Aquaculture Medicine

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Control of epibionts with chemical disinfectants in the phyllosoma larvae of the spiny lobster, *Panulirus homarus* (Linnaeus)

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1. Introduction

Phyllosoma larvae of spiny lobsters pass through several stages, each involving one or more moults, before metamorphosing to puerulus, the lobster post-larva. The protracted larval life, extending even up to one year, and diverse feed requirements make rearing of phyllosoma difficult compared to other decapods. Few temperate and subtropical species of spiny lobsters have been reared to puerulii in the hatchery (Kitaakka, 1997), but the maximum success achieved in rearing a tropical spiny lobster was up to the sixth stage in *Panulirus homarus* (Radhakrishnan and Vijayakumaran, 1995; Vijayakumaran and Radhakrishnan, 1986). Artemia nauplii were used as feed to rear *P. homarus* phyllosoma, even though other feeds like *Sagitta* spp. and chopped mussel and clam meat were also found to be suitable for rearing the larvae. Heavy infestation by micro-epibionts, especially when chopped meat is fed, restricted the movement of the larvae and resulted in mortality. Many microorganisms like, diatoms, filamentous bacteria and stalked ciliates were found attached to the surface of the phyllosoma and the most damaging one was the stalked ciliate, *Zoothamnium* spp. *Zoothamnium* sp. multiplies rapidly when the organic load in the rearing water increases, especially when chopped meat is fed. This paper describes the use of chemical disinfectants in controlling *Zoothamnium* sp. infestation during rearing of phyllosoma larvae of *P. homarus*.

2. Materials and methods

Four disinfectants, malachite green, light green, potassium permanganate and formalin were tried to control *Zoothamnium* sp. infestaion in phyllosoma lobster of the spiny lobster *P. homarus*. Since the stalked ciliates were found to be highly resistant to light green in preliminary trials, this chemical was not tested further. Freshly hatched, healthy phyllosoma larvae of *P. homarus* were exposed to different concentrations - 1, 5, 10, 20, 30, 50, 70 and 100 ppm of malachite green, potassium permanganate and formalin to assess the toxicity of these chemicals to phyllosoma. Fifteen larvae in 200 ml of filtered seawater in glass containers were exposed to the different concentrations of chemicals. Triplicate trials were maintained for each treatment. The activity of the larvae was continuously monitored and was recorded at 30 minutes interval till 4 hours. The ability of the larvae to recover fully when released back to fresh seawater was also recorded at different intervals.

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Zoothamnium sp. attached to the phyllosoma larvae were then exposed to the same concentrations of chemicals. The experimental design was the same as described for the phyllosoma but the observation time was limited to 60 minutes and the activity was recorded at 5 minutes interval. The behaviour of the ciliate to exposure to chemicals and its ability to recover when released back to fresh seawater were also studied.

3. Results and discussion

The phyllosoma larvae were very active up to 30 minutes in the maximum concentration of all the three chemicals tried (Table 1). Larvae were active even after 4 hours at 1 ppm concentration of malachite green, while it became lethargic after 90 minutes at 5 ppm and after 60 minutes at 10 ppm. At 100 ppm concentration all larvae died within 60 minutes. In potassium permanganate, 10 ppm concentration did not affect the activity of phyllosoma even after 4 hours, but the larvae became inactive after 2 hours and died in 4 hours at concentrations 20 and 30 ppm. At 50 and 70 ppm the larvae were active up to 60 minutes and more than 50 per cent died after 90 minutes. At maximum concentration of 100 ppm, 50 per cent of the larvae died after 60 minutes exposure. Formalin treated larvae were active even after 4 hours of exposure at 25 ppm. When the inactive larvae were released back to fresh seawater, the larvae could regain full activity within 30 minutes. In 50 ppm, larvae were active after 2 hours but gradually settled at the bottom and died after 3 hours. In 100 ppm, larvae were active upto 60 minutes and thereafter gradually become inactive.

The concentration of 100 ppm formalin was not effective in killing *Zoothamnium* sp. even after 30 minutes of exposure. The cup-like structure of the ciliates were found detached from the stalk after 3 hours when exposed to 50 ppm and in 25 ppm, all the ciliates died after 4 hours of exposure. Exposure for 45 minutes at 10 and 20 ppm

Table 1. Effect of chemical disinfectants on phyllosoma larvae of the spiny lobster, *Panulirus homarus*

Disinfectant	Concentration (ppm)	Activity of phyllosoma [a]		
Malachite green	1 to 100	100 ppm	:	All active up to 30 minutes
		10-70 ppm	:	All inactive after 60 minutes
		5 ppm	:	All inactive after 90 minutes
		1 ppm	:	All active up to 4 hours
Potassium permanganate	1 to 100	100 ppm	:	All active up to 30 minutes
		50-70 ppm	:	50% died after 90 minutes
		20-30 ppm	:	All inactive after 2 hours
		1-10 ppm	:	All active up to 4 hours
Formalin	1 to 100	100 ppm	:	All active upto 60 minutes
		50 ppm	:	All active upto 120 minutes
		25 ppm	:	All active beyond 4 hours

[a] Active - Normal swimming activity
Inactive - Settling at the bottom of the container

Table 2. Effect of chemical disinfectants on *Zoothamnium* sp. attached to phyllosoma larvae

Disinfectant	Concentration (ppm)	Activity of *Zoothamnium* sp.
Malachite green	1 to 100	100 ppm : All died instantly 10-70 pm : All died with in 10 minutes 10 ppm : All died after 10 minutes
Potassium permanganate	1 to 100	100 ppm : All died in 5 minutes 70 ppm : All died in 15 minutes 10-50 ppm : All died after 45 minutes
Formalin	1 to 100	100 ppm : All died in 90 minutes 50 ppm : All died after 3 hours 25 ppm : Died after 4 hours

and 15 minutes of exposure at 70 ppm of potassium permanganate killed all the ciliates, while at 100 ppm 5 minutes exposure was enough to kill the ciliate. Malachite green was the most effective disinfectant to control *Zoothamnium* sp. At the highest concentration tried (100 ppm), all ciliates were killed instantly, while at 10 ppm, the ciliates died with in 10 minutes of exposure.

When *Zoothamnium* sp. is exposed to the chemicals, it temporarily withdraws its cilia and assumes a round shape. It remains in this position for few minutes, depending on the concentration of chemicals, and die if the exposure is prolonged. The ciliate can regain its activity within minutes if released back to fresh seawater after initial exposure. It is extremely important to ensure that the concentration of chemicals and exposure time are enough to kill the ciliates while attempting to eradicate them by short-term dip/bath treatment.

Malachite green has been used as an effective hatchery disinfectant in controlling fungus infection of the eggs of salmon and trout (Scot, 1993) and also to control fungus infections in crustaceans (Lightner, 1993). The present study proves that the best treatment to control epibionts in phyllosoma larvae is to give a dip treatment in 100 ppm malachite green on a short bath treatment for 10 minutes in 10 ppm. Potassium permanganate also can be used for bath treatment, 45 minutes in 10 ppm or 15 minutes in 70 ppm or 5 minutes in 100 ppm. Long term exposure (5 hours) at a lower concentration (25 ppm) seem to be feasible, as at higher concentrations formalin is lethal to the larvae.

Daily feeding with live feed like *Artemia* nauplii is likely to introduce *Zoothamnium* to the rearing system and this can be prevented by giving dip treatment to the *Artemia* nauplii (dip in 100 ppm or bath for 10 minutes in 10 ppm) in malachite green. The nauplii have to be cleaned with fresh seawater before feeding to avoid introduction of malachite green to the rearing system. The same authors (unpublished) have proved that the *Artemia* nauplii can withstand exposure to malachite green at the suggested concentration.

Acknowledgements

We thank Mr. K. Shahul Hameed, Technical Assistant (retd.), Central Marine fisheries Research Institute; Chennai for the help rendered for this study.

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