UNIVERSIDADE FEDERAL DO RIO GRANDE - FURG
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PENETRAÇÃO DA RADIAÇÃO UV NA COLUNA D'ÁGUA DO ESTUÁRIO DA LAGOA DOS PATOS E SEUS EFEITOS SOBRE CÉLULAS E LARVAS DE PEIXES

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RESUMO

Os objetivos desta tese foram conhecer a incidência sazonal de UVA e UVB na coluna d'água do estuário da Lagoa dos Patos e seus efeitos biológicos em diferentes níveis organizacionais: linhagem celular estabelecida e larvas de peixe. Como indicador biológico celular, foi utilizada a linhagem de eritroforoma GEM-81 e como indicador larval, larvas do peixe-rei *Odonthestes argentinensis*. No estudo, foram feitas saídas de campo para coleta de dados ambientais, coleta de larvas de peixe e técnicas de cultura celular associadas a ferramentas fluorimétricas para detecção de possíveis danos causados pelas radiações ultravioleta (UV). Os resultados indicam que a radiação UV penetra na coluna d'água do estuário até 1 m de profundidade, associada a eventos de intrusão salina, comum nos meses de outono e verão, quando temos águas mais claras. A linhagem celular GEM-81 mostrou que doses ambientais de UVA inibem a proliferação celular e a atividade do sistema de defesa antioxidante (TOSC), mas, em contrapartida, aumentam a concentração de ROS (espécies reativas de oxigênio) e o conteúdo de lipídios peroxidados (LPO). UVB causou citotoxicidade na maior dose utilizada e foi capaz de induzir dano de DNA nas células irradiadas com a menor dose. Além disso, as larvas do peixe-rei *O. argentinensis* apresentaram diminuição da taxa de crescimento, quando exposta ao UVA, e no sistema de defesa antioxidante, quando exposta ao UVB. O conjunto dos resultados permite sugerir que organismos planctônicos que vivem em regiões superficiais da coluna d'água do estuário da Lagoa dos Patos estão sujeitos aos efeitos da exposição continuada à radiação UV. O conhecimento deste impacto é relevante para o entendimento de futuras

mudanças na dinâmica de populações de organismos planctônicos e suas implicações ecológicas e econômicas para a região sul do Brasil.

Palavras-chaves: UVA, UVB, Lagoa dos Patos, GEM-81, estresse oxidativo, dano de DNA.

ABSTRACT

The objective of this thesis was to study the seasonal incidence of both UVA and UVB in the water column of the Patos Lagoon estuary, and the effects of UV radiation at different organizational levels. GEM-81 eritroforoma cells and larvae of silverside *Odonthestes argentinensis* were used as biological indicators. Field work was conducted to collect environmental data and fish larvae, and techniques of cell culture combined with tools for fluorimetric detection to assess possible damage caused by ultraviolet (UV) radiation. The results indicate that UV radiation penetrates the water column of the estuary up to 1 m, associated with events of salt water intrusion, common during autumn and summer, when water is clear. GEM-81 cell lineage revealed that doses similar to the environment inhibit cell proliferation, increase the concentration of ROS (reactive oxygen species), the peroxide lipids content (LPO) and lower the activity of the antioxidant defense system (TOSC). UVB caused cytotoxicity at the highest dose and was able to induce DNA damage in cells irradiated with lower doses. Silverside larvae showed low growth rate when exposed to UVA and antioxidant capacity when exposed to UVB. Based on these results it is suggested that planktonic organisms that live close to surface water column in

the Patos Lagoon estuary are subject to the effects of continued exposure to UV radiation. Knowledge of this impact is relevant to understand future changes in the populations dynamics of planktonic organisms, and its ecological and economic implications to southern Brazil.

Keywords: UVA, UVB, Patos Lagoon, GEM-81, oxidative stress, DNA damage.

1. INTRODUÇÃO

A radiação ultravioleta (UV) compreende uma faixa de comprimentos de onda eletromagnética que varia entre 200 e 400 nm. As células, sejam procarióticas ou eucarióticas, possuem inúmeros cromóforos endógenos para a radiação UV, tornando-a capaz de causar danos em diferentes alvos celulares. Considerando essa capacidade, a radiação UV foi dividida, para fins biológicos, em três faixas de comprimento de onda: UVA (320 a 400 nm), UVB (290 a 320 nm) e UVC (200 a 290 nm). As radiações UVA e UVB também são chamadas de UV solar e podem causar uma variedade de efeitos nos organismos vivos. Já a radiação UVC, também chamado de UV curto, é totalmente absorvida na estratosfera pelo oxigênio e pelo ozônio, sendo ecologicamente irrelevante (Chapman *et al.*, 1995), com exceção em grandes altitudes, como nos Andes e Himalaia.

Vários são os fatores que interferem na magnitude dos efeitos biológicos do UV solar, como a espessura da camada de ozônio, o espaço percorrido pela radiação até atingir o alvo, as condições atmosféricas cada vez mais alteradas pela poluição, e ainda a capacidade de reflexão dos comprimentos de onda pelo solo (Parrish *et al*., 1978; Häder & Worrest, 1991).

A diminuição da camada de ozônio em altas e médias latitudes, particularmente no continente antártico e sul-americano (Stolarski *et al.*, 1992; Gleason *et al.*, 1993; Manney *et al.*, 1994; Kirchhoff *et al*., 1997; Kirchhoff & Echer, 2001) tem sido correlacionada com o aumento do perigo biológico do UVB, uma vez que é o ozônio o principal agente capaz de barrar parcialmente essa radiação (Madronich, 1992; Kerr & McElroy, 1993). Estuários localizados

em latitudes médias da América do Sul e Uruguai podem ser especialmente suscetíveis à radiação UV devido à redução no ozônio estratosférico (Sze *et al.,* 1989). De fato, baixas concentrações de ozônio, durante curtos períodos de tempo, têm sido detectadas na América do Sul devido ao transporte de massas de ar com pouco ozônio trazidas pelo vórtex Antártico (Kirchhoff *et al*., 1996).

No ambiente marinho, ao contrário do que se pensava (Jerlov, 1950), a radiação UV pode penetrar profundamente na coluna d'água (Hargreaves, 2003), tendo importantes efeitos sobre os organismos que aí vivem, como bactérias, cianobactérias e fitoplâncton (Häder & Sinha, 2005). O fitoplâncton, particularmente, é bastante vulnerável a radiação UV, pois estes organismos vivem na superfície da água em áreas costeiras e oceânicas, recebendo intensa radiação solar. Häder e colaboradores (1998) viram que a radiação UV prejudica o fitoplâncton a nível molecular, celular e em nível de população e comunidade, podendo ter sérias conseqüências sobre a cadeia alimentar.

A transmissão de UV no ambiente aquático pode variar com mudanças na estabilidade e propriedades da coluna d'água devido à variação sazonal da radiação solar, florações de fitoplâncton ou variações no material em suspensão e dissolvido (Kirk, 1994). As mudanças na atenuação da radiação UV podem ter importantes efeitos biológicos sobre os organismos aquáticos, como invertebrados, macrófitas e peixes, afetando sua taxa de crescimento e reprodução (Häder & Sinha, 2005).

Os efeitos biológicos deletérios da radiação UV vão desde danos moleculares e celulares a efeitos em nível de população (Wake, 1991; Kerr & McElroy, 1993; Blaustein *et al.*, 1994; Bothwell *et al*., 1994; Hofer & Mokri,

2000; Ravanat *et al*., 2001; Armstrong *et al*., 2002). Os danos moleculares incluem a deterioração de importantes moléculas biológicas, como proteínas, lipídeos e DNA (Sinha & Häder, 2002; Gouveia *et al*., 2005).

A exemplo do que acontece com as radiações ionizantes, a radiação UV ataca moléculas orgânicas essenciais por mecanismos direto e indireto. Danos estruturais de DNA podem ser causados diretamente pelo UVB, com a formação de dímeros de ciclobutano de pirimidina (CPD) (Cadet *et al*. 1997), sendo o câncer de pele um exemplo clássico deste impacto; e indiretamente pelo UVA, onde moléculas intermediárias, preferencialmente a água, são irradiadas e, como conseqüência, espécies reativas de oxigênio (ROS) são produzidas que, por sua vez, podem gerar estresse oxidativo e, desta forma, alterar moléculas orgânicas como proteínas, lipídeos e DNA (Ravanat *et al.,* 2001). São vários os trabalhos demonstrando que a radiação UVA provoca preferencialmente danos celulares não nucleares, ao contrário das radiações UVB e UVC (Kochevar, 1990; Beer *et al.*, 1993).

O estresse oxidativo ocorre quando a formação de ROS excede a capacidade de defesa antioxidante ou perturba os caminhos de sinalização redox, afetando a funcionalidade celular (Jones, 2006). Para combater esses danos, as células possuem proteínas que desintoxicam as moléculas de ROS antes que elas possam danificar o DNA ou outros componentes celulares (Ridley *et al*., 2009).

Armeni & colaboradores (2004) viram um aumento no sistema de defesa antioxidante em queratinócitos humanos, 4 h depois da irradiação com UVA, sugerindo que esta resposta foi decorrência de algum dano celular. Outros

autores, estudando o mesmo tipo de células, evidenciaram um aumento na formação de micronúcleos (Phillipson *et al*., 2002). Também fibroblastos irradiados com UVA mostraram um aumento na formação de ROS (Camera *et al*., 2008). Peak & Peak (1990) mostraram que o UVA pode danificar o DNA e também outros alvos, como lipídios de membrana em células P3 humanas, levando a mudanças na permeabilidade e funcionamento celular através da peroxidação lipídica (Gaboriau *et al.,* 1993). Os produtos da peroxidação, como hidroperóxidos lipídicos e radicais peroxil podem reagir com outros lipídios, levando a uma reação em cadeia (Tyrell, 1995; Punnonen *et al*., 1991; Girotti, 2001).

A compreensão das respostas induzidas pela radiação UV nos organismos, bem como seus possíveis meios de defesa contra esta radiação, são de fundamental importância. Em função de várias adaptações encontradas nos diferentes organismos (compostos protetores, mecanismos de reparo de DNA, alterações morfológicas, mudanças comportamentais, etc.) a biota pode conviver com a exposição à radiação UV. Entretanto, há um limite, no qual a proteção pode ser efetiva para a defesa dos organismos (Hasen *et al.,* 2003).

Eritroforoma (GEM-81) é uma linhagem celular estabelecida derivada de tumores espontâneos de pele do peixe teleósteo *Carassius auratus* (Matsumoto *et al.,* 1980). Sob tratamento com indutores de diferenciação e soro de peixe, células GEM-81 apresentam uma heterogeneidade clonal e, em muitos casos, células melanizadas (com ou sem a habilidade de movimento dos melanossomas) ou, em proporções menores, escamas e outros tipos celulares podem ser observados (Matsumoto *et al.,* 1981, 1984, 1989).

Alguns efeitos da radiação UV têm sido descritos para organismos marinhos encontrados próximos à superfície, onde altos fluxos desta radiação estão presentes (Dey *et al.,* 1988), como uma maior taxa de mortalidade em salmões *Oncorhyncus nerka* (Bell & Hoar, 1950), em larvas de anchoíta *Engraulis mordax* (Hunter *et al*., 1979, 1981), em ovos de *Perca flavescens* (Williamson *et al*., 1997) e em larvas de *Esox lucius* (Vehniäinen *et al.,* 2007). Outros autores também têm mostrado um aumento significativo no dano de DNA em ovos, embriões e larvas de ouriços do mar *Strongylocentrotus droebachiensis, Sterechinus neumayeri, Evechinus chloroticus, Sphaerechinus granularis* e *Paracentrotus lividus* (Lesser *et al.,* 2006, Nahon *et al*., 2008; Nahon *et al.*, 2009), em caranguejos *Chasmagnathus granulata* (Gouveia *et al.,* 2005), em embriões de salamandra *Ambystoma maculatum* (Lesser, 2001) e do bacalhau *Gadus morhua* (Malloy *et al*., 1997; Lesser *et al*., 2001).

Apesar da relevância ecológica de estuários ou marismas, existem poucos estudos sobre os fatores que controlam a atenuação da radiação UV nestes ecossistemas (Piazena & Häder, 1994; Kuhn *et al.,* 1999). Segundo Conde e colaboradores (2000), os impactos podem ser diminuídos através de substâncias que absorvem ou dispersam a radiação incidente. Neste sentido, alguns estudos demonstraram que a troca entre água doce e marinha, com alta e baixa concentração de MOD (material orgânico dissolvido), respectivamente, é o principal processo que coordena a atenuação da radiação UV nestes sistemas (Kuhn *et al*., 1999; Conde *et al*., 2002).

Os ambientes estuarinos são caracterizados por abrigar uma baixa diversidade, mas alta abundância de algumas espécies, incluindo muitos

organismos juvenis (Jaureguizar *et al.,* 2004). Na Lagoa dos Patos, os dois principais ambientes estuarinos são constituídos por baías costeiras rasas e protegidas, e pelo corpo de água central (Bonilha & Asmus, 1994). Esta lagoa está conectada ao oceano através de um único canal relativamente estreito, o que a caracteriza como uma lagoa do tipo “estrangulado”, controlada mais pela força dos ventos do que pela maré (Kjerfve, 1986; Soares & Möller Jr, 2001).

Uma elevada variabilidade interanual da precipitação pluviométrica e um complexo padrão de fluxo dos rios, na bacia de drenagem, resultam em processos hidrográficos altamente variáveis e dinâmicos (Asmus, 1998). Essa dinâmica e a diversidade de hábitats proporciona um abundante suprimento de alimento e proteção contra predadores, o que faz deste estuário um ambiente próprio para o desenvolvimento de ovos e larvas (Weiss & Souza 1977 a, b, Muelbert 1986, Muelbert & Weiss 1991). A maioria destes organismos apresenta grande tolerância às condições ambientais flutuantes, típicas destes sistemas. Os principais parâmetros conhecidos por afetar a estrutura espacial das comunidades estuarinas, são a salinidade e profundidade (Whitfield, 1999). Entretanto, fatores como temperatura, turbidez e capacidade de penetração da radiação UV também podem influenciar estes organismos.

Neste estudo, buscamos conhecer a incidência sazonal de UVA e UVB na coluna d’água do estuário da Lagoa dos Patos (ELP) e seus efeitos em células de eritroforoma GEM-81 e em larvas do peixe-rei *Odonthestes argentinensis*, uma das espécies dominantes na ictiofauna estuarina.

1.1 HIPÓTESES

Os antecedentes descritos acima permitem a formulação das seguintes hipóteses que serão investigadas nesta Tese:

- A penetração de UVA e UVB na coluna d'água do ELP é influenciada pela transparência da água, salinidade, concentração de séston e clorofila-a;

- a viabilidade celular de eritroforoma (GEM-81) será alterada quando exposta a doses ambientais de UVA ou UVB;

- células expostas ao UVA sofrerão estresse oxidativo;

- células expostas ao UVB sofrerão dano de DNA;

- a incidência de radiação UV em nossa região causa danos oxidativos nas fases iniciais do desenvolvimento larval de peixes e influencia seu crescimento e sobrevivência.

1.2 OBJETIVOS

Objetivo Geral:

Conhecer a incidência sazonal de UVA e UVB na coluna d'água do ELP e seus principais fatores atenuantes, e estudar os efeitos dessas radiações UV em células e larvas de peixes.

Objetivos específicos:

1- Monitorar a incidência de UVA e UVB na coluna d'água do ELP;

2- estudar a interferência de variáveis ambientais (transparência da água, salinidade, concentração de séston e clorofila-a) na penetração de radiação UV na coluna d'água do ELP;

3- determinar a viabilidade celular em culturas celulares estabelecidas da linhagem GEM-81 (eritroforoma), submetidas às radiações UVA ou UVB;

4- avaliar a produção de espécies reativas de oxigênio (ROS) e a resposta do sistema de defesa antioxidante (TOSC) na linhagem celular GEM-81, exposta ao UVA;

5- avaliar possíveis alterações nos níveis de lipídios peroxidados (LPO) na linhagem celular GEM-81 exposta ao UVA;

6- demonstrar possíveis danos de DNA na linhagem celular GEM-81, exposta ao UVB;

7- determinar possíveis alterações no crescimento e mortalidade larval do peixe-rei (*O.argentinensis*), ocasionada pela exposição a doses ambientais destas radiações;

8- analisar a capacidade do sistema de defesa antioxidante (TOSC) em larvas de peixe-rei (*O.argentinensis*), ocasionados pela exposição a doses ambientais de UVA ou UVB.

1.3 ESTRUTURA DA TESE

Os objetivos específicos desta Tese são abordados em estudos independentes que se encontram nos anexos sob forma de trabalhos submetidos ou em processo de submissão para publicação. Os objetivos 1 e 2 compõem o trabalho intitulado "The influence of abiotic and biotic factors on

UVA and UVB penetration in the water colunm of a South West Atlantic subtropical estuary" (anexo 5.1). Os objetivos 3 a 6 são estudados no trabalho "Effects of the UVA and UVB radiation on GEM-81 goldfish erythrophoroma cells" (anexo 5.2). Por último, os objetivos 7 a 9 compõem o trabalho "Efeitos das radiações UVA e UVB em larvas de peixe *Odontesthes argentinensis*" (anexo 5.3).

Nestes trabalhos, encontram-se os detalhes metodológicos, resultados sucintos e discussão de cada objetivo. Uma síntese dos principais aspectos metodológicos, resultados, discussão e conclusão é apresentado a seguir como corpo principal desta Tese.

2. SÍNTESE DO ESTUDO

2.1 Avaliação da influência de fatores ambientais na penetração de UVA e UVB na coluna d'água do estuário da Lagoa dos Patos (anexo 5.1)

O monitoramento da radiação UV foi realizado em uma estação localizada na região interna (32°01'S 52°06'W) ("es tação 1"), e outra na região próxima a desembocadura do ELP (32°08'S 52°06'W) (" estação 2"). As medições foram feitas durante 15 meses, 2 vezes por semana, através de uma fotocélula (radiômetro/fotômetro IL 1400 A, International Light, MA, USA) presa a uma haste e submersa na superfície, 25, 50, 75 e 100 cm de profundidade. O coeficiente de atenuação foi calculado assumindo uma diminuição exponencial da irradiância. Entretanto, para facilitar a visualização, utilizamos Z_{10}, que representa a profundidade correspondente a 10% da irradiância de superfície. As medições foram realizadas ao meio dia para minimizar influências devidas à variação na posição do Sol. Dados de temperatura, salinidade e transparência da água (Disco de Secchi) também foram obtidos para cada amostragem. Amostras da água de superfície foram coletadas, filtradas e analisadas para determinação de clorofila-a e conteúdo de séston. Os dados foram tratados através de análise de variância (ANOVA) e análise de regressão múltipla.

A maior penetração de radiação UVA ocorreu na estação próxima ao oceano, durante os meses de verão. A variação na profundidade de penetração foi controlada principalmente pela transparência e influenciada pela salinidade da água. Na estação localizada no interior do estuário, UVA atingiu regiões mais profundas no outono e mais rasas na primavera sendo, esta estação do

ano, significativamente mais rasa do que o verão, outono e inverno e coincidindo com águas menos transparentes, de baixa salinidade e alta concentração de séston e clorofila-a. Na estação próxima ao oceano, a penetração de UVA na coluna d'água no verão foi significativamente mais profunda do que na primavera. Na primavera, a transparência e a salinidade da água foram baixas e o conteúdo de clorofila-a foi alto, diminuindo a penetração da radiação UVA. A radiação UVB não apresentou variação entre as estações do ano nem entre as estações de amostragem.

Nossos resultados apóiam a hipótese de que a penetração de radiação UV na coluna d'água está condicionada a eventos de intrusão salina que são comuns nos meses de verão e outono, quando temos águas mais claras no estuário. Esta condição sugere que organismos que vivem em regiões superficiais da coluna d'água estão sujeitos aos perigosos efeitos da exposição continuada à radiação UV neste período. Em especial durante o verão, quando espécies meroplanctônicas como peixes e crustáceos utilizam o ELP como área de criação (Muelbert & Weiss, 1991; Calazans, 1992). Entretanto, não podemos desconsiderar o fato de que em outras estações do ano também ocorrem dias com altos índices de UV na água e que os organismos também sofram danos causados pela exposição esporádica à radiação UV.

2.2 Efeitos das radiações UV utilizando como indicador biológico células de eritroforoma GEM-81 (anexo 5.2)

Considerando que ocorre a penetração da radiação UV na coluna d'água do estuário, é pertinente testar seus efeitos em organismos vivos, utilizando como modelo experimental a linhagem celular GEM-81, eritroforoma do peixe *Carassius auratus* (peixe dourado).

As células GEM-81 foram crescidas em meio Ham F-10, suplementado com 10% de soro fetal bovino e 1% de antibiótico e antimicótico, à 28℃. Numa densidade de 5,0 X 10^5 cél/mL, as células foram irradiadas em PBS, com diferentes doses de UVA (0,78; 1,56; 3,13; 4,69 e 6,26 $J.cm^{-2}$) ou UVB (0,007; 0,014 e 0,07 $J.cm^{-2}$). Depois de irradiadas, as células foram novamente colocadas em meio de cultura e a viabilidade celular foi avaliada pela técnica de exclusão por azul de tripan, em diferentes tempos de observação. Níveis de ROS, LPO e TOSC foram testados nas células irradiadas com UVA (3,13 $J.cm^{-2}$), 48 h após a irradiação. Nas células irradiadas com as maiores doses de UVB, utilizamos o ensaio cometa para detecção de possíveis danos de DNA.

As células expostas às maiores doses de UVA tiveram uma diminuição significativa na proliferação celular, 48 h após a irradiação, ocorrendo uma recuperação em 72 h, quando comparadas ao grupo controle. Os níveis de ROS e do conteúdo de LPO, foram significativamente maiores nas células expostas ao UVA, enquanto que a área relativa invertida do TOSC foi menor, indicando baixa competência em neutralizar radicais peroxil. Apesar disso, a recuperação na proliferação celular em 72 h é indicativa de que a dose utilizada não foi citotóxica para esta linhagem celular.

Nas células irradiadas com UVB, não houve diferença significativa entre o número de células do grupo controle e irradiado com a menor dose. Entretanto, o grupo exposto à maior dose de UVB apresentou diferença significativa quando comparado ao seu controle já nas 24 h após a irradiação. A partir de 48 h, os grupos que receberam as demais doses mostraram diferença significativa no número de células viáveis em relação ao seu grupo controle. UVB causou citotoxicidade na maior dose utilizada e foi capaz de induzir dano de DNA nas células irradiadas com uma dose intermediária. Assim, vimos que a linhagem celular de eritroforoma GEM-81 foi sensível a doses mais elevadas de radiação UVA, apresentando inibição de proliferação celular no tempo de 48 h após a exposição. No entanto, a rápida recuperação na proliferação de células nas próximas 24 h sugere que as doses ambientais de UVA utilizadas não são citotóxicas. Por outro lado, as células GEM-81 irradiadas com a maior dose de UVB apresentaram morte celular já nas 24 h após a irradiação, sendo indicativa de fototoxicidade.

Estes resultados confirmam as hipóteses postuladas de que a viabilidade celular de eritroforoma (GEM-81) será alterada quando exposta a doses ambientais de UVA ou UVB. Ficou evidente também de que células expostas ao UVA sofrem estresse oxidativo, e de que células expostas ao UVB tem dano de DNA. Apesar do uso de doses ambientais de UVA e UVB, não se pode desconsiderar o fato de que, no ambiente, outros comprimentos de onda são emitidos pelo Sol, o que pode causar um efeito sinergístico ou até protetor nas respostas dos organismos .

2.3 Efeitos das radiações UVA ou UVB em larvas do peixe-rei *Odontesthes argentinensis* (anexo 5.3)

O fato de doses de radiação UV equivalentes as que ocorrem no ambiente estuarino induzir danos oxidativos e de DNA em células GEM-81, permite hipotetizar que também haja danos em nível de organismo. Em especial, este efeito deve ser mais expressivo em organismos planctônicos, que vivem próximos à superfície da coluna d'água. Assim, esta etapa do estudo investiga os efeitos desta radiação em larvas do peixe-rei *O. argentinensis.*

Ovos do peixe-rei *O. argentinensis* foram coletados na beira da praia do Cassino, Rio Grande, RS e levados para a Estação Marinha de Aquacultura (EMA) da FURG. Após a eclosão, as larvas foram transferidas para aquários, com troca d'água diária, antes da exposição às radiações UVA ou UVB. Após a exposição, era então fornecido o alimento, que se consistiu de *Artemia sp* "*ad libitum".* Diariamente foi feita a medição e o acompanhamento da mortalidade das larvas de O. *argentinensis.* Testes para avaliar o sistema de defesa antioxidante (TOSC) foram feitos após o 12º dia de exposição com UVA e após o 3º dia de irradiação com UVB.

Nossos resultados mostraram que organismos irradiados com UVA cresceram significativamente menos do que aqueles que não foram irradiados. Os experimentos de mortalidade mostraram que as larvas irradiadas com UVB sobreviveram menos do que aquelas do grupo controle. Problemas com o experimento de UVA, possivelmente devido a condições fisiológicas dos animais selecionados, não possibilitaram resultados conclusivos sobre os efeitos desta radiação na mortalidade larval. A área relativa invertida de TOSC

apresentou uma tendência não significativa de diminuição nas larvas irradiadas com UVA durante 12 dias. Nas irradiadas com UVB por 3 dias, houve uma diminuição significativa na competência antioxidante, sugerindo que estes organismos estavam em situação de estresse oxidativo.

Neste sentido, os resultados corroboram a hipótese que doses ambientais de radiação UV encontradas na região sul do Brasil causam efeitos deletérios em larvas de *O. argentinensis.* Isto sugere que, organismos planctônicos, que vivem próximo à superfície da coluna d'água, podem sofrer efeitos danosos destas radiações. Em especial, durante o verão quando a maioria das espécies de peixes desova e dispersa seus ovos e larvas nas águas do estuário da Lagoa dos Patos (Muelbert & Weiss, 1991). Entretanto, experimentos adicionais são necessários para concluir sobre os efeitos destas radiações em outros organismos planctônicos.

3. CONSIDERAÇÕES FINAIS E RECOMENDAÇÕES

Os resultados desta Tese contribuem para o melhor entendimento dos efeitos que radiações UV ambientais podem causar nos organismos vivos no meio aquático. Considerando a nossa localização geográfica, em particular, este estudo demonstra que, apesar do estuário da Lagoa dos Patos ter águas túrbidas, estas radiações podem penetrar consideravelmente na coluna d'água quando há intrusão de águas salgadas oriundas do oceano em especial durante o verão. Este fato é preocupante, uma vez que o estuário é considerado um berçário para muitas espécies de importância comercial em nossa região, e que durante este estágio de sua vida estas espécies vivem perto da superfície.

Além disso, a Tese estabeleceu que a radiação UV pode causar danos em linhagens celulares e que estas são hábeis em reparar estes danos. As células pigmentares utilizadas neste trabalho são oriundas de tumores espontâneos do peixe dourado e se mostraram resistentes à radiação UV ambiental.

Para estudar o efeito em organismos, experimentos com larvas de peixe-rei (*O. argentinensis*) foram conduzidos com doses de exposição equivalentes as ambientais. Os resultados mostraram que estes organismos são sensíveis à radiação UV, o que leva a sugerir que organismos planctônicos no estuário da Lagoa dos Patos, que vivem próximo à superfície da coluna d'água, podem sofrer com os efeitos deletérios destas radiações.

No entanto, os experimentos com larvas de peixe-rei precisam ser reavaliados, uma vez que apenas um experimento com baixo número amostral

foi realizado. Este relato demonstra a dificuldade na realização de experimentos controlados com os primeiros estágios de vida de peixes, e aponta para necessidade de estudos futuros para testar com maiores detalhes a hipótese da influência do aumento da incidência de radiação UV em organismos que utilizam águas superficiais em suas fases iniciais de desenvolvimento no sul do Brasil.

4. LITERATURA CITADA

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5. ANEXOS: manuscritos na forma original de submissão

Anexo 5.1 - Gouveia, GR; Gambetá-Leite, MRS; Nery, LEM; Trindade, GS & Muelbert, JH. 2009. The influence of abiotic and biotic factors on UVA and UVB penetration in the water colunm of a South West Atlantic subtropical estuary. Submetido para a revista *Marine Environmental Research.*

THE INFLUENCE OF ABIOTIC AND BIOTIC FACTORS ON UVA AND UVB PENETRATION IN THE WATER COLUNM OF A SOUTH WEST ATLANTIC SUBTROPICAL ESTUARY

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Abstract

Our goal was to determine which environmental variables like transparency, salinity, seston and chlorophyll a (Chl a) concentration and if marine/freshwater exchange influences the penetration of UVR in the water column. UVR was monitored at two sampling sites in the estuary of Patos Lagoon. At station 1, UVA penetration was deeper in autumn and shallower in spring, being the later season significantly smaller than the summer, autumn and winter and coinciding with less transparent waters, low salinity and high concentration of seston and Chl a. At station 2, UVA penetration in the water column was significantly different in the spring and summer. In spring, the transparency and water salinity were low and the Chl a was high. UVB did not present significant variation between seasons at both sampling stations. The variability in UVA penetration could be mainly explained by alterations in the water transparency and salinity and, in summer months, also by the Chl a concentration.

KEYWORDS: UVA, UVB, water attenuation, Patos Lagoon, salinity, water transparency.

1. Introduction

In the last decades, decreases in stratospheric ozone over high and mid latitudes, and the regular incidence of polar ozone holes in the Antarctic and Arctic have been observed. As a consequence solar ultraviolet radiation (UVR) reaching the Earth's surface has increased (Crutzen, 1992; Kerr and McElroy, 1993; Kerr, 1994; Manney et al., 1994; Lubin and Jensen, 1995; Weatherhead and Andersen, 2006).

The electromagnetic spectrum of UVR is usually divided into three bands: UVA (400-320 nm), UVB (320-290 nm) and UVC (290-200 nm). While UVC is essentially absorbed by oxygen and is ecologically irrelevant, the longer UVB and UVA can have a variety of effects on many organisms. The deleterious effects of UVR range from molecular and tissue damage to population level effects (Wake, 1991; Kerr and McElroy, 1993; Blaustein et al, 1994; Bothwell et al, 1994; Hofer and Mokri, 2000; Ravanat et al, 2001; Armstrong et al, 2002) including alteration of relevant biological molecules such as proteins, lipids and DNA (Sinha and Häder, 2002; Gouveia et al, 2005).

In the marine environment, UVR can penetrate deeper in the water column (Hargreaves, 2003) than previously expected (Jerlov, 1950), and can have important effects on organisms including bacteria, cyanobacteria and phytoplankton (Häder and Sinha, 2005). Particularly phytoplankton, that lives in the top layers of the oceans and coastal areas and receives intense solar radiation, has been considered vulnerable to UVR. Results showed that solar UVR damages phytoplankton at the molecular, cellular, population and

community levels, which may have consequences for the food web (Häder et al, 1998).

Despite several behavioral and physiological adaptations that prevent or counteract the damaging effect of high ambient UVR, many studies have shown that organisms that live close to the surface of the water column may be suffering the damage of hazardous UVR. Recent studies on phytoplankton have showen photoinhibition effects induced by UVR (Sommaruga and Augustin, 2006; Gao et al., 2007; Villafañe et al., 2008; Hessen et al., 2008; Scott et al., 2009). Other studies demonstrated that embryos of the spotted salamander, cod larvae, anchovy larvae, fish and krill showed DNA damage after exposure to UVB (Malloy et al., 1997; Lesser, 2001; Lesser et al., 2001), high rate of mortality or abnormal behavior (Vehniäinen et al., 2007). Obermüller et al. (2007), saw that UVA and UVB induced an alteration of the respiratory intensity rates in polar amphipods, suggesting that occur probably due to radiation avoidance, shelter seeking and from cellular repair processes. Studies in mesocosmos Belzile et al. (2006) revealed that an increase in UVR can significantly impact the food web structure due to different sensivity of planktonic organisms.

These impacts can be diminished in eutrophic freshwater and coastal areas of the oceans where absorbing and scattering substances attenuate the incident radiation (Conde et al, 2000). Despite the ecological relevance of estuaries and other shallow brackish systems, relatively few studies have been devoted to reveal the factors that control the UVR attenuation in these ecosystems (Piazena and Häder, 1994; Kuhn *et al.,* 1999). Estuaries are

characterized by intense exchange of waters with different concentrations of dissolved organic material (DOM) that absorb UVR (Stedmon et al., 2000). Early studies demonstrated that exchange between freshwater and marine waters with high and low DOM concentration, respectively, is the main process governing the underwater UVR attenuation in estuarine systems (Kuhn et al., 1999; Conde et al., 2002).

Estuaries in the mid-latitudes of South America and Uruguay may be susceptible to UVR due to significant reduction of stratospheric ozone (Sze et al., 1989). In fact, low ozone concentrations during short periods of time have already been detected in South America up to 30°S, due to the transport of low ozone air masses following the Antarctic vortex break-up (Kirchhoff et al., 1996).

Estuaries are ecologically important environments (Dyer, 1997). In southern Brazil, the Patos Lagoon estuary is characterized by a single entrance channel and wind forced hydrology (Kjerfve, 1986; Soares and Möller Jr, 2001). Because of relatively high annual precipitation in southern Brazil, Patos Lagoon is mainly an oligohaline-freshwater river ecosystem, and the high variability inherent to estuaries is restricted to its southern area (Odebrecht et al., 2005; Seeliger, 2001). Approximately 80% of the estuarine region is shallower than 2 m and constitute important nursery grounds for many commercially relevant fish and shrimp species (Calazans, 1984; Muelbert and Weiss, 1991). In order to reach the nursery areas, planktonic organisms enter the estuary through this single channel (Sinque and Muelbert, 1997).

The main objectives of the present study are to assess the role of marine/freshwater exchange on UVR penetration in the water column and to determine the amount of UVR in the water column on the basis of environmental variables like transparency, salinity, seston and chlorophyll a (Chl a) concentration.

2. Material and methods

2.1 Study area

The Patos Lagoon located in the southern Brazilian coastline between 30–32°S and 50–52°W, is the largest choked coastal lagoon in the world (Kjerfve, 1986). With a length of 250 km and average width of 40 km, the lagoon covers an area of 10.360 km^2, and is connected to the South Atlantic Ocean via a 5 km long and less than 1 km wide inlet. This single entrance channel, allows a perennial exchange of water, sediment, dissolved materials and organisms between the estuary and the open sea (Fernandes et al., 2004). The region has small tidal influence and it is dynamically dependent on wind and freshwater discharge (Möller et al., 1996). The rivers flowing into the lagoon have a total catchments area of 201.626 km^2. They exhibit a typical mid-latitude pattern of high discharge in late winter and early spring, followed by low to moderate discharge through summer and autumn. They also have a large year-to-year variation in discharge rates (Möller et al., 1996). A balance between the wind and freshwater inflow controls the direction and intensity of the currents in the system as well as its residence time.

2.2 Sampling

UVR was monitored at two sampling sites in the estuary of Patos Lagoon from December 2005 to February 2007. One site was located in the interior of the estuary, with depth of approximately 2 m (32º01'S 52º06'W – station 1), and the other near the lagoon mouth, with depth of approximately 14 m, (32º08'S 52º06'W – station 2) (Fig. 1). One hundred and three samplings were made and classified according to the weather condition as: cloudy (13); partially cloudy (24); and, sunny (66). For this study, only the 66 measurements taken at sunny conditions are considered.

2.3 Irradiance measurements

UVR was measured around noon time to minimize influence of solar position. A radiometer/photometer (model IL 1400A, International Light, MA, USA) was used, and the photocell was fixed to a pole and submerged in the water column at surface, 25, 50, 75 and 100 cm depth. The attenuation coefficients (K_d) were calculated assuming an exponential decrease of irradiance with depth, according to Kirk (1994):

$$E_d(Z) = E_d(0)\, e^{-K_d Z}$$

where E_d (Z) is the value of irradiance at depth Z (m), E_d (0) is the value of irradiance just below the surface. To facilitate visualization, we use the 10% irradiance depth ($Z10 = 2/3\ K_d$) which represents the depth corresponding to 10% of the irradiance just below the surface (Whitehead et al., 2000).

2.4 Water parameters

Water temperature and salinity were measured using a thermosalinometer (Model 30-50 FT, YSI Incorporated – Yellow Springs, Ohio, USA) and water transparency using a Secchi disk.

2.5 Chlorophyll a and seston concentration

Duplicate surface water samples were collected to determine Chl a and seston concentration. Concentration of Chl a was determined from 10-50 mL aliquotes filtered through a Whatman GF/F 55 mm diameter, and extracted with acetone 90% v/v over 24 hours at low temperature (-16℃) in the dark. Chl a concentration ($\mu g.L^{-1}$) was measured with a calibrated fluorometer (Turner TD-700) without acidification (Welschmeyer, 1994). For seston ($mg.L^{-1}$), 70-600 mL aliquotes were filtered (Whatman GF/F 55 mm diameter) with a previously dried (60℃, 24 h) and weighted filter.

2.6 Statistical analysis

Factorial analysis of variance (ANOVA) was used to test differences in variables among seasons as well as differences in penetration of UVA and UVB into the water column between sampling stations. ANOVA assumptions (data normality and homogeneity of variances) were previously checked. When a significant difference was observed among treatments, means were compared using the Tukey's test. Stepwise multiple linear regressions were performed to evaluate the dependence of the $Z10_{UVA,\ UVB}$ on the water transparency, salinity, Chl a and seston concentration. In all cases, the significance level adopted was 5% ($p<0.05$).

3. Results

3.1 Intensity of UVA and UVB on land and on the surface of the water column

The values of land UVA intensity in the interior of the estuary (station 1) from 88.78 ± 5.10 to 140.10 ± 4.69 $W.m^{-2}$, corresponding to winter and spring, respectively (Fig. 2A). Closer to the ocean (station 2), the values were from 82.11 ± 5.16 to 136.00 ± 4.77 $W.m^{-2}$, corresponding also to winter and spring, respectively. UVA intensity during spring and summer was significantly higher than autumn and winter in both stations ($p<0.05$) (Fig. 2A). The values of UVA intensity on the surface of the water column in the interior of the estuary (station 1) from 58.98 ± 5.98 to 100.40 ± 3.05 $W.m^{-2}$, corresponding to winter and summer, respectively (Fig. 2B). Closer to the ocean (station 2), the values were from 52.09 ± 5.30 to 95.58 ± 3.83 $W.m^{-2}$, corresponding also to winter and summer, respectively. UVA intensity during spring was significantly different to summer and summer was significantly higher than winter in both stations ($p<0.05$) (Fig. 2B). The values of land UVB intensity in the interior of the estuary (station 1) from 0.92 ± 0.12 to 3.19 ± 0.29 $W.m^{-2}$, corresponding to winter and spring, respectively (Fig. 3A). Closer to the ocean (station 2), the values were from 0.95 ± 0.11 to 3.08 ± 0.14 $W.m^{-2}$, corresponding also to winter and spring, respectively (Fig. 3A). UVB intensity during spring and summer was significantly higher than autumn and winter in both stations ($p<0.05$) (Fig. 3A). The values of UVB intensity on the surface of the water column in the interior of the estuary (station 1) from 0.53 ± 0.07 to 1.73 ± 0.10 $W.m^{-2}$, corresponding to winter and summer, respectively. The summer months were significantly different to autumn and winter months ($p<0.05$) (Fig. 3B). Closer to the ocean (station 2),

the values were from 0.37 ± 0.07 to 1.51 ± 0.10 $W.m^{-2}$, corresponding also to winter and summer, respectively. UVB intensity during summer was significantly different to winter months ($p<0.05$) (Fig. 3B).

3.2 Penetration of UVA and UVB into the water column

In the interior of the estuary (station 1), the depth corresponding to 10% of the irradiance at the surface (Z10) ranged from 0.39 ± 0.03 to 0.70 ± 0.03 m for UVA, corresponding to spring and autumn, respectively (Fig. 4A). $Z10_{UVA}$ during spring was significantly shallower than summer, autumn and winter ($p<0.05$) (Fig. 4A). UVB values were similar over the year, and ranged from 0.22 ± 0.01 to 0.33 ± 0.02 m during winter and summer, respectively (Fig. 4B). Closer to the ocean (station 2), UVA penetration ranged from 0.51 ± 0.03 m during spring to 0.82 ± 0.07 m in summer (Fig. 4A). $Z10_{UVA}$ during summer was significantly deeper than in spring ($p<0.05$) (Fig. 4A). UVB values of 0.26 ± 0.01 m were registered in autumn and 0.42 ± 0.03 m during summer (Fig. 4B). During summer, UVA penetration was significantly deeper closer to the ocean than in the inner estuary ($p<0.05$) (Fig. 4A). $Z10_{UVB}$ was statistically similar among seasons and between sampling stations ($p>0.05$) (Fig. 4B).

3.3 Water transparency

Water closer to the ocean (station 2) showed higher tranparency values than the interior of the estuary (station 1), in wich Secchi disk depth ranged from 0.48 ± 0.09 to 0.91 ± 0.13 m in spring and summer, respectively. Closer to the ocean water column during spring was significantly less transparent than summer at ($p<0.05$) (Fig. 5). In the inner estuary, Secchi disk depth ranged from

0.29 ± 0.05 to 0.90 ± 0.15 m in spring and winter months, respectively. The water during spring months was significantly less transparent than autumn and winter months ($p<0.05$) (Fig. 5).

3.4 Salinity and temperature

In the interior of the estuary, mean seasonal salinity varied from 6.60 ± 2.12 to 24.80 ± 3.12, for spring and autumn. Closer to the ocean, mean seasonal salinity varied from 11.60 ± 2.06 to 28.30 ± 2.49, corresponding to spring and autumn. Although on average salinity was higher closer to the ocean than inside the estuary, differences were not significant ($p>0.05$) (Fig. 6).

In the interior of the estuary, mean temperature between seasons varied from 14.73 ± 0.67 to 25.03 ± 0.30 for winter and summer, respectively. Closer to the ocean, mean seasonal temperature varied from 15.31 ± 0.87 to 24.41 ± 0.21, corresponding to winter and summer, respectively. Although on average temperature was higher closer to the ocean than inside the estuary differences were not significant ($p>0.05$) (Fig. 7).

3.5 Chlorophyll a and seston concentration

Station 1 showed the highest Chl a concentration, with mean values ranging from 5.56 ± 0.84 to 13.02 ± 1.33 $\mu g.L^{-1}$ in winter and spring, respectively. Chl a at spring was significantly higher than other seasons ($p<0.05$) (Fig. 8). At station 2, Chl a mean concentration varied from 2.81 ± 0.35 to 8.65 ± 0.93 $\mu g.L^{-1}$, also corresponding to winter and spring. During spring Chl a was significantly higher than the other seasons ($p<0.05$) (Fig. 8).

In the inner estuary (station 1), seston concentration during spring (1219.77 ± 130.00 $mg.L^{-1}$) was significantly different ($p<0.05$) from summer (815.82 ± 72.25 $mg.L^{-1}$). Despite the high seston concentration during autumn (1136.33 ± 70.34 $mg.L^{-1}$), it was not statistically different from the other seasons ($p>0.05$). Closer to the ocean, mean seston concentrations were 952.87 ± 91.22; 784.09 ± 115.63 and 1083.90 ± 95.56 $mg.L^{-1}$, corresponding to spring, summer and autumn months. Seston was not significantly different among seasons neither between sampling stations ($p>0.05$) (Fig. 9).

3.6 Relationships between $Z10_{UVA, UVB}$ and environmental variables

In the interior of the estuary, $Z10_{UVA}$ was significantly influenced by water salinity ($p<0.05$) but not by transparency ($p<0.18$) when those variables were analysed separately. However, when combined in the multiple linear regression model salinity and transparency can explain $Z10_{UVA}$ ($r^2 = 0.12$, $p<0.01$) (Table 1). $Z10_{UVB}$ was not significantly correlated to any analyzed variable ($r^2 = 0.02$, $p<0.20$).

Closer to the ocean, when the variables were analysed separately, $Z10_{UVA}$ was significantly influenced by water transparency ($p<0.05$) and salinity ($p<0.03$). When combined in the multiple linear regression model, these two variables maintained positively influencing $Z10_{UVA}$ ($r^2 = 0.14$, $p<0.01$) (Table 2). $Z10_{UVB}$ was significantly correlated only with water transparency ($r^2 = 0.34$, $p<0.03$) (Table 2). During spring and summer months, when $Z10_{UVA}$ was significantly different from other seasons, spring $Z10_{UVA}$ was positively correlated to salinity ($p<0.04$) and negatively to water transparency ($p<0.04$),

but not Chl a ($p<0.25$) when analysed separately. Nevertheless, when combined in the model, the variables were not significantly correlated to $Z10_{UVA}$ ($r^2 = 0.13$, $p<0.13$). During summer, $Z10_{UVA}$ was positively influenced by Chl a concentration ($r^2 = 0.26$, $p<0.01$) (Table 3). $Z10_{UVB}$ was positively correlated to transparency ($p<0.01$), but not to water salinity ($p<0.09$) and seston ($p<0.21$) when analysed separately. When combined in the model, all variables were significantly correlated to $Z10_{UVB}$ ($r^2 = 0.27$, $p<0.05$) (Table 3).

4. Discussion

Subtropical estuaries are located in mid-latitudes and sporadically receive high doses of UVR due to the influence of periodic events of low ozone concentrations from its proximity to the Antarctic polar vortex (Kirchhoff et al., 1996). Our results show that

UVA and UVB incidence on the land is greater during the spring, while on the surface of the water column, the highest values occurring during summer.

Z10 values ($Z10_{UVA}$ = 0.39 to 0.82 m and $Z10_{UVB}$ = 0.22 to 0.42 m) are similar to the range reported for other coastal waters of the Southern Atlantic ($Z10_{UVA}$ = 0.33 to 1.1 m and $Z10_{UVB}$ = 0.09 to 0.31 m) (Conde et al., 2000; Conde et al., 2002), with UVA penetrating deeper into the water column than UVB.

In this study, UVR penetration was at deeper the station closer to the ocean than in the interior of the estuary, particularly during summer months. Conde et al., (2000) and Conde et al. (2002), report that during events of marine water intrusion, the UV attenuation coefficient in the brackish zone decreases up to 70% of the values observed under the influence of the freshwater discharge. In

the present work, in the inner station UVA penetration is deeper only during autumn months, when high salinity is observed inside the estuary. In fact, saltwater enters the estuary exactly in summer and autumn, when southwest winds, associated to atmospheric frontal system passages over the region, increases the residence time with high evaporation and low precipitation (Abreu et al., 2009).

In the inner estuary, transparency and water salinity accounted most for changes in UVA. Closer to the ocean, Chl a also revealed to be important in the penetration of UVA during summer months, whereas UVB is only dependent on the transparency of the water. Some studies have shown significant linear correlation between Chl a and UVA in coastal waters (Stambler et al., 1997; Figueroa, 2002; Tedetti and Semperé, 2006). Conde et al. (2000) also showed significant linear correlation between Chl a (6.0 $\mu g.L^{-1}$, approximately) and UVA. In our study, although Chl a values are higher in the interior of the estuary than closer to the ocean, multiple linear regression analysis indicated a negative relationship between $Z10_{UVA}$ and Chl a concentration only in summer months closer to the ocean. During this period, mean Chl a value was 4.06 $\mu g.L^{-1}$, which is within the range in which previous studies have shown an influence on UVA penetration. Moreover, some studies suggest that substances which absorve in this range and covary with Chl a, such as yellow substances, or components like mycosporine-like aminoacids within the phytoplankton, may account for decreasing UVA penetration in the water column (Karentz et al., 1991; Karentz, 1994; Sinha et al., 2001).

Seston was not related to penetration of UVR, except in summer when multiple linear regression model showed association at closer to ocean. All components, including water molecules, DOM, non-algal particles and phytoplankton are known to attenuate UVR in the water column. According to Sommaruga and Augustin (2006), some empirical relationships associate greater UVR penetration in different lakes, when the concentrations of DOC (dissolved organic carbon) are below 2 mg. L^{-1}. During a study about UVR penetration in Alpine lakes, they saw that the concentration of DOC is not an accurate to predictor of UVR in the water column and that changes in penetration, particularly in deeper layers, are mainly controlled by phytoplankton growth, which also influencies the transparency and the level of Chl a concentration in the water. However, Conde et al. (2000) found that in mid-latitude estuaries, attenuation coefficient is significantly correlated to both DOC and Chl a and when the variables were combined in the multiple linear regression model, UVB was better explained by DOC and OSS (organic suspended solids) concentrations, while UVA was associated with DOC and Chl a.

The level of UVR penetration is seasonal, with the deepest $Z10_{UVA,\ UVB}$ occuring during summer months, with highest temperatures. According to Scott et al. (2009), the high temperatures in summer may increase the rate of photoenzimatic repair of organisms, as reported for zooplankton (MacFadyen et al., 2004). Therefore, damaging effects due to an increase in the incidence of UVR during summer may be compensated by use of other defense

mechanisms like the ability of photoprotection by mycosporine-like aminoacids, pigment cells or by migrating downward in the water column.

As emphasized earlier, the Patos Lagoon estuary is an important nursery ground for many commercially relevant fish and shrimp species (Calazans, 1984). The growth of these organisms inside the estuary is essential for the preservation of the local and coastal fishing activity. In addition, it is very important to consider that these organisms enter the estuary during their planktonic stages (Muelbert and Weiss, 1991), when they occupy the water surface layer and are thus susceptible to UVR.

However, an adequate evaluation of the effect of UVR on specific environment has to consider all the relationships between the different variables that compose this environment. Certainly the organism responses are a result of the interaction of several environmental factors that, in an integrated way, contribute significantly to the production of these effects. Furthermore, it has to be considered that the environment is exposed not only to UVR but to the other ranges of solar radiation such as visible and infrared, which may interact with UVR, and these, in turn, with the components of the environment (Udagawa and Nagasawa, 2000), reducing the radiation damage.

5. Conclusion

UVR was shown to penetrate the water column of the Patos Lagoon estuary, and the level and depth of penetration is mainly controlled by marine/freshwater exchange, since salinity and transparency were the variables that most influenced UVR penetration. Therefore, despite all the mechanisms

that can be developed by the organisms to protect themselves from damage caused by these radiations, we can not ignore the fact that they may be suffering from exposure to UVR.

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Figure Legends

Figure 1 – The Patos Lagoon location an the estuary detail showing the sampling sites (■). (Modified from Martins *et al.*, 2007).

Figure 2 – Seasonal variation in the UVA intensity. A – UVA intensity on land. B – UVA intensity on the surface of the water column. ■ – station 1; □ – station 2. Values are expressed as mean +1 standard error of the mean. Different letters indicate significant difference between seasons ($p<0.05$).

Figure 3 – Seasonal variation in the UVB intensity. A – UVB intensity on land. B – UVB intensity on the surface of the water column. ■ – station 1; □ – station 2. Values are expressed as mean +1 standard error of the mean. Different letters indicate significant difference between seasons ($p<0.05$).

Figure 4 – Seasonal variation of depth corresponding to 10% of the irradiance at the surface (Z10). A – $Z10_{UVA}$. B - $Z10_{UVB}$. ■ – station 1; □ – station 2. Values are expressed as mean +1 standard error of the mean. Different letters indicate significant difference between seasons. Circles indicate significant difference between sampling stations ($p<0.05$).

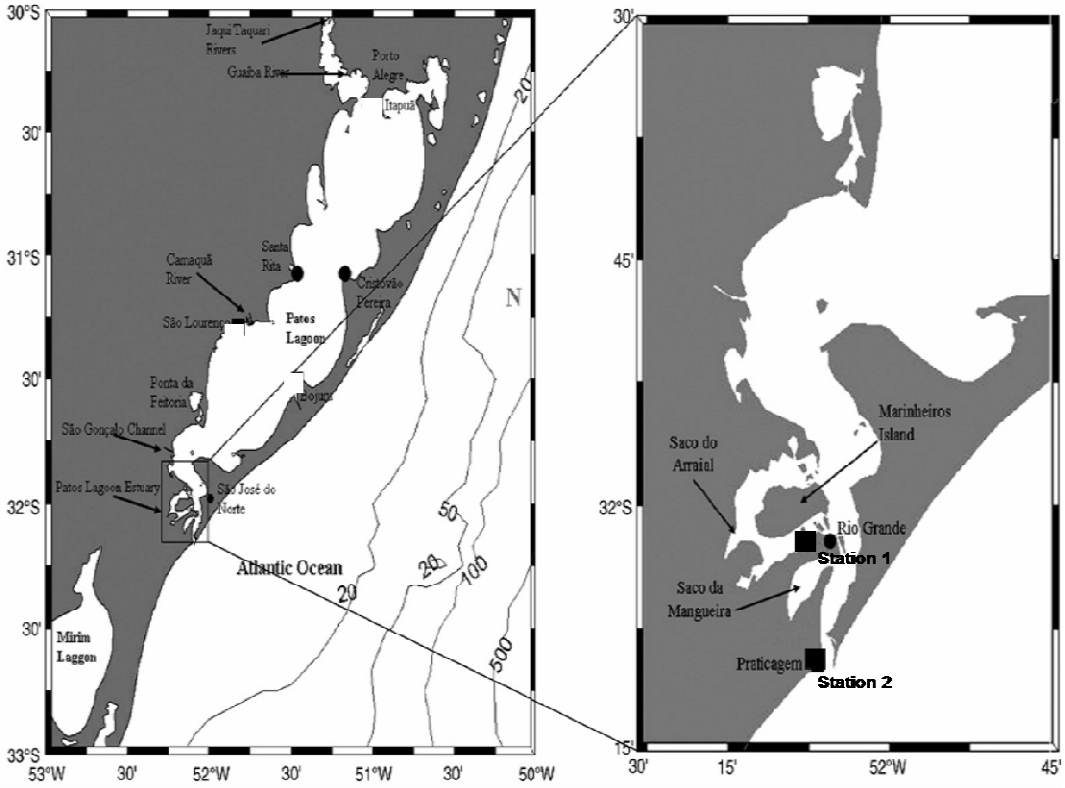
Figure 5 – Seasonal variation of Secchi disk depth. ■ – station 1; □ – station 2. Values are expressed as mean +1 standard error of the mean. Different letters indicate significant difference among seasons ($p<0.05$).

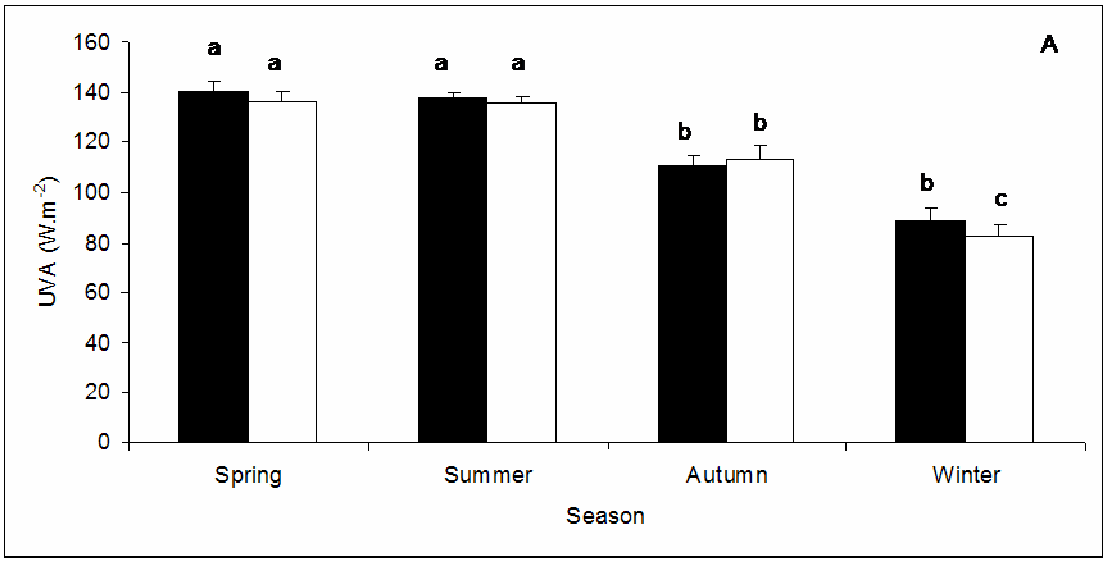
Figure 6 – Seasonal variation of water salinity. ■ – station 1; □ – station 2. Values are expressed as mean +1 standard error of the mean. Different letters indicate significant difference among seasons ($p<0.05$).

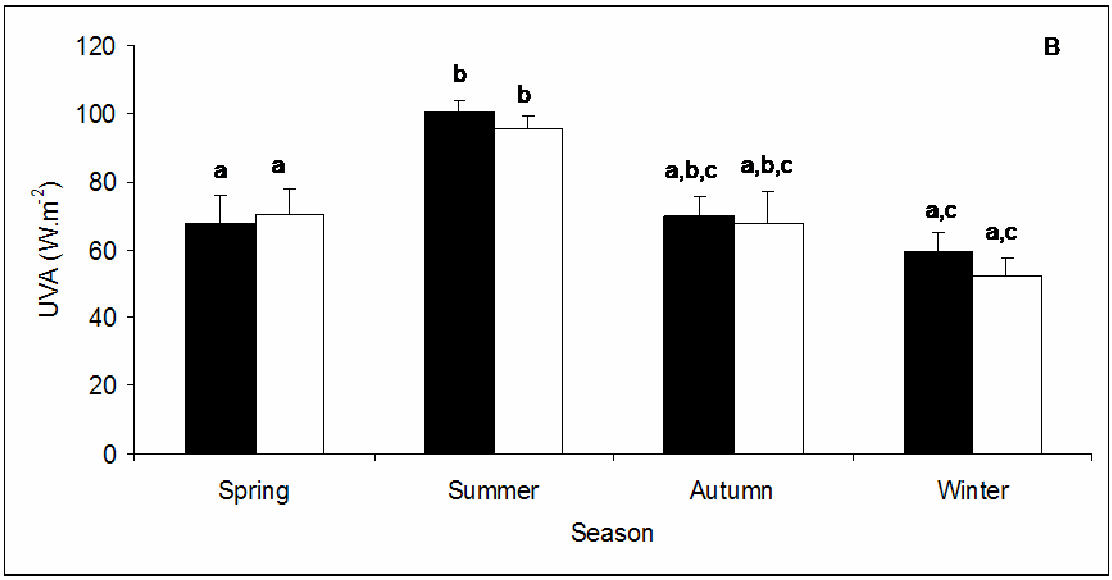
Figure 7 – Seasonal variation of water temperature. ■ – station 1; □ – station 2. Values are expressed as mean +1 standard error of the mean. Different letters indicate significant difference among seasons ($p<0.05$).

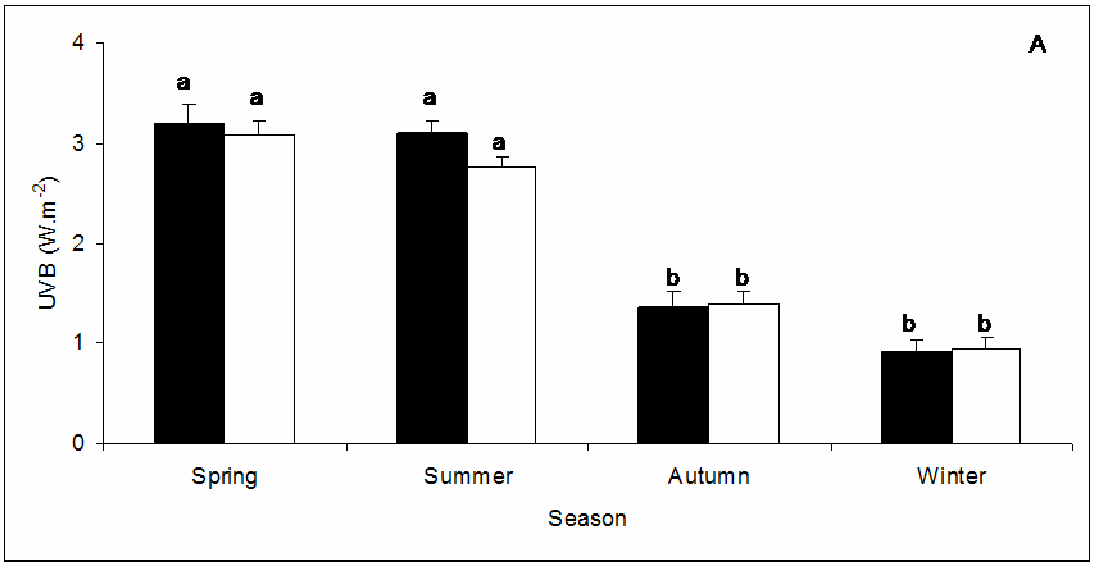
Figure 8 – Seasonal variation of Chl a concentration. ■ – station 1; □ – station 2. Values are expressed as mean +1 standard error of mean. Different letters indicates significant difference among seasons. Circles indicate significant difference between sampling stations ($p<0.05$).

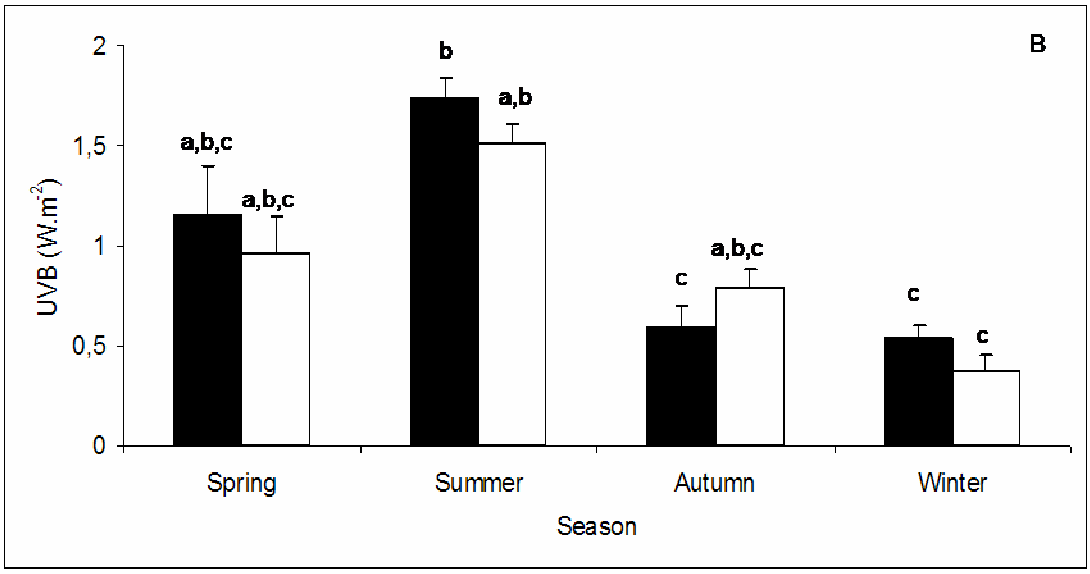
Figure 9 - Seasonal variation of seston concentration. ■ – station 1; □ – station 2. Values are expressed as mean +1 standard error of mean. Different letters indicates significant difference among seasons ($p<0.05$).

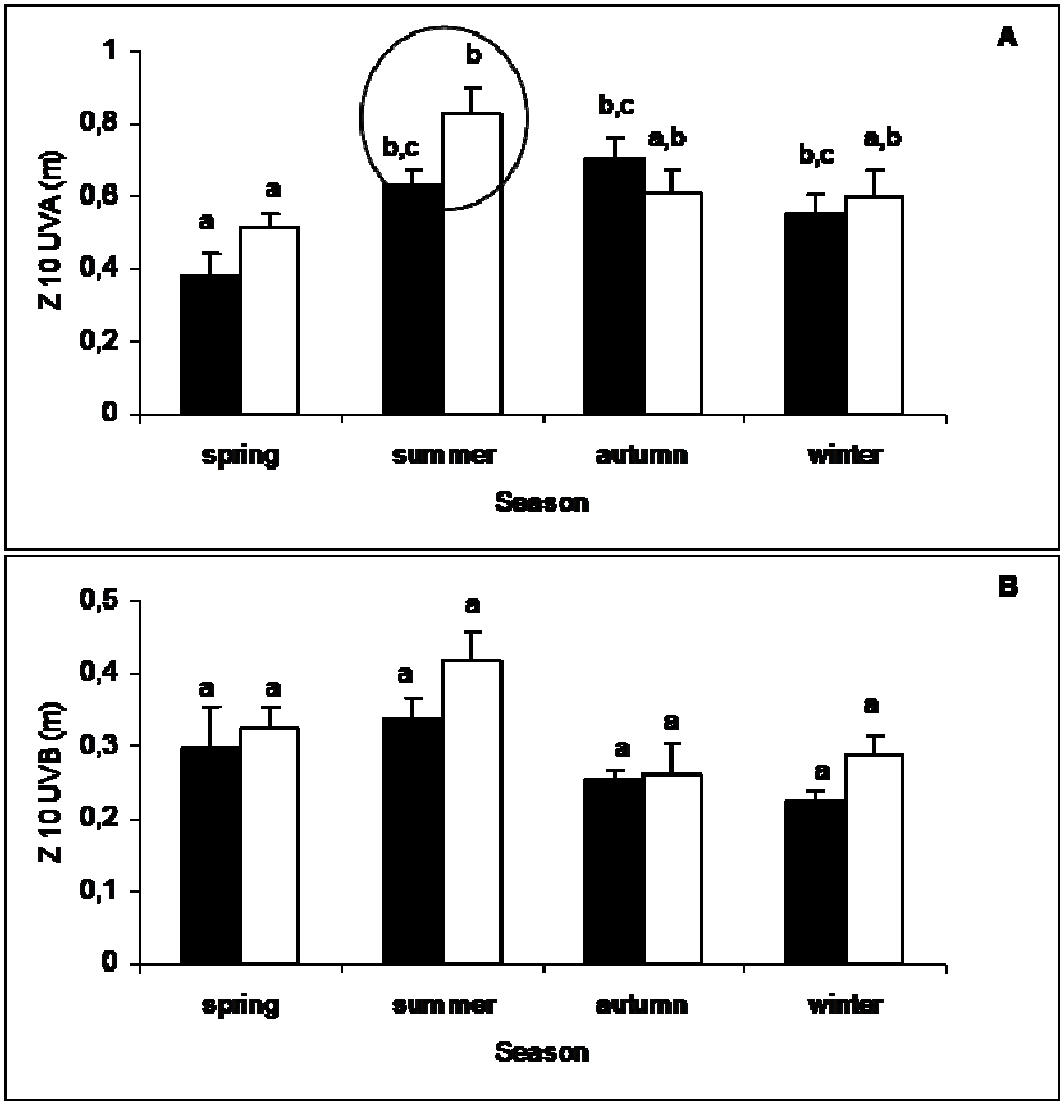

30°S
30'
31°S
30'
32°S
30'
33°S
53°W
30'
52°W
30'
51°W
30'
50°W
Jacuí Taquari Rivers
Gaúba River
Porto Alegre
Itapuã
Camaquã River
Santa Rita
Cristóvão Pereira
São Lourenço
Patos Lagoon
Rio Grande
Porta da Feitoria
São Gonçalo Channel
Patos Lagoon Estuary
São José do Norte
Atlantic Ocean
Mirim Laggon
20
50
100
500
N
30'
45'
15'
30'
15'
52°W
45'
Marinheiros Island
Saco do Arraial
Rio Grande
Station 1
Saco da Mangueira
Praticagem
Station 2

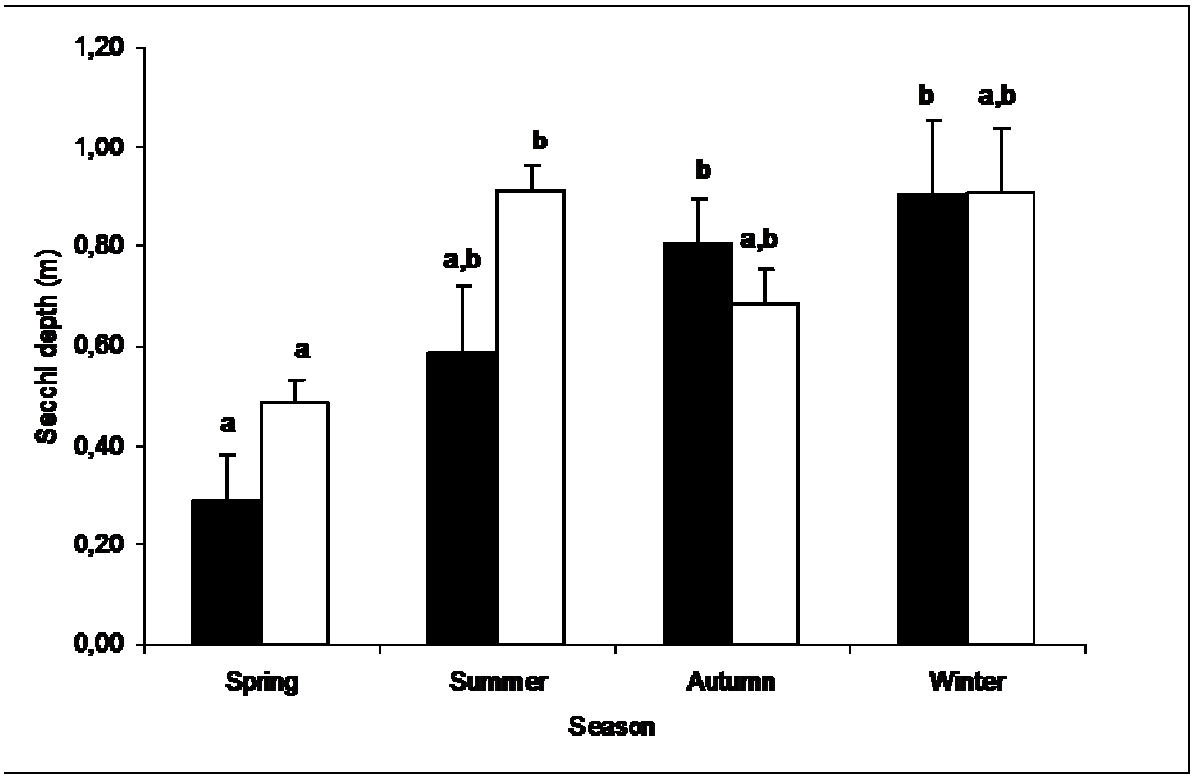

A
160
140
120
100
80
60
40
20
0
UVA (W.m-2)
a
a
a
a
b
b
b
c
Spring
Summer
Autumn
Winter
Season

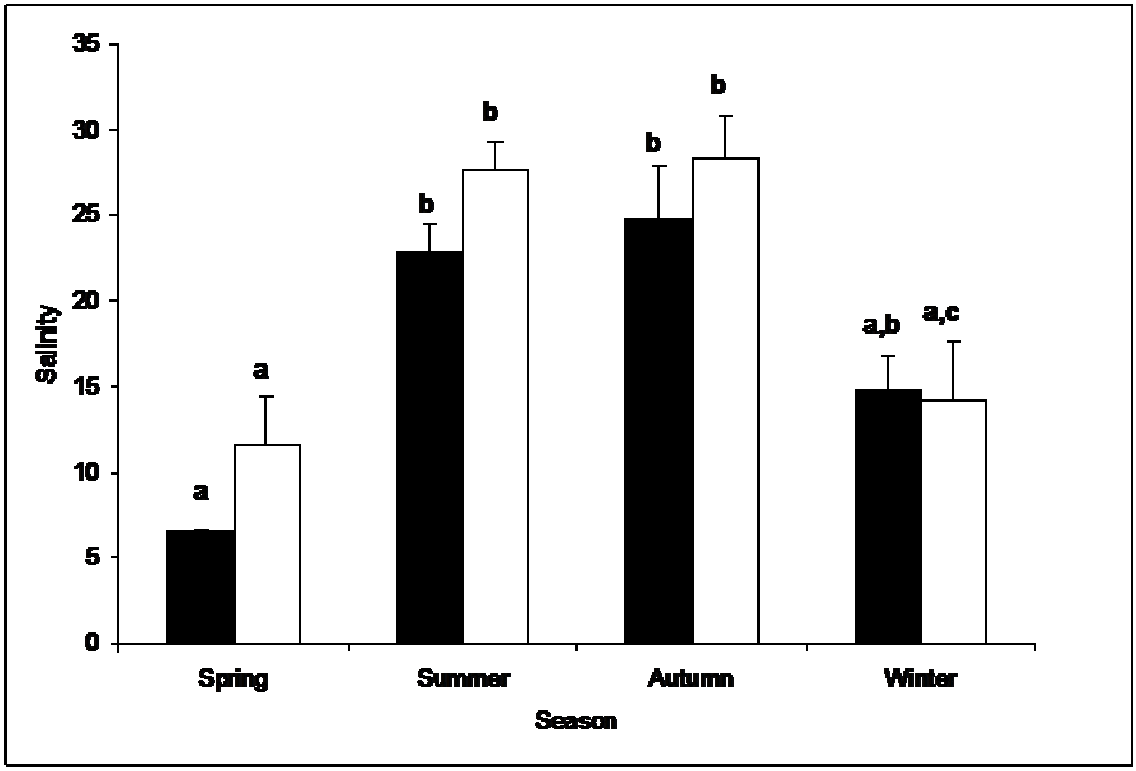

B
120
100
80
60
40
20
0
UVA (W.m-2)
a
a
b
b
a,b,c
a,b,c
a,c
a,c
Spring
Summer
Autumn
Winter
Season

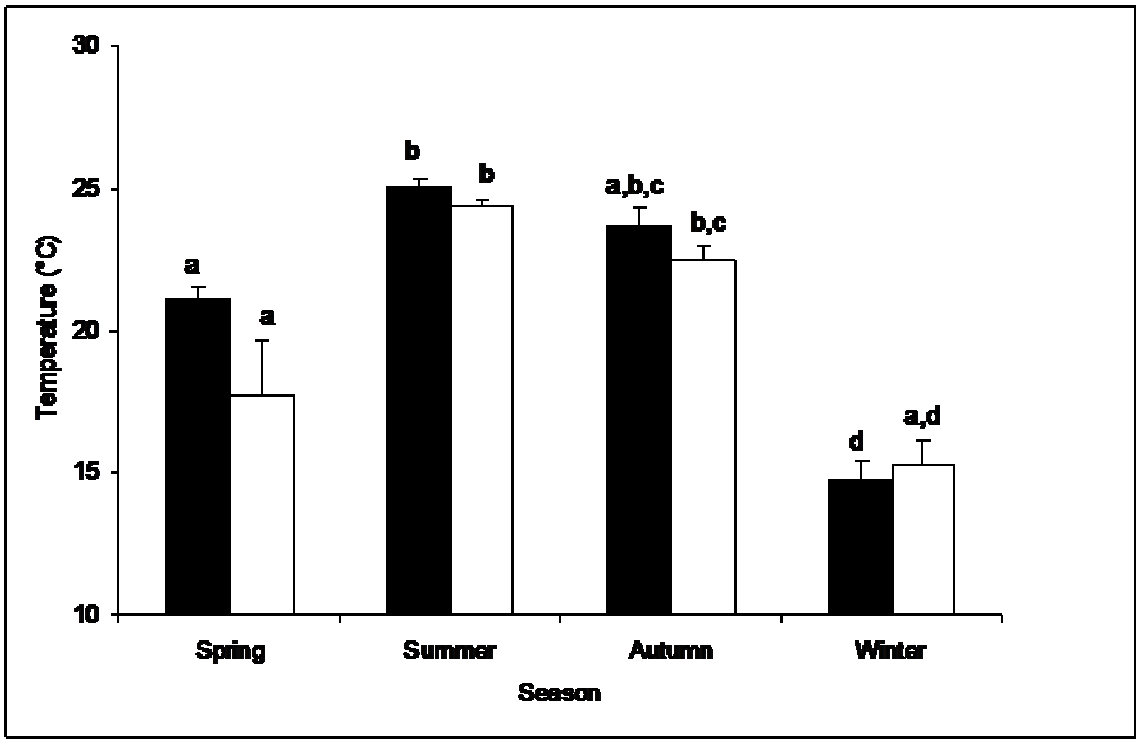

A
UVB (W.m-2)
4
3
2
1
0
a
a
a
a
b
b
b
b
Spring
Summer
Autumn
Winter
Season

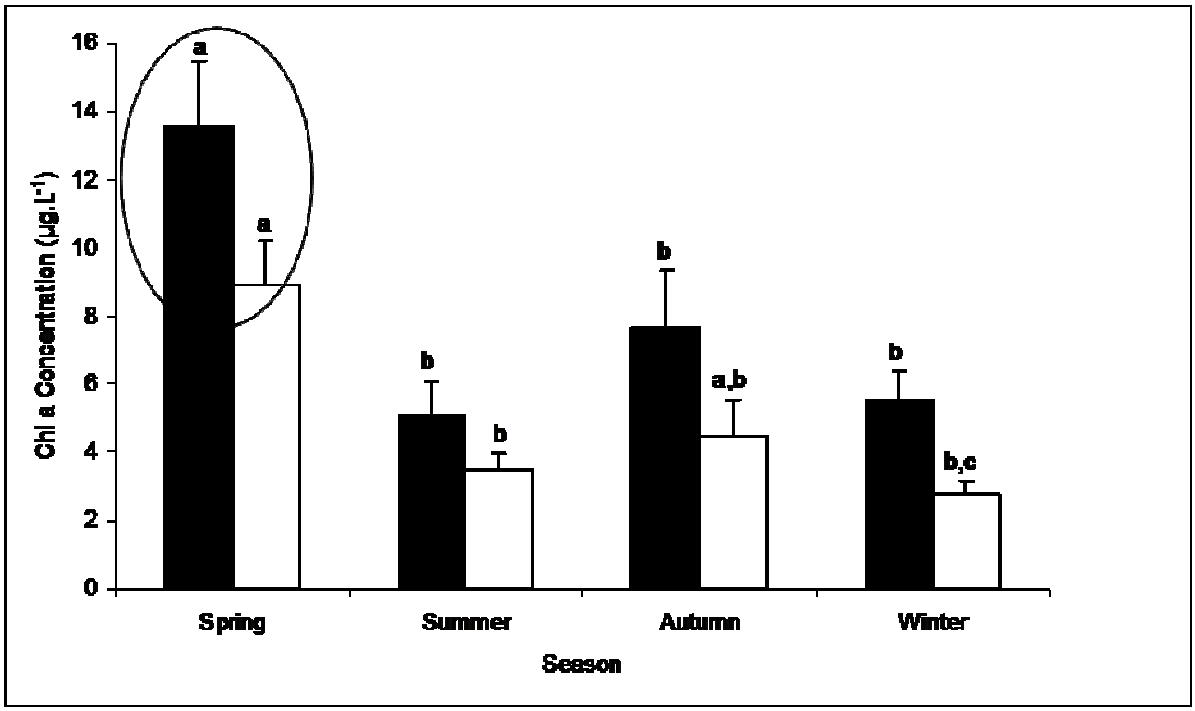

B
UVB (W.m-2)
2
1,5
1
0,5
0
a,b,c
a,b,c
b
a,b
c
a,b,c
c
c
Spring
Summer
Autumn
Winter
Season


A
Z 10 UVA (m)
1
0,8
0,6
0,4
0,2
0
a
a
b,c
b
b,c
a,b
b,c
a,b
spring
summer
autumn
winter
Season
B
Z 10 UVB (m)
0,5
0,4
0,3
0,2
0,1
0
a
a
a
a
a
a
a
a
spring
summer
autumn
winter
Season


1,20
1,00
0,80
0,60
0,40
0,20
0,00
Secchi depth (m)
a
a
a,b
b
b
a,b
b
a,b
Spring
Summer
Autumn
Winter
Season


35
30
25
20
15
10
5
0
Salinity
a
a
b
b
b
b
a,b
a,c
Spring
Summer
Autumn
Winter
Season


30
25
20
15
10
Temperature (°C)
a
a
b
b
a,b,c
b,c
d
a,d
Spring
Summer
Autumn
Winter
Season


Chl a Concentration (µg.L-1)
16
14
12
10
8
6
4
2
0
a
a
b
b
b
a,b
b
b,c
Spring
Summer
Autumn
Winter
Season

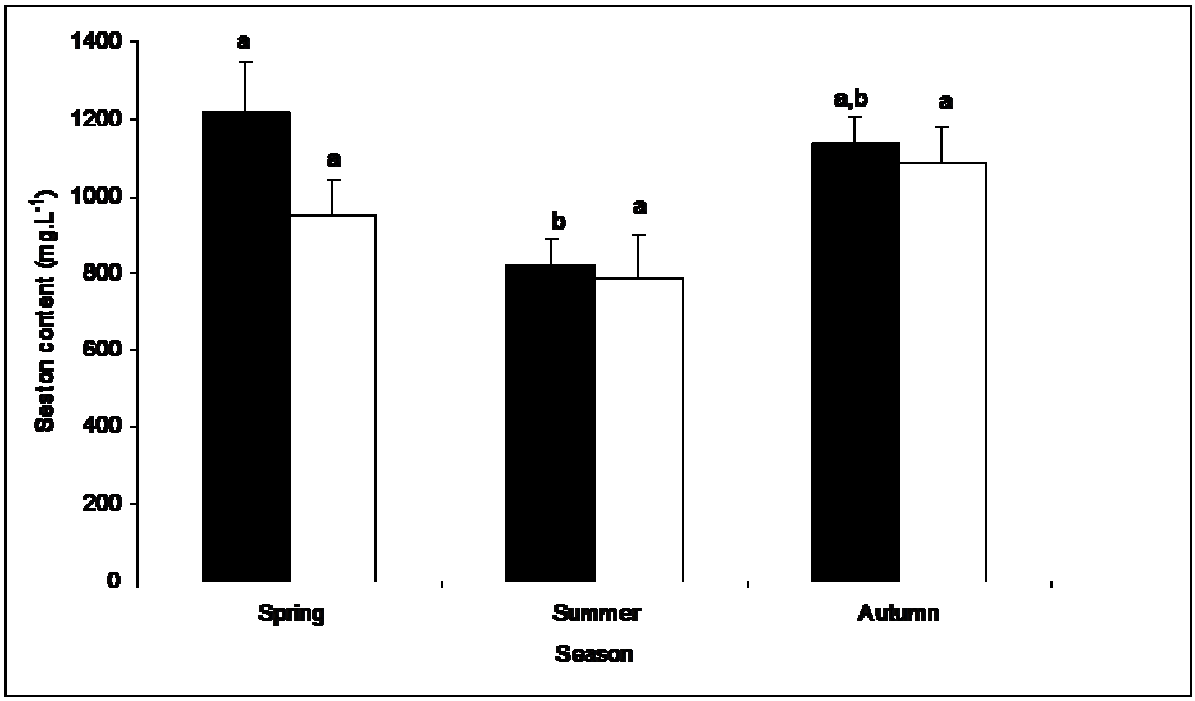

1400
1200
1000
800
600
400
200
0
Seston content (mg.L-1)
a
a
b
a
a,b
a
Spring
Summer
Autumn
Season

Table 1 – Summary of multiple linear regression model relating 10% of the irradiance at the surface (Z10) for UVA to different attenuation factors at station 1 (n =56).

Dependent variable	Independent variables	B	Adjusted r^2	R	Model p	SE of estimate
$Z10_{UVA}$	Salinity	0.01				
	Secchi depth	0.16				
			0.12	0.38	0.01	0.19

Table 2 – Summary of multiple linear regression model relating 10% of the irradiance at the surface (Z10) for UVA (n = 56) or UVB (n = 42) to different attenuation factors at station 2.

Dependent variable	Independent variables	B	Adjusted r^2	R	Model p	SE of estimate
$Z10_{UVA}$	Salinity	0.01				
	Secchi depth	0.13				
			0.14	0.42	0.01	0.26
$Z10_{UVB}$	Secchi depth	0.11	0.09	0.99	0.02	0.14

Table 3 – Summary of multiple linear regression model relating 10% of the irradiance at the surface (Z10) for UVA (n = 20) or UVB (n = 19) to different attenuation factors at station 2 during the summer.

Dependent variable	Independent variables	B	Adjusted r^2	R	Model p	SE of estimate
$Z10_{UVA}$	Chl a	0.08				
			0.26	0.54	0.01	0.28
$Z10_{UVB}$	Secchi depth	0.26				
	Salinity	0.01				
	Seston	0.00				
			0.27	0.62	0.05	0.15

Anexo 5.2 - Gouveia, GR; Félix Jr, P; Podewils, T; Silva-Júnior, FMR; Trindade, GS & Muelbert, JH. 2009. Effects of UVA and UVB radiation on GEM-81 goldfish erythrophoroma cells. Em preparação para a revista *Toxicology in Vitro.*

EFFECTS OF UVA AND UVB RADIATION ON GEM-81 GOLDFISH ERYTHROPHOROMA CELLS

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ABSTRACT

Our goal was to assess the effect of environmental doses of ultraviolet radiation (UVR) on GEM-81 cells. Our results show that 3.13 $J.cm^{-2}$ of UVA inhibits cell proliferation, increases the concentration of ROS (reactive oxygen species) and the lipid peroxide content (LPO), and lowers the activity of the antioxidant defense system (TOSC). For UVB, 0.07 $J.cm^{-2}$ causes cytotoxicity, and 0.014 $J.cm^{-2}$ induces DNA damage in cells. These results suggest that planktonic organisms that live close to the surface and are exposed to similar doses are subject to the damaging effects of continued exposure to UV radiation. Knowledge of this impact is relevant to understand possible future changes in the dynamics of populations of planktonic organisms.

1. Introduction

Ultraviolet radiation (UVR) is an important component of the solar spectrum and it is generally subdivided into UVA (400-320 nm), UVB (320-280 nm) and UVC (280-200 nm) (Fleischmann, 1989). Of these three bands of wavelength, only UVA and UVB reach the atmosphere. The decrease in stratospheric ozone, main screening factor for UVB radiation, and the regular occurrence of polar ozone holes in the Antarctic and Arctic has increased solar UVR reaching Earth's surface (Crutzen, 1992; Kerr and McElroy, 1993; Kerr, 1994; Manney et al., 1994; Lubin and Jensen, 1995; Weatherhead and Andersen, 2006) to levels that can be harmful and mutagenic to organisms (Ravanat et al., 2001).

The deleterious effects of UVR range from molecular and tissue damage to population level effects (Wake, 1991; Kerr and McElroy, 1993; Blaustein et al, 1994; Bothwell et al, 1994; Hofer and Mokri, 2000; Ravanat et al, 2001; Armstrong et al, 2002) and include alteration of relevant biological molecules such as proteins, lipids and DNA (Sinha and Häder, 2002; Gouveia et al, 2005).

In fact, DNA damage effects after exposure to UVB were already detected in several studies with different organisms, as in eggs, embryos and larvae of sea urchins (Lesser et al., 2006, Nahon et al., 2007; Nahon et al., 2009), crabs (Gouveia et al., 2005), embryos of the spotted salamander (Lesser, 2001) and cod larvae (Malloy et al., 1997; Lesser et al., 2001). Structural damage to DNA can be caused directly by UVB and indirectly by UVA radiation through generation of reactive oxygen species (ROS). The cyclobutane pyrimidine dimer (CPD) is the predominant photoproduct of direct

UVB exposure, while UVA wavelengths indirectly cause damage to DNA through the photodynamic production of hydroxyl radicals causing strand breaks and DNA cross-links (Peak and Peak, 1990). In addition, oxidative stress can occur when ROS formation exceeds antioxidant defense capability or disrupt redox signaling, affecting cell functionally (Jones, 2006).

The changes induced by UVA and UVB in cells isolated from mammals are already well established. Studies with human keratinocytes irradiated with UVR showed an increase in antioxidant defense system or the micronuclei formation (Phillipson et al, 2002, Armeni et al., 2004). Fibroblasts irradiated with UVA showed an increase in the ROS formation (Camera et al., 2008). Peak and Peak (1990) showed that UVA can damage DNA and other targets, such as the lipid membrane, leading to changes in permeability and cell function through lipid peroxidation (Gaboriau et al., 1993). Products of peroxidation such as lipid hydroperoxides and peroxil radicals can react with other lipids, leading to a chain reaction (Tyrell, 1995; Punnonen et al., 1991; Girotti, 2001).

There are few studies that show the effects of UVR in cells fish. The erythrophoroma GEM-81 is a permanent cell line derived from spontaneous skin tumors from the teleost *Carassius auratus* (Matsumoto et al., 1980). Upon treatment with differentiation inductors and heterologous fish serum, GEM-81 cells present a clonal heterogeneity, and in most cases melanized cells (with or without ability for melanosome movements) or, in minor proportions, scales, lens and others cell types can be observed (Matsumoto et al., 1981, 1984, 1989).

Recent studies detected levels up to 103 $W.m^{-2}$ of UVA and 1.9 $W.m^{-2}$ of UVB in estuarine water column surface (Gouveia et al., submitted). Considering that UVA may produces ROS and interferes in antioxidant capacity while UVB may induce DNA damages, the aim of this study was to assess the possible effects of environmental UVR doses in undifferenciated GEM-81 cells. This will enable us to investigate the possible effects of UVR on water surface leaving organism from the plankton in the region.

2. Material and methods

2.1 Cells and culture conditions

Erythrophoroma cell line (GEM-81) were maintained in Ham's F-10 (Cultilab, Campinas – SP, Brasil), supplemented with 10% fetal bovine serum (Gibco), 2 g/L $NaHCO_3$, and 15mM N-[2-hydroxyethyl]piperazine-N0-[2-ethanesulfonic acid], 1% of antibiotic - antimycotic (Gibco - penicillin – 10.000 U/mL, streptomycin – 10.000 µg/mL and 25 µg/mL amphotericin B) pH 7.2, in plastic flasks, at 28ºC.

2.2 UV irradiation conditions

GEM-81 cells were seeded at 5 x 10^5 cells/well in 24 well plates 24 h before UVR exposure. Cells were irradiated with UVA (VL - 115 L, 30 W) or UVB (VL - 115 C, 30 W; Vilber Lourmat, France) lamps. UVA and UVB radiation was monitored using a radiometer/photometer (model IL 1400A, International Light, MA, USA). UVA lamp produced 87.00 $W.m^{-2}$ of UVA and 0.03 $W.m^{-2}$ of UVB, with contamination of 0.007 $W.m^{-2}$ of visible light. UVB lamp produced

2.33 $W.m^{-2}$ of UVA and 5.53 $W.m^{-2}$ of UVB, with contamination of 0.003 $W.m^{-2}$ of visible light. The environmental doses were calculated from the integration of data from UVR intensity monitored in the environment during sunny days. Control cells were maintained without visible light exposure. Different doses of UV (0.78; 1.56; 3.13; 4.69 and 6.26 $J.cm^{-2}$ of UVA and 0.007; 0.014 and 0.07 $J.cm^{-2}$ of UVB) were obtained using different exposure times (15; 30; 60; 90 and 120 min of UVA and 0.5; 1 and 5 min of UVB). Prior to UV exposure, Ham's F-10 was removed from the plates and the cells were irradiated in 1 mL phosphate buffered saline (PBS). After irradiation, cells were suspended in Ham's F-10 during 72 h for UVA and 96 h for UVB. To evaluate cell viability, cells were harvested with trypsin 0,125% (Gibco) and counted by trypan blue (Gibco) exclusion. Counts were made immediately, 24, 48 and 72 h after UVA exposure; and immediately, 24, 48, 72 and 96 h after UVB exposure.

2.3 Assessment of intracellular ROS formation

Suspension (4.0×10^5 cells/mL) of both cell cultures, control and treated with 3.13 $J.cm^{-2}$ of UVA after 48 h of exposure, were washed twice with PBS. Cell cultures were incubated for 30 min at 28℃ with the fluorogenic compound, 2',7' – dichlorofluorescin diacetate (H_2DCF-DA, Molecular Probes) at final concentration of 40 µM. Each treatment was performed in quadruplicate. After loading the cells with H_2DCF-DA, they were washed twice with PBS and suspended in fresh PBS. Aliquots of 160 µL of each sample (three replicates) were placed onto a white 96 wells microplate and the fluorescence intensity determined during 90 min at 28℃, using a fluorometer (Victor 2, Perkin Elmer),

with excitation and emission wavelengths of 488 and 525 nm, respectively. ROS levels were expressed in terms of fluorescence area, after fitting fluorescence data to a second order polynomial and the estimated functions integrated between 0 and 90 min.

2.4 Assessment of antioxidant capacity against peroxyl radicals

Suspension (5.0 x 10^5 cells/mL) of both cell cultures, control and treated with 3.13 J.cm^{-2} of UVA after 48 h of exposure, were washed twice with PBS and frozen at -80℃ until use. Each pellet was then suspended with PBS, triturated in ice and centrifuged at 10.000g for 20 min at 4℃. Fifteen microliters of the supernatant were pipeted into the wells of a white 96 wells microplate with 120 µl buffer (Hepes, KCl, MgCl2 and water), and 10 µl of water or 2,2´-azobis(2 metilpropionamidina) (ABAP) and 20 µl of H2DCF-DA. The fluorescence intensity was determined during 60 min at 37ºC, using a fluorometer (Victor 2, Perkin Elmer), with an excitation and emission wavelength of 485 and 520 nm, respectively. The fluorescence data was fit to a second order polynomial and integrated between 0 and 60 min in order to obtain the fluorescence area. Total fluorescence production was calculated according to Amado et al. (2009). The inverse relative difference between ROS area with and without ABAP was considered as a measure of antioxidant capacity, with high area difference meaning high antioxidant capacity.

2.5 Assessment of lipid peroxidation

Lipid peroxidation (LPO) was determined according to Hermes-Lima et al. (1995). Samples were homogenized (10% w/v) in 100% cold (4℃) methanol. The homogenate was centrifuged at 1000 g, for 10 min at 4℃. The supernatant was used for LPO determinations (580 nm). Cumene hydroperoxide (CHP; Sigma) was employed as standard.

2.6 Assessment to alkaline single cell electrophoresis (comet) assay

The comet assay was performed in according of Singh et al. (1988) and Steinert et al. (1998) with some modifications. Microscope slides were covered with 1.5% normal melting point agarose diluted in PBS. Suspension of both cell lines (4.0 x 10^5 cells/mL), control and treated with 0.014 and 0.07 J.cm^{-2} of UVB, were harvested with trypsin 0,125% (Gibco) immediately after irradiation. Sixty microliters of cellular suspension was added to 80 µL of 1.0% low melting point agarose diluted in PBS, added onto the frosted slide prepared previously, and covered with a cover slip. After gel solidification (5 min at 4℃), slides were submitted to lysis solution (2.5 M NaCl, 100 mM EDTA, 10 mM Tris–HCl, 10% dimethyl sulfoxide and 1% Triton X-100) and kept at 4℃ for 2 h. To unwind DNA strands, slides were transferred to chambers filled with electrophoresis and unwinding buffer (10 N NaOH and 200 mM EDTA). After leaving it undisturbed for 30 min, electrophoresis was carried out for 15 min at 25 V and 300 mA. Slides were washed three times in 0.4 M Tris (pH 7.5). Preparations were stained with 30 µL of Sybr Gold (1:10,000; Molecular Probes) and analyzed using a fluorescence microscope (Olympus BX50) with a CCD camera (Pro-Series, High Performance) coupled to a commercial software for image

acquisition. A total of 50 nuclei were selected and photographed randomly in each slide. The nuclei were analyzed by CASP software (Konca et al., 2003) and DNA damage was represented by % DNA in tail.

2.7 Statistical analysis

Data were expressed as mean +1 standard error and analysed with ANOVA. Differences between mean values of control and irradiated groups were compared using the Tukey's test. In all cases, the significance level adopted was 5% ($P<0.05$).

3. Results

3.1 Effects of UVA or UVB on the cell proliferation

Exposure of GEM-81 cells to 3.13, 4.69 and 6.26 $J.cm^{-2}$ UVA radiation induced a significant decrease in cell proliferation after 48 h ($p<0.05$). However, in 72 h has no more significant differences when compared to control group ($p>0.05$). Minor doses (0.78 and 1.56 $J.cm^{-2}$) of UVA did not significantly affect the proliferation of this GEM-81 line ($p>0.05$) (Fig. 1 A). After 48 h of irradiation, all doses of UVB were significantly different from the control group ($p<0.05$). However, 72 h after irradiation, the irradiated group with the minor dose (0.007 $J.cm^{-2}$) showed recovery and continued prolifering up to 96 h of observation, without significantly difference from the control group ($p>0.05$). Cell proliferation, in the group irradiated with intermediate dose (0.014 $J.cm^{-2}$ of UVB), decreased significantly from the control group 48 h after irradiation, and maintained this difference until 96 h of observation ($p<0.05$). However, this

decrease does not mean cytotoxic effect but proliferation inhibition because the cells showed a lower growth when compared with the control cells growth (Fig 1B). The highest dose UVB radiation (0.07 $J.cm^{-2}$) induced cytotoxicity, and reduced significantly cell proliferation up to the 96 h of observation.

3.2 Analyses of oxidative stress

The ROS concentration in GEM-81 cells was evaluated 48 h after UVA irradiation (3.13 $J.cm^{-2}$), because this was the lowest dose to cause decreased cell proliferation precisely in time of 48 h. ROS concentration was significantly higher in the treated group than the control group ($p<0.05$) (Fig. 2). Assessment of the antioxidant capacity revealed that cells exposed to UVA showed low inversed relative area, that meant lower competence against peroxyl radicals than compared to the control group ($p<0.05$) (Fig. 3). The capability of LPO induction by UVA was significantly higher than the control group ($p<0.05$) (Fig. 4).

3.3 Analyses of DNA damage

The DNA damage in GEM-81 cells was evaluated immediately after UVB irradiation. The levels of DNA strand breakage in GEM-81 cells irradiated with UVB (0.014 $J.cm^{-2}$) were significantly higher than the control group ($p<0.05$) (Fig. 5-6). At the highest UVB dose (0.07 $J.cm^{-2}$), the nuclei were not visible in the slides (data not show).

4. Discussion

According to our study, low doses of UVA (0.78 and 1.56 $J.cm^{-2}$) did not affect the GEM-81 cells proliferation, but higher doses (3.13, 4.69 and 6.26 $J.cm^{-2}$) decreased viable cells number. Ray et al. (2008) showed that there was no phototoxicity in mouse fibroblast (L-929 cell line) irradiated with doses up to 2.7 $J.cm^{-2}$ of UVA till 60 min exposure. However, higher doses induced a time and dose-dependent decrease cell viability in 90 min after irradiation.

UVB radiation (0.014 $J.cm^{-2}$) effectively decreased cell proliferation compared to the control group. However, this dose was not phototoxic since cells continued to grow but at a slower rate than the control group. This pattern was similar to the cells irradiated with minor dose (0.007 $J.cm^{-2}$). The results with highest UVB radiation (0.07 $J.cm^{-2}$) was indicative of phototoxicity, since it reduced cell proliferation significantly. Corroborating our results, Sandrini et al. (2009) showed that irradiation of zebrafish hepatocytes with UVB (0.07 $J.cm^{-2}$) reduced the number of viable cells. Only the lowest dose (0.01 $J.cm^{-2}$) was not able to significantly reduce viable cells number. According to Ray et al. (2008), UVB doses L-929 cells from 0.72 to 5.04 $J.cm^{-2}$ were not phototoxic up to 60 min exposure. Decreased cellular viability was observed after 90 min of exposure. It is important to point out that in this study, the authors follow the UVR effects on cellular viability over a short time observation compared to the 96 h used in our study. Misra et al. (2005) also used approximately the same UVB doses, and found dose-dependent toxicity in human erythrocytes. Youn et al. (2007), studying UVB effects in human retinal pigment epithelial cells,

observed dose and time-dependent decreases in cellular viability up to 48 h after irradiation.

The levels of UVR used in this study are approximately equivalent to the environmental levels in the water-column of a mid-latitude (32°S) estuary (Gouveia et al. submitted). However, our results showed that when minor doses of UVR were followed for longer periods, GEM-81 cells were shown to be sensitive to UVR. Although, other wavelengths are present in the environment, and a synergystic effect in the organism responses or some protection effect can be expected. During our study, UVA radiation induced a significant decrease in cell proliferation 48 h after irradiation, concomitant tests of oxidative stress was indispensable.

The use of GEM-81 cells as biological model revealed that both ROS concentration and LPO content were higher in cells irradiated with UVA when compared with the control group after 48 h of exposure. This suggests a condition of oxidative stress, since there was low competence to neutralize peroxyl radicals. Although cells possess an antioxidant system to diminish UVR induced oxidative stress (Trindade et al., 1999), extensive and chronic exposure to UVR can exceed antioxidant capacity of the cells, leading to oxidative damage.

DNA damage was higher in GEM-81 cells irradiated with UVB (0.014 $J.cm^{-2}$), indicating that despite cellular growth, there was possibly an activation of the cells repair system. At the highest dose of UVB (0.07 $J.cm^{-2}$), nuclei in the slides of the comet assay were not visible, suggesting that there may be a

synergistic effect of UVB radiation and comet assay protocol, destroying the cell (Gouveia et al., 2005).

Our results demonstrate that UVR doses similar to the environment can cause oxidative and DNA damages in GEM-81 cells. Results also revealed that these cells were able to repair the damage caused by UVR. Thus, we suggest that organisms that live near the surface of the water column may suffer damage caused by hazardous UVR and that, especially in the range of UVB, may be an important limiting ecological factor for the survival of organisms. While considering that some of the damage observed in this study could be repaired, it should be noted that some damage are permanent for the doses and periods of observation used. Furthermore, future changes in the ozone layer can lead to higher incidence of UVR. Therefore, environmental doses higher than those used in this work may cause unrepairable damage to cells and organisms.

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LEGEND FIGURES

Fig. 1 – Viable GEM-81 cells number (x 10^4). **A -** Control group and submitted to different doses of UVA (0.78; 1.56; 3.13; 4.69 and 6.26 J.cm^{-2}), followed immediately, 24, 48 and 72 h after irradiation. **B -** Control group and submitted to different doses of UVB (0.007; 0.014 and 0.07 J.cm^{-2}), followed immediately, 24, 48, 72 and 96 h after irradiation. Data are expressed as mean +1 standard error. * Indicates significant diferences as compared to control in a given time of observation ($p<0.05$).

Fig. 2 - Quantification of ROS production (fluorescence area) in GEM-81 cells evaluted 48 h after UVA irradiation (3.13 J.cm^{-2}). Data are expressed as mean +1 standard error. * Indicates significant diferences as compared to control group ($p<0.05$).

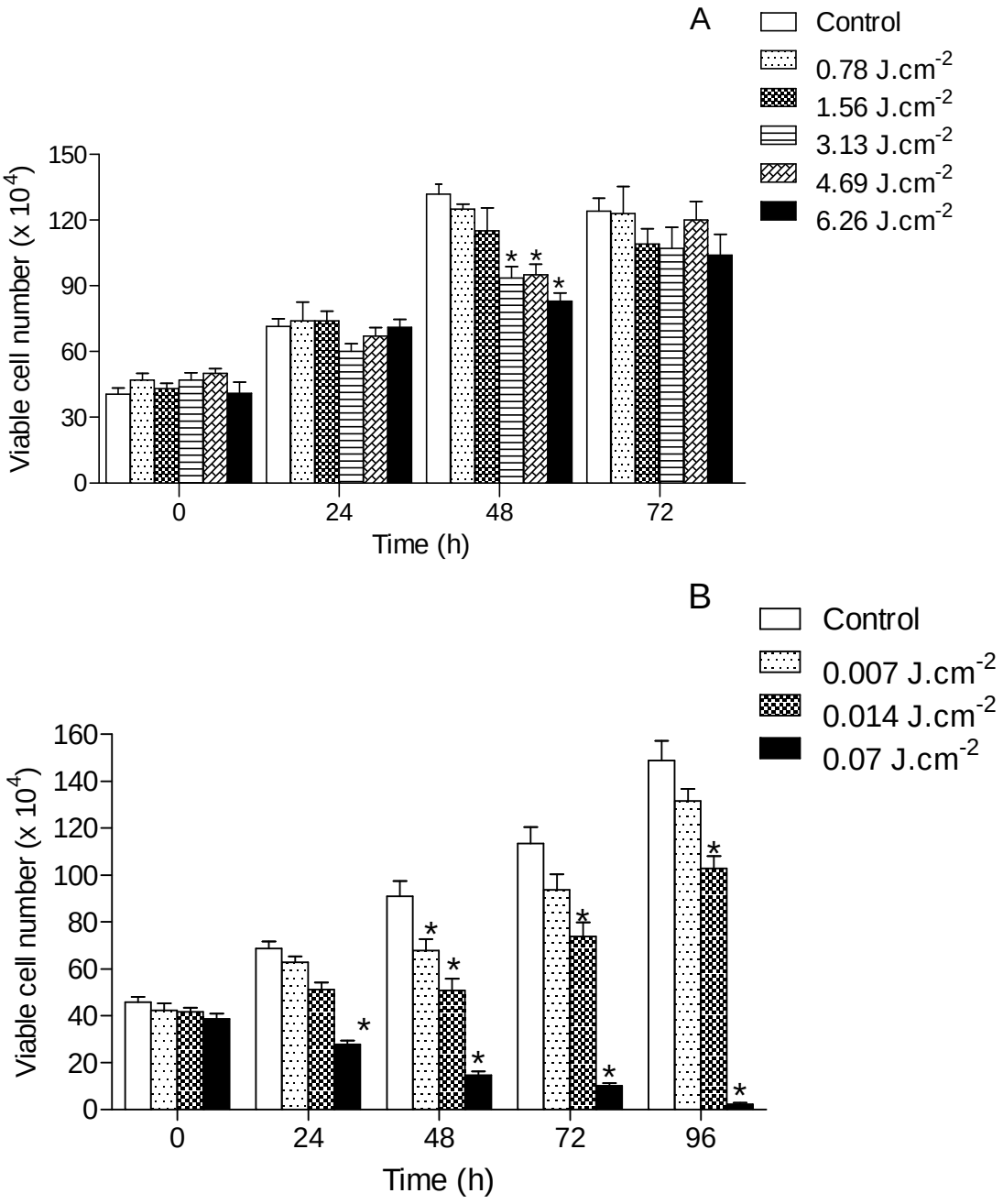
Fig. 3 – Total antioxidant capacity against peroxyl radicals (inversed relative area) in GEM-81 evaluted 48 h after UVA irradiation (3.13 J.cm^{-2}). Data are expressed as mean +1 standard error. * Indicates significant diferences as compared to control group ($p<0.05$).

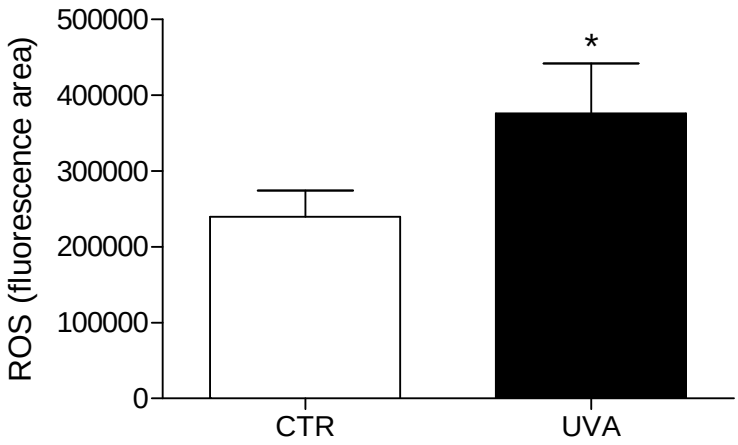
Fig. 4 – LPO content (nmol CHP/mg pellet) in GEM-81 cells evaluted 48 h after UVA irradiation (3.13 J.cm^{-2}). Data are expressed as mean +1 standard error. * Indicates significant diferences as compared to control group ($p<0.05$).

Fig. 5 – Levels of DNA damage in GEM-81 cells irradiated with UVB (0.014 J.cm^{-2}) immediately after exposition. Data are expressed as mean +1 standard error. * Indicates significant diferences as compared to control group ($p<0.05$).

Fig. 6 – Illustrative DNA damage in GEM-81 cells irradiated or no with UVB. **A-** Control. **B-** Dose to 0.014 J.cm^{-2} of UVB.

FIGURES


A
Control
0.78 J.cm-2
1.56 J.cm-2
3.13 J.cm-2
4.69 J.cm-2
6.26 J.cm-2
Viable cell number (x 10^4)
150
120
90
60
30
0
0
24
48
72
Time (h)
B
Control
0.007 J.cm-2
0.014 J.cm-2
0.07 J.cm-2
Viable cell number (x 10^4)
160
140
120
100
80
60
40
20
0
0
24
48
72
96
Time (h)


ROS (fluorescence area)
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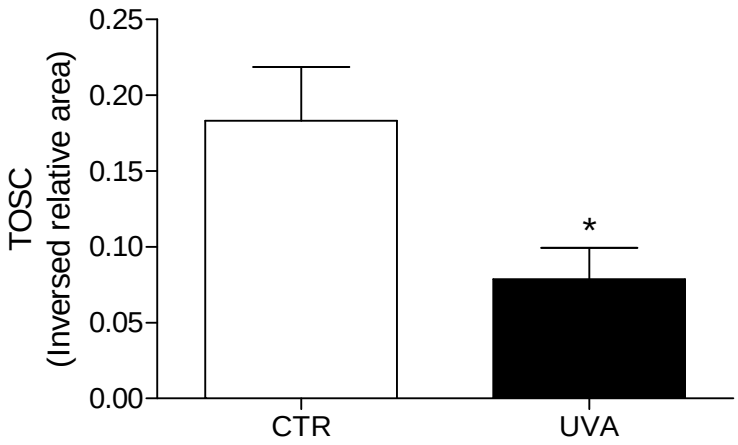
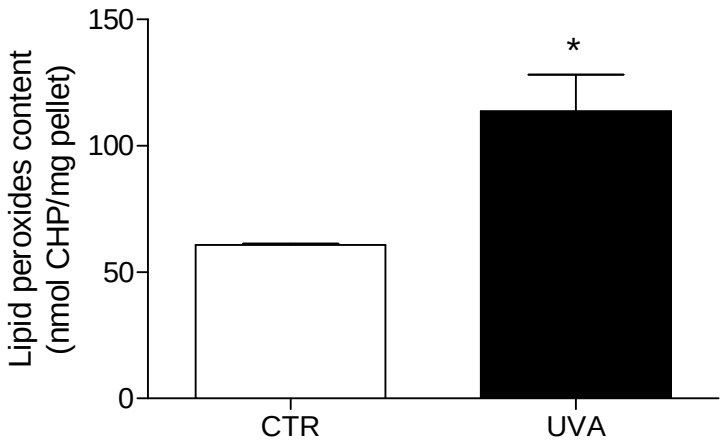
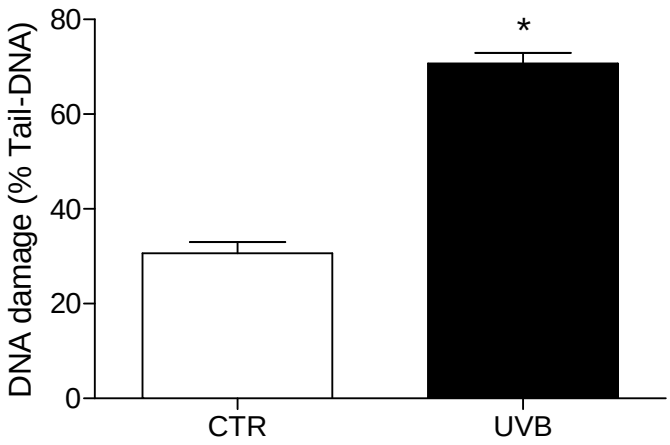
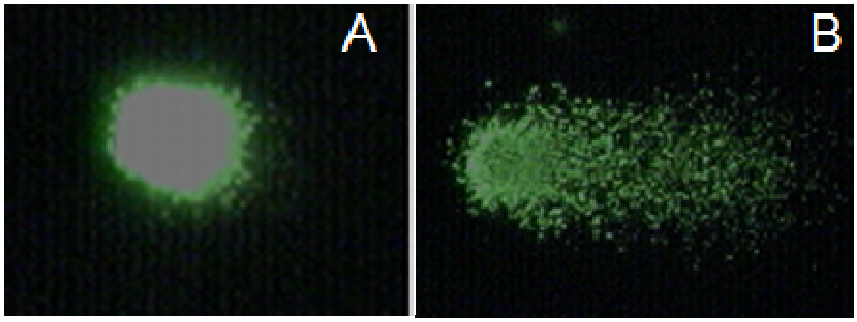
400000
300000
200000
100000
0
*
CTR
UVA


TOSC
(Inversed relative area)
0.25
0.20
0.15
0.10
0.05
0.00
*
CTR
UVA


Lipid peroxides content
(nmol CHP/mg pellet)
150
100
50
0
*
CTR
UVA


DNA damage (% Tail-DNA)
80
60
40
20
0
*
CTR
UVB


A
B

Anexo 5.3 - Gouveia, GR; Tesser, MB; Sampaio, LAN; Trindade, GS & Muelbert, JH. 2009. Efeitos das radiações UVA e UVB em larvas do peixe-rei *Odontesthes argentinensis.* Em preparação.

EFEITOS DAS RADIAÇÕES UVA E UVB EM LARVAS DO PEIXE-REI Odontesthes argentinensis

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1. Introdução

A radiação ultravioleta (UV) compreende uma faixa de comprimentos de onda eletromagnética que varia entre 200 e 400 nm. Considerando sua capacidade de causar danos em diferentes alvos celulares, a radiação UV foi dividida, para fins biológicos, em três faixas de comprimento de onda: UVA (320 a 400 nm), UVB (290 a 320 nm), também chamados de UV solar, e UVC (200 a 290 nm), também chamado de UV curto. UVA e UVB podem causar uma variedade de efeitos nos organismos vivos; por outro lado, UVC é totalmente absorvida pelo oxigênio e pelo ozônio na atmosfera, sendo ecologicamente irrelevante.

Alguns efeitos da radiação UV têm sido descritos para organismos marinhos encontrados próximos à superfície, onde altos fluxos desta radiação estão presentes (Dey *et al.,* 1988). Entre estes efeitos, é possível citar uma maior taxa de mortalidade em salmões *Oncorhyncus nerka* (Bell & Hoar, 1950), em larvas de anchoíta *Engraulis mordax* (Hunter *et al*., 1979, 1981), em ovos de *Perca flavescens* (Williamson *et al*., 1997) e em larvas de *Esox lucius* (Vehniäinen *et al.,* 2007). Outros autores também têm mostrado um aumento significativo no dano de DNA em ovos, embriões e larvas de ouriços do mar *Strongylocentrotus droebachiensis, Sterechinus neumayeri*, *Evechinus chloroticus, Sphaerechinus granularis* e *Paracentrotus lividus* (Lesser *et al.,* 2006, Nahon *et al*., 2008; Nahon *et al.*, 2009), em caranguejos *Chasmagnathus granulata* (Gouveia *et al.,* 2005), em embriões de salamandra *Ambystoma maculatum* (Lesser, 2001) e do bacalhau *Gadus morhua* (Malloy *et al*., 1997; Lesser *et al*., 2001).

Entre os efeitos clássicos destas radiações, principalmente aqueles provocados pela radiação UVA, podemos citar o estresse oxidativo, que pode ocorrer quando a formação de ROS (espécies reativas de oxigênio) excede a capacidade de defesa antioxidante ou perturba os caminhos de sinalização redox, afetando a funcionalidade celular (Jones, 2006). Para combater esses danos, as células possuem proteínas que detoxificam as moléculas de ROS antes que elas possam danificar o DNA ou outros componentes celulares (Ridley *et al*., 2009; Gouveia *et al.*, *em preparação*).

A compreensão das respostas induzidas pela radiação UV nos organismos e seus possíveis meios de defesa contra esta radiação são de fundamental importância. Em função de várias adaptações (compostos protetores, mecanismos de reparo de DNA, alterações morfológicas, mudanças comportamentais, etc.) a biota pode coexistir com a exposição à radiação UV. Entretanto, há um limite, no qual a proteção pode ser efetiva para a defesa dos organismos (Hasen *et al.,* 2003).

A diminuição da camada de ozônio em altas e médias latitudes, particularmente no Continente Antártico e Sul-americano (Stolarski *et al.*, 1992; Gleason *et al.*, 1993; Manney *et al.*, 1994; Kirchhoff *et al*., 1997; Kirchhoff & Echer, 2001) tem sido correlacionada com o aumento do perigo biológico do UVB, uma vez que é o ozônio o principal agente capaz de barrar parcialmente essa radiação (Madronich, 1992; Kerr & McElroy, 1993).

Estuários localizados em latitudes médias do Brasil, Argentina e Uruguai podem ser especialmente suscetíveis à radiação UV, devido à redução no ozônio estratosférico (Sze *et al.,* 1989). A cada 1% de redução no ozônio

estratosférico no Rio Grande do Sul, por exemplo, aumenta em 1% a radiação UV (Guarnieri *et al.*, 2004). De fato, baixas concentrações de ozônio durante curtos períodos de tempo têm sido detectados na América do Sul devido ao transporte de massas de ar, com ozônio rarefeito, trazidas pelo vórtex Antártico (Kirchhoff *et al*., 1996). O estuário da Lagoa dos Patos, localizado em médias latitudes, possui uma elevada variabilidade interanual da precipitação pluviométrica e um complexo padrão de fluxo dos rios na bacia de drenagem, resultando em processos hidrográficos altamente variáveis e dinâmicos (Asmus, 1998). Essa dinâmica, associada à diversidade de hábitats proporciona um abundante suprimento de alimento e proteção contra predadores, o que faz deste estuário um ambiente próprio para o desenvolvimento de ovos e larvas (Weiss & Souza 1977 a, b; Muelbert, 1986; Muelbert & Weiss, 1991). Durante a primavera e verão, ovos e larvas de peixes costeiros e estuarinos são liberados na coluna d'água e transportados para o interior do estuário (Muelbert & Weiss, 1991; Sinque & Muelbert, 1997).

Neste estuário, valores de até 103 $W.m^{-2}$ de UVA e 1,9 $W.m^{-2}$ de UVB podem penetrar nas camadas superficiais da coluna d'água (Gouveia *et al.*, *submetido*). Também foi possível demonstrar que estas doses produzem efeitos deletérios em células GEM-81, linhagem de eritroforoma do peixe *Carassius auratus* (peixe dourado) (Gouveia *et al., em preparação).*

Assim, considerando que a radiação UV pode influenciar no crescimento, mortalidade e capacidade antioxidante dos organismos, o objetivo deste trabalho é estudar os efeitos destas radiações em organismos planctônicos que habitam o estuário da Lagoa dos Patos. Assim, o modelo

utilizado neste estudo são larvas do peixe-rei *Odonthestes argentinensis*, comuns na região costeira do sul do Brasil (Vieira e Castello, 1996).

2. Material e métodos

2.1 Captura dos animais e condições de manutenção

Ovos do peixe-rei *Odontesthes argentinensis* foram coletados na beira da praia do Cassino, Rio Grande, RS, e levados para o Laboratório de Piscicultura da FURG. No laboratório, os ovos foram colocados em baldes com água do mar (salinidade 35) e aeração constante até a eclosão. À medida que ocorria a desova, as larvas eram transferidas para tanques com água do mar na mesma salinidade e aeração constante. A partir do terceiro dia de eclosão, as larvas foram transferidas cuidadosamente para aquários com as mesmas condições de manutenção. A troca d'água foi diária e ocorria antes da exposição ao UVA ou UVB. As larvas foram alimentadas com náuplios de Artemia *ad libitum.*

2.2 Condições de irradiação

As larvas (n=50) foram irradiadas com Lâmpadas de UVA (VL - 115 L, 30 W) ou UVB (VL - 115 C, 30 W; Vilber Lourmat, França). A intensidade de irradiação das lâmpadas foi monitorada utilizando um radiômetro/fotômetro (modelo IL 1400A, International Light, MA, USA). A lâmpada de UVA emitiu 11,41 $W.m^{-2}$ de UVA e 0,05 $W.m^{-2}$ de UVB. A lâmpada de UVB produziu 3,05 $W.m^{-2}$ de UVA e 6,65 $W.m^{-2}$ de UVB. Os grupos controle foram mantidos com luz visível. As doses utilizadas no experimento foram de 2,05 e 4,08 $J.cm^{-2}$ de UVA e 0,598 $J.cm^{-2}$ de UVB, e foram obtidas utilizando-se diferentes tempos

de exposição (30 e 60 min de UVA diários, durante 12 dias de exposição e 15 min diários de UVB, durante 3 dias de exposição).

2.3 Influência das radiações UVA e UVB no crescimento e sobrevivência das larvas de O. argentinensis

Diariamente, foi feita a medição das larvas de O. *argentinensis*, com o auxílio de um papel milimetrado fixado a uma lupa estereoscópica. Foi retirado, ao acaso, apenas um exemplar de cada tratamento para este fim, que foi sacrificado após a medição. Também foi feito um acompanhamento diário da mortalidade das larvas de O. *argentinensis.* Devido a uma maior mortalidade no grupo controle dos animais tratados com UVA, apenas o tratamento com UVB será relatado.

2.4 Capacidade antioxidante contra radicais peroxil (TOSC)

Para avaliação da capacidade antioxidante, foram montados "*pools*" de 6 larvas, que foram homogeneizadas (1:9 - p/v) com um tampão Tris-HCl (100 mM, pH 7,75) com EDTA (2 mM) e Mg^{2+} (5 mM) (Gallagher *et al*., 1992). Os homogeneizados foram centrifugados a 10.000g durante 20 min (4℃) e o sobrenadante foi utilizado nas medições. O conteúdo de proteínas totais foi determinado através do método de Biureto, em triplicata. A placa foi lida a 550 nm em fluorímetro (Victor 2, Perkin Elmer). Cada amostra foi diluída com o tampão de homogeneização até ficar com uma concentração de 3,32 mg de proteína/mL. A capacidade antioxidante total contra radicais peroxil foi avaliada através da determinação de ROS em amostras tratadas ou não com um radical gerador (Amado *et al*., 2009). Em uma placa branca de 96 poços, foi adicionado 127,5 µL do tampão de reação contendo 30 mM HEPES (ph 7,2),

200 mM KCL e 1 mM $Mgcl_2$ e 10 µL do sobrenadante da amostra. Em três dos seis poços de cada amostra foi adicionado 7,5 µL de 2,2'-azobis 2 dihidroclorido de metilpropionamidina (ABAP 4 mM, Aldrich). Nos três restantes, foi adicionado o mesmo volume de água MilliQ e a placa foi levada para leitura em fluorímetro (Victor 2, Perkin Elmer), à 37℃. Imediatamente antes da leitura, foi adicionado aos poços 20 µL de H_2DCF-DA, que é clivado pelas esterases presentes na suspensão celular. Assim, o composto não fluorescente H_2DCF é oxidado pelas ROS a um composto fluorescente DCF, que é detectado em comprimentos de onda de 488 e 525 nm, para excitação e emissão, respectivamente. A decomposição térmica do ABAP e formação de ROS foi monitorada durante 60 min, com leituras a cada 5 min. A produção fluorescente total foi calculada segundo Amado *et al.* (2009). A diferença relativa invertida entre a área de ROS com e sem ABAP foi considerada como medida da capacidade antioxidante.

2.5 Análises estatísticas

Os dados foram expressos como ±1 erro padrão da média. A taxa de crescimento e mortalidade foi determinada através de regressão linear e a comparação entre as curvas foi feita pela análise de covariância. As possíveis diferenças estatísticas na capacidade antioxidante foram determinadas através de ANOVA. Em todos os casos, o nível de significância adotado foi de 5% ($p<0,05$).

3. Resultados

Não houve diferença significativa entre as taxas de mortalidade para os grupos irradiados com UVB (-17,6 %/dia) quando comparado ao grupo controle (-8,4 %/dia) ($F = 5,34$, $p>0,05$) (Fig. 1). Após 12 dias de experimento, a taxa de crescimento foi significativamente maior no grupo controle (0,36 mm/dia) do que nos grupos irradiados com UVA (UVA_1 = 0,25 mm/dia e UVA_2 = 0,15 mm/dia) ($F= 9,97$, $p<0,05$) (Fig. 2).

Nas larvas irradiadas durante 12 dias, a área relativa invertida de TOSC para o grupo controle (0,35 ± 0,12) foi maior do que para os grupos irradiados com UVA (UVA_1 = 0,17 ± 0,02; UVA_2 = 0,15 ± 0,04). Entretanto, não houve diferença significativa entre os grupos analisados ($p>0,05$) (Fig. 3). Já para o grupo irradiado durante 3 dias com UVB, a área relativa invertida foi significativamente diferente entre o grupo controle (0,16 ± 0,00) e o grupo irradiado (0,09 ± 0,01) (Fig. 4).

4. Discussão

Os resultados aqui obtidos não apresentaram diferença significativa nas taxas de mortalidade entre os animais irradiados com UVB e os animais controle. Contrário a estes resultados, larvas de *Esox lucius* apresentaram distúrbios comportamentais e mortalidade quando expostas a baixas doses de radiação UVA e UVB (Vehniäinen *et al*., 2007). Bell e Hoar (1950) também encontraram maior taxa de mortalidade em ovos e larvas de salmões *Oncorhyncus nerka*, o que também foi visto em larvas de anchoíta *Engraulis*

mordax (Hunter *et al*., 1979, 1981) e em ovos de *Perca flavescens* (Williamson *et al*., 1997). O pouco tempo de acompanhamento do experimento deve ter contribuído para esta diferença entre o resultado do presente estudo e a literatura.

As larvas de *O. argentinensis* irradiadas com UVA e UVB apresentaram uma diminuição da competência antioxidante contra radicais peroxil. Apesar das larvas expostas ao UVA não apresentarem diferença significativa na capacidade antioxidante com relação ao grupo controle, houve uma clara tendência à diminuição deste sistema. No grupo exposto ao UVB, a baixa área relativa invertida, nos mostra uma diminuição da competência antioxidante destas larvas expostas a radiação UV, indicando que, possivelmente, estes animais estão em situação de estresse oxidativo. Corroborando com estes resultados, Krapp *et al.,* (2009) viram uma baixa capacidade antioxidante contra radicais peroxil e hidroxil em anfípodas *Gammarus wilkitzkii,* expostos a estas radiações.

De acordo com o presente estudo, a radiação UVA pode interferir no crescimento de larvas *O. argentinensis.* Possivelmente, estes organismos diminuam suas taxas metabólicas quando expostos ao UVA, como também demonstrado em peixes ciclídeos *Cichlasoma nigrofasciatum* (Winckler e Fidhiany, 1996) ou em larvas do peixe zebra *Danio rerio* (Dong *et al*., 2007).

Organismos planctônicos possuem diversos mecanismos de defesa que evitam ou reduzem os danos causados pelas radiações UV. Estudos prévios indicam que o zooplâncton pode utilizar diferentes estratégias para proteção ou são mais tolerantes que outros organismos (Souza *et al*., 2007). Entretanto,

este trabalho demonstrou que estas estratégias são muitas vezes vencidas em função dos danos produzidos.

Neste sentido, embora estejamos considerando resultados preliminares, eles são suficientes para sugerir que larvas de *O. argentinensis* apresentam respostas danosas frente à radiação UV em doses semelhantes àquelas registradas na superfície da coluna d'água do estuário da Lagoa dos Patos (Gouveia *et al.*, submetido). Larvas de *O. argentinensis* fazem parte do ictioplâncton e vivem próximas a superfície (Muelbert & Weiss, 1991), o que permite sugerir que estes organismos possam sofrer prejuízos com essa exposição. Em especial, estas doses são mais comuns durante o verão, quando a maioria dos organismos meroplanctônicos, como peixes e crustáceos, têm seus primeiros estágios de vida depositados na coluna d'água e transportados para o interior do estuário (Muelbert & Weiss, 1991; Sinque & Muelbert, 1997). Assim, experimentos com diferentes espécies e com maior número amostral permitirão avançar no conhecimento da influência destas radiações em organismos planctônicos.

5. Referências

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Figuras

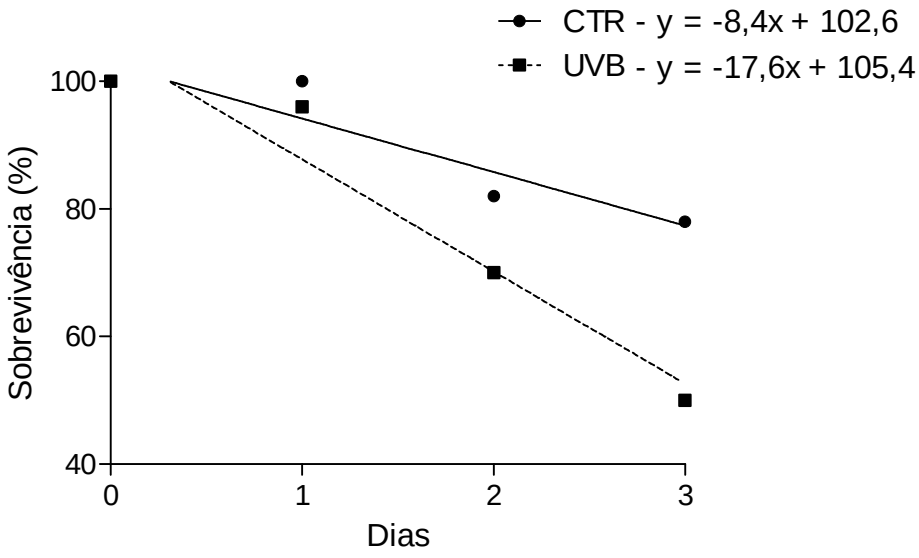

Figura 1 - Mortalidade de larvas de O. *argentinensis* (expressa em porcentagem) expostas ao UVB (0,598 J.cm^{-2}).

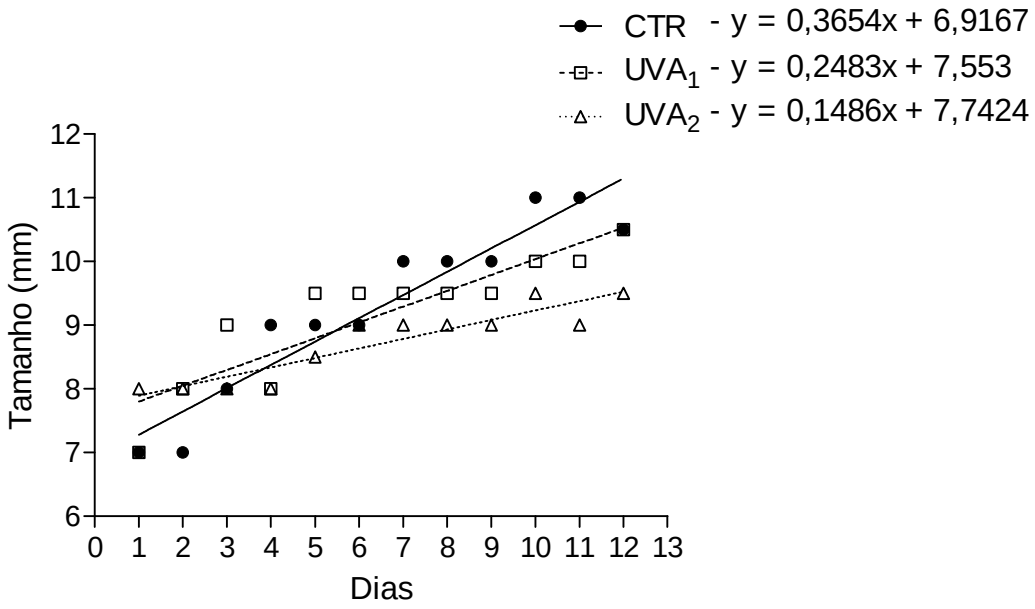

Figura 2 **-** Crescimento de larvas de O. *argentinensis* expostas ao UVA (UVA_1 - 2,05 J.cm^{-2} e UVA_2 - 4,08 J.cm^{-2}) e no grupo controle (CTR).

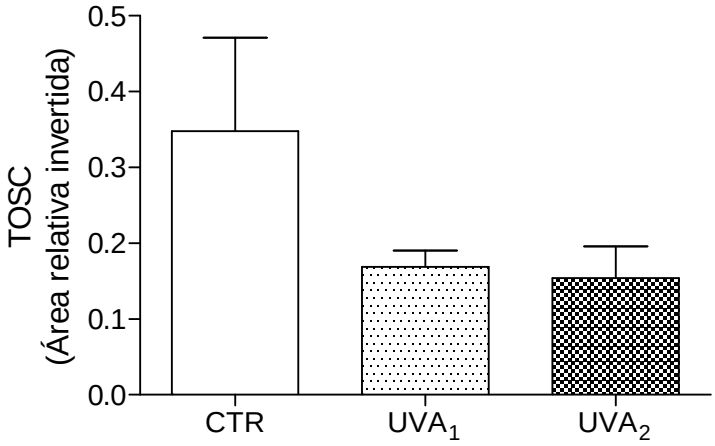

Figura 3 – Capacidade antioxidante total contra radicais peroxil (em termos de área relativa invertida) em larvas de *O. argentinensis* expostas ao UVA (UVA_1 - 2,05 e UVA_2 - 4,08 $J.cm^{-2}$) durante 12 dias de irradiação. Os resultados foram expressos como média +1 erro padrão.

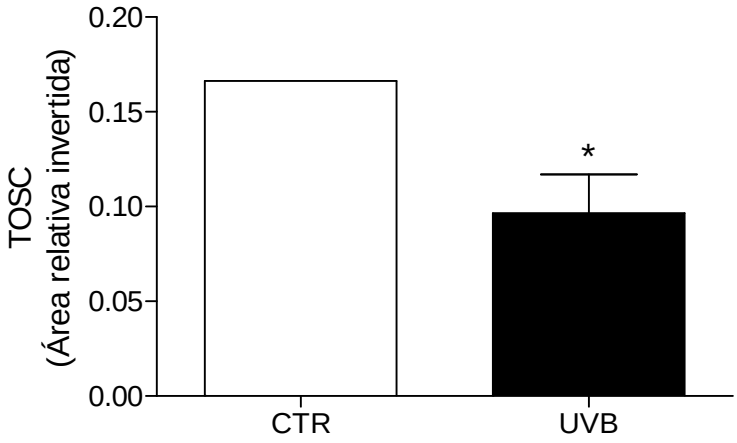

Figura 4 **–** Capacidade antioxidante total contra radicais peroxil (em termos de área relativa invertida) em larvas de *O. argentinensis* expostas ao UVB (0,598 $J.cm^{-2}$) durante 3 dias de irradiação. Os resultados foram expressos como média +1 erro padrão. * Indica diferença significativa quando comparado ao grupo controle.