Response of *Acanthaster planci* (Linnaeus, 1758) to an acetic-acid control protocol

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Abstract. Crown-of-thorns sea star, *Acanthaster planci* (Linnaeus, 1758), naturally feeds on reef-building corals and severely disrupts their growth. In the Philippines, *A. planci* outbreaks have been seen in isolated reefs on the western shore of Samal. Island, Tubbataha Reefs and Natural Park, and in Sogod Bay, Southern Leyte. Various methods are used to control outbreaks of *A. planci,* but the most popular is via injection of household vinegar, which achieves 100% mortality in 48 h. In this study, a series of experiments was conducted to determine the effects of the popular outbreak control method for *A. planci* by injection of household vinegar. The seastars were categorized into three groups: household vinegar, seawater injection, puncture only, and control, with 5 replicates each. The number of seastars that spawned, the density of egg cells, and the number of mortalities for each group were recorded and tested for significant differences using the Kruskal-Wallis statistical test. Household vinegar was effective in killing *A. planci* with a 100% mortality within 10 h. However, the *A. planci* were also observed to spawn within an hour after the injection of vinegar. There was a significant difference in the number of sea stars that spawned ($p < 0.05$) between treatment groups and the control, where no injections were administered. Using to control population outbreaks of *A. planci* is effective but may trigger a secondary outbreak or a new outbreak in an adjacent reef. Based on the results of this study, it is advisable to avoid the use of vinegar injections and utilize the traditional collection method of *A. planci*, as it may minimize in-water spawning.
Key Words: crown-of-thorns sea star, spawning, vinegar injection, outbreak control, mortality.

Introduction. According to Fabricius (2013), the Crown-of-thorns sea star, *Acanthaster planci* (Linnaeus, 1758), is a large coral-eating asteroid that has gained notoriety due to the enormous amount of loss it can inflict on coral reefs and to its large population fluctuations. The *A. planci* can be seen throughout the world's tropical and subtropical reefs, excluding the Atlantic Ocean. *A. planci* naturally feed on reef-building corals and severely disrupt their growth (Kayal et al 2012). Earlier studies found that a major cause of coral degradation throughout the Pacific can be attributed to the corallivorous *A. planci*. One of the causes of coral mortality in the Indo-Pacific region was *A. planci* outbreaks (e.g. Halmera and Aceh în Indonesia (Baird et al 2013), which resulted in an extensive and rapid deterioration of the coral reef environments (Pratchett et al 2014).

Although overfishing and anthropogenically elevated nutrients were reported to contribute to outbreaks, the bases remain debatable. In the Chagos Archipelago, where the location has restricted anthropological activities, the reef system was seen with high densities of *A. planci* (Roche et al 2015). Now, the reduction of global pressures is greatly recognized since coral reefs around the world are deteriorating. Surveys conducted from 1985 to 2012 (2,258 surveys of 214 reefs) showed a drastic decline in coral cover from 28 to 13.8%, a 50.7% loss of primary coral cover (De'ath et al 2012). The loss of corals by *A. planci* predation was estimated at 42%. Finding alternative measures to lessen *A. planci* populations may prevent coral reduction and boost the Great Barrier Reef's surroundings (De'ath et al 2012).

Immense coral mortality with prolonged recovery, which contributes to the steady and ongoing coral cover decline around the Indo-Pacific, is due to *A. planci* population outbreaks. The quick increase in the *A. planci* population is due to their short maturity

timespan, within two years, and a high level of fecundity, generating more than 100 million oocytes for a single sea star in a season (Caballes & Pratchett 2017).

Outbreak control methods and protocols have been studied to reduce the impact of *A. planci* outbreaks occurring in the Indo-Pacific region (Rivera-Posada et al 2013, 2014; Moutardier et al 2015). Several studies have proven the efficacy of injecting low pH solutions into the coelomic cavity of *A. planci* in inducing mortalities and providing snapshot solutions to *A. planci* outbreaks (Boström-Einarsson & Rivera-Posada 2016; Boström-Einarsson et al 2018; Yamamoto & Otsuka 2013; Rivera-Posada et al 2014; Moutardier et al 2015). However, these studies did not consider the potential effects the protocols would have on the organism, given that *A. planci* response to physical injury/stress is to spawn (Lawrence & Herrera 2000).

The spawning response of the *A. planci* to injury or physical stress have been noted in the literature (Lawrence & Herrera 2000; Caballes & Pratchett 2016), as well as anectdotes from fisherfolks actively participating in *A. planci* clean-ups (D. Innocencio, personal communication March 14, 2023), urging the utmost care when handling the organism, however, no actual tests were done to confirm this.

Since there is a lack of observation regarding the effects of vinegar, such as the increase or decrease of *A. planci* populations, after being injected into *A. planci*, this study aims to find out whether or not the injection of vinegar to control *A. planci* populations brings more harm to reef systems. It is important because control methods used, especially in third-world coastal communities, use vinegar to control *A. planci* population outbreaks. This study aims to determine if household vinegar triggers the spawning behavior of *A. planci*.

Material and Method

Collection site. The collection was done within the municipal waters of Barangay Bantayan on August 13, 2023, where local *Bantay Dagat* reported an outbreak of *A. planci*. A total of 40 *A. planci* specimens ranging from 24-35 cm in size (Yamaguchi 1974) were collected along the coast of Dumaguete City. The collection of samples was done by SCUBA and freediving. A metal tong with a silicone tip was used to safely pick up the specimens which were contained in a plastic bucket filled with seawater. The specimens were then transported to the Silliman University Institute of Environmental and Marine Sciences (SU-IEMS) in Dumaguete City, Negros Oriental, Philippines. The specimens were left to acclimatize for 48 to 72 hours in seawater tanks with fresh seawater pumped through them. *Caulerpa lentillifera*, a species of green algae, was used as a food source for *A. planci* specimens. Specimens damaged during the course of the collection were disposed (Rivera-Posada et al 2014).

Treatment of samples. Ten specimens were used for control, which were not subjected to any injury or injection. For the treatments, ten specimens each were allocated to the treatment groups: 25 mL injection of seawater, 25 mL injection of household vinegar, and a puncture at the central disk of the specimen was done using a 16-gauge needle from a continuous variable syringe gun. The specimens were transferred to 35 L rectangular plastic containers with individual aeration and maintained in a water bath to stabilize temperature, followed by another round of acclimatization (48 to 72 h).

Eight plastic containers, each with an individual sea star, were tagged after treatments were administered in no particular order, hence the randomized block design. Experiments were done in five 48-hour series, with 2 specimens per treatment for each series. The limited number of specimens per series is due to the size of the circular tanks for the water bath. Markers attached to the containers were used to identify treatment and control specimens. Control and treatment experiments were done simultaneously and monitored hourly for 48 hours from the time of injection or puncture. The elapsed time of spawning after injections was noted. Ten mL water samples were collected from the control and three treatment containers, which were immediately observed under a compound microscope with the use of glass slides, every hour for 48 hours or until gametes were seen. Only gametes released immediately after injection and injury were

counted. The sex of individuals was not possible since dissection of the specimen was needed for determination. Further studies are needed regarding the viability of the gametes since it lies beyond the scope of this study.

Statistical analysis. The data collected was subjected to normality and homogeneity tests and other exploratory statistical tools. As the data was not normally distributed and did not fit the criteria for homogeneity, Kruskal-Wallis was used to determine whether there was a significant difference in the spawning of *A. planci* between control and treatment groups as well as the number of mortalities between control and treatment groups. The same statistical test was done for the density of gametes as the data was imbalanced and was not normally distributed. The Dunn's Test was used as a post-hoc test to determine where the significant differences in spawning were between control group and treatments.

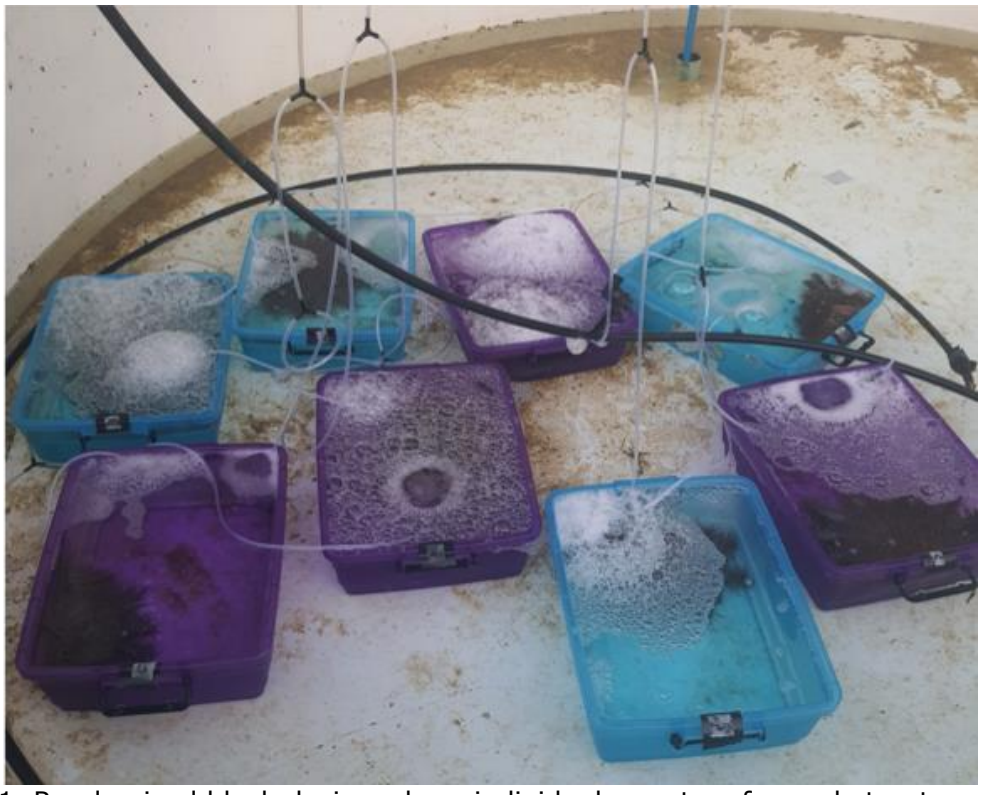

Figure 1. Randomized block design where individual sea stars for each treatment were placed.

Results. All sea stars spawned from the vinegar and puncture treatment, 9 out of 10 sea stars spawned from the seawater treatment, while only three sea stars spawned from the control group. The Kruskal-Wallis test showed a $p = 0.003$, which showed that there is a significant difference in spawning time between control and treatments. The Dunn's Test further showed that the most significance was seen for the vinegar group at $p = 0.010$ and the puncture group at $p = 0.008$. This indicates that there is indeed a significant difference between gametes in the water samples between the control and treatment groups.

The overall percentage of spawning for the treatment group (vinegar, seawater, and puncture) was 96.66% (29 out of 30). Vinegar and puncture groups had 100% probability of spawning, while the seawater group had a 90% probability. The control group had a 30% probability of spawning. For the vinegar group, spawning occurred between 1 and 14 h, which had an average spawning time of 5.2 h (±1.298). Puncture group had spawning occur between 1-8 h, averaging 4.8 h (±0.952). The seawater group had spawning occur between 1-16 h with an average of 4.5 h (±1.558).

Table 1

Kruskal-Wallis results for spawning

Kruskal-Wallis test	*h*	*df*	*p-value*	*Dunn's test p-value*
Spawning	0.05	3	0.003	0.010 (Control - Vinegar)
Spawning	0.05	3	0.003	0.008 (Control - Puncture)

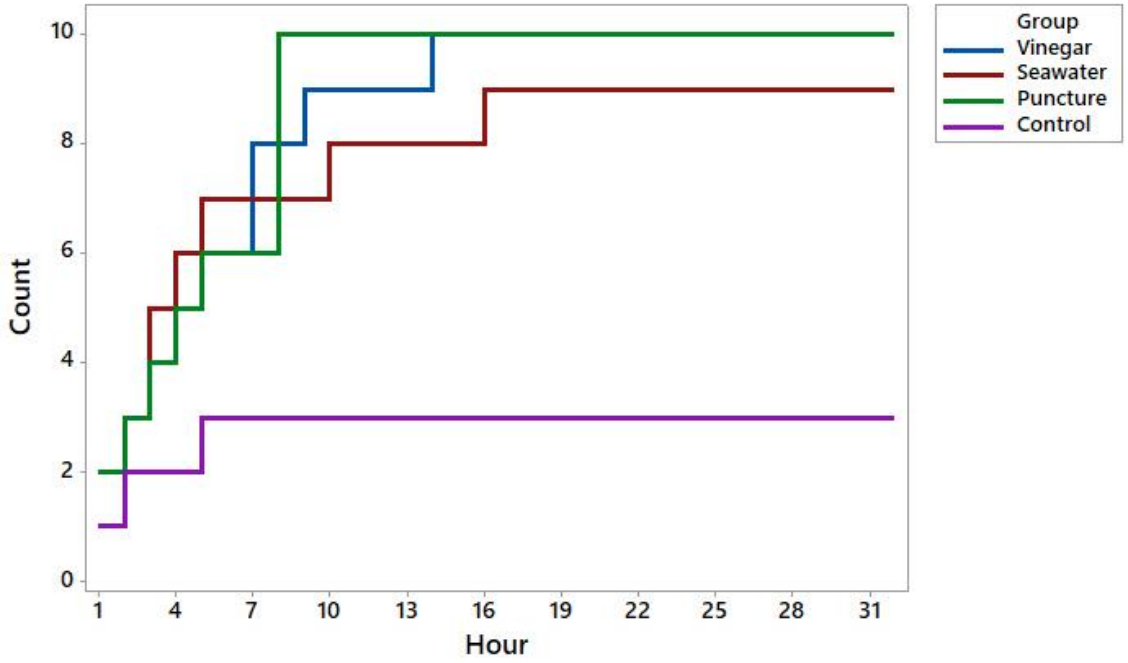

Figure 2. Spawning count of individuals over 48 hours.

Gametes in the water sample. Gametes were found in all treatment groups. For the vinegar treatment, six out of ten water samples were observed to have egg cells, while the rest of the water samples had sperm cells. For the puncture treatment, six water samples out of the ten samples were observed to have egg cells with fewer counts, while four water samples had sperm cells. For seawater treatment, one of the seastars spawned before the experiment and was removed from the test. Six out of nine water samples had egg cells, and three out of nine had sperm cells. For the control group, unfortunately, three samples from the control group spawned all released egg cells with significantly lower counts compared to the treatment groups. The trigger of spawning for the seastars in the control group was not identified, but can be attributed to other stressors, such as handling. The number of egg cells in the water samples showed a significant difference between the control and treatment groups ($p = 0.021$). The Dunn's test showed that the number of egg cells in the water samples from the vinegar treatment was significantly different from the control ($p = 0.012$).

Table 2

Kruskal-Wallis results for gametes in the water sample.

Kruskal-Wallis Test	*h*	*df*	*p-value*	*Dunn's test p-value*
Number of egg cells	0.05	3	0.021	0.012 (Control - Vinegar)

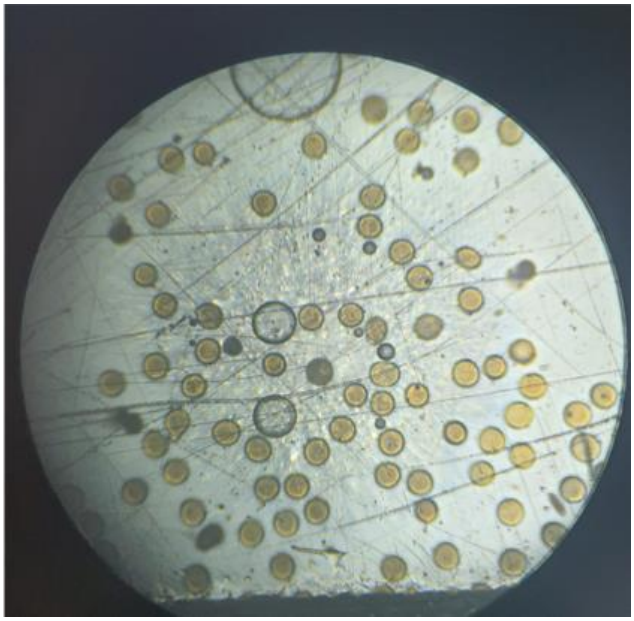

Figure 3. Female gametes seen under the microscope.

Mortality in different treatments. The vinegar treatment had 100% mortality within 11-18 h. The puncture treatment had four mortalities within 16-31 h. The seawater treatment had three mortalities within 17-29 h. The control group had two mortalities at the 27 and 32 h mark. For the first replicate, both specimens for vinegar treatment achieved 100% mortality in 11 h, one specimen from the puncture treatment in 24 h, and one specimen from seawater treatment in 17 h. Unfortunately, one specimen from the control group died in 32 h. For replicate two, both specimens for vinegar treatment died in 16 h, both specimens for puncture treatment in 16 and 31 h, and none for seawater treatment. Unfortunately, this replicate's control group also had one specimen that died in 27 h. For replicate three, both specimens for vinegar treatment died in 12 and 18 h, one specimen from the puncture treatment in 29 h, and both specimens from the seawater treatment in 29 h as well. For replicate four, only the vinegar treatment had specimens that died in 11 h and 18 h, respectively. For replicate five, mortalities were only recorded in the vinegar treatment at 10 and 14 h. The Kruskal-Wallis test showed a p-value of 0.022, showing a significant difference in the number of mortalities between the control and treatment groups. Further, results of Dunn's Test showed that significant differences were between the control and vinegar group.

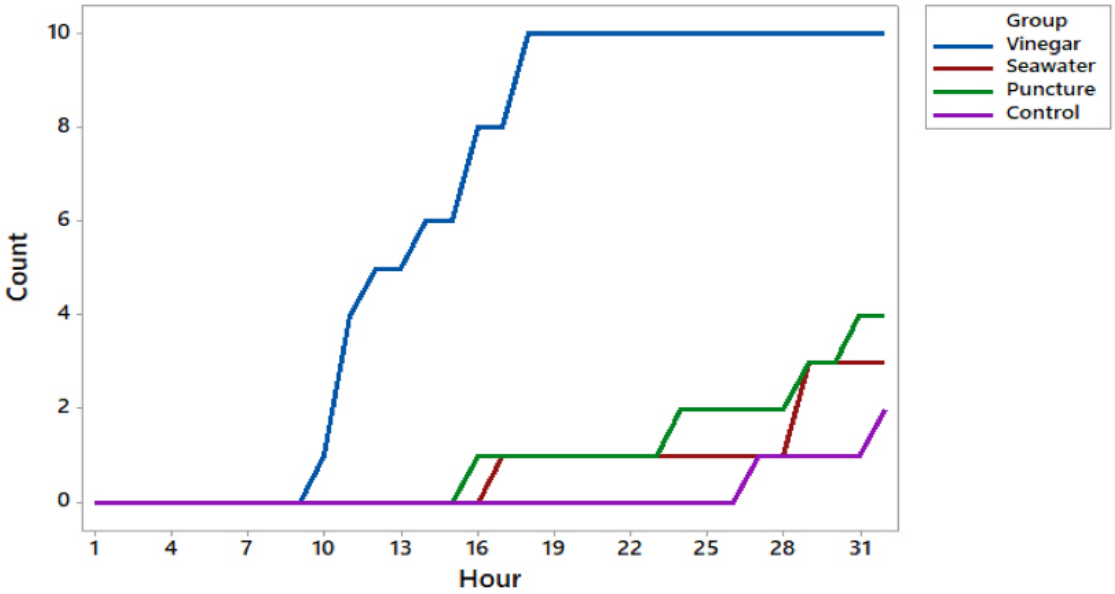

Figure 4. Mortality count of individuals over a 48 h-period.

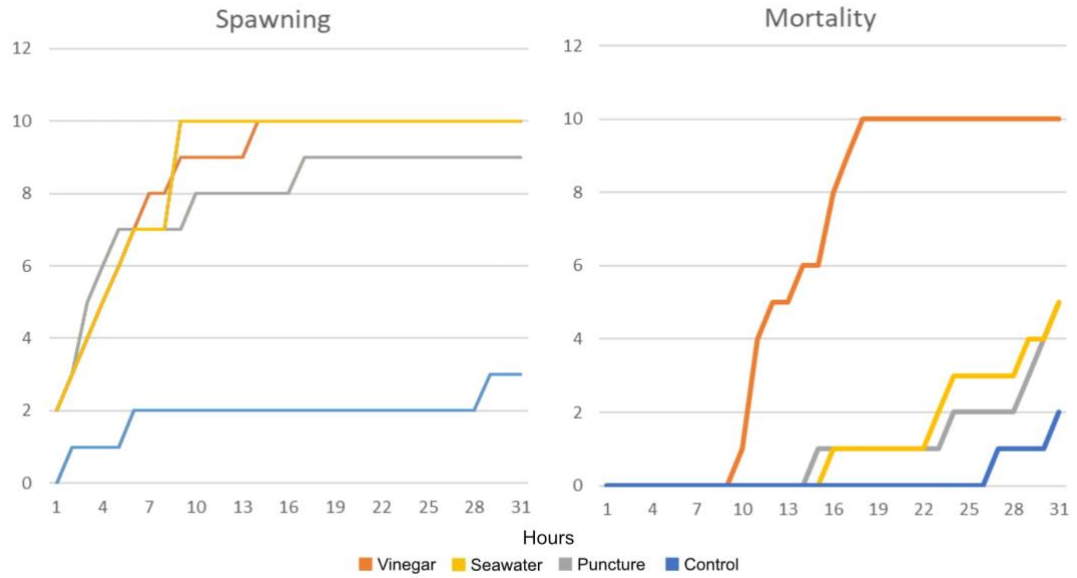

Figure 5. Mortality count of individuals over a 48 h-period.

Table 3

Kruskal-Wallis result for mortality

Kruskal-Wallis test	*h*	*df*	*p-value*	*Dunn's test p-value*
Mortality	0.05	3	0.022	0.009 (Control - Vinegar)

Discussion. Vinegar was successful as an outbreak control protocol for *A. planci* as well as a low-cost alternative to TCBS and bile salts. *A. planci* injected with 25 mL of vinegar on the base of the arm, exhibited functional mortality within 24 h and 100% mortality in 48 h (Rivera-Posada et al 2014; Moutardier et al 2015; Boström-Einarsson & Rivera-Posada 2016). Dilute acetic acid was also seen to be effective when injected with 5 mL around the central part, having a 100% mortality in 72 h with 25 out of 40 samples surviving (Yamamoto & Otsuka 2013). Results from the study showed that the seastars injected with 25 mL of vinegar died faster compared to previous studies. Signs of stress (e.g., increased mobility, effort to escape containers, and arching behavior) were observed within minutes after injection of vinegar, which may be caused by the significant decrease in pH level of the specimens (Lawrence & Herrera 2000). However, between the time of injection and mortality, spawning had occurred in all specimens treated with vinegar (Boström-Einarsson & Rivera-Posada 2016). It was made sure that each *Acanthaster* had its own container to avoid contamination of pheromones, which would cause spawning to nearby specimens, in the ambient water (Beach et al 1975). According to Lawrence (1990) and Lawrence & Herrera (2000), *A. planci* has a low tolerance for disturbance, and stress may lead to deviant reproduction. The stress caused by the injection of vinegar, which lowered the pH level of the sea star, and injury from the puncture alone are stressors that made deviant or aberrant spawning possible. It is also important to note that due to the water-miscible nature of acetic acid, it penetrates the seastar's tissue, which disintegrates it from the inside out (Yamamoto & Otsuka 2013). Alternatively, the injury from the injection alone may have been enough to cause stress to the sea star. The arching behavior seen during spawning may not only be a feeding behavior but also a cue of spawning behavior (Pratchett et al 2017). Even if vinegar is a low-cost alternative to bile salts and achieves 100% mortality in as fast as 10 h for *A. planci* seen in Dumaguete City, there is a high chance for it to spawn, which would affect the adjacent coral reefs since gametes would be carried by the current. Manual collection of *A. planci* as a control method induces less stress to the organism since no chemical reaction is happening. Spawning is kept to a minimum since the

organism is killed on the surface, as compared to using household vinegar, where the organism is kept underwater, possibly secreting pheromones before spawning, which allows other sea stars to spawn and fertilize the gametes. Another *A. planci* outbreak would cause coral mortality and an rise of *A. planci* populations.

Little is known about why stress induces spawning, but from observations from this experiment, it may be due to the preservation of the species. Echinoderms are known to be osmoconformers that match their body fluid to the medium's concentration. Vinegar contains chloride, and its addition due to the introduction of vinegar causes osmodysregulation. This is due to an increase in coelomic fluid, which suggests that the sea star is concentrating on one or more osmolytes, which may affect the physiology of the water vascular system (Wahltinez et al 2020). The increase of pH in the coelomic cavity, as the acetic acid found in vinegar penetrates the tissues of *Acanthaster*, alters multiple physiological functions as well as the failure of immune and reproductive functions (Yamamoto & Otsuka 2013; Moutardier et al 2015). This may be the reason as to why *A. planci* samples were able to spawn between the time of injection and mortality. It may also be the reason for the increase in density of female gametes compared to the control group, puncture, and seawater treatments. Present evidence points to Sea Star Wasting Syndrome being caused by bacterial infection rather than being a virus (Konar et al 2019). Stress induced by the increase of pH may also trigger immune responses, which may have imbalanced the *Vibrio* bacteria found in the sea star, since a 1,200-fold increase of the said bacteria was seen post-mortality in sea stars seen with Sea Star Wasting Syndrome (Hutchinson 2018; McCracken et al 2023). Further studies are needed regarding the relationship between stress, which causes the alteration of physiological processes of the sea star, and spawning.

Local anecdotes from the fishermen and Bantay Dagat stationed on the coastline of the city suggested that the manual collection of *A. planci,* locally known as "Dap-ag", would be the better choice rather than the widely used control method of using vinegar, as it lessens the stress caused to the sea star.

Conclusions. The study confirmed that both mechanical injury and the injection of vinegar and seawater successfully triggered the spawning response in *A. planci*. Although the vinegar injections are successful in achieving 100% mortalities within 48 hours, there is an inherent risk of triggering a spawning event. The amount of gametes produced in the process may cause a secondary outbreak to occur within the site. Additionally, the spawning event caused by the injections may become a potential seed source for a new outbreak in adjacent reefs as the currents carry the gametes. The link established between injury and spawning necessitates an urgent shift in population control protocols. Methods that do not involve organism injury must be explored and adopted to mitigate the risk of stress-induced gamete release, thereby protecting unimpacted coral reefs. Until non-injurious methods are validated, manual collection is the suggested interim control strategy to minimize the risk of stress-induced spawning. Implementing this strategy is crucial for both coral preservation and the long-term livelihood of coastal communities in areas like Dumaguete City.

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Conflict of interest. The authors declare that there is no conflict of interest.

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