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Citric Acid Injections: An Accessible and Efficient Method for Controlling Outbreaks of the Crown-of-Thorns Starfish *Acanthaster* cf. *solaris*

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Abstract: Outbreaks of the crown-of-thorns starfish (*Acanthaster* cf. *solaris*, COTS) are one of the primary causes of coral decline in the Indo-Pacific region. Effective methods to control COTS outbreaks may therefore be one of the most direct and immediate ways to reduce coral loss. However, the cost and logistical challenges associated with current control methods have undermined the effectiveness of many control efforts. In this study, we tested the feasibility of using powdered citric acid, which is widely available and low-cost, as an injection chemical for COTS control. We tested what combination of concentration, number of injections, volume, and water type were most efficient at killing COTS. All COTS injected in two or four sites died, irrespectively of the concentration of citric acid used, while single injections failed at reaching 100% mortality. The fastest combination was the injection of 150 $g \cdot L^{-1}$ citric acid solution in four injection sites (5 mL per site), which killed the starfish in 26.4 $\pm$ 4 h. These results suggest that injections of powdered citric acid are an effective, economical, and widely available alternative to current COTS control methods.

Keywords: COTS; outbreak; control methods; pest control; coral reefs; injections

1. Introduction

Periodic outbreaks of *Acanthaster* cf. *solaris* (crown-of-thorns starfish, COTS) represent one of the single biggest threats to tropical coral reefs in the Indo-Pacific [1,2]. The corallivorous starfish can cause extensive damage when present at outbreak densities on coral reefs. For example, a large outbreak in Guam reduced live coral cover by more than 90% over a period of 2–3 years [3]. On Australia's Great Barrier Reef (GBR), mean live coral cover has halved between 1985 and 2012, and ~40% of that coral loss is attributed to COTS [4,5]. Preventing and managing COTS outbreaks is therefore a major priority for environmental science and resource management, and may be the most immediate and effective mechanism to prevent ongoing coral loss across the Indo-Pacific [6]. Management of most anthropogenic disturbances that threaten coral reefs (e.g., climate-induced disturbances and declining water quality) require large-scale interventions and international policy changes [7]). In contrast, management of localised outbreaks of COTS are potentially feasible and may significantly increase the resilience of coral reef communities [5]. However, finding efficient, cheap, and safe methods that can be applied at large scales and across developed and developing nations has proven challenging [8].

Historically, a wide range of methods have been employed to cull COTS [8], however, most have been inefficient or damaging to the marine environment. Currently, large-scale control programs for COTS involve a single injection of a bile salt solution, which effectively kills COTS in less than 24 h [9,10]. However, bile salts can be expensive, are only accessible through specialised suppliers, and

carry quarantine restrictions that impede international operations [11]. Thus, for many communities in remote areas of the Indo-Pacific that experience recurrent COTS outbreaks, it can be difficult to obtain bile salts. To overcome these challenges, recent research has been investigating the potential use of alternate chemicals that are cheap, readily available, easy to deploy, safe to both the marine environment and to humans, and equally efficient at killing COTS as bile salts. To date, injections with cooking salt solution, vinegar, and lime juice have been demonstrated to be both lethal to COTS and safe for the environment [12–17]. However, not all of these chemicals are viable for large-scale control programs in remote coral reef regions. For instance, the high quantities of cooking salt and limes required during extensive control programmes may be challenging to transport in remote locations [16]. Limes are not readily available in all seasons nor in all locations, which may increase their price. In addition, juice extraction is labour-intensive, adding labour costs to control programmes, and the juice may deteriorate if not rapidly used [15]. Instead, this study examines the viability of using powdered citric acid, which can be purchased locally from grocery stores, is inexpensive, readily available, and has a long shelf life.

For a large-scale COTS control programme to be efficient, it is critical that any method employed achieves 100% COTS mortality. Achieving complete mortality depends not only on the efficiency of the injected chemical, but also on the technique used when administering the injection. Recent studies have revealed the importance of the number of injections [12,15], the size of the needle [10,12], location of injection site [10], and the volume or concentration injected [12,13,15,18]. For example, a single injection of 20 mL of lime juice did not kill 100% of treated COTS, while splitting the volume between two injection sites did [15]. Furthermore, a single 20 mL injection of vinegar with a 16-gauge (1.2 mm inner diameter) needle resulted in 100% mortality, but using a 4 mm diameter needle reduced mortality to 87% [12]. The ideal injection method hinges on finding the optimal combination of techniques, using chemicals readily available in the local area.

The aim of this study was, therefore, to investigate the potential of using powdered citric acid as an efficient and cost-effective method to cull COTS. To achieve this, we aimed to determine: (1) the lowest effective concentration and number of injection sites needed to obtain 100% mortality, (2) the most effective injection volume, and (3) the efficiency of untreated seawater compared to distilled water as solvent for the citric acid solution. Finally, we compared the efficiency of citric acid injections to other chemical products in order to provide management recommendations.

2. Materials and Methods

2.1. Collection Site and Maintenance Conditions

The study was carried out at Lizard Island (14°40′S, 145°28′E), Northern GBR, Queensland, Australia in February 2015. Adult *A.* cf. *solaris* were collected from reefs around Lizard Island and transported to Lizard Island Research Station in plastic aquaria (64 × 41 × 40 cm, max. 20 COTS per aquaria) with aerators. Then, COTS were allowed to acclimatise for at least 24 h in two large aerated holding tanks (160 cm diameter × 50 cm deep) with flow-through ambient seawater. Injured and weak specimens were discarded. Specimens were then measured from the tip of one randomly selected arm to the tip of the diametrically opposite arm (mean size 28.2 ± 0.4 cm SE) and placed individually in aquaria (40 × 30 × 25 cm) with flow-through ambient seawater.

This study was conducted in accordance with James Cook University ethical guidelines and the Queensland Animal Care and Protection Act 2001.

2.2. Experiment 1: Concentration and Number of Injection Sites

To test whether different concentrations and number of injection sites influenced the effectiveness in killing COTS, we conducted a factorial experiment. We tested three different concentrations of citric acid in seawater solution (90, 120, and 150 $g \cdot L^{-1}$) crossed with three different numbers of injection sites (one, two, and four injection sites, hereafter I.S.). Six replicate COTS were injected in each of the

nine treatment combinations (three citric acid concentrations × three injection site levels) using a total of 54 individual COTS. In addition, five COTS per I.S. treatment level were injected with 20 mL of seawater to control for the injection itself. All treatments were run simultaneously, except the three 90 $g \cdot L^{-1}$ treatments and the 4 I.S., 120 $g \cdot L^{-1}$ treatments, where COTS were injected in bunches of three (each still in individual tanks). The second bunch was injected ~24 h after the first one to keep the start of the experiment at a similar time of day, therefore minimizing any variation in water temperature.

A 16-gauge stainless steel needle mounted on a 25 mL disposable syringe was chosen to perform the injection in order to minimise puncture size and leakage postinjection [12]. Injections were performed at the base of the arms [10], targeting the hydrovascular system of the starfish [19]. Single injections were administered at a randomly selected arm, double injections at opposing arms, and four injections in different quartiles. For each treatment, a total of 20 mL of solution was evenly split between injection sites (one I.S. treatment = 20 mL injected in one arm, two I.S. treatments = 10 mL per arm, and four I.S. treatments = 5 mL per arm).

The temporal progression of the treatments was recorded every 4 h (three observations per day) for up to one week or until death. The clinical signs recorded were: (1) no response vs hyperactivity, (2) matting of spines, (3) swelling and loss of turgor, (4) appearance and increased production of mucus, (5) appearance of bacterial films, (6) loss of any arms or splitting, (7) immobility, and (8) death [cf. 9]. The primary response variables measured were mortality, time to immobility, and time to death, also recorded every 4 h (three observations per day). Immobility was defined as the inability to cling to the walls of the tank or move [12], while death was determined when all tube feet completely stopped moving [10,20,21]. Time to immobility was recorded because it represents the ecological death of the starfish, due to their inability to feed when immobile. In some cases, time to death was delayed by up to 38 h past the time of immobility as individual tube feet remained motile.

2.3. Experiment 2: Volume

A second experiment was performed to evaluate whether increasing the injected volume could improve the efficacy of the single injection method in achieving 100% mortality. Here, six COTS were injected with 30 mL of 120 $g \cdot L^{-1}$ solution via two simultaneous injections at the base of one arm. Injections of 15 mL of solution from two syringes were performed simultaneously, with the needles held approximately 5 mm apart (considered one I.S.). Two needles were needed to accommodate the increased volume, as larger syringes were not readily available. A maximum of 30 mL was used, as it is the capacity of the most commonly used gun used in the field to inject sodium bisulphate and bile salts [10]. The effect of 30 mL injections on mortality, time to immobility, and time to death was compared to the effect of 20 mL injections of 120 $g \cdot L^{-1}$ at one I.S. (performed in Experiment 1).

2.4. Experiment 3: Seawater vs Distilled Water

To compare the effect of the solvent on citric acid efficacy, we compared COTS mortality and response times when using seawater as the solvent versus distilled water. Distilled water was used to test whether a hypoosmotic reaction induced by low salinity and lower pH of the solvent would have increased the efficacy of the citric acid solution, increased percentage of mortality, and/or reduced time to immobility or to death. Distilled water was expected to induce an osmotic shock in COTS tissues, thereby increasing the efficiency of the citric acid injections, in two ways. First, by accentuating acidosis, because distilled water (pH 7.0) is less alkaline than seawater (pH 7.5–8.4) and should lower COTS coelomic pH after citric acid injection. Second, by causing a hypoosmotic shock, because COTS are unable to tolerate drastic changes in internal salinity or osmotic pressure [13,16]. Distilled water was used as the solvent in one treatment (one I.S., 20 mL of 120 $g \cdot L^{-1}$ citric acid solution) repeated on six individuals. The effect of distilled water on mortality, time to immobility, and time to death was compared to the effect of 20 mL injections of 120 $g \cdot L^{-1}$ at one I.S. (performed in Experiment 1).

2.5. Statistical Analysis

For Experiment 1, differences between times to immobility and to death were analysed using two-way fixed factor analysis of covariance (ANCOVAs). Because only treatments that reached 100% mortality were included in these models, none of the single injection site treatments were included in the statistical tests for Experiment 1. Additionally, for the 150 g·L^{-1}, 4 I.S., 20 mL seawater treatment, specific hours of immobility and death were not attained because all COTS reached immobility and died overnight. Therefore, death was assumed to have occurred at the same time as immobility, and both times were scored at the following morning observation. For all analyses, assumptions of normality among residuals were analysed using the Shapiro–Wilk test (time to immobility: W = 0.787, $p < 0.001$; time to death: W = 0. 772, $p < 0.001$). Homogeneities of variances between concentrations and number of injection sites were analysed with Levene's test. Time to immobility and to death measurements were subsequently log transformed to meet assumptions of normality: log(time to immobility): W = 0.958, $p = 0.317$; log(time to death): W = 0.950, $p = 0.118$. The dependent variables were time to immobility and to death (hours), analysed separately, and the independent fixed factors were the concentrations of the solution (three levels: 90, 120, 150 g·L^{-1}) and the number of injection sites (two levels: two and four I.S.). To control for the effect of body size, we used the diameter of the starfish as covariate. Because the interaction terms among size, concentration, and number of injection sites were not significant for time to immobility and to death, the full models were rerun without the interaction terms to increase the power of the tests. Tukey's post hoc tests were used to analyse statistical differences between concentration groups and I.S. treatments.

For Experiments 2 and 3, differences between times to immobility and to death (dependent variables) were analysed separately using one-way ANCOVAs with volume and water type as independent fixed parameters, while using the diameter of the starfish as covariate. Assumptions of normality were analysed with the Shapiro–Wilk test of normality (Experiment 2, time to immobility: W = 0.978, $p = 0.954$, time to death: W = 0.901, $p = 0.226$; Experiment 3, time to immobility: W = 0.876, $p = 0.144$, time to death: W = 0 .878, $p = 0.152$) and homogeneity of variances were analysed with Levene's tests. No transformations were required. Because the interaction terms between size and volume and size and water type were not significant for time to immobility and to death, the full models were rerun without the interaction terms to increase the power of the tests. Statistical analyses were conducted using *TIBCO Spotfire S+*® 8.2 Programmer's Guide, *TIBCO* Software Inc. (Palo Alto, CA, United States) Technical Support.

3. Results

3.1. Experiment 1: Concentration and Number of Injection Sites

All treatments using either two or four injection sites had 100% mortality, regardless of the citric acid concentration (Figure 1) or of COTS size (Table 1). Concentration significantly affected time to immobility (Table 1), with the 120 g·L^{-1} treatments being almost twice as fast (32.2 ± 8.2 h SEM) than the 90 g·L^{-1} treatments (69.9 ± 18.7 h). In contrast, the concentration of citric acid did not have a significant impact on time to death (Table 1), although the 150 g·L^{-1} treatments were, on average, 1.8 times faster than the 90 g·L^{-1} ones (respectively, 46.8 ± 7.4 h and 84.4 ± 18.7 h). Time to death was instead significantly affected by the number of injection sites (Table 1), as COTS with more injections died faster. On average, four injections halved the time to death compared to two injections (44.1 ± 6.7 h and 79.2 ± 12.1 h, respectively). In contrast, time to immobility was not affected by number of injection sites (Table 1), although four injections immobilised COTS twice as fast as two injections (respectively, 32.8 ± 6.2 h and 59.6 ± 12.8 h). The 150 g·L^{-1}, four I.S. treatment was the quickest overall at killing COTS (26.4 ± 4 h), and was 4.3 times faster than the 90 g·L^{-1}, double injection. The latter was the slowest treatment that still achieved 100% mortality and took 112.8 ± 31.8 h (Figure 1).

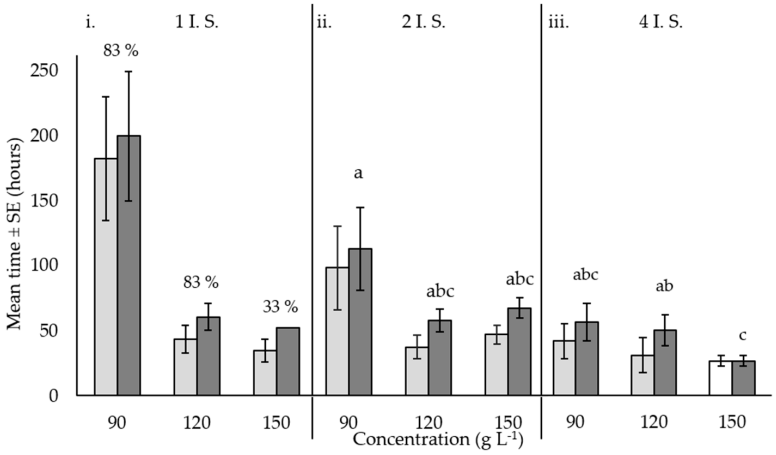

Figure 1. The effects of citric acid concentration and the number of injections. Mean time to immobility (□) and to death (■) ± standard error for *Acanthaster* cf. *solaris* injected with 20 mL of citric acid and seawater solution at concentrations of 90, 120, and 150 g·L^{-1} in one (**i**), two (**ii**) and four (**iii**) injection sites (I.S.). Numbers above bars represent total percent mortality; where no numbers are shown, mortality was equal to 100%. For the 150 g·L^{-1}, 4 I.S. treatment, time to immobility was assumed to equal time to death (□). Six replicates per treatment combination were used. Letter notations above bars (a, b, c) indicate Tukey's post-hoc groupings between injection site treatments and concentrations for time to death. Different letters indicate significant differences among treatments.

When a single injection was administered, mortality depended on the concentration used. At 90 and 120 g·L^{-1}, mortality was 83% (five out of six starfish died), while at 150 g·L^{-1} mortality decreased to 33% (only two out of six starfish died, Figure 1). However, increasing the concentration reduced time to immobility by 5-fold and time to death 4-fold. At 90 g·L^{-1}, COTS were immobile in 182.8 ± 47.1 h and died in 200 ± 50 h, while at 150 g·L^{-1} they were immobile in 35 ± 8.5 h and died in ~50 h.

Behaviour and Macroscopic Progression

Immediately after injection with citric acid, COTS showed symptoms of stress through hyperactivity, swelling, and increased mucus production. Approximately one day after injection, mucus production, matting of the spines, and localised necrosis on at least one arm were common symptoms. Additionally, many specimens, particularly those treated with higher concentrations, were already partly immobile or dead and others had split in half or thirds. In some cases, tissue decomposition progressed over the central disc, exposing internal organs. After two days, the first dense colonies of bacteria started forming, creating orange-red films around decomposing parts of COTS. Matting of the spines was usually body-wide, and some spines had been dropped or were bleached. Starfish motility was usually very low, with most animals completely immobile or dead. Three to four days after injection, tissue necrosis and bacterial decomposition progressed. By this time, most COTS were considered unrecoverable, completely immobile, or dead. However, in some cases where the starfish had split, one half was completely dead and decomposing while the other half, or a few roaming arms, remained alive for up to 10 d postinjection, before eventually dying. These individuals (eight) were scored as survivors in our analyses, because they were still alive during the observation period.

3.2. Experiment 2: Volume

Increasing the volume of citric acid solution from 20 to 30 mL did not increase the percentage of mortality in the starfish injected once with the 120 g·L^{-1} solution, which remained at 83% (five out of six COTS died in both treatments) (Figure 2). However, increasing the volume accelerated the mean

time to immobility by around ~20%, from 43.8 ± 10.3 h (20 mL) to 37.2 ± 10.1 h (30 mL), although this difference was not statistically significant (Table 1). Time to death was also not significantly affected by changes in the injected volume (Table 1), although higher volumes resulted in a 27% decrease in time to death, passing from 60.7 ± 10.3 h (20 mL) to 47.8 ± 9.3 h (30 mL) (Figure 2).

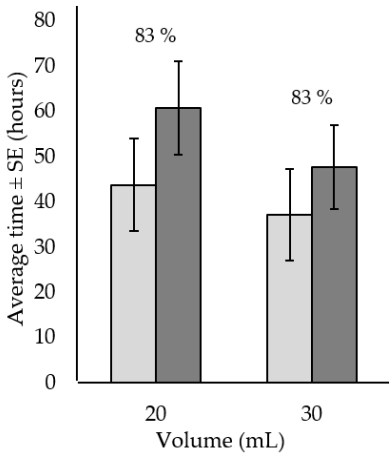

Figure 2. The effect of volume on response to citric acid injections. Mean time to immobility (□) and to death (■) ± standard error for *Acanthaster* cf. *solaris* injected in one site with either 20 mL or 30 mL of 120 g·L^{-1} citric acid and seawater solution. Numbers above bars represent total percent mortality for each treatment. Six replicates per treatment were used.

3.3. Experiment 3: Water Type

Seawater appeared to be a better solvent than distilled water, as it resulted in (1) higher mortality and (2) ~30% reduction in time to immobility and to death, although these differences were not significant (Figure 3, Table 1). Seawater killed five out of six COTS (or 83%), while distilled water only killed four out of six COTS (or 67%). Additionally, the seawater treatment caused immobility in 43.8 ± 10.3 h, while the distilled water solvent took 59.4 ± 22.5 h (Figure 3). Similarly, COTS injected with seawater solution died in 60.7 ± 10.3 h, while those injected with the distilled water solution died in 77.8 ± 22.6 h.

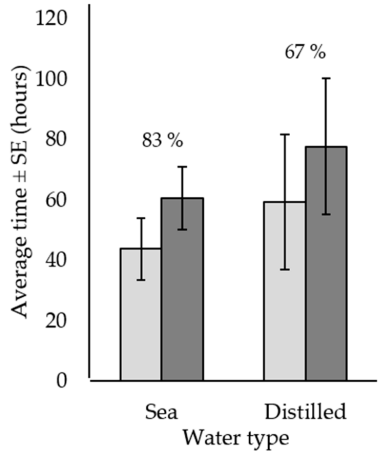

Figure 3. The effect of solvent on responses to citric acid injections. Mean time to immobility (□) and to death (■) ± standard error for *Acanthaster* cf. *solaris* injected in one site with 20 mL of 120 g·L^{-1} citric acid and either seawater or distilled water solution. Numbers above bars represent total percent mortality for each treatment. Six replicates per treatment were used.

Table 1. Results of the analysis of covariance (ANCOVA) on time to immobility and to death for *Acanthaster* cf. *solaris* injected with citric acid. Experiment 1 tests the response to acid concentration (90, 120, or 150 g·L^{-1}) and number of injection sites (two or four). Experiment 2 tests the effect of injection volume (20 mL or 30 mL). Experiment 3 tests the effect of distilled water or seawater as solvent for the citric acid. Body diameter was used as covariate in all models. Data for Experiment 1 were log transformed. * indicates significance at $P < 0.05$.

Source	DF	SS	MS	F	P
Experiment 1: two-way ANCOVA					
Log(Time to Immobility)					
Size	1	0.20	0.20	0.18	0.677
Concentration	2	8.94	4.47	3.91	0.031 *
Injection sites	1	1.74	1.74	1.53	0.226
Error	30	34.23	1.14		
Log(Time to Death)					
Size	1	0.01	0.01	0.02	0.882
Concentration	2	1.35	0.68	2.49	0.100
Injection sites	1	2.82	2.82	10.37	0.003 *
Error	30	8.16	0.27		
Experiment 2: one-way ANCOVA					
Time to Immobility					
Size	1	196.60	196.60	0.34	0.579
Volume	1	23.44	23.44	0.04	0.847
Error	7	4070.46	581.49		
Time to Death					
Size	1	709.08	709.08	1.43	0.270
Volume	1	96.74	96.74	0.20	0.671
Error	7	3454.80	493.54		
Experiment 3: one-way ANCOVA					
Time to Immobility					
Size	1	61.44	61.44	0.05	0.836
Water type	1	800.55	800.55	0.61	0.465
Error	6	7878.63	1313.11		
Time to Death					
Size	1	367.11	367.11	0.30	0.603
Water type	1	1241.10	1241.10	1.01	0.352
Error	6	7333.14	1222.19		

4. Discussion

This study demonstrated that injections of citric acid powder and seawater solution represent an efficient, economical, and easy-to-use method to cull crown-of-thorns starfish (COTS). We found that injecting 20 mL of seawater and citric acid powder at a concentration of 90–150 g·L^{-1} in two or four opposing arms effectively kills 100% of COTS, with times to death comparable to that of bile salts, vinegar, and lime juice (Table 2). Although time to death was higher for the two injections method (~58 h, [120 g·L^{-1} citric acid) compared to four (~26 h, [150 g·L^{-1} citric acid]), we argue that the reduced handling time makes the two injections method the most cost and time effective for operations in the field.

Mortality rates were significantly affected by both the number of injections and the concentration used. Indeed, for the single injection treatments, increasing the concentration from 90–120 g·L^{-1} to 150 g·L^{-1} reduced mortality from 83% to 33%. This concentration-related decrease in mortality may have occurred because the high localised dose of acid induced rapid, local necrosis of the injected area, with consequent loss of one or more arms, while the rest of the body survived. In contrast, all the multiple injection treatments reached 100% mortality, regardless of the concentration used, perhaps

because the acidic solution was more evenly spread throughout the starfishes' bodies. While the precision of mortality rates within each of our treatments is quite low (with six replicates, the error is 1/6 or 16.6%) multiple injections were conclusively effective at culling all COTS thus treated. All 36 specimens treated with multiple injections died (18 double and 18 quadruple injections, with a combined sampling error for multiple injections of 5.5%).

For the treatments that reached 100% mortality (double and quadruple injections), time to immobility significantly decreased with increasing concentration, dropping from 70 ± 19 h for the 90 $g \cdot L^{-1}$ to 37 ± 5 h for the 150 $g \cdot L^{-1}$ solution. Contrarily, time to death was not affected by concentration, but by the number of injections: four injections halved the time to death compared to two (respectively, 44 ± 7 h and 79 ± 12 h). However, due to the low number of replications for each treatment in this study, specific times to immobility and to death should only be used as guidelines for which is the most effective (fastest) method, and not as accurate indicators of true times to immobility and to death.

Another determinant factor affecting times to immobility and to death in this study was the incredible resistance of some roaming arms, which in some cases survived for up to 10 days postinjection. However, although COTS can regenerate from extensive tissue loss [22,23], it is unlikely that these roaming parts would have caused further coral loss, because of the absence of central disc and pyloric caeca, and the increased predation caused by chemoattraction of predators to injured COTS [24,25]. Therefore, we consider the lag time in reaching death, found herein, to be inconsequential.

Because none of the single injection treatments of Experiment 1 reached 100% mortality, we evaluated whether using distilled water as solvent or increasing the injected volume could improve the efficiency of citric acid single injections. Distilled water was expected to induce an osmotic shock in COTS tissues, thus increasing the efficiency of the citric acid injections. On the contrary, it reduced mortality and slowed time to immobility and to death. This may have happened because the hypoosmotic conditions caused by distilled water activated the opening of the water vascular system in attempt to restore the physiological osmolarity [26], leading to the exchange of water with the environment and consequently flushing out the citric acid. In contrast, COTS injected with seawater may not have had the possibility of doing so, lacking the hypoosmotic triggering factor. Therefore, we argue that distilled water should not be used as a solvent for the citric acid injections. Similarly, increasing the volume of a single injection of citric acid from 20 to 30 mL did not increase the percent mortality, although it moderately reduced time to immobility and to death. Likewise, a single injection of 10, 15, and 20 mL of lime juice failed to achieve 100% mortality and is therefore not an effective method to cull COTS [15]. We conclude that single injections of citric acid should not be performed on COTS in the field, given that mortality with single injection methods only reached 83%.

COTS death by citric acid injections is most likely induced by chronic pH stress caused by the low pH of the solution injected, which ranged between ~1.6 and 1.7. Similar mechanisms of death were proposed for vinegar (pH 2.2) and lime juice (pH 1.8) [12,15]. Indeed, echinoderms are poor acid–base regulators [27], and citric acid, like acetic acid, is both water-soluble and lipid-soluble, so it can easily perfuse into COTS tissues, where the low tissue pH causes protein degeneration and tissue necrosis [12,17].

This study showed that injection with citric acid is an efficient way of culling COTS, and thus of potentially controlling localised outbreaks. But why should this method be used over other current alternatives like sodium bisulphate, bile and cooking salts, vinegar, or lime juice? Compared to the single injection of bile salts [10] and vinegar [12], this method may be slightly more time consuming, because, from the data available, at least two injections per starfish are required to achieve 100% mortality. Nevertheless, citric acid has several characteristics that make it a valid alternative to those control methods (Table 2). First, compared to both sodium bisulphate and bile salts, it is generally available for purchase from a variety of stores. Indeed, rapid intervention, which is crucial to the successful control initiatives against COTS outbreaks [1,28,29], is possible only with a reliable and easy access to the chemical product. However, due to quarantine restrictions and high importation

cost, bile salts may be inaccessible for some remote island communities that experience recurrent COTS outbreaks throughout the Indo-Pacific. Secondly, citric acid has a favourable ecological profile (compared, for example, to sodium bisulphate) and is unlikely to accumulate in soil or sediment, as it is rapidly degraded by naturally occurring bacteria [30–32]. Additionally, predators feeding upon decomposing COTS would not be expected to suffer from ingestion of acidic tissues. Investigations on the environmental side effects of similar natural acidic products (lime juice and vinegar) have found no evidence of an impact on other marine organisms [12,15,17]. Third, in contrast to vinegar and lime juice, citric acid is easily transportable as a lightweight powder which can be mixed on site with seawater, reducing transportation costs and volumes on land. It also has a long shelf life, allowing storage in remote areas where fresh citrus juice may not be available or rapidly deteriorate. Additionally, compared to extracting juice from fresh limes, using powdered citric acid is far less labour intensive and more readily available (limes are seasonal and their price can vary greatly).

Until effective measures to prevent COTS outbreaks are found, having a collective workforce and/or volunteers "adopt a reef" may be the most efficient way of deploying COTS treatments and thereby preventing further mass coral predation. However, some important considerations need to be made. Firstly, permits need to be obtained from relevant local authorities. Secondly, safety guidelines for operators are needed to avoid spiking hazards from COTS spines and syringe needles, and for correct handling of citric acid powder to avoid skin, eye, and respiratory inflammations. Thirdly, further studies should aim at developing a single injection protocol for citric acid and investigate the efficiencies of injecting citric acid in different parts of COTS bodies (i.e., distal and medial portion of the arm and central disc). Finally, considering that the objective of COTS control programmes is prevention of extensive coral mortality and not the eradication of the species, injections should be carried out only if more than four to five COTS are counted during a 15 min swim, considered active outbreaking density [11]. If an outbreak is identified, citric acid solution [~90–150 $g \cdot L^{-1}$] can be easily prepared by mixing the solid powder with seawater; 20 mL of solution can then be administered with double or quadruple injections using any syringe attached to a veterinary 16 Ga × 1/2" needle, both easily available from any chemist store. It is important to note, however, that we caution against the use of citric acid until large-scale field trials have been undertaken. Citric acid is a promising alternative to existing COTS control techniques due to the ease of access, transport, storage, handling, and delivery compared to current methods. As such, it provides a new option to combat COTS outbreaks that is especially useful in remote locations and developing countries.

Table 2. Comparison of the chemicals currently available for injections of *Acanthaster* cf. *solaris* (crown-of-thorns starfish (COTS)).

	Absolute Lethal Dose (LD_{100})	Time to Death	Advantages	Disadvantages
Sodium bisulphate	Multiple injections of up to 180 mL [33] of 140 $g \cdot L^{-1}$ solution [8,9]	Unreported	-Highly effective	-Multiple injections required -Potent oxygen scavenger [8,9]
Bile salts	1 × 10 mL injection of 8 $g \cdot L^{-1}$ solution [10]	~28 h [10]	-Single injection -No known environmental side effects	-Not readily available in remote areas -Quarantine restrictions on access -<0.05 to 0.29 USD per injection [10,15,18,33]
Cooking salt	2 × 10 mL injections of 400 $g \cdot L^{-1}$ solution [13,16]	~48 h [16]	-Readily available -No known environmental side effects -<0.05 USD per COTS [13]	-High quantities required (8 kg/1000 COTS) -Solution preparation requires heating -Precipitation and crystallization [16]
Vinegar	2 × 10 mL injections [12,18] or 1 × 25 mL injection [15]	~30 h [15], ~40 h [12]	-Single injection -Readily available -No known environmental side effects -<0.05 USD per COTS [15]	-High quantities required (20–25 L/1000 COTS)
Lime juice	2 × 10 mL injections [15,18]	~20 h [15]	-No known environmental side effects [15]	-High quantities required (20 L/1000 COTS) -Laborious process for juice extraction -Seasonal and not ubiquitously cheap -Perishable
Powdered Citric acid [a]	2 × 10 mL or 4 × 5 mL injections of 90–150 $g \cdot L^{-1}$ solution	~26 h [b]	-Readily available, long shelf life -No known environmental side effects -<0.05 USD per COTS [a] -Easily transportable (180–300 g/1000 COTS)	Multiple injections required

[a] This study. [b] Four injections of 150 $g \cdot L^{-1}$ solution of citric acid.

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