Eradicating and preventing the spread of the invasive alga *Caulerpa taxifolia* in NSW

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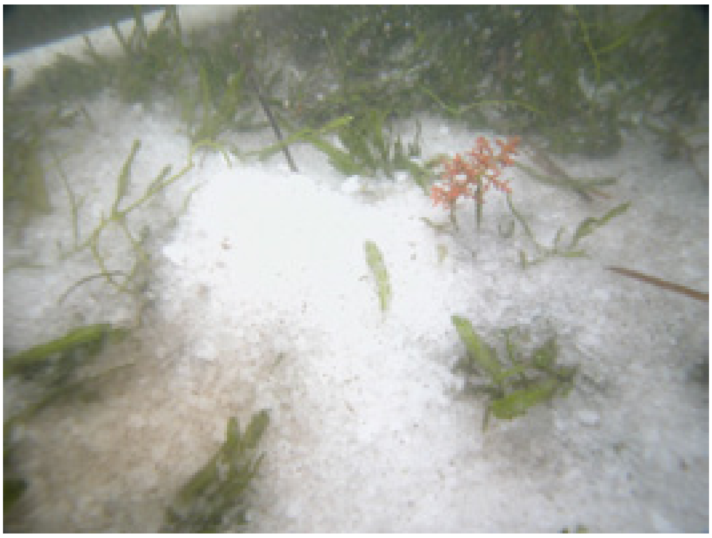

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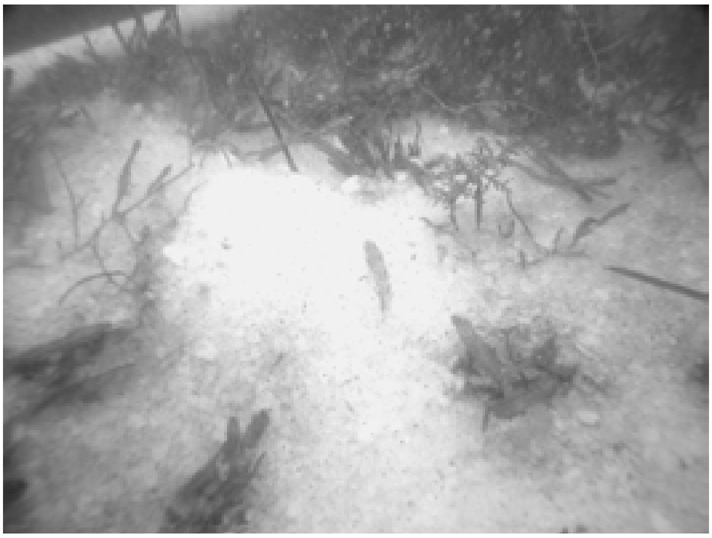

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The successful completion of this project is due, in no small part, to the unstinting efforts of the aquatic pest managers at NSW Fisheries. Bill Talbot, ably supported at various stages by Michelle Belcher, Rebecca Chapman, Kylie Russell and Graham White, championed the need for coordinated, practical solutions to the potential risks posed by this invasive seaweed in NSW, and promoted the need for sound research to underpin management actions.

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EXECUTIVE SUMMARY

This joint project between NSW Fisheries and the University of Wollongong had 3 objectives:

1 To investigate patterns of dispersal, recruitment and growth of the invasive alga *Caulerpa taxifolia* and provide information on spread within NSW estuaries
2 To investigate the vectors that may transfer *C. taxifolia* to new locations
3 To develop environmentally benign ways of removing *C. taxifolia* which might eventually lead to its elimination from whole sites or regions

The research undertaken to address these objectives provided a good understanding of the population ecology of *C. taxifolia* in NSW estuaries, allowed the evaluation of several control techniques and underpinned the development of a ‘Control Plan for *Caulerpa taxifolia* in NSW’ based on a preliminary assessment of risks. The control plan can be found at http://www.fisheries.nsw.gov.au/thr/species/fn-caulerpa.htm

To date, *C. taxifolia* has been found in 9 separate locations. All are estuaries or sheltered embayments and the seaweed has not yet been found on exposed coasts. It occurs in water 0.5–10 metres deep. *C. taxifolia* is capable of growing extremely quickly; stolons can extend by up to 13 mm per day in optimal conditions. Vegetative growth is the primary means by which the alga has invaded these NSW waterways, covering over a total of 4-8 km^2 by mid 2004. *C. taxifolia* reproduces asexually through a process of fragmentation, dispersal and eventual anchoring of drifting fragments which are negatively buoyant and move across the seafloor in bottom currents. Large numbers of fragments were found within existing beds of *C. taxifolia*, and experiments showed that they could be trapped within seagrass beds or other structures on the seafloor. Once trapped, even small fragments can attach to the seafloor and grow into new plants. Infestations of *C. taxifolia* in NSW range from sparse distributions of scattered runners to dense beds 40 cm thick. Several other marine organisms may occur within beds of *C. taxifolia*, but most herbivorous species avoid eating it. Only two species of opisthobranch molluscs appear to readily feed on it.

A boat-mounted mapping system was developed to document the extent and spread of *C. taxifolia* in NSW waterways. A procedure whereby all known infestations are comprehensively mapped twice a year, in mid summer and in mid winter, has now been implemented. This mapping has accurately documented the continued spread of *C. taxifolia* in most of the estuaries where it occurred at the start of the project. Large-scale die-offs, however, occur in shallow water (0.5–2 m) in most waterways in NSW during winter and this was particularly evident after heavy rainfall. This die-back may be a consequence of decreased temperature, decreased salinity, increased turbidity or some combination of these.

There are several natural vectors that aid the fragmentation and translocation of *C. taxifolia*; storms, and the increased wave action associated with them, were found to be particularly important. These vectors become increasingly significant as the amount of *C. taxifolia* at a site expands, and they probably overshadow human-mediated vectors when infestations cover large areas such as in Lake Conjola and Botany Bay. Commercial activities on waterways infested by *C. taxifolia* such as commercial fishing, aquaculture, dredging or the building/maintenance of foreshore structures such as wharves, jetties or boat ramps can potentially cause increased fragmentation. Most such activities are now banned or strictly controlled at sites with *C. taxifolia.* Many human leisure activities may also generate, trap and transport fragments of *C. taxifolia*, including passive pursuits such as swimming, diving and more active pursuits such as boating, water skiing, anchoring or recreational fishing. Abundances of fragments were higher in areas of human use, and experiments showed that boat anchors, in particular, were readily able to remove significant amounts of the seaweed from beds of *C. taxifolia*. Additional experiments showed that fragments caught this way could survive for 1-2 days out of water in conditions that mimicked the anchor well on a small boat and might constitute a major risk for transferral to other waterways.

Removing *C. taxifolia* by either hand-picking or using underwater suction devices was found to be effective for very small patches at shallow sites with sandy bottoms and good underwater visibility. Many of the infested waterways in NSW, however, are muddy and often turbid, making detection of all plants difficult and increasing the risk of accidentally releasing fragments. A scoping exercise was done for using a commercial dredging vessel to remove large areas of the seaweed, but the logistics proved too difficult. Experiments with various types of smothering materials, particularly jute matting, were also reasonably effective at killing most *C. taxifolia* in small-scale trials. Their use for areas larger than a few hundred square metres, however, created more difficulties than they provided solutions.

The use of osmotic shock showed the most promise in preliminary trials. The addition of a layer of salt directly onto the plants killed them within hours. Trials using salt delivered from a specially designed punt were very successful at scales of several hundred square metres, but results of larger scale salting were mixed. For example, single applications of salt to numerous outbreaks at one location resulted in the apparent removal of almost 5200 m^2 of *C. taxifolia*, whereas repeated salting of a 3000 m^2 infestation at another site led to a considerable reduction in the density of *C. taxifolia*, but no overall change to the extent of the infestation. Salt rapidly dissolves in seawater and therefore has little residual impact on the marine environment. Although salt may kill other marine organisms directly covered by it, experiments showed that the seagrass, *Zostera marina*, and invertebrate infauna which often co-occur with *C. taxifolia*, recover after 6 months if salt is applied at 50 kg salt per square metre. The use of this salting technique has now been adopted as a major component of the NSW *Caulerpa* Control Plan for the targeted control of new outbreaks or high risk infestations.

Because there is now more *C. taxifolia* in NSW waterways than can be effectively treated with salt, eradication does not seem feasible at this time. It is hoped, however, that the control procedures outlined in this report and in the NSW *Caulerpa* Control Plan will prevent the spread of the alga to locations where it is not currently found. A better understanding of the biology and patch dynamics of *C. taxifolia* will also assist in minimizing its impact on native biodiversity and the sustainable use of marine resources in NSW estuaries.

1. INTRODUCTION & BACKGROUND

1.1. Invasive *Caulerpa taxifolia*

Caulerpa taxifolia (Vahl) C. Agardh is a marine, green macroalga (Plate 1) that is endemic to tropical and sub-tropical regions around the world. It is primarily a subtidal species that has running stolons and feather-like fronds and can grow on hard and soft substrata. Species of *Caulerpa* are coenocytic, meaning that each plant consists of one, multinucleate cell. In Australia, native populations of *C. taxifolia* are found in the Northern Territory, Queensland, Western Australia and on Lord Howe Island (Phillips and Price 2002 and references therein).


Courtesy Alan Millar, RBG

Courtesy Alan Millar, RBG

Plate 1. A comparison of native *C. taxifolia* from northern Queensland (lower) and invasive *C. taxifolia* from NSW (upper). Photographs courtesy of Alan Millar, Royal Botanic Gardens, Sydney.

The alga came to international attention 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 subtidal hard and soft substrata in the Mediterranean (Meinesz 2002). This invasive strain became known as the "aquarium strain", because it was presumed to have been introduced from marine aquaria in which it was (and still is) used as a decorative plant (Jousson *et al*. 1998, 2000; Fama *et al*. 2002). Molecular evidence supports this notion, demonstrating that the invasive strain in the Mediterranean is genetically identical to a strain of *C. taxifolia* widely cultivated in aquaria for at least 15 years prior to its appearance in Monaco (Jousson *et al*. 1998). From Monaco, *C. taxifolia* spread to coastal localities in France, Spain, Italy, Croatia and Tunisia (Meinesz *et al*. 2001). By the end of 2000, the alga covered approximately 131 km^2 of seafloor in the Mediterranean (Meinesz *et al*. 2001).

Infestations of *C. taxifolia* were also reported from California at about the same time as they were recorded in NSW (Jousson *et al*. 2000; and see below). More recently it has been recorded from West Lakes and the Port River in South Australia (Cheshire *et al*. 2003). It is unclear whether the same invasive strain has colonised all these locations, and there is debate about the origin of the invasive aquarium strain first found in the Mediterranean. Numerous studies have indicated that the aquarium strain in the Mediterranean is genetically similar to populations of *C. taxifolia* that 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, it is almost certain that the invasive strain of *C. taxifolia* that colonised the Mediterranean did not originate from southern NSW, as suggested by Meinesz *et al*. (2001), but rather from sub-tropical areas in Queensland (north-eastern Australia). The supposed cold-tolerance of the invasive *C. taxifolia* is also under question as native populations of the alga from Queensland can tolerate water around 10°C (Chisholm *et al*. 2000; Wright ms in review).

Vegetative fragmentation seems to be the primary mode of reproduction of *C. taxifolia* (Meinesz *et al*. 1993; Smith and Walters 1999), as it is for most other species of *Caulerpa* (Jacobs 1994). Sexual reproduction has been documented for native tropical *C. taxifolia* (Meusnier *et al*. 2002), but successful sexual reproduction has not been observed in invasive *C. taxifolia* in the Mediterranean (Zuljevic and Antolic 2000) or elsewhere (A. Millar pers. comm). *C. taxifolia* can grow extremely quickly and vegetative growth is the primary mode by which the alga has colonised large areas of seafloor in the Mediterranean and elsewhere. Species of *Caulerpa* are capable of regenerating from small pieces of stolon or frond (Jacobs 1994), so fragments are an effective means of dispersal (Belsher and Meinesz 1995; Ceccherelli and Cinelli 1999a).

C. taxifolia rapidly reaches high abundance in places it invades (Meinesz *et al*. 1995, Ceccherelli and Cinelli 1998; Williams and Grosholsz 2002). It has been listed as one of the world's top 100 worst invasive species because it can potentially invade seagrass beds (Ceccherelli and Cinelli 1999), modify organic and inorganic components of the sediment (Chisholm and Moulin 2003) and threaten biodiversity (Meinesz 2002). Experiments in the Mediterranean have shown that fragments of *C. taxifolia* can establish on the edges of beds of seagrass during the warmer months of the year (Ceccherelli and Cinelli 1999a). It is not yet clear how *C. taxifolia* may interact with seagrass, but it has been suggested that dense patches of seagrass might be resistant to invasion by *C. taxifolia*, whereas sparse seagrass might be susceptible to invasion (Villèle and Verlaque 1995; Ceccherelli and Cinelli 1999b; Jaubert *et al*. 1999).

1.2. *Caulerpa taxifolia* infestations in NSW

The first confirmed sighting of *C. taxifolia* in NSW was in Fisherman's Bay, Port Hacking, on the southern outskirts of Sydney (Figure 1.1), in April 2000. *C. taxifolia* was found growing in beds of the seagrass *Posidonia australis* in this bay and outbreaks were discovered subsequently in several other bays in the estuary. Also in April 2000, *C. taxifolia* was found 180 km south of Sydney in Lake Conjola (Figure 1.1). There is, however, anecdotal information that the alga may have invaded Lake Conjola between 1987 and 1995 and that the Port Hacking invasions may have occurred in 1998 or earlier (Grey 2001).

Subsequent to the initial introductions, infestations were confirmed in 6 further estuaries in NSW: Careel Bay in Pittwater, 25 km north of Sydney in December 2000, followed by Lake Macquarie (90 km north of Sydney) in February 2001, Burrill Lake (200 km south of Sydney) in March 2001, Narrawallee Inlet (230 km south of Sydney) and Botany Bay (20 km south of Sydney) in April

2001 and the northern part of Sydney Harbour in April 2002 (see Figure 1.1). Again, the dates that *C. taxifolia* was first recorded in these estuaries may be many years after it actually arrived in the waterway. As this report was being prepared, a ninth location, St Georges Basin (see Figure 1.1) was also confirmed as containing significant infestations of *C. taxifolia.*

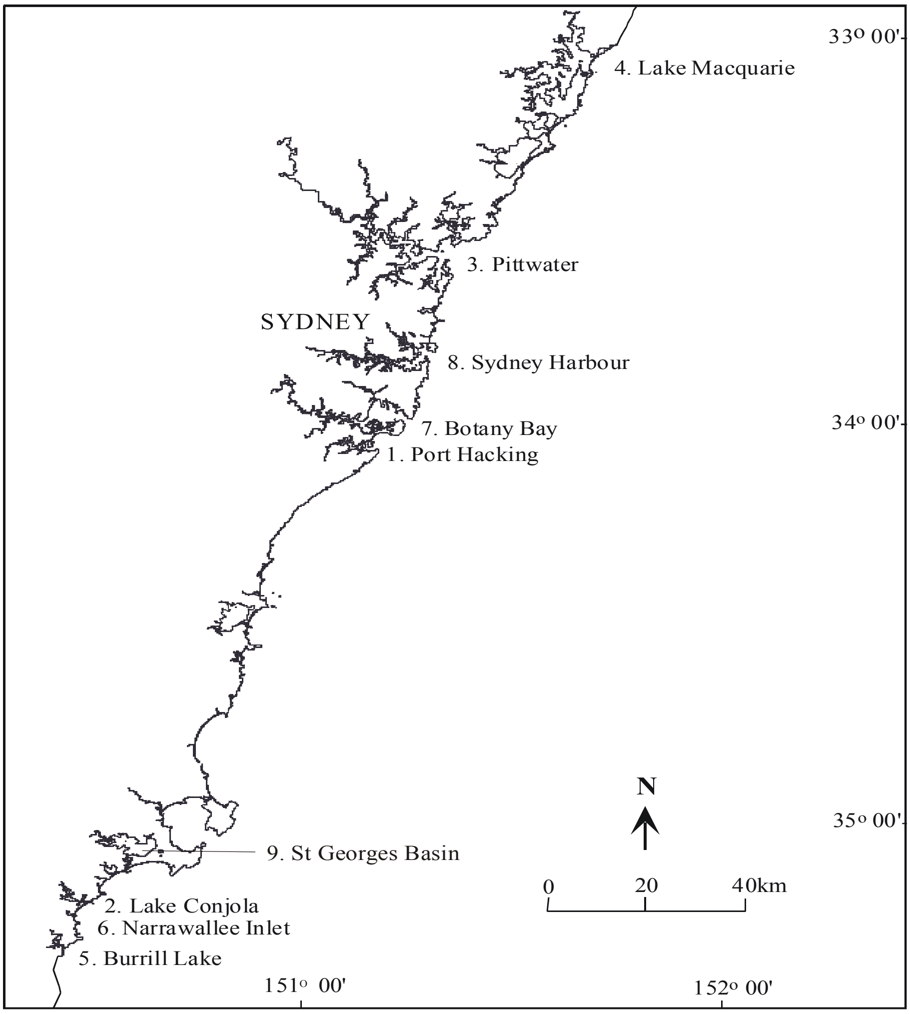


Figure 1.1. Sites where invasive *C. taxifolia* occurs in NSW (as at May 2004). Sites are numbered according to the order in which infestations were confirmed.

All outbreaks of the alga in NSW have been in bays and estuaries for which no previous record of the species exists. There is still uncertainty about the source of outbreaks of *C. taxifolia* in NSW (Phillips and Price 2002), but genetic evidence suggests that the various populations of *C. taxifolia* may have come from multiple sources, including native Queensland populations in Moreton Bay (Schaffelke *et al*. 2002; Murphy and Schaffelke 2003). Not only are there subtle genetic variations, but there is also considerable variability in the morphology of *C. taxifolia* from different waterways in NSW (Wright ms in review). It is highly unlikely that outbreaks in NSW represent a natural, southward range extension along the eastern Australian coastline (Millar 2002). This is because the 600 km break between the most northerly NSW population in Lake Macquarie and the southernmost populations in Queensland (Figure 1.1) is considered too great a distance for the natural transport of viable fragments.

The arrival of *C. taxifolia* in NSW has generally been attributed, as it was in the Mediterranean, to release from aquaria (Grey 2001). For example, at one heavily infested site in Port Hacking (Gunnamatta Bay) there was an aquarium shop that had stocked and sold *C. taxifolia*. Such an introduction mechanism could readily account for the subtle genetic differences described by Schaffelke *et al*. 2002 if multiple releases had occurred from different aquarium stocks. However, it seems unlikely that the infestations in all 8 NSW estuaries were the result of separate releases from aquaria. Rather, there has probably been some translocation among NSW estuaries by other human vectors such as boating and fishing activities, as has also apparently been the case in the Mediterranean Sea (Relini *et al*. 2000). All waterways in NSW where *C. taxifolia* is now found are either frequently used anchorage sites or popular fishing spots, as has been reported for *C. taxifolia* infestations in other countries (e.g. Boudouresque *et al*. 1995). Several sites in Lake Macquarie, Lake Conjola and Narrawallee Inlet where *C. taxifolia* is found, for example, were commercial hauling grounds prior to the lakes being closed to commercial fish netting from May 2002. The alga is also found in areas previously used as hauling and trawling grounds in Botany Bay and Port Jackson. There is, however, no correlation between commercial fishing and the introduction of *C. taxifolia* in Port Hacking as this waterway has been closed to commercial fishing for many decades although it is a very popular recreational boating area. It is therefore likely that a combination of vectors has contributed to the arrival and spread of *C. taxifolia* in NSW.

As soon as *C. taxifolia* was discovered in NSW, controls were put in place by NSW Fisheries to limit the risk of translocation to other locations. Measures included the deployment of marker buoys to restrict access by boats and minimise anchoring in beds of *C. taxifolia*, fishing closures to prevent hauling or mesh netting, and restrictions on the aquarium industry necessitating permits to import or sell the species or to keep it in anything other than a fully-contained, private aquarium. An emergency declaration of *C. taxifolia* as noxious marine vegetation was followed in October 2001 by the alga being officially declared a noxious species under the NSW Fisheries Management Act 1994. A public education campaign also was initiated, involving public meetings, information brochures, website information, the erection of warning signs in popular boating areas, and a reporting hotline.

1.3. This report

There are four interrelated parts to this project, each of which is directly linked to more effective management of invasive *C. taxifolia* in NSW. As such, this project was done in conjunction with the development by NSW Fisheries of a statewide control plan for invasive *C. taxifolia* in NSW. This plan is available on the NSW Fisheries website at http://www.fisheries.nsw.gov.au/thr/species/fn-caulerpa.htm

Chapter 2 describes the distribution of *C. taxifolia* in NSW. It is important to accurately document the spatial extent of any translocation to new localities and the spread of the alga within already infested waterways. Techniques were developed, therefore, to obtain quantitative estimates of the location and coverage of all *C. taxifolia* in NSW waterways. Mapping has been done on several occasions since 2002 to provide a time series of the alga's increases and decreases in extent at all known sites. These data have aided the process of prioritising sites for control work and of evaluating the success of any eradication trials.

Control of *C. taxifolia* in NSW will ultimately depend on a sound scientific understanding of the ecology of invasive *C. taxifolia,* including its life history characteristics, growth potential, mechanisms of dispersal and susceptibility to marine herbivores. Preliminary investigations of these ecological parameters were done at the University of Wollongong and are described in Chapter 3 and Davis *et al*. (ms in review). Complementary research, funded by the Australian Research Council, is ongoing at the University of Wollongong and will be reported elsewhere (eg Wright, ms in review).

Chapter 4 describes an assessment, using field and laboratory experiments carried out by researchers at the University of Wollongong, of boating and other human vectors that may contribute significantly to the generation of fragments of *C. taxifolia* and the transportation of those fragments to other localities.

The main focus of the work done during this project was the development and trial of environmentally benign ways of removing *C. taxifolia* from NSW waterways. Such ‘eradication’ techniques, in combination with other management controls, might eventually lead to the elimination of this invasive alga from whole sites or regions. These ‘eradication’ trials are described and evaluated in Chapter 5.

2. MAPPING THE SPREAD OF *CAULERPA TAXIFOLIA* IN NSW

Macrophytes such as mangroves, seagrasses and saltmarshes are a conspicuous biological feature of many estuaries, harbours and sheltered coastal embayments. They are widely recognised as serving a number of important functions including sediment stabilisation and the provision of vital habitats for many marine invertebrates and coastal fishes. Quantifying the extent of these estuarine macrophytes is often undertaken to assess medium to long-term changes in their distribution and abundance, and measures such as the amount of seagrass often provide key performance indicators of estuarine health in 'State of the Environment' reports and other similar documents (Ward *et al.* 1998). Mapping of these macrophytes is typically done by remote sensing, and the technique most often used involves aerial photographs which are converted into images by scanning, ortho-rectifying and using image enhancement to optimise the features of interest (Walford & Williams 1998, Williams *et al*. 2003). Images are then mapped by onscreen digitising via a Geographical Information System (GIS).

Field mapping of *C. taxifolia* was undertaken to follow the change in size of known infestations and to locate any new infestations. Because recent aerial photographs of the estuaries under consideration were not available at the start of this project, a more direct approach was needed. Further, aerial photographic images do not give reliable resolution of underwater features deeper than approximately 1-2 m (G. West, pers. comm.), and *C. taxifolia* often occurs in water deeper than 2m. Even for *C. taxifolia* in shallow water, its spatial extent was likely to change much faster than for seagrasses, necessitating a system that could provide maps on a shorter time frame than would be possible using aerial photographs.

2.1. Field survey methods for *Caulerpa taxifolia*

The mapping protocol that we developed was designed as a rapid assessment technique and was not meant to be a comprehensive survey. It was restricted to NSW estuaries that were known to contain *C. taxifolia.* Further, because most of these estuaries were very large, it was not feasible to survey them in their entirety as it would have taken many months to do that, reducing the effectiveness of the rapid assessment approach. Only Narrawallee Inlet, the smallest estuary infested by *C. taxifolia*, was thoroughly searched each time a mapping survey was done there. For the other 7 estuaries, a field survey technique was developed which relied on the known or expected status of infestation. The technique was adaptive in that it was based on prior knowledge and risk assessment. Sampling effort could therefore be adjusted according to results from previous surveys. Prior to starting a particular survey, sections of the infested waterways to be mapped were classified according to three risk categories:

1. areas where *C. taxifolia* was known, had been reported, or had previously occurred
2. areas that were considered susceptible to invasion
3. areas not likely to be affected

For category 1, the assessment was based on reports from field researchers or members of the general public. All reports of new infestations were usually investigated within 2-4 weeks of the report being made. Spot investigations involved divers thoroughly searching in the vicinity of the report. If the presence of *C. taxifolia* was confirmed, that site was accorded a risk category of 1 and added to the schedule for the next mapping survey of that waterway.

For category 2, the assessment was based on knowledge acquired by field researchers (either NSW Fisheries or University of Wollongong) during other parts of the research program (see Chapters 3-5). Sites were considered susceptible if they were in shallow water (<6 m depth), and/or had sparse seagrass (especially if these bordered on dense seagrass), and/or were along sandy or mud shorelines with a gentle slope, and/or were in areas with boat moorings or common anchoring sites. The most susceptible areas were those in close proximity to existing *C. taxifolia* beds and that lay in the direction of the prevailing wind or current.

Category 3 included rocky foreshore that steeply dropped into water greater than 6 m depth, places with strong currents and anywhere deeper than 10 m. The areas identified as not likely to become affected were generally not included in surveys. However, if an area with these characteristics was adjacent to a known infestation (i.e. within approximately 500 m) it would be searched if time permitted.

Field surveys were done from a small boat equipped with a specially designed, habitat mapping system (Plate 2) that was based on a similar system designed to validate habitat maps of estuarine macrophytes and in-stream riverine features (G. West, pers. comm.). The system had two position-locating instruments: a Magellan Meridian handheld global positioning system (GPS) and a Trimble GPS Pathfinder Pro XR with digital global positioning system (DGPS) capability. The Trimble GPS has a positional accuracy of ±1 m when using a DGPS signal (provided free by the Australian Maritime Safety Authority). The Magellan system is accurate to ±5 m. Both GPS units output NMEA (National Marine Electronics Association) data that were logged to an on-board laptop computer that recorded latitude and longitude. The mapping system also included a Garmin Fishfinder 240 operating frequency 200kHz and temperature and speed sensors. The sounder also outputs NMEA data directly to the laptop.

Plate 2. The on board mapping system developed to document the spatial extent of *C. taxifolia* infestations in NSW waterways.

The system logged data at two-second intervals to a text file that had been set up with fields for all the measured variables along with a field for 'habitat category'. The operator on the boat could annotate this field with habitat information, one row at a time for every logged point, using predetermined key codes on the laptop. The way these habitat data were collected depended on water clarity and depth. In clear, shallow water, observations from the boat were adequate to estimate the extent of small infestations of *C. taxifolia* and its density. In deeper, more turbid water or for large infestations, SCUBA divers or underwater video was used. The software program 'ARCPAD' locates the boat's position and provides a current display of that position. When doing repeat surveys of an area, the previous map was loaded into the laptop to help locate previously affected areas. Survey methods for each of the three risk categories are detailed below.

2.1.1. Areas already affected by C. taxifolia

If *C. taxifolia* occurred as a small bed (< 10 m in all directions), a single GPS point was taken approximately corresponding to the centre of the bed, the size of the bed was estimated by eye and the density of *C. taxifolia* in the area noted. Density of the bed was scored as either dense (> 50% cover) or sparse (<50% cover) and the presence of other vegetation (seagrass or other algae) or other relevant features were noted. For medium sized beds (10-20 m in any direction), a diver swam around the margin of the bed and dropped weighted floats. GPS points were recorded by the mobile mapping unit while the boat followed the marker floats. If *C. taxifolia* was very extensive (> 20 m in any direction), an underwater video camera was towed through the area in a tight zig-zag pattern parallel to the shoreline. An observer in the boat noted the relevant data as seen on the video and relayed them to the operator of the mapping system. Divers were still often used in this latter situation to double-check video interpretations and to swim past the mapped boundaries to ensure that they truly represented the limit of the *C. taxifolia*.

2.1.2. Areas likely to be affected by C. taxifolia

An underwater camera was towed through the area, generally at depths of 2 m and then 4 m in a tight zig-zag pattern parallel to the shoreline. An observer watched from the boat and noted what was being seen on the video screen. Alternatively a diver was towed behind the boat instead of the camera and gave rope signals for presence and density of *C. taxifolia*. If the alga was found, the boat was stopped and a diver verified its presence. Relevant data were recorded as described above.

2.1.3. Areas considered unlikely to be affected by C. taxifolia

No surveys were undertaken unless adjacent to already affected areas. If time permitted, wide zig-zag searches were made with the towed video camera or by SCUBA divers.

2.2. Creating final maps

Once back in the office, the dataset saved on the laptop was downloaded to the NSW Fisheries server at the Port Stephens Fisheries Centre. The data set was then checked for extraneous data, cleaned if necessary and converted to a point coverage using ESRI ARCMAP Version 8.3. A polygon coverage of *C. taxifolia* boundaries was generated using the point data, and overlaid with depth contours (from a NSW Waterways data layer) and coverage of seagrasses or other relevant biological data (i.e. pre-existing NSW Fisheries layers).

2.3. Sites with *C. taxifolia*

Descriptions of the 8 NSW estuaries that were affected by *C. taxifolia* during this study are presented below (in order from north to south) and summarized in Table 2.1. The alga was found in April 2004 in a ninth estuary, St Georges Basin (see Figure 1.1), where it covered approximately 7.5 hectares.

2.3.1. Lake Macquarie

C. taxifolia was first discovered in Lake Macquarie at Crangon Bay in April 2001. It was removed from that site soon after by hand-picking (see Chapter 5) and has not been found there since. Subsequently, it was found on the northern and eastern shores of Pulbah Island, the southern shore of the Wangi Wangi peninsula, at Pearl Beach and at Mannering Park. At the time of the last mapping exercise, January 2004, it was recorded in living beds only at Mannering Park. However, there have been recent public reports and positive identification of fragments of *C. taxifolia* occurring on beaches along Wangi Wangi, which suggests that it may still be patchily distributed along the peninsula.

Mannering Park is located in the southwestern end of Lake Macquarie and has a northeasterly aspect, making it exposed to the predominant summer winds. All of the *C. taxifolia* occurs patchily in water less than 1 m deep and amongst dense *Z. capricornii*. The substratum consists of fine estuarine mud mixed with sand and is susceptible to suspension during episodes of strong winds and associated choppy conditions.

The Wangi Wangi peninsula is approximately 3km long and has a southwesterly aspect, making it exposed to the predominant southerly winds experienced during winter. Historically, *C. taxifolia* has occurred in several dense beds along this peninsula in depths up to 7 m. Most of these beds were well defined, occupying areas of previously bare sediment in deeper water than *Z. capricornii* usually occurs. There were occasionally small patches occurring amongst *Z. capricornii* in water less than 1 m deep, but only in summer.

2.3.2. Pittwater

C. taxifolia is confirmed in two locations in Pittwater (southern arm of Broken Bay). A small, sparse bed, first discovered in June 2001, is patchily distributed in mud in 1-4 m of water in Careel Bay, adjacent to the marina (Plate 3). A band of *Z. capricornii* occupies the shallows, with the majority of the *C. taxifolia* occurring immediately offshore of this band. There are small patches of *Posidonia australis* adjacent to the infestation, with significant beds of *P. australis* located approximately 200m to the north. The deep edge of the *C. taxifolia* is growing under permanent boat moorings. Careel bay has a northwest aspect making it a sheltered area for most of the year.

The second infestation was found and mapped in March 2004 in clean sand to the west of the Barrenjoey peninsula. A few small patches were also located along the eastern shore between Palm Beach and Careel Bay. In these 'new' areas, *C. taxifolia* typically occurs in the sandy patches between beds of *P. australis*, *Z. capricornii* and *Halophila ovalis*. Most *C. taxifolia* occurs in 1-4 m of water, although some attached plants were found as deep as 8 m. Due to its proximity to the heads of Broken Bay, the area is subject to tidal currents and is exposed to northerly weather.

2.3.3. Port Jackson

C. taxifolia was first discovered here in March 2003 and has been confirmed growing in three areas since then. Very small patches (<2 m diam.) or individual plants have been found in 1-3 m of water at Clontarf Marina, Clontarf Beach (500 m SE of marina) and along the beach immediately south of Parriwi Head (750 m SW of marina). In most of these areas, *C. taxifolia* is growing on clean sand, occasionally amongst sparse *Z. capricornii* and *H. ovalis*.

In the Manly area, the most significant infestation is at Little Manly Cove, where there is a large area of sparse, scattered *C. taxifolia* growing amongst sparse *Z. capricornii* and *H. ovalis*. The area has a southwesterly aspect exposing it to strong winter southerlies. Some individual plants have been discovered off Quarantine Beach (immediately inside Cannae Point). Small-scattered patches have also been found at Forty Baskets Beach (Western Manly Cove). The depth range of infestations is 2-7 m, mostly beneath permanent boat moorings and on clean sand.

In Chowder Bay (Clifton Gardens) sparse *C. taxifolia* grows on clean marine sand in 1-7 m of water amongst sparse *Z. capricornii, P. australis* and *H. ovalis*. There are small, dense patches growing adjacent to rocky reef in the northern corner of the bay. Chowder Bay faces southeast, making it exposed to most weather patterns.

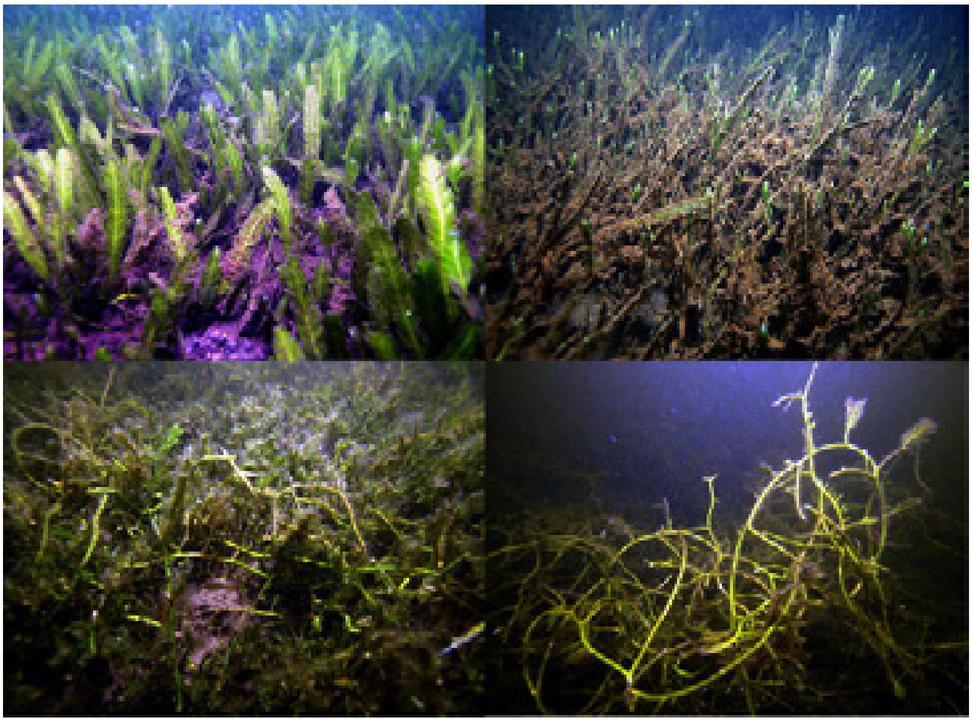


Plate 3. Examples of *C. taxifolia* growing in situ in NSW estuaries. Top left: Botany Bay, bottom left: Port Hacking, top right: Burrill Lake shallow, bottom right: Burrill Lake deep.

2.3.4. Botany Bay

The first (December 2001) and most significant infestation in Botany Bay occurs in Quibray Bay, where there is a large area of dense *C. taxifolia* (Plate 3) growing in fine mud in 0.5-3 m of water adjacent to substantial beds of *P. australis* and scattered *Z. capricornii* and *H. ovalis*.

A large area along the southern shores of Botany Bay is affected by sparse *C. taxifolia* growing in 1-2 m water depth amongst *Z. capricornii, P. australis* and *H. ovalis* on clean sand. Other areas in Botany Bay affected by sparse *C. taxifolia* with similar characteristics are Lady Robinson's Beach (easterly aspect, very exposed) and an area between the third runway and Port Botany (southerly aspect).

2.3.5. Port Hacking

C. taxifolia was first found at this locality in early 2000 at Fishermans Bay near Maianbar. However, the dense bed growing adjacent to extensive patches of the seagrass *Posidonia australis* disappeared following very heavy rainfall in the winter of 2003. It has not been encountered at this site since then. Currently, the worst affected area is Gunnamatta Bay, which has an extensive, dense bed of *C. taxifolia* in 1-4 m water depth along the northwestern shore in the end of the bay (Plate 3). An area of sparse *C. taxifolia, P. australis* and *Z. capricornii* surrounds the dense bed.

The bay is well protected and the substratum is mostly fine mud. The more exposed entrance to Gunnamatta bay can have an extensive area of sparse *C. taxifolia* growing on a clean marine sand bottom, but its occurrence here is very intermittent.

There are several other areas with beds of sparse *C. taxifolia* with similar characteristics to the entrance of Gunnamatta Bay: Simpson's Bay (directly opposite Gunnamatta Bay), which has mostly *Z. capricornii* as the associated seagrass; Burraneer Bay which has both *P. australis* and *Z. capricornii* and Jibbon Beach that has predominantly *P. australis*. Small, intermittent patches of *C. taxifolia* have been found in the western part of Port Hacking around Gogerleys Point. The most exposed location is Jibbon Beach, which is open to northerly winds, and swells which are common during the summer months. It is also adjacent to the open ocean making it more exposed to oceanic conditions than the other locations.

2.3.6. Lake Conjola

First discovered on the southern shores of Lake Conjola in April 2000, *C. taxifolia* has now spread to cover almost 28% of the shoreline of this lake and Berringer Lake (Table 2.1). At the time of initial discovery, there was estimated to be almost 100 hectares infested by *C. taxifolia* (Table 2.2). The alga grows on substrata ranging from fine mud to clean sand in 1-10 m water depth. With the exception of some scattered *Z. capricornii* and *H. ovalis*, there is little associated seagrass at present, although both seagrasses were formerly much more abundant (Meehan 2001). *C. taxifolia* is now the predominant aquatic plant in the system.

2.3.7. Narrawallee Inlet

The characteristics of Narrawallee Inlet make it unique compared to other estuaries affected by *C. taxifolia*. It is a small estuarine creek entering the ocean through a narrow mouth guarded by a shallow sand bar. The sandy channel is only approximately 150 m wide at its widest point and experiences strong tidal currents. The banks of the channel have dense beds of *Z. capricornii*, which decrease in density with water depth. *H. ovalis* co-occurs in these areas of lower density *Z. capricornii*. The central region of the channel is largely bare sand but often has large mats of entangled brown algae (*Ecklonia radiata* and *Sargassum* sp.), washed in from the ocean and from the rocky reef at the mouth.

C. taxifolia was first found here in April 2001. It is usually patchily distributed in 1-4 m of water amongst the *Z. capricornii* and *H. ovalis* along the banks of the channel. The middle of the channel can have scattered individual plants growing in the sand, but patches of *C. taxifolia* rarely occur here. Fragments of *C. taxifolia* are often found in the entangled mats of brown algae, which move along the bottom with the tide. The most well established beds have been found in a deep hole (4 m) on a bend in the channel, which appears to escape the effects of strong tidal flow and may act as a sink and/or source for fragments.

2.3.8. Burrill Lake

Patches of sparse and dense *C. taxifolia* affect most of the muddy, northern and western shorelines of the main body of Burrill Lake, having spread considerably since its initial discovery in March 2001. The dense patches are usually in deeper water (up to 7 m) where the stolons are often unattached to the substratum (see Plate 3). These patches are located offshore from areas of sparse *C. taxifolia* mixed with *Z. capricornii* and, to a lesser extent, *H. ovalis*. There is also a large area of sparse *C. taxifolia* in 1-5 m depth on the southwestern shore of the southwestern arm of the lake (Plate 3). *Z. capricornii* predominates here in the shallows, with *C. taxifolia* occupying vacant space in the seagrass bed.

Table 2.1. Characteristics of NSW sites affected by *C. taxifolia*. Further data on the locations can be found at http://www.dlwc.nsw.gov.au/care/water/estuaries/Inventory.html

Location	Waterway area (ha)	Depth range (m)	Associated seagrasses	Sediment type	Aspect of beds	% affected by *C. taxifolia*
Lake Macquarie	12000	0.5 – 7	*Z. capricornii* *H. ovalis*	Fine estuarine mud	NE, S	0.002
Pittwater	1730	0.5 – 12	*Z. capricornii* *P. australis* *H. ovalis*	Fine estuarine mud, Clean marine sand	NW, W	2.9
Port Jackson	4970	1 – 7	*Z. capricornii* *H. ovalis*	Clean marine sand	SW, E, S	0.07
Botany Bay	8000	0.5 – 3	*P. australis* *Z. capricornii* *H. ovalis*	Fine estuarine mud Clean marine sand	N, E, S	6.2
Port Hacking	1100	1 – 4	*P. australis* *Z. capricornii* *H. ovalis*	Fine estuarine mud Clean marine sand	N, S	5.0
Lake Conjola	590	1 – 10	*Z. capricornii* *H. ovalis*	Fine estuarine mud Clean marine sand	All aspects	28.0
Narrawallee Inlet	40	1 – 4	*Z. capricornii* *H. ovalis*	Clean marine sand	N	0.5
Burrill Lake	410	1 – 7	*Z. capricornii* *H. ovalis*	Fine estuarine mud	All aspects	6.5

2.4. Temporal changes in the extent of *C. taxifolia*

The mapping system was first trialed in the small Narrawallee Inlet in May 2001. Further refinement and trials were done in 2002 in Narrawallee, Lake Macquarie and Pittwater. Close to complete coverage of all estuaries (for areas with risk categories 1 or 2) was first done in summer 2003 (February and March). Complete surveys were then repeated in winter 2003 (August) and summer 2004 (Table 2.2; Appendix 1).

For large waterways with several large, but widely spread infestations (Botany Bay and Lake Conjola), the estimates obtained (especially the initial ones in summer 2003) are not likely to be very accurate. Several hundred hectares of *C. taxifolia* now grow in these two waterways. For the other mapped estuaries, the estimates presented in Table 2.2 are considered to be reasonably accurate. From summer 2003 to summer 2004, some estuaries experienced dramatic increases of over 200% (Botany Bay and Burrill Lake). The coverage increased by approximately 70% in Port Hacking and by lesser amounts in Port Jackson (30%) and Lake Conjola (6%). The summer coverage also increased in Pittwater, but this was due to the discovery of a large, but previously undocumented, bed at Palm Beach (Table 2.2). Coverage at Careel Bay remained virtually unchanged during this time. At the other two locations, Lake Macquarie and Narrawallee Inlet, the spatial extent of *C. taxifolia* decreased from high values in 2002 and 2003 respectively; for Lake Macquarie the decline was substantial (Table 2.2). These decreases were associated with large scale control operations in these two waterways (see Chapter 5).

Many southern locations showed a decrease in the area covered by *C. taxifolia* between summer and winter (Table 2.2). In Port Hacking this decrease was 50% in 2003 (from 32 to 16.6 ha), in Lake Conjola 30% in 2003 (155 to 111 ha), in Burrill Lake 65% in 2003 (6.3 to 2 ha) and in Narrawallee 48% in 2002 (3.9 to 2 ha). In addition, areas classified as having dense cover of *C. taxifolia* in summer often were classified as having sparse cover in winter. Observations during the surveys confirmed that *C. taxifolia* disappeared completely from many sites over winter, particularly in areas of shallow water.

2.5. Discussion

Regular mapping of infestations in NSW and extensive field observations have supported findings from the Mediterranean that beds of *C. taxifolia* reach their greatest extent at the end of summer and decrease in size during winter (Meinesz *et al*. 1995; Ceccherelli and Cinelli 1999). In most waterways, the shapes and sizes of beds of *C. taxifolia* are very dynamic and sometimes areas of *C. taxifolia* can disappear over relatively short periods of time (weeks – months). The exact causes for such changes are unclear, but they likely involve a variety of factors such as decreased temperature or salinity, increased turbidity or lack of nutrients (Vicente *et al*. 1993). *C. taxifolia* beds may also seemingly disappear because they are smothered by sediments, as happened in Fishermans Bay in Port Hacking and Narrawallee Inlet (Glasby *et al*. ms in review).

Other methods of mapping *C. taxifolia* in NSW could be explored. For example, researchers in the Mediterranean have recently used imagery obtained from a Compact Airborne Spectral Imager (CASI) mounted on board a small aircraft to obtain more precise estimates of the spatial coverage of the alga off the coast of France (Jaubert *et al*. 2003). Interestingly, they concluded that the actual extent of *C. taxifolia* in the areas they mapped was substantially less than had previously been claimed. This highlights the need, irrespective of what remote sensing technique is used (e.g.. aerial photographs or spectral imagery) for comprehensive ground truthing.

Mapping of the spatial extent of beds is only one tool for documenting and understanding the seasonal dynamics and spread of *C. taxifolia* in NSW waterways. Starting in 2004, additional field information will be routinely collected during mapping surveys. Quantitative data on the cover and amount of *C. taxifolia* will be recorded from two sites for each of two habitats in each affected waterway: *C. taxifolia* only and *C. taxifolia* mixed with seagrass. Data will be collected by divers sampling several replicate quadrats (50 x 50 cm) strung with 100 points to sample % cover of *C. taxifolia* and other habitats (e.g. bare, *Posidonia, Zostera* or *Halophila*). This will provide more accurate estimates of the density of *C. taxifolia* and seagrasses through time (i.e. rather than the two coarse categories used to date) and the average height of *C. taxifolia* beds. Samples of *C. taxifolia* will also be collected from each quadrat to provide additional information on plant morphology, which differs considerably between locations (Plate 3, see also Chapter 3). Finally, water quality measurements will be taken at each site.

Table 2.2. Mapped coverage (hectares) of dense (> 50% cover) and sparse (< 50% cover) *C. taxifolia* in 8 NSW estuaries, 2001-2004.

Estuary	Date	Dense	Sparse	Total	Comments
Lake Macquarie	Aug 2002	0.21	3.78	3.99	Earliest GPS estimate; prior to salt treatment
	Mar 2003	0.004		0.004	Isolated patches at Wangi & Mannering Park
	Aug 2003	0.01		0.01	Isolated patches only at Mannering Park
	Jan 2004		0.25	0.25	Isolated patches only at Mannering Park
Pittwater	Feb 2002	0.23		0.23	Earliest GPS estimate; C. tax. at Careel Bay
	Oct 2002	0.29		0.29	C. tax. at Careel Bay only
	Aug 2003		0.27	0.27	C. tax. at Careel Bay only
	Mar 2004		48.98	48.98	New infestation at Palm Beach (48.72 ha)
Port Jackson	Aug 2003		2.66	2.66	Earliest GPS estimate; C. tax. in 3 areas
	Mar 2004	0.03	3.46	3.49	
Botany Bay	Feb 2003	23.15	122.62	145.77	Earliest GPS estimate; not all sites surveyed
	Aug 2003	16.11	282.54	298.65	
	Mar 2004	16.93	482.15	499.08	First, complete summer survey
Port Hacking	Feb 2003	3.36	28.55	31.91	Earliest GPS survey of whole estuary
	Aug 2003	0.57	16.01	16.58	
	Mar 2004	6.37	48.73	55.1	
Lake Conjola	2000	63.27	32.87	96.14	Rough estimation from diver survey
	Feb 2003	57.52	98.19	155.71	Compilation of partial maps & diver surveys
	Aug 2003	51.21	60.49	111.70	Earliest GPS survey of whole estuary
	Feb 2004	148.12	17.32	165.44	
Narrawallee Inlet	May 2001		3.94	3.94	Earliest GPS survey
	Sept 2002		2.05	2.05	
	Feb 2003		6.21	6.21	Prior to salt treatment (see chapter 5)
	Aug 2003		0.19	0.19	
	Feb 2004	0.27	4.81	5.08	
Burrill Lake	Feb 2003	0.32	5.99	6.31	Earliest GPS survey
	Aug 2003		1.96	1.96	
	Feb 2004	3.11	23.38	26.49	

3. DISPERSAL, RECRUITMENT AND GROWTH OF *C. TAXIFOLIA* IN NSW

Effective management is based on sound scientific information. It is important to identify attributes that lead to successful invasion in order to better understand the invasion process and to assist in the management of the invasive species. High population growth rates, high reproductive output as well as good dispersal and recruitment are key life history characteristics that characterise successful marine invasive species.

C. taxifolia is capable of growing extremely quickly and vegetative growth seems to be the primary mode by which the alga has invaded large areas of seafloor in NSW and in other countries (Meinesz *et al*. 1993; Smith and Walters 1999). Evidence to date indicates that invasive *C. taxifolia* in the Mediterranean rarely, if ever, reproduces sexually (Žuljević and Antolić 2000). Species of *Caulerpa* are capable of regenerating from small pieces of stolon or frond (Jacobs 1994), so fragments have the potential to be an effective means of dispersal (Belsher and Meinesz 1995; Ceccherelli and Cinelli 1999a).

The success of *C. taxifolia* has largely been attributed to its ability to reproduce asexually. *C. taxifolia* reproduces asexually through a process of fragmentation, dispersal, recruitment of drifting fragments and subsequent vegetative growth of thalli. Marine invasive species that are capable of fragmentation tend to have an advantage because one single fragment (see Plate 5) can start a new colony.

The overall goal of this component of the project was to investigate the dispersal, recruitment and growth characteristics of *C. taxifolia* to provide ecological information about its spread within NSW estuaries.

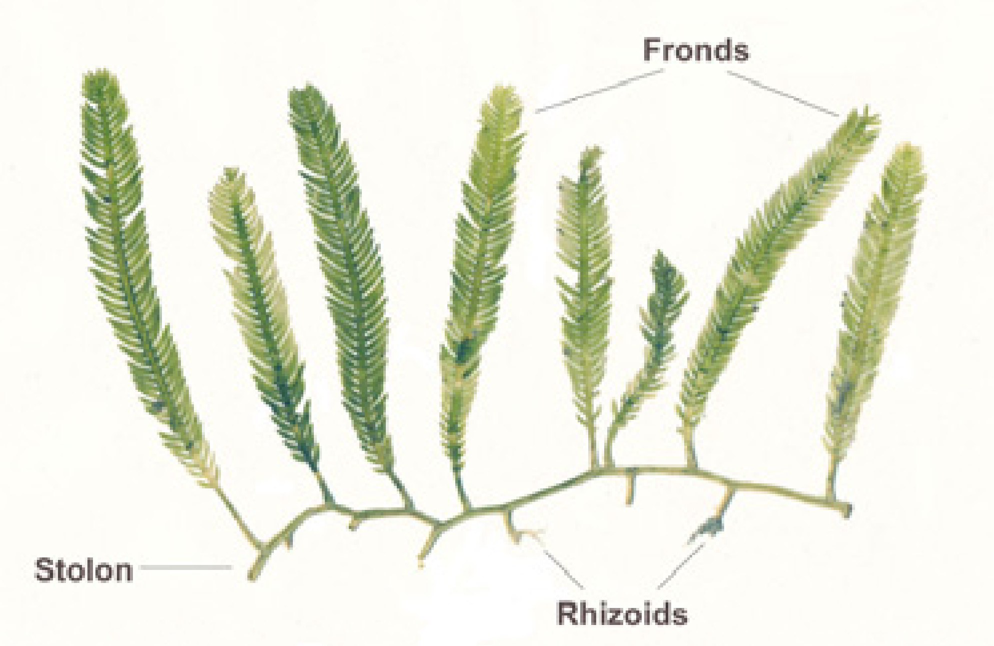


Plate 5. Thallus fragment of *Caulerpa taxifolia*, indicating the frond, stolon and rhizoid section of the alga.

3.1. The role of fragments in contributing to spread

Field investigations were undertaken to examine the role of fragments in contributing to the spread of *C. taxifolia*. This was done in three parts. First, the biomass of *C. taxifolia* fragments in Lake Conjola was monitored over a range of spatial and temporal scales to document the availability of fragments in an estuary within extensive, well-established beds of the alga. Second, the abundance of fragments within beds of two native seagrass species in Port Hacking was measured to assess the potential capacity for invasion into beds of seagrass. Finally, manipulative experiments were done to estimate the rate of accumulation of *C. taxifolia* fragments into existing vegetation.

3.1.1. Spatial and temporal patterns of abundance and biomass of C. taxifolia fragments

3.1.1.1. Methods

As fragments are a key means of spreading *C. taxifolia*, detailed information on the presence of fragments at a variety of temporal scales was sought. Spatial and temporal patterns of abundance and biomass of fragments initially were quantified to examine natural variability in the occurrence of fragments at two sites within each of two locations in Lake Conjola (West Conjola and Roberts Point). Two sites within each location were examined to provide a picture of finer scale spatial variation. A third site was added at West Conjola in Nov 2002 as consistently poor visibility had hindered sampling at one of the sites at this location. At each site, unattached *C. taxifolia* fragments were collected by hand from 25 haphazardly placed quadrats (0.25 m^2), returned to the laboratory at the University of Wollongong, classified into categories, counted and weighed individually (g wet weight). In addition, the percentage cover of attached *C. taxifolia* in each quadrat was estimated and the heights of five haphazardly chosen fronds were measured. These latter measurements were later averaged to provide a mean frond height for each sampled quadrat. Field sampling was done on five occasions between June 2002 and August 2003 (Table 3.1).

Table 3.1 Sampling schedule at three sites within Lake Conjola between June 2002 and August 2003.

Location	Site	June 02-July02	Sept 02	Nov02-Jan03	Mar 03	Aug 03
West Conjola	1	x	x	x	x	x
	2	x	x	x	x	x
	3			x	x	x
Roberts Point	1	x	x	x	x	x
	2	x	x	x	x	x
Adder Bay	1			x	x	x
	2			x	x	x

3.1.1.2. Results

Unattached fragments were widely distributed within Lake Conjola (Figures 3.1-3.3). The mean number of fragments ranged from 4 to 260 m^2. Fragments were present at all sites at all times of sampling, with no obvious seasonal trends apparent (Figures 3.1-3.3). Relatively large abundances of fragments were correlated with significant storm events during the course of this study. Storms occurred in September 2002 and August 2003. The effects of the September 2002 storm were restricted to Roberts Point, although Adder Bay was not being investigated at this time. During September 2002, fragments were four times more abundant at Roberts Point (Figure 3.2) than at West Conjola (Figure 3.1) and were at least twice as abundant as at other times of sampling at this locality. There was a heavy chop at this time created by strong winds, but this was much more pronounced at Roberts Point than in West Conjola (A. Ferguson, pers. obs.). Similarly, in August 2003 all three sites at West Conjola and one site at Adder Bay had recently experienced rougher sea conditions than elsewhere and the abundance of fragments increased 3 to 5 fold. These correlations provide evidence that natural processes may cause significant levels of fragmentation. Similar observations have been made at Lake Macquarie, where large amounts of material were dislodged from shallow water area following strong onshore winds and cast up onto the shoreline along the Wangi peninsula (J. Sakker, NSW Fisheries, pers. obs.).

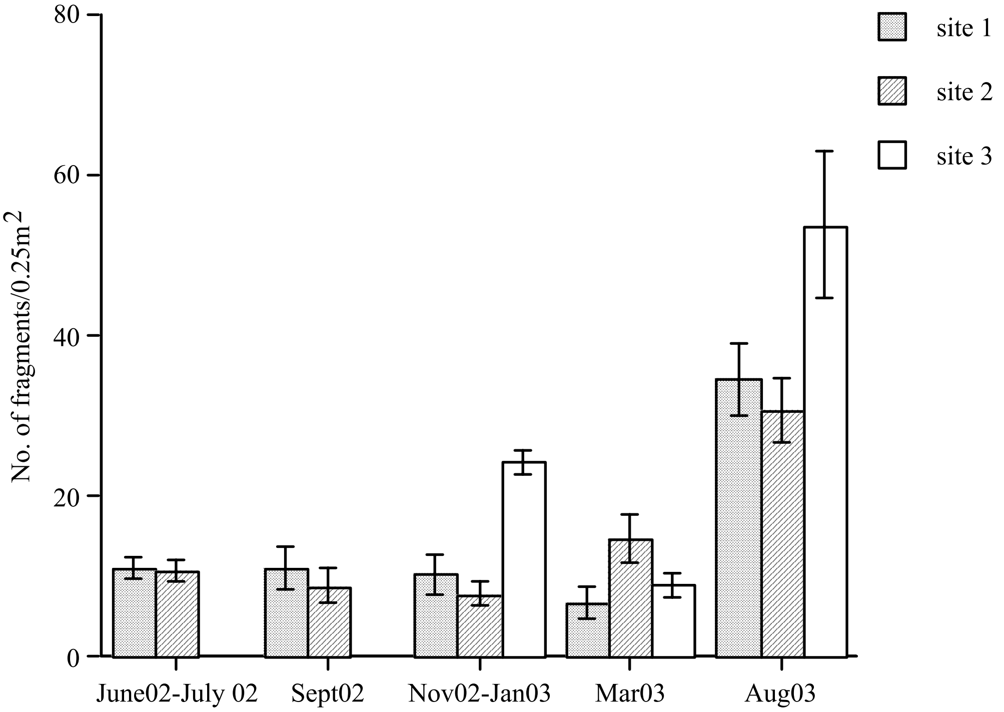


Figure 3.1. Mean (±se) number of fragments per quadrat of *C. taxifolia* fragments at West Conjola, Lake Conjola between June 2002 and August 2003. n=25.

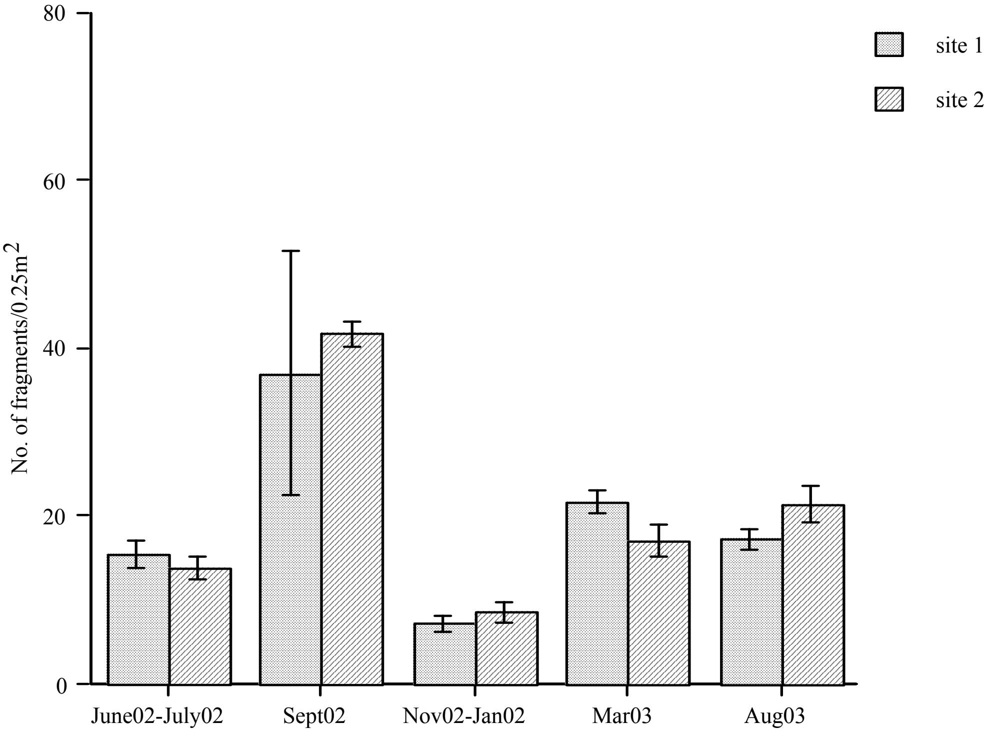


Figure 3.2. Mean (±se) number of fragments per quadrat of *C. taxifolia* fragments at Roberts Point, Lake Conjola between July 2002 and August 2003. n=25.

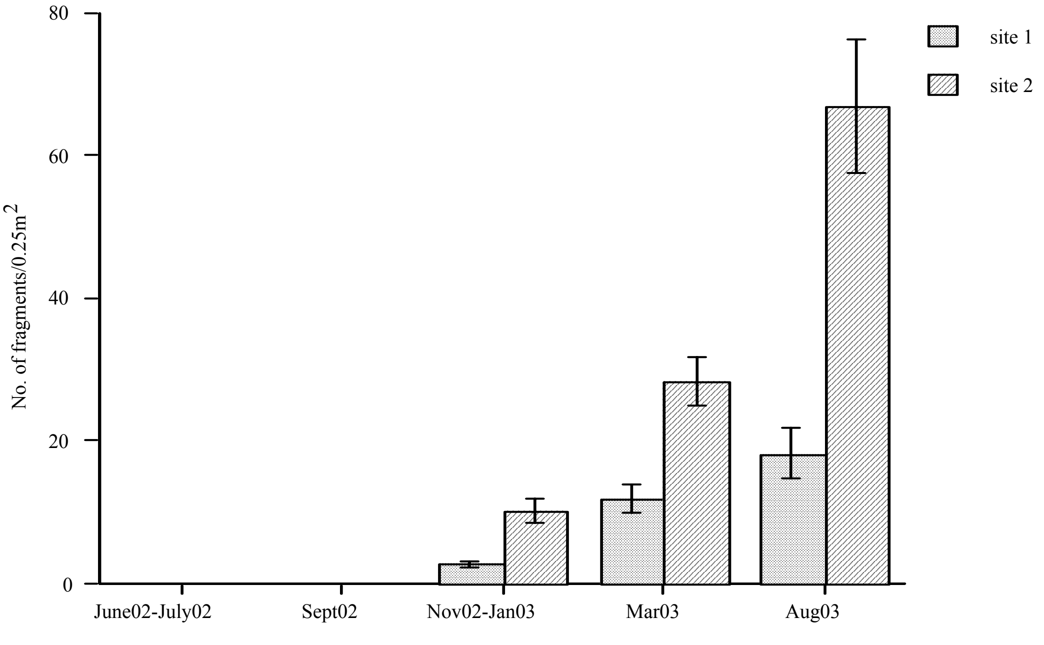


Figure 3.3. Mean (±se) number of fragments per quadrat of *C. taxifolia* fragments at Adder Bay, Lake Conjola between July 2002 and August 2003. n=25.

Lengths of individual fragments were compared to wet weight of individual fragments to determine an appropriate measure of size to use for future sampling and analyses. There was a significant positive association between the length and wet weight of individual fragments (Figure 3.4). Therefore, wet weight of fragments was used in all subsequent experiments because it was quicker to measure.

Relationships of wet weight of fragments with structural characteristics of *C. taxifolia* beds were examined by regressing fragment weight against the cover of *C. taxifolia* and the mean frond height of *C. taxifolia* growing within quadrats. There were no consistent relationships. Rather, there were significant positive relationships at some locations at some times, significant negative ones at some locations at some times and no relationships at all for other places and times. For example, the biomass of fragments within quadrats showed a positive relationship with the average height and with the percentage cover of *C. taxifolia* within each quadrat in June 2002 at West Conjola (Figure 3.5) and in July 2002 at Roberts Point (Figure 3.6). Three of these four relationships were statistically significant.

Spatial and temporal patterns were examined in more detail for each of three fragment types (frond only, stolon only, thallus – stolon plus frond) and wet weight (divided into appropriate size classes). Individual fragments varied in weight from 0.001 g to over 1 g. Thallus fragments (consisting of stolons and fronds) were the most abundant type of fragment on most sampling occasions. These thallus fragments were generally larger than the other types of fragments. Frond fragments were smallest, with most being under 0.01 g. Fragments of stolon and of thallus were more frequently in the smallest size class (0.0-0.99 g) whereas frond fragments showed no consistent pattern with respect to size class.

Here we present data for the three locations within Lake Conjola at times corresponding to storms and sampling periods just prior to storms. Storms resulted in dramatic increases in the number of fragments but did not alter the size frequency distributions (Figures 3.7-3.9).

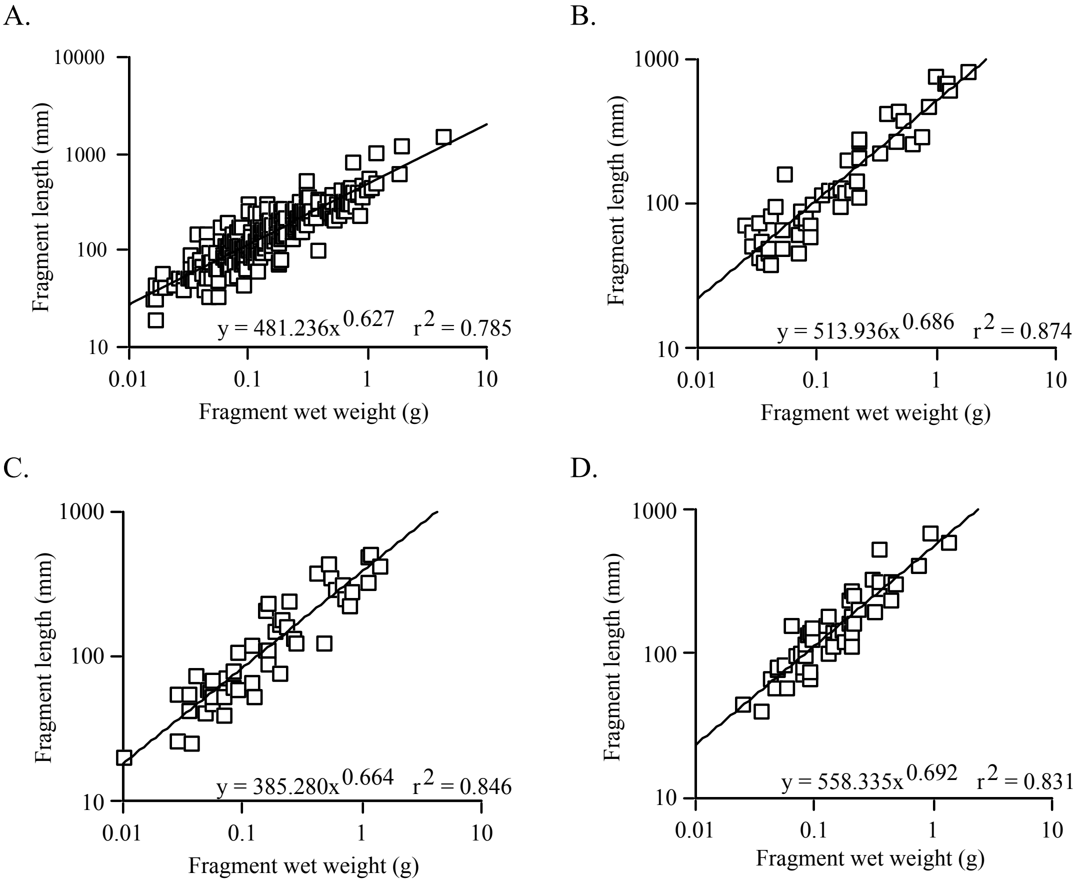
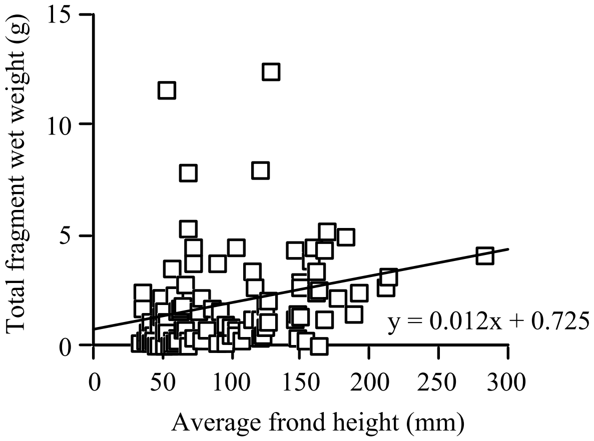


Figure 3.4. Relationships between lengths of *C. taxifolia* fragments and their wet weights at West Conjola site 1 (A) and site2 (B) and at Roberts Point site 1 (C) and site 2 (D).

A.



B.

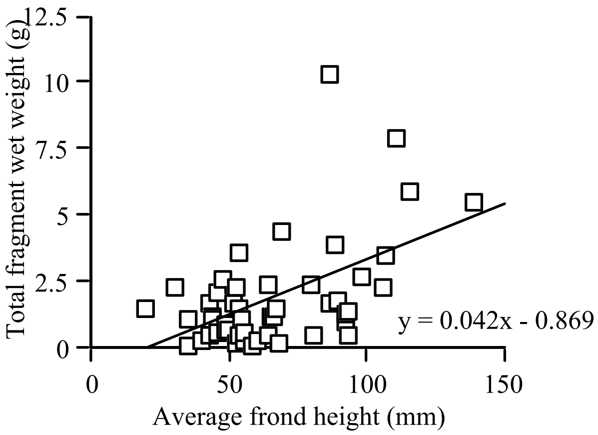
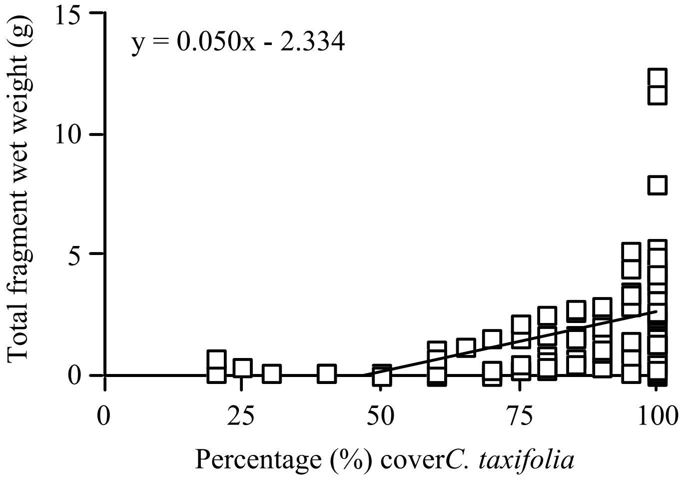


Figure 3.5. Relationships between total biomass of fragments and average frond height within quadrats in June/July 2002. West Conjola (A) r=0.27, n=100, P<0.01; Roberts Point (B) r=0.53, n=50, P<0.001.

A.



B.

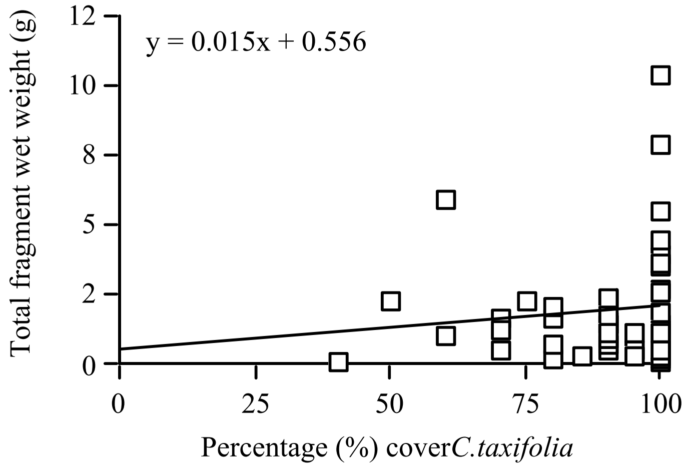


Figure 3.6. Relationships between total biomass of fragments and percent cover within quadrats in June/July 2002. West Conjola (A) r=0.43, n=100, P<0.001; Roberts Point (B) r=0.11, n=50, P>0.05.

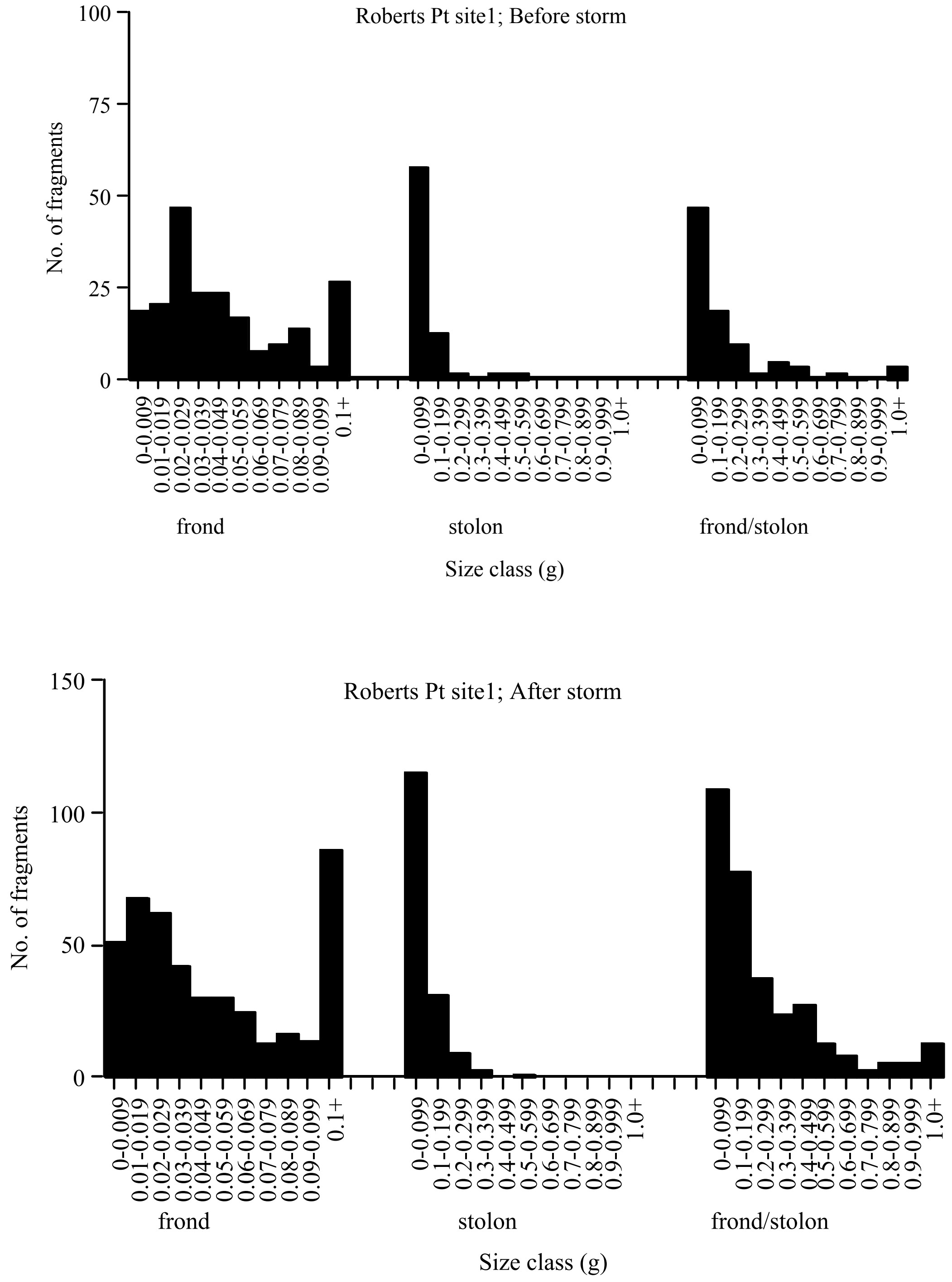
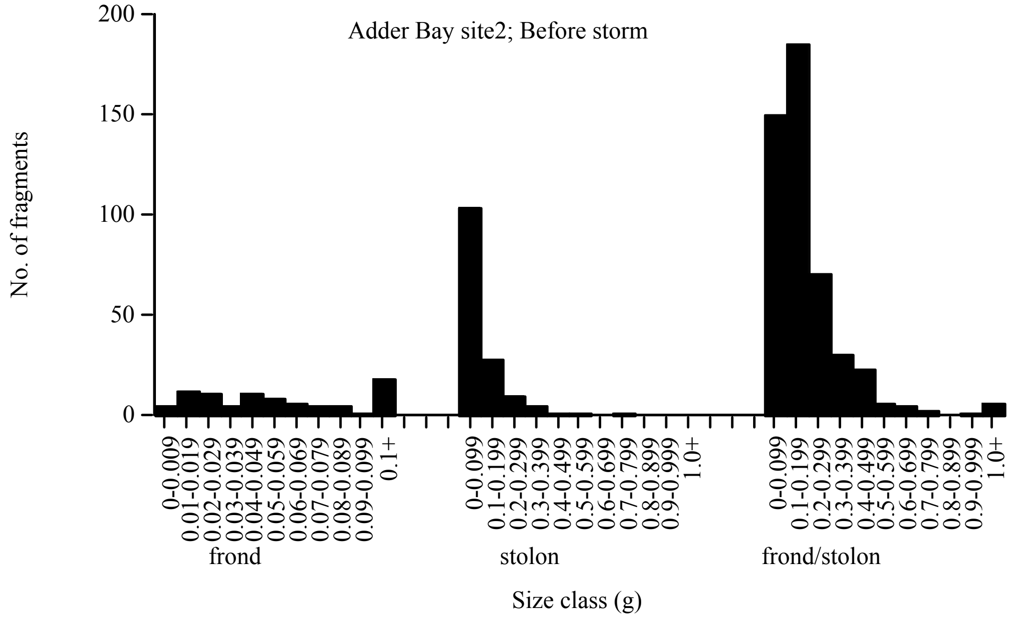


Figure 3.7. Size frequencies of fragments collected Before (upper) and After (lower) storm activity at Roberts Point, site 1.



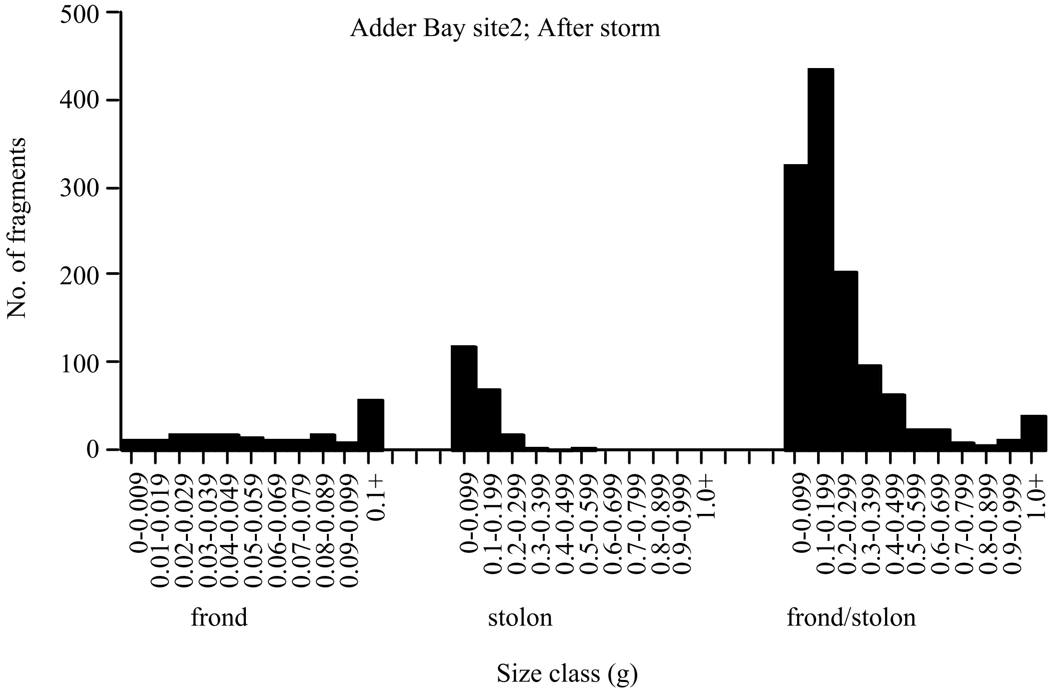
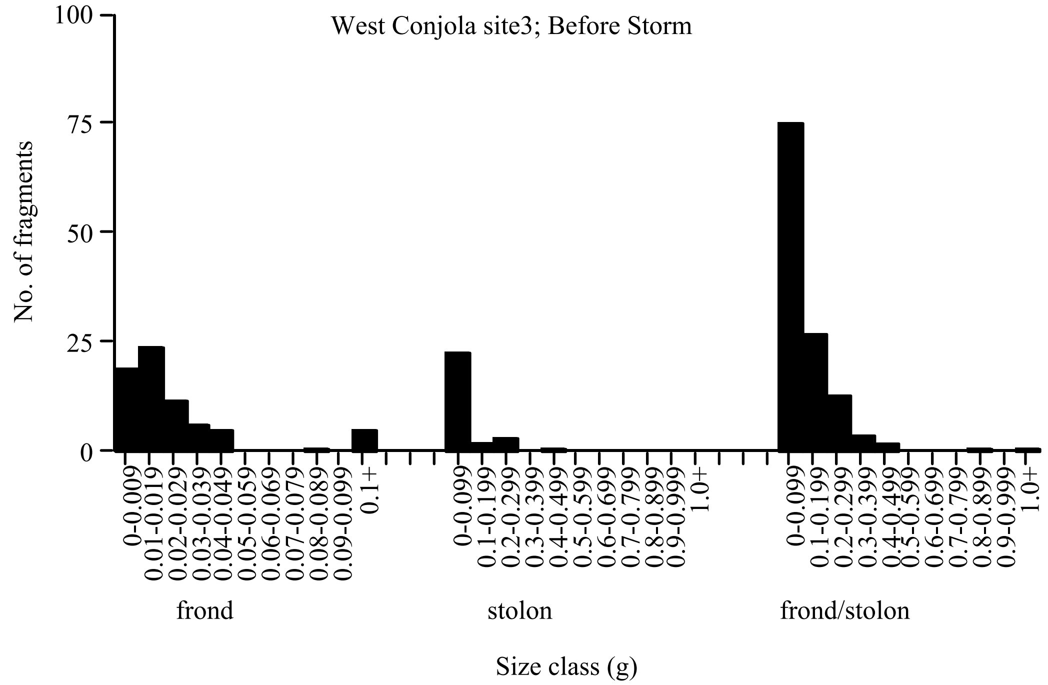


Figure 3.8. Size frequencies of fragments collected Before (upper) and After (lower) storm activity at Adder Bay, site 2.



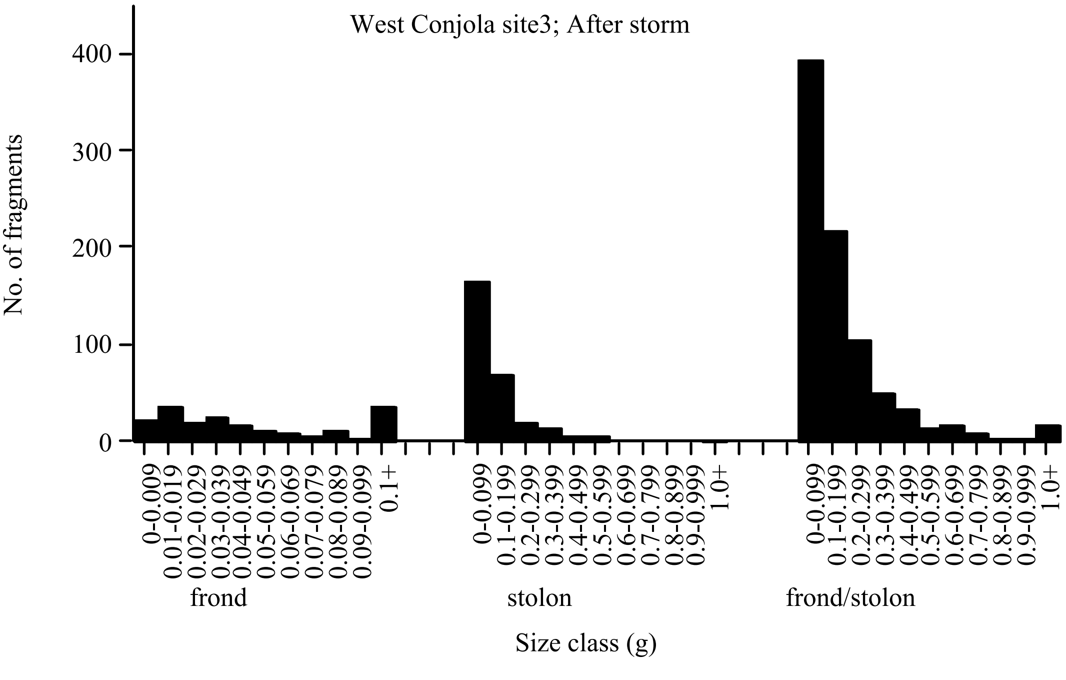


Figure 3.9. Size frequencies of fragments collected Before (upper) and After (lower) storm activity at West Conjola, site 3.

3.1.2. *Preliminary assessment of abundance and biomass of fragments within seagrass beds*

3.1.2.1. *Methods*

The presence and abundance of fragments of *C. taxifolia* within seagrass beds may have important implications for subsequent competitive interactions between this alga and seagrass. The abundance and biomass of *C. taxifolia* fragments were examined within two types of native seagrass beds, *Posidonia australis* and *Zostera capricorni,* at Port Hacking in March 2003. Each type of seagrass was sampled at each of two locations, Gunnamatta Bay and Maianbar. Sampling of fragments within *P. australis* beds at Maianbar had to be abandoned, however, because low visibility compromised the collection of data. In beds of *P. australis*, *C. taxifolia* fragments were collected from each of 10 quadrats (0.25 m^2) within each of three zones defined as follows:

- Zone 1 was dominated by *C. taxifolia* (but some sparse *P. australis* was present at Maianbar),
- Zone 2 was a transition zone consisting of *C. taxifolia* in the understorey with a canopy of *P. australis*,
- Zone 3 had *P. australis* only.

For *Z. capricorni*, there was no obvious gradation between zones of differing density and all beds represented a mixture of native *Z. capricorni* seagrass and invading *C. taxifolia*. Fragments were collected from each of 25 quadrats (0.25 m^2), returned to the laboratory at the University of Wollongong, counted and weighed (g wet weight). Measurements were also taken of the seagrass leaves in each quadrat to derive a measure of the structure of the seagrass bed at that point. For each seagrass species, leaf density was counted and the heights of five haphazardly chosen leaves were measured. These latter measurements were averaged to provide a mean leaf height for each sampled quadrat.

3.1.2.2. *Results*

Fragments of *C. taxifolia* occurred infrequently in beds of *Z. capricorni.* Although present in low numbers, abundance patterns were consistent at both sites (Figure 3.10). Patterns of fragment abundance in or near *P. australis* beds produced very clear patterns in Gunnamatta Bay. Fragments were abundant in the *C. taxifolia* zone adjacent to *P. australis* and in the transition zone in which *C. taxifolia* formed an understorey (Figure 3.11). Fragments were not found within beds of *P. australis* and therefore may play a limited role, if any, in the incursion of *C. taxifolia* into beds of this seagrass.

To test for a relationship between the wet weight of fragments and the structure of the seagrass beds from which they were collected, regression analyses were done between the wet weight of *C. taxifolia* fragments and leaf density and average leaf length for each of the two seagrass species. However, there was substantial variability among quadrats and no significant relationships were found.

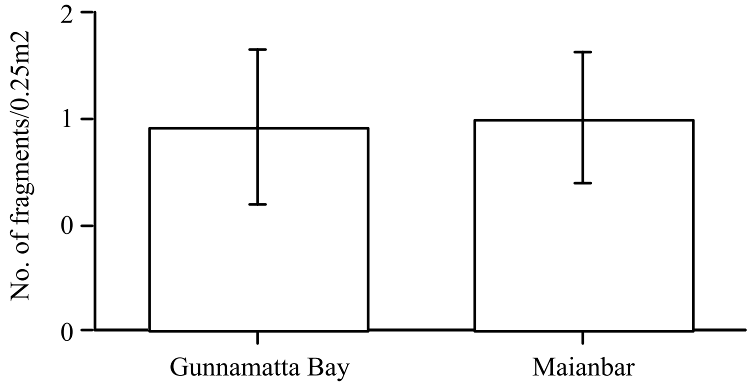


Figure 3.10. Mean (±se) number of *C. taxifolia* fragments per quadrat within *Zostera* beds at two sites within Port Hacking in May 2003. n=25.

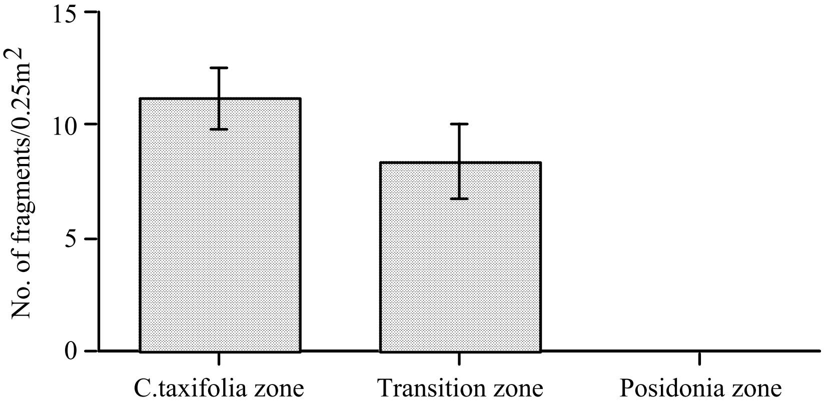


Figure 3.11. Mean (±se) number of *C. taxifolia* fragments per quadrat within 3 zones of *Posidonia* beds at two sites in Port Hacking in May 2003. n=10.

3.1.3. The effect of bed structure on the abundance and biomass of C. taxifolia fragments

3.1.3.1. Methods

The following experiment was based on the positive relationships found between the abundance of naturally occurring *C. taxifolia* fragments and the presence and height of *C. taxifolia* beds found in Lake Conjola (see section 3.1.1; Figures 3.5 and 3.6). Manipulative experiments were done in Lake Conjola to examine the hypothesis that patches of *C. taxifolia* accumulate more fragments thereby enhancing establishment. Such a mechanism would act to continually sustain *C. taxifolia* beds by augmenting growth with regular recruitment of 'new' plants. It was predicted that: 1) the presence of *C. taxifolia* would increase the accumulation of fragments, and 2) that taller fronds within a bed would increase the amount of fragments collected.

Artificial structures were used to mimic patches of *C. taxifolia*. These consisted of strips of polypropylene packing tape (6mm wide) attached to 1 m^2 steel reinforcing mesh frames (mesh size of 6 cm x 6 cm) (Figure 3.12). The placement of the strips onto the mesh was based on rough estimates of the distance between fronds along a stolon of naturally growing *C. taxifolia*. Comparisons were made among three treatments:

- Control plots, consisting of four stakes at each corner of a 1 m^2 bare patch of substratum
- Plots with short artificial fronds, consisting of a mesh structure with 5 cm long strips
- Plots with long artificial fronds, consisting of a mesh structure with 20 cm long strips.

In addition, to test for an artefact of using a steel mesh, a fourth treatment (consisting of a mesh frame with no strips attached) was included (Figure 3.12). The experiment was repeated at each of three locations in Lake Conjola; Picnic Bay, Roberts Point and Roberts Bay. Within each location, 4 replicates of each treatment were haphazardly positioned within approximately 2 m of *C. taxifolia* beds. *C. taxifolia* fragments were collected from the plots after 4 weeks, returned to the laboratory at the University of Wollongong, counted and weighed (g wet weight). The experiment was done on two occasions, December 2003 and February 2004.

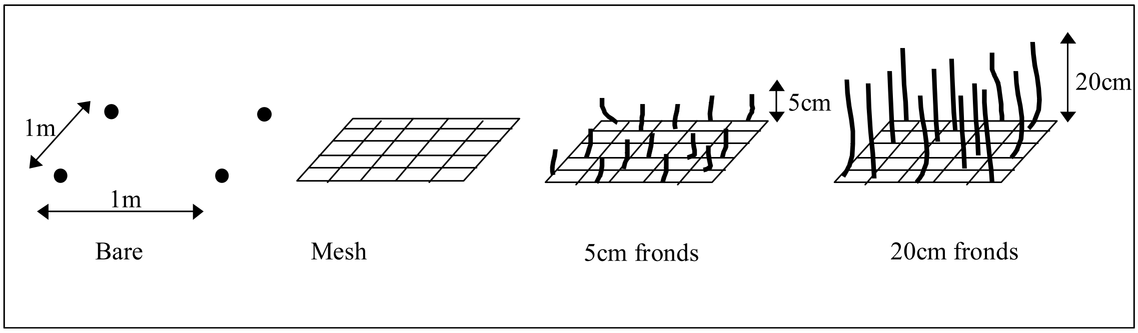


Figure 3.12. Treatments (bare, mesh, 5cm fronds and 20cm fronds) used to test for the effect of bed structure on the accumulation of *C. taxifolia* fragments.

3.1.3.2. Results

The number and weight of fragments differed dramatically among locations (Figure 3.13). In December 2003, artificial structures accumulated low numbers of fragments at Picnic Bay and Roberts Bay relative to controls. At Roberts Point, significantly more fragments accumulated in the 5cm and 20cm treatments compared to the control treatments, at each time (Table 3.2). In contrast, there were no significant differences among treatments at either time for Roberts Bay or Picnic Bay (Table 3.2).

In February 2004, there appeared to be an artefact of the presence of the frame itself, as this treatment collected reasonable numbers of fragments at Roberts Bay and Roberts Point and an average of approximately 2 g at Roberts Point (Figure 3.14). However, there was still a larger weight of fragments among the 20cm treatment compared to the control treatments (Figure 3.14). In summary, it appears that the presence of structure can dramatically increase the accumulation of fragments, however this is dependant on the location and time. The presence of polypropylene strips at Roberts Point at both times played an important role in fragment accumulation, but their height did not appear to make a difference (Figures 3.13, 3.14).

Table 3.2. Results of ANOVAs for testing hypotheses about effects of experimental treatments on the number and/or weight of *C. taxifolia* fragments accumulated on artificial structures at Roberts Point (RP), Picnic Bay (PP) and Roberts Bay (RB).

Source	*df*	**Number**			**Weight**			**F versus**
		MS	**F**		**MS**	**F**		
Time	1	32.54	9.34		46.8065	6.33		Ti x Si
Site	2	55.67	15.99		85.2861	11.54		Ti x Si
Treatment	3	25.39	0.00		42.4703	0		NO TEST
Ti x Si	2	3.48	2.43		7.3886	2.15		Res
Ti x Tr	3	4.01	1.95		16.3644	4.13		Ti x Si x Tr
Si x Tr	6	11.12	5.41	*	20.4482	5.16	*	Ti x Si x Tr
Ti x Si x Tr	6	2.06	1.43	ns	3.9599	1.15	ns	Res
Residual	72				3.4408			
Total	95	1.43						
Transformation			Sqrt (X+1)			none		
Cochran's			ns			*		
SNK								
Si x Tr		RB: Bare=frame =5cm=20cm PP: Bare=frame =5cm=20cm RP: Bare=frame <5cm=20cm			RB: Bare=frame =5cm=20cm PP: Bare=frame =5cm=20cm RP: Bare=frame <5cm=20cm			

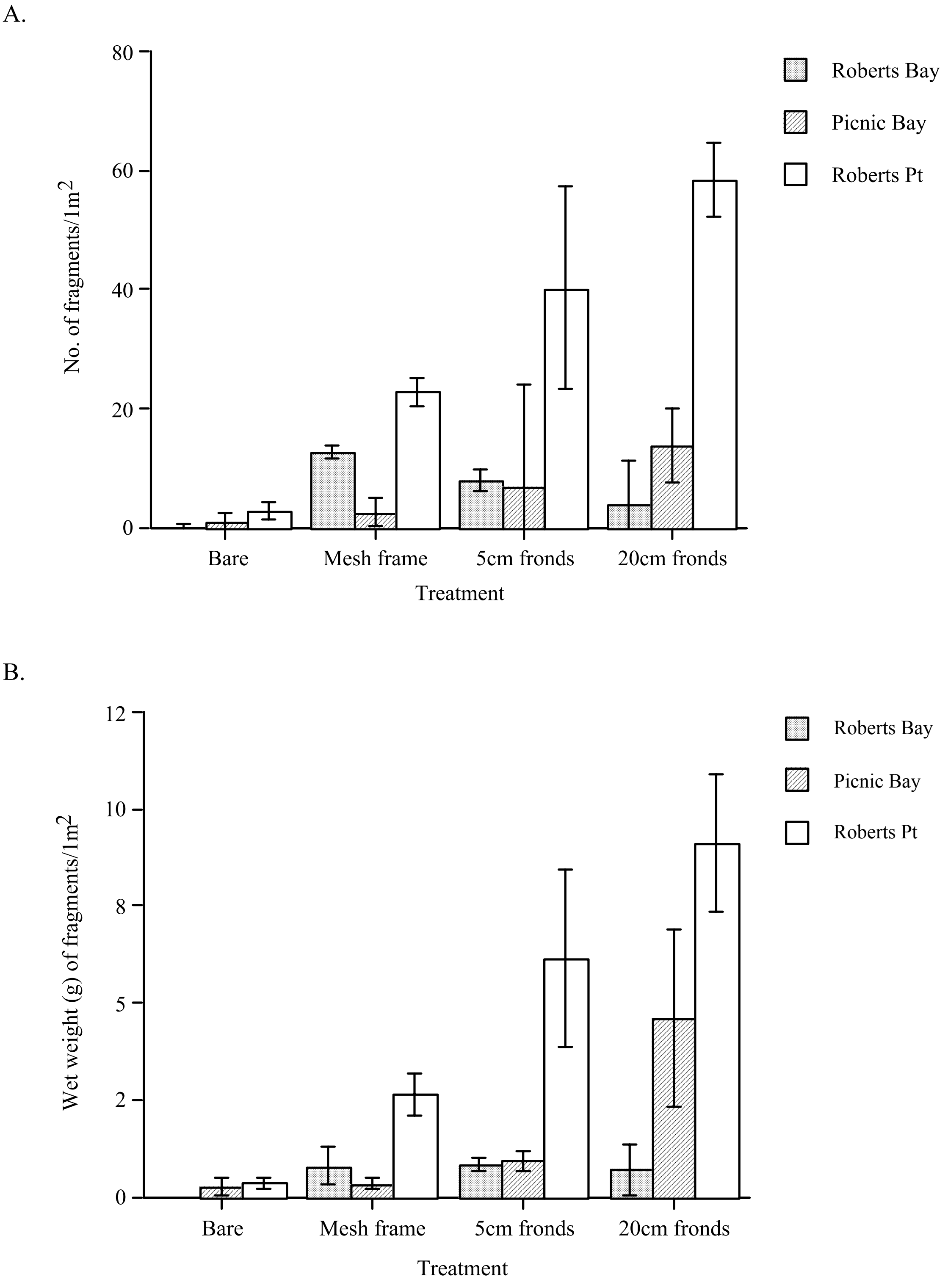
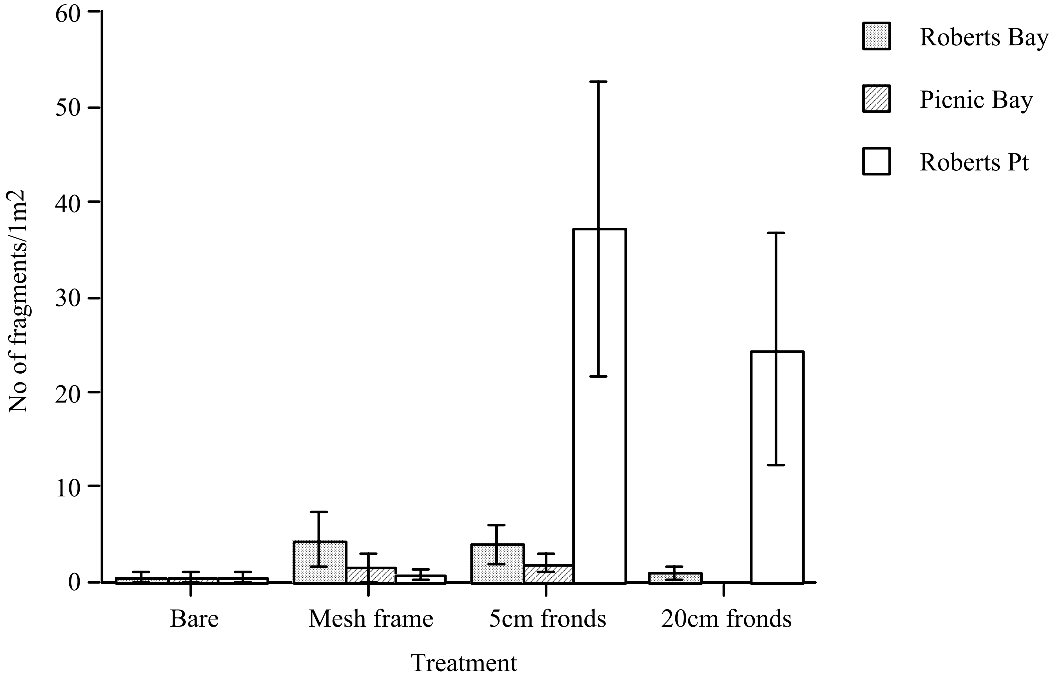


Figure 3.13. Mean (±se) number (A) and wet weight (B) of *C. taxifolia* fragments accumulated in each of four treatments in 3 sites at Lake Conjola between 13/01 and 12/02 2004. n=4 replicates.

A.



B.

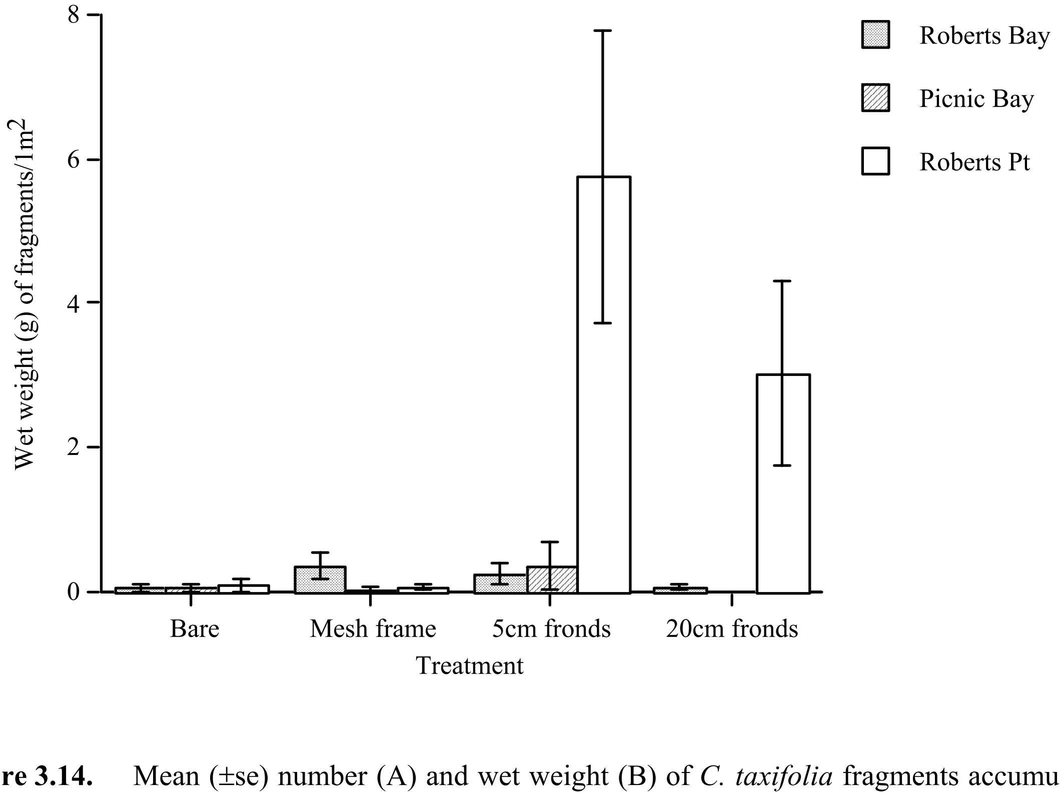


Figure 3.14. Mean (±se) number (A) and wet weight (B) of *C. taxifolia* fragments accumulated in each of four treatments: bare, frame only, short artificial fronds (5 cm) and long artificial fronds (20 cm) in 3 sites at Lake Conjola between 10/12/2003 and 13/01/2004. n=4.

3.2. Persistence and growth of newly established plants

A series of laboratory and field experiments was done to examine the persistence and growth of *C. taxifolia.* The growth of *C. taxifolia* in the field was measured in established patches and in patches adjacent to native seagrass. The coastal lakes and estuarine environments where *C. taxifolia* has established in NSW (see Chapter 2) are subject to significant, and often rapid changes in salinity. Thus, the response of fragments of *C. taxifolia* to a range of salinities was also examined.

3.2.1. Stolon extension within and on the edge of established C. taxifolia beds

3.2.1.1. Methods

Stolon growth of *C. taxifolia* was examined at three locations within Lake Conjola (West Conjola, Roberts Point and Adder Bay), each location containing two sites. This experiment was done to determine the rate of stolon extension within the interior and on the edge of established patches of *C. taxifolia*. At each site, stakes were used to mark out two permanent transects, one on the edge where stolons extended over bare substrata and one in the interior where stolons were within thick patches. Along each transect, 50 stolons were tagged with small numbered cable ties. The length (mm) of tagged stolons was measured approximately every month, for a period of one year (July 2002 until July 2003). Stolon growth was standardised to mm per day. If tags were not recovered during monthly sampling, new stolons were tagged and measured, so that a sample size of approximately 50 plants was maintained.

3.2.1.2. Results

Considerable fragmentation occurred at Roberts Point, which resulted in most tags being lost and no data being recorded for this location. Subsequently, this location was abandoned in October 2002, and tagging at two sites in Adder Bay began in December 2002. Overall, *C. taxifolia* had large and continuous stolon extension. Zero stolon growth was not recorded on any occasion or at any site, indicating that *C. taxifolia* is capable of some growth at all times of year. The highest mean stolon growth was 13mm per day at Adder Bay in January 2003.

C. taxifolia had high stolon growth from December to March and low growth from June to September (Figures 3.15, 3.16). Rapid growth in summer was associated with the warmer water temperatures recorded during these months (up to 26°C; A. Ferguson, pers. comm.). Stolons extending over bare substrata on the edge of patches grew faster than stolons amongst thick infestations in the interior of the *C. taxifolia* patches, but only during summer months (Figures 3.15, 3.16).

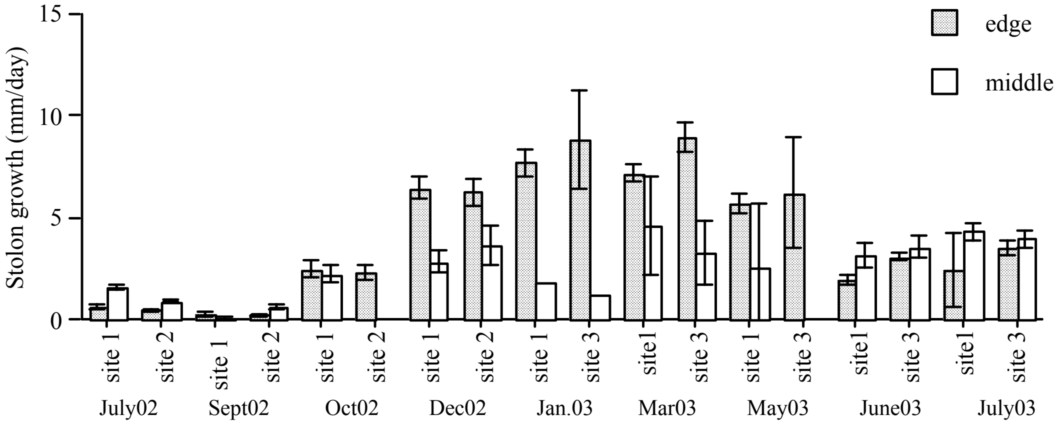


Figure 3.15. Average stolon growth (±se) within the interior and on the edge of established *C. taxifolia* patches at West Conjola, Lake Conjola between July 2002 and July 2003.

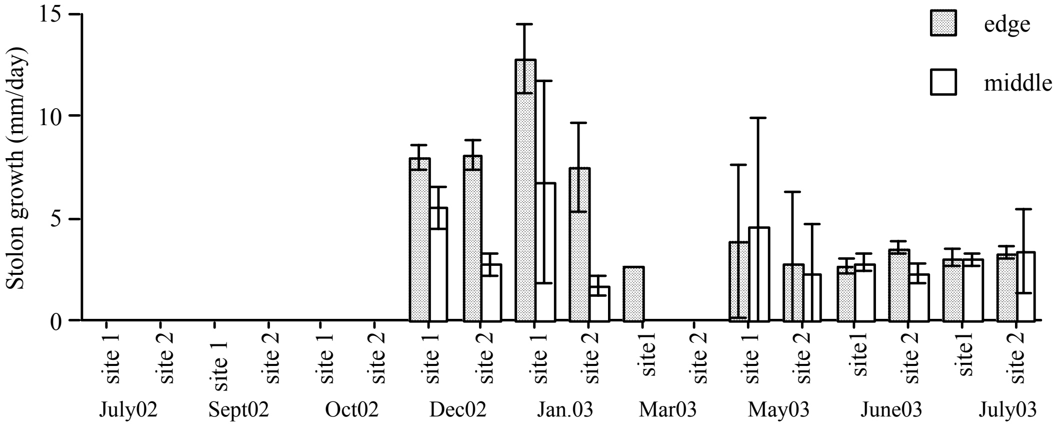


Figure 3.16. Average stolon growth (±se) within the interior and on the edge of established patches of *C. taxifolia* at Adder Bay, Lake Conjola between December 2002 and July 2003.

3.2.2. *Growth of C. taxifolia into Posidonia seagrass beds*

3.2.2.1. *Methods*

The spread of *C. taxifolia* adjacent to seagrass beds of *P. australis* was investigated within Port Hacking, at two locations, Gunnamatta Bay and Maianbar. Each location was divided into 3 zones as previously described (see section 3.1.3). At each location, 6 permanent transects were staked out perpendicular from the shore, through the 3 zones (Figure 3.17). Transects were approximately 2m apart and 15m and 10m in length at Gunnamatta Bay and Maianbar respectively. The distance of each of the three zones along each transect was recorded.

Six permanent quadrats ($0.25m^2$), 2 within each zone, were also sampled along each transect (Figure 3.17). Within each quadrat the percentage cover of *C. taxifolia*, the number of *P. australis* sheaths and the height (mm) of 5 haphazardly selected *C. taxifolia* fronds and 5 haphazardly selected *P. australis* shoots were recorded. Locations were sampled 9 times between January 2003 and February 2004 at Gunnamatta Bay and between March 2003 and March 2004 at Maianbar.

3.2.2.2. *Results*

At Gunnamatta Bay over the 13 month sampling period, the extent of the *C. taxifolia* zone increased along the transects and the extent of the transition zone decreased with limited change to the width of the *P. australis* zone (Figure 3.18). This suggests that the transition zone shrank because any sparse *P. australis* within it disappeared, subsequently resulting in a larger *C. taxifolia* zone. Conversely, the dense seagrass within the *P. australis* zone did not show any deterioration and the zone was not invaded by *C. taxifolia*.

At Maianbar, there was no significant change in the width of any of the three zones from March 2003 to June 2003. In June 2003, a storm event caused large amounts of freshwater input into Maianbar. By July 2003, *C. taxifolia* had disappeared from all six transects and it did not re-establish in the nine months after that (Figure 3.19). The absence of *C. taxifolia* in what was previously the 'transition' zone meant that any part of a transect which contained any *P. australis* was now classified as '*Posidonia*' zone, resulting in an apparent increase in the width of this zone (Figure 3.19).

The dieback of *C. taxifolia*, which was evident throughout the whole of this location may have been a result of the large freshwater input. If so, this observation provides evidence that lowered salinity can cause significant mortality (A. Ferguson, pers. obs.). After July 2003, two other seagrasses, *Z. capricorni* and *Halophila* sp., were observed colonising the areas where *C. taxifolia* had died out.

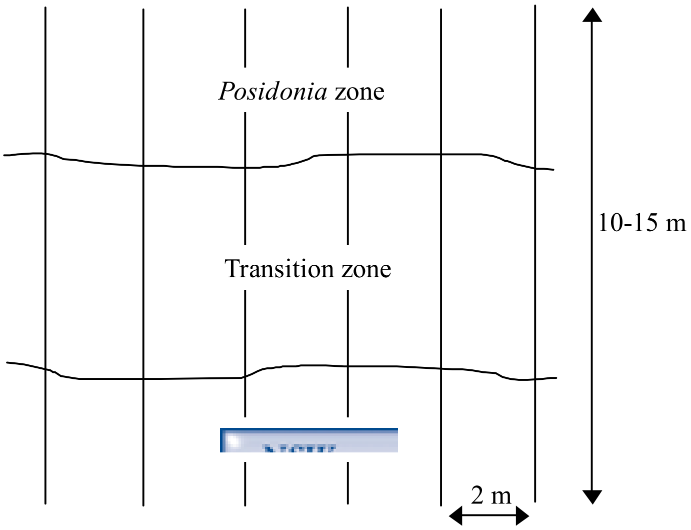


Figure 3.17. Diagrammatic representation of the experimental design to examine the spread of *C. taxifolia* among three zones at the boundary between the seagrass *Posidonia australis* and *C. taxifolia*.

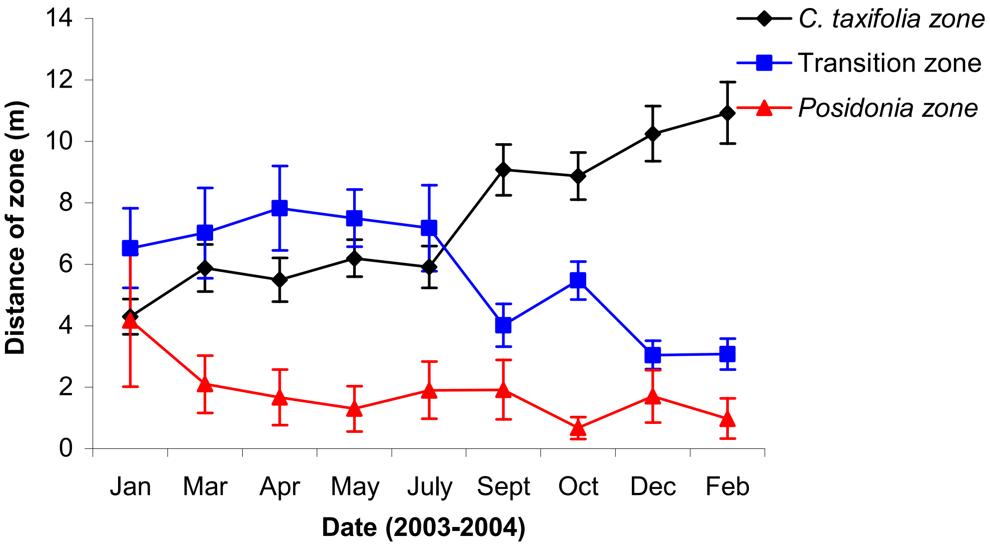


Figure 3.18. Average distance (±se) occupied by each of three zones along transects in Gunnamatta Bay between January 2003 and February 2004.

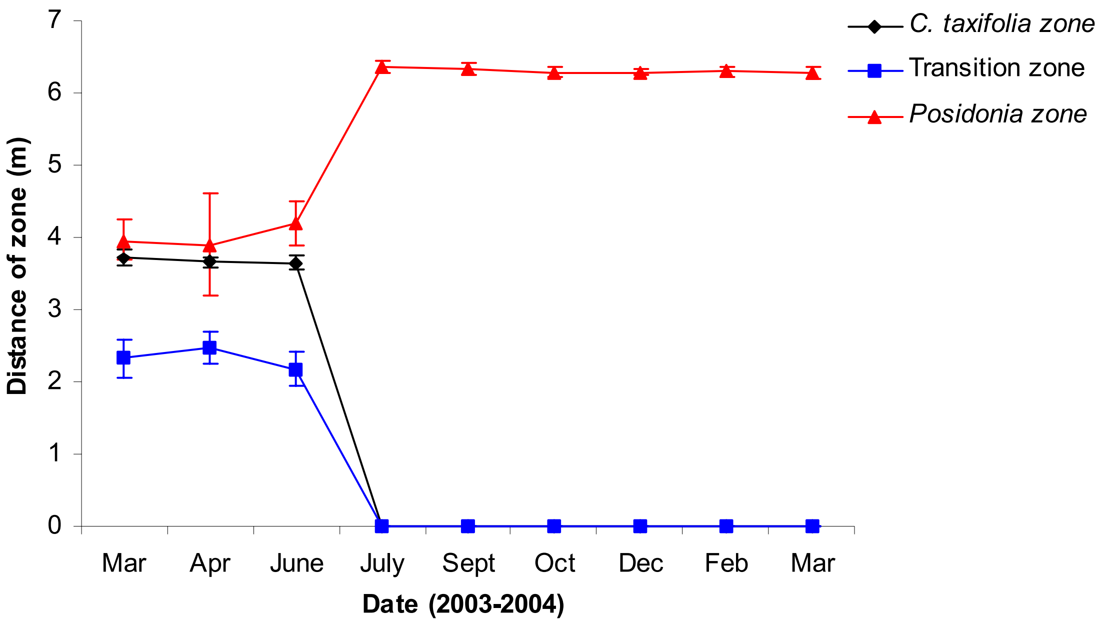


Figure 3.19. Average distance (±se) occupied by each of three zones along transects at Maianbar, between March 2003 and March 2004.

3.2.3. Survivorship of fragments in response to salinity

3.2.3.1. Methods

A laboratory experiment was established to examine fragment mortality following exposure to reduced salinity. Experiments were done in a temperature-controlled laboratory at the University of Wollongong and illuminated with triphosphate fluorescent tubes. *C. taxifolia* and seawater were collected from Lake Conjola, transported to the laboratory and placed in seawater (30ppt) for 2 days prior to experiments. Fragment portions were cut to include a frond (<5 cm) and a short piece of stolon. Twenty fragments were placed in each of thirty-six plastic containers containing 2 L of water. Containers were randomly assigned one of the three different salinity treatments; 10 ppt, 15 ppt and 30 ppt (control) based on salinity levels recorded in the coastal estuaries it has invaded. Six replicate containers of each salinity treatment were exposed for periods of each of 24 hrs (pulse) and 7 days (press), after which time salinity was maintained at 30 ppt. After 10 days, the percentage of fragments that were bleached was recorded (as a measure of mortality). Analysis of covariance tests were used to test for significant differences, with the light received by each replicate being the covariate.

3.2.3.2. Results

Mortality was highest in fragments exposed to 15ppt for one week (Figure 3.20). Fragments showed similar levels of mortality when exposed to either 10ppt or 15ppt for 24 hours, but there was a higher mortality of fragments held at 15ppt than at 10 ppt when exposed for one week. Fragments exposed to 15ppt had a higher mortality when exposed for one week than those exposed for 24 hours. In contrast, fragments exposed to 30ppt and 10ppt had significantly higher mortality when exposed for 24 hours than those exposed for one week (Figure 3.20). Two factor ANCOVA confirmed that there was a significant interaction between salinity and duration of exposure. The covariate (light received) was not significant (Table 3.3). Overall, exposure to low salinity increased the mortality of *C. taxifolia* compared to the control treatments.

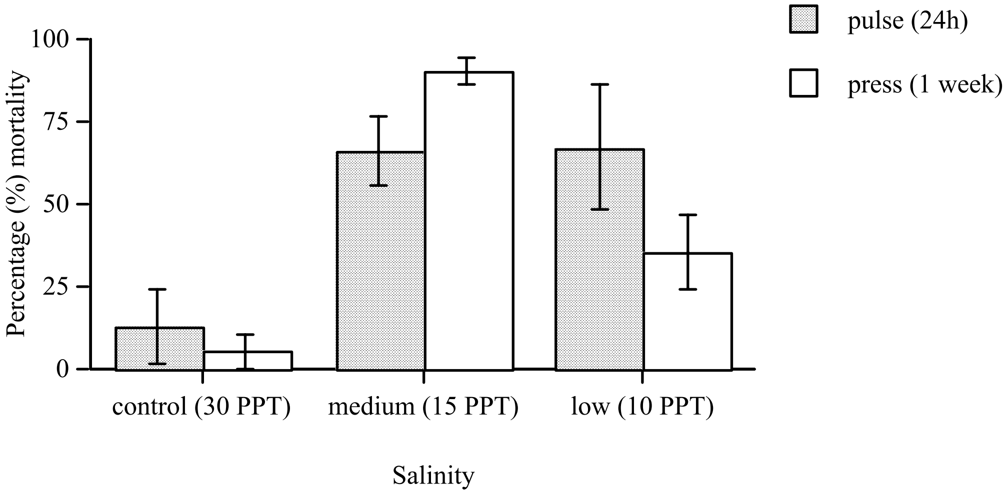


Figure 3.20. Average mortality (±se) of *C. taxifolia* fragments under three different salinity regimes (30ppt, 15ppt and 10ppt) for pulse (24hrs) and press (1 week) time periods. n=6.

Table 3.3. Two factor ANCOVA of exposure of fragments of *C. taxifolia* to reduced salinity. n = 6, with 20 fragments per replicate.

Source	df	Sum of Squares	F Ratio	Prob >F
salinity	2 29214.51	17.70	0.00	
treatment	1 93.91	0.11	0.74	
treatment*salinity	2 4409.05	2.6719	0.09	
Covariate (kilolux/sec)	1.00	231.06	0.28	0.60
Error	29 23927.27			
C Total	35.00	57755.56		

3.3. Predation on *C. taxifolia*

Biological control has often been proposed for controlling invasive pests (Van Driesche and Bellows 1996) including marine algae, but some authors have urged caution in the application of biological control in marine systems (e.g. Secord 2003). The great taxonomic diversity and complexity of marine systems, combined with the fact that biological control is in its infancy in marine systems, means that the introduction of yet another species rarely will be given serious consideration in the marine environment. Rather than seeking exotic biological control agents, enhancing populations of native predators might be a less perilous approach (Chang and Karieva 1999). The possible use of this technique (sometimes called 'augmentative biocontrol') for invasive *C. taxifolia* requires detailed information on the responses of native consumers to this alga. Previous research into interactions between herbivores and *Caulerpa* spp. was done in tropical waters (Hay 1984; Paul & Fenical 1987). Research undertaken at the University of Wollongong, partly in conjunction with this project, investigated which native species might eat *Caulerpa* spp. in temperate Australian waters and evaluated their possible application in augmentative biocontrol.

Previous research on two *Caulerpa* spp. that had become locally very prevalent in some areas of NSW (*C. filiformis* and *C. scalpelliformis*) was augmented by similar trials on *C. taxifolia*. The focus was on large, common, reef-dwelling molluscs, urchins and fish, as these generalist consumers can, through their feeding activities, control the structure of shallow, subtidal, algal assemblages in temperate Australia. Solvent and aqueous extracts of the three *Caulerpa* spp. were incorporated into palatable agar discs and offered to the grazers in laboratory tanks and field trials. Discs were used as it was considered too risky to use living fronds of these invasive species in the field because they can regrow from very small fragments which might have established new populations in the study area. Responses of the large grazers to extracts of *Caulerpa* spp. were evaluated to infer their ability to control the persistence or spread of these algae. This research has been written up and submitted as a manuscript to the scientific journal (see Appendix 2a). None of the herbivores examined in that study particularly liked eating *Caulerpa* spp. and they were considered to have no potential for curbing the rapid expansion of *C. taxifolia* or the other two species investigated.

A second study documented the assemblages of small invertebrates found on four species of *Caulerpa* (including *C. taxifolia*) from a variety of locations and habitats in NSW and examined the feeding of four common herbivorous gastropods on *C. filiformis*. Twenty-nine species of invertebrates were recorded from *C. taxifolia* from sites in Lake Conjola and Port Hacking. The small sacoglossan mollusc, *Oxynoe viridis*, was found on all *Caulerpa* spp. although rare on *C. taxifolia*. This species was observed consuming *C. taxifolia* in laboratory feeding trials, and has also been seen actively feeding on living *C. taxifolia* in lake Conjola (J. Wright, pers. comm.) and Lake Macquarie (P. Gibson, pers. comm.). A summary of this Honours project (Edwards 2003) is given in Appendix 2b. The feeding biology of *O. viridis* and other opisthobranchs that feed on *Caulerpa* spp. (such as *Elysia tomentosa* which is very common on *C. taxifolia* in Port Hacking and Botany Bay; T. Glasby pers. obs.) probably warrants further investigation.

Finally, the interaction between potential herbivores and *C. taxifolia* was studied in Lake Conjola. This study revealed that four abundant and widespread mesograzers within this coastal estuary: the fish, *Girella tricuspidata*; the sea hare *Aplysia dactyomela*; the polychaete *Platynereis dumerilii antipoda*; and the amphipod *Cymadusa setosa* were occasionally found on, or in close proximity to, *C. taxifolia.* The latter two species strongly avoided *C. taxifolia* in feeding preference experiments, preferring instead species of brown algae. The amphipod would only eat *C. taxifolia* when it was the only food item available, and the sea hare also strongly avoided *C. taxifolia*. Luderick (*G. tricuspidata*) have been observed to bite on *C. taxifolia* fronds, and fragments of this alga have been found in the guts of fish speared in Lake Conjola (J. Sakker, pers. comm.). However, Gollan's work and subsequent experiments suggest that *C. taxifolia* in Lake Conjola is experiencing only weak grazing pressure from luderick and other native herbivores. They are unlikely, therefore, to modify the persistence or spread of invasive *C. taxifolia*. A summary of this Honours project (Gollan 2003) is given in Appendix 2c.

3.4. Discussion

3.4.1. Asexual Reproduction: fragmentation and stolon extension

In the absence of evidence for sexual reproduction, it appears that asexual reproduction via fragments and stolon growth contribute importantly to the establishment and spread of *Caulerpa taxifolia*. Large numbers of unattached fragments are always present in or near infestations of *C. taxifolia* and have the potential to disseminate and produce new infestations. It has been suggested that detached fragments are capable of wide natural dispersal; for example, drifting fragments were observed from a submarine at depths of 45-100m in the Mediterranean (Belsher and Meinesz 1995). Work in the Mediterranean also confirms that drifting fragments can attach and successfully establish, although their subsequent success (i.e. continued growth and expansion) shows considerable spatial and temporal variability (Ceccherelli and Cinelli 1999a). These authors attributed the observed differences to a number of factors including temperature, type of substratum and water flow. The means by which fragments are generated by anthropogenic activities and subsequently transported to new locations are considered in chapter 4.

Field observations strongly suggest that water movement associated with storms has enormous potential to create fragments of *C. taxifolia* and this may account for the spatial and temporal variation observed in fragment abundance. The extent to which natural fragmentation contributes to the overall fragment abundance remains unclear, but these experiments showed that fragments were at least twice and up to six times as abundant after a storm than at other times of sampling. Although creating fragments, storms did not appear to alter the size range of fragments observed. Numbers of all types of fragments increased following storms, but their relative sizes remained the same.

This study has not examined the fate of drifting fragments and so the relative importance of fragment type (frond, stolon and thallus) and size in establishing new plants is unknown in the field setting in NSW. Laboratory experiments confirm that all fragment types were capable of regrowing from very small fragments and thereby have the potential to establish new infestations. Once an infestation is established stolon growth leads to the rapid cover of the substratum by the alga. Stolon growth was strongly seasonal, peaking during summer at rates of up to 13 mm per day. The growth rates of stolons were faster over bare substrata than in the middle of dense patches of *C. taxifolia*. Experiments removing stolons and adding fragments provide evidence that stolon growth rather than the presence of fragments contributes most significantly to increases in biomass as infestations spread. As this work was done under the auspices of an ARC Postdoctoral Fellowship it will be reported elsewhere.

Interactions with seagrasses

Experiments and monitoring reveal that fragments of *C. taxifolia* are often positively correlated with structural heterogeneity. Given these findings, the biogenic structure that seagrasses impart onto these habitats would be expected to trap fragments and thereby heighten interactions among these organisms. Preliminary data from seagrass beds indicate that fragments of *C. taxifolia* are frequently spread throughout beds of *Zostera capricorni,* but are not present in dense beds of *Posidonia australis.* Instead fragments accumulate at the edges of the beds of this slow growing seagrass. Stolons of *C. taxifolia* can extend beneath the canopy of *Posidonia australis* and, over a 14 month period, we documented some seagrass loss, although the mechanism by which this occurs is not known and is deserving of much closer attention.

Interactions with herbivores

The responses of native herbivores to *C. taxifolia* indicate that they are unlikely to intercede in the spread or control of this invader. Laboratory and field feeding trials show avoidance of fronds of *C. taxifolia* or solvent extracts of this alga; in field trials, palatable agar feeding discs were used to assess the responses of native herbivores to extracts of *C. taxifolia*, ensuring no chance of disseminating the alga. In “no choice” feeding trials herbivores often consumed *C. taxifolia* or its extracts, but when offered a choice of algae, *C. taxifolia* was ranked low in preference. Small *saccoglossan* molluscs will consume *C. taxifolia*, but their distribution is extremely patchy and hence their utility in ‘augmentative biocontrol’ remains to be established.

4. VECTORS THAT MAY TRANSFER THE WEED TO NEW LOCATIONS WITHIN NSW

The generation of fragments and their subsequent attachment and regrowth are accepted as being the processes by which invasive *C. taxifolia* reproduces, disperses and establishes new infestations (Belsher and Meinesz 1995; Ceccherelli and Cinelli 1999a; Millar 2002). We identified several mechanisms, both natural and human associated, that might generate fragments from established patches of *C. taxifolia* and which could assist in the transportation of those fragments to other sites, either within the same bay or estuary or to more distant locations (Table 4.1).

Table 4.1. Potential mechanisms for generating and transporting fragments of *C. taxifolia* and their relative importance in NSW estuaries.

Vector	Places of greatest risk	NSW examples (as at January 2004)
1. Human mediated		
Commercial fishing nets	Estuaries where commercial hauling is undertaken	Pittwater, North Sydney Harbour. Other estuaries with *C. taxifolia* are now closed to commercial fishing
Recreational fishing gear (lines or nets)	Estuaries that are popular fishing locations	Most currently infested sites
Diving equipment (e.g. wetsuits, fins)	Estuaries that are popular swimming or spear-fishing locations	Lake Macquarie, Narrawallee Inlet
Boat propellers or hulls, water skis, trailers	*Sites adjacent to boat ramps, permanent mooring sites or marinas*	Lake Conjola, Port Hacking
Anchors / anchor chains	Sheltered bays that are popular anchoring locations for casual use	Most currently infested sites except Narrawallee
Release from aquaria	Sites where aquarium stores or public aquaria hold *C. taxifolia* and discharge seawater directly to the sea	None known. The possession of *C. taxifolia* in the coastal fringe is now illegal in NSW
Aquaculture	*Estuaries where oyster farming is done*	Burrill Lake, Lake Conjola, Botany Bay
2. Natural		
Ocean currents, tides & wave action	Large, open areas with considerable wave fetch or strong currents	Lake Macquarie, Botany Bay, Western Lake Conjola (wind); Narrawallee (tidal currents)

There are several natural vectors that may aid the fragmentation and translocation of *C. taxifolia*, such as storms (see Chapter 3.1.1), currents, disturbance of the seafloor by feeding animals such as fish and rays or the feeding activities of herbivores such as sacoglossan molluscs that are eating the alga (Coquillard *et al*. 2000; Thibaut *et al*. 2000; Thibaut *et al*. 2001). These natural processes are most likely to contribute to spread within a location such as an estuary. These vectors become increasingly important as the amount of *C. taxifolia* at a site expands, and they may overshadow the importance of human associated vectors when infestations cover large areas such as in Lake Conjola and Botany Bay in NSW (see Chapter 2). Natural processes associated with water movement, such as the ripping out of plants, rapid transport of fragments or the burial of plants under sediments (e.g. Glasby *et al*, in review) may occur periodically (e.g. storms) or on a more regular basis where there are strong tidal currents (eg in Narrawallee inlet. Spread to a new location could potentially occur if fragments are washed out to sea, carried by currents along the coast, and then swept into a new bay or estuary. However, this risk is considered slight in NSW (Millar 2002). Vectors associated with human activities are considered much more likely to cause the transportation of fragments to more distant locations.

Caulerpa taxifolia is a popular marine aquarium plant throughout the world, including Australia, and it has been sold for many years within the aquarium industry. It appears that *C. taxifolia* has been introduced into several waterways in the Mediterranean, California and Australia, accidentally or otherwise, from aquaria. Plants may escape directly from large aquaria with flow-through seawater systems (eg aquarium shops, public display tanks, research institutions) where these are situated immediately adjacent to coastal waterways. Introductions may also occur if the unwanted contents of smaller aquaria that contain fragments of *C. taxifolia*, such as household fish tanks, are tipped into brackish creeks, estuaries or sheltered coastal embayments.

Many commercial activities on waterways infested by *C. taxifolia* can be significant in generating and spreading fragments of the alga. These include commercial fishing (eg shore-based hauling, trawling in estuaries), dredging or sand extraction, building or maintenance of foreshore structures such as wharves, jetties or boat ramps and the deployment of channel markers, boat moorings or other floating devices that need to be anchored to the seafloor. These mechanisms are likely to be most important where infestations of *C. taxifolia* occur adjacent to large urban areas or near commercial ports. Examples in NSW are Pittwater, Sydney Harbour, Botany Bay and Port Hacking (see Figure 1.1).

Finally, there are human leisure activities that may generate, trap and transport fragments of *C. taxifolia*, including passive pursuits such as swimming, snorkelling or diving and more active pursuits such as boating, water skiing, anchoring or recreational fishing. These mechanisms are likely to be most important where infestations of *C. taxifolia* occur in smaller or more isolated areas. Examples in NSW are the southern lakes such as Conjola, Burrill and Narrawallee (see Figure 1.1). When fragments of *C. taxifolia* removed from the water are kept moist for several days (eg in fishing nets or the anchor wells of boats), this provides a potential vector for transportation to another estuary. It is activities associated with human leisure pursuits in these southern lakes that are investigated in this chapter in an attempt to quantify their likely contribution to the spread of *C. taxifolia*.

4.1. Patterns of *C. taxifolia* fragmentation

Spatial patterns of abundance and biomass of fragments were investigated within Lake Conjola and Port Hacking, between locations with different levels of anthropogenic activity. The hypothesis was that there would be more fragments in locations with high levels of anthropogenic activity.

Locations, approximately 900 m^2 and with > 90% cover of *C. taxifolia,* were classed as having either ‘high’ or ‘low’ anthropogenic activity, based on a series of observations. Locations designated “high activity” locations were adjacent to boat ramps and/or were observed to have more human activity (boats, fishers and swimmers) compared to low activity locations. At each location, *C. taxifolia* fragments were collected in 10 replicate quadrats and their abundance and biomass estimated. The number of locations that were infested with *C. taxifolia* and the intensity of anthropogenic activities were different between Lake Conjola and Port Hacking, therefore different experimental designs were used for each estuary.

At Lake Conjola, three locations of high activity were compared to three locations of low activity. High and low activity locations were sampled within Lake Conjola on two occasions, March and June 2003. The hypothesis that the abundance and/or biomass of fragments were significantly larger at locations of high compared to low anthropogenic activity was tested using a three factor ANOVA. Interactions of Time x Location (Activity) and Time x Activity were examined first. When there were no significant interactions, the main effects of Activity and Time were then examined. Post-hoc pooling or elimination of factors was used when possible (when $p > 0.25$) to construct an appropriate test.

At Port Hacking, one location of "high activity" was compared to two locations of "low activity" in March 2003. This design was necessary because few locations in Port Hacking were infested with *C. taxifolia.* Only one location in Port Hacking had much more anthropogenic activity compared to other locations invaded by *C. taxifolia*. Because the experiments in Port Hacking involved the comparison of one location of high anthropogenic activity to two locations of low anthropogenic activity, asymmetrical analyses were used.

4.1.1. Results

In Lake Conjola, the mean (± s.e.) abundances of fragments of *C. taxifolia* (per 1250 cm^2 quadrat) in the locations of high and low anthropogenic activity were 8.1 (± 3.1) and 1.5 (± 0.2) respectively, on the first sampling occasion, and 18.7 (± 3.8) and 7.9 (± 1.0), on the second sampling occasion.

There were significantly more fragments of *C. taxifolia* in locations of high anthropogenic activity compared to locations of low anthropogenic activity, on both sampling occasions (Figure 4.1; Table 4.2). Abundances of fragments were also more variable among locations of high anthropogenic activity than locations of low anthropogenic activity on both sampling occasions (Figure 4.1; Table 4.2). There were no significant interactions between any of the factors tested. Patterns of difference and variation remained similar for both sampling occasions, although overall there were significantly more fragments on the second sampling occasion (Figure 4.1; Table 4.2).

Similar trends in patterns of differences were observed for biomass. Mean (± s.e.) biomass (g dry weight) of fragments of *C. taxifolia* (per 1250 cm^2 quadrat) in the locations of high and low anthropogenic activity were 0.33 (± 0.12) and 0.05 (± 0.01) respectively, on the first sampling occasion, and 0.64 (± 0.12) and 0.17 (± 0.03), on the second sampling occasion. However, there were no significant differences in the biomass of *C. taxifolia* fragments between locations of high anthropogenic activity compared to those with low anthropogenic activity. There was more variability among locations of high anthropogenic activity compared to low (Figure 4.1; Table 4.2). There were no significant interactions between any of the factors tested. Overall, there were significantly higher biomasses of *C. taxifolia* fragments on the second sampling occasion, but patterns of difference and variation remained similar for both sampling occasions (Figure 4.1; Table 4.2).

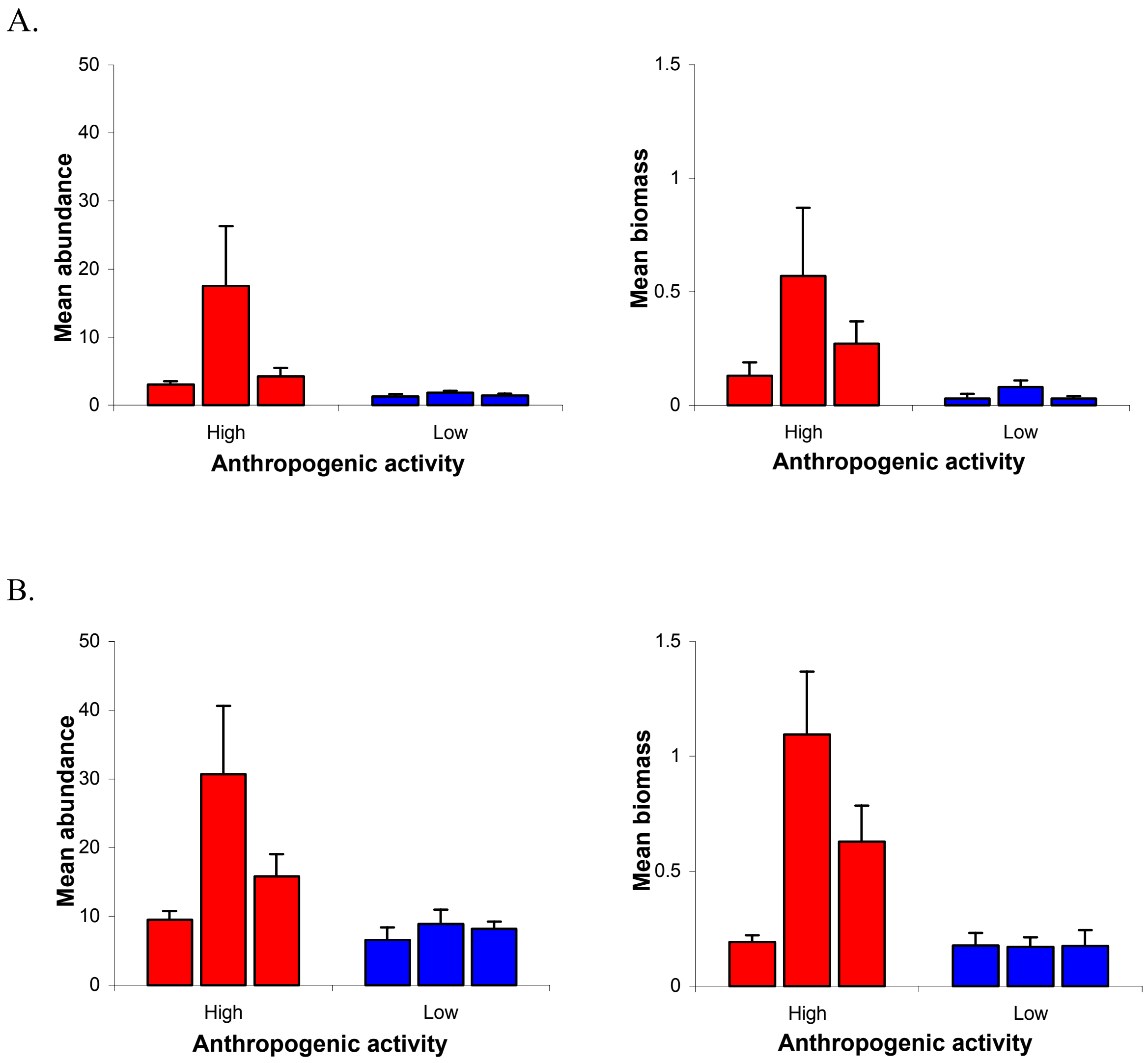


Figure 4.1. Mean (+s.e.) abundance (left) and biomass (g dry weight) (right) per quadrat, of *C. taxifolia* fragments sampled in Lake Conjola at three locations with high anthropogenic activity and three locations with low anthropogenic activity, on two sampling occasions, (A) 26th March 2003 and (B) 20th June 2003. n=10.

Table 4.2. Results of ANOVAs and SNK tests, for testing hypotheses about patterns of distribution of abundance and/or biomass of *C. taxifolia* fragments in Lake Conjola. Note: F-ratios in bold were calculated after non-significant (P>0.25) interactions were pooled or eliminated to test for main effects. ns=not significant; * <0.05; ** <0.01; *** <0.001.

Source	***df***	**Abundance**			**Biomass**			**F versus**
		MS	**F**		**MS**	**F**		
Time	1	34.04	161.79	***	0.75	14.88	*	Ti x Lo(Ac)
Activity	1	18.15	**8.77**	*	1.51	**4.77**	**ns**	**Lo(Ac)**
Location(Ac)	4	2.07	9.83	**	0.31	6.28	***	Ti x Lo(Ac)
Ti x Ac	1	0.03	0.13	ns	0.09	1.74	ns	Ti x Lo(Ac)
Ti x Lo(Ac)	4	0.21	0.37	ns	0.05	0.95	ns	Res
Residual	108	0.57		0.05				
Total	119							
Transformation			Ln (X+1)			Ln (X+1)		
Cochran's			ns			**		
SNK								
Time		T1<T2			T1 <T2			
Activity		High>Low						
Location(Ac)		High: L1=L3<L2 Low: L1=L2=L3			High: L2>L1; L2=L3; L2=L1 Low: L1=L2=L3			

In Port Hacking, the mean (± s.e.) abundances of fragments of *C. taxifolia* (per 1250 cm^2 quadrat) at locations with high and low anthropogenic activities were 49.3 (±4.9) and 8.8 (±1.1), respectively. However, because the control locations were significantly different from each other and the analysis had very few degrees of freedom, a significant difference between high and low activity could not be detected (Figure 4.2; Table 4.3).

The mean (± s.e.) biomass (g dry weight) of fragments per quadrat at the locations with high and low anthropogenic activity in Port Hacking were 1.79 (±0.34) and 0.10 (±0.01), respectively. The biomass of fragments was significantly larger at the location with high anthropogenic activity compared to the two locations with low anthropogenic activity (Figure 4.2; Table 4.3).

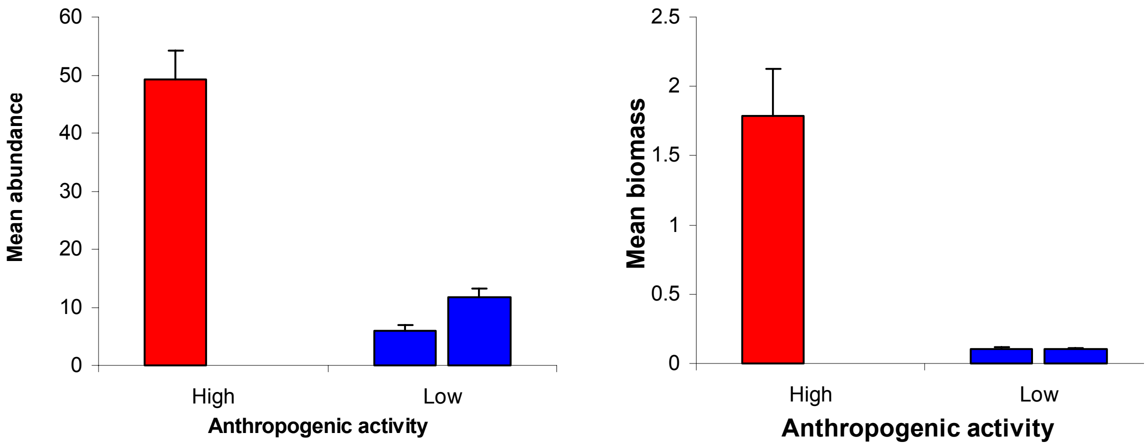


Figure 4.2. Mean (+s.e.) abundance (left) and biomass (g dry weight) (right) per quadrat of *C. taxifolia* fragments sampled in Port Hacking at one location with high anthropogenic activity and two locations with relatively low anthropogenic activity, sampled on 20th March 2003. n=10.

Table 4.3. Results of asymmetrical ANOVAs for testing hypotheses about patterns of distribution of abundance and/or biomass of *C. taxifolia* fragments in Port Hacking. ns not significant; * <0.05; ** <0.01; *** <0.001.

Source	***df***	**Abundance**			**Biomass**			**F versus**
		MS	**F**		**MS**	**F**		
Location	2	10.77			2.55			
Impact vs Control	1	19.44	9.31	ns	5.09	50921.00	***	B. Cs
Between Cs	1	2.09	13.09	**	0.00	0.00	ns	Res
Residual	27	0.16			0.04			
Total	29							
Transformation		Ln (X+1)			Ln (X+1)			
Cochran's		ns			**			

4.2. Generation of *C. taxifolia* fragments by boat propellers

4.2.1. Methods

Two manipulative experiments were done to examine the impact of boating activity on the creation of *C. taxifolia* fragments in Lake Conjola. The first experiment was done in June 2003, to test the hypothesis that the movement of boats creates fragments of *C. taxifolia.* Three 30m x 1m transects were haphazardly positioned approximately 10m apart in shallow water (<1m deep) in a dense bed of *C. taxifolia*. Abundance (total number) and biomass (dry weight) of fragments were estimated in each of six randomly placed 25cm x 50cm quadrats along each transect (Figure 4.3). A small motorboat, similar to those extensively used in the lake, was then driven along one transect. This transect was defined as the 'boat' impacted transect, while the other two without boating activity were 'control' transects. Abundance and biomass of fragments were then estimated again in each of six distinct randomly placed quadrats along each transect. Positions of all quadrats in each transect were chosen before sampling to avoid the same place being re-sampled before and after. Asymmetrical ANOVAs were used to test for significant interactions of abundance and/or biomass of fragments between the boat and control transects from before to after the impact.

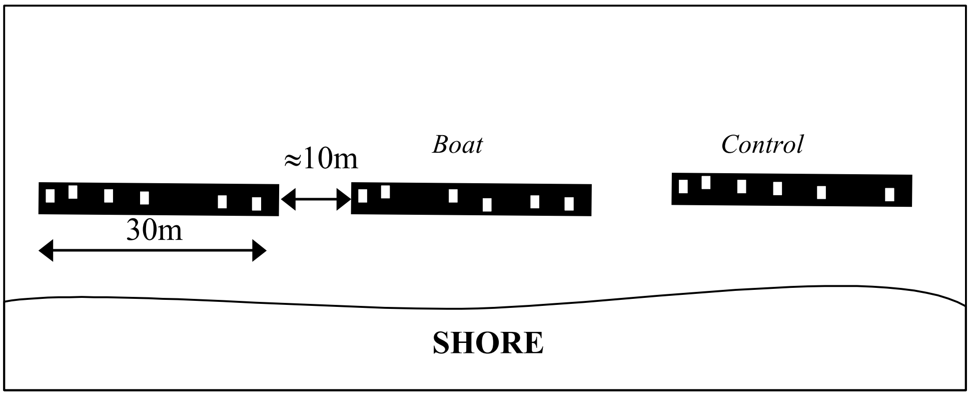


Figure 4.3. Diagrammatic representation of the experimental design to examine fragmentation along a boat-impacted transect and control transects on 4 June 2003. Fragment abundance and biomass were measured on each transect 'before' and 'after'.

A second experiment was done within a dense bed of *C. taxifolia* in Lake Conjola in September 2003. This experiment expanded on the previous experiment by testing for effects of boats at two different depths, shallow (approximately 1m) and deep (approximately 1.5 m). Four 30m x 1m transects, two 'boat' and two 'control,' were haphazardly positioned within each of these two depths (Figure 4.4). Abundance (total number) and biomass (g dry weight) of fragments were estimated in each of six replicate 25cm x 50 cm quadrats along each transect before and then again after the boat was driven along the 'boat' transects. Balanced, 4 factor ANOVAs were used to test for significant interactions of abundance and/or biomass of fragments between the boat and control transects from before to after the impact.

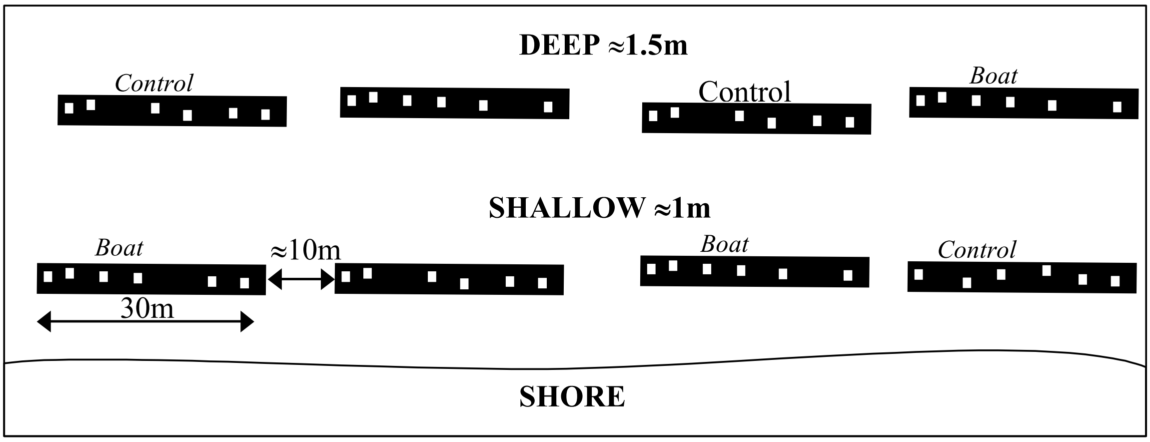


Figure 4.4. Diagrammatic representation of the experimental design to examine fragmentation along boat-impacted transects and control transects on 24th September. Fragment abundance and biomass were measured on each transect ‘before’ and ‘after’.

4.2.2. Results

In the first experiment (June 2003), the abundance and biomass of fragments were, on average, higher in the transect with boat activity (Figure 4.5). The abundance of fragments increased by 10 fold and the biomass of fragments increased by 4 fold. These apparent increases, however, were not always statistically significant when compared to control locations. Asymmetrical ANOVA tests showed that there was a significant ($p< 0.001$) increase in the biomass of fragments along the transect which had boat activity compared to control transects, but no significant difference in abundance of fragments (Figure 4.5; Table 4.4). The latter statistical test had few degrees of freedom, and caution should be used in interpreting this result (Table 4.4) because of low statistical power.

Results for the second experiment (September 2003) showed the same general patterns. Although there were small increases in the mean abundance of *C. taxifolia* fragments in the shallow transects after boating activity (Figure 4.6), there were no statistically significant increases in abundance at either depth (Table 4.5). Again, there were significant increases in biomass of *C. taxifolia* fragments from before to after boating activity in the boat transects compared to the controls at both depths (Figure 4.6; Table 4.5).

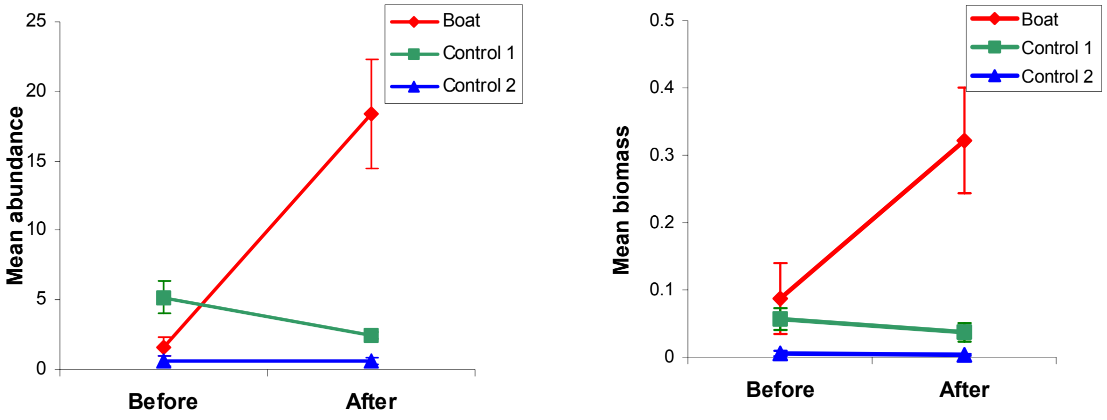


Figure 4.5. Mean (± s.e.) abundance (left) and biomass (g dry weight) (right) per 50x50cm quadrat of *C. taxifolia* fragments on one boat and two control transects before and after the impact of boat activity. Experiment was done in Lake Conjola on 4th June 2003. n=6.

Table 4.4. Results of asymmetrical ANOVAs for testing hypotheses about impact of boat activity on abundance and/or biomass of *C. taxifolia* fragments. Note: F-ratios in bold were calculated after non-significant (P>0.25) interactions were pooled or eliminated to test for main effects. ns not significant; * <0.05; ** <0.01; *** <0.001.

Source	***df***	**Abundance**				**Biomass**			
		MS	**F**		**F versus**	**MS**	**F**		**F versus**
Before vs After	1	0.46				0.055			
Location	2	1.12				0.032			
Impact vs Control	1	1.97				0.064			
Between Cs	1	0.28				0.001			
B v A x L	2	10.06				0.067			
B v A x I v C	1	19.70	46.00	ns	BvA x B.C.	0.130	25.12	***	Res
B v A x Between Cs	1	0.43	1.78	ns	Res	0.000	0.08	ns	Res
Residual	24	0.24				0.005			
Total	29								
Transformation			Ln (X+1)				Ln (X+1)		
Cochran's			ns				**		

Table 4.5. Results of ANOVAs for testing hypotheses about impact of boat activity at two separate depths on abundance and/or biomass of *C. taxifolia* fragments. ns not significant; * <0.05; ** <0.01; *** <0.001.

Source	***df***	**Abundance**			**Biomass**			
		MS	**F**		**MS**	**F**		**F versus**
Time	1	13.50			0.07			
Activity	1	63.38			0.16			
Depth	1	1204.17			5.07			
Transect (De x Ac)	4	53.94			0.19			
De x Ac	1	2.67			0.09			
De x Ti	1	0.04			0.05			
Ac x Ti	1	4.17	6.45	ns	0.14	10.56	*	Ti xTr (DexAc)
Ti xTr (De x Ac)	4	0.65	0.07	ns	0.01	0.31	ns	Res
De x Ac x Ti	1	2.04	3.16	ns	0.02	2.01	ns	Ti xTr (DexAc)
Residual	80	9.81			0.04			
Total	95							
Transformation			none			none		
Cochran's			ns			ns		
SNK								
Ac x Ti					Boat: T1<T2 Control: T1=T2			

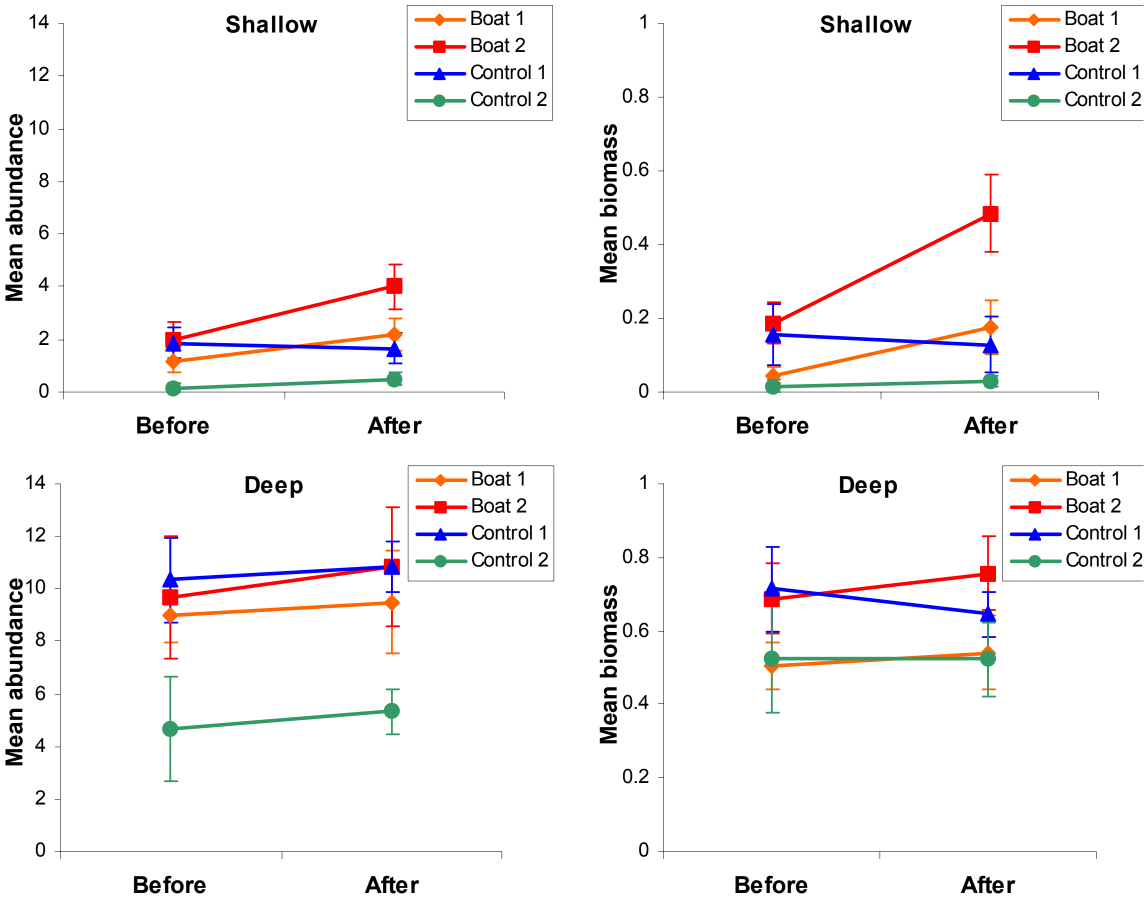


Figure 4.6. Mean (± s.e.) abundance (left) and biomass (g dry weight) (right) per 50x50 cm quadrat of *C. taxifolia* fragments on two boat and two control transects before and after the impact of boat activity, within two separate depth, shallow (upper) and deep (lower). n=6 replicates.

4.3. Generation of fragments by anchors

4.3.1. Methods

Manipulative experiments were done at Lake Conjola to test for differences in the amount of *C. taxifolia* removed from the lake by different types of anchors (rock or sand) and attachments (rope or chain) combinations (Plate 6). A total of six anchors (3 replicate sand anchors and 3 replicate rock anchors) were combined with four types of attachment (2 replicate ropes and two replicate chains). Each of the 6 possible combinations of anchor and attachment was lowered from the boat to the lake bottom and retrieved six times (i.e., there were 6 replicates for each trial). Each time, all fragments of *C. taxifolia* brought into the boat on the anchor and attachment were placed into zip-lock bags and taken back to the laboratory where the total biomass was determined. During the experiment the boat was allowed to drift over the bed of *C. taxifolia* to ensure that the anchor lowered was not on the same spot. To account for spatial and temporal variation, this experiment was done twice, May and July 2003, at two different locations. Four factor, partially nested ANOVAs were done to test for significant differences in the biomass of fragments associated with the type of anchor, type of attachment or combination of these.

Plate 6. Sand and rock anchors used in experiments examining the effect of anchors. Two replicate lengths of chain and two replicate lengths of rope were attached, in separate trials, to each anchor.

4.3.2. Results

On almost all occasions during both runs of the experiment, several fragments of *C. taxifolia* were caught on the anchors themselves. There were also fragments caught on chain attachments but rarely on rope attachments. Both experiments gave very similar results. The quantity of *C. taxifolia* brought to the surface was quite variable. While there were, on average, greater biomasses of fragments on sand anchors compared to rock anchors (Figures 4.7, 4.9), these data were highly variable and differences were not significant (Table 4.6). Regardless of the type of anchor used (sand or rock), significant differences were found with respect to the type of anchor attachment (chain or rope); there was significantly greater biomass of fragments on chains compared to ropes (Figures 4.8, 4.10; Table 4.7).

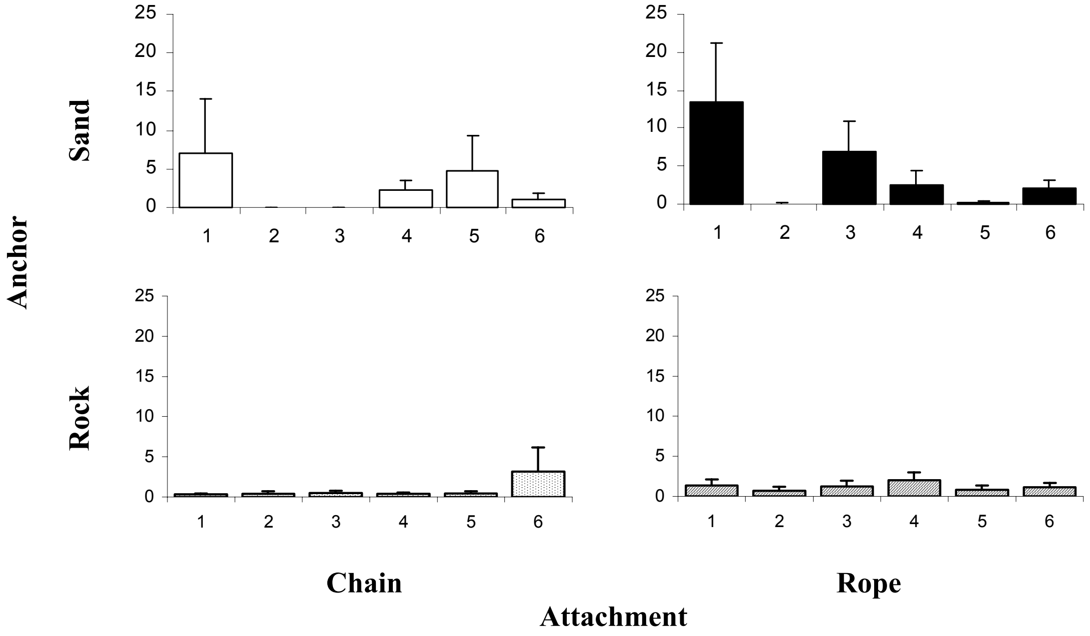


Figure 4.7. Mean (+s.e.) biomass (g dry weight) of *C. taxifolia* fragments removed from Lake Conjola on anchors. There were two types of anchor (with 3 replicates of each) and two types of attachments (with 2 replicates of each). This gives a total of 6 treatments along the x- axis. Experiments were done in Lake Conjola on 27th May 2003. n=6 trials with each combination of anchor and attachment.

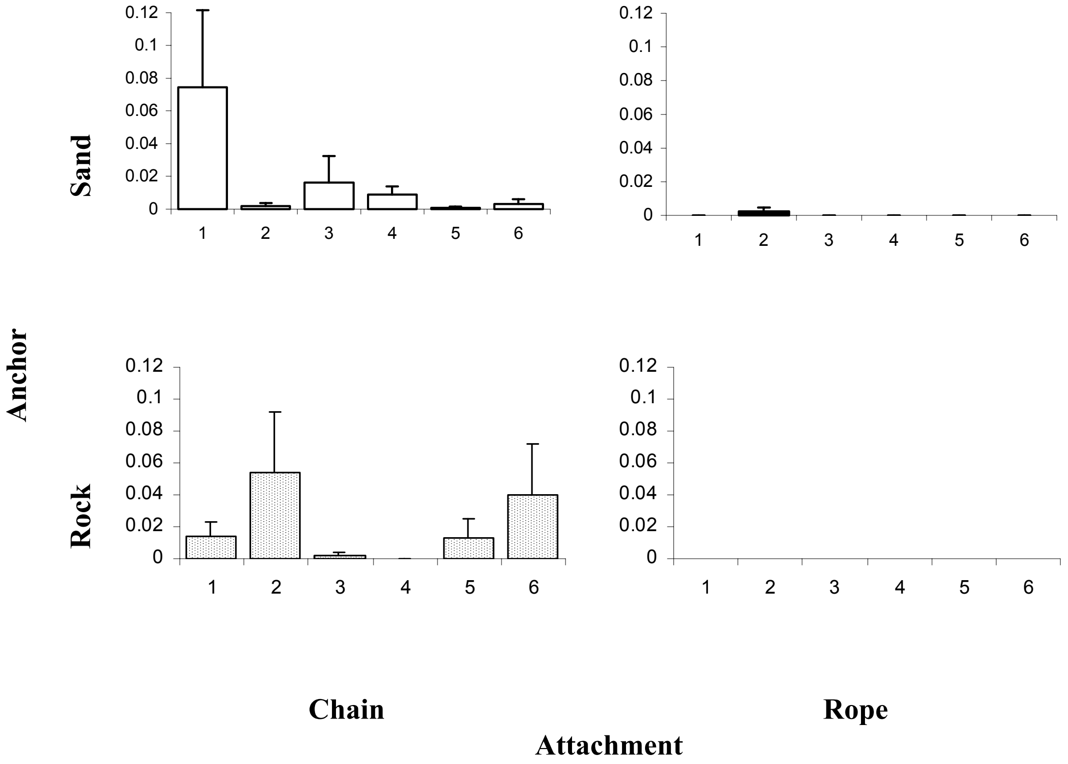


Figure 4.8. Mean (+s.e.) biomass (g dry weight) of *C. taxifolia* fragments removed from Lake Conjola on anchor attachments. Experimental design as in Figure 4.7. Experiments were done in Lake Conjola on 27th May 2003.

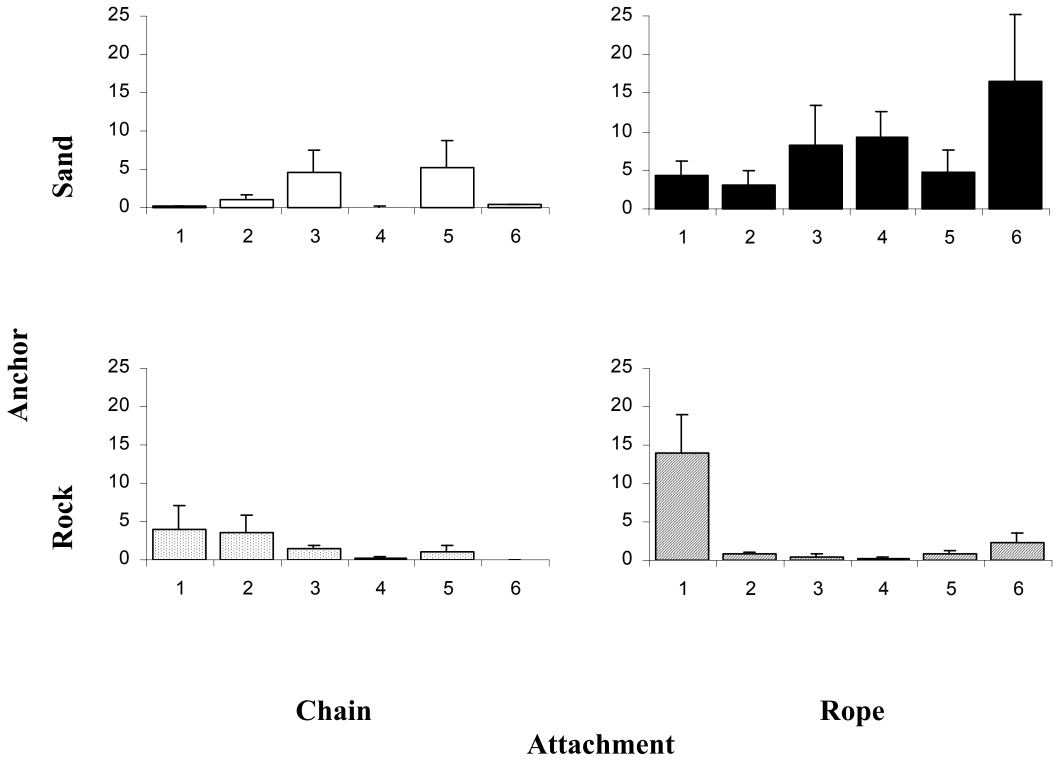


Figure 4.9. Mean (+s.e.) biomass (g dry weight) of *C. taxifolia* fragments removed from Lake Conjola on anchors. Experimental design as in Figure 4.7. Experiments were done in Lake Conjola on 28th July 2003.

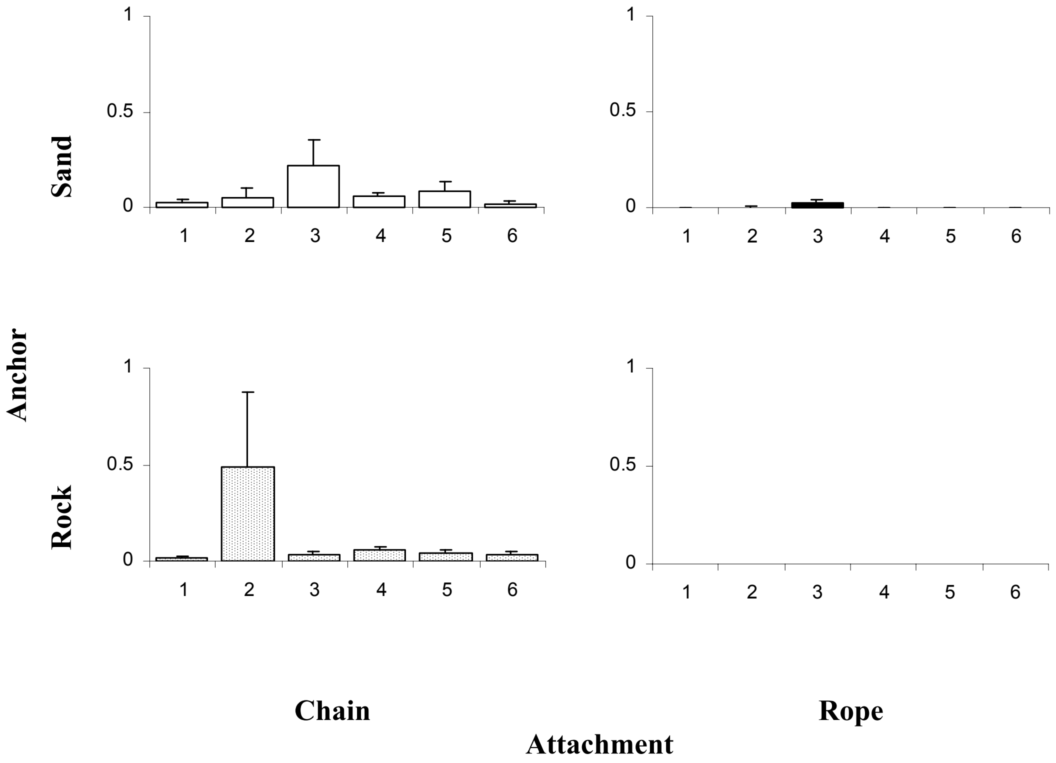


Figure 4.10. Mean (+s.e.) biomass (g dry weight) of *C. taxifolia* fragments removed from Lake Conjola on anchor attachments. Experimental design as in Figure 4.7. Experiments were done in Lake Conjola on 28th July 2003.

Table 4.6. Results of ANOVAs to test for differences in biomass of *C. taxifolia* fragments removed by anchors. Note: F-ratios in bold were calculated after non-significant (P>0.25) interactions were pooled or eliminated to test for main effects. ns not significant; * <0.05; ** <0.01; *** <0.001.

Source	***df***	**Experiment 1**			**Experiment 2**		
		MS	**F**	**F versus**	**MS**	**F**	**F versus**
Anchor	1	0.95	**0.49 ^{ns}**	**R(An)**	4.09	**0.01 ^{ns}**	**R(An)**
Replicate Anchor (An)	4	1.94	5.95*	R(At)xR(An)	0.87	0.43 ^{ns}	R(At)xR(An)
Attachment	1	3.74	**3.70 ^{ns}**	**AtXR(An)**	12.25	**9.28 ^{ns}**	**R(At)**
Replicate attachment (At)	2	0.85	2.62 ^{ns}	R(At)xR(An)	1.13	0.56 ^{ns}	R(At)xR(An)
An x At	1	0.17	**0.16 ^{ns}**	**At x R(An)**	5.27	**0.38 ^{ns}**	**AnxR(At)**
An x R(At)	2	0.75	2.30 ^{ns}	R(At)xR(An)	3.43	1.72 ^{ns}	R(At)xR(An)
At x R(An)	4	1.01	3.10 ^{ns}	R(At)xR(An)	1.97	0.98 ^{ns}	R(At)xR(An)
R(At) x R(An)	8	0.33	0.49 ^{ns}	Res	2.00	1.67 ^{ns}	Res
Residual	120	0.66			0.75		
Total	143						
Transformation			Ln (X+1)			Ln (X+1)	
Cochran's			*			ns	

Table 4.7. Results of ANOVAs to test for differences in biomass of *C. taxifolia* removed from Lake Conjola by the attachments, chain or rope, of anchors. Note: F-ratios in bold were calculated after non-significant ($P>0.25$) interactions were pooled or eliminated to test for main effects. ^{ns} not significant; * <0.05; ** <0.01; *** <0.001

Source	***df***	**Experiment 1**			**Experiment 2**		
		MS	**F**	**F versus**	**MS**	**F**	**F versus**
Anchor	1	0.000	0.07^{ns}	R(An)	0.000	0.38^{ns}	R(An)
Replicate Anchor (An)	4	0.002	0.88^{ns}	R(At)xR(An)	0.013	0.65^{ns}	R(At)xR(An)
Attachment	1	0.011	6.41*	R(At)	0.142	9.28 *	R(At)
Replicate attachment (At)	2	0.002	0.36^{ns}	R(At)xR(An)	0.015	0.79^{ns}	R(At)xR(An)
An x At	1	0.000	0.13^{ns}	AnxR(At)	0.000	0.38^{ns}	R(An)
An x R(At)	2	0.001	0.45^{ns}	R(At)xR(An)	0.001	0.04^{ns}	R(At)xR(An)
At x RA(An)	4	0.002	0.94^{ns}	R(At)xR(An)	0.011	0.56^{ns}	R(At)xR(An)
R(At) x RA(An)	8	0.002	1.58^{ns}	Res	0.020	1.25^{ns}	Res
Residual	120	0.001			0.009		
Total	143						
Transformation			Ln(X+1)			Ln(X+1)	
Cochran's			**			**	
SNK							
Attachment			Chain>Rope			Chain>Rope	

4.4. The ability of *C. taxifolia* to survive exposure to air

Experiments in the Mediterranean have indicated that *C. taxifolia* could survive for many days once removed from the water (Sant *et al.* 1996). The so called 'boat transportation hypothesis' is consistent with the appearance of new infestations at sites quite some distant from established populations. Here, the role that boats may play in transporting *C. taxifolia* once removed from the water was assessed under conditions prevailing in NSW. Exposure to air is thought to be the main factor affecting the viability of fragments once onboard a vessel. It has also been suggested that *C. taxifolia* may avoid desiccation if covered by wet rope, attached to wet fishing gear, or placed in shaded areas, such as in anchor boxes or in the bow of a boat. It is also possible that the size of clumps of *C. taxifolia* may influence viability under desiccating conditions. An experiment was designed to examine the survival of *C. taxifolia*, out of water, under a variety of conditions and for several periods of time.

4.4.1. Method

C. taxifolia was collected from Lake Conjola in October, 2003, transported to the laboratory and divided into clumps of three sizes. Each clump of *C. taxifolia* was placed in a 30 cm x 15 cm aluminium tray randomly positioned in one of four large fibreglass containers (1 m x 2 m). The fibreglass containers were positioned in direct sunlight. To test if wet rope prolonged the survivorship of *C. taxifolia,* small piles of rope, previously soaked in seawater, were placed on half of the fragments in each large container. To test the effects of shade, two of the containers were completely covered with a piece of wood, placed 50 cm over the trays (to allow airflow). The shading treatment was designed to simulate anchor box conditions at the bow of a boat. Consequently, 3 factors were investigated; period of desiccation, presence/absence of wet rope, and shade vs exposure to direct sunlight.

Two replicate fragments from each combination of factors were collected after each of 3 periods of time; 1 hour; 1 day; and 3 days of desiccation. Once collected, they were placed into re-circulating seawater aquaria and their viability was determined. Fragments were recorded as being viable if they had any green colouration after one week in the aquaria; non viable fragments were colourless and either limp or in the process of disintegrating. The desiccation time intervals were chosen to represent realistic periods that *C. taxifolia* may be kept out of water during normal boating activities. For example, 1 hour may represent the period of time an angler takes to move between fishing locations within an estuary. One to three days may represent the period of time it takes for a boat to be transported between estuaries.

4.4.2. Results

All large clumps of *C. taxifolia*, and some medium and small clumps, were viable after one hour out of water under all experimental conditions (Table 4.8). Some medium and large clumps of *C. taxifolia* remained viable after the 1 day period of desiccation, but all clumps, regardless of size or treatment, were dead after the 3 day period of desiccation (Table 4.8). These preliminary data indicate that *C. taxifolia* fragments that are lodged in anchor wells or on fishing gears may survive short periods of desiccation which would allow them to be transported to new sites within an estuary or to new sites in nearby estuaries.

Table 4.8. Number of small, medium and large clumps of fragments that were viable after 1 hour, 1 day and 3 days of desiccation under experimental conditions. Note that each treatment combination initially had four viable clumps of fragments.

Clump size			**Small**	**Medium**	**Large**
1 Hour	**Sun**	**Rope**	2	4	4
		No Rope	1	2	4
	Shade	**Rope**	3	4	4
		No Rope	1	3	4
1 Day	**Sun**	**Rope**	0	1	1
		No Rope	0	0	0
	Shade	**Rope**	0	1	3
		No Rope	0	1	1
3 Days	**Sun**	**Rope**	0	0	0
		No Rope	0	0	0
	Shade	**Rope**	0	0	0
		No Rope	0	0	0

4.5. Public awareness of boating activity as a potential vector – a preliminary study

A questionnaire was designed by a student at Wollongong University to examine the types of boating activity done in the south coast lakes of NSW and to identify if members of the boating public had observed the alga. This questionnaire was trailed in a preliminary survey in March 2003. The responses from this preliminary survey (see Appendix 3) were used to design a more comprehensive questionnaire that could be used to further quantify recreational vectors that might potentially transfer *C. taxifolia* to new locations (Appendix 3).

It is recommended that managers adopt this revised questionnaire to gain a broader understanding of community knowledge and potential vectors. It is important that a broad range of people who use the south coast lakes for recreational activities is sampled to allow as many transport vectors as possible to be identified. In order to do this, a suggested sampling protocol would be a combination of face-to-face surveys and questionnaires placed in local shops and businesses. Face-to-face sampling would best be done at weekends or during school holiday periods in summer (i.e. at Christmas and Easter). Questionnaires could be placed in local shops and businesses, such as the local takeaway and bottle shop at Conjola or on the Port Hacking Ferry Service and marina in Port Hacking. This would involve the production of leaflets to explain what *C. taxifolia* is and why people should fill out the questionnaire. A box or other collection point could be provided, perhaps in co-operation with the local business owners, in which people could place their completed surveys.

4.6. Discussion

The monitoring undertaken in Port Hacking and Lake Conjola has confirmed that a variety of anthropogenic activities are capable of generating fragments of *C. taxifolia*. Large quantities of unattached fragments were observed year round in the vicinity of infestations (section 3.1.1). These fragments were significantly more abundant in locations with high levels of anthropogenic activity, particularly where boats were operating in the vicinity. The results from the experiments strongly suggest that boats are an important mechanism for creating fragments as they move over established beds of *C. taxifolia*, particularly in shallow water. Here, dramatic increases in the presence of fragments was found. Although this is of concern in the shallow estuarine locations in which *C. taxifolia* has established, the addition of fragments to well established beds which are not exposed to strong water movement does not appear to significantly increase the biomass of the invader (Wright and Davis, in prep.). As this work was done under the auspices of an ARC Postdoctoral Fellowship it will be reported elsewhere.

The anchoring of vessels also has the potential to create fragments and move these fragments onto vessels for translocation within and between estuaries. Sand and rock anchors will both remove significant quantities of fragments, although rope when used as a means of attaching anchors had little impact on removing *C. taxifolia* from the water. Importantly, anchor chain removes fragments and, as these fragments are relatively small, they are likely to be missed by boaters and not removed from the anchor gear. These small fragments attached to chain have the potential to be translocated to other locations within estuaries, but are unlikely to remain viable for translocation to other estuaries. It is clear from the desiccation experiments that only relatively large clumps of *C. taxifolia*, particularly those that are shaded and covered with damp anchor warp, are likely to survive translocation between estuaries. The survival times recorded in our experiments are much less than those reported from a similar study in the Mediterranean where *C. taxifolia* fragments could apparently survive for one week when kept emerged in dark and humid conditions (Sant *et al.* 1996).

Our findings are generally supported by overseas studies that also suggest that anchors and anchor chains have been important in creating and translocating fragments of *C. taxifolia* (Meinesz *et al.* 1993; Boudouresque 1996). Taken together, these findings support the practice, already adopted in many infested areas, of using educational signage to alert the public to the possibility that fragments may be attached to their boating equipment and that they may be inadvertently assisting in the spread of *C. taxifolia* to new locations. It is recommended that additional information about the general public's awareness of these risks should be obtained, perhaps by the use of questionnaires used in conjunction with other advisory material (as outlined in the NSW control plan; http://www.fisheries.nsw.gov.au/thr/species/fn-caulerpa.htm). The results of our research also support the practice of establishing 'no-anchoring' zones in infested areas. These exclusion zones should prevent anchors from generating new fragments and, more importantly, should prevent their translocation to other locations. Where *C. taxifolia* grows in very shallow water at, or adjacent to, public boat ramps or other boat launching areas, propellers, oars or the boat hulls themselves may damage *C. taxifolia* plants and release fragments. Rather than also excluding boating activity from these sites, treatment of the seaweed is recommended (as described in Chapter 5).

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

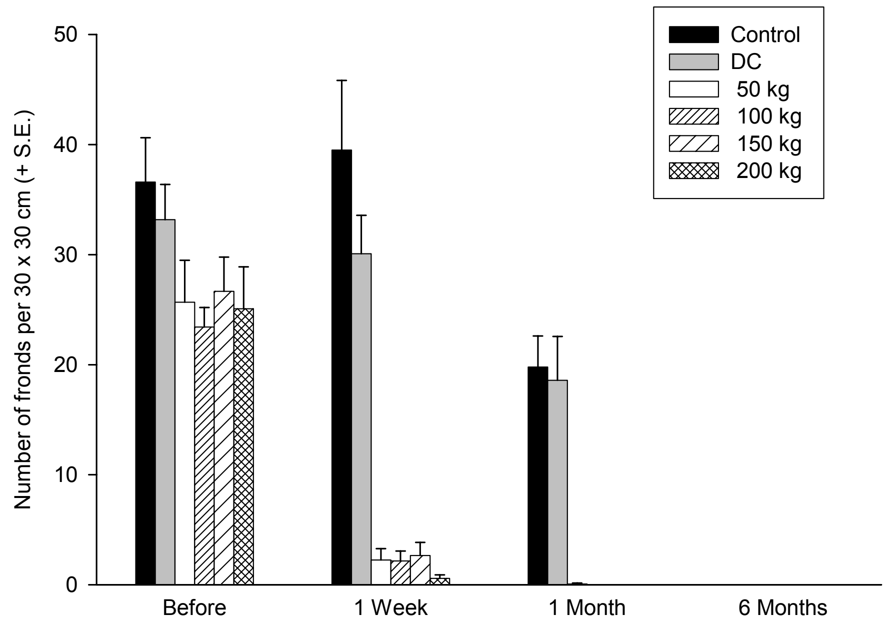
The salt generally dissolved within 2-3 hours of application and after 1 day, *C. taxifolia* became limp and started to lose colour. All concentrations of salt had a dramatic effect on the density of *C. taxifolia*. Prior to salting, there was no significant difference in the cover of *C. taxifolia* 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*.

(a) *Caulerpa* frond density



(b) *Zostera* shoot density

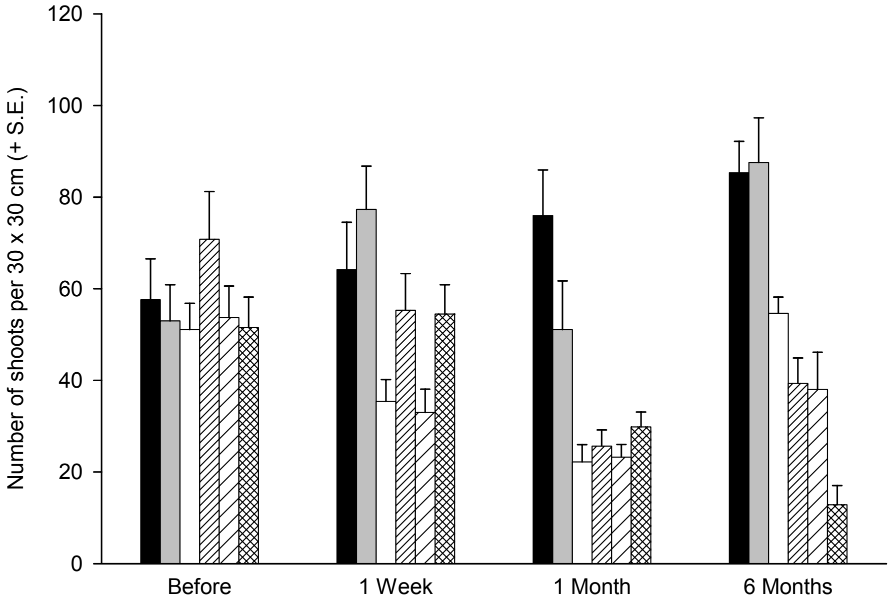
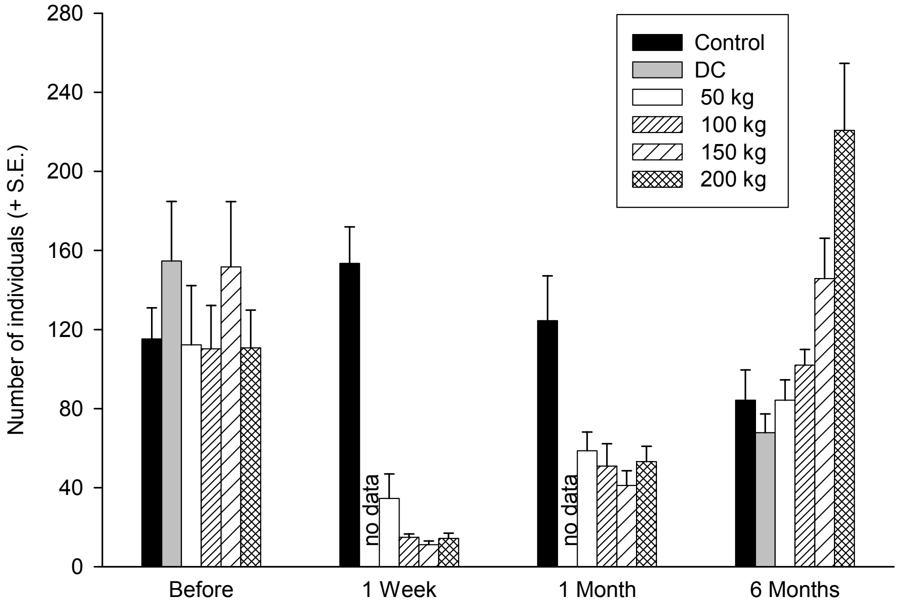


Figure 5.1. Mean number of fronds of *C. taxifolia* (a) and Zostera capricorni (b) in plots that were either not salted (close control (Control) & distant control (DC)), or salted at concentrations of 50, 100, 150 or 200 kg/m2. Data for each treatment are averaged across replicates, plots and sites.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

(a) Total abundance



(b) Total number of taxa

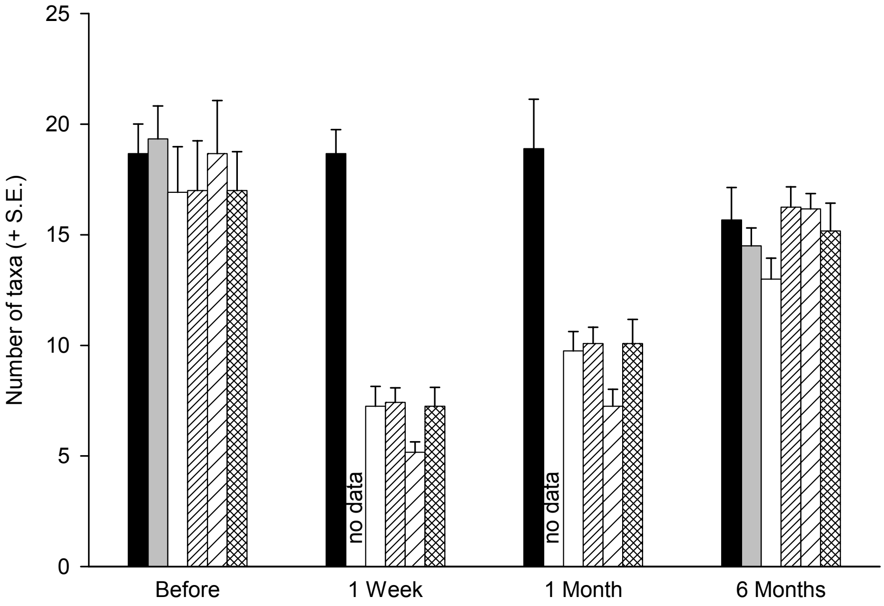


Figure 5.2. Mean number of soft sediment invertebrates (a) and infaunal taxa (b) in plots that were either not salted (close control & distant control), or salted at concentrations of 50, 100, 150 or 200 kg/m2. Data for each treatment are averaged across replicates, plots and sites. The experiment was 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

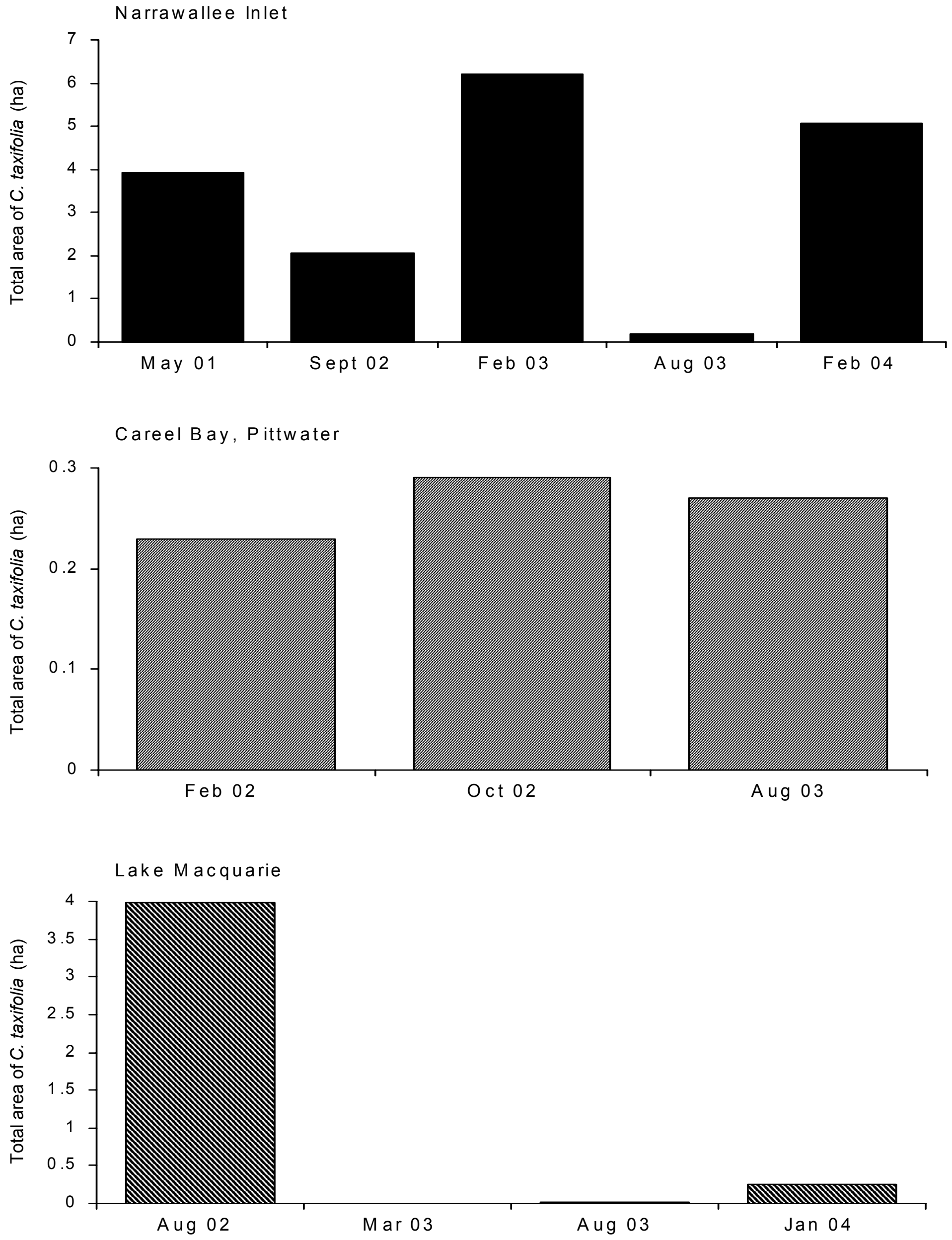


Figure 5.3. Estimated coverage (hectares) of *C. taxifolia* before and after complete treatment of salt in the summer of 2002-2003 at three NSW localities. Major salt treatment was done in Nov.–Dec. 2002 in Narrawallee Inlet (top), Feb.-Mar. 2003 in Careel Bay (centre) and Sept. 2002 to Mar. 2003 in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

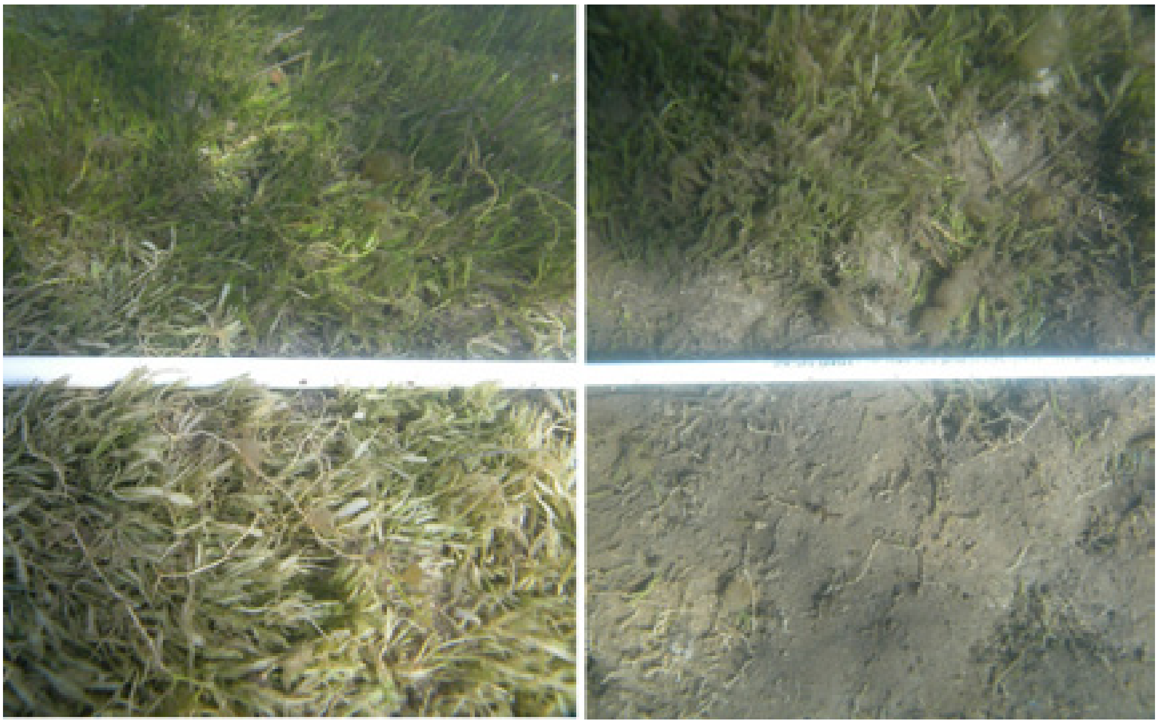


Plate 9. Effectiveness of salt treatments applied to beds of *C. taxifolia* at a concentration of 50kg per m^2. Short-term (3 days) on left, longer-term (3 weeks) on right. Control at top and treated area at bottom.

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).

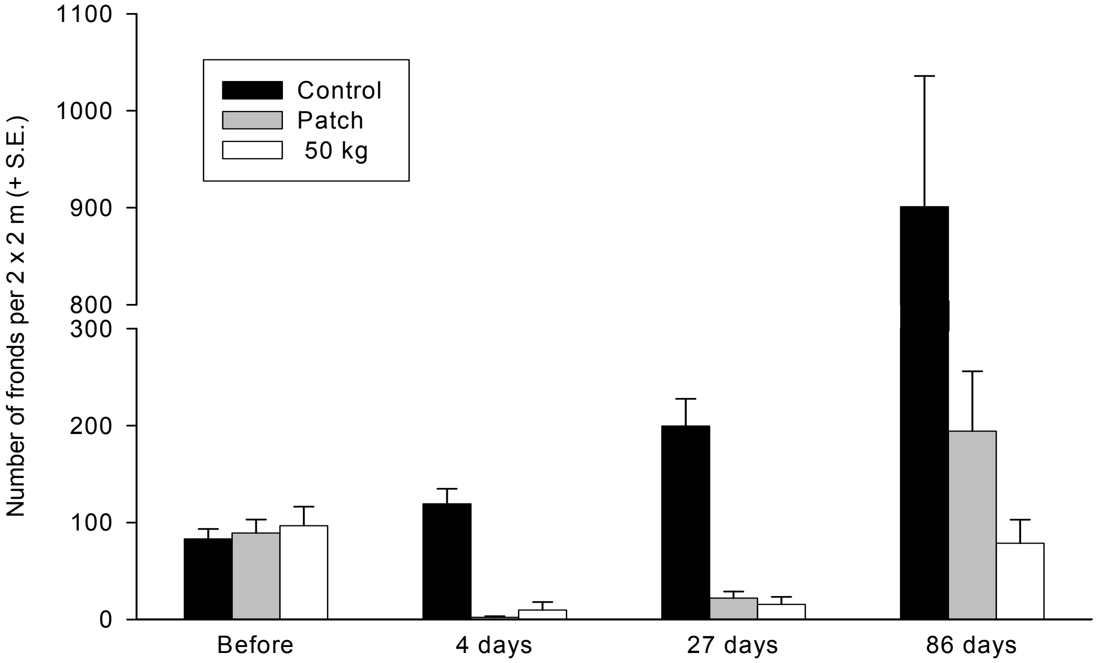


Figure 5.4. Mean number of fronds (+ S.E.) of *C. taxifolia* in plots that were either not salted (controls), 'patch' salted, or covered with 50kg/m2 of salt. Note that the y-axis has been split. The experiment was started in February 2003 in Narrawallee Inlet.

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.

6. GENERAL DISCUSSION

To date, *C. taxifolia* in NSW 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, adjacent to or in beds of seagrass or in previously unvegetated sediments. In only a few places is the alga found on hard surfaces such as pier pilings, concrete mooring blocks or shallow rocky reef.

During winter, the cover of *C. taxifolia* in NSW waterways generally decreases and the alga is typically much smaller, which is consistent with findings in other countries (Meinesz *et al*. 1995, Ceccherelli and Cinelli 1999a). Large-scale die-off occurs in shallow water (0.5–2 m) in most waterways during the cooler months and this has been particularly evident after heavy rainfall. Thus, it is possible that die-back may be a consequence of decreased temperature, decreased salinity, increased turbidity or some combination of these factors. This information, and a better understanding of the basic biology of the species in NSW, has been used to help determine when control work is likely to be most effective.

Eliminating or controlling the spread of *C. taxifolia* with osmotic shock techniques (application of salt in NSW or the use of freshwater in South Australia) has been shown to be reasonably effective and cost-efficient. Any control technique, however, will be scale dependent – things that work well at small scales may not be feasible at larger scales. Thus, in NSW, the application of salt can be very effective at scales up to a few hectares. 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. If natural phenomena assisted with the removal of *C. taxifolia* from Lake Macquarie, as seems likely, a focus of future research needs to be on patterns of change in established populations of *C. taxifolia* and the causes of those fluctuations. *Caulerpa* spp. worldwide often undergo patterns of rapid expansion followed by dramatic declines (Jaubert *et al*. 2003 and references therein), and it may be that *C. taxifolia* will show the same phenomenon in NSW. Until the reasons for any 'natural' fluctuations are known, however, work on elimination or controlling the further spread of the alga is imperative.

For larger infestations such as those in many NSW estuaries, salt treatment can only be used in localised sites. Additional technologies such as species-specific biocides may provide one avenue for further investigation. The development of such technologies, however, will take a considerable time (R. Thresher, pers. comm.) and their utility will still then have to be evaluated in field situations. Given this time frame, it is unlikely that any technology will be able to completely eradicate *C. taxifolia* from NSW waterways in the near future. This realisation means that no single approach to the management of this marine invader will suffice. Rather, an integrated plan incorporating multiple approaches is required.

The investigations described in this report have informed the process of developing a statewide control plan for invasive *C. taxifolia* in NSW. This plan, officially released in February 2004, contains a preliminary risk assessment that considers the risks of transport of *C. taxifolia* to new estuaries or areas within estuaries, the risks of environmental and socio-economic impacts of the alga and the process by which priorities are set for control activities. This plan is available on the NSW Fisheries website at http://www.fisheries.nsw.gov.au/thr/species/fn-caulerpa.htm

Future directions

A great deal more research is needed on natural fluctuations in abundance of *C. taxifolia* and on correlations between various biotic and abiotic parameters and abundances of *C. taxifolia*. Research of this kind is likely to be more effective for managing the species than is continued control work which is expensive and often logistically difficult. Importantly, it is not yet known whether *C. taxifolia* is having negative impacts on estuarine ecosystems and thus it is unclear whether vast resources should be allocated to the control of the species. Future research, therefore, should be more directed towards understanding the potential impacts of *C. taxifolia* and investigating natural fluctuations in abundance of the alga. Control work will continue, but efforts will be prioritised because the treatment of *C. taxifolia* with salt in all invaded estuaries in NSW is not logistically feasible and prohibitively expensive. Key priority areas for control have been identified in the NSW Fisheries *Caulerpa* control plan and include seagrass beds (which may be vulnerable to invasion), boat ramps and popular fishing spots (from where *C. taxifolia* may be spread) and outbreaks close to the open coast. Some resources should also be devoted to investigating other methods for control.

Various research projects on the potential impacts of *C. taxifolia* are currently underway in collaboration with universities. These include comparisons of *C. taxifolia* and native seagrasses as habitats for fishes and invertebrate epifauna and infauna. NSW Fisheries has also begun investigating the interactions between *C. taxifolia* and seagrasses in an attempt to determine how vulnerable beds of seagrass are to invasion. As described in this report, research at the University of Wollongong has focussed on the life history characteristics, growth and spread of *C. taxifolia*, in addition to associations between marine herbivores and the alga and more research is planned along these lines. Future research by NSW Fisheries may include studies of the invasiveness of *C. taxifolia* in different waterways in NSW versus that of native *C. taxifolia* in Queensland. Given that results of genetic studies regarding the affiliations of different populations of *C. taxifolia* are still inconclusive, research on the invasive characteristics of populations may be an indirect way of determining whether different populations of *C. taxifolia* are related whilst addressing one of the most important issues, namely the ability of different strains to invade. Additional effort will also be devoted to identifying the types of places that *C. taxifolia* is most likely to invade so that preventative action can be made more effective.

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APPENDIX 1

The spatial extent of *C. taxifolia* infestations in eight NSW estuaries mapped at three times: summer 2003, winter 2003 and summer 2004. Estuaries are presented in order from north to south, and from most recent to the first in the sequence.

(compiled by Alan Genders)

Distribution of *Caulerpa taxifolia* Lake Macquarie, January 2004

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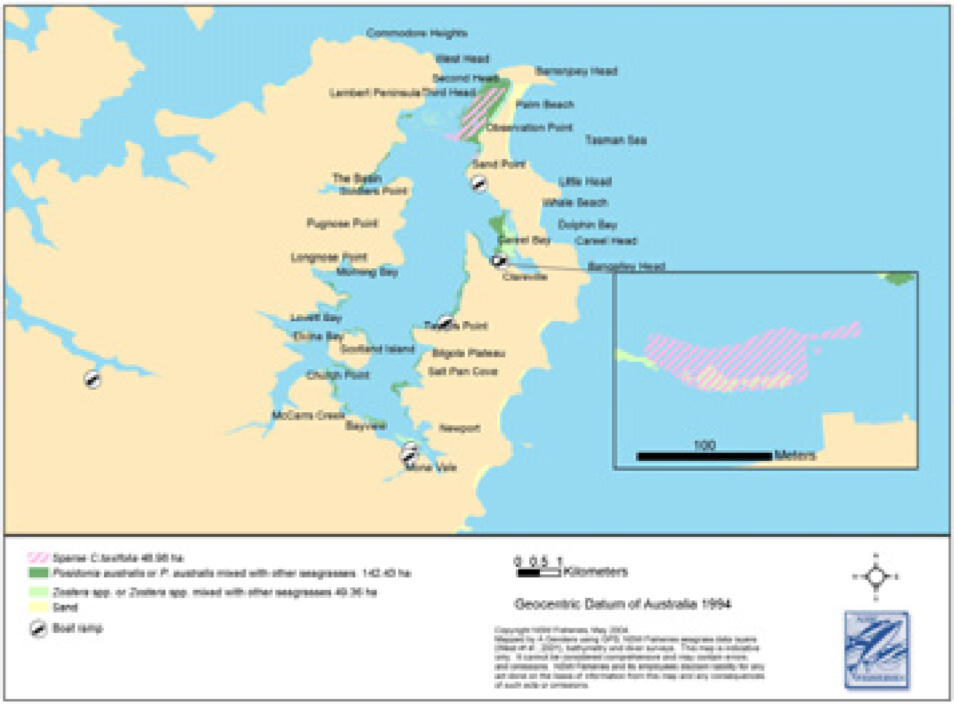
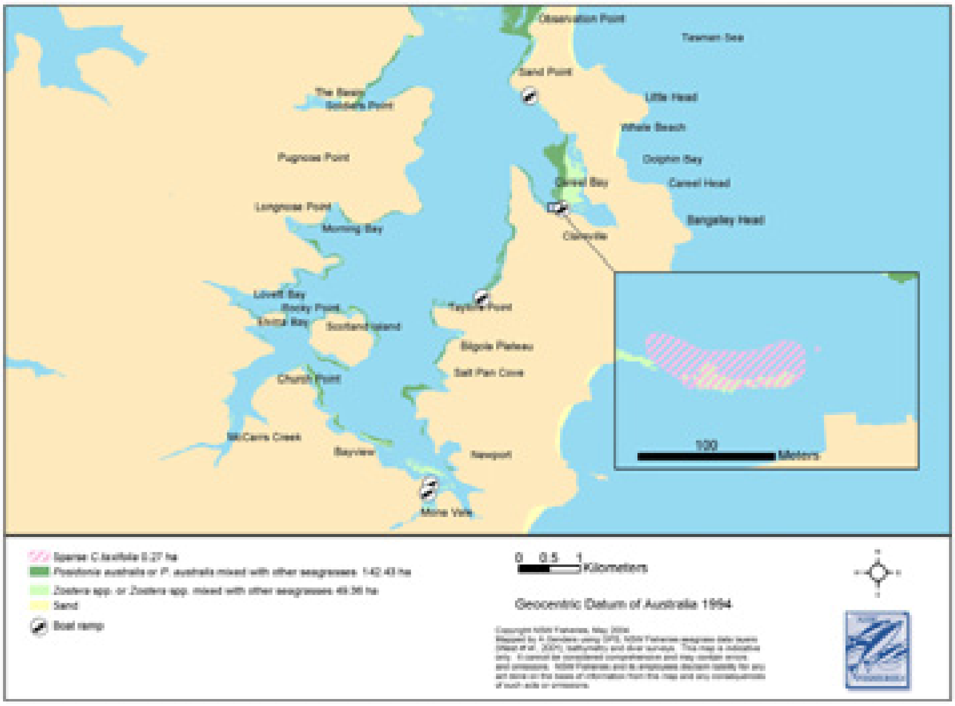
Distribution of *Caulerpa taxifolia* Lake Macquarie, August 2003

Distribution of *Caulerpa taxifolia* Lake Macquarie, March 2003 (after salting)

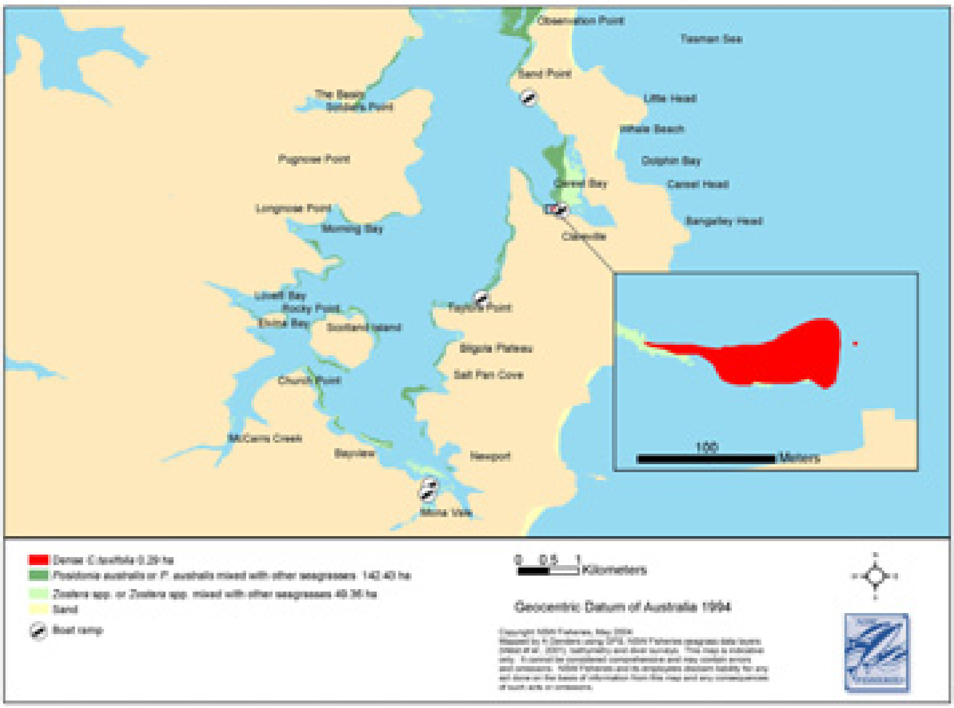
Distribution of *Caulerpa taxifolia* Careel Bay, Pittwater, March 2004



Distribution of *Caulerpa taxifolia* Careel Bay, Pittwater, August 2003



Distribution of *Caulerpa taxifolia* Careel Bay, Pittwater, October 2002



Distribution of *Caulerpa taxifolia* Port Jackson, March 2004

Distribution of *Caulerpa taxifolia* Port Jackson, August 2003

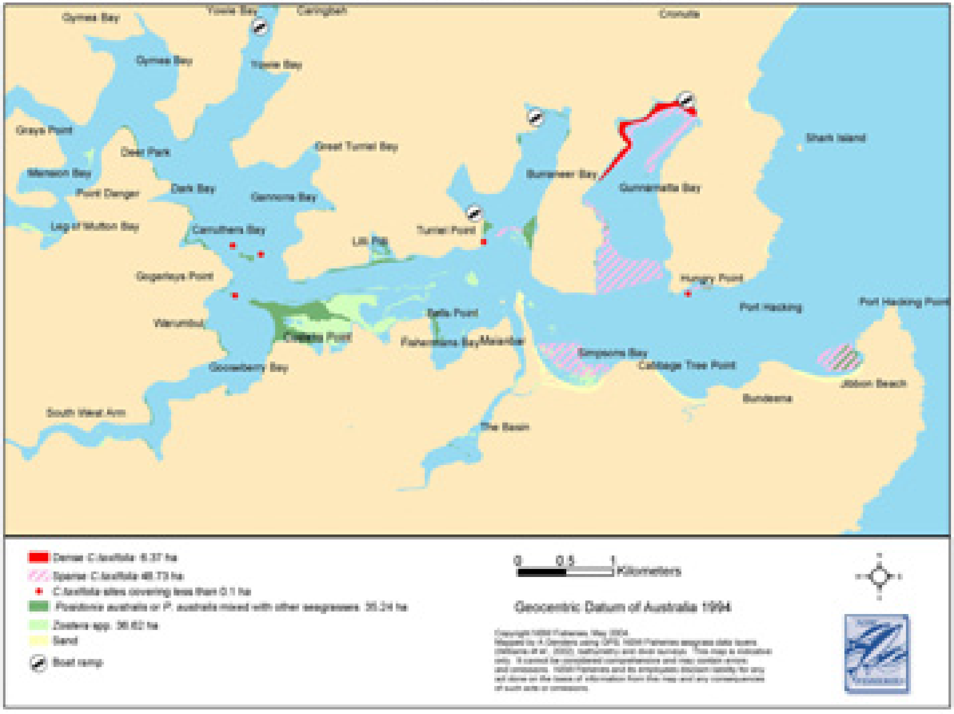
Distribution of *Caulerpa taxifolia* Port Jackson, March 2003

Distribution of *Caulerpa taxifolia* Botany Bay, March 2004

Distribution of *Caulerpa taxifolia* Botany Bay, August 2003

Distribution of *Caulerpa taxifolia* Botany Bay, February 2003

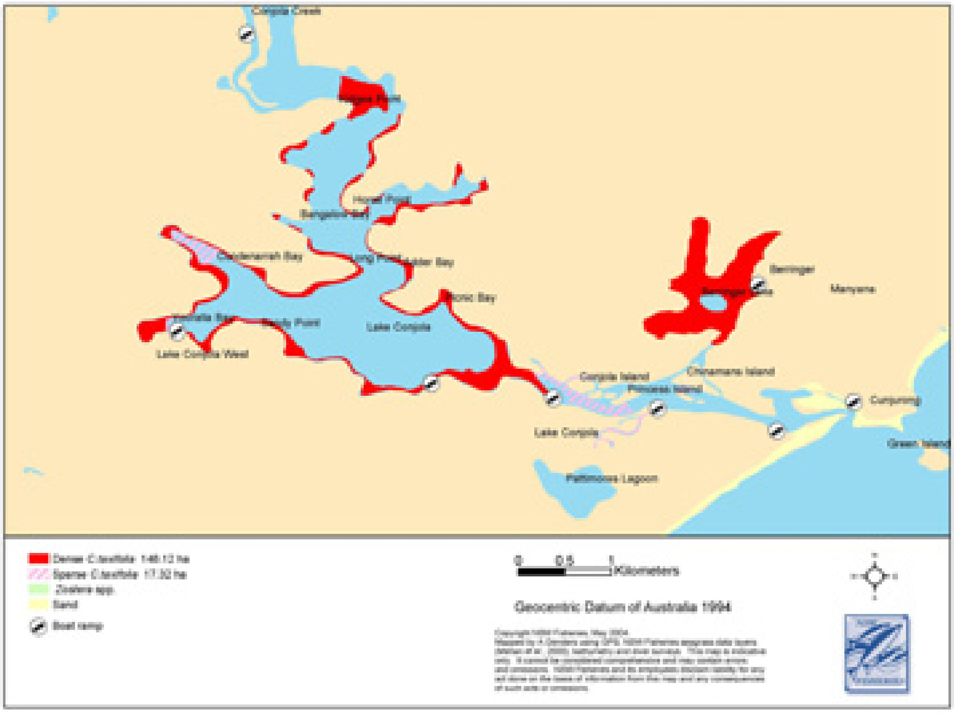
Distribution of *Caulerpa taxifolia* Port Hacking, March 2004



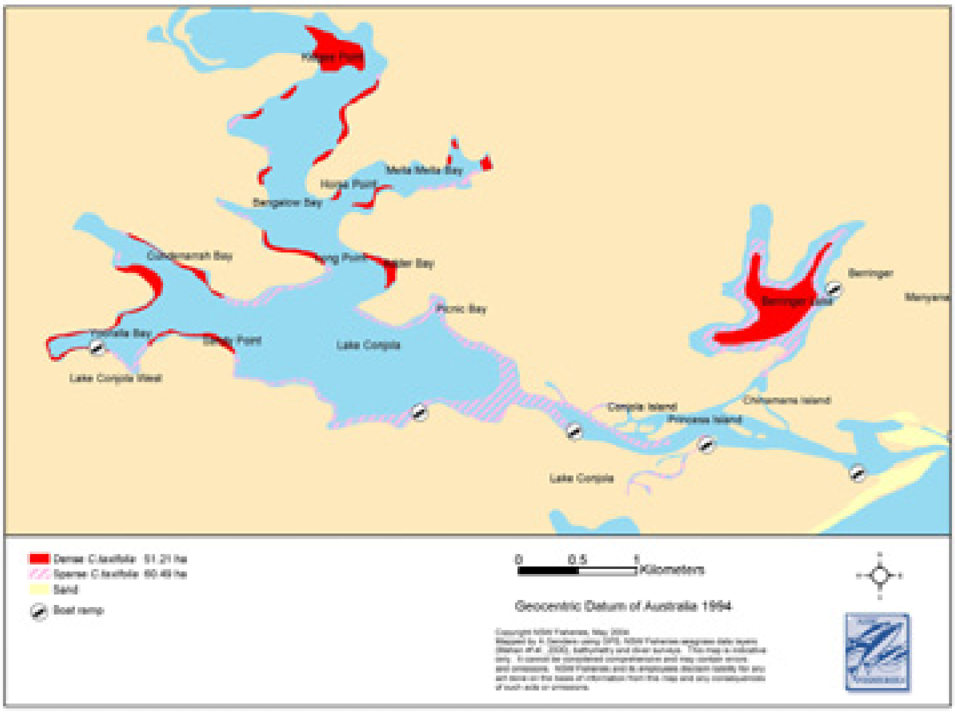
Distribution of *Caulerpa taxifolia* Port Hacking, August 2003

Distribution of *Caulerpa taxifolia* Port Hacking, February 2003

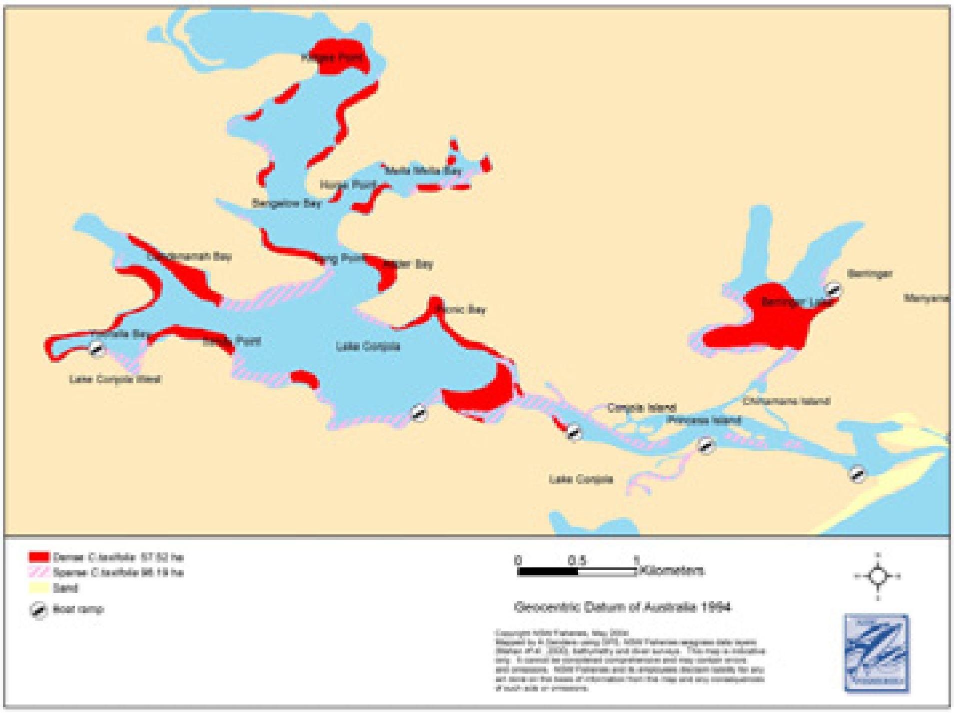
Distribution of *Caulerpa taxifolia* Lake Conjola, February 2004



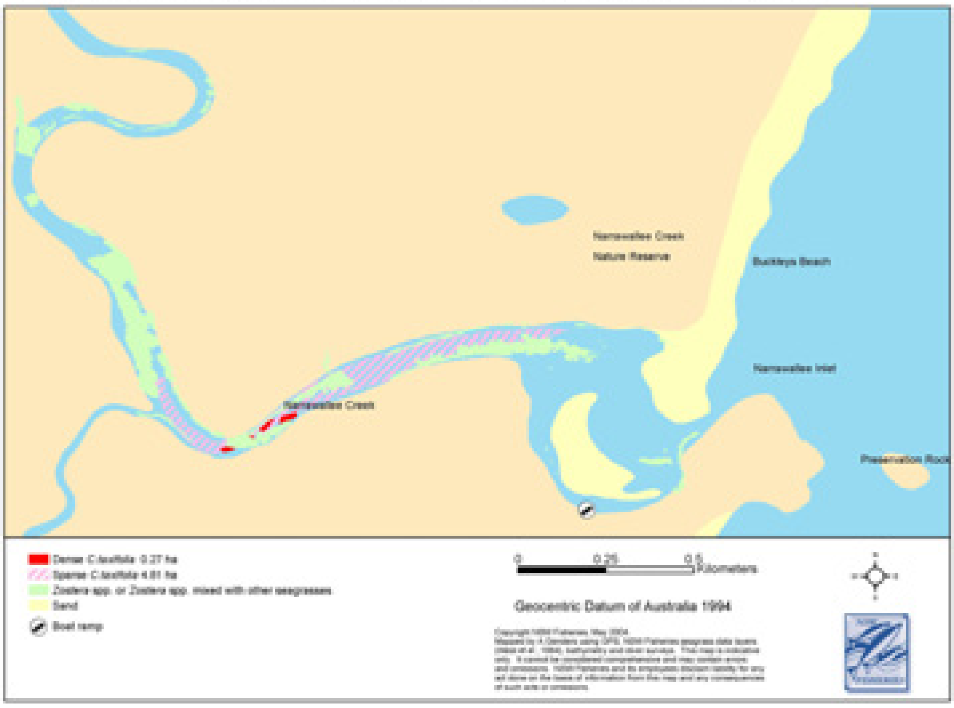
Distribution of *Caulerpa taxifolia* Lake Conjola, August 2003



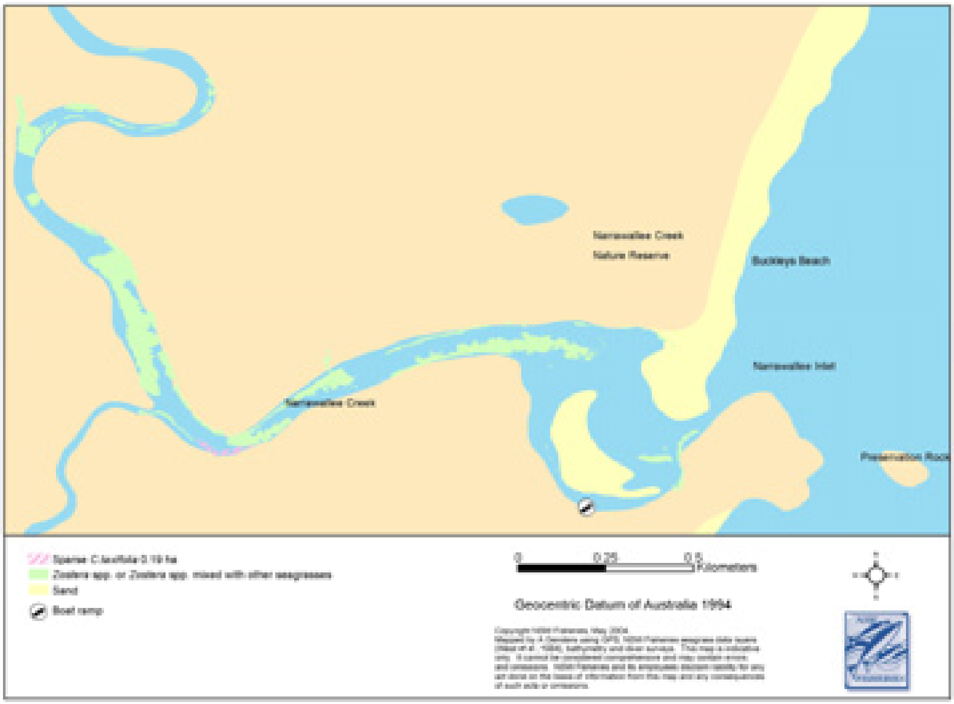
Distribution of *Caulerpa taxifolia* Lake Conjola, February 2003



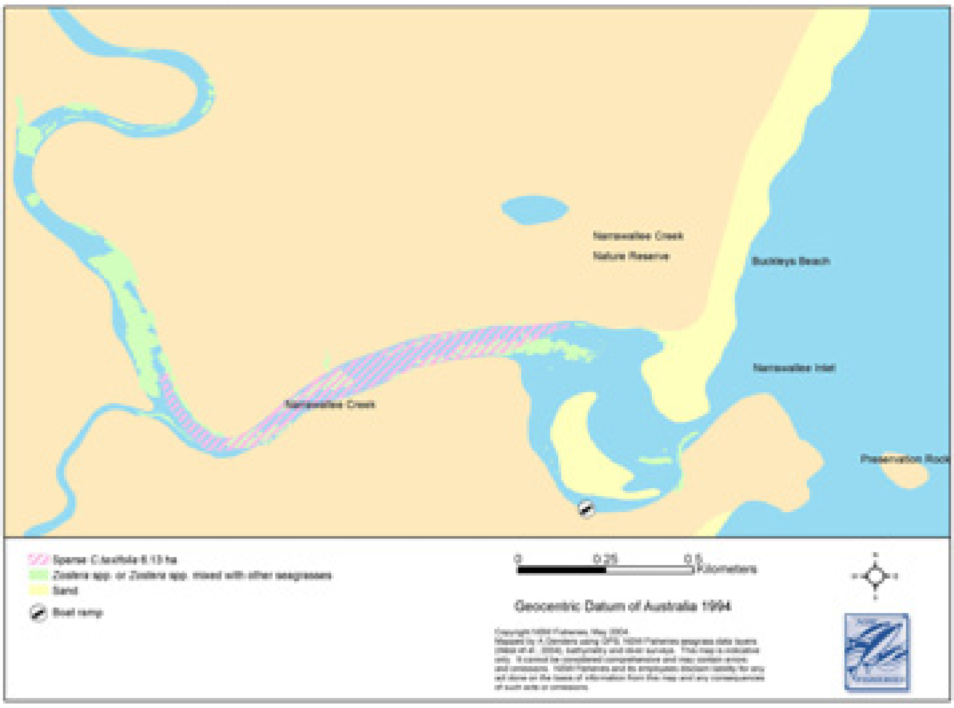
Distribution of *Caulerpa taxifolia* Narrawallee Inlet, February 2004



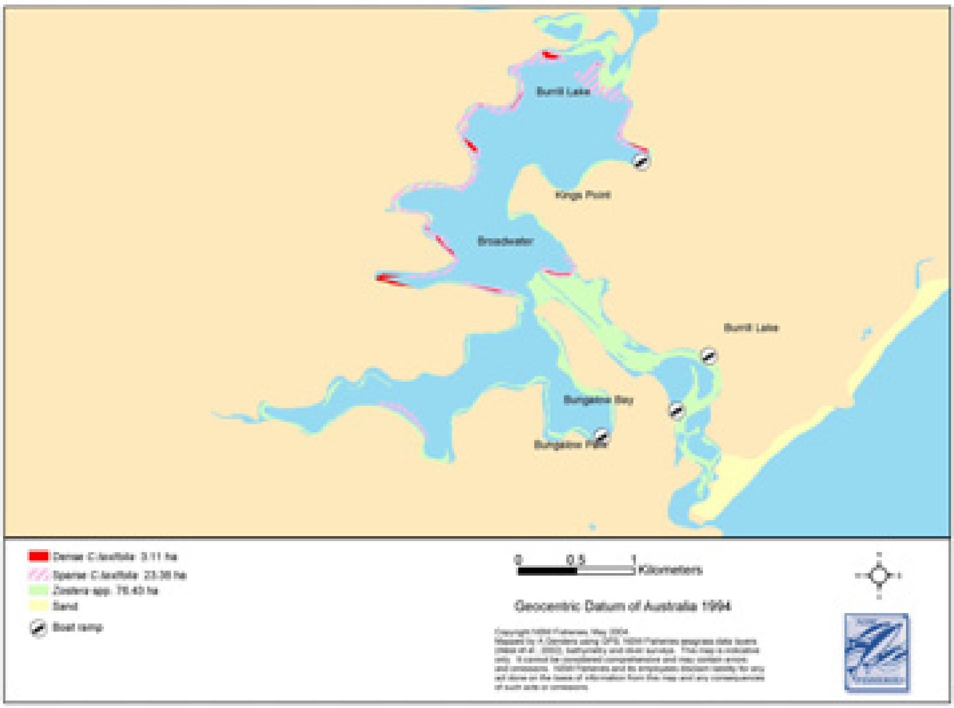
Distribution of *Caulerpa taxifolia* Narrawallee Inlet, August 2003



Distribution of *Caulerpa taxifolia* Narrawallee Inlet, February 2003



Distribution of *Caulerpa taxifolia* Burrill Lake, February 2004



Distribution of *Caulerpa taxifolia* Burrill Lake, August 2003

Distribution of *Caulerpa taxifolia* Burrill Lake, February 2003

APPENDIX 2

Summaries of additional research done at the University of Wollongong on the interactions between potential herbivores and *C. taxifolia*.

Appendix 2A

Responses of common SE Australian herbivores to three invasive *Caulerpa* spp.

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ABSTRACT

We sought to determine whether common intertidal and shallow subtidal zone grazers would consume extracts or fronds of three invasive *Caulerpa* spp. all of which are now resident in southern New South Wales, Australia. We examined the responses of herbivorous fishes, echinoderms and molluscs to *C. filiformis*. The responses of a subset of these organisms to extracts of the highly invasive *C. scalpelliformis* and *C. taxifolia* were also examined. Most feeding trials were conducted in the field using palatable agar discs containing extracts to prevent any chance of spreading these algae. Algal extracts were produced by macerating algal fronds in ethanol or seawater. Polar extracts of *C. filiformis* deterred a single herbivore, *Aplysia sydneyensis*, but confirmed that the biological activity reported from some *Caulerpa* spp. is not restricted to the lipophilic fractions. The large turbinid *Turbo torquatus* was dissuaded from feeding on agar discs containing ethanol extract of *C. filiformis*, while in contrast the small congener *T. undulatus* demonstrated a significant preference for ethanol extracts of *C. filiformis* relative to controls. However, when *T. undulatus* were offered a choice of fronds from six algal species in a laboratory experiment they readily consumed *Ulva* spp. and *Sargassum* sp., showing the lowest preference for *C. filiformis*. Solvent extracts of *C. scalpelliformis* and *C. taxifolia* did not significantly dissuade any grazers. However, the overall trend was for deterrence by the discs containing solvent extracts of these seaweeds. Indeed, for the large urchin *Centrostephanus rodgersii* and in the fish trials these effects were very near significant ($P<0.06$). We conclude that common herbivores associated with hard substrata are highly unlikely to intercede in the spread or control of these invasive algae.

Appendix 2B

Plant – herbivore interaction in an estuary invaded by *Caulerpa taxifolia…..*
Estimating the impacts of native herbivores on their food resources

By John Gollan

This thesis is submitted in partial fulfilment of the requirements for the degree of Bachelor of Science (Honours)

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University of Wollongong, NSW, Australia
October 2003

ABSTRACT

Invasive species are among the greatest threats to ecosystems worldwide. Frequently invasive species occur in very high densities in the invaded regions and one theory as to why these species do so well is that they suffer less from the effects of natural enemies in their new environments. Herbivory is an important ecological force shaping most plant communities, altering plant abundance, species diversity and/or species distribution. Consequently, herbivory or its absence, can facilitate invasion by exotic plants. Herbivory is a particularly important ecological force in most marine communities and is likely to have an important role in the invasion of exotic marine plants.

One of the most notorious marine invasive plants is the tropical macroalga *Caulerpa taxifolia*, which is suspected to experience low levels of herbivory in recipient communities. In this study I examined the entire assemblage of herbivores in Lake Conjola, NSW, which is probably the location most heavily infested by *C. taxifolia* in temperate Australia. Specifically, I examined patterns of distribution and abundance of herbivores in Lake Conjola, their feeding rates, habitat choice and their survivorship on native plants vs. *C. taxifolia.*

Surveys during the year revealed that there were only four abundant herbivores in Lake Conjola: the fish, *Girella tricuspian;* the macrograzer, *Aplysia dactylomela*; and two mesograzers, *Platynereis dumerilii antipoda* and *Cymadusa setosa*. Densities of both *G. tricuspidate* and *A. dactylomela* varied significantly in space and time and there was no relationship between their abundance and the abundance of *C. taxifolia*. Densities of both mesograzers also varied significantly in space and time and among five host plants. Most notably, there were consistently low densities of both mesograzers on *C. taxifolia* and a significant negative relationship between the total number of these grazers and biomass of *C. taxifolia*. Highest densities of these mesograzers mostly occurred on two brown algae; *Cystophylum onustum* and *Sargassum* sp. although at some times and sites other hosts had similar or greater densities. Feeding trials to examine the consumption rates of these herbivores on native plants vs. *C. taxifolia* failed to gain useful data for both G. tricuspidate and *A. dactylomela*. However, food selectivity by *A. dactylomela*, assessed by comparing gut content with field abundance, indicated a strong preference for the red alga, *Laurencia* spp. and strong avoidance of *C. taxifolia*. A no-choice feeding experiment comparing native plants vs. *C. taxifolia* showed feeding by *C. setosa* was lowest on *C. taxifolia* and highest on *C. onustum* and *Sargassum* sp. The aversion for *C. taxifolia* displayed by *C. setosa* was further examined using starved and unstarved animals and indicated this species will only eat *C. taxifolia* when hunger stressed.

Conversely, *P. antipoda* showed no aversion for *C. taxifolia* and had similar feeding rates on *C. taxifolia* to all other native seaweeds. Estimated impacts of both mesograzers (calculated by combining field densities with feeding rates on different hosts) were lowest for *C. taxifolia* and highest for *C. onustum* and *Sargassum* sp. Both mesograzers strongly avoided *C. taxifolia* in feeding preference experiments and preferred the two brown algae. Survivorship of *C. setosa* was lower on *C. taxifolia* vs. *C. onustum* but there was no difference between those fed *C. taxifolia* and those that were fed no food. In contrast, the survivorship of *P. antipoda* to 41 days was 100% on *C. taxifolia, C. onustum* and no food.

Overall, the results from this study support the suggestion that *C. taxifolia* is experiencing weak grazing pressure from native herbivores. However the absence of feeding data for two of these herbivores, in particular for *Girella tricuspidata*, constrain this conclusion and future research on the feeding and impacts of this herbivore will contribute to understanding its role in the invasion of *C. taxifolia* in temperate Australia.

Appendix 2C

Fauna associated with *Caulerpa* spp.; potential Biological Control of *C. taxifolia*

Amanda Edwards BSc

This thesis is submitted in partial fulfilment of the requirements for a Bachelor of Science degree

(Honours) from the University of Wollongong

Department of Biology
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ABSTRACT

Marine ecosystems worldwide are being altered by the invasion of non-indigenous species. *Caulerpa taxifolia* is an alga that has invaded the Mediterranean, California and now Australia. There is great concern about its potential impacts and consequently there is a need to understand more on its ecology to be able to manage or control it.

This study had 2 main aims: 1) to determine patterns of variation in species richness, abundance and community structure in the fauna associated with 4 *Caulerpa* spp. (particularly herbivores) commonly found along the eastern coastline of NSW, Australia. They were: *Caulerpa filiformis*, *C. taxifolia, C. flexilis* and *C. cactoides*. One particular area of interest in the study was the presence of the native herbivores occurring on *Caulerpa* in south eastern Australia and their potential to act as a biological control on *C. taxifolia*. The 4 species were compared across 3 locations and sites within locations. Two species (*C. filiformis* and *C. taxifolia*) were examined 3 times to assess temporal patterns. 2) To examine the feeding of 4 common gastropod herbivores on *C. filiformis* and the feeding of a common sacoglossan on *C. taxifolia*.

The hierarchical sampling design revealed significant differences in species richness and abundance of fauna on small scales: between locations and between sites within locations ($F = 3.13$, $p = 0.0143$; $F = 4.97$, $p<0.001$; respectively for species richness). This data suggests that ‘patchiness’ in fauna species richness and abundance occurs on small scales. No differences in species richness or abundance occurred between *Caulerpa* spp. Temporal variation between times for different sites (*C. filiformis* and *C. taxifolia*) were also detected, hence, patchiness on temporal as well as spatial scales.

Although there was no variation in fauna numbers between *Caulerpa* spp., the community structure for each was not the same. In total, 61 species were found on the 4 *Caulerpa* spp. *C. filiformis* was comprised mainly of herbivorous gastropods (eg. *Canthrindella picturata* and *Oxynoe viridis*), while the other three species contained mainly amphipods and bivalves (>50% contribution). The fact that samples for each *Caulerpa* spp. were taken from different locations in different habitats may be an important factor contributing to differences in the composition of fauna.

The 4 most abundant herbivores found on *C. filiformis* were used to examine which herbivores consumed it. Of the 4 herbivores, only *Oxynoe viridis* consumed *C. filiformis*. This was based on 3 different measurements of consumption: weight loss, percentage bleached fronds and the photosynthetic ability of the alga. *O. viridis* also consumed the invasive *C. taxifolia* (based on weight loss and percentage bleached fronds). However, consumption of *C. taxifolia* by *O. viridis* as weight loss was relatively low when compared to percent bleaching measurements. *O. viridis* are suctorial feeders and this may affect frond health, shown by large percentage bleaching of fronds following grazing, but may not have an impact on frond weight. The fact that *O. viridis* consumes the invasive *C. taxifolia* at several locations is encouraging when considering the sacoglossan as a potential biological control agent against *C. taxifolia* in Australia.

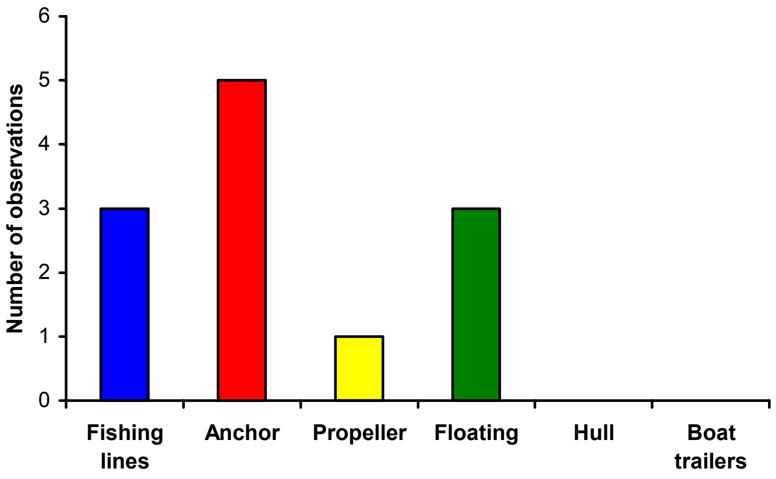
APPENDIX 3

Preliminary survey of boat users in Lake Conjola, March 2003

(compiled by Elizabeth West)

The preliminary survey involved 11 people, who were using Lake Conjola for recreational purposes - fishing, skiing and swimming. All people surveyed were using boats approximately 2-6m in length, eight of these people had outboard motors and three had inboard motors. Six people noted that they had sand anchors and four people had rock anchors. Three people mentioned that they had rope attachments and seven people had chain attachments.

Of the eleven people surveyed, nine people where aware of the *Caulerpa taxifolia* infestation within Lake Conjola and had observed it during their day's activities either floating, on anchors, on fishing line or on their boat propeller (see figure below). The other two people had never heard of *Caulerpa* and had not noticed it.



Number of observations of *Caulerpa taxifolia* on fishing lines anchors, propellers or floating, made by nine recreational users of Lake Conjola, on 25th March 2003 (12 observations).

Initial questionnaire used in a trial face-to-face survey at Lake Conjola, March 2003.

Date___________Time__________

Please circle the appropriate response

Boat size <2m 2.1-3m 3.1-4m 4.1-5m 5.1-6m >6m

Type of motor none inboard outboard

Number of motors 1 2

Capacity (if known) ________________________________

Did boat users observe *Caulerpa* yes no

Where did they observe it anchor fishing lines propellor other

if other specify ________________________________

Activities undertaken fishing skiing diving other

if other specify ________________________________

Presence of *Caulerpa* none interior of vessel exterior of vessel

if interior anchor locker within hull other

if exterior propellor trailer other

if other specify ________________________________

Complete sample collected yes no

Sample placed in bag no. ________________________________

Attitude of boat owner/operator : cooperative neutral uncooperative aggressive

Postcode of owner/operator ________________________________

Initials of Assessor ________________________________

A revised questionnaire suggested for future use at sites with *C. taxifolia* infestations and significant recreational activity is shown below. The questionnaire could be used in face-to-face interviews or given out to people to fill in and return to a central location.

Location: **Date:** **Time:**

Please circle the appropriate answer and give detail where necessary.

1. What recreational activities have you done?

Boating Swimming Fishing Canoeing Skiing

Other (please specify)? ………………………………………………………………

2. For each recreational activity, what equipment has been used?

Boating:	Outboard motor	Inboard motor	Paddle boat	Anchor
Swimming:	Wetsuit	Mask & Snorkel		
Fishing:	Rod & Line	Nets	Hooks	Sinker
Skiing:	Water-skis	Donut	Wake board	

Other (please describe)……………………………………………………………………

3. How often would you do these recreational activities?

Daily Twice a week Once a week Fortnightly Monthly

4. Where else do you do these recreational activities?……………………………….

5. Did you know *Caulerpa taxifolia* occurs in this waterway?……………………………..

6. Have you seen *Caulerpa taxifolia* (see information sheet)? Yes No

7. Where did you see it?

Propeller Fishing gear anchor

Other (please specify)?…………………………...

8. What is you postcode?…………………………………………………………………...

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