Life history and distribution of mysids (Tenagomysis spp.) in estuaries of southern New Zealand: an eco-physiological approach

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Dedicated to my father Mr. Supriya Paul and mother Mrs. Kajal Paul
Abstract

Physiological flexibility influences the life-history strategies and spatial-temporal distribution of species. Spatial and temporal variation in salinity, temperature, nutrients, and the opening and closing regime of estuaries make estuarine systems physiologically challenging environments to inhabit. Estuarine species may adapt their body size to buffer physiological challenges associated with osmoregulation. However, adjusting body size may have consequences for life-history parameters, including growth, fecundity, timing of maturity, and eventually influence distribution. I investigated the interplay between these life-history conflicts using the dominant estuarine mysids in New Zealand, i.e. *Tenagomysis chiltoni* and *T. novaezealandiae*.

Southern New Zealand estuaries are cold in winter and comprise a mix of intermittently and permanently open systems. *T. chiltoni* is more dominant in tidal lakes and upper reaches of estuaries, whereas *T. novaezealandiae* occurs more in lower reaches and in intermittently open estuaries. The adults of these species differ significantly in body size, with *T. chiltoni* growing larger than *T. novaezealandiae*. This fuelled my interest to find multiple working hypotheses for my thesis, in which I explored the tolerance, osmoregulation capacity, respiration, growth and fecundity of these two species. My aim was to clarify the energetic, growth and fecundity trade-offs, and the mechanisms driving key life history features that determine where these species live.

My results showed that the salinity tolerance and osmoregulation of *Tenagomysis chiltoni* and *T. novaezealandiae* were poor in a cold, low salinity environment, especially for juveniles. This might have different implications for the two *Tenagomysis* species, depending on their habitat. In cold hypo-saline habitats, *T. chiltoni* is possibly more restricted by osmoregulation than by food, and forced to prolong growth and delay maturity to achieve a larger body size to survive winter. Hence its breeding period is limited, and juveniles can only be released in warmer seasons to reduce mortality from cold. However, its larger body may also have advantages over *T. novaezealandiae*. First, it keeps the per unit mass metabolic expenditure lower, which is crucial to meet the higher osmotic cost in upper reaches of estuaries, which is possibly difficult for *T. novaezealandiae*. Further, *T. chiltoni* females carry larger broods than *T.
novaezealandiae. However, *T. novaezealandiae* grows better in brackish (i.e. salinities 10 to 20), and nutrient-rich intermittently open estuaries (nutrient rich due to eutrophication associated with limited marine exchange) in southern New Zealand. By taking advantage of such productive, often euryhaline environments, *T. novaezealandiae* can reach maturity at smaller body size and produce multiple cohorts, compensating for lower individual fecundity than *T. chiltoni*. This strategy may enable *T. novaezealandiae* to attain very high densities, and allow them to out-compete and exclude *T. chiltoni* from the lower reaches of estuaries.

Results suggest mysids and similar crustaceans in temperate estuaries may be constrained by osmoregulation, especially in winter months. Such physiological constraints could explain some aspects of habitat-specific life-history specialisation, distribution and seasonal changes in many estuarine communities. However, in future, synergistic changes in the estuarine environment (natural and anthropogenic) may trigger responses that are non-linear, and less predictable.
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Chapter 1: General Introduction

1.1 Use of eco-physiology for studying animals

Eco-physiologists generally analyse how individuals, populations or communities adapt physiologically to their environment, relationships that may influence their life histories (Stren 1992; Nielson 1997; Ricklefs and Wikelski 2002). Consequently, they examine how closely related species may have small but critical variations in their physiology (e.g. in tolerance, bioenergetics) for uniquely exploiting natural resources of their habitats (e.g. temperature, salinity, light, nutrients) (Nielson 1997). Further, eco-physiology can detect stress responses to fluctuating and/or extreme environments, which leads to the contraction or expansion of ecological niches (Angilletta 2009; Stillman 2003; Miller and Stillman 2012). For example, a species may show a different spatial and/or temporal niche depending on the latitude or altitude it inhabits (Nielson 1997; Miller and Stillman 2012). In my thesis, I used eco-physiology as a tool for clarifying the underlying mechanisms driving life histories, which may then help explain the geographic distribution of species with examples of mysid fauna from southern estuaries of New Zealand.

1.2 Importance of physiology and body size in animal distribution

The physiological flexibility of a species influences its optimal life-history strategies and ecological distribution in a specific habitat (Stren 1992; Nielson 1997; Krebs 2009; Miller and Stillman 2012). For example, aquatic animals in cold habitats may develop life-history strategies to overcome ‘winter mortality’ (Conover 1992). They may protect delicate stages of their life cycle from harsh environmental conditions (for example, by breeding in favourable seasons when temperatures are moderate, nutrient availability is higher etc.), or release juveniles in benign and productive habitats, but the efficacy of such strategies depends on the underlying physiological plasticity of that animal (Stern 1992; Nielson 1997; Stillman 2003; Miller and Stillman 2012). For example, polar and desert animals exhibit...
remarkable cold/heat tolerance due in part to changes in their dermal and circulatory systems, and aquatic animals equipped with efficient osmoregulatory mechanisms are able to invade brackish habitats (Merkt and Taylor 1994; Nielson 1997; Towle 1997). Consequently, physiological flexibility, specialisation, or an inability to adapt, contributes to animal diversity, invasion of new habitats and, at times, the extinction of populations (Stillman 2003; Miller and Stillman 2012). Animal body size often plays a critical role in that regard (Nielson 1984).

Body size is critical for an animal because it can influence life history and geographic distribution (Nielson 1984; Woodward et al. 2005). Consequently, animals across taxa alter their body size through different growth and maturation strategies (Nielson 1984; Gotthard 2001). For example, greater body volume could be an advantage for osmoregulation in fresh water because it buffers against higher osmotic costs by changing the surface to volume ratio of an animal (Nielson 1984, 1997). A larger body possibly provides more scope for survival through utilization of stored energy, and is often cited as the primary reason for animals being large bodied in temperate latitudes (Nielson 1984; Gotthard 2001). Gradients of body size contribute towards different life-history strategies such as growth, fecundity, and parental care (Shine 1988; Stern 1992; Gotthard 2001); physiological adjustments such as in energetic, osmoregulation, respiration, and tolerating cold or heat (Nielson 1984); and ecological interactions like predator-prey, host-parasite, and niche segregation (Woodword et al. 2005; Krebs 2009). All of these directly or indirectly influence an animal’s geographic distribution (Woodward et al. 2005).

1.3 Influence of habitats on animal physiology and life histories

Habitats demand physiological adjustments, an insight that suggests the origin of different life-history strategies (Stern 1992; Nielson 1997; Krebs 2009). For instance, animals that invaded land, freshwater, and brackish habitats had to make adjustments in circulation and respiration physiology, primarily to prevent desiccation, perform osmoregulation, and sustain energetic demands (Anger 1995; Nielson 1997). A demanding physiological process in aquatic animals is osmoregulation; changes in salinity and temperature often force animals to alter their internal ionic conditions in relation to their environment by expending metabolic energy (Charmantier 1996; Nielson 1997). However, this requires sophisticated molecular
mechanisms like Na+/K+ ATPase pumps, and specific protein structures for membrane permeability for inwards/outwards movement of water and ions (Towle 1997). Often physiological efficiency depends on the coordination of multiple molecular structures inside an animal's body, which eventually determines the fate of an individual or population in a specific habitat (Miller and Stillman 2012). However, a fluctuating climate puts stress on this molecular apparatus, triggering physiological responses which can be non-linear (Towle 1997; Agard 1999). Measuring the tolerance, energetic, and efficiency of a body’s molecular mechanisms under varying but plausible habitat conditions is thus necessary for establishing physiological thresholds of populations (Nielson 1997; Miller and Stillman 2012). This may help us understand the life-history trade-offs that species may accommodate to avoid such thresholds (Stillman 2003; Miller and Stillman 2012). Many aquatic species may, for instance, avoid habitats during certain seasons of a year where/when osmoregulation can be difficult, and have life-history strategies to either resist or circumvent such constraints (Charmantier 1996).

Life-history strategies may arise from various physiological constraints of habitats (Stern 1992; Ricklefs and Wikelski 2002); the synchronised life histories of numerous animals from temperate latitudes bear the testimony to that (Conover 1992; Atkinson 1995; Gotthard 2001). One reason could be that the threshold of physiological tolerance breaks down at certain times of year (e.g. in winter), or at certain life-history stages (e.g. larvae and juveniles) when some animals are more vulnerable to adverse conditions than others (Stern 1992; Angilletta 2009; Stillman 2003; Miller and Stillman 2012). To overcome such constraints, species adopt diverse strategies such as prolonging growth to build large bodies, seeking faster routes to maturity for early reproduction, breeding in favourable climates, or providing more parental care to their offspring to improve their chance of survival (Smith and Fretwell 1974; Conover 1992; Stern 1992; Atkinson 1995; Nelson 1997; Gotthard 2001). However, the extent of their success depends on the how well a species can utilise the natural resources of its habitat and/or the intensity of species competition (Stern 1992; Fox and Czesak 2000; Krebs 2009). A species may, for example, successfully complete its life history by adjusting its physiology and timing of life stages, but its distribution may still shrink if the competition from closely related species is intense, or expand if the competition is less and/or absent (Krebs 2009; Buckley et al. 2010).
1.4 Estuaries

1.4.1 Classification and characteristics

Estuaries are dynamic ecosystems that have serviced millions if not billions of people since prehistoric times (Lotze et al. 2006). For their ecosystem services, estuaries are amongst the most valuable ecosystems in the world (Costanza et al. 1997; Barbier et al. 2011). Estuaries occur at all latitudes apart from Poles, for example tropical estuaries in India, Bangladesh, Malaysia and Amazon, or warm temperate estuaries in South Africa, Spain, or cold temperate estuaries in Northern Europe, Japan, Tasmania and Southern New Zealand (Hume and Herdendorf 1988; Roy et al. 2001; Lill 2010). Estuaries have been defined and classified by numerous authors based on different geological, morphological, and hydrological characteristics, but each classification has its own unique merits and demerits (Hume and Herdendorf 1988; Roy et al. 2001). From these studies, estuaries may be defined as semi-enclosed water bodies in the coastal zone where sea water is measurably diluted by the inflow of freshwater (Hume and Herdendorf 1988; Roy et al. 2001; Lill 2010). I worked in southern New Zealand estuaries both ‘intermittently open/closed’ (if the spatial-temporal connection to sea/ocean is not continuous) and ‘open’ (if the spatial-temporal connection to sea/ocean is continuous) which present contrasting environment for ecological communities (Lill 2010).

1.4.2 Contrasting environment

Estuaries often have fluctuating gradients of physical-chemical characteristics which put estuarine communities under physiological and ecological stress (Ysebaert and Herman 2002). Ecological communities of intermittently open/closed and open estuaries are different because of contrasting physical-chemical gradients, a lot of which are driven by the either periodic open close of estuary mouth and or fresh water inputs and or presence of sandbar at the mouth which may restrict recruitment of primarily marine breeders (Roy et al. 2001; Perissinotto et al. 2002; Lill et al. 2013). For example, salinity in estuaries has been suggested as the primary factor that structures these communities, limiting diversity and density, and even determining the presence and absence of many animals, irrespective of estuary type (Remane and Schlieper 1971; Attrill 2002; Ysebaert and Herman 2002). In open estuaries, spatial gradients of salinity, temperature, and nutrients can change with every tidal cycle, giving rise to a very complex and dynamic environment within a short time. However,
such gradients are possibly more predictable in the long term. In contrast, in intermittently open/closed estuaries short to medium term spatial variation may be limited, but sudden and quite drastic changes in salinity, temperature and a host of other parameters may suddenly occur when the system opens to the sea (Roy et al. 2001; Perissinotto et al. 2002; Lill 2010). For a species which inhabits both types of estuaries, physiological mastery of such high environmental variation is highly challenging. In that case, depending on its physiological plasticity, it may need different life-history strategies to survive, strategies that may relate to different niches and distributions (Stren 1992; Attrill 2002; Attrill and Power 2004). Although a multitude of ecological studies have been conducted in estuaries, few have been undertaken at a regional scale (Lill 2010). Looking at the vulnerability of many ecological communities in estuaries in temperate regions, I aimed to provide a deeper insight of the aforesaid ideas by studying estuaries of southern New Zealand and their mysid inhabitants.

1.4.3 New Zealand estuaries

New Zealand's temperate estuaries are a mix of ‘intermittently open/closed’ and ‘open’ types (McLay 1976; Hume and Herdendorf 1988; Lill 2010). There are approximately 300 estuaries in New Zealand out of which 138 in South Island and majority of them (around 61) have intermittent connection to the seas (McLay 1976). Estuaries in the northern New Zealand are relatively warm, like those of South Africa, but estuaries in the South Island are generally cold, like estuaries in the Baltic Sea region, Japan, and Tasmania (Lill 2010). According to Drake et al. (2002), temperate estuaries in Europe exhibit similar characteristics in temperature and salinity with maximum values of both variables in summer and minima in winter. Apart from large estuaries like Avon Heathcote which receives continuous monitoring (www.niwa.co.nz) for water quality, environmental data of almost small inlets, creeks and lagoons of southern New Zealand are potentially illusive but overall they do not become too saline because of rainfall throughout the year which may vary 1000 to 2000 mm (Kirk and Lauder 2000). Like many temperate estuaries worldwide levels of available nutrients (nitrates-nitrites) and productivity are skewed towards spring-summer, and affects the community structure, size, and diversity of New Zealand estuaries too (Drake et al. 2002; Whitfield et al. 2006; Thrush et al. 2008; Lill 2010) but the variable which is chiefly responsible for such contrast in bio-physical environment is, the ‘berm elevation’ near the estuary mouths (Lill et al. 2013). In Otago and Southland of southern New Zealand, one can find ‘open’ estuaries like Clutha River, Taieri River, Tokomairiro River, Kakanui River,
Pleasant River, and also small ‘intermittently open/closed’ estuaries like Kaikorai Lagoon, Sawmill Creek, Brighton Estuary, and Tomahawk Lagoon (Lill et al. 2011, 2013). Salinity of these estuaries possibly depend on their connection to the sea rather the fresh water inflows, for example Lill et al. (2011) found intermittently closed estuaries are less saline (mean 7.8 PSU) than permanently open estuaries (mean 23.9 PSU) in the Otago region. As off Otago regional Council Report (2010), the median concentrations of nitrite, nitrate and other nitrogenous contents vary between 25-50 mg/L and the dissolve reactive phosphates has a median of 25 mg/L which the report concluded not alarming. Findings of Scallenberg et al. (2010) showed periodic open close of Otago estuaries may influence their primary productivity by varying the quantity of chlorophyll-a. However I critique these findings because these results did not emerge from any continuous monitoring of local estuaries. Thus I tried to provide some supportive evidence, especially of nutrient loads of Otago and southland estuaries those I studied (Fig 1.1), details of which could be found in Chapter 5 of this thesis.

Estuaries around the world and those of southern New Zealand are vulnerable to climatic and anthropogenic impacts (McLay 1976; Lotze et al. 2006; Lill 2010; Bierschenk et al. 2012). For example the survey of McLay (1976) revealed the number and the magnitude of polluted estuaries are higher in North Island compared to south. Study of Bierschenk et al. (2012) found estuaries with developed catchments in South Island to have higher salinities and dissolve reactive phosphorus than those with pristine catchments. Increasing sedimentaion has been indentified as threat to the biodiversity of New Zealand estuaries along the fresh to brackish water continuum both in North and South Island (McLay 1976; Thrush et al. 2008; Lill 2010; Bierschenk et al. 2012). Furthermore, the climatic shift in temperature (+ 0.6 to 1 °C) and sea level rise 18 to 59 cm by 2100 in the region (www.niwa.co.nz) may create higher complexity which is yet to be understood. One way to study such synergistic stresses on estuaries is to study their communities for example the zooplankton.
1.4.4 Estuarine communities of New Zealand

Temperate estuaries worldwide, harbour diverse communities such as microbial, benthic, pelagic and avifaunal elements (Attrill 2002; Lotze et al. 2006; Lill et al. 2013). The structure of such communities often differs between seasons, latitudes, estuaries and even between upper and lower reaches of a single estuary (Froneman 2003). The reasons for such contrasts can be many, including physiological plasticity, habitat productivity, different life-history strategies, and presence or absence of predators (Maes et al. 1998; Whitfield et al. 2006; Jeong et al. 2007). Within communities, crustaceans, including crabs, mysids, amphipods, and copepods can form large biomasses in small intermittently open/closed temperate estuaries in the Southern Hemisphere (Maes et al. 1998; Drake et al. 2002; Froneman 2003; Lill et al. 2011). Of these, mysid shrimps can be dominant (Drake et al. 2002; Lill 2005). In southern New Zealand, mysid communities are abundant in small intermittently open/closed
estuaries, possibly because of limited top-down control on these communities (Lill et al. 2010, 2011, 2012). However, the seasonal influence is strong as the communities bloom during spring to autumn but diminish during winter (Lill 2005; Lill et al. 2010). Among mysids, the genus *Tenagomysis* is highly abundant in estuaries of southern New Zealand (Greenwood et al. 1985; Jones et al. 1989; Lill 2005; Lill et al. 2011, 2012; Paul et al. 2013). Given their importance in estuarine food webs (i.e. link between benthic and pelagic life, and form large biomass), I decided to study their eco-physiology to gain insights into the factors driving mysid abundance in temperate regions.

### 1.5 Estuarine mysid communities

Mysid shrimps (Crustacea: Mysida) (Mauchline 1980) are possibly the most abundant hyper-benthic macro-invertebrates in estuaries and coastal waters around the world, linking the benthic and pelagic food webs (Drake et al. 2002; Lill et al. 2010). Numerous mysid species inhabit tropical and temperate estuaries, and at times are seen as ecological indicators of estuarine water quality (Roast et al. 1998; Vilas et al. 2009; Lill et al. 2010). In temperate estuaries, mysids can form large biomasses which are often a food source for coastal fish (Drake et al. 2002). In order to better understand how dynamic and contrasting habitats influence the physiological ecology of these animals, I provide a short review of mysid biology and ecology.

#### 1.5.1 Mysid behaviour

Mysid shrimps belong to the crustacean class Malacostraca. Some of them are marine inhabitants but others have invaded brackish and fresh water habitats (Mauchline 1980). Mysids are also called ‘opossum shrimps’ because the females have a ventral brood pouch where the eggs are deposited and develop, and the young resemble the adults when they are released from the pouch (Mauchline 1980). Mysids are omnivores, and to some extent opportunistic feeders, feeding mostly on detritus, but can at times prey on smaller zooplankton like copepods (Wilhelm et al. 2002). Tides in estuaries pose challenges for the orientation, position maintenance and swimming ability of mysids. Previous authors observed such behaviour as species-specific and the role of climatic variables in their behaviour were possibly less important (Hough and Naylor 1992; Ritz 1997). Further, mysids are generally
nocturnal, and can be particularly abundant near full moon, which possibly influences their abundance in estuaries (Mauchline 1980; Azeiteiro et al. 1999; Sutherland and Closs 2001).

1.5.2 General life history and distributional patterns

Mysid life histories have been studied widely across the globe (Lill 2005). Mysids can remain in estuaries throughout the year, but population density fluctuates with seasons and/or events like floods and droughts (Toda et al. 1982; Fenton 1992; Mees et al. 1994; Baldo et al. 2001; Winkler and Greve 2002; Hanamura et al. 2009; Lill et al. 2010). Breeding is somewhat dependent on latitude, as tropical and subtropical mysids breed continuously whereas temperate mysids have discrete cohorts only every year (Toda et al. 1982; Baldo et al. 2001; Hanamura et al. 2009; Lill et al. 2010). Variations in physical-chemical quality of estuarine environments are likely responsible for these life-history contrasts (Mees et al. 1994; Baldo et al. 2001; Winkler and Greve 2002; Hanamura et al. 2009). For example, temperature and salinity changes often lead to high mortality among juveniles, affect growth rates and maturity, and the brood size of gravid females (Bhattacharaya 1982; Bremer and Vijverberg 1982; McKenney and Celestial 1995; Winkler and Greve 2002; Fockedey et al. 2006).

In cold temperate estuaries, mysids like Neomysis spp., Acanthomysis spp., Mysis relicta, and Tenagomysis spp. exhibit commonalities in their life histories (Mauchline 1980; Toda et al. 1982; Fenton 1992; Mees et al. 1994; Fockedey et al. 2006; Lill et al. 2010). For example, populations’ peak in spring-summer-autumn and nearly collapse during winters (Lill et al. 2010). In spring, the over-wintering cohort starts breeding, which may then continue in to summer – autumn, but ceases in winter (Toda et al. 1982; Mees et al. 1994; Lill et al. 2010). Breeding can be exhaustive for both the sexes so semelparity is common among mysids (Mauchline 1980; Lill et al. 2010). Adults of summer-autumn cohorts are generally smaller in body size, mature earlier, but carry smaller broods than overwintering cohorts, which often have a larger body size, possibly a result of a prolonged period of growth (Mees et al. 1994; Lill et al. 2010). However, mysid studies have never integrated multiple lines of evidence to help understand the underlying mechanisms driving mysid life-history strategies, and the expansion or contraction of their niches depending on habitat variation.
The distribution of mysids in estuaries has been studied in detail in relation to single or synergistic changes in salinity and temperature, most often by testing tolerance and osmoregulation (Paul et al. 2013) and recommended narrower range of salinities where mysids can actually be found despite showing a broader range of tolerance in laboratory (McKenney and Celestial 1995). For example adults and juveniles of *Mesopodopsis orientalis* exhibit a broader salinity tolerance in the laboratory than in the field, but the author doubts if the juveniles could survive the low saline monsoon waters of Western India (Bhattacharaya 1982). Studies of Vilas et al. (2009) exhibited how a narrow difference in osmoregulation can lead to a separation of niches among the mysid species in estuaries of South Africa, whereas the work of Paul et al. (2013) showed that differences in osmoregulation and salinity tolerance within life history stages of *Tenagomysis* spp. may influence how they distribute themselves within an estuary. It has often been speculated that differences in such physiological abilities, if any, may have effects on niche partitioning, intra-inter specific competition, and distribution (Webb et al. 1997; Vilas et al. 2009). Along with variables related to climate, sediment type (particle size, utilization of sediment as food resource), estuary bed, and mouth conditions (open-close), mysid behavior such as predation can also impact distribution (Hough and Naylor 1992; Azeiterio et al. 1999; Baldo et al. 2001; Boscarino et al. 2007).

### 1.5.3 Estuarine mysid communities in New Zealand

Mysids of the genera *Tenagomysis* and *Gastrosaccus* inhabit New Zealand estuaries, and possibly form the dominant secondary biomass in numerous small and intermittently open estuaries in the region (Lill 2010). Of the *Tenagomysis* species, *T. chiltoni*, *T. novaezealandiae*, and *T. macropsis* have received some attention for their environmental tolerances, life histories, distribution, and food preferences (Tattersall 1923; Bary 1956; Kirk 1983; Greenwood et al. 1985; Jones et al. 1989; Southerland and Closs 2001; Wilhelm et al. 2002; Lill et al. 2010, 2012; Paul et al. 2013, 2014). These studies have provided some insights into the biology of *Tenagomysis*, but have not clarified the physiological and life-history constraints on them, or the distribution of temperate mysids at regional scale. In view of the critical position of mysids in estuarine food webs, and their large biomass, I thought they warranted close examination. Consequently I chose the mysids *T. chiltoni* and *T. novaezealandiae* for this purpose. I used eco-physiology as a tool to understand how mysid communities may react to the chronic and complex outcomes of natural, human and climate
induced stresses in the estuaries in the region. I integrated multiple lines of evidence by studying their physiological tolerances, bio-energetic and life-history parameters such as growth and fecundity, to clarify their distributional patterns in the region, and to shed light on their life-history strategies and distribution of temperate mysids in general, some of which may be applicable to other estuarine crustacea.

### 1.6 Response of mysid communities to varying salinity

The estuarine literature has traditionally emphasized the importance of varying salinity on the physiology, life history as well as ecology of numerous crustaceans (e.g. crabs, isopods, copepods), including mysids (Remane and Schlieper 1971; Attrill 2002). Studies on mysid communities in Northern Europe, South Africa, and Australia have also identified salinity to be one of the major environmental factors to influence life history and distribution of mysids (Toda et al. 1982; Jones et al. 1989; Fenton 1994; Mees et al. 1994; Web et al. 1997; Fokedey et al. 2006; Hanamura et al. 2009). Experiments exploring the physiology of different mysid species in this regard have played a major role, in advancing understanding of the mechanisms such as osmoregulation, survival, respiration, growth and fecundity which determine how species may choose specific micro-habitats within estuaries (Web et al. 1997; Marshall et al. 2003; Fokedey et al. 2006; Vilas et al. 2009). For example experiments on salinity tolerance, energetics and growth of mysids in the laboratory in relation to varying salinity have often highlighted the mechanisms behind their behaviour, choice of life history strategies, field distribution (Bhattacharaya 1982; Hough and Naylor 1992; McKenney and Celestial 1995; Marshall et al. 2003; Vilas et al. 2009). These studies have often emphasised the importance of chronic effects of environmental changes on the interaction within and or between mysid species leading to separation of niche (Fokedey et al. 2005, 2006; Vilas et al. 2006, 2009). However the ecophysiology of New Zealand mysids has received little attention consequently the physiological constraints on their life histories and field distribution are mostly unknown (Paul et al. 2013, 2014). This literature gap has influenced my decision to choose the thesis topic, focusing on salinity as the most likely primary limiting variable.
1.7 Aims and structure of the thesis

My PhD thesis aimed to explain how contrasting conditions in temperate estuaries can affect the physiology (tolerances and energetic) of mysids and may thereby force them to adjust their life-history strategies (i.e. for growth and fecundity), and how this may cause expansion or shrinkage of their niches under variable habitat conditions. I studied the mysids *Tenagomysis chiltoni* and *Tenagomysis novaezealandiae* in southern New Zealand estuaries to explore this. The questions that I explored were as follows; however, the exact hypotheses are presented in each chapter separately.

1. Are osmoregulation and survival of mysids in cold estuaries of the Southern Hemisphere dependent on synergistic changes in salinity, temperature and life stages?

2. Can energetics of mysids in terms of varying salinity and body size reveal more about habitats?

3. Is growth of temperate mysids influenced by their micro-habitats; if so, then what are the possible life-history implications?

4. Do seasonal influence on life histories and contrasting micro-habitats influence fecundity of temperate mysids?

5. Why the mysid *Tenagomysis chiltoni* is large bodied than *T. novaezealandiae*?

6. What is possibly governing the distribution of mysids in temperate estuaries?

I used laboratory experiments and field surveys to investigate the above questions. Chapter 1 introduces physiological-ecology as a tool to study the ecological fate of temperate estuarine communities through the example of mysids. Chapters 2 and 3 investigate the role of body size, life stage on osmoregulation, survival capability and energetics of mysids to examine the physiological threshold. These aspects are investigated using *Tenagomysis chiltoni* and *T. novaezealandiae* under a combination of salinities and temperatures. Chapter 4 examines the
growth under different salinities in high or low nitrogen-rich environments to evaluate growth strategies. Chapter 5 reports on a field survey to evaluate fecundity strategies of *Tenagomysis chiltoni* and *T. novaezealandiae*. A general discussion in Chapter 6 integrates the results from Chapters 2 to 5 to shed more light on life-history constraints and distribution of temperate mysids and similar crustaceans, and a limited assessment of their vulnerabilities. Some chapters were written as stand-alone research articles for publications, some of which are already published (Chapter 2 and 4). However in all cases I am primarily responsible, for experimental designs, data collection and analysis, interpretation of results, the possible discussion, and preparation of all the thesis chapters i.e. from Chapter 1 to Chapter 6.
Chapter 2: Osmoregulation and survival of *Tenagomysis chiltoni* and *T. novaezealandiae*

A paper based on this chapter, authored by Sourav Paul, Martin Krkosek, Keith Probert and Gerry Closs has been published in *Marine and Freshwater Research* 2013, 64 (4), 340–347

[http://dx.doi.org/10.1071/MF12316](http://dx.doi.org/10.1071/MF12316)
2.1 Abstract

The mysid shrimps *Tenagomysis chiltoni* and *T. novaezealandiae* are abundant in southern New Zealand estuaries. I observed their short to medium term ability of osmoregulation and survival under salinities of 0, 5, 10, 15, 20, 25, 30, and 33 at 5°C and 20°C, to evaluate if salinity limits their distribution. *Tenagomysis chiltoni* and *T. novaezealandiae* maintained species-specific hemolymph concentrations across the salinities tested. Their osmoregulatory capacity can be most parsimoniously modelled through the combined effects of salinity and temperature change. Mortality of *T. chiltoni* and *T. novaezealandiae* increased towards the extremes of fresh (salinity within 0 to 1) and salt water (salinity 33 to 34) but was lower in intermediate salinities (10 to 25). Juveniles suffered high mortality if the changes in salinity are beyond their tolerance. The ability of these species to osmoregulate and survive were limited at 5°C, but improved at 20°C. Salinity may have acted non-linearly causing variations in survival of the two species of *Tenagomysis*. I concluded that salinity could influence osmoregulation and survival, depending on temperature and life stage. This finding partly explains occurrences of both *Tenagomysis* species in intermediate salinities and the higher prevalence of *T. chiltoni* in the upper reaches of southern New Zealand estuaries, but not of *T. novaezealandiae* in lower reaches.

2.2 Introduction

Estuaries exhibit wide spatial-temporal variation in physical-chemical conditions, often exacerbated by anthropogenic and climate mediated changes, and which can trigger eco-physiological responses among resident communities (Maes *et al.* 1998; Lill 2005; Lotze *et al.* 2006). Such variation in estuarine environments creates physiologically challenging situations for individual survival and community stability (Remane and Schlieper 1971; Attrill 2002). Variations of salinity and temperature play a key role in structuring estuarine communities by influencing survival because they require a highly responsive osmoregulatory capacity (Maes *et al.* 1998; Attrill 2002; Vilas *et al.* 2009), a trait that is common among estuarine crustaceans (Delisle and Roberts 1987). However, osmoregulation varies with multiple, potentially interacting bio-physical factors such as seasonal changes in climate (i.e. salinity and temperature) and life stage, which can affect survival in estuaries (McKenney and Celestial 1995; Greenwood 2007; Hanamura *et al.* 2009; Vilas *et al.* 2009).
Mysids are studied widely because they are considered to be an important part of estuarine food webs (Drake et al. 2002), linking the benthic and pelagic food chains (Roast et al. 1998). Estuarine mysids are typically osmoregulators, exhibiting high survival across a broad range of salinities (McKenney 1994; McKenney and Celestial 1995). However, published literature has a strong Northern Hemisphere bias, with few studies of Southern Hemisphere mysid communities (Webb et al. 1997), and none on the osmoregulation and survival capacity of Tenagomysis spp. The mysid genus Tenagomysis contains 16 species, some of which are estuarine and form a significant biomass in intermittently to permanently open estuaries of southern New Zealand (Lill et al. 2010). For example, T. chiltoni is more common in upper reaches of estuaries, whereas T. novaezealandiae is usually most abundant near estuarine mouths, with seasonal changes in the extent of their distribution (Lill et al. 2010). They differ in their body size, with T. chiltoni (gravid adults > 10 mm) growing larger than T. novaezealandiae (gravid adults < 10 mm), but have overlapping fundamental niches within estuaries (Jones et al. 1989; Jocque and Blom 2009; Lill et al. 2010). These studies suggest their osmoregulation, and hence survival, may be influenced by salinity and temperature variation in estuaries.

My study primarily addresses osmoregulation and survival of T. chiltoni and T. novaezealandiae under salinities of 0, 5, 10, 15, 20, 25, 30, and 33 at 5°C and 20°C, under controlled laboratory conditions. This is aimed to better understand their distribution in estuaries of New Zealand based on physiological flexibilities of these two species. The experimental treatments reflect the range of salinities regularly encountered in estuaries, and the likely seasonal temperature extremes that occur in southern New Zealand (Jones et al. 1989; Lill et al. 2010). I hypothesised that changes in salinity and temperature will change the hemolymph concentration of T. chiltoni and T. novaezealandiae, and thereby influence survival.
2.3 Materials and methods

2.3.1 Mysid collection

Tenagomysis chiltoni and T. novaezealandiae were collected using a 500-µm-mesh sweep net from Kaikorai Lagoon (see Lill et al. 2010 for details) which is intermittently open in nature, Dunedin (45°8550′S, 170°8230′E) (Fig 2.2). Mysids were collected in January–February and July–August of the year 2011 to capture contrasting seasonal patterns, and summer and winter populations of T. chiltoni and T. novaezealandiae (Lill et al. 2010). Laboratory experiments for osmoregulation and survival were completed immediately after the sampling, with the mysids being maintained and manipulated at either the high or low temperature corresponding to the season in which they were collected. Tenagomysis chiltoni was mostly collected from a location in the upper reaches (45°9140′S, 170°4043′E) of the lagoon (salinity 1 to 5), and T. novaezealandiae at a location near the lagoon mouth (45°3276′S, 170°3907′E) (salinity 10 to 32), because populations of each species are typically highest in these zones (Lill et al. 2010). Mysids were immediately transported to the laboratory in 20 Litre (L) sealed buckets filled with water from the sampling sites. They were sorted according to species, and life-history stages as follows: juveniles (3 to 5 mm, for both species) and adults (12 to 15 mm for Tenagomysis chiltoni; 7–10 mm for T. novaezealandiae) (Jocque and Blom 2009).
2.3.2 Laboratory maintenance

In the laboratory, *T. chiltoni* and *T. novaezealandiae* were tested for osmoregulation and survival under salinities of 0, 5, 10, 15, 20, 25, 30 and 33. For each species, the sample was halved for two different laboratory experiments (osmoregulation and survival), which proceeded simultaneously after the mysids were collected. Mysids were acclimated to the experimental salinities (0, 5, 10, 15, 20, 25, 30, and 33) for 3 days. Mysids collected in summer were acclimated at 20°C and those in winter at 5°C. Those mysids that survived the acclimation period were then subsampled for the experiments. Groups of 20 mysids were housed in different 2 L sterilised PVC containers and exposed to salinities of 0, 5, 10, 15, 20, 25, 30, and 33. Experiments were conducted in the 2 L containers at 5°C and 20°C (± 0.1) to represent typical extreme winter and summer temperatures for estuaries of southern New Zealand (Kattel and Closs 2007). Salinities of 0, 5, 10, 15, 20, 25, 30, and 33 were prepared in the laboratory by diluting natural seawater (salinity 33 to 34) with spring water (salinity within 1), with the water being filtered with filter paper (1 µm) before use in both cases. Mysids were fed daily with freshly hatched *Artemia salina* Leach nauplii, cultured in the laboratory, to satiation during the day period. One-third of the water was exchanged every day of the experimental period, maintaining the water volume at 2 L. Each day, dead individuals, excess *Artemia* and any other visible wastes were removed to maintain water quality. Photoperiod for experiments done at 20°C was set at 14h: 10h of light: dark condition and for 5°C at 10h: 14h of light: dark to mimic the typical natural conditions of southern New Zealand.

2.3.3 Osmoregulation experiment

The first half of the sample was used to measure the change in the concentration of hemolymph, following protocols of Webb *et al.* (1997). About 50 to 60 adult mysids were housed in each 2 L container and exposed to salinities of 0, 5, 10, 15, 20, 25, 30, and 33, and kept either at 5°C or 20°C for 24 h (depending upon the temperature at which the final experiments were carried out.). There were three replicates for each combination of salinity and temperature. Only adults were included in the osmometry study because extraction of sufficient hemolymph from juveniles for testing proved impossible. Because of the rapid (within 8 h of experimental time) mortality of both the species in fresh water (salinity 0 to 1),
Osmoregulation and survival of mysids

Mysids were exposed to this salinity for 8 h instead of 24 h. Mysids were not fed during this exposure period to avoid any confounding factor affecting the environment or osmolality of the hemolymph. At the end of the exposure period, each mysid was individually removed and washed in Milli-Q water to remove any surface ions. Each mysid was then blotted with filter paper disks for 1 min to remove excess surface water, snap frozen on dry ice, and then placed in Eppendorf tubes and stored at –70°C for future analysis. Prior to the measurement of body fluid concentrations, the mysids from each replicate were partly defrosted inside the Eppendorf tubes. To extract the body fluid from the tissue, mysids (50 to 60 individuals in total) were homogenised and centrifuged for 10 min at 20200 relative centrifugal forces (rcf) to extract a minimum of 10 µL of supernatant. The 10µL sample of supernatant was then analysed in a WESCORE 5100 vapour pressure osmometer (WESCORE Inc, U.S.A), which was periodically recalibrated and thermally balanced after every 10 measurements. Water samples were also collected from corresponding treatments and measured for environmental osmolality.

### 2.3.4 Survival experiments

Survival experiments were run with the second half of the collected sample, with conditions maintained as described above, but in this case juveniles were included with adults. For the respective life-stages (adults and juveniles) of each species, three replicates were used. For all juveniles (tested at 5°C and 20°C), survival was recorded at 24 hours intervals for a maximum of 14 days or until 50% mortality, whichever occurred earlier. Adults at 5°C were also monitored for a maximum of 14 days or until 50% of mortality, whichever was first. Only adults at 20°C were monitored for a maximum of 24 days or until 50% of the test population died because of relatively low mortality at high temperature. Mysids were fed *Artemia salina* nauplii *ad libitum* daily, to avoid death as a result of starvation.
2.3.5 Data analysis

To test the osmoregulatory capacity of *T. chiltoni* and *T. novaezealandiae* under salinities 0, 5, 10, 15, 20, 25, 30, and 33 and temperatures (5°C and 20°C), linear models were built where temperature and species variables were treated as categorical variables, and salinity as a continuous variable. Throughout the analysis, group means of individuals within each replicate were used in the analyses to reduce variance related to individual variation among replicates. There was no evidence that housing mysids in groups had an impact on any of the assessed response variables. The candidate models were aimed to test whether the salinity, temperature, or their combinations and interactions could explain variation in the osmoregulation of *Tenagomysis* spp., started from the most parsimonious. I tested if salinity as a single predictor can explain variations in osmoregulation of both *Tenagomysis* species. From that regression equation I derived parameter estimates which I later used to estimate the iso-osmotic points (where hemolymph osmolality nears or equals environmental osmolality) of both species at 5°C and 20°C. I used the equation of a straight line to calculate the iso-osmotic points, as follows:

\[ Y = m \cdot X + c \] (where \( m \) is the slope, \( c \) is the intercept, \( X \) and \( Y \) are environmental osmolality and hemolymph osmolality), and

\[ Z = c / (1 - m) \] (where \( Z \) is the iso-osmotic point, and \( c \) and \( m \) are the parameter estimates gained from (linear regression (Osmoregulation vs. Salinity) for each species).

To obtain the standard error on \( Z \), the iso-osmotic point, I used simulations (10000 times) based on the parameter estimates and standard errors of \( m \) and \( c \) from the linear regressions (intercept and slope of linear regression (Osmoregulation vs. Salinity), using random normal function in software R.3.0.1 for Windows (R Development Core Team 2014). The resulting mean and standard error of \( Z \) were used to calculate 95% confidence intervals for iso-osmotic points at each temperature for each species.

To evaluate survival of *T. chiltoni* and *T. novaezealandiae* under different salinities, survival analysis was performed. Data were fitted with a mixed-effects Cox proportional-hazards model (Therneau 2012), by using the ‘Coxme’ package under survival function. The data were right-censored because some of the mysids lived beyond the experimental timeframe. The data were also hierarchical because groups of 20 mysids were held in multiple replicate containers for each treatment combination of salinity, temperature and life stage. That is, because individuals were nested within containers, and containers were replicated for each
treatment, a random-effects structure was needed to model the nested structure of the data. Random effects were included to allow for the variation in survival among replicates. A higher order of salinity was included in some of the models because of the observed quadratic nature of the survival data. I used Akaike information criterion (AIC) to select the most parsimonious model, i.e. the model with the lowest AIC value among the candidates (Burnham and Anderson 2002), based on the negative log-likelihood. Models with a ΔAIC value of more than 10 have literally no support for data (Burnham and Anderson 2002), so I restricted my discussion to the models that were more plausible among candidates. I did not include three-way interactions or interactions that included higher orders in the models, to keep them biologically less complex to interpret. All confidence intervals were set at 95% and statistical analyses were performed using software R 3.0.1 for Windows.

2.4 Results

2.4.1 Osmoregulation under different salinities

Results indicated that the osmoregulatory capacity of *T. chiltoni* and *T. novaezealandiae* (Table 2.1) could be explained by combinations of variables such as salinity, temperature and species. Both species exhibited some degree of hyper- to hypo-osmoticity (Fig 2.2) because the ambient media changed from a diluted (0, 5, 10, 15, and 20) to a more concentrated (25, 30 and 33) solution. Both species found it more difficult to osmoregulate at 5°C than at 20°C, as indicated by the steeper slope of the regressions (Fig 2.2) at 5°C. *T. chiltoni* deviated further from the iso-osmotic line than did *T. novaezealandiae* at 5°C at the salinity extremes (Fig 2.2). Mortality was very rapid in the salinity 0 to 1, preventing accurate measurement of the hemolymph osmolality. Consequently, I did not include that treatment in the regression analysis (Fig 2.2). Among the linear models listed in Table 2.1, Model 1, which explains changes in hemolymph concentration of each *Tenagomysis* species, combined effects of salinity, and temperature, was the most parsimonious (model with the lowest AIC value). The iso-osmotic points for *T. chiltoni* and *T. novaezealandiae* varied with the temperature of the medium (Fig 2.2), being lower at 20°C than at 5°C (Table 2.2). The 95% CI of the iso-osmotic points of both species varied within the range of 374–483 mOsm kg⁻¹, again depending on temperature (Table 2.2).
Table 2.1 Models on osmoregulation of *Tenagomysis chiltoni* and *T. novaezealandiae* under salinities 0 to 33 at 5°C and 20°C. Sal = salinity, including 0, 5, 10, 15, 20, 25, 30 and 33; Temp = temperature (5°C and 20°C); Sp = species; AICc = Akaike information criterion (corrected for small sample size); Δ AICc = difference in AIC value of the model from the lowest AIC value of the model among the candidates; + = additive effect; * = interactive effects; All replicates were included in the data analysis.

<table>
<thead>
<tr>
<th>Model</th>
<th>Factor(s)</th>
<th>AICc</th>
<th>Δ AICc</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Sal + Temp + Temp * Sp</td>
<td>1055.1</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>Sp + Temp + Sal * Temp</td>
<td>1072.2</td>
<td>17.1</td>
</tr>
<tr>
<td>3</td>
<td>Sal + Temp + Sal * Temp</td>
<td>1076.6</td>
<td>21.5</td>
</tr>
<tr>
<td>4</td>
<td>Sal + Temp</td>
<td>1085.9</td>
<td>30.8</td>
</tr>
</tbody>
</table>

![Fig 2.2 Regression models on osmoregulation of *Tenagomysis chiltoni* and *T. novaezealandiae* under salinities 5 to 33 at 5°C and 20°C. Number of replicates was three; replicates of salinity 0 to 1 were not presented and included for data analysis because of 100% mortality within very short time.](image)
Table 2.2 Iso-osmotic points of *Tenagomysis chiltoni* and *T. novaezealandiae* at 5°C and 20°C.

<table>
<thead>
<tr>
<th>Species</th>
<th>Temperature (°C)</th>
<th>Iso-osmotic point (mOsm kg(^{-1}))</th>
<th>95% CI* (mOsm kg(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>T. chiltoni</em></td>
<td>5</td>
<td>448</td>
<td>430–466</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>406</td>
<td>375–438</td>
</tr>
<tr>
<td><em>T. novaezealandiae</em></td>
<td>5</td>
<td>447</td>
<td>411–483</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>404</td>
<td>374–434</td>
</tr>
</tbody>
</table>

*CI = confidence intervals

2.4.2 Survival under different salinities

There was 100% mortality recorded for *T. chiltoni* and *T. novaezealandiae* in fresh water (0 to 1) within 24 h of exposure (Fig 2.3) and a failure to acclimatise. In other salinities after the acclimation period, 50 to 100% of juveniles died within 14 days, with generally higher rates of mortality under cold conditions (Fig 2.3). Survival of both species of *Tenagomysis* exhibited a quadratic relationship with salinity (Fig 2.3), with fewer individuals surviving in the extremes of fresh and sea water, whereas survival was higher in the intermediate salinities (i.e. in salinities 15, 20, and 25). *T. chiltoni* exhibited slightly higher relative survival in lower salinities (Fig 2.3) of 5 to 15, whereas *T. novaezealandiae* exhibited higher survival at salinities towards the seawater end (Fig 2.3). Juveniles suffered more in low temperature than their adults (Fig 2.3). Among the models listed in Table 2.3, Model 6, which explains variation in survival of *Tenagomysis* spp. through non-linear impacts of salinity, was the most parsimonious (model with the lowest AIC value) among the candidates. According to the AIC model-selection statistic, salinity when combined with either changes in temperature or life history stage can be highly explanatory in describing the survival of *Tenagomysis* species (Models 3 and 4 of Table 2.3).
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Fig 2.3 Mortality of *Tenagomysis chiltoni* and *T. novaezealandiae* under salinities 0 to 33 at 5°C and 20°C. Each replicate started with 20 individual mysids; Experiments juveniles and adults ran for 14 days or until 50% of mortality, whichever occurred earlier (except for adults at 20°C for which the period was 24 days because the mortalities were very low in most of the treatments).

Table 2.3 Survival models (Cox mixed-effects) of *Tenagomysis chiltoni* and *T. novaezealandiae* salinities 0 to 33 at 5°C and 20°C. Sal = Salinity (0, 5, 10, 15, 20, 25, 30, and 33); Sal$^2$ = Quadratic effects of variable salinity; Temp = Temperature, including 5°C and 20°C; LS = Life stage, Sp = Species; AICc = Akaike information criterion (Corrected for the smaller data sets); ∆AICc = difference in AIC value of the model from the lowest AIC value of the model among the candidates; + = additive effect; All replicates were included in the data analysis.

<table>
<thead>
<tr>
<th>Model</th>
<th>Factor(s)</th>
<th>AICc</th>
<th>∆AICc</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Sal + Sal$^2$ + Temp + LS + Sp</td>
<td>42256.46</td>
<td>7.56</td>
</tr>
<tr>
<td>2</td>
<td>Sal + Sal$^2$ + Temp + LS</td>
<td>42254.44</td>
<td>5.54</td>
</tr>
<tr>
<td>3</td>
<td>Sal + Sal$^2$ + LS</td>
<td>42251.06</td>
<td>2.16</td>
</tr>
<tr>
<td>4</td>
<td>Sal + Sal$^2$ + Temp</td>
<td>42250.62</td>
<td>1.72</td>
</tr>
<tr>
<td>5</td>
<td>Sal + Sal$^2$ + Sp</td>
<td>42250.48</td>
<td>1.58</td>
</tr>
<tr>
<td>6</td>
<td>Sal + Sal$^2$</td>
<td>42248.90</td>
<td>0.00</td>
</tr>
</tbody>
</table>
2.5 Discussion

2.5.1 Role of salinity and temperature on mysid physiology

Our study showed that variation in salinity resulted in non-linear changes to the survival of *T. chiltoni* and *T. novaezealandiae*. The survival of these species was high in intermediate salinities, but decreased at the salinity extremes. Changes in salinity, when combined with temperature and life-history stage, were also highly explanatory for the observed variation in their survival. For osmoregulation, salinity never individually emerged as the most explanatory predictor; however, when combined with temperature, it was the most parsimonious, suggesting that the combined effects of environmental variables had a larger impact on osmoregulation than did individual variables alone. Our findings did not completely agree with Webb *et al.* (1997) who found species-specific responses to variation in salinity among various mysid species; however, they did not evaluate the combined effect of salinity and temperature. Because euryhalinity is common among estuarine mysids, the finding that both species of *Tenagomysis* are osmoregulators is also consistent with the wider literature where osmoregulation has been seen as a response of combined changes in salinity and temperature (Delisle and Roberts 1987).

Variation in salinity elicits a non-linear response to survival of *T. chiltoni* and *T. novaezealandiae* because survival was high in brackish zones, but decreased at the salinity extremes and lowest in near fresh waters. Survival trends of *T. chiltoni* and *T. novaezealandiae* agree with observations for *Mysidopsis bahia* which also exhibited non-linearity in survival that could be influenced by the interaction of salinity, temperature and life stage (McKenney 1994; McKenney and Celestial 1995). The observed non-linearity for survival of both the species of *Tenagomysis*, is consistent with the field observations of Lill *et al.* (2010) who found these mysids in a broad range of salinities at Kaikorai Lagoon in southern New Zealand, indicating that survival in near fresh water or sea water is possible for at least short periods, although may be not be for longer terms (Carrasco and Perissinotto 2011). These survival patterns possibly have significance for habitat use of *Tenagomysis* species (Delisle and Roberts 1986; McKenney and Celestial 1995; Vilas *et al.* 2009).
2.5.2 Impact on distribution of mysids

Estuaries are challenging environments where osmoregulation ability can influence survival and thereby the distribution of mysids (Greenwood 2007; Allen et al. 2008; Muylaert et al. 2009, Primo et al. 2009; Lill et al. 2010). I found low temperature can reduce the ability of *T. chiltoni* and *T. novaezealandiae* to osmoregulate and survive, especially if the conditions are hypo-saline, and particularly for juveniles. These findings may partly explain the very low density and abundances of *T. chiltoni* and *T. novaezealandiae* observed in winters in upper reaches of estuaries in the region (Jones et al. 1989; Lill et al. 2010). However in warmer conditions, *T. chiltoni* and *T. novaezealandiae* survived relatively better in intermediate salinities (10 to 20) which were to be expected as they were closer to their iso-osmotic points (McKenney and Celestial 1995; Webb et al. 1997; Vilas et al. 2009). This could explain their co-existence in brackish zones of various local estuaries in the region (Lill et al. 2010).

Spatial-temporal variation in salinity and temperature has been identified as a key factor structuring estuarine communities, given its major influence on species distribution including mysids (Greenwood 2007). The distribution of different mysid species has been related to the variation in salinity, based on their ability to osmoregulate (Vilas et al. 2009). Further, salinity interacting with daily or seasonal changes in temperature, and life-history stage, has been shown to influence the distribution of mysids (Greenwood 2007; Hanamura et al. 2009). However, contrasting views are presented by authors who concluded that salinity was not a driver of mysid distribution and attributed their distribution to other bio-physical variation (Webb et al. 1997). Some authors indicated that short-term survival and osmoregulation under varying salinities may be possible, although its energetic costs may become critical over longer time and with subsequent implications on the patterns of distribution (Vilas et al. 2006, 2009). Life-history stage has also been shown to have an impact on mysid osmoregulation, survival and, subsequently, distribution (McKenney and Celestial 1995). I suggest that a study of the energetics of mysids under varying salinity and temperature conditions could further clarify the factors controlling their distribution in temperate estuaries.
Chapter 3: Oxygen consumption by *Tenagomysis chiltoni* and *T. novaezealandiae* in relation to body size and salinity

3.1 Abstract

Animal body size influences rates of respiration, which may result in habitat-specific eco-physiological adaptations. The mysids *Tenagomysis chiltoni* and *T. novaezealandiae* differ in adult body size (average gravid females of *T. chiltoni* > 10 mm and *T. novaezealandiae* < 10 mm), and inhabit New Zealand estuaries. I hypothesized that mysid body size influences rates of oxygen consumption (µmol. O$_2$ hr$^{-1}$ mg$^{-1}$ DW) in relation to variation in salinity. Mysids were exposed to salinities of 0, 5, 10, 15, 20, 25, 30, and 34 at 5ºC or 20ºC in a controlled laboratory environment. Differences in mass-specific oxygen consumption rates under varying salinities were not significant, indicating a high degree of saline acclimation within certain thermal ranges. However, the larger individuals of *Tenagomysis* spp., especially of *T. chiltoni*, had significantly lower mass-specific oxygen consumption rates than smaller mysids, which possibly convey an energetic advantage in the often low salinity environments of upper reaches of intermittently open/closed temperate estuaries.

3.2 Introduction

A large body and respiratory flexibility can strongly influence the ecology of crustaceans in habitats such as estuaries where salinity is the dominant environmental variable (Kinne 1971; Remane and Schlieper 1971; Nielson 1997). Estuarine crustaceans are mainly euryhaline, adjusting to salinity gradients that may change with every tidal cycle; however, some osmotic stress is inevitable (Delisle and Roberts 1987; Pe´queux 1995; Vilas et al. 2006). Crustaceans
in estuaries often use body shape and size to alter surface to volume ratios, which can buffer against osmotic stress (Prosser and Brown 1961; Nielson 1984; Charmantier 1996). Besides such adaptations, they often occupy microhabitats that correspond with iso-osmotic conditions (i.e. where the concentration of ions within and outside a cell is equal or nearly equal) (Prosser and Brown 1961; Web et al. 1997; Vilas et al. 2009). Moving beyond their preferred isotonic microhabitat incurs additional metabolic cost (Kinne 1971), which can be measured as a temporal change in oxygen consumption (Marshall et al. 2003; Vilas et al. 2006). However, this response may also vary with other factors such as salinity and temperature (McKenney and Celestial 1995; Marshall et al. 2003).

In estuaries, mysids often link the benthic and pelagic food webs (Roast et al. 1998), so work on their osmoregulation and respiration has attracted attention (Marshall et al. 2003; Paul et al. 2013). Previous studies of aquatic crustaceans found their specific oxygen consumption rates (OCR) decreased with increasing body size and the slopes of such relationships generally remained below 1, indicating that as the individuals grow larger in body they possibly become more efficient in utilizing the metabolic energy for respiration (Zeuthen 1953; Jawed 1973; Emmerson 1985; Marshall et al. 2003). However, this change in the specific OCR of crustaceans including mysids may also depend on the salinity in estuaries, with increases or decreases in OCR as salinity increases (Kinne 1971; Simmons and Knight 1975; Vlasblom and Elgershuizen 1977). Like many other crustaceans an increase in temperature generally increases the metabolic rates of mysids too, but when temperature interacts with salinity the impact on the specific OCR of can be antagonistic (McKenney and Celestial 1995). For example, previous studies on Neomysis integer and Gastrosaccus brevifissura, and brackish-water caridean shrimps found inconsistent responses in the OCR in relation to changes in the estuarine environment, and concluded that interactions between salinity and temperature were having antagonistic influences on specific OCR (Marshall et al. 2003; Vilas et al. 2009).

Tenagomysis is a mysid genus of southern Pacific coastal waters (Lill et al. 2010). Of the eighteen species of Tenagomysis, T. chiltoni and T. novaezealandiae are endemic to New Zealand; the former inhabits brackish waters and tidal lakes, whereas the later frequents the lower reaches of intermittently open/closed estuaries (Jones et al. 1989; Lill et al. 2010). Gravid females of these two species differ in their maximum body size (T. chiltoni > 10 mm, T. novaezealandiae <10 mm) (Lill et al. 2010). Tenagomysis chiltoni is slightly more tolerant
of hypo-osmotic (salinity > 10) and *T. novaezealandiae* of hyper-osmotic (salinity < 30) conditions, possibly because of relatively better osmoregulatory abilities in those contrasting environments (see details in Chapter 2). Whilst both species have similar patterns of tolerance to salinity, small differences in the energetics of osmoregulation due to differences in size between the two species may have long-term consequences for growth, reproduction and distribution (Jones *et al.* 1989; Lill *et al.* 2010; Paul *et al.* 2013). Consequently, I decided to study mass-specific OCR of *Tenagomysis chiltoni* and *T. novaezealandiae* across salinities (0 to 34) in temperatures typical of summer and winter conditions (Paul *et al.* 2013). I predicted, irrespective of salinity, that large bodied individuals of the two *Tenagomysis* species, especially of *T. chiltoni*, would exhibit lower mass-specific OCR than small bodied individuals.

### 3.3 Method

#### 3.3.1 Mysid collection

Gravid females of *Tenagomysis chiltoni* and *T. novaezealandiae* were collected respectively from the upper and lower reaches of Kaikorai Lagoon, southern New Zealand (for details of collection methods, Kaikorai estuary see Lill *et al.* 2010; Paul *et al.* 2013), and transported to the laboratory in 20 Liter (L) sealed buckets filled with water from the respective sampling sites. Mysids were collected in late spring of 2011 and in mid-summer of 2012. Sampling was done only in these seasons to capture the contrasting temperature, and high density of gravid females in the Kaikorai estuary (Lill *et al.* 2010). Mysids collected in spring were kept and tested only at 5°C, and summer cohorts only at 20°C (± 0.1), thus reflecting the likely field temperatures the animals had recently experienced. The mysids were sorted to species and separated into different batches for acclimatization. Only gravid females of similar age (represent similar cohorts of a year) were used for ease of species identification relative to males and juveniles.
3.3.2 Acclimatisation of mysids

Before the oxygen consumption experiments, mysids were acclimated to the experimental salinities of 0, 5, 10, 15, 20, 25, 30, and 34, either at 5°C or 20°C for three days. For each treatment, sub-groups of 10 mysids were housed in 2 L PVC containers. The different salinities were prepared in the laboratory by filtering (with 1 µm filter papers) and diluting natural seawater with spring water. Mysids were fed daily with freshly hatched Artemia (Johns et al. 1981) that were cultured in the laboratory. One-third of the water was exchanged daily in each container, and any detritus, feces, excess food, and dead individuals removed. Ammonia concentrations were also monitored daily using an Aqua One (NH₃) test kit (http://www.aquaone.co.uk); however, there was no evidence of ammonia accumulation which might cause stress to the mysids (Miranda-Filho et al. 2009). Dark-light cycles typical of late spring (12h: 12h) and mid-summer (10h: 14h) conditions in the Otago region of southern New Zealand were used. After three days, mysids found to be healthy were used for the respiration experiments.

3.3.3 Oxygen consumption experiments

Mass-specific oxygen consumption rates of Tenagomysis chiltoni and T. novaezealandiae were measured at the aforementioned salinities and temperatures in a controlled laboratory environment using an YSI ProODO (YSI Corporation, USA) oxygen meter which had an optical probe and a magnetic stirrer to ensure continuous gentle mixing of the medium inside the respiratory chamber. I mostly followed the protocols of Marshall et al. (2003). For each experimental trial, a gravid mysid with brood pouch was placed inside a 1 ml glass incubation chamber. These chambers were acid washed and the openings were constructed so as to provide an airtight seal (with Vaseline) when the probe was inserted into the chamber. Chambers containing water of known salinity (0, 5, 10, 15, 20, 25, 30, and 34) were placed inside a water bath to maintain a temperature of 5°C or 20°C (± 0.1°C). Further, the entire experiment was conducted in temperature-controlled rooms that matched the temperature (± 0.3°C) of the water bath. For each salinity-temperature combination I ran 3 to 5 replicates of each species (depending upon the availability of healthy gravid females). Oxygen consumption was measured after 30 minutes of placing the mysid inside the respirometry chamber to avoid initial stress and changes in oxygen concentration due to chamber opening. Each experimental period lasted for 1hr until the oxygen concentration in the respirometry
chamber was depleted to 80% of the initial condition, whichever occurred first. The volumes (less than 1ml) of water were measured after each trial as the mysid displaced some water depending on their body volume, and the volume used to calculate oxygen consumption was adjusted accordingly. Oxygen readings were also taken from identical control chambers without mysids. Changes in dissolved oxygen (mL⁻¹) in the respirometry chamber and from controls were taken at the start and end of the experimental period. The difference in the oxygen concentration in the respirometry chamber within this period was assumed to be due to resting metabolic rates (oxygen consumption rates were measured when mysids were sitting on the floor) of the enclosed mysid and measured as µmol O₂ hr⁻¹ mg⁻¹ DW. Mysids were not fed for 24 hours prior to and during the experimental period to avoid any confounding factors (i.e. activity levels, nutritional state) that could have affected the oxygen concentration within the respiratory chamber, as well as their resting metabolic rates of mysids. After each trial, the gravid mysid was removed from the respirometry chamber with fine forceps, quickly frozen on dry ice, and stored at -70°C in an Eppendorf tube for later measurement of body size (length defined as the distance from the tip of the rostrum to the posterior end of the sixth abdominal somite) and its dry weight (DW). During processing, mysids were quickly thawed, and gently blotted with tissue for 1 minute to remove surface water. A single mysid was then measured to the nearest ± 0.1 mm (mid of the eye stalk to end of sixth somite) under a microscope fitted with an eyepiece micrometer. After measuring wet weight (WW) to the nearest ± 0.1 mg, the mysid was placed in an oven at 60º C for 24 hours, cooled to room temperature, desiccated (for 2 hours) with silica gel, and then weighed to obtain dry weight (DW). This procedure was followed for each mysid used in the experiment.

3.3.4 Statistical analysis

I used generalized linear models (GLM) to evaluate if the mass-specific OCR of T. chiltoni and T. novaezealandiae varied with respect to salinity (0 to 34), body weight and temperature (5°C or 20°C). While performing GLMs, I treated the response variable mass-specific OCR and the predictors such as ‘Body weight’ and ‘Salinity’ as continuous, but ‘Temperature’ and ‘Species’ as categorical. Data set had a nested structure, so a random variable was added at the intercept of each GLM to capture the individual variation. Confidence intervals were set at 95%, and analysis done using statistical software R.3.0.1 for Windows (R Development Core Team 2014).
3.4 Results

The average body sizes of gravid *Tenagomysis chiltoni* and *T. novaezealandiae* used for respirometry were 10.18 ± 2.2 mm and 7.23 ± 1.3 mm, respectively. A preliminary GLM (on pooled data) indicated that the mass-specific OCR of *T. chiltoni* and *T. novaezealandiae* were not statistically different (*P* = 0.81), so the data sets for each species of *Tenagomysis* were analyzed separately. I observed that the mass-specific OCR of gravid *T. chiltoni* declined significantly (*P* = 0.001) with increasing body size, especially for those with body length exceeding 10 mm, but only a weak trend was apparent for *T. novaezealandiae* (*P* = 0.06) (Fig. 3.1 and Table 3.1). Interactions between variables ‘Salinity’, ‘Temperature’, and ‘Body weight’ yielded no statistically significant i.e. *P* > 0.05 results (Figs. 3.1, 3.2, 3.3, and Table 3.1).

![Fig 3.1 Relationship between mass specific oxygen consumption rates of mysids *Tenagomysis chiltoni* and *T. novaezealandiae* under salinities 0 to 34 at 5°C and 20°C. Relationships between mass specific oxygen consumption rates and body length of *T. novaezealandiae* were not statistically significant (i.e. *P* > 0.05), so linear regression lines were not drawn. For every plot, number of individuals is n ≥ 30.](image-url)
Oxygen consumption of mysids

Fig 3.2 Variation in mass-specific oxygen consumption rates of *Tenagomysis chiltoni* and *T. novaezealandiae* under salinities 0 to 34 at 5°C and 20°C. Salinity = 0, 5, 10, 15, 20, 25, 30, and 34; N = 73 (for each species); bars represent mean ± 95% confidence intervals of mass specific oxygen consumption rates of individual mysids.

Fig 3.3 variation in mass specific oxygen consumption rates of *Tenagomysis chiltoni* and *T. novaezealandiae* between 5°C and 20°C. Box plots exhibit the mean ± 95% confidence intervals (where n ≥ 35 for every plot)
Table 3.1 Probable generalized linear models describing the variations of mass-specific oxygen consumption rates of *Tenagomysis chiltoni* and *T. novaezealandiae* under salinities 0 to 34 at 5°C and 20°C. Salinity = Sal (0, 5, 10, 15, 20, 25, 30, and 34); Temperature = Temp (i.e. 5°C and 20°C); Body dry weight = DW (mg); *T.ch = T. chiltoni* and *T.nz = T. novaezealandiae*; * = interaction between predictors; N = 73 (for each species). A random variable at the intercept is used for capturing individual variation within the data sets, but only the static variables are reported.

<table>
<thead>
<tr>
<th>Predictor (s)</th>
<th><em>T.ch</em> Z-values</th>
<th><em>T.nz</em> Z-values</th>
<th><em>T.ch</em> P-values</th>
<th><em>T.nz</em> P-values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sal</td>
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<td>0.03</td>
<td>0.47</td>
<td>0.89</td>
</tr>
<tr>
<td>Temp</td>
<td>1.3</td>
<td>1.12</td>
<td>0.08</td>
<td>0.11</td>
</tr>
<tr>
<td>DW</td>
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<td>1.52</td>
<td>0.001</td>
<td>0.06</td>
</tr>
<tr>
<td>Sal * Temp</td>
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<td>0.05</td>
<td>0.64</td>
<td>0.85</td>
</tr>
<tr>
<td>Sal * DW</td>
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<td>1.45</td>
<td>0.36</td>
<td>0.07</td>
</tr>
<tr>
<td>Temp * DW</td>
<td>1.16</td>
<td>1.21</td>
<td>0.11</td>
<td>0.09</td>
</tr>
<tr>
<td>Sal * Temp * DW</td>
<td>1.41</td>
<td>0.19</td>
<td>0.07</td>
<td>0.66</td>
</tr>
</tbody>
</table>

### 3.5 Discussion

#### 3.5.1 Oxygen consumption, body size and estuarine environment

Oxygen consumption rates of *Tenagomysis chiltoni* were affected by body weight, with a significant linear negative relationship being observed between the mass-specific OCR and body size of larger mysid individuals. This trend is common among mysids *Neomysis integer* and *Gastrosaccus brevifissura*, brackish-water caridean shrimps, and estuarine crustacean communities (Emmerson 1985; Toda *et al.* 1987; Marshall *et al.* 2003). Large gravid individuals of *T. chiltoni* had lower mass OCR than their smaller counterparts, possibly indicating a higher tolerance to stress, which might have arisen from osmoregulation (Nielsen 1984; Marshall *et al.* 2003) at salinities beyond their preferred zones (see Lill *et al.* 2010; Paul *et al.* 2013). Changes in the metabolic efficiency of *T. novaezealandiae* were probably very small across the limited change in weight, which probably resulted in a weak relation between body weight and variation in mass-specific OCR.
Results showed that the mass-specific OCR of *Tenagomysis* spp., varied independently of salinity, which is not unexpected among estuarine crustaceans (Kinne 1971; Simmon and Knights 1975; Mauchline 1980). For example, the effect of salinity on the oxygen consumption of the mysid *Americamysis bahia* was found to be negligible when studied under various salinity-temperature combinations (Modlin and Froelich 1997). However, oxygen consumption of *Neomysis* spp. increased or decreased with increasing or decreasing salinity (Roast *et al.* 1999; Vilas *et al.* 2006), but that was not the case for *T. chiltoni* and *T. novaezealandiae*.

I found temperature had no significant effect on mass-specific OCR of *Tenagomysis chiltoni* and *T. novaezealandiae*. From the results it could be easily forecast that the $Q_{10}$ was less than 1 (Prosser and Brown 1961; Nielson 1997), which is somewhat surprising, but this result has been observed for other mysid species (Jawed 1973; Allan *et al.* 2006). *Tenagomysis chiltoni* and *T. novaezealandiae* collected for the experiments were from Kaikorai Estuary which often remains closed for long periods (Lill *et al.* 2010), potentially giving these mysids ample scope to acclimatize to the prevailing environment. Several authors have argued that temperature tends to increase mass-specific OCR, but salinity decreases it at the same time, thus buffering crustaceans against the rapid changes in salinity and temperature that can occur in estuarine environments (Kinne 1971; Jawed 1973; Vlasblom and Elgershuizen 1977; Newell 1980; Roast *et al.* 1999; Marshall *et al.* 2003; Vilas *et al.* 2006). The synergistic effects of temperature and salinity did not influence OCR of the two *Tenagomysis* species significantly; this is possibly a result of aforementioned cause. However in future an investigation on the non-linear dynamics of the molecular mechanisms involved (e.g. enzymes like Na+/K+ ATPase and membrane permeability) in mysid respiration may shed more light on it.

### 3.5.2 Possible influence of oxygen consumption on ecology of *Tenagomysis*

Specific OCR often has subtle implications for the ecology of mysids (Roast *et al.* 1999; Marshall *et al.* 2003; Vilas *et al.* 2009). My results suggest that if intermittently open/closed estuaries remain closed and stagnant for extended periods (Lill *et al.* 2010), mysids may acclimatize well energetically to the conditions of their specific microhabitats, but the response may be different if habitats experience frequent changes, like open estuaries. For example at times of the estuary mouth opening or closing and during flash floods, rapid
changes and extreme salinities are likely (Marshall et al. 2003); this might affect mysids such as *T. novaezealandiae* which has a smaller body size by limiting their osmoregulation ability compared with mysids species with larger bodies (see also Delisle and Roberts 1987; Pe’queux 1995; Marshall et al. 2003). This reason may explain the higher survival rates of large bodied mysid species, such as *Tenagomysis chiltoni*, in the hypo-saline environment and in open estuaries (Jones et al. 1989) which potentially demands higher osmoregulatory costs from these mysids (Paul et al. 2013). In conclusion this Chapter shows that respiration of temperate mysids may not be severely affected if the mysids are not exposed to abrupt changes in their micro-habitat, and in stable environments the large bodied mysids may gain some energetic advantage over smaller ones. However, further research on growth and or fecundity may shed more light if large bodied mysids are using their possible metabolic advantages for ecological purposes.
“You’ll never know everything about anything, especially something you love.”
- Julia Child

Chapter 4: Effects of salinity and food quality on the growth of subadult *Tenagomysis* spp.

Measuring growth (length (mm)) of mysids at the laboratory

4.1 Abstract

In estuaries, the somatic growth of crustacean mysids may be influenced by spatial and temporal variation in salinity and food resources. I tested such a possibility in a laboratory experiment, where somatic growth of sub-adult mysids *Tenagomysis chiltoni* and *T. novaezealandiae* was studied in intermediate salinities (10 to 20) with contrasting high or low (based on carbon and nitrogen richness) food qualities. Different salinity and/or food quality combinations interacted to produce contrasting growth trajectories between the two species. Growth of sub-adult *T. chiltoni* was more influenced by salinity, whereas the growth of *T. novaezealandiae* more strongly affected by food quality. My results suggest that small environmental changes to salinity and food regimes may give rise to different growth trajectories among sub-adult mysids which may have a cascading influence on their life histories and patterns of coexistence with other species in small intermittently open/closed estuaries in temperate regions.

4.2 Introduction

The growth of aquatic crustaceans can influence their distribution where multiple species have overlapping niches (Delgado *et al.* 2011). In estuaries, crustaceans are commonly euryhaline (DeLisle and Roberts 1987; Charmantier 1996), but their growth can vary because of spatial-temporal variation in environmental gradients such as salinity, temperature, nutrients (McKenney and Celestial 1995), resulting indifferent adult body sizes and shapes at maturity (Fockedey *et al.* 2005, 2006; Xue *et al.* 2013). Apparently small variations in growth, body size, and time to maturity are important to overcome the challenges of osmoregulation, avoiding predators and parasites, and capturing prey in respective microhabitats within estuaries (Nielson 1984; Conover 1992; Atkinson 1995; Gotthard 2001). Growth and time to maturity may also depend on specific feeding habits (e.g. scavenger, predator, and opportunist), food preferences, quality and quantity of nutrients in a habitat, and metabolic efficiency (Amarasinghe *et al.* 1997; Fockedey *et al.* 2005). Previous studies on estuarine Crustacea have shown the importance of food quality on the early phases of growth (Urbina *et al.* 2010). These intrinsic and extrinsic factors act together to alter the growth trajectories, with cascading implications for how different crustacean species partition their resources to maintain coexistence and or distribute themselves within and across estuaries (Winkler and Greve 2002; Carrasco and Perissinotto 2011).
Estuarine crustacea, such as mysids, often sustain a high biomass, are key elements of food webs, and are frequently taken as prey by fish (Roast et al. 1998). Field and laboratory studies on mysid growth are overwhelmingly dominated by studies from the northern hemisphere; few are focused on warmer small intermittently open/closed estuaries in southern hemisphere (Astthorsson and Ralph 1984; Fenton 1996a; Winkler and Greve 2002; Fockedey et al. 2005, 2006; Hanselmann et al. 2011). These studies often found that the growth could be restricted by feeding habits, species-specific life stages, gradients of salinity, and temperature which may act together (McKenney and Celestial 1995). For example osmotic stress at zones beyond their preferred microhabitat may limit the growth of juvenile mysids (Astthorsson and Ralph 1984; McKenney and Celestial 1995; Winkler and Greve 2002; Fockedey et al. 2005) such as Neomysis spp. Laboratory studies on the growth of mysid genera Neomysis spp. and Mysidopsis spp. have received attention in literature but growth of the genus Tenagomysis spp. is unstudied (Fenton 1996a; Lill et al. 2011).

In the southern hemisphere, mysids of Tenagomysis spp. inhabit estuaries in Tasmania and in New Zealand. There are studies on salinity tolerance, osmoregulation, life history and field distributions of different Tenagomysis species (Jones et al. 1989; Fenton 1996a; Lill et al. 2010; Paul et al. 2013), but none have taken an experimental approach to understand growth to further advance understanding of their life history which might influence their distribution in estuaries. The mysids T. chiltoni and T. novaezealandiae coexist in many small estuaries in the Otago region of southern New Zealand (Lill et al. 2010). Tenagomysis chiltoni generally dominates hypo-saline, less productive upper estuaries, whereas T. novaezealandiae is generally more abundant in brackish and more productive habitats in the lower reaches of estuaries (Jones et al. 1989; Lill et al. 2010). Previous work evaluated their life histories and found adults of T. chiltoni to have a significantly larger maximum body size than T. novaezealandiae, both species produce multiple cohorts every year, with the autumn, and summer cohorts reaching maturity faster (2 to 3 months) than winter cohorts (6 to 8 months) (Lill et al. 2010). Previous work on osmoregulation and survival of these species across salinities (0 to 34) found that if the conditions are warmer at intermediate salinities (10 to 20), inter-specific differences are relatively minor, but for both the species osmoregulation becomes increasingly difficult at high and low salinities (25 > Salinity > 10) which causes high mortality especially among juveniles and sub-adults (Paul et al. 2013).
In this Chapter, I investigated the growth trajectories of *T. chiltoni* and *T. novaezealandiae* over three weeks in salinities of 10, 15, and 20 with access to either high or low quality food resources (high/low carbon and nitrogen). Estuarine mysids are opportunistic feeders, utilising detritus when access to higher quality animal-based food is limited (Rudstam *et al.* 1999; Wilhelm *et al.* 2002). Given that these species live in habitats with contrasting patterns of variation in salinity and productivity (Lill *et al.* 2010), I presumed that the growth of *T. chiltoni* would be more strongly influenced by varying salinity (highest possibly towards low salinity because of its preferred habitats) than food quality. However the growth of *T. novaezealandiae* would be influenced more strongly by variation in food quality than salinity (because it survives well across a broad range of intermediate salinities, but is most abundant in highly productive, relatively eutrophic intermittently open/closed estuaries (Lill *et al.* 2010)).

### 4.3 Methods

#### 4.3.1 Mysid collection, acclimatisation and growth trials

*Tenagomysis chiltoni* and *T. novaezealandiae* were collected from up and downstream reaches of the Kaikorai Lagoon respectively in autumn (March-April) of 2013 (for details of Kaikorai estuary, and mysid collection method see Lill *et al.* (2010) and Paul *et al.* (2013)). During sampling, water temperatures (13 ±1°C) and salinities (ranged 8 to 21) were measured with a hand held YSI multi probe meter (YSI Corporation, USA) to determine conditions for acclimatisation and run growth trials in conditions comparable to those found in the Kaikorai Lagoon. On return to the laboratory, mysids were left for a day to recover from sampling stress in 20L plastic buckets. From there sub-adults of a similar length (estimated by eye) of both species were randomly selected for the experimental trials (Jocque and Blom 2009). These individuals were then given a week to acclimatize to the experimental conditions and kept in different 2L plastic containers. Each container was provided with either high or low quality food (for details, refer to next section) at salinities of 10, 15, and 20 at a controlled laboratory environment. Experiments in more extreme salinity regimes (10 < salinity ≤ 25) and with juveniles were not conducted due to the high rates of mortality that occur in such conditions (for details see Paul *et al.* 2013). Different salinities were produced by diluting seawater (salinity 32 to 34) with spring water (salinity 0 to 0.5) to an accuracy of ± 0.2.
Nearly 50 mysids were initially housed in each container for acclimatisation. On the final day of acclimatization, 10 individuals were sub-sampled randomly from each container (which holds 45 to 50 mysids) for measurement of their weight (dry weight); this was done to establish an average reference weight at Time = 0 (i.e. dry weight of *T. chiltoni*: 0.28 ± 0.19 mg and *T. novaezealandiae*: 0.21 ± 0.1 mg), from which relative change in growth was calculated. The remaining individuals, comprising not less than 35 to 40 in each container, were used in the growth experiment. Growth experiments were run for the next three weeks. For each species, three replicates of each treatment (salinity-food combination) were prepared. At the end of the third week (T), 20 to 25 individuals were randomly removed from each replicate for the measurement of growth relative to T₀ (i.e. dry weight T-T₀). Once removed, mysids were immediately placed in Eppendorf tubes and frozen at -20°C. Later at laboratory batch of mysids were dried within an oven at 60°C for 24 hours and then weighed with a fine balance (± 0.001 mg) for their dry weight (DW).

### 4.3.2 Preparation of food and laboratory maintenance

Mysids of *Tenagomysis* spp. primarily consume detritus and may also be classified as opportunistic scavengers and predators (Wilhelm *et al.* 2002), which suggests they can access food of widely differing quality (nitrogen and organic carbon content). To determine the effects of food quality on growth of *T. chiltoni* and *T. novaezealandiae*, mysids were fed with what was considered to be either a high quality or a low quality food. The high quality food comprised ground salmon feed pellets ([https://www.crt.co.nz](https://www.crt.co.nz)), the main ingredients of which were fish meal, fish oil and plant proteins (such as soy). Such a food was intended to represent the quality of food that mysids scavenging on dead organisms or predating on other organisms might consume. The low quality food comprised organic and inorganic silt collected from Kaikorai estuary, which was dried by baking at 60°C for three days and then ground to produce a powdered food. The quality of both types of food was assessed by analysing their carbon and nitrogen content using the protocol of Preston and Owen (1983). Analysis of food quality indicated that the salmon pellets (high quality food) had a nitrogen and organic carbon content (providing a relative indication of protein and energy content) of approximately 8% and 40% respectively, whereas the detritus had a nitrogen and organic carbon content of 0.2% and 2% respectively.
Throughout the experiment, air temperature in the experimental room was continuously recorded (average of 13.44°C ± 0.92 SD) and water temperature was measured daily (13 ± 0.15 SD); the temperature throughout was such that mortality would be expected to be low (see Paul et al. 2013). Excess food, faeces, and dead individuals were removed every day to keep the containers clean and minimise fouling. One third of the water in each container was changed every day and the salinities were kept within ± 0.2 of the experimental treatment concentrations. Ammonia concentrations were also monitored daily using an Aqua One (NH₃) test kit, (http://www.aquaone.co.uk), but never exceeded 0.5 mg/L in any replicate. Water samples were taken randomly from different replicates each day to test water quality using a Skalar SANPlus segmented flow analyser (Skalar Analytical B.V., Breda, The Netherlands); these samples also showed no evidence of ammonia accumulation which could have been detrimental to mysid health (Miranda-Filho et al. 2009). The average total N concentration in the water of the high food quality treatments was 1760 ± 20 (SD) µg/L and in the low food quality treatments was 812 ± 90 (SD) µg/L, and did not exceed the total nitrogen concentration of the water from which the mysids were collected (2200 ± 50 (SD) µg/L). Mysids were maintained under a photoperiod typical of autumn (11h: 13h) in Otago, southern New Zealand, and all the replicate containers were spatially randomized to avoid spatial confounding of growth due to position.

4.3.3 Data analysis

To compare growth of *Tenagomysis chiltoni* and *T. novaezealandiae* under the different salinity (10, 15, and 20) and food quality (high and low) regimes, a three-way ANOVA was performed followed by pair-wise *t*-tests. Experiments had a nested structure, so group means were used to reduce variance (if any, arising from individual variation within each replicate). I used dry-weight (mg) as the response variable because length-mass regression for these two species is very accurate (Lill *et al.* 2011) and to avoid any error due to discontinuous increases associated with moults. Data from all of the replicates were included and confidence intervals were set at 95% for analyses, which were performed in R.3.0.1 for Windows (http://cran.r-project.org).
4.4 Results

Three-way ANOVA indicated that the growth of *Tenagomysis* spp. (pooled data) was reduced under increased salinity ($F = 12.11; P = 0.0016$), and that *T. chiltoni* grew faster than *T. novaezealandiae* ($F = 6.19; P = 0.019$) (Fig 4.1 and Table 4.1). Although the two-way interactions were not significant at $p = 0.05$, the salinity*species and food*species interactions were both $P < 0.08$ suggesting some level of species-specific responses to treatments may be present (Table 4.1). Pair wise $t$-tests suggested that the overall growth of *T. chiltoni* had a negative relationship with increasing salinity; growth in salinity 10 and 15 was not significantly different ($P = 0.36$) but was higher ($p = 0.0017$ and $0.0011$) than salinity 20, with food having a minimal effect. In contrast, whilst the growth of *T. novaezealandiae* was not limited by salinity ($P = 0.226$) (Fig 4.1), growth was significantly (dataset separated for each species) reduced ($t = -3.7, P = 0.0019$) in the low quality food regime (Fig 4.1).

<table>
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<th>Predictor(s)</th>
<th>DF</th>
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<th>P-value</th>
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</tr>
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</tr>
<tr>
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<td>3.36</td>
<td>0.08</td>
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<tr>
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<td></td>
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</table>
Fig 4.1 Growth of *Tenagomysis chiltoni* and *T. novaezealandiae* under salinities 10, 15, and 20 either at high or low food quality (Carbon: Nitrogen %). The changes in growth were relative to the reference point T₀ (i.e. dry weight measured for *T. chiltoni* (0.28 ± 0.19 mg) and *T. novaezealandiae* (0.21 ± 0.1 mg)) and the end of the experimental period (i.e. three weeks after T₀); error bars represent the standard deviations between replicates of each treatment i.e. salinity-food combinations.

4.5 Discussion

4.5.1 Growth of *Tenagomysis* spp. in varying salinities

Growth of the sub-adults of *T. chiltoni* and *T. novaezealandiae* responded differently in environments of contrasting salinity and food quality. The growth of *T. chiltoni* declined with increasing salinity but not *T. novaezealandiae*. Studies by McKenney and Celestial (1995) and Yamada *et al.* (1995), on *Mysidopsis bahia* and *Acanthomysis mitsukurii* respectively, suggested that growth can be reduced due to osmotic stress under either hyper or hypo-osmotic conditions. Fockedey *et al.* (2005) commented that either hyper or hypo osmotic stress can affect the survival and development of juvenile *Neomysis integer*. Paul *et al.* (2013) found that the survival of juvenile *T. chiltoni* and *T. novaezealandiae* is low at extreme salinities; however at intermediate salinities inter-specific differences are minor. As the
salinities in this experiment were typical of the salinities in intermittently open estuaries in the region (Lill 2010; Bierschenk et al. 2012), so the results demonstrate the ability of sub-adult *T. chiltoni* and *T. novaezealandiae* to grow well in habitats where they are most abundant (Lill et al. 2010).

### 4.5.2 Growth of *Tenagomysis* spp. in different food quality regimes

Mysids, including different species of *Tenagomysis*, are generally opportunistic feeders, feeding mostly on detritus and plankton (Fenton 1996b; Rudstam et al. 1999; Wilhelm et al. 2002). However, based on the variation within their microhabitats and across seasons, variation in the quality and availability of food might affect their growth, development, length and time to maturity of mysids (Fenton 1996b; Fockedey et al. 2005, 2006). Results for sub-adults of *T. novaezealandiae*, which exhibited higher growth under a high quality (nitrogen and carbon rich) food regime, correspond with studies on *Mysis mixta* which grew faster and larger when fed with zooplankton rather benthic detritus (Lethiniemi et al. 2002). The typical habitat of *T. novaezealandiae* (i.e. lower reaches of intermittently open/closed estuaries) is often nutrient enriched by urban and agricultural runoff (Bierschenk et al. 2012). This often results in the production of an abundant and diverse range of decaying organic materials that varies between seasons, potentially creating a rich food resource for mysids. Thus it is not surprising that *T. novaezealandiae* was better able to utilise high as well as low quality of food to enhance growth (Viherluoto et al. 2000; Lethiniemi et al. 2002). However, dietary results for sub-adults of *T. chiltoni* are less clear to me; it appeared that their growth may not be limited by food quality, perhaps related to an ability to sort food particles more efficiently of in detritus of varying quality (Sterner and Robinson 1994; Fenton 1996b; Viherluoto et al. 2000; Wilhelm et al. 2002). For *T. chiltoni* the low quality food treatment at salinity 10 registered higher growth which is not surprising because it prefers such hyposaline habitats (Lill et al. 2010, Paul et al. 2013). However the high variation in the aforementioned treatment is hinting other nutrients and or texture of food particles apart from C: N richness might also affect feeding efficiency of mysids and give rise to varying growth trajectories.
4.5.3 Possible influence of growth on *Tenagomysis* spp.

Inter-specific differences in somatic growth of sub-adults of *Tenagomysis* spp. in this experiment were statistically significant, and could lead to contrasting population dynamics and life histories even when environmental changes are relatively small (Fenton 1996a; Wilhelm *et al.* 2002; Fokedey *et al.* 2005). Whilst the short-term survival of *T. chiltoni* and *T. novaezealandiae* is not significantly different at intermediate salinities (Paul *et al.* 2013), my results suggest that even slight differences in salinity and food resources generate changes to growth trajectories that have the potential to alter life history options. For example, reduced summer growth in *T. chiltoni* may limit their survival under cold hypo-saline winter conditions (Paul *et al.* 2013), whereas reduced summer growth in *T. novaezealandiae* could reduce the number of summer generations produced (Lill *et al.* 2010). Overall, my results suggest that the growth of sub-adult *Tenagomysis* spp. is sensitive to small changes in their microhabitats which could influence population and life history dynamics, and patterns of coexistence.
“When we try to pick out anything by itself, we find it hitched to everything else in the universe.” - John Muir

Chapter 5: Fecundity of temperate mysids: Why does *Tenagomysis chiltoni* have a larger body than *T. novaezealandiae*?

Working in the estuaries in Otago region of New Zealand
5.1 Abstract

Habitat may influence body and brood size of a female, leading to adjustments in fecundity which may cascade into species life history. I evaluated such possibilities by studying the body-brood size relationship of the mysids *Tenagomysis chiltoni* and *T. novaezealandiae* in different habitats (i.e. open or intermittently open/closed estuaries) across their breeding seasons. *Tenagomysis chiltoni* is a large bodied species, dominant in hypo-saline reaches of estuaries, with one cohort (occasionally two) per year, whereas *T. novaezealandiae* is a small bodied species, dominant in brackish zones of estuaries, with 2 to 3 cohorts per year. I sampled gravid females of these two species from 15 estuaries of southern New Zealand in their main breeding seasons (i.e. during spring (2011) and autumn (2012)). I found intermittently open/closed estuaries to have relatively higher nutrient levels than open estuaries, possibly due to the influence of agricultural and urban land use in their catchments. Body size of gravid *T. chiltoni* had a significant positive relationship with brood size amongst individuals from intermittently open/closed estuaries, but for *T. novaezealandiae* the significance of body-brood size relationship contrastingly varied between habitats in breeding seasons. I discuss if the observed results were consequences of nutrient levels in respective habitats, or influence of latitude that limits life history options, forcing females to have either a small body and multiple cohorts or a large body and single spawning life history strategy.

5.2 Introduction

In temperate regions, key features of life histories, such as body size at maturity, fecundity (e.g. brood size), and timing of breeding, are often adjusted to suit variable seasonal conditions (Conover 1992; Silby and Atkinson 1994; Atkinson 1995; Gotthard 2001; Angilletta *et al.* 2004). At higher latitudes an animal’s growth is typically slower, but many taxa continue to grow to a larger size; a life-history puzzle that has received wide attention, including for crustaceans (Gotthard 2001; Angilletta *et al.* 2004). Females may earn a ‘fecundity advantage’ through a large body size (Shine 1988), but overcoming ‘winter mortality’ has often been suggested as a more plausible explanation for prolonged growth and the larger body of ectotherms in higher latitudes (Atkinson 1995; Abrams and Rowe 1996; Gotthard 2001). Many of these animals synchronize their life-history stages by delaying or
accelerating maturity at smaller or larger size, to maximise use of favourable seasonal conditions (for example higher productivity in warmer seasons) which may affect the breeding frequency (Sibly and Atkinson 1994; Jeong et al. 2007). Synchronizing life history with seasons may provide advantages such as larger maternal body size by prolonging growth so females can carry more eggs, and or provide better parental care (Stern 1992; Gotthard 2001). However, altering time of maturity can force females to choose between more or fewer broods, larger or smaller eggs, and altered breeding frequency (Smith and Fretwell 1974; Fleming and Gross 1990). For decades the consequences of these alternative life history strategies have been explored for a range of taxa including aquatic crustaceans, but with few studies from temperate estuaries (Fukui and Wada 1986; Shine 1988; Stern 1992; Jeong et al. 2007).

At higher latitudes (＞40°), environmental gradients in space and time in estuaries are often extreme because of seasonal changes in temperature and salinity, and can be exacerbated by anthropogenic stress such as nutrient run-off from adjacent catchments (Lotze et al. 2006; Statham 2012). Conditions vary greatly not only between estuaries but also within estuaries; for example, in winter the upper reaches of estuaries are often relatively cold and less productive, while conditions are reversed in the lower reaches (Attrill 2002; Statham 2012; Lill 2010). Further the geomorphic characteristics of estuaries such as periodic opening and closing of estuary mouths influence marine and freshwater exchange which often results in dynamic levels of nutrients in space and time (Statham 2012). Thus estuaries which open/close periodically are often stable environments over short to medium term periods, but conditions can change suddenly when estuarine berms are breached (Lill et al. 2013) whereas open estuaries are highly dynamic over short time periods, with continuous but otherwise relatively predictable changes with every tidal cycle (Attrill 2002; Cornell and Klarer 2008). Between and within estuaries, such contrasts create different microhabitats, which possibly have implications for life histories such as size at maturity, brood size, and the breeding frequency of resident communities; crustacean communities which inhabit estuaries of temperate regions are no exception (Fukui and Wada 1986; Silby and Atkinson 1994; Jeong et al. 2007).

The life-history literature on crustaceans that addresses adaptations on body size of females at maturity, brood size and breeding frequency in relation to seasonal change in estuarine salinity, temperature, and productivity are rich (Fonds 1979, Fukui and Wada 1986; Stern
Fecundity of mysids

1992; Hall and Burns 2001; Jeong et al. 2007; Yamada et al. 2007, Guay et al. 2011). For example, a positive relationship between brood and maternal body size in crustaceans such as mysids and crabs which change with seasons and productivity of micro-habitats are not uncommon (Mauchline 1980; Hines 1982; Jeong et al. 2007; Burdloff et al. 2002). However, extracting a general pattern from the life histories of any community is difficult because it at least demands undertaking regional scale studies, which has never been conducted on any mysid shrimp species.

In estuaries, mysid shrimps often constitute a very large proportion of the epibenthic biomass, and their life histories have been examined from tropical to temperate estuaries around the world (Mees et al. 1994; Ramarn et al. 2012). For example, temperate mysids like Neomysis spp., Mysis relicta, Tenagomysis spp. have distributions which span considerable latitudinal gradients, but no studies have been conducted across regional scales (Mees et al. 1994; Vainola and Vainio 1998; Fockedey et al. 2005, Lill et al. 2010). From previous work, it appears they have distinct cohorts; seasonal variations in size at maturity; females breed mostly in spring-summer; and life cycles usually annual but some living for > 1 year (Toda et al. 1982; Mees et al. 1994; Vainola and Vainio 1998; Fockedey et al. 2005; Lill et al. 2010). Detailed work on mysid fecundity has revealed that variation in brood size can some extent be explained through variation in maternal body size, temperature and nutrient level of estuaries (Mauchline 1980; Toda et al. 1982; Mees et al. 1994; Takahashi and Kawaguchi 2004). However, I have found no regional scale study on any mysid species which explained body-brood size variations, nor have any integrated the findings across species to advance our understanding of the various fecundity strategies of temperate mysids.

The mysids Tenagomysis chiltoni and T. novaezealandiae occur at high densities in coastal lakes, intermittently open/closed, and open estuaries around New Zealand (Lill et al. 2010). Tenagomysis chiltoni occurs predominantly in hypo-saline habitats, but the counterpart T. novaezealandiae typically inhabits the more saline lower reaches, and forms extremely high densities especially in intermittently open/closed estuaries (Jones et al. 1989; Lill et al. 2010). Previous studies have established their euryhalinity and high vulnerability of juveniles to cold conditions which possibly have consequences on their population dynamics in winter (Lill et al. 2010; Paul et al. 2013). Studies also demonstrated that T. chiltoni has the potential to grow faster than T. novaezealandiae given the conditions in their microhabitats are similar (Paul et al. 2014). However it is possible that contrasting microhabitats of T. chiltoni and T.
Fecundity of mysids

*M. novaezealandiae* have influence on their growth trajectories, giving rise to significantly different body sizes of gravid *T. chiltoni* and *T. novaezealandiae* at maturity (see Jones *et al.* 1989; Lill *et al.* 2010). This might have implications for brood size, i.e. number of eggs and different stages of larvae that females of *T. chiltoni* and *T. novaezealandiae* can carry in their brood pouch, spawning seasons and frequency (*T. chiltoni* mainly once in spring but *T. novaezealandiae* 2 to 3 times in spring-summer-autumn (Lill *et al.* 2010)); all of these variations possibly influence fecundity strategies of *T. chiltoni* and *T. novaezealandiae* (Lill *et al.* 2010). However this cannot be inferred from the very limited field observation of Jones *et al.* 1989 and Lill *et al.* (2010), Hence, I conducted a field survey on body-brood size relationships of *T. chiltoni* and *T. novaezealandiae* in their breeding seasons across 15 estuaries in southern New Zealand with contrasting conditions related to either for mouth opening and closing frequency and/or nutrient loading.

I had multiple working hypotheses: (1) the larger body size of *T. chiltoni* will facilitate carrying larger broods than *T. novaezealandiae*; (2) the spring cohort of *T. novaezealandiae* will carry more broods than the autumn cohort due to higher nutrient levels in estuaries in that time of the year studied by Lill (2010), and (3) *Tenagomysis* spp., which inhabits intermittently open/closed estuaries will carry more broods than the mysids in open estuaries because intermittently open/closed estuaries are possibly more nutrient enriched than open estuaries. Based on the results and literature review, I wanted to present a perspective of the different fecundity strategies which *T. chiltoni* and *T. novaezealandiae* might be following, and which might be more widely applicable to mysid communities in temperate.

### 5.3 Methods

#### 5.3.1 Field work

Gravid females of *Tenagomysis chiltoni* and *T. novaezealandiae* were collected from 15 (open and intermittently-open) estuaries along the Otago coast of southern New Zealand (Fig 1.1 and Table 5.1) in 2011–12 during their main breeding seasons in the austral spring and autumn (Lill *et al.* 2010). Upstream and downstream reaches of each estuary were sampled. Gravid *T. chiltoni* females were very sparse in autumn compared to spring so were not included in any data analysis. Gravid females (irrespective of species) were also less abundant in permanent rather than in intermittently open estuaries, hence sampling effort was
increased accordingly to ensure near even numbers of gravid females were collected at each site. At the sampling sites, individuals were immediately fixed in formalin (10%), transported to the laboratory in 1 L sampling bottles, and stored at -20°C for later processing. Further to have some estimation of possible relationships between estuarine productivity and the fecundity of mysids, water samples from each site (i.e. randomly ten samples of total nitrogen and total phosphorus were taken, see Fig 5.1) were collected in 150 ml Falcon tubes and subsequently analysed to estimate the Total Nitrogen (TN) and Total Phosphorus (TP). Water samples were also stored at -20°C for later processing.

Table 5.1 Sampled estuaries and catchment land-use patterns (%). Estuaries are arranged from the north to south of Otago region in the southern New Zealand.

<table>
<thead>
<tr>
<th>Estuaries</th>
<th>Character</th>
<th>Agriculture</th>
<th>Forest</th>
<th>Urban</th>
<th>Water</th>
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</tbody>
</table>
5.3.2 Laboratory work

5.3.2.1 Estimating fecundity of mysids

Population fecundity estimates (i.e. ‘brood’) were based on a randomly selected sub-sample of gravid individuals from each sampling site within and across estuaries. Individuals were first sorted into species (Jocque and Blom 2009), dissected, and all eggs, larvae, and eyed larvae inside the brood pouch were counted. All eggs, larvae, and eyed larvae were then summed to estimate the ‘brood’ of each individual mysid. Standard length (SL= mid of eye stock to end of 6th abdominal somite) of these individuals was recorded (± 0.01 mm) under a microscope fitted with an eyepiece micrometer. Later the relationship between the maternal body size and brood size was measured for each species between seasons among estuaries. Initially egg sizes were also measured for individuals of each species collected from the sampled respective sample sites. This was done to evaluate relationships between egg size vs. brood and body size across seasons and between estuaries. However such relationship yielded no patterns (in trial statistical analysis), so were not carried out extensively, nor included in the final data analysis and discussion.

5.3.2.2 Indication of estuarine productivity from water quality and GIS analysis

At the laboratory, I used a Skalar SANPlus segmented flow analyser (Skalar Analytical B.V., Breda, The Netherlands) to estimate the total nitrogen (TN) and total phosphorus (TP) concentrations (µg/L) water sample collected from sampling sites. I followed established methods of Valderrama (1981) and Ebina et al. (1983) for such analysis. Results were used for a rough indication (because point estimates are not as reliable as continuous measurements) of nutrient enrichment and productivity of each site within and across estuaries. As Otago estuaries are not continuously monitored for their nutrient dynamics, such data are potentially illusive. Therefore I used the prevailing patterns of land use within the catchment of each estuary as proxy to estimate possible nutrient levels. I gathered such information using a GIS approach. During sampling, the location of each sampling site was identified with a handheld GPS device (GRAMINMAPS 60CX, Kansas USA). These GPS points were used to manually draw the catchments upstream of each sampled estuary by using Google Earth 7 (www.google.com/earth, March 2013). Furthermore, the catchment land use and estuary mouth condition (open or closed) were recorded during the sampling time as well as from the databases of the Ministry for the Environment (www.mfe.govt.nz,
Fecundity of mysids

March 2013) and ‘Land Information New Zealand’ (www.linz.govt.nz, February 2013). Different land use classifications (Table 5.1) were combined into four broader land-use categories, namely native forest, agricultural land, urban, and industrial, for their influence on estuarine productivity. All these analyses were completed in ArcGIS education version 10. All of these can only be taken as supportive evidence of the likely productivity of Otago estuaries that gravids of T. chiltoni and T. novaezealandiae inhabit.

5.3.2.3 Statistical analysis

Mixed effect generalised linear models (GLMM) were used to evaluate if fecundity (‘brood size’ = number of (eggs + larvae) inside the brood pouch) of T. chiltoni and T. novaezealandiae vary with season (‘spring’ and ‘summer’), maternal body size (mm), and estuary type (‘open’ or ‘intermittently open/closed’). I used ‘estuary type’ as a proxy variable to indicate productivity (for an indication of productivity GIS and water sample analysis were used together as a proxy variable). While doing GLMs, the response variable ‘Brood Size’ exhibited an over-dispersed Poisson distribution. Further a random effect was needed on the intercept of GLMs for the hierarchical nature of data. Therefore a mixed effect GLM was necessary with quasi-Poisson family which would have been ideal for analysis. In absence of such a function in statistical software R.3.0.1 for Windows (R Development Core Team 2014), I had to use a negative binomial distribution. This was done with rationale that negative binomial family approximates a quasi-Poisson distribution, where the extra parameter corresponds to the scale parameter in a quasi-Poisson model. I used ADMB package compatible with R.3.0.1 for Windows. Models were compared with each other by following AIC model selection statistics and a summary of which is presented in Table 5.2 (Table 5.2 includes only 1st three probable models for each species, which are picked based on guidance of Brunham and Anderson (2002)). For testing the relationship between hydrological parameters and body size I again used the lme4 package and mixed effect GLMs, but this time with ‘Gaussian’ family. I further used linear models (LMs) to analyse variation in body size between seasons and types of estuaries. Too few mysids were caught from the Clutha River, hence this system was not considered for the analysis. Similarly T. chiltoni females caught during autumn were too few (a total of only four gravids were caught from 15 estuaries sampled), so the comparison between autumn and spring cohorts of T.
chiltoni was not done. All the confidence intervals were set at 95% and analyses were performed using R.3.0.1 for Windows.

5.4 Results

5.4.1 Possible indication of productivity in estuaries

Of the 15 estuaries sampled, 7 were open and 8 were intermittently open/closed (Fig 1.1 and Table 5.1). GIS analysis revealed the catchment land uses of the sampled estuaries to be dominated (> 50%) by agricultural activities, and the catchments of intermittently open/close estuaries e.g. Tomahawk Lagoon (14%), Hawkesbury Lagoon (11%) and Kaikorai Lagoon (13%) had a significant urban component (Table 5.1). In autumn the TN level of intermittently open/closed estuaries 887.9 ± 542.4 (SD) (µg/L) which was almost two times higher than for open estuaries at 461.94 ± 152.2 (SD) and so was the TP level (µg/L) (i.e. 103.6 ± 90.2 (SD) and 56.06 ± 41.3 (SD) respectively) (Fig 5.1, Table 5.1). In spring the TN level (µg/L) of intermittently open/closed estuaries was 1167.06 ± 602.56 (SD) and for open estuaries it was 359.13 ± 228.94 (SD); TP levels (µg/L) were 99.37 ± 68.75 (SD) and 43.26 ± 40.02 (SD) respectively (Fig 5.1).

5.4.2 Body and brood sizes between seasons and estuary types

Body sizes of the sampled gravid T. chiltoni (11.94 ± 2.11 mm; only the spring sample) were significantly larger (lm (Body Size ~ Species), slope \( T_{ch} \) = 4.08, 0.18 (SE), \( t = 21.75, P < 0.001 \)) than those of T. novaezealandiae (7.85 ±1.07 mm) individuals (Fig 5.2, 5.3). Brood sizes of T. chiltoni ranged from 1 to 25 (median = 14.5) in open estuaries, and from 3 to 39 (median = 18) in intermittently open/closed estuaries (Fig 5.3, 5.4). Samples (i.e. gravid T. chiltoni) collected from intermittently open/closed estuaries had significantly larger brood size if their body sizes were larger (intercept = 4.17 (1.57 SE), slope = 1.12 (1.03 SE), \( Z = 3.87, P = 0.00011 \)), but this trend was not evident (\( Z = 0.22, P = 0.82 \)) in case the samples were collected from open estuaries (Fig 5.5). When GLMs were compared with each other based on AIC method, it is the estuary type (i.e. a proxy variable for the possible nutrient load of an estuary) which emerged as the most parsimonious model (\( \Delta \text{QAICc} = 0 \)) (Table 5.2).
The mean body size of gravid *T. novaezealandiae* was 8.03 ± 1.21 mm in spring, but only 7.50 ± 0.63 mm in autumn which was significantly different (t = 6.16, \( P < 0.01 \)) (Fig 5.2, 5.3). For *T. novaezealandiae* in open estuaries brood size ranged from 1 to 30 (median = 9) in spring and 1 to 14 (median = 5.5) in autumn, whereas the ranges in intermittently open/closed estuaries were 1 to 35 (median = 12) in spring, and 0 to 23 (median = 8) in autumn (Fig 5.3, 5.4). Individuals of *T. novaezealandiae* that were collected from intermittently open/closed estuaries during spring had significantly positive relationships (intercept = 4.52 (1.41 SE), slope = 1.14 (1.03 SE), \( Z = 3.56, \ P = 0.00038 \)) for their brood vs. body size but his trend was absent (\( Z = 1.65, \ P = 0.09 \)) in case *T. novaezealandiae* were collected from open estuaries (Fig 5.5, 5.6). In autumn, samples that were collected from open estuaries had significantly (intercept = 0.36 (2.03 SE), slope = 1.42 (1.08 SE), \( Z = 4.34, \ P = 0.00014 \)) larger brood sizes if their body sizes were larger but that was not the case (\( Z = 1.33, \ P = 0.18 \)) if they were collected from intermittently open/closed estuaries (Fig 5.5, 5.6). According to AIC method as a predictor estuary type (i.e. a proxy variable for the possible nutrient load of an estuary) was found to be most parsimonious (\( \Delta QAICc = 0 \)) when compared with other GLM models.

Fig 5.1 Indication of total nitrogen (TN) and total phosphorus (TP) levels (µg/L) in the upper and lower reaches of Otago estuaries. Open estuaries: 1 = Kakanui River, 2 = Pleasant River, 3 = Waikouaiti River, 4 = Waitati River, 5 = Taieri River, 6 = Tokomairiro River, 7 = Clutha River; Intermittently open / closed estuaries: 1 = Orore Creek, 2 = Terapuke Creek, 3 = HAwksbury Lagoon, 4 = Tomahawk Lagoon, 5 = Kaikorai Lagoon, 6 = Ocean View, 7 = Brighton Estuary; 8 = Sawmill Creek.

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Fig 5.2 Body sizes of *Tenagomysis chiltoni* and *T. novaezealandiae* in estuaries of the Otago region. Error bars represent standard deviation; for each column number of individual mysids = 20; data not presented for estuaries where samples sizes are too low.
Fig 5.3 Variations in brood and body size of *Tenagomysis chiltoni* and *T. novaezealandiae* in estuaries in the Otago region. Box plots are exhibiting median of brood size but mean of body size (mm); number of open estuaries = 7 and intermittently open/closed estuaries = 8; number of sites within one estuary = 2 (upper and lower reaches of respective estuaries) and number of gravid mysids of each species collected from each site ~ 10.
Fig 5.4 Brood size of *Tenagomysis chiltoni* and *T. novaezealandiae* in the estuaries of the Otago region. Error bars represent variations in brood size of species in an estuary; for each column number of individual mysids = 20; data not presented for estuaries where samples sizes are too low; total Brood Size = sum of (Number of eggs + larvae in different stages of life history) within brood pouch of a gravid mysid.
Fig 5.5 Body vs. brood size of *Tenagomysis chiltoni* and *T. novaezealandiae* during spring in the estuaries of Otago region. Dotted lines represent the regression equations in exponential scale.

Table 5.2 Probable generalized linear models describing the observed variations in brood sizes of *Tenagomysis chiltoni* and *T. novaezealandiae*. $\Delta$ AICc = Quasi-Akaike Information Criterion (corrected); $\Delta$ AICc = difference of QAIC values from the model with least AICc; Season = spring and autumn but only applicable for *T. novaezealandiae* because the main cohort of *T. chiltoni* is in spring only; Estuary Type = open or intermittently open/closed in character; + additive effect.

<table>
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<th>Predictor (s)</th>
<th>$\Delta$QAICc</th>
<th>Model (S)</th>
<th>Predictor (s)</th>
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</tr>
</tbody>
</table>
5.5 Discussion

Body vs. brood size positive relationship was evident if gravid mysids of *T. chiltoni* were collected from intermittently open/close estuaries. Brood and body sizes of gravid *T. novaezealandiae* were larger in spring than in autumn. These results are consistent with mysid and crustacean literature; in temperate regions females of the overwintering cohort often have larger bodies and produce larger broods in spring than autumn cohorts especially if they are in small intermittently open/closed estuaries where nutrient levels can be high after winter (Fukui and Wada 1986; Mees *et al.* 1994, Atkinson 1995, Jeong *et al.* 2007; Yamada *et al.* 2007; Viegas *et al.* 2012). During spring, high levels of nutrients often enable mysid females to reach maturity at their maximum body size consequently they could carry larger broods in their pouch (Fockedey *et al.* 2005; Jeong *et al.* 2007). However *T. novaezealandiae*, if collected from intermittently open/closed estuaries in autumn carried smaller broods than...
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Mysids in open estuaries. In autumn primary production of intimately open/closed estuaries generally speed up, so is their dependent communities in intermittently open/close estuaries consequently become nitrogen limited (Golber *et al.* 2005). In such conditions crustacea, including mysids, may reach maturity earlier but at a smaller size which may limit their brood size, assuming constant egg size (Fockedey *et al.* 2005; Jeong *et al.* 2007; Lill *et al.* 2010). Looking at these results I suggest it is possibly that seasonal change in nutrient levels at specific habitats responsible for variation in relationship of body vs. brood sizes of temperate mysids.

Differences in nutrient levels between open and intermittently open/closed estuaries in the Otago region may be partly driven by anthropogenic stresses which may influence the fecundity strategy of *T. chiltoni* and *T. novaezealandiae*. I found intermittently open/closed estuaries in the region had higher nitrogen and phosphorus loads than open estuaries, possibly because of human induced modifications in their catchments (Lill 2010; Bierschenk *et al.* 2012). High nutrient loading in any estuary has the potential to boost brood production, generating more food for mysids, potentially influencing fecundity and life history strategies (Ramarn *et al.* 2012). While sampling I found evidence of a limited second cohort of *T. chiltoni* in few intermittently open/closed estuaries in autumn, i.e. Kaikorai Lagoon and Tomahawk Lagoon, These two estuaries are often eutrophic due to run-off from urban and intensively managed agricultural area (Lill 2010). In South African nutrient enriched intermittently open/closed estuaries, a large zooplankton biomass can be generated by individuals of various species reaching maturity earlier at a smaller body size in warm conditions (Drake *et al.* 2002; Froneman 2003). Similar to my survey, Lill *et al.* (2010) and Jones *et al.* (1989) in their limited field survey at Kaikorai Lagoon and Avon Heathcote Estuary found that the spring cohorts of *T. novaezealandiae* are larger in body size but may delay maturity. Whereas the autumn cohorts reach maturity earlier than spring cohorts, especially if they are from nutrient rich intermittently open/closed estuaries but at smaller body size (Lill *et al.* 2010). Thus I suggest it is possible that by taking advantage the nutrient rich conditions of small intermittently open/closed estuaries in warmer seasons *T. novaezealandiae* reaches maturity early to spawn more frequently but with a smaller brood in their pouch. However it is possible that reduced individual fecundity is compensated by high spawning frequency resulting in a high population density in some estuaries of the Otago region (Lill *et al.* 2010).
Like numerous other ectotherms in temperate regions worldwide, the life history strategies of *T. chiltoni* and *T. novaezealandiae* depend on habitat condition (Conover 1992; Stren 1992; Lill *et al.* 2010). Previous study showed that *T. chiltoni* has the potential to grow faster than *T. novaezealandiae* if the environment in their microhabitats is similar (Paul *et al.* 2014). Despite that *T. chiltoni* has a longer growth period to maximum body (6 to 8 months) than *T. novaezealandiae* (3 to 4 months), and generally has only a single (occasional two) breeding season in late spring (Lill *et al.* 2010). It could be the variation of severity of winter survival in temperate (Conover 1992; Atkinson 1995) estuaries which may be forcing these two species to adopt contrasting fecundity strategies. Winter survival is possibly more difficult for *T. chiltoni* than for *T. novaezealandiae* as the former occupies habitats that are more hypo-saline and struggles for osmoregulation if conditions are cooler (Paul *et al.* 2013). Thus a greater focus on winter survival is needed by *T. chiltoni*; one possible way out to do that is by attaining a large body size (Gotthard 2001) but this requires delayed maturity and reproduction until usually in spring (Gotthard 2001). Such a constrained life cycle precludes a second generation for *T. chiltoni* within the same year unless estuaries are warm and particularly productive. This life cycle is not uncommon among mysids (e.g. *Mysis relicta, Neomysis* spp.) which inhabit estuaries in higher latitudes (Mees *et al.* 1994; Vainola and Vainio 1998). However, I suggest that *T. chiltoni* females can to some degree possibly compensate for the constrained spawning opportunities by producing an increased number of eggs per individual given their larger size at maturity (see Smith and Fretwell 1974; Stern 1992; Shine 1988; Jones *et al.* 1989; Lill *et al.* 2010).

In temperate regions, animals often have discrete cohorts because of short and season-specific breeding periods, prolonged growth, and large size body at maturity (Atkinson 1995; Gotthard 2001; Jeong *et al.* 2007). Around the world temperate mysids such as *Neomysis* spp., *Mysis relicta, Tenagomysis* spp. show breeding patterns with close similarities to those of *T. chiltoni* and *T. novaezealandiae*, where mysids in warmer seasons reach maturity at smaller body size to spawn early (Toda *et al.* 1982; Jones *et al.* 1989; Fenton 1992; Mees *et al.* 1994; Vainola and Vainio 1998; Fockedey *et al.* 2005; Yamada *et al.* 2007; Lill *et al.* 2010). However, the actual mechanisms that result in single or multiple cohorts per year are rarely considered. I suggest that such patterns could be the combination of more than one factor. For example, inefficient osmoregulation in winter may prevent mysid spawning in cold conditions (Paul *et al.* 2013). However, if the estuaries are nutrient rich, then such
physiological restrictions on fecundity may some extent be lessen, giving mysids chances to go for multiple cohorts with smaller body and brood sizes. I predict that more frequent spawning by *T. chiltoni* and *T. novaezealandiae* will occur at lower, warmer latitudes, a prediction that could be readily tested by comparison of populations along extended latitude.
"After climbing a great hill, one only finds that there are many more hills to climb." - Nelson Mandela

Chapter 6: General Discussion

6.1 Key findings of the thesis

My research aimed to present an eco-physiological perspective on the dominant taxa of the southern New Zealand estuarine zooplankton community, i.e. mysids of Tenagomysis spp.

- Chapter 1, introduced concepts of how contrasting estuarine environments can force species to adjust their physiology and life histories for ecological success, with particular focus on mysids in cold temperate estuaries. For exploring the topic I’ve taken examples of the two mysid species of Tenagomysis spp., which inhabit a wide range (where spatial-temporal gradients of environments are contrasting) of estuaries in New Zealand.

- Chapter 2 showed that the salinity tolerance and osmoregulation of T. chiltoni and T. novaezealandiae may be poor under winter conditions, especially if the environment is hypo-saline, causing high mortality of juveniles, but adults were well adapted to the environmental changes if these were not too extreme. This result demonstrates the varying vulnerability of different life history stages of mysids which may arise from seasonal cycles. Simultaneously it shows the resistance of adults (or larger body size) to the chronic changes in estuarine environment that might indicate the lesser vulnerability of mysids inhabiting temperate estuaries around the world if the climate-mediated changes are not too abrupt.

- The experiment described in Chapter 3 showed respiratory/metabolic physiology of adult T. chiltoni and T. novaezealandiae can acclimatise well against within limited salinity and thermal gradients, and the metabolic advantage is well pronounced if a mysid body is larger than average.
Chapter 4 showed that increasing salinity limits the growth of *T. chiltoni*, whereas low quality (lower Carbon: Nitrogen %) of food limits growth of *T. novaezealandia*, and *T. chiltoni* grew faster than *T. novaezealandia* when the conditions are similar. Life history work by Lill *et al.* (2010) on these mysids showed their different life history strategies; for example, *T. novaezealandia* reach early maturity to reproduce multiple times within a year compared to *T. chiltoni*. This experiment showed that despite physiological similarities, contrasting microhabitats within temperate estuaries may force mysids to follow different growth trajectories which may have implications for their ecology.

The field survey in Chapter 5 found that, compared to large open estuaries the small intermittently open/closed estuaries of the Otago region are rich in nutrients (especially nitrogen), possibly a result of high agricultural and some urban runoff from catchments and limited marine exchange. Such contrasting environments may be responsible for the variation of growth and maturity time of mysids and the maximum body sizes which mysids can attain in a particular season. Variation in body size possibly contributes to the variations of brood carrying capacity of gravid *T. chiltoni* and *T. novaezealandia*. However to overcome the possible constraints of preferred micro-habitats *T. chiltoni* and *T. novaezealandia* possibly adjust to different fecundity strategies for maintaining their populations.

This final chapter (Chapter 6) attempts to integrate the preceding results into an eco-physiological model of life-history strategies of mysids in temperate estuaries with respect to latitudinal change. A qualitative review of myid life histories indicates that mysids from higher latitudes are possibly larger in body size than tropical mysids but unlike tropical mysids which breed throughout their life cycle, temperate mysids can only afford limited number of cohorts.
6.2 Life history and distribution of *T. chiltoni* and *T. novaezealandiae*

Tenagomysis chiltoni and *T. novaezealandiae* may be more vulnerable in winter because of poor osmoregulation ability than in summer, especially in hypo-saline habitats; this possibly affects their life histories. They have a synchronized life cycle, so that growth, maturity and reproduction are restricted to warmer seasons in New Zealand (Lill *et al.* 2010); this is typical of numerous ectotherms in temperate latitudes (Stern 1992). For example, *T. chiltoni* generally reaches maturity (and large body size) before winter (Lill *et al.* 2010). Their large body may represent an adaptation for ‘winter survival’ because it reduces per unit mass metabolic expenditure. Consequently, that can buffer the high energetic costs of osmoregulation in a cold, hypo-saline environment which *T. chiltoni* often inhabits (Jones *et al.* 1989; Lill *et al.* 2010). The limited osmoregulatory ability of *T. chiltoni* in cold fresh water habitats suggests a physiological constraint in its early life history (i.e. high juvenile mortality). Juveniles need a longer growth period in summer to ensure a large body for winter survival and efficient osmoregulation (Nielsen 1997). The physiological advantages of the larger body of *T. chiltoni* possibly explain its dominance, albeit at relatively low densities, in tidal lakes (e.g. Lake Waihola) and hypo-saline reaches of open estuaries (e.g. Avon Heathcote Estuary, Taieri River, Tokomairiro River), where *T. novaezealandiae* is generally absent (Jones *et al.* 1989; Wilhelm *et al.* 2002; Lill *et al.* 2011). Further, a large body has an associated benefit of allowing for greater individual fecundity, thus possibly compensating for a limited reproduction time (Toda *et al.* 1984; Mees *et al.* 1994; Lill *et al.* 2010). However, less reproduction time may have consequences for distribution in the face of intense competition from species like *T. novaezealandiae* that has an overlapping niche, and might be a reason why *T. chiltoni* is less frequent in lower reaches of estuaries (Stren 1992; Krebs 2009; Lill *et al.* 2010).

In the lower reaches of estuaries *T. novaezealandiae* reaches maturity earlier and at a smaller body size (Lill *et al.* 2010), a common life-history strategy that results in faster growth to maturity and early reproduction (Stern 1992; Atkinson 1995; Gotthard 2001). Mysids from warm nutrient-rich estuaries at lower latitudes can use this strategy given that osmoregulation across a wider range of conditions is possible; however it becomes severely limiting when cold conditions are encountered (Mees *et al.* 1994; Lill *et al.* 2010; Paul *et al.* 2013). *Tenagomysis chiltoni* can possibly adopt a similar life history strategy to that of *T.
Tenagomysis novaezealandiae may be compensating for low individual fecundity by achieving multiple cohorts every year, so maximising the reproductive output of the entire population; this can be seen as a ‘fecundity advantage’ (Shine 1988; Lill et al. 2010). ‘Fecundity advantage’ may be behind their overwhelming density in local estuaries and they may exclude *T. chiltoni* from lower reaches by this competitive mechanism (Lill et al. 2010), a hypothesis worth exploring in the future. *T. novaezealandiae* thrives in brackish environments in mid to lower reaches of permanently open estuaries (e.g. Taieri River, Kakanui River, Tokomairiro River) and in intermittently open estuaries (Brighton Estuary, Sawmill Creek, Terapuke Creek, Tomahawk Lagoon) in the Otago region (Jones et al. 1989; Lill et al. 2011). In summer-autumn when osmoregulation is relatively easier than in winter, survival chances remain high (Paul et al. 2013). With the possibilities of faster growth and more reproduction in warmer seasons (Toda et al. 1982), they boost their density and broaden their distribution (Jones et al. 1989; Lill et al. 2010), even at times venturing into the upper reaches of estuaries in southern New Zealand (Lill et al. 2010). However, they are excluded from these cold hypo-saline habitats when temperatures are low over winter.

In conclusion, from the physiology and life histories of *T. chiltoni* and *T. novaezealandiae*, I suggest their distribution may depend on the physiological tolerances and species-specific features of their life histories. Larger body size and the sustained energetic advantage that comes with it, possibly provides *T. chiltoni* with an ecological advantage over *T. novaezealandiae* in the upper reaches of open estuaries. However, the latter, due to its fast paced life-history strategies, may be better adapted to small coastal ecosystems in southern New Zealand. Fig 6.1 summarises the possible life-history strategies of these mysids in their respective habitats, taking into consideration the regional conditions of estuaries and seasons that are typical to South Island, New Zealand.
6.3 Life history and distribution of mysids in temperate estuaries

Life cycles in temperate climates may pose restrictions on the distribution of aquatic ectotherms such as fish and crustaceans because their growth, maturity and reproduction are often synchronised with warmer seasons (Stern 1992; Atkinson 1995). Various authors have speculated that a prolonged growth period is needed to grow a large body that can buffer against extreme winter conditions and an increased risk of mortality (Roff 1992; Stearn 1992; Atkinson 1995). However, exactly how a large body might buffer against extreme winter conditions is not always considered, especially in estuaries. The present study shows that efficient osmoregulation in hypo-saline or brackish water can be difficult under winder conditions, and can contribute to high mortality. This could be a limitation on successful completion of the life history in estuaries in higher latitudes. A large body in that case can buffer against some energetic costs of osmoregulation, so individuals may be less constrained for survival, but still incur a toll on fecundity because their reproductive seasons are shortened (Conover 1992; Stern 1992; Gotthard 2001).
Fig 6.1 Possible life histories of mysids in brackish (A) and/or hypo-saline habitats (B). The thematic diagrams are drawn based on the observed life cycle of *Tenagomysis* spp. (Seasonal duration represents the typical annual climate in the South Island, New Zealand).
Temperate mysids share similarities in growth patterns, time to maturity, and breeding seasons (see Toda et al. 1984; Fenton 1992; Mees et al. 1994; Fockedey et al. 2005, Lill et al. 2010). However, no unified explanation of these commonalities can be found in the mysid literature. I suggest that osmoregulation at cold temperatures could be a constraint. Consequently, mysids and other ectotherms at higher latitudes achieve winter survival by growing large. The higher the latitude is, the longer growth period required for growing a large body size (Mees et al. 1994; Vainola and Vainio 1998); this results in a longer time to maturity and time-constrained reproductive periods (Stern 1992; Atkinson 1995; Gotthard 2001). If that is plausible, then at very high latitudes e.g. Arctic region, the breeding period may not be long enough for maintaining a viable population (Stern 1992; Gotthard 2001), even with large bodied females that can carry a large number of eggs. Based on this scenario, I suggest that mysids and similar crustaceans may have a limited distribution in estuaries at higher latitudes, especially in winter, although some unusual mysids, such as Mysis relicta, can survive in these habitats.

Mysids (like Mysis relicta) in higher latitudes may adjust to a multi-year life cycle, with prolonged growth in the first season and then reproduction, but that requires specialised physiology for osmoregulation at low temperatures (Hakala 1978; Vainola and Vainio 1998). Also, a multi-year life cycle may increase the risk of predation from fish and larger macro-invertebrates that prey on mysids (Mauchline 1980; Stern 1992; Silby and Atkinson 1995; Roast et al. 1998; Gotthard 2001). Such a life-history strategy may be too risky and I am not aware of many mysids which have such a strategy. However, a prerequisite of such a life history could be an osmoregulatory physiology that is cold-tolerant (Pe’queux 1995; Nielsen 1997). Mysids and other estuarine crustaceans are generally euryhaline (Charmantier 1996), but the synergistic effects of temperature-salinity often makes osmoregulation harder under cold conditions which may inflict high mortality among estuarine mysids (Paul et al. 2013). As estuarine crustaceans often have a marine origin, their osmoregulatory capacity is most often limited in brackish and or fresh water habitats (for example efficiency of enzymes like Na+/K+-ATPase and membrane permeability can vary with changes in salinity and temperature) (Mauchline 1980; Towle 1997; Lee and Bell 1999). As a result, I suggest that they will possibly struggle to inhabit higher latitudes if the estuaries are / become less saline. Future research on the molecular mechanism of osmoregulation of mysids under challenging osmotic conditions would be interesting and potentially advance understanding of mysid life cycle evolution significantly.
A warmer environment possibly presents fewer osmoregulatory challenges for mysids, so may facilitate more life history and distributional opportunities, a pattern also applicable to similar crustaceans (Bhattacharaya 1982; Web et al. 1997; Vilas et al. 2009). The life histories of mysids and other planktonic crustaceans in tropical estuaries in India and Malaysia, and in warm temperate estuaries of South Africa, Japan and Australia have similarities. For example, they often have faster growth to maturity at smaller size, and continuous breeding, leading to multiple cohorts every year (Winkler and Greve 2002; Hanamura et al. 2009). A possible explanation is less restriction on osmoregulation and reduction in the challenges of winter survival. In addition, the higher productivity of such habitats can support greater densities of zooplankton such as mysids, which is often the case in many estuaries in South Africa (Drake et al. 2002). But such conditions are rare at higher latitudes, except during spring–autumn periods when estuaries remain nutrient rich (Lill 2010). For these reasons I suggest that, regionally, it is not salinity alone, but temperature interacting with salinity which controls mysid and other zooplankton distributions in temperate estuaries. In future, more inclusive and multivariate research regionally would be useful for vulnerability assessment of temperate estuarine communities.

6.4 Future of mysid communities in the region

Locally seasonal fluctuations, anthropogenic stresses, and climate change together have implications for the physiology of estuarine populations which can be non-linear at times so hard to predict (Kennish 1992; Maes et al. 1998; Flemer and Champ 2006; Lotze et al. 2006; IPCC 2007). For example, in southern New Zealand, the Otago region may experience a temperature increase of 0.6 to 1.1°C by 2040 (www.mfe.govt.nz), and possible sea-level rise of 18 to 59 cm by 2100 (www.niwa.co.nz). Synergistic changes in seasonal temperature and salinity, anthropogenic stresses such as nutrient runoffs, release of hot/cold water, sedimentation due to development activities and dredging together with climatic changes are possibly shifting microhabitats of estuarine mysids towards extreme (Woolridge 1991; Attrill 2002; Froneman 2003; Thrush et al. 2004, 2008). It will not be surprising it the ecophysiological adjustment of mysids and other crustaceans under such stressful environment are non-linear (Thrush et al. 2004, 2008), with possibilities of population collapse. This thesis has shown that the synergistic effects of salinity, temperature and
nutrient loads on *Tenagomysis* spp. could affect osmoregulation, survival, growth and fecundity of *Tenagomysis* spp. in estuaries of the Otago region of southern New Zealand. In that scenario, I predict to see changes favouring more *T. novaezealandiae* perhaps because of increased eutrophication and temperatures seem to suit that species, which may give *T. chiltoni* a tougher inter-specific competition than at present. Unfortunately we often have estuarine models which are too abstract, limited research at the regional scale (Lill 2010), and a lack of multivariate research at the local scale. These major gaps in our understanding raise serious concerns about the fate of mysid communities in the region.

6.5 Limitations and future work on mysids

Unlike temperate Northern Hemisphere (Mckenny and Celetal 1995; Fockey et al. 2005, 2006), South Africa (Marshall *et al.* 2003), New Zealand mysid communities have never been studied from an ecophysiological angle. This led me to initially predict that salinity is the dominant variable responsible for mysid distribution in New Zealand estuaries. Chapter 1 provided the context of the habitats and the properties of the taxa I studied. However the absence of water quality data of New Zealand estuaries has made it difficult to pin down the exact spatial-temporal environment which mysids face and or are likely to face. Thus I recommend monitoring of salinity, temperature, dissolved oxygen level and preparation of nutrient map by studying nutrients in relation to mysid abundance (Hagy *et al.* 2004; Johnes 2007). Patterns of productivity in relation to salinity, temperature, latitude, catchment land uses, connection to the sea and biodiversity richness would advance understanding of the role, food supply and quality may play in determining mysid communities. Estuarine-ecology research in New Zealand is relatively new, so understanding of ecological relationships is limited (Thrush *et al.* 2004, 2008; Lill 2010, Lill *et al.* 2013); my thesis is just a beginning, and long term environmental monitoring of New Zealand estuaries will encourage future researchers to explore relationships between environment and community structure in New Zealand estuaries.

In Chapter 2 I found osmoregulation is difficult for mysids if the conditions are hypo saline and cold. This may have profound effect among large number of estuarine animals (Lee and Bell 1999; Sanchez-Fernandez 2010), which may have wide implications commercial coastal fisheries as mysids and great many zooplankton are often important food web elements
(Roast et al. 1998). There was an indication that with increasing body size the demands of osmoregulation are reduced so the large adults survive much better than juveniles in cold hypo saline environments. Thermal acclimatisation studies have been used to explain distribution of mysids (McKenney and Celestial 1995; Yamada et al. 2007; Penk and Minchin 2014), but I suggest a laboratory study on osmoregulation under controlled temperatures with mysids across a range of body size to find out the critical size at which the osmoregulatory constraints would be useful. Such study can further build up under varying nutritional environment which might affect the work efficiency of molecular enzymes such Na+/K+ ATPase, and membrane permeability as evident in fresh water shrimps, crabs and prawns and of interest for aquaculture (Furriel et al. 2000; Romano et al. 2012; Garçon et al. 2013).

In Chapter 3 and 4, I considered energetics and growth of mysids; however the experiments are short in nature and level of acclimatisation did vary (McKenney and Celestial 1995, Fockedey et al. 2005, 2006; Vilas et al. 2006). For example numerous crustacean literatures, including mine, considered respirometry work in captive environment which are far from reality (Newell and Branch 1980; Emmerson 1985; Marshal et al. 2003; Vilas et al. 2006). More precise measurement of oxygen consumption across a range of activity levels and conditions would provide insights into how much energy is being consumed across their entire life-cycle, with implication for long term effects on growth and or fecundity of individual mysids (Torres et al. 2011). Results can be cross checked with field surveys as conducted by Fenton (1996) and Lill et al. (2011), which looked into growth rates by studying different age structure of mysid populations. In situ cage field experiments examining competition between different mysid species in contrasting conditions could reveal the long-term impacts of contrasting life history strategies on inter-specific population dynamics, with implications for our understanding of the contribution of mysids to estuarine ecology, coastal fisheries and aquaculture (Roast et al. 1998).

In Chapter 5, I have only partially answered why, despite its larger body and higher individual fecundity, *T. chiltoni* fails to overwhelm small bodied *T. novaezealandiae* in lower reaches of New Zealand estuaries (Lill et al. 2010, 2011; Paul et al. 2013, 2014). However the hypothesis that the ‘extreme high density of *T. novaezealandiae* (Lill et al. 2011) is preventing *T. chiltoni* to successfully colonise lower reaches of southern New Zealand estuaries’ can be explored through agent based models under simulated environments
(Batchelder and Miller 1989; Cianelli et al. 2012) to look at competitive exclusion scenarios. From Chapter 2 and 4 one can calculate growth and survival rates of *Tenagomysis* spp. which can be checked with growth rates from the field studies of Lill *et al.* (2011) and Fenton (1996). Fecundity and body size relationships are also available in Chapter 5. All of these can be integrated as properties of the model agents. Tentative temperature scenarios can be derived from IPCC 2007 global climate prediction models applicable for South Island of New Zealand. An extensive field survey on the population density, age stratification needs to be done across the region to ratify the findings of the plausible models. Thus far I have not found any such comparable work on mysids worldwide.

Chapter 6 hints that latitude may be responsible for the fecundity strategies, life span, and body size of temperate mysids worldwide; however present the literature is too limited to complete such an analysis (Miyashita and Danil 2014). A quantitative meta-analysis on mysid distribution across latitudinal gradients in that case would be useful, and such literature exists on estuarine fish, crabs, and copepods but not on mysids (Hines 1982; Sainte-Marie 1991; Conover 1992). Thus the priority is to take more regional scale mysid research like Chapter 5 of my thesis or the work of (Miyashita and Danil 2014) which can be integrated later. Quantitative data on life histories of tropical and subtropical mysids e.g. *Mysidopsis* spp. (Bhattacharaya 1982), temperate mysids e.g. *Neomysis* spp., *Tenagomysis* spp. (Fenton 1992; Vilas *et al.* 2006; Fockedey *et al.* 2005, 2006; Lill *et al.* 2011), and even from arctic e.g. *Mysis relicta* (Vainola and Vainio 1998) will help to build unified theory of how and why mysids are adjusting to different body sizes at maturity, juvenile release time, and number of cohorts per year. I hypothesized that at higher latitudes mysids will produce less number of cohorts per year but be larger in body to overcome ‘winter mortality’.
References:


References


References


