5. CONTROL TECHNIQUES FOR *CAULERPA TAXIFOLIA*

It is always very difficult to effect the total eradication of marine invasive species once they have become established in a new locality and few attempts have been made (Kuris 2003), particularly for seaweeds (Curiel *et al*. 2001; Williams and Schroeder 2004). Successful eradication is thought to involve three things (McEnnulty *et al*. 2001): early detection, the availability of effective techniques to kill the invader *in situ* and rapid implementation of those techniques. In the Mediterranean, the control of *C. taxifolia* has involved a number of methods at a number of locations, but efforts apparently have merely slowed the rate of spread at some places rather than achieving complete elimination (G. Ceccherelli, pers. comm.). The descriptions of most of these attempts are contained in anecdotal accounts or conference proceedings rather than in peer reviewed scientific journals or creditable technical reports (Williams & Schroeder 2004), making rigorous evaluation of the success or otherwise of the trials difficult.

C. taxifolia was already so widespread in NSW when first encountered (Chapter 2) that total eradication was never a realistic goal. However, the development of methods to kill the alga in the field in a cost-effective and environmentally benign way might provide a way of controlling its spread by eliminating it from localized areas and thereby limiting its spread to new locations. In examining control techniques that might be of value in NSW, accounts of techniques used in Australia or overseas for either *C. taxifolia* or other marine invasive species were evaluated (see McEnnulty *et al*. 2001). Potential control techniques considered for *C. taxifolia* were:

- Handpicking – physical underwater removal by divers
- Diver-operated suction dredging
- Cutter/suction dredging – mechanical removal using a commercial dredging vessel
- Smothering – laying various materials over the *C. taxifolia* to cut off light
- Chemicals such as chlorine, metal cations or herbicides
- Osmotic shock – the use of very high or very low salinity water
- Temperature shock – the use of very hot or very cold water
- Biocontrol – the use of herbivores to eat the alga

These techniques are considered below, and any trial work done in NSW is described and evaluated. The last prospective technique, biocontrol, has previously been examined in Chapter 3.3.

5.1. Removal by hand

Often, the first instinct when encountering a potentially invasive species in a new location for the first time is to pull it out. Manual removal of *C. taxifolia* is usually the first control technique that is attempted because it can be done straightaway and requires few resources. In the Mediterranean, hand-picking has been performed at several sites in Italy and Spain, but the alga still occurs at those places. For example, a month long operation by divers apparently removed most *C. taxifolia* spread over 1 hectare in Cala D'Or, Spain in 1992. Subsequent visits in 1993 and 1994 apparently removed all remaining plants, but a much larger infestation was found nearby in 1995 (Grau *et al*. 1996; see also McEnnulty *et al*. 2001). In France, an annual exercise involving an underwater dive club removes *C. taxifolia* from a small marine protected area on the French coast. Again, however, total eradication has not been achieved, merely arresting its rate of spread (G. Ceccherelli, pers. comm.).

Attempts at physical removal by hand picking in NSW have proved partially effective for areas with small isolated patches of *C. taxifolia*. The first plants found in Lake Macquarie, in the shallows of Crangon Bay in March 2001, were effectively removed by hand-picking. Similarly, some isolated plants were removed from very shallow water in Myuna Bay in Lake Macquarie using this technique. The alga has not since been recorded at these sites, although it is doubtful that all plants were located and destroyed in these single, one-off exercises. Rather, it is more likely that plants in these shallow localities were growing in suboptimal places anyway, and would have eventually died off naturally, perhaps during periods of low water temperature over winter. In contrast, attempts to remove some isolated plants from a site on the western shore of Burrill Lake in April 2002 made no impact, with larger patches recorded there during subsequent visits. Attempts to clear large patches were soon adjudged to be futile. In 2000, a trial in Lake Conjola resulted in a patch (4 m^2) being manually cleared by two divers in 1 hour, but this patch grew back in 6 months (M. Miller, NSW Fisheries, pers. comm.).

The usefulness of this technique on a broader scale is extremely limited as it is very labour-intensive. As the alga itself is fragile and often has an extensive attachment system, it is difficult to completely remove especially if it is growing amongst seagrass. Further, handpicking will have limited applicability in areas of fine muddy sediments because visibility will soon be reduced to levels where it becomes impossible to find remaining *C. taxifolia* plants. However, single plants growing in clean sand can be efficiently removed by hand-picking and, if the infestation at that place is in its very early stages, it should be possible to effect something approaching total elimination.

5.2. Removal by diver-operated suction devices

There are anecdotal reports of total or partial removal of *C. taxifolia* from sites in Croatia using a suction pump (treated areas were 350 and 250 m^2) (Zuljevic and Antolic 1999a, 1999b). Re-surveys of the areas showed no or only sporadic regrowth of C. *taxifolia* one year later.

Preliminary trials in NSW using various designs of "underwater vacuum dredges" or "slurp guns" were undertaken in Lake Conjola and Port Hacking in 2000 (see Millar 2002). A further trial was done in July 2001 in Narrawallee Inlet which has clean, sandy sediments (see Chapter 2). When divers encountered *C. taxifolia*, they "weeded" the alga out with dive knives while simultaneously vacuuming with slurp guns. Care was taken to ensure that all fragments and debris were captured and retained in the attached catch bags. After bags became full they were taken to the surface where the support person in the boat replaced them with new bags. An area of 760 m^2 was effectively cleared of *C. taxifolia* using this method in a period of 6.5 dive hours.

A second trial was conducted in September 2001 at Careel Bay in Pittwater using a team of commercial divers. Removal was effected by a diver using Surface Supplied Breathing Apparatus (SSBA) who operated a venturi-driven, hand-held suction apparatus. All materials were collected into a geotextile bag that was attached to the end of the dredging pipe. When the bag became full, which was indicated by a marked decrease in suction capacity, the diver returned to the surface where a new bag was fitted. When full, the geotextile bags could potentially hold up to 300 kg of material (sediment + plant material). The maximum amount obtained before the filter bag clogged with the fine, muddy sediment that is characteristic of this site, however, was only 15 kg of material. It took 5 minutes to dredge up this amount of material. A total of 3 bags were used in this trial, producing a total of only 40 kg of material dredged. This trial was then judged to be ineffective and further attempts were abandoned. It was concluded that hand-held dredging equipment would only be a viable option for removal of *C. taxifolia* from muddy sites if a suitable mesh size could be found for the containment bags. This would probably have to be done for each and every locality, because sediment characteristics tend to be site specific.

Although diver-operated devices do remove *C. taxifolia*, their use would only be cost effective in NSW for very small, isolated patches because the technique is very slow and painstaking. In addition, it is only feasible at sites where the sediments are primarily sandy (such as Narrawallee Inlet), because water clarity is rapidly reduced where the sediments are muddy, severely reducing underwater visibility and hence efficiency. One way to avoid problems with rapid clogging would be to pump material suctioned off the bottom by divers directly up onto a barge for storage in a bin for later disposal on land rather than into individual "catch bags". Alternatively, materials could be collected over a suitable mesh grid on the surface vessel that would allow water and fine sediments to drain away. Again, the appropriate mesh size would need to be determined to allow adequate runoff (and hence avoid accumulating large volumes of water and fine sediment), while minimising the release of *C. taxifolia* fragments back into the water. A procedure apparently similar to this was used in 2003-04 in the Port River in South Australia to remove small infestations of *C. taxifolia* (J. Gilliland, pers. comm.).

5.3. Large scale removal by commercial dredges

The use of divers in the water allows small patches or individual *C. taxifolia* plants to be accurately targeted during suction dredging operations. For extensive areas of *C. taxifolia*, however, especially in fine, muddy sediments, such a procedure would not be practicable. Instead, the use of commercial dredging vessels was suggested as a means of dramatically reducing the biomass of invasive *C. taxifolia* in such areas.

It had been hoped to use a planned dredging of the boating channels into Port Hacking using a commercial cutter suction dredge as a way of testing the effectiveness of large scale dredging to effectively remove *C. taxifolia*. Extensive discussions with the NSW Department of Land & Water Conservation (DLWC) and Sutherland Shire Council led to a proposal to place any sediments dredged from areas containing *C. taxifolia* into large, geotextile 'sausages' which could be later offloaded into deeper water off Cronulla Beach (i.e., outside the sheltered waters where *C. taxifolia* grows). The plan was that the *C. taxifolia* would die within the 'sausages' and that these could be slit open by divers after a couple of months so that sediments could be redistributed by natural processes. Extensive underwater surveys were done to ascertain places where the proposed dredge path intersected beds of *C. taxifolia*, and several test sites were identified After much debate, however, DLWC decided that the costs were too high and the logistics too difficult for this to be attempted. As it turned out, by the time the boating channels were actually dredged (March-April 2003), much of the *C. taxifolia* in Port Hacking had naturally died back and there was none growing within or adjacent to the dredge paths.

5.4. Killing *C. taxifolia* by smothering

Control by smothering has been trialed overseas with some apparent but localized success. For example, an area of *C. taxifolia* covering 512 m^2 at a site in Croatia was covered with black PVC plastic, reportedly leading to the almost complete eradication of the alga with only sporadic regrowth (Zuljevic and Antolic 1999a, 1999b). Black plastic tarpaulins have also been used on the infestations of *C. taxifolia* in southern California, initially to contain liquid chlorine that was pumped under them to kill the alga. The relative effectiveness of the tarpaulins vs the chlorine has not been experimentally determined (Williams and Schroeder 2004).

Several trials using smothering techniques were attempted in Lake Macquarie and Careel Bay, NSW. In the first trial, sixteen small plots (1m x 1m) were set up within two beds of *C. taxifolia* at Mannering Park in October 2001 (Plate 7). Plots were separated by several metres from neighbouring plots. There were 4 treatments in the experiment with 4 replicates of each:

- controls (i.e., plots left uncovered)
- biodegradable jute matting
- bags made from biodegradable geotextile cloth and filled with sand
- rubber conveyor belting.

The smothering materials were anchored down where needed, and the edges covered by sediment to limit the growth of adjacent *C. taxifolia* underneath them. Prior to placement of the smothering materials, the plots were mapped and photographed. All plots were checked after 1 and 2 months, and the presence of any apparently living algae noted. All trial materials remained in place during the duration of the trial, although additional weights had to be added to the edges of some of the rubber matting. Although some stolons of *C. taxifolia* were still recognisable under the covers after 1 month, there was no evidence of any living plant tissue underneath the central part of the treatments after 2 months. The sediment was black and anoxic, and no living seagrass or benthic animals were found. However, some organisms, including some *C. taxifolia,* plants were present under the edges of the treatment squares. The trials were considered successful enough at this small scale to warrant further investigation at a larger scale.

Plate 7. Trials in NSW to evaluate the feasibility of killing *C. taxifolia* by smothering it with various materials. Top left: jute matting about to be laid at Mannering Park in Lake Macquarie; top right: jute matting at Careel Bay after several months on the bottom; bottom left and right: rubber conveyor belting being laid in Lake Macquarie.

Because large amounts of used, unwanted rubber conveyor belting was locally available around Lake Macquarie a large scale trial of this material was initiated in November 2001. Sections of belting (each 10 m x 2 m) were lowered from a commercial barge into the water (Plate 7). Commercial divers on SSBA then rolled them out underwater and fixed them in place with stainless steel rods hammered through pre-drilled holes around the edges of the belts. Three belts were laid side by side with their edges overlapping in dense beds of at each of 2 sites off Wangi Wangi Peninsula. This gave two areas of approximately 60 m^2. Underwater video footage was taken prior to the deployment and again after 4 months when the belts were raised from the seabed. 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 killed many other species on the seafloor including all seagrass.

Jute matting was then trialed because it was cheap, relatively light and easy to handle and was expected to biodegrade completely after 2 years. A single roll was placed on part of a dense *C. taxifolia* bed in an area without seagrass off Wangi Wangi Peninsula, Lake Macquarie in April 2002. The rolled out mat (approximately 15 m^2) was easily deployed and fixed to the bottom by bamboo stakes around its edge. Underwater video footage was taken prior to the deployment and again at intervals of 2 and 4 months after deployment by lifting up one edge of the mat. As with the previous trials, the *C. taxifolia* under the central part of the mat was completely killed, as were other marine organisms. Some plants, however, were growing under the edges of the mat where it had lifted away from the seafloor or in places where the matting had been torn or punctured.

The trial with jute matting in Lake Macquarie was followed by a larger trial in Careel Bay in November 2002. The trial involved 50 rolls of heavy duty matting being laid, with the assistance of navy divers, over the entire bed of *C. taxifolia* which covered approx. 2,000 m^2. A heavier, more robust variety of matting was used in an attempt to more effectively cover the algal bed. Unfortunately, it turned out to be more buoyant than the variety previously used in Lake Macquarie and needed to be weighted down (second-hand roof tiles were used), especially where adjacent rolls overlapped. This made its deployment awkward. Most vegetation under the matting was killed after several months (ascertained by lifting sections of the matting and taking samples of the anoxic sediments underneath them) as were most other marine organisms. Eventually, *C. taxifolia* was found growing in between the joins of the jute and through any tears that occurred during deployment (Plate 7). An additional problem was that fragments were created during the process of deploying the matting and many of these settled on top of the mat. Although divers attempted to manually remove these fragments, this was not totally possible and many fragments started growing on this new surface.

While most of the smothering techniques trailed in NSW proved effective in killing the plants under the material, the process of laying and stabilising the covering material was very labour intensive and expensive, and the process did not work in areas of uneven bottom or on rocky substrata. The conveyor belting had the added disadvantage that it is not biodegradable and had to be removed after 6 months, whereas the other two coverings trialed naturally degraded over several months. The trials showed that it was vital for any smothering material to completely cover all of the plant material and also a surrounding “buffer” area. Otherwise, *C. taxifolia* quickly grew out from under the edges of the material. The trials also showed the importance of treating entire beds of *C. taxifolia* rather than patches within beds, because encroachment invariably occurred from surrounding areas and the new stolons eventually grew over the top of the smothering material. Finally, fragments are almost invariably generated during the process of deploying the smothering material, and these may settle and establish nearby or, in the case of jute matting or other fibrous material, on top of the very thing that is supposed to kill them.

5.5. Killing *C. taxifolia* with chemicals

The use of chemicals in order to eradicate incursions of marine pests has been trialed or used throughout the world, with varying success. One of the most spectacular successes was the use of chlorine and copper sulphate to completely eradicate the invasive mussel *Mytilopsis sallei* from three locked marinas in Darwin in northern Australia (McEnnulty *et al*. 2001). For macroalgae, toxic compounds that have been trailed as an eradication or control technique have included copper or other metal compounds, acetic acid, chlorine, commercial herbicides, antifoulants, lime and hydrogen peroxide. A major problem with chemical treatments in the marine environment is that any toxic compound is readily diluted and concentration is rapidly decreased, increasing the contact time required for complete mortality. Hence, many attempts to kill invasive macroalgae with chemicals have not resulted in eradication (McEnnulty *et al*. 2001). In most of these cases, initial aquarium tests showed promise, but field application was often difficult or unsuccessful (e.g., Boudouresque *et al*. 1996).

The method chosen to eradicate *C. taxifolia* in southern California initially involved placing plastic tarpaulins over infested areas (to prevent rapid dilution of the chemical toxicant) and injecting liquid chlorine (in the form of sodium hypochlorite) under them (Withgott 2002; Williams and Grosholz 2002). Later, slow-release chlorine blocks were used instead of liquid chlorine in an attempt to cut costs (Williams & Schroeder 2004). The chlorine apparently killed all living things under the tarpaulins, including the *C. taxifolia.* Estimates suggests that up to 99% of the original biomass of alga was effectively removed using this technique over a three year period (Withgott 2002), but there is still uncertainty about the extent of successful control and the (Williams & Schroeder 2004).

Copper ions have also been applied to C. *taxifolia* using a range of different application methods. The effect of copper depends on concentration and application time. Several techniques have been tested for applying copper ions to control/eradicate *C. taxifolia* in the Mediterranean (reviewed in McEnnulty *et al*. 2001). Laboratory experiments have shown a copper concentration of > 10 ppm applied for 30 minutes caused complete mortality. Concentrations of copper ions required to obtain 100% mortality were up to 10,000 times lower than those of potassium and sodium ions (Uchimura *et al*. 2000). More recent laboratory trials have focused on the use of aluminium ions (Thake *et al*. 2003). One ongoing use of copper ions to control *C. taxifolia* has been reported from the French Mediterranean coast where mats soaked in copper sulphate (called ion-exchange textile covers) are placed in beds of the alga (Uchimura *et al*. 2000). Copper ions apparently leach out of the mats and effectively kill large amounts of *C. taxifolia* each year (J. Ceccherelli, pers. comm.).

A preliminary experiment was undertaken using ‘Coptrol’, a chelated copper compound registered for use in potable water. Coptrol is commonly used in Australia as an algacide in freshwater. The laboratory experiment consisted of two control aquaria where only seawater was added, two aquaria with Coptrol added at the recommended dose (a 10% solution) and two aquaria with Coptrol added at twice recommended dose (a 20% solution). Ten fragments of *C. taxifolia* were added to each aquarium. After several weeks, *C. taxifolia* in the control treatments showed no sign of bleaching, whereas the other four treatments had mortality rates of 90-95%. As Coptrol was not 100% effective, even at a very high dose rate, it was not considered worthy of further research. Similarly, preliminary laboratory trials with lime produced inconclusive results and use of this compound was not pursued.

Although few published studies on the effect of herbicides on *C. taxifolia* are available, it has been reported that the alga is highly resistant to a number of herbicides and is therefore difficult to kill (Anderson 2002). Tests have been conducted in Tasmania and New Zealand on another invasive alga, *Undaria Pinnatifida*, using commercial herbicides and lime applied in various ways, including injection into the stipe or midrib, applying a gel formulation, attaching a sponge saturated with active substance to the thallus, and applying compounds inside a bag enclosing the thallus. All these methods were ineffective in killing *U. pinnatifida* and were labour-intensive and expensive (Sanderson 1996).

A major difficulty with chemical controls is containing the chemical in the desired area, both to reduce impacts on non-target species and areas, and to optimise the concentration on or around the target pest species. The high concentrations often needed and the dispersal of toxicants away from the target usually means that most, if not all, other marine organisms in the area are killed as well. Another problem is that chemicals, especially metal ions such as copper, can persist for a long time and may continue to have residual toxic effects long after the target invader has been eliminated. For these and other logistical reasons, no field trials using toxic chemicals were undertaken in NSW.

Table 5.1. Measures to remove or kill *C. taxifolia* (other than use of salt) undertaken by NSW Fisheries in 2001-2003 at a variety of sites in NSW.

Method	Location and scale of trials	Positives	Difficulties encountered
Smothering with used conveyor belting	Pebbly Beach in Lake Macquarie; two replicate plots of $30m^2$. Mannering Park, Lake Macquarie; experimental plots of $1m^2$	Kills covered *C. taxifolia* within 1 mo. Inexpensive to buy, but expensive to deploy	Expensive Deployment & retrieval is labour intensive Requires flat substratum Collateral damage high (all other organisms covered are killed) Non-biodegradable
Smothering with sand filled geo-textile bags	Mannering Park, Lake Macquarie; experimental plots of $1m^2$	Kills covered *C. taxifolia* within 1 mo. Biodegradable. Inexpensive.	Deployment labour intensive Collateral damage high (all other organisms covered killed) *C. taxifolia* may encroach from sides if not adequately covered, colonising the bag.
Smothering with jute matting	Mannering Park, Lake Macquarie; experimental plots of $1m^2$ Pebbly Beach in Lake Macquarie; 1 roll covering approx. $15m^2$ In Careel Bay	Kills covered *C. taxifolia* within 1 mo. Biodegradable Inexpensive Relatively easy to deploy in small quantities	Collateral damage high (all other organisms covered killed) Requires pegging down or weighting down, especially for large areas *C. taxifolia* may encroach from sides if not adequately covered, colonising the matting.
Dredging using small diver operated air lift device	Continuous bed of $10m^2$ in Lake Conjola 760 m^2 in Narrawallee Scattered plants in $15m^2$ area at Careel Bay	Moderate expense Effective over sandy substrata Suitable for small discrete patches	Easy to miss some plants Requires repeat treatments to ensure all *C. taxifolia* is removed. May fragment/distribute *C. taxifolia*. Non selective (vacuum will take in other organisms and sediment) Very time consuming
Hand picking	Crangon Bay in Lake Maquarie; one small patch completely removed Area 1 in Burrill Lake Narrawallee Inlet	Inexpensive Ideal for small individual plants.	Easy to miss some plants May require repeat treatments May fragment and further distribute *C. taxifolia.* Very time consuming Requires good visibility.

5.6. Killing *C. taxifolia* by osmotic shock

The addition of salt (a treatment believed to have been trialed first in France) or freshwater has been advocated as a way of controlling *C. taxifolia* because it relatively cheap and is considered to have minimal environmental effects. Each *C. taxifolia* plant is a simple single-celled organism with very limited osmoregulatory capacity. Observations during laboratory trials at the Port Stephens Fisheries Centre in 2001 found that osmotic stress from significant changes in salinity (either raised or lowered), even over the short-term, would result in the rupture or collapse of the cell wall and death of the plant. If the salinity of the immediate environment of *C. taxifolia* is lowered sufficiently, for example by flooding with freshwater, plants are rapidly killed. This response to freshwater led to the successful removal of *C. taxifolia* from West Lakes, one of two infested waterways in South Australia (Gilliland, pers. comm.). Preliminary observations in the laboratory also suggested that a short-term increase in salinity (achieved by adding crystals of pool salt) killed *C. taxifolia* in a matter of hours but had limited impacts on other marine biota kept in the same aquaria. Further comprehensive field trials were then done using salt, and these are described below. Due to the urgent need for a method of controlling *C. taxifolia* in NSW, the studies were limited in spatial and temporal replication, but nevertheless provided important information.

5.6.1. Qualitative trials

A series of preliminary trials and observations carried out in summer 2001/02 indicated that salt could effectively kill *C. taxifolia* in infested estuaries in NSW. The results from these trials in Lake Macquarie and Careel Bay showed that a salt concentration of between 100 kg/m^2 and 150 kg/m^2 eliminated 100% of *C. taxifolia* from an area of seabed. The method of deployment of salt in all these trials was from 25 kg bags opened underwater by SCUBA divers.

Although no quantitative measurements were made during these trials, repeated observations in plots treated with salt revealed no obvious short-term impacts on seagrass, *Zostera capricorni*, which often co-occurs with *C. taxifolia*. Use of salt as a control agent at the scale of hundreds of square metres was first trialed in Careel Bay (December 2001 and February 2002), an area with very little seagrass and very sparse animal life. This successfully killed large areas of *C. taxifolia*. Before using large amounts of salt in other estuaries, however, it was considered necessary to investigate how salt might affect co-occurring seagrass, surface-dwelling macroinvertebrates and benthic infauna.

Plate 8. Trials in NSW to evaluate the feasibility of killing *C. taxifolia* by spreading salt on it. Top left: navy divers assist in Careel Bay; top right: experimental 1m^2 plot covered with salt; bottom left and right: deploying large quantities of salt in Lake Macquarie.

5.6.2. *Quantitative experiments on effectiveness of salt and its colateral impact*

An initial experiment to measure the impact of salt on benthic organisms was conducted in Lake Macquarie in March 2002. The salt concentration used on this occasion was approximately 250 kg/m^2. This concentration was much higher than previously used, and was a consequence of the method of deployment – dumped from the surface in one tonne bags rather than being evenly spread underwater from 25 kg bags. Because it was virtually impossible to control the spread of salt once the large bags were opened, it was deposited very thickly on all of the experimental plots (four 4 x 4 m areas at each of 2 sites). The results of this experiment indicated that the salt on the treatment plots effectively eliminated all plant life on the substratum. Large bivalves such as cockles, razor clams and mussels also perished, and preliminary results from sorting of benthic samples showed that the abundance of benthic infauna (primarily worms) was greatly reduced after the addition of salt at 250 kg/m^2. A second experiment was designed, therefore, to thoroughly address the issues surrounding salt concentrations and their effects on *C. taxifolia*, *Z. capricorni*, macro-invertebrates and benthic infauna and to evaluate whether the large scale use of salt was a viable option for controlling *C. taxifolia* in NSW.

5.6.2.1. Methods

Experiments were set up at two sites, approximately 1 km apart, in Lake Macquarie in March 2002. Coarse sea salt (99.5% NaCl; mean particle size 2.7mm; Cheetham Salt Ltd) was used for the experiment. In each site, twelve plots (1m x 1m) were marked out in areas that contained *C. taxifolia* and the seagrass *Zostera capricorni* at depths of 1–3 m. Two replicate plots were established for salt concentrations of 50 kg/m^2, 100 kg/m^2, 150 kg/m^2 and 200 kg/m^2. There were also 2 control plots positioned close to the salt treatments and 2 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 x 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 plot, the numbers of shoots of *Z. capricorni* and of fronds of *C. taxifolia* were counted in 3 smaller quadrats (30 x 30cm) which were placed haphazardly. Benthic infauna was sampled using 3 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 3 factor ANOVAs and 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 all times.

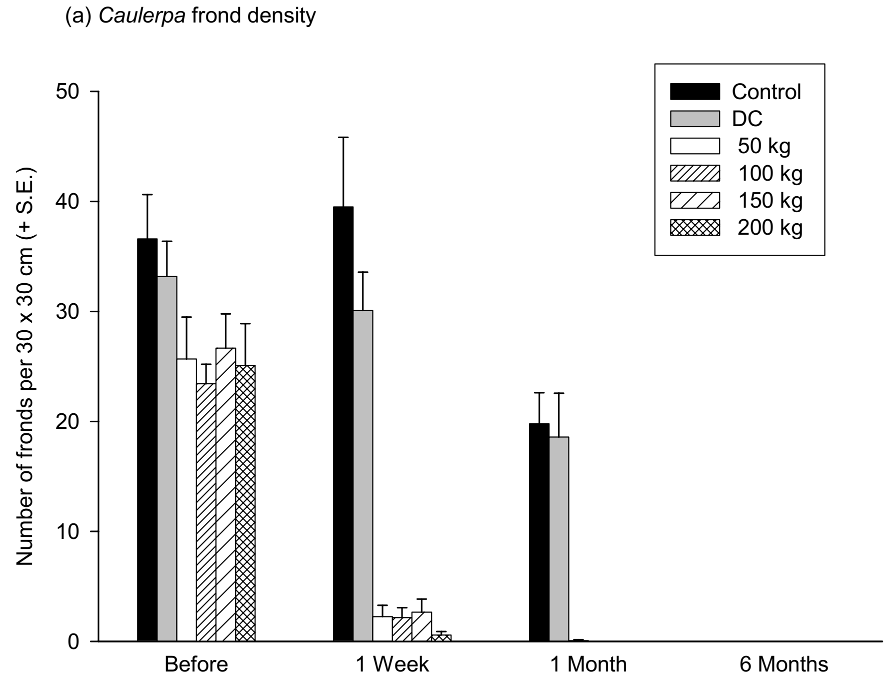
5.6.2.2. Results

The salt generally dissolved within 2-3 hours of application and after 1 day, *C. taxifolia* became limp and 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 smaller than in controls ($F = 20.6$, $p < 0.001$; Figure 5.1a). These differences were consistent between sites. After 1 month, *C. taxifolia* fronds had essentially disappeared from all salted plots (Figure 5.1a), and no fronds had reappeared after 6 months. in these plots. Frond density decreased in the control plots over the course of the experiment (Figure 5.1a). This result is consistent with the earlier trials (and with studies done in the Mediterranean; Meinesz *et al*. 1995) which have shown that the density of *C. taxifolia* can decrease greatly over the winter period (May–September), particularly in shallow water (see Chapter 2). Thus, it was clear that a concentration of 50 kg/m^2 of salt could remove all *C. taxifolia* fronds for a period of at least 6 months if applied during autumn. Subsequent control programs have demonstrated that this concentration of salt is also effective when applied during the warmer months, but possibly not as effective as in autumn/winter.

The density of the seagrass *Zostera 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. 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 ($F = 2.6$, $p > 0.05$; Figure 5.1b). 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 (Figure 5.1b). By 6 months, differences still occurred among treatments ($F = 4.5$, $p < 0.05$), but seagrass density had increased in the 50 kg/m^2 salt plots and had returned to the pre-salting density (Figure 5.1b). It is not known whether salt may have had any other effects on seagrass (e.g., detrimental impacts on productivity). Seagrass that had been treated with salt at concentrations greater than 50 kg/m^2 did not recover as well. There was no evidence that salt applied to a 1 x 1 m area had any effects on seagrass nearby because densities of seagrass in the "close controls" were never significantly less than those in the "distant controls" (Figure 5.1b). 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. 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 (Figure 5.2 a,b). There was, however, some indication that the disturbance caused by the application of salt at higher concentrations resulted in increased numbers of some infauna. Taxa which increased greatly in abundance from the 1 month sampling period to the 6 month sample in the 150 kg/m^2 and 200 kg/m^2 salt treatments were tanaids, nematodes, capitellid polychaetes and gammarid amphipods.

In summary, this experiment provided good evidence that salt applied at a concentration of 50 kg/m^2 during autumn removes *C. taxifolia* for at least 6 months, whilst having limited short-term effects on *Z. capricorni* and infauna. *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*.



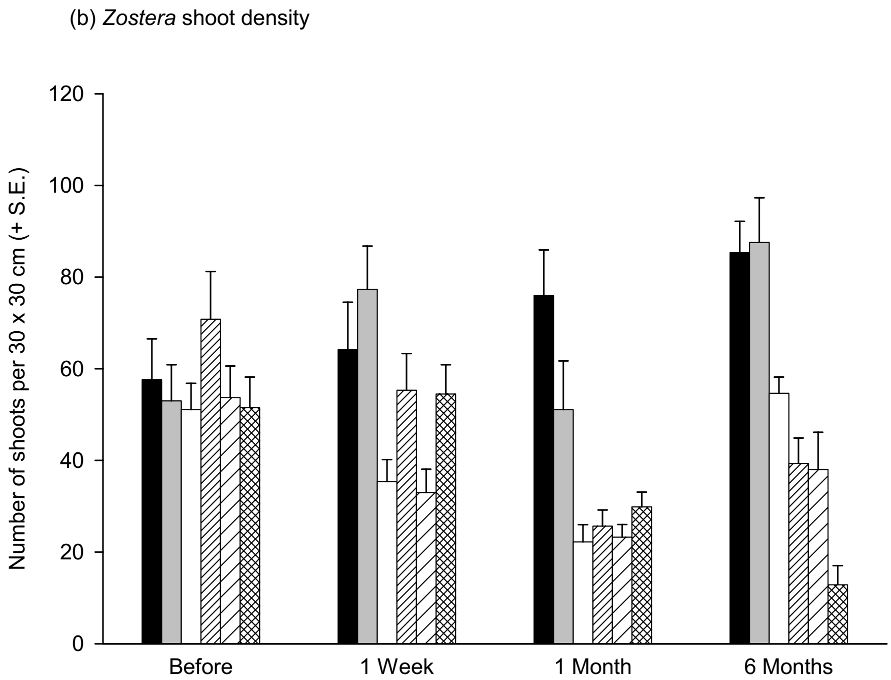
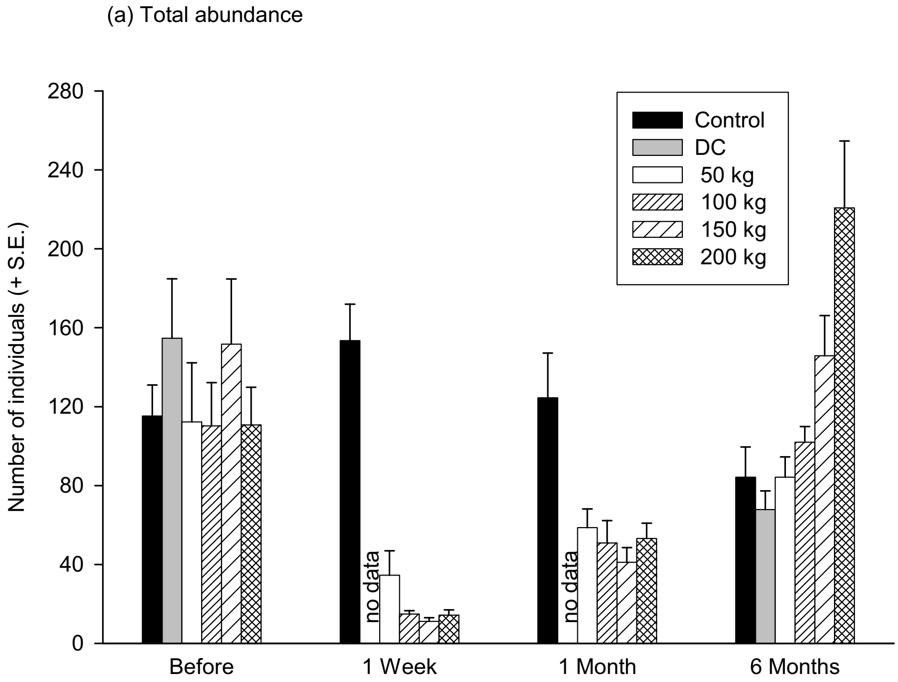


Figure 5.1. Mean number of fronds of *C. taxifolia* (a) and Zostera capricorni (b) in plots that were either not salted (close control (Control) & distant control (DC)), or salted at concentrations of 50, 100, 150 or 200 kg/m2. Data for each treatment are averaged across replicates, plots and sites. The experiment was initiated in March 2002 in Lake Macquarie.



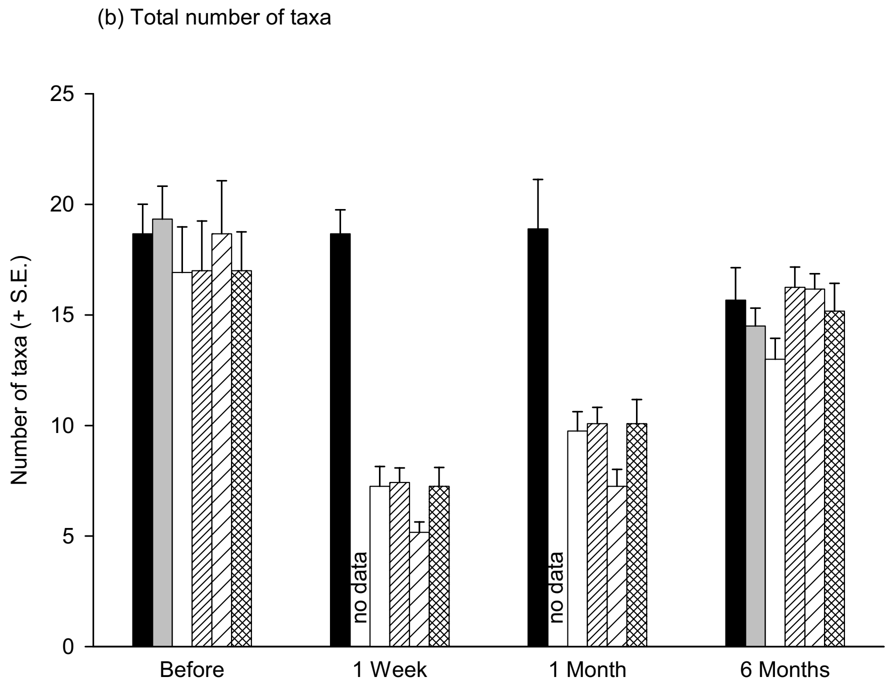


Figure 5.2. Mean number of soft sediment invertebrates (a) and infaunal taxa (b) in plots that were either not salted (close control & distant control), or salted at concentrations of 50, 100, 150 or 200 kg/m2. Data for each treatment are averaged across replicates, plots and sites. The experiment was initiated in March 2002 in Lake Macquarie. No data were collected for the distant controls after 1 week and 1 month.

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

Once it had been established that 50 kg/m^2 of salt would be effective for controlling *C. taxifolia*, an effective and efficient method of application needed to be developed. Small patches of the alga could be treated by SCUBA divers using 25 kg bags of salt which were spread by hand (Plate 8). It was difficult to apply a precise concentration of salt, but divers were trained to apply salt at a thickness of about 4cm, thereby approximating a salt concentration of 50 kg/m^2. Large areas of *C. taxifolia* need to be salted quickly in order for this control method to be cost effective, so a hopper was designed to deliver salt from a 7m long, flat-bottomed boat. The hopper holds 1 tonne of salt and another 1 tonne bag sits in the middle of the boat as ballast. 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 (Plate 8). The exact amount of salt dumped on each patch varies according to the speed of the boat and the depth of the water, but in calm weather conditions in water < 5m, it is possible to get a good coverage of salt over the alga at ~50 kg/m^2. This method of application is not as effective in water deeper than 5m because the salt disperses too much before reaching the substratum. It may be possible, however, to treat deeper areas using this system by adding a chute to deliver the salt directly to beds of *C. taxifolia*. Another effective method (although relatively expensive) involved a large commercial barge lowering 1 tonne bags of salt into the water (Plate 8) where commercially-trained divers opened the bags underwater and spreading the salt by hand. This technique was only ever used in Lake Macquarie where a suitable vessel was available to do the work..

During the summer of 2002-03, nearly 1100 tonnes of salt were deployed, covering areas of *C. taxifolia* in excess of 4 hectares (Table 5.2). Largest applications were in Lake Macquarie where 680 tonnes were spread over a total area of 2-3 hectares accounting for all infestations known at that time. The delivery of salt here was mostly done using the specially constructed punt with the hopper or with the commercial barge. Follow up surveys, and mapping in winter 2003 and summer 2004, confirmed that *C. taxifolia* had been severely reduced from most of the treated areas in Lake Macquarie (Chapter 2, Figure 5.3). It seems that natural phenomena may have assisted with the removal of *C. taxifolia* from Lake Macquarie although these have not been identified.

Complete salt treatment of all known *C. taxifolia* infestations was also done in Narrawallee in early summer (November to December 2002), and in Careel Bay in late summer (February to March 2003) (Table 5.2). The infestation in Narrawallee was greatly reduced in the month immediately following treatment with 34 tonnes of salt applied underwater by divers from 25 kg bags. New infestations in the form of scattered patches, however, began to reappear in late summer/early autumn 2003. These re-emerging patches were treated again with salt as part of some additional experimental trials (see Section 5.6.3), and very little remained when remapped in winter 2003 (Chapter 2, Figure 5.3). The alga reappeared again in the summer of 2003/2004 (Appendix 1; Figure 5.3) indicating that a single salt treatment here had not been effective in long-term control.

Repeated salting of an infestation that covered approximately 2500 m^2 in Careel Bay in Pittwater led to a considerable reduction in the density of the alga, but no overall change in the boundaries of the infestation (Figure 5.3). The 183 tonnes of salt applied here in the summer of 2003, all via the special punt, was laid on top of and around the edges of the jute matting that had been put down the previous summer (see section 5.4).

Table 5.2. Summary of salt distributed in each estuary (tonnes) to control *C. taxifolia*, December 2001 to March 2003. Complete treatment was only accomplished in Lake Macquarie, Narrawallee Inlet and Careel Bay (see text for details).

Date	Botany Bay	Burrill Lake	Lake Conjola	Lake Macquarie	Narrawallee Inlet	Careel Bay	Port Hacking	Sydney Harbour	Total
Dec 01						9.6			9.6
Feb 02						67.2			67.2
Mar 02				24					24
Apr 02								0.625	0.625
May 02				2.4					2.4
Jun 02									0
Jul 02				1.2					1.2
Aug 02									0
Sep 02		1.2	1.2	140			5		147.4
Oct 02				12		2.4			14.4
Nov 02				80	18	6			104.0
Dec 02			36	100	12				148
Jan 03				72	4				76
Feb 03	3.6	48				147	3.6	12.4	214.6
Mar 03				252		36			288
Total	3.6	49.2	37.2	683.6	34.0	268.2	8.6	13.025	1,097.425

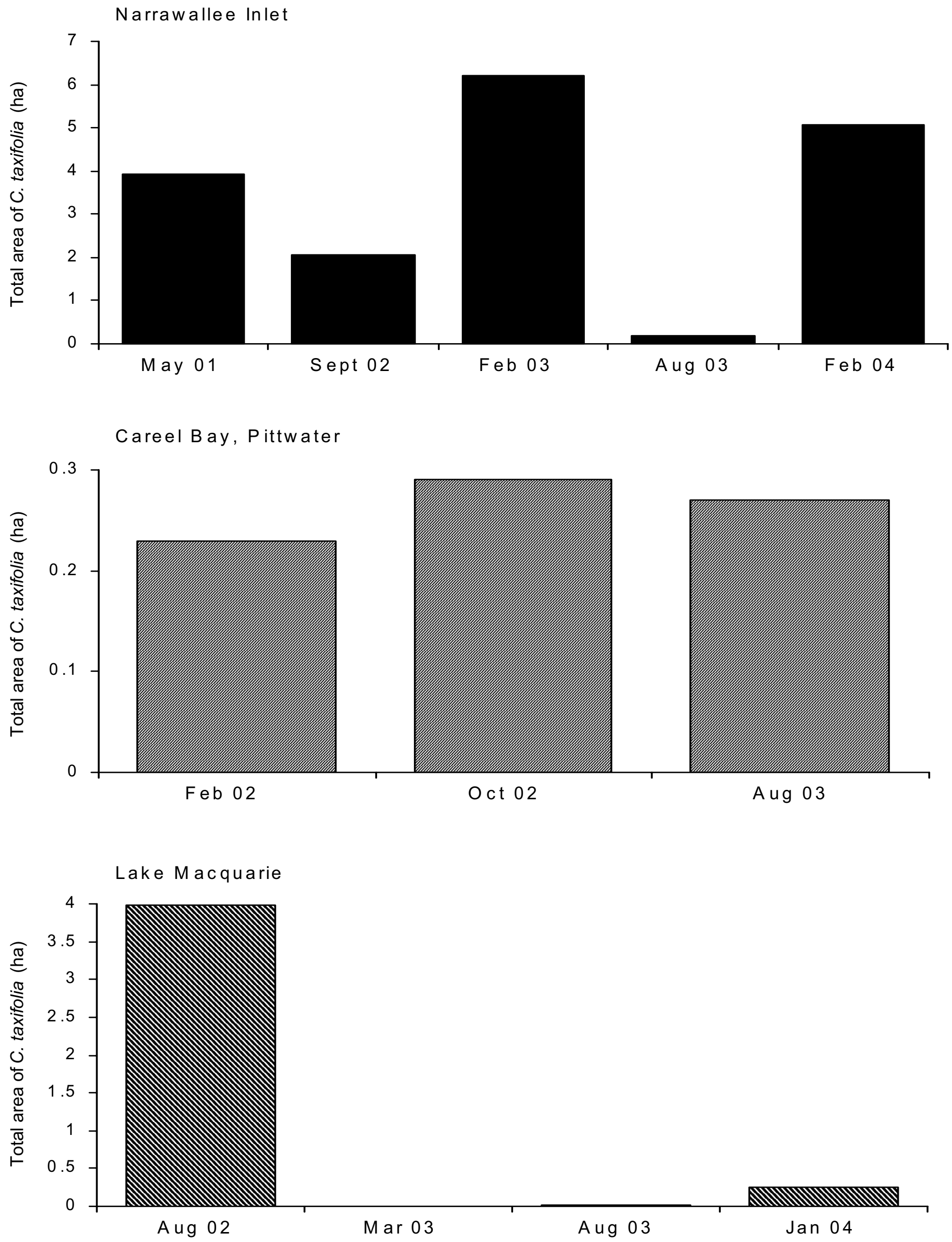


Figure 5.3. Estimated coverage (hectares) of *C. taxifolia* before and after complete treatment of salt in the summer of 2002-2003 at three NSW localities. Major salt treatment was done in Nov.–Dec. 2002 in Narrawallee Inlet (top), Feb.-Mar. 2003 in Careel Bay (centre) and Sept. 2002 to Mar. 2003 in Lake Macquarie (bottom). Subsequent bars represent remapping of the areas at various time intervals after salting.

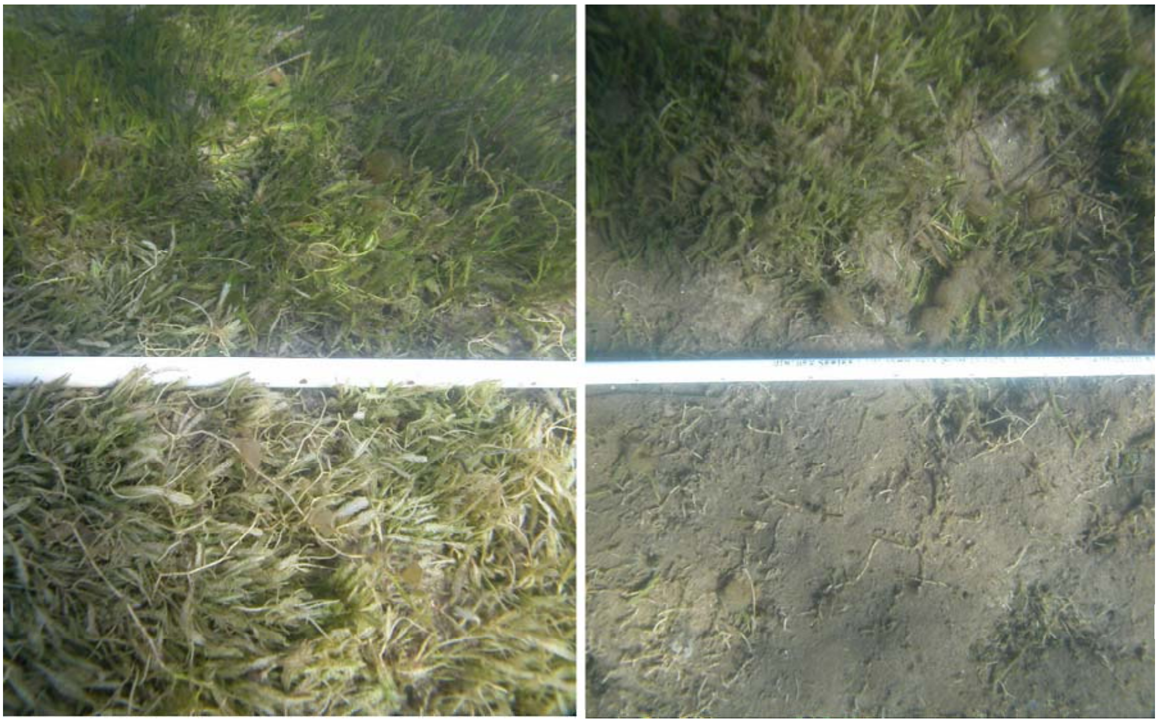


Plate 9. Effectiveness of salt treatments applied to beds of *C. taxifolia* at a concentration of 50kg per m^2. Short-term (3 days) on left, longer-term (3 weeks) on right. Control at top and treated area at bottom.

5.6.4. Fine tuning the use of salt as a control technique

In some waterways, such as Narrawallee Inlet (Figure 1.1), *C. taxifolia* tends to grow in small isolated patches, rather than in large dense beds. In this waterway, it is not possible to use the boat-mounted hopper because large quantities of salt cannot be delivered to the shores of the estuary. Instead, salt application was done by hand (from 25 kg bags) to individual patches of the alga at this site (Table 3.2). A trial during the summer of 2002/2003 appeared to have been successful, with extensive die-off of *C. taxifolia* after a few days. Six weeks after the application of salt, however, there was little sign that the salting had 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 rather than entire sections of substratum being salted as would occur when using the purpose-built hopper. 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, but mimicking the way salt is applied by the hopper). The experiment was done in 4 sites separated by ~ 100 m and located within ~ 500 m of the mouth of the inlet. Each site was at a depth of 1–2 m and contained *C. taxifolia*, seagrass (*Zostera capricorni*, *Halophila ovalis*) and areas of unvegetated sediment. At each site, six plots (2 x 2 m) 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 (Figure 5.4).

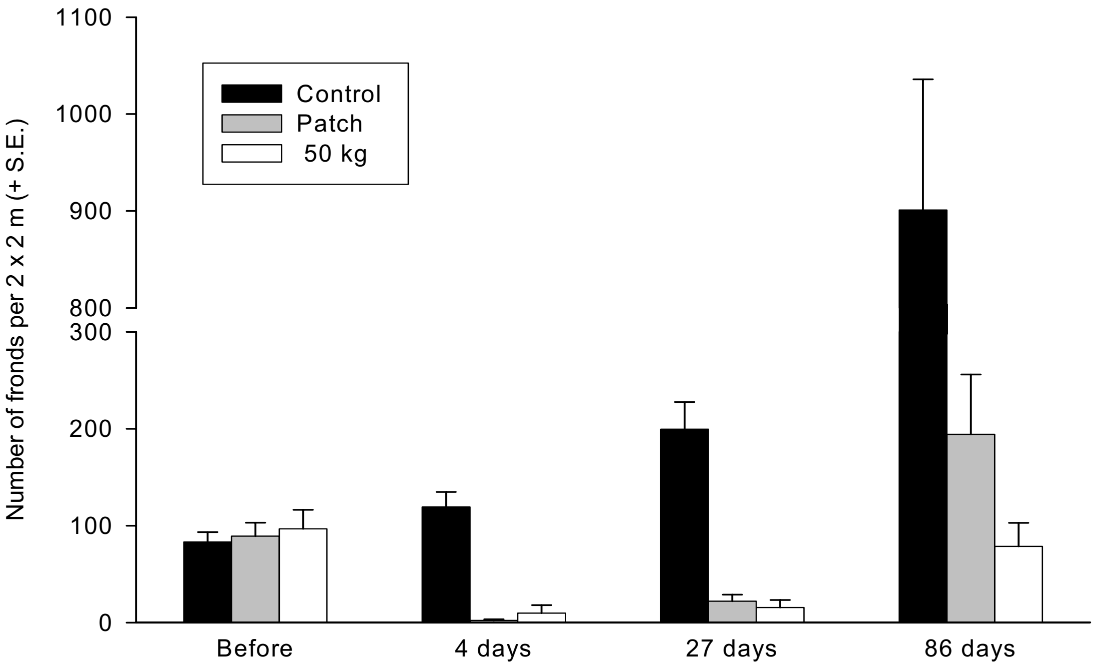


Figure 5.4. Mean number of fronds (+ S.E.) of *C. taxifolia* in plots that were either not salted (controls), 'patch' salted, or covered with 50kg/m2 of salt. Note that the y-axis has been split. The experiment was started in February 2003 in Narrawallee Inlet.

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 (Figure 5.4). 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% (Figure 5.4). Thus, in the longer term, salting individual patches of *C. taxifolia* was not as effective as covering the entire plot with salt, suggesting that stolons under the sediment had escaped the 'patch' salting treatment. Moreover, the 50 kg/m^2 salt treatment in this experiment did not appear to be as effective as that in the previous experiment conducted over winter in Lake Macquarie which resulted in the complete removal of *C. taxifolia* after just 1 month (Figure 5.3).

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 (Figure 5.4). 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 2003, the water temperature had dropped to around 17°C and there had been 368 mm of rain in the month 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. All control plots were sampled destructively, therefore, 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, but a search of the remainder of the waterway revealed a few scattered patches of *C. taxifolia* in the deepest section of the waterway (5–6 m) (see Figure 5.3a). Their fronds 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).

5.7. Discussion

Trials of methods to control the spread of *C. taxifolia* using salt have generally been successful at small scales. For example, infestations of the order of a few square metres were effectively eliminated using salt soon after they were first discovered at Clontarf in Sydney Harbour. Limited resources have meant that larger outbreaks cannot be treated using this technique. Also, the success of large-scale salting treatments have been mixed. Salt was effectively used to eliminate substantial amounts of *C. taxifolia*, to the point of near elimination, from only one major locality (Lake Macquarie). Even these “large scale trials” were restricted to infestations covering no more than a couple of hectares. The salting protocols developed during this project rely heavily 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 at a site so that large quantities of salt can be efficiently delivered, loaded and then spread on the *C. taxifolia* beds. There are severe infrastructure limitations in places like Lake Conjola and Burrill Lake and it is not possible to bring in large barges to carry salt from the few access points to the sites where it is needed. Salting trials at these locations (Table 5.2) have been restricted to key priority areas such as boat ramps and popular fishing spots. Although the treated *C. taxifolia* was killed at these sites too, a comprehensive control program for the large estuaries is currently not logistically feasible and would be prohibitively expensive.

It appears that salting is most effective in winter, soon after the fastest growing period of *C. taxifolia*. At this time, effects on seagrass are likely to be minimised because species such as *Z. capricorni* tend to die-back naturally. *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 ~ 50 kg/m^2 are effective in significantly reducing the density of *C. taxifolia*, but some stolons generally 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.

To be most successful, salting needs to be done in a blanket fashion, rather than by application to individual patches of *C. taxifolia*, because stolons (and perhaps other parts of the alga) may be hidden under the sediment and so not treated when salt is applied by hand in this way. Delivering salt from the surface of the water is most effective for relatively shallow infestations (< 5m), but divers can apply salt to deeper areas. 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 in future control work (see NSW *Caulerpa* Control Plan; http://www.fisheries.nsw.gov.au/thr/species/fn-caulerpa.htm).

As yet, *C. taxifolia* has not been discovered on the open coast of NSW 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 NSW waterways is unlikely, it is hoped that the control procedures developed during this project will prevent further spread of the alga.