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The Effect of UV Irradiation on *Perkinsus marinus* and its Potential Use to Reduce Transmission Via Shellfish Effluents

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Introduction. Interstate transport of infected oysters for market, hatchery production, research, or even personal consumption can serve as a vector to spread oyster parasites and exacerbate local disease pressure. The protozoan oyster pathogen *Perkinsus marinus* has been indentified in oysters from every state between Maine and Texas (Ford and Tripp 1996). This parasite causes Perkinsiosis or Dermo disease in oysters, which can lead to extensive oyster mortality (Andrews 1988). Given the ease, frequency and economic demand for interstate transport of oysters, protocols that eliminate or minimize *P. marinus* in effluents from shellfish operations should be employed whenever possible.

Bushek et al. (1997) investigated several methods to kill *P. marinus*. Results indicated that chlorination, a common disinfection method, works but requires that 300 ppm or more sodium hypoclorite (bleach) be added to overcome chlorine demands in marine and estuarine waters. This level of chlorination is not practical at the scale needed to treat effluents and may lead to other environmental problems if the chlorine is not properly neutralized. Desiccation, heat, and osmotic shock also effectively killed *P. marinus*, but, like chlorination, these methods are impractical for treating large volumes of effluent.

Ultraviolet irradiation (UV) provides a practical and potentially economically viable alternative. UV irradiation has been shown to kill a variety of microorganisms including viruses, bacteria, protozoa, dinoflagellates, yeasts and molds (SAIC 1996). Moreover, the technology to treat large volumes of water in a flow-through system is readily available from a variety of companies (e.g., Emperor Aquatics, Pottstown, PA). In fact, UV irradiation is widely used by the aquaculture industry to kill microbes as they enter a facility or to depurate shellfish of bacterial populations. Although virtually every organism tested has been shown to be susceptible to UV irradiation, no studies have examined the amount of UV light needed to kill *P. marinus*. This document summarizes some recent studies designed to determine the levels of UV irradiation required to kill *P. marinus*.

The principle behind UV sterilization is based upon its effect on DNA. Ultraviolet light of approximately 254 nm causes photochemical fusion of adjacent pyrimidines (i.e., thymine and cytocine) into pyrimidine dimers (Watson et al. 1987, SAIC 1996 – attached excerpt). This creates a lesion in the DNA that prevents replication. A cell that cannot replicate its DNA cannot reproduce. Natural UV irradiation from the sun leads to the formation of pyrimidine dimers that can accumulate and kill living organisms.

Skin cancer is one potential outcome in humans, for example. The accumulation of these dimers can be prevented by a process called photoreactivation in which visible light catalyses a reaction between the dimer and the enzyme photolyase to reverse dimer formation (Watson et al. 1987). Under high amounts of UV irradiation, however, photoreactivation is overwhelmed and the cell(s) will become sterile and die.

Methods and Materials. An ultraviolet exposure apparatus and an IL1400A light meter with an SEL240/TD detector was provided for this study by Emperor Aquatics (2229 Sanatoga St. Rd., Pottstown, PA 19464). The detector was fitted with filters and calibrated to measure UV light at 254 nm. This is within the range of wavelengths commonly referred to as UVA. The exposure system mimicked those used in many aquaculture systems to treat incoming seawater. It consisted of a UV bulb enclosed in a quartz sleeve within a three inch pvc pipe that was sealed on both ends. The center section was fitted with a 3 x 2 inch Tee to provide an exposure portal for experiments. The two inch opening effectively functioned as a colliminating tube beneath which in vitro cultured *P. marinus* (isolate ATCC 50764) were exposed to UV irradiation.

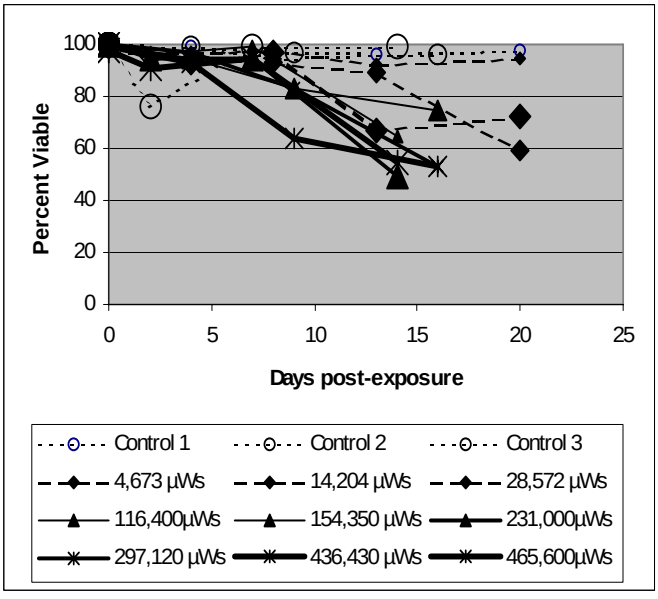

Figure 1. Percent viability of in vitro cultured *P. marinus* following exposure to various dosages of UV irradiation while in culture medium.

The parasites were enumerated with a hemacytometer and percent viability determined based on the uptake of the vital stain neutral red (0.04% w/v solution in FSW) prior to each UV exposure trial. A sample of 200 µl was then diluted in 8 ml of fresh culture medium (see recipe in Bushek et al. 2000) or 1 µm filtered seawater (FSW) in a 60 mm sterile petri dish and exposed to UV irradiation. UV dosage was adjusted by controlling exposure time. Twenty measurements of UV irradiation intensity ($\mu W/cm^2/s$) were made immediately before and after each exposure and the average used to provide an estimate of exposure intensity. This number was then multiplied by the number of seconds of exposure time to determine the UV dosage. Zero second exposure controls were run for each trial. Immediately following exposure, and continuing for a period of two to three weeks, percent viability and the number of viable cells was determined as described above. Both measures were tracked through time to determine whether or not surviving cells could proliferate.

Results. To determine if UV light could destroy in vitro cultured *P. marinus* cells approximately 3.01 x 10^5 cells were exposed to 522 $\mu W/cm^2$ for one hour. The resulting dosage of 1,879,200 $\mu Ws/cm^2$ destroyed virtually all of the parasites. Only 4 x 10^4 cells remained intact and of those only 25% were potentially viable. This trial clearly indicated that UV light can destroy cultured *P. marinus*.

When parasites were still in culture medium, viability declined over time at UV irradiation doses greater than 14,000 µWs (Figure 1). At lower dosages viability remained greater than 90%, similar to controls. After 2 weeks, only controls began to proliferate (Figure 2). Cells exposed to UV did not proliferate and in many cases began to die within two weeks following exposure.

To determine if organic compounds and other chemicals in the culture medium inhibited the effect of UV irradiation, a second set of exposure trials was conducted. Exposure times were identical, but minor differences in wattage emitted from the UV lamp resulted in slight differences in dosage. In addition, bacterial contamination in the controls and the 145,680 µWs dosage precluded following those

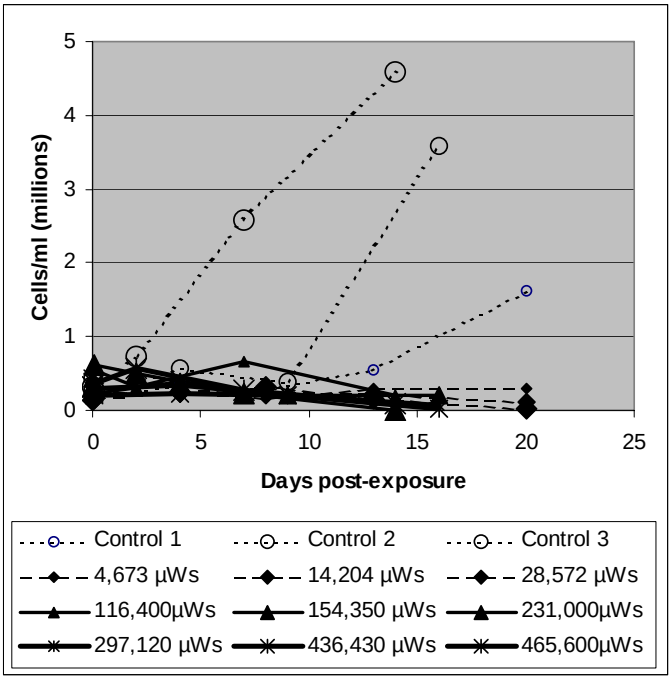

Figure 2. Proliferation of in vitro *cultured P. marinus* in culture medium following exposure to various dosages of UV irradiation while in culture medium.

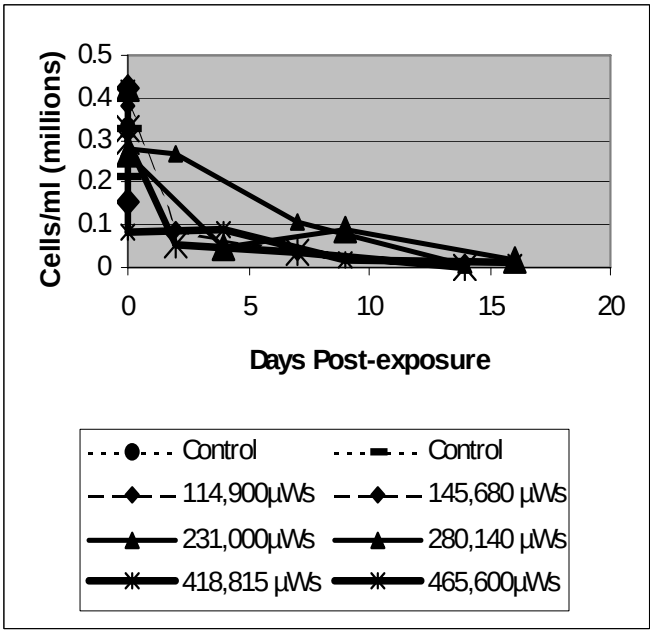

Figure 4. Proliferation of in vitro *cultured P. marinus* in culture medium following exposure to various dosages of UV irradiation while in FSW. Note that survival of controls was confounded by bacterial contamination which was killed in UV exposure treatments.

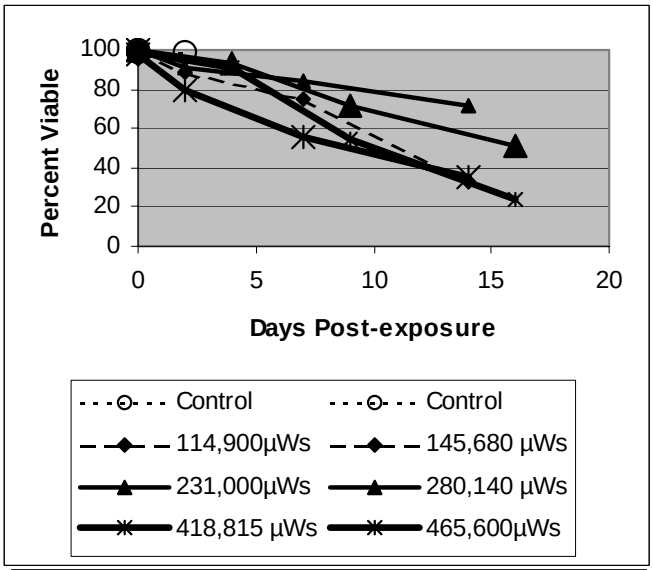

Figure 3. Percent viability of in vitro cultured *P. marinus* following exposure to various dosages of UV irradiation while in FSW. Note that survival of controls was confounded by bacterial contamination which was killed in UV exposure treatments

treatments through time. Nonetheless, results indicate that the UV irradiation was more effective in FSW than in culture medium (compare Figure 3 to Figure 1 and Figure 4 to Figure 2). As in the culture media exposures, viability declined with time, but the decline was steeper in the filtered seawater (Figure 3). Similarly, proliferation not only stopped, but the number of cells clearly declined with time (Figure 4), indicating that cell mortality was occurring. Interestingly, bacteria was only present in the controls and one of the lower dosage exposures even though the same source of filtered seawater was used. This indicates that the UV irradiation was also killing bacteria that were present in the filtered water. In all UV treatments, cell numbers declined with time, but did so most rapidly at the higher dosages and in FSW.

Discussion and Conclusions. In an attempt to prevent or minimize Dermo disease in local oyster populations, many states have created regulations that virtually ban importation of oysters from outside state waters. For example, Chapter 24 of the Maine Department of Marine Resources indicates that shellfish from specified growing areas outside of Maine will be presumed to carry infectious disease and therefore prohibits importation of shellfish from these areas without evidence that the shellfish are disease free. The interpretation and enforcement of this and similar laws and regulations threatens depuration, wet storage and other facilities from processing shellfish from outside the state. In the absence of methodologies to treat potentially infected oysters or potentially contaminated effluents, such strict regulations may be a reasonable means to protect local populations. The impact on the local

industry, however, can be stifling, particularly when local shellfish production declines. Furthermore, distant shellfish producers are impacted by the loss of a market for their shellfish product. UV irradiation of effluents may alleviate the problem by providing a mechanism to prevent the transmission of *P. marinus* (and other parasites and pathogens) into local waters via shellfish processing operations

Based on the data presented, low to moderate doses of UV irradiation (4,000 to 14,000 μWs/cm^2) will inhibit proliferation of *P. marinus*. Higher doses (> 28,000 μWs/cm^2) will lead to parasite mortality. The higher the dosage, the more effective the sterilization. *Perkinsus marinus* kills oysters via massive proliferation within the host that leads to sloughing of tissues and embolisms (Mackin 1951). Hence, even a treatment that only prevents the parasite from proliferating will serve as an effective treatment to prevent the spread of Dermo disease.

Many aquaculture equipment suppliers currently stock and sell UV sterilizer units that operate at 30,000 μWs/cm^2. These and other units can be easily configured to operate at much higher dosages. Engineering these systems to treat effluents from processing plants, depuration facilities, hatcheries, laboratories, etc., could serve as a practical and economical means to prevent or at least minimize the transmission of *P. marinus* to local oyster populations from infected oysters that are held in these facilities.

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References

Andrews, J. D. 1988. Epizootiology of the disease caused by the pathogen *Perkinsus marinus* and its effects on the oyster industry, pp 47-63. Fisher (ed.), Disease Processes in Marine Bivalve Molluscs. Amer. Fish. Soc. Spec. Publ., 18. Bethesda, MD.

Bushek, D., R. Holley, and M. Kelly. 1997. Treatment of *Perkinsus marinus*-contaminated materials. Nat'l Shellfisheries Assoc. Ann. Mtg., Fort Walton Beach, FL. Apr. 20-24, 1997. *J. Shellfish Res.* 16(1):330.

Bushek, D., R. A. Holley and K. S. Reece. 2000 Use of Micromanipulation and "Feeder" Cultures to Clone the Protozoan Oyster Pathogen *Perkinsus marinus. J. Eukaryotic Microbiol*., 47(2):164-166

Ford, S.E., and M.R. Tripp. 1996. Diseases and defense mechanisms. Pages 581-660, in A.F. Eble, V.S. Kennedy, R.I.E. Newell, eds. The eastern oyster, *Crassostrea virginica*. Maryland Sea Grant College Park, MD.

Mackin, J.G. 1951. Histopathology of infection of *Crassostrea virginica* (Gmelin) by *Dermocystidium marinum* (Mackin, Owen, and Collier). Bull. Mar. Sci. Gulf Carib., 1:72-87

Science Applications International Corporation (SAIC). 1996. Utraviolet light disinfection technology in drinking water application – an overview. U.S. Environmental Protection Agency, Office of Groundwater and Drinking Water, 401 M Street SW, Washington DC 20460. EPA 811-R-96-002. 208 pp.

Watson, J.D., N.H. Hopkins, J. W. Roberts, J. Argetsinger Steitz, A.M. Weiner. 1987. Molecular Biology of the Gene. 4th ed.

Benjamin/Cummings Publishing Co., Inc.
Menlo Park, CA. pp. 345-346.