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Chemical eradication methods for aquarium strains of *Chaetomorpha*

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ABSTRACT

Biological invasions by plants released from home aquaria into natural environments are an issue of global concern. The best-documented of these invasions, those by the macroalga *Caulerpa taxifolia*, have affected the ecology and economy of the Mediterranean coast, Australia, and California. As California boasts the only successful eradication of *C. taxifolia*, we sought to proactively identify effective eradication methods for future invasions by aquarium macroalgae. The genus *Chaetomorpha* is currently the most popular macroalgal group for saltwater aquaria, used by over 50% of surveyed hobbyists. As such, the opportunity for its introduction is considerable and poses a serious threat to marine and estuarine systems. We tested five chemical treatments—chlorine bleach, rock salt, copper sulfate, acetic acid, and fluridone—to determine the best techniques for rapid eradication or control of an invasion by aquarium *Chaetomorpha*. We conducted factorial experiments on 1-cm fragments cut from algal thalli from three retail purchases of *Chaetomorpha* (included *C. crassa, C. spiralis, C. linum*) exposed to experimental concentrations of each chemical for differing durations, rinsed the fragments, and then resubmerged them in chemical-free artificial seawater. We monitored survival and growth for 4 wk after the end of chemical exposure; of the treatments tested, only acetic acid was consistently effective at inducing full mortality at tested exposure levels (at least 2% for 4 min). Chlorine bleach results were inconsistent for tested species. Rock salt and copper sulfate did not significantly reduce survival but effectively limited growth of *Chaetomorpha* fragments. As tested, fluridone did not significantly limit survival or growth.

Key words: *Caulerpa taxifolia,* chlorine bleach, copper sulfate, rock salt, acetic acid, sonar, fluridone

INTRODUCTION

Aquarium release is a worldwide concern as a vector for the introductions of nonnative species to natural environments (Padilla and Williams 2004). These introductions happen intentionally by direct release of aquarium pets and plants or unintentionally through aquarium breakage, escape, or the disposal of aquarium water that contains living organisms (Calado and Chapman 2006). Additionally, aquarists who release organisms or water down storm-water drains may not be aware that they lead to natural waterways. The ease and low cost of obtaining nonnative species through the aquarium trade, combined with the number of reasons and ways they can be released into natural environments, make mitigation for this risk an important task (Padilla and Williams 2004, Walters et al. 2006).

The best-documented aquarium release invasions are those of the green feather alga *Caulerpa taxifolia* (Walters 2009). *Caulerpa taxifolia* has cost millions of dollars in economic damage, as well as modified the aquatic ecosystems of the Mediterranean, California, and South Australia (Boudouresque et al. 1996, Relini et al. 1998, Williams and Smith 2007). California saw the only successful eradication by staking black tarps over algal beds and injecting chlorine bleach, although it was unclear whether the light deprivation, chemical exposure. or the combination was responsible for the die-off (Anderson 2005). The implementation and monitoring of this chemical eradication, however, cost over 7 million dollars (Anderson 2007). Particularly when considering that macroalgal reproduction by vegetative fragmentation can increase the ability for secondary spread, rapid response to invasions is essential in limiting their detrimental effects and in minimizing the cost of controlling the invasion (Smith and Walters 1999).

Since the detrimental invasions by *C. taxifolia* became public knowledge through books, documentaries, and outreach campaigns, members of the aquarium hobby industry began using different genera of macroalgae that lacked the notoriety of invasive species. Most popular among these was *Chaetomorpha*, a green macroalgal genus (Kutz.), used by more than 50% of the hobbyists we surveyed in 2010 (Walters et al. 2011). Because it possesses many of the same qualities that enabled the invasions by *Caulerpa taxifolia*, we sought to mitigate the invasion risk posed by *Chaetomorpha* if introduction events extend to these aquarium strains. Species in this genus have been shown to have wide environmental tolerances, strong competitive abilities through nutrient uptake, and a propensity for reproducing vegetatively from fragments as small as 0.5 mm in length (Lavery and McComb 1991, Xu and Lin 2008, Caputo et al. 2010, Odom and Walters 2014). These properties could enable unintentional introductions and promote the potential for establishment and spread. Introduction of this genus may be of particular threat to coastal waters, especially in highly developed areas, where nutrient levels are more likely to cause eutrophication, as Lapointe (1997) documented that *C. linum* responds to eutrophication with bloom-forming growth.

When a nonnative species is introduced, rapid response is crucial to effectively eradicating or managing the invader.

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For this reason, we proactively evaluated chemicals used in the management and eradication of invasive *Caulerpa taxifolia* to determine their effectiveness on aquarium *Chaetomorpha* if it is introduced to natural waterways. We tested chlorine bleach (sodium hypochlorite), the chemical used for *Caulerpa* eradication in California (Williams and Schroeder 2004); rock salt, the most popular management method for Australia's invasive *Caulerpa* populations (Uchimura et al. 2000, Glasby et al. 2005); copper sulfate,[1] an effective method for inducing mortality in a variety of marine organisms (Uchimura et al. 2000); acetic acid[2] (Anderson 2007, Forrest et al. 2007); and fluridone[3] (Anderson 2007) at various concentrations and exposure durations that were relevant to *C. taxifolia*.

MATERIALS AND METHODS

The genus *Chaetomorpha* is a uniseriate, unbranched, multinucleate green macroalga that can be found loose, entangled, or attached to hard substrates in estuarine and coastal waters. The taxonomy of *Chaetomorpha* is primarily based on cell dimensions and species richness is not well resolved; the ITIS dDatabase (2013) lists 11 species in the genus, whereas AlgaeBase lists 66 species as currently accepted (Guiry and Guiry 2013). Although it has not yet been documented as introduced into novel ranges, when one considers its popularity among aquarium hobbyists, *Chaetomorpha* is a likely candidate to be disposed of into natural waterways. *Chaetomorpha* reproduces sexually by isogamy by means of biflagellate gametes, and is extremely robust with vegetative fragmentation (Guiry and Guiry 2013). As long as a single cell of any of multiple species of *Chaetomorpha* remains intact and undamaged, it is able to survive and continue growing (Odom and Walters 2014).

Chaetomorpha was purchased from three aquarium retailers; each purchase was kept in a 10-gallon aquarium at 31 parts per thousand (ppt) artificial seawater (made with Instant Ocean™ sea salts), the optimal salinity according to Xu and Lin (2008), with 12h : 12h light : dark cycle for at least 1 wk before commencing experiments. *Chaetomorpha* from each monospecific purchase was identified to the species level on the basis of cellular dimensions and stiffness of the thalli (Abbott and Hollenberg 1976, Littler and Littler 2000). Purchased species were *C. crassa*, *C. linum*, and *C. spiralis*. *Chaetomorpha crassa* resembles loosely tangled monofilament fishing line with cylindrical cells averaging 300 to 1,000 μm in both diameter and length, whereas *C. linum* resembles more tightly packed steel wool with cells that are 100 to 375 μm in diameter and 300 to 800 μm in length (Littler and Littler 2000). The third species, *C. spiralis*, has cells with diameters that are greater than their lengths (diameter: 170 to 250 μm) (EOL 2013). According to AlgaeBase global distribution maps, *C. crassa*, *C. linum*, and *C. spiralis* all have global distributions encompassing the Eastern and Western hemispheres as well as tropical to artic/subarctic conditions (Guiry and Guiry 2013).

From each purchase, filaments were randomly selected and cut with a razor blade at both ends to 1 cm in length (Williams and Schroeder 2004, Odom and Walters 2014). Blotted dry, wet weights for 1-cm fragments of *Chaetomorpha* ranged from 4.1 to 5.7 mg (mean ± standard error: 4.9 ± 0.1 mg; $n = 20$). For the chlorine bleach, salt, copper sulfate, and acetic acid experiments, fragments were placed individually in closed petri dishes (5.5-cm diameter) with 10 ml of chemical solution (diluted with 31 ppt Instant Ocean artificial seawater) for the designated exposure times (Table 1).

TABLE 1. EXPERIMENTAL CONCENTRATIONS AND EXPOSURE DURATIONS FOR EACH CHEMICAL TESTED.

Chemical	Tested Concentrations	Exposure Durations
Chlorine bleach	0, 50, 125, 250 ppm	30, 60, 90, 120 min
Rock salt	0, 10, 20, 30 g L^{-1}	30, 60, 90, 120 min
Copper sulfate	0, 10, 20, 50 mg L^{-1}	30, 60, 90, 120 min
Acetic acid	0, 1, 2, 4%	1, 2, 3, 4 min
Fluridone	0, 10, 20, 50 ppb	2, 4, 6, 8 wk

For the fluridone trials, experimental fragments from one purchase (*C. linum*) were placed individually in acrylic 1.25-L aquaria with 1 L of artificial seawater, as we were unable to accurately measure and move small enough quantities of the solid chemical to deploy in the 10-ml design. Fluridone was weighed in halves of petri dishes, and the dish was deployed into the aquarium. Initial water levels were marked for each aquarium, and deionized water was added weekly to replace water lost by evaporation.

For all experiments, we used five replicate 1-cm fragments per treatment combination per species and completely randomized locations of all dishes or aquaria used for that chemical. All experiments were conducted at room temperature (22 C) under standard laboratory lighting of overhead fluorescent bulbs (23 μmol m^{-2} s^{-1} photosynthetically active radiation).

After exposure to chemicals, fragments were rinsed for 5 s with deionized water and resubmerged in new petri dishes with 10 ml of artificial seawater. Fragment survival and growth were monitored at 4 wk after the end of chemical exposure. Fragments were considered surviving if at least one cell was alive; mortality was determined by discoloration and the withdrawal of the cell membrane from the cell wall (Odom and Walters 2014). Final survival was analyzed for each purchase with logistic regression in the statistical program R, version 2.11.1 (64-bit), with survival predicted by the product of chemical concentration multiplied by exposure duration. Growth was analyzed with linear regression in R, where final growth (total length of live tissue minus initial length of 10 mm) was predicted by the same concentration by duration units. In our data analysis, we treated our three species together because of the uncertainty of the taxonomy at the genus level and the knowledge that aquarium hobbyists do not distinguish among species of *Chaetomorpha*.

RESULTS AND DISCUSSION

At tested exposures, chlorine bleach was only effective at inducing mortality in *C. crassa*; however, it did significantly decrease growth in *C. crassa* and *C. spiralis* (Figures 1, 2, Tables 2, 3). Although these exposure levels were sufficient for killing *Caulerpa taxifolia* fragments, no tested exposure level resulted in consistent mortality or complete preven-

Table 2. *P*-values for logistic regressions where concentration by duration predicted survival. Significant *P*-values indicate that increasing exposure to chemical treatment decreased survival.

Chemical	*Chaetomorpha crassa*	*Chaetomorpha spiralis*	*Chaetomorpha linum*
Chlorine bleach	$P = 0.0028$*	$P = 0.3820$	$P = 0.4358$
Rock salt	$P = 0.0609$	$P = 0.1254$	$P = 0.2000$
Copper sulfate	$P = 1.0000$	$P = 0.9977$	$P = 0.1676$
Acetic acid	$P = 0.0003$*	$P < 0.0001$*	$P = 0.0004$*

*A significant *P*-value ($P < 0.05$)

Table 3. *P*-values for linear regressions where concentration by duration predicted growth. Significant *P*-values indicate that increasing exposure to chemical treatment decreased growth.

Chemical	*Chaetomorpha crassa*	*Chaetomorpha spiralis*	*Chaetomorpha linum*
Chlorine bleach	$P < 0.0001$*	$P = 0.0004$*	$P = 0.8590$
Rock salt	$P < 0.0001$*	$P < 0.0001$*	$P < 0.0001$*
Copper sulfate	$P < 0.0001$*	$P = 0.0997$	$P = 0.0005$*
Acetic acid	$P < 0.0001$*	$P < 0.0001$*	$P < 0.0001$*

*A significant *P*-value ($P < 0.05$)

tion of growth for *Chaetomorpha* (Table 4; Williams and Schroeder 2004). As such, we would not recommend using it for eradication of aquarium *Chaetomorpha* in the event of an invasion.

Application of rock salt did not significantly reduce survivorship in any of the three tested species (Figure 1, Table 2). This chemical treatment did limit growth effectively in all three species (Figure 2, Table 3), decreasing the percentage of fragments that produced new growth from 92% in control (0 g L^{-1}) fragments to 48% in fragments exposed to salt. Specifically, mean growth among the three species was reduced from 9.8 mm (nearly doubling in size) for control treatments to 0.1 mm in salted treatments during the 4-wk trial. Members of the genus *Chaetomorpha* are known for having high tolerance to hypersaline conditions (Xu and Lin 2008, Caputo et al. 2010). Salting may be useful for maintaining the current biomass of *Chaetomorpha* if it is introduced but is unlikely to be useful for eradication at the exposure levels considered by Uchimura et al. (2000) and Glasby et al. (2005) for management of *Caulerpa taxifolia*.

Copper sulfate was ineffective at inducing mortality for *Chaetomorpha* fragments across all species at exposure levels considered for eradication of *Caulerpa taxifolia* (Figure 1, Tables 2, 4; Uchimura et al. 2000). Exposure to copper significantly reduced growth in *Chaetomorpha crassa* and *C. linum* ($P < 0.001$) and was marginally effective in limiting growth in *C. spiralis* ($P = 0.0997$; Figure 1, Table 3). Although copper exposure only reduced the number of fragments producing new growth from 82 to 61%, mean growth was reduced from 7.3 mm (control) to 0.7 mm. Use of copper sulfate solution may be successful in limiting the spread of an introduction of *Chaetomorpha* rather than eradication. In experimental situations, copper can have negative impacts on sensitive, nontarget species such as the minnow *Pimephales promelas* and the motile microcrustacean *Ceriodaphnia dubia* (e.g., Murray-Gulde et al. 2002). Potential harm to nontarget species and limited success in our eradication assays lead us to discourage the use of this chemical technique in the event of an invasion by aquarium *Chaetomorpha*.

Acetic acid was the only chemical tested that consistently reduced survivorship and growth of fragments of aquarium *Chaetomorpha* (Figures 1, 2, Tables 2, 3). Full mortality was achieved with exposure to 2% acetic acid for 4 min or 4% acetic acid for 1 min. Applications of acetic acid have been shown to have detrimental effects on nontarget estuarine species at concentrations of 5% (Locke et al. 2009); thus if this chemical is used, it should be applied in the lowest effective concentration possible. As the exposure durations we tested were considerably lower than in other chemical treatments, further research should be conducted to examine the effects of lower concentrations with extended durations to determine the lowest concentration of chemical needed for eradication.

Application of fluridone did not affect survival of fragments of *Chaetomorpha*; 100% survival was observed for all treatment combinations. Furthermore, exposure to fluridone was insignificant ($P = 0.499$) in deterring growth (Figure 3). These results were not surprising, considering that fluridone inhibits a photosynthetic pathway primarily in higher plants, although it has been effective in select species of macroalgae (e.g., Anderson 2007). Had it been effective, a selective chemical such as fluridone would have been desirable for limiting detriment to nontarget species. However, this chemical was not effective at tested concentrations in eradicating invasions by aquarium *Chaetomorpha*, as it neither induced mortality nor limited growth.

Concentrations required to induce mortality in aquarium *Chaetomorpha* were consistently higher than what studies have reported necessary for treatment of *Caulerpa taxifolia* (Table 4). High chemical concentrations may increase risk to nontarget species. In the event of an introduction, the potential nontarget effects of any chemical eradication tool should be weighed against that chemical's effectiveness in limiting survival and growth of these competitive algal strains. In addition to considerations of negative side effects, McEnnulty et al. (2001) note that even though laboratory experiments show promise, *in situ* application is often ineffective. As such, we recommend testing any of these chemicals on an introduced population before widespread application.

There are many ways to build on our *Chaetomorpha* data set. First, we used small, 1-cm fragments of *Chaetomorpha* that were newly cut on both ends to maximize our chances of seeing effects and to represent the size fragments generated by hobbyists when transporting this macroalga or when tank cleaning (Odom and Walters 2014). The critical burden of a chemical to kill cells of *Chaetomorpha* should be higher for larger fragments with new damage to only one end of the fragment or to attached filaments with no damage. Additionally, if *Chaetomorpha* was tested as it is regularly found as an entwined clump rather than individual fragments, this may also slow down chemical exposure and uptake; these contact time variables warrant further testing. Second, we have only tested compounds in laboratory assays. Mesocosm data that include information on resi-

Table 4. Comparison of chemical exposure required for mortality of invasive *Caulerpa taxifolia* versus aquarium strains of *Chaetomorpha*.

Chemical	*Chaetomorpha*	*Caulerpa taxifolia*	Reference (*C. taxifolia*)
Chlorine bleach	Not effective at tested concentrations (up to 250 ppm for 120 min)	125 ppm for 30 to 60 min 50 ppm for 90+ min	Williams and Schroeder 2004
Rock salt	Not effective at tested concentrations (up to 30 g L^{-1} for 120 min)	24 g L^{-1} for 30 min 18 g L^{-1} for 60+ min	Uchimura et al. 2000
Copper sulfate	Not effective at tested concentrations (up to 50 mg L^{-1} for 120 min)	20 mg L^{-1} for 30 min 5 mg L^{-1} for 60+ min	Uchimura et al. 2000
Acetic acid	4% for 1 min 2% for 4 min	1% for 60+ min	Anderson, pers. comm.
Fluridone	Not effective at tested concentrations (up to 50 ppb for 8 wk)	Partial chlorosis at 50 ppb for 12 d	Anderson 2007

dence times in the water column are also required before forecasting performance in field situations.

Considering the high rates of aquarium-release introductions, the recent popularity of *Chaetomorpha* use among aquarium hobbyists, and its potential for invasion success, this supposedly "safe" aquarium alternative to *Caulerpa taxifolia* merits attention for its invasion risk. Although these techniques present a guide for chemical eradication if

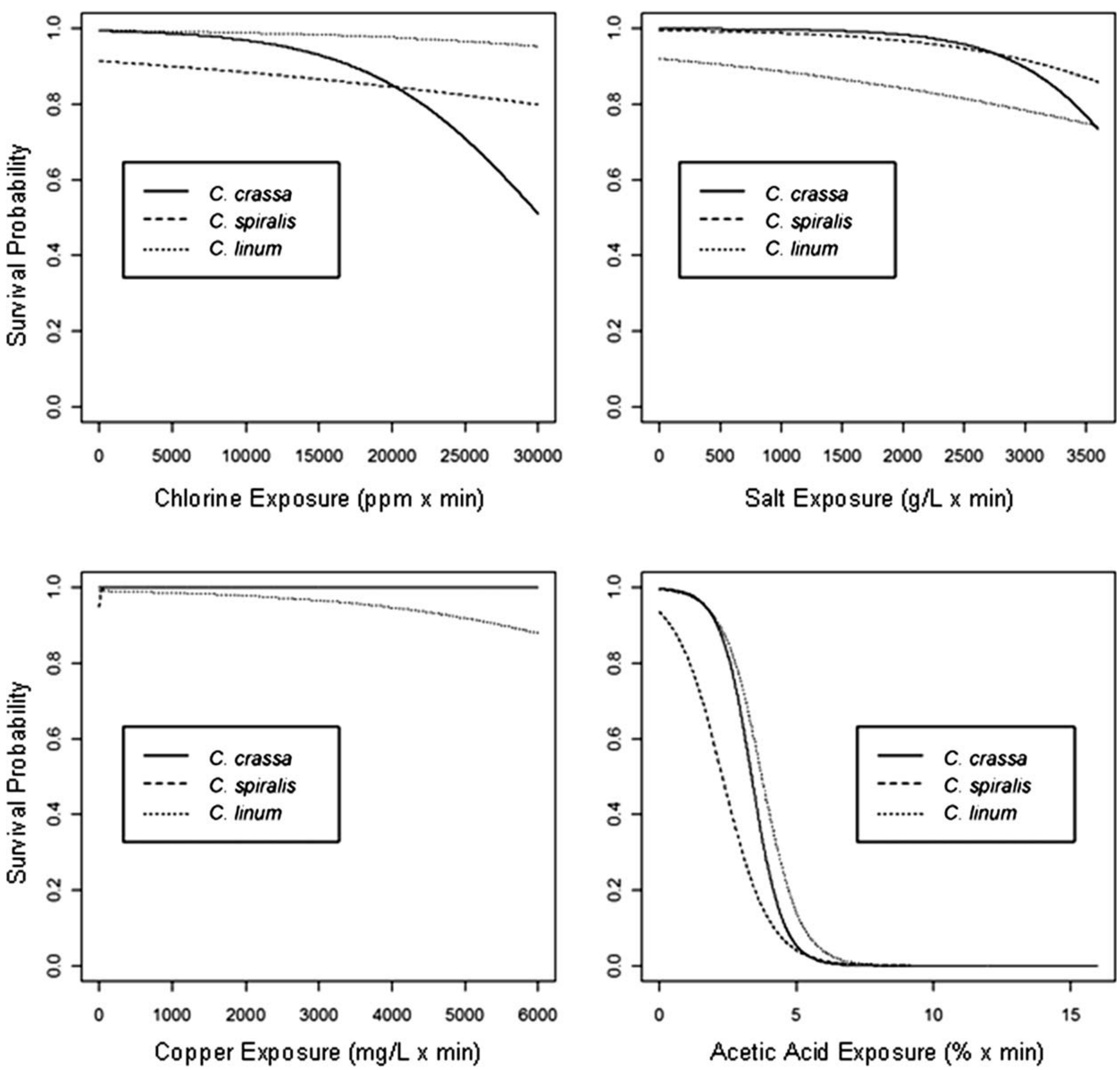

Figure 1. Logistic regressions of survival for three purchased species of aquarium *Chaetomorpha* exposed to chlorine bleach, rock salt, copper sulfate, and acetic acid. Survivorship shown was observed at 4 wk after chemical exposure ended. See Table 1 for concentration by duration unit information for each chemical.

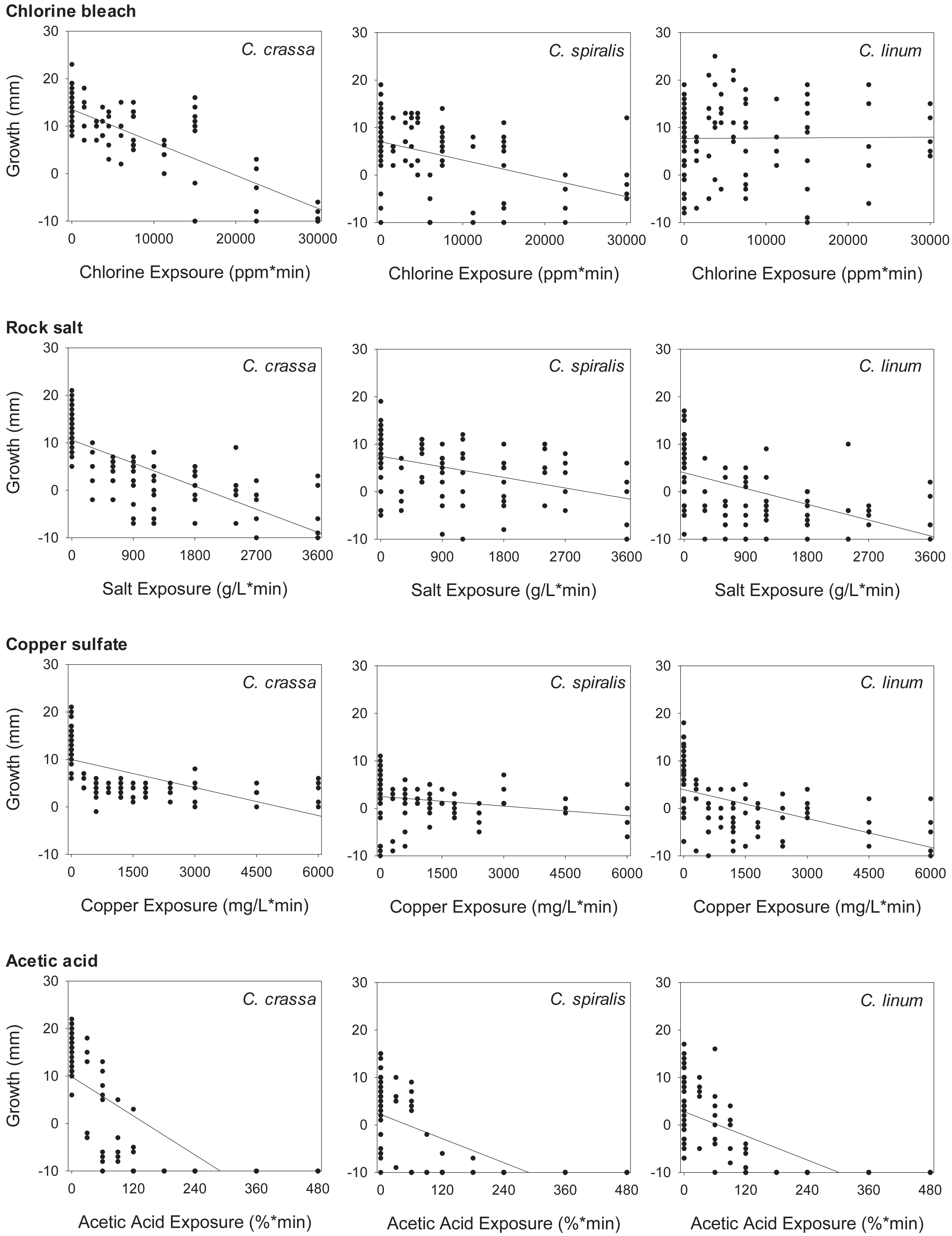

Figure 2. Linear regressions of final growth, as determined by concentration by duration units, for the three species of *Chaetomorpha* exposed to chlorine bleach, rock salt, copper sulfate, and acetic acid treatments. Negative growth values indicate partial (–1 to –9 mm) and full (–10 mm) mortality.

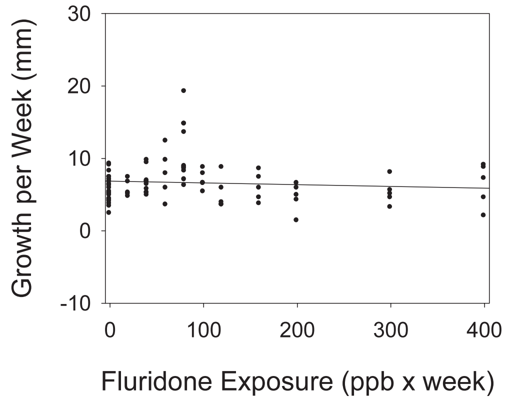

Figure 3. Linear regression of final growth for *Chaetomorpha* exposed to fluridone for 2 to 8 wk ($P = 0.499$).

aquarium *Chaetomorpha* is introduced into natural environments, the best management strategy is prevention (Sepulveda et al. 2012, Odom et al. 2014). This goal can be achieved through continued outreach efforts—to aquarium hobbyists and professionals, aquaculturists, and the general public—to increase awareness of the dangers of releasing captive organisms and to offer effective and convenient disposal alternatives to such introductions.

SOURCES OF MATERIALS

[1]Copper sulfate, catalog number SC9203-1, Fisher Scientific, Pittsburgh, PA 15275.

[2]Acetic acid, catalog number A38-500, Fisher Scientific, Fair Lawn, NJ 07410.

[3]Sonar™, model F2231, Chem Service, P.O. Box 599, West Chester, PA 19381.

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