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Lethal doses of oxbile, peptones and thiosulfate-citrate-bile-sucrose agar (TCBS) for *Acanthaster planci*; exploring alternative population control options

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ABSTRACT

Effective control of outbreaks of *Acanthaster planci* represents the most immediate and practical intervention to reverse sustained declines in coral cover on reefs in the Indo-Pacific. This study explored the minimum doses of oxbile, oxgall, and thiosulfate-citrate-bile-sucrose agar (TCBS) that result in reliable and comprehensive mortality when injected into adult *A. planci*. The minimum doses required to induce 100% mortality among starfish ($n = 10$) were 4 g l^{-1} of oxbile, 8 g l^{-1} of oxgall and 22 g l^{-1} of TCBS. Moreover, there was no evidence of unintended side effects for other coral reef organisms (e.g., scleractinian corals, echinoderms and fishes) when using oxbile, oxgall, or TCBS at minimum doses. The effectiveness of peptones in killing crown-of-thorns starfish was also tested, but inconsistency in the results revealed that these proteins are unreliable.

1. Introduction

Outbreaks of the crown-of-thorns starfish, *Acanthaster planci*, represent one of the most significant biological disturbances on coral reefs (Kayal et al., 2012). Despite recent increases in the prevalence of climate induced coral bleaching and coral disease (Yakob and Mumby, 2010), outbreaks of *A. planci* remain one of the principal causes of coral loss in the Indo-Pacific (Rivera-Posada et al., 2012). On Australia's Great Barrier Reef (GBR), for example, it is estimated that 40% of coral loss recorded over the last 27 years is due to reef-wide outbreaks of *A. planci* (De'ath et al., 2012). Given widespread declines in coral cover (Bellwood et al., 2004; Bruno and Selig, 2007) and associated degradation of coral reef ecosystems (Pratchett et al., 2009), there is an urgent need to identify immediate and practical interventions that will reduce or reverse sustained declines in coral cover.

Outbreaks of *A. planci*, rank alongside climate change, severe tropical storms and increasing prevalence of coral disease, as one of most significant threats to coral reefs (De'ath et al., 2012), but of these threats, outbreaks of *A. planci* are probably the only threat that is amenable to direct intervention. In the last few decades, it is estimated that >17 million starfishes have been killed or removed from coral reefs in the Indo-Pacific (Pratchett et al., 2013). Control measures have been costly, largely ineffective, and often involved dangerous side effects. Currently the most efficient technique to kill *A. planci* is to inject individual sea stars with lethal doses of chemicals. A variety of chemicals have been used since 1960s to control *A. planci* but are noxious to the marine environment. For example, formaldehyde (CH_2O) is well known for his flammable, explosive, and carcinogenic properties; copper sulfate ($CuSO_4$) is highly toxic to fish and aquatic invertebrates, such as crabs, shrimps, and oysters (Yanong, 2010). Sodium hypochlorite (NaClO), ammonia (NH_3), ammonium hydroxide (NH_4OH) and many other toxic organic solvents have been also used in past control efforts (Birkeland and Lucas, 1990; Harriott et al., 2003). Sodium bisulfate is currently considered the best option to kill COTS *in situ*. However, this requires careful administration of solution into multiple areas of oral disk and arms, otherwise starfish experience only localized tissue damage and persist. Moreover, high concentrations (140 g l^{-1}) and volumes (60 ml of solution per sea star) of sodium bisulfate are used in controlling outbreak populations, which may comprise in excess of 53,750 sea stars per km^{-2} (Kayal et al., 2011). In addition, sodium bisulfate is a strong oxygen scavenger widely used to inhibit corrosion and remove traces of residual oxygen or chlorine in the brine recirculation systems of desalination plants at doses of just 0.5 mg l^{-1} (Abuzinada et al., 2008; Lattemann and Höpner, 2008). Current best practice is time consuming, expensive and difficult to accomplish in large areas.

Other control techniques include hand collection of sea stars for disposal on land, cutting up and construction of physical barriers.

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Hand collection limits the potentially deleterious effects of poisoning, but is very expensive, labor intensive and time consuming. Numerous boats must be on hand for the estimated number of participants, pre and post-surveys are required, there is a high risk of serious spiking of divers and people involved in the transfers in and out of the boat. Cutting sea stars into pieces was one of the first methods implemented in the late 1960s and is still used in the Gulf of Oman (Mendonça et al., 2010). However, it is not recommended due to the regeneration capabilities of the sea star creating an even bigger problem (Messmer et al., 2013). Similarly, installing fences in tourism areas to prevent movement of adult sea stars was used in the 1980s. However fences (1) cannot stop migration of the sea star's larvae or small juveniles; (2) are expensive, especially when maintenance is taken into account; (3) difficult to construct in rugged areas as the bottom of the fences must be in close contact with the substrate and there are many different topographic features in the reef; and (4) they are prone to damage in heavy seas and cyclones (Harriott et al., 2003; Rivera-Posada et al., 2012). While few of these control programs have been effective in ending outbreaks or preventing subsequent coral loss at small scales (Birkeland and Lucas, 1990), the problem lies mostly with inherent inefficiencies in the methods used. Developing more effective and less harmful methods to control *A. planci* outbreaks is therefore vital to minimize coral loss and allow affected coral reefs to recover.

Rivera-Posada et al. (2012) demonstrated that single injections of low concentrations of proteins contained in the TCBS formula induced rapid death of *A. planci*, representing a novel and potentially much more efficient method for population control. They found that four out of nine TCBS medium culture ingredients induced disease and death in *A. planci*. Oxgall and peptone were reported as the most effective inducing 100% mortality in injected sea stars, but several factors need to be considered before field testing these potential control methods. The purpose of this study was to (1) establish the minimum dose of oxbile, peptone and TCBS that will reliably and comprehensively kill *A. planci*; and (2) explore possible side-effects associated with the use of these chemicals, testing for any evidence of disease or ill-health in other coral reef organisms (e.g., corals, fishes and other echinoderms) that feed on or are in close contact with dying *A. planci*.

2. Materials and methods

2.1. A. planci collection

A total of 397 adult *A. planci* specimens were collected at the Tandayag Marine Sanctuary in Amlan, Negros Oriental, central Philippines (9° 27′ 10.12″ N, 123° 14′ 14.81″ E) by local fishermen who were freediving up to 15 m depth and collected starfish using improvised bamboo tongs. Specimens were transported to the Institute of Environmental and Marine Sciences of Silliman University (SU-IEMS) in Dumaguete, Negros Oriental, Philippines and kept in 2 m^3 concrete tanks with flow-through ambient seawater and left to acclimatize for 3 days. Weak and damaged individuals were discarded.

2.2. Chemical reagents tested

Peptones, bile derivatives, TCBS, and yeast were tested to determine lethal doses (Table 1). Peptones used were bacteriological peptone, proteose peptone, special peptone, peptone EHCK, peptone 2400, and peptone 2382. Bacteriological peptone is mixed pancreatic and papaic digest of different animal proteins containing a wide molecular weight distribution of peptides. Proteose peptone is enzymatic digest of animal proteins with high content of low molecular weight proteoses used to create an environment beneficial to the maintenance of virulence and the elaboration of bacterial by-products. Special peptone is prepared from meat, plant and yeast digest which contains the widest spectrum of peptide structures available in any peptone. Peptones EHCK, 2400 and 2382 are pancreatic digest of casein and whey (milk derivatives) with different molecular weights. Oxgall is dehydrated fresh bovine bile while bile salts N3 may be effective at less than one-third of the normal concentration of bile salts and are usually added as selective inhibitory agents in culture media.

2.3. Lethal dose experiments

Ten 95-l plastic bins were placed inside a large concrete tank, which served as a water bath. The depth of seawater in the concrete tank was set to 20 cm, about half of the depth inside the plastic bins to maintain ambient temperature (28.5 °C) within each individual bin. Each plastic bin was supplied with constant flow of fresh seawater (40 l/min). Ten seemingly healthy sea stars (15–25 cm) were haphazardly selected from the stock and placed in individual bins. Ten ml of each chemical at different concentrations (Table 1) were injected to each sea star using a 21-gauge syringe. There were 10 replicates for each chemical tested except for bacteriological peptone (200 g l^{-1}), peptone EHCK (100 g l^{-1}), peptone 2400 (200 g l^{-1}), and peptone 2382 (200 g l^{-1}), where only 5 replicates were used because of the inefficacy and variability in results displayed by those types of peptones.

The reaction of sea stars was evaluated at 1 h, 8 h, 24, and 48 h after injection. The following clinical signs of disease or reactions were monitored: (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, following Rivera-Posada et al. (2011).

2.4. Disease transmission

Fish, corals, and other invertebrates (Table 2) were collected from Bantayan Reef, Dumaguete (9° 19′ 56.1″ N, 123° 18′ 38.06″ E) across the SU-IEMS Marine Laboratory. Fish were collected by local fishermen using hand nets and fish traps. Experiments were conducted using four concrete tanks (3 m long × 1 m wide × 0.5 m deep) with flow-through seawater at ambient conditions (mean temperature = 28 °C, salinity = 33 ppt, pH = 8.3). Half of each coral colony was enclosed in a wire cage to ensure that a portion of every coral survived despite feeding activities of newly introduced *A. planci* (Fig. 1). Coral fragments and colonies (~15 cm L × W × H) were arranged in a way that the least preferred species were closest to the seawater inlet and the injected sea stars, while the most preferred species were farthest (Pratchett, 2007). Fish and mobile invertebrates were also placed in the tanks. Eight sea stars were separated in pairs and one *A. planci* was injected with 10 ml oxgall (8 g l^{-1}), oxgall (4 g l^{-1}), peptone (20 g l^{-1}), and TCBS (44 g l^{-1}) at day 1 and the remaining one at day 4. All starfish were placed near the seawater inlet of Tanks 1–4, respectively. Interaction between all the animals in the tank was recorded for 4 h in the morning and 4 h in the afternoon using a GoPro Hero 2 HD video camera. Signs of disease such as darkened coloration to the skin and fins, erythema, changes to the eyes such as distension and cloudiness, periorbital swelling, haemorrhagic septicaemia and mortality were monitored every 8 h for 12 days.

3. Results

3.1. Lethal doses

Mortality rates were highest in individuals injected with bile derivatives (bile salts, oxgall) and TCBS, while mortality rates in

Table 1
Chemical components tested and their doses in g l^{-1}. TCBS standard concentration is based on prescribed amounts of each component per liter as specified in manufacturer's formulation.

Chemical components tested		TCBS standard (g l^{-1})	DOSE (g l^{-1})
Peptones	Bacteriological peptone[a,b]	10	200, 20, 10, 5
	Proteose peptone[c]	10	20, 10, 5
	Special peptone[a]	10	20, 10, 5
	Peptone EHCK[d]	10	100
	Peptone 2400[d]	10	200
	Peptone 2382[d]	10	200
Bile derivatives	Bile salts N3[a]	8	8, 4, 2
	Oxgall[c]	8	4, 2
Others	TCBS[a]	88	44, 22
	Yeast[c]	5	10, 5

[a] Oxoid, Hampshire, UK.
[b] Himedia, Mumba, India.
[c] Difco, MD, USA.
[d] Tatua, Morrinsville, NZ.

Table 2
Animals used in disease transmission experiments. Values indicate number of fragments (corals) or individuals (fish, other invertebrates) used per tank. Tank 1 (T1): Oxgall 8 g l^{-1}; Tank 2 (T2): Oxgall 4 g l^{-1}; Tank 3 (T3): Peptone 20 g l^{-1}; Tank 4 (T4): TCBS 44 g l^{-1}.

Category		Species	T1	T2	T3	T4
Fish	Balistidae	*Balistoides viridescens* (Bloch and Schneider 1801)	1	1	1	1
		Abalistes stellatus (Anonymous 1798)	0	1	1	1
	Tetraodontidae	*Arothron hispidus* (Linnaeus 1758)	1	1	1	1
		Arothron mappa (Lesson 1831)	0	1	0	0
		Arothron caeruleopunctatus (Matsuura 1994)	1	0	0	0
	Pomacentridae	*Chromis caerulea* (Cuvier 1830)	5	4	5	5
		Pomacentrus moluccensis (Bleeker 1853)	4	5	4	4
	Chaetodontidae	*Chaetodon auriga* (Forsskål 1775)	1	0	0	0
Corals	Acroporids	*Acropora aspera* (Dana 1845)	1	1	1	1
		Acropora nasuta (Dana 1846)	1	1	1	1
		Acropora palifera (Lamarck 1816)	2	2	2	2
	Pocilliporids	*Pocillopora damicornis* (Linnaeus 1758)	1	1	1	1
		Seriatopora hystrix (Dana 1846)	1	1	1	1
	Fungids	*Fungia fungites* (Linnaeus 1758)	2	2	2	2
	Poritids	*Porites cylindrica* (Dana 1846)	1	1	1	1
		Porites lutea (Milne Edwards and Haime 1860)	2	2	2	2
Other invertebrates	Asteroidea	*Linckia laevigata* (Linnaeus 1758)	2	2	2	2
		Protoreaster nodosus (Linnaeus 1758)	1	1	1	1
	Holothuroidea	*Holothuria atra* (Jaeger 1833)	2	2	2	2
		Pearsonothuria graeffei (Semper 1868)	1	1	1	1
	Echinoidea	*Diadema setosum* (Leske 1778)	2	2	2	2
		Diadema savignyi (Audouin 1829)	1	1	1	1

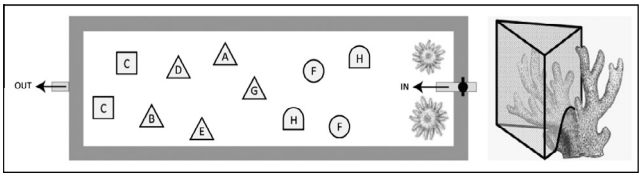

Fig. 1. Set-up of coral fragments (branching △, columnar □) and colonies (massive ⌓, solitary ○) in tank. Half of branching fragments was exposed to feeding *A. planci*, while the other half was enclosed in a wire mesh cage (1 cm × 1 cm mesh size). Letters represent coral species: A = *A. aspera*, B = *A. nasuta*, C = *A. palifera*, D = *P. damicornis*, E = *S. hystrix*, F = *F. fungites*, G = *P. cylindrica*, H = *P. lutea*.

peptones were moderate and only increased when concentrations were raised to 10–20× the standard concentration based on manufacturer formulation of TCBS (Fig. 2). Severity of clinical signs, mentioned hereafter, will range from low (i.e. localized to site of injection) to high (i.e. spread to more than 50% of the sea star).

3.1.1. Bacteriological peptone

At the TCBS standard concentration of 10 g l^{-1}, there was 0% mortality up to 48 h using Oxoid brand and only one 1 out of 10 *A. planci* died using Himedi brand. Most *A. planci* showed localized loss of turgor, matting, and mucus secretion. At half the TCBS standard concentration (5 g l^{-1}), 50% of the sea stars showed loss of turgor and swelling after 8 h, but all recovered after 48 h and there was 0% mortality. At twice (20 g l^{-1}) the TCBS standard concentration, 4 out of 10 exhibited localized tissue necrosis and 2 out of 10 sea stars showed medium severity necrosis at 8 h. After 24 h, 6 out of 10 showed medium severity necrosis and 1 out of 10 with localized necrosis. However, only 1 out of 10 and 2 out of 10 died after 24 h and 48 h, respectively. At 10× the TCBS standard concentration, there was severe loss of turgor, matting of spines, and tissue necrosis at 24 h, where 2 out of 5 died. All sea stars challenged at this concentration died after 48 h.

3.1.2. Proteose peptone

There was 0% mortality at all tested concentrations (0.5×, 1×, 2×). All specimens only showed localized loss of turgor and swelling 8–24 h after injection but eventually recovered after 48 h.

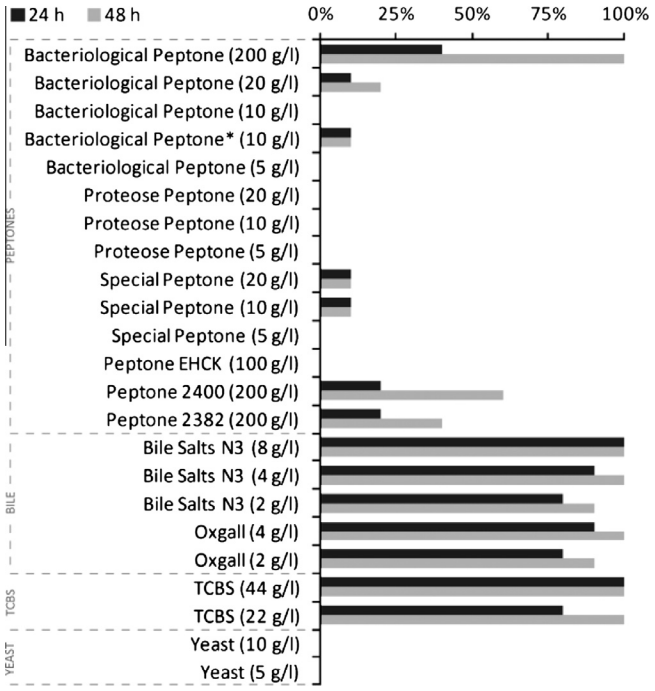

Fig. 2. Mortality of *A. planci* challenged with different concentrations of culture media components 24 h (black bars) and 48 h (gray bars) after injection.

3.1.3. Special peptone

There was 10% mortality of *A. planci* injected with 1× and 2× the TCBS standard concentration, 24 h and 48 h after injection. Mortality was at 0% when the concentration was lowered to 0.5× the standard. Clinical signs of disease at mid to high severity were mainly observed in individuals that died, while only localized swelling, matting, or lesions were observed in a few individuals, which recovered 48 h after injection.

3.1.4. Other peptones

Peptone EHCK at 10× the TCBS standard concentration showed localized tissue necrosis after 24 h, secretion of mucus, swelling and matting of spines, but did not result in any mortality. Peptone 2400 at 20× the TCBS standard concentration showed moderate loss of skin turgor, matting of spines, necrosis at the site of the injection and killed 3 out of 5 *A. planci* in 72 h. Peptone 2382, also used at 20× the TCBS standard concentration, showed similar patterns as peptone 2400 in terms of severity levels of mucus secretion, loss of turgor, matting of spines, and tissue necrosis. Peptone 2382 killed two out of five *A. planci* in 48 h. We observed one specimen discarding tissues that were starting to decompose, while half of what was left recovered after 72 h.

3.1.5. Bile salts

At the standard TCBS concentration (8 g l^{-1}), *A. planci* already started exhibiting low to medium severity loss of skin turgor, swelling, matting of spines, and tissue necrosis after 8 h. One out of 10 died 8 h after injection and there was 100% mortality after 24 h, half of these sea stars were already dead after 12 h. Dead sea stars were almost completely decomposed after 36 h. Even when lowered to 0.5× and 0.25× the TCBS standard concentration, there was 90% and 80% mortality after 24 h, then 90% and 100% mortality after 48 h, respectively. Severity of signs (loss of turgor, collapsed spines, and tissue necrosis) ranged from low to medium after 8 h, but were mostly high after 24 h. Mucus secretion were mostly absent in all specimens tested.

3.1.6. Oxgall

At half (4 g l^{-1}) the TCBS standard concentration, *A. planci* exhibited low to medium severity of swelling, matting of spines, and tissue necrosis after 8 h, and severity increased after 24 h. There was 90% mortality after 24 h and 100% mortality after 48 h. Even at 0.25× the TCBS standard concentration, there was 80% mortality after 24 h and 90% mortality 48 h after injection. The same pattern of severity as those injected with 0.5× concentration was observed in these *A. planci*. Mucus secretions were mostly absent in all specimens tested.

3.1.7. TCBS

There was 100% mortality after 24 h at 0.5× the concentration per liter specified by TCBS manufacturers. Eight hours after injection, severity of mucus secretion, loss of turgor, matting of spines, and tissue necrosis ranged from low to medium. There was an increase in severity of these signs after 24 h. Even at 0.25× the standard concentration, severity of mucus secretion, loss of turgor, matting of spines, and tissue necrosis ranged from medium to severe after 24 h and resulted in 80% mortality. All sea stars were dead 48 h after injection.

3.1.8. Yeast

There was 0% mortality at the TCBS standard concentration (5 g l^{-1}) and also when this concentration was doubled. Disease signs were not exhibited except for localized swelling and tissue necrosis at the site of injection.

3.2. Transmission

Twelve days after exposure to *A. planci* injected with oxgall (8 g l^{-1}, 4 g l^{-1}), peptone (20 g l^{-1}), and TCBS (44 g l^{-1}), none of the fish, corals, and mobile invertebrates exhibited any signs of disease. No signs of bacterial disease such as cloudy eyes, fin rot, pop eyes and changes in skin color were observed in any of the fish tested. There were also no spots, bands, or discoloration observed in corals that were constantly in contact with floating *A. planci* particles in the water. It is important to mention that corals were not attacked by *A. planci* and there was minimal movement of the starfish one hour after injection with oxbile. Mobile invertebrates remained active each night and there was no loss of spines observed in sea urchins and no lesions in sea stars and sea cucumbers (Fig. 3).

4. Discussion

Bile derivatives (i.e. oxgall, bile salts) have consistently resulted in high mortality rates in previous studies (Rivera-Posada et al., 2012) and in this study. Bile salts are added in media culture formulations to inhibit the growth of gram-positive bacteria and isolate resistant strains. Bile is a natural digestive enzyme produced by all vertebrates to aid in the digestion of lipophilic nutrients. In addition, bile is an important route of elimination of environmental toxins, carcinogens, hormones, drugs and their metabolites and may control the growth of bacteria in the small intestine (Nathanson and Boyer, 1991).

Two well-known mechanisms of cell death are triggered by bile acids: necrosis at higher concentrations and apoptosis at lower concentrations (Palmeira and Rolo, 2004; Rolo et al., 2004). Several studies indicate that impairment of mitochondrial oxidative phosphorylation is an early and critical event in the mechanism of bile acid cytotoxicity. Apoptosis induction is dependent on the bile acid, its concentration, or its conjugation state. Toxic bile acid-induced apoptosis involves both extrinsic (death receptor-mediated apoptosis) and intrinsic (direct targeting to mitochondria)

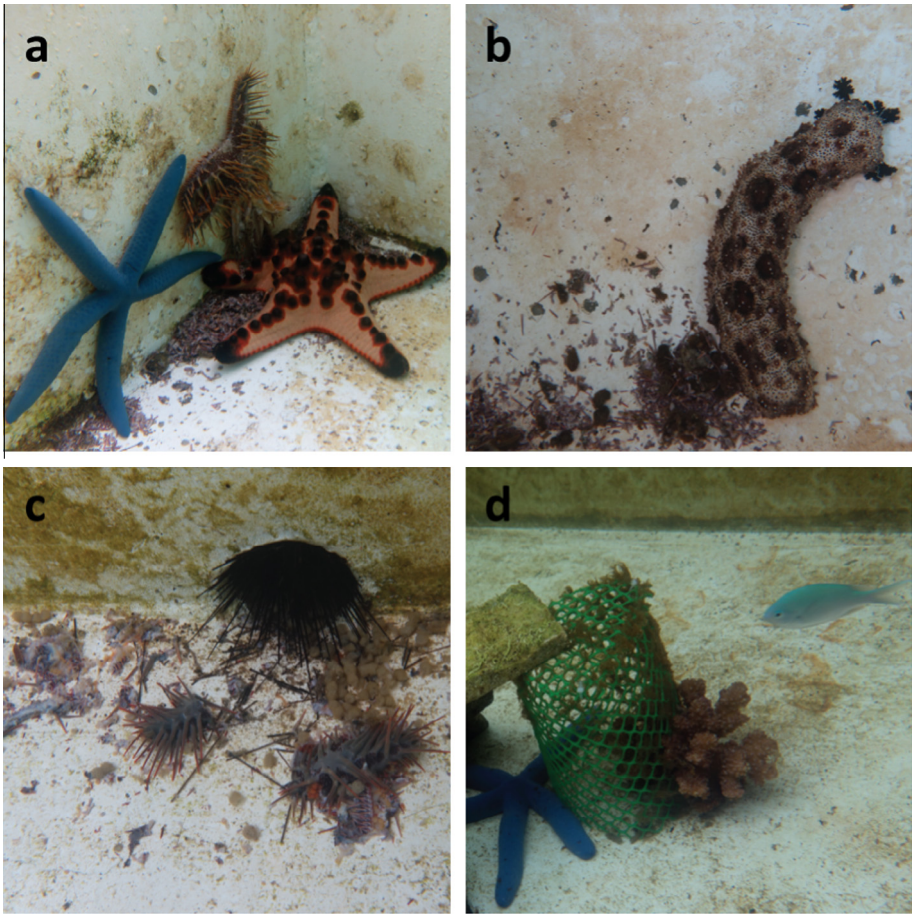

Fig. 3. Disease transmission experiments showing animals in contact with dying and decomposing *A. planci*: (a) with *L. laevigata* and *P. nodosus*; (b) with *P. graeffei*; (c) with *D. setosum*; (d) *L. laevigata*, *P. damicornis* partially caged, and*C. caerulea.*

apoptotic pathways. Bile acids induce alterations in membrane fluidity associated with impairment of mitochondrial respiration and mitochondrial depolarization. Upon exposure to low bile acid concentrations, there is release of apoptogenic factors, activation of caspases and DNA fragmentation which entails apoptotic cell death.

Additionally and besides direct targeting to mitochondria, apoptotic cell death is also induced by bile acids through receptor-dependent-mechanisms which could explain why *A. planci* did not attack corals in the tank and showed minimal movement 1 h after injection. On the other hand, high concentrations of bile also cause a bioenergetic catastrophe culminating in the disruption of plasma membrane integrity. Mitochondrial calcium homeostasis is severely impaired (Palmeira and Rolo, 2004; Rolo et al., 2004). Both necrosis and apoptosis could be the mechanisms involved in the induction of disease and death of COTS after bile injections. Necrosis is evident in *A. planci* tissues 24 h after injection with oxbile. Apoptosis could be also involved in the induction of disease because is the mechanism normally triggered by low concentrations of bile salts as those used in this study.

There is a direct relationship between sodium chloride concentration and bile salts cytotoxicity. It has been shown that increasing NaCl concentrations lowers the critical micellar concentration (CMC) of bile salts and increase bile toxicity (Morgan et al., 1998) which could explain why very small doses of oxbile are required to kill *A. planci*. We hypothesize that a synergistic effect between seawater (high NaCL concentration) and bile salts (unnatural proteins) could trigger a fulminating allergic reaction (cytotoxicity) inducing apoptosis and necrosis of *A. planci* tissues, but this needs to be confirmed by analysis of sea star tissues through histology (HandE and Gram stains), SEM and TEM to confirm that injection of bile do not induce a transmissible bacterial disease. There is however, always the possibility that there will be secondary infection induced by opportunistic pathogens once the immune system collapses.

Effectiveness of peptones varied greatly between the different types of peptones tested, and rarely caused high mortality of injected *A. planci* even at 20× concentration based on TCBS standard concentration of 10 g l^{-1}, except for bacteriological peptone. Aside from the dose-dependent nature of the effectiveness of peptone in killing *A. planci* (Rivera-Posada et al., 2012), peptone commonly exhibits batch-to-batch variability in chemical composition and culture performance even between the same brand (Taylor, 1981; Murakami et al., 1982) which could lead to inconsistent results. In addition, peptones are produced from many different sources such as plants (cotton seed, soya beans), meat (bovine and porcine, heart, liver, brain, meat), casein (milk of mammals) all of which have different properties.

Other parameters that influence the final product are the raw materials used in the preparation of the media (i.e. materials contaminated with copper ions show a very low growth rate of microorganisms). Sterilization parameters such as the time autoclaving and the quantity of media sterilized during the heat treatment of culture media may result in its nutrient destruction either by direct thermal degradation or by reactions between components. Other parameters such as temperature, volume, pressure, and gel

strength also influence the final product (Basu et al., 2005). Interestingly, some brands of TCBS use proteose peptone (e.g. Difco) in their formulation, while others use bacteriological peptone (e.g. Oxoid). In this study there was 0% mortality with proteose peptone at all tested concentrations meanwhile bacteriological peptone induced severe loss of turgor, matting of spines, and tissue necrosis at the same concentrations and 100% mortality at 20×. The variability showed between peptones could be related with the difficulties in inducing transmission of disease through the injection of TCBS in some instances.

Disease induced by TCBS injections have been shown to have a potential for interspecific transmission in previous studies (Caballes et al., 2012). In this study, no animals were reported sick after 12 days of exposure and contact with sick and decomposing *A. planci* and repeated consumption of *A. planci* remains by fishes. Only one *Pomacentrus moluccensis* died, but several bite marks on its body and fins indicate that mortality was not related to disease or infection. Rivera-Posada et al. (2012) demonstrated that peptone toxicity is concentration dependent and the TCBS concentration employed in this study was only 44 g l^{-1} which is half the concentration used in previous studies. In addition there was no manipulation of physical parameters such as pH, salinity and temperature that are key factors in promoting growth of bacteria. Another important aspect to consider is the water volume and maintenance conditions. Caballes et al. (2012) used small plastic aquariums while this study used 2 m^3 tanks with high water flow. In small spaces and in the absence of predators that feed on remains, bacteria concentration is higher due to the large amount of decomposing tissues in the water.

For fishes that feed on dead remains of *A. planci*, the risk of secondary toxicity or disease is low. Their digestive and immune system will help to halt toxicity of the remaining tissues by degrading tissues and bile salts. Initially, *A. planci* tissues will be degraded by chloridic acid and powerful enzymes that are responsible for the breakdown of food in the stomach (killing bacteria that overgrow during disease and after death and structurally decomposing the remaining tissue). Subsequently, *A. planci* remains will pass to the intestines of scavenging fish, which also breaks down tissues using enzymes released by the pancreas and bile from the liver (Hofmann and Hagey, 2008; Bodo, 2011). Peristalsis also is at work in this organ, moving food through and mixing it with digestive secretions from the pancreas and liver. The intestine is largely responsible for the continuous breaking-down process and for absorption of nutrients into the bloodstream. Contents in intestine start out semi-solid, and end in a liquid form after passing through the organ. Water, bile, enzymes, and mucous contribute to the change in consistency. Once the nutrients have been absorbed and the leftover-food residue liquid has passed through the small intestine, it then moves onto the large intestine for expulsion. Lastly bile toxicity has been related to apoptosis and necrosis, not bacteria infection which has been demonstrated in several studies.

The absence of evidence of disease transmission to other reef organisms is promising for field testing. The doses of oxbile required to kill *A. planci*, concentration 4 g l^{-1} at 10 ml volume (single injection) are very low compared to doses used when injecting sodium bisulfate, 140 g l^{-1} concentration at 60 ml volumes (multiple injections) (Kayal et al., 2011). The amount of oxbile injected is only 0.04 mg/sea star which is distributed in *A. planci* tissues and it will be attacked, englobed and partially degraded by the sea star immune cells and expelled through the water vascular system as part of the regular functions of the immune system. More importantly, many bacteria are capable of transforming and degrading bile in the digestive tract and in the environment (Hofmann and Hagey, 2008; Bodo, 2011).

Bacterial bile salt transformation and degradation is of high ecological relevance and also essential for the biotechnological production of steroid drugs (Bodo, 2011). Thus, *A. planci* remains containing bile salts will be constantly degraded by different mechanisms. Normally, considerable amount of bile salts is released into the environment with faeces and urine of vertebrates. Bile salts cholate, glycocholate, deoxycholate and glycodeoxycholate are also produced and degraded by marine bacteria (Bode et al., 2003; Maneerat et al., 2005; Kim et al., 2007). Moreover, aerobic bacteria are able to grow with bile salts as sole source of carbon and energy. For energy conservation, these bacteria oxidase steroid compounds completely to CO_2. In the water column, petromyzonol sulfate which is the major bile salt in sea lampreys is subject to microbial degradation (Hagey et al., 2010).

On the GBR, eradication of outbreaks populations of *A. planci* are predicted to reverse the current trend of declining coral cover (De'ath et al., 2012). The low doses (concentration and volume) and limited risk of unintended casualties make oxbile and oxgall good candidates for field testing as a novel control method for *A. planci*. This new approach, coupled with strategic measures to improve water quality, could mitigate the effects of *A. planci* on coral communities and enable gradual recovery of coral assemblages and reef ecosystems.

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