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Improving management of nuisance midges (Chironomidae: Diptera) in Perth (Western Australia) wetlands based on their response to temperature and seditment type

Kirsty Suffell
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Improving management of nuisance midges
(Chironomidae: Diptera) in Perth (Western
Australia) wetlands based on their response to
temperature and sediment type.

Kirsty Suffell

A Thesis submitted in partial fulfilment of the requirement for the award of:
Bachelor of Science (Environmental Management) Honours at the School of
Natural Sciences, Edith Cowan University, Joondalup.

Date of Submission: 22nd November 2002.

Supervisor: Mark Lund

USE OF THESIS

The Use of Thesis statement is not included in this version of the thesis.

ABSTRACT

The eutrophication of wetlands has created ideal breeding and growth conditions for certain species of Chironomidae (midge). In urban areas, high abundances can cause adult midges to become a nuisance to nearby residents. The shallow and often eutrophic wetlands of Perth (Western Australia) provide ideal conditions for the growth of certain Chironomid species, resulting in common nuisance problems.

To control nuisance plagues a range of management options are available, including light traps, biological controls, insect growth regulators and pesticides. In Australia, Abate® is the only registered pesticide for the control of midges. Little is known about the life histories of nuisance species. Current management approaches rely on threshold larval densities as an indication of optimum spray times (with Abate®). This ignores the possibility of exploiting vulnerabilities within the life cycle to provide good control with minimum environmental impact. This project aims to determine the effects of two key factors, temperature and sediment type on growth and survival of two common nuisance species.

Eggs of *Chironomus alternans* and *Polypedilum nubifer* were collected from the field within 24 hours of laying and reared at 15, 20, 25 and 30°C. Hatching times for *C.alternans* were 5-5.2, 3-3.5, 2-3 and 2 days for 15°C through to 30°C respectively. Hatching times for *P.nubifer* were slightly longer at 6, 4, 3 and 2.67 days for 15°C through to 30°C respectively. All eggs in this experiment hatched at 15°C to 30°C, implying that the eggs do not act as a resting stage to survive harsh conditions.

There were three containers per replicate and three replicates per temperature for the main and sediment experiments. Each container at each replicate was terminated over the course of the experiment. Terminations occurred at 2.5, 3.5 and 4.5-6 weeks for the main experiment and 4.5-6 weeks for the sediment experiment. Terminations consisted of separating the living larvae from the

sediment, preserving them and then measuring their head widths and body lengths.

Chironomus alternans had highest survival at 20°C followed by 15°C, with very low survival at 25°C and 30°C. Emergence occurred at >51, 32.5, 28 and 26 days at 15°C through to 30°C respectively. In the field, *Chironomus alternans* emerges in large numbers in spring. Faster larval development resulted in low survival levels. Some larvae were able to survive at 25°C and 30°C, enabling larvae to survive through to the next season.

Polypedilum nubifer had highest survival at 20°C followed by 25°C with no larvae surviving at 15°C and 30°C. Emergence only occurred at 30°C at 19 days for *P.nubifer*. Eggs of *P.nubifer* were not available for the majority of the experiment, thus preliminary results are presented and further research needs to be done on this summer species.

Sediment type and particle size did not affect the survival of larvae. The sediment experiment showed higher survival rates in the coarse (99% ± 0.005) and fine sand (88% ± 0.12) compared to metaphyton (62% ± 0.082) (Figure 6.3.1). Average time to emergence was fastest in the coarse sand, implying that larvae preferred those conditions.

Although current pesticide usage is adequate to reduce nuisance problems, this study has highlighted some potentially new approaches to the timing of spraying that may increase effectiveness and reduce the number of sprays required. Ultimately nutrient control remains the only long-term way of eliminating or reducing the problem.

DECLARATION

I certify that this thesis does not, to the best of my knowledge and belief: incorporate, without acknowledgment, any material previously submitted for a degree or diploma in any institution of higher education; contain any material previously published or written by another person except where due reference is made in the text.

Signature.....

Date.....4/03/03.....

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TABLE OF CONTENTS

TITLE.....	I
ABSTRACT	II
DECLARATION	IV
ACKNOWLEDGMENTS.....	V
TABLE OF CONTENTS	VI
LIST OF FIGURES.....	VIII
LIST OF TABLES.....	X
CHAPTER 1: INTRODUCTION	1
1.1 BACKGROUND.....	1
1.2 RESEARCH AIMS	12
CHAPTER 2: SITE DESCRIPTION	14
2.1 SITE DESCRIPTION	14
CHAPTER 3: HATCHING EXPERIMENT.....	17
3.1 INTRODUCTION	17
3.2 METHODS.....	18
3.3 RESULTS	20
3.4 DISCUSSION	23
CHAPTER 4: PILOT STUDY	24
4.1 INTRODUCTION	24
4.2 METHODS.....	26
4.3 RESULTS	30
4.4 DISCUSSION	34

CHAPTER 5: MAIN EXPERIMENT	35
5.1 INTRODUCTION	35
5.2 METHODS.....	36
5.3 RESULTS	39
5.4 DISCUSSION	55
CHAPTER 6: SEDIMENT EXPERIMENT.....	60
6.1 INTRODUCTION	60
6.2 METHODS.....	61
6.3 RESULTS	63
6.4 DISCUSSION	66
CHAPTER 7: TRANSFERRING EXPERIMENT	68
7.1 INTRODUCTION	68
7.2 METHODS.....	69
7.3 RESULTS	70
7.4 DISCUSSION	72
CHAPTER 8: SYNTHESIS.....	73
8.1 FURTHER EXPERIMENTS.....	73
8.2 TEMPERATURE	76
8.3 SEDIMENT TYPE AND PARTICLE SIZE	77
8.4 CONTROL OF MIDGES	78
8.5 FUTURE.....	81
REFERENCES	83

LIST OF FIGURES

Figure 1.1.1: Typical life cycle of a midge.	3
Figure 1.1.2: Times in the year when <i>C.alternans</i> has the highest densities.	7
Figure 1.1.3: Times in the year when <i>P.nubifer</i> has the highest densities.	7
Figure 1.1.4: Comparison between the egg mass shapes of <i>P.nubifer</i> (A) and <i>C.alternans</i> (B).	8
Figure 2.1.1: Map of Lake Joondalup in relation to Western Australia and Perth. A = Banyandah Boulevard, B = Neil Hawkins Park, C = Edgewater Drive, D = McCubbin Boulevard (Adapted from UBD, 2000).	16
Figure 3.2.1: Photograph of logs used to collect midge eggs.	19
Figure 3.3.1: Mean (\pm SE) hatching rates for <i>C.alternans</i> at 15-30°C. n = number of replicates.	21
Figure 3.3.2: Mean (\pm SE) hatching rates for <i>C.alternans</i> at 15-30°C in the main experiment. n = number of replicates. S.E. = standard error.	21
Figure 3.3.3: Mean (\pm SE) hatching rates for <i>P.nubifer</i> at 15-30°C. n = number of replicates.	22
Figure 4.2.1: Photograph of the pilot study.	29
Figure 4.3.1: Survival rates at 15-30°C for the pilot study. n = replicates.	31
Figure 4.3.2: Frequencies of head widths for the pilot study. 14 = instar stages.	31
Figure 4.3.3 Survival compared between tap water and lake water for <i>C.alternans</i> at 20°C.	32
Figure 4.3.4 Survival compared between milk powder and TetraMin® fish food for <i>C.alternans</i> at 20°C.	32
Figure 4.3.5 Survival compared between metaphyton and sterilised propagating sand for <i>C.alternans</i> at 20°C.	33
Figure 5.2.1: Photograph of the main experiment.	38
Figure 5.2.2: Photograph of an emergence bottle.	38
Figure 5.3.1: Survival rates of <i>C.alternans</i> at 15-30°C in the main experiment. n = number of replicates.	40
Figure 5.3.2: Survival rates of <i>P.nubifer</i> at 15-30°C in the main experiment. n = number of replicates.	40
Figure 5.3.3: Survival rates of <i>C.alternans</i> at the three terminations at 15-30°C in the main experiment. 1, 2, 3 = terminations.	41

Figure 5.3.4: Head widths and body lengths for <i>C.alternans</i> in the main experiment. 2-4 = instar stages.	41
Figure 5.3.5: Head widths and body lengths through three termination intervals for <i>C.alternans</i> at 15°C in the main experiment. 2-4 = instar stages.	43
Figure 5.3.6: Head widths and body lengths through three termination intervals for <i>C.alternans</i> at 20°C in the main experiment. 2-4 = instar stages.	44
Figure 5.3.7: Head widths and body lengths through three termination intervals for <i>C.alternans</i> at 25°C in the main experiment. 2-4 = instar stages.	45
Figure 5.3.8: Head widths and body lengths through three termination intervals for <i>P.nubifer</i> at 20°C in the main experiment. 2-4 = instar stages.	46
Figure 5.3.9: Comparison of the average head widths between the pilot study and the main study at 15-30°C.	48
Figure 5.3.10: Box plots of NO _x concentrations at 15-30°C (Plots with the same symbol were not significantly different).	48
Figure 5.3.11: Box plots of NH ₄ concentrations at 15-30°C (Plots with the same symbol were not significantly different).	50
Figure 5.3.12: Box plots of FRP concentrations at 15-30°C.	50
Figure 5.3.13: Box plots of O ₂ concentrations at 15-30°C (Plots with the same symbol were not significantly different).	51
Figure 5.3.14: Box plots of pH concentrations at 15-30°C (Plots with the same symbol were not significantly different).	51
Figure 5.3.15: Survivorship curves for <i>C.alternans</i> larvae at 15-30°C in the main experiment.	54
Figure 6.2.1: Photograph of the coarse sand and fine sand.	62
Figure 6.3.1: Survival rates between the coarse sand, fine sediment and metaphyton.	64
Figure 6.3.2: Head widths and body lengths for the sediment experiment. 2-4 = instar stages	65
Figure 7.2.1: Photograph of transferring experiment.	69
Figure 7.3.1: Survival rates for the transferring experiment.	71
Figure 7.3.2: Head widths and body lengths for the transferring experiment. 2-4 = instar stages.	71
Figure 8.4.1: Larval densities and the occurrence of spraying at Lake Joondalup in 2001.	80

LIST OF TABLES

Table 5.3.1: Emergence times for <i>C.alternans</i> and <i>P.nubifer</i> in the main experiment.....	49
Table 5.3.2: Life history table for larvae of <i>C.alternans</i> at 15-30°C in the main experiment.....	53
Table 6.3.1: Emergence times in the metaphyton, coarse sand and fine sand.	63
Table 7.3.1: Emergence in the control and treatment in the transferring experiment.....	70

CHAPTER 1: INTRODUCTION

1.1 Background

La Verne Stovicek (2000, cited in Tanji, 2002) complained, "It's like being a prisoner in your own apartment... You cannot go out or they'll come in." The Stovicek family (2000, cited in Tanji, 2002) said "But it gets unbearable... When we had a light in our front doorway, we wouldn't get into the house without a cloud of them all over our heads, you had to keep waving your arms and they still got in with you." These comments are from residents living near wetlands that have nuisance levels of midges.

Chironomidae (Diptera) are some of the most widely distributed and abundant freshwater invertebrates (Armitage, Cranston & Pinder, 1995; Hirabayashi & Wotton, 1998). The ability of many Chironomidae to adapt to the local environment enables them to proliferate in harsh conditions (Armitage *et al.*, 1995). Chironomids are an important functional component of wetlands (Ali, 1991; Armitage *et al.*, 1995; Hirabayashi, Kubo, Yamaguchi, Fujimoto, Murakami & Nasu, 1998; Hirabayashi & Okino, 2000). Larvae are a food source for higher trophic levels (Hirabayashi *et al.*, 1998) and are important in detrital processing (Ali, 1991).

Chironomids are often the most abundant fauna in lotic and lentic systems, comprising up to 40% of the total fauna (Armitage *et al.*, 1995; Edward, 1986). There are approximately 15,000 species of Chironomidae worldwide (Cranston, 1995a), with 4,000 being described and less than 100 being pestiferous (Ali, 1995). Chironomids have been found from the Antarctic mainland (Cranston, 1995a) to 5,600 metres above sea level in the Himalaya (Saether and Willassen 1987, cited in Armitage *et al.*, 1995). The wide-spread distribution of

chironomids allows managers to use them as indicators of water quality (Ali, 1995; Armitage *et al.*, 1995; Davis & Christidis, 1997; Hilsenoff, 1977, 1982, 1987, cited in Williams & Feltmate, 1992). Historical environmental change can be determined through the assessment of preserved larval head capsules (Armitage *et al.*, 1995; Williams & Feltmate, 1992).

Chironomidae are non biting midges that belong to the order Diptera 'True Flies' (Armitage *et al.*, 1995; Lamb, 2001). They are free living holometabolous insects (Armitage *et al.*, 1995).

Chironomidae species have four life stages: egg; larva; pupa and adult (Figure 1.1.1). The larval stage is the longest and ranges from two weeks to six months (Hein & Mahadeva, 1992). There are four larval instar stages, which consist of the larvae shedding the outer exoskeleton to increase in size (Pinder, 1995). Instar stages can be separated by head width and body length measurements (Maier, Kosalwat & Knight, 1990). The larvae of the midge are often referred to as bloodworms as they possess a red to brown colouring from haemoglobin (Hein & Mahadeva, 1992). The larvae can be free living, or they can construct tubes that are buried in the sediment. Larvae predominantly eat algae (Armitage *et al.*, 1995; Edward, 1964; Lamb, 2001).

The pupal stage is short lived; occurring in the larval tube over two to three days (Edward, 1964; Hein & Mahadeva, 1992; Maier *et al.*, 1990; Pinder, 1986). Once ready to emerge the pupae swim to the surface of the water and in seconds break free from the pupal case (Edward, 1964; Lamb, 2001; Lund, Brown & Lee, 2000; Maier *et al.*, 1990; Pinder, 1986).

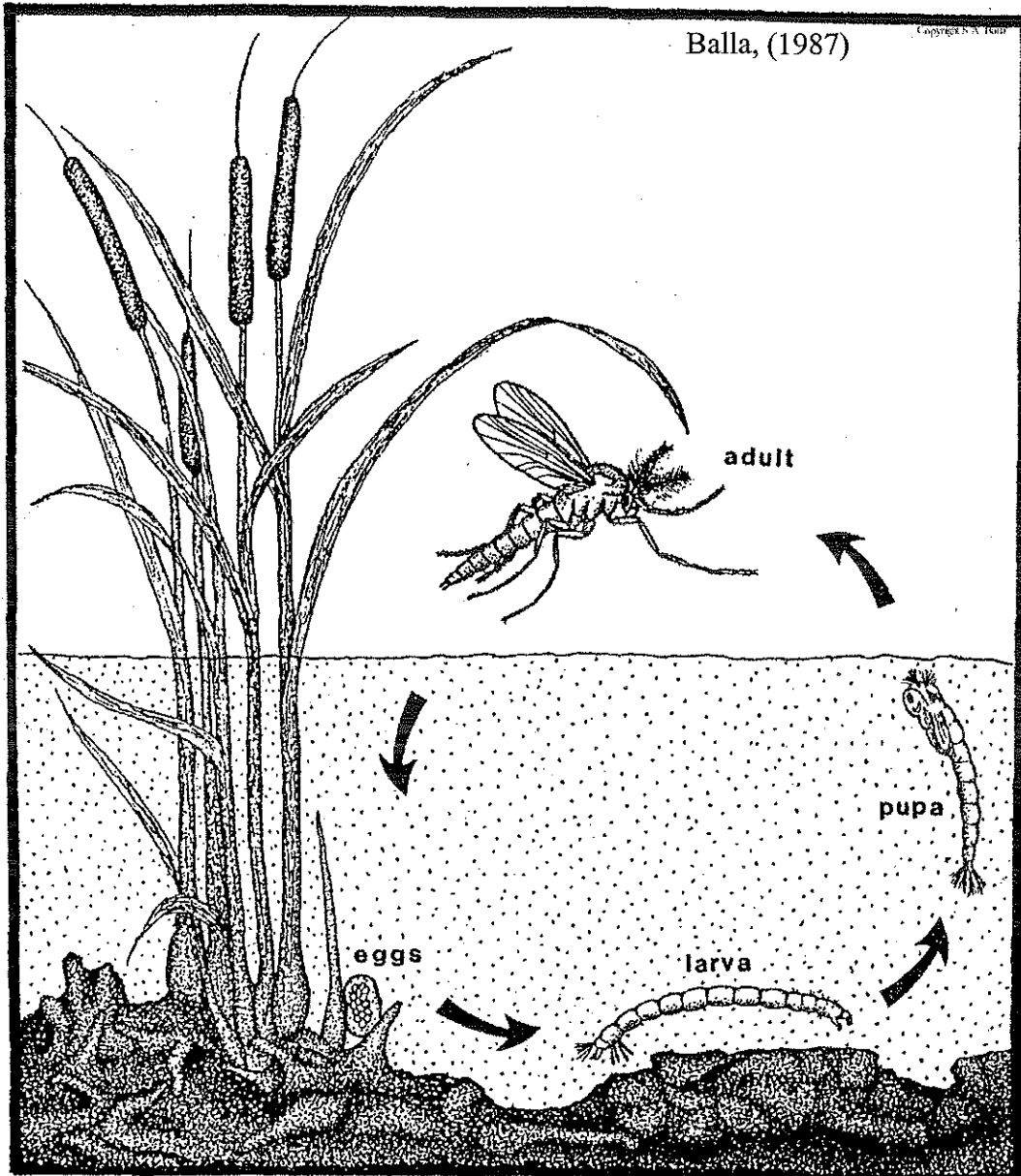


Figure 1.1.1: Typical life cycle of a midge.

Learner *et al.* (1990, cited in Armitage *et al.*, 1995) suggested that emergence patterns were species specific whereas Hilsenhoff (1966, cited in Maier *et al.*, 1990) and Pennak (1978, cited in Maier *et al.*, 1990) said that emergence was more common at dusk, but could occur at any time. Adult midges survive between two to three weeks and is the only stage that is not aquatic (Pinder, 1986). The males form mating swarms above the water (Hein & Mahadeva, 1992) and the females are attracted to the sound of wings beating (Hirabayashi & Nakamoto, 2001; Hirabayashi & Ogawa, 1999; 2000). Once females have mated they lay their eggs on floating substrate or near the shore (Edward, 1964; Lamb, 2001). The females can lay between 20 to 2,000 eggs, which are protected by a gelatinous sheath (Pinder, 1995).

Many species of chironomids show synchronous emergence and a swarming behaviour (Pinder, Trayler & Davis, 1991). It has been estimated that thousands (Pinder *et al.*, 1991) to millions (Armitage, 1995; Lund *et al.*, 2000) of midges occur in these swarms.

Adult midges are poor fliers thus wind direction probably has a great influence on their final location (Lund *et al.*, 2000). In Perth, the strong summer sea breeze blows these swarms in a north-easterly direction. When these mass swarms end up in urban areas they become a nuisance to local residents (Ali, Stanley & Chaudhuri, 1986; Hirabayashi *et al.*, 1998; Hirabayashi & Okino, 2000). Midges are attracted to bright lights and can cause an annoyance by congregating around barbecue areas and outside houses. Certain midge species, such as *Polypedilum nubifer* are small enough to fly through screen doors (Ali, 1991; Tabaru, Moriya & Ali, 1987).

There are gaps in the knowledge of the life cycle of chironomids (Edward, 1964; 1986; Lamb, 2001; Pinder, 1986), in particular how different environmental factors influence midge populations. Lamb (2001) and Edward (1964) suggested that temperature ranges should be studied, to allow for predictive models to be established and temperature ranges determined for nuisance species.

High abundances of certain chironomid taxa is a problem throughout the world (Ali, 1991). Japan (Hirabayashi & Watanabe, 1996), Italy (Ali, 1991), England (Gibson, 1945, cited in Armitage, 1995) and the United States of America (Ali *et al.*, 1986) are some of the countries that have encountered midge plagues. Unusually in Japan, problems began when regulations were put into place to clean up waterways, which were so degraded that very few taxa could survive. Since regulations were implemented and the water quality improved, chironomid problems started to occur (Tabaru *et al.*, 1987).

In Australia, there are scattered reports of nuisance midges from Brisbane, South Australia (Brisbane City Council, 2002) but Western Australia has a history of problems (Pinder *et al.*, 1991). The Perth metropolitan area has more than 450 wetlands (ranging from 21 ha to 592 ha) with 30 of these experiencing problems with midges (Midge Research Group, 2002). Shallow, eutrophic wetlands dominate Perth; many of these provide ideal conditions for Chironomidae species (Davis & Christidis, 1997; Edward, 1986; Hill, Semeniuk, Semeniuk & Del Marco, 1996).

Eutrophication occurs when nutrient enrichment results in plant growth such as phytoplankton or aquatic macrophytes (Cullen, 1986). Eutrophication is a significant problem for urban wetlands (Kinnear, Garnett, Bekle & Upton, 1997). The main cause is pollutants and nutrients from surface run-off and groundwater (Kinnear *et al.*, 1997). Eutrophication can result in poor water quality, which can favour the growth of tolerant species (Ali, 1995). Chironomid larval densities rapidly increase in these environments (Davis, Harrington & Pinder, 1988; Harper, 1992; Pinder *et al.*, 1991). In severely polluted systems chironomids may be the only insect present (Armitage *et al.*, 1995).

Residents located on the fringes of wetlands can experience the problems associated with nuisance midges (Coffman, 1995). Some medical problems have been associated with midges, such as itching of the skin, conjunctivitis and allergic rhinitis (hay fever and asthma) (Cranston, 1995b). Chironomid haemoglobin has been found to be the major allergen associated with these

immunological responses to midges (Baur, 1982 cited in Armitage *et al.*, 1995). Perth has experienced no documented medical problems associated with midges (Cranston, 1995b).

Seven subfamilies of Chironomidae are found in Australia: Telmatogetoninae, Podonominae, Diamesinae, Aphroteniinae, Tanypodinae, Orthocladiinae and Chironominae (Edward, 1986); the last three of these are found on the Swan Coastal Plain (Davis & Christidis, 1997; Edward, 1986). Within these subfamilies *Polypedilum nubifer* (Skuse) (De Deckker & Williams, 1986; Lamb, 2001; Pinder *et al.*, 1991), *Chironomus alternans* (Walker) (Edward, 1964; Lamb, 2001; Pinder *et al.*, 1991), *Procladius villosimanus* (Kieffer) (Lamb, 2001; Pinder *et al.*, 1991) and *C. occidentalis* (Skuse) (Lamb, 2001) (*Syn. C. australiensis* and *C. australis*; Edward, 1964) are pestiferous in Perth wetlands.

Chironomus alternans is a winter species; high numbers of eggs are laid between April and September and adults become prevalent from June to October (Davis, Harrington & Pinder, 1989; Edward, 1964) (Figure 1.1.2). In favourable conditions the larval cycle ranges from 7 to 10 weeks (Edward, 1964). *Chironomus alternans* has been found in at least 32 Perth wetlands (Davis & Christidis, 1997).

Polypedilum nubifer is a summer species able to tolerate temperatures up to 34.5°C (Edward, 1964). It is typically the dominant species from December to March (Davis *et al.*, 1989), but occurs from July to March (Figure 1.1.3). The larval cycle ranges from 3 to 4 weeks at warm, summer temperatures (Davis *et al.*, 1988; Edward, 1964) and 8 to 11 weeks at low, winter temperatures (Edward, 1964). *Polypedilum nubifer* has been found in 30 Perth wetlands (Davis & Christidis, 1997). Edward (1964) found high larval densities in the shallow, warm waters of Lake Monger with no larvae present in the deeper sections of the lake. Predation and temperature were excluded from influencing this distribution (Edward, 1964). Maher and Carpenter (1984, cited in Davis *et al.*, 1988) also found *P. nubifer* to prefer shallower depths. Edward (1964) found that adults did not breed in rearing cages as *P. nubifer* needed to swarm for mating to occur.

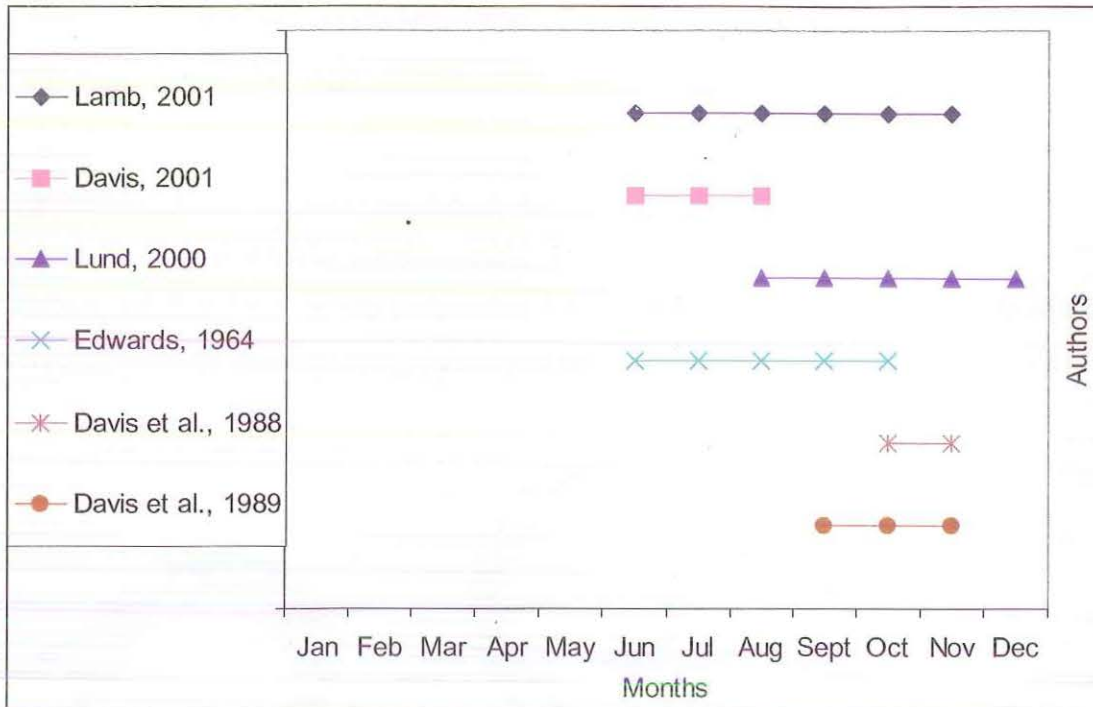


Figure 1.1.2: Times in the year when *C. alternans* has the highest densities.

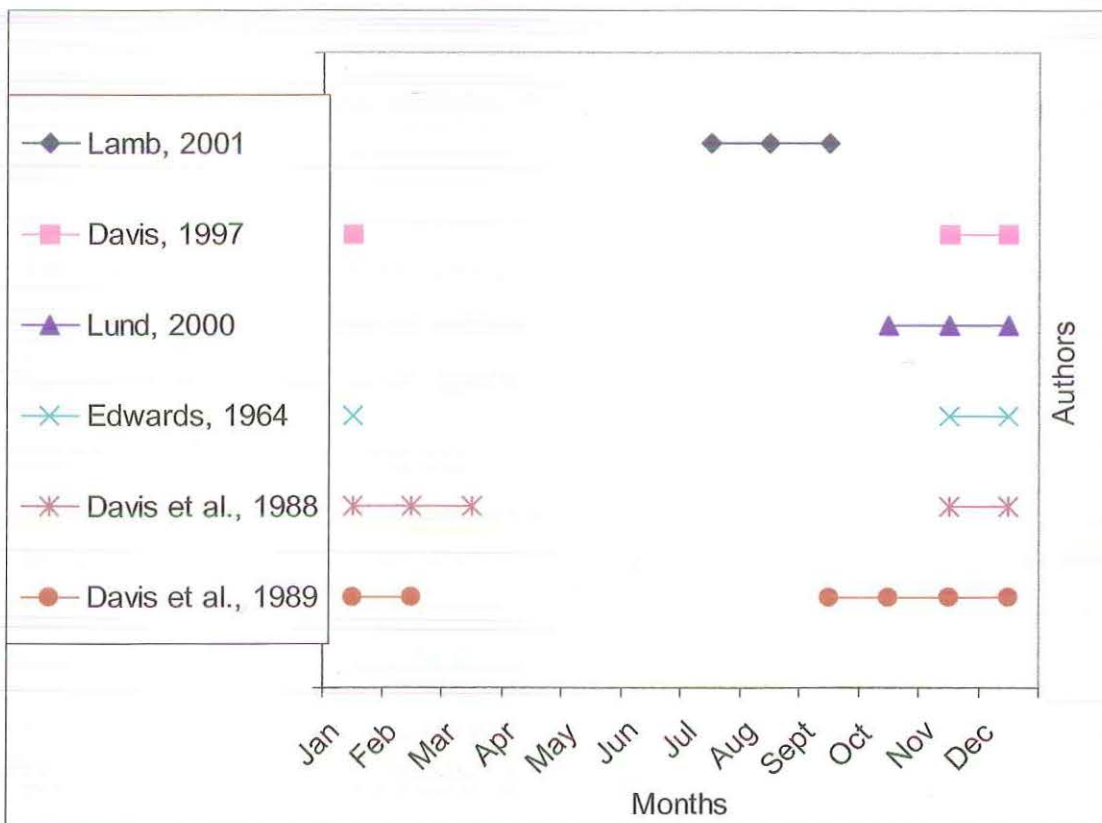


Figure 1.1.3: Times in the year when *P. nubifer* has the highest densities.

Chironomus alternans and *P. nubifer* are multivoltine, having around six to seven generations per year (Edward, 1964). Egg mass shapes can be used to identify species; *P. nubifer* egg masses are 'coil' shaped whereas *C. alternans* are 'globular' (Figure 1.1.4). *Chironomus alternans* and *P. nubifer* are sediment dwelling species, both building tubes out of sediment and algae for protection and feeding (Edward, 1964). They are active swimmers and Edward (1964) observed *P. nubifer* larvae swimming for up to three hours. The life cycle of both species occurs throughout the year and no diapause is evident (Edward, 1964).

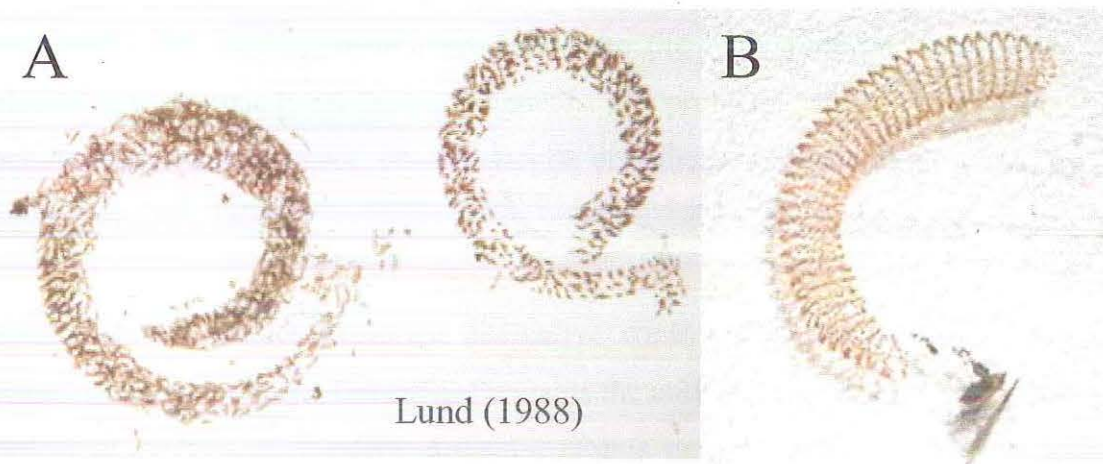


Figure 1.1.4: Comparison between the egg mass shapes of *P. nubifer* (A) and *C. alternans* (B)

Long-term strategies involve re-establishing fringing vegetation around wetlands. This reduces the number of midges flying off the lake, or being blown into residential areas (Lund *et al.*, 2000). Buffer zones around wetlands can reduce the amounts of nutrients in groundwater reaching the wetland (Lund *et al.*, 2000). Reduction in nutrient levels within wetlands appears able to ultimately bring midge numbers back to normal (non-nuisance) levels by reducing potential food sources.

Adult midges are attracted to high intensity lights. Bright lights with traps can be placed in unpopulated locations to distract midges from urban areas (Ali, 1995). Light traps are a short-term solution that may not have a significant impact on

midge numbers. Light traps can malfunction when large swarms of adult midges clog the zappers (Ali, 1995).

Biological controls consist of viruses, fungal pathogens and predators such as water mites and fish. Davis, Christidis, Wienecke, Balla and Rolls (1987) did not regard biological controls as feasible. More research needs to be performed on the effects of biological controls on the natural environment (Ali, 1995).

Davis *et al.* (1988) found that tekmar liquid (*Bacillus thuringiensis* var. *israelensis*) and insect growth regulators were the more preferred controls in relation to their toxicity on the environment. The problems with these strategies include the severe impact on non-target organisms and the high costs of application (Ali, 1995).

Despite several studies exploring alternative control mechanisms for midges, Abate® (active ingredient Temephos) remains the only registered pesticide for the control of midges in Western Australia (Davis *et al.*, 1988). Abate® is an organophosphate that is applied in pellet form to target the larval phase of the midge. Problems associated with chemical controls are the resistance developed over time by the target organism (Ali, 1995; Lund, 2002), the toxicity on the local environment and the effects on non-target organisms.

Chironomids are tolerant to a wide range of environmental conditions (Armitage *et al.*, 1995; Davis *et al.*, 1987; Davis *et al.*, 1988; Pinder *et al.*, 1991; Tokeshi, 1995). The main factors affecting the survival and abundance of Chironomid species are dissolved oxygen (Armitage *et al.*, 1995; Nakazato, Hirabayashi & Okino, 1998; Pinder, 1986; Real, Rieradevall & Prat, 2000), salinity (De Deckker & Williams, 1986; Pinder, 1986), food (Armitage *et al.*, 1995; Edward, 1964; Giberson & Rosenberg, 1992; Hauer & Benke, 1991; Lamb, 2001; Maier *et al.*, 1990; Nakazato *et al.*, 1998; Pinder, 1986; Real *et al.*, 2000), sediment (Edward, 1964; Lobinske, Ali & Frouz, 2002; Pinder, 1986; Real *et al.*, 2000) and temperature (Armitage *et al.*, 1995; Cranston, 1994; Davis & Christidis, 1997;

Davis, Christidis, Wienecke, Balla & Rolls, 1987; Edward, 1964; 1986; Lamb, 2001; Nakazato & Hirabayashi, 1998; Nakazato *et al.*, 1998; Pinder, 1986; Real *et al.*, 2000).

Although salinity can vary in Perth wetlands on a seasonal basis from fresh to brackish (Davis *et al.*, 1987; Lamb, 2001), it is not believed to substantially influence Chironomid abundances within problem wetlands (Lund, 2002; Lund *et al.*, 2000). Chironomid species in Western Australia have been exposed to saline waters for a long time and have the widest recorded tolerance in Australia (Edward, 1986). Most nuisance midge species in the south-west of Western Australia contain haemoglobin, which enables them to tolerate low levels of dissolved oxygen (Armitage *et al.*, 1995; Edward, 1964). Therefore salinity and dissolved oxygen concentrations are not factors needing immediate investigation (Edward, 1964).

Quality of food, rather than quantity appears the most important factor influencing the distribution of larvae (Giberson & Rosenberg, 1992; Lamb, 2001; Nakazato & Hirabayashi, 1998; Real *et al.*, 2000). The effects of temperature on food resources have been studied on several occasions (Edward, 1964; Giberson & Rosenberg, 1992) along with the effects of food quality on growth rates (Cranston, 1994; Edward, 1964; Giberson & Rosenberg, 1992; Nakazato & Hirabayashi, 1998). Lamb (2001) suggested that food did not limit larval development.

There have been contradictory findings as to whether particle size is important for larval tube building. Brennan and McLachlan (1979, cited in Pinder, 1986) found larvae to be unselective with particle sizes, whereas others have found larval distribution to be affected (Dudgeon, 1994, cited in Lamb, 2001; Pinder, 1986). The nutritional benefit of sediments also impacts larval development (Pinder, 1986; Real *et al.*, 2000). Lobinske *et al.* (2002) identified the need for further research on the variables affecting sediment conditions and the impacts this has on larval distribution.

Temperature has been widely reported as affecting the abundance of midge species (Armitage *et al.*, 1995; Edward, 1964; Lamb, 2001; Lund *et al.*, 2000; Pinder, 1986; Real *et al.*, 2000). The effects of temperature on growth rates has not been studied in detail (De Deckker & Williams, 1986; Edward, 1964; Lamb, 2001; Pinder *et al.*, 1991). This is despite Pinder *et al.* (1991) demonstrating that temperature was a good predictor of nuisance plagues in some Perth wetlands.

The life cycle of midge species is impacted by many factors. Different species adapt and thrive in different conditions. Edward (1986) identified the need to document how the life cycle of chironomids responded to local climates.

1.2 Research Aims

The aim of this study is to examine the effects of temperature and sediment type on selected nuisance Chironomidae species in Perth wetlands, to improve the management of nuisance problems.

Specifically, the aims are:

- to determine suitable rearing conditions for *C.alternans* and *P.nubifer*,
- to determine the survival rates and duration of *C.alternans* and *P.nubifer* at different life cycle stages at a range of temperatures, and
- to determine the effects of sediment type and particle size on survival rates and tube building capabilities of *C.alternans*.

The species used in the study were determined through the availability of eggs. *Chironomus alternans* was used in all the experiments due to large quantities of eggs being laid throughout the study. *Polypedilum nubifer* was only used in the hatching experiment and the main experiment, as eggs could only be collected at the end of the study.

Chapter 3 describes an experiment, which examined the hatching rate and survival of eggs for *C.alternans* and *P.nubifer*.

The pilot study (Chapter 4) investigated suitable rearing methods for larvae. The overall research question associated with rearing midges in this study was 'does temperature affect the life cycle of nuisance chironomid species?' Conditions in the pilot study needed improvement to allow temperature to be the main factor affecting survival rates.

Chapter 5 describes an experiment that tested the effects of temperature on survivorship and growth rates. This experiment incorporated the rearing methods developed from the pilot study. An opportunistic collection method was

developed for *P.nubifer*. Due to it being a summer species, and the study being predominantly through winter, the chances of collecting egg masses were low. Enough egg masses were collected at the end of the study to obtain some results for the main experiment.

Chapter 6 details an experiment that determined the effects of sediment type and particle size on growth rates and tube building capabilities of the larvae. Sterilised propagating sand was used and results were compared to the main experiment, which used metaphyton collected from Lake Joondalup. This experiment explored the basic effects of two sediment types.

Chapter 7 describes an experiment that determined whether survival rates changed due to handling of the larvae during the experiment.

CHAPTER 2: STUDY SITE DESCRIPTION

2.1 Study Site Description

Lake Joondalup is situated north of Perth on the Swan Coastal Plain (Figure 2.1.1). Perth experiences a Mediterranean climate, which is characterised by hot, dry summers and cool, wet winters. Lake Joondalup is located in the Yellagonga Regional Park on the Spearwood Dune System, which is characterised by calcareous sands. It is one of the largest freshwater wetlands in the Perth region, being approximately 450 hectares in area (Kinnear *et al.*, 1997). The predominant fringing flora are *Melaleuca raphiophylla* and *Baumea articulata* (Armitage *et al.*, 1995; Kinnear *et al.*, 1997; Lamb, 2001).

Lake Joondalup is a permanent wetland that is both surface and groundwater fed. The water table of the Gnangara Mound's unconfined aquifers feeds into Lake Joondalup and groundwater is fed from surrounding shallow aquifers (Kinnear *et al.*, 1997; Upton, 1996). The southern section of the lake dries out in summer months.

Since European settlement vegetation surrounding Lake Joondalup has been cleared for urbanisation and agriculture (Kinnear *et al.*, 1997). A high usage of fertilisers on land, to increase the production of food crops and to beautify urban and parkland gardens has caused nutrient levels to increase in wetlands (Upton, 1996). A decreased buffer zone around wetlands increases nutrient seepage into these water bodies (CALM, 1980, cited in Upton, 1996). A study of Lake Joondalup has shown it to be 'excessively eutrophic' (Congdon, 1986).

An increase in nutrient rich waters from surface run off, groundwater and stormwater drains results in an increase in the occurrence of algal blooms (Congdon, 1986). Biological diversity is lost with habitat removal and nutrient

enrichment (Davis & Cheal, 1995, cited in Upton, 1996), resulting in the proliferation of tolerant species.

Midges have become a problem to humans since urbanisation has infringed on Lake Joondalup. Urbanisation has focused on development around 'beautiful' water bodies, with little respect to the natural processes occurring in these ecosystems (Kinnear *et al.*, 1997; Lund *et al.*, 2000). Increased nutrient levels provide ideal conditions for midge species to proliferate as it results in increased algae, which larvae use as a food source and habitat (Ali, 1995; Kinnear *et al.*, 1997; Ward, 1992). Lake Joondalup was chosen for the study due to recent midge problems.

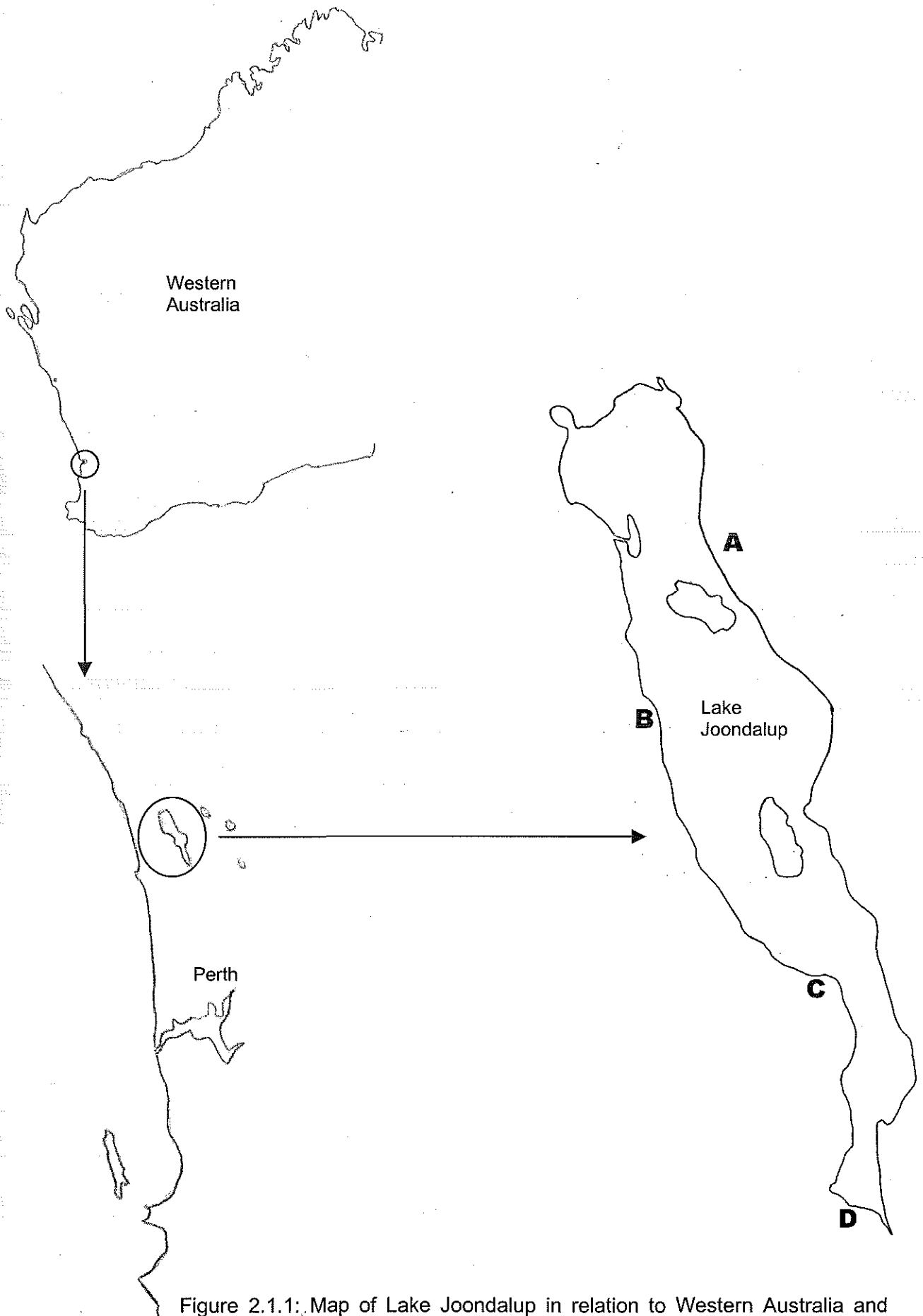


Figure 2.1.1: Map of Lake Joondalup in relation to Western Australia and Perth. A = Banyandah Boulevard, B = Neil Hawkins Park, C = Edgewater Drive, D = McCubbin Boulevard (Adapted from UBD, 2000).

CHAPTER 3: HATCHING EXPERIMENT

3.1 Introduction

Apart from the influences of temperature, embryonic chironomids have near-to-ideal conditions for development; eggs supply protection and a food source (Kokkinn & Williams, 1988, cited in Pinder, 1995). Embryonic development rates are not a good indicator of total development times for chironomids (Pinder, 1995), but need to be known to improve management.

Some species have a diapause phase in the egg stage. Temperature and photoperiod are seen as the main factors controlling diapause (Tokeshi, 1995a). Diapause is a benefit to chironomids as it synchronises adult emergence and allows species to exploit a range of habitats (Tokeshi, 1995a).

Knowing the conditions that are associated with diapause for different species is important for managing midges. The control used for midges in Perth is a larvicide, if a species shows diapause in the egg phase at a critical stage in the control program, resources could be wasted.

This experiment aimed to determine the influences of temperature on the hatching rates of *C.alternans* and *P.nubifer* eggs. The experiment was performed twice for *C.alternans* to ensure results were consistent. *P.nubifer* eggs were in short supply and the experiment could only be done once.

3.2 Methods

Eggs were collected using two Oregon Pine logs (0.3m x 0.1m x 0.045m). Logs were placed into the lake attached to a two metre long rope and secured to the shore (Figure 3.2.1). This has been shown to be a useful method for collecting eggs (J. Vaisey, Water Corporation, pers. comm., 2002). The logs were placed near the shore as this has been found by Pinder (1995) to be where the females lay eggs. The logs were left overnight. Each morning eggs were collected and placed into a jar containing lake water. Once all the eggs were collected the logs were scrubbed to remove any unseen egg masses before reuse. Logs were placed at Banyandah Boulevard (A), Neil Hawkins Park (B), Edgewater Drive (C) and McCubbin Boulevard (D) (Figure 2.1.1).

Egg masses were identified according to shape within one hour of collection (Figure 1.1.2) and were checked under a dissecting microscope for evidence of hatching. Single egg masses were individually placed into a petri dish containing 5mm of filtered lake water (filtered through a 63µm sieve to remove macroinvertebrates and detritus). The petri dishes were kept in incubators set at 15°C, 20°C, 25°C and 30°C. These temperatures cover typical water temperatures in Perth wetlands in summer (20-30°C), autumn (15-25°C), winter (15°C) and spring (15-20°C) (Bureau of Meteorology, 2002).

There were at least three replicates per temperature. The incubators were set at a light:dark regime of 12:12 hours. The eggs were checked daily to detect hatching. Once one egg in each mass started to hatch the number of days since the collection was recorded.

Data analysis was performed on SPSS V11.0 (SPSS Inc., 2002). Data were tested for homogeneity of variance using the Levene's Test. Homogeneous data were then analysed with a oneway ANOVA followed by a Tukey's post hoc test (for significant results only) to determine where the difference lay.



Figure 3.2.1: Photograph of logs used to collect midge eggs.

3.3 Results

A significant difference was found between hatching rates across all temperatures ($p < 0.05$; $f = 100.547$). Hatching times decreased as temperatures increased. Exact time of egg laying was not known due to logs being placed in the water the previous day therefore an additional 24 hours was incorporated into the calculations.

Chironomus alternans eggs hatched at an average (\pm SE) of 5.2 ± 0.2 , 3.5 ± 0.19 , 3 ± 0 and 2 ± 0 days for 15°C to 30°C respectively (Figure 3.3.1). This was the first experiment determining the effects of temperature on hatching rates and there were 8-10 replicates at each temperature.

Eggs at 25°C and 30°C hatched on the second day of incubation for *C.alternans* in the main experiment (Figure 3.3.2). At 20°C and 15°C eggs hatched on the third and fifth days respectively. Standard error was zero and the number of replicates at each temperature was three.

Polypedilum nubifer eggs hatched at an average (\pm SE) of 6 ± 0 , 4 ± 0 , 3 ± 0 and 2.67 ± 0.33 days for 15°C to 30°C respectively (Figure 3.3.3). There were three replicates per temperature.

Overall the average number of eggs per egg mass for *C.alternans* was $602.5 (\pm 32.057)$. Numbers of eggs per mass were not counted for *P.nubifer*.

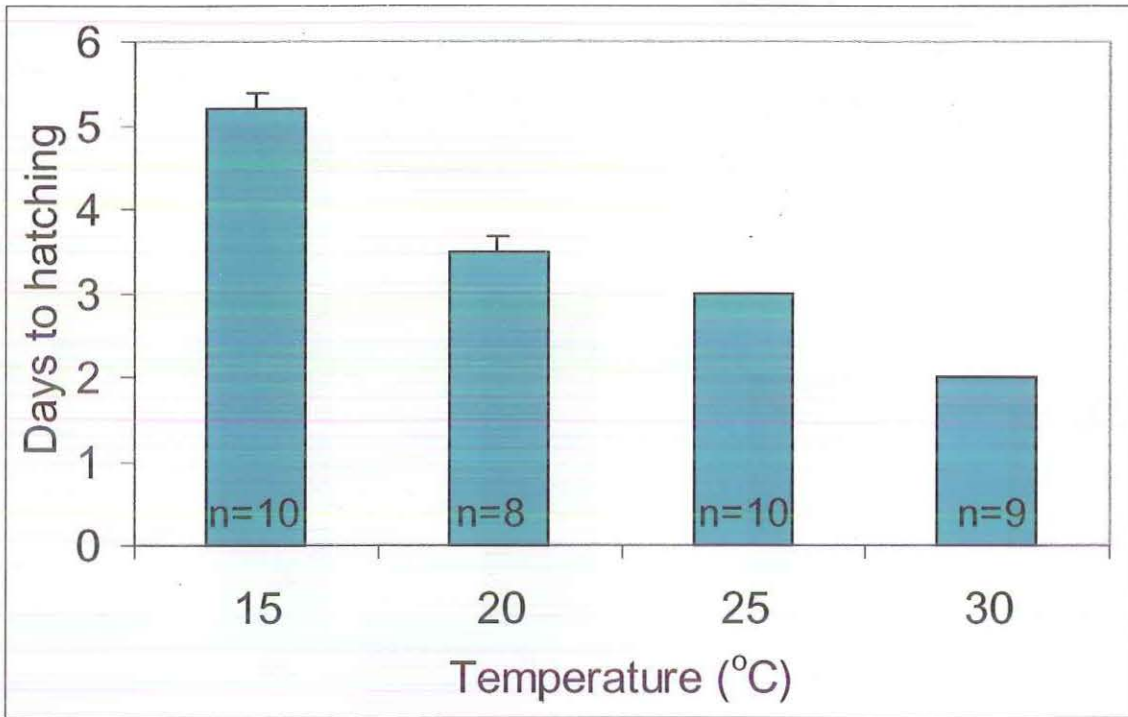


Figure 3.3.1: Mean (\pm SE) hatching rates for *C.alternans* at 15-30°C. n = number of replicates.

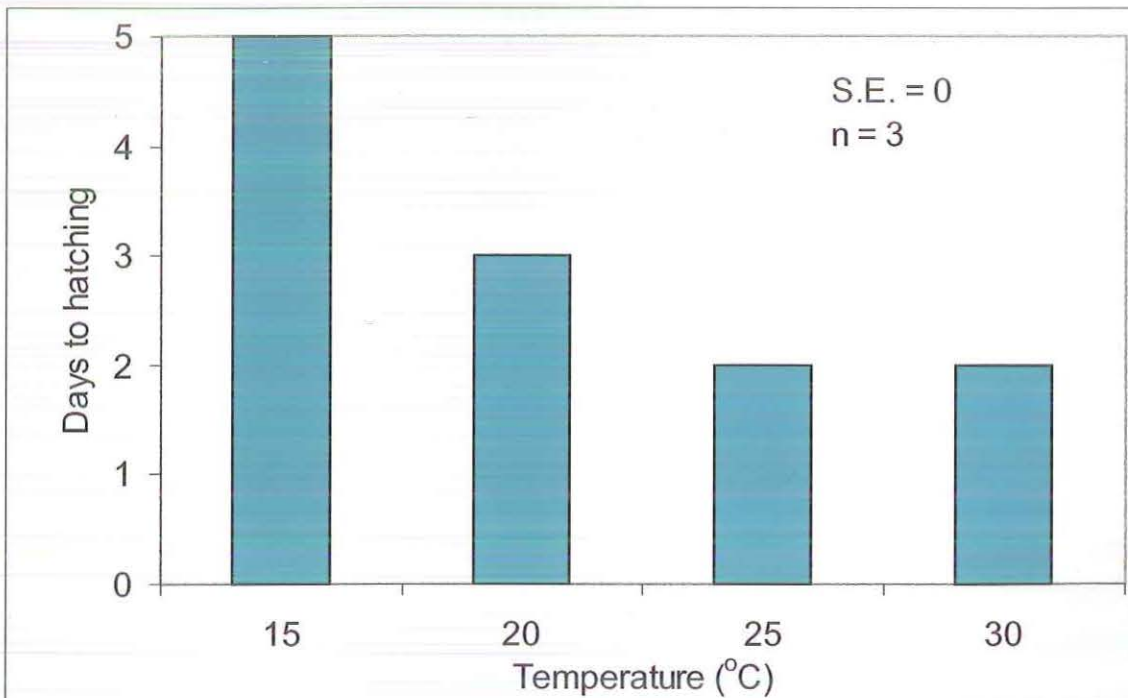


Figure 3.3.2: Mean (\pm SE) hatching rates for *C.alternans* at 15-30°C in the main experiment. n = number of replicates. S.E. = standard error.

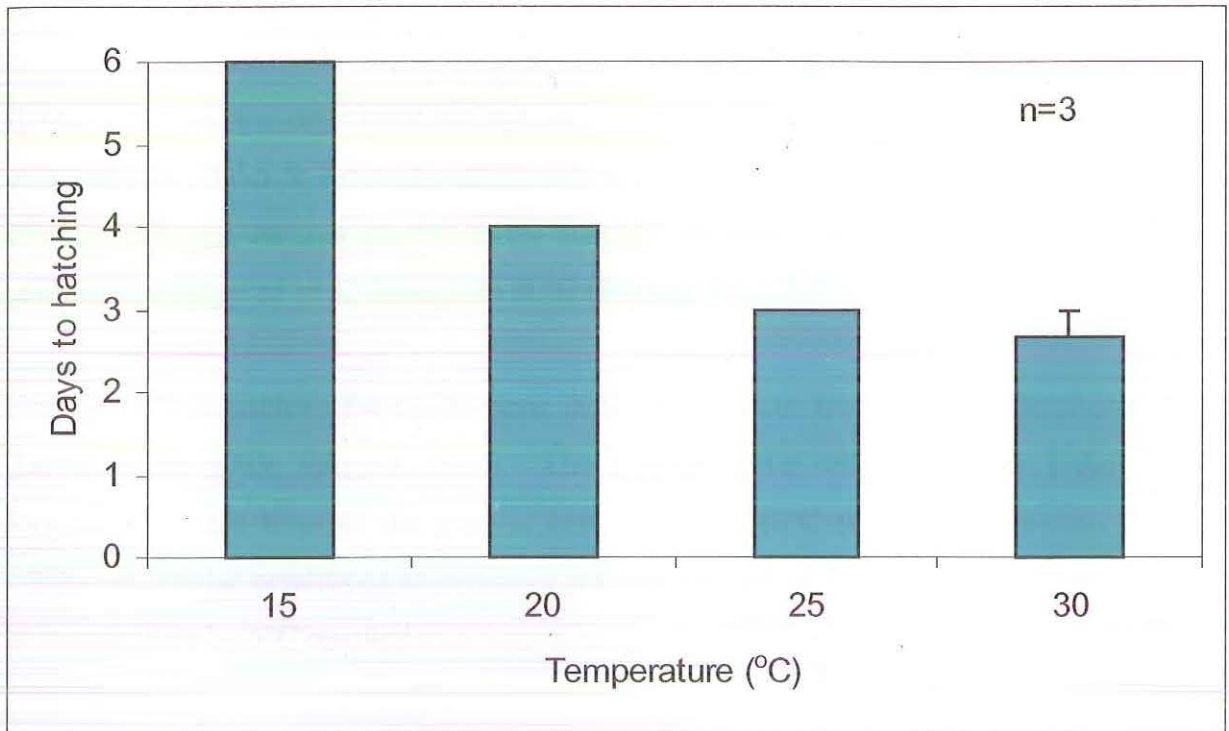


Figure 3.3.3: Mean (\pm SE) hatching rates for *P. nubifer* at 15-30°C. n = number of replicates.

3.4 Discussion

Hatching times are dependent on the temperature with shorter times at higher temperatures (Balci & Kennedy, 2002; Edward, 1964; Maier *et al.*, 1990; Pinder, 1995). All egg masses in this study hatched between two to six days at a temperature range of 15°C through to 30°C (Figures 3.3.1-3.3.3).

Ideal growth conditions for *C.alternans* in the field occur from June to October (Davis *et al.*, 1989; Edward, 1964). The average water temperature for Lake Joondalup at this time of the year is approximately 18°C (City of Wanneroo, 2002). In similar conditions at 20°C egg masses hatched at 3.5 days. A decrease in temperature by 5°C resulted in 1.5 days being added to the hatching time.

Hatching time at 15°C was 5 days for *P.nubifer*. As temperatures increased hatching times became similar between *C.alternans* and *P.nubifer*. *Polypedilum nubifer* prefers summer temperatures (Davis *et al.*, 1989; Edward, 1964). These results indicate that in ideal conditions (~25°C), *P.nubifer* hatches out within two to three days.

Some Chironomidae species have a resting period in the egg, larval or pupal stage of their life cycle (Tokeshi, 1995a). This allows them to survive through harsh conditions while not compromising development. Several studies have shown eggs from specific species to lie dormant at low temperatures (Hilsenhoff, 1966, cited in Maier *et al.*, 1990). All egg masses in this experiment hatched, even at winter temperatures, indicating that for *C.alternans* and *P.nubifer* survival over winter occurs in the larval phase.

Edward (1964) found the number of eggs per mass for *C.alternans* to range from 600 to 830, with an average of 721, which was higher than the 602.5 found in this study. Small species of chironomids have 20 to 30 eggs per mass while large species can have up to 2,000 eggs per mass (Pinder, 1995). In comparison, *P.nubifer* has an average of 567 eggs per mass (Edward, 1964).

CHAPTER 4: PILOT STUDY

4.1 Introduction

There are a range of techniques that have been used to rear midges in the laboratory. The areas of difference include the type of water, aeration, food, photoperiod and substrate type. A range of water types have been successfully used: creek or well water (Jackson & Sweeney, 1995; Stanko-Mishic, 1999), tap water (Balci & Kennedy, 2002; Giberson & Rosenberg, 1992; Maier *et al.*, 1990) and deionised water (Giberson & Rosenberg, 1992). Some studies changed the rearing water (Giberson & Rosenberg, 1992; Hein & Mahadeva, 1992; Maier *et al.*, 1990) while others added water to compensate for evaporation (Balci & Kennedy, 2002; Giberson & Rosenberg, 1992). Several studies used airstones to aerate the water (Balci & Kennedy, 2002; Hein & Mahadeva, 1992; Maier *et al.*, 1990; Stanko-Mishic, 1999). Martin (2002) found that airstones increased the chances of contamination and algal growth, therefore used a hypodermic needle to aerate the water.

Jackson and Sweeney (1995) and Martin (2002) fed larvae algae, while Hein and Mahadeva (1992) used the microbes associated with the organic matter as a food source. Other foods that have been used include a blend of dog food and cerophyll (Maier *et al.*, 1990), pulverised laboratory chow (Giberson & Rosenberg, 1992) and milk powder (Lund, 1988). TetraFin® (Stanko-Mishic, 1999), TetraMin B® (Giberson & Rosenberg, 1992), TetraMin® (Balci & Kennedy, 2002), K9 Fish Food (Frouz, Ali & Lobinske, 2002) and Janos® trout starter (Martin, 2002) were fish foods used to rear larvae.

Photoperiod used in incubators or temperature rooms for rearing larvae were inconsistent between studies. Light to dark regimes ranged from 24:0 hours (Giberson & Rosenberg, 1992; Jackson & Sweeney, 1995) to 16:8 hours (Maier *et al.*, 1990; Stanko-Mishic, 1999) to 14:10 hours (Frouz *et al.*, 2002) to 12:12

hours (Balci & Kennedy, 2002; Jackson & Sweeney, 1995; Martin, 2002) to a variable light regime (Martin, 2002).

Sterilised sands were used as a substrate type in some studies (Balci & Kennedy, 2002; Giberson & Rosenberg, 1992; Jackson & Sweeney, 1995; Stanko-Mishic, 1999). Hein and Mahadeva (1992) used organic matter as a substrate as the associated microbes acted as a food for larvae. Maier *et al.* (1990) used shredded paper towels, as sand was found to erode mouthparts. Martin (2002) used shredded Kleenwipes and toilet paper.

Two studies have previously reared Western Australian species in the laboratory (Edward, 1964; Lund, 1988). Lund (1988) used white sand and tap water in the rearing containers. Larvae were fed milk powder; when the water was clear 0.1g was fed (Lund, 1988). It was found that aeration was unnecessary (Lund, 1988).

A pilot study was conducted to determine the most suitable rearing conditions to use for a Western Australian nuisance species. Several techniques incorporated in the pilot study were improved for the subsequent experiments. This experiment aimed to determine suitable rearing conditions for *C.alternans* and to determine the effects of temperature on the life cycle of *C.alternans*.

4.2 Methods

Three replicate sets of five jars (70ml) filled with 5mm of sterilised propagating sand and tap water were prepared and kept at 15, 20, 25 and 30°C. Eggs were collected as in the hatching experiment (Chapter 3). All the larvae hatching from a single egg mass were split into five jars. Transferring did not take place until the majority of the eggs had hatched.

One jar from each replicate was randomly selected over four intervals to determine survivorship and larval progression through the instars. The fifth jar was kept to allow the larvae to pupate (Figure 4.2.1). The intervals between terminations were estimated from published information about the probable length of the larval cycle (Davis *et al.*, 1989; Edward, 1964). A random number was selected from a random number table to determine which jar to terminate. To determine the suitability of these intervals, random jars were terminated at more frequent intervals to determine larval instar stage. When analysing data, terminations were split into four termination groups: <28days; 29-35 days; 36-42 days; and >43 days. This allowed terminations two and three from the main experiment to be compared to the pilot study.

Water was added to rearing containers to compensate for evaporation. Aeration of the rearing containers was not needed.

Larvae were fed 0.1g of milk powder (1/4 teaspoon of milk powder to 30ml of water) per day (developed from Lund, 1988). Feeding continued through the larval phase and was based on the clearness of the water; the more turbid the water the more milk powder was present, and less was fed.

A light to dark ratio of 12:12 hours was used in the temperature incubators. Three incubators were available for use and were set at 15°C, 25°C and 30°C. A temperature room was available at 20°C. Temperatures were monitored regularly to ensure they remained constant.

To improve rearing conditions an additional experiment was developed to determine the best type of water to use. Lake water was collected from Lake Joondalup, filtered and compared to dechlorinated tap water. Three replicates were put at 20°C. The remaining conditions were the same as the initial part of the pilot study.

To determine whether the feeding regime impacted on larval success, another commonly used food type TetraMin[®] fish food was trialed (Giberson & Rosenberg, 1992). A small pilot study was conducted to determine whether milk powder (Lund, 1988) or TetraMin[®] fish food had higher survival rates. Milk powder and TetraMin[®] fish food were used as methods in the literature gave exact amounts to feed larvae. The feeding regime for milk powder was the same as the initial section of the pilot study. The TetraMin[®] fish food had five grams of flakes ground with a mortar and pestle; this was added to 100mls of MilliQ deionised water (DDI) (Balci & Kennedy, 2002). Each week fish food solution was made up and 0.5ml was fed to larvae on Mondays and Wednesdays and 1ml was fed on Fridays. Three replicates were put at 20°C. The remaining conditions were the same as the initial part of the pilot study.

The third pilot study involved determining the effects of sediment type on larval survivorship. Sterilised propagating sand was compared to a combination of metaphyton and sand. The sterilised propagating sand was bought from a nursery. Metaphyton is a natural habitat and source of food for larvae (Lamb, 2001). The metaphyton and sand combination was collected from Lake Joondalup at Edgewater drive (Figure 2.1.1). A dip net was swept over the top of the sediment and the contents were placed into a bucket. The sediment was air-dried, eliminating chironomids and other moisture dependent organisms. Five millimetres of each sediment type was added to each of three replicate jars at 20°C. The remaining methods were the same as the initial part of the pilot study.

When terminating an experiment all surviving larvae were separated from the sediment and preserved in five percent Formaldehyde. Larval head widths were measured using an oculi graticule lense. At each magnification level it was determined how much the measurements on the oculi graticule lense were in millimetres by using a measuring graticule slide. Head capsules are hard and remain a constant size for each larval instar, thus can be measured to determine which instar stage the larvae belongs to (Lund, 1988). Once all larvae were measured, the largest was selected and mounted on a slide for identification. Identification was confirmed by reference to the voucher collection of Mark Lund at Edith Cowan University and according to Davis and Christidis (1997).

Data analysis was performed on SPSS V11.0 (SPSS INC., 2002). All data were percentages, and therefore were arcsine transformed. Data were tested for homogeneity of variance using the Shapiro-Wilk test. If data were homogeneous then an independent-group t-test was used to determine if a significant difference existed. If data were not homogeneous, then a non-parametric test (Mann-Whitney U Test) was used to determine significance.



Figure 4.2.1: Photograph of the pilot study.

4.3 Results

There was a significant difference between temperature and survival rates for *C.alternans* ($p<0.05$; $\chi^2=16.463$) (Figure 4.3.1). The highest survival rate was 40.5% (± 0.01) at 15°C. Survival was low at 20°C (8.8% ± 0.07), and then gradually increased at 25°C and 30°C (9.9% ± 0.05 and 16.6% ± 0.08 respectively).

Head widths were grouped into instar stages, to depict the four larval stages of the midge; instars one to four had a range of 0-0.12mm, 0.12-0.22mm, 0.22-0.38mm and >0.38mm respectively (Lund, 1988). All instar stages for *C.alternans* were represented in the pilot study (Figure 4.3.2). The majority of larvae had head widths in the second and third instar stages. The highest survival rate was at 15°C therefore the greatest number of larvae measured were from that temperature.

Survival rates for lake water were much higher than for tap water at 65.27% (± 0.075) and 1% (± 0.006) respectively ($p<0.05$; $F=4.757$) (Figure 4.3.3). Survival rates for TetraMin® fish food and milk powder at 59.13% (± 0.054) and 10.13% (± 0.017) respectively (Figure 4.3.4) were significantly different ($p<0.05$; $F=12.162$). Larvae in metaphyton had higher survival rates than those in sand. There was a significant difference between survival rates for metaphyton (22.05%, ± 0.047) and sterilised propagating sand (5.5%, ± 0.038) ($p<0.05$; $F=3.825$) (Figure 4.3.5).

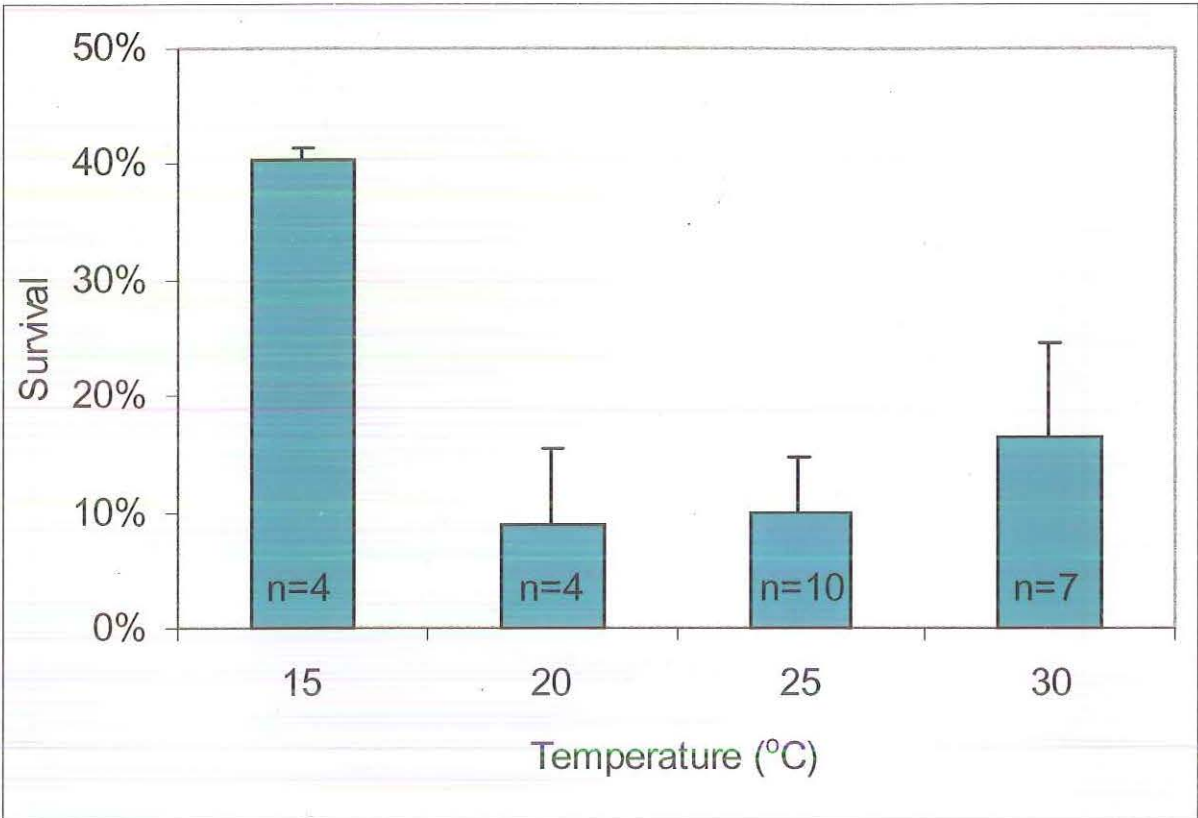


Figure 4.3.1: Survival rates at 15-30°C for the pilot study. n = replicates.

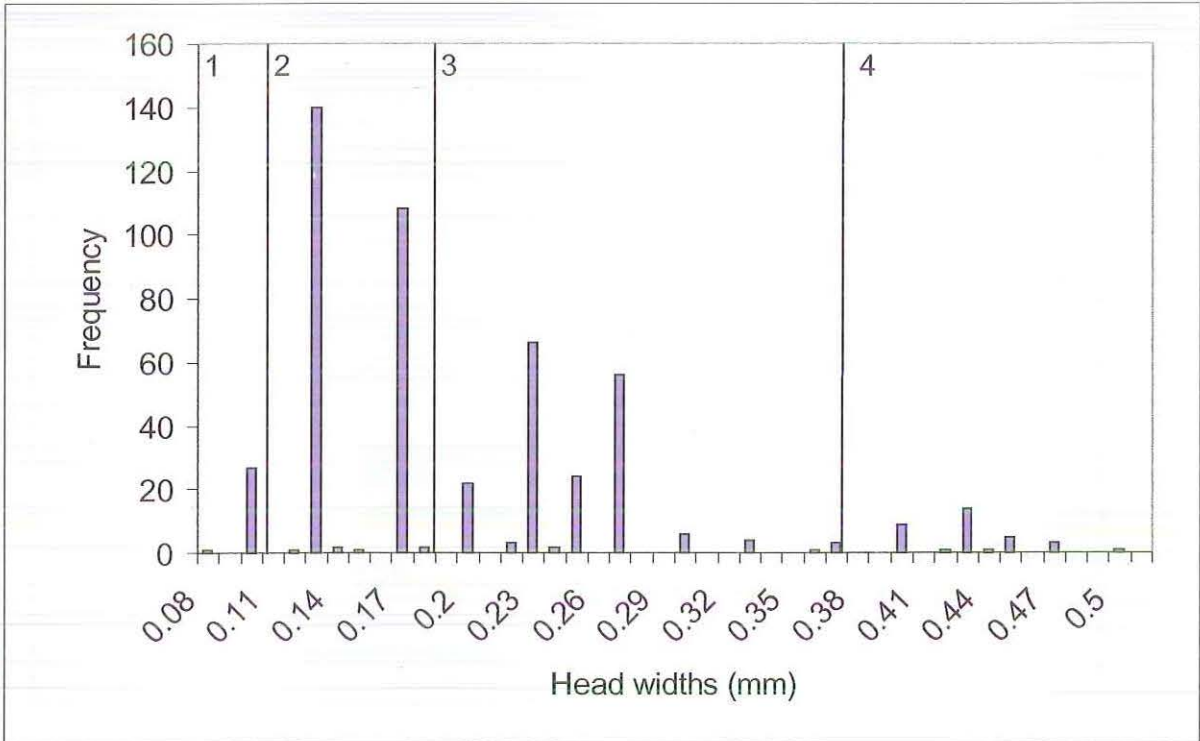


Figure 4.3.2: Frequencies of head widths for the pilot study. 1-4 = instar stages.

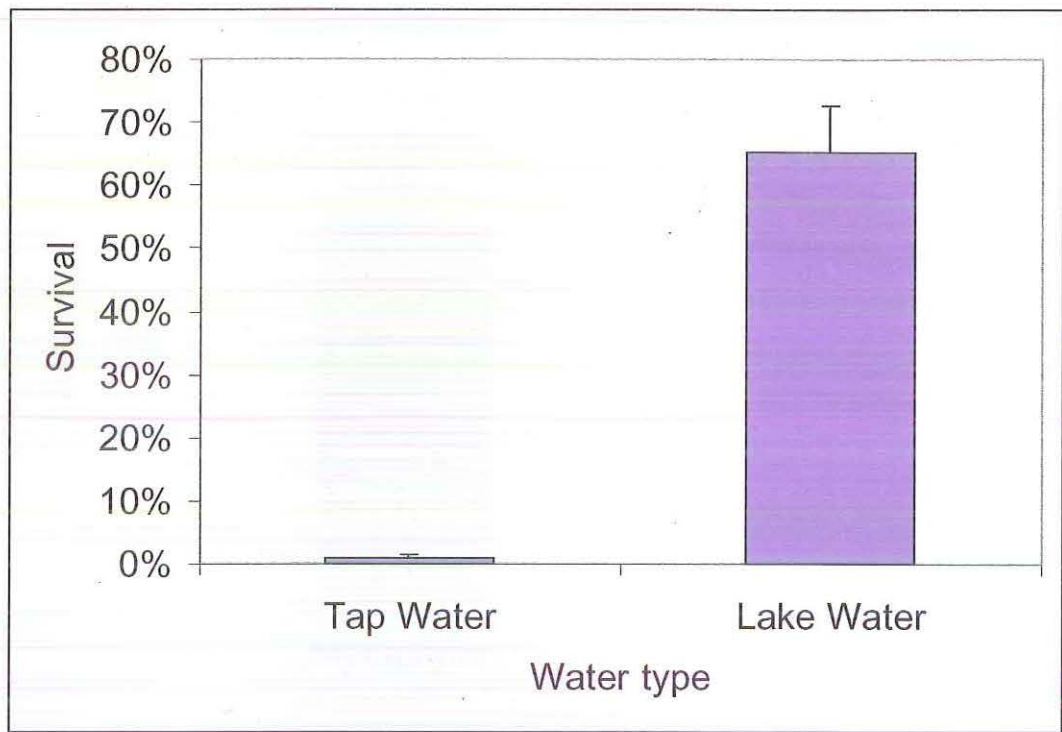


Figure 4.3.3 Survival compared between tap water and lake water for *C.alternans* at 20°C.

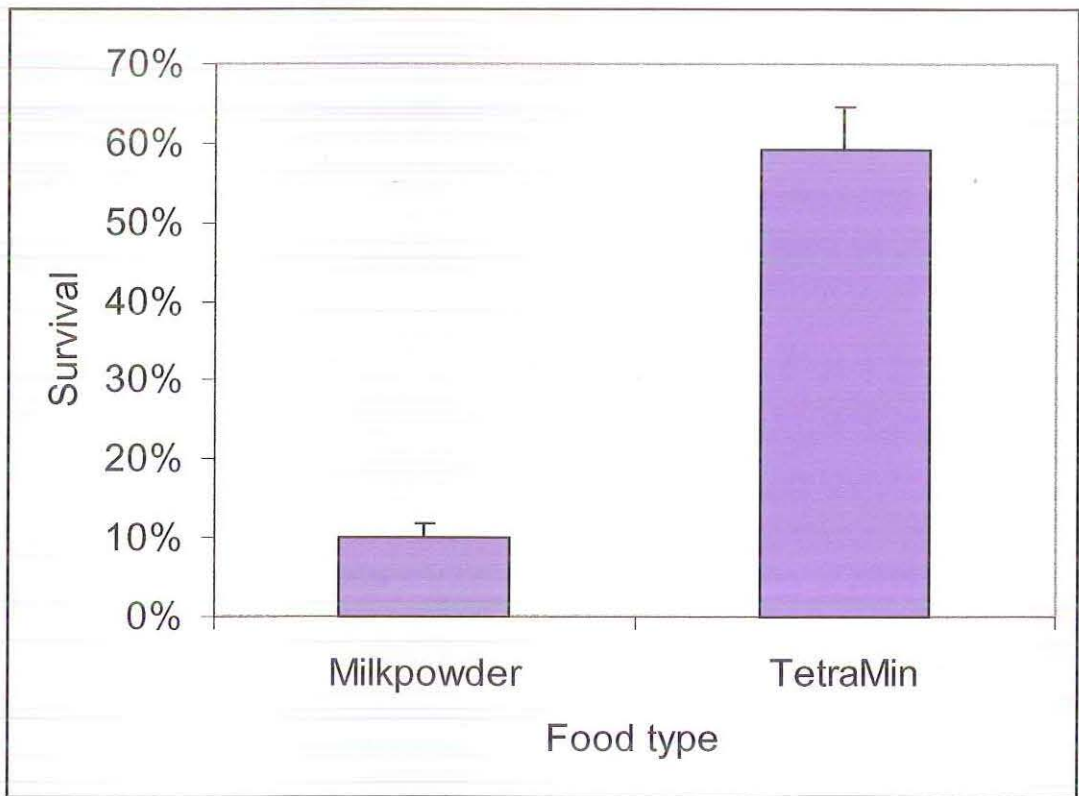


Figure 4.3.4 Survival compared between milk powder and TetraMin[®] fish food for *C.alternans* at 20°C.

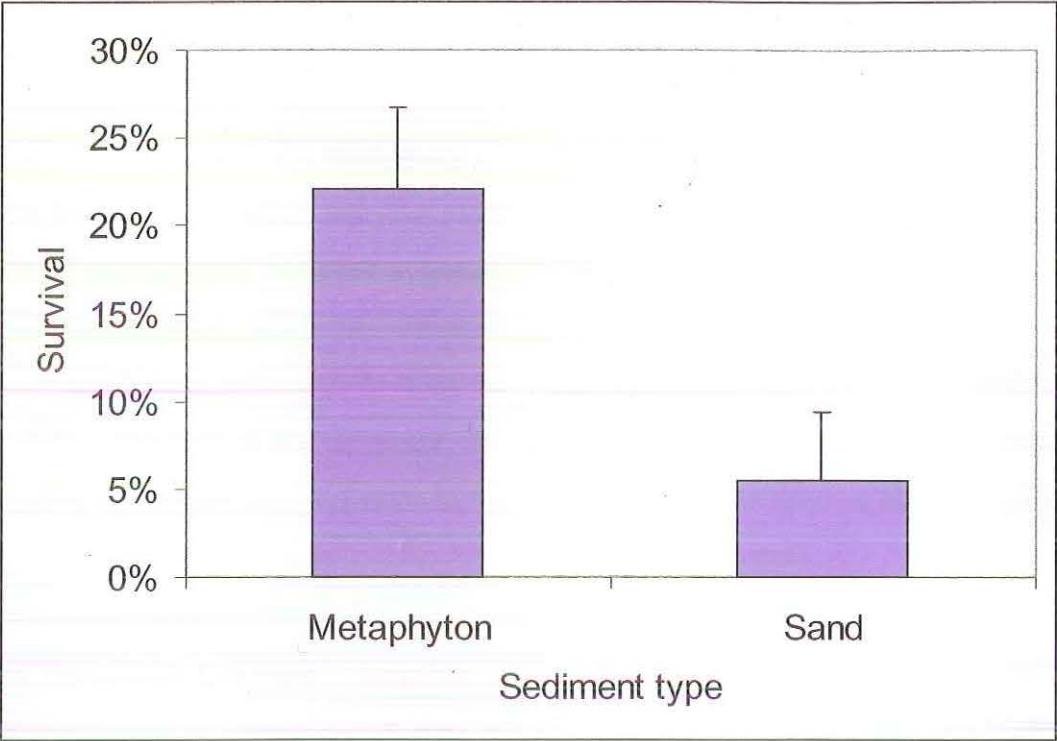


Figure 4.3.5 Survival compared between metaphyton and sterilised propagating sand for *C.alternans* at 20°C.

4.4 Discussion

Low survival rates in the pilot study (Figure 4.3.1) indicated that conditions may have been unfavourable for the growth of *C.alternans*. Specifically, results showed that survival was poor at temperatures greater than 15°C.

Hatching success was high in both tap water and lake water but after hatching mortality was high in the tap water, the larvae did not make it past the first instar stage. Due to high survival rates in the lake water, it was used in all subsequent experiments (Figure 4.3.3).

The Tetramin® fish food had the highest survival rates and was therefore used in the subsequent experiments (Figure 4.3.5). It is unknown whether the higher survival in the TetraMin® fish food related to the nutritional value of the food itself, the particulates or the fungi and bacteria associated with the fish food. A natural food source such as algae should be used in future studies. This would enable food levels to be compared to the field.

Larvae only grew for a short period of time in the propagating sand before they died (Figure 4.3.4). The silty metaphyton allowed larvae to build tubes, which is a natural habit for *C.alternans* (Edward, 1964). Success was greater in the metaphyton/sand combination and was used subsequently. It is suspected that particle size may be an important factor accounting for these results.

Densities were around 12,000 larvae per m² in the pilot study. When mass emergence occurs and adult midges first become a nuisance to humans, the density in the lake sediment on average is >2000 larvae per m² (Lund, 2002). High densities may have limited resources and increased competition, this could have affected survival rates. In all subsequent experiments a density of 2,000 larvae per m² was used. To achieve this density and still rear around 100 larvae per rearing container, larger containers were used in subsequent experiments.

CHAPTER 5: MAIN EXPERIMENT

5.1 Introduction

Temperature has been found to be one of the main environmental factors influencing the growth of aquatic insects (Vannote & Sweeney, 1980, cited in Ward, 1992). Temperature can influence the availability of food through decomposition rates (Ward, 1992). Growth rates can be influenced by temperature through ingestion and assimilation rates (Williams & Feltmate, 1992).

Various species respond to different temperature regimes. *Chironomus alternans*, a cool weather species (Edward, 1964) may show high mortality and fast growth rates at high temperatures, whereas, *P.nubifer*, a warm weather species (Edward, 1964) may show low mortality and fast growth rates at high temperatures. Environmental temperatures may directly affect adult size and fecundity (Vannote & Sweeney, 1980, cited in Williams & Feltmate, 1992). An increase in temperature may result in quicker emergence and smaller individuals (Maier *et al.*, 1990). Distribution can also be affected by temperature. *Polypedilum nubifer* is found in shallow areas of wetlands as water temperatures are higher than in the deep areas (Edward, 1964).

This experiment aims to determine the survival and duration of *C.alternans* and *P.nubifer* at different life cycle stages at a range of temperatures.

5.2 Methods

Metaphyton and sand were collected from Lake Joondalup as per the pilot study (Chapter 4), the sediment was dried at 70°C for three days, then sterilised in an autoclave for 20 minutes at 121°C. The metaphyton and sand were ground to a consistent size (0.125mm). Each container (105 x 165 x 65mm) contained 100 (± 10) larvae, 10mm of sediment and 500ml of filtered lake water (Figure 5.2.1). Evaporation rates were high and DDI was added to containers as required to maintain levels. The fish food TetraMin® Tropical Flakes was made up weekly and fed to larvae (refer to pilot study, Chapter 4).

If and when pupation began the pupae were transferred into a jar with a plastic bottle connected to the top (Figure 5.2.2). The bottles had panels cut out of the sides and fly wire secured around the panels to allow for ventilation. The adults were able to fly into the bottle, preventing them from drowning. Adults, larvae and pupa terminated through the experiment were preserved in 70% ethanol.

Eggs were collected as in the hatching experiment (Chapter 3). All other methods were the same as the pilot study (Chapter 4).

There were three containers per replicate and three replicates per temperature. Each container at each replicate was terminated over the course of the experiment. Terminations occurred at 2.5, 3.5 and 4.5-6 weeks; the final termination was determined by adult emergence. These termination intervals were developed from the pilot study (Chapter 4). Once all living larvae were removed from the container the water was filtered through a 0.45 μ m glass fibre filter paper (Metriguard™), then frozen for analysis. Terminated larvae had their head widths and body lengths measured. Head widths were measured at the widest section of the head capsule. Body lengths were measured from the tip of the head capsule to the anal tubules.

The water samples were later analysed for ammonium (NH_4), nitrate/nitrite (NO_x) and filterable reactive phosphorous (FRP). Methods for NH_4 analyses were as per Holmes, McClelland, Sigman, Fry and Peterson (1998). The working reagent and stock solution were made up three weeks prior. When making up solutions for matrix effects, dilutions of the samples occurred after the standard was added.

Nitrate/nitrite and FRP methods were as per the American Public Health Association (1995). Methods were modified to work on a 'Skalar Segmented Flow Analyser'. Samples were corrected for dilution, baseline drift and standard drift.

Data analysis was performed on SPSS V 11.0 (SPSS Inc., 2002). Data were tested for homogeneity of variance using the Levene's Test. If data were homogenous then a oneway ANOVA with a post hoc test was used. Tukey's post hoc test was used to determine where differences lay.

Any heterogenous data were transformed by $\log_{10}(x+1)$. All percentages were transformed before analysis using Arcsine. If data were not homogeneous after transformation non-parametric tests were used. A Kruskal-Wallis test was used and if a significant difference was found a Mann-Whitney U Test was conducted to determine where the difference lay.

A correlation was used to determine if there was a significant relationship between head width and body length measurements.



Figure 5.2.1: Photograph of the main experiment.

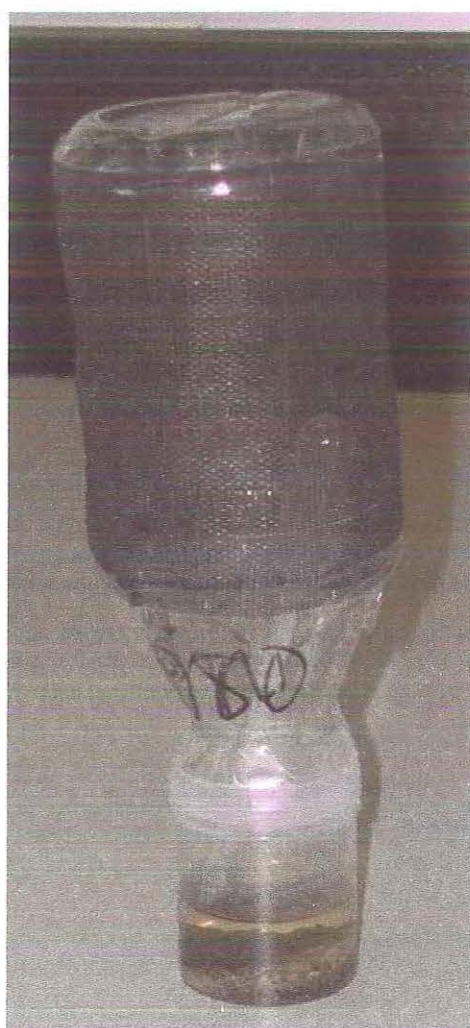


Figure 5.2.2: Photograph of an emergence bottle.

5.3 Results

For both *C.alternans* and *P.nubifer*, there was a significant difference in survival between temperatures ($p<0.05$; $\chi^2=28.048$). Statistical analysis showed a significant difference between survival rates for *C.alternans* between 15°C and 25°C, 15°C and 30°C, 20°C and 25°C, 20°C and 30°C ($p<0.05$). Differences for *P.nubifer* were between 20°C and the remaining temperatures ($p<0.05$).

Average survivorship for *C.alternans* was highest at 20°C with 78% (± 0.05), compared with 62% (± 0.07), 9% (± 0.1) and 4% (± 0.007) at 15, 25 and 30°C respectively (Figure 5.3.1). Survivorship for *P.nubifer* was highest at 20°C with 22.5% (± 0.07). An average survival rate of 0.20% (± 0.02) occurred at 25°C, while 15°C and 30°C had no surviving larvae (Figure 5.3.2).

Survival rates of *C.alternans* at 15°C changed from 57% to 30.2% to 73% from termination one to three respectively, indicating high variability between containers (Figure 5.3.3). Survival rates at 20°C decrease with time (88%, 79.2% & 62% respectively). The first two terminations for 25°C have surviving larvae with termination one having 13% survival and termination two having 1.3% survival. Survival at 30°C only occurred in the first termination with 3% of larvae surviving.

There was a strong relationship between head widths and body lengths of *C.alternans* ($p<0.05$; $r^2=0.823$) (Figure 5.3.4). Large body lengths had large head widths. Larvae were in the second, third and fourth instar stages, with the majority of larvae in the fourth instar.

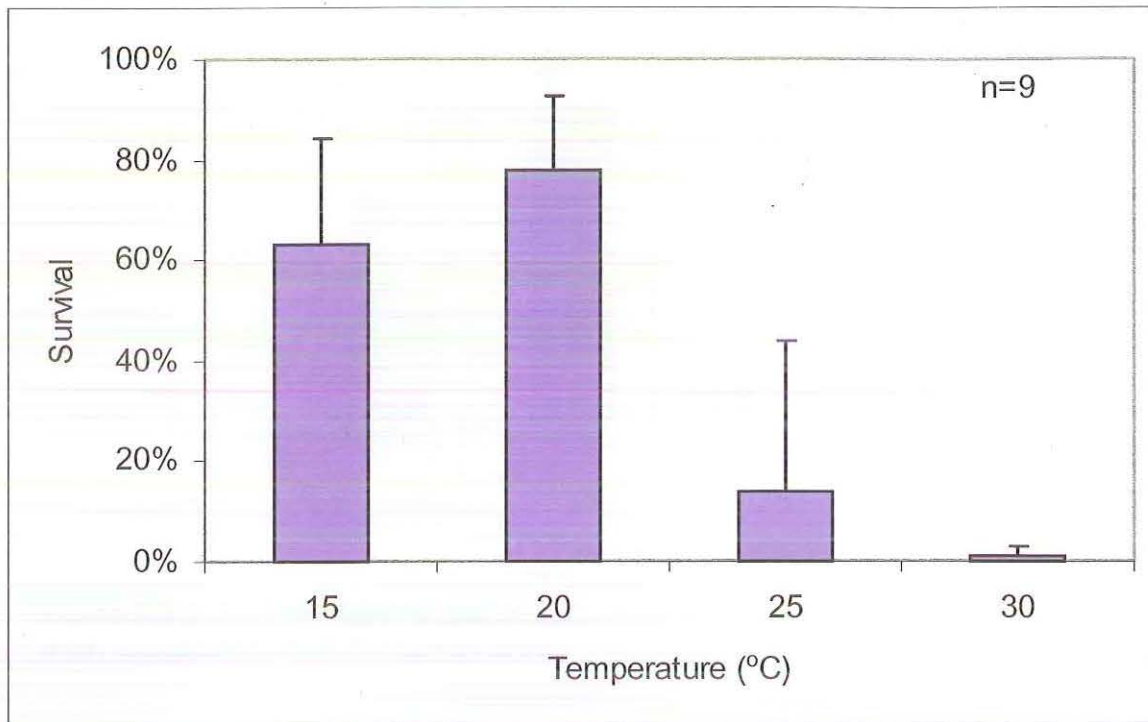


Figure 5.3.1: Survival rates of *C.alternans* at 15-30°C in the main experiment. n = number of replicates.

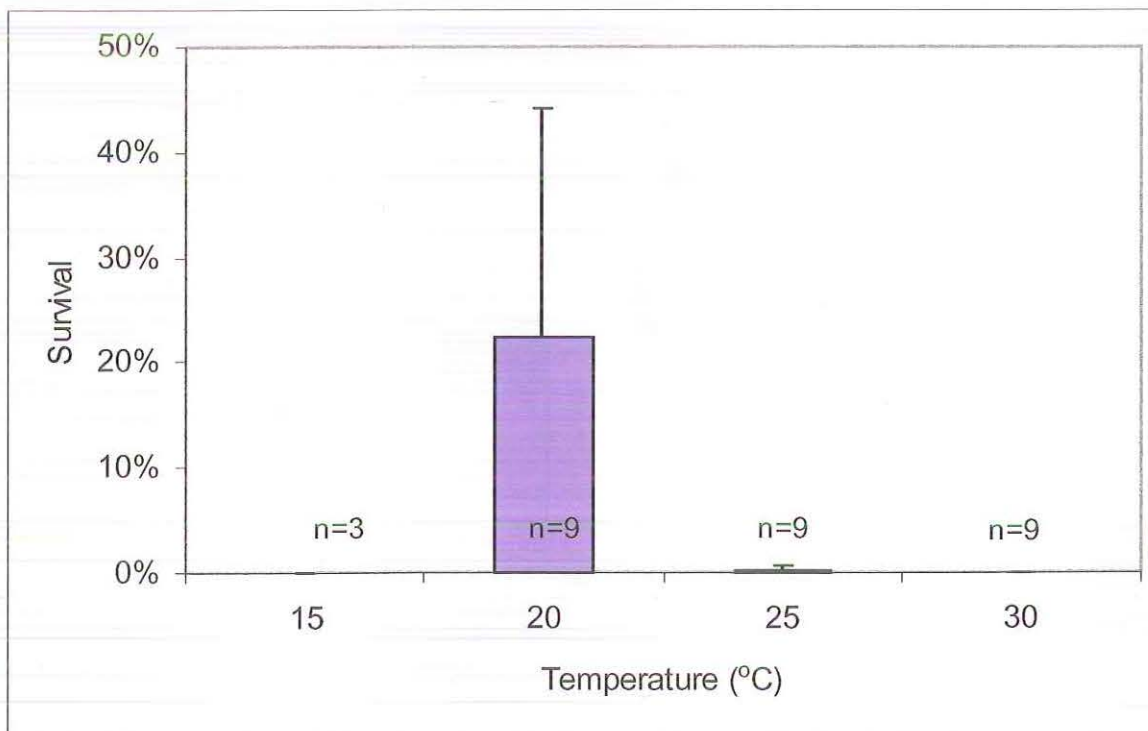


Figure 5.3.2: Survival rates of *P.nubifer* at 15-30°C in the main experiment. n = number of replicates.

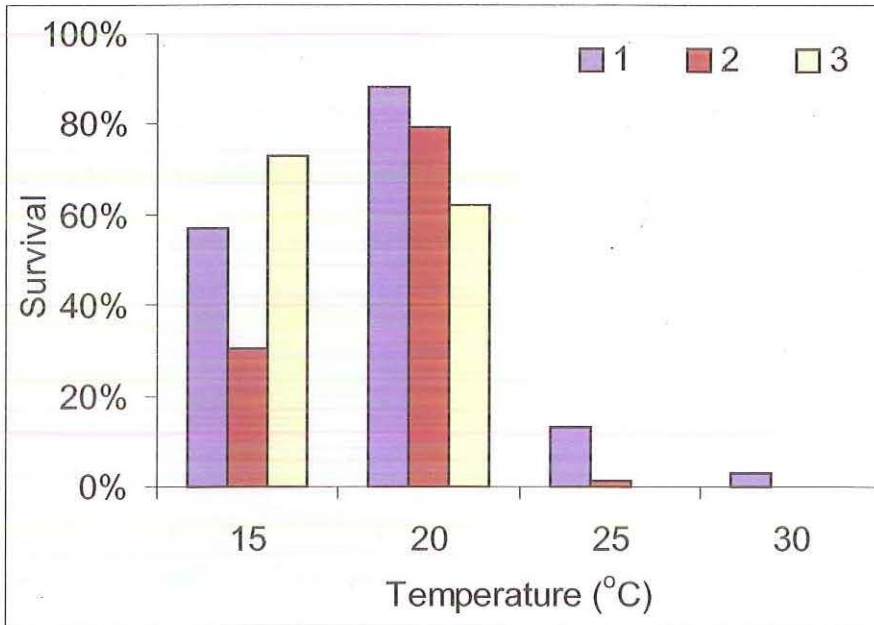


Figure 5.3.3: Survival rates of *C. alternans* at the three terminations at 15-30°C in the main experiment. 1, 2, 3 = terminations.

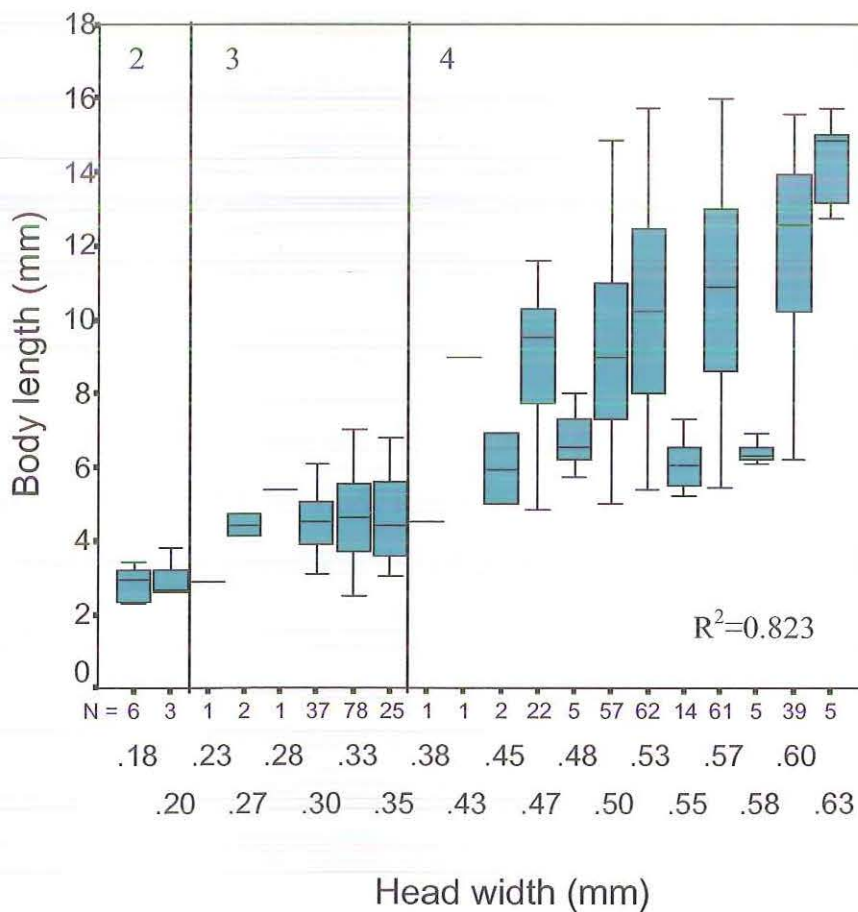


Figure 5.3.4: Head widths and body lengths for *C. alternans* in the main experiment. 2-4 = instar stages.

Larval head widths at 15°C ranged from 0.18mm to 0.63mm, while body lengths ranged between 2.27mm to 15.71mm (Figure 5.3.5). The first and second terminations contained multiple but similar instar stages, with the first termination having second and third instars and the second termination having third and fourth instars. The third termination had larvae in the fourth instar.

Larval head widths at 20°C ranged from 0.3mm to 0.6mm, while body lengths ranged from 3.37mm to 15mm (Figure 5.3.6). The first and second terminations had larvae in the second and third instar stages while the third termination had larvae in the fourth instar.

All larvae at 25°C were in the fourth instar at the first termination (Figure 5.3.7). Measurements were only taken for two terminations, as larvae did not survive past the second termination. Head widths for 25°C ranged from 0.47mm to 0.53mm and body lengths ranged from 6.14mm to 13.57mm.

There were low survival rates at 30°C in the first termination and zero survival in the second and third terminations so comparisons between terminations could not be made.

Slow growth rates were evident at 15°C (Figure 5.3.5) compared to 20°C (Figure 5.3.6) and 25°C (Figure 5.3.7). Body length measurements in the final termination for 15°C were generally larger than the other temperatures. Overall, the majority of larvae were in the fourth instar stage.

Comparisons between terminations could only be made at 20°C for *P.nubifer* (Figure 5.3.8). The other temperatures had very low to no survival through the terminations so comparisons could not be made. *Polypedilum nubifer* were classified into first to fourth larval instars according to head widths of <0.1mm, 0.1-0.19mm, 0.19-0.31mm and >0.31mm respectively (Davis *et al.*, 1988; 1989; Edward, 1964). Larvae measured represented the second to fourth larval instars.

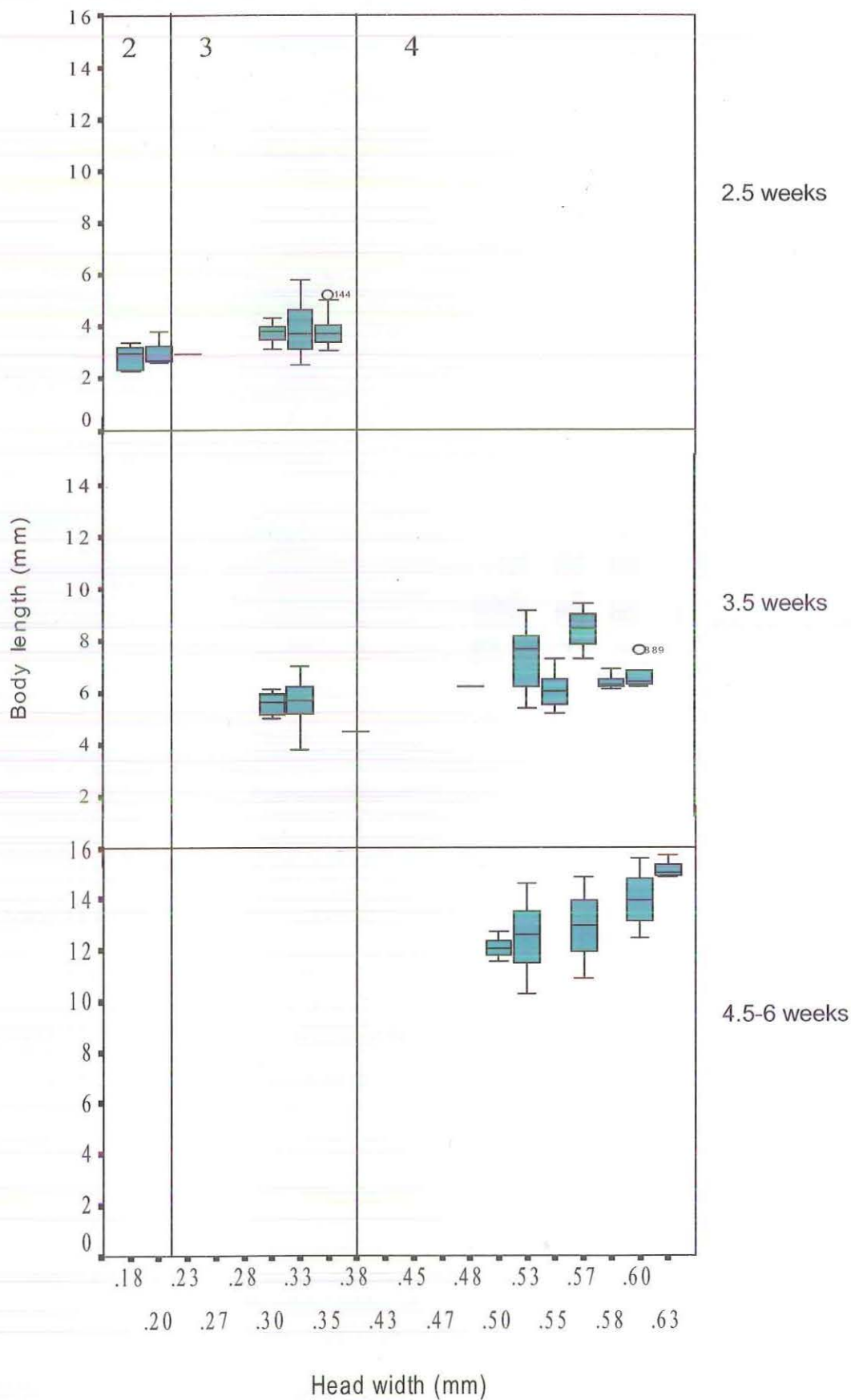


Figure 5.3.5: Head widths and body lengths through three termination intervals for *C.alternans* at 15°C in the main experiment. 2-4 = instar stages.

Main Experiment

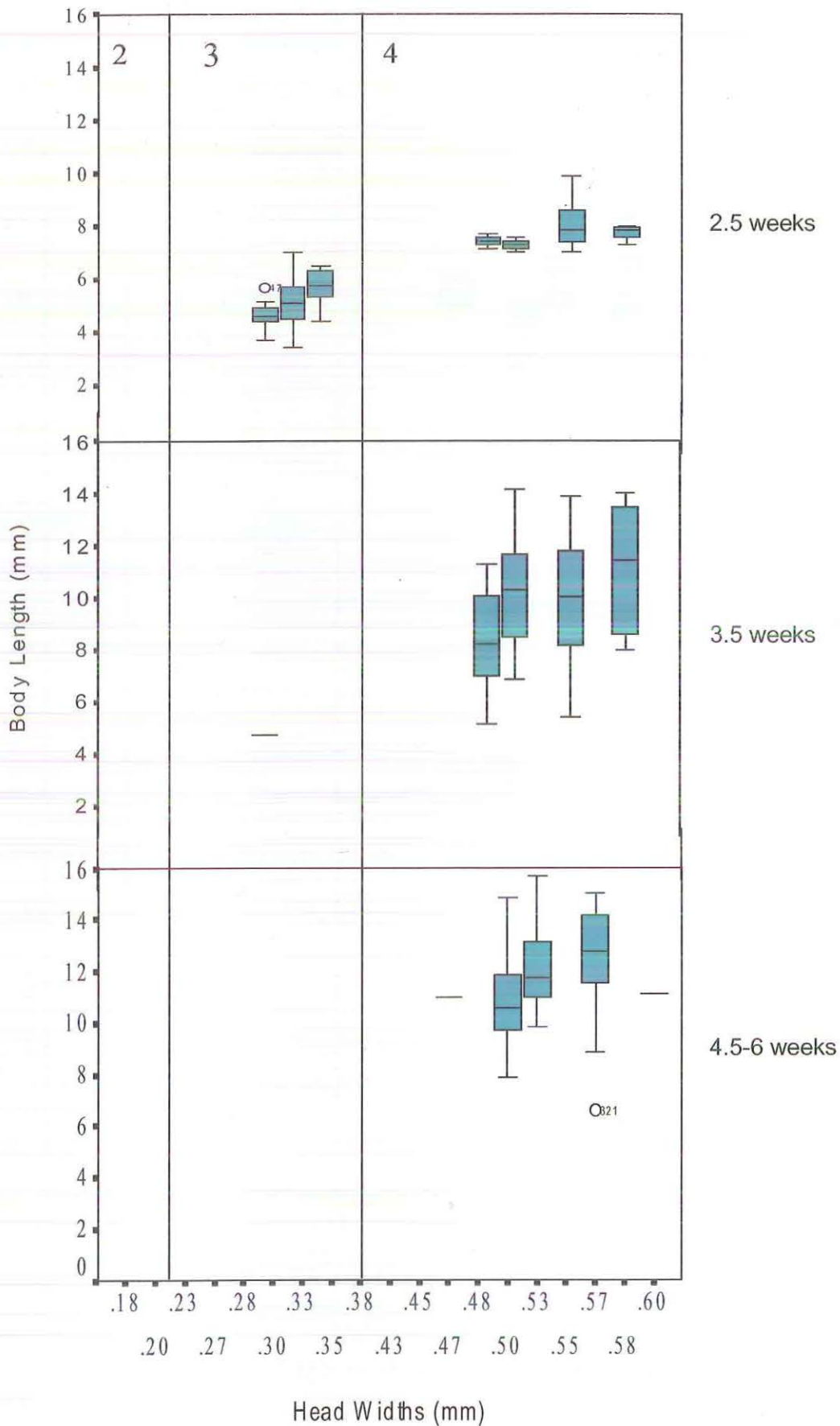


Figure 5.3.6: Head widths and body lengths through three termination intervals for *C. alternans* at 20°C in the main experiment. 2-4 = instar stages.

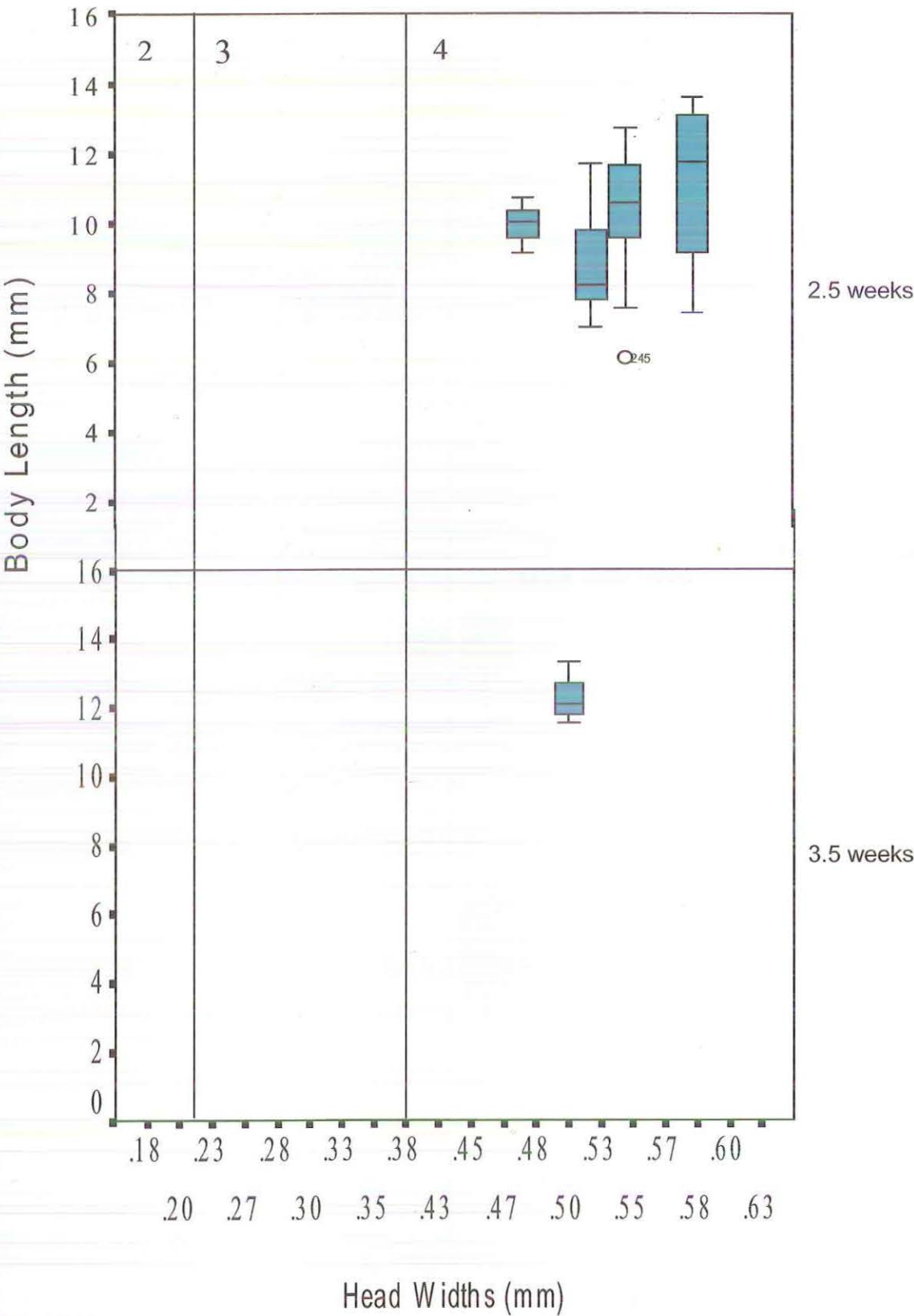


Figure 5.3.7: Head widths and body lengths through three termination intervals for *C.alternans* at 25°C in the main experiment. 2-4 = instar stages.

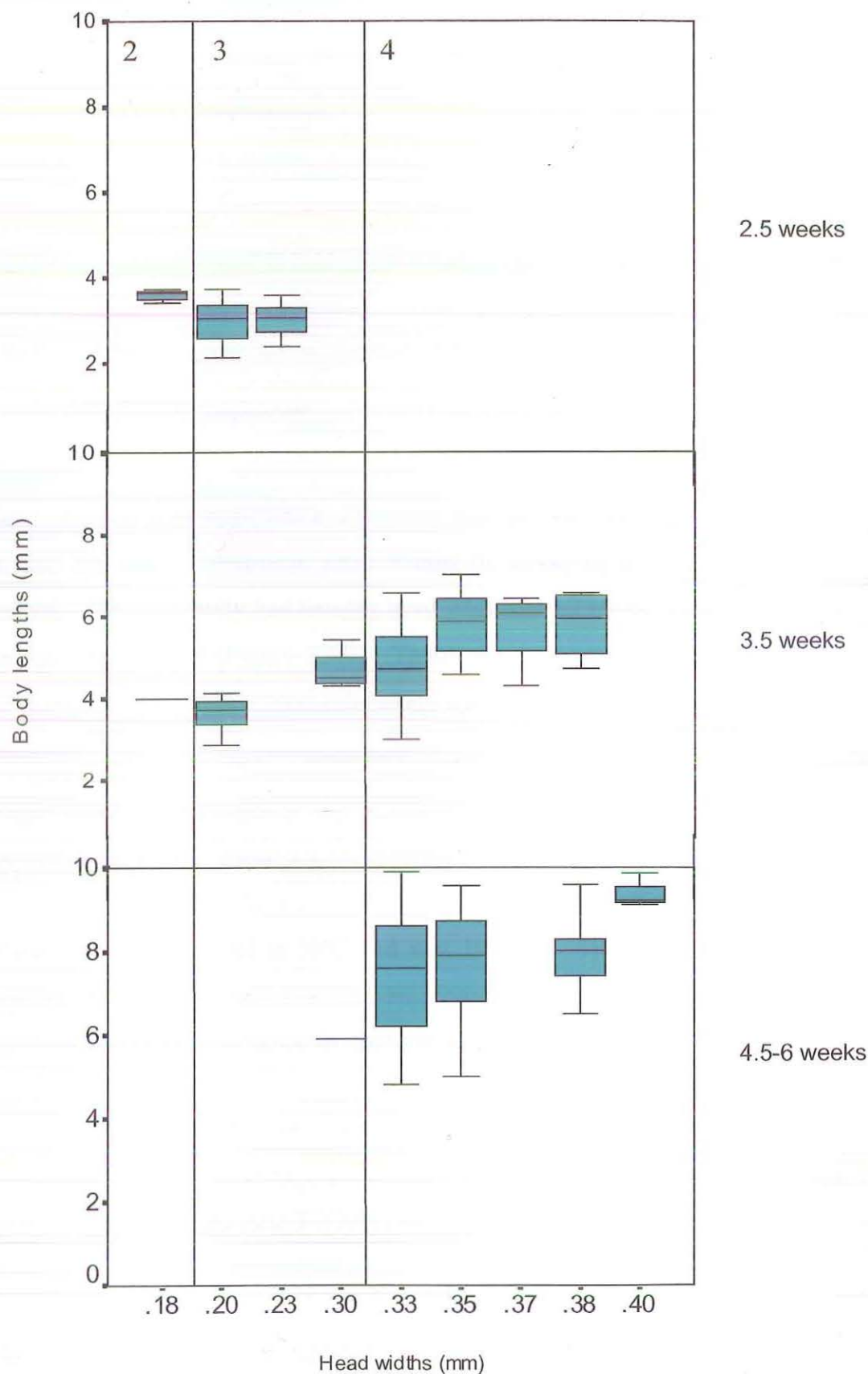


Figure 5.3.8: Head widths and body lengths through three termination intervals for *P.nubifer* at 20°C in the main experiment. 2-4 = instar stages.

Head widths and body lengths for *P.nubifer* increased with time. Termination two (3.5 weeks) had the largest range of head widths, encompassing both the third and fourth instars. Termination one (2.5 weeks) included only third larval instars and termination three (4.5 to 6 weeks) contained fourth larval instars.

Statistical analysis was used to determine whether there was a difference between the three termination intervals (progression through time) and survivorship at each termination. There were no significant differences found for either species ($p > 0.05$; $f = 0.109$ for *C.alternans*; $\chi^2 = 5.341$ for *P.nubifer*).

To determine whether there was a difference with growth rates between the pilot study and the main experiment, head widths of larvae of the same age, were compared. The pilot study had smaller head widths at all temperatures compared to the main experiment (Figure 5.3.9). There was an average difference of head width sizes of 0.2mm between the pilot study and main experiment.

Average times to emergence for *C.alternans* and *P.nubifer* decreased as temperatures increased (Table 5.3.1). Average time to emergence for *C.alternans* was >51, 32.5, 28 and 26 days at 15°C through to 30°C respectively. Emergence for *P.nubifer* only occurred at 30°C and was 19 days. The temperatures where emergence is >51 days indicates that emergence did not occur before the last termination, therefore emergence would have occurred after 51 days.

Nutrient concentrations did not have a significant effect on survival ($p > 0.05$; $f = 0.787$ (NH_4), $\chi^2 = 5.557$ (NO_x), 2.755 (FRP)) or between termination intervals (time) ($p > 0.05$; $f = 0.088$ (NH_4), 0.074 (NO_x), 0.436 (FRP)).

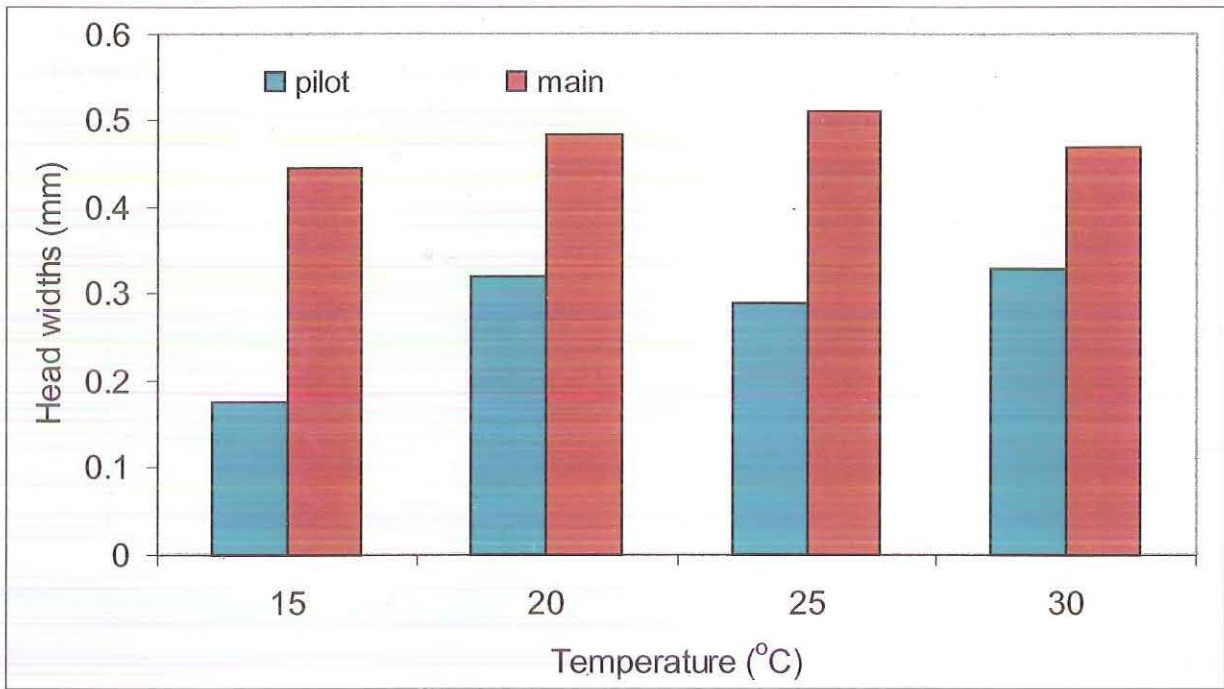


Figure 5.3.9: Comparison of the average head widths between the pilot study and the main study at 15-30°C for *C. alternans*.

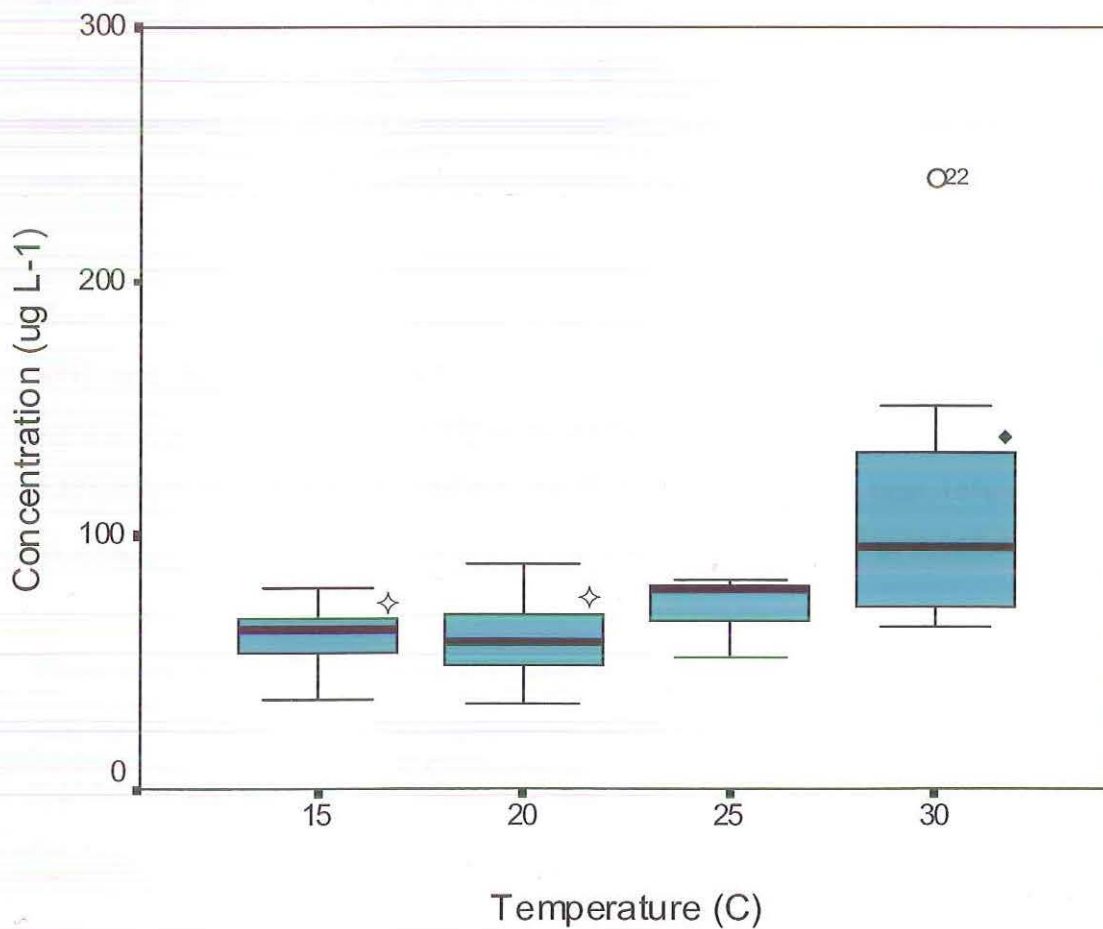


Figure 5.3.10: Box plots of NO_x concentrations at 15-30°C (Plots with the same symbol were not significantly different).

Table 5.3.1: Emergence times for *C.alternans* and *P.nubifer* in the main experiment.

Species	Temperature (°C)	Average time to emergence (days)
<i>C.alternans</i>	15°C	>51
	20°C	32.5
	25°C	28
	30°C	26
<i>P.nubifer</i>	15°C	>51
	20°C	>51
	25°C	>51
	30°C	19

There was a significant difference with NO_x concentrations between 15°C and 30°C and between 20°C and 30°C ($p < 0.05$; $f = 6.292$) (Figure 5.3.10). Both 15°C and 20°C had lower concentrations compared to 30°C. The largest range of concentrations was at 30°C with $64\text{--}151\mu\text{g L}^{-1}$. Ranges at 15°C through to 25°C were $35.3\text{--}79.3\mu\text{g L}^{-1}$, $33.7\text{--}89.1\mu\text{g L}^{-1}$ and $52.1\text{--}82\mu\text{g L}^{-1}$ respectively.

There was a significant difference with NH_4 concentrations between 15°C and 20°C and between 15°C and 30°C ($p < 0.05$; $f = 4.319$) (Figure 5.3.11). The highest concentration was at 15°C with $10,200\mu\text{g L}^{-1}$. The ranges for 20°C ($351.4\text{--}9,471.9\mu\text{g L}^{-1}$) and 30°C ($3,346.4\text{--}8,256.9\mu\text{g L}^{-1}$) were larger than 15°C ($7,177.9\text{--}10,200\mu\text{g L}^{-1}$). Concentrations at 25°C ranged from $91.4\mu\text{g L}^{-1}$ to $9,983.4\mu\text{g L}^{-1}$.

There were no differences between temperature and FRP concentrations ($p > 0.05$; $f = 0.701$). Ranges for 15°C through to 30°C for FRP were $2,953\text{--}5,250\mu\text{g L}^{-1}$, $2,464\text{--}7,263\mu\text{g L}^{-1}$, $2,534\text{--}5,792\mu\text{g L}^{-1}$ and $2,554\text{--}6,205\mu\text{g L}^{-1}$ respectively (Figure 5.3.12).

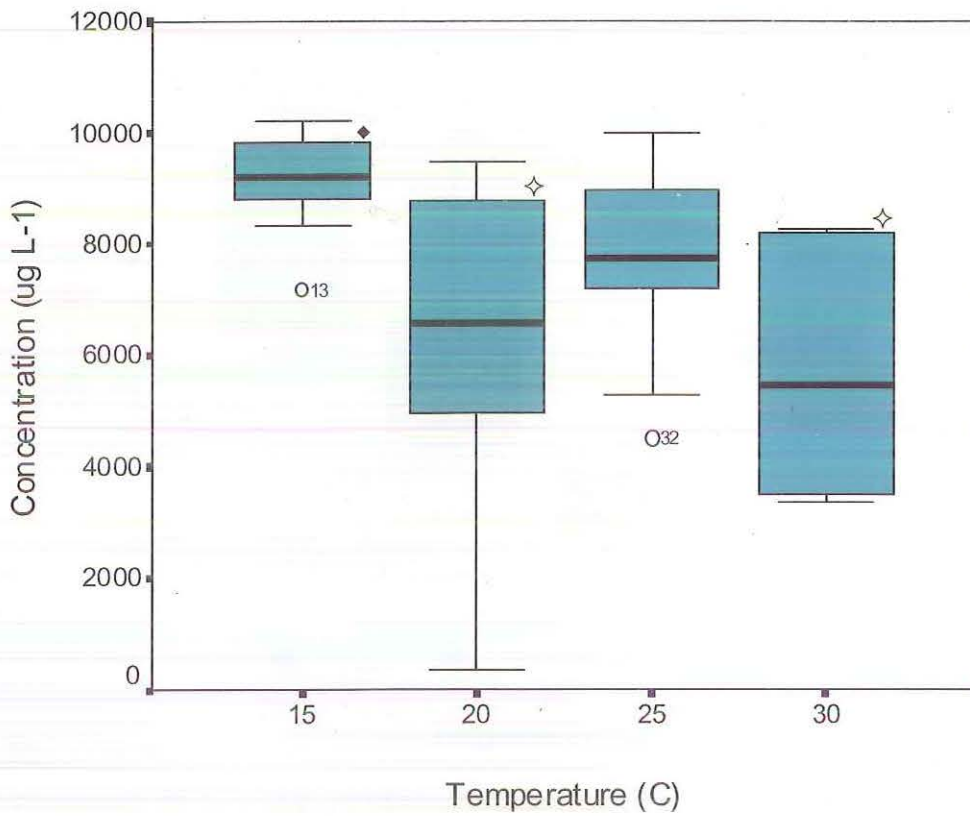


Figure 5.3.11: Box plots of NH_4 concentrations at 15-30°C (Plots with the same symbol were not significantly different).

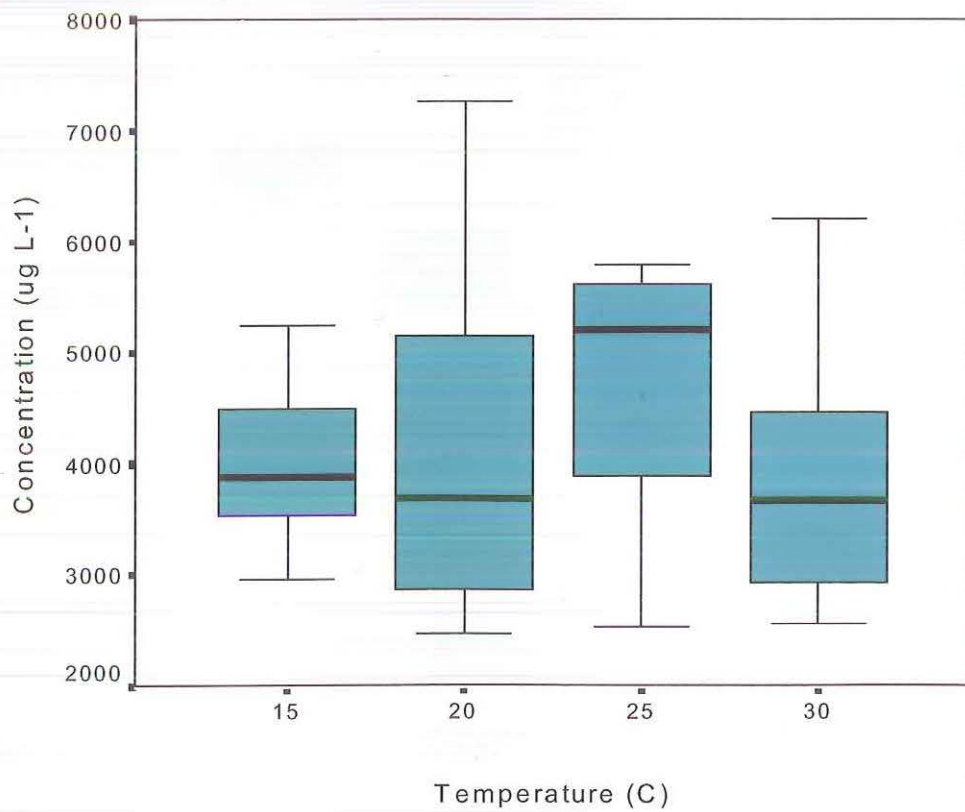


Figure 5.3.12: Box plots of FRP concentrations at 15-30°C.

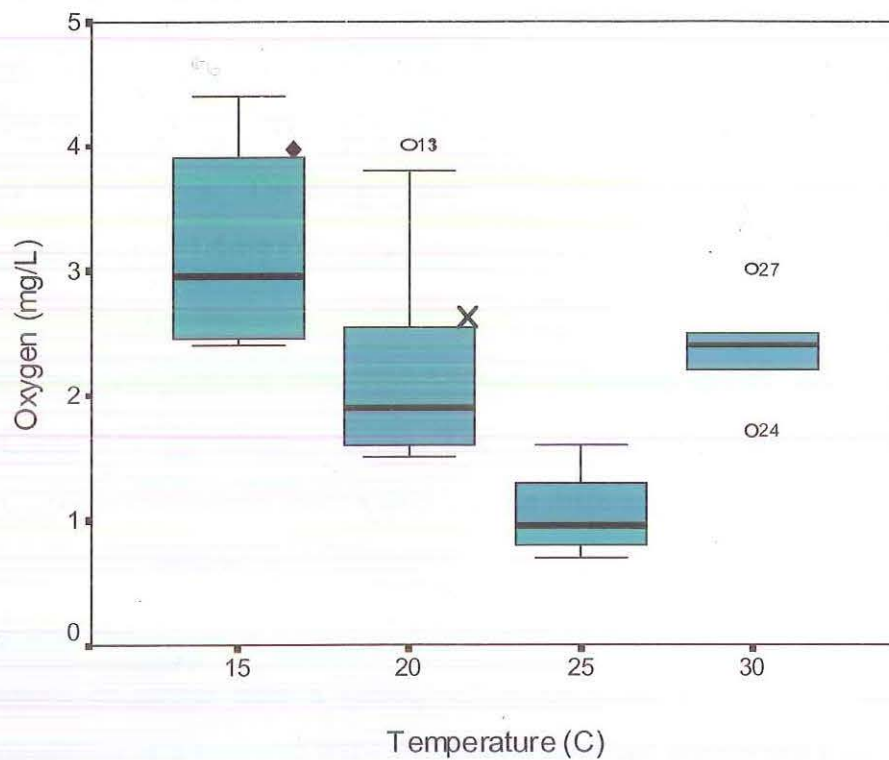


Figure 5.3.13: Box plots of NH_4 concentrations at 15-30°C (Plots with the same symbol were not significantly different).

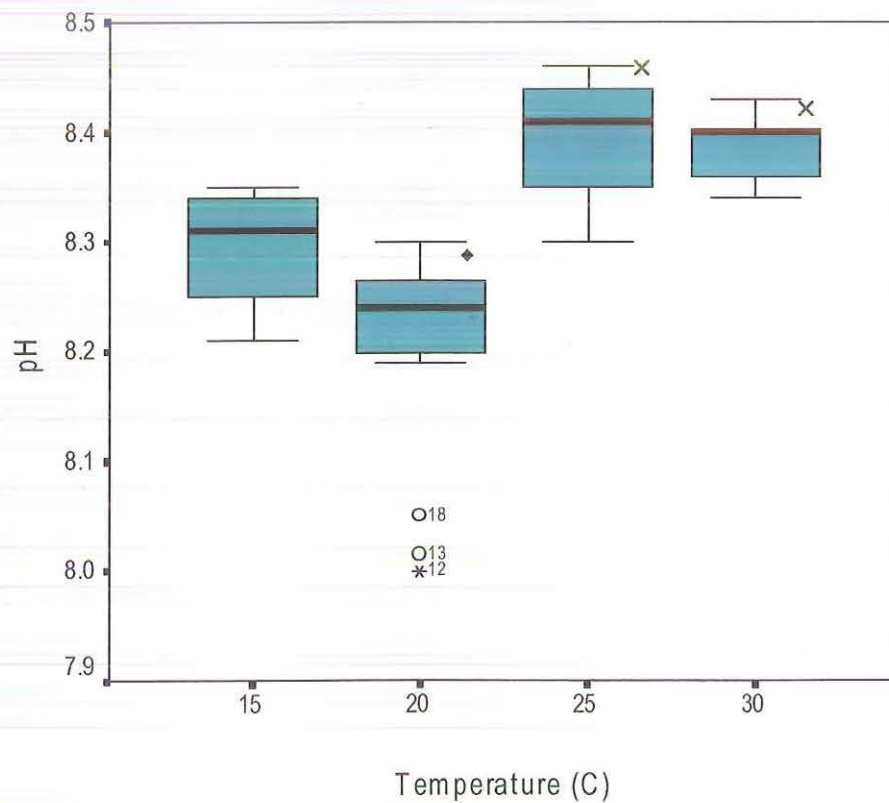


Figure 5.3.14: Box plots of pH concentrations at 15-30°C (Plots with the same symbol were not significantly different).

There was a significant difference between oxygen levels at 15°C and 20°C ($p < 0.05$; $f = 4.437$). The range of concentrations at 15°C was higher than the other temperatures. The ranges from 15°C through to 30°C were 2.4-4.4mg L⁻¹, 1.5-4mg L⁻¹, 0.7-1.6mg L⁻¹ and 1.7-3mg L⁻¹ respectively (Figure 5.3.13).

There was a significant difference between pH levels at 20°C and 25°C as well as 20°C and 30°C ($p < 0.05$; $f = 9.49$). The ranges at 25°C and 30°C were higher than 20°C. pH levels ranged from 8 to 8.5 at the different temperatures (Figure 5.3.14).

The life history table (Table 5.3.2) for *C.alternans* shows the population age structure of larvae over a variety of temperatures (15-30°C). This table is a combination of a series of static life tables. The age classes are from eggs through to the last termination at each temperature. It was assumed that 95% of eggs hatched at all temperatures.

As terminations occurred independently, continuous or cohort tables could not be presented. Limitations arose when unusually high survival rates were incurred at 15°C at the age of 29-51 days; the n_x value for adults at 15°C is affected and is meaningless. Instar stages for each age group are shown in Figures 5.3.5 to 5.3.7 for 15°C through to 30°C respectively. Mortality is highest in the third age group for all temperatures. There were no individuals alive at the fourth age group for 25°C and 30°C implying that no larvae progressed into the adult stages. The adult stage was based on the assumption that all larvae surviving in the fourth age group would emerge as adults. The largest number of adults emerging would occur at 20°C, with the least at 25°C and 30°C.

Table 5.3.2: Life history table for larvae of *C.alternans* at 15-30°C in the main experiment.

Temp (°C)	Age Interval (days) (x)	Number surviving at beginning of age interval (n _x)	Number surviving as a fraction of newborn (l _x)	Number dying during age interval (d _x)	Average mortality rate per day (q _x)	Average number of individuals alive during each age interval (L _x)	Number of larval years to be lived by all individuals (T _x)	Average number of days of life remaining at beginning of age interval (e _x)
15	0-5	1000	0.95	50	0.01	975	2687	2.69
	5-22	950	0.57	380	0.03	760	1712	1.80
	22-29	570	0.30	268	0.10	436	952	1.67
	29-51	302	0.73	-428	0.01	516	516	1.71
	Adult	730	-	-	-	-	-	-
20	0-3.5	1000	0.95	50	0.01	975	3432	3.43
	3.5-18	950	0.88	70	0.01	915	2457	2.59
	18-27	880	0.79	88	0.03	836	1542	1.75
	27-37	792	0.62	172	0.02	706	706	0.89
	Adult	620	-	-	-	-	-	-
25	0-3	1000	0.95	50	0.01	975	1593	1.59
	3-18	950	0.13	820	0.05	540	618	0.65
	18-27	130	0.01	117	0.14	71.5	78	0.60
	27-37	13	0	13	0.05	6.5	6.5	0.50
30	0-2	1000	0.95	50	0.01	975	1480	1.48
	2-19	950	0.03	920	0.06	490	505	0.53
	19-26	30	0	30	0.14	15	15	0.50
	26-36	0	-	-	-	-	-	-

According to Krohne (2001) mortality trends are easier to interpret when they are plotted as survivorship curves. The age groups one to three represent the three terminations, these are related to the second to fourth age groups on the life table as the egg phase is taken as the first age group (Figure 5.3.15). It was assumed that the survivorship of hatchlings was the same across the temperatures. After hatching, larvae at 30°C died rapidly. If mortality were high in the hatching phase at 30°C, then the curve would be concave. Larvae at 25°C died rapidly after hatching, though not as fast as 30°C. Mortality was low at 15°C, the greatest amount of deaths were in the third age group. This could be attributed to the limitations explained for 15°C in the life table. Survival was high at 20°C compared to the other temperatures; death rates occurred evenly over the age groups.

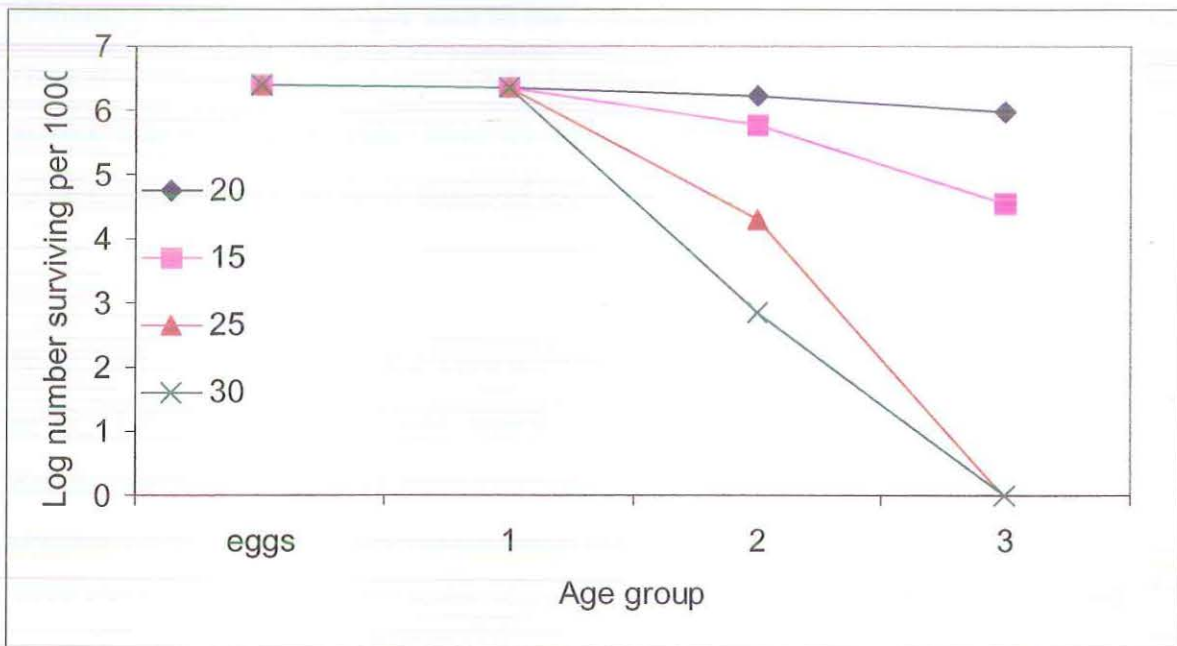


Figure 5.3.15: Survivorship curves for *C.alternans* larvae at 15-30°C in the main experiment.

5.4 Discussion

Average ambient temperatures for each season in 2001 were 20-30°C, 15-25°C, 15°C and 15-20°C for summer, autumn, winter and spring respectively (Bureau of Meteorology, 2002). According to the results, ideal temperatures for *C.alternans* occurred in spring (15-20°C). Larval growth rates, as seen from the head width measurements, decrease at 15°C but survivorship stays high. Summer temperatures have increased growth rates but low survival. It can be seen that larvae persist through summer, but are unable to proliferate (Figures 5.3.1 & 5.3.7).

Due to *C.alternans* being used in the pilot study, rearing conditions were based on their requirements. Therefore, conditions may not have been ideal for *P.nubifer*, as illustrated by low survival levels. *Polypedilum nubifer* is a summer species and prefers high temperatures (Edward, 1964). This was not evident in this experiment, as the highest survival rate occurred at 20°C (Figure 5.3.2). Generally, *P.nubifer* emerges four to six weeks after the mean water temperature rises above 22°C (Pinder *et al.*, 1991). Emergence time in this study for *P.nubifer* at 30°C occurred at just under three weeks. Egg masses for *P.nubifer* were unable to be collected until the final stages of the main experiment; therefore a full study was not performed.

Head widths and body lengths were used to determine larval development (Maier *et al.*, 1990) and to determine which was the best measure of development. Results showed a strong relationship between the two measurements ($r^2=0.823$) (Figure 5.3.4). Head widths were a better measure of larval development as there were clear divisions between instar stages. Body length measurements overlapped and did not give a clear indication of instar stage.

Body lengths in the third termination at 15°C for *C.alternans* were the largest found at any temperature. As 20°C appears to be the optimum temperature for *C.alternans*, the longer time spent at an instar at 15°C may result in larger sizes.

Polypedilum nubifer had smaller head widths and body lengths compared to *C.alternans*. Ali (1991) and Tabaru (1987) have acknowledged the problem with *P.nubifer* creating a nuisance to residents by flying through screen doors into houses.

Results show progression through the larval phase until the fourth instar (Figures 5.3.4, 5.3.5-5.3.8), where larvae wait and emerge when conditions are favourable (Edward, 1986). Other studies have found conflicting results with which instar development can halt. Pinder (1986) found larvae halted progression in the first and second instar stages, while other authors (Armitage, 1995a; Davis *et al.*, 1988; Edward, 1986; Maier *et al.*, 1990) found that larvae progressed through to the fourth instar. Larvae expend energy when metamorphosing; therefore need ideal conditions for adults to have high survival rates (M.Lund, Edith Cowan University, pers. comm., 2002).

Conditions improved from the pilot study to the main experiment. There were higher growth rates through the larval phase in the main experiment (Figures 5.3.5-5.3.7 & 5.3.9). Conditions from the main experiment need further improvement to allow for the appropriate analysis of the effects of temperature.

Certain temperature and photoperiod regimes act as a trigger for aquatic insects to emerge (Ward, 1992). Temperatures that are ideal for emergence may not be ideal for high growth rates (Heiman & Knight, 1975, cited in Williams & Feltmate, 1992). Results for the average time to emergence were different to those predicted; 7-10 weeks for *C.alternans* and 3-4 weeks for *P.nubifer* (Edward, 1964). Emergence times at different temperatures can be related to seasonal variations. Summer temperatures have the fastest time to emergence for both species, though survivorship is very low. At the optimum temperature for *C.alternans* (20°C), average time to emergence was 32.5 days. These results can be incorporated into control mechanisms, which can be altered to accommodate different response times for different species. If managers monitor temperature

ranges through the year and put control mechanisms into place before the time of highest abundance, effectiveness of controls could be increased.

Chironomidae are tolerant of very poor water quality conditions (Chessman, 1995). According to the SIGNAL (Stream Invertebrate Grade Number-Average Level) index developed by Chessman (1995; 1997) Chironomidae have a rating of 1, with ratings falling between 1 and 10 with the former being most tolerant to severe conditions. The water quality standards for protection of aquatic ecosystems are $320\text{--}2300\mu\text{g L}^{-1}$ for NH_4 and $17\text{--}17,000\mu\text{g L}^{-1}$ for NO_x (ANZECC, 1992). Nutrient concentrations in the experiments were very high compared to studies undertaken at Lake Joondalup by Lund (2002) and Kinnear (1997). Combining the natural levels found at Lake Joondalup (Kinnear, 1997; Lund, 2002) NO_x , NH_4 and FRP concentrations should range from 4 to $30\mu\text{g L}^{-1}$, 15 to $200\mu\text{g L}^{-1}$ and 23 to $160\mu\text{g L}^{-1}$ respectively. Concentrations in this study were probably too high and may have had a limiting affect on larval survival. No significant differences ($p>0.05$) were shown between survival rates at different nutrient concentrations. This could be due to the overall high concentrations throughout the temperatures. It would be expected that with acceptable nutrient levels, similar trends may be seen in survivorship, although the percentages would be higher.

Nutrients were classified into groups according to concentration (NO_x : 0-100, 100-1000, $>1000\mu\text{g L}^{-1}$; NH_4 : 0-4000, 4000-8000, $>8,000\mu\text{g L}^{-1}$; FRP: 0-3000, 3000-5000, $>5000\mu\text{g L}^{-1}$). Naturally occurring ranges for all nutrients were well below those seen in the rearing containers, therefore trends may not have been seen in the statistical analysis.

Ammonium and FRP levels may have been high due to the accumulation of wastes from the larvae (Brewer, 1994; Maier *et al.*, 1990). High concentrations of NO_x may have been attributed to nitrification, which would have been occurring in rearing containers with suitable oxygen levels (Brewer, 1994). The accumulation of food may have also been contributing to high nutrient concentrations.

Dissolved oxygen concentrations and pH levels in the rearing containers need to be maintained at the same levels that naturally occur in wetlands. From studies undertaken at Lake Joondalup (Kinnear *et al.*, 1997; Lund, 2002), dissolved oxygen concentrations were on average between 6.5mg L⁻¹ and 8.8mg L⁻¹, while pH levels were between 8.1 & 9.3. pH levels in this study were consistent with those found in Lake Joondalup during 1997 and 2002 (Kinnear *et al.*, 1997; Lund, 2002). pH levels in Lake Joondalup are naturally alkaline due to the underlying limestone formations (Kinnear *et al.*, 1997).

Survivorship curves were used to visually detect changes in survival rates (Brewer, 1994). A Type I curve was shown for 20°C, which is typical of mammals (Krohne, 2001). Type I curves are characterised by high initial survivorship followed by late mortality, few large young and parental caring of the young (Starr & Taggart, 1998). Both 25°C and 30°C showed similar trends to a type II curve, which represents lizards, small mammals and some birds (Starr & Taggart, 1998). Type II curves are characterised by constant death and animals are highly influenced by environmental conditions (Brewer, 1994). Normally, invertebrates are represented by a type III curve (Krohne, 2001), which is characterised by many offspring, no parental care and high mortality in the early stages of the life cycle.

Limiting conditions in the rearing containers may explain unusual findings with the survivorship curves. The lack of naturally occurring processes, such as predation and competition in the rearing containers could have attributed to unusual survivorship curves in this study. Low levels of competition could have allowed early instars to flourish. As the larvae grew, competition for space and food could have increased, resulting in only the fittest surviving. This could be related to the highest mortality rates in the third age group for all temperatures.

Early instars were present when nutrient levels were low and food and wastes had not accumulated. This may attribute to the high success rate early in the life cycle of the larvae.

It must be noted that there were a lot of limitations associated with laboratory trials. This study had constant temperatures; light and dark regimes, not variable as associated with dawn and dusk; a constant feeding regime of a foreign food source; no competition from other macroinvertebrates; no predation and high nutrient levels. These factors could have impacted on survival levels, growth rates, duration of the larval stage and emergence times. When relating findings to the field these factors need to be considered. Conditions were kept as natural as the scope of the project allowed for.

CHAPTER 6: SEDIMENT EXPERIMENT

6.1 Introduction

Sediment type and particle size have been found to be important factors affecting the distribution of aquatic insects as sediment acts as a food source and habitat (Ward, 1992).

The effects of sediment type on survival were initially explored in the pilot study. Due to low survival rates in the sterilised propagating sand, this experiment was developed to determine whether it was the sediment type that resulted in low survival levels or the poor rearing conditions associated with the pilot study. It was also developed to explore the effects of particle size.

Sediment type and particle size has been shown to affect larval distribution in wetlands (Dudgeon, 1994, cited in Lamb, 2002). If larval distribution over a wetland can be determined, then managers can restrict controls to the areas of highest abundance. Lamb (2002) and Lund (2000) conducted spatial surveys of larval distributions at Lake Joondalup. Neither study found any link between sediment type and larval distribution.

The sediment experiment aimed to determine whether sediment type and different particle sizes had significant impacts on survival rates for *C.alternans*.

6.2 Methods

The sediment experiment had three treatments: metaphyton, coarse sand and fine sand (Figure 6.2.1). Sterilised propagating sand was bought from a nursery and was dried at 70°C for one day and sterilised in an autoclave for 20 minutes at 121°C. The metaphyton at 20°C in the main experiment was used for this experiment. There were three treatments each with three replicates.

In the pilot study, larvae were unable to thrive in jars containing sterilised propagating sand. To determine whether it was the particle size that affected the building of tubes, half the sand was ground to the same size as the metaphyton (0.125mm) and the new feeding regime with TetraMin[®] fish food was adopted (refer to Chapter 4).

Chironomus alternans larvae were only reared at 20°C due to its fast growth rates and space restrictions in the temperature cabinets. Termination occurred at 4.5-5 weeks, due to high adult emergence at this time. All other conditions were the same as the main experiment. The third termination of the metaphyton in the main experiment at 20°C was compared to the sand due to termination times being the same.

The effect of particle size on tube building in the larval phase was an observational part of the experiment. In the pilot study tubes were not built in the sterilised propagating sand. To determine whether this was due to particle size, observations were made throughout this experiment.

Data analysis was performed on SPSS V11.0 (SPSS Inc., 2002). Before analysis all percentages were transformed using Arcsine. Data were tested for homogeneity of variance using the Levene's Test of Normality. Data were not homogeneous after transformation therefore a non-parametric test was used (Kruskal-Wallis test) to determine significance.



Figure 6.2.1: Photograph of the coarse sand and fine sand

6.3 Results

A significant difference was not found between the three treatments ($p > 0.05$; $\chi^2 = 4.414$). The sediment experiment showed higher survival rates in the coarse (99% ± 0.005) and fine sand (88% ± 0.12) compared to metaphyton (62% ± 0.082) (Figure 6.3.1).

Larvae in the fine and coarse sand were in the third and fourth instar stages, while larvae in the metaphyton were in the fourth instar (Figure 6.3.2). There were slightly more larvae in the fourth instar in the coarse sand compared to the fine sand. Body lengths increased as head widths increased.

Emergence in the sediment experiment occurred between 30 and 35 days (Table 6.3.1). The coarse sand had the fastest larval development (31.7) and the metaphyton had the slowest (34.3).

Table 6.3.1: Emergence times (days) in the metaphyton, coarse sand and fine sand for *C. alternans*.

Replicates	Metaphyton	Coarse Sand	Fine Sand
1	35	30	31
2	34	33	34
3	34	32	33
Average	34.3	31.7	32.7

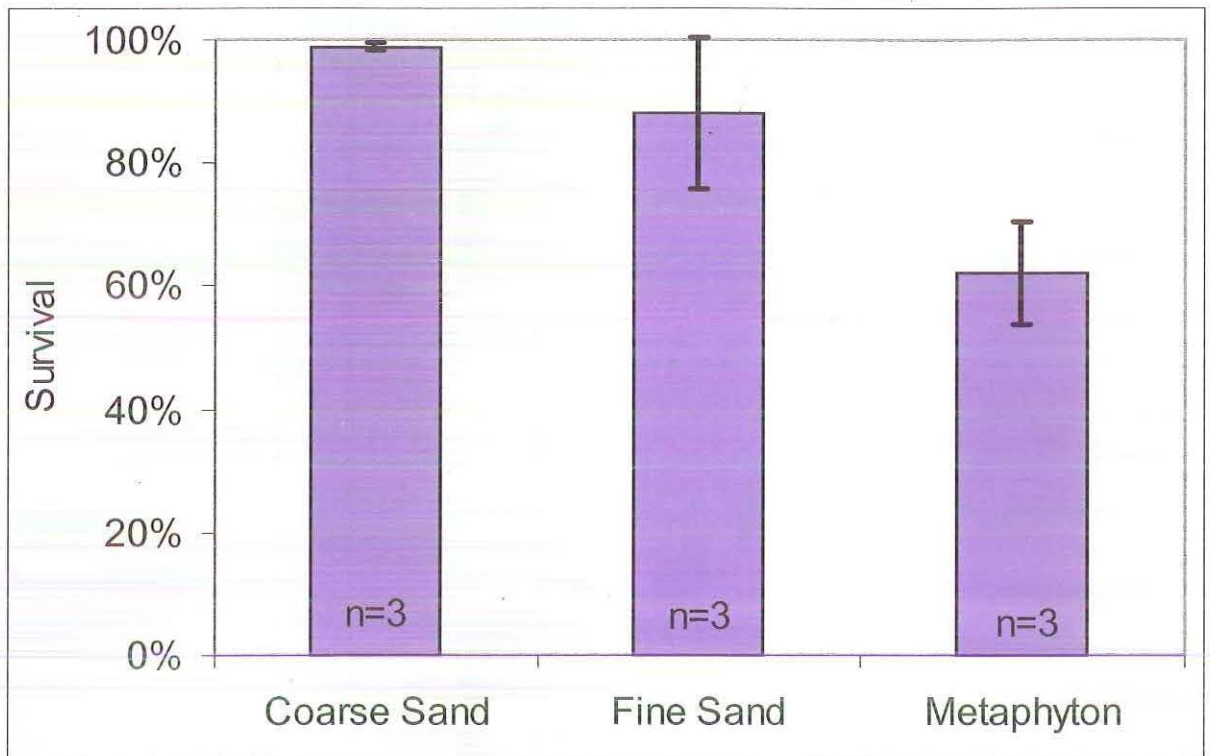


Figure 6.3.1: Survival rates between the coarse sand, fine sediment and metaphyton.

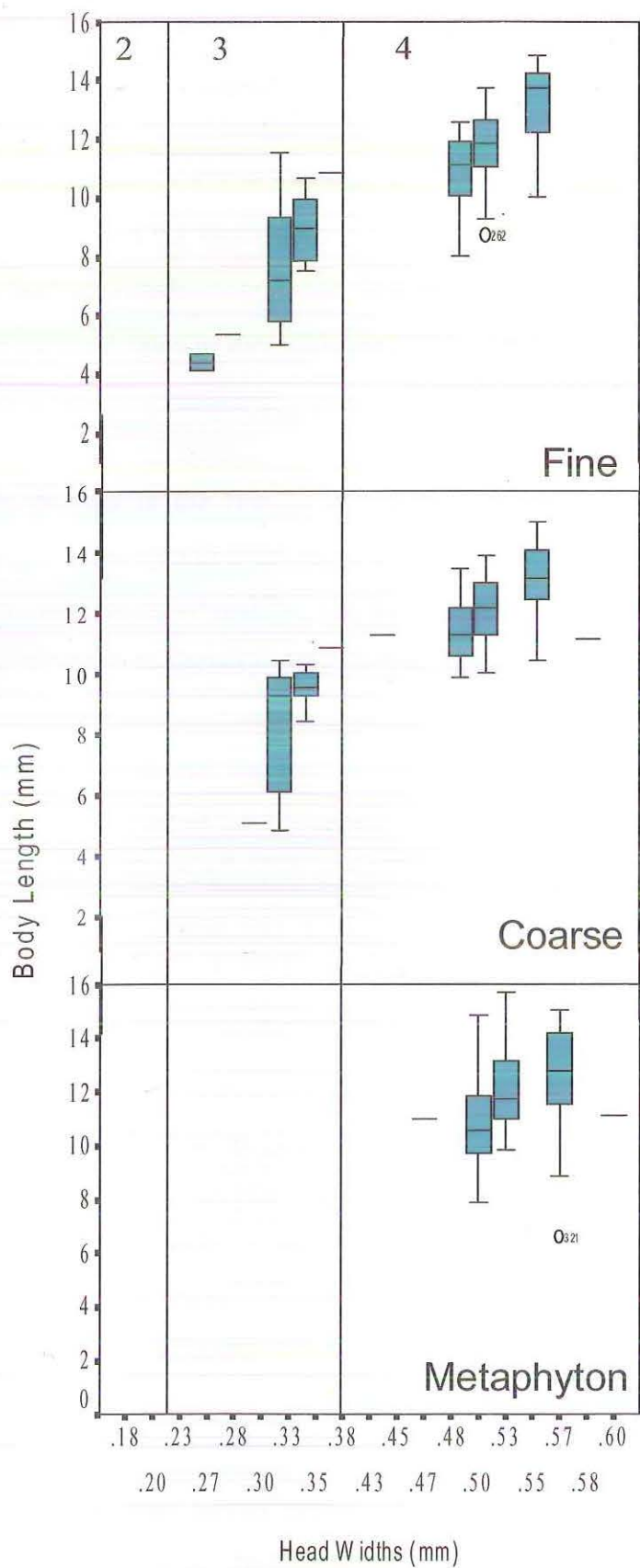


Figure 6.3.2: Head widths and body lengths for the sediment experiment. 2-4 = instar stages.

6.4 Discussion

To determine whether food was limiting, survival rates were compared between the sterilised sand and the metaphyton. Sand does not contain any material that larvae can feed on, while metaphyton is a potential source of food for larvae. Due to high survival rates in the sand treatments, the food that was supplied was not limiting.

Tubes were stronger in the fine sand and metaphyton compared to the coarse sand. Stronger tubes should offer more protection for larvae, however this does not appear to relate to survivorship. The particle sizes in the coarse sand were too large for the larvae to construct tightly packed tubes.

Larvae continually explored the rearing containers by swimming. Some tubes were attached to the sides of the containers. Any disturbance in the vicinity of the experimental containers resulted in swimming larvae quickly descending to the sediment and burrowing until they were not visible. It often took an hour before they would emerge again and continue swimming.

High survival rates in the sterilised propagating sand can be compared to a study undertaken at Lake Jeondalup (Lamb, 2001) that found *C.alternans* to prefer sandy sediments. This is despite the fact that Lamb's (2001) results conflicted with Ali and Mulla's (1976, cited in Lamb, 2001) findings that *Chironomus* species preferred fine sands covered in algae. This study indicated that distribution was not affected when sterilised sand and metaphyton were compared.

It has been shown that larvae prefer to feed on the algae associated with metaphyton (Lamb, 2001). In this study the metaphyton was dead, possibly rendering it unusable by larvae. Larval feeding habits may be related to the bacteria associated with the TetraMin[®] fish food, instead of the sediment type.

Third and fourth larval instars were present in this experiment, which indicated that under the same conditions larvae developed at different rates, as has been also observed by Davis *et al.* (1988). Emergence occurred in all the rearing containers before terminations began. High survival and fast development in the coarse sand implies that those conditions were the most favourable. With fast emergence and high survival rates it indicates that sediment type did not affect development. High survival in this experiment indicates that 20°C is an ideal temperature for *C.alternans*.

This experiment indicated that factors other than sediment type, attributed to low survival rates in the pilot study.

Nutrient concentrations through all experiments were discussed in Chapter 5. Nutrient concentrations were high in the sand experiment. This indicates that nutrient release from the metaphyton was not the main cause of high nutrient levels. Other sources, such as food and faeces, may have been contributing to the nutrient levels in the water.

CHAPTER 7: TRANSFERRING EXPERIMENT

7.1 Introduction

Many factors contributed to low survival rates in the pilot study. The effects of transferring larvae were not known. Once the eggs had hatched the larvae were counted and transferred into their rearing container. Ntote (1995, cited in Frouz, Ali & Lobonske, 2002) noted that manipulation could affect development of chironomids (Frouz, *et al.*, 2002).

The aim of this experiment was to determine the effects that transferring *C.alternans* larvae had on survivorship.

7.2 Methods

Two containers with three replicates each were set up at 20°C with one *C.alternans* egg mass in each (Figure 7.2.1). A divider was placed across the centre of all containers. The containers had 10mm of metaphyton and sand from Lake Joondalup and 500ml of filtered lake water.

Once all the eggs had hatched, the dividers were removed from the control containers. In the treatment containers all larvae were transferred to the other side of the container and then the dividers were removed.

Termination occurred at 4.5 weeks. Water was filtered and frozen for analysis, as described in Chapter 5. The remaining conditions were the same as the main experiment.



Figure 7.2.1: Photograph of transferring experiment.

7.3 Results

Survival rates between the control and treatment in the transferring experiment at 20°C for *C.alternans* were 0.17% and 6.77% respectively (Figure 7.3.1).

On termination of the experiment, all surviving larvae were in the fourth instar (Figure 7.3.2). A total of 102 larvae were measured. The average body length was 11.65mm and the average head width was 0.54mm.

Average time to emergence was faster in the control (28) compared to the treatment (30) (Table 6.2.3.1). Emergence occurred in one control rearing container and two treatment containers, with both treatment containers having emergence at 30 days.

Table 7.3.1: Emergence in the control and treatment in the transferring experiment.

	Control	Treatment
Days	28	30

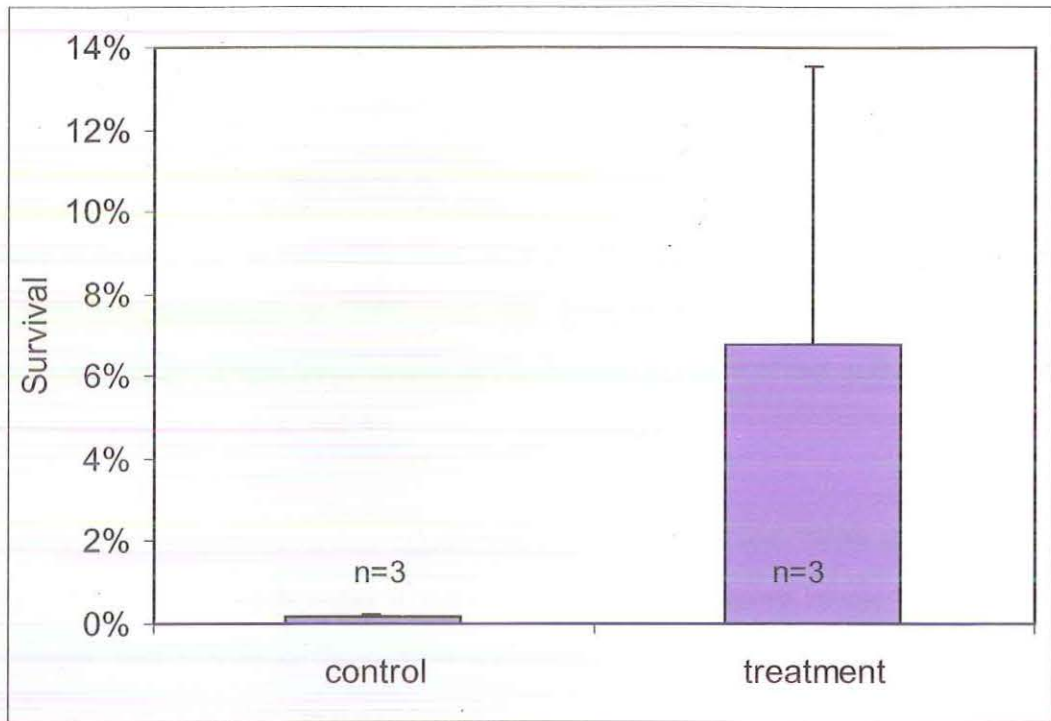


Figure 7.3.1: Survival rates for the transferring experiment.

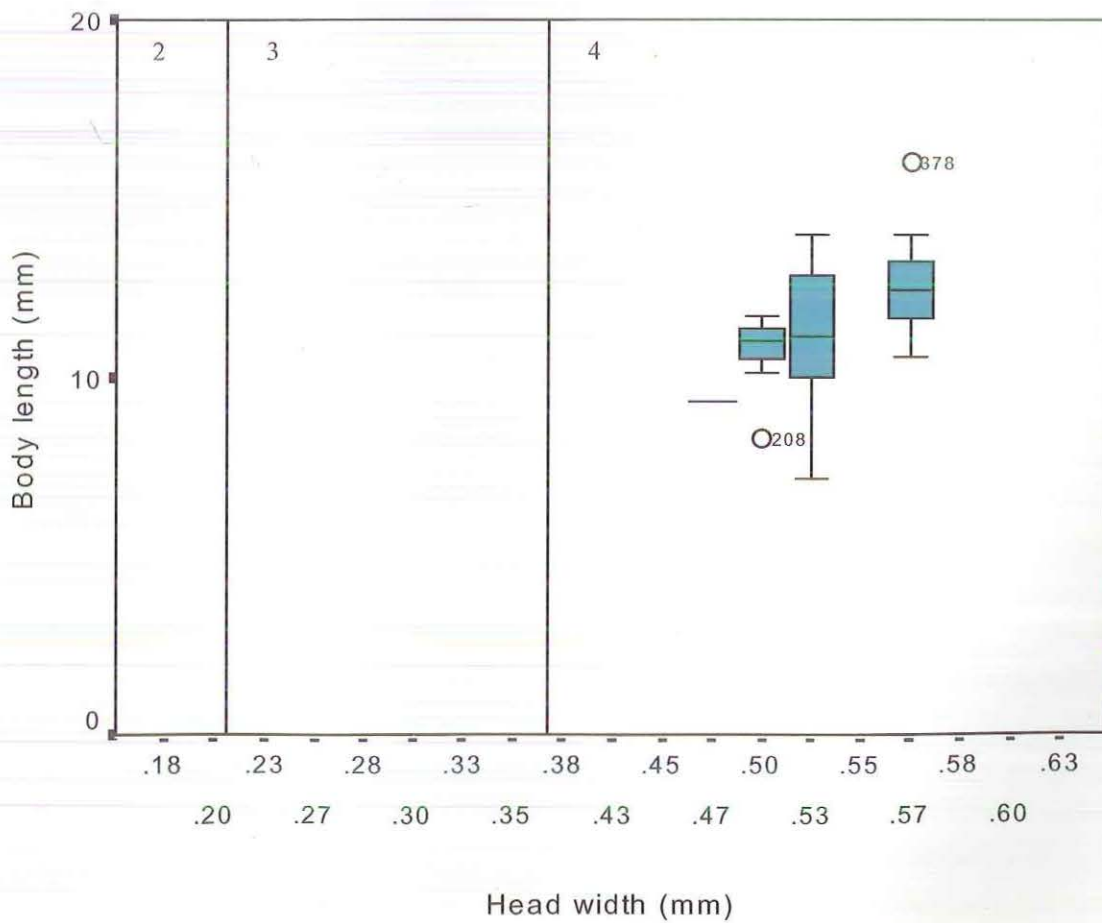


Figure 7.3.2: Head widths and body lengths for the transferring experiment. 2-4 =instar stages

7.4 Discussion

Low survival rates in the controls and the treatments indicated that other factors were affecting larval survival. The only difference between this experiment and the main experiment at 20°C was the transferring. Higher survival in the treatment showed that transferring larvae had no adverse effect and results for the other experiments were valid.

Emergence occurred in a few containers at 28 and 30 days. With the larvae only occurring in the fourth instar it indicates that the remaining larvae were ready to emerge. Emergence in the control and treatment for the transferring experiment were quicker than the main experiment.

CHAPTER 8: SYNTHESIS

8.1 Further experiments

The pilot study was developed to determine suitable rearing conditions and to use the conditions for the subsequent experiments. Through the remaining experiments, limitations in rearing techniques were evident. Further improvements need to be made for future studies.

Jackson and Sweeney (1995) used natural foods when they reared larvae. Limitations were associated with preferences of different species (Jackson & Sweeney, 1995). Using a natural food such as algae would enable closer-to-natural conditions to be present in the laboratory situation. It would enable an experiment to be conducted to determine the effects of different feeding regimes. Limiting food levels could be determined and related to the abundance of algae in a natural system. Manipulating nutrient levels in a wetland, which alters algal composition and abundance could then be related to larval success (Ali, 1990).

Constant temperatures were used in a few studies (Jackson & Sweeney, 1995; Maier *et al.*, 1990) but this does not allow patterns to be compared between night and day. Diel temperature changes have been shown to have a greater impact on the life cycle compared to absolute and predictable temperatures (Hynes, 1961, cited in Williams & Feltmate, 1992). Variable temperatures and photoperiod regimes would mimic natural temperatures and light levels and improve comparability of the experiment to the field. Light levels and temperatures could be gradually changed over a period of one to two hours. A variable temperature and photoperiod regime would allow larval behaviour to be compared between dawn/dusk, day and night. For example emergence times could be monitored to determine midge emergence levels.

Egg masses need to be divided into rearing containers before they hatch (Frouz *et al.*, 2002), this would decrease disturbances to the larvae. Problems occur when survivorship levels are assessed, as it would not be known if all eggs hatched.

Maintaining acceptable dissolved oxygen concentrations decreases stress levels for larvae. Most studies used airstones to maintain the dissolved oxygen in the water (Balci & Kennedy, 2002; Hein & Mahadeva, 1992; Maier *et al.*, 1990; Stanko-Mishic, 1999), although algal growth in the rearing containers needs to be controlled (Martin, 2002). Average dissolved oxygen concentrations should be related to seasonal variations in Lake Joondalup.

To avoid the accumulation of nutrients, rearing water should be changed on a regular basis, as did Maier *et al.* (1990). Nutrient levels need to correspond to those found at Lake Joondalup over the year.

High densities can affect larvae through competition, limited food and decreased growth rates (Giberson & Rosenberg, 1992). Densities in rearing containers need to be related to field densities (Giberson & Rosenberg, 1992). Various density levels need to be studied in relation to larval success. An experiment needs to be developed with three to four different densities as the treatment. The density levels would range between 1,000 and 10,000 larvae per m². Larval success would be monitored through three termination intervals to determine which larval instar is most affected by increasing densities. The different densities would be compared to find the maximum threshold for different species. This would be comparable to natural increases in larval numbers.

To determine the length of each stage of the life cycle, a closely monitored experiment should be established. The hatching experiment would be used but modified. Hatching would be monitored twice daily and the time between the first to last eggs hatched would be determined. This would show variability within egg masses as it would be related to the variability in larval development.

Frouz *et al.* (2002) reared individual larvae in petri dishes that contained rearing solution. The petri dishes allowed larvae to be observed under a dissecting microscope and to determine changes between instar stages (Frouz *et al.*, 2002). Larvae would be reared under varying conditions (ranges of temperatures) and monitored closely to determine changes between instar stages. Pupal and adult phases would be monitored and time spent at each of these phases determined. This would enable resting periods to be identified.

The period of midge nuisance to humans is related to the longevity of the adult phase. The ability of adults to feed needs to be determined and related to the duration and persistence of the adult stage (Armitage, 1995a). An experiment using a control (without food) and a treatment (with food) would determine whether adults feed and if they do feed, whether it prolongs the adult phase.

The experiments need to be conducted throughout one year. This would allow egg masses of all nuisance species to be collected. Larval densities and the identification of instars over one year could be determined in wetlands. Exact occurrences of nuisance species over one year would be valuable data for control programs. Environmental parameters such as ambient temperatures, water temperatures, photoperiod, rainfall, physico-chemical parameters and nutrient concentrations should be collected over the year to relate variations to species occurrences.

Rearing conditions were improved through the pilot study and further limitations were found in the subsequent experiments. Any further studies involving rearing macroinvertebrates need to incorporate the above-mentioned factors for a more successful result.

8.2 Temperature

Determining the impact of environmental factors on life histories of midges is important for ecological studies (Berg & Hellenthal, 1992). Temperature is a main factor influencing the growth and development of aquatic insects (Berg & Hellenthal, 1992). Unless there is an understanding of the effects of temperature on the life cycle of different species, predictions about the occurrence of a species could be wrong (Edward, 1964).

Chironomus alternans was found, in this study, to have high survival and fast development rates in spring in laboratory conditions. This may coincide with the quality of food in wetland systems in spring (Frouz *et al.*, 2002). Berg and Hellenthal (1992) stated that increases in temperatures associated with spring resulted in higher quality foods in wetland systems, thus development rates are increased. It has been shown that *P.nubifer* abundances are related to the breakdown of summer algal blooms (Davis *et al.*, 1988; 1989).

Temperature is a main factor affecting emergence patterns for midges. An increase in temperature either results in increased time to emergence (Maier *et al.*, 1990) or high larval mortality thus reducing numbers emerging (Maier *et al.*, 1990). Although increased temperature sometimes results in quicker emergence, it often results in smaller individuals (Maier *et al.*, 1990). Adult size impacts on mating success and the number of eggs laid by the female (Pinder, 1995a). Temperature needs to be monitored throughout the year to determine different species thresholds and to predict when the best times are to spray (Pinder *et al.*, 1991).

8.3 Sediment type and particle size

This study showed that survival of *C.alternans* was not affected by two sediment types. Though it was a small study, it emphasises that chironomids are highly adaptable.

Lamb (2001) found that *C.alternans* preferred bare sediments at Lake Joondalup. Bare sediments are thought to have a low supply of food, though food was not limiting at Lake Joondalup (Lamb, 2001). From a study conducted at Lake Joondalup (Lamb, 2001), it was found that metaphyton, bare sediment and sediment covered in *Chara* species did not affect the distribution of larvae.

From observations, particle size affected the construction of larval tubes; this did not impact on survival rates. Particle sizes at Lake Joondalup are suitable for tube building (Lamb, 2001).

A preference to a particular sediment type may reflect food needs or shelter requirements (Ward, 1992). Chironomids have been found by Barton and Hynes (1978, cited in Ward, 1992) to be the most abundant insect in sandy substrates. Higher larval densities associated with metaphyton in Lake Joondalup (Lamb, 2001) may not be related to the algal composition in the metaphyton, but the bacteria and particulates associated with the metaphyton. The bacteria and particulates in the water column may have a greater impact on distribution. Microorganisms (bacteria) are nutritionally more beneficial than organic matter (Tokeshi, 1995c). The importance of bacteria for larvae changes according to the trophic state of the wetland as it is related to the breakdown of phytoplankton (Perg & Hellenthal, 1992).

8.4 Control of midges

Managers currently measure the larval densities in wetland sediments to predict nuisance plagues. Twenty sites are monitored over Lake Joondalup and when an average of 2000 larvae per m² occurs the lake is sprayed (Lund, 2002). Using larval densities as a trigger to apply pesticides results in a number of individuals not being affected by the spray as they have already progressed to adults and are able to breed and lay eggs (Lund, 2002).

In 2001, Lake Joondalup experienced high abundances of midges, predominantly *C.alternans* and *C.occidentalis*. The council sprayed the lake four times from September to December (Figure 8.4.1). The first spray was ineffective as larval densities were already declining. The larval densities decreased after spraying, though colonisation was fast and levels increased shortly after. All peaks experienced after spraying resulted in nuisance plagues around residential housing. The spraying was ineffective in reducing larval densities to a manageable level over the long-term.

Determining how seasonal variations impact on the life cycle of nuisance midge species is important in determining when they will become most prevalent. From this study it has been shown that *C.alternans* has the highest survival rate at 20°C and completes the larval cycle in just over four weeks at this temperature. Relating the spraying information from 2001 and based on a four week larval stage, spraying needs to be conducted over shorter intervals to be more effective (Lund, 2002). A two and a half to three week spraying regime needs to be adopted. It is known that *C.alternans* emerge around June. Once temperatures have reached those favoured by *C.alternans*, larval densities in the sediment need to be checked. If they correspond with increasing numbers, then hotspots are sprayed. Continual monitoring of larval densities and adult emergence is conducted throughout the problem time. Two and a half to three weeks later another spray could be applied. This could have a greater impact on reducing larval densities and it could hopefully take midges more generations to build-up

their numbers. If it does have a major impact on midges then subsequent spraying may not be needed (Lund, 2002).

A three-week spraying regime has the potential to reduce larval densities and the number of adults emerging. When determining how long it takes for midges to recolonise the lake after spraying, the short-term effects of pesticides become evident. There needs to be an average of 2,000 larvae per m² at Lake Joondalup for spraying to occur, which equals 900 million larvae across the whole lake. It has been estimated that 1 in 50 (Pinder *et al.*, 1991) to 1 in 500 (Lund *et al.*, 2000) larvae emerge per night. At the lowest ratio of 1 in 500, at a larval density of 2000 per m² approximately two female adults emerge per m² per night (assuming the sex ratio is 1:1). As *C.alternans* females lay around 600 eggs per mass, it would take a maximum of two days for the midges to recolonise the lake. This assumes a survival of 78% in the larval phase.

Re-colonisation of the lake in two days seems feasible when considering the large numbers of midges associated with Lake Joondalup. Adults emerging the night prior to spraying and adults emerging as a consequence of spraying are able to lay eggs that will not be affected by the spray. Eggs layed in the days prior to this also need to be considered. First and second instar larvae are known to be planktonic (Lellak, 1968, cited in Pinder, 1995), therefore they may avoid the effects of the larvicide. Considering the potential of the midge to recolonise the lake after spraying, a three-week spraying regime may substantially reduce numbers, and the potential for re-colonisation could be decreased.

Recommending any amount of spraying raises a lot of management questions associated with resistance to the pesticide (Ali, 1995; Lund, 2002), the effect on non-target organisms and the toxicity on the environment. It must be emphasised that this is a short-term approach and long-term methods need to be implemented to remove the need for spraying.

Appropriate monitoring programs need to be developed and sustained through any spraying or control program. This enables methods to be refined and their feasibility reviewed (Pinder *et al.*, 1991). A large monitoring program needs to be established to determine environmental parameters that trigger the emergence of midges. Though it has been suggested that temperature is the primary factor triggering emergence, other factors may be important. Determining these factors would allow predictions to be accurate and control mechanisms to be more effective. If there is lack of understanding of Chironomid life histories then sampling and monitoring programs can be impacted (Berg & Hellenthal, 1992)

Along with monitoring environmental parameters, the effect of the pesticide needs to be closely monitored to determine its impact on the environment. The toxicity and persistence of the pesticide along with its effects on non-target organisms and midges needs to be closely watched.

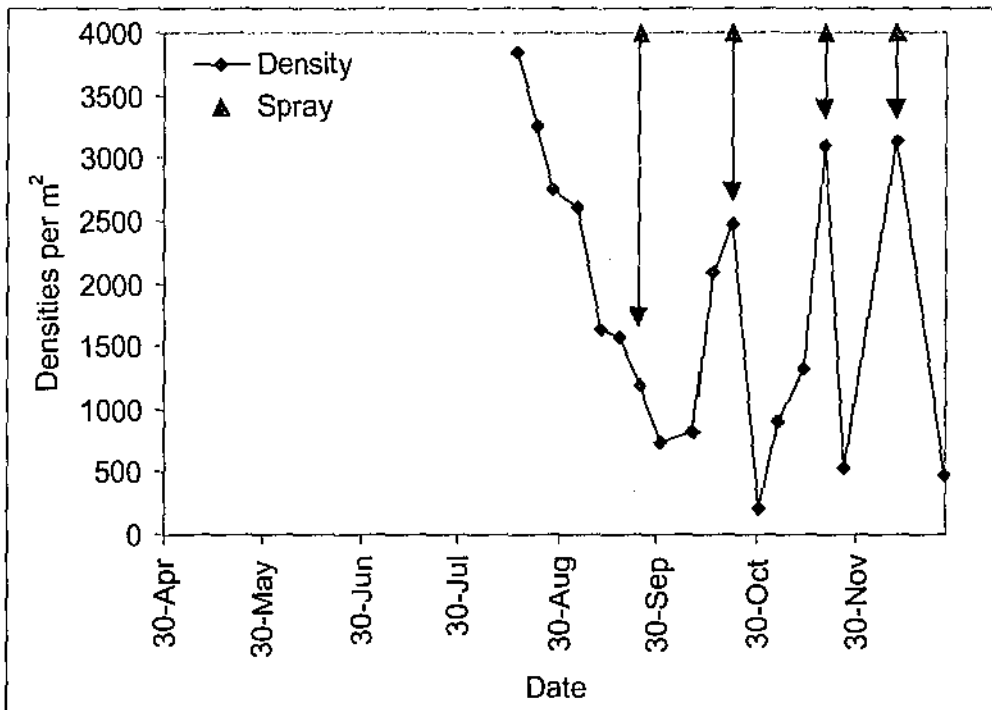


Figure 8.4.1: Larval densities and the occurrence of spraying at Lake Joondalup in 2001.

8.5 Future

Various control mechanisms for midges have been investigated over the years (Ali, 1995; Davis *et al.*, 1987). Continual application of pesticides is evident. The use of light traps around wetlands and in isolated areas to distract midges from residential areas is currently being used. Lighting needs to be discussed at the planning level of residential areas as bright street and house lights attract adults.

Long-term strategies are being formulated for Lake Joondalup. An 'all encompassing' catchment management plan needs to be implemented to improve water quality (Davis & Christidis, 1997). Vegetation surrounding wetlands plays important roles in the functioning of these ecosystems. Vegetation reduces the amount of light reaching the water by both shading areas and contributing colour (gilvin) to the water. Shading keeps temperatures down and both shading and high gilvin reduce the availability of light to algae, thus reducing their growth (Davis & Christidis, 1997; Ward, 1992). Roots aerate the soil and filter nutrients flowing into the wetland (Davis & Christidis, 1997). Revegetating the fringes of wetlands along with reducing the nutrient input into the lake needs to be implemented. Reinstating near-natural conditions in Lake Joondalup will relieve managers of having to use pesticides.

Managers associated with Lake Monger have effectively reduced nutrient concentration. The impact of the reduction of nutrients on midge numbers has been evident (M. Lund, Edith Cowan University, pers. comm., 2002).

Managers need to increase public awareness (Davis *et al.*, 1987), with special focus on residents and business owners in the surrounding catchment (Davis *et al.*, 1988; 1989). Informing residents will show them the enormous task that is involved in controlling midges. Residents need to be told what they can do to contribute to the reduction in midge numbers; this is especially needed in reducing nutrient inputs into wetlands.

Chironomidae are an important component of the food chain in wetland ecosystems (Davis *et al.*, 1987) and must not be eradicated from these systems. To understand the processes affecting midge abundance, a better understanding of chironomid ecology is needed (Davis *et al.*, 1987).

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