Short communication

Experimental validation of dilute acetic acid solution injection to control crown-of-thorns starfish (*Acanthaster planci*)

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希酢酸注射によるオニヒトデ薬殺の実験検証

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Abstract: Major outbreaks of crown-of-thorns starfish (*Acanthaster planci*) at tropical and subtropical ocean areas have been reported. These phenomena cause a serious problem because of their coral bleaching behavior. We have validated a dilute acetic acid injection method to save labor and cost in controlling them and result was encouraging.

I. Introduction

Coral reef bleaching has been manifested by crown-of-thorns starfish (*Acanthaster planci*) extensively throughout the tropical and subtropical areas in the Indian and Pacific Oceans (Moran, 1986). Japanese ocean areas such as the Ryukyu Islands, Ashizuri-Uwakai and Kushimoto have also been no exceptions (Yamaguchi, 1986).

Several trials to control *Acanthaster* infestation have been done at Great Barrier Reef and Okinawa Islands. Injection of copper sulfate at Great Barrier Reef was considered unsuccessful and expensive (Johnson, 1990). Sodium bisulfate injection has been reported as effective at Papua New Guinea (Baine, 2006).

We have searched for a drug preliminary which is effective, inexpensive and safe for divers and environment. Among candidate drugs, dilute acetic acid solution seemed best fitted for these objectives. We here report the results of experimental validation of dilute acetic acid solution injection comparing with sodium bisulfate.

A part of this experiment was reported to "Marine worker project" (Biological Institute on Kuroshio, 2011).

II. Materials and methods

The experiment was conducted at the sea front of Nishidomari, Otsuki, Hada, Kochi in Japan. The sea water temperature was between 25.6 to 27.8 °C and its specific gravity was between 1.024 and 1.025.

*Acanthaster* of 25 cm to 35 cm in diameter, used in this experiment, was collected at the area where this experiment was performed. The number of *Acanthaster* used for control with sea water was ten and for treatment with each of the two chemicals was 40. Each group was kept in separate cage with net on the bottom of the ocean as shown in Fig. 1.

Acetic acid and sodium bisulfate were obtained (Nacalai tesque), which were diluted to 15%, or dissolved to 14%, with sea water, respectively. Variable syringe STV with needle was used for

Fig. 1. Cage used for the experiments.
injection (Simcro) as shown in Fig. 2. Five milliliters of sea water, the acetic acid solution, or the sodium bisulfate solution was injected twice to each subject around its central part. Mortalities were judged every 24 hours to 72 hours.

Data were analyzed by Long-rank (Mantel-Cox) test, using GraphPad Prizm5 (GraphPad software). Differences at $P < 0.05$ were considered statistically significant.

III. Results
All of the subjects injected with sea water as control survived at the end of the experiment. Injection of sodium bisulfate showed only slight effect after 48 hours, which was not statistically significant ($P = 0.1999$) at 72 hours as shown in Table 1 and Fig. 3.

Many of the subjects injected with 15% acetic acid presented with symptoms such as shrinking roundly within 24 hours. The number of final survivors was 25 with statistical significance ($P = 0.0137$) as shown in Fig. 4. These results were shown in Table 1 and Fig. 5.

IV. Discussion
We showed effectiveness of injection of dilute acetic acid to control Acanthaster. As acetic acid has water-soluble and lipid-soluble nature, we consider that it penetrates into tissues of Acanthaster and cause necrotic death. Even when this method is used extensively, the amount of acetic acid to be used will be small in terms of organics, compared with that of Acanthaster itself and the chemical will be easily diffused and decomposed by microbes.

The injection method will save the labor to recover victims to the ship and avert envenomation during operation. Our concern left for this method is a leakage of injected substance due to its low viscosity. We have tried adding hydroxyethyl cellulose or hydroxymethyl cellulose into dilute acetic acid to increase viscosity in tank experiments and achieved much better results both in efficacy and potency. Thick solution should be considered for the practical application.

V. Acknowledgements
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VI. References

山本俊政・大塚隆尚：希酢酸注射によるオニヒトデ薬殺の実験検証

要約
熱帯および亜熱帯の海域でのオニヒトデ（Acanthaster planci）の異常繁殖が報告されている。この現象は深刻な珊瑚礁の白化問題となっている。我々は省力化とコスト低減を目的として希酢酸注射法によるオニヒトデ薬殺の実験検証を行い良好な結果を得た。

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