in Spanish, if the article is written in that language) one below the other.** Long titles may need subtitles. **It must be typed in capital letters, alignment centralized.** Sources of support in the accomplishment of the work should be informed as a footprint note in the first page, by using an asterisk at the end of the title.

NAME OF THE AUTHOR(S): Full names should be presented in direct order (first name and surname). Only the surname under which the author(s) may be identified should be typed in capital letters. The author(s)'s affiliation, complete mailing address and e-mail should be placed at the bottom of the first page, identified by Arabic numbers separated by commas when necessary.

ABSTRACT + KEY WORDS: Mandatory in any kind of paper. The abstract should state, concisely, the study's purposes, basic procedures, results and conclusion. It must be limited to 250 (two hundred and fifty) words for Research and Review Articles; 150 (one hundred and fifty) words for Short Communication and Case Report. The key words must be limited to 6 (six), including scientific names if necessary. **Abstract + Key words in Portuguese and English are mandatory**, independently of the language in which the paper is written.

INTRODUCTION: Should explain the theme and objective of the study based on references from the literature.

MATERIAL AND METHODS: Description **RESULTS:** May be presented as tables and/or figures when necessary. Tables should be numbered with Arabic numerals, and the respective title should be put at the top. Data should not be duplicated in graphs and tables, unless when absolutely necessary. Charts, drawings, maps, photographs, etc., no larger than 16X21 cm, should fit in the text, be mentioned as a figure and numbered consecutively in Arabic numerals, with a self-explanatory title below. Drawings, maps and photographs should be sent in their original forms and in separate file(s), preferably in “tiff” digital format. E.g.: *name of the file.tif*. The publication of colored figures is restricted to situations where the colors are essential.

DISCUSSION: Results and Discussion may constitute a single chapter.

CONCLUSIONS: Discussion and Conclusions may also constitute a single chapter.

ACKNOWLEDGEMENTS: The acknowledgements are optional.

REFERENCES:

1- IN THE TEXT

- Use the system Author/Date: the author(s)' last name in capital letters and the year in which the article was published.

For example:

One author: MIGHELL (1975) observed...; According to AZEVEDO (1965), the “piracema”...; These statements were confirmed by later studies (WAKAMATSU, 1973).

Two authors: ROSA JÚNIOR and SCHUBART (1980), researching... (If the cited article **has been written** in Portuguese, use “e” linking the authors' surnames; **if it has been written** in English, use “and”; if in Spanish, use “y”).

Three or more authors: The first author's surname should be followed by “*et al*”. Thus, SOARES *et al.* (1978) verified... or Such fact was verified in Africa (SOARES *et al.*, 1978).

- For the same author published in different years, the chronological order should be followed, separated by comma. Ex : SILVA (1980, 1985)

To quote several authors sequentially, respect the chronological order of the year of publication and separate them by “;” Example: in commercial nurseries (SILVA, 1980; FERREIRA, 1999; GIAMAS and BARBIERI, 2002).

When it is absolutely necessary to refer an author cited in a consulted paper, the name of that author should be inserted only in the text (lower case). The name of the consulted paper's author, which will be included in the reference list, should be written between commas, and preceded by the latin word *apud*. Example: “According to Gulland, *apud* SANTOS (1978), the coefficients...”.

2- ON THE REFERENCE LIST

2.1. Printed documents

- For two authors, list the papers referred in the text with last names and initials of all authors separated by e, and or y, **when the text is written** in Portuguese, English or Spanish, respectively. If more than two authors, they should be separated by a semicolon.

In case of more than one article with the same entry, the chronological order should be considered. After that, use the alphabetical order of the third element of the reference.

Examples:

a) Periodical article

BARBIERI, G. e SANTOS, E.P. dos 1980 Dinâmica da nutrição de *Geophagus brasiliensis* (Quoy e Gaimard, 1824), na represa do Lobo, Estado de São Paulo, Brasil. *Ciência e Cultura*, São Paulo, 32(1): 87-89.

WOHLFARTH, G.W.; MOAY, R.; HULATA, G. 1983 A genotype-environment interaction for growth rate in the common carp, growing in intensively manured ponds. *Aquaculture*, Amsterdam, 33: 187-195.

b) Dissertation, thesis, paper presented in order to take Bachelor's degree, etc.

GODINHO, H.M. 1972 *Contribuições ao estudo do ciclo reprodutivo de Pimelodus maculatus Lacépède, 1803 (Pisces, Siluroidei) associado a variações morfológicas do ovário e a fatores abióticos*. São Paulo. 94p. (Tese de Doutorado. Instituto de Ciências Biomédicas, USP).

EIRAS, A.C. 1991 *Células sanguíneas e contagem diferencial de leucócitos de 13 espécies de teleósteos do rio Paraná - PR*. São Paulo. 95p. (Trabalho para obtenção do título de Bacharel em Ciências Biológicas. Organização Santamarense de Educação e Cultura).

c) Book, leaflet, etc.

GOMES, F.P. 1978 *Curso de estatística experimental*. 8ªed. Piracicaba: Escola Superior de Agricultura "Luiz de Queiroz". 430p.

ENGLE, R.F. and GRANGER, C.W.J. 1991 *Long-run economic relationship: readings in cointegration*. New York: Oxford University Press. 301p.

d) Book chapter, publication in collective work, congress, meeting or seminar annals, etc.

MACKINNON, J.G. 1991 Critical values for cointegration tests. In: ENGLE, R.F. and GRANGER, C.W.J. *Long-run economic relationship: readings in cointegration*. New York: Oxford University Press. p.267-276.

AMORIM, A.F. e ARFELLI, C.A. 1977 Contribuição ao conhecimento da biologia e pesca do espadarte e agulhões no litoral sul sudeste do Brasil. In: CONGRESSO PAULISTA DE AGRONOMIA, 1., São Paulo, 5-9/set./1977. *Anais...* São Paulo: Associação de Engenheiros Agrônomos. p.197-199.

ÁVILA-DA-SILVA, A.O.; CARNEIRO, M.H.; FAGUNDES, L. 1999 Gerenciador de banco de dados de controle estatístico de produção pesqueira marítima - ProPesq. In: CONGRESSO BRASILEIRO DE ENGENHARIA DE PESCA, 11.; CONGRESSO LATINO AMERICANO DE ENGENHARIA DE PESCA, 1., Recife, 17-21/out./1999. *Anais...* v.2, p.824-832.

2.2. *Electronic references* (documents consulted online, CD-ROM, etc.)

- The rules for *printed documents* should be followed, adding the electronic address at which the document has been consulted.

Examples:

FLORES, S.A. y HIRT, L.M. 2002 Ciclo reproductivo y fecundidad de *Pachyurus bonariensis* (Steindachner, 1879), Pisces, Scianidae. *B. Inst Pesca*, São Paulo, 28(1): 25-31. Available at: <http://www.pesca.sp.gov.br/publicações.shtml>. Access on: 26 August 2004.

CASTRO, P.M.G. (no date) *A pesca de recursos demersais e suas transformações temporais*. Available at: <http://www.pesca.sp.gov.br/textos.php>. Access on: 3 September 2004.

SILVA, R.N. e OLIVEIRA, R. 1996 Os limites pedagógicos do paradigma da qualidade total na educação. In: CONGRESSO DE INICIAÇÃO CIENTÍFICA DA UFPE, 4., Recife, 1996. *Anais eletrônicos...* Available at: <http://www.propesq.ufpe.br/anais/anais.htm>. Access on: 21 January 1997.

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OBSERVATIONS:

1. The manuscripts should be typed in Word for Windows, font Book Antiqua, size 11, 1.5 spacing between the lines. Important: the lines should be numbered sequentially from first to last page and not more than 20 pages in A4 paper size, including figure(s) and/or table(s).
2. The paper should be submitted in one printed copy and identified CD-ROM with the file(s).
3. The papers will be analysed by the Comitê Editorial do Instituto de Pesca-CEIP (Fishery Institute Editorial Board-FIEB) and by scientific referees following chronological order. They may be sent back to the author(s) for modifications. The authors have a deadline of 30 (thirty) days to return the paper to the CEIP (FIEB), after which it will be automatically cancelled.
4. The papers not accepted for publication will be sent back to the author(s).
5. The author(s) will receive, in total, 20 (twenty) offprints of the paper. In case there is need of a larger number of offprints, they may be sent at the author(s)' expense.

6. The papers not originated from the Instituto de Pesca will be sent to the Comitê Editorial do Instituto de Pesca-CEIP at: Av. Francisco Matarazzo, 455 – CEP: 05001-900 - São Paulo - SP - Brazil Fax number: (0xx11) 3871-7568 or 3871-7525 e-mail: ceip@pesca.sp.gov.br / home page: www.pesca.sp.gov.br

7. Only the papers prepared in strict accordance with these rules will be analysed by the Comitê Editorial do Instituto de Pesca-CEIP (FIEB). Otherwise, they will be sent back to the author(s).