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THE NEXT “KILLER” ALGAE?
ASSESSING AND MITIGATING INVASION RISK FOR AQUARIUM STRAINS OF THE
MARINE MACROALGAL GENUS *CHAETOMORPHA*

by

RACHEL L. ODOM
B.S. University of Central Florida, 2004

A thesis submitted in partial fulfillment of the requirements
for the degree of Master of Science
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in the College of Sciences
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Major Professor: Linda J. Walters

ABSTRACT

Biological invasions threaten the ecological integrity of natural ecosystems. Anthropogenic introductions of non-native species can displace native flora and fauna, altering community compositions and disrupting ecosystem services. One often-overlooked vector for such introductions is the release of aquarium organisms into aquatic ecosystems. Following detrimental aquarium-release invasions by the “killer alga” *Caulerpa taxifolia*, aquarium hobbyists and professions began promoting the use of other genera of macroalgae as “safe” alternatives. The most popular of these marine aquarium macroalgae, the genus *Chaetomorpha*, is analyzed here for invasion risk. Mitigation strategies are also evaluated. I found that the propensity for reproduction by vegetative fragmentation displayed by aquarium strains of *Chaetomorpha* poses a significant invasion threat—fragments of aquarium *Chaetomorpha* are able to survive from sizes as small as 0.5 mm in length, or one intact, live cell. Fragments of this size and larger are generated in large quantities in online and retail purchases of *Chaetomorpha*, and introduction of these fragments would likely result in viable individuals for establishment in a variety of geographic and seasonal environmental conditions.

Mitigation of invasion risk was assessed in two ways—rapid response to a potential introduction by chemical eradication and prevention through safe hobbyist disposal. I tested the effectiveness of five chemicals used as algicides and found that acetic acid was highly effective at limiting survival and growth of aquarium *Chaetomorpha*. Chlorine bleach, copper sulfate and rock salt were effective at limiting growth but were inconsistent or ineffective in reducing survival of algal fragments. The algicide Sonar limited neither survival nor growth. If aquarium strains of

Chaetomorpha are released, chemical eradication presents a viable management strategy, particularly through the use of acetic acid. A more cost-effective strategy, however, would be preventing introductions; thus safe alternatives to release were determined for hobbyist disposal of unwanted or excess aquarium *Chaetomorpha*. Here I present the minimum exposure durations necessary to induce full mortality of aquarium *Chaetomorpha* through boiling, microwaving, freezing, desiccation and exposure to freshwater. Hobbyist disposal by any of these methods would constitute safe alternatives to introduction of the alga into natural environments. Such preventative measures will inform outreach campaigns in order to limit the potential for aquarium-release introduction.

ACKNOWLEDGMENTS

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CHAPTER 1: GENERAL INTRODUCTION

Biological invasions are a leading threat to biodiversity (Wilcove et al. 1998). By displacing native flora and fauna through competition or direct interference, non-native invasives are able to disrupt ecosystem structure and function (e.g., Lockwood et al. 2007). For these reasons, invasive species are the primary threat to at least 42% of currently listed threatened or endangered species in the United States (Pimentel et al. 2005).

In the marine realm, there are several common vectors for the introductions of non-native species, including ballast water, hull fouling, escape from aquaculture, intentional introductions for fisheries, biological control species and escaped ornamental aquarium organisms (Ruiz et al. 2000; Williams and Smith 2007). This work focuses on the latter—aquarium release as a vector for introductions of invasive species. This topic is particularly relevant to the warm waters of Florida, as Semmens et al. (2004) found 16 species of non-native fishes in south Florida coral reefs that were most likely introduced by aquarium release. They thus identified south Florida as a “hot spot” for aquarium releases. This number has since been increased to 28 species (Zajicek et al. 2009). Aquarium release invasions are further evident through the presence of the lionfish, *Pterois volitans* and *P. miles*, in the waters of the southeastern United States and Caribbean (Morris et al. 2009). As a testament to the profound ecological effects that invasive species can have on native communities, Albins and Hixon (2008) demonstrated that a single lionfish on one experimental coral reef reduced recruitment of juvenile fishes by up to 79% over a period of five weeks.

The best-documented invasion from aquarium releases, however, has been that of *Caulerpa taxifolia*. *C. taxifolia* is a tropical-to-subtropical siphonous green alga that commonly

reproduces sexually and asexually, although invasive strains have only been documented to reproduce asexually via vegetative fragmentation (Wright 2005). It has been introduced to the waters of the Mediterranean, South Australia, California, and Japan. It was successful in establishing and spreading in all of these temperate regions with the exception of Japan (Komatsu et al. 2003). California has seen the only successful eradication, a feat that has cost over 7 million dollars to date (Anderson 2005). In the Mediterranean Sea and South Australia, *C. taxifolia* has displaced native flora and fauna and resulted in virtual monocultures stretching hundreds of kilometers in length (Walters 2009).

Aquarium hobbyists use seaweeds to absorb excess nutrients in their aquaria, to act as food for herbivores, and to serve as habitat for juvenile fishes and invertebrates. In the wake of the *C. taxifolia* invasions, the aquarium industry in the United States began promoting the use of another group, a genus of filamentous green macroalgae, *Chaetomorpha*, as a “safe” alternative. When my colleagues and I surveyed home aquarists in 2008 and 2009, 67% indicated that they were using *Chaetomorpha* in their tanks.

Originally described by Kutzing (1845), *Chaetomorpha* is a genus of uniseriate filamentous macroalgae of the phylum Chlorophyta (green algae). There is currently general disagreement as to the taxonomy of this genus: The Integrated Taxonomic Information System (ITIS) database reports 11 species within this genus, while Algaebase lists 59 accepted species (Guiry and Guiry 2012). In addition, John et al. (2004), among others, cited *C. linum* and *C. aerea* as synonymous, while Price (1967) and Parke and Dixon (1976) have suggested that *C. linum* and *C. crassa* are synonymous (Dawes and Mathieson 2008).

Members of this filamentous genus can be found as either free-floating thalli or attached via a basal disc; for some species, both forms can be found (e.g. *C. linum*; Godward et al. 1966). John et al. (2002) suggested that the two diplohaplontic life stages, sporophyte and gametophyte, may be responsible for free-floating versus attached forms. Perhaps these two life stages may also reflect some of the taxonomic uncertainty within the genus.

Members of this genus have high nutrient uptake rates and high growth rates with most growth occurring through elongation and intercalary cell division (Krause-Jensen et al. 1999; Lavery and McComb 1991; McGlathery et al. 1997; McGlathery and Pedersen 1999; Van Den Hoek et al. 1995; Wehr and Sheath 2003). 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. Furthermore, the potential for detrimental effects has been demonstrated experimentally—dense mats of *C. linum* promoted sulfide uptake and thus declines of seagrass (Holmer and Nielsen 2007).

Distributions of *C. linum* and *C. crassa* are cosmopolitan; they occur on all continents except Antarctica (Guiry and Guiry 2012). With a lack of regulation on the trade of aquarium plants (Walters et al. 2006), strains of this species in use in the aquarium hobby may be from a very wide variety of locations. Individuals introduced from non-local populations may behave differently than local populations once placed in the novel environment and could thus prove problematic even in systems where *Chaetomorpha* naturally occurs. Furthermore, the culturing process may lead to unintentional artificial selection for traits desirable in an aquarium, such as wide environmental thresholds, high nutrient uptake rates, and strong competitive abilities. As

was the case with *Caulerpa taxifolia*, aquarium culturing may lead to the breeding of “super-organisms” (Kitajima et al. 2006; Wright 2005); release of these populations could aggravate invasion threat. For these reasons, I questioned whether members of the genus *Chaetomorpha* should really be considered “safe” as a replacement for the use of *Caulerpa taxifolia*.

CHAPTER 2: A SAFE ALTERNATIVE TO INVASIVE *CAULERPA TAXIFOLIA*? ASSESSING AQUARIUM-RELEASE INVASION POTENTIAL OF AQUARIUM STRAINS OF THE MACROALGAL GENUS *CHAETOMORPHA*

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Introduction

The introduction of aquarium organisms into natural environments is an ongoing threat that endangers the ecological integrity of these ecosystems (Padilla and Williams 2004). The best known example of invasion resulting from aquarium release is the chlorophyte *Caulerpa taxifolia*, which successfully invaded the waters of the Mediterranean, South Australia, and California (Jousson et al. 2000; Meinesz and Hesse 1991; Walters et al. 2006; Wiedenmann et al. 2001). The only successful eradication of this species occurred in California with bleach as an algicide but at a price tag of over \$7 million (Anderson et al. 2005; Williams and Schroeder 2004). In these areas, this “killer alga” changed benthic floral and associated faunal communities and has achieved secondary spread (Meinesz et al. 2001; Relini et al. 1998). Several congeners of *C. taxifolia* are known to be invasive, including *C. racemosa* in the Mediterranean, *C. brachypus* in Florida, and *C. scalpelliformis* in Australia (Davis et al. 1997; Schrope 2003; Verlaque et al. 2003; Walters 2009).

Macroalgae are used in aquaria primarily for biological filtration but can also function as ornamentals or habitat structure for invertebrates and small fishes. Aquarium macroalgae are

thus considered desirable if they have high nutrient uptake rates, are easy to maintain due to wide environmental tolerances, and if they are easy and inexpensive to obtain and share. Indeed, many hobbyists regularly prune their macroalgae and share these trimmings through local or regional clubs and conventions. Following the publicity of the *Caulerpa* invasions, aquarium hobbyists began promoting the use of other genera of macroalgae; in particular, the genus *Chaetomorpha* was expounded as a preferable alternative, as it demonstrates many of the aforementioned desirable characteristics but without the reputation and stigma of being an invasive species (Walters et al. 2011).

Chaetomorpha, a genus of unbranched filamentous green macroalgae (phylum Chlorophyta) found in marine and estuarine environments, is currently the most commonly used macroalgae among aquarium hobbyists with tropical or subtropical reef aquaria (Walters et al. 2011). It is characterized by high nutrient uptake rates and thus high growth rates, and it has a wide range of environmental tolerances (Lavery and McComb 1991). For example, *C. linum* can be found in subarctic and temperate climate zones as well as the tropics (e.g., Guiry and Guiry 2012; Lapointe 1997; Littler et al. 2008; Nielsen and Gunnarsson 2001). It has a wide tolerance to light levels and salinity (Xu and Lin 2008); aquarium-cultured strains of *C. linum* can even survive at a salinity of 80 ppt in laboratory conditions for up to 2 months (personal observation). For these reasons, it is considered very “hardy” by aquarium hobbyists. In addition, the ability to reproduce by vegetative fragmentation makes *Chaetomorpha* easy to share among hobbyists, a factor contributing to its recent popularity among aquarists. Unfortunately, broad environmental tolerances are also characteristics that promote invasiveness in aquatic plants and invertebrates (e.g., Nyberg and Wallentinus 2005; Yuan et al. 2010).

The propensity for vegetative reproduction was one of the major contributing factors to the invasion success of *Caulerpa taxifolia* (Walters 2009). This invader was found to produce large numbers of fragments (Ceccherelli and Cinelli 1999). Fragments as small as 10 mm in length are viable (Smith and Walters 1999); thus, algal introductions could occur easily if used tank water containing small yet viable fragments was released into natural waterways. To gauge whether this same threat is posed by aquarium strains of *Chaetomorpha*, I tested the survival and growth of small fragments of *Chaetomorpha* purchased from aquarium retailers.

I hypothesized that smaller fragments would show lower survival and growth due to the strain placed on intact cells when nearby cells were ruptured to create new fragments. Furthermore, I conducted these experiments at optimal thermal conditions in addition to temperatures representative of extreme warm and cold conditions found in Florida's coastal waters to gain a more robust understanding of invasion risk. In addition, I monitored the numbers of fragments generated in each purchase, since aquarium hobbyists usually dispose of the fragments generated during shipping along with the shipping water. I predicted that online purchases would generate more fragments than local purchases as a result of the larger amount of time spent in transit. I thus sought to quantify the expected propagule pressure, a major contributor to invasion success as identified by Lockwood et al. (2005), as well as the number of viable individuals which may establish if these fragments were released.

Methods and Materials

Experimental samples of aquarium *Chaetomorpha* were purchased from 5 local stores in central Florida and 5 online aquarium retailers. Upon receipt of each purchase, the shipped

specimen was identified to the species level (Abbott and Hollenberg 1976; Littler and Littler 2000). Fragment generation resulting from transport and handling was assessed for 9 of these purchases by isolating fragments (filaments separated from the ball of entangled filaments when dipped in 31 ppt artificial seawater made from Instant Ocean™ salts and deionized water) and haphazardly selecting 300 of these fragments, which were measured for length. The 300 measured fragments were collectively weighed, as well as the mass of unmeasured fragments. Total fragment generation was estimated using the following formula:

$$N_E = N_M + (M_U/M_M)*N_M$$

where N_E is the number of estimated fragments generated, N_M is the number of measured fragments, M_U is the mass of unmeasured fragments, M_M is the mass of measured fragments, and N_M is the number of measured fragments. Where 300 or fewer fragments were generated, actual values are reported. Two-way ANOVA was used to test for differences in fragment generation between purchase types (local, online) and among species using the statistical program R, version x64 2.11.1. I also ran a linear regression in R, predicting numbers of fragments generated by distance of the aquarium retailer from the University of Central Florida. Distances were generated by using the top recommendation for directions with Google Maps™.

Experimental fragments were cut with single-edge razor blades from haphazardly selected filaments (one fragment per filament) to the experimental treatment lengths (0.5, 1, 2, 4, 8, and 10 mm) and placed randomly into individual wells of 12-well Costar well plates within a 0.1 m³ light exclusion box at 5, 22, or 30 °C with 24 replicate fragments per treatment combination. Fragments were kept in 31 ppt artificial seawater in well plates suspended from a wire rack 18 cm above 2 fluorescent strip lights (mean PAR 20.82 μmol/m²/s ± 8.46 SD). As

differences in lighting can confound growth patterns of *Chaetomorpha*, I measured irradiance levels for each well plate well and analyzed the effects on survival and growth (McGlathery and Pedersen 1999). Since light was not significant ($p>0.05$) in determining either of these variables for any of the purchases, it was excluded from the analyses presented here.

After 2 weeks, fragment survival and growth were measured. Survival was determined under 8x magnification with a dissecting microscope. Fragments were classified as dead only if all cells showed signs of mortality; if the cellular membrane containing the cytosol and organelles retracted from the cell wall or if the cell showed complete loss of coloration, the cell was considered dead. Survival data were analyzed with negative binomial logistic regressions in R. Growth of live fragments was assessed by measuring the total length of live tissue for each fragment. Growth for surviving fragments was analyzed with ANCOVA in PASW Statistics 18, release version 18.0.2, with initial size as the covariate and temperature as the categorical variable.

Results

From the 10 purchases, I received 4 batches of *Chaetomorpha linum*, 4 of *C. spiralis*, and 2 of *C. crassa* with no occurrences of mixtures of species (Table 1). I found no significant differences in the numbers of fragments generated between online and local purchases ($P=0.654$), nor were there significant differences among species ($P=0.704$) (Table 2). Shipping distance was also insignificant in determining fragment generation ($P=0.410$). Numbers of fragments received in a purchase ranged from 28 to an estimated 6266 (Table 1).

Table 1 Estimated numbers of fragments generated by size class in each purchase. Purchase 1 is excluded, as fragment generation was assessed beginning with Purchase 2.

Purchase	Purchase type	Species	Total no. fragments generated	<1 mm	1-1.5 mm	2-3.5 mm	4-7.5 mm	8-9.5 mm	10+ mm
2	online	<i>C. spiralis</i>	6266	0	74	441	1175	1346	3230
3	local	<i>C. spiralis</i>	223	13	8	31	68	32	71
4	online	<i>C. spiralis</i>	28	0	0	8	11	1	8
5	online	<i>C. crassa</i>	90	0	11	14	19	2	44
6	online	<i>C. crassa</i>	1208	0	9	25	77	81	1016
7	local	<i>C. linum</i>	672	0	5	29	50	25	563
8	online	<i>C. linum</i>	704	0	17	99	227	73	288
9	local	<i>C. linum</i>	1917	0	0	45	224	103	1545
10	local	<i>C. linum</i>	857	0	0	6	29	43	779

Values fewer than 300 total fragments represent actual, not estimated values.

Table 2 ANOVA table showing number of fragments generated predicted by species (*Chaetomorpha crassa*, *C. spiralis* and *C. linum*) and purchase type (online, local).

Source	df	MS	F-ratio	P-value
Purchase Type	1	1223311	0.233	0.654
Species	2	2009005	0.383	0.704
Type x Species	1	4005147	0.763	0.432
Residuals	4	5245975		

In my laboratory assays, I found that *Chaetomorpha* can survive and produce new growth from fragments as small as 0.5 mm in length, provided that a single cell remains intact and undamaged (Figure 1, Figure 2). Furthermore, 9 of 10 purchased populations showed no effect of fragment size on survival but a strong effect of temperature (Figure 1, Table 3). In no cases did a significant interaction exist between temperature and initial size in determining survival, although interactions were significant in determining growth for 5 of the 10 purchases, including all species tested (Table 3). For growth, I saw consistently across these populations that larger fragments grew significantly more with growth occurring primarily through elongation and

intercalary division (Figure 2). Initial size significantly affected growth in these 9 purchases, with few instances of significant difference between the 22° and 30° temperature treatments (Figure 2, Table 3). Fragments from these purchases generally experienced mortality at 5°; 3 of the 24 fragments from Purchase 2, however, were able to withstand the cold temperature (Figure 1).

Table 3 *P* values for logistic regressions of survival and ANCOVA analyses of growth.

Purchase	Species	Survival			Growth		
		Size	Temperature	SxT	Size	Temperature	SxT
1	<i>C. spiralis</i>	<i>P</i> =0.659	<i>P</i> <0.001*	<i>P</i> =0.712	<i>P</i> =0.018*	<i>P</i> =0.121	<i>P</i> <0.001*
2	<i>C. spiralis</i>	<i>P</i> =0.415	<i>P</i> <0.001*	<i>P</i> =0.738	<i>P</i> =0.004*	<i>P</i> =0.010*	<i>P</i> =0.530
3	<i>C. spiralis</i>	<i>P</i> =0.691	<i>P</i> <0.001*	<i>P</i> =0.970	<i>P</i> =0.002*	<i>P</i> =0.012*	<i>P</i> =0.171
4	<i>C. spiralis</i>	<i>P</i> =0.383	<i>P</i> <0.001*	<i>P</i> =0.498	<i>P</i> <0.001*	<i>P</i> =0.029*	<i>P</i> =0.465
5	<i>C. crassa</i>	<i>P</i> =0.002*	<i>P</i> =0.662	<i>P</i> =0.814	<i>P</i> =0.154	<i>P</i> =0.006*	<i>P</i> <0.001*
6	<i>C. crassa</i>	<i>P</i> =0.930	<i>P</i> <0.001*	<i>P</i> =1.000	<i>P</i> =0.017*	<i>P</i> =0.540	<i>P</i> <0.001*
7	<i>C. linum</i>	<i>P</i> =0.886	<i>P</i> <0.001*	<i>P</i> =0.770	<i>P</i> <0.001*	<i>P</i> =0.082	<i>P</i> =0.068
8	<i>C. linum</i>	<i>P</i> =0.970	<i>P</i> <0.001*	<i>P</i> =0.948	<i>P</i> =0.010*	<i>P</i> =0.446	<i>P</i> <0.001*
9	<i>C. linum</i>	<i>P</i> =0.186	<i>P</i> <0.001*	<i>P</i> =0.434	<i>P</i> <0.001*	<i>P</i> =0.077	<i>P</i> =0.690
10	<i>C. linum</i>	<i>P</i> =0.579	<i>P</i> <0.001*	<i>P</i> =0.723	<i>P</i> =0.050*	<i>P</i> =0.182	<i>P</i> <0.001*

*A significant *P* value at *P*≤0.05 level

The anomalous population (Purchase 5) demonstrated high survival at all temperatures and a significant effect of fragment size on survivorship (*P*=0.002) with low survival rates for fragments less than 2 mm in length (Figure 1). Consistently lower growth rates at 5° than in the warmer treatments resulted in a non-significant effect (*P*=0.154) of initial fragment size as well as a significant interaction term (Figure 2).

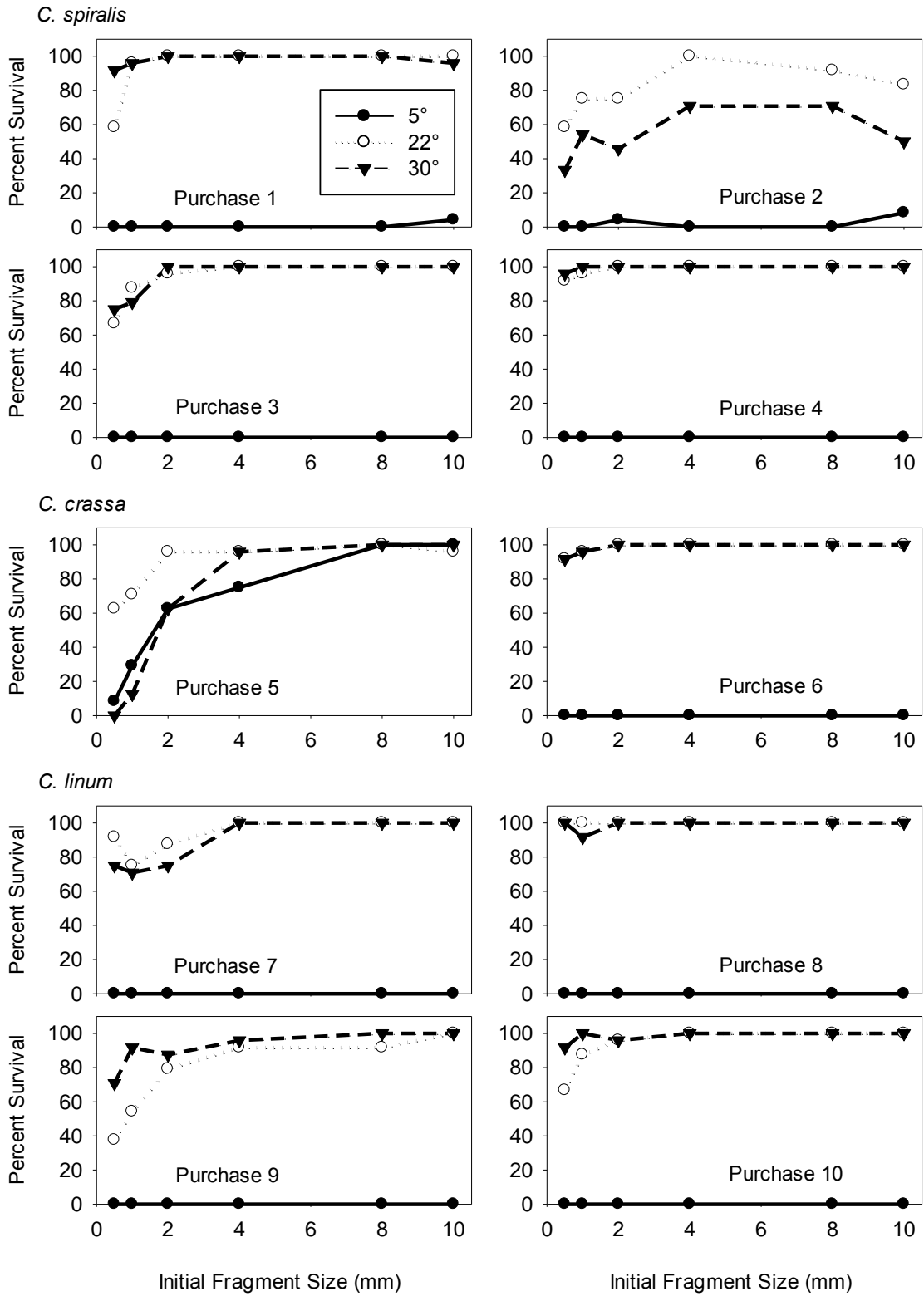


Figure 1 Survival of experimental fragments cut to 0.5-10 mm and exposed to 5, 22 or 30°C.

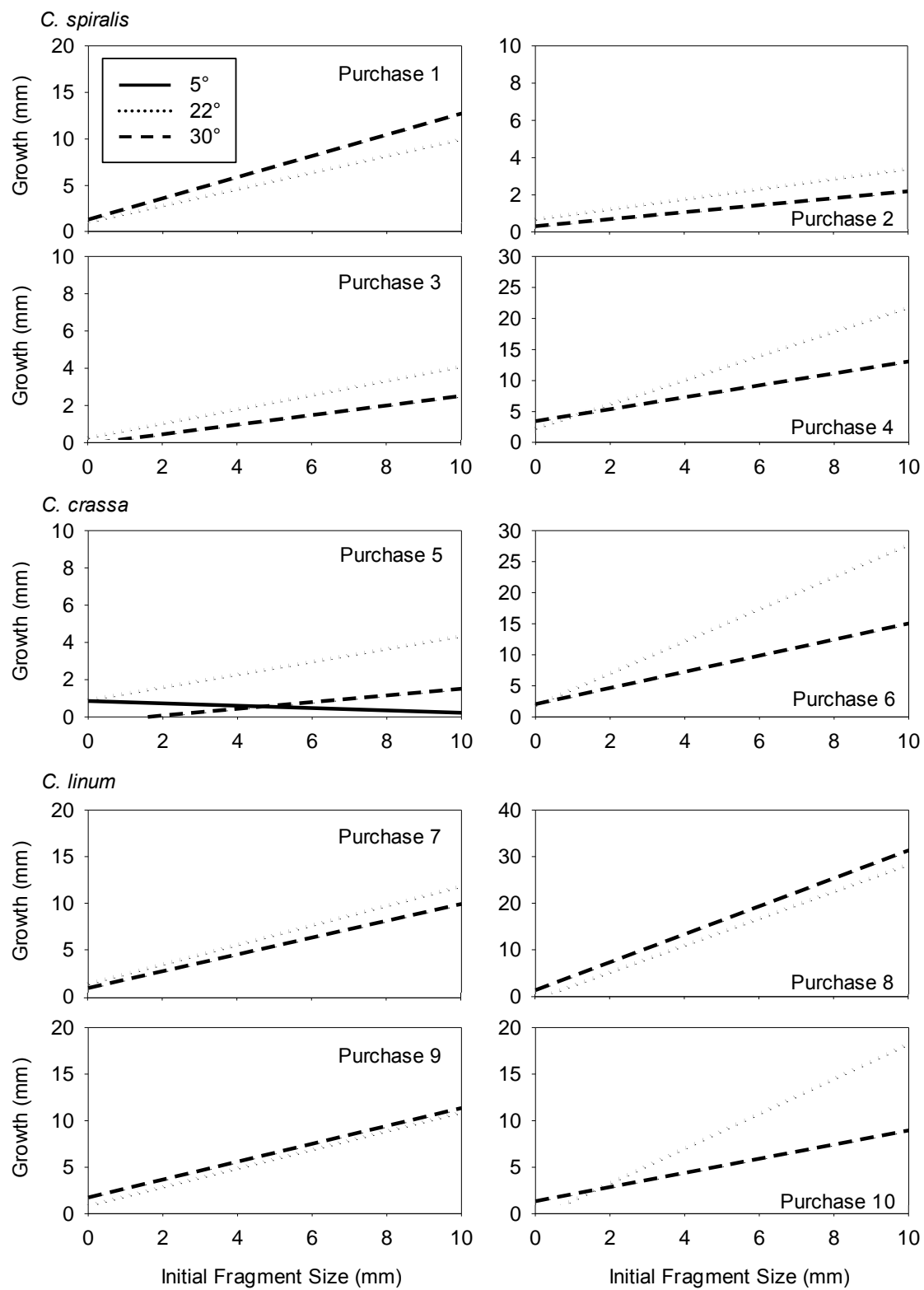


Figure 2 Growth of experimental fragments cut to 0.5-10 mm and exposed to 5, 22 or 30°C.

Discussion

Neither of my hypotheses, that online purchases would generate more fragments than local purchases nor that small starting size would limit fragment success, were supported. On the contrary, I found that an introduction of fragments from either an online or a local purchase would result in a large propagule pressure and that even the smallest fragments (0.5 mm) could pose an invasion threat if introduced. With *Caulerpa taxifolia*, the ability to survive from fragments as small as 10 mm in length was suggested to have enabled both its introduction and secondary spread in invaded areas (Smith and Walters 1999). High incidence of survival and growth from even smaller fragments observed with *Chaetomorpha* could make for unintentional introductions and easy spread. Furthermore, I note that these results demonstrate a conservative estimate of potential invasiveness, as no nutrients were supplied during experimentation and fragments were kept under moderate light levels, well below their optimum growing conditions around $80 \mu\text{mol}/\text{m}^2/\text{s}$ (Xu and Lin 2008). Differences in growth among purchases may therefore be attributable to not only inherent differences among strains, but also to different nutrient conditions where the populations were kept prior to my purchasing them, as this genus is known for having long-term nutrient storage abilities (Lavery and McComb 1991). Survival and growth of fragments of *Chaetomorpha*, if introduced into natural waterways, may thus have nutrient supplies available for exploitation, enhancing their establishment capabilities.

The high survivorship at cold temperatures exhibited by Purchase 5 suggests that the initial source of this aquarium strain may have been more cold-adapted and thus may have originated from a more northern geographical location. It is also possible that this strain has become more cold-adapted during generations of culturing, as has been suggested with the

invasive aquarium strain of *Caulerpa taxifolia* (e.g., Meinesz and Boudouresque 1996). This idea, however, has been refuted by Glasby and Glisson (2007) and Phillips and Price (2002) for *C. taxifolia*. In addition, the significant effect of fragment size on the survival of fragments from this population was likely due to the longer cell length typical of *Chaetomorpha crassa*, described as 300-1400 μm compared with the usual 50-750 μm length found in *C. linum*, which made it difficult to find cells to isolate that fit into the smaller size classes (Taylor 1967). These cells likely represented post-mitotic cells that may have been less stable than cells longer in length.

Release of aquarium macroalgae poses an additional threat of introducing “hitchhiker” species that live within the tangled filaments of *Chaetomorpha*. I found a wide variety of hitchhiker invertebrates arrive with the purchases—the calcareous tubeworm *Spirorbis* sp., the polychaete worm *Eurythoe complanata*, the sea star *Asterina* sp., the anemone *Aiptasia* sp., the banded brittle star *Ophioderma appressum*, the snail *Collonista* sp., the miniature ark *Acar* sp. and numerous herbivorous amphipods—as well as a range of macroalgal types. I found 1 occurrence each of *Halimeda goreau* (Purchase 8) and *Ulva lactuca* (Purchase 9). More commonly found were members of the genus *Caulerpa*, including *C. microphysa* (Purchases 1, 5 and 6), *C. brachypus* (Purchases 1 and 6) and *C. racemosa* (Purchase 6; Figure 3). The latter 2 of these species have been documented as invasive in Florida and the Mediterranean, respectively (Lapointe and Bedford 2010; Verlaque et al. 2000). If aquarium hobbyists release *Chaetomorpha* into natural environments, they may inadvertently introduce these species as well.

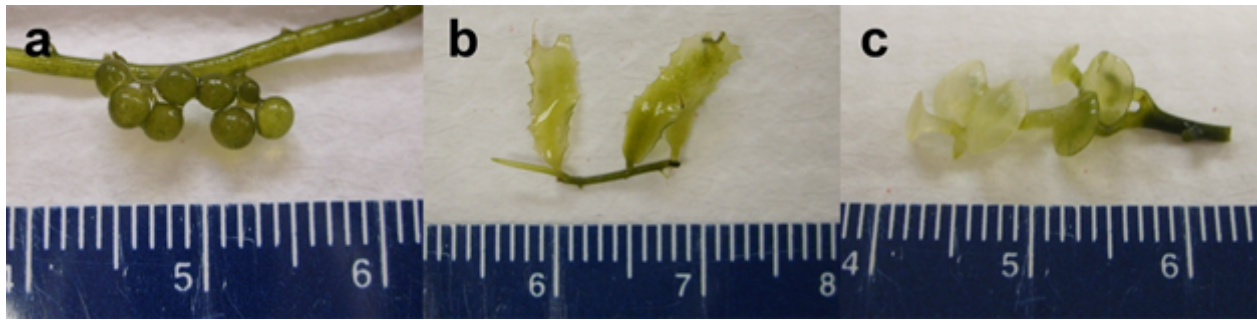


Figure 3 Photos of 3 species of *Caulerpa* found hitchhiking on purchases of aquarium *Chaetomorpha*. (a) *Caulerpa microphysa*, (b) *Caulerpa brachypus*, (c) *Caulerpa racemosa*.

The popularity of *Chaetomorpha* among aquarium hobbyists supports the potential for introduction events (Walters et al. 2011), and even a single introduction event could impose a strong propagule pressure with many of those individuals viable for establishment (Table 4). The local purchases included both *C. linum*, which can be found in Florida's coastal waterways, as well as *C. spiralis*, which is not found in Florida, demonstrating the ease with which macroalgal species are transported throughout the country in this unregulated industry (Walters et al. 2006). Even release into areas where these species of *Chaetomorpha* naturally occur could be detrimental, as cultivar selection can alter the properties of a population once it is removed from its natural environment and cultivated over generations (Kitajima et al. 2006).

Table 4 Fragments generated during shipping and predicted numbers surviving under tested thermal conditions.

Purchase	Total no. fragments	Species	Predicted to survive at 5°	Predicted to survive at 22°	Predicted to survive at 30°
2	6266	<i>C. spiralis</i>	288	5487	3643
3	223	<i>C. spiralis</i>	0	216	218
4	28	<i>C. spiralis</i>	0	28	28
5	90	<i>C. crassa</i>	72	84	74
6	1208	<i>C. crassa</i>	0	1208	1208
7	672	<i>C. linum</i>	0	667	663
8	704	<i>C. linum</i>	0	704	703
9	1917	<i>C. linum</i>	0	1880	1902
10	857	<i>C. linum</i>	0	858	859

Predicted survivorship was determined by multiplying the number of fragments found within each size class by the observed survival probabilities for that size class within each purchase.

The viability of fragments as small as 0.5 mm in length arouses another conservation concern—the release of aquarium tank water can be harmful as well (Padilla and Williams 2004). Such small algal fragments may not be noticeable to aquarium hobbyists in the water they dispose of during water changes and lead to unintentional introductions. In addition, tank water release may introduce foreign pathogens, which could invade native hosts without an evolutionary history to prompt an adequate immune response (Stewart 1991). Hobbyists releasing tank water into natural environments (or storm drains that lead to natural waterways) may not even realize that they have introduced fragments of *Chaetomorpha* or non-native microorganisms.

Several species of *Chaetomorpha* have a proclivity for forming mats of entangled filaments which may drift or become trapped in benthic communities (Littler and Littler 2000). Holmer and Nielsen (2007) demonstrated that dense mats of *C. linum* can negatively affect benthic flora by sequestering dissolved oxygen and promoting sulfide invasion into the tissues of

nearby seagrasses. Such nutrient sequestration has also been suggested to limit nearby pelagic biodiversity by creating resource-limited conditions in the water column above (McGlathery et al. 1997). Furthermore, the demonstrated competitive abilities of *C. linum* under nutrient-limited conditions contribute to its potential invasion threat (Lavery and McComb 1991; McGlathery and Pedersen 1999); this trend was observed for *Caulerpa taxifolia* with efficient nutrient uptake encouraging invasion success into *Cymodocea nodosa* beds (Ceccherelli and Cinelli 1997). These characteristics indicate that it is inappropriate to label *Chaetomorpha* as a safe alternative to *Caulerpa*, as both seem to possess similar qualities of competitive exclusion.

It is therefore imperative that the scientific community continues outreach campaigns to aquarium hobbyists and the general public warning of the dangers of releasing live organisms into natural environments. Rather than the traditional focus on shifting hobbyist use from known invasive species to those proposed as “safe” alternatives, I recommend that outreach strategies promote safe behaviors for disposing of unwanted or excess aquarium water, plants and pets to prevent all potential invasions resulting from these types of introductions (Walters et al. 2011).

CHAPTER 3: ALTERNATIVES TO RELEASE: EFFICIENT METHODS FOR DISPOSAL OF EXCESS OR UNWANTED AQUARIUM MACROALGAE

Introduction

In aquatic environments, the major vectors for introductions of non-native species are ballast water, boat hull fouling, aquaculture escape, fisheries stocking, biological control agents, and aquarium release (Ruiz et al. 2000; Williams and Smith 2007). Aquarium release, however, has been underrepresented in biological research despite its commonality as an introduction vector and lack of regulation on sale and trade of these species (Padilla and Williams 2004; Walters et al. 2006; Walters 2009). Invasions by organisms introduced from aquariums encompass a variety of taxa, including invertebrates, fishes and plants, comprised of both freshwater and saltwater species.

There are numerous examples of non-native fishes in freshwater systems that were most likely introduced in this manner (Franch et al. 2008; Van Kleef et al. 2008; Zidana et al. 2009). Lintermans (2004) stated that in Australia, 22 of the 34 freshwater fish invasions were likely due to the aquarium industry. In 2007, Copp and colleagues reported that the ornamental aquarium industry was the most important introduction vector for freshwater fishes in Europe. Furthermore, a model by Gertzen et al. (2008) estimated that approximately 10,000 fish are released annually by hobbyists in Montreal alone. As for invertebrates, 5 of the 7 non-native freshwater gastropods found in Texas by Karatayev et al. (2009) were either documented as or most likely introduced by the aquarium industry.

Aquarium releases are a pressing issue in the marine realm as well. Semmens et al. (2004) identified 16 species of non-native fishes on Florida's coastal reefs that were likely the result of aquarium releases. Calado and Chapman (2006) and Zajicek et al. (2009) increased this number to 28 species. Included in these figures are the two species of invasive Indo-Pacific lionfishes, *Pterois volitans* and *P. miles*, which are currently established in waters of the southeastern United States and throughout the Caribbean (Morris et al. 2009; Ruiz-Carus et al. 2006). Despite their growing infamy, lionfish remain popular among the aquarium ornamental trade; surveys of aquarium hobbyists at the 2010 Marine Aquarium Conference of North America revealed that although few (4.3%) reported keeping lionfish as pets, 88.8% stated that their local fish stores sell these known invaders (Walters et al. 2011).

Freshwater and marine plant invasions have also been identified as resulting from aquarium releases. Freshwater examples include fanwort (*Cabomba caroliniana*) in Canada (Wilson et al. 2007) and the Amazonian burhead (*Echinodorus grandiflorus*) in Floridian waters (Lehtonen 2009). Maderia et al. (2000) indicated that escaped aquarium ornamentals were responsible for initial introductions of invasive *Hydrilla* species in the United States, which cost approximately \$14.5 million per year for management and more than \$10 million annually in economic losses in Florida (Pimentel et al. 2005). The best-documented aquarium-release invader from saltwater aquaria has been *Caulerpa taxifolia*, which invaded the waters of the Mediterranean, South Australia and California (Walters 2009). This invasion cost the United States over \$7 million for eradication and management (Anderson 2005). *C. taxifolia* displaced native benthic flora and associated fauna in its invaded ranges (Walters 2009), earning it a classification by the IUCN as one of the world's 100 worst invasive species (Lowe et al. 2004).

Three of its congeners have also been anthropogenically introduced to the waters of the Mediterranean, Florida and Australia (Davis et al. 1997; Glardon 2006; Verlaque et al. 2003).

Between 2006 and 2010, my colleagues and I provided information to aquarium hobbyists on the invasions by *Caulerpa taxifolia* and its congeners as well as the dangers of releasing aquarium organisms to natural environments. During these outreach efforts, I conducted surveys on the use of aquarium organisms and practices that revealed that while hobbyists' understanding of the problems associated with aquarium dumping has increased, the use of invasive *Caulerpa* species has not declined significantly since its invasions. Simultaneously, other macroalgal species have become more common; specifically, use of macroalgae in the genus *Chaetomorpha* was reported in more than 50% of surveys of conference-going saltwater aquarium hobbyists, making it the most commonly used type of macroalgae among home saltwater aquariums (Walters et al. 2011).

Chaetomorpha, or “Chaeto” as aquarium hobbyists often call it, is a genus of filamentous green macroalgae known to have broad environmental tolerances as well as high nutrient uptake and growth rates (Lavery and McComb 1991; Xu and Lin 2008). It is desired by many aquarium hobbyists for use in sumps and refugia as a means of biological filtration and nutrient uptake. *Chaetomorpha* is preferred by many hobbyists over *Caulerpa taxifolia* as it is easily obtained and grown and is less likely to reproduce sexually, which can degrade water quality in tanks (Walters et al. 2011). When considering its popularity among aquarium hobbyists, it is a likely candidate to be disposed of into natural waterways. I thus sought to determine effective methods for disposing of unwanted or excess algal tissue in order to prevent introductions of *Chaetomorpha* into natural environments. I tested several reported household techniques for

algal disposal and determined the required amounts of time for each method to be completely effective.

Methods and Materials

Aquarium strains of *Chaetomorpha* were purchased from 3 retailers and placed in aerated aquaria upon receipt with deionized water mixed to 31 ppt with Instant Ocean™ sea salt, the optimal salinity found by Xu and Lin (2008) for culturing *C. linum*. These aquaria were lit with 65 watt power compact lighting in the 10K spectrum. Each of the following experiments was performed with 3 iterations, one with each aquarium purchase.

For all fragment experiments, filaments were selected haphazardly and cut at both ends to create 1-cm long fragments. For clump experiments, multiple filaments were haphazardly pinched to form clumps of mass between 0.01 and 1.5 g (mean=0.28 g, S.D.=0.28) that were blotted dry, weighed and deployed into treatments of boiling, microwaving, freezing, desiccation while exposed to air, desiccation within a closed environment or exposure to freshwater under no-light conditions. At the end of each treatment application, experimental units (fragments, clumps) were submerged in petri dishes with 10 mL of 31 ppt artificial seawater and closed lids. Dishes were kept at room temperature (22 °C) with standard overhead fluorescent lighting (23 $\mu\text{mol}/\text{m}^2/\text{s}$ PAR). Survivorship was monitored immediately, 24 and 48 hours after treatment. Replicates were considered surviving if at least a single cell in the fragment or clump survived because members of this genus can survive from single-cell fragments (Chapter 2). Cell mortality was determined by separation of the cell contents from the cell wall or disruption of the cell wall (Chapter 2).

Final percent survival for fragment experiments was calculated within each replicate. These data were arcsine square-root transformed and analyzed with nested ANOVA in PASW Statistics 18, release version 18.0.2, with treatment durations nested within purchases. For clump experiments, survival was analyzed using Pearson's chi square test.

Following experimentation, all fragments and clumps of *Chaetomorpha* were disposed of in the trash. Aquarium water was strained through a 1-mm mesh aquarium net; algal tissue was deposited into the garbage and the strained water was poured down the sink drain, which leads to Orange County wastewater treatment facilities.

Boiling Treatment

Fragments were placed into boiling deionized water in a teflon-coated cooking saucepan for 0, 1 and 5 minute durations. 10 fragments were boiled simultaneously with 5 replicate iterations. For clumps, 20 replicate clumps were individually boiled.

Microwave Treatment

Blotted dry fragments were placed into dry petri dishes with 10 fragments per dish (n=5) and microwaved in a 1000 watt Emerson model MW1161SB microwave for 0 or 15 second durations. As fragments had the tendency to break apart during microwaving, the following formula was used to determine the number of surviving fragments per dish for analysis, rounded up to a whole number:

$$S = I_S + [(P_S/P_T)*(10-I_T)]$$

where S is the total number of surviving fragments, I_S is the number of intact fragments that survived, P_S is the number of pieces that survived, P_T is the total number of pieces and I_T is the total number of intact fragments. Clumps were blotted dry and microwaved in individual dry petri dishes ($n=20$) for the same treatment durations. No formula was needed for calculating survival, as a single living cell in the clump led to classification of that clump as having survived.

Freezing Treatment

Fragments were blotted dry and placed into empty, 10.5 x 16.5 cm plastic zip-top bags in the freezer of a Crosley model CT 21A5W kitchen refrigerator/freezer with a mean temperature of -19.5°C (range -24.5° to -9° , S.E.=0.35) for 0, 12, 24 or 48 hours. For each treatment duration, 5 replicate bags were used, each containing 10 fragments. Clumps were similarly dried and exposed to the same durations with 20 replicates (one experimental clump per plastic bag).

Desiccation Treatment

Blotted dry fragments were placed into dry petri dishes at room temperature (22°C) with 10 fragments per dish ($n=5$) either with no lid (exposed) or with the lid on (closed) for 0, 3 or 6 hours. Clumps ($n=35$) were blotted dry and exposed to the same treatment factors (lid, no lid) and exposure durations with one clump per petri dish. These experiments were supplemented with an additional test for clumps ($n=20$) in petri dishes with closed lids with durations extended to 0, 3 and 6 days in order to determine the minimum time needed for 100% mortality.

Freshwater Treatment

Fragments were placed in closed-lid petri dishes at room temperature (22°) with 10 fragments per dish (n=5) with 10 mL of deionized water for 0, 2 or 4 days. These dishes were kept under a light exclusion box to simulate conditions similar to household pipes and storm-water drains. Clumps (n=20) were exposed to the same treatment durations with one clump per petri dish.

Results and Discussion

All disposal treatments presented here represent safe alternatives to releasing aquarium strains of *Chaetomorpha* with sufficient exposure times. All treatments significantly reduced survival for both fragments and clumps (Tables 5, 6). Boiling was highly effective at inducing mortality—all fragments and clumps boiled for at least 1 minute died (Figure 4A). Microwaving also led to 100% mortality of fragments and clumps at a duration of 15 seconds (Figure 4B). Freezing was equally effective when samples were exposed for durations of at least 24 hours (Figure 4C). Aquarists utilizing these methods will not introduce viable algal tissue.

Table 5 Fragment survival. Nested ANOVA table for analyses of arcsine square-root transformed fragment survival percentages for 3 purchases, each with 5 replicates of 10 fragments per treatment duration. Treatments not shown (boiling, microwave, desiccation while exposed, freshwater) displayed no variance within treatment groups and were incompatible with analyses of variance; all control groups showed 100% survivorship, while all treated groups showed 100% mortality.

Treatment	df	MS	F-ratio	<i>P</i> value
Freezing				
Purchase	2	0.016	6.000	0.005*
Duration (purchase)	9	3.016	1165.165	<0.001*
Residuals	48	0.003		
Desiccation (closed)				
Purchase	2	0.492	16.288	<0.001*
Duration (purchase)	6	3.292	1240.957	<0.001*
Residuals	36	0.030		
Freshwater				
Purchase	2	0.017	1.244	0.300
Duration (purchase)	6	3.709	277.540	<0.001*
Residuals	36	0.013		

* A significant *P* value at $P < 0.05$ level.

Table 6 Clump survival. *P* values for Pearson's chi square test analyses of clump survival.

Treatment	df	Purchase 1		Purchase 2		Purchase 3	
		X^2	<i>P</i> value	X^2	<i>P</i> value	X^2	<i>P</i> value
Boiling	2	40.00	<0.0001	40.00	<0.0001	40.00	<0.0001
Microwave	1	20.00	<0.0001	20.00	<0.0001	20.00	<0.0001
Freezing	3	60.00	<0.0001	60.00	<0.0001	60.00	<0.0001
Desiccation (exposed)	3	29.10	<0.0001	29.16	<0.0001	32.12	<0.0001
Desiccation (closed)	2	9.80	0.0074	34.35	<0.0001	6.72	0.0347
Freshwater	2	28.00	<0.0001	22.89	<0.0001	19.42	<0.0001

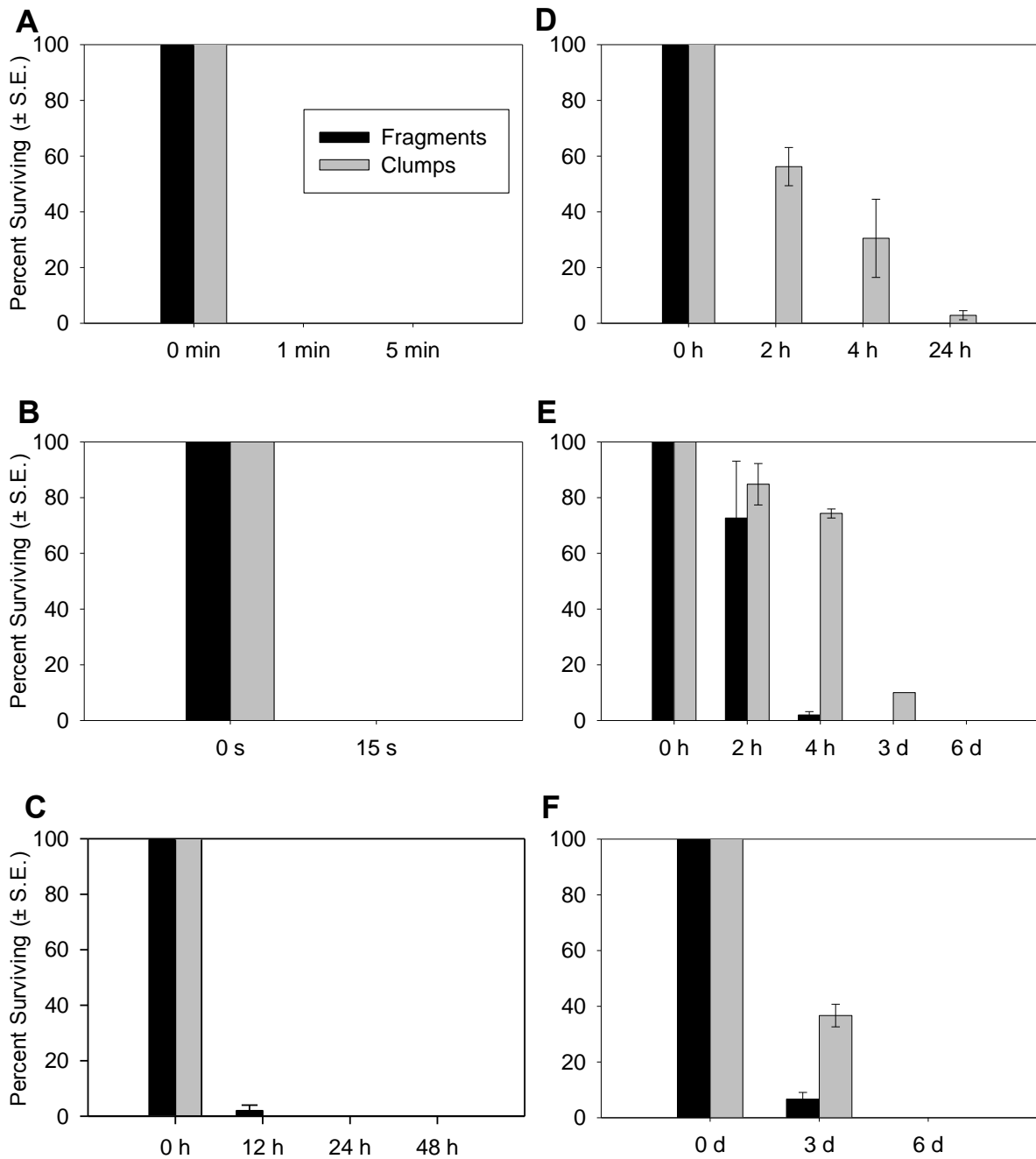


Figure 4 Mean survivorship across all purchases for fragments and clumps treated with (A) boiling, (B) microwave, (C) freezing, (D) desiccation (exposed to air), (E) desiccation within a closed container and (F) exposure to freshwater under light exclusion.

For desiccation, 2 hours was sufficient to induce 100% mortality in fragments if left exposed to air at room temperature (Figure 4D). For clumps, I observed 94.3%, 97.1%, and 100% mortality when exposed to air for 24 hours (Figure 4D). When desiccated in a closed container, the duration required for 100% mortality increased to 6 days for clumps and more than 4 hours for individual fragments (Figure 4E). Specifically, fragments desiccating in a closed container experienced 96%, 98%, and 100% mortality at 4 hours (Figure 4E). I recommend that aquarists disposing of *Chaetomorpha* allow it to desiccate for longer than 24 hours if exposed to air or at least 6 days if in a closed container. Furthermore, since larger clumps tended to have higher survival in the middle exposure times (2, 4 hours), I recommend extending desiccation times for larger quantities of discarded algae or breaking up clumps to increase surface area of exposure. Disposal through garbage should be sufficient if it is destined for a landfill.

Freshwater exposure required 6 days for 100% mortality of both individual fragments and clumps (Figure 4F). Since larger clumps demonstrated higher survival rates at moderate freshwater exposure (3 days), extra caution should be taken when disposing of quantities larger than 1.5 grams, as larger amounts may require additional exposure time to ensure 100% mortality. While indoor plumbing (e.g., sinks and toilets) may provide adequately long freshwater exposure to prevent introduction of viable individuals, I strongly caution against disposal of aquarium macroalgae via storm-water drains, which lead to natural waterways without undergoing sewage treatment. Particularly in coastal areas, storm-water drains often lead to marine or estuarine environments that may be favorable for the establishment of *Chaetomorpha*.

Even disposal of aquarium water following water changes or purchase water may introduce fragments of *Chaetomorpha*. This alga can survive and produce new growth from fragments as small as 0.5 mm in length; these fragments may be undetectable by hobbyists and aquaculturists disposing of water (Chapter 2). Additionally, purchase water and used tank water may contain non-native microorganisms that pose an invasion risk if the water is dumped into a natural waterway or storm-water drain (Duggan 2010).

Although biological invasions generally refer to non-native species, I further caution against introducing aquarium strains of *Chaetomorpha* into any waterways, including areas where this genus is naturally found. In captivity, aquarium macroalgae may undergo strong selective pressures that alter the traits of the population; such compositional changes have been suggested for the invasive aquarium strain of *Caulerpa taxifolia* (e.g., Meinesz and Boudouresque 1996). Release of an aquarium strain altered by this artificial, or “cultivar,” selection could introduce a population that responds differently in the new location than the native population. For example, the invasive population of the ornamental shrub *Artisia crenata* displays differences in seed production and root structure than the wild populations in its native range; this allowed the cultivated invading strain to develop superior competitive abilities (Kitajima et al. 2006). In addition, genetic pollution has been documented among congeneric invasions by hybridization and introgression in a variety of terrestrial plants (e.g., Barbour et al. 2002; Smith and Sytsma 1990). Furthermore, released aquarium-cultivated macroalgae may also harbor non-native microbes and non-native hitchhiker invertebrates and plants (Chapter 2; Stewart 1991). In summary, release of cultivated organisms poses invasion risks regardless of where it is introduced.

Despite a lack of documentation for introduction into novel ranges, aquarium strains of *Chaetomorpha* pose a substantial invasion risk if aquarists release live algal tissue into marine ecosystems or storm-water drains that lead to such environments. This is especially true for *Chaetomorpha*, as it has similar characteristics to *Caulerpa taxifolia* that enabled establishment and spread of this invasive species. With proper precautions, however, use of any aquarium macroalgae can be safe. Inducing mortality of aquarium algae by boiling, microwaving, freezing or desiccation, or disposing by garbage or indoor plumbing, can prevent detrimental introductions of these species and limit future biological invasions (Table 7). Furthermore, promoting “Don’t Release” literature and conservation messages through aquarium retail stores and commercial aquariums will help to combat potential invasions of all aquarium organisms.

Numerous published articles advocate the promotion of safe disposal alternatives to aquarium release (e.g., Chang et al. 2009; Morrissey et al. 2011); however, experimental assessment of the effectiveness of disposal techniques has received little attention in the literature. While a variety of chemical, mechanical and biological disposal methods have been tested for eradication of introduced species in natural environments, there is a definite lack of science-based knowledge in how to dispose of aquarium organisms prior to introduction. Even with the ecologically devastating invasions of *Caulerpa taxifolia* and the extensive outreach campaigns that have followed, aquarist disposal recommendations have been based on anecdotal evidence and well-reasoned assumptions about treatment methods and necessary durations to ensure 100% mortality of discarded aquarium organisms. Further research is needed in determining safe disposal methods for the wide variety of known and potential nuisance species

used in private and commercial aquaria if aquarists and aquaculturists hope to move toward a sustainable industry.

Table 7 Recommended methods and minimum durations for disposal of *Chaetomorpha*.

Method	Duration	Disposal Application
Boiling	1 minute	Boiling purchase water containing algae or tank water after water change
Microwave	15 seconds	Microwaving prior to disposal
Freezing	24 hours	Freezing in plastic bags prior to disposal
Desiccation (exposed to air)	24+ hours	Desiccation prior to disposal
Desiccation (closed container)	6 days	Disposal via garbage cans (destined for landfill)
Freshwater	6 days	Disposal via plumbed sinks or drains, NOT recommended via storm-water drains

CHAPTER 4: CHEMICAL ERADICATION METHODS FOR AQUARIUM STRAINS OF *CHAETOMORPHA*

Introduction

Aquarium release is a worldwide concern as a vector for the introductions of non-native 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 (Chapter 3). The ease and low cost of obtaining non-native 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*. Having invaded the waters of the Mediterranean, California and South Australia, *C. taxifolia* has cost millions of dollars in economic damage, as well as modifying the ecosystems it has invaded (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 (Walters 2009). 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 *Caulerpa 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 the genus of filamentous green macroalgae, *Chaetomorpha*, 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 (Chapter 2; Caputo et al. 2010; Lavery and McComb 1991; Xu and Lin 2008). These properties could enable unintentional introductions and promote the potential for establishment and spread.

When a non-native species is introduced, rapid response is crucial to effectively eradicating or managing the invader. For this reason, we proactively evaluated chemicals used in the management and eradication of invasive *Caulerpa taxifolia* to determine their effectiveness as algicides if aquarium *Chaetomorpha* is introduced to natural waterways. We tested chlorine bleach (sodium hypochlorite), the preferred algicide in the United States for *Caulerpa* invasions (Williams and Schroeder 2004); rock salt, the most popular management method for Australia's invasive *Caulerpa* populations (Glasby et al. 2005; Uchimura et al. 2000); copper sulfate, a

severe but effective method for inducing mortality in a variety of marine organisms (Uchimura et al. 2000); acetic acid (Anderson 2007; Forrest et al. 2007); and fluridone, the active ingredient in Sonar™ (Anderson 2007) at various concentrations and exposure durations that were relevant to *Caulerpa taxifolia*.

Materials and Methods

Samples of aquarium *Chaetomorpha* were purchased from 3 aquarium retailers. Each purchase was kept in a 10-gallon aquarium at 31 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 one week prior to commencing experiments. From each purchase, filaments were haphazardly selected and cut with a razor blade at both ends to 1 cm in length (Chapter 2; Williams and Schroeder 2004). For the chlorine bleach, salt, copper sulfate and acetic acid experiments, fragments from all 3 purchases 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 8).

Table 8 Experimental chemical concentrations and exposure durations for each algicide 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	30, 60, 90, 120 min
Copper sulfate	0, 10, 20, 50 mg/L	30, 60, 90, 120 min
Acetic acid	0, 1, 2, 4%	1, 2, 3, 4 min
Sonar™	0, 10, 20, 50 ppb	2, 4, 6, 8 weeks

For Sonar treatments, experimental fragments from one purchase were placed individually in acrylic 1.25 L aquaria with 1 L of artificial seawater, as we were unable to accurately measure low enough quantities to deploy in the 10 mL design. Sonar 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 5 replicate fragments per treatment combination per purchase 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 $\mu\text{mol m}^{-2}\text{s}^{-1}$ PAR).

Following exposure to chemicals, fragments were rinsed for 5 seconds with deionized water and resubmerged in new petri dishes with 10 mL artificial seawater. Fragment survival and growth were monitored at 4 weeks following 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 (Chapter 2). 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 x duration units.

Results and Discussion

Chlorine bleach was only effective at inducing mortality in *Chaetomorpha* for Purchase 1; however, it did significantly decrease growth in Purchases 1 and 2 (Figures 5, 6; Tables 9, 10). Although these exposure levels were sufficient for killing *Caulerpa taxifolia* fragments, no tested exposure level resulted in consistent mortality nor complete prevention of growth for *Chaetomorpha* (Figures 5, 6; Table 11; Williams and Schoeder 2004). As such, I 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 3 purchases (Figure 5, Table 9). This chemical treatment did limit growth effectively in all three purchases (Figure 6, Table 10), decreasing the numbers of fragments that produced new growth from 92% in control (0 g/L) fragments to 48% in fragments exposed to salt. Specifically, mean growth among the three purchases was reduced from 9.8 mm (nearly doubling in size) for control treatments to 0.1 mm in salted treatments during the 4 week trial. Members of the genus *Chaetomorpha* are known for having high tolerance to hypersaline conditions (Caputo et al. 2010; Xu and Lin 2008). Salting may be useful for managing *Chaetomorpha* if it is introduced but is unlikely to be useful for eradication at the exposure levels considered by Glasby et al. (2005) and Uchimura et al. (2000) for management of *Caulerpa taxifolia*.

Copper sulfate was ineffective at inducing mortality for *Chaetomorpha* fragments across all purchases at exposure levels considered for eradication of *Caulerpa taxifolia* (Figure 5; Tables 9, 11; Uchimura et al. 2000). Exposure to copper significantly reduced growth in Purchases 1 and 3 ($P < 0.001$) and was marginally effective in limiting growth in Purchase 2 ($P = 0.0997$; Figure 6; Table 10). 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 managing an introduction of *Chaetomorpha* rather than eradication. Copper, however, has negative impacts on a wide range of taxa (e.g., Murray-Gulde et al. 2002). Potential harm to non-target 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*.

The use of acetic acid was the only chemical tested that consistently reduced survivorship and growth of fragments of aquarium *Chaetomorpha* (Figures 5, 6; Tables 9, 10). Full mortality was achieved with exposure to 2% acetic acid for 4 minutes or 4% acetic acid for 1 minute. Applications of acetic acid have been shown to have detrimental effects on non-target estuarine species at concentrations of 5% (Locke et al. 2009); thus if this chemical is used as an algicide, it should be applied in the lowest 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 in order to determine the lowest concentration of chemical needed for eradication.

Application of Sonar did not affect survival of fragments of *Chaetomorpha*; 100% survival was observed for all treatment combinations. Furthermore, exposure to Sonar was insignificant ($P=0.422$) in determining growth (Figure 7). These results were not surprising, considering that fluridone, the active ingredient in Sonar, 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 algicide such as Sonar would have been desirable for limiting detriment to non-target species. However, this chemical would not be

effective at tested concentrations in eradicating invasions by aquarium *Chaetomorpha*, as it neither induces mortality nor limits growth.

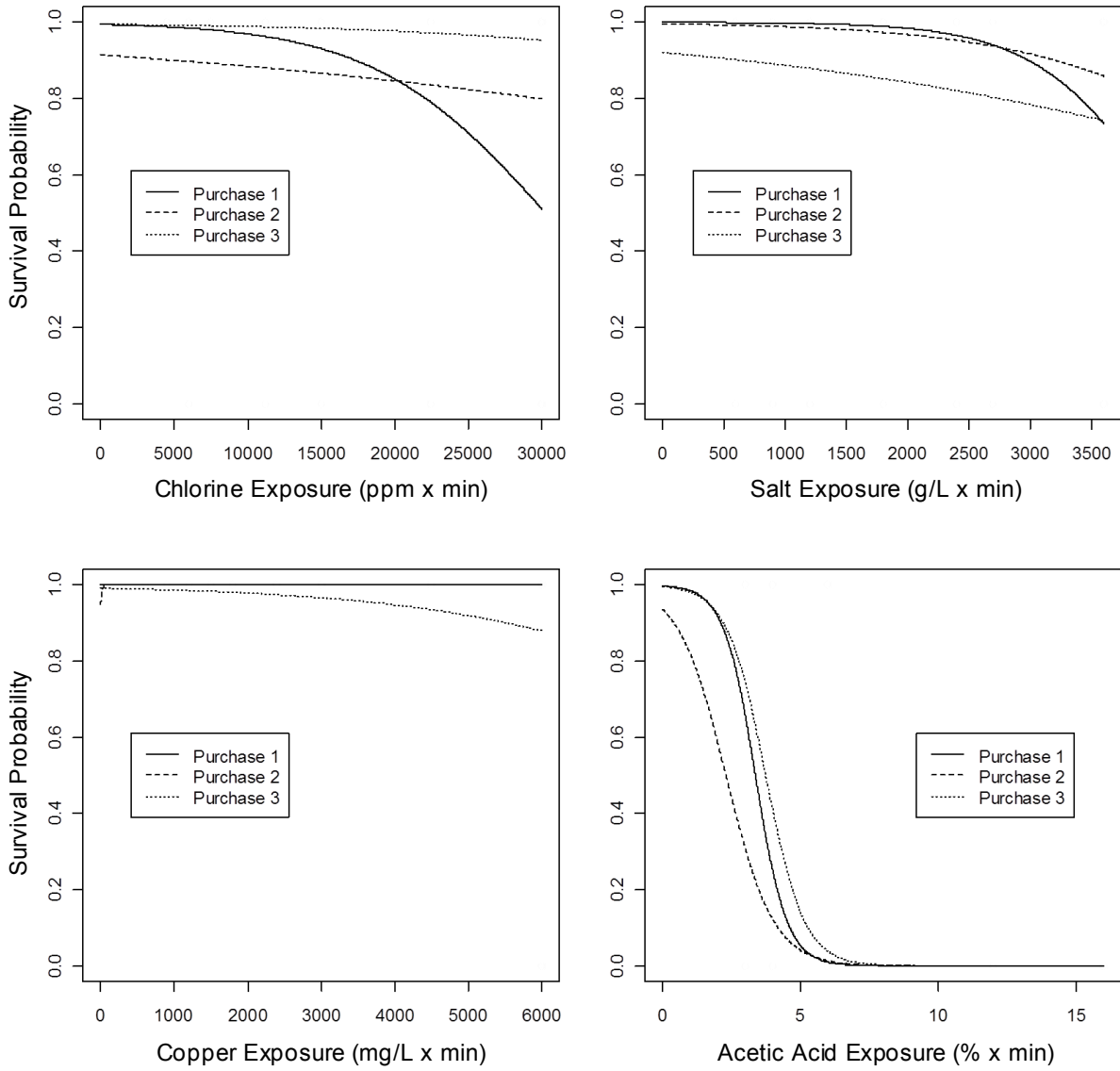


Figure 5 Logistic regressions of survival for 3 purchases of *Chaetomorpha* exposed to chlorine bleach, rock salt, copper sulfate and acetic acid treatments. Survivorship shown was observed at 4 weeks after chemical exposure ended.

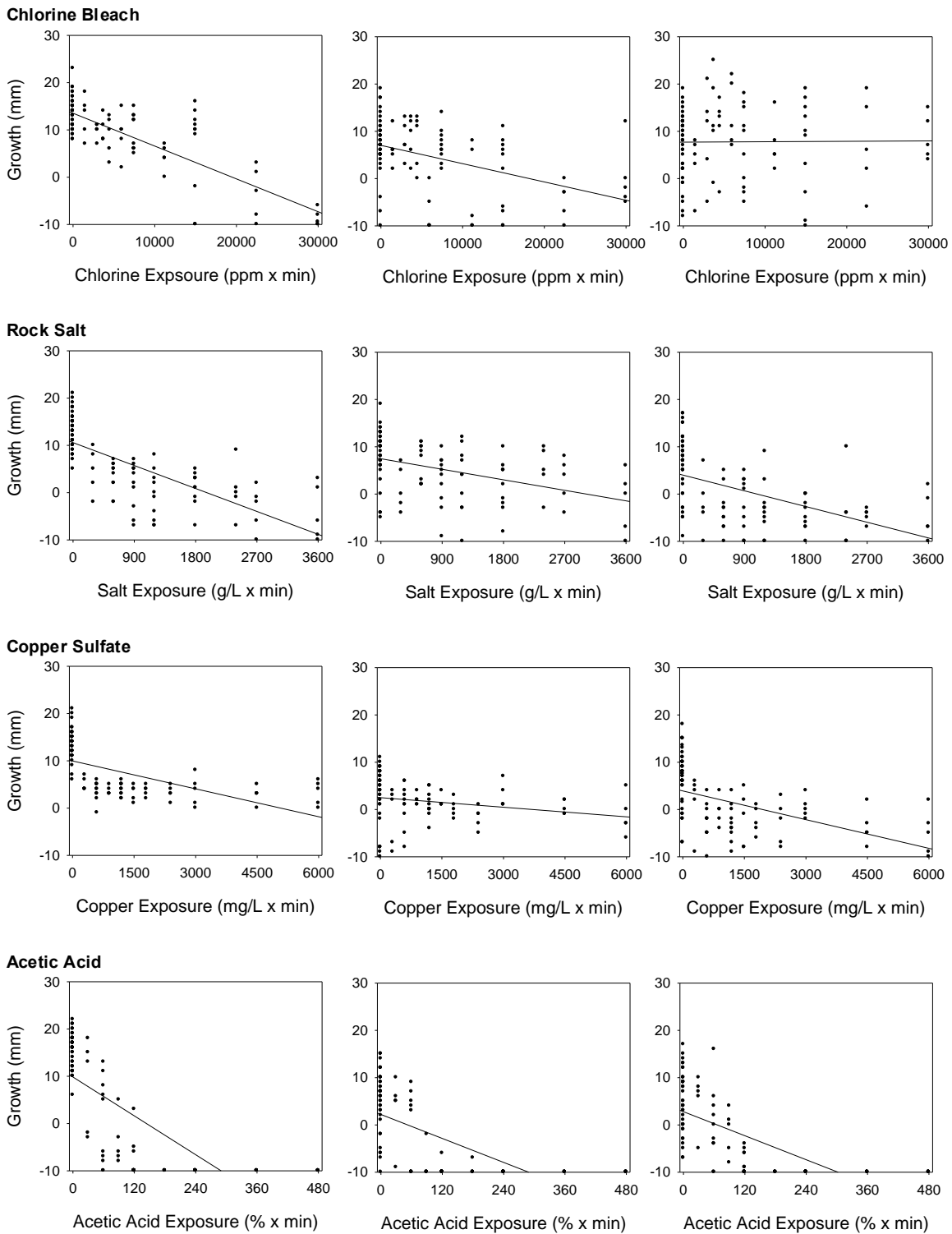


Figure 6 Linear regressions of final growth, as determined by concentration x duration units, for 3 purchases exposed to chlorine bleach, rock salt, copper sulfate and acetic acid treatments. Negative growth values indicate partial (-1 to -9 mm) and full (-10 mm) mortality.

Table 9 *P* values for logistic regressions where concentration x duration predicted survival. Significant *P* values indicate that increasing exposure to chemical treatment decreased survival.

Chemical	Purchase 1	Purchase 2	Purchase 3
Chlorine bleach	<i>P</i> =0.0028*	<i>P</i> =0.3820	<i>P</i> =0.4358
Rock salt	<i>P</i> =0.0609	<i>P</i> =0.1254	<i>P</i> =0.2000
Copper sulfate	<i>P</i> =1.0000	<i>P</i> =0.9977	<i>P</i> =0.1676
Acetic acid	<i>P</i> =0.0003*	<i>P</i> <0.0001*	<i>P</i> =0.0004*

*Significant *P* value (*P*<0.05)

Table 10 *P* values for linear regressions where concentration x duration predicted growth. Significant *P* values indicate that increasing exposure to chemical treatment decreased growth.

Chemical	Purchase 1	Purchase 2	Purchase 3
Chlorine bleach	<i>P</i> <0.0001*	<i>P</i> =0.0004*	<i>P</i> =0.8590
Rock salt	<i>P</i> <0.0001*	<i>P</i> <0.0001*	<i>P</i> <0.0001*
Copper sulfate	<i>P</i> <0.0001*	<i>P</i> =0.0997	<i>P</i> =0.0005*
Acetic acid	<i>P</i> <0.0001*	<i>P</i> <0.0001*	<i>P</i> <0.0001*

*Significant *P* value (*P*<0.05)

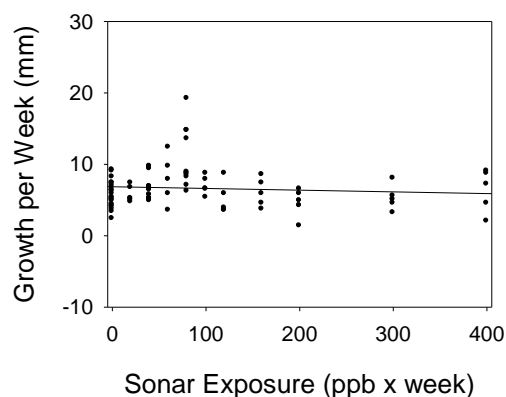


Figure 7 Linear regression of final growth for one purchase exposed to Sonar for 2 - 8 weeks (*P*=0.422).

Concentrations required to induce mortality in aquarium *Chaetomorpha* were consistently higher than what experimentation has determined necessary for treatment of *Caulerpa taxifolia* (Table 11). High chemical concentrations may amplify declines in non-target species. In the event of an introduction, the potential non-target 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 lab experiments show promise for an algicide, *in situ* application is often ineffective. As such, I recommend *in situ* testing for any of these algicides on an introduced population before widespread application.

Table 11 Comparison of algicide exposure required for mortality of invasive *Caulerpa taxifolia* versus aquarium strains of *Chaetomorpha*.

Chemical	<i>Chaetomorpha</i>	<i>Caulerpa taxifolia</i>	Reference (<i>C. taxifolia</i>)
Chlorine bleach	Not effective at tested concentrations (up to 250 ppm for 120 min)	125 ppm for 30-60 min 50 ppm for 90+ min	Williams and Schroeder 2004
Rock salt	Not effective at tested concentrations (up to 30 g/L for 120 min)	24 g/L for 30 min 18 g/L for 60+ min	Uchimura et al. 2000
Copper sulfate	Not effective at tested concentrations (up to 50 mg/L for 120 min)	20 mg/L for 30 min 5 mg/L for 60+ min	Uchimura et al. 2000
Acetic acid	4% for 1 min 2% for 4 min	1% for 60+ min	Anderson, personal communication
Sonar™	Not effective at tested concentrations (up to 50 ppb for 8 weeks)	50 ppb for 12 days (partial loss of color)	Anderson 2007

The costs of these chemicals also vary considerably. Chlorine bleach and rock salt, both available at grocery stores, are very inexpensive; bleach retails for less than \$1 per liter, and rock

salt, sold as ice cream salt, costs approximately \$1 per kilogram. Aqueous copper sulfate and glacial acetic acid are available through chemical supply companies at moderate prices. Based on quantities purchased, these chemicals cost between \$15 and \$140 per liter. Sonar, on the other hand, is incredibly expensive; Fisher Scientific sells the powdered form of this herbicide in a maximum quantity of 100 mg, for which the company charges approximately \$35 retail.

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 aquarium *Chaetomorpha* is introduced into natural environments, the best management strategy is prevention (Chapter 3; Sepulveda et al. 2012). 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 safe disposal alternatives to such introductions.

CHAPTER 5: GENERAL DISCUSSION

Aquarium strains of *Chaetomorpha* pose a significant invasion risk. These macroalgal strains demonstrate many characteristics that enabled the establishment, spread and detriment of invasive *Caulerpa taxifolia*. Fragments of *Chaetomorpha* are viable at sizes as small as 0.5 mm in length; a single live cell represents an individual capable of persisting if introduced into a favorable environment. High numbers of such fragments—often reaching into the thousands—are generated by a single retail purchase of aquarium *Chaetomorpha*.

Previous research has demonstrated that this genus possesses strong competitive abilities with rapid nutrient uptake and growth; broad thresholds for favorable abiotic environmental conditions; and the ability to disrupt normal ecosystem function, especially in areas anthropogenically driven to eutrophication, where *Chaetomorpha* can form blooms (Holmer and Nielsen 2007; Lapointe 1997; Lavery and McComb 1991; Xu and Lin 2008). Combining these insights with the experimental results presented here demonstrate that if aquarium *Chaetomorpha* is released into natural environments, a strong likelihood exists that it will be able to establish, spread, and cause ecological damage.

If an invasion occurs, rapid response is key for eradicating or managing introduced populations of non-native species. Several of the chemical algicides screened here may be effective at limiting establishment and spread of aquarium *Chaetomorpha*. Acetic acid shows promise as a potential eradication agent, limiting both survival and growth of this alga, although future research should optimize recommended concentrations for longer exposure durations. Chlorine bleach, rock salt and copper sulfate may be effective in managing an invasion by *Chaetomorpha*, as these chemicals limited growth in my trials. More research should be

conducted to proactively determine potential eradication methods for all ornamental species deemed to be of high invasion risk. *A priori* knowledge of how to manage an invasion would enable quick, effective removal of introduced species and thus limit the negative consequences of that introduction.

Prevention, however, is the most effective and lowest-cost way to ameliorate the effects of biological invasions. Outreach campaigns that highlight changing disposal behaviors, rather than banning risky species, are better aligned with aquarium hobbyist goals and the livelihoods of professionals in the aquarium industry. Safe alternatives to release must be provided for an effective “Don’t Release” campaign, and those recommendations should be guided by science, as was presented here, with necessary exposure durations for disposal treatments such as boiling, microwave, freezing, desiccation and freshwater. It is therefore important that experimental determination of safe disposal methods becomes a research priority in aquaculture and aquarium science, rather than a novelty.

Furthermore, these conservation messages need to be brought to the attention of high-risk groups. Aquarium hobbyists involved in organizations like the Marine Aquarium Society of North America (MASNA) and professional aquaculturists are usually aware of the risks of releasing aquarium organisms into natural environments, and outreach goals for these groups should highlight lesser-known facets of the problem (e.g., introduction of aquarium tank water into natural ecosystems). Higher risk groups include casual aquarium hobbyists and families with children that may believe releasing aquarium organisms is an ethically responsible behavior. These groups should be targeted with outreach materials, such as public service announcement videos, that are broad-reaching.

Identifying high-risk areas for introductions, typically areas of high human population density, will provide geographic regions to target for such outreach campaigns. Ecologically disturbed areas such as human population centers are also more likely to experience successful establishment of a non-native species once it has been introduced. Future research should highlight determining high-risk species and preventing introductions. Rather than banning the sale of these species, however, I recommend requiring warning materials to be issued with the purchase, much as Material Safety Data Sheets (MSDS) are included with chemical purchases. Investing resources into risk assessment, proactive eradication screening and invasion prevention will limit the negative effects that human activities have on already-declining natural systems.

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- After one year+6 months, the thesis will become publicly available in electronic format
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I am happy to discuss this matter over the phone. I attempted to call earlier today, July 5 at 17:15 CEST (11:15 my time) and left a voice message. If you could tell me the hours of your office, I will try to phone again tomorrow, July 6. My phone number is a US cellular/mobile phone, (011) 850 525 4851.

The thesis submission deadline is approaching, and I thank you greatly for your assistance in resolving this matter quickly.

Rachel Odom
Coastal and Estuarine Ecology Lab
Department of Biology
University of Central Florida
rachel.odom@knights.ucf.edu

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Sent: Sunday, 24 June, 2012 8:44 PM

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I have attached a letter to this email that meets the guidelines for my university's thesis submission process. I would greatly appreciate if you could complete this form or refer me to the appropriate person.

Best regards,

Rachel Odom
Coastal and Estuarine Ecology Lab
Department of Biology
University of Central Florida
rachel.odom@knights.ucf.edu

REFERENCES

- Abbott IA, Hollenberg GJ (1976) Marine algae of California. Stanford University Press, Stanford
- Albins MA, Hixon MA (2008) Invasive Indo-Pacific lionfish *Pterois volitans* reduce recruitment of Atlantic coral-reef fishes. *Mar Ecol Prog Ser* 367:233-238
- Anderson LWJ (2005) California's reaction to *Caulerpa taxifolia*: a model for invasive species rapid response. *Biol Invasions* 7:1003-1016
- Anderson LWJ, Tan WL, Woodfield R, Mooney R, Merkel K (2005) Use of sediment bioassays to verify efficacy of *Caulerpa taxifolia* eradication treatments. *J Aquat Plant Manage* 43:1-9
- Anderson LWJ (2007) Control of invasive seaweeds. *Bot Mar* 50:418-437
- Barbour RC, Potts BM, Vaillancourt RE, Tibbits WN, Wiltshire RJE (2002) Gene flow between introduced and native *Eucalyptus* species. *New Forest*. 23:177-191
- Boudouresque CF, Lemee R, Mari X, Meiesz A (1996) The invasive alga *Caulerpa taxifolia* is not a suitable diet for the sea urchin *Paracentrotus lividus*. *Aquat Bot* 53:243-250
- Calado R, Chapman PM (2006) Aquarium species: deadly invaders. *Mar Pollut Bull* 52:599-601
- Caputo E, Ceglie V, Lippolis M, La Rocca N, De Tullio M (2010) Identification of a NaCl-induced ascorbate oxidase activity in *Chaetomorpha linum* suggests a novel mechanism of adaptation to increased salinity. *Environ Exp Bot* 69:63-67
- Ceccherelli G, Cinelli F (1997) Short-term effects of nutrient enrichment of the sediment and interactions between the seagrass *Cymodocea nodosa* and the introduced green alga *Caulerpa taxifolia* in a Mediterranean bay. *J Exp Mar Biol Ecol* 217:165-177
- Ceccherelli G, Cinelli F (1999) Effect of temperature, salinity, and light intensity on the growth of the green macroalga, *Chaetomorpha linum*. *Mar Ecol Prog Ser* 182:299-303
- Chang AL, Grossman JD, Spezio TS, Weiskel HW, Blum JC, Burt JW, Muir AA, Piovio-Scott J, Veblen KE, Grosholz ED (2009) Tackling aquatic invasions: risks and opportunities for the aquarium fish industry. *Biol Invasions* 11:773-785
- Copp GH, Templeton M, Gozlan RE (2007) Propagule pressure and the invasion risks of non-native freshwater fishes: a case study in England. *J Fish Biol* 71:148-159

- Davis AR, Roberts DE, Cummins SP (1997) Rapid invasion of a sponge-dominated deep reef by *Caulerpa scalpelliformis* (Chlorophyta) in Botany Bay, New South Wales. *Aust J Ecol* 22:146-150
- Dawes CJ, Mathieson AC (2008) *The seaweeds of Florida*. University Press of Florida, Gainesville, Florida
- Duggan IC (2010) The freshwater aquarium trade as a vector for incidental invertebrate fauna. *Biol Invasions* 12:3757-3770
- Forrest BM, Hopkins GA, Dodgshun TJ, Gardner JPA (2007) Efficacy of acetic acid treatments in the management of marine biofouling. *Aquaculture* 262:319-332
- Franch N, Clavero M, Garrido M, Gaya N, Lopez V, Pou-Rovira Q, Qeral JM (2008) On the establishment and range expansion of oriental weatherfish (*Misgurnus anguillicaudatus*) in NE Iberian Peninsula. *Biol Invasions* 10:1327-1331
- Gertzen E, Familiar O, Leung B (2008) Quantifying invasion pathways: fish introductions from the aquarium trade. *Can J Fish Aquat Sci* 65:1265-1273
- Gardon C (2006) Predicting risks of invasion of *Caulerpa* species in Florida. Ph. D. dissertation, University of Central Florida, Department of Biology, 99 pp
- Glasby TM, Creese RG, Gibson PT (2005) Experimental use of salt to control the invasive marine alga *Caulerpa taxifolia* in New South Wales, Australia. *Biol Conserv* 122:573-580
- Glasby TM, Glisson PT (2007) Limited evidence for increased cold-tolerance of invasive versus native *Caulerpa taxifolia*. *Mar Biol* 152:255-263
- Godward M, Puisieux-Dao S, Leedale GF, Dodge JD, Roberts M, Evans LV, Dixon PS (1966) *The chromosomes of the algae*. St Martin's Press, New York
- Guiry MD, Guiry GM (2012) *AlgaeBase*. World-wide electronic publication, National University of Ireland, Galway. <http://www.algaebase.org>; searched on 02 January 2012
- Holmer M, Nielsen RM (2007) Effects of filamentous algal mats on sulfide invasion in eelgrass (*Zostera marina*). *J Exp Mar Biol Ecol* 353:245-252
- John DM, Whitton BA, Brook AJ (2002) *The freshwater algal flora for the British Isles*. Cambridge University Press, Cambridge

- John DM, Prud'homme van Reine WF, Lawson GW, Kostermans TB, Price JH. 2004. A taxonomic and geographical catalogue of the seaweeds of the western coast of Africa and adjacent islands. *Nova Hedwigia Beih* 127:1-339
- Jousson O, Pawlowski J, Zaninetti L, Zechman FW, Dini F, Di Guiseppe G, Woodfield R, Millar A, Meinesz A (2000) Invasive alga reaches California. *Nature* 408:157-158
- Karatayev AY, Burlakova LE, Karatayev VA, Padilla DK (2009) Introduction, distribution, spread, and impacts of exotic freshwater gastropods in Texas. *Hydrobiol* 619:181-194
- Kitajima K, Fox AM, Sato T, Nagamatsu D (2006) Cultivar selection prior to introduction may increase invasiveness: evidence from *Ardisia crenata*. *Biol Invasions* 8:1471-1482
- Komatsu T, Ishikawa T, Yamaguchi N, Hori Y, Ohba H (2003) But next time?: Unsuccessful establishment of the Mediterranean strain of the green seaweed *Caulerpa taxifolia* in the Sea of Japan. *Biol Invasions* 5:275-278
- Krause-Jensen D, Christensen PB, Rysgaard S (1999) Oxygen and nutrient dynamics within mats of the filamentous macroalga *Chaetomorpha linum*. *Estuaries* 22:31-38
- Lapointe BE (1997) Nutrient thresholds for bottom-up control of macroalgal blooms on coral reefs in Jamaica and southeast Florida. *Limnol Oceanogr* 42:1119-1131
- Lapointe BE, Bedford BJ (2010) Ecology and nutrition of invasive *Caulerpa brachypus* f. *parvifolia* on coral reefs off southeast Florida, USA. *Harmful Algae* 9:1-12
- Lavery PS, McComb AJ (1991) The nutritional eco-physiology of *Chaetomorpha linum* and *Ulva rigida* in Peel Inlet, Western Australia. *Bot Mar* 34:251-260
- Lehtonen S (2009) On the origin of *Echinodorus grandiflorus* (Alismataceae) in Florida ("*E. floridanus*"), and its estimated potential as an invasive species. *Hydrobiol* 635:107-112
- Lintermans M (2004) Human-assisted dispersal of alien freshwater fish in Australia. *N Z J Freshw Mar Res* 38:481-501
- Littler DS, Littler MM (2000) Caribbean reef plants. Offshore Graphics, Washington, D.C.
- Littler DS, Littler MM, Hanisak MD (2008) Submersed plants of the Indian River Lagoon. Offshore Graphics, Washington, D.C.
- Locke A, Doe KG, Fairchild WL, Jackman PM, Reese EJ (2009) Preliminary evaluation of effects of invasive tunicate management with acetic acid and calcium hydroxide on non-target marine organisms in Prince Edward Island, Canada. *Aquat Invasions* 4:221-236

- Lockwood JL, Cassey P, Blackburn T (2005) The role of propagule pressure in explaining species invasions. *Trends Ecol Evol* 20:223-228
- Lockwood JL, Hoopes M, Marchetti M (2007) *Invasion Ecology*. Blackwell Publishing, Malden, Massachusetts
- Lowe SJ, Browne M, Boudjelas S (2004) 100 of the World's Worst Invasive Alien Species. IUCN/SSC Invasive Species Specialist Group (ISSG). Auckland, New Zealand
- Madeira PT, Jacono CC, Van TK (2000) Monitoring hydrilla using two RAPD procedures and the nonindigenous aquatic species database. *J Aquat Plant Manag* 38:33-40
- McEnnulty FR, Bax NJ, Schaffelke B, Campbell ML (2001) A literature review of rapid response options for the control of ABWMAC listed species and related taxa in Australia. *Cent Res Introd Mar Pests*. 95 pp.
- McGlathery KJ, Krause-Jensen D, Rysgaard S, Christensen PB (1997) Patterns of ammonium uptake within dense mats of the filamentous macroalga *Chaetomorpha linum*. *Aquat Bot* 59:99-115
- McGlathery KJ, Pedersen MF (1999) The effect of growth irradiance on the coupling of carbon and nitrogen metabolism in *Chaetomorpha linum* (Chlorophyta). *J Phycol* 35:721-731
- Meinesz A, Hesse B (1991) Introduction et invasion de l'algue tropicale *Caulerpa taxifolia* en Méditerranée nord-occidentale. *Oceanol Acta* 14(4):415-426
- Meinesz A, Boudouresque CF (1996) On the origin of *Caulerpa taxifolia* in the Mediterranean Sea. *Cr Acad Sci III-Vie* 319:603-613
- Meinesz A, Belsher T, Thibaut T, Antolic B, Mustapha KB, Boudouresque CF, Chiaverini D, Cinelli F, Cottalorda JM, Djellouli A, El Abed A, Orestano C, Grau AM, Ivesa L, Jaklin A, Langar H, Massuti-Pascual E, Peirano A, Tunesi L, DeVaugelas J, Zavodnik N, Zuljevic A (2001) The introduced green alga *Caulerpa taxifolia* continues to spread in the Mediterranean. *Biol Invasions* 3:201-210
- Morris JA, Akins JL, Barse A, Cerino D, Freshwater DW, Green SJ, Munoz RC, Paris C, Whitfield PE (2009) Biology and ecology of the invasive lionfishes, *Pterois miles* and *Pterois volitans*. *Proc Gulf Caribb Fish Inst* 61:409-414
- Morrisey D, Inglis G, Neil K, Bradley A, Fittridge I (2011) Characterization of the marine aquarium trade and management of associated marine pests in Australia, a country with stringent import biosecurity regulation. *Environ Conserv* 38:89-100

- Murray-Gulde CL, Heatley JE, Schwartzman AL, Rodgers JH (2002) Algicidal effectiveness of Clearigate, Cutrine-Plus, and copper sulfate and margins of safety associated with their use. *Arch Environ Contam Toxicol* 43:19-27
- Nielsen R, Gunnarsson K (2001) Seaweeds of the Faroe Islands. An annotated checklist. *Fróðskaparrit* 49: 45-108
- Nyberg CD, Wallentinus I (2005) Can species traits be used to predict marine macroalgal introductions?. *Biol Invasions* 7:265-279
- Padilla DK, Williams SL (2004) Beyond ballast water: aquarium and ornamental trades as sources of invasive species in aquatic ecosystems. *Front Ecol Environ* 2:131–138
- Parke MW, Dixon PS (1976) Check-list of British marine algae-third revision. *J Mar Biol Assoc UK* 56:527-594
- Phillips JA, Price IR (2002) How different is Mediterranean *Caulerpa taxifolia* (Caulerpales: Chlorophyta) to other populations of the species?. *Mar Ecol Prog Ser* 238:61-71
- Pimentel D, Zuniga R, Morrison D (2005) Update on the environmental and economic costs associated with alien-invasive species in the United States. *Ecol Econ* 52:273-288
- Price WM (1967) Some aspects of the biology and taxonomy of the unbranched Cladophorales. Dissertation, University of Liverpool
- Relini G, Relini M, Torchia G (1998) Fish biodiversity in a *Caulerpa taxifolia* meadow in the Ligurian Sea. *Ital J Zool* 65(Sup):465-470
- Ruiz GM, Fofonoff PW, Carlton JT, Wonham MJ, Hines AH (2000) Invasion of coastal marine communities in North America: apparent patterns, processes, and biases. *Annu Rev Ecol Syst* 31:481-531
- Ruiz-Carus R, Matheson RE, Roberts DE, Whitfield PE (2006) The western Pacific red lionfish, *Pterois volitans* (Scorpaenidae), in Florida: evidence for reproduction and parasitism in the first exotic marine fish established in state waters. *Biol Conserv* 128:384-390
- Schrope M (2003) Coral smothering “green tide” seaweed spreading on Florida reefs. Press Release, January 23, 2003. Harbor Branch Oceanographic Institute, Fort Pierce, FL
- Semmens BX, Buhle ER, Salomon AK, Pattengill-Semmens CV (2004) A hotspot of non-native marine fishes: evidence for the aquarium trade as an invasion pathway. *Mar Ecol Prog Ser* 266:239-244

- Sepulveda A, Ray A, Al-Chokhachy R, Muhlfeld C, Gresswell R, Gross J, Kershner J (2012) Aquatic invasive species: Lessons from cancer research. *Am Sci* 100:234-242
- Smith CM, Walters LJ (1999) Fragmentation as a strategy for *Caulerpa* species: fates of fragments and implications for management of an invasive weed. *Mar Ecol* 20:307-319
- Smith RL, Sytsma KJ (1990) Evolution of *Populus nigra* (sect. *Aigeiros*): introgressive hybridization and the chloroplast contribution of *Populus alba* (sect. *Populus*). *Am J Bot* 77:1176-1187
- Stewart JE (1991) Introductions as factors in diseases of fish and aquatic invertebrates. *Can J Fish Aquat Sci* 48(Sup1):110-117
- Taylor WR (1967) Marine algae of the eastern tropical and subtropical coasts of the Americas. University of Michigan Press, Ann Arbor
- Uchimura M, Rival A, Nato A, Sandeaux R, Sandeaux J, Baccou JC (2000) Potential use of Cu²⁺, K⁺ and Na⁺ for the destruction of *Caulerpa taxifolia*: differential effects on photosynthetic parameters. *J Appl Phycol* 12:15-23
- Van Den Hoek C, Mann DG, Jahns HM (1995) Algae: an introduction to phycology. Cambridge University Press, Cambridge
- Van Kleef H, Van der Velde G, Leuven RSEW, Esselink H (2008) Pumpkinseed sunfish (*Lepomis gibbosus*) invasions facilitated by introductions and nature management strongly reduce macroinvertebrate abundance in isolated water bodies. *Biol Invasions* 10:1481-1490
- Verlaque M, Boudouresque CF, Meinesz A, Gravez V (2000) The *Caulerpa racemosa* complex (Caulerpales, Ulvophyceae) in the Mediterranean Sea. *Bot Mar* 43:49-68
- Verlaque M, Durand C, Huisman JM, Boudouresque CF, Le Parco Y (2003) On the identity and origin of the Mediterranean invasive *Caulerpa racemosa*. *Eur J Phycol* 38:325-339
- Walters LJ, Brown KR, Stam WT, Olsen JL (2006) E-commerce and *Caulerpa*: unregulated dispersal of invasive species. *Front Ecol Environ* 4:75-79
- Walters L (2009) Ecology and management of the invasive marine macroalga *Caulerpa taxifolia*. In: Inderjit K (Ed). Management of invasive weeds. Springer, Dordrecht, pp 287-318
- Walters L, Odom R, Zaleski S (2011) The aquarium hobby industry and invasive species: has anything changed?. *Front Ecol Environ* 9:206-207

- Wehr JD, Sheath RG (2003) Freshwater algae of North America: ecology and classification. Academic Press, San Diego, California
- Wiedenmann J, Baumstark A, Pillen TL, Meinesz A, Vogel W (2001) DNA fingerprints of *Caulerpa taxifolia* provide evidence for the introduction of an aquarium strain into the Mediterranean Sea and its close relationship to the Australian population. *Mar Biol* 138:229-234
- Wilcove DS, Rothstein D, Dubow J, Phillips A, Losos E (1998) Quantifying threats to imperiled species in the United States. *Bioscience* 48:607-615
- Williams SL, Schroeder SL (2004) Eradication of the invasive seaweed *Caulerpa taxifolia* by chlorine bleach. *Mar Ecol Prog Ser* 272:69-76
- Williams SL, Smith JE (2007) A global review of the distribution, taxonomy, and impacts of introduced seaweeds. *Annu Rev Ecol Evol Syst* 38:327-59
- Wilson CE, Darbyshire SJ, Jones R (2007) The biology of invasive alien plants in Canada. 7. *Cabomba caroliniana* A. Gray. *Can J Plant Sci* 87:615-638
- Wright JT (2005) Differences between native and invasive *Caulerpa taxifolia*: a link between asexual fragmentation and abundance in invasive populations. *Mar Biol* 147:559-569
- Xu Y, Lin J (2008) Effect of temperature, salinity, and light intensity on the growth of the green macroalga, *Chaetomorpha linum*. *J World Aquacult Soc* 39:847-851
- Yuan W, Walters LJ, Schneider KR, Hoffman EA (2010) Exploring the survival threshold: A study of salinity tolerance of the nonnative mussel *Mytella charruana*. *J Shellfish Res* 29:415-422
- Zajicek P, Hardin S, Watson C (2009) A Florida marine ornamental pathway risk analysis. *Rev Fish Sci* 17:156-169
- Zidana H, Turner GF, Van Oosterhout C, Hanfling B (2009) Elevated mtDNA diversity in introduced populations of *Cynotilapia afra* (Gunther 1894) in Lake Malawi National Park is evidence for multiple source populations and hybridization. *Mol Ecol* 18:4380-4389