



## Effect of hyposalinity on coral bleaching and survival in a hard (*Pocillopora damicornis*) and soft coral (*Sarcophyton* sp.)

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### Abstract

This study investigates the effect of hyposalinity on coral bleaching in a species of hard coral (*Pocillopora damicornis*) and a soft coral (*Sarcophyton* sp.). Corals were exposed to ambient seawater (32 psu) and reduced-salinity seawater (15 psu and 25 psu) for 32 days. Both the hard coral and soft coral were highly affected by the salinity level of 15 psu. Fragments of *Pocillopora damicornis* were 90% bleached within four days and died after five days of exposure to salinity levels of 15 psu. Similarly, *Sarcophyton* sp. was 50%–95% bleached within four days and died after seven days of exposure. Both corals were less impacted by exposure to salinity levels of 25 psu and 32 psu; *P. damicornis* and *Sarcophyton* sp. were partially bleached but survived until the end of the experiment. We report that *P. damicornis* is more susceptible to hyposalinity than *Sarcophyton* sp. The results of this study can serve as basic information for managers regarding the release of freshwater into areas of the ocean with coral reefs. The study also suggests that salinity monitoring during rainy seasons or flooding may allow prediction of future coral bleaching caused by these low-salinity events.

**Keywords:** rainfall, bleaching, scleractinian, soft corals, hyposalinity

### Introduction

Today, there are many anthropogenic forces impacting the climate, particularly the volume of greenhouse gases being released into the atmosphere from our daily activities. The increase of greenhouse gases leads to temperature fluctuations and anomalies in ocean currents as well as changing rainfall (Hoegh-Guldberg, 2011). Over the last 50 years, monthly mean sea temperature in Phuket has shown a significant decadal increase of 0.126 °C (Brown, 1997), global temperature has been rising steadily, and mass bleaching of corals has frequently been observed in different parts of the world's oceans (Wilkinson, 2000; Eakin, Sweatman, & Brainard, 2019). An extended period of record-breaking temperatures brought us the most severe, widespread, and longest-lasting global-scale coral bleaching on record (Eakin et al., 2019). This dramatic change in climate makes it extremely difficult for corals to adapt. The heat stress on coral reefs has increased over the past three decades, and coral bleaching events were caused in the Gulf of Thailand by elevated sea surface temperature in 2010, and by low salinity due to heavy flooding in 2011 (e.g., Kongjandtre & Chankong, 2015; Pongsakun et al., 2019).

Coral bleaching is a stress response that results in the loss of intracellular symbiotic dinoflagellates (Family Symbiodiniaceae) and their pigments (Hoegh-Guldberg, 1999) from their coral hosts. Environmental stressors, such as changes in temperature, salinity and solar radiation, as well as anthropogenic stressors, such as chemical dispersants are contributing factors to bleaching of corals (Brown, 1997; Hoegh-Guldberg et al., 2007; Studivan, Hatch, & Mitchelmore, 2015; Ainsworth et al., 2016; Hughes et al., 2018). Global climate change is threatening the world's ecologically and economically important tropical reef ecosystems, most notably the scleractinian corals (Eakin et al., 2019). Increases in sea surface temperature anomalies are of global concern.



Major rainfall events are also common in tropical regions, further exacerbating the effects of global climate change. During heavy rains, coral may experience significant reduction in salinity.

Seawater temperature and salinity have been recognized as limiting environmental factors for survival and growth of reef corals (Coles & Jokiel, 1992). While temperature thresholds have been widely studied in efforts to understand the effect of climate change, salinity thresholds have received relatively little attention from researchers (e.g., Jokiel, Hunter, Taguchi, & Watarai, 1993; Kerswell & Jones, 2003; Chavanich, Viyakarn, Loyjiw, Pattaratamrong, & Chankong, 2009; Berkelmans, Jones & Schaffelke, 2012). Freshwater runoff is a major cause of coral mortality, especially on coastal reefs close to major river systems (e.g., Jokiel et al., 1993; van Woerik, De Vantier, & Glazebrook, 1995).

The Gulf of Thailand (GoT) is a semi-enclosed, shallow coastal basin situated on the northwestern part of the Sunda Shelf, measuring approximately 400 km by 800 km. It can be divided into the upper and lower GoT. Coral reefs are found in coastal areas of the upper GoT (Chon Buri Province) and along the eastern part of the lower GoT (Rayong, Chanthaburi and Trat). Rivers with relatively large volumes (Chao Phraya) as well as smaller rivers (Bangpakong, Tha Chin and Mae Klong) discharge into the upper GoT (Wattayakorn, 2006; Saramul & Ezer, 2014). In the upper GoT, seasonal changes cause surface temperature to range from 26 °C in January to 31 °C in May, and result in a minimum salinity of 16–17 psu in October and maximum of 33–34 psu in July (Buranapratheprat, Yanagi, & Matsumura 2008; Buranapratheprat, Luadnakrob, Yanagi, Morimoto, & Qiao, 2016). In some years, Thailand is impacted by La Nina, which causes lower seawater temperatures than average and higher rainfall (Saramul & Ezer, 2014). During heavy rainfall, a large amount of fresh water enters the river and is discharged into the sea, causing the salinity of the seawater to fall and potentially affect marine organisms locally. Chavanich et al. (2009) reported mass bleaching of the soft corals *Sarcophyton* spp. in the upper GoT from June to October in 2006 and 2007. They also performed acute tests of temperature and salinity, and found that at 40 °C and 20 psu, *Sarcophyton* spp. were completely bleached after 57 hours, and died after 204 hours. The authors concluded that elevated temperatures have a greater effect on bleaching of *Sarcophyton* spp. than salinity.

Low salinity is one of the known causes of coral bleaching. However, we were interested in discovering the lower limits of salinity tolerance in hard corals and soft corals that inhabit low-salinity conditions such as occur in the upper GoT. Therefore, in this study we 1) investigate the effect of low salinity on coral bleaching in *Pocillopora damicornis* (a hard coral species widely distributed across habitats) and *Sarcophyton* sp. (a widely distributed soft coral in the upper GoT) and 2) test the salinity dose-time threshold for the selected species based on the salinity range found in the upper GoT.

## Methods and Materials

### Coral collection and preparation

The experiment was conducted in the hatchery building, Department of Aquatic Science, Faculty of Science, Burapha University, Chon Buri Province. Mature colonies of hard coral (*Pocillopora damicornis*) and soft coral (*Sarcophyton* sp.) specimens used in this study were produced through asexual reproduction in a research hatchery at the Bangsaen Institute for Marine Science, Burapha University. Fragments from four colonies of *P. damicornis* (colony diameter  $\geq$  20 cm) were used as replicates for the experiment. Three vertically oriented



fragments (8–10 cm long) were isolated from the center of each parent colony (1 fragment per salinity test x 4 colonies) using surgical bone forceps. Fragments from three *Sarcophyton* sp. colonies (diameter  $\geq$  20 cm) were used as soft coral replicates. Three fragments (8–10 cm long) were cut from each parent colony (1 fragment per salinity test x 3 colonies). Coral fragments were acclimatized in a tank with salinity of 32 psu and water temperature of 27–31 °C for one week prior to the experiment. Light intensity and water temperature were recorded with an Onset® HOBO Pendant® Temp/Light data logger.

Solutions of the desired salinity were made by diluting water from a seawater system (32 psu) with freshwater. Fresh solutions were made to 32 psu, 25 psu and 15 psu and kept in a closed system before the experiment. Each tank was a closed system which re-circulated water through a pump and filter system, with separate aeration for each treatment (Figure 1). The 32 psu ambient salinity tank served as a control. The salinity in each tank was measured daily using a refractometer, and freshwater was added if the salinity had risen due to evaporation. Alkalinity and pH were measured throughout the experiment using IMPACT Alkalinity and pH kits following the manufacturer's instructions.

### **Experimental design**

To examine the chronic response to low-salinity stress, *P. damicornis* and *Sarcophyton* sp. were exposed to ambient seawater (32 psu) or reduced-salinity seawater (25, 15 psu) for 32 days. One tank was used for each treatment, and each tank contained four *P. damicornis* and three *Sarcophyton* sp. fragments (Figure 1).

To quantify the level of coral bleaching, a Coral Health Chart ([www.ReefQuest.org](http://www.ReefQuest.org)) was used to record the changing color of the colonies. Observations were made three days per week (Monday, Wednesday and Friday) for one month. The level of bleaching was recorded as the difference between the color of a particular coral fragment at the beginning and the end of the experiment. The percentage bleaching was estimated from the bleached area and total surface area of coral fragment (English, Wilkinson & Baker, 1997).

The effect of salinity on the percentage bleaching of hard and soft corals was tested using analysis of variance (ANOVA), with fixed factors of day of exposure, salinity and coral taxon. Student-Newman-Keul's tests were performed to assess differences among salinity levels and days of exposure.

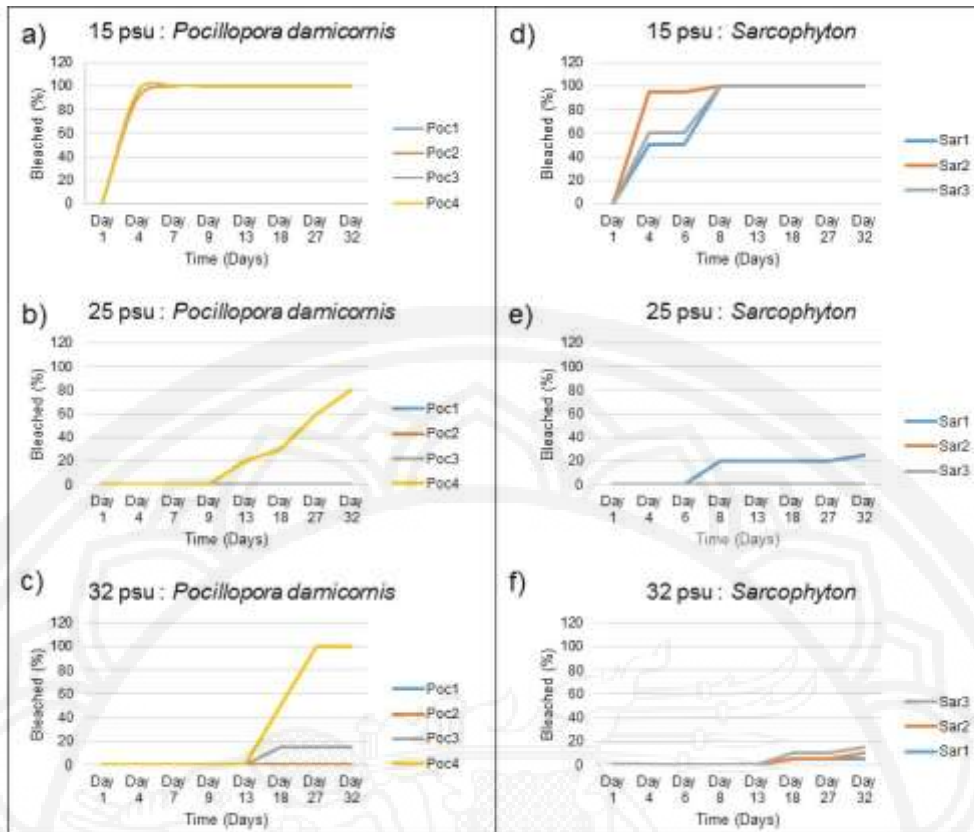


**Figure 1** Experimental design and aquaria setting for *Pocillopora damicornis* and *Sarcophyton* sp. Salinities tested were 32 psu, 15 psu and 25 psu (left to right). Each tank contained a set of fragments from four *P. damicornis* colonies and three *Sarcophyton* sp. colonies (one fragment per colony)

## Results

Results from the salinity experiment revealed that *P. damicornis* was more vulnerable to bleaching at low salinity than *Sarcophyton* sp. ( $P=0.022$ ) (Figures 2-5). The bleaching was significantly affected by salinity ( $P=0.000$ ), day of exposure ( $P=0.000$ ) and their interaction ( $P=0.000$ ). Of the salinity levels tested in our experiment, 15 psu significantly produced the greatest bleaching effect. For the treatments of 25 psu and 32 psu (control), coral fragments showed partial bleaching but survived until the end of the experiment.

*Pocillopora damicornis* responded to hyposalinity by first becoming paler, then fully bleached, and finally the tissue sloughed away from the limestone structure. *P. damicornis* at 15 psu became completely bleached within seven days and all fragments died after 7-9 days of exposure (Figures 2a-3). Partial bleaching was observed in one fragment at 25 psu from day 13 until the end of the experiment, while at 32 psu, partial bleaching began on day 18. It is possible that some *P. damicornis* colonies were more sensitive than others, as fragments were bleached in both 25 and 32 psu (Figure 2b-c, Poc4).



**Figure 2** Percentage of bleached tissues of *Pocillopora damicornis* and *Sarcophyton* sp. fragments at three salinities



**Figure 3** Changes in colony color in *Pocillopora damicornis* during one month of exposure to three salinities. Day of exposure, colony number, color chart label and percentage bleaching are indicated on each image



**Figure 4** Changes in colony color of *Sarcophyton* sp. during one month of exposure to three salinities. Day of exposure, colony number, color chart label and percentage bleaching are indicated on each image

In the experiment, *Sarcophyton* sp. responded to hyposalinity of seawater (15 psu) by becoming paler in color, shrinking in overall size, and with withered skin and peeling tissue (Figures 2d, 4). The other treatments with *Sarcophyton* sp. showed that they are able to acclimatize to levels of 25–32 psu. A slight change in color and partial bleaching were observed in only one colony at 25 psu beginning from day 8, and in two colonies at 32 psu beginning from day 18. All colonies in these two treatments survived until the end of the experiment (Figures 2e–f, 4).

During the experiment, daytime and nighttime water temperature ranged from 26.2–31.8 °C, with an average of 29.2 °C (Figure 5). At the beginning of the experiment, alkalinity varied according to salinity, but then became similar for all treatments (Figure 6).

### Average seawater temperature

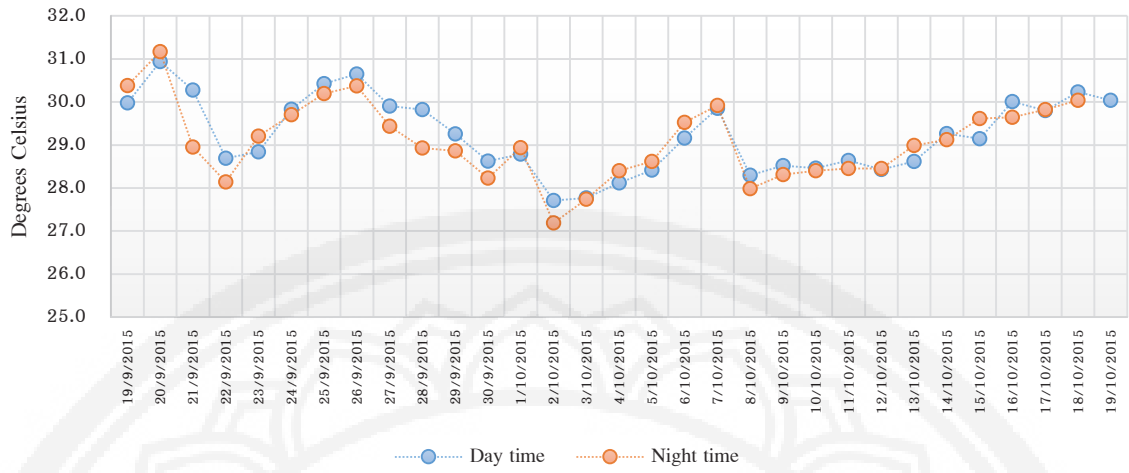


Figure 5 Seawater temperature during month-long salinity exposure experiment on hard and soft corals

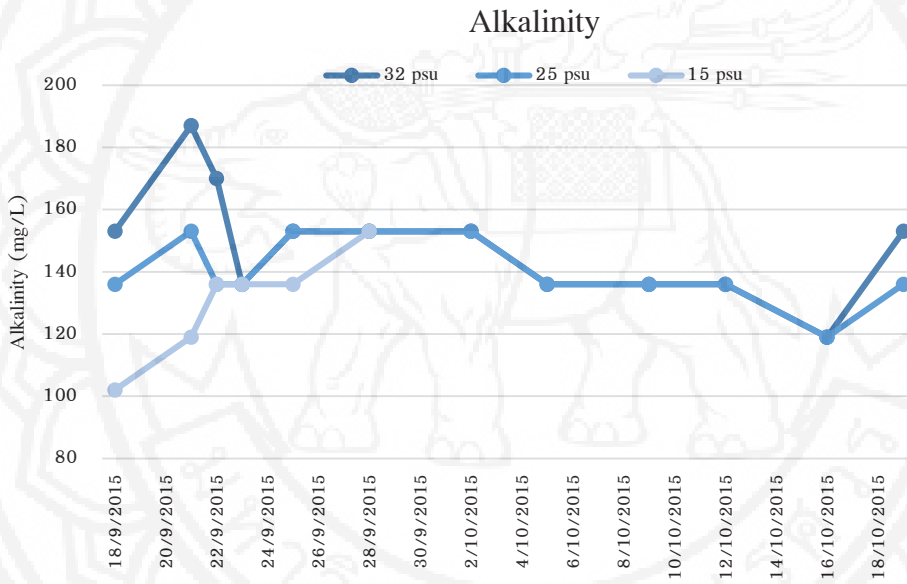


Figure 6 Alkalinity of seawater during month-long salinity exposure experiment on hard and soft corals



## Discussion

This study revealed significantly different vulnerability to low salinity between a hard coral and a soft coral. A salinity of 15 psu was lethal to both taxa in this study; however, *P. damicornis* appears more vulnerable to bleaching at low salinity than *Sarcophyton* sp. Prolonged periods of reduced salinity during flooding or monsoons can produce acute to sub-lethal effects in corals of inshore reef communities near rivers. Most research on the effects of freshwater influx has focused on lethal levels of hyposalinity in indicator species of corals (Table 1). Recently, Pengsakun et al. (2019) reported salinity at Ko Khang Khao in the inner GoT ranging from 30.74–31.45 in July 2010 and 11.18–17.78 psu in August 2011, which lower (by as much as 11 psu) than the lethal threshold. They showed that *Acropora millepora*, *Favia favaus*, *Favites abdita*, *Goniopora columna*, *Pavona decussate*, *P. damicornis*, *Platygyra sinensis*, *P. pini* and *Porites lutea* were highly susceptible to coral bleaching by hyposalinity. In contrast, the most tolerant corals were *Galaxea fascicularis*, *Fungia fungites*, *Pavona frondifera*, *Oulastrea crispata* and *Symphyllia recta*.

In general, salinity tolerance of corals can be determined by the ambient salinity of the coral's normal environment. The same coral species may exhibit different ranges of salinity tolerance in different habitats. For example, for *Porites compressa* in the Arabian Gulf, some of the colonies survived in salinities as high as 49 psu, while all specimens died at 21 psu and were bleached or died at 23 and 25 psu. On the other hand, *P. compressa* from Hawaii and *P. porites* from Florida died at 45 psu and partial mortality occurred at 40 psu, with a lower tolerance limit of 20 psu (Coles, 1992).

**Table 1** Experimental studies assessing the effect of low salinity on reef corals in various locations

Taxa	Ambient salinity, Location, Exposure time	Salinity treatment (psu)	Response	Reference
<i>Pocillopora damicornis</i>	32 psu, Chon Buri, 32 d	15, 25, 32	Dead: 15 psu–after 7–9 d Pale: 25–32 psu–partial bleaching after 13–18 d but survived/recovered	<b>This study</b>
<i>Pocillopora damicornis</i>	10–30, Thailand, 1.5 hrs	10, 20, 30	40% decline in Gross production: Respiration from 30 to 20 psu; 74% decline from 30 to 10 psu	Moberg, Nystrom, Kautsky, Tedengren, and Jarayabhan (1997)
<i>Pocillopora damicornis</i>	32–35, Kaneohe Bay, Hawaii, USA, 20 d	15, 20, 25, 30, 35, 40, 45	Dead: 15 psu– after 1d; 20 psu– after 10 d Pale: 25 psu–bleached but alive after 20 d except 1 specimen; 35 psu– 50% pale after 20 d	Coles (1992)
<i>Porites compressa</i>			Dead: 45 psu Partial mortality: 40 psu	
<i>Montipora verrucosa</i>			Dead: 15 psu– after 1 d; 20 psu– after 10 d	



**Table 1** (Cont.)

Taxa	Ambient salinity, Location, Exposure time	Salinity treatment (psu)	Response	Reference
			Pale: 25 psu–bleached but alive; 35, 30 psu–partial bleaching–fully pigmented	
<i>Porites porites</i>	Florida, USA, 20 d	15, 20, 25, 30, 35, 40, 45	Dead: 45 psu Partial mortality: 40 psu Pale: 20 psu bleached but 60% survived Normal: 25, 30, 35 psu	
<i>Porites compressa</i>	40–43 psu, Saudi Arabia, 20 d	Ex1: 19, 25, 31, 37, 43, 49, 53 Ex2: 21, 23, 25, 27, 40 (ambient), 45, 47, 49 and 51	Stressed within 48 hrs at 19, 21, 51 and 53 psu. Dead: 19 psu–within 3 d; 51, 53 psu– within 4 d Dead: 49 psu–within 7 d; 21 psu– 9 d; 51 psu–4 d Pale: 23,25 psu–within 2–4 d; 27, 31 psu–within 7 d Normal: 37, 40, 43, 45 psu	
<i>Stylophora pistillata</i>		Ex1: 19, 25, 31, 37, 43, 49, 51	Dead: 19, 51 psu–within 2 d; 49 psu–within 10 d; 25 psu–after 20 d Normal: 31, 37, 43 psu	
<i>Porites lutea</i>	10–30, Thailand, 1.5 hrs	10, 20, 30	25% decline in Gross production: Respiration from 30 to 20 psu, 52% decline from 30 to 10 psu	Moberg et al. (1997)
<i>Stylophora pistillata</i>	37 psu, Heron Is, Australia 12 hrs exposure, 12 d to recovery	37, 33.5, 29.5, 26, 22, 20.5, 18.5, 15 37, 31.5, 26, 20.5 (6 d)	Dead: 15 psu 1 d after exposure for 12 h Pale: Corals exposed to salinity ranging from 26 to 18 psu for 12 h remained alive but discolored within 3 d of exposure. Zoox density ok at 33.5 and 29.5 (NS); 90% decline at 26, 22 and 18.5 psu Significant reduction in mean dark-adapted Fv/Fm in coral exposed to salinity level at 26 and 20.5 psu.	Kerswell and Jones (2003)
<i>Sarcophyton</i> sp.	32 psu, Chon Buri, 32 d	15, 25, 32	Dead: 15 psu–withered skin and peeling tissue after 8 d Normal: 25–32 psu–able to acclimatize	<b>This study</b>
<i>Sarcophyton</i> sp.	30–31 psu, Thailand, 12 d	20, 30, 40	Dead: 20 psu–completely bleached and dead within 204 h Normal: 40 psu–survived	Chavanich et al. (2009)

**Table 1** (Cont.)

Taxa	Ambient salinity, Location, Exposure time	Salinity treatment (psu)	Response	Reference
			Chronic: all colonies died when salinity dropped to 10 psu (276 h after the experiment) and at 49 psu (264 h after the experiment)	

Ambient salinity levels for corals tested in this study were similar to those observed in Kaneohe Bay, Hawaii, where storm-generated floods in 1987 reduced salinity to 15 psu in the surface water. The event resulted in mass mortality of coral reef organisms in shallow water, particularly *P. damicornis* (Coles, 1992; Jokiel et al., 1993). Coles & Jokiel (1992) reported estimates of 15 psu to 20 psu as the lower lethal salinity for coral reefs. Similarly, Jokiel et al. (1993) reported that all *P. damicornis* were killed after exposure of two days to a salinity of 15 psu.

Moberg et al. (1997) showed that when *P. damicornis* was exposed to sudden salinity reductions from the ambient level (30 psu) to 20 and 10 psu, the gross production to respiration ratio ( $Pg:R$ ) was significantly lowered, but *Porites lutea* was less affected. *Pocillopora damicornis* is more stenohaline than most other corals, in particular juvenile colonies. Kuanui, Chavanich, Viyakarn, Omori, and Lin (2015) measured chlorophyll fluorescence ( $Fv/Fm$ ), which is used for detecting stress in plants, and found that juvenile colonies of *P. damicornis* were more sensitive to low salinity than older colonies. Fluorescence was not detected in six-month-old *P. damicornis* exposed to 27 psu seawater. Different coral species at different ages did not display the same physiological response to changes in salinity.

This study revealed that *Sarcophyton* sp. can tolerate a salinity of 15 psu for up to eight days. Similarly, Chavanich et al. (2009) found that *Sarcophyton* sp. specimens were completely dead within eight days during an acute salinity treatment of 20 psu. Chavanich et al. (2009) suggested that stress from hyposalinity can lead to prolonged coral death after bleaching to a greater degree than high temperature.

Heavy rainfall/flooding events not only affect the coral community but can also impact reproductive success of hard corals and the successful recruitment pulses of many species if the wet monsoon is delayed. True (2012) found that fertilization success, larval survivorship and settlement success varied among coral genera. The observed taxa included *Platygyra* spp., *Acropora hyacinthus* and *Favites abdita*, of these, *Acropora* was the most susceptible species. Duration of exposure at 22–25 psu was a critical transition factor for survivorship.

### Conclusion and Suggestions

Different coral species can display different vulnerability responses to hyposalinity. A coral species acclimated to ambient salinity at one location may not exhibit the same response to low salinity events as it does elsewhere. In this study, the lethal threshold for *P. damicornis* and *Sarcophyton* sp. was 15 psu at the exposure time of 7–9 days. Increases in coastal runoff from land-based activities and climate change may have more impact on coral communities than has been previously recognized. Thus, more studies focused on hyposalinity are needed to



investigate the complex interactions among coral communities, fertilization success, larval survivorship and settlement success in common taxa found at sites vulnerable to hyposaline conditions.

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