Identification of Environmental Factors, With Particular Reference to Acid Sulfate Soil Runoff, Causing Production Losses in Sydney Rock Oysters (*Saccostrea glomerata*)

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GLOSSARY OF TERMS AND ABBREVIATIONS

AASS – actual acid sulfate soil
Adductor muscle – translucent organ for the purpose of closing the oyster shell
Aerobic – free oxygen present
AHD – Australian height datum; 0 AHD = 0.46 m below mean sea level
Anaerobic – free oxygen absent
Anterior – the hinge end of the oyster shell
ANZECC – Australian and New Zealand Environment and Conservation Council. ANZECC compiled the 'Australian Water Quality Guidelines for Fresh and Marine Waters' which are recommendations for managing Australian water resources in a sustainable way.
APHA – American Public Health Association
ASS – acid sulfate soils; refers to both actual acid sulfate soils and potential acid sulfate soils in this study
Balzers Carbon Evaporator – apparatus used for the preparation of scanning electron microscope samples
Bioassay – a measure of the strength of a biologically active substance as it acts on living organisms
Biodeposits – oyster faeces; comprised of true faeces and pseudofaeces
CI – confidence interval
Cilia – short hair-like structures arranged in groups that beat rhythmically together to create water currents, remove particles from suspension or transport particles
Condition index – the ratio of the dry soft tissue weight and the internal shell cavity capacity
Davidson's fixative – solution used for the chemical preservation of oyster soft tissue
Demibranch – part of the oyster gill composed of two marginally joined lamellae
DLWC – Department of Land and Water Conservation
DO – dissolved oxygen (units = percentage saturation or mg L^{-1})
EC – electrical conductivity (units = dS m^{-1})
EDS - energy dispersive x-ray spectrometer
Epithelium – the cellular tissue covering surfaces, forming glands and lining most cavities of the body
ETOH - ethanol
Excessive gaping – oyster valve separation beyond the range of normal feeding
Faeces production – total true faeces production per unit of time
Feeding activity – the rate of true faeces and pseudofaeces production
Filament – a component of each lamella and are arranged in groups to form a plica
Filtration rate – amount of particles cleared from a volume of water per unit of time
Floc – another word for floccule
Flocculation – the aggregation of suspended particles
Formalin (10% sea water) – a standard fixative used to preserve oyster soft tissue for pathology
GF/C – Whatman glass microfibre filters
Greenspan Smart Sonde Model SD300 – brand of submersible data logger manufactured by Greenspan Technical Services Pty. Ltd., Warwick, Queensland
H&E – haematoxylin and eosin
Haematoxylin and eosin – general tissue thin section stain abbreviated as H&E
Haemocytes – blood cells of bivalve molluscs; haemocytes have a role in inflammation, wound repair, encapsulation and phagocytosis

Haemolymph – fluid containing haemocytes
HCl – hydrochloric acid
HDPE – high-density polyethylene
Hinge – the pivot point of the left and right valve located at the anterior of the oyster
Histopathology – the study of tissue changes using light microscopy and stained thin sections
Holocene – the second and most recent epoch of the Quaternary period, which began approximately 10,000 years ago at the end of the Pleistocene
ICPAES - Inductively Coupled Plasma Atomic Excitation Spectroscopy; used for determining the ionic composition of water samples
Inflammation or Inflammatory response – the accumulation of exudate and haemocyte cells in irritated tissues to protect from further injury; may be acute or chronic
Interlamellar junctions – tissue connections that join filaments at regular intervals
Labial palps – large, soft flaps at the gills anterior (mouth) used to control the amount of food ingested as well as sort food before ingestion
Lamella – a single arm of the demibranch, which is composed of vertical filaments
LS – Limeburners syndrome
Leica/Cambridge S-360 – brand and model of a scanning electron microscope
Lesion – an area of tissue with impaired function due to damage by wounding or disease
Mantle – a fleshy fold that covers the internal organs of a bivalve; also called a pallium
Mudworm – an oyster disease caused by a spionid polychaete worm (*Polydora websteri*)
Necrosis – death of cells in an organ or tissue caused by disease, physical or chemical injury, or interference with the blood supply
NSW – New South Wales
Overcatch – settlement of oyster spat on oysters
PASS – potential acid sulfate soil
Periostracum – a thin organic veneer covering the external surface of the shell; easily removed by abrasion
Perls' Prussian Blue – thin section stain specific for ferric iron abbreviated as PPB
pH – a measure of how acidic or alkaline (basic) an aqueous solution is. It is a measure of the hydrogen ion concentration (H^+).
PIM – particulate inorganic matter
Plica – a gill fold composed of filaments
POM – particulate organic matter
Posterior – the valve end of the shell
PPB – Perls' Prussian Blue
ppt – parts per thousand, units used for salinity
Pseudofaeces – particles filtered from suspension by the gills and rejected from the pallial cavity before ingestion
PVC – polyvinyl chloride
Pyrite – a common mineral that occurs in ASS (iron disulfide: FeS_2); the structure contains S_2^{2-} species
QX – stands for 'Queensland unknown': a disease that affects the Sydney rock oyster and is caused by the protozoan parasite *Marteilia sydneyi*

Rejection rate – total pseudofaeces production per unit of time
Salinity – the amount of salt which estuarine waters contain measured in parts per thousand (ppt). (Salinity in ppt = Electrical Conductivity in dS m^{-1} x 0.64)
SDL – submersible data logger; used for continuous or 'spot' measuring of water quality variables such as pH, EC, DO and temperature.
SEM – scanning electron microscopy
Seston – suspended material (or particles)
Sinus – wide channel containing blood (haemolymph)
Sloughing – refers to the detachment of tissue layers
Spat – larval bivalve molluscs
SPSS – statistical software package by SPSS Inc., Chicago
TPM – total particulate matter; it is measured in mg L^{-1} and is equivalent to the dietary abundance for oysters
True faeces –particles that are filtered, ingested and move through the digestive tract
Umbo – the shell above the hinge constituting the apex of the valve
Valves – an oyster shell has a left valve and a right valve; in the Sydney rock oyster the left valve is cupped and the right valve is flat.
Winter mortality – a disease that impacts Sydney rock oysters caused by *Mikrocytos roughleyi* which is proctoctistan parasite
Yeo-Kal 611 Intelligent Water Quality Analyser – brand name of a hand held submersible data logger manufactured by Yeo-Kal Electronics, Brookvale, NSW.

1996/285	**Identification of environmental factors, with particular reference to acid sulfate soil runoff, causing production losses in Sydney rock oysters**

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OBJECTIVES:

1. To identify associations between water quality conditions (with particular reference to acidified water and toxic metals), other environmental factors and reduced growth rates and disease outbreaks/mortalities in oysters at selected sites on the Hastings, Manning and Richmond Rivers;
2. To identify specific environment and management related risk factors for reduced growth rates at selected sites;
3. To identify environmental and management risk factors for specific diseases with particular reference to QX on the Richmond River; and,
4. To effectively communicate the findings of this study to the oyster industry and relevant agencies.

NON-TECHNICAL SUMMARY:

OUTCOMES ACHIEVED

The study has confirmed that estuarine acidification, associated with drainage of acid sulfate soils, reduces growth rates and survival in Sydney rock oysters leading to significant production losses. The work has also demonstrated that acidification is not a factor in outbreaks of QX disease. The findings have raised greater awareness of the environmental and economic impacts of estuarine acidification, and have influenced environmental decision making at local and state government levels. The oyster industry is now recognised as an important stakeholder in the management of acid sulfate soils and their impacts. Reactive and proactive strategies to manage acidification now consider the impacts on the oyster industry whereas prior to the study the industry concerns and needs were largely ignored. The industry is now represented on key management and advisory committees responsible for management of acid sulfate soils. The research has enabled oyster farmers to minimise stock losses through improved risk and stock management in parts of the estuary impacted by acidification. The study has provided a basis for more accurate diagnosis of acid-related oyster mortalities and important baseline information for environmental impact assessment in coastal development.

Production of the Sydney rock oyster (*Saccostrea glomerata*) has experienced a significant downturn over the last thirty years due to a range of known and unknown

environmental risk factors. Estuarine acidification, associated with drainage of acid sulfate soils, has recently emerged as a major environmental management issue in eastern Australia. The oyster industry in eastern Australia utilises reaches of estuaries that are fringed by rural and urban development on acid sulfate soils.

The current study was prompted by concerns from oyster farmers that acidic plumes, passing over once productive oyster leases, were causing poor growth rates and increased mortalities in farmed Sydney rock oysters. Unexplained oyster mortalities in reaches of the Hastings River also led farmers to suspect that either an unidentified pathogen or declining water quality caused poor growth and lower survival rates on affected leases. Farmers had discounted many known causes of poor productivity because affected oysters did not present the clinical signs of diseases that normally cause poor growth and mortality. QX disease, a known cause of declining oyster productivity, was also putatively linked to acid on the assumption that acidity could increase susceptibility to this disease. Previous studies had discounted acid as a necessary factor but had not tested whether acid could increase susceptibility of individual oysters in QX-affected estuaries.

The overall objective of this study was to investigate the role of water quality, particularly acid and associated toxic metals, in oyster mortality, poor growth, disease induction, feeding activity and shell changes. Field-based studies involving a comparison of acid-impacted and pH-neutral oyster leases identified several significant impacts on oysters. Oyster growth rates were lower at acid-impacted leases than at pH-neutral leases. Negative growth was also recorded at acid-impacted leases due to the loss of recent shell growth and shell dissolution; this tended to occur in areas affected by regular low tide acid outflows or chronic acidification. Mortality rates at acid-impacted sites were also significantly greater than at pH-neutral sites, particularly in juvenile oysters.

Oysters exposed to severe acidity experienced shell degradation that was characterised by the dissolution and eventual breakdown of the protective outer layer of shell. Affected shells were bleached white and brittle, and in some cases the shells perforated exposing the oyster tissue to acid. Mortality rates were highest in groups of oysters with severe shell dissolution and associated perforation. The presence of iron precipitates in drainage waters often coated oysters and also entered the shells and accumulated on the gills. The iron precipitates can be transported for up to 15 km downstream from their origin and can affect oysters in neutral waters. Affected oysters are unsaleable due to the red stain.

Interestingly, there was no significant difference in the condition index of surviving oysters at acid-impacted and pH-neutral sites due to the ability of oysters to remain closed during acidic conditions. This behaviour is known as avoidance reaction and helps to protect oysters from acute acid events. Under chronic or longer-term acid exposure, shell perforation eventually exposes the vulnerable soft tissue to the toxic and injurious effects of acid and metals. Laboratory experiments also showed that oysters exposed to gradual changes in pH do not demonstrate avoidance reaction and will open during toxic conditions. By contrast, a sudden drop in pH and salinity, as occurs during low tide outflows from floodgates, causes oysters to close.

Laboratory investigations on the effects of acid and metals demonstrated that soft tissue damage occurred in oysters exposed to acid and metals mobilised from acid sulfate soils. Aluminium increased the toxicity of acidic water causing more severe lesions than in oysters exposed to acid without any aluminium. The target organs for acid and metal toxicity were the gills, mantle and digestive glands. Under natural conditions, aluminium is usually always present in acidic outflows and is in its most toxic form around pH 5 - a weakly acidic pH value that commonly occurs in estuaries after major rainfall. Iron accumulation occurred on the gills, mantle, rectum and digestive glands of oysters exposed to acid sulfate soil outflows. Histopathological examinations showed that oysters ingest iron precipitates. Although iron accumulation was prevalent in acid-exposed oysters, it did not appear to induce lesions. Acidic conditions also reduced feeding activity in oysters. At pH 6.5, a very weakly acidic pH value, there was a reduction in the total faeces, true faeces and pseudofaeces compared to oysters in normal estuarine waters of about pH 7.9. At pH 5.5 there was a significant reduction in feeding activity. The field and laboratory experiments demonstrated, unequivocally that acidified outflows are a threat to the oyster industry and can cause poor growth, increased mortality and shell degradation in exposed oysters.

Field studies on the lower Richmond River found no association between acidification and outbreaks of QX disease. The present study showed that exposure to acid does not increase susceptibility of oysters to QX disease. The severity of outbreaks is therefore, independent of acidified water. The most severe case of QX measured by the study occurred at a site unaffected by acidified water.

Investigations of unexplained production losses, unrelated to acidification, in Limeburners Creek on the Hastings River indicate the possibility of a microcell disease. Gross examination of moribund oysters revealed yellowish pustules in the soft tissue of oysters particularly the gills, mantle, labial palps, digestive gland and gonads. This condition was also accompanied by staining of the internal shell surface and minor shell deformity. Further work is required to investigate the possible role of an infective agent, its pathogenesis and environmental risk factors.

KEYWORDS: acid sulfate soils, estuarine acidification, Sydney rock oyster, oyster mortalities, aluminium, iron, low pH.

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SECTION I

INTRODUCTION AND PILOT INVESTIGATION

1 INTRODUCTION

1.1 BACKGROUND

Oyster production is the largest aquaculture industry in NSW and relies on healthy estuaries for a sustainable future. Over the last 20 years the oyster industry has experienced significant declines in productivity that have been blamed on disease and altered environmental processes triggered by coastal development. Although disease events are an established cause of some productivity declines, other environmental risk factors, in particular poor water quality, have only been putatively linked to acute and more chronic production problems. Estuarine acidification caused by drainage of acid sulfate soils (ASS), has emerged as one of the greatest threats to the recreational, commercial and conservational value of eastern Australian estuaries (Sammut *et al*., 1995; 1996a; White *et al*., 1996) and is thought to be the cause of recurrent oyster mortalities and low farm yields.

The proposition that acid causes production losses emanated from studies on the effects of acid on fish which demonstrated, unequivocally, that acid and dissolved metals, displaced from drained ASS, cause fish kills and trigger fish diseases in eastern Australian estuaries (Callinan, 1997a; Callinan *et al.*, 1996; Sammut *et al.*, 1995; 1996a; Sammut, 1998). Oyster production losses are most commonly reported from NSW estuaries that contain expansive areas of ASS, in particular barrier estuaries such as the Richmond, Hastings, Manning and Clarence River systems. Coastal development on these soils is responsible for the oxidation of pyrite that leads to severe soil and groundwater acidification. Following major rainfall events, hundreds of tonnes of acidic water can be displaced into estuarine reaches, including oyster-growing areas (Sammut *et al*., 1996a). Oyster farmers have reported that episodic acid outflows from floodgates draining ASS pass over leases causing poor growth rates, iron staining of shells and increased mortality that appear unrelated to other known risk factors.

There have been very few scientific studies undertaken on the effects of estuarine acidification on bivalves and specifically the Sydney rock oyster. Overseas studies have investigated the impacts of acidified water on a small number of bivalve species (Bamber, 1987; 1990; Calabrese and Davis, 1966; Kuwatani and Nishii, 1969; Loosanoff and Tommers, 1947) but the severity and the extent of waterway acidification experienced in eastern Australian estuaries are orders of magnitude greater than reported in the overseas literature. To date there have been no studies that show direct links between poor oyster health and ASS. The hypothesis that estuarine acidification is affecting oyster production is plausible in view of the proven impacts of acidified water on mobile species such as fish.

Not all production problems are attributable to estuarine acidification. There are known production risks associated with oyster diseases and other unrelated environmental factors. Likewise, the climatic conditions that trigger estuarine acidification are also responsible for a variety of other water quality changes that may affect oysters (for example low dissolved oxygen, low electrical conductivities and increased nutrient loadings). This study specifically investigates estuarine acidification but in doing so, measures a range of other water quality variables in the estuarine environment to examine other environmental risk factors for production of the Sydney rock oyster, *Saccostrea glomerata*.

The present study was undertaken to determine if estuarine acidification and associated changes in water quality cause the production impacts reported by oyster farmers. This will establish the context of the problem to underpin environmental decision making, to ultimately develop more appropriate reactive and proactive strategies for oyster farming in acid-impacted estuaries, and to improve the investigation and diagnosis of oyster mortalities and production problems in estuaries.

Oyster farming is one of the most vulnerable aquaculture industries in eastern Australia due to the reliance on estuarine waters that are shared with other users and impacted by land-based activities. Oyster leases are also close to the most urbanised sections of a catchment where population and development are most intense. Oyster farmers have limited control over the movement of their stock due to the impracticalities of removing and storing stock during poor water quality events. The sustainability of oyster farming is, in part, controlled by farming practices, but largely affected by the quality of estuarine waters.

Korringa (1976) identified hydrographic and biological conditions, predators (such as porcupine fish, bream, toad fish and stingrays), parasites, diseases and competitors as the primary known risk factors for oyster production. Nell (1993) categorised heat kill, floods and pollution as environmental hazards, mudworm, winter mortality disease and QX disease as disease risks and Pacific oysters, mussels and barnacles as the principal competitors. Risk factors associated with environmental hazards are not always clearly understood due to synergistic effects of some hazards, lag effects and a lack of information on their direct and indirect impacts on oysters.

In recent years the industry has inferred a link between estuarine acidification and productivity declines based on established associations between acidification and fish kills and fish diseases (Sammut *et al*., 1995; 1996a; Callinan *et al*., 1996). Estuarine acidification in eastern Australia is largely caused by acid produced in drained or excavated ASS (Sammut *et al*., 1996b). Acid is exported into estuarine waters via artificial drains and floodgates. The acid events are characterised by elevated concentrations of metals, and pHs that are generally less than 5.5 (Sammut *et al.*, 1996b). Oyster farmers observed that sudden production crashes often coincided with major rainfall events and plumes of acidic waters passing over or near leases. Impacted leases are often coated with a film of iron, a by-product of the oxidation of pyrite and the dissolution of iron from the acidified soils of the catchment. These acute events appeared to be unrelated to diseases or management practices. Chronic production problems, such as poor growth rates, were linked to regular low-tide releases of acidified water from floodgates that act as temporary stores for acid in drained floodplains. Poor growth rates, iron staining, shell bleaching, low survival rates and poor meat condition have all been reported from leases affected by acid outflows.

On the far north coast of NSW, oyster farmers postulated that acidified waters might be a stress factor in oysters, predisposing them to QX disease. Past research (Anderson *et al*., 1994; Wesche, 1995) on associations between ASS and QX outbreaks showed that QX outbreaks could occur without acidification of a lease. The studies were not designed to test specifically for increased susceptibility to QX

through exposure to acidic water. Consequently, the oyster farming community remains concerned that acidified waters may contribute to outbreaks.

Estuarine acidification has been investigated in detail on the Richmond River (Sammut *et al.*, 1996a; Sammut, 1998) and the Hastings River (Johnston, 1995). Poor water quality directly associated with ASS outflows affects large areas of the Richmond River, Manning and Hastings River estuaries. An extensive network of flood-gated drains dissect areas mapped as high risk ASS (Figures 3.1 and 3.2) and have the potential to discharge acidified water resulting in widespread estuarine acidification.

Research for this report was conducted on the mid north coast and north coast regions of NSW. Research examining links between water acidification and QX disease outbreaks in rock oysters was undertaken on the Richmond River (Northern NSW). The Hastings and Manning Rivers (mid north coast, NSW) were selected to examine associations between ASS outflows and poor oyster performance and health. These estuaries are classified as mature "barrier estuaries" (Roy, 1984) and contain extensive deposits of ASS adjacent to the estuary (Figures 3.1 and 3.2).

1.1.1 Oyster Production in NSW

NSW oyster production peaked in 1976/77 when close to 150,000 bags of bottle and plate oysters were harvested. This figure has declined to around 50,000 bags of bottle and plate grade oysters produced in 1999/00 (NSW Fisheries, 2001). Figure 1.1 illustrates the changes in production that have occurred since the late 1970s.

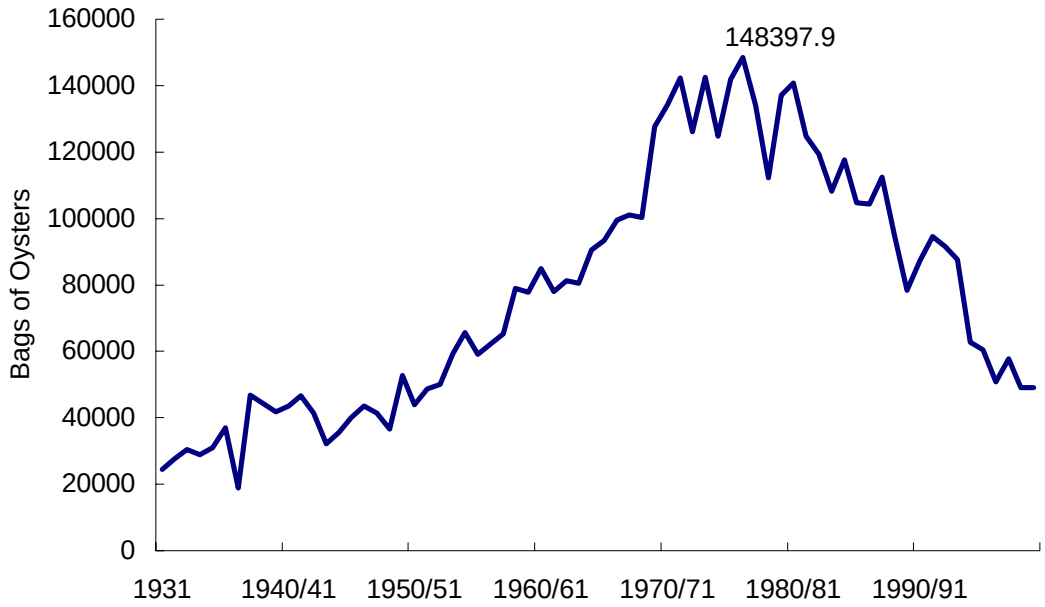


Figure 1.1 Number of bags of bottle and plate grade oysters produced in NSW (*Source*: NSW Fisheries Unpublished Data)

The Hastings and Manning River estuaries (Figure 1.2) are the primary experimental sites in this study, and the Richmond River was used for a sub-project on QX disease. The Hastings River was the eighth largest producer of oysters in NSW for the 1999/00 financial year, contributing approximately 3% of the State's total. The Hastings River produced predominately bottle-grade oysters for the 1999/00 period. In 1999/00 the total value of oyster sales was $204,825 (NSW Fisheries, 2001).

Production methods used to mature oysters on the Hastings River include rack-tray culture, raft culture, rack-stick culture, and baskets. The rack tray method dominates oyster production on the Hastings River and is used extensively in Limeburners Creek and Big Bay (Figure 3.5). Plastic slats placed in spat fall areas is the main method of spat collection in this system. The spat attach to the plastic slats and after approximately 4 months are used to stock trays or sold to farmers in other estuaries for single seed production. The sale of single seed oysters is an important component of the industry and the Hastings River has consistently been the largest supplier in NSW. For example, the Hastings oyster industry supplied over 10 million single seed oysters in 1998/99 (46% of the State's total) to other NSW estuaries (NSW Fisheries, 2000).

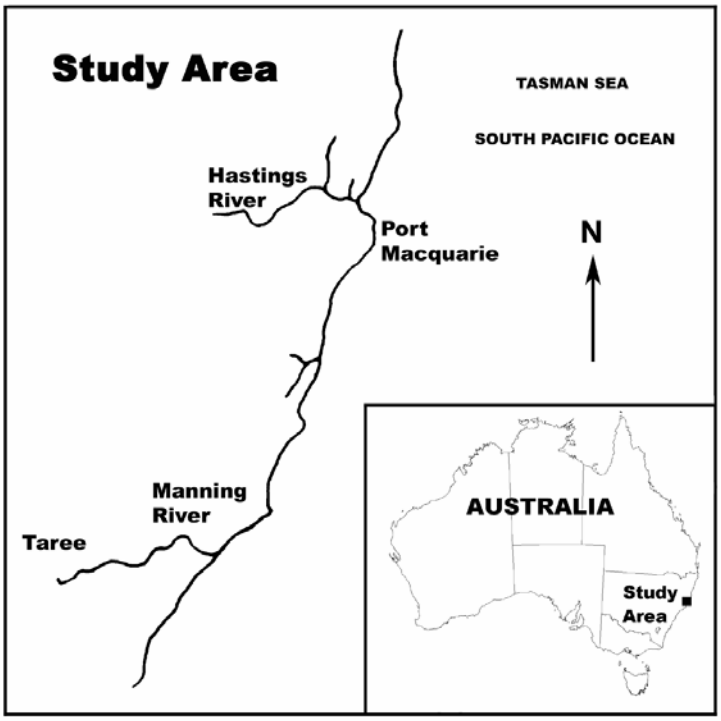


Figure 1.2 Location of the Hastings and Manning River estuaries.

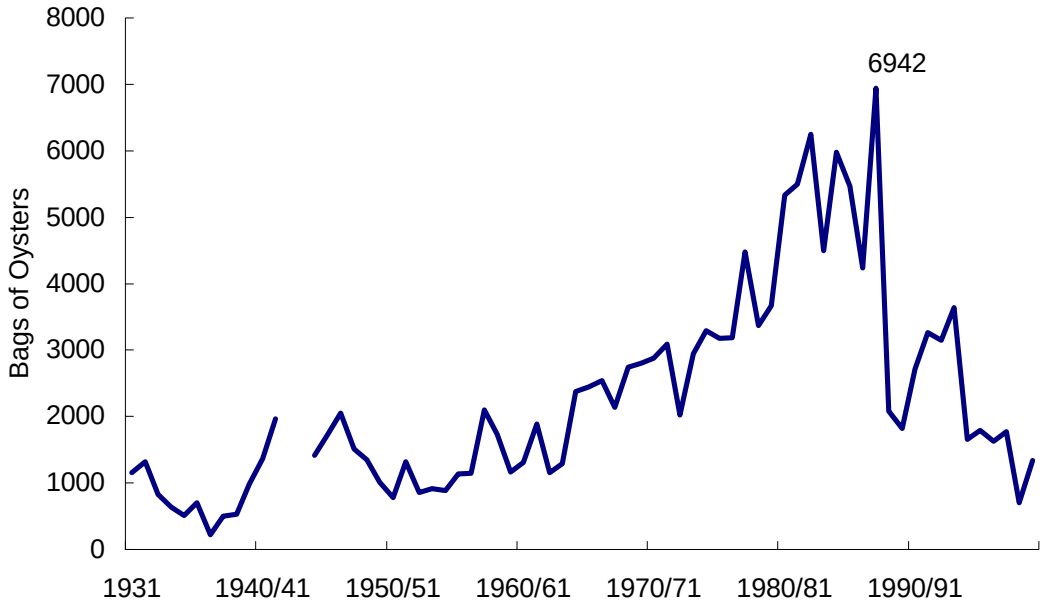


Figure 1.3 Hastings River oyster production (bottle and plate oysters) (*Source*: NSW Fisheries Unpublished Data).

Figure 1.3 highlights the variability in oyster production from year-to-year on the Hastings River. Production peaked in 1987/88 when 6,942 bags of plate and bottle oysters were produced. However, only 2,080 bags of plate and bottle oysters were harvested in the following year. Recurrent episodes of mortality and slow growth have been experienced in the Hastings estuary and are discussed in this report.

The Manning River was the ninth largest producer of Sydney rock oysters in New South Wales, contributing 2.6% of the State's total in 1999/00. Rack-tray culture is the main method used for oyster production on the Manning River. Other production methods used for maturing oysters include rack-stick culture, baskets, raft culture and dredge beds. The Manning River mainly produces premium-grade plate oysters. In 1999/00 the total value of oyster sales was $797,756, compared to $600,053 for the 1998/99 season (NSW Fisheries, 2001; 2000).

Export of oysters to other estuaries is another key component of the Manning River oyster industry. In 1998/99 the Manning oyster industry supplied 5% (1.1 million) of the State's single seed oysters, and 7% of tray-farmed oysters for inter-estuary transfer (NSW Fisheries, 2000).

Oyster production figures for the Manning River indicate harvests vary considerably from year-to-year (Figure 1.4). Production peaked in the early 1960s, when over 6,500 bags of plate and bottle oysters were produced. In recent years, there has been a marked decline in the numbers of plate and bottle oysters harvested. For example, in 1991/92 4,652 bags of plate and bottle oysters were produced, compared with only 1,270 bags in 1998/99. Seasonal dips in production are, in part, now attributed to acid

water outflows and associated declines in oyster health and mortalities, which are investigated in this report.

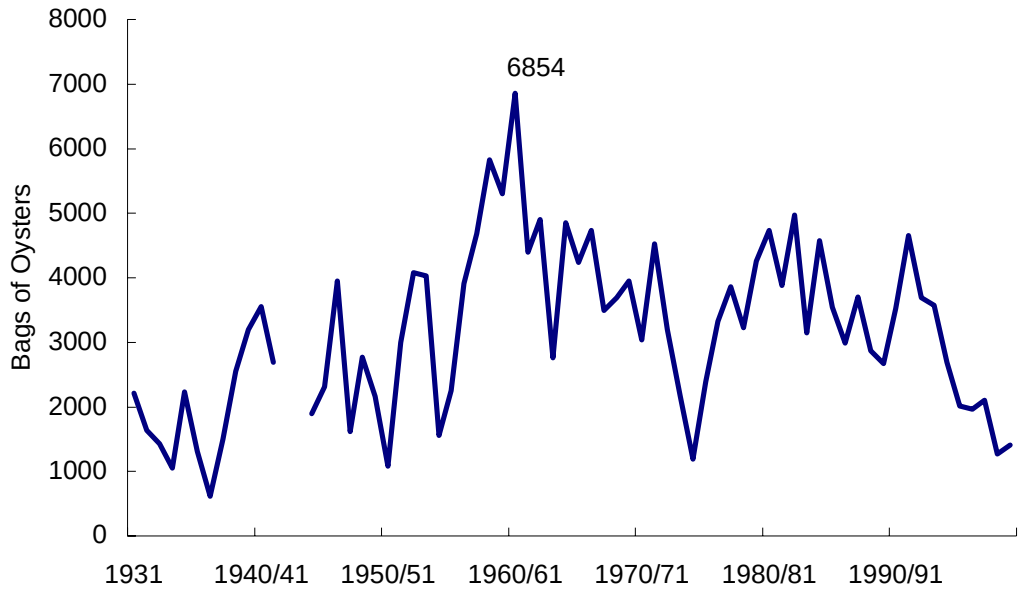


Figure 1.4 Manning River oyster production (bottle and plate oysters) (*Source*: NSW Fisheries Unpublished Data).

1.1.2 Past Studies on the Effects of Acidified Water on Oysters

Prior to this project, research on the effects of ASS-affected waters on the Sydney rock oyster was limited to bioassays using embryos (Wilson and Hyne, 1997). Wilson and Hyne (1997) investigated the toxicity of leachate from ASS, pH-adjusted seawater and aluminium to early embryonic development of Sydney rock oysters. Wilson and Hyne (1997) concluded that abnormal embryonic development resulted when larvae were exposed to treatments containing > 3.3% acid sulfate leachate in seawater and when pH dropped below 6.75 in pH-adjusted seawater containing no ASS leachate. A significant decrease in early embryonic development also occurred when aluminium was elevated above 150 µg L^{-1} in pH neutral conditions.

Overseas studies on the effects of acidified water on other bivalves do not involve ASS but give insight to the probable impacts of ASS-affected waters on Sydney rock oyster production. Loosanoff and Tommers (1947) recorded increased pumping rates at pH values between 7.0 and 6.75, but when the pH dropped below 6.5 pumping rates dramatically decreased in adult *Ostrea virginica*. Loosanoff and Tommers (1947) also observed abnormal shell movements when pH was less than 6.5. Calabrese and Davis (1966) found growth was inhibited at pH < 6.75 and abnormal development occurred at pH values below 6.0 in *Cassostrea virginica* larvae.

Bamber (1987) measured feeding inhibition and a significant reduction in tissue and shell growth for the species *Venerupis decussata* at pH < 7.0. He also found that at pH values below 6.5, mortality dramatically increased during the experiment with smaller clams being more sensitive to the acidic conditions. Bamber (1990) investigated the effects of acidic conditions on *Crassostrea gigas*, *Mytilus edulis* and *Ostrea edulis* and concluded that a pH less than or equal to 7 is detrimental to these bivalve molluscs. Significant mortalities occurred at pH < 6 in *C. gigas* after 30 days exposure, pH 6.6 in *M. edulis* after 30 days exposure and pH 6.9 in *O. edulis* after 60 days exposure (Bamber, 1990). For *C. gigas*, feeding inhibition, shell growth reduction and flesh weight reduction occurred below the critical pH of 7.0 and behavioural inhibition was observed below pH 6.5 (Bamber 1990).

Overseas studies have demonstrated that shell dissolution can occur when perturbations in pH are only very minor. Kuwatani and Nishii (1969) discovered shell dissolution starts to occur at a pH of 7.6 in the Japanese Pearl Oyster (*Pinctada fucata*). Work carried out by Bamber (1987; 1990) discovered shell dissolution occurs at a pH of 7.5 in carpet shell clams (*V. decussata*), and at pH 7.0 for the native oyster (*O. edulis*), the Pacific oyster (*C. gigas*) and the native mussel (*M. edulis*). ASS outflows are capable of decreasing the pH of the estuary to levels significantly lower than the critical pH values stated in the overseas studies (Sammut *et al.*, 1996b; White *et al*., 1996).

The behavioural response of oysters exposed to acidified water is important for the design of experimental work and to interpret field and laboratory data. Laboratory experiments under which pH, salinity and temperature can be manipulated, whilst observing valve movements of individual oysters, will allow an understanding of behaviour for this species under acidic conditions.

It is unknown whether exposure to low pH is injurious to the Sydney rock oyster soft tissue. Exposure of the soft tissue to acidified water is dependant upon whether the valves are open or closed at reduced pH levels. Also, ASS-affected waters are characterised by elevated concentrations of aluminium and iron that may be detrimental to the soft tissue of the Sydney rock oyster. Laboratory experimental exposures are the most effective way of determining the impacts of these metals as they can be tested separately and at known concentrations.

There is no information regarding the resilience of developed Sydney rock oysters exposed to ASS-affected waters. Studies on the impacts of acid on fish suggest that the gills are a target organ for acid and metal toxicity (Sammut, 1998). It is therefore plausible that oysters, in their sedentary existence, may experience gill and other soft tissue injuries from exposure to ASS-affected waters. Oyster farmers believe that mass mortalities and poor growth rates in Sydney rock oysters are linked to episodic and long-term exposure to acidified outflows (pH < 5). Many formerly productive oyster leases have been decommissioned in areas receiving acidified water originating from excavated ASS.

1.1.3 Water Acidification and QX Outbreaks

QX disease, caused by the protozoan parasite *Marteilia sydneyi*, triggers serious, seasonally recurrent mortalities in farmed and wild Sydney rock oyster in eastern Australian estuaries.

Haysom (1978), Lester (1986) and Wesche (1995) reported occurrence of QX disease outbreaks after significant rainfall events. In eastern Australian estuaries, such rainfall events are often followed by acidification (Wesche, 1995; Sammut *et al.*, 1995) and/or de-oxygenation (Callinan, 1997a) of tributaries and parts of the main channels on the lower flood plains, including sites with large oyster populations. There is evidence that exposure to adverse environmental conditions impairs the health and productivity of oysters (Fisher and Tamplin, 1988) and that exposure to pollutants renders them more susceptible to infectious disease (Chu and Hale, 1994).

Two prior studies have examined possible relationships between outbreaks of QX disease and exposure of rock oysters to acidified water. Anderson *et al.* (1994) described two outbreaks of *M. sydneyi* infection in oysters near the mouth of the Brisbane River. Before the first outbreak the pH fell slightly, but before the second outbreak it remained unchanged. Changes in salinity and temperature were minor. The results indicated that QX disease outbreaks were not correlated with fluctuations in pH, salinity and temperature of water in close proximity to the oysters. In a study on the Pimpama River, southeast Queensland, Wesche (1995) reported a QX disease outbreak in oysters not apparently exposed to acidified water and no evidence of QX disease in oysters exposed to a minor (0.6 unit) reduction in pH.

The present study was designed to test the hypothesis that exposure to acidified water increases the susceptibility of Sydney rock oysters to QX disease outbreaks. The study was conducted on the Richmond, rather than the Tweed River because of proximity to Regional Veterinary Laboratory (RVL), Wollongbar and consequent cost savings.

1.2 NEED

Prior to the present study, associations between ASS and poor oyster production were based on observations made by farmers, and comparisons to the effects of acid on other gilled organisms. Despite having raised concerns over the potential impacts of ASS on oyster production, the industry concerns were largely ignored and the management paradigm of the time did not address its needs. There is a need to prove or disprove the role of acid in oyster mortalities and production problems in order to:

1. Develop more effective diagnostic and investigative protocols for oyster mortality events and chronic production problems at leases;
2. Increase awareness of the acid sulfate soil hazard and acidification risk amongst oyster farmers, developers and environmental managers to improve environmental decision making, the environmental impact assessment process for coastal development, and other planning processes so that the problem is not exacerbated through ignorance;
3. Improve reactive management strategies by the industry and government agencies so that impacts can be reduced or eliminated; and,
4. Enable farmers to improve the day-to-day management of high-risk leases.

1.3 OBJECTIVES

The overall objective of this study is to investigate risk factors for poor oyster production with a particular emphasis on acidification due to drainage of acid sulfate

soils. Other water quality and environmental factors are considered in the field-based components of the study.

The specific objectives of the study are:

1. To identify associations between water quality conditions (with particular reference to acidified water and toxic metals), other environmental factors and reduced growth rates and disease outbreaks/mortalities in oysters at selected sites on the Hastings and Manning Rivers;
2. To identify specific environment and management related risk factors for reduced growth rates at selected sites;
3. To identify environmental and management risk factors for specific diseases with particular reference to QX on the Richmond River; and
4. To effectively communicate the findings of this study to the oyster industry and relevant agencies.

The first objective was modified to include the Manning River and remove the Tweed River. The Manning River was included because early investigations of production problems on the Hastings River identified a potentially new or undescribed disease that could confound field experiments on the effects of acid. The Tweed River was removed from this objective because of the possibility of a complete collapse of the industry during the study; this could have impacted the field experiments through loss of experimental sites and field support by the local industry.

The second objective focussed on the effects of "cooking" practices to remove fouling following the recommendations of a linked study by Lake (1997) and concerns from farmers that this practice may stress oysters and increase their susceptibility to injury or infection. Other management practices, such as drying and stock movement were too difficult to test experimentally.

The third objective was originally related to QX outbreaks on the Tweed River. For similar reasons discussed above, the study was moved to the Richmond River where QX is prevalent and acidification is a regular event.

2 PILOT INVESTIGATION

2.1 INTRODUCTION

A pilot investigation was necessary for the present study due to the limited information on the effects of acidification on the Sydney rock oyster. The pilot study was designed to provide a platform on which more rigorous experiments could be based. To investigate links between ASS-affected water and poor oyster production, Sydney rock oysters were experimentally exposed to acidified water under field and laboratory conditions.

The purpose of the pilot study was to: describe gross impacts to oysters caused by exposure to acidified water; determine the behavioural response of oysters exposed to acidified water; determine whether ASS-affected waters cause oyster mortality; and, examine the response of oyster soft tissues to acidified water.

A laboratory experiment was used to examine the effects of low pH on oyster soft tissues and behaviour. Observations of oyster behaviour (valve activity and feeding) were needed to determine whether the soft tissues of oysters were exposed to acidified water. A field experiment was used to expose oysters directly to ASS-affected waters under natural conditions to examine oyster shell impacts and calculate oyster survival. More specific details of the methodologies and the experiments described in this section can be found in Dove (1997).

2.2 METHODS

2.2.1 Laboratory Experiment

2.2.1.1 Experimental Apparatus

Oysters were exposed to naturally and artificially acidified waters in a 30 L aquarium. Water was recirculated through the aquarium to observe feeding and other oyster behaviour. The experimental apparatus consisted of a header tank (70 L) which gravity fed water to the aquarium. The aquarium overflowed into a 70 L tank. Water was returned to the header tank using two 2,000 L h^{-1} bilge pumps and 20 mm diameter food-grade hosing. The aquarium was constructed from high-density polyethylene (HDPE) and all other tanks were food-grade, stabilised plastic so that no reaction could occur with the acidic water. A flow velocity of ~ 0.01 m s^{-1} was maintained in the aquarium during all of the treatments. Each aquarium was cleaned with a dilute nitric acid solution and rinsed with deionised water between each treatment.

An air stone was used to aerate the treatment waters. The water temperature, dissolved oxygen concentration (DO), salinity, electrical conductivity (EC) and pH of the experimental waters were measured at 10-minute intervals using a Yeo-Kal 611 Intelligent Water Quality Analyser. The Yeo-Kal 611 Intelligent Water Quality Analyser was calibrated with standard, certified calibration solutions before the start of each treatment. The stocking density of the tank for the four treatments was ~ 0.5 oysters L^{-1} (15-17 oysters in total).

2.2.1.2 Treatment Water

Oysters were exposed to four treatment waters. Three treatments contained seawater that was artificially acidified and the fourth treatment contained seawater that was naturally acidified using ASS-affected water. The seawater used in the four

treatments was collected at Cronulla (Treatments 1, 2 and 3) and Camden Haven (Treatment 4).

The seawater used in Treatments 1 to 3 was diluted with deionised water to a salinity between 24 and 26 ppt. Analar hydrochloric acid (HCl) (0.1 M) was added to the overflow tank to acidify the treatment water. HCl was used instead of H_2SO_4 to acidify treatments to avoid unstable aluminium-sulfate complexes that can decouple changing the aluminium species present in the treatment water (Sammut, 1998). HCl was also routinely used as an acidifying agent by other studies (Loosanoff and Tommers, 1947; Kuwatani and Nishii, 1969; Calabrese and Davis, 1966; Allan and Maguire, 1992).

Seawater was diluted with deionised water in Treatment 4 to the same salinity as Treatments 1 to 3. ASS-affected waters were collected from Fernbank Creek (Figure 3.3) on the day of the experiment to acidify the seawater. A water sample was collected every 2 hours during each treatment to test for trace metals that could interfere with the experiment.

A description of the four treatments used to monitor behaviour and expose the soft tissue to acidified water is provided below.

Treatment 1:
Treatment 1 had a varying pH range (8.2 - 2.0) and constant salinity (~ 26 ppt) to determine oyster behaviour over a range of acidified conditions.

Treatment 2:
Treatment 2 exposed oysters to an artificially acidified treatment before oysters were removed and preserved for histopathology. Oyster behaviour was monitored during this treatment. Treatment 2 involved a rapid and dramatic pH change. Seventeen oysters were placed into the aquarium at pH 7.6 and salinity 26 ppt and allowed time to adjust and feed normally. The aquarium was then acidified to pH 3 with 0.1 M HCl.

Treatment 3:
Treatment 3 exposed oysters to moderate acidic conditions in an artificially acidified aquarium before oysters were removed and preserved for histopathology. Oyster behaviour was monitored during this treatment. Fifteen oysters were introduced to the aquarium at pH 7.8 and allowed time to open and feed. The aquarium was then acidified to pH 5.7 using 0.1 M HCl. Salinity was kept constant at 26 ppt.

Treatment 4:
Treatment 4 exposed oysters to ASS-affected waters. Oyster behaviour was monitored before oysters were removed for histopathology. The aquarium water was acidified by adding the ASS-affected water collected from Fernbank Creek. ASS-affected waters were used in this treatment to simulate estuarine acidification under laboratory conditions. Oysters were placed into the aquarium at pH 8 and a salinity of 27 ppt. ASS-affected water acidified the treatment water to pH 3.8. The addition of ASS-affected water caused the salinity to decrease to 8 ppt.

2.2.1.3 Behaviour

Oyster behaviour was assessed by observing and recording the movements of oyster valves during the four treatments. This was performed under laboratory conditions to allow continual and close observation of individual oysters. Behaviour was observed and recorded during the four treatments described in Section 2.2.1.2.

2.2.1.4 Soft Tissue

Treatments 2, 3 and 4 exposed oysters to acidic conditions and oyster soft tissue was preserved for histopathological investigation. Oysters collected from Limeburners Creek, Hastings River were exposed to treatments of artificially and naturally acidified water described in Section 2.2.1.2 for varied time intervals before being removed and placed in Davidson's fixative.

For Treatment 2, one oyster was removed prior to acidification of the aquarium, then oysters were removed randomly at 5, 10, 15, 30, 60, 120, 180, 360 and 720 minutes of exposure to the acidic treatment. Oysters were removed at these times because no background data existed to determine the duration of exposure for detrimental effects to occur.

For Treatment 3, one oyster was removed and preserved for histopathology before the aquarium was acidified to pH 5.7 and oysters removed at the same time intervals listed above. This pH value was used because it was commonly measured in the Hastings River estuary after rainfall (Johnston, 1995).

For Treatment 4, oysters were removed and preserved for histopathology using the same procedure as the previous treatments. Water samples were collected from each of the treatments to ensure that there was no interference from high concentrations of metals.

2.2.2 Field Experiment

A field study involving 50 small (average height 40.83 mm), and 50 large (average height 58.61 mm) oysters was used to determine the impact of long-term exposure to ASS-affected waters in a tidal creek. Oysters were placed into Fernbank Creek and observed over an eight-week period.

The small and large oysters were deployed in two plastic mesh baskets and fixed above the bed-level of the creek and below the low tide height so the baskets remained permanently submerged for the duration of the experiment. A Greenspan Technical Services submersible data logger (SDL) affixed to a nearby floodgate by Hastings Council monitored tide, water temperature, EC and pH at one hour intervals during the experiment.

The oysters were observed at day 1, 7, 14, 26, 39, 57 and 87 due to field accessibility. The number of dead oysters was counted and oyster shell samples were collected for descriptions and analysis. Fernbank Creek was chosen for the exposure site due to frequent, long duration, severely acid events which were regularly below pH 3.

The oyster shell surface of oysters removed from Fernbank Creek was examined and compared to oysters from non-acid impacted sites in the Hastings River using scanning electron microscopy (SEM). Shell specimens were rinsed in deionised water and air-dried after they were removed from Fernbank Creek (Figure 3.3). The shells were drilled and broken to remove small pieces (approximately 5 mm x 5 mm) of shell from the middle of the left valve. Each shell fragment was fastened onto a carbon shield, and then glued to a 13 mm diameter aluminium stub using colloidal graphite solution. The carbon shield prevented aluminium spikes occurring on the x-ray spectra. Shells were dried at 44 ^{O}C for 30 minutes and then carbon coated using a Balzers Carbon Evaporator which applies a light carbon film onto the specimen.

Specimens were placed into a Leica/Cambridge S-360 Scanning Electron Microscope interfaced with an energy dispersive x-ray spectrometer (EDS). EDS allows rapid detection of all elements simultaneously contained in the surface layer of the shell. This information was then processed using Iridium software to produce x-ray spectra for each shell.

2.3 RESULTS

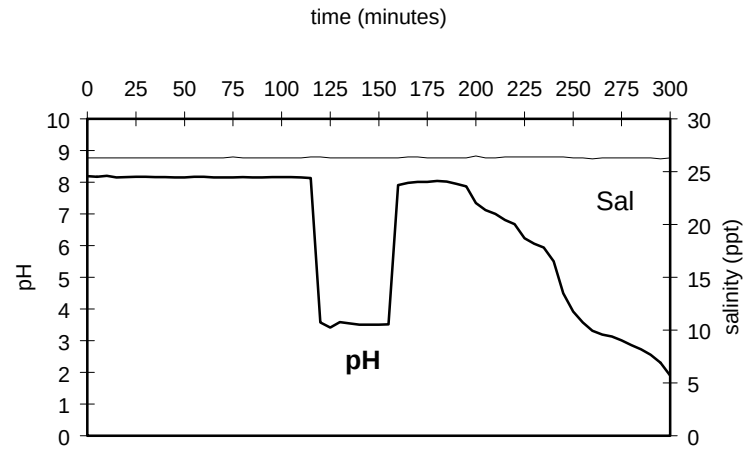
2.3.1 Behaviour of Oysters to Acidified Water

Figure 2.1 summarises the behaviour of oysters during Treatment 1. A total of 13 oysters out of 15 had open valves at a pH of 8.16 and salinity of 26.3 ppt. The rapid pH decline caused all open oysters to close their valves. The pH value at which this occurred was between 3 and 4. The pH was suppressed at 3.5 during which time the oysters remained closed. The pH was then returned to above neutral conditions and the original 13 oysters that had open valves at the start of the treatment re-opened their valves. Subsequently the pH was gradually reduced and oysters commenced to close their valves at a pH of 6.2. As the pH reduced further, more oysters began to close their valves. Some oysters responded by clomping (rapid opening and closing of their valves) before remaining closed. Two oysters remained open while the pH decreased to pH 2.7.

The behaviour of oysters for Treatments 2 and 3 is displayed in Figures 2.2 and 2.3, respectively. Figure 2.2 summarises observations on oyster behaviour as well as pH and salinity for the duration of this treatment. Oysters remained open until the aquarium was acidified, at which time the rapid and dramatic decrease in pH caused the oysters to immediately close their valves.

Figure 2.3 shows oyster behaviour as well as the pH and salinity for the duration of Treatment 3. All oysters were open whilst the aquarium was acidified and abnormal behaviour was observed during the course of this treatment. Figure 2.4 shows a dramatic drop in both pH and salinity when the ASS-affected waters were introduced into the aquarium. The decrease of these variables caused all open oysters to respond by immediately closing their valves. Oyster valves then remained closed for the duration of this treatment.

A.



B.

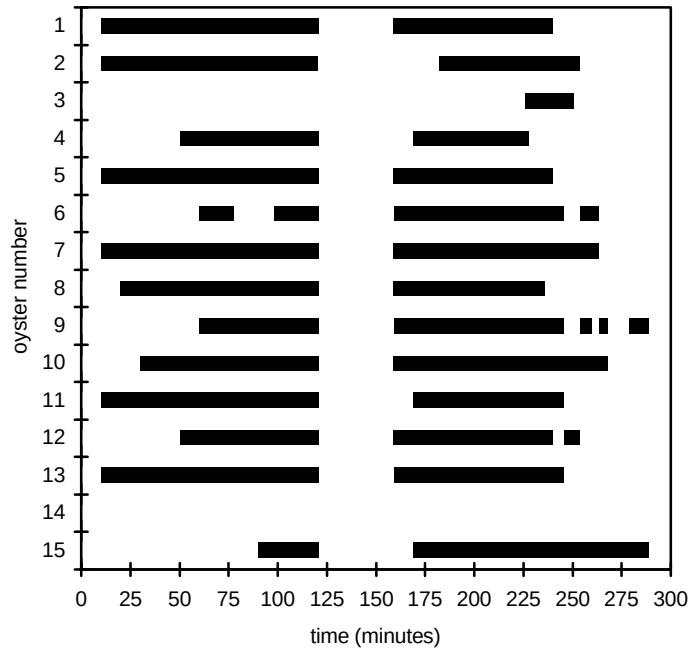
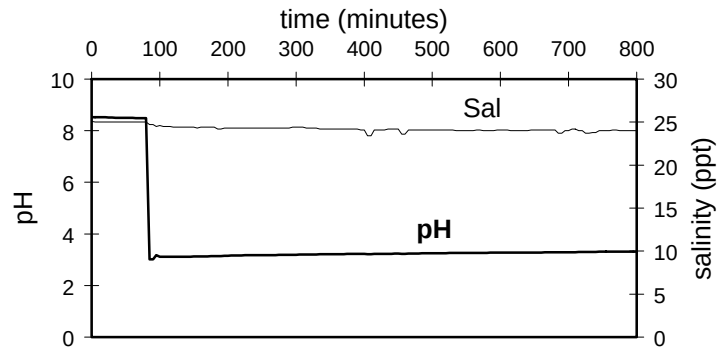


Figure 2.1 Treatment 1 summary: (A) pH and salinity of artificially acidified treatment water; and, (B) oyster status (solid bar indicates open oyster) during the treatment (*Source*: Dove, 1997).

A.



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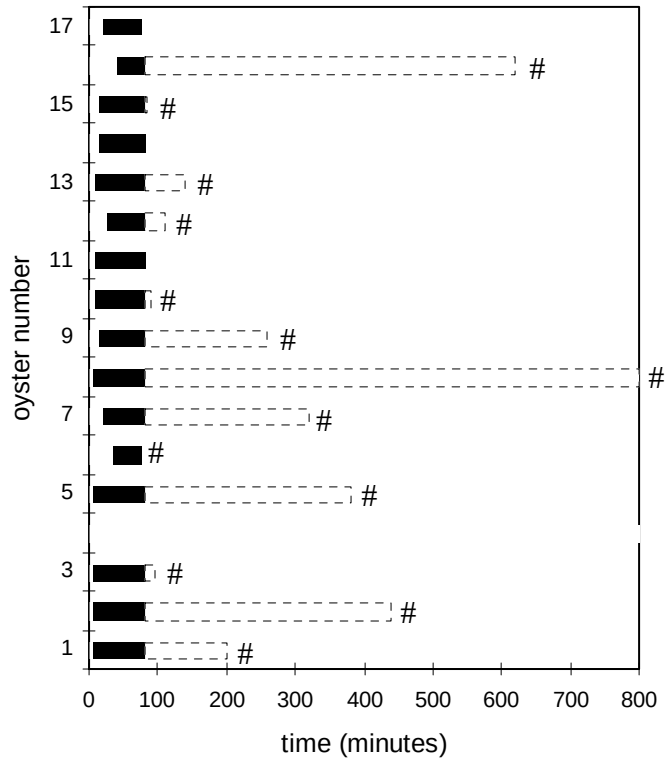
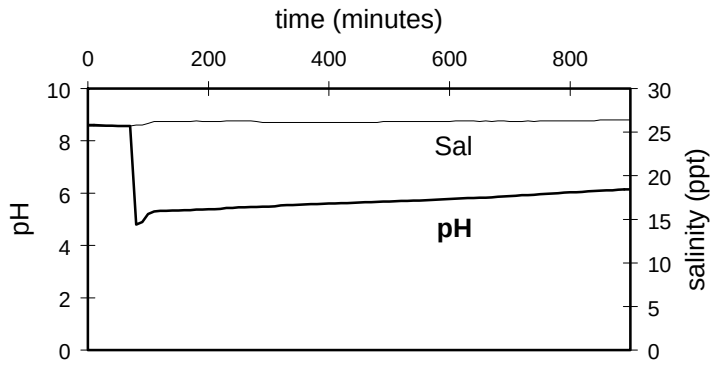


Figure 2.2 Treatment 2 summary: (A) pH and salinity of artificially acidified treatment water; and, (B) oyster status (solid bar indicates open oyster, open bar indicates closed oyster and # indicates oyster was removed for histopathology) (*Source*: Dove, 1997).

A.



B.

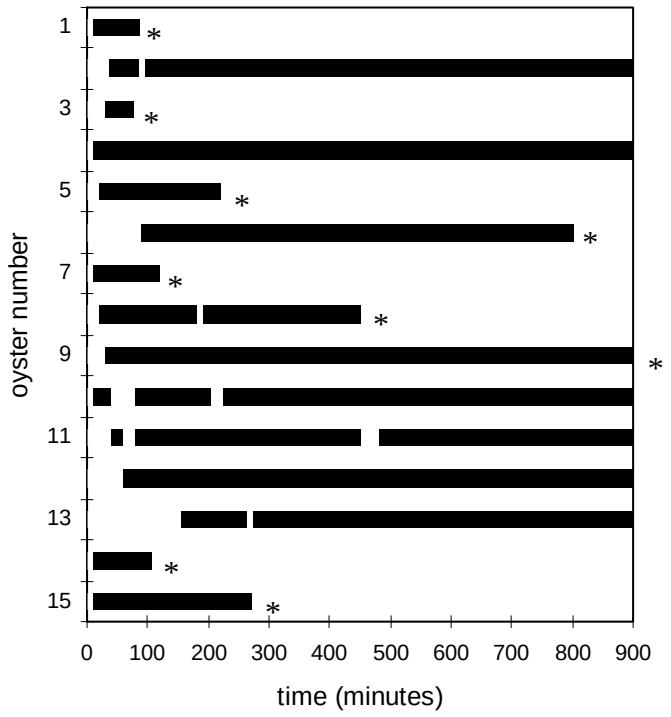
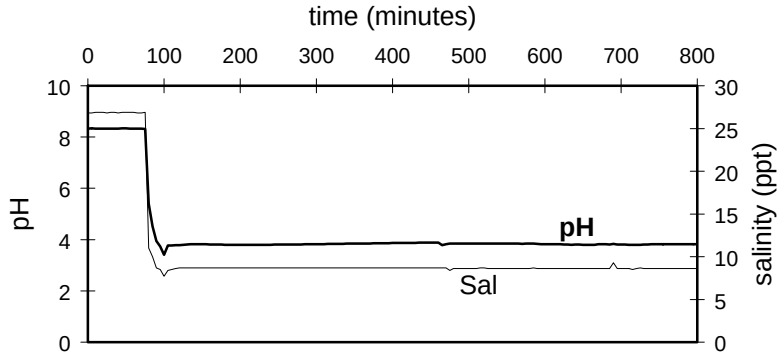


Figure 2.3 Treatment 3 summary: (A) pH and salinity of artificially acidified treatment water; and, (B) oyster status (solid bar indicates open oyster and * indicates oysters removed for histopathology) (*Source*: Dove, 1997).

A.



B.

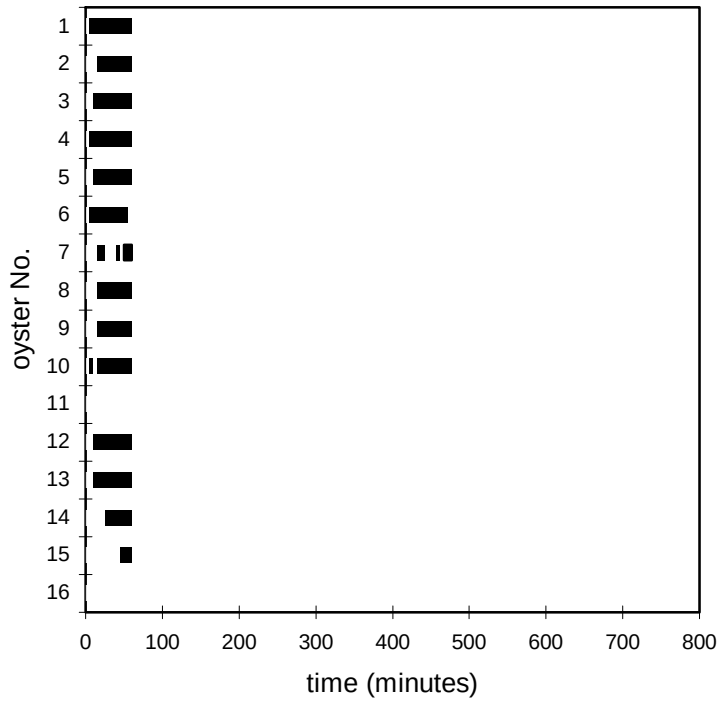


Figure 2.4 Treatment 4 summary: (A) pH and salinity of naturally acidified treatment water; and, (B) oyster status (solid bar indicates open oyster and oysters 1, 2, 4, 5, 6, 8, 10, 12, 13 and 14 were removed for histopathology) (*Source*: Dove, 1997).

2.3.2 Histopathology

The histopathology data from oysters exposed to acidified water are summarised below from Dove (1997):

Treatment 2:

- Seventeen oysters remained open until the aquarium was acidified.
- Acidification of the aquarium resulted in valve closure in all oysters.
- Histopathology showed background inflammatory cells predominately in the mantle consistent with Callinan's (1997a) findings of non-specific inflammatory changes only, with no evidence of a causative agent. This condition is referred to as Limeburners syndrome (LS) (Callinan, 1997a) and is discussed further in Chapter 6 of this study.
- Artefact from this background condition made it difficult to distinguish actual tissue damage from exposure to the acidified treatment.
- Oysters did not show any consistent pattern of tissue damage.
- Some oysters did show epithelial necrosis and sloughing of the mantle that was not consistent with LS.
- Water samples from this treatment showed no elevated metal concentrations.

Treatment 3:

- All oysters were open in the acidified treatment water.
- Particular oysters displayed signs of excessive gaping and responded slowly to tactile stimulation.
- Histopathology data indicated the presence of inflammatory cells consistent with Callinan's (1997a) description of non-specific inflammatory changes only, with no evidence of a causative agent.
- Inconsistent patterns of response of soft tissues were observed.
- Necrosis and sloughing of the mantle epithelium was patchy and not as severe as observed in Treatment 2.
- Analysis of water samples revealed that concentrations of trace metals were not high enough to interfere with this treatment.

Treatment 4:

- Fifteen oysters were exposed to a dramatic drop in pH as well as salinity when naturally acidified water was introduced to the aquarium.
- The reaction of all of the oysters to this change in water quality conditions was to immediately close their valves.
- Histopathology revealed that tissue responses were not as severe as observed in Treatments 2 and 3. However, an inconsistent picture of acid-induced soft tissue response was once again observed.
- Mantle epithelium sloughing and necrosis were minor to moderate in all samples.
- Water sample analysis indicated elevated concentrations of iron (mean concentration 2.49 mg L^{-1}) and aluminium (mean concentration 2.54 mg L^{-1}).

2.3.3 Survival

The water quality measurements collected from Fernbank Creek during the field experiment showed prolonged acidic conditions that were partially neutralised during high tides. The pH fluctuated considerably over the tidal cycle with higher pH levels measured during the latter stages of the flood tide which increased the EC of the water. Spot measurements of pH and results of laboratory analysis of the physical water samples are displayed in Table 2.1. Analysis of the water samples shows high concentrations of aluminium, iron and manganese. Alkalinity levels in these samples were very low or undetectable (Table 2.1).

The percentage survival of 50 large and 50 small oysters placed into Fernbank Creek is displayed in Figure 2.5. Dramatic mortality for smaller sized oysters commenced at day 40. This coincided with shell perforation in the anterior of the left valve in these oysters. Shell perforation was more prevalent after 40 days of exposure. All oysters affected by shell perforation were found dead. Shell perforation commenced in larger oysters after day 87. All oysters removed from Fernbank Creek were coated in a thick layer of iron precipitates. Iron precipitate was also observed grossly on the gills and soft tissues of dead oysters, causing the soft tissue to appear a red/brown colour. Underneath the iron precipitate coating was a severely bleached and brittle oyster shell. Shell degradation is discussed further in Section 2.3.4.

Table 2.1 Summary of selected water quality parameters measured in Fernbank Creek during the survival field exposure experiment (*Source*: Dove, 1997).

Day	pH	Al (mg L^{-1})	Fe (mg L^{-1})	Mn (mg L^{-1})	Alkalinity (mg L^{-1})
0	2.77	12.25	18.53	0.84	nd
1	3.08	13.39	23.57	0.99	nd
7	2.68	9.64	20.13	0.66	nd
14	2.71	11.26	23.47	0.83	13
39	2.61	11.16	17.44	0.81	nd
56	3.19	13.84	17.09	1.03	8

nd = not detectable

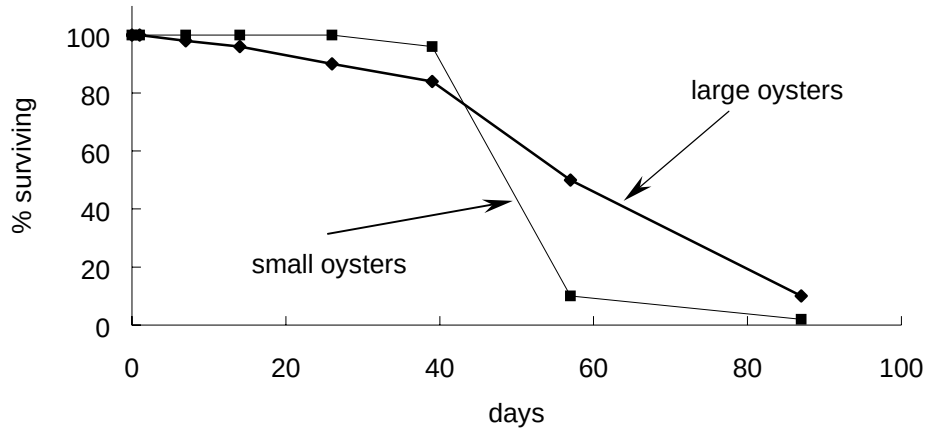


Figure 2.5 Percentage survival for large and small oysters placed in Fernbank Creek (*Source*: Dove, 1997).

2.3.4 Oyster Shell Impacts

Impacts to the oyster shell resulting from exposure to ASS-affected waters were assessed using gross examination and SEM analysis. SEM was effective in examining micromorphological changes in structure of external shell layers and was used in combination with EDS analysis to show chemical changes in the outer shell layers.

There were marked differences in the appearance of oyster shells exposed to ASS-affected waters compared to oysters from circumneutral waters. Oysters removed from Fernbank Creek were uniformly coated with a bright red/brown, iron precipitate (Plate 2.1). Oyster growers considered iron stained oysters as unmarketable due to the discolouration of the product. Underneath the iron precipitate veneer, shells were bleached white (Plate 2.2). Shells exposed to regular acid outflows were also very brittle and friable, and the external surface layer was typically smooth with no evidence of recent growth. By contrast, oyster shells from circumneutral waters were: free of iron precipitate; had a normal grey to black colour; and, showed normal shell growth lines and new shell growth extending from the mantle fringe.

SEM analysis of the shell surface of oysters provided both visual and elemental information on the effects of exposure to ASS-affected waters. Oysters that had been exposed to naturally acidified water for 7 days were collected from Fernbank Creek and compared with oysters selected from non-acidified areas of the Hastings River.

Plate 2.3 displays the differences between a healthy oyster shell and an oyster shell that has been exposed to acidic conditions. Healthy oyster shells are characterised by a rough surface texture with evident prismatic scales. Scanning electron micrographs of an acid exposed oyster (Plate 2.3) reveals an almost featureless surface that is typically smooth. At increased magnifications (Plate 2.3D) there is evidence of fragmentation of the outer shell surface layers revealing the underlying shell matrix.

Plate 2.1 A severely bleached and degraded left valve of a Sydney rock oyster exposed to ASS-affected waters for 39 days (iron coating has been removed).

Plate 2.2 Photograph of iron coated oyster shells with perforation in the anterior of the left valve resulting from internal and external shell dissolution. The diameter of the coin = 28.4 mm.

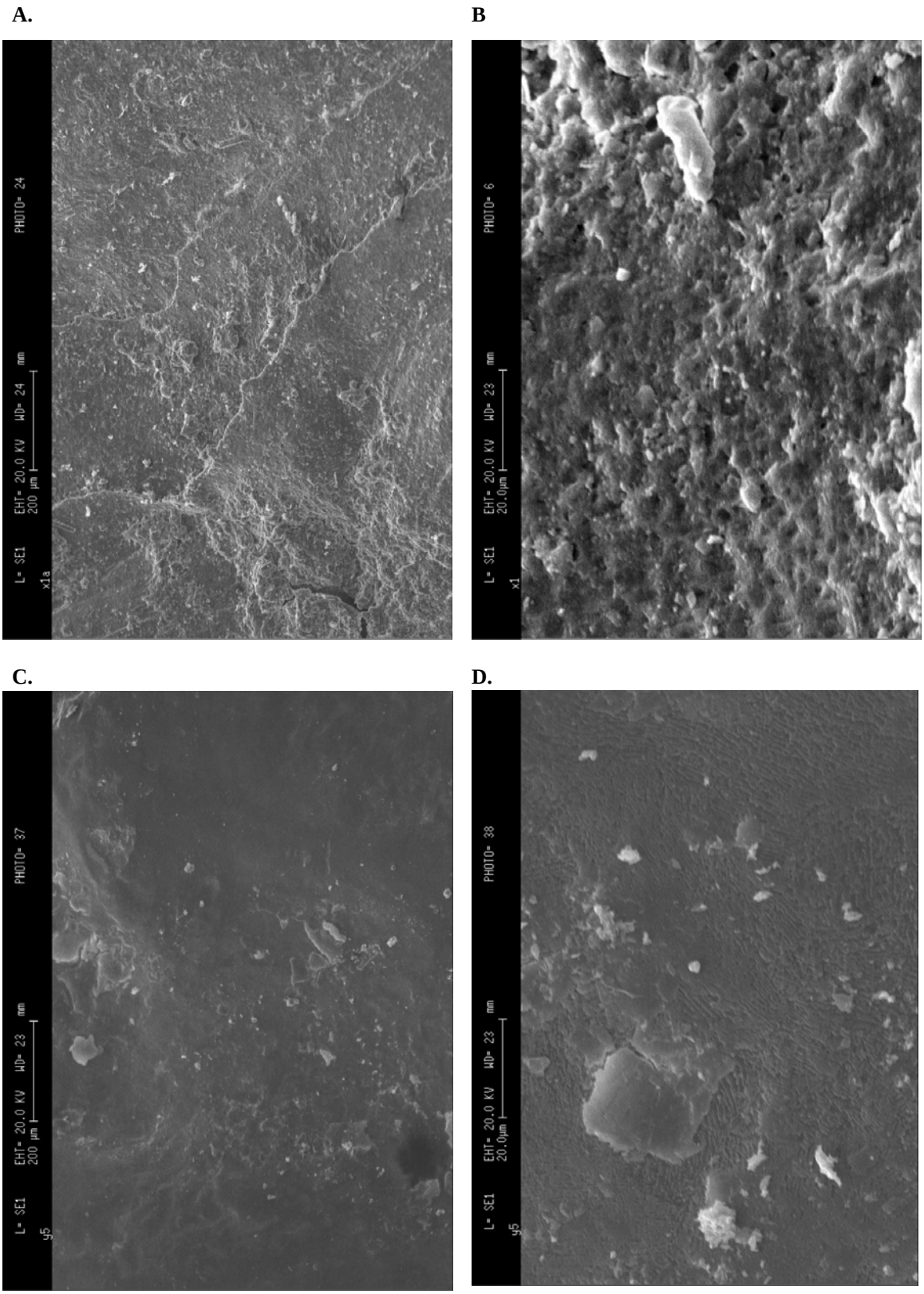


Plate 2.3 Scanning electron micrographs showing: (A and B) a healthy oyster shell with a rough textured surface layer; and, (C and D) an oyster shell impacted by ASS-affected waters with a smooth surface and exposed underlying shell layers (*Source*: Dove, 1997).

SEM-EDS spectrum provided information on the shell surface chemistry of oysters. The results showed shell surface layer variations between acid and non-acid exposed oysters. Figure 2.6 is the x-ray spectral profile from a healthy oyster shell. There are noticeable peaks for the elements Ca, Si and Al, and smaller peaks for Ti, Fe, Mo. Figure 2.7 is the x-ray spectral profile for an oyster shell exposed to ASS-affected waters in Fernbank Creek for 7 days. The SEM-EDS spectrum shown in Figure 2.7 has lower counts of all elements shown in Figure 2.6 with the exception of Ca. The high peak of Ca was due to the removal of other elements contained in the surface layers of the shell. Other elements were stripped away by ASS-affected water, resulting in higher x-ray feedback from the residual Ca.

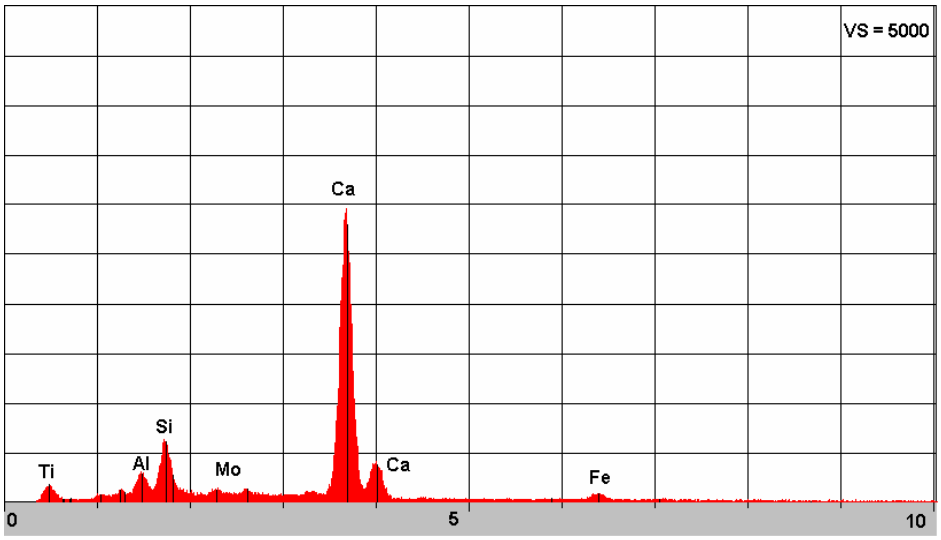


Figure 2.6 SEM-EDS spectrum obtained from the surface layer of the left valve of an oyster that has not been exposed to ASS-affected waters (*Source*: Dove, 1997).

Figures 2.6 and 2.7 confirm that the strong mineral acidity of ASS-affected waters causes shell dissolution. When oysters are exposed to recurrent and prolonged exposure to ASS-affected waters the shell perforates in the anterior of the left valve. This process takes approximately 40 to 60 days and is dependant on the size of the oyster as well as the severity and duration of the exposure. As to be expected, shell perforation occurs first in smaller oysters due to their lower shell density.

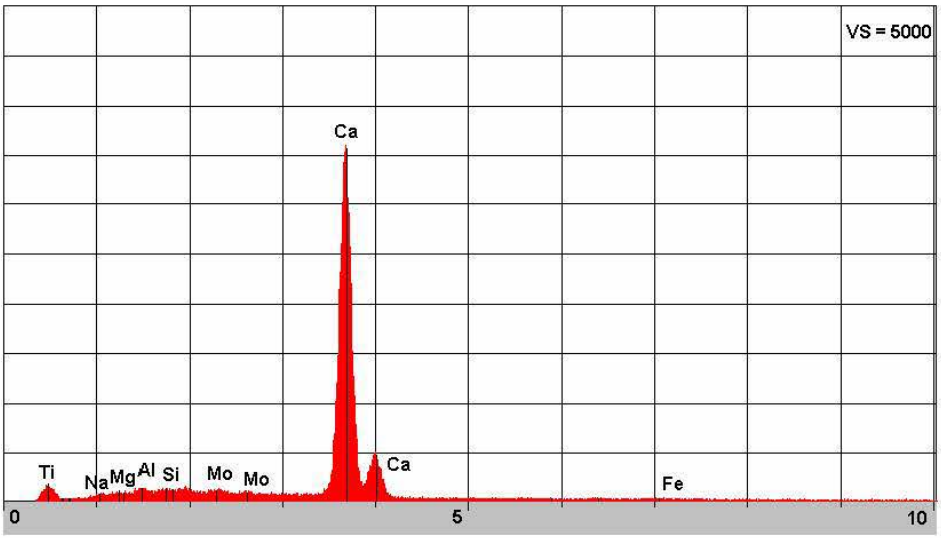


Figure 2.7 SEM-EDS spectrum obtained from the surface layer of the left valve of an oyster exposed to ASS-affected waters for 7 days (*Source*: Dove, 1997).

2.4 DISCUSSION

The pilot investigation detailed in this chapter was the first study to investigate impacts caused by ASS-affected waters on developed Sydney rock oysters. The study showed that under moderate to severe acidity, shell condition is degraded and oysters experience mortality. Additionally, oyster behaviour is altered by changes in pH.

Bamber (1987; 1990) investigated the effects of acidified treatments on oyster survival. Bamber (1987; 1990) found the critical pH for significant mortality after 30 days exposure ranged from 6.6 for *M. edulis* down to 6.0 for *C. gigas*. Bamber (1987; 1990) also described changes in bivalve behaviour when exposed to acidified water including excessive gaping. Based on the results form this pilot investigation and Bamber's (1987; 1990) earlier work, oyster behaviour and survival under acidic conditions will be investigated in greater detail for this present study.

Shell dissolution has been found to occur in other species of bivalves when decreases in pH were only very minor. For example, Kuwatani and Nishii (1969) discovered shell dissolution starts to occur at a pH of 7.6 in the Japanese pearl oyster (*P. fucata*). Bamber (1987; 1990) showed that shell dissolution occurs at a pH of 7.5 in carpet shell clams (*V. decussata*), and at pH 7.0 for the native oyster (*O. edulis*), the Pacific oyster (*C. gigas*) and the native mussel (*M. edulis*). The pH values at which shell dissolution can occur reported by these studies are at least 1,000 times less acid than the conditions in estuaries affected by ASS outflows (Sammut *et al.*, 1996a). It is, therefore, not surprising that shell bleaching and deterioration occurred in the oysters exposed to acid in the field experiment.

A process of internal shell dissolution can also be induced by prolonged exposure to ASS-affected waters. Prolonged valve closure caused by acidified water prevents feeding. In this work it was shown that oysters respond to acidified water by remaining closed for the duration of exposure. The stress induced by the oyster's inability to feed alters the chemistry of the fluid contained within the shell. When valves are closed, the carbon dioxide produced by an oyster decreases the pH of the mantle liquid; to regulate this pH decline in their body fluid, calcium ions from the shell are used by the oyster to buffer the acid (Dwyer and Burnett, 1996). This is described as shell decalcification and it results in internal shell dissolution. This process, when combined with external shell dissolution from acidified water, eventually leads to shell breakthrough in the rear of the left valve of the oyster (Plate 2.1). This part of the shell is thinnest in single seed oysters and is, therefore, the area of the shell that is most susceptible to breakthrough. Once shell perforation has occurred the soft tissue has no protection and is in turn exposed to the ASS-affected waters.

The SEM analysis showed that the external layer of shell, also known as the periostracum, was dissolved by the acid and exposed the underlying shell matrix. Bamber (1987) observed very similar effects in the soft shell clam, *V. decussata*. This protective layer of the shell also includes the pigment of the shell. The loss of this pigmented layer and the effects of acid on the exposed underlying shell matrix explains the shell bleaching observed at all acid impacted experimental sites. SEM-EDS analysis showed the principal elements stripped from the shell and SEM micrographs demonstrate the associated breakdown of the periostracum and the exposure of the smooth underlying shell matrix.

Internal shell dissolution when combined with external shell dissolution has implications for the ability of the oyster to protect its soft tissue from ASS-affected waters. Once shell perforation has occurred, there is no mechanism by which an oyster can protect its soft tissue from the injurious effects of the ASS outflows resulting in the eventual mortality of the oyster.

2.5 CHAPTER SUMMARY

The pilot investigation identified a number of impacts on Sydney rock oysters caused by exposure to ASS-affected waters. This work is limited and shows that more research is needed to better understand these impacts. Therefore, on the basis of results obtained from this pilot investigation it is suggested that further research into the impacts of ASS-affected waters on Sydney rock oysters focus on:

- Sydney rock oyster survival when exposed to different concentrations of ASS-affected waters in the field;
- the effects of ASS-affected waters on Sydney rock oyster growth and condition;
- acid-induced responses of Sydney rock oyster soft tissues and the influence of aluminium and iron; and
- changes to oyster feeding behaviour as a result of lowered pH.

In order to achieve this, field observation and exposure experiments combined with laboratory experiments will be carried out. An understanding of the nature and spatial extent of acidification events in the study areas is also a vital component of this research. Therefore water quality monitoring in the form of continual long-term data

logging combined with discrete *in-situ* measurements of water quality at impacted and non-impacted sites will be carried out.

Section II (Field Investigations) follows this chapter and details the field methods used and the results of water quality investigations and exposure experiments conducted in areas of the estuary impacted by ASS-affected waters.

SECTION II

FIELD INVESTIGATIONS

3 WATER QUALITY OF THE HASTINGS AND MANNING RIVER ESTUARIES

3.1 INTRODUCTION

An understanding of the environmental conditions at oyster leases is important to this study to confirm oyster growers claims that ASS-affected waters impact areas used for oyster production and to gather information on the range of water quality conditions oysters are exposed to.

Department of Land and Water Conservation (DLWC) ASS risk mapping shows that ASS are present in the Hastings and Manning River floodplains and these soils have been extensively drained (Smith, 1999; Figures 3.1 and 3.2). Past studies have confirmed estuarine acidification occurs in the Hastings and Manning River estuaries (Johnston, 1995; Sonter, 1999; Manly Hydraulics Laboratory (MHL), 1997). Additionally, there are abandoned oyster leases in tributaries that are regularly affected by acidification in both of these estuaries. However, ASS-induced water quality changes in oyster producing areas of these estuaries are not well documented as past studies have investigated acidification in discrete areas of the Hastings and Manning Rivers. These factors make the Hastings and Manning Rivers an ideal study area to investigate characteristics of acidification in areas of the estuary used for oyster production and to expose oysters to acidification under realistic conditions.

In this present study, a sampling program was undertaken to measure the extent of acidification in the Hastings River and Manning River catchments to select experimental sites and to ascertain if recurrent production problems were related to acid events. Measurements were made at drain outflow locations and in the centre of the main channels of both estuaries following heavy rainfall. A long-term water quality study was also conducted in the lower Hastings River/Limeburners Creek area. This part of the estuary is responsible for the majority of oyster production in the Hastings River estuary and has experienced recurrent oyster production problems including poor oyster health, slow growth and high mortalities for over a decade.

Areas impacted by acidification in the Hastings River estuary include the Maria River, Connection Creek, Pipers Creek and Fernbank/Partridge Creek (Johnston, 1995). Acidic conditions have been measured in the Manning River estuary in the Pipeclay/Cattai Creek area, the Lansdowne River/Ghinni Ghinni Creek area and North Oxley Island (Lawrie, 1996; Webb, McKeown and Associates, 1997; Silcock, 1998; Sonter, 1999). Sonter (1999), in a related study, investigated the spatial characteristics of estuarine acidification in Cattai Creek. This study concluded that acidification was a major problem within Cattai Creek and contributed pyrite oxidation products to downstream reaches of the Manning River.

The Manning River water quality data presented in this chapter were collected in collaboration with a related project (Smith and Dove, 2001), which investigated drain management options on North Oxley Island, Taree, and areas affected by acidification in the Manning River.

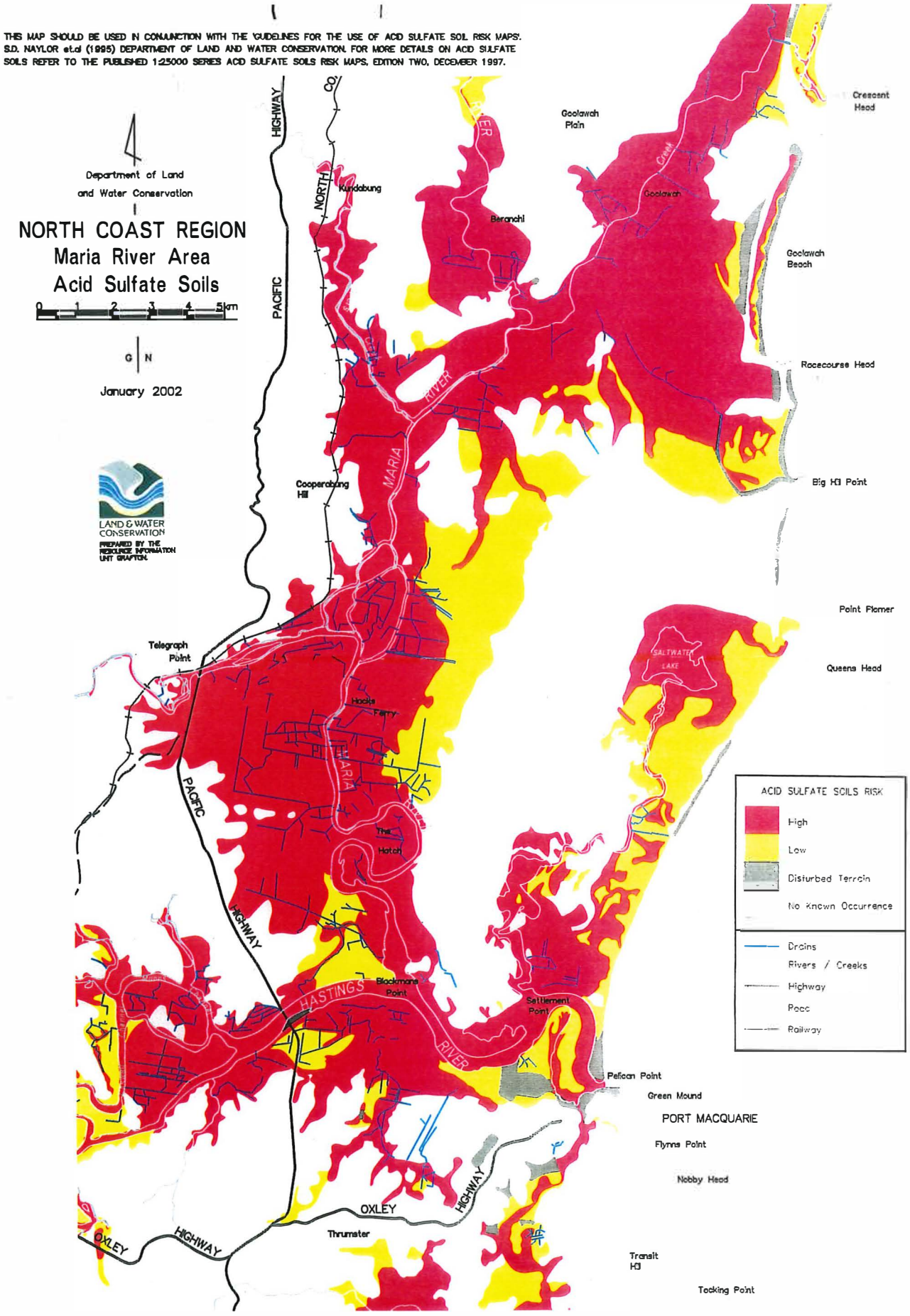


Figure 3.1 Distribution of ASS in the Hastings River region (*Source*: DLWC Resource Information Unit, Grafton).

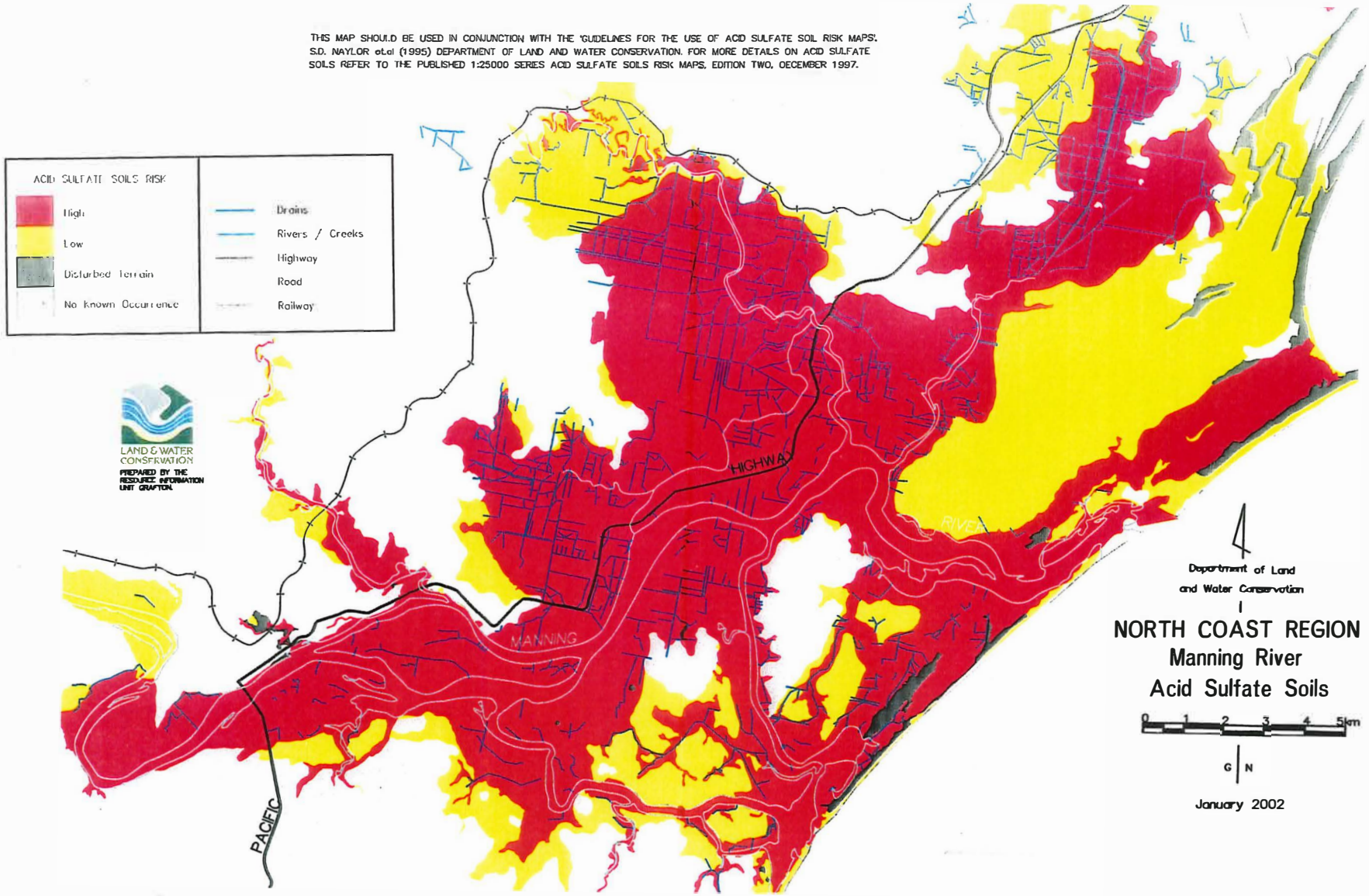


Figure 3.2 Distribution of ASS in the Manning River region (*Source*: DLWC Resource Information Unit, Grafton).

The purpose of this chapter was three-fold: firstly, to characterise estuarine acidification in the two study areas to understand the range of water quality conditions oysters can potentially be exposed to; secondly, to obtain data and conduct field observations that enables selection of reference and exposure sites for field experiments outlined in Chapter 4 and to design laboratory experiments detailed in Chapter 6; and, thirdly, to investigate water quality conditions in Limeburners Creek to determine if acidification is associated with poor oyster production in this area.

3.2 WATER QUALITY MONITORING OBJECTIVES

The specific objectives of water quality monitoring for this study were:

- to characterise estuarine acidification in both study areas,
- to obtain water quality data that will be useful for the selection of experimental field sites and design of the laboratory experiments used in the latter chapters of this study; and,
- to investigate water quality in an area of the estuary recurrently impacted by oyster production problems.

3.3 METHODS

This section details the methods used to collect water quality information and shows the locations of water quality monitoring sites in the Hastings and Manning Rivers.

3.3.1 Water Quality Monitoring Techniques and Analyses

A Yeo-Kal Intelligent Water Quality Analyser calibrated with certified, standard solutions was used for all *in situ* field measurements of water temperature, pH, EC and DO. Surface measurements were made at a depth of 0.1 m and bed measurements were taken 0.2 m above the bottom substrate. A Palin Test Photometer (Model 5000) was used to measure alkalinity of selected water samples in the field.

Water samples requiring laboratory analysis were collected in acid-washed 0.5 L plastic containers and filtered through 0.22 μm cellulose nitrate filter paper to remove particulates and colloids before sample preservation. Surface water samples were 'gulp' sampled and bed water samples were collected with a train of three biological-oxygen-demand bottles in series (Boyd, 1979). Samples were analysed for Na, K, Mg, Ca, Si, Zn, Cu, B, S, Al, Fe, and Mn using Inductively Coupled Plasma Atomic Excitation Spectroscopy (ICPAES). Sulfate was determined using a modified version of the Turbidimetric Method (APHA, 1998). The Potentiometric Method (APHA, 1998) was used to determine chloride concentration. Total metal concentrations in bioassay water quality samples was analysed using the Nitric Acid Digestion Method detailed in APHA (1998).

Continuous time series water quality measurements of pH, EC and temperature were collected using a Greenspan Technical Services Smart Sonde (Model SD300) SDL or a Yeo-Kal 611 Intelligent Water Quality Analyser SDL. SDLs monitored water quality for 2 to 3 week periods before they were retrieved for data downloads, probe inspection and recalibration using standard, certified solutions. EC and pH were the principal variables reported on in this study and the results of other measured variables were selectively used.

3.3.2 Estuarine Acidification and Water Quality Measurements

To determine the spatial extent of estuarine acidification in the Hastings and Manning estuaries, opportunistic centre-channel transects and drain water quality monitoring was carried out after high rainfall events in the lower catchment areas. Sampling was conducted during the final stages of the ebb tide. The sampling sites and dates for each estuary are detailed in the following sections.

3.3.2.1 Hastings River Sampling Sites and Dates

Smith (1999) identified major drains on the Hastings River floodplain. Hastings River drain locations are displayed on Figures 3.1 and 3.3. A field inspection of drains displayed in these figures was undertaken to establish if the drains contained acidic water or displayed characteristics of previous acid water outflows (e.g. iron precipitate coating or corroded concrete on the floodgate structure). Drains identified as likely sources of acid outflows were sampled on the 18-19/6/99, 29-30/11/99, 1-2/12/00 and 12-13/2/01. Surface water quality measurements were taken at drain locations. In addition to drain measurements, centre-channel surface and bed water quality measurements of pH and EC were recorded in the Hastings River, Maria River, Pipers Creek and Connection Creek (Figure 3.3) on the 18/6/99, 29/11/99, 1/12/00 and 13/2/01.

Drains with floodgate structures were sampled on the downstream (estuary) side where accessible and a water sample was collected and preserved for laboratory analysis in instances where outflow waters were very acidic (~ pH < 4). Drains were identified by the coding system used in Smith (1999). This system assigned each drain a code based on the initials of the tributary name, followed by the distance upstream from the river entrance and ended with the letter "L" or "R" to denote whether the drain was situated on the left or right bank (Smith, 1999). For example, a drain located on the left bank of the Maria River, 45 kilometres from the Hastings River entrance would be coded as: MR45.0L. Centre-channel water quality measurements were made at the same sites on all four sampling occasions and were coded by the initials of the tributary name, followed by the distance upstream from the Hastings River entrance in a similar way to drain sites. Water quality sampling sites are displayed in Figure 3.3. Results of the water quality sampling on the Hastings River estuary are detailed in Section 3.4.2.

3.3.2.2 Manning River Sampling Sites and Dates

Limited information existed on the location of potentially acidic drains with floodgates on the lower Manning River floodplain at the time of this study. Topographical and ASS risk maps were used to identify drain outflow locations that were potentially acidic. Sampling of the Manning River was conducted on the 27/5/98 and 9/5/99.

Centre-channel surface and bed water quality measurements of pH and EC were recorded in Cattai Creek, Lansdowne River, Ghinni Ghinni Creek and Dickensons Creek (Figure 3.4). Drains with floodgate structures were surface sampled on the downstream (estuary) side where accessible and a water sample was collected and preserved for laboratory analysis when ASS-affected waters were very acidic (~ pH < 4).

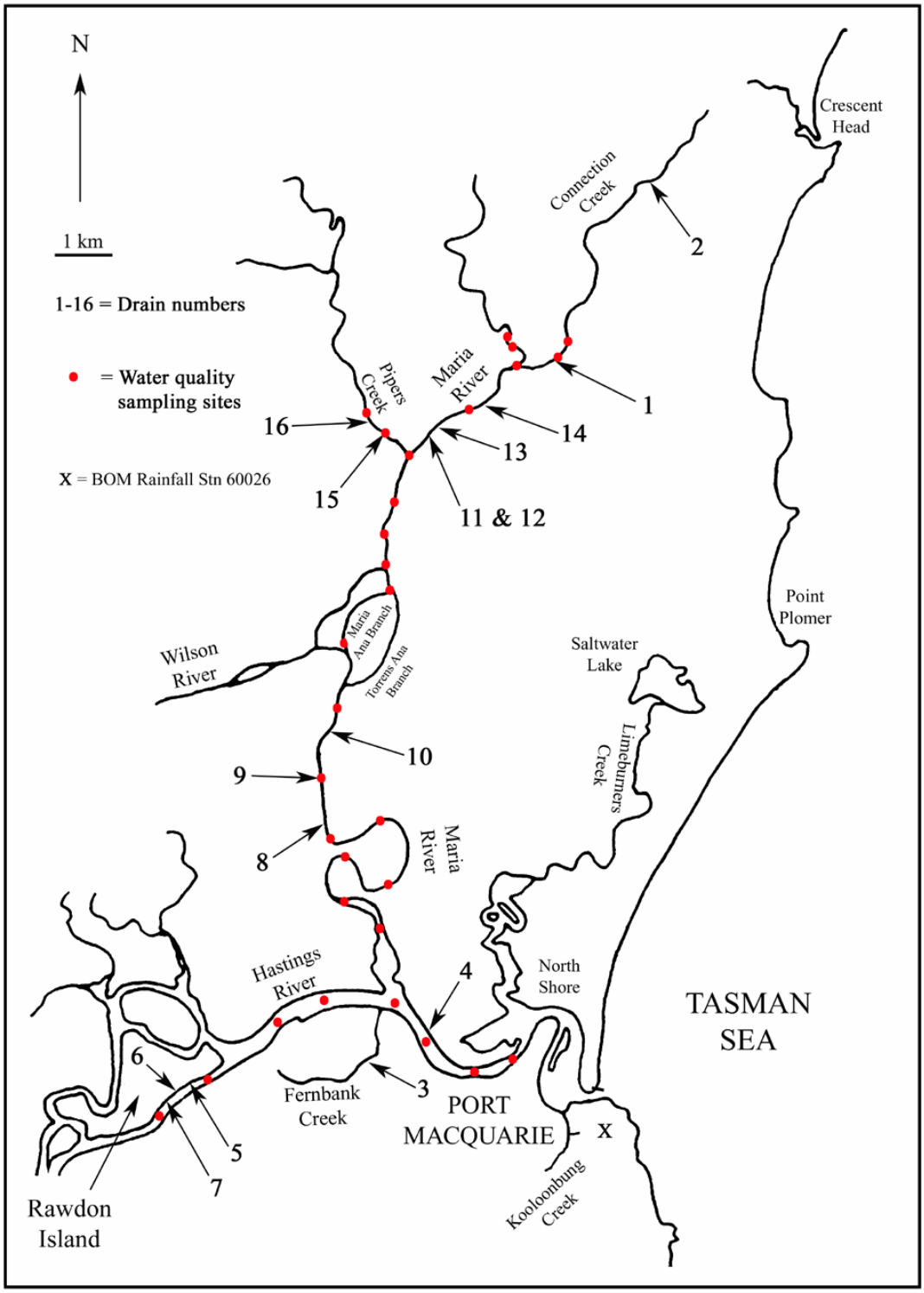


Figure 3.3 The Hastings River estuary and water quality sampling locations. Numbers 1 to 16 show the locations of drains listed in Table 3.1.

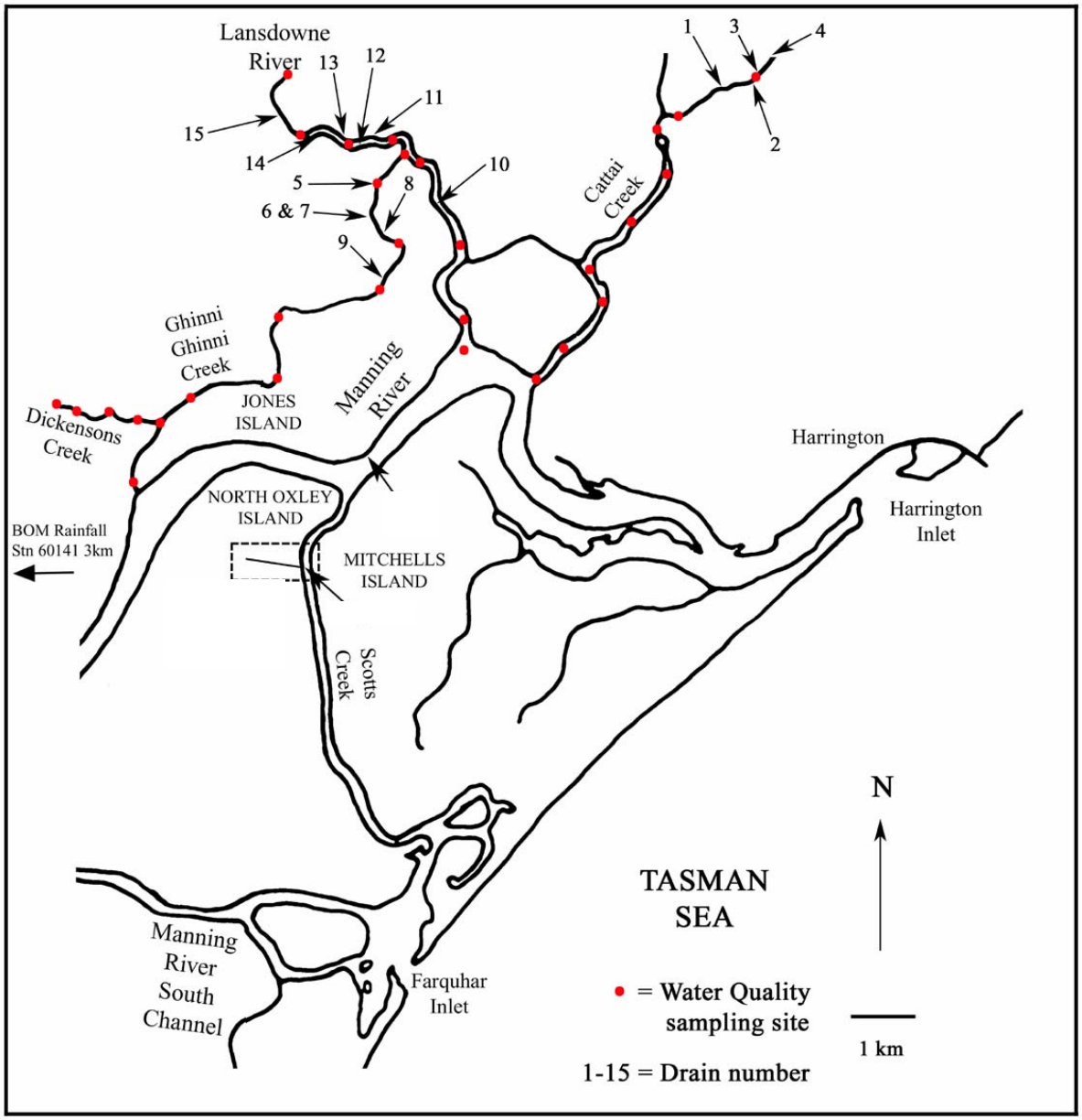


Figure 3.4 The Manning River estuary and water quality sampling locations. Numbers 1 to 15 show the locations of drains listed in Table 3.2.

All drains monitored were coded using the methodology applied to Hastings River drains detailed in Smith (1999) and explained in Section 3.2.3.1. Likewise, in-channel sampling locations on the Manning River were coded using the initials of the tributary, followed by the distance upstream from the Manning River entrance at Harrington. Drain and channel water quality sampling sites are displayed on Figure 3.4.

3.3.2.3 Water Quality Investigation of Limeburners Creek

An objective of this study was to identify risks to Sydney rock oyster production. Lake (1997) raised several hypotheses requiring further investigation in the lower Hastings River area. Specific hypotheses relate to landuse in areas adjacent to Limeburners Creek, freshwater inflows into Limeburners Creek and oyster cultivation practices resulting in oyster kills. Therefore, an investigation of water quality conditions in the lower Limeburners Creek and Hastings River was undertaken to determine if noticeable changes in water quality was associated with oyster production problems. Regular water quality transects measuring pH, EC, temperature and DO of surface and bed waters at 19 locations (Figure 3.5) were conducted in Limeburners Creek and Hastings River to investigate variations in water quality conditions and estuarine acidification.

The sampling period was from 17/11/97 to 30/3/99. Sampling was conducted on the following dates: 17/11/97; 4/12/97; 20/3/98; 25/3/98; 27/3/98; 2/4/98; 17/4/98; 27/4/98; 4/5/98; 15/5/98; 2/6/98; 5/6/98; 21/7/98; 31/7/98; 10/8/98; 17/8/98; 31/8/98; 15/9/98; 1/10/98; 16/10/98; 9/11/98; 7/12/98; 25/1/99; 4/2/99; 4/3/99; and, 30/3/99 (Figure 3.8). A Greenspan Technical Services SDL measuring pH, EC and temperature was deployed at Site A (Figure 3.5) to provide long-term, continuous time series data at a depth of 0.5 m for the periods: 6/11/97 to 2/12/97; 4/12/97 to 28/12/97; 9/4/98 to 25/5/98; and, 11/8/98 to 31/8/98.

Water quality measurements were made using the Yeo-Kal 611 Intelligent Water Quality Analyser in the centre of the main channel of the Hastings River and Limeburners Creek adjacent to areas where Sydney rock oysters were cultivated. At four sites (Sites 1, 4, 12 and 19) a surface and bed water quality sample was collected for laboratory analysis on each sampling date. The Greenspan Technical Services Smart Sonde Model SD300 was deployed in an area of intensive oyster cultivation to provide continuous time series data at a depth of 0.5 m for periods during the study.

The water quality sampling locations are numbered 1 to 19 and are shown in Figure 3.5. Approximately 10,000 oysters were placed at three oyster lease sites that had reported production problems. Sites A and B were located in Limeburners Creek and Site C was in Big Bay (Figure 3.5). Oysters were inspected fortnightly between the 6/11/97 to 31/7/98 to detect an oyster kill. Approximately 3,000 oysters were placed into 10 covered, plastic trays and randomly distributed on intertidal racks at Sites A, B and C. No atypical oyster mortality was observed during the water quality sampling period. However, an oyster kill did occur in September 2000 and details are included in Chapter 5 of this present study.

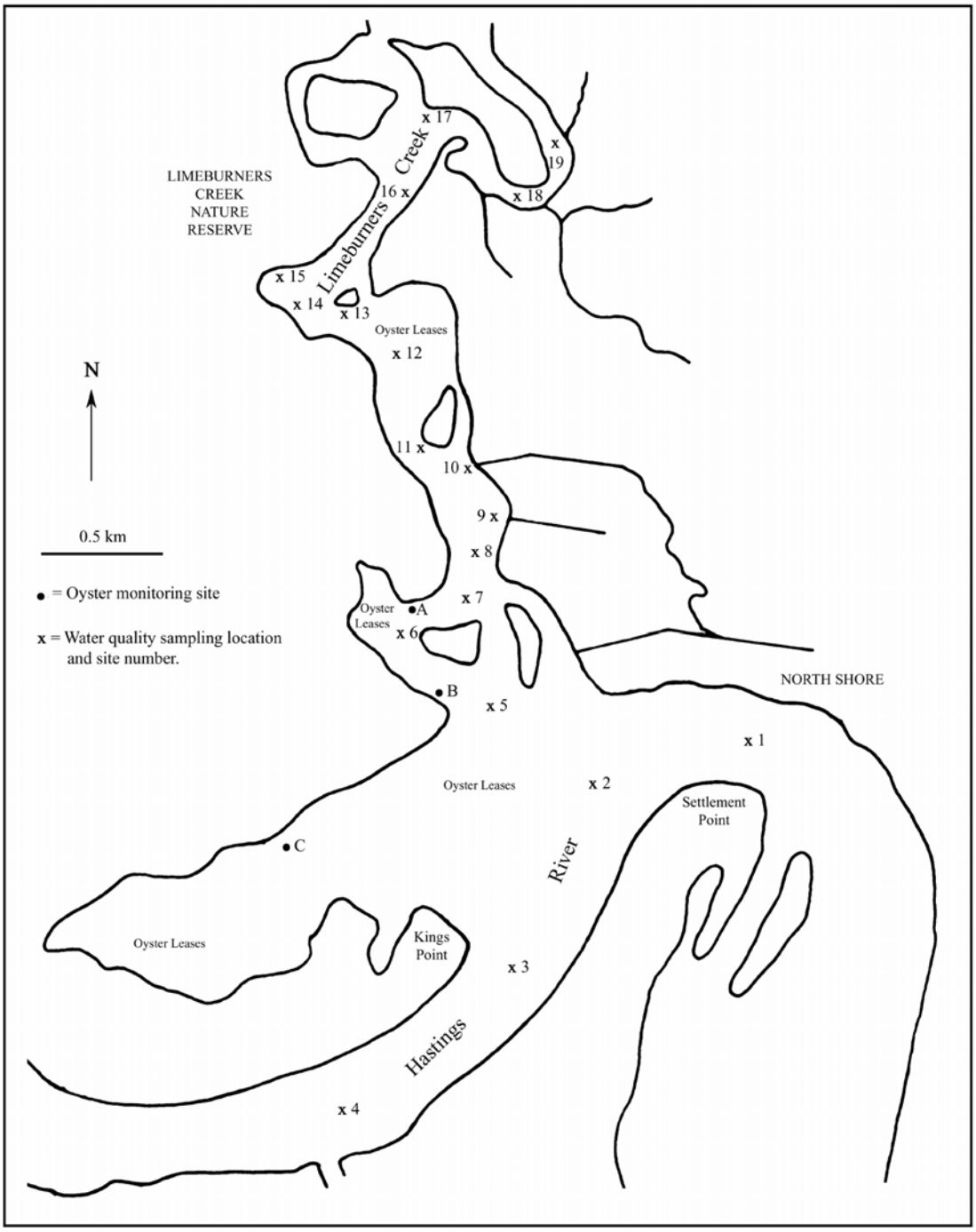


Figure 3.5 Map of the lower Hastings River and Limeburners Creek showing water quality monitoring sites.

3.4 RESULTS

3.4.1 Rainfall

Heavy rainfall flushes ASS oxidation products from the floodplain and into estuaries (Sammut *et al.*, 1996a; Johnston, 1995). Therefore, rainfall information is important to this study. Rainfall data for this section were obtained from the Bureau of Meteorology (Station Numbers 60026 and 60141) and were collected at Hill Street, Port Macquarie (Figure 3.3) and Taree Airport (Figure 3.4). These rainfall stations were considered the most representative for the study areas. Rainfall information related to acidification of the Hastings and Manning River are displayed in Figure 3.6 and 3.7, respectively. The rainfall information relevant to the lower Hastings River and Limeburners Creek water quality sampling is displayed in Figure 3.8.

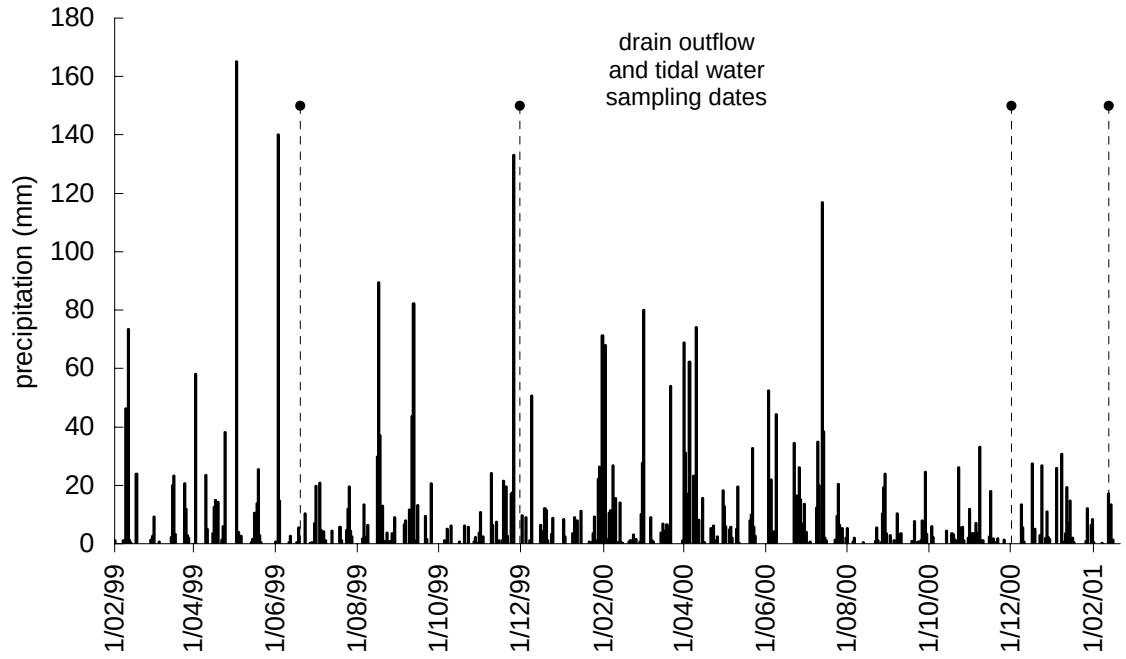


Figure 3.6 Rainfall recorded at Port Macquarie for the period 1/2/99 to the 16/2/01 (*Source*: Bureau of Meteorology Station Number 60026) and tidal water and drain outflow sampling dates.

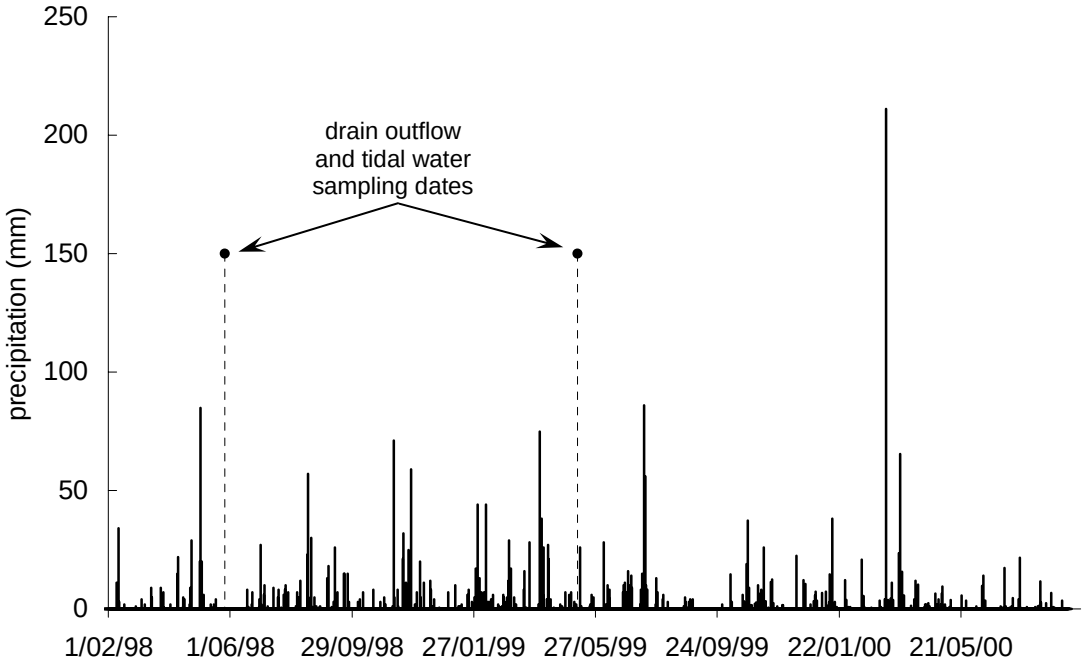


Figure 3.7 Rainfall recorded at Taree Airport for the period 1/2/98 to the 31/5/99 (*Source*: Bureau of Meteorology Station Number 60141) and water sampling dates.

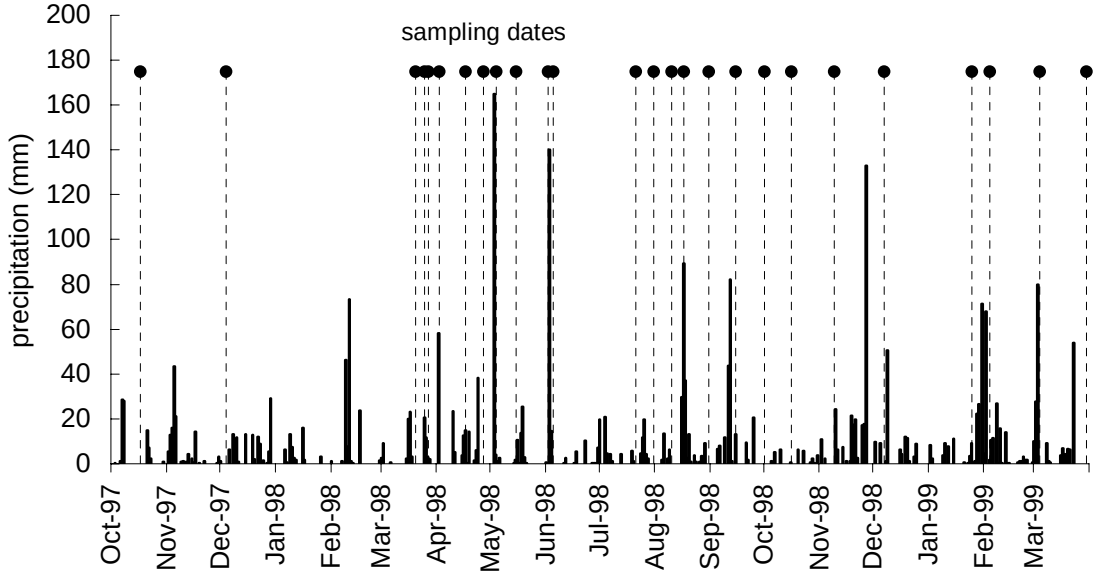


Figure 3.8 Rainfall for the lower Hastings River and Limeburners Creek (*Source*: Bureau of Meteorology Station Number 60026) and sampling dates for the study period.

3.4.2 Hastings River Estuary

3.4.2.1 Drain Outflow Water Quality Following High Rainfall

Table 3.1 presents pH, EC, iron, aluminium, manganese, silicon, zinc and $Cl:SO_4$ data for selected drains that outflow into tributaries of the Hastings River. The concentrations of iron, aluminium, manganese, silicon and zinc are presented in this table because these dissolved species are commonly measured in ASS-affected waters at elevated concentrations (Sammut *et al.*, 1996a; Sonter, 1999).

Drain outflow water quality data presented in Table 3.1 shows that strongly acidic water containing elevated concentrations of metals flows into the Hastings River estuary following high rainfall events. The pH data of drains discharging into the Hastings and Maria Rivers collected during the four sampling occasions are also displayed on Figures 3.9 and 3.10, respectively as drain outflow pH. Drain water quality measurements collected on the four sampling occasions are included in Appendices 3A and 3B.

Table 3.1 Water quality of selected Hastings River estuary drain outflows. For location of drains refer to Figure 3.3.

Drain No.	Drain ID	Date	pH	EC (dS m^{-1})	Fe (mg L^{-1})	Al (mg L^{-1})	Mn (mg L^{-1})	Si (mg L^{-1})	Zn (mg L^{-1})	$Cl:SO_4$
Connection Creek										
1	CC38.4R	13/02/01	3.19	0.9	7.75	2.27	0.38	6.39	0.10	1.1
2	CC44.1R	13/02/01	3.58	1.0	36.70	1.37	0.53	6.52	0.10	0.5
Fernbank Creek										
3	FC11.6L	13/02/01	3.28	1.3	35.90	1.84	0.40	10.30	0.06	1.3
Hastings River										
4	HR08.1R	13/02/01	4.10	1.1	12.20	0.46	0.16	4.60	0.03	3.8
5	HR16.0R	12/02/01	2.81	5.9	48.10	9.53	1.45	15.80	0.23	1.6
6	HR16.5R	18/06/99	3.37	2.7	4.03	8.34	1.22	12.15	ND	2.8
7	HR16.8R	12/02/01	3.48	8.8	33.90	19.40	2.30	17.90	0.47	4.4
Maria River										
8	MR21.7L	01/12/00	3.19	1.6	1.27	7.37	0.58	8.98	0.11	2.4
9	MR23.0L	30/11/99	3.64	3.5	2.54	2.73	0.66	5.28	ND	3.6
10	MR24.2R	12/02/01	3.83	3.2	0.98	2.19	0.34	6.44	0.05	3.0
11	MR33.8R(A)	13/02/01	3.06	1.9	8.02	5.34	0.56	5.84	0.13	1.0
12	MR33.8R(B)	02/12/00	2.77	2.4	11.40	4.43	0.57	2.18	0.12	1.1
13	MR34.1R	02/12/00	3.20	2.4	3.22	20.70	1.47	3.65	0.25	0.9
14	MR35.5R	02/12/00	2.91	5.3	15.30	2.08	0.31	5.51	0.05	2.6
Pipers Creek										
15	PC34.5L	12/02/01	3.47	1.1	2.80	1.58	0.36	4.36	0.06	2.1
16	PC34.7L	18/06/99	4.29	0.7	0.32	1.60	0.13	10.10	ND	2.2

ND = Not detected

All drains listed in Table 3.1, with the exception of HR16.8R have a low pH combined with a $Cl:SO_4$ ratio of less than 4 indicating that the outflow water has originated from oxidised pyrite contained in the drained floodplain soils. A $Cl:SO_4$ ratio of less than 4 and pH values less than 4 indicate mineral acidity rather than naturally occurring humic acids due to the release of sulfate during pyrite oxidation (Mulvey, 1993). Sulfate, released from pyrite oxidation, reduces the $Cl:SO_4$ although secondary acidification from metal hydrolysis may also drive the pH down (Sammut *et al*., 1996b).

The drain coded as HR16.8R has extremely high concentrations of aluminium (19.40 mg L^{-1}), iron (33.90 mg L^{-1}), manganese (2.30 mg L^{-1}) and zinc (0.47 mg L^{-1}). These concentrations exceed the threshold values of the Australian and New Zealand Environment and Conservation Council (ANZECC) guidelines for the maintenance of water quality for biological systems (ANZECC, 2000). This drain flows into the main channel of the Hastings River at a point where the channel is wide and deep and drainage density is low. Therefore acidic flows would be quickly neutralised by estuarine waters when ECs are high or diluted when the river has low ECs.

The drainage density and drainage volume are high in the upper reaches of the Maria River (> 20 km upstream from the Hastings River entrance) and Connection Creek. The artificial drains in these areas enable more efficient export of large quantities of fresh, acidic water containing elevated concentrations of iron and aluminium into the main channel.

3.4.2.2 Tidal Water Quality Following High Rainfall

The pH and EC data collected in the Hastings River and Maria River were plotted against distance upstream from the Hastings River entrance (Figures 3.9 and 3.10). The pH measured at major drain outflow locations in Hastings River and Maria River were also plotted on Figures 3.9 and 3.10.

Water pH and EC decrease with increasing distance upstream in the Hastings River channel (Figure 3.9). This decrease in pH and EC is attributable to neutral, poorly-buffered, freshwater inflows from the upper catchment in combination with acidic outflows from floodplain drains 15-17 kilometres upstream from the Hastings River entrance (Figure 3.9). The pH drops below neutral in the surface waters at the centre of the main channel close to the drain outflow locations.

Figure 3.10 shows very low EC values present in the Maria River system following the June rainfall event due to the dominance of floodwaters in the system. The decrease of pH in the main channel of the Maria River is pronounced and is caused by acidic water discharging from the numerous drains on the Maria River floodplain (Figure 3.1). Additional tidal water data collected on the four sampling occasions are listed in Appendix 3C.

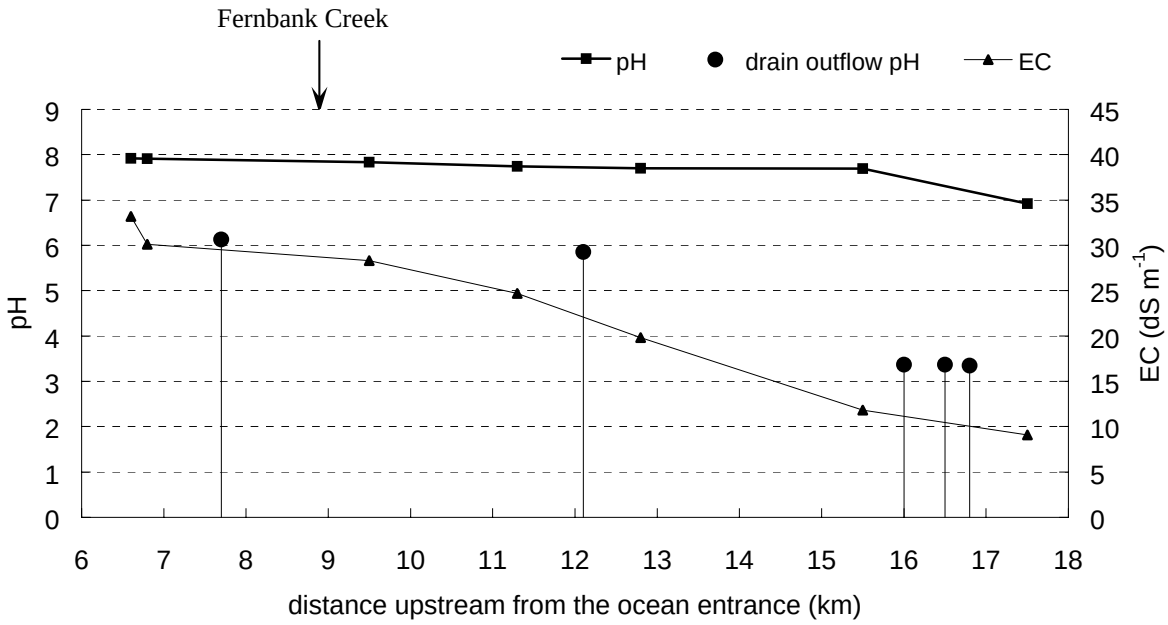


Figure 3.9 Hastings River surface water pH, EC and drain outflow pH measured on the 18 and 19/6/99.

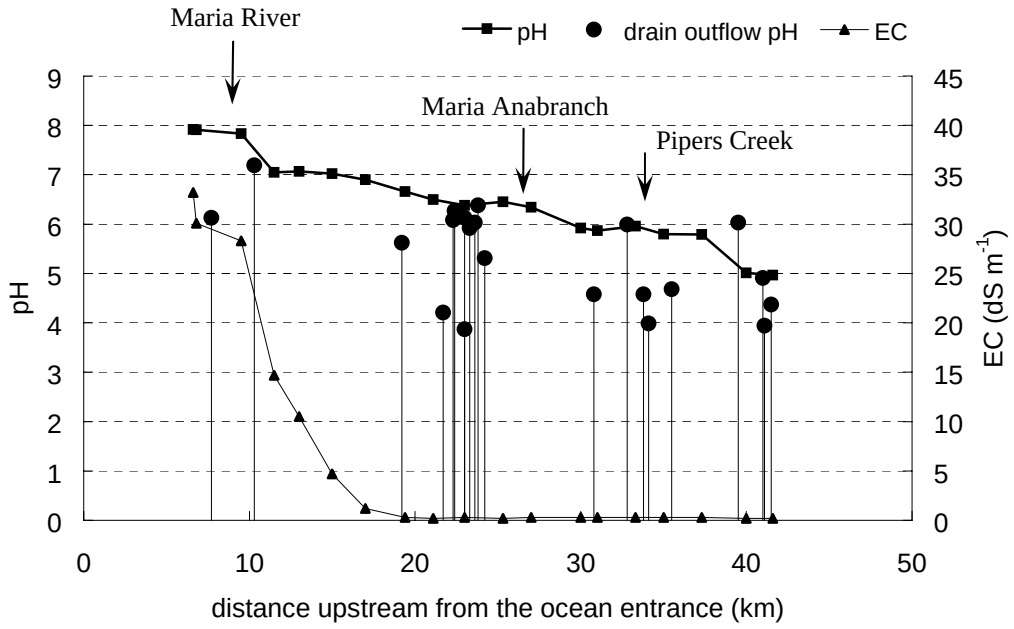


Figure 3.10 Hastings/Maria River surface water pH, EC and drain outflow pH measured on the 18 and 19/6/99.

3.4.2.3 Metal Precipitate Distribution

The extent to which iron and aluminium precipitates were distributed in the system was observed during each sampling date and on other occasions following rainfall. The primary sources of iron flocs entering the main channel of the Hastings River were from Fernbank Creek, Maria River, drains located on Rawdon Island (Figure 3.3) and drain HR08.1R (Table 3.1). Aluminium flocs were commonly observed in Fernbank Creek outflows.

Iron flocs contaminated the near-shore downstream reach of the Hastings River for a distance of 0.2 km at Rawdon Island (Figure 3.3) and 2.5 km downstream of Fernbank Creek after high rainfall. Similarly, iron flocs originating from drains in the Maria River smothered the northern bank of the Hastings River for a distance of 6.1 kilometres from the Hastings River ocean entrance on the 20/3/01 (the Hastings River Maria River confluence is located 9.5 kilometres upstream from the ocean entrance). This occurred after a flood event where 187 mm of rainfall was recorded in 10 days.

Iron flocs were transported distances greater than 15 kilometres from their source. Similar observations have been made on the Richmond River in northern NSW, where plumes of neutral but iron contaminated water affects the main channel of the river for 3 km downstream of the source (Sammut *et al*., 1996a). Plates showing the extent of metal precipitate mobilisation are provided in Section 3.5.

3.4.3 Manning River Estuary

3.4.3.1 Drain Outflow Water Quality Following High Rainfall

Figure 3.2 displays drains that intersect high-risk ASS in the lower Manning River catchment. The pH, EC, iron, aluminium, manganese, silicon, alkalinity and $Cl:SO_4$ data for selected drains in the Manning River estuary sampled on the 27/5/98 and 9/5/99 are listed in Table 3.2. Table 3.2 shows that low pHs and elevated metal concentrations are present in drain outflow water on the two sampling dates which were conducted following high rainfall. Drains sampled on the 27/5/98, typically have higher EC values yet the pH and metal concentrations of all listed drain outflows on this date exceed recommended guidelines as stipulated in ANZECC (2000).

The $Cl:SO_4$ ratios listed in Table 3.2 indicate that the drain outflow waters on the 9/5/99 have interacted with oxidised sediments in the floodplain (Mulvey. 1993). Low pH drain outflows containing elevated metal concentrations are originating from the Cattai-Pipeclay area and the lower Lansdowne-Moto-Ghinni Ghinni Creek area. These two areas are listed as ASS priority management areas on the lower Manning River floodplain in Tulau (1999).

Table 3.2 Water quality of selected Manning River drain outflows measured on the 27/5/98 and 9/5/99. For the locations of drains refer to Figure 3.4.

Drain No.	Drain ID	Date	pH	EC (dS m^{-1})	Fe (mg L^{-1})	Al (mg L^{-1})	Mn (mg L^{-1})	Si (mg L^{-1})	$Cl:SO_4$	ALK (mg L^{-1})
Cattai Creek										
1	CC15.7L	27/05/98	4.11	15.2	0.14	1.53	ND	2.55	6.82	13
2	CC16.1L	09/05/99	3.55	1.1	4.90	1.05	0.24	2.65	6.57	NS
3	CC16.5L	09/05/99	3.04	1.8	9.82	17.71	2.13	12.83	2.10	NS
4	CC16.9L	27/05/98	3.87	10.9	ND	2.64	0.06	1.85	24.32	8
Ghinni Ghinni Creek										
5	GG15.0R	09/05/99	3.46	1.5	4.02	1.74	0.85	6.47	5.17	NS
6	GG15.4R	27/05/98	3.58	21.2	0.32	5.11	0.57	3.10	9.74	21
7	GG15.5R*	27/05/98	3.21	22.3	2.17	11.28	1.41	6.76	7.79	8
8	GG15.8L	09/05/99	3.31	11	2.71	2.84	2.57	12.00	4.21	NS
9	GG16.6R*	27/05/98	3.45	15.4	2.82	32.57	4.03	16.07	5.19	8
Lansdowne River										
10	LR13.1L	09/05/99	3.22	10.2	4.74	2.14	1.10	9.05	2.97	NS
11	LR15.1R	09/05/99	3.2	3.6	38.40	3.38	1.00	8.29	3.05	NS
12	LR15.4L	09/05/99	3.07	7.6	3.09	0.76	0.43	5.91	3.31	NS
13	LR15.9R	09/05/99	2.97	9.3	4.83	5.35	1.06	10.67	1.47	NS
14	LR16.1L*	27/05/98	3.47	15	2.87	9.62	0.99	7.87	6.74	8
15	LR16.6L*	27/05/98	4.91	15.3	3.37	7.04	1.18	12.36	7.44	0

* = Drain measurement collected upstream from floodgate
ND = Not detected
NS = Not sampled

3.4.3.2 Tidal Water Quality Following High Rainfall

The pH and EC data collected in Cattai Creek and Lansdowne River are displayed in Figures 3.11 and 3.12, respectively. The pHs of drain outflows in Cattai Creek, and Lansdowne River are also plotted on these figures as drain outflow pH.

Figure 3.11 shows poor water quality conditions in Cattai Creek on the 9/5/99. The surface water is increasingly acidic and fresh upstream from the junction with the Manning River. The four drains discharging into Cattai Creek in the upstream section of this transect contain acidic water which has a pronounced influence on the channel pH and EC values. The drain coded as CC16.5L has a pH of 3.04 and very high concentrations of aluminium (17.71 mg L^{-1}), iron (9.82 mg L^{-1}) and manganese (2.13 mg L^{-1}) (Table 3.2). Appendix 3D lists the water quality data of drains discharging into Cattai Creek on the 27/5/98 and 9/5/98.

Surface water pH and EC as well as drain pH data for the Lansdowne River on the 9/5/99 are shown in Figure 3.12. The surface water is increasingly acidic and fresh upstream from the junction with the Manning River to approximately 15 kilometres from the Manning River entrance. The minimum pH and EC measured in the Lansdowne on the 9/5/99 was 6.01 and 8.7 dS m^{-1}, respectively. Figure 3.12 indicates that floodplain drain outflows, 15-16 kilometres upstream from the Manning River entrance, reduces the pH and EC of surface waters in Lansdowne River. The drains

are very acidic (pH < 4.0) and contain elevated concentrations of aluminium, iron and manganese (Table 3.2 and Appendix 3D).

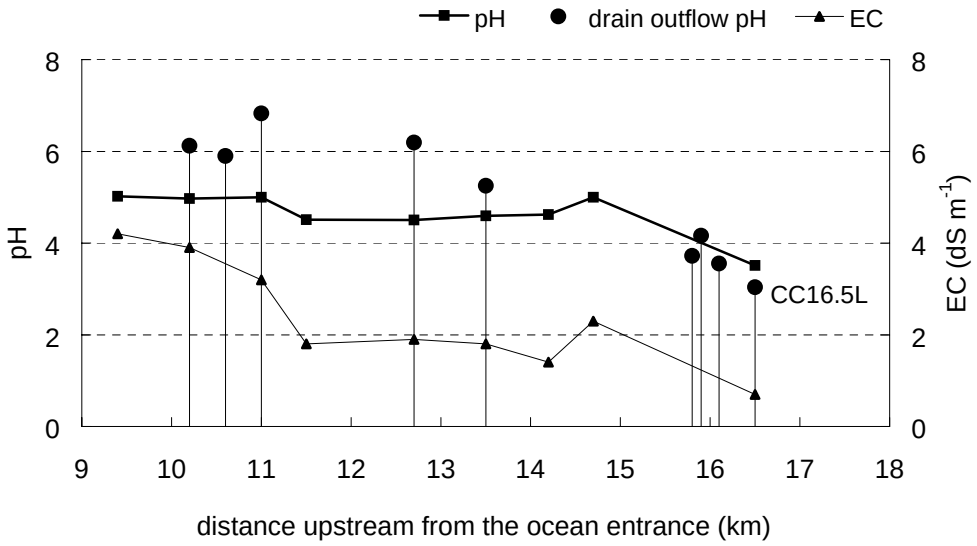


Figure 3.11 Cattai Creek surface water pH, EC and drain outflow pH measured on the 9/5/99.

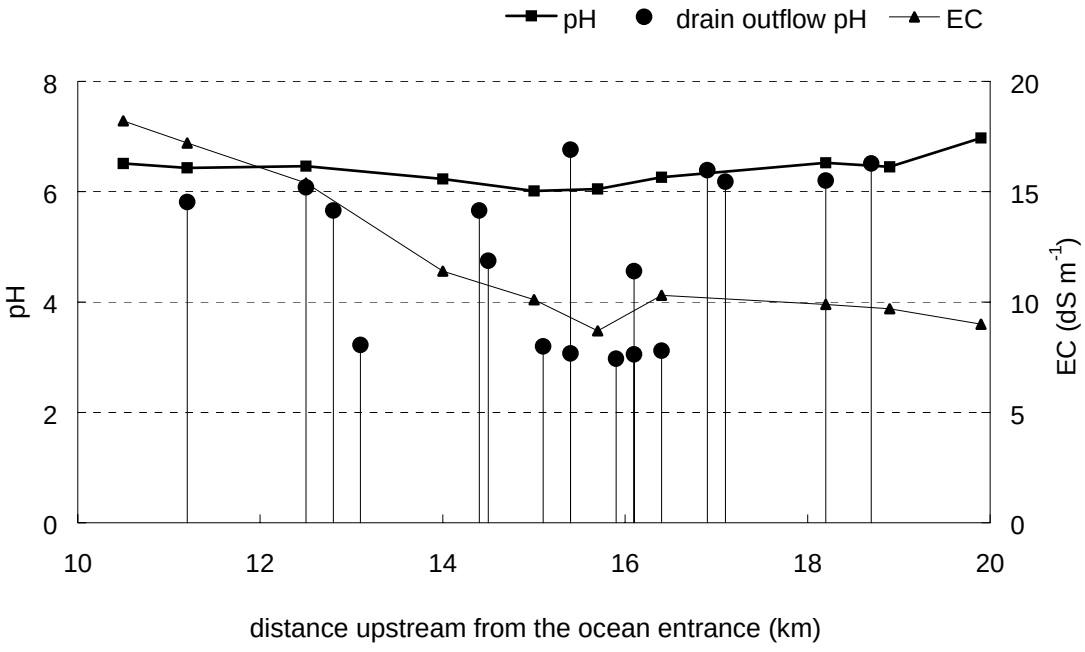


Figure 3.12 Lansdowne River surface water pH, EC and drain outflow pH measured on the 9/5/99.

Surface water pH and EC as well as drain pH data for the Lansdowne River on the 9/5/99 are shown in Figure 3.12. The surface water is increasingly acidic and fresh upstream from the junction with the Manning River to approximately 15 kilometres from the Manning River entrance. The minimum pH and EC measured in the Lansdowne on the 9/5/99 was 6.01 and 8.7 dS m^{-1}, respectively. Figure 3.12 indicates that floodplain drain outflows, 15-16 kilometres upstream from the Manning River entrance, reduces the pH and EC of surface waters in Lansdowne River. The drains were very acidic (pH < 4.0) and contained elevated concentrations of aluminium, iron and manganese (Table 3.2 and Appendix 3D).

The pH and EC data collected in Ghinni Ghinni Creek and Dickensons Creek are displayed in Figures 3.13 and 3.14, respectively. The pHs of drain outflows into Ghinni Ghinni Creek are also plotted on Figure 3.13 as drain outflow pH. Ghinni Ghinni Creek connects the Lansdowne River to the Manning River creating Jones Island (Figure 3.4). The rapid increase in pH to normal estuarine conditions at the > 21 km point (Figure 3.13) is due to the mixing and neutralising effects of the main channel tidal waters. The appearance of the water in Ghinni Ghinni Creek on this date was a milky green/blue colour from the presence of suspended aluminium flocs, which are precipitated by the neutralisation process.

Dickensons Creek was another tributary of the Manning River affected by acidification on the 9/5/99 (Figure 3.14). The surface waters of the entire creek were acidified below pH 6.16. The minimum pH and EC measured in Dickensons Creek on the 9/5/99 was 3.95 and 9.4 dS m^{-1}, respectively. Dickensons Creek water also had a milky green/blue appearance from elevated concentrations of suspended aluminium flocs. Additional water quality data collected from acidified Manning River tributaries on the 27/5/98 and 9/5/99 are presented in Appendix 3E.

3.4.3.3 Metal Precipitate Distribution

The extent to which iron and aluminium precipitates were distributed through the system was recorded during both sampling dates and on other occasions following high rainfall. The main sources of iron and aluminium flocs entering the main channel of the Manning River were from Cattai Creek, Lansdowne River, Ghinni Ghinni Creek and a drain located on North Oxley Island (Figure 3.4). Flocs from Cattai Creek were observed extending for distances in excess of 1 kilometre downstream of its confluence with the Manning River. Flocs from the Lansdowne River extended more than 700 metres and flocs from Ghinni Ghinni Creek extended more than 500 metres from the confluence of each system and the Manning River. Plates showing the extent of metal precipitation are provided in Section 3.5.

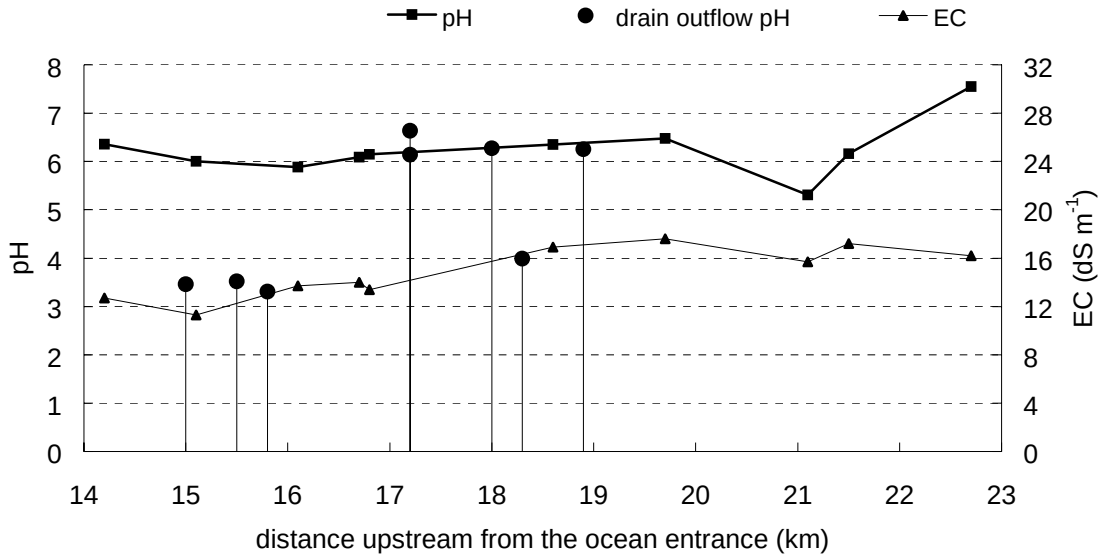


Figure 3.13 Ghinni Ghinni Creek surface water pH, EC and drain outflow pH measured on the 9/5/99.

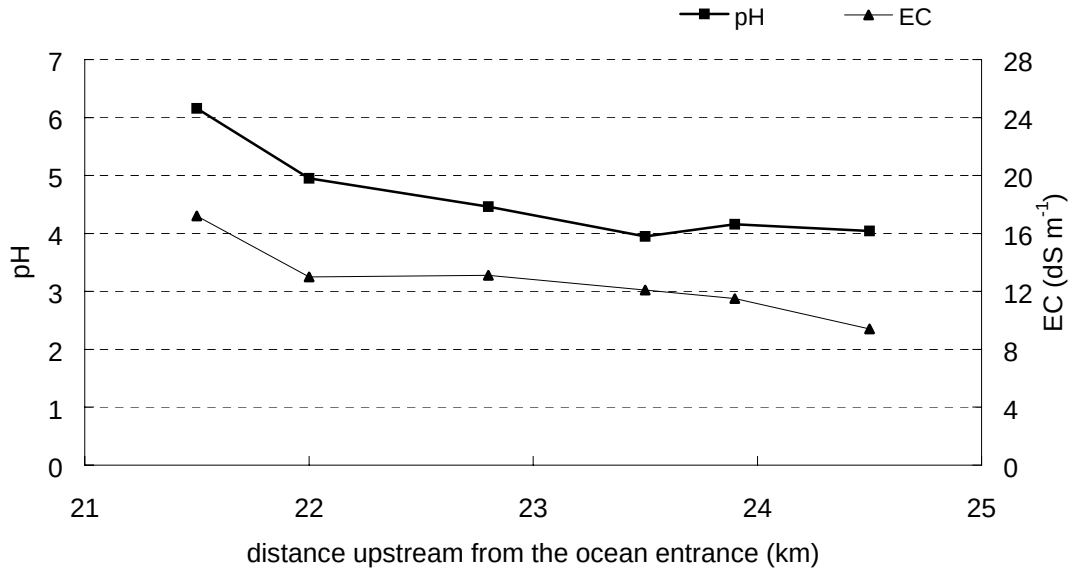


Figure 3.14 Dickensons Creek surface water pH and EC measured on the 9/5/99.

3.4.4 Lower Hastings River and Limeburners Creek Water Quality

3.4.4.1 pH

The minimum pH measured in the surface waters during the study was 6.31 at Site 19 on the 5/6/98. The minimum pH for bed waters was 6.57 at Site 17 measured on the same date. This occurred immediately after a large rainfall event where 150 mm of rainfall was recorded in two days (Figure 3.8). This also resulted in EC levels in Limeburners Creek being depressed in both surface and bed waters. Figure 3.15 displays the pH and EC conditions during a period of low rainfall where both the pH and EC values are high and consistent with distance upstream. However, immediately after rainfall both EC and pH decrease with increasing distance upstream in Limeburners Creek (Figure 3.16).

The minimum pH measured in surface waters of the main channel of the Hastings River was 7.05 on the 15/9/98. Figure 3.17 shows the difference in pH between surface and bed waters resulting from density stratification. The water quality data collected during this study showed that estuarine acidification caused by ASS outflows did not occur in the lower Limeburners Creek and lower Hastings River areas during the data collection period. $Cl:SO_4$ ratios were greater than 5 and the minimum pH values measured were greater than 6 on all occasions during the study period which indicates that the estuarine waters had not interacted with FeS_2 oxidation products contained in ASS (Mulvey, 1993). However, pH was reduced to a level below neutral conditions (pH 7) after high rainfall (Figure 3.16). This pH decrease was attributed to inflows of large quantities of humic acids that originate in the adjoining Limeburners Creek Nature Reserve (Figure 3.5). Following high rainfall, natural drainage courses in the nature reserve allow humic acids to enter Limeburners Creek which results in suppressed pH levels for short durations. The pH data for all of the sampling sites and dates are listed in Appendix 3F.

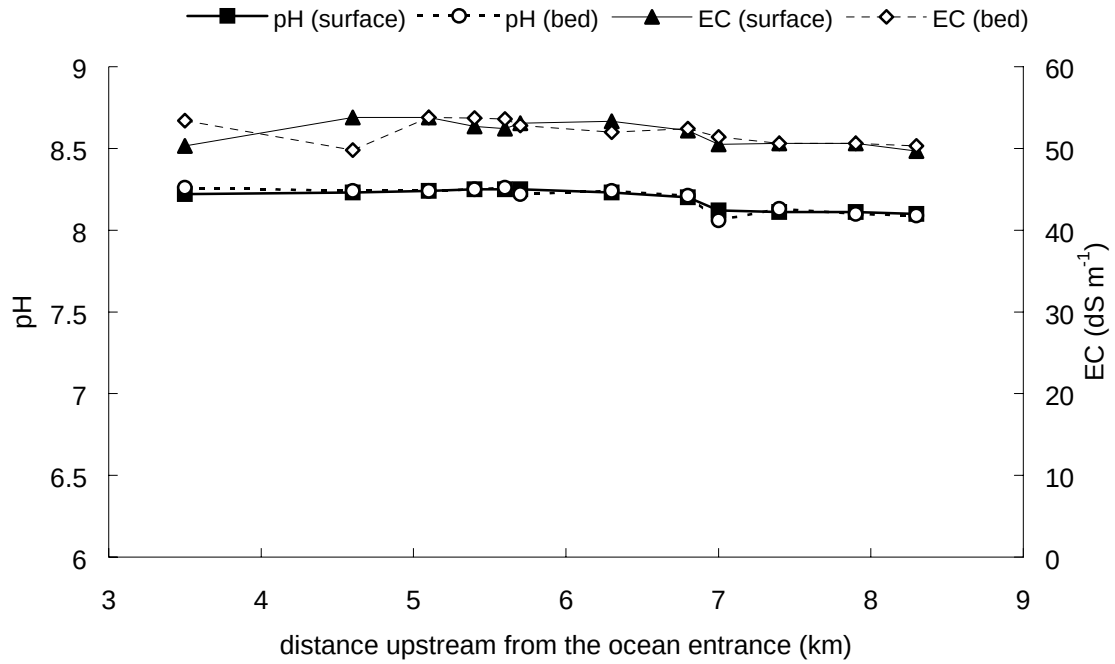


Figure 3.15 EC and pH in Limeburners Creek surface water and bed water prior to high rainfall on the 4/12/97.

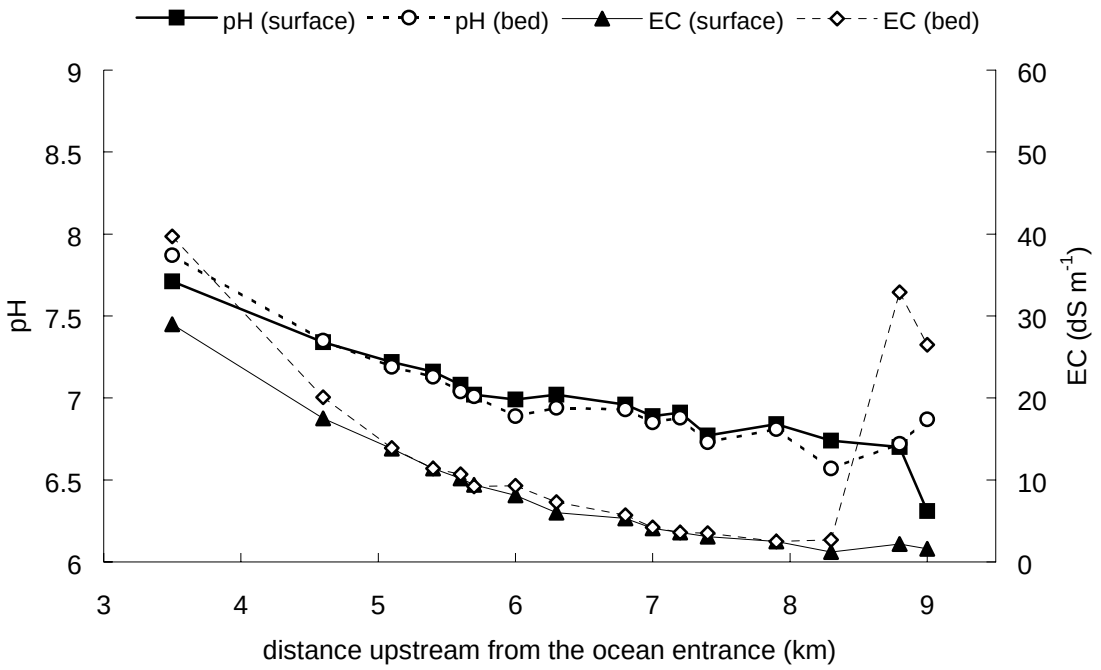


Figure 3.16 EC and pH in Limeburners Creek surface water and bed water after high rainfall on the 5/6/98.

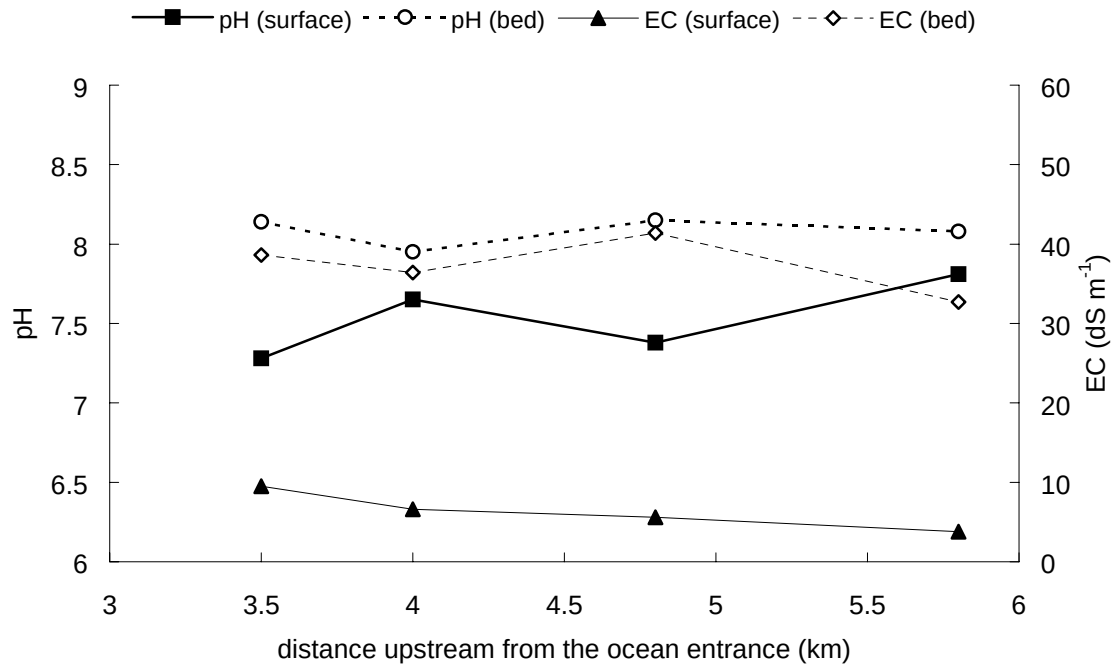


Figure 3.17 EC and pH stratification in the Hastings River prior to high rainfall on the 17/8/98.

3.4.4.2 EC

EC at all sites was influenced by rainfall and runoff. The minimum EC level measured in Limeburners Creek surface and bed waters occurred on the 5/6/98 (Figure 3.16) and the minimum EC level measured in the Hastings River occurred on the 17/8/98 (Figure 3.17) for surface waters and 2/6/98 for bed waters. Maximum EC for all sites occurred on the 7/12/98. Stratification resulting from EC differences between surface and bed waters was typical in the main channel of the Hastings River and is displayed in Figure 3.17. Stratification was not as pronounced in Limeburners Creek as it was in the main channel of the Hastings River. The EC data for all of the sampling sites and dates are listed in Appendix 3F.

3.4.4.3 DO and Temperature

The lowest DO measurement was 20.8% saturation in the bed waters at Site 19 on the 31/8/98. The maximum was at Site 8 on the 31/3/99 when the bed waters had a DO saturation of 142.1% (measured in the early evening). Water temperatures at the 19 sample sites ranged from a minimum of 10.63^{O} C at Site 16 on the 5/6/98 to 29.58^{O} C at Sites 18 and 19 on the 4/3/99. The minimum temperature was measured in the bed waters and the maximum temperature was measured in the surface waters. The DO and temperature data for all of the sampling sites are listed in Appendix 3F.

3.4.4.4 Water Sample Analysis

Analysis of the surface and bed water samples did not show any unusually high concentrations of dissolved ions (Fe, Al, Ca, Mn, K, Mg, SO_4, As, Cu, Si and Zn) in Limeburners Creek or the Hastings River. Appendix 3G lists the concentrations of dissolved ions measured in surface and bed waters at Sites 1, 4, 12 and 19.

3.4.4.5 SDL Measurements

The pH, EC and temperature data collected by the SDL located at Site A are displayed in Figures 3.18 and 3.19 and are summarised in Table 3.3. The median pH, EC and temperature value at Site A was 8.07, 44.25 dS m^{-1} and 22.03^{O} C, respectively (Table 3.3).

Figures 3.18 and 3.19 show the variation in pH and EC at Site A. Figure 3.18 A and B are typical displays of dry conditions in Limeburners Creek catchment. In these two figures, EC is close to oceanic levels and the pH range was 7.5 to 8.5. Figure 3.18 C and D, as well as Figure 3.19 A, C and D, show reduced EC levels due to the influx of fresh water following rainfall. This also caused a decrease in pH to below 7 in two circumstances during data collection. There is rapid recovery of EC levels in Limeburners Creek after short dry periods due to the close proximity of this area to the ocean entrance and the small catchment area of Limeburners Creek. Diurnal variation in temperature was also evident in Figures 3.18 and 3.19. Fresh water inflows do not have a large influence on the temperature variation in Limeburners Creek. These data show that estuarine acidification resulting from the disturbance of ASS was not a problem in lower Limeburners Creek during the study period.

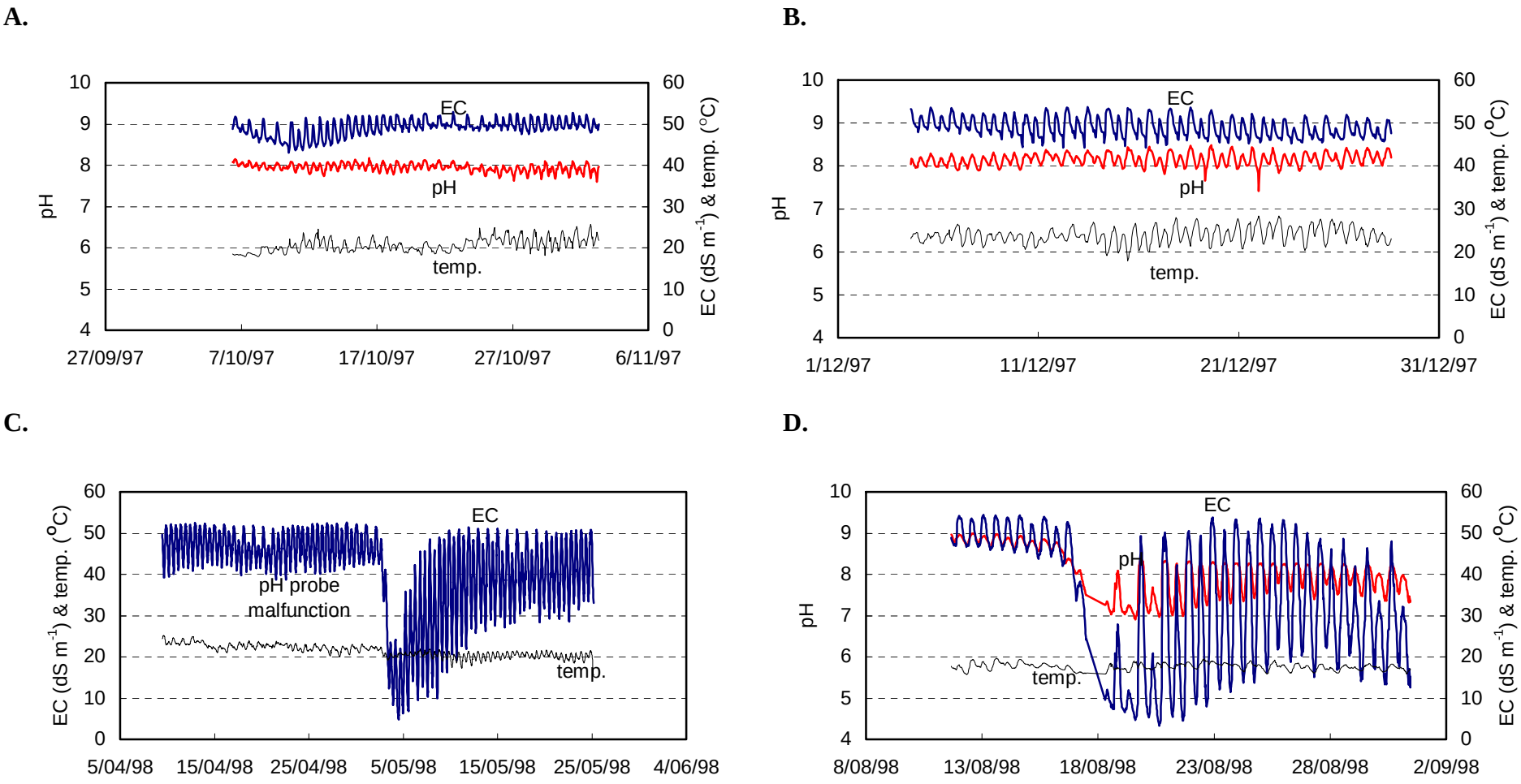


Figure 3.18 pH (red), EC (blue) and temperature (black) at Site A, Limeburners Creek: (A) 6/10/97 to 2/11/97; (B) 4/12/97 to 28/12/97; (C) 9/4/98 to 25/5/98; and (D) 11/8/98 to 31/8/98.

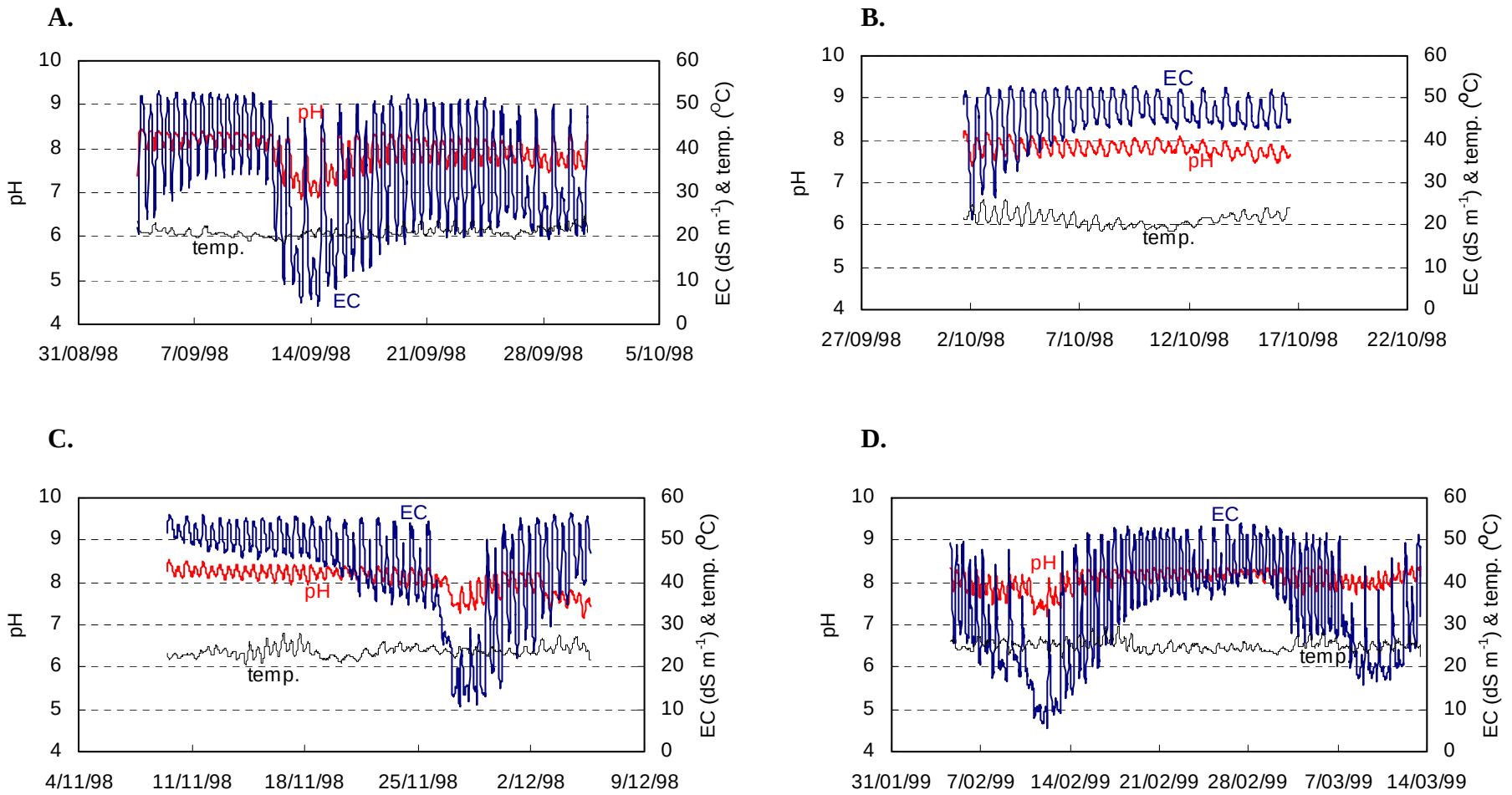


Figure 3.19 pH (red), EC (blue) and temperature (black) at Site A, Limeburners Creek: (A) 3/9/98 to 30/9/98; (B) 1/10/98 to 16/10/98; (C) 9/11/98 to 5/12/98; and (D) 4/2/99 to 13/3/99.

Table 3.3 Summary of pH, EC and temperature data collected by the SDL at Site A.

	pH (n = 10,396)	EC (dS m^{-1}) (n = 13,692)	Temperature (^{O}C) (n = 13,687)
Minimum	6.86	3.30	15.32
Maximum	9.00	56.25	29.64
Median	8.07	44.25	22.03
Mean	-	39.72	22.00
Standard Dev.	0.32	12.26	2.58

3.5 DISCUSSION

3.5.1 Hastings River Water Quality

Water quality data collected from the Hastings River estuary after rainfall events clearly show poor water quality conditions present in the Maria River, Connection Creek and Fernbank Creek. Water discharging from floodplain drains is acidified and has elevated concentrations of toxic metals that exceed the acceptable limits for aquatic ecosystems (ANZECC, 2000; Sammut *et al.*, 1995; 1996a). The spatial extent of acidification on the Hastings River is displayed in Figure 3.20A. This figure shows surface tidal waters acidified to pH 6.5 or below on the 18/6/99 and the extent of iron coating on the tributary banks in the Hastings River estuary.

Widespread acidification is facilitated by floodgates and artificial drainage systems that increase acid production and export, and attenuate or completely restrict tides that would otherwise buffer water pH in the drains. Sammut *et al*. (1996a) and Johnstone (1995) showed that floodgates can store acidified water in floodplain drains for prolonged periods. The stored acid can be released during low tide when the hydraulic head of the drains is higher than the tidal reach; the floodgate opens when this occurs and closes on high tide when the pressure of the tidal water forces the gates to close. Under dry weather conditions, when estuary waters are well buffered, the low tide pulses of acidic water are neutralised close to the outflow point, but iron flocs can nevertheless travel for many kilometres downstream. By contrast, wet weather can deplete the acid neutralising capacity of tidal waters and increase acid outflows from floodgates leading to extensive acidification of tidal reaches (Sammut *et al.*, 1996b). Acid may also move up and down the river during wet weather due to plug-flow displacement from the drains. Density differences between acid water and tidal water can result in an upstream and downstream movement of an acid plug (Sammut *et al.*, 1996a).

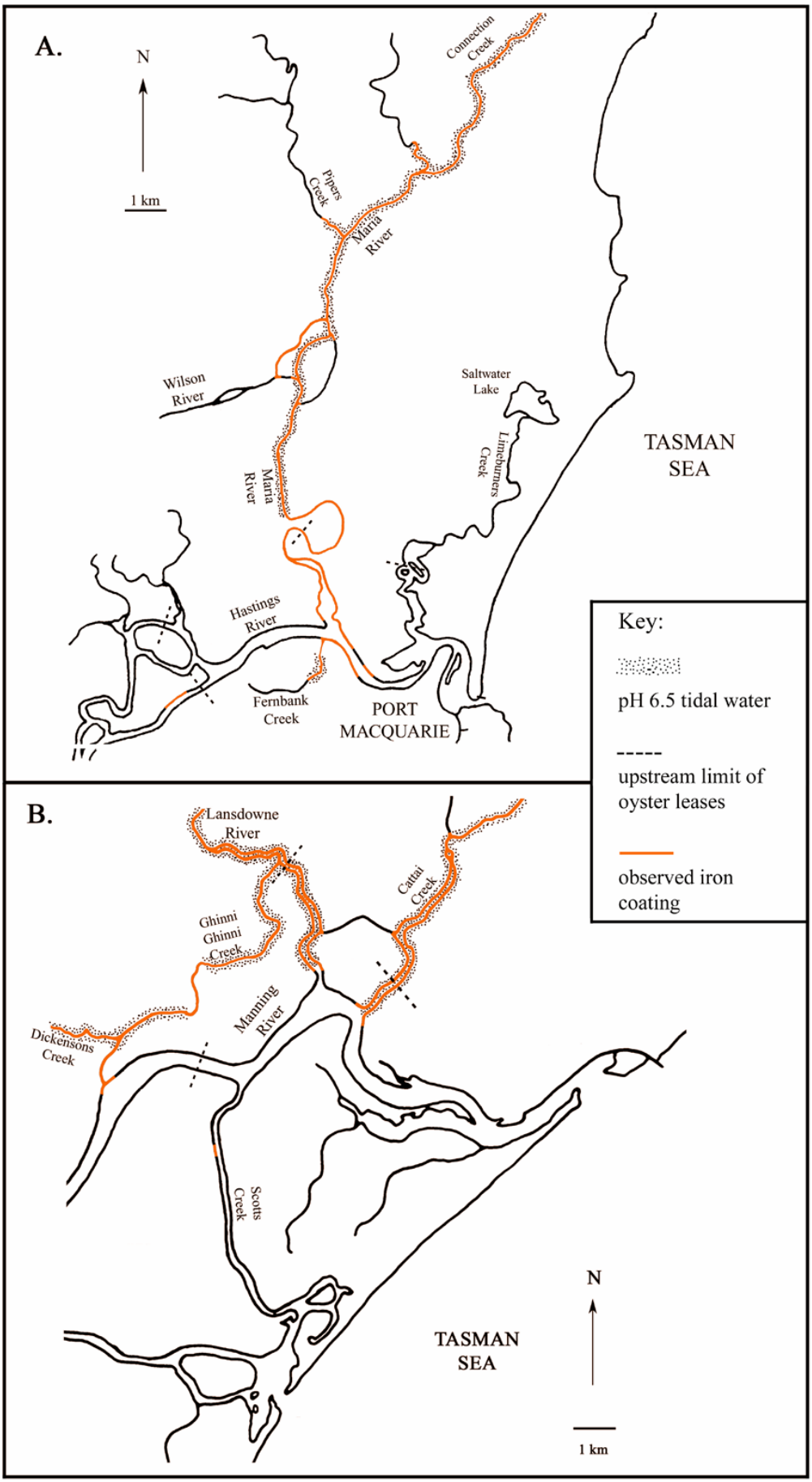


Figure 3.20 Spatial extent of acidification and iron precipitate coating in the Hastings River (A) and Manning River (B) estuaries measured on the 18/6/99 and 9/5/99, respectively.

This widespread acidification of the Hastings River estuary after rainfall events has profound implications for the aquatic ecosystems in these river and creek systems. The extent of the acid encompasses parts of the river system where oyster losses and poor production occurs. Oyster leases immediately upstream of Fernbank have largely been abandoned and the remaining infrastructure is coated with iron flocs. Oyster production in the Maria River has also ceased.

It has been estimated that Fernbank Creek contributes, on average, 400 tonnes of sulfuric acid into the Hastings River per year (White, 1998). Johnston (1995) estimated the ASS in the Maria River catchment are capable of producing approximately 1.8×10^9 m^3 of dilute sulphuric acid at pH 3.5.

The temporal persistence of acid events in Maria River and Fernbank Creek is dependant upon the intensity of the rainfall event and the duration of the interim dry (or low rainfall) period (Johnston, 1995; MHL, 1997; ERM Mitchell McCotter, 1997). Johnston (1995) reported that acidic conditions (< pH 5.5) persist for 4 to 6 weeks in the upper reaches of the Maria River. MHL (1997) measured a pH of approximately 6.6 continuing from October 1994 to January 1995 in the Maria River at Green Valley and in Connection Creek (32.2 and 42.5 km upstream from the Hastings River entrance respectively). Data collected by Hastings Municipal Council shows Partridge Creek drain can have ASS-affected water outflows with a pH of < 3.5 entering into Fernbank Creek every day at low tide for a period of over two months. This study and previous studies (ERM Mitchell McCotter, 1997; Johnston, 1995) have measured extremely high concentrations of iron and aluminium. Johnston (1995) measured an aluminium concentration of 3.06 mg L^{-1} and ERM Mitchell McCotter measured 20 mg L^{-1} of aluminium and 14 mg L^{-1} of iron at the Partridge Creek drain discharging into Fernbank Creek. The maximum concentrations of iron and aluminium measured at Fernbank Creek during this study were 35.90 mg L^{-1} and 13.84 mg L^{-1} respectively.

Plumes of acidic water were observed emanating from Fernbank Creek and moving upstream along the southern bank of the Hastings River for a distance of approximately 2 kilometres after a rain event in July 1998. Plate 3.1 shows milky blue/green water from high concentrations of suspended aluminium and Plate 3.2 shows red coloured water caused by high concentrations of suspended iron. The acidic outflows in both of these plates are discernable from the saline and well-buffered Hastings River water. Plumes of acidic water originating from the Maria River typically extend along the northern bank of the Hastings River and are characterised by high concentrations of suspended iron flocs. Plate 3.3 shows a plume of fresh, acidic water containing suspended iron flocs mixing with saline, well buffered Hastings River water at the confluence of these two river systems.

Plate 3.1 A milky blue/green coloured acidic plume from Fernbank Creek affecting the Hastings River.

Plate 3.2 A red coloured acidic plume (due to high concentrations of suspended iron) from Fernbank Creek affecting the Hastings River.

Plate 3.3 Acidic plume characterised by high concentrations of suspended iron from the Maria River affecting the main channel of the Hastings River.

Water quality investigations in the lower Hastings River and Limeburners Creek did not measure estuarine acidification caused by ASS outflows at the 19 monitoring sites during the study period. Regular drain water quality monitoring was also conducted throughout the study and did not detect any acidified water in drains connected to Limeburners Creek. Circumneutral pH values were measured in Limeburners Creek and Hastings River after high rainfall. EC suppression was generally for short periods of time and brackish estuary conditions rapidly returned after the rainfall had ceased. The water quality data measured during this investigation did not reveal any water quality variable tested that could be identified as a potential problem for oyster production in this area.

Although this lower region of the Hastings River estuary is not impacted by estuarine acidification, water quality data from Section 3.4.2 of this chapter showed that leases located further upstream were being affected by acidification. Therefore, acidic conditions are not commonplace in the lower Hastings River and Limeburners Creek areas due the close proximity to the ocean entrance which increases tidal mixing and is the source of strongly-buffered oceanic water.

3.5.2 Manning River Water Quality

Water quality data collected from the Manning River estuary after rainfall events clearly highlights the poor water quality conditions present in the Cattai Creek, Lansdowne River, Ghinni Ghinni Creek and Dickensons Creek areas. Inflows of acidified water from these tributaries have caused acidification of the main channel of the Manning River. Water discharging from floodplain drains was acidified and has elevated concentrations of toxic metals.

The spatial extent of acidification on the Manning River is displayed in Figure 3.20B. This figure shows surface tidal waters acidified to pH 6.5 or below on the 9/5/99 and the extent of iron coating on the tributary banks in the Manning River estuary. Widespread estuarine acidification after rainfall events in the Manning River estuary has profound implications for the aquatic ecosystems in these creek systems and has caused many oyster leases to be abandoned in affected areas.

Acidic conditions in Ghinni Ghinni persist for long periods after rainfall because acidic water becomes trapped in this area by fast flowing currents in the Manning River and Lansdowne River. Fish and other aquatic fauna become trapped in this area when the Lansdowne River and Dickensons Creek are acidic. Fish and eels were observed at the surface gulping air, swimming slowly and behaving erratically in both Ghinni Ghinni Creek and Dickensons Creek on the 9/5/99. The DO levels in surface and bed waters in Ghinni Ghinni Creek were as low as 18.2% and 5.2% saturation, respectively, on this date. Sammut (1998) found that this behaviour was related to blood hypoxia in fish with damaged gills, either from aluminium and acid induced lesions, or the accumulation of metal flocs in the lamellar spaces of the gills, and could occur at higher DO.

Plumes of acidic water impact the main channel of the Manning River after large rainfall events and are shown in Plates 3.4 to 3.6. These oblique aerial photographs taken of the Manning River after rainfall in July and August 1998 further highlight the extent of the acidification problem.

Plate 3.4 Acidified water originating from Ghinni Ghinni Creek and Dickensons Creek affecting the Manning River.

Plate 3.5 Acidified water originating from the Lansdowne River affecting the Manning River.

Plate 3.6 Acidified water originating from Cattai Creek affecting the Manning River and passing through a number of oyster leases.

3.6 CHAPTER SUMMARY

Water quality monitoring on the Hastings and Manning estuaries confirmed that estuarine acidification is widespread following rainfall events and impacts oyster producing areas, particularly leases downstream of large floodgate structures and tributaries draining extensive ASS. Drain water quality is unacceptable in relation to the specifications outlined in current guidelines (ANZECC, 2000). Drain waters have low pHs and elevated concentrations of metals that cause a variety of impacts to estuarine ecosystems. ASS oxidation products were efficiently exported downstream and into areas used for oyster production.

Water quality monitoring conducted in the lower Hastings River and Limeburners Creek area did not detect acidification associated with drained ASS. Intensive water quality sampling under a range of environmental conditions did not identify a decline in any of the measured water quality variables likely to cause the production problems observed in this area by oyster growers. Water quality conditions in this area of the estuary were well-buffered by brackish water with strong oceanic influence. However, salt stratification was commonly measured following rainfall in the catchment due to fresh waters derived from land drainage overlying more saline waters.

Water quality data relating to estuarine acidification of the Hastings and Manning Rivers is used in the experimental design of field exposure experiments discussed in Chapter 4.

4 FIELD EXPOSURE OF SYDNEY ROCK OYSTERS TO ASS-AFFECTED WATERS

4.1 INTRODUCTION

Chapter 3 demonstrated that estuarine acidification impacts areas of the Hastings and Manning Rivers used for oyster production. This chapter examines the effects of acidification on oyster survival and growth at field sites located on the Manning River. The Manning River was selected for the field exposure experiments because there were:

- a number of suitable experimental sites that were exposed to ASS-affected waters;
- two related studies (Sonter, 1999; Smith and Dove, 2001) conducted at or near the experimental sites; and,
- no reported outbreaks of LS or QX disease which could potentially interfere with the interpretation of the results.

Oyster survival, growth and condition index were monitored at four sites that had a high probability of exposure to ASS-affected waters and three sites that had a very low probability of exposure to ASS-affected waters. The selection of the seven experimental sites was based on the water quality information collected in the Manning River and presented in Chapter 3.

The pilot study showed that exposure of Sydney rock oysters to ASS-affected waters caused high mortalities after approximately 40 days. It was also discovered during this work that smaller oysters experienced higher mortality rates compared to larger oysters. This was mostly attributed to shell degradation, caused by the acidic conditions, which perforated the shells of smaller oysters more rapidly than larger oysters. This first experiment was conducted at a small scale, only used 2 sites (a site exposed to ASS-affected waters and a site isolated from ASS-affected waters) and did not use replicate groups of oysters. This chapter presents and discusses a much larger experiment that used the information from the pilot investigation to design two field exposure experiments that examined oyster survival, growth and condition index at sites impacted by acidification and sites isolated from acidification. The first experiment (Experiment 1) examined oyster survival and growth and the second experiment (Experiment 2) focussed on oyster condition index. Water quality was also monitored over both experimental periods and is also presented in this chapter. The sites selected were in areas of the Manning River estuary designated for oyster production.

Condition index was monitored in this study to investigate whether oyster quality and health are impacted by acidification caused by ASS outflows. Condition index is a very sensitive indicator of oyster growth and environmental stress. Condition index was not measured in Experiment 1 because it required sampled oysters to be sacrificed.

4.2 EXPERIMENTAL DESIGN

4.2.1 Experiment 1

4.2.1.1 Experimental Sites

The locations of the seven sites selected to monitor oyster survival and growth are displayed in Figure 4.1. Three sites (1 to 3) were situated in areas of the estuary that had a very low probability of exposure to ASS-affected waters and these sites are collectively referred to as "reference sites". Four sites (4 to 7) were located in areas of the estuary that had a high probability of exposure to ASS-affected waters after high rainfall and these sites are collectively referred to as "impacted sites". All sites were located on an oyster lease with the exception of Site 5. Descriptions of the seven sites are provided in Table 4.1.

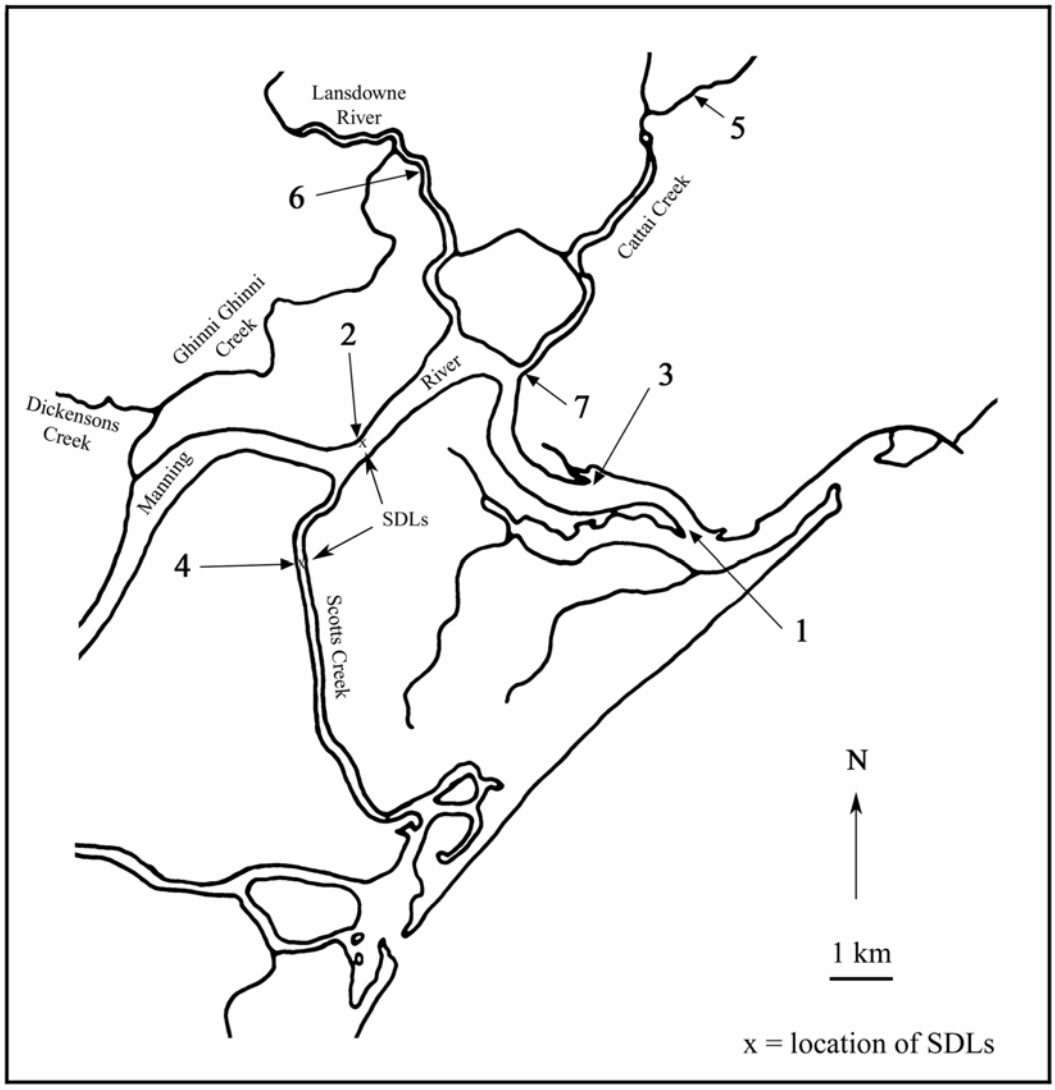


Figure 4.1 Locations of oyster and water quality monitoring Sites 1 to 7 on the Manning River.

Table 4.1 Descriptions of Sites 1 to 7.

Site Number	Location	Oyster Lease Number	Distance Upstream* (km)	Comments
1	Manning River	85-210	5.1	Very Low probability of exposure to ASS-affected waters
2	Manning River	84-176	11.9	Very Low probability of exposure to ASS-affected waters
3	Manning River	69-137	6.3	Very Low probability of exposure to ASS-affected waters
4	Scotts Creek	79-182	14.3	Located 5 m from a large floodgate structure
5	Cattai Creek	-	15.1	Extended periods of acidic and fresh conditions
6	Lansdowne River	85-220(1)	13.5	Area of declining oyster productivity
7	Manning River	59-174	9.3	Situated downstream from the mouth of Cattai Creek

* From the Manning River entrance at Harrington

4.2.1.2 Sampling Dates

Water quality measurements and a count of the number of dead oysters were performed fortnightly for a 14-week period starting on the 1/6/99. Three subsequent sampling dates were performed on the 1/10/99, 15/11/99 and the 10/1/00 to extend the experiment into a period of lower rainfall. The sampling dates and the measurements performed on each date are listed in Table 4.2.

Table 4.2 Sampling dates and field measurements.

Sampling Dates	Measurements
01/06/99	Water quality, surviving oysters and whole oyster weight
15/06/99	Water quality, surviving oysters and shell dimensions
28/06/99	Water quality, surviving oysters and whole oyster weight
12/07/99	Water quality, surviving oysters and shell dimensions
26/07/99	Water quality, surviving oysters and whole oyster weight
09/08/99	Water quality, surviving oysters and shell dimensions
23/08/99	Water quality, surviving oysters and whole oyster weight
06/09/99	Water quality, surviving oysters and shell dimensions
01/10/99	Water quality, surviving oysters and whole oyster weight
15/11/99	Water quality, surviving oysters, whole oyster weight and shell dimensions
10/01/00	Water quality, surviving oysters, whole oyster weight and shell dimensions

4.2.1.3 Water Quality

On all sampling dates, *in-situ* surface water quality measurements close to the experimental oysters were performed at the seven sites using a Yeo-Kal 611 Intelligent Water Quality Analyser. The Palin Test Photometer (Model 5000) was used to measure alkalinity at each site on the sampling dates and a surface water sample was also collected for ICPAES and chloride analysis. Water sampling and analysis methods and techniques are detailed in Section 3.3.1.

The Greenspan Technical Services Smart Sonde Model SD300 and the Yeo-Kal 611 Intelligent Water Quality Analyser were placed at Sites 2 and 4, respectively. This provided continuous measurements of pH, EC and temperature to demonstrate temporal differences in water quality conditions at these two sites.

4.2.1.4 Experimental Oysters

Manning River Sydney rock oysters were used for Experiment 1. All oysters were inspected to ensure that they displayed no clinical signs of LS (Section 6.3). Oysters were acclimated at Site 2 for 14 days before being placed at each site on the 1/6/99. Oysters used in Experiment 1 were single-seed stock originally removed from PVC (polyvinyl chloride) catching slats.

Four replicate groups of 50 large oysters which had a mean weight (± 95% confidence interval (CI)) of 29.1 ± 0.4 g and 50 small oysters which had a mean weight (± 95% CI) of 5.1 ± 0.1 g were placed in plastic baskets at Sites 1 to 7. Each basket was attached to 7 mm nylon rope and suspended approximately 0.3 m below the water surface. Four foam floats were used for buoyancy at the experimental sites.

4.2.1.5 Oyster Survival

Oyster survival for Experiment 1 was determined at each experimental site on the dates listed in Table 4.2. Oyster survival was measured as percentages and calculated using the formula:

$$\textit{Survival Rate} = (\textit{No. of living shell}/[\textit{No. of living shell} + \textit{No. of dead shell}]) \times 100$$

To investigate differences among the impacted sites and the reference sites, a three factor analysis of variance (ANOVA) was used. The factors were Acid (fixed factor), Size (fixed factor) and Site (nested within Acid – random factor). The ANOVA was performed on the oyster survival data from two sampling dates (23/8/99 and 10/1/00) during Experiment 1. The 23/8/99 (day 83) sampling was after a period of high rainfall where 379 mm of precipitation was recorded from the start of the experiment (1/6/99) at the Bureau of Meteorology Station Number 60141. The 10/1/00 (day 224) was the final sampling date for Experiment 1.

4.2.1.6 Instantaneous Growth Rate

Instantaneous growth was expressed as the percent increase in whole weight per day (Rheault and Rice, 1996; Toro *et al.*, 1995). Growth rate was determined for the same two periods detailed in Section 4.2.1.5 during Experiment 1 (i.e. from the 1/6/99 to 23/8/99 (day 0 to 83), which corresponded to a period of high rainfall and from 1/6/99 to 10/1/00 (day 0 to 224), which represents the growth rate for the entire experiment). Instantaneous growth was calculated using the formula (Ricker, 1975; Rheault and Rice, 1996):

$$\%\ \textit{increase per day} = (\ln[W_t/W_o]/t) \times 100$$

Where W_o is the initial mean wet weight in grams and W_t is the mean weight at time "t" in days.

The instantaneous growth rate was calculated using the mean weight of the total number of oysters alive at each site on the 23/8/99 and 10/1/00. Pooling of the oyster whole weights was necessary due to the high mortality at particular sites exposed to ASS-affected waters. The mean instantaneous growth rates were plotted for the two time intervals (day 0 to 83 and day 0 to 224). Bootstrap was used to approximate the 95% CIs of the plotted means.

4.2.2 Experiment 2

4.2.2.1 Experimental Sites

The sites used in Experiment 2 were the same sites used in Experiment 1. However, oysters were not placed at Site 5 because of the high mortality rate measured at this location during Experiment 1. The location of Sites 1, 2, 3, 4, 6 and 7 are displayed in Figure 4.1 and described in Table 4.1.

4.2.2.2 Experimental Oysters

Five hundred and fifty single-seed Sydney rock oysters (mean CI ± 95% = 19.7 ± 0.6 g) were collected from Sites 1, 2 and 3 and acclimated at Site 2 for 30 days. On the 1/2/00 oysters were randomly placed at Sites 1, 2, 3, 4, 6 and 7 (Figure 4.1) in plastic baskets using the same system described in Section 4.2.1.4.

4.2.2.3 Oyster and Water Quality Sampling Dates

The experimental period commenced in February 2000 and concluded in January 2001. Twelve oysters were sampled from Site 1, 2, 3, 4, 6 and 7 on the 6/4/00, 9/6/00, 7/8/00, 23/10/00 and 10/1/01. Sampling dates are shown on Figure 4.3. Water samples and water quality measurements were collected on all sampling dates using the same methods and techniques detailed in Section 4.2.1.3 for Experiment 1.

4.2.2.4 Condition Index

Within six hours of collection oysters were cleaned of fouling and commensal organisms, washed, blotted dry and their whole weight was measured using a weight balance. Oysters were opened from the hinge and the soft tissue was removed from the valves with a scalpel. Shells were washed in deionised water, blotted dry and weighed. The soft tissue was also washed in deionised water to remove any shell debris and dried for 48 hours at 80 ^{O}C. The dried soft tissue was placed in desiccators to cool and then weighed to determine the dry soft tissue weight.

The gravimetric method recommended by Crosby and Gale (1995) was used to calculate condition index. The following condition index formula was used:

CI = dry soft tissue weight (g) x 1000 / internal shell cavity capacity (g)

Where:
internal shell cavity capacity = whole weight (g) – shell weight (g)
(Lawrence and Scott, 1982).

To investigate differences in oyster's condition indices at acid-impacted sites and reference sites, a three factor ANOVA was used. The factors were Acid (fixed factor), Date (random factor) and Site (nested within Acid – random factor).

4.3 RAINFALL (EXPERIMENTS 1 AND 2)

As demonstrated in Chapter 3, rainfall in the lower Manning River catchment was a key factor in the mobilisation of acidified water and other oxidation products from the floodplain into particular areas of the estuary. Daily rainfall during Experiment 1 and Experiment 2 are displayed in Figure 4.2 and 4.3, respectively. Sampling dates for Experiments 1 and 2 are also displayed on these two figures.

Figure 4.2 shows a high rainfall event where 186 mm of rainfall was recorded between the 11/7/99 and the 17/7/99. This caused flooding of the lower Manning River floodplain. The first five months of 1999 were particularly wet and this resulted in Sites 4, 5, 6 and 7 being acidified prior to the start of Experiment 1 (1/6/99).

During Experiment 2, 1,059 mm of rainfall was measured and March was the wettest month with 373 mm of rainfall being recorded in this month (Figure 4.3). September was the driest month with only 9 mm of rainfall recorded in the 30 days. More rainfall was recorded during Experiment 1 compared to Experiment 2.

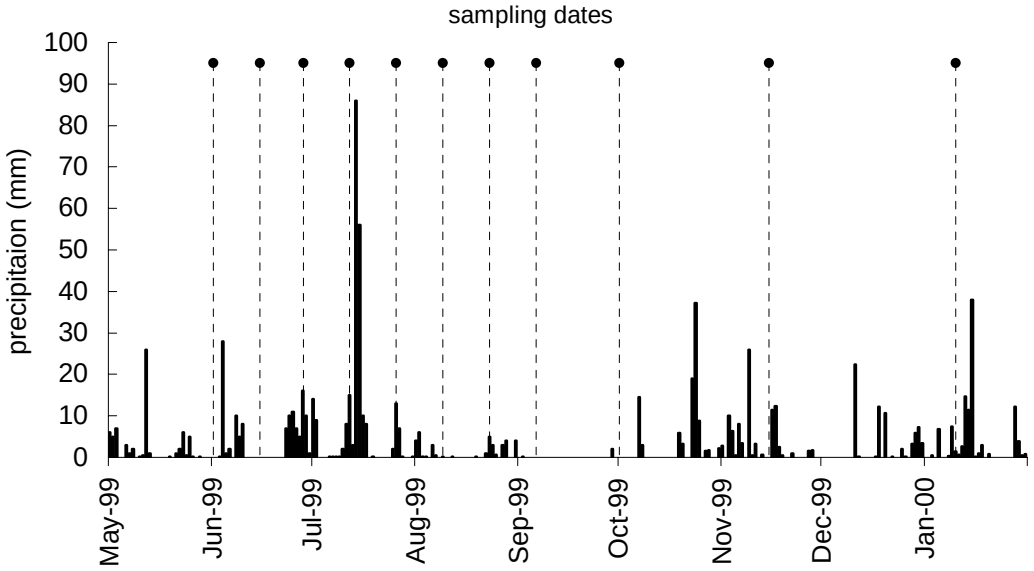


Figure 4.2 Experiment 1 rainfall (Source: Bureau of Meteorology, Station Number 60141) and sampling dates.

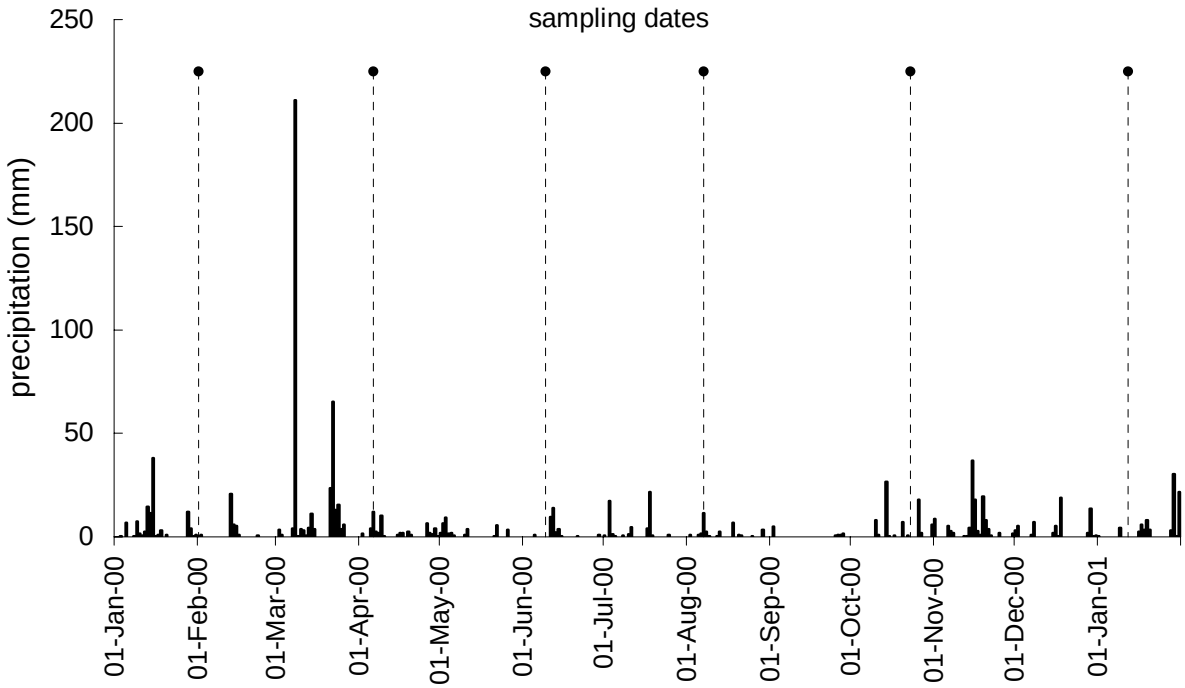


Figure 4.3 Experiment 2 rainfall (*Source*: Bureau of Meteorology Station Number 60141) and sampling dates.

4.4 RESULTS: EXPERIMENT 1

4.4.1 Water Quality Conditions

4.4.1.1 pH and EC

Rainfall influenced the EC levels at Sites 1, 2 and 3 and both EC levels and the pHs at Sites 4, 5, 6 and 7. Sites 1, 2 and 3 were characterised by higher ECs with the measured mean EC values being greater than 18.7 dS m^{-1} for these sites. Sites 4, 5, 6 and 7 had mean EC values less than 16.4 dS m^{-1}. Graphs of pH and EC measured on each sampling date are presented with the oyster data in Sections 4.3.4 and 4.3.5 (refer to Figures 4.5 to 4.11).

A summary of pH and EC data collected at the seven sites on the 11 site visits during Experiment 1 is included in Tables 4.3 and 4.4, respectively. Table 4.3 highlights the variation in pH that was measured at the seven sites. Sites 1, 2 and 3 had higher pHs on the ebb and flood tides than Sites 4, 5, 6 and 7.

The EC data displayed in Table 4.4 shows that Sites 1, 2 and 3 have higher EC values than Sites 4, 5, 6 and 7. Experimental sites located further upstream (Table 4.1 and Figure 4.1) have reduced EC values. Appendix 3H contains additional water quality data collected during each site visit. Alkalinity levels measured at Sites 4, 5, 6 and 7 were commonly lower than Sites 1, 2 and 3. SDLs were used to gather additional pH, EC and temperature data at Sites 2 and 4. A display of the pH and EC data between the 26/6/99 and 27/7/99 is included in Figure 4.4.

Table 4.3 Summary of pH data for Sites 1 to 7 for Experiment 1 (calculated from data collected on the 11 sampling visits).

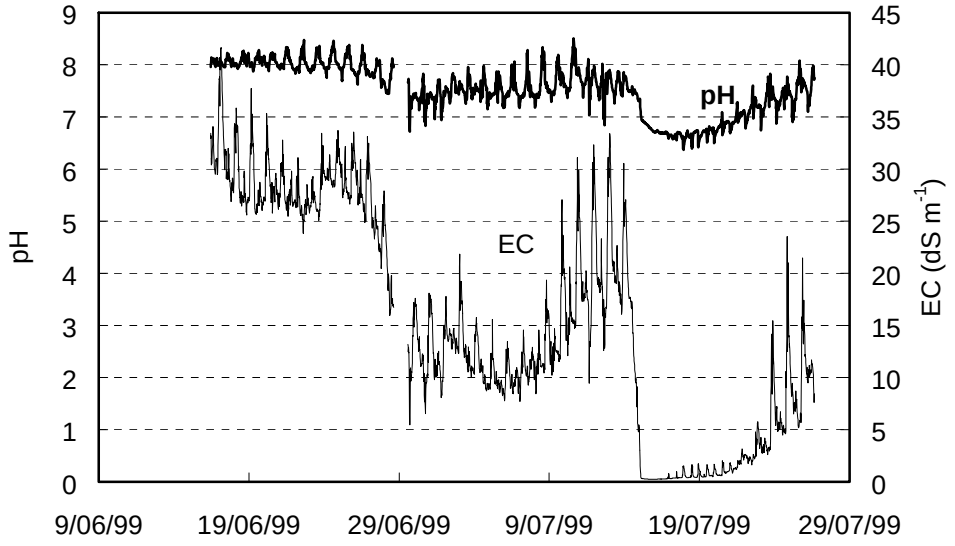
EBB TIDE				FLOOD TIDE			
Site	Median pH	Min pH	Max pH	Site	Median pH	Min pH	Max pH
1	8.00	7.53	8.78	1	8.12	7.41	8.67
2	7.92	7.21	8.65	2	7.89	7.42	8.40
3	7.95	7.59	9.04	3	8.10	7.64	8.67
4	5.92	3.51	7.81	4	7.58	5.72	8.34
5	5.47	4.45	7.36	5	5.64	4.46	7.33
6	6.84	5.51	7.01	6	6.95	6.19	7.75
7	6.65	5.27	7.90	7	7.48	5.89	8.00

Table 4.4 Summary of EC data for Sites 1 to 7 for Experiment 1 (calculated from data collected on the 11 sampling visits).

EBB TIDE				FLOOD TIDE			
Site	Median EC (dS m^{-1})	Min EC (dS m^{-1})	Max EC (dS m^{-1})	Site	Median EC (dS m^{-1})	Min EC (dS m^{-1})	Max EC (dS m^{-1})
1	30.4	14.5	44.4	1	41.1	20.2	50.5
2	21.5	5.9	31.4	2	23.0	8.7	33.4
3	27.9	13.8	40.0	3	38.6	16.2	51.9
4	15.9	1.6	27.9	4	19.8	6.2	32.3
5	3.3	0.3	26.8	5	4.1	1.1	28.1
6	12.9	2.3	21.7	6	18.4	3.9	25.1
7	16.0	1.9	28.3	7	23.3	1.9	34.1

Figure 4.4B highlights the large variability in pH at Site 4 due to the influence of the floodgate and acidified drainage water. Site 2 experienced slightly decreased pHs because of the reduced buffering capacity in the river caused by the massive influx of fresh flood waters during this period. This caused a decrease in the pH at Site 2 to pH 6.5. This was the minimum pH recorded by the SDL at this site during Experiment 1. The water quality data collected at the seven sites over the experimental period shows the varying degree by which each site is impacted by ASS outflows.

A.



B.

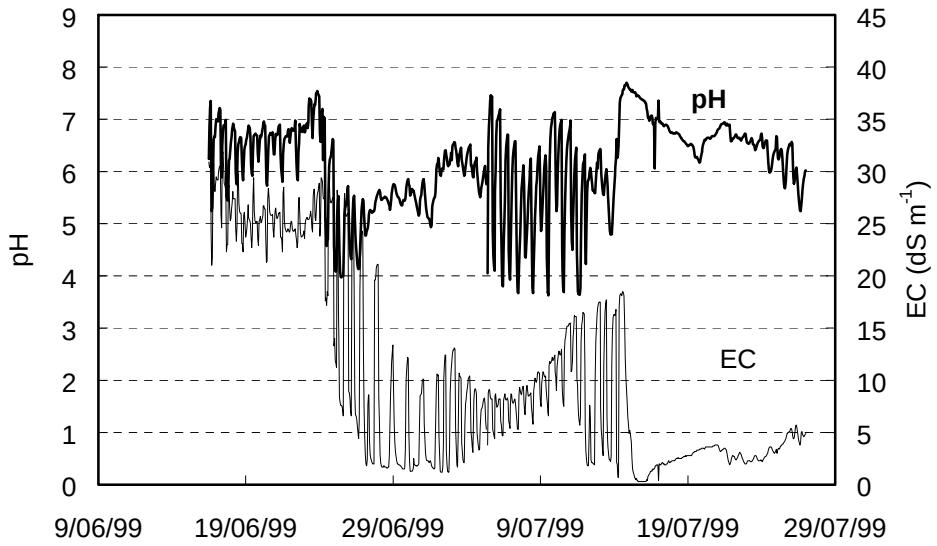


Figure 4.4 pH (bold line) and EC (thin line) at Site 2 (A) and Site 4 (B) for the period: 26/6/99 to 26/7/99.

4.4.1.2 Dissolved Metals

Table 4.5 lists the maximum concentrations of iron, aluminium and manganese measured at the seven experimental sites. Elevated levels of iron, aluminium and manganese were measured at Site 4 after the large rainfall event in mid-July. These maximum-recorded values are considerably greater than the values measured at other sites. This is mainly due to the close proximity of this site to the acid outflow source which reduces the amount of dilution and neutralisation of the outflow before it impacts oysters at this particular site. Red and pearly-white flocs were commonly observed in the water during the initial three months of the experiment at Sites 4, 5, 6 and 7. This indicates high concentrations of suspended iron and aluminium.

Table 4.5 Maximum concentrations of Fe, Al and Mn at each site measured on the ebb tide during Experiment 1.

	EBB TIDE		
Site	Fe ($mg\ L^{-1}$)	Al ($mg\ L^{-1}$)	Mn ($mg\ L^{-1}$)
1	0.03	0.07	0.16
2	0.02	0.1	0.08
3	0.04	0.06	0.19
4	25.95	9.95	8.34
5	0.72	2.02	0.45
6	BD	0.08	0.61
7	BD	0.14	0.22

n = 11, BD = below detection limits

4.4.2 Reference Sites: Oyster and Water Quality Data

Sites 1 to 3 were characterised by high oyster survival rates with very little variation between and amongst large and small oysters at all three sites (Figures 4.5 to 4.7). Weight loss in large oysters was measured on the 26/7/99 at Sites 1, 2 and 3. This can be attributed to low ECs caused by flooding and subsequent reduced feeding opportunities for oysters. Small oyster weight gain at Sites 1, 2 and 3 was negligible or non-existent between 12/7/99 and 26/7/99 (Figures 4.5 to 4.7). During this period water temperatures were low and affected by the mid-July flood. The lowest EC levels detected at Sites 1, 2 and 3 during Experiment 1 were measured on the 26/7/99 and were also due to the mid-July flood during which freshwater inputs dominated the estuary flows. The pH values measured at Sites 1, 2 and 3 on this date were all > 8. The lowest pH levels at Sites 1, 2 and 3 were measured before the flood and did not fall below circumneutral conditions. Aluminium and iron concentrations did not exceed the ANZECC (2000) guidelines at Sites 1, 2 and 3 (Table 4.5).

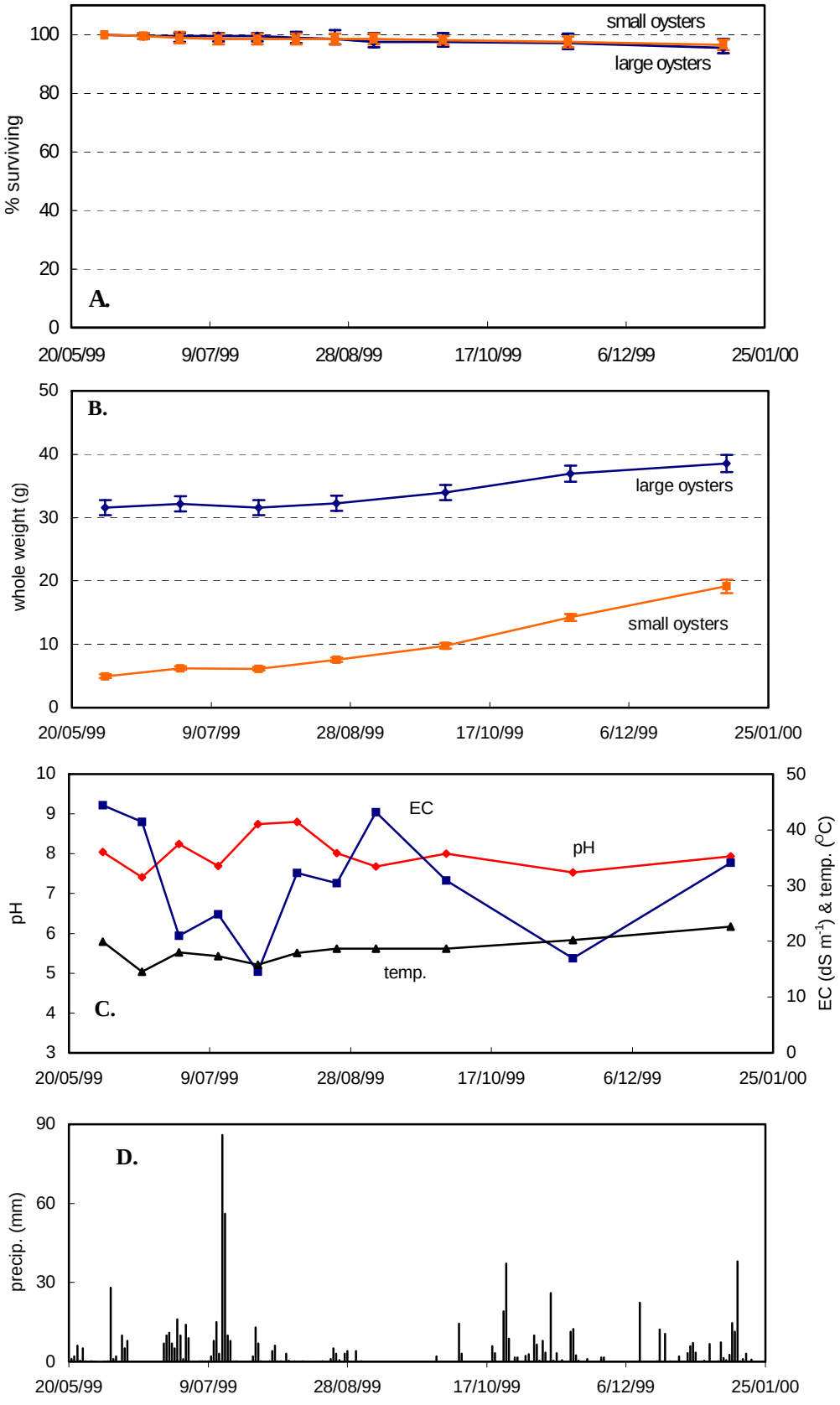


Figure 4.5 Site 1 summary display: (A) mean percentage survival (± 95% CIs); (B) mean whole weight (± 95% CIs); (C) pH, EC and temperature; and, (D) rainfall.

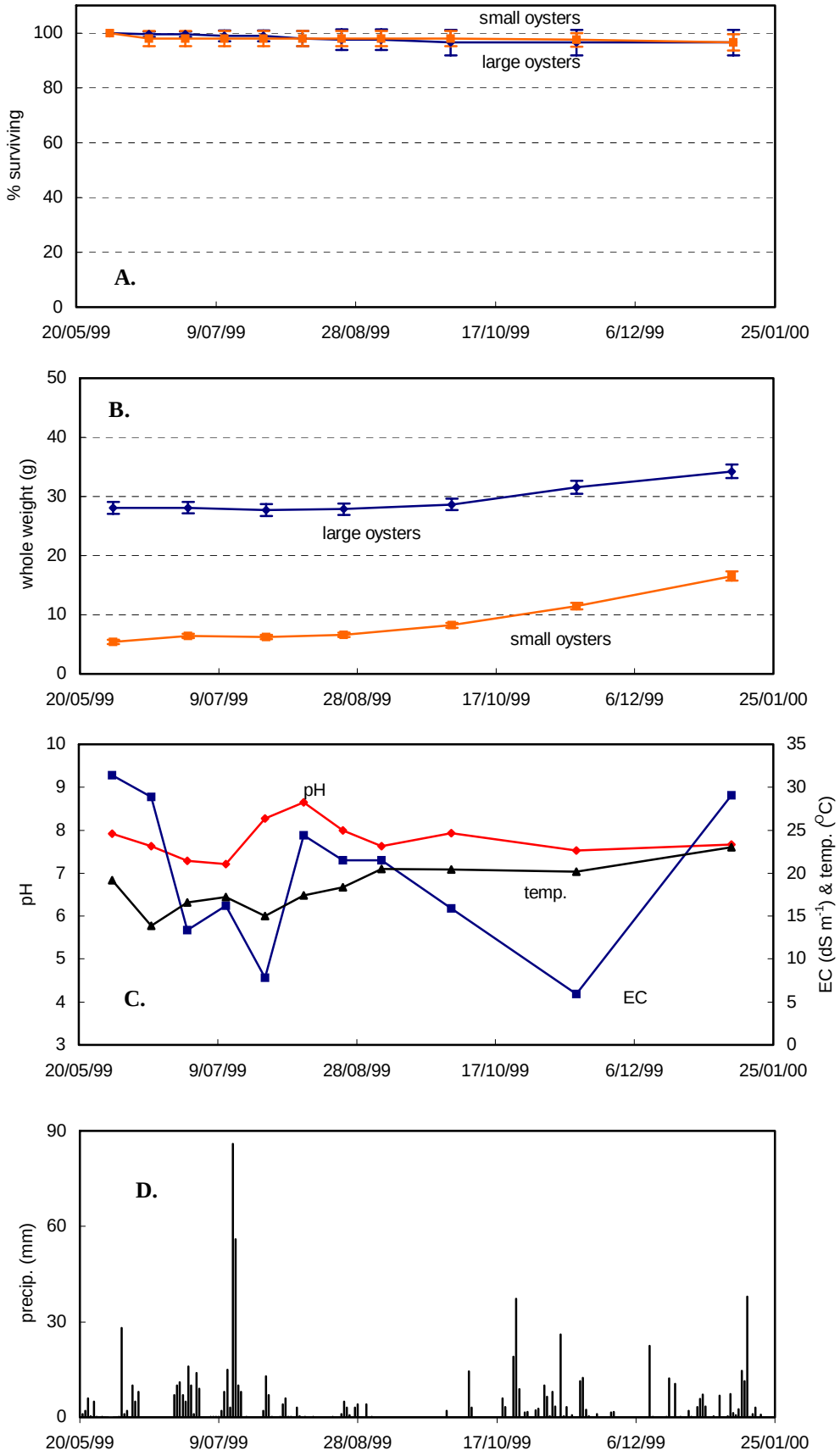


Figure 4.6 Site 2 summary display: (A) mean percentage survival (± 95% CIs); (B) mean whole weight (± 95% CIs); (C) pH, EC and temperature; and, (D) rainfall.

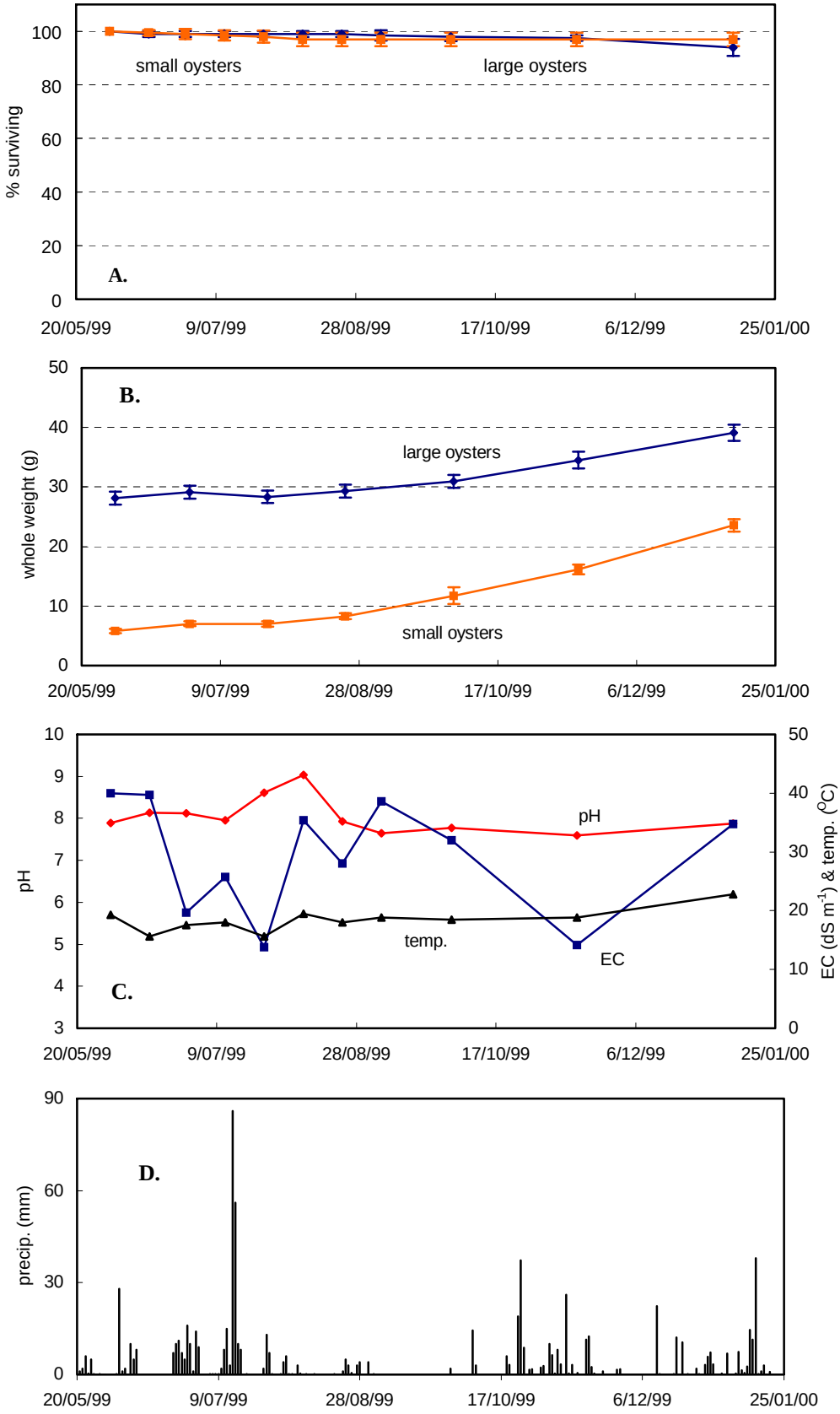


Figure 4.7 Site 3 summary display: (A) mean percentage survival (± 95% CIs); (B) mean whole weight (± 95% CIs); (C) pH, EC and temperature; and, (D) rainfall.

4.4.3 Impacted Sites: Oyster and Water Quality Data

There was a considerable difference between large and small oyster survival at Site 4 after the 26/7/99 (Figure 4.8). Small oysters experienced an increased mortality rate following this date. More than 70% of small oysters removed from baskets at Site 4 after the 9/8/99 had shell perforation (Plate 4.1). The water quality data collected at this site shows a high variability in both pH and EC induced by rainfall events (Figure 4.4). On the 9/8/99, 9.95 mg L^{-1} of dissolved aluminium and 25.95 mg L^{-1} of dissolved iron were measured at this site (Table 4.5).

Site 5 was characterised by dramatic mortality in large and small oysters after 12/7/99 (Figure 4.9), which corresponded to the date when shell perforation in small oysters was first noted. More than 85% of dead small oysters had shell perforation on the 9/8/99. At Site 5, all of the small oysters that had a flat section at the anterior of the left valve, caused by the PVC catching slat, exhibited shell perforation. Small oysters that had developed a rounded anterior section in their left valve had a much lower incidence of shell perforation. Plate 4.1 shows this difference in shell morphology. Whole weights in large and small oysters decreased from the start of the experiment at Site 5. Oysters were not weighed after the 26/7/99 due to high mortality at this site. Low pH and EC levels were measured at Site 5 during all site visits (Tables 4.3 and 4.4). The highest values of pH and EC were recorded on the 1/10/99 following a short dry period, which allowed brackish estuary water to neutralise the acidity at this site. However, by this date there were very few oysters still alive at Site 5.

Rainfall in late October through to the end of the experiment caused pH and EC to drop 2.6 units and 25 dS m^{-1}, respectively at Site 5. The maximum dissolved aluminium and iron concentrations measured at Site 5 were 2.02 mg L^{-1} and 0.72 mg L^{-1}, respectively (Table 4.5). However, suspended iron was observed in the water during all site visits and formed a thick coating on all oysters and baskets located at this site.

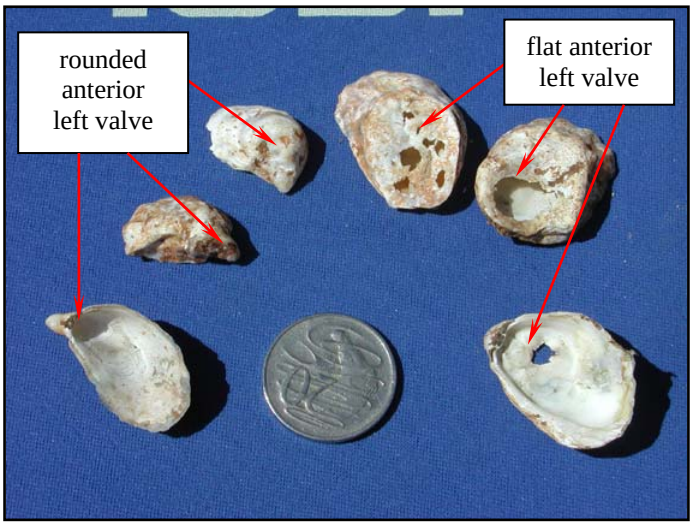


Plate 4.1 Variation in the shell morphology in the anterior of the left valve of small oysters. Oysters that have a flat section on their left valve (right hand side) also display shell perforation.

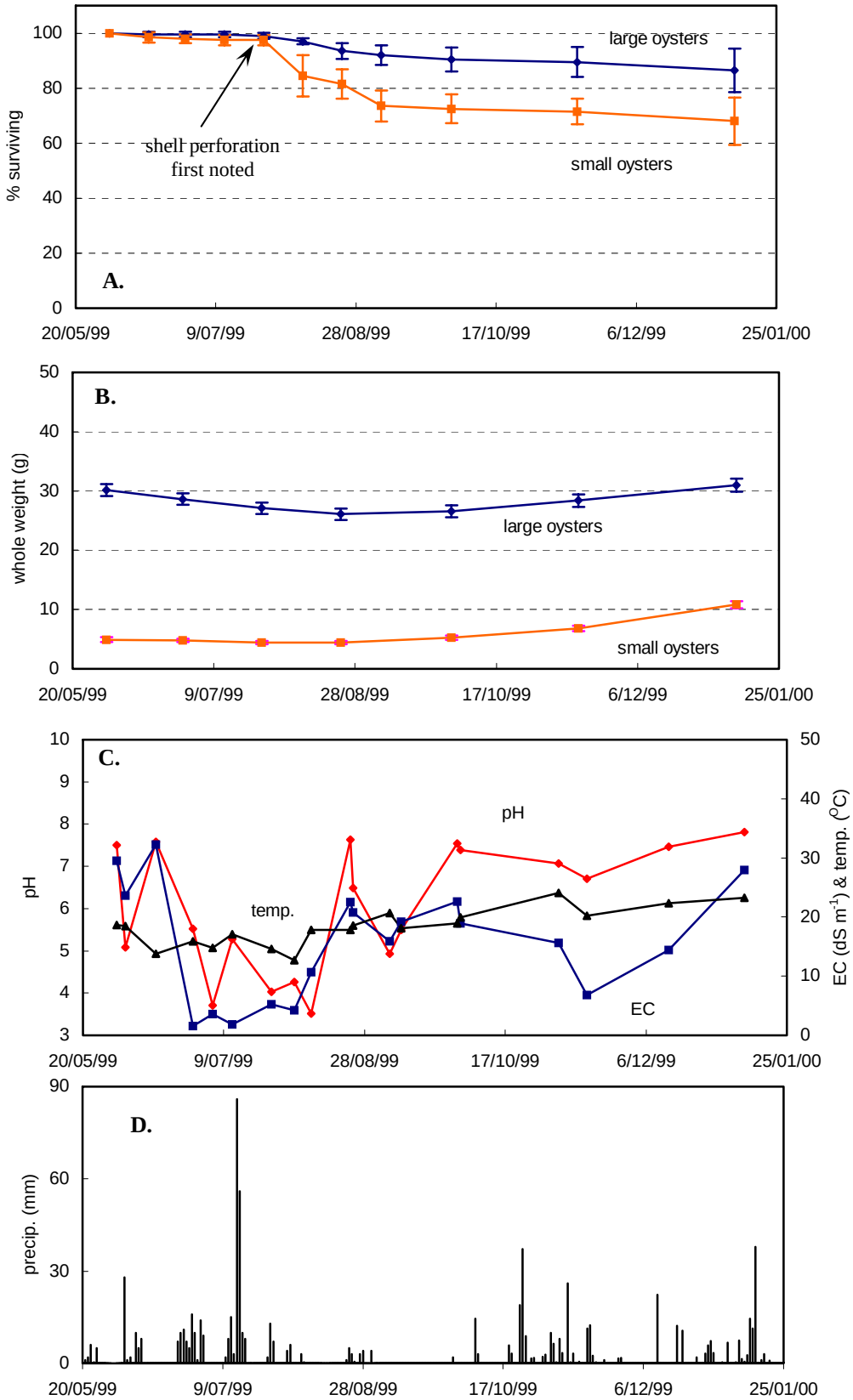


Figure 4.8 Site 4 summary display: (A) mean percentage survival (± 95% CIs); (B) mean whole weight (± 95% CIs); (C) pH, EC and temperature; and, (D) rainfall.

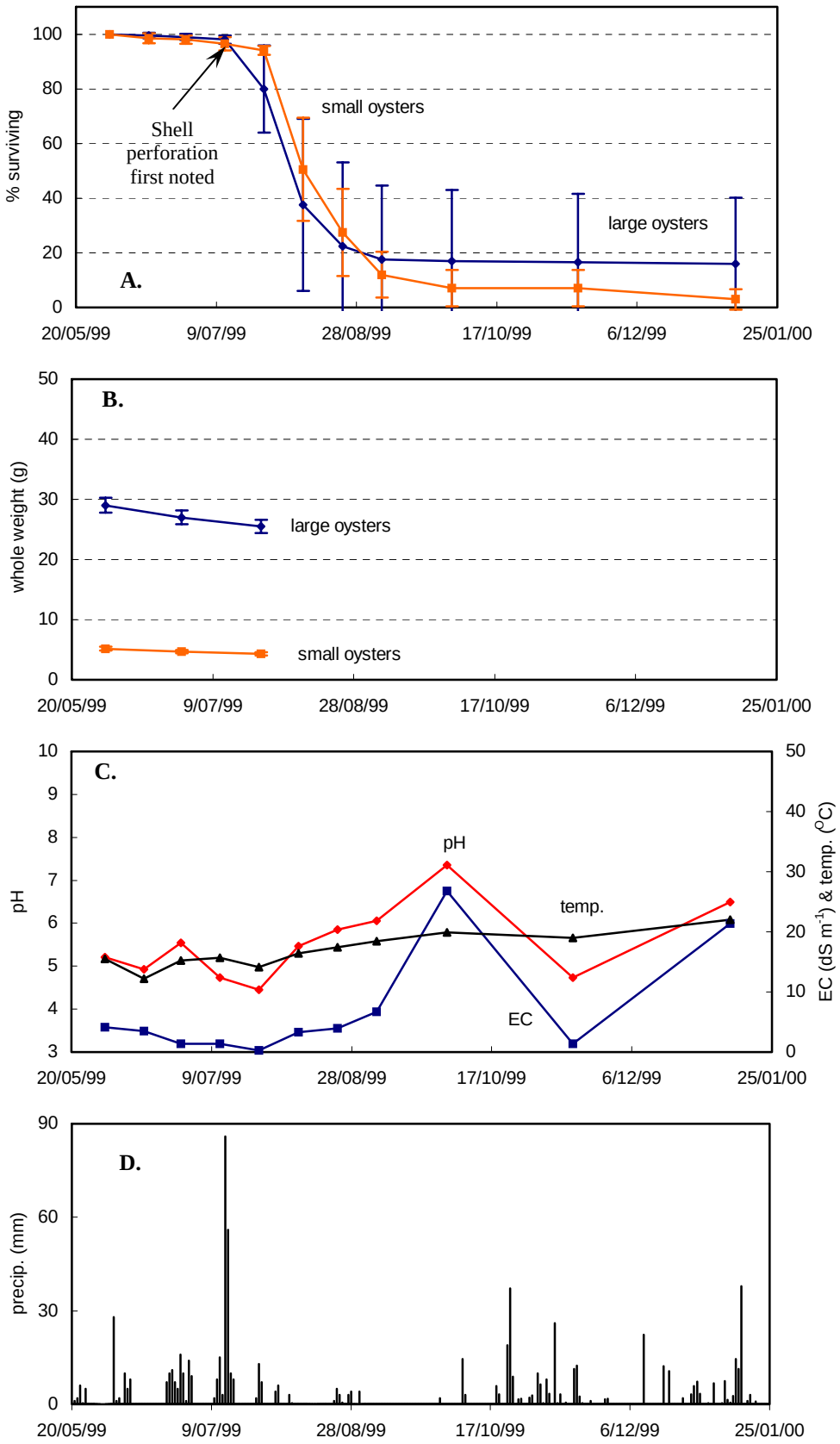


Figure 4.9 Site 5 summary display: (A) mean percentage survival (± 95% CIs); (B) mean whole weight (± 95% CIs); (C) pH, EC and temperature; and, (D) rainfall.

Small oysters at Site 6 experienced high mortality in comparison to large oysters during the period 12/7/99 through to the 6/9/99 (Figure 4.10). All small oysters with flat sections (from PVC catching slats) in the rear of their shell (Plate 4.1) were dead at Site 6 after 26/7/99. All of these oysters had evident shell perforation. Weight loss was measured in large oysters during the first three months of the experiment at Site 6 (Figure 4.10). Low pH and EC values at this site in the period before the flood event were due to high rainfall throughout June and July. Low EC values persisted at Site 6 over most of the experimental period. No dissolved iron or aluminium was detected by ICPAES analysis at this site. However, iron flocs were observed in high concentrations in the water and it formed a thick coating on both the oysters and baskets. ICPAES sample preparation removed these colloidal species of iron and aluminium from the water samples.

Site 7 was the only experimental oyster lease exposed to ASS-affected waters and located in the main channel of the Manning River (Figure 4.1). There was also a large difference in the mortality rate between large and small oysters at this site (Figure 4.11). Shell perforation was noticed in large and small oysters at Site 7 after the 9/8/99. Whole weight loss was measured in large oysters between the dates: 28/6/99 and 26/7/99.

The pH and EC at Site 7 was strongly influenced by rainfall in the Cattai Creek catchment (Sonter, 1999). The pH and EC varied considerably at this site ranging from 5.27 to 7.90 and 1.9 to 33.8 dS m^{-1}, respectively (Tables 4.3 and 4.4). This variation can be attributed to the strong combined influence of Cattai Creek and the Manning River at this location. Dissolved aluminium and iron were measured at this site only in low concentrations. However, suspended iron flocs were commonly observed at this site and formed a thick coating on the oysters and baskets, especially following high rainfall.

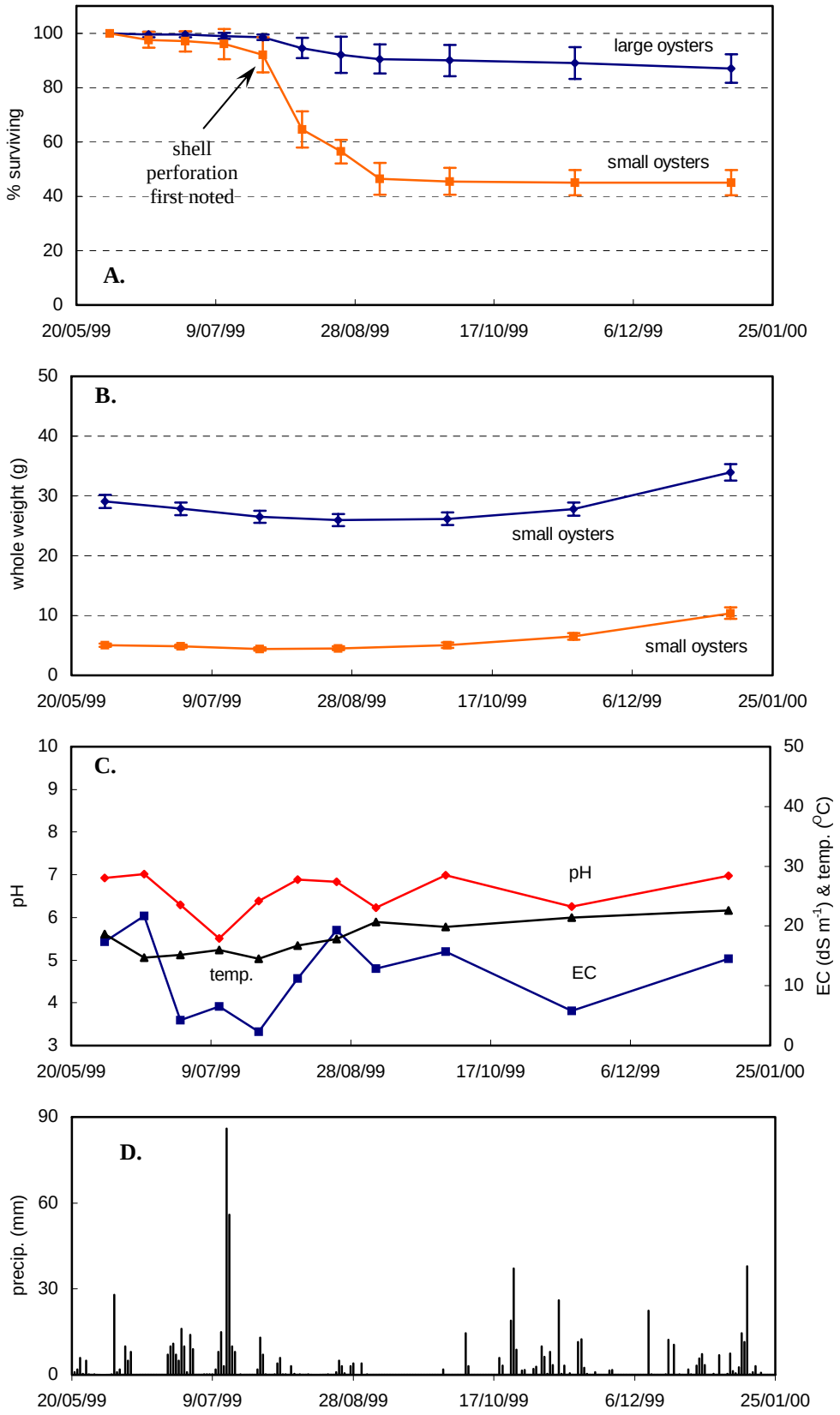


Figure 4.10 Site 6 summary display: (A) mean percentage survival (± 95% CIs); (B) mean whole weight (± 95% CIs); (C) pH, EC and temperature; and, (D) rainfall.

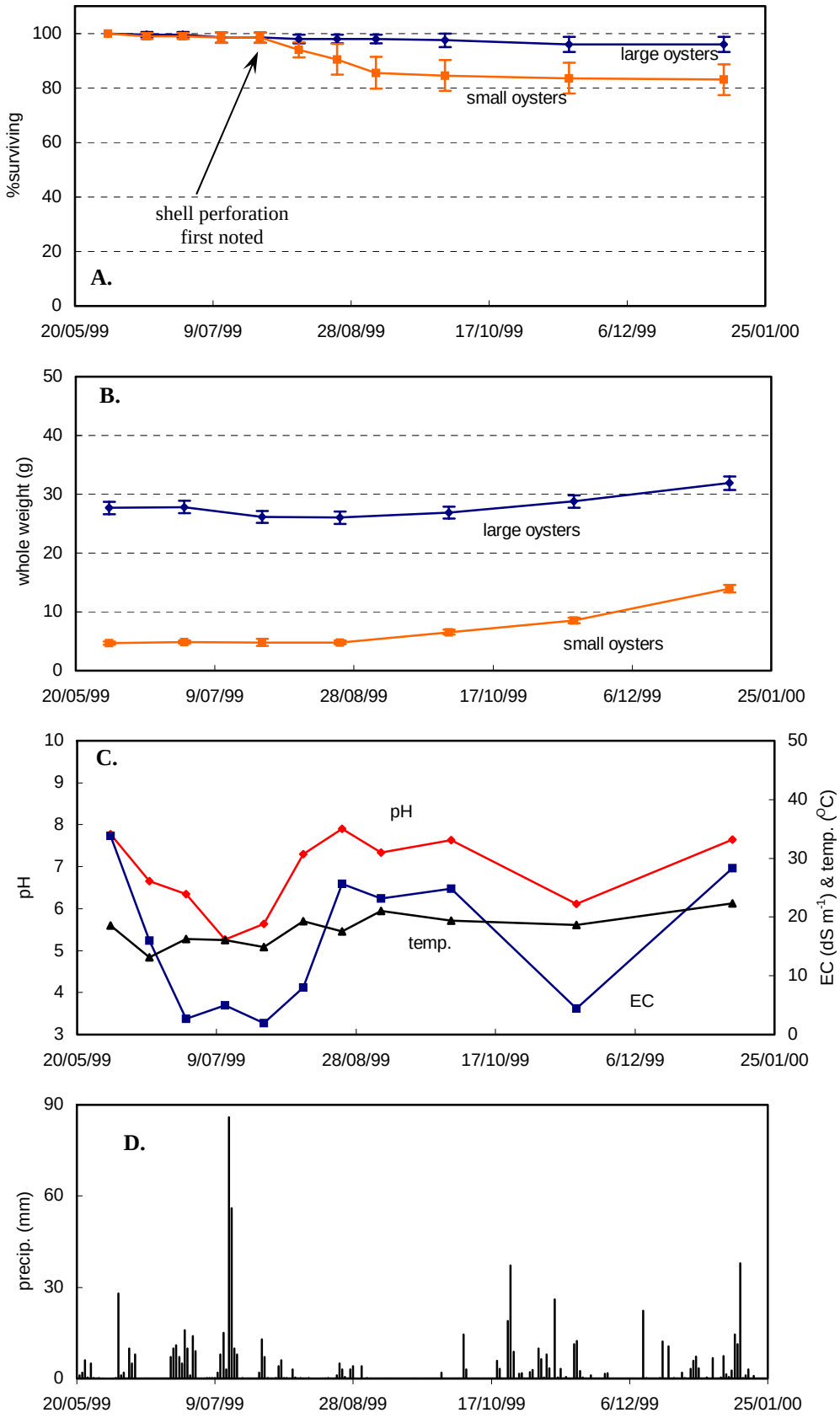


Figure 4.11 Site 7 summary display: (A) mean percentage survival (± 95% CIs); (B) mean whole weight (± 95% CIs); (C) pH, EC and temperature; and, (D) rainfall.

4.4.4 Oyster Survival

Figures 4.12 and 4.13 show the mean survival of large and small oysters at the seven experimental sites. Survival of small and large oysters at Sites 1, 2 and 3 were high and very similar on all sampling dates (Figures 4.5, 4.6, 4.7, 4.12 and 4.13). The mean (± 95% CI) survival percentage for large and small oysters located at Sites 1, 2 and 3 for the period between 1/6/99 and 10/1/00 was 95.3 ± 2.0% and 96.7 ± 1.3%, respectively.

Figures 4.5A to 4.11A display the difference in survival between large oysters and small oysters at each individual site. An increase in mortalities of large and small oysters at Sites 4, 6 and 7 after the 26/7/99 can be seen on Figures 4.12 and 4.13. Mortalities at Site 5 in large oysters started to occur after the 12/7/99 sampling and small oysters at Site 5 experienced high mortality after the 26/7/99 sampling (Figures 4.9 and 4.12). The increase in mortality at Site 5 is dramatic in comparison to the other acidified sites. At Sites 4, 6 and 7, large oysters had a better survival rate than small oysters at the same site between the 28/6/99 and 10/1/00.

The results of the three factor ANOVA for the comparison of acid sites to the reference sites on the 23/8/99 and 10/1/00 are listed in Table 4.6. The survival data measured at all sites on the 23/8/99 and the 10/1/00 are listed in Appendix 3I.

Table 4.6 Summary of the three factor analysis of variance results for the comparison of acidified sites to the reference sites on the 23/8/99 and 10/1/00.

Source of Variation	df	23/08/99			10/01/00		
		Mean Square	F	p	Mean Square	F	p
Acid	1	10624.381	2.192	0.199	17222.625	2.272	0.187
Site (Acid)	5	4699.867	13.599	0.006	6005.275	25.958	0.001
Size	1	757.786	1.516	0.443	1944.643	1.038	0.495
Acid x Size	1	493.714	1.429	0.286	1807.149	7.812	0.038
Size x Site(Acid)	5	345.600	3.389	0.012	231.342	3.527	0.009
Error	42	101.976			65.595		

The results listed in Table 4.6 indicate that there was a significant difference in the mean survival rates among experimental sites on the 23/8/99 and 10/1/00. There was no significant difference between the mean survival rate of small oysters and mean survival rate of large oysters on the 23/8/99 and 10/1/00 across all sites.

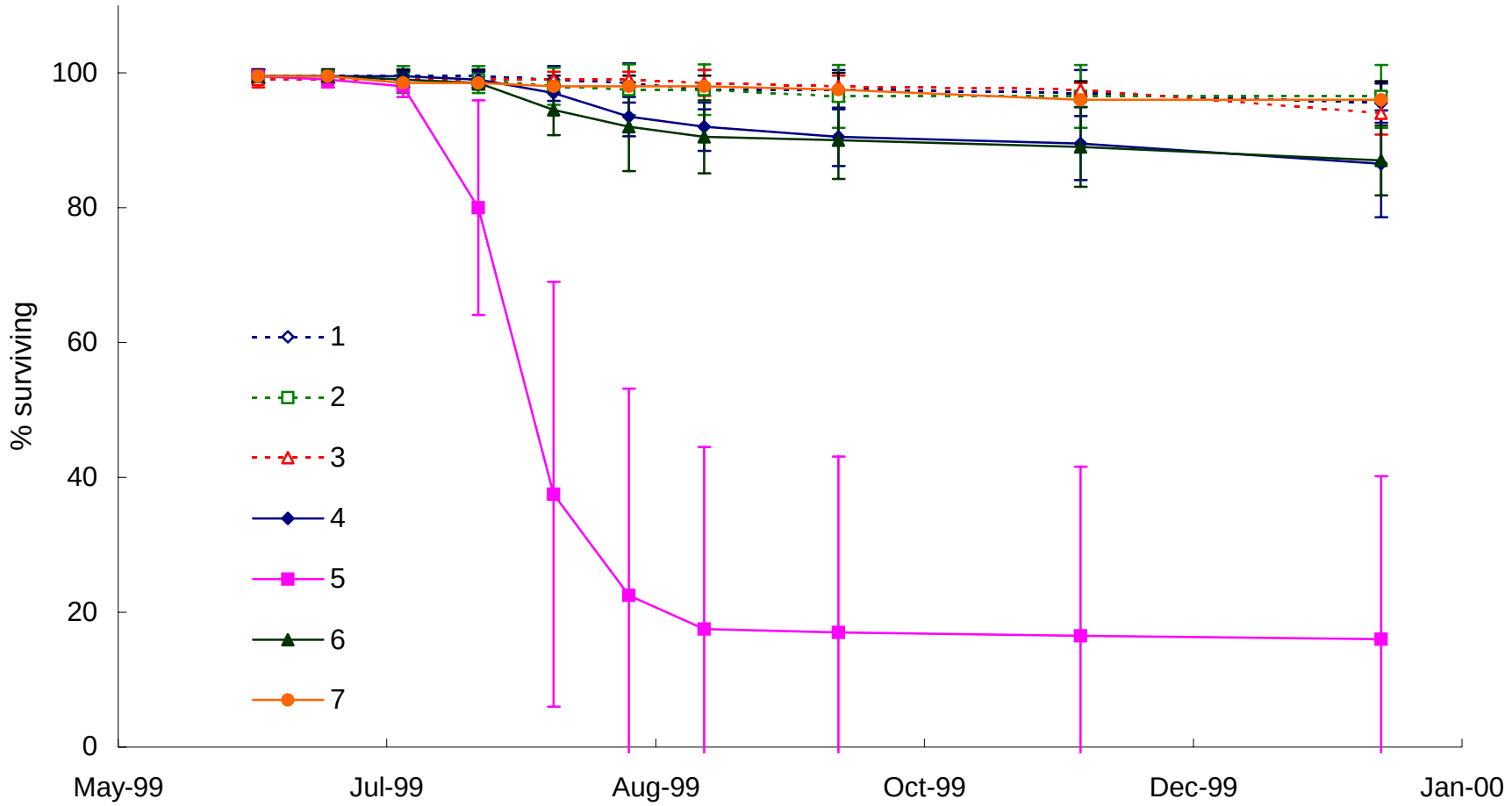


Figure 4.12 Mean survival (± 95% CIs, n=4) of large oysters at experimental sites.

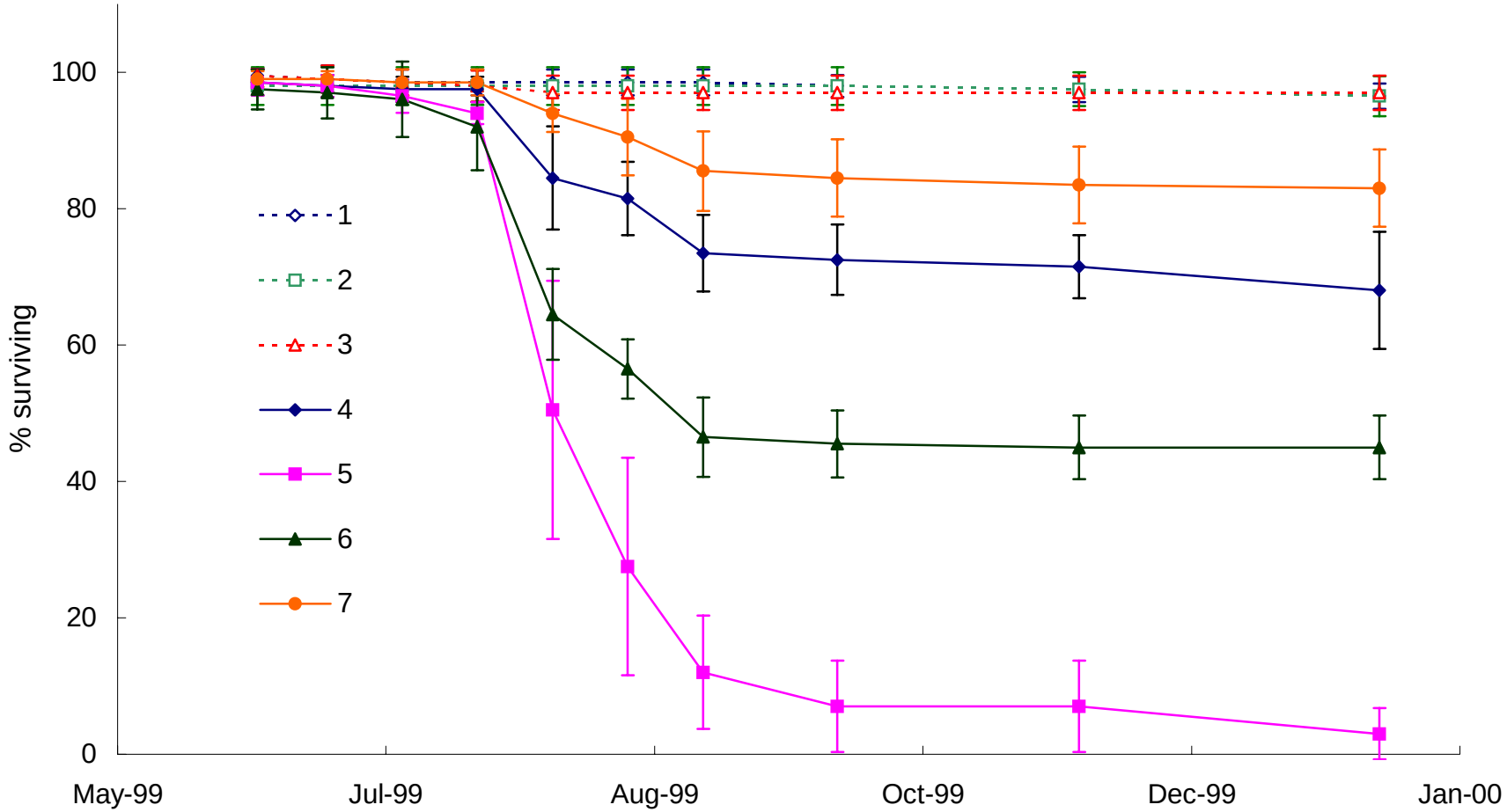


Figure 4.13 Mean survival (± 95% CIs, n=4) of small oysters at experimental sites.

There was no interaction between the factors Acid and Size on the 23/8/99 but there was an interaction between these two factors on the 10/1/00. There was an interaction between the factors Site (Acid) and Size on both the 23/8/99 and the 10/1/00.

Post hoc analysis using a Tukey HSD test of the interaction between the factors Size and Site(Acid) was performed for the dates 23/8/99 and 10/1/00 to examine differences between sites for large and small oysters and between large and small oysters at different sites. The results of the *post hoc* analysis are displayed in Appendix 3I and confirmed that small oysters at ASS-affected sites experienced significantly higher mortalities than large oysters at the same sites on the 10/1/00. However, there was not a significant difference between large and small oyster survival at the sites isolated from ASS-affected waters on this same date.

Therefore, from the results listed in Table 4.6 and displayed graphically in Figures 4.8 to 4.13 and Appendix 3I, at the conclusion of the experiment small oyster survival was significantly lower than large oyster survival at the sites exposed to ASS-affected waters. Also, sites isolated from ASS-affected waters had significantly higher survival percentages of large and small oysters compared to sites exposed to ASS-affected waters with the exception of Site 7.

4.4.5 Oyster Growth Rate

The growth rate of oysters at Site 5 was not calculated because of the high mortality rate experienced at this site during Experiment 1. Growth rates were calculated using oyster whole weight during a period of high rainfall (day 0-83) and over the entire experiment (day 0 to 224). The percent per day weight increases for oysters are displayed in Figures 4.14 and 4.15.

4.4.5.1 Growth Rates During High Rainfall

Figure 4.14 displays the percent per day mean weight increase calculated from whole weight of large and small oysters during a period of high rainfall at the start of Experiment 1. Percent per day mean weight increase for small oysters located at Sites 1, 2 and 3 were substantially greater than at Sites 4, 6 and 7 (Figure 4.14). Small oysters at Sites 4 and 6 lost weight during this period and therefore returned a negative result. Additionally, small oysters at Sites 1, 2 and 3 gained considerably more weight than large oysters located at the same sites (Figure 4.14).

Sites 1 and 3 were the only locations at which large oysters experienced mean weight increases during the high rainfall period (Figure 4.14). Sites 1 and 3 are the closest to the Manning River ocean entrance at Harrington allowing faster recovery from 'fresh' or low EC conditions following rainfall compared to all other sites. The average weight of large oysters located at Sites 2, 4, 6 and 7 decreased during this period. Water quality measurements collected at Site 2 did not detect acidic water, however the recorded EC values were low during this period because of recurrent rainfall events in the Manning River catchment. Site 2 was located 11.9 kilometres upstream (Figure 4.1 and Table 4.1), which would mean that recovery from fresh conditions would take longer than at Sites 1 and 3 because of tidal attenuation and proximity to freshwater base flows.

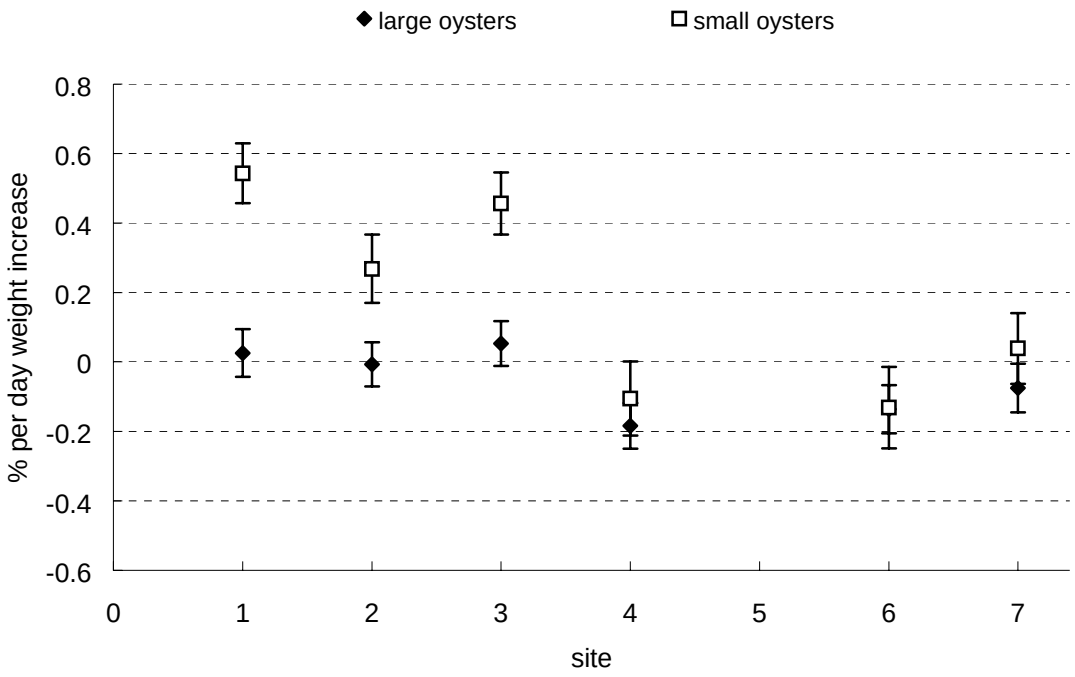


Figure 4.14 Instantaneous growth for small and large oysters presented as percentage increase per day in weight for each site during the first 83 days of the experiment (mean growth rates plotted with ~ 95% CIs).

4.4.5.2 Growth Rates From June to January

Figure 4.15 displays the mean instantaneous growth rates of large and small oysters for the entire duration of Experiment 1 (i.e. 1/6/99 to 10/1/00). The mean instantaneous growth rates of large and small oysters at all sites were positive in this period. Large and small oysters at Sites 1, 2 and 3 gained more weight than the large and small oysters at Sites 4, 6 and 7. At all sites, small oysters had a faster mean growth rate than the large oysters located at the same sites (Figure 4.15). Sites 1 and 3 had the best growth performance in small oysters over the entire experiment (day 0 to 224), as was the case during the high rainfall period. Figure 4.15 indicates that sites exposed to ASS-affected waters experience good growth in periods of lower rainfall. This is more evident in the growth data recorded from large oysters at the experiment sites displayed in Figure 4.15. In this instance, the difference between sites exposed to ASS-affected waters and those sites isolated from ASS-affected waters was much less apparent when compared to the period of high rainfall. However, all of the oysters exposed to ASS-affected waters at Sites 4, 6 and 7 during this experiment had a mean instantaneous growth rate value that was lower than was measured in oysters of the same size at the reference sites (Sites 1, 2 and 3).

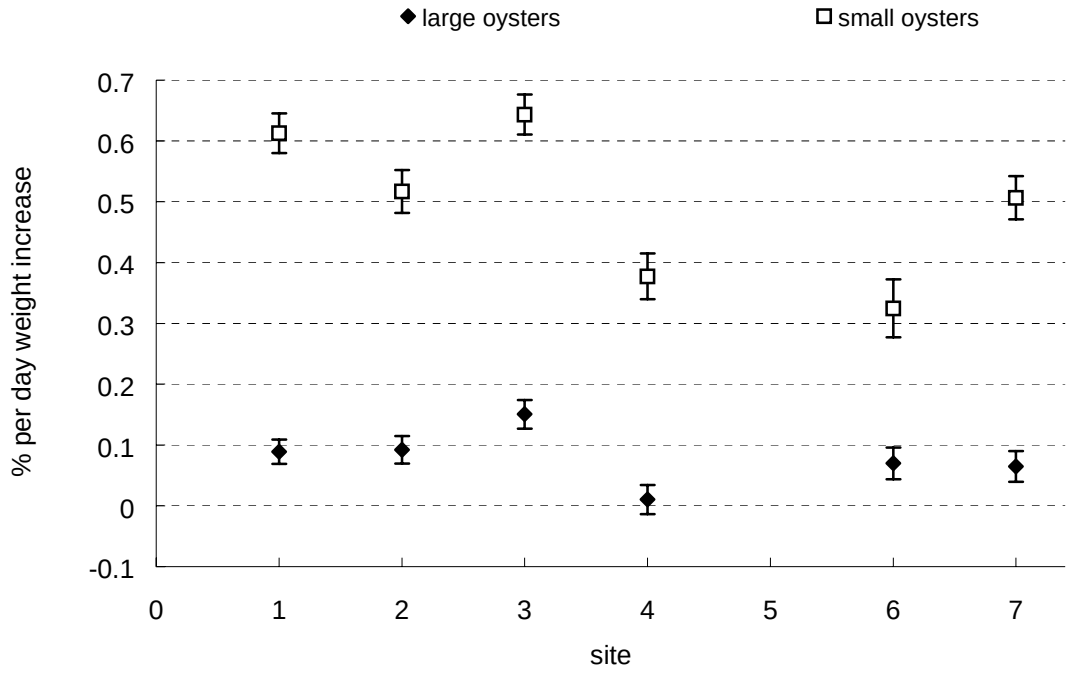


Figure 4.15 Instantaneous growth for small and large oysters presented as percentage increase per day in weight for each site measured over the entire experiment (224 days) (mean growth rates plotted with ~ 95% CIs).

4.5 RESULTS: EXPERIMENT 2

4.5.1 Water Quality Conditions

Water quality monitoring during Experiment 2 was not carried out as frequently as for Experiment 1. A different rainfall pattern was evident from Figures 4.2 and 4.3 between the two experiments. The data from the SDL located at Site 4 shows that recurrent rainfall events throughout July 1999 (Figure 4.2) caused pHs to fall below 5 and resulted in highly variable EC levels at this site (Figure 4.4). Table 4.7 displays the pH, EC, temperature and alkalinity data measured during Experiment 2. Additional water quality data measured during Experiment 2 are tabulated in Appendix 3J.

The lowest pH measured during Experiment 2 was 5.32 at Site 6 on the 7/8/00 (Table 4.7). This was the only date that moderately acidified waters were detected at the experimental sites, with the exception of Site 4. However, high concentrations of suspended iron flocs were observed causing the water to appear a deep red/burgundy colour on the 6/4/00, 9/6/00 and 7/8/00 at Sites 4, 6 and 7. On these dates the alkalinity levels measured at Sites 4, 6 and 7 were lower than Sites 1, 2 and 3, with the exception of Site 4 on the 6/4/00 (Table 4.7). Temperature has a significant influence on oyster growth and temperatures at all sites were typically low throughout the winter months.

Table 4.7 Water quality at Sites 1, 2, 3, 4, 6 and 7 measured during Experiment 1.

Sampling Date	Site	pH	EC (dS m^{-1})	Temp (^{O}C)	Alkalinity (mg L^{-1})
01/02/00	1	8.18	44.6	22.88	103
	2	8.02	32.7	23.47	76
	3	8.29	51.7	22.73	73
	4	7.93	28.7	23.21	76
	6	7.46	23.9	23.74	65
	7	7.96	33.4	23.41	76
06/04/00	1	8.02	34.2	22	76
	2	7.84	19.7	22.26	73
	3	8.08	34.6	22.2	80
	4	7.84	20.9	22.49	76
	6	6.88	13	22.35	60
	7	7.36	20	22.43	60
09/06/00	1	8.12	45.4	14.06	95
	2	8.05	34.4	13.15	80
	3	8.18	45.8	14.39	85
	4	7.93	34.6	12.33	65
	6	7.65	35.1	12.59	73
	7	6.76	30.6	11.45	47
07/08/00	1	7.93	34	16.37	73
	2	7.75	25	16.06	85
	3	7.79	36.7	16.55	76
	4	5.64	21.7	17.18	47
	6	5.32	24.2	14.21	NS
	7	6.97	10.8	14.13	30
23/10/00	1	8.19	48.2	20.01	76
	2	7.67	35	26.69	73
	3	8.31	46.4	21.09	65
	4	7.84	33.8	21.59	90
	6	NS	NS	NS	NS
	7	8.05	37.5	21.14	73
12/01/01	1	7.99	40.5	23.41	80
	2	7.73	33.3	27.12	100
	3	7.91	39	23.96	95
	4	7.81	35.2	24.42	90
	6	7.58	37.4	24.74	90
	7	7.82	36.3	24.07	80

NS = not sampled

4.5.2 Oyster Condition Index

The initial mean (± 95% CIs, n = 71) condition index of oysters at the start of Experiment 2 (on the 1/2/00) was 109.7 ± 4.9. Site 6 experienced high oyster mortality rates and all of the experimental oysters were dead at this site by the 9/6/00. Experimental oysters were missing at Site 1 on the 12/01/01 which prevented sampling on this date. The mean condition index measured at each site on the six sampling dates is displayed in Figure 4.16. The oyster condition index data is included in Appendix 3K.

The mean condition index of oysters from Site 6 on the 6/4/00 was greater than all other sites (Figure 4.16). The pH and EC at Site 6 on this date was 6.88 and 13 dS m^{-1}, respectively. A decrease in the mean condition index was measured from the start of the experiment to the first sampling date on the 6/4/00 at all sites. High rainfall occurred during mid-March where more than 240 mm of rainfall was measured over 9 days in the lower Manning River catchment. It is probable that the oysters spawned between the 1/2/00 and the 6/4/00 which would account for the decline in condition index. During this 60-day period there were small rainfall events (Figure 4.3) and water temperatures were low. From the 7/8/00 to the 23/10/00 mean condition index increased at all sites. Likewise, all sites recorded increases in condition index for the period 23/10/00 to 12/1/01, with the exception of Site 2. The decrease in condition index at Site 2 may be attributed to early spawning by the oysters. All sites show a rapid increase in condition between 7/8/00 and the 23/10/00, probably due to gonad development. Sites 2 and 3 had the most pronounced increases with the condition index at Site 3 increasing from 80.9 to 140.9 and the condition index at Site 2 increasing from 77.9 to 129.7 during this period.

Table 4.8 summarises the results of the three factor ANOVA analysis. The mean condition indices measured at the reference sites were not significantly higher than the condition indices measured at sites exposed to ASS-affected waters. However, there was a significant difference between the mean condition indices measured on the five sampling dates. The ANOVA results indicate that there was an interaction between the two factors Site(Acid) and Date and also Acid and Date.

Table 4.8 Summary of the three factor analysis of variance results for the comparison of condition index at acidified sites and reference sites.

Source of Variation	df	Mean Square	F	p
Acid	1	5484.035	0.677	0.443
Date	4	41781.080	8.396	0.033
Acid x Date	4	4886.695	3.429	0.039
Site(Acid)	4	3548.349	2.549	0.094
Site(Acid) x Date	12	1373.557	3.862	0.000
Error	260	355.656		

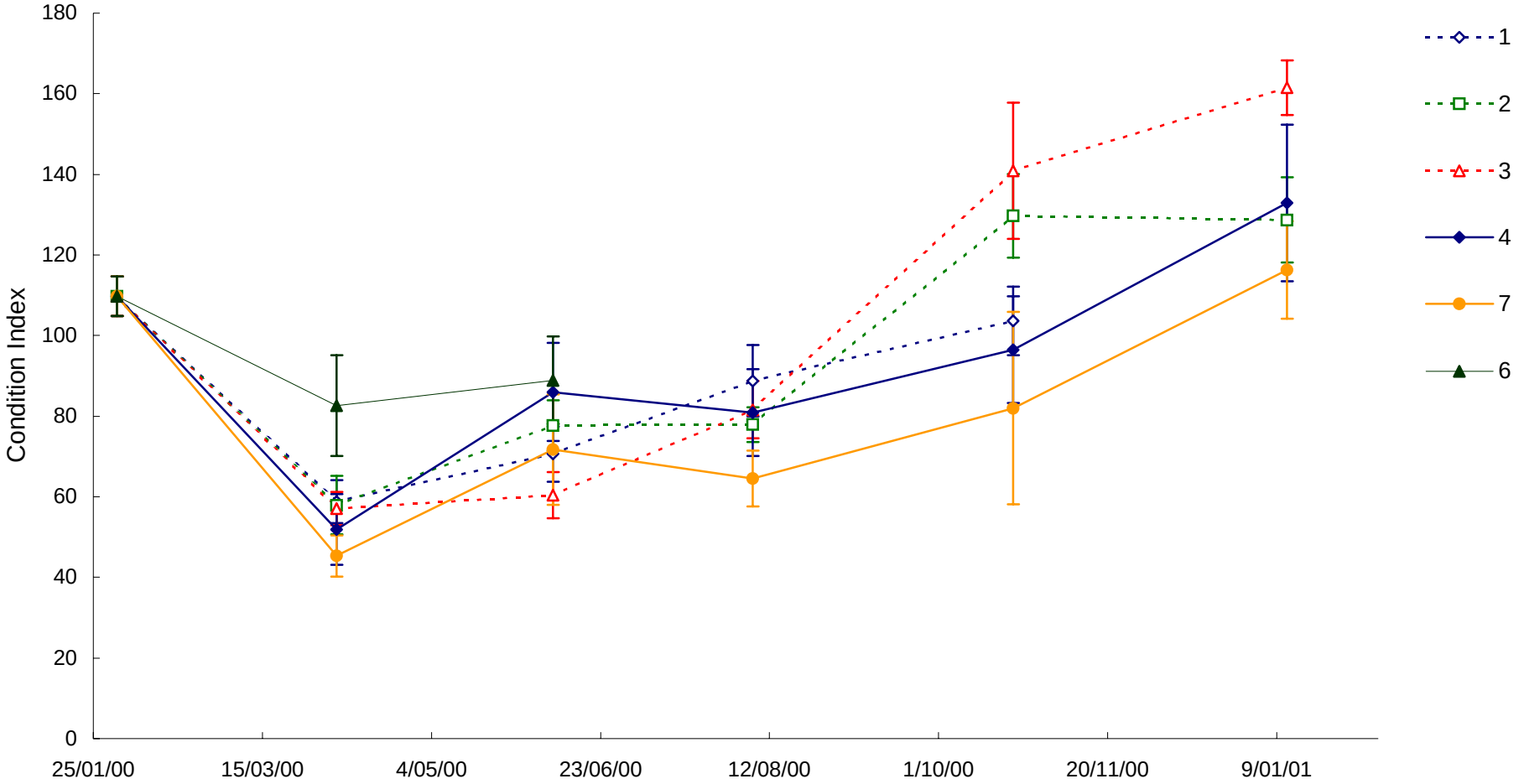


Figure 4.16 Mean condition index (95% CIs, n = 12) measured at Sites 1, 2, 3, 4, 6, and 7.

4.6 DISCUSSION

4.6.1 Water Quality

The water quality monitoring at the seven experimental sites has measured extended periods of acidic conditions at sites exposed to ASS-affected waters (Sites 4, 5, 6 and 7). Sites that had a low probability of exposure to ASS-affected waters (Sites 1, 2 and 3) had circumneutral pHs for the entire experimental period.

The pH and EC levels at locations impacted by ASS-affected waters fluctuated dramatically and were dependant upon antecedent climatic conditions, predominately rainfall. Elevated concentrations of iron, aluminium and manganese were measured at the sites exposed to ASS-affected waters and were not detected at the sites where pHs were circumneutral. Suspended iron flocs were commonly observed at all of the sites impacted by ASS-affected waters, particularly after rainfall. These results are consistent with the findings relating to estuarine acidification presented in Chapter 3.

Lease sites that were located close to the acid outflow sources, especially in areas of the estuary that are well-flushed (e.g. Site 4), experienced widely fluctuating pH levels, EC levels and dissolved metal concentrations. Water quality for such leases was dependent on the tidal stage and the nature of the drain outflow water.

4.6.2 Oyster Survival

Small and large oysters at sites exposed to ASS-affected waters experienced significantly higher mortality than small and large oysters at sites that were not exposed to ASS-affected waters at the conclusion of the experiment (10/1/00). ASS-affected waters also impacted the survival of small oysters significantly more than large oysters at the same sites on this date.

Oyster leases located in tidally attenuated areas of the estuary suffered the highest mortality rates due to the acidic and fresh conditions persisting for prolonged periods. Tidally attenuated areas typically have reduced brackish water mixing and capacity to neutralise the acidity.

Sites that are well flushed and exposed to ASS-affected waters had dramatic and wide-ranging variations in pH and EC. This provided oysters with short periods of more favourable water quality conditions, mostly during the final stages of the flood tide. The flood tide waters not only neutralised the acidity but also increased the salinity which gave oysters an opportunity to actively feed.

Oyster growing areas situated in tributaries that have an extensive backswamp system that has had drainage modifications are more susceptible to problems associated with estuarine acidification. The Cattai Creek and Lansdowne River catchments contain extensive backswamp areas (Birrell, 1987; Naylor *et al.*, 1995). Cattai Creek and the Lansdowne River both receive the drainage waters of heavily modified floodplains (Sonter, 1999; Tulau, 1999) and the experimental sites located in or near these systems experienced low oyster survival rates during Experiment 1.

The primary cause of mortality in small oysters was exposure to acidified water that entered through a perforated left valve. Oysters are able to protect their soft tissue from the direct effects of acid by closing their valves (Chapter 2 and Dove, 1997).

Once the shell is breached through a combination of internal and external shell dissolution, acidified water directly impacts on the oyster tissue. Small oysters are more susceptible to shell perforation because their shells have not fully developed and are therefore thinner. Small oysters had a significantly higher mortality rate than larger oysters at the same sites and this high mortality rate was directly related to the prevalence of shell perforation in smaller oysters. However, dead oysters of both sizes with no shell perforation were consistently found at all of the sites exposed to ASS-affected waters.

Death in oysters that did not experience shell perforation was attributed to the acidic conditions and elevated concentrations of iron and aluminium. It is highly likely that these factors have a synergistic impact on oyster survival.

Bamber (1987; 1990) found the critical pH for significant mortality after 30 days exposure ranged from 6.6 for *M. edulis* down to 6.0 for *C. gigas*. The minimum pH values measured during Experiment 1 were dramatically lower than these levels. The time taken for dramatic mortality in small Sydney rock oysters in this present study was greater than 30 days and ranged between 42 days and 70 days.

Very high concentrations of iron were measured and observed during the field investigations of this study. Winter (1972) demonstrated that iron at neutral pH levels significantly reduced the survival of *M. edulis*. Cruz (1969) showed that when iron was absorbed in large quantities by the digestive tract it caused mortality and internal lesions in fish. The effects of iron are investigated in further detail in Chapter 7 of this present study. The concentrations of aluminium at sites exposed to ASS-affected waters were also elevated and aluminium cannot be discounted as a factor for the high mortality rates measured at these locations. Chapter 7 of this study also investigates the effects of aluminium and iron at pH 5.1 on oyster soft tissue to experimentally elucidate the possible causes for mortalities in oysters resulting from exposure to ASS-affected waters.

In conclusion, sites exposed to ASS-affected waters experienced low pH levels, reduced EC levels and increased concentrations of dissolved and suspended metals, namely iron and aluminium. Increased mortality rates in large and small oysters were measured at sites that recurrently experienced these conditions following high rainfall. Sites that are well-flushed and distant from ASS outflows experienced low mortality rates when compared to sites in well-flushed areas that are close to the ASS outflows. It is recommended to avoid areas that are acidified after high rainfall or relocate oysters, if practicable, in acid-prone areas in the event of high rainfall. Cultivation of smaller oysters in areas affected by ASS outflows is strongly discouraged.

4.6.3 Oyster Growth

There was an association between reduced oyster growth rates and sites that were exposed to ASS-affected waters. Small and large oysters at sites exposed to ASS-affected waters had reduced growth rates when compared to the same size oysters at the experimental sites not exposed to ASS-affected waters.

All of the sites exposed to ASS-affected waters during Experiment 1 showed a reduced growth rate (in terms of weight gain and shell height increases) compared to sites isolated from ASS-affected waters. Minor or negative growth rates in small and

large oysters were measured during periods of high rainfall, which also corresponded to reduced EC levels and/or poor water quality conditions at these sites. During this period, sites that were isolated from ASS-affected waters had a marginally better growth rate in large oysters and strong growth in small oysters even though water temperatures were low. The variation that existed between sites impacted by ASS-affected waters and sites not impacted was most evident when growth rates were calculated for the entire experiment. In this instance growth rates at sites that experienced acidification following rainfall showed a reduced growth rate in terms of whole weight and shell height. This difference becomes even more apparent when comparing the growth data for small oysters at the impacted sites to the three reference sites.

Reduced growth rates in oysters were chiefly attributed to low pH and EC levels allowing for fewer feeding opportunities for oysters at sites exposed to ASS-affected waters. Shell dissolution (Bamber, 1987; 1990) and high concentrations of iron (Winter, 1972) are also believed to contribute to the poor growth performance measured in oysters at sites exposed to ASS-affected waters. Additionally, geomorphic and hydrological factors mentioned in Section 4.6.2, which increase the probability of survival, are also likely to enhance growth rates based on the results obtained from this experiment.

4.6.4 Oyster Condition Index

The condition index data highlights the variability that exists between different oyster growing areas of the Manning River. The combined mean condition indices for the entire experiment were lower at sites exposed to ASS-affected waters relative to the reference sites. Throughout the autumn and winter months oyster condition at all sites were similar. Differences in condition index were not apparent until spring where oysters at Sites 2 and 3 had rapid increases in condition as their gonads developed. In the middle of summer, oysters at Sites 3, 2, 4 and 7 had comparable condition indices. Late winter and early spring was characterised by low rainfall and there were small recurrent rainfall events throughout late spring and early summer. Water quality data indicates that conditions were good at all sites during this period.

On the 6/4/00, the mean oyster condition index was high at Site 6 compared to other sites. The decrease in condition at all other sites between the 1/2/00 and the 6/4/00 was attributed to spawning in the oysters. A very high mortality rate was recorded at Site 6 on the 6/4/00 and no oysters remained after the 9/6/00. It is likely that the large rainfall event in early March (Figure 4.3) caused a decline in water quality conditions at Site 6 (Section 4.5.1). High concentrations of colloidal iron were observed at this site on the 6/4/00 suggesting the mobilisation of ASS oxidation products into the Lansdowne River.

Dead oysters removed from Site 6 did not show any evidence of shell perforation, however oyster shells were smothered in iron flocs and the soft tissue appeared an ochre red colour. High concentrations of colloidal iron were observed during Experiment 1 at this site.

Bamber (1987; 1990) showed that tissue growth was reduced at pH values less than 7.0 in *V. decussata, O. edulis, C. gigas and M. edulis.* For example, the shell area and flesh weight of *C. gigas* decreased as pH was decreased from 8 to 5.5 (Bamber,

1990). Pronounced flesh weight reductions were also measured in *V. decussata* (Bamber, 1987). These findings are relevant to the current study and help explain the overall lower condition index values measured in oysters at the sites exposed to ASS runoff. The effect of acidic treatments containing elevated iron and aluminium levels on oyster soft tissue is examined in Chapter 7 of this study.

4.7 CHAPTER SUMMARY

The water quality data showed that oysters located at Sites 4, 5, 6 and 7 were exposed to prolonged periods of acidification, particularly during the ebb tide, caused by ASS outflows during the experimental period. The water quality data also showed that oysters at sites 1, 2 and 3 experienced circumneutral pH levels during the same period.

The results of the field exposure experiments clearly demonstrated that ASS-affected waters have a detrimental impact to Sydney rock oysters and are the cause of reduced survival and growth. ASS-affected waters were also the cause of reductions in oyster condition index. A significant finding from Experiment 1 was the extent that ASS-affected waters impact small oysters, which is consistent with Bamber's (1987; 1990) findings.

These experiments have provided considerable evidence that supports the observations of oyster growers and helps to explain the production problems that they experience in certain areas of the Hastings River and Manning River. The laboratory investigations presented in Section III of this present study were designed to better understand oyster behaviour, examine the effects of acidification on oyster feeding processes and soft tissue. The following chapter examines associations between estuarine acidification and QX disease outbreaks in Sydney rock oysters.

5 THE EXAMINATION OF A POSSIBLE RELATIONSHIP BETWEEN ESTUARINE ACIDIFICATION AND QX DISEASE OUTBREAKS IN SYDNEY ROCK OYSTERS *Saccostrea glomerata*

5.1 INTRODUCTION

This chapter examines a possible relationship between water acidification at lease sites and QX disease outbreaks in Sydney rock oysters. This addresses Objective 3 of this study which was to 'identify environmental and management risk factors for specific diseases with particular reference to QX on the Tweed River'. The study was transferred to the Richmond River due to the possibility that the Tweed leases would close down during the study and affect field experiments.

Two prior studies have examined possible relationships between outbreaks of QX disease and exposure of Sydney rock oysters to acidified water. Anderson *et al.* (1994) described two outbreaks of *M. sydneyi* infection in oysters near the mouth of the Brisbane River. Before the first outbreak the pH fell slightly, but before the second outbreak it remained unchanged. Changes in salinity and temperature were minor. The results indicated that QX disease outbreaks were not correlated with fluctuations in pH, salinity and temperature of water in close proximity to the oysters. In a study on the Pimpama River, southeast Queensland, Wesche (1995) reported a QX disease outbreak in oysters not apparently exposed to acidified water and no evidence of QX disease in oysters exposed to a minor (0.6 unit) reduction in pH.

The study reported below was designed to test the hypothesis that exposure to acidified water increases the susceptibility of Sydney rock oysters to QX disease outbreaks. The study was conducted on the Richmond, rather than the Tweed, River because of proximity to RVL Wollongbar and consequent cost savings.

5.2 MATERIALS AND METHODS

Between December 1997 and May 1998, Sydney rock oysters were placed weekly at three commercial oyster leases on North Creek, a tributary of the lower Richmond River which drains extensive ASS areas. The three leases (Figure 5.1) were selected on the basis of the likely variability in exposure to acidified runoff, i.e. the furthest upstream lease (lease N: relatively high probability of exposure to acidified upper catchment runoff), the furthest downstream lease (lease S: relatively low probability of exposure to acidified upper catchment runoff because of increased tidal exchange) and a central lease (lease B: intermediate probability of exposure).

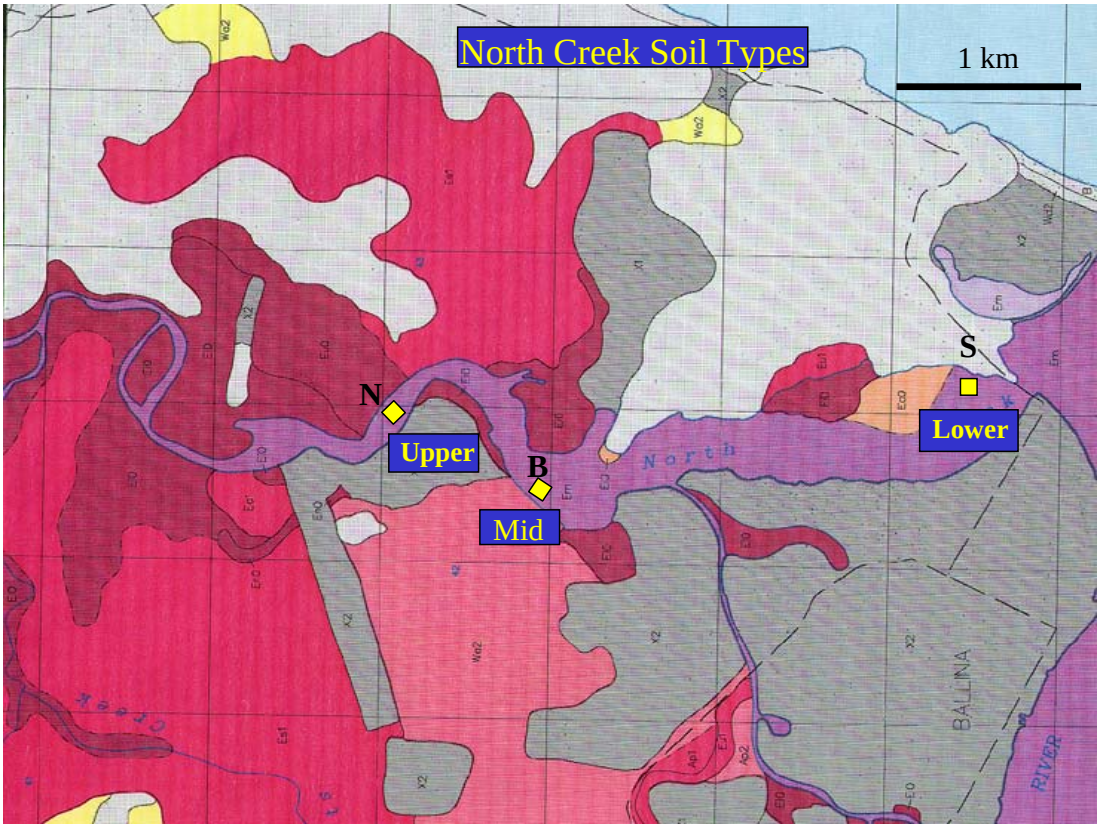


Figure 5.1 Location of sites N (upper), B (mid) and S (lower), North Creek, Richmond River (Adapted from DLWC, ASS Risk Map Series).

Sentinel (experimental) oysters were obtained from the Hastings River NSW, where QX disease has never been detected (Callinan and Smith, unpublished data). Typically, 100 oysters (50+ oysters in each of two sealed mesh baskets) were inserted at approximately 1-week intervals at each lease at the usual growing height. Oysters were left in place for five weeks before being removed and examined for evidence of QX disease. Early in the study period, when the risk of QX disease occurrence was low, oyster batches were inserted less frequently and left in place for longer periods.

Using a Horiba U10 Water Quality Checker (Australian Scientific, Kotara, NSW), water quality was measured periodically (usually weekly) at low tide, adjacent to the oysters at each lease. The following water quality variables were measured: temperature, pH, dissolved oxygen concentration, conductivity. The batches of oysters were removed after approximately five weeks and each surviving oyster (up to a total of 100 from each lease) was examined microscopically for evidence of QX disease as follows.

5.2.1 Digestive Gland Impression Smear

Oysters were washed to remove external mud and opened to expose the ventral surface of the gonad. The digestive gland was then exposed by cutting through the gonad with a clean scalpel blade. A sample of digestive gland approximately 1 x 3 x 3 mm was excised and, after blotting its surfaces to remove excess fluid, a cluster of

approximately 10 impression smears of the sample surfaces was made on a clean glass slide. The smears were air dried and stained with Hemacolor before being mounted under a cover slip. Smears were then examined under oil immersion using a compound microscope for evidence of *M. sydneyi* plasmodia, secondary cells and later stages, all of which stain differentially. An oyster was considered positive for QX if two or more parasites were seen in its smear cluster.

5.2.2 Histopathology

A 'blind' study was conducted to compare the sensitivity and specificity of the impression smear examination with histopathological examination of the digestive gland; the latter is generally considered to be the 'gold standard' test for QX disease. After impression smear preparation as above, 100 individually identified oysters collected from lease S during a period of high QX disease prevalence were fixed in 10% neutral buffered formalin. A transverse section was cut through the middle part of the digestive gland, embedded in paraffin wax, sectioned at 4 μm and stained with haematoxylin and eosin. Sections, labeled so as to conceal their relationship with the corresponding impression smears, were then examined using a compound microscope for evidence of *M. sydneyi* infection. The diagnostic criteria listed in Table 5.1 were used for assessment of sections.

Table 5.1 Diagnostic criteria for assessment of sections.

	Digestive gland connective tissue	**Digestive gland epithelium**
Negative QX	Not more than 2 interstitial inflammatory cell aggregations	No change
Probable QX	Multifocal or locally extensive interstitial inflammatory cell aggregations	No change
Positive QX	Multifocal, locally extensive or diffuse interstitial inflammatory cell aggregations	Cellular degeneration, necrosis or proliferation (*M.sydneyi* are rarely seen at 5 wks exposure)

5.3 RESULTS

5.3.1 Relationships Between QX Disease Outbreaks and Acidification

Severe QX outbreaks (with up to 95% of oysters infected) occurred at all three leases between mid-January and mid-May 98. Results are summarised in Table 5.2. There was no evidence of significant acidification at the downstream lease (Lease S) during the study period. However, acidification occurred at the upstream lease (Lease N) and the middle lease (Lease B), where pH values fell to 4.9 and 6.7, respectively, for a period of less than one week immediately after heavy rain on 16 April (Table 5.3). No significant variations in water temperatures or dissolved oxygen concentrations were detected at any of the leases during the study.

Table 5.2 QX disease prevalence in batches of sentinel oysters placed in three leases in North Creek (n d : not done). Refer to Figure 5.1 for location of sites.

			QX Disease Prevalence (%)		
Insertion	**Date in**	**Date out**	**Lease N**	**Lease B**	**Lease S**
1	05-Dec-97	07-Jan-98	0	n d	0
2	19-Dec-97	22-Jan-98	16	n d	1
3	24-Dec-97	06-Feb-98	64	n d	9
4	07-Jan-98	13-Feb-98	32	2	33
5	15-Jan-98	21-Feb-98	55	3	49
6	22-Jan-98	28-Feb-98	60	16	70
7	02-Feb-98	08-Mar-98	88	18	80
8	06-Feb-98	14-Mar-98	66	15	59
9	13-Feb-98	22-Mar-98	80	38	50
10	21-Feb-98	29-Mar-98	94	61	73
11	28-Feb-98	05-Apr-98	95	51	77
12	08-Mar-98	13-Apr-98	75	22	42
13	14-Mar-98	19-Apr-98	24	0	5
14	22-Mar-98	26-Apr-98	2	1	27
15	29-Mar-98	03-May-98	33	10	39
16	05-Apr-98	10-May-98	0	0	19

Table 5.3 pH and conductivity values at the three monitored lease sites during the study period (n a : not available).

	pH			**Conductivity (dS/m)**		
	Lease N	**Lease B**	**Lease S**	**Lease N**	**Lease B**	**Lease S**
18-Jan-98	8.0	8.2	8.3	51.7	51.8	53.3
22-Jan-98	7.9	8.3	8.3	49.9	51.8	51.7
2-Feb-98	7.8	8.0	8.3	46.5	51.0	52.6
6-Feb-98	7.9	8.5	8.3	47.1	51.3	52.1
13-Feb-98	7.2	8.1	n a	40.0	38.5	43.6
21-Feb-98	7.5	7.9	8.0	37.0	37.1	40.8
28-Feb-98	7.6	7.9	8.0	43.8	46.1	48.2
8-Mar-98	7.7	8.0	8.1	46.0	46.0	46.2
14-Mar-98	7.5	8.1	8.1	46.1	48.9	48.5
22-Mar-98	7.5	7.9	8	43.6	44.7	44.6
29-Mar-98	7.2	7.2	7.2	45.5	46.1	45.6
5-Apr-98	7.5	7.8	8.1	42.1	43.3	45.7
13-Apr-98	7.8	8.1	8.1	44.5	45.2	45.5
16-Apr-98	7.4	7.9	8	28.3	35.6	42.8
19-Apr-98	4.9	6.7	7.7	7.2	19.6	33.7
26-Apr-98	6.6	8.1	8.1	17.1	35.1	42.1
3-May-98	6.8	7.8	8	31.2	37.7	41.3
10-May-98	6.4	8.2	8	14.0	31	40

At the time of the mid-April acidification, QX disease prevalence at each lease was declining, and this decline continued at essentially the same rate at the significantly acidified, upstream lease, the moderately acidified middle lease and the non-acidified downstream lease. QX disease prevalence increased at all three leases approximately 10 days after the rain event, with the highest increase occurring at the non-acidified downstream lease (Figure 5.2).

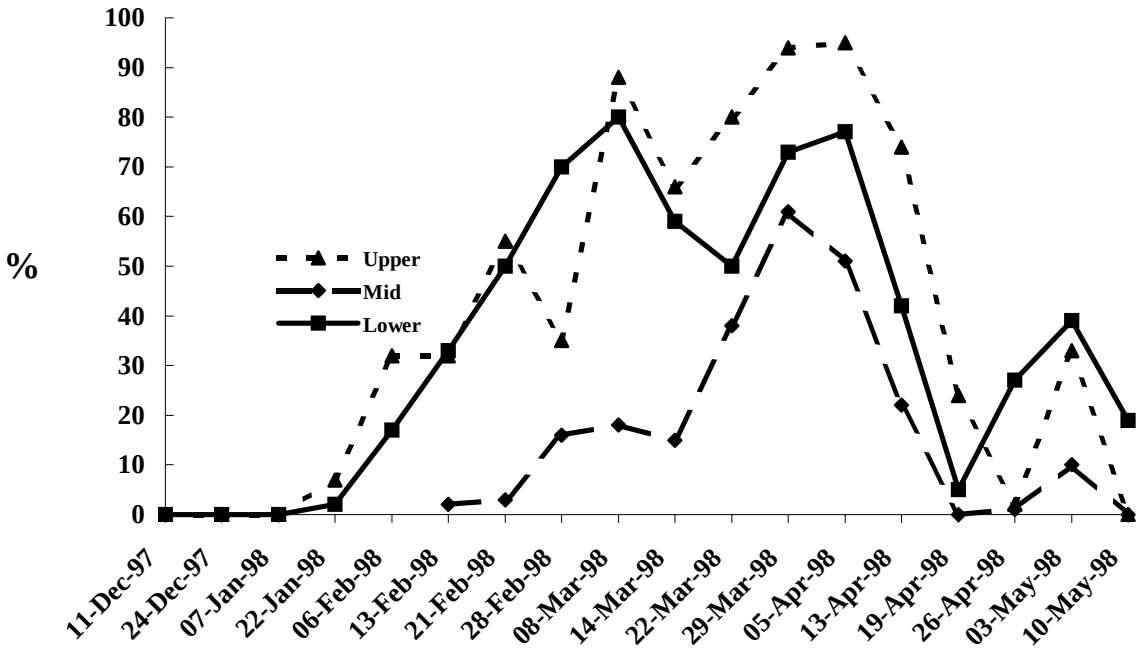


Figure 5.2 QX prevalence in sentinel oysters at North Creek leases after 5 weeks exposure.

5.3.2 Test Comparisons

Examination of digestive gland impression smears from 100 individually identified sentinel oysters from Insertion 5 (Table 5.2), Lease S, using the test criteria described above, indicated 49% were infected with *M. sydneyi*. Comparisons of these impression smear findings with histopathological diagnosis of QX disease, using the test criteria described above, in these oysters are shown in Table 5.4.

Table 5.4 Comparisons of QX disease diagnoses on 100 individually identified oysters from Insertion 5, Lease S, using digestive gland impression smear (imprint) and histopathology (section). (+ : positive QX disease, - : negative QX disease, P : doubtful QX disease)

Oyster	Imprint	Section
1	-	+
2	+	+
3	+	+
4	+	+
5	-	+
6	+	+
7	-	+
8	+	+
9	+	+
10	-	+
11	-	+
12	-	+
13	-	+
14	-	-
15	+	+
16	-	-
17	-	-
18	+	+
19	-	-
20	+	+
21	-	+
22	+	+
23	-	+
24	+	+
25	+	+
26	+	+
27	-	+
28	-	+
29	+	+
30	+	+
31	-	P
32	+	+
33	-	+
34	+	+
35	-	+
36	-	+
37	-	-
38	+	+
39	+	+
40	+	+
41	+	+
42	+	+
43	+	+
44	+	+
45	-	+
46	-	+
47	+	+
48	+	+
49	-	+
50	-	-

Oyster	Imprint	Section
51	+	+
52	-	-
53	-	+
54	+	+
55	-	-
56	-	+
57	+	+
58	+	+
59	+	+
60	-	-
61	-	-
62	-	+
63	+	+
64	-	+
65	+	+
66	+	+
67	-	+
68	-	-
69	-	-
70	+	P
71	-	+
72	-	+
73	+	+
74	-	-
75	-	+
76	+	+
77	-	P
78	-	-
79	-	+
80	-	+
81	-	+
82	+	+
83	-	+
84	-	-
85	-	-
86	+	+
87	-	+
88	-	+
89	+	+
90	-	P
91	+	+
92	+	+
93	+	P
94	+	+
95	-	+
96	+	+
97	+	+
98	+	+
99	+	+
100	+	+
Total +	49	80

The test comparison can be summarised as follows:

	Positive	**Probable**	**Negative**
Histopathology	80	6	14
Impression smear	49	-	51

These results indicate that, relative to the putative 'gold standard' histopathology test for QX disease, the digestive gland impression smear test has a sensitivity of approximately 60% (i.e. 49/80) and a specificity of close to 100% (i.e. 16/16, depending on the true status of oysters 70 and 93, which tested positive by impression smear but returned only a 'probable' QX result on histopathology).

5.4 DISCUSSION

The sensitivity of a diagnostic test may be defined as the proportion of true positive cases identified as positive by the test. Study results showed that the impression smear test used has a sensitivity of approximately 60%, and it is therefore likely that the true prevalence of QX disease in sentinel oysters during the study was significantly higher than that detected. However, it is also likely that the times of initiation, severity and duration of outbreaks were, for purposes of this study, accurately identified.

Severe QX outbreaks (with up to 95% of oysters infected) occurred at all three leases between mid-January and mid-April 1998. Importantly, there was no acidification detected at any of the leases during this period. Study findings therefore confirm the findings of Anderson *et al.* (1994) and Wesche (1995) that QX outbreaks can occur in the absence of acid exposure. Study findings also show that, even if acid exposure does occur, as at Lease N and, to a lesser extent at Lease B during mid-April, it does not increase the severity of an outbreak. This is shown by the finding that relatively minor QX outbreaks (maximum prevalence 39%) occurred at all three leases in early May, but the most severe of these occurred at Lease S, which did not receive acidified water.

5.5 CHAPTER SUMMARY

Investigations on the association of QX outbreaks confirm the findings of Anderson *et al.* (1994) and Wesche (1995) which is that QX outbreaks can occur in the absence of acid exposure. Study findings also show that, even if acid exposure does occur, it does not increase the severity of an outbreak. This is shown by the finding that relatively minor QX outbreaks (maximum prevalence 39%) occurred at all three experimental leases in early May, and the most severe outbreak occurred at a site which did not receive acidified water.

6 INVESTIGATION OF HASTINGS RIVER OYSTER KILLS

6.1 INTRODUCTION

Oyster growers in areas of the Hastings River have experienced heavy oyster losses for more than a decade that were only recently attributed to a new condition known as Limeburners Syndrome (LS) (Callinan, 1997b). The areas affected by oyster mortalities include Limeburners Creek, Big Bay and the Maria River (Figures 6.1 and 3.3) (Steen, 1996). LS-induced oyster kills cause large financial losses to oyster growers and are seasonally recurrent (Callinan, 1997b; Steen, 1996). LS lesions in Hastings River oysters confounded the interpretation of histopathological data for this study (Chapter 2) and oyster growers suspect that LS is an acid-induced condition, however no direct link has been established.

This chapter presents information obtained from investigating Hastings River oyster kills that have different clinical signs to acid induced mortalities, described in Chapter 4 of this study. An oyster kill, which was reported in August 2000, was investigated; the clinical signs and gross pathology of affected oysters are described in this chapter.

Hastings River oyster kills have been detected prior to this study and were investigated (Callinan, 1997b; Steen, 1996; Adlard, 1993; Desmarchelier, 1993). Callinan's (1997b) study showed that outbreaks of LS generally occurred between September and December in dry periods, which were characterised by high salinities, and on leases with low flow conditions. Callinan's (1997b) histopathology data did not identify a causative agent but showed a severe but non-specific inflammatory response in affected oysters. The mortalities were associated with a yellow algal growth on the external shell surface of affected oysters and the algae were identified as a common diatom (*Melosira* sp.) not known to be toxic (Callinan, 1997b). A surface-water algal material (commonly referred to as 'scum' by oyster growers) was reported to be in contact with the oysters during mortality events and analysis of the algae revealed a mix of non-toxic species (Callinan, 1997b). Callinan (1997b) also states that there was no substantial evidence before the study to suggest oyster mortalities were from exposure to estuarine acidification. There was also no clear relationship between dredging and oyster mortality. Oyster growers were concerned that channel dredging was a risk factor.

Adlard (1993) investigated the cause of reduced growth rates in Hastings River Sydney rock oysters. Adlard (1993) concluded that the mortalities were not a result of winter mortality or QX disease. Histology of oysters found spherical bodies of unknown origin. A small number of oysters were also tested for the aetiological agent responsible for Brown Ring Disease (*Vibrio* spp.) due to similar clinical signs in the affected oysters from the Hastings River (Desmarchelier, 1993). However, the bacteria responsible for Brown Ring Disease were not detected (Desmarchelier, 1993).

Steen (1996) identified the leases where oyster mortalities occurred in Limeburners Creek between 1992 and 1995 (Figure 6.1). Steen (1996) found that most oyster mortalities were reported from oyster leases immediately downstream and upstream of the Limeburners Creek road bridge (Figure 6.1). This particular area represents the most intensively farmed location on the Hastings River estuary which may account for the high number of reported oyster kills at this site.

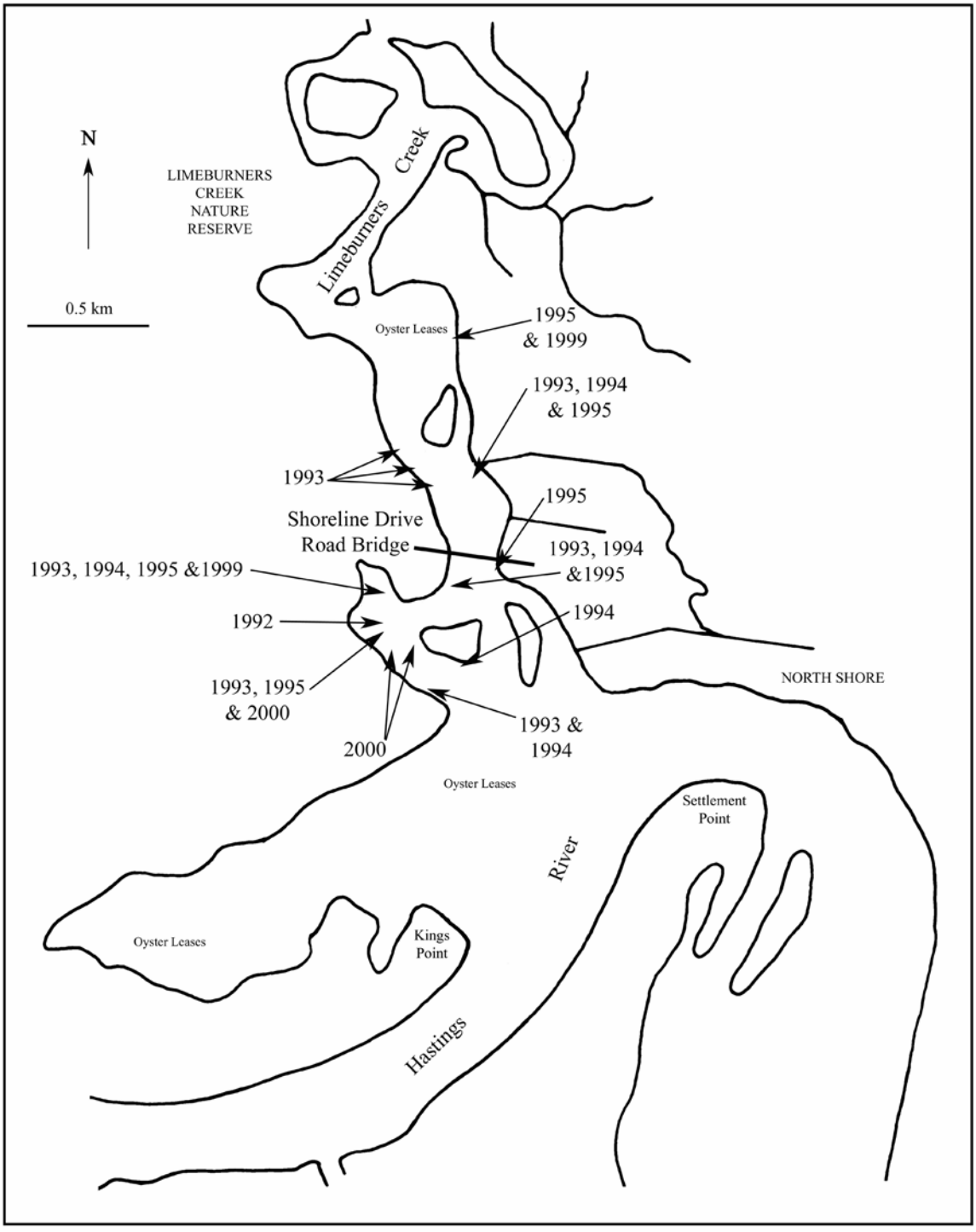


Figure 6.1 Locations of reported oyster mortalities in Limeburners Creek (*Source*: adapted from Steen, 1996).

The most recent investigation into Hastings River oyster production problems was conducted by Lake (1997). Lake's (1997) epidemiological study suggested putative risk factors for production problems occurring in the Hastings River but did not test the suggested causal links. The aim of the study was to identify plausible associations and potential risk factors for oyster production. The study defined a case for poor oyster production on the Hastings River, identified putative risk factors for production using farmer interviews, and identified areas of the estuary most affected by poor production.

The study proposed that production problems were potentially caused by several interrelated problems with the ultimate controlling factor being location (Lake, 1997). Lake (1997) also separated production problems into 'oyster kills', which relates to mortality of oysters and 'oyster *degeneratus*', which relates to slow growth in affected oysters. Putative risk factors for oyster kills and oyster *degeneratus* include: climatic variation; lease location; cultivation methods and practices; and, surrounding landuse activities.

Lake (1997) proposed the following hypotheses:

1. Present landuse in Limeburners Creek has inhibited oyster production through altered environmental processes;
2. Excessive drainage work in the Maria River has meant it is currently unable to sustain oyster production due to acidic drain outflows;
3. Siltation in Big Bay has reduced the estuarine habitat suitable for oyster production;
4. Freshwater influxes in disturbed acidic landscapes will result in oyster kill; and,
5. The *ad hoc* nature of cooking methodology results in unnecessary oyster kill.

Findings from Chapter 4 of this study support hypotheses 2 and 4.

Recent testing by Macquarie University, NSW and Queensland Museum for *M. sydneyi*, has discovered that it is present in the Hastings River estuary (NSW Fisheries, 2003). Polymerase chain reaction (PCR) testing of oysters from the Hastings River has found low levels of *M. sydneyi* organisms in the gills. During the present study, the clinical signs of QX disease were not evident but no testing for the pathogen was conducted.

These studies and oyster growers' lay knowledge suggest that an unidentified agent (or agents) is episodically impacting the Hastings River Sydney rock oyster industry and causes oyster kills in combination with reduced growth and poor health in affected oysters. The affected oysters display clinical signs which are different to oysters that have been exposed to acidification. Problems generally occur between September and December in dry periods that result in high salinity conditions in Limeburners Creek and the Hastings River (Callinan, 1997b). Observations from previous oyster kills report that the commonly observed yellow material on the soft tissue of affected oysters and mortality was primarily a feature in older oysters (Manton, 1993; Langton, 1993). Limeburners Creek road bridge (Figure 6.1) is the area most often affected by these unexplained production problems. The following sections detail and discuss an oyster kill that was detected in Limeburners Creek in August 2000.

6.2 OYSTER KILL INVESTIGATION

6.2.1 Date and Location

The oyster kill investigated during this study was identified in late August 2000 in the lower Limeburners Creek area. The location is shown on Figure 6.1 (labelled as '2000'). Figure 6.1 also displays the locations of oyster kills that occurred between the years of 1992 and 1995 (Steen, 1996). Two oyster kills were detected in Limeburners Creek in June and December 1999 during the present study and are labelled as '1999' on Figure 6.1.

No atypical oyster mortality occurred in the experimental oysters located at Sites A, B and C (Figure 3.5) between the 6/11/97 and the 31/7/98. All experimental oysters had to be removed on the 31/7/98 because of excessive overcatch. This prevented oyster mortality monitoring during the time when cases of LS are commonly reported (September to December). However, water quality monitoring continued until the 30/3/99 in the circumstance that an outbreak in cultivated oysters was identified. An oyster kill was detected in late August 2000 in Limeburners Creek and oysters from this event were examined grossly and using histopathology.

6.2.2 Rainfall

Figure 6.2 shows that in the two-month period before the oyster kill no heavy rain events were measured at the Bureau of Meteorology Station Number 60026 located at Hill Street, Port Macquarie (Figure 3.3). The largest rainfall was in mid July when 33 mm was recorded. All other daily rainfall events were less than 25 mm. Late winter to early spring is a typically dry period on the mid north coast of NSW. Water quality monitoring results presented in Chapter 3 (Section 3.4.4) show that rainfall of this magnitude and intensity is not sufficient to cause acidification in the lower Limeburners Creek area.

Salinity data obtained from the NSW Shellfish Quality Assurance Program showed that the dry conditions during this time resulted in high salinities in Limeburners Creek due to negligible influence from freshwater catchment inflows. Salinity levels measured on the 13/7/00, 27/7/00, 23/8/00 and 31/8/00, recorded by the NSW Shellfish Quality Assurance Program, were all above 30 ppt.

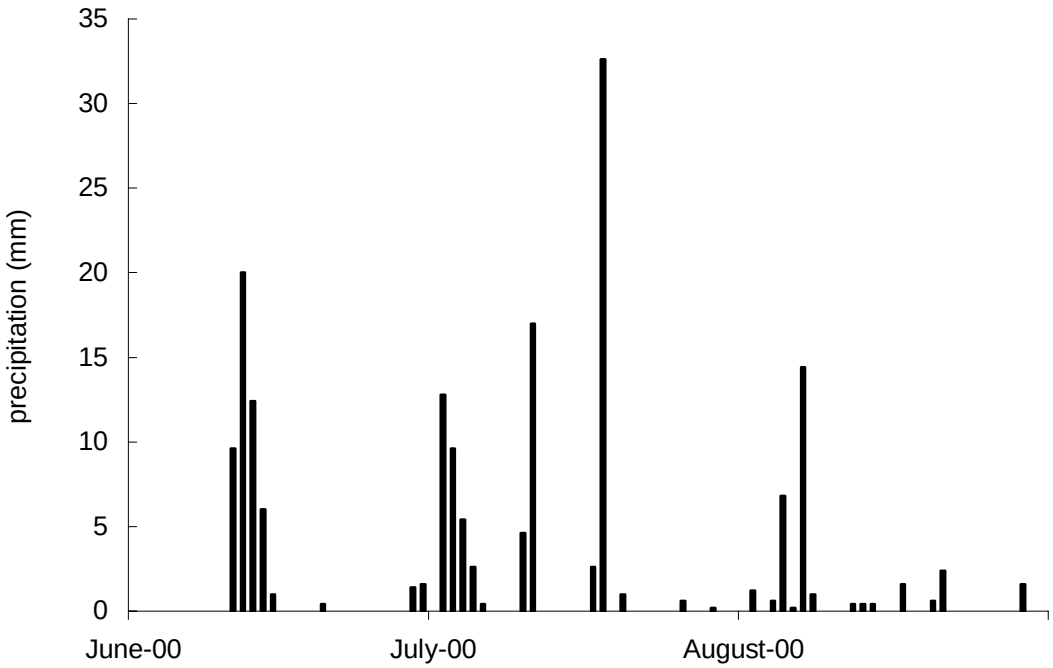


Figure 6.2 Rainfall recorded in June, July and August 2000 (*Source*: Bureau of Meteorology, Station 60026 – Hill Street, Port Macquarie).

6.2.3 Characteristics of Affected Oysters

This section describes the pattern of oyster mortality and gross pathology of moribund Sydney rock oysters sampled during the oyster kill, which was detected in August 2000. Moribund animals from the oyster kill were sampled on the 1/9/00 and preserved for histopathology.

Moribund oysters were randomly sampled from amongst adjacent oyster leases at the locations labelled as '2000' on Figure 6.1. Oysters cultivated by both rack-tray and pontoon methods were collected. Oysters cultivated using the rack-tray method suffered higher mortalities than the oysters cultivated using pontoons.

Moribund oysters could be identified by their shell appearance. The lip of the right valve of the affected oysters indicated growth had ceased and was very brittle, deformed and showed signs of mantle recession on the inward side. There were distinct patches of dead oysters centrifugally spread from a focus. The patches of dead oysters were diffuse across several leases in this area of Limeburners Creek (Figure 6.1). Moribund oysters were collected from the perimeter of the patches of dead oysters. Plates 6.1 to 6.4 are typical examples of moribund oysters collected from Limeburners Creek following an oyster kill on the 1/9/00 (Plates 6.1 and 6.2) and the 1/6/99 (Plates 6.3 and 6.4), which are included for comparative purposes.

Plate 6.1 Shell discolouration and shell deposits in the anterior of the left valve (specimen collected on the 1/9/00).

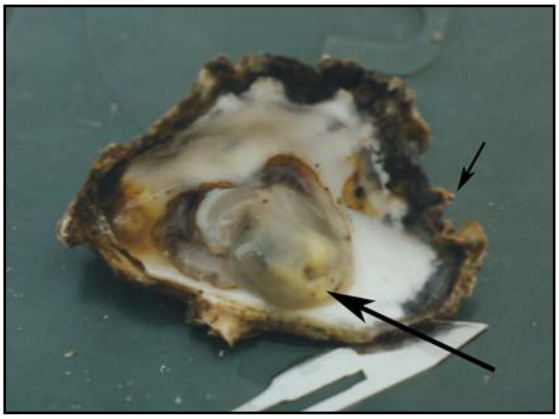


Plate 6.2 Yellow pustules in the gonad (large arrow) and the valves of affected oysters were brittle (small arrow) (specimen collected on the 1/9/00).

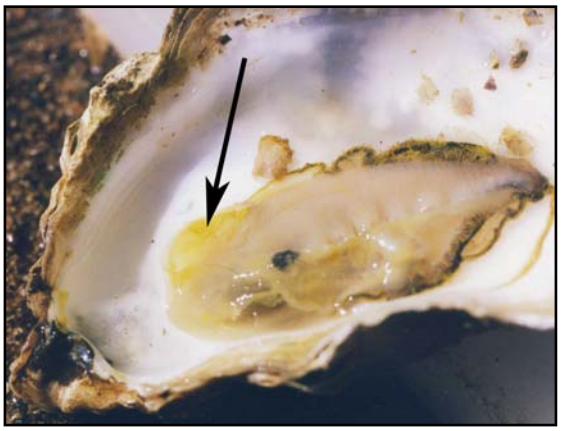


Plate 6.3 Yellow pustules in the labial palps (specimen collected on the 1/6/99).

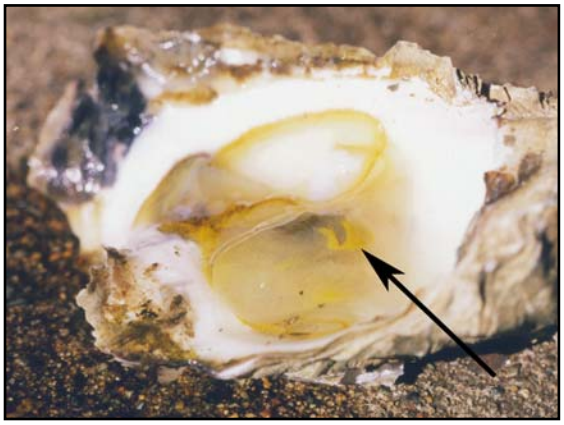


Plate 6.4 Example of an oyster in poor condition with yellow pustules in the mantle (specimen collected on the 1/6/99).

Gross examination of moribund oysters detected pustules in the soft tissues of oysters. 'Pustules' were small abscesses filled with puss and were: yellow to orange in colour; an irregular shape; had a soft and pasty consistency; and, ranged in size from 1 mm to 7 mm. Pustules were observed on and in the gills, mantle, labial palps, digestive gland, adductor muscle and gonad (Plates 6.1 to 6.4). Yellow/orange to brown pustules were also evident on the internal surface of the oyster shell and stained the internal shell. The most common location of the stain was in the anterior of the left valve (Plates 6.1 and 6.2). In some instances oysters had created a new shell layer over the pustule to isolate it within the shell.

The general condition of moribund oysters' soft tissue ranged from very poor to a normal appearance. In particular moribund animals: the soft tissue was grey and watery to the extent of being translucent; the mantle soft tissue was thin, weak and easily torn; and, oysters were very easy to open. Mudworm (*P. websteri*), indicated by a tubular blister located under the gills and mantle, had also infested a high proportion of oysters sampled.

6.2.4 Histopathology Data From the Oyster Kill

Oysters sampled from this oyster kill were examined using histopathology. Lesions in moribund oysters were characterised by foci of inflammatory cells located in the mantle and digestive gland. Myocardial necrosis associated with accumulations of haemocytes was observed in the heart. Histopathological diagnosis on representative oysters from this kill reported that the animals had experienced limited food availability or other factors that prevented ingestion of a normal ration (R. Elston, Aqua Technics, USA, personal communication, 2002).

An active infectious process was observed in one oyster based on intense multifocal haemocytosis. This oyster was sent to the Department of Microbiology and Parasitology to be tested for QX disease using *in situ* hybridisation. The test indicated that the oyster was not infected with *M. sydneyi*. Several morphologically equivalent microcell type organisms were observed in the digestive gland, which suggested that the infection could be due to *Mikrocytos roughleyi* (R. Callinan, NSW Fisheries, personal communication, 2000; R. Elston, Aqua Technics, USA, personal communication, 2002).

6.3 DISCUSSION

Evidence obtained from this and previous studies (Callinan, 1997b; Steen, 1996; Lake 1997) point to an unidentified agent that is causing production problems in areas of the Hastings River and there is need for further and more detailed investigation. Information obtained from oyster growers regarding oyster mortalities, poor health and slow growth reveal that the clinical signs are seasonally recurrent and coincide with high salinities in Limeburners Creek and the lower Hastings River (Callinan, 1997b; Steen, 1996; Lake, 1997). Low pH conditions are unlikely to occur under these conditions.

Clinical signs of affected oysters from this investigation were:

- a deformed, weak and friable shell void of any evidence of growth;
- grey, watery soft tissue containing yellow pustules in the gills, mantle, labial palp, adductor muscle and digestive gland;

- yellow/orange staining of the internal shell surface particularly in the anterior of the left valve; and,
- older oysters displayed the above clinical signs more frequently than younger oysters located on the same lease.

These clinical signs are similar to the observations of Manton (1993), Langton (1993) and Callinan (1997b). The clinical signs displayed by affected oysters, the nature of the oyster kills and the associated slow growth and poor health, so far appear unique to the Hastings River. The evidence collected from this study is insufficient to suggest a cause or causes for the mortality.

Water quality data presented in Section 3.4.4 did not detect acidification caused by the oxidation of pyrite in Limeburners Creek or the lower Hastings River. This strongly suggests that acid is not a necessary factor for LS. The gross clinical signs of mortalities, slow growth and shell deformities are the only similarities that LS bears with production problems caused by exposure to ASS-affected waters (Table 6.1).

Table 6.1 Gross clinical signs displayed by oysters exposed to ASS-affected waters and affected by LS. X indicates an observation based on data from the present study.

	slow growth	mortality	poor condition	pustules	weak adductor muscle	mantle recession	shell deposits	shell deformities	shell degradation	shell bleaching	shell perforation	iron coating on shell	iron on soft tissue
Oyster impacted by ASS-affected waters	X	X	X	-	-	-	-	X	X	X	X	X	X
LS in oysters	X	X	X	X	X	X	X	X	-	-	-	-	-

There is need for further study of LS-induced oyster production problems to identify the causes and reduce or prevent future oyster kills. This study has enabled future studies to concentrate on the two separate oyster production problems that occur on the Hastings River.

6.4 CHAPTER SUMMARY

This chapter examined an oyster kill that was detected in Limeburners Creek in August 2000, in order to investigate oyster production problems on the Hastings River. Water quality investigations conducted on the lower Hastings River and Limeburners Creek areas (Chapter 3) suggest that acidification of these areas is unlikely. Sampling of moribund oysters on the 1/9/00 confirmed that there were a number of similarities between other oyster kills that have been detected in this area prior to this current study that have been attributed to LS. An exact cause or causes for oyster kills and oyster *degeneratus* has not been identified in previous

investigations or by the present study. The recurrent nature and the widespread extent of this problem is of particular concern for the Hastings River oyster industry as it threatens not only their livelihood but also the viability of oyster production in particular parts of this estuary. Further study of this problem is necessary to identify the exact cause or causes.

Section III follows this chapter and details the laboratory investigations conducted for this present study. The laboratory investigations were essential to the study to investigate effects of acidified waters that are difficult and impractical to measure in the field and to enable more accurate interpretation of the field observation experiment data.

SECTION III

LABORATORY INVESTIGATIONS

7 EXPERIMENTAL EXPOSURE OF OYSTERS TO ACIDIFIED WATER AND EFFECTS ON OYSTER FEEDING AND SOFT TISSUE

7.1 INTRODUCTION

The results from Chapter 4 showed that oysters exposed to extended periods of ASS-affected waters experienced reduced survival and growth rates. Laboratory-based experimental exposures were required for this study because of the variability of water quality at field experiment sites and the inability to test for the separate and combined effects of metals under field conditions. The purpose of this chapter is to outline the design, methods and materials required to investigate the physiological and histopathological effects of ASS-affected waters on the Sydney rock oyster and present and discuss the findings from these experiments.

Overseas research that investigated the effects of acidification on bivalves demonstrated, using laboratory experiments, that exposure to pH values < 7 reduces feeding activity (Bamber 1987; 1990) and pumping rates (Loosanoff and Tommers 1947). The evidence from field investigations from this present study and overseas studies suggest that it is very probable that ASS-affected water will reduce feeding rates in Sydney rock oysters.

Sydney rock oysters experimentally exposed to ASS-affected waters were examined using histopathology to investigate changes to the gill and mantle soft tissues. Histopathological investigation will provide further information to help understand: poor oyster health observed at field sites exposed to ASS-affected waters; iron accumulation in/on oysters at field sites exposed to ASS-affected waters; and, changes in oyster filtration rate caused by exposure to ASS-affected waters.

The following section provides background information on: oyster valve movements under acidic conditions; methods to assess feeding processes in oysters; and, the function of the oyster gill and mantle. Following this background information, the experimental design and the materials and methods used for the laboratory experiments are detailed. The results from the laboratory experiments are then presented followed by the discussion section and the chapter summary.

7.2 BACKGROUND

7.2.1 Oyster Behaviour Under Acidic Conditions

Studies on the effects of acidic water on bivalves reported that acidic conditions elicit abnormal valve responses, including excessive gaping and behavioural inhibition (Bamber, 1987; 1990; Loosanoff and Tommers, 1947). Using behaviour descriptions from these studies, the following valve responses in Sydney rock oysters exposed to artificially acidified treatments were observed in laboratory experiments conducted in Chapter 2 (Sections 2.3.1 and 2.3.2):

- excessive gaping – valve separation beyond the range of normal feeding;
- clomping – shell adductions (used to eject water and to remove faecal material);
- no activity – oyster valves remain closed and inactive for long periods; and,
- open valves – valves are separated a normal distance and the oyster exposes the mantle and gills to the test water.

Observations for these valve responses were recorded during both of the laboratory experiments conducted for this chapter.

7.2.2 Oyster Feeding

Two studies have investigated the effect of acidification on feeding activity in *V. decussata*, *O. edulis*, *C. gigas*, *M. edulis* (Bamber, 1987; 1990) and one study investigated the effect of acidification on pumping rates in *O. virginica* (Loosanoff and Tommers, 1947).

Loosanoff and Tommers (1947) recorded increased pumping rates at pH values between 7.0 and 6.75, but when the pH dropped below 6.5 pumping rates dramatically decreased in adult *O. virginica*. Loosanoff and Tommers (1947) also observed abnormal shell movements when pH was less than 6.5. Bamber (1987) measured feeding inhibition and a significant reduction in tissue and shell growth for the species *V. decussata* at or below pH 7.0. Bamber (1990) investigated the effects of acidic conditions on feeding activity in *C. gigas*, *M. edulis* and *O. edulis*. For *C. gigas*, suppression of feeding activity occurred below pH 7.0 and behavioural inhibition was observed below pH 6.5. Feeding activity was reduced at or below pH 7.2 for *O. edulis* and *M. edulis*. In these studies (Loosanoff and Tommers, 1947; Bamber, 1987; 1990), artificially acidified test waters were used in their experiments and acidification was caused by inflows of slightly acidic fresh water or industrial pollution as opposed to ASS outflows.

Bamber (1987; 1990) quantified oyster feeding activity in acidified bioassays by collecting, drying and weighing all true faeces and pseudofaeces produced by oysters in a specified period when exposed to a range of pH levels. Loosanoff and Tommers (1947) defined pumping rate as the volume of water pumped through the mantle cavity per unit of time (Iglesias *et al.*, 1998). Direct measurements of pumping rates are difficult to perform and can inhibit pumping (Iglesias *et al.*, 1998; Newell and Langdon, 1996). Clearance rate is a measure of oyster feeding and is favoured over the direct measurement of pumping rates (Iglesias *et al.*, 1998).

Clearance rate (L h^{-1}) and filtration rate (mg h^{-1}) are essentially the same measure using different units and is the volume of water cleared of particles per unit time multiplied by the particle concentration (Iglesias *et al.*, 1998). Pseudofaeces are made up of mucus-coated material rejected from the palps and the marginal food groove of the gill (Newell and Langdon, 1996). The mantle moves this material using cilia to the ventral free edge, adjacent to the labial palps. Ciliary action or 'clomping' (rapid closure and opening of the valves) ejects the material as 'pseudofaeces' from within the valves (Newell and Langdon, 1996).

Feeding in bivalves can be determined through measurements of suspended particles and biodeposit production using the biodeposition method (Iglesias *et al.*, 1998). Iglesias *et al*. (1998) identifies two assumptions that underpin the biodeposition methodology. The first is that the organic matter to inorganic matter ratio is similar for both the available "food" and the actual material filtered by oysters. The second is that both the pseudofaeces and true faeces are based on oysters filtering the same source of total particulate matter (TPM).

The biodeposition method can be used in this present study to quantify food processing rates in Sydney rock oysters when exposed to ASS-affected waters, however, the two assumptions detailed above must be addressed. In order to address the first assumption natural silt collected from the surface of the deposited sediment in the estuary was used as the diet in all treatments. To address the second assumption, a flow-through experimental apparatus was designed to deliver constant TPM levels and regular testing of TPM was performed throughout all treatments to ensure a constant constitution. The flow-through experimental apparatus was based on Widdows' (1985) apparatus for the measurement of clearance rate, which allows quantification of the composition of suspended particles as well as true faeces and pseudofaeces of individual animals.

7.2.3 Function of the Oyster Gill and Mantle

The gills collect food particles and, together with the mantle to a lesser extent, are used for gas exchange (Newell and Langdon, 1996). The gills achieve this by creating a water current and filtering suspended food particles which are then sorted and separated from the other materials in suspension (Galtsoff, 1964). The gills are also used to disperse and separate sex cells during spawning (Galtsoff, 1964). The labial palps are located at the anterior of the gills. The function of the labial palps is to control the amount of food ingested as well as sort food before ingestion.

The mantle, or pallium, is a fleshy fold of tissue that covers the internal organs (Galtsoff, 1964; Eble and Scro, 1996). The main role of the mantle is shell formation (Galtsoff, 1964). The mantle is involved in other functions which include (Galtsoff, 1964): receiving and conveying sensory stimuli to the nervous system; shedding and dispersing eggs during spawning; respiration by providing direct exchange of gases between the surface tissues of the oyster and the surrounding water; storage of reserve materials such as glycogen and lipids; and, secretion of mucus. The mantle also aids in excretion by discarding blood cells containing waste products (Galtsoff, 1964).

7.3 EXPERIMENTAL DESIGN

Two laboratory experiments were conducted to expose oysters to artificially and naturally acidified treatments. The purpose of the first experiment was to investigate behavioural and soft tissue response whilst the second was used to examine oysters' feeding rates. The first experiment is referred to as the 'Behaviour Experiment' and the second as the 'Feeding Experiment'. A total of eight treatments were used in the two experiments and oysters were exposed to pH levels ranging from 5.1 to 8.0. Table 7.1 lists the type of treatment water, purpose and target pH and EC used in the Behaviour and Feeding Experiments. As stated previously, it was necessary to establish oysters' behavioural response to acidified treatments before the Feeding Experiment could be undertaken to ensure oysters remain open and feed in weak acid treatments.

The laboratory experiments were designed to resemble realistic environmental conditions. Data from the SDL situated at Site 4 (Figure 4.1) were used as a guide to select the minimum pH and maximum EC levels for both experiments to ensure that laboratory conditions were similar to estuarine waters affected by ASS. Sydney rock oysters typically open their valves at salinities greater than 15 ppt (23.4 dS m^{-1}) (Holliday, 1995). Between the 4/6/99 and the 12/6/99 low pH values (pH 4.41) were measured at EC levels that exceeded 23.4 dS m^{-1} at Site 4 (Figure 7.1). Based on this

information, a minimum pH of 5 and a maximum EC of 31 dS m^{-1} were used for the two laboratory experiments.

Table 7.1 Behaviour Experiment and Feeding Experiment details.

Exp.	Purpose	Treatment Number	Treatment Water	Target pH / EC (dS m^{-1})	No. of Oysters
Behaviour Experiment	behaviour and soft tissue repsonse	1	seawater + deionised water	8 / 29	24
		2	seawater + deionised water + 0.1 M HCl	5.1 / 29	24
		3	seawater + deionised water + Al + 0.1 M HCl	5.1 / 29	24
		4	seawater + deionised water + Fe + 0.1 M HCl	5.1 / 29	24
		5	seawater + ASS-affected water + 0.1 M HCl	5.1 / 29	24
Feeding Experiment	feeding measurements	6	seawater + deionised water + natural silt	8 / 29	18
		7	seawater + ASS-affected water + natural silt + 0.1 M HCl	6.5 / 29	18
		8	seawater + ASS-affected water + natural silt + 0.1 M HCl	5.5 / 29	18

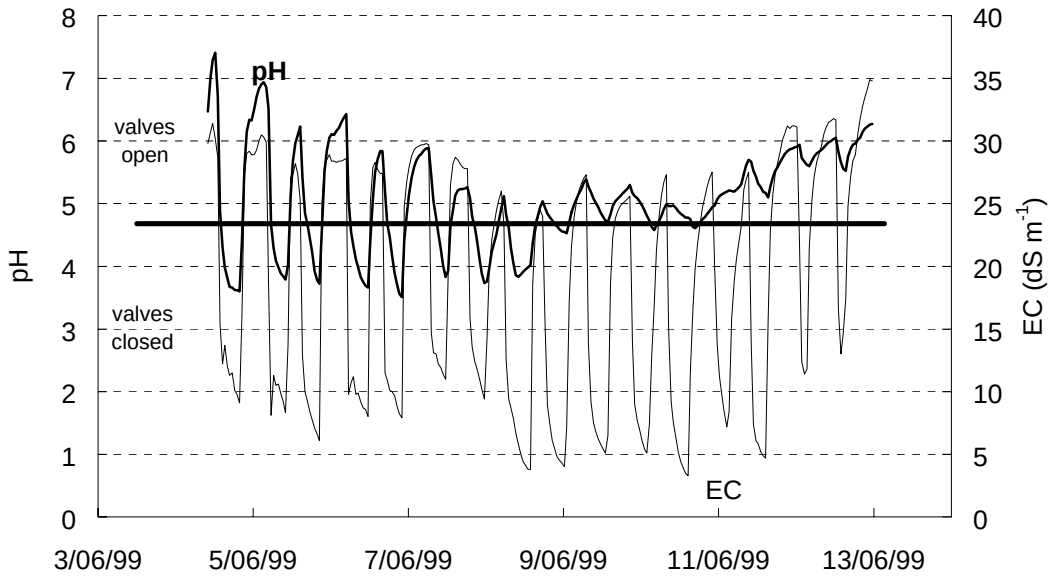


Figure 7.1 EC (thin line) and pH (bold line) conditions at Site 4 (Figure 4.1) between the 4/6/99 and the 12/6/99. The solid black line indicates the EC value of 23.4 dS m^{-1}.

7.3.1 Behaviour Experiment

Behaviour was observed and recorded to ensure that oysters had open valves and were feeding at pH 5.1 at the selected EC level (29 dS m^{-1}). Five treatments (numbered 1 to 5 and detailed in Table 7.1) were conducted and twenty-four oysters were observed for behavioural responses during each treatment. The duration of exposure was six-hours, commencing from the moment an oyster opened its valves. Six hours was chosen for the duration of exposure because it was the length of time that oysters were exposed to acidic conditions in one tidal cycle in the estuary (Dove, 2003). The valve movements listed in Section 7.2.1 were recorded during Treatments 1 to 5.

The Behaviour Experiment was also designed to examine short-term, sub-lethal effects of weak acidity (pH 5.1) on the gills and the mantle soft tissues of Sydney rock oysters. Histopathology was used to examine the response in the soft tissues. Aluminium and iron were added to Treatments 3 and 4, respectively and ASS-affected water was added to Treatment 5. The pH, iron and aluminium concentrations used in these treatments were based on actual field data obtained during this present study from oyster leases (Chapter 3).

To avoid the problem of LS confounding the histopathology data in the Behaviour Experiment, all oysters were sourced from the Manning River after it was established that there were no clinical signs of LS (Section 6.3) in these oysters. The duration of exposure in all treatments for the Behaviour Experiment was six-hours, commencing from the moment an oyster opened its valves. Of the 24 oysters used in Treatments 1 to 5, 12 oysters were preserved for histopathology and 12 oysters were returned to Site 2 (Figure 4.1) for monitoring of post-experiment survival.

7.3.2 Feeding Experiment

The Feeding Experiment used three treatments referred to as Treatment 6, Treatment 7 and Treatment 8. Treatment 6 contained no ASS-affected water and the pH was maintained at 7.96 for the entire experiment. ASS-affected waters were used to acidify the test waters to pH 6.5 in Treatment 7 and pH 5.5 in Treatment 8. Details relating to the experimental apparatus used to expose oysters to acidified water are presented in Section 8.9.3.

7.4 MATERIALS AND METHODS

7.4.1 Experimental Oysters

For the Behaviour Experiment, 150 Sydney rock oysters were randomly collected from oyster leases in the Manning River isolated from areas impacted by ASS-affected waters (i.e. Sites 1, 2 and 3 shown in Figure 4.1). Oysters were acclimated at Site 2, a non-acid impacted site, from the 1/2/00 for a minimum of 30 days before transfer to the laboratory. The mean shell height (± 95% CI) of all of the oysters used in the Behaviour Experiment was 51.89 ± 0.72 mm. A Greenspan Smart Sonde SDL was installed at Site 2 and recorded pH, EC and temperature at this site during the acclimation period to ensure that oysters were not exposed to acidic conditions prior to the experimental work.

Eighty Sydney rock oysters (mean shell height ± 95% CI = 57.94 ± 1.39 mm) were acclimated for a minimum period of 30 days at Site 2 (Figure 4.1) from the 1/10/00

for the Feeding Experiment. Once again, the Greenspan Technical Services Smart Sonde was installed at Site 2 during this period to ensure that oysters were not exposed to acidification prior to the Feeding Experiment. Eighteen oysters were used in each of the three treatments. Physical attributes of the oysters collected for the Feeding Experiment are summarised in Table 7.2.

Table 7.2 Shell heights, whole weights and soft tissue dry weights of experimental oysters (all values listed are the means ± 95% CI, n =18).

Treatment	Shell Height (mm)	Whole Weight (g)	Soft Tissue Dry Weight (g)
6	58.77 ± 2.16	18.875 ± 2.059	0.658 ± 0.097
7	54.74 ± 2.25	17.581 ± 2.131	0.752 ± 0.112
8	59.66 ± 2.47	21.297 ± 1.633	0.901 ± 0.148

7.4.2 Set-up of the Experimental Apparatus

A flow-through system was used to maintain a stable pH in the test trays and to encourage oysters to feed by controlling flow. The flow-through system used recirculated water and was designed to expose oysters to eight different test waters (Treatments 1 to 8). The aquarium is illustrated in Figure 7.2 and the design was based on Widdow's (1985) apparatus for measurement of clearance rate.

The apparatus used in this present study consisted of a 60 L header tank which gravity fed ten 2.9 L trays (120 mm x 300 mm x 80 mm). A baffle was placed in each tray to reduce turbulence in the trays. A flow rate of 0.5 L min^{-1} was delivered to each tray for the duration of each treatment. This flow rate was selected for reasons detailed below. The header tank and the eight trays overflowed into a 200 L reservoir where pH, EC, DO and temperature were continuously monitored using a Yeo-Kal 611 Intelligent Water Quality Analyser. Water was intermittently pumped to the header tank from the reservoir using two 2,000 L h^{-1} pumps. A third pump ran continuously to stir the reservoir water and to prevent sedimentation. All components of the experimental apparatus were made from food-grade or stabilised plastic to prevent any reaction with the acidic test water.

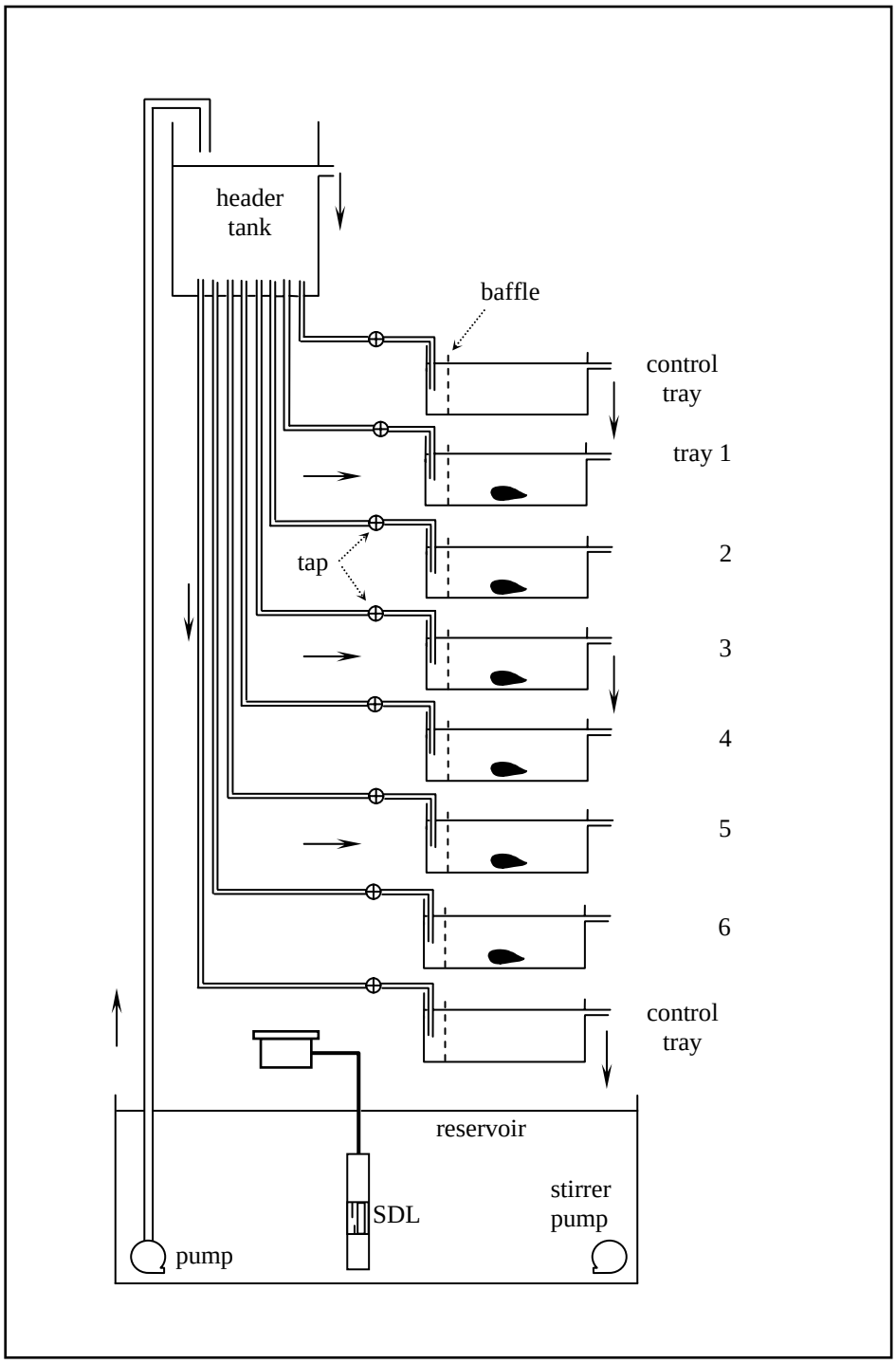


Figure 7.2 Apparatus for exposure of oysters to acidified water (modified from Widdows, 1985).

For the Behaviour Experiment, six trays each contained four oysters and the control trays were used to collect water samples for chemical analyses. However, oysters were placed individually into trays 2 to 6 for the Feeding Experiment and trays 1 and 8 (Figure 7.2) were used as control trays to collect water samples for measurement of suspended particles. Test runs were conducted before the Feeding Experiment to determine an appropriate flow rate to ensure: biodeposits were not being resuspended by water current flows; sedimentation of suspended particles was not occurring on the tray bottoms; and, that the ratio of particulate organic matter (POM) to particulate inorganic matter (PIM) did not vary during and between treatments (Iglesias *et al*., 1998).

7.4.3 Source and Composition of Test Waters

The main constituents of the treatment water used in Treatments 1 to 8 are listed in Table 7.1. Seawater was used in all treatments and was collected offshore from Port Macquarie (31^{O} 25' 30" S, 152^{O} 55' 20" E). ASS-affected waters were mixed with seawater in Treatments 5, 7 and 8 (Table 7.1) and were collected from Fernbank Creek (Figure 3.3) immediately before the start of these exposures. The pH was stabilised in Treatments 2, 3, 4, 5, 7 and 8 using 0.1 M Analar hydrochloric acid (HCl). A Yeo-Kal 611 Intelligent Water Quality Analyser was used to measure pH, EC, DO and temperature for both experiments. The methods used to determine other water quality variables in the treatment waters, and the measured levels of each variable, are detailed in the respective sections below.

7.4.3.1 Behaviour Experiment

Three artificially acidified test waters and one naturally acidified test water were used to investigate the effects of acidified water on oyster soft tissue and behaviour. Oysters were also exposed to pH 8.0, which was a mixture of seawater and deionised water. This was done to ensure that the oyster sampling and handling procedure did not cause lesions in the soft tissue and that oyster behaviour was not a reaction to the aquarium environment. A summary of the five treatments that includes pH, EC, DO and temperature conditions is provided in Table 7.3.

Table 7.3 pH, EC and temperature values (means are ± 95% CI) of Treatments 1 to 5.

Treatment Number	Treatment Water	Time (h)	Mean pH	pH Range	Mean EC (dS m^{-1})	Mean Temp (^{O}C)
1	Seawater + Deionised H_2O	6	8.02 ± 0.009	7.99 - 8.12	29.3 ± 0.01	26.76 ± 0.045
2	Seawater + Deionised H_2O + 0.1 M HCl	6	5.11 ± 0.005	5.05 - 5.18	29.3 ± 0.02	24.86 ± 0.064
3	Seawater + Deionised H_2O + Al + 0.1 M HCl	6	5.12 ± 0.007	5.04 - 5.18	29.3 ± 0.02	25.42 ± 0.073
4	Seawater + Deionised H_2O + Fe + 0.1 M HCl	6	5.08 ± 0.007	5.01 - 5.16	30.8 ± 0.01	22.28 ± 0.035
5	Seawater + ASS-Affected Water + 0.1 M HCl	6	5.09 ± 0.008	5.01 - 5.18	28.9 (no shift)	20.85 ± 0.038

A stock solution of aluminium chloride was added to Treatment 3 and a stock solution of iron chloride was added to Treatment 4. The iron and aluminium chloride were AR grade and were used because sulfate derivatives of divalent cations were found to

evoke an inflammatory response in mussels (Sunila, 1988). HCl was used instead of H_2SO_4 to acidify treatments to avoid unstable aluminium-sulfate complexes that can decouple changing the aluminium species present in the treatment water (Sammut, 1998). Also, HCl has been used to acidify treatments in a number of other studies (Loosanoff and Tommers, 1947; Kuwatani and Nishii, 1969; Calabrese and Davis, 1966; Allan and Maguire, 1992). The concentrations of dissolved and suspended iron and aluminium for each experiment are listed in Table 7.4. Total metal concentrations were determined using the Nitric Acid Digestion method detailed in APHA (1998).

Table 7.4 Concentrations of dissolved and suspended iron and aluminium measured in Treatments 1 to 5.

Treatment Number	Treatment Water	Dissolved		Suspended	
		Fe (mg L^{-1})	Al (mg L^{-1})	Fe (mg L^{-1})	Al (mg L^{-1})
1	Seawater + Deionised H_2O	ND	ND	ND	ND
2	Seawater + Deionised H_2O + 0.1 M HCl	ND	ND	ND	ND
3	Seawater + Deionised H_2O + Al + 0.1 M HCl	ND	1.4	ND	6.24
4	Seawater + Deionised H_2O + Fe + 0.1 M HCl	ND	ND	7.71	ND
5	Seawater + ASS-Affected Water + 0.1 M HCl	0.201	0.292	13.25	5.86

ND = Not detectable (Fe not detectable when < 0.04 mg L^{-1}, Al not detectable when < 0.02 mg L^{-1})

7.4.3.2 Feeding Experiment

The selected pH values for the three treatments were 7.96 (Treatment 6), 6.5 (Treatment 7) and 5.5 (Treatment 8). Table 7.1 lists the treatments and includes the target pH and provides details relating to the treatment water. Treatment water was obtained by mixing seawater with deionised water (Treatment 6) or ASS-affected water (Treatments 7 and 8). Treatment water was pre-filtered to 11 µm before the Feeding Experiment.

The diet in the Feeding Experiment consisted of natural silt which was collected from the intertidal mud flats adjacent to Site 2 (Figure 4.1). Natural silt collection, storage and filtration were conducted according to the methodology outlined in Bayne *et al.* (1999a). Silt was scraped from surface sediments to a depth of 2-3 mm and stored at 4^{O} C prior to each treatment. Silt was sieved through 140 µm and 11 µm nylon mesh, left to stand for 60 minutes and then decanted into the reservoir of the experimental apparatus.

Regular measurements of pH, EC, DO and temperature were performed throughout Treatments 6, 7 and 8. The mean pH, EC, DO and temperature values measured during each treatment are listed in Table 7.5. Table 7.5 indicates that pH, EC, DO and temperature were stable and similar in the three treatments.

Table 7.5 Treatment water pH, EC, DO and temperature (values displayed are means ± 95% CI).

Treatment Number	n	pH	EC (dS m^{-1})	DO (% Sat.)	Temp. (^{O}C)
6	69	7.96 ± 0.017	29.2 ± 0.02	88.7 ± 0.14	25.63 ± 0.153
7	89	6.50 ± 0.002	29.3 ± 0.02	88.1 ± 0.67	25.55 ± 0.097
8	102	5.50 ± 0.003	29.3 ± 0.02	85.6 ± 1.51	26.22 ± 0.172

A water sample was collected before each treatment commenced and analysed to determine the concentration of aluminium, iron, manganese, zinc and silicon. Table 7.6 lists the concentrations of these metals measured in Treatments 6, 7 and 8. Analysis of samples from all of the treatments did not show elevated concentrations of dissolved iron. However, iron flocs were visible on the GFC filters in Treatments 7 and 8 and the experimental water in Treatment 8 appeared orange suggesting that iron was precipitating out of solution. An elevated concentration of dissolved aluminium was measured in Treatment 8 compared to Treatments 6 and 7 (Table 7.6).

Table 7.6 Concentrations of dissolved Al, Fe, Mn, Zn and Si measured in Treatments 6 to 8.

Treatment Number	Al (mg L^{-1})	Fe (mg L^{-1})	Mn (mg L^{-1})	Zn (mg L^{-1})	Si (mg L^{-1})
6	ND	ND	0.02	0.02	0.22
7	ND	0.01	0.20	0.05	3.20
8	0.11	0.03	0.15	0.03	3.92

ND = Not detectable

During Treatments 6 to 8, water samples were collected from trays 1 and 8 for quantitative measurement of seston concentration or TPM using the gravimetric method (Iglesias *et al.*, 1998). TPM is measured in mg L^{-1} and is the dried suspended matter, which is equivalent to dietary abundance for oysters (Hawkins *et al.*, 1996). The organic component of TPM represents the dietary or "food" quality (Hawkins *et al.*, 1996; Bayne *et al.*, 1987).

The samples were filtered onto pre-ashed and pre-weighed glass microfibre filters (Whatman GFC, Catalogue Number 1822 047). These filters were ashed at 450^{O} C for 4 to 6 hours, weighed and placed in a desiccator before use. A one-litre aliquot of the treatment water was filtered through the glass microfibre filter and the filter was

rinsed with 10 ml of 0.9% ammonium formate to remove any salts (Bayne *et al.*, 1999b). Deionised water was also filtered onto pre-ashed and pre-weighed glass microfibre filters to ensure that filters were not contaminated during drying or weighing (Widdows, 1985).

The filters were oven dried for a minimum of 12 hours at 80^{O} C to a constant weight before weighing. The final step involved ashing the filters at 560^{O} C for 4 to 6 hours before being placed in a desiccator to cool and then weighed for the final time (B. Bayne, personal communication, 2000). This was done to calculate the concentration of PIM and POM in the water samples. Forceps were used when handling filters.

The Feeding Experiment diets for Treatments 6, 7 and 8 are displayed in Figure 7.3. The mean TPM concentration for Treatments 6 (pH 7.96) and 7 (pH 6.5) are similar, however the organic content is slightly greater in Treatment 7. Treatment 8 (pH 5.5) had a greater mean TPM concentration and this was attributed to ASS oxidation products (iron and aluminium) in the treatment water being in a suspended state. This is reflected by the high PIM value. Attempts were made to remove iron precipitates from the treatment water by filtration and allowing flocs to settle in a sedimentation tank before the ASS-affected water was added to Treatments 7 and 8 to achieve similar compositions of suspended particles for all treatments. However, this was not effective in removing the dissolved iron from the treatment water as can be seen in Figure 7.3.

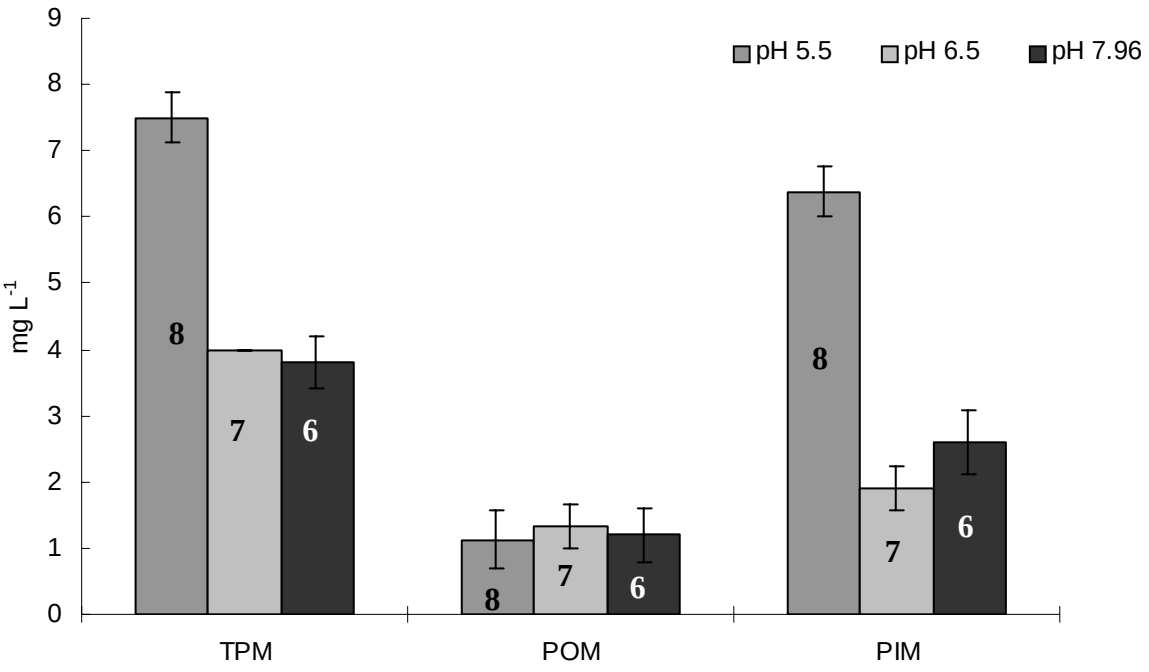


Figure 7.3 Mean TPM, POM and PIM measured in Treatments 6 (pH 7.96), 7 (pH 6.5) and 8 (pH 5.5) (means are ± 95% CIs).

The increased concentrations of TPM and PIM measured in Treatment 8 have implications for the oyster diet and the calculation of filtration rates. The two assumptions of the biodeposition method were (Iglesias *et al*., 1998):

- the organic matter to inorganic matter ratio must be similar for both the available "food" and the actual material filtered by the oysters, and
- the pseudofaeces and true faeces are based on the oysters filtering the same source of total particulate matter (TPM).

These assumptions were addressed for Treatments 6 and 7, but not for Treatment 8. Therefore this difference in available food levels experienced in Treatment 8 was taken into consideration in the interpretation of the filtration rate results.

7.4.4 Oyster Behavioural Response

The experimental apparatus allowed close inspection of oysters. Observations of individual oysters for the four behavioural traits detailed in Section 7.2.1 were performed in all treatments. The observations and descriptions of oyster behaviour for Treatments 1 to 5 are presented in the following chapter.

7.4.5 Feeding Rates

7.4.5.1 Biodeposit Sampling and Analysis

Trays 2 to 7 were used to measure oyster true faeces and pseudofaeces and trays 1 and 8 were used as controls (Figure 7.2). Water samples were collected from the control trays to measure the concentration of suspended particles. One oyster was placed into each of the 6 trays and the time taken for oysters to open their valves was measured. Oysters were then left undisturbed for a period of 2 to 3 hours to allow passage of all material that was in their gut before the experiment. A measurement of true faeces and pseudofaeces was then performed.

A wide-mouth pipette was used to sample the true faeces and pseudofaeces from the trays. All of the pseudofaeces and true faeces produced in a one hour time period were collected from the trays and filtered onto pre-ashed and pre-weighed Whatman GFC filters. A second measurement of pseudofaeces and true faeces was performed immediately after the first measurement for the same period of time to obtain an average value for the weight of biodeposits produced. This entire procedure was repeated three times to expose 18 oysters to each treatment.

The filtered samples of oyster true faeces and pseudofaeces were analysed using the same methodology as suspended particles. After the biodeposits were filtered through the GFC filter, it was rinsed with 10 ml of 0.9% ammonium formate and was oven dried for no less than 12 hours at 80^{O} C before being re-weighed (Bayne *et al.*, 1999b). The filters were then ashed at 560^{O} C for 4 to 6 hours, placed in a desiccator to cool and then weighed for the final time (B. Bayne, personal communication, 2000). This was done to calculate the organic component of the true faeces and pseudofaeces.

Once all measurements of biodeposits had been conducted, the oysters were weighed and the dimensions of height, length and width were recorded with digital vernier callipers. The mean shell height and whole weights for all of the Feeding Experiment oysters are listed in Table 7.2. The oysters were shucked and the soft tissue of

individual oysters were dried at 80^{O} C for 12 hours before being placed in a desiccator to cool and then weighed to determine soft tissue dry weight.

The variables measured during the three treatments were: total suspended particulate matter; particulate organic matter; total faeces; faeces organic matter; total pseudofaeces; and, pseudofaeces organic matter. These data were then used to calculate rejection rate, faeces production, feeding activity and filtration rate. The calculations used to determine each of these components are listed in Table 7.7. The weight of true faeces and pseudofaeces produced by each oyster in the three treatments is provided in Appendix 3L.

Table 7.7 Definitions and calculations of oyster feeding behaviour components (*Source*: Bayne *et al*., 1999a).

Measured Variable	Derived Variable	Description/Calculation
Total suspended particulate matter		TPM (mg L^{-1}): Suspended matter dried at 80^{O} C
Particulate organic matter		POM (mg L^{-1}): TPM ashed at 560^{O} C for 4 h
	Particulate inorganic matter	PIM (mg L^{-1}): TPM-POM
	Particulate organic content	OC (fraction): POM/TPM
Total faeces		Faeces prod^{n} (mg h^{-1}): Faeces dried at 80^{O} C
Faeces organic matter		FOM (mg h^{-1}) : Faeces ashed at 560^{O} C for 4 h
	Faeces inorganic matter	FIM (mg h^{-1}) : Faeces prod^{n}-FOM
	Faeces organic content	FOC (fraction): FOM/Faeces prod^{n}
Total pseudofaeces		Rejection rate, RR (mg h^{-1}): Pseudofaeces dried at 80^{O} C
Pseudofaeces organic matter		PsOM (mg h^{-1}): Pseudofaeces ashed at 560^{O} C
	Pseudofaeces inorganic matter	PsIM (mg h^{-1}): RR-PsOM
	Pseudofaeces organic content	PsOC (fraction): PsOM/RR
	Filtration rate	FR (mg h^{-1}): (FIM+PsIM)x(TPM/PIM)
	Clearance rate*	CR (L h^{-1}): (FIM+PsIM)/PIM
	Feeding activity**	Feeding activity (mg h^{-1}): Faeces prod^{n}+RR

* Estimate
** Bamber (1987;1990)
N.B. Drying time for suspended particulates and biodeposits was > 12 hours

7.4.5.2 Correction for Body Size

Body size of the experimental oysters is an important variable affecting most physiological responses (Widdows, 1985). There were slight differences in the dry body mass of oysters used in the three treatments (Table 7.2). The Feeding Experiment was designed to test for the variance of feeding behaviour amongst individuals that is not weight dependent but due to exposure to ASS-affected waters. The variations in dry body mass can be removed by correcting feeding rates to a standard body size using the allometric equation (Bayne and Newell, 1983):

$$Y = aX^b$$

or $$\log_{10} Y = \log_{10} a + b \log_{10} X$$

Where Y = measured feeding variable, X = dry body mass in grams, and a is the intercept. The slope, b is the allometric exponent in the equation which describes the physiological rate as a function of body size (Bayne *et al.*, 1999a). Mean dry body mass (± 95% CI, n = 54) of the experimental oysters was 0.77 ± 0.07 g. This mean body mass was used as the standard body mass in place of a standard 1 g animal and the corrections for weight differences were calculated using the following equation (Widdows, 1985):

$$\text{Log } Y_c = \log Y_o - (b \log X_o - b \log X_c)$$

Where Y_c is the corrected value for a standard body mass (X_c) and Y_o and X_o are the individual's measured rate and body mass, respectively. The weight-exponent was taken from Bayne's *et al.* (1999b) study that measured clearance rate in Sydney rock oysters and estimated b as 0.641.

Single factor ANOVA was used to test for differences between Treatments 6, 7 and 8 for weight-corrected feeding activity, faeces production, rejection rate and filtration rate data. SPSS Version 11.0.0 (SPSS Inc.) statistical software package was used to perform each single factor ANOVA. *Post hoc* pairwise comparisons of the results were made using the Least Significant Difference test.

7.4.6 Gross Pathology

Oysters removed from the Behaviour Experiment were observed for any gross changes in their soft tissue appearance following the six hours of exposure. In particular, any visible accumulation of iron or aluminium in their shell liquid or on their soft tissue was noted.

7.4.7 Handling and Fixation of Oysters

Twelve oysters were removed from each treatment of the Behaviour Experiment after six hours of exposure to the treatment water. The time of exposure commenced from the first instance that individual oysters opened their valves. After oysters were removed from the aquarium, the soft tissue of the animal was immediately excised from the valves. To do this, oysters were opened from the hinge and a sterile scalpel was used to cut away the adductor muscle from the right and left valves. The soft tissue was rinsed in deionised water to remove shell fragments and three incisions were made into the digestive gland to allow penetration of the fixative. Oysters infected by mudworm were discarded.

Formalin (10% sea water) was used to preserve oyster soft tissue for histopathology (Howard and Smith, 1983). Howard and Smith (1983) describe Formalin (10% sea water) as a good general fixative for bivalves. The ingredients for this fixative are provided in Appendix 3M. Formalin (10% sea water) was used for this study in preference to Davidson's fixative because acid fixatives interfere with iron (Howard and Smith, 1983).

Oysters were placed in formalin (10% sea water) fixative for 24 to 48 hours, at room temperature, before being stored in 70% ETOH solution (Howard and Smith, 1983). Additional information on the preparation, processing and staining of sections is provided below and in Appendix 3M.

7.4.8 Cutting and Staining of Histological Sections

Two transverse tissue cross sections were taken, the first through the intestine, digestive diverticula, stomach and labial palps and the second through the adductor muscle, kidney and gills. Processing of the specimens involved replacing water in the tissue with wax at 60^{O} C under vacuum to give the tissue enough stability to be cut. The tissue was processed in a Shandon Hypercentre XP Tissue Processing System. Tissue was then embedded into molten Paraplast wax using a Tissue-Tek Embedding Console System.

Sections were cut at 5 µm using Feather S35 Microtome Blades on a Microm HM 330 Microtome. Once cut, sections were floated on a water bath of boiled deionised water. Sections were picked up on acid washed glass slides and dried overnight at 58 ^{O}C. All oysters were stained with haematoxylin and eosin (H&E). Oysters from Treatments 1, 4 and 5 were also stained with Perls' Prussian Blue (PPB). The staining procedure for both stains is listed in Appendix 3M. Oyster sections stained with H&E stain were examined using light microscopy for changes to the gills and mantle resulting from exposure to the test waters. PPB is a stain specific for ferric iron (Howard and Smith, 1983) and these sections were examined for iron accumulation also using light microscopy.

7.5 RESULTS

7.5.1 Oysters Behavioural Response to Acidified Water

The observed behavioural traits in the five treatments were: open valves (observed in all treatments), excessive gaping (observed in Treatments 3 and 5); clomping (observed in Treatment 5); and, no activity (observed in Treatments 4 and 5). The proportion of oysters displaying each behavioural trait described above in each treatment is listed in Table 7.8. Five oysters in Treatment 5 and two oysters in Treatment 4 were inactive for the entire exposure period (Table 7.8). In Treatments 1, 2 and 3, all oysters opened their valves and produced true faeces and pseudofaeces. Excessive gaping was only observed in the acidified test waters. Clomping occurred in Treatment 5 and was attributed to the high concentrations of suspended particles (Table 7.4) in the treatment water. The time taken for individual oysters to open their valves in each treatment varied between 1 and 272 minutes.

Table 7.8 Summary of oysters' behavioural response in Treatments 1 to 5.

Treatment Number	Treatment Water	Mean pH	Behaviour Trait: Open Valves	Excessive Gaping	Clomping	No Activity
1	Seawater + Deionised H_2O	8.0	24/24	-	-	-
2	Seawater + Deionised H_2O + 0.1 M HCl	5.1	24/24	-	-	-
3	Seawater + Deionised H_2O + Al + 0.1 M HCl	5.1	24/24	7/24	-	-
4	Seawater + Deionised H_2O + Fe + 0.1 M HCl	5.1	22/24	-	-	2/24
5	Seawater + ASS-Affected Waters + 0.1 M HCl	5.1	19/24	1/24	8/24	5/24

Proportion displaying behaviour trait

Bamber (1987; 1990) found that oysters were slow to respond to stimuli in acidified treatments. Oysters were prodded every hour to assess their response to a tactile stimulus. Oysters in Treatments 2 to 5 were slower to react after prodding, especially in the latter stages (i.e. 4 to 6 hours of exposure). The results obtained from the Behaviour Experiment show that oysters actively feed at pH 5.1. The results from the Feeding Experiment are presented in the following section.

7.5.2 Effect of ASS-affected Waters on Oyster Feeding Behaviour

7.5.2.1 Feeding Activity

Feeding activity is the amount of true faeces and pseudofaeces produced by individual oysters over a designated period of time (Bamber, 1987; 1990). The mean feeding activity data from the Feeding Experiment are displayed in Figure 7.4. Figure 7.4 shows an increasing decline in feeding activity as pH is reduced. The feeding activity at pH 5.5 (Treatment 8) was significantly lower than the feeding activity measured at pH 6.5 and 7.96 (Treatment 7 and 6, respectively) (Figure 7.4).

The results of the single factor ANOVA for feeding activity, faeces production, rejection rates and filtration rates are tabulated in Appendix 3N. Also included in Appendix 3N are the results from the Least Significant Difference *post hoc* multiple comparisons.

7.5.2.2 Faeces Production

Faeces production is the amount of true faeces produced by an individual oyster per hour (Table 7.7) (Bayne *et al.*, 1999a). The mean faeces production data from the Feeding Experiment are displayed in Figure 7.5. This figure shows that faeces production decreases as pH is reduced. The mean faeces production at pH 5.5 was significantly lower than at pH 6.5 and 7.96.

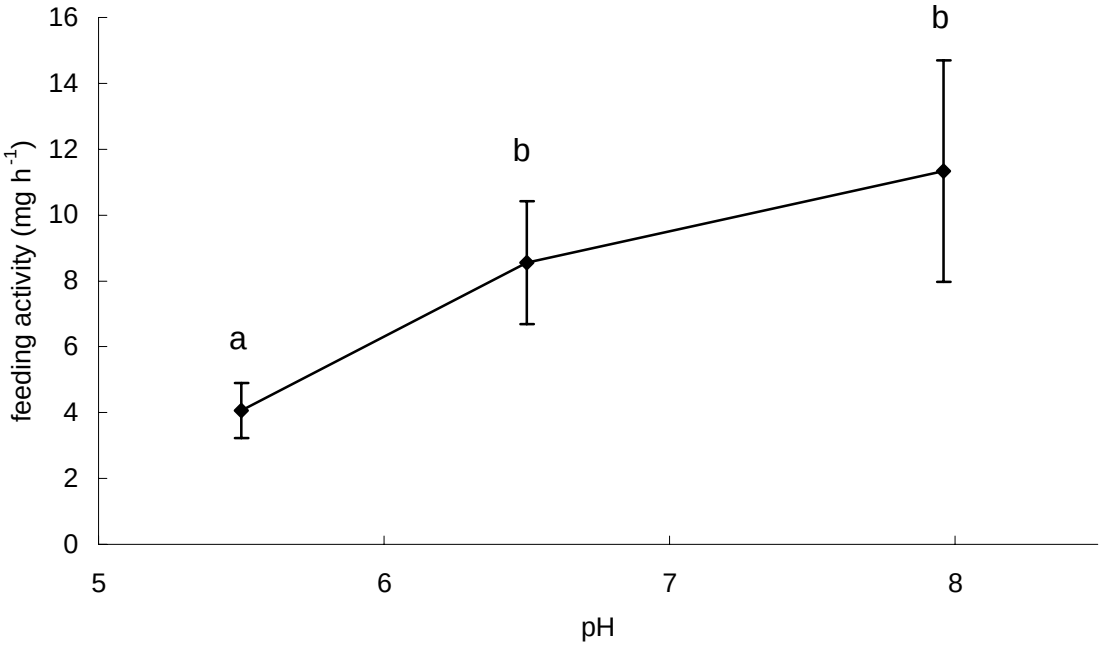


Figure 7.4 Mean (± 95% CIs, n = 18) feeding activity over a range of pH. Means sharing letters are not significantly different (P > 0.05).

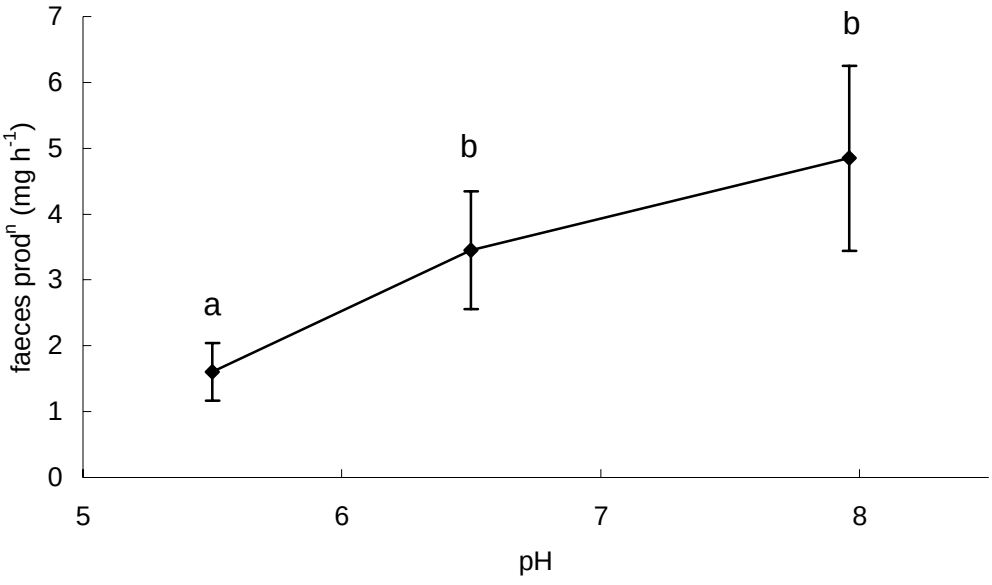


Figure 7.5 Mean (± 95% CIs, n = 18) faeces prod^{n} over a range of pH. Means sharing letters are not significantly different (P > 0.05).

7.5.2.3 Rejection Rate

Rejection rate is the amount of pseudofaeces production by individual oysters in an hour and was quantified by collecting and measuring the total amount of material egested (Bayne *et al.*, 1999a). The mean rejection rate data from the Feeding Experiment are displayed in Figure 7.6. Figure 7.6 shows that the rejection rate also decreases as pH decreases. As was the case in the previous feeding traits, the rejection rate at pH 5.5 is significantly lower than at pH 6.5 and 7.96.

7.5.2.4 Filtration Rate

The mean filtration rate data obtained from the Feeding Experiment are displayed in Figure 7.7. A significant reduction in the filtration rate was measured at pH 5.5 compared to pH 6.5 and 7.96. Filtration rate is dependent on the TPM to PIM ratio. Figure 7.3 shows that the TPM to PIM ratio was lower in Treatment 8 (pH 5.5) compared to Treatment 6 (pH 8.0) and 7 (pH 6.5).

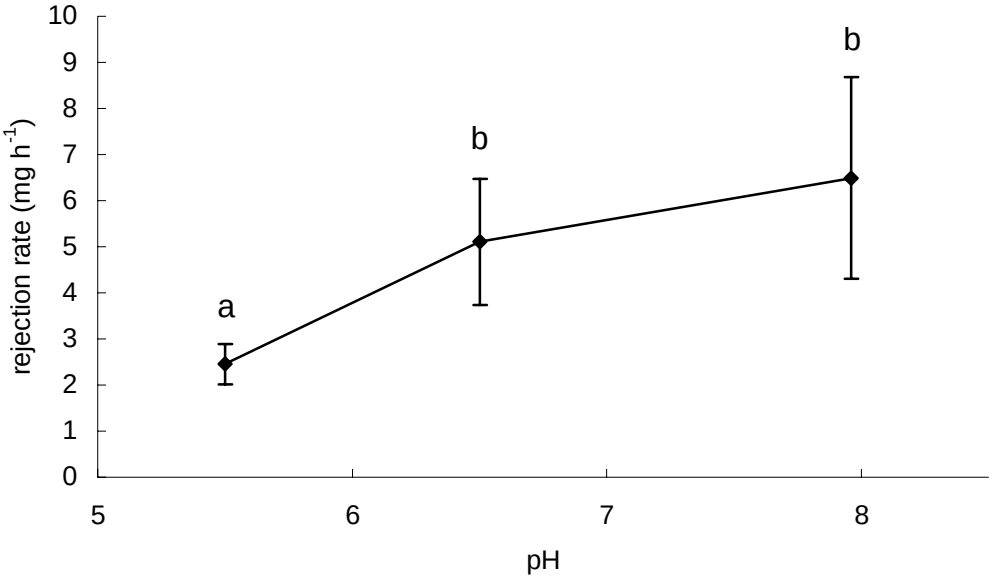


Figure 7.6 Mean (± 95% CIs, n = 18) rejection rate over a range of pH. Means sharing letters are not significantly different (P > 0.05).

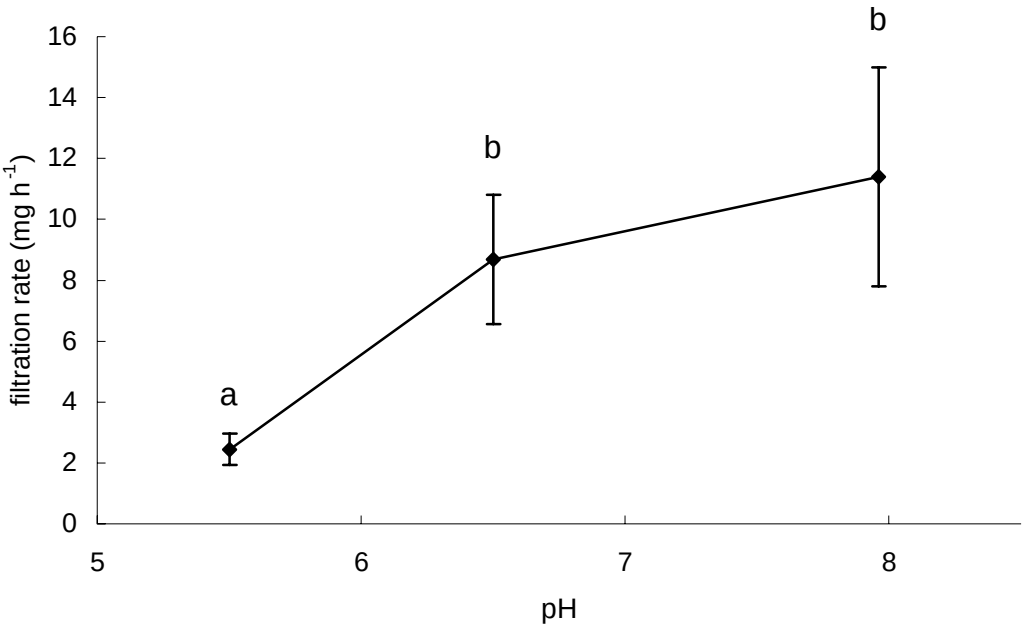


Figure 7.7 Mean (± 95% CIs, n = 18) filtration rate over a range of pH. Means sharing letters are not significantly different (P > 0.05).

7.5.3 Post Experiment Oyster Survival

Twelve oysters from Treatments 1 to 5 of the Behaviour Experiment were replaced in the estuary at the conclusion of each treatment and monitored for survival over a four-week period at Site 2 (Figure 4.1). Monitoring of post experiment oyster survival was undertaken to determine if short-term exposure to the five treatments were lethal. No mortalities were recorded in the oysters from the five treatments four weeks after exposure suggesting that farmed oysters can recover from short-term acid exposure. This finding has implications for modified management practices for farmed oysters in acid-impacted estuaries.

7.5.4 Oyster Soft Tissue Response to Acidified Water

This section details the results of the short-term effects of exposure of Sydney rock oysters to: acidified water; acidified water containing aluminium or iron; and, ASS-affected waters. The tissue and cell changes observed in Treatments 2 to 5 of the Behaviour Experiment were compared to the 12 oysters from Treatment 1 to ensure that the changes were a result of exposure to the treatment waters. The histopathology data for Treatments 1 to 5 are detailed in the following sections. Examples of the soft tissue responses are shown in Plate 7.1.

7.5.4.1 Treatment 1 (pH 8.0, No Added Iron or Aluminium)

Histopathology examination did not reveal any significant aggregations of haemocytes in the gills or mantle soft tissues of oysters from this treatment. However, there was focal necrosis of the frontal and lateral cells of the ordinary filaments in particular oysters. Two oysters had very mild, focal accumulations of haemocytes located in the gills. This response was not typical of the other oysters from Treatment 1. Due to the limited histological information available for the

Sydney rock oyster, data from Treatments 2 to 5 were compared to the data derived from Treatment 1.

7.5.4.2 Treatment 2 (pH 5.1, No Added Iron or Aluminium)

Oysters from Treatment 2 typically had increased haemocyte activity in the gills when compared to oyster sections from Treatment 1. There were mild, focal aggregations of haemocytes located in the interlamellar junctions and the haemolymph sinuses of plicae and ordinary filaments of the gill of particular oysters (Plate 7.1A). Frontal and lateral cell necrosis was observed to a greater extent in Treatment 2 oysters than was observed in Treatment 1, however, it could not be determined if this was due to the acidity. No significant findings were observed in the mantle soft tissue of the 12 oysters from Treatment 2.

7.5.4.3 Treatment 3 (pH 5.1, 7.6 mg L^{-1} of Aluminium)

Oysters from Treatment 3 had extensive haemocyte activity throughout the gills. Large accumulations of haemocytes were observed in the interlamellar junctions and haemolymph sinuses of plicae and ordinary filaments of the gill. There were gill lesions present in all oysters from this treatment. The most common lesion was in the haemolymph sinuses of plicae. Rupturing of this sinus caused infiltrations of haemocytes into the adjacent water tube (this occurred in 11 oysters) (Plate 7.1C). There were infiltrations of haemocytes into the pallial cavity through necrotic frontal and lateral cells of ordinary filaments (Plate 7.1B). This response was observed in six oysters. Haemocytes were commonly observed in the junctions between adjacent filaments, congesting the gills. There was also necrosis and sloughing of mantle epithelial cells predominately on the pallial surface in oysters from Treatment 3.

7.5.4.4 Treatment 4 (pH 5.1, 7.7 mg L^{-1} of Iron)

There were mild to moderate, focal aggregations of haemocytes located in the interlamellar junctions and the haemolymph sinuses of plicae and ordinary filaments of oysters from Treatment 4. There was necrosis and sloughing of the mantle epithelial cells on the pallial surface in two oysters. Corresponding thin sections stained with PPB showed iron at the sites where mantle necrosis and sloughing was occurring. The degree of haemocyte activity throughout the gills in this treatment was comparable to that observed in Treatment 2.

7.5.4.5 Treatment 5 (ASS-Affected Waters Adjusted to pH 5.1)

Treatment 5 contained 13.5 mg L^{-1} of dissolved and suspended iron and 6.2 mg L^{-1} of dissolved and suspended aluminium, which was from the added ASS-affected water. Oysters from Treatment 5 had mild to moderate haemocyte activity throughout the gills. Moderate aggregations of haemocytes were observed in the interlamellar junctions and haemolymph sinuses of plicae and ordinary filaments. Focal necrosis and sloughing of the mantle epithelial cells on the pallial surface was observed in the thin sections as well (Plate 7.1D).

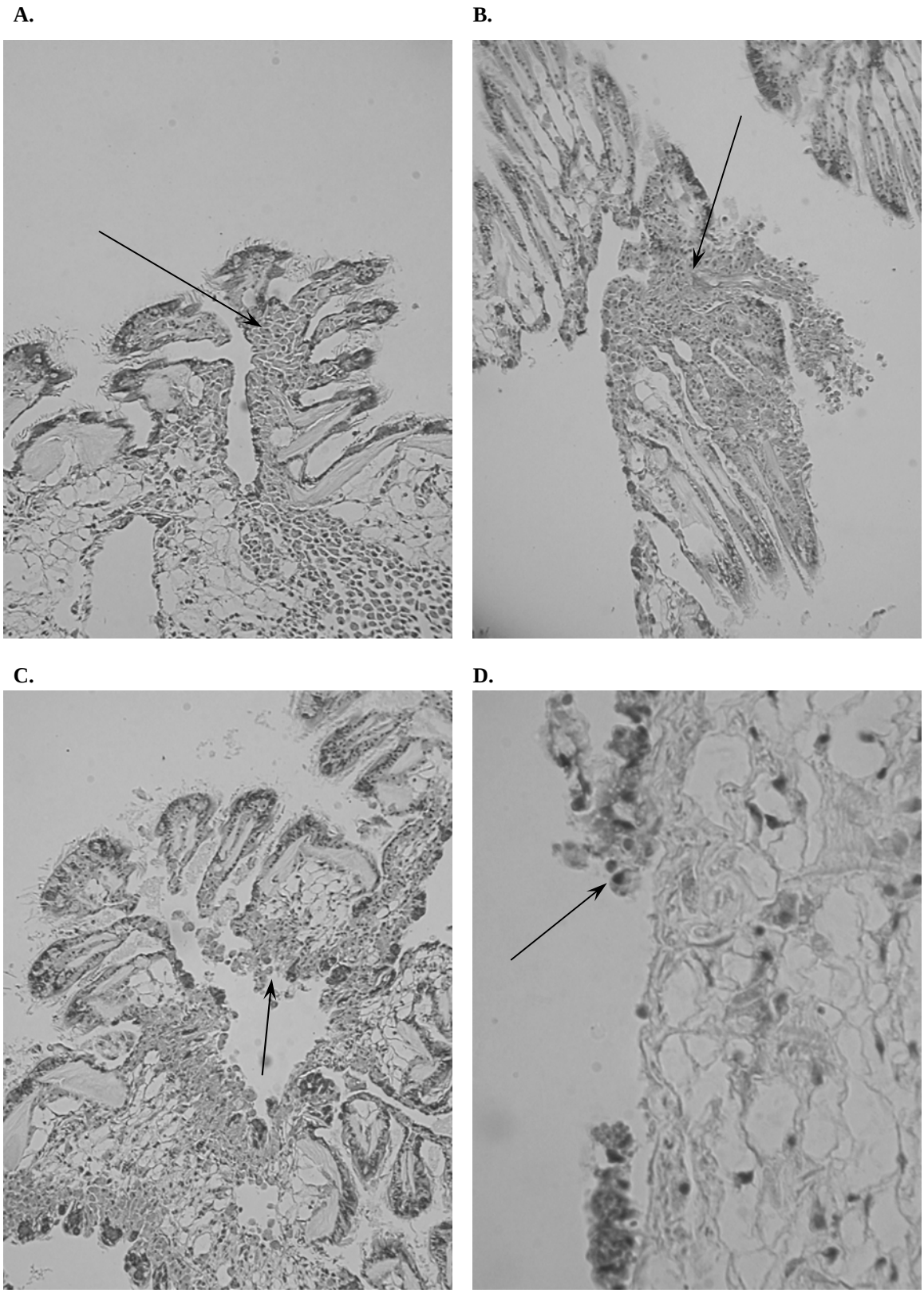


Plate 7.1 Soft tissue responses in Behaviour Experiment oysters: (A) haemocyte infiltration into the haemolymph sinuses of the plica and filaments (Treatment 2, x 40); (B) haemocyte infiltration into the haemolymph sinuses of the plica and filaments with rupture of the ordinary filaments (Treatment 3, x 40); (C) rupture of the haemolymph sinus of plica (Treatment 3, x 40); and, (D) necrosis and sloughing of mantle epithelial cells (Treatment 5, x 160).

As was observed in the previous treatments, there was necrosis of the frontal cells and lateral cells of particular gill filaments. Haemocytes in the sinuses of the ordinary filaments were escaping into the pallial cavity through necrotic frontal cells and lateral cells of these filaments. This was observed in 5 oysters from Treatment 5. Corresponding thin sections stained with PPB showed iron had accumulated at sites of necrosis and sloughing of mantle epithelial cells. The soft tissue response in Treatment 5 was not as severe as was observed in oysters from Treatment 3 even though the aluminium concentrations were similar.

7.5.5 Effects of Iron Precipitates

The water used for Treatments 4 and 5 appeared orange and suspended iron flocs were clearly visible. Water samples were collected during these treatments and analysed using ICPAES for the concentration of dissolved and suspended iron. The results from ICPAES analysis (Table 7.4) show that there was: 7.71 mg L^{-1} of suspended iron in Treatment 4; and, 0.201 mg L^{-1} and 13.25 mg L^{-1} of dissolved and suspended iron, respectively, in Treatment 5. These iron concentrations were commonly measured at acidified field sites during the present study (Chapter 3) and are consistent with levels measured in other studies investigating estuary acidification in eastern Australia (Sammut *et al.*, 1996a; Sammut, 1998; Sonter, 1999).

All oysters removed from Treatments 4 and 5 displayed gross signs of iron flocs in the shell fluid (Plate 7.2) and on the gill surface (Plate 7.3). Twelve oysters removed from Treatments 1, 4 and 5 were fixed in formalin (10% sea water), processed for histopathology and stained with PPB to investigate the extent of iron precipitate accumulation on the soft tissues. PPB stains iron blue, nuclei appear red and the background appears a pale red colour. Table 7.9 lists the presence and extent of iron accumulation on and in the soft tissues of Behaviour Experiment oysters.

No iron was observed in any of the histopathology sections from oysters removed from Treatment 1 (Table 7.9 and Plate 7.4). However, histopathology data revealed that oysters removed from Treatments 4 and 5 had extensive accumulations of iron on and in their soft tissues (Table 7.9).

Iron precipitates were observed: on the gill epithelium; on the mantle epithelium; in the stomach; in the intestine; and, in the rectum of oysters removed from Treatment 4 (Table 7.9). Similarly, iron precipitates were observed: on the gill epithelium (Plate 7.5A); on the mantle epithelium and in the pallial cavity (Plate 7.5B and 7.5C); in the stomach (Plate 7.5D); in the intestine (Plate 7.6A); in the digestive gland ducts (Plate 7.6B); in the digestive tubules (Plate 7.6C); and, in the rectum (Plate 7.6D) of oysters removed from Treatment 5.

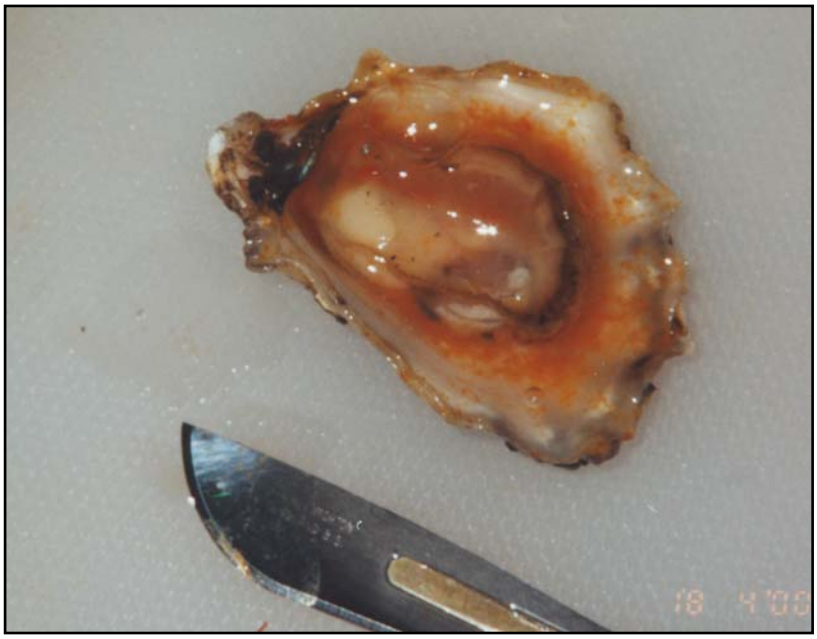


Plate 7.2 Oyster with the right valve removed showing iron flocs in the shell fluid. Treatment 4 oyster after 6 hours of exposure to acidified water with added iron chloride.

Plate 7.3 Oyster with the left valve cut-away showing extensive accumulation of iron flocs on the gills. Treatment 5 oyster after 6 hours exposure to ASS-affected waters.

Table 7.9 List of iron accumulation on the gills, on the mantle, in the stomach, in the digestive gland tubules and in the rectum of Sydney rock oysters.

			Iron Accumulation					
Exp.	Oyster	Iron Present	Gills	intestine	Stomach	Digestive Gland Tubules	Rectum	Mantle
1	1	No	-	-	-	-	-	-
	2	No	-	-	-	-	-	-
	3	No	-	-	-	-	-	-
	4	No	-	-	-	-	-	-
	5	No	-	-	-	-	-	-
	6	No	-	-	-	-	-	-
	7	No	-	-	-	-	-	-
	8	No	-	-	-	-	-	-
	9	No	-	-	-	-	-	-
	10	No	-	-	-	-	-	-
	11	No	-	-	-	-	-	-
	12	No	-	-	-	-	-	-
4	1	Yes	D	-	B	-	A	C
	2	Yes	D	A	B	-	A	C
	3	Yes	D	-	A	-	-	B
	4	Yes	D	B	A	-	A	C
	5	Yes	D	C	A	-	A	D
	6	Yes	D	D	-	-	A	C
	7	Yes	D	-	-	-	-	C
	8	Yes	D	-	-	-	-	C
	9	Yes	D	B	A	-	-	C
	10	Yes	C	-	A	-	-	C
	11	Yes	D	B	B	-	-	C
	12	Yes	D	C	B	-	A	C
5	1	Yes	D	-	A	-	-	A
	2	Yes	D	D	C	C	-	B
	3	Yes	D	-	B	B	-	A
	4	Yes	D	-	-	A	-	C
	5	Yes	C	C	-	-	C	C
	6	Yes	C	D	B	C	-	A
	7	Yes	D	D	C	-	-	B
	8	Yes	D	B	B	-	-	B
	9	Yes	D	-	B	-	-	C
	10	Yes	D	-	A	-	A	C
	11	Yes	C	B	A	-	-	C
	12	Yes	C	A	A	-	A	B

A = very minor accumulation
B = minor accumulation
C = moderate accumulation
D = extensive accumulation

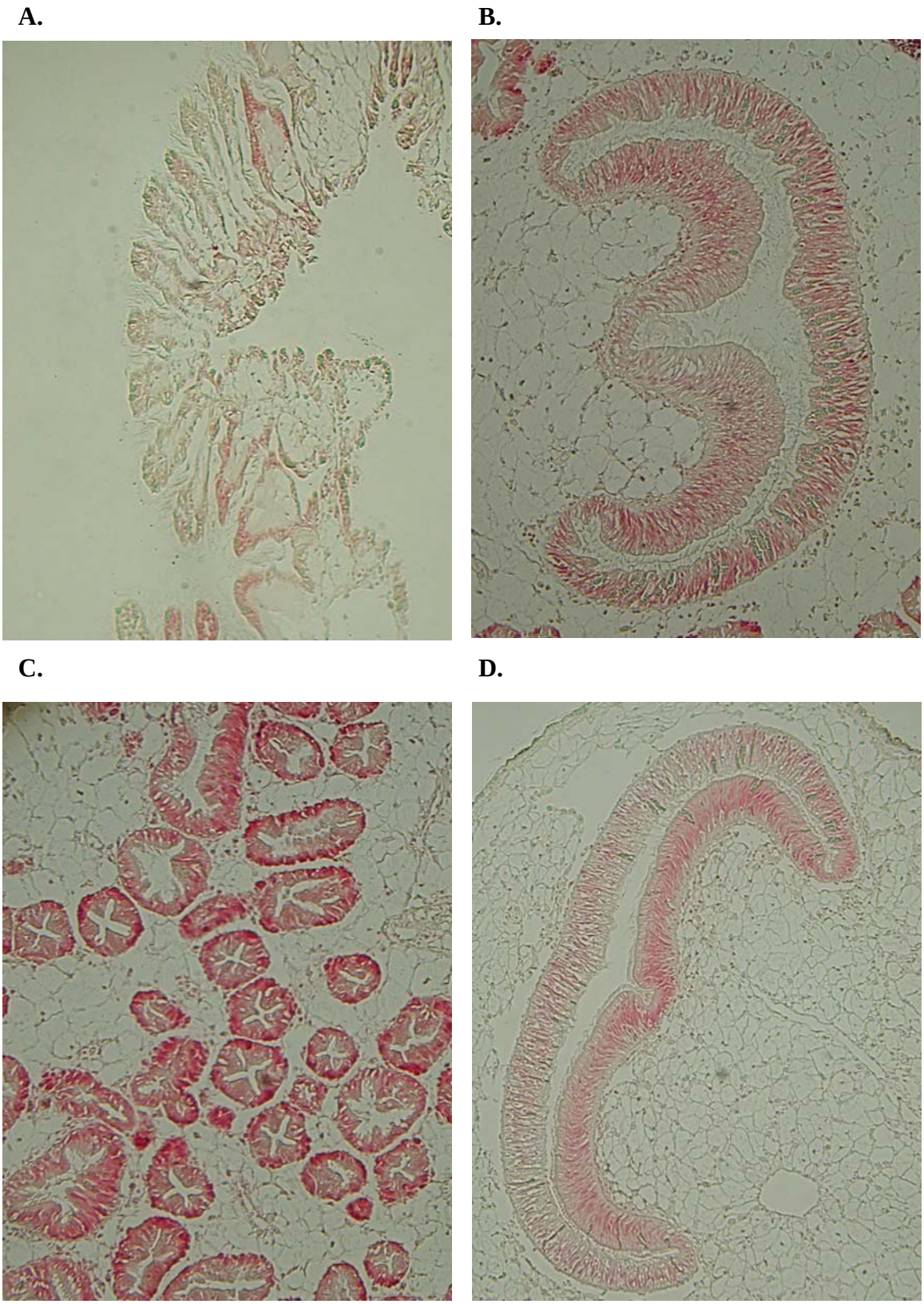


Plate 7.4 Treatment 1 Behaviour Experiment oysters stained with PPB showing: (A) a gill plica (x 40); (B) the intestine (x 40); (C) digestive tubules (x 40); and, (D) the rectum (x 40).

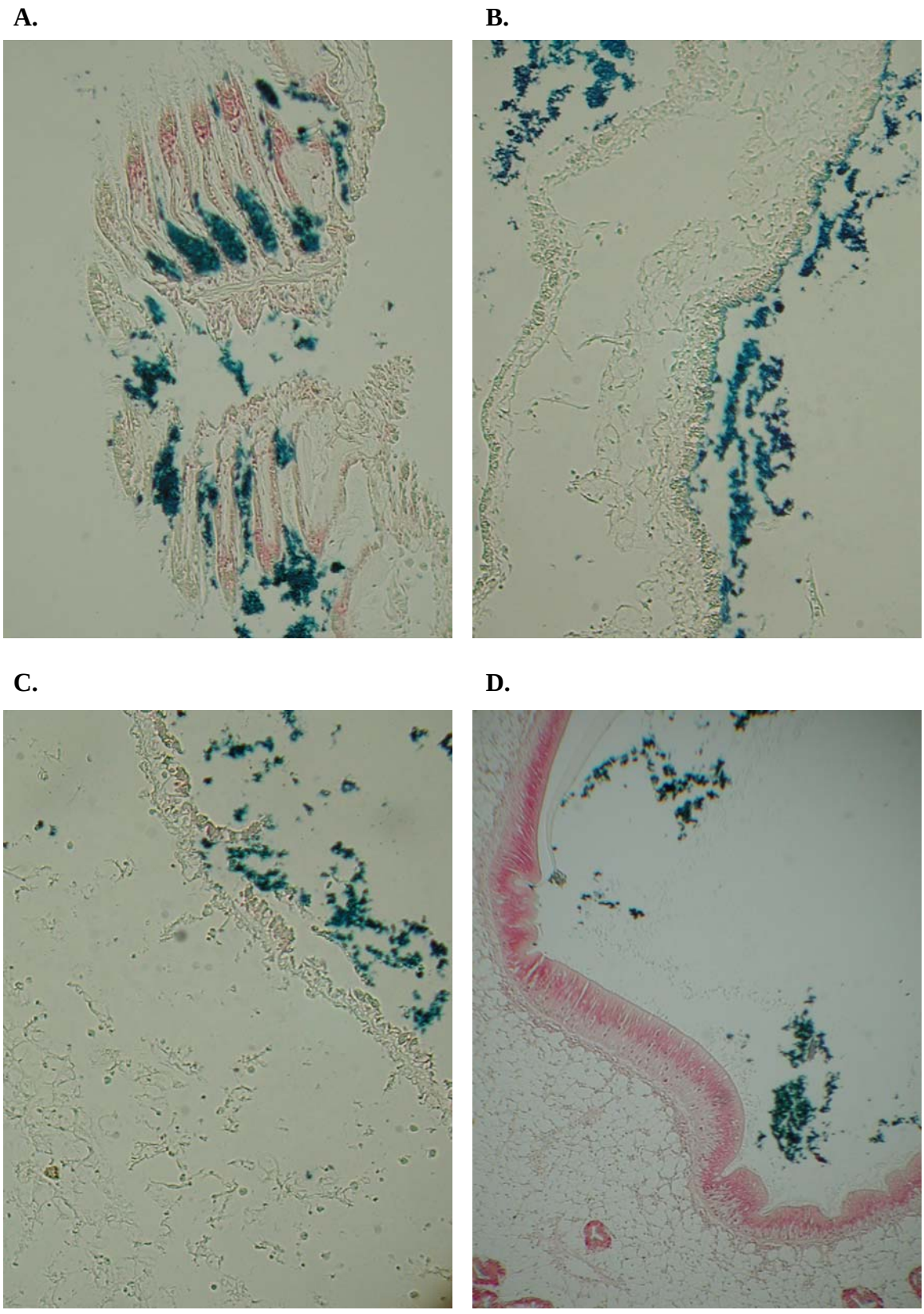


Plate 7.5 Treatment 5 Behaviour Experiment oysters stained with PPB showing: (A) iron on a gill plica (x 40); (B and C) iron on the mantle and in the pallial cavity (x 40); and, (D) iron in the stomach (x 15).

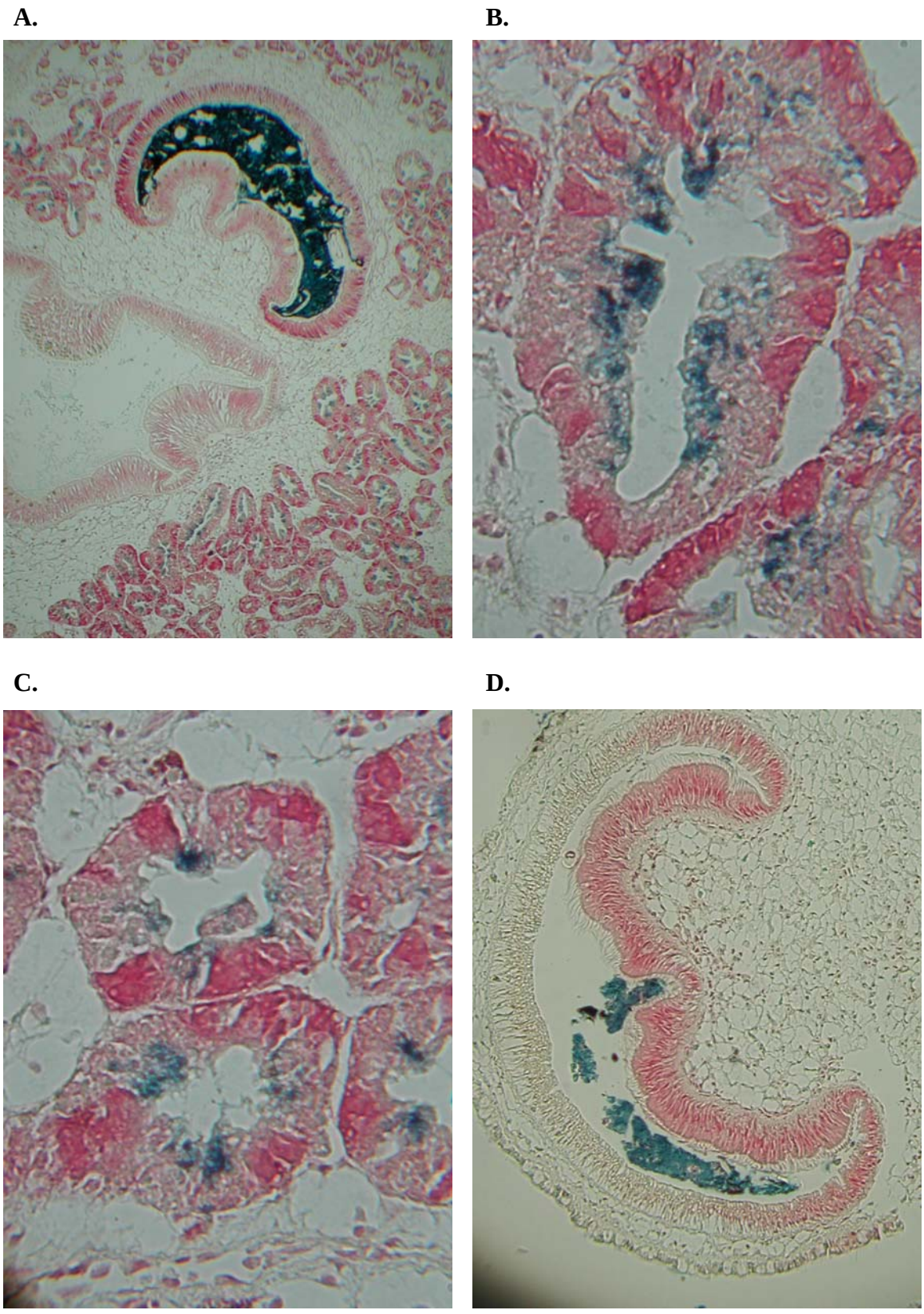


Plate 7.6 Treatment 5 Behaviour Experiment oysters stained with PPB showing: (A) iron in the intestine (x 15); (B and C) iron in the secretory-absorptive cells of digestive gland tubules (x 160); and, (D) iron in the rectum (x 40).

7.6 DISCUSSION

7.6.1 Oyster Behavioural Response to Acidified Water

Observations of oyster behaviour from Treatments 1 to 5 indicate that oysters attempt to feed under acidic conditions providing salinity conditions are satisfactory (i.e. above 15 ppt). This directly exposes oyster soft tissue to conditions that are potentially injurious. Additionally, this finding permitted feeding rates to be quantified as oysters produced true faeces and pseudofaeces under acidic conditions. Exposure to acidified water (pH 5.1) containing aluminium or ASS-affected waters caused abnormal valve movements in a small proportion of experimental oysters.

7.6.2 Feeding Behaviour

Feeding activity, faeces production, rejection rate and filtration rate (Figures 7.4 to 7.7) were significantly reduced at pH 5.5. The large difference in all feeding traits (feeding activity, faeces production, rejection rate and filtration rate) between Treatments 6 and 7 and Treatment 8 can be attributed to the presence of ASS-affected waters. Treatment 8 had a higher concentration of TPM and PIM compared to Treatments 6 and 7. This was attributed to the presence of oxidation products contained in ASS-affected waters, namely iron and aluminium. However, it cannot be established whether the significant reduction in feeding at pH 5.5 was due to a reduction in pH alone or from the influence of the oxidation products contained in ASS-affected waters. The results obtained from Treatments 6 and 7 indicate that the reduction in feeding behaviour traits is likely to be mostly influenced by pH as the experimental conditions were similar in all other respects apart from pH.

The findings from the Feeding Experiment are consistent with overseas studies investigating feeding and pumping in other species of bivalves exposed to acid (Bamber, 1987; 1990; Loosanoff and Tommers, 1947). The results from this study support Loosanoff and Tommers' (1947) finding that pumping rate dramatically decreases at pH values below 6.5 in *O. virginica*. This is a significant finding and helps to explain poor growth rates measured in the field studies detailed in Chapter 6.

Results from the Feeding Experiment also highlighted the change in the dietary abundance of food available to oysters in ASS-affected waters. High concentrations of colloidal iron and aluminium alter the ratio between the inorganic component and the organic component of ASS-affected waters. ASS-affected waters cause conditions that consist of a small proportion of food and a large proportion of non-utilisable matter within the available seston (Hawkins *et al.*, 1996; Bayne *et al.*, 1987). Therefore, the nutritional quality of ASS-affected waters is low when quality is expressed as organic content per unit volume of diet.

Attempts were made to remove the iron from the treatment waters in Treatments 7 and 8 to address the assumptions that underpin the biodeposition methodology. This was unsuccessful in the case of Treatment 8, however it highlights the realistic environmental problem caused by high concentrations of suspended iron to bivalves. Furthermore, the histopathology data clearly showed the accumulation of iron flocs on the gills and mantle and in the digestive gland and rectum.

The results from Treatment 8 strongly suggest that a combination of low pH and iron is impairing feeding in oysters. Further investigation is still required to elucidate the

effects of low pH alone from the other constituents of ASS-affected waters on oyster feeding.

7.6.3 Response of Oyster Soft Tissues

This study identified changes in the gill and mantle soft tissues resulting from exposure to acidic waters relative to oysters exposed to pH neutral waters. Changes were most noticeable in acidic treatments containing added iron, aluminium or ASS-affected waters. Lesions in the gills were observed after only 6 hours of exposure to acidified treatments containing aluminium. The extensive inflammatory response and gill lesions observed in Treatment 3 suggests that the presence of aluminium in combination with low pH causes a more intense response in the gill and mantle soft tissues than water of a low pH with no added aluminium (Treatment 2). It is likely that this was only the initial stage of changes in the soft tissue as exposure time was only for a short duration and oysters were immediately fixed in formalin after exposure.

Comparison of corresponding thin sections stained with H&E and PPB revealed aggregations of inflammatory cells were not only associated with iron accumulation. Further research is required to examine the effects of suspended iron precipitates at neutral and alkaline pH levels. The mobilisation of suspended iron precipitates can be several kilometres from the ASS outflow location (Chapter 3). Iron flocs were observed grossly in oysters removed from the field exposure experiments detailed in Chapter 4. Based on the results from the laboratory investigations, it is highly probable that the high concentrations of iron at acidified field sites were contributing to the high mortality rates and negative growth rates measured during field exposure experiments.

Other studies have confirmed that iron is not toxic to bivalves at neutral pH levels. Sunila (1988) found that ferric iron did not cause a toxic reaction in the gills of *M. edulis*. Also, it has been established that not all of the iron that enters the gut is absorbed. George *et al.* (1976) estimated that 30% of the iron presented to the gut is not absorbed and is passed via the faeces in the mussel *M. edulis*. An interesting finding from the Behaviour Experiment was that iron chloride contained in Treatment 4 was not observed in digestive tubules, however iron in the treatment containing ASS-affected water was observed in the secretory-absorptive cells of digestive tubules. Iron transformations in ASS-affected waters are likely to be different than in the artificial test waters due to the reaction of iron with other pyrite oxidation products and other elements present in the natural waters. The resulting iron chemical species are therefore, likely to be different to those in the artificial test waters. It is also likely that the species of aluminium contained in Treatment 3 was different to the aluminium in Treatment 5 for the same reasons. This would account for the more intense response in soft tissues in Treatment 3 oysters. Further work should model iron and aluminium speciation in the treatment waters to account for metal transformations.

Soft tissue responses observed in oysters from Treatment 4 were likely to be induced by the combination of acidity and iron as opposed to the iron alone. Although there is no evidence of direct iron toxicity in Sydney rock oysters, it is very probable that iron impairs gill function by congesting the ciliary junctions thereby affecting feeding processes and gas exchange. The long-term implications of iron accumulation on the soft tissues of the Sydney rock oyster are unknown.

7.7 CHAPTER SUMMARY

This chapter demonstrated that exposure of Sydney rock oysters to acidified water alters their valve movements, inhibits their feeding behaviour and causes changes to their gill and mantle soft tissues. Feeding activity, true faeces production, rejection rate and filtration rate were dramatically reduced in weakly acidified treatments (pH 5.5) that contained ASS-affected water. However, it could not be established if the reduction in feeding was a result of the acidity alone or due to the presence of oxidation products contained in the ASS-affected waters. The data from the Feeding Experiment and other studies (Bamber, 1987; 1990; Loosanoff and Tommers, 1947) strongly suggests that the reduction in pH caused by the addition of ASS-affected water is the main factor that inhibits oyster feeding.

This chapter has confirmed that acidified water containing aluminium causes a degenerative soft tissue response in the gills and, to a lesser extent, the mantle of the Sydney rock oyster after only a short period of exposure. Injuries to the gills of oysters were a result of the combined effect of low pH and aluminium. Histopathology has revealed that iron is extensively accumulated on the gill and mantle and in the intestine, stomach digestive tubules and rectum of oysters exposed to ASS-affected waters.

ASS outflows dramatically alter the biochemical composition of suspended particles in the estuarine waters that it affects. The chemical and physical nature of suspended particles in areas of the estuary impacted by ASS-affected waters is different to the properties of suspended particles that are present under normal estuarine conditions.

The results from these laboratory investigations aid in the explanation of decreased growth performance measured at sites recurrently exposed to ASS-affected waters. Also, this chapter highlights the deleterious effects of high concentrations of iron precipitates contained in ASS-affected waters to oyster health. Chapter 8 is the final chapter and details the benefits, further developments and conclusions arising from this present study.

8 BENEFITS, FURTHER DEVELOPMENT AND CONCLUSION

8.1 BENEFITS

The project findings directly benefit the oyster industry in several ways. Firstly, the study has confirmed that ASS are a threat to the industry; this confirmation has enabled the industry to seek and achieve a greater commitment from local and state governments to ameliorate and manage acidification of estuarine waters. Acidification can only be realistically managed at its source. Oyster farmers are now in a better position to influence the setting of environmental goals for water quality management in estuary systems. There is now an increased awareness of the threats of acidification to the oyster industry by: NSW Fisheries; NSW EPA; NSW Agriculture; The Department of Infrastructure, Planning and Natural Resources (formerly Planning NSW and DLWC); and, Public Works. Oyster farmers have reported increased responsiveness to acidification problems from environmental managers.

Secondly, farmers are now able to make better risk and stock management decisions based on the location of their leases in relation to high ASS risk areas. Leases at risk of high frequency or chronic acidification can be avoided, or an understanding of acidification processes and their relationship with rainfall and floodplain hydrology can guide decisions on stock movement.

Thirdly, the study has enabled farmers to diagnose acid-related problems on leases and to differentiate acid-induced losses from other risk factors such as disease. An ability to identify acidification as a factor, where it occurs, is important to farmers in order to implement reactive strategies. For example, farmers may be able to manage the impacts of ASS by moving oysters to a refuge area at the onset of an acid event. This work has shown that oyster can recover from short-term exposure to acidified water.

The fourth objective of this study was to effectively disseminate the findings of the study. This objective was successfully met. The findings of the project have already been taken into consideration by environmental decision makers at the local and state government level. For example, the Hasting Council now recognises oyster farmers as stakeholders when approving developments in areas of the Hastings River catchment that are mapped as an acid sulfate soil hazard. The council has also appointed an Acid Sulfate Soil Officer due in part to the increased local awareness of ASS raised by this project and lobbying by the Hastings River Oyster Growers. The research findings have also empowered the oyster industry in the public participation stage of the environmental impact statement process. The research findings have enabled the industry to assess EISs more effectively and to make comment on elements of developments that may cause potential harm to their activities. Similarly, oyster farmers are now recognised as an "interest" group and stakeholder in ecological risk assessment. An oyster-farming representative was appointed to the Acid Sulfate Soil Management Advisory Committee (ASSMAC) due to the now widespread recognition of the threat of acidification to the oyster farming industry.

The research findings have also been disseminated to a range of government departments involved in ASS management in NSW and Queensland through the ASSAY newsletter, meetings of both the NSW and Queensland ASS Technical Committees, and ASSMAC and QASSMAC. "An Introduction to ASS", co-authored by the Principal Investigator, was re-issued with a section on oysters, by ASSMAC. The research team members also presented findings at regular oyster grower association meetings and field days. Farmers who participated on the project were able to disseminate the findings to their peers.

During the course of the study, the research findings were an impetus for a successful public forum on ASS on the Hastings River, and a gathering of Water Reform CEOs to examine more closely the impacts of ASS on the oyster industry and other water users.

The study has significantly added to the knowledge on the environmental impacts of ASS enabling environmental managers and consenting authorities to address "uncertainty" more effectively, and to improve planning processes and proactive management strategies.

8.2 FURTHER DEVELOPMENT

This work was mainly focused on the identification and characterisation of an environmental problem that impacts on oyster productivity. Further research should focus on the toxicology of acidified waters to set acceptable water quality criteria for sustainability of the industry. Research on acid sulfate soil management and amelioration is now a priority in eastern Australia, but environmental goals are often poorly defined. The findings of the present study should continue to be promoted so that remediation studies can set discharge criteria that will benefit the oyster industry. The application of the Precautionary Principle can be made more effective through wider dissemination of the research findings.

Investigations of oyster kills in Limeburners Creek have led to the hypothesis that a microcell disease or unknown environmental factor, not identified by the present study, may be present in the Hastings River system. The current study was not resourced to investigate this hypothesis and we recommend further research on this condition.

8.3 CONCLUSIONS

The overriding objective of this study was to identify environmental risk factors for production losses in the Sydney rock oyster industry with a particular emphasis on the role of acidification. The first objective of the study was met through a series of field experiments and laboratory assays that confirmed that acidification causes poor growth rates and higher mortality rates in oysters. Environmental factors such as high rainfall and associated decreases in salinity were identified as contributing factors. High rainfall increases acid export into estuarine waters and also reduces the acid neutralising capacity of the receiving waters leading to spatially extensive and temporally persistent acidic conditions in estuaries. The second objective of the study was refocused as a result of Lake's (1997) study and other field investigations. Poor growth rates observed on the Hastings River system were associated with the loss of recent shell growth and

degenerative changes in the soft tissue of oysters. This led to negative growth due to the reduced shell dimensions. The possibility of a microcell disease was postulated but requires further investigation to define the condition, identify a pathogen, and test for environmental controls on outbreaks.

The third objective of the study was to identify risk factors for QX disease. The study showed that acidification and other measured water quality variables were not factors in outbreaks. Acid did not trigger or increase the severity of QX outbreaks on the Richmond River.

The final objective of the study was to effectively communicate the findings of the study to the oyster industry and relevant agencies. This was progressively achieved through the study; the project team participated in regular oyster grower association meetings, field days, workshops, ASSMAC technical committees and presented findings to environmental managers at intergovernmental meetings and other forums. The media was also used to communicate the findings to the community. Additionally, a Fishnote entitled 'Oysters and Acid Sulfate Soil pollution was produced in collaboration with NSW Fisheries to provide a reference tool for the oyster industry and communicate the findings to the general public.

The risk of estuarine acidification to oyster production is not restricted to the Richmond, Manning and Hastings Rivers. DLWC ASS mapping of the NSW coastline has identified deposits of ASS occurring in the catchment of every estuary used for the production of the Sydney rock oyster with the greatest deposits occurring in the barrier estuaries. Environmental managers must tackle estuary acidification at its source i.e. in the heavily engineered and modified coastal lowlands that fringe estuaries. Both reactive and proactive strategies for management are required to address existing soil acidification and prevent the development of new problems.

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APPENDICES

Appendix 1. INTELLECTUAL PROPERTY

No patents or commercial in confidence material emerged from this research. The core experiments were submitted as part of the fulfilment of a PhD degree by Michael Dove:

> Dove, M.C. (2003). Effects of Estuarine Acidification on the Sydney Rock Oyster *Saccostrea glomerata*. Unpublished PhD thesis, Geography Program, The Faculty of the Built Environment, The University of New South Wales, Sydney.

Each chapter will be progressively published in scientific and industry journals and in educational materials.

Appendix 2. PROJECT PERSONNEL

Dr Jesmond Sammut	Principal Investigator
Dr Richard B. Callinan	Principal Investigator
Mr Michael C. Dove	Co-Investigator (and formerly Research Assistant, funded under project)
Ms Kavita Gosavi	Casual Research Assistant (funded under project)
Loren Ravenscroft	Casual Research Assistant (funded under project)
Dorothy Yu	Soil and Water Analyst
Chris Myers	Senior Laboratory Manager

Other Contributors:

Sarah Kleeman and Stephen Weche - Contributed to the field component of the QX project.

Appendix 3A. Listing of field and analytical water quality data following rainfall for Hastings River estuary drains.

HASTINGS RIVER ESTUARY DRAIN WATER QUALITY

Drain ID	Date	pH	EC (dS m^{-1})	DO (mg L^{-1})	Temp (^oC)	Cl:SO_4	Lab. pH	Fe (mg L^{-1})	Al (mg L^{-1})	Ca (mg L^{-1})	Mn (mg L^{-1})	K (mg L^{-1})	Mg (mg L^{-1})	S (mg L^{-1})	SO_4 (mg L^{-1})	As (mg L^{-1})	Cu (mg L^{-1})	Si (mg L^{-1})	Zn (mg L^{-1})
BC19.1L	18/06/99	5.89	11.1	2.4	8.72	8.8	5.64	0.04	0.02	72.96	0.56	67.08	226.00	128.53	385.59	<0.35	<0.005	4.89	<0.02
CC38.4R	19/06/99	5.05	0.3	4.2	11.27	3.7	4.28	0.84	0.13	5.40	0.06	2.60	8.75	11.40	34.20	<0.35	<0.005	3.64	<0.02
CC38.4R	30/11/99	3.66	1.3	3.9	20.74	3.7	3.49	3.11	0.38	16.10	0.13	6.17	30.20	38.40	115.20	<0.09	0.007	1.280	<0.04
CC38.4R	13/02/01	3.19	0.9	1.8	23.38	1.1	3.19	7.75	2.27	15.70	0.38	9.14	27.10	55.90	167.70	<0.12	<0.004	6.39	0.10
CC39.1R	13/02/01	3.91	0.9	1.3	23.84	1.0	3.77	0.98	0.47	32.10	0.82	6.34	26.80	64.40	193.20	<0.12	<0.004	4.99	0.06
CC44.1R	13/02/01	3.58	1.0	0.6	22.50	0.5	3.37	36.70	1.37	18.90	0.53	7.21	24.00	65.10	195.30	<0.12	<0.004	6.52	0.10
CC44.1R	02/12/00	3.98	2.8	4.1	21.51	2.6	3.72	1.42	0.52	44.10	0.31	17.70	81.80	81.70	245.10	<0.14	<0.004	5.39	0.06
CC44.8R	19/06/99	4.96	0.3	2.3	12.64	4.2	4.69	0.03	0.14	8.94	0.11	2.43	8.20	13.22	39.66	<0.35	<0.005	3.30	<0.02
FC11.6L	26/05/98	3.00	0.9	11.6	8.18	2.5	3.59	1.31	2.31	4.46	<0.002	2.76	10.66	22.10	66.30	<0.40	<0.004	1.24	<0.01
FC11.6L	19/06/99	4.18	0.3	2.3	10.83	3.8	3.76	2.38	0.69	4.54	0.13	2.79	6.93	18.09	54.26	<0.35	<0.005	7.62	<0.02
FC11.6L	30/11/99	3.48	1.1	2.7	21.83	4.3	3.20	6.30	0.15	4.38	0.11	2.19	12.40	21.10	63.30	<0.09	<0.006	0.651	<0.04
FC11.6L	13/02/01	3.28	1.3	2.1	22.49	1.3	3.17	35.90	1.84	11.70	0.40	6.66	26.50	60.20	180.60	<0.12	<0.004	10.30	0.06
FC11.6L	02/12/00	3.09	1.4	3.6	21.32	2.1	3.05	6.58	1.06	11.80	0.26	6.54	26.10	48.30	144.90	<0.14	0.01	5.81	0.07
HR16.0R	18/06/99	3.37	1.5	10.5	9.02	2.3	3.28	5.45	8.69	19.15	1.01	13.61	32.42	80.96	242.87	<0.35	<0.005	12.40	<0.02
HR16.0R	12/02/01	2.81	5.9	2.5	22.56	1.6	2.77	48.10	9.53	44.00	1.45	28.30	97.90	193.00	579.00	<0.12	<0.004	15.80	0.23
HR16.5R	18/06/99	3.37	2.7	10.7	9.75	2.8	3.32	4.03	8.34	28.48	1.22	18.83	56.05	88.66	265.98	<0.35	<0.005	12.15	<0.02
HR16.5R	12/02/01	3.23	8.5	3.1	22.74	7.0	3.22	5.33	6.10	52.20	1.03	40.90	131.00	142.00	426.00	<0.12	0.01	11.70	0.12
HR16.8R	18/06/99	3.35	2.2	10.9	9.42	2.6	3.21	4.80	3.64	28.77	1.04	18.24	51.22	91.29	273.87	<0.35	<0.005	12.17	<0.02
HR16.8R	12/02/01	3.48	8.8	3.0	22.37	4.4	3.25	33.90	19.40	77.20	2.30	47.70	175.00	212.00	636.00	<0.12	0.01	17.90	0.47
HR8.1R	13/02/01	4.10	1.1	4.5	20.23	3.8	3.90	12.20	0.46	9.01	0.16	6.43	21.20	31.70	95.10	<0.12	<0.004	4.60	0.03
HR8.1R	19/06/99	6.13	5.0	0.3	9.79	7.8	5.11	5.00	0.13	31.55	0.07	29.71	93.64	67.51	202.54	<0.35	<0.005	4.41	<0.02
MA29.6L	18/06/99	5.99	0.5	2.8	9.71	26.8	6.22	1.28	0.09	4.79	0.02	5.81	10.74	7.33	21.98	<0.35	<0.005	4.11	<0.02
MR19.2R	19/06/99	5.62	0.1	8.5	10.94	17.0	6.02	0.49	0.25	2.80	0.02	2.41	5.50	4.73	14.20	<0.35	<0.005	2.79	<0.02
MR21.7L	12/02/01	3.53	0.9	6.3	24.70	0.6	3.46	1.46	3.00	12.90	0.38	6.65	21.40	43.80	131.40	<0.12	<0.004	7.74	0.09
MR21.7L	18/06/99	4.21	0.2	6.9	10.00	2.9	4.29	0.31	1.17	4.99	0.12	2.78	6.66	15.19	45.57	<0.35	<0.005	5.72	<0.02
MR21.7L	01/12/00	3.19	1.6	-	25.07	2.4	3.40	1.27	7.37	30.50	0.58	18.00	64.80	96.10	288.30	<0.14	0.01	8.98	0.11
MR23.0L	19/06/99	3.87	0.3	2.5	10.38	1.4	3.80	0.23	2.65	7.46	0.49	3.94	8.75	28.01	84.02	<0.35	<0.005	8.44	<0.02

Appendix 3A. (Continued)

HASTINGS RIVER ESTUARY DRAIN WATER QUALITY

Drain ID	Date	pH	EC (dS m^{-1})	DO (mg L^{-1})	Temp (^oC)	Cl:SO_4	Lab. pH	Fe (mg L^{-1})	Al (mg L^{-1})	Ca (mg L^{-1})	Mn (mg L^{-1})	K (mg L^{-1})	Mg (mg L^{-1})	S (mg L^{-1})	SO_4 (mg L^{-1})	As (mg L^{-1})	Cu (mg L^{-1})	Si (mg L^{-1})	Zn (mg L^{-1})
MR23.0L	30/11/99	3.64	3.5	5.4	20.30	3.6	3.48	2.54	2.73	21.30	0.66	13.10	58.20	67.60	202.80	<0.09	0.010	5.280	<0.04
MR23.0L	01/12/00	3.72	2.2	-	24.60	2.4	3.79	0.70	3.08	34.00	0.62	13.70	52.80	81.10	243.30	<0.14	<0.004	10.00	0.12
MR23.0L	12/02/01	4.04	1.2	2.6	24.13	4.5	3.89	0.63	2.44	16.40	0.49	7.69	28.00	43.60	130.80	<0.12	<0.004	7.82	0.09
MR24.2R	12/02/01	3.83	3.2	4.8	23.53	3.0	3.71	0.98	2.19	24.10	0.34	15.40	57.90	72.90	218.70	<0.12	<0.004	6.44	0.05
MR30.8R	19/06/99	4.58	2.4	7.0	10.61	2.2	4.24	1.87	0.74	50.08	0.15	9.55	48.02	79.13	237.39	<0.35	<0.005	8.72	<0.02
MR32.8L	18/06/99	5.99	0.5	8.3	8.44	7.6	5.60	0.58	0.13	5.22	0.03	4.39	9.50	8.91	26.73	<0.35	<0.005	3.10	<0.02
MR33.8R(A	19/06/99	4.58	0.1	3.8	9.91	4.5	4.48	0.84	0.26	2.33	0.04	1.02	3.97	5.90	17.71	<0.35	<0.005	1.88	<0.02
MR33.8R(A	13/02/01	3.06	1.9	2.6	23.80	1.0	3.00	8.02	5.34	22.40	0.56	7.64	35.90	78.50	235.50	<0.12	0.01	5.84	0.13
MR33.8R(A	02/12/00	3.22	3.1	2.4	21.05	2.5	3.42	2.22	1.86	24.40	0.33	9.63	44.10	72.80	218.40	<0.14	0.01	2.44	0.06
MR33.8R(B	30/11/99	3.66	0.5	2.8	21.08	2.0	3.34	2.76	0.21	3.92	0.10	2.84	7.45	15.10	45.30	<0.09	0.02	0.13	<0.04
MR33.8R(B	13/02/01	2.91	1.6	3.2	24.71	1.4	3.00	8.95	5.77	16.90	0.53	5.47	29.20	75.40	226.20	<0.12	<0.004	5.36	0.13
MR33.8R(B	02/12/00	2.77	2.4	2.9	22.15	1.1	2.89	11.40	4.43	19.30	0.57	5.77	31.50	80.60	241.80	<0.14	<0.004	2.18	0.12
MR34.1R	19/06/99	3.99	0.5	6.4	10.88	1.8	3.93	0.37	4.50	14.91	0.26	2.54	13.35	29.45	88.35	<0.35	<0.005	2.20	<0.02
MR34.1R	30/11/99	3.71	0.8	3.8	19.29	1.0	3.52	1.30	5.81	16.50	0.47	2.33	23.60	42.10	126.30	<0.09	<0.006	0.104	<0.04
MR34.1R	13/02/01	3.38	1.6	1.7	23.14	0.9	3.28	4.20	8.82	37.20	0.86	8.81	40.70	89.60	268.80	<0.12	<0.004	5.70	0.18
MR34.1R	02/12/00	3.20	2.4	2.8	20.42	0.9	3.25	3.22	20.70	53.10	1.47	10.70	64.40	144.00	432.00	<0.14	0.01	3.65	0.25
MR35.5R	19/06/99	4.68	0.2	2.7	11.06	4.5	4.39	0.01	0.26	3.56	0.05	2.60	4.56	7.12	21.35	<0.35	<0.005	1.78	<0.02
MR35.5R	30/11/99	3.41	2.0	1.5	20.74	1.0	3.11	24.40	1.47	26.20	0.36	7.80	51.50	82.10	246.30	<0.09	<0.006	2.70	<0.04
MR35.5R	13/02/01	4.43	2.3	0.8	22.80	4.1	4.08	22.30	0.33	19.30	0.27	14.30	43.40	47.10	141.30	<0.12	<0.004	5.19	0.03
MR35.5R	02/12/00	2.91	5.3	1.2	20.47	2.6	2.97	15.30	2.08	35.70	0.31	17.30	83.50	118.00	354.00	<0.14	<0.004	5.51	0.05
MR41.0R	18/06/99	4.91	0.2	2.4	11.50	4.2	4.86	0.33	0.42	4.56	0.10	1.55	6.70	9.70	29.10	<0.35	<0.005	4.44	<0.02
MR41.1R	18/06/99	3.94	2.6	1.9	10.04	3.5	4.68	0.60	0.52	4.45	0.11	1.59	6.81	10.19	30.57	<0.35	<0.005	4.64	<0.02
MR41.5L	18/06/99	4.37	0.4	4.6	10.11	2.4	4.05	0.16	1.07	8.29	0.21	1.91	12.05	21.58	64.73	<0.35	<0.005	4.89	<0.02
PC34.5L	18/06/99	3.86	0.4	6.6	8.47	2.2	3.70	0.18	1.21	6.83	0.17	2.81	10.31	23.81	71.42	<0.35	<0.005	4.42	<0.02
PC34.5L	12/02/01	3.47	1.1	1.4	23.80	2.1	3.37	2.80	1.58	12.40	0.36	7.71	21.30	35.50	106.50	<0.12	<0.004	4.36	0.06
PC34.6R	18/06/99	5.26	0.4	4.4	10.00	3.3	4.91	0.01	0.08	6.68	0.07	3.08	8.61	14.70	44.10	<0.35	<0.005	6.93	<0.02
PC34.7L	18/06/99	4.29	0.7	2.4	9.49	2.2	4.26	0.32	1.60	10.15	0.13	4.07	15.16	31.46	94.38	<0.35	<0.005	10.10	<0.02

Appendix 3B. Field data for Hastings River estuary drains.

HASTINGS RIVER ESTUARY DRAIN WATER QUALITY (PHYSICO-CHEMICAL PARAMETERS)

	18-19/6/99				29-30/11/1999				1-2/12/2000				12-13/02/2001			
Drain ID	pH	EC (dS m^{-1})	DO (% Sat.)	Temp (^oC)	pH	EC (dS m^{-1})	DO (% Sat.)	Temp (^oC)	pH	EC (dS m^{-1})	DO (% Sat.)	Temp (^oC)	pH	EC (dS m^{-1})	DO (% Sat.)	Temp (^oC)
CC38.4R	5.05	0.3	4.2	11.27	3.66	1.3	3.9	20.74	5.40	3.8	3.4	21.58	3.19	0.9	1.8	23.38
CC38.8R	6.14	0.5	6.3	11.36	6.11	1.2	5.8	20.66	6.20	4.1	3.1	20.74	6.23	2.2	1.7	23.34
CC39.1R	6.12	0.3	4.3	10.49	6.15	0.7	3.5	20.84	6.67	0.9	0.7	21.91	3.91	0.9	1.3	23.84
CC41.0R	6.06	0.5	5.8	11.24	6.40	1.0	0.1	17.46	6.64	1.6	0.0	19.85	6.45	2.1	0.2	23.08
CC42.5R	6.15	0.6	5.6	11.02	6.02	2.2	3.1	21.26	6.34	3.6	4.0	21.12	6.53	3.2	0.2	22.45
CC44.1R	5.73	0.4	0.3	10.95	4.11	1.9	5.0	20.74	3.98	2.8	4.1	21.51	3.58	1.0	0.6	22.50
CC44.8R	4.96	0.3	2.3	12.64	3.93	1.0	0.7	20.07	3.84	1.2	1.3	22.80	6.35	0.4	0.2	24.19
CC46.4R	5.56	0.5	1.4	11.36	6.24	3.6	3.5	21.66	5.55	8.7	1.4	23.01	6.15	0.9	0.7	23.79
FC10.1R	6.61	22.4	6.5	9.51	-	-	-	-	-	-	-	-	-	-	-	-
FC10.2R	6.74	9.9	7.7	9.49	-	-	-	-	-	-	-	-	-	-	-	-
FC10.4R	5.68	6.4	6.3	8.82	-	-	-	-	-	-	-	-	-	-	-	-
FC11.1R	6.02	1.6	6.9	8.52	-	-	-	-	-	-	-	-	-	-	-	-
FC11.3R	6.19	16.7	0.7	10.87	6.21	31.6	2.3	21.90	7.05	16.5	1.1	19.77	6.74	10.6	1.6	20.99
HR4.0R	7.64	26.0	6.4	9.58					7.09	40.7	-	20.77	7.24	39.6	0.9	23.81
HR7.7R	6.13	5.0	0.3	9.79	4.73	0.4	3.0	20.89	4.30	0.7	-	17.75	4.10	1.1	4.5	20.23
HR12.1R	5.85	17.7	7.1	11.91	6.94	28.2	6.8	18.23	6.05	21.0	-	23.21	5.25	15.5	6.4	23.42
HR16.0R	3.37	1.5	10.5	9.02	3.25	3.1	8.0	17.40	2.72	6.3	-	21.97	2.81	5.9	2.5	22.56
HR16.5R	3.37	2.7	10.7	9.75	3.55	11.4	6.1	17.48	4.75	11.8	-	21.88	3.23	8.5	3.1	22.74
HR16.6R	-	-	-	-	6.38	22.3	3.4	19.82	5.93	14.5	-	24.35	4.54	5.6	5.5	23.27
HR16.8R	3.35	2.2	10.9	9.42	6.61	27.8	0.9	22.36	6.13	13.0	-	23.91	3.48	8.8	3.0	22.37
LC5.7R	7.52	16.6	5.2	11.21	-	-	-	-	7.09	37.0	-	18.25	-	-	-	-
LC5.9R	8.00	47.6	5.9	17.12	-	-	-	-	7.20	38.1	-	20.07	-	-	-	-
MA29.6L	5.99	0.5	2.8	9.71	6.48	0.5	6.7	22.86	6.15	4.2	-	25.84	5.51	5.1	2.8	24.12
MR10.3L	7.19	20.0	10.2	12.00	-	-	-	-	6.92	14.1	-	24.70	6.88	7.3	4.3	24.09
MR19.2R	5.62	0.1	8.5	10.94	7.50	7.4	7.4	22.29	6.62	8.6	3.8	21.28	6.63	1.8	5.7	24.23
MR20.1L	-	-	-	-	-	-	-	-	6.59	8.7	-	22.41	5.87	7.9	3.1	23.21
MR21.4L	-	-	-	-	6.95	9.0	7.7	16.22	-	-	-	-	6.52	1.5	1.1	21.96
MR21.7L	4.21	0.2	6.9	10.00	6.09	6.4	6.9	21.29	3.19	1.6	-	25.07	3.53	0.9	6.3	24.70
MR22.3R	6.08	1.1	2.2	11.39	6.73	9.6	3.1	19.94	7.01	3.2	1.9	19.69	6.77	1.7	3.5	23.17
MR22.4R	6.27	0.7	5.9	10.02	6.73	14.5	0.6	18.89	7.08	5.1	0.6	18.90	6.89	1.1	0.9	21.80
MR23.0R	6.13	0.9	9.2	10.42	-	-	-	-	6.90	2.5	1.4	20.62	6.49	3.8	4.3	23.66
MR23.3R	5.92*	1.3*	7.4*	10.54*	-	-	-	-	6.53	5.8	3.3	19.10	6.45	5.6	3.6	23.26
MR23.6R	6.03	0.4	7.4	12.24	6.68	7.5	0.1	21.31	6.62	2.4	2.1	20.73	6.08	7.7	4.0	22.35
MR23.8R	6.38	0.9	2.5	11.36	7.19	2.9	8.4	21.40	6.82	3.3	4.7	21.86	4.98	4.4	6.9	23.64
MR24.2R	5.31	1.2	7.3	10.39	6.69	2.0	7.0	19.88	-	-	-	-	3.83	3.2	4.8	23.53
MR30.8R	4.58	2.4	7.0	10.61	6.25	0.6	7.0	23.48	-	-	-	-	-	-	-	-
MR32.8L	5.99	0.5	8.3	8.44	4.23	0.8	3.5	20.14	5.14	1.4	-	23.55	6.35	3.2	3.3	23.34
MR33.8R(A)	4.58	0.1	3.8	9.91	4.05	0.6	3.1	19.42	3.22	3.1	2.4	21.05	3.06	1.9	2.6	23.80
MR33.8R(B)	-	-	-	-	3.66	0.5	2.8	21.08	2.77	2.4	2.9	22.15	2.91	1.6	3.2	24.71
MR39.5R	6.03	0.6	2.4	10.03	-	-	-	-	-	-	-	-	-	-	-	-
PC34.5L	3.86	0.4	6.6	8.47	3.16	1.6	2.3	19.49	3.01	1.3	-	24.54	3.47	1.1	1.4	23.80
PC34.6R	5.26	0.4	4.4	10.00	5.98	0.7	5.4	19.30	5.59	3.1	-	22.82	6.17	1.4	2.9	23.71
PC34.7L	4.29	0.7	2.4	9.49	5.75	1.0	3.0	18.22	3.89	1.3	-	22.73	4.07	1.1	1.1	24.17
TA28.7R	6.3*	0.3*	6.7*	11.06*	-	-	-	-	-	-	-	-	-	-	-	-
WR29.3L	-	-	-	-	-	-	-	-	-	-	-	-	5.76	3.0	4.9	23.73
WR29.5L	-	-	-	-	-	-	-	-	-	-	-	-	6.76	0.3	5.7	23.42
WR29.7L	-	-	-	-	-	-	-	-	-	-	-	-	5.27	1.1	8.5	26.09
WR30.6L	-	-	-	-	-	-	-	-	-	-	-	-	6.30	0.6	2.1	22.95

* = Drain measurement collected upstream from floodgate

Appendix 3C. Estuary pH and EC transect data for the Hastings River following rainfall.

HASTINGS RIVER ESTUARY CHANNEL TRANSECT DATA

18/06/99				
Channel ID	pH		EC (dS m^{-1})	
	surface	bed	surface	bed
HR5.6	7.92	8.12	33.2	49.7
HR6.8	7.91	8.05	30.1	40.6
HR9.5	7.83	8.08	28.3	42.1
HR11.3	7.74	7.95	24.7	34.7
HR12.8	7.70	7.91	19.8	33.1
HR15.5	7.69	7.69	11.8	21.3
HR17.5	6.92	6.99	9.1	11.4
MR11.5	7.05	7.08	14.7	15.9
MR13.0	7.07	7.03	10.5	10.6
MR15.0	7.02	6.92	4.7	5.6
MR17.0	6.90	6.78	1.2	1.3
MR19.4	6.66	6.50	0.3	0.4
MR21.1	6.50	6.42	0.2	0.2
MR23.0	6.38	6.30	0.3	0.3
MR25.3	6.45	6.38	0.2	0.2
MR27.0	6.34	6.31	0.3	0.3
MR30.0	5.92	5.88	0.3	0.3
MR31.0	5.87	5.81	0.3	0.3
MR33.3	5.96	5.85	0.3	0.3
MR35.5	5.80	5.81	0.3	0.3
MR37.3	5.79	5.76	0.3	0.3
MR38.0	5.90	5.89	0.4	0.4
MR40.0	5.01	4.97	0.2	0.2
MR41.6	4.97	4.92	0.2	0.2

29/11/99				
Channel ID	pH		EC (dS m^{-1})	
	surface	bed	surface	bed
HR5.6	7.98	8.26	29.0	51.0
HR6.8	7.98	8.16	25.3	43.6
HR9.5	7.89	8.07	22.8	41.0
HR11.3	7.84	7.98	21.8	35.7
HR12.8	7.77	8.02	18.6	39.3
HR15.5	7.76	7.88	7.0	30.1
HR17.5	7.67	7.66	4.5	23.8
MR11.5	7.61	7.68	27.8	28.4
MR13.0	7.43	7.50	22.9	23.0
MR15.0	7.44	7.42	15.3	15.6
MR17.0	7.42	7.40	13.6	13.9
MR19.4	7.37	7.28	6.5	8.2
MR21.1	7.41	7.22	4.1	4.8
MR23.0	6.80	6.81	2.0	2.7
MR25.3	7.10	7.06	1.0	1.0
MR27.0	7.13	7.08	0.6	0.8
MR29.1	6.72	6.72	0.5	0.5
MR31.0	6.49	6.44	0.6	0.6
MR33.3	6.28	6.08	0.6	0.7
MR35.0	5.74	5.80	0.9	0.9
MR37.3	5.77	5.80	1.0	1.0
MR38.7	5.55	5.53	0.8	0.8

1/12/00				
Channel ID	pH		EC (dS m^{-1})	
	surface	bed	surface	bed
HR5.6	7.74	7.98	17	43.6
HR6.8	7.67	8.01	12	47.4
HR9.5	7.6	7.98	16.7	41.1
HR11.3	7.42	7.75	8	29.4
HR12.8	7.44	7.9	8.2	38.6
HR15.5	7.32	7.38	2.7	9.9
HR17.5	7.32	7.47	1	19.1
MR11.5	7.08	7.1	11.4	12.3
MR13.0	6.98	6.97	7.8	8.2
MR15.0	6.85	6.83	4.6	4.7
MR17.0	6.86	6.82	2.8	2.8
MR19.4	6.85	6.79	1.9	1.9
MR21.1	6.78	6.75	1.8	1.8
MR23.0	6.5	6.56	1.6	1.7
MR25.3	6.56	6.58	1.3	1.3
MR27.0	6.49	6.51	1.7	1.7
MR29.1	6.15	6.14	4.1	4.1
MR31.0	5.95	5.94	4.3	4.3
MR33.3	5.88	5.77	4.2	4.3
MR35.0	5.95	5.93	4.2	4.2
MR37.3	6.01	6.02	3.4	3.7
MR38.7	6.01	6	2.5	2.5
MR39.0	6	5.99	2.5	2.5

12/02/01				
Channel ID	pH		EC (dS m^{-1})	
	surface	bed	surface	bed
	12/02/01			
HR4.9	7.71	8.15	20.4	44.7
HR6.8	7.7	8.21	18.5	48.6
HR8.0	7.59	8.25	16.5	49.3
HR9.5	7.58	8.11	14.7	36
HR11.3	7.43	7.96	8.7	29.7
HR12.8	7.35	7.92	5.3	26.5
HR15.5	7.27	7.29	1	2.2
HR17.5	7.26	7.24	0.3	0.3
MR11.5	6.87	6.92	4.5	5.6
MR13.0	6.72	6.71	1.7	1.7
MR15.0	6.63	6.62	0.9	0.9
MR17.0	6.56	6.55	0.6	0.6
MR19.4	6.51	6.5	0.5	0.5
MR21.1	6.51	6.49	0.4	0.4
MR23.0	6.4	6.41	0.5	0.5
MR25.3	6.45	6.47	0.4	0.4
MR27.0	6.39	6.38	0.6	0.6
MR29.1	5.8	5.81	1.1	1.1
MR30.0	5.58	5.58	1.1	1.1
MR32.0	5.35	5.35	1	1.1
MR33.3	5.3	5.1	1	1.2
MR35.0	5.43	5.41	1.2	1.2
MR37.3	5.49	5.48	0.8	0.9
MR39.0	5.65	5.64	0.5	0.5

Appendix 3D. Listing of field and analytical water quality data following high rainfall for Manning River estuary drains.

MANNING RIVER ESTUARY DRAIN WATER QUALITY

Drain ID	Date	pH	EC (dS m^{-1})	DO (% Sat.)	Temp. (^{o}C)	Alk. (mg L^{-1})	Cl:SO_4	LAB pH	Fe (mg L^{-1})	Al (mg L^{-1})	Ca (mg L^{-1})	Mn (mg L^{-1})	K (mg L^{-1})	Mg (mg L^{-1})	S (mg L^{-1})	SO_4 (mg L^{-1})	As (mg L^{-1})	Cu (mg L^{-1})	Si (mg L^{-1})	Zn (mg L^{-1})
CC15.7L	27/05/98	4.11	15.2	75.1	9.94	13	6.8	4.42	0.14	1.53	30.18	<0.002	29.37	88.12	105.16	315.47	<0.40	<0.004	2.55	<0.01
CC16.1L	09/05/99	3.55	1.1	49.9	16.52	NS	6.6	3.71	4.90	1.05	7.26	0.24	6.38	18.82	29.20	87.61	<0.35	<0.005	2.65	<0.02
CC16.1L	27/05/98	4.93	18.8	99.4	6.44	21	32.3	4.95	<0.01	0.27	38.30	<0.002	43.36	108.12	79.97	239.91	<0.40	<0.004	0.96	<0.01
CC16.5L	09/05/99	3.04	1.8	67.0	18.28	NS	2.1	3.22	9.82	17.71	20.81	2.13	7.89	40.75	114.27	342.81	<0.35	<0.005	12.83	<0.02
CC16.9L	27/05/98	3.87	10.9	108.0	5.18	8	24.3	4.31	<0.01	2.64	18.46	0.06	19.13	51.16	43.72	131.16	<0.40	<0.004	1.85	<0.01
DC22.9L	27/05/98	6.39	26.1	84.9	8.95	42	30.9	6.51	<0.01	<0.05	54.97	<0.002	58.41	150.81	95.19	285.57	<0.40	<0.004	0.49	<0.01
DC23.4L	27/05/98	6.22	25.2	87.4	8.62	38	12.6	6.43	<0.01	<0.05	121.08	0.27	113.16	328.64	222.16	666.48	<0.40	<0.004	1.23	<0.01
DC24.3R	27/05/98	5.75	23.5	96.8	7.05	26	25.4	5.92	<0.01	<0.05	58.19	0.10	56.10	156.51	103.68	311.04	<0.40	<0.004	1.09	<0.01
GG15.0R	09/05/99	3.46	1.5	33.2	18.36	NS	5.2	3.52	4.02	1.74	13.04	0.85	11.55	23.47	47.53	142.59	<0.35	<0.005	6.47	<0.02
GG15.4R	27/05/98	3.58	21.2	101.8	4.05	21	9.7	3.89	0.32	5.11	57.94	0.57	47.22	148.82	157.32	471.96	<0.40	<0.004	3.10	<0.01
GG15.5R*	27/05/98	3.21	22.3	110.5	4.56	8	7.8	3.56	2.17	11.28	134.84	1.41	105.29	340.24	287.35	862.06	<0.40	<0.004	6.76	<0.01
GG15.8L	09/05/99	3.31	11.0	46.5	18.98	NS	4.2	4.07	2.71	2.84	94.30	2.57	63.80	225.94	239.19	717.58	<0.35	<0.005	12.00	<0.02
GG16.6R*	27/05/98	3.45	15.4	121.5	7.94	8	5.2	3.76	2.82	32.57	151.32	4.03	78.27	306.58	329.72	989.16	<0.40	<0.004	16.07	<0.01
LR13.1L	09/05/99	3.22	10.2	27.4	16.42	NS	3.0	3.51	4.74	2.14	41.24	1.10	24.10	92.64	186.10	558.30	<0.35	<0.005	9.05	<0.02
LR15.1R	09/05/99	3.20	3.6	42.1	17.27	NS	3.1	3.32	38.40	3.38	15.13	1.00	7.86	33.48	98.39	295.17	<0.35	<0.005	8.29	<0.02
LR15.4L	09/05/99	3.07	7.6	143.5	24.19	NS	3.3	3.72	3.09	0.76	36.30	0.43	22.94	71.23	125.44	376.31	<0.35	<0.005	5.91	<0.02
LR15.4L	27/05/98	6.22	21.3	86.1	8.70	21	12.1	6.39	<0.01	<0.05	40.32	<0.002	40.21	112.74	98.48	295.45	<0.40	<0.004	0.23	<0.01
LR15.9R	09/05/99	2.97	9.3	64.6	18.92	NS	1.5	3.41	4.83	5.35	38.06	1.06	26.95	94.63	175.90	527.69	<0.35	<0.005	10.67	<0.02
LR16.1L	09/05/99	3.05	2.9	84.2	20.42	NS	7.3	3.39	2.07	2.34	14.28	0.59	12.14	24.56	43.16	129.48	<0.35	<0.005	4.14	<0.02
LR16.1L*	27/05/98	3.47	15.0	107.5	8.13	8	6.7	3.69	2.87	9.62	55.13	0.99	37.22	133.15	127.51	382.54	<0.40	<0.004	7.87	<0.01
LR16.6L	09/05/99	3.07	5.9	35.0	18.17	NS	5.5	3.41	2.63	4.06	29.57	0.98	25.11	61.10	81.86	245.59	<0.35	<0.005	7.69	<0.02
LR16.6L*	27/05/98	4.91	15.3	95.9	9.14	0	7.4	4.92	3.37	7.04	70.37	1.18	52.78	177.80	153.23	459.70	<0.40	<0.004	12.36	<0.01
LR18.7L	09/05/99	6.51	6.1	63.3	19.58	NS	6.1	6.30	0.01	<0.05	57.49	0.34	51.93	158.14	124.21	372.64	<0.35	<0.005	2.79	<0.02
SC14.3R*	27/05/98	6.40	25.6	102.8	9.38	30	12.6	5.45	<0.01	0.01	76.23	0.72	60.98	184.81	174.02	522.06	<0.40	<0.004	1.91	<0.01

* = Drain measurement collected upstream from floodgate

Appendix 3E. Manning River estuary pH and EC data for surface and bed waters.

MANNING RIVER ESTUARY CHANNEL TRANSECT DATA

Channel ID	Date	pH		EC (dS m^{-1})	
		surface	bed	surface	bed
CC9.5	27/05/98	6.74	NS	28.0	NS
CC11.6	27/05/98	6.20	6.92	20.6	30.1
CC14.0	27/05/98	5.23	6.41	15.9	31.3
CC15.1	27/05/98	4.89	5.02	17.2	18.0
DC22.5	27/05/98	6.30	NS	25.8	NS
DC23.6	27/05/98	6.02	NS	24.6	NS
DC25.0	27/05/98	4.74	NS	18.6	NS
GG15.8	27/05/98	5.59	5.85	21.3	21.6
GG22.7	27/05/98	7.45	7.62	25.9	40.9
CC10.2	09/05/99	4.97	7.18	3.9	30.1
CC11.0	09/05/99	5.00	7.29	3.2	33.1
CC11.5	09/05/99	4.51	6.28	1.8	27.1
CC12.7	09/05/99	4.50	6.37	1.9	28.6
CC13.5	09/05/99	4.59	6.33	1.8	28.5
CC14.2	09/05/99	4.62	6.37	1.4	28.6
CC14.7	09/05/99	5.00	6.29	2.3	27.5
CC16.5	09/05/99	3.51	NS	0.7	NS
CC9.4	09/05/99	5.02	7.78	4.2	37.3
DC21.5	09/05/99	6.16	6.24	17.2	19.8
DC22.0	09/05/99	4.95	7.05	13.0	32.5
DC22.8	09/05/99	4.46	6.71	13.1	32.1
DC23.5	09/05/99	3.95	6.38	12.1	29.5
DC23.9	09/05/99	4.16	5.99	11.5	22.8
DC24.5	09/05/99	4.04	5.02	9.4	16.7
GG14.2	09/05/99	6.36	6.61	12.7	24.0
GG15.1	09/05/99	6.00	6.16	11.3	16.1
GG16.1	09/05/99	5.88	6.27	13.7	21.0
GG16.7	09/05/99	6.09	6.16	14.0	15.6
GG16.8	09/05/99	6.15	6.15	13.4	14.4
GG18.6	09/05/99	6.35	6.42	16.9	17.0
GG19.7	09/05/99	6.48	6.55	17.6	19.1
GG21.1	09/05/99	5.31	6.12	15.7	18.2
GG21.5	09/05/99	6.16	6.24	17.2	19.8
GG22.7	09/05/99	7.55	7.61	16.2	33.2
LR10.5	09/05/99	6.51	7.60	18.2	32.1
LR11.2	09/05/99	6.43	7.63	17.2	35.4
LR12.5	09/05/99	6.46	7.62	15.4	36.1
LR14.0	09/05/99	6.23	7.60	11.4	37.7
LR15.0	09/05/99	6.01	7.53	10.1	36.5
LR15.7	09/05/99	6.05	7.43	8.7	35.5
LR16.4	09/05/99	6.26	7.24	10.3	34.7
LR18.2	09/05/99	6.52	7.19	9.9	34.1
LR18.9	09/05/99	6.45	7.19	9.7	33.1
LR19.9	09/05/99	6.97	7.01	9.0	31.6

NS = not sampled

Appendix 3F. Lower Hastings River and Limeburners Creek pH, EC, DO and temperature data.

SITE: 1

	pH		Elec. Cond. (mS cm^{-1})		Dissolved Oxygen (% Saturation)		Temperature (^{o}C)	
Date	surface	bed	surface	bed	surface	bed	surface	bed
17/11/97	8.17	8.22	52.1	53.2	81.0	80.6	17.40	17.00
04/12/97	8.22	8.26	50.3	53.4	55.0	60.8	22.10	22.00
20/03/98	NS	NS	NS	NS	NS	NS	NS	NS
25/03/98	8.13	8.15	52.0	52.4	NS	NS	22.17	21.78
27/03/98	NS	NS	NS	NS	NS	NS	NS	NS
02/04/98	8.14	8.22	50.1	51.9	NS	NS	23.53	23.51
17/04/98	8.22	8.07	54.0	49.1	NS	NS	22.85	22.57
27/04/98	8.20	8.21	46.7	47.1	NS	NS	22.11	22.10
04/05/98	7.53	8.29	13.4	43.6	NS	NS	20.44	21.51
15/05/98	7.73	7.88	37.1	47.0	81.2	83.3	14.71	16.14
02/06/98	7.82	7.89	36.4	25.4	88.5	89.3	13.48	15.66
05/06/98	7.71	7.87	29.0	39.7	94.6	90.5	12.89	14.94
21/07/98	8.20	8.26	44.1	48.1	85.4	83.7	17.31	18.14
31/07/98	8.21	8.20	46.0	45.7	87.1	86.9	15.20	15.18
10/08/98	8.14	8.17	49.0	49.9	91.5	96.7	16.72	17.08
17/08/98	7.28	8.14	9.5	38.6	108.5	93.1	15.69	16.79
31/08/98	7.46	7.91	24.8	46.6	98.9	95.9	17.60	17.86
15/09/98	7.05	7.87	13.1	46.2	98.9	89.6	19.31	18.49
01/10/98	NS	NS	NS	NS	NS	NS	NS	NS
16/10/98	7.75	7.76	54.5	54.9	94.8	96.3	19.83	19.55
09/11/98	8.08	8.11	52.3	53.0	78.4	82.4	20.27	19.83
07/12/98	7.92	7.94	62.7	63.2	96.8	99.9	24.45	24.81
25/01/99	8.07	8.15	46.7	49.1	89.0	109.3	26.43	25.51
04/02/99	8.16	8.39	27.9	48.5	115.7	126.0	25.92	25.15
04/03/99	8.16	8.52	20.0	44.0	126.5	113.4	25.61	24.13
30/03/99	7.80	7.82	43.8	46.1	91.8	98.4	24.31	24.36
Summary:								
N of cases	23	23	23	23	18	18	23	23
Minimum	7.05	7.76	9.5	25.4	55.0	60.8	12.89	14.94
Maximum	8.22	8.52	62.7	63.2	126.5	126.0	26.43	25.51
Median	8.08	8.15	46.0	48.1	91.7	91.8	20.27	19.83
Mean	7.92	8.10	39.8	47.7	92.4	93.1	20.01	20.18
Standard Dev.	0.33	0.20	15.2	7.1	15.4	14.2	4.12	3.50

NS = Not sampled

SITE: 2

	pH		Elec. Cond. (mS cm^{-1})		Dissolved Oxygen (% Saturation)		Temperature (^{o}C)	
Date	surface	bed	surface	bed	surface	bed	surface	bed
17/11/97	8.14	8.19	52.8	53.2	81.4	79.7	17.20	17.00
04/12/97	NS	NS	NS	NS	NS	NS	NS	NS
20/03/98	8.08	8.13	51.8	52.7	NS	NS	24.91	24.22
25/03/98	8.15	8.16	52.3	52.0	NS	NS	22.01	21.68
27/03/98	NS	NS	NS	NS	NS	NS	NS	NS
02/04/98	8.12	8.22	48.9	51.7	NS	NS	23.53	23.47
17/04/98	8.22	8.04	54.1	48.2	NS	NS	22.74	22.65
27/04/98	8.12	8.29	44.4	48.8	NS	NS	22.09	22.22
04/05/98	7.45	8.15	13.1	33.7	NS	NS	20.36	20.90
15/05/98	7.61	7.86	33.3	48.1	79.2	78.1	14.29	16.27
02/06/98	7.81	7.90	36.6	43.1	87.1	87.4	13.54	15.81
05/06/98	7.62	7.89	27.5	43.5	94.8	88.5	12.60	16.06
21/07/98	8.20	8.26	43.5	48.6	83.7	82.5	17.18	18.25
31/07/98	8.19	8.21	44.4	45.6	87.2	85.7	14.95	15.17
10/08/98	8.12	8.17	47.9	49.6	92.2	93.3	16.62	16.61
17/08/98	7.65	7.95	6.6	36.4	108.9	92.5	15.67	16.73
31/08/98	7.49	7.90	24.1	46.8	107.4	95.1	17.59	17.81
15/09/98	7.25	7.77	11.8	44.9	98.3	87.4	19.27	18.53
01/10/98	NS	NS	NS	NS	NS	NS	NS	NS
16/10/98	7.73	7.79	52.3	55.0	94.3	95.6	20.88	19.62
09/11/98	8.03	8.11	51.3	52.5	76.9	80.1	20.69	19.86
07/12/98	7.90	7.93	62.6	64.5	92.6	91.7	24.88	23.41
25/01/99	8.10	8.15	47.4	49.2	103.5	99.2	26.41	25.70
04/02/99	8.17	8.42	27.2	52.2	115.1	123.7	25.98	24.88
04/03/99	8.03	8.43	17.9	35.1	124.4	124.5	25.69	25.38
30/03/99	7.82	7.83	37.8	44.7	129.4	110.5	24.35	24.47
Summary:								
N of cases	23	23	23	23	17	17	23	23
Minimum	7.25	7.77	6.6	33.7	76.9	78.1	12.60	15.17
Maximum	8.22	8.43	62.6	64.5	129.4	124.5	26.41	25.70
Median	8.03	8.13	44.4	48.6	94.3	91.7	20.69	19.86
Mean	7.91	8.08	38.7	47.8	97.4	93.9	20.15	20.29
Standard Dev.	0.28	0.19	15.7	6.8	15.4	13.9	4.33	3.52

NS = Not sampled

Appendix 3F. (Continued)

SITE: 3

	pH		Elec. Cond. (mS cm^{-1})		Dissolved Oxygen (% Saturation)		Temperature (^{o}C)	
Date	**surface**	**bed**	**surface**	**bed**	**surface**	**bed**	**surface**	**bed**
17/11/97	8.14	8.15	53.0	53.2	77.6	77.6	17.20	17.00
04/12/97	8.11	8.23	51.7	53.8	58.4	64.1	21.80	21.80
20/03/98	NS	NS	NS	NS	NS	NS	NS	NS
25/03/98	8.12	8.16	51.3	49.4	NS	NS	22.76	21.85
27/03/98	NS	NS	NS	NS	NS	NS	NS	NS
02/04/98	8.10	8.23	48.9	52.1	NS	NS	23.62	23.42
17/04/98	8.21	8.04	51.2	48.2	NS	NS	22.63	22.57
27/04/98	8.13	8.25	44.2	50.1	NS	NS	22.11	21.82
04/05/98	NS	NS	NS	NS	NS	NS	NS	NS
15/05/98	7.57	7.87	32.6	49.9	81.4	80.1	14.19	16.76
02/06/98	7.80	7.90	35.8	43.6	87.8	86.6	13.30	16.06
05/06/98	7.60	7.90	27.4	41.2	93.7	90.4	12.58	15.73
21/07/98	8.19	8.26	42.9	50.1	85.3	82.5	17.10	18.41
31/07/98	8.09	8.18	38.3	46.9	90.5	83.9	14.36	15.48
10/08/98	8.10	8.16	46.5	48.7	93.4	92.8	16.65	16.59
17/08/98	7.38	8.15	5.6	41.4	107.3	90.4	15.65	17.10
31/08/98	7.32	7.89	20.6	47.3	105.3	95.1	17.77	17.86
15/09/98	7.20	7.94	10.5	46.9	98.1	88.9	19.39	18.48
01/10/98	NS	NS	NS	NS	NS	NS	NS	NS
16/10/98	7.72	7.78	50.9	54.1	87.9	94.1	20.64	19.93
09/11/98	8.05	8.12	51.5	53.0	79.0	82.6	20.54	19.89
07/12/98	7.86	7.90	62.5	64.7	93.6	95.4	25.01	23.27
25/01/99	8.03	8.14	43.9	49.1	99.4	94.0	27.04	25.48
04/02/99	8.14	8.42	23.4	52.4	116.3	123.0	26.10	24.87
04/03/99	8.12	8.54	19.2	49.7	126.5	115.0	25.41	23.96
30/03/99	7.78	7.83	31.9	44.8	114.7	100.1	24.28	24.19
Summary:								
N of cases	22	22	22	22	18	18	22	22
Minimum	7.20	7.78	5.6	41.2	58.4	64.1	12.58	15.48
Maximum	8.21	8.54	62.5	64.7	126.5	123.0	27.04	25.48
Median	8.07	8.15	43.4	49.6	93.5	90.4	20.59	19.91
Mean	7.90	8.09	38.4	49.6	94.2	90.9	20.01	20.11
Standard Dev.	0.31	0.20	15.2	5.0	16.1	13.2	4.43	3.30

NS = Not sampled

SITE: 4

	pH		Elec. Cond. (mS cm^{-1})		Dissolved Oxygen (% Saturation)		Temperature (^{o}C)	
Date	**surface**	**bed**	**surface**	**bed**	**surface**	**bed**	**surface**	**bed**
17/11/97	NS	NS	NS	NS	NS	NS	NS	NS
04/12/97	NS	NS	NS	NS	NS	NS	NS	NS
20/03/98	8.01	8.01	49.8	50.0	NS	NS	25.12	24.76
25/03/98	8.08	8.15	50.4	52.4	NS	NS	23.28	21.88
27/03/98	NS	NS	NS	NS	NS	NS	NS	NS
02/04/98	8.10	8.11	48.4	49.7	NS	NS	23.59	23.62
17/04/98	8.18	7.99	52.5	46.5	NS	NS	22.49	22.98
27/04/98	8.10	8.24	42.6	49.4	NS	NS	22.18	21.80
04/05/98	NS	NS	NS	NS	NS	NS	NS	NS
15/05/98	7.59	7.84	33.3	45.0	79.2	81.7	14.14	15.71
02/06/98	7.80	7.92	38.7	48.3	88.7	85.5	12.97	16.21
05/06/98	7.58	7.90	26.6	40.5	95.0	89.7	12.48	15.79
21/07/98	8.16	8.19	42.0	44.1	85.3	84.7	16.94	17.17
31/07/98	8.13	8.20	39.6	44.9	88.8	83.4	14.42	15.40
10/08/98	8.10	8.19	43.6	49.1	93.5	91.8	16.91	16.55
17/08/98	7.81	8.08	3.8	32.7	93.3	106.7	16.50	15.61
31/08/98	7.51	7.97	19.3	47.5	104.0	92.4	18.25	17.88
15/09/98	7.06	7.90	9.5	48.9	97.2	85.3	19.24	18.42
01/10/98	NS	NS	NS	NS	NS	NS	NS	NS
16/10/98	7.73	7.74	49.9	50.1	91.9	93.7	20.63	20.57
09/11/98	8.06	8.08	52.6	52.9	84.5	86.1	20.05	19.85
07/12/98	7.82	7.86	62.5	64.8	94.2	90.9	24.98	23.18
25/01/99	8.02	8.12	43.4	47.2	107.1	96.4	27.17	25.99
04/02/99	8.07	8.39	19.2	48.8	115.8	121.2	26.14	25.01
04/03/99	8.05	8.46	19.4	41.5	116.0	114.7	25.34	24.01
30/03/99	7.79	7.84	38.7	46.0	108.1	95.3	24.27	24.11
Summary:								
N of cases	21	21	21	21	16	16	21	21
Minimum	7.06	7.74	3.8	32.7	79.2	81.7	12.48	15.40
Maximum	8.18	8.46	62.5	64.8	116.0	121.2	27.17	25.99
Median	8.02	8.08	42.0	48.3	93.9	91.4	20.63	20.57
Mean	7.89	8.06	37.4	47.6	96.4	93.7	20.34	20.31
Standard Dev.	0.28	0.19	15.6	6.0	10.9	11.3	4.61	3.69

NS = Not sampled

Appendix 3F. (Continued)

SITE: 5

	pH		Elec. Cond. (mS cm^{-1})		Dissolved Oxygen (% Saturation)		Temperature (^{o}C)	
Date	surface	bed	surface	bed	surface	bed	surface	bed
17/11/97	8.22	8.25	50.1	53.1	83.6	84.9	17.20	17.20
04/12/97	8.23	8.24	53.8	49.8	62.5	64.6	21.40	21.40
20/03/98	8.10	8.13	52.3	52.8	NS	NS	24.71	24.30
25/03/98	8.08	8.08	51.8	51.7	NS	NS	22.55	22.56
27/03/98	8.21	8.21	52.6	52.9	NS	NS	24.40	24.40
02/04/98	8.12	8.13	49.6	49.7	NS	NS	23.67	23.67
17/04/98	NS	NS	NS	NS	NS	NS	NS	NS
27/04/98	8.21	8.23	48.2	48.6	NS	NS	22.28	22.35
04/05/98	7.61	7.63	11.8	20.8	NS	NS	20.71	20.42
15/05/98	7.67	7.70	36.0	37.1	78.6	77.4	14.84	14.66
02/06/98	7.72	7.74	36.0	36.6	82.9	82.2	13.61	13.77
05/06/98	7.34	7.35	17.5	20.1	94.6	92.8	12.30	12.43
21/07/98	8.10	8.12	43.0	43.7	81.1	81.4	17.94	18.01
31/07/98	8.15	8.17	43.0	44.4	88.5	87.7	14.89	15.04
10/08/98	8.11	8.12	47.9	48.1	97.3	97.5	17.09	17.10
17/08/98	7.69	7.70	19.7	25.5	111.2	102.3	15.93	15.75
31/08/98	NS	NS	NS	NS	NS	NS	NS	NS
15/09/98	NS	NS	NS	NS	NS	NS	NS	NS
01/10/98	7.14	7.30	12.0	22.0	89.9	75.3	27.33	23.84
16/10/98	NS	NS	49.8	52.1	91.9	93.6	20.63	20.21
09/11/98	8.07	8.08	45.1	52.8	78.5	81.1	19.99	19.82
07/12/98	7.97	7.97	62.1	62.1	103.3	105.8	25.33	25.32
25/01/99	8.07	8.10	44.5	48.0	94.6	109.4	27.03	25.88
04/02/99	8.33	8.34	41.9	42.2	128.7	131.5	26.39	26.39
04/03/99	8.40	8.49	35.4	38.5	134.5	135.3	26.43	26.41
30/03/99	7.78	7.79	40.8	42.6	108.9	106.1	24.29	24.26
Summary:								
N of cases	22	22	23	23	17	17	23	23
Minimum	7.14	7.30	11.8	20.1	62.5	64.6	12.30	12.43
Maximum	8.40	8.49	62.1	62.1	134.5	135.3	27.33	26.41
Median	8.09	8.11	44.5	48.0	91.9	92.8	21.40	21.40
Mean	7.97	7.99	41.1	43.3	94.7	94.6	20.91	20.66
Standard Dev.	0.32	0.31	13.7	11.6	18.3	19.0	4.63	4.37

NS = Not sampled

SITE: 6

	pH		**Elec. Cond. (mS cm^{-1})**		**Dissolved Oxygen (% Saturation)**		**Temperature (^{o}C)**	
Date	**surface**	**bed**	**surface**	**bed**	**surface**	**bed**	**surface**	**bed**
17/11/97	8.23	8.26	52.7	52.8	85.0	85.0	17.40	17.40
04/12/97	8.24	8.24	53.8	53.8	63.0	66.5	21.40	21.40
20/03/98	8.08	8.12	49.6	50.8	NS	NS	25.48	24.91
25/03/98	NS	NS	50.1	50.0	NS	NS	23.56	23.56
27/03/98	8.21	8.21	52.9	52.8	NS	NS	24.42	24.40
02/04/98	8.10	8.14	49.2	50.0	NS	NS	23.74	23.70
17/04/98	8.08	8.16	49.3	51.1	NS	NS	22.67	23.08
27/04/98	NS	NS	NS	NS	NS	NS	NS	NS
04/05/98	7.57	7.45	8.3	12.5	NS	NS	20.53	20.35
15/05/98	7.60	7.59	33.8	33.9	76.3	74.2	14.38	14.36
02/06/98	7.67	7.67	34.0	34.0	82.7	82.7	13.27	13.27
05/06/98	7.22	7.19	13.8	13.9	102.7	101.9	11.99	11.97
21/07/98	8.05	8.08	41.7	41.9	80.1	80.8	17.84	17.86
31/07/98	8.16	8.16	43.1	43.3	90.1	90.1	14.94	14.96
10/08/98	NS	NS	NS	NS	NS	NS	NS	NS
17/08/98	7.52	7.56	20.2	22.4	109.9	103.2	15.51	15.61
31/08/98	7.10	7.22	16.3	22.2	103.8	104.6	17.32	16.64
15/09/98	7.00	7.18	14.1	21.8	97.8	100.2	19.34	18.84
01/10/98	7.41	7.44	22.2	24.0	87.3	88.6	24.94	24.66
16/10/98	7.59	7.60	45.8	46.0	83.1	90.9	21.37	21.23
09/11/98	8.03	8.04	52.6	52.5	84.7	84.8	19.97	19.97
07/12/98	7.97	7.97	61.9	61.9	101.8	106.7	25.45	25.45
25/01/99	8.00	8.01	46.8	46.4	81.5	94.5	26.71	26.72
04/02/99	8.33	8.36	47.5	48.3	109.2	112.5	26.43	26.12
04/03/99	8.30	8.30	32.2	32.2	131.1	129.1	26.72	26.72
30/03/99	7.74	7.78	39.0	40.9	123.2	111.0	24.35	24.28
Summary:								
N of cases	23	23	24	24	18	18	24	24
Minimum	7.00	7.18	8.3	12.5	63.0	66.5	11.99	11.97
Maximum	8.33	8.36	61.9	61.9	131.1	129.1	26.72	26.72
Median	8.00	8.01	44.5	44.7	88.7	92.7	21.39	21.32
Mean	7.83	7.86	38.8	40.0	94.1	94.9	20.82	20.73
Standard Dev.	0.40	0.38	15.3	14.1	17.2	15.3	4.58	4.56

NS = Not sampled

Appendix 3F. (Continued)

SITE: 7

	pH		Elec. Cond. (mS cm^{-1})		Dissolved Oxygen (% Saturation)		Temperature (^{o}C)	
Date	**surface**	**bed**	**surface**	**bed**	**surface**	**bed**	**surface**	**bed**
17/11/97	8.25	8.28	52.8	52.8	86.8	85.6	17.60	17.60
04/12/97	8.25	8.25	52.7	53.7	60.0	62.6	21.40	21.50
20/03/98	7.95	8.08	50.0	51.4	NS	NS	25.77	25.05
25/03/98	7.95	7.96	50.0	50.0	NS	NS	23.78	23.78
27/03/98	8.21	8.21	52.8	52.6	NS	NS	24.26	24.22
02/04/98	8.07	8.08	48.0	48.6	NS	NS	23.34	23.40
17/04/98	8.12	8.14	50.3	50.9	NS	NS	23.05	22.98
27/04/98	8.11	8.11	44.6	44.6	NS	NS	22.06	22.06
04/05/98	7.73	7.53	5.2	5.5	NS	NS	20.62	20.38
15/05/98	7.55	7.55	32.6	32.7	77.0	76.6	14.26	14.38
02/06/98	7.64	7.64	32.9	32.9	81.5	82.6	13.18	13.17
05/06/98	7.16	7.13	11.4	11.4	101.7	101.2	11.80	11.79
21/07/98	8.06	8.06	41.6	41.5	80.6	81.3	17.86	17.85
31/07/98	8.05	8.11	40.7	43.0	89.1	88.5	14.29	14.85
10/08/98	8.08	8.11	47.7	47.7	95.1	96.0	16.97	16.98
17/08/98	7.48	7.49	19.2	20.2	111.8	109.3	15.51	15.50
31/08/98	7.08	7.08	11.7	15.7	103.2	103.2	17.18	16.61
15/09/98	6.88	NS	8.1	NS	94.0	NS	20.33	NS
01/10/98	7.56	7.58	26.7	27.1	92.0	92.3	24.19	24.11
16/10/98	7.52	7.59	45.6	ND	87.7	94.8	22.37	21.52
09/11/98	7.97	8.06	49.6	51.8	82.8	81.2	21.25	20.32
07/12/98	7.95	7.96	61.7	61.7	99.1	103.0	25.68	25.68
25/01/99	7.96	7.97	47.6	47.8	78.5	90.5	26.82	26.77
04/02/99	8.35	8.35	44.1	44.2	104.5	92.7	26.33	26.38
04/03/99	8.27	8.28	31.6	31.6	132.9	130.3	26.84	26.85
30/03/99	7.68	7.70	37.3	37.5	126.0	126.4	24.38	24.45
Summary:								
N of cases	26	25	26	24	19	18	26	25
Minimum	6.88	7.08	5.2	5.5	60.0	62.6	11.80	11.79
Maximum	8.35	8.35	61.7	61.7	132.9	130.3	26.84	26.85
Median	7.96	8.06	44.4	44.4	92.0	92.5	21.73	21.52
Mean	7.84	7.89	38.3	39.9	93.9	94.3	20.81	20.73
Standard Dev.	0.39	0.36	15.7	14.8	17.3	16.6	4.53	4.56

NS = Not sampled

SITE: 8

	pH		Elec. Cond. (mS cm^{-1})		Dissolved Oxygen (% Saturation)		Temperature (^{o}C)	
Date	**surface**	**bed**	**surface**	**bed**	**surface**	**bed**	**surface**	**bed**
17/11/97	8.26	8.28	52.8	52.8	85.3	84.9	17.60	17.60
04/12/97	8.25	8.26	52.4	53.6	59.4	62.0	21.50	21.50
20/03/98	8.02	8.06	49.9	50.8	NS	NS	25.74	25.31
25/03/98	7.95	7.95	50.1	50.1	NS	NS	23.84	23.83
27/03/98	8.20	8.21	52.7	52.7	NS	NS	24.19	24.21
02/04/98	8.06	8.09	47.6	48.5	NS	NS	23.31	23.46
17/04/98	8.12	8.13	49.2	50.4	NS	NS	23.38	22.97
27/04/98	8.09	8.09	44.0	44.0	NS	NS	22.02	22.03
04/05/98	7.29	7.24	4.9	4.8	NS	NS	20.40	20.33
15/05/98	7.53	7.52	31.4	31.8	76.2	75.3	14.17	14.13
02/06/98	7.61	7.61	31.8	32.0	82.8	82.5	13.03	13.05
05/06/98	7.08	7.04	10.2	10.7	101.0	99.6	11.70	11.80
21/07/98	8.04	8.05	41.1	41.2	80.6	81.2	17.83	17.82
31/07/98	8.01	8.08	41.1	34.5	88.4	90.4	14.41	14.58
10/08/98	8.09	8.09	47.5	47.5	98.7	98.7	16.97	16.93
17/08/98	7.46	7.47	18.4	19.9	110.8	108.7	15.46	15.51
31/08/98	7.00	7.00	10.3	12.1	100.9	101.2	16.87	16.85
15/09/98	6.88	6.87	5.8	6.9	94.1	93.6	20.05	20.41
01/10/98	7.62	7.64	28.0	28.4	94.5	95.1	23.97	23.89
16/10/98	7.56	7.57	45.3	45.2	88.5	91.9	21.75	21.78
09/11/98	8.00	8.05	50.0	51.3	81.4	81.3	21.05	20.55
07/12/98	7.95	7.96	61.5	61.5	105.2	105.5	25.84	25.82
25/01/99	7.92	7.94	47.9	48.0	80.4	89.7	26.89	26.87
04/02/99	8.33	8.37	41.5	44.2	104.4	114.9	26.51	26.25
04/03/99	8.23	8.25	31.2	31.4	129.5	128.6	27.03	26.99
30/03/99	7.68	7.69	37.0	37.2	123.1	142.1	24.41	24.42
Summary:								
N of cases	26	26	26	26	19	19	26	26
Minimum	6.88	6.87	4.9	4.8	59.4	62.0	11.70	11.80
Maximum	8.33	8.37	61.5	61.5	129.5	142.1	27.03	26.99
Median	7.98	8.01	42.8	44.1	94.1	93.6	21.63	21.64
Mean	7.82	7.83	37.8	38.1	94.0	96.2	20.77	20.73
Standard Dev.	0.41	0.43	16.0	15.9	16.7	18.7	4.58	4.52

NS = Not sampled

Appendix 3F. (Continued)

SITE: 9

	pH		Elec. Cond. (mS cm^{-1})		Dissolved Oxygen (% Saturation)		Temperature (^{o}C)	
Date	**surface**	**bed**	**surface**	**bed**	**surface**	**bed**	**surface**	**bed**
17/11/97	8.24	8.27	52.6	52.7	86.1	85.0	17.80	17.80
04/12/97	8.25	8.22	53.1	52.8	58.5	62.1	21.60	21.90
20/03/98	NS	NS	NS	NS	NS	NS	NS	NS
25/03/98	7.95	7.95	49.8	49.8	NS	NS	23.85	23.85
27/03/98	8.20	8.21	52.5	52.5	NS	NS	24.19	24.19
02/04/98	8.04	8.06	47.8	48.2	NS	NS	23.39	23.42
17/04/98	8.10	8.12	49.2	50.1	NS	NS	23.15	22.95
27/04/98	8.08	8.08	43.7	43.6	NS	NS	22.01	22.01
04/05/98	7.22	7.19	4.5	5.0	NS	NS	20.38	20.39
15/05/98	7.51	7.49	31.3	31.1	77.0	76.0	14.11	14.14
02/06/98	7.60	7.60	31.7	31.6	82.5	82.1	13.04	13.03
05/06/98	7.02	7.01	9.4	9.2	98.3	98.3	11.65	11.59
21/07/98	8.03	8.05	41.2	41.2	79.7	80.7	17.87	17.84
31/07/98	8.08	8.09	40.9	41.3	89.1	89.2	14.34	14.40
10/08/98	8.06	8.09	47.4	47.4	94.4	95.6	16.88	16.97
17/08/98	7.43	7.44	18.1	18.5	110.0	109.3	15.48	15.44
31/08/98	7.01	7.00	9.9	10.2	106.7	102.2	16.61	16.49
15/09/98	6.86	6.84	5.4	7.0	91.8	93.5	20.21	19.97
01/10/98	7.67	7.69	29.0	31.0	96.6	96.9	23.84	23.50
16/10/98	7.53	7.54	45.4	45.4	87.9	90.4	22.08	22.07
09/11/98	8.00	8.02	50.2	50.4	76.9	79.9	21.05	20.92
07/12/98	7.92	7.95	61.3	61.4	103.6	105.0	26.01	25.90
25/01/99	7.90	7.91	47.8	47.8	83.1	87.8	26.90	26.83
04/02/99	8.32	8.34	41.0	42.6	97.5	107.6	26.37	26.22
04/03/99	8.21	8.23	31.0	31.2	129.4	128.6	27.09	27.09
30/03/99	7.66	7.68	36.1	36.5	131.5	130.0	24.38	24.39
Summary:								
N of cases	25	25	25	25	19	19	25	25
Minimum	6.86	6.84	4.5	5.0	58.5	62.1	11.65	11.59
Maximum	8.32	8.34	61.3	61.4	131.5	130.0	27.09	27.09
Median	7.95	7.95	41.2	42.6	91.8	93.5	21.60	21.90
Mean	7.80	7.80	37.2	37.5	93.7	94.7	20.57	20.53
Standard Dev.	0.42	0.44	16.3	16.2	17.7	16.9	4.57	4.55

NS = Not sampled

SITE: 10

	pH		Elec. Cond. (mS cm^{-1})		Dissolved Oxygen (% Saturation)		Temperature (^{o}C)	
Date	**surface**	**bed**	**surface**	**bed**	**surface**	**bed**	**surface**	**bed**
17/11/97	8.25	8.27	51.3	52.8	87.7	86.5	18.00	18.00
04/12/97	NS	NS	NS	NS	50.9	61.8	21.80	21.80
20/03/98	7.98	8.03	49.6	49.8	NS	NS	26.07	25.59
25/03/98	7.94	7.97	49.9	50.3	NS	NS	23.87	23.70
27/03/98	8.20	8.20	52.4	52.4	NS	NS	24.17	24.17
02/04/98	8.06	8.06	48.0	47.9	NS	NS	23.42	23.41
17/04/98	8.05	8.09	48.3	49.3	NS	NS	23.31	23.02
27/04/98	8.06	8.13	43.2	44.2	NS	NS	22.00	22.07
04/05/98	7.17	7.16	4.7	4.6	NS	NS	20.47	20.43
15/05/98	7.48	7.49	30.4	31.0	77.3	76.3	14.16	14.04
02/06/98	7.57	7.60	30.7	31.6	82.6	82.6	12.92	13.09
05/06/98	6.99	6.89	8.1	9.3	97.0	98.1	11.47	11.72
21/07/98	8.00	8.07	40.7	41.9	79.0	79.8	17.78	17.97
31/07/98	8.04	8.05	40.2	40.3	89.9	90.0	14.05	14.07
10/08/98	8.12	8.12	47.3	47.5	98.3	100.1	17.16	17.20
17/08/98	7.37	7.37	15.2	17.4	110.0	109.5	15.50	15.44
31/08/98	7.02	7.00	8.7	9.8	104.0	102.9	16.67	16.40
15/09/98	6.85	6.85	4.9	5.4	92.1	93.0	20.17	19.89
01/10/98	7.70	7.66	33.2	14.9	97.5	99.4	23.10	22.88
16/10/98	7.45	7.46	45.7	45.7	88.7	89.6	22.49	22.50
09/11/98	7.96	7.97	49.5	49.2	78.1	80.5	21.34	21.34
07/12/98	7.92	7.94	61.0	61.1	104.8	104.8	26.24	26.14
25/01/99	7.88	7.89	47.7	47.7	84.5	88.5	26.87	26.84
04/02/99	8.28	8.34	37.8	41.5	90.8	122.4	26.91	26.39
04/03/99	8.16	8.26	30.4	31.8	129.7	129.3	27.23	27.21
30/03/99	7.65	7.66	35.2	35.2	120.7	126.5	24.35	24.36
Summary:								
N of cases	25	25	25	25	19	19	26	26
Minimum	6.85	6.85	4.7	4.6	50.9	61.8	11.47	11.72
Maximum	8.28	8.34	61.0	61.1	129.7	129.3	27.23	27.21
Median	7.94	7.97	40.7	41.9	90.8	93.0	21.90	21.94
Mean	7.77	7.78	36.6	36.5	92.8	95.9	20.83	20.76
Standard Dev.	0.42	0.45	16.4	16.8	17.3	17.6	4.67	4.58

NS = Not sampled

Appendix 3F. (Continued)

SITE: 11

	pH		Elec. Cond. (mS cm^{-1})		Dissolved Oxygen (% Saturation)		Temperature (^{o}C)	
Date	**surface**	**bed**	**surface**	**bed**	**surface**	**bed**	**surface**	**bed**
17/11/97	8.22	8.25	52.2	52.2	82.7	85.4	18.20	18.20
04/12/97	8.23	8.24	53.3	52.0	50.9	61.8	21.80	21.80
20/03/98	NS	NS	NS	NS	NS	NS	NS	NS
25/03/98	7.90	7.90	49.1	49.1	NS	NS	24.18	24.16
27/03/98	8.19	8.20	52.1	52.3	NS	NS	24.05	24.12
02/04/98	7.99	8.04	45.1	47.1	NS	NS	23.19	23.34
17/04/98	8.06	8.06	47.0	48.3	NS	NS	23.37	22.90
27/04/98	8.01	8.03	41.9	42.1	NS	NS	22.00	22.01
04/05/98	7.28	7.23	4.1	4.1	NS	NS	20.43	20.41
15/05/98	7.43	7.45	28.8	30.0	76.2	75.3	13.99	13.93
02/06/98	7.49	7.54	28.9	30.2	82.2	82.0	12.72	12.88
05/06/98	7.02	6.94	6.0	7.3	96.7	96.7	11.22	11.37
21/07/98	7.97	8.02	40.1	40.7	79.0	79.5	17.76	17.80
31/07/98	8.03	8.03	39.5	39.5	91.7	91.1	13.95	13.95
10/08/98	8.02	8.02	46.8	46.8	93.0	93.2	16.72	16.72
17/08/98	7.36	7.37	15.7	17.2	111.8	109.5	15.37	15.42
31/08/98	6.93	6.93	6.5	7.2	101.0	101.4	17.19	16.40
15/09/98	6.84	6.83	4.5	5.2	91.4	93.2	19.92	19.86
01/10/98	7.63	7.74	25.6	37.3	94.6	97.6	24.51	22.37
16/10/98	7.38	7.41	45.8	45.6	84.0	89.1	22.86	22.95
09/11/98	7.85	7.92	47.7	48.5	74.6	76.1	21.93	21.73
07/12/98	7.86	7.88	60.5	60.5	95.4	103.0	26.70	26.66
25/01/99	7.85	7.86	47.5	47.5	75.6	92.9	27.00	26.99
04/02/99	8.19	8.25	33.7	37.7	87.7	114.3	26.56	26.13
04/03/99	8.05	8.14	29.4	30.4	124.2	124.0	27.36	27.25
30/03/99	7.64	7.64	34.4	34.3	121.3	123.7	24.35	24.34
Summary:								
N of cases	25	25	25	25	19	19	25	25
Minimum	6.84	6.83	4.1	4.1	50.9	61.8	11.22	11.37
Maximum	8.23	8.25	60.5	60.5	124.2	124.0	27.36	27.25
Median	7.86	7.90	40.10	40.70	91.40	93.20	21.93	21.80
Mean	7.74	7.76	35.4	36.5	90.2	94.2	20.69	20.55
Standard Dev.	0.42	0.43	16.8	16.4	17.1	16.3	4.76	4.68

NS = Not sampled

SITE: 12

	pH		Elec. Cond. (mS cm^{-1})		Dissolved Oxygen (% Saturation)		Temperature (^{o}C)	
Date	**surface**	**bed**	**surface**	**bed**	**surface**	**bed**	**surface**	**bed**
17/11/97	8.20	8.22	51.1	50.4	84.9	85.0	18.60	18.60
04/12/97	8.20	8.21	52.2	52.4	61.2	63.3	22.20	22.10
20/03/98	7.97	7.97	49.1	49.1	NS	NS	26.23	26.17
25/03/98	7.89	7.89	49.0	49.0	NS	NS	24.21	24.21
27/03/98	8.13	8.13	51.6	51.3	NS	NS	23.74	23.74
02/04/98	8.00	8.01	45.5	46.1	NS	NS	23.21	23.24
17/04/98	8.03	8.05	44.2	48.0	NS	NS	23.59	22.86
27/04/98	7.98	7.99	40.9	41.5	NS	NS	22.03	21.94
04/05/98	7.08	7.07	3.8	3.8	NS	NS	20.35	20.33
15/05/98	7.33	7.34	27.4	28.2	76.8	77.0	13.84	13.78
02/06/98	7.43	7.44	27.8	28.7	80.7	80.7	12.60	12.83
05/06/98	6.96	6.93	5.3	5.7	95.0	95.3	11.35	11.15
21/07/98	7.96	7.98	39.6	40.2	79.1	79.4	17.73	17.88
31/07/98	8.02	8.03	38.1	38.2	89.9	90.0	13.77	13.76
10/08/98	8.00	8.01	46.6	30.5	94.6	95.5	16.74	16.76
17/08/98	7.36	7.33	14.4	16.0	110.0	109.0	15.39	15.36
31/08/98	6.88	6.88	5.8	6.6	104.6	102.7	16.45	15.95
15/09/98	6.83	6.79	3.6	4.6	91.4	92.9	19.96	19.68
01/10/98	7.72	7.75	38.9	41.5	100.6	100.9	22.08	21.73
16/10/98	7.35	7.36	45.2	45.2	88.9	89.7	23.14	23.12
09/11/98	7.84	7.85	47.9	47.7	73.7	73.0	21.96	21.95
07/12/98	7.84	7.85	60.2	60.3	101.1	101.2	26.92	26.82
25/01/99	7.83	7.84	47.4	47.5	79.9	82.5	26.97	26.90
04/02/99	8.12	8.17	31.0	32.9	99.2	100.8	26.73	26.52
04/03/99	8.03	8.05	29.1	29.3	124.3	123.2	27.45	27.41
30/03/99	7.60	7.61	33.4	33.4	119.8	121.8	24.29	24.30
Summary:								
N of cases	26	26	26	26	19	19	26	26
Minimum	6.83	6.79	3.6	3.8	61.2	63.3	11.35	11.15
Maximum	8.20	8.22	60.2	60.3	124.3	123.2	27.45	27.41
Median	7.87	7.87	40.25	40.85	91.40	92.90	22.06	21.95
Mean	7.71	7.72	35.7	35.7	92.4	92.8	20.83	20.73
Standard Dev.	0.42	0.44	16.7	16.4	15.9	15.6	4.80	4.78

NS = Not sampled

Appendix 3F. (Continued)

SITE: 13

	pH		Elec. Cond. (mS cm^{-1})		Dissolved Oxygen (% Saturation)		Temperature (^{o}C)	
Date	**surface**	**bed**	**surface**	**bed**	**surface**	**bed**	**surface**	**bed**
17/11/97	NS	NS	50.8	50.9	83.3	83.7	19.10	19.00
04/12/97	8.12	8.06	50.5	51.4	60.3	66.3	23.10	22.50
20/03/98	NS	NS	NS	NS	NS	NS	26.55	26.35
25/03/98	7.89	7.89	48.9	48.9	NS	NS	24.28	24.28
27/03/98	8.11	8.11	51.4	51.3	NS	NS	23.64	23.62
02/04/98	7.97	7.99	43.0	40.1	NS	NS	22.91	23.14
17/04/98	8.00	8.00	43.3	46.5	NS	NS	23.34	22.80
27/04/98	7.96	7.96	40.2	40.2	NS	NS	22.08	22.09
04/05/98	7.11	7.06	3.5	3.7	NS	NS	20.38	20.33
15/05/98	7.36	7.29	26.3	26.6	76.4	76.1	13.71	13.69
02/06/98	7.39	7.39	26.8	26.8	81.1	81.1	12.58	12.54
05/06/98	6.89	6.85	4.1	4.2	94.7	94.8	11.03	11.03
21/07/98	7.92	7.94	39.4	39.4	78.3	79.1	17.71	17.70
31/07/98	7.93	7.97	36.3	37.2	88.5	88.7	13.67	13.70
10/08/98	7.93	7.94	46.4	46.4	94.7	94.8	16.68	16.67
17/08/98	7.25	7.25	13.1	13.3	110.6	110.2	15.28	15.27
31/08/98	6.73	6.64	4.1	6.1	102.4	100.4	15.28	16.52
15/09/98	6.82	6.79	3.2	4.1	91.8	92.9	19.88	19.61
01/10/98	7.68	7.76	39.5	45.6	99.4	100.5	22.08	21.12
16/10/98	7.24	7.26	44.8	44.8	86.4	86.4	23.10	23.03
09/11/98	7.76	7.82	46.8	47.6	70.2	70.2	22.15	22.03
07/12/98	7.82	7.83	59.9	60.0	99.6	100.7	27.16	27.12
25/01/99	7.81	7.79	47.3	47.4	74.3	76.9	27.16	26.97
04/02/99	8.05	8.09	28.9	30.2	89.9	108.8	26.79	26.64
04/03/99	7.99	8.00	29.0	29.0	132.7	128.8	27.58	27.57
30/03/99	7.57	7.57	32.3	32.4	125.0	126.2	24.27	24.27
Summary:								
N of cases	24	24	25	25	19	19	26	26
Minimum	6.73	6.64	3.2	3.7	60.3	66.3	11.03	11.03
Maximum	8.12	8.11	59.9	60.0	132.7	128.8	27.58	27.57
Median	7.82	7.83	39.50	40.10	89.90	92.90	22.12	22.06
Mean	7.64	7.64	34.4	35.0	91.6	93.0	20.83	20.75
Standard Dev.	0.43	0.45	17.0	16.9	17.9	17.3	4.94	4.84

NS = Not sampled

SITE: 14

	pH		Elec. Cond. (mS cm^{-1})		Dissolved Oxygen (% Saturation)		Temperature (^{o}C)	
Date	**surface**	**bed**	**surface**	**bed**	**surface**	**bed**	**surface**	**bed**
17/11/97	NS	NS	NS	NS	NS	NS	NS	NS
04/12/97	NS	NS	NS	NS	NS	NS	NS	NS
20/03/98	7.87	7.92	49.0	49.0	NS	NS	26.55	26.35
25/03/98	7.89	7.89	48.9	48.9	NS	NS	24.30	24.30
27/03/98	8.10	8.11	51.1	51.2	NS	NS	23.51	23.53
02/04/98	NS	NS	NS	NS	NS	NS	NS	NS
17/04/98	NS	NS	NS	NS	NS	NS	NS	NS
27/04/98	NS	NS	NS	NS	NS	NS	NS	NS
04/05/98	NS	NS	NS	NS	NS	NS	NS	NS
15/05/98	NS	NS	NS	NS	NS	NS	NS	NS
02/06/98	7.36	7.36	26.1	26.4	81.9	81.1	12.59	12.59
05/06/98	6.91	6.88	3.6	3.6	95.2	95.3	10.99	10.99
21/07/98	7.94	7.95	39.2	39.2	79.8	80.2	17.70	17.70
31/07/98	NS	NS	NS	NS	NS	NS	NS	NS
10/08/98	NS	NS	NS	NS	NS	NS	NS	NS
17/08/98	7.22	7.22	12.2	12.6	111.9	109.7	15.27	15.27
31/08/98	6.72	6.74	3.1	3.8	99.2	100.7	15.94	15.00
15/09/98	6.81	6.81	2.5	2.5	93.4	93.4	19.55	19.45
01/10/98	7.72	7.74	47.6	48.4	104.2	107.5	20.78	20.64
16/10/98	7.22	7.23	44.7	44.7	83.1	84.8	23.13	23.13
09/11/98	7.80	7.79	47.4	47.4	71.1	71.7	22.03	22.08
07/12/98	7.77	7.80	59.8	59.9	100.2	101.9	27.27	27.20
25/01/99	7.78	7.78	47.4	47.4	77.2	79.4	27.19	27.03
04/02/99	8.00	8.02	27.7	28.3	96.5	97.3	26.95	26.77
04/03/99	8.01	8.01	28.8	28.9	123.6	123.4	27.67	27.63
30/03/99	7.56	7.56	31.8	31.8	123.1	123.4	24.34	24.34
Summary:								
N of cases	17	17	17	17	14	14	17	17
Minimum	6.72	6.74	2.5	2.5	71.1	71.7	10.99	10.99
Maximum	8.10	8.11	59.8	59.9	123.6	123.4	27.67	27.63
Median	7.77	7.78	39.20	39.20	95.85	96.30	23.13	23.13
Mean	7.57	7.58	33.6	33.8	95.7	96.4	21.52	21.41
Standard Dev.	0.45	0.45	18.7	18.6	16.3	16.0	5.36	5.39

NS = Not sampled

Appendix 3F. (Continued)

SITE: 15

	pH		Elec. Cond. (mS cm^{-1})		Dissolved Oxygen (% Saturation)		Temperature (^{o}C)	
Date	**surface**	**bed**	**surface**	**bed**	**surface**	**bed**	**surface**	**bed**
17/11/97	8.17	8.19	49.1	49.9	78.4	82.3	19.50	19.50
04/12/97	8.11	8.13	50.6	50.6	62.9	63.3	22.90	22.90
20/03/98	7.83	7.90	49.2	49.1	NS	NS	26.51	26.36
25/03/98	7.89	7.90	48.9	48.9	NS	NS	24.34	24.32
27/03/98	8.10	8.11	51.0	51.0	NS	NS	23.64	23.56
02/04/98	7.94	7.94	43.1	43.3	NS	NS	22.80	22.82
17/04/98	7.95	7.96	44.2	44.7	NS	NS	22.80	22.74
27/04/98	7.95	7.96	39.2	39.7	NS	NS	22.01	22.13
04/05/98	6.99	6.95	2.6	2.7	NS	NS	20.38	20.36
15/05/98	7.28	7.26	24.7	25.3	76.7	76.9	13.63	13.71
02/06/98	7.32	7.32	25.2	25.7	81.1	80.9	12.78	12.70
05/06/98	6.77	6.73	3.1	3.5	95.4	95.1	11.02	11.20
21/07/98	7.89	7.93	38.8	39.2	79.9	81.1	17.79	17.79
31/07/98	NS	NS	NS	NS	NS	NS	NS	NS
10/08/98	7.88	7.92	46.1	46.3	94.8	97.5	16.67	16.88
17/08/98	7.18	7.18	11.1	11.8	110.2	108.8	15.26	15.27
31/08/98	6.67	6.69	2.6	3.1	99.6	100.5	15.27	14.81
15/09/98	6.82	6.79	2.1	2.4	94.0	93.7	19.58	19.80
01/10/98	7.63	7.65	48.2	50.5	104.6	106.0	20.69	20.27
16/10/98	7.15	7.16	44.3	44.3	81.6	82.5	23.41	23.41
09/11/98	7.72	7.73	47.1	45.7	66.8	65.0	22.17	22.17
07/12/98	7.80	7.83	59.7	60.0	99.8	99.9	27.36	27.07
25/01/99	7.76	7.76	47.2	47.3	76.8	87.7	27.62	26.93
04/02/99	7.99	8.01	26.4	27.3	94.0	108.5	27.21	26.87
04/03/99	8.03	8.01	28.8	28.8	129.4	126.8	27.99	27.69
30/03/99	7.57	7.57	31.0	30.7	123.5	126.8	24.41	24.41
Summary:								
N of cases	25	25	25	25	18	18	25	25
Minimum	6.67	6.69	2.1	2.4	62.9	63.3	11.02	11.20
Maximum	8.17	8.19	59.7	60.0	129.4	126.8	27.99	27.69
Median	7.80	7.83	43.10	43.30	94.00	94.40	22.17	22.17
Mean	7.62	7.62	34.6	34.9	91.6	93.5	21.11	21.03
Standard Dev.	0.46	0.47	17.9	17.8	18.0	18.0	4.88	4.78

NS = Not sampled

SITE: 16

	pH		Elec. Cond. (mS cm^{-1})		Dissolved Oxygen (% Saturation)		Temperature (^{o}C)	
Date	**surface**	**bed**	**surface**	**bed**	**surface**	**bed**	**surface**	**bed**
17/11/97	8.13	8.15	47.6	48.5	79.5	85.2	20.30	20.00
04/12/97	8.11	8.10	50.6	50.6	59.2	59.9	23.00	23.00
20/03/98	7.80	7.86	49.2	49.1	NS	NS	26.43	26.40
25/03/98	7.87	7.87	48.6	48.6	NS	NS	24.33	24.32
27/03/98	8.09	8.10	51.0	51.0	NS	NS	23.39	23.40
02/04/98	7.92	7.92	41.9	41.9	NS	NS	22.62	22.62
17/04/98	7.91	7.91	43.3	43.5	NS	NS	22.85	22.68
27/04/98	7.90	7.91	37.7	37.5	NS	NS	21.86	21.86
04/05/98	6.91	6.88	2.2	2.2	NS	NS	20.43	20.42
15/05/98	7.27	7.22	23.7	23.8	76.8	76.9	13.64	13.67
02/06/98	7.26	7.26	24.4	24.5	80.7	80.5	12.95	12.94
05/06/98	6.84	6.81	2.5	2.5	96.0	95.6	10.64	10.63
21/07/98	7.82	7.86	37.7	37.8	80.3	80.7	17.84	17.79
31/07/98	NS	NS	NS	NS	NS	NS	NS	NS
10/08/98	7.80	7.85	45.5	45.9	91.7	92.5	16.57	16.58
17/08/98	7.18	7.15	9.9	11.2	109.3	106.6	15.28	15.35
31/08/98	6.77	6.76	1.5	2.5	100.9	100.0	15.93	14.98
15/09/98	6.82	6.79	1.7	1.8	93.1	93.5	19.58	19.55
01/10/98	7.55	7.56	48.7	51.6	106.6	107.9	20.64	20.00
16/10/98	7.13	7.13	44.0	44.0	80.7	83.2	23.86	23.69
09/11/98	7.67	7.69	46.8	46.8	67.8	65.1	22.27	22.25
07/12/98	7.78	7.80	59.2	59.4	98.7	102.2	27.79	27.62
25/01/99	7.75	7.76	47.2	47.2	82.5	86.7	27.62	27.16
04/02/99	7.84	7.90	24.5	26.2	93.5	94.8	26.98	26.94
04/03/99	8.01	8.02	28.7	29.0	130.5	125.1	27.42	27.31
30/03/99	7.57	7.56	30.3	30.4	134.3	133.8	24.44	24.48
Summary:								
N of cases	25	25	25	25	18	18	25	25
Minimum	6.77	6.76	1.5	1.8	59.2	59.9	10.64	10.63
Maximum	8.13	8.15	59.2	59.4	134.3	133.8	27.79	27.62
Median	7.78	7.80	41.90	41.90	92.40	93.00	22.27	22.25
Mean	7.59	7.59	33.9	34.3	92.3	92.8	21.15	21.03
Standard Dev.	0.43	0.45	18.0	18.0	19.5	18.6	4.84	4.84

NS = Not sampled

Appendix 3F. (Continued)

SITE: 17

	pH		Elec. Cond. (mS cm^{-1})		Dissolved Oxygen (% Saturation)		Temperature (^{o}C)	
Date	**surface**	**bed**	**surface**	**bed**	**surface**	**bed**	**surface**	**bed**
17/11/97	8.09	8.12	46.8	49.6	81.2	81.7	20.50	19.50
04/12/97	8.10	8.09	49.7	50.3	58.7	61.6	23.10	23.10
20/03/98	NS	NS	NS	NS	NS	NS	NS	NS
25/03/98	7.85	7.85	48.3	48.3	NS	NS	24.37	24.37
27/03/98	8.00	8.07	50.8	51.3	NS	NS	23.26	23.42
02/04/98	7.92	7.91	41.6	41.6	NS	NS	22.59	22.58
17/04/98	7.87	7.87	42.3	42.4	NS	NS	22.66	22.61
27/04/98	7.86	7.95	35.4	39.4	NS	NS	21.80	21.71
04/05/98	6.89	6.85	2.1	2.0	NS	NS	20.78	20.71
15/05/98	7.20	7.17	20.7	21.7	78.8	78.0	13.61	13.58
02/06/98	7.23	7.27	23.6	25.3	81.0	80.4	13.05	12.95
05/06/98	6.74	6.57	1.2	2.7	93.8	93.9	10.43	10.63
21/07/98	7.72	7.82	36.6	37.0	80.0	80.2	17.84	17.80
31/07/98	NS	NS	NS	NS	NS	NS	NS	NS
10/08/98	7.72	7.75	45.3	45.4	90.0	91.3	16.51	16.47
17/08/98	7.42	7.23	8.8	13.9	102.6	104.5	15.35	15.42
31/08/98	6.84	6.75	1.9	2.8	99.9	100.2	16.19	15.66
15/09/98	6.87	6.73	0.9	2.0	92.7	92.9	19.82	19.66
01/10/98	7.40	7.41	48.6	52.8	100.9	105.3	20.60	19.70
16/10/98	7.11	7.11	43.7	43.7	84.9	86.8	24.27	24.20
09/11/98	7.64	7.65	46.4	46.3	65.5	63.9	22.28	22.27
07/12/98	7.74	7.81	58.5	59.9	100.8	100.1	28.41	27.15
25/01/99	7.72	7.74	47.3	46.9	80.4	90.9	27.68	27.17
04/02/99	7.79	7.87	23.6	26.5	95.1	96.1	27.09	26.83
04/03/99	7.98	7.99	28.0	28.2	130.8	128.7	27.73	27.74
30/03/99	7.58	7.55	29.1	31.1	137.0	131.2	24.48	24.37
Summary:								
N of cases	24	24	24	24	18	18	24	24
Minimum	6.74	6.57	0.9	2.0	58.7	61.6	10.43	10.63
Maximum	8.10	8.12	58.5	59.9	137.0	131.2	28.41	27.74
Median	7.72	7.75	39.10	40.50	91.35	92.10	22.04	21.99
Mean	7.55	7.55	32.6	33.8	91.9	92.7	21.02	20.82
Standard Dev.	0.42	0.48	18.2	18.0	19.4	18.3	4.89	4.79

NS = Not sampled

SITE: 18

	pH		Elec. Cond. (mS cm^{-1})		Dissolved Oxygen (% Saturation)		Temperature (^{o}C)	
Date	**surface**	**bed**	**surface**	**bed**	**surface**	**bed**	**surface**	**bed**
17/11/97	8.13	8.11	47.5	49.5	81.0	81.2	20.30	19.50
04/12/97	NS	NS	NS	NS	NS	NS	NS	NS
20/03/98	NS	NS	NS	NS	NS	NS	NS	NS
25/03/98	7.84	7.89	48.5	49.1	NS	NS	24.59	24.40
27/03/98	8.03	8.10	50.2	50.8	NS	NS	23.14	23.08
02/04/98	7.88	7.80	37.5	48.7	NS	NS	22.34	23.44
17/04/98	7.78	7.89	39.0	46.6	NS	NS	22.88	22.41
27/04/98	7.97	8.09	40.5	44.7	NS	NS	21.96	21.17
04/05/98	6.87	6.72	2.1	6.1	NS	NS	21.56	20.26
15/05/98	7.28	7.58	22.5	37.6	77.4	70.3	13.63	14.33
02/06/98	7.24	7.64	23.9	35.8	82.7	78.3	12.84	13.58
05/06/98	6.70	6.72	2.2	32.9	92.3	44.8	10.88	13.44
21/07/98	7.78	8.03	36.1	45.1	80.4	74.3	17.89	17.80
31/07/98	NS	NS	NS	NS	NS	NS	NS	NS
10/08/98	7.63	7.69	45.7	46.1	89.8	89.5	16.37	16.01
17/08/98	7.42	7.76	10.1	29.4	108.2	107.2	15.13	15.84
31/08/98	6.90	6.97	3.4	13.3	102.0	82.1	15.96	16.82
15/09/98	6.81	6.79	1.4	2.6	92.2	92.8	19.58	18.74
01/10/98	7.19	7.19	51.4	53.9	110.1	109.8	20.10	19.51
16/10/98	7.03	7.07	43.9	43.7	80.1	83.7	24.55	24.27
09/11/98	7.54	7.55	43.6	44.7	62.5	59.3	22.32	22.32
07/12/98	7.68	7.88	59.0	61.4	99.2	98.9	28.00	25.87
25/01/99	7.71	7.72	47.1	47.6	78.5	74.9	27.51	27.06
04/02/99	7.78	8.02	24.2	29.3	93.0	89.7	27.03	26.12
04/03/99	7.91	8.03	26.4	31.4	131.6	118.6	29.58	24.85
30/03/99	7.51	7.67	30.1	36.8	134.8	114.4	24.81	23.49
Summary:								
N of cases	23	23	23	23	17	17	23	23
Minimum	6.70	6.72	1.4	2.6	62.5	44.8	10.88	13.44
Maximum	8.13	8.11	59.0	61.4	134.8	118.6	29.58	27.06
Median	7.63	7.72	37.50	44.70	92.20	83.70	21.96	21.17
Mean	7.50	7.60	32.0	38.6	93.9	86.5	21.00	20.62
Standard Dev.	0.42	0.46	17.9	14.8	19.1	19.6	5.10	4.19

NS = Not sampled

Appendix 3F. (Continued)

SITE: 19

	pH		Elec. Cond. (mS cm^{-1})		Dissolved Oxygen (% Saturation)		Temperature (^{o}C)	
Date	**surface**	**bed**	**surface**	**bed**	**surface**	**bed**	**surface**	**bed**
17/11/97	NS	NS	NS	NS	NS	NS	NS	NS
04/12/97	NS	NS	NS	NS	NS	NS	NS	NS
20/03/98	7.80	7.80	49.0	49.0	NS	NS	27.78	26.57
25/03/98	7.86	7.87	48.3	48.8	NS	NS	24.46	24.45
27/03/98	8.00	8.03	50.1	50.1	NS	NS	23.24	23.17
02/04/98	7.83	7.81	36.5	48.7	NS	NS	22.57	23.51
17/04/98	7.75	7.89	37.3	46.4	NS	NS	22.91	22.52
27/04/98	7.98	8.08	40.0	45.3	NS	NS	21.95	21.20
04/05/98	6.83	7.43	2.0	3.2	NS	NS	21.39	20.96
15/05/98	7.16	7.53	23.6	38.9	75.0	64.9	13.35	14.54
02/06/98	7.24	7.62	23.5	37.4	80.1	75.8	12.63	13.79
05/06/98	6.31	6.87	1.6	26.5	93.3	94.6	11.03	12.90
21/07/98	7.71	8.11	37.1	47.6	79.7	69.8	18.02	18.04
31/07/98	7.72	7.69	26.3	30.6	92.1	90.8	13.06	12.97
10/08/98	7.49	7.56	44.7	45.4	88.2	87.2	16.48	15.73
17/08/98	7.07	7.85	7.7	31.8	106.7	97.1	15.37	15.89
31/08/98	6.68	6.70	3.1	27.0	102.4	20.8	15.93	17.41
15/09/98	6.78	6.73	1.3	38.8	92.7	31.4	19.65	18.78
01/10/98	6.90	6.92	53.2	54.1	111.4	111.6	19.57	19.41
16/10/98	6.92	7.01	43.4	44.1	82.2	74.4	25.00	22.43
09/11/98	7.54	7.53	43.7	46.1	65.4	61.7	22.61	23.02
07/12/98	7.68	7.81	59.3	60.6	94.5	97.9	27.74	26.56
25/01/99	7.66	7.69	45.8	47.8	77.1	85.3	27.75	26.95
04/02/99	7.78	7.80	23.6	24.3	89.9	95.6	27.00	27.01
04/03/99	7.98	8.04	27.6	29.2	133.8	128.6	29.58	27.09
30/03/99	7.58	7.67	30.0	37.5	143.9	122.3	24.80	23.22
Summary:								
N of cases	24	24	24	24	17	17	24	24
Minimum	6.31	6.70	1.3	3.2	65.4	20.8	11.03	12.90
Maximum	8.00	8.11	59.3	60.6	143.9	128.6	29.58	27.09
Median	7.62	7.69	36.80	44.70	92.10	87.20	22.26	21.82
Mean	7.43	7.59	31.6	40.0	94.6	82.9	20.99	20.76
Standard Dev.	0.48	0.43	17.8	12.3	20.4	28.3	5.46	4.72

NS = Not sampled

Appendix 3G. List of analytical data for surface and bed waters for Sites 1, 4, 12 and 19 located in Limeburners Creek and the lower Hastings River.

SITE: 1

Date	Measurement	Alkalinity ($mg\ L^{-1}$)	Cl:SO_4	Lab. pH	Fe ($mg\ L^{-1}$)	Al ($mg\ L^{-1}$)	Ca ($mg\ L^{-1}$)	Mn ($mg\ L^{-1}$)	K ($mg\ L^{-1}$)	Mg ($mg\ L^{-1}$)	S ($mg\ L^{-1}$)	SO_4 ($mg\ L^{-1}$)	As ($mg\ L^{-1}$)	Cu ($mg\ L^{-1}$)	Si ($mg\ L^{-1}$)	Zn ($mg\ L^{-1}$)
25/03/98	surface	85	12.0	7.81	<0.01	<0.05	347.67	<0.002	400.48	986.12	720.45	2161.34	<0.40	<0.004	<0.03	<0.01
02/04/98	surface	65	12.7	7.93	<0.01	<0.05	171.55	<0.002	178.50	500.80	507.66	1522.97	<0.40	<0.004	0.01	<0.01
02/04/98	bed	56	37.3	7.95	<0.01	<0.05	121.53	<0.002	148.94	351.38	277.88	833.64	<0.40	<0.004	<0.03	<0.01
17/04/98	surface	52	15.1	7.80	<0.01	<0.05	187.47	<0.002	209.63	560.89	499.34	1498.01	<0.40	<0.004	0.18	<0.01
17/04/98	bed	65	14.5	7.89	<0.01	<0.05	207.28	<0.002	220.46	596.52	571.14	1713.42	<0.40	<0.004	0.01	<0.01
27/04/98	surface	56	25.9	7.93	<0.01	<0.05	99.31	<0.002	101.06	291.11	313.73	941.18	<0.40	<0.004	<0.03	<0.01
27/04/98	bed	56	21.6	7.83	<0.01	<0.05	138.75	<0.002	153.52	418.90	443.11	1329.33	<0.40	<0.004	0.08	<0.01
04/05/98	surface	38	24.0	7.78	<0.01	<0.05	14.89	<0.002	14.40	44.52	61.94	185.81	<0.40	<0.004	<0.03	<0.01
04/05/98	bed	38	23.3	6.79	<0.01	<0.05	20.61	<0.002	22.91	67.52	80.26	240.77	<0.40	<0.004	<0.03	<0.01
20/05/98	surface	52	28.1	7.54	<0.01	<0.05	98.47	<0.002	98.36	287.31	191.95	575.84	<0.40	<0.004	0.16	<0.01
20/05/98	bed	56	NS	7.72	<0.01	<0.05	NS	<0.002	NS	NS	NS	NS	<0.40	<0.004	<0.03	<0.01
02/06/98	surface	60	12.5	7.71	<0.01	<0.05	287.41	<0.002	330.29	829.59	597.85	1793.55	<0.40	<0.004	0.87	<0.01
02/06/98	bed	73	9.4	7.71	<0.01	<0.05	298.38	<0.002	345.69	861.42	620.93	1862.80	<0.40	<0.004	0.76	<0.01
05/06/98	surface	65	7.3	7.47	<0.005	<0.03	265.99	0.002	271.87	796.27	503.28	1509.85	<0.15	0.018	1.36	<0.02
05/06/98	bed	56	7.9	7.60	<0.005	<0.03	283.83	0.001	291.21	849.08	500.36	1501.07	<0.15	0.006	0.77	<0.02
21/07/98	surface	80	9.1	7.98	<0.005	<0.03	469.44	0.001	508.09	1379.88	686.25	2058.75	<0.15	0.004	0.86	<0.02
31/07/98	surface	65	10.6	7.86	<0.005	<0.03	379.20	0.000	402.73	1114.03	647.95	1943.84	<0.15	0.003	0.75	<0.02
31/07/98	bed	60	10.4	7.90	<0.005	<0.03	379.42	0.000	403.91	1109.51	659.26	1977.78	<0.15	0.003	0.66	<0.02
10/08/98	surface	73	13.6	7.98	<0.005	<0.03	413.86	0.001	446.80	1206.11	641.02	1923.05	<0.15	0.006	0.42	<0.02
10/08/98	bed	76	12.8	7.94	<0.005	<0.03	385.67	0.000	410.86	1123.13	613.65	1840.94	<0.15	0.004	0.65	<0.02
17/08/98	surface	52	8.5	6.87	0.005	<0.03	103.89	0.001	93.96	295.67	128.21	384.64	<0.15	0.005	2.56	<0.02
17/08/98	bed	52	9.5	7.00	0.005	<0.03	140.62	0.001	133.62	412.15	221.66	664.97	<0.15	0.004	2.06	<0.02
31/08/98	surface	56	9.3	7.23	0.005	<0.03	182.85	0.001	179.04	538.65	298.94	896.83	<0.15	0.001	1.93	<0.02
31/08/98	bed	65	7.5	7.45	<0.005	<0.03	255.91	0.002	260.59	756.47	413.34	1240.01	<0.15	0.002	1.50	<0.02
16/10/98	surface	73	16.5	8.08	<0.005	<0.03	436.60	0.003	480.01	1279.14	650.63	1951.88	<0.15	0.003	0.28	<0.02
16/10/98	bed	60	16.9	8.09	<0.005	<0.03	492.57	0.004	545.57	1431.25	654.29	1962.86	<0.15	0.004	0.21	<0.02
07/12/98	surface	NS	8.5	7.89	<0.007	<0.05	285.87	<0.001	341.14	833.31	557.86	1673.58	<0.35	<0.005	0.53	<0.02
07/12/98	bed	NS	8.1	7.92	<0.007	<0.05	336.89	<0.001	402.07	974.50	657.90	1973.70	<0.35	<0.005	0.51	<0.02
04/01/99	surface	NS	8.1	7.81	<0.007	<0.05	197.28	<0.001	223.68	567.72	386.93	1160.80	<0.35	<0.005	2.33	<0.02
04/01/99	bed	NS	7.7	7.81	<0.007	<0.05	215.84	<0.001	246.85	624.41	418.91	1256.73	<0.35	<0.005	2.11	<0.02
25/01/99	surface	NS	8.8	7.96	<0.007	<0.05	265.37	<0.001	319.72	751.05	513.85	1541.55	<0.35	<0.005	0.93	<0.02
25/01/99	bed	NS	9.2	7.98	<0.007	<0.05	264.38	0.01	319.23	755.76	503.57	1510.71	<0.35	<0.005	0.82	<0.02
04/03/99	surface	NS	11.4	7.71	<0.007	<0.05	84.20	0.01	92.31	243.42	157.20	471.61	<0.35	<0.005	3.54	<0.02
30/03/99	surface	NS	5.3	8.21	<0.007	<0.05	251.19	<0.001	275.38	737.34	519.84	1559.52	<0.35	<0.005	1.51	<0.02
30/03/99	bed	NS	10.2	8.25	<0.007	<0.05	199.36	<0.001	228.85	584.72	379.26	1137.78	<0.35	<0.005	1.81	<0.02

NS = Not sampled

Appendix 3G.(Continued)

SITE: 4

Date	Measurement	Alkalinity ($mg\ L^{-1}$)	Cl:SO_4	Lab. pH	Fe ($mg\ L^{-1}$)	Al ($mg\ L^{-1}$)	Ca ($mg\ L^{-1}$)	Mn ($mg\ L^{-1}$)	K ($mg\ L^{-1}$)	Mg ($mg\ L^{-1}$)	S ($mg\ L^{-1}$)	SO_4 ($mg\ L^{-1}$)	As ($mg\ L^{-1}$)	Cu ($mg\ L^{-1}$)	Si ($mg\ L^{-1}$)	Zn ($mg\ L^{-1}$)
25/03/98	surface	76	10.1	7.93	<0.01	<0.05	179.91	<0.002	189.54	524.25	553.88	1661.65	<0.40	<0.004	0.02	<0.01
02/04/98	surface	56	8.7	7.96	<0.01	<0.05	205.47	<0.002	218.51	594.59	644.65	1933.95	<0.40	<0.004	0.18	<0.01
02/04/98	bed	52	14.9	7.95	<0.01	<0.05	200.86	<0.002	212.65	577.82	528.86	1586.58	<0.40	<0.004	0.08	<0.01
17/04/98	surface	60	11.0	7.82	<0.01	<0.05	268.00	<0.002	287.47	763.42	651.15	1953.46	<0.40	<0.004	0.38	<0.01
17/04/98	bed	52	9.5	7.85	<0.01	<0.05	214.71	<0.002	224.42	616.81	631.87	1895.62	<0.40	<0.004	0.44	<0.01
27/04/98	surface	60	15.5	7.71	<0.01	<0.05	130.71	<0.002	134.30	389.98	407.77	1223.32	<0.40	<0.004	0.26	<0.01
27/04/98	bed	60	28.8	7.79	<0.01	<0.05	74.22	<0.002	84.87	222.06	266.36	799.08	<0.40	<0.004	<0.03	<0.01
20/05/98	surface	56	11.1	7.70	<0.01	<0.05	189.23	<0.002	215.56	554.63	422.72	1268.17	<0.40	<0.004	0.43	<0.01
20/05/98	bed	56	53.3	7.68	0.04	0.12	40.27	<0.002	35.53	114.15	91.88	275.65	<0.40	<0.004	0.41	<0.01
02/06/98	surface	73	10.1	7.70	<0.01	<0.05	266.75	<0.002	302.53	768.22	565.07	1695.21	<0.40	<0.004	1.02	<0.01
02/06/98	bed	65	11.6	7.69	<0.01	<0.05	287.65	<0.002	329.78	827.44	601.77	1805.30	<0.40	<0.004	0.77	<0.01
21/07/98	surface	65	9.2	7.80	<0.005	<0.03	405.24	0.000	433.68	1193.77	658.00	1973.99	<0.15	0.003	1.13	<0.02
31/07/98	surface	73	10.8	8.10	<0.005	<0.03	322.86	0.001	336.28	946.97	551.65	1654.95	<0.15	0.008	0.71	<0.02
31/07/98	bed	76	11.1	7.81	<0.005	<0.03	329.00	0.000	344.10	965.26	570.08	1710.23	<0.15	0.006	0.96	<0.02
10/08/98	surface	76	12.5	7.84	<0.005	<0.03	361.00	0.001	380.71	1048.42	567.42	1702.26	0.031	0.006	0.85	<0.02
10/08/98	bed	60	12.6	7.95	<0.005	<0.03	416.32	0.001	447.04	1210.24	651.92	1955.77	<0.15	0.002	0.37	<0.02
17/08/98	surface	21	8.5	6.48	<0.005	<0.03	26.88	0.001	21.48	67.97	46.48	139.45	0.029	0.004	2.74	<0.02
17/08/98	bed	52	9.0	6.90	0.005	<0.03	97.49	0.001	89.58	283.16	167.38	502.14	0.014	0.008	2.35	<0.02
31/08/98	surface	56	8.9	7.41	<0.005	<0.03	155.65	0.001	153.16	459.48	256.12	768.36	0.025	0.006	2.06	<0.02
31/08/98	bed	56	7.6	7.35	0.007	<0.03	219.84	0.001	221.30	648.10	352.10	1056.31	<0.15	0.007	1.80	<0.02
15/09/98	surface	26	4.6	6.82	0.091	<0.03	66.13	0.004	60.26	192.30	113.79	341.38	0.023	0.006	2.83	<0.02
16/10/98	surface	52	16.9	8.07	<0.005	<0.03	421.20	0.007	458.91	1235.47	576.64	1729.92	<0.15	0.003	0.55	<0.02
16/10/98	bed	65	17.0	8.06	<0.005	<0.03	427.60	0.007	469.93	1251.11	597.83	1793.48	<0.15	0.004	0.57	<0.02
07/12/98	surface	NS	8.1	7.86	<0.007	0.01	303.73	0.01	359.27	864.90	583.72	1751.15	<0.35	<0.005	1.06	<0.02
07/12/98	bed	NS	7.9	7.98	0.01	<0.05	356.21	<0.001	433.05	1022.37	688.19	2064.56	<0.35	<0.005	0.37	<0.02
04/01/99	surface	NS	8.7	7.76	<0.007	<0.05	91.32	<0.001	103.46	266.15	216.97	650.92	<0.35	<0.005	2.50	<0.02
04/01/99	bed	NS	2.9	7.61	0.01	<0.05	296.12	<0.001	349.18	851.61	552.96	1658.88	<0.35	<0.005	1.17	<0.02
25/01/99	surface	NS	10.4	7.94	<0.007	0.01	165.33	<0.001	202.17	485.56	311.82	935.46	<0.35	<0.005	0.86	<0.02
25/01/99	bed	NS	10.1	7.61	<0.007	<0.05	218.97	<0.001	269.15	637.37	387.68	1163.03	<0.35	<0.005	0.90	<0.02
04/03/99	surface	NS	9.2	7.73	<0.007	<0.05	123.76	<0.001	135.16	361.60	237.67	713.00	<0.35	<0.005	3.06	<0.02
30/03/99	surface	NS	7.3	8.25	<0.007	0.03	250.83	0.01	275.72	735.65	499.62	1498.86	<0.35	<0.005	2.30	<0.02
30/03/99	bed	NS	7.6	8.22	<0.007	0.02	246.85	<0.001	277.53	711.16	491.83	1475.49	<0.35	<0.005	1.74	<0.02

NS = Not sampled

Appendix 3G.(Continued)

SITE: 12

Date	Measurement	ALK (mg L^{-1})	Cl:SO_4	Lab. pH	Fe (mg L^{-1})	Al (mg L^{-1})	Ca (mg L^{-1})	Mn (mg L^{-1})	K (mg L^{-1})	Mg (mg L^{-1})	S (mg L^{-1})	SO_4 (mg L^{-1})	As (mg L^{-1})	Cu (mg L^{-1})	Si (mg L^{-1})	Zn (mg L^{-1})
25/03/98	surface	56	12.0	7.95	<0.01	<0.05	374.91	<0.002	432.62	1059.07	749.03	2247.09	<0.40	<0.004	0.18	<0.01
02/04/98	surface	65	20.3	7.93	<0.01	<0.05	55.03	<0.002	64.17	164.36	237.33	711.98	<0.40	<0.004	<0.03	<0.01
02/04/98	bed	56	18.1	7.91	<0.01	<0.05	134.92	<0.002	163.64	392.76	364.75	1094.25	<0.40	<0.004	0.01	<0.01
17/04/98	surface	56	12.7	7.81	<0.01	<0.05	132.58	<0.002	138.29	386.72	432.27	1296.81	<0.40	<0.004	0.20	<0.01
17/04/98	bed	47	16.7	7.92	<0.01	<0.05	140.86	<0.002	146.91	411.14	448.23	1344.68	<0.40	<0.004	0.07	<0.01
27/04/98	surface	56	35.4	7.71	<0.01	<0.05	57.80	<0.002	57.94	172.23	205.66	616.98	<0.40	<0.004	<0.03	<0.01
27/04/98	bed	56	20.8	7.71	<0.01	<0.05	86.48	<0.002	85.23	254.84	308.42	925.27	<0.40	<0.004	0.08	<0.01
04/05/98	surface	13	36.3	6.63	<0.01	<0.05	12.16	<0.002	12.93	34.99	27.41	82.24	<0.40	<0.004	<0.03	<0.01
04/05/98	bed	8	12.8	6.37	<0.01	<0.05	20.40	<0.002	19.69	57.14	43.38	130.13	<0.40	<0.004	0.16	<0.01
15/05/98	surface	73	21.1	7.39	<0.01	<0.05	100.36	<0.002	103.21	299.47	229.13	687.38	<0.40	<0.004	0.81	<0.01
15/05/98	bed	52	55.7	7.44	<0.01	<0.05	25.97	<0.002	26.05	77.02	72.30	216.90	<0.40	<0.004	<0.03	<0.01
02/06/98	surface	65	9.1	7.45	<0.01	<0.05	209.29	<0.002	228.84	610.92	448.84	1346.51	<0.40	<0.004	0.95	<0.01
02/06/98	bed	52	8.8	7.32	<0.01	<0.05	218.36	<0.002	240.02	637.08	465.81	1397.43	<0.40	<0.004	0.92	<0.01
21/07/98	surface	65	9.0	7.74	<0.005	<0.03	413.91	0.001	441.97	1222.33	608.21	1824.64	<0.15	0.011	0.28	<0.02
31/07/98	surface	76	9.5	7.54	<0.005	<0.03	339.50	0.001	355.63	995.29	561.97	1685.91	<0.15	0.003	0.82	<0.02
31/07/98	bed	65	9.9	7.53	<0.005	<0.03	367.43	0.001	388.22	1076.61	557.99	1673.98	<0.15	0.003	0.83	<0.02
10/08/98	surface	73	12.0	7.90	<0.005	<0.03	400.35	0.001	426.15	1169.48	617.98	1853.93	<0.15	0.002	0.52	<0.02
10/08/98	bed	76	12.4	7.89	<0.005	<0.03	389.73	0.001	414.28	1139.40	622.59	1867.76	<0.15	0.001	0.55	<0.02
17/08/98	surface	52	12.6	6.99	0.048	<0.03	121.89	0.001	119.24	357.09	171.72	515.15	<0.15	0.010	1.02	<0.02
17/08/98	bed	52	9.8	6.96	0.051	<0.03	116.76	0.001	113.47	342.68	194.78	584.34	<0.15	0.006	1.02	<0.02
31/08/98	surface	42	10.6	6.91	0.259	<0.03	42.31	0.001	38.71	119.05	69.01	207.02	<0.15	0.006	1.27	<0.02
31/08/98	bed	38	9.2	6.83	0.309	<0.03	45.92	0.001	42.08	128.67	78.28	234.83	<0.15	0.004	1.33	<0.02
15/09/98	surface	13	3.5	6.73	0.331	<0.03	23.54	0.001	21.71	61.99	37.52	112.55	<0.15	0.006	1.53	<0.02
16/10/98	surface	52	14.4	7.75	<0.005	<0.03	366.75	0.009	391.62	1073.33	560.09	1680.28	<0.15	0.005	0.85	<0.02
16/10/98	bed	73	16.2	7.78	<0.005	<0.03	369.34	0.009	391.77	1080.78	518.23	1554.68	<0.15	0.004	0.80	<0.02
07/12/98	surface	NS	6.6	7.80	<0.007	<0.05	294.19	<0.001	336.14	839.10	581.63	1744.88	<0.35	<0.005	1.07	<0.02
07/12/98	bed	NS	7.2	7.77	<0.007	<0.05	295.34	<0.001	341.02	855.08	578.85	1736.54	<0.35	<0.005	1.09	<0.02
04/01/99	surface	NS	8.6	7.56	<0.007	<0.05	177.07	<0.001	204.61	508.03	339.21	1017.64	<0.35	<0.005	1.39	<0.02
04/01/99	bed	NS	8.8	7.72	<0.007	<0.05	213.63	<0.001	247.10	612.69	408.22	1224.65	<0.35	<0.005	1.49	<0.02
25/01/99	surface	NS	8.4	7.82	<0.007	<0.05	296.52	<0.001	361.60	859.61	559.86	1679.58	<0.35	<0.005	0.99	<0.02
25/01/99	bed	NS	8.2	7.80	<0.007	<0.05	321.59	<0.001	387.92	924.23	597.85	1793.54	<0.35	<0.005	1.07	<0.02
04/03/99	surface	NS	10.4	7.72	0.01	<0.05	147.20	<0.001	168.91	431.34	283.93	851.80	<0.35	<0.005	1.32	<0.02
30/03/99	surface	NS	12.5	8.14	<0.007	<0.05	139.86	0.01	158.01	406.96	256.32	768.97	<0.35	<0.005	1.41	<0.02
30/03/99	bed	NS	8.7	8.11	0.01	<0.05	203.79	0.01	224.35	578.86	374.20	1122.59	<0.35	<0.005	2.05	<0.02

NS = Not sampled

Appendix 3G.(Continued)

SITE: 19

Date	Measurement	Alkalinity (mg L^{-1})	Cl:SO_4	Lab. pH	Fe (mg L^{-1})	Al (mg L^{-1})	Ca (mg L^{-1})	Mn (mg L^{-1})	K (mg L^{-1})	Mg (mg L^{-1})	S (mg L^{-1})	SO_4 (mg L^{-1})	As (mg L^{-1})	Cu (mg L^{-1})	Si (mg L^{-1})	Zn (mg L^{-1})
25/03/98	surface	76	11.1	7.65	<0.01	<0.05	353.57	<0.002	398.31	999.31	745.70	2237.11	<0.40	<0.004	0.63	<0.01
02/04/98	surface	47	10.9	7.67	<0.01	<0.05	74.98	<0.002	75.85	224.90	295.99	887.96	<0.40	<0.004	<0.03	<0.01
02/04/98	bed	52	11.6	7.68	<0.01	<0.05	210.65	<0.002	231.93	605.05	579.84	1739.51	<0.40	<0.004	0.30	<0.01
17/04/98	surface	60	29.8	7.75	<0.01	<0.05	75.42	<0.002	76.04	222.00	196.92	590.76	<0.40	<0.004	0.04	<0.01
17/04/98	bed	60	15.6	7.66	<0.01	<0.05	134.55	<0.002	136.04	393.92	398.52	1195.56	<0.40	<0.004	0.15	<0.01
27/04/98	surface	47	25.2	7.61	<0.01	<0.05	72.82	<0.002	72.11	215.53	262.82	788.45	<0.40	<0.004	0.01	<0.01
27/04/98	bed	52	22.1	7.71	<0.01	<0.05	75.75	<0.002	79.78	234.00	302.99	908.97	<0.40	<0.004	<0.03	<0.01
04/05/98	surface	26	11.7	6.84	0.08	<0.05	13.62	<0.002	13.35	34.94	30.19	90.57	<0.40	<0.004	0.25	<0.01
04/05/98	bed	60	8.9	7.69	<0.01	<0.05	113.44	<0.002	173.64	493.93	487.16	1461.48	<0.40	<0.004	0.46	<0.01
15/05/98	surface	47	16.7	7.37	<0.01	<0.05	66.27	<0.002	62.38	194.02	179.54	538.62	<0.40	<0.004	0.31	<0.01
15/05/98	bed	47	21.6	7.31	<0.01	<0.05	71.60	<0.002	70.25	209.71	174.53	523.58	<0.40	<0.004	0.15	<0.01
02/06/98	surface	56	8.8	7.27	<0.01	<0.05	157.32	<0.002	163.71	451.40	325.51	976.52	<0.40	<0.004	1.19	<0.01
02/06/98	bed	76	9.4	7.30	<0.01	<0.05	287.20	<0.002	328.97	829.33	589.90	1769.71	<0.40	<0.004	0.82	<0.01
05/06/98	surface	26	14.4	6.74	0.166	0.044	14.41	0.002	13.32	34.44	22.18	66.53	<0.15	0.009	2.04	<0.02
05/06/98	bed	90	8.0	7.43	0.004	<0.03	327.93	0.001	338.34	981.72	590.31	1770.93	<0.15	0.002	1.19	<0.02
21/07/98	surface	60	8.7	7.80	0.003	<0.03	327.90	0.001	340.01	969.80	572.84	1718.53	<0.15	0.017	0.28	<0.02
31/07/98	surface	65	9.4	7.31	0.056	<0.03	204.88	0.001	203.69	601.79	369.73	1109.18	<0.15	0.011	1.38	<0.02
31/07/98	bed	65	8.0	7.48	0.018	<0.03	256.12	0.000	260.58	754.78	464.32	1392.96	<0.15	0.005	0.98	<0.02
10/08/98	surface	60	11.2	7.69	<0.005	<0.03	366.57	0.001	388.33	1071.79	608.63	1825.88	<0.15	0.006	0.56	<0.02
10/08/98	bed	65	12.3	7.77	<0.005	<0.03	354.02	0.000	371.40	1033.26	590.39	1771.17	<0.15	0.006	0.56	<0.02
17/08/98	surface	38	10.2	6.88	0.065	<0.03	74.36	0.000	73.16	218.47	131.58	394.73	<0.15	0.007	1.28	<0.02
17/08/98	bed	65	11.9	7.49	0.002	<0.03	360.01	0.000	375.80	1046.09	485.63	1456.88	<0.15	0.003	0.78	<0.02
31/08/98	surface	30	9.3	6.78	0.334	<0.03	33.23	0.001	29.69	89.19	42.23	126.70	<0.15	0.005	1.31	<0.02
31/08/98	bed	76	9.8	7.11	0.025	<0.03	187.58	0.001	182.18	547.28	299.28	897.84	<0.15	0.001	1.66	<0.02
15/09/98	surface	21	8.6	6.73	0.402	0.037	12.14	0.001	10.87	27.22	15.18	45.54	<0.15	0.003	1.34	<0.02
16/10/98	surface	52	12.6	7.47	0.001	<0.03	349.98	0.014	362.61	1021.41	525.97	1577.91	<0.15	0.005	0.97	<0.02
16/10/98	bed	52	14.9	7.53	<0.005	<0.03	437.23	0.013	464.76	1272.93	523.95	1571.84	<0.15	0.004	0.87	<0.02
07/12/98	surface	NS	6.3	7.62	0.01	<0.05	263.32	0.01	284.72	755.32	533.02	1599.06	<0.35	<0.005	1.25	<0.02
07/12/98	bed	NS	6.7	7.66	0.00	<0.05	271.03	<0.001	302.01	776.90	524.73	1574.19	<0.35	<0.005	1.33	<0.02
04/01/99	surface	NS	9.3	7.55	0.01	<0.05	149.32	<0.001	166.53	435.39	303.16	909.47	<0.35	<0.005	1.61	<0.02
04/01/99	bed	NS	8.4	7.48	0.01	<0.05	152.78	<0.001	168.96	440.31	310.58	931.75	<0.35	<0.005	1.70	<0.02
25/01/99	surface	NS	12.8	7.69	0.00	<0.05	143.98	<0.001	159.28	408.49	284.71	854.14	<0.35	<0.005	2.50	<0.02
25/01/99	bed	NS	7.4	7.72	0.00	<0.05	323.90	<0.001	384.92	926.15	613.77	1841.30	<0.35	<0.005	1.18	<0.02
04/03/99	surface	NS	10.8	7.61	0.02	<0.05	146.14	<0.001	169.84	430.57	273.32	819.96	<0.35	<0.005	1.28	<0.02
30/03/99	surface	NS	9.7	7.94	0.01	<0.05	168.50	0.01	186.04	482.80	301.31	903.92	<0.35	<0.005	2.03	<0.02
30/03/99	bed	NS	9.4	8.09	0.00	<0.05	173.09	0.01	191.95	498.35	320.40	961.20	<0.35	<0.005	1.99	<0.02

NS = Not sampled

Appendix 3H. Experiment 1 (Chapter 4) water quality data for Sites 1 to 7.

SITE: 1

DATE	pH	DO (% Sat.)	EC (dS m^{-1})	Temp (^{O}C)	Alkalinity (mg L^{-1})	NO_2 - N (mg L^{-1})	PO_4 - P (mg L^{-1})	NH_3 - N (mg L^{-1})	NO_3 -N (mg L^{-1})	Cl:SO_4	Al (mg L^{-1})	As (mg L^{-1})	Ca (mg L^{-1})	Cu (mg L^{-1})	Fe (mg L^{-1})	K (mg L^{-1})	Mg (mg L^{-1})	Mn (mg L^{-1})	Na (mg L^{-1})	S (mg L^{-1})	Si (mg L^{-1})	Zn (mg L^{-1})
01/06/99	8.04	121.9	44.4	19.91	73	0.000	0.26	0.19	0.085	11.4	<0.05	<0.35	298.12	<0.005	<0.007	341.81	862.23	<0.001	sat.	603.16	1.29	<0.02
15/06/99	7.41	101.2	41.4	14.52	85	0.000	0.12	0.63	0.040	11.5	<0.05	<0.35	241.15	<0.005	<0.007	274.89	692.64	<0.001	sat.	483.72	1.27	<0.02
28/06/99	8.25	95.3	21.0	18.05	76	0.000	0.46	0.15	0.076	38.3	<0.07	<0.17	110.69	0.01	<0.01	114.52	318.77	0.03	sat.	194.11	2.70	<0.16
12/07/99	7.69	102.1	24.9	17.31	65	0.004	0.54	0.38	0.040	2.6	<0.07	<0.17	137.43	0.05	<0.01	155.46	403.19	0.05	148.54	286.95	2.34	<0.16
26/07/99	8.74	129.9	14.5	15.77	65	0.014	0.28	0.30	0.120	10.0	<0.04	<0.11	105.49	<0.007	<0.17	131.79	307.19	<0.002	sat.	247.25	3.56	<0.02
09/08/99	8.80	136.7	32.3	17.96	73	0.004	0.16	0.03	0.250	11.0	<0.04	<0.11	127.89	<0.007	<0.17	181.34	389.72	0.04	sat.	316.37	1.59	<0.02
23/08/99	8.02	117.8	30.4	18.67	60	0.003	0.06	0.36	0.055	13.5	<0.04	<0.11	145.63	<0.007	<0.17	190.43	444.92	0.00	sat.	371.09	2.22	<0.02
06/09/99	7.68	68.8	43.2	18.66	65	0.000	0.12	0.25	0.180	11.0	<0.04	<0.11	201.47	<0.007	<0.17	256.67	561.25	0.06	sat.	527.53	1.65	<0.02
01/10/99	8.00	95.1	30.9	18.69	80	0.000	0.00	0.11	0.200	11.4	<0.04	<0.11	193.75	<0.007	<0.17	238.42	574.23	0.03	sat.	529.37	1.77	<0.02
15/11/99	7.53	45.2	17.0	20.26	76	0.000	0.10	0.04	0.470	18.3	0.07	<0.12	112.26	<0.06	0.03	119.22	344.04	0.01	sat.	264.77	4.83	<0.11
10/01/00	7.93	78.0	34.1	22.62	80	0.003	0.10	0.03	0.190	2.6	<0.02	<0.09	302.000	<0.006	<0.04	414.000	506.000	0.159	sat.	998.000	1.200	<0.04

SITE: 2

DATE	pH	DO (% Sat.)	EC (dS m^{-1})	Temp (^{O}C)	Alkalinity (mg L^{-1})	NO_2 - N (mg L^{-1})	PO_4 - P (mg L^{-1})	NH_3 - N (mg L^{-1})	NO_3 -N (mg L^{-1})	Cl:SO_4	Al (mg L^{-1})	As (mg L^{-1})	Ca (mg L^{-1})	Cu (mg L^{-1})	Fe (mg L^{-1})	K (mg L^{-1})	Mg (mg L^{-1})	Mn (mg L^{-1})	Na (mg L^{-1})	S (mg L^{-1})	Si (mg L^{-1})	Zn (mg L^{-1})
01/06/99	7.92	115.9	31.4	19.14	73	0.003	0.26	0.26	0.085	12.5	<0.05	<0.35	178.97	<0.005	<0.007	196.40	519.49	0.01	sat.	361.72	2.33	<0.02
15/06/99	-	-	-	-	80	0.004	0.16	0.11	0.110	14.5	<0.05	<0.35	158.82	<0.005	<0.007	179.72	462.73	0.02	sat.	308.21	1.84	<0.02
28/06/99	7.29	91.2	13.4	16.59	76	0.007	1.09	0.23	0.080	6.2	<0.07	<0.17	102.60	0.01	<0.01	105.65	291.62	0.04	sat.	175.51	3.19	<0.16
12/07/99	7.21	119.1	16.2	17.22	56	0.025	0.40	0.71	0.072	2.9	<0.07	<0.17	82.51	0.02	<0.01	85.34	237.00	0.05	sat.	161.71	3.41	<0.16
26/07/99	8.27	129.2	7.8	14.99	52	0.011	0.38	0.16	0.012	6.0	0.10	<0.11	83.39	0.01	<0.17	100.39	228.95	0.02	sat.	175.57	6.45	<0.02
09/08/99	8.65	136.7	24.4	17.38	76	0.004	0.14	0.02	0.080	7.8	<0.04	<0.11	211.23	0.01	<0.17	289.63	632.80	0.08	sat.	521.63	4.41	<0.02
23/08/99	7.99	123.5	21.5	18.36	76	0.001	0.20	0.19	0.000	12.7	<0.04	<0.11	137.20	<0.007	<0.17	172.45	400.30	0.03	sat.	340.24	2.72	<0.02
06/09/99	7.63	113.5	21.5	20.49	65	0.000	0.22	0.47	0.350	7.0	<0.04	<0.11	255.15	<0.007	<0.17	324.30	732.09	0.08	sat.	693.84	3.09	<0.02
01/10/99	7.93	106.0	15.9	20.46	65	0.003	0.44	0.06	0.350	9.2	<0.04	<0.11	101.55	<0.007	<0.17	115.82	295.30	0.02	sat.	352.30	3.31	<0.02
15/11/99	7.53	87.5	5.9	20.18	60	0.003	0.14	0.63	1.000	26.4	0.06	<0.12	48.34	<0.06	0.02	46.07	139.05	0.06	1069.93	99.66	6.24	0.04
10/01/00	7.67	77.0	29.1	23.00	90	0.004	0.14	0.04	0.220	2.8	0.040	<0.09	159.000	<0.006	<0.04	222.000	360.000	0.072	2350.000	690.000	0.979	<0.04

Appendix 3H.(Continued).

SITE: 3

DATE	pH	DO (% Sat.)	EC (dS m^{-1})	Temp (^{o}C)	Alkalinity (mg L^{-1})	NO_2 - N (mg L^{-1})	PO_4 - P (mg L^{-1})	NH_3 - N (mg L^{-1})	NO_3 -N (mg L^{-1})	Cl:SO_4	Al (mg L^{-1})	As (mg L^{-1})	Ca (mg L^{-1})	Cu (mg L^{-1})	Fe (mg L^{-1})	K (mg L^{-1})	Mg (mg L^{-1})	Mn (mg L^{-1})	Na (mg L^{-1})	S (mg L^{-1})	Si (mg L^{-1})	Zn (mg L^{-1})
01/06/99	7.89	114.2	40.0	19.31	65	0.027	0.28	0.17	0.018	13.5	<0.05	<0.35	215.35	<0.005	<0.007	247.17	629.44	<0.001	sat.	434.03	1.37	<0.02
15/06/99	8.14	141.4	39.7	15.66	80	0.006	0.07	0.44	0.059	18.8	<0.05	<0.35	147.39	<0.005	<0.007	171.42	430.11	0.01	sat.	329.92	0.81	<0.02
28/06/99	8.12	94.9	19.7	17.53	65	0.003	0.16	0.11	0.068	14.6	<0.07	<0.17	105.27	0.00	<0.01	109.94	301.80	0.04	sat.	176.68	2.72	<0.16
12/07/99	7.95	140.4	25.7	18.00	73	0.004	0.18	0.11	0.072	1.8	<0.07	<0.17	155.12	0.01	<0.01	168.62	453.78	0.05	sat.	299.71	3.16	<0.16
26/07/99	8.61	126.6	13.8	15.62	65	0.016	0.38	0.11	0.150	6.4	0.06	<0.11	139.90	<0.007	<0.17	187.66	406.44	<0.002	sat.	321.20	6.61	<0.02
09/08/99	9.04	174.1	35.4	19.48	73	0.003	0.14	0.02	0.080	16.1	<0.04	<0.11	164.83	<0.007	<0.17	230.40	479.16	<0.002	sat.	393.59	1.67	<0.02
23/08/99	7.93	109.7	28.0	18.03	80	0.000	0.14	0.11	0.170	8.7	<0.04	<0.11	262.52	<0.007	<0.17	350.52	768.11	0.04	sat.	651.25	3.19	<0.02
06/09/99	7.64	78.9	38.6	18.82	60	0.000	0.20	0.05	0.063	9.5	<0.04	<0.11	244.88	<0.007	<0.17	319.04	711.07	<0.002	sat.	640.66	1.97	<0.02
01/10/99	7.78	71.1	32.0	18.43	65	0.000	0.10	0.03	0.072	9.3	<0.04	<0.11	204.85	<0.007	<0.17	260.25	578.89	0.19	sat.	583.20	1.53	<0.02
15/11/99	7.59	62.3	14.2	18.83	80	0.001	0.12	0.00	0.500	19.2	0.03	<0.12	92.53	<0.06	0.02	98.51	283.22	0.04	0.00	206.02	4.58	<0.11
10/01/00	7.88	75.6	34.7	22.75	73	0.006	0.12	0.03	0.150	8.9	<0.02	<0.09	267.000	<0.006	0.042	402.000	470.000	0.121	2530.000	543.000	1.010	<0.04

SITE: 4

DATE	pH	DO (% Sat.)	EC (dS m^{-1})	Temp (^{o}C)	Alkalinity (mg L^{-1})	NO_2 - N (mg L^{-1})	PO_4 - P (mg L^{-1})	NH_3 - N (mg L^{-1})	NO_3 -N (mg L^{-1})	Cl:SO_4	Al (mg L^{-1})	As (mg L^{-1})	Ca (mg L^{-1})	Cu (mg L^{-1})	Fe (mg L^{-1})	K (mg L^{-1})	Mg (mg L^{-1})	Mn (mg L^{-1})	Na (mg L^{-1})	S (mg L^{-1})	Si (mg L^{-1})	Zn (mg L^{-1})
05/05/99	6.38	97.5	22.9	19.47	-	-	-	-	-	3.5	<0.05	<0.35	132.36	<0.005	<0.007	116.44	351.58	1.03	sat.	287.02	1.04	<0.02
01/06/99	7.51	99.2	29.5	18.62	85	0.006	0.30	0.19	0.085	7.2	<0.05	<0.35	209.71	<0.005	<0.007	230.36	615.81	0.02	sat.	420.67	2.83	<0.02
04/06/99	5.09	81.7	23.6	18.51	-	-	-	-	-	2.2	2.04	<0.35	133.17	<0.005	0.32	103.48	333.85	1.67	sat.	383.95	8.53	<0.02
15/06/99	7.58	96.2	32.3	13.75	76	0.000	0.20	0.57	0.043	11.4	<0.05	<0.35	132.86	<0.005	<0.007	151.06	385.51	0.03	sat.	259.36	1.46	<0.02
28/06/99	5.52	46.9	1.6	15.86	21	0.007	0.54	0.51	0.089	0.4	0.15	<0.17	39.79	0.01	<0.01	19.70	53.47	1.17	256.32	74.97	0.45	<0.16
05/07/99	3.71	9.6	3.6	14.78	-	-	-	-	-	0.1	5.09	<0.17	70.28	0.01	0.28	20.51	96.51	2.61	445.53	157.82	11.77	0.09
12/07/99	5.28	43.3	1.8	17.10	0	0.006	0.38	1.00	0.032	0.3	<0.07	<0.17	46.78	0.01	<0.01	26.73	75.49	1.41	422.81	100.19	1.09	0.05
26/07/99	4.03	66.0	5.2	14.59	8	0.032	0.34	0.45	0.028	2.1	9.71	<0.11	169.12	0.04	3.95	70.59	275.43	8.34	1327.57	480.36	26.64	0.26
03/08/99	4.26	67.1	4.2	12.71	26					4.9	2.41	<0.12	76.16	<0.06	1.75	22.33	104.82	2.67	527.13	225.32	8.01	0.03
09/08/99	3.51	101.4	10.7	17.84	26	0.001	0.26	0.06	0.055	3.4	9.95	<0.11	216.45	<0.007	25.95	137.58	446.73	6.00	sat.	659.12	29.30	0.22
23/08/99	7.63	113.2	22.6	17.86	65	0.001	1.30	0.00	0.000	8.9	<0.04	<0.11	222.76	<0.007	<0.17	280.51	622.97	0.11	sat.	539.58	4.08	<0.02
24/08/99	6.49	98.7	20.8	18.60	-	-	-	-	-	6.1	<0.04	<0.11	155.68	<0.007	<0.17	165.76	428.62	0.61	sat.	600.31	2.84	<0.02
06/09/99	4.93	110.1	15.9	20.68	13	0.000	0.07	0.03	-	10.2	1.60	<0.11	123.31	<0.007	1.04	101.20	297.03	1.95	sat.	445.87	5.61	<0.02
10/09/99	5.49	83.2	19.2	18.13	42	-	-	-	-	11.1	0.15	<0.12	134.62	<0.06	0.17	116.07	376.24	1.60	sat.	382.23	1.64	<0.11
30/09/99	7.54	87.6	22.6	18.91	73	-	-	-	-	14.5	0.04	<0.12	165.48	<0.06	<0.01	172.27	498.56	0.31	sat.	400.74	2.25	0.10
01/10/99	7.39	61.2	18.9	19.91	60	0.001	0.30	0.06	0.130	11.0	<0.04	<0.11	91.24	<0.007	<0.17	97.91	257.92	0.17	sat.	310.57	2.24	<0.02
05/11/99	7.06	112.0	15.6	24.06	80	-	-	-	-	19.4	0.04	<0.12	114.40	<0.06	<0.01	116.58	341.60	0.05	sat.	248.05	5.47	<0.11
15/11/99	6.70	77.2	6.8	20.22	42	0.001	0.06	0.00	0.600	17.2	0.06	<0.12	62.26	<0.06	0.02	51.17	157.72	0.74	1124.95	152.92	4.55	<0.11
14/12/99	7.47	71.5	14.4	22.35	-	-	-	-	-	8.1	<0.02	<0.09	197.000	<0.006	<0.04	263.000	409.000	0.114	2430.00	397.000	1.140	<0.04
10/01/00	7.81	78.1	27.9	23.21	76	0.001	0.16	0.03	0.110	2.3	<0.02	<0.09	174.000	<0.006	<0.04	237.000	374.000	0.003	2330.00	776.000	1.580	<0.04
26/01/00	7.36	59.3	24.7	22.42	76	-	-	-	-	8.3	<0.02	<0.09	165.000	<0.006	0.060	215.000	371.000	0.005	sat.	377.000	0.919	<0.04

Appendix 3H.(Continued).

SITE: 5

DATE	pH	DO (% Sat.)	EC (dS m^{-1})	Temp (^{O}C)	Alkalinity (mg L^{-1})	NO_2 - N (mg L^{-1})	PO_4 - P (mg L^{-1})	NH_3 - N (mg L^{-1})	NO_3 -N (mg L^{-1})	Cl:SO_4	Al (mg L^{-1})	As (mg L^{-1})	Ca (mg L^{-1})	Cu (mg L^{-1})	Fe (mg L^{-1})	K (mg L^{-1})	Mg (mg L^{-1})	Mn (mg L^{-1})	Na (mg L^{-1})	S (mg L^{-1})	Si (mg L^{-1})	Zn (mg L^{-1})
01/06/99	5.21	71.5	4.1	15.48	8	0.003	0.06	0.10	0.009	14.3	0.24	<0.35	28.62	<0.005	0.72	30.29	82.55	0.23	700.98	67.65	3.97	<0.02
15/06/99	4.93	62.9	3.5	12.23	65	0.001	0.02	0.30	0.015	24.0	0.51	<0.35	13.15	<0.005	0.62	16.94	38.09	0.11	338.58	30.71	2.36	<0.02
28/06/99	5.54	33.5	1.4	15.23	0	0.003	0.12	0.06	0.043	0.6	<0.07	<0.17	11.17	0.01	<0.01	11.66	28.36	0.14	243.60	20.78	0.53	<0.16
12/07/99	4.73	69.9	1.4	15.73	8	0.001	0.08	0.10	0.059	0.5	0.08	<0.17	5.56	0.01	<0.01	6.20	14.03	0.11	108.47	12.30	1.37	<0.16
26/07/99	4.45	98.3	0.3	14.13	0	0.007	0.46	0.03	0.006	2.9	2.02	<0.11	9.45	0.06	0.42	6.45	18.39	0.45	128.41	32.95	4.27	0.08
09/08/99	5.47	104.5	3.3	16.46	26	0.000	0.26	0.26	0.160	18.1	0.36	<0.11	16.44	0.01	0.47	11.63	44.34	0.24	333.93	52.41	1.36	<0.02
23/08/99	5.85	85.9	3.9	17.40	13	0.001	3.20	0.00	0.032	15.3	<0.04	<0.11	22.98	<0.007	<0.17	20.75	69.22	0.14	570.89	86.44	0.54	<0.02
06/09/99	6.06	96.3	6.7	18.45	13	0.000	0.10	0.02	0.170	26.1	<0.04	<0.11	19.61	<0.007	<0.17	17.42	58.33	0.12	515.68	87.81	0.27	<0.02
01/10/99	7.36	79.1	26.8	19.87	56	0.000	0.12	0.05	0.120	9.4	<0.04	<0.11	169.58	<0.007	<0.17	202.17	493.60	0.13	sat.	495.73	1.52	<0.02
15/11/99	4.73	37.7	1.4	18.95	8	0.001	0.08	0.06	0.089	20.3	1.09	<0.12	11.89	<0.06	0.09	11.14	33.43	0.29	247.44	35.64	5.40	0.19
10/01/00	6.49	60.5	21.4	22.01	56	0.000	0.02	0.22	0.120	9.7	0.047	<0.09	146.000	<0.006	<0.04	198.000	356.000	0.261	2020.000	293.000	0.951	<0.04

SITE: 6

DATE	pH	DO (% Sat.)	EC (dS m^{-1})	Temp (^{O}C)	Alkalinity (mg L^{-1})	NO_2 - N (mg L^{-1})	PO_4 - P (mg L^{-1})	NH_3 - N (mg L^{-1})	NO_3 -N (mg L^{-1})	Cl:SO_4	Al (mg L^{-1})	As (mg L^{-1})	Ca (mg L^{-1})	Cu (mg L^{-1})	Fe (mg L^{-1})	K (mg L^{-1})	Mg (mg L^{-1})	Mn (mg L^{-1})	Na (mg L^{-1})	S (mg L^{-1})	Si (mg L^{-1})	Zn (mg L^{-1})
01/06/99	6.93	81.3	17.4	18.62	52	0.011	0.12	0.23	0.063	10.6	<0.05	<0.35	121.01	<0.005	<0.007	121.46	348.35	0.23	sat.	246.86	3.85	<0.02
15/06/99	7.01	84.4	21.7	14.73	26	0.006	0.12	0.34	0.240	7.9	<0.05	<0.35	143.22	<0.005	<0.007	149.50	412.01	0.16	sat.	293.86	3.90	<0.02
28/06/99	6.30	66.8	4.2	15.13	8	0.009	0.22	0.63	0.059	0.8	<0.07	<0.17	29.48	0.01	<0.01	27.79	78.25	0.15	652.03	48.76	1.14	<0.16
12/07/99	5.51	82.8	6.5	15.98	21	0.000	0.08	0.00	0.047	9.7	<0.07	<0.17	45.69	0.00	<0.01	41.59	123.92	0.38	987.99	92.37	1.18	<0.16
26/07/99	6.39	116.2	2.3	14.56	21	0.006	0.64	0.14	0.068	5.9	<0.04	<0.11	16.37	0.02	<0.17	14.25	41.79	0.23	355.28	49.86	0.90	<0.02
09/08/99	6.89	103.5	11.2	16.77	52	0.004	0.14	0.30	0.110	6.2	<0.04	<0.11	136.46	0.01	<0.17	155.56	381.26	0.61	sat.	343.40	3.24	<0.02
23/08/99	6.84	108.9	19.3	17.83	52	0.003	0.26	0.06	0.700	13.6	<0.04	<0.11	108.15	<0.007	<0.17	123.45	318.68	0.20	sat.	294.39	1.38	<0.02
06/09/99	6.23	90.9	12.9	20.66	30	0.000	0.12	0.06	0.170	7.9	<0.04	<0.11	153.33	<0.007	<0.17	173.72	438.85	0.50	sat.	411.69	1.36	<0.02
01/10/99	6.99	76.7	15.7	19.87	60	0.004	0.07	0.05	0.170	9.9	<0.04	<0.11	134.19	<0.007	<0.17	152.44	387.54	0.22	sat.	358.68	3.98	<0.02
15/11/99	6.25	49.9	5.8	21.44	26	0.001	0.14	0.06	0.580	16.5	0.08	<0.12	42.56	<0.06	<0.01	39.22	121.17	0.41	905.05	103.03	3.01	<0.11
10/01/00	6.97	56.3	14.5	22.65	60	0.004	0.12	0.57	0.250	11.8	<0.02	<0.09	86.000	<0.006	<0.04	106.000	233.000	0.139	1590.000	185.000	0.642	<0.04

Appendix 3H.(Continued).

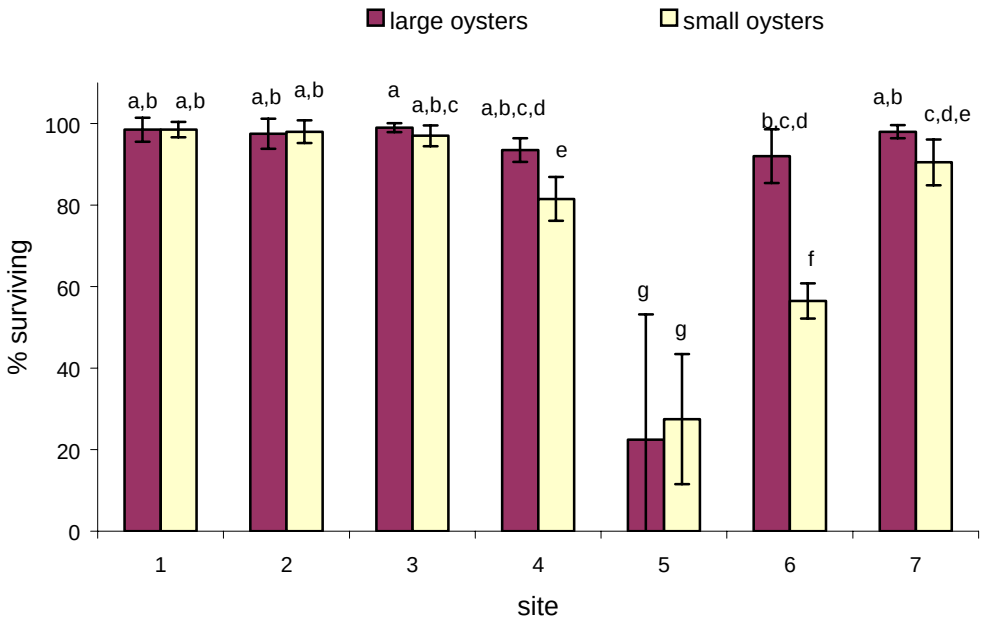
SITE: 7

DATE	pH	DO (% Sat.)	EC (dS m^{-1})	Temp (^{O}C)	Alkalinity (mg L^{-1})	NO_2 - N (mg L^{-1})	PO_4 - P (mg L^{-1})	NH_3 - N (mg L^{-1})	NO_3 -N (mg L^{-1})	Cl:SO_4	Al (mg L^{-1})	As (mg L^{-1})	Ca (mg L^{-1})	Cu (mg L^{-1})	Fe (mg L^{-1})	K (mg L^{-1})	Mg (mg L^{-1})	Mn (mg L^{-1})	Na (mg L^{-1})	S (mg L^{-1})	Si (mg L^{-1})	Zn (mg L^{-1})
01/06/99	7.78	111.5	33.8	18.59	65	0.001	0.12	0.26	0.059	14.1	<0.05	<0.35	238.45	<0.005	<0.007	261.72	694.85	0.03	sat.	472.92	2.42	<0.02
15/06/99	6.65	92.4	16.0	13.15	52	0.003	0.04	0.29	0.140	9.6	<0.05	<0.35	114.56	<0.005	<0.007	119.35	336.79	0.14	sat.	228.41	2.98	<0.02
28/06/99	6.35	52.3	2.7	16.24	8	0.009	0.20	0.14	0.330	14.6	<0.07	<0.17	20.60	0.01	<0.01	21.98	57.47	0.10	504.08	31.77	0.54	<0.16
12/07/99	5.27	81.0	5.0	16.09	21	0.000	0.08	0.06	0.024	0.8	<0.07	<0.17	28.53	0.01	<0.01	28.22	81.43	0.21	669.29	58.72	2.23	<0.16
26/07/99	5.64	119.7	1.9	14.93	0	0.003	0.22	0.06	0.015	3.8	0.14	<0.11	19.69	0.01	<0.17	18.55	54.30	0.22	449.63	54.63	0.44	<0.02
09/08/99	7.30	145.3	8.0	19.31	42	0.004	0.26	0.00	0.130	8.6	<0.04	<0.11	76.38	<0.007	<0.17	87.72	222.48	0.22	sat.	201.27	1.55	<0.02
23/08/99	7.90	115.5	25.6	17.60	80	0.003	0.20	0.12	0.094	8.5	<0.04	<0.11	248.12	<0.007	<0.17	320.11	729.43	0.08	sat.	623.75	3.26	<0.02
06/09/99	7.33	121.9	23.2	21.01	60	0.000	0.24	0.04	0.260	8.0	<0.04	<0.11	216.96	<0.007	<0.17	265.87	599.36	0.11	sat.	576.54	1.59	<0.02
01/10/99	7.63	75.1	24.8	19.43	73	0.001	0.12	0.03	0.240	9.6	<0.04	<0.11	166.43	<0.007	<0.17	198.75	476.33	0.05	sat.	465.63	1.96	<0.02
15/11/99	6.11	63.8	4.4	18.64	26	0.000	0.06	0.00	0.380	21.2	0.14	<0.12	28.43	<0.06	0.00	26.84	85.16	0.22	662.47	69.64	3.23	0.07
10/01/00	7.65	75.4	28.3	22.31	85	0.003	0.10	0.14	0.160	5.6	<0.02	<0.09	195.000	<0.006	<0.04	290.000	400.000	0.019	2320.000	419.000	1.720	<0.04

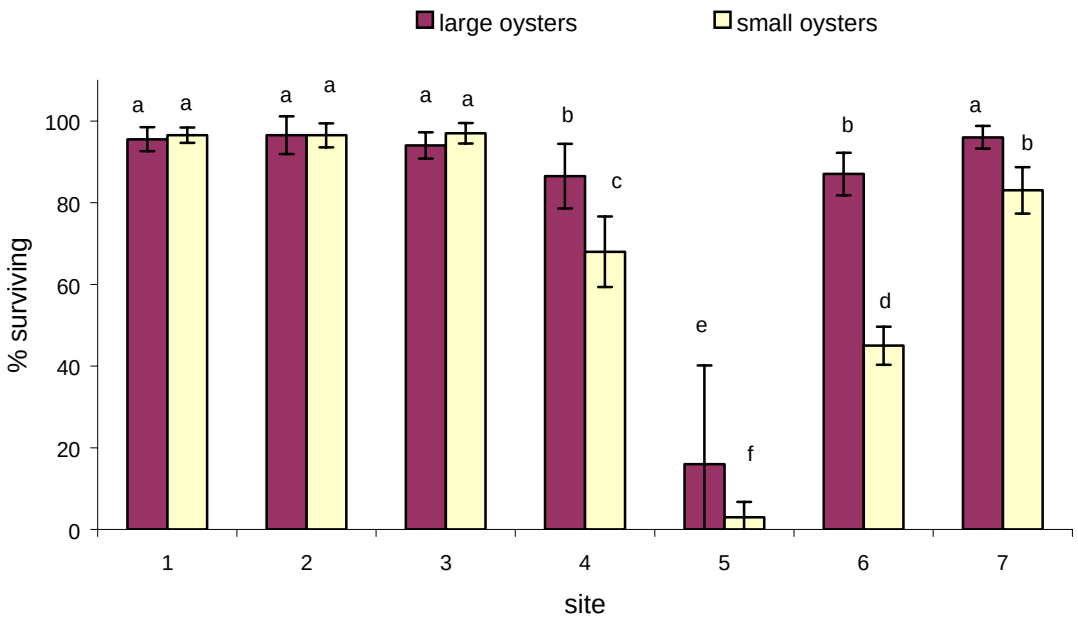
Appendix 3I. Oyster survival data measured on the 23/8/99 and 10/1/00 at Sites 1 to 7 and results of *post hoc* analyses.

ACID	SITE	SIZE	% SURV(23/8/99)	% SURV(10/1/00)	CODES
R	1	L	94	94	R = Reference
R	1	L	100	98	A = Acid
R	1	L	100	92	L = Mature oysters
R	1	L	100	98	S = Juvenile oysters
R	1	S	100	98	
R	1	S	100	96	
R	1	S	98	98	
R	1	S	96	94	
R	2	L	100	100	
R	2	L	100	100	
R	2	L	92	90	
R	2	L	98	96	
R	2	S	100	98	
R	2	S	94	92	
R	2	S	98	98	
R	2	S	100	98	
R	3	L	100	94	
R	3	L	100	94	
R	3	L	98	98	
R	3	L	98	90	
R	3	S	98	98	
R	3	S	94	94	
R	3	S	100	100	
R	3	S	96	96	
A	4	L	92	78	
A	4	L	96	90	
A	4	L	90	82	
A	4	L	96	96	
A	4	S	88	78	
A	4	S	78	64	
A	4	S	84	72	
A	4	S	76	58	
A	5	L	68	52	
A	5	L	18	12	
A	5	L	4	0	
A	5	L	0	0	
A	5	S	22	8	
A	5	S	34	0	
A	5	S	46	0	
A	5	S	8	4	
A	6	L	100	94	
A	6	L	84	82	
A	6	L	90	84	
A	6	L	94	88	
A	6	S	58	48	
A	6	S	54	48	
A	6	S	52	38	
A	6	S	62	46	
A	7	L	100	98	
A	7	L	96	92	
A	7	L	98	96	
A	7	L	98	98	
A	7	S	94	90	
A	7	S	94	82	
A	7	S	92	84	
A	7	S	82	76	

Appendix 3I. (Continued)



Percentage survival on the 23/8/99 at the seven sites (*post hoc* analysis results for the factor Size x Site(Acid) are displayed as letters indicating means which are not significantly different, $p > 0.05$).



Percentage survival on the 10/1/00 at the seven sites (*post hoc* analysis results for the factor Size x Site(Acid) are displayed as letters indicating means which are not significantly different, $p > 0.05$).

Appendix 3J. Tables of field and analytical water quality data collected during Experiment 2 (Chapter 4).

DATE	SITE	pH	EC ($dS\ m^{-1}$)	DO (% Sat.)	Temp (^{o}C)	Alkalinity ($mg\ L^{-1}$)	NH_3 - N ($mg\ L^{-1}$)	NO_2 - N ($mg\ L^{-1}$)	NO_3 -N ($mg\ L^{-1}$)	PO_4 - P ($mg\ L^{-1}$)
01/02/00	1	8.18	44.6	102.6	22.88	103	0.61	0.004	0.14	0.42
01/02/00	2	8.02	32.7	94.4	23.47	76	0	0.003	0.28	0.2
01/02/00	3	8.29	51.7	117.4	22.73	73	0.09	0.003	0.42	0.14
01/02/00	4	7.93	28.7	93.3	23.21	76	0.13	0	0.63	0.22
01/02/00	6	7.46	23.9	87	23.74	65	0.2	0	0.072	0.2
01/02/00	7	7.96	33.4	89.7	23.41	76	0.29	0	0.27	0.18
10/03/00	4	6.14	1.1	0.4	21.41	13	NS	NS	NS	NS
31/03/00	4	6.22	3.6	44.4	23.65	73	NS	NS	NS	NS
06/04/00	1	8.02	34.2	89.4	22	76	0.19	0.009	0.17	0.26
06/04/00	2	7.84	19.7	80.9	22.26	73	0.16	0.011	0.072	0.2
06/04/00	3	8.08	34.6	94.8	22.2	80	0.16	0.006	0.063	0.72
06/04/00	4	7.84	20.9	76.7	22.49	76	0.08	0.007	0.53	0.32
06/04/00	6	6.88	13	56.5	22.35	60	0.3	0.007	0.055	0.18
06/04/00	7	7.36	20	73.7	22.43	60	0.28	0.006	0.1	0.24
19/04/00	4	6.55	18.2	NS	22.02	NS	NS	NS	NS	NS
07/05/00	4	6.55	22.3	90.6	19.62	NS	NS	NS	NS	NS
30/05/00	4	7.98	30.6	99.4	12.76	NS	NS	NS	NS	NS
09/06/00	1	8.12	45.4	82.4	14.06	95	0.19	0.004	0.18	0.22
09/06/00	2	8.05	34.4	95.6	13.15	80	0.2	0.003	0.12	0.18
09/06/00	3	8.18	45.8	84.4	14.39	85	0.3	0.004	0.072	0.2
09/06/00	4	7.93	34.6	88.1	12.33	65	0.11	0.001	0.032	0.24
09/06/00	6	7.65	35.1	84.7	12.59	73	1	0	0.1	0.14
09/06/00	7	6.76	30.6	76.2	11.45	47	0.13	0.014	0.047	0.08
20/06/00	4	5.77	27.8	97.5	15.64	NS	NS	NS	NS	NS
10/07/00	4	5.8	27.5	123.8	16.26	52	NS	NS	NS	NS
07/08/00	1	7.93	34	85.2	16.37	73	0.08	0	0.14	0.07
07/08/00	2	7.75	25	90.4	16.06	85	0	0.001	0.1	0.16
07/08/00	3	7.79	36.7	88.7	16.55	76	0	0.001	0.1	0.12
07/08/00	4	5.64	21.7	72.4	17.18	47	0.07	0.006	0.021	0.08
07/08/00	6	5.32	24.2	67.7	14.21	NS	NS	NS	NS	NS
07/08/00	7	6.97	10.8	49.8	14.13	30	0	0.001	0.021	0.07
18/08/00	4	6.6	31.6	99.1	14.88	NS	NS	NS	NS	NS
23/10/00	1	8.19	48.2	NS	20.01	76	0.44	0	0.047	0.1
23/10/00	2	7.67	35	NS	26.69	73	0.37	0.006	0.021	0.4
23/10/00	3	8.31	46.4	NS	21.09	65	0.11	0	0.063	0.1
23/10/00	4	7.84	33.8	NS	21.59	90	0.23	0.001	0.032	0.07
23/10/00	6	NS	NS	NS	NS	NS	NS	NS	NS	NS
23/10/00	7	8.05	37.5	NS	21.14	73	0.16	0	0.089	0.06
12/01/01	1	7.99	40.5	67.4	23.41	80	0.42	0.003	0.11	0.3
12/01/01	2	7.73	33.3	76.5	27.12	100	0.06	0.007	0.055	0.26
12/01/01	3	7.91	39	73.9	23.96	95	0.05	0.003	0.12	0.14
12/01/01	4	7.81	35.2	68.4	24.42	90	0.1	0.006	0.04	0.32
12/01/01	6	7.58	37.4	62	24.74	90	0	0.003	0.036	0.06
12/01/01	7	7.82	36.3	63	24.07	80	0.02	0.004	0.032	0.28

NS = Not Sampled

Appendix 3J. (Continued)

DATE	SITE	$Cl:SO_4$	Al (mg L^{-1})	As (mg L^{-1})	Ca (mg L^{-1})	Cu (mg L^{-1})	Fe (mg L^{-1})	K (mg L^{-1})	Mg (mg L^{-1})	Mn (mg L^{-1})	Na (mg L^{-1})	S (mg L^{-1})	Si (mg L^{-1})	Zn (mg L^{-1})
01/02/00	1	10.38	0.131	<0.09	327.000	<0.006	<0.04	561.000	503.000	0.141	2670.000	653.000	1.310	<0.04
01/02/00	2	8.63	<0.02	<0.09	280.000	<0.006	<0.04	460.000	477.000	0.037	sat.	565.000	1.700	<0.04
01/02/00	3	9.97	<0.02	<0.09	354.000	<0.006	<0.04	577.000	528.000	<0.001	sat.	727.000	1.010	<0.04
01/02/00	4	7.65	<0.02	<0.09	224.000	<0.006	<0.04	314.000	422.000	0.004	2390.000	484.000	1.710	<0.04
01/02/00	6	4.93	<0.02	<0.09	231.000	<0.006	<0.04	351.000	431.000	0.090	2390.000	519.000	2.370	<0.04
01/02/00	7	6.03	<0.02	<0.09	296.000	<0.006	<0.04	492.000	477.000	0.008	2580.000	634.000	1.540	<0.04
10/03/00	4	7.25	0.185	<0.09	10.800	<0.006	0.271	7.220	19.000	0.424	112.000	23.600	0.317	<0.04
31/03/00	4	5.36	<0.02	<0.09	27.400	<0.006	<0.04	15.800	60.800	0.615	382.000	57.900	0.877	<0.04
06/04/00	1	8.77	<0.02	<0.09	241.000	<0.006	<0.04	358.000	436.000	<0.001	2400.000	530.000	1.880	<0.04
06/04/00	2	11.44	<0.02	<0.09	86.900	<0.006	<0.04	94.500	228.000	<0.001	1560.000	204.000	2.710	<0.04
06/04/00	3	8.82	<0.02	<0.09	182.000	<0.006	<0.04	257.000	375.000	<0.001	2220.000	463.000	1.670	<0.04
06/04/00	4	5.61	<0.02	<0.09	189.000	<0.006	<0.04	266.000	378.000	<0.001	2230.000	427.000	3.060	<0.04
06/04/00	6	7.70	<0.02	<0.09	86.300	<0.006	<0.04	91.700	224.000	0.172	1540.000	190.000	2.230	<0.04
06/04/00	7	11.40	<0.02	<0.09	81.200	<0.006	<0.04	88.500	214.000	0.003	1490.000	201.000	1.740	<0.04
19/04/00	4	5.14	<0.02	<0.09	196.000	<0.006	<0.04	233.000	392.000	1.330	2350.000	417.000	2.180	<0.04
07/05/00	4	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
30/05/00	4	7.37	<0.009	<0.14	226.00	0.02	0.01	203.00	694.00	0.07	5840.00	495.00	2.16	0.22
09/06/00	1	5.10	<0.009	<0.14	451.00	<0.004	0.06	431.00	1410.00	0.01	12000.00	1040.00	0.94	0.01
09/06/00	2	7.24	<0.009	<0.14	247.00	0.00	0.01	229.00	776.00	0.02	6660.00	578.00	1.77	<0.007
09/06/00	3	8.83	<0.009	<0.14	283.00	<0.004	<0.005	276.00	899.00	0.01	7730.00	675.00	0.85	<0.007
09/06/00	4	7.12	<0.009	<0.14	245.00	<0.004	0.01	229.00	763.00	0.01	6560.00	566.00	1.02	0.01
09/06/00	6	8.87	<0.009	<0.14	257.00	<0.004	<0.005	241.00	802.00	0.05	6870.00	563.00	1.78	<0.007
09/06/00	7	10.05	<0.009	<0.14	162.00	<0.004	0.01	149.00	509.00	0.20	4380.00	363.00	1.06	0.02
20/06/00	4	6.02	<0.009	<0.14	226.00	0.02	0.01	184.00	650.00	1.46	5320.00	535.00	0.87	0.24
10/07/00	4	6.04	<0.009	<0.14	234.00	0.01	0.01	189.00	669.00	1.49	5450.00	549.00	0.97	0.14
07/08/00	1	4.82	<0.009	<0.14	141.00	0.00	0.03	131.00	452.00	0.02	4100.00	525.00	0.76	<0.007
07/08/00	2	5.78	<0.009	<0.14	258.00	0.01	0.01	241.00	813.00	0.01	6870.00	577.00	2.21	<0.007
07/08/00	3	9.26	<0.009	<0.14	243.00	<0.004	0.01	234.00	774.00	0.02	6600.00	544.00	1.16	<0.007
07/08/00	4	6.75	0.08	<0.14	193.00	0.01	0.37	143.00	528.00	1.70	4120.00	457.00	0.80	0.08
07/08/00	6	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
07/08/00	7	6.97	<0.009	<0.14	80.50	0.01	0.10	78.20	267.00	0.31	2150.00	192.00	0.45	0.04
18/08/00	4	6.82	0.07	<0.14	217.00	0.01	0.01	193.00	661.00	0.42	5600.00	544.00	0.91	0.04
23/10/00	1	8.12	<0.009	<0.14	356.00	<0.004	0.01	347.00	1120.00	0.01	9600.00	786.00	0.70	<0.007
23/10/00	2	6.06	<0.009	<0.14	337.00	0.00	0.01	322.00	1050.00	0.00	8920.00	743.00	1.51	0.01
23/10/00	3	9.35	<0.009	<0.14	317.00	0.00	0.01	309.00	1000.00	0.01	8470.00	692.00	0.57	0.01
23/10/00	4	8.76	<0.009	<0.14	240.00	0.01	0.01	225.00	746.00	0.05	6390.00	530.00	1.34	0.01
23/10/00	6	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
23/10/00	7	6.77	<0.009	<0.14	347.00	0.00	0.01	329.00	1080.00	0.02	9120.00	764.00	0.89	0.01
12/01/01	1	8.35	<0.009	<0.14	304.00	0.01	0.01	292.00	959.00	0.01	8110.00	675.00	1.38	<0.007
12/01/01	2	8.80	<0.009	<0.14	238.00	0.01	0.01	222.00	743.00	0.01	6260.00	525.00	2.19	<0.007
12/01/01	3	9.05	<0.009	<0.14	291.00	0.01	<0.005	280.00	910.00	0.01	7570.00	625.00	1.18	0.01
12/01/01	4	9.38	<0.009	<0.14	253.00	0.00	0.01	239.00	786.00	0.01	6670.00	551.00	1.93	0.01
12/01/01	6	7.53	<0.009	<0.14	310.00	<0.004	<0.005	296.00	972.00	0.04	8150.00	675.00	0.97	<0.007
12/01/01	7	8.41	<0.009	<0.14	290.00	0.00	<0.005	274.00	907.00	0.02	7690.00	642.00	1.71	<0.007

NS = Not Sampled

Appendix 3K. Oyster condition index measured at Sites 1, 2, 3, 4, 6 and 7.

6/04/00		9/06/00		7/08/00		23/10/00		12/01/01	
Site	Condition Index	Site	Condition Index	Site	Condition Index	Site	Condition Index	Site	Condition Index
1	59.38	1	63.64	1	74.78	1	103.97	2	123.67
1	49.79	1	95.03	1	90.69	1	68.17	2	148.48
1	55.42	1	63.01	1	79.35	1	116.51	2	121.08
1	62.57	1	64.77	1	88.48	1	113.28	2	122.34
1	69.34	1	62.17	1	81.14	1	117.70	2	151.97
1	62.25	1	73.09	1	91.55	1	97.91	2	154.87
1	59.97	1	66.59	1	116.29	1	110.01	2	111.56
1	51.04	1	77.34	1	102.30	1	112.85	2	105.92
1	56.42	1	69.73	1	61.79	1	105.92	2	121.60
1	60.97	2	76.43	1	80.05	1	100.68	2	125.98
1	41.07	2	84.42	1	113.21	1	114.17	3	165.49
1	77.68	2	63.78	1	85.69	1	81.53	3	170.10
2	54.39	2	66.46	2	77.81	2	113.01	3	171.53
2	55.82	2	83.03	2	81.74	2	136.99	3	155.78
2	31.76	2	72.66	2	78.27	2	141.99	3	153.30
2	70.72	2	83.93	2	85.79	2	123.01	3	153.03
2	56.90	2	58.60	2	85.04	2	130.33	4	152.57
2	57.12	2	81.70	2	83.18	2	154.17	4	124.66
2	58.51	2	83.52	2	76.25	2	125.71	4	82.67
2	62.69	2	78.37	2	83.55	2	114.64	4	157.25
2	76.18	2	99.73	2	72.87	2	161.64	4	90.53
2	55.02	3	52.83	2	70.34	2	107.29	4	159.67
3	47.89	3	63.71	2	62.01	2	143.36	4	122.92
3	65.32	3	66.69	3	85.59	2	104.16	4	158.78
3	48.08	3	61.99	3	81.76	3	204.51	4	147.33
3	62.99	3	82.15	3	86.09	3	127.60	7	66.15
3	52.31	3	58.29	3	102.23	3	119.20	7	117.92
3	64.31	3	60.06	3	98.94	3	91.21	7	130.27
3	59.27	3	51.42	3	66.25	3	146.76	7	115.96
3	48.60	3	50.42	3	83.71	3	129.32	7	128.07
3	59.62	3	59.27	3	70.09	3	169.22	7	139.15
3	64.96	3	72.25	3	64.05	3	127.13	7	116.84
3	55.06	3	45.24	3	82.17	3	162.92	7	121.03
4	25.69	4	61.69	3	77.26	3	128.72	7	115.77
4	62.03	4	53.80	4	72.08	3	143.48	7	112.27
4	66.33	4	101.80	4	92.56	4	59.00		
4	34.71	4	85.51	4	57.55	4	96.29		
4	44.93	4	108.27	4	110.66	4	140.15		
4	71.04	4	57.33	4	98.01	4	91.66		
4	78.00	4	97.16	4	59.35	4	91.04		
4	58.73	4	86.72	4	82.03	4	85.62		
4	47.10	4	109.74	4	90.54	4	63.15		
4	43.20	4	79.39	4	108.71	4	104.29		
4	40.15	4	104.36	4	64.26	4	113.14		
4	51.15	7	49.71	4	74.83	4	118.07		
7	46.95	7	69.67	4	60.05	4	98.65		
7	34.93	7	55.38	7	73.66	7	65.90		
7	43.34	7	72.08	7	57.78	7	82.40		
7	27.49	7	70.80	7	86.12	7	69.79		
7	44.73	7	65.88	7	52.99	7	75.16		
7	56.15	7	79.46	7	60.37	7	98.93		
7	42.57	7	62.94	7	42.27	7	72.95		
7	45.24	7	53.06	7	57.38	7	203.33		
7	46.70	7	140.71	7	77.22	7	75.55		
7	62.01	7	55.30	7	68.51	7	46.18		
7	41.65	7	85.27	7	74.26	7	49.49		
7	52.01	6	105.85	7	66.67	7	99.53		
6	82.80	6	111.96	7	56.75	7	45.30		
6	84.69	6	83.48						
6	99.26	6	70.90						
6	43.83	6	110.16						
6	63.47	6	74.54						
6	103.56	6	90.71						
6	118.63	6	94.84						
6	109.73	6	87.14						
6	74.33	6	59.01						
6	81.52								
6	60.51								
6	69.38								

Appendix 3L. Feeding Experiment data.

Treat.	Shell Height (mm)	Shell Length (mm)	Shell Width (mm)	Whole Weight (g)	Soft Tissue Dry Mass (g)	Mean True Faeces Production (mg h^{-1})	Mean Rejection Rate (mg h^{-1})	Feeding Activity (mg h^{-1})
6	53.73	40.16	15.87	18.948	0.861	6.2	13.0	19.1
6	54.48	33.33	13.94	15.732	0.487	7.7	12.2	19.9
6	61.17	50.69	14.68	22.473	0.790	8.6	13.2	21.8
6	46.65	35.45	14.17	12.596	0.837	10.7	8.8	19.4
6	56.55	39.26	13.02	16.167	0.501	7.2	11.2	18.4
6	49.41	44.95	14.87	15.986	0.786	3.5	7.0	10.5
6	51.25	39.16	13.98	13.832	0.830	6.8	3.6	10.3
6	61.98	44.18	19.15	19.696	0.630	2.4	4.0	6.4
6	59.80	41.69	13.20	17.157	1.088	2.8	6.8	9.6
6	54.30	35.76	15.56	15.159	0.464	5.3	2.9	8.1
6	54.71	41.94	14.00	16.908	0.390	1.8	6.2	8.0
6	63.76	45.67	16.39	24.936	0.498	1.7	2.8	4.6
6	53.74	40.45	18.77	20.487	0.903	2.6	1.5	4.1
6	53.88	44.15	15.59	20.509	0.796	1.9	1.9	3.8
6	47.74	37.46	12.14	11.311	0.413	2.3	1.9	4.2
6	56.84	34.23	11.88	12.270	0.345	1.7	1.9	3.6
6	56.75	36.38	16.59	16.462	0.630	2.3	2.3	4.6
6	49.79	41.42	15.07	16.403	0.586	2.2	2.4	4.6
7	60.84	44.88	16.54	21.466	0.598	3.5	6.0	9.5
7	68.49	43.79	15.73	24.851	1.041	3.0	7.0	10.0
7	54.79	37.96	14.16	15.918	0.477	4.0	3.0	7.0
7	46.03	39.75	14.01	13.900	0.594	2.5	6.0	8.5
7	67.17	44.36	14.48	23.394	1.446	1.0	5.0	6.0
7	71.52	52.61	18.98	26.483	0.884	4.5	4.0	8.5
7	60.72	43.05	14.49	18.420	0.818	6.0	5.5	11.5
7	57.64	40.48	15.47	20.044	0.690	1.5	7.5	9.0
7	58.19	41.55	18.08	17.612	0.849	2.0	3.5	5.5
7	69.99	49.73	15.13	25.924	0.510	3.0	2.5	5.5
7	53.71	33.99	14.84	13.960	0.659	6.0	7.5	13.5
7	59.70	48.12	16.02	24.717	0.499	2.5	9.5	12.0
7	63.09	42.86	13.29	21.047	0.513	2.6	1.6	4.2
7	55.10	41.37	17.69	16.276	0.976	4.9	2.7	7.5
7	61.23	48.96	21.71	27.470	0.799	7.4	7.4	14.8
7	52.31	35.57	14.99	11.926	0.612	2.3	5.2	7.5
7	60.02	36.69	12.41	15.817	0.712	0.9	1.9	2.9
7	60.35	39.67	17.47	18.841	0.857	0.8	0.5	1.3
8	70.87	39.79	17.92	29.617	1.365	1.4	2.4	3.7
8	57.25	42.92	17.90	19.815	0.802	0.8	2.1	2.9
8	67.89	44.13	17.34	25.534	0.775	2.0	2.5	4.5
8	56.28	40.82	16.70	17.491	0.910	1.3	3.8	5.1
8	57.65	35.50	17.52	23.088	0.848	4.5	5.1	9.6
8	59.96	40.66	14.40	18.569	0.773	2.2	3.3	5.4
8	55.91	41.72	16.18	20.709	1.017	2.6	3.0	5.7
8	50.24	37.95	15.50	18.373	0.721	2.4	3.3	5.7
8	60.84	44.57	16.73	18.485	0.846	1.6	2.8	4.4
8	68.10	38.56	18.29	25.871	1.888	1.6	2.4	4.0
8	63.61	40.65	16.59	20.084	0.818	1.3	2.0	3.3
8	57.79	38.31	19.31	18.583	0.644	1.1	2.0	3.2
8	59.54	40.18	22.21	22.805	0.821	0.9	1.7	2.6
8	54.13	40.14	16.19	16.344	0.539	2.5	2.4	5.0
8	55.44	39.60	20.34	18.660	0.544	0.5	0.8	1.4
8	62.86	40.06	20.85	24.297	0.996	1.3	2.6	3.9
8	55.38	40.09	17.32	20.277	0.720	1.1	2.9	4.0
8	60.17	45.13	18.94	24.386	1.196	1.4	1.6	3.0

Appendix 3M. Histopathology details.

(A) Fixation of Oyster Soft Tissue

Formalin (10% sea water) (Lillie, 1965; C.A. Farley, personal communication, cited in Howard and Smith, 1983) for oysters comprises:

1. 10 ml 37-40% formaldehyde
2. 90 ml filtered ambient sea water

(B) Preparation, Processing and Staining of Sections

The School of Pathology, UNSW, provided the notes below on the preparation, processing and staining of sections for histopathology.

HAEMATOXYLIN AND EOSIN STAIN

1. Dewax Tissue Sections
2. Stain in Harris' Haematoxylin 4 minutes
3. Wash in Water
4. Differentiate in Acid Alcohol 1 dip
5. Immediately Wash in Water
6. Blue Sections in Scott's Blue 10 dips (ensure sections are blue)
7. Wash in Water
8. Stain in Eosin 3 minutes
9. Blot Excess Stain
10. Dehydrate, Clear and Mount.

Results:

- Nuclei- Blue/Black
- Cytoplasm- Pink
- Muscle Fibres- Deep Pink/Red
- Collagen- Pale Pink/Red
- Red Blood Cells- Orange/Red
- Fibrin- Deep Pink

Appendix 3M. (Continued).

IRON STAIN

1. Bring Sections to Distilled Water
2. Mix Equal Parts of 2% HCl and 2% Potassium Ferrocyanide solutions and Filter
3. Incubate Sections 30 minutes
4. Wash in Water
5. Counterstain with 1% Neutral Red 5 minutes
6. Wash in Water
7. Dehydrate, Clear and Mount

Results:

- Haemosiderin (ferric iron salts)- Blue
- Nuclei- Red
- Background- Pale Red.

Appendix 3N. ANOVA and multiple comparison results from the Feeding Experiment

Table 1 One-way ANOVA of feeding activity by treatment and results of Least Significant Difference *post hoc* analyses.

Source of Variation	SS	df	MS	F	P-value
Between Groups	486.2621	2	243.131	10.03945	0.000211
Within Groups	1235.095	51	24.21756		
Total	1721.357	53			

Multiple Comparisons

Dependent Variable: FA

LSD

(I) EXP	(J) EXP	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
1	2	2.788	1.6404	.095	-.505	6.081
	3	7.284*	1.6404	.000	3.991	10.577
2	1	-2.788	1.6404	.095	-6.081	.505
	3	4.496*	1.6404	.008	1.203	7.789
3	1	-7.284*	1.6404	.000	-10.577	-3.991
	2	-4.496*	1.6404	.008	-7.789	-1.203

*. The mean difference is significant at the .05 level.

Table 2 One-way ANOVA of faeces production by treatment and results of Least Significant Difference *post hoc* analyses.

Source of Variation	SS	df	MS	F	P-value
Between Groups	95.47346	2	47.73673	10.27687	0.000178
Within Groups	236.8983	51	4.645065		
Total	332.3718	53			

Multiple Comparisons

Dependent Variable: FP

LSD

(I) EXP	(J) EXP	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
1	2	1.400	.7184	.057	-.042	2.842
	3	3.247*	.7184	.000	1.805	4.689
2	1	-1.400	.7184	.057	-2.842	.042
	3	1.847*	.7184	.013	.405	3.289
3	1	-3.247*	.7184	.000	-4.689	-1.805
	2	-1.847*	.7184	.013	-3.289	-.405

*. The mean difference is significant at the .05 level.

Appendix 3N. (Continued)

Table 3 One-way ANOVA of rejection rate by treatment and results of Least Significant Difference *post hoc* analyses.

Source of Variation	SS	df	MS	F	P-value
Between Groups	151.464	2	75.73202	7.111553	0.001887
Within Groups	543.1068	51	10.64915		
Total	694.5708	53			

Multiple Comparisons

Dependent Variable: RR
LSD

(I) EXP	(J) EXP	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
1	2	1.388	1.0878	.208	-.796	3.572
	3	4.037*	1.0878	.001	1.853	6.221
2	1	-1.388	1.0878	.208	-3.572	.796
	3	2.649*	1.0878	.018	.465	4.833
3	1	-4.037*	1.0878	.001	-6.221	-1.853
	2	-2.649*	1.0878	.018	-4.833	-.465

*. The mean difference is significant at the .05 level.

Table 4 One-way ANOVA of filtration rate by treatment and results of Least Significant Difference *post hoc* analyses.

Source of Variation	SS	df	MS	F	P-value
Between Groups	758.3511	2	379.1755	13.71156	0.00002
Within Groups	1410.339	51	27.65372		
Total	2168.691	53			

Multiple Comparisons

Dependent Variable: FR
LSD

(I) EXP	(J) EXP	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
1	2	2.714	1.7529	.128	-.805	6.233
	3	8.951*	1.7529	.000	5.432	12.470
2	1	-2.714	1.7529	.128	-6.233	.805
	3	6.237*	1.7529	.001	2.718	9.756
3	1	-8.951*	1.7529	.000	-12.470	-5.432
	2	-6.237*	1.7529	.001	-9.756	-2.718

*. The mean difference is significant at the .05 level.

N.B. for all multiple comparisons tables in Appendix N: Treatment 6 = 1, Treatment 7 = 2 and Treatment 8 = 3.