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Lisa Boström-Einarsson & Jairo Rivera-Posada

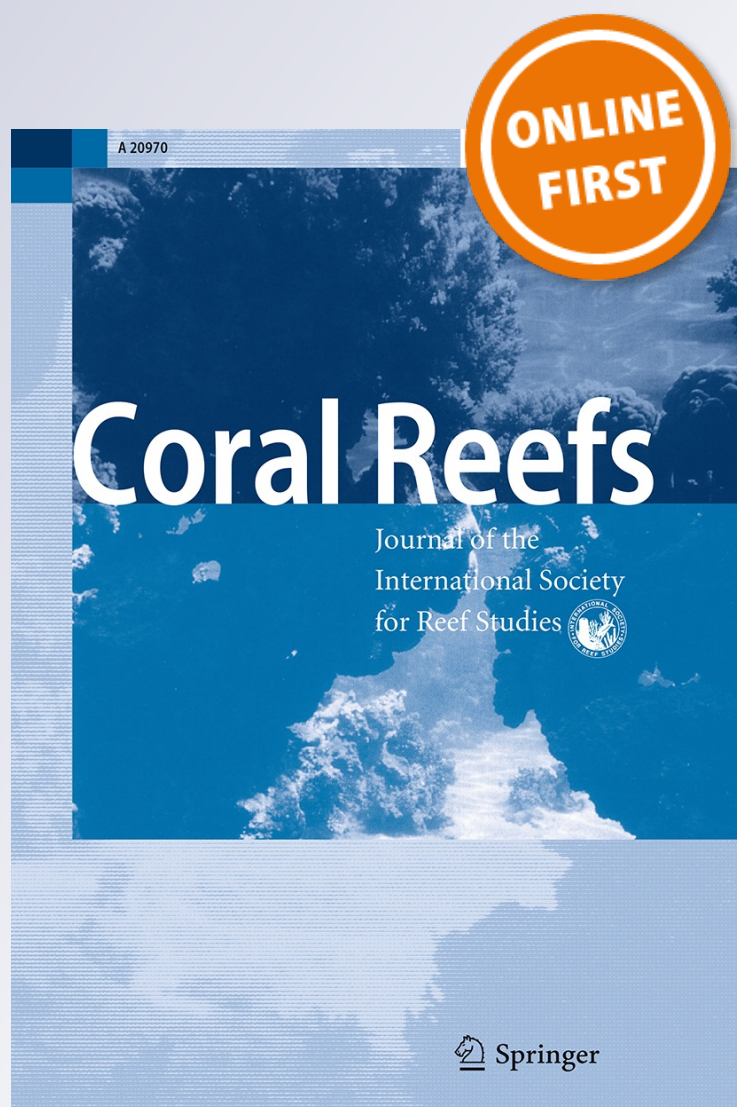
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NOTE

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Lisa Boström-Einarsson^{1,2}  · Jairo Rivera-Posada^{1,3}

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Abstract Outbreaks of the destructive coral-eating crown-of-thorns starfish, *Acanthaster planci*, present a considerable threat to coral reefs worldwide, and mitigating their impact has proven challenging. The most effective methods to control *A. planci* require injecting individual starfish with lethal chemicals. While some of these are highly effective, their administration often requires permits, training and access to specialised equipment. We aimed to identify a widely available and highly efficient alternative. We discovered that common household vinegar is lethal to *A. planci* individuals when injected at the base of one of their arms. A single injection of 25 ml vinegar induced functional mortality in <24 h and 100 % mortality in <48 h. These results demonstrate that vinegar is an effective alternative to currently used chemicals. Vinegar is a viable alternative in the toolkit of methods that can control and eradicate local outbreaks of COTS on coral reefs.

Keywords COTS · *Acanthaster planci* · Control · Injection · Vinegar · Acetic acid

Introduction

Population outbreaks of the coral-eating crown-of-thorns starfish (COTS, *Acanthaster planci*) are a major cause of live coral loss on Indo-Pacific coral reefs (Bruno and Selig 2007; Pratchett et al. 2014). On Australia's Great Barrier Reef (GBR), COTS outbreaks have been identified as one of the primary causes of live coral declines in the past few decades (Osborne et al. 2011; De'ath et al. 2012). The effects of outbreaks on reef environments are often devastating and widespread, sometimes reducing live coral cover by 90 % or more (Chesher 1969; Kayal et al. 2012). The loss of coral habitat also leads to a marked decline in the abundance of reef-associated fishes due to a reduction in their primary food source (Bouchon-Navaro et al. 1985; Kayal et al. 2012), loss of shelter space (Holbrook and Schmitt 2002) or changes in behaviour (McCormick 2012; Boström-Einarsson et al. 2014). While other stressors affecting coral reefs may be difficult to mitigate, COTS outbreaks are one threat to coral reefs that could feasibly be controlled (De'ath et al. 2012). Ideally, COTS outbreaks should be controlled at the population level; however, current knowledge gaps in the exact cause of outbreaks have prevented the development of such controls to date (Pratchett et al. 2014). Until such methods are developed, direct control remains the most effective weapon in our limited toolkit.

Previous direct control methods include physical removal of individual starfish (Barnes 1966; Yamaguchi 1986) and cutting individuals into multiple pieces. These methods are relatively slow, ineffective and carry a high

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✉ Lisa Boström-Einarsson
lisa.bostromenarsson@my.jcu.edu.au

¹ ARC Centre of Excellence for Coral Reef Studies, James Cook University, Townsville, Australia

² College of Marine and Environmental Sciences, James Cook University, Townsville, Australia

³ Environmental Corporation, University of Antioquia, Medellin, Colombia

risk of injury from contact with the venomous spines of *A. planci*. The most common and widely adopted control methods to date involve lethal injections with a range of chemicals (Johnson et al. 1990). Early injection methods involved chemicals that were toxic to both the injecting SCUBA diver and other marine organisms beside the starfish themselves (e.g., formaldehyde, copper sulphate) or required multiple injections (e.g., sodium bisulphate: 10–15 injections; Birkeland and Lucas 1990). Recently, a highly effective method was developed using injections of diluted bile salts, which kills *A. planci* in <24 h with a single injection (Rivera-Posada et al. 2013, 2014). However, the use of bile salts on *A. planci* is restricted by permits in Australia and the USA, and the salts can be subject to quarantine regulations when transported across borders.

Most current control methods require dilutions and mixing and can only be accessed through relatively specialised outlets (e.g., abattoirs, industrial chemical supply shops). While this may not limit government-funded control programs in Australia and the US Pacific islands, these issues may limit adoption in remote communities. We therefore aimed to develop an alternative injection method using a ubiquitous and affordable chemical without the need for dilutions or mixing. Here, we report the efficiency, safety and applicability of lethal injections using a common household product: vinegar. While vinegar has previously been tested with limited success in Japan (Yamamoto and Otsuka 2013), we demonstrate improved injection methods that were tested on two separate outbreak populations of COTS.

Methods

Detailed methods are available in the Electronic Supplementary Material.

The efficiency of vinegar injections to kill *A. planci* was tested on two separate populations, in Kimbe Bay, Papua New Guinea (PNG), and on Lizard Island, GBR, Australia. In both studies, *A. planci* were collected from reefs and placed in holding tanks. The effectiveness of the number of injection sites and total injection volume (treatments) was compared to each other and to control *A. planci* injected with seawater. Multiple injection sites were evenly spread around the central disc, and the total volume was evenly split among injection sites.

Trial 1: PNG

A total of 58 *A. planci* were collected from inshore reefs in Kimbe Bay, PNG (5°25'S, 150°05'E). A total of either 15 or 1.5 ml household vinegar (acetic acid content unknown)

was injected at the base of the arm using a 29-gauge hypodermic needle in either two or four injection sites (Fig. 1). Starfish were monitored twice daily, approximately 7 h apart. A starfish was considered to be dead when no signs of movement were detected. Recorded times to mortality represent a maximum, as starfish could have died at any time between monitoring intervals.

Trial 2: GBR, Australia

A total of 99 *A. planci* were collected from reefs around Lizard Island, Australia (14°40'S, 145°28'E) in September 2014 and February 2015. Injections were administered using a syringe with a 16-gauge stainless steel needle. Individuals were injected with a total of 25, 20, 15 or 1.5 ml vinegar (4 % stated acetic acid content), split between one, two or four injection sites. From Trial 1, we observed that immobility, meaning that the starfish has lost turgor and is unable to move and feed (i.e., functionally dead), can occur hours or days before complete death, when all podia have ceased moving. We therefore measured the effectiveness of vinegar using three measures: (1) time to immobility; (2) time to death; and (3) proportion of starfish killed. To investigate whether a larger needle size might be an explanation for previous failures of using vinegar, a further eight starfish were injected with 25 ml vinegar using a 4-mm needle tip (Fig. 2).

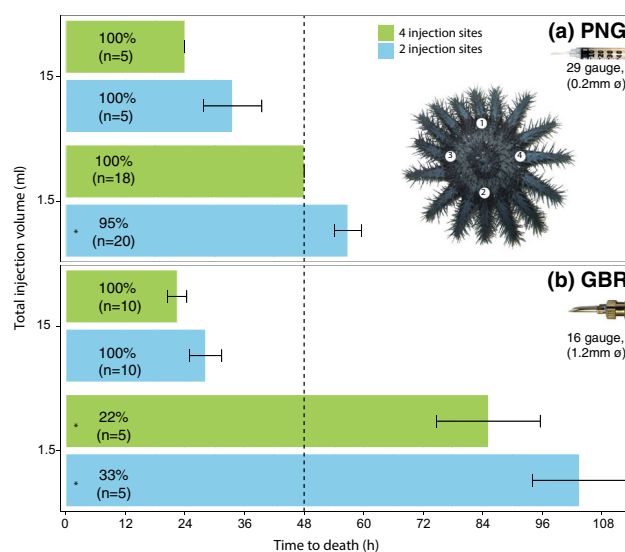


Fig. 1 Average time to death following vinegar injections of *A. planci* from two outbreak populations in Papua New Guinea (a) and the Great Barrier Reef (b). Injection volumes consisted of two or four injections (blue or green, respectively) of either 15 or 1.5 ml of vinegar. Percentages indicate total mortality, treatments with <100 % mortality indicated by asterisk. Insets illustrate location of injection sites on *A. planci* and needle sizes used. Dashed line marks 48 h post-injection, and error bars represent standard errors

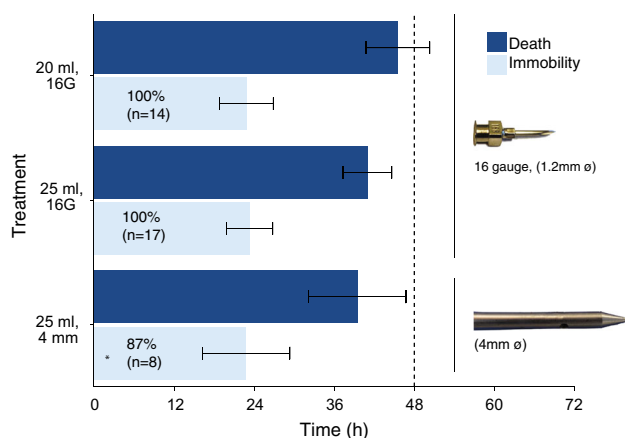


Fig. 2 Average time to immobility (i.e., stopping of movement and feeding) and death (all tube feet stopped moving) of *A. planici* after a single injection of vinegar. Injections were administered with a 1.3-mm (16-gauge) or 4-mm needle in volumes of 20 or 25 ml. Percentages indicate total mortality, treatments with <100 % mortality indicated by asterisk. Dashed line marks 48 h post-injection, and error bars represent standard errors

Transmission trials

To test whether *A. planici* injected with vinegar are a threat to other marine organisms, 27 reef species were placed in tanks containing either two injected (treatment, 25 ml vinegar) or control *A. planici* (Table 1). Species were selected from families that have previously been recorded consuming and interacting with decaying COTS, making them vulnerable to any adverse effects from vinegar. Organisms were kept in each treatment for a minimum of 7 d, video monitored for 2 h daily in the first 3 d to record interactions with *A. planici*, and visually inspected three times daily for any signs of disease or stress.

Results and discussion

Vinegar caused 100 % mortality in <48 h when injected into *A. planici* in both PNG and GBR populations (Fig. 1). There was no mortality or tissue necrosis in control *A. planici* injected with seawater in any of the treatments. This demonstrates that regular household vinegar can be used as an effective lethal injection of the coral-eating COTS, providing an alternative to existing control methods. However, the number of injection sites and injected total volume affected the time to death and immobility. A single shot of 25 ml of this widely available chemical stopped the starfish from moving and feeding in <24 h (22.9 ± 3.3 h SE; Fig. 2), with complete mortality in <48 h (40.3 ± 3.5 h; Fig. 2). A reduction in volume by 5 ml yielded 100 % mortality and did not affect time to immobility (23.3 ± 4.3 h); however, time to death increased by 13.5 % (46.6 ± 5 h; Fig. 2). Mortality rates

and time to functional mortality of *A. planici* are similar to those found using bile salts, the most effective product currently in use (Rivera-Posada et al. 2014), although twice the volume is required. While the two chemicals are comparable in terms of efficiency, vinegar is widely available and does not require dilution or mixing prior to use.

Vinegar and acetic acid have been tested previously, but this is the first time they have been demonstrated to induce 100 % mortality in <48 h with a single injection. Previous tests included larger needle sizes (2 and 4 mm inner diameter) rather than the 29- and 16-gauge needles used here (0.2 and 1.2 mm, respectively), which may allow small quantities of vinegar to leak through the multiple injection holes (Yamamoto and Otsuka 2013). This theory is supported by our experiment where 25-ml injections using a larger needle (4 mm) reduced mortality to 87 %, while time to death and immobility were similar to the 16-gauge needle injections (time to immobility 22.7 ± 6.5 h, time to death 39.4 ± 7.2 h; Fig. 2). A loss of vinegar through leakage may explain the lower mortality and longer time to death described here and in previous studies (KBRF 2012; Yamamoto and Otsuka 2013; Rivera-Posada et al. 2014).

While a reduction in injected total volume of vinegar reduced the mortality in one-shot treatments, our study suggests an increase in injection sites can compensate for volume reductions. Vinegar injections of 15 ml split among either two or four injection sites resulted in 100 % mortality of both PNG and GBR populations in <48 h (Fig. 1). A higher number of injection sites resulted in a 27 and 21 % decrease in average time to mortality in PNG and GBR populations, respectively (Fig. 1). However, there were differences between populations in response to volume reductions. In the PNG population, one-tenth the volume of injected vinegar (1.5 ml) split among four injection sites still resulted in 100 % mortality. This is in contrast with GBR starfish, which only suffered 22 and 33 % mortality with two or four injections of 1.5 ml, respectively (Fig. 1). While it is unclear why these differences between populations occurred, it might be due to the smaller size of the needle used in PNG.

Starfish injected with vinegar displayed a matting of the spines in the first few hours post-injection (Fig. 3a), followed by immobility and tissue necrosis (Figs. 3b, c). Decaying *A. planici* were often covered in a bacterial film in <24 h post-injection (Fig. 3d). These clinical signs were similar to those described when *A. planici* were injected with either *Vibrio* spp. bacteria culture or bile salts (Rivera-Posada et al. 2011a, b) and raise the possibility of pathogen transmission to other reef inhabitants. Given that an alkaline environment is conducive to *Vibrio* growth (Rivera-Posada et al. 2011a, b), and vinegar is commonly used as

Table 1 Reef organisms used in transmission tank experiments

Taxon	Species	Control <i>n</i>	Treatment <i>n</i>	Adverse effects Yes/No
Ballistidae	<i>Balistoides viridescens</i> (juv)	3	3	N ^a
	<i>Rhinecanthus aculeatus</i> (adult)	3	3	N ^a
	<i>Rhinecanthus aculeatus</i> (juv)	3	3	N ^a
Chaetodontidae	<i>Chaetodon auriga</i>	2	2	N
	<i>Chaetodon vagabundus</i> (juv)	3	3	N ^a
Labridae	<i>Halichoeres melanurus</i>	2	2	N ^a
	<i>Thalassoma lunare</i>	3	3	N ^a
Nemipteridae	<i>Scolopsis bilineatus</i>	3	3	N
Pomacanthidae	<i>Centropyge bicolor</i>	3	3	N
Pomacentridae	<i>Acanthachromis polyacanthus</i>	3	3	N ^a
	<i>Chromis viridis</i>	5	5	N
	<i>Pomacentrus chrysurus</i>	3	3	N
	<i>Pomacentrus moluccensis</i>	3	3	N
Tetraodontidae	<i>Arothron hispidus</i>	1	1	N ^{a,b}
	<i>Arothron nigropunctatus</i>	1	1	N
Acroporidae	<i>Acropora nasuta</i>	1	1	N
	<i>Acropora sarmentosa</i>	1	1	N
	<i>Acropora spatulata</i>	1	1	N
	<i>Stylophora pistillata</i>	1	1	N
Faviidae	<i>Goniastrea retiformis</i>	1	1	N
Pocilloporidae	<i>Pocillopora damicornis</i>	1	1	N
Poritidae	<i>Porites porites</i>	1	1	N
Holothuridae	<i>Holothuria atra</i>	3	3	N ^c
	<i>Holothuria edulis</i>	3	3	N ^c
	<i>Stichopus chloronotus</i>	3	3	N ^c
Asteroidea	<i>Linckia laevigata</i>	3	3	N ^c
	<i>Echinaster luzonicus</i>	3	3	N ^c

Species were placed in a holding tank containing either two healthy COTS (control) or two COTS injected with vinegar (treatment)

^a Observed feeding on injected *A. planci*

^b Observed feeding on control *A. planci*

^c Observed in contact with injected *A. planci*

an organic disinfectant, we do not expect individuals injected with vinegar (acetic acid ~2.6 pH) to spread bacterial infections. Indeed, our tank trials demonstrate that it is unlikely that injected *A. planci* pose a threat to reef organisms that may be feeding or interacting with decaying starfish. Although multiple species were seen touching or consuming *A. planci*, there were no mortalities or signs of adverse effects in any organisms in either the treatment (containing two injected COTS) or control tank (two healthy COTS) (Table 1). Glynn (1984) suggested that exposure of internal tissues of *A. planci* will considerably increase the likelihood of attacks by a broad array of predators, reducing the time COTS remains will persist on reefs.

While we are unsure of the exact reason for the rapid tissue necrosis in this study, Rivera-Posada and Owens

(2014) demonstrated that drastic changes in osmolarity induced rapid tissue shrinking or swelling in *A. planci*, leading to cell membrane damage followed by death. Given that echinoderms are poor acid–base regulators (Wittmann and Pörtner 2013), it is likely that the drastic decrease in pH induced acidosis, causing tissue walls to necrose, eventually leading to death. While large numbers of COTS injected in a small area may lower the pH of surrounding waters, the vinegar is likely to quickly be diluted as the starfish decompose and are consumed by scavengers. Though the outcomes of the transmission experiment are promising, we suggest that larger trials in the reef environment are needed to fully test potential effects on organisms other than *A. planci*.

While any direct control method is unlikely to stop an ongoing outbreak, sustained efforts can protect specific

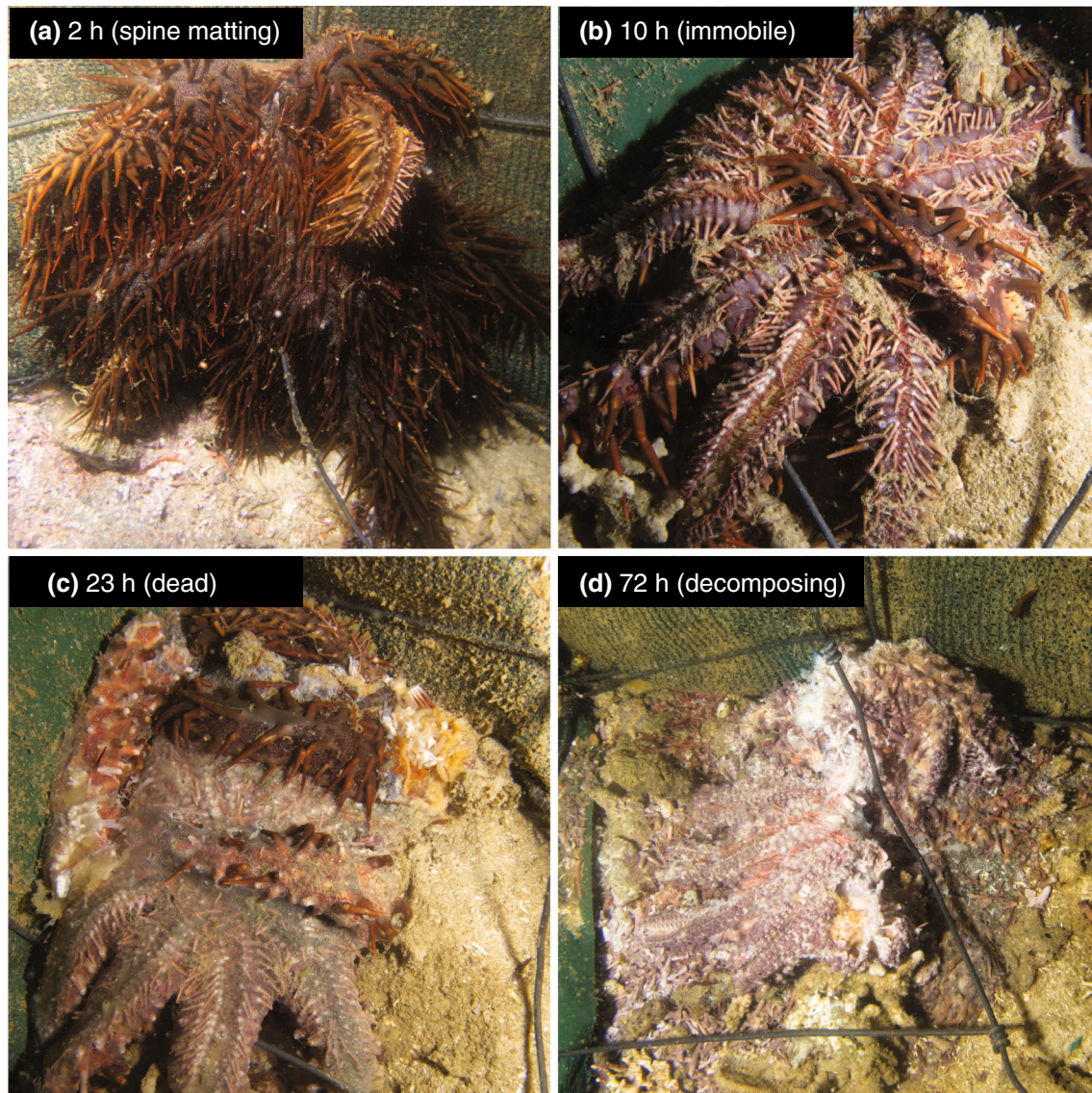


Fig. 3 Sequence of tissue necrosis of *A. planci* injected with 25 ml of vinegar: **a** Spine matting, 2 h post-injection; **b** *A. planci* immobile but some tube feet still moving, 10 h post-injection; **c** dead, all tube feet

ceased moving, 23 h post-injection; **d** bacterial film and decomposition, 72 h post-injection

reefs of high ecological, aesthetic and/or commercial value. This study highlights vinegar as a promising alternative to current methods, providing a safe, inexpensive and widely available method for killing COTS. This method removes significant obstacles to the adoption of previous methods, in terms of accessibility, affordability and effectiveness of culling programs worldwide. Vinegar is ideal for adoption in remote communities and developing countries where access to bile salts and other control chemicals may be restricted. While adverse effects to other reef organisms are unlikely, the authors would argue for caution in using this method until further field trials have been conducted.

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