Journal of Coastal Life Medicine

journal homepage: www.jclmm.com

Document heading doi:10.12980/JCLM.2.2014J53

Osmotic shock as alternative method to control *Acanthaster planci*

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PEER REVIEW

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Comments

This is an interesting but only preliminary research work in which authors have demonstrated that the osmotic shock could be a promising alternative method to control *A. planci* outbreaks. However, the authors honestly admit that this variety of chemicals employed as osmotic stressors also demonstrates several defects that could represent a huge logistical challenge for dive operators running large scale control programs.
Details on Page 105

ABSTRACT

Objective: To test six osmotic stressors as alternative methods to control *Acanthaster planci* (*A. planci*) outbreaks by exploiting their incapacity to tolerate drastic changes in osmolarity. Finding more effective ways to control *A. planci* outbreaks is one of the most immediate and effective ways by which to reverse rapid declines in the abundance of live coral cover in the Indo–Pacific.

Methods: A total of 10 mL of each of the following chemicals: sodium chloride, ethylenediaminetetraacetic acid, sodium carbonate, sodium cholate, sodium deoxycholate, urea and mannitol were injected into individual healthy sea stars to examine which chemicals induced disease and death.

Results: Four out of six chemicals used in this study induced disease. Sodium chloride, sodium cholate, sodium deoxycholate and ethylenediaminetetraacetic acid are capable of inducing death in injected sea stars offering an alternative option to control *A. planci* outbreaks.

Conclusions: Hyperosmotic stress is a viable alternative to control *A. planci* outbreaks as massive cell death results when acute hypertonicity exceeds a certain level.

KEYWORDS
COTS, Pest control, Outbreaks, Hyperosmotic stress, Cell permeabilisation

1. Introduction

Acanthaster planci (*A. planci*), the crown of thorns sea star (COTS) is the major natural enemy of reef–building corals amenable to direct intervention[1]. In recent years, COTS have had a detrimental effect on the already stressed and less resilient coral communities and caused more than 90% decline in coral cover in affected areas[2]. Cascading effects of *A. planci* outbreaks spread to the entire reef ecosystem and commonly lead to increases in benthic algae, loss of coral–feeding assemblages, collapse of reef structural complexity, and a decline in biodiversity and productivity[3]. As a result, control efforts are often carried out by local fishermen, dive clubs, non–governmental organisations, private resorts and management authorities to eradicate *A. planci* from reefs.

Attempts to control *A. planci* outbreaks have involved numerous techniques, including manual collection followed by burial ashore, cutting up, crushing COTS *in situ*, underwater fences, putting COTS in bags and injecting *A. planci* with a variety of chemicals that are noxious to the marine environment. For example, sodium hypochlorite (NaClO) reacts with organic matter when added to water, producing organic chlorine compounds

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Foundation Project: Supported by the Department of Sustainability, Environment, Water, Population & Communities – National Environmental Research Program – Tropical Ecosystems Hub. Grant–Research on early intervention options for outbreaks of crown–of–thorns starfish on the Great Barrier Reef.

Article history:
Received 23 Dec 2013
Received in revised form 2 Jan, 2nd revised form 12 Jan, 3rd revised form 17 Jan 2014
Accepted 5 Feb 2014
Available online 28 Feb 2014

such as AOX (halogenated organic compounds), which are highly toxic for aquatic organisms and are persistent environmental contaminants[4]; formaldehyde (CH_2O) is flammable, explosive, and carcinogenic; sodium bisulfate ($NaHSO_4 \cdot H_2O$) is a strong oxygen scavenger[5]; copper sulfate ($CuSO_4$) is highly toxic to fish and aquatic invertebrates[6]; ammonia (NH_3), ammonium hydroxide (NH_4OH) and many other toxic organic solvents have also been used in past control efforts[5,7-9]. In addition, control measures have been costly, largely ineffective in either eradicating the coral-feeding sea star or preventing further coral mortality, and *A. planci* outbreaks are increasing in frequency and intensity[2,10-15]. Against this backdrop, finding more efficient methods to control outbreaks is essential.

A. planci, similar to many invertebrates, which inhabit the marine environment, are stenohaline osmoconformers unable to tolerate drastic variations in environmental salinity, a circumstance that might provide novel opportunities to control COTS outbreaks. Their body fluids are similar to seawater in osmolarity, thus they are easily permeable to water and gain or lose water depending upon the concentration of the medium. When the protoplasm of these animals becomes slightly diluted, they can survive and carry on their metabolic functions. However, drastic changes in osmolarity cannot be tolerated. Amino acids and ions cannot be mobilised rapidly enough to compensate for sudden increases in salinity (hyperosmotic stress); the body volume is not regulated and often these animals either swell or shrink in proportion to their solute concentration[16]. For example, the body weight of *Golfingia* decreases or increases on transfer to high or low concentrations and reaches equilibrium in a few hours. Normally, the original body volume of these invertebrates is restored once they return to normal seawater[17]. However, drastic changes in osmolarity induce rapid tissue shrinking or swelling leading to damage of cell membranes and organelles, followed by death[18]. Hyperosmotic stress induces cell cycle arrest and apoptosis, increases DNA strand breaks, elevates reactive oxygen species, inhibits transcription and translation and induces mitochondrial depolarisation[19]. This is why *A. planci* is not found in estuaries, or river mouths where fresh and salt water meet and the salinity fluctuates greatly. In echinoderms in altered salinities, cell volume and isosmotic intracellular regulation is affected first by mobilisation of Na^+ and Cl^- followed by amino acids; intracellular K^+ and Ca^{2+} levels are conserved. Intracellular osmoregulation produces an altered intracellular ionic environment that affects membrane potentials and enzyme activities[16].

The aim of this study was to test several osmotic stressors (sodium chloride, ethylenediaminetetraacetic acid (EDTA), sodium carbonate, sodium cholate, sodium deoxycholate, urea and mannitol) as alternative methods to control *A. planci* outbreaks by exploiting their incapacity to tolerate drastic changes in osmolarity.

2. Materials and methods

2.1. Specimen collection and study site

A total of 40 *A. planci* specimens were collected at the Tandayag Marine Sanctuary in Amlan, Negros Oriental, Central Philippines (9°27.544′ N, 123°14.017′ E) (Figure 1). Local fishermen were free diving between 5-15 m depth and collected specimens by using improvised bamboo tongs. Specimens were immediately transported to the Institute of Environmental and Marine Sciences of Silliman University in Dumaguete city, Negros Oriental, Philippines. Sea stars were kept in large concrete tanks at a depth of 0.5 m with flow-through ambient seawater and left to acclimatise for 3 d. At Day 4, *A. planci* were distributed in eight groups of five sea stars and individually placed in 95 L plastic containers with flow-through seawater at ambient conditions (mean temperature 28 °C, pH=8.3).

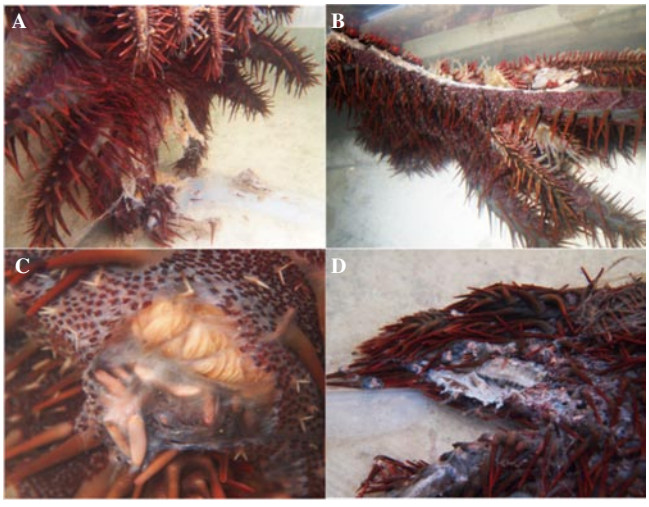

Figure 1. Clinical signs induced by osmotic stressors.
(A) *A. planci* showing large areas of necrotic tissue after sodium carbonate injections. (B) Sea star splitting in two after sodium chloride injections. (C) Localised lesion at the site of injection induced by urea. (D) Dead *A. planci* 24 h after sodium cholate/sodium deoxycholate injection.

2.2. Osmotic stressors tested

Six chemicals were used (Table 1). A total of 10 mL of each solution were injected with a 21-gauge syringe into individual healthy sea stars to examine which chemicals could induce osmotic shock followed by disease and death. COTS were individually placed in separate aquaria to observe their behavior, clinical signs, latency period of the disease and time to death (every 4 h). The clinical signs of disease evaluated were: (1) hyperactivity, (2) mucus production, (3) loss of turgor and swelling, (4) matting and loss of spines, (5) necrosis/blisters/lesions/exposed organs and (6) time to death. Severity indices of clinical signs of disease were assigned as: 1=low, 2=moderate and 3=strong reaction for each factor evaluated.

Table 1
Osmotic stressors tested and their concentrations in g/L.

Groups	Chemical components tested	Concentration (g/L)
Group 1	Sodium chloride (NaCl)[c]	300.0
Group 2	Sodium chloride (NaCl)[c]	400.0
Group 3	Ethylenediaminetetraacetic acid (EDTA)[a]	120.0
Group 4	Sodium cholate ($C_{24}H_{39}O_5$ Na) and Sodium deoxycholate ($C_{24}H_{40}O_4$) – (bile salts)[b]	8.0
Group 5	Sodium carbonate (Na_2CO_3)[c]	400.0
Group 6	Urea ($CO(NH_2)_2$[b]	400.0
Group 7	Mannitol 20% infusion ($C_6H_8(OH)_6$)[d]	0.2
Group 8	Control sea stars	

[a]Sigma-Aldrich, MO, USA. [b]Oxoid, Hampshire, UK. [c]HiMedia, Mumbai, India. [d]Sahar Pharmaceuticals, Paranaque, Philippines.

3. Results

Four out of six osmotic stressors tested in this study induced disease and death in injected *A. planci*. Sodium chloride 400 g/L, sodium cholate and sodium deoxycholate 8 g/L and EDTA 120 g/L induced 100% mortality. Sodium carbonate 400 g/L induced death in three out of five sea stars injected. The two survivoring *A. planci* showed strong areas of necrosis that covered half their body size but started recovering after 5 d. Sodium chloride at 300 g/L also induced death in three out of five sea stars injected. However, those that survived the injection of cooking salt split in two and started recovering after 5 d. Urea 400 g/L induced localised lesions at the site of injection and mannitol 20 g/L did not induce any kind of allergic reactions or lead to symptoms of disease (Figure 1).

3.1. Sodium chloride

Cooking salt 400 g/L induced death in all injected COTS. Two sea stars died at 24 h post-injection and the remaining three at 48 h. Loss of turgor/swelling was low to moderate at 24 h. COTS injected with NaCl 400 g/L also displayed moderate amount of blisters/lesions and exposed organs at 24 and 48 h. There was low production of mucus in all sea stars tested. On the other hand, NaCl 300 g/L only induced death in 60% of injected sea stars (three out of five). Time to death was 48 h in average requiring double amount of time to induce death when compared to COTS injected with sodium cholate and deoxycholic acid 8 g/L. The amount of blisters/lesions/

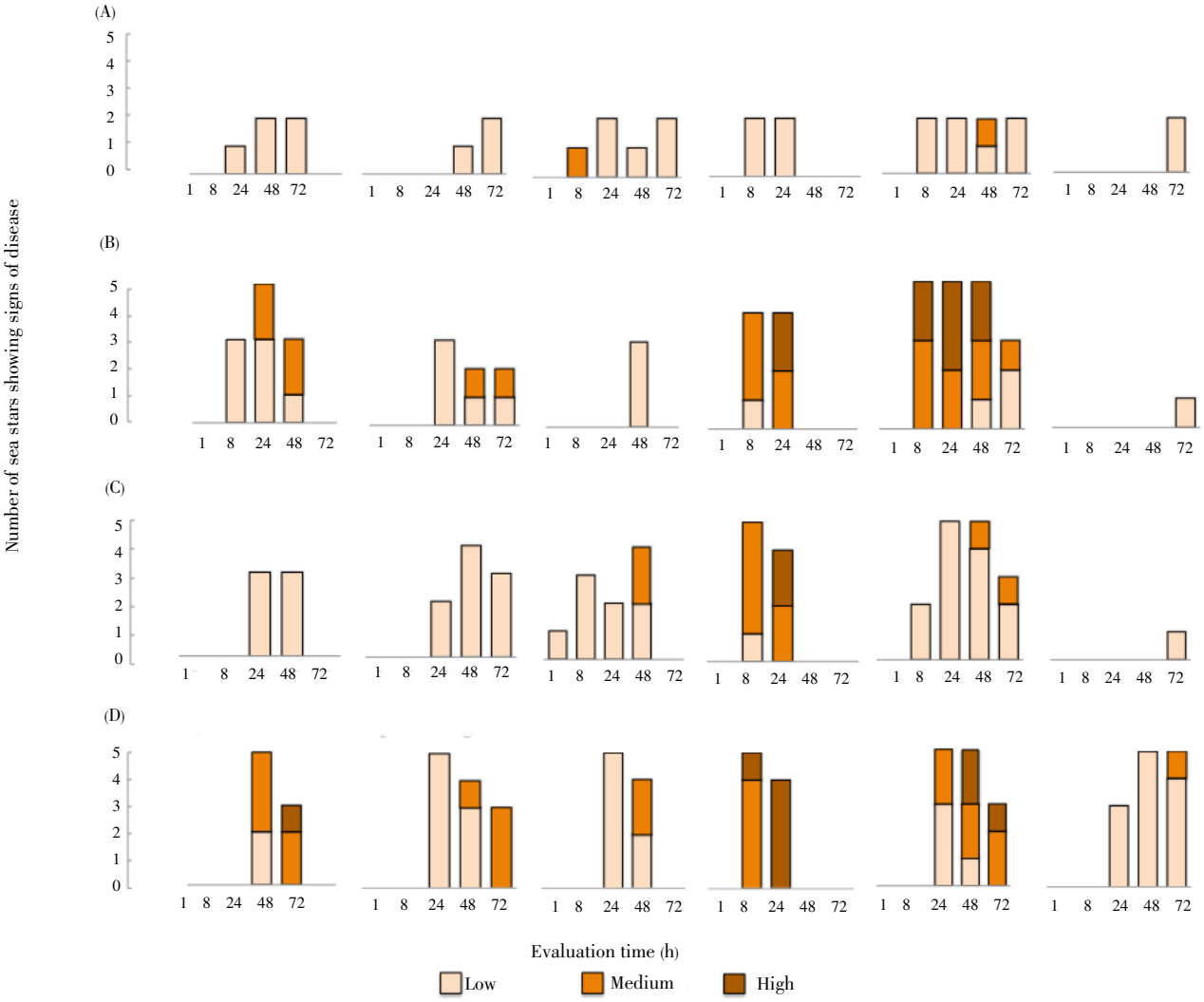

Figure 2. Time of appearance, severity, and number of individuals showing signs of disease.
(A) mucus secretion, (B) loss of body turgor and swelling, (C) matting of spines, (D) blisters, lesions, exposed organs. Levels of severity ranged from low to high (*i.e.* localized to widespread manifestation of clinical signs of disease).

exposed organs was low at 24 and 48 h and increased to moderate at 72 h. Forty percent of the sea stars (two out of five) survived the injections of sodium chloride 300 g/L (Figures 2 and 3). Survival COTS split in two after 48 h and started recovering after 5 d.

3.2. Sodium cholate and sodium deoxycholate

Bile salts produced 100% mortality in injected sea stars. One sea star died at 8 h and the other four at 24 h after the injection. COTS injected with sodium cholate and sodium deoxycholate died faster (24 h) and with lower doses (8 g/L) when compared to other chemicals used in this study such as NaCl, Na_2CO_3, $CO(NH_2)_2$ (400 g/L) that also required double or triple amount of time (up to 72h) to induce death in injected *A. planci*. All sea stars showed moderate hyperactivity post-injection; moderate loss of body turgor and matting of spines were observed at 8 and 24 h. There was low mucus production in all injected *A. planci*.

3.3. EDTA

EDTA induced 100% mortality in all injected sea stars. One *A. planci* died at 24 h and the other four at 48 h. Loss of turgor/swelling was observed in three out of five *A. planci* at 48 h post-injection. Matting/loss of spines and blisters/lesions/exposed organs were moderate in two out of five sea stars injected with EDTA (Figure 3).

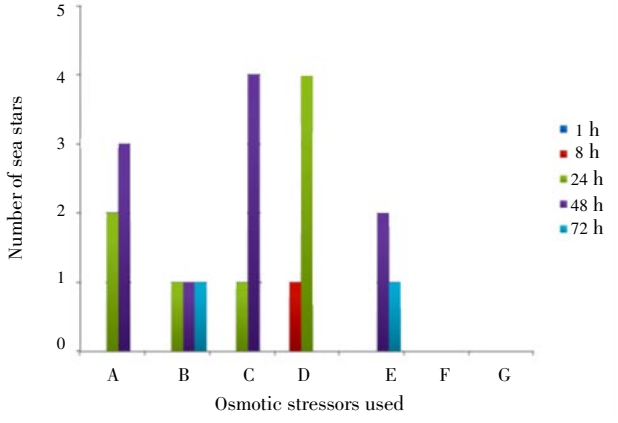

Figure 3. Mortality of *A. planci* challenged with osmotic stressors. (A) NaCl 400 g/L, (B) NaCl 300 g/L, (C) EDTA 120g/L, (D) sodium cholate-deoxycholate, (E) Na_2CO_3 400 g/L, (F) Urea 400 g/L, (G) Mannitol.

3.4. Sodium carbonate

Washing soda caused death in 60% of *A. planci* challenged (three out of five). All *A. planci* injected with sodium carbonate left a white precipitate behind them when they started moving around the tank after injection and marked swelling. Matting of spines was low in four out of five sea stars at 48 h. Large amounts of necrotic tissue, blisters and exposed organs were observed in three out of five *A. planci*. Mucus production was low.

3.5. Urea

Carbamide did not induce death in any *A. planci* tested. All COTS injected with urea showed small areas of necrosis at the site of injection (Figure 1) and low levels of hyperactivity. *A. planci* start recovering after 48 h.

3.6. Mannitol

$C_6H_8(OH)_6$ did not induce any signs of disease in injected *A. planci* which could be related to the low dose used in this study (Figure 3).

4. Discussion

Adjustment of amino acids is essential for intracellular ionic regulation, a process that is as important to the short-term distribution of echinoderms in altered salinities as cell volume regulation[20]. For echinoderms transferred to altered salinity, the adjustment of intracellular osmolarity occurs initially as inorganic ions move out of, or into, the cells, but the change in osmolarity is sustained by the change in free amino acids. The coordinated adjustment of different types of solutes allows for cell volume regulation as well as the recovery or accumulation of K^+ or Ca^{2+}, the intracellular concentrations of which are critical to metabolic and neuromuscular function and can adversely affect physiological processes, such as absorption efficiency, growth and reproduction[16,21].

4.1. Sodium chloride

NaCl (cooking salt) is hypertonic and it is considered the principal determinant of tonicity of the extracellular fluid. Na greatly influences the distribution of water among the different organs[19]. In this case, hyperosmotic stress induced by injections of NaCl-drew water out of the cells through osmosis, which inhibits the transport of substrates and cofactors into the cell inducing osmotic shock and cell damage. NaCl as alternative *A. planci* control method offers several advantages. It is a 100% natural product available anywhere in the world and no permits or special handling procedures are required. Another extended advantage is that cooking salt is cheap and can be obtained through the process of evaporation of seawater on the beach. This is a critical aspect of all control efforts, especially in developing countries where funds for controlling *A. planci* outbreaks are limited. NaCl is a good option in those cases of incipient outbreaks or in areas where locals want to keep the normal sea star densities in check. However, NaCl also demonstrates several inherent drawbacks such as:

(1) The high concentration of salt that is required to kill COTS (400 g/L) represents a huge logistical challenge for dive

operators running large scale control programs, due to the immense amount of salt that is required to be transported in their boats. It is important to recognise that COTS outbreaks may comprise in excess of 151 650 sea stars/km^{-2}[3].

(2) The solution reaches the saturation point of NaCl during preparation of the solution, making it necessary to heat the solution to increase solubility (*i.e.* stoves required).

(3) Precipitation and formation of crystals was observed at the bottom of the flasks 48 h after preparation when the temperature of the solution drops. Thus needles, syringes and guns could become blocked. More importantly, the concentration of injected salt could become lower, due to precipitation of NaCl. In this study 40% of sea stars injected with 300 g/L split in two and began recovering after 5 d, which could potentially create an even bigger problem.

(4) Sodium chloride is well known for its corrosive properties, and therefore there is an intrinsic risk that the process of corrosion of the equipment is accelerated. In addition, measures to avoid salt hardening due to the high humidity at sea should be implemented (*e.g.* long trips to remote areas).

(5) Time to death is another key factor that should be considered. NaCl injections induced death in approximately 2-3 d, which will represent a logistic challenge for managers and boat operators who will need to wait for that period to confirm the successful cleaning of the reef, or return to the same spot after several days which will increase enormously the cost of control efforts.

4.2. Sodium cholate and deoxyclolic acid

These bile salts each demonstrate unique properties that enable manipulation (disruption or formation) of hydrophobic-hydrophilic interactions among molecules in biological samples. In biological research, these detergents are used to lyse cells (release soluble proteins), solubilise membrane proteins and lipids, control protein crystallisation, prevent non-specific binding in affinity purification and immunoassay procedures, and as additives in electrophoresis[22]. Bile salts induce alterations in membrane fluidity associated with impairment of mitochondrial respiration and mitochondrial depolarisation leading to apoptosis or necrosis. Necrosis is best characterised by cell death via swelling and lysis which releases inflammatory cytokines[23]. This study showed that the best option to kill *A. planci* is bile salts. Low doses are required to induce 100% mortality when compared to the other chemicals used in this study. In addition, these chemicals offer the advantage of easy transport, rapid death (<24 h) and the mechanism of induction of disease is related to necrosis and/or apoptosis, and not transmissible disease. Moreover, bile salts can be easily degraded by many bacteria (energy source) and it is transformed and degraded in the digestive tract of fishes and other vertebrates that feed on *A. planci* remains[24].

4.3. EDTA

EDTA is a man-made amino acid chelating (binding) agent that has been used extensively as a food additive to sequester trace metals that catalyse the oxidation of oils, vitamins, and unsaturated fats that cause rancidity, flavour changes, and discoloration. Permissible levels of EDTA calcium disodium salt in food range from 25-800 mg/L, and an acceptable daily intake of 2.5 mg/kg was established by the Joint FAO/WHO Expert Committee on Food Additives in 1973. EDTA salts are soluble in water, have low sorption to soil and sediments, and have a biodegradation half-life of weeks to months[25]. EDTA has also been used in medicine for chelation therapy in cases of lead poisoning, in which EDTA renders the toxic ions present in the body non-bioavailable, essentially harmless. The EDTA is administered intravenously and pulls toxic heavy metals detected in the bloodstream towards itself and then attaches itself to these metal ions. This attachment forms a compound that can be excreted from the body through urine, not allowing them to bind to enzymes and cytochromes[26]. Its ability to sequester metal ions such as Ca^{2+} and Fe^{3+} that are necessary for the proper functioning of the body are likely to be involved in the induction of disease and death of *A. planci*. Intracellular concentrations of Ca^{2+} and K^{+} are critical to metabolic and neuromuscular functions in echinoderms[21]. If, instead, these minerals were bound to EDTA, then deficiencies of these trace ions will adversely affect physiological processes resulting in disease[21,27]. In echinoderms, it has been shown that failure of K^{+} and Ca^{2+} to be accumulated led to deterioration of metabolic processes, despite cell volume regulation. Tissues of *Luidia clathrata* and *Isostichopus badionotus* tended to deteriorate upon exposure to higher salinity but not to lower salinity[27].

4.4. Sodium carbonate

It is synthetically produced in large quantities from salt (sodium chloride). Sodium carbonate is used as a water softener in laundering because it competes with the magnesium and calcium ions in hard water and prevents them from bonding with the detergent being used. In the process of dyeing, it is used to ensure proper chemical bonding of the dye with cellulose fibres. The induction of disease and death in injected *A. planci* could be related to the precipitation properties of the sodium carbonate. All *A. planci* injected with sodium carbonate left a white sand precipitate behind them when they started moving around the tank after injection and showed a marked swelling (loss of skin turgor). The sodium carbonate test is widely used to distinguish between different metal ions *e.g.*, a white precipitate indicates the presence of Ca as opposed to other metal ions.

4.5. Urea

High concentrations of urea are stressful to cells, altering their function or even killing them by apoptosis[19,28]. Urea produces rapid arrest in all phases of the cell cycle and oxidative DNA damage with elevation of reactive oxygen species[29,30]. However in this study, urea was found to be not lethal and only induced tissue damage at the site of injection. The low tissue damage induced by urea injections could be related to the mutually protective effects of high urea and high sodium chloride. Their effects are not additive and one can protect against the other, *e.g.*, pretreatment with NaCl protects MDCK cells from high urea-induced apoptosis. The experiments were carried out on cells conditioned by increasing osmolality from 290-600 mosmol/kg H_2O by adding NaCl. Upon subsequent exposure to an additional 600 mmol/L urea in the medium for 24 h, 90% of the osmotically conditioned cells, but only 15% of non-conditioned cells survive[31]. In this case, the high concentration of salt provided by seawater could neutralise the effect of high concentrations of urea.

4.6. Mannitol

It is one of the most abundant energy and carbon storage molecules in nature, produced by a plethora of organisms, including bacteria, yeasts, fungi, algae, lichens, and many plants. Mannitol is used clinically in osmotherapy to reduce acutely raised intracranial pressure after head trauma by increasing water and Na^+ excretion, thereby decreasing extracellular fluid volume. Mannitol is also used for the treatment of acute glaucoma in veterinary medicine. It is administered as a 20% solution intravenously to dehydrate the vitreous humor and, thus, lowers the intraocular pressure[32]. In this study, mannitol did not produce any clinical signs of disease in injected COTS, which could be related to the low doses used when compared to the other osmotic stressors.

Here we showed that hyperosmotic stress is a viable alternative to control *A. planci* outbreaks. Massive cell death results when acute hypertonicity exceeds a certain level. Studies in which osmolality is increased by 300 mosmol/kg H_2O or more showed that hyperosmolality lead to apoptosis[27,33]. Hypertonicity-dependent apoptosis triggers components of both the intrinsic and extrinsic pathway. Through the intrinsic pathway hypertonicity causes a rapid mitochondrial dysfunction that precedes apoptosis[28] and via the extrinsic pathway they produce their own death ligand and self-destruct[34].

4.7. Other options

4.7.1. Chemical permeabilisation

Chemical methods have been employed in order to extract intra-cellular components from micro-organisms by permeabilising the outer-wall barriers. It can be achieved with organic solvents that act by creation of canals through the cell membrane: toluene, ether, phenylethyl alcohol, dimethyl sulfoxide, methanol, chloroform, and benzene work in this way and some of them have been previously used to kill COTS[7]. However, it is important to consider their toxicity, *e.g.*, ethanol in surface water results in complete depletion of dissolved oxygen, as evidenced by the fish kill documented in Lawrenceburg, KY, USA in 2000. In addition, ethanol drastically reduces water pH-inhibiting the bioremediation processes in the ocean[35]. Moreover, C_2H_6O (ethanol) is flammable and conducts electricity, thus electrocution and ignition hazards are present during operations involving the use of ethanol. Nonetheless, chemical permeabilisation can also be achieved by the use of many other substances such as antibiotics, thionins, surfactants (Triton, Brij, Duponal), chaotropic agents, and chelates. The chelating agent EDTA that was used in this study is one example. EDTA is widely used for permeabilisation of Gram-negative micro-organisms in laboratories. Its effectiveness is a result of its ability to bond the divalent cations Ca^{++} and Mg^{++} that stabilise the structure of outer membranes, by bonding the lipopolysaccharides to each other. Once EDTA sequesters these cations, the lipopolysaccharides are removed, resulting in increased permeability areas of the outer walls inducing cell death. Chaotropic agents, such as urea (also tested in this study) and guanidine are capable of bringing some hydrophobic compounds into aqueous solutions. They accomplish this by disrupting the structure of water, making it a less hydrophilic environment and weakening the hydrophobic interactions among solute molecules.

4.7.2. Enzymatic permeabilisation

Enzymes can also be employed to permeabilise cells, but this method has been limited to releasing periplasmic or surface enzymes in laboratories. They often use EDTA in order to destabilise the outer membrane of Gram-negative cells, making the peptidoglycan layer accessible to the enzymes used. Some of the enzymes used for enzymatic permeabilisation are: beta(1-6) and beta (1-3) glycanases, proteases, and mannase.

Exploring more effective methods to control *A. planci* outbreaks is a critical step in reversing coral loss and reef degradation. Like all echinoderms, crown-of-thorns sea stars are highly susceptible to changes in osmolarity, which may be exploited to improve the effectiveness of population controls. In this study, the induction of disease and death in *A. planci* is related to cell membrane damage caused by hyperosmotic stress, rather than hypo-osmotic exposure, which only decreases organismal activity in asteroids[36,37]. Glycine and sodium bicarbonate do not induce death in *A. planci* because the sea star lives in an environment

of high salinity. Thus *A. planci* are capable of easily re-establishing their normal osmolarity through their water vascular system.

Acknowledgments

Funding for this project was provided by the Department of Sustainability, Environment, Water, Population & Communities – National Environmental Research Program – Tropical Ecosystems Hub. Grant- Research on early intervention options for outbreaks of crown-of-thorns starfish on the Great Barrier Reef.

We would like to thank Mr. Job Tagle and Mr. Dionaldo Omole of the Amlan Municipal ENRO for organising local fishermen led by Mr. Virgilio Aviso to help collect specimens; we also wish to thank Ciemon Caballes and Dr. Janet S. Estacion for laboratory and logistical assistance at the Silliman University Institute of Environmental and Marine Sciences.

Conflict of interest statement

We declare that we have no conflict of interest.

Comments

Background

Outbreaks of *A. planci*, represent one of the most significant biological disturbances on reef ecosystems. Removals of *A. planci* are crucial initiatives in limiting the damage to coral communities and enable gradual recovery of reef ecosystems. Despite more than 3 decades of research control methods have been unsuccessful as well as costly, time consuming and difficult to accomplish.

Research frontiers

The present research work shows that single injections of several osmotic stressors, primarily sodium salts that are not noxious to the marine environment, are capable of inducing death in *A. planci* and could offer an alternative option to other techniques employed in the past to control *A. planci* outbreaks.

Related reports

Echinodermata is considered one of the only stenohaline phyla in the animal kingdom. The aim of this research was to test several osmotic stressors as alternative methods to control *A. planci* outbreaks by exploiting their incapacity to tolerate drastic changes in osmolarity.

Innovations and breakthroughs

In the past, attempts to control *A. planci* outbreaks have involved numerous techniques, including injecting this species with a variety of chemicals that are noxious to the marine environment. As far as injection of NaCl as alternative method is concerned it offers several advantages. It is a natural and cheap product available anywhere in the world. However, it also demonstrates several difficult problems to solve.

Applications

This study shows that single injections of several osmotic stressors primarily sodium could offer an option to control *A. planci* outbreaks, but the method obviously requires a number of improvements to be considered a viable alternative to other current control methods that were in any case ineffective in either eradicating the coral-feeding starfish or preventing further coral mortality.

Peer review

This is an interesting but only preliminary research work in which authors have demonstrated that the osmotic shock could be a promising alternative method to control *A. planci* outbreaks. However, the authors honestly admit that this variety of chemicals employed as osmotic stressors also demonstrates several defects that could represent a huge logistical challenge for dive operators running large scale control programs.

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