ECOPHYSIOLOGY OF *NEOMYSIS INTEGER* (MYSIDACEA: PERACARIDA)

By

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ABSTRACT

Neomysis integer (Leach) (Peracarida: Mysidacea) is a common component of the hyperbenthos of Western European estuaries, and this study focused on the ecophysiology and ecotoxicology of this mysid. Behavioural and physiological responses of *N. integer* to physical and chemical variables (current velocity, substratum type, temperature and salinity) were measured in the laboratory to gain better understanding of how this mysid maintains position in estuaries. Current velocity was the most important factor controlling position maintenance, and *N. integer* had a maximum swimming speed of *c.* 9cm s^{-1}. Position maintenance was facilitated by a muddy substratum and was not affected by salinity. Oxygen consumption and feeding rates of *N. integer* increased with increasing temperature but, whilst oxygen consumption decreased with increasing salinity, feeding rate increased. Male mysids consumed oxygen at a higher rate than females, however, there was no difference in the feeding rates of males and females. The acute toxicity of two organophosphate pesticides (chlorpyrifos and dimethoate) to *N. integer* was examined, and 96h LC_{50} values of 0·13µg chlorpyrifos L^{-1} and 0·54mg dimethoate L^{-1} were estimated. Sub-lethal exposure to chlorpyrifos led to an increased rate of oxygen consumption and decreased feeding rates compared with control animals. In addition, chlorpyrifos exposure led to disrupted behaviour of *N. integer*, including hyperactivity and decreased maximum swimming speed. The results are discussed in terms of the behaviour and physiology of *N. integer* in response to natural and anthropogenic physical and chemical variables in the natural environment. The potential of *N. integer* as a toxicity testing species, and the sub-lethal responses used in the study, are critically assessed.

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Author's Declaration

'At no time during the registration for the degree of Doctor of Philosophy has the author been registered for any other University award.'

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CHAPTER 1

GENERAL INTRODUCTION

The contents and theme of this chapter formed the basis of a talk presented at the international conference: 'The Ecology of Estuaries and Soft Sediment Habitats' at the School of Aquatic Sciences and Resource Management, Deakin University, Warrnambool, Victoria, Australia (February 1997). The talk, presented to some 120 delegates, was entitled 'Mysids and environmental monitoring - the case for their use in estuaries'.

1.1 INTRODUCTION

Mysid crustaceans (Malacostraca: Peracarida: Mysidacea) are shrimp-like animals, often called 'opossum shrimps' because ovigerous females carry the developing embryos in a ventral brood pouch. Mysids are distributed from 80°N to 80°S, and occur in most aquatic environments (lentic and lotic) including freshwater, brackish, estuarine, coastal and oceanic (Tattersall & Tattersall, 1951; Mauchline & Murano, 1977; Mauchline, 1980). Since the last published world list of mysids [which described 765 species distributed between approximately 120 genera (Mauchline & Murano, 1977)] approximately 130 new species and 6 new genera have been described. The number of described species is ever increasing as sampling techniques are improved and new habitats are investigated. For example, many mysids are members of the hyperbenthos (the association of small animals living in the water layer close to the bottom) and are difficult to sample effectively, however, improved design of sampling gear has resulted in many new hyperbenthic species being described (Mees & Jones, 1997). It has also been hypothesised that the few species which have been described as pelagic are, in fact, hyperbenthic species caught during inaccurate sampling (Mauchline and Murano, 1977). Twenty-nine mysid species are found in British waters (Mauchline & Murano, 1977).

1.2 ECOLOGICAL ROLE OF MYSIDS IN ESTUARIES

Although Order Mysidacea has a global distribution, individual species are often endemic to particular environments. This is particularly so for estuaries, where specific mysids may occupy discrete sections of the salinity gradient (Greenwood *et al.*, 1989; Moffat & Jones, 1993). As is typical of most of the estuarine biota, the mysid fauna is reduced compared with marine systems. Most estuarine mysids are hyperbenthic, although some species make diurnal migrations into the water column (Dauvin *et al.*, 1994). Mysids are important in the food webs of estuaries as consumers and producers (Mauchline, 1980). Many mysids are carnivores and feed selectively (by size and/or species) on zooplankton, mainly when suspended material is reduced, while other mysids filter feed on phytoplankton (Mauchline, 1980). Such selective feeding has the potential for structuring zooplankton communities (Fulton, 1982), and influencing the structure of phytoplankton and meiofaunal communities (Mees & Jones, 1997). Estuarine mysids are mostly omnivores, feeding on a wide range of items including detritus, phytoplankton and zooplankton (Mauchline, 1980).

Most ingest organic detritus and are responsible for the remineralisation of a large proportion of refractile detritus (Fockedey & Mees, 1997). Occurring in large numbers, mysids contribute significantly to the omnivore standing stock of estuaries. For example, *Schistomysis spiritus* constituted 76% of the omnivore biomass and 43% of the total annual integrated omnivore standing stock of the Bristol Channel (UK) (Williams & Collins, 1984).

Despite the inadequacies of most sampling gear, mysids have been caught in high densities in many estuaries (Mees & Jones, 1997). Due to recruitment, mysid numbers in temperate estuaries usually peak during the summer and the following densities have been reported: 31,250 $100m^{-3}$ in the Westerschelde Estuary (The Netherlands) (Mees *et al.*,1994), 1500 $100m^{-3}$ in the Avon-Heathcote Estuary (New Zealand) (Greenwood *et al.*, 1989), and >100,000 $100m^{-3}$ for the River Tamar Estuary (UK) (Moffat & Jones, 1992). Such high densities lead to high productivity and *N. integer* has an estimated productivity of 300mg ash free dry weight m^{-2} yr^{-1} (Mees *et al.*, 1994). Mysid production is reviewed briefly by Mees & Jones (1997). High densities, an omnivorous diet and the fact that mysids are preyed upon by fish and wading birds (Moffat, 1996), implicate mysids as an important link in the energy transfer to higher trophic levels of estuaries (Mees *et al.*, 1994). Unfortunately, there are limited data on their contribution to such food fluxes (Mees & Jones, 1997).

1.3 MYSIDS IN ENVIRONMENTAL MONITORING

Mysids have been used in laboratory toxicity testing from the early 1970s (Anderson *et al.*, 1974; Jacobs & Grant, 1974) and the development of entire life-cycle tests with *Americamysis bahia* enhanced this use (Nimmo *et al.*, 1977). *Americamysis bahia* is more sensitive to toxic substances than many other marine species (Table 1.1), and is one of the standard test organisms adopted by the United States Environmental Protection Agency (USEPA) for marine and estuarine environments (USEPA, 1987). As a result, there is a relatively large amount of published information on the sensitivity to toxicants of this, and related species [*A. bigelowi* and *A. almyra* (Nimmo *et al.*, 1978; Nimmo & Hamaker, 1982)], and relatively limited toxicity data for other mysid species. The available evidence suggests that mysids *sensu lato* are very sensitive to toxic substances (Brandt *et al.*, 1993;

Wildgust & Jones, 1997), and there is growing interest in using local, indigenous mysids in monitoring rather than the standard American species (Emson & Crane, 1994; Langdon *et al.*, 1996).

Table 1.1: 96h LC_{50} data for the toxicity of some contaminants to marine and estuarine organisms. (Source: [a] WHO, 1992; [b] WHO, 1990; [c] WHO, 1991; [d] Wildgust and Jones, 1997.)

Organism	Cadmium [a]		Tributyl Tin [b]		Lindane [c]	
	mg l^{-1}	age/size	µg l^{-1}	age/size	µg l^{-1}	age/size
BIVALVIA						
Mytilus edulis	25	4g	38	adult	-	-
M. galloprovincialis	-	-	-	-	5·5	*c.* 60mm
COPEPODA						
Eurytemora affinis	0·06	nauplii	-	-	-	-
Acartia tonsa	-	-	1·0	sub adult	-	-
MALACOSTRACA						
Americamysis bahia	0·017	adult	1·1	1d	6·3	adult
Neomysis integer	3·9	adult [d]	-	-	-	-
Carcinus maenas	4·1	5.9g	10	larva	-	-
Pagarus longicarpus	-	-	-	-	5·0	*c.* 0·3g
Palaemonetes pugio	-	-	-	-	10	*c.* 0·5g
OSTEICHTHYES						
Cyprinidon variegatus	50	1.1g	25·9	sub adult	104	1·1g
Onchorynchus kisutch	1·5	smolt	-	-	41	*c.* 1g
Solea solea	-	-	2·1	larva	-	-

In terms of their use in environmental monitoring, mysids are considered to be particularly useful because they are sensitive to extremely low levels of many toxicants and at levels that are likely to occur in the environment (Odenkirchen & Eisler, 1988). Most data for mysid sensitivity to contaminants are based upon acute toxicity testing (e.g. Table 1.1) and there is considerable debate as to the value of such data for environmental monitoring. While acute lethal tests are useful indicators of relative contaminant toxicity, they do not reveal effects which may be deleterious to natural populations and acute lethal data are very difficult to extrapolate to the field situation. There is a growing demand for tests which measure sub-lethal effects of contaminant exposure at environmentally-realistic

concentrations and which take into account other key factors operating in the natural environment (Depledge *et al.*, 1995). Even so, it is widely accepted that single-species laboratory toxicity tests will continue to be one of the most widely used methods for regulatory evaluation of chemical hazards (Forbes & Forbes, 1994). The following criteria have been established for organisms used in laboratory toxicity studies (e.g. Reish, 1973; Stebbing *et al.*, 1980; Hutchinson *et al.*, 1995): (1) they are available when required; (2) they are already adapted to laboratory conditions, eliminating an (expensive) conditioning phase; (3) collection for laboratory testing will not decimate field populations (or destroy habitat during collection - an increasingly important point now that many field populations are under threat from anthropogenic activities); (4) their diet is known and readily controlled; (5) the life history is short, making it possible to study the effects of a pollutant on various aspects of reproduction; (6) specimens are easily transported; and (7) they are of ecological importance. Mysids fulfil all these criteria. Specimens are available in high densities, generally throughout the year, and are easy to collect since mysids often aggregate into shoals. Collection should not deplete field populations since mysids occur in such large numbers. Mysids are easily maintained and cultured in the laboratory, and readily feed on brine shrimp nauplii [*Artemia spp* (Collins *et al.*, 1991)]. In addition, mysids have a relatively short life cycle [*c.*28d for *Americamysis bahia* (Nimmo *et al.*, 1978)] and ovigerous females carry their developing embryos in a ventral brood pouch, allowing various aspects of their reproduction to be studied. The role of mysids in food chains clearly identifies their ecological importance.

When assessing the environmental impacts of chemicals, however, there are additional considerations to take into account in test design. For example, tests should: (1) be designed for making predictions about a particular kind of community or habitat and should be based upon field studies in that community or habitat; (2) be capable of predicting mortalities amongst the key species of the community; (3) address the effects upon mortalities of such factors as seasonal or behavioural variation; and (4) be based on a knowledge of the ecological consequences of the pollutant-induced mortalities (Baker & Crapp, 1974; Forbes & Forbes, 1994). Again, mysids can be used in ways which address these points. For example, in an estuary, animals are exposed to continually changing tidal and river currents, which lead, amongst other things, to variations in salinity. Although estuarine mysids are typically euryhaline, many show behavioural adaptations which

maintain their position within distinct salinity ranges or water current speeds (Hough & Naylor, 1992; Schlacher and Wooldridge, 1994). In addition, mysid aggregations are interpreted as a protection from predation (O'Brien & Ritz, 1988; Ritz, 1994). Exposure to sub-lethal contaminant concentrations may disrupt this behaviour, resulting in displacement from their zone of "optimal performance" or in the breakdown of their ability to maintain their schools. In either case, such disruption may result in high mortalities with significant ecological consequence.

1.3.1 Why use *Neomysis integer* ?

Neomysis integer (Plate 1.1), reported from all the European coasts of the Atlantic ocean mostly in estuaries (Tattersall & Tattersall, 1951), dominates the hyperbenthic fauna of the low salinity regions of Western European estuaries, both in terms of density and biomass (Mees & Hamerlynck, 1992; Mees *et al.,* 1995). Individuals aggregate in swarms close to the water's edge, facilitating the easy capture of large numbers. Preliminary work has suggested that *N. integer* is a possible alternative testing species to the widely-used *Americamysis bahia* in laboratory toxicity testing (Emson & Crane, 1994; Wildgust & Jones, 1997).

Plate 1.1: *Neomysis integer*. The plate shows and ovigerous female mysid of *c.* 18mm long. The marsupium, containing eggs, can be seen clearly.

1.3.2 General biology and ecology of *Neomysis integer*

Although generally considered a brackish-water species, populations of *Neomysis integer* have been described from freshwaters, usually as a result of brackish-water habitats becoming cut-off from saline inputs (e.g. Angus, 1996). *Neomysis integer* is extremely euryhaline (Moffat, 1996), and is a hyper/hypo osmoregulator, regulating its haemolymph at a relatively constant osmotic level over the range <1 - 36‰ [although not in fresh water (Ralph, 1965)]. Osmoregulation is achieved by controlling the amounts of free amino acids in the haemolymph, and by oral and anal drinking (Ralph, 1965; Moffat, 1996). In the natural environment, *N. integer* is omnivorous, utilising mesozooplankton and detritus carbon pools by feeding on copepods, rotifers, cladocerans, macrophytal detritus and amorphous detritus from sediment flocs (Mauchline, 1980; Fockedey & Mees, 1997). In the laboratory, *N. integer* feeds readily on phytoplankton, copepods, cladocerans and *Artemia* nauplii (e.g. Lucas, 1936, Astthorsson, 1980; Irvine *et al.*, 1993), and sediment (*pers. obs*). Populations of *N. integer* tend to have three generations per year (Mauchline, 1971; Mauchline, 1980; Moffat, 1996), with adults attaining an average length of *c.*18mm (Plate 1.1; Mauchline & Murano, 1977). As with all mysids, the eggs are laid in a marsupium, and the maximum brood size of *N. integer* (72 eggs) is one of the largest recorded for coastal mysids (Mauchline, 1971; 1973). There is evidence that spawning occurs simultaneously within mysid populations (Mauchline, 1973). The basic life cycle of *N. integer* is as follows: juveniles, released in early autumn, form a slow-growing, overwintering population; as water temperatures increase during the spring, the overwintering population reproduces and the offspring form the (late) spring generation; this latter generation is faster growing, and reaches maturity and reproduces (at a smaller size than the overwintering population) in mid-summer; the summer generation is usually the fastest growing generation, and reproduces at a similar size to the spring generation; juveniles are released in late summer/early autumn and the offspring become the next overwintering population (Mauchline, 1971).

1.3.3 Why use a pesticide as a reference toxicant ?

Pesticides, used in agricultural production and wood and textile preservation, are amongst the most toxic and persistent pollutants entering the marine environment (WWF, 1993). It is estimated that >190,000 tonnes of agricultural pesticides are released into the

environment of North Sea states per annum (WWF, 1993). Diffuse pollution by pesticides has been recognised as a problem for several years in the United Kingdom (Croll, 1991). In 1991, 22·5 thousand tonnes of pesticide were applied to agricultural land in the United Kingdom (a market value of *c.*£415 million), representing an average application of 8·5 pesticide active ingredients to all crops (WWF, 1993). Approximately 90% of this agricultural application never reaches its target organisms but is, instead, dispersed through the air, soil and water (Moses *et al.*, 1993). Although the spillage of pesticides into the environment (point-source contamination) affects local systems (e.g. Boreham & Birch, 1987), diffuse contamination through agricultural run-off affects larger systems and is more difficult to control (Croll, 1991). Laboratory studies have shown that pesticides can be acutely or chronically toxic to estuarine species and there is growing concern that pesticides pose a serious threat to coastal wetlands receiving direct pesticide applications or rainfall run-off from agricultural land (Clark *et al.*, 1993). The toxicity, environmental persistence and bio-accumulation properties of organochlorine pesticides (e.g. DDT and dieldrin) are well documented, and their usage has been decreasing for several years (Croll, 1991). Pesticides used currently in the United Kingdom, however, are applied at higher concentrations than the organochlorines, and their increased solubility in water lends them increased potential to leach into surface and groundwaters (Croll, 1991). In addition to the lethal toxicity of pesticides to non-target organisms, sub-lethal effects include behavioural disruption, dysfunctioning of the immune system and reproductive impairment (WHO, 1986; Lyons, 1996). Biota at risk from pesticide contamination include planktonic, pelagic, epifaunal and infaunal organisms, which may be resident or migratory in estuaries (Clark *et al.*, 1993). Mysids are particularly at risk as they are reported to be comparatively sensitive to pesticides (Table 1.1). There is a paucity of knowledge on the sub-lethal effects of pesticide contamination and there is an increasing demand for toxicity studies showing the effects of pesticides on ecologically-important species (Lyons, 1996).

1.4 AIMS

The aims of the current study were two-fold. Firstly, to establish the responses of *Neomysis integer* (swimming behaviour, respiration and feeding) to the natural environmental variables (current flow, temperature and salinity) of estuaries. Secondly, to examine the effects of an organophosphate pesticide on these aspects of mysid behaviour and

physiology. Establishing the sensitivity of *N. integer* to lethal and sub-lethal pesticide exposure, allows further assessment of the use of *N. integer* as an environmental toxicity testing species for European estuaries.

1.5 OUTLINE OF THESIS

Using a novel annular flume, Chapter 2 investigates the effect of current velocity, salinity and type of substratum on the swimming behaviour of *Neomysis integer*. Current velocity, salinity and substratum are major physical variables encountered by *N. integer* in estuaries, and their relative importance in controlling mysid position maintenance was examined in the flume and compared with mysid distribution in the natural environment. In Chapter 3 oxygen consumption, and in Chapter 4, feeding rate and absorption efficiency are examined in response to temperature and salinity. *Neomysis integer* is a eurythermal and euryhaline species, however, the effect of temperature and salinity on the physiology of male and female *N. integer* over tidal and seasonal cycles is unknown. The effect of anthropogenic toxicants (the pesticides chlorpyrifos and dimethoate) on *N. integer* is examined in Chapter 5. Acute toxicity tests, examining the lethal toxicity of these pesticides to adult and juvenile *N. integer*, were conducted at the Brixham Environmental Laboratory (ZENECA Limited), in order to assess which pesticide, and at which concentrations, would be used in the ensuing sub-lethal tests. Having identified the ecophysiology and behavioural responses of *N. integer* in relation to physical and chemical variables in the natural environment (Chapters 2, 3 and 4), and the acute toxicity of pesticides to *N. integer* (Chapter 5), the effects of sub-lethal exposure to chlorpyrifos on the swimming behaviour, oxygen consumption, feeding rate and absorption efficiency of *N. integer* are investigated in Chapter 6. In addition, the scope for growth of mysids exposed to chlorpyrifos was calculated. Chapter 7 discusses the results of the thesis in terms of mysid ecophysiology and sub-lethal bioassays using *Neomysis integer* as a toxicity testing species.

CHAPTER 2

THE POSITION MAINTENANCE BEHAVIOUR OF *NEOMYSIS INTEGER* IN RESPONSE TO CURRENT VELOCITY, SUBSTRATUM AND SALINITY

The main findings of this chapter have been accepted for publication as:

"The position maintenance behaviour of *Neomysis integer* (Peracarida: Mysidacea) in response to current velocity, substratum and salinity."

Roast, S.D., Widdows, J. & Jones, M.B. (1998)

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2.1 ABSTRACT

The mysid *Neomysis integer* (Leach) is a common member of the hyperbenthos of the upper reaches of European estuaries. How this species maintains its position against the conflicting influence of tides and river flow forms the underlying question addressed in this study. The swimming behaviour of *N. integer* was examined in the laboratory by means of a novel annular flume with variable water current velocity. A range of experimental conditions was examined, including water current velocity, substratum type and salinity. Variations in behaviour (e.g. positioning in the water column, swimming speed and orientation to the flow) were recorded with video equipment. Current velocities, salinity and distribution of *N. integer* over a range of tides (including seasonal variations as well as spring and neap tides) were measured at the collection site at the East Looe River Estuary (Cornwall, England). *Neomysis integer* was found to sustain a swimming speed of 6cm s^{-1} in the laboratory and showed better position maintenance with a muddy rather than sandy substratum. These findings agree with field observations at the East Looe River Estuary, where *N. integer* was found at the periphery of the water channel where flow velocity was *c.*8cm s^{-1}. No clear behavioural trends were observed in relation to variations in salinity. In the field, *N. integer* occurred in salinities of 1 - 34‰.

2.2 INTRODUCTION

Aquatic organisms inhabiting lotic environments continually encounter the problems of maintaining their position and preventing their displacement by the flowing water. In general, there are three main controls of the maintenance of positioning by pelagic invertebrate populations in estuarine systems: reproductive compensation of seaward losses, behavioural adaptations and hydrodynamic processes (Schlacher & Wooldridge, 1994). Reproductive compensation is shown by mysids, where gravid females produce relatively large numbers of juveniles per brood, for example, 72 embryos have been recorded in the marsupium of *Neomysis integer* (Mauchline, 1973). All offspring are released together from the marsupium and then aggregate in a shoal to improve survival (Mauchline, 1980). In addition, spawning is frequently synchronised within a population, resulting in female mysids releasing their broods simultaneously (Mauchline, 1973). Behavioural adaptations, such as altering swimming activity at different tidal phases, have been shown to control invertebrate positioning in both coastal and estuarine environments (e.g. Warman *et al.*, 1991; Hough & Naylor, 1992). Finally, hydrodynamic processes may control the distribution of copepods, for example the distribution of *Eurytemora affinis hirundoides* in the Gironde River Estuary has been shown to relate directly to patterns of water circulation and particulate transport (Castel & Veiga, 1990). Mysids are common components of the hyperbenthos of estuaries and are, therefore, subject to the position maintenance problems described. Much attention has been focused on mysid behaviour but, until recently, this has tended to concentrate on their swarming behaviour (e.g. Steven, 1961; Clutter, 1969; Mauchline, 1971; Wittman, 1977; O'Brien *et al.*, 1986). Research attempting to relate shoaling behaviour to how mysids actually maintain their position in the estuary is limited. Although hyperbenthic organisms are difficult to sample quantitatively due to the paucity of reliable sampling gear (Mauchline, 1980; Mees & Jones, 1997), attempts at sampling the distribution of estuarine mysids over an entire tidal cycle have been made, allowing their abundance in areas of differing physico-chemical attributes, and at different tidal phases, to be related to the behavioural characteristics which control their distribution. Hough & Naylor (1992) reported that *Neomysis integer* maintained its position in the tidally-mixed Conwy Estuary by increased swimming on the flood tide to counter seaward displacement on the ebb tide. This is similar to the strategy observed for *Mesopodopsis slabberi* (Wooldridge & Erasmus, 1980). On the ebb tide, *M. slabberi* remains close to the substratum where water movement is reduced, however, as the flood tide commences, *M. slabberi* rises up into the water column where the faster flowing water pushes

it upstream (Wooldridge & Erasmus, 1980). An alternative strategy is shown by *Gastrosaccus brevifissura* in the Gamtoos River Estuary (South Africa). This mysid makes axial migrations over the tidal cycle, thereby, reducing displacement by remaining in slower moving waters (Schlacher & Wooldridge, 1994). As well as water current speed, other physical or chemical variables, including salinity and temperature (Williams & Collins, 1984; Moffat & Jones, 1993), and substratum type (Mauchline, 1980; Schlacher & Wooldridge, 1994) may control mysid distribution.

In addition to studies of mysid distribution in the natural environment, several workers have examined mysid behaviour in the laboratory. Swimming behaviour of deep-sea species [e.g. *Gnathophausia sp.* (e.g. Quetin & Childress, 1980)] has been examined in aquaria since observations in the natural deep-sea environment are difficult. Fossa (1986) examined the swimming behaviour of deep-sea hyperbenthic mysids in an aquarium, and reported their vertical zonation and position relationships with the substratum. Flumes, with a generated water current, are particularly useful to study the swimming behaviour of riverine or estuarine organisms (e.g. Hughes & Richard, 1973).

2.2.1 Aim

The aim of this chapter was to investigate how the swimming behaviour of *Neomysis integer* responded to current velocity, salinity and type of substratum to determine whether mysid distribution is controlled by behaviour or hydrodynamic processes.

2.3 METHODS

2.3.1 Site description

Inter-population variability in the physiological responses of peracarid crustaceans has been reported (Maltby & Crane, 1994). To prevent this variability a single mysid population was studied. All mysids used in these experiments were collected from the south side of Terras Bridge, East Looe River Estuary [4° 27' W, 50° 21' N (Fig. 2.1)]. Located approximately 2km from the estuary mouth, this site is at the upper tidal limit and has a salinity range of *c.*1 - 34‰, and a mean tidal range of *c.*2m (*pers. obs.*). Although high tidal salinity approaches that of normal seawater, much of the time the site is dominated by low salinity

water due to the influence of the river flow (Plates 2.1 and 2.2). The catchment of the East Looe River is mainly grazing land, and the river has been used in other studies as a control site for the collection of uncontaminated crustacean specimens [e.g. amphipods (Jones & Johnson, 1992) and mysids (e.g. NRA, 1993)]. In addition to the mysid population, the aquatic macrofauna at Terras Bridge comprises amphipods (e.g. *Gammarus spp.*), decapod crustaceans (e.g. *Carcinus maenas* and *Crangon crangon*), and unidentified flat fish (*pers. obs.*).

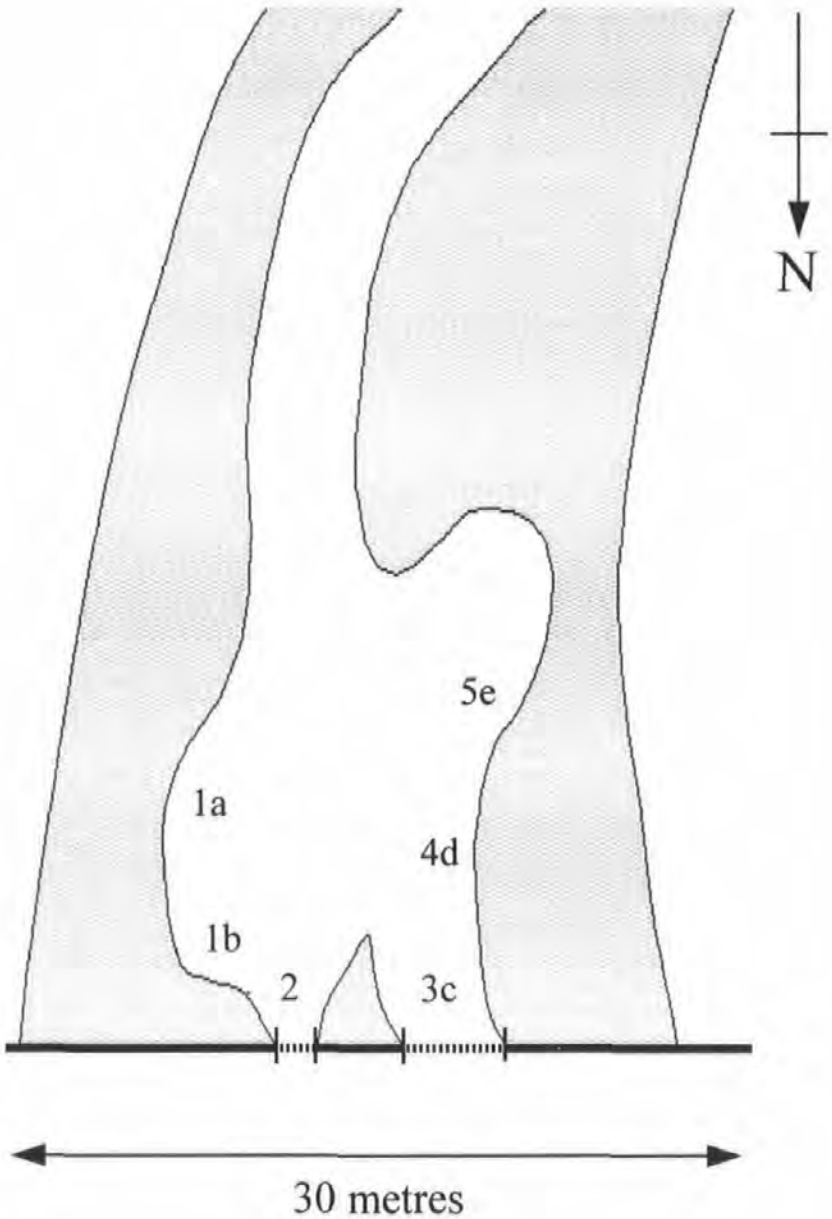


Figure 2.1: The river channel at Terras Bridge, East Looe River Estuary, Cornwall (UK) at low tide. The shaded area represents the intertidal area. Sites 1b-5e are the locations of current velocity measurements. Sites a - e are the locations from which surface sediment samples were taken.

Plate 2.1: View from Terras Bridge (looking south) of the East Looe River Estuary at high tide (upper plate) and low tide (lower plate).

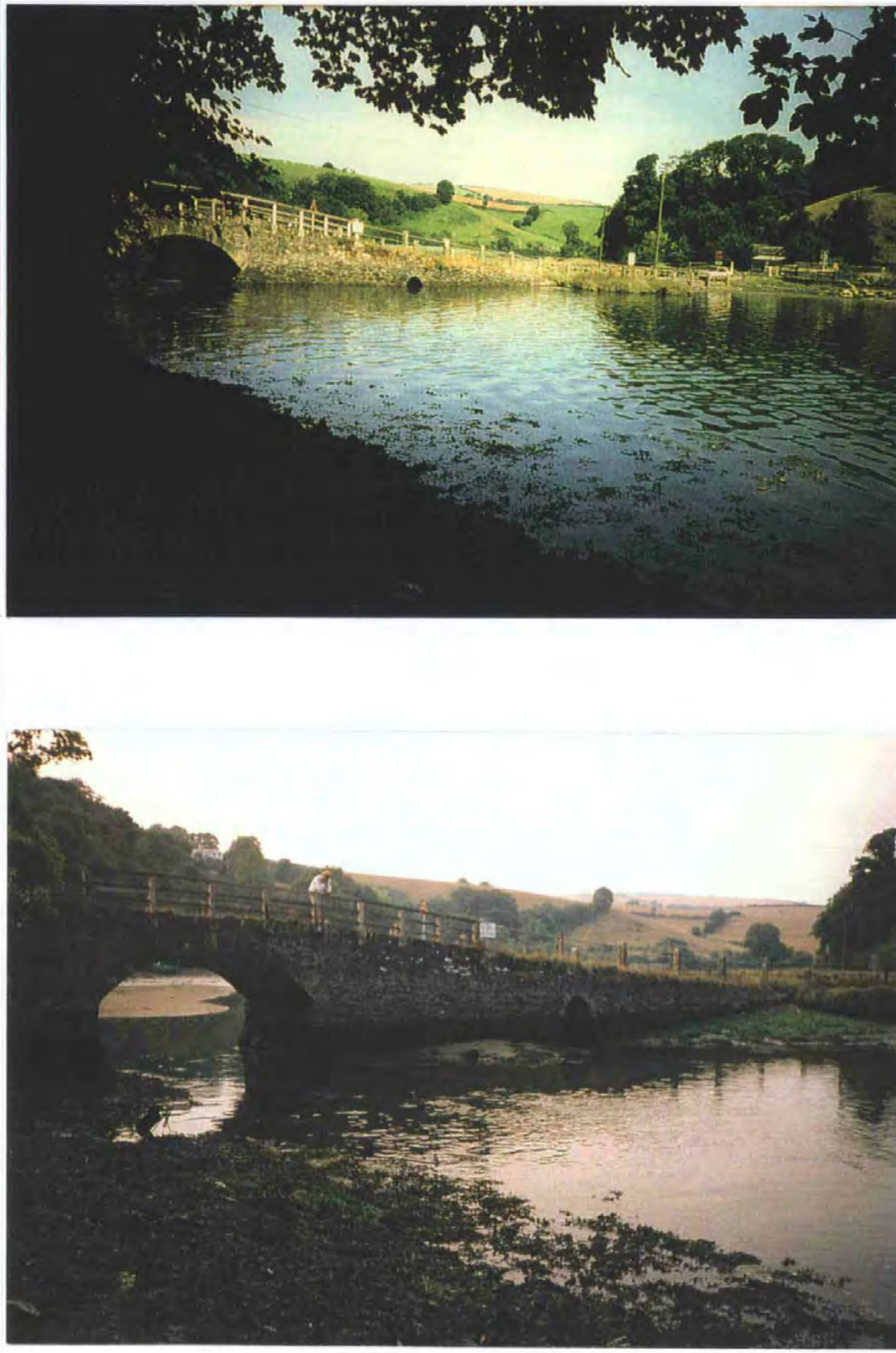


Plate 2.2: View of Terras Bridge and the East Looe River Estuary (looking north) at high tide (upper plate) and low tide (lower plate).

2.3.2 Collection and maintenance of mysids

During January and February (1995), mysids were collected at low tide from the southern side of Terras Bridge, by sweeping an FBA dip net (1mm mesh) along the water's edge. At the time of sampling, the population comprised overwintering mature males and females, and juveniles of various sizes (Chapter 1). Each sweep of the net lasted <3s so that mysids were not stressed excessively. Mysids were placed in a 10L bucket containing low salinity water (*c.*1‰) from the estuary, transported by car to the laboratory and placed in a 20L holding tank. In the holding tank, water was maintained at 12 ±1°C (achieved by placing the holding tank in a large water bath cooled with a Hetofrig dip-cooler) and 10 ±1‰ (made by combining filtered seawater and double distilled, de-ionised water), and was recirculated through an Eheim (Model 2213) filter. An under-gravel filter was also used to maintain water quality. Lighting, provided by overhead fluorescent lights, was at ambient laboratory levels. Mysids were fed twice daily on <48h old *Artemia salina* larvae, such that nauplii were always in excess (Lussier *et al.*, 1988; ASTM, 1990).

2.3.3 Measurement of physical variables at Terras Bridge

Current velocities were measured at 5 points (1b-5e) across the channel at Terras Bridge (Fig. 2.1) using a Valeport Model 800-175 electromagnetic (EM) current flow meter (Plate 2.3). The EM flow meter was attached to a tripod and measured flow 10cm above the substratum. Flow velocities were measured over the entire tidal cycle on spring and neap tides, and during different seasons. The presence or absence of mysids at each flow measurement point was recorded to compare with laboratory mysid swimming results. Salinity measurements were also made over the tidal cycle using a Y.S.I. Instruments salinity meter. A sample of the surface sediment (the top 1cm) was taken at each of 5 sites (a-e; Fig. 2.1) at low tide to establish granulometric composition. In the laboratory, sediment samples were sieved through a series of sieves (2000, 1000, 710, 500, 355, 250, 120, 80 and 63μm mesh sizes) and each fraction was oven-dried to establish percentage composition by weight (Buchanan, 1984).

Plate 2.3: Measuring low tide current velocity with the Valeport EM current meter at Terras Bridge, East Looe River Estuary.

2.3.4 Swimming behaviour

Swimming behaviour was studied using an annular flume with adjustable free-stream current velocities between <1 to >50cm s^{-1} [Fig. 2.2; Plate 2.4 (Widdows *et al.*, 1997)]. One of the major advantages of using annular flume designs is that the constant channel geometry, and infinite flow length, result in a fully developed boundary layer above the sediment (Amos *et al.*, 1992). Consequently, there are not the hydrodynamic problems associated with the entrance and exit conditions of straight open-channel flumes, which then limit the size of the 'test section'. The annular flume has the added advantage over other re-circulating flumes in that there are no pumps to damage the hyperbenthic organisms and the entire flow volume can be contained over a bed of sediment. In the annular flume, the water current is generated by a motor-driven, rotating drive-plate located 5cm below the water surface which creates flow by friction with its smooth surface. A digital counter records the speed of the drive plate in revolutions per minute (r.p.m.), where 5r.p.m. corresponds to a free-stream current velocity of 3cm s^{-1} (Widdows *et al.*, 1997). Measurements of current velocity in the flume with the

EM current meter, and video-tracking of neutrally-buoyant particles, showed a constant free-stream velocity down the water column to within 1cm of the substratum. Consequently, there is a logarithmic decline in current velocity within the 1cm boundary layer (see Fukada & Lick, 1980).

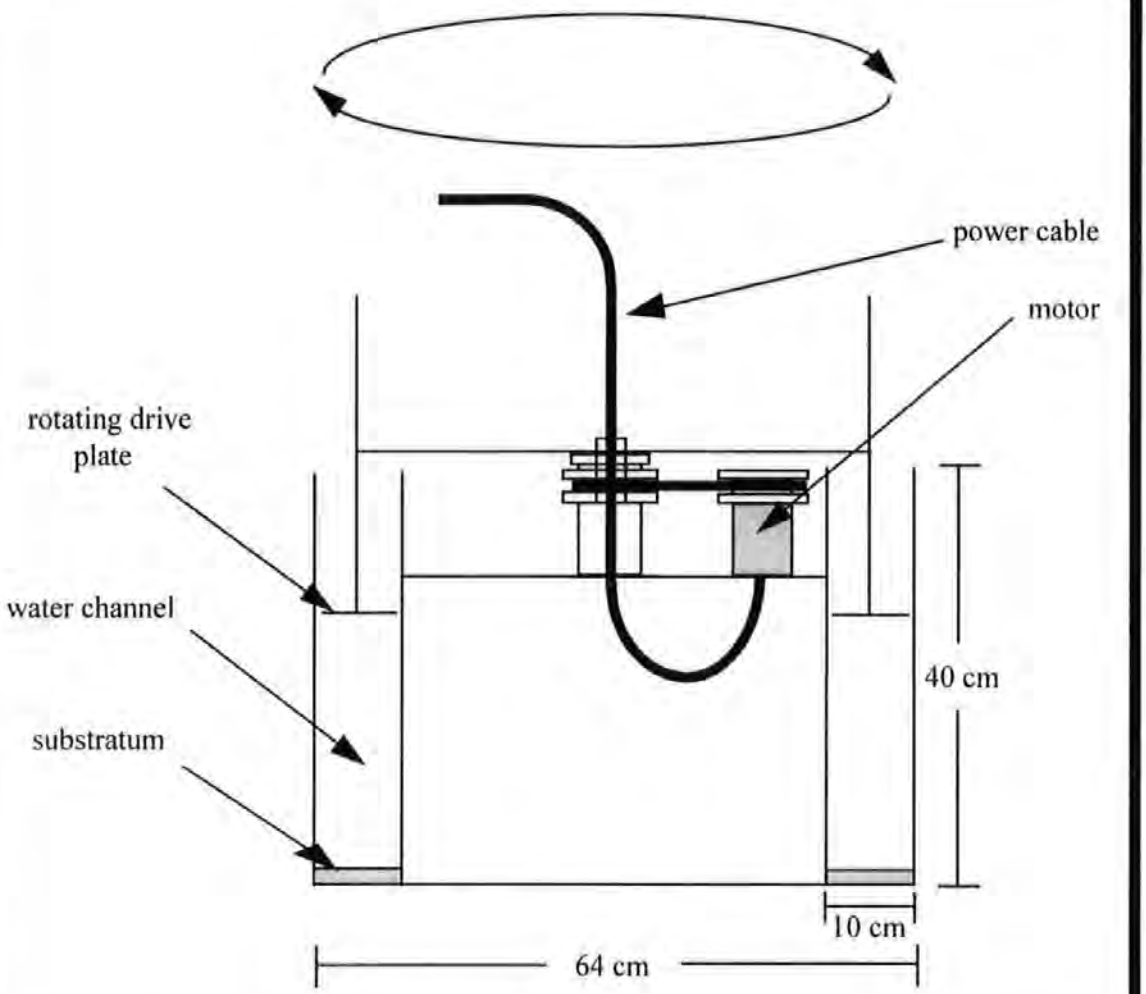


Figure 2.2: The annular flume. The diagram represents a vertical section through the centre of the flume. Circular arrows indicate direction of flow.

For each experiment, 40 *Neomysis integer* of similar body length (15mm ±1mm from anterior margin of rostrum to tip of telson) were used. Mysids, placed in the flume 24h in advance of any experimental work to allow them to acclimate to the flume environment, were fed *ad libitum* on <48h old *Artemia*. Feeding was stopped 2h prior to each experiment so that swimming behaviour was not influenced by feeding behaviour. All experiments were conducted during the day at 15 ±1°C and with continuous fluorescent lighting (temperature,

salinity and pH were monitored daily). An Eheim filter (Model 2213), placed in a temperature-controlled water bath, was used to maintain water quality and temperature, however, this was switched off whilst experiments were conducted since the pump creates a water current. Mysid activity was recorded through the side of the flume via a Sony Hi-8 Camcorder (Model CCD-TR2000E) onto video cassette, and visual observations of swimming behaviour were also noted throughout the experiments.

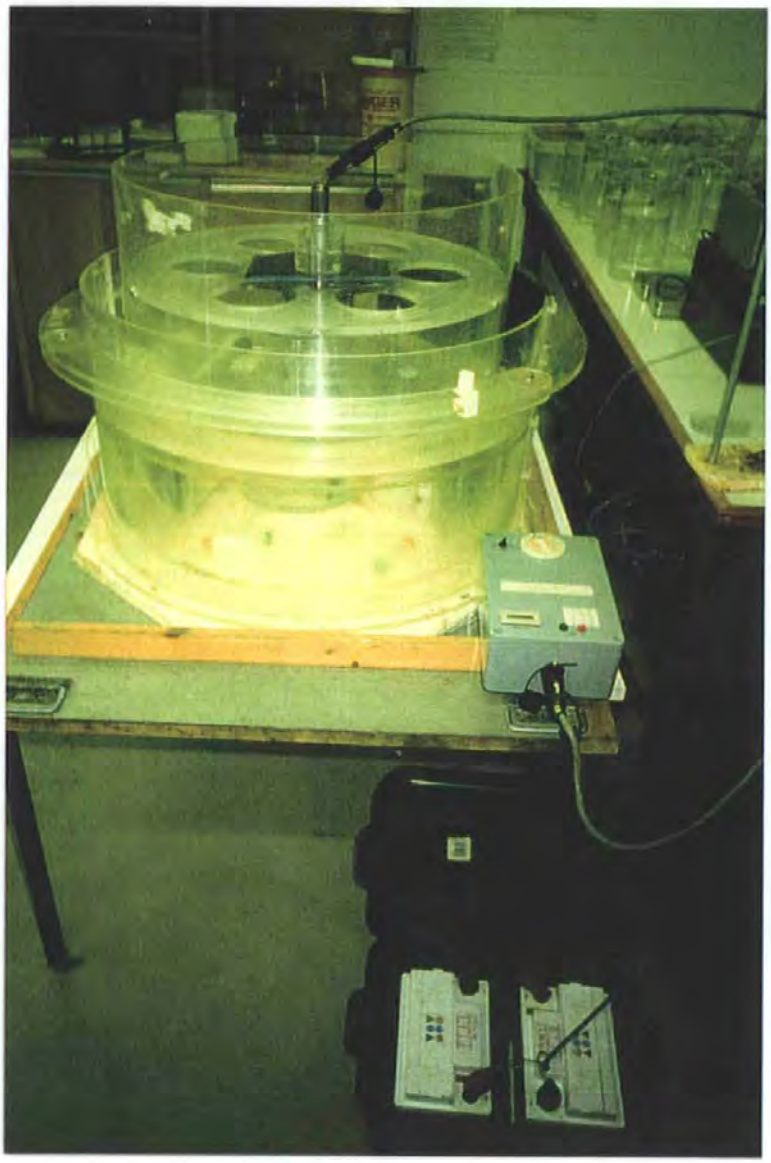


Plate 2.4: The annular flume [scale: diameter = 64cm (Fig. 2.2)].

Effect of current velocity and substratum type on swimming behaviour

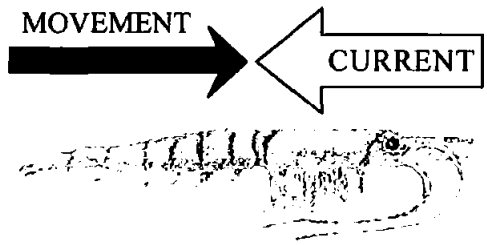
The methods for studying the effects of current velocity and substratum type were the same, repeating each velocity experiment using different substrata. The two substrata used were: sand (*c.*1mm diameter) and mud (<180μm diameter). In all experiments, sediment depth was *c.*1cm and salinity was maintained at 10 ±1‰ (achieved by combining filtered seawater and double distilled, de-ionised water). An initial current speed of 3cm s^{-1} was used and swimming activity was recorded for 15min. The flow velocity was then increased to 6cm s^{-1} for a further 15min, and so on, increasing the velocity in units of 3cm s^{-1} up to a final velocity of 21cm s^{-1} (a maximum speed of 15cm s^{-1} was used with the muddy substratum since re-suspension of the silt prevented accurate vision into the flume). Eight replicates were made for each substratum, using different mysids for each replicate.

Effect of salinity on mysid swimming behaviour

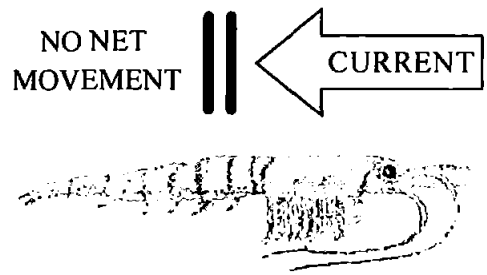
The effect of salinity was examined at a constant current velocity and with the sandy substratum only. Flow velocities of 6cm s^{-1} and 9cm s^{-1} were used (having been defined as important speeds from the previous experiments) and water of different salinities was made by combining filtered seawater with freshwater collected from the study site at Looe. An initial salinity of 1 ±1‰ was established in the flume and mysids were allowed 24h to acclimate to this environment. Swimming behaviour was monitored for 15min at 6cm s^{-1}, followed by 15min at 9cm s^{-1}. Salinity was then increased rapidly to 10 ±1‰ and swimming behaviour monitored as before. This procedure was repeated at salinities of 20 and 30 ±1‰. The mysids were allowed 24h to rest and feed (<24h *Artemia*) whilst held at 30 ±1‰, and the experiment was repeated, but in descending salinity order. Four replicates, using different mysids, were performed for these experiments.

Analysis of swimming behaviour

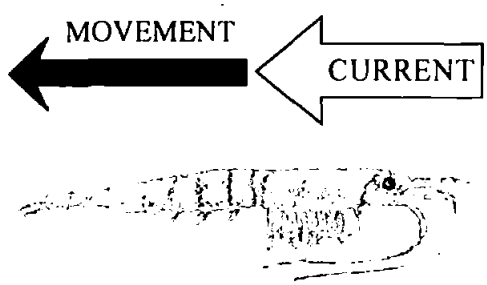
The video recordings of swimming behaviour were analysed visually and mysid swimming behaviour categorised into four different behavioural types (Fig. 2.3).

1). 

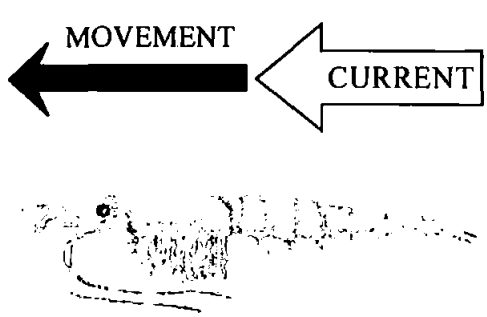
The mysid is facing into the current and swimming faster than the flow velocity, therefore, it moves forward.

2). 

The mysid is facing into the current and swimming at the same speed as the flow velocity, therefore, it maintains its position.

3). 

The mysid is facing into the current and swimming slower than the flow velocity, therefore, it is swept with the current.

4). 

The mysid is facing the same direction as current movement, therefore, it moves with the current. The mysid may, or may not, be swimming.

Figure 2.3: The four types of swimming behaviour for *Neomysis integer* recorded in the flume.

Thus, mysids were categorised according to orientation and swimming speed (i.e. whether net movement was up- or down-current, or whether position was maintained). Frequency of each behavioural type was recorded at each height level in the water column up to 15cm above the substratum. Mysids swimming >15cm above the substratum were classified as one height class.

2.3.5 Statistical treatment of results

Mysid behaviour frequency data were converted to proportions and arcsine square-root transformed to account for small proportions. The transformed data were then examined statistically by one- and two-way analysis of variance (ANOVA).

2.4 RESULTS

2.4.1 Field measurements

At Terras Bridge, there was a time delay for tidally-based water movements. For example, on winter spring tides (e.g. 23 January 1996), incoming seawater did not reach the bridge until *c.*4h after the start of the coastal flood tide, however, the water started to withdraw immediately after the predicted time of the coastal high tide and had completely withdrawn several hours before coastal low tide. The maximum flow velocities measured at Terras Bridge for winter and summer neap and spring tides are shown in Table 2.1. Ebb tide maxima correspond to river flow which occurred for *c.*10h of the tidal cycle (from *c.*1h after the start of the ebb tide to *c.*4·5h after the start of the next flood tide). Peak flow occurred during winter spring tides, resulting from increased freshwater run-off (Table 2.1). Similarly, minimum river flow occurred during summer neap tides when run-off was reduced. Differences in flow rates due to spring and neap tides were small, however, spring tides lead to slightly faster river flow, presumably due to the reduced damming effect caused by the lower water of spring tides. On all occasions, the fastest flow occurred at sites 2 and 3, where the flow was unidirectional through the bridge arches; mysids were never found at either of these sites (Table 2.1; Fig. 2.4). Flow was greatly reduced at the periphery of the main water channel where flow direction was more variable and mysids were found at various densities in these peripheral areas (Table 2.1; Fig. 2.4).

Table 2.1: Current flow velocities and salinity recorded at Terras Bridge over neap and spring tidal cycles. All measurements were recorded 10cm above the substratum. Site numbers correspond to those given in Figure 2.1; + corresponds to downstream water movement (i.e. river flow on ebb tide), and - corresponds to upstream water movement (i.e. tidal flow on flood tide).

			Flood Tide		Ebb Tide	
Date	Tide	Site No.	Flow (cm s^{-1})	Salinity	Flow (cm s^{-1})	Salinity
15 Jan. 1996	Neap	1b	- 60	33	+ 10	0
		2	- 71	33	+ 56	0
		3c	- 73	33	+ 54	0
		4d	- 66	33	+ 30	0
		5e	- 64	33	+ 17	0
23 Jan. 1996	Spring	1b	- 78	33	+ 14	0
		2	- 82	33	+ 59	0
		3c	- 92	33	+ 61	0
		4d	- 76	33	+ 37	1
		5e	- 68	33	+ 19	1
7 Aug. 1995	Neap	1b	- 50	33	+ 4	0
		2	- 57	33	+ 31	0
		3c	- 65	33	+ 41	0
		4d	- 54	33	+ 10	0
		5e	- 55	34	+ 11	0
14 Aug. 1995	Spring	1b	- 66	34	+ 4	0
		2	- 76	33	+ 32	0
		3c	- 80	34	+ 38	0
		4d	- 70	32	+ 12	1
		5e	- 66	34	+ 13	1

Since the incoming seawater did not reach Terras Bridge until *c.*4·5h after the start of the flood, tidal flow only occurred for *c.*2h. On the flood tide, there was less variation in flow velocity between the five sample sites on each recording date since the whole area was flooded (e.g. 50 - 65cm s^{-1}, 7 Aug. 1995; Table 2.1). Maximum flow was recorded on winter spring tides. Tide tables (Admiralty Tide Tables, 1996) showed larger tidal ranges in winter than in summer, presumably accounting for the seasonal differences in flow. Again, flow rates were higher at sites 2 and 3 than at other sites due to the constricted flow through the bridge arches and mysids were not found here (although some solitary animals were seen to get washed through). Maximum flow velocities occurred only for *c.*15min duration (*c.*1h before the time of predicted high tide). As the time of predicted high tide approached, velocity decreased to a slower, more consistent flow. It is assumed that these maxima correspond to a pulse of incoming tidal water as the site first floods. On the flood tide, mysids stayed at the periphery of both sides of the water channel, where flow rates remained low (*c.*8 - 15cm s^{-1}; Fig. 2.4).

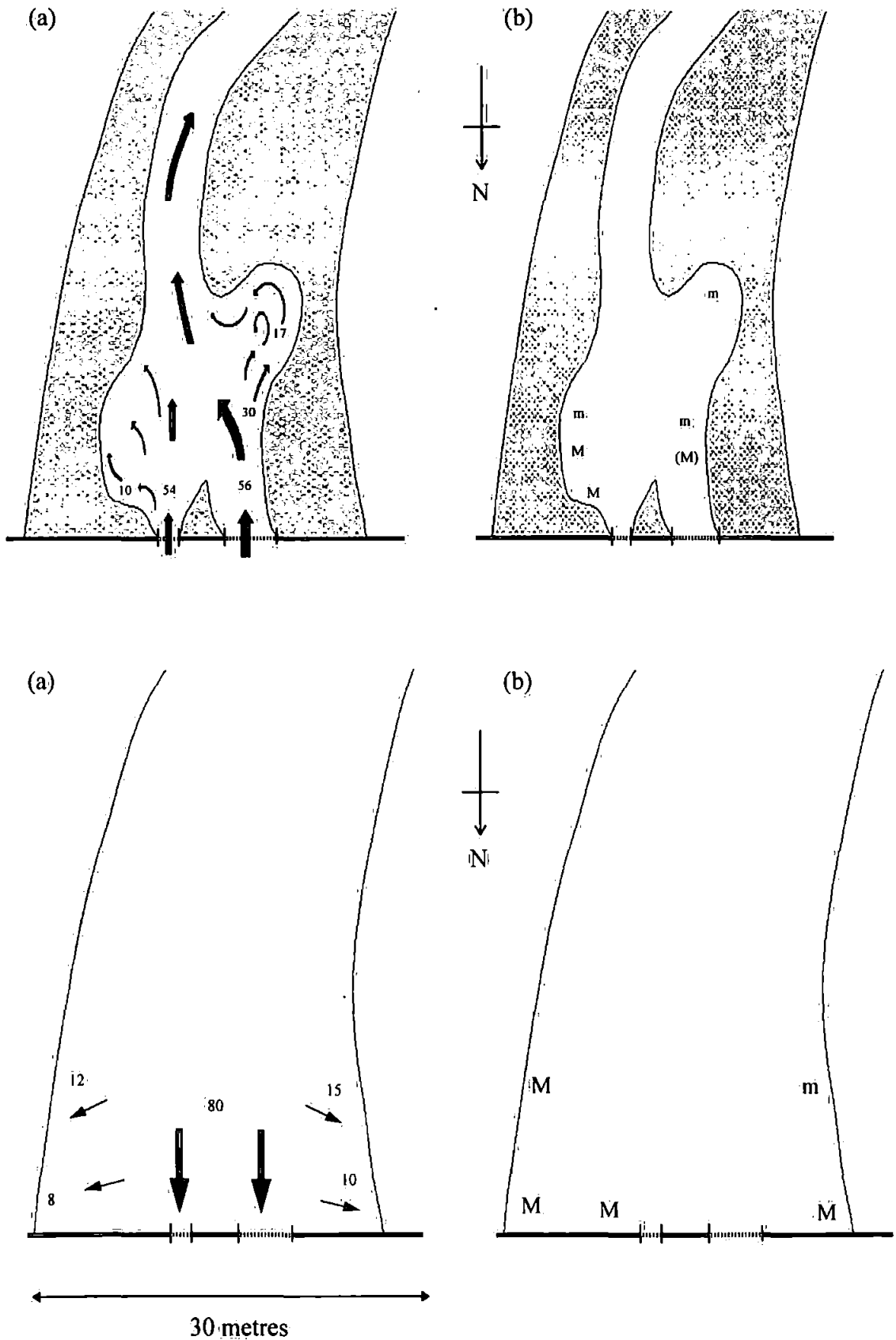


Figure 2.4: Physical variables and mysid occurrence at Terras Bridge at low tide [upper figures] and high tide [lower figures] during winter (23/1/96). The shaded area represents the intertidal area. (a) Current: arrows show the direction of flow, weight of arrow denotes current speed. Figures correspond to measured current velocities (cm s^{-1}), (b) Presence of *Neomysis integer*: M denotes the presence of a swarm of mysids, m denotes the presence of solitary mysids. Brackets denote that the mysids were found only during the summer.

Sediment composition was variable (Fig. 2.6). At sites 1a and 1b, the substratum was mostly fine sand/silt (<250μm), whereas at sites 3c and 4d, the substratum was more variable with a larger sand (>355μm) component. The substratum at site 5e was of intermediate composition. Differences in substratum granulometry may be attributed to differences in current flow velocities at the 5 sites.

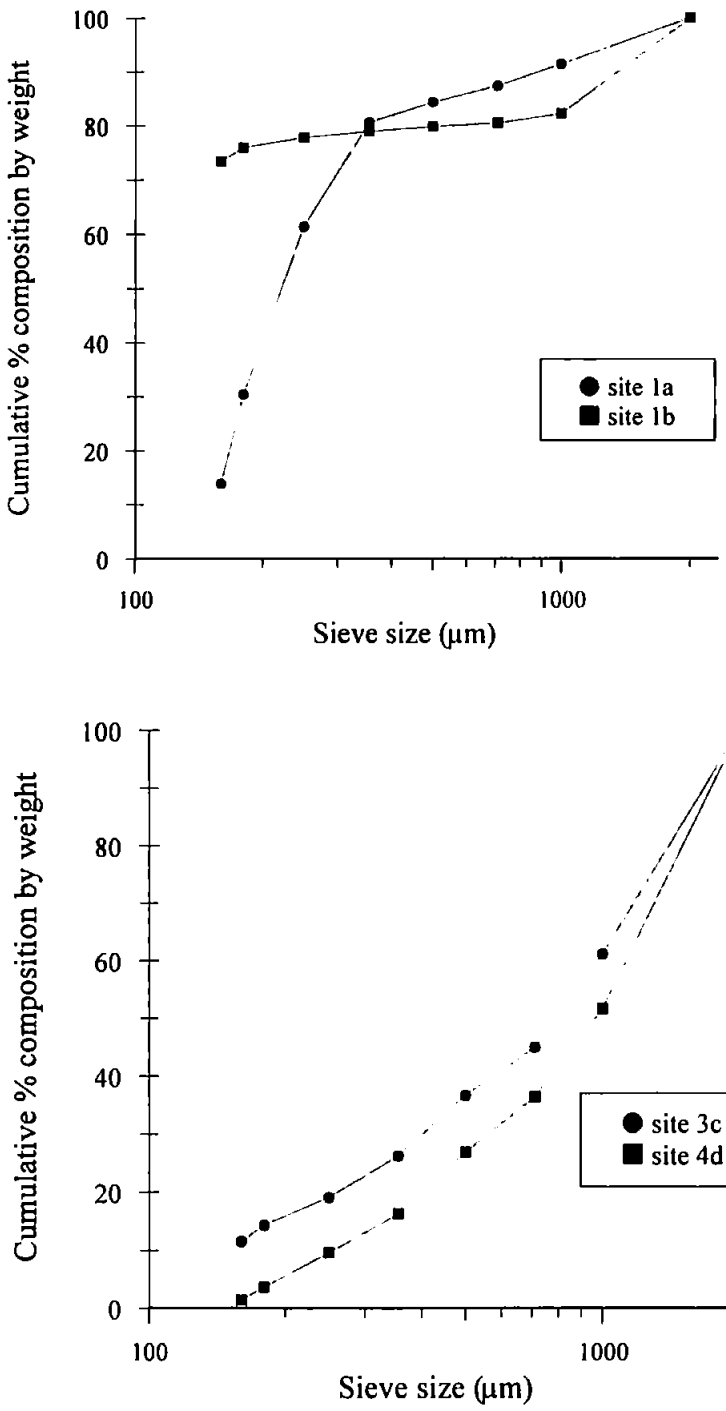


Figure 2.5: Granulometric curves for surface sediment samples taken from the East Looe River Estuary.

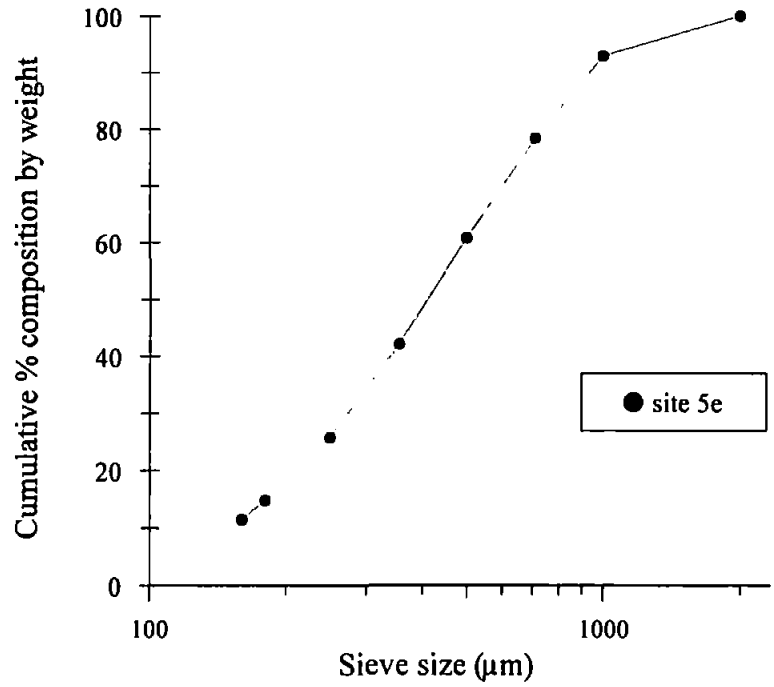


Figure 2.5 (cont'd): Granulometric curves for surface sediment samples taken from the East Looe River Estuary.

2.4.2 Laboratory studies

In all experiments, most individuals displayed positive rheotaxis even in the absence of any flow generated by the flume (the mysids showed positive rheotaxis in response to the small current created by the Eheim filter pump used to maintain water quality; the velocity of the current created by the pump was not recorded, but was in the order of 1cm s^{-1}). Whilst swimming against the filtration current, most mysids remained within 1 or 2cm of the substratum.

The effect of current speed and substratum on swimming behaviour

Most animals showed a positive rheotaxic behaviour, with an inverse relationship between current velocity and proportion of animals swimming forward into the current. At low current speeds (e.g. 3cm s^{-1}), the majority of animals swam forward into the current. As the current velocity increased (i.e. >6cm s^{-1}), the frequency of animals swimming forward into the current decreased over both the sandy and muddy substrata (Fig. 2.6a) [ANOVA, f=11·2 (sand) and 11·7 (mud), d.f.=6 (sand) and 4 (mud), $p<0{\cdot}01$; Table 2.2]. There was a marked

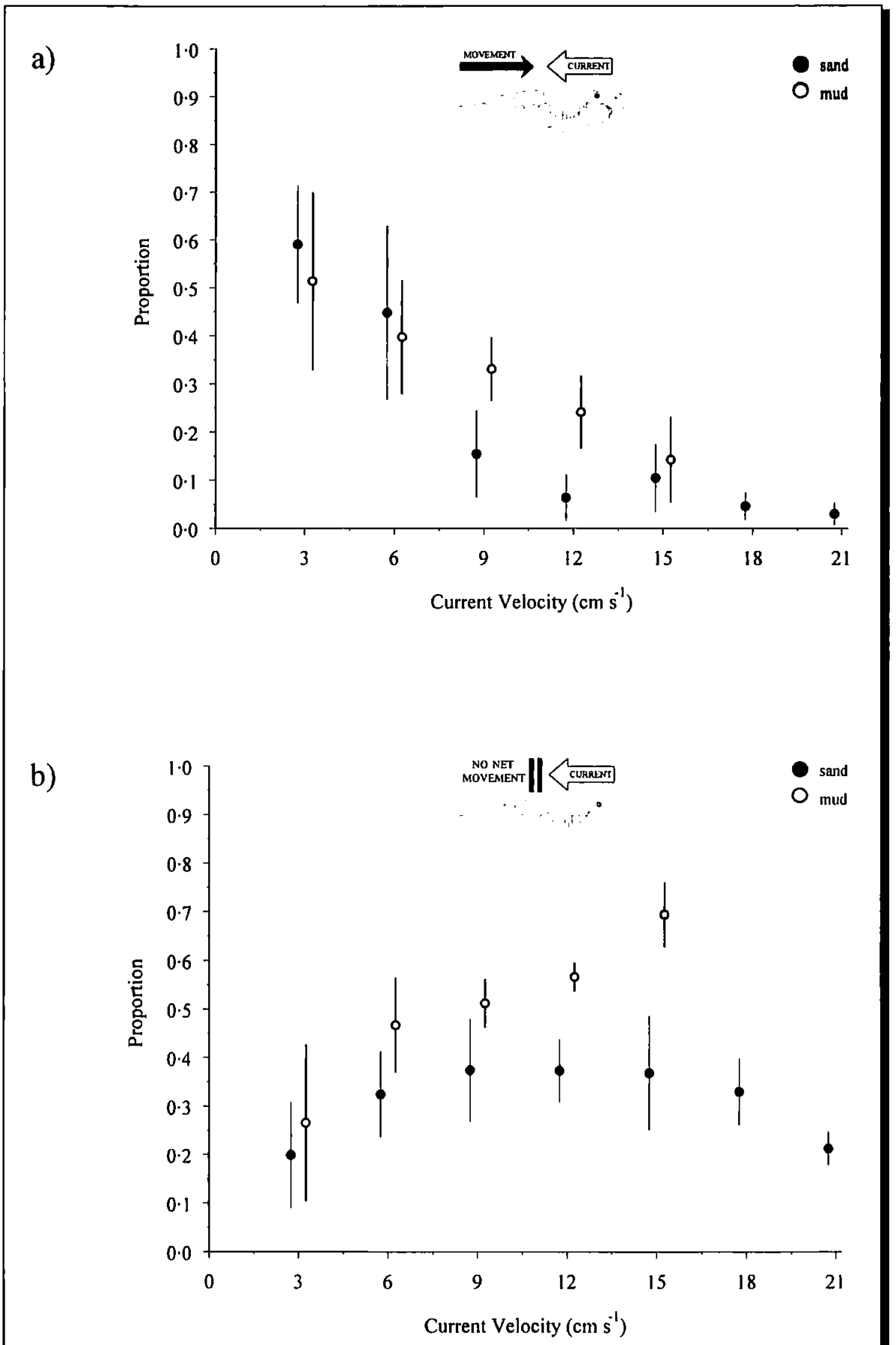


Figure 2.6: Effect of increasing current velocity on the swimming behaviour of *Neomysis integer*. (a) proportion of animals swimming forward into the current, (b) proportion of animals maintaining position. All data (including those mysids in boundary layer) are shown. n=320 for each substratum. Error bars correspond to ±95% confidence intervals.

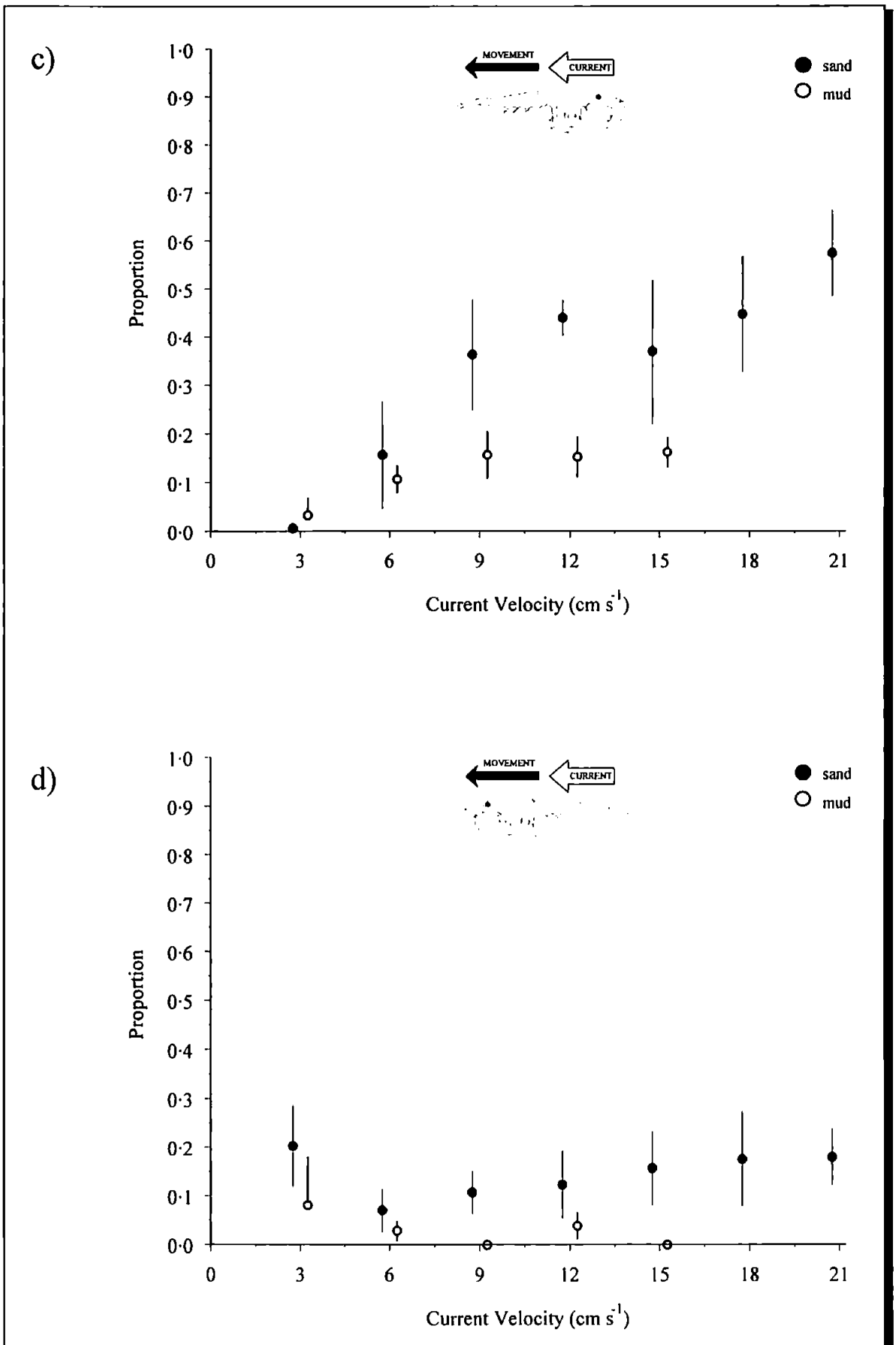


Figure 2.6 (contd.): Effect of increasing current velocity on the swimming behaviour of *Neomysis integer*. (c) proportion of animals facing into the current but being displaced, (d) proportion of animals swimming with the current. All data (including those mysids in boundary layer) are shown. n=320 for each substratum. Error bars correspond to ±95% confidence intervals.

decrease in the frequency of this behaviour at 9cm s^{-1} for mysids swimming over sand (95% confidence intervals, $p<0{\cdot}05$; Fig. 2.6a). With a sandy substratum, current velocities of 9cm s^{-1} and 12cm s^{-1} were greater than the maximum sustainable swimming speed of most mysids, which was 6cm s^{-1} (Fig. 2.6a). With a muddy substratum, however, the maximum sustained swimming speed of *N. integer* was increased to 12cm s^{-1} (Fig. 2.6a).

Table 2.2: One-way analysis of variance (ANOVA) examining the effect of increasing current velocity on the swimming behaviour of *Neomysis integer* swimming over two types of substratum. n=40 for each substratum; critical f=2·57 (sand) and 3·06 (mud); d.f.=6 (sand) and 4 (mud); n.s.=not significant ($p>0{\cdot}05$). All data analysed.

Behaviour Category	Substratum	f statistic	p value	significance
Swimming forward into current	sand	11·2	$1{\cdot}21 \times 10^{-5}$	$p<0{\cdot}01$
	mud	11·7	$1{\cdot}62\times 10^{-4}$	$p<0{\cdot}01$
Maintaining position	sand	2·12	0·09	n.s.
	mud	6·79	1.2×10^{-3}	$p<0{\cdot}01$
Facing into the current, being displaced	sand	12·7	$4{\cdot}71\times 10^{-6}$	$p<0{\cdot}01$
	mud	4·74	0·01	$p<0{\cdot}05$
Swimming with the current	sand	0·63	0·70	n.s.
	mud	2·31	0·11	n.s.

Type of substratum also affected the ability of *N. integer* to maintain its position. With sand, there was no significant effect of current velocity on the proportion of mysids swimming forward into the current (Fig. 2.6b) [ANOVA, f=2·12, d.f.=6, $p>0{\cdot}05$; Table 2.2]. There was a general trend, however, of increased frequency of mysids maintaining position as current velocity was increased to 9cm s^{-1}, and decreased numbers of mysids maintaining position with increasing current velocities >15cm s^{-1} (Fig. 2.6b). With mud, however, there was a significant increase in the frequency of position maintenance with increasing current velocities (Fig. 2.6b) [ANOVA, f=6·79, d.f.=6, $p<0{\cdot}01$; Table 2.2]. Frequency of position maintenance behaviour with mud was still increasing at the highest flow velocity used (*c.*0·7 at 15cm s^{-1}; Fig. 2.6b). With increasing current velocity, there was an increase in the number of animals facing into the current but being displaced by the current (Fig. 2.6c) [ANOVA, f=12·7 (sand) and 4·74 (mud), d.f.=6 (sand) and 4 (mud), $p<0{\cdot}05$; Table 2.2]. The effect of

substratum on the frequency of this behaviour was unclear. With sand, the frequency of animals facing into, but being displaced by, the current increased with increasing current velocity up to 9cm s^{-1}, plateaued between 9 and 15cm s^{-1}, and continued to increase at current velocities >15cm s^{-1}. With mud, although the frequency of animals facing into the current but being displaced was generally lower than with sand, a similar response occurred with a plateau occurring between current velocities of 6 and 15cm s^{-1} (95% confidence intervals, $p<0{\cdot}05$; Fig. 2.6c). As the experiment was not continued beyond 15cm s^{-1}, it is not known whether the frequency of this behaviour would increase at velocities >15cm s^{-1} (Fig. 2.6c). Frequency of animals facing with the flow and being carried by the current showed no clear relationship with flow velocity and, in all cases, frequency was relatively low (< 0·2; Fig. 2.6d) [ANOVA, f=0·63 (sand) and 2·31 (mud), d.f.=6 (sand) and 4 (mud), $p>0{\cdot}05$; Table 2.2].

For both types of substratum, there was a significant effect of current velocity on the vertical distribution of *Neomysis integer* (Fig. 2.7) [ANOVA, f=7·96 (sand) and 7·47 (mud); d.f.=6 (sand) and 4 (mud); $p<0{\cdot}01$; Table 2.3]. However, the type of response was affected by the type of substratum. Although there were significant differences in the frequency of mysids swimming at different heights in the water column, generally these are difficult to discern (Table 2.3; Fig. 2.7). The most noticeable effect of current velocity on the vertical distribution of *N. integer* was the change in number of mysids swimming on, or within 1cm, of the substratum (Table 2.3; Fig. 2.7). With sand, mysids showed a clear affinity for the substratum at low current velocities, however, as current velocity was increased the number of animals distributed on the substratum decreased (Fig. 2.7a) [ANOVA, f=7·96; d.f.=6; $p<0{\cdot}01$; Table 2.3]. The number of mysids swimming at 1cm above the sand also decreased with increasing current velocity (Fig. 2.7a) [ANOVA, f=7·96; d.f.=6; $p<0{\cdot}01$; Table 2.3]. With mud, however, the opposite occurred. Again, *N. integer* showed a clear affinity for the substratum, but more mysids were located on the substratum at high current velocities (Fig. 2.7b) [ANOVA, f=4·60; d.f.=4; $p<0{\cdot}05$; Table 2.3]. As the current velocity was increased from 3 to 9cm s^{-1}, the number of mysids recorded on the mud increased, but decreased as the current velocity was increased to 15cm s^{-1} (Fig. 2.7b) [ANOVA, f=4·60, d.f.=4, $p<0{\cdot}05$; Table 2.3]. The number of mysids swimming 1cm above the mud decreased with increasing current velocity up to 9cm s^{-1}, but decreased at velocities >9cm s^{-1} (Fig. 2.7b) [ANOVA, f=7·47; d.f.=4; $p<0{\cdot}01$; Table 2.3].

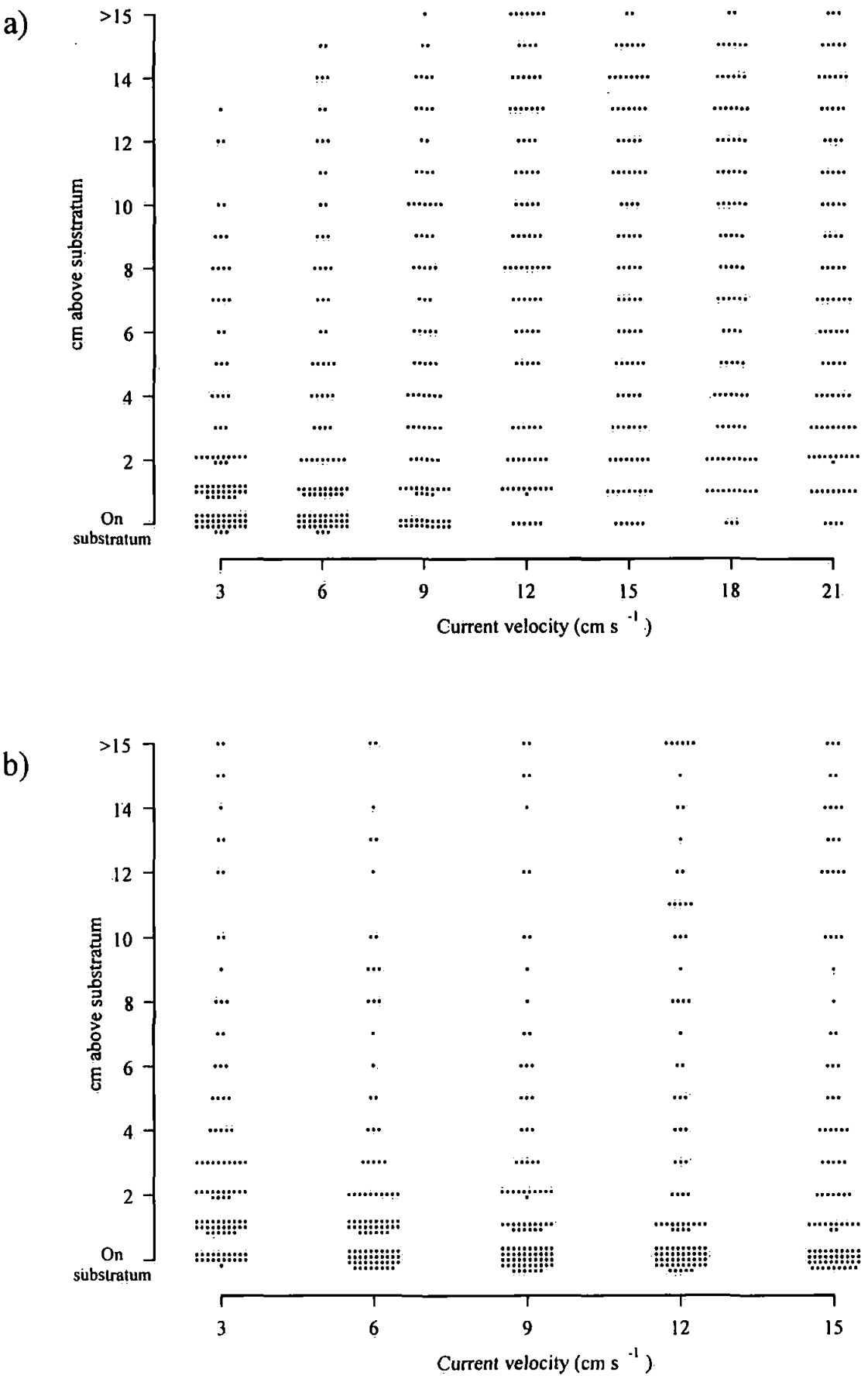


Figure 2.7: Effect of increasing current velocity on the vertical distribution of *Neomysis integer*. (a) with sandy substratum and (b) with muddy substratum. Each dot represents 1% mysid occurrence. n=320 for each substratum.

Table 2.3: One-way analysis of variance (ANOVA) examining the effect of increasing current velocity on the vertical distribution of *Neomysis integer* swimming over two types of substratum. n=40 for each substratum, critical f=2·57 (sand) and 3·06 (mud), d.f.=6 (sand) and 4 (mud), n.s.=not significant (p>0·05).

Substratum Type	Height (cm)	f statistic	p value	significance
Sand	On substratum	7·96	$1·5 \times 10^{-4}$	p<0·01
	1	3·35	0·02	p<0·05
	2	1·93	0·12	n.s.
	3	5·02	$2·5 \times 10^{-3}$	p<0·01
	4	1·97	0·12	n.s.
	5	3·06	0·03	p<0·05
	6	6·33	$6·4 \times 10^{-4}$	p<0·01
	7	3·67	0·12	n.s.
	8	1·13	0·38	n.s.
	9	2·1	0·10	n.s.
	10	0·98	0·47	n.s.
	11	1·23	0·33	n.s.
	12	0·79	0·59	n.s.
	13	0·53	0·78	n.s.
	14	0·72	0·64	n.s.
	15	0·75	0·62	n.s.
	16	0·87	0·51	n.s.
Mud	On substratum	4·60	0·01	p<0·05
	1	7·47	$1·6 \times 10^{-3}$	p<0·01
	2	4·18	0·02	p<0·05
	3	2·35	0·10	n.s.
	4	0·83	0·53	n.s.
	5	0·24	0·91	n.s.
	6	3·42	0·02	p<0·05
	7	0·63	0·65	n.s.
	8	1·67	0·21	n.s.
	9	0·19	0·94	n.s.
	10	0·25	0·91	n.s.
	11	1·58	0·23	n.s.
	12	0·60	0·67	n.s.
	13	0·97	0·51	n.s.
	14	0·81	0·49	n.s.
	15	0·76	0·57	n.s.
	16	4·55	0·01	p<0·05

These results highlight another behavioural response, that of mysids utilising the substratum. At low current velocities (e.g. 3 or 6cm s^{-1}), most mysids remained within a few centimetres of the sandy substratum (Fig. 2.7a). As current velocity increased, mysids were swept off the sand and, although they tried frequently to resettle on the sand, they were unable to. In contrast, mysids were able to settle on and burrow into the muddy substratum as the current velocity was increased (Fig 2.7b). Burrowing into the substratum accounted for the differences in frequency of mysid position maintenance described, since mysids burrowing into the substratum were given the behavioural score of maintaining position. Burrowing behaviour was not observed over sand, presumably because the particles were too large for the mysids to move and burrow into. At the higher speeds (e.g. >18cm s^{-1}), there was significant transport of the sand, and mysids were seen to settle on the downstream side of 'sand waves' and use them for shelter. Although mud was re-suspended at high speeds (e.g. 15cm s^{-1}), the surface of the mud remained relatively flat.

Since *Neomysis integer* displayed a significant affinity for the boundary layer, the effect of current velocity (up to 18cm s^{-1}) on mysids swimming above this region was analysed (i.e. data for mysids swimming in the boundary layer were removed from the analysis). Variance in the frequency of each behavioural type for these mysids was large (Fig. 2.8), but the same general trends were apparent as described when all the data were analysed. Thus, the proportion of animals swimming forward into the current decreased as current velocity was increased (Fig. 2.8a) [ANOVA, f=12·35 (sand) and 16·8 (mud), d.f.=6, $p<0·01$; Table 2.4], and number of animals facing into but being displaced by the current increased with increasing current velocity (Fig. 2.8c) [ANOVA, f=4·57 (sand) and 9·05 (mud), d.f.=6, $p<0·01$; Table 2.4]. The number of animals maintaining position above the boundary layer was not significantly affected by current velocity (Fig. 2.8b) [ANOVA, f=0·43 (sand) and 1·39 (mud), d.f.=6, $p>0·05$; Table 2.4]. The number of mysids swimming with the current above the boundary layer increased with increasing current velocity for mysids swimming above mud (Fig. 2.8d) [ANOVA, f=8·39, d.f.=6, $p<0·01$; Table 2.4], but the frequency of this behaviour type for mysids swimming over sand was not affected by current velocity (Fig. 2.8d) [ANOVA, f=0·48, d.f.=6, $p>0·05$; Table 2.4].

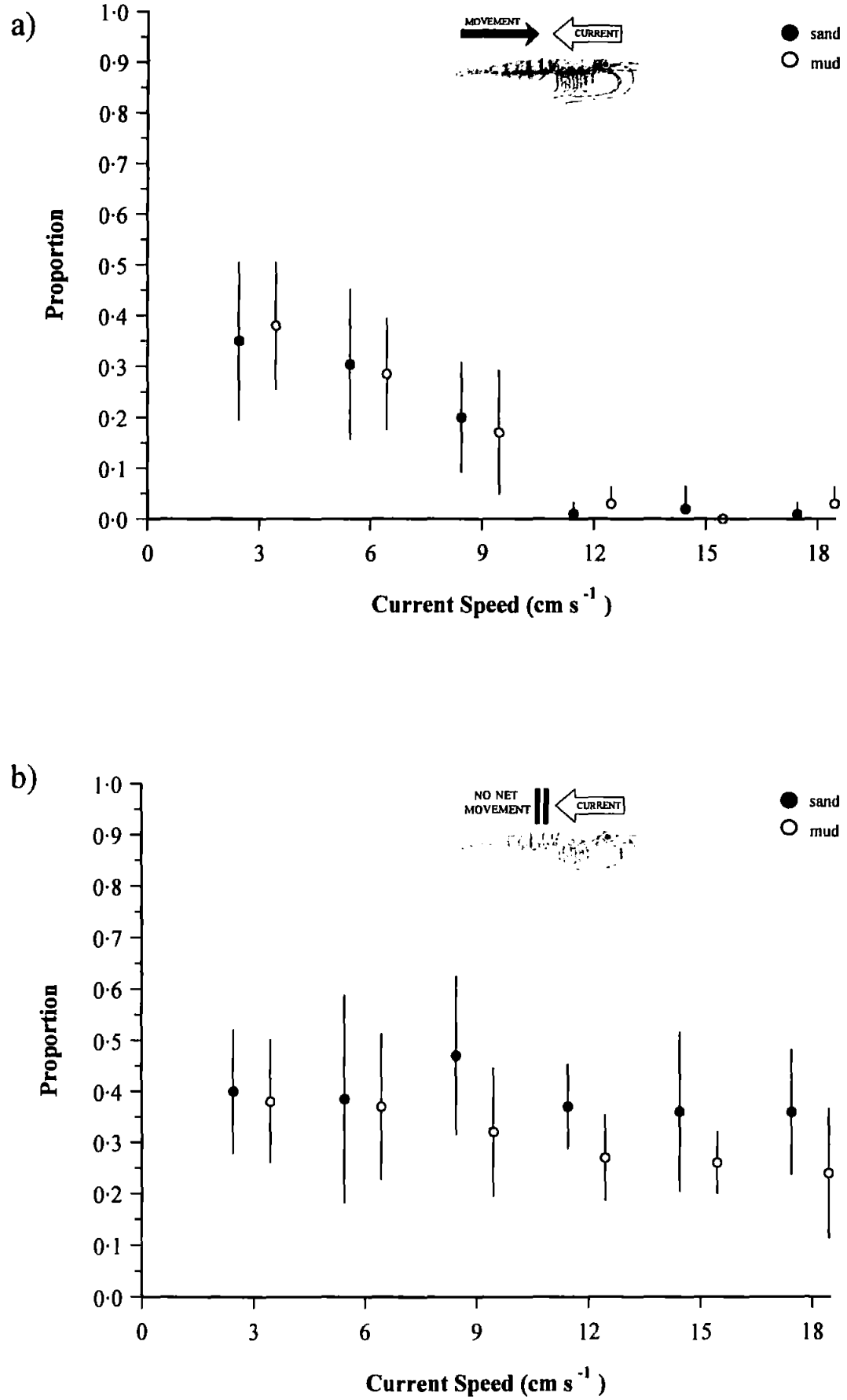
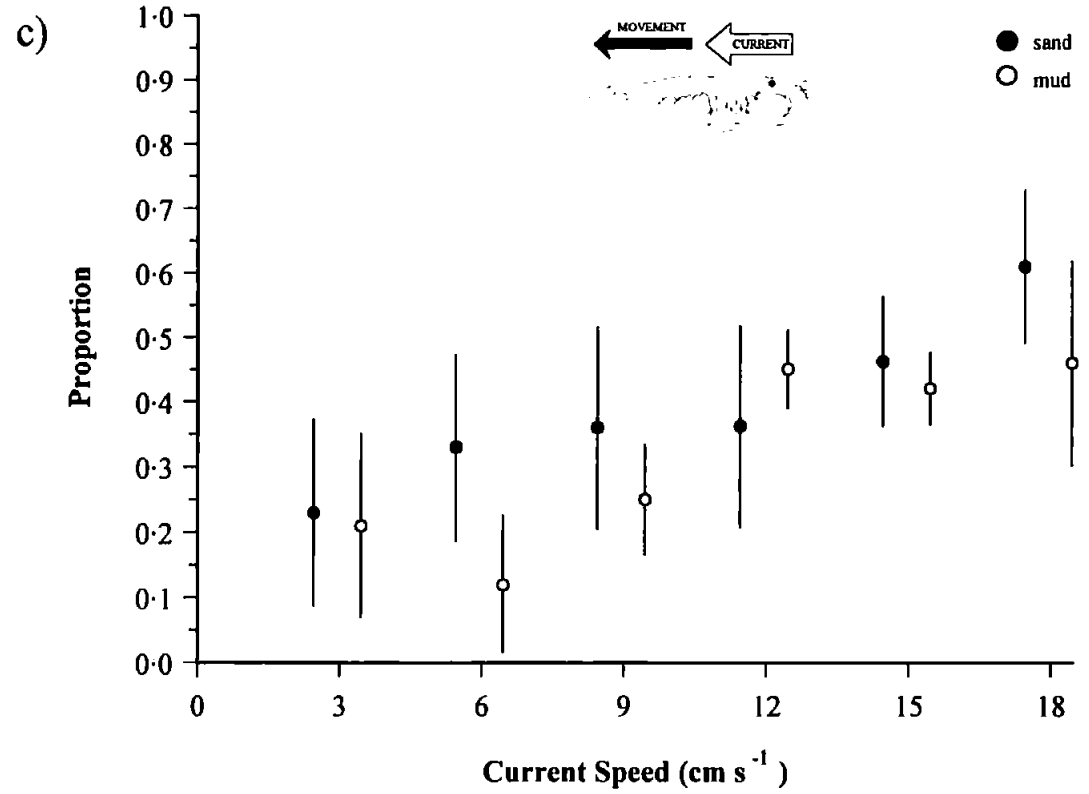


Figure 2.8: Effect of increasing current velocity on the swimming behaviour of *Neomysis integer*. (a) proportion of animals swimming forward into the current, (b) proportion of animals maintaining position. Data for mysids in boundary layer have been removed. n = variable for each substratum. Error bars correspond to ±95% confidence intervals.



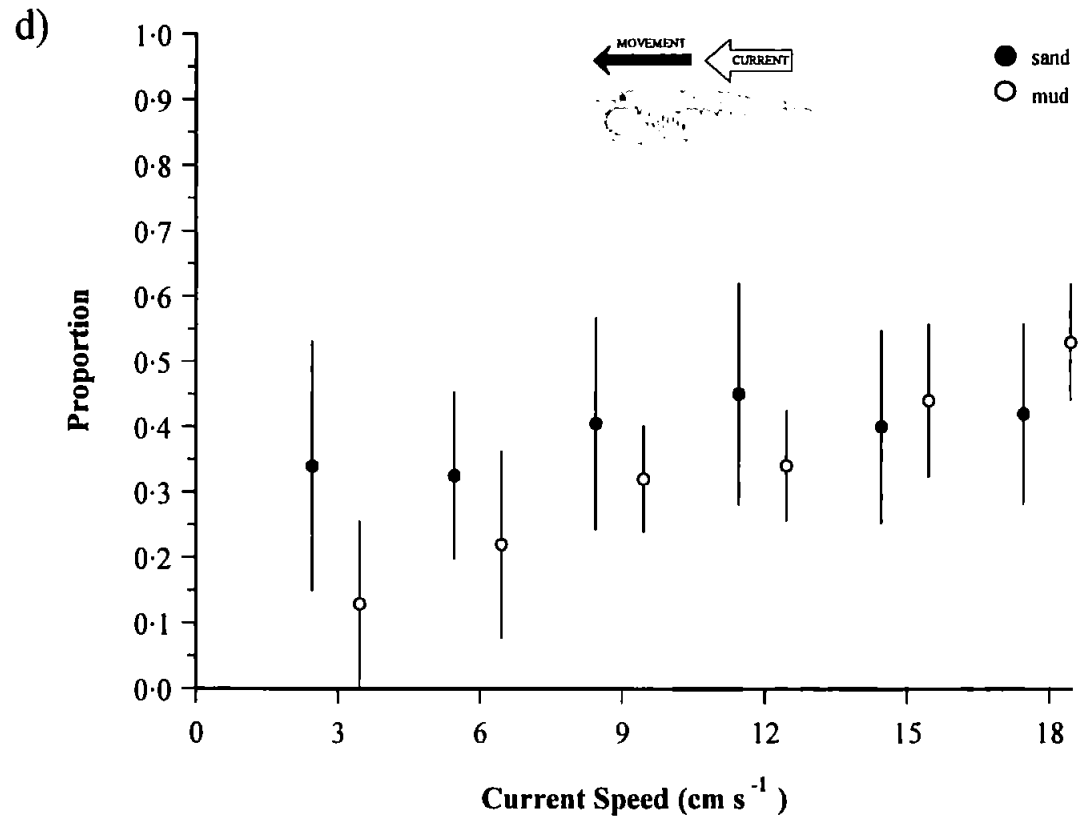


Figure 2.8 (contd.): Effect of increasing current velocity on the swimming behaviour of *Neomysis integer*. (c) proportion of animals facing into the current but being displaced, (d) proportion of animals swimming with the current. Data for mysids in boundary layer have been removed. n = variable for each substratum. Error bars correspond to ±95% confidence intervals.

Table 2.4: One-way analysis of variance (ANOVA) examining the effect of increasing current velocity on the swimming behaviour of *Neomysis integer* swimming over two types of substratum. n=40 for each substratum, critical f=2·38, d.f.=6, n.s.=not significant (p>0·05). Data for mysids in boundary layer removed.

Behaviour Category	Substratum	f statistic	p value	significance
Swimming forward into current	sand	12·35	$5{\cdot}3 \times 10^{-10}$	p<0·00
	mud	16·8	$5{\cdot}3 \times 10^{-8}$	p<0·00
Maintaining position	sand	0·43	0·83	n.s.
	mud	1·39	0·24	n.s.
Facing into the current, being displaced	sand	4·57	$1{\cdot}5 \times 10^{-3}$	p<0·00
	mud	9·05	$2{\cdot}7 \times 10^{-6}$	p<0·00
Swimming with the current	sand	0·48	0·79	n.s.
	mud	8·93	$3{\cdot}1 \times 10^{-6}$	p<0·00

Effect of salinity on swimming behaviour

There was no significant effect of increasing salinity on the swimming behaviour of *Neomysis integer* exposed to current velocities of 6 and 9cm s^{-1} (Fig. 2.9) [ANOVA, $f \leq 3{\cdot}41$ (6cm s^{-1}) and $\leq 1{\cdot}32$ (9cm s^{-1}), d.f.=3, p>0·05; Table 2.5]. The results were highly variable and no clear trends were discerned. For most behavioural categories at each current velocity, there were behavioural similarities between mysids exposed to 1 and 20‰, and between mysids exposed to 10 and 30‰ (Fig. 2.9).

Table 2.5: One-way analysis of variance (ANOVA) examining the effect of increasing salinity on the swimming behaviour of *Neomysis integer* at two current velocities. n=40 for each salinity, critical f=3·49, d.f.=3, n.s.=not significant (p>0·05).

Behaviour Category	Current velocity (cm s^{-1})	f statistic	p value	significance
Swimming forward into current	6	2·42	0·12	n.s
	9	1·14	0·37	n.s
Maintaining position	6	3·41	0·05	n.s
	9	1·32	0·31	n.s
Facing into the current, being displaced	6	1·24	0·34	n.s
	9	0·32	0·81	n.s
Swimming with the current	6	0·87	0·49	n.s
	9	0·37	0·78	n.s

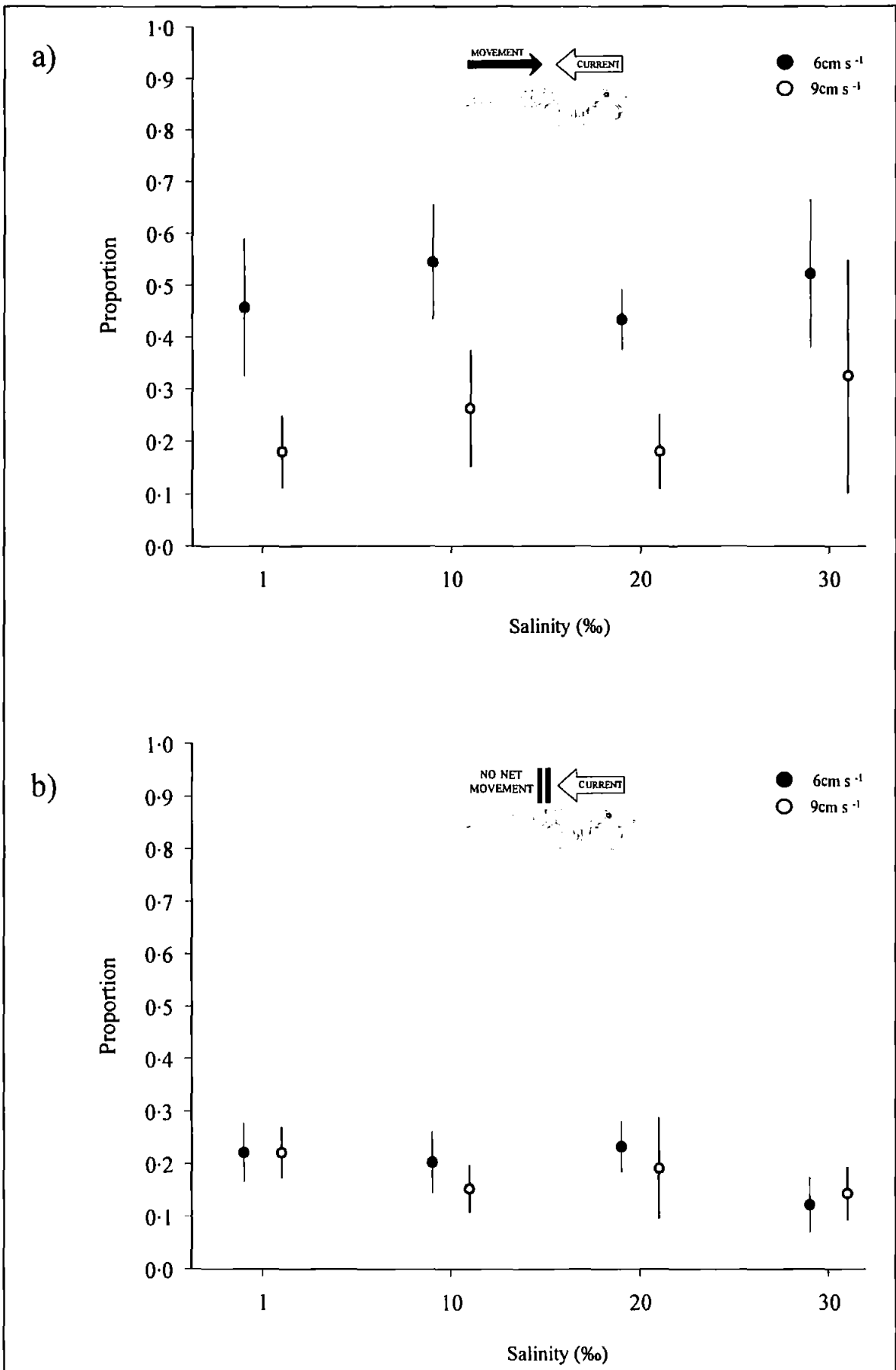


Figure 2.9: Effect of increasing salinity on the swimming behaviour of *Neomysis integer*. (a) proportion of animals swimming forward into the current, (b) proportion of animals maintaining position. n=160 for each velocity. Error bars correspond to ±95% confidence intervals.

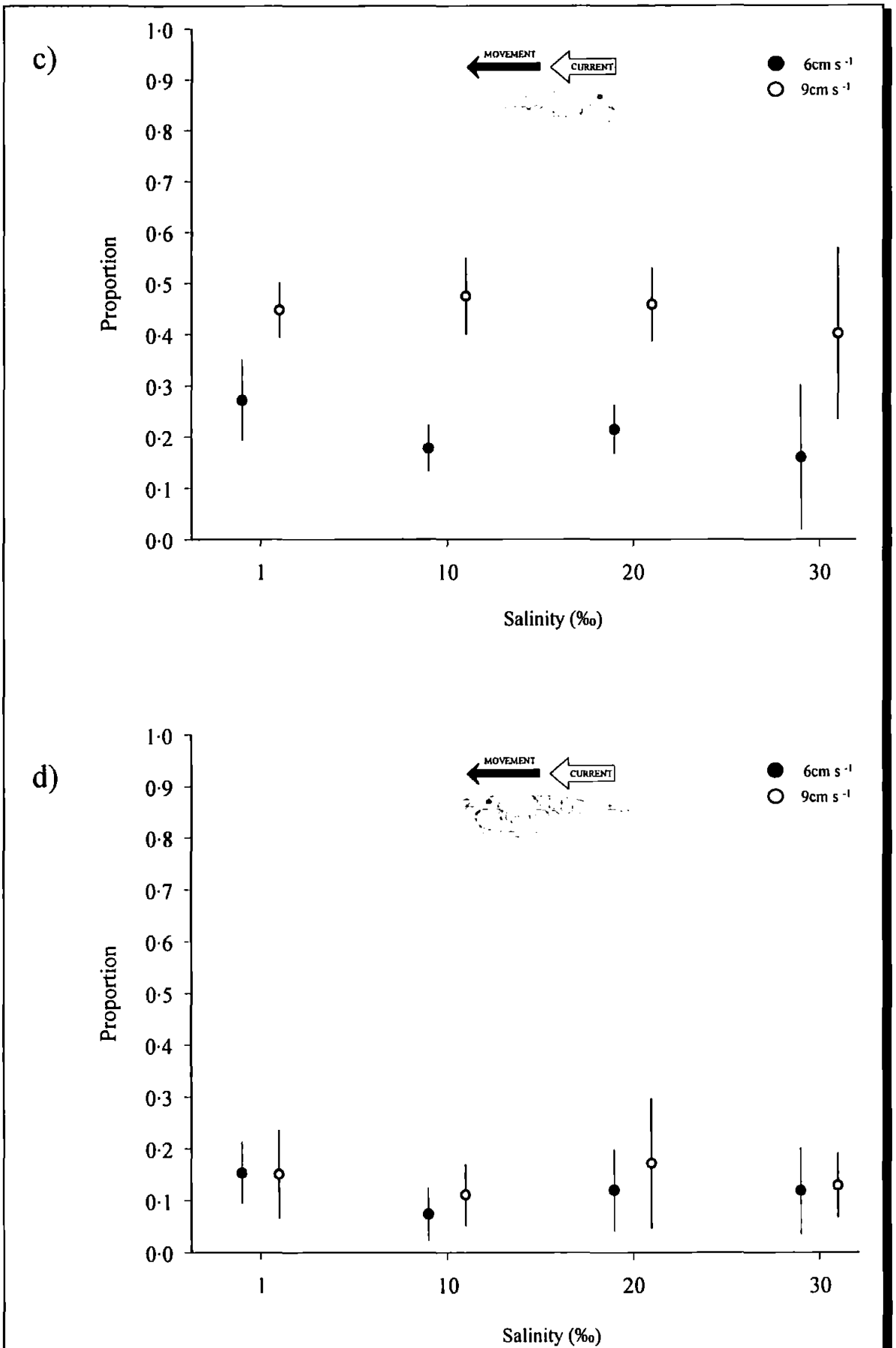


Figure 2.9 (contd.): Effect of increasing salinity on the swimming behaviour of *Neomysis integer*. (c) proportion of animals facing into the current but being displaced, (d) proportion of animals swimming with the current. n=160 for each velocity. Error bars correspond to ±95% confidence intervals.

Decreasing salinity had no significant effect on mysid swimming behaviour at either current velocity (Fig. 2.10) [ANOVA, $f \leq 3{\cdot}06$ (6cm s^{-1}) and $\leq 0{\cdot}78$ (9cm s^{-1}), d.f.=3, $p > 0{\cdot}05$; Table 2.6]. With decreasing salinity, few obvious trends appeared in the data. In contrast to the swimming response to increasing salinity there was no similarity in the frequency of each behavioural type exposed to 1 and 20‰, and 10 and 30‰ (Fig. 2.10).

Table 2.6: One-way analysis of variance (ANOVA) examining the effect of decreasing salinity on the swimming behaviour of *Neomysis integer* at two current velocities. n=40 for each salinity, critical f=3·49, d.f.=3, n.s.=not significant ($p > 0{\cdot}05$).

Behaviour Category	Current velocity (cm s^{-1})	f statistic	p value	significance
Swimming forward into current	6	0·39	0·76	n.s.
	9	0·59	0·63	n.s.
Maintaining position	6	3·06	0·07	n.s.
	9	0·35	0·79	n.s.
Facing into the current, being displaced	6	0·78	0·53	n.s.
	9	0·78	0·52	n.s.
Swimming with the current	6	1·08	0·40	n.s.
	9	0·57	0·65	n.s.

For all salinities, there was a very clear vertical spatial distribution of mysids in the water column, with most animals staying close to the substratum, however, there was no significant effect of salinity on the vertical distribution of mysids (Fig. 2.11) [ANOVA, $f \leq 2{\cdot}77$, d.f.=3, $p > 0{\cdot}05$; Tables 2.7 and 2.8].

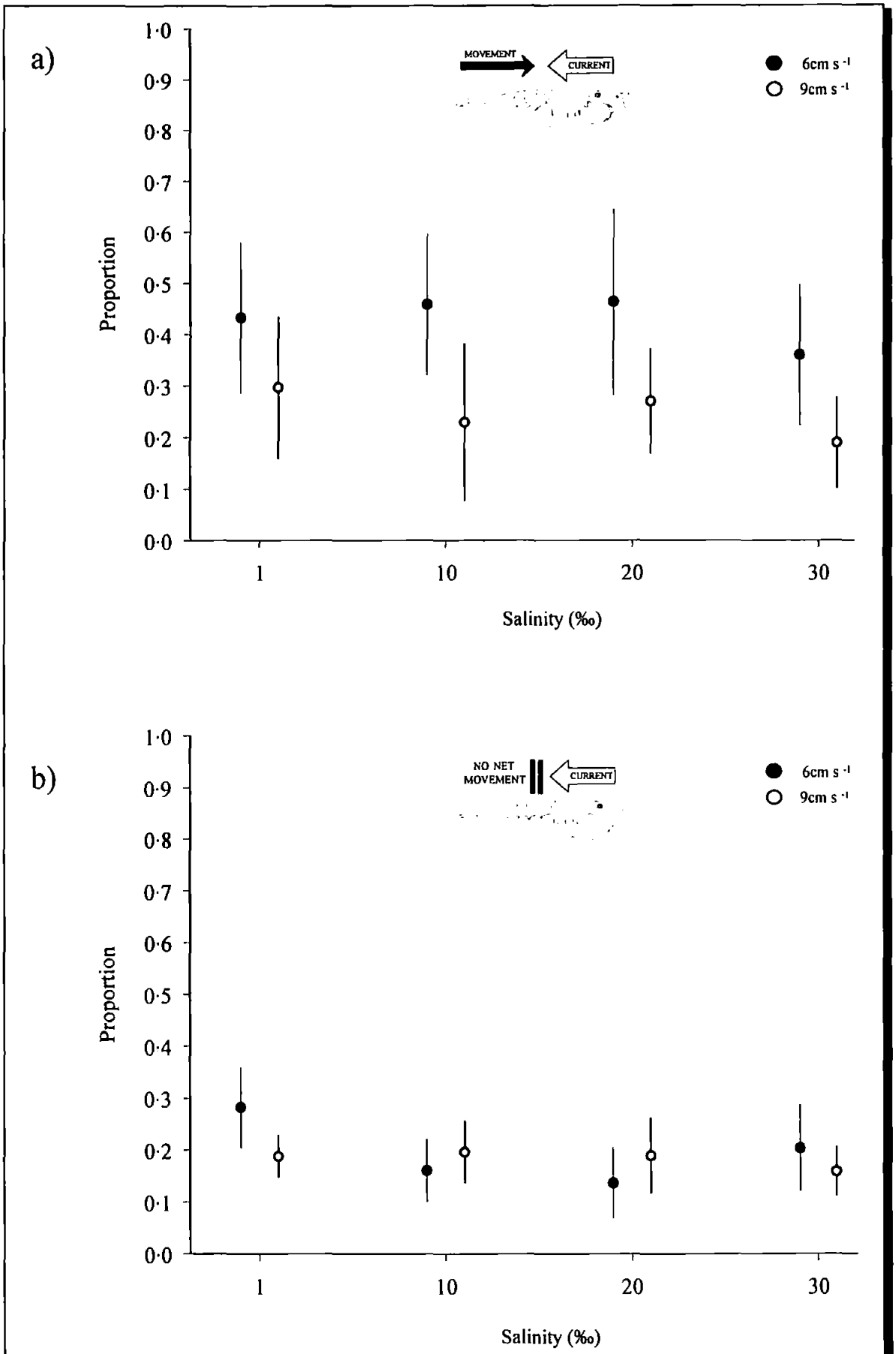


Figure 2.10: Effect of decreasing salinity on the swimming behaviour of *Neomysis integer*. (a) proportion of animals swimming forward into the current, (b) proportion of animals maintaining position. n=160 for each velocity. Error bars correspond to ±95% confidence intervals.

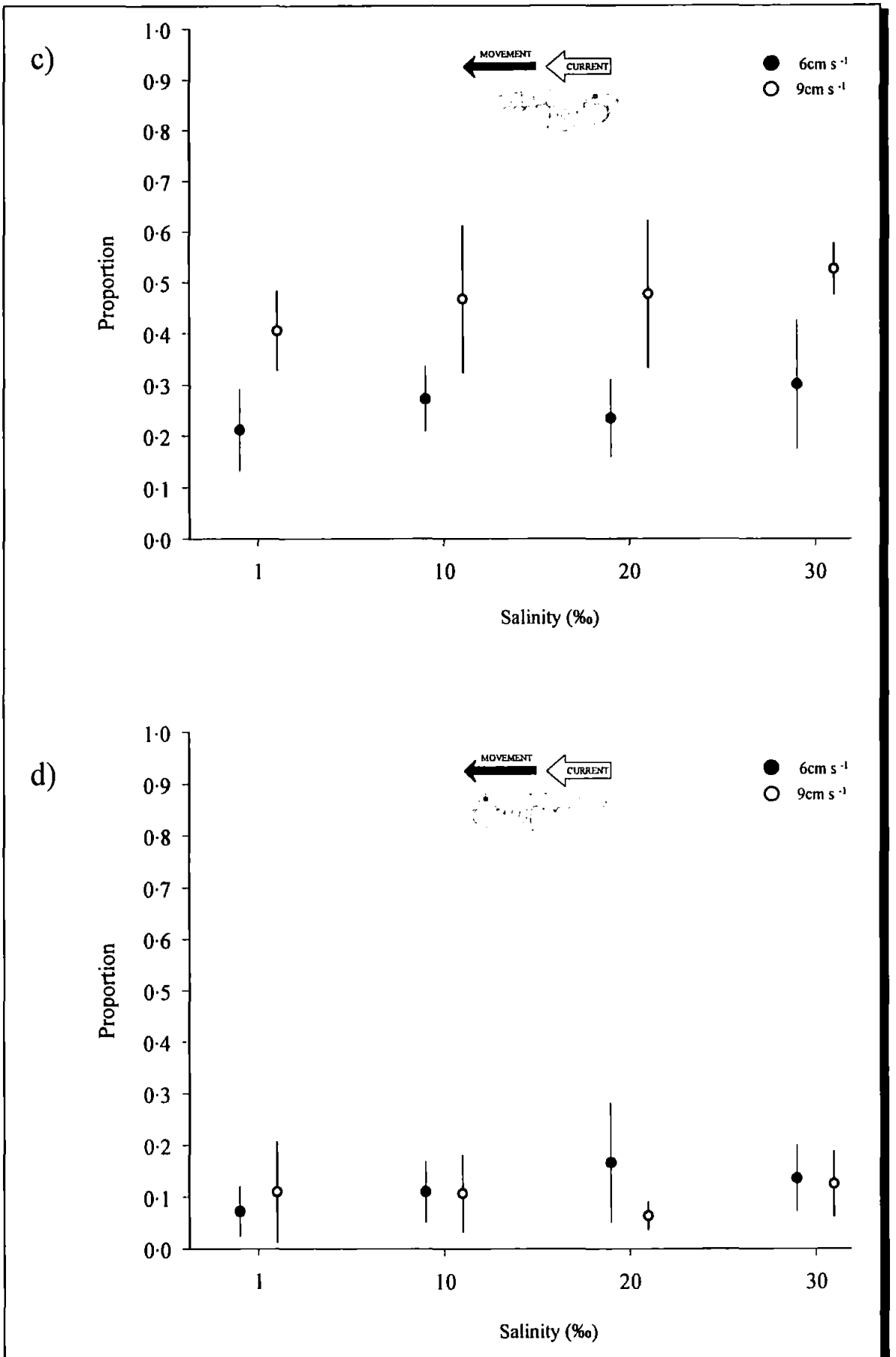


Figure 2.10 (contd.): Effect of decreasing salinity on the swimming behaviour of *Neomysis integer*. (c) proportion of animals facing into the current but being displaced, (d) proportion of animals swimming with the current. n=160 for each velocity. Error bars correspond to ±95% confidence intervals.

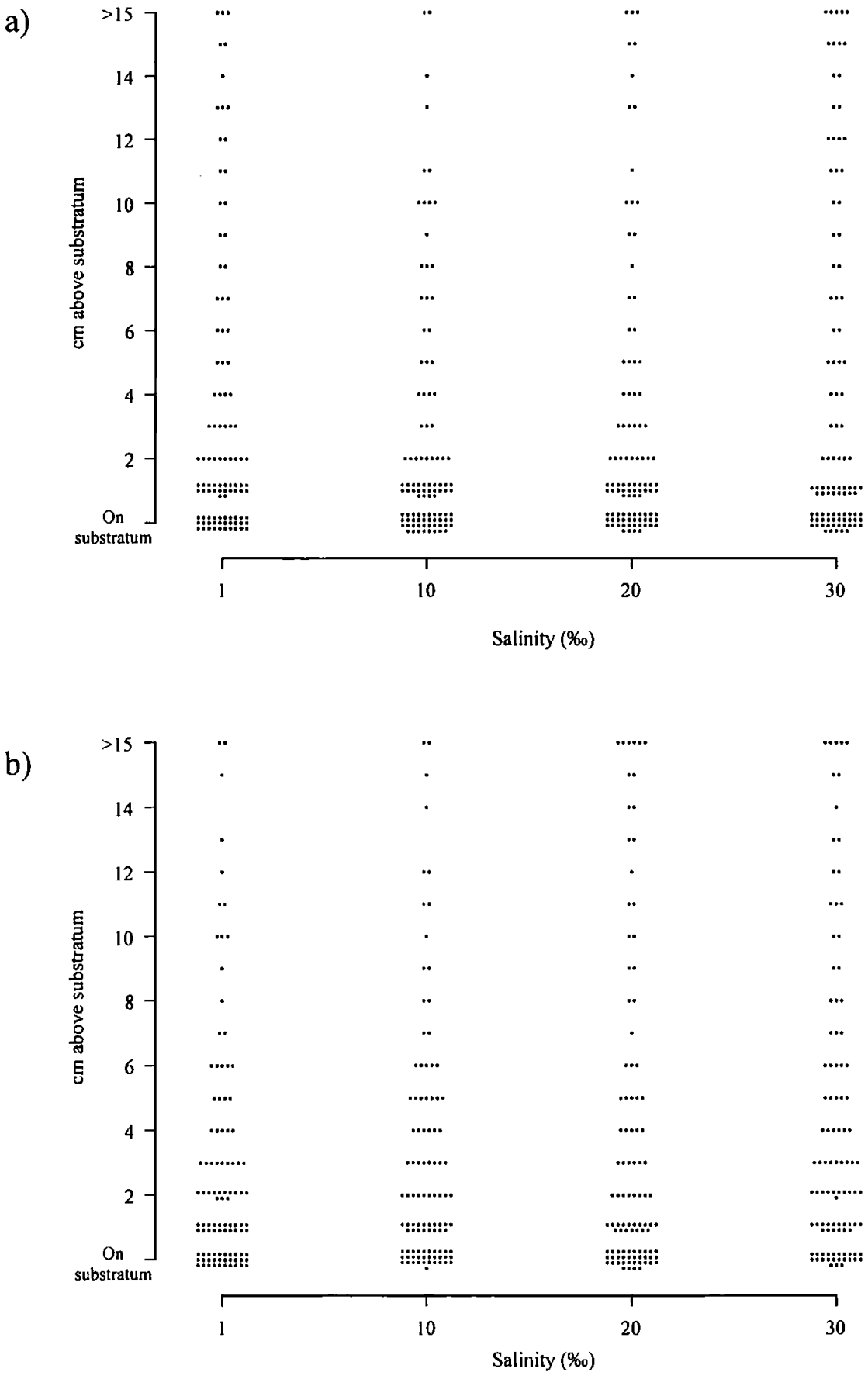


Figure 2.11: Effect of salinity on the vertical distribution of *Neomysis integer* exposed to a current velocity of 6cm s^{-1}. (a) with increasing salinity and (b) with decreasing salinity. Each dot represents 1% mysid occurrence. n=160.

Table 2.7: One-way analysis of variance (ANOVA) examining the effect of salinity on the vertical distribution of *Neomysis integer* exposed to a current velocity of 6cm s^{-1}. n=40 for each salinity, critical f=3·49, d.f.=3, n.s.=not significant (p>0·05).

Salinity Change	Height (cm)	f statistic	p value	significance
Increasing	On substratum	0·76	0·54	n.s.
	1	0·46	0·72	n.s.
	2	2·77	0·09	n.s.
	3	1·26	0·33	n.s.
	4	1·71	0·22	n.s.
	5	1·28	0·32	n.s.
	6	0·39	0·76	n.s.
	7	0·69	0·58	n.s.
	8	0·58	0·64	n.s.
	9	0·54	0·67	n.s.
	10	1·54	0·25	n.s.
	11	0·38	0·77	n.s.
	12	1·95	0·18	n.s.
	13	2·30	0·13	n.s.
	14	0·12	0·94	n.s.
	15	1·64	0·23	n.s.
	16	1·47	0·27	n.s.
Decreasing	On substratum	0·54	0·67	n.s.
	1	0·90	0·47	n.s.
	2	0·68	0·58	n.s.
	3	0·59	0·64	n.s.
	4	0·77	0·53	n.s.
	5	0·66	0·59	n.s.
	6	1·75	0·21	n.s.
	7	0·39	0·76	n.s.
	8	1·83	0·19	n.s.
	9	0·68	0·58	n.s.
	10	1·54	0·25	n.s.
	11	0·99	0·43	n.s.
	12	1·64	0·23	n.s.
	13	1·98	0·17	n.s.
	14	0·67	0·59	n.s.
	15	2·12	0·15	n.s.
	16	1·83	0·19	n.s.

Table 2.8: One-way analysis of variance (ANOVA) examining the effect of salinity on the vertical distribution of *Neomysis integer* exposed to a current velocity of 9cm s^{-1}. n=40 for each salinity, critical f=3·49, d.f.=3, n.s.=not significant (p>0·05).

Salinity Change	Height (cm)	f statistic	p value	significance
Increasing	On substratum	0·39	0·77	n.s.
	1	0·16	0·92	n.s.
	2	0·67	0·58	n.s.
	3	0·28	0·84	n.s.
	4	0·31	0·82	n.s.
	5	0·43	0·74	n.s.
	6	0·64	0·60	n.s.
	7	0·75	0·54	n.s.
	8	0·52	0·68	n.s.
	9	1·14	037	n.s.
	10	1·41	0·29	n.s.
	11	0·16	0·92	n.s.
	12	0·69	0·58	n.s.
	13	0·10	0·95	n.s.
	14	0·70	0·57	n.s.
	15	0·15	0·93	n.s.
	16	1·55	0·25	n.s.
Decreasing	On substratum	1·18	0·36	n.s.
	1	0·73	0·56	n.s.
	2	0·69	0·57	n.s.
	3	0·49	0·70	n.s.
	4	0·27	0·84	n.s.
	5	0·14	0·93	n.s.
	6	1·76	0·21	n.s.
	7	0·21	0·89	n.s.
	8	0·52	0·68	n.s.
	9	0·46	0·72	n.s.
	10	1·07	0·40	n.s.
	11	0·77	0·53	n.s.
	12	0·89	0·47	n.s.
	13	0·61	0·62	n.s.
	14	0·38	0·77	n.s.
	15	0·38	0·77	n.s.
	16	0·40	0·75	n.s.

2.5 DISCUSSION

The swimming behaviour of *Neomysis integer* was significantly affected by current velocity. The data suggest, however, that rather than orientation behaviour of the mysids being altered by the flow velocity, it was their net movement. At each current velocity, mysids showed positive rheotaxis but, at the highest current velocities were unable to overcome the flow and, therefore, the frequency of each 'behavioural type' was significantly altered by flow velocity. Rheotaxic behaviour *per se* appeared to be unaltered, and these data imply that position maintenance in unidirectional flow is dictated by the maximum sustainable swimming speed of the mysid, although utilisation of the substratum was also found to be important. Behaviour of mysids above the boundary layer was variable, however, the same general trends were observed as when the behaviour of all mysids was analysed. Salinity had no effect on the swimming behaviour of *N. integer*.

Mysid swimming behaviour has been shown to vary according to several physical and chemical stimuli (for review see Mauchline, 1980); however, it is worth noting that under some laboratory conditions mysids become "accustomed" to certain stimuli and do not alter their behaviour (Zelickman, 1974). Most reports, including this study, have shown that mysids exhibit rheotaxic behaviour, although some swimming behavioural patterns have been observed in static water. For example, *Neomysis mirabilis* orientated into the corners of rectangular tanks or radially in a circular basin (Zelickman, 1974). Although light intensity and direction were thought to be important controls of *N. mirabilis* orientation, perhaps the most important observation was the degree of organisation of mysid groups, which displayed an extremely orderly distribution with regards to both orientation and spacing (Zelickman, 1974). Aggregation of mysids is thought to play an important role in mysid behaviour, where proximity or 'distance to nearest neighbour' is thought to be an important regulator in the formation and maintenance of mysid swarms (Steven, 1961; O'Brien, 1989). Solitary mysids often show random orientation even when exposed to a number of stimuli (Zelickman, 1974). In the present study, all animals displayed rheotaxic behaviour (mostly positive rheotaxis) and, since all animals may be considered as 'solitary' because shoaling was not observed, it is suggested that rheotaxis is very important in controlling mysid distribution. It is likely that shoaling behaviour is also a very important response since it occurs so regularly in the natural environment, however, mysids may get displaced from the shoal and need to respond as individuals to environmental factors such as water currents.

Maximum swimming speed achievable by the mysid is important since this dictates in which flow velocities it can maintain its position. Current velocities of 6cm s^{-1} and 9cm s^{-1} appear to be tolerated by the population from Terras Bridge, although some mysids were able to swim at faster velocities. Some individuals were seen to swim at speeds of up to 27cm s^{-1} but they could only sustain these speeds for a few seconds. These findings are in agreement with other published swimming speeds for mysids. Walesby (1973) found that *Neomysis integer* maintained its position at velocities between 3 - 12cm s^{-1}, but at 16cm s^{-1} displacement started to occur, and at 18cm s^{-1} all mysids were displaced (although as described in this study a few would swim at this speed for a few seconds until they became fatigued). These swimming speeds correlate well with the distribution of mysids at the Terras Bridge study site, where mysids were found consistently in slower moving water (< 15cm s^{-1}) and were absent in faster flowing water (> 20cm s^{-1}). The swimming speed of *c.*12cm s^{-1} recorded in this study for *N. integer* is similar to that of *Mesopodopsis slabberi* (Walesby, 1973). In the Gamtoos Estuary, *Gastrosaccus brevifissura*, slightly smaller than *N. integer* at 12mm long (Mauchline & Murano, 1977), inhabited areas with velocities up to 22cm s^{-1} (Schlacher & Wooldridge, 1994). In a straight channel experimental flume, the sub-tropical mysid *Americamysis bahia* was reported to have a maximum sustained speed of 12cm s^{-1} (Cripe *et al.*, 1981).

If swimming speed is an important factor in the position maintenance of estuarine mysids, it is clearly beneficial for them to utilise any available shelter to conserve energy. Selection of sheltered environments was observed in this study where mysids used the sediment to reduce swimming effort. By attaching to the substratum (mud or sand), mysids enter the boundary layer and are thus exposed to flows less than the free-stream velocities (Widdows *et al.*, 1997). Furthermore, burrowing into the sediment is an effective means of position maintenance in moving waters and is, therefore, a common behaviour for mysids inhabiting areas subject to tidal disturbance. This type of behaviour was very common in the flume but has also been observed in the natural environment, particularly on the ebb tide (*pers. obs.*). Similarly, the isopod *Eurydice pulchra* burrows into the sand on the ebb tide to prevent displacement (Warman *et al.*, 1991). When swimming above a sandy substratum, *N. integer* was observed to shelter on the downstream side of 'sand waves/ripples', or in the shelter of algae, thus reducing the swimming effort. *Neomysis integer* has been observed settling on and 'clamping' itself to the substratum in previous flume studies (Walesby, 1973), and this

behaviour was also seen in the present study. At Terras Bridge, the sediment consisted generally of a fine sand/mud (<250μm), although a sandy substratum occurred in the faster flowing regions. On most sampling occasions, the majority of *N. integer* was found on or above muddy substrata. Other workers have described sediment-specific associations of mysid distributions. For example, *Gastrosaccus brevifissura* was found primarily in sandy areas (Schlacher & Wooldridge, 1994), although the advantages of choosing this substratum were attributed to feeding. *Neomysis integer* was also seen to be feeding on/ingesting muddy sediment in the flume, so the reason for being associated with this substratum in the natural environment is probably related to both feeding and selection of regions of slower currents. In both the flume and the estuary, *N. integer* was found most frequently in the hyperbenthon (an area of lower current velocities and a source of food), similar to *N. integer* in other systems (e.g. Hough & Naylor, 1992; Mees *et al.*, 1993).

Behavioural adaptations, utilising water motion, have been observed in several crustaceans. *Eurydice pulchra* makes vertical migrations into the water column to control its distribution (Warman *et al.*, 1991). On the flood tide, this isopod migrates to the faster moving surface waters, facilitating its movement inshore. At high tide, *E. pulchra* migrates to the hyperbenthon where it swims with the ebb tide (to prevent stranding above tide level) before burrowing into the sediment. Utilisation of tidal water movements by zooplankton is most commonly described in large, two-layer flow systems (Schlacher & Wooldridge, 1994). For example, Orsi (1986) described *Neomysis mercedis* making use of surface currents in the entrapment zone of the Sacramento River on flood tides; any mysids displaced from the population migrated down to the bottom water layer to be transported upstream. More recent studies have demonstrated that, although water movements are less clearly defined in tidally-mixed estuaries, mysids may still use currents as a position maintenance mechanism. For example, in the Conwy Estuary (Wales), *Neomysis integer* demonstrated circa-tidal swimming activity, peaking on the ebb tide (Hough & Naylor, 1992). If tidal currents were being used in this way, it would be expected that *N. integer* would swim with the current as salinity was increased, since this would represent the flood tide. However, this was not observed in the flume, again indicating that current velocity is a greater stimulus. In contrast to the unidirectional flows in rivers and flumes, however, the estuarine environment has periods of complex counteracting two-way flow created by the combined effects of river flow and tidal flow which might influence swimming behaviour.

Several workers have related differences in mysid distribution to environmental variables such as temperature, salinity and turbidity (e.g. Ralph, 1965; Mauchline, 1971; Williams & Collins, 1984; Hamerlynck & Mees, 1991; Laprise & Dodson, 1994; Moffat, 1996). Williams & Collins (1984) speculated that salinity and temperature acted in combination to affect the numerical and seasonal abundance of mysids. *Schistomysis spiritus* occurs in a well-defined salinity envelope (Williams & Collins, 1984), however, many mysid species are extremely euryhaline (Tattersall & Tattersall, 1951; Mauchline, 1980). For example, both *M. slabberi* and *N. integer* are able to tolerate large fluctuations in salinity (Moffat & Jones, 1992), and it would appear that salinity alone is not responsible for mysid distribution. The present study shows *N. integer* to be extremely tolerant of large, acute salinity fluctuations (1 - 30‰), and no changes in swimming behaviour when exposed to large variations in salinity were identified. It appears, therefore, that water movement is a stronger stimulus than salinity for affecting the swimming behaviour of this mysid. One interesting observation was that when salinity was changed, by introducing water of different salinity to the flume, mysids would aggregate and orientate themselves towards the entry point of the new water. The reason for this is unclear and rheotaxis occurred immediately the current was generated. Changes in salinity also had little effect on mysid distribution in the field, where mysids were found consistently at the water's edge at Terras Bridge, even though the salinity in this region varied from *c.*1 to 34‰ over the tidal cycle. The Looe River Estuary is tidally mixed and salinity is consistent across the entire width of the river at Terras Bridge. Current velocity is not consistent and mysids occurred at the periphery of the river where flow is slowest. Temperature varied seasonally and tidally from *c.*3°C (winter low tide) to *c.*15°C (summer high tide), however, this did not appear to affect mysid distribution, which was remarkably consistent at the study site. Abundance of *N. integer* did vary throughout the year, numbers peaking in July/August and reaching a minimum in January/February, with juveniles being most abundant at all times (*pers. obs.*). This may be due to a number of factors, but temperature and current velocity are thought to be most important. Temperature is a major factor in the reproductive cycle of *N. integer* which usually produces two broods in spring/summer and one in autumn (Chapter 1). Current velocity increases greatly in winter as run-off increases and this is thought to disrupt aggregations of mysids. Seasonal differences in mysid abundance are thought to reflect degrees of aggregation/disaggregation, rather than major changes in individual numbers (Mauchline, 1971). It is, therefore, possible that reduced numbers of mysids at Terras Bridge during winter are due to increased water flow displacing

numbers of mysids at Terras Bridge during winter are due to increased water flow displacing animals from the population. Circadian rhythms and diurnal migrations are reported commonly for marine (e.g. Mauchline, 1980) and also for estuarine (e.g. Hough & Naylor, 1992) species, however, no obvious migrations were observed at Terras Bridge. *Neomysis integer* migrated axially as river width increased with the flood tide, always remaining in the slowest moving areas. This agrees with other mysid populations (e.g. Dadswell, 1975; Schlacher & Wooldridge, 1994).

2.6 SUMMARY

In summary, current flow velocity and substratum type were found to be the most important controls of mysid position maintenance in the flume. Positive rheotaxis was the most common swimming behaviour. At high current velocities, *N. integer* utilised the substratum in an attempt to prevent displacement. Salinity had no clear effect on swimming behaviour. Therefore, current flow appears to represent a stronger control than salinity changes on the distribution of *N. integer*. In the natural environment, mysids were found in areas of flow comparable to those current velocities in the flume where mysids were able to maintain position and sustain swimming.

CHAPTER 3

EFFECT OF ABIOTIC (TEMPERATURE AND SALINITY) AND BIOTIC (SEX) FACTORS ON THE OXYGEN CONSUMPTION OF *NEOMYSIS INTEGER*

3.1 ABSTRACT

The euryhaline mysid *Neomysis integer* (Peracarida: Mysidacea) is a common member of the hyperbenthos of the upper reaches of European estuaries. In the East Looe River Estuary, Cornwall (UK), this species has been recorded from areas experiencing a salinity range of *c.*1 - 34‰. In addition, this population of *N. integer* experiences diurnal and seasonal temperature changes covering the range *c.*3 - 15°C. The acute effect of changes in temperature (5, 10 and 15°C) and salinity (1, 10, 20 and 30‰) on the oxygen consumption of *N. integer* was addressed in this study. Oxygen consumption of male and female *N. integer* increased with increasing temperature, and Q_{10} values ranged from *c.*1·8 - 2·5. In contrast, increasing salinity was found to suppress oxygen consumption rates of male and female mysids. At any given temperature/salinity combination, male mysids consumed oxygen at a higher rate than females. Temperature and salinity interacted significantly to affect the oxygen consumption by *N. integer*. The oxygen consumption ofby *N. integer* was most sensitive to changes in temperature at high salinity and was most sensitive to salinity changes at low temperatures. In addition, females were slightly more sensitive to temperature change at high salinity than males. The results are discussed in terms of the predicted changes in oxygen consumption by *N. integer* in relation to the diurnal and seasonal fluctuations of these variables experienced by the natural mysid population.

3.2 INTRODUCTION

In aquatic invertebrates, the rate of oxygen consumption is dependent upon abiotic (temperature, salinity and season) and biotic (weight and sex of animal) factors (Kinne, 1971; Burggren & Roberts, 1991). In the Mysidacea, most respiratory experiments have been made using species of the sub-order Mysida, comparatively little work has been done with members of the sub-order Lophogastrida. Of the two sub-orders, only the lophogastrids have gills for gaseous exchange (Tattersall & Tattersall, 1951; Mauchline, 1980). Species of the sub-order Mysida have no obvious organ of gaseous exchange, however, their carapace has a very rich supply of blood, with many blood spaces and sinuses, and it is thought that gaseous exchange occurs through the thin, non-chitinous wall of the carapace (Tattersall & Tattersall, 1951). A complex series of currents, resulting from movements of the thoracic limbs for locomotory purposes, facilitate oxygen exchange. Locomotion occurs by movement of the exopods of the thoracic limbs, which produces two powerful currents passing laterally from the anterior to the posterior of the mysid (Tattersall & Tattersall, 1951). The movement of these two currents along the body leaves a small area of static water in the mid-dorsal and mid-ventral longitudinal axes (Tattersall & Tattersall, 1951), and it is this water that is used for gaseous exchange. The respiratory current is created by the movement of the epipod of the first thoracic limb in a 'respiratory chamber' formed by the joining of the carapace to the thorax. This respiratory chamber is situated anterior to the dorsal area of static water, so the movement of the epipod flushes out water from the chamber anteriorly, drawing in water from the dorsal static water area to replace it (Tattersall & Tattersall, 1951). Hence, a rapid flowing respiratory current is created passing anteriorly between the carapace and body wall, where gaseous exchange can occur. It is suggested that the thin walls of the body in the thoracic region might also provide an area for gaseous exchange (Tattersall & Tattersall, 1951). Since species of the sub-order Lophogastrida have gills on the epipods of their thoracic limbs, it is assumed that the movement of the epipods creates an adequate water current for respiration, although this may be supplemented by movement of the thoracic exopods (Tattersall & Tattersall, 1951).

Most of the circulatory system of mysids has been described in detail, although the fate of the very fine blood vessels is largely unknown (Mauchline, 1980). The heart contains a single chamber and is situated dorsally in the posterior region of the thorax (Mauchline, 1980). The heart is cylindrical and is formed from a dilation of the dorsal artery, from

which it is separated by a valve at each end. The dorsal artery extends both anteriorly, to form the *aorta cephalica* (which supplies blood to the intestine and cardiac stomach), and posteriorly as the *aorta abdominalis* (which provides blood for the abdomen, including the telson). The ventral side of the thorax is also supplied with a major blood vessel since this is where the legs are located (this artery is joined vertically to the posterior of the heart, via the sternal artery). Descending vertically from the anterior of the heart are a pair of hepatic arteries which are thought to supply blood to the stomach [although their exact destination is unknown (Mauchline, 1980)]. A further dilation of the dorsal artery occurs at the anterior of the *aorta cephalica* to form the frontal heart, which divides anteriorly to supply the cerebral ganglia and the eyes (Mauchline, 1980). This frontal heart beats rhythmically, more or less in time with the main heart (Belman & Childress, 1976). Once at the destination tissue, each artery sub-divides into arterioles, which exude blood from their ends into a vast complex of sinuses. It is thought that it is in the sinuses under the carapace that oxygenation of the blood occurs (Mauchline, 1980).

3.2.1 Temperature effects

The effect of temperature on the respiration rate of aquatic organisms is well documented. In general, increasing temperature leads to increased metabolism and respiration (e.g. Burggren & Roberts, 1991; Schmidt-Nielsen, 1997). Temperature is considered to be the single most influential factor affecting the respiration rates of mysids (Mauchline, 1980; Weisse & Rudstam, 1989) and the general effect is one of increased oxygen uptake at higher temperatures (Table 3.1). Thus, *Archaeomysis grebnitzkii* and *Neomysis awatschensis* increased their oxygen consumption from *c.*0·20µl O_2 mg wet wt^{-1} h^{-1} at 5°C to >0·30µl O_2 mg wet wt^{-1} h^{-1} at 15°C (Jawed, 1973). Similarly, the oxygen consumption of *Neomysis intermedia* increased from 0·056 to 0·28µl O_2 mg wet wt^{-1} h^{-1} as temperature was increased from 5 to 25°C (Toda *et al.*, 1987), and that of *N. integer* from *c.*0·30µl O_2 mg wet wt^{-1} h^{-1} at 5°C to *c.*0·50µl O_2 mg wet wt^{-1} h^{-1} at 15°C (Vlasblom & Elgershuizen, 1977). The effect of temperature on oxygen consumption may usually be described by linear regression (eg. Simmons & Knight, 1975; Toda *et al.*, 1987). From the data given in Table 3.1, it is possible to calculate the temperature coefficients, or Q_{10} (the factor by which the respiration rate will increase for every 10°C increase in water temperature), for some mysid species. The estimated Q_{10} values for *Neomysis americana*, *N. awatschensis* and *Archaeomysis grebnitzkii* were 1·6, 1·7 and 2·9 respectively. Clearly, the higher the Q_{10}

Table 3.1: Oxygen consumption of mysids.

Species	Temperature (°C)	Salinity (‰)	Sex / Age	Oxygen consumption ($\mu l\ O_2$ mg wet $wt.^{-1}\ h^{-1}$)	Reference
LOPHOGASTRIDA					
Gnathophausia ingens	5	seawater	immature, non-sexed	0·02	Childress (1971)
	5	seawater	immature, non-sexed	0·03	Quetin *et al.* (1978)
MYSIDA					
Archaeomysis grebnitzkii	5	30	-	0·18	Jawed (1973)
	5	22	-	0·21	"
	10	30	-	0·27	"
	10	22	-	0·20	"
	15	30	-	0·49	"
	15	22	-	0·36	"
Metamysidopsis elongata	14	seawater	ovigerous female	0·52	Clutter & Theilacker (1971)
	14	seawater	male	0·71	" "
Mysis relicta	5	freshwater	male + female	0·40	Lasenby & Langford (1972)
	4	freshwater	male, female + juvenile	0·36	Foulds & Roff (1976)
Neomysis awatschensis	5	30	-	0·23	Jawed (1973)
	5	22	-	0·21	"
	10	30	-	0·37	"
	10	22	-	0·33	"
	15	30	-	0·32	"
	15	22	-	0·29	"
N. integer	5	2	mixed adults	0·31	Vlasblom & Elgershuizen (1977)
	5	16	" "	0·25	" "
	15	2	" "	0·56	" "
	15	16	" "	0·53	" "
N. intermedia	5	freshwater	-	0·056	Toda *et al.* (1987)
	15	freshwater	-	0·12	"
	25	freshwater	-	0·28	"

value the more sensitive is the respiration rate to changes in temperature. Mysids, however, are remarkably adaptable to changes in their environment and acclimation to high temperatures may lead to a lower respiration rate than expected (Mauchline, 1980). To accompany the increase in respiration rate at higher temperatures, the rate of heart beat of mysids also increases [a linear relationship exists between heart rate and temperature for *Mysis relicta* (Mauchline, 1980)].

3.2.2 Salinity effects

Kinne (1971) defined four types of respiratory response to changes in salinity for aquatic invertebrates. For euryhaline organisms, oxygen uptake increases in sub-normal salinities and/or decreases in supra-normal salinities (type 1), or increases in sub- and supra-normal salinities (type 2). The respiration rate of stenohaline animals usually decreases in sub- and supra-normal salinities (type 3), and the respiration rate of holeuryhaline invertebrates remains basically unaffected by salinity (type 4). Altered oxygen consumption rates due to changes in salinity have been reported for mysids (Table 3.1). *Archaeomysis grebnitzkii* and *Neomysis awatschensis* showed decreased oxygen consumption at low compared with high salinity (Jawed, 1973). At 30‰, the oxygen consumption of *A. grebnitzkii* was 0·49µl O_2 mg dry wt $^{-1}$ h^{-1} compared with 0·36µl O_2 mg dry wt $^{-1}$ h^{-1} at 22‰ (Jawed, 1973). A smaller decrease in oxygen consumption rate was reported for *N. awatschensis* [0·32 and 0·29µl O_2 mg dry wt $^{-1}$ h^{-1} at 30 and 22‰ respectively (Jawed, 1973)]. In contrast, *Neomysis integer* showed increased oxygen consumption with decreasing salinity [from 0·25 to 0·31 µl O_2 mg dry wt $^{-1}$ h^{-1} at 16 and 2‰ respectively (Vlasblom & Elgershuizen, 1977)]. The results of Vlasblom & Elgershuizen (1977) indicate that some mysids show the type 1 response suggested by Kinne (1971). The effect of salinity on the oxygen consumption of *Neomysis mercedis*, however, is more complex than any of the responses defined by Kinne (1971) would suggest. Although a large increase in salinity led to a decrease in oxygen consumption by *N. mercedis*, oxygen consumption was stable at salinities <10‰, and between 10 and 20‰ (Simmons & Knight, 1975). The data suggest that the oxygen consumption of *N. mercedis* was independent of small salinity changes.

3.2.3 Weight effects

Since an organism's weight affects some physiological rates (Schmidt-Nielsen, 1997), oxygen consumption rates are given frequently as weight-specific rates. For mysids, an

increase in weight leads to a larger absolute oxygen consumption rate (e.g. Vlasblom & Elgershuizen, 1977), however, oxygen consumption per milligram of mysid body weight decreases with increasing mysid body length (e.g. Smith & Hargreaves, 1985). When calculating oxygen consumption rates, weight may be accounted for in two ways. Firstly, the absolute oxygen consumption rate may be divided by the weight of the organism or, secondly, a weight correction exponent may be applied. Dividing the oxygen consumption rate by the weight of the organism gives a simple weight-specific rate per unit weight of organism. Large animals, however, respire at a slower rate per gram body weight than do small animals and weight-specific rates calculated by dividing by body weight decrease with increasing body weight with a slope of *c.*-0·25 (Schmidt-Nielsen, 1997). Therefore, simply dividing by the weight of an organism makes comparison of the oxygen consumption of two different organisms difficult if they are of different body weights. When the oxygen consumption of a single species is plotted for various weights of organism, the data fall on a straight line with a slope of *c.*0·67, irrespective of species (Schmidt-Nielsen, 1997). A more rigorous way of accounting for size in respiration measurements, therefore, is to express oxygen consumption rate for a 'standard body size' using the allometric equation:

$$Y = a X^{b} \qquad (\text{or } \log Y = \log a + b \log X)$$

where: Y = physiological rate (e.g. oxygen consumption rate), X = dry body mass (g), a = intercept and b = slope of line. The exponent b (usually = *c.*0·67) is used for conversion of oxygen consumption to an appropriate weight-specific rate (e.g. for a 1g animal). Much of the published data for mysids are, unfortunately, calculated[1] by dividing oxygen consumption by body weight. Simmons & Knight (1975) reported an increase in oxygen consumption with increasing mysid weight. Interestingly, the linear regression coefficient for weight was >1, suggesting that weight alone did not explain the increased oxygen consumption (i.e. life stage or hormone levels also affected oxygen consumption). Most data reporting the effects of various factors on the oxygen consumption of mysids are presented for animals of similar size, allowing valid comparisons within each publication.

3.2.4 Sex effects

Sex may also affect oxygen consumption (Burggren & Roberts, 1991), most frequently due to the reproductive status of the organism being tested (Simmons & Knight, 1975). Most

workers studying mysid oxygen consumption have ignored the sex of the test mysid, using mixed sex groups when taking measurements (Table 3.1). Those workers who have studied the oxygen consumption of male and female mysids have shown that males tend to consume more oxygen than females. For example, male *Neomysis mercedis* consumed more oxygen than females (Simmons & Knight, 1975), and male *N. americana* had a higher weight-specific oxygen consumption rate than females (Smith & Hargreaves, 1985). In addition, ovigerous female *Metamysidopsis elongata* consumed less oxygen than male *M. elongata* [0·52 and 0·71µl mg wet wt^{-1} h^{-1} respectively (Clutter & Thielacker, 1971)]. Care must, therefore, be taken when referring to literature data that comparisons are made only for mysids of the same sex.

3.2.5 Factor interactions

Perhaps the most comprehensive investigation of mysid respiration is that of Simmons & Knight (1975) who showed that temperature, salinity, weight, sex and season interacted to affect the oxygen consumption of *Neomysis mercedis* according to a multiple linear regression equation. Within the overall relationship described, the various factors interacted with each other in various ways. For example, temperature effects were more pronounced for larger mysids, and the intensity of the response to salinity was moderated by temperature and the sex score for the test mysid (Simmons & Knight, 1975). In general, changes in temperature had a greater effect (as described in Section 3.2.1) on oxygen consumption by mysids at low salinity and changes in salinity had a greater effect (as described in Section 3.2.2) on oxygen consumption at low temperatures (Mauchline, 1980). The investigation by Simmons & Knight (1975) is the only study of mysid oxygen consumption rate where environmental parameters (temperature, salinity and season) and physiological factors (weight and sex) have been considered together.

Several workers have examined the combined effects of two or more of the variables examined by Simmons & Knight (1975). Jawed (1973) reported that temperature and salinity interacted significantly to affect the oxygen consumption of *Archaeomysis grebnitzkii* and *Neomysis awatschensis* (although the individual effects of each factor could not be ascertained). No salinity effects occurred at 5°C for either *A. grebnitzkii* or *N. awatschensis,* although salinity-dependent decreases in oxygen consumption did occur at 10 and 15°C (Jawed, 1973). The findings of Jawed (1973) were in contrast to the results of

Simmons & Knight (1975), where salinity effects on oxygen consumption were greatest at low temperatures. Temperature and salinity interacted significantly to affect the oxygen consumption of *Praunus flexuosus* and *Neomysis integer* (Vlasblom & Elgershuizen, 1977). Differences in the oxygen consumption of male and female *N. americana* were predicted to be greatest at high temperatures [i.e. >15°C (Smith & Hargreaves, 1985)].

3.2.6 Aim

The aim of this Chapter was to establish the effects of temperature and salinity on the oxygen consumption by male and female *Neomysis integer*.

3.3 METHODS

3.3.1 Animal collection and maintenance

During January and February (1996), mysids were collected and maintained as described in Chapter 2 (Section 2.2.1). At the time of sampling, the population comprised overwintering mature males and females, and juveniles of various sizes (Chapter 1).

3.3.2 Oxygen consumption measurements

There are various methods for measuring oxygen consumption, but the 'Winkler method' and oxygen electrodes are used most frequently. The 'Winkler method' involves drawing off a sample of test water followed by titration against sodium thiosulphate and has been used for mysids (e.g. Lasenby & Langford, 1972; Morioka *et al.*, 1987). Electrodes, however, have advantages over the chemical method because they allow continuous monitoring of dissolved oxygen (DO) concentrations and are less time consuming than titrations, and have, therefore, been used more frequently in mysid respiration studies (e.g. Jawed, 1973; Quetin *et al.*, 1978). The oxygen electrode allows *in situ* measurement of oxygen depletion by placing a polarographic electrode in the test-chamber itself. A consideration when measuring oxygen consumption is whether to use closed or flow-through test-chambers (Widdows & Salkeld, 1993). A flow-through system prevents the decline of DO to critical levels, and the build up of carbon dioxide and excretory products in the test-chamber, factors which can affect respiration rate if they accumulate beyond critical concentrations (Widdows & Salkeld, 1993). Problems of oxygen decline and accumulation of toxic waste need not be a problem, however, if the duration of oxygen measurement is limited. Closed chambers were chosen for use in the present study, and

duration of oxygen measurements were kept to a minimum to ensure that oxygen concentrations did not decline to critical levels and that there was limited build up of toxic wastes.

The oxygen consumption of individual mysids was measured using Strathkelvin 1302 'Clark-type' polarographic electrodes connected to Strathkelvin 781b oxygen meters. These were used in accordance with the manufacturers instructions (the membrane was changed every two weeks and the meter was zeroed daily with sodium tetraborate solution). Oxygen consumption measurements were made in perspex respiration chambers (Fig. 3.1). The chambers were designed for 'closed' oxygen measurement. Each chamber comprised a 25ml cylinder with a magnetic follower in the base to maintain a homogeneous DO concentration. A 2mm mesh covered the follower to prevent the mysids from being disturbed or damaged (Fig. 3.1). The respiration chambers were placed in a water bath (temperature-controlled by a heater offset by a dip-cooler) which kept the water temperature in the test chambers constant (± 0·01°C).

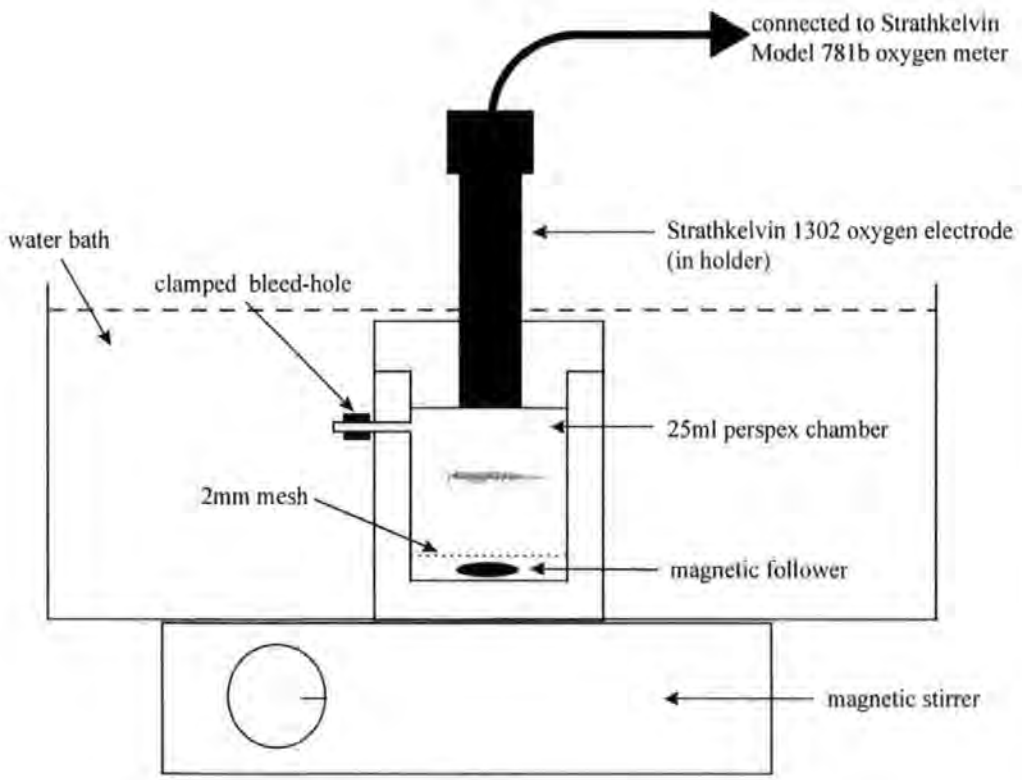


Figure 3.1: Diagram of a respiration chamber.

The sides of the water bath were opaque and, therefore, shielded the mysids in the test chambers from visual disturbance. Three respiratory chambers were used simultaneously within the one water bath. Dissolved oxygen readings from the oxygen meters were recorded by hand. All experiments were carried out during the day, with ambient lighting from fluorescent laboratory lights. Preliminary experiments showed that oxygen consumption by mysids in the morning was not significantly different from mysid oxygen consumption in the afternoon (Student's *t*-test, t=1·14, d.f.=18 , p>0·05; Table 3.2).

Table 3.2: Effect of time of day on oxygen consumption by *Neomysis integer* (10°C, 10‰, ambient laboratory lighting). n=20, critical t=2·10, calculated t=1·14, d.f.=18, p>0·05 (CI=confidence interval).

	Oxygen consumption (µl O_2 mg wet wt.$^{-1}$ h^{-1})	
Time	A.M.	P.M.
Replicates	0·271	0·263
	0·281	0·279
	0·264	0·275
	0·283	0·286
	0·283	0·271
	0·278	0·267
	0·284	0·261
	0·273	0·281
	0·286	0·278
	0·264	0·263
Mean	0·277	0·272
Lower 95% CI	0·271	0·267
Upper 95% CI	0·281	0·278

3.3.3 Effect of temperature and salinity on mysid oxygen consumption

Oxygen consumption was measured at various temperature/salinity combinations (temperatures of 5, 10 and 15°C; salinities of 1, 10, 20 and 30‰). Variations in water temperature were achieved by altering the temperature of the water bath. Filtered seawater (10µm filter) was microwaved and combined with double-distilled, de-ionised water to establish water of the correct salinity (determined by an Atago hand-held refractometer). Solutions were aerated in the water bath for 15min to allow the water to reach the correct

temperature and to ensure that the water was fully saturated with oxygen. The chambers were filled with the aerated water and sealed, and the electrode placed gently in the electrode holder such that the chamber was completely air-tight. Rapid changes in pressure can rupture the membrane of the electrode, therefore, the test-chamber design incorporates a 'bleed-hole' so that when the electrode is placed in the chamber the pressure can come to equilibrium before the bleed-hole is gradually sealed (Fig. 3.1). With the electrode in place, the sealed chamber was allowed to equilibrate in the water bath for *c.*60min until the oxygen meter reading was stable for a period of >5min (slight temperature and pressure changes due to setting up cause the DO reading to drift). Once the DO reading was stable, the oxygen meter was calibrated by setting the digital display to show the theoretical saturated DO level taken from tables (Green & Carrit, 1967). The dissolved oxygen tables give values for 100% oxygen saturation for different temperatures and salinities, however, not all the combinations used in these experiments were listed in the tables, therefore, appropriate saturation values were calculated from the equation:

$$\text{Saturation (ml } O_2 \text{ } L^{-1}) = [a - ((b \div c) \times d)] \times 1{\cdot}428$$

where: a = table value for closest chlorinity value lower than chlorinity of sample, b = chlorinity of sample (‰), c = difference between table chlorinity values either side of sample chlorinity value, and d = difference between table DO readings for chlorinity values either side of sample chlorinity.

$$\text{Chlorinity (‰)} = [\text{salinity (‰)} \div 1{\cdot}805] - 0{\cdot}3 \text{ (Green \& Carritt, 1967).}$$

Having calibrated the oxygen meter, the electrode was removed gently from the electrode holder and a single mysid was introduced, via the electrode holder, into the chamber. A Pasteur pipette with the narrow tip removed was used to gently suck up and move single mysids. The electrode was then replaced gently in the holder and the chamber was left to equilibrate for 1h. The bleed-hole was not used to reduce pressure changes whilst mysids were in the chamber to prevent possible damage to the animal. Initial experiments showed that DO levels decreased rapidly after replacement of the electrode, and that within 30min DO decreased more slowly and at a more uniform rate. Therefore, it was assumed that the chamber was at equilibrium after 1h. Readings from the first hour were ignored whilst the mysid became accustomed to the chamber and the oxygen electrode equilibrated again.

Dissolved oxygen concentration was recorded at 5min intervals for 90min. Twelve replicate mysids of similar size (15 ± 1mm, measured from anterior margin of rostrum to tip of telson) were used for each temperature/salinity combination, having first been acclimated to the appropriate temperature and salinity conditions for 2h. After recording for 90min, each mysid was removed from the respiration chamber, blotted on tissue paper and wet weighed (±0·01mg) using a Sartorius R200-D balance. After each experiment, the respiration chambers were soaked overnight in 5% DECON solution and rinsed thoroughly in double distilled, de-ionised water. This ensured that any microbes on the inside of the chamber were removed prior to re-use (unexplained drift of the DO readings in initial experiments was assumed to be caused by microbial metabolic activity).

3.3.4 Statistical treatment of results

Decline in DO was plotted against time and oxygen consumption was calculated from the corresponding regression equation. Two-way analysis of variance (ANOVA) was applied to the data to determine the significance of any temperature, salinity or sex effects, and to establish factor interactions. Multiple linear regression analysis was applied to the data as a whole, and to sub-sections of the data, to determine the effects of temperature, salinity and sex of animal on mysid oxygen consumption (Simmons & Knight, 1975). Slope of the line and intercept describe the effects of each variable on respiration rate. Temperature coefficients (Q_{10}) were calculated from the rates of oxygen consumption at 5 and 15°C. All statistical analyses were made using the Minitab (Version 7.1), Figure P for Windows (Version 1.0) and Quattro Pro for Windows (Version 5.0) computer programmes.

3.4 RESULTS

The oxygen consumption of *Neomysis integer* at various temperature/salinity combinations is shown in Table 3.3.

Table 3.3: Mean oxygen consumption rates for male and female *Neomysis integer* at various temperature/salinity combinations. n=12 for each temperature/salinity combination.

Sex	Temperature (°C)	Salinity (‰)	O_2 Consumption (µl O_2 / mg wet wt. / h)	95% Confidence Intervals minimum	maximum
Male	5	1	0·232	0·227	0·237
		10	0·211	0·204	0·218
		20	0·167	0·160	0·174
		30	0·161	0·155	0·168
	10	1	0·282	0·275	0·285
		10	0·271	0·264	0·277
		20	0·263	0·256	0·271
		30	0·245	0·237	0·252
	15	1	0·412	0·402	0·423
		10	0·402	0·392	0·413
		20	0·388	0·373	0·402
		30	0·398	0·383	0·413
Female	5	1	0·220	0·213	0·227
		10	0·203	0·196	0·210
		20	0·154	0·147	0·161
		30	0·143	0·136	0·149
	10	1	0·274	0·267	0·281
		10	0·254	0·247	0·261
		20	0·241	0·234	0·249
		30	0·212	0·205	0·219
	15	1	0·380	0·369	0·392
		10	0·377	0·367	0·388
		20	0·369	0·358	0·380
		30	0·358	0·345	0·371

These data show general trends of increasing oxygen consumption by *N. integer* with increasing temperature and decreasing oxygen consumption with increasing salinity (Table 3.3; Fig. 3.2). Males consumed more oxygen than females of similar size.

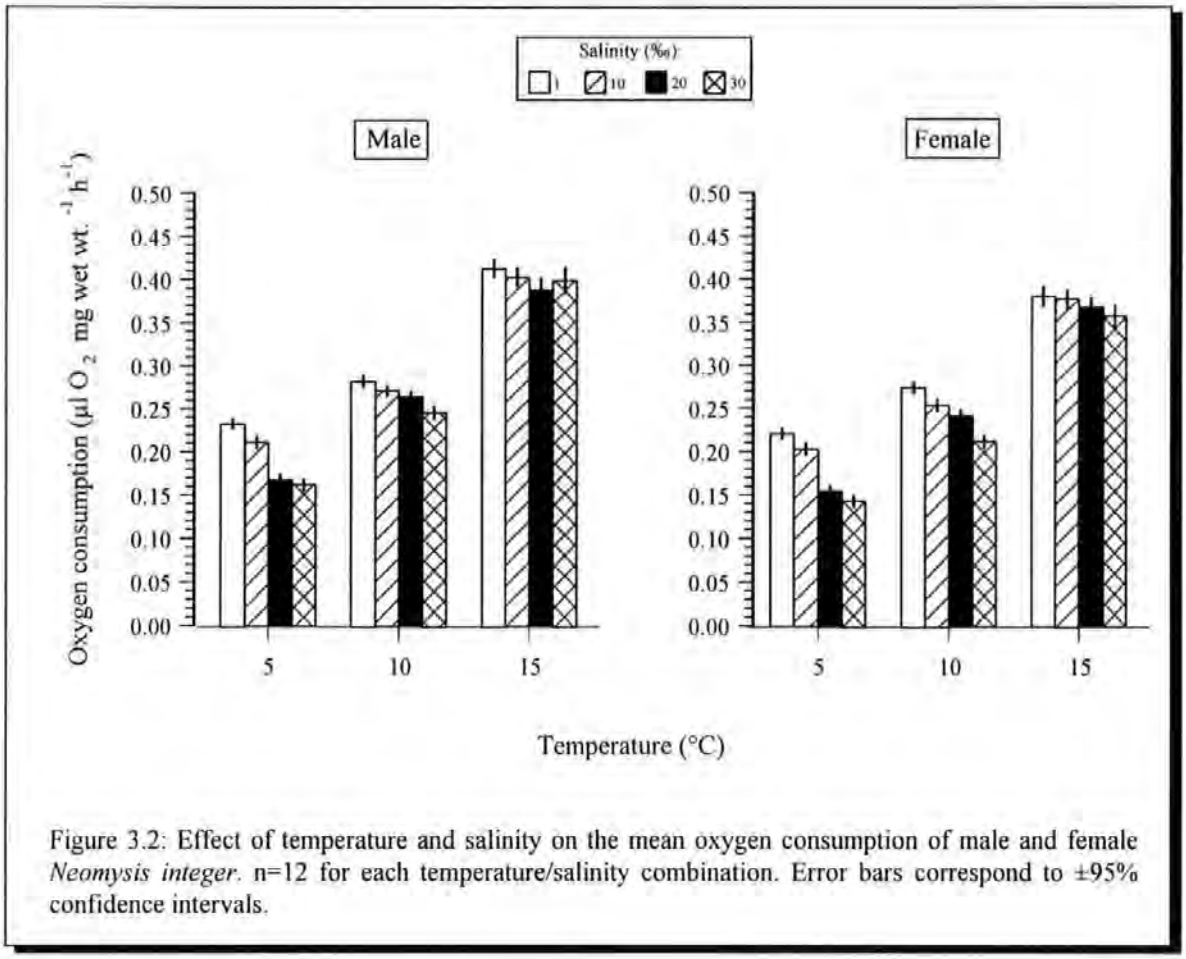


Figure 3.2: Effect of temperature and salinity on the mean oxygen consumption of male and female *Neomysis integer*. n=12 for each temperature/salinity combination. Error bars correspond to ±95% confidence intervals.

3.4.1 Combined effects of temperature, salinity and sex on oxygen consumption

Multiple linear regression analysis showed that the oxygen consumption of *Neomysis integer* was modified by temperature, salinity and sex according to the equation:

$$R = 0{\cdot}1127 + 0{\cdot}0199\,T - 0{\cdot}0017\,Sal - 0{\cdot}0207\,S$$

where: R = respiration rate (µl O_2 mg wet wt.$^{-1}$ h^{-1}), T = temperature (°C), Sal = salinity (‰), and S = score for sex (males = 0, females = 1). Oxygen consumption was, therefore, positively related to temperature (positive regression coefficient), and inversely related to salinity and sex score (negative regression coefficients). Table 3.4 shows the multiple linear regression statistics for the relationship between oxygen consumption and temperature, salinity and mysid sex.

Table 3.4: Statistical values for multiple linear regression analysis of the effects of temperature, salinity and sex on the oxygen consumption of *Neomysis integer*. n=12 for each temperature/salinity combination, r^2=0·95.

Variable	Coefficient	Standard Error	*t* - statistic	significance	95% Confidence Interval	
					Lower	Upper
y intercept	0·1127	0·0043	26.1	p<0·01	0·1220	0·1448
temperature	0·0199	0·0003	59.6	p<0·01	0·00193	0·0206
salinity	-0·0017	0·0001	-13.5	p<0·01	-0·0019	-0·0014
sex	-0·0207	0·0027	-7.59	p<0·01	-0·0261	-0·0153

Oxygen consumption increased significantly with increasing temperature (linear regression, t=19·54, d.f.=3, p<0·01; Table 3.4) and decreased significantly with increasing salinity (linear regression, t=-4·42, d.f.=3, p<0·01; Table 3.3). Differences between the oxygen consumption of male and female mysids were significant (linear regression, t=-7·59, d.f.=3, p<0·05; Table 3.4). Two-way analysis of variance (ANOVA) showed a significant interaction between temperature and salinity on mysid oxygen consumption (ANOVA, f=12·25, d.f.=6, p<0·01; Table 3.5). Temperature interacted with mysid sex when modifying mysid oxygen consumption (ANOVA, f=2·99, d.f.=2, p<0·05; Table 3.5) but salinity did not (ANOVA, f=0·13, d.f.=3, p>0·05; Table 3.5).

Table 3.5: Two-way analysis of variance (ANOVA) testing for interactions between the effects of temperature, salinity and sex on the oxygen consumption of *Neomysis integer*. n=12 for each temperature/salinity combination; n.s.=not significant (p>0·05).

Interaction	d.f.	*f* statistic	critical *f*	significance
temperature + salinity	6	12·25	2·89	p<0·01
sex + temperature	2	2·99	2·30	p<0·05
sex + salinity	3	0·13	1·37	n.s.

Having defined the overall relationship between temperature, salinity and sex on the oxygen consumption of *N. integer*, the effects of individual factors, and the various interactions, will be described in more detail.

3.4.3 Effect of temperature on oxygen consumption

For male and female *Neomysis integer*, oxygen consumption increased with increasing temperature (Fig. 3.3). Oxygen consumption rates were significantly different at 5, 10 and 15°C for each salinity (Fig. 3.3) [ANOVA, f=2518 (male) and 2357 (female) *N. integer*, d.f.=2, p<0·01; Table 3.6]. The increase in oxygen consumption rate was most significant between 10°C and 15°C, indicated by the steeper gradient of the lines (Fig. 3.3).

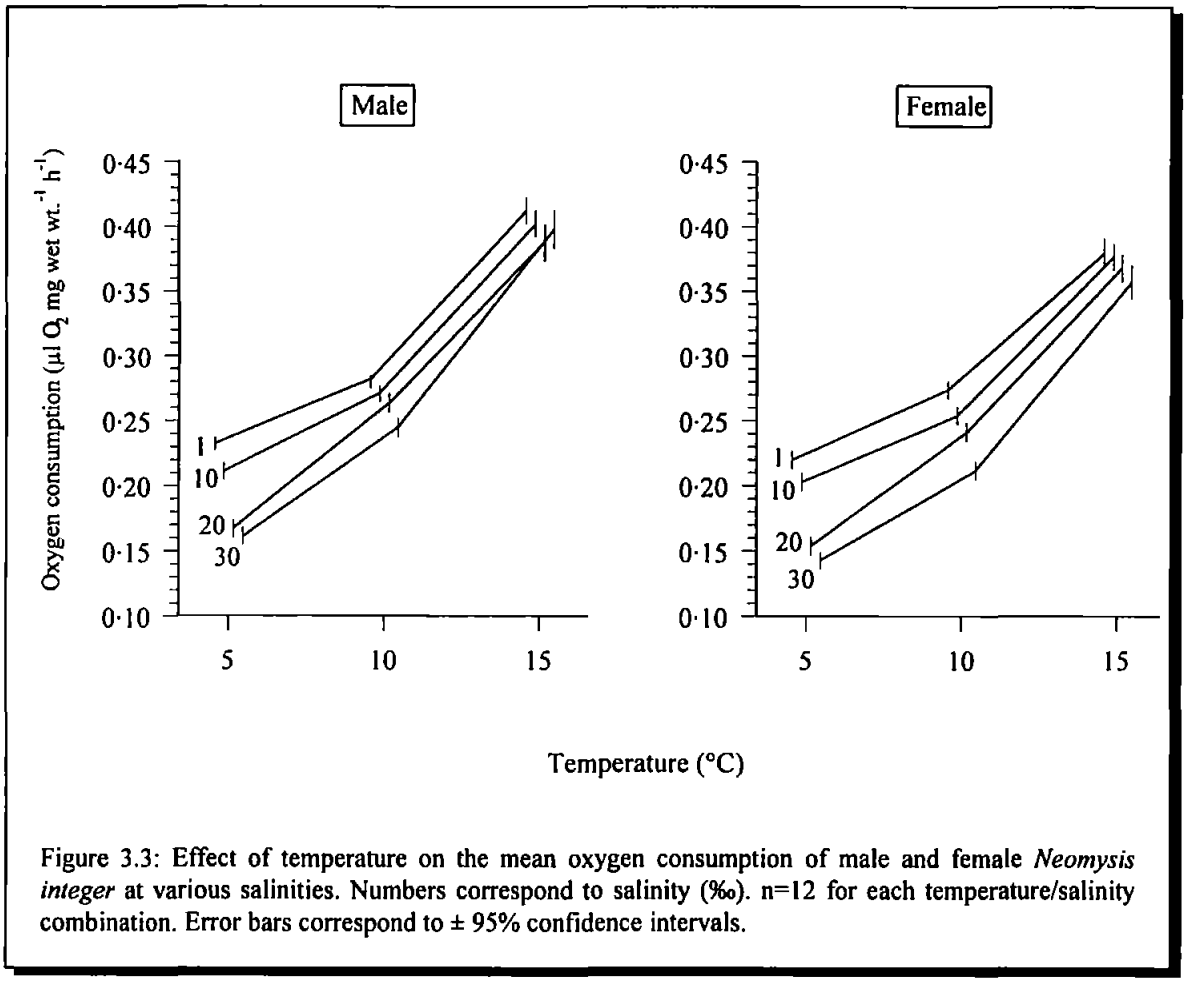


Figure 3.3: Effect of temperature on the mean oxygen consumption of male and female *Neomysis integer* at various salinities. Numbers correspond to salinity (‰). n=12 for each temperature/salinity combination. Error bars correspond to ± 95% confidence intervals.

Two-way ANOVA revealed that temperature and salinity interacted significantly to affect the oxygen consumption of male and female *N. integer* [ANOVA, f=12·4 (male) and 13·7 (female) d.f.=6, p<0·01; Table 3.6). The interaction between temperature and salinity can be seen in Figure 3.3 (by the lines not running parallel as temperature is increased). At 5°C, oxygen consumption of male and female mysids was dependent upon salinity, and mysids consumed more oxygen at lower salinities. Oxygen consumption by male *N. integer* at 5°C was significantly higher at 1‰ than at 10‰ (95% confidence intervals, p<0·05; Fig. 3.3).

Oxygen consumption by male mysids was significantly lower at 20 and 30‰ than at 1 or 10‰ (95% confidence intervals, $p<0{\cdot}05$; Fig 3.3), however, there was no significant difference in oxygen consumption by male mysids at 20 and 30‰ (95% confidence intervals, $p>0{\cdot}05$; Fig. 3.3). The same relationship between salinity and oxygen consumption at 5°C occurred for female *N. integer* (Fig. 3.3).

Table 3.6: Two-way analysis of variance (ANOVA) comparing the effects of temperature and salinity on the oxygen consumption of male and female *Neomysis integer*. n=12 for each temperature/salinity combination, critical f=3·06 (temperature), 2·67 (salinity) and 2·17 (interaction).

Sex	Source	d.f.	f statistic	p values	significance
Male	temperature	2	2523	$6{\cdot}6 \times 10^{-106}$	$p<0{\cdot}01$
	salinity	3	61·8	$4{\cdot}6 \times 10^{-25}$	$p<0{\cdot}01$
	interaction	6	12·4	$4{\cdot}5 \times 10^{-11}$	$p<0{\cdot}01$
Female	temperature	2	2402	$1{\cdot}6 \times 10^{-104}$	$p<0{\cdot}01$
	salinity	3	111	$7{\cdot}1 \times 10^{-36}$	$p<0{\cdot}01$
	interaction	6	14·3	$1{\cdot}9 \times 10^{-12}$	$p<0{\cdot}01$

At higher temperatures, the effect of salinity on oxygen consumption by *N. integer* was reduced (Fig. 3.3). At 15°C, the only significant difference in oxygen consumption of male *Neomysis* was between 1 and 20‰ (95% confidence intervals, $p<0{\cdot}05$; Fig. 3.3). For female *Neomysis* there was a decrease in oxygen consumption at 15°C as salinity increased, although the only significant difference was between oxygen consumption at 1 and 30‰ (95% confidence intervals, $p<0{\cdot}05$; Fig. 3.3). Influence of salinity at 10°C was intermediate of the effects recorded at 5 and 15°C (Fig. 3.3).

Two-way ANOVA showed that there was a significant interaction between temperature and sex to affect oxygen consumption rates (ANOVA, f=2·99, d.f.=2, $p<0{\cdot}05$; Table 3.7). The interaction occurred at 10 and 15°C. There was a greater increase in oxygen consumption between 10 and 15°C for male than for female *N. integer*, indicated by the steeper gradient of the line between these two temperatures for males (Fig. 3·3).

Table 3.7: Two-way analysis of variance (ANOVA) comparing the effects of temperature and mysid sex on the oxygen consumption of *Neomysis integer*. n=12 for each temperature/salinity combination, critical f=4·61 (temperature), 6·63 (sex) and 2·30 (interaction).

Source	d.f.	f statistic	p value	significance
temperature	2	1463·8	$1·5 \times 10^{-150}$	p<0·01
sex	1	42·4	$4·9 \times 10^{-11}$	p<0·01
interaction	2	2·99	0·03	p<0·05

Increases in the intercept of the y-axis from multiple linear regression equations describing effects of salinity and sex on mysid oxygen consumption at 5, 10 and 15°C confirm that oxygen consumption increased with increasing temperature (Table 3.8). With increasing temperature, the salinity regression coefficients decrease in magnitude, indicating that salinity effects were greatest at low temperatures. The increased magnitude of the coefficient for sex with increasing temperatures showed that differences between male and female oxygen consumption rates were greatest at high temperatures (ANOVA, f=2·99, d.f.=2, p<0·05; Table 3.7).

Table 3.8: Multiple linear regression relationships for the effects of salinity and sex on the oxygen consumption of *Neomysis integer* at various temperatures. n=12 for each temperature/salinity combination.

	Regression Coefficients				
Temperature (°C)	Salinity	Sex	Y-Intercept	Standard Error	r^2
5	-0·0028	-0·0130	0·2531	0·0131	0·92
10	-0·0016	-0·0201	0·2901	0·0121	0·90
15	-0·0007	-0·0291	0·4105	0·0192	0·90

Temperature coefficients for male and female *Neomysis integer* over the temperature range 5-15°C were between *c.*1·7 and 2·5 depending on salinity (Table 3.9; Fig 3.4). The interaction between temperature and salinity on oxygen consumption can be seen by the Q_{10} values which increased with increasing salinity, indicating that temperature effects

were greatest at high salinity (Table 3.9; Fig 3.4). There appeared to be little difference between the Q_{10} values of male and female mysids at any salinity (Table 3.9; Fig 3.4).

Table 3.9: Temperature coefficients (Q_{10}) for the oxygen consumption of male and female *Neomysis integer* at different salinities. n=12 for each temperature/salinity combination.

Sex	Salinity (‰)	Q_{10} Value
Male	1	1·78
	10	1·91
	20	2·33
	30	2·48
Female	1	1·73
	10	1·87
	20	2·41
	30	2·52

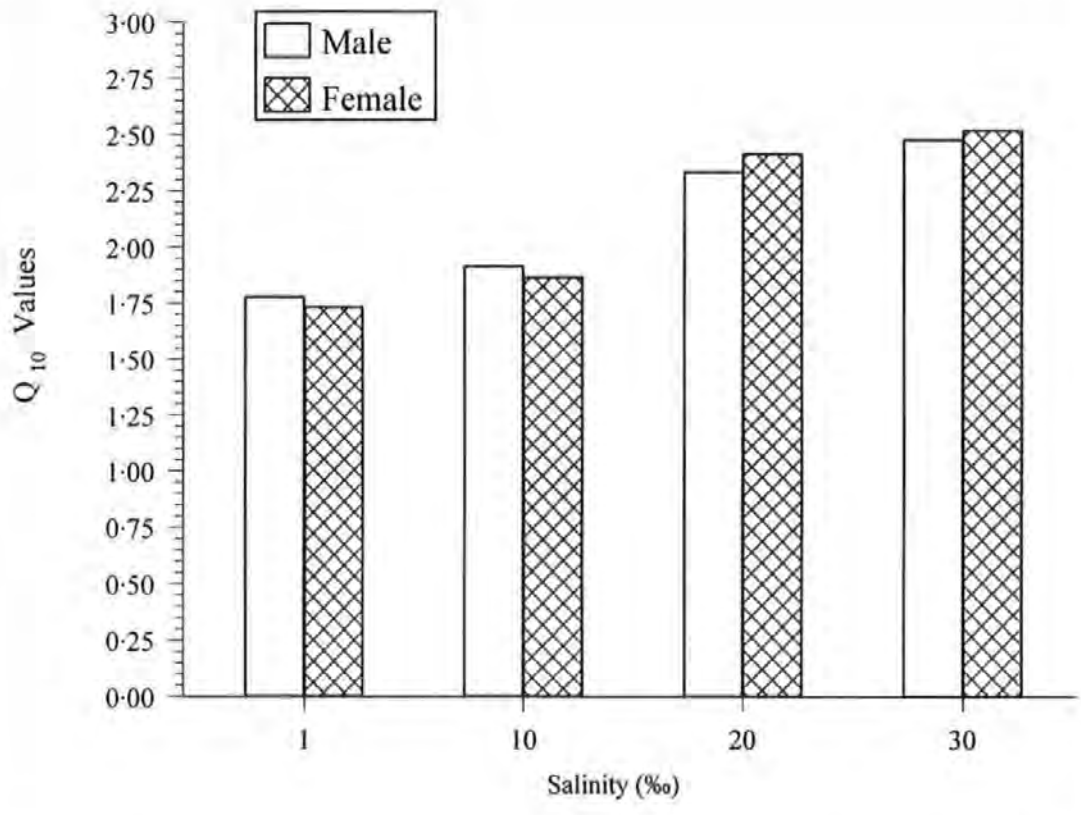


Figure 3.4: Temperature coefficients (Q_{10}) for the oxygen consumption of male and female *Neomysis integer* at different salinities. n=12 for each temperature/salinity combination.

3.4.4 Effect of salinity on oxygen consumption

At each temperature, there was a general trend of decreasing oxygen consumption with increasing salinity (Fig. 3.5). This salinity effect on oxygen consumption was significant for males and females [ANOVA, f=62 (male) and 109 (female), d.f.=3, $p<0{\cdot}01$; Table 3.6]. A highly significant interaction between salinity and temperature (see previous section), however, indicated a complex effect of these two variables on oxygen consumption. The change in oxygen consumption with changing salinity was greatest at 5°C, where oxygen consumption rates at 30‰ were *c.*30 and 35% lower (for males and females respectively) than at 1‰ (Table 3.3; Fig. 3.5). Consumption of oxygen at 5°C varied significantly between 1, 10 and 20‰, the largest change occurring between 10 and 20‰ (95% confidence intervals, $p<0{\cdot}05$; Fig 3.5). There was no significant difference in oxygen consumption between 20 and 30‰ (95% confidence intervals, $p>0{\cdot}05$; Fig. 3.5). This relationship between salinity and oxygen consumption at 5°C was the same for male and female mysids (Fig. 3.5). At 15°C, salinity had no effect on the oxygen consumption of male and female mysids (95% confidence intervals, $p>0{\cdot}05$; Fig. 3.5).

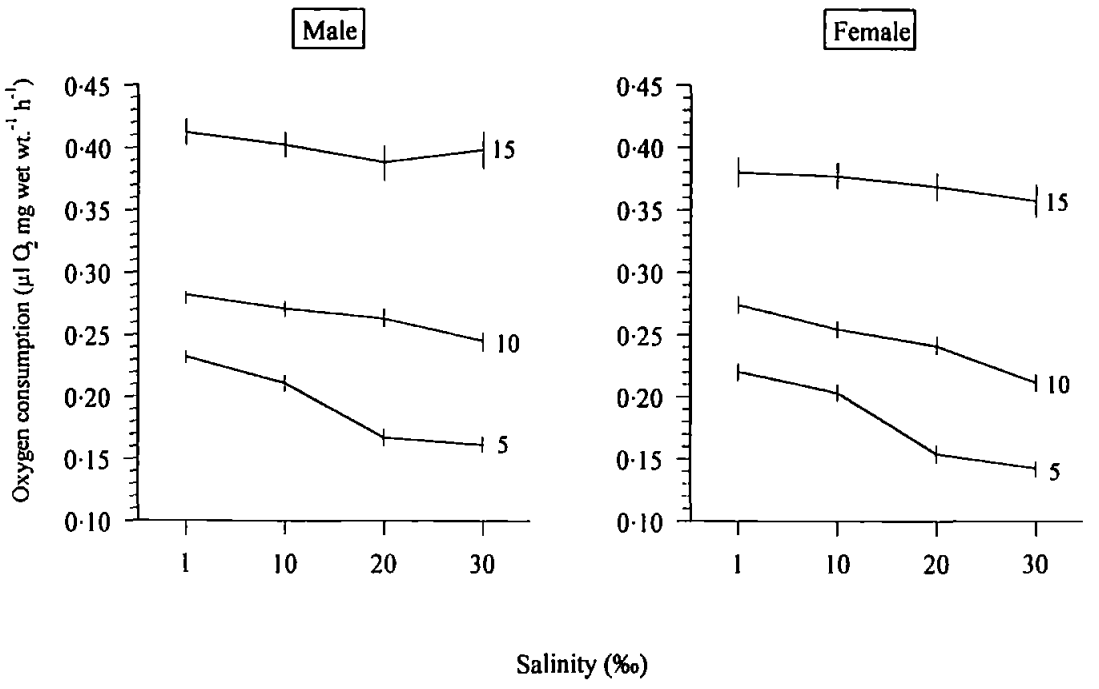


Figure 3.5: Effect of salinity on the mean oxygen consumption of male and female *Neomysis integer* at various temperatures. Numbers correspond to temperature (°C). n=12 for each temperature/salinity combination. Error bars correspond to 95% confidence intervals.

Effect of salinity on oxygen consumption at 10°C was intermediate between 5 and 15°C (see temperature effects). At 10°C, the difference in oxygen consumption at 10 and 20‰ was not significant, however, there were significant differences between the oxygen consumption at 1‰ and 30‰ (95% confidence intervals, $p<0{\cdot}05$; Fig. 3.5). Two-way ANOVA showed that there was no interaction between salinity and sex on oxygen consumption of *N. integer* (ANOVA, $f=0{\cdot}13$, d.f.=3, $p>0{\cdot}05$;Table 3.10).

Table 3.10: Two-way analysis of variance (ANOVA) comparing the effects of salinity and mysid sex on the oxygen consumption of *Neomysis integer*. n=12 for each temperature/salinity combination, critical $f=3{\cdot}78$ (salinity), 3·84 (sex) and 1·37 (interaction), n.s.=not significant ($p>0{\cdot}05$).

Source	d.f.	f statistic	p value	significance
salinity	3	4·23	$4{\cdot}0 \times 10^{-3}$	$p<0{\cdot}01$
sex	1	3·86	0·04	$p<0{\cdot}05$
interaction	3	0·13	0·95	n.s.

With increasing salinity, there was a decrease in the multiple linear regression intercept, indicating that oxygen consumption was negatively correlated with salinity (Table 3.11). As salinity increased, the temperature regression coefficient increased, indicating that temperature effects were greatest at high salinities. Temperature effects were similar at 1 and 10‰, and at 20 and 30‰ (Table 3.11). Effect of sex on oxygen consumption was variable, however, the coefficient was greatest at 30‰, indicating sex effects were most important at high salinity (Table 3.11).

Table 3.11: Multiple linear regression relationships for the effects of temperature and sex on the oxygen consumption of *Neomysis integer* at various salinities. n=12 for each temperature/salinity combination.

	Regression Coefficients				
Salinity (‰)	Temperature	Sex	Y-Intercept	Standard Error	r^2
1	0·0170	-0·0172	0·1386	0·0212	0·92
10	0·0183	-0·0168	0·1117	0·0220	0·93
20	0·0218	-0·0181	0·0548	0·0166	0·97
30	0·0226	-0·0308	0·0422	0·0237	0·94

3.4.5 Effect of sex on oxygen consumption

At all temperature/salinity combinations, female *Neomysis integer* had lower rates of oxygen consumption than males (Table 3.2; Fig. 3.6). At most combinations, these differences in oxygen consumption were significant (95% confidence intervals, $p<0{\cdot}05$; Fig. 3.6). There was a significant interaction between sex and temperature (ANOVA, $f=2{\cdot}99$, d.f.=2, $p<0{\cdot}05$; Table 3.7) but not between sex and salinity (ANOVA, $f=0{\cdot}13$, d.f.=3, $p>0{\cdot}05$; Table 3.10). Multiple linear regression equations for male and female mysid oxygen consumption confirmed that temperature effects were greatest for male mysids (indicated by the higher coefficient; Table 3.12) and that there was no interaction between the effects of salinity and mysid sex (indicated by similar coefficients; Table 3.12).

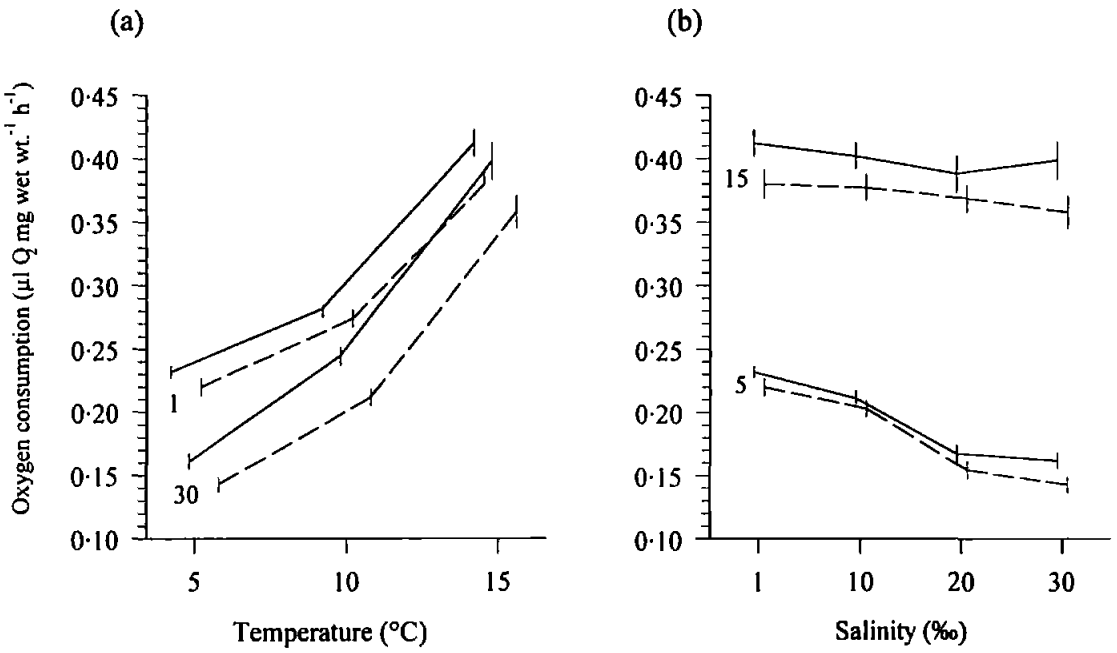


Figure 3.6: Effect of sex on the mean oxygen consumption of *Neomysis integer* at (a) different temperatures (numbers correspond to different salinities, ‰) and (b) different salinities (numbers correspond to temperatures, °C). Males = solid line, females = dotted line. n=12 for each temperature/salinity combination. Error bars correspond to 95% confidence intervals.

Table 3.12: Multiple linear regression relationships for the effects of temperature and salinity on the oxygen consumption of male and female *Neomysis integer*.n=12 for each temperature/salinity combination.

	Regression Coefficients				
Sex	Temperature	Salinity	*Y*-Intercept	Standard Error	r^2
Male	0·0207	-0·0015	0·1034	0·0234	0·93
Female	0·0191	-0·0019	0·1013	0·0224	0·93

3.5 DISCUSSION

The oxygen consumption rates recorded for *Neomysis integer* in the present study are similar to those reported previously for this and other mysid species (Table 3.13).

Table 3.13: Rates of oxygen consumption for various *Neomysis* species under various experimental conditions.

Species	Temp (°C)	Salinity (‰)	Sex	O_2 consumption (µl O_2/ mg wet wt. / h)	Reference
N. americana	5	seawater	-	0·26 - 0·38	Raymont & Conover (1961)
	15	"	-	0·40	Smith & Hargreaves (1984)
N. awatschensis	5	30	-	0·23	Jawed (1973)
	5	22·5	-	0·21	"
	10	30	-	0·37	"
	10	22·5	-	0·33	"
	15	30	-	0·32	"
	15	22·5	-	0·29	"
N. integer	10	9	female	0·15 - 0·46	Raymont *et al.* (1966)
	5	2	mixed	0·31	Vlasblom & Elgershuizen
	15	2	"	0·25	"
	5	16	"	0·56	"
	5	**1**	**male**	**0·23**	**This study**
	15	**1**	**male**	**0·41**	**"**
	5	**20**	**male**	**0·17**	**"**
	15	**20**	**male**	**0·39**	**"**
N. intermedia	5	freshwater	-	0·056	Toda *et al.* (1987)
	15	freshwater	-	0·12	"
	25	freshwater	-	0·28	"

Factors known to influence mysid oxygen consumption include temperature, salinity, weight, season and sex (Simmons & Knight, 1975). In the current study, the effects of weight and season on mysid oxygen consumption were not investigated. As all experiments were carried out during the spring, and all mysids used were of similar size, it is unlikely that variations in oxygen consumption due to season and weight occurred in these results. Absolute oxygen consumption values, however, may be specific to the season and weight of mysid used in the experiments. When comparing the present data with other oxygen

consumption rates in the literature, it should be remembered that the data presented here were recorded during the spring and that mysid wet weight was *c.* 10mg.

3.5.1 Effect of temperature

Temperature had a significant effect on the oxygen consumption of *Neomysis integer*. The Q_{10} values ranged from *c.* 1·8 to 2·5, depending on the experimental salinity (lower Q_{10} values occur at lower salinities). The three experimental temperatures (5, 10, and 15°C) were within the normal range of temperatures encountered by *N. integer* in the East Looe River Estuary (see Chapter 2). At low tide, *N. integer* concentrated at the periphery of the river, where salinity and temperatures were low (due to the freshwater input). Mysids migrated axially with the flood tide, so that at high tide they were in high salinity water of a higher temperature (due to the seawater input). Over a summer tidal cycle, for example, *N. integer* experienced temperature changes from *c.* 5°C to 15°C, and back to 5°C again. The data from the current study imply that the oxygen consumption of *N. integer* at Terras Bridge increased on the flood tide, peaked at high tide, and then decreased as the tide ebbed and the mysids were exposed to the cooler freshwater. High tide lasted for only *c.* 2h in the upper reaches of the East Looe River Estuary (Chapter 2) and, for most of the time (*c.* 18-20h day^{-1}), *N. integer* inhabited the cooler freshwater, implying that the oxygen consumption of *N. integer* was at the reduced rate. Water temperature varied seasonally, such that river and seawater temperatures peaked during the summer and were reduced during the winter. Oxygen consumption of *N. integer* would, therefore, be at a maximum rate during the summer.

The effects of temperature on the oxygen consumption of *N. integer* recorded in this investigation are similar to those recorded by other workers. Weisse & Rudstam (1989) found a rise in temperature (from 6 to 16°C) significantly increased the oxygen consumption of *N. integer*. *Neomysis integer* tolerated higher temperatures than *Praunus flexuosus*, although the respiratory rate of *N. integer* was greater than that of *P. flexuosus* at 5 and 15°C (Vlasblom & Elgershuizen, 1977). At 5°C (2‰), *N. integer* consumed 0·31µl O_2 mg wet $wt.^{-1}$ h^{-1} whilst at 15°C (2‰) the rate was 0·56µl O_2 mg wet $wt.^{-1}$ h^{-1}. These values are slightly higher than those recorded here (0·23 and 0·41µl O_2 mg wet $wt.^{-1}$ h^{-1} for male *N. integer* at 5 and 15°C respectively), although the relationship between temperature and oxygen consumption is similar, indicated by similar Q_{10} values (*c.* 1·8). Astthorsson

(1980) recorded increased oxygen consumption by *N. integer* with increasing temperature. For the winter generation of *N. integer,* oxygen consumption was *c.*0·10, 0·17 and 0·37µl O_2 mg wet wt.$^{-1}$ h^{-1} at 2, 9 and 16°C respectively (Astthorsson, 1980). The mysid population used in the present investigation was also a winter generation and the oxygen consumption values compare extremely well (Table 3.11). Raymont *et al.* (1966) found the oxygen consumption of *N. integer* from Southampton Water ranged between 0·15 and 0·46µl O_2 mg wet wt.$^{-1}$ h^{-1} at 10°C. These values again compare well with the data from this study (Table 3.11). Oxygen consumption varied according to time of year, with lowest rates in September and highest rates in March, even though the experimental temperature was kept constant at 10°C (Raymont *et al.*, 1966).

Several investigations have shown that the oxygen consumption of mysids varies with temperature (Mauchline, 1980). Oxygen consumption of *Neomysis mercedis* increased by up to *c.* 300% as temperature was increased from 6 to 21°C (Simmons & Knight, 1975). Toda *et al.* (1987) showed that the oxygen consumption of *N. intermedia* increased from 0·056µl O_2 mg wet wt.$^{-1}$ h^{-1} at 5°C to 0·28µl O_2 mg wet wt.$^{-1}$ h^{-1} at 25°C. This corresponds to a Q_{10} of 1·9 to 2·1 (Toda *et al.*, 1987), which agrees with the Q_{10} values calculated in the current study. Jawed (1973) showed that the oxygen consumption of *Archaeomysis grebnitzkii* and *Neomysis awatschensis* was temperature dependent. As temperature was increased from 5 to 15°C, the oxygen consumption of *A. grebnitzkii* increased from 0·18 to 0·49µl O_2 mg wet wt.$^{-1}$ h^{-1}, whilst that of *N. awatschensis* increased from 0·23 to 0·32µl O_2 mg wet wt.$^{-1}$ h^{-1} (Jawed, 1973). The Q_{10} values for *A. grebnitzkii* and *N. awatschensis* were 2·7 and 1·4 respectively. Increasing temperature led to increased oxygen consumption in *Americamysis bahia* (Modlin & Froelich, 1997). Oxygen consumption rates were *c.*350% higher at 24°C than at 20°C. This relationship was dependent upon salinity, the largest temperature effect was recorded at 26‰ (Modlin & Froelich, 1997). Oxygen consumption by *A. bahia* was similar to that reported for *N. integer*, however, the conclusion of Modlin & Froelich (1997) that the oxygen consumption of these two mysids was similar because of similar size is untrue. *Americamysis bahia* is approximately half the size of *N. integer,* with adult body lengths of *c.*8 and 17mm respectively (Mauchline & Murano, 1977). The experiments of Modlin & Froelich (1997) also revealed that *A. bahia* was highly acclimated to the culture conditions (20°C and 22‰). Oxygen consumption was lowest at this temperature/salinity combination (Modlin & Froelich, 1997). Oxygen consumption

rates of *Mysis relicta*, from two different environments (with mean annual temperatures of 0·9 and 5·3°C), were not significantly different over an experimental temperature range of 2 - 8°C (Lasenby & Langford, 1972). These data suggest that temperature acclimation did not occur in *M. relicta* (Lasenby & Langford, 1972).

3.5.2 Effect of salinity

The oxygen consumption of *N. integer* decreased with increasing salinity. This response is similar to that reported for other euryhaline invertebrates (Kinne, 1971). In the East Looe River Estuary, *N. integer* was exposed to diurnal changes in salinity from *c.* 1 to 30‰ over the tidal cycle (Chapter 2). The oxygen consumption data imply, therefore, that oxygen consumption decreases significantly at high tide. At Terras Bridge, however, high tide lasted for less than 2h, since the site flooded and ebbed extremely quickly (Chapter 2). Most of the time, *N. integer* was exposed to water of *c.* 1‰, and, therefore, was likely to have a higher rate of oxygen consumption, which would decrease to a minimum for the 2h duration of high tide. Seasonally-based fluctuations in salinity at Terras Bridge were not recorded (Chapter 2).

The effect of salinity on the oxygen consumption of *N. integer* in this study agrees with the results of other workers. Vlasblom & Elgershuizen (1977) reported that an increase in experimental salinity led to a decrease in the oxygen consumption of *N. integer*. At 5°C, oxygen consumption of *N. integer* was 0·31µl O_2 mg wet wt.$^{-1}$ h^{-1} at 2‰, and 0·25µl O_2 mg wet wt.$^{-1}$ h^{-1} at 16‰ (Vlasblom & Elgershuizen, 1977). Although the actual values were slightly lower in this study (Table 3.11), the general trend is the same. There is evidence from the literature that salinity affects the oxygen consumption of other mysid species (Mauchline, 1980). For example, Simmons & Knight (1975) showed that increasing salinity led to a general decrease in the oxygen consumption of *N. mercedis*. The effect of salinity on *N. integer* in the present study was, therefore, similar to that described by Simmons & Knight (1975) for *N. mercedis*. Both *Archaeomysis grebnitzkii* and *Neomysis awatschensis* showed an increase in oxygen consumption with increasing salinity (Jawed, 1973). In contrast, salinity had no significant overall effect on the oxygen consumption of *Americamysis bahia* (Modlin & Froelich, 1997).

There is frequently an interaction between the effects of salinity and temperature on mysid oxygen consumption rate (Jawed, 1973; Simmons & Knight, 1975; Vlasblom & Elgershuizen, 1977; Modlin & Froelich, 1997). A statistically significant temperature/salinity interaction was also apparent for *Neomysis integer* in this study. At 5°C, increasing the salinity from 1 to 30‰ led to a decrease of 45% in the oxygen consumption of *N. integer*. At 15°C, the decrease was only 5%. This relationship is contradictory to the hypothesis of Kinne (1971) that "supra-normal temperatures tend to increase salinity dependent variations in metabolic rate, while sub-normal temperatures frequently reduce them". Simmons & Knight (1975) found a temperature/salinity interaction, similar to that described here for *N. integer,* caused variation in oxygen consumption by *N. mercedis*. Accordingly, changes in oxygen consumption due to changing salinity were greatest at low temperatures (Simmons & Knight, 1975). Vlasblom & Elgershuizen (1977) found that at higher temperatures at all test salinities, the oxygen consumption of *Praunus flexuosus* frequently exceeded the rate recorded at the acclimation salinity (16‰). This relationship was not seen in *N. integer* which showed better tolerance to low salinities at high temperatures. It was concluded that *N. integer* was better adapted to environments of low salinity, particularly at high temperatures, than *P. flexuosus* (Vlasblom & Elgrshuizen, 1977). A relatively high tolerance to changes in salinity and temperature might be expected for *N. integer* at Terras Bridge, since this population is exposed to large fluctuations in temperature and salinity. In the present study, *N. integer* was more responsive to salinity change at low than high temperature, however, the reason for this interaction between salinity and temperature is unknown. As stated, the population at Terras Bridge spent most of the time in low salinity water and was only exposed to changes in salinity as the site flooded or ebbed. Given that the incoming seawater was always of a higher temperature than the out-flowing freshwater, the change in salinity on the flood tide occurred at slightly higher temperatures. If temperature and salinity effects on oxygen consumption are considered together, the data imply that changes in oxygen consumption as the site flooded were minimised. The opposite would occur on the ebb tide, when salinity decreased as the seawater withdrew and the mysids were exposed to the lower temperature of the freshwater. On a seasonal scale, the data indicate that changes in oxygen consumption due to salinity fluctuations are likely to be greatest in winter.

As with temperature, however, there is evidence that some mysid species may become acclimated to a certain salinity if maintained at that salinity for a period of time (e.g. Vlasblom & Elgershuizen, 1977; Modlin & Froelich, 1997). The relevance of such acclimation is unclear, as different species show different responses after acclimation (e.g. Vlasblom & Elgershuizen, 1977; Modlin & Froelich, 1997).

3.5.3 Effect of sex

Irrespective of temperature and salinity, the oxygen consumption of male and female *Neomysis integer* was significantly different, and there was a general trend that male mysids consumed more oxygen than females. Usually, differences in the metabolism between male and female organisms are related to physiological differences related to reproductive status (Simmons & Knight, 1975). Raymont *et al.* (1966) suggested that the investment of body lipids into egg production by female *N. integer* led to a decrease in the metabolism of ovigerous females. In the present study, only non-ovigerous females were used, however, they were mature and, since the study was carried out in early spring, it is possible that egg production was commencing although not visible by eye. This might explain the lower oxygen consumption of female *N. integer* compared with males. It has been suggested that the lower rate of oxygen consumption by female mysids is an artefact of the difference in weight between males and females of similar size due to the presence of the brood pouch (Smith & Hargreaves, 1984). The brood pouch is made of chitin and is not metabolically active tissue (Mauchline, 1980), however, it does contribute to the weight, therefore, reducing the weight specific oxygen consumption rates of female mysid when compared to males (Smith & Hargreaves, 1984). By the same argument, however, mature male mysids have an elongated 4^{th} pleopod which would contribute non-metabolically active tissue weight to male mysid overall weight. Although males were reported to consume more oxygen than females in the present study, both sexes responded in similar ways to changes in abiotic factors (i.e. temperature and salinity), indicating the importance of abiotic factors in the respiratory physiology of *Neomysis integer*.

3.6 SUMMARY

In summary, the oxygen consumption of *N. integer* was similar to that reported for other mysid species. The oxygen consumption of *Neomysis integer* was affected by temperature, salinity and sex according to the equation:

$$R = 0{\cdot}1334 + 0{\cdot}0199\,T - 0{\cdot}0017\,Sal - 0{\cdot}0207\,S$$

where: R = respiration rate (µl O_2 mg wet wt.$^{-1}$ h^{-1}), T = temperature (°C), Sal = salinity (‰), and S = score for sex (males = 0, females = 1). Oxygen consumption of *N. integer* was most susceptible to changes in temperature. Temperature interacted with salinity to modify the oxygen consumption of by *N. integer*. Temperature effects were most significant at high salinities and salinity effects were most significant at low temperatures. Male *N. integer* consumed oxygen at a higher rate, and were more susceptible to temperature changes, than females.

CHAPTER 4

EFFECT OF ABIOTIC (TEMPERATURE AND SALINITY) AND BIOTIC (SEX) FACTORS ON THE FEEDING RATE AND ABSORPTION EFFICIENCY OF *NEOMYSIS INTEGER*

4.1 ABSTRACT

In the East Looe River Estuary, Cornwall (UK) *Neomysis integer* (Peracarida: Mysidacea) experiences fluctuating temperatures and salinities due to diurnal and seasonal cycles. Although some mysids migrate to avoid unfavourable temperatures and salinities, the population at Terras Bridge, East Looe River Estuary remained permanently in areas exposed to temperatures of *c.*3 - 15°C, and salinities of *c.*1 - 34‰. In this study, the effect of temperature (5, 10 and 15°C) and salinity (1, 10, 20, 30‰) changes on the feeding rate of adult *N. integer* was measured over an 18h period. The rate of faecal production was used as a measure of feeding rate and detritus was used as a natural food source. Faecal production increased with increasing temperature and Q_{10} values ranged between *c.*1·9 and 2·3. In general, feeding rate increased with increasing salinity. For any given temperature/salinity combination, male and female mysid feeding rates were not significantly different. Temperature and salinity interacted significantly to modify the feeding rate of *N. integer*. At low temperatures, feeding rate increased with increasing salinity over the range 1 - 30‰, but at high temperatures (≥10°C), feeding rate was suppressed at 30‰. Changes in temperature had the greatest effect on mysid feeding rate at 10 and 20‰. Absorption efficiency of *N. integer* remained constant at *c.*0·35 for each temperature/salinity combination and it is assumed, therefore, that responses of digestive processes to temperature/salinity changes are similar to those recorded for feeding rate. The results are discussed in terms of possible changes in the feeding rate of natural *N. integer* populations in response to diurnal and seasonal fluctuations of these variables.

4.2 INTRODUCTION

Mysids inhabit a wide range of environments (Chapter 1) and encounter a wide diversity of food resources on which they feed readily (Mauchline, 1980). Due to this diversity of food types, large variation in the form of the mysid feeding appendages has developed. Although the functional role of many of the different types of feeding appendage are unknown, the forms have been comprehensively described by Mauchline (1980). Typically, members of the two orders of mysid (Lophogastrida and Mysida) employ two types of feeding method and either take large pieces of food or, more commonly, filter feed on small particles suspended in the water column (Tattersall & Tattersall, 1951). Large pieces of food are usually carrion (e.g. dead mysids). Using the filter feeding process, mysids may feed on either microscopic planktonic organisms (phyto- and zooplankton) or detrital organic matter (Tattersall & Tattersall, 1951; Mauchline 1980).

Large pieces of food are eaten frequently whilst the mysid is swimming. The food is held in the thoracic endopods just below the mouthparts, manipulated into position by the carpopodites of the thoracic limbs, and then lifted up to the mouthparts where the mandibular palps grip the food item and press it over the mandibles (Tattersall & Tattersall, 1951). Here, the incisor processes of the mandible, and the distal endites of the maxillules, bite pieces off the food mass and, because the mandibles are arranged asymmetrically, the food is passed automatically to the *laciniae mobiles* and finally to the molar processes. Here, the food is ground into smaller pieces before entering the mouth, where the asymmetrical mandibles interlock and pass the food to the dorsal molar processes. These molar processes grind the food into smaller pieces which are sucked into the oesophagus by a peristaltic action (Tattersall & Tattersall, 1951). In the laboratory, mysids are seen frequently to eat other mysids in this manner, carrying the dead mysid whilst swimming and feeding simultaneously (*pers. obs.*). When predatory feeding, there is a sudden concerted outstretching of the second to eighth pairs of endopods, which suck the prey into the capturing limbs [i.e. the second and third endopods (Metillo & Ritz, 1993)].

Filter feeding is probably the more common mode of feeding in mysids, and the complicated series of filtration and feeding movements were described in detail by Tattersall & Tattersall (1951). A brief summary of the general filter feeding process is presented here. The main food groove is a ventral tube which passes forward from the thoracic appendages, expanding into a food basin at the mouthparts. When swimming,

movement of the thoracic limbs creates a static area of water in the median ventral area close to the body and it is from this static area of water that the maxillae create an anteriorly-directed water current into the food groove by a 'suction pump' action. This main food stream is joined by water from subsidiary food streams from other limb movements, aided by the exhalent respiratory current (Chapter 3). A comb of setae on the maxillal endite filters food particles from the food stream, and the food is pushed into the mouth by setae of the maxilla and the first thoracic limbs. Here, the asymmetrical mandibles interlock and pass the food to the dorsal molar processes, where it is ground into smaller pieces and sucked into the oesophagus by peristalsis (Tattersall & Tattersall, 1951). The existence of either a forwardly or backwardly directed ventral filtration current in *Hemimysis lamornae* and *Praunus flexuosus* (and by inference other species), however, has been contested (Attramadal, 1981).

Of particular importance to this chapter is the method by which many mysids feed on detritus from the substratum. Many mysids are hyperbenthic, spending much of the time within a few centimetres of the substratum (Mees & Jones, 1997; Chapter 2). Occasionally, mysids make excursions into the substratum, where the continuous movements of the exopods stir up the substratum so that small detrital particles are re-suspended (Tattersall & Tattersall, 1951). This detritus is then filtered in the manner described above. Another type of sediment feeding has been reported for *Neomysis mercedis* which was found to prey on meiobenthic harpacticoid copepods (Johnston & Lasenby, 1982). In static environments, where food material drops rapidly out of suspension to collect on the substratum, mysids assume a position which can best be described as 'standing on their heads' on the substratum. In this position, the usual filter feeding movements of the thoracic limbs draw fine detritus particles off the substratum and into suspension, where the particles can be fed upon (Cannon & Manton, 1927). Furthermore, *Hemimysis lamornae* [the species studied by Cannon & Manton (1927)] was observed to swim along the substratum-water interface, inclined head-down at an angle of *c.*25°, ploughing up the substratum with its antennal scales. As *H. lamornae* swam along, the stirred-up detritus was collected by the thoracic endopods and passed to the mouth (Cannon & Manton, 1927). It was whilst observing *Neomysis integer* in the annular flume with a silt substratum (Chapter 2) that a similar feeding mechanism to that described for *H. lamornae* was noticed. In the absence of the usual *Artemia* nauplii for food, *N. integer* would readily settle into the substratum to feed on the detritus therein. *Neomysis integer* would swim relatively fast, and settle quickly on

the substratum so that it sank slightly into the mud. Here, repeated movements of the thoracic exopods stirred up the local substratum, allowing the mysid to sink further into the sediment such that approximately half the depth of the carapace was submerged. After a few seconds, the mysid would rise out of the substratum and resume swimming, having collected a 'ball' of sediment which it would carry in the thoracic endopods. As the mysid swam along, it (apparently) sorted or sieved the collected sediment, discarding unwanted matter. Suitable detritus sieved from the sediment was presumably passed to the mouth. This observation led to the use of sediment as a novel food source in the experiments described in this chapter.

In the natural environment, mysids feed on a wide range of food types, as indicated by stomach content analysis (Mauchline, 1980; Fockedey & Mees, 1997). As with many crustaceans, mysids macerate their food, making identification of stomach contents difficult (or impossible), and Mauchline (1980) listed 'detritus' in the stomachs of 80% of the species he reviewed. Tattersall & Tattersall (1951) described *Neomysis integer* primarily as a filter feeder, living on organic detritus and microscopic animals and plants; however, Mauchline (1980) reported detritus, algae, diatoms, rotifers, copepods, amphipods, other crustacea, carrion, terrigenous material, and insect material from the stomach of *N. integer*. Although the relative amounts of each food item was not specified, only the detritus, algae, diatoms, rotifers and copepods could be considered to be microscopic (and, therefore, fed upon by the filtering mode of feeding). Presumably, the other food categories would have been fed upon as described for large food items. Currently, *N. integer* is considered to be omnivorous (Mees & Jones, 1997). In the Elbe, Westerschelde and Gironde Estuaries (Europe), the diet of *N. integer* was dominated by calanoid copepods supplemented with rotifers and cladocerans (Fockedey & Mees, 1997). Amounts of phytoplankton and benthic organisms were negligible, but macrophytal detritus was very abundant in mysid stomachs. Unidentifiable, amorphous material, originating from suspended sediment flocs in the maximum turbidity zone, was also an abundant food item (Fockedey & Mees, 1997).

However, although there is a large amount of information in the literature on the diet of mysids (usually determined by stomach content analysis), there is a paucity of data on mysid feeding rates (Toda *et al.*, 1987; Webb *et al.*, 1987). In particular, there are very few data on the effects of environmental variables on mysid feeding. In general, the feeding

response of marine invertebrates (both in terms of feeding rate and food selection) varies with abiotic (e.g. light, temperature, salinity and food density) and biotic factors (e.g. size and sex) (Lucas, 1936; Kinne, 1963; 1964; 1970; 1971; Newell & Branch, 1980; Ladd Prosser & DeVillez, 1990).

4.2.1 Temperature effects

In general, the feeding rate of aquatic invertebrates increases with increasing temperature over the optimum temperature range, but often decreases suddenly near the upper lethal temperature limit as the organism suffers from heat coma (Kinne, 1963, 1970). Examples for molluscs include a significant increase in the clearance rate of *Phaeodactylum* with increasing temperatures by the filter-feeding gastropod *Crepidula fornicata* (Newell & Kofoed, 1977) and increased filtration at higher temperatures by the oyster *Ostrea edulis* (Newell *et al.*, 1977). Crustaceans also tend to exhibit feeding rate increases with increasing temperature. For example, barnacles increased their cirral activity with increasing temperature, although variance was extremely high at the higher test temperatures (Ritz & Foster, 1968). Kondzela & Shirley (1993) measured the feeding rate of juvenile Dungeness crabs (*Cancer magister*) at different temperatures and showed that the amount of mussel ingested increased from 2·5 to 10g mussel as temperature was increased from 5 to 15°C. These differences in feeding rate were considered to be due to deficiencies in the diet and temperature stress (Kondzela & Shirley, 1993).

Most investigations into the effect of temperature on the feeding rate of mysids have concentrated on freshwater species feeding on the cladoceran *Daphnia magna* (e.g. Cooper & Goldman, 1982; Chigbu & Sibley, 1994). In contrast to the results of other crustacean feeding experiments, mysid predation does not always appear to be affected by changes in temperature. Cooper & Goldman (1982), examining the predation rate of *Mysis relicta* on the calanoid copepods *Diaptomus sp.* and *Epischura sp.* at different temperatures, reported an increase in predation rate at low compared with high temperatures. The authors concluded, however, that the increased predation rate was an artefact of the slower escape response of *Diaptomus* at the lower temperature and that the actual feeding response of the mysid was unaffected by temperature changes (Cooper & Goldman, 1982). Predation by the freshwater mysid *Neomysis mercedis* was unaffected by small changes in temperature [e.g. 10 and 14°C (Chigbu & Sibley, 1994)]. In contrast, estuarine mysid species such as *Neomysis integer* (Astthorsson, 1980) and *N. intermedia* (Toda *et al.*, 1987) have been

reported to increase their predation rate at increased temperatures. The results of Cooper & Goldman (1982), however, highlight an inherent problem in using predation as a measure of feeding rate under variable environmental conditions. That is, any alteration of environmental parameters will affect both the predator and the prey. Interpretation of temperature effects on predation rate, therefore, is complicated (without determining the effects of temperature on the escape response of the prey, it is impossible to define whether temperature affects the predatory feeding rate). Non-predation based experiments have shown that mysid feeding rate increases with temperature. For example, when fed Tetramin powder (a food for aquarium fish), the feeding rate of *Leptomysis lingvura* increased by 100 - 200% as temperature was increased from 10 to 18°C (Gaudy *et al.*, 1991).

4.2.2 Food density effects

Another variable which has been shown to affect the predatory feeding of mysids is prey density. In general, predation rates increase with increasing prey density, and increase also with increasing mysid size and/or decreasing prey size (Fulton, 1982; Cooper & Goldman, 1982; Irvine *et al.*, 1993; Chigbu & Sibley, 1994). *Mysis relicta* consumed more small *Daphnia magna* at high prey densities, but increasing the density of large *Daphnia* did not affect mysid predation rate (Chigbu & Sibley, 1994). *Neomysis integer* consumed more prey (the copepod *Eurytemora*) at high prey densities and small prey (i.e. nauplii) were preyed upon more than large prey [i.e. copepodites and adults (Irvine *et al.*, 1993)]. Wooldridge & Webb (1988) described a linear relationship between the predation rate of the estuarine mysid *Rhopalophthalmus terranatilis* and the density of its prey (the mysid *Mesopodopsis slabberi). Neomysis integer* showed increased predation on *Artemia* nauplii with increasing prey density up to 400 nauplii 500 ml^{-1} (Astthorsson, 1980). In contrast, *Neomysis americana* and *Americamysis bigelowi* consumed less copepods at high compared with low prey densities (Fulton, 1982).

Filter-feeding, used frequently by many mysids (Mauchline, 1980), is also affected by food density. Filter feeding is most efficient at high food densities. Lucas (1936) reported that when *N. integer* was supplied with a diatom culture of *c.*1,000 *Nitzschia closterium* cells mm^{-3}, an average of >1 million (and a maximum of 6·5 million) cells were consumed $mysid^{-1}$ h^{-1}. In 3h, *Mesopodopsis slabberi* consumed up to 10 million cells of the diatom *Anaulus birostratus* at diatom densities of 350,000 cells ml^{-1}, five times the number

consumed at 50,000 cells ml^{-1} (Webb *et al.*, 1987). When presented with a phytoplankton mixture, *M. slabberi* fed selectively on the numerically dominant diatom (Webb *et al.*, 1987). In the Baltic Sea, the filtration rate of *Mysis mixta* is so efficient that, even at annual maximum zooplankton densities, *M. mixta* is food limited (Mohammadian *et al.*, 1997).

4.2.3 Salinity effects

Few investigations have included the effects of salinity on the feeding rate of aquatic invertebrates. From the limited literature, it appears that salinity has various effects on feeding rate. The feeding rate of the oligochaete *Nais elinguis* was unaltered by changes in salinity (Little, 1984); however, the feeding rate of the blue crab (*Callinectes sapidus*) was significantly lower at 25‰ than at 2·5‰ (Guerin & Stickle, 1995). Unfortunately, there are no literature data for the effects of salinity on mysid feeding rate. Although Toda *et al.* (1987) highlighted the effect of temperature on physiological processes of estuarine mysids, they did not study salinity effects. Morris (1971), however, investigated the biochemical and dietary relationships of *Neomysis integer* at different salinities and found no effect of salinity on lipid composition (although fatty-acid composition of mysids changed with environmental temperature).

4.2.4 Sex and weight effects

Differences in feeding rates have been reported for male and female mysids. Male *Mesopodopsis slabberi* ingested more cells of the diatom *Asterionella glacialis* than females (Webb *et al.*, 1987). At 10°C, female *Leptomysis lingvura* consumed approximately twice as much Tetramin fish food as males, although there was no difference between male and female feeding rate at 14°C, and males consumed more food at 18°C than females (Gaudy *et al.*, 1991). As with oxygen consumption (Chapter 3), feeding rate may vary according to mysid size, and the weight-specific ingestion rate of mysids is greater for small than for large individuals (Toda *et al.*, 1987). The advantages and disadvantages of the various methods for expressing weight-specific physiological processes have been addressed in Chapter 3 (Section 3.1.3).

4.2.5 Aim

The aim of this chapter was to investigate the effects of temperature and salinity on the feeding rate and absorption efficiency of *Neomysis integer*.

4.3 METHODS

4.3.1 Animal collection and maintenance

In May and June (1996), mysids were collected and maintained as described in Chapter 2 (Section 2.3.2). At the time of sampling, the population comprised mature males, ovigerous and non-ovigerous females, and juveniles of various sizes from the spring generation (Chapter 1).

4.3.2 Measurement of feeding rate

Although *Artemia* nauplii are used frequently as a food source for maintaining mysids in the laboratory (e.g. ASTM, 1990), and *Artemia* has been used by previous workers to measure feeding rate of *N. integer* (Astthorsson, 1980), *Artemia* are not representative of the natural *N. integer* diet (Fockedey & Mees, 1997). Quantitative measurements of feeding rate (e.g. clearance rates) of mysids fed on *Artemia* are difficult to establish because mysids are 'messy' eaters when feeding on *Artemia* and frequently do not consume the whole animal (T.D. Williams, *pers. comm.*). Detritus is a more representative natural food item of *N. integer* than *Artemia* (Mauchline, 1980, Fockedey & Mees, 1997) and, in the flume (Chapter 2), *N. integer* fed readily on detrital material from the muddy substratum (this Chapter, Section 4.2). Ingestion of detrital material from consolidated sediment is difficult to quantify since the volume ingested by each mysid is extremely small compared with the amount of initial sediment supplied (making gravimetric analysis difficult). Whilst feeding *Neomysis* with *Artemia* nauplii in the annular flume (Chapter 2), mysid faecal material was observed to collect on the mesh covering the intake of the Eheim filter. These *N. integer* faeces were relatively robust and withstood the action of gentle washing to remove them from the mesh. From these observations, it was concluded that collection of mysid faecal material was relatively easy. Measurement of faecal production has been used by previous workers to calculate crustacean feeding rate (e.g. Gaudy, 1974; Reeve *et al.*, 1977). The weight of faeces produced from mysids feeding from the sediment was, therefore, used as measure of feeding rate. Mysid egestion rates have been shown to be highly positively correlated with mysid ingestion rates (Murtaugh, 1984), allowing this approach.

Feeding rate was calculated by measuring the weight of faeces produced in 18h. Surface sediment (the top 10mm layer) was collected at low tide from the south-east side of Terras

Bridge, East Looe River Estuary (Chapter 2, Fig. 2.1; Sites 1a and 1b). Mysids swarmed in this area, and granulometric analysis showed the sediment in this part of the estuary consisted mainly of mud and silt (Chapter 2). Sediment was collected by scraping a plastic shovel across the surface of the mud. After removing large pieces of organic matter (such as decaying leaf litter), the sediment was placed in a 10L plastic bucket with low salinity water (*c.*1‰) from the estuary, returned to the laboratory and stored in the dark in a refrigerator (*c.*2°C). Immediately prior to each feeding experiment, the sediment was sieved through a 63μm sieve into a 20L plastic aquarium. Low salinity water (*c.*10‰, made by combining filtered seawater with double-distilled, de-ionised water) was used to wash the sediment particles through the sieve. After 60min, when most of the sediment particles had settled to the bottom of the aquarium, the water used whilst sieving was decanted off. The slurry of sediment (<63μm diameter size) left on the bottom of the aquarium was used as mysid food. The slurry was shaken and stirred vigorously to ensure all sediment particles were re-suspended. Approximately 100ml of the slurry was collected using a 50ml plastic syringe and injected into each of twelve 500ml plastic pots (*c.*110mm diameter × 70mm depth). The sediment slurry was shaken prior to filling each test vessel, ensuring that each had similar sized particles (if this was not done, by the time the sediment was drawn off to fill the 12th vessel, the larger, heavier sediment particles had already settled out of suspension). The sediment was left for another hour to settle out of suspension onto the floor of the test vessels. Water of the correct experimental salinity, made by combining filtered seawater (10μm) with aerated mains water, was decanted carefully into the twelve test vessels. Test salinities were 1, 10, 20 and 30‰ (± 1‰), giving three test chambers at each salinity. A 100mm diameter circle cut from a 'bubble wrap' packaging sheet was placed carefully on top of the sediment prior to the addition of the test water. Water was poured slowly into the centre of this disc, which floated on the rising water surface so that re-suspension of the consolidated sediment was kept to a minimum (and tended to only occur around the edge of the test chamber).

All experiments, run in a Sanyo MLR-350HT growth cabinet with programmable temperature and photoperiod, were carried out with a 16h light: 8h dark photoperiod with dawn and dusk sequence (i.e. gradual increases and decreases of light intensity). The effect of temperature on mysid feeding rate was investigated at three test temperatures (5, 10 and 15°C). The effect of the four salinities on mysid feeding rate was, therefore, investigated

simultaneously at each temperature. The test vessels were placed inside the growth cabinets and aerated for 2h prior to placing mysids into the experimental vessels. This period was sufficient to allow the water temperature to equilibrate with the cabinet temperature. Once at equilibrium, one adult mysid was placed into each test chamber and left for 18h (from 16·30h until 09·30h the following day). All mysids were mature adults of similar size (12 ±1mm measured from the front margin of the rostrum to the tip of the telson); ovigerous females were excluded. After 18h, the mysids were removed from the test chambers, placed in a freezer (*c.*-18°C) for 6h, and freeze-dried for 24h (removal of the mysids from the test chambers was made as quickly as possible to ensure that variation in the amount of time spent by mysids in the test chambers was kept to a minimum). Freeze-dried mysids were weighed (±0·01mg) using a Sartorius R200-D balance.

Following mysid removal, the water in the test chambers was shaken gently to re-suspend the sediment, taking care not to break up the faecal material. The resultant slurry was sieved through a 128µm sieve. This allowed all the loose sediment to pass through the sieve but retained the faeces on the mesh (faecal material from *N. integer* was *c.*1·5mm long and cylindrical). The faeces were washed gently by spraying with double-distilled, de-ionised water and rinsed from the sieve into labelled beakers. Faecal material was poured gently from the beaker onto pre-weighed, ashed Whatman GF/F filter papers, attached to a water suction pump to gently collect the faecal material. A summary of the experimental procedure is shown in Figure 4.1.

The filter papers were frozen for 6h, freeze-dried for 24h and re-weighed (± 0·01mg) to calculate the weight of faecal material. Even using extreme care when handling and processing, the Whatman GFF filter papers show significant changes in weight, presumably due to daily changes in humidity (Widdows & Salkeld, 1993), therefore, blank GFF filter papers were weighed at each weighing to correct for any weight change. All filter papers were kept in individually-labelled petri dishes, and freeze-dried mysids were stored in labelled Eppendorf tubes (with a hole pierced in the top to allow freeze-drying). Filter papers, freeze-dried mysids and dried faecal samples were stored in glass desiccators, replacing the silica gel regularly to ensure negligible humidity.

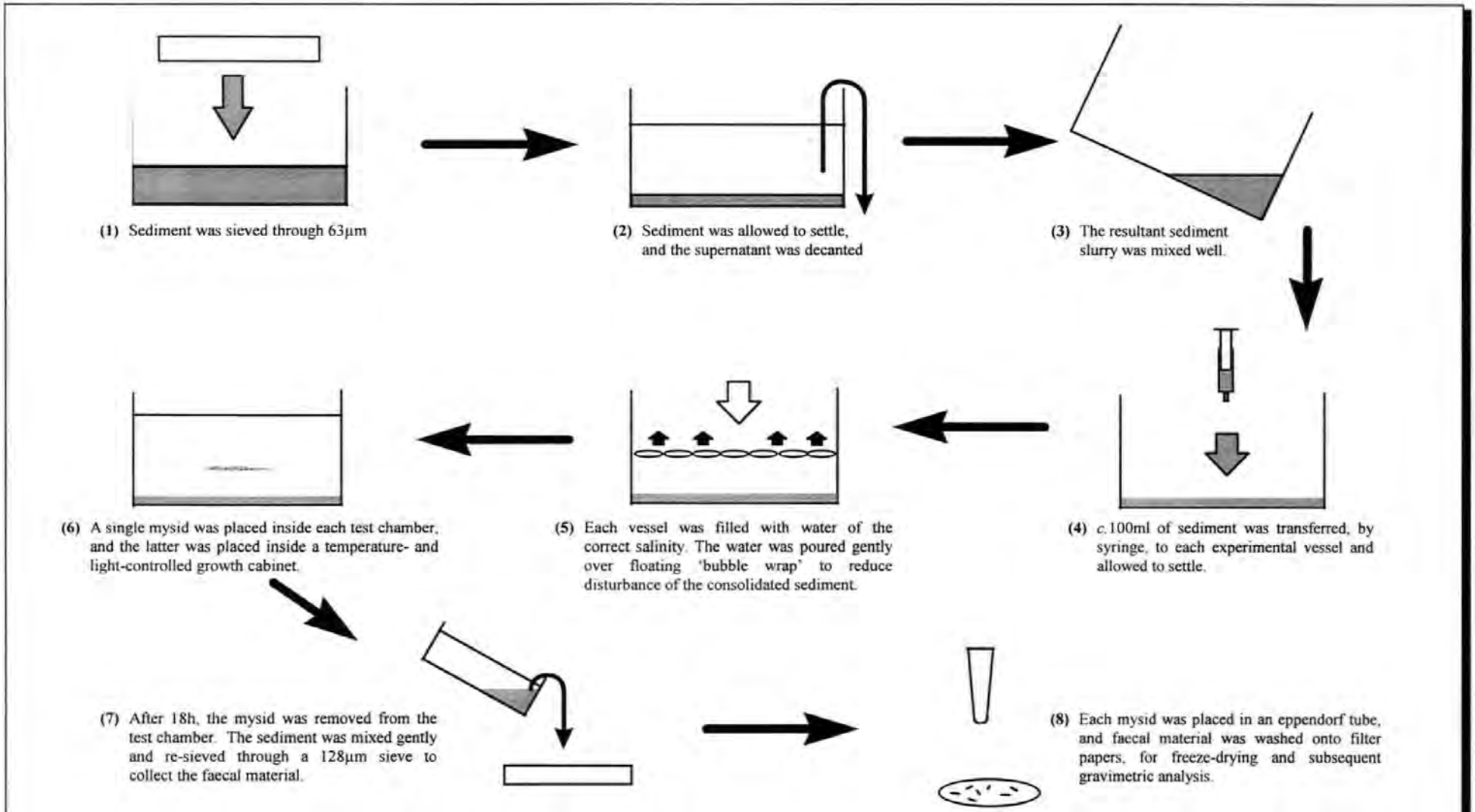


Figure 4.1: The experimental protocol for measurement of mysid faecal production.

4.3.3 Food absorption efficiency

Absorption efficiency was calculated by analysing the faecal material for organic carbon content. Dry faecal material was removed carefully from the filter papers and placed in pre-weighed, ashed aluminium-foil containers. Since the weight of the faecal material was small, and any weight changes due to ashing were likely to be extremely small, the 9 replicates at each temperature/salinity combination were combined. The foil containers (with faecal material) were re-ashed at 450°C for 2h to ensure that all the organic matter was combusted fully, and re-weighed (±0·01mg) using a Sartorius R200-D balance so that the ash content of the faeces was known. At all stages, blank foil containers were weighed to correct for any residual weight change.

The food absorption efficiency of *N. integer* was calculated according to the ash-ratio method of Conover (1966), which represents the efficiency with which organic material is absorbed from the ingested food material as:

$$\text{Absorption Efficiency} = (F - E) \div [(1 - E) \times F]$$

where F= ash-free dry weight: dry weight ratio of the food, and E = ash-free dry weight: dry weight ratio of the faeces.

4.3.4 Statistical treatment of results

To facilitate comparison of temperature, salinity and sex effects on mysid feeding rate with the same effects on oxygen consumption rates, statistical analyses of faecal production were identical to those applied to the oxygen consumption data (i.e. 95% confidence intervals, two-way ANOVA, multiple linear regression and Q_{10}; Chapter 3). However, since the faeces were pooled to calculate the absorption efficiency, resulting in only one measurement for each temperature/salinity combination, statistical analysis of absorption efficiencies was not possible.

4.4 RESULTS

The data presented in Table 4.1 report the effects of temperature, salinity and sex on the faecal production of *Neomysis integer*; and these data are displayed graphically in Figure 4.2.

Table 4.1: Mean faecal production rates for male and female *Neomysis integer* at various temperature/salinity combinations. n=9 for each temperature/salinity combination.

Sex	Temperature (°C)	Salinity (‰)	Faecal Production (mg / mg dry wt / h)	95% Confidence Intervals minimum	maximum
Male	5	1	0·0169	0·0150	0·0188
		10	0·0188	0·0168	0·0208
		20	0·0226	0·0200	0·0251
		30	0·0237	0·0214	0·0260
	10	1	0·0277	0·0255	0·0299
		10	0·0328	0·0303	0·0354
		20	0·0383	0·0359	0·0407
		30	0·0324	0·0296	0·0353
	15	1	0·0356	0·0325	0·0386
		10	0·0441	0·0411	0·0472
		20	0·0490	0·0456	0·0420
		30	0·0452	0·0420	0·0484
Female	5	1	0·0172	0·0154	0·0190
		10	0·0191	0·0174	0·0208
		20	0·0224	0·0202	0·0246
		30	0·0242	0·0217	0·0267
	10	1	0·0278	0·0252	0·0304
		10	0·0335	0·0305	0·0364
		20	0·0392	0·0366	0·0417
		30	0·0325	0·0294	0·0356
	15	1	0·0323	0·0294	0·0353
		10	0·0394	0·0366	0·0423
		20	0·0467	0·0434	0·0495
		30	0·0463	0·0432	0·0493

Increase in temperature increased the weight of faecal material produced per hour by *N. integer* (Table 4.1; Fig. 4.2). Increasing the salinity also tended to increase faecal production, although not at the highest salinity [30‰ (Table 4.1; Fig. 4.2)]. Male and female mysids had similar rates of faecal production (Table 4.1; Fig. 4.2).

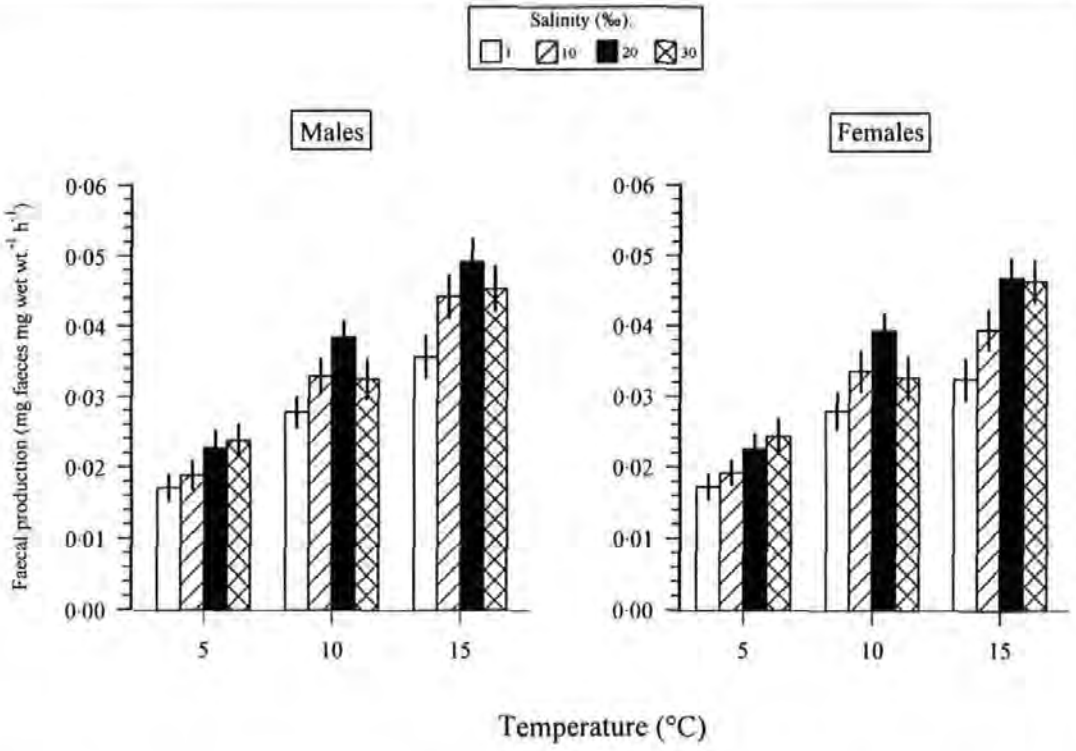


Figure 4.2: Effect of temperature and salinity on the mean faecal production of male and female *Neomysis integer*. n=9 for each temperature/salinity combination. Error bars correspond to ± 95% confidence intervals.

4.4.1 Combined effects of temperature, salinity and sex on faecal production

Multiple linear regression analysis showed that faecal production of *N. integer* was modified by temperature, salinity and sex according to the equation:

$$F = 0{\cdot}0061 + 0{\cdot}0022\,T + 0{\cdot}0003\,Sal - 0{\cdot}0005\,S$$

where F = faecal production (mg faeces mg dry wt.$^{-1}$ h^{-1}), T = temperature (°C), Sal = salinity (‰), and S = score for sex (males = 0, females = 1). The regression statistics are given in Table 4.2. Faecal production was positively correlated with temperature and salinity (indicated by positive coefficient values). Faecal production increased significantly with increasing temperature and salinity (linear regression, t=29·7 (temperature) and 10·5 (salinity), d.f.=2 (temperature) and 3 (salinity), $p<0{\cdot}01$; Table 4.2), with no significant difference between males and females (linear regression, t=-0·91, d.f.=1, $p>0{\cdot}05$; Table 4.2).

Table 4.2: Statistical values for multiple linear regression analysis for effects of temperature, salinity and sex on the faecal production of *Neomysis integer*. n=9 for each temperature/salinity combination, r^2=0·90, f=331, d.f.=3, p<0·01.

Variable	Coefficients	Standard Error	d.f.	t - statistic	p value	95% Confidence Interval	
						Lower	Upper
y intercept	0·0061	0·0009	3	6·47	$6·4 \times 10^{-10}$	0·0042	0·0091
temperature	0·0022	0·0001	2	29·7	$3·2 \times 10^{-52}$	0·0020	0·0023
salinity	0·0003	0·0006	4	10·5	$8·4 \times 10^{-19}$	0·0002	0·0003
sex	-0·0005	0·0000	1	-0·91	0·36	-0·0017	0·0006

Temperature and salinity interacted significantly to affect mysid faecal production (ANOVA, f=8·34, d.f.=6, p<0·01; Table 4.3). There were no significant interactions between temperature and sex (ANOVA, f=1·45, d.f.=2, p>0.05; Table 4.3), or salinity and sex (ANOVA, f=0·09, d.f.=3, p>0·05; Table 4.3) on faecal production.

Table 4.3: Two-way analysis of variance (ANOVA) testing for interactions between the effects of temperature, salinity and sex on the faecal production of *Neomysis integer*. n=9 for each temperature/salinity combination; n.s.=not significant (p>0·05).

Interaction	d.f.	f statistic	critical f	significance
temperature + salinity	6	8·34	2·80	p<0·01
sex + temperature	2	1·45	3·03	n.s.
sex + salinity	3	0·09	2·65	n.s.

Having defined the overall relationship between temperature, salinity and sex on mysid faecal production, the effects of individual factors, and the various interactions, will be described in more detail.

4.4.2 Effect of temperature on faecal production

For male and female *N. integer* at each experimental salinity, faecal production increased with increasing temperature (Fig. 4.3). Production of faeces increased significantly as temperature was increased from 5 to 15°C [ANOVA, f=401 (male) and 328 (female), d.f.=2, p<0·01; Table 4.4]. In general, the increase in faecal production was greater between 5 and 10°C than between 10 and 15°C, indicated by the steeper gradient between the lower two temperatures (Fig. 4.3).

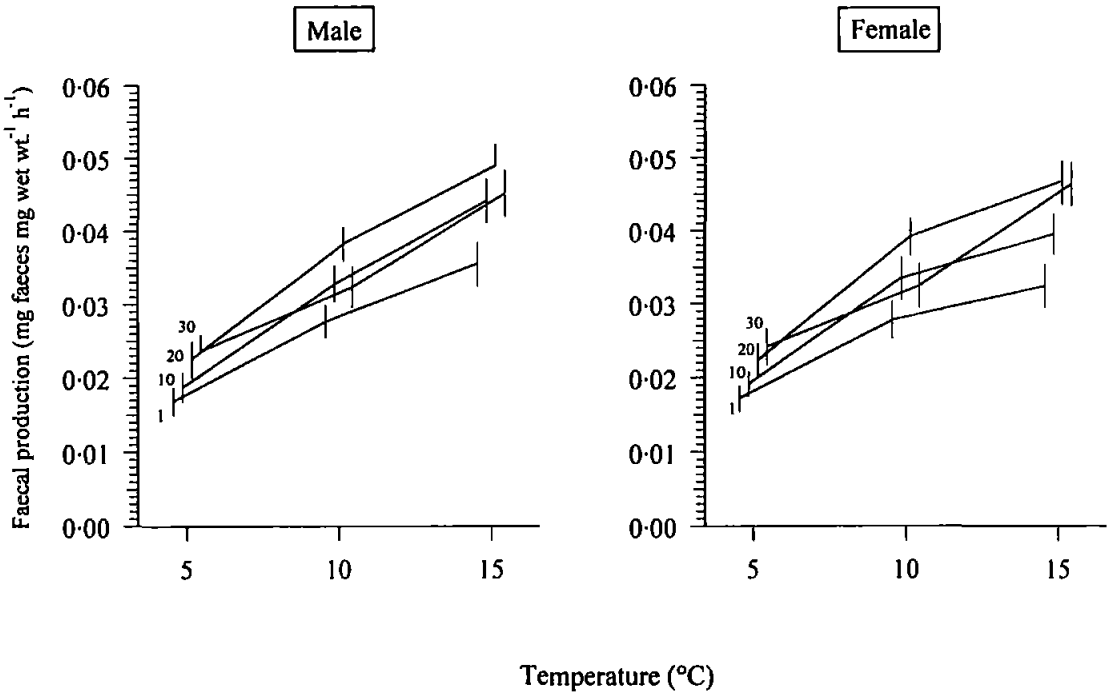


Figure 4.3: Effect of temperature on the mean faecal production of male and female *Neomysis integer* at various salinities. Numbers correspond to salinity (‰). n=9 for each temperature/salinity combination. Error bars correspond to 95% confidence intervals.

Table 4.4: Two-way analysis of variance (ANOVA) comparing the effects of temperature and salinity on the faecal production of *Neomysis integer*. n=9 for each temperature/salinity combination, critical f=3·09 (temperature), 2·70 (salinity) and 2·19 (interaction).

Sex	Source	d.f.	f statistic	p value	significance
Male	temperature	2	399	3×10^{-47}	p<0·01
	salinity	3	39·3	$1{\cdot}3 \times 10^{-14}$	p<0·01
	interaction	6	3·42	$4{\cdot}2 \times 10^{-3}$	p<0·01
Female	temperature	2	328	$1{\cdot}1 \times 10^{-43}$	p<0·01
	salinity	3	47·9	$4{\cdot}5 \times 10^{-19}$	p<0·01
	interaction	6	5·94	$2{\cdot}5 \times 10^{-5}$	p<0·01

Temperature and salinity interacted significantly to affect the faecal production of male and female *N. integer* [ANOVA, f=3·42 (males) and 5·94 (females), d.f.=6, p<0·01; Table 4.4]. The effect of the temperature/salinity interaction can be seen clearly in Figure 4.3 (by the lines not running parallel as temperature is increased). At 5°C, faecal production of male and female mysids was significantly lower at 1‰ than at 20 and 30‰ (95% confidence intervals, p<0.05; Fig. 4.3), but not at 10‰ (95% confidence intervals, p>0·05; Fig. 4.3). At 15°C, however, faecal production at 1‰ was significantly lower than at all other salinities (Fig. 4.3). In addition, at 15°C, females showed significantly lower faecal production at 10‰ than at either 20 or 30‰ (95% confidence intervals, p<0.05; Fig. 4.3). At 10°C, there was no obvious trend for salinity effects on faecal production, however, increasing salinity from 1 to 20‰ caused significant increases in faecal production; faecal production at 30‰ was decreased and was significantly lower than at 20‰ (95% confidence intervals, p<0·05; Fig. 4.3). At 15°C, the faecal production of mysids at 30‰ was not significantly different from that at 20‰ (95% confidence intervals, p>0·05; Fig. 4.3).

There was no significant interaction between temperature and sex on mysid feeding rate (ANOVA, f=1·45, d.f.=2, p>0·05; Table 4.5).

Table 4.5: Two-way analysis of variance (ANOVA) comparing the effects of temperature and mysid sex on the faecal production of *Neomysis integer*. n=9 for each temperature/salinity combination, critical f=3·03 (temperature), 3·89 (sex) and 3·03 (interaction), n.s.=not significant (p>0·05).

Source	d.f.	f statistic	p value	significance
temperature	2	299	$3·8 \times 10^{-62}$	p<0·00
sex	1	0·56	10·46	n.s.
interaction	2	1·45	0·24	n.s.

Multiple linear regression analysis for effects of salinity and sex on mysid faecal production at 5, 10 and 15°C cannot clarify the temperature and salinity interaction further because the overall relationship for salinity and sex effects on faecal production at each temperature is non-linear, as indicated by the low r^2 values (0·50, 0·16 and 0·49 at 5, 10 and 15°C respectively; Table 4.6). The regression equations for each temperature are shown in Table 4.6.

Table 4.6: Multiple linear regression relationships for the effects of salinity and sex on the faecal production of *Neomysis integer* at various temperatures. n=9 for each temperature/salinity combination.

Temperature (°C)	Regression Coefficients		*Y*-Intercept	Standard Error	r^2
	Salinity	Sex			
5	0·0002	0·0002	0·0167	0·0027	0·50
10	0·0002	0·0004	0·0298	0·0047	0·16
15	0·0004	-0·0023	0·0370	0·0048	0·49

The temperature coefficients for feeding tended to increase from 1‰ to maxima at 10 or 20‰, and decreased from 20 to 30‰ (Table 4.7; Fig. 4.4). Coefficients were larger for male than female mysids, except at 30‰ when they were identical. Although these data imply, therefore, that males were more sensitive to temperature change than females, there was no significant interaction between temperature and sex (ANOVA, f=1·45, d.f.=2, p>0·05; Table 4.5).

Table 4.7: Temperature coefficients (Q_{10}) for the faecal production of male and female *Neomysis integer* at various salinities. n=9 for each sex at each temperature/salinity combination.

Sex	Salinity (‰)	Q_{10} Value
Male	1	2·10
	10	2·35
	20	2·17
	30	1·91
Female	1	1·88
	10	2·06
	20	2·09
	30	1·91

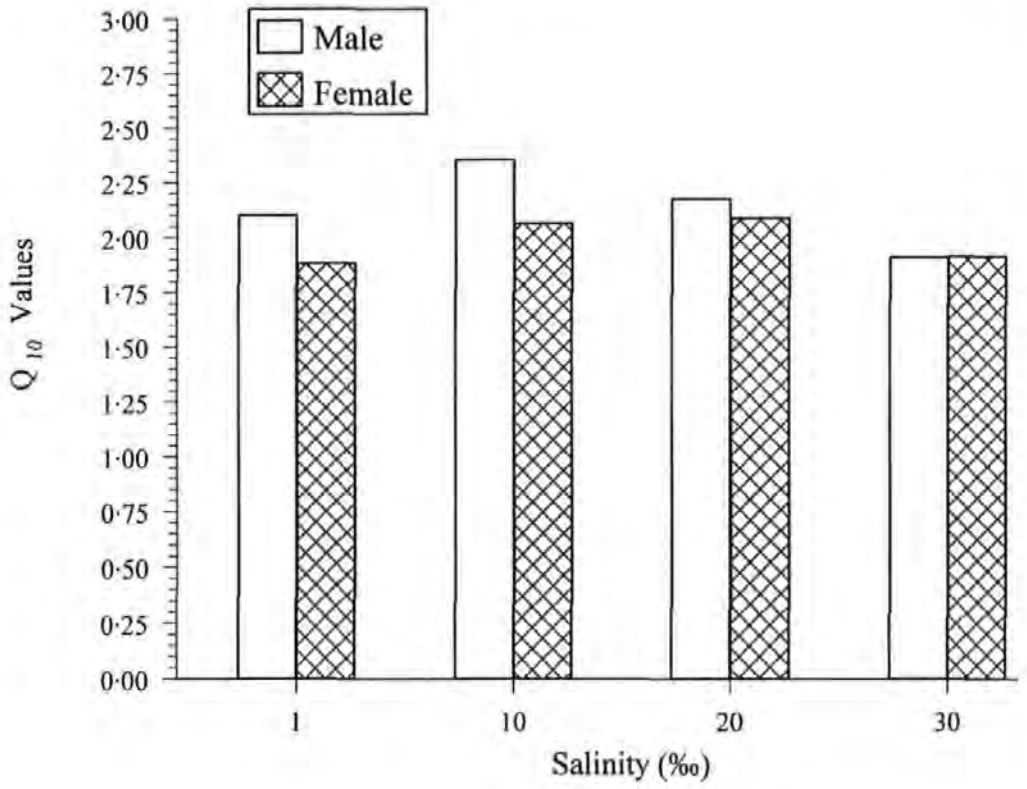


Figure 4.4: Temperature coefficients (Q_{10}) for the faecal production of male and female *Neomysis integer* at various salinities. n=9 for each sex at each temperature/salinity combination.

4.4.3 Effect of salinity on faecal production

Salinity had a significant effect on the faecal production of male and female *N. integer* [ANOVA, f=39·3 (male) and 47·9 (female), d.f.=3, $p<0·01$; Table 4.4]. In general, at each temperature, faecal production increased with increasing salinity from 1 to 20‰ (Fig. 4.5). A variable response was recorded at 30‰, when feeding rate increased at low temperature but decreased at high temperatures (Fig. 4.5). The effect of salinity on faecal production was not simple as indicated by a significant interaction between salinity and temperature (see previous section; Table 4.3). At 5°C, faecal production of male and female *N. integer* increased as salinity increased from 1 to 30‰; however, significant differences in faecal production (95% confidence intervals, $p<0·05$; Fig 4.5) occurred only when salinities differed by ≥20‰ (95% confidence intervals, $p<0·05$; Fig. 4.5). At 10 and 15°C, faecal production also increased significantly with increasing salinity up to 20‰ (95% confidence intervals, $p<0·05$; Fig. 4.5). At 30‰, however, faecal production was lower than at 20‰

[although only significantly at 10°C (95% confidence intervals, $p<0{\cdot}05$; Fig. 4.5)]. There was no significant interaction between salinity and sex affecting faecal production by *N. integer* (ANOVA, $f=1{\cdot}37$, d.f.=3, $p>0{\cdot}05$;Table 4.8).

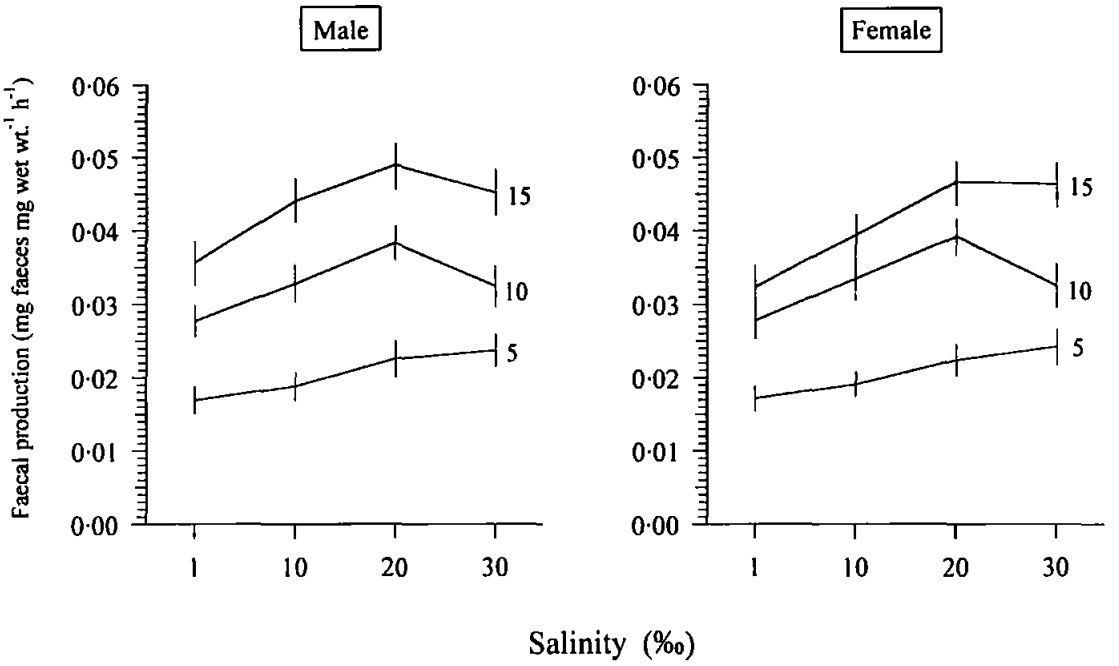


Figure 4.5: Effect of salinity on the mean faecal production of male and female *Neomysis integer* at various temperatures. Numbers correspond to temperature (°C). n=9 for each temperature/salinity combination. Error bars correspond to 95% confidence intervals.

Table 4.8: Two-way analysis of variance (ANOVA) comparing the effects of salinity and mysid sex on the faecal production of *Neomysis integer*. n=9 for each temperature/salinity combination, critical f=2·65 (salinity), 3·89 (sex) and 2·65 (interaction), n.s.=not significant ($p>0{\cdot}05$).

Source	d.f.	f statistic	critical f	significance
salinity	3	10·5	$1{\cdot}7 \times 10^{-6}$	$p<0{\cdot}01$
sex	1	0·17	0·68	n.s.
interaction	3	0·09	0·97	n.s.

Multiple linear regression analysis confirmed that temperature interacted with salinity (Table 4.9). Faecal production was least affected by temperature at 1‰ and most affected at 20‰, indicated by the magnitude of the temperature coefficients (Table 4.9). Temperature effects at 10, and 30‰ were similar. Regression coefficients for changes in

salinity with male and female mysids were not significantly different from zero ($t \leq \pm 1{\cdot}22$, d.f.=1, $p>0{\cdot}05$), indicating no effect of sex on mysid faecal production.

Table 4.9: Multiple linear regression relationships for the effects of temperature and sex on the faecal production of *Neomysis integer* at various salinities. n=9 for each sex at each temperature/salinity combination.

	Regression Coefficients				
Salinity (‰)	Temperature	Sex	*Y*-Intercept	Standard Error	r^2
1	0.0017	-0.0010	0.0098	0.0034	0.91
10	0.0023	-0.0012	0.0091	0.0037	0.93
20	0.0025	-0.0005	0.0112	0.0038	0.94
30	0.0022	0.0006	0.0120	0.0038	0.93

4.4.4 Effect of sex on faecal production

At each temperature/salinity combination, there were no significant differences between male and female faecal production (95% confidence intervals, $p>0{\cdot}05$; Table 4.2; Fig 4.6). Similar coefficients for multiple linear regression analysis of male and female faecal production rates at different temperatures and salinities confirmed this finding (Table 4.10).

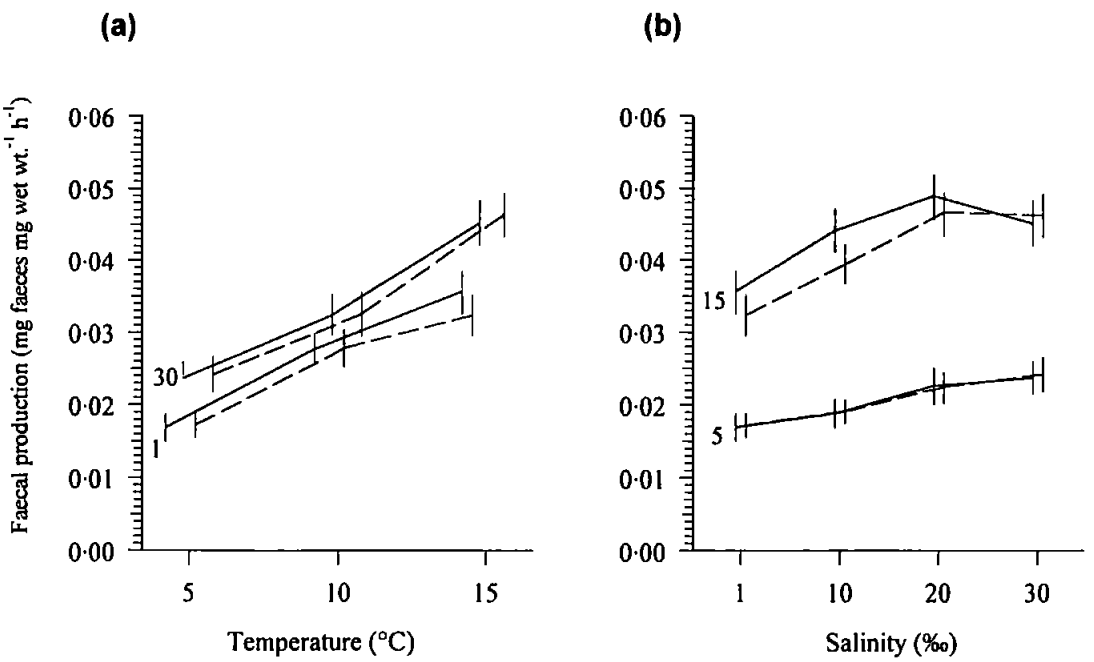


Figure 4.6: Effect of sex on the mean faecal production of *Neomysis integer* at (a) different temperatures (numbers = ‰), and (b) different salinities (numbers = °C). Males = solid line, females = dotted line. n=9 for each temperature/salinity combination. Error bars correspond to 95% confidence intervals.

Table 4.10: Multiple linear regression relationships for the effects of temperature and salinity on the faecal production of male and female *Neomysis integer*.n=9 for each temperature/salinity combination.

Sex	Regression Coefficients		*Y*-Intercept	Standard Error	r^2
	Temperature	Salinity			
Male	0·0023	0·0003	0·0053	0·0043	0·92
Female	0·0020	0·0003	0·0064	0·0044	0·90

4.4.5 Absorption efficiency

Absorption efficiency was extremely consistent in all experiments (Table 4.11). Due to the extremely small weight changes due to ashing, all material for each treatment was pooled. Each value, therefore, is a mean of 9 replicates, making statistical analysis of these results impossible. At each temperature/salinity combination, the absorption efficiency of male and female *Neomysis integer* was *c.*0·35.

Table 4.11: Mean absorption efficiencies, calculated by the ratio method (Conover, 1966), for *Neomysis integer*. n=9 for each temperature/salinity combination. (*F* and *E* = ash-free dry-weight : dry-weight ratios of food and faeces respectively).

Sex	Temperature (°C)	Salinity (‰)	Food value (*F*)	Faeces value (*E*)	Absorption efficiency
Male	5	1	0·920	0·883	0·344
		10	0·911	0·870	0·346
		20	0·921	0·867	0·343
		30	0·909	0·884	0·346
	10	1	0·921	0·885	0·343
		10	0·917	0·878	0·349
		20	0·918	0·880	0·345
		30	0·918	0·881	0·341
	15	1	0·916	0·878	0·342
		10	0·909	0·868	0·342
		20	0·917	0·879	0·344
		30	0·920	0·882	0·350
Female	5	1	0·918	0·879	0·352
		10	0·919	0·882	0·343
		20	0·917	0·878	0·349
		30	0·918	0·881	0·340
	10	1	0·920	0·883	0·342
		10	0·921	0·885	0·341
		20	0·918	0·881	0·340
		30	0·918	0·880	0·344
	15	1	0·917	0·879	0·344
		10	0·918	0·881	0·342
		20	0·919	0·880	0·354
		30	0·917	0·879	0·342

4.5 DISCUSSION

Due to the paucity of published data on the feeding rates of mysids, it is not possible to compare the feeding data from this study with those from other investigations. Most of the limited publications reporting on factors affecting the clearance rates of predatory mysids, are for freshwater species (e.g. *Mysis relicta* or *Neomysis mercedis*) feeding on *Daphnia* or copepods (e.g. Cooper & Goldman, 1982; Chigbu & Sibley, 1994). The mechanism of predatory feeding by mysids is very different from the mechanism of feeding on detritus by *Neomysis integer* in the current study (Tattersall & Tattersall, 1951). Previous workers have used faecal production to study mysid diet or to measure mysid feeding rates (e.g. Murtaugh, 1984; Gaudy *et al.*, 1991; Carola *et al.*, 1993). In the present study, the experimental protocol worked well and, although the high variability of crustacean gut residence times has implications on the results, mysid egestion rates have been shown to be highly positively correlated with mysid ingestion rates (Murtaugh, 1984). The rate of faecal production of *Neomysis integer* is, therefore, considered here to be proportional to the feeding rate of *N. integer*. A comparison of some mysid feeding rates is shown in Table 4.12.

4.5.1 Effect of temperature on feeding rate

In the current study, feeding rate increased with increasing experimental temperature (response type was the same at all salinities, and male and female mysids responded in the same way). In his review of the effects of temperature on aquatic invertebrates, Kinne (1970) stated that feeding rates tended to increase with increasing temperature over most of the species-specific temperature tolerance range. The data from the present study support this statement. Newell & Branch (1980) defined three types of temperature-dependent metabolic response in aquatic invertebrates. With changes in environmental temperature, an organism will make adjustment to its feeding rate and metabolic expenditure (type 1), or make adjustment to its feeding rate but no adjustment of its metabolic expenditure (type 2). The third type of response is to make no adjustment to its feeding rate, but to compensate metabolic expenditure (type 3). In the present study, the response of *N. integer* agrees with response types 1 and 2 as defined by Newell & Branch (1980). Further definition of response [i.e. whether *N. integer* exhibits a type 1 or type 2 response according to Newell & Branch (1980)] is not possible without considering the total metabolic energy

expenditure. Effect of temperature on metabolic expenditure has been addressed partly in Chapter 3 (using oxygen consumption as a measure of respiration).

Table 4.12: Feeding rates of mysids under different experimental conditions.

Species	Food	Condition / Variable	Feeding Rate	Reference
Americamysis bigelowi	*Acartia tonsa*	5mm long mysid	14 copepods mysid^{-1} d^{-1}	Fulton (1982)
		10mm long mysid	20 copepods mysid^{-1} d^{-1}	
Mesopodopsis slabberi	*Anaulus birostatus*	20, 000 cells ml^{-1}	65, 000 cells mysid^{-1} h^{-1}	Webb *et al.* (1987)
		350, 000 cells ml^{-1}	5·6 million mysid^{-1} h^{-1}	
		males	1·8 million cells mysid^{-1} h^{-1}	
		females	2·8 million cells mysid^{-1} h^{-1}	
Neomysis americana	*A. tonsa*	5mm long mysid	8 copepods mysid^{-1} d^{-1}	Fulton (1982)
		10mm long mysid	24 copepods mysid^{-1} d^{-1}	
N. integer	*Nitzschia closterium*	≥ 2000 cells ml^{-1}	1·3 million cells mysid^{-1} h^{-1}	Lucas (1936)
		1000 - 2000 cells ml^{-1}	0·7 million cells mysid^{-1} h^{-1}	
		> 500 cells ml^{-1}	0·04 million cells mysid^{-1} h^{-1}	
	Eurytemora affinis	9·0mm long mysid	38 nauplii mysid^{-1} h^{-1}	Irvine *et al.* (1993)
		9·4mm long mysid	26 nauplii mysid^{-1} h^{-1}	
		9·0mm long mysid	53 copepodites mysid^{-1} h^{-1}	
		9·4mm long mysid	34 copepodites mysid^{-1} h^{-}	
	Daphnia magna	8·9mm long mysid	15 *Daphnia* mysid^{-1} h^{-1}	
		9·5mm long mysid	4·4 *Daphnia* mysid^{-1} h^{-1}	
	sediment	**5°C (10‰, males)**	**0·019mg faeces mg dry wt.$^{-1}$ h^{-1}**	**This study**
		15°C (10‰, males)	**0·044mg faeces mg dry wt.$^{-1}$ h^{-1}**	
		1‰ (10°C, males)	**0.028mg faeces mg dry wt.$^{-1}$ h^{-1}**	
		30‰ (10°C, males)	**0.032mg faeces mg dry wt.$^{-1}$ h^{-1}**	
N. mercedis	*D. magna*	10°C	29 *Daphnia* mysid^{-1} 12h^{-1}	Chigbu & Sibley (1994)
		14°C	30 *Daphnia* mysid^{-1} 12h^{-1}	
		9mm long mysid	21 *Daphnia* mysid^{-1} 12h^{-1}	
		13·5mm long mysid	30 *Daphnia* mysid^{-1} 12h^{-1}	
Rhopalophthalmus terrantalis	*Mesopodopsis slabberi*	8mm long mysid	0·3 *M. slabberi* mysid^{-1} d^{-1}	Wooldridge & Webb (1988)
		14mm long mysid	1·0 *M. slabberi* mysid^{-1} d^{-1}	
	Pseudodiaptomus hessei	4mm long mysid	1·6 copepods mysid^{-1} d^{-1}	
		8mm long mysid	2·9 copepods mysid^{-1} d^{-1}	

Increased rate of feeding in response to increased temperature, as described for *N. integer* in this study, is similar to that recorded for other aquatic invertebrates. Feeding rate of the water mite *Hygrobates longipalpis* increased 2 to 3 times as water temperature was increased from 12 to 24°C (Davids *et al*, 1981). Petraitis (1992), working with three intertidal gastropods (*Austrocochlea constricta, Cellana tramoserica* and *Nerita*

atramentos), reported increased grazing rates with increasing temperature. The feeding rate of the estuarine oligochaete *Nais elinguis* increased linearly with increasing temperature (Little, 1984). Many crustacean species show this increased feeding response with increasing temperature. Grass shrimp (*Palaemonetes pugio)* showed an exponential increase in its consumption of epiphytes of sea grass (*Halodule wrightii*) as temperature was increased from 14 to 30 °C (Morgan, 1980); and the feeding rate of the Pacific white shrimp (*Penaeus vannemei*) has been shown to be directly related to temperature [feeding rate increased with increasing temperatures (Wyban *et al.*, 1995)]. Temperature coefficients for *Neomysis integer* feeding rate (i.e. 1·9 - 2·3) are similar to those of other invertebrates. The Q_{10} value for the oligochaete *Nais elinguis* was *c.*2·0 between 5 - 25°C at a salinity of 1·6‰ (Little, 1984). Small pacific white shrimp (*P. vannemei*) had a feeding rate Q_{10} of *c.*1·66 between 23 and 30°C, whilst large shrimp had a Q_{10} of *c.* 2·0 (Wyban *et al.*, 1995).

Mysids are reported to have a variable response to temperature change when feeding (Table 4.12). The rate of predation by *Neomysis integer* on *Artemia* nauplii was elevated at 16°C compared with 9°C (Astthorsson, 1980). Although the specific ingestion rate of *Daphnia* by *N. intermedia* increased between 3 and 20°C, feeding at 29°C was not significantly different from that at 20°C (Toda *et al.*, 1987). *Leptomysis lingvura*, feeding on powdered fish food (used for aquarium fish), showed an increased feeding response with increasing temperature to a maximum of 18°C (Gaudy *et al.*, 1991). At temperatures greater than 18°C, feeding rate decreased, presumably due to thermal shock (Gaudy *et al.*, 1991). In other investigations, however, mysid feeding rates have been reported to be unaffected by temperature changes. Chigbu & Sibley (1994) reported similar predation rates (*c.* 30 *Daphnia* $12h^{-1}$) for *Neomysis mercedis* feeding on *Daphnia* at 10 and 14°C (Table 4.12). Predation rates of *Mysis relicta* were also unaffected within the range 5 - 15°C (Cooper & Goldman, 1982). The different temperature response of predatory mysids compared with *Neomysis integer* in this study may possibly be accounted for by different feeding methods. In the current experiments, the inert detrital food source remained unaffected by temperature, therefore, changes in feeding rate are a direct measure of the effect of temperature on *N. integer*. When measuring predation rates, the effect of temperature on the prey must be considered in addition to the effects on the predator since feeding rate is affected by both mysid feeding activity and activity of prey (Cooper & Goldman, 1982). O'Brien (1979) reported that Arctic grayling were more successful at capturing copepods at

5°C than at 15°C, and suggested that the increased success of prey capture was correlated with a decrease in the escape ability of the copepods at low temperatures. The temperature response of prey was cited also to explain the increased capture rate of *Diaptomus* by *Mysis relicta* [the evasive ability of the copepods was suppressed at 5°C (Cooper & Goldman, 1982)]. Effectiveness of neuromuscular transmission is decreased at lower temperatures (Stephens, 1985) and the smaller size of the copepod made it more susceptible to temperature changes than the larger mysid predator.

In the natural environment, higher feeding rates are observed frequently during the warmer spring and summer months than during the winter, as is the case with mesozooplankton of the Mediterranean Sea (Christou & Moraitou-Apostolopoulou, 1995). In the present study, increased feeding of *Neomysis integer* with increasing temperature is in agreement with the findings of Kinne (1963) who reported that the feeding rate of *N. integer* increased during the spring and summer. The mysid population at Terras Bridge experienced seasonal and diurnal variation in temperature (Chapter 2). The minimum winter seawater temperature was *c.*8°C and the summer maximum was *c.*15°C; whilst temperature fluctuated daily due to tidal movements [lower temperatures were associated with the low tide, freshwater input, and higher temperatures were recorded at high tides due to the seawater input being of higher temperature (Chapter 2)]. The results of the present study suggest, therefore, that *N. integer* feeding rate increased at Terras Bridge as the temperature increased from winter through to summer, and also increased with temperature on the flood tide. Overall increase in production was also likely to occurr as the environmental temperature increases during the summer, and this appeared to be the case, reflected in the increased reproductive output of *N. integer*. The population of *N. integer* at Terras Bridge appeared to have three generations per year: a slow growing, winter generation, and faster growing spring and summer generations (Chapter 1). Given the high reproductive output during the spring and summer, it is likely that feeding rate also increased with the higher environmental temperatures of summer. Magnitude of temperature variations over the tidal cycle were similar [during the summer, the temperature ranged from *c.*7°C at low tide to *c.*15°C at high tide, and during the winter the range was *c.*3° to 8°C (Chapter 2)]. Changes in feeding rate due to diurnal temperature changes are, therefore, likely to be independent of seasonal temperature changes.

4.5.2 Effect of salinity on feeding rate

Although there is comprehensive literature dealing with the effect of salinity on the survival of mysids, literature on the effects of salinity on feeding rates is very limited. In the current study, the feeding rate of *Neomysis integer* increased with increasing salinity, except for 30‰ where the feeding rate decreased at higher temperatures (10 and 15°C). In contrast with the findings of this study, the feeding rate of the estuarine oligochaete *Nais elinguis* was not significantly different over a wide salinity range (1 - 25‰), but was significantly reduced at 30‰, a salinity beyond the maximum salinity experienced by *N. elinguis* in the natural environment (Little, 1984). Ingestion rates of the rotifer *Brachionus plicatilis* (feeding on yeast cells and unicellular algae) were also unaffected by exposure to a wide salinity range [i.e. 5 - 30‰ (Lebedeva & Orlenko, 1995)]. The feeding rate of colonies of the hydroid *Cordylophora caspia*, however, decreased in the order 16·7‰ > 30‰ > freshwater (Kinne, 1964). Feeding rate (measured as the time taken from first contact of a worm with the hydroid tentacle to its complete disappearance in the gastral cavity) was 13, 16 and 22min at 16·7‰, 30‰ and freshwater respectively (Kinne, 1964). As these literature data are from invertebrates other than crustaceans, their relevance to the current study is limited. However, the feeding rate of the blue crab (*Callinectes sapidus*) was significantly suppressed at high compared with low salinity [i.e. 25‰ than at 2·5‰ (Guerin & Stickle, 1995)].

Results from the current study suggest that *N. integer* at Terras Bridge would increase their rate of feeding on the flood tide (i.e. when they were exposed to increasing salinity between 1 and 30‰). At the low winter temperatures, however, feeding rate would be reduced at high tide [*c.*34‰ (Chapter 2)]. Lowest feeding rates would occur at low tide (*c.*1‰). At Terras Bridge, low tide formed the significant part of the tidal cycle [*c.* 18h per day (Chapter 2)], so the feeding rate of mysids at 1‰ is probably representative of the 'normal' feeding rate of *N. integer*. Flocculation of sediment into large particles in estuaries is greatest at salinities of *c.*1 - 5‰, and the area where this occurs is called the maximum turbidity zone [MTZ (Fockedey & Mees, 1997)]. Sediment flocs (e.g. clay minerals) have been reported as abundant food items in the diet of *N. integer*, although their energetic value is unclear (Fockedey & Mees, 1997). The occurrence of *N. integer* in areas of low salinity (Chapter 2) and high turbidity suggests that *N. integer* was feeding on sediment flocs. Flocculation decreases as salinity increases, and *N. integer* may have to increase

feeding rate to compensate for such a decrease. Feeding rate was only significantly different between mysids kept at salinities differing by >10‰, indicating that feeding rate may be independent of small changes in salinity.

4.5.3 Effect of sex on feeding rate

There was no significant difference between the feeding rates of male and female *Neomysis integer* at any of the temperature/salinity combinations. In contrast with the current results, Webb *et al.* (1987) reported that female *Mesopodopsis slabberi* ingested more cells of the diatom *Asterionella birostratus* than did males. At 16°C and 33‰, male *M. slabberi* consumed *c.*2·8 million cells of *A. birostratus* at a diatom concentration of 100,000 cells ml^{-1}, compared with females which consumed *c.* 3·8 million (Webb *et al.*, 1987). Although there was no significant difference between male and female feeding rates in the present study, the lower Q_{10} values of females (maximum Q_{10}=2·09) compared with males (maximum Q_{10}=2·35) indicate that the feeding rate of male *N. integer* was more sensitive to temperature change than that of females.

4.5.4 Absorption efficiency

The absorption efficiency values of *Neomysis integer* from the present study are comparable with those for other crustaceans feeding on detritus. *Neomysis integer* feeding on detritus had ash-ratio absorption efficiencies of 0·10 - 0·47, and the amphipod *Hyallela azteca* had ash-ratio absorption efficiencies of 0·15 and 0·07 when feeding on surface and sub-surface detritus respectively (Astthorsson, 1980). When feeding on animal or plant matter, absorption efficiencies of crustaceans are higher than when feeding on detritus. For example, *Mysis relicta* had an ash-ratio absorption efficiency of 0·84 to 0·90 when feeding on *Daphnia pulex* (Lasenby & Langford, 1973). *Neomysis integer* had an ash-ratio absorption efficiency of 0·68 to 0·92, and 0·59 to 0·90 when feeding on zoo- and phytoplankton respectively (Astthorsson, 1980). The lower absorption efficiency of *N. integer* feeding on sediment in the current study (compared to that recorded by other workers when feeding on animal matter), indicates that feeding rates need to be higher for *N. integer* feeding on sediment than on plant or animal matter to obtain the same amount of energy.

There appeared to be no effect of temperature, salinity or mysid sex on the absorption efficiency of *N. integer*. As feeding rate was unaffected by mysid sex, it is no surprise that

absorption efficiency was also unaffected. Temperature and salinity did affect feeding rate but had no effect on the absorption rate. Widdows & Bayne (1971) showed that the absorption efficiency of *Mytilus edulis* decreased with increasing temperatures (from 5 to 20°C). The decrease in absorption efficiency of *M. edulis* was attributed to temperature-related increases in food ingestion and egestion, such that food was passing through the digestive system too fast to be digested properly (Widdows & Bayne, 1971). In the present study, egestion rate (and by inference ingestion rate) increased with increasing temperature but absorption efficiency remained unchanged, indicating that there was a corresponding increase in the rates of digestion and absorption. In crustaceans which increase their feeding rates with increasing temperature, there is evidence of an increase in the rates of digestive processes (e.g. Krylov *et al.*, 1996). There was a highly significant increase in the digestive enzyme activity (protease activity) of cyclopoid copepods after water temperature was increased from 4 to 20°C (Krylov *et al.*, 1996). Furthermore, activity of various proteinases of *Penaeus japonicus* increased with increasing temperature from 17 to 29°C (Galgani, 1985). There are no data available on the digestive kinetics of mysids, however, the increased rates of digestion and absorption inferred from the absorption efficiencies recorded in this study suggest that the activity of digestive enzymes of *N. integer* is also increased by increasing temperature.

4.6 SUMMARY

In summary, the rate of faecal production (≡ feeding rate) was increased significantly with increases in temperature and salinity. Feeding rate of male and female mysids was not significantly different at any temperature/salinity combination. The overall relationship between faecal production and temperature, salinity and mysid sex was represented by the multiple linear regression relationship:

$$F = 0{\cdot}00665 + 0{\cdot}00217\ T + 0{\cdot}00029\ Sal - 0{\cdot}00054\ S$$

where: F= faecal production (mg faeces mg dry wt.$^{-1}$ h^{-1}), T = temperature (°C), Sal = salinity (‰), and, S = score for sex (males = 0, females =1) . Absorption efficiency was not affected by temperature, salinity or mysid sex. Since absorption efficiency remained constant, whilst feeding rate increased with increasing temperature, it is inferred that digestive processes also increased with increasing temperature and salinity.

CHAPTER 5

ACUTE TOXICITY TESTING OF TWO PESTICIDES TO DETERMINE THEIR SUITABILITY FOR USE IN SUB-LETHAL EXPERIMENTS

5.1 ABSTRACT

The sub-tropical mysid species *Americamysis bahia* is used routinely by regulatory authorities (e.g. the United States Environmental Protection Agency) to assess the toxicity of pollutants in the aquatic environment and to predict the potential hazards of such pollutants to natural aquatic ecosystems. The widespread use of *A. bahia* in toxicity testing has extended to Europe, however, there is increasing interest in using locally important, indigenous species for predicting toxicity hazards for European waters instead of the standard American species. *Neomysis integer* (Peracarida: Mysidacea) is the most common member of the hyperbenthic fauna of Western European estuaries and this study investigated the suitability of this mysid species as an alternative species for toxicity testing to *A. bahia*. Adult *N. integer*, collected from the East Looe River Estuary, Cornwall, UK, were exposed to chlorpyrifos and dimethoate (both widely used organophosphate pesticides) in flow-through and semi-static acute toxicity tests. *Neomysis integer* was most sensitive to chlorpyrifos, indicated by the 96hLC_{50} estimates of 0·13µg chlorpyrifos L^{-1} and 0·54mg dimethoate L^{-1}. Juvenile *N. integer*, collected within 24h of release from ovigerous females, were equally tolerant to chlorpyrifos exposure as adult mysids (96h LC_{50} 0·19µg chlorpyrifos L^{-1}). Analysis of test water samples by gas chromatography revealed that considerable degradation of chlorpyrifos (*c.*50%) occurred within 24h in the semi-static exposure vessels, highlighting the advantages of flow-through testing facilities. The *N. integer* LC_{50} for chlorpyrifos is higher than that of *A. bahia* (96h LC_{50} 0·035µg chlorpyrifos L^{-1}), but lower than those of other frequently used testing species. The results highlight the potential of *N. integer* as a suitable alternative to *A. bahia* for the testing of toxicants and evaluation of pollution hazards to European estuaries.

5.2 INTRODUCTION

This chapter describes the method development for the selection of the pesticides considered for use in the ensuing sub-lethal toxicological studies. The two candidate pesticides, chlorpyrifos and dimethoate, were chosen for consideration because they are widely used organophosphate (more specifically organophosphorothioate) pesticides which have toxic effects on non-target organisms at field application concentrations. In addition, mysids are particularly sensitive to organophosphate pesticides (WHO, 1986; 1989; 1991).

As with all organophosphorothioate pesticides, the mode of action for the acute toxicity of chlorpyrifos and dimethoate is by inhibiting the enzyme acetylcholinesterase (AChE) from hydrolysing acetylcholine (a neural transmitter) at nerve synapses and neuro-muscular junctions (WHO, 1986). When an impulse arrives at the synapse, acetylcholine is released from cholinergic nerve endings and carries the signal across the synaptic cleft to a receptor on the post-synaptic membrane (Walker *et al.*, 1996). Acetylcholine then reacts with the receptor which creates a signal so that the impulse carries on, or causes contraction of muscle fibres. It is important that, having generated the signal, the acetylcholine is broken down rapidly so that the signal is terminated and no more impulses are transmitted. The mode of action of organophosphates is to prevent this breakdown of acetylcholine by inhibiting the enzyme responsible (AChE) (Fig. 5.1). Thus, the receptor is stimulated repeatedly to continue the impulse, when it should have ceased. Eventually, if acetylcholine levels continue to increase, the synapse will become blocked (Walker *et al.*, 1996). If AChE activity is inhibited by more than 50%, toxic effects will be seen in the organism, however, the inhibition is reversible (Fig. 5.1) (Barron & Woodburn, 1995; Walker *et al.*, 1996).

In terms of European legislation on maximum admissible concentrations (MACs) in drinking water, all pesticides are covered by European Community directive 80/778/EEC (Council of European Communities, 1980). This directive states MACs of $0{\cdot}1\mu g\ L^{-1}$ of any one pesticide, and of $0{\cdot}5\mu g\ L^{-1}$ total pesticides, although these figures are arbitrary and are not based on any specific scientific study (Urech, 1996). The Department of the Environment of the United Kingdom (DoE) has issued guidelines for the interpretation of the EC directive which includes a list of toxicologically derived, interim maximum concentrations admissible in drinking water (DoE, 1989). Many of the DoE maxima are

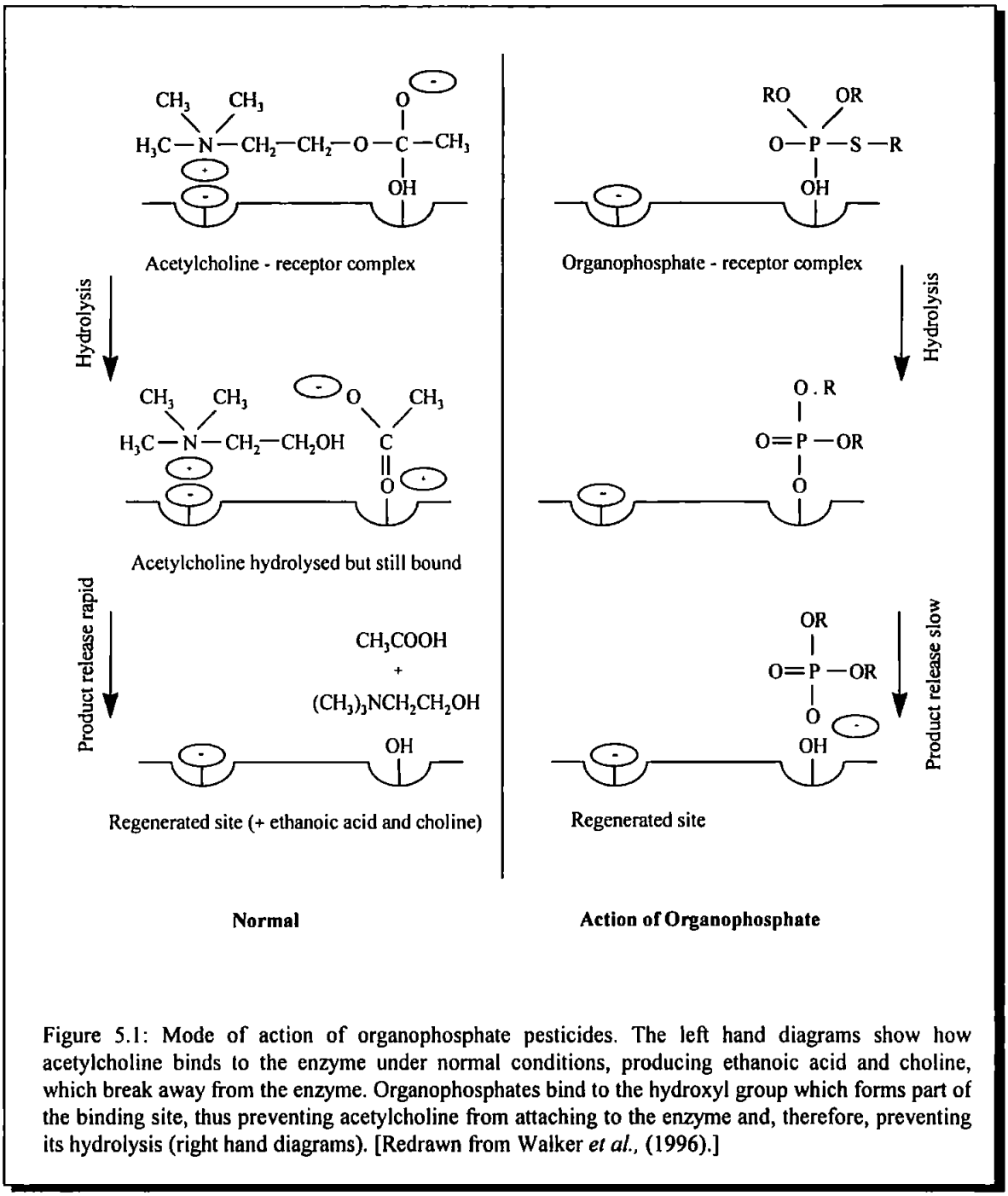


Figure 5.1: Mode of action of organophosphate pesticides. The left hand diagrams show how acetylcholine binds to the enzyme under normal conditions, producing ethanoic acid and choline, which break away from the enzyme. Organophosphates bind to the hydroxyl group which forms part of the binding site, thus preventing acetylcholine from attaching to the enzyme and, therefore, preventing its hydrolysis (right hand diagrams). [Redrawn from Walker *et al.*, (1996).]

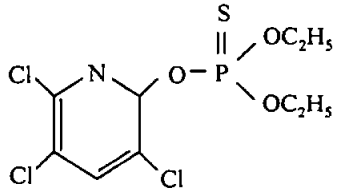
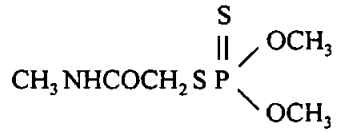
above the EC requirements (0·1μg L^{-1}), however, measures are being made to comply with European legislation. Unfortunately, neither chlorpyrifos nor dimethoate are monitored routinely in the UK, although levels of monitored pesticides in groundwaters are always (and in surface waters almost always) lower than the MACs defined by the DoE (Croll, 1991). For example, in a study of pesticide levels in groundwaters of the Anglian Water Region, levels of up to 0·2μg dimethoate L^{-1} were found compared with the DoE maximum of 3μg L^{-1} (Croll, 1991). Since the hydrolysis half-lives of chlorpyrifos and dimethoate are

relatively fast (*c.*1·5d and 12d respectively, Table 5·1) it is unlikely that run-off from agricultural land will lead to persistently high levels in aquatic ecosystems, although heavy rainfall may lead to pulses of highly concentrated pesticide run-off (Williams *et al.*, 1994). High pesticide levels are encountered most frequently after accidental spills (e.g. Boreham and Birch, 1987) and as discharge from pesticide production plants (e.g. Bormann *et al.*, 1993).

5.2.1 Chlorpyrifos

Chlorpyrifos (*O,O,*-diethyl *O*-3,5,6-trichloro-2-pyridyl phosphorothioate) has an O-alkyl phosphorothioate phosphate group (i.e. the same type of phosphate group as compounds such as bromophos, dichlorofenthion, fenthion and parathion) and is used as a broad spectrum, non-systemic insecticide by contact, stomach and vapour action (Table 5.1). Known more commonly under the trade name of Dursban, chlorpyrifos was first registered in 1965 by the Dow Chemical Company (now DowElanco) and has been used to control a wide range of pests including aphids, beetles, caterpillars, mites, moths and weevils, in a range of applications, mostly for fruit and vegetable crops in the UK (British Crop Protection Council and The Royal Society of Chemistry, 1995; CAB International & British Crop Protection Council, 1997). Chlorpyrifos is used also to control household pests (e.g. cockroaches and mosquitoes), and leatherjackets on golf courses, and is applied to soils beneath building foundations to prevent termite invasion. In the USA, it has widespread use in the control of mosquitoes (Odenkirchen & Eisler, 1988). Formulations of chlorpyrifos, including emulsifiable concentrates, granules, powders, pellets, microencapsulates and impregnated materials, are applied as foliar or soil treatments. In the UK, most chlorpyrifos products have 480g active ingredient L^{-1} and maximum residue levels (MRL, i.e. the maximum allowable concentration) in food crops range from 0·01 to 0·5mg residue per kg of food (British Crop Protection Council and The Royal Society of Chemistry, 1995; CAB International & British Crop Protection Council, 1997). Acute toxicity tests of the effects of chlorpyrifos on non-target organisms have shown that several freshwater and marine species are sensitive to this pesticide (Table 5.2). Of the invertebrates, crustaceans are more sensitive than molluscs, with mysids being particularly sensitive (Table 5.2).

Table 5.1 Chemical and physical properties of the pesticides chlorpyrifos and dimethoate.

Common Name	chlorpyrifos	dimethoate
Chemical name	*O,O*-diethyl *O*-3,5,6-trichloro-2-pyridyl phosphorothioate	*O,O*-diethyl *S*-methylcarbamoylmethyl phosphorodithioate
Official Code*	OMS 971; ENT 27 311	OMS 94; OMS 111; ENT 24 650
Trade names	*Dursban*, (DowElanco), *Piridane* (Diachem), *Pyrinex* (Makhteshim-Agan), *Silrifos* (Siapa), *Spannit* (Pan Britannica), *Talon* (FCC), *Tricel* (Excel).	*Cygon* (Cyanamid), *Perfekthion* (BASF), *Rogor* (Isagro), *Roxion* (Cyanamid), *Chimigor* (Diachem), *Danadim* (Cheminova).
Molecular Weight	350·63	229·2
Empirical Formula	$C_9H_{11}Cl_3NO_3PS$	$C_5H_{12}NO_3PS_2$
Structural formula		
Physical State (25°C)	Colourless crystals	Colourless crystals
Melting Point	41·5 to 43·5°C	49°C
Vapour Pressure (25°C)	2·7mPa	1·1mPa
log K_{ow}	4·7	0·704
Solubility (25°C)	In water *c.*1·4mg L^{-1}	In water 23·8g L^{-1} (pH 7)
	In alcohols, benzene and ketones >450g kg^{-1}	In alcohols, benzene, ketones, dichloromethane >300g kg^{-1}
Stability	Hydrolysis DT_{50} *c.*1·5d (water, pH8, 25°C).	Hydrolysis DT_{50} 12d (water, pH9). Decomposes on heating.

*OMS = World Health Organisation code; ENT = Entomological Society of America code

Table 5.2. Acute toxicity (96h unless stated otherwise) of chlorpyrifos to some aquatic organisms (modified from Odenkirchen & Eisler, 1988).

Organism	Species	Habitat	LC_{50} ($\mu g\ L^{-1}$)	Reference
CRUSTACEANS				
Mysid	*Americamysis bahia*	brackish	0·035	Schimmel *et al.* (1983)
Amphipod	*Gammarus lacustris*	freshwater	0·11	Sanders (1969)
Cladoceran	*Daphnia magna*	freshwater	1.0[a]	Marshall & Roberts
Grass shrimp	*Palaemonetes pugio*	brackish	1·5[b]	Marshall & Roberts
Crayfish	*Orconectes immunis*	freshwater	6·0	Phipps & Holcombe
MOLLUSCS				
Snail	*Lanistes carinatus*	freshwater	2,710	Karim *et al.* (1985)
FISH				
Fathead minnow	*Pimephales promelas*	freshwater	0·13	Jarvinen & Tanner (1982)
Rainbow trout	*Onchorhynchus*	freshwater	9·0	Phipps & Holcombe

[a] 6·6h LC_{50} [b] 48h LC_{50}

Sub-lethal effects of chlorpyrifos have been reported for several aquatic species, and these include inhibition of cholinesterase (ChE) activity levels, disruption of motor co-ordination, delayed maturation and growth, reproductive impairment and reduced feeding rate (Odenkirchen & Eisler, 1988; Barron & Woodburn, 1995). These sub-lethal effects may manifest themselves at extremely low toxicant concentrations. For example, the reproduction of *Daphnia magna* is impaired at 0·08μg chlorpyrifos L^{-1}, leading to reduced offspring numbers (USEPA, 1985). As it is lipophilic, chlorpyrifos should be bioconcentrated by invertebrates, yet no detectable chlorpyrifos residues were found after 12 days in 10 species of estuarine invertebrates (including oligochaetes, molluscs and crustaceans) following treatment with 46g chlorpyrifos ha^{-1} (Marganian & Wall, 1972). The isopod *Asellus aquaticus*, however, has been shown to bioconcentrate chlorpyrifos (Montañés *et al.*, 1995). After a single dose of 5μg chlorpyrifos L^{-1} to outdoor experimental ditches, *A. aquaticus* accumulated the pesticide to levels as high as 200mg chlorpyrifos kg lipid $weight^{-1}$ of isopod; tissue residues reached their maximum after 2 days and decreased subsequently in accordance with prediction models (Montañés *et al.*, 1995). Lack of detection of chlorpyrifos bioconcentrated by the organisms investigated by Marganian & Wall (1972), therefore, may have resulted from the fact that any chlorpyrifos accumulated had been depurated by the time the authors took their measurements (after 12 days).

Several accidental spills of chlorpyrifos to the environment have been recorded but quantitative assessment of their environmental effects is limited (Odenkirchen & Eisler, 1988). A comprehensive study of the environmental effects after a chlorpyrifos spill was made in England (April 1985) when a truck overturned, shedding some 500L into an adjacent stream (Boreham & Birch, 1987). The contamination caused decreases in abundance and diversity of the macrobenthic organisms, which lasted approximately 6 months before recovery was complete. Decreased species diversity resulted in a population of unusually abundant chlorpyrifos-resistant benthic organisms. In the USA, recommended application concentrations of chlorpyrifos to control mosquitoes range between 0·28 and 0·56kg chlorpyrifos ha^{-1}, equivalent to 9 to 18μg chlorpyrifos L^{-1} (Marshall & Roberts, 1978). Although no adverse effects have been recorded in mammals, amphibians or reptiles at these application concentrations, various fish, and non-target invertebrate species have shown deleterious effects, including reduced survival and reproduction (Odenkirchen & Eisler, 1988). In a lake ecosystem, a 0·004kg chlorpyrifos ha^{-1} application led to the insect and snail populations being reduced by up to 40% and 10% respectively after 24h (Moore & Breeland, 1967). A single aerial application of 0·56kg emulsible chlorpyrifos concentrate ha^{-1} to a salt marsh caused 35% mortality in caged fish and 84% in caged shrimp after 48h (Wall & Marganian, 1971). In a different salt marsh, a single spraying of 0·56kg chlorpyrifos ha^{-1} resulted in the deaths of all caged fiddler crabs (*Uca sp.*), while white shrimp (*Penaeus setiferus*) populations were reduced and large numbers of blue crabs (*Callinectes sapidus*) died (Fisheries Wildlife Service, 1967). One month after spraying, the shrimp and blue crab populations had recovered to normal levels, but fiddler crabs were still absent (Fisheries Wildlife Service, 1967).

5.2.2 Dimethoate

Dimethoate (*O,O*-dimethyl *S*-methylcarbamoylmethyl phosphorodithioate) is another organophosphorothioate insecticide with a contact and non-systemic mode of action (Table 5.1). Major manufacturers of dimethoate include BASF, Cheminova and Cyanamid. It was first introduced in 1956, and is used to control a large variety of agricultural pests (especially aphids and spider mites) and houseflies (WHO, 1989; British Crop Protection Council and The Royal Society of Chemistry, 1995; CAB International & British Crop Protection Council, 1997). Most frequently, formulation is liquid and aerial application is used for cereal, potato and pea crops. Most dimethoate formulations have a concentration

of 400g active ingredient L^{-1}, and MRLs are between 0·05 and 2mg residue per kg of food (British Crop Protection Council and The Royal Society of Chemistry, 1995; CAB International & British Crop Protection Council, 1997). Both chlorpyrifos and dimethoate may be mixed with other pesticides, and a chlorpyrifos/dimethoate mix is manufactured by Atlas to control aphids, rootfly and leatherjackets in brassica crops (CAB International & British Crop Protection Council, 1997).

Although dimethoate is used regularly in the USA, the National Estuarine Monitoring Program conducted by the United States Environmental Protection Agency (USEPA) has no published data on the effects of dimethoate on estuarine species (United States Department of Agriculture, 1981). Given its widespread use in toxicity tests by the USEPA, it is surprising that there are no available literature toxicity data for dimethoate effects on the standard bioassay species *Americamysis bahia* (Crustacea: Mysidacea). There are data for other species and some of these are presented in Table 5.3. With most of the values listed being milligrams per litre, it is clear that dimethoate is less toxic than chlorpyrifos (toxic at micrograms per litre, Table 5.2). The pure chemical form of dimethoate is frequently less toxic than the emulsible concentrate of commercially available dimethoate pesticide (Buesen & Neven, 1989). No mortalities for the guppy (*Poecilia reticulata*) were recorded after 96h exposure to 26mg pure dimethoate L^{-1}, although the 96h LC_{50} for guppy exposed to 10% emulsible concentrate was 13mg dimethoate L^{-1} (Buesen & Neven, 1989). It is likely that the solvent used in emulsible concentrates (frequently xylene) is also toxic to test organisms and, therefore, decreases the LC_{50}. The 48h LC_{50} for xylene toxicity to *Daphnia magna* has been estimated at 9·5mg xylene L^{-1} (Bobra *et al.*, 1983). It is important, therefore, when considering these data to know the formulation of the test chemical (all the data in Table 5.3 are from tests using pure dimethoate).

In addition to the use of dimethoate as a pesticide for terrestrial agricultural systems, dimethoate has been used to control crustacean grazers of commercially grown seaweed. The peracarid crustaceans *Gammarus oceanis, Idotea baltic* and the gastropod *Lacuna vincta* showed almost complete mortality at application concentrations of 1-10mg dimethoate L^{-1} (Murgatroyd & Patel, 1994). As with the acute toxicity data, there are comparatively few literature data for the chronic effects of dimethoate on aquatic invertebrates. The midgut glands of post-larval prawns (*Penaeus monodon*) have been

shown to be severely damaged following exposure to 1mg dimethoate L^{-1}, with histopathological effects being noticeable before any behavioural effects of pesticide contamination (Vogt, 1987).

Table 5.3 Acute toxicity (96h LC_{50} unless stated otherwise) of dimethoate to some aquatic organisms.

Organism	Species	Habitat	LC_{50} (mg L^{-1})	Reference
CRUSTACEANS				
Amphipod	*Gammarus lacustris*	freshwater	0·2	Sanders (1969)
Cladoceran	*Daphnia magna*	freshwater	1·7[a]	Buesen & Neven (1989)
Freshwater prawn	*Machrobrachium lamerri*	freshwater	2·6	Murgatroyd & Patel (1994)
Brown shrimp	*Crangon crangon*	brackish	0·3 - 1·0[a]	Murgatroyd & Patel (1994)
FISH				
Guppy	*Lebistes reticulata*	freshwater	4·64	Ramana *et al.* (1992)
Rainbow trout	*Onchorhynchus mykiss*	freshwater	27[a]	Sanderson & Edson (1964)

[a] 48h LC_{50}

5.2.3 Aim

The aim of this chapter was to investigate the toxicity of chlorpyrifos and dimethoate to *Neomysis integer*. The relative toxicity of each pesticide, stability of the compounds and ease of use in the laboratory were the criteria used to decide which chemical would be selected as the reference toxicant for the ensuing sub-lethal studies (Chapter 6).

5.3 METHODS

5.3.1 Animal collection and maintenance

During May (1996), mysids were collected as described in Chapter 2. At the time of sampling, the population comprised mature adults (including ovigerous females) of the spring generation and juveniles of the summer generation. Mysids were transferred into a 10L polythene bucket, containing water from the collection site (*c.* 1‰), transported by car to the Brixham Environmental Laboratory (ZENECA Limited) and separated randomly into two 15L holding tanks. The Brixham Environmental Laboratory has flow-through facilities with programmable photoperiod and air temperatures. The holding tanks were linked to one of the flow-through 'rigs' and received 50L h^{-1} of diluted sea water (7 ± 1‰). Air and

water temperatures were maintained at 12 ± 1°C, and lighting was programmed for an 18h light:6h dark photoperiod with dawn and dusk sequences. Holding water, of the appropriate salinity (established by combining natural seawater from an offshore intake in Tor Bay with de-chlorinated mains water), was passed through a 10μm filter. Mysids were fed twice daily on <72h old *Artemia* nauplii hatched from dry cysts. The USEPA and the American Standards for the Testing of Materials (ASTM) procedures for toxicity testing with mysids (*Americamysis bahia*) recommend a daily supply of 150 *Artemia* nauplii per mysid (Lussier *et al.*, 1988; ASTM, 1990), although 50 nauplii per mysid has proven to be sufficient (Langdon *et al.*, 1996). Although the number of added nauplii was not counted, the feeding regime ensured that nauplii were always in excess (when examined by eye *Artemia* nauplii were always present in the test vessels).

5.3.2 Good laboratory practice

At the Brixham Environmental Laboratory (ZENECA Limited), all experimental work was carried out in accordance with ZENECA's 'Good Laboratory Practice' (GLP) policy [as defined by the Department of Health's United Kingdom Compliance Programme (Department of Health, 1989)]. Good laboratory practice involves making detailed plans for the proposed study, defining appropriate control and storage of test substances and, in particular, using all laboratory equipment according to the Standard Operating Procedures (SOP) defined by laboratory managers. Following GLP procedures, therefore, ensures that results from different laboratories are of the same technical quality and reliability, and allows the use of generated data by Government Regulatory and Registration Authorities, both in the UK and internationally. The research study numbers for the work with chlorpyrifos and dimethoate were AC0375/A and AC0376/A respectively. Where ZENECA Standard Operating Procedures have been used and quoted in the text, the SOP code number is given (with the version number given in brackets).

5.3.3 Test solutions

All test solutions were made in the same way. Two-litre conical flasks were marked accurately at 2L by the addition of 2L of water from a measuring cylinder (a magnetic follower was first added to the flask). Approximately 1·5L of dilution water (test solution without toxicant) were added to the flask, which was then placed on a stirrer plate operating at high speed to generate a vortex. The correct amount of toxicant stock for each

test concentration was injected into the centre of the vortex using a Hamilton glass syringe. The solution was allowed to mix for 5min at high speed before stirring was stopped and dilution water was added to the 2L mark. The test solution was stirred for a further 5min prior to distribution into the test vessels.

5.3.4 Range-finding tests

Prior to running a definitive 96h LC_{50} test, a 96h range-finding test was carried out. Suitable ranges of concentrations for chlorpyrifos and dimethoate were chosen with reference to LC_{50} literature values for other crustaceans (in particular the mysid *Americamysis bahia*). A semi-logarithmic increase (1·0, 3·2, 10, 32) of concentrations was used. The 96h LC_{50} for chlorpyrifos toxicity to *A. bahia* is 0·035µg L^{-1} (Shimmel *et al*, 1983). An LC_{50} value for dimethoate and mysids was not found in the literature, however, dimethoate was assumed to be less toxic than chlorpyrifos based on LC_{50} data for other species [e.g. chlorpyrifos and dimethoate 96h LC_{50} estimates for the amphipod *Gammarus lacustris* are 0·11µg chlorpyrifos L^{-1} and 0·2mg dimethoate L^{-1} (Sanders, 1969)]. Therefore, the initial concentrations used were:

chlorpyrifos: 0·01, 0·032, 0·1, 0·32, 1·0µg L^{-1}

dimethoate: 0·032, 0·1, 0·32, 1·0, 3·2mg L^{-1}

The method used for the semi-static range finding tests was the ZENECA SOP *BA 206 (2)* (Determination of the acute toxicity (LC_{50}) to the mysid shrimp *Americamysis bahia* using a static test procedure) modified to incorporate a semi-static approach where test solutions were renewed every 24h. Technical points of a semi-static approach were taken from various ZENECA SOPs for semi-static toxicity tests [e.g. *AQ 97 (03)* (Determination of the acute toxicity of a chemical to larvae of *Chironomus riparius* under semi-static test conditions)]. Due to the relatively low solubility and stability of chlorpyrifos, initial chlorpyrifos stock solutions were made up in acetone. Solvent carriers, such as acetone, are used frequently when testing toxicants with low solubility in water, rather than dissolving the toxicant directly into the dilution water and risking precipitation (e.g. Kersting, 1995). A requirement of the SOP is that not more than 0·1ml L^{-1} solvent is used in any test solution, therefore, high concentrations of chlorpyrifos stock were needed. An initial stock solution of 1000mg chlorpyrifos L^{-1} was made up and subsequently diluted in series to meet the required concentrations for the test stock solutions (100, 10 and 1mg chlorpyrifos L^{-1}). Since much higher concentrations of dimethoate were needed, it was not possible to

use a solvent carrier without exceeding the 0·1ml L^{-1} maximum solvent concentration. A dimethoate stock of 100mg dimethoate L^{-1} was made up in double distilled, de-ionised water. Although dimethoate is relatively stable in aqueous solutions, a new stock solution was made each day to ensure that decomposition was minimal.

Two litres of each test solution were prepared daily by combining known volumes of stock solutions with dilution water from the rig; 2L of dilution water were prepared as a control. Since chlorpyrifos was dissolved in an organic carrier, a solvent control containing 100µl acetone L^{-1}, was also prepared (100µl acetone L^{-1} corresponding to the highest concentration of solvent used in the test). Equalising volumes of acetone were added to each chlorpyrifos test solution so that the concentration of solvent was standardised (i.e. all test solutions contained 100µl acetone L^{-1}). Equalising volumes of distilled water were added to each of the dimethoate test solutions. One and a half litres of each exposure concentration, and the two controls, were distributed randomly amongst 2000ml glass beaker test vessels. Eight mysids of similar size (15 ±1mm from the anterior margin of the rostrum to the tip of the telson) were added to each test vessel; ovigerous females were excluded. Exposure water was aerated gently with filtered compressed-air. Mysids were fed twice daily on <72h *Artemia salina* nauplii, ensuring that nauplii were in excess.

Exposure solutions were replaced every 24h with freshly prepared solutions. A minimum of 1400ml was drained off before the vessel was re-filled to 1500ml with new test solution. Mortalities of the mysids were recorded in each vessel at 24h intervals, and all dead individuals were removed. Mortality was defined as absence of response when mechanically stimulated. In addition, other overt symptoms of toxicity (e.g. altered swimming ability or behaviour, or repeated escape response movements) were recorded.

A major problem with using mysids in toxicity tests is cannibalism. Although ensuring an excess of *Artemia* helped to reduce cannibalism, *N. integer* preyed on each other (post-moult individuals were especially vulnerable to predation). Obviously, such deaths would affect the estimated LC_{50}, so it was important to discriminate such mortalities from the mortalities resulting from toxicity. This was not difficult, since moult deaths usually disappeared from the test vessel (they were eaten). In contrast, toxicant-related deaths remained in the test-chamber. Moult deaths were, therefore, removed from the analysis by

ignoring animals that disappeared from the test vessels (i.e. the initial *n* number is reduced for every animal that disappeared).

The dissolved oxygen concentration, pH and temperature of each test concentration was measured at the start of the test and at the end of every 24h (using a YSI Model 58 Oxygen Meter, Corning Model 240 pH Meter and mercury thermometer, respectively). The water temperature of an additional vessel without mysids, and air temperature, were measured and recorded automatically by computer at hourly intervals.

Mysid mortality data were analysed by computer (ZENECA LC_{50} Programme, Version 5.04), and the median lethal concentration (LC_{50}) and corresponding 95% confidence limits were calculated. The computer programme calculated the LC_{50} by three methods: binomial, average moving angle and PROBIT analysis (for review see Hoekstra, 1991). All data presented here were calculated by the average moving angle method (the binomial calculation is only a basic method for a rough estimate and the rigorous statistical constraints of the PROBIT analysis were not met in all tests). A useful feature of the computer programme is that it will calculate LC_{50} data for each of the 24h durations (i.e. 24h, 48h etc.), although 96h data are most frequently reported in the literature. The 96h LC_{50} data were calculated using nominal concentrations (96h LC_{50} estimates of *c.*0·15µg chlorpyrifos L^{-1} and 0·37mg dimethoate L^{-1}; Table 5.4). The results from these range finding experiments were used to choose appropriate test concentration ranges for the definitive tests.

Table 5.4: Estimated 96h LC_{50} values from semi-static range-finding tests. n=40 per test.

Test Substance	96h LC_{50}	95% Confidence Intervals
Chlorpyrifos	0·147µg L^{-1}	0·079 - 0·311µg L^{-1}
Dimethoate	0·366mg L^{-1}	0·137 - 1·093mg L^{-1}

5.3.5 Definitive tests

A definitive test allows a more accurate estimation of the LC_{50} by using a narrower range of test concentrations. One of the major problems with static or semi-static toxicity tests, however, is the uncertainty of whether the test organism is exposed to a constant, known

concentration of the toxicant. Although chemical analysis of the test solutions allows comparison with nominal concentrations, the toxicant concentration may decrease over time for various reasons (e.g. bioaccumulation, hydrolysis, precipitation or adsorption to the sides of the test vessel). Therefore, at the end of a 96h static test, toxicant levels in the test-chambers may have decreased considerably from initial concentrations. Clearly, semi-static tests reduce the problem and ensure that the exposure concentration is returned to initial concentrations at 24h intervals. If the toxicant concentration decreases significantly over 24h, however, the test organism receives pulses of high contaminant concentrations which decrease gradually with time. A more reliable method of maintaining exposure concentrations (and one which is more environmentally-realistic) is to use a flow-through system, whereby the test solution drains to waste and is replaced constantly by fresh solution flowing into the chamber. A flow-through system was used for the definitive 96h test for chlorpyrifos. Due to the high concentration of pesticide needed, and the constraints of the appropriate SOP for acute testing, however, this approach was not feasible for the dimethoate test and a definitive semi-static test was conducted.

Flow-through test (chlorpyrifos definitive test)

At the Brixham Environmental Laboratory, the flow-through system is designed to allow the test solution to mix with dilution water and drain into the test-chamber by gravity (Fig. 5.2; Plate 5.1). Toxicant stock was pumped (1·36µl min^{-1}) into the mixing cell where it was mixed with diluted seawater (7 ±1‰) flowing from the rig (340ml min^{-1}), giving a dilution factor of 250,000 (Fig. 5.2; Plate 5.2). The toxicant stocks used were 4·5, 8, 14, 24, 45 and 80mg chlorpyrifos L^{-1}, resulting in test solutions of 0·018, 0·032, 0·056, 0·100, 0·180 and 0·320µg chlorpyrifos L^{-1} respectively. The test-chamber was kept at constant volume by an overflow draining from the opposite end of the chamber (Fig. 5.2; Plate 5.3). Hence, the test-chamber constantly received fresh solution at the rate of *c.*20·5L h^{-1}. At the start of each test, 20 adult mysids of similar size (15 ±1mm) were placed in each test-chamber; ovigerous females were excluded from the test. Mysids were fed with <48h old *Artemia* nauplii twice a day (an excess of food is required since some drains away with the flow). Mortalities were recorded every 24h (with "moult deaths" being noted and excluded from the analysis). Other overt signs of toxic disruption, such as irregular swimming behaviour, were noted also.

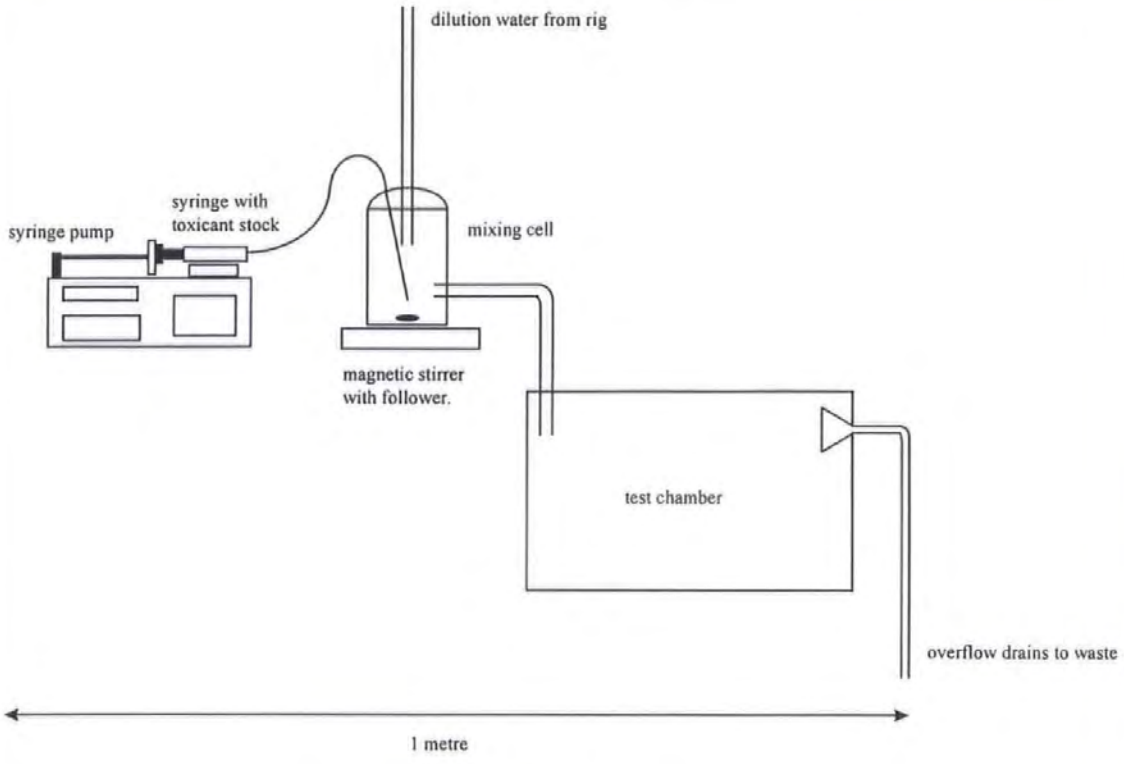
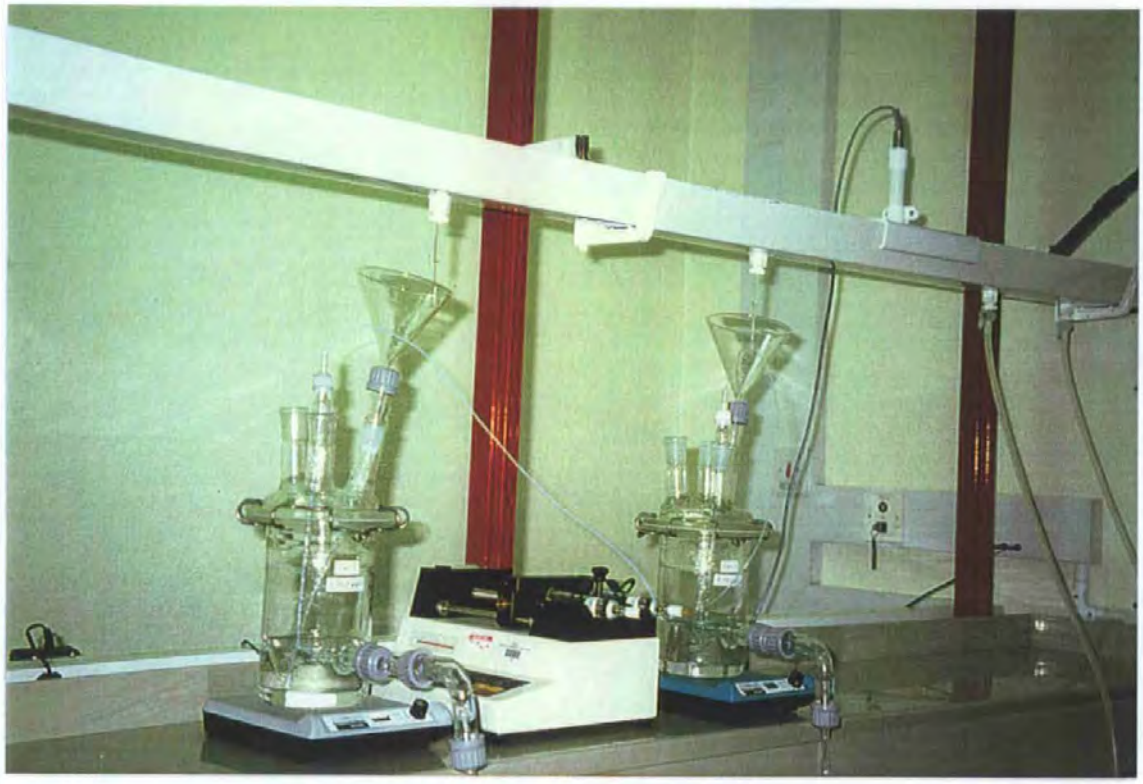


Figure 5.2: Schematic representation of the flow-through rig.

Plate 5.1: The flow-through rig at the Brixham Environmental Laboratory (ZENECA Limited).



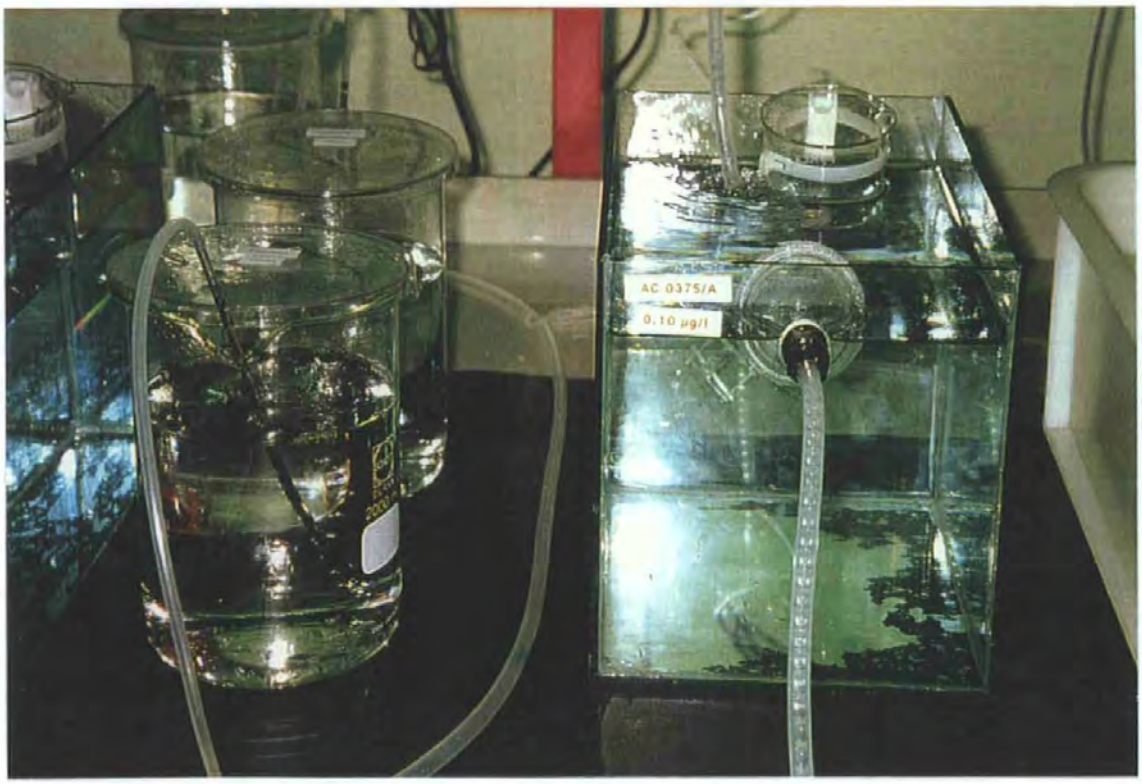


Plate 5.2 (upper plate):Flow-through dosing appartus (syringe pumps and mixing cells).
Plate 5.3 (lower plate): Exposure vessels: 2L beaker (semi-static test), 15L aquarium (adult flow-through test and 250ml beaker (juvenile flow-through test).

Regulatory tests often use <24h old test organisms, as these are thought to be less variable, and often more sensitive in response, than adult animals. Sensitivity of juvenile *N. integer* was examined simultaneously with the adults by placing a juvenile test-chamber in each of the flow-through test-chambers. The juvenile test-chamber comprised a 250ml glass beaker with a 0·5mm mesh covering a 50mm diameter hole cut from the side of the beaker to allow exchange of solution (Fig. 5.3, Plate 5.3). Each juvenile test-chamber was suspended such that the mesh-covered hole was immediately next to the in-flowing test solution, providing maximum water exchange (water flowing directly into the top of the juvenile test-chamber created too much turbulence for the animals to survive). At the start of the test, 10 juveniles (<24h old, released in the laboratory by ovigerous females caught in the field) were placed into each juvenile test-chamber. Juveniles were fed <48h old *Artemia* twice daily. Mortalities were recorded at 24h intervals, together with any other overt signs of intoxication (e.g. irregular swimming behaviour).

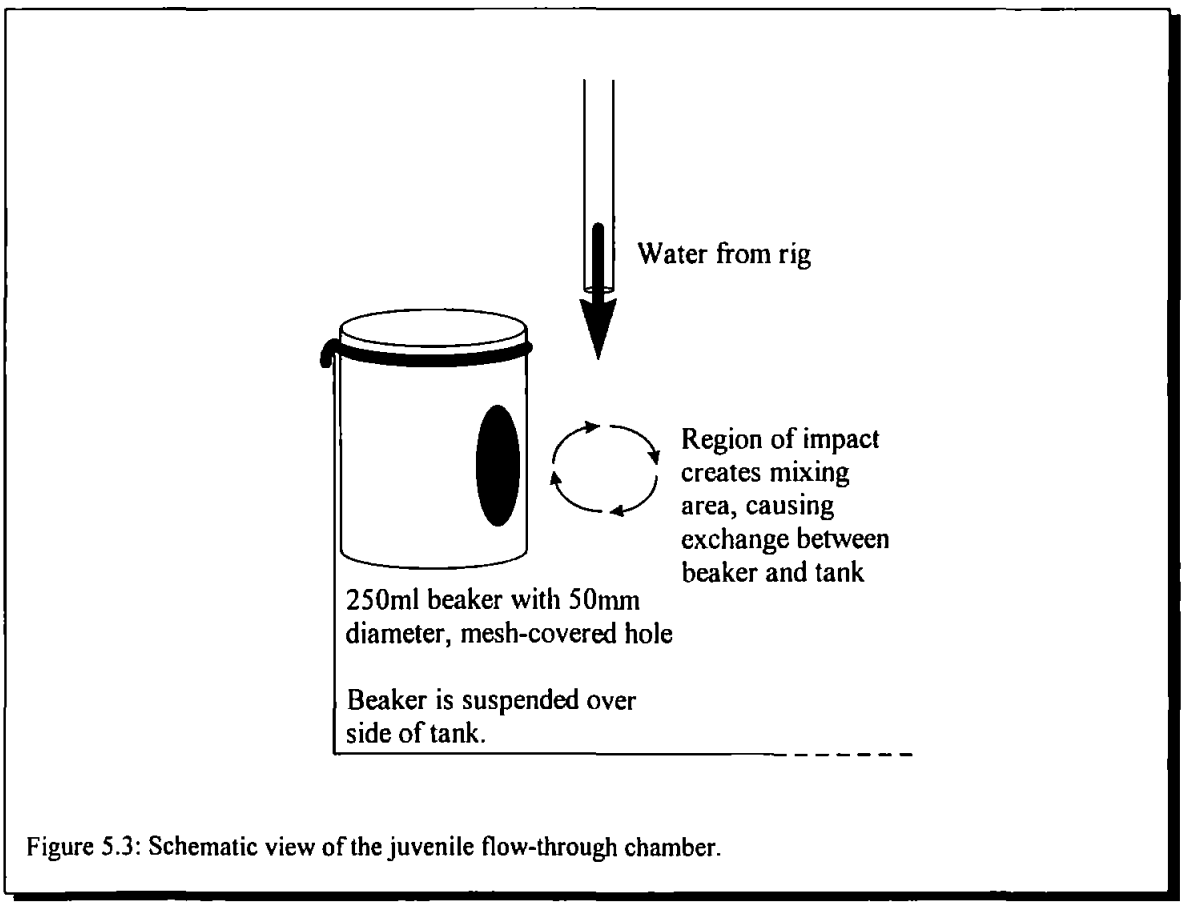


Figure 5.3: Schematic view of the juvenile flow-through chamber.

The temperature, pH, salinity and dissolved oxygen of the out-flowing water from each test-chamber was recorded every 24h (using a mercury thermometer, Corning Model 240 pH Meter and Y.S.I. Salinity and Oxygen Meters respectively). Flow rates from the rig into each mixing vessel were measured with a measuring cylinder and approximate readings of flow from the syringe were recorded daily to ensure that toxicant flow/dilution was correct. A sample of out-flowing water from each test vessel was collected once after 96h and frozen for chemical analysis of chlorpyrifos concentration at a later date. As a surplus of chlorpyrifos stock was left after 96h in the flow-through test, this experiment was allowed to run for 8d (i.e. until the toxicant stock had been used).

Semi-static test (dimethoate definitive test)

The methods and SOP for the definitive semi-static test were the same as those described for the range finding test, except that 10 mysids were placed in each test vessel. Based on the LC_{50} derived from the range-finding test (Table 5.4), the concentrations used were: 0·28, 0·50, 0·88, 1·56, 2·81, and 5·00mg dimethoate L^{-1}. These concentrations were too high to use a solvent carrier, so fresh stocks were made up in double-distilled, de-ionised water every 24h. At the start of the test, 10 mysids of similar size (15 ±1mm) were placed into each test-chamber (ovigerous females were excluded) and mortalities were recorded at 24h intervals. The feeding regime and water analysis was identical to that described earlier for the range finding tests (Section 5.2.4). The 96h LC_{50} values (and corresponding 95% confidence intervals) were calculated using the ZENECA LC_{50} programme.

5.3.6. Chemical stability of chlorpyrifos and dimethoate

A final consideration regarding the possibility of degradation of chlorpyrifos and dimethoate was examined by conducting semi-static tests using test solutions that had been made up 24h prior to being used (allowing decomposition of the two pesticides).

Test solutions of chlorpyrifos and dimethoate were made up and placed in 2L glass beakers (as in the definitive tests). The solutions were aerated for 24h and 10 animals of similar length (15 ±1mm) were placed into each test-chamber. The test followed the same procedure as described earlier for a definitive semi-static test, with the exception that all test solutions were made 24h prior to their use. The feeding regime and water analysis was as described earlier for the range finding tests (Section 5.2.4). The 96h LC_{50} values (and

corresponding 95% confidence intervals) were calculated by computer. The LC_{50} data from this test were compared with those obtained using new test solutions, thereby, establishing the degree of degradation of the chlorpyrifos and dimethoate.

5.3.7 Analysis of chlorpyrifos concentrations in the test solutions

Extraction of chlorpyrifos from test solutions

Due to the extreme sensitivity to contaminants of the gas chromatograph (GC), all glassware was thoroughly washed in DECON, rinsed with double distilled, de-ionised water, oven-dried and then rinsed in solvent (hexane). Due to the very low concentrations of chlorpyrifos in the test solutions, the pesticide needed to be extracted and concentrated so that it could be detected by the GC. Chlorpyrifos is extremely hydrophobic, therefore, extraction was made into organic solvent (Distol grade hexane). One litre samples of test solution were placed into 1L volumetric flasks and 5ml of hexane was added. The flask was shaken vigorously for exactly 2min to transfer the chlorpyrifos from aqueous solution into the solvent. It was important to ensure that the vigour and duration of shaking was the same for all solutions, since this was likely to affect extraction efficiency (P. Donkin, *pers. comm.*). The flask was allowed to stand for 5min, allowing the less dense hexane to rise to the top of the flask. The hexane was transferred into a glass vial using a Pasteur pipette, taking care not to transfer any water. This procedure was repeated on two further occasions, so that 15ml of chlorpyrifos/hexane extract was collected. To remove any water that had been accidentally collected, the samples were stored in a deep-freeze (*c.*-18°C) overnight (at least 6h is recommended) and transferred to clean vials, leaving the frozen water behind. The resultant extract was reduced to approximately 1ml by placing the vial on a warm hot-plate with oxygen-free nitrogen blowing gently on the solution. Finally, the extract was reduced to exactly 1ml by transferring to small vials and reduction by evaporation to a pre-marked 1ml line. Again, duration of evaporation may affect extract concentration (e.g. by loss of chlorpyrifos to the air), so this procedure was standardised for all extracts, requiring approximately 20min for reduction from 15ml to 1ml. This procedure resulted in the chlorpyrifos being concentrated down from $\mu g\ L^{-1}$ to $\mu g\ ml^{-1}$. Extraction of all test solutions, and some control solutions, was made in this way. All concentrates were stored in a freezer (*c.*-18°C) until GC analysis. Since the stability of chlorpyrifos in hexane is unknown, all samples were analysed as soon as possible (within 48h). Standards of relevant exposure concentrations were made up in Distol grade hexane and frozen until

analysis. Standard concentrations were 0·1, 0·05 and 0·025µg chlorpyrifos ml^{-1}, covering the expected range of extract concentrations.

Gas chromatography

Extracted samples were analysed for chlorpyrifos using a Hewlett-Packard 5890 (Series II) Gas Chromatograph with a ^{63}Ni cell electron capture detector (ECD). Gas chromatography requires a specific detector type for various compounds, and the ECD (selective for gas-phase electrophores) is used most frequently for pesticides due to its high sensitivity for halogen atoms (Buffington & Wilson, 1987).

The initial GC conditions and temperature programme were based on the recommendations of Green *et al.* (1996) [Table 5.5]. These initial conditions gave a retention time for chlorpyrifos of 22·7min. After replacement of the expired helium cylinder, however, this combination failed to give consistent results, assumed to be caused by gaseous contamination (even though both the make-up and carrier gases were pure grade and were both passed through filters). A replacement helium cylinder was used, and the GC conditions and programming were modified (Table 5.5).

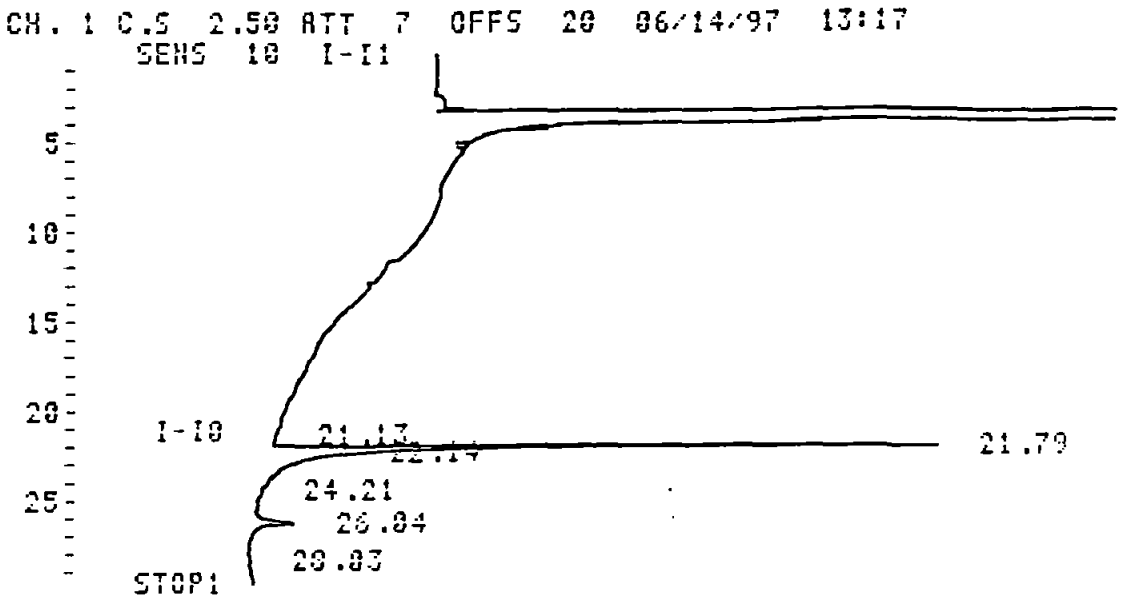
Table 5.5: Initial and final set-up conditions used for Hewlett-Packard 5890 (Series II) gas chromatograph. Pure-grade helium was used as a carrier gas, with pure-grade nitrogen being used for the make-up gas.

Set up	Column	Temperature Programme	Injection
Initial	30m x 0·32mm ID DB-5 fused silica capillary column fitted with a 2m x 0·53 ID quartz retention gap	1min held at 150°C, followed by increases of 20°C per minute to 220°C, slowed to increases of 5°C per minute to 300°C. Detector temperature was set to 350°C.	2·5µl, manual injection using Hamilton 5µl syringe.
Final	As above, but 10m retention gap fitted	2min held at 70°C, followed by increases of 40°C per minute to 130°C, slowed to increases of 5°C per minute to 260°C, followed by 3min equilibrium period. Detector temperature was set to 350°C.	2·5µl, manual injection using Hamilton 5µl syringe.

Since problems had been encountered previously with the gases, special attention was paid to the gas pressures. Cylinder pressures of 55 - 60psi were used for the helium and nitrogen, with the injector pressure set at 7psi at room temperature. The ECD make-up gas was supplied at *c.*60ml min^{-1}. Data from the GC were analysed by a Merck-Hitachi integrator, which plots chromatograms and generates peak data as either peak height or peak area. With this set up and temperature programme, the retention time of chlorpyrifos was approximately 21·80min (an auto sampler was not available, therefore, injections of sample into the GC and initiation of the integrator were made manually, accounting for slight differences in retention times). The time program was continued beyond the chlorpyrifos retention time, through to 29·5min, to see if any peaks due to decomposition of chlorpyrifos occurred. Under these conditions, and using hexane as the carrier solvent, a large peak occurred at *c.*3·5min and a smaller peak occurred at *c.*26min (Fig. 5.4). Analysis of solvent blanks (hexane only) revealed peaks of similar sizes at similar retention times, indicating that both peaks were solvent effects. Initial replications of standard injections showed that reproducibility of GC readings was good (± 1S.D ≤ 4mm for peak height readings). To test the efficiency of the extraction method, extractions were made from fresh test solution (i.e. the chlorpyrifos was extracted within 15min of making the solution). Extractions were then made from several of the exposure vessels after 24h.

5.3.8 Statistical treatment of results

The LC_{50} programme has no facility for comparing different values for significance, however, comparisons may be made using the 95% confidence intervals since these give more information than some hypothesis tests (Kirk, 1990). Differences are significant at the 95% level ($p<0·05$) if the 95% confidence intervals do not overlap. Gas chromatography data are presented as means with corresponding 95% confidence intervals.



CHLORPYRIFOS EXP 1997(S.ROAST)
GC-ECD 5890+DB 30COL+2m RETGAP

D-2000 06/14/97

2 CALIB. OF 2

SAMPLE: CHL'FOS TAG: 3 CH: 1

FILE: 1 CALC-METHOD: EXT-STD TABLE: 2 CONC: HEIGHT

NO.	RT	HEIGHT	UG/ML	FACTOR1	BC	NAME	GC
3	21.79	74882	0.025	0.382	BV	CPFS	1

Figure 5.4: Hewlett Packard 5890 (Series II) GC trace for a chlorpyrifos standard (0·1µg chlorpyrifos ml^{-1}), using hexane as the solvent carrier. Retention time for chlorpyrifos is 21·79min. Peak height is 94mm at attenuation 7 (188mm at attenuation 6). Peaks at *c.*3·5 and 26min are solvent effects. Although the chromatography is good at the start of the chlorpyrifos peak (i.e. the peak rises straight up from the baseline), the chromatography is poor at the end of the peak, leaving a noticeable tail. Peak height is, therefore, used as an indication of chlorpyrifos, and justification of peak height use is given in the text.

5.4 RESULTS

5.4.1 Toxicity tests

From the range-finding semi-static test (the only experimental set-up used for both pesticides) chlorpyrifos was more toxic to *Neomysis integer* than dimethoate (Table 5.6).

Table 5.6: Summary of 96h LC_{50} values (and 95% confidence intervals) for *Neomysis integer* exposed to two organophosphorothioate pesticides.

Test	Chlorpyrifos	Dimethoate
Range finding semi-static (n=40)	0·147µg L^{-1} (0·079 - 0·311µg L^{-1})	0·366mg L^{-1} (0·137 - 1.093mg L^{-1})
Definitive semi-static (n=60)	—	0·543mg L^{-1} (0·403 - 0·814mg L^{-1})
Flow-through: Adults (n=120)	0·134µg L^{-1} (0·112 - 0·164µg L^{-1})	—
Flow-through: Juveniles (n=60)	0·187µg L^{-1} (0·157 - 0·226µg L^{-1})	—

The definitive LC_{50} for dimethoate was higher than the range finding estimate, demonstrating the need for a narrow range of exposure concentrations in these tests (also indicated by the reduction in the width of the 95% confidence intervals in the definitive tests). The test conditions for the range-finding and definitive tests with chlorpyrifos were different (semi-static and flow-through respectively), however, there was no significant difference between the 96h LC_{50} values generated (Table 5.6; Fig 5.4). In addition, the 96h LC_{50} for chlorpyrifos was not significantly different for adults and juveniles (Table 5.6, Fig 5.5).

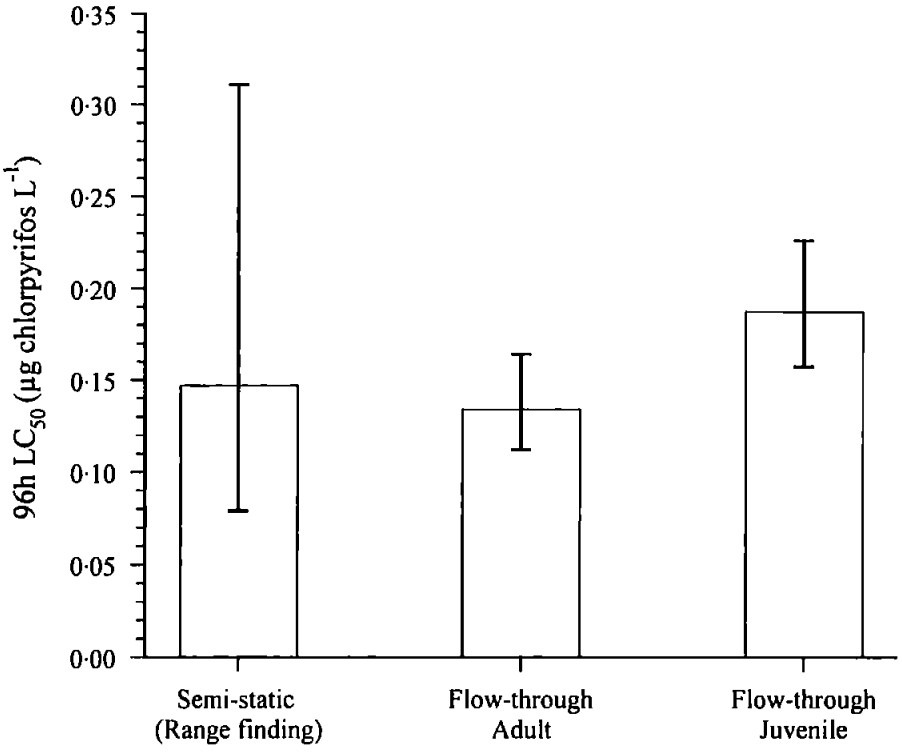


Figure 5.5: Effect of experimental procedure on the 96h LC_{50} estimates for toxicity of chlorpyrifos to *Neomysis integer*. n=40 (semi-static), 120 (adult flow-through) and 60 (juvenile flow-through). Error bars correspond to ±95% confidence intervals.

Table 5.7 compares the LC_{50} data for dimethoate when the moult deaths were removed from the computer analysis with values obtained when all deaths were included (whether they were toxicant or moult related). If deaths due to moulting are not separated from pesticide deaths, the estimated LC_{50} value is lower than if moults are removed from the analysis, however, this is not significant (95% confidence intervals, $p>0{\cdot}05$). Interestingly, no moult deaths were observed in any of the flow-through experiments.

Table 5.7 Effect of mortalities due to moulting on estimation of the 96h LC_{50} for *Neomysis integer* exposed to dimethoate (figures in brackets correspond to 95% confidence intervals). Variable n.

Test Type	Moult Deaths Removed	Moult Deaths Included
Semi-Static Definitive	0·543 mg L^{-1} (0·403 - 0·683 mg L^{-1})	0·474 mg L^{-1} (0·361 - 0·606 mg L^{-1})

As there was chlorpyrifos stock solution left after the intended 96h period, the flow-through tests were continued for 192h (8d). There was no significant difference between the sensitivity of adult and juvenile *N. integer* (95% confidence intervals, $p>0{\cdot}05$; Tables 5.8 and 5.9; Fig. 5.6). However, these data show a general trend of initial greater sensitivity of juveniles to chlorpyrifos than the adults; however, after 48h, adults were more sensitive to chlorpyrifos than juveniles (Fig. 5.6) At 144h, juveniles were again more sensitive.

Table 5.8: LC_{50} values for adult *Neomysis integer* exposed to chlorpyrifos for various test durations. n=120.

Time (hours)	LC_{50} ($\mu g\ L^{-1}$)	95% Confidence Intervals ($\mu g\ L^{-1}$)	
		Minimum	Maximum
48	0·302	0·251	0·496
72	0·175	0·148	0·215
96	0·137	0·118	0·160
120	0·107	0·090	0·128
144	0·090	0·074	0·112
168	0·084	0·070	0·104
192	0·072	0·059	0·088

Table 5.9: LC_{50} values for juvenile *Neomysis integer* exposed to chlorpyrifos for various test durations (the juvenile test was started 48 after the adult test, therefore, it only ran for 144h). n=60.

Time (hours)	LC_{50} ($\mu g\ L^{-1}$)	95% Confidence Intervals ($\mu g\ L^{-1}$)	
		Minimum	Maximum
48	0·267	0·234	0·324
72	0·240	0·202	0·308
96	0·187	0·157	0·226
120	0·187	0·157	0·226
144	0·080	0·045	0·156

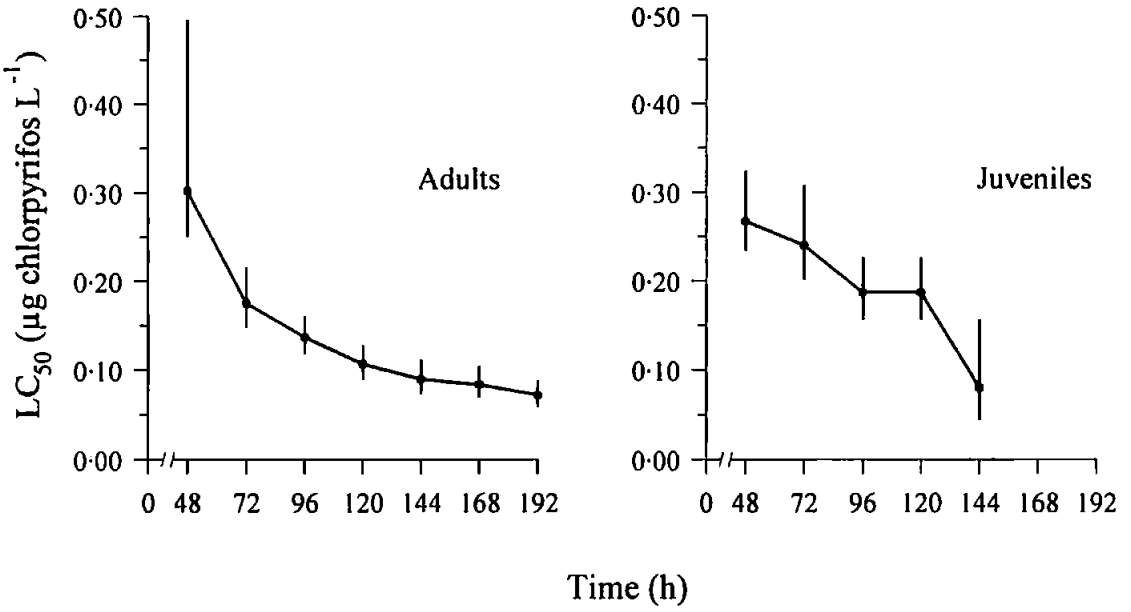


Figure 5.6: Effect of test duration on the LC_{50} estimates for adult and juvenile *Neomysis integer* exposed to chlorpyrifos. n=120 (adults) and 60 (juveniles). Error bars correspond to 95% confidence intervals.

The shape of the LC_{50} graphs for juvenile and adults was different (Fig. 5.6). Adult LC_{50} estimates decreased every 24h, producing a negatively exponential curve (Fig. 5.6). The decrease in the LC_{50} value per 24h was not significant (95% confidence intervals, $p>0{\cdot}05$; Fig. 5.6) except for the change between 48h and 72h (95% confidence intervals, $p>0{\cdot}05$; Fig 5.6). However, the change in LC_{50} per 48h was significant (95% confidence intervals, $p<0{\cdot}05$; Fig. 5.6) until after 144h. It may be assumed that the LC_{50} value for adults does not change significantly with increasing test durations beyond 144h (95% confidence intervals, $p>0{\cdot}05$; Fig. 5.6). In contrast to the adults, juvenile LC_{50} values decreased with increasing test duration in a linear relationship (Fig. 5.6). There were significant decreases in the LC_{50} value every 48h (95% confidence intervals, $p<0{\cdot}05$; Fig. 5.6) right through to 144h. Unfortunately, the juvenile flow-through test only ran for 144h, so it is not possible to estimate at which test duration the LC_{50} becomes stable.

With freshly-made test solution, the estimated adult semi-static 96h LC_{50} for chlorpyrifos was $0{\cdot}147\mu g\ L^{-1}$ (Table 5.10). It was not possible to calculate an LC_{50} for 24h old test

solution because, excluding deaths attributable to moulting, there was 100% survival at the highest exposure concentration of 0·32µg chlorpyrifos L^{-1} (at this concentration, however, the mysids were clearly affected since they were extremely active when compared with control mysids). These data suggest that there was a large degree of degradation of chlorpyrifos in the first 24h. Since the upper 95% confidence limit for fresh chlorpyrifos solution is 0·311µg L^{-1}, the difference between the 96h LC_{50} estimates for fresh and 24h old solution is significant. Dimethoate samples were not analysed on the GC, so the only indication of the stability of dimethoate in the test vessels was the LC_{50} estimate from 24h old test solution. Fresh dimethoate test solution resulted in a 96h LC_{50} value of 0·543mg L^{-1} and the 24h old solution a value of 0·642mg L^{-1}, indicating that there was *c.*20% degradation of the pesticide during the first 24 - 48h. (Table 5.10). The LC_{50} values from fresh and 24h old dimethoate solutions, however, were not significantly different (95% confidence intervals, $p>0·05$).

Table 5.10: Effect of 24h ageing of test solutions on 96h LC_{50} values for *Neomysis integer* exposed to chlorpyrifos and dimethoate. Values in brackets correspond to 95% confidence intervals. n=60 for each test except chlorpyrifos fresh-test solution (n=40).

Age of Test Solution	Chlorpyrifos	Dimethoate
'Fresh'	0·147µg L^{-1}	0·543mg L^{-1}
	(0·079 - 0·311 µg L^{-1})	(0·403 - 0·683mg L^{-1})
24h Old	>0·32µg L^{-1}	0·642 mg L^{-1}
	unknown	(0·503 - 0·814mg L^{-1})

5.4.2 Gas chromatography

Despite several modifications to the GC and the temperature programme, the gas chromatography did not give 'classical' shaped peaks as expected. As shown in Figure 5.4 (a standard sample), although the chromatography was accurate at the start of the chlorpyrifos peak, a large 'tail' was generated at the end of the peak. Therefore, peak area should give the most accurate representation of chlorpyrifos concentration (if the chromatography was perfect, the tail would not be present and the peak would be much higher). When the test solutions were analysed, the peak was split so the area could not be

used as an indicator of chlorpyrifos concentration (even combining the areas of the two split peaks would be an underestimate of the concentration due to baseline tracking problems experienced with the integrator). If peak area is used as a measure of pesticide concentration, recovery of chlorpyrifos from freshly-made test solution is only 66% (Table 5.11). Given the hydrophobic nature of chlorpyrifos, 66% recovery is lower than expected (P. Donkin, *pers. comm.*). In contrast, if peak height is used as a measure of pesticide concentration, analysis of extractions from freshly-made solutions showed that recovery of chlorpyrifos was in the order of 99%. Therefore, peak height has been used in these analyses. Since peak height data show 99% recovery, the 24h old extract concentrations may be read directly from the standard calibration graph (Fig. 5.7).

Table 5.11: Gas chromatography analysis of relative chlorpyrifos concentrations in fresh and 24h old test solution extracts. Standard values are shown for comparison. All data are for 2·5µl injections. n=5 for each sample.

Sample	Concentration (nominal)	Area (integrator units)	Height (mm) (at attenuation 6)	95% Confidence Intervals Lower	Upper
Standard	0·100	839 128	186	182	190
	0·050	468·922	100	97	103
	0·025	225 449	48	45	50
Fresh Extract	0·100	552 966	184	181	187
	0·038	183·953	66	64	68
24h Old Extract	0·100	214 135	86	82	89
	0·038	111 137	35	33	37

The linear relationship $y = 4{\cdot}84 + 1821{\cdot}72x$ (r^2=0·99) gives the chlorpyrifos concentrations in extracts from fresh-test solutions at *c.*99% of nominal concentrations and at *c.*44% of nominal concentrations after 24h (Table 5.12). Since the extraction process was almost 100% efficient, it can be assumed that the decreased concentration is due entirely to chemical breakdown of the compound (although it is not known whether this is due to photolysis, adsorption to glassware or some other mechanism).

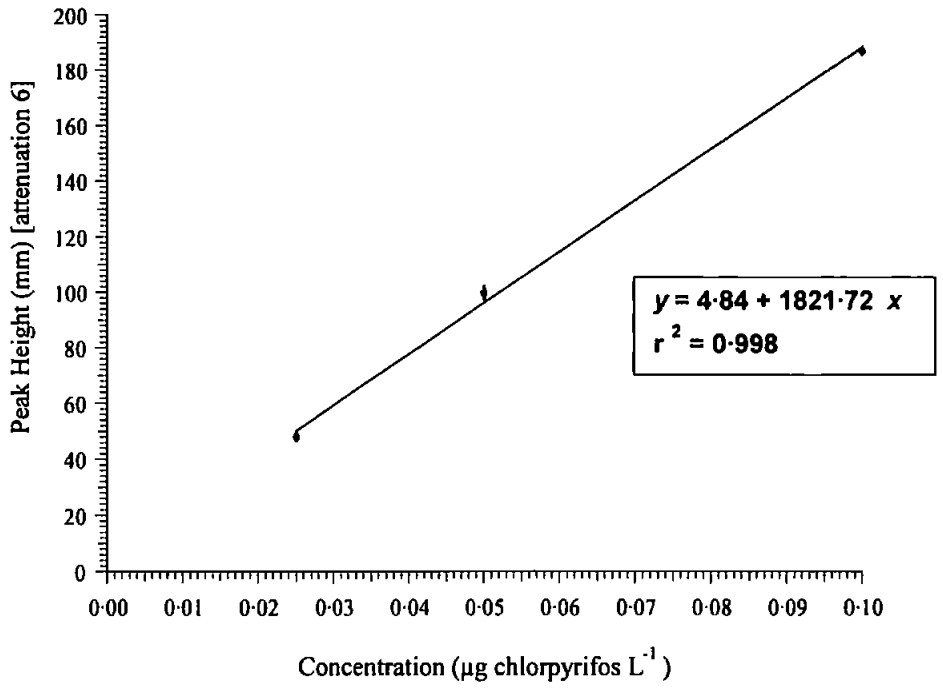


Figure 5.7: Calibration graph for estimation of chlorpyrifos in test solutions from peak height (at attenuation 6) calculated by GC. Error bars correspond to 95% confidence intervals, n=5 for each concentration. The regression equation and r^2 value are also given.

Table: 5.12: Comparison of nominal and GC measured chlorpyrifos concentrations in test solutions. n=5 for each sample concentration.

Sample	Nominal (µg L^{-1})	Measured (µg L^{-1})	95% Confidence Intervals	
			Lower	Upper
Fresh Extract	0·100	0·099	0·095	0·101
	0·038	0·034	0·031	0·036
24h Old Extract	0·100	0·044	0·042	0·047
	0·038	0·017	0·015	0·019

If 44% degradation within the first 24 is taken into account, the expected LC_{50} value for the 24h old test experiment conducted at the Brixham Environmental Laboratory would be *c.*0·212µg chlorpyrifos L^{-1} compared with the >0·32µg chlorpyrifos L^{-1} value listed earlier (Table 5.10). It must be remembered, however, that the pesticide concentration would have been 44% of nominal at the start of the test and the degradation of chlorpyrifos within the second 24h is unknown (i.e. the period of mysid exposure). This may account for the

discrepancy between the two estimates (although the initial LC_{50} is also estimated for a solution that is degrading). Analysis of the test solutions taken from the flow-through rig at Brixham showed that the chlorpyrifos test concentrations were approximately 5% higher than the nominal concentrations. These samples were treated differently from the other samples (e.g. extraction was made from only 0·5L of test solution which had been in the deep-freeze for 12 months). Also, the source of the chlorpyrifos used at Brixham was different (different suppliers are used at the Brixham Environmental Laboratory and the Plymouth Marine Laboratory).

5.5 DISCUSSION

Clearly, chlorpyrifos is more toxic to *Neomysis integer* than dimethoate, indicated by 96h LC_{50} values of 0·137µg chlorpyrifos L^{-1} and 0·543mg dimethoate L^{-1}. Comparison of these values with literature LC_{50} values shows that *N. integer* is equally sensitive to chlorpyrifos as other peracarid crustaceans used frequently in toxicity testing and more sensitive than decapods (Table 5.13). *Neomysis integer* is less sensitive to chlorpyrifos than *Americamysis bahia*. Dimethoate is equally toxic to *N. integer* as it is to other Crustacea (Table 5.13), although there are no literature data for dimethoate toxicity to *A. bahia*. From these data, chlorpyrifos appears a better choice to use as a reference toxicant than dimethoate, since much lower concentrations are needed, reducing the amount of pesticide used.

Table 5.13: Comparison of chlorpyrifos and dimethoate 96h LC_{50} estimates for *Neomysis integer* with LC_{50} values for other species of Crustacea.

Pesticide	Organism	Species	LC_{50}	Reference
Chlorpyrifos	**Mysid**	***Neomysis integer***	**0·137µg L^{-1}**	**This study**
	Mysid	*Americamysis bahia*	0·035µg L^{-1}	Schimmel *et al.* (1983)
	Amphipod	*Gammarus lacustris*	0·11µg L^{-1}	Sanders (1969)
	Crayfish	*Orconectes immunis*	6·01µg L^{-1}	Phipps & Holcombe (1985)
Dimethoate	**Mysid**	***Neomysis integer***	**0·543mg L^{-1}**	**This study**
	Amphipod	*Gammarus lacustris*	0·2 mg L^{-1}	Sanders (1969)
	Freshwater prawn	*Machrobrachium lamerri*	2·6 mg L^{-1}	Murgatroyd & Patel (1994)
	Brown shrimp	*Crangon crangon*[a]	0·3 - 1·0mg L^{-1}	" "

[a] 48h LC_{50}

Unfortunately, due to the chemical analysis of the pesticides being delayed for 12 months, dimethoate levels in the test water were not determined. It is, therefore, not possible to conclude which pesticide has the better chemical stability, recoverability or detection by GC (although 24h old LC_{50} data imply that dimethoate is much more stable than chlorpyrifos). Although the toxicity of any substance is the most important factor in toxicity tests, knowledge of the chemistry of the test substance is also important if toxicity data are to be interpreted fully. Chemical stability of the toxicant is, perhaps, the most critical aspect, since an LC_{50} estimate could be grossly underestimated if the toxicant is degraded significantly during the course of the test. Nominal test concentrations are currently acceptable for toxicity data, however, it is becoming increasingly important to state the actual test concentration measured analytically (test design is also important, for example the advantages of flow-through tests, and these will be discussed later). Chemical stability data are in the literature (e.g. CAB International & British Crop Protection Council, 1997), although such data are likely to be specific to certain conditions. Chemical analysis should, therefore, be made for every test solution, however, it is equally important to know the accuracy of any chemical analysis made. Regarding the chemical analysis made in this study, there are two important steps for which it is important to know the accuracy. Firstly, the chlorpyrifos has to be extracted from the test solution into organic solvents because the actual test solution cannot be passed through the GC. Secondly, test concentrations were very low, therefore, concentration of the extract was needed to enable pesticide detection by the GC. It was, therefore, important to know the efficiency and repeatability of the extraction and concentration techniques. Chlorpyrifos is very hydrophobic [hydrolysis half-life 1·5d (CAB International & British Crop Protection Council, 1997)] and should be extracted easily into hexane. This was tested by comparison with standards made up directly in solvent and, using peak height as a measure of chlorpyrifos concentration, the results showed that recovery was almost 100% (98·8%). Therefore, the extraction and concentration technique used is extremely efficient. When working optimally, the GC should record narrow, discrete peaks (i.e. they should not have 'tails'). Although initial problems with the GC were rectified by altering the column and run conditions, all traces of chlorpyrifos standards had tailed peaks. Peak height was, therefore, used as a measure of chlorpyrifos and gave repeatable results. The degradation of chlorpyrifos was in the order of 50% per day which is slightly faster than literature values. This discrepancy may be due to adsorption onto the test vessel, hydrolysis or aeration of the

test solution facilitating evaporation. Although the stability of chlorpyrifos in the test solution was not particularly good, the analyses and extraction of the pesticide were reliable, so that actual concentrations are known. Based on these chemical parameters, chlorpyrifos appears to be a suitable toxicant for further toxicological studies.

The problem of toxicant degradation can be significantly reduced by altering the test conditions (i.e. using a flow-through system). The GC analysis of the test solution showed that there was approximately 50% breakdown after 24h under semi-static exposure conditions (after which time the solution was changed, therefore, restoring chlorpyrifos levels to nominal concentrations). In the flow-through system, the actual chlorpyrifos concentrations were within 5% of the nominal concentrations. It would be expected, therefore, that the LC_{50} of the flow-through test would be lower than that for the semi-static. This was not the case and there was no significant difference between the LC_{50} estimates for the two types of test (0·147 and 0·134µg chlorpyrifos L^{-1} for the semi-static and flow-through tests respectively). Considering the instability of chlorpyrifos, the similarity of the two LC_{50} values was unexpected, however, the semi-static test was only a range-finding test and, therefore, the 95% confidence intervals were extremely wide (0·079 - 0·311µg chlorpyrifos L^{-1}). A definitive semi-static test should generate a better 96h LC_{50} estimate which may be different from the flow-through value. It may be that the pulsed effect of the semi-static test (i.e. restoring the chlorpyrifos concentration to nominal levels every 24h) is equally toxic to *N. integer* as continuous, flow-through exposure. This might be the case if uptake of the pesticide is rapid, such that pesticide is taken up before significant degradation occurs. Flow-through tests are preferred by the USEPA or the ASTM for regulatory testing (e.g. ASTM, 1990). In the current experiments, the only difference between the two types of test was that there were no deaths attributable to moulting in the flow-through test. In terms of pesticide exposure in the natural environment, the flow-through test is more relevant because the pulsed exposure effect of the semi-static test is extremely unlikely to occur [although it can happen after heavy rain showers (Williams *et al.*, 1994)]. The similarity of the semi-static LC_{50} and the flow-through LC_{50} was encouraging, however, as further experiments to examine the effects of sub-lethal concentrations of chlorpyrifos were carried out under semi-static exposure conditions since flow-through facilities were not available.

Neomysis integer appears to be an extremely good organism for acute toxicity testing. Individuals of *N. integer* collected from the East Looe River Estuary were easy to maintain in the laboratory and seemed unaffected by handling (mysid mortality in the holding tanks, although not monitored, was low). The only disadvantage of using *N. integer* for toxicity tests was their inherent cannibalism, especially feeding on post-moult mysids. When provided with sufficient food, however, mysid cannibalism was kept to a minimum. Any animals that died from intoxication were not eaten (*pers. obs.*) and were distinguishable from deaths resulting from cannibalism. With increasing interest in using mysid species representative of local populations (e.g. Martin *et al.*, 1989; Brandt *et al.*, 1993; Harmon & Langdon, 1996), *N. integer* appears to be a good species for European estuarine toxicity testing.

For regulatory testing purposes, it is usual that juvenile mysids (<24h) are used (ASTM, 1990), however, the current experiments show that there was no significant difference between the sensitivity of adults and <24h juveniles. The difference between adults (96h LC_{50} 0·137µg chlorpyrifos L^{-1}) and juveniles (96h LC_{50} 0·187µg chlorpyrifos L^{-1}) was, however, close to being significant and the 95% confidence intervals overlapped by only 0·003µg chlorpyrifos L^{-1}, indicating that adults were more sensitive. In general, juvenile crustaceans are thought to be more sensitive to toxicants than adults (ASTM, 1990), however, there is little evidence to support this. Green *et al.* (1996) found that the nauplii of the infaunal copepod *Amphiascus tenuiremis* had a 96h LC_{50} of 40µg chlorpyrifos kg $sediment^{-1}$, which was significantly lower than the 96hLC_{50} of either copepodites or adults (74µg and 66µg chlorpyrifos kg $sediment^{-1}$ respectively). Other researchers, however, have found juveniles to be less sensitive than adults. Key & Fulton (1993) recorded a 96h LC_{50} of 0·44µg chlorpyrifos L^{-1} for 18d old larvae of grass shrimp (*Palaemonetes pugio*); the corresponding LC_{50} for adult *P. pugio* was 0·37µg chlorpyrifos L^{-1}. Sensitivity of juveniles and adults may be species specific, toxicant specific or both. Since there was no significant difference between the sensitivity of adult and juvenile *N. integer* to chlorpyrifos, it would be interesting to test whether ovigerous females are the most sensitive life stage. Unfortunately, ovigerous females were not tested here due to time availability at the Brixham Environmental Laboratory.

Although the relative toxicity of chlorpyrifos and dimethoate to *N. integer* was an important criterion for deciding which pesticide to use for further sub-lethal work, consideration was also paid to the ecological importance of the two pesticides. Apart from maintaining the health of aquatic ecosystems, the main priority of environmental agencies responsible for the monitoring of water quality is to ensure that toxicants do not enter water that is to be used for human consumption (or at least to ensure that toxicant levels are non-harmful to humans). Most countries have their own guidelines for pesticide levels in water. For example, Canadian water quality guidelines for the protection of aquatic life allow 4μg L^{-1} of pesticide in natural waters (Struger *et al.*, 1995). European Community (EC) legislation states that no more than 0·1μg L^{-1} of any one pesticide, and no more than 0·5μg L^{-1} total pesticide loading, be present in human drinking water (Council of European Communities, 1980). In the United Kingdom, efforts are being made to comply with European legislation, however, the Department of the Environment (DoE) has produced interim maximum levels for certain pesticides (Croll, 1991). Most of the DoE maxima are higher than the 0·1μg L^{-1} specified by the EC. For example, the Environmental Quality Standards (EQS) for the protection of aquatic life and potable water sources are not to exceed 1μg dimethoate L^{-1} in freshwater or 0·2μg dimethoate L^{-1} in potable waters (Murgatroyd & Patel, 1994). These EQS are, however, annual average values and there are currently no EQS for seawater (Murgatroyd & Patel, 1994). The EC legislative maxima are not based on scientific information, but are arbitrary and based instead on 'worse case scenarios' (Urech, 1996). In terms of pesticide hazards to aquatic life, dimethoate and chlorpyrifos clearly show the problem with such arbitrary maxima. If pesticide levels had to be maintained according to EC legislation (i.e. 0·1μg L^{-1} for individual pesticides), dimethoate would not pose too much of a threat to *N. integer* since the 96hLC_{50} is 0·5mg dimethoate L^{-1}. Dimethoate toxicity to other aquatic Crustacea, for example amphipods, is also at the milligram level (Sanders, 1969). Chlorpyrifos, however, is toxic to *N. integer* at much lower concentrations, so that the EC maximum allowable level is close to the 96h LC_{50} (0·13μg chlorpyrifos L^{-1}), and, therefore, clearly a threat to mysid populations (particularly as chlorpyrifos is toxic to other crustaceans at similar concentrations). A mixture of 0·1μg L^{-1} each of dimethoate and chlorpyrifos would also be hazardous to mysids, although these concentrations are within the EC's 0·5μg L^{-1} maximum. Although neither chlorpyrifos nor dimethoate are monitored routinely in the UK (Raven & George, 1989; Williams *et al.*, 1994), the available data for these two pesticides indicate that

average annual levels are usually within the DoE recommendations (Croll, 1991). In running waters, pesticide maxima occur at, or just prior to, peak flow rates after rainfall (Williams *et al.*, 1994). After heavy rainfall, many pesticides are flushed out from agricultural soils and drainage waters may reach concentrations in excess of legislative guidelines, therefore, becoming harmful to aquatic fauna (Williams *et al.*, 1994).

Acute exposure of non-target organisms to high pesticide concentrations occurs more often following accidental spills than from agricultural run-off. In 1985, there was a large spill of Dursban 4E in Essex (England) when 2570L (carrying 1230kg chlorpyrifos active ingredient) were spilt, of which 500L (240kg chlorpyrifos) entered the local river system (Raven & George, 1989). Within 40h, the Dursban had travelled 26km downstream, killing 90% of the fish stock and all the arthropods. These immediate mortalities are not surprising, since initial concentrations of chlorpyrifos were found to be 14mg chlorpyrifos L^{-1} at the point of entry to the river, 2·5mg chlorpyrifos L^{-1} 16km downstream, and >0·3mg chlorpyrifos L^{-1} over the entire 26km stretch (Raven & George, 1989). If these concentrations are compared with the LC_{50} values shown in Table 5.2, they are clearly highly toxic to arthropods and fish. Even the tidal reaches of the river had a chlorpyrifos concentration of *c.*30µg chlorpyrifos L^{-1}, which is highly toxic to arthropods (Raven & George, 1989). Such accidental spills of chlorpyrifos (and other pesticides) may be hazardous to aquatic fauna, especially to *N. integer,* for long periods of time. After 10 weeks, water concentration was still *c.*10µg chlorpyrifos L^{-1} and intertidal silt was still contaminated (4 - 15µg chlorpyrifos kg $sediment^{-1}$) 100 weeks after the spill (Raven & George, 1989). Despite increasing criticism of laboratory tests in the literature (e.g. Depledge *et al.,* 1995), effects of this spill were extremely similar to those predicted from previous ecotoxicological studies (Raven & George, 1989).

5.6 SUMMARY

This chapter, describing acute toxicity testing at the Brixham Environmental Laboratory (ZENECA Limited), has shown *Neomysis integer* to be a suitable species for toxicity testing in the estuarine environment. *Neomysis integer* appears to be an appropriate alternative to the frequently used *Americamysis bahia* for acute toxicity testing in European

estuaries. The 96h LC_{50} data for adult *N. integer* were 0·134μg chlorpyrifos L^{-1} and 0·054mg dimethoate L^{-1}. There was no difference between the sensitivity of juvenile and adult *N. integer* to chlorpyrifos. Based on the relative toxicities of chlorpyrifos and dimethoate to *N. integer*, chlorpyrifos was chosen as a representative contaminant for the investigations into sub-lethal effects on respiration, feeding and swimming of *N. integer*.

CHAPTER 6

EFFECT OF EXPOSURE TO CHLORPYRIFOS ON THE SWIMMING BEHAVIOUR AND PHYSIOLOGY OF *NEOMYSIS INTEGER*

6.1 ABSTRACT

Mysids are used routinely by regulatory authorities for conducting acute toxicity tests (e.g. 96h LC_{50} tests) to evaluate the potential hazards of pollutants to aquatic ecosystems. Recently, the common European mysid *Neomysis integer* (Peracarida: Mysidacea) has been suggested by the Environment Agency (UK) as a suitable alternative species to the frequently used American species, *Americamysis bahia*, for testing the toxicity of chemical contaminants to European estuarine biota. Although acute lethal toxicity tests are used frequently by regulatory bodies there is a growing demand to develop toxicity tests which are more representative of the effects of contaminants on natural populations and which provide results which are more readily extrapolated to natural ecosystems. The present study investigated the effect of exposure to the organophosphate pesticide chlorpyrifos on the swimming behaviour, oxygen consumption and feeding rate of *N. integer*. The maximum swimming speed of *N. integer* was reduced and mysids became hyperactive following exposure to 0·038μg chlorpyrifos L^{-1}. Exposure to organophosphate pesticides is, therefore, predicted to disrupt the position maintenance of natural mysid populations. Following exposure to 0·038μg chlorpyrifos L^{-1}, mysid oxygen consumption was increased; feeding rate was suppressed at 0·072μg chlorpyrifos L^{-1}. Scope for growth (an integrated approach to measure the health or physiological status of an organism) was reduced (and was negative in some cases) following exposure to 0·072μg chlorpyrifos L^{-1}. Oxygen consumption was considered the physiological process most sensitive to chlorpyrifos exposure, but the response in feeding rate was more representative of pesticide effects on natural mysid populations. Swimming behaviour was equally sensitive to pesticide contamination, and has large implications for natural populations. The disruption of behaviour and physiological processes by exposure to chlorpyrifos is discussed in relation to how natural mysid populations might be affected by pesticide exposure.

6.2 INTRODUCTION

In the natural environment, concentrations of contaminants are generally much lower than the LC_{50} values estimated in the laboratory (Gaudy *et al.*, 1991). Measurement of some aspect of an organism's physiology or behaviour, following contaminant exposure, provides a better understanding of the likely environmental consequences of the toxicity of a contaminant than lethal effects, particularly if the toxicant is at environmentally realistic concentrations. Physiological parameters used frequently in toxicological bioassays include respiration (e.g. Gaudy *et al.*, 1991), feeding (e.g. Crane & Maltby, 1991), excretion (e.g. McKenney & Edwards, 1990), growth (e.g. Toda *et al.*, 1987) and reproduction (e.g. McKenney & Celestial, 1996). Behavioural responses used to determine the sensitivity of an organism to a toxic contaminant include swimming speed (e.g. Wilson, 1996) and activity (e.g. Sørensen *et al.*, 1995). Integrated sublethal bioassays, combining two or more of these individual responses, are also used frequently in environmental monitoring [e.g. 'scope for growth' (Widdows, 1993)]. In the current study, the effects of toxicants on the behaviour, oxygen consumption and feeding rate of aquatic invertebrates are of particular interest.

6.2.1 Effects of toxic contaminants on behaviour

Several behavioural responses have been used to assess toxicant effects on invertebrates, including distance travelled, travel velocity, frequency of changes in direction and response to light (e.g. Lang *et al.*, 1981; Bayley & Baatrup, 1996; Wilson, 1996). Significant changes in activity, average velocity, frequency of movement, frequency of turning and distance travelled were reported for woodlice (*Porcellio scaber*) after application of the organophosphate dimethoate to soil, and the type of response was dependent upon the concentration of dimethoate applied (Bayley & Baatrup, 1996). Similar responses in locomotory behaviour were reported for the collembolan *Folsomia candida* (Sørensen *et al.*, 1995). In aquatic organisms, the behavioural response used most frequently in toxicity studies is swimming velocity [sometimes termed 'critical swimming velocity' (e.g. Brett, 1964)]. Teleost fish have been used most frequently in swimming speed experiments. For example, the critical swimming speed of juvenile rainbow trout (*Onchorhynchus mykiss*) was significantly reduced following exposure (24h) to aluminium (Wilson & Wood, 1992). Comparatively few data are available on the effects of contaminants on the swimming speed of invertebrates. Following a 96h exposure to copper (20 - 160µg L^{-1}), the

spontaneous mean linear velocity of barnacle nauplii (*Balanus improvisus*) decreased (Lang *et al.*, 1981). Exposure to similar concentrations of cadmium also significantly reduced the mean linear velocity of barnacle nauplii, although not to the same extent as copper (Lang *et al.*, 1981). There is a large literature on the swimming behaviour of mysids (Chapter 2), although there have been relatively few investigations of the disruption of mysid behaviour by toxicants. The swimming activity (defined as the number of cm travelled in one minute) of *Mysidopsis juniae* was significantly increased by exposure to 5µl benzene L^{-1}, but decreased following exposure to 20µl benzene L^{-1} (Martinez *et al.*, 1992). Exposure to the organophosphate pesticides phorate and methyl parathion caused a general suppression of the maximum swimming speed of *Americamysis bahia*, however, only at the highest concentration of phorate (0·18µg phorate L^{-1}) was the effect significantly different from the controls (Cripe *et al.*, 1981). Other crustacean behavioural responses affected by toxicants include reduced phototaxic response of barnacle nauplii exposed to cadmium (Lang *et al.*, 1981) and reduced tail-flip escape responses in grass shrimp *Palaemonetes pugio* exposed to the organophosphate pesticide parathion (Farr, 1977).

6.2.2 Effects of toxic contaminants on oxygen consumption

The oxygen consumption of any organism exposed to a chemical contaminant may change directly due to metabolic stress or indirectly due to the metabolic demands of detoxification (Smith & Hargreaves, 1985; Gaudy *et al.*, 1991). For aquatic invertebrates, oxygen consumption may change as a result of toxicant exposure or remain unaffected. For example, the oxygen consumption of juvenile blue crabs (*Callinectes sapidus*) decreased following exposure to 100µg cadmium L^{-1}, a concentration below the LC_{50} for this species (Guerin & Stickle, 1995). In contrast, oxygen consumption of the amphipod *Gammarus pulex* was unaffected by sub-lethal (0·1mg L^{-1}) exposure to zinc (Maltby & Naylor, 1990).

Trace metals, pesticides and oil fractions have been reported to affect the oxygen consumption of mysids (Table 6.1). The respiration rate of the coastal species *Leptomysis lingvura* decreased significantly after exposure to 0·1mg cadmium L^{-1} (Gaudy *et al.*, 1991). Juvenile *Americamysis bahia* increased their oxygen consumption following exposure to the thiocarbamate herbicide 'thiobencarb', with oxygen consumption rates increasing as the concentration was increased from 100 to 700ug thiobencarb L^{-1} (McKenney, 1985). A similar increase in respiration rate with increasing contaminant concentration was reported

Table 6.1: The response of mysids to toxicants as measured by oxygen consumption.

Species	Contaminant	Conditions	Concentration	Control	Exposed	Reference
				(µl oxygen mg wet wt.$^{-1}$ h^{-1})		
Americamysis bahia	Fenthion (pesticide)	exposure from hatching; 25°C; 24h old juvenile	300ng fenthion L^{-1}	0·25	0·41	McKenney & Matthews (1990)
	Thiobencarb (pesticide)	exposure from hatching; 25°C; 24h old juvenile	0·2mg thiobencarb L^{-1}	0·23	0·31	McKenney (1985)
		,, ,,	0·8mg thiobencarb L^{-1}	0·23	0·27	
Leptomysis lingvura	Cadmium (metal)	exposure period not stated; 18°C; female	0·10mg cadmium L^{-1}	0·11	0·10	Gaudy *et al.* (1991)
		,, ,,	0·25mg cadmium L^{-1}	0·11	0·09	
Neomysis americana	naphthalene (hydrocarbon)	acute exposure; predicted mean; 15°C; female	0·2mg naphthalene L^{-1}	0·33	0·44	Smith & Hargreaves (1985)
		,, ,,	1·0mg naphthalene L^{-1}	0·33	0·54	
N. integer	light fuel oil (hydrocarbon)	1d exposure; 6°C	unspecified	*c.* 0·17	*c.* 0·19	Laughlin & Lindén (1983)
		15d exposure; 6°C	,,	*c.* 0·20	*c.* 0·19	

for juvenile *A. bahia* exposed to the organophosphate insecticide 'fenthion' [i.e. 50 - 300ng fenthion L^{-1} (McKenney & Matthews, 1990)]. Smith & Hargreaves (1985), using the aromatic hydrocarbon naphthalene as a model of petroleum toxicity, reported that the oxygen consumption of *Neomysis americana* was altered depending on the concentration of naphthalene to which the mysids were exposed. Exposure to *c.*1·1mg naphthalene L^{-1} caused an increase in oxygen consumption, whilst exposure to *c.*0·1mg naphthalene L^{-1} reduced oxygen consumption (Smith & Hargreaves, 1984). In contrast, the oxygen consumption of *Neomysis integer* was unaffected by exposure to water soluble fractions of light fuel oil (Laughlin & Lindén, 1983).

6.2.3 Effects of toxic contaminants on feeding

Feeding (measured by a variety of methods) is affected frequently by exposure to many toxic contaminants and is often used as an endpoint in toxicity bioassays (e.g. Crane & Maltby, 1991; Bitton *et al.*, 1996). A one hour toxicity test has been developed based on whether or not the cladoceran *Ceriodaphnia dubia* will feed on yeast (stained with fluorescent dye) after exposure to a toxicant (Bitton *et al.*, 1996). More specifically, feeding rate is measured to determine toxicological disturbance (Table 6.2). The feeding rate of the blue crab (*Callinectes sapidus*) was significantly reduced after exposure to 100µg cadmium L^{-1} (Guerin & Sickle, 1995), however, there was no significant concentration effect (Guerin & Sickle, 1995). Feeding rate of *Gammarus pulex*, measured *in situ*, was affected by anthropogenic inputs to the natural environment, but varied also between different control populations (Crane & Maltby, 1991). For example, *G. pulex* located downstream of effluent discharges from a sewage treatment works, a quarry and a paper mill all showed decreased feeding rates compared with control populations upstream of the discharges (Crane & Maltby, 1991). Trace metals also affect the feeding of *G. pulex* (Maltby & Naylor, 1990). Exposure to 0·1mg zinc L^{-1} significantly reduced the amount of energy absorbed by *G. pulex* [the authors did not report whether the decrease in energy absorbed was due to reduced food consumption, reduced absorption efficiency, or both (Maltby & Naylor, 1990)].

There are few data on the effect of chemical toxicants on the feeding rate of mysids, however, toxicant-induced changes on mysid feeding rate have been reported. Making the assumption that the number of faecal pellets produced by small crustaceans is proportional to food intake, Gaudy *et al.* (1991) reported that exposure to 0·1mg cadmium L^{-1}

Table 6.2: Some effects of toxicant exposure on the feeding rates of some peracarid crustaceans. Feeding rate data are mg food eaten mg dry wt^{-1} day, except for mysid data (number of faecal pellets $mysid^{-1}$ h^{-1}).

Species	Contaminant	Conditions	Concentration	Control	Exposed	Reference
				mg food mg dry wt^{-1} h^{-1}		
AMPHIPODA						
Gammarus pulex	Malathion 60 (pesticide)	*in situ* measurement; caged 'below bed'	600ml *c.*4% solution Malathion 60 sprayed over $900m^{-3}$	0·041	0·023	Crane *et al.* (1995)
		in situ measurement; caged 'below pool'	" "	0·039	0·055	" "
	sewadge sludge	effluent discharge to river	unspecified	*c.* 0·075	*c.*0·050	Crane & Maltby (1991)
	quarry effluent	" "	" "	*c.* 0·25	*c.* 0·15	" "
	paper mill effluent	" "	" "	*c.* 0·25	*c.* 0·08	" "
MYSIDACEA						
Leptomysis lingvura	cadmium (metal)	exposure period not stated; 14°C; female	0·1mg cadmium L^{-1}	100	60	Gaudy *et al.* (1991)

suppressed the feeding rate of *Leptomysis lingvura*. At high temperatures (>20°C), however, exposure to cadmium led to increased feeding rates (Gaudy *et al.*, 1991).

6.2.4 Scope for growth

Rate of growth is a fundamental measure of physiological fitness/performance and provides one of the most sensitive measures of stress in an organism (Widdows, 1993). Measurement of growth potential (or scope for growth) has proved to be particularly useful in assessing the biological effects of pollution (Widdows & Johnson, 1988; Widdows & Donkin, 1992). Scope for growth (SFG) provides a rapid instantaneous measurement of the energy status of an organism (i.e. without the time-consuming measurement of actual growth rates), and provides insight into the underlying mechanisms of toxicity and the factors which affect growth rate (Widdows, 1993). The energy budget of an animal represents a basic integration of the basic physiological responses such as feeding, food absorption, respiration, excretion and production (Fig. 6.1) [Widdows, 1993; Widdows & Salkeld, 1993]. Scope for growth (SFG), therefore, is determined by measurement of respiration rate, feeding rate, absorption efficiency and excretion rate of an organism followed by transformation of these rates into energy equivalents (Joules h^{-1}) [Widdows & Salkeld, 1993]. Energy lost through metabolism and excretion is subtracted from the energy absorbed from feeding, and any excess energy is then available for growth and reproduction. Scope for growth may range from maximum positive values under optimum conditions, declining to negative values when the organism is severely stressed and utilising body reserves (Widdows, 1993).

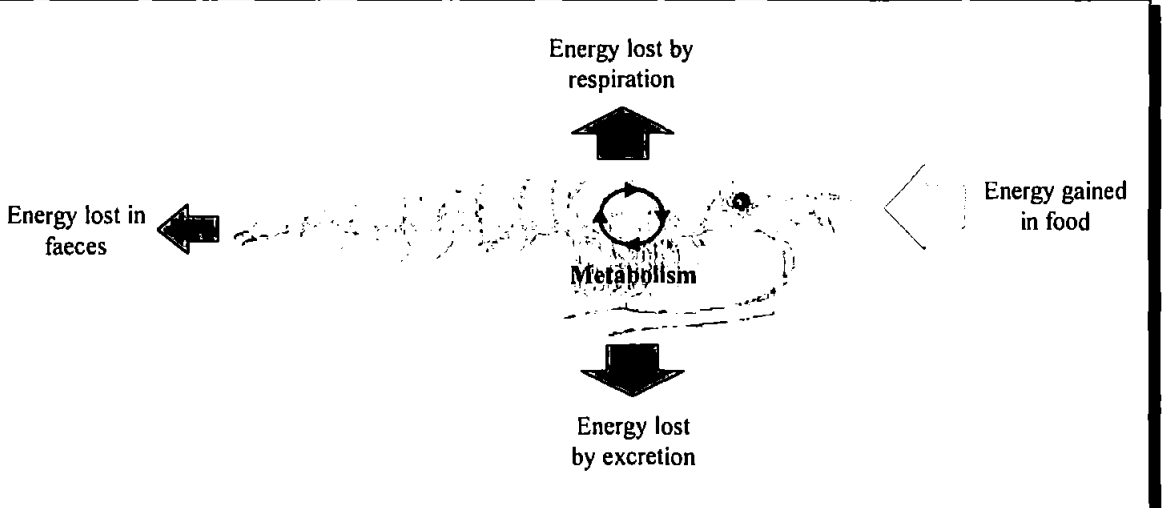


Figure 6.1: A schematic representation of the energy balance for a mysid, highlighting the physiological parameters measured for scope for growth.

Scope for growth has been used widely in pollution studies, particularly with bivalve molluscs [e.g. the blue mussel, *Mytilus edulis* (Widdows & Johnson, 1988, Widdows & Donkin, 1992)], and there is increasing interest in using this bioassay to study toxicant effects on other aquatic invertebrates (e.g. Naylor *et al.*, 1989). Species, other than bivalve molluscs, used in scope for growth bioassays include teleost fish (e.g. Malloy & Targett, 1994), polychaete worms (e.g. Ropert *et al.*, 1996), cladocerans (e.g. Baillieul *et al.*, 1996), copepods (e.g. Carlotti & Nival, 1991), corals (e.g. Edmunds & Davies, 1989), amphipods (e.g. Maltby & Naylor, 1990) and decapod crustaceans (e.g. Wang & Stickle, 1988). To date, scope for growth *per se* (i.e. as described by Widdows & Salkeld, 1993) has not been investigated for mysids, although investigations into other aspects of their energy budget have been made (e.g. Carr *et al*, 1985; Toda *et al.*, 1987).

Scope for growth is considered a better indicator of physiological condition than feeding rate (the most sensitive component of the scope for growth assay) because SFG is relatively independent of seasonal variations in feeding and/or respiration rates (e.g. Widdows *et al.*, 1995). Scope for growth may also eliminate any individual variation in physiological processes. Reduction in feeding rate is accompanied frequently by a reduction in respiration rate, therefore, scope for growth remains relatively unaffected. In contrast, if feeding of the same individual is considered in isolation, the reduction of feeding rate would imply that the organism is challenged energetically.

6.2.5 Aim

Having measured the swimming behaviour, oxygen consumption and feeding responses of *Neomysis integer* to naturally occurring estuarine variables (Chapters 2, 3, and 4), and established the acute toxicity of the organophosphorus insecticide chlorpyrifos to *N. integer* (Chapter 5), the effect of exposure to sub-lethal concentrations of chlorpyrifos on the swimming behaviour, oxygen consumption and feeding of *N. integer* is examined in this chapter. In addition, the integrated response of scope for growth was calculated.

6.3 METHODS

6.3.1 Animal collection and maintenance

From autumn 1996 to spring 1997, mysids were collected from the East Looe River Estuary as described in Chapter 2. Over this sampling period, the population comprised adults and juveniles of various generations (Chapter 1). Mysids were returned to the Plymouth Marine Laboratory and placed in a shallow, 15L holding tank at a salinity of 10 ±1‰ (made by combining filtered seawater and double distilled, de-ionised water) in a constant-temperature room (15 ±1°C). An under-gravel filter was used to maintain water quality. Lighting was provided at ambient laboratory levels by overhead fluorescent lights connected to a time-switch producing a 16h light:8h dark photoperiod. Unfortunately, a gradual increase and decrease of light intensity ('dawn-dusk sequence') was not possible. Mysids were fed as described in Chapter 2.

6.3.2 Exposure to chlorpyrifos

Mysids were exposed to various concentrations of chlorpyrifos for various time periods (48, 96 and 168h) prior to their use in experiments. Based on LC_{50} estimates (Chapter 5), concentrations of 0·038, 0·056, 0·072, and 0·100µg chlorpyrifos L^{-1} were chosen for these sub-lethal experiments. Most of these concentrations were below the 7d LC_{50} of 0·084µg chlorpyrifos L^{-1} (Chapter 5). Exposure to 0·100µg chlorpyrifos L^{-1} was included because the estimated 7d LC_{50} was for a flow-through test (Chapter 5), whereas the exposure regime for these sub-lethal tests was semi-static and, therefore, the 7d LC_{50} was likely to be higher [flow-through test are considered to be more sensitive than semi-static ones (Chapter 5)].

The protocol for exposing the mysids to the pesticide was modified from the semi-static toxicity tests (Chapter 5) as follows. An initial pesticide stock solution was made by dissolving 10mg of chlorpyrifos in 10ml of analytical grade acetone producing an initial concentration equivalent to 1g L^{-1}. Three serial dilutions were made from this stock solution to make a final experimental stock solution of 1mg L^{-1}. The other stock solutions used in the dilution series were stored in a freezer (*c.*-18°C) until they were required to make more 1mg L^{-1} stock (all solutions stored in the freezer were allowed 30min prior to use for equilibration to room temperature). Exposure solutions were made by mixing the required amount of pesticide stock solution with dilution water (Chapter 5). Three 10L

plastic buckets were filled daily with water of the correct salinity (10 ±1‰, made by combining filtered seawater and mains water), and aerated overnight to ensure oxygen saturation and to remove any chlorine from the mains water prior to use. One litre of dilution water was poured into clean 2L conical flasks, pre-marked accurately at 2L by the addition of 2000ml from a measuring cylinder. The water was stirred at high speed by magnetic followers to create a vortex into which the correct amount of pesticide stock was injected via an adjustable Gilson pipette. The volumes of stock injected were 76, 112, 144, and 200µl of the 1mg chlorpyrifos L^{-1} stock, and equalising volumes of acetone (124, 88, 54 and 0µl respectively) were added to the exposure vessels to standardise the volume of acetone (Chapter 5). This protocol resulted in the desired test exposure concentrations of 0·038, 0·056, 0·072, and 0·100µg chlorpyrifos L^{-1} respectively, after each conical mixing flask had been topped up to the 2L mark with dilution water. In addition to the four pesticide exposure vessels, an acetone control was set up. It had already been established that exposure to 100µl acetone L^{-1} did not adversely affect mysid survival (Chapter 5), however, it was unknown whether the acetone would cause sub-lethal effects. A full control (containing only dilution water) was also included. The test solutions were mixed for 5min, to ensure that the pesticide was mixed homogeneously with the dilution water, and 1·5L of each concentration was poured into separate exposure vessels. The exposure vessels were 2L tall-form glass beakers, with filtered compressed air providing gentle aeration via silicone tubing and glass pipettes. The basic exposure apparatus (Plate 6.1) comprised four replicate exposure vessels at each test concentration (number of vessels set up depended upon how many mysids were required for each experiment). Ten mysids of equal length (12 ±1mm from anterior margin of rostrum to tip of telson), chosen randomly from the holding tank, were added to each exposure vessel. More mysids were exposed to each test concentration than were required to compensate for any mortalities during the exposure period. Transferral and general handling of mysids, made with a 1mm cotton mesh hand net, was kept to a minimum and any mysid that was dropped or injured during the handling procedures was removed from the experiment. Mysids were fed twice daily with <48h old *Artemia* nauplii supplied in sufficient quantity to ensure that there was always an excess of nauplii. Exposure solutions were replaced daily and the number of mortalities (defined as a lack of response when mechanically stimulated, Chapter 5) in each vessel was recorded at this time. Pesticide exposure was made in a controlled-temperature room (15 ±1°C) with lighting provided from fluorescent tube lighting controlled with a

time-switch to produce a 16h light:8h dark photoperiod (without a dawn and dusk sequence).

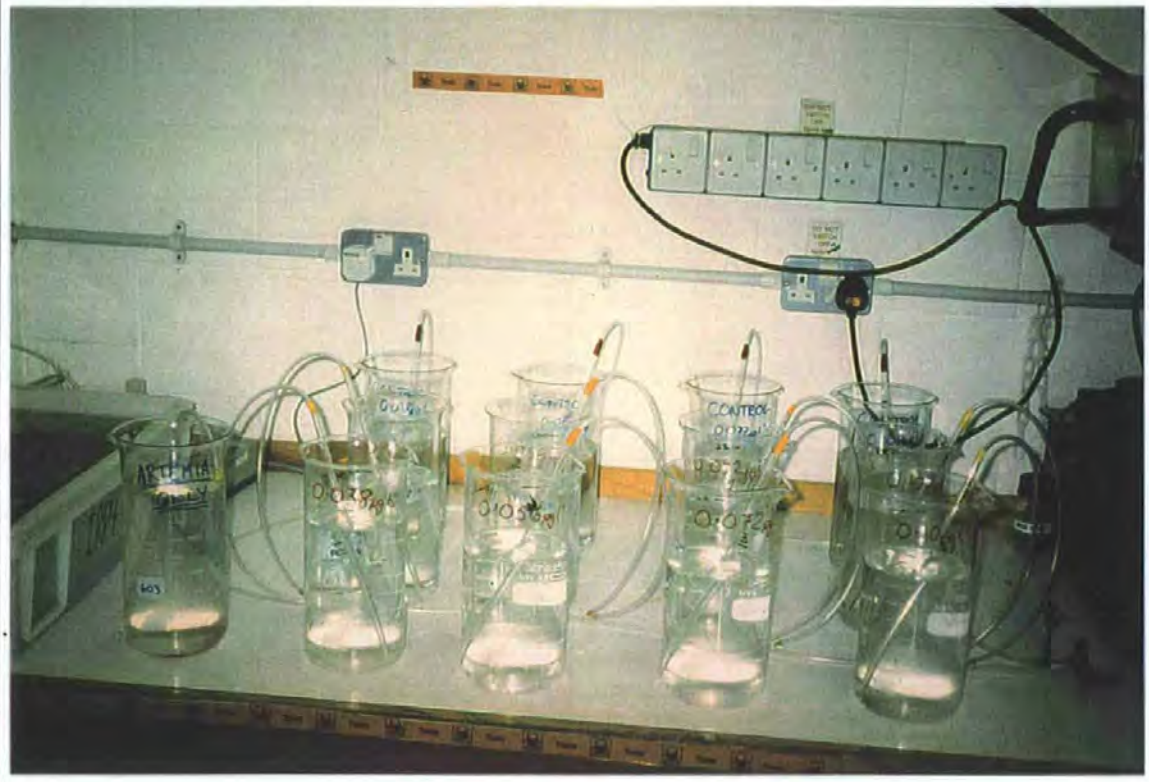


Plate 6.1: Apparatus for the semi-static exposure of *Neomysis integer* to chlorpyrifos.

Mysids were exposed to chlorpyrifos for various periods (48, 96 and 168h) prior to their use in the experiments. Animals which showed obvious signs of pesticide poisoning (e.g. irregular swimming, or repetitive and exaggerated tail-flip escape responses) were not used in the experiments. Whilst exposing mysids to chlorpyrifos collected during the autumn, however, a problem of high mysid mortality became apparent. During the pesticide exposure, a significant number of control mysids (in some tests 100%) died. High mortalities were recorded also in the pesticide-exposed mysids, with no apparent concentration-dependent response or obvious trend in the mortality data. An investigation into water quality (using seawater from a different source) and exposure conditions (altering the temperature and photoperiod) did not explain the high mortalities recorded in all exposure vessels. High mortalities occurred for *c.*4 weeks, however, mysids collected from Looe after this period survived well (i.e. 100% of control animals survived), and

showed a concentration-dependent survival response when exposed to chlorpyrifos. These latter *N. integer* were smaller (*c.*12 ±1mm from anterior margin of rostrum to tip of telson) than those used initially (*c.*16 ±1mm). It was concluded that the larger mysids from the summer were probably dying due to age-related factors. The smaller mysids of the overwintering and spring generations were, therefore, adopted for use in these chlorpyrifos experiments to ensure that mysid responses were a direct result of pesticide exposure.

6.3.3 Swimming experiments

Modifications made to the protocol developed in Chapter 2

Mysids used in the swimming experiments were from the over-wintering population (Chapter 1) and were of standard length (12 ±1mm from anterior margin of rostrum to tip of telson). The general experimental protocol to examine the effect of chlorpyrifos on the swimming behaviour of *Neomysis integer* was similar to that described in Chapter 2, except for the following modifications.

To remove the risk of chlorpyrifos contaminating the flume, all experiments were conducted under 'standard' conditions using water devoid of chlorpyrifos. Water of 10 ±1‰, made by combining filtered seawater with mains water, was added to the flume and recirculated through an Eheim Filter (Model 2213). Mysids, exposed for 7d to each chlorpyrifos concentration, were placed in the flume and tested. As each experiment was completed within 4h of mysid placement in the flume, it was assumed that there was negligible depuration of chlorpyrifos from the mysids. All flume experiments were conducted in a controlled-temperature room (±15°C). The second modification was to remove the sediment from the flume. As substratum type significantly influenced mysid swimming behaviour (Chapter 2), the pesticide swimming experiments were carried out devoid of substratum, leaving the perspex base of the flume bare. It was assumed that lack of substratum (for the mysids to hold on to or bury into) would allow easier monitoring of swimming behaviour. Removal of the substratum had the additional advantage that the flume base remained uniform at high current speeds (i.e. re-suspension or movement of the sediment could not occur). The silicone sealant was removed from the side and base of the flume, and replaced with a smaller and smoother layer of silicone sealant. The latter reduced the ability of mysids to hold on to the small irregularities in the surface of the sealant. Despite these modifications, at high current speeds, some mysids were still able to

cling onto the silicone sealant, scratches or other irregularities in the surface of the flume's perspex base and sides. The third major modification of the protocol was that the video camera was not used. Instead, mysid swimming behaviour was recorded by eye. Although the video camera provided a permanent record of the experiment, the camera filmed only a small segment (*c.*25%) of the flume at any one time, requiring direct observation to supplement mysid behaviour recordings (Chapter 2). To record mysid swimming behaviour by eye, a score sheet was produced with boxes for each behaviour category (defined in Chapter 2) at heights of 0 to 15cm above the substratum. Mysids swimming >15cm above the substratum were counted as one height group (although each behavioural category was recorded). At each current velocity, all mysids were observed and a tick was placed in the appropriate box (i.e. behaviour type and height above substratum). This method ensured that all mysids were counted and that the same mysid was not recorded twice.

Examination of swimming behaviour after exposure to chlorpyrifos

Following the 7d pesticide exposure period, 20 mysids, chosen at random from the 4 exposure vessels, were placed in the flume and left for 1h to recover from handling stress and become accustomed to the flume. After this acclimation period, the drive plate was used to create a current speed of 3cm s^{-1}. Following 5min acclimation to the new current speed, mysid swimming behaviour was monitored. The current speed was then increased to 6cm sec^{-1} and the mysids were left for a further 5mins to acclimate to the faster current speed before their behaviour was recorded. This protocol was repeated for current velocities of 9, 12, 15, and 18cm s^{-1}. The mysids were then removed from the flume. This procedure was repeated 10 times for mysids from each chlorpyrifos exposure concentration and for mysids from the acetone control (i.e. 200 mysids were tested at each pesticide concentration and the two controls).

6.3.4 Oxygen consumption experiments

For the respiration experiments, mysids were from the spring generation (Chapter 1) and of standard length (12 ±1mm from anterior margin of rostrum to tip of telson). Measurements of oxygen consumption were made after mysids had been exposed to the four chlorpyrifos concentrations (0·038, 0·056, 0·072 and 0·100µg chlorpyrifos L^{-1}), the acetone control (100µl acetone L^{-1}) and the full control for 48, 96 and 168h. As the oxygen consumption of male and female mysids was shown to differ significantly (Chapter 3), only adult males

were used in these experiments. Nine mysids were tested for each exposure period at each pesticide concentration.

Oxygen consumption was measured using the protocol described in Chapter 3. Strathkelvin 1302 'Clark-type' polarographic electrodes were used to measure the decline in dissolved oxygen from 25ml perspex respiration chambers. Each oxygen electrode was connected to a Strathkelvin 781b Oxygen Meter, coupled to a multi-channel chart recorder (in contrast to Chapter 3 where readings were taken manually from the oxygen meters). Oxygen consumption was measured in freshly-made solutions of the same pesticide concentration to which the mysid had been exposed. Each chlorpyrifos test solution (made according to the methods for pesticide exposure; Section 6.2.2) was aerated in a water bath at 15 ±1°C for 30mins to allow the solution to reach thermal equilibrium and become oxygen saturated. The respiration chambers were filled with the pesticide-spiked seawater, a single mysid was added to each chamber and the oxygen electrodes were placed in the holders (Chapter 3). Mysid oxygen consumption was recorded over 90min. Following removal from the respiration chamber, mysids were blotted dry and wet-weighed (±0·01mg) for weight-specific expression of oxygen consumption (Chapter 3).

6.3.5 Feeding experiments

For the feeding experiments, mysids were from the spring generation (Chapter 1) and of standard length (12 ±1mm from anterior margin of rostrum to tip of telson). Feeding rate was measured after mysids had been exposed to the four chlorpyrifos concentrations (0·038, 0·056, 0·072 and 0·100µg chlorpyrifos L^{-1}), the acetone control (100µl acetone L^{-1}) and the full control for 48, 96 and 168h. Although faecal production [≡ feeding rate (Chapter 3)] of male and female mysids did not differ significantly (Chapter 4), to enable scope for growth of *N. integer* to be calculated, only adult males were used (i.e. the same as for oxygen consumption measurements).

Method development

Measurement of feeding rate was based on the protocol developed for the collection of faecal pellets (Chapter 4). It was necessary to modify the details of the protocol, however, due partly to equipment availability and partly to the incorporation of the pesticide into the experiments which led to variable results. To overcome the latter, several experiments were

made consecutively to develop, and further improve, the protocol and reproducibility of results. Development of the new protocol is described in this section.

The initial protocol was similar to that described in Chapter 4, apart from changes made due to pesticide use and equipment differences (these experiments were conducted at the Plymouth Marine Laboratory as opposed to the University of Plymouth). Instead of plastic jars (used in Chapter 4), 2L tall-form glass beakers were used as the test vessels since these were considered to be easier to decontaminate after containing chlorpyrifos solution. Sieved sediment slurry (100ml) was added to 20 exposure vessels and, once the sediment had consolidated (after *c.*1h), 1L of chlorpyrifos-spiked seawater was added to each vessel, pouring over a 'bubble-wrap' disc to prevent disturbance of the sediment (Chapter 4). One mysid was placed in each vessel and allowed to feed for 24h. The mysid was then removed, oven-dried (24h at *c.*100°C) and weighed (±0·01mg) using a Sartorius R200-D balance. Faecal material was washed with 0·14M ammonium formate solution to remove any seawater salts. At 0·14M, ammonium formate is approximately isosmotic with the experimental salinity (10‰) and provided a means of washing the faeces without inducing osmotic stress (Widdows & Salkeld, 1993). Faeces were transferred into glass test tubes and 5ml of 0·14M ammonium formate solution was injected by pipette, resulting in the re-suspension and agitation of the faecal material, which was then allowed to settle to the bottom of the tube. The supernatant was drawn off with a Pasteur pipette, and a further 5ml of ammonium formate was injected into the tube and the supernatant drawn off. Following the addition of a third 5ml injection of ammonium formate, the solution and faecal material was pipetted carefully into marked, ashed and pre-weighed individual aluminium trays. The aluminium trays were placed in a Gallenkamp drying oven (Model 2) at *c.*100°C for 48h to ensure that the material was dry and that all the ammonium formate salts had been removed. The aluminium trays were re-weighed to calculate the dry weight of faeces, ashed at 450°C for 1h in a muffle furnace, allowed to cool and weighed again to calculate the ash-free dry weight. Since trace amounts of ammonium formate residue still remained after 48h at 100°C, blank aluminium trays containing equal amounts (5ml) of ammonium formate solution were also dried to enable weight correction for any salt residues.

The feeding rates of 20 mysids were measured at each pesticide concentration. The feeding rates of mysids exposed to different pesticide concentrations, and mysids from the two

controls, were measured on different days. Dry-weight, and ash-free dry-weight, of faeces were converted to weight-specific data by dividing through by the appropriate mysid dry-weight. The results obtained by this protocol showed large variability (Fig. 6.2) and the protocol was considered to be inaccurate for distinguishing responses to the different experimental conditions.

bar?

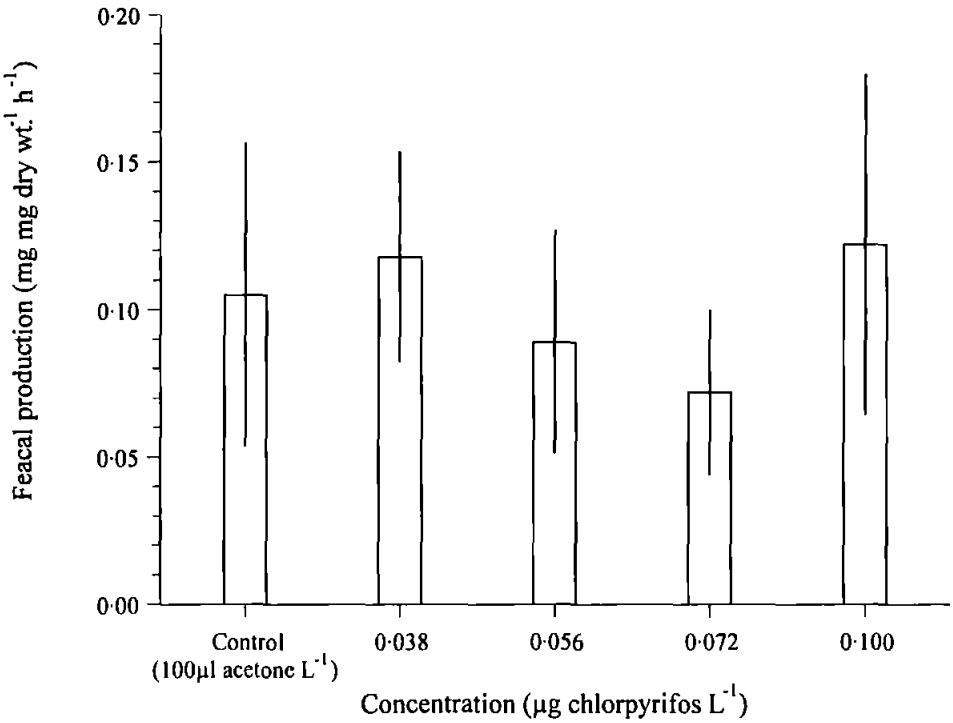


Figure 6.2: Faecal production of mysids following exposure to chlorpyrifos, highlighting the variability in results encountered using the initial experimental protocol.

The reason for the variance was unknown, however, there were several possibilities. Firstly, although blank aluminium trays were used to account for any salt residue, visual inspection of the aluminium trays revealed that the formate residue did not appear to be uniform in all trays. Secondly, although the trays were weighed accurately (±0·01mg), the faecal material was extremely small compared with the weight of the aluminium trays (*c.*2g). Any changes in weight of the aluminium tray could, therefore, greatly affect the weight of faecal material calculated by subtracting tray weight. Several modifications to the initial protocol were made in an attempt to rectify these problems.

The exposure regime and the setting up of the feeding experiment was kept the same as described for the initial protocol, but changes to the collection and analytical stages of the protocol were made. Washing the faeces in ammonium formate caused an unquantifiable residue to appear in the aluminium trays, so it was decided to revert to washing the faecal material in double distilled, de-ionised water as used in the protocol described in Chapter 4. Since the aluminium trays were considered to be too heavy, relative to the material collected, smaller drying vessels were used. Aluminium cups were made by moulding aluminium foil into a thimble shape (*c.*10mm diameter × 15mm deep). Faecal material was added to pre-ashed and weighed aluminium cups, oven dried (*c.*100°C for 48h) and weighed (±0·01mg). Again the results were highly variable (Fig. 6.3) and it became apparent that there was considerable variation of faecal production, even of control mysids, on a daily basis. It was also apparent that a residue was collecting on the inside of the aluminium cups which was affecting considerably their weight.

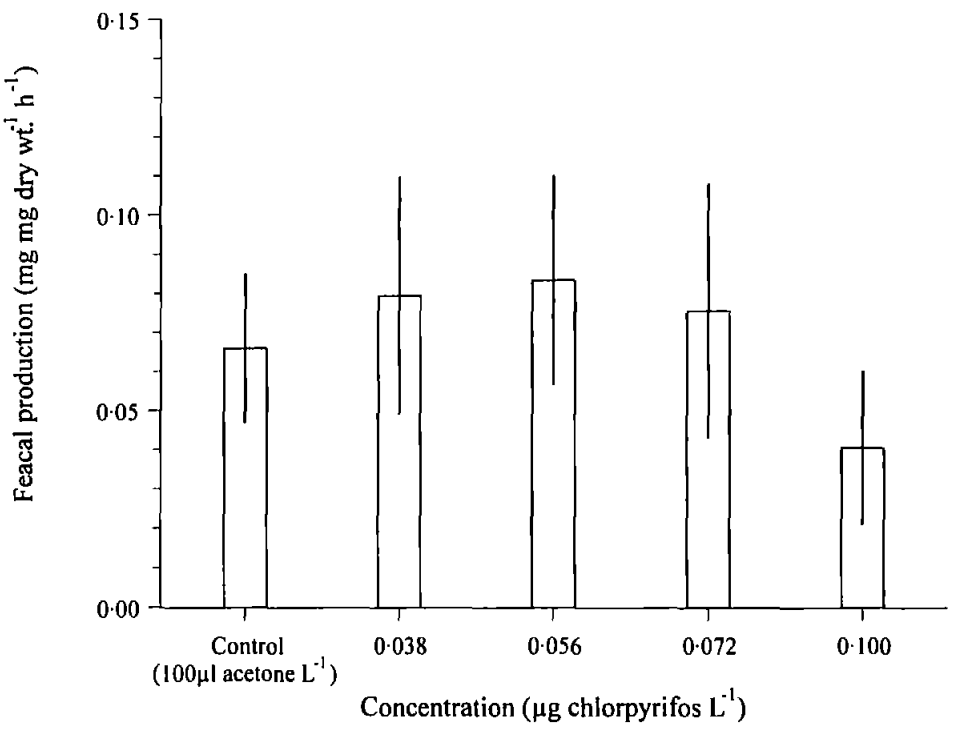


Figure 6.3: Faecal production of mysids following exposure to chlorpyrifos, highlighting the variability in results encountered using the revised experimental protocol.

The latter was due possibly to oxidation of the aluminium by the water in the cups, as it only appeared in the portion of the cups where the water had been. The weight change due to this oxidation residue was difficult to quantify even using blank cups filled with just water and was not correlated with any particular parameter of the aluminium cup (e.g. weight or height). The protocol was, therefore, modified further. Due to the frequent problem of weight change in the drying vessels, it was decided that a chemically-inert container should be used when drying the faeces. Plastic Eppendorf tubes were chosen to replace the aluminium cups (after testing that they were stable at 80°C, a lower drying temperature being chosen due to the plastic nature of the Eppendorf tubes). Faecal material was rinsed with double distilled, de-ionised water on the sieve (as before) and washed into clean glass test-tubes, from which they were transferred into dry, pre-weighed and labelled Eppendorf tubes. The Eppendorf tubes were oven-dried at *c.*80°C for 48h and re-weighed to calculate the dry weight of faecal material. Blank Eppendorf tubes (containing just double distilled de-ionised water) were treated in the same manner to account for any weight change due to drying effects or daily humidity changes. The Eppendorf tubes proved to be very stable and weight changes due to drying or humidity were never greater than 0·02mg, allowing accurate calculation of faecal material. Due to the Eppendorf tubes being plastic, the faecal material did not adhere to the sides of the tubes so faecal material could be removed easily (a necessity for ashing at 450°C). Eppendorf tubes were, therefore, adopted for the final experimental protocol.

Preliminary feeding experiments

Having improved the experimental procedure, several preliminary experiments were conducted, without pesticide exposure, to examine some important factors which may affect mysid feeding. Since relatively large variation in faecal production was encountered in control mysids even with the revised protocol, the effect of sediment preparation on feeding was studied. In addition, daily variation in faecal production of the same mysid was recorded. The hypothesis tested was that daily variations in the quality of the sediment supplied as food was influencing faecal production. Sediment could not be analysed chemically, so the following experiment was conducted. Twenty 2L tall-form glass beakers were set up with 100ml of sediment slurry and 1L of dilution water (10 ±1‰) as described above. A single mysid was added to each vessel and allowed to feed for 24h. The faecal material produced by each mysid was collected, dried and weighed. The same mysids were

then placed into different beakers containing sediment that had been prepared differently. Ten mysids were placed in beakers containing fresh sediment prepared in the usual way, and the other 10 mysids were placed in beakers containing excess sediment sieved the previous day which had been stored in a cold room (*c.*2°C). Comparison of the feeding rates of the same mysids measured each day would demonstrate the variability in the feeding rate of control mysids and identify whether the variance was due to differences in the sediment type. For both experimental mysid groups, variance in faecal production was relatively large, and there was no significant difference between the faecal production of mysids fed on the two types of sediment (Fig. 6.4).

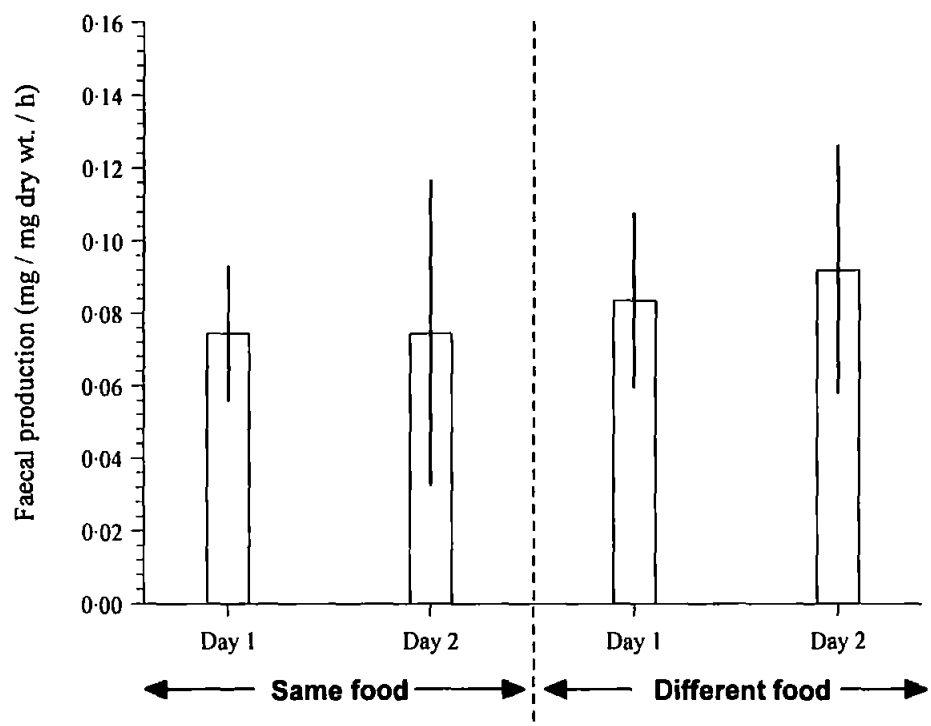


Figure 6.4: Investigation into daily variation of control mysid feeding rates (n=10 mysids for each food type).

The high daily variability of feeding rate in this first experiment led to feeding rates measured in subsequent experiments being expressed as a percentage of control mysid feeding rates. In a second experiment, a comparison was made between the faecal material produced by the same individual over 16 and 24h to identify whether feeding occurred at a constant rate over 24h, or whether there was any diurnal cycle (in Chapter 4, faecal

production was measured over 18h from mysids exposed to a 10h light:8h dark photoperiod). When calculated as an hourly rate, faecal production was significantly higher for measurements taken during the night compared with total production over 24h (95% confidence intervals, $p<0{\cdot}05$; Fig. 6.5). These results imply that *Neomysis integer* is a nocturnal feeder. Since there was a diurnal variation in mysid feeding rate, the use of a 24h protocol for the main experiments was justified since diurnal variation is accounted for in measurements taken over a 24h period.

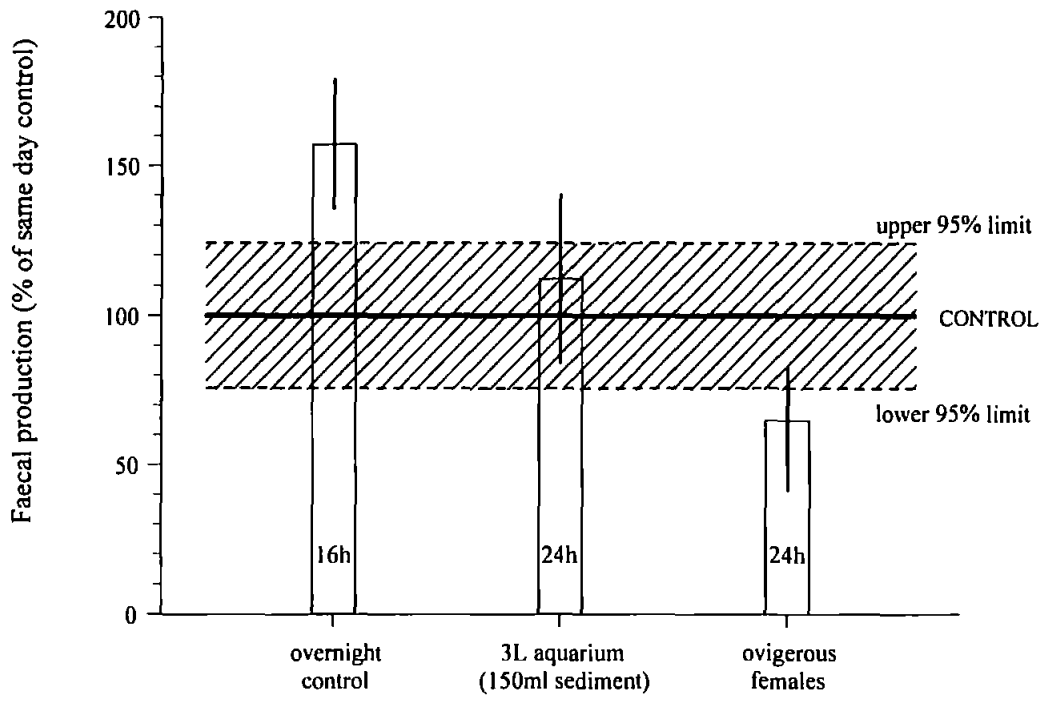


Figure 6.5: Additional feeding experiments: effect of experiment duration on hourly rate of faecal production (i.e. 16h), effect of experimental vessel size and amount of sediment supplied on faecal production, and faecal production of ovigerous females. Mean values plotted as a percentage of faecal production of control animals measured for 24h in 2L tall-form glass beakers with 100ml sediment; n=10. Error bars correspond to 95% confident intervals.

Since the volume of experimental vessel affects the feeding rate of aquatic invertebrates (Astthorsson 1980; Peters 1984), a third experiment investigated the effect of the basal surface-area of the container (≡ amount of food) on mysid faecal production. Faecal production of 10 mysids placed in 2L tall-form beakers (100mm diameter) with 100ml of

sediment was compared with faecal production of 10 mysids place in 3L rectangular tanks (150mm × 200mm base area) with 150ml of sediment slurry. Faecal production was measured at 10 ±1‰ and 15 ±1°C over 24h. Volume of test vessel, and amount of sediment supplied, had no effect on the mysid faecal production (Fig. 6.5). A fourth experiment measured faecal production of ovigerous females (over 24h). Ovigerous females were not used in any of the original feeding experiments (Chapter 4), however, the sample of mysids collected contained sufficient numbers of ovigerous females to investigate their feeding rate relative to other adult mysids. Faecal production of ovigerous females was not significantly different from the faecal production of other adults (Fig. 6.5). The lower mean value measured can be accounted for by the heavier weight of the ovigerous females due to the eggs or nauplii in the brood pouch (which add to the weight but do not contribute to the feeding rate).

Final method for measurement of faecal production

Taking into consideration the findings of the protocol development and preliminary experiments, the final protocol for testing the effects of chlorpyrifos on faecal production (≡feeding) of *Neomysis integer* was as follows. Mysids were exposed to chlorpyrifos as described in Section 6.2.2 for various durations (48, 96 and 168h). Sediment, suspended in water of 10‰, was passed through a 63µm sieve three times, and allowed to settle so that the supernatant could be siphoned off. Using a plastic syringe, 100ml of the concentrated sediment slurry was added to each 2L tall-form glass beaker. After 1h, 1L of chlorpyrifos-spiked exposure water (0·10, 0·072, 0·056 or 0·038µg chlorpyrifos L^{-1}) was added to each of 10 beakers, poured over bubble wrap to prevent disturbance of the consolidated sediment (Plate 6.2), and a single mysid from the pesticide exposure corresponding to the test concentration was added to each vessel. The other 10 beakers had 1L of acetone control water (100µl acetone L^{-1}) and a single mysid from the acetone control exposure added. Mysids were left for 24h to feed (15 ±1°C; 10 ±1‰; 16h light:8h dark photoperiod; Plate 6.3), after which they were removed and placed in pre-weighed Eppendorf tubes, oven dried (*c.*80°C) and weighed (±0·01mg) using a Sartorius R200-D balance. Faecal material produced by each mysid was collected by agitating the sediment in the beaker carefully so that it was re-suspended and passing the resultant slurry through a 120µm sieve. Faeces were rinsed in double distilled, de-ionised water, washed from the sieve into glass test-tubes and pipetted into individual, dry and pre-weighed Eppendorf tubes. Faecal material was oven dried (*c.*80°C) for 48h and weighed (±0·01mg) using a Sartorius R200-D

Plate 6.2 (upper plate): addition of exposure water over bubble-wrap to prevent disturbance of consolidated sediment.
Plate 6.3 (lower plate): experimental set-up for measurement of mysid faecal production.

balance. All dried material was covered and stored in a glass desiccator (with silica crystals) to prevent dust and moisture contamination. Mysid and faecal dry-weights were calculated by subtracting the weight of the empty Eppendorf tube from the total weight.

Measurement of absorption efficiency

Measurement of absorption efficiency was made by the ratio method (Conover, 1966) as in Chapter 4. Due to the extremely low weight of mysid faeces, faecal material from several mysids was pooled for the calculation of absorption efficiency (Chapter 4). Faecal material was removed from the Eppendorf tubes and placed into ashed and pre-weighed aluminium foil cups [*c.*10mm deep × 10mm diameter (ashing of dry aluminium did not create the residue described in Section 6.2.5 for wet foil)].

Starvation tolerance of *Neomysis integer*

The hypothesis that exposure to chlorpyrifos would affect feeding rate has implications for the results of the acute toxicity testing described in Chapter 5. If energy reserves of the test organism are low, mortalities from acute toxicity tests might be caused by toxicant-induced depression of feeding rate. A preliminary investigation was, therefore, made into the energy reserves of *N. integer* by estimating the LT_{50} (median lethal time) for starvation. Twenty 2L tall-form glass beakers were set up with 1L of aerated control water (10 ±1‰), and a single control mysid was added to each. Mysids were starved and mortalities (defined as absence of movement when mechanically stimulated) were recorded each day. To maintain water quality, and to remove any faecal material (mysids have been shown to exhibit coprophagy), water was changed each day by siphoning off at least 90% of the water and replacing it with clean water. The LT_{50} for starvation was calculated by linear regression.

6.3.6 Statistical treatment of results

Swimming experiments

Frequency data for mysids exhibiting each type of behaviour, and swimming at each height in the water column, were converted into proportions and arcsine square-root transformed to account for low proportions (Chapter 2). The data were examined statistically by one- and two-way analysis of variance [ANOVA (Chapter 2)].

Oxygen consumption experiments

Mean weight-specific rates of oxygen consumption (calculated with 95% confidence intervals) were examined by one- and two-way analysis of variance (ANOVA). Multiple linear regression analysis was applied to the data to describe the relationship between oxygen consumption and pesticide concentration/exposure period.

Feeding experiments

Amount of faecal material was made weight-specific by dividing by the appropriate mysid dry-weight, and converted to an hourly rate (divided by test duration, i.e. 24h). There was large daily variation even with control animals, therefore, rate of faecal production is expressed as a percentage of control mysid faecal production measured on the same day. Since this is a derived statistic, further statistical analysis was not possible. However, 95% confidence intervals, calculated for each set of data, have been transformed to percentages and these are shown in the figures. Similarly absorption efficiency was from pooled samples preventing statistical analysis (Chapter 4).

Scope for growth

Scope for growth (SFG) of *Neomysis integer* was calculated by converting oxygen consumption and feeding rates into energy equivalents (J h^{-1}), and calculating the net energy gain/loss due to these physiological processes from the equation:

$$P = A - (R + U)$$

where: P = SFG (J mg dry wt^{-1} h^{-1}), A = energy absorbed (J mg dry wt^{-1} h^{-1}), R = energy respired (J mg dry wt^{-1} h^{-1}) and U = energy excreted (J mg dry wt^{-1} h^{-1}) (Widdows & Salkeld, 1993).

The relative components were calculated and transformed into energy equivalents as follows. Energy absorbed was calculated using the equation:

$$A = C \times \text{Absorption efficiency}$$

where: A = energy absorbed (J mg dry wt^{-1} h^{-1}), and C = energy consumed (J mg dry wt^{-1} h^{-1}). The energy content of particulate organic matter is *c.*23J mg ash-free dry wt^{-1} (Widdows *et al.*, 1979). Energy consumed (C) was calculated, therefore, by multiplying

sediment consumed (mg ash-free sediment mg dry wt^{-1} h^{-1}) by 23 (Joules). Absorption efficiency was calculated by the ash-ratio method (Conover, 1966) as described previously (Chapter 4).

Energy respired was calculated using the equation:

$$R = \text{oxygen consumption } (\mu l\ O_2 \text{ mg dry } wt^{-1}\ h^{-1}) \times 0{\cdot}02008$$

where R = energy respired (J mg dry wt^{-1} h^{-1}), oxygen consumption rates were converted to dry-weight specific rates by multilpying by 5 [mysid dry wt = *c.*20% mysid wet wt. (Mauchline, 1980)], and the heat equivalent of oxygen uptake = 0·02008 J $(\mu l\ O_2)^{-1}$ (Gnaiger, 1983). Mean dry-weight of mysids used in the present experiments was *c.*1·5mg.

The rate of ammonia excretion is usually closely correlated with respiration rate and, contributing usually <5% of metabolic energy expenditure, is usually omitted from the calculation of SFG (Widdows & Salkeld, 1993). Ammonia excretion was not measured in the current study and SFG was calculated from the equation P = A - R (units defined above).

6.4 RESULTS

For all experiments, there was no significant difference between the responses of mysids in the acetone control (exposed to 100µl acetone L^{-1}) and mysids in the full control (exposed only to dilution water). All results are discussed, therefore, in terms of deviation from the response of acetone control mysids, since this is the more representative control of chlorpyrifos effects (i.e. a positive control).

6.4.1 Effect of chlorpyrifos on swimming behaviour

Mysid swimming behaviour was modified by both pesticide concentration and current velocity [ANOVA, $f \geq 4{\cdot}19$ (concentration) and 16·4 (velocity), d.f.= 4 (concentration) and 5 (velocity), $p<0{\cdot}01$; Table 6.3]. The effects of current velocity on mysid swimming behaviour have been described in Chapter 2 and are not addressed in this chapter. However, there was a significant interaction between pesticide concentration and current velocity (ANOVA, $f \geq 5{\cdot}57$, d.f.=20, $p<0{\cdot}01$; Table 6.3). The effects of exposure to chlorpyrifos are, therefore, complex and significant only for certain pesticide

concentrations at certain current velocities. An exhaustive description of the results would be lengthy and confusing, therefore, only the most obvious trends in response are described here.

Table 6.3: Two-way analysis of variance (ANOVA) comparing the effects of chlorpyrifos and current velocity on the proportion of mysids exhibiting each behaviour type. 10 replicates of 20 mysids per experiment. Critical f=2·41 (concentration), 2·25 (velocity) and 1·61 (interaction). All data, including animals in boundary layer, analysed.

Behaviour	Source	d.f.	f value	p	significance
Swimming forward into the current	concentration	4	19·7	3×10^{-14}	p<0·01
	velocity	5	509	4×10^{-135}	p<0·01
	interaction	20	13·2	$6·7 \times 10^{-30}$	p<0·01
Maintaining position	concentration	4	35·5	$8·5 \times 10^{-24}$	p<0·01
	velocity	5	16·4	$1·7 \times 10^{-16}$	p<0·01
	interaction	20	7·42	$1·2 \times 10^{-16}$	p<0·01
Facing into the current, but being displaced	concentration	4	4·19	$2·6 \times 10^{-3}$	p<0·01
	velocity	5	125	$4·1 \times 10^{-68}$	p<0·01
	interaction	20	5·57	$5·9 \times 10^{-512}$	p<0·01
Swimming with the current	concentration	4	30·7	$4·5 \times 10^{-21}$	p<0·01
	velocity	5	109	8×10^{-14}	p<0·01
	interaction	20	8·16	$1·9 \times 10^{-14}$	p<0·01

The type of response to chlorpyrifos on the proportion of animals swimming forward into the current was dependent upon current velocity (Fig. 6.6a) [ANOVA, f=13·2, d.f.=20, p<0·01; Table 6.3]. Exposure to low concentrations of chlorpyrifos (<0·100µg chlorpyrifos L^{-1}) caused significantly more mysids to swim forward into the current at low current velocities (i.e. 3 and 6cm s^{-1}) compared with control mysids (Fig 6.6a) [ANOVA, f=37·8, d.f.=4, p<0·01; Table 6.4]. Significantly more mysids exposed to 0·38, 0·56 and 0·072µg chlorpyrifos L^{-1} swam forward into a current velocity of 3cm s^{-1} compared with control mysids (95% confidence intervals, p<0·05; Fig. 6.6a), but exposure to 0·100µg chlorpyrifos L^{-1} had no significant effect on the frequency of this behaviour type (95% confidence intervals, p>0·05; Fig. 6.6a). The number of animals swimming forward into the current at 3cm s^{-1} was concentration-dependent, and the frequency of this behaviour was inversely

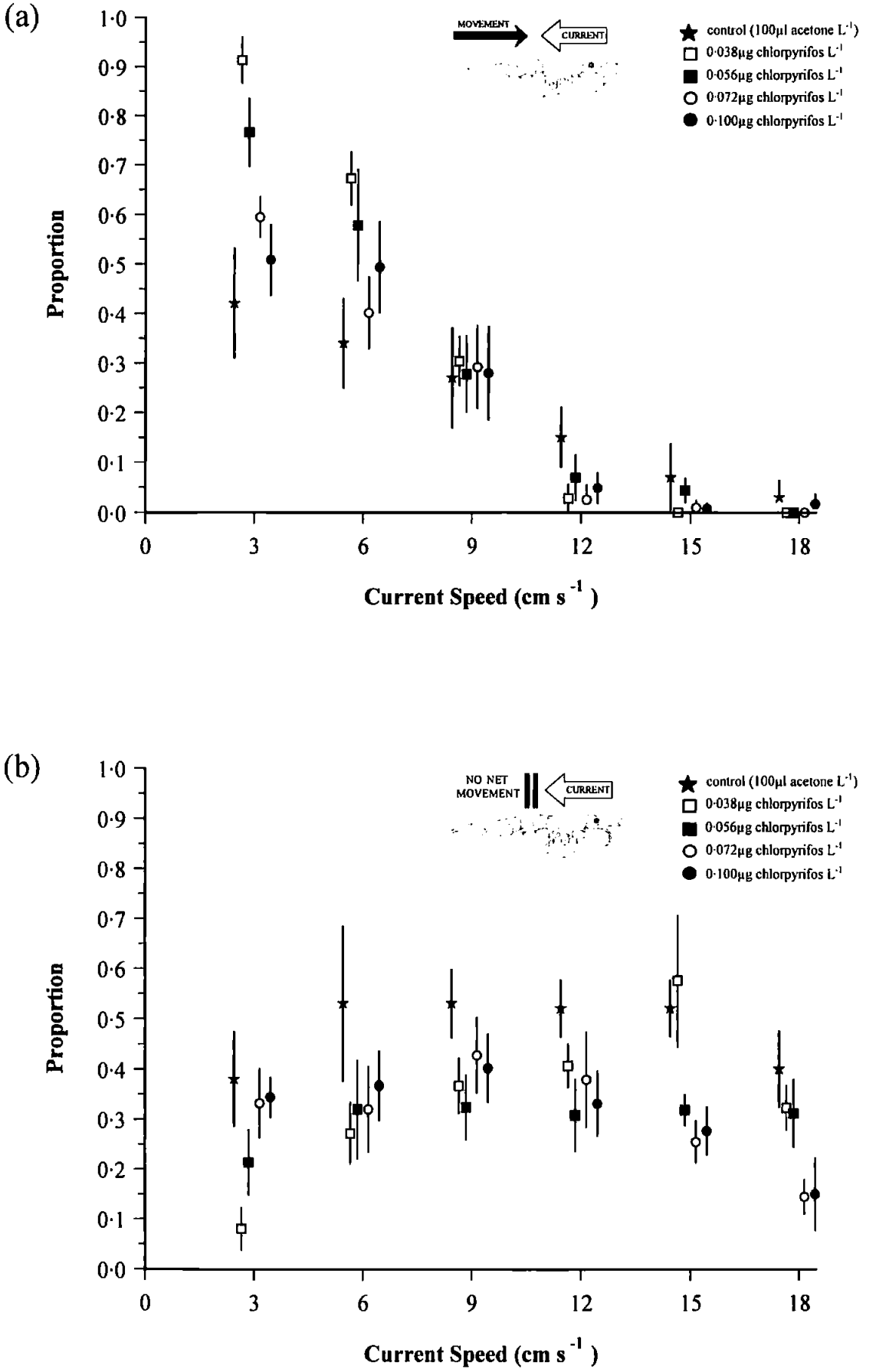


Figure 6.6: Effect of chlorpyrifos exposure on the mean swimming behaviour of *Neomysis integer* with increasing current velocity. (a) swimming forward into the current; (b) facing into the current an maintaining position. Data for all mysids, including those in the boundary layer. n=200 for each pesticide concentration. Error bars correspond to 95% confidence intervals.

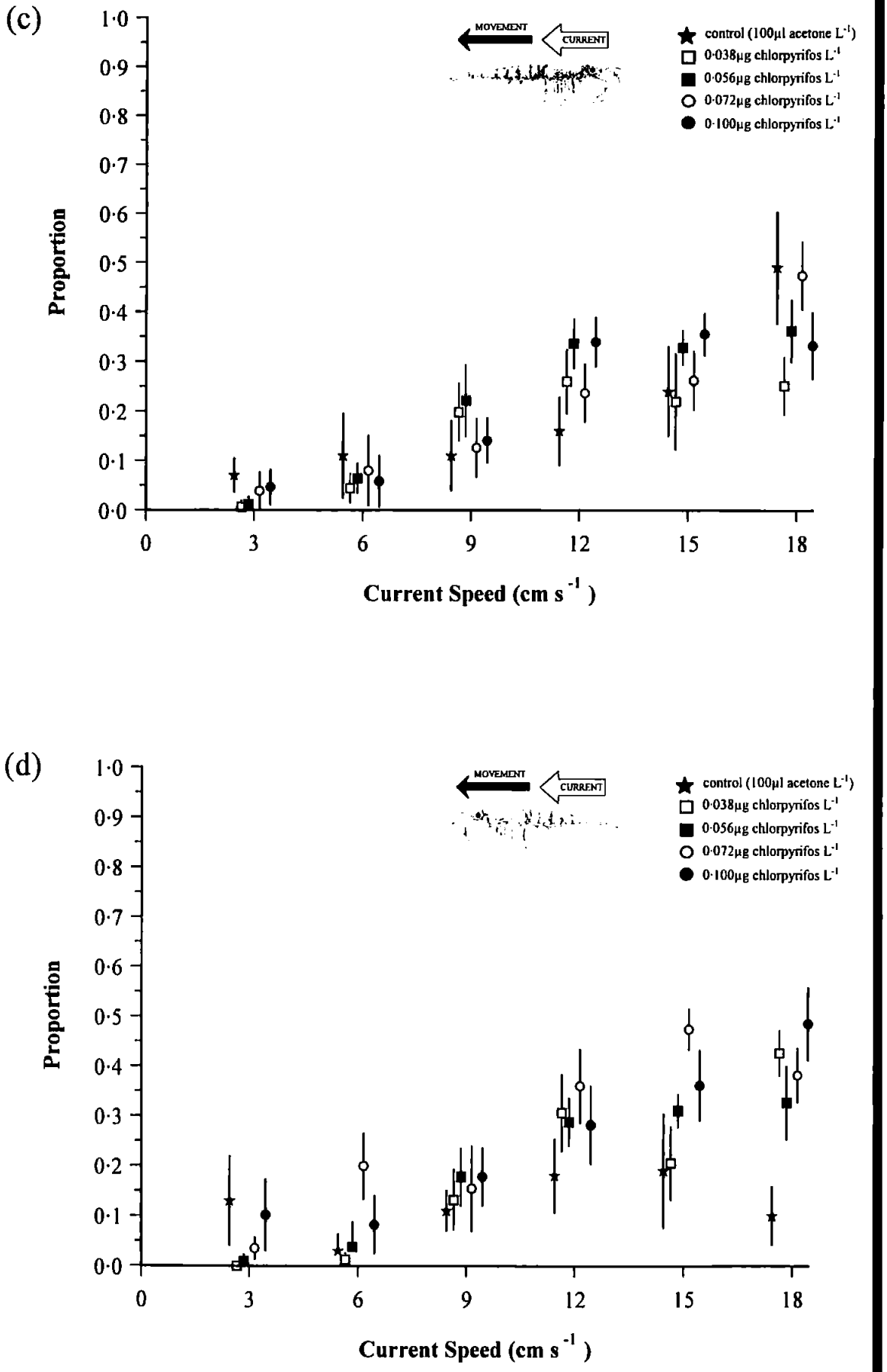


Figure 6.6 (cont'd): Effect of chlorpyrifos exposure on the mean swimming behaviour of *Neomysis integer* with increasing current velocity. (c) facing into the current but being displaced; (d) swimming with the current. Data for all mysids, including those in the boundary layer, are shown. n=200 for each pesticide concentration. Error bars correspond to 95% confidence intervals.

Table 6.4: One-way analysis of variance (ANOVA) examining the effect of chlorpyrifos concentration on proportion of mysids demonstrating each of the four behavioural types with increasing current velocity, 10 replicates of 20 mysids per experiment, critical f=2·58, d.f.=4. All data, including animals in boundary layer, analysed.

Behaviour Type	Current Speed (cm s^{-1})	f value	p value	significance
Swimming forward	3	37·8	$7·5 \times 10^{-14}$	p<0·01
into the current	6	11·6	$1·5 \times 10^{-6}$	p<0·01
	9	0·12	0·98	n.s.
	12	7·96	$6·1 \times 10^{-5}$	p<0·01
	15	4·07	0·007	p<0·01
	18	3·30	0·019	p<0·05
Maintaining	3	17·2	$1·3 \times 10^{-8}$	p<0·01
position	6	5·41	0·002	p<0·01
	9	6·86	$2·1 \times 10^{-4}$	p<0·01
	12	7·32	$1·2 \times 10^{-4}$	p<0·01
	15	11·8	$1·2 \times 10^{-6}$	p<0·01
	18	17·2	$1·3 \times 10^{-8}$	p<0·01
Facing into the	3	4·06	0·007	p<0·01
current, but being	6	0·96	0·438	n.s.
displaced	9	3·07	0·026	p<0·05
	12	8·13	5×10^{-5}	p<0·01
	15	3·61	0·012	p<0·05
	18	8·12	$5·1 \times 10^{-5}$	p<0·01
Swimming with	3	6·08	$5·2 \times 10^{-4}$	p<0·01
the current	6	12·5	$6·3 \times 10^{-7}$	p<0·01
	9	1·15	0·344	n.s.
	12	4·25	0·005	p<0·01
	15	13·5	$2·6 \times 10^{-7}$	p<0·01
	18	26·8	$2·1 \times 10^{-11}$	p<0·01

related to pesticide concentration (95% confidence intervals; Fig. 6.6a). Exposure to 0·038 and 0·056μg chlorpyrifos L^{-1} led also to increased numbers of mysids swimming forward into the current at 6cm s^{-1} (95% confidence intervals, $p<0·05$; Fig. 6.6a). In contrast, exposure to 0·038, 0·072 and 0·100μg chlorpyrifos L^{-1} caused fewer mysids to swim forward into the current at high current velocities (i.e. 12 and 15cm s^{-1}), compared with control mysids (95% confidence intervals, $p<0·05$; Fig. 6.6a). At 18cm s^{-1}, no animals swam forward into the current (Fig. 6.6a).

At each current velocity, exposure to chlorpyrifos had a significant effect on the proportion of mysids maintaining position (ANOVA, $f \geq 5·41$, d.f.=4, $p<0·01$; Table 6.4), resulting in fewer pesticide-exposed mysids maintaining position compared with control mysids (Fig. 6.6b). Chlorpyrifos-related disruption of position maintenance behaviour is dependent upon current velocity (Fig. 6.6b) [ANOVA, $f=7·42$, d.f.=20, $p<0·01$; Table 6.3]. Exposure to low pesticide concentrations (e.g. 0·038 and 0·056μg chlorpyrifos L^{-1}) caused fewer mysids to maintain position at low current speeds (e.g. 3 and 6cm s^{-1}) compared with control mysids (Fig. 6.6b) [ANOVA, $f=17·2$, d.f.=4, $p<0·01$; Table 6.4]. Exposure to high pesticide concentrations (e.g. 0·072 and 0·100μg chlorpyrifos L^{-1}) had no significant effect on position maintenance at these low velocities (95% confidence intervals, $p>0·05$; Fig. 6.6b). In contrast, fewer mysids exposed to high pesticide concentrations (e.g. 0·072 and 0·100μg chlorpyrifos L^{-1}) maintained position at high current speeds (e.g. 15 and 18cm s^{-1}) compared with control mysids (Fig. 6.6b) [ANOVA, $f=17·2$, d.f.=4, $p<0·01$; Table 6.4].

At all current velocities except 6cm s^{-1}, exposure to chlorpyrifos significantly affected the frequency of mysids facing into the current but being displaced (Fig. 6.6c) [ANOVA, $f=0·96$, d.f.=4, $p>0·05$; Table 6.4]. Again, the response is complex and concentration effects were dependent upon current velocity (Fig 6.6c) [ANOVA, $f=5·57$, d.f.=20, $p<0·01$; Table 6.3]. At 12cm s^{-1}, exposure to chlorpyrifos caused an increase in the frequency of animals facing into, but being displaced, by the current (compared to control mysids), however, chlorpyrifos caused a decrease in the frequency of this behaviour at 18cm s^{-1} (95% confidence intervals, $p<0·05$; Fig. 6.6c). Exposure to chlorpyrifos caused increased numbers of mysids to swim with the current at high current velocities (e.g. >9cm s^{-1}) compared with control mysids (Fig. 6.6d) [ANOVA, $f \geq 4·25$, d.f.=4, $p<0·01$; Table 6.3]. At

18cm s^{-1}, only *c.*10% of the control mysids swam with the current, compared with >30% of the exposed mysids (95% confidence intervals, $p<0{\cdot}05$; Fig. 6.6d).

Effect of exposure to chlorpyrifos on the swimming behaviour of mysids above the boundary layer was less consistent and, at many velocities, there was no significant effect of chlorpyrifos. There was a significant interaction between pesticide concentration and current velocity for all behavioural types (ANOVA, $f \geq 2{\cdot}26$; d.f.=20, $p<0{\cdot}01$;Table 6.5).

Table 6.5: Two-way analysis of variance (ANOVA) comparing the effects of chlorpyrifos and current velocity on the proportion of mysids exhibiting each behaviour type. 10 replicates of 20 mysids per experiment. Critical f=2·41 (concentration), 2·25 (velocity) and 1·61(interaction). Data for animals in the boundary layer have been removed from the analysis.

Behaviour	Source	d.f.	f value	p	significance
Swimming forward into the current	concentration	4	13·82	$2{\cdot}9 \times 10^{-10}$	p<0·01
	velocity	5	124	$1{\cdot}2 \times 10^{-67}$	p<0·01
	interaction	20	7·25	$3{\cdot}2 \times 10^{-16}$	p<0·01
Maintaining position	concentration	4	6·9	$2{\cdot}5 \times 10^{-5}$	p<0·01
	velocity	5	11·9	$2{\cdot}1 \times 10^{-10}$	p<0·01
	interaction	20	5·59	$5{\cdot}1 \times 10^{-12}$	p<0·01
Facing into the current, but being displaced	concentration	4	3·86	$4{\cdot}6 \times 10^{-3}$	p<0·01
	velocity	5	32·0	$1{\cdot}4 \times 10^{-25}$	p<0·01
	interaction	20	2·26	$1{\cdot}9 \times 10^{-3}$	p<0·01
Swimming with the current	concentration	4	5·22	$5{\cdot}0 \times 10^{-4}$	p<0·01
	velocity	5	20·5	$2{\cdot}4 \times 10^{-117}$	p<0·01
	interaction	20	3·81	$2{\cdot}3 \times 10^{-17}$	p<0·01

Significant modification of swimming behaviour due to chlorpyrifos, therefore, occurred only at certain current velocities (Table 6.6; Fig. 6.7). In general, exposure to chlorpyrifos caused similar responses in those mysids swimming above the boundary layer as those described above when all data are analysed (Table 6.6; Fig. 6.7). Chlorpyrifos caused more mysids to swim forward into the current at low current velocities (Fig. 6.7a) [ANOVA, f=15·9, d.f.=4, $p<0{\cdot}01$; Table 6.6] and more mysids to swim with the current at high current speeds (Fig. 6.7d) [ANOVA, f=11·6, d.f.=4, $p<0{\cdot}01$; Table 6.6], compared

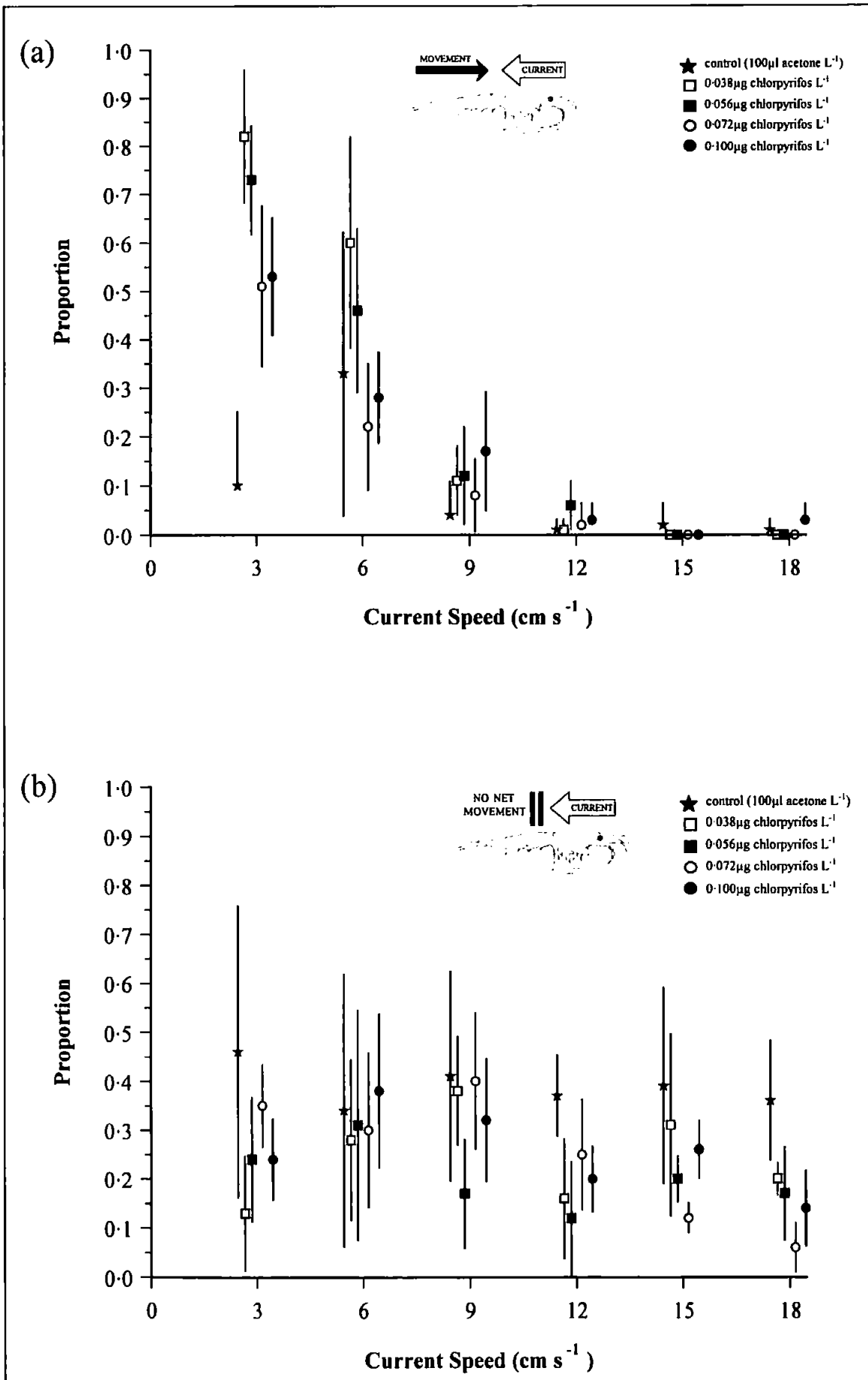


Figure 6.7: Effect of chlorpyrifos exposure on the mean swimming behaviour of *Neomysis integer* with increasing current velocity. (a) swimming forward into the current; (b) facing into the current an maintaining position. Data for mysids swimming in the boundary layer are removed. n = variable for each pesticide concentration. Error bars correspond to 95% confidence intervals.

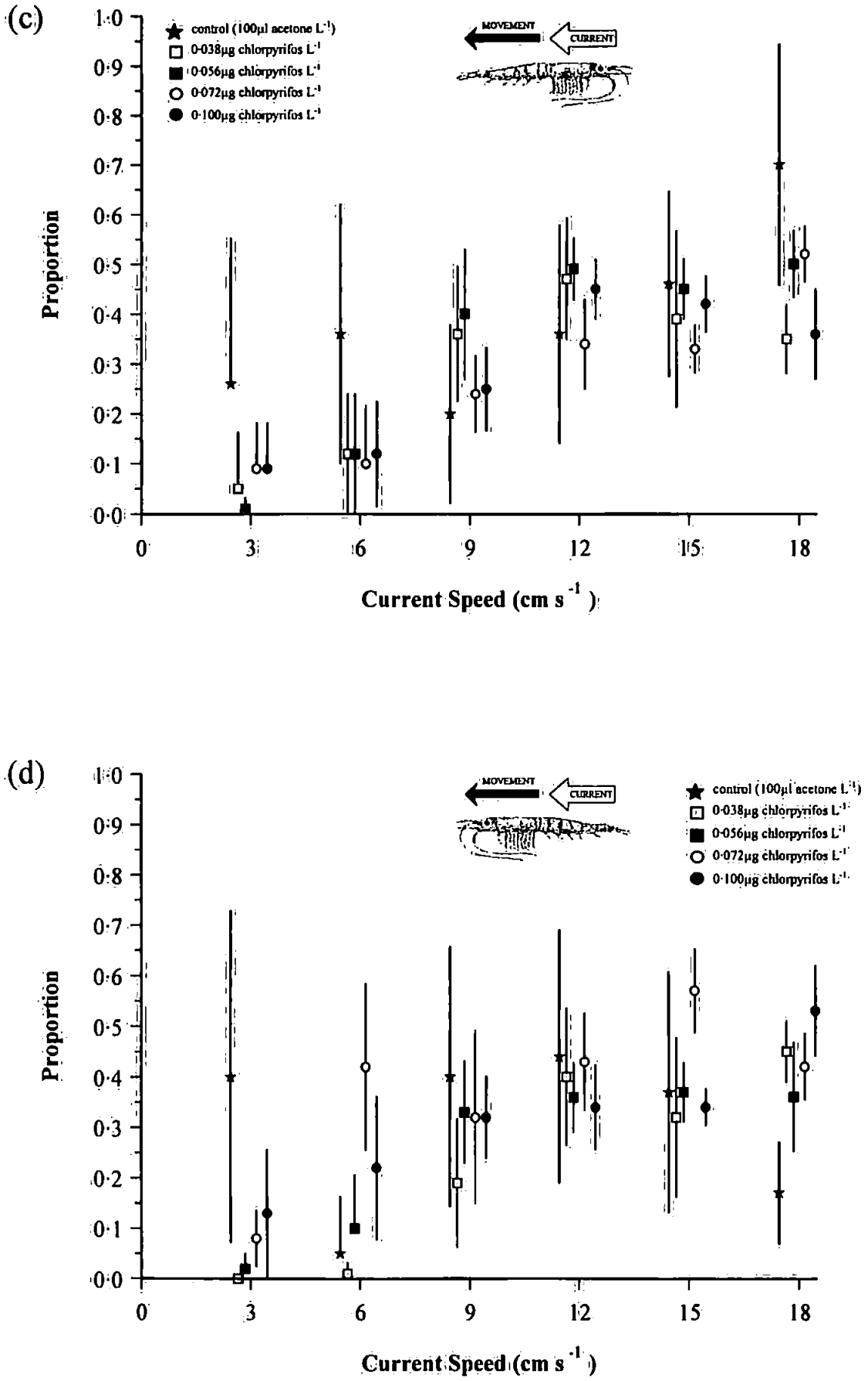


Figure 6.7 (cont'd): Effect of chlorpyrifos exposure on the mean swimming behaviour of *Neomysis integer* with increasing current velocity. (c) facing into the current but being displaced; (d) swimming with the current. Data for mysids swimming in the boundary layer are removed. n = variable for each pesticide concentration. Error bars correspond to 95% confidence intervals.

Table 6.6: One-way analysis of variance (ANOVA) examining the effect chlorpyrifos concentration on proportion of mysids demonstrating each of the four behavioural types with increasing current velocity, 10 replicates of 20 mysids per experiment, critical f=2·58, d.f.=4. Mysids in boundary layer removed from analysis.

Behaviour Type	Current Speed (cm s^{-1})	f value	p value	significance
Swimming forward	3	15·9	$3·4 \times 10^{-8}$	p<0·01
into the current	6	2·69	0·03	p<0·05
	9	1·49	0·222	n.s.
	12	1·62	0·185	n.s.
	15	1	0·417	n.s.
	18	2·55	0·052	n.s.
Maintaining	3	3·39	0·016	p<0·05
position	6	0·31	0·867	n.s.
	9	2·04	0·105	n.s.
	12	4·44	0·004	p<0·01
	15	2·28	0·075	p<0·01
	18	9·22	$1·6 \times 10^{-5}$	p<0·01
Facing into the	3	2·19	0·085	n.s.
current, but being	6	2·77	0·039	p<0·05
displaced	9	2·31	0·072	n.s.
	12	0·75	0·562	n.s.
	15	0·97	0·433	n.s.
	18	5·80	0·001	p<0·01
Swimming with	3	5·04	0·002	p<0·01
the current	6	5·59	$1·1 \times 10^{-5}$	p<0·01
	9	1·40	0·249	n.s.
	12	0·75	0·560	n.s.
	15	1·99	0·111	n.s.
	18	11·6	$1·4 \times 10^{-6}$	p < 0·01

with control mysids. Exposure to chlorpyrifos significantly reduced the ability of mysids to maintain position at a current velocity of 18cm s^{-1} compared with control mysids (Fig. 6.7b) [ANOVA, f=9·22, d.f.=4, p<0·00; Table 6.6].

Chlorpyrifos had a variable effect on the vertical distribution of *Neomysis integer* and significant differences due to pesticide exposure were identified only at certain heights (Table 6.7; Fig 6.8). Exposure to 0·038 and 0·072µg chlorpyrifos L^{-1} caused significantly more mysids to remain on the substratum at 15cm s^{-1}, compared with control animals which were more evenly distributed throughout the water column (Fig.6.8e) [ANOVA, f=5·40, d.f.=4, p<0·00; Table 6.7]. Exposure to 0·100µg chlorpyrifos L^{-1} caused more mysids to swim above the substratum at current velocities ≥15cm s^{-1}, compared with control animals (Fig 6.8e,f). Exposure to various concentrations of chlorpyrifos also significantly affected the number of mysids swimming 3, 5, 10, 14 and >15cm above the substratum (ANOVA, f≥2·69, d.f.=4, p<0·01; Table 6.7), although there was no obvious pesticide-related trend (Fig. 6.8).

Table 6.7: One-way analysis of variance (ANOVA) examining the effect of chlorpyrifos concentration on the vertical distribution of *Neomysis integer*. 10 replicates of 20 mysids per experiment, critical f=2·65, d.f.=4.

Height above substratum (cm)	f value	p value	significance
On substratum	5·40	0·001	p<0·01
1	2·08	0·104	n·s.
2	1·61	0·189	n.s.
3	3·06	0·029	p<0·05
4	1·88	0·133	n.s.
5	6·59	$5·1 \times 10^{-4}$	p<0·00
6	2·25	0·083	n.s.
7	1·78	0·152	n.s.
8	2·65	0·050	n.s.
9	0·75	0·525	n.s.
10	2·69	0·047	p<0·05
11	1·88	0·133	n.s.
12	2·39	0·070	n.s.
13	0·46	0·710	n.s.
14	3·90	0·010	p<0·05
15	1·99	0·116	n.s.
>15	5·02	0·002	p<0·01

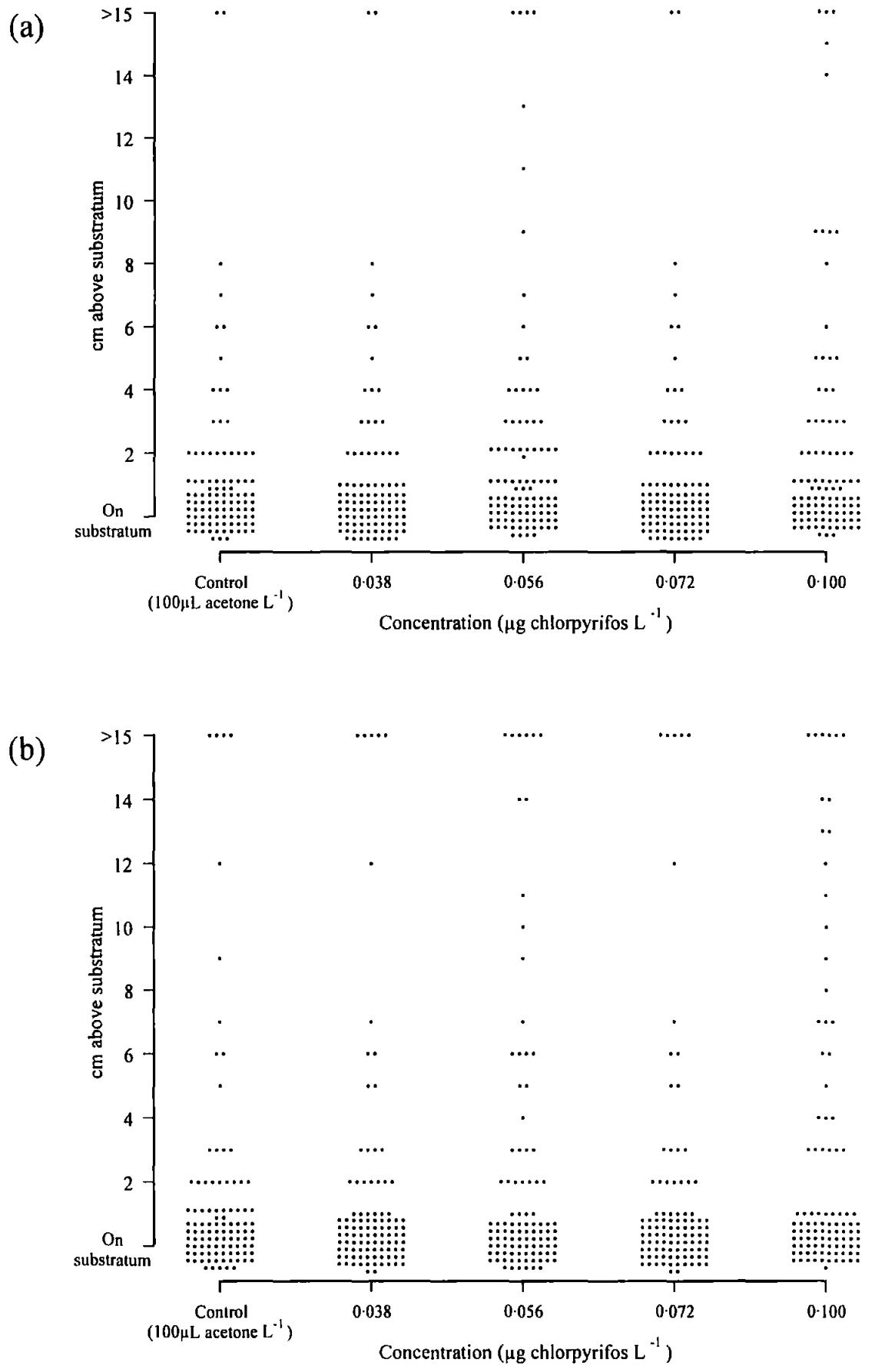


Figure 6.8: Effect of exposure to chlorpyrifos on the vertical distribution of mysids exposed to increasing current velocities. (a) 3cm s^{-1} and (b) 6cm s^{-1}. Each dot represents 1% mysid occurrence, n=200 for each pesticide concentration.

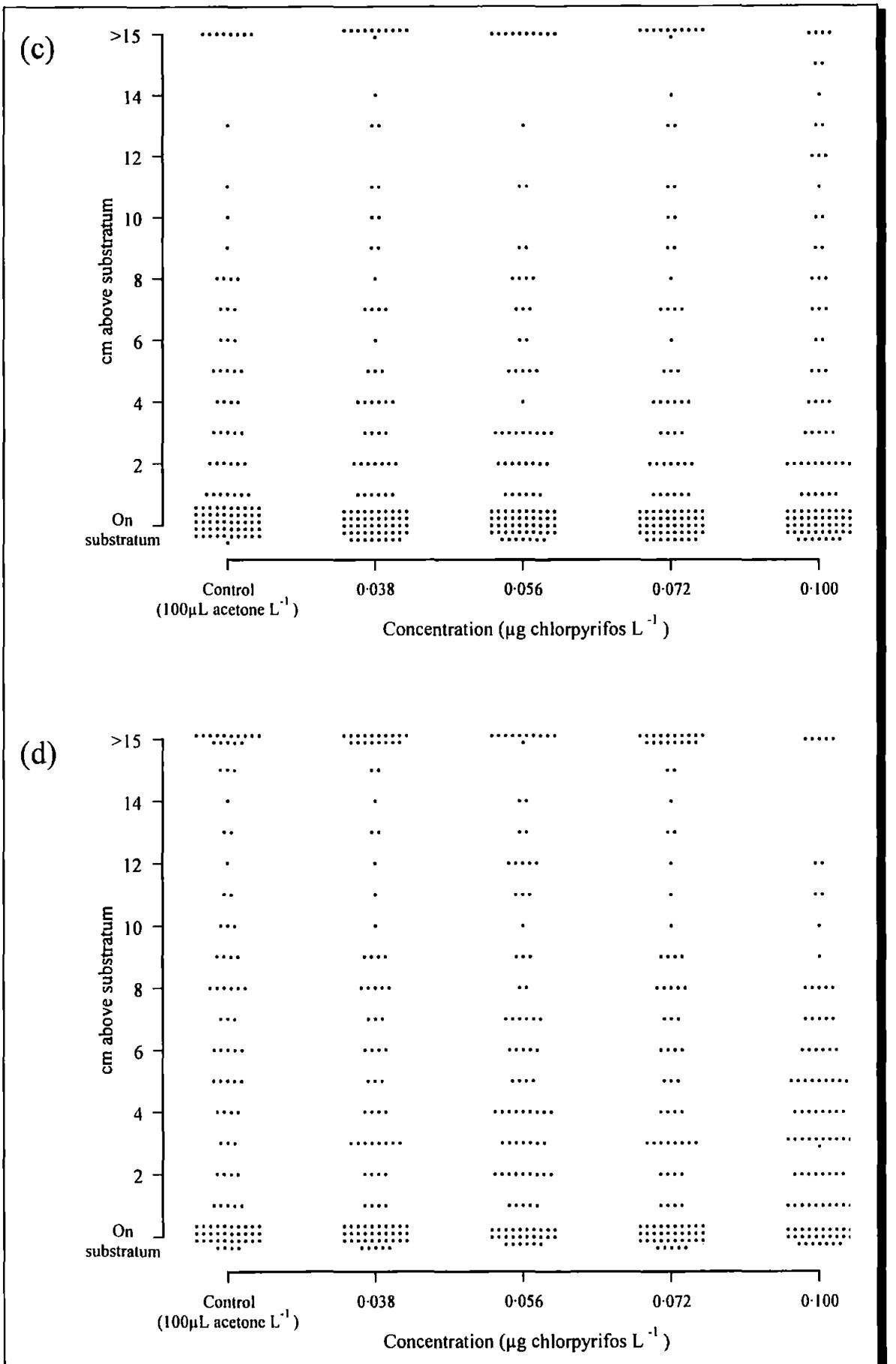


Figure 6.8 (contd.): Effect of exposure to chlorpyrifos on the vertical distribution of mysids exposed to increasing current velocities. (c) 9cm s^{-1} and (d) 12cm s^{-1}. Each dot represents 1% mysid occurrence, n=200 for each pesticide concentration.

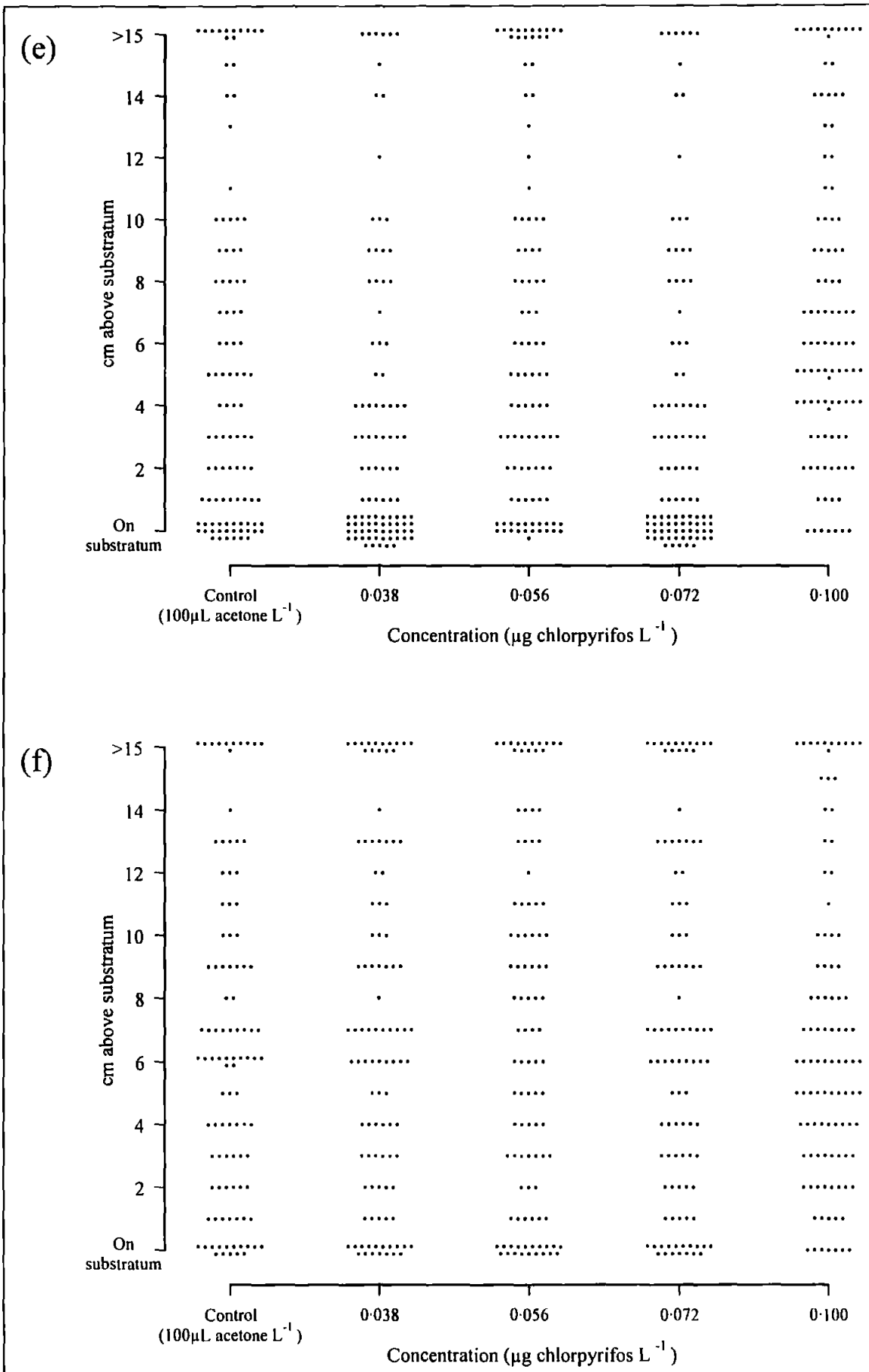


Figure 6.8 (contd.): Effect of exposure to chlorpyrifos on the vertical distribution of mysids exposed to increasing current velocities. (e) 15cm s^{-1} and (f) 18cm s^{-1}. Each dot represents 1% mysid occurrence, n=200 for each pesticide concentration.

6.4.2 Effect of chlorpyrifos on oxygen consumption

Exposure to chlorpyrifos caused elevated oxygen consumption by *Neomysis integer*. In general, oxygen consumption was correlated directly to pesticide concentration for each exposure duration. Highest oxygen consumption rates were recorded for mysids exposed to chlorpyrifos for 48h (Table 6.8).

Table 6.8: Effect of chlorpyrifos on the mean oxygen consumption of *Neomysis integer*. n=9 for each pesticide exposure concentration/duration combination.

Chlorpyrifos Exposure:		Oxygen	95% Confidence	
Concentration	Duration	Consumption	Intervals	
($\mu g\ L^{-1}$)	(h)	($\mu l\ O_2\ mg\ wet\ wt.^{-1}\ h^{-1}$)	Minimum	Maximum
Control	48	0·428	0·417	0·440
(100µl acetone L^{-1})	96	0·417	0·402	0·430
	168	0·427	0·418	0·436
0·038	48	0·577	0·538	0·617
	96	0·495	0·474	0·516
	168	0·471	0·462	0·479
0·056	48	0·723	0·694	0·757
	96	0·602	0·582	0·622
	168	0·612	0·591	0·632
0·072	48	0·863	0·832	0·893
	96	0·698	0·674	0·722
	168	0·665	0·629	0·702
0·100	48	0·943	0·906	0·980
	96	0·856	0·825	0·888
	168	0·869	0·822	0·917

For each exposure duration, increases in chlorpyrifos concentration caused increases in oxygen consumption (95% confidence intervals, $p<0·05$; Fig 6.9). This general response of increased oxygen consumption at higher pesticide concentrations occurred following all pesticide exposure periods (ANOVA, $f>200$, d.f.=4, $p<0·05$; Table 6.9). There was,

however, no significant difference between the oxygen consumption of mysids exposed to 0·056 and 0·072 for 168h (95% confidence intervals; p>0·05; Figure 6.9).

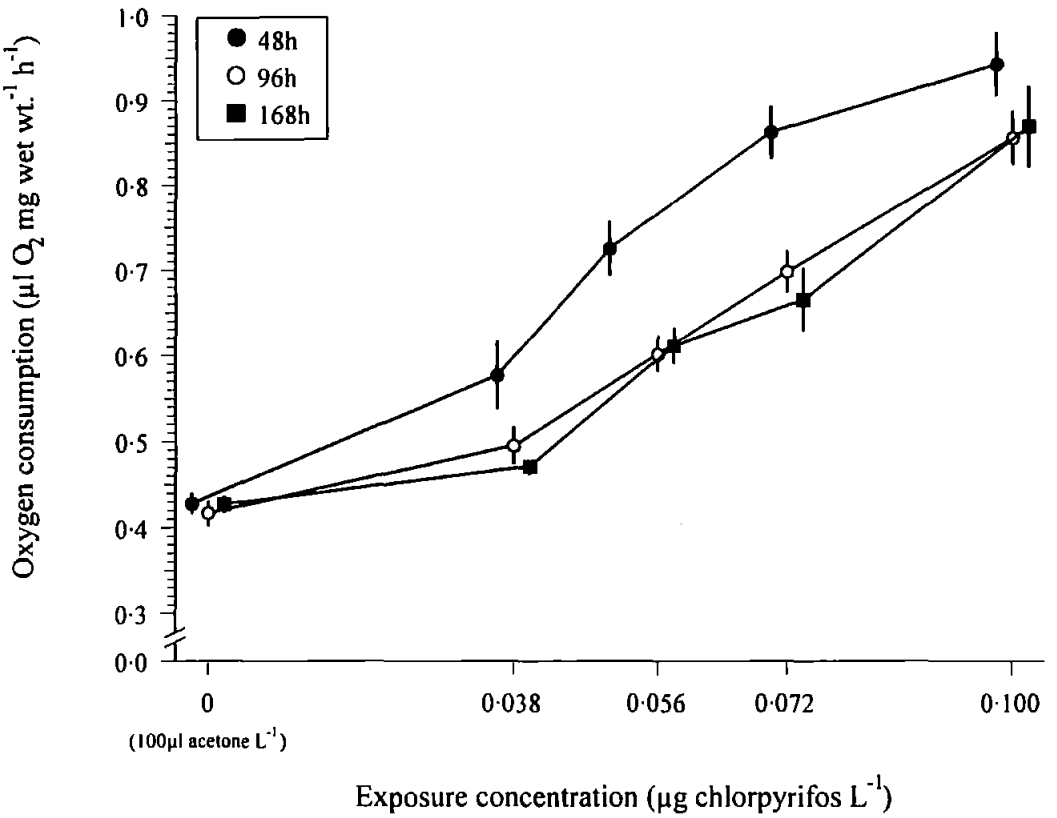


Figure 6.9: Effect of chlorpyrifos concentration on the mean oxygen consumption of *Neomysis integer* exposed for various durations; n=9 for each pesticide exposure concentration/duration combination. Error bars correspond to 95% confidence intervals.

Table 6.9: One-way analysis of variance (ANOVA) examining the effect of chlorpyrifos concentration on the mean oxygen consumption of *Neomysis integer* following exposure for 48, 96 and 168h. n=9 for each pesticide exposure concentration/duration combination, critical f=2·58.

Duration	d.f.	f statistic	p value	significance
48h	4	226	$3{\cdot}74 \times 10^{-29}$	p<0·00
96h	4	295	$1{\cdot}2 \times 10^{-31}$	p<0·00
168h	4	236	$1{\cdot}51 \times 10^{-29}$	p<0·00

Effect of exposure duration on oxygen consumption

There was a significant effect of length of exposure to chlorpyrifos on oxygen consumption of mysids exposed to each chlorpyrifos concentration (ANOVA, f>7, d.f.=2, p<0·01; Table 6.10). In general, for any given chlorpyrifos concentration, mysids exposed for 48h consumed significantly more oxygen than mysids exposed for 96 and 168h (95% confidence intervals; p<0·05; Fig 6.10). For each chlorpyrifos concentration, exposure duration had no significant effect on mysid oxygen consumption for exposure periods >48h (95% confidence intervals, p>0·05; Fig. 6.10). Oxygen consumption of acetone control mysids was unaffected by exposure duration (Fig. 6.10) [ANOVA, f=1·47, d.f.=2, p>0·05; Table 6.10].

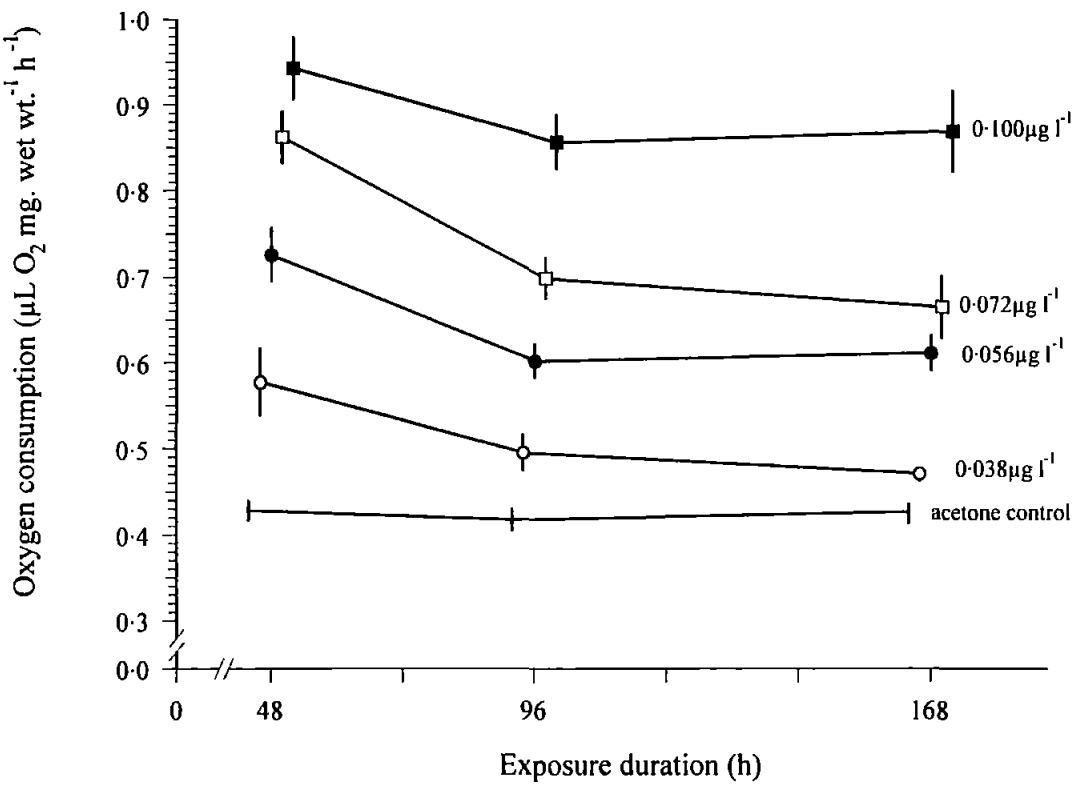


Figure 6.10: Effect of exposure duration on the mean oxygen consumption of *Neomysis integer* exposed to various concentrations of chlorpyrifos. n=9 for each pesticide exposure concentration/duration combination. Error bars correspond to 95% confidence intervals.

Table 6.10: One-way analysis of variance (ANOVA) examining the effect of exposure duration (to various exposure concentrations) on the oxygen consumption of *Neomysis integer*. n=9 for each pesticide exposure concentration/duration combination, critical f=3·35, n.s.= not significant (p>0·05).

Concentration	d.f.	f statistic	p value	significance
acetone control	2	1·47	0·246	n.s.
0·038µg L^{-1}	2	23·1	$1·4 \times 10^{-6}$	p<0·01
0·056µg L^{-1}	2	39·8	$9·0 \times 10^{-9}$	p<0·01
0·072µg L^{-1}	2	85·8	$1·9 \times 10^{-12}$	p<0·01
0·100µg L^{-1}	2	7·3	0·003	p<0·01

Two-way ANOVA revealed that there was a significant interaction between exposure concentration and duration (ANOVA, f=4·65, d.f.=8, p<0·01; Table 6.11), however, individual effects of concentration and duration were more significant (higher f values; Table 6.11).

Table 6.11: Two-way analysis of variance (ANOVA) comparing the effects of chlorpyrifos concentration and exposure duration on the oxygen consumption of *Neomysis integer*. n=9 for each pesticide exposure/concentration/duration, critical f=3·41 (concentration), 4·71 (duration) and 2·96 (interaction).

Source	d.f.	f statistic	critical f	significance
concentration	4	718	$8·5 \times 10^{-90}$	p<0·01
duration	2	109	$7·2 \times 10^{-29}$	p<0·01
interaction	6	4·65	$3·7 \times 10^{-11}$	p<0·01

Multiple linear regression analysis confirmed that oxygen consumption increased with increasing concentration of chlorpyrifos (regression coefficient 4·8) and decreased with exposure duration [regression coefficient -0·001 (Table 6.12)].

Table 6.12: Multiple linear regression analysis of the effects of chlorpyrifos concentration and exposure duration on the oxygen consumption of *Neomysis integer*. n=9 for each pesticide exposure concentration/duration combination, r^2=0·90.

Variable	Coefficients	95% Confidence Interval		t statistic	significance
		Lower	Upper		
y intercept	0·466	0·438	0·494	32·9	p<0·01
concentration	4·811	4·514	5·107	32·1	p<0·01
duration	-0·001	-0·001	-0·001	-7·5	p<0·01

Oxygen consumption by mysids exposed to chlorpyrifos was, therefore, described by the regression equation:

$$R = 0{\cdot}466 + 4{\cdot}811C - 0{\cdot}001D$$

where:- R = respiration rate (µl O_2 mg wet wt.$^{-1}$ h^{-1}), C = exposure concentration (µg chlorpyrifos L^{-1}), D = exposure duration (h).

6.4.3 Effect of chlorpyrifos on faecal production

Exposure to chlorpyrifos significantly affected the rate of faecal production of *N. integer* (Table 6.13).

Table 6.13: Effect of chlorpyrifos on the faecal production of *Neomysis integer*. Results expressed as percentages of faecal production of control mysids (acetone control; 100µl acetone L^{-1}) measured on the same day. Overall mean faecal production rate of controls (i.e.100%) = 0·044mg mg dry wt.$^{-1}$ h^{-1}. n=10.

Exposure concentration (µg chlorpyrifos L^{-1})	Exposure duration (h)	Faecal production (% of control)	95% Confidence Intervals	
			Lower	Upper
0·038	48	115	93	137
0·056	"	146	110	182
0·072	"	48	26	70
0·100	"	35	14	56
0·038	96	146	94	183
0·056	"	141	113	172
0·072	"	52	28	81
0·100	"	38	19	51
0·038	168	142	102	181
0·056	"	149	110	187
0·072	"	58	26	88
0·100	"	34	14	45

Exposure to the lowest pesticide concentrations (0·038 and 0·056µg chlorpyrifos L^{-1}) had no significant effect on faecal production rate of *N. integer* at each of the three exposure durations (95% confidence intervals, p>0·05; Fig. 6.11a - Fig 6.11c). Exposure to 0·072µg chlorpyrifos L^{-1} for 48h resulted in significantly reduced faecal production compared with control mysids (95% confidence intervals, p<0·05; Fig. 6.11a). Longer exposure periods (96 and 168h) to this pesticide concentration had no significant effect on faecal production (95% confidence intervals, p>0·05; Figs. 6.11b - 6.11c). At each exposure duration, 0·100µg chlorpyrifos L^{-1} significantly reduced the rate of faecal production relative to control mysids (95% confidence intervals, p<0·05; Figs. 6.11a -6.11c). The general trend in feeding response was of increased faecal production (relative to the control) after exposure to 0·038 and 0·056µg chlorpyrifos L^{-1}, and decreased faecal production (relative to the control) after exposure to 0·072 and 0·100µg chlorpyrifos L^{-1} (Table 6.13; Fig 6.11).

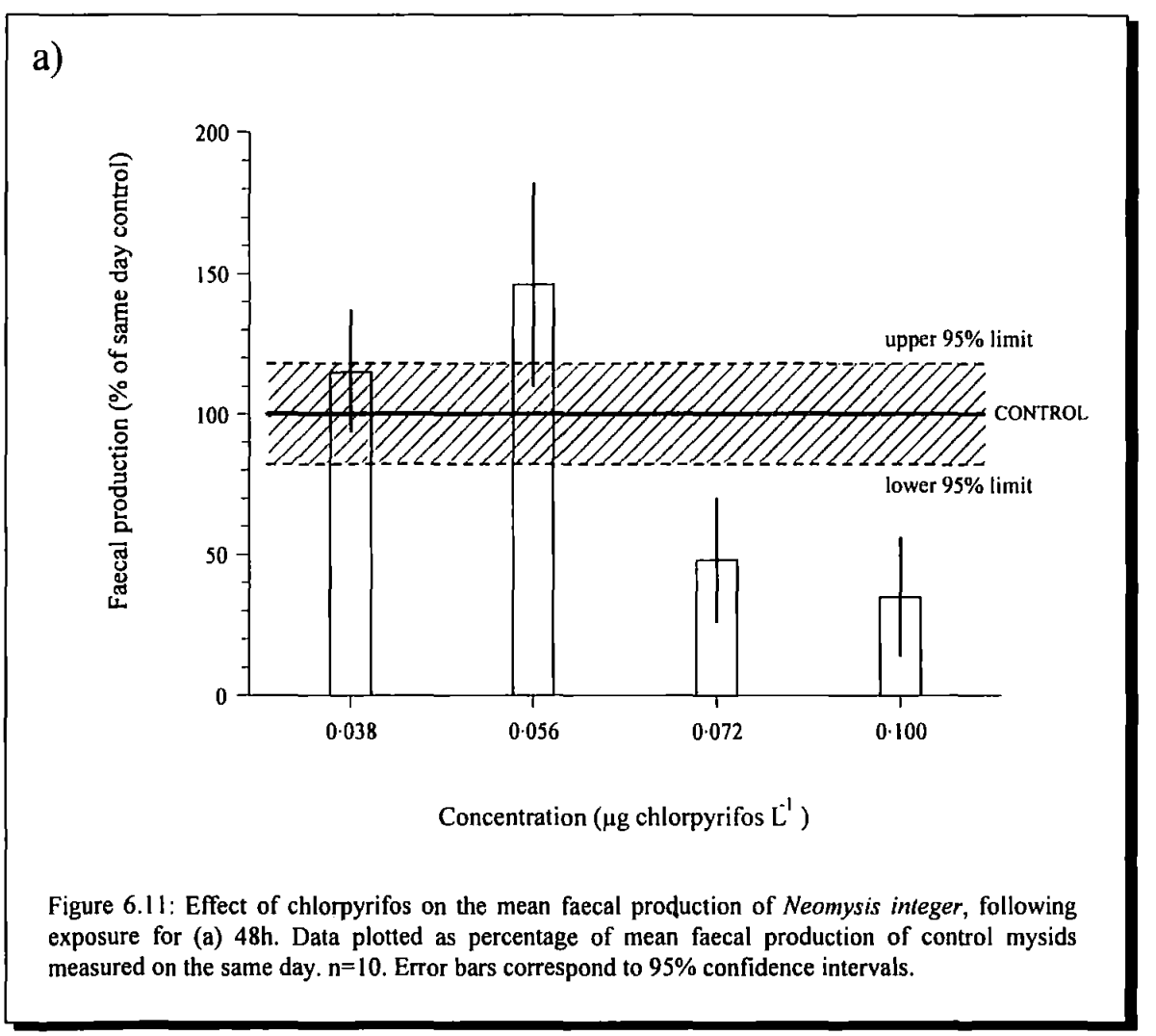
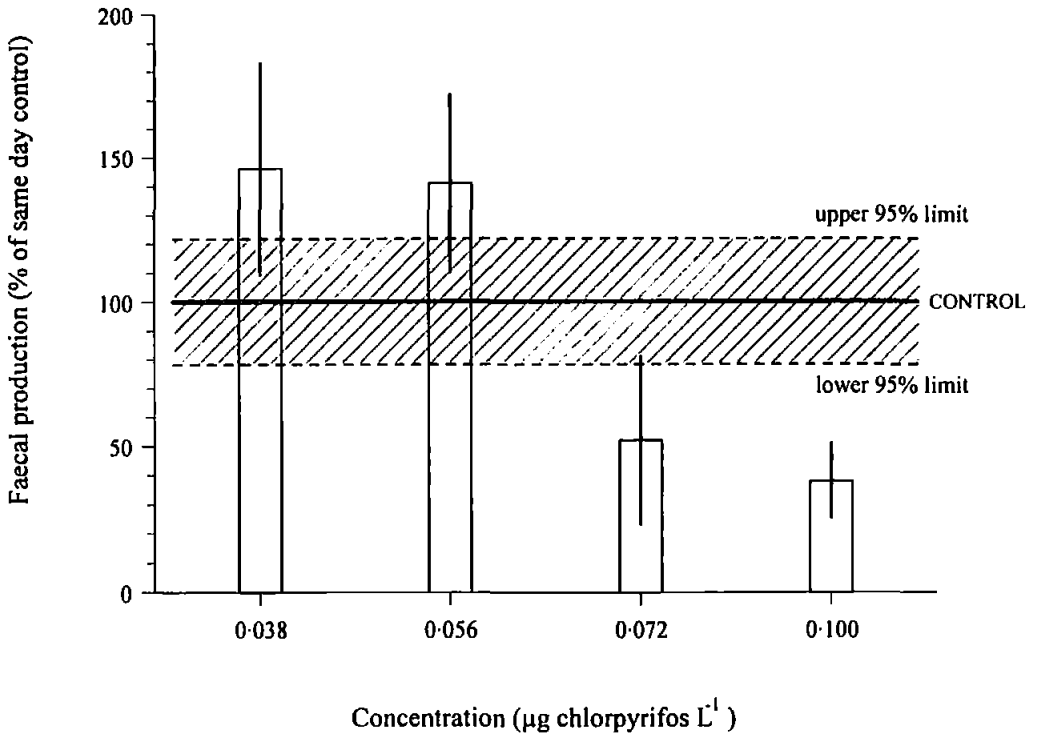


Figure 6.11: Effect of chlorpyrifos on the mean faecal production of *Neomysis integer*, following exposure for (a) 48h. Data plotted as percentage of mean faecal production of control mysids measured on the same day. n=10. Error bars correspond to 95% confidence intervals.

b)



c)

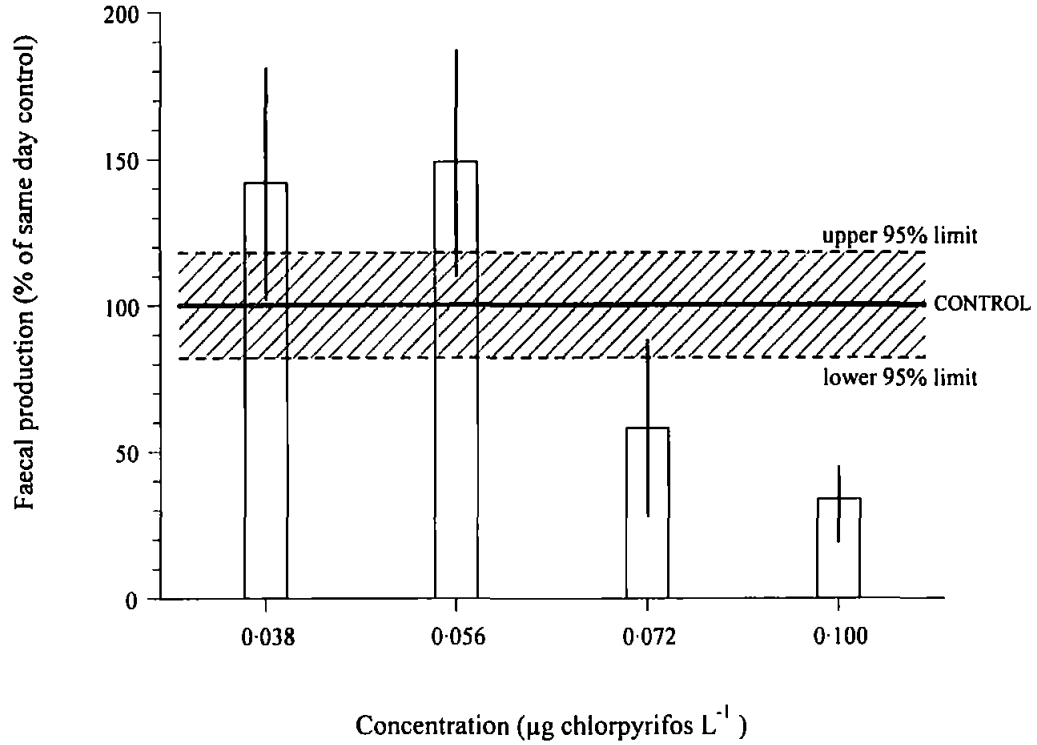


Figure 6.11 (cont'd): Effect of chlorpyrifos on the mean faecal production of *Neomysis integer*, following exposure for (b) 96h and (c) 168h. Data plotted as percentage of mean faecal production of control mysids measured on the same day. n=10. Error bars correspond to 95% confidence intervals.

Effect of chlorpyrifos on food absorption efficiency

Irrespective of exposure concentration and duration, the absorption efficiency of *Neomysis integer* was unaffected by chlorpyrifos (Table 6.14). Faecal material from the ten mysids exposed to each pesticide concentration/exposure duration was pooled preventing statistical analysis. There was, however, *c.*1% variation about the mean value calculated for the ash-free dry weight of the natural sediment (used in the calculation of the absorption efficiency). The absorption efficiency data for each chlorpyrifos exposure concentration was consistently within *c.*1% (i.e. the natural variation within the food source) of the absorption efficiency of control mysids (Table 6.14) and it is assumed, therefore, that any small variations in absorption efficiency were not due to exposure to chlorpyrifos.

Table 6.14: Effect of chlorpyrifos on the absorption efficiency of *Neomysis integer*. Values calculated from faecal material pooled from 10 mysids for each pesticide exposure concentration/duration combination.

Exposure Concentration (μg chlorpyrifos L^{-1})	Exposure Duration (h)	Absorption Efficiency
Control (100µl acetone L^{-1})	48	0·356
	96	0·362
	168	0·357
0·038	48	0·348
	96	0·352
	168	0·349
0·056	48	0·347
	96	0·349
	168	0·35
0·072	48	0·342
	96	0·353
	168	0·345
0·100	48	0·342
	96	0·344
	168	0·342

Starvation

Mysid mortalities occurred within the first 24h of being starved and the LT_{50} for starvation was *c.*70h, depending on which data points are used in the calculation of LT_{50} (Fig. 6.12).

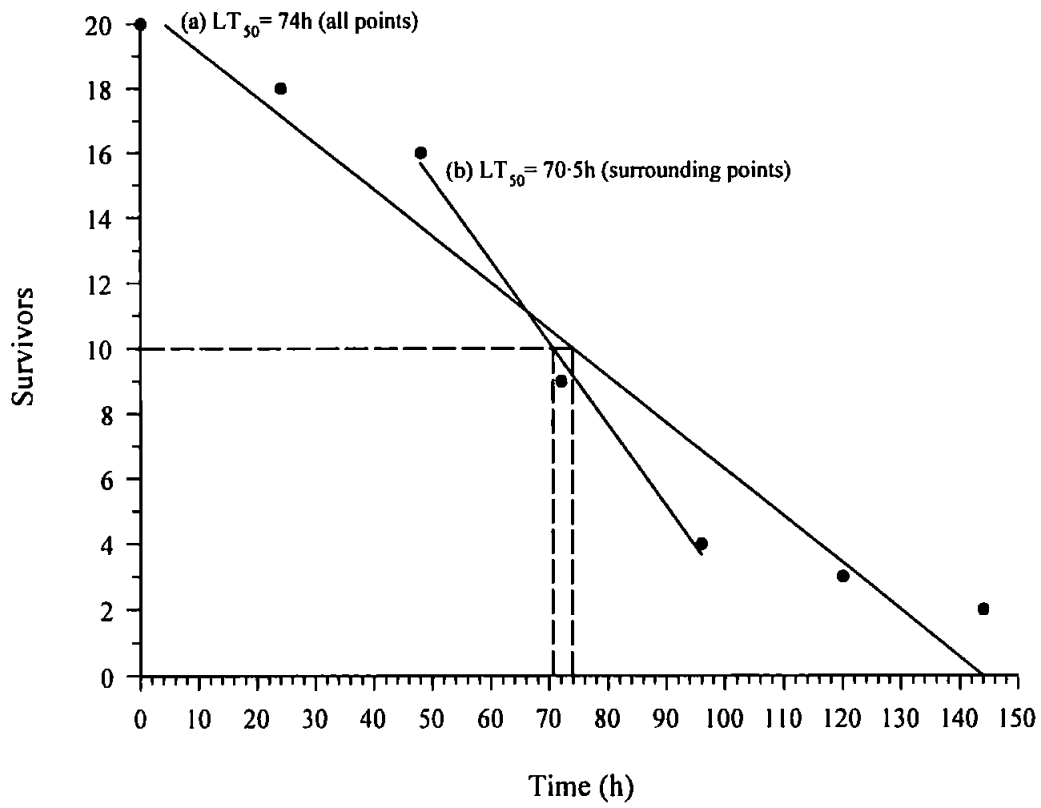


Figure 6.12: Lethal median time (LT_{50}) for the starvation tolerance of *Neomysis integer*. LT_{50} values calculated from regression lines (a) for all data points [y = 20·57 - 0·14x (r^2=0·94)] and (b) limited to those data points surrounding 50% mortality (y = 27·7 - 0·25x (r^2=0·99)].

Linear regression analysis was applied to the data and the LT_{50} calculated from the regression equation (Fig.6.12). If the regression analysis is applied to all the data points, the LT_{50} for starvation is estimated at 74h. The data have a sigmoidal distribution, however, and the regression equation is affected by relatively small changes in mortality before 48h and after 120h, causing a decrease in the gradient of the regression line (Fig. 6.12). An estimated LT_{50} value of 74h is, therefore, too high (e.g. LT_{50}=74h compared with 55% mortality after 72h). Linear regression analysis was, therefore, applied only to the data points closest to the 50% data (i.e. the datum closest to 50%, or 10 mortalities, and those data points on either side), resulting in an LT_{50} for mysid starvation of 70·5h (Fig. 6.12).

Scope for growth

Chlorpyrifos had a significant effect on the scope for growth (SFG) of *Neomysis integer* (ANOVA, f=61·45, d.f.=4, p<0·01). The SFG of *N. integer* decreased significantly relative to control mysids after exposure to 0·072 and 0·100µg chlorpyrifos L^{-1} (Table 6.15; Fig. 6.13). At 0·100µg chlorpyrifos L^{-1}, the lower 95% confidence interval has a negative value, indicating that some mysids may be utilising more energy than they are assimilating (Table 6.15; Fig. 6.13). The SFG of mysids exposed to 0·038 and 0·056µg chlorpyrifos L^{-1} increased relative to the control, but this was not significant (95% confidence intervals, p>0·05; Table 6.15; Fig. 6.13).

Table 6.15: Scope for growth for *Neomysis integer* exposed to chlorpyrifos. n=3 for each chlorpyrifos concentration.

Chlorpyrifos concentration (µg chlorpyrifos L^{-1})	Scope for Growth (J mg dry wt^{-1} h^{-1})	95% Confidence intervals	
		lower	upper
control (100µl acetone L^{-1})	0·321	0·216	0·425
0·038	0·431	0·285	0·593
0·056	0·450	0·358	0·541
0·072	0·108	0·047	0·169
0·100	0·032	-0·023	0·086

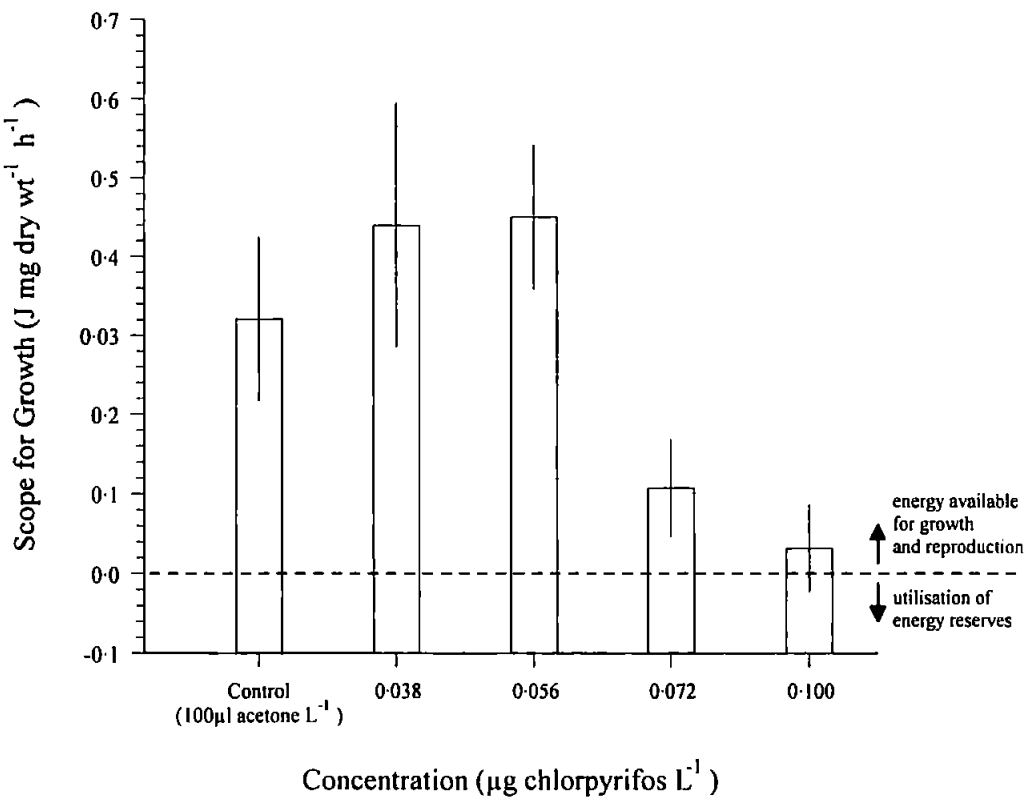


Figure 6.13: Effect of chlorpyrifos on the mean scope of growth for *Neomysis integer*; n=3. Error bars correspond to 95% confidence intervals.

6.5 DISCUSSION

6.5.1 Swimming behaviour

In general, significant differences in the swimming behaviour of *Neomysis integer* were recorded after exposure to each test concentration of chlorpyrifos (0·038 - 0·100µg chlorpyrifos L^{-1}). Pesticide exposure had the most significant effects at the lowest (i.e. 3cm s^{-1}) and highest (i.e. 18cm s^{-1}) current velocities. The effect of chlorpyrifos on the swimming behaviour of *N. integer* interacted with current velocity effects and, therefore, was very complex. Many responses were due to single concentration effects at individual current velocities, and the relevance of these variable responses in the natural environment is unknown. Only major trends in the data, therefore, are discussed.

Current velocity is an important factor controlling the distribution of *Neomysis integer* in the East Looe River Estuary (Chapter 2). At Terras Bridge, *N. integer* was limited to areas where the current velocity did not exceed *c.* 12cm s^{-1}. Mysid distribution, therefore, appears to be controlled primarily by the ability of mysids to maintain their position against a current (Chapter 2). Any disruption of this ability may cause the displacement of mysids to unfavourable regions of the estuary or make them more vulnerable to predation. At low current velocities (e.g. 3 and 6cm s^{-1}), relatively low pesticide concentrations (e.g. 0·038 and 0·056µg chlorpyrifos L^{-1}) caused increased swimming activity and elevated numbers of mysids swam forward into the current. Increased swimming activity is consistent with the mode of action of chlorpyrifos (Chapter 5, Section 1.1), resulting from the accumulation of acetylcholine at neural junctions over-stimulating the peripheral nervous system. Some literature support the present results. For example, *Porcellio scaber* (Crustacea: Isopoda) and *Folsomia candida* (Hexapoda: Collembola) showed increased activity after exposure to the organophosphate pesticide dimethoate (Sørensen *et al.*, 1995; Bayley & Baatrup, 1996). Similarly, following exposure to 5µl benzene L^{-1}, the mysid *Mysidopsis junaie* swam twice as far in one minute as did control mysids (Martinez *et al.*, 1992). The mode of action of benzene on mysid behaviour is unknown, however, although the increased activity of *M. junaie* may be an avoidance response.

Results from Chapter 2 suggest that increased swimming activity would cause the population of *N. integer* at Terras Bridge to move further upstream at low tide and further downstream on the flood tide. The implications of such displacement are unknown but may

include movement towards areas of faster water flow (resulting in increased displacement from optimum conditions), displacement from regions of optimum feeding [e.g. movement away from the maximum turbidity zone (Chapter 4)], or movement into areas where predator density is increased. Even if increased swimming activity does not cause *N. integer* to move into less favourable regions of the estuary, it does cause increased metabolic activity (the faster a mysid swims the more oxygen it requires). The increased activity, and energy expenditure, of chlorpyrifos-exposed *N. integer* may cause utilisation of energy reserves that may be required for other purposes. For example, energy utilisation by rainbow trout (*Onchorhynchus mykiss*) required to swim at any given current velocity was increased in fish exposed to trace metals compared with unexposed fish swimming at the same speed (Waiwood & Beamish, 1978; Wilson *et al.*, 1996). If the same was true for mysids exposed to chlorpyrifos, they would be able to swim for shorter durations than unexposed mysids, again leading to disruption of position maintenance.

Exposure to 0·038, 0·072 and 0·100μg chlorpyrifos L^{-1} caused a decrease in the maximum swimming speed of *N. integer*, such that mysids were unable to swim forward into the current at 15cm s^{-1}. There are clear implications of such reduced ability to swim against this current velocity for *N. integer* in the East Looe River Estuary (mysids would be displaced, leading to the possible deleterious consequences described earlier). In general, the mysid population at Terras Bridge was distributed in areas where the current velocity was <15cm s^{-1} (Chapter 2), however, single mysids may move into areas where the current velocity is ≥15 cm s^{-1} and need to swim to regain their position with the rest of the population. Reduction in the maximum swimming speed after exposure to toxicants has been reported frequently for fish (e.g. Wilson & Wood, 1992; Taylor *et al.*, 1996). Exposure to 0·47μmol copper L^{-1} caused a significant reduction in the critical swimming speed of brown trout [*Salmo trutta* (Taylor *et al.*, 1996)]. The critical swimming speed of juvenile rainbow trout (*Oncorhynchus mykiss*) was reduced following exposure to 'acid' and 'acid + aluminium' water (Wilson, 1996). There are limited data, however, on the effects of contaminants on crustacean swimming speeds. Exposure to 20μl benzene L^{-1} caused a decrease in the swimming activity of *Mysidposis junaie* (Martinez *et al.*, 1992) although swimming speed *per se* was not examined. Cripe *et al.* (1981) reported that exposure to two organophosphate pesticides (phorate and methyl parathion) reduced the swimming speed of *Americamysis bahia*. Following 96h exposure to the pesticides, the

maximum sustained speed of *A. bahia* was reduced from *c.*12cm s^{-1} (in control mysids) to *c.*1 and 7cm s^{-1} for mysids exposed to phorate and methyl parathion respectively (Cripe *et al.*, 1981). The results from the current study are, therefore, in agreement with these effects of organophosphate pesticides on *A. bahia.*

Another noticeable effect was that chlorpyrifos significantly elevated the number of mysids swimming with the current at high current velocities (i.e. >15cm s^{-1}). There are two possible explanations for this response. Exposure to chlorpyrifos may have caused disruption of the sensory capabilities of *N. integer*, leading to disruption of the usual rheotaxic response (Chapter 2). More likely, exposure to chlorpyrifos exerted increased energetic expenditure on *N. integer*, causing mysids to travel with the current whilst resting. If exposure to chlorpyrifos had disrupted the rheotaxic response of *N. integer*, increased numbers of animals swimming with the current would be expected at all current velocities. Exhaustion of chlorpyrifos exposed mysids is, therefore, a better explanation for the increased frequency of this behaviour at 18cm s^{-1}. Although chlorpyrifos exposure may have led to increased energy expenditure, this has little relevance in terms of mysid position maintenance at Terras Bridge. Similar proportions of control and pesticide exposed mysids were swept by the current at 18cm s^{-1} (only direction facing was altered by pesticide exposure). In the East Looe River Estuary, therefore, most mysids would be not be able to maintain position against a current velocity of 18cm s^{-1}, but contaminated individuals would try to swim against this current speed for a shorter duration.

6.5.2 Oxygen consumption

Exposure to chlorpyrifos resulted in increased oxygen consumption by *Neomysis integer*, and there was a direct correlation between oxygen consumption and pesticide concentration. Mysids exposed to 0·100µg chlorpyrifos L^{-1} consumed oxygen at twice the rate of control mysids. These results are in agreement with the effects of other toxic contaminants on the oxygen consumption of mysids. The oxygen consumption of *Neomysis americana* increased following exposure to naphthalene (an aromatic hydrocarbon) compared with control mysids (Smith & Hargreaves, 1985). Exposure to the pesticides thiobencarb and fenthion resulted in increased oxygen consumption by *Americamysis bahia*, which was dependent upon pesticide concentration (McKenney, 1985; McKenney &

Matthews, 1990). In contrast, exposure to the water soluble fraction of fuel oil did not significantly affect the oxygen consumption of *N. integer* (Laughlin & Lindén, 1983), and exposure to cadmium caused a significant concentration-dependent decrease in the oxygen consumption by *Leptomysis lingvura* (Gaudy *et al.*, 1991). For aquatic organisms, it appears that the oxygen consumption response to contaminant exposure is dependent upon the type of chemical. Following sub-lethal trace metal poisoning, the oxygen consumption of aquatic invertebrates may be increased [e.g. *Acartia clausi* (Moraitou-Apostolopoulou & Verriopoulos, 1979)], decreased [e.g. *Callinectes sapidus* (Guerin & Stickle, 1995)], or remain unchanged [e.g. *Gamarus pulex* (Maltby & Naylor, 1990)]. Organic compounds tend to increase the rate of oxygen consumption [e.g. *Neomysis americana* (Smith & Hargreaves (1985)], particularly organophosphate pesticides [e.g. *Americamysis bahia* (McKenney & Matthews, 1990)].

Exposure to low concentrations of chlorpyrifos (i.e. 0·038 and 0·056μg chlorpyrifos L^{-1}) led to hyperactivity of *N. integer*, and this would lead to increased requirements for oxygen. The fact that mysids exposed to higher pesticide concentrations (i.e. 0·072 and 0·100μg chlorpyrifos L^{-1}) consumed oxygen at the highest rate implies that oxygen consumption is related more directly to metabolic effects of chlorpyrifos than mysid activity alone. Extrapolation of the effects of chlorpyrifos on oxygen consumption to natural mysids populations suggests increased energy expenditure due to increased oxygen consumption. In addition, increased oxygen consumption by mysids at rest (as they were for oxygen consumption measurements), implies that swimming mysids consume more oxygen, leading to reduced swimming ability or stamina. The implications of this have already been described (Section 6.4.1).

In the present study, length of exposure duration at any given concentration of chlorpyrifos significantly affected the oxygen consumption of *Neomysis integer*. Irrespective of chlorpyrifos concentration, oxygen consumption was highest in mysids exposed for 48h, and decreased at 96 and 168h. The reason for this difference is unclear, however, it may be linked with detoxification mechanisms. At 48h, mysids may be responding to the physiological stress of acute pesticide poisoning, whereas at 96h and 168h, detoxification mechanisms (e.g. mixed function oxidases) may have been stimulated, resulting in more efficient metabolism of the pesticide. These data imply an ability to recover from toxic

effects if exposure to pesticides is prolonged. In contrast to the findings of the present study, the oxygen consumption of the blue crab (*Callinectes sapidus*) did not recover during prolonged cadmium exposure (Guerin & Stickle, 1995). In the natural environment, therefore, mysids may show some recovery following chronic pesticide exposure, but would be most vulnerable during the first 48h of contamination. Despite metabolic recovery by mysids following pesticide exposure for ≥96h, however, oxygen consumption rates for mysids exposed to ≥0·038µg chlorpyrifos L^{-1} after 168h were still significantly greater than control rates. Mysids exposed to ≥0·038µg chlorpyrifos L^{-1} in the natural environment are, therefore, likely to have increased energetic demands.

6.5.3 Feeding rate

The feeding rate of mysids exposed to ≥0·072µg chlorpyrifos L^{-1} was significantly reduced compared with control mysids, and chlorpyrifos effects at these concentrations were concentration-dependent. In addition to a clear concentration-response, the feeding rate was generally most affected at the shortest exposure period (i.e. 48h). For example, although significantly reduced at 48h, the feeding rate of mysids exposed to 0·072µg chlorpyrifos L^{-1} was not significantly affected by pesticide exposures longer than 48h. The exception was at the highest pesticide concentration (0·100µg chlorpyrifos L^{-1}) which significantly reduced the feeding rate of *Neomysis integer* after 48, 96 and 168h exposure periods. The effect of exposure duration on the toxic effects of chlorpyrifos on *N. integer* are unknown, but may be due to metabolism of the pesticide after 48h (Section 6.5.2). *Neomysis integer*, therefore, appears unable to efficiently metabolise chlorpyrifos at concentrations >0·072g L^{-1}. The increase in feeding rate of mysids exposed to 0·038 and 0·056µg chlorpyrifos L^{-1} may be accounted for by the hyperactivity of these mysids requiring increased energy intake. In contrast, exposure to chlorpyrifos concentrations >0·056µg L^{-1}, although causing mysid hyperactivity, appears to cause other metabolic disruption and feeding suppression.

The results of the current study are in agreement with other workers who have reported a general decrease in the feeding rates of Crustacea following exposure to toxicants (e.g. Crane & Maltby, 1991; Gaudy *et al.*, 1991; Crane *et al.*, 1995; Guerin & Stickle, 1995). Amphipod feeding rate has been proposed as a sensitive bioassay for highlighting the contamination of freshwater environments (Maltby & Crane, 1994). The feeding rate of the

contamination of freshwater environments (Maltby & Crane, 1994). The feeding rate of the freshwater amphipod *Gammarus pulex* feeding *in situ* downstream of effluent discharges from a sewage treatment works (processing domestic and industrial waste), a quarry and a paper mill decreased relative to control animals feeding *in situ* upstream of the discharge points (Crane & Maltby, 1991). Following application of an organophosphate insecticide (Malathion 60) to water cress farms, the feeding rate of *G. pulex* kept in cages below the water cress beds decreased by almost 50% [although the feeding rates of *G. pulex* in cages in the settling pool increased by almost 50% (Crane *et al.*, 1995)]. Following exposure to >50μg cadmium L^{-1}, the feeding rate of the blue crab (*Callinectes sapidus*) was significantly suppressed (Guerin & Stickle, 1995). Few studies have investigated the effect of contaminant exposure on mysid feeding rate. Following exposure to 0·1mg cadmium L^{-1}, the feeding rate of *Leptomysis lingvura* decreased by almost 50% (Gaudy *et al.*, 1991). In the natural environment, exposure of *Neomysis integer* to ≥0·072μg chlorpyrifos L^{-1}, and subsequent suppression of the feeding rate, would cause a net loss in energy acquisition, leading to reduced stamina (e.g. for position maintenance) and growth.

Absorption efficiency

The absorption efficiency of *Neomysis integer* was unaffected by exposure to any of the concentrations of chlorpyrifos, or by the duration of the exposure period. In contrast to the findings of the current study, the absorption efficiency of *Leptomysis lingvura* exposed to cadmium was *c.*9% lower than that of control mysids (Gaudy *et al.*, 1991). In addition, the absorption efficiency of the blue crab *Callinectes sapidus* was reduced after exposure to 0·50mg cadmium L^{-1} (Guerin & Stickle, 1995). The stability of the absorption efficiency of *N. integer* may be related to the low organic content of the sediment used as food, which was likely to have a high refractile component (Chapter 4). Absorption efficiency is generally less sensitive to contaminant exposure than ingestion rate. Disruption of the latter is considered to be the most significant energy acquisition process and is, therefore, used most frequently in toxicological studies. For example, a decrease in the absorption efficiency affected total energy acquisition by <25% in *Leptomysis lingvura* (Gaudy *et al.*, 1991) and <30% in *Callinectes sapidus* (Guerin & Stickle, 1995). When combined, the decrease in feeding rate (*c.*50%), and the decrease in absorption efficiency (*c.*9%), meant an overall 43% decrease in the energy acquisition of *L. lingvura* exposed to cadmium. The

results from the current study suggest up to 66% decrease in the energy acquisition of *N. integer* exposed to chlorpyrifos, and this is due solely to reduced ingestion rate.

Starvation

Neomysis integer was able to withstand starvation for approximately 70h (LT_{50} for starvation=70·5h). Mortalities occurred within the first 24h, and 80% of mysids died within 144h of starvation. These results are comparable with those of Linford (1963) who reported that *N. integer* survived starvation for 6 or 7 days (144h or 168h). In the current study, the 96h and 168h LC_{50} values for chlorpyrifos to *N. integer* were 0·137 and 0·084µg chlorpyrifos L^{-1} respectively (Chapter 5). The feeding experiments suggest, therefore, that suppressed feeding and starvation of *N. integer* during LC_{50} tests may make a significant contribution to mysid mortality. Following 48h exposure to 0·100µg chlorpyrifos L^{-1}, feeding rate was reduced to 38% of normal feeding rate and, since a concentration-response was apparent, it may be assumed that at 0·137µg chlorpyrifos L^{-1} feeding rate was suppressed to <38% of the normal rate. Starvation caused 50% mortality of control mysids after *c.*70h, therefore, it appears that suppression of feeding or starvation is probably a major factor influencing mysid mortality in the 96 and 168h LC_{50} tests. Increased oxygen consumption and, therefore, energy utilisation, by pesticide-exposed mysids supports this assumption. Food availability during acute toxicity tests significantly affected the estimated LC_{50} values for four chemicals on *Americamysis bahia* (Cripe *et al.*, 1989). When fed a ration of 10 *Artemia* nauplii $mysid^{-1}$ day^{-1}, *A. bahia* was significantly more sensitive to carbophenothion and malathion (organophosphate insecticides), cypermethrin (a synthetic pyrythroid insecticide) and 4-(*tert*-Octyl) phenol (an ionic component of detergents) than mysids fed a ration of 60 *Artemia* nauplii $mysid^{-1}$ day^{-1} (Cripe *et al.*, 1989). Reduced food rations also reduced the survival of sheepshead minnow (*Cyprinidon variegatus*) chronically exposed to 3·0µg chlorpyrifos L^{-1} (Cripe *et al.*, 1986). It is likely that mortalities in the LC_{50} tests are a combination of the direct toxicity of chlorpyrifos and the utilisation of all energy reserves through cessation or suppression of feeding, and increased respiration. The relative importance of each factor is unknown. Tolerance to starvation, however, has important implications for LC_{50} tests, and species which cannot withstand starvation well are desirable. There is a need, therefore, to have good knowledge of the biology of proposed test species.

6.5.4 Scope for growth

The previous discussion has highlighted that chlorpyrifos exposure increases the energy expenditure of *Neomysis integer*. Increased oxygen consumption reflects increased energy expenditure [the heat equivalent of oxygen uptake is 0·456J μMol oxygen^{-1} (Gnaiger, 1983)] and decreased feeding rates result in decreased energy acquisition. The calculation of the scope for growth (SFG) of *N. integer* combines these physiological responses and demonstrates the overall physiological effect of chlorpyrifos on the organism.

The SFG of *N. integer* was dependent upon the concentration of chlorpyrifos above a threshold of 0·056μg chlorpyrifos L^{-1}. For control *N. integer* (at 15°C and 10‰), the SFG was 0·32J mg dry wt^{-1} h^{-1}. Exposure to 0·038 and 0·056μg chlorpyrifos L^{-1} had no significant effect on SFG. At these concentrations, oxygen consumption increased, but energy acquired through increased feeding was greater than the extra energy required for increased respiration and a net energy gain occurred, as seen in the slightly elevated SFG. At 0·072 and 0·100μg chlorpyrifos L^{-1}, however, mysids had reduced SFG, caused by increased respiration coupled with decreased food intake. The SFG of mysids exposed to 0·100μg chlorpyrifos L^{-1} was close to zero, and the lower 95% confidence interval was negative indicating that some mysids might be utilising energy reserves (i.e. have negative SFG).

In general, SFG declines in organisms exposed to toxicants. Bivalve molluscs are used most frequently in SFG studies and there are many reports of the SFG of the blue mussel (*Mytilus edulis*) decreasing after exposure to toxic contaminants (e.g. Widdows & Donkin, 1992; Widdows and Page, 1993; Widdows *et al.*, 1995). The SFG approach has been used rarely for crustaceans. The SFG of blue crabs (*Callinectes sapidus*) was significantly reduced as the exposure concentration and/or exposure duration of cadmium increased (Guerin & Stickle, 1995). The SFG of *C. sapidus* also decreased following exposure to the water soluble fractions of crude oil (Wang & Stickle, 1988). Following exposure to 0·3mg zinc L^{-1}, the SFG of ovigerous female *Gammarus pulex* was significantly reduced, primarily due to reduced feeding and energy absorption (Maltby & Naylor, 1990). In a previous study, the SFG of adult male *G. pulex* was reduced after exposure to 0·5mg zinc L^{-1} (Maltby *et al*, 1990) and it was concluded that brooding females were more sensitive to toxicants than adult males (Maltby & Naylor, 1990). Although SFG has not been calculated

previously for mysids, the reduced food consumption, and reduced absorption efficiency of *Leptomysis lingvura* following exposure to cadmium (Gaudy *et al.*, 1991), suggests that the SFG of this mysid species was reduced due to cadmium contamination.

The SFG of *Neomysis integer* exposed to toxicants in the natural environment may be reduced more than these laboratory results suggest. In the present study, *N. integer* has increased oxygen consumption and decreased food consumption after exposure to 0·072 and 0·100µg chlorpyrifos L^{-1}, leading to a significantly reduced SFG. These results, however, were for mysids at rest. Increased oxygen consumption of active mysids (i.e. swimming mysids) is likely, so mysids swimming to maintain their position in the estuary are likely to respire at a higher rate, decreasing their SFG further. In addition, pesticide disruption of swimming behaviour is predicted to cause mysids to lose position in the estuary and may cause them to move into less favourable feeding regions. Therefore, energy acquisition (and SFG) may decrease. If the predicted increase in energy demands of swimming activity, and decreased energy acquisition, are considered together, the SFG of *N. integer* at Terras Bridge may decrease to a value much lower than that measured in the laboratory.

6.6 SUMMARY

Neomysis integer was sensitive to pesticide exposure at concentrations below those which cause lethal effects. Swimming behaviour and oxygen consumption were significantly affected by exposure to ≥0·038µg chlorpyrifos L^{-1}, and feeding rate and SFG were significantly affected by exposure to ≥0·072µg chlorpyrifos L^{-1}. The general responses and SFG of *N. integer* following exposure to chlorpyrifos are summarised in Table 6.16. In general, the responses of *N. integer* in the present study are similar to those of other Crustacea exposed to organophosphate pesticides (e.g. Cripe *et al.*, 1981; Gaudy *et al.*, 1991; Crane & Maltby, 1991; Bayley & Baatrup, 1996).

Table 6.16: General responses, and scope for growth, of *Neomysis integer* in three bioassays following exposure to chlorpyrifos. All responses described relative to control mysids.

Response	Minimum concentration causing response (µg chlorpyrifos L^{-1})	Response of exposed mysids
Swimming	0·038	At low current velocities (3cm s^{-1}), increased number of mysids swam forward into the current.
	0·072	At high current velocities (15cm s^{-1}), fewer mysids swam forward into the current.
	0·038	At all current velocities, fewer mysids maintained position.
	0·038	At high current velocities (18cm s^{-1}), increased numbers of mysids swam with the current.
Oxygen consumption rate	0·038	Mysids increased their rate of oxygen consumption. Oxygen consumption was related directly to pesticide concentration.
Feeding rate	0·072	Feeding rate of mysids had reduced feeding rates. Feeding rate was related directly to pesticide concentration.
Scope for Growth (SFG)	0·072	SFG was reduced (possibility of negative SFG at 0·100µg chlorpyrifos L^{-1}). SFG was related directly to pesticide concentration.

CHAPTER 7

GENERAL DISCUSSION

7.1 ECOPHYSIOLOGY OF *NEOMYSIS INTEGER*

Results from laboratory studies examining the swimming behaviour, oxygen consumption and feeding rate of *Neomysis integer* (Chapters 2, 3 and 4) are consistent with the ecological distribution of *N. integer* at Terras Bridge, East Looe River Estuary, Cornwall. At Terras Bridge, *N. integer* does not appear to make migrations over the tidal cycle and was always found in the vicinity of the bridge. Other workers, however, have reported that *N. integer* makes tidally-based migrations in response to salinity (e.g. Hough & Naylor, 1994). Salinity had no effect on the swimming behaviour of *N. integer* in the flume experiments and it is concluded that current velocity is the main determining factor controlling the distribution of *N. integer* at Terras Bridge. Under high current velocities, *N. integer* maintains position by moving to the slower moving water at the periphery of the river. The same behaviour was described for *Gastrosaccus brevifissura* in the Gamtoos River Estuary, South Africa (Schalacher & Wooldridge, 1994). Although euryhaline [in previous studies, *N. integer* has been shown to respond to changes in salinity within 2h (Moffat, 1996)], *N. integer* shows physiological responses to changes in salinity. Based on the results from the current laboratory studies, and measurements of physico-chemical variables at Terras Bridge, the oxygen consumption and feeding rates of *N. integer* respond to tidally-based salinity fluctuations as illustrated in Figure 7.1a. Oxygen consumption and feeding will be stable over most of a typical tidal cycle, only increasing for the *c.*2h period when the site floods (Fig. 7.1a). It is assumed that these changes in oxygen consumption and feeding rate are within the usual physiological range tolerated by this species. Changes in salinity are accompanied by tidally-based temperature changes [seawater was *c.*5°C warmer than the fresh river water at Terras Bridge (Fig. 7.1a)]. This tidally-based temperature change will also affect respiration and feeding rates (Figure 7.1a).

In addition to the short-term responses of *Neomysis integer* to tidally-based changes in salinity and temperature, there will be a seasonally-dependent temperature response, with physiological processes increasing during the warmer months of spring and summer (Fig. 7.1b). This seasonally-based temperature response is likely to be important in controlling mysid productivity. Increased temperatures will lead to increased increased feeding, and, if food is available, increased scope for growth (Fig. 7.2), resulting in the observed high reproductive output during the spring and summer (Chapter 1). In the current study, scope for growth was very closely coupled with feeding rate. Hence, feeding rates alone may serve as a good indicator of mysid productivity. Feeding rate is often the most sensitive

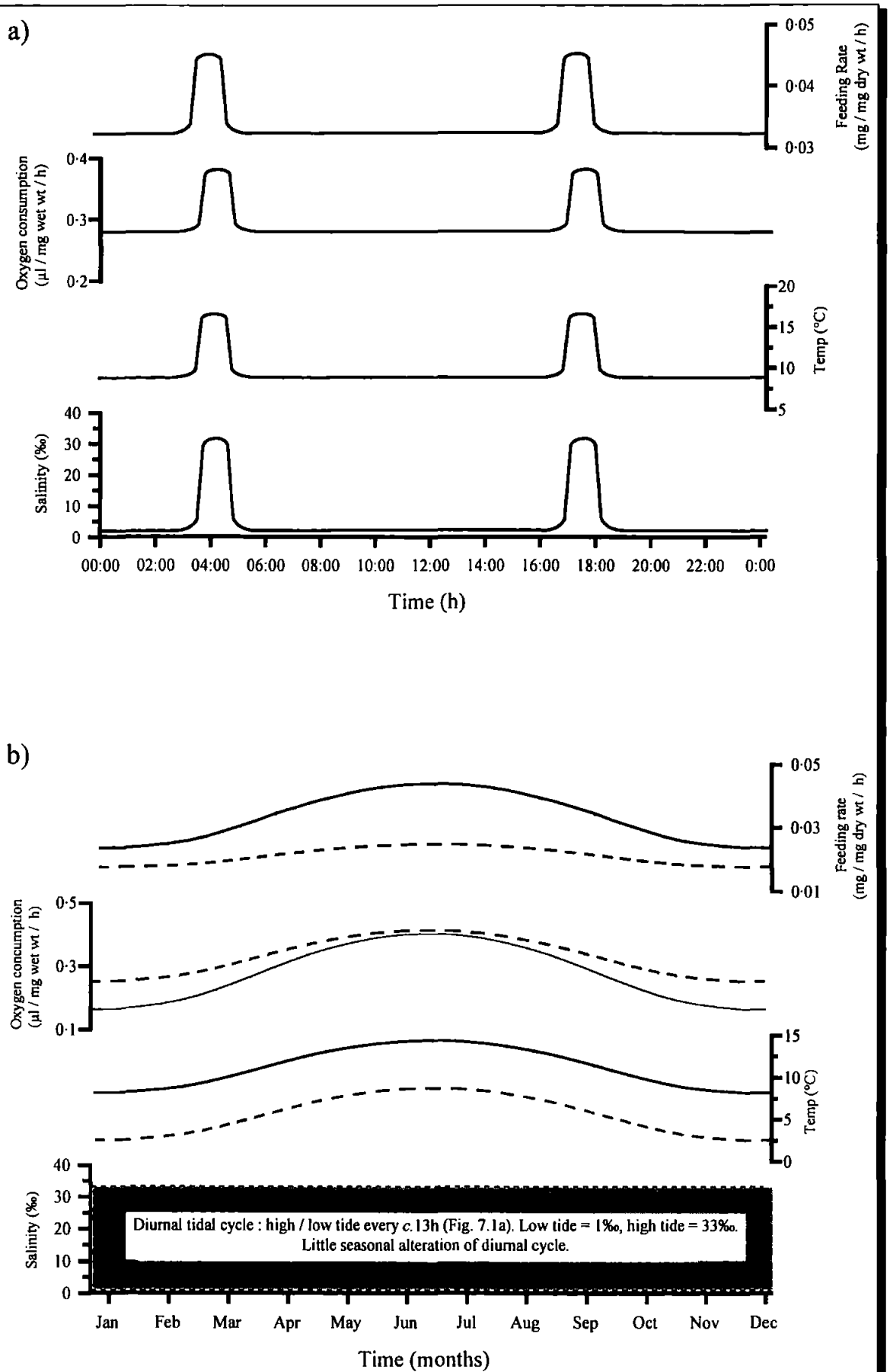


Figure 7.1: Schematic representation of how the rates of oxygen consumption and feeding of *Neomysis integer* might change in response to environmental variables (salinity and temperature) over (a) a typical summer tidal cycle and (b) a typical year, at Terras Bridge, East Looe River Estuary, Cornwall. Solid line = high tide data; dashed line = low tide data.

physiological response in estimates of scope for growth, and is frequently used in isolation (e.g. Crane & Maltby, 1991)]. It has been suggested that *N. integer* makes seasonal migrations, moving seawards during the winter, possibly to stay in the warmer regions of the estuary (Ralph, 1965). Seasonal migration did not appear to occur in the mysid population at Terras Bridge, where mysids were found all year round except on sampling occasions immediately after heavy rainfall when river flow velocities were extremely high (>1m s^{-1}), presumably 'flushing' mysids further downstream.

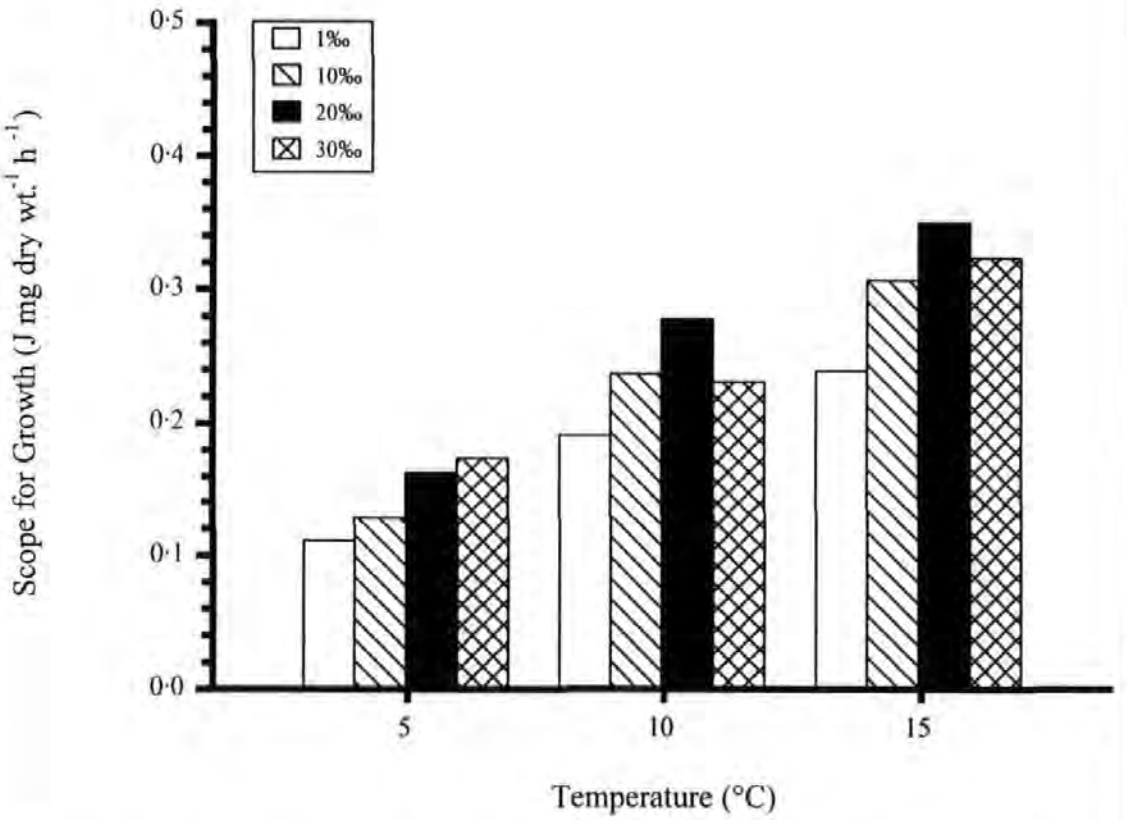


Figure 7.2: Mean scope for growth of male *Neomysis integer* from Terras Bridge, East Looe River Estuary, Cornwall, in response to salinity and temperature. n=9 at each temperature/salinity combination.

7.2 ENVIRONMENTAL TOXICOLOGY

Neomysis integer has proven to be relatively sensitive to the organophosphate pesticides chlorpyrifos and dimethoate. Acute median lethal concentrations (96h LC_{50}) for these two compounds (0·137µg L^{-1} and 0·543mg L^{-1} for chlorpyrifos and dimethoate respectively) are similar to those of other organisms used frequently in toxicity tests (WHO, 1990; Barron &

Woodburn, 1994). There is increasing interest in using indigenous species important to local ecosystems for toxicity testing, since their sensitivity to contaminants is more representative in predicting pollutant effects on local ecosystems. The mysid *Americamysis bahia* is one of the most frequently used organisms in toxicity testing for the estuarine environment (e.g. ASTM, 1990), even though it is a sub-tropical species and its relevance to temperate European estuaries is dubious. The current study has shown *N. integer* to be sensitive to chlorpyrifos and, given its widespread and abundant occurrence in European estuaries (Fockedey & Mees, 1997), *N. integer* is considered to be a better choice than *A. bahia* for toxicity testing for European estuaries. Preliminary work by other authors has led to the same conclusion (e.g. Emson & Crane, 1992; NRA, 1993).

In addition to its acute, lethal sensitivity to chlorpyrifos, *Neomysis integer* showed sub-lethal responses to chlorpyrifos exposure. Swimming behaviour, oxygen consumption and feeding rates were affected at pesticide concentrations below those causing mortality in acute tests. Effects of exposure to chlorpyrifos, at lethal and sub-lethal exposure concentrations, are summarised in Figure 7.3. There is growing demand for bioassays which better demonstrate effects on natural populations and one of the most popular of these is the integrated approach of scope for growth (SFG). The SFG assay incorporates contaminant effects on several physiological processes and gives results in an easily understood, ecologically important manner (i.e. how much, if at all, an organism may grow and reproduce). From an ecological perspective, each of the responses examined in the present study has significant implications for natural mysid populations exposed to toxic contaminants and these have been discussed (Chapter 6). A critical appraisal of the various sub-lethal responses examined in this thesis and their potential use for field and laboratory toxicity testing, however, has not yet been made. The merits of using oxygen consumption as a sensitive indicator of toxicant exposure lie in the good reproducibility of results and detection of relatively low contaminant concentrations (significant effects were recorded at the lowest pesticide exposure concentration). In terms of regulatory testing, oxygen consumption was also the easiest and least time-consuming assay to perform. Although mysid oxygen consumption increased after exposure to each pesticide concentration, increased oxygen consumption had a negligible effect on the SFG of *N. integer* [which increased slightly at the two lowest pesticide concentrations (Chapter 6)]. Disruption of mysid oxygen consumption by contaminants, therefore, has less impact on natural mysid populations than disruption of mysid feeding (the other major component of the SFG

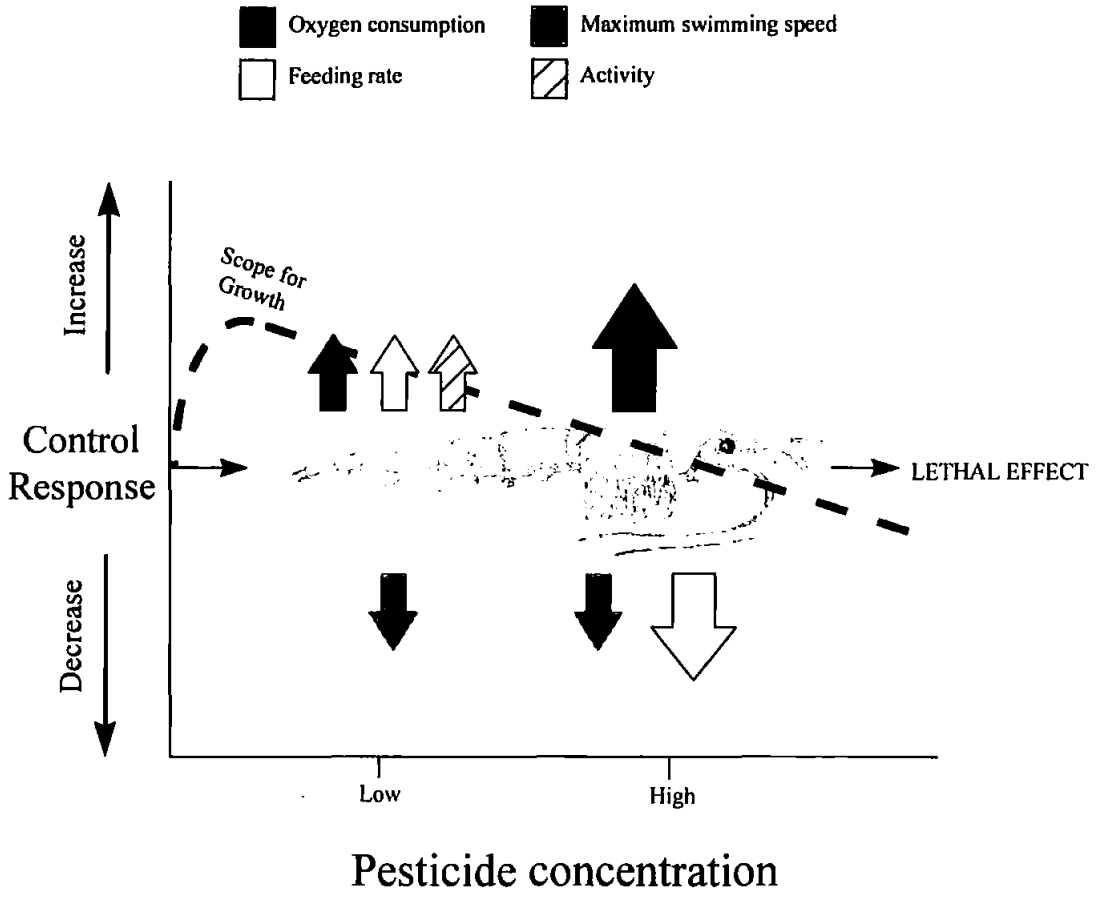


Figure 7.3: Schematic representation of the physiological and behavioural responses of *Neomysis integer* exposed to an organophosphate pesticide [based on the responses of *N. integer* to chlorpyrifos (Chapter 6)].

calculation). The response of mysid feeding rate was not as sensitive as oxygen consumption. For example, feeding rate was not significantly affected following exposure to the two lowest pesticide exposure concentrations. Only following exposure to the two highest concentrations of chlorpyrifos was the feeding rate of *Neomysis integer* significantly reduced (Chapter 6). Feeding rate was also extremely variable and there is a need to improve the protocol further before its use can be recommended. In addition, this assay was more complicated and time-consuming than oxygen consumption. Although mysid feeding was less sensitive to toxicant exposure than oxygen consumption, the former physiological response is more representative of factors likely to affect natural mysid populations because disruption of feeding has a larger impact on SFG. The SFG of *N. integer* increased slightly for mysids exposed to the two lowest pesticide concentrations, but decreased following exposure to the two highest concentrations. The SFG response,

therefore, mirrors very closely the feeding rate response and does not reveal any additional effects of chlorpyrifos exposure on *N. integer*. Scope for growth does, however, translate the decrease in feeding rate into ecologically-meaningful units, revealing that following exposure to 0·100μg chlorpyrifos L^{-1} some mysids had to utilise energy reserves, preventing energy from being directed into growth and/or reproduction. Feeding rate alone, therefore, may be used to predict pollutant effects on natural mysid populations, even though, as a bioassay *per se*, oxygen consumption has proven to be more sensitive. Whichever physiological response is used is dependent upon what questions are asked. If a lowest observed effect concentration (LOEC) is sought, oxygen consumption would be the better bioassay to use. If predictions of impacts on natural populations are required, then feeding rate or SFG are preferable. Finally, for pelagic or hyperbenthic organisms, swimming behaviour or speed may be used as a bioassay of contaminant exposure. Swimming speed and behaviour of *Neomysis integer* was affected following exposure to each pesticide concentration, however, many responses were complex, difficult to interpret and difficult to extrapolate to natural populations. Mysid swimming with the current at high speed was significantly affected by pesticide exposure and this response might be used as a bioassay to highlight pesticide contamination. This response, however, has little relevance to mysid position maintenance in the natural environment (Chapter 6). Reduced maximum swimming speed and/or hyperactivity of pesticide exposed mysids is a more significant response with regards to pollutant effects on the natural mysid population and would be the preferred bioassay. Again, which aspect is used is likely to depend upon what criteria are required by the investigator(s).

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