

EFFECTS OF UVC RADIATION ON SURVIVAL OF ARTEMIA CYSTS AND NAUPLII (CRUSTACEA, BRANCHIOPODA)

Marija Crnčević^{1*}, Melita Peharda², Jakša Bolotin³, Slaven Dobrović⁴, Adam Benović³, Ana Bratoš¹, Valter Kožul³, Nikša Glavić³, Pero Tutman², Josip Lovrić¹, Nikola Ružinski⁴

¹ Collegium Ragusinum, Dubrovnik, Croatia - * marijca1@netscape.net

² Institute of Oceanography and Fisheries, Split, Croatia

³ Institute of Oceanography and Fisheries, Dubrovnik, Croatia

⁴ Faculty of Mechanical Engineering and Naval Architecture, University of Zagreb, Croatia

Abstract

The objective of this research was to examine the potential of UVC radiation (254 nm) to kill organisms in ballast water, using brine shrimp *Artemia* nauplii and their resistant resting cysts as model organisms. Brine shrimp nauplii could be killed using UV radiation, while cysts were found to be more resistant.

Key words: Artemia, ballast water, UV radiation

Introduction

The movement of ship's ballast water is currently the most important source of marine species transfers throughout the world, posing a serious threat to biodiversity, economy and human health (1, 2, 3). Land-locked Adriatic Sea can be influenced by such introductions (4). Authorities of some Croatian ports are looking into possibilities of expanding their current activities and there are indications that a vast quantities of ballast waters will be discharged in the North Adriatic Sea in a very near future.

The most effective solution for reducing transfer of alien species is treatment of ballast water (5). Ultraviolet radiation (UV) is an environmentally benign approach (6) that produces no significant residual toxicants. It is already present at many re-circulating water aquaculture operations as a means of improving water quality (7). This study was designed to evaluate the potential of UV to kill cysts and nauplii of brine shrimp *Artemia*.

Material and methods

The experimental assembly, consisted of batch type UV disinfection units with centrally placed source of UV light, protected from water with quartz tube. The light source of every unit was single low-pressure mercury lamp at 254 nm. UV intensity was kept constant (40 $\mu\text{W}/\text{cm}^2$) and the UV dose was calculated as the product of intensity ($\mu\text{W}/\text{cm}^2$) and exposure time (s). Absorbance at 254 nm was $A=0.06$ and was measured in spectrophotometer HP 8453 using 1 cm quartz cuvette. Exposure columns were made of 50 cm tall glass cylinders (8.8 cm inner diameter). In the experiment, simultaneously run in triplicate, nauplii (6 ind/ cm^3) were exposed to different UV dose levels: 2.4, 7.2, 12, and 19.2 mWsec/ cm^2 . Control nauplii received air, at the same flow rate and exposure times, as the treatment nauplii. Survival rates were estimated from manual counts of nauplii in six replicate 1 cm^3 samples, collected from each exposure column 0, 60, 120 and 1440 min after UV exposure, to look at long-term survival.

In experiments with cysts, *Artemia* cysts (2g/ dm^3) were placed in each of two 2 dm^3 exposure columns (one treatment and one control). Cysts were exposed to different UV dosages: 24, 72, 144, 432, 720, and 1008 mWsec/ cm^2 . After exposure, cysts were cultured at 27°C and 15 psu for 24 h. Cyst survival rates were estimated from manual counts of hatched nauplii in 10 replicate 1 cm^3 samples collected from each exposure column.

Difference in survival among different treatments was tested using Kruskal Wallis nonparametric analysis of variance.

Results and discussion

All experiments were performed at 23.5 - 24°C, 37.4 psu, and pH 8.15 - 8.18. Cyst viability ranged between 72.02% for UV dose 24 mWs/ cm^2 and 11.78% for UV dose 1008 mWs/ cm^2 . Statistically significant differences were found for survival of cysts exposed to different doses of UV radiation ($p < 0.001$, $n = 60$).

During preliminary experiments, 100 % brine shrimp nauplii mortality was achieved by 24 mWs/ cm^2 UV dose. Survival of nauplii after UV exposure decreased significantly with increasing time after exposure. The mean percent survival for an exposure time of 1min (UV dose 2.4 mWs/ cm^2) was 73.3%; immediately after exposure; while after 24 hours of exposure it was only 21.1% (Table 1). There

was a statistically significant difference ($p < 0.001$) in the long-term survival of nauplii exposed to different UV dose levels.

Significant differences ($p < 0.001$) in survival of nauplii were found among treatments for different exposure time immediately after treatment, 1 hour after treatment and 2 hours after treatment. There was no significant difference in survival among treatments for different exposure times 24 hours after ($p = 0.157$). The effectiveness of UV in inactivating *Artemia* depends on the life stage of the organism. It has been shown that UV has a potential to kill nauplii while cysts of *Artemia* are much more resistant.

This research could be applied in future investigation of methods for inactivation of different target organisms and their resting stages in the ship's ballast water. Investigation of UV treatment in real ballast tank would be necessary because there may be mechanisms that can change the possibility of UV species inactivation.

Table 1. Long - term survival * (mean \pm SE) of brine shrimp nauplii exposed to UV radiation

UV dose mWsec/ cm^2	Time after UV exposure (min)			
	0	60	120	1440
2.4	73.3 \pm 6.63	67.5 \pm 7.10	73.3 \pm 12.48	21.1 \pm 6.44
7.2	64.2 \pm 7.36	82.5 \pm 12.44	90.8 \pm 17.09	6.8 \pm 3.03
12	53.3 \pm 7.60	5.0 \pm 2.10	5.0 \pm 1.72	9.0 \pm 4.52
19.2	2.5 \pm 1.36	3.3 \pm 1.94	0.8 \pm 0.83	4.5 \pm 2.86

* Percent survival = mean of 100 X percent survival of each replicate/mean of treatment control percent survival

References

- 1 - Carlton J.T., 1985. Transoceanic and interoceanic dispersal of coastal marine organisms: the biology of ballast water. *Oceanogr. Mar. Biol. Ann. Rev.*, 23: 313-371.
- 2 - Golani D., Orsi-Relini L., Massuti E., and Quignard J.-P., 2002. CIESM Atlas of Exotic species in the Mediterranean. Vol.1. Fishes. (F. Briand, ed.). CIESM Publ., Monaco, 256 p.
- 3 - Hallegraeff G.M., Bolch C.J., Bryan J. and Koerbin B., 1990. Microalgal spores in ships' ballast water: a danger to aquaculture. Pp. 475-480. In: Graneli E. et al. (ed.), Toxic Marine Phytoplankton. Elsevier, New York.
- 4 - Benović A., Lovrić J. and Ružinski N. 1995. Ballast waters: problems and perspectives. Proceedings of the VII Congress of the International Maritime Association of Mediterranean, pp. 440-446.
- 5 - Oemcke D., 1999. The treatment of ships' ballast water. Ecoports Monograph Series No. 18 (Ports Corporation of Queensland, Brisbane), 102 p.
- 6 - Sutherland T.F., Levings C.D., Elliott C.C. and Hesse W.W., 2001. Effect of a ballast water treatment system on survivorship of natural populations of marine plankton. *Mar. Ecol. Prog. Ser.*, 210: 139-148.
- 7 - Lilved H., Hektoen H., and Efraimsen H., 1995. Inactivation of bacterial and viral fish pathogens by ozonation or UV irradiation in water of different salinity. *Aquacult. Eng.*, 14: 107-122.