UNIVERSIDADE FEDERAL DO RIO GRANDE – FURG
PROGRAMA DE PÓS-GRADUAÇÃO EM AQUICULTURA

Efluentes da Carcinocultura e seus Efeitos sobre o Sistema Estuarino da Lagoa dos Patos

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Tese apresentada ao programa de Pós-graduação em Aquicultura da Universidade Federal do Rio Grande – FURG, como requisito parcial à obtenção do título de Doutor.

Orientador: Dr. Luis Henrique da Silva Poersch

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DEDICATÓRIA

A Deus, razão do meu ser

“Conheço teu medo, a tua felicidade e os teus sonhos. Conheço tua estrada
e sei exatamente o teu destino...

E sem que tu tenhas que me pedir, eu entendo o que tu queres. Conheço o
teu sorriso, e sei tudo que está dentro do teu coração. Conheço e te
reconheço em qualquer lugar…

Sei do teu amor, da tua saudade, dos sonhos que movimentam a tua vida e
da esperança que te faz lutar…

Acompanho-te desde sempre! Estou ao teu lado mesmo quando pensas que
te abandonei…

Conheço-te, porque eu te criei.”

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A todos os meus amigos.

ÍNDICE

RESUMO GERAL

A aquicultura é o setor de produção de alimentos de origem animal que mais cresce no mundo. A carcinocultura, como parte desse setor, tem gerado diversos benefícios em termos sócio-econômicos, mas também tem gerado uma atenção especial aos aspectos ambientais envolvidos com a atividade. A produção em cativeiro do camarão branco do Pacífico *Litopenaeus vannamei* vem se consolidando cada vez mais no entorno do estuário da Lagoa dos Patos, região sul do Brasil. Esse estuário possui importância ecológica, industrial, portuária, agrícola e pesqueira, o que torna necessário a compreensão do potencial impacto poluidor da atividade nessa região. Nesse estudo, parâmetros de qualidade da água, composição e abundância do fitoplâncton, protozooplâncton, mesozooplâncton e macrozoobentos foram avaliados espaço-temporalmente no corpo d`água receptor dos efluentes de uma fazenda produtora de camarões da espécie *Litopenaeus vannamei* localizada as margens do estuário da Lagoa dos Patos. Foram avaliados ainda os teores de carbono orgânico total (COT), nitrogênio total (NT), Cobre (Cu) e Zinco (Zn) do sedimento do viveiro de cultivo, da bacia de sedimentação e do estuário receptor dos efluentes. Foram avaliadas ainda as concentrações de Cu e Zn do tecido dos camarões cultivados ao final do ciclo produtivo. As amostras foram tomadas antes da descarga dos efluentes (AD) e; 1 dia (1 PD), 5 dias (5 PD), 10 dias (10 PD), 20 dias (20 PD), 30 dias (30 PD), 60 dias (60 PD) e 90 dias (90 PD) após a descarga dos efluentes. Vale ressaltar que cada estudo contou com um cronograma próprio e assim nem todas as análises citadas acima tiveram coletas em todas as datas mencionadas. Especificamente para a avaliação do macrozoobentos, um segundo ciclo de produção foi acompanhado para realização de amostragens. Os pontos de coleta no estuário distribuíram-se desde a desembocadura do canal de lançamento dos efluentes até uma distância de 250 m do mesmo. Dados de temperatura, oxigênio dissolvido, pH, salinidade, clorofila *a,* nitrogênio amoniacal total (NAT), nitrito, nitrato, fósforo total, sólidos suspensos totais e turbidez da água foram mensurados. Além da temperatura e salinidade que sofrem variações sazonais em ambientes estuarinos, NAT, clorofila *a* e turbidez foram os parâmetros de qualidade de água que sofreram alterações mais marcadas em decorrência do lançamento dos efluentes. No entanto, de maneira geral, essas alterações foram restritas a uma distância de até 20 m do canal de descarga dos efluentes por um período de tempo de no máximo 5 dias. Diatomáceas, cianobactérias, clorofíceas e ciliados foram identificados e quantificados. A comunidade

predominante foi a de clorofíceas, seguido das diatomáceas, cianobactérias e ciliados. Houve um aumento na concentração dos diferentes grupos no primeiro dia pós-descarga (1 PD). Entretanto, esse aumento pode estar relacionado ao enriquecimento de nutrientes das águas estuarinas ocasionado por chuvas abundantes, e não propriamente ao despejo dos efluentes. A abundância meso-zooplanctônica foi baixa e representada exclusivamente por copépodos, na sua grande maioria da espécie *Acartia tonsa*. A única exceção foi a amostragem 30 PD que sofreu um acréscimo expressivo, sem no entanto apresentar relação com os parâmetros abióticos avaliados. Apesar de diferenças significativas ($p < 0,05$) terem sido observadas em algumas amostragens, essas não aparentam ter nenhuma relação com o lançamento dos efluentes. Sete grupos macrozoobentônicos foram observados (Polychaeta, Tanaidacea, Isopoda, Gastropoda, Bivalvia, Malacostraca e Ostracoda) ao longo de dois ciclos produtivos (2012 e 2013). A densidade e a riqueza de espécies sofreram pequena variabilidade ao longo dos pontos amostrais do estuário para ambas as etapas. Já ao longo do tempo, em 2012 pode-se observar um aumento desses índices no inverno comparativamente ao verão. Ao longo das coletas de 2013, a densidade do macrobentos teve um padrão oposto e a riqueza de espécies sofreu pouca variabilidade. A concentração de COT variou de 0,12 a 0,67% e a concentração de NT ficou abaixo do limite de detecção do equipamento (<0,1 ppm) em todos os locais e campanhas amostrais. Os teores de Cu e Zn no sedimento foram quantificados através de extração fraca (fração de metais lábeis ou potencialmente biodisponível) e extração semi-forte (fração de metais mais fortemente adsorvida). O Cu na fração lábil variou de 0,12 a 1,27 µg/g e o Zn variou de 0,52 a 3 µg/g, enquanto que a fração mais fortemente adsorvida de Cu variou de 0,3 a 2.65 µg/g e o Zn de 30,44 a 121,4 µg/g. Diferenças significativas (ANOVA, $p < 0,05$) foram observadas nos resultados de COT entre alguns pontos amostrais nas campanhas 1PD e 10PD, sem no entanto haver relação com o lançamento dos efluentes. A análise de correlação de Pearson não mostrou relação entre o lançamento dos efluentes e aumento nos valores de COT, NT, Cu ou Zn no sedimento, com exceção da relação encontrada entre os valores de COT e Cu e Zn no sedimento no primeiro dia pós-descarga (1 PD). Ao final do ciclo, exemplares de camarões coletados de dois viveiros de engorda revelaram concentrações médias de 6,63±0,2 µg/g de Cu e 19,76±0,2 µg/g de Zn no viveiro 1, e 7,6±0,51 µg/g de Cu e 19,13±0,32 µg/g de Zn no viveiro 2. Apesar de alguns parâmetros de qualidade de água terem sofrido variações em decorrência do lançamento dos efluentes, esses efeitos foram agudos e pontuais, restritos a uma distância de até 20m da margem e observados até os 5 dias após o descarte dos efluentes. Além disso, os valores ficaram dentro da faixa exigida pela Resolução ambiental vigente. O fitoplâncton, protozooplâncton, mesozooplâncton e o macrozoobentos sofreram apenas variações decorrentes das oscilações naturais dos parâmetros abióticos que ocorrem em sistemas estuarinos. Por fim, os teores de COT, NT, Cu e Zn no sedimento e Cu e Zn no tecido dos camarões ficaram dentro dos valores máximos estipulados na legislação, mostrando que o lançamento dos efluentes provenientes da produção de camarões no entorno do estuário da Lagoa dos Patos não trouxe efeitos adversos ao meio ambiente.

GENERAL ABSTRACT

Aquaculture is the food producing sector of higher growth worldwide. The shrimp farming, as part of this sector, has generated several socio-economic benefits. However, it has also developed particular attention to environmental aspects involved with the activity. The production of Pacific white shrimp *Litopenaeus vannamei* is increasing in areas adjacent to Patos Lagoon estuary, Southern Brazil. This estuary has importance on several areas as ecology, industry, ports, agriculture and fisheries, which makes the understanding of the potential pollution impact of the shrimp farm in this ecosystem extremely necessary. In this study, the water quality, composition and abundance of phytoplankton, protozooplankton, meso-zooplankton and macrozoobenthos were spatio-temporally evaluated in the Patos Lagoon estuary, which received effluents from a commercial shrimp farm. Total organic carbon (TOC), total nitrogen (TN), copper (Cu) and zinc (Zn) content in sediment of pond, sedimentation basin and the estuary were also analyzed. Moreover, concentrations of Cu and Zn in shrimp tissues were measured at the end of the growth out cycle. Samples were taken before the effluents discharge (BD) and; 1 day (1 PD) 5 days (5 PD), 10 days (10 PD), 20 days (20 PD), 30 days (30 PD), 60 days (60 PD) and 90 days (90 PD) after the effluents discharge. Only for the macrozoobenthos analysis, samples were collected in two cycle's period. The sampling sites in the estuary were distributed across the effluents discharge channel (EDC), 20m, 30m, 100m and 250m from the channel. Temperature, dissolved oxygen, pH, salinity, chlorophyll a, total ammonia nitrogen (TAN), nitrite, nitrate, total phosphorus, total suspended solids and turbidity were measured. Temperature and salinity suffered seasonal influences and TAN, chlorophyll a and turbidity showed the most marked changes because of the effluents discharge. However, these changes were restricted to a distance of 20 m from the EDC for a period of 5 days. Diatoms, cyanobacteria, chlorophyceae and ciliates were identified and quantified. The predominant community was chlorophytes, followed by diatoms, cyanobacteria and ciliates. There was an increase in the abundance of different groups on the 1 PD sampling. However, this may be related to nutrient enrichment of estuarine waters caused by rains, and not necessarily to the effluents discharge. The meso-zooplankton abundance was low and was represented exclusively by copepods, mostly *Acartia tonsa* species. The only exception was the sampling 30 PD, when the abundance was higher, but there was no relationship

with the evaluated abiotic parameters. Seven macrozoobenthic groups were observed (Polychaeta, Tanaidacea, Isopoda, Gastropoda, Bivalvia, Malacostraca and Ostracoda) over two cycles period (2012 and 2013). Spatially, the density and species richness suffered little variability over the sampling sites in the estuary for both campaigns. Temporarily, it was observed an increase in these indices in winter compared to summer in 2012. Over the 2013 samples, the macrozoobenthos density had an opposite pattern and species richness was little variable. The TOC concentration ranged from 0.12 to 0.67% and the concentration of TN was below the equipment detection limit (<0.1 ppm) in all sampling sites. The contents of Cu and Zn in the sediment were quantified by weak extraction (labile fraction or potentially bioavailable) and semi-strong extraction (more strongly adsorbed fraction). Cu in the labile fraction ranged from 0.12 to 1.27 µg g^{-1} and Zn ranged from 0.52 to 3 µg g^{-1}, while in the more strongly adsorbed fraction, Cu ranged from 0.3 to 2.65 µg g^{-1} and Zn from 30.44 to 121.4 µg g^{-1}. Significant differences (ANOVA, $p < 0.05$) were observed in the TOC values among sites in samplings 1PD and 10PD, but not related to the effluents discharge. The Pearson correlation analysis showed no relationship between the effluents discharge and increase in TOC, TN, Cu or Zn in the sediment, except for the relation found between the values of TOC, Cu and Zn in the sediment on 1 PD sampling. At the end of the cycle period, shrimps were collected from two ponds and after the tissue analysis it revealed mean concentrations of 6.63 ± 0.2 µg g^{-1} of Cu and 19.76 ± 0.2 µg g^{-1} of Zn in pond 1 and 7, 6 ± 0.51 µg g^{-1} of Cu and 19.13 ± 0.32 µg g^{-1} of Zn in pond 2. Although some water quality parameters have suffered variations due to the effluents discharge, these effects were acute and punctual, restricted to a distance of 20m from the EDC and observed until 5 days after the discharge. In addition, all values were within the limits stipulated by standard guidelines and available Brazilian legislation. Phytoplankton, protozooplankton, meso-zooplankton and the macrozoobenthos showed natural fluctuations related to abiotic parameters in estuarine systems. Finally, TOC, TN, Cu and Zn in the sediment and Cu and Zn in the shrimp tissue were within the maximum amounts stipulated by standard guidelines, showing that the effluents discharge from the commercial shrimp farm in Patos Lagoon estuary did not cause negative impacts on the environment.

INTRODUÇÃO GERAL

Crescimento da aquicultura

A aquicultura marinha e costeira representam um importante componente na cadeia produtiva de alimentos (Webb et al., 2012). O crescimento populacional e a estabilização das capturas selvagens têm permitido que essa atividade cresça a elevadas taxas, sendo assim considerada de grande potencial para suprir a demanda mundial por proteína de alta qualidade (Brander, 2007). América Latina e Caribe representam atualmente a segunda posição no ranking mundial de crescimento, com taxas médias que alcançaram 10%/ano entre 2000-2012 (FAO, 2014). Ainda segundo a FAO (2014), o Brasil ocupa a décima segunda posição mundial na produção de pescados através da aquicultura, com um montante total de mais de 700 mil Ton/ano. Dentro desse contexto, apenas a produção de crustáceos representa mais de 70 mil Ton/ano, o que demonstra a expressividade dessa atividade no Brasil.

Ao longo dos seus 8.500 km de linha de costa, o Brasil apresenta grande potencial para o desenvolvimento da aquacultura marinha e costeira (Barroso et al., 2007). No extremo sul do Brasil, a carcinicultura começou a se instalar no final da década de 1990 com o cultivo de *Farfantepenaeus paulensis* e posteriormente se consolidou com a criação do camarão branco do pacífico *Litopenaeus vannamei*. Com sua importância cada vez mais expressiva frente ao mercado, a carcinocultura tem ganhado foco por parte das autoridades ambientais no que diz respeito ao uso de água e emissão de efluentes ao meio adjacente. Isso porque a aquicultura ainda tem uma participação expressiva na poluição orgânica, eutrofização, lançamento de nutrientes e efluentes ao ecossistema, floração de algas tóxicas, depleção de oxigênio e mudanças na macrofauna bêntica (Naylor et al., 1998; Páez-Osuna, 2001; Anderson et al., 2002; Aubin, 2006; Olsen et al., 2008).

Diversos incidentes até então mal compreendidos, causaram o colapso de diversas fazendas de criação de camarões ao redor do mundo. Atualmente os motivos são claros, entre eles a falta de manejo e a super-intensificação sem controle dos sistemas de criação. A intensificação dos sistemas de criação favorece ainda mais o potencial poluidor dessa atividade uma vez que aumenta-se a quantidade de fertilizantes utilizados, o uso de dietas balanceadas ricas em nitrogênio (N) e fósforo (P) e, consequentemente, os produtos decorrentes da excreção dos animais. Esses fatores são apontados como os principais agentes potenciais para a eutrofização de zonas costeiras (Cho et al., 1994; Jackson et al., 2004). A ração é citada como a principal responsável pela deterioração da qualidade da água, uma vez que apenas 15-30% do alimento ofertado é assimilado pelos animais (Barbieri & Ostrensky, 2002). De acordo com Boyd & Tucker (1998), do montante oferecido pelas dietas, cerca de 25-45% do N, 20-30% do P e 10-15% do C é absorvido e assimilado pelos camarões. O restante permanece dentro dos viveiros na forma de fezes ou matéria orgânica em decomposição até ser liberada para o meio ambiente através dos efluentes ao final do ciclo produtivo.

Islam (2005) reporta a importância das práticas de manejo na regulação da saída de nutrientes para o meio ambiente. A troca de água dos viveiros ao longo do ciclo produtivo é uma prática comum em sistemas de aquicultura. Essas são realizadas a fim de controlar a concentração de fitoplâncton e de nutrientes nos viveiros, evitando que os compostos nitrogenados e o oxigênio dissolvido alcancem níveis que possam ser prejudiciais aos camarões (Lemonnier & Faninoz, 2006). A carga de nutrientes originados durante um ciclo de criação é variável de acordo com o tempo de cultivo, o sistema adotado e as técnicas de manejo empregadas. Primavera (1997) destaca ainda que o efeito do efluente sobre o corpo d'água receptor depende de fatores como magnitude de descarga, composição do efluente, taxa de diluição e tempo de residência. A capacidade assimilativa de um determinado ambiente para o enriquecimento de nutrientes é finita e a quantidade excessiva de nutrientes pode alterar a composição de espécies, diversidade e dinâmica da comunidade biótica (Kennish, 1992). Além de afetar diretamente a qualidade da água e os sedimentos, os resíduos orgânicos particulados podem também ser utilizados diretamente por animais consumidores no ecossistema receptor, podendo levar a mudanças nas comunidades planctônicas e bentônicas (Roditi et al., 2000).

Um sistema que vêm sendo amplamente utilizado no tratamento de águas residuais é a bacia de sedimentação. A utilização dessa tecnologia tem crescido desde os anos 1970 nos mais diversos setores, entre eles a aquicultura. Diversos processos bióticos e abióticos ocorrem nessas bacias, como mineralização microbiana, nitrificação/desnitrificação, absorção de nutrientes pela vegetação, deposição do material particulado, entre outros. Uma vez que os efluentes permaneçam um tempo suficientemente longo na bacia de decantação, torna-se possível a precipitação de todos os sólidos em suspensão, inclusive os mais finos (Boyd, 1990). Isso proporciona a liberação de uma água para o ambiente com melhores parâmetros de qualidade. Lin et al. (2005) reportam remoção de 55-66% dos sólidos suspensos e redução de 91-99% da turbidez dos efluentes de um cultivo intensivo de *L. vannamei* através do uso dessa tecnologia. As propriedades físico-químicas da água são usadas como indicadores ambientais tanto na água dos viveiros como nos efluentes da carcinocultura. Esses parâmetros permitem um melhor entendimento na avaliação dos impactos dos efluentes sobre o meio ambiente receptor (Chua et al., 1989). Um dos grandes desafios da carcinocultura é superar as preocupações ambientais para melhorar a eficiência econômica, através do desenvolvimento e implementação de uma abordagem integrada para a redução de resíduos nitrogenados (Jackson et al., 2003). Assim, o monitoramento dos efluentes e dos parâmetros físico-químicos da água do ambiente receptor torna-se de extrema importância para assegurar a viabilidade de um sistema de cultivo.

Efeito do enriquecimento orgânico sobre o sedimento

O sedimento tem um papel crucial na ciclagem de nutrientes. Em ecossistemas costeiros rasos, como é o caso da Lagoa dos Patos, o sedimento pode atuar como a principal fonte de nutrientes para a coluna d'água (Warnken et al., 2002). Os nutrientes e matéria orgânica produzidos nos viveiros de cultivo de camarões estão presentes na forma dissolvida e suspensa na água, e uma importante porção se acumula no sedimento ou volatiliza para a atmosfera (Páez-Osuna et al., 1999). O material orgânico presente no fundo dos viveiros da aquicultura é proveniente da produção primária do fitoplâncton, dos restos de ração e dos produtos de excreção dos animais (Funge-Smith & Briggs, 1998; Steeby et al., 2004). A matéria orgânica por sua vez tende a se acumular no sedimento, mantendo-se mesmo após o final do ciclo de produção. Esse acúmulo pode trazer impactos negativos ao ambiente biológico e químico dos viveiros (Suplee & Cotner, 1996) ou do ambiente receptor dos efluentes, uma vez que essas grandes quantidades de nutrientes tornam o sedimento um local favorável para o desenvolvimento microbiano pela disponibilidade de matéria orgânica.

O incremento de matéria orgânica ao ecossistema pode causar eutrofização. Sistemas eutrofizados geralmente revelam presença de carbono orgânico particulado (Pelletier et al., 2011), o que torna esse composto um bom indicador de enriquecimento em sedimentos (Hyland et al., 2005). O Nitrogênio orgânico por sua vez exerce um importante papel como fonte de nutrientes (Fütterer, 2000). Esse nutriente provém principalmente da proteína das rações, por meio da excreta na forma de amônia e por meio das fezes na forma de nitrogênio orgânico (Henry-Silva & Camargo, 2007). Briggs & Funge-Smith (1994) avaliando viveiros de criação de camarão na Tailândia concluíram que 31% do N que entra no sistema é absorvido pelo sedimento.

A aquicultura é muitas vezes reportada como uma fonte potencial de metais traço, os quais estão presentes como componentes naturais de rações, impurezas em fertilizantes ou como princípio ativo de pesticidas (Tacon & Forster, 2003). Sedimentos podem aprisionar metais introduzidos no sistema aquático servindo como bons indicadores de poluição e permitindo uma avaliação consistente de contaminação espacial e temporal (Salomons & Förstner, 1984; Buchman, 1989). O cobre (Cu) é um elemento traço presente nas dietas essencial para os camarões sintetizarem hemocianina na sua hemolinfa (Cuzon, 2004) e o zinco (Zn) é um cofator em diversos sistemas de enzimas (Davis et al., 2002). O conteúdo residual de metais pode se acumular no sedimento, servindo assim como indicadores dos efluentes da aquicultura (Chou et al., 2002). A remobilização do sedimento favorece a liberação da fração lábil do metal para a coluna d`água, o que pode causar efeitos tóxicos para os organismos (Wallner-Kersanach et al., 2009). Metais traço que estão adsorvidos ao sedimento podem ser liberados em decorrência de mudanças nas condições físico-químicas, principalmente no pH e no potencial-redox (Cappuyns & Swennwn, 2005).

Além dos efeitos deletérios do acúmulo dos metais traço no sedimento, eles são também conhecidos graças a sua potencialidade em se concentrar em órgãos de organismos aquáticos. O excesso desses metais quando inseridos nas dietas além do requerimento nutricional necessário, pode fazer com que o camarão *L. vannamei* possa absorver e acumular no tecido ou dispersar para a água ou sedimento quando não digerido (Wu & Yang, 2011). A análise de metais traço de organismos aquáticos pode prover informações importantes do grau de contaminação ambiental e do potencial impacto do consumo desse alimento (Ip et al., 2005).

Efeitos sobre a biodiversidade

Comunidades planctônicas

Diversos estudos têm acompanhado os fatores que afetam a variabilidade do fitoplâncton nas áreas rasas do estuário da Lagoa dos Patos (Abreu et al., 1995; Bergesch & Odebrecht, 1997; Fujita & Odebrecht, 2007; Abreu et al., 2010). Nesse ambiente, a produtividade fitoplanctônica parece ser limitada pela luz, temperatura da água e disponibilidade de nutrientes (Abreu et al., 1994). Entretanto, a abundância fitoplanctônica também é dependente das condições hidrológicas locais, já que em locais com alta hidrodinâmica a diluição dos nutrientes pode ser mais rápida do que sua produção. Estudos prévios já têm demonstrado que a descarga de efluentes provenientes de cultivo de camarões no estuário da Lagoa dos Patos podem causar mudanças de curto prazo no ambiente receptor (Cardozo e Odebrecht, 2012). O *input* de nutrientes nos viveiros de cultivo provenientes das dietas formuladas e das fertilizações pode contribuir para o lançamento de efluentes ricos em nitrogênio e fósforo (Naylor et al., 1998). Isso pode colaborar para o processo de enriquecimento orgânico do ecossistema, processo conhecido como eutrofização. Esse fato deve ser observado com atenção já que existe uma relação direta entre o aumento de nutrientes no meio ambiente e o aumento da biomassa fitoplanctônica em ambientes oligotróficos e pouco eutróficos, como é o caso desse estuário (Hargrave, 1995). Assim, o monitoramento das comunidades fitoplanctônicas representam uma importante ferramenta de manejo de forma a reduzir riscos ambientais (Abreu et al., 2010).

Da mesma forma, o zooplâncton pode ser utilizado como indicador da qualidade da água em viveiros de criação de camarões e das condições ambientais do ambiente receptor dos efluentes. Esses organismos respondem a baixos níveis de oxigênio dissolvido, altos níveis de nutrientes, contaminantes tóxicos, predação, entre outros (Casé et al., 2008). Assim como na maioria dos estuários temperados, a ocorrência e a abundância de espécies de zooplâncton são determinadas principalmente por variações sazonais de salinidade e de temperatura (Montú, 1980). O protozooplâncton por sua vez pode exercer um papel chave na transferência de carbono da teia alimentar microbiana para níveis tróficos mais elevados (Yang et al., 2012).

Comunidade macrozoobentônica

A entrada de nutrientes no meio proveniente dos efluentes da aquacultura pode gerar mudanças físico-químicas significativas no sedimento, o que pode diminuir a diversidade biológica e favorecendo o aparecimento de espécies oportunistas (Johannssen et al., 1994). Esse enriquecimento orgânico pode levar a severas modificações na estrutura das comunidades bênticas, levando a seleção de poucas espécies tolerantes. Quando as taxas de acumulação de matéria orgânica ultrapassam a capacidade de assimilação do sedimento, zonas anaeróbicas podem surgir, aumentando assim a produção e liberação de compostos tóxicos reduzidos, como amônia, sulfeto de hidrogênio e metano (Alongi et al., 1999; Islam, 2005). Essas condições extremas prejudicam ainda mais a qualidade do sedimento e a estrutura da comunidade bentônica (Islam, 2005), podendo trazer como consequência o desaparecimento da fauna bêntica e a criação de uma zona anóxica (Heilskov & Holmer, 2001). Segundo Belan (1970) o aparecimento de organismos oportunistas pode ser usado como indicativo do enriquecimento do sedimento.

Organismos bentônicos desempenham um papel crucial no suprimento e mineralização da matéria orgânica (Heilskov & Holmer, 2001), o que faz com que o acompanhamento espaço-temporal das comunidades gere dados precisos na avaliação da qualidade ambiental de um ecossistema receptor de nutrientes. Diversos trabalhos têm sido desenvolvidos com vistas a avaliar e mitigar o impacto dos nutrientes liberados pela aquicultura sobre a comunidade bentônica (Bartoli et al., 2001; Soares et al., 2004; Carvalho et al., 2009; Aguardo-Giménez et al., 2011). Em regiões estuarinas, os organismos bentônicos são o principal link entre os produtores primários e os níveis tróficos superiores (Foreman et al., 1995), além de desempenharem um papel crucial no suprimento e mineralização da matéria orgânica (Heilskov & Holmer, 2001). No estuário da Lagoa dos Patos, a composição e abundância do macrozoobentos tem sido alvo de diversos estudos (Bemvenuti et al., 1978; Capítoli et al., 1978; Rosa e Bemvenuti, 2006; Colling et al., 2007), mas o acompanhamento dessa comunidade exposta ao lançamento dos efluentes da carcinocultura são escassos.

A hipótese testada é a de que o lançamento de efluentes proveniente do cultivo de camarões em sistema semi-intensivo possa causar alterações espaço-temporais sobre parâmetros de qualidade de água, o plâncton e nos níveis de carbono, nitrogênio e metais traço no sedimento do ambiente estuarino receptor.

Local de estudo

A Lagoa dos Patos é a maior laguna costeira estrangulada do mundo (Kjerfve, 1986), estende-se por 270 km de linha de costa e localiza-se entre as latitudes 30°S e 32°S (Möller et al. 1996). A hidrodinâmica é regulada principalmente pelas relações entre descarga fluvial e ação dos ventos, e, secundariamente, pelo efeito da maré (Möller et al. 2009; Seeliger, 2010). Sua formação está associada a múltiplas barreiras de areia complexas e pode ser dividida em três unidades biológicas: setor Norte (com a cidade de Porto Alegre e o Rio Guaíba como principais tributários), o setor Médio (pouco populoso e com entrada de água doce do Rio Camaquã) e o setor Sul (caracterizado como a região estuarina) (Seeliger et al., 1997). Possui importância ecológica, industrial, portuária, agrícola e pesqueira, tornando o aporte de nutrientes de origem antrópica a principal causa de eutrofização desse sistema aquático (Reis & D'Incao, 2000; Seeliger, 2010; Niencheski et al., 2014). Este estudo foi realizado nas proximidades de uma fazenda comercial de criação de camarões da espécie *Litopenaeus vannamei* localizada as margens do estuário da Lagoa dos Patos, sul do Brasil (Rio Grande do Sul, Brasil - 31º56'04S, 52º00'11W) (Figura 1).

Figura 1: Localização da fazenda de cultivo e dos respectivos pontos de coleta. V1 = Viveiro, V2 = Viveiro 2 e BS = Bacia de Sedimentação.

OBJETIVO GERAL

Avaliar espaço-temporalmente os possíveis impactos dos efluentes de uma fazenda de criação de camarões marinhos sobre a qualidade da água, o plâncton, o macrozoobentos e os sedimentos do ambiente estuarino receptor.

Objetivos específicos

- Determinar a variabilidade do plâncton e da qualidade da água antes e após a liberação dos efluentes;
- Identificar a influência do cultivo de camarões sobre a comunidade macrozoobentônica estuarina ao longo de dois ciclos produtivos;
- Determinar a variabilidade da concentração de carbono orgânico total, nitrogênio total, cobre e zinco no sedimento do viveiro, bacia de sedimentação e do estuário antes e após a liberação dos efluentes; determinar ainda os teores de cobre e zinco nos camarões ao final do ciclo produtivo.

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CAPÍTULO 1

VARIABILIDADE DO PLÂNCTON E DA QUALIDADE DA ÁGUA EM UM ESTUÁRIO ANTES E APÓS O LANÇAMENTO DE EFLUENTES DA CARCINOCULTURA: IMPACTOS E REGENERAÇÃO

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PLANKTON AND WATER QUALITY VARIABILITY IN AN ESTUARY BEFORE AND AFTER THE SHRIMP FARMING EFFLUENTS: POSSIBLE IMPACTS AND REGENERATION

PLANKTON AND WATER QUALITY VARIABILITY SUBJECT TO SHRIMP FARM EFFLUENT

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ABSTRACT

Water quality, chlorophyll a, phytoplankton, proto and mezo-zooplankton abundance were spatiotemporally evaluated in an estuary receiving effluents from a Pacific white shrimp *Litopenaeus vannamei* farm in Patos Lagoon estuary, Southern Brazil. Samples were taken before (BD) and; 1 day (1 PD) 5 days (5 PD), 10 days (10 PD), 20 days (20 PD) and 30 days (30 PD) after the effluents discharge. Some water quality parameters were affected by the effluents discharge; however, these changes were restricted to a distance of 20 m from the effluent discharge channel for a period of 5 days. The microbial community was dominated by chlorophyceae, followed by diatoms, cyanobacteria and ciliates. There was an increase in the abundance of different groups on the 1 PD sampling compared to BD. The zooplankton abundance was low in practically all sites, except for 30 PD sampling. The meso-zooplanktonic organisms were represented by copepods, mostly *Acartia tonsa*. Despite some effects on water quality and phytoplankton and protozooplankton abundance until 5 PD sampling, these alterations dissipated in a short period of time. We conclude that the environment quickly assimilated the effluents discharge, and the water quality parameters remained within the limits stipulated by standard guidelines.

RESUMO

Parâmetros de qualidade da água, composição e abundância do fitoplâncton e do proto e meso-zooplâncton foram avaliadas espaço-temporalmente no estuário receptor de efluentes de uma fazenda produtora de camarão *Litopenaeus vannamei* na Lagoa dos Patos, Brasil. As amostras foram tomadas em sete pontos antes (BD) e; 1 dia (1 PD), 5 dias (5 PD), 10 dias (10 PD), 20 dias (20 PD) e 30 dias (30 PD) após a descarga dos efluentes. Alguns dos parâmetros de qualidade de água sofreram alterações devido ao lançamento dos efluentes, restritos a 20 m do canal de descarga dos efluentes e por um período máximo de 5 dias. A comunidade microbiana sofreu variação em 1 PD comparativamente a BD. A abundância zooplanctônica foi baixa na maioria das amostras, com exceção de alguns pontos em 30 PD. Os copépodos foram o único grupo encontrado no meso-zooplâncton, na sua grande maioria a espécie *Acartia tonsa.* Apesar de modificações em alguns parâmetros avaliados nos primeiros dias após o lançamento dos efluentes, essas alterações foram assimiladas em um curto período. Os parâmetros de qualidade de água mantiveram-se dentro dos limites estipulados pela legislação e o ambiente assimilou de maneira rápida as mudanças ocorridas.

Descriptors: Aquaculture, Environmental Impact, Plankton, Effluent.

Descritores: Aquicultura, Impacto Ambiental, Plâncton, Efluente.

INTRODUCTION

The global seafood consumption has been increasing mainly due the world's growing population, fisheries stagnation and consumption habits improvement (FAO, 2012). Shrimp is still the largest single commodity in value terms, representing about 15 percent of the total value of internationally traded fishery products (FAO, 2014). The Brazilian shrimp farming started in 1970 in the Northest region, raising different *Penaeus* species (MOLES; BUNGES, 2002). In the Southest, facilities started to work in the late 1990 with the native shrimp *Farfantepenaeus paulensis*, and later with the Pacific white shrimp *Litopenaeus vannamei* around the Patos Lagoon estuary. The Patos Lagoon is the world's largest chocked lagoon (KJERFVE, 1986) with 270 km of coastline between latitudes 30°S and 32°S (MÖLLER et al., 1996). The importance of this lagoon remains on its ecology, industries, ports, agriculture and fisheries, and the anthropogenic impact by the nutrient input is the major cause of eutrophication of this aquatic system. (REIS; D'INCAO, 2000; SEELIGER, 2010; NIENCHESKI et al., 2014).

The shrimp farming, as an anthropic activity, can contribute to the nutrient input in adjacent ecosystems by the release of effluents loaded with nitrogen and phosphorous (NAYLOR et al., 1998). These nutrients come from fertilizes use, shrimps' excretion and unconsumed aquafeeds (CHO et al., 1994; BURFORD, 1997; JACKSON et al., 2004; HERBECK et al., 2013). The organic matter and nutrient accumulation in shrimp ponds lead to large phytoplankton blooms (ALONSO; OSUNA, 2003), increasing the effluents pollution potential and generating anoxia conditions in the receiving water body (BURFORD; WILLIAMS, 2001; JACKSON et al., 2004).

The phytoplankton is considered a sensitive biological indicator that responds to the anthropogenic stress (COUTINHO et al., 2012). According to HARGRAVE (1991), there is a direct relationship between increased nutrients in coastal waters and the enlargement of phytoplankton biomass in oligotrophic environments as Patos Lagoon. Several studies have been conducted in the Patos Lagoon estuary shallow areas to evaluate its environmental variability (ABREU et al., 1995; BERGESCH; ODEBRECHT, 1997; FUJITA; ODEBRECHT, 2007; ABREU et al., 2010). ABREU et al. (2010) reported that the most significant variations in chlorophyll *a* in this estuary occur within days to weeks. As stated by OLSEN et al. (2008), the assimilation capacity of the water column is mediated by two main mechanisms: nutrient assimilation and hydrodynamics. The nutrient uptake and assimilation by the phytoplankton transfers energy and materials to higher trophic levels and the hydrodynamics involve the transport and dilution of nutrients and planktonic organisms.

Therefore, the nutrients input can increase not only the phytoplankton, but also all food web. Protozooplankton play an essential role in the carbon transfer of the microbial food web to higher trophic levels (YANG et al., 2012). The ciliates are sensitive to environmental alterations, and their community fluctuations can affect the food web and the energy transfer (CHEN et al., 2009). The zooplankton is a group of organisms that can also be used as biological indicators, once they respond to low dissolved oxygen, high nutrient levels and toxic contaminants (CASÉ et al., 2008). In the Patos Lagoon the protozooplankton is comprised of flagellates, dinoflagellates and ciliates (ABREU; ODEBRECHT, 1998). The largest biomass of this group is found during spring, while the smallest occurs during winter (ABREU et al., 1992). The zooplankton species in this lagoon is strictly related to local hydrological conditions (MONTÚ et al., 1998), and the most abundant organisms are copepods of *Acartia* genus, only the *Acartia tonsa* occurring in high densities (> 40.016 organisms/m^3) (MONTÚ et al., 1997).

Previous studies showed that the shrimp farm effluents discharge can cause short term changes in Patos Lagoon (CARDOZO; ODEBRECHT, 2012). However, no detailed analyzes on spatial-temporal variation of water quality and plankton organisms were available. Thus, this study aim to evaluate the *L. vannamei* farm effluents effects on the water quality, phytoplankton, proto and meso-zooplankton in an estuary in Southern Brazil.

MATERIAL AND METHODS

Study site and sampling procedures

This study was conducted in the Patos Lagoon estuary adjacent to a commercial *L. vannamei* shrimp farm (Rio Grande do Sul, Brazil - 31º56'04S, 52º00'11W). The earthen ponds were filled with water from the estuary, and before stocking it was proceeded the fertilization with 270 kg of urea ($(NH_2)_2 CO$) and 28 Kg of calcium triple superphosphate ($Ca (H_2PO_4)_2$) to stimulate phytoplanktonic growth. The facility works in a semi-intensive system, and the stocking density was 12 shrimp/m² with a total area of 3.75 ha. Shrimps were fed with commercial pellets (35% of crude protein). The cycle lasted 112 days; there was no effluent discharge during the culture period; water was only added in order to compensate the evaporation and soil infiltration. Both ponds share a 1 ha vegetated sedimentation basin, which was empty until the end of the cycle.

The sampling sites were assigned as follows: across the EDC (1); in 20 m from the channel (2 and 3); 30 m from the channel (4 and 5) and two control sites located at 100 m and 250 m from channel (C1 and C2, respectively) (Figure 1). In addition to these sites, the ponds and the sedimentation basin were also sampled. This study was conducted from February to April 2012, and samplings were taken before the discharge (BD), 1-day post-discharge (1 PD), 5 days post-discharge (5 PD), 10 days post-discharge (10 PD), 20 days post-discharge (20 PD) and 30 days post-discharge (30 PD).

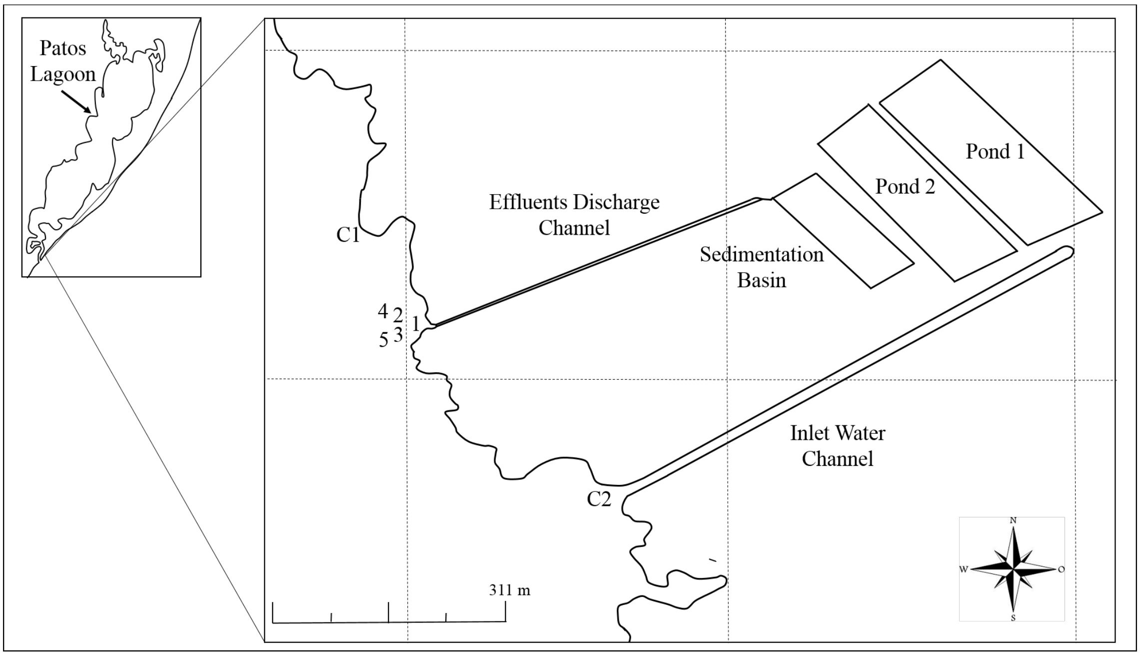


Figure 1: Sampling sites in the shrimp farming. C1 = Control 1, C2 = Control 2, 1 = Effluents discharge channel, 2 and 3 = 20m from the channel, 4 and 5 = 30m from the channel, P1 = Pond 1 and P2 = Pond 2.

Water quality

Temperature (WTW Oxi 3205), dissolved oxygen (WTW Oxi 3205), pH (YSI 60) and salinity (refractometer) were measured *in situ*, with three replicates. Meteorological data were obtained from the Meteorological Station - Federal University of Rio Grande. At each site, three replicates were taken from the water surface for chlorophyll *a*, total ammonia nitrogen (UNESCO, 1983), nitrite (BENDSCHNEIDER; ROBINSON, 1952), nitrate and total phosphorous (AMINOT; CHAUSSEPIED, 1983).

At these same sites water was collect from the surface and placed in amber glass flasks containing formaldehyde 4% solution for further evaluation of phytoplankton and protozooplankton identification and density estimative. The aliquots were analyzed in sedimentation chamber (2.1 ml) under inverted microscope following the Utermöhl method (HASLE, 1978). Ciliates and diatoms were counted in all chamber's area under 20X magnification. Chlorophyceae and cyanobacteria were counted in random fields under 10X magnification until <30% coefficient of variation was reached.

Samples for zooplankton analysis were collected using cylindrical-conical net with 30cm in diameter (150 µm mesh) fitted with a mechanical flow meter attached to the net mouth. The net was hauled at surface covering an average distance of 15 meters. The collected material was transferred to 100 mL glass flasks containing formaldehyde 4% solution. Sub-samples were then transferred to Bogorov chambers and analyzed under a stereoscopic microscope (BOLTOVSKOY, 1981). The zooplankton specimens were identified to major groups.

For the chlorophyll *a*, 25 mL aliquotes were filtered (Whatman GF/F) and extracted with acetone 90% in the dark and the concentration was estimated measuring the fluorescence in a calibrated fluorometer (Turner TD-700). (WELSCHMEYER, 1994).

Statistics

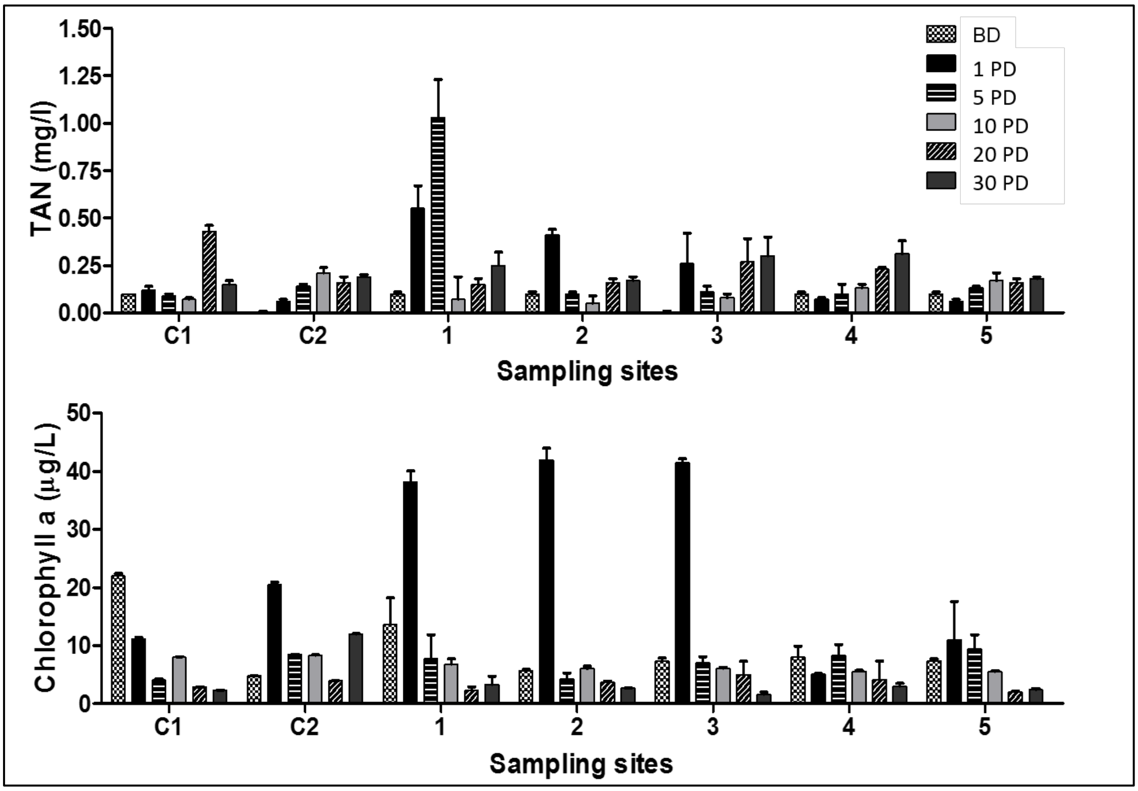
Zooplankton data were compared with the non-parametric Kruskal-Wallis test, with 5% significance (SOKAL; ROHLF, 1995).

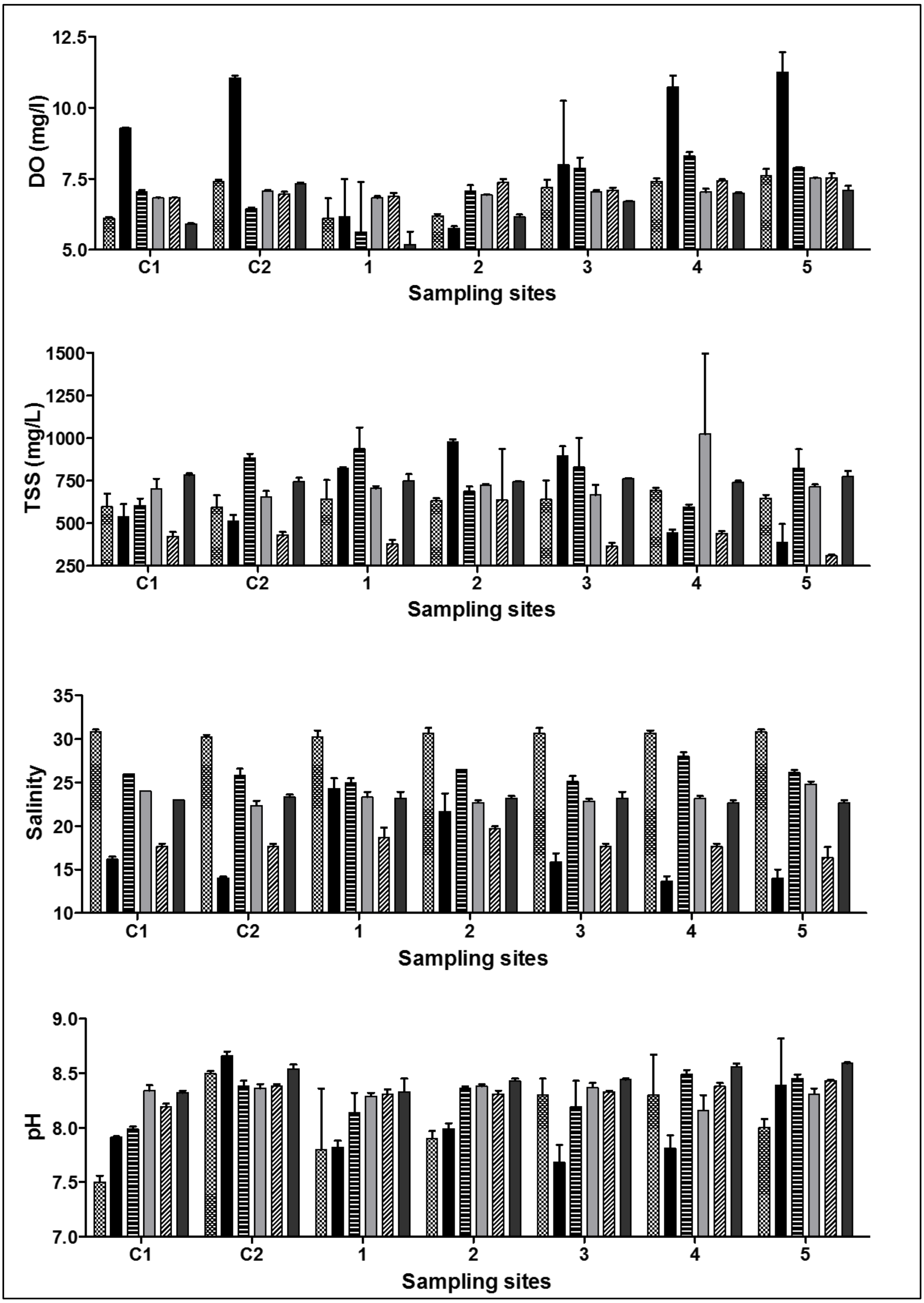
RESULTS

Water quality in the ponds and in the receiving estuary

Water quality parameters, as well as the chlorophyll a values are shown in Figure 2. The total ammonia nitrogen (TAN) had small variation over the sampling period and sites, showing only two peaks observed in sample 1 PD (0.55 mg / L) and 5 PD (1.03 mg / L) in site 1. The nitrite, nitrate and phosphate had null values in all samples, both in the ponds and in the estuary. The only exception was the low nitrite concentration observed on the first day after the discharge (1 PD) in sites 1 (0.01 ± 0mg / L), 2 (0.01 ± 0.01mg / L) and in the sedimentation basin (0.02 ± 0.01mg / L).

The chlorophyll a concentration in ponds and sedimentation basin are shown in Table 1. Over the different sites, it ranged from 1.8 to 20 mg / L, except for the first day post discharge, where the sites 1, 2 and 3 showed mean values of 38.17 ± 1.89, 41.98 ± 1.97 and 41.47 ± 0.66 mg / L, respectively. The dissolved oxygen ranged from 5.19 to 11.25 mg / L, showing little spatio-temporal variation, except in sampling 1 PD, where it was observed lower values at the effluent discharge channel. The total suspended solids (TSS) ranged from 308.3 to 1023.3 mg / L, and concentrations increased in PD 1 and 5 PD with the plume reaching 20 m away from the effluent channel discharge. The salinity varied from 13.33 to 31. There was an increase in the sites next to the margin as a result of ponds water (that had higher salinity) discharge. The pH ranged between 7.47 and 8.56, having no apparent relation with the release of effluents. The water turbidity was 1.93 to 43.83 NTU, and it can be noticed an increase in sites 1, 2 and 3 on 1 PD and site 1 on 5 PD (Fig. 2). The temperature had only seasonal variation between sampling stations (20.67 ° C to 30.8 ° C).





DO (mg/l)
12.5
10.0
7.5
5.0
C1
C2
1
2
3
4
5
Sampling sites
TSS (mg/L)
1500
1250
1000
750
500
250
C1
C2
1
2
3
4
5
Sampling sites
Salinity
35
30
25
20
15
10
C1
C2
1
2
3
4
5
Sampling sites
pH
9.0
8.5
8.0
7.5
7.0
C1
C2
1
2
3
4
5
Sampling sites

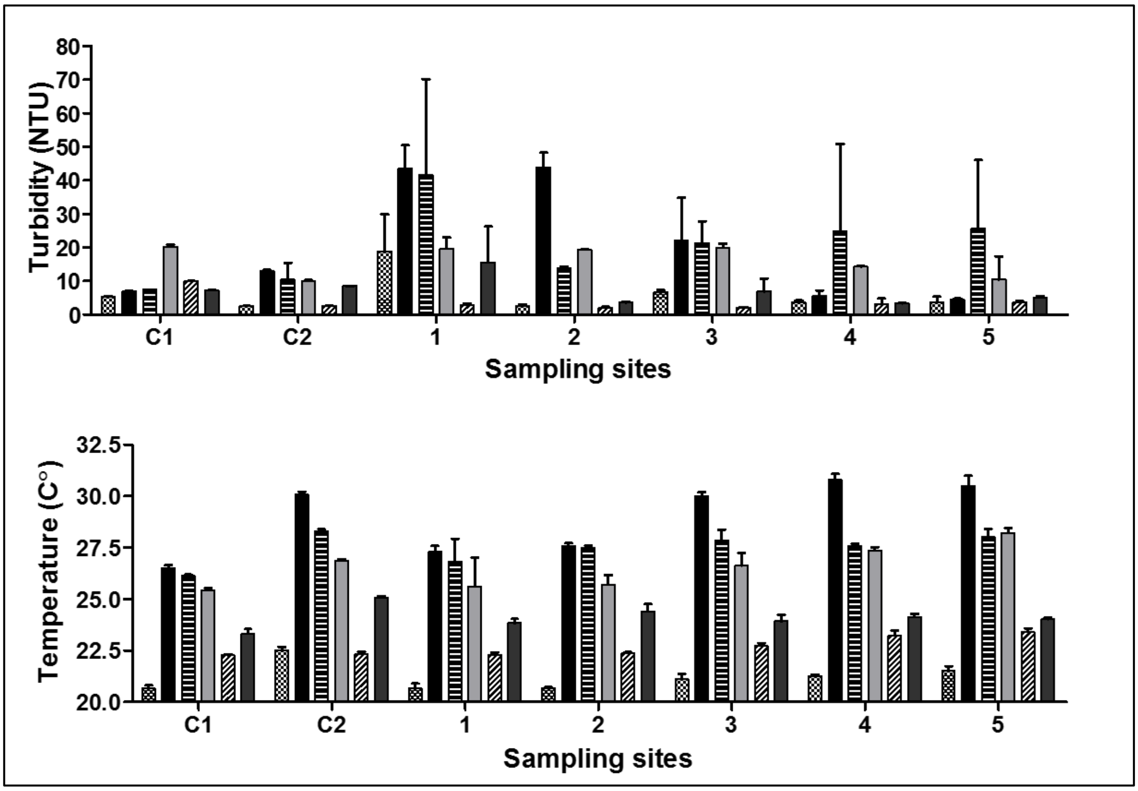


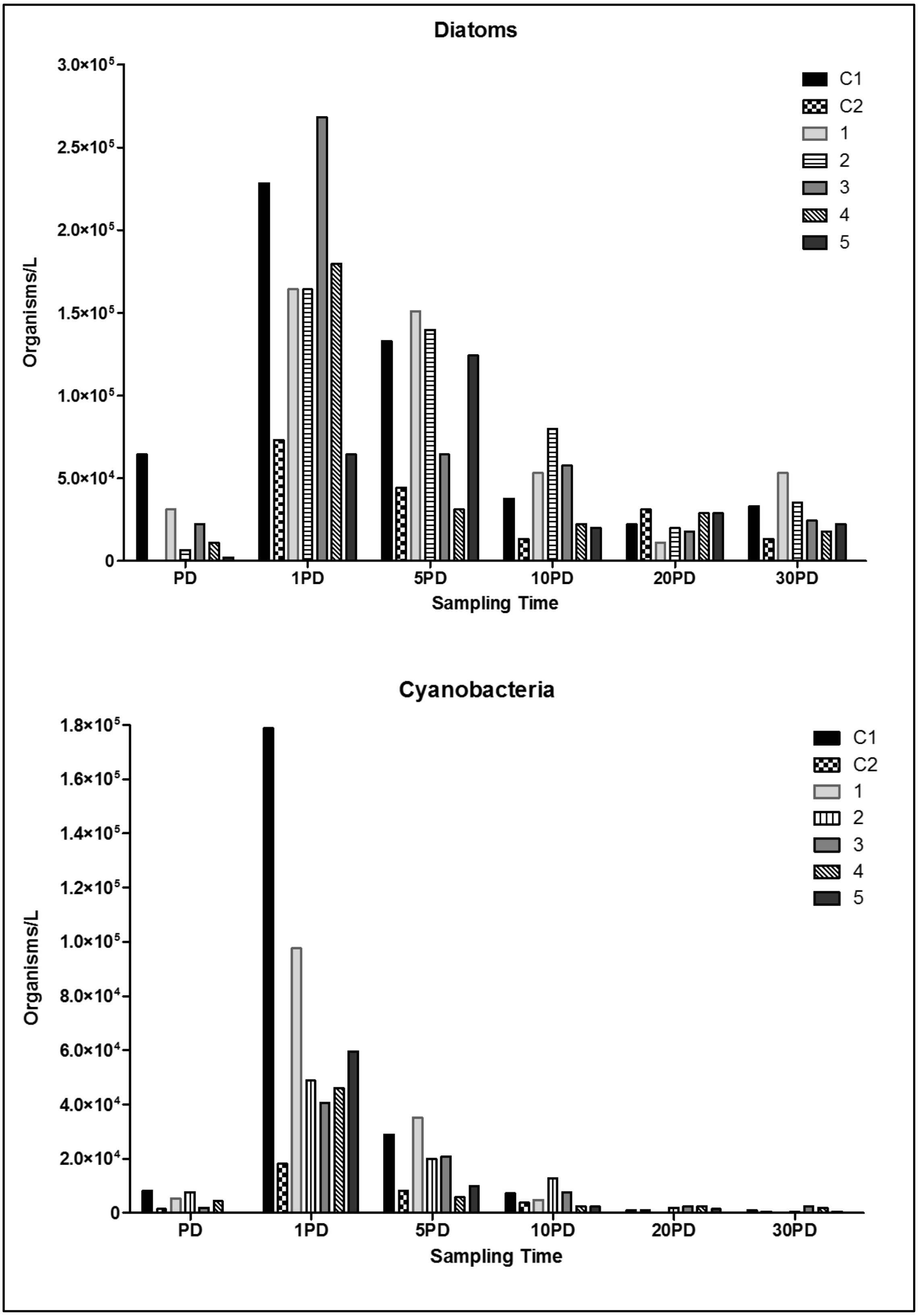
Figure 2. Water quality (mean ± SD) over sampling period in sites control (C1 and C2), effluents channel discharge (1), 20 m (2 and 3) and 30 m (4 and 5) from the effluents channel discharge. TAN = Total Ammonia Nitrogen; DO = Dissolved Oxygen; TSS = Total Solid Suspended.

Table 1. Water quality values (mean ± SD) from ponds 1 and 2 and sedimentation basin in the moment of shrimp harvest.

	Pond 1	**Pond 2**	**Sedimentation Basin**
Total ammonia nitrogen (mg/L)	0,94±0,05	0,69±0,03	1,1±0,03
Chlorophyll a (µg/L)	32,96±7,14	36,47±2,01	40,75±0,55
Dissolved oxigen(mg/L)	3,73±0,02	4,50±0,03	3,34±0,02
Total solid suspended (mg/L)	580±62,45	635±108,28	821,67±79,11
Salinity	24,17±0,29	23±0,00	23±0,00
pH	7,79±0,05	7,89±0,01	7,67±0,04
Turbidity (NTU)	25,8±0,17	26,17±0,06	8,24±0,01
Temperature (°C)	26,1±0,10	26,43±0,12	26,43±0,21

Phytoplankton and protozooplankton communities

The phytoplankton and protozooplankton communities in the estuary were analyzed through the identification and quantification of the following groups: chlorophyceae, diatoms, cyanobacteria and ciliates. Their spatio-temporal distribution is shown in Figure 3. The predominant group was the chlorophyceae, followed by diatoms, cyanobacteria and ciliates. Diatoms, cyanobacteria and ciliates were more abundant at all points on 1 PD and there was also an increased concentration of Chlorophyceae on 1 PD comparing to pre-harvest sampling.



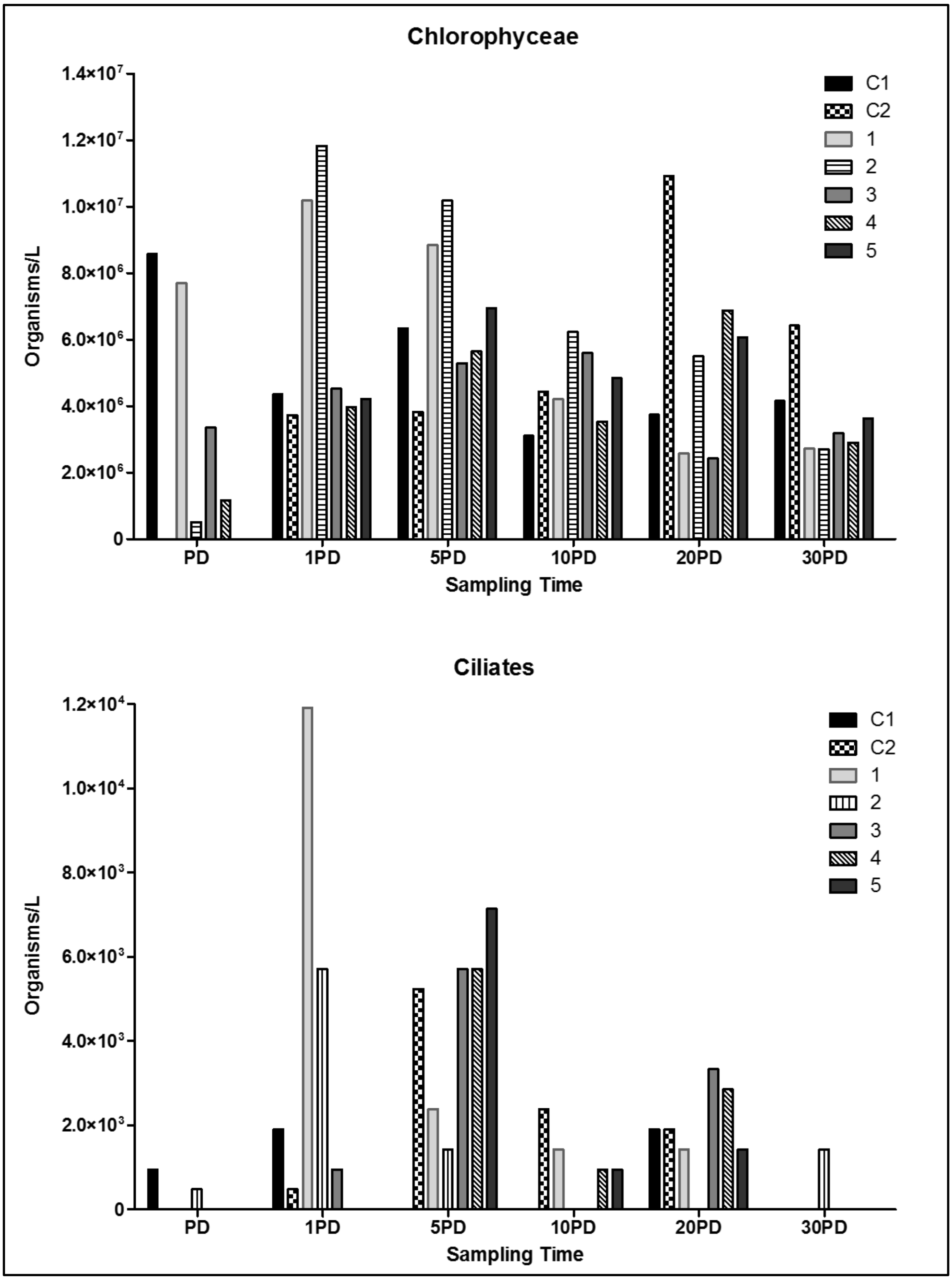


Figure 3. Phytoplankton and protozooplankton communities spatio-temporal variation in sites control (C1 and C2), in front of effluents discharge channel (1), 20 m (2 and 3) and 30 m (4 and 5) from the effluents discharge channel.

Zooplankton abundance was low in almost all samples, except in 30PD when some sites showed peaks (Fig. 4). Copepods were dominants, mainly *Acartia tonsa* species.

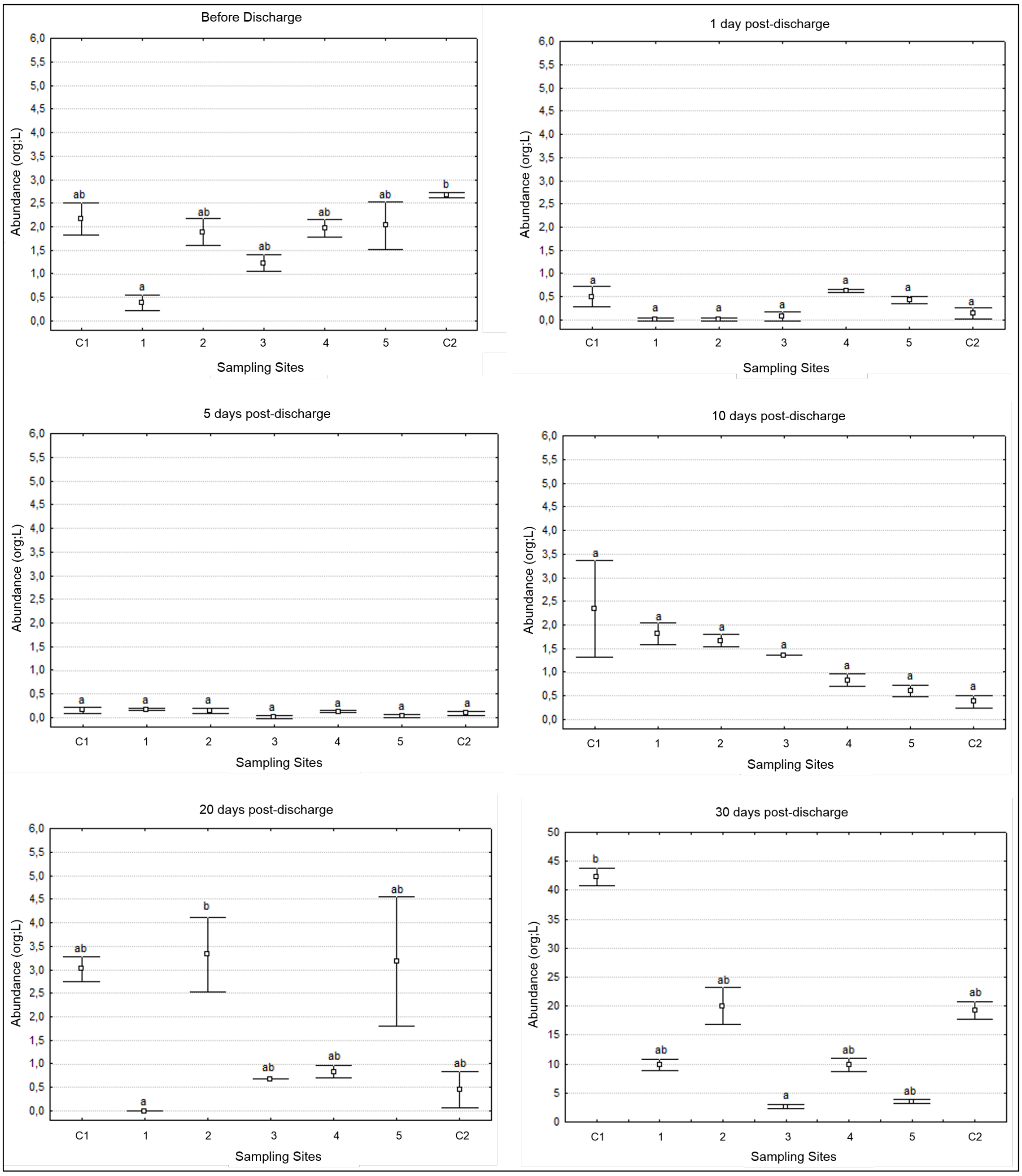


Figure 4: Total abundance of meso-zooplankton (org/L) over different sites distributed spatiotemporally. * Figures in different scales.

DISCUSSION

The main nutrient sources in shrimp farming come from aquafeeds and organic and inorganic fertilizers used to increase the *production* of phytoplankton

(HARGREAVES, 1998). Only the commercial feeds are responsible for the input of 76% of nitrogen and 83.4% of the phosphorus in the system, depending on management strategies (PÁEZ-OSUNA et al., 1997). Hence, the pond water becomes richer in nutrients, suspended solids, plankton and oxygen demand, when compared to coastal receiving water bodies (SCHWARTZ; BOYD, 1994). As there were no water exchange during the cycle period, nutrient absorption and plankton production dynamics were gradually increasing until the end of the grow out period when the feed intake was higher.

All ponds water is discharged in a vegetated sedimentation basin prior to reach the estuary water receiving. The sedimentation basin improves effluents water quality through the transport of suspended solids, nutrient cycling, biomass production, nutrient absorption by plants and animals, and distribution of organic matter and oxygen (SHPIGEL et al., 2013). JACKSON et al. (2003) report a reduction of 60% of TSS, 23% of the total nitrogen and 35% of total phosphorus in the effluent after the passage in the sedimentation basin. However, even with the basin, part of nutrients and plankton eventually reach the estuary. These compounds may favor an increase in the natural productivity of the water body receptor (TACON; FORSTER, 2003) or may be quickly diluted.

Accordingly, the local hydrodynamics and the environment carrying capacity can advantage the low residence time of the exported material. The Patos Lagoon is characterized by its strong hydrological interaction between wind and river discharge (KJERFVE, 1986; MÖLLER; FERNANDES, 2010). MÖLLER et al. (1996) also report that in shallow areas of the Patos Lagoon estuary predominate NE winds, which induce water exchanges and low residence times. The low estuarine salinity observed in 1 PD may be related to the receding water flows caused by wind and / or rain, which may also have diluted the nutrients and organisms from the effluents. The results indicate that water quality parameters had little variation during the trial, except for the temperature. Total ammonia nitrogen, chlorophyll a and turbidity were the most variable parameters. However, these alterations occurred only in the first days after the effluent discharge and they were restricted to 20m away from the EDC, showing that the estuary had a quick recovery.

The nutrients load is often used as an indicator of water quality. The sampling sites 1, 2 and 3 exhibited the higher TAN values in 1 PD, whereas nitrite, nitrate and phosphate in the effluent were practically null. The phytoplankton community play an important role in the nitrogen absorption preventing the accumulation to toxic levels in shrimp ponds (BURFORD, 1997; GLIBERT; BURFORD, 1999), and consequently in the effluent. Similar values are reported to the Patos Lagoon estuary (KANTIN; BAUMGARTEN 1982; ABREU et al, 1995) and to areas close to shrimp farms (BRIGGS; FUNGE-SMITH, 1994; BURFORD, 1997; CARDOZO et al, 2011).

The concentration of chlorophyll a also showed little variability over the sample period, and the values are close to those reported in the literature for the region (ABREU et al, 2010; CARDOZO et al 2011), despite the higher value in 1 PD sample. The highest concentrations were noted next to the effluents discharge channel, and values were elevated until sample 5 PD. This is probably due the high levels also found in the ponds and in the sedimentation basin, not being specifically an increase of the primary productivity in the environment due to the effluents release.

In general, cyanobacteria, diatoms and ciliates showed similar pattern in space and time, having their density increased in 1 PD sample, not necessarily related to the discharge of effluents. In addition to the effects on the water quality, restricted to 20 m away from the effluents discharge channel, freshwater input by rains may have been an important nutrients source for phytoplankton and protozooplankton development. However, no data on water quality between samples 20 PD and 30 PD is available to prove the hypothesis of increased levels of nutrients in the water. ABREU et al. (2010) found no relationship between chlorophyll a levels in Patos Lagoon estuary and abiotic factors, but there was a significant relationship between the mean annual values of chlorophyll a and average rainfall in the estuary.

The salinity of Patos Lagoon estuary have a defined pattern: more saline waters when SO winds are predominant and have low river discharge, and less saline waters in ebb from the increase in river discharge (MÖLLER; FERNANDES, 2010). CARDOZO; ODEBRECHT (2012) also reported that major differences in salinity between the pond and the environment result in lower levels of chlorophyll a and primary production, because the immediate inhibiting on microalgae growth. Thus, it may also have inhibited the growth of phytoplankton and protozooplankton released through effluents.

The ciliates abundance and diversity has been used as a water quality indicator and ecosystems dynamics (FOISSNER, 1988). Heterotrophic ciliates feed on microorganisms in aquatic ecosystems and they are important in the energy flow (SHERR; SHERR, 1988; DECAMP et al., 2003). These organisms serve as food for fish larvae, playing a key role in the link between the microbial loop and the higher trophic levels (FUKAMI ET AL., 1999). YANG et al. (2011) showed that the community and abundance of protozooplankton usually follows the spatial dynamics and abundance of phytoplankton, which was observed in this study.

Nonetheless, there is another hypothesis on phytoplankton and protozooplankton increased abundance on sample 1 PD. The local hydrodynamics may have dispersed the effluent plume, which could indicate that the chosen control sites were not effectively far enough from the EDC. No sample of ponds was collected to analyze the composition and abundance of these organisms, being impossible to determine the main groups exported to the environment by the discharge of effluents. In culture ponds the occurrence of phytoplankton species can be temporary or long-term (ALONSO-RODRÍGUEZ; PÁEZ-OSUNA, 2003). But there is no consent on the dominance of a certain group since variability depends on several factors such as light, water temperature, nutrient availability and predation by meso-zooplankton (BURFORD, 1997; FUJITA; ODEBRECHT, 2007; ABREU et al., 2010). Nevertheless, even if the effluent has been responsible for the increase in phytoplankton and protozooplankton abundance in 1 PD, the lack of favorable conditions for their development dispersed these organisms.

The zooplankton community was dominated almost exclusively by the copepod *Acartia tonsa*. This is a dominant estuarine species that depends partly on tide transport mechanisms (MONTÚ et al., 1998). This species is significant in the secondary production of estuary food web, and several studies have focused in the Acartiidae family (IRIGOIEN; CASTLE, 1995; ARA, 2001). The zooplankton concentration was low over the sampling sites and showed few oscillations, except for sampling 30 PD. Low zooplankton abundance is reported by CARDOZO et al. (2011) for the same region. Despite the increase in the abundance of cyanobacteria, diatoms and ciliates in sample 1 PD, there was no increase in zooplankton abundance. That could be expected because of the greater availability of food. The occurrence and abundance of zooplankton species in Patos Lagoon is mainly determined by seasonal variations of salinity, temperature, wind direction and intensity, freshwater discharge and food supply (MCLAREN; CORKETT, 1981;. MONTÚ et al, 1998; MUXAGATA et al 2012). However, it was not possible to identify the relationship between the increased zooplankton abundance in sample 30 PD and the tested parameters that could affect these organisms. HIRST; BUNKER (2003) report that the chlorophyll a can be used as a good indicator of food availability for copepods. A constant concentration of chlorophyll a shows that herbivory action was not markedly present, different from the results of CARDOZO et al. (2011), which reported an opposite pattern between chlorophyll a and zooplankton density levels. The estuarine water temperature, which seems to influence the reproduction of *A. tonsa* (MONTÚ et al., 1997), showed little variation during the sampling period. Likewise, temporal data on wind strength (INMET) shows that the wind was constant and had low speed during the sample period. The input of saline water into the estuary favors marine species such as *A. tonsa* to dominate the zooplankton community (MONTÚ et al., 1997). Analysis on salinity data between samples 20 PD and 30 PD also revealed no salt wedge from entering the estuary that could be related to this abundance increase. One possible explanation is the decrease in predation action since several species of fish larvae and juveniles depend on Patos Lagoon estuary to feed (VIEIRA et al., 1998). Sampling 30 PD coincided with the transition between summer and autumn, and the estuary temperature has great influence on the ichthyoplankton, where the greatest abundance of fish eggs and larvae occurs in summer (SINQUE; MUELBERT 1998).

In addition to the environmental variables favorable to disperse the effluents, other factors may have contributed to the low pollution potential of the effluents. According to ALONSO-RODRÍGUEZ; PÁEZ-OSUNA (2003), feeding management and the use of appropriate technology can limit shrimp farming environmental impact. Despite the changes observed in some water quality parameters, the levels of chlorophyll a and phytoplankton, protozooplankton and meso-zooplankton abundance in the first days after the release of effluents suggest that the carrying capacity of environment was not exceeded.

The biotic and abiotic parameters analyzed in this study indicate that the proper management and the favorable environmental conditions were extremely important on generating low potential impact effluent in the shrimp farm. All parameters were in accordance with the environmental standards set out in Brazilian legislation, having minimal environmental impacts on Patos Lagoon estuary.

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CAPÍTULO 2

IMPACTO DOS EFLUENTES DA CARCINOCULTURA SOBRE A COMUNIDADE MACROZOOBENTÔNICA DA LAGOA DOS PATOS, BRASIL

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IMPACT OF SHRIMP FARM EFFLUENT ON MACROZOOBENTHOS COMMUNITY IN PATOS LAGOON ESTUARY, SOUTHERN BRAZIL

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Keywords: Macrozoobenthos, Effluents, Sediment, *Litopenaeus vannamei.*

SHRIMP FARM IMPACT ON MACROZOOBENTHOS IN BRAZIL

Seção: Ciências Agrárias (Aquicultura).

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ABSTRACT

The Pacific white shrimp *Litopenaeus vannamei* production in earth ponds has been increasing in the surrounding of the Patos Lagoon estuary, Southern Brazil. This study evaluated the spatio-temporal effects of shrimp farming effluent on macrozoobenthos community over two cycles period (2012 and 2013). The samples were collected before and after the effluents discharge in the estuary receiving and the sampling sites were assigned as follows: across the effluents discharge channel (EDC), 20 m, 30 m, 100 m and 250 m from the EDC. Collected sample were sieved through a 500µm mesh size, sorted, identified to lowest possible taxonomic level and counted. Seven groups were recorded (Polychaeta, Tanaidacea, Isopoda, Gastropoda, Bivalvia, Malacostraca and Ostracoda) along the sampling campaigns. Spatially, the density and species richness had little variability over the sampling sites in both campaigns. Temporally, during 2012 campaign, these indices increased over time in winter compared to summer, unlike 2013 campaigns, where the density decreased in colder months and species richness suffer little variability. The results show that the effluents discharge not influenced the macrozoobenthos community and the statistical differences recorded ($p < 0.05$) were probably caused by natural fluctuations of environmental parameters, natural in estuarine ecosystems.

INTRODUCTION

The artisanal fishery of *Farfantepenaeus paulensis* shrimp is a significant activity in the Patos Lagoon estuary with socio-economic importance. The production is extremely variable and depends on environmental conditions (Castello and Möller 1978; D'Incao and Reis 2002). The aquaculture of Pacific white shrimp *Litopenaeus vannamei* in this estuary emerged as an alternative to meet the market demand of this seafood. However, as a growing agribusiness its environmental sustainability should be conducted carefully. Several studies evaluated the potential environmental impacts of aquaculture around the world (Carroll et al. 2003; Crawford et al. 2003; Forchino et al. 2011), and the aquatic environmental degradation by effluents discharge is a main concern.

The aquaculture effluents are usually rich in nutrients and organic solids (Burford et al. 2003) from fertilizers, phytoplankton, unconsumed feed and animal excretion (Jackson et al. 2004). This particulate organic matter input acts as a source of organic carbon for a diverse community of filtering and decomposers organisms (Albertelli et al. 1999). These organisms are susceptible to environmental fluctuations and disorders that impact them in different time scales (Gray and Christie 1983). In some cases, the disturbance caused by the effluents can cause physical and chemical changes in the sediment. Thus, it can reduce the biological diversity and favor opportunistic species (Johannssen et al. 1994), which affects the richness and the density of the macrozoobenthic community (Heip 1995). Anaerobic zones may occur and toxic compounds such as ammonia, hydrogen sulfide and methane are released when the organic accumulation rates exceed the carrying capacity of the substrate (Alongi et al. 1999). These extreme conditions lead to the destruction of the environmental quality and community structure (Islam 2005), conducting to the impoverishment / disappearance of fauna (Heilskov and Holmer 2001).

Several studies have focused on the evaluation and mitigation of the impacts of aquaculture effluents on benthic communities (Bartoli et al. 2001; Carvalho et al. 2009; Aguado-Giménez et al. 2011). These communities are used as a sensitive tool to detect environmental effects (Carroll et al. 2003), and they can be useful to monitor the impacts of aquaculture effluents. Benthic organisms are the main link between primary producers and higher trophic levels in estuarine regions (Foreman et al. 1995).

Moreover, they play a crucial role in the supply and re-mineralization of organic matter (Heilskov and Holmer 2001).

The composition and abundance of macrozoobenthos were examined in several studies in Patos Lagoon estuary (Bemvenuti et al. 1978; Capitol et al. 1978; Rosa and Bemvenuti 2006; Colling et al. 2007). However, studies analyzing the impact of shrimp farming effluents on estuarine macrozoobenthos are scarce. This study aims to evaluate the macrozoobenthos subjected to shrimp farm effluents in the Patos Lagoon estuary, analyzing the spatio-temporal variations of the density and species richness.

Material and Methods

Study area and shrimp farm characteristics

This study was conducted in a commercial *L. vannamei* farm located in the Patos Lagoon estuary, Southern Brazil (Rio Grande do Sul - 31º55'S, 52º00'W - Fig. 1). The ponds with 3.75 ha area was stocked with 12 shrimps/m² (semi-intensive system). Because of the low temperatures during winter, this farm works on a 1-cycle per year, and after each cycle the ponds are drained and dried. During the cycle period there was no effluents discharge, and water was added in the ponds only to compensate the evaporation and infiltration. Before the discharge, the effluents were transferred to a vegetated sedimentation basin in order to decrease the load of nitrogen, phosphorous and suspended solids.

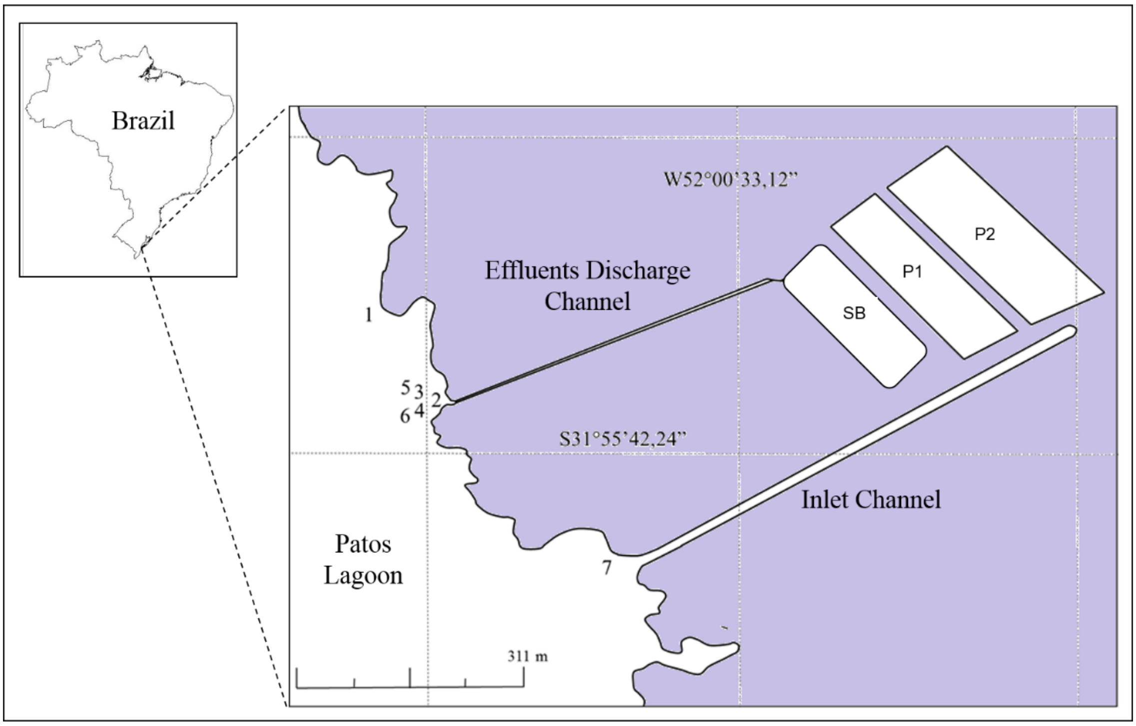

Figure 1: Sampling sites in the shrimp farming. 1 = Control 1, 7 = Control 2, 2 = Effluents discharge channel, 3 and 4 = 20m from the channel, 5 and 6 = 30m from the channel, P1 = Pond 1, P2 = Pond 2 e SB = Sedimentation basin.

Sampling plan

Sampling was conducted based on BACl (Before-After-Control-Impact) design with some modifications (Underwood 1994). Samples were taken between summer and winter during 2 cycles periods (2012 and 2013), in a total of six campaign per year. Seven sites were previously chosen to water quality analysis and macrozoobenthos sampling (Fig. 1): two control sites, located in 100 m and 250 m from the EDC (1 and 7); site 2, in front of the EDC; sites 3 and 4, located 20 m from the EDC; and sites 5 and 6, 30 m away from the EDC. Between February and July 2012 the sampling was conducted as follows: prior to the release of the effluents (BD) and 5, 10, 30, 60 and 90 days after the effluents discharge (5 PD, 10 PD, 30 PD, 60 PD e 90 PD, respectively). Due to an emergency shrimp harvest caused by bacterial outbreak in 2013, no BD sample from this year is available. Thus, between February and May 2013 the samples were performed immediately after the discharge (1 PD) and as the previous year: 5 PD, 10 PD, 30 PD, 60 PD and 90 PD.

Water quality parameters and macrozoobenthos

Temperature (WTW Oxi 3205), dissolved oxygen (WTW Oxi 3205), salinity (refractometer) and pH (YSI 60) were measured *in situ* (n=3) in all sampling sites. At each site, three macrozoobenthos sub-samples were taken using a corer PVC (0,008 m^2, 20cm depth). Sample were sieved *in situ* through a 500µm mesh size, fixed in 4% buffered formaldehyde to wich Rose Bengal stain added. In laboratory, samples were sorted and identified at lowest possible taxonomic level and counted. Samples were also taken in surface layer (interface substrate/water) to analyze sediment characteristics. The granulometry composition were obtained through sieving (> 0,063 mm) and pipetting analysis (< 0,063 mm) according Suguio (1973).

Statistical analysis

The data were tested for normal distribution before choosing parametric or non-parametric statistical methods. To assess significant spatial and temporal differences in density and richness species of monitored points, Analysis of Variance (ANOVA 1 way) and post-hoc Tukey tests were performed ($P < 0.05$). When the assumptions for normality and equal variance were not observed, Kruskal-Wallis followed by post-hoc Dunn was used to determine significant differences.

RESULTS

The water column depth during the sampling period ranged from 10 to 100 cm, but it has not found a relationship between the season and the highest or lowest level of water column. Particle size analysis revealed predominantly sandy substrates (43-51%) with low percentages of silt / clay, with small spatial variations. Temporal variations in temperature (due to seasonal variations) and salinity (caused by flood flows / ebb and / or rain) were observed (Table 1). The dissolved oxygen and pH values remained high throughout the sample period and showed little variability.

Table 1: Abiotic parameters (mean±SD) in different sites through the sampling period (2012 and 2013) in Patos Lagoon estuary before and after shrimp farm effluents discharge. *n.a. = not available.

		Temperature (°C)	**Dissolved Oxygen (mg/L)**	**Salinity**	**pH**
2012	**BD**	29,07±1,77	6,92±0,75	30,59±0,49	8,00±0,43
	5PD	26,51±1,11	7,03±1,13	26,00±1,09	8,26±0,21
	10PD	22,58±0,41	7,02±0,22	23,37±0,88	8,31±0,07
	30PD	21,26±0,71	6,44±0,82	23,00±0,51	8,44±0,12
	60PD	15,68±0,50	7,18±0,82	20,06±0,17	7,88±0,04
	90PD	11,87±0,74	8,65±0,77	15,34±0,32	7,64±0,13
2013	**1PD**	27,38±0,91	6,21±1,12	23,37±0,50	7,52±0,36
	5PD	24,5±0,23	8,11±0,63	30,00±1,11	7,99±0,10
	10PD	21,31±0,31	8,89±0,13	14,53±1,04	7,98±0,14
	30PD	22,83±0,95	8,63±0,41	30,53±0,63	8,08±0,27
	60PD	16,21±0,1	9,55±0,09	13,50±0,44	7,49±0,24
	90PD	*n.a.	n.a.	7,31±1,77	7,61±0,07

Three major taxonomic groups were recorded along the sampling campaigns: Malacostraca, Polychaeta, phylum Mollusca and Ostracoda. The most diverse class was Malacostraca (6 taxa), followed by Polychaeta (5 spp.), phylum Mollusca (two species of bivalves and a Gastropoda), and Ostracoda (Table 2).

Table 2: Mean density (ind. m^{-2}) of macrozoobenthos identified in 2012 and 2013.

Taxa	Density (ind. m^{-2})	
	2012	**2013**
Polychaeta (Class)		
Laeonereis acuta	1.905	965
Heteromastus similis	1.674	1.094
Alitta succinea	1.259	375
Paraprionospio pinnata	176	0
Nephtys fluviatilis	172	74
Malacostraca (Class)		
Kalliapseudes schubartii	87	290
Ampithoe sp.	77	1
Farfantepenaeus paulensis	1	0
Cassidinidea fluminensis	0	1
Sphaeromopsis mourei	1	55
Callinectes sapidus	7	1
Mollusca (Phylum)		
Heleobia australis	7	41
Tagelus plebeius	3	0
Erodona mactroides	2	0
Ostracoda	47	0

In 2012 sampling, eight taxa accounted for > 99% of the total recorded density, including the polychaete *Laeonereis acuta* (35.1%), *Heteromastus similis* (30.9%), *Alitta succinea* (23.2 %), *Paraprionospio pinnata* (3.2%), *Nepthys fluviatilis* (3.1%), crustaceans *Kalliapseudes schubartii* (1.6%), *Ampithoe* sp. (1.4%) and Ostracoda (0.9%). Spatially, species richness (Kruskal-Wallis) and the density of macrozoobenthos (ANOVA) showed no significant differences over the sampling sites ($P > 0.05$); however, the total density and species richness over time were significantly affected (Kruskal-Wallis, $P < 0.05$). It can be observed a pattern of increase in both attributes during winter when compared to summer (Fig. 2A, 2C).

On the 2013 production cycle, seven species accounted for > 99% of the total recorded density. A number of 2.848 individuals were sampled - approximately 3 times smaller than the previous year: *H. similis* (37.8%), *L. acuta* (33.3%), *A. succinea* (12.9%), *K. schubartii* (10%), *N fluviatilis* (2.6%), *Sphaeromopsis mourei* (1.9%) and *Heleobia australis* (1.4%). The density of organisms differed significantly ($p < 0.05$) between sites in 5 PD, 30 PD, 60 PD and 90 PD, but this occurred in a random way without relationship with effluents discharge. Over time, the only significant difference found occurred between 1 PD and 90 PD samples ($P < 0.05$) (Fig. 2B). As in 2012, macrozoobenthic diversity did not show significant spatial differences ($P > 0.05$), only temporal ($P < 0.05$) (Fig. 2D).

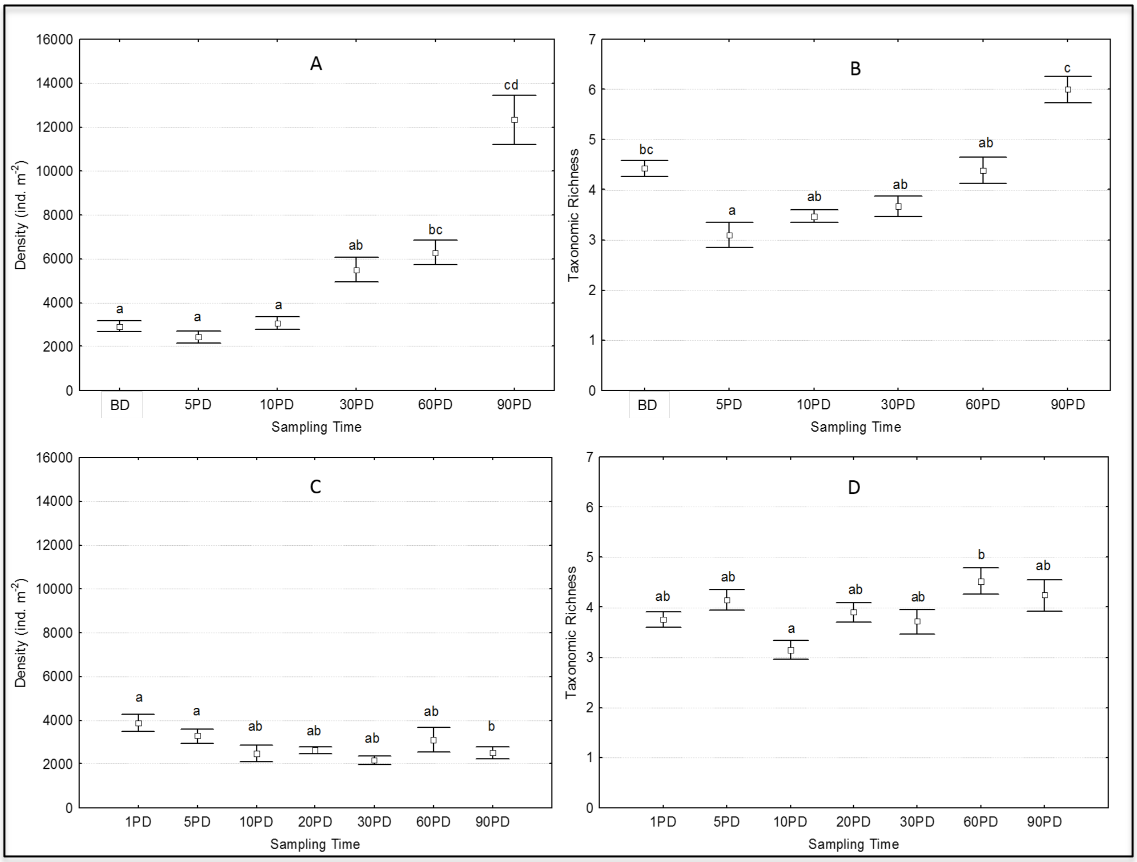

Figure 2: Macrozoobenthos parameters (mean value ± SE) over sampling campaigns: Density (ind. m^{-2}) and Taxonomic richness in 2012 (A and B) and 2013 (C and D). Different letters denote significant ($p < 0,05$) differences. BD = Before discharge; 1PD = 1 day post-discharge; 5PD = 5 days post-discharge; 10PD = 10 days post-discharge; 20PD = 20 days post-discharge; 30PD = 30 days post-discharge; 60PD = 60 days post-discharge and 90PD = 90 days post-discharge.

DISCUSSION

The Patos Lagoon is characterized by high temperatures in summer and low rainfall rates, which favor the income of seawater into the estuary, increasing the salinity (Rosa and Bemvenuti 2006). These authors report that in winter the opposite situation occurs, which reduces the salinity. In this study, temperature and salinity showed visible seasonal patterns over the sample period. The macrozoobenthos in this lagoon has their spatial and temporal distribution affected by many factors, including water quality, substrate characteristics, and biological interactions (Bemvenuti and Colling 2010). However, environmental parameters such as temperature and salinity may have a significant influence on this community that inhabits subtropical and temperate regions with marked seasonal fluctuations (Gray and Elliott 2009). The macrozoobenthos community did not show a spatial variability in both sampling campaigns (2012 and 2013), since the differences were punctual and not related to the effluents release due to their random nature.

The 2012 sampling began in the summer and finished in winter. In 2013, the sample campaign began in the summer and ended in the autumn. Temporally, the macrozoobenthos variability was quite marked during 2012 and less evidenced in 2013. In 2012 cycle period, the density and species richness was lower in summer when compared to winter, and an opposite situation occurred in 2013. A pattern usually observed for macrozoobenthos in Patos Lagoon estuary is the higher densities in summer than in the winter (Bemvenuti 1987; Rosa and Bemvenuti 2006) due to an increase in reproductive activities of many benthic species, despite the predation pressure from higher trophic levels (Bemvenuti 1998). The presence/absence of predators is an important factor to be considered, since many macrozoobenthic species are important food items to organisms such as the blue crab *Callinectes sapidus*, the corvina *Micropogonias furnieri* and the shrimp *Farfantepenaeus paulensis* (Bemvenuti 1987, 1997). In fact, benthic animals are directly and indirectly involved in most physical and chemical processes that occur in estuaries (Reish 1980), which makes it difficult to predict what exactly causes this change in macrozoobenthos pattern.

Water pollution from intensive prawn farms negatively affects adjacent ecosystems (Ellison 2008). A reduction of biological diversity is the first consequence of coastal degradation by nutrients enrichment (Mouillot et al. 2005) and accordingly benthonic organisms, particularly polychaetes have been used as stress indicator in environmental impact studies (Pocklington et al. 1994). Generally, environments with high accumulation of organic matter (dissolved and/or particulate) possess substrates containing acids and hypoxic/anoxic sediment, which promotes the anaerobic decomposition increasing the production/release of toxic compounds. Thence, these compounds can also change the benthic communities structure (Jackson et al. 2004). In reduced environments is observed a decrease in benthic diversity (Lorenzen et al. 1987), a process that favors the settlement and/or re-colonization by opportunistic species due to the lack of competitors (Rosa and Bemvenuti 2006). In fact, the organic enrichment can cause sudden changes in community structure, reducing macrozoobenthic community assembly to some tolerant species (Pearson and Rosenberg 1978). Benthic macroinvertebrates can be used as indicators of the changes and variability in estuarine ecosystems since they have relatively long life-spans, the different species exhibit tolerances to stress, play an important role in cycling nutrients and materials and they are fundamental providing links to higher levels (Dauvin 2007).

It was observed that in both sample campaigns there was a predominance of polychaetes, which play an important role in benthic communities acting recycling and reworking the nutrients, linking the trophic web (Hutchings 1998). They have being widely used as enrichment indicators (Ansari et al. 1986) because organic enrichment can decrease their richness and favor opportunistic species (Tomassetti and Porrello 2005). Dominance of polychaetes in terms of density and species composition is also reported by Murugesan et al. (2009) in a study evaluating the impact of shrimp farming in India. Among the five species found in this class, *L. acuta*, *H. similis* and *A. succinea* were the most abundant. *A. succinea* and *H. similis* are commonly recorded in the estuarine-lagoon complex of temperate and subtropical regions of the Atlantic Southwest coast (Pagliosa and Barbosa 2006). These species behavior allow the maintenance of high densities through escape mechanisms, such as burial capacity, high mobility and reproductive strategies that ensure a rapid recolonization of the environment. However, according Bemvenuti et al. (1997) the abundance of these species tends to decrease considerably after environmental disturbances events.

The polychaete *H. similis* is a subsurface deposit-feeder that has no clear seasonal pattern and suffers low predation effects (Rosa and Bemvenuti 2006). Its burial ability is considered the main strategies of tolerance to disturbances in the substrate and escape predation, since many epifaunal predators act in the surface layers of the sediment (Bemvenuti, 1988). *L. acuta* is a deposit-feeder species with high abundance and biomass that inhabits mixoaline waters (Pagliosa and Barbosa 2006) from the Northeast Brazil to Southern Argentina (Omena and Amaral 2001). This species is found in regions of muddy substrate with high organic matter content (Pagliosa and Barbosa 2006) and it feeds on this content in the substrate (Olivier 1995), increasing the degradation rate (Heilskov and Holmer 2001). The tanaidacea *K. schubartii* was recorded in significant densities and with little temporal variability, particularly over 2013. This species occurs in high densities in sandy-muddy substrates, showing preference for sediment containing higher percentages of silt and clay (Capitoli et al. 1978) since it digs U-shape tubes with 15 cm depth (Rosa-Filho and Bemvenuti 1998). Several studies indicate a significant density for the species in this estuary (> 20,000 ind. m^{-2}), and the marked seasonal influence. Higher salinity and high water temperatures affect the *K. schubartii* recruitment during summer and early autumn , which suggested a continuous reproduction of the species (Fonseca and D'Incao 2006). As long as the population is not subject to salinities close to zero for extended periods - which may cause gaps in their recruitment - the success of *K. schubartii* recruitment is also determined by the size of the population prior to the reproductive process, indicating a density-dependence for the species (Colling et al. 2007).

Generally, a decreasing trend in macrozoobenthic density was observed in 2013 when compared to 2012 samplings. Nevertheless, it cannot be observed relation between abiotic parameters over the two years of sampling and the result obtained. Some species have undergone major variations in density along the sampling campaigns, as *P. pinnata*, *Amphitoe sp* and Ostracoda, that almost disappear in 2013 and *K. schubartii* and *S. mourei* that increased the density in the same year. In this way, many factors can affect its variability. These fluctuations are influenced by the conditions which favor growth and development of pelagic larvae (Kastoro et al. 1989). Senales et al. (2007) report that climatic events (*El Niño*) and the effects of competition and predation by other species may have caused fail in *P. pinnata* recruiting in Chile.

In this study the shrimp farm effluent not contributed to a reduced species richness, which could indicate that there was no accumulation of organic matter enough to cause a restructuring of macrozoobenthic community, different of the results reported by Canary et al. (2009) and Rodríguez-Gallego et al. (2008) that found changes in macrobenthic structure in environments next to shrimp farms. In general, no significant differences over the sampling sites show an absence of disturbances in macrozoobenthic community by the effluents release, since there was no clear distribution change of macrofaunal assembly. This study showed that the effects of effluent discharge on some attributes of macrozoobenthic community (density and richness) were less significant than the natural environmental variation, since the estuarine invertebrate communities in temperate regions have significant seasonal variations.

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RESUMO

A produção em cativeiro do camarão branco do Pacífico Litopenaeus vannamei vem se consolidando cada vez mais no entorno do estuário da Lagoa dos Patos, região sul do Brasil. Nesse estudo, foram avaliados os efeitos espaço-temporais dos efluentes de uma fazenda de cultivo de camarões sobre o macrozoobentos (> 500µm) ao longo de dois ciclos produtivos (2012 e 2013). As amostragens ocorreram em sete diferentes pontos do ambiente receptor, sendo dois pontos controle e cinco pontos distribuídos em frente a desembocadura do canal de lançamento dos efluentes. As coletas ocorreram em diferentes escalas temporais. Sete grupos foram observados (Polychaeta, Tanaidacea, Isopoda, Gastropoda, Bivalvia, Malacostraca e Ostracoda) ao longo das duas campanhas amostrais. Espacialmente, a densidade e a riqueza de espécies tiveram uma variabilidade pouco expressiva em ambas as campanhas amostrais. Já ao longo do tempo, na campanha de 2012 observou-se um aumento desses índices no inverno comparativamente ao verão, oposto do que foi constatado em 2013. Os resultados sugerem que o macrobentos não sofreu influência do lançamento dos efluentes e que as diferenças estatísticas ($P < 0,05$) observadas foram decorrentes das oscilações naturais dos parâmetros abióticos que ocorrem em sistemas estuarinos.

Palavras-chave: Densidade, Riqueza de espécies, Sedimento, *Litopenaeus vannamei.*

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CAPÍTULO 3

IMPACTOS DOS EFLUENTES DA CARCINOCULTURA SOBRE O SEDIMENTO

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Impact of Shrimp Farming Effluent: TOC, TN, Cu and Zn Levels in the Sediment

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Abstract

The unconsumed food in shrimp farm systems is responsible for increasing the organic compounds in sediment and it is a potential source of trace metals. The aim of this study was to evaluate the total organic carbon (TOC), total nitrogen (TN), copper (Cu) and zinc (Zn) concentrations in sediments from control sites, shrimp pond, sedimentation basin and receiving ecosystem effluents of a semi-intensive *Litopenaeus vannamei* farm in southern Brazil. The sediment samples were taken before the effluent discharge (BD), 1-day post-discharge (1PD), 10 days post-discharge (10PD) and 30 days post-discharge (30PD). Shrimps were also collected to analyze Cu and Zn concentrations in the tissue. The TOC concentration ranged from 0.12 to 0.67% and the TN concentration was <0.07% in all samples. Labile Cu and Zn concentrations ranged from 0.12 to 1.27µg g^{-1} and 0.51 to 3.07µg g^{-1}, respectively, while the more strongly adsorbed fraction Cu ranged from 0.3 to 2.65µg g^{-1} and Zn 30.44 to 121.4µg g^{-1}. Some significant differences ($p<0.05$) were observed among the sites, but not related to the effluent discharge. Pearson correlation analysis showed no relationship between the effluent discharge and increase in TOC, TN, Cu or Zn values in the sediment, except in 1PD. The Cu and Zn concentrations in shrimps' tissue were 6.63±0.2µg g^{-1} and 19.76±0.2µg g^{-1} in pond 1, and 7.6±0.51µg g^{-1} and 19.13±0.32µg g^{-1} in pond 2. All parameters were within the safe levels stipulated in the Brazilian legislation, showing that this shrimp production did not bring adverse effects to the environment.

Keywords: copper, *Litopenaeus* vannamei, total nitrogen, total organic carbon, zinc.

Abbreviations

TOC – Total organic carbon
TN – Total nitrogen
Cu – Cooper
Zn – Zinc
BD – Before discharge
1PD – 1-day post-discharge
10PD – 10 days post-discharge
30PD – 30 days post-discharge

Introduction

The aquaculture in coastal areas is always inquired about the possible environmental impacts of the activity (Biao et al. 2004). The uncontrolled growth of shrimp farming has led to negative environmental impacts in many countries (Páez-Osuna 2001a; Páez-Osuna 2001b), which increased the concern on the activity expansion. Culture systems become more intensive as new technology packages are available; intensive systems usually have greater concentrations of nutrients and organic matter in the culture environment (Lemonnier and Faninoz 2006), thus increase the need of water exchanges. Therefore, the effluents from these systems are loaded with nitrogen, carbon, phosphorus and suspended solids (Paez-Osuna et al. 1997; Biao et al. 2004).

According to Boyd & Teicher-Coding (1995), less than fifty percent of carbon and nitrogen from food is converted into shrimps biomass. Briggs & Funge-Smith (1998) state that approximately 18-27% of nitrogen and 6-11% of carbon from the feeds is assimilated by shrimps, and the remainder is available for plankton, volatilizes or stays entrapped in the sediment. The organic and inorganic dissolved nutrients have an indirect ecological impact by increasing primary phytoplankton production, which decease and increase the organic matter sedimentation (Olsen et al. 2008). Thus, the organic matter in aquaculture ponds bottom derives from phytoplankton, unconsumed feed and shrimps excretion (Funge-Smith and Briggs 1998; Steeby et al. 2004). This accumulation can cause biological and chemical impacts to pond environment (Suplee and Cotner 1996) and to receiving waters environment, since such large amounts of nutrients promote microbial growth by the availability of organic matter. This increase of organic matter can cause eutrophication of the ecosystem. Eutrophic systems usually have particulate organic carbon (Pelletier et al. 2011), which is a good indicator of enriched sediment (Hyland et al. 2005). Organic nitrogen also plays an important role as a source of nutrients (Fütterer 2000), and thus is an important element to be evaluated.

Sediment can trap metals in the aquatic system serving as a good pollution indicator, allowing a consistent evaluation of spatial and temporal contamination (Solomons & Förstner 1984; Buchman 1989). Aquaculture is often reported as a potential source of trace metals, which are present as natural components in feed, fertilizer or as impurities of pesticides (Tacon & Forster 2003). The copper (Cu) is a trace element present in shrimp a diets essential to synthesize hemocyanin in the hemolymph (Cuzon 2004) and zinc (Zn) is a cofactor in many enzyme systems (Davis et al. 2002). The quantification of these trace metals has already been evaluated in salmon farming, serving as indicators of aquaculture wastewater (Chou et al. 2002). The sediment, when revolved, releases the labile metal fraction to the water column, which can have toxic effects on organisms (Wallner-Kersanach et al. 2009) by inducing changes in physicochemical conditions, especially changes in pH and redox-potential (Cappuyns & Swennwn 2005). It is necessary to consider that shrimp farming effluents can contribute to trace metals to the adjacent coastal environment, depending mainly on the amount of feed used during the production cycle (Lacerda et al. 2006).

Trace metals can be also quantified in aquatic organisms' tissues, since the excess of trace metals in unbalanced diets for shrimp *L. vannamei* promotes the accumulation of these compounds in their tissues (Yang and Wu 2011) and the analysis of trace metals in aquatic organisms can provide important information on the degree of environmental contamination and the potential impact of this food consumption (Ip et al. 2005).

Literature concerning the TOC, TN and trace metals in the shrimp farms sediments are scarce. Therefore, this study evaluated the total organic carbon (TOC), total nitrogen (TN), Cu and Zn in the pond sediment, sedimentation basin and the receiving waters. Additionally, we determined the concentrations of both metals in the tissue of farmed *L. vannamei*.

Materials and Methods

Study area and sampling

This study was conducted in a *L. vannamei* shrimp farm located in the coastal area of Patos Lagoon estuary (Rio Grande do Sul, Brazil - 31º56'04S, 52º00'11W). The area does not suffer anthropogenic effect and the shrimp farm have one cycle per year, where after each cycle the ponds are drained and dried. The culture period began in December 2011 and ended in May 2012 when the shrimps were harvested. Outflowing waters are driven into a sedimentation basin before flowing into the receiving body water. The stocking density was 12/m² shrimp in two ponds, with a total area of 3.75 ha. The facility works in a semi-intensive farming system and there was no effluent discharge during the culture period; water was only added in order to compensate the evaporation and soil infiltration.

Sediment samples were collected between February and May 2012. For the TOC and TN analysis, seven sampling sites were chosen: two control sites (C1 and C2) in the estuary, shrimp pond (P1), sedimentation basin (SB) and three sites across the effluents discharge channel (in front of the channel - 1, 20 meters from the channel - 2 and 30 m from the channel - 3) (Figure 1). For the analysis of Cu and Zn in sediment the sampling sites were the control site (C1), shrimp pond (P1), sedimentation basin (SB) and a site in front of the effluent outflow channel (1) (Figure 1). Temporal samplings at these sites for analysis were taken according to effluents discharge, as follows: sample before the discharge (BD), 1-day post-discharge (1PD), 10 days post-discharge (10PD) and 30 days post-discharge (30PD).

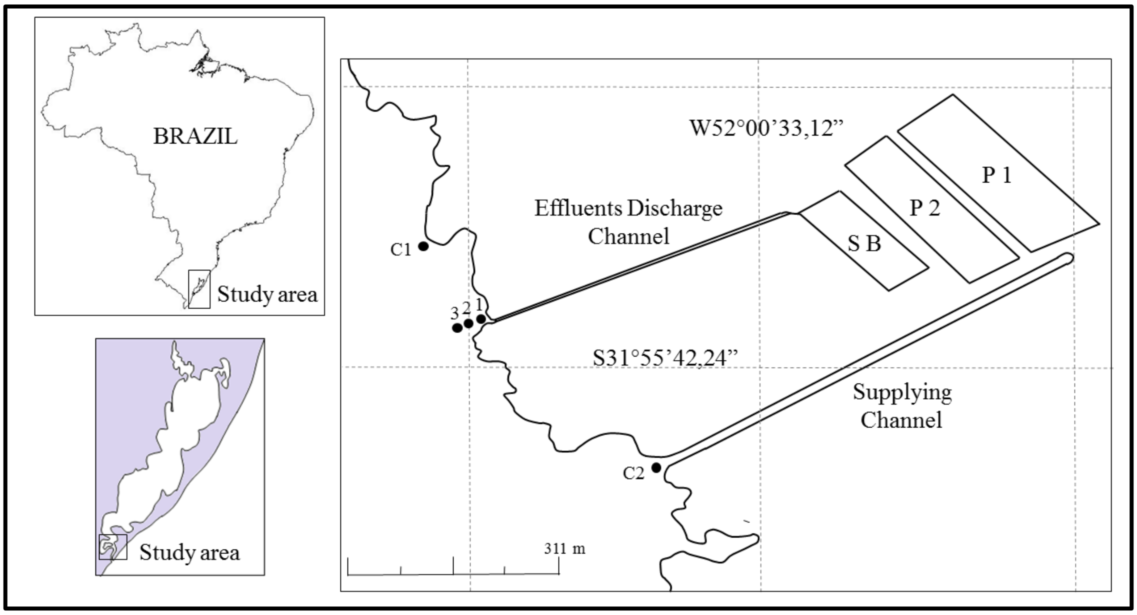

Figure 1: Sampling sites in the shrimp farming. C1 = Control 1, C2 = Control 2, 1 = Effluents discharge channel, 2 = 20m from the channel, 3 = 30m from the channel, P1 = Pond 1, P2 = Pond 2 e SB = Sedimentation basin.

The glassware used in this study was cleaned and stored according to methodology described by Baumgarten et al (2010). At each sampling site, three sediment samples were collected with a 5-cm-diameter PVC pipe. Sub-samples were separated with plastic spatula and stored in plastic bags for Cu and Zn determination. With a stainless steel spatula, sediment samples of surface layer were collected for TOC and TN analysis from each site and stored in 50 ml glass vials. In the same sites sediment samples were also collected to determine the proportions of sand, silt and clay by sieving (> 0.062 mm diameter) and pipetting (<0.062 mm diameter) as described by

Suguio (1973). At the end of the culture period, shrimp specimens were collected from two shrimp ponds (P1 and P2) for Cu and Zn tissue analysis.

Analysis of Cu and Zn in the sediment and shrimps muscle tissues

Two methods of extraction of Cu and Zn of sediment were performed: low extraction, which provides the labile fraction (potentially bioavailable) and semi-strong extraction (more strongly adsorbed). All the material used for trace metals analysis was previously washed with solution of 20% (v / v) nitric acid. The semi-strong extraction values were compared to Brazilian legislation about these metals.

For the Cu and Zn labile extraction (n = 2), 1 g of sediment samples was previously macerated and dried in an oven at 60 °C and then re-weighed. The samples were digested in 0.1M hydrochloric acid (v / v) Suprapuro® (Merck, Germany) for 24 hours under continuous stirring, in a solid / liquid ratio of 1:20 (g / ml). The leaching products rest for 5 hours to sedimentation, and then they were filtered through Whatman # 44 filter paper in a slow rate at ambient pressure. The extracts obtained were transferred to 25ml volumetric flasks and filled with 0.1M HCl solution (Li et al. 2009, modified).

The semi-strong extraction analysis of Cu and Zn (n = 2) was conducted by weighing 1g of dry sediment and digesting it with concentrated nitric acid and hydrogen peroxide (Suprapuro®, Merck, Germany) at4:1 volume proportion. The digestion process occurred in a microwave (CEM Model X-Press Model Mars) for 45 minutes at 180 °C and 1600W. Subsequently, the samples were filled with 50 mL of Milli-Q® water.

Trace metals determination in shrimps' tissues was carried out with pools of 30 specimens from each shrimp pond and the analysis was performed in triplicate. In the procedure, 5 mL of concentrated nitric acid Suprapuro® (Merck, Germany) was added in 0.5g of dry muscle previously weighed, followed by microwave digestion as in the sediment samples. The samples were then filled with 25 mL of Milli-Q® water. The Cu and Zn analysis in sediment and shrimp tissue was performed by Inductively Coupled Plasma Optical Emission Spectrometry (2100DV Model, ICP-OES).

Statistical Analysis

All data were submitted to the analysis of variance (ANOVA) followed by a Tukey test (mean comparison). Possible relations between the TOC, TN, copper and zinc values (for both extraction methods) was determined using the Pearson correlation analysis. The significance level used for all tests was 5% (Sokal and Rohlf 1995).

Results

The sediment was mainly composed by fine sand (2.5 Phi) in all sampling sites (43.64 to 48.28%). The percentage of TOC showed variations among sampling sites (Figure 1). In the BD and 30PD samplings, no significant differences were observed (*P* < 0.05). The shrimp pond in BD and 1PD had the highest mean values of TOC. The shrimp pond in 1PD differed significantly (*P* < 0.05) from the sedimentation basin and the sites 1 (in front of the effluents discharge channel), Control 1 and Control 2. In the 10PD, the C2 site differed significantly (*P* < 0.05) from 2 and 3 sites. TN concentrations were below the detection limit of the equipment (< 0.07 %) for all sampling sites analyzed.

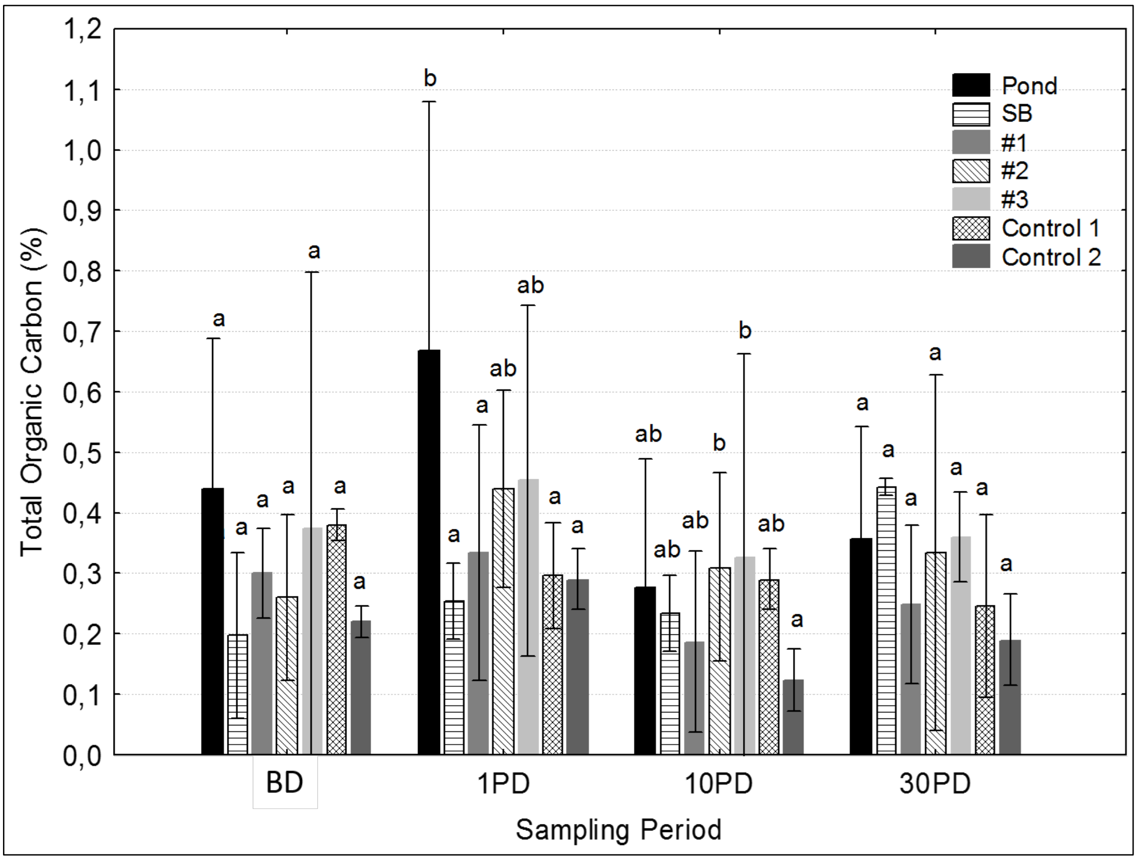

Figure 1: Spatio-temporal TOC percent variations in sediment (mean ± SD, n = 3). Different letters denote significantly differences (*P* < 0.05) through the different periods of effluents

discharge: sample pre-discharge (BD), 1-day post-discharge (1PD), 10 days post-discharge (10PD) and 30 days post-discharge (30PD). SB = Sedimentation Basin

The mean concentration of Cu and Zn in the labile fraction ranged from 0.12 to 1.27µg g^{-1} and 0.52 to 3.07µg g^{-1}, respectively (Figure 2). The Pearson's correlation between TOC concentration and the metals indicated a significant and moderate correlation ($P < 0.05$; r = 0.68) with the labile fraction of Cu and significant and strong correlation ($P < 0.05$; r = 0.90) with the labile fraction of Zn in the sediment sample DP. The highest value of Cu (1,27 µg / g) was found at the effluent outflow channel in BD sampling. It is noteworthy that no effluent was released at this date. The sedimentation basin showed the highest Zn value (3,07 µg g^{-1}) in 30PD.

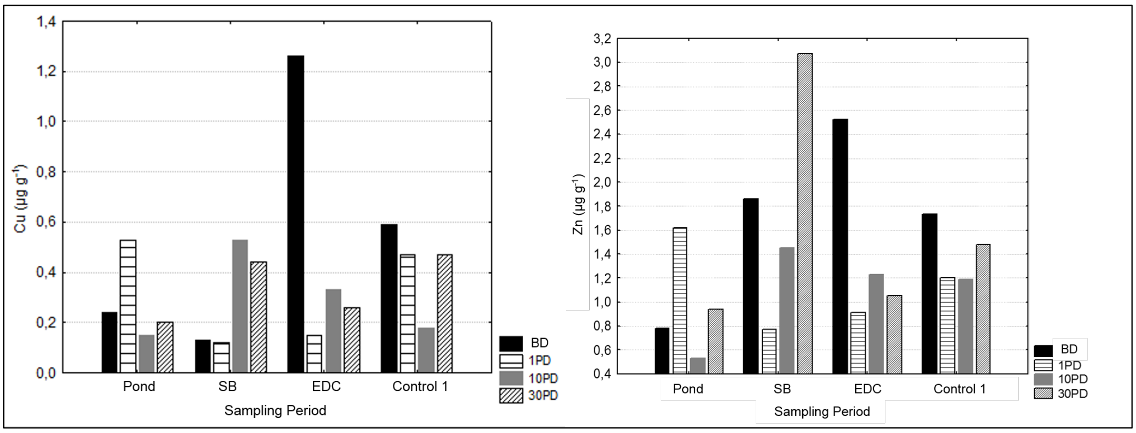

Figure 2: Mean concentration (n=2) of Cu e Zn (µg/g) in sediment through weak acid extraction (bioavailable) in different sampling sites. SB = Sedimentation Basin; EDC = Effluents Discharge Channel. *Figure in different scales.

The semi-strong extraction of Cu and Zn in the sediment showed variations between 0.3 to 2.65µg g^{-1} for Cu and 30.44 to 121.4µg g^{-1} for Zn (Figure 3). Unlike the labile fraction, when Pearson's correlation was applied only Cu indicated a strong correlation ($P < 0.05$; r = 0.70) with TOC in 1PD.

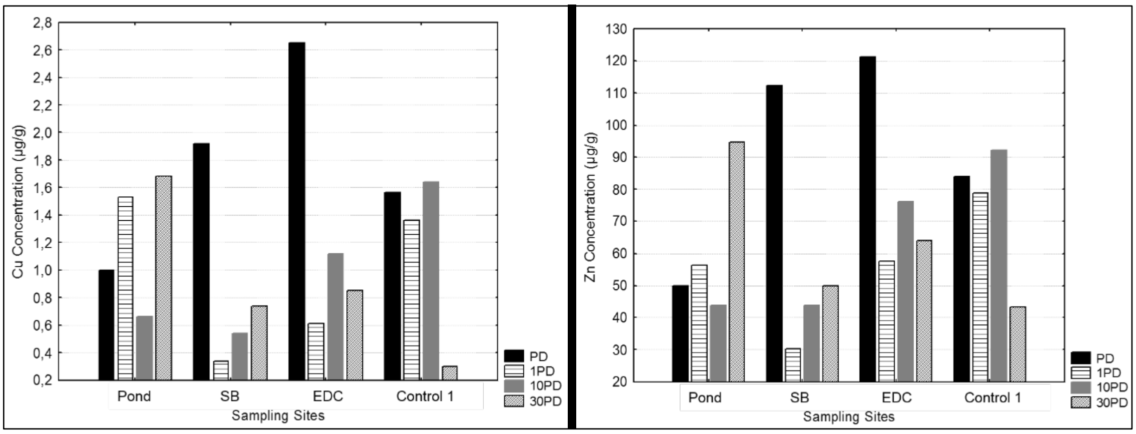

Figure 3: Mean concentration (n=2) of Cu e Zn (µg/g) in sediment through semi-strong extraction in different sampling sites. SB = Sedimentation Basin; EDC = Effluents Discharge Channel. *Figure in different scales.

The mean concentration of Cu and Zn in the tissue of the shrimp were 6.63 ± 0.2µg g^{-1} of Cu and 19.76 ± 0.2µg g^{-1} of Zn in pond 1 and 7.6 ± 0.51µg g^{-1} of Cu and 19.13 ± 0.32µg g^{-1} of Zn in pond 2.

Discussion

The TOC in this study was low and had little variation (0.12 to 0.67%). Several factors may have contributed to this result, and the low stocking density of shrimp during the culture period is an important fact, as mentioned in another study for Vinatea et al (2006). Organic carbon is originated from the dead phytoplankton, organic fertilizer, unconsumed feed and animal faeces with soil particles (Olsen et al. 2008). The low stocking densities of shrimp prevent that large nutrient inputs are inserted through the feed, and the appropriate management of ponds' water quality prevents algal blooms. Burford & Williams (2001) report that phytoplankton blooms have deleterious effects on water quality and sediment.

The grain size of the sediment is another determining factor in organic material content of estuarine sediments (Pelletier et al. 2011). The predominantly sandy and fine content found in this study, in addition with constant artificial water aeration during the culture period, may have improve the decomposition of organic matter, avoiding the carbon accumulation in sediment. Sutherland et al (2007) found a strong correlation between the porosity of the sediment and organic content, where sandy sediments were characterized by low organic content. In BD and 30PD sampling, no significant differences were observed of TOC between the sampling sites, unlike occurred in 1PD and 10PD. However, these differences did not appear to be related to the effluent discharge effects.

The shrimp pond presented the highest percentage of TOC in BD and 1PD sampling, and these values decreased after shrimp harvest (10PD and 30PD sampling). This is due to the pond drainage and sun-drying, which increases the soil aeration and accelerate the organic matter decomposition (Ayub et al. 1993; Boyd 1995). Boyd et al (2010) evaluated 233 commercial aquaculture ponds and found TOC concentrations in sediment ranging from 1.08 to 7.01%. The authors report that there was not a clear relationship between TOC concentration and the species or stocking density. Another study evaluating ponds sediment in six *L. vannamei* shrimp farms in southern Brazil identified average concentrations of TOC ranging between 0.9 and 2.15% (Vinatea et al. 2006). Smith (1996) also reported organic carbon values between 0.96% and 2.49% in a *Penaeus monodon* farm. In Mirim Lagoon, a lake connected to Patos Lagoon estuary, showed TOC concentrations in sediment ranging from 0.21 to 2.4% (Santos et al. 2003).

The low concentrations of TN found in the sediment (< 0.07 %) may indicate that there was no excess of bacterial activity to decompose organic matter. Studies have shown that the microbial community (Burford and Williams 2001), phytoplankton (Hargreaves 1998; Burford and Glibert 1999) and the constant aeration throughout the culture period play an important role in the removal of nitrogenous compounds in culture ponds (Sanares et al. 1986). However, several studies have shown that the effluent from shrimp farming can contribute to an increase of nitrogen in the sediment. Anh et al (2010) reported 0.07% to 0.17% of NOT in the sediment of *P. monodon* culture ponds; Lemonnier and Faninoz (2006) found higher levels (0.15 to 0.22%) of TN in semi-intensive shrimp culture ponds in New Caledonia. Teichert-Codding and Boyd (1995) also reported that nitrogen may be converted into gaseous form and lost to the atmosphere. The volatilization of ammonia in semi-intensive shrimp farming in Mexico was estimated at 27.4% (Páez-Osuna et al. 1997).

This study showed low values of labile (potentially bioavailable) Cu (0.12 – 1.27μg g^{-1}) and Zn (0.51 - 3μg g^{-1}) concentrations in sediment. Labile Cu and Zn concentration increased at 1PD and were significantly ($P < 0.05$) correlated the higher levels of organic carbon found in the sediment. The Pearson's correlation analysis demonstrated higher levels of organic carbon when Cu and Zn were increased in the labile extraction on 1PD. This may be related to the availability of non-consumed feed and faeces shrimps, since this correlation was not found at 10 and 30 days post-discharge. Organic matter has a strong affinity for Cu and Zn (Lin and Chen 1998), and Frías-Espericueta et al (2006) found a positive correlation between organic matter level in the sediment of shrimp farm and the Cu, Zn and Ni concentration. However, the content of C and N was low despite the positive correlation, which explain the low levels of Cu and Zn also found in this study. Even though the high concentration of Cu and Zn have been found in the effluent outflow channel in BD, the sampling occurred prior to effluent discharge. This was probably due to poor water circulation in effluent discharge channel, once the concentrations of both elements decreased after culture period.

The content of labile Cu and Zn concentrations in sediment of the present study were low when compared to other studies (Table 1). An trace metal assessment in sediment of several sites of Patos Lagoon estuary showed mean values of 4.5 – 19.2μg g^{-1} of Cu and 18.5 – 62.5μg g^{-1} of Zn, both in the labile fraction <0.63 μm (silt and clay), in an area with low anthropogenic impact (Costa et al., unpublished data). The dominance of fine sand and the short period of use of the ponds promote low environmental disturbance, different of the estuarine area of the Patos Lagoon.

In the same way, when comparing the semi-strong acid extraction of Cu and Zn (0.3-2.65μg g^{-1} from 30.44 to 121.4μg g^{-1}, respectively), the values were in general lower than in other studies, except for the Zn concentration (121.4μg g^{-1}) found in the effluent discharge channel (EDC) on pre-discharge sample (PD). Russel et al (2011) evaluated the concentrations of this metals in the sediment next to marine fish farms in Scotland, and found mean values of 35.8 and 89.1 μg g^{-1} of Cu and Zn, respectively. A study in a *Penaeus monodon* farm reports concentrations up to 45μg g^{-1} of Cu and up to 85μg g^{-1} of Zn in ponds sediment and concentrations below 2μg g^{-1} of Cu and 64μg g^{-1} of Zn in effluent sediment (Smith 1996). Moreover, another study evaluated the amount of Cu and Zn in a shrimp farm and it was found a negative imbalance between the quantities added via feed and fertilizer and the amounts found in soil, suggesting that these metals may have been immobilized by conversion to organic form (Ritvo et al. 1998). Brazilian law does not have specific regulations for these trace metals concentrations in aquaculture facilities, but there are established guidelines for the management of dredged sediment in brackish waters, reports as safe values to the biota 34μg g^{-1} of Cu and 150μg g^{-1} of Zn (CONAMA 454/2012). Guidelines on soil quality is also available, reporting safe values of 60μg g^{-1} of Cu and 300μg g^{-1} of Zn in soil

(CONAMA 420/2009). This shows that the results obtained in this work are in accordance to the guidelines cited above.

Table 1: Trace metal concentrations (µg g^{-1} dry weight, mean values) in the sediment and shrimp tissue of this study area compared with different locations.

Locations		Fraction	Extraction	Cu	Zn	References
Patos Lagoon, Brazil[a]		Total	HCl	0.12-1.27	0.52-3.07	Present study
Patos Lagoon, Brazil[d]		<63µm	HCl	4.5-19.2	18.5-62.5	Unpublished data
New Brunswick, Canada[a]		<63µm	HNO_3	21.2	71.5	Chou et al. 2002
Western Isles, Scotland[a]		<63µm	HNO_3	35.8	89.1	Russel et al. 2011
Cultured shrimp[b]	Pond 1		HNO_3	6.63	19.76	Present study
	Pond 2		HNO_3	7.6	19.13	
Arabian Sea, Pakistan[c]			HNO_3	4.55	7.11	Jaffar et al. 1993
Pearl River estuary[c]			n.a.*	1.28	2.60	Wei et al. 2002
Zhanjiang Harbour, China[b]			HNO_3	24.26	171.56	Wu and Yang 2011
Patos Lagoon, Brazil[c]			HNO_3	0.45	3.77	Pinto et al. 2013

[a] adjacent to aquaculture farms; [b] shrimp farm cultured; [c] wild shrimp tissue; [d] Patos Lagoon without pollutant source; * n.a. - not available.

The analysis of Cu and Zn concentrations in the muscle on a dry weight basis resulted in low mean values of Cu (7.1µg g^{-1}) and Zn (19.4 µg g^{-1}). Table 1 shows Cu and Zn values found in the present study compared to other studies. Trace metals in the tissues present in the pink shrimp *Farfantepenaeus paulensis* from fisheries in Patos Lagoon estuary were quantified and the values found were 12.9µg g^{-1} of Cu and 32.3µg g^{-1} of Zn (Pinto et al. 2013). Another study assessing the concentrations of Cu and Zn in *L. vannamei* tissue from intensive shrimp farm in China resulted in mean levels of 24.26µg g^{-1} of Cu and 171.56µg g^{-1} of Zn (Wu & Yang 2011). Some decapod crustaceans are able to regulate bodily concentration of trace metals such as Cu and Zn, while allowing the necessary requirements for their biochemical necessities and detoxifying the excess (Rainbow 1988; Rainbow et al. 1999). In general, the values in this study were low probably because the area of shrimp farming is an area of the estuary without contribution of urban and industrial effluents. Other factors to consider are the grain size of sediment in the ponds, type of feed and management used. The concentrations found in this study are in accordance with national regulation.

These results suggest that the semi-intensive shrimp farm of *L. vannamei* was performed with appropriate management which resulted in low values of TOC, TN, Cu and Zn exported to the estuary sediment and low Cu and Zn concentrations in shrimps' tissues. Therefore, it can be concluded that this shrimp *L. vannamei* culture period had excellent handling conditions, and did not generate a significant organic or trace metals load to sediment and shrimp.

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DISCUSSÃO GERAL

Dentre os parâmetros abióticos avaliados, espacialmente o lançamento dos efluentes causou um efeito agudo e pontual. Nitrogênio amoniacal total (NAT), clorofila *a* e turbidez da água foram os parâmetros que sofreram alterações mais evidentes após a descarga dos efluentes. Concentrações semelhantes as encontradas no presente estudo de compostos nitrogenados e fosfatados (Kantin & Baumgarten 1982; Briggs & Funge-Smith 1994; Abreu et al. 1995; Burford 1997; Cardozo et al. 2011) e clorofila *a* (Abreu et al. 2010; Cardozo et al. 2011) são reportadas para o estuário da Lagoa dos Patos e para proximidades de cultivos de camarões. Apesar das alterações observadas nos parâmetros de qualidade de água, essas ocorreram apenas nos primeiros dias após a descarga dos efluentes (até a amostragem 5 PD) e ficaram restritas até os primeiros 20m de distância do canal de lançamento dos efluentes. Ao longo do tempo amostral, os parâmetros abióticos também se mostraram pouco variáveis, com exceção da temperatura e da salinidade, fatores mais susceptíveis a sofrerem variações em ambientes estuarinos.

A hidrodinâmica local e a capacidade suporte do ambiente podem ter sido de suma importância para garantir o baixo tempo de residência do material exportado dos viveiros. A Lagoa dos Patos é caracterizada pela hidrologia forçada pelas relações entre vento e descarga fluvial (Kjerfve 1986; Möller & Fernandes 2010), favorecendo as trocas de água e o baixo tempo de residência induzidos pelos ventos predominantes de NE (Möller et al. 1996). A baixa salinidade observada na amostragem 1 PD pode ser um indicativo de fluxo de vazante derivado do aumento da descarga fluvial (Möller & Fernandes 2010), favorecendo a rápida dispersão do material.

A comunidade fitoplanctônica e protozooplanctônica apresentaram um padrão semelhante espaço-temporalmente, onde foi observado um acréscimo na abundância desses organismos na amostragem 1 PD. A única exceção foi a classe das clorofíceas, que se mantiveram em densidades estáveis ao longo de todo o período amostral, sem apresentar oscilações marcadas. Esse aumento ocorrido no primeiro dia pós-descarga pode estar relacionado ao *input* de nutrientes ocasionado pelas chuvas, e não necessariamente a um incremento da produtividade primária ou um possível espalhamento da pluma dos efluentes por todo o local amostral.

Abreu et al. (2010) encontraram uma relação significativa entre os valores médios anuais de clorofila *a* e a quantidade de chuva na região estuarina, mostrando que esse fator tem grande influência sobre essa comunidade. A abundância e diversidade de ciliados tem sido utilizada como indicador da qualidade de água e dinâmica de ecossistemas (Foissner 1988), uma vez que esses organismos exercem um importante papel no fluxo de energia (Sherr & Sherr 1988; Decamp et al. 2003). Esses organismos servem de alimento para larvas de peixes, exercendo uma função essencial no link entre o *microbial loop* e os níveis tróficos superiores (Fukami et al. 1999). Yang et al. (2012) demonstraram que a comunidade e o tamanho da estrutura do protozooplâncton geralmente segue a dinâmica espacial e o tamanho da estrutura do fitoplâncton, fato que pode ser observado no presente estudo.

Com exceção da amostragem 30 PD, a concentração zooplanctônica foi baixa e sofreu poucas oscilações espaço-temporalmente, com algumas poucas diferenças estatísticas ($p < 0,05$) observadas entre os pontos amostrais. Baixas concentrações zooplanctônicas são reportadas na literatura para a região também por Cardozo et al. (2011). A ocorrência e abundância de espécies zooplanctônicas no estuário da Lagoa dos Patos é determinada principalmente por variações sazonais de salinidade, temperatura, direção e intensidade do vento, descarga de água doce e oferta de alimentos (McLaren & Corkett 1981; Montú et al. 1998; Muxagata et al. 2012). Apesar de a clorofila *a* poder ser usada como indicador de oferta de alimento para copépodos (Hirst & Bunker 2003), as concentrações constantes de clorofíceas e clorofila *a* demonstram que a ação de herbivoria não foi marcante. Dentre os parâmetros abióticos avaliados não foi possível encontrar uma relação para o aumento de densidade ocorrido na amostragem 30 PD.

Além das variáveis ambientais favoráveis a dispersão do material exportado pelos efluentes, o manejo alimentar e o uso de tecnologia adequados podem limitar o impacto ambiental da carcinocultura (Alonso-Rodríguez & Páez-Osuna 2003). Em áreas rasas com baixa capacidade de diluição, os resíduos provenientes da atividade poderão sedimentar próximo ao local de descarga dos efluentes, maximizando o potencial poluidor dos efluentes. Em sistemas de cultivo extensivos as descargas de efluentes são escassas, diferente do que acontece em sistemas semi-intensivos, onde quantidades mais significativas são descarregadas (Alonso-Rodríguez & Páez-Osuna 2003). As dietas artificiais e os fertilizantes orgânicos e inorgânicos são as principais fontes de *input* de nutrientes em sistemas de criação de camarões, o que causa reflexo direto na composição dos efluentes. Do total de N e P que entram em um sistema semi-intensivo de cultivo de camarões, 76% do N e 83,4% do P são provenientes da ração (Páez-Osuna et al. 1997). Desse total, apenas 25-30% do N e P aplicados na forma de rações e fertilizantes são retirados na forma de camarões ao final do ciclo (Boyd & Tucker 1998). Dessa forma, geralmente altas concentrações de sólidos suspensos orgânicos, carbono, nitrogênio e fósforo proveniente do alimento não consumido e dos produtos de excreção estão presentes nos viveiros de cultivo e consequentemente nos efluentes (Burford & Williams 2001), favorecendo um aumento da produtividade natural do corpo d'água receptor (Tacon & Forster 2003).

O percentual de carbono orgânico total (COT) variou entre 0,12 e 0,67% e o percentual de nitrogênio orgânico total (NT) manteve-se < 0,07%. O carbono orgânico é proveniente do fitoplâncton morto, dos fertilizantes orgânicos, da ração não consumida e das fezes dos animais misturados com as partículas do solo (Olsen et al. 2008). As baixas densidades evitam que grandes *inputs* de nutrientes sejam inseridos através da ração, e o manejo adequado da qualidade da água dos viveiros impedem *blooms* algais.

Os valores de COT encontrados no presente estudo estão abaixo dos reportados na literatura para viveiros de criação de camarões (Smith 1996; Vinatea et al. 2006; Boyd et al. 2010) e para o estuário da Lagoa dos Patos (Santos et al. 2003). As baixas concentrações de NT podem ser um indicativo de que não ocorreu excesso de matéria orgânica para ser decomposta no meio. Estudos têm demonstrado que a comunidade microbiana (Burford & Williams 2001), o fitoplâncton (Hargreaves 1998; Burford & Glibert 1999) e o uso contínuo de aeradores de pás ao longo do ciclo produtivo (Sanares et al., 1986) desempenham um importante papel na remoção de compostos nitrogenados em viveiros de cultivo. No entanto, outros estudos têm demonstrado que o efluente do cultivo de camarões pode aportar quantidades significativas de nitrogênio no sedimento (Lemonnier & Faninoz 2006; Anh et al. 2010). A granulometria do sedimento é outro fator determinante no conteúdo orgânico de sedimentos estuarinos (Pelletier et al. 2011). O teor predominantemente arenoso e fino encontrado no presente estudo juntamente com a aeração constante da água durante o ciclo de produção, podem ter auxiliado na decomposição da matéria orgânica evitando assim o acúmulo de carbono no sedimento. Sutherland et al. (2007) encontraram uma forte correlação entre a porosidade do sedimento e o conteúdo orgânico, onde sedimentos arenosos foram caracterizados por apresentarem baixo conteúdo orgânico.

A bacia de sedimentação presente na fazenda, é uma tecnologia que age na melhora da qualidade da água dos efluentes no que diz respeito ao transporte dos sólidos suspensos totais, ciclagem de nutrientes, produção de biomassa, absorção de nutrientes pelas plantas e animais e distribuição da matéria orgânica e oxigênio (Shpigel et al., 2013). Vários processos bióticos e abióticos regulam a remoção de poluentes nesses locais, como mineralização microbiana, nitrificação-desnitrificação, absorção por macrófitas, precipitação química, sedimentação e adsorção pelo substrato (Lin et al. 2005). Jackson et al. (2003) reportam uma redução da carga dos efluentes de viveiros de cultivo de camarões de 60% do SST, 23% do nitrogênio total e 35% do fósforo total. A correta escala da bacia de sedimentação pode auxiliar na retenção de fósforo e amônia dos viveiros reduzindo os possíveis efeitos sobre a comunidade planctônica (Cardozo & Odebrecht. 2012). Nesse sentido, a bacia pode ter agido de forma positiva no controle do potencial poluidor dos efluentes.

A macrofauna bentônica apresentou pequena variabilidade espacial em ambas as campanhas amostrais (2012 e 2013). Já ao longo do tempo, essa variabilidade foi bastante marcada ao longo das coletas de 2012 e menos variável nas coletas de 2013. Em 2012, maior densidade e maior riqueza de espécies foram observadas nos meses mais frios comparativamente aos meses mais quentes, situação oposta à que ocorreu durante a campanha amostral de 2013. Bemvenuti (1987) e Rosa & Bemvenuti (2006) reportam aumento da densidade do macrozoobentos no verão quando comparado ao inverno, assim como ocorreu em 2013. Isso porque em períodos de altas temperaturas ocorre aumento da atividade reprodutiva e consequente controle populacional através de predadores infaunais e baixas taxas de recrutamento e consequente baixas taxas de predação nos meses mais frios (Bemvenuti, 1998). Por outro lado, mudanças nos padrões de temperatura e salinização do estuário pode causar o insucesso dos recrutamentos. Existe uma forte influência entre padrões de abundância do macrobentos e flutuações de salinidade em ambientes estuarinos (Holland et al., 1987). Ambientes poluídos com acúmulo de matéria orgânica reduzem a diversidade bentônica (Lorenzen et al., 1987), favorecendo a recolonização por organismos oportunistas após tais eventos de perturbação (Rosa & Bemvenuti, 2006). Esse acúmulo pode tornar os sedimentos ácidos e pobres em oxigênio, facilitando as vias de decomposição anaeróbicas e aumentando a produção e liberação de compostos tóxicos reduzidos, o que pode alterar por fim a estrutura da comunidade bentônica (Jackson et al., 2004).

Em fato, o enriquecimento orgânico pode causar mudanças bruscas na estrutura da comunidade reduzindo a assembléia a algumas poucas espécies tolerantes, diminuindo assim a densidade e a riqueza de espécies (Pearson & Rosenberg, 1978). O *input* orgânico no sedimento leva a mudanças nos parâmetros físicos e químicos

(Schaanning 1994) o que pode gerar efeitos diretos e indiretos na comunidade faunal (Pearson & Rosemberg 1978). Os macroinvertebrados bentônicos podem ser usados como indicadores das modificações e variabilidade em ecossistemas estuarinos uma vez que são relativamente sedentários, tem vida longa, as diferentes espécies exibem tolerâncias ao stress, tem um importante papel na ciclagem de nutrientes e materiais e no link de transferência de energia para níveis tróficos superiores (Dauvin, 2007). Os resultados desse estudo demonstram que a riqueza de espécies sofreu pouca variação, o que corrobora com os baixos níveis orgânicos encontrados no sedimento.

Observou-se durante as campanhas amostrais (2012 e 2013) que a densidade do macrozoobentos foi predominantemente constituída por poliquetas. Esses organismos representam um importante papel no funcionamento das comunidades bentônicas (Hutchings, 1998) e são bastante utilizados como indicadores de enriquecimento orgânico (Ansari et al., 1986). As espécies mais abundantes foram *Alitta succinea, Heteromastus similis* e *Laeonereis acuta.* O hábito dessas espécies permite a manutenção de densidades elevadas através de mecanismos de escape a predação, como capacidade de enterramento, mobilidade e estratégias reprodutivas que garantem uma rápida recolonização do ambiente após perturbações. Esses organismos não sofreram redução na densidade após o lançamento dos efluentes. Segundo Bemvenuti et al. (1997), a abundância dessas espécies tende a diminuir consideravelmente após eventos de distúrbios ambientais.

O conteúdo de Cu (0.12 – 1.27µg g^{-1}) e Zn (0.51 - 3µg g^{-}1) lábeis encontrados no presente estudo foram baixos quando comparados a outros trabalhos. Um estudo realizado em diversos locais do estuário da Lagoa dos Patos indicou valores médios de 4,5 – 19,2 µg g^{-1} de Cu e 18,5 – 62,5 µg g^{-1} de Zn, ambos na fração lábil no sedimento de fração <0,63 µm (silte e argila), em uma área com baixo impacto antrópico. (Costa et al., dados não publicados). No que diz respeito à fração de Cu e Zn mais adsorvida ao sedimento (0,3 - 2.65µg g^{-1} e 30,44 - 121,4µg g^{-1}, respectivamente), Russel et al. (2011) avaliando as concentrações de Cu e Zn no sedimento próximo de fazendas marinhas de peixes na Escócia, encontraram valores médios de 35,8 e 89,1µg g^{-1}, respectivamente. Já Smith (1996) avaliando cultivos de *Penaeus monodon* reporta concentrações de até 45µg g^{-1} de Cu e até 85µg g^{-1} de Zn nos sedimentos de viveiros e concentrações abaixo de 2µg g^{-1} de Cu e 64µg g^{-1} de Zn no sedimento dos efluentes. Ritvo et al. (1998) avaliando a quantidade de Cu e Zn em um sistema de cultivo de camarões observaram um desequilíbrio negativo entre as quantidades que foram adicionados através da ração e dos fertilizantes e as quantidades encontradas no solo, sugerindo que esses elementos podem ter sido imobilizados pela conversão para forma orgânica.

A legislação brasileira não possui regulamento específico para as concentrações máximas permitidas desses metais traço em instalações aquícolas. A Resolução CONAMA 454/2012, que estabelece as diretrizes para o gerenciamento de material a ser dragado em aguas salobras sob jurisdição nacional, reporta como limiar abaixo do qual há menor probabilidade de efeitos adversos a biota, concentrações de 34μg g^{-1} de Cu e 150μg g^{-1} de Zn. Já a Resolução CONAMA 420/2009, que dispõe valores orientadores menos restritivos quanto a qualidade de solo, apresenta valores de prevenção 60μg g^{-1} de Cu e 300μg g^{-1} de Zn em solos. Isso demonstra que os resultados aqui obtidos encontram-se abaixo do limiar permitido nas resoluções vigentes.

A análise dos teores de Cu e Zn no músculo em base seca, resultou em uma concentração média de 6,63 ± 0,2μg g^{-1} de Cu e 19,76 ± 0,2μg g^{-1} de Zn nos animais do Viveiro 1 e, 7,6 ± 0,51μg g^{-1} de Cu e 19,13 ± 0,32μg g^{-1} de Zn nos animais do Viveiro 2 (n=6). Pinto et al. (2013) quantificaram os metais presentes no camarão-rosa *Farfantepenaeus paulensis* proveniente da pesca extrativa no estuário da Lagoa dos Patos, e até 12,9μg g^{-1} de Cu e 32,3μg g^{-1} de Zn foram detectados no tecido muscular. Outro estudo avaliando as concentrações de Cu e Zn no tecido do camarão *L. vannamei* proveniente de cultivos intensivos na China resultou em valores médios de 24,26μg g^{-1} de Cu e 171,56μg g^{-1} de Zn (Wu & Yang, 2011). As concentrações encontradas no presente estudo estão bem abaixo do permitido pela legislação nacional e do encontrado na média internacional. Alguns crustáceos decápodes são capazes de regular a concentração corpórea de metais como Cu e Zn, disponibilizando o necessário para suas necessidades bioquímicas e detoxificando o excedente (Rainbow, 1988; Rainbow et al., 1999).

De maneira geral, os resultados obtidos neste estudo sugerem que o cultivo semi-intensivo do camarão *L. vannamei* causou um impacto agudo e pontual em alguns dos parâmetros de qualidade de água avaliados, uma vez que as alterações não excederam a capacidade suporte do ambiente e o sistema assimilou em um curto espaço de tempo (1 a 5 dias) o efluente liberado. Não foi observado impacto do lançamento dos efluentes sobre o fitoplâncton, protozooplâncton, mesozooplâncton, macrozoobentos, ou mesmo sobre a concentração de COT, NT, Cu e Zn no sedimento (frações lábeis e mais fortemente adsorvida), assim como do Cu e Zn no tecido dos camarões. As diferenças observadas nos parâmetros abióticos avaliados foram decorrentes de oscilações naturais que ocorrem em sistemas estuarinos. Dessa forma, pode-se concluir que o ciclo de produção de camarão *L. vannamei* nas condições de cultivo que está sendo aplicada demonstra ótimas condições de manejo, por não gerar de forma significativa efluentes com alta carga orgânica ou metais pesados para o ambiente natural.

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ANEXO

Canal de abastecimento dos viveiros.

Viveiro de cultivo no momento do esvaziamento prévio a despesca.

Momento da despesca.

Saída do efluente da bacia de sedimentação para o canal de lançamento.

Bacia de sedimentação seca.

Chegada do efluente no estuário e coleta de macrozoobentos ao fundo.

Encontro do canal de lançamento dos efluentes com o estuário.

Material de apoio utilizado durante as coletas.

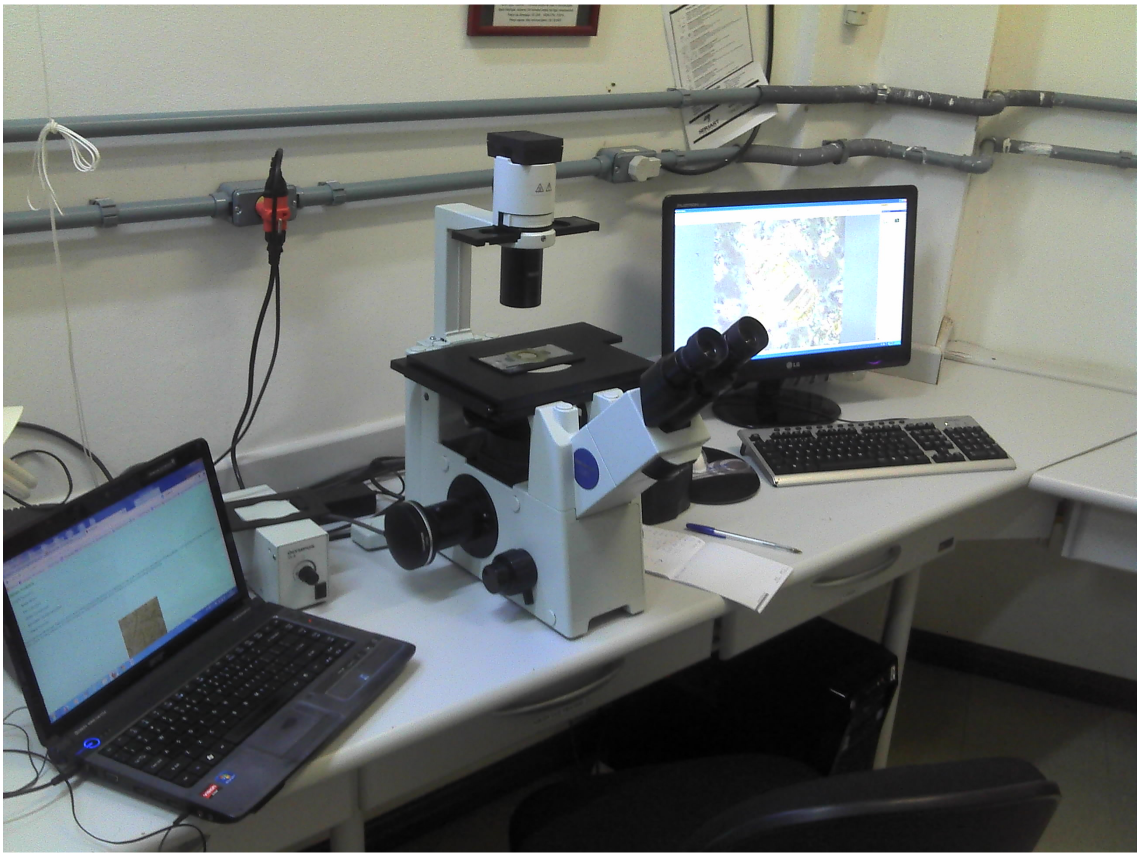
Coleta de dados de oxigênio dissolvido e temperatura com auxílio de um oxímetro.

Momento da coleta do zooplâncton com auxílio de rede cilindro-cônica.

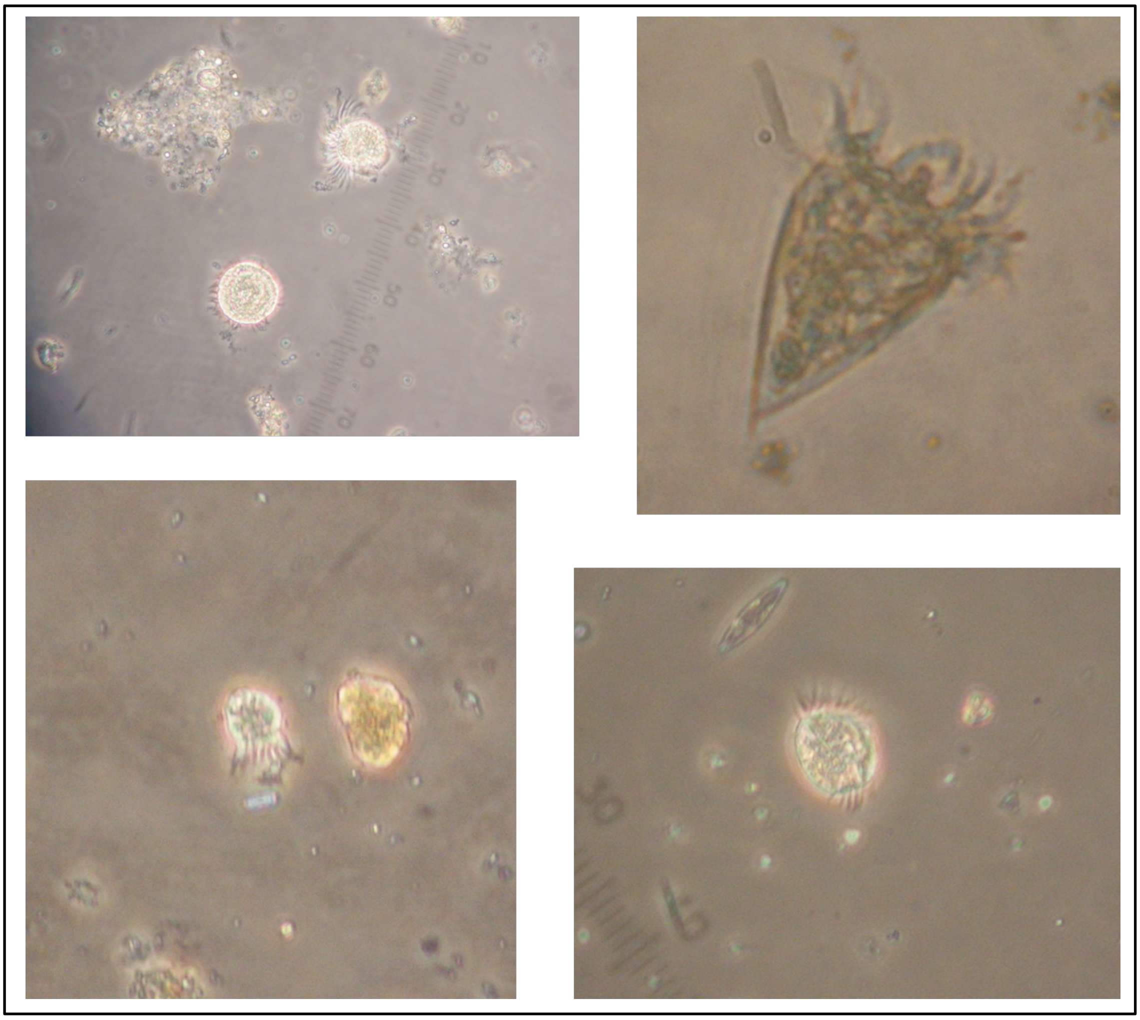
Coleta de macrozoobentos com auxílio de *corer* de PVC.

Peneiramento do macrozoobentos em tela com abertura de 500µm.

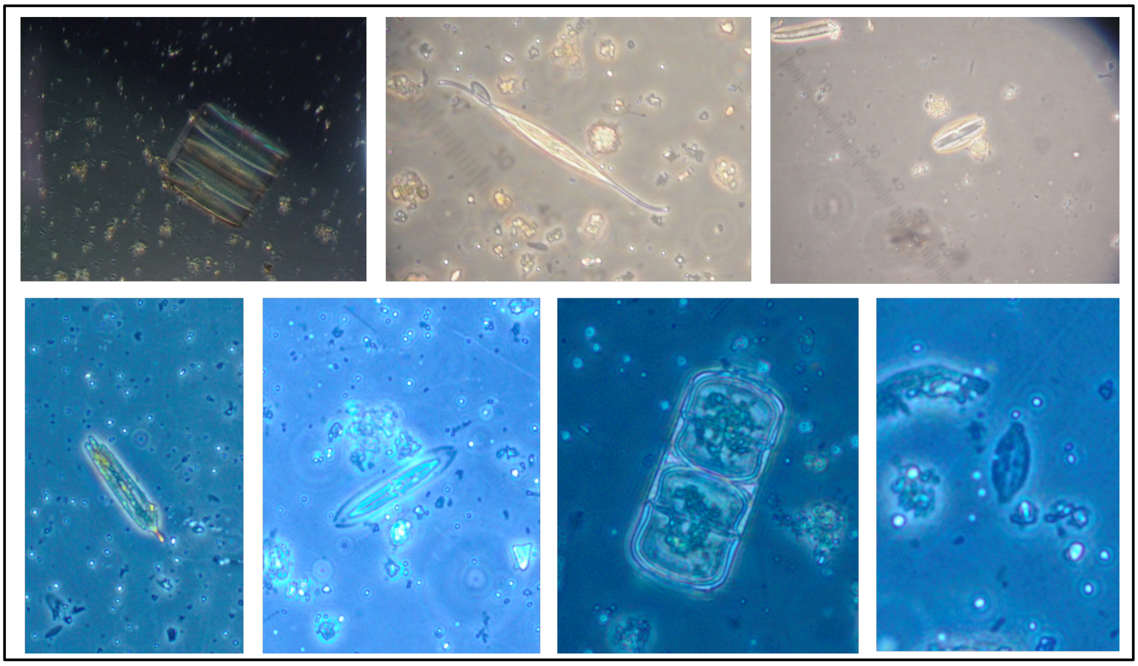
Amostra de fitoplâncton e protozooplâncton armazenada em frasco âmbar.



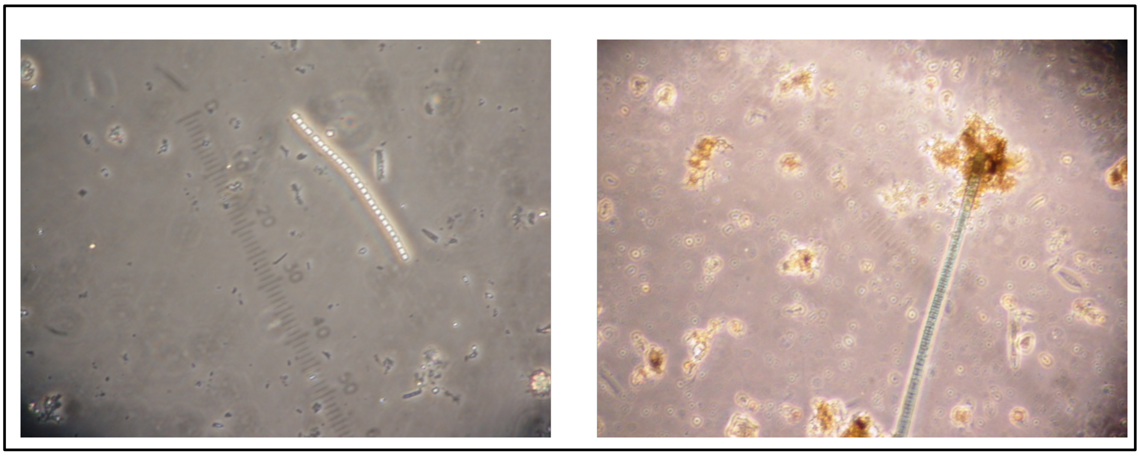
Material utilizado para identificação e captura de imagens de organismos fitoplanctônicos e protozooplanctônicos. Detalhe para o microscópio invertido.



Espécimes de ciliados encontrados.



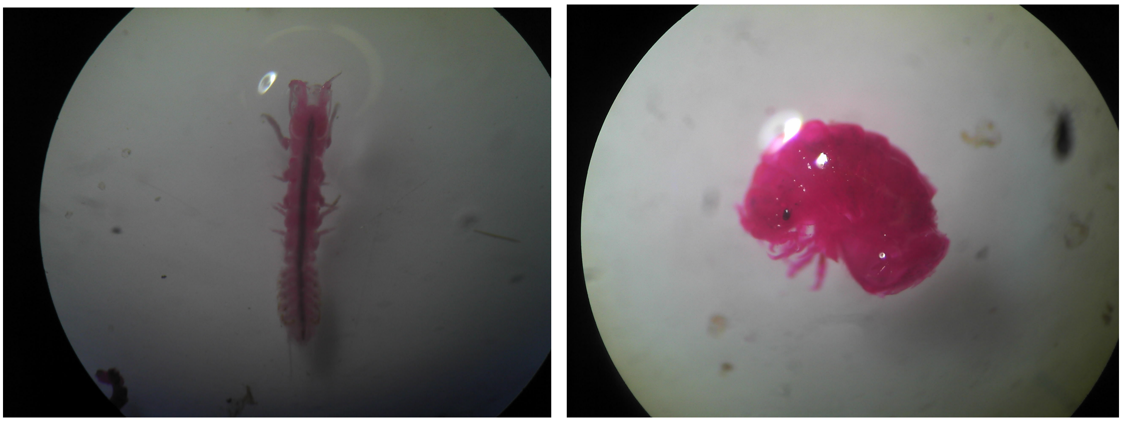
Espécimes de diatomáceas encontradas.



Espécimes de cianobactérias encontradas.

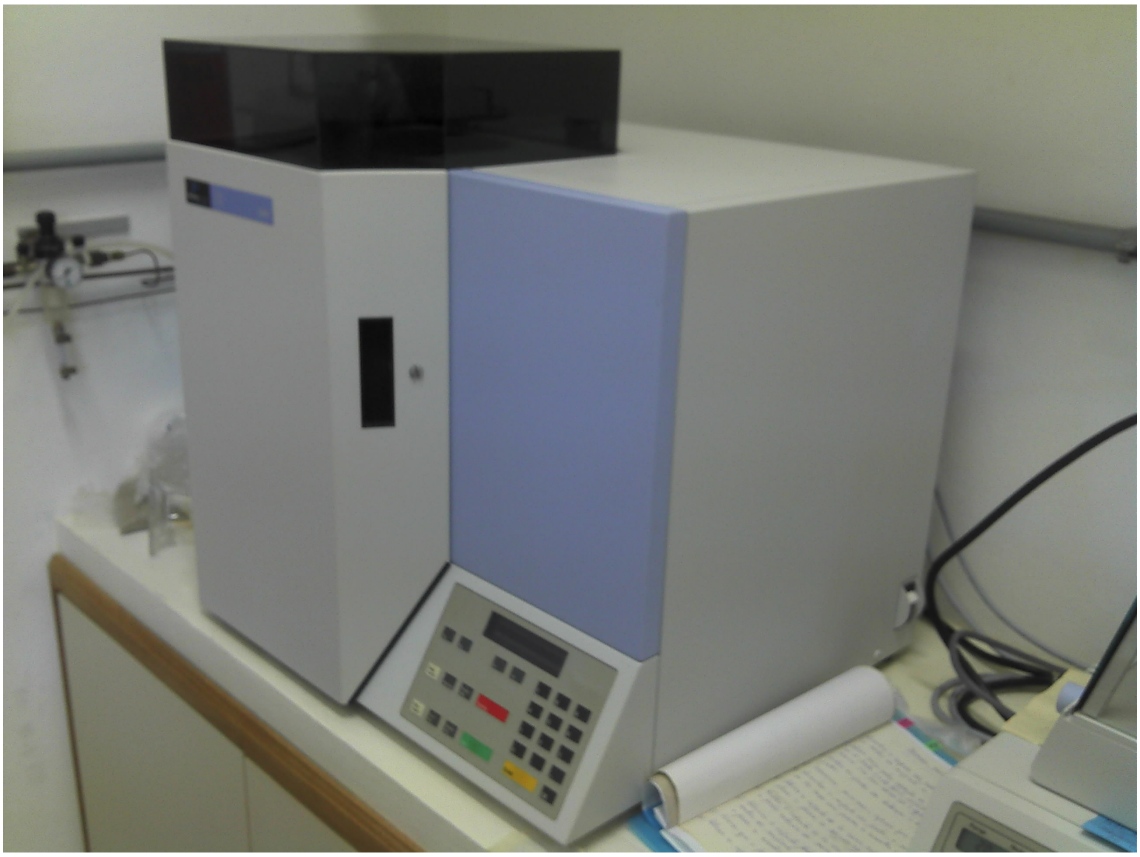
Amostras de macrozoobentos fixados em solução formaldeído 4% coradas com rosa de bengala, previamente a identificação.

Lupa utilizada na identificação do macrozoobentos.



Exemplares de macrozoobentos identificados – *Kalliapseudes schubartii* a direita e *Sphaeromopsis mourei* a esquerda.

Balança utilizada para pesagem de sedimento durante análise de C e N.



Analisador elementar CHNS/O Série 2400 da Perkin Elmer utilizado para determinação do teor de C e N no sedimento.