

Research Article

Use of sodium hypochlorite as a control method for the non-indigenous coral species *Tubastraea coccinea* Lesson, 1829

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Editor's note:

This study was first presented at the 9th International Conference on Marine Bioinvasions held in Sydney, Australia, January 19–21, 2016 (<http://www.marinebioinvasions.info/previous-conferences>). Since their inception in 1999, ICMB series have provided a venue for the exchange of information on various aspects of biological invasions in marine ecosystems, including ecological research, education, management and policies tackling marine bioinvasions.

Abstract

The scleractinian coral *Tubastraea coccinea* is native to the Pacific Ocean, and it is the first documented hard coral to have invaded the Western Atlantic Ocean. Along the Brazilian coast, this species was documented in the late 1980s on artificial substrates, but currently, *T. coccinea* is also observed in the natural environment. Previous studies reported that *T. coccinea* can alter the structure of the native community and can cause social and economic impacts. However, relatively little information is available about control methods and strategies focusing on this coral as the target species. This study aims to evaluate the effectiveness of sodium hypochlorite (NaClO) exposure on *T. coccinea* colony mortality and to determine the lowest concentration required to kill this species. The experiments were conducted in controlled laboratory conditions. Colonies were exposed to sodium hypochlorite solutions (2.5% active chlorine) at concentrations of 2, 20, 50, 100, 150 and 200 ppm. The control treatment exposed colonies to only seawater. Colonies were monitored over seven days or until death. Concentrations equal to or higher than 20 ppm were harmful to *T. coccinea*, causing several types of damage and, eventually, mortality of the colonies. The time needed to kill all the colonies was 108 hours in 20 ppm sodium hypochlorite solution, 72 hours in 50 and 100 ppm, 5 hours in 150 ppm and 3 hours in 200 ppm. Our results showed that the sodium hypochlorite solution was effective for killing *T. coccinea* colonies. In addition, at 150 ppm and 200 ppm we obtained the best results since the colonies achieved 100% mortality in a short period of time. Therefore, sodium hypochlorite is a potential option to be applied in the management and control of this invasive coral in restricted areas, in both artificial and natural substrates.

Key words: Sun coral, orange cup coral, bioinvasion, management, bleach, mortality

Introduction

The rate of biological invasions has increased in recent decades and is considered a major threat to marine biodiversity (Ruiz and Carlton 2003). This is

recognized to be mainly due to the increased shipping traffic, of commercial and recreational vessels that transport species around the world in ballast water and by hull fouling (Carlton 1987; Carlton 2001; Carlton and Geller 1993; Endresen et al. 2004). Other human-mediated activities, such as the movement of

dry docks and exploratory drilling platforms, aquaculture and the aquarium trade also act as vectors of species introduction to non-native areas (Williams et al. 2013). In addition, the number of artificial structures, such as sea walls, breakwaters, docks, jetties, pilings, bridges and offshore platforms, are increasing in coastal urbanized areas. These structures are suitable for the establishment of non-indigenous species (NIS) and can act as dispersion corridors, facilitating their spread (Bulleri and Airoidi 2005; Airoidi et al. 2015).

Non-indigenous species cause many ecological impacts in new habitats and the extent of these impacts on the native biota may vary widely, especially in areas where their abundances heavily increased, becoming dominant species in the community (Ruiz et al. 1997). The invaders may be harmful to a single native species, but they can also alter the structure of the entire native community, causing changes in the entire ecosystem (Ruiz et al. 1997; Grosholz 2002). Moreover, encrusting NIS can cause social and economic impacts by fouling man-made structures and smothering of commercially important organisms (Coutts and Forrest 2007; Denny 2008; Ojaveer et al. 2015).

The scleractinian coral *Tubastraea coccinea* Lesson, 1829, native to the Pacific Ocean, was the first NIS of hard coral documented in the western Atlantic Ocean (Cairns 2000). In Brazil, this species has been documented since the 1980s, first in artificial substrates and later expanding to natural substrates (De Paula and Creed 2004). Previous studies showed that *T. coccinea* can alter the structure of the native community (Creed and De Paula 2007) and cause social and economic impacts (Mantelatto and Creed 2014). Although it is considered invasive to the Brazilian coast (Lopes et al. 2009), few studies have been conducted to develop strategies to control this species (e.g., Moreira et al. 2014; Mantelatto et al. 2015).

Recently, some methods of control have been developed for treatment of encrusting invasive species in man-made structures and in aquaculture systems. Some of these methods include the immersion in freshwater (Carver et al. 2003; Forrest and Blakemore 2006; Denny 2008); immersion in hot water (Bax et al. 2002; Carver et al. 2003; Forrest and Blakemore 2006); treatment with chemical compounds, such as sodium hypochlorite (Rajagopal et al. 2002a; Carver et al. 2003; Denny 2008; Roche et al. 2015), chlorine dioxide, copper sulphate, detergents (Bax et al. 2002), acetic acid (Carver et al. 2003; Denny 2008; Roche et al. 2015; Atalah et al. 2016), calcium oxide (Carver et al. 2003; Denny 2008), silicic acid and caustic soda (Denny 2008); water blasting (Forrest and

Blakemore 2006); air drying (Forrest and Blakemore 2006); and the construction of encapsulation systems (Roche et al. 2015; Atalah et al. 2016).

Sodium hypochlorite is a disinfectant with a broad anti-microbial action, and it is considered a low-toxicity chemical compound in low concentrations (Taylor 2006). The household bleach (sodium hypochlorite solution) is commonly used for the treatment of drinking water (in a proportion of 1 ml per liter) and to sanitize food. Usually, household bleach contains from 2% to 8% sodium hypochlorite. Sodium hypochlorite, as well as other chlorine compounds, has been extensively applied in the management and control of freshwater (Jenner et al. 1998; Rajagopal et al. 2002b) and marine invasive species (Jenner et al. 1998; Rajagopal et al. 2002b; Denny 2008; Roche et al. 2015), especially by electric power plants and by aquaculture industry stations. Chlorination is a promising method for application in the control of NIS in marine environment, because the free oxidants formed when chlorine is added to seawater are even more effective as a biocide than those formed when it is added to freshwater (Taylor 2006).

The use of chlorine was successful in the control of the invasive freshwater mussel *Dreissena polymorpha* (Pallas, 1771) (Cope et al. 2003). In the Great Lakes (North America), sodium hypochlorite was effective in the treatment of residual sediment from ballast tanks, inhibiting hatching of diapausing eggs (Gray et al. 2006). In Darwin, North Australia, chlorine was applied for the eradication of *Mytilopsis* sp. in three marinas where this species was detected (Bax et al. 2002). Sodium hypochlorite was toxic to the mussels *Mytilopsis leucophaea* (Conrad, 1831) and *Mytilus edulis* Linnaeus, 1758, considered invasive in Europe (Rajagopal et al. 2002a). Sodium hypochlorite was also lethal to the colonial ascidian *Didemnum vexillum* Kott, 2002, a nuisance in mussel farms in Shakespeare Bay, New Zealand, that recently was detected in ports in the United Kingdom and the United States (Coutts and Forrest 2007; Denny 2008; Lambert 2009; Forrest and Hopkins 2013; Roche et al. 2015). Sodium hypochlorite was also used to eradicate the invasive macroalgae *Caulerpa taxifolia* (M. Vahl) C. Agardh, 1817 in an estuary in California, USA (Williams and Schroeder 2004; Anderson 2005).

This study aimed to investigate the effectiveness of chlorination as a method to control and eradicate the invasive coral *T. coccinea* and to establish the lowest sodium hypochlorite (NaClO) concentration required to kill the colonies. Here, we conducted experiments under controlled laboratory conditions to evaluate the effect of sodium hypochlorite at different concentrations on *T. coccinea* mortality.

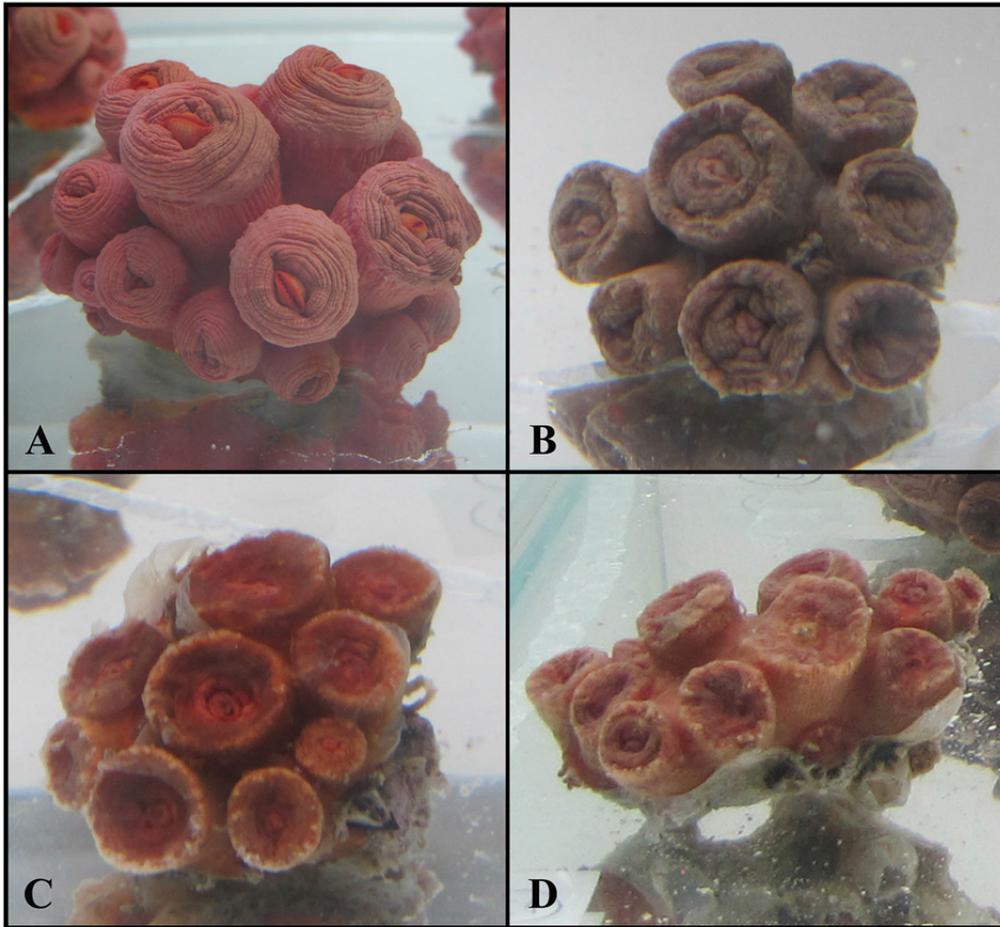


Figure 1. *Tubastraea coccinea* colonies exposed to different sodium hypochlorite concentrations were evaluated according to a damage scale: (A) healthy; (B) changes in tissue colour; (C) tissue degradation and (D) apparent skeleton. Photographs by Luciana Altwater.

Materials and methods

Coral sampling

Colonies of *Tubastraea coccinea* were collected by scuba diving using a spatula and a hammer from a rocky shore located at Porcos Island, Arraial do Cabo, Rio de Janeiro, Brazil (−22.965843; −41.993323). Immediately after sampling, colonies were placed individually in plastic bags with seawater and brought to laboratory in thermic containers. In the laboratory, colonies were cleaned to remove all the epibionts and were acclimated in 250 L tanks (temperature = 23 °C; salinity = 35 psu) for 48 hours.

Laboratory procedures

Different concentrations of bleach (sodium hypochlorite solution with 2.5% active chlorine) were tested to evaluate the effectiveness of sodium hypo-

chlorite (NaClO) on *T. coccinea* colony mortality and to determine the lowest concentration required to eliminate this species. Colonies (N = 15 in each treatment) were exposed to bleach at concentrations of 2, 20, 50, 100, 150 and 200 ppm (for details about the dilutions see Supplementary material Table S1) and monitored for seven days or until the death of the colonies. The control treatment was the immersion in filtered seawater (35 psu). Colonies were considered dead when there was no response to tactile stimulation and polyps showed tissue degradation.

The experimental design consisted of a system with 20 aquaria installed in a 23 °C acclimatized room. Each concentration was replicated five times and each aquarium received three colonies. The colonies were arranged with 10 cm between each other in each aquaria and identified. Aquaria were maintained with aeration, and partial changes of water were carried out every 48 hours to maintain the target

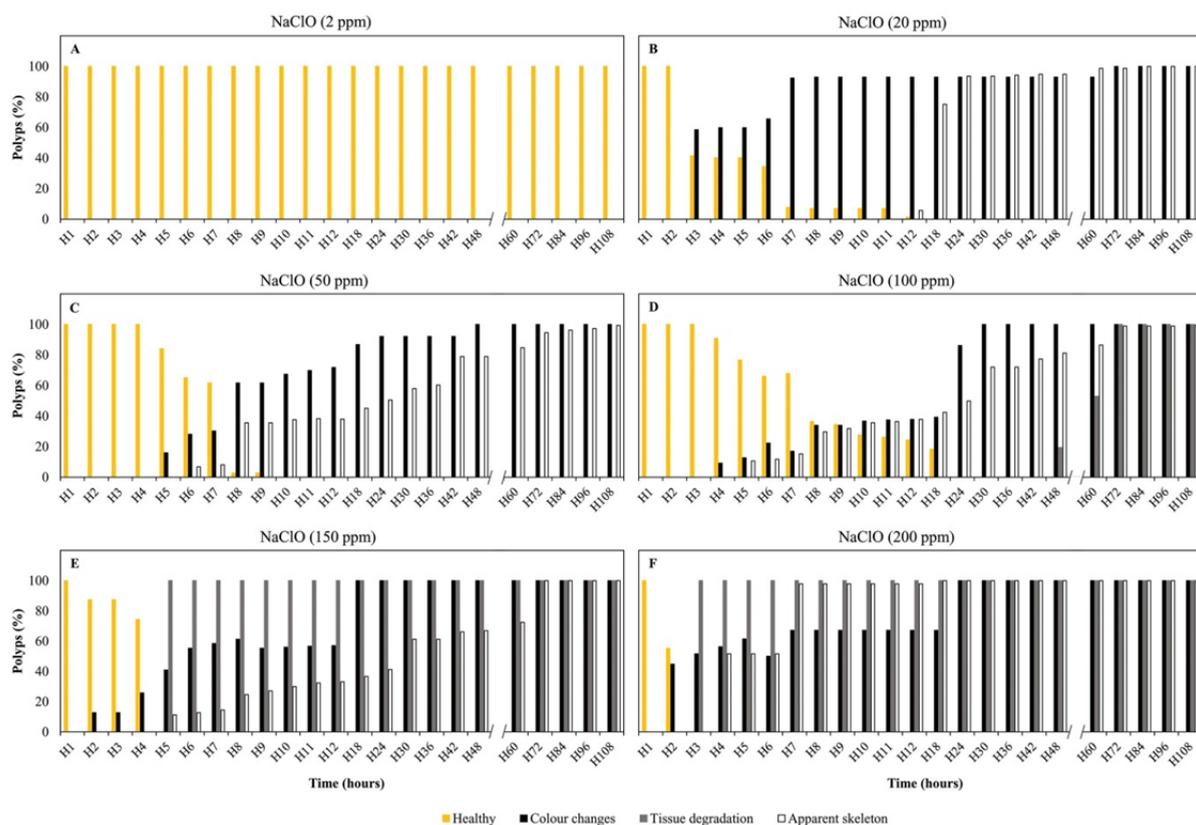


Figure 2. Percentage of polyps that were healthy or exhibited the evaluated types of damage (changes in tissue colour, tissue degradation and apparent skeleton) after exposure to sodium hypochlorite (NaClO) solution at concentrations of 2 ppm (A); 20 ppm (B); 50 ppm (C); 100 ppm (D); 150 ppm (E) and 200 ppm (F) for 108 hours (H).

bleach concentration. Temperature was monitored daily and varied between 22.3 °C and 23.5 °C. Experiments were conducted in two steps. In the first step, the concentrations of 2, 20 and 200 ppm were tested and in the second step, the concentrations of 50, 100 and 150 were tested. Control condition was set in both steps. Colonies used in two steps were obtained from the same population.

Colonies were monitored hourly during the first twelve hours of exposure, every 6 hours until 48 hours of exposure, and, after that, every twelve hours until the end of the experiment (168 hours; 7 days). Polyps were evaluated based on a damage scale to estimate the proportion of polyps with the following characteristics: (1) healthy; (2) colour changes; (3) tissue sloughing; and (4) apparent skeletons (Figure 1). Dead colonies were quantified to establish mortality proportion vs. time in the treatments that caused mortality. A colony was only considered dead when 100% of the polyps were dead.

Statistical analysis

A one-way ANOVA was applied to identify the significant differences in time to death (survival time) of the colonies among sodium hypochlorite concentrations in which mortality was observed and the Tukey post-hoc test was used to identify these differences. Because the data (time until reach mortality) did not meet the assumptions of normality and homogeneity of variances, they were $\log(x+1)$ transformed. The significance level was set to $\alpha = 0.05$. The statistical analysis was performed using the software Statistica 8 (StatSoft 2007).

Results

No colonies in the control condition (sea water), nor did any of the colonies exposed to the 2 ppm concentration of sodium hypochlorite died before the end of the experiment (seven days). The polyps of the colonies immersed under these conditions remained

healthy during the entire experiment, despite the extrusion of mesenteric filaments by the colonies. Filamentous extrusion was observed in polyps at all sodium hypochlorite concentrations tested.

At concentrations of 20 ppm, 50 ppm, 100 ppm, 150 and 200 ppm, polyps presented changes in tissue colour (darkening or paling), and the skeleton became apparent (Figure 2). Additionally, when exposed to concentrations of 100 ppm, 150 ppm and 200 ppm NaClO, tissue degradation was observed (Figure 2).

At concentrations equal to or higher than 20 ppm, sodium hypochlorite was harmful to *T. coccinea*, causing damage to the polyps and, finally, colony death. At concentrations of 20 ppm and 50 ppm NaClO, colonies started to die after 60 hours of exposure. Colonies achieved 100% mortality after 108 hours of exposure to 20 ppm and 72 hours at 50 ppm (Figure 3). After 30 hours of exposure to the 100 ppm concentration, the mortality was approximately 20%, and 100% mortality was achieved after 72 hours (Figure 3). At concentrations of 150 and 200 ppm NaClO, all colonies died within the first 5 and 3 hours of the exposure, respectively (Figure 3).

A significant difference was observed among the survival times of the colonies exposed to concentrations in which mortality was observed (ANOVA $F_{(4,70)} = 1207.23$; $p < 0.001$). The shortest survival time was documented for the 200 ppm concentration; followed by 150 ppm; 100 and 50 ppm, which did not differ from each other; and 20 ppm (Figure 4).

Discussion

In this study, we proved the effectiveness of sodium hypochlorite (household bleach) concentrations in the control or eradication of the invasive coral *T. coccinea* under controlled conditions. In Brazil, studies about invasive species are recent, especially in what concerns their control. The chlorination is already applied to control invasive species in hydroelectric power plants. However, chlorine, as well as other chemical compounds, were never applied before in the treatment of marine NIS in Brazil. Our findings will allow the development of new methodologies for large scale marine invasive species treatment.

Based on the present results, the lowest tested sodium hypochlorite concentration (2 ppm) does not seem to be harmful to *T. coccinea* colonies. However, previous studies have shown that even lower concentrations of chlorine are capable of killing invasive organisms, if they are exposed to these concentrations over a longer period of time. A concentration of 0.5 mg/L (equivalent to 0.5 ppm) caused 100% mortality in the freshwater mussel *D. polymorpha*

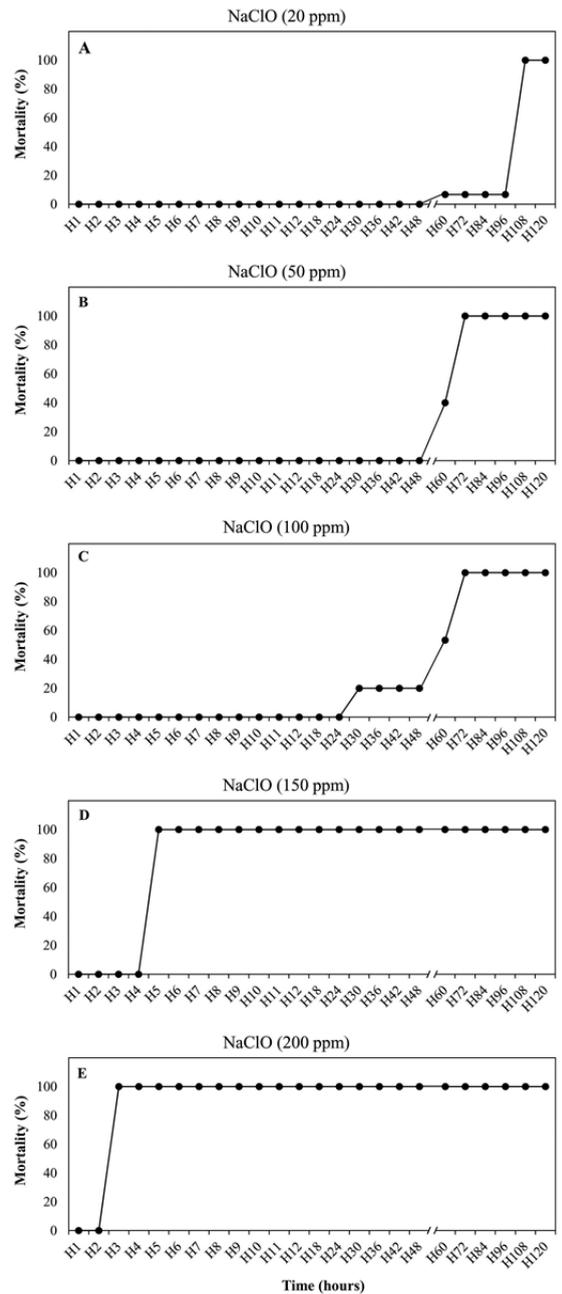


Figure 3. Mortality percentage curve of *Tubastraea coccinea* colonies exposed to sodium hypochlorite (NaClO) solution at concentrations of 20 ppm (A); 50 ppm (B); 100 ppm (C); 150 ppm (D) and 200 ppm (E) up to 120 hours (H). Note: There was no mortality under the control condition (seawater) or at a concentration of 2 ppm NaClO.

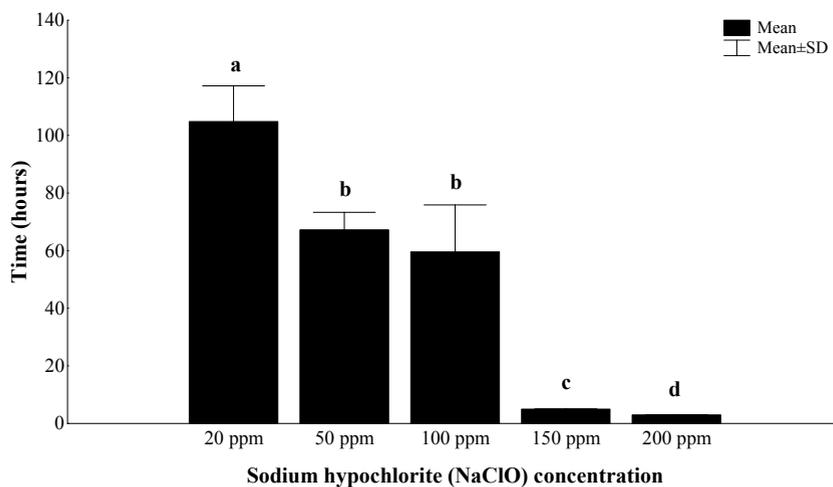


Figure 4. Time to death (mean \pm standard deviation) of the invasive coral *Tubastraea coccinea* in a sodium hypochlorite (NaClO) solution at different concentrations (20, 50, 100, 150 and 200 ppm). Significant differences are indicated by different letters.

after 882 hours of exposure (Rajagopal et al. 2002b). Even a concentration as low as 0.25 mg/L killed *M. edulis* and *M. leucophaeata*, but the time required to cause 100% mortality was greater than 75 days and 100 days, respectively (Rajagopal et al. 2002a).

The duration of our experiment may not have been sufficient to kill colonies at a concentration of 2 ppm NaClO. However, when the focus is establishing control methods, the method with the most rapid time-to-death may be the best option to decision makers. In general, it is not feasible to keep the organisms immersed in a solution for periods longer than just a few hours. For this reason, concentrations capable of causing death in a shorter period of time will be the most applicable.

On the other hand, the hypochlorite solution was lethal to the colonies at all concentrations equal and higher than 20 ppm. Sodium hypochlorite acts by dissolving the organic tissue of organisms. In this study, the damages caused by sodium hypochlorite to the polyps included changes in the colour of the coenenchyme, tissue degradation and consequently, parts of the skeleton became visible.

Nevertheless, the time needed to reach 100% of mortality was longer when the polyps were exposed to 20 ppm NaClO (108 hours), 50 ppm (72 hours) and 100 ppm (72 hours). Concentrations of 150 ppm and 200 ppm were the most effective, killing the colonies after five and three hours of exposure, respectively. In other words, the time-to-death of the colonies decreased as the sodium hypochlorite concentration increased. Our results were similar to those found by Rajagopal et al. (2002a) and Rajagopal et al. (2002b) using the mussels *D. polymorpha*, *M. edulis* and *M. leucophaeata* and Bax et al. (2002) for *Mytilopsis* sp. Time to reach 100% mortality on mussels decreased with increasing chlorine concentration.

However, in the studies of Rajagopal et al. (2002a) and Rajagopal et al. (2002b), the maximum concentration tested was much lower (5 ppm) than the maximum concentration in our study (200 ppm). At a concentration of 2 ppm, equivalent to the lowest concentration tested in our study, the time to reach 100% mortality on mussels was higher than 20 days (Rajagopal et al. 2002a). At concentrations 12 ppm and 24 ppm, Bax et al. (2002) registered survival times of 111 hours and 90 hours, respectively, to *Mytilopsis* sp., similar to the registered in our study to 20 ppm (108 hours).

Sodium hypochlorite is a promising alternative to control *T. coccinea*, as well as other invasive species, especially in floating pontoons, recreational and commercial vessels, and offshore platforms. To increase effectiveness, chlorination can be combined with other methods, such as temperature variation (Rajagopal et al. 2002a), encapsulation systems (Roche et al. 2015) and the wrap technique (Atalah et al. 2016). Whether applied within an enclosed system, as a decontamination berth (Roche et al. 2015) or other wrap techniques (Inglis et al. 2012; Atalah et al. 2016; Morrissey et al. 2016), the adequate concentration of sodium hypochlorite could be easily achieved. In addition, the use of encapsulation systems reduces the release of this chemical compound in the environment and facilitates its appropriated discharge (Morrissey et al. 2016).

Future studies concerning the control of *T. coccinea* should consider using acetic acid, as it is also commonly used to control invasive species. The effectiveness of this compound, present in domestic vinegar, has never been tested against corals, and low concentrations of acetic acid, as well as sodium hypochlorite, do not represent an environmental risk and can be safely applied. It should be noted that

acetic acid has not been effective against all target species. It is effective against a variety of cosmopolitan taxa (e.g., algae, solitary ascidians and some species of colonial ascidians) (Carver et al. 2003; Forrest et al. 2007) but has limited effectiveness against more resistant species (the serpulid polychaete *Hydroides elegans* and the colonial ascidian *Didemnum vexillum*) (Forrest et al. 2007; Denny 2008).

Our results showed that a sodium hypochlorite solution was effective in killing *T. coccinea* colonies. Concentrations higher than 20 ppm were lethal from sixty hours of exposure, however, 150 ppm was the lowest sodium hypochlorite concentration that caused the death of 100% of the colonies within few hours (≤ 5 hours). According to these findings, concentrations higher than 150 ppm are the best option to be employed in the management and control of the invasive coral *T. coccinea* in restricted areas, in both natural and artificial substrates. In Brazil, the discussion and implementation of eradication programs for non-indigenous species is still incipient. Only recently, after fifteen years of the first detection, an action that involves the manual removal of colonies was set by policy makers and many researchers, to reduce and control the expansion of *Tubastraea* species (Silva et al. 2014). However, its efficacy remains unknown. Therefore, other methods of control should be considered and the use of sodium hypochlorite is a promising alternative to be applied in the eradication of this invasive species.

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Supplementary material

The following supplementary material is available for this article:

Table S1. The volume of seawater (salinity 35) and bleach (sodium hypochlorite (NaClO) solution; 2,5% active chlorine) used in each treatment.

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