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.

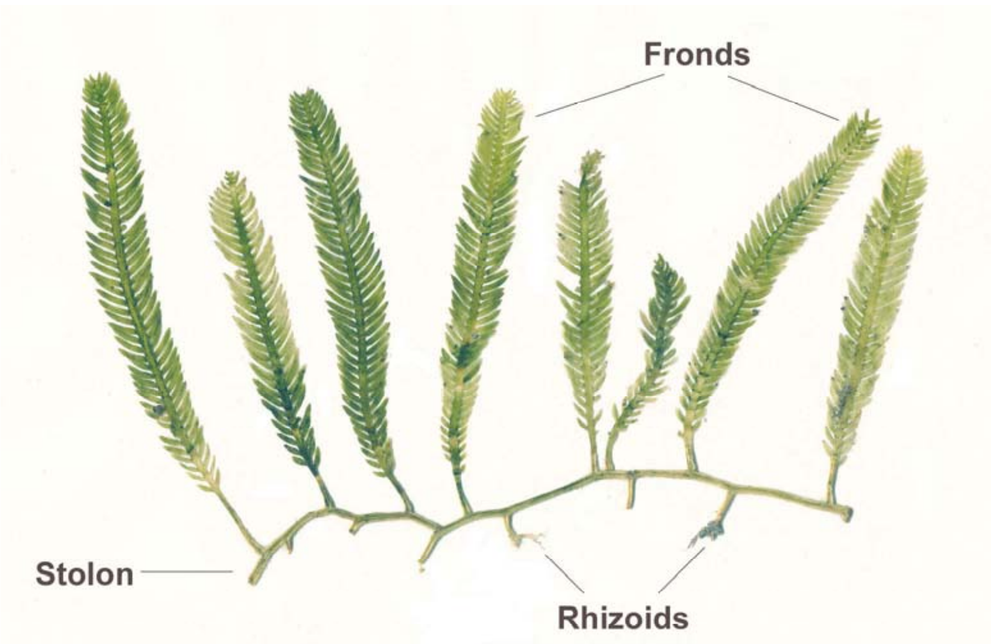


Plate 5. Thallus fragment of *Caulerpa taxifolia*, indicating the frond, stolon and rhizoid section of the alga.

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.).

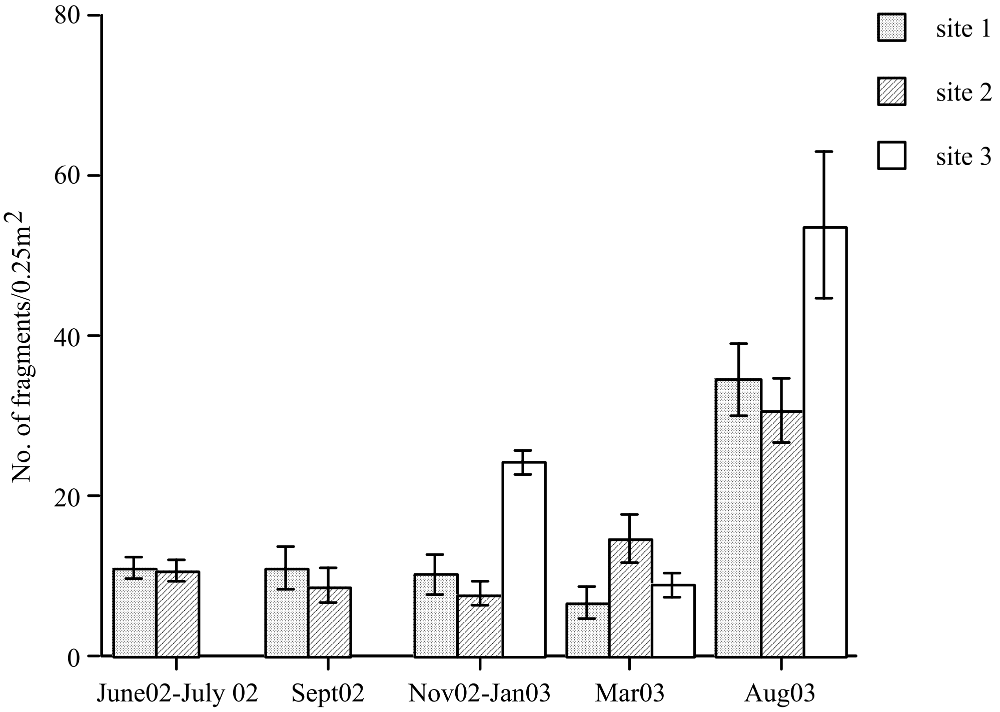


Figure 3.1. Mean (±se) number of fragments per quadrat of *C. taxifolia* fragments at West Conjola, Lake Conjola between June 2002 and August 2003. n=25.

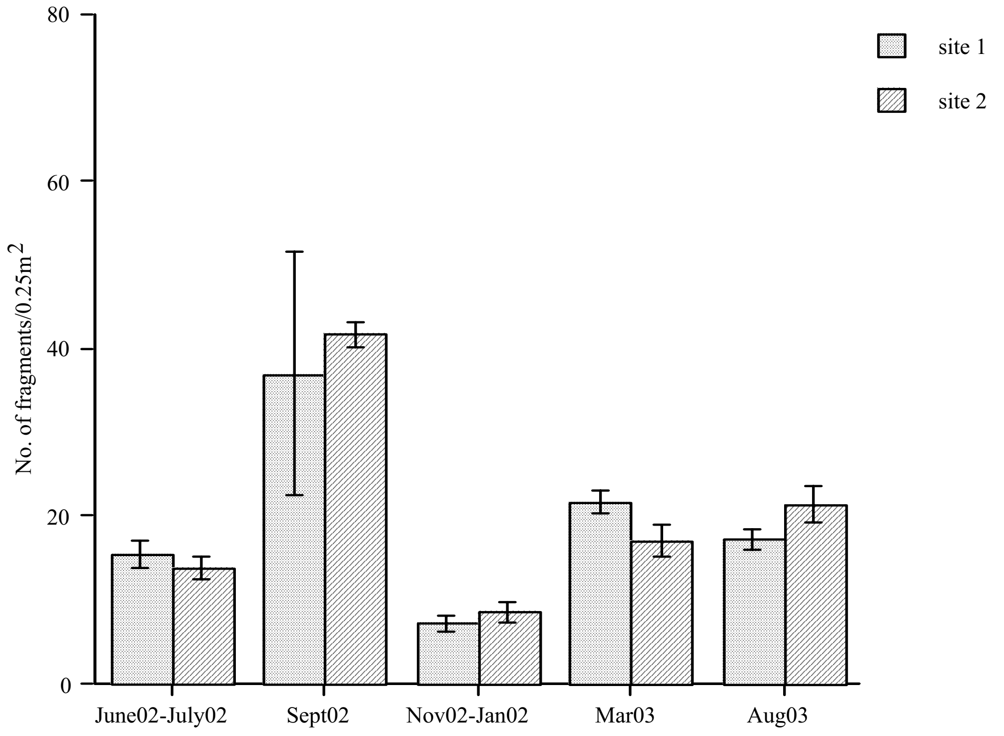


Figure 3.2. Mean (±se) number of fragments per quadrat of *C. taxifolia* fragments at Roberts Point, Lake Conjola between July 2002 and August 2003. n=25.

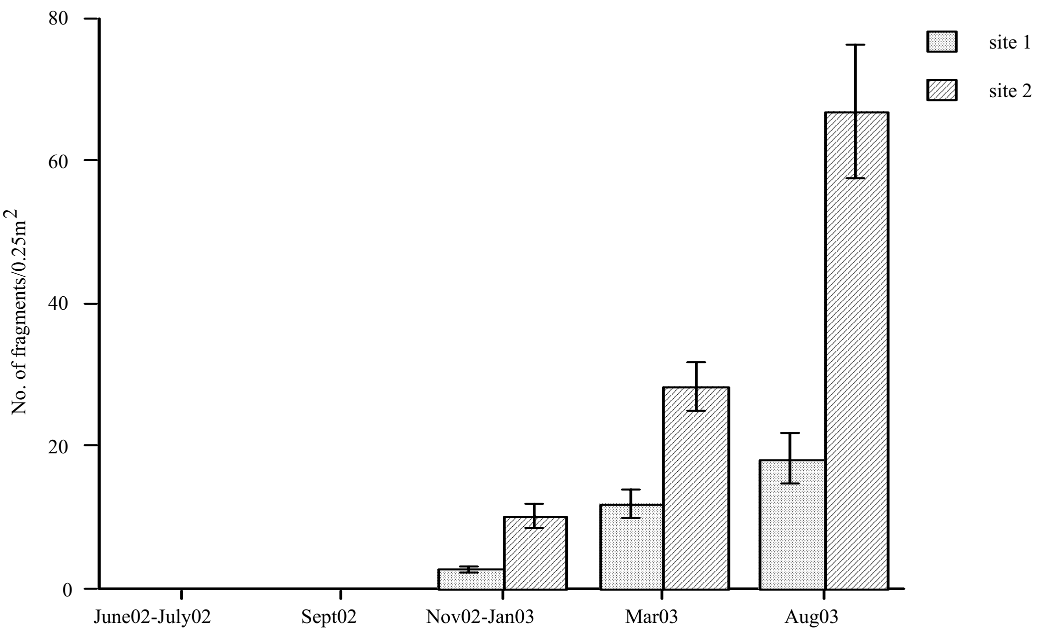


Figure 3.3. Mean (±se) number of fragments per quadrat of *C. taxifolia* fragments at Adder Bay, Lake Conjola between July 2002 and August 2003. n=25.

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).

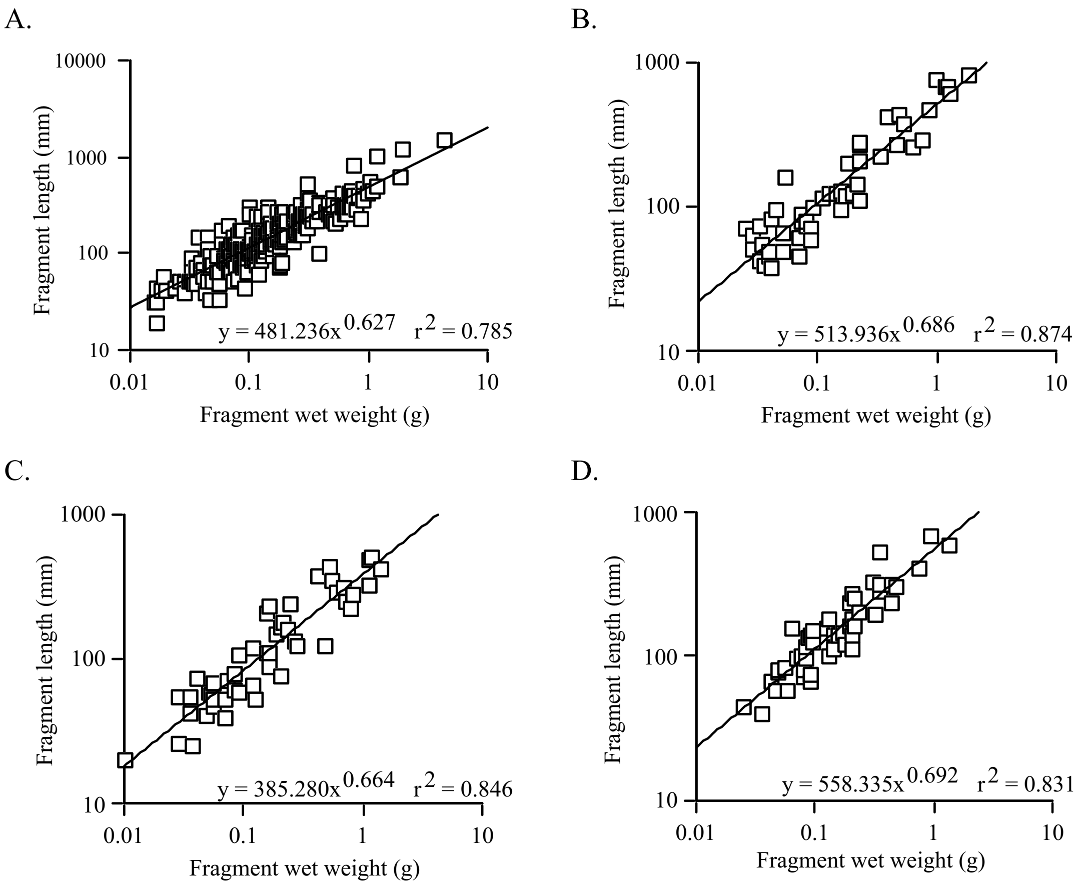
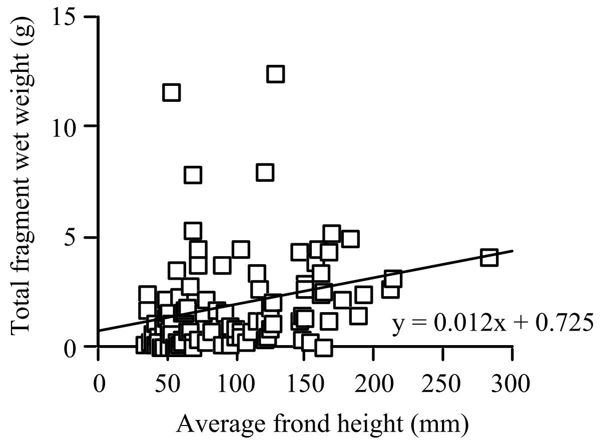


Figure 3.4. Relationships between lengths of *C. taxifolia* fragments and their wet weights at West Conjola site 1 (A) and site2 (B) and at Roberts Point site 1 (C) and site 2 (D).

A.



B.

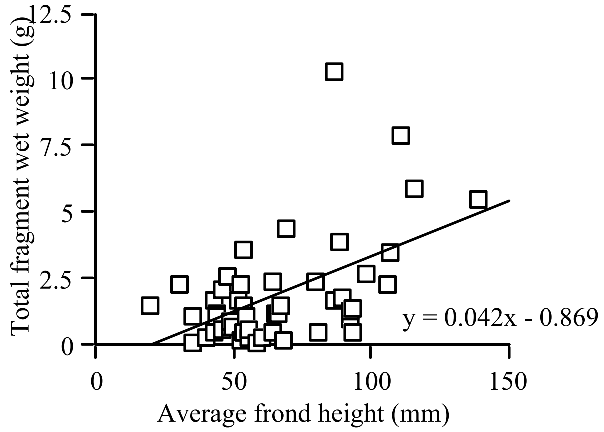
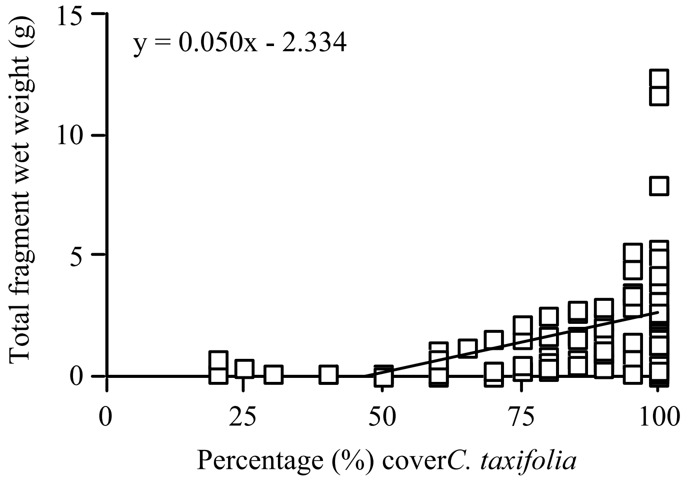


Figure 3.5. Relationships between total biomass of fragments and average frond height within quadrats in June/July 2002. West Conjola (A) r=0.27, n=100, P<0.01; Roberts Point (B) r=0.53, n=50, P<0.001.

A.



B.

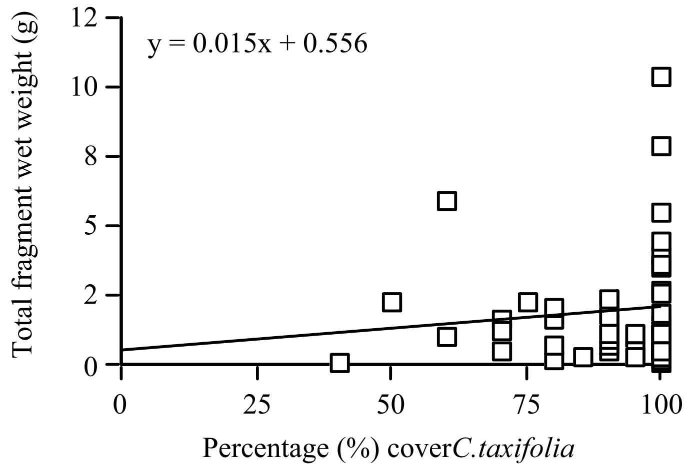


Figure 3.6. Relationships between total biomass of fragments and percent cover within quadrats in June/July 2002. West Conjola (A) r=0.43, n=100, P<0.001; Roberts Point (B) r=0.11, n=50, P>0.05.

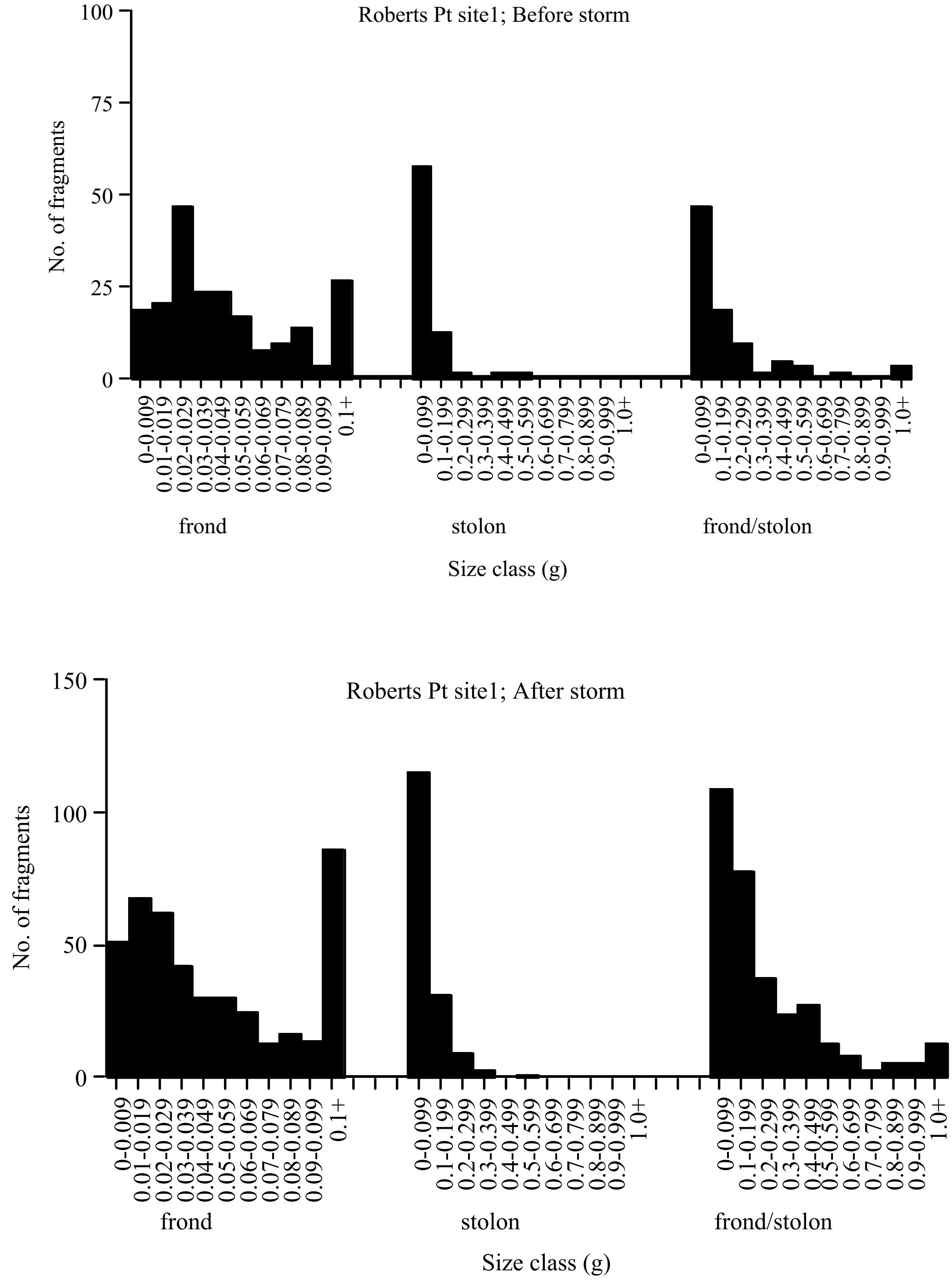
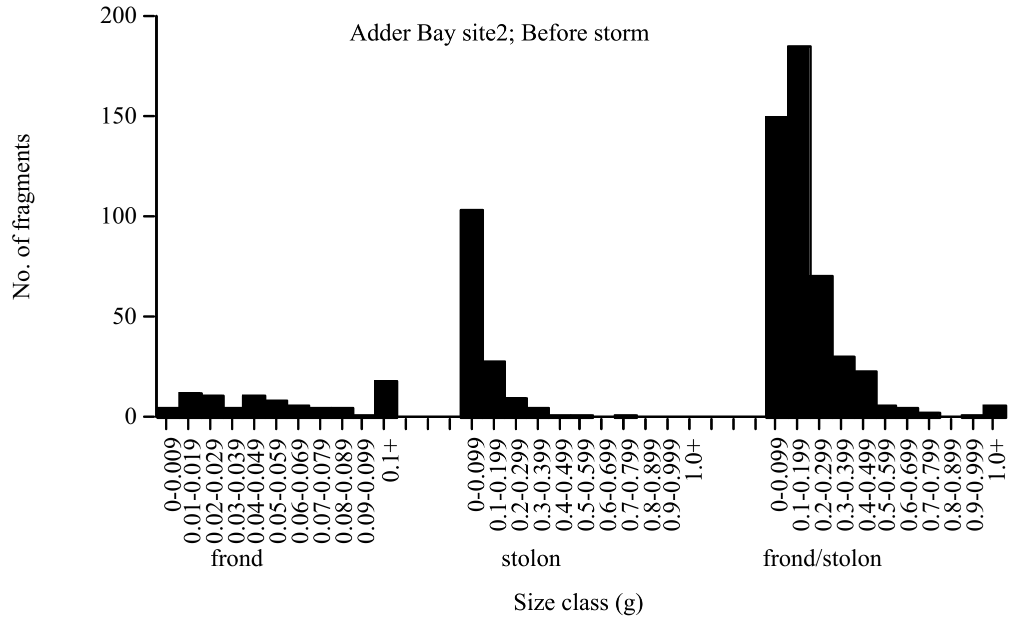


Figure 3.7. Size frequencies of fragments collected Before (upper) and After (lower) storm activity at Roberts Point, site 1.



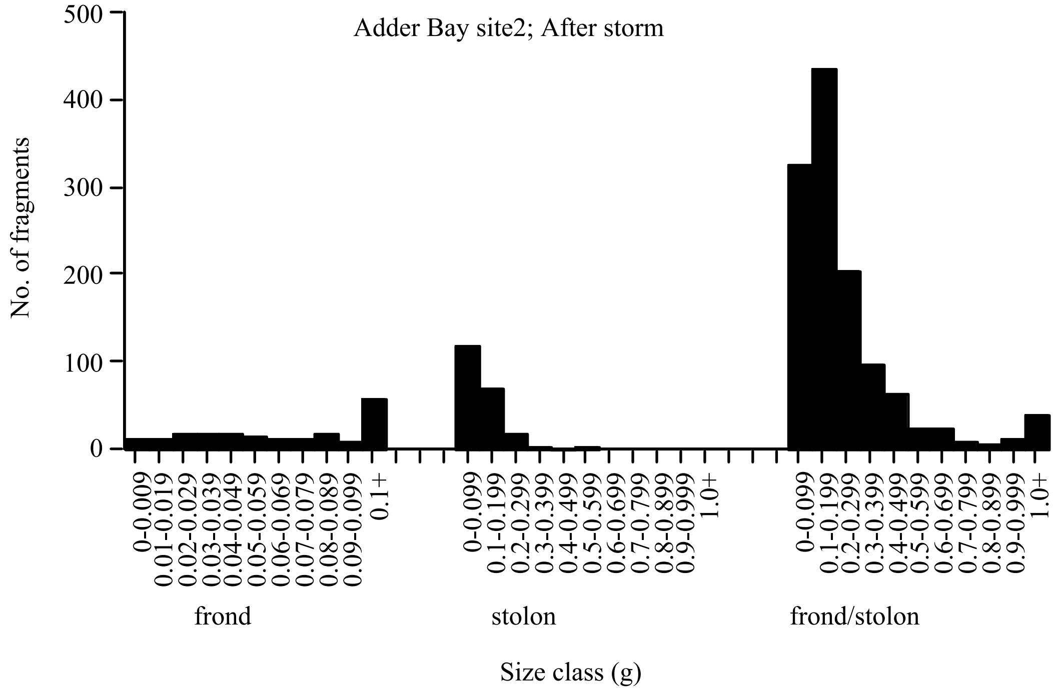


Figure 3.8. Size frequencies of fragments collected Before (upper) and After (lower) storm activity at Adder Bay, site 2.

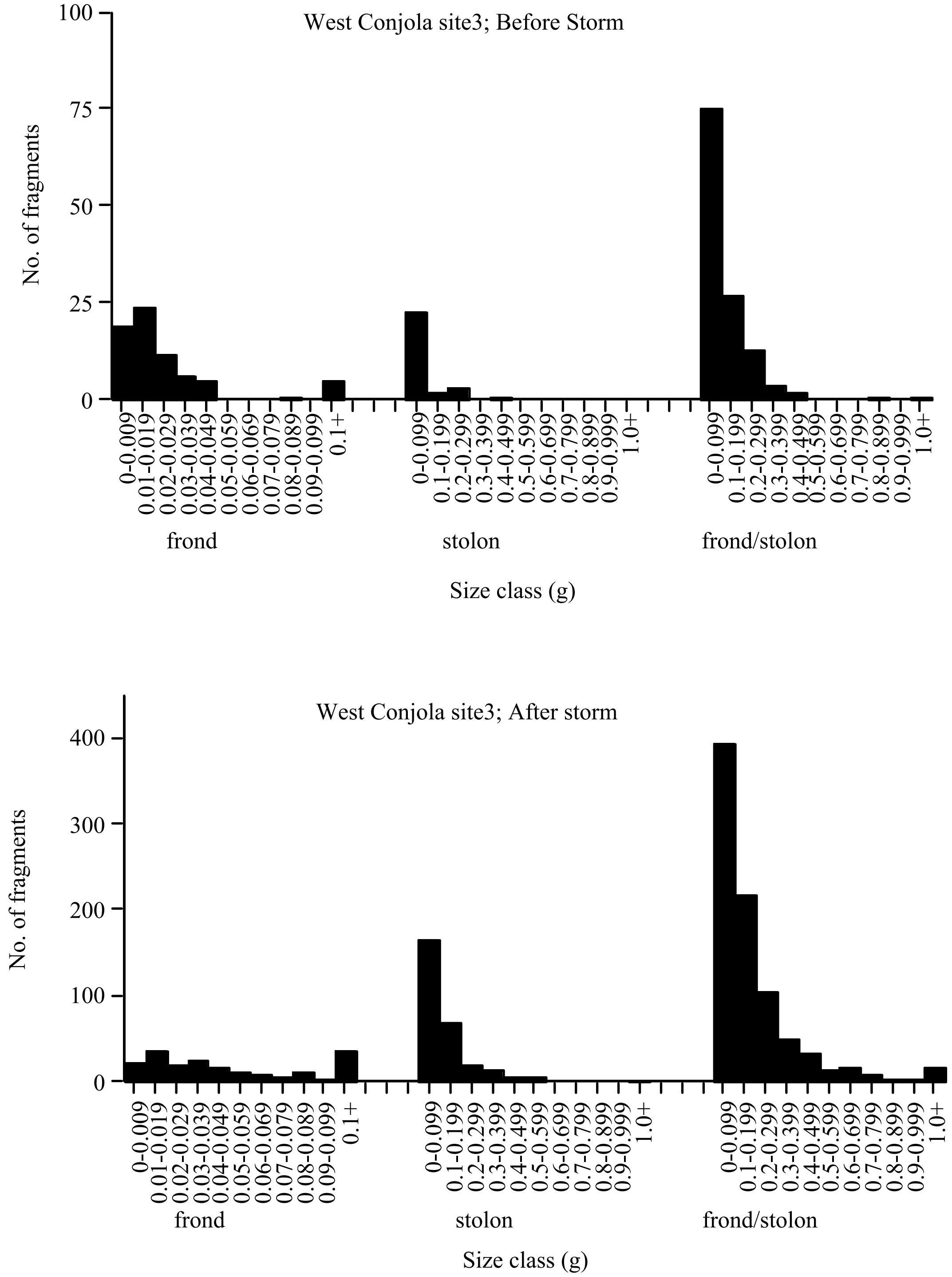


Figure 3.9. Size frequencies of fragments collected Before (upper) and After (lower) storm activity at West Conjola, site 3.

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.

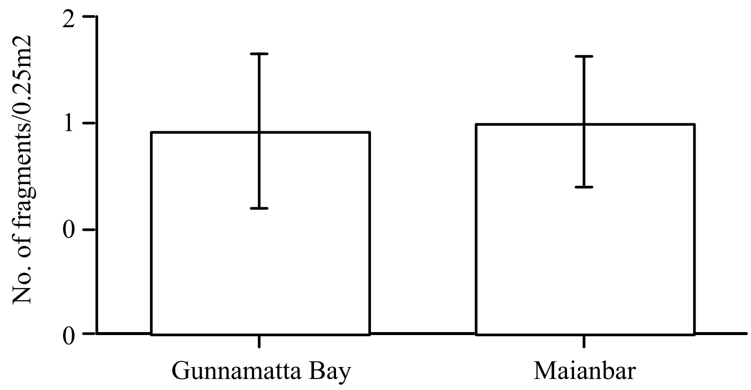


Figure 3.10. Mean (±se) number of *C. taxifolia* fragments per quadrat within *Zostera* beds at two sites within Port Hacking in May 2003. n=25.

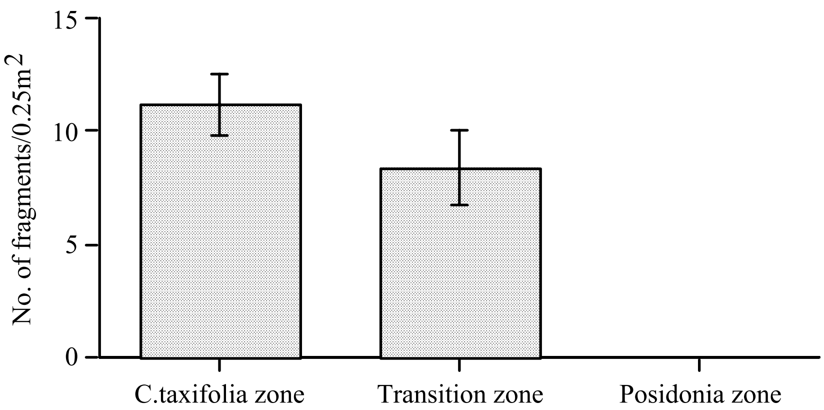


Figure 3.11. Mean (±se) number of *C. taxifolia* fragments per quadrat within 3 zones of *Posidonia* beds at two sites in Port Hacking in May 2003. n=10.

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.

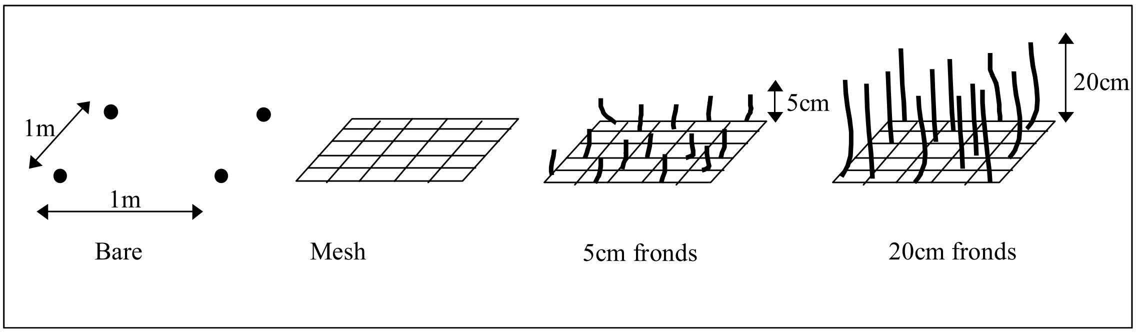


Figure 3.12. Treatments (bare, mesh, 5cm fronds and 20cm fronds) used to test for the effect of bed structure on the accumulation of *C. taxifolia* fragments.

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			

A.

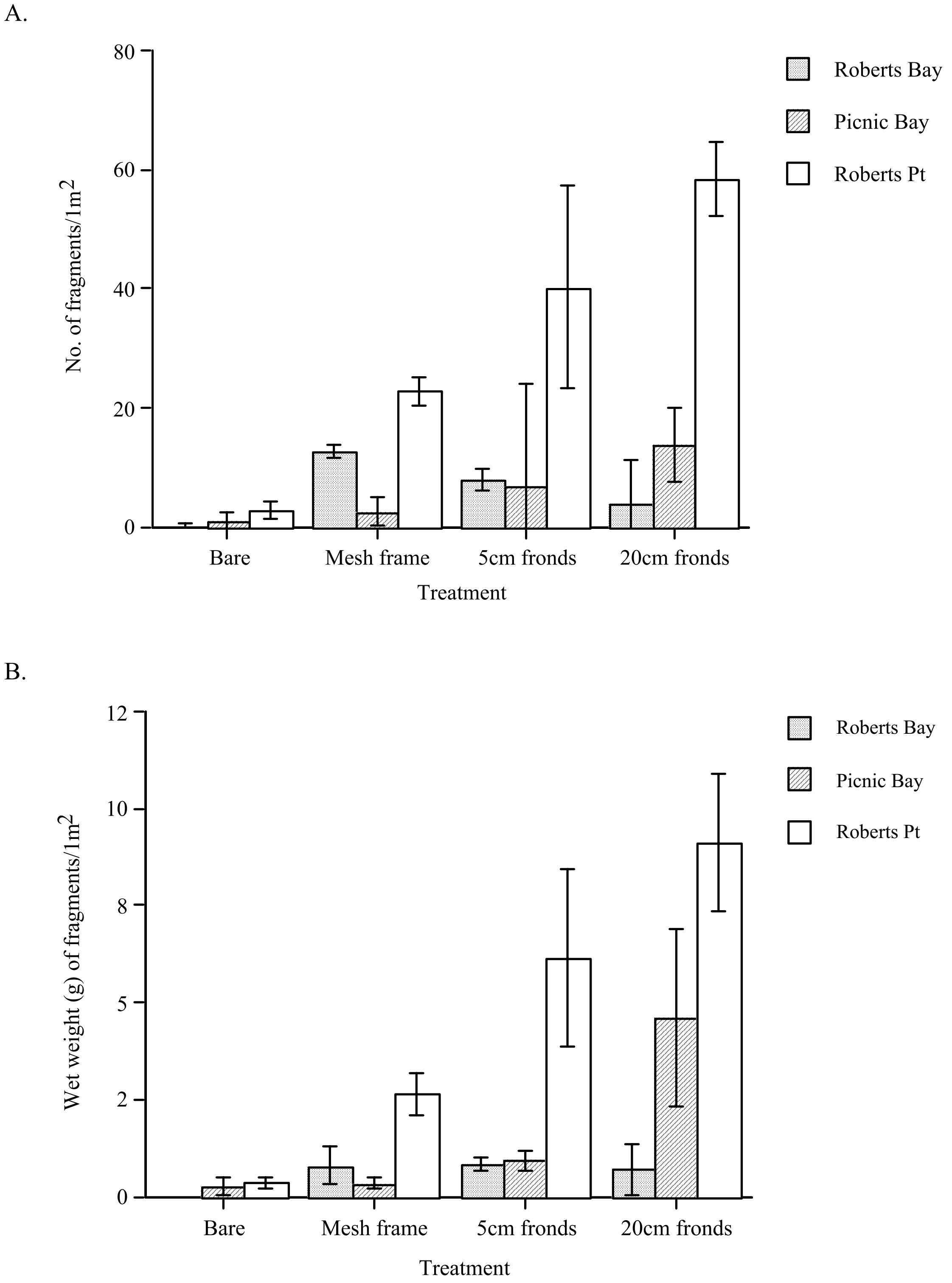
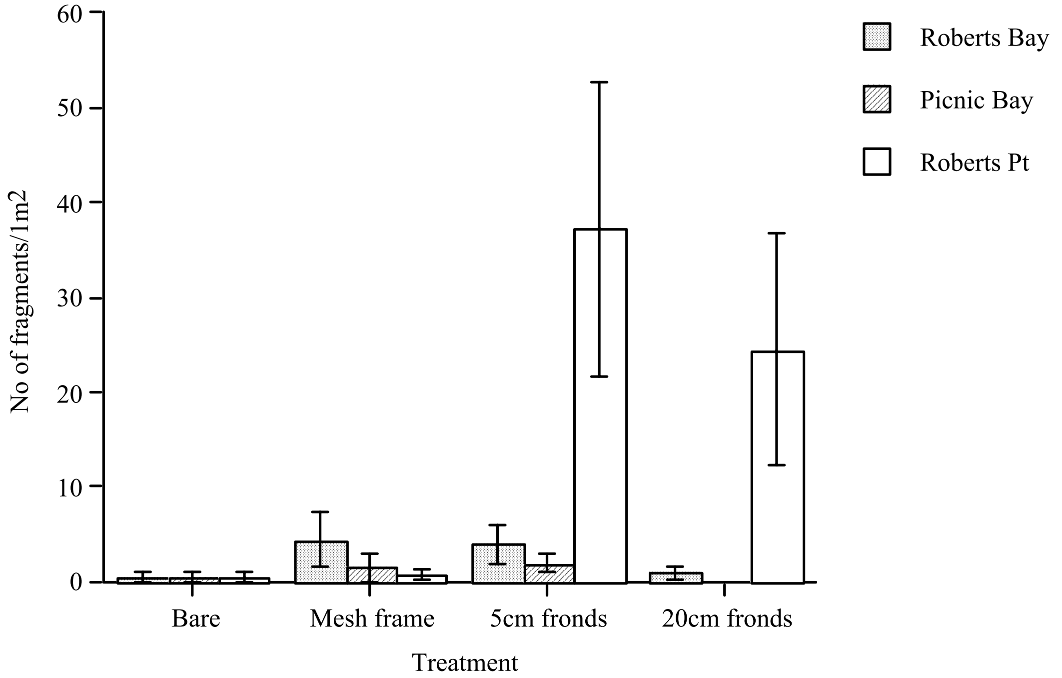


Figure 3.13. Mean (±se) number (A) and wet weight (B) of *C. taxifolia* fragments accumulated in each of four treatments in 3 sites at Lake Conjola between 13/01 and 12/02 2004. n=4 replicates.

A.



B.

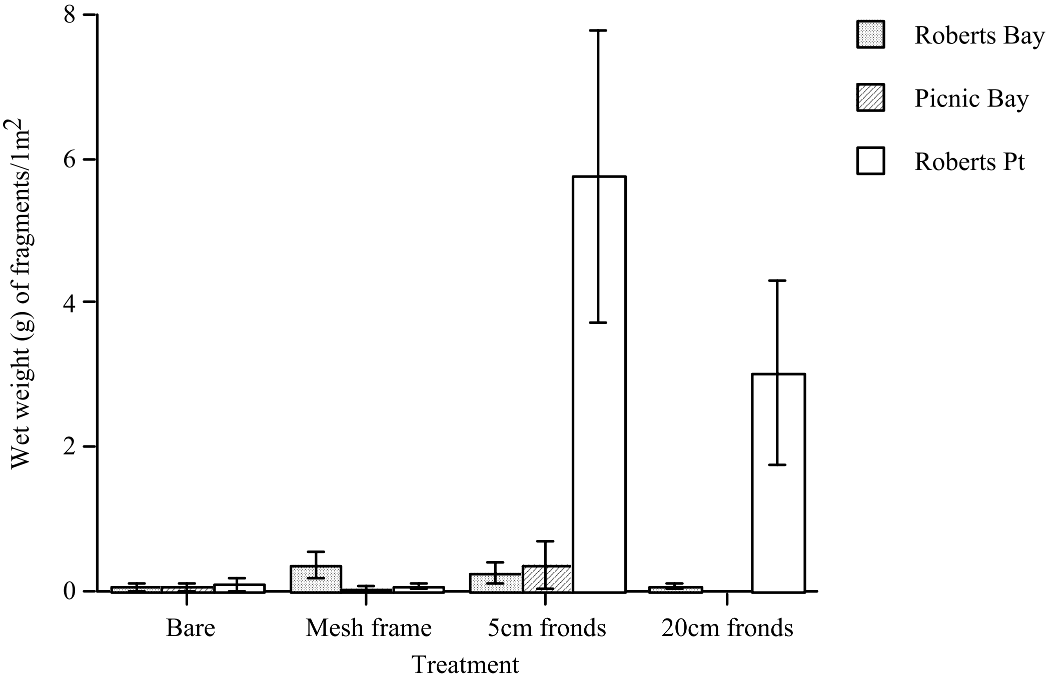


Figure 3.14. Mean (±se) number (A) and wet weight (B) of *C. taxifolia* fragments accumulated in each of four treatments: bare, frame only, short artificial fronds (5 cm) and long artificial fronds (20 cm) in 3 sites at Lake Conjola between 10/12/2003 and 13/01/2004. n=4.

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).

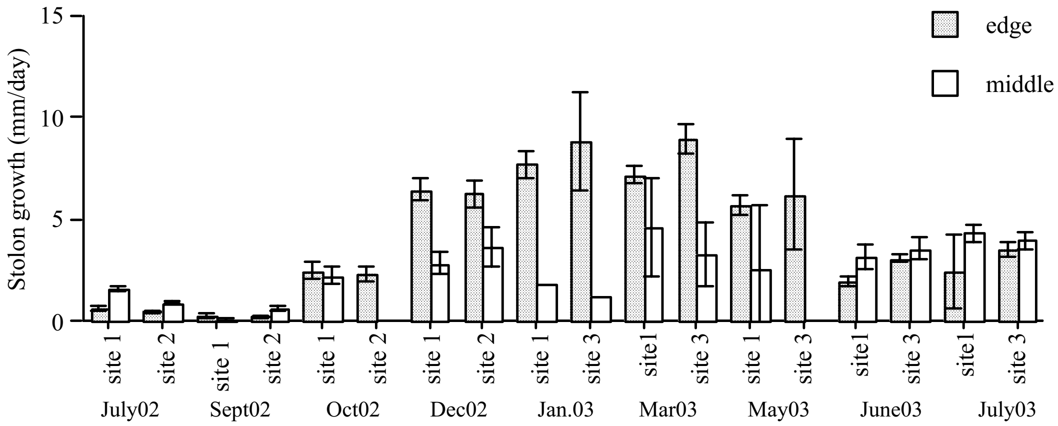


Figure 3.15. Average stolon growth (±se) within the interior and on the edge of established *C. taxifolia* patches at West Conjola, Lake Conjola between July 2002 and July 2003.

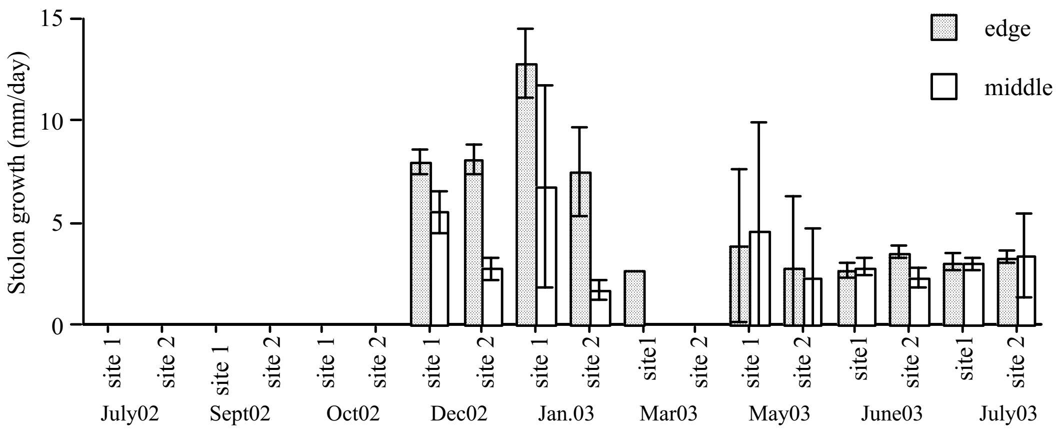


Figure 3.16. Average stolon growth (±se) within the interior and on the edge of established patches of *C. taxifolia* at Adder Bay, Lake Conjola between December 2002 and July 2003.

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.

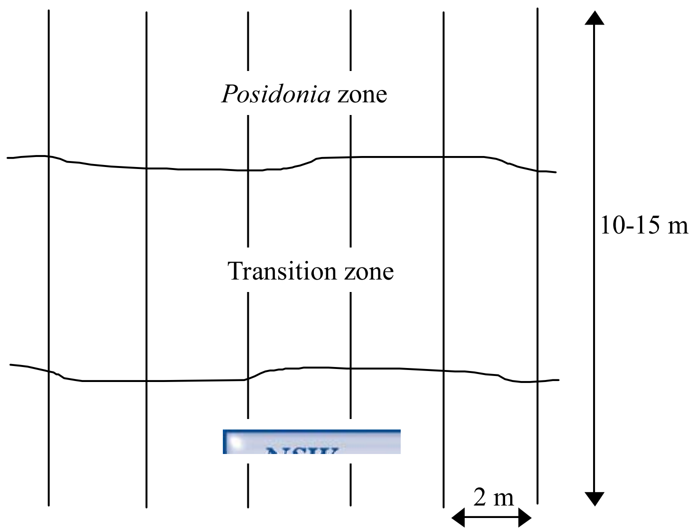


Figure 3.17. Diagrammatic representation of the experimental design to examine the spread of *C. taxifolia* among three zones at the boundary between the seagrass *Posidonia australis* and *C. taxifolia*.

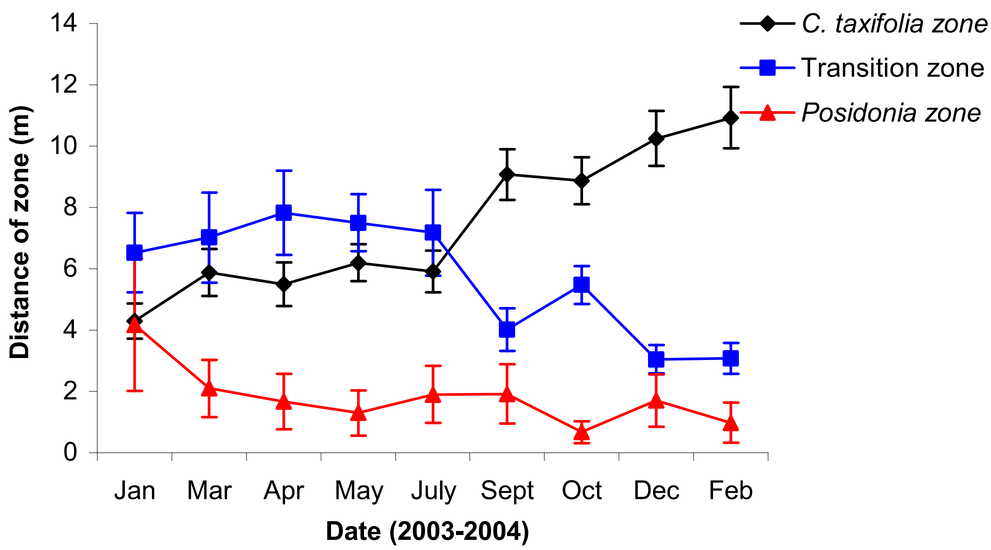


Figure 3.18. Average distance (±se) occupied by each of three zones along transects in Gunnamatta Bay between January 2003 and February 2004.

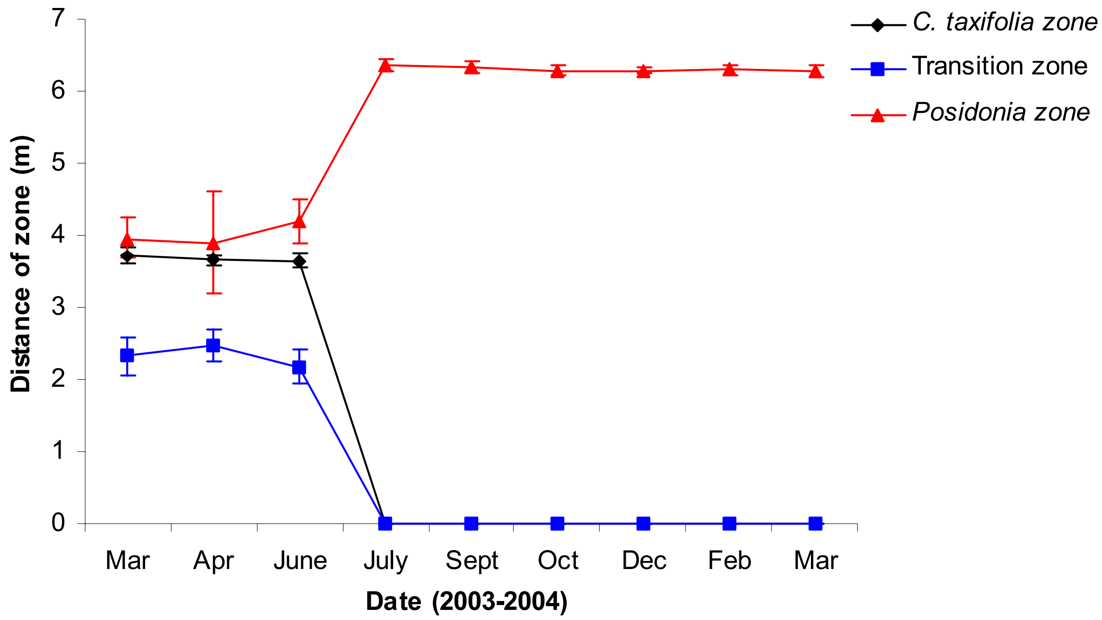


Figure 3.19. Average distance (±se) occupied by each of three zones along transects at Maianbar, between March 2003 and March 2004.

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.

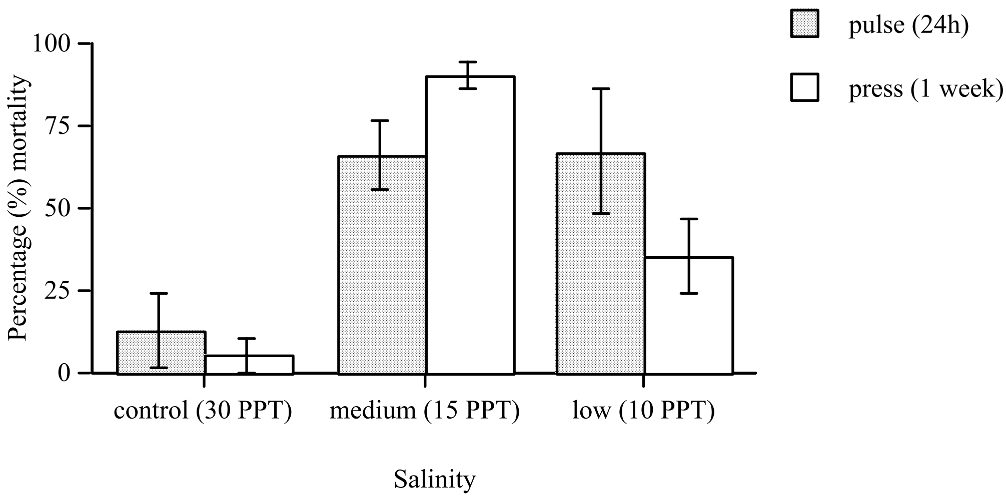


Figure 3.20. Average mortality (±se) of *C. taxifolia* fragments under three different salinity regimes (30ppt, 15ppt and 10ppt) for pulse (24hrs) and press (1 week) time periods. n=6.

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.