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Experimental use of salt to control the invasive marine alga *Caulerpa taxifolia* in New South Wales, Australia

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Abstract

Caulerpa taxifolia was first discovered in New South Wales, Australia, in April 2000 and is now present in nine waterways. Infestations, which range from sparse distributions of scattered runners to dense beds 40 cm thick, covered approximately 8.1 km^2 by mid 2004. Various methods for controlling the alga were trialed. The application of coarse sea salt at a concentration of 50 kg/m^2 was found to be the most effective method as it rapidly killed the alga, had relatively minor effects on native biota (seagrass and infauna) and was relatively inexpensive. In small-scale trials, frond density of *C. taxifolia* had decreased by 70–95% one week after salting and no fronds were present after 1 or 6 months. Seagrass and infauna were also affected by salt, but abundances had generally recovered after 6 months. The effectiveness of salting at larger scales depended on the method of application and salting appeared to be most successful in the cooler months when *C. taxifolia* dies back naturally. Results of large-scale salting trials were mixed. In one waterway, a single application of salt resulted in the apparent removal of almost 5200 m^2 of *C. taxifolia*; in another, repeated salting of a 3000 m^2 infestation led to a considerable reduction in the density of the alga, but no overall change in the boundaries of the infestation. Eradication of *C. taxifolia* from New South Wales is unlikely, but local control measures, extensive monitoring and experimentation are continuing in an attempt to limit the impact of this invasive alga.

Keywords: Control; Experimental; Introduced species; Management; Seaweed

1. Introduction

Caulerpa taxifolia is a marine green macroalga that is endemic to tropical and sub-tropical regions around the world. It is primarily a sub-tidal species that has running stolons, feather-like fronds and rhizoids that can anchor to hard or soft substrata. Species of *Caulerpa* are coenocytic, meaning that each alga consists essentially of one, multinucleate, cell. In Australia, native populations of *C. taxifolia* are found in the Northern Territory, Queensland, Western Australia and around Lord Howe Island (see Phillips and Price, 2002 for a comprehensive overview). The alga became a public issue in 1984 when an invasive strain was discovered in the Mediterranean Sea in front of the Monaco Oceanographic Museum (Meinesz and Hesse, 1991). This infestation rapidly colonised thousands of hectares of sub-tidal soft and hard substrata (Meinesz, 2002), but it has been suggested recently that estimates of its coverage have been greatly overestimated (Jaubert et al., 2003).

The invasive strain of *C. taxifolia* was dubbed the "aquarium strain" because it was presumed to have been introduced from marine aquaria in which it was (and still is) used for decoration. Molecular evidence has since supported this notion, demonstrating that the invasive strain in the Mediterranean is genetically identical to a strain of *C. taxifolia* cultivated in aquaria 15 years prior to its appearance at Monaco

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(Jousson et al., 1998). Numerous studies have indicated that the aquarium strain in the Mediterranean is genetically similar to populations of *C. taxifolia* which are native to Queensland (Benzie et al., 2000; Wiedenmann et al., 2001; Famà et al., 2002; Meusnier et al., 2002; but see Murphy and Schaffelke, 2003). Thus, the invasive strain of *C. taxifolia* that colonised the Mediterranean did not originate from southern New South Wales, as suggested by Meinesz et al. (2001), but rather from sub-tropical areas in Queensland (north-eastern Australia).

The first confirmed sightings of *C. taxifolia* in New South Wales were in Port Hacking, on the southern outskirts of Sydney, and Lake Conjola (200 km south of Sydney) in April 2000. There is, however, anecdotal information that the alga first invaded Lake Conjola between 1987 and 1995 and that the Port Hacking invasions may have occurred in 1998 (Grey, 2001). *C. taxifolia* is now present in nine waterways in New South Wales, covering approximately 8.1 km^2. Measures have been put in place to limit the risk of translocation of the alga and various methods for control have been investigated. There is little published information on methods of control of *C. taxifolia* and even less about the potential effects of control methods on native biota (but see Williams and Grosholz, 2002). The control methods trialed in New South Wales included physical removal by hand or using a suction pump, smothering with heavy rubber conveyer belts and jute matting (hessian), and osmotic shock using salt. Chlorine has been used elsewhere to control *C. taxifolia* (Williams and Schroeder, 2004), but this method was not investigated in New South Wales.

Physical removal of *C. taxifolia* in New South Wales was very labour intensive, slow and often resulted in reduced visibility (due to suspended sediment), thus making finding the alga difficult. Moreover the alga often became fragmented during the removal process and any fragments could spread and re-establish. Smothering *C. taxifolia* with conveyer belts killed the alga, but was very labour intensive because it was difficult to deploy and even more so to remove. This treatment also appeared to kill many other species on the substratum. Jute matting was then trialed because it was expected to biodegrade completely after 2 years. Small-scale trials with jute matting were successful, but in a larger trial the matting was positively buoyant and so needed to be weighted down, making its deployment awkward. Most vegetation under the matting was killed as were many macroinvertebrates. Eventually, *C. taxifolia* was found growing between the joints of the jute and through any tears that had occurred during deployment. Salting (a treatment believed to have been trialed first in France) proved to be the most effective control. Here we describe studies that investigated the effectiveness of different concentrations of salt at killing *C. taxifolia*, the potential impacts of salt on infauna and seagrass, and the effectiveness of applying salt in different ways.

2. Methods

2.1. Description of study areas

To date, *C. taxifolia* in New South Wales has been found only in relatively sheltered embayments in water ~0.5–10 m deep and never on exposed coasts. Comprehensive water quality data are not available for the various waterways and depths in which *C. taxifolia* is present, but based on data from numerous sources, salinity in these areas (at depths >0.5 m) may range from ~27–36 ppt and temperature ranges can be from ~12–25 °C. In all waterways, *C. taxifolia* is growing primarily on soft sediments, often adjacent to or in beds of seagrass, but also in previously unvegetated sediments. In a few places, the alga is found on hard surfaces, such as pier pilings, concrete mooring blocks and rocky reefs. During winter, the cover of *C. taxifolia* generally decreases and the alga is typically much smaller, which is consistent with findings in other countries (Meinesz et al., 1995; Ceccherelli and Cinelli, 1999).

The first trial of salt was set up in Lake Macquarie, a 115 km^2 wave-dominated lagoon situated 100 km north of Sydney. The waterway has been heavily modified by human activity. Experimental sites were established in areas that contained *C. taxifolia* and the seagrass *Zostera capricorni*. The second salting experiment was done in Narrawallee Inlet which is a narrow, 4.7 km long (0.3 km^2) waterway, 200 km south of Sydney. It is a relatively pristine waterway and experimental sites contained *C. taxifolia* and seagrasses (*Z. capricorni* and *Halophila ovalis*).

2.2. Effects of salt on C. taxifolia, seagrass and benthic infauna

Experiments were set up at two sites, approximately 1 km apart, in Lake Macquarie in March (autumn) 2002. Coarse sea salt (99.5% NaCl; mean particle size 2.7 mm; Cheetham Salt Ltd) was used for the experiment. In each site, twelve 1 × 1 m plots were marked out at depths of 1–3 m. Two replicate plots were established for salt concentrations of 50, 100, 150 and 200 kg/m^2. There were also two control plots positioned close to the salt treatments and two distant control plots positioned 50 m from the salt treatments. The distant control plots were used to test whether salt might have effects that extended further than the individual 1 × 1 m plots (e.g. because salt dispersed during application to a plot). All plots were sampled prior to applying salt and then 1 week, 1 month and 6 months after salt was applied, although some plots were lost and so could not be sampled at all times. In each 1×1 m plot, the numbers of shoots of *Z. capricorni* and of fronds of *C. taxifolia* were counted in three smaller quadrats (30×30 cm) which were placed haphazardly. Benthic infauna was sampled using three replicate cores (65 mm diameter, 100 mm deep) per plot. Sediment samples were preserved in 10% formaldehyde, stained with Biebrich scarlet then sieved over a 500 μm mesh. Remaining coarse material was elutriated with running fresh water to suspend any animals. Sieved and elutriated parts of each sample were examined under a dissecting microscope and all benthic infauna was identified, generally to the level of family.

Data were analysed using three factor ANOVAs with the factors Site (random, orthogonal), Treatment (fixed, orthogonal) and Plot (random, nested in the Site × Treatment interaction). Homogeneity of variances was tested prior to each analysis and if transformations could not stabilise variances, α was reduced from 0.05 to 0.01 to minimise the chances of Type I error (Underwood, 1997). *Post-hoc* pooling was used to increase the power of tests when interaction terms were non-significant ($p > 0.25$) (Underwood, 1997). Student–Newman–Keuls tests were used to compare means of factors that were significant. Separate ANOVAs were done for each time of sampling because the experimental design varied among times due to replicates being lost, or because particular taxa were not present at each time.

2.3. Application of salt to beds of *C. taxifolia*

The initial method of application of salt involved SCUBA divers spreading 25 kg bags of salt by hand over patches of *C. taxifolia*. Although time consuming, this method was considered appropriate in waterways where *C. taxifolia* grew in small isolated patches, rather than in large dense beds. A trial using this salting method was done in Narrawallee Inlet during the summer of 2002/2003 and appeared to have been successful, with extensive die-back of *C. taxifolia* after a few days. Six weeks after the application of salt, however, there was little sign that the salting had any effect and the alga seemed to be thriving and covering more area than before. The apparent failure was possibly due to stolons under the sediment not being treated with salt, because only the obvious stolons and fronds were treated by divers. If this were the reason for the reappearance of *C. taxifolia*, then salting larger areas should prove to be more effective than salting discrete patches.

An experiment was set up in Narrawallee Inlet in February 2003 to compare the effectiveness of salting small patches by hand, versus completely covering large areas with salt (also by hand). The experiment was done in four sites separated by ~100 m and located within ~500 m of the mouth of the inlet. Sites were 1–2 m deep and at each, six 2×2 m plots were marked out and two plots were assigned randomly to each of the following treatments: (i) 50 kg/m^2 salt covering the entire plot, (ii) patch salt treatment where salt was applied only to visible *C. taxifolia* patches in the plot and, (iii) control (no salt). The numbers of live *C. taxifolia* fronds were counted in each plot prior to applying any salt and then again 4 days, 27 days and 86 days after application.

3. Results

3.1. Effects of salt

Salt generally dissolved completely within 10 h of application and after 1 day, *C. taxifolia* was limp and had started to lose colour. All concentrations of salt had a dramatic effect on the density of *C. taxifolia*. Prior to salting, there was no significant difference in the cover of *C. taxifolia* among treatments ($F = 1.8$, $p > 0.05$), but just 1 week after the salt was applied, mean frond density in salted treatments had decreased by 70–95% and was significantly less than in controls ($F = 20.6$, $p < 0.001$; Fig. 1(a)). These differences were consistent between sites. After 1 month, *C. taxifolia* fronds had essentially disappeared from all salted plots (Fig. 1(a)). No fronds had reappeared after 6 months in these plots, but it is important to note that frond density also decreased in the control plots over the course of the experiment (Fig. 1(a)). This is consistent with our other studies (and studies in the Mediterranean, e.g. Meinesz et al., 1995) which have shown that the density of *C. taxifolia* can decrease greatly over the winter period (May–September in the southern hemisphere), particularly in shallow water.

The shoot density of the seagrass *Z. capricorni* was also affected adversely by salt, but less so than *C. taxifolia*, and there were clear signs of recovery after 6 months for most salt concentrations (Fig. 1(b)). Densities of this seagrass did not differ among treatments prior to the application of salt ($F = 0.4$, $p > 0.05$), nor 1 week after salt had been applied ($F = 2.6$, $p > 0.05$; Fig. 1(b)). After 1 month, however, differences became apparent among treatments ($F = 3.8$, $p < 0.05$) and it was clear that seagrass density had decreased in all salted plots (Fig. 1(b)). By 6 months, differences still occurred among treatments ($F = 4.5$, $p < 0.05$), and seagrass density in all treatments was still significantly less than in controls. Of the salted treatments, seagrass density was greatest in the 50 kg/m^2 plots and doubled from 1 to 6 months to be similar to the pre-salting density (Fig. 1(b)). Seagrass that had been treated with salt at concentrations greater than 50 kg/m^2 did not recover as well (Fig. 1(b)). There was no evidence that salt applied to a 1×1 m area had any effects on seagrass a few metres away because densities of seagrass in the close controls were never

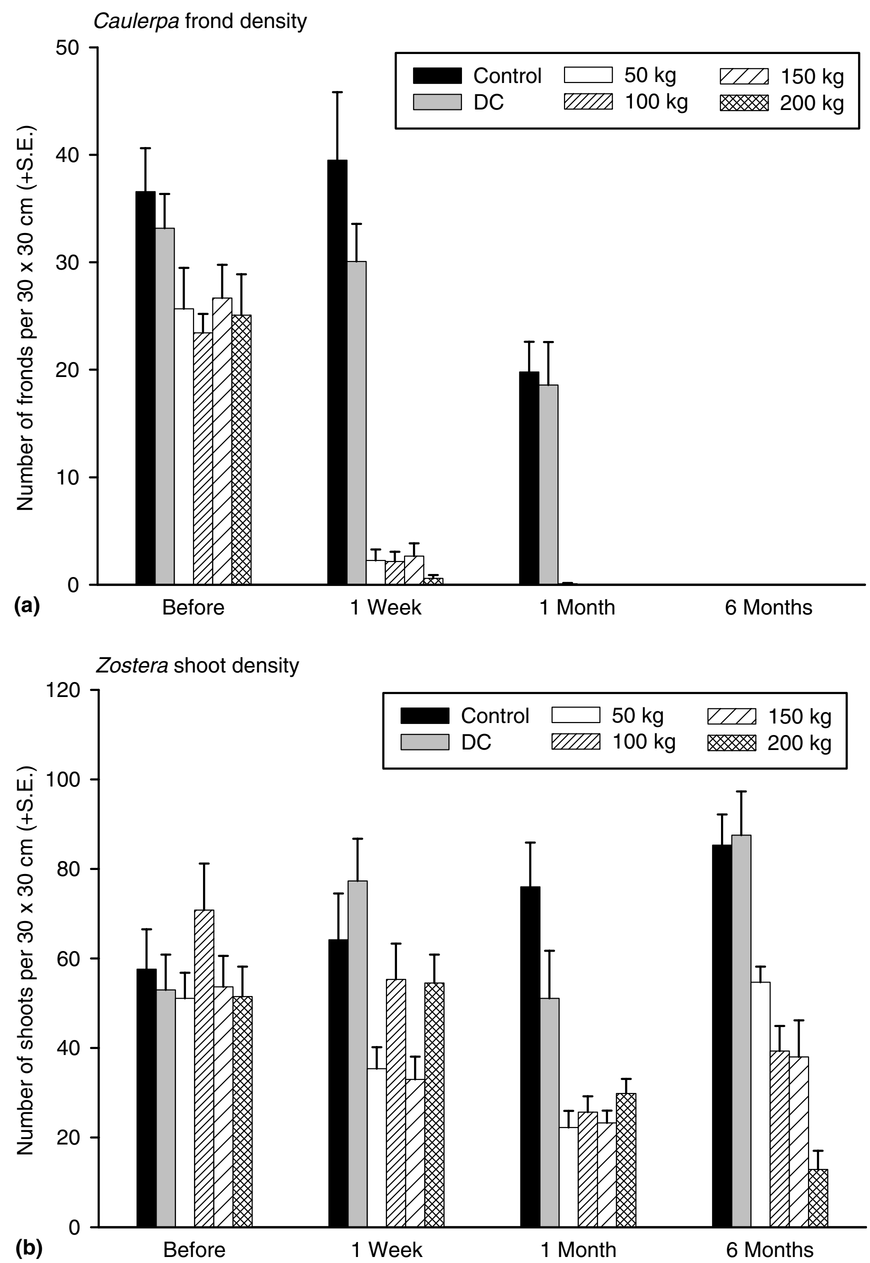

Fig. 1. (a) Mean number of fronds of *C. taxifolia* and (b) *Z. capricorni* in 1 × 1 m plots that were either not salted (close control (Control) and distant control (DC)), or salted at concentrations of 50, 100, 150 or 200 kg/m^2. Data for each treatment are averaged across replicates, plots and sites (so total $n = 12$). The experiment was initiated in March (autumn) 2002 in Lake Macquarie.

significantly less than those in the distant controls (Fig. 1(b)). Again, the patterns among treatments did not differ significantly between sites.

The abundance and the diversity of infauna were similar among treatments prior to the salt being applied ($F = 0.8$, $F = 0.5$ respectively, $p > 0.05$ for both). One week after the application of salt, significant differences appeared among treatments for abundance ($F = 43.0$, $p < 0.001$) and diversity ($F = 40.2$, $p < 0.01$) of infauna. It is clear from Fig. 2(a) and (b) that these differences were due to significant reductions in all salted treatments relative to the nearby controls (distant controls were not sampled at this or the subsequent time). These differences were still evident 1 month after salting. As for seagrass, the infauna began to recover after 1 month and by 6 months, abundances and diversity of infauna in the 50 kg/m^2 plots were comparable to those in the controls (Fig. 2(a) and (b)). There was, however, evidence that the application of salt at 150 and 200 kg/m^2 resulted in increased numbers of some infauna after 6 months

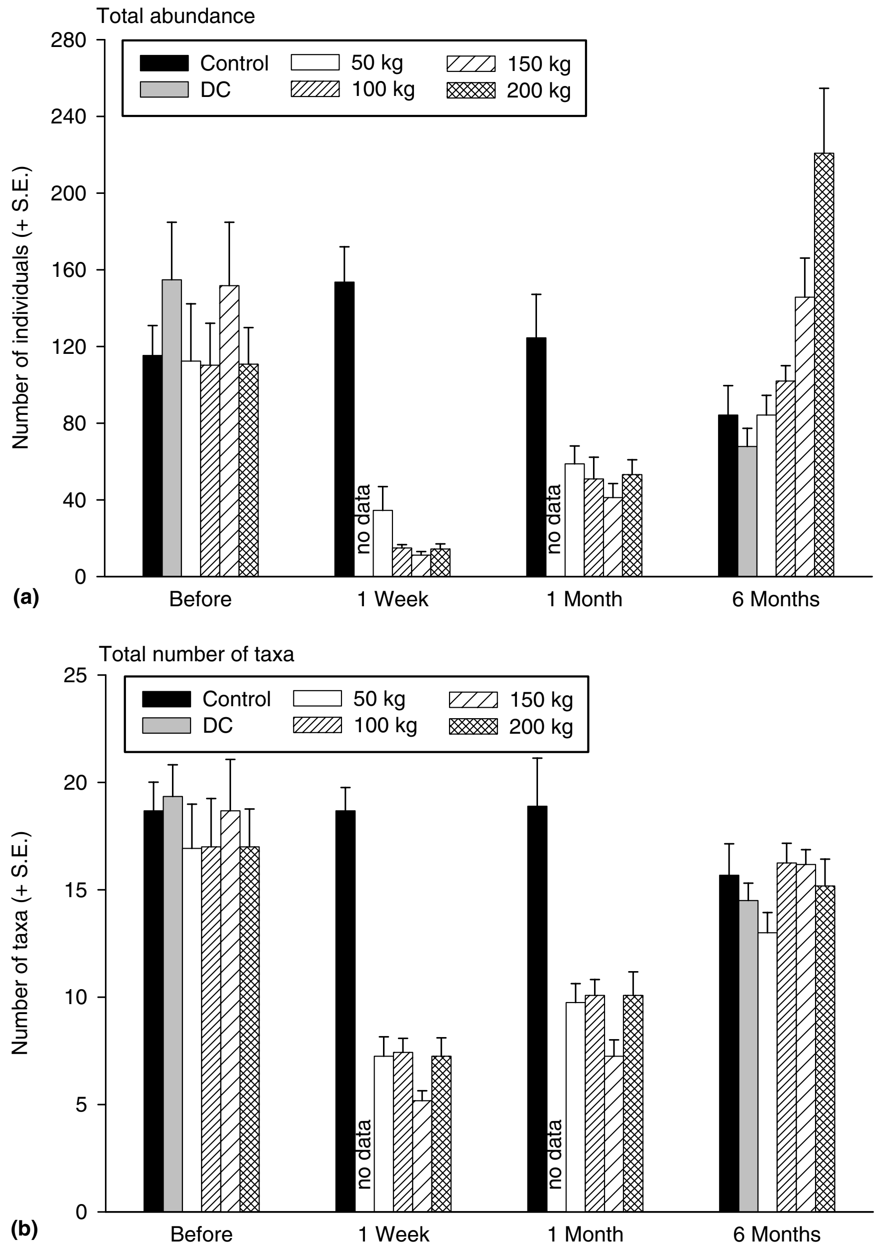

Fig. 2. (a) Mean number of soft sediment invertebrates (infauna) and (b) infaunal taxa in 1 × 1 m plots that were either not salted (close control and distant control), or salted at concentrations of 50, 100, 150 or 200 kg/m^2. Data for each treatment are averaged across replicates, plots and sites (total $n = 12$). The experiment was initiated in March (autumn) 2002 in Lake Macquarie. No data were collected for the distant controls after 1 week and 1 month.

(Fig. 2(a)), in particular tanaids, nematodes, capitellid polychaetes and gammarid amphipods.

3.2. Patch salt vs. total coverage

The numbers of *C. taxifolia* fronds were reduced dramatically in both salting treatments after 4 days and this effect was still evident after 27 days (Fig. 3). By 86 days, it was apparent that the 50 kg/m^2 salt treatment had reduced the density of *C. taxifolia* by ~90% relative to unsalted control plots, whereas the patch salt treatment reduced the frond density by ~20% (Fig. 3). Thus, in the longer term, salting individual patches of *C. taxifolia* was not as effective as covering the entire plot with salt.

The most striking finding during the experiment in Narrawallee Inlet was the 10-fold increase in the density of *C. taxifolia* fronds in control plots during autumn (Fig. 3). An even more dramatic result occurred in July 2003, 56 days after the last time of sampling, when all *C. taxifolia* had disappeared from every experimental plot. By July, the water temperature had dropped to around 17 °C and there had been 368 mm of rain in the month

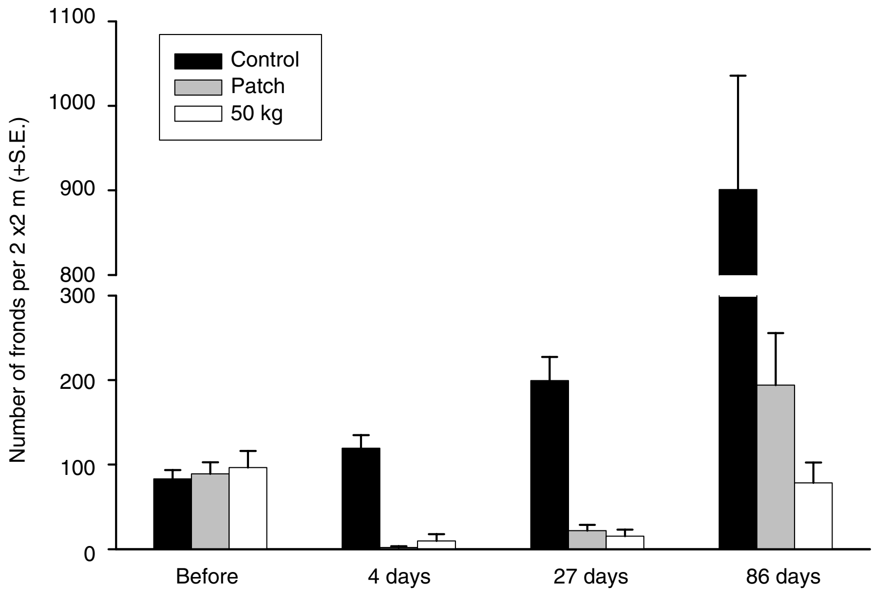

Fig. 3. Mean number of fronds of *C. taxifolia* in 2 × 2 m plots that were either not salted (controls), patch salted, or covered with 50 kg/m^2 of salt. Note that *y*-axis has been split. The experiment was initiated in February 2003 (summer) in Narrawallee Inlet. Data for each treatment are means (+SE) of two experimental plots per site at each of four sites (total $n = 8$).

following the last sampling date (the greatest monthly rainfall in the region for 8 years and over twice the average for that time of year; Bureau of Meteorology, Sydney). By September 2003 (when only 47 mm of additional rain had fallen), there was still no sign of *C. taxifolia* in the experimental area, so all control plots were sampled destructively to search for remains of the alga which had been so abundant 4 months earlier. Divers sifted through the top 10 cm of sediment in each control plot and also collected sub-samples of sediment which were sieved over a 1 mm mesh. No traces of the alga were found, indicating that the sediments might not be a refuge for *C. taxifolia* as suggested by Williams and Schroeder (2004). A search of the remainder of the waterway revealed only a few scattered patches of *C. taxifolia* in the deepest section of the waterway (5–6 m). The fronds of these algae appeared healthy and showed no signs of senescence. It remains unclear why the alga survived only in this restricted part of the waterway, but perhaps it was isolated from decreases in salinity (due to freshwater runoff) which could kill the alga (perhaps in combination with decreased temperatures).

4. Discussion

The salt concentration experiment provided good evidence that salt applied at 50 kg/m^2 during autumn removes *C. taxifolia* for at least 6 months, whilst having limited short-term effects on the densities of *Z. capricorni* and infauna. It is not known whether salt may have had any other effects on seagrass (e.g. effects on productivity). *Z. capricorni* tends to die back naturally over winter and its peak growing and flowering period is spring–summer (Larkum et al., 1984). Thus, it is likely that the cover of seagrass would have increased considerably if the experiment had been run for another 1–2 months as this would have incorporated the expected growing season of *Z. capricorni*. Abundances of infauna in 50 kg/m^2 treatments were not different from controls after 6 months, but were elevated in plots salted with higher concentrations. This latter result is perhaps not surprising given that disturbances, specifically organic enrichment and pollution, have been demonstrated to increase abundances of some soft sediment infauna (e.g. Rosenberg, 1972; Grassle and Grassle, 1974; Pearson and Rosenberg, 1978).

No quantitative studies were done with salt concentrations less than 50 kg/m^2, but the effectiveness of 25 kg/m^2 of salt was assessed qualitatively. This lower concentration killed *C. taxifolia* effectively but, due to a much sparser coverage of salt on the substratum, many fragments of *C. taxifolia* were not salted and so survived. Survival of unsalted *C. taxifolia* also appeared to be a problem when obvious patches of the alga were salted rather than using a blanket cover of salt on the substratum. Over a period of 86 days, salting individual patches of *C. taxifolia* did not kill *C. taxifolia* as effectively as did covering entire plots with salt, suggesting that stolons under the sediment had escaped the ‘patch’ salting treatment. It is also noteworthy that the 50 kg/m^2 salt treatment in this experiment in Narrawallee Inlet did not appear to be as effective as that in the previous experiment conducted in Lake Macquarie at a similar time of year, which resulted in the complete removal of *C. taxifolia* after just 1 month. It is possible that different physical conditions in the two waterways led to the differing results. For example, currents are generally stronger in Narrawallee Inlet, meaning that salt was probably dispersed or dissolved faster than in Lake Macquarie.

In order to make the control technique cost effective, a method of delivering salt to large areas relatively quickly needed to be developed. A hopper was designed to deliver 1 tonne of salt from a 7 m long flat-bottomed boat, whilst carrying another 1 tonne bag in the middle of the boat as ballast. To deliver the salt, the boat is manoeuvred over areas of *C. taxifolia* that have been marked out with buoys and a small slot running along the bottom of the hopper is opened using a lever so that a stream of salt is dumped into the water. The exact amount of salt dumped varies according to the speed of the boat and the depth of the water, but in calm weather conditions in water <5 m, it is possible to get a good coverage of salt over the alga at $\sim$50 kg/m^2 (but difficult to deliver a lesser concentration due to the speed at which salt exits the hopper). The average cost of using this technique, including salt and labour, is \$A7 per m^2. Delivering salt from a hopper is not generally effective in water deeper than 5 m because the salt disperses too much before reaching the substratum. It may be possible to treat deeper areas using this technique by adding a chute to deliver the salt directly to beds of *C. taxifolia*. Another effective method (although expensive, at \$A30 per m^2) involved a barge lowering 1 tonne bags of salt into the water and commercially trained divers spreading the salt by hand. This latter technique was used to remove approximately 1700 m^2 of *C. taxifolia* from water 3–7 m deep in Lake Macquarie during late spring 2002.

It appears that salting is most effective in winter, soon after the fastest growing period of *C. taxifolia*. *C. taxifolia* is, however, more difficult to see during winter because it is less abundant, tends to become smaller and often gets covered with epiphytes. The best control strategy would entail detailed mapping during the warmer months, followed by repeated salting of these mapped infestations during winter. Salt concentrations of $\sim$50 kg/m^2 are effective in significantly reducing the density of *C. taxifolia*, but some stolons survive just one application of salt. Repeated salting is likely to increase the effectiveness of the treatment. The longer term (>6 months) effects of salting have not yet been studied in detail and it will be important to examine the effects of repeated salting on native biota. It is possible that repeated short-term (or pulse) disturbances due to salting could have long lasting (or press) effects on native organisms (Glasby and Underwood, 1996).

Since the first confirmed reports of *C. taxifolia* in two New South Wales estuaries in April 2000, the alga has been discovered in seven other waterways. Trials of methods to control the spread of *C. taxifolia* using salt have generally been successful at small scales. Limited resources have meant that many outbreaks have not yet been treated and the results of large-scale salting have been mixed. For example, in Lake Macquarie, single applications of salt to numerous outbreaks have resulted in the apparent removal of almost 5200 m^2 of *C. taxifolia*, whereas repeated salting of a 3000 m^2 infestation in Careel Bay in Pittwater has led to a considerable reduction in the density of the alga, but no overall change in the boundaries of the infestation. It seems likely that natural phenomena assisted with the removal of *C. taxifolia* from Lake Macquarie. More research is needed on natural fluctuations in abundance of *C. taxifolia* and testing for correlations between various biotic and abiotic parameters and abundances of *C. taxifolia*.

Delivering salt from the surface of the water is most effective for relatively shallow infestations (<5 m), but divers can apply salt to deeper areas. To date in New South Wales, salt has been used only on small infestations of *C. taxifolia* (<1 ha) growing in soft sediments. Moreover, to treat large infestations, the technique relies on suitable infrastructure (good roads, boat ramps, wharves with loading facilities, etc.) and maritime equipment (large barges to carry tonnes of salt around an estuary) being available so that large quantities of salt can be efficiently delivered, loaded and applied to beds of *C. taxifolia*. At present, the treatment of *C. taxifolia* with salt in all invaded estuaries in New South Wales is not logistically feasible and prohibitively expensive (estimated at >\$A60 million for one application of salt, assuming that all areas could be treated using the cheapest method, and excluding any follow-up monitoring). In areas where large-scale salting is not possible, key priority areas such as seagrass beds (which may be vulnerable to invasion), boat ramps and popular fishing spots (from where *C. taxifolia* may be spread) will be targeted. As yet, *C. taxifolia* has not been discovered on the open coast of New South Wales and a priority for future control work will be to eradicate any outbreaks close to the mouths of estuaries to limit the possibility of the alga spreading to the open coast. While total eradication of *C. taxifolia* from New South Wales waterways is unlikely, it is hoped that the use of salt as outlined in this paper will help prevent further spread of the alga.

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