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VÉRONIQUE BOILY

EFFETS DE LA QUALITÉ ET DE L'INTENSITÉ DU RAYONNEMENT ULTRAVIOLET SUR LES LARVES DE PERCHAUDE (*PERCA FLAVESCENS*)

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COMITÉ D'ÉVALUATION

Directeur

Pierre Magnan (Ph. D.)
Chaire de recherche du Canada en écologie des eaux douces
Département de chimie-biologie
Université du Québec à Trois-Rivières
Trois-Rivières (Québec)
G9A 5H7

Co-directeur

Andrea Bertolo (Ph. D.)
Chaire de recherche du Canada en écologie des eaux douces
Département de chimie-biologie
Université du Québec à Trois-Rivières
Trois-Rivières (Québec)
G9A 5H7

Membres du comité d'évaluation

Hélène-Marie Thérien (Ph. D.)
Directeur
Département de chimie-biologie
Université du Québec à Trois-Rivières

Jean-Jacques Frenette (Ph. D.)
Centre de recherche sur les bassins versants et les écosystèmes aquatiques (RIVE)
Département de chimie-biologie
Université du Québec à Trois-Rivières

Hélène Glémet (Ph. D.)
(En remplacement de M. Jean-Jacques Frenette)
Centre de recherche sur les bassins versants et les écosystèmes aquatiques (RIVE)
Département de chimie-biologie
Université du Québec à Trois-Rivières

AVANT-PROPOS

Ce mémoire présente la synthèse des résultats pour mes travaux effectués au printemps 2008 dans le cadre du programme de maîtrise en sciences de l'environnement. Ce projet avait pour but de vérifier expérimentalement les effets du rayonnement ultraviolet sur les larves de perchaude à l'aide du spectre solaire naturel.

Ce document est constitué de deux chapitres ainsi que d'une annexe. Le premier chapitre est un résumé substantiel comprenant une introduction, les matériels et méthodes, les résultats et la discussion et le second, mes résultats sous la forme d'un article scientifique. Ce chapitre a pour titre: « Les effets du rayonnement ultraviolet sur les larves de perchaude (*Perca flavescens*) » et est présenté selon les normes de la revue Limnology & Océanography, le périodique dans lequel cet article sera soumis pour publication. Je suis première auteure de cet article suivi de M. Andrea Bertolo et M. Pierre Magnan comme troisième auteur. L'annexe contient le guide pour les auteurs de la revue Limnology & Oceanography.

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LISTE DES ABRÉVIATIONS

RUV : Radiation ultraviolette (angl. UVR : ultraviolet radiation)

COD : Carbone organique dissous (angl. DOC : dissolved organic carbon)

CPD : Cyclobutan pyrimidine dimer

ADN : Acide désoxyribonucléique (angl. DNA: desoxyribonuclease acid)

ROS: Reactive oxygen species

SOD : Superoxide dismutase

ELISA : Enzyme-linked immunosorbent assay

ABTS : 2,2'-Azino-bis(3ethylbenzothiazoline-6-sulfonicacid)diammonium salt

PBSN : Phosphate buffer soluble nitrogen (Voir annexe II)

PBSNT : Phosphate buffer soluble nitrogen + Tween-20 (Voir annexe II)

AICc : Critère d'information théorique de type Akaike corrigé pour de petits échantillons (angl: Aikaike information criterion corrected for small samples)

VIF: Facteur de l'augmentation de la variance (angl: variance inflation factor)

RÉSUMÉ

L'objectif de cette étude est de déterminer expérimentalement les effets de la qualité (PAR-UVA-UVB) et de l'intensité de la lumière sur la survie des larves de perchaude ainsi que sur le stress oxydant via le dosage de l'activité d'un antioxydant, la SOD. Nous avons aussi estimé la quantité de photo-dommages dans les larves de perchaude par l'utilisation de dosimètres à ADN brut. Des larves fraîchement écloses ainsi que des dosimètres à ADN brut ont été incubés sous des combinaisons de filtres sélectifs (opaques aux UVB ou UVB+UVA) et de densité neutre (moustiquaire de fenêtre) offrant différents niveaux de protection des rayons ultraviolets (RUV). Les résultats indiquent qu'à elles seules, les radiations UVA ont un effet négatif sur la survie des larves de perchaude à même titre qu'en présence d'UVA + UVB. Au contraire, (i) les résultats des dosimètres à RUV montrent que seuls les UVB sont responsable du photo-dommage dans l'ADN, alors que le stress oxydant est maximum en présence d'UVA et d'UVB. Bien que nos résultats reliés au photo-dommage et au stress oxydant confirment que les radiations UVB sont un facteur clé dans la compréhension des effets du RUV sur la mortalité des larves, (i) ils montrent aussi l'importance de prendre en compte les effets des UVA tout comme ceux des UVB et (ii) qu'il est préférable d'utiliser le spectre solaire naturel plutôt qu'un éclairage artificiel lors de telles études.

Mots clés : UVB, UVA, photo-dommage, stress oxydant, larve de perchaude

CHAPITRE I

RÉSUMÉ SUBSTANTIEL

Introduction

Les rayons ultraviolets (RUV) ont le potentiel d'induire des dommages aux organismes terrestres (Caldwell et al. 1998) et aquatiques (Bancroft et al. 2007) entraînant des impacts tant au niveau de la population que de l'écosystème (Williamson 1995; Zagarese et Williamson 2001). La majorité des recherches effectuées en milieux aquatiques mettent surtout l'emphase sur les effets des UVB, ces derniers étant considérés comme étant les rayons les plus dommageables (Williamson 1995; Bancroft et al. 2007). À titre d'exemple, Häkkinen et al. (2004) ont montré par une étude en laboratoire que les UVB induisent des dommages au système nerveux du grand brochet du Nord (*Esox lucius*), alors que Bell et Hoar (1950) et Kouwenberg et al. (1999) ont montré que les UVB avaient un impact négatif sur la survie des œufs de saumon (*Oncorhynchus nerka)* et de morue (*Gadus morhua*). La cause la plus fréquente de mortalité engendrée par les UVB est le photo-dommage (e.g. Kouwenberg et al. 1999), tel que l'accumulation de photo-produits dans l'ADN comme le CPD (Olson et al. 2006). La production de CPD dépend majoritairement de l'intensité des UVB mais son accumulation dépend de la présence de processus enzymatiques de protection engendrés par les UVA et la lumière visible (Vetter et al. 1999). Répondant à un cycle diurne, la production de CPD tend à s'accumuler au cours de l'avant-midi à la suite d'une augmentation de l'exposition aux UVB et à diminuer graduellement en après-midi par l'activation des processus de protection, à la condition que les organismes aient été préalablement exposés aux UVA et à la lumière visible (Vetter et al. 1999). La balance entre ces deux processus (photo-production vs. photo-réparation) détermine la quantité de photo-dommages. L'accumulation de CPD dans l'ADN des organismes vivants permet de mesurer les effets d'une exposition aux UVB *in vivo* tandis que cette même mesure dans de l'ADN purifié permet d'estimer le

dommage maximal potentiel (sans les mécanismes de photo-réparation) causé par les UVB (Vetter et al. 1999; Olson and Mitchell 2006).

Les UVA contribuent, au moyen de mécanismes indirects reliés au stress cytoplasmique, à la production de dommages à l'ADN. Les agents oxydants sont produits par l'absorption des RUV, majoritairement les UVA, par des agents photosensibles qui se trouvent à l'intérieur et à l'extérieur de la cellule (Vincent et Neale 2000). Le radical superoxide ($O_2^{\cdot}$) est un oxydant très puissant pouvant entraîner du stress oxydant par le débalancement du ratio oxydants/antioxydants (Monaghan et al. 2009). Son action est minimisée par sa dismutation par la superoxide dismutase (SOD), un enzyme antioxydant, en composés moins oxydants (ref. dans Häkkinen et al. 2004). Le dosage de l'activité de cet enzyme permet de connaître indirectement la quantité d'agents oxydants dans l'organisme et ainsi le stress oxydatif induit par les RUV. Cependant, les UVA peuvent aussi avoir un effet inverse puisqu'ils contribuent, à une certaine dose, à inhiber la SOD.

Les organismes aquatiques développent plusieurs stratégies afin de se protéger des RUV. L'évitement des zones à risque, les protections métaboliques (par ex. la pigmentation) et la photo-réparation sont parmi les stratégies adoptées par les poissons (Vincent et Neale 2000). Des preuves de l'adoption de telles stratégies ont été montrées pour la perchaude (*Perca flavescens*) notamment (Williamson et al. 1997; Huff et al. 2004); cette dernière adaptant sa profondeur de ponte aux dépens de conditions optimales pour le développement de ses œufs.

Au stade prolarvaire, les larves de perchaude sont complètement transparentes (Mansueti 1964; Scott et Crossman 1974), ce qui les rend potentiellement vulnérables aux RUV. Aussi, elles sont reconnues pour adopter un comportement de phototropisme positif (Mansueti 1964), entraînant un

mouvement vers la surface où le risque d'exposition aux RUV augmente. Malgré une relation positive entre le recrutement des jeunes perchaudes de l'année et l'augmentation en carbone organique dissous (i.e. réduction de la pénétration des RUV; Steinberg et al. 2006) (Bertolo et Magnan 2007), peu d'informations sont connues à propos de la vulnérabilité des larves de perchaude. Comme les embryons de perchaude sont hautement sensibles aux RUV (Williamson et al. 1995), nous croyons que les larves de perchaude pourraient montrer une réponse similaire. Ces dernières constatations font donc de la perchaude un excellent modèle pour observer et comprendre les effets des RUV sur les organismes aquatiques.

L'objectif de cette étude est de déterminer les effets de la qualité (UVA vs. UVA + UVB) et de l'intensité des RUV sur :

(i) la survie des larves de perchaude,

(ii) le stress oxydant via le dosage de l'activité d'un antioxydant, la SOD, et

(iii) la quantité de photo-dommages dans de l'ADN brut (i.e. photo-dommage maximum potentiel).

En nous basant sur la littérature, nous prédisons que :

(a) les effets négatifs des RUV sur les larves de perchaude seront majoritairement induits par les UVB via le photo-dommage et, avec une importance moindre, par l'augmentation du stress oxydant (Kouwenberg et al. 1999; Vetter et al. 1999),

(b) les UVA seront associés à une augmentation du stress oxydant et, avec une moins grande importance, au photo-dommage dans l'ADN (Dong et al. 2007; Zagarese et Williamson 2001), mais que

(c) les UVA auront un impact moins important sur la variation de la survie des larves à cause de leur rôle photo-réparateur (Vetter et al.1999).

Contrairement à la plupart des études, ces prédictions seront évaluées sous le spectre solaire naturel, ce qui permettra une meilleure compréhension de l'impact des RUV naturels sur les organismes aquatiques.

Matériels et méthodes

Site d'échantillonnage

Les œufs de perchaude ont été collectés dans l'archipel des îles de Sorel-Berthier, fleuve Saint-Laurent (Québec, Canada) (46°04'52.06' N 73°10'00.98' O), du 1er au 5 mai 2008, quelques jours avant l'éclosion. Les œufs ont ensuite été incubés jusqu'à l'éclosion à une température de 14°C au Laboratoire de Recherche sur les Communautés Aquatiques de l'Université du Québec à Trois-Rivières. Afin de maximiser l'oxygénation des œufs, chaque ponte a été étirée et maintenue individuellement sur un filet dans un aquarium. Les aquariums étaient dotés d'un système de recirculation de l'eau et d'un bulleur, afin de garantir de bonnes conditions pour les œufs et les larves. À partir du moment où nous étions capables d'observer les larves bouger à l'intérieur des œufs, les pontes ont été brassées délicatement afin de faciliter l'éclosion. Après résorption du sac vitellin, les larves ont été nourries avec de la moulée pour alvins (Tétramin®).

Incubation sous le spectre solaire complet

Les larves fraîchement écloses, prélevées au hasard parmi les pontes, ont été incubées dans des contenants en plastique (prof. : 6 cm, dia. :11 cm) et placées à l'intérieur d'une glacière de styrofoam® (présentée comme un « bloc» dans la suite du texte) avec re-circulation d'eau. Le fond des contenants de plastique (présentés comme un « incubateur » dans la suite du texte) a été remplacé par un filet de maille fine (~200 µm) permettant la circulation de l'eau. Chacun des blocs contenait neuf incubateurs. Deux expériences d'une durée de sept jours ont été effectuées à une semaine d'intervalle, soit du 7 au 14 mai 2008 et du 21 au 27 mai 2008. Chaque expérience comptait cinq blocs. L'irradiance a été mesurée en continu lors des deux expériences à une fréquence de cinq Hertz à l'aide d'un radiomètre (PUV-2545/2546, *Biospherical instruments Inc.*) pour les longueurs d'ondes de 313nm (UVB), 320nm (UVB), 340nm (UVA) et de 400 à

700nm (lumière visible). Afin de tester différents niveaux de qualité et d'intensité de la lumière sur les larves, les incubateurs ont été recouverts aléatoirement d'une des neuf combinaisons de trois filtres neutres (moustiquaires de fenêtre) (Huggins et al. 2004) et de trois filtres sélectifs :

Whirlpak® : spectre solaire complet identifié comme « UVB + UVA + lumière visible ». Ce matériel a été utilisé afin de prendre en compte l'effet du plastique des filtres présent dans les autres traitements.

Mylar-*d*® : bloque les UVB (Vetter et al. 1999) : identifié comme « UVA + lumière visible » et

Jroll® : bloque les UVB et les UVA (Olson et al. 2006) : identifié comme « lumière visible ».

Dans le but d'obtenir un plan factoriel complet (3 X 3), nous avons croisé les trois niveaux de filtres neutres avec les trois niveaux de filtres sélectifs. Les neuf traitements ont été randomisés à l'intérieur de chaque bloc. Le pourcentage de transmission de chaque filtre sélectif a été calculé pour toutes les longueurs d'ondes mentionnées plus haut à partir du ratio de l'irradiance sous le filtre (mesurée à l'aide du radiomètre recouvert de chaque filtre sélectif) et de l'irradiance totale (mesurée avec le radiomètre non recouvert). Afin de s'assurer de la stabilité des propriétés optiques des filtres, des mesures d'absorbance ont été effectuées avant et après les expérimentations à l'aide d'un spectrophotomètre (Ultrospec 3100 *pro*). La transmittance des filtres sélectifs a aussi été mesurée à un nanomètre d'intervalle entre 280 à 700nm à l'aide d'un spectrophotomètre (Ultrospec 3100 *pro*).

Chaque jour, au coucher du soleil, les larves mortes ont été dénombrées et retirées des incubateurs. La température dans les glacières a été contrôlée à l'aide d'un bain thermostatique permettant de recréer des variations journalières semblables à celles retrouvées en milieu naturel. Au cours de la première expérience, la température variait de minimum 10,8° C ± 2,2 (moyenne inter-jour ± E.T.) à 7h30 am à maximum 18,8°C ± 1,3 à 13h30 pm et de minimum 11,2°C ± 2,3 à 7h30 am à maximum 20,4°C ± 2,9 à 13h30 pm pour la deuxième

expérience. À la fin de la deuxième expérience, les larves vivantes ont été triées et conservées à -80°C pour le dosage de la SOD. Pour des raisons de logistique, aucune analyse de la SOD n'a été réalisée pour la première expérience. Lors du tri, certaines larves étaient étendues au fond de l'incubateur (inactives) alors que d'autres nageaient encore (actives), laissant supposer que certaines larves avaient subi plus de dommages que d'autres. Afin de considérer l'état des larves, ces dernières ont été triées selon deux groupes (actives ou inactives) et analysées séparément.

Photo-dommages dans l'ADN brut : dosimètres à ADN

Afin d'évaluer les dommages potentiels à l'ADN, nous avons mesuré la production de CPD dans des dosimètres faits d'ADN brut (*Sigma Chemical Company*). Les dosimètres ont été fabriqués à partir de cuvettes de spectrophotométrie en méthacrylate permettant le passage des RUV. Chaque cuvette a été remplie d'une solution d'ADN brut de salmonidé (100 µg d'ADN $\cdot ml^{-1}$ d'eau doublement distillée) (Olson et al. 2006). Un dosimètre à ADN a été placé à l'intérieur de chaque incubateur et retiré à la fin de l'expérience. Afin de détecter la présence de dommages sur l'ADN (CPD) dans les dosimètres, nous avons utilisé une méthode immunoenzymatique de type ELISA (Enzyme-Linked Immunosorbent Assay) adapté de Kobayashi et al. (2001). Un sous-échantillon (100 µl) de chaque dosimètre irradié a été déposé dans un des 96 puits d'une microplaque (Costar®, *Fisher Scientific*). La plaque a été incubée 12 heures à 4°C pour permettre à l'ADN d'adhérer correctement aux parois du puits. Toutes les étapes suivantes ont été faites à la température de la pièce. Par la suite, les sites de liaison non-spécifique potentiellement disponibles sur les parois des puits ont été bloqués à l'aide de protéines ne pouvant pas réagir lors de la réaction de détection immunologique (lait en poudre). Un anticorps spécifique au CPD, ou « anti-CPD » (1 µg d'anti-CPD $\cdot$ mL^{-1} de tampon neutre, le PBSNT) induit chez les souris (*KAMIYA Biomedical company, États-Unis*) a ensuite été ajouté dans le puits et incubé pendant une heure. Le PBSNT est un tampon

phosphate neutre additionné d'un détergent (Tween-20) qui permet une meilleure adhésion des anticorps. L'étape suivante a consisté en l'ajout d'un anticorps anti-immunoglobulines de souris ($1\mu g \cdot ml^{-1}$ de PBSNT) marqué à la peroxidase (*Sigma*) : il a été nécessaire de laisser agir une heure. La peroxidase est un enzyme qui catalyse l'oxydation de l'ABTS (*2,2'-Azino-bis(3éthylbenzothiazoline-6-sulfonicacid)diammonium salt)*, le réactif colorant utilisé, au moyen d'une molécule d'oxygène du peroxyde. Enfin, nous avons ajouté le réactif colorant ($0,4mg \cdot ml^{-1}$) en solution avec du peroxyde (0,015%) et un tampon citrate-phosphate ($0,1\ m \cdot L^{-1}$, pH 4.0). Afin de laisser réagir le colorant, la plaque a incubé pendant 30 minutes dans l'obscurité. L'absorbance des échantillons a été mesurée à une longueur d'onde de 405nm. Entre chaque étape, cinq lavages de cinq minutes chacun ont été faits avec un tampon phosphate neutre, le PBSN, afin de retirer tous les anticorps n'ayant pas réagi au cours des périodes d'incubation.

Activité de la SOD

L'activité de la SOD a été dosée à l'aide du « SOD assay kit-WST » (Dojindo Molecular Technology ®, *Rockville, U.S.A*). Ce protocole permet de doser l'activité de la SOD par la coloration des produits créés à la suite de l'action anti-oxydante de la SOD et ainsi, d'évaluer la variation de l'activité spécifique (activité/mg de protéine). Une concentration de $3\mu g \cdot puits^{-1}$ de protéine pour chaque échantillon a été nécessaire pour effectuer le dosage.

Méthode statistique

Un protocole expérimental de type factoriel complet avec blocs complètement randomisés a été utilisé afin de contrôler la variabilité inter-blocs (nuisance éventuelle), pour laquelle nous n'avions pas d'estimations préalables. Afin de modéliser adéquatement ce protocole expérimental, un modèle linéaire mixte généralisé a été utilisé pour comparer les effets des RUV sur la survie des

larves. Cette approche permet de prendre en compte la non-indépendance des données recueillies à l'intérieur de chaque bloc et chaque incubateur. Ce modèle généralisé a aussi permis d'ajuster la variable dépendante selon un modèle de régression logistique afin de prendre en compte la distribution binomiale de l'état des larves (morte/vivante). Six modèles expliquant la variation de la survie des larves ont été construits en se basant sur la simplification du modèle le plus complexe, soit celui comprenant toutes les variables pouvant expliquer la variation de la survie des larves (qualité de la lumière, intensité de la lumière, interaction entre la qualité et l'intensité, variabilité entre les deux expériences et effet des blocs) (Burnham and Anderson 2002).

Un modèle linéaire mixte a été utilisé afin d'analyser les effets des RUV sur la quantité de photo-dommages dans les dosimètres. Ce modèle diffère du précédent en raison de la nature de la variable dépendante (continue et non binomiale); il n'a donc pas été nécessaire de l'ajuster. Six modèles comprenant les mêmes variables que celles de l'analyse de la survie ont été testés. Les modèles construits afin d'expliquer le potentiel de photo-dommage dans les dosimètres comprennent les effets de la qualité et de l'intensité des RUV, de l'interaction entre la qualité et l'intensité des RUV, la variabilité entre les deux expériences et l'effet des blocs.

À cause d'un protocole expérimental incomplet, dû à un patron de mortalité non aléatoire des larves entre les traitements, il n'a pas été possible de traiter les donnés sur l'activité de la SOD selon le protocole de type factoriel complet avec blocs complètement randomisés. Ces données ont donc été analysée selon le gradient de la dose cumulative reçue pour les différentes longueurs d'ondes (i.e. 313, 320 et 340nm) à titre de variables dépendantes. La longueur d'onde de 320nm a été retirée du modèle en raison de sa grande colinéarité avec les autres variables (313 et 340 nm), qui aurait pu influencer l'estimation des paramètres du modèle. La colinéarité a été mesurée à l'aide du VIF (*angl :*

variance inflation factor), lequel est considéré comme non acceptable lorsqu'il est supérieur à dix (Kutner et al. 2004). Les modèles ont donc été construits en fonction des doses cumulatives reçues à 313 et 340nm comme variables fixes et de l'effet bloc comme variable aléatoire.

Afin de comparer et d'ordonner les modèles selon leur plausibilité, un critère d'information théorique de type Aikaike corrigé pour les petits échantillons (AIC_c) a été utilisé (Burnham and Anderson 2001). Les modèles ont été ordonnés selon le $\Delta_i = AIC_i - AIC_{min}$ où AIC_i est la valeur du modèle à comparer et AIC_{min} est la plus petite valeur d'AIC donnée pour l'ensemble des modèles (Burnham and Anderson 2001). Le modèle ayant la plus petite valeur d'AIC_i ($\Delta_i = 0$) étant le modèle plus plausible parmi les modèles comparés. Le pourcentage de variation expliqué par chaque modèle a été calculé selon la méthode utilisée par Deschênes et Rodríguez (2007).

Résultats

Transmittance des filtres

Contrairement à nos attentes, le Whirlpak® (UVB + UVA + lumière visible) n'était pas complètement transparent aux RUV. Environ 50,04% ± 1,93 (moyenne ± E.T) des UVB (longueurs d'ondes comprises entre 280 et 315nm) et 59,19% ± 2,96 des UVA (longueurs d'ondes comprises entre 316 et 400nm) passent à travers le Whirlpak®. Le Mylar-*d*® (UVA + lumière visible) a une transmittance moyenne 1,34% ± 4,12 pour les UVB et de 76,71% ± 10,26 pour les UVA. La transmittance moyenne du Jroll® est de 0,11% ± 0,04 pour les UVB et de 6,30% ± 11,17 pour les UVA. Les trois filtres sélectifs sont considérés comme transparents à la lumière visible (longueurs d'ondes entre 400 et 800nm), cependant le Whirlpak® est légèrement plus opaque à la lumière visible (transmittance : 73,36% ± 4,36) que le Mylar-*d* (UVA + lumière visible) et le

Jroll® (lumière visible) (transmittance : 88,06% ± 0,98 et 86,58% ± 4,27 respectivement). Un niveau de filtre de densité neutre bloque 38,4% ± 0,47 de l'irradiance totale, alors que deux niveaux en bloquent 66,23% ± 0,56.

Survie des larves de perchaude

Sur la base du Δi, le modèle B se classe comme celui expliquant le mieux la survie des larves de perchaude exposées à différentes conditions de RUV. Ce modèle inclut toutes les variables reliées aux RUV (qualité, intensité et interaction entre les deux) et explique 81% de la variation de la survie des larves. Parmi tous les modèles, seul le modèle A semble une alternative raisonnable au modèle sélectionné ($\Delta_i < 4$). En comparaison avec le modèle B, le modèle A a un terme supplémentaire (effet aléatoire « expérience »), alors que les coefficients des autres termes sont essentiellement similaires. L'interprétation des deux modèles reste essentiellement la même. La survie des larves est presque maximale (80%) dans tous les traitements avec lumière visible seulement. Au contraire, cette dernière diminue avec une augmentation de l'intensité pour les traitements avec (UVB + UVA + lumière visible) et (UVA + lumière visible). Ces différences sont clairement reflétées dans le modèle par le terme d'interaction.

Photo-dommage sur l'ADN

Peu importe l'intensité de la lumière, le photo-dommage est maximum sous les traitements avec (UVB + UVA + lumière visible) et minimum sous les traitements avec lumière visible seulement. Parmi les différents modèles, le modèle E est de loin le meilleur modèle pour expliquer le photo-dommage dans l'ADN brut. Le modèle E inclut la qualité de la lumière comme composante fixe et l'effet bloc comme composante aléatoire et explique 76% de la variation du photo-dommage avec les RUV.

Activité de la SOD

Tout comme le photo-dommage, le stress oxydant pour les larves inactives est maximum sous les traitements avec UVB + UVA + lumière visible, peu importe l'intensité de la lumière, intermédiaire sous les traitements avec UVA + lumière visible et minimum sous la lumière visible. Les résultats pour les larves actives sont cependant beaucoup moins clairs tel que le suggère la variation expliquée par le modèle sélectionné. Cependant, en raison du peu de larves actives et inactives sous les traitements de forte intensité, les effets en lien avec l'intensité de la lumière ne peuvent pas être explorés en profondeur.

Autant pour les larves actives qu'inactives, le modèle C est le meilleur modèle basé sur le Δ_i pour expliquer le stress oxydant. Cependant, bien que ce modèle explique 70% de la variabilité du stress oxydant pour les larves inactives, ce même modèle l'explique seulement à 22% pour les larves actives. Ce modèle inclut la dose d'UVB (313nm) comme composante fixe et l'effet bloc comme composante aléatoire. Les coefficients du modèle montrent une relation positive entre le stress oxydant et les UVB (313nm) pour les larves inactives mais une relation négative pour les larves actives. Parmi les différents modèles pour les larves actives, seul le modèle B semble être une bonne alternative ($\Delta i<4$). Le modèle B a un terme de plus que le modèle C, soit l'effet fixe 340nm. Tous les modèles non sélectionnés pour les larves inactives montrent un $4<\Delta_i<10$, suggérant que le modèle C est l'unique modèle à considérer mais que les UVA (effet fixe 340nm) ne peuvent pas être définitivement mis de côté comme mécanisme potentiel.

Discussion

Contrairement à nos prédictions, nos résultats montrent que les UVA peuvent être au moins aussi dommageables que les UVB pour les larves de perchaude exposées sous le spectre solaire naturel. L'absence d'UVB n'améliore pas la

survie des larves, cependant, l'absence d'UVB + UVA l'augmente grandement et ce, même sous une forte intensité (i.e. eaux claires très peu profondes). Nos résultats confirment aussi que les UVB sont le plus important facteur dans la production de photo-dommages mais qu'ils n'expliquent pas complètement la mortalité des larves. Étonnamment, les UVB sont associés à un effet plus fort que les UVA sur le stress oxydant. Le lien incomplet entre la mortalité, le photo-dommage et le stress oxydant suggèrent que des voies additionnelles devraient être considérées afin de bien comprendre les effets des RUV sur la mortalité des larves de poissons.

Contrairement à nos résultats, la plupart des études sur les effets des RUV (Charron et al. 2000; Bancroft et al. 200) suggèrent que les UVB sont majoritairement responsables de la mortalité des organismes aquatiques. Il est à noter que la majorité de ces études ont été effectuées en laboratoire, à l'aide de lampes ne recréant pas parfaitement les conditions naturelles du spectre solaire (Häkkinen et Oikari 2004). Bien que dans notre étude les traitements sous forte intensité montrent une irradiance plus forte que la réalité, les autres traitements représentent des conditions d'irradiance près de la réalité. Les traitements d'intensités moyenne et faible représentent les conditions d'irradiance retrouvées dans les 30 premiers cm d'eau d'un système tel le lac Saint-Pierre (Frenette et al. 2006), où les larves de perchaude sont habituellement observées (Magnan et Bertolo, observation personnelle). L'utilisation du spectre solaire naturel permet d'avoir une interprétation plus réaliste de l'impact des RUV et souligne le rôle des UVA. Cependant nos résultats ne reflètent que partiellement ceux des autres études obtenus sous la lumière naturelle. Béland et al. (1999) ont utilisé un plan d'échantillonnage semblable au nôtre afin d'évaluer l'impact des RUV sur les embryons de morue. Cependant, ils n'ont observé aucun effet des UVA sur la mortalité. Williamson et al. (1997) ont montré que la survie des œufs de perchaude est nulle lorsqu'ils sont exposés, en eaux claires, aux UVB + UVA + lumière visible. Bien que les embryons exposés aux UVA + lumière visible survivent plus longtemps que

ceux exposés aux UVB + UVA + lumière visible, la mortalité après dix jours d'irradiation est de 100% dans les deux traitements. Bien que la mortalité varie avec la qualité et l'intensité de la lumière, le photo-dommage varie avec la qualité seulement. Nos résultats sont cohérents avec la littérature et indiquent que le photo-dommage augmente avec les UVB (Vetter et al. 1999; Kobayashi et al. 2001; Torizawa et al. 2004). Notre étude a suggère aussi que le photo-dommage peut être induit par les UVA.

Sans exclure le rôle des UVA, nos résultats, en lien avec l'activité de la SOD, suggèrent que les UVB sont majoritairement responsables du stress oxydant, autant pour les larves actives qu'inactives. Ce résultat est contraire à la littérature qui suggère que le stress oxydant est induit principalement par les UVA (Zagarese et Williamson 2001). Cependant, seulement quelques études utilisent la SOD comme marqueur du stress oxydant dans les poissons (Charron et al. 2000; Häkkinen et al. 2004). À notre connaissance, seulement Häkkinen et Oikari (2004) ont utilisé l'activité de la SOD pour évaluer l'impact des RUV sur le terrain. Ces derniers n'aient pas trouvé de réponse significative chez les larves de poissons, alors que nos résultats suggèrent que l'activité de la SOD est potentiellement un bon marqueur de stress oxydant.

Nos résultats sur le stress oxydant ont aussi montré l'importance de prendre en compte l'état des larves afin d'obtenir une réponse plus claire. Les résultats sont relativement peu concluants pour ce qui concerne les larves actives; la grande variabilité observée au sein des larves actives pourrait être induite par un mélange d'individus montrant différents niveaux de stress à l'intérieur d'un même traitement. Au contraire, la variabilité était plus faible pour les larves inactives, suggérant que ces dernières étaient probablement dans un état moribond. Ainsi, nous croyons qu'il serait utile de prendre en compte l'état des larves lors d'évaluation des effets des RUV, en couplant des analyses de stress oxydant avec des marqueurs comme des protéines apoptotiques (Franco et al.

2009), qui pourraient donner des indications quant à l'état physiologiques des larves.

La concentration en carbone organique dissous (COD) peut réduire la pénétration des RUV dans la colonne d'eau et ainsi agir comme un écran protecteur pour les organismes aquatiques (Steinberg et al. 2006). En montrant la corrélation positive entre l'augmentation du COD et l'abondance de jeunes perchaudes de l'année, Bertolo et Magnan (2007) ont suggéré que le COD avait un effet protecteur contre les RUV. Ceci est en accord avec les résultats de Geddes (2009) qui a montré que la fraction colorée du COD, malgré ses effets toxiques sur les organismes aquatiques, a un effet global positif sur la faune aquatique. Nos résultats supportent cette voie en montrant que les RUV peuvent être un facteur important de mortalité pour les larves de perchaude. De plus, en montrant le rôle important des UVA, nos résultats suggèrent que l'approche commune mettant l'emphase sur les marqueurs des UVB peut sous-estimer le rôle des RUV sur les organismes. Finalement, puisque nos résultats sont obtenus à partir d'irradiation sous le spectre solaire naturel, notre étude a l'avantage de permettre une évaluation réaliste de l'impact des RUV sur les organismes.

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CHAPITRE 2

Effects of ultraviolet radiation quality and intensity on yellow perch larvae (*Perca flavescens*)

Véronique Boily, Andrea Bertolo, Pierre Magnan

(En préparation)

Effects of ultraviolet radiation quality and intensity on yellow perch larvae (*Perca flavescens*)

Véronique Boily, Andrea Bertolo[1], Pierre Magnan

[1] Corresponding author

Research Centre for Watershed - Aquatic Ecosystem Interactions

Université du Québec à Trois-Rivières

C.P. 500, Trois-Rivières (Québec)

Canada G9A 5H7

e-mail: andrea.bertolo@uqtr.ca

tel: + 1 819 376-5011 #3302

fax: + 1 819 376-5084

Running head: UVR effects on yellow perch larvae

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33

Abstract

The main purpose of this study was to experimentally determine the effects of both the quality (UVA vs. UVA + UVB) and the intensity of natural ultraviolet radiation (UVR) on the survival of yellow perch (*Perca flavescens*) larvae as well as on the oxidative stress in the cytoplasm, which we use as a proxy of cellular damage. We also estimated the potential accumulation of photodamage in yellow perch larvae using UV dosimeters with purified DNA. Both freshly hatched yellow perch and UV dosimeters were incubated in controlled conditions under a factorial combination of selective (opaque to UVB only or to both UVA + UVB) and non-selective (window screen) filters offering different levels of UVR protection. The results indicate that not only do UVA + UVB radiation increase larval mortality—which was expected—but also that exposure to UVA alone has negative effects on larval survival. In contrast, (i) the results of UV dosimeters with purified DNA showed that UVA + UVB had the most effect compared to UVA alone which suggest that only UVB exposure was responsible for DNA photodamage, whereas (ii) oxidative stress was highest when larvae were exposed to both UVB and UVA. Our results on photodamage and on SOD activity confirmed that UVB is an important factor in larval mortality, but highlight that UVA is a more important factor in mortality than previously expected. Because these results were obtained under natural solar light, they should give a more realistic idea of the impact of UVR on aquatic organisms.

Introduction

Solar ultraviolet radiation (UVR) has the potential to induce damage to terrestrial (Caldwell et al. 1998) and aquatic (Bancroft et al. 2007) organisms, with potential consequences at both the population and ecosystem levels (Williamson 1995; Zagarese and Williamson 2001). Most research on aquatic organisms has focussed on the effects of UVB radiation since this fraction of UVR is considered to be the most damaging (Williamson 1995; Bancroft et al. 2007). For example, Häkkinen et al. (2004) showed experimentally that UVB can induce damage to the nervous system of northern pike (*Esox lucius*), whereas Bell and Hoar (1950) and Kouwenberg et al. (1999) showed that UVB has a negative impact on egg survival in both salmon (*Oncorhynchus nerka)* and cod (*Gadus morhua*). The most frequently cited cause of mortality brought about by UVB is photodamage (e.g., Kouwenberg et al. 1999), such as the accumulation of photoproducts like cyclobutan pyrimidine dimer (CPD) in DNA (Olson et al. 2006). The production of CPD in the DNA is a direct function of the intensity of UVB, but its accumulation also depends on the presence of enzymatic photorepair mechanisms, which are enhanced mostly by UVA and visible light (Vetter et al. 1999). During the diel cycle, the CPD products tend to accumulate in cells in the morning due to the increase in UVB exposure and gradually decrease in the afternoon because of the repair action of photoenzymatic processes, if exposure to UVA and visible light occurred (Vetter et al. 1999). The balance between these two mechanisms (production vs. photoreparation) determines the amount of photodamage. The accumulation of CPD in the DNA of living organisms is a common way to measure the effect of short-term UVR exposure (i.e., the balance between UVB and UVA). In contrast, quantifying CPD accumulation in purified DNA allows an estimation of the maximum potential damage

due to UVB in the absence of any repair mechanisms (Vetter et al. 1999; Olson and Mitchell 2006).

Even through it is less frequently considered, UVA can also contribute to DNA damage by indirect mechanisms related to cytoplasmic stress. Reactive oxygen species (ROS) are produced following the absorption of UVR (mostly UVA) by intermediate compounds (photosensitizing agents) found both inside and outside the cells (Vincent and Neale 2000). ROS such as superoxide radicals (O_2^-) are highly energetic oxidant compounds that may cause an oxidative stress due to an imbalance in the ROS/antioxidant ratio (Monaghan et al. 2009). Superoxide dismutase (SOD) is an antioxidant enzyme that minimizes the action of superoxide radicals (Monaghan et al. 2009). SOD catalyzes the dismutation of superoxide anion into less damaging ROS (see references in Häkkinen et al. 2004). Therefore, measuring SOD activity can be an indirect measure of the oxidative stress induced by UVR. . However, it is important to mention that UVA can have antagonistic effect since they may eventually contribute to inhibit, rather than activate the SOD (Pourzand et Tyrrell 1999).

Aquatic organisms develop different protection strategies to counteract the effects of UVR. Avoidance of risky zones, metabolic protection (e.g., pigmentation), and photoreparation are some of the strategies adopted by fishes (Vincent and Neale 2000). Guttiérrez-Rodriguez and Williamson (1999) showed that bluegill (*Lepomis macrochirus*) use different spawning depths in lakes with different water transparencies. This is probably an adaptation to protect eggs from UVR in waters with high transparency. Williamson et al. (1997) and Huff et al. (2004) showed that yellow perch (*Perca flavescens*) spawn in relatively deep waters in lakes with low dissolved organic carbon (DOC) concentrations and high UVR penetration in the water column, leading to

103 slower egg and larval development because of suboptimal temperatures. After hatching,
104 yellow perch larvae are nearly completely transparent (Mansueti 1964; Scott and
105 Crossman 1974), making them vulnerable to UVR. Furthermore, newly hatched yellow
106 perch larvae are known to adopt a positive phototropic behaviour (Mansueti 1964), in
107 which individuals tend to move close to the surface where the risks of UVR exposition
108 are highest. Taken together, these factors might contribute to UVR-induced mortality in
109 nature. Even though a positive relationship has been found between increases in DOC
110 concentrations (i.e., reduced UVR penetration) and young-of-the-year yellow perch
111 abundance in Canadian Shield lakes (Bertolo and Magnan 2007), little is known about
112 the vulnerability of yellow perch larvae to natural UVR. Since we know that embryos of
113 this species are highly sensitive to UVB-induced damage (Williamson et al. 1997), we
114 expect yellow perch larvae to show a similar response. This species is thus a good
115 model to address the effects of both UVB and UVA on fish larvae under natural light
116 conditions.

117 The goal of the present study is to determine the effects of both the quality (UVA vs.
118 UVA + UVB) and the intensity of UVR (i) on survival in yellow perch larvae, (ii) on the
119 oxidative stress in larval cells by assaying SOD activity, and (iii) on the quantity of
120 photoproducts in purified DNA (i.e., maximum potential photodamage). Based on the
121 literature, we predicted that (a) the negative effects on yellow perch survival will be
122 mainly due to UVB, mostly through the accumulation of photodamage and, to a lesser
123 extent, to an increase in cytoplasmatic stress (SOD activity) (Kouwenberg et al. 1999;
124 Vetter et al. 1999), (b) UVA will be associated mostly with an increase in SOD activity
125 and also to the accumulation of potential photodamage in DNA (Dong et al. 2007;
126 Zagarese and Williamson 2001), but (c) UVA will have a lesser impact on larval

127 survival than UVB because of its expected photorepair mechanisms (Vetter et al. 1999).
128 In contrast to most of the previously published work, these predictions were assessed
129 under the natural solar spectrum, explicitly taking into account both UVA and UVB.
130 This allows a better understanding of the impact of natural UVR on aquatic organisms.
131

Materials and methods

Sampling site

Yellow perch egg strands were collected in the Sorel–Berthier island archipelago, St. Lawrence River (Quebec, Canada) (46°04'52.06″ N 73°10'00.98″W), from 1 to 5 May 2008, a few days before hatching. Eggs were incubated at 14°C until hatching in the Aquatic Communities Research Laboratory of the Université du Québec à Trois-Rivières. Each strand of eggs was gently stretched on a net and maintained in an aquarium to maximize egg oxygenation. Each aquarium was equipped with both water circulation and oxygenation systems. When larvae were seen moving within the eggs, the egg strand was gently stirred to facilitate hatching. During the experiment, larvae were fed with Tetramin® flakes only after reabsorption of the yolk sack.

Incubation under natural solar light

Newly hatched larvae were incubated in plastic bowls (6cm depth, 11 cm dia.) placed inside a Styrofoam® tank (hereafter referred to as a "block") with water circulation. The bottom of each plastic bowl (hereafter referred to as the "incubator") was cut and replaced with a net (mesh size ~200 μm) to allow water circulation. Each block contained nine incubators. Two experiments were run for one week, each with five blocks: one from 7 to14 May 2008 and a second, from 21 to 27 May 2008. Surface irradiance during both periods was measured at a frequency of five Hertz with a radiometer (PUV-2545/2546, *Biospherical Instruments Inc.*) at wavelengths of 313 nm (UVB), 320 nm (UVB), 340 nm (UVA), and 400-700 nm (hereafter referred to as "visible light") wavelengths. To test the effects of both quality and intensity of light on yellow perch larvae, the incubators were randomly covered with nine combinations of three selective wavelength filters (Whirlpak®: full solar spectrum, hereafter UVB +

156 UVA + visible light; Mylar-*d*®: opaque to UVB, hereafter UVA + visible light (Vetter et
157 al. 1999); Jroll®: opaque to both UVA and UVB, hereafter visible light (Olson et al.
158 2006)) and three neutral density filters (window screens; Huggins et al. 2004). To obtain
159 a complete 3 x 3 factorial plan, three levels of selective wavelength filters (Whirlpak®,
160 Mylar-*d*® and Jroll®) were crossed with three levels of neutral density filters (none, one,
161 and two window screens). Treatments were completely randomized within each block.
162 Percent transmission of each selective filter was calculated using the radiometer at the
163 four wavelengths mentioned above from the ratio of the irradiance under the filter
164 (measured with the radiometer covered with the different plastic films) and the total
165 irradiance (measured with the uncovered radiometer). The stability of the filters' optical
166 properties was checked by measuring absorbance before and after the experiments. The
167 transmittance of selective filters for the whole spectrum between 280 and 800 nm was
168 measured with a spectrophotometer at 1 nm intervals (Ultrospec 3100 *pro*).
169 Dead larvae were counted and removed from the incubators every day at dusk.
170 Temperature was controlled in each block with a thermostatic bath to mimic the cyclic
171 thermal conditions found in nature. The daily cycle of water temperature oscillated
172 between a minimum of 10.8°C ± 2.2 (inter-daily mean ± SD) at around 7:30 am and a
173 maximum of 18.8 ± 1.3°C at around 1:30 pm during the first experiment and between
174 11.2°C ± 2.3 at around 7:30 am and 20.4°C ± 2.9 between 2:00 pm and 5:00 pm during
175 the second. Larvae still alive at the end of the second experiment were collected and
176 frozen at –80°C for SOD analyses. For logistic reasons, SOD analyses were not
177 conducted in the first experiment. Since some larvae were lying on the bottom of the
178 incubators (inactive) while others were still swimming (active), some individuals might
179 have suffered more damage than others. To account for this possible bias and to consider

180 larval state, they were sorted into two groups (active or inactive) that were analyzed
181 separately.

182 *Photodamage in purified DNA: UV dosimeters*

183 We measured the photoproduction of CPD in UV dosimeters with purified DNA
184 (*Sigma Chemical Company*) to evaluate DNA photodamage. UV dosimeters were made
185 of methacrylate spectrophotometry cuvettes transparent to UVR. Each cuvette was filled
186 with a solution of purified salmonid DNA (100 µg purified DNA·L^{-1} double distilled
187 water) (Olson et al. 2006). UV dosimeters were put in each incubator and sampled at the
188 end of the experiments. In order to detect CPD on DNA, we adapted the protocol used
189 by Kobayashi et al. (2001), which used an enzyme-linked immunosorbent assay
190 (ELISA). A subsample (100 µl) of each irradiated UV dosimeter was put into one of the
191 96 wells of a micro-plate (Costar®, *Fisher Scientific*). The plate was incubated for 12 h
192 at 4°C to fix DNA to the wells' surface. The next steps of the protocol were made at
193 room temperature. Afterwards, all the potential binding sites remaining in each well
194 were saturated with unrelated proteins that could not contribute to the immunoenzymatic
195 detection (powdered milk). A mouse antibody specific to CPD (anti-CPD) was then
196 added (1 µg anti-CPD·$L^{-1}$of PBSNT) (*KAMIYA Biomedical Company, USA*) and
197 incubated for an hour. PBSNT is a neutral phosphate buffer added with a detergent
198 (Tween-20) that allows a better adhesion of antibodies. An antibody marked with
199 peroxidase (1 µg antibody· $L^{-1}$of PBSNT) specific to mouse immunoglobulins (*Sigma*)
200 was added. Peroxidase is an enzyme that catalyzes the oxidation of a colourant, which
201 here is the ABTS (2,2'-Azino-bis (3ethylbenzothiazoline-6-sulfonicacid) diammonium
202 salt) with a peroxide molecule. After an hour of incubation, the colourant was added
203 (ABTS 0,4mg·ml^{-1}) in a solution of peroxide (0.015%) and citrate-phosphate buffer (0,1

m·L^{-1}, pH 4.0). The plates were incubated for 30 minutes in darkness to let the colourant react. The absorbance of each well was then measured at 405 nm. Between each step, every well was washed five times for five minutes with a neutral phosphate buffer (PBSN) to remove all antibodies that had not reacted.

SOD activity

SOD activity was tested with the WST SOD assay kit (Dojindo Molecular Technology®, *Rockville, USA*). This kit shows the activity of SOD via the colouration of product after the antioxidation action of SOD. The same concentration of protein (3 µg ·well^{-1}) was used for each sample to test the SOD activity. Tests were made in 96 wells microplates. Three blanks were included on each plate; blank 1 shows 100% of the reaction without antioxidation action, blank 2 shows the colour of the samples without the enzyme solution, and blank 3 shows the noise related to the buffer used in the test. SOD activity was computed from the following equation (Dojindo Molecular Technology®, *Rockville, USA*):

$$\% \text{ SOD activity} = \left(\frac{[(A_{blank1} - A_{blank3}) - (A_{sample} - A_{blank2})] \text{ X } 100}{(A_{blank1} - A_{blank3})} \right)$$

Statistical analysis

A generalized linear mixed modelling approach was used to compare the effects of UVR on yellow perch larval survival. This allowed us to adequately model the factorial plan with a completely randomized block design (CRB). The models were adjusted with a logit function because of the binomial distribution of yellow perch state (dead or alive). Six models explaining yellow perch survival were compared (Table 1a). The models were built on the simplification of the most complex model (i.e., the one

228 containing all variables that could predict yellow perch survival: light quality and
229 intensity, interaction between light quality and intensity, experiment and block effect;
230 Burnham and Anderson 2002).

231 To analyze the effect of UVR on the quantity of photodamage in UV dosimeters,
232 linear mixed modelling was used assuming a linear function between the dependent
233 variable (quantity of photodamage) and both light quality and intensity. Models built to
234 explain potential photodamage included the effect of UVR quality and intensity, the
235 interaction between UVR quality and intensity, and the experiment and the block effect
236 (Table 1b). Because of an unbalanced design due to larval mortality, we analyzed the
237 SOD data using the gradient of cumulative dose for the different wavelengths as
238 independent variables (i.e., 313, 320, and 340 nm) instead of using the factorial design.
239 Given its high collinearity with the other independent variables, the 320 nm wavelength
240 was not included in the model. Collinearity was measured by the variance inflation
241 factor (VIF), which is considered as not acceptable when larger than 10 (Kutner et al.
242 2004). Therefore, models were built using only the cumulative doses received at 313 nm
243 (UVB) and 340 nm (UVA) as fixed variables, and the block effect as a random variable
244 (Table 2).

245 We used an information–theoretic framework to compare and rank the models
246 (Burnham and Anderson 2001). Akaike Information Criterion (AIC) is based on the
247 principle of parsimony by selecting the best model to explain variation with the fewest
248 variables (Burnham and Anderson 2001). Models are ranked using $\Delta_i = AIC_i - AIC_{min}$
249 where AIC_i is the value for the selected model and AIC_{min} is the smallest value given for
250 the set of models. A model has a substantial support when $\Delta_i \leq 2$, considerable support
251 when $4 \leq \Delta_i \leq 7$ and less support when $\Delta_i \geq 10$ (Burnham and Anderson 2001). Since the

252 ratio between the sample size and the number of parameters in the model is lower than
253 40, we used the AIC corrected for small size samples (AIC_c) for model selections
254 (Burnham and Anderson 2001). The percent of variation explained by each model was
255 calculated by squaring the Pearson correlation between observed values and values
256 predicted by the full model following Deschênes and Rodriguez (2007).
257

Results

Transmittance of filters

Contrary to our expectations, the Whirlpak® filter (UVB +UVA + visible light) was not completely transparent to UVR, nearly half of both UVB and UVA passed through: on average, 50.04% ± 1.93 (mean ± SD) of UVB (wavelengths comprised between 280 nm and 315 nm), and 59.19% ± 2.96 of UVA (wavelengths between 316 nm and 400 nm) (Fig 1a). Mylar-*d*® (UVA + visible light) had a mean transmittance of 1.34% ± 4.12 for UVB and 76.71% ± 10.26 for UVA (Fig.1b). Jroll® (visible light) mean transmittance was 0.11% ± 0.04 for UVB and 6.30% ± 11.17 for UVA. (Fig.1c). The three selective filters were essentially transparent to visible light (wavelengths between 400 nm and 800 nm), although the Whirlpak® (UVB + UVA + visible light) was slightly more opaque to these wavelengths (73.36% ± 4.36 transmittance) than either Mylar-*d*® (UVA + visible light) or Jroll® (visible light) (respectively 88.06 ± 0.98 and 86.58% ± 4.27). One level of neutral density filters blocked 38.4% ± 0.47 of total irradiance at each wavelength while two levels blocked 66.23% ± 0.56. Based on these values and the irradiance measures, we calculated the cumulative doses received in each treatment for each wavelength separately (Table 3).

Survival of yellow perch larvae

The model selection based on Δ_i ranked model B as the best to explain the survival of yellow perch larvae exposed to different UVR conditions (Table 1a). This model, which includes all variables associated with UVR (quality, intensity and their interaction term), explained 81% of the variation in larvae survival. Within the set of candidate models, only model A seemed to be a reasonable alternative to the selected model ($\Delta_i <$ 4). In comparison to model B, model A had one additional term (the random effect

282 "experiment") whereas the coefficients for the other terms were essentially the same
283 (Table 4). Therefore, conclusions based on the two models are very similar. Larval
284 survival was always close to the maximum (ca. 80%) in all visible light treatments,
285 whatever the light intensity (Fig. 2). In contrast, larval survival decreased in both the
286 UVB + UVA + visible light and UVA + visible light treatments going from low to high
287 light intensity (Fig. 2). These differences are clearly reflected by the inclusion of the
288 interaction term in the selected model.

289 *DNA photodamage*

290 Whatever the light intensity, photodamage was highest under UVB + UVA + visible
291 light, intermediate under UVA + visible light, and lowest under the visible light only
292 (Fig. 3). Within the set of candidate models, model E was by far the best to explain the
293 photodamage in purified DNA, as revealed by Δ_i (Table 1b; all the others $\Delta_i > 10$).
294 Model E includes light quality (intercept: 0.033 ± 0.034; visible light: 0.033 ± 0.034;
295 UVA + visible light: 0.064 ± 0.036; UVB + UVA + visible light: 0.298 ± 0.036) as a
296 fixed component and the block as a random component; it explained 76% of the
297 variation in the photodamage.

298 *SOD activity*

299 As was the case for photodamage, oxidative stress for inactive larvae was highest
300 under UVB + UVA + visible light, whatever the light intensity (Fig. 4a), intermediate
301 under UVA + visible light, and lowest under the visible light. As suggested by the lower
302 variation explained by the selected model, results for active larvae were less clear than
303 for inactive ones and showed no clear pattern among the treatments (Fig. 4b). However,
304 because few individuals survived in the high intensity treatments for both active and

305 inactive larvae (except for the visible light treatment), the effects along the light
306 intensity gradient cannot be fully explored.

307 For both active and inactive larvae, model C best explained oxidative stress based on
308 Δ_i (Table 2). However, whereas this model explained a large fraction (70%) of the
309 oxidative stress variability for inactive larvae, it explained only 22% of the variability
310 for active ones. This model included the UVB (313 nm) dose received as a fixed
311 component and the block effect as a random component. The coefficients of the model
312 show a positive relationship between oxidative stress and UVB (313 nm) in inactive
313 larvae (intercept: 19.81 ± 3.17; 313 nm: 0.68 ± 0.50) whereas the relationship was
314 negative for active larvae (intercept: 17.77 ± 2.33; 313nm: -0.80 ± 0.71). Within the set
315 of candidate models, only model B seemed to be a reasonable alternative to the selected
316 model for active larvae ($\Delta_i < 4$). Model B had one more term (the fixed effect "340 nm")
317 than model C. For active larvae, the coefficient for UVA (340 nm) was positive whereas
318 the coefficient for UVB (313 nm) was negative, as in model C (intercept: 16.62 ± 2.62;
319 313 nm: -1.45 ± 0.98; 340nm: 0.14 ± 0.15). All the non-selected models for inactive
320 larvae had $\Delta_i > 4$, suggesting that model C had to be preferred to the others. However, as
321 for active larvae, Δ_i was < 10 in most cases (i.e., models including UVA), suggesting
322 that UVA cannot be ruled out unequivocally as a potential mechanism.
323

Discussion

Contrary to our predictions, our results show that UVA can be at least as damaging as UVB for yellow perch larvae exposed to natural solar light. Filtering out only UVB did not improve larval survival in our experiments, whereas filtering out both UVB and UVA greatly improved it, even under the highest irradiance conditions (i.e., at a very shallow depth in clear water). Our results also confirmed that UVB is the most important factor inducing photodamage in DNA, but this factor alone cannot completely explain our mortality results. Unexpectedly, UVB was also associated with a stronger effect on oxidative stress than UVA, suggesting another potential pathway leading to larval mortality. The incomplete match between mortality and both photodamage and oxidative stress suggests that additional pathways should be considered to better understand the link between UVR and fish larval mortality.

In contrast to our results, most studies on UVR effects (Charron et al. 2000; Bancroft et al. 2007) have suggested that UVB is mostly responsible for the mortality of aquatic organisms. It is noteworthy that these experiments were done in the laboratory under UVR lamps. Even though these lamps were designed to reproduce natural light conditions, they do not adequately produce realistic UVB/UVA ratios (Häkkinen and Oikari 2004). Our study using natural light avoids this problem; although treatments without non-selective screens were probably too harsh for larvae, conditions in the other treatments were close to what is potentially found in the field. The irradiance received under the low and medium intensity treatments are in fact close to the light conditions found in the first 30 cm of the water column of some sections of the St. Lawrence River (Frenette et al. 2006), where yellow perch larvae are commonly found (P. Magnan and A. Bertolo, personal observations). The use of natural solar radiation allowed us to get a

348 more realistic interpretation of the impact of UVR in a natural environment and allowed
349 us to pinpoint the role of UVA. However, our results only partially match those of other
350 studies conducted under natural light. The effect of UVR, mostly UVB, on mortality in
351 embryos and larvae are well covered in the literature (Charron et al. 2000; Häkkinen et
352 al. 2001; Olson et al. 2006). Béland et al. (1999) used an experimental design similar to
353 ours to evaluate the impact of UVR on cod embryos. However, they did not observe any
354 effect of UVA on mortality. Williamson et al. (1997) showed that yellow perch egg
355 survival is null when exposed in clear water to UVB + UVA + visible light. Even though
356 embryos exposed to UVA + visible light survived longer than those exposed to UVB +
357 UVA + visible light, mortality was 100% after ten days in both treatments. While larval
358 mortality varied clearly with both UVR and intensity, photodamage seems to vary with
359 light quality only. Although it is difficult to explain why photodamage does not vary
360 with UVR intensity, it is relatively simple to explain our results on UVR quality. Even
361 though different light sources were used, our results on photodamage are consistent with
362 those found in the literature and indicate that the concentration of CPD increases with
363 UVB dose with both artificial and natural light (Vetter et al. 1999; Kobayashi et al.
364 2001; Torizawa et al. 2004). Our study showed that photodamage could also be
365 associated with UVA.

366 Without excluding a potential role for UVA, our SOD activity results suggest that
367 UVB are mostly responsible for the oxidative stress in both active and inactive yellow
368 perch larvae, even though the results are by far clearer on the latter. This result is unlike
369 what is reported in the literature, which suggests that oxidative stress is mostly induced
370 by UVA (Zagarese and Williamson 2001). However, only a few studies used SOD as a
371 proxy for oxidative stress in fish (Charron et al. 2000; Häkkinen et al. 2004). To our

knowledge, only Häkkinen and Oikari (2004) used SOD activity to evaluate the impact of UVB in field conditions. Whereas Häkkinen and Oikari (2004) did not find any significant response in fish larvae, our results suggest that SOD activity is a good marker for oxidative stress in natural conditions.

Our results on oxidative stress also showed the importance of considering the larval state when examining the responses to oxidative stress. The high variability observed in oxidative stress for active larvae could be related to the presence of a mixture of individuals with different levels of non-lethal stress within each treatment. In contrast, variability was lower for inactive larvae, suggesting that these individuals were probably all closer to death and exhibited a more uniform response to stress. Therefore, it sems that the physiological state of organisms used in experiments on UVR must be taken into account. One could do this by coupling analyses like SOD activity with markers such as apoptosis proteins (Franco et al. 2009). In the presence of environmental stressors, such as UVR exposure, apoptosis (i.e., programmed cellular death) is in fact induced to eliminate damaged cells. Quantifying its importance could help to reduce sources of variability following UVR exposure.

Factors such as the concentration of dissolved organic carbon (DOC) can reduce UVR penetration in the water column of natural systems and therefore could act as protective screens against these damaging wavelengths (Steinberg et al. 2006). By showing a correlation between the increase in DOC concentration and the abundance of yellow perch larvae in 22 Canadian Shield lakes, Bertolo and Magnan (2007) suggested DOC protection against UVR as a potential mechanism behind their results. This is in agreement with the results of Geddes (2009), who showed that the coloured fraction of DOC, despite its potential toxicity for aquatic organisms, has overall positive effects on

396 living biota by intercepting damaging UVR. Our results give support to this view by
397 showing that natural UVR can be an important mortality factor for yellow perch larvae.
398 By showing the key role of UVA, these results suggest that the common approach of
399 focussing only on markers of UVB might underestimate the effect of UVR on
400 organisms. Since our results were obtained under conditions of natural solar light, this
401 study gives a realistic view of the potential impact of UVR on organisms in natural
402 systems.
403

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503 Table1: Set of candidate logistic models to explain (a) yellow perch larval survival and
504 (b) the quantity of photodamage in raw DNA. Model selection was based on the Aikaike
505 information criterion corrected for small samples (AICc; see text for details). μ =
506 intercept, x1 = light quality, x2 = light intensity, x3 = block, x4 = experiment. Selected
507 models are indicates in bold.

Model			
a) Survival: generalized linear models		AICc	Δ_i
A	Logit(y) = μ + αx1 + βx2 + φx1x2 + cx3 + dx4	901,9	2,2
B	**Logit(y) = μ + αx1 + βx2 + φx1x2 + cx3**	**899,7**	**0,0**
C	Logit(y) = μ + αx1 + βx2 + cx3 + dx4	1015,4	115,7
D	Logit(y) = μ + αx1 + βx2 + cx3	1013,3	113,5
E	Logit(y) = μ + αx1 + cx3	1401,3	501,5
F	Logit(y) = μ + βx2 + cx3	1615,1	715,4
b) Photodamage: linear mixed models			
A	y = μ + αx1 + βx2 + φx1x2 + cx3 + dx4	-35,8	30,7
B	y = μ + αx1 + βx2 + φx1x2 + cx3	-35,5	31,0
C	y = μ + αx1 + βx2 + cx3 + dx4	-55,7	10,8
D	y = μ + αx1 + βx2 + cx3	-55,4	11,1
E	**y = μ + αx1 + cx3**	**-66,5**	**0,0**
F	y = μ + βx2 + cx3	-13,0	53,5

508

509 Table 2: Set of candidate logistic models to explain SOD activity in active and inactive
510 yellow perch larvae. Model selection (in bold) was based on the Akaike information
511 criterion corrected for small samples (AICc; see text for details). μ = intercept, x1 = 313
512 nm, x2 = 340 nm, x3 = block.

Models		Active		Inactive	
SOD activity : linear mixed models		AICc	Δ_i	AICc	Δ_i
A	$y = \mu + \delta x1 + \varepsilon x2 + \varphi x1x2 + cx3$	220,0	8,2	232,5	10,9
B	$y = \mu + \delta x1 + \varepsilon x2 + cx3$	214,8	3,0	225,8	4,2
C	$\mathbf{y = \mu + \delta x1 + cx3}$	**211,8**	**0.0**	**221,6**	**0,0**
D	$y = \mu + \varepsilon x2 + cx3$	216,8	5,0	225,8	4,2

513 Table 3: Cumulative doses received by each treatment for the two experiments, as estimated by continuous irradiance measurements

514 (PUV-2545/2456) and calculated transmittance of the different filters. Light intensity: High =no screen, Medium = one screen and

515 Low = two screens.

			Cumulative dose								
			Whirlpak			Mylar-*d*			Jroll		
λ(nm)	Units	Exp.	High	Medium	Low	High	Medium	Low	High	Medium	Low
313 (UVB)	µW/cm2*nm	1	22,7	13,8	7,6	5,5	2,9	1,5	0,1	0,1	0,0
		2	18,4	11,2	6,1	4,5	2,4	1,2	0,1	0,1	0,0
340 (UVA)	µW/cm2*nm	1	101,6	62,9	34,8	100,1	59,7	29,7	1,7	1,0	0,7
		2	80,2	49,6	27,5	79,0	47,1	23,5	1,4	0,8	0,6

Table 4: Fixed component coefficients (± S.E.) for the selected models used to explain the effect of light quality and intensity on yellow perch larval survival Light intensity: High = no screen, Medium = one screen and Low = two screens. Light quality: J = Jroll, M = Mylar-*d* and W = Whirlpak.

Components	Coefficients	S.E.
Intercept	-1.586	0.18
(visible light) (J)	-1.586	0.18
(UVA + visible light) (M)	0.942	0.141
UVB + UVA + visible light (W)	0.812	0.144
No screen (High)	-1.586	0.18
One screen (Medium)	0.651	0.144
Two Screens (Low)	0.22	0.15
Interaction (M * 1)	0.408	0.189
Interaction (M * 2)	0.299	0.191
Interaction (W * 1)	1.621	0.199
Interaction (W * 2)	1.925	0.206

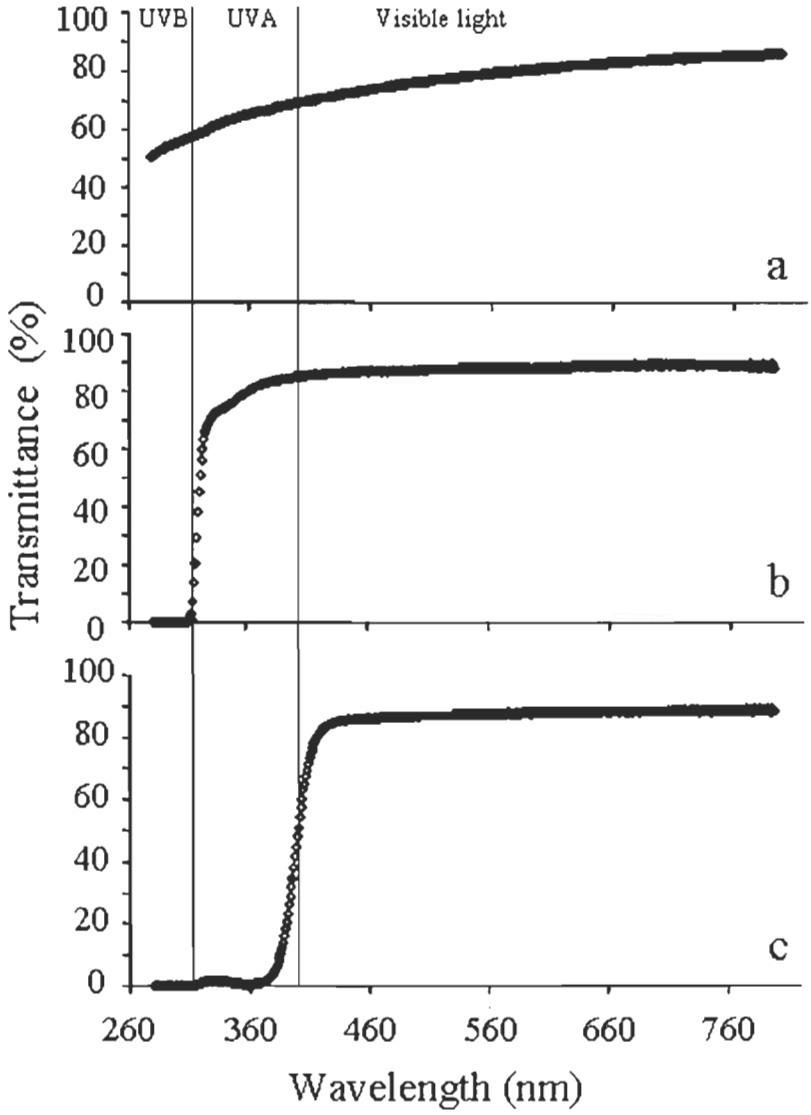
Figure captions

Figure1: Percent transmittance of filters: (a) Whirlpak® (UVB + UVA + visible light), (b) Mylar-*d*® (UVA + visible light), and (c) Jroll® (visible light).

Figure 2: Effects of UVR quality and intensity on yellow perch larval survival. Data are pooled for the two experiments. Whirlpak® (UVB + UVA + visible light), Mylar-*d*® (UVA + visible light), Jroll® (visible light).

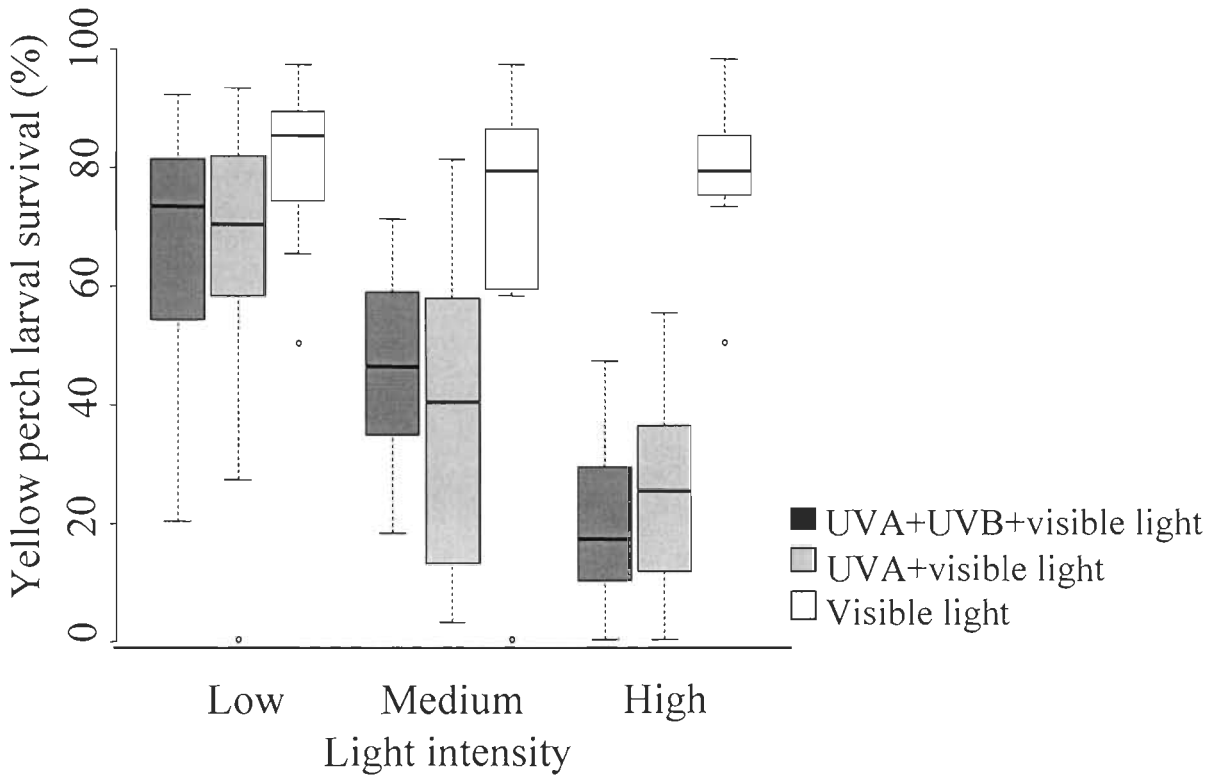
Figure 3: Effects of UVR quality and intensity on the quantity of photodamage (CPD concentration) in UV dosimeters. Data are pooled for the two experiments. Whirlpak® (UVB + UVA + visible light), Mylar-*d*® (UVA + visible light), and Jroll® (visible light).

Figure 4: Effects of UVR quality and intensity on percent SOD activity of (a) inactive and (b) active yellow perch larvae. Data are from the second experiment only. Whirlpak® (UVB + UVA + visible light), Mylar-*d*® (UVA + visible light), Jroll® (visible light).



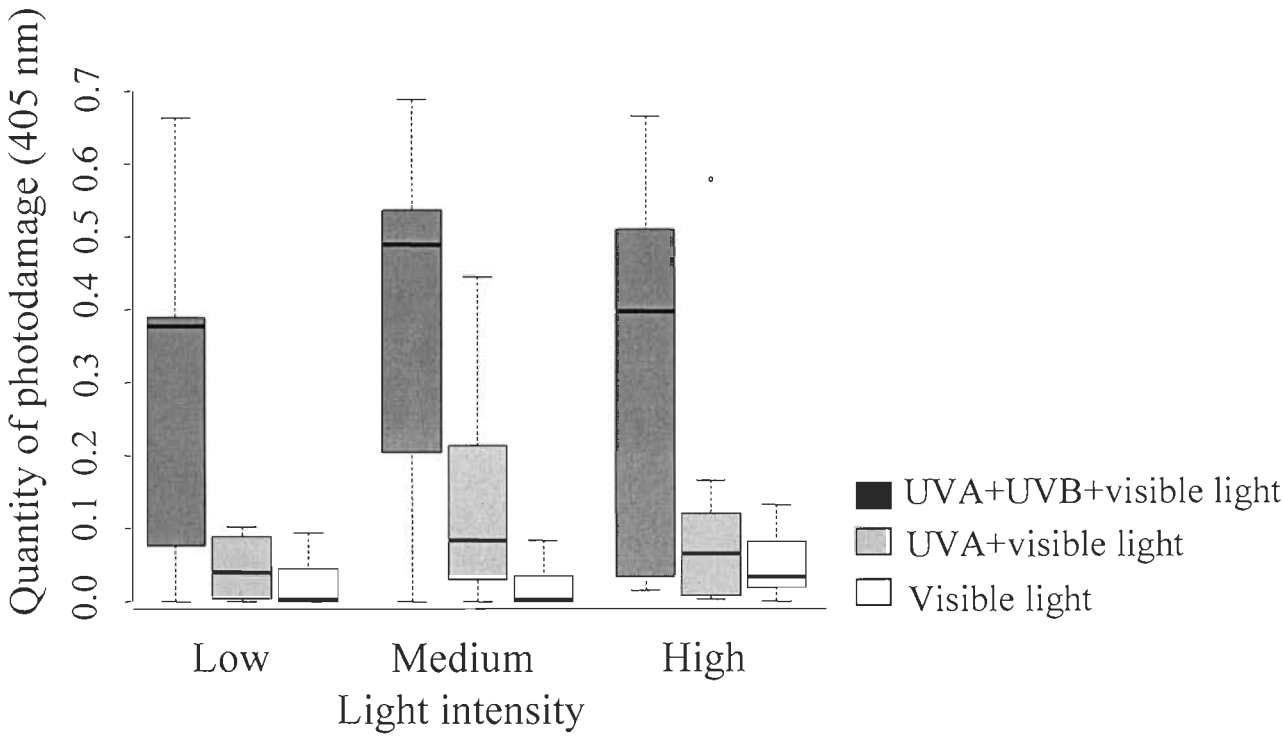
534

535 Figure 1



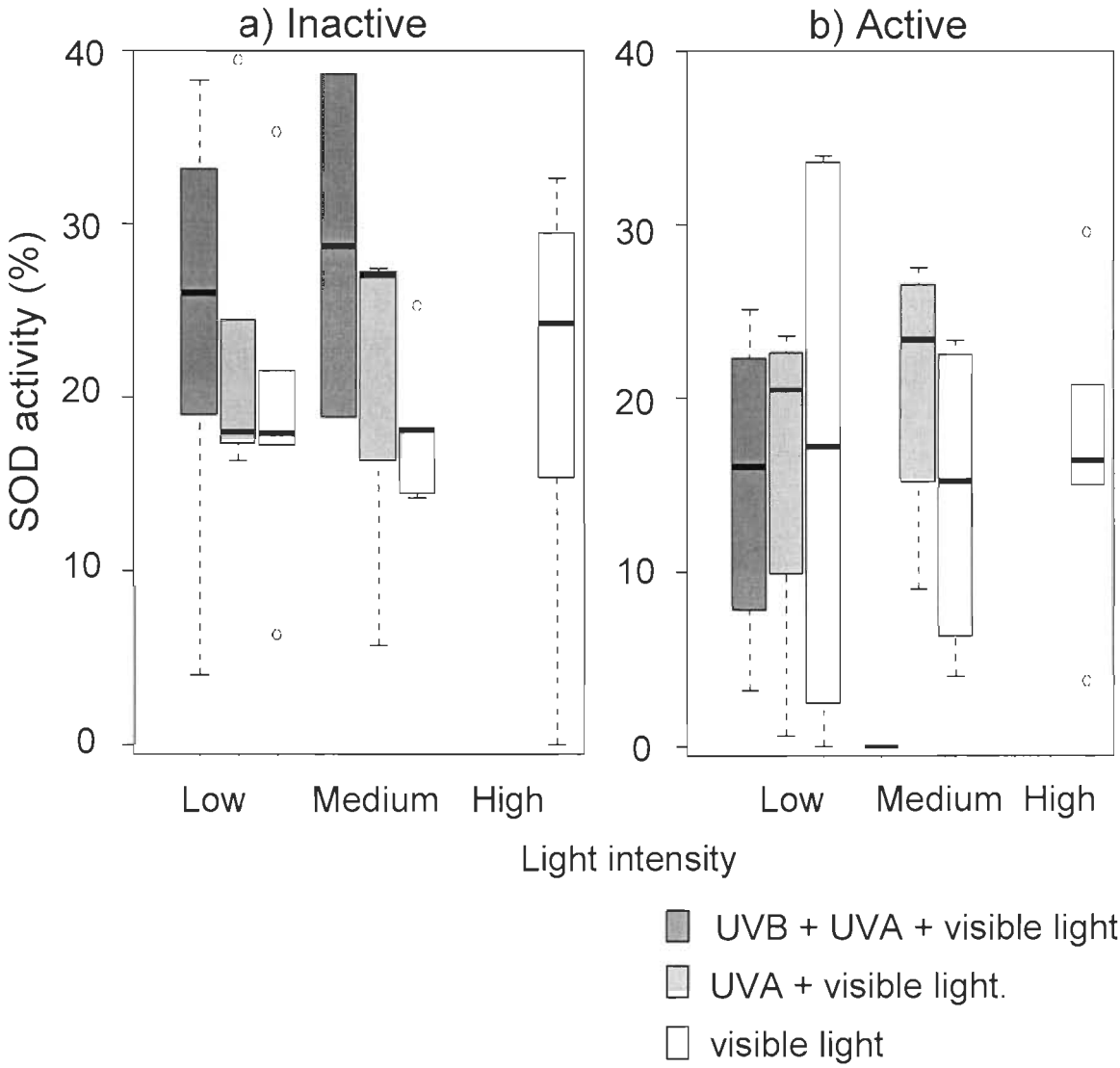
536

537 Figure 2



538

539 Figure 3



540

541 Figure 4

ANNEXE I

Author Instructions

General points

The American Society of Limnology and Oceanography (ASLO) publishes six regular issues of *Limnology and Oceanography* (*L&O*) (ISSN 0024-3590). In addition, Special Issues that deal with a topic that is both timely and of general interest to the ASLO membership are published occasionally. For further information regarding Special Issues, and the requirements for publishing a Special Issue, click here.

L&O (ISSN 0024-3590) publishes original articles, including scholarly reviews, about all aspects of limnology and oceanography. (Click here for a description of the various kinds of papers that L&O publishes.) The journal's unifying theme is the understanding of aquatic systems. Submissions are judged on the originality of their data, interpretations, and ideas, and on the degree to which they can be generalized beyond the particular aquatic system examined. Laboratory and modeling studies must demonstrate relevance to field environments; typically this means that they are bolstered by "real-world" data. Authors are strongly advised to include theoretical contributions in more complete papers that use the new theory to elucidate important features of actual aquatic systems. Papers that focus on methods should be submitted to *L&O*'s sister journal *Limnology and Oceanography: Methods*. If you are unsure about appropriateness for L&O, please contact the Editor-in-chief (lo-editor@aslo.org) before submission.

Submissions to *Reviews in Limnology and Oceanography* should be clearly labeled as such. Originality of data is not required, but originality and generality of interpretations and ideas are of paramount importance. Reviews will not be considered without a statement of why you believe your synthesis to be original and how you expect it to affect interpretation and practice.

Conditions for publication

ASLO holds copyright of any material published in *L&O* or on its website. *L&O* submissions may not contain material published elsewhere (including online publication) or currently under consideration by another journal; see the *L&O* Editorial Comments web page for a discussion of what constitutes dual publication.

Submissions will not be considered unless results are amenable to independent verification. If a manuscript contains data from a biological strain isolated from nature, originating from the author's laboratory, and not available from a public collection, the author must honor in a reasonable time all bona fide requests for samples of the culture or deposit specimens in a public culture collection. Similar expectations apply to results obtained using new antibodies originating from the author's laboratory. Authors of submissions reporting research that includes new nucleotide or amino acid sequences must submit the sequence information to a publicly accessible archive (e.g., GenBank or EMBL) and provide the accession numbers in the part of the manuscript that describes the research methods. Manuscripts that use existing sequences from GenBank/EMBL must cite accession numbers and original literature references to them (if they exist). Publication of an article in *L&O* implicitly binds authors to these conditions.

Authors are responsible for supplying complete bibliographic information—editors do not perform library research. They do edit for brevity and clarity. The Editorial Office is not liable for editorial or printing errors or errors in the technical content of the manuscript.

Communication with the Editorial Office at all points of the publication process is encouraged. Send correspondence to:

Everett Fee, Editor-in-chief
lo-editor@aslo.org

or

Lucille Doucette, Journals Manager
lo-manager@aslo.org

L&O Editorial Office
343 Lady MacDonald Crescent
Canmore, Alberta T1W 1H5
CANADA
office: (403) 609-2456
fax: (403) 609-2400

Authorship

Every person listed as an author must have: 1) contributed substantially to the study's conception, data acquisition, or analysis; 2) contributed substantially to drafting the manuscript; and 3) approved the final submitted manuscript. All three conditions must be met. Acquisition of funding, the collection of data, or general supervision of the research group, by themselves, do not justify authorship.

Publication Charges

Authors are responsible for paying the following publication charges:

Color figures are $500 for one figure, and $50 for each subsequent figure to a maximum of 8 figures. If you have more than 8 color figures contact the Editorial Office for a price.

$50 per typeset page (including web appendices), if either the lead author or the corresponding author is an ASLO member. If neither lead or corresponding author is an ASLO member, the charge is $75 per page.

Tips to successful publication in *L&O*

The most common reasons for manuscript rejection are flawed study design or lack of detail in methods. Rejection is also likely if the writing is unclear, the manuscript is poorly organized, or incomplete. Authors should be aware that *L&O* permits only one major revision of any submission; i.e., if the revision of a paper is still not scientifically acceptable the manuscript will be rejected and resubmission will not be allowed. To prevent reviewers from dwelling on issues of style in the first round of review and overlooking substantive issues that subsequently result in the rejection of the revision, authors are advised to submit only fully polished manuscripts. In rare instances, the editor may invite an author to resubmit a paper for consideration as a new manuscript after, e.g., further research has been done. Such a revision is expected to be so profound that the revision will truly be a new manuscript. Under no circumstances will more than one such resubmission be allowed.

Every year the number of submissions from authors whose first language is not English increases. While we welcome these submissions, a distressing nurnber of them contain serious grammatical errors; such papers are typically given short shrift by our reviewers, which prevents the science that they contain from being fairly assessed. We lack the resources that would be needed to bring such manuscripts up to the *L&ampO* standard for English grammar. If you cannot get a colleague or friend who is fully competent in English to help you we recommend that you make use of a professional scientific copyediting service (e.g., http://www.journalexperts.com/, http://www.teceditor.com). Also, if you are new to the game or get back reviews saying that your paper is disorganized or difficult to understand you should take a bit of time to learn how to write paragraphs in a scientific paper, as described succinctly on the website http://lgmacweb.env.uea.ac.uk/lequere/communication/communication.html.

How to submit a manuscript

Proposals (for *Reviews* only)

Because space is limited and because *Reviews in Limnology and Oceanography* are intended to serve multiple purposes, including education and outreach, we strongly encourage submissions of proposals for reviews. These proposals will be formally reviewed by experts in the field, with the explicit function of providing recommendations for improvement of the eventual review. Proposals should be limited to no more than five double-spaced pages. Each should include the following:

a provisional title, along with a fuller explanation of material to be covered and excluded;

a list of authors and roles, including all institutional affiliations (We particularly encourage mentee-mentor collaborations in which a junior researcher who would find a review most useful engages a senior researcher with recognized perspective on a field.);

a statement indicating why the review is both timely and needed (e.g., the citation for the most recent review on the same or a closely related topic and a summary of the significant advances after its publication);

an explanation of the approach to be taken (e.g., a focus on a new piece of theory or a novel interpretation of past results);

an explanation of the overall novelty of the approach and its likely impact on practice or thought; and,

a description of the companion materials planned for the ASLO Teaching Tools web page (e.g., PowerPoint lectures on the review topic or editable vector graphics files of figures for educational use). Such materials are not required but are strongly encouraged as means to enhance the broader impact of the review.

All submissions

Do not submit a revision of a manuscript that was rejected by *L&O* unless you were specifically invited to do so! Uninvited resubmissions of rejected manuscripts will be turned away without review.

Send all submissions, including revisions, to the *L&O* Editorial Office, i.e., *never* send a manuscript directly to an *L&O* Associate Editor because doing so defeats our tracking system and will delay processing.

Be sure that the cover letter contains the corresponding author's surface and E-mail addresses, and telephone and fax numbers.

Manuscripts must be double-spaced throughout (i.e., including references and figure legends) and must be printed on only one side of each page (i.e., single sided). Table captions must be double-spaced, but not the tables themselves. Start each section of the manuscript on a new page, and put these in the following order: title page, acknowledgments, abstract (omitted if the submission is a Comment), text, references, tables (each on a separate page), figure legends, and figures (each on a separate page). If your submission is a Comment, do **not** attempt to mimic the format of this types of paper in the printed journal.

Hard submissions

Include one copy of the cover letter.

Include one copy of the manuscript.

If a customs declaration is required, declare the contents to be "Educational materials, no commercial value" (otherwise the *L&O* office will have to pay a customs brokerage fee, which will be charged back to the author).

Electronic submissions

We encourage authors to submit new manuscripts and revisions electronically. Acceptable electronic formats are Adobe PDF and MS-Word. The manuscript must be in a *single* file that contains all text, tables, and figures.

To ensure that reviewers and editors can print your manuscript, all fonts must be embedded in PDF files, and PDF files must NOT contain security settings. If you are unsure how to create an acceptable PDF file, submit your manuscript as an MS-Word document and let us make the PDF file for you.

To submit electronically, attach two files (1: the cover letter, which must include the response to reviews if the submission is a revision; and 2: the manuscript) to an e-mail message addressed to the Editor-in-chief. If you have any difficulties or concerns with submitting by e-mail send a short e-mail message (text only) to the Editor-in-chief, who will send instructions for submitting by FTP to the L&O internet server. If you do not receive an acknowledgment of manuscript receipt within four days please enquire about the status of your submission by sending an e-mail message (text only) to the Editor-in-chief.

Do *not* send hard copy unless specifically instructed to do so by the L&O office.

Original submissions

The cover letter must contain the names and complete addresses (including E-mail) of **four** people who the authors believe to be qualified reviewers for the paper. Suggested reviewers must be free of any potential conflict of interest. Any of the following situations may constitute a conflict of interest, so persons with these potential conflicts should be omitted from your list:

someone with whom you or a co-author have had a significant and acrimonious disagreement with at any time in the past;

a co-investigator with either you or a co-author on a current research project;

a co-author with your or with one of your co-authors on the current manuscript in an article published within the past 5 years;

a close friend of yours or of a co-author's;

someone who works at your institution (or that of a co-author); or,

someone who has seen and commented on the manuscript prior to its submission to L&O.

To provide balance and avoid overworking particular reviewers, L&O will probably go outside the list of reviewers you provide. Thus you may wish to make other potential conflicts known to us.

Clearly indicate in the cover letter whether the submission is intended as an Article, Review, or Comment (the Editorial Comments website describes how these manuscript types differ). Include a statement that the manuscript contains only original data (i.e., no data in it are already published or currently submitted for review to another journal), and a statement that publication charges will be paid if the paper is accepted for publication in *L&O*. Finally, briefly identify the novel contribution of this work and how it will affect interpretation and practice in aquatic sciences.

If any data in the manuscript were previously published or are used in another manuscript presently under consideration elsewhere, describe the extent of the overlap in the cover letter and include copies of the relevant papers. Similarly, it will speed review if you include copies of related manuscripts that are in press, submitted to another journal, or that reviewers are likely to have difficulty locating. We prefer to receive copies of all such manuscripts electronically (as PDF files).

Revisions

The cover letter must contain detailed responses to the Reviewers' and Editor's comments. Describe how you modified the manuscript in response to each comment or outline your reasoning carefully if you disagree with the comment.

Final Submissions

Send **one** complete hard copy of the final manuscript, including figures. You must include a cover letter where you state in detail how the manuscript was changed in response to the editor's letter and reviews (if any).

We also need an electronic copy. The preferred format for the text and references is Microsoft Word. (PDF files are not accepted at this stage.) Tables can be embedded in the file as MS-Word tables, but not as pictures (e.g., .pic, .gif, or .tif formats). If you cannot insert them as Word tables, then send all tables in one MS-Excel file. Send the figures in one PDF file created using the "Press Quality" Acrobat setting unless otherwise instructed.

The cover letter and any extra material (e.g., web appendices) should be in separate files.

The *L&O* Style

As you prepare your paper, refer to a recent issue of *L&O* for examples of the journal's style. The ASLO Journals Manager (lo-manager@aslo.org) will be happy to answer any questions that you cannot resolve in this way or by referring to the detailed *L&O* style specifications below.

The order of the different parts of a submission should be:

Title page

Acknowledgments page

Abstract page

Text

References

Tables

Figure Legends

Figures

Web Appendices

General style:

Use a 12-point font (Times Roman preferred), double-spaced on one side of non-glossy A4 or "letter" (8-1/2x11 inch; 21.6x28 cm) paper throughout the manuscript. Use 1-inch (2.5-cm) margins on all sides.

Number all pages, starting with 1 on the title page. If the software used to prepare the manuscript can do so, number all lines of text (making it easier for reviewers to comment on the manuscript).

Do not justify (i.e., align text) on the right-hand margin.

Do not break (hyphenate) words over lines.

Indent the first line of each paragraph. Do not put a blank line between paragraphs.

The only allowable footnotes are for author addresses on the title page or when they are unavoidable in tables.

L&O does not publish printed appendices. We do, however, publish electronic appendices on the L&O website. Such appendices may contain materials that cannot be printed in L&O (e.g., videos) or tables that would take up too much space in the printed journal. The reviewers and editor must agree that this material is essential to understanding the associated L&O paper; i.e., L&O Web Appendices are not intended to be used to archive raw data. Submit material intended for publication on the *L&O* website as separate electronic files and refer to the material as "see the Web Appendix". The first reference to each such appendix must include the URL; e.g., see Web Appendix: www.aslo.org/lo/toc/vol_xx/issue_x/xxxxa1.pdf

Do not number or letter sections of the manuscript.

Use an italic font for lower case Greek letters; but use a regular font (i.e., not italic) for upper case Greek letters.

Thoroughly proofread and spell-check the manuscript with a computer program.

Use a single serifed font (Times New Roman preferred); if special mathematical or Greek symbols not available in that font are needed, use the Symbol font. Note: superscripts, subscripts, italic, boldface, underline, and changes of font size are not considered to be different fonts.

Cite all figures and tables in the text and number them in the order that they appear in the text.

Do not use punctuation (commas or periods) in numbered equations.

Cite literature in the text in chronological, followed by alphabetical, order and formatted like these examples: "Campbell (1983, 1987*b*)," "(Smith et al. 1984; Karl and Craven 1988; Korobi 1997, 1998)." In the *References* section, list citations in alphabetical, followed by chronological, order.

Manuscripts must be written in English. Before submission, the manuscript should be proofread by a person fluent in English.

Order the manuscript as: title page, acknowledgments page, abstract page (not required for a Comment), manuscript body, references, tables, figure legends, and figures. All papers should be formatted in this way, i.e., do NOT place author names at the end of the manuscript, which is how Comments are formatted in the journal.

Use only SI units (metric and Celsius; for detailed SI specifications, click here). The following are required formats for situations that are commonly formatted incorrectly:

Use exponents to indicate multiplication or division in units (slashes are not allowed).

Use mol L^{-1} for molar concentrations ('M' is not acceptable).

Use mol quanta for photosynthetically available radiation (PAR) (Einsteins is not acceptable).

Use × for multiplication (* is not acceptable).

To indicate a power of 10, write, e.g., 5×10^{-8} (5E-8 is not acceptable).

Do not italicize common Latin terms and abbreviations such as i.e., e.g., in situ, in vivo, and et al.

The Title page:

Capitalize only the first word, proper nouns, and acronyms in the title. I.e., *Do not* capitalize all words nor use all capitals for the entire title.

Do not use abbreviations in the title (e.g., use 'iron', not 'Fe'; and 'southeast', not 'SE').

List the names of all authors in a single continuous character string below the title. Use footnotes to indicate the corresponding author (if different than the first author listed) and author addresses; these addresses should be those where the authors resided *at the time that the work presented in the paper was done* (use separate footnotes for current addresses, if different). Spell out state or province names in full. Double-space all footnotes on the title page.

For Articles, provide a condensed *running head* of no more than 40 characters (including spaces) at the bottom of the page.

The Acknowledgments page:

Include brief statements about granting agencies, important aid received from institutions, and any potential conflicts of interest (as detailed in the *L&O* Ethics statement section 3.4 and 3.4.1).

Thank anyone who made a substantial contribution to the work (e.g., data collection, analysis, or writing or editing assistance) but who did not fulfill the authorship criteria, along with their specific contributions.

You are responsible for ensuring that all persons named in the Acknowledgments section know and agree to being identified there (since it may be interpreted as endorsement of the data or conclusions).

The Abstract:

A single paragraph of no more than 250 words (15 to 17 lines of text in a 12-point, Times New Roman font, where the line width is 17 cm [=6.5 in]). State what you did and what you found; omit 'introductory' statements that summarize previous work and avoid statements that do not identify actual findings (e.g.,

"The implications of these results are investigated with a dynamic model.") Summarize rather than advertise important findings and their significance. (In the jargon of scientific writing, L&O abstracts must be informative rather than indicative.

See http://www.southernct.edu/~brownm/inform_ab.html for further explanation of these terms.) Because the abstract must stand on its own, it cannot include references. Comments have no abstracts.

Text:

Follow all directions given in the General style section above. Articles must be structured in the conventional format (Introduction => Methods => Results => Discussion). There should be no "Conclusions" section at the end of the Discussion; this type of summary information belongs in the Abstract.

Describe statistical methods in enough detail to enable a knowledgeable reader with access to the original data to verify the reported results. Give degrees of freedom for *F*-tests as subscripts (e.g., $F_{3,4}$); for other statistics, report degrees of freedom as "df=*n*" following the test result (e.g., *t*=3.4, df=20). Use italics for symbols representing a statistic: *p* for probability level, *n* for the sample size, *r* for the correlation coefficient, R^2 to denote the coefficient of determination. (r^2 = R^2 only for a linear regression.)

Use the same font for the same mathematical symbol regardless of where it appears in the manuscript (text, displayed equations, tables, figures, or figure legends).

Use periods after all abbreviations except for metric measures, compass directions, and time (s, min, h, d, yr; do not abbreviate 'week' or 'month'). Use hh:mm h or hh:mm:ss h for time of day. Do not use a.m. or p.m. E.g., 09:30 h, 18:24:44 h.

Provide the full expansion of all acronyms on first use (even common ones like DNA).

Format dates like "15 June 1999" throughout the text, figures, and tables. If it is necessary to conserve space, abbreviate month names to the first 3 letters of the month name (no period) and the year to the last two digits.

Do not abbreviate names of states, provinces, or cities. Abbreviate names of countries only after defining on first use, e.g., United Kingdom (U.K.), United States of America (U.S.A.)

References:

The ratio of pages of references to pages of text must be less than 1:4.unpubl. (See the editorial commentary Web page for reasons.) For Reviews only, the ratio of references to text may be relaxed at the discretion of the editor. Nevertheless, Reviews should limit citations to prior reviews and key papers published since the last review or omitted from prior reviews. Exhaustive bibliographies (annotated or not) may be useful and can be submitted to the ASLO Teaching Tools web page.

All references cited in the text must appear in the *References*, and vice versa.

No more than 3 references can be cited to support any statement. (See the editorial commentary Web page for reasons.)

Double check the spelling of author names and years of publication. All author names must be given--even if there are more than eight (the copyeditor will abbreviate the list to 'and others' if appropriate).

Manuscripts in preparation, submitted, unpublished theses, or other inaccessible sources should be cited in text by giving the author(s) initial(s), last name(s), and

'pers. comm.' or 'unpubl.' For example, (A. B. Jones unpubl.) Such materials must **NOT** appear in the *References*.

References to manuscripts that are available via an online discussion forum (e.g., the EGU *Discussions* journals) must be refered to as 'pers. comm.' These manuscripts must **NOT** appear in the *References*.

Verify all references against original sources; check especially journal titles, accents, diacritical marks, and spelling in languages other than English.

Make sure that each citation is complete, according to the following examples:

Article:

Fenchel, T. 1986. Protozoan filter feeding. Prog. Protistol. **1:** 65-113.

Articles with a Digital Object Identifier (DOI):

Many older papers that were originally published with page numbers have been retroactively assigned DOI's while some newer electronic journals assign article identifiers instead of page numbers (HTML being the primary form of publication). Thus, a paper with a DOI may contain page numbers, an article identifier, or both, and at least one of these is needed to complete the reference.

De Pol-Holz, R., O. Ulloa, L. Dezileau, J. Kaiser, F. Lamy, and D. Hebbeln. 2006. Melting of the patagonian ice sheet and deglacial perturbations of the nitrogen cycle in the eastern South Pacific. Geophys. Res. Lett. **33**: L04704, doi:10.1029/2005GL024477

If there are page numbers, the last part would be **33:** 15-32, doi:10.1029/2005GL024477

If there are both page numbers and an article identifier, the last part would be **33:** 15-32, L04704, doi:10.1029/2005GL024477

Check that every DOI that you cite is correct via the doi system website. Note that references with a doi *do not* have a period at the end; this facilitates electronic lookup (doi's terminated with a period fail when sent to doi resolver websites).

Book:

Stumm, W., and J. Morgan. 1981. Aquatic chemistry, 2nd ed. Wiley.

Chapter:

Codispoti, L. A. 1983. Nitrogen in upwelling systems, p. 513-564. *In* E. J. Carpenter and D. G. Capone [eds.], Nitrogen in the marine environment. Academic.

Thesis:

Kimmance, S. A. 2001. The interactive effect of temperature and food concentration on plankton grazing and growth rates. Ph.D. thesis. Univ. of Liverpool.

Papers which are unconditionally accepted for publication but for which exact publication data are not yet available should be formatted according to the above examples but with the phrase "In press" appearing instead of the year of publication.

Use mixed case (upper and lower case OR caps and small caps) for all text in the *References* section. In particular, do not use all capital letters for author

names because doing so makes it impossible to for the copyeditor to properly typeset names like "MacKenzie".

For abbreviations of journal names refer to Chemical Abstracts Service Source Index (CASSI) or Biosis.

Do not include part (issue) numbers after volume numbers unless each part of the volume is paginated separately.

Websites. A websites may be referred to only if it is sponsored by an organization that is committed to maintaining it in perpetuity. Personal or university-based websites are *not* allowed in L&O because such websites are prone to disappear when the scientist who created them moves or loses interest in material. Websites are referred to only in the text and are not included in the list of references.

Tables:

Start each table on a new page.

Format tables so that they will fit on the printed page: A 1-column table can be up to 60 characters wide, and a 2-column table up to 130.

Type table legends as double-spaced paragraphs at the top of each table.

Figure Legends:

Group figure legends together on the page(s) preceding the figures; one paragraph per figure.

Explain all panels in each figure (A), (B), ...

Symbols used in the figure (e.g., circles, squares, ...) must be explained on the figure itself (i.e., not in the figure legend). No special symbols are allowed in the figure legend.

Figures:

Do not put figure legends on the figures. Put only "Fig. #." on the figure.

Number figures with Arabic numerals in the order of their citation in the text. If panels of a figure are labeled (A, B, ...) use the same case when referring to these panels in the text (A, B, ..., not a, b,...).

If a figure consists of multiple panels, put all panels on one page and repeat axes titles on each panel only if they are different.

Put scale bars on the figure, NOT in the figure legend.

Use the Times New Roman font for all text and numerals on figures. Font sizes size should be from 9 to 11 points. If mathematical or Greek symbols are not available in Times New Roman, use the Symbol font.

Page layout: See page layout diagram.

Submit figures at the intended print size. The *L&O* column width is 8.9 cm (3.5 in) and full page width is 18.4 cm (7.25 in). The maximum size for a figure is 18.4 x 23.2 cm (7.25 x 9.125 in).

Make figures as simple as possible. For example, avoid grid lines and boxes around symbol definitions.

Maps must include latitude and longitude, an indication of compass direction, and a thin line as a border. All markings must be legible.

Color figures:

See detailed instructions.

Web Appendices:

L&ampO has never published appendices in the journal itself. But in 2003 (Volume 45, Issue 1) we began publishing electronic appendices. These are reviewed as rigorously as the paper they support and are copyrighted by ASLO. Their purpose is to make available material that cannot be printed (e.g., videos) or essential data such as a table that would take up too much space in the journal. The reviewers and the Associate Editor must agree that the material in a Web Appendix is essential to understanding the associated L&O paper; i.e., Web Appendices are not intended to be a way to archive raw data or to make an *L&ampO* article shorter. Since there are many people who still read the printed journal, we try to minimize the need to go the Web to obtain essential information. We therefore have a strict policy of not allowing Web Appendices to be used for materials such as simple graphs or short tables that could be printed in the journal.

ANNEXE II

Solutions tampon utilisées pour le protocole ÉLISA

- Tampon PBSN (Phosphate buffer saline Na 0.1M. pH 7.4)
  11.5g Na_2HPO_4 anhydre
  2.28g NaH_2PO_4 anhydre
  9g NaCl
  Ajuster le volume à 1000ml

- Tampon PBSNT
  PBSN
  0.1% de Tween 20

- Tampon sodium-carbonate 0.1M. pH 4-5)
  1.475g acide citrique anhydre
  1.37g de Na_2HPO_4 anhydre
  Ajuster le volume à 250ml