Response of *Acanthaster planci* (Echinodermata: Asteroidea) to hypersaline solution: Its potential application to population control

Homer Hermes Y. de DIOS*1,2, Filipina B. SOTTO2, Danilo T. DY2, and Anthony S. ILANO2

1 Southern Leyte State University-Bontoc Campus, Bontoc, Southern Leyte, Philippines
2 Marine Biology Section, University of San Carlos, Cebu City, Philippines

* Corresponding author: Homer Hermes Y. de Dios  
E-mail: homeryray@yahoo.com

Communicated by Saki Harii (Ecology Editor)

**Abstract** The response of *Acanthaster planci* or crown-of-thorns starfish (COTS) to intracoelomic injection of hypersaline solutions was investigated to establish its potential application as *A. planci* population control especially during an outbreak. Adult *A. planci* when inverted had a normal righting response time (in seconds) of 163 ± 1 (mean ± SE; N = 298). Injection of concentrated salt solutions led to negative effects on adult *A. planci*. The spines bent down, body collapsed and the starfish became comatose and died after 24-hours. The higher the salt concentration, the higher is the % comatose individuals. In addition, comatose specimens failed to recover with time. Mortality was highest at the highest treatment concentration with LC90 of 285.3 and LC99 of 383.2 ppt. The effects of time, salt concentration and its interaction (salt concentration x time) were significant (p = 0.00). The ionic and osmotic disruption of the coelomic fluid can seriously affect physiological functions of the organism including neuromuscular activity. Thus, in the control of *A. planci*, concentrated salt solution can be used as a cheaper alternative to dry acid, acetic acid or ammonium hydroxide, all of which are expensive, may not be locally available and not environment friendly.

**Keywords** mortality rate, comatose, crown-of-thorns starfish, Philippines

**Introduction**

Infestation of *Acanthaster planci* (Linnaeus 1758) (crown-of-thorns starfish) is one of the most destructive biological disturbances in tropical coral reefs (Birkeland and Lucas 1990; Pratchett 2005; Pratchett et al. 2009; Pratchett 2010; Timmers et al. 2011). When *A. planci* occur at very high densities (>1500 starfish/km²) termed as “outbreak” (Moran and De’ath 1992), it can cause coral mortality up to 90% (Pratchett 2005; Pratchett et al. 2009) with consumption rate varying from 0.4 to 1 m²/month/animal (Pearson and Endean 1969; Glynn 1973). Without *A. planci*, coral cover at Great Barrier Reef and other coral reefs in Indo-Pacific region could increase 0.89%/y despite losses due to cyclones and bleaching (De’ath et al. 2012). Thus, reducing *A. planci* outbreak populations, by developing alternative control measures, could significantly prevent further coral decline (De’ath et al. 2012).

Several *A. planci* outbreak occurred across the Philippine archipelago. Outbreaks or severe infestation were reported in Inopacan, Leyte (Benliro et al. 1999), in
Southern Leyte (de Dios et al. 2014) and in Apo Reef and Puerto Galera of Mindoro, in Tubbataha reefs of Sulu Sea (Bos 2010) and in Samal Island, Davao del Norte (Bos et al. 2013). Seemingly, *A. planci* outbreak is becoming a regular menace to Philippine coral reefs hence attention to control their population outbreak should be immediate.

A number of methods have been used to control the outbreak of *A. planci*. The use of injection gun with chemical poison like dry acid, formalin, copper sulphate and ammonium hydroxide, compressed air, collection and bury on land, fencing, cutting into pieces, killing by using quick lime and bounty system are but the few methods being used to control *A. planci* (Birkeland and Lucas 1990; Lassig 1995). Most of these control measures are either expensive to implement, time consuming especially for a developing country such as the Philippines and maybe harmful or toxic to coral reefs.

Control efforts against *A. planci* outbreak are still ongoing. Biological control using thiosulfate-citrate-bile sucrose agar to induce a transmissible vibrio-related disease in the coral eating *A. planci* (Rivera-Posada et al. 2011) may led to detrimental effects of other reef organisms. Furthermore, Rivera-Posada and Owens (2014) described the importance of osmotic shock as alternative method to control *A. planci* using sodium chloride as one of the chemicals used as osmotic stressor to the organism. They found 100% mortality of *A. planci* at saturated concentration of sodium chloride, however, they did not examine the effect of NaCl at concentrations lower than 300 g/L. On the other hand, manual removal and dry acid injection of *A. planci* are the common practice of controlling *A. planci* outbreak, but it may endanger the health of the diver/collector because the spines of *A. planci* carry a hematoxin called plancitoxins that are potentially hepatotoxic (Watanabe et al. 2009). Puncture wound from a spine is intensely painful and causes oedema, erythema and infection of the surrounding areas (Rivera-Posada et al. 2011). Limiting the exposure of divers/collector to spines by injecting the organism with cheaper and environmental friendly chemicals (i.e. concentrated salt solution) would be safer.

Knowledge on the physiological characteristics (i.e. osmo- and ion regulation) of *A. planci* is key to population control. Echinoderms lack excretory organs with poor ability to osmo- and ion regulate (Binyon 1961, 1972; Stickle and Diehl 1987). Hence, they are considered osmoconformer, isosmotic and mostly isoionic animals (Stickle and Diehl 1987; Vidolin et al. 2007; Santos and Freire 2007; Freire et al. 2011) unable to withstand significant ionic gradient with respect to ambient seawater or are unable to regulate the osmotic pressure of the coelomic fluid (Binyon 1972; Diehl 1986; Stickle and Diehl 1997; Santos and Freire 2007; Freire et al. 2011).

The response of *A. planci* when injected with different salt concentrations or to hypersaline condition has not been investigated as a potential application to population control. Although *A. planci* can survive in higher salinity (40 ppt) like in the Red Sea (Ormond et al. 1973), sudden changes in the salt concentration of the coelomic fluid would be stressful due to its lack of ion- or osmo-regulatory mechanisms. Thus, injecting a hypersaline solution inside the coelomic cavity of *A. planci* should lead to physiological stress, and eventually to death of the organism. To determine the effectivity of this method, we conducted a manipulative experiment using the righting response of *A. planci* as a dependent variable. Righting is a characteristic behavior of asteroids that involves neuromuscular coordination. Such righting response of echinoderms has been used as an indicator of sub-lethal stress (Lawrence and Cowell 1996; Ubaldo et al. 2007). In this study we propose a better less harmful alternative population control compared to dry acid (sodium bisulphate), copper sulphate, TCBS and acetic acid, all of which are very prohibitively expensive in a developing economy such as the Philippines.

### Materials and methods

#### Collection of samples

More than 700 *A. planci* (diameter in cm: 26.14±0.176; mean±SE) were collected in Tankaan, Padre Burgos, Sogod Bay, Southern Leyte from February to March 2013. *Acanthaster planci* were transported immediately (distance <9 km) to the experimental site in San Juan, Padre Burgos, Southern Leyte. The collected individuals were acclimated for 24 hours in a fish cage located offshore at depth of 10 meters. After acclimatization, the righting
time of each individual were measured to check their fitness for the experiment. The normal righting response time of *A. planci* was 163±1 seconds; mean±SE (n = 298). Specimens which did not right themselves in less than 163 seconds were excluded from the study.

**Experimental design**

The experiment followed a Randomized Complete Block Design (RCBD) with blocks corresponding to ten replicates per treatment level with 10 individuals per each treatment. There were seven treatment levels as followed: (1) puncture only, (2) injection with ambient seawater (33–34 ppt), (3) 48 ppt, (4) 145 ppt, (5) 242 ppt salt solutions and (6) 345 ppt saturated salt solution. The response variable was % comatose and % mortality using 10 *A. planci* per treatment level. Salt solutions were prepared using rock salts available from the wet market (example: 100 ppt = 100 g of salts dissolved in 1 L of freshwater). We further analysed the final salt concentration by drying the salt in the oven at 60°C for 24 hours and another 24hrs in the desiccators jar. Salts were weighed using Ohaus digital balance with 0.01 accuracy. Table 1 showed the initial and final salt concentration.

For each treatment, ten *A. planci* were placed in a plastic container filled with 100 L seawater (33–34 ppt) and provided with constant aeration for 24 hours. The water was regularly changed every 8 hours. Each starfish was injected five times (10 mL/injection) at the aboral region using a disposable syringe (Fig. 1).

The righting time of individual *A. planci* was observed five times (1, 4, 8, 12 and 24 hours after injection). *Acanthaster planci* was inverted and the time it took the animals to return to its original position were measured using a stopwatch and backed up with video camera. Each observation was stopped when the organism remained inverted after 15 minutes and was considered comatose (i.e. when some parts of the animals are moving) and considered dead if no response was visibly detected 24 hours after injection. Percentage comatose *A. planci* was determined as the number of organisms that failed to turn into normal position after 15 minutes divided by the total number of *A. planci* observed multiplied by 100%. Mortality rate was measured 24 hours after injection by counting dead *A. planci* divided by the total number of *A. planci* observed multiplied by 100%. A starfish was considered dead when there’s no sign of any movement to any parts of the body.

**Table 1** Final salt concentration (ppt) after 48 hrs drying in the oven and dessicator jar (N=5)

<table>
<thead>
<tr>
<th>Initial salt concentration</th>
<th>Final salt concentration (Mean ± SE)</th>
</tr>
</thead>
<tbody>
<tr>
<td>50</td>
<td>48.00 ± 0.17</td>
</tr>
<tr>
<td>150</td>
<td>145.00 ± 0.75</td>
</tr>
<tr>
<td>250</td>
<td>242.14 ± 0.48</td>
</tr>
<tr>
<td>350</td>
<td>344.68 ± 1.46</td>
</tr>
</tbody>
</table>

*Fig. 1* Administering concentrated salt solution into the aboral region of *A. planci*. Site of injection (10 mL/injection x 5 sites = 50 mL).

**Statistical analysis**

The effects of salt concentrations (48, 145, 242 and 345 ppt) and time (1, 4, 8 and 12 hrs) post injection on the
percent comatose *A. planci* were tested using two-way analysis of variance with repeated measures (ANOVAR). Mauchly’s test of sphericity was initially used. Since the Mauchly’s test of sphericity was significant (*p* < 0.05), the univariate test or within-subject effects were used. The epsilon (ɛ) values of Greenhouse-Geisser and Huynh- Feldt were used to calculate the appropriate adjustment to the degrees of freedom of F-test. Since all univariate tests were significant, Bonferroni test was used as pairwise comparisons of the main effects. On the other hand, the effect of salt concentration on the mortality rate of *A. planci* was tested using one-way ANOVA-Model 1 (fixed factor). The data were normally distributed and the variance was homoscedastic (Levene’s Test). Comparison of the main effects was tested using post hoc analysis (i.e. Bonferroni test). Significance level was set at *p*=0.05.

The lethal salt concentration (i.e. LC50, LC90 and LC99) was estimated using probit analysis (Finney 1952).

**Results**

Most of the *A. planci* injected with concentrated salt solution (except for puncture only, injection with ambient seawater and 48 ppt) failed to right themselves in the normal position and they remained indefinitely inverted for more than 15 minutes. This condition was considered comatose even if some parts of the body were moving (i.e. tube feet). There was a significant effect (*p*=0.00) on the percent comatose starfish with time (Table 2). The effect of salt concentration on the percent comatose starfish was also significant (*p*=0.00). The interaction of time and salt concentration was also significant (*p*=0.00; Table 1). Increasing the salt concentration significantly shorten the time it took the *A. planci* to become comatose (see Fig. 2). Thus, the animals got weaker over time. The greatest change in % comatose *A. planci* with time was 8 hours at salt concentrations 145 and 242 ppt. The greatest change in % comatose *A. planci* with saturated salt concentration was 345 ppt.

<table>
<thead>
<tr>
<th>Source</th>
<th>SS</th>
<th>df</th>
<th>MS</th>
<th>F</th>
<th><em>p</em></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Within -subjects Effects</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Time after injection</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sphericity Assumed</td>
<td>6014.583</td>
<td>3</td>
<td>2004.861</td>
<td>151.593</td>
<td>0.00*</td>
</tr>
<tr>
<td>Greenhouse-Geisser</td>
<td>6014.583</td>
<td>1.713</td>
<td>3511.426</td>
<td>151.593</td>
<td>0.00*</td>
</tr>
<tr>
<td>Huynh-Feldt</td>
<td>6014.583</td>
<td>1.927</td>
<td>3120.772</td>
<td>151.593</td>
<td>0.00*</td>
</tr>
<tr>
<td>Time after injection x Salt concentration</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sphericity Assumed</td>
<td>8367.917</td>
<td>15</td>
<td>557.861</td>
<td>42.181</td>
<td>0.00*</td>
</tr>
<tr>
<td>Greenhouse-Geisser</td>
<td>8367.917</td>
<td>8.564</td>
<td>977.069</td>
<td>42.181</td>
<td>0.00*</td>
</tr>
<tr>
<td>Huynh-Feldt</td>
<td>8367.917</td>
<td>9.636</td>
<td>868.368</td>
<td>42.181</td>
<td>0.00*</td>
</tr>
<tr>
<td>Error (Time after injection)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sphericity Assumed</td>
<td>2142.500</td>
<td>162</td>
<td>13.225</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Greenhouse-Geisser</td>
<td>2142.500</td>
<td>92.494</td>
<td>23.164</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Huynh-Feldt</td>
<td>2142.500</td>
<td>104.073</td>
<td>20.587</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Between-subjects Effects</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Salt concentration</td>
<td>294257.083</td>
<td>5</td>
<td>58851.417</td>
<td>990.795</td>
<td>0.00*</td>
</tr>
<tr>
<td>Error</td>
<td>3207.500</td>
<td>54</td>
<td>59.398</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* significant at 0.05
Twenty four hours after injection, a number of *A. planci* were dead at higher salt concentration. The percent mortality of *A. planci* ranged from 2.0% ± 1.3 (mean ± SD) to 99% ± 1.0 (mean ± SE). Injection of concentrated salt solution has a significant effect ($F_{(6,63)} = 1028.15, p = 0.00$) on the mortality rate of *A. planci* (Fig. 3). Percent mortality at 345 ppt saturated salt solution was significantly higher ($p = 0.00$). Goodness of fit test showed that the observed mortalities fitted the probit model ($P = <0.0001; X^2 = 48.06; R^2 = 0.737$). The mean 24-hr LC$_{50}$, LC$_{90}$ and LC$_{99}$ for *A. planci* were 165.1, 285.3 and 383.2, respectively (Fig. 4).

**Discussion**

The righting response of starfish is a good indicator of fitness. This was done by inverting the asteroid and the time was measured when the animal returns to its normal position. Our use of righting response to test the effect of hypersaline solution injection on *A. planci* is based on previous assessments. Lawrence (1973) and Ubaldo et al. (2010) noted that echinoids, asteroids and ophiuroids (i.e. *Luidia clathrata*, *Protoreaster nodosus*, *Echinometra oblongata* and *Ophiocoma scolopendrina*) which fail to right themselves after 15 minutes and remained inverted indefinitely were considered comatose. Our results showed that concentrated salt solutions (i.e. 345 ppt) had a significant negative effect on the condition or well-being of *A. planci* corroborating the results obtained by Rivera-Posada and Owens (2014). The higher the salt concentration the higher the percent comatose starfish with time, leading to higher mortality rates. The interactive effect of both time and salt concentration suggested that the percent comatose *A. planci* changed over time and reacted in different ways. The animals got weaker over time at higher salt concentrations (i.e. 145 and 242 ppt).
The ionic and osmotic regulation of solutes between the intracellular and extracellular fluids is physiologically important to the survival of echinoderms. Osmotic pressure plays a vital role in all biological processes that involves diffusion of solutes (i.e. sodium chlorides) or transfer of fluids through membranes. Energy is required to remove excess water that enters by osmosis in order to maintain homeostasis, thus preserving the balance of salts (i.e. \(Na^+\) and \(Cl^-\)) in the tissues and cells of the organisms (Prusch 1983).

Sodium is the main cation of the extracellular medium and together with chlorides represents a major fraction of the extracellular osmotic effector (Herrera and Plaza 1981). Prusch (1983) explained that the cells maintain a relatively low sodium concentration by virtue of membrane impermeability to sodium. Further, he explained that the maintenance of the sodium gradient across the cell membrane offsets the driving force for the entry of water into the cell (i.e. volume regulation).

*Acanthaster planci* has no physiological capability to regulate ionic gradients and osmotic pressure or has no capability to withstand very high salt concentrations (i.e. >300 ppt). Hence, it was expected that *A. planci* are dependent on their water vascular system, and when injected with concentrated salt solutions will lead to bending of their spines, body collapse and the starfish became comatose and later died after 24-hours. The injection of salt solution in essence led to unbalanced ionic concentration and osmolarity between coelomic fluid and its internal organs (i.e. pyloric caeca) and the coelomocytes. In order to get rid of the excess ionic solutes inside the coelom, energy must be expended (Beadle 1931) and it would be stressful for the organism. The sudden change of salinity in the coelomic cavity can adversely affect their functioning (Talbot and Lawrence 2002).

Echinoderms are not normally subjected to great changes in either the osmotic pressure or ionic composition of the environment (Binyon 1961). The coelomic fluid approximate the composition of seawater (Binyon 1961, 1972), making the members of the echinoderms isosmotic/isionic animals (Vidolin et al. 2007; Santos and Freire 2007; Freire et al. 2011). Hence, they are unable to withstand significant ionic or osmotic gradient with respect to ambient seawater (Diehl 1986; Binyon 1972; Santos and Freire 2007; Freire et al. 2011). This physiological characteristic is what makes the injection of hypersaline solutions very effective in killing *A. planci*. However, *A. planci* can still tolerate injections of 48 ppt saline solution with very low mortality (<2%) and is not significantly different from the two control experiments (Fig. 3). *Acanthaster planci* can tolerate an ambient salinity of 40 ppt salinity as observed in the Red Sea (Ormond et al. 1973) and can also survive at 19 to 29 ppt in running seawater (Glynn 1974). However, Yamaguchi, (1986) observed that *A. planci* ceased feeding activities at 40 ppt and resumed feeding when returned to 34 ppt, *A. planci* also feed during rainy seasons when the salinity is below 25 ppt (Glynn 1974). Moran (1990) stated that *A. planci* is a marine species inhabiting the Indo-Pacific coral reefs with ideal salinity range of 30–33 ppt. The previous studies indicate that *A. planci* can be a euryhaline osmoconformer species and can survive at lower salinity (i.e. 19–29 ppt) and higher salinity (i.e. 40–50 ppt) than the ambient seawater. Our experiment showed that *A. planci* cannot survive or is not capable of maintaining large ionic gradient and large osmotic pressure at hypersaline concentrations (i.e. 145–429 ppt).

In general, table salt (NaCl) is cheap and readily available in wet and dry markets worldwide and is a natural product from the sea. It is processed via evaporation of concentrated brine water in salt ponds. Salt solution of more than 300 ppt using improvised injection gun or syringe can be a better alternative to dry acid (sodium bisulphate), acetic acid, copper sulphate, TCBS and ammonium hydroxide which are potentially expensive and harmful to the environment when applied in high quantity. For the control of *A. planci* during an outbreak we suggest the filling of hypersaline solution in the improvised injection gun as an alternative to manual collection. To be successful, the use of hypersaline solution as *A. planci* population control should be coupled with *A. planci* control program/strategies (i.e. assessment of the distribution and abundance of *A. planci* population outbreak).
Acknowledgments

We thank the anonymous reviewers for their comments and suggestions. The authors sincerely thank the following agencies for financial support: The Philippine Council for Agriculture and Aquatic Resources for Research and Development (PCAARRD) and Philippine Council for Marine and Aquatic Research Development (PCMARD) of Department of Science and Technology (DOST) for the scholarship grant of HHYDD. We thank Southern Leyte State University (SLSU) - Bontoc Campus and the University of San Carlos (USC), Department of Biology for the logistic support. We are grateful to Arnel Beslig from the local government unit of Padre Burgos, Southern Leyte and the fisherfolks for helping the authors in the collection of Acanthaster planci and assistance during the conduct of the study. This study is a marine science contribution of DOST, SLSU and USC.

References

Pratchett MS (2005) Dynamics of an outbreak population of Acanthaster planci at Lizard Island, northern Great Barrier
Selective coral mortality associated with outbreaks of Acanthaster planci L. in Bootless Bay, Papua New Guinea.
Talbot TD, Lawrence JM (2002) The effect of salinity on respiration, excretion, regeneration and production in Ophio


Received: 13 September 2013
Accepted: 25 May 2015

Ⓒ Japanese Coral Reef Society