Journal of Environmental Biology
©Triveni Enterprises, Lucknow (India)
Free paper downloaded from: www.jeb.co.in

31 251-254 (**May** 2010)
For personal use only
Commercial distribution of this copy is illegal

Radiation induced effects on viability and antioxidant enzymes of crustaceans from different habitats

D. Mukherjee[1], M. Manna[2], S. Selvaraj[3], S. Bhattacharya[4], S. Homechoudhury[4] and A. Chakraborty*[3]

[1]Department of Environmental Science, University of Calcutta, Kolkata - 700 019, India
[2]Department of Zoology, Bethune College, Kolkata - 700 006, India
[3]UGC- DAE Consortium for Scientific Research, Calcutta Center, Kolkata - 700 098, India
[4]Department of Zoology, University of Calcutta, Kolkata - 700 019, India

(Received: May 06, 2008; Revised received: March 13, 2009; Re-revised received: July 25, 2009; Accepted: August 07, 2009)

Abstract: *The paper describes differential tolerance of two fresh water crustaceans Mesocyclops hyalinus and Allodiaptomus satanus to ^{60}Co gamma radiation. Mesocyclops hyalinus is dominant species at site 1, near a Thermal Power Plant at Kolaghat East Midnapore where fly ash deposition is a regular phenomenon. Allodiaptomus satanus is dominant species at site 2 at Kolkata, Ballygung where anthroponotic activities are more pronounced. M. hyalinus is naturally exposed to more stressful situation than A. satanus as revealed by comparing the hydrological parameters of the two habitats. Experimental exposure to ionizing radiation resulted in differential changes in viability, morphology and antioxidant enzyme activities in the two selected species. Survival experiments showed greater tolerance of M. hyalinus compared to A. satanus up to 8Gy (absorbed dose) after which it showed drastic fall in survival. More pronounced morphological changes were observed in A. satanus as compared to that in M. hyalinus. The pattern of changes in antioxidant enzyme activity is distinctly opposite in the two radiation exposed species. While in M. hyalinus stimulation in activity of both CAT (excepting at 10Gy absorbed dose) and SOD was observed A. satanus showed decrease in activity of both the enzymes when compared to their unirradiated counterparts.*

Key words: *Radiation, Zooplanktons, Oxidative stress, Viability, Antioxidant enzymes*
PDF of full length paper is available online

Introduction

Aquatic ecosystem, a key component of the earth's biosphere (Hader *et al.*, 2003) is exposed to different sources of radioactivity in environment including naturally occurring radionuclides, fallout from the atmosphere *etc*. Depending upon the element and the chemical form, radionuclides may accumulate in the bottom sediment or remain in the water column in the dissolved state (Blaylock *et al.*, 1993). From either location, they can subsequently accumulate in biota and be transferred through the aquatic food chain. Zooplankton, being a very important link in aquatic food web (Banse, 1995; Padmanabha and Belagali, 2008) including humans, thus deserves considerable attention to analyze potential risk of radiation exposure. Some reports from earlier workers have documented significant effect on viability of different zooplankton exposed to UV radiation and changes in oxidative stress parameters, (Borgeraas and Hessen, 2000) but little is known regarding the effect of gamma radiation on the freshwater zooplankton. Resistances towards solar UV radiation have been observed in some copepods due to the presence of protective Mycosporine like amino acids (MAAs) (Helbling *et al.*, 2002), while in some amphipods tolerance response towards natural radiation is mediated via enzymatic antioxidant defense (Obermuller *et al.*, 2005). As zooplankton is sensitive and reactive to external perturbations (Harris *et al.*, 2000) and is consequently an indicator of environmental change, the present work has been designed to focus on to study the change in morphology if any, of the gamma exposed zooplankton and change in their antioxidant enzyme profiles.

* **Corresponding author:** ani@alpha.iuc.res.in

Materials and Methods

Sampling sites and collection of specimens: Fresh samples of copepod *M. hyalinus* and *A. satanus* were collected from Kolaghat, site 1, near Kolaghat Thermal Power Plant, 22°272 N, 87°502 E and Kolkata, site 2 at Ballygung, 22°312 N, 88°212 E respectively, during the winter of 2005. The physical distances between two sites are almost sixty kilometers.

Zooplankton samples were collected with plankton net to determine the Shannon-Weaver diversity index (Shannon, 1948), of two water bodies. For this purpose samples were preserved in 4% formaldehyde and plankton enumeration was done by drop count method following the standardized protocol (APHA, 1995). Different physicochemical parameters of water including transparency by Secchi disk method (Secchi disk diameter: 20 cm) and chlorophyll-a content (Arnon, 1949) of water from the two selected sites were analyzed as outlined by APHA (1992) following the methods described by Brower *et al*. (1997).

Irradiation: The zooplanktons were exposed to a ^{60}Co gamma source for irradiation at UGC-DAE Consortium for Scientific Research, Kolkata, India. Zooplankton species (10 individuals) were taken in the micro centrifuge tube containing 0.5 ml distilled water. The range of absorbed doses used for irradiation of the plankton was 2-10Gy (14Gy min^{-1}). The dose rate was measured with Fricke Dosimeter (Spinks and Woods, 1976).

Mortality determinations: The viability /survival of the exposed zooplankton was analysed immediately after irradiation following the method of Goncalves *et al.* (2002). This method estimates mortality as the relationship between the number of dead and initial number of organisms. Viability of zooplanktons were determined immediately after irradiation under a dissecting microscope. Morphological changes were observed and photographed using Fluorescence microscope (Leica DC 300FX, Wetzler, Germany). All experiments were repeated five times and control (unirradiated) was run in parallel.

Enzyme assay: After sonicating the planktons (using B –Braun Melsungen AG model) in Tris-HCL buffer they were subjected to centrifugation at 15000 rpm (using cold centrifuge machine, floor Model SORVALL RC5B plus) for 30 minutes at 4°C to get cytosolic enzyme in the supernatant. The extract was kept at -20°C till use.

Catalase activity in cytosolic extracts was measured according to the method of Aebi (1984). Briefly, 1ml reaction mixture contained 50 mM KH_2PO_4/Na_2HPO_4 buffer, pH 7.00 and 30 mM H_2O_2. Catalase activity was calculated from the turnover time of hydrogen peroxide (H_2O_2) resulting in an absorbance decrease at 240 nm. Assay temperature was 20°C. The initial absorbance was approximately A= 0.500.

Superoxide dismutase (SOD) activity was measured by the method described by Paoletti *et al*. (1986). 1 unit of enzyme activity inhibits the rate of superoxide anion mediated NADH oxidation by 50% is determined by linear regression of a standard curve of rate of NADH oxidation (% of control) versus the concentration of pure SOD (ng). Total protein has been estimated following the standard protocol mentioned by Lowry *et al.* (1951). All chemicals used for the experiments were procured from Sigma St. Louis, USA.

Statistical analysis: Students't-test was performed for statistical analysis of the data obtained. Level of significance considered at $p \leq 0.05$.

Results and Discussion

Results of our present investigation clearly show distinct difference in the zooplankton diversity index of the two sample sites (0.645 at site 1 and 0.662 at site 2). Clear difference has also been observed in the species dominance of the two sites considered. While *M. hyalinus* was noted to be dominant species at site 1, *A. satanus* was observed to be dominant at site 2. Data from the survival experiments reflect dose dependent mortality of both*, M. hyalinus* and *A. satanus* (Fig. 1). Though *A. satanus* reflect greater sensitivity as compared to that of *M. hyalinus*, at an absorbed dose of 8Gy *M. hyalinus* showed drastic fall in survival in comparison to *A. satanus.*

The dose-dependant increases in mortality of the two selected planktonic species exposed to gamma irradiation indicate that these

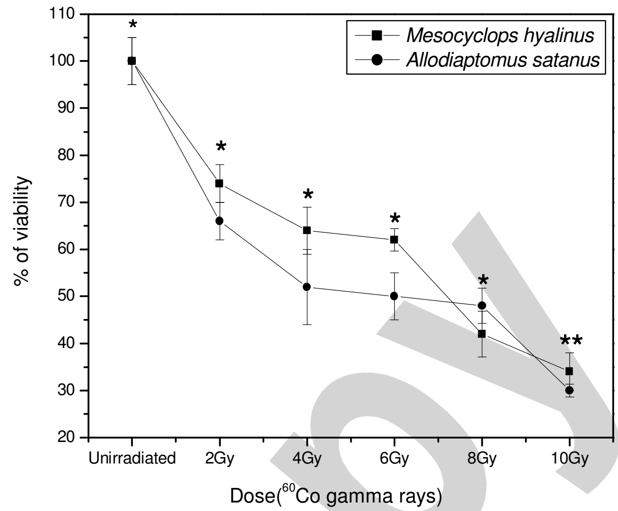

Fig. 1: Viability of two zooplanktons (*Allodiaptomus satanus* and *Mesocyclops hyalinus*) against different doses of ^{60}Co gamma irradiation (*p<0.0001; **p<0.001)

species from freshwater bodies of the chosen sites at Kolaghat and Kolkata are sensitive to ionizing radiation. However, in contrast to *Boeckella titicacae* which show resistance to high levels of radiation (Helbing *et al*., 2002) or *B. gracilipes* that has been documented to be sensitive even under relatively low radiation doses (Zagarese *et al*., 1997), both the selected species in our present investigation can be considered as moderately sensitive towards gamma exposure as recorded from their mortality in the chosen range of dose (Fig.1). Difference in the frequency of survival between the two types of radiation-exposed planktons could be attributed to either species–specific radio-sensitivity or due to influence of differential habitat of the two species. Similar habitat-dependent differential response in viability of radiation-exposed planktonic species have been reported by host of earlier workers who documented differences in mortality of species under different environment ranging from polar to tropical to temperate (Zagarese *et al*., 1998; Newman *et al*., 1999; Rocco *et al*., 2002).

Table 1 shows different water parameters of the two sites selected for the present study. While $(NO_3)^-$ and Ca^{++} content, hardness and dissolved oxygen of site 2 are higher when compared to that of site 1, the latter is very rich with phosphate content due to fly ash deposition from the nearby thermal plant. Water transparencies analyzed from the two sites show correlation with the chlorophyll-a content of the water. Though the present study has not been designed to evaluate diversity or vulnerability of the planktonic species as a function of seasonal variation, some earlier groups have shown correlation of water quality index on population diversity and seasonal fluctuation in ostracods (Padmanabha and Belagalli, 2008).

Radiation induced morphological alterations were noted to be more pronounced in *A. satanus* when compared to that in *M. hyalinus*. Increase in absorbed dose showed remarkable changes in antennae and appendages of *A. satanus*. At an absorbed dose of 10Gy disintegration of the antennae and degradation of cephalothoracic region were noted in *A. satanus* (Fig. 2c). However

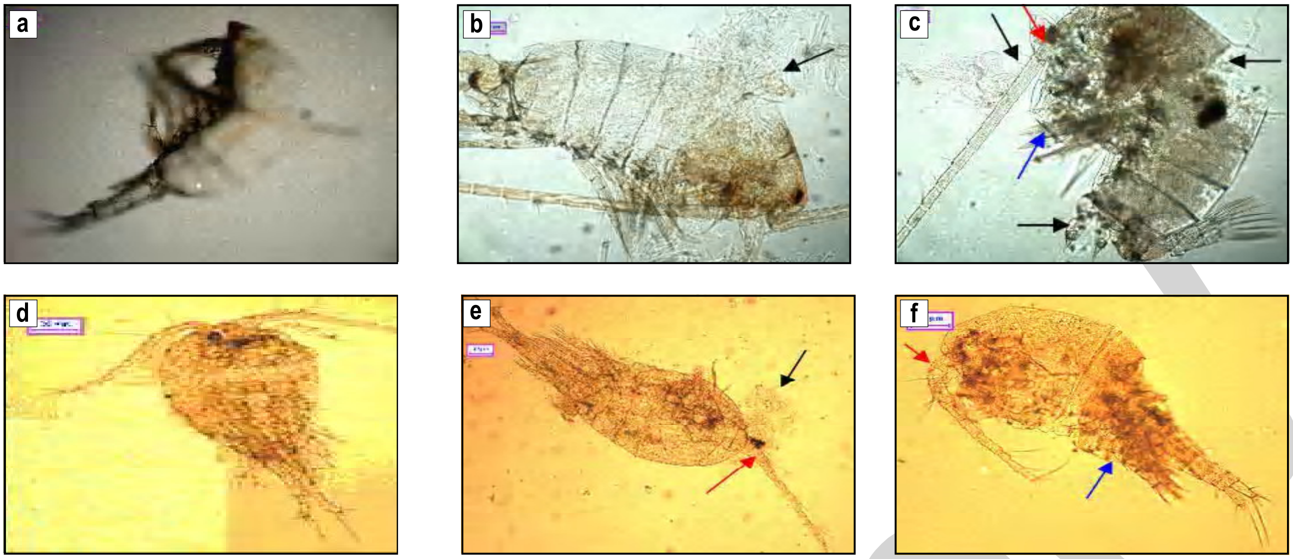

Fig. 2: 2a-2c, 2d-2f shows morphological alteration of two zooplanktons (*Allodiaptomus satanus* and *Mesocyclops hyalinus*) respectively, both in unirradiated and irradiated conditions. Arrow indicate damaged antenna (red arrow), rupture of the body surface and liberation of body exodus (black arrow), loss of appendages (blue arrow) (2a: Unirradiated, 2b: 6Gy, 2c: 10Gy, 2d: Unirradiated, 2e: 6Gy; 2f: 10Gy)

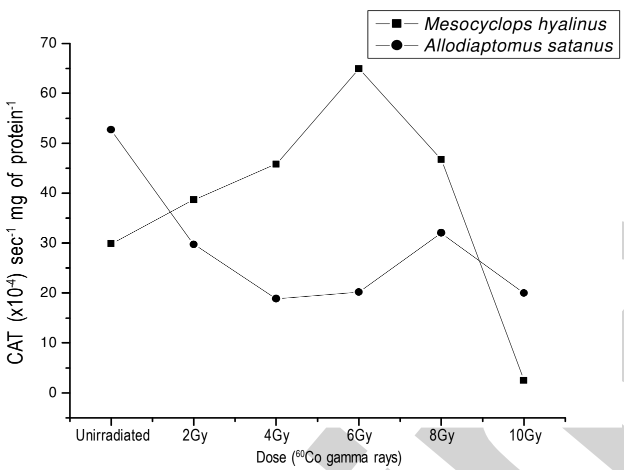

Fig. 3: Specific activity of catalase against different doses of ^{60}Co gamma irradiation for two zooplankton species (*Allodiaptomus satanus* and *Mesocyclops hyalinus*)

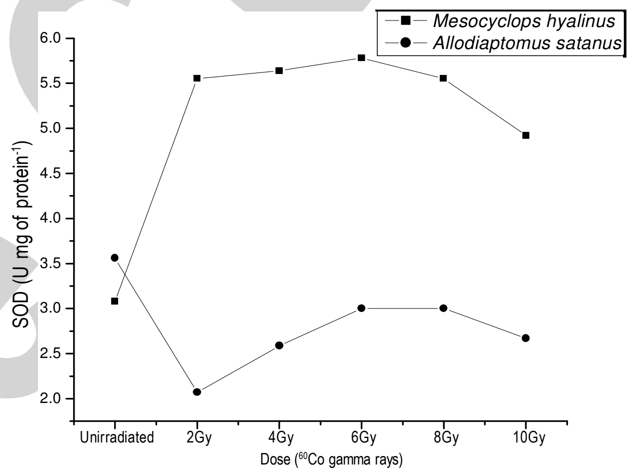

Fig. 4: Specific activity of superoxide dismutase against different doses of ^{60}Co gamma irradiation for two zooplankton species (*Allodiaptomus satanus* and *Mesocyclops hyalinus*)

Table - 1: Physico-chemical characteristic of water from two different sites

Sampling sites	Temperature (°C)	Transparency (cm)	Chlorophyll-a (mg g^{-1} tissue)	Nitrate $(NO_3)^-$(mg l^{-1})	Phosphate $(PO_4)^{3-}$(mg l^{-1})	Calcium (Ca^{++})(mg l^{-1})	Hardness ppm	Dissolved oxygen (mg l^{-1})
Site 1 (Kolaghat)	26 ± 2	75 ± 1.8	1.4 ± 0.29	6.5 ± 0.54	3 ± 0.06	36.07 ± 3.7	200 ± 14	4.32 ± 0.5
Site 2 (Kolkata)	26 ± 2	90 ± 1.5	2.14 ± 0.07	17.5 ± 0.97	0.12 ± .008	54.10 ± 4.2	300 ± 17	6.081 ± 0.7

Data represents mean ± S.E. of n = 5

at this dose *M. hyalinus* exhibited disintegration of posterior portion of the body (Fig. 2f).

The observed difference in water parameters between the two sites (Table 1) may also be responsible for variation in response towards stress as has been proposed by Hader *et al.* (2003) that greater proportion of organic constituents in the ambient water reduces natural radiation. Thus the observed water parameters Kolkata site (site 2) possibly made *A. satanus,* the dominant species of this site more vulnerable when exposed to further stress by gamma irradiation. This further advocates the earlier documentation of Goncalves *et al.* (2002) who proposed that response of organisms depends not only on the conditions they exposed to, but also combination of factors namely, the type and effectiveness of the strategy they employ to cope with the changed environment.

Results of the present investigation reveal significant changes in activities of the antioxidant enzymes in both the planktonic species. Gamma irradiation induced gradual increase in catalase activity in *M. hyalinus* (Fig. 3). At an exposure of 6Gy maximum induction in CAT (about 1.17 fold) of *M. hyalinus* was noted while absorbed dose of 10Gy resulted in drastic fall in activity (91.56%). On the contrary, pattern of change in CAT activity was totally different in case of gamma irradiated *A. satanus* (Fig. 3). This species when irradiated at 4Gy experienced 64.21% decrease in CAT when compared to that of the unirradiated control group. This was followed by an increase in CAT at 6 -8Gy (with respect to the 4Gy Group), however at 10Gy CAT again was dropped down by 62.08% from that of the control.

The trend of change in superoxide dismutase activity is also observed to be opposite in the two planktonic species. While a stimulation of SOD activity was observed in *M. hyalinus* when irradiated at an absorbed dose of 2Gy, the same dose induced a decrease in SOD activity in *A. satanus.* Irradiation at higher doses resulted in a gradual fall of SOD activity in *M. hyalinus* in comparison to the 2Gy-irradiated group, however in case of *A. satanus* an increase in such activity was observed (Fig. 4).

Differential response of the two plankton species in enzyme activity might be mediated via specific metabolic pathway as per the requirement of the concerned species. This possibly reflects presence of some distinct systems responsible for combating the generated superoxide and hydrogen peroxide as seen in other planktonic species (Daekyung *et al*., 2007). This finds support from the work of Irato *et al.* (2007) who reported species-specific antioxidant responses (activities of SOD, CAT and Se-GPX) of the two bivalve species towards variation in their habitat conditions. Specific response of antioxidant systems have been even documented at interspecific and intraspecific levels in different groups of crustacea (Borgeraas and Hessen, 2002; Obermuller *et al.,* 2005).

Acknowledgments

Authors would like to thank Chemical Science Division, Saha Institute of Nuclear Physics, Kolkata, India for providing the use of ^{60}Co source for gamma irradiation and Indian Institute of Chemical Biology, Kolkata India for providing some instrumental facilities needed for the work. Fund support for the work obtained from UGC-DAE consortium for Scientific Research, Kolkata Centre, India is also thankfully acknowledged.

References

Aebi, H.: Catalase *in vitro*. *In*: Methods in Enzymology. Oxygen Radicals in Biological systems (*Ed.*: L. Packer). Academic Press, Orlando, FL., 105, 121-126 (1984).

APHA: Standard Methods for the Examination of Water and Wastewater. (*Eds*: A.B. Eaton, L.S. Clescreri, E.W. Rice and A.E. Greenberg). Washington DC (1995).

APHA: Standard Methods for the Examination of Water and Wastewater. 18th Edn.. Washington DC (1992).

Arnon, D.I.: Copper enzyme in the isolated chloroplast, polyphenol oxidase in *Beta vulgaris*. *Plant Physiol*., **24**, 1-15 (1949).

Banse, K.: Zooplankton: pivotal role in the control of ocean production. *ICES J. Marine Sci.,* **52**, 265-277 (1995).

Blaylock, B.G., M.L. Frank and B.R.O' Neal: Methodology for estimating radiation dose rates to fresh water biota exposed to radionuclides in the environment. Report prepared for the U.S. Department of Energy. Office of Environmental Management (1993).

Borgeraas, J. and O.D. Hessen: UV-B induced mortality and antioxidant enzyme activities in Daphnia magna at different oxygen concentrations and temperature. *J. Plankton Res.*, **22**, 1167-1183(2000).

Borgeraas, J. and O.D. Hessen: Variations of antioxidant enzymes in Daphnia species and populations as related to ambient UV exposure. *Hydrobiologia*, **477**, 15-30 (2002).

Brower, J.E., J.H. Zar and C.N.V. Ende: Analysis of aquatic habitat. *In*: Field and laboratory methods for General Ecology (4th Edn.). Chapter 2d, WCB Mc Graw-Hill (1997).

Daekyung, K., N.Takuji, M.Yukihiko, N. Yoshimi, Y. Kenichi and O.Tatsuya: Presence of the distinct systems responsible for superoxide anion and hydrogen peroxide generation in red tide phytoplankton *Chattonella marina* and *Chattonella ovata. J. Plankton Res.,* doi: 10.1093/plankt/fbm011 (**2007).**

Goncalves, R.J., V.E. Villafañe and E.W. Helbling: Photorepair activity and protective compounds in two freshwater zooplankton species (*Daphnia menucoensis* and *Metacyclops mendocinus*) from Patagonia, Argentina. *Photochem. Photobiol. Sci*., **1**, 996-1000 (2002).

Hader, Donat-Peter, H.D. Kumar, R.C. Smith, C. Ray and Robert C. Worrest: Aquatic ecosystems: Effects of solar ultraviolet radiation and interactions with other climatic change factors. *Photochem. Photobiol. Sci.*, **2**, 39-50 (2003).

Harris, R.P., P.H. Wiebe, J. Lenz, H.R. Skjoldal and M. Huntley: ICES Zooplankton Methodology Manual. Academic Press, San Diego, CA. p. 648 (2000).

Helbling, E.W., F. Zaratti, L.O. Sala, E.R. Palenque, C.F. Menchi and V.E. Villafane: Mycosporine like amino acids protect the copepod *Boeckella titicacae* against high levels of solar UVR. *J. Plankton Res.*, **24**, 225-234 (2002).

Irato, Paola, Ester Piccinni, Arnaldo Cassini and Gianfranco Santovito: Antioxidant responses to variations in dissolved oxygen of *Scapharca inaequivalvis* and *Tapes philippinarum*, two bivalve species from the lagoon of Venice. *Marine Pollut. Bull.*, **54**, 1020-1030 (2007).

Lowry, O.H., N.J. Rosebrough, A.L. Farr and RJ. Randall: Protein measurement with the Folin-phenol reagents. *J. Biol. Chem.*, **193**, 265-275 (1951).

Newman, S.J., S. Nicol, D. Ritz and H.J. Marchant: Susceptibility of Antarctic krill (*Euphausia superba* Dana) to ultraviolet radiation. *Polar Biol.*, **22**, 50-55 (1999).

Obermüller, B., U. Karsten and D. Abele: Response of oxidative stress parameters and sun screening compounds in Arctic Amphipods during experimental exposure to maximal natural UVB radiation. *J. Exp. Mar. Biol. Ecol.,* **323**, 100-117 (2005).

Padmanabha, B. and S.L. Belagali: Ostracods as indicators of pollution in the lakes of Mysore. *J. Environ. Biol*., **29**, 711-714 (2008).

Paoletti, F., D. Aldinucci, A. Mocali and A. Caparrini: A sensitive spectrophotometric method for the determination of superoxide dismutase activity in tissue extracts. *Anal. Biochem.*, **154**, 536-541 (1986).

Rocco, V.E., O. Oppezzo, R. Pizarro, R. Sommaruga, M. Ferraro and H.E. Zagarese: Ultraviolet damage and counteracting mechanisms in the freshwater copepod *Boeckella poppei* from the Antarctic Peninsula. *Limnol. Oceanogr.*, **47**, 829-836 (2002).

Shannon, C.E.: A mathematical theory of communication. *Bell System Tech. J.*, **27**, 379-423, 623-656 (1948).

Spinks, J.W.T. and R.J. Woods: An Introduction to radiation chemistry, 2nd Edn., Wiley, New York (1976).

Zagarese, H.E., M. Feldman and C.E. Williamson: UV-B induced damage and photoreactivation in three species of *Boeckella* (Copepoda, Calanioda). *J. Plankton Res.*, **19**, 357-367 (1997).

Zagarese, H.E., B. Tartarotti, W.R. Cravero and P. Gonzalez: UV damage in shallow lakes: The implications of water mixing. *J. Plankton Res.*, **20**, 1423-1433 (1998).