Part 1

Effects of flooding and turbidity on water plant germination and establishment from wetland seed banks

Chapter 2: Introduction and Methods

Chapter 3: Results

Chapter 4: Discussion

Chapter 2 Effects of flooding and turbidity on water plant germination and establishment from wetland seed banks

2.1 Introduction

Most researchers agree that water plants, once established, stabilize a system and maintain water clarity (Moss 1990, 1998, Scheffer *et al.* 1994, Moss *et al.* 1996, Coops and Doef 1996, Faafeng and Mjelde 1998, Van den Berg *et al.* 1998). Certainly there is no clear cause and effect relationship between the presence of water plants and turbidity levels. There are two current hypotheses that describe the interaction of water plants and turbidity. One is that submerged plant establishment occurs as the result of reduced turbidity (Moss *et al.* 1996) while the second is that plants reduce turbidity through many factors previously discussed in Chapter 1.6 (Crawford 1979, Wium-Anderson *et al.*. 1982, Moss 1984, Hart 1986, Scheffer *et al.* 1994). This 'chicken and egg' problem is complicated by the findings that in many studies water plants were already present and increased their dominance (Moss *et al.* 1996, Van den Berg *et al.* 1998). This introduction details some of the more specialised and technical concepts that will be used throughout Chapters 2 to 8 that were not discussed in the literature review in Chapter 1.

2.2 Water plants

2.2.1 Water plants and flooding

Many European and North American physiological studies have been done on submerged plants and their adaptions to flooding, germination and reproduction underwater (Haag 1983, Crawford 1987, Frankland *et al.* 1987, Davy *et al.* 1990, Hartleb *et al.* 1993, Kimber *et al.* 1995). However in Australia, few permanent lakes exist and water plants have adapted differently to large variations in water depth and frequent drying out. As a result of this environmental variability, European studies are often not relevant to Australian conditions and the studies on Australian species are fairly recent (Brock and Casanova 1997). Changes to water regimes caused by weirs and damming has created the situation where permanent water conditions are more common and water plants do not experience the same variation in water regime (Brock *et al.* 1994a). Australian studies into water regime have been on edge and emergent species (Chambers and McComb 1992, Rea and Ganf 1994 a,b, Smith and Brock 1996) and more rarely on submerged plants. *Vallisneria* sp. and charophytes have

been found to colonize permanently flooded conditions in man-made dams created by mining operations (Brock and Britton 1995, John and Ward 1996) but literature on the establishment of submerged water plants under Australian conditions is scarce (Blanch 1997).

2.2.2 Seed bank

Sediments can be a valuable source of seeds for the re-vegetation of degraded wetlands (Grillas *et al.* 1992, Brock and Britton 1995, Brock 1997). In 1859 Charles Darwin discovered 6¾ ounces (198 g) of sediment from a pond contained 537 water plant seeds (Darwin 1859). Sediments from wetlands in the Northern Tablelands, including farm dams, with different water regimes have been found to contain diverse seed banks with high numbers of wetland water plant seeds and high numbers of charophyte oospores (Bell 1991, Casanova 1993, Brock and Britton 1995, Casanova and Brock 1990, 1999b). Lists of plants that germinated from two seed banks that will be used in the experiment discussed in this chapter can be found in Casanova (1993), Britton and Brock (1995) and more recently, Crosslé (1998). Having a list of plants is an advantage in that donor seed banks can be chosen on the basis of species richness or composition but this does not guarantee germination of these plants will always occur. An explanation of the dispersion and subsequent germination of water plant seeds from submerged sediments can be found in various books and papers (Haag 1983, Cook 1987, Frankland *et al.* 1987, Ernst 1990, Kimber *et al.* 1995). A difference in species composition is known to occur when germinating seed bank under field and glasshouse conditions (Brock and Britton 1995). These differences may be caused by seasonal and physiological differences, the absence of dormancy breaking conditions, grazers, variation in temperature, light and depth. To estimate seed bank composition of a wetland, taking many small samples allows for more precise estimates of species richness than a few large ones (Grillas *et al.* 1992, Brock *et al.* 1994 b).

2.2.3 Functional groups

The use of functional groups to group plants into guild-oriented models to predict plant responses to different environmental situations has become accepted practice in conservation (Boutin and Kennedy 1993). Such groupings are useful when the taxonomic placement of a species is not as important as its morphology and response to environmental conditions. A 'functional classification system' that identifies water plants through where they germinate, grow and reproduce in response to water presence and absence has been developed (Brock and Casanova 1997) (Table 2.1). This system groups water plants into three bands; terrestrial, amphibious and submerged. The submerged plants, the main focus of this thesis, are fully aquatic and cannot tolerate desiccation. Although amphibianious plant groups are also very important to farm dams, time constraints on this study and the fact that other

students were in the process of studying these groups determined the decision that such data was not to be collected (Sansom 1997, Crossle' 1998). Submerged plants are more likely to control the functions of aquatic ecosystems discussed in Chapter 1.6 although amphibious plants, by reducing edge disturbance and wave action, would also reduce water turbidity (Moss *et al.* 1996). Amphibious plant groups, because of their position above or just beneath the water line, are not affected by turbidity to the extent of the fully submerged groups. The results of Brock and Casanova's (1997) study on 'functional groups' were published after Part 1 of this research work was completed, so discussions on the results have been adjusted in light of this recent information.

A permanently flooded water regime, as found in farm dams, has the potential to change plant communities towards dominance by particular functional groups e.g. amphibious responder and submerged species, and in doing so reduce species richness (Brock and Casanova 1997). The use of this existing functional grouping will be followed in the discussion of the results from PATN to identify any changes that might be the result of turbidity. The use of seed banks in which most species have been identified and classified using this model made this easier.

2.3 Aims

The experiments that were undertaken in Part 1 aimed to:

a) map and evaluate the light climate (PAR) under four turbidity and three depth treatments to see if underwater light was limited and was preventing water plants from colonising turbid farm dams and

b) assess the suitability of the secchi disc for measuring secchi depth versus turbidity, depth and light climate in ponds for later use in field work with landowners where turbidity may be high.

c) assess the suitability of the source of seed bank and species present in two wetlands, one temporary and one permanent, to use in the revegetation of turbid farm dams.

d) record changes in germination, biomass and length in water plants that germinate and establish from these seed banks to determine the effect of turbidity on plant responses which may indicate adaption or tolerance of turbidity in these plants.

Scheffer *et al.*'s (1994) observation that 'plants reduce turbidity through many factors' assumes plants are present in the waterbody in the first place. Many waterbodies, e.g. man-made dams are often turbid but once they have been colonised by water plants they have

'cleared' (pers.obs.). The interpretation which can be made from this observation is that some submerged water plants (and also emergent floating plants seen in turbid lakes and dams), do not need clear water for their germination and establishment.

Three hypotheses are put forward to explain how certain species of water plants colonize turbid waters:

(1) Turbidity does not prevent water plant seeds from germinating. Plants do not need clear water in which to germinate and establish.

(2) Turbidity affects the species richness and biomass of submersed plants.

(3) Flooding and turbidity tolerant species are able to respond to a changing depth and light climate by altering their morphology, such as petiole length.

Specifically the underlying null hypotheses being tested are:

1) There is no difference in germination and establishment from the seed bank under turbid and clear conditions.

2) There is no difference in species richness and biomass of plants in turbid and clear conditions.

3) The depth of flooding in combination with high turbidity has no effect on plant morphology.

2.4 Methods

2.4.1 Experimental design

This experiment to undertake the three aims outlined in the last chapter were set up as follows:

• Two wetland soil seed banks were used, one from a temporary and one from a permanent wetland. (Discussion in Chapters 2.4.2 and 2.4.3)

• Four turbidity treatments were applied including a control in replicated pairs of ponds: (clear water control: 0-3 NTU, low: 20-50 NTU, medium: 50-100 NTU, high: 100-160 NTU. (Turbidity in Nepthelometric turbidity units, NTU) (Discussion in Chapter 2.5.2).

• Three depths for the submersion of the seed bank were 5, 10 and 20 cm (Discussion in Chapter 2.4.3.)

The 500 litre ponds were laid out in a simple block design with a non-random arrangement of the ponds with different turbidities (Plate 1a). This non-randomness would not have affected the results as direct sunlight was available to all ponds for more than 10 hours per day and pots were placed at random in these ponds. Ponds were topped up with rainwater so that the depth was stable and constant. Turbidity, secchi disc transparency (Z_{SD}) and temperature was monitored for a week before adding the pots and was shown to vary little over the time and only needed re-mixing after 5 days. Description of turbidity generation and how the ponds were set up is discussed in Chapter 2.5

2.4.2 Study sites and sources of seed bank

Pond, glasshouse and laboratory experiments were carried out at the University of New England, Armidale, New South Wales, from July 1996 to July 1998. Two wetlands in the Northern Tablelands area of New South Wales; Racecourse Lagoon (30^0 39′S; 151^0, 30′E), Dumaresq Dam (30^0 26′S; 151^0, 36′E), were used as a source of wetland plant seeds. Sediment from these wetlands had been used in previous experiments and was known to contain viable seeds (Casanova and Brock 1996, 1999a). Seed bank from the wetlands had been previously collected and dried on tarpaulins, broken up and sieved through a rough sieve and mixed thoroughly with a shovel.

Both wetlands had several submerged and rooted floating plant species that were of interest in this experiment. (The latter have been observed in the deeper, turbid waters of farm dams and drainage channels near Griffith, N.S.W., Australia (pers.obs.)) (Table 2.1). Germination in water plants occurs in spring and autumn with the latter season seen as more favourable for germination and establishment (Brock and Britton 1995). (For complete species lists of these wetlands see Casanova (1993), Brock and Britton (1995). For more recent publications with species lists: Casanova and Brock 1997, Sansom 1997, Crosslé 1998).

Racecourse Lagoon

Racecourse Lagoon is a temporary lake and is a deflation basin on granitic soil at an elevation of 1030 metres above sea level. It has a surface area of 20.1 ha and a small catchment area of 65.4 ha. It fills for about two years in five and has a maximum depth of 2.75 metres (Casanova 1993). The groundwater table and run-off from rainfall affect the water regime. Submerged and attached floating water plant species in the seed bank are listed in Table 2.1

Dumaresq Dam

A man-made embankment built on Dumaresq Creek, which flows through Armidale, created Dumaresq Dam. It is a permanent water storage in granite soil country with a catchment area of 20.5 km^2, a maximum depth of 9.1m and a surface area of 12.1 ha (Banens 1990), and is used mainly for weekend recreation and a source of water for a number of downstream users. The depth of the storage can fluctuate by 1 to 2m in dry years (Casanova and Brock 1999a). This man-made lake supports a lower total species richness than Racecourse Lagoon but still has a diverse submerged and floating water plant community (Table 2.1)

2.4.3 Installing pots with seed bank in ponds

Dumaresq Dam or Racecourse Lagoon soil containing the seed bank was added in a 2 cm layer to the top of 7 cm diameter pots (area 227 cm^2) filled with river sand. Soil was dampened with rainwater before adding to the ponds so soil and seed did not disperse and contaminate other pots. One control of washed river sand was placed in each pond at 5 cm to detect any seed transfer between pots. Pots were randomly arranged in the ponds at three depths with 5, 10 and 20 cm between the soil surface and the water/air interface. The pots were suspended in the water from chains hung from hooks attached to a wire frame erected horizontally over the ponds. The links in the chain could thus be used to immerse the pots at the three different depths required. This arrangement gave four replicates of each seed bank per depth per treatment, and 8 replicates per treatment placed at the three depths. There were 13 pots per pond including control pots; a total of 104 pots. The 1-metre diameter ponds allowed for water movement around the pots. Pots placed at 20 cm would have been partly

shaded at some time during the day by the pots at 5 and 10 cm depth and the pots at 10 cm shaded by the pots at 5 cm. However the random placement of pots prevented a consistent bias in the effects of shading from other pots.

Table 2.1 Species of submerged and attached floating water plants found in two wetlands: Racecourse Lagoon and Dumaresq Dam (Casanova 1993). Functional groups from Brock & Casanova 1997. Ate = amphibious fluctuation-tolerators, emergent; ARp = Amphibious fluctuation responders, morphologically plastic; ARf = Amphibious fluctuation responders, floating/ stranded; S = submerged.

Species	Racecourse Lagoon	Dumaresq Dam
Crassula helmsii (ARp)		√
Elatine gratioloides (ARp)		√
Lilaeopsis polyantha (ATe)	√	
Marsilea hirsuta (ARf)	√	√
Myriophyllum lophatum (ARp)	√	
Myriophyllum variifolium (ARp)	√	
Myriophyllum verrucosum (ARp)	√	
Najas tenuifolia (S)	√	
Nymphoides montana (ARf)		√
Potamogeton ochreatus (S)	√	√
Potamogeton tricarinatus (ARp)	√	
Vallisneria gigantea (S)	√	√
Chara australis (S)	√	√
Chara muelleri (S)	√	√
Chara globularis (S)	√	√
Chara preissii,(fibrosa) (S)	√	√
Nitella cristata (S)	√	√
Nitella hookeri (S)	√	
Nitella sonderi (S)	√	√
Nitella subtilissima (S)	√	
Total	17	12

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2.5 Description of turbidity generation and experimental design

2.5.1 Turbidity generation

To simulate the light conditions found in local farm dams, clay suspensions were used in 500 litre outdoor ponds. This first step involved making up a stock suspension of clay dug from clay-rich deposit (60% kaolin) collected in Armidale, NSW. To make the stock, clay was mechanically agitated to slurry with ion-free rainwater using an electric hand drill with mixer attachment. The thick stock solution was then filtered through a 500μm, 250μm, 125μm and then a 38μm sieve and the filtrate thoroughly mixed. This stock was too concentrated for its turbidity to be read on a turbidimeter (Hach 2100A). The clay stock was stored in 4 x 50 litre sealed plastic containers in a darkened cold room (10 °C to inhibit algal and bacterial activity). The relative protection from flocculation is the result of repulsion between the negatively charged clay particles, which prevents them from coming together to cohere. The majority of the clay particles in this clay were in the 2 to 5μm diameter range (D. McLeod, pers. comm.). The size of the fraction meant these particles could remain suspended, similar to clays in many turbid farm dams in the Tablelands.

To obtain the required turbidity measured amounts of clay concentrate (5, 10, 15, 20, 25 ml) were added to rain water in vitro and made up to 1000 ml. Turbidity readings were made after 12 hours to allow the 6 to 38 μm fraction to settle out. Because turbidity and concentration (the amount of clay added to 1000 ml) are positively correlated (Gippel 1989) the amount of stock clay to be used was simply extrapolated for the 500 litre tanks. The use of low conductivity rainwater instead of high conductivity tap water in the ponds greatly reduced flocculation so submersible pumps were not needed to keep the clay particles in suspension. A drill-operated mixing plate was found adequate for mixing the clay concentrate when it was first added. This mixer was simple and consisted of a rod with attached aluminium propeller that fitted into an ordinary battery operated carpenter's drill.

Rainwater from storage tanks at UNE was used to fill the eight 500 litre ponds used in the experiment

2.5.2 Setting up turbid ponds

Before addition of the pots of seed bank the 0.6 metre deep by 0.95 metre wide, 500 litre paired ponds had measured amounts of clay stock agitated into the rainwater using the battery operated mixer. An amount of water was first removed equal to the amount of stock to be added. Two ponds were designated Treatment 1, control ponds, no clay stock was added to these and the water clarity was maintained at all times. The three other turbidity treatments were designated Treatment 2) low, Treatment 3) medium and Treatment 4) high (Table 2.2). An addition of 0.5 litre of clay stock to 500 litres was equivalent to an increase in turbidity of 10 NTU so a measured amount could be added when necessary to bring the turbidity of a pond into the desired range. For 5 days before pots were added, measurements of turbidity, secchi depth, air and water temperature were taken in triplicate to observe if turbidity, and hence underwater light conditions, could be maintained in these experimental ponds.

Table 2 2 Rule of thumb for generating turbidity (NTU) using clay stock (approximate).

Turbidity	Stock (l in 500 l)	Nephelometric Turbidity Units (NTU) Range
Treatment 1) Clear	0	0-3
Treatment 2) Low	2.5	20-50
Treatment 3) Mid	5.0	50-100
Treatment 4) High	10	100-160

After the pots containing the seed bank were placed in the ponds turbidity measurements were done daily, and a measured amount of clay concentrate was agitated by hand into the surface of the water column twice a week in the evening. This enabled the fraction 6 to 38 μm to settle out overnight (16 hours) before turbidity and light measurements were taken the next morning and noon respectively. It was decided not to use the mechanical stirrer to ensure that the establishing seedlings would not be uprooted or disturbed, but to gently mix

the clay concentrate in by hand. There was some settling of fine clay into the pots but not enough to smother the seeds.

2.5.3 Testing the consistency of turbidity readings at depth

A small side experiment was undertaken before removing samples of water from the the ponds to investigate the ideal depth of sampling for turbidity readings and if sampling was affected by settling out of the clay suspension over time. This experiment was done in replicated glass aquariums in the glasshouse to negate any effects of wind mixing in outdoor ponds. Identical solutions were made by adding one part well-stirred clay concentrate to 5 parts of water. Turbidity was measured daily at depths of 1, 3, 5 and 7 cm with a portable turbidimeter (Jenway 6035) and compared to readings on another turbidimeter (Hach 2100A). This was to determine if the portable turbidimeter could be reliably used for both the university ponds and in the field. Using a ruler to measure depth from the water surface, multiple samples of turbid water were taken from each depth using a large 20 ml syringe and transferred to a sample bottle where they were then read after re-mixing gently by hand.

2.6 Data collection:

Measurements of underwater light and other parameters were made to investigate the environment for germination and establishment of water plants in turbid water. Nitrate-nitrogen (NO_3-N) and soluble reactive phosphate (SRP) but not total phosphate (TP) were measured in all ponds at the start of the pond trial. The phytoplankton community was sampled using one scoop of a 20 μm net and identified before adding the pots to indicate if any algae were present in the clay solution. There was no attempt to sample for Chlorophyll-$_a$, so no differentiation was made between the biotic and abiotic contributions to turbidity. Pots were placed in ponds on 19th November 1996 and after 8 weeks the experiment was terminated when most plants were well established but before they could shade plants in the lower pots. Pots were removed at random times and all plants were harvested between 13th and 17th January, 1997. Table 2.3 gives a list of physical, chemical and biological measurements used in Chapter 2 to 4 (pond experiment) and Chapter 5 to 7 (farm dam experiment and sediment germination experiment in the glasshouse). Methods are taken from Wetzel and Likens 'Limnological Analysis', 1991; and American Public Health Association. 'Standard methods for the examination of water and wastewater', 1989).

2.6.1 Light irradiance

A (LI-185B) Quantum radiometer-photometer with a LI-COR Underwater quantum sensor (LI-192SB) was used to measure the average underwater downwards irradiance, Ed, and upward irradiance, Eu, of the photosynthetically available radiation flux (PAR). Zm was the

depth from the surface to the sediments in the pots so pots were positioned so that light was measured at depths (Zm) of 5, 10 and 20 cm (Z_5, Z_{10}, Z_{20}) below the water-air interface. Ed and Eu readings were made randomly at these depths, twice a week at the same time around noon when light intensity was focused downward. Readings for each of the three depths and for each of the ponds were averaged. The collection of light intensity data was to indicate what light conditions occurred at shallow depths in a turbid waterbody, so as to determine why there was often no observed water plant growth in farm dams at these shallow depths. Readings were not made on extremely overcast or rainy days due to the high variation in light irradiance in air, so readings were biased towards the maximum intensity that would be received at that depth and turbidity. A rod attached to the wire frame allowed the sensor to be moved up and down and to be stabilized at each depth.

Light irradiance in air (Io) was also measured before and after the underwater readings were made when light intensity was most directly downward using a separate quantum sensor (LI-190SB) attached to the radiometer/photometer.

Results of Ed and Eu readings were plotted over the period of the experiment for the three depths and the replicates of the four turbidities. Table 2.4 shows a description of the measurements, the symbols and units used in light studies and in this thesis.

Table 2.3 List of physical, chemical and biological measurements used in Chapter 2-4 (pond experiment) and Chapter 5-7 (farm dam experiment and the sediment germination experiment in the glasshouse).

Measurement	Method used	Ponds	Farm Dams
PAR (μmol $m^{-2}s^{-1}$) (LI-185B) Quantum radiometer/ Photometer	Underwater quantum sensor (LI-192SB)	Yes	No
I_o (μmol $m^{-2}s^{-1}$) (LI-185B) Quantum radiometer/Photometer	Air Quantum sensor (LI-190SB)	Yes	No
Secchi depth (m)	Secchi disc 20 cm diameter	Yes	Yes
Turbidity (NTU)	Portable turbidimeter (Jenway 6035)	Yes	Yes (and glasshouse)
Conductivity (μScm^{-1})	Beta 81 conductivity meter	No	Yes (and glasshouse)
SRP (mg L^{-1})	Molybdate Blue method, read 705nm	Initial	Yes
TP (mg L^{-1})	Persulphate Digestion method	No	Yes
Nitrate-Nitrogen (mg L^{-1})	Cadmium reduction method, read 543nm	Initial	No
Chlorophyll-$_a$ ($\mu g\ L^{-1}$)	Trichromatic method for chl a,b,c , read 664, 665, 647, 630nm.	No	Yes
pH	Orion 200 pH meter temp calibrated	Yes	Yes (and glasshouse)
Phytoplankton	Identification using Wild Heerbrugg and Leitz Laborlux high magn. Microscope	Initial	Yes

Biomass (gm)	Scales – to 0.0000 gm	Yes	No
Germination (Nos)	Zeiss Dissecting microscope, magnifn. glass over tray	Yes	Yes (and glasshouse)
Presence-absence Waterplant identification.	Casanova 1993, Sainty and Jacobs 1981, 1994; Harden 1990-1993, Woods and Imahori 1964	Yes	Yes (and glasshouse)

Table 2 4 Measurements, symbols and units commonly used in light studies.

Symbol	Description	Units
PAR	Photosynthetically available radiation 400-700nm.	μmol m^{-2} s^{-1}
Io	Irradiance: radiant flux per unit area of surface in air	μmol m^{-2} s^{-1}
Ed(z)	downwelling irradiance of PAR, at depth z, diminishes with depth	μmol m^{-2} s^{-1}
Eu(z)	Upwelling irradiance of PAR at depth z	μmol m^{-2} s^{-1}
(Ed(z) +Eu(z))	*Total incident radiant flux on unit area at depth Z_m	μmol m^{-2} s^{-1}
Z_m	Underwater depth from surface to sediment to depth m	metres
Z_{SD}	Secchi depth transparency	metres
Z_{eu}	**Euphotic depth:where Ed falls to 1% of the subsurface value	metres
NTU	Turbidity	Nepthelometric turbidity units

*Most relevant light measurement for photosynthesis

**Z_{eu} = 2 X Z_{SD} or 3 X Z_{SD} in turbid water. Rough rule of thumb (Kirk 1994, Boulton and Brock 1999)

2.6.2 Physical parameters

Ponds were monitored daily over the experimental period. Three replicated measurements of secchi disc transparency (Z_{SD}) and turbidity for each pond was taken. Z_{SD} measurements were taken with a 20cm diameter secchi disc. Water samples were taken from a depth of 10-20 cm and turbidity read with a portable turbidimeter (Jenway 6035). Maximum-minimum submersible thermometers were placed in each tank and similar thermometers placed in air, one in the open and one in shade and measurements taken daily for both air and water. pH was measured daily with a portable meter (Orion 200). Results of Z_{SD}, turbidity and

underwater irradiance were measured to compare between ponds and relate them to each other and calculate Z_{eu} (Chapter 3, Table 3.2)

The secchi disc transparency was seen as a particularly important measure as it was expected this would be used to convey the significance of light to submerged water plants when undertaking extension work with landowners. Secchi disc transparency is a measure of water transparency and approximates the greatest depth at which rooted aquatic plants will grow. Euphotic depth, (Z_{eu}) is taken as approximately twice that (2 X Z_{SD}) and is given as the lower limit for plants including phytoplankton (Moss *et al.* 1996), but it may be 3 X Z_{SD} in turbid waters (Boulton and Brock 1999). This is because light scattering may be more intense in turbid (or alga dense) waters as Eu is reflected downwards again (Kirk 1994). For practical purposes when dealing with turbid farm dams, as a rough rule of thumb the difference between 2 times and 3 times the Secchi disc depth may not be all that large.

2.6.3 Water plant collection, identification and measurement

For taxonomic identification of water plant species in Part 1 and 2 the following were used: Harden G.J. (1990-1993), Sainty and Jacobs (1981, 1994). For functional groupings see Table 2.1 (from Brock & Casanova 1997). For identification of charophytes: Casanova (1993) was used. For identification of algae: Prescott (1978), Belcher and Swale (1976) and Bold and Wynne (1978), Entwistle, Sonneman and Lewis (1997), and Canter-Lund and Lund (1995) were utilised.

Photographs were taken of *Chara* and *Nitella* grown under low and high turbidities using a Wild M-400 photomicroscope using automatic exposure. A presence-absence and abundance score was carried out weekly for all pots for up to 6 weeks after submersion to determine if significant numbers of water plants died before they could be harvested. Water levels were kept topped up until plants were harvested. Harvesting entailed dividing the soil layer into 4 equal sectors with a knife, taking one quarter at a time, soaking this apart and separating the plants gently in a flat tray. If charophyte germinations were too abundant to count within an hour, one quarter of the pot was counted and the results extrapolated. Both below-ground and above-ground biomass was gathered for total biomass weights.

All plants were placed in paper bags for drying at 85 ^{0}C for three weeks. The morphological features of each plant species and the numbers which germinated initially determined the method of scoring and counting individual plants (i.e submerged charophytes were identified as SC, submerged 'others' were identified as SO (even when apical shoots emerged with reproductive parts and floating leaves), and emergents as E. Soon after this data was collected and analysed, the functional classification system was introduced. This is a multivariate analysis that identifies plants with a key by their germination, growth and

reproduction in relation to water regime (Brock and Casanova 1997). With this system plants were finally identified by their place in relation to the water regime which gave a focus for future discussions. To make use of this model and simplify further discussion, plants have been listed under the functional classification system as submerged, S; amphibious fluctuation responders (plastic or floating), ARp and ARf; amphibious fluctuation tolerators (emergent) ATe; and terrestrial damp, Tda. The use of two keys may be initially confusing but the placement of the water plant with respect to water regime has not changed. Some plants that were initially listed as 'submerged other ' but under the new key are listed as Arp were merely responding to constant flooding. All submerged charophytes that were SC are now listed with the key S (submerged) under the new functional classification system. The functional classification system will be used throughout.

The results of the species that germinated, their original identification (in brackets) and their new functional groupings are listed in Appendix I.

Data collected (for Racecourse Lagoon and Dumaresq Dam) were number of species, (species richness), number of plants that germinated per species and in total (as plants per 17 cm diameter pot then converted to germinations m^2) and biomass. Dried samples for biomass estimations for both species and total were weighed to four decimal places, the bag weight deleted and weights presented as biomass g 227 cm^2 which was converted to biomass g m^2.

Changes in morphology with depth and turbidity were measured by taking the longest stem or leaf length (cm) of each *Vallisneria gigantea* and *Myriophyllum variifolium* plant and each of the charophyte plants (*N. sonderii, N. subtilissima, C. fibrosa* and *C. muelleri*) and averaging them. This data was recorded from all treatments at the greatest submerged depth (20 cm). The number of apical shoots was counted in *M. variifolium* and also the width of *V. gigantea* leaves. Due to the lower numbers of plants of all species that were harvested at 20 cm from Dumaresq Dam seed bank, it was not possible to analyse the differences in length as was done for Racecourse Dam seed bank from 20 cm (Results are in Figure 3.6)

2.7 Data analysis

2.7.1 Measures of light and physical parameters

Results of Ed and Eu readings were averaged and graphed for the period of the experiment for the three depths and the four turbidities. The measures of the physical parameters secchi disc transparency, turbidity, temperature (air and water) and pH were averaged and graphed over time. Initial SRP, TP and NO_3-N readings were sampled from each pond and measured

using methods in Table 2.3. The relationship between Z_{SD}, Ed +Eu, Z_{eu} and turbidity were found by plotting the points from the averaged data from the paired ponds.

2.7.2 Biological parameters – Germinations and Biomass

The data for species presence and absence (species richness), numbers of plants (germinations) and total dry weight (biomass) of each species were entered into Excel and statistical analyses were undertaken on transferred data using Minitab (release 9) for analysis of variance (ANOVA) and regression. The species richness data were normally distributed so no transformations were necessary. Multivariate analysis (association and classification) using the program 'Pattern Analysis Package', PATN (Belbin 1991) was used to find similar 'groups of pots' depending on the species which germinated.

All species were treated as of equal value and species germination data from Racecourse and Dumaresq Dam seed banks were initially grouped and analysed together and then analysed individually separately. The clustering of pots from all depths in all treatment ponds was done for Racecourse Lagoon and Dumaresq Dam to determine similarities among depth and turbidity treatments with rows denoting treatment pots at different depths (objects of primary importance) and columns representing the numbers of germinations of species (objects of secondary importance).

Association measures were made between all pairs of rows in the data file with number of species weighed similarly using an unweighed pair group method using by convention the ß value of –0.25. The hierarchical-agglomerative-polythetic clustering method (FUSE) was used to cluster species because it displays the results as a dendrogram (DEND) and groups the pots according to their degree of similarity in species germination.

2.7.3 Germination and biomass of charophytes and other species under different treatments and depths

In the comparisons of submerged germination and biomass, charophytes were visually dominant in one seed bank and *V. gigantea* in the other. As charophyte germination and biomass appeared to have increased under increasing turbidity, it was decided to compare the germination and biomass of charophytes with that of the angiosperms. This resulted in a comparison of one seed bank in which charophytes were dominant to another in which angiosperms were dominant. The dry biomass of each charophyte species, submerged species (angiosperms), and edge emergent species were counted individually for biomass and germination. Emergents in this case were not presented as they only occurred in smaller numbers and their biomass was insignificant.

Vallisneria was also compared as a separate submerged group to the other angiosperms because of its numbers and complete submersion to see if its biomass was significantly different under clear and turbid conditions. A comparison of germination numbers and total biomass of charophytes and 'other species' was plotted between Racecourse and Dumaresq Dam seed bank.

Data was entered in an Excel file with species biomass in rows and treatment pots in columns and copied to Minitab for statistical analysis and analysis of variance (ANOVA) done on treatments (depth and turbidity) versus germination and biomass. Seed banks from Racecourse Lagoon and Dumaresq Dam were analysed separately. Germinations (plants m^2) and dry biomass (g m^2) for charophytes and other species were plotted against each turbidity treatment.

2.7.4 Lengths

For the Racecourse Lagoon seed bank, lengths were measured on plants taken from 20 cm depth. All plants of *Myriophyllum variifolium, Chara muelleri, C. fibrosa (C. preissi), Nitella sonderi* and *N. subtillisima*, the five species that germinated in each of the pots of Racecourse Lagoon seed bank at depth 20 cm, were measured using a fine grade calibration ruler. These data were analyzed for significant differences between treatments using ANOVA.

For the Dumaresq Dam seed bank, at 20 cm depth there were insufficient numbers of each species for statistical comparison.

Chapter 3 Results: Effects of flooding and turbidity on water plant germination and establishment from pond trials with wetland seed banks

3.1 Environment of ponds: light, chemical and physical parameters

Four turbidity treatments (Table 2.2) were imposed on two seed banks to test germination and plant establishment and maintained for the 8 weeks duration of the experiment. Baseline soluble reactive phosphate (SRP) and nitrate–nitrogen (NO_3-N) readings are shown in Table 3.1. These are the average of two water samples taken from each pond. Post-experiment readings were not done. The increase in water pH after 8 weeks was slightly higher in the clear water and low turbidity ponds than in the medium and high turbidity ponds. All ponds responded to the environmental variation of the site in the same way as seen by the similarity in the maximum and minimum temperatures (Table 3.2) and light at 5 cm (Figure 3.1).

In the glass aquariums in the glasshouse, the initial tests on the stability of the generated turbidity without the further addition of clay concentrate showed that the heavier particles settled out a few hours after the concentrated clay was added, leaving the fine particles in suspension (See Chapter 2.5.3). Data from these tests are not given here but turbidity measurements were very stable 1 cm from the water-air surface and dropped by an average of 10 NTU over 4-5 days. Samples taken at each of the three depths were similar with small differences in turbidity readings (± 0.5 NTU). Photosynthetic bacterial activity (but not blue green algae) was sometimes present in the top 0.5 cm with increased turbidity readings so samples were not taken at this depth. These measurements showed that turbidity in a water column could be measured at all depths (except directly at the surface). Clay concentrate should be added at night when the light to the water plants would not be affected by the larger particles which settled out before the morning readings were taken.

Few algae were present in the ponds at the start of the experiment but iron-oxidising and photosynthetic bacteria were observed with increasing additions of clay stock that created a slight film at the surface. In a second survey of algae three weeks later, there was more diversity in the clear water ponds where numbers of blue-green algae were present as epiphytes on plants. Algal scums established in the turbid ponds a few days after the pots and plants were removed for harvesting.

Table 3 1 Initial readings of SRP and NO_3-N and initial and final pH in all turbidity treatments. (n = 2, identical results).

Turbidity	Clear	Low	Medium	High
Base SRP (mg ml^{-1})	0.056	0.057	0.078	0.099
Base NO3-N (mg ml^{-1})	0.006	0.006	0.006	0.006
Initial pH	5.9	5.8	5.8	5.8
Final pH	8.75	8.5	7.65	7.25

Light irradiances in Armidale over spring-summer are given in Appendix II. Air temperatures over spring and summer in Armidale, New South Wales during the experiment ranged from a minima of 3 to 17 ^{0}C to a maxima of 22 to 39 ^{0}C (Appendix III).

The underwater light environment at three depths in the four turbidity treatments is given in Figure 3.1. Raw data for PAR, secchi disc depth and turbidity are given in Appendix IV.

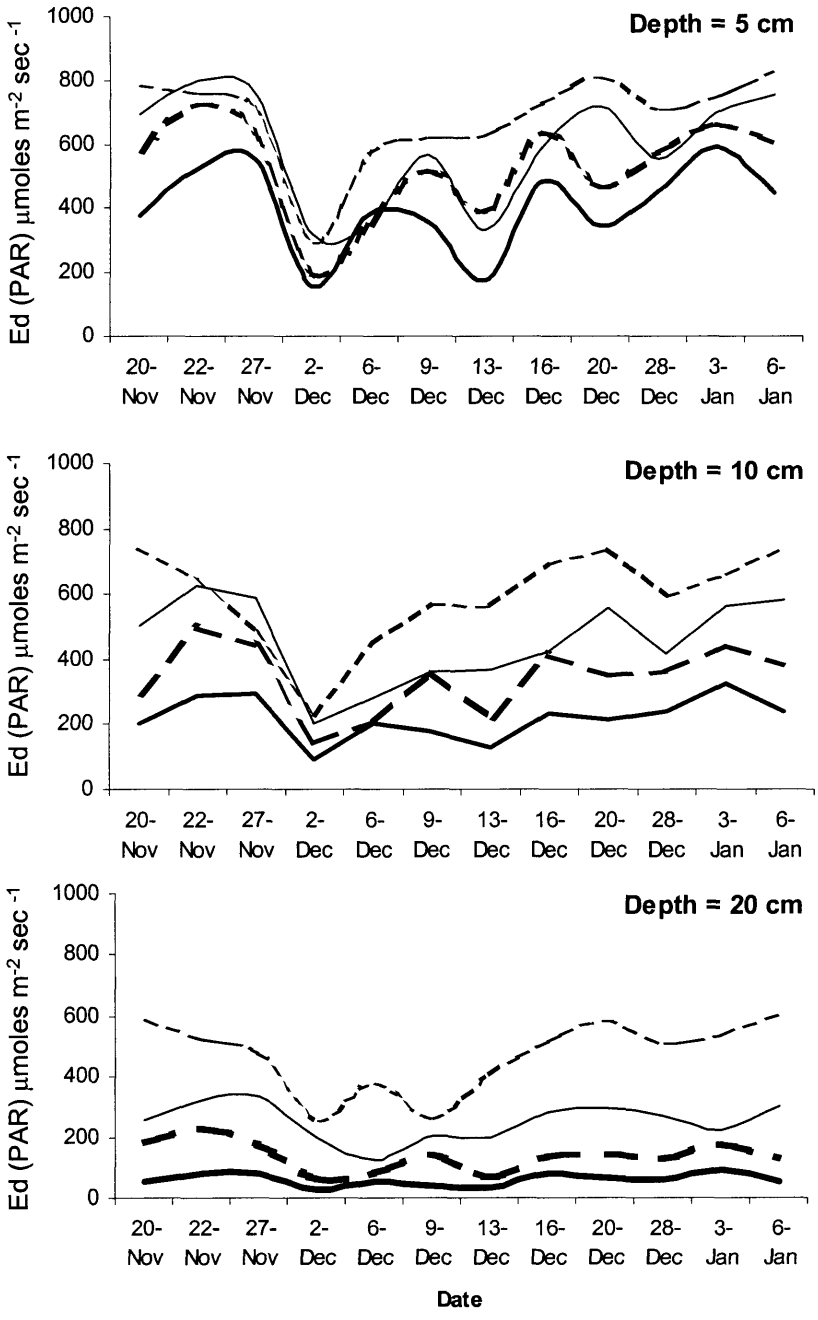


Figure 3-1 Downwelling irradiance, Ed (PAR) in µ mol m^{-2} s^{-1}, measured in four waters of differing turbidities (clear and low NTU represented as light dashed and continuous lines and medium and high NTU represented as bold dashed and continuous lines) at three depths from the water surface. Top graph = 5 cm, Middle graph = 10 cm, Bottom graph = 20 cm. Light was measured around noon when light intensity was most directly downward.

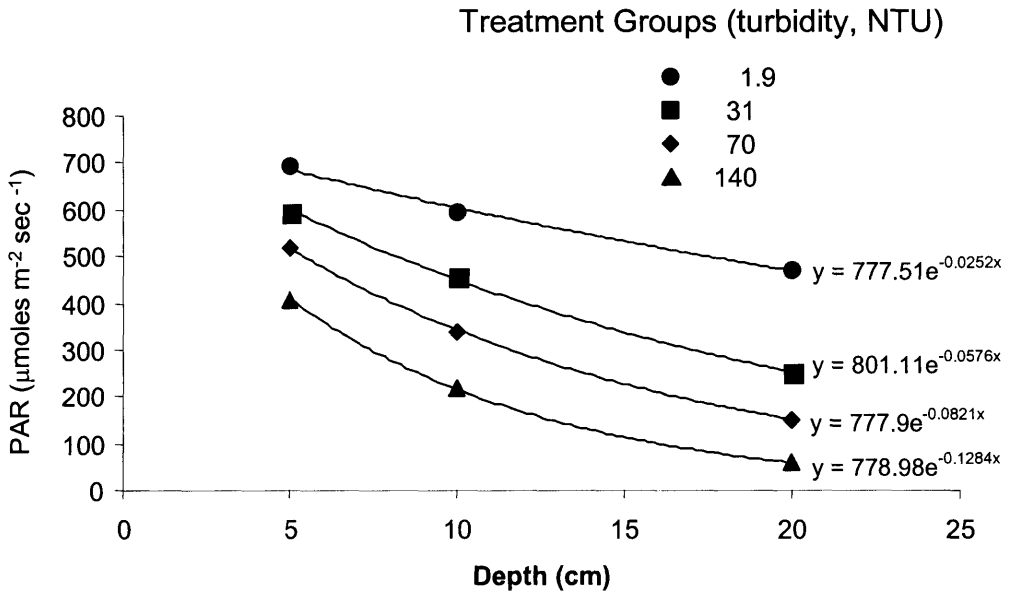


Figure 3-2 Downwards irradiance, Ed (PAR) (µ mol m^{-2} s^{-1}), measured at three depths, 5, 10 and 20 cm, for the four treatment groups (2 ponds per treatment). Turbidities 1.9 NTU = clear, 31 NTU = low, 70 NTU = medium, 140 NTU = high. Each point represents the mean of two ponds. Each pond was measured on 12 separate occasions twice weekly over a period of 6 weeks. An exponential trendline has been fitted to each series. Complete data is given in Appendix IV.

Attenuation of light from above and reflected light from below both increased with increasing turbidity as expected and Ed (PAR) decreased with depth in waters having different concentrations of turbidity (Figure 3.2). The average results for turbidity, Z_{SD}, and daily incident radiation flux (Ed (z) + Eu (z) in µmol $m^{-2}s^{-1}$), the maximum and minimum temperatures and calculated euphotic depth for the ponds are shown in Table 3.2. Each data point equals the average of 32 readings.

A graph of secchi depth readings taken over time is given in Figure 3.3. The correlation relationship between secchi depth and turbidity was r = – 0.79

Table 3 2 Results of average turbidity, secchi depth (Z_{SD}); total incident radiation flux, [Ed (z) +Eu (z)], euphotic depth (Z_{eu}) (calculated) and temperature (^{0}C) in four turbidity treatments in ponds. Depth, z, at which Ed and Eu readings taken = 0.20 m., readings are averaged over 8 weeks. (b) Approximate calculated range (not averaged) of Z_{eu} (m) = 2 X Z_{SD} to 3 X Z_{SD}

Treat-ment	NTU ± sd	Z_{SD} (m) ± sd	[Ed(z)+Eu(z)] µmol $m^{-2}s^{-1}$ (a)	Z_{eu} (m) Range (b)	Temp Max (^{0}C) ± sd	Temp Min (^{0}C) ± sd
1) Clear	1.9 ± 0.4	>1	472 + 28	>2-3	28.6 ± 1.8	17.2 ± 1.6
2) Low	31.1 ± 4.4	0.30 ± 0.05	252 + 24.	0.60-0.90	29.3 ± 1.8	17.0 ± 2.1
3) Med.	70.3 ± 7.7	0.18 ± 0.02	151 + 26	0.37-0.55	29.0 ± 2.2	17.5 ± 2.2
4) High	134.9 ± 12.1	0.13 ± 0.01	59 + 13	0.26-0.39	28.5 ± 1.2	17.0 ± 2.0

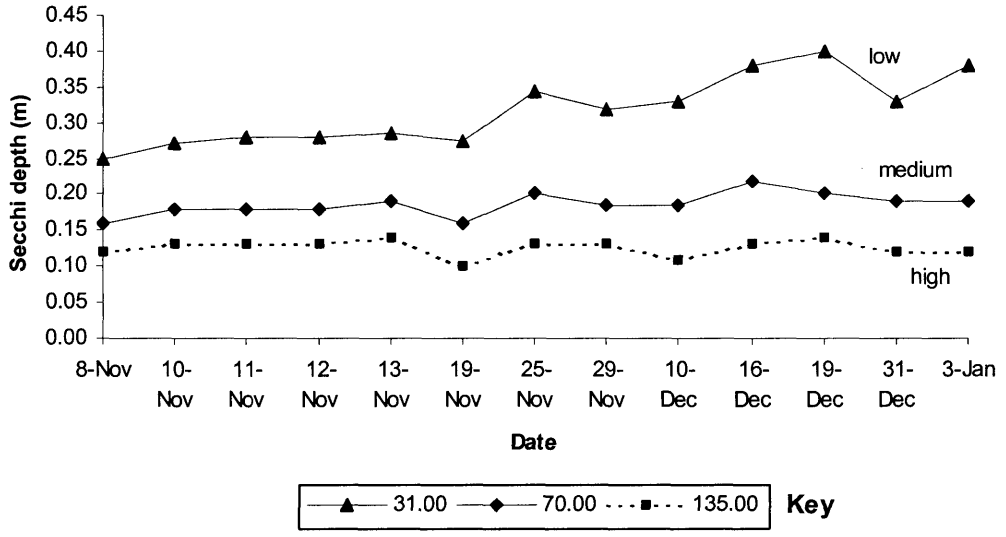


Figure 3-3 Average secchi disc transparency (Z_{SD}) in metres in paired ponds with varying turbidity indicating stability of visual readings at higher turbidities. Z_{SD} was maintained with clay concentrate. For Treatment 1 (control) Clear water, Z_{SD} > 1m (= depth of pond) so line not shown. (Assume 10's of metres (Kirk, 1994)). σ= Treatment 2, low turbidity; ♦= Treatment 3, medium turbidity; ® = Treatment 4, high turbidity. For simplicity standard deviations are not shown on the graph but were ± 0.05 m. in the low, ± 0.02 m. in the medium and ± 0.01 m. in the high turbidity ponds. Increase in secchi depth over time in the low turbidity may be due to flocculation of suspended solids by bacteria, algae or plants.

3.2 Water plants

3.2.1 Functional species from two wetlands

Nineteen species germinated and established from Racecourse Lagoon seed bank and twenty five species from Dumaresq Dam seed bank under four turbidity treatments and three

constant depths; conditions which were imposed for 8 weeks. A list of plant species that germinated and established from the two seed banks are given in Appendix 1. In this study species which grew underwater (submerged) were found to belong to Brock and Casanova's (1997) Arp and S groups.

Some species germinated and died before they could be identified. Other species such as *Utricularia* (possibly *U. australis*) from Racecourse Lagoon seed bank germinated and grew for a period then disintegrated, but these species are not listed in Appendix 1 as they did not last until the time of harvest. Complete lists of species that have germinated from these seed banks in a previous study can be found in Casanova (1993). Charophyte species made up one third and one fifth of the submerged species that germinated from Racecourse Lagoon and Dumaresq Dam seed banks respectively. *M. variifolium*, which invariably germinated and established successfully, behaved as an emergent at shallower depths. *Vallisneria gigantea* and *Elatine gratioloides* were the only other species from these two seed banks that showed abundant germination and establishment.

Appendix V lists the total number of species, charophyte and 'other' species that germinated and established at the three depths in the four treatments. The transfer of seeds between pots was low to negligible (0-2 charophyte plants) from observation of the control pots placed in each pond. Species common to both wetlands which germinated and established in a range of turbidities are listed below (Table 3.3).

Table 3 3 Species of water plant common to two seed banks which germinated and established in a range of turbidities from clear to high (0-140 NTU) at three depths (5, 10 and 20 cm).

Family	Species
Characeae	*Chara fibrosa*
	Chara muelleri
	Nitella cristata
	Nitella sonderi
	Nitella subtilissima
Potamogetonaceae	*Potamogeton ochreatus*
Haloragaceae	*Myriophyllum variifolium*
Hydrocharitaceae	*Vallisneria gigantea*
	Ottelia ovalifolia
Scrophulariaceae	*Limosella australis*
	Glossostigma diandrum
Cyperaceae	*Eleocharis pusilla*
	Eleocharis acuta
	Eleocharis dietrichiana
Elatinaceae	*Elatine gratioloides*
Gramineae	*Echinochloa crus-galli*

The presence of both male and female stoloniferous plants of *V. gigantea* from Dumaresq Dam indicated that sexual and vegetative reproduction was occurring in this dioecious species from this wetland. *Chara muelleri* had both oogonia and antheridia and was monoecious, *N. sonderi* and *N. subtilissima* were dioecious. *C. fibrosa* was dioecious and for this reason should probably be recorded as *C. preissi*. (information on classification of charophytes is given in Casanova, 1993, pp 79-80). Many oogonia on the charophytes had already been fertilised and formed oospores after 8 weeks in the ponds.

3.2.2 Species richness, germination and biomass

There was no significant reduction in species richness with increasing depth or turbidity from either seed bank (Figure 3.4 (a, b). The mean number of species that germinated and established from both seed banks from each depth and at each turbidity is given in Appendix V and sorts them into charophyte and 'other' (angiosperm) species. Charophyte species often made up more than half the species in the Racecourse Lagoon seed bank (Plate 1(b, c, d, e, f)) while angiosperm species dominated in Dumaresq Dam seed bank.

Germination and biomass was unique for each seed bank so the results obtained from each seed bank are discussed separately. Charophytes and *M. variifolium* dominated in pots with Racecourse Lagoon seed bank while *V. gigantea*, *M. variifolium* and *Elatine gratioloides* were found in pots with Dumaresq Dam seed bank.

The germination of charophyte oospores from Racecourse Lagoon seed bank was ten times higher than from Dumaresq Dam seed bank (Figure 3.5 (a, c)) and the charophyte biomass was up to 3 times higher (Figure 3.5 (b, d)). Germination of charophytes and angiosperms displayed different patterns, so the results are segregated in the figures. Angiosperms are grouped here as 'other submerged species'. Germination of angiosperms was higher from Dumaresq Dam seed bank than for charophytes, but there was also an increase in charophyte germination and biomass with an increase in turbidity as in the Racecourse Lagoon seed bank. The general trend was for the germination (Figure 3.5 (a) and (c)) and total biomass of charophytes (the sum of the three depths) (Figure 3.5 (b) and (d)) from each seed bank to increase with increasing turbidity. One discrepancy is that the dry biomass in the medium turbidity ponds was less than in the low and high turbidity ponds (Figure 3.5 (b) and (d)).

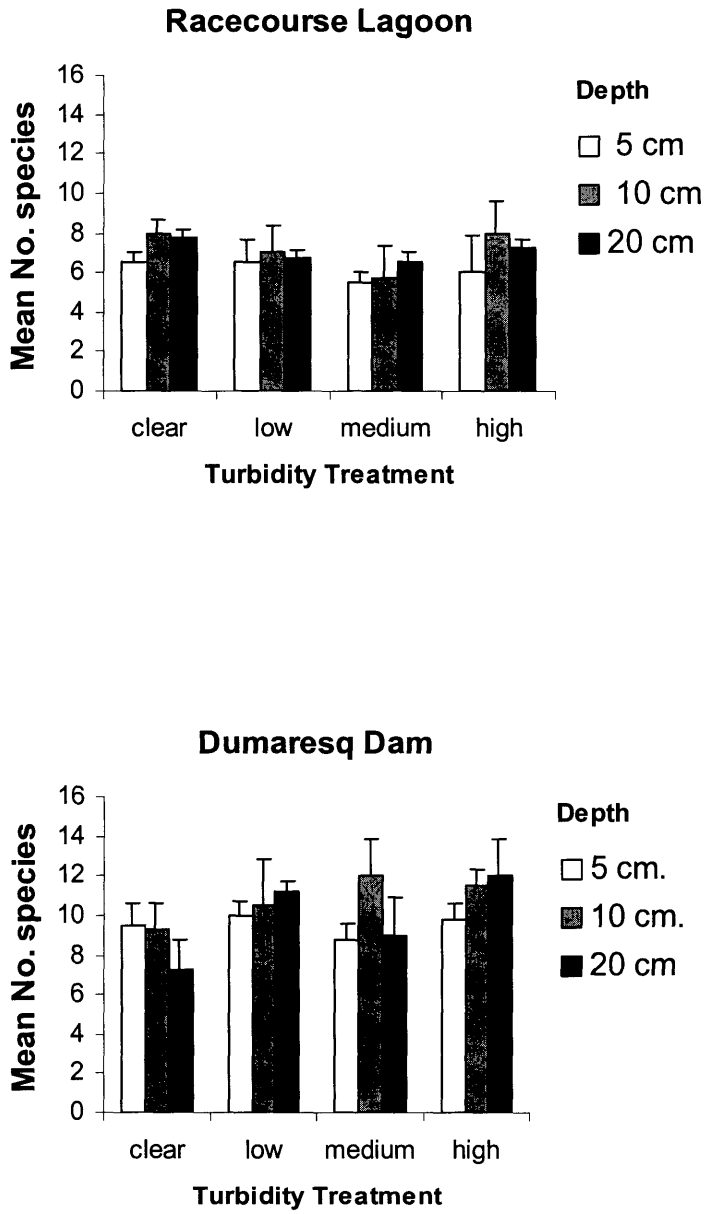


Figure 3-4 Mean number of species which germinated from two seed banks from (a) Racecourse Lagoon (top) and (b) Dumaresq Dam (bottom) under three depth (5,10 and 20 cms) and four turbidity treatments (1)clear, 2) low, 3) medium and 4) high). Error bars represent standard deviations from the mean.

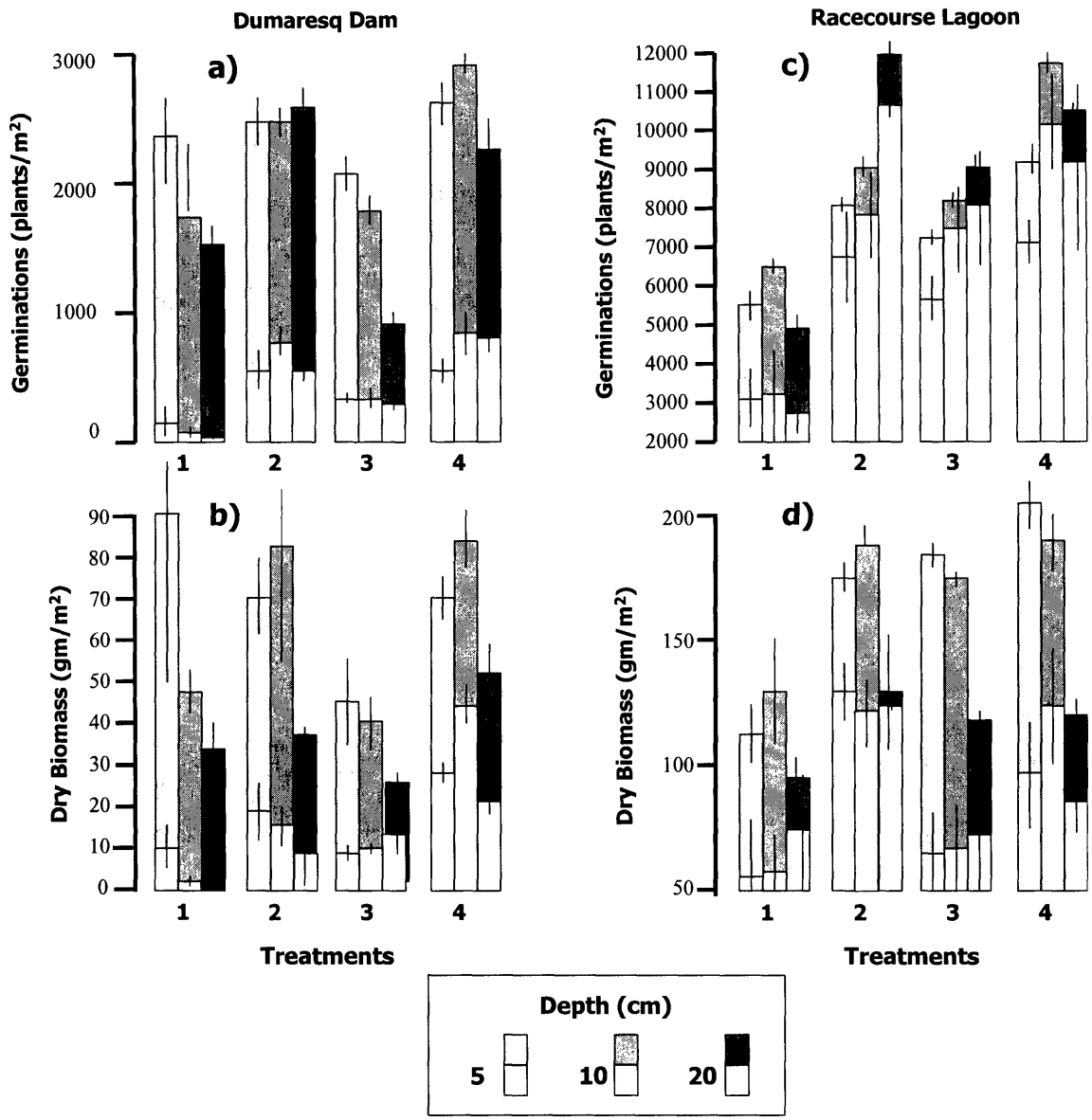


Figure 3-5 Germination (plants m^2) and dry biomass (g m^2) for Dumaresq Dam (a, b) and Racecourse Lagoon (c, d) seed banks at 5cm, 10cm and 20 cm depths for the four experimental turbidity treatments (1 = clear (control), 2 = low, 3 = medium, 4 = high). Charophyte species are indicated by the height of the clear bars and all other submerged species are indicated by the total height of the grey bars. Error bars represent S.E.M. Note differences of scale between Dumaresq Dam and Racecourse Lagoon.

Racecourse Lagoon

Of the 19 species from Racecourse Lagoon seed bank, 7 were submerged species (5 charophytes), 6 were amphibious fluctuation responders, (of which 5 were morphologically plastic species ARp; and one was a rooted, floating species, ARf), 5 were amphibious fluctuation tolerators, ATe, (emergents) and 1 was a terrestrial damp species, Tda (Appendix I). Of the amphibious fluctuation responders, ARp, three (*Elatine gratioloides, Glossostigma diandrum* and *Limnosella australis*) behaved as submerged sediment covering species. *Chara fibrosa* and *C. muelleri* sometimes dominated the germination and produced a large amount of biomass in pots at 10 and 20cm depths respectively. Average germination (plants m^2) and average total dry weight (biomass as g m^{-2}) for Racecourse Lagoon seed bank at 5 cm, 10 cm and 20 cm depths for the four experimental turbidity treatments are given in Figure 3.5 (c, d).

Germination:

As noted in Chapter 3.2.2, charophytes and *M. variifolium* dominated germination from this seed bank. The maximum (extrapolated) number of charophyte plants to germinate was 10,500 m^2 at 20 cm in the low turbidity ponds followed by 10,030 m^2 at 10 cm in the high turbidity ponds. Using two way ANOVA's with turbidity and depth as predictors, the total numbers of germinations was found significantly different between the clear water and turbid ponds ($p < 0.005$) and for numbers of charophyte germinations was significant at $p < 0.001$. Depth was significant for the total number of germinations ($p < 0.001$) but not for charophytes or 'others' separately.

Biomass:

The establishment of charophytes, particularly *Nitella sonderi* and *N. subtilissima,* added significantly to the total dry biomass, reaching a maximum of 130 g m^2 at 5 cm depth in the low turbidity treatment (Figure 3.5(d)). Simple Pearson correlations showed charophyte biomass was positively and significantly correlated to turbidity ($r = + 0.406$) but negatively correlated to depth ($r = - 0.504$). Two-way ANOVA's of turbidity and depth against biomass of charophytes and angiosperms showed turbidity was a significant predictor of total and charophyte biomass ($p < 0.005$) but not that of angiosperm biomass. Depth was not a significant predictor for charophyte biomass but was a significant predictor for the reduction in the total and angiosperm biomass especially in the 20cm depth treatment.

Simple one way ANOVA's of charophyte and angiosperm biomass on total biomass showed charophytes to be significant predictors of total dry weight (biomass) ($r =+ 0.85$, $p<0.005$, Figure 3.5(d)) but angiosperms were not.

While charophyte species differed in their response to increasing turbidity, *C. muelleri* significantly increased in length in response to increasing turbidity, whereas *C. fibrosa* decreased its length as turbidity increased (Chapter 3.2.4, Figure 3.6).

Dumaresq Dam

The number of species that germinated in Dumaresq Dam seed bank appeared to increase slightly with depth but was not significant (Figure 3.4 (b)). Of the 25 species from Dumaresq Dam, eight were submerged (5 charophytes), seven were amphibious fluctuation responders (6 morphologically plastic, ARp; 1 rooted floating, ARf), and seven were amphibious fluctuation tolerators, (7 emergent, Ate) and three were terrestrial damp species, (Tda) (Appendix I). The submerged angiosperm *Vallisneria gigantea* dominated in Dumaresq Dam seed bank followed in order of abundance by the amphibious fluctuation responders *M. variifolium, Elatine gratioloides, Gratiola latifolia* and *Limnosella australis.*

There were more emergent ATe and ARp germinating from Dumaresq Dam seed bank than Racecourse Lagoon seed bank, irrespective of the planting depth. These were *Typha orientalis, Gratiola latifolia, Eleocharis pusilla, E. dietrichiana, E. acuta, Echinochloa crus–galli, E. sphacelata, Marsilea sp.* There were two terrestrial species (Tda), *Alteranthera sp.* and *Rumex sp.* that behaved like ATe emergents. Average germination (plants m ^{2}) and average total dry weight (biomass as g m^{-2}) for Dumaresq Dam seed bank at 5 cm, 10 cm and 20 cm depths for the four experimental turbidity treatments are given in Figure 3.5 (a, b).

Germination:

Charophyte germination was lower than that of other submerged species from Dumaresq Dam seed bank (Figure 3.5 (a)). Of the 25 species that germinated only five were charophytes and the rest were either amphibious or emergent species. Both charophytes and angiosperms were more abundant under turbid conditions but charophytes did not respond to the same extent as the angiosperms. The maximum total number of germinating plants (3,000 plants m^2) was in the high turbidity ponds from 10 cm depth, one third of which were charophytes. Using two way ANOVA with turbidity and depth as predictors, the number of Chara germinations was significantly higher for more germinations with increasing turbidity ($p < 0.001$) but there was no significant response to depth (Figure 3.5 (a))

Biomass:

The maximum dry biomass (90 gm^2) was from the clear water ponds at 5 cm depth and was contributed by the angiosperms *M. variifolium, V. gigantea, P. ochreaus, O. ovalifolia, E. gratioloides* and *G. latifolia.*

The floating species, *Ottelia ovalifolia*, germinated in both clear and turbid water but it did not contribute significantly towards numbers of germinations (11 plants). Similarly to *Potamogeton ochreatus*, which was also uncommon, their growth form and bulk added considerably to the final biomass of a pot. *Eleocharis acuta* contributed biomass to the pots at 5 cm but its growth declined below that depth.

The emergent growth of emergent *M. variifolium* and the submerged *V. gigantea* contributed more to total biomass per pot than charophytes in this seed bank (Figure 3.5 (d)) but at 20 cm depth the submerged species, *V. gigantea,* contributed more to the biomass than did *M. variifolium.* At this depth *M. variifolium* showed its plasticity by changing its leaf form (heterophylly) to softer, feathery underwater leaves which had less rigidity and bulk. There were only two charophyte plants in the four pots in the clear water ponds at 20 cm depth but these individuals of *N. sonderi* and *N subtilissima* contributed significantly to the biomass in the low to high turbidities (Figure 3.5 (d)). The highest charophyte biomass was at depth 10 cm in the high turbidity pond.

Total dry weight biomass from Dumaresq Dam seed bank (pooled depths) was positively correlated with the dry weight of water plants such as *V. gigantea,* and/or *M. variifolium* and other angiosperms in the three turbidity treatments (clear, $r = + 0.989$; low, $r = + 0.948$; medium, $r = + 0.929$). In the high turbidity treatment, this correlation between total biomass and angiosperms was reduced ($r = + 0.826$) when charophytes represented half of the biomass ($r = + 0.795$). Two way ANOVA's of treatment against biomass of charophytes and of *V. gigantea* in Dumaresq Dam pots showed that turbidity was a significant predictor of *Chara* biomass ($p < 0.001$) but not of the total biomass or of *V. gigantea* biomass (Figure 3.5 (d)). Depth was also not a significant predictor of either charophyte, total or *V. gigantea* biomass for this seed bank.

3.2.3 Effect of depth on germination and biomass

The biomass of plants at 20 cm depth in both seed banks was slightly less than that of shallower depths (Figure 3.5 (b, d)). Although *V. gigantea* was only one of the eight submerged species that established from Dumaresq Dam seed bank, numbers of *V. gigantea* plants were higher with depth at 20 cm (Table 3.4). The biomass of *V. gigantea* was weakly positively correlated with depth ($r = + 0.174$). The results are reported as plants m^{-2} from results recorded as plants per pot by multiplying by a factor of 44. In comparison very few *V. gigantea* plants germinated in pots from Racecourse Lagoon seed bank. The effect of turbidity and depth on *Myriophyllum variifolium* is reported in Chapter 3.2.5.

Table 3 4 Average number of *Vallisneria gigantea* plants m^2 (converted from plants per pot by multiplying by a factor of 44) that established from Dumaresq Dam seed bank under three depth and four turbidity treatments (clear, low, medium high). Numbers include vegetatively produced clones of parent plant.

Depth (cm)	Clear	Low	Medium	High	Average plants $m^2 \pm$ s.d.
5	132	66	44	88	82.5 ± 32
10	154	110	308	154	181 ± 75
20	484	198	66	264	253 ± 151

3.2.4 Effect of turbidity on the length of water plants

Changes were recorded in the average length of water plants as a result of the influence of turbidity on Racecourse Lagoon (but not Dumaresq Dam) seed bank at the same depth (20 cm) (Figure 3.6). Plants from 20 cm were harvested and their length measured. This indicates the maximum growth or reduction in length that could be obtained under the most stable light climate (See Figure 3.1, 20 cm). Limitations in the time available for harvesting all the pots did not allow for the measurements of the plants at all depths.

Using one-way ANOVA's, comparisons of lengths within species as light irradiance decreased were made on plants taken from 20cm depth. The *Nitella* species, *N. sonderi* and *N. subtilissima* increased their length between the clear water ponds and high turbidity ponds by about 2 cm. The change in length between the clear and high turbidities was significant for *N. sonderi* ($p < 0.005$, $F = 6.12$, $n = 169$, $DF = 3$) and *N. subtilissima* ($p < 0.001$, $F = 10.65$, $n = 160$, $DF = 3$). The *Chara* species, *C. fibrosa* and *C. muelleri* responded quite differently to each other. *C. fibrosa* significantly reduced its length by 3 cm between the clear and high turbidities ($p < 0.05$, $F = 4.37$, $n = 163$, $DF = 3$), compared to *C. muelleri* which doubled its length. Plants reached a maximum of 29.6 cm in the highest turbidity so that the apical points of the shoots were often floating just below the surface of the water. The difference in length between the clear and high turbidities was significant ($p < 0.001$, $F = 14.60$, $n = 111$, $DF = 3$). *M. variifolium* from Racecourse Lagoon declined in length with turbidity but this was not significant.

Plants of *Vallisneria gigantea* from Dumaresq Dam seed bank from 20 cm depth in different turbidities were measured for length and width, and were found to become slightly longer and narrower with increasing turbidity. No data on the effect of turbidity on length are presented here for Dumaresq Dam as the number of charophyte, *V. gigantea* and *M.*

variifolium plants which germinated at 20 cm from that seed bank was not enough for analysis. (Similar work on *Vallisneria americana* has been reported in Blanch (1997) which indicated there was an elongation of the leaves with increasing turbidity).

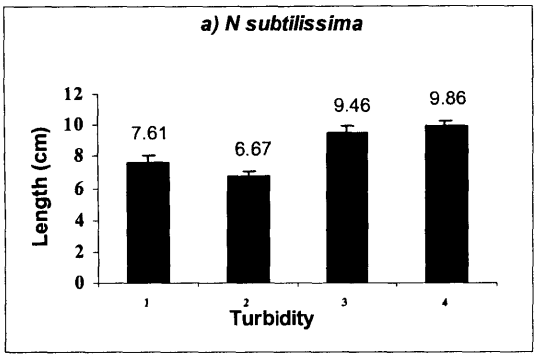
(Note: there were gradual changes observed in chlorophyll pigmentation from olive-green towards purple-violet in *V. gigantea* as light irradiance decreased. This chromatic adaption also appeared in the charophytes with chlorophyll pigmentation changing from dark green to yellow-green between the clear water and turbid ponds. The mucus on the top whorls of *Nitella sonderi* was often tinted with a brown or orange colour (Plate 1 (e).))

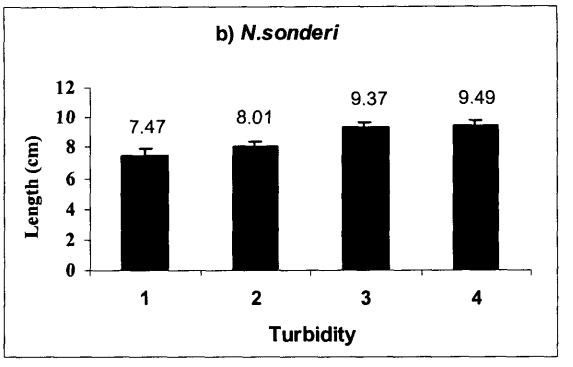
3.2.5 Effect of turbidity on Myriophyllum variifolium

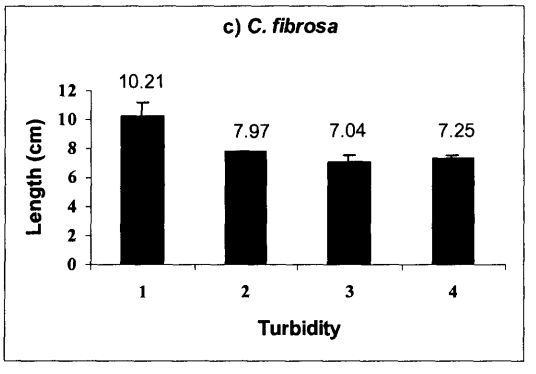
The first plants to germinate from both of the seed banks were *Myriophyllum variifolium* which dominated the pots and had submerged feathery leaves. The numbers of plants were observed to be higher in the clear water ponds and the growth of these *M. variifolium* plants from 20 cm depth was more robust. Samples were only measured and analysed for Racecourse Lagoon seed bank due to fewer numbers of plants in Dumaresq Dam seed bank. *Myriophyllum* plants from Dumaresq Dam seed bank, which were fewer in number than from Racecourse Lagoon seed bank, compensated for their deficiency with a more robust growth form consisting of secondary and tertiary branching. The apical tips emerged at the surface after 28 days.

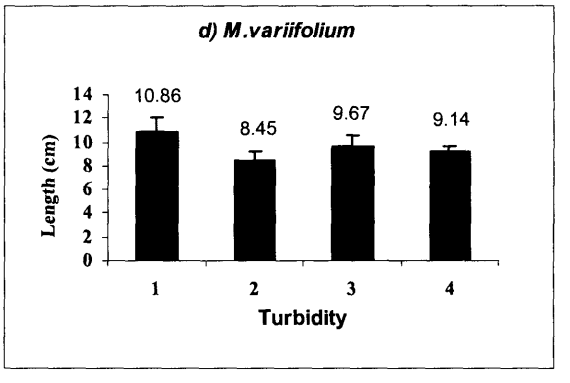
The maximum length of plants taken from 20 cm depth in the clear ponds was 35 cm (mean 10.86 cm ± 1.14 SEM) from the base of the stem to the tip (Figure 3.6 (d)). The growth in pots at 20 cm in the highly turbid ponds was less robust with delicate, feathery submerged leaves and stems. The maximum length was 28.4 cm (mean 9.14 cm ± 0.26 SEM). (Figure 3.6 (d)). The small reduction in length of *M. variifolium* with increasing turbidity was not statistically significant, but the changes observed in stem and leaf form suggest increasing turbidity was detrimental to the health of the plants.

The reduction in average biomass in grams per 17 cm diameter pot at three depths is seen in *M. variifolium* from both seed banks. Biomass was reduced by 4 to 5 times in each seed bank between 5 and 20 cm as a result of fewer emergent shoots reaching the surface. When plants reached the surface biomass production increased as plants obtained enough light to increase photosynthesis. (Table 3.5).









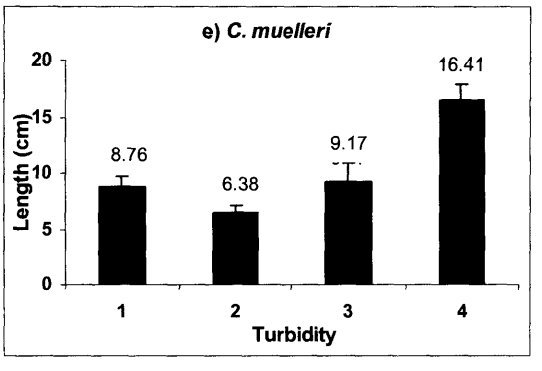


Figure 3-6 Changes in the length of different submerged plants at 20 cm depth with turbidity (1 = 1.9, 2 = 31, 3 = 70, 4 = 135 NTU). (a) *Nitella subtilissima*, (b) *Nitella sonderi*,(c) *Chara fibrosa* (d) *Myriophyllum variifolium* (e). *Chara muelleri.* The seed bank was collected from Racecourse Lagoon. Average length in cm and the SEM are shown on top of columns.

Table 3 5 Reduction in the average total dry biomass grams per pot (diam 17 cm) of *M. variifolium* grown from two seed banks, Racecourse Lagoon and Dumaresq Dam, when placed at three depths; 5, 10 and 20 cm underwater (using data pooled from four turbidity treatments). (Different numbers of plants were weighed from each wetland but there was a similar reduction in biomass with depth.)

Depth of seed bank underwater (cm)	Racecourse Lagoon (g ± s.d.)	Dumaresq Dam (g ± s.d.)
5	1.142 ± 0.459	0.507 ± 0.328
10	0.723 ± 0.525	0.367 ± 0.229
20	0.232 ± 0.242	0.165 ± 0.136

3.2.6 Groupings of pots according to species determined by depth and turbidity

For each seed bank the pots containing plants were grouped using multivariate cluster analysis according to their similarity based on the extent to which the pots had species (attributes) in common and equal weighting was given to all measured attributes. The results of the cluster analysis for Racecourse Lagoon (Figure 3.7) and Dumaresq Dam (Figure 3.8) seed banks are illustrated by separate dendrograms. The level of similarity between groups is indicated at a point where the branches of the dendrogram fuse.

Groupings of pots from Racecourse Lagoon

This cluster analysis of number of plants germinating from pots with Racecourse Lagoon seed bank divides them into two main groups of water plants that prefer clear or turbid water. Subgroups are usually determined by depth. *M. variifolium* is found in all pots in all groups.

Group 5 at the top of the dendrogram contained 6 pots or half of the 12 pots in total from the clear water ponds. Group 5 contained pots with low numbers of *N. sonderi* and *N. subtilissima*, high numbers of *M. variifolium* and numbers of *Limosella australia* and *Elatine gratioloides*. Although the measured turbidity and downwelling light was the same in Pond 2 as in Pond 1, it is possible that there may have been an unknown effect, possibly of higher epiphytic growth as pots from Pond 2 were grouped with the larger group (in Groups 4, 3 and 2).

The large group contained 42 pots from mixed turbidities which was subdivided into four subgroups: Groups 1, 2, 3 and 4. This group is made up of pots containing charophytes subgrouped according to depth and turbidity.

Group 1 (24 pots) is divided into three subgroups (1A, 1B and 1C), which are similar in that they are mainly in the medium to high turbidities and have very high numbers of *N. sonderi* and *N. subtilissima*.

Group 1A - pots at 10 and 20 cm depth, which have high numbers of *C. muelleri* and *C. fibrosa*

Group 1B - are pots at mainly 5 cm - have low numbers of *C. muelleri* and *C. fibrosa*

Group 1C - are pots at 5cm - have (usually) no *C. muelleri.*

Group 2 (4 pots) is made up of two subgroups (2A, 2B) containing 2 pots each from 20 cm depth in clear to medium turbidities. Generally the two species of Nitella occur together but in these pots they do not.

Group 2A - contains *N. sonderi* (but no *N. subtilissima*) and very high numbers of *C. fibrosa* and *C. muelleri*

Group 2B - contains *N. subtilissima* (but no *N. sonderi*) and high numbers of *C. fibrosa* and *C. muelleri* .

Group 3 contains 6 pots in a mixed range of turbidity treatments (clear to high) at 10-20 cm (but not 5 cm).

C. fibrosa, *C. muelleri* , *N. sonderi*, *N. subtilissima*, *Limosella australis* and *Elatine gratioloides* grew equally well.

Group 4 contains 10 pots all which have *C. muelleri* plants at shallow depth and mainly low turbidities. (6RA1 and 6RB2 appear to be anomalies but in these cases *C. muelleri* may be getting enough light in the high turbidity ponds through stem elongation.) Some pots from clear water are in this group because of the presence of *N. sonderi* and *N. subtilissima.* This group is only grouped separately because the large *Chara* species grew in shallow water and the smaller *Nitella* species grew in clear water.

Groupings of pots from Dumaresq Dam

This clustering analysis of pots with Dumaresq Dam seed bank shows five groupings of pots according to the species that germinated in these pots, with most similar groupings to the most dissimilar reading from left to right. The broad picture shows that Groups 3, 4 and 5 are pots from clear water ponds while Groups 1 and 2 are pots from turbid water ponds.

Group 1 – 18 pots from mixed turbidity (medium-high) with the majority at 20 cm depth which have *C.muelleri, C. fibrosa, V. gigantea* and the *Nitella* species.

Group 2 – 15 pots from mixed turbidity (low-high), with ten 10 pots from 5 cm and five from 10 cm depth. There are no pots from 20 cm. Half the pots have *V. gigantea*. The pots also contain *N. sonderi* and *N. subtilissima.* The *Nitella* species can survive in shallow turbid water but there are no *Chara muelleri* or *Chara fibrosa* in this group.

In groups 3, 4, 5 (15 pots) the majority are clear water over a range of depths and half the pots had *V. gigantea.* 8DA2 appears to be an anomaly at 5 cm depth in highly turbid pond but as for previous anomalies, may be obtaining enough light.

M. *variifolium, N. sonderi* and *N. subtilissima* were present but did not germinate in the high numbers as from Racecourse Lagoon seed bank. *Elatine gratioloides* was more common in Dumaresq Dam seed bank.

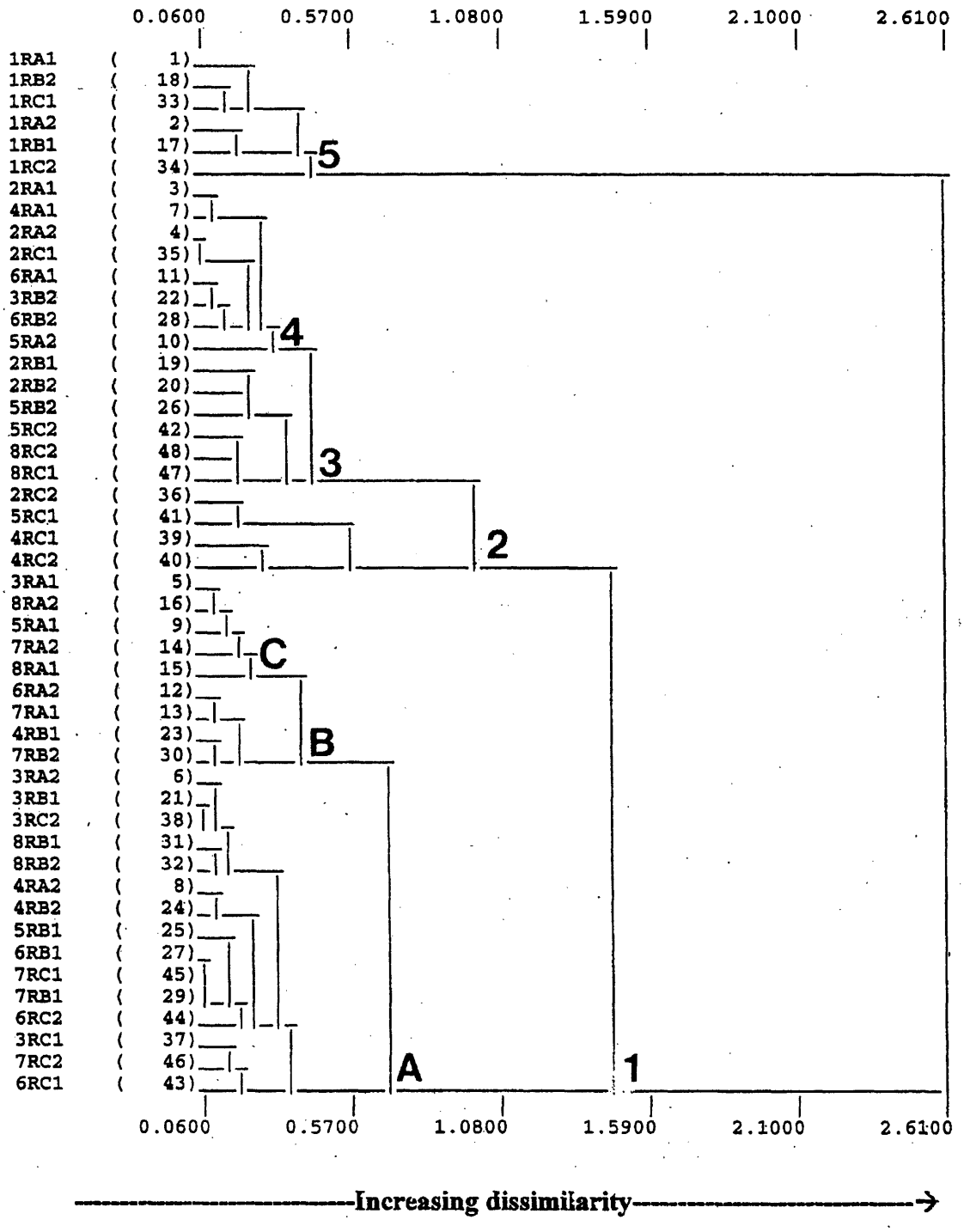


Figure 3-7 Dendrogram showing the grouping of pots containing Racecourse Lagoon seed bank from different depths and turbidity treatments after cluster analysis using PATN (Belbin, 1991). Pots were clustered according to the species and numbers of germinations from the seed bank. Left hand number 1 to 8 represents treatment pond (see below); R =Racecourse Lagoon; A, B or C represents 5, 10 or 20 cm depth respectively; right hand number 1 or 2 represents replicate in pond. Ponds 1 & 2 = Clear water treatment, ponds 3 & 4 = low turbidity treatment, Ponds 5 & 6 = medium turbidity treatment, Ponds 7 & 8 = high turbidity treatment

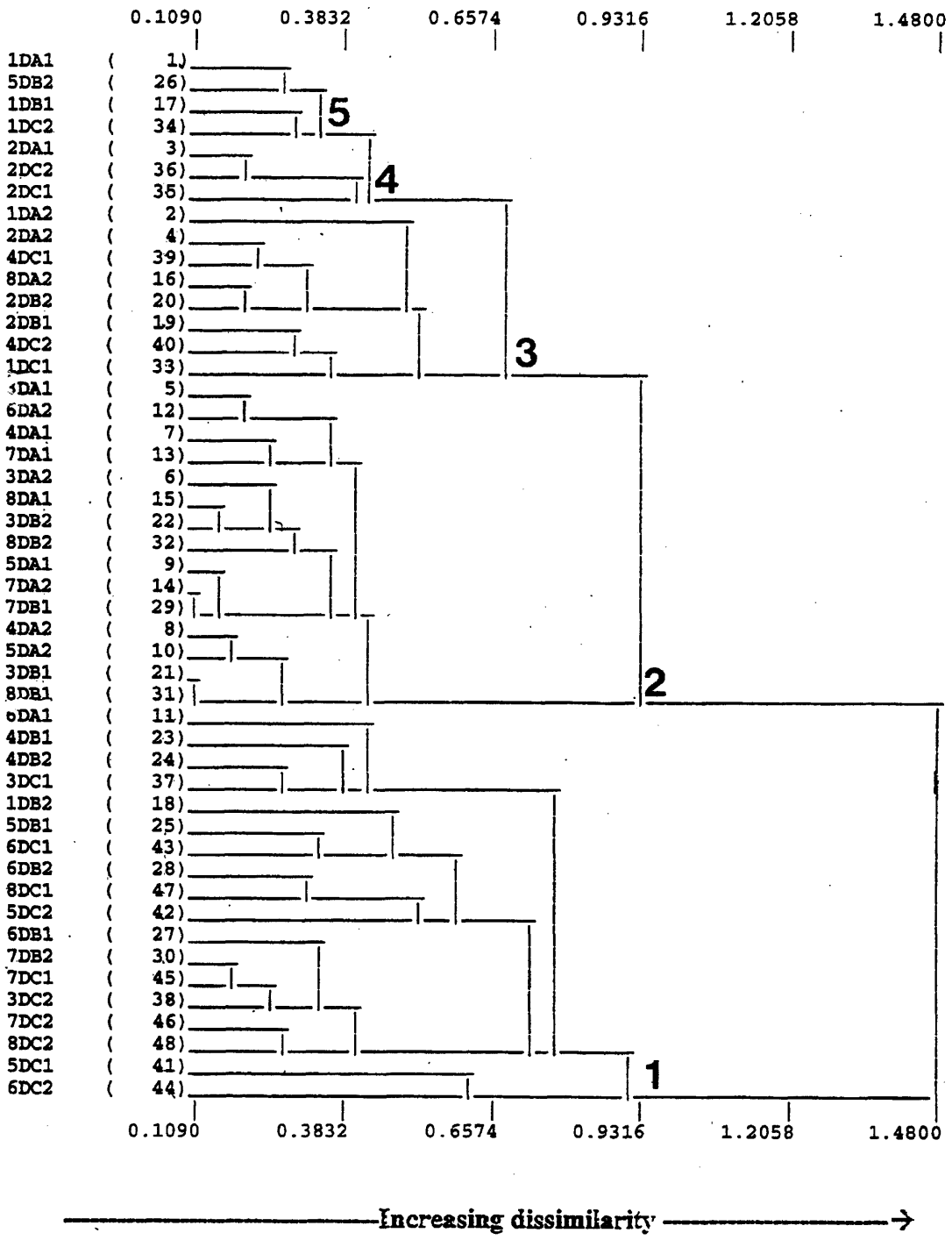


Figure 3-8 Dendrogram showing the grouping of pots containing Dumaresq Dam seed bank from different depths and turbidity treatments after cluster analysis using PATN (Belbin, 1991). Pots were clustered according to the species that germinated and established from the seed bank. Left hand number represents treatment pond (see below); D = Dumaresq Dam; A, B or C represents depth 5, 10 or 20 cm respectively; right hand number 1 or 2 represents replicate in pond. Treatment ponds 1 & 2 = Clear water treatment , Ponds 3 & 4 = low turbidity treatment, Ponds 5 & 6 = medium turbidity treatment, Ponds 7 & 8 = high turbidity treatment.