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The physiological ecology of the specialist lagoon amphipod, *Gammarus insensibilis*

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ABSTRACT

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THE PHYSIOLOGICAL ECOLOGY OF THE SPECIALIST LAGOON AMPHIPOD, *GAMMARUS INSENSIBILIS*

By Andrew Russell Gates

Coastal lagoons are habitats of conservation importance. The characteristic fauna of lagoons includes a number of specialist species, some of which are scheduled for protection. Work on the conservation of coastal lagoons has suggested that detailed information on the ecology of lagoon specialist species is essential to ensure management strategies are relevant to the lagoonal species. This study addresses this issue by providing information about the monthly reproductive investment and energy-balance as well as interactions with parasites for the specialist lagoon amphipod, *Gammarus insensibilis* (Stock). For comparison information is also presented on the reproductive investment of the lagoonal isopod *Idotea chelipes* (Pallas).

Gilkicker lagoon on the south coast of England, UK, from which the majority of samples were taken, demonstrated environmental variability characteristic of coastal lagoons. Annually, temperature varied between 2 and 28 °C and salinity fluctuated between extremes of 24 and 39. The Lymington-Keyhaven lagoons, also on the south coast of England, demonstrated similar variation. This has implications for *Gammarus insensibilis,* and effects on reproductive investment were evident. The amphipod was shown to employ a continuous reproductive strategy but while the overall investment, expressed as clutch volume, remained relatively stable over the course of the year, the individual components varied. In the summer, when weight-specific embryo number was at its highest with a mean of 13 embryos mg dry wt.$^{-1}$ the mean size of the individual embryos was small (0.032 mm^3). The opposite was true in the winter months, with mean brood sizes as low as 6 embryos mg dry wt.$^{-1}$ while mean embryo volume was larger, at 0.04 mm^3. *Idotea chelipes* demonstrated a more seasonal reproductive strategy in which winter reproductive output was low.

The environmental variability also had effects on the metabolic rates of individual amphipods with temperature related increases in feeding and respiration rates in the summer months. Scope For Growth (SFG), a measure of net energy availability to the organism, was variable during the year and was unrelated to temperature and salinity. Lowest SFG occurred in spring in conjunction with peak reproductive output. This suggested that the amphipods were well adapted to the environmental variation of the lagoon and that it was reproductive investment that represented an important cost to the organism.

Eighty four percent of *G. insensibilis* from Gilkicker were shown to be infected by microphallid trematode parasites. Reproductive investment was negatively affected, with a 36.6 % reduction in weight-specific brood size associated with higher degrees of infection. Respiration rates were also reduced in the infected organisms. The high prevalence of the parasites and the related effects on the host suggest that trematode infection should be considered, alongside the characteristic habitat variability, as an important aspect of the ecology of lagoons.

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Graduate School of the National Oceanography Centre, Southampton

PhD. Dissertation

by Andrew Russell Gates

Declaration of Authorship

The work presented in this thesis is my own, and was done wholly whilst in candidature for a research degree at the University of Southampton. Where I have quoted or consulted from the published work of others, the source is always clearly attributed. I have acknowledged all the main sources of help. None of this work has been published before submission.

Signed: ………………………………………………………

Date: ……………………………………………………...

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Chapter 1

General Introduction

1.1. Brief introduction

Saline lagoons are EU priority habitats of international conservation importance. In the UK they provide habitats to a number of scheduled specialist species, found only in lagoons. The nature of these habitats induces high levels of environmental variability, particularly in temperature and salinity, providing a stressful habitat for the essentially subtidal species present. Despite the fact that many of these organisms are scheduled, very little is known about their biology and ecology, so relevant conservation measures are not implemented. This study examined populations of the specialist lagoon amphipod *Gammarus insensibilis* Stock, 1966 and to a lesser extent the lagoon isopod *Idotea chelipes* (Pallas, 1772) in order to provide information on these organisms in order to provide data to implement and support lagoon conservation.

1.2. Lagoons

This study focuses on organisms from coastal lagoons in the UK. However, the term lagoon refers to two features of the marine environment; coastal lagoons and lagoons associated with coral reefs or atolls. These two features are formed in different ways and found in different geographical regions.

Coastal lagoons are defined as shallow inland marine waters, usually orientated parallel to the coast, separated from the ocean by a barrier, but connected to it by one or more restricted inlets (Castanares and Phleger 1969), a similar definition is offered by Kjerfve (1994). Lagoons associated with coral reefs or atolls are also bodies of water separated from the ocean by a barrier; the barrier in this case being the reef or atoll itself. In order to differentiate between coastal lagoons and those associated with coral reefs and atolls, (Bird 1994) extends the definition, defining coastal lagoons as areas of relatively shallow water that have been partly or wholly separated from the sea by the formation of depositional barriers, usually of sand or shingle, built up above high tide level by wave action. The most important difference between coastal lagoons and coral-associated lagoons is demonstrated by their complimentary geographic distribution (Barnes 1980). Coastal lagoons are dependent on the deposition of sediment to form the enclosing barrier. The deposited material essential to the presence of coastal lagoons would prevent the growth of corals, which require firm substratum and sediment free water.

The two types of lagoon have different characteristics relating to how they are formed. Lagoons associated with coral systems are linked to the open sea to a greater extent than coastal lagoons. The barrier is overtopped by seawater at virtually all states of the tide because reef forming corals are intolerant of prolonged exposure to the air. Coral lagoons must be considered as an integrated part of the overall reef system because of this exchange, whereas coastal lagoons can be studied as separate systems in their own right (Barnes 1980).

Although coastal lagoons are found throughout the world, they are most common in the north Atlantic. The high-energy, macrotidal seas mean that they are not enclosed by sand barriers, like elsewhere in the world, but are formed from the offshore shingle deposited after past glacial action. Frequently North Atlantic coastal lagoons have no inlet/outlet channel so rely on overtopping and percolation for exchange (Barnes 1991).

1.2.1. The coastal lagoon environment

There are several important factors that influence the environment of coastal lagoons. Most importantly they are separated from the sea by a barrier. Coastal lagoons are therefore subject to reduced tidal influence meaning that they are essentially sublittoral habitats. They are generally small. In England the largest lagoon is The Fleet in Dorset which is 480 ha in area, more typically they are much smaller with the average size being 10 ha (Bamber et al. 2000). Lagoons of important conservation concern are often smaller again, for example Gilkicker lagoon in Hamphire, England is 3.7 ha (Al-Suwailum, 1991), while Swanpool lagoon in Cornwall, England has an area of 8 ha (Dorey et al. 1973).The barrier, along with their small size, means that wave action is also limited. As a result of these factors the organisms in lagoons are protected from both forms of exposure that characterize most near-shore marine habitats. Coastal lagoons are also generally shallow; Gilkicker lagoon does not exceed 0.75 m in depth (Al-Suwailum, 1991). Lagoons therefore have their own characteristic environmental conditions. Their proximity to terrestrial influences and their generally small, shallow nature mean they are subject to greater environmental variation than other sublittoral habitats. Factors such as temperature, salinity, pH, dissolved oxygen and nutrient levels vary within and between lagoons so it is difficult to classify these systems (Barnes 1980; Bamber et al. 2000).

The variability of environmental parameters in coastal lagoons in comparison to the adjacent sea creates a physiologically demanding habitat. Despite this there are organisms found in great numbers in lagoons. Lagoon fauna however, is characterized by low diversity, but those species that are present can be abundant (Bamber et al. 2000), for example 4 species dominate the fauna associated with macrophytes in Gilkicker lagoon with up to counts of up to 265 animals per gram dry weight of plant material. The ability to prosper despite the environmental stress may explain why certain species are abundant where congeners are absent.

Coastal lagoons are listed as priority habitats under the European Union Habitats Directive (Council of the European Communities1992) and are frequently designated as, or lie within, Sites of Special Scientific Interest (SSSI), National Nature Reserves, Ramsar sites and Special Protection Areas (SPAs) (Bamber et al. 2000). Lagoons are of particular conservation importance because they are uncommon habitats (important physiographic features in their own right), and they frequently support plant and animal species and communities absent or rare in other saline habitats, including a number specialist lagoonal species (Barnes 1980; Barnes 1991; Bamber et al. 1992). Specialist lagoon species are those which are “distinctly more characteristic of lagoons and lagoon-like habitats than of other habitats” (Bamber et al. 2000). Resulting from the rarity of lagoons these species tend to be uncommon nationally, there are 13 specialist lagoon species of priority concern under the UK biodiversity action plan.

In addition to their rarity and specialist biota, coastal lagoons also provide important habitats and feeding grounds for many species of seabirds and waders. After decline to the point of extinction as a regularly breeding British species in the 17th Century, *Platalea leucorodia* (spoonbill) breeding activity has increased in the UK since recolonization from the Netherlands and has been closely associated with saline lagoons, flooded marshes and estuaries (Bamber et al. 2000). *Recurvirostra avocetta* (avocet) is more closely associated with coastal lagoons than other coastal habitats (Hill et al. 1993). It was extinct in Britain until it re-colonized in 1947 but in 2000 there were 600 breeding pairs at 23 sites with 80-90% of pairs associated with non-tidal saline lagoons (Bamber et al. 2000). *Larus melanocephalus* (Mediterranean gull), *Sterna albifronus* (little tern) and *Sterna sandvicensis* (sandwich tern) are other birds of conservation concern associated with coastal lagoons (Bamber et al. 2000). This importance of the lagoon habitat to shore birds is highlighted by the creation and management of lagoons by the Royal Society for the Protection of Birds (RSPB), for example at Freiston Shore in Lincolnshire, UK where a lagoon has been created (RSPB 2004).

To ensure the conservation of lagoons is relevant to both the habitat and the biota it is important that the interactions between the two are understood. Although there is some information about the biology, life cycles and reproductive strategies of some lagoon organisms (Karakiri and Nicolaidou 1987; Barnes 1994; Sheader 1996; Porter et al. 2001; Pearson et al. 2002), it is limited. Conservation surveys have been conducted on many coastal lagoons and lagoon systems in the UK, but the overall findings have invariably stated that there is not enough information on the basic biology of the organisms of conservation concern to implement effective management strategies (Bamber et al. 2000; Bamber 2004; Bamber et al. 2004). Detailed studies relating the biology of the animals to the environment are required to fully understand lagoon ecosystems.

1.2.2. Lagoon Organisms

Lagoon fauna comprises six groups of organisms (Bamber et al. 1992); I freshwater species, II stenohaline marine lagoonal specialists, III euryhaline lagoonal specialists, IV estuarine species tolerant of lagoons, V estuarine species incidental in lagoons and VI under-recorded species. With the exception of the lagoon specialists these organisms utilize lagoons opportunistically and, as species, are more successful in their marine, estuarine or freshwater habitats. This study is concerned with the lagoon specialists. Bamber et al. (1992) name 46 species of organism found in studies of lagoons in the United Kingdom and state that 25 of these are lagoon specialists (Table 1.1), although one of these (*Edwardsia ivelli*) may now be extinct. The lagoon specialists are not all found at the same sites and there are seven lagoon specialist biotopes included in the Joint Nature Conservation Committee biotope classification system (Bamber et al. 2000). Biotopes describe the combination of the physical environment and the distinctive assemblages of conspicuous species of any habitat to enable description and comparison of different sites. The different lagoon biotopes provide habitats for different combinations of organisms and are determined by the predominant macrophytes and sediment types. In the classification they are described by the ENLag abbreviation.

Table 1.1: Specialist lagoon species found in UK lagoons, after Bamber et al. (2000)

	Species	**Common name**
Plants		
Chlorophyta	*Chaetomorpha linum*	Green seaweed
	Lamprothamnium papulosum	Foxtail stonewort
	Tolypella nidifica	Bird's nest stonewort
Tracheophyta	*Ruppia maritima*	Beaked tasselweed
	Ruppia cirrosa	Spiral tasselweed
Animals		
Cnidaria	*Clavopsella navis*	A hydroid
	Edwardsia ivelli	Ivell's sea anemone
	Nematostella vectensis	Starlet sea anemone
Bryozoa	*Conopeum seurati*	Lagoon sea mat
	Victorella pavida	Trembling sea mat
Annelida	*Armandia cirrhosa*	Lagoon sandworm
Mollusca	*Hydrobia ventrosa*	Lagoon mud snail
	Hydrobia acuta	Neglected lagoon mud snail
	Onoba aculeus	A rissoid snail
	Cerastoderma glaucum	Lagoon cockle
	Caecum armoricum	De Folin's snail
Crustacea	*Cyprideis torosa*	An ostracod
	Idotea chelipes	Lagoon slater
	Lekanosphaera hookeri	An isopod
	Gammarus insensibilis	Lagoon sand shrimp
	Gammarus chevreuxi	An amphipod (sand shrimp)
	Corophium insidiosum	An amphipod
Insecta	*Geranomyia bezzia*	A cranefly
	Glyptotendipes barbipes	A chironomid midge
Aves	*Recurvirostra avocetta*	Avocet

One of the most important interactions in the lagoon environment, and characteristic of habitats conducive to some lagoon specialists is that of the filamentous alga *Chaetomorpha linum* (Chlorophyta) and the peracarid crustaceans found in lagoons (Figure 1.1). The amphipod *Gammarus insensibilis* and the isopod *Idotea chelipes* are commonly associated with the alga, which forms an important component of their diets (Sheader and Sheader 1985; Bamber et al. 2000). Sheader and Sheader (1985) suggest that the enclosed nature of the habitat allows the development and retention of *C. linum*, which forms extensive floating mats (Figure 3.2, p. 42) which host an important contingent of the lagoon fauna including some of the lagoon specialist species (Table 1.2). The presence of the animals may further increase the extent of the alga by their feeding actions. Pieces of *C. linum* that have been broken off the main plant continue to grow forming the dense mats witnessed in lagoons. The importance of this particular aspect of coastal lagoon habitats is recognized by its inclusion in the biotope classification system under the abbreviation, ENLag.Veg.

Table 1.2 The animals associated with *C. linum* in coastal lagoons (Sheader and Sheader 1985)

Fauna associated with *Chaetomorpha linum*	
Specialist species	**Non-specialist species**
Gammarus insensibilis (Amphipoda)	*Sphaeroma rugicauda* (Isopoda)
Idotea chelipes (Isopoda)	*Corophium insidiosum* (Amphipoda)
Nematostella vectensis (Anthozoa)	*Chironomus salinarius* (Chironimidae)
Cerastoderma glaucum (Bivalvia)	*Halocladia varians* (Chironomidae)
Hydrobia ventrosa (Gastropoda)	*Hydrobia ulvae* (Gastropoda)
	Littorina saxatalis (Gastropoda)

1.2.3. Adaptations to the lagoon environment

The recognition of specialist lagoon species implies that there may be particular adaptations to the lagoon environment that enable these species to prosper where others are excluded. However there have been few comparative studies between lagoon species and their marine, estuarine or freshwater congeners.

One organism for which there is some information is the lagoon cockle (*Cerastoderma glaucum*). It demonstrated lower respiration rates than the closely related estuarine species *Cerastoderma edule*. The temperature coefficient (Q_{10}) was also relatively low within its normal temperature range suggesting adaptation to a wider range of environmental conditions

(Wilson and Elkaim 1997). *Cerastoderma glaucum* also appears to have a greater ability to regulate its metabolism than *C. edule*, with much reduced energy expenditure being reported at high temperatures (Wilson and Elkaim 1997). This regulation ability has been suggested to be a selective advantage for *C. glaucum* over *C. edule* in the lagoon environment, where in the summer months the animals may be subjected to prolonged periods of elevated temperatures, one of the factors that may separate the distributions of the two species.

Examples of lagoon adaptations may be uncommon but the fragmented, isolated nature of lagoons provides conditions conducive to allopatric speciation. Intraspecific variability has been demonstrated in populations of the bryozoan *Alcyonidium gelatinosum* from inside and outside The Fleet, Dorset. The colonies showed differences in zooid size, tentacle number, embryo colour, reproductive season and substratum preference (Porter et al. 2001). The lagoon population appeared to be reproductively isolated owing to differences in timing of recruitment. In the lagoon this occurred in January to February and was directly related to a substrate free of vegetation and silt. In the sea population, recruitment occurred in the autumn. The larvae of *A. gelatinosum* originally from the sea, that were relocated to the lagoon as a result of tide action were unlikely to find a substrate because of the absence of their preferred *Fucus serratus*.

This essentially highlights the problem for the conservation of the lagoonal habitat. The limited data suggest specific lagoonal adaptations but there is not sufficient information to provide relevant strategies for the management of lagoons.

1.3. Study organisms

The majority of the work in this study was carried out on the amphipod *Gammarus insensibilis* (Crustacea: Amphipoda) and there is some consideration of the isopod *Idotea chelipes* (Crustacea: Isopoda) (Figure 1.1), both are members of the Superorder Peracarida. The Peracarida includes the orders, Amphipoda, Isopoda, Cumacea, Tanaidacea, Spelaeogriphacea, Mictacea and Thermosbaenacea (Johnson et al. 2001), which are characterized by direct development of their offspring through a number of distinguishable embryonic stages, in a marsupium or brood chamber. Breeding begins with formation of precopula in which the females are held beneath the male. After copulation, the fertilized eggs are deposited in the marsupium where they remain until development of juveniles is complete, at the latest by the next moult of the female (Kroer 1989; Johnson et al. 2001).

Gammarus insensibilis and *Idotea chelipes* are abundant in the saline lagoons of the UK, particularly in association with *C. linum*, for example *I. chelipes* was present at numbers of up to 21,743 individuals in a 380 g sample of *C. linum*. *G. insensibilis* is protected under the Wildlife and Countryside Act 1992, while *I. chelipes* is considered nationally scarce. These species are therefore directly relevant for conservation interest and represent an integral part of the lagoon system.

Peracarids, particularly isopods and gammarid amphipods are useful experimental animals. Their size makes them relatively manageable in physiological experiments, but the most important factor is the direct development of their young in the marsupium. This means that reproductive investment can be readily quantified. Therefore the reproductive status of animals used in physiological experimentation is apparent without invasive procedures which may stress the individual. Also, gammarid amphipods and idoteid isopods are generally easy to maintain under laboratory conditions. Finally, peracarids are often important components of many marine and freshwater habitats, their importance is evidenced by their use in toxicity testing in both environments (McCahon and Pascoe 1988; Schlekat et al. 1995).

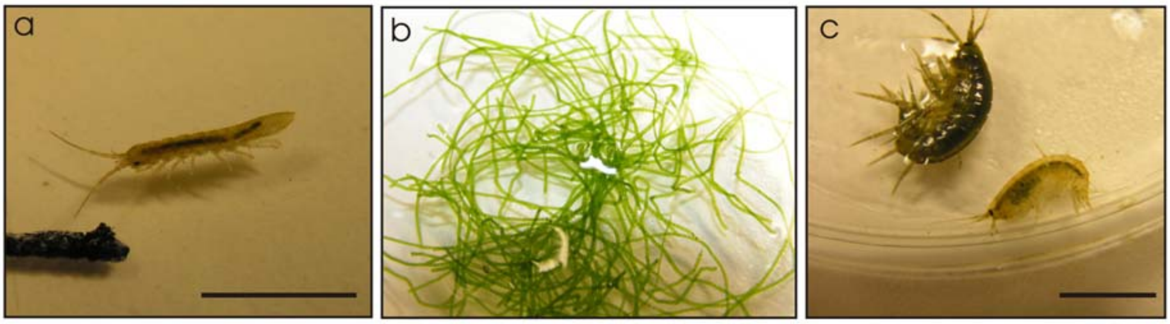

Figure 1.1: *Chaetomorpha linum* (b) and its inhabitants, *Idotea chelipes* (a) and *Gammarus insensibilis* (c) – black lines represent 10 mm.

1.3.1. Gammarus insensibilis (Stock 1966)

The lagoon sand shrimp, *Gammarus insensibilis* (Figure 1.1c) is found in the Mediterranean, Black Sea, and on the Atlantic coast of Europe, its northern limit being the Humber on the east coast of England. In England, *G. insensibilis* has not been recorded west of the Fleet in Dorset. It tolerates reduced salinity and can be locally abundant in brackish pools and saline lagoons (Lincoln 1979; Sheader and Sheader 1985; Sheader and Sheader 1987; Gilliland and Sanderson 2000). Notably *G. insensibilis* is not found sympatrically with the morphologically similar *G. locusta* (common in fully marine environments). The only recorded fully marine habitats of *G. insensibilis* are in the Mediterranean, where *G. locusta* is absent (Sheader and Sheader 1987). Since *G. insensibilis* is so similar to *G. locusta*, RAPD DNA fingerprinting has been used to differentiate the species taxonomically (Costa et al. 2004).

The limited published information on the biology of *G. insensibilis* suggests continuous recruitment and a variable (female dominant) sex ratio throughout the year with reproductive output greatest in the winter months (Karakiri and Nicolaidou 1987). An inverse relationship between egg size and temperature has been recorded (Sheader 1996), suggesting greater investment in individual eggs in the winter months to aid survival of juveniles.

Gammarus insensibilis is the intermediate host for a number of trematode parasites including *Maritrema subdolum*, *Microphallus hoffmanni*, *Microphallus papillorobustus* and *Levinseniella propinqua* (Kostadinova and Mavrodieva 2005a). *M. papillorobustus* manipulates the behaviour of *G. insensibilis*, increasing vulnerability to predation by the avian definitive host. The cerebral metacercariae induce positive phototaxis, negative geotaxis and aberrant evasive behaviour, (Helluy 1984; Thomas et al. 1996b; Brown et al. 2003). The parasite life cycle is described in full in Figure 1.2 (p.26).

1.3.2. Idotea chelipes (Pallas, 1772)

The majority of the work in this study concentrates on *G. insensibilis* but in the Chapter 9 there is some consideration of the lagoon isopod *Idotea chelipes* (Pallas). *Idotea chelipes* (Figure 1.1 a), formerly *Idotea viridis*, is a benthic isopod that tolerates a range of brackish to fully marine environments with salinity ranging from 4 – 39 (Horlyck 1973). It is primarily herbivorous and is known to feed on *Enteromorpha* sp. and *Chaetomorpha* sp. (Bamber et al. 2000; Lapucki et al. 2005).

In the UK the isopod is found in saline lagoons on the southern and eastern coasts of England (Al-Suwailem 1991; Bamber et al. 2000) but its range extends as far south on the Atlantic coast as Morocco and is also known from the Mediterranean and the Baltic seas. It has been described as a polytypic species, with the UK populations of the subspecies *I. chelipes chelipes* (Charfi-cheikhrouha 1996).

1.4. The reproductive biology of peracarid crustaceans

For conservation purposes it is important to understand how populations function. One of the most important aspects in determining the success of a particular group of organisms is the reproductive success of the individuals. To understand the interactions between lagoon species and their environment it is useful to determine how environmental factors affect their reproductive investment and success. For the reasons previously outlined the peracarid crustaceans used in this study are useful for measuring such factors.

The Peracarida are a successful Superorder of the Crustacea. They are characterized by the direct development of their young in a marsupium (Johnson et al. 2001). The Amphipoda and Isopoda are particularly successful orders having colonized certain terrestrial and most aquatic habitats from freshwater to fully marine and from alpine lakes to the deep sea. The success of the peracarids may be considered surprising given the conservative nature of their reproductive strategies. However, within the constraints of direct marsupial development there is variability both intra- and interspecifically in many of the reproductive parameters. In addition the mode of reproduction (direct development of the embryos in a marsupium) enables the relative ease of study of various aspects of their reproductive investment.

1.4.1. Physico-chemical effects

Temperature is one of the most important factors affecting invertebrates but under natural, coastal circumstances it is frequently closely linked to salinity. General trends include relatively more, smaller embryos in warmer conditions but fewer larger embryos in the winter. *Gammarus duebeni* from freshwater produced larger eggs than those from brackish water (Hynes 1954) while winter animals produced larger eggs than summer animals (Steele and Steele 1969; Sheader 1983). *Gammarus lawrencianus* also produced larger eggs at low salinity but Steele and Steele (1991) report reduced viability of these eggs, possibly the result of extra-embryonic fluid becoming isotonic and swelling of the eggs. Despite equivalent brood volumes owing to fewer embryos, the larger eggs, probably the result of osmotic swelling, are not thought to be an adaptation to low salinity as they do not hatch.

There were several temperature-related effects on reproductive parameters in *Echinogammarus marinus* from the Mondego estuary. A 5 to 10 °C increase in temperature resulted in a decrease in duration of embryonic development by approximately 15 days, however salinity had no noticeable effect on embryonic development time (Maranhao and Marques 2003). The highest production of juveniles occurred at 10°C and the lowest at 20 °C, under laboratory conditions; again there were no salinity effects. This contradicts the usual amphipod trend where fewer larger eggs are produced in the winter months. It was suggested that the stable conditions of the laboratory, with unlimited food, may induce higher egg production.

Other less-commonly-studied factors include photoperiod and exposure of intertidal organisms to the air. Photoperiod can influence the sex ratio of *Gammarus duebeni*. Exposure to short days (<13-14 h light) leads to a female-dominated population while long days (>13-14 h light) will result in a predominance of males, in experiments carried out at 15 °C (Watt 1994). *Chaetogammarus marinus* eggs were larger than those of other amphipods. This was supposedly the result of a trend for the production of larger eggs at higher intertidal levels (Borowsky 1980), possibly an adaptation to the reduced feeding opportunities imposed by the extended periods of time exposed to the air.

1.4.2. Geographic factors

Several geographic effects have been reported both intra- and interspecifically on reproductive biology of peracarids. However, geographic effects are essentially combinations of the physico-chemical effects previously discussed. For example latitude is a combination of the effects of temperature and photoperiod, but there are reports of effects on amphipod reproduction. Generally, interspecifically, increased latitude is associated with decreased voltinism (number of generations per year), larger body size and fewer, larger embryos (Highsmith and Coyle 1991; Saintemarie 1991; Costa and Costa 1999) while, intraspecifically, similar trends occur. The size of mature female *Ampelisca brevicornis* increased and brood size decreased between 43°N (Marseille) and 54°N (Isle of Man) (Highsmith and Coyle 1991) and longer embryonic development times are evident in *Echinogammarus marinus* between Portuguese populations from the Mondego estuary (Maranhao and Marques 2003) and the Oosterschelde estuary (Southwest Netherlands) (Vlasblom 1969).

There has also been discussion of altitude effects on amphipod reproduction. Again this is a combination of other factors (temperature and oxygen concentration). The freshwater amphipod *Gammarus lacustris* (Sars) from a series of lakes along an elevation gradient (decreasing water temperature with increased altitude) in western Canada showed variation in investment into individual eggs by females in alpine lakes (Wilhelm and Schindler 2000). Females produced fewer, larger eggs in the lakes at higher altitude than the lower (prairie) lakes. The eggs produced by females inhabiting lakes at intermediate altitude were intermediate in size and number. Development time of eggs was shown to decline with increase in incubation temperature. Wilhelm and Schnidler (2000) concluded that the high phenotypic plasticity of reproductive traits in *G. lacustris* contributes to the success of the organism in a range of habitats.

The low pressure at high altitudes reduces the solubility of oxygen in water so organisms from alpine lakes are subjected to lower oxygen concentrations. The maximum length of amphipods from Lake Titicaca was 2-4 times smaller than those from the Caspian Sea and Lake Baikal, a result of reduced growth rates owing to low lower oxygen availability (Peck and Chapelle 2003). Although not a direct effect on reproduction increased body size is adaptive for both males and females in amphipods because brood size is directly related to female size and larger males are able to guard larger females (Watt and Adams 1993).

1.4.3. Parasitism

Parasites affect their hosts in a variety of ways and can, through effects on survival or fecundity, to a certain extent regulate the populations of their hosts (Thomas et al. 2005b). As previously discussed, mate choice in *Gammarus insensibilis* can be assortative, when infected by microphallid trematodes. Infected animals are more likely to pair with similarly infected amphipods because of their location in the water column as a result of their altered behaviour (Thomas et al. 1995). If trematode infection incurs an energetic cost there could be reproductive implications resulting from lowered energy available for reproduction.

There are several documented factors which may affect the reproductive biology of peracarids. Sex-ratio distortion by vertically transmitted feminizing parasites, e.g. *Gammarus duebeni* infected with microsporidian parasites (Dunn et al. 1993; Hatcher et al. 2000; Kelly et al. 2001) has the potential to affect the population dynamics of the host. The pairing success of *Gammarus lacustris* was significantly reduced when infected by *Polymorphus paradoxus* and *Polymorphus marilis* as a result of physiological costs to the hosts (Zohar and Holmes 1998).

1.5. Stress

Lagoonal organisms are subjected to far greater variation in environmental parameters such as temperature, salinity, pH and dissolved oxygen than the majority of marine organisms, suggesting that lagoons are stressful habitats (Barnes 1980, Bamber et al. 2000). These conditions present physiological challenges to their inhabitants. The ability to tolerate extremes of environment is thought to explain, to a certain extent, the distribution of organisms in their habitat ranges (Bayne et al. 1985; Randall et al. 1997; Tait and Dipper 1998). Stress response is a vital factor in understanding ecosystem dynamics but as an area of study is far reaching. Stress can be considered from a causative point of view, at the molecular or cellular level, while the effects of stress may be evident at the individual, population, community or ecosystem level (Maltby 1999). There has also been much debate in the scientific literature on the use of the term “stress”.

A single definition for stress has been a matter for debate in the study of environmental physiology, owing to the interchanging usage of the word to explain cause and effect. Stress has been defined as ‘the physiological response of an organism to a stressor’, ‘the collective non-specific physiological reactions of an organism against some kind of attack’ and ‘a state produced by an environmental or other factor which extends the adaptive responses of an animal beyond the normal range, or which disturbs the normal functioning to such an extent that the chances of survival are significantly reduced’ (Brett 1958; Lucas 1996), i.e. stress results from a stimulus. It has also been described as the cause of a response in an organism e.g. ‘the external force or stimulus that elicits generalized adaptive responses in organisms’ (Stebbing 1981), ‘the environmental stimulus which by exceeding a threshold value, disturbs normal animal function’(Bayne et al. 1985) or ‘external constraints limiting the rates of resource acquisition, growth or reproduction of organisms’ (Grime 1989). In this thesis the term ‘stress’ will be used to describe external factors that induce a physiological response in organisms, to ensure continued survival.

According to this definition stress is a stimulus that causes an animal to have a reduced chance of survival. Stebbing (1981) explains that agents bearing no physico-chemical resemblance to one another can induce similar responses in organisms, for example extremes of environmental temperature, dissolved oxygen, pH or salinity can induce similar responses in animals, as can many forms of anthropogenic stressor in the form of pollution. It can be concluded that stress is a factor that induces a general adaptive response.

1.5.1. Stress Resistance and the stress response

For populations to survive periods of environmental stress there must be individuals that can respond to the stress. There are several ways in which organisms respond to stress including the process acclimatization[1]. This describes changes to the physiological phenotype (Spicer and Gaston 1999), such as changes in enzyme production rates and cell membrane structure. More extreme short term stress invokes a more rapid response known as hardening, which may also involve changes in membrane composition and the use of energy reserves but more specifically invokes the stress response (Sorensen et al. 2003).

The stress response is the complex series of behavioural and physiological processes that occur when an organism senses the presence of a stressor. It is a highly conserved reaction; the same molecules are involved from invertebrates through to human beings, despite the lack of the main organs involved in mammalian stress response in invertebrates (Ottaviani and Franceschi 1996).

The stress response has primary, secondary and tertiary stages. Initially the stress is detected leading to the primary, *alarm reaction*. The secondary stress response is termed *the resistance stage* followed by the *stage of exhaustion* (Ottaviani and Franceschi 1996).These stages are comparable to the primary, secondary and tertiary effects of stress; the primary effect is the release of hormones, the secondary effects are changes in blood chemistry including depletion of nutrients and other metabolic changes and tertiary effects are reduced production (growth and reproduction) and immunosuppresion (Koehn and Bayne 1989).

Ecologically, the essential consideration of the stress response is its energetic cost. Energy balance of stressed organisms may be upset because of one of two changes in the energy equation. There may be reduced energy uptake because of lower feeding (or assimilation) rate or increased energy loss resulting from increased respiration rate (Lucas 1996). Higher respiration rate may result from increased locomotor activity, increased rates of cellular activity or utilization of reserves.

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[1] The terms acclimation and acclimatization refer to changes in organisms related to changes in environmental conditions. Although they are frequently used interchangeably acclimation refers to a change in a single parameter, such as temperature, under laboratory conditions while acclimatization refers to the short term, often reversible (but not necessarily) changes in more than one parameter, characteristic of environmental change in a more natural situation.

1.5.1.1 Behavioural responses

Perhaps the most basic way in which organisms can respond to stress is to physically avoid it, this may involve movement away from the source in motile animals, or in the case of sessile animals such as anemones or bivalves simply closing up until the stress has passed. For example the freshwater gastropod *Bithynia tentaculata* is inactive under temperature stress (Hahn 2005). If it is not possible to avoid the stress there are several physiological responses that organisms can make.

1.5.1.2 Heat Shock Proteins

The induction of the synthesis of heat shock protein is one of the main factors in response to a stressor. Heat shock proteins (hsps) are a subset of the molecular chaperone proteins, involved in the general maintenance of the cell, including transport, folding and unfolding, assembly and disassembly of multistructured units and degradation of misfolded or aggregated proteins. In addition to these functions there are the more typical heat shock protein functions; the prevention of proteins becoming inappropriately aggregated (Sorensen et al. 2003). Under normal conditions these proteins are important but under stress their action is essential to prevent the damage that may be caused to a cell by problems with protein organisation.

Despite the name, the production of hsps is induced by all known stressors (Feder and Hofmann 1999). Hsps are produced by organisms when environmental stress causes the denaturation of membrane proteins. They bind damaged and non-functioning proteins so they can re-attain the natural state or prevent/minimise accumulation of non-functioning or toxic aggregations of protein molecules. As molecular chaperones hsps are also important in the general maintenance of homeostasis in cells as shown by inbreeding experiments with *Drosophila;* there was greater production of *hsp70* in inbred *Drosophila* under no environmental stress than outbred flies, interpreted as a response to more deleterious recessive alleles causing increased levels of incorrectly folded proteins (Wheeler 1999; Kristensen et al. 2002; Sorensen et al. 2003).

Despite their highly conserved nature hsp induction is dependent on the ecological niche of the specific organism; for example Antarctic organisms initiate the heat shock response at approximately 5 °C while thermophillic bacteria may not demonstrate a response below 100 °C (Sorensen et al. 2003).

1.5.1.3 Endocrine responses

There is a large body of literature describing hormonal responses to various stressors in vertebrates (Ottaviani and Franceschi 1996; Bonga 1997). Until relatively recently this area had been widely ignored in the study of invertebrates. The importance of stress to the commercial culture of shellfish has prompted some work in this area but endocrine responses to stress are clearly important considerations in the study of the bioenergetics of any organism.

Much like the vertebrate system, invertebrates release Corticotrophin-releasing hormone (CRH) and Adrenocorticotropic hormone (ACTH) when affected by a stressor. In molluscs catecholamines (notably noradrenaline and dopamine) are released from the haemocytes to the haemolymph in response to increased levels of CRH and ACTH in the haemolymph. Cytokines (immune hormones) are also involved in the release of catecholamines (Ottaviani and Franceschi 1996).

These responses are demonstrated by increases in haemolymph levels of catecholamines in oysters (*Crassostrea gigas*) in response to common stressors – salinity, temperature and mechanical disturbance (Lacoste et al. 2001b). These changes in catecholamine levels may have important consequences for the general health of the organisms as the hormones have important roles in feeding, locomotion, respiration and reproduction (Lacoste et al. 2001b) as well as potentially reducing the effectiveness of the immune response (Lacoste et al. 2002). Lacoste et al (2001b) observed that catecholamine levels reduced to pre-stress levels shortly after the mechanical stress that the oysters were subject to was over. They proposed that the CA were degraded by enzymes when no longer needed because of the associated costs.

1.5.2. Differential susceptibility to stress

Research has shown that certain individuals are more resistant to stress than others. This can result from effects at the individual (previous experience to stress) or population level (inherent genetic factors). Previous experience is an important factor in the consideration of stress. Hypertrophy (accumulated stress resistance mechanisms) occurs in response to previous exposures to a stressor, allowing animals with a history of exposure to be less sensitive to it on subsequent exposures (Stebbing 1981; Maltby 1999).

Genetic factors are also associated with susceptibility to stress. Heterozygosity has been suggested to be correlated with lower maintenance costs. It was suggested that the greater the degree of heterozygosity the broader the range of temperatures over which organisms are able to maintain positive energy balance (Koehn and Bayne 1989). It has been suggested that natural environmental stress increases heterozygosity (Cimmaruta et al. 2003). In a comparison of a coastal lagoons in Italy Cimmaruta et al. (2003) found that fish from lagoons characterized by natural stress (temperature and salinity) showed values of heterozygosity twice as high as anthropogenically polluted lagoons. They proposed that anthropogenic stressors are rapid and can dramatically reduce population sizes and therefore genetic variability while populations affected by natural stressors have developed the genetic variation that allows them to withstand the extremes they are subject to. It would be expected therefore that populations that have historically been restricted to naturally stressful habitats would be highly heterozygous and therefore have low maintenance costs and a broad range of tolerance.

In the case of UK coastal lagoons, there may be an increased proportion animals homozygous at certain alleles, resulting from the fragmented nature of the individual habitats. Pearson (2003) showed that under the variable environmental conditions of the lagoon, homozygosity increased in several populations of the lagoon amphipod *Gammarus insensibilis*. At many loci values were close to 1. It was suggested that high homozygosity may be the result of population bottlenecks leading to reduced allele frequencies and therefore higher chance of homozygosity (Pearson 2003).

1.5.3. Energetic costs of stress resistance and trade-offs

The aspect of stress that is of most interest ecologically is the trade off that occurs between stress response and energetic cost. The ability to respond to stress is clearly an essential attribute to any organism, but an energetically costly one. There is a trade off between resistance to stress and growth and reproduction in unstressed environments, essentially this is a trade off between production and mortality (Sibly and Calow 1989).

For example, studies on *Drosophila melanogaster* have invariably shown that the expression of heat shock proteins increases thermotolerance. However, there are deleterious aspects associated with the production of hsps, including lower reproductive rates in animals that produced hsps under no thermal stress (Krebs and Loeschcke 1994); reduced survival to adulthood in unstressed *D. melanogaster* larvae with high *hsp* levels (Krebs and Feder 1997); reduced cellular growth rates of *D. melanogaster* associated with production of *Hsp70* under no thermal stress (Feder et al. 1992); maternal expression of *hsp70* caused a decrease in egg hatch proportion (Silbermann and Tatar 2000).

These costs result from the shut-down of normal cell functions during the stress response (disruption of the cellular processes of development), the extensive use of energy (reduced allocation to reproduction), and toxic effects (lower viability of oocytes) associated with high hsp concentrations (Silbermann and Tatar 2000; Sorensen et al. 2003).

As hsps may attack sites of high rates of protein synthesis it appears that some animals have the ability stop their production during reproduction and growth especially at early stages of development when cell division is at its most rapid (Krebs and Loeschcke 1994; Feder and Hofmann 1999), leading to potential stress susceptibility at times of increased growth. To control the levels of hsps they become concentrated into granules rather than the usual diffuse levels of the protein throughout the cell, presumably to as a mechanism to control the protein's activity (Feder et al. 1992).

Stress-induced catecholamine production also has costs. Noradrenaline and dopamine production in response to stress can inhibit immune functions such as haemocyte phagocytosis and production of reactive oxygen species (respiratory burst) (Lacoste et al. 2002; Li et al. 2005). For example, mortality in the giant prawn *Macrobrachium rosenbergii* infected with the bacterial pathogen *Lactococcus garvieae* increased when they were injected with dopamine, suggesting immunosuppression after catecholamine production in response to stress (Li et al. 2005). While the pacific oyster (*Crassostrea gigas*) mobilized immunoactive haemocytes into certain tissues when the animals were under stress (Lacoste et al. 2002). This was thought to be to increase resistance to pathogens when stressed but the authors also found that under the same stress, the animals were more susceptible to bacterial infection (Lacoste et al. 2001a). They suggested that the redirection of haemocytes may be related to another function of haemocytes, specifically the transportation of nutrients, to maintain energy balance while the animals are stressed.

1.6. Bioenergetics, energy balance and the energy equation

The environmental variability evident in saline lagoons implies that lagoonal organisms must have a means by which they are able to tolerate extremes of various environmental parameters. It would be expected that such tolerance would represent a cost to the organism. A method by which the condition of individual organisms can be monitored is through the use of bioenergetics and energy balance.

The stress response is presumably an important component of the ability of lagoon organisms to prosper in their environment. When induced, however, it requires resources. All organisms have finite resources from assimilated or stored energy that are allocated to the various functions. This can be described by the concept of energy balance. The energy balance of an organism describes all the functions that require energy and those that gain energy. Over the lifetime the energy equation must balance or the organism cannot function (Table 1.3; Equation 1.1). All energy consumed is transferred, initially in the food not assimilated (faeces), then subsequently in metabolic processes (respiration and ammonia excretion), secretions, growth and reproduction (Lucas 1996).

Table 1.3: The gains, losses and balances apparent in the energy balance sheet of all organisms. Italicized abbreviations apply to the energy balance equation (Equation 1) after (Lucas 1996)

Gains	**Losses**	**Balance**
Food consumed C	Excreta $F + U$	Tissues formed P_G
	Eliminated tissue P_E	Residual secretions (e.g. shell) P_{S1}
	Reproductive products P_R	
	Non-residual secretions P_{S2}	
	Respiration R	

$$C - (F + U + P_E + P_R + P_{S2} + R) = P_G + P_{S1}$$

Equation 1.1: The energy balance equation (abbreviations are described in Table 1.3)

The energy balance equation can be simplified into measurable metabolic equivalents. In this case P is the combined energy for somatic growth and reproduction can then be used as a measure of an animal’s condition.

$$C - F = A = R + U + P$$

$$\textit{or}$$

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

Equation 1.2: Simplified energy balance equation. Where C = energy consumed, F = energy lost in faeces, A = assimilated energy, R = loss through respiration and U = loss through excretion and P = production or scope for growth.

1.6.1. Scope for growth and stress

Based on the energy balance equation (Equation 1.2), the Scope for Growth assay (SFG) monitors the condition of organisms by assessing the energy available for growth and reproduction (Widdows 1993). The term SFG is equivalent to P in Equation 1.2. Generally energy intake is greater than energy loss so energy is available for somatic growth or reproduction i.e. SFG or P is a positive value; times of stress (or use of resources for growth or reproduction) can be identified as negative SFG. Scope for Growth has been developed as a toxicity test for detecting pollution in fresh and marine ecosystems frequently using peracarid crustaceans as test organisms (Naylor et al. 1989; Maltby and Naylor 1990; Maltby et al. 1990; Maltby 1992; Roast et al. 1999a; Roast et al. 1999b).

By negatively affecting SFG, stress may reduce the zone of tolerance of an organism (Koehn and Bayne 1989). If an organism invests its energy in high levels of growth and reproduction at the expense of potential stress response it may be successful under constant environmental conditions. Therefore it would be expected that organisms that inhabit variable environments would have the physiological mechanisms to respond to stress and have a generally lower SFG. It would be expected that specialist lagoon species like *Gammarus insensibilis* have greater ability to respond to the stressors apparent in the lagoon habitat than congeners but have a lower scope to reproduce at a rate that would enable them to be an ecologically competitive species outside of lagoons.

1.6.2. Factors affecting scope for growth

Most work on SFG has been carried out on intertidal bivalve molluscs, notably *Mytilus edulis* (Mollusca: Bivalvia) with respect to anthropogenic stressors and commercial culture of shellfish. The earliest of this work was extensively reviewed by (Bayne et al. 1985). Since then SFG has been developed as a toxicity test using peracarids to detect pollution in freshwater and marine ecosystems (Naylor et al. 1989; Maltby and Naylor 1990; Maltby et al. 1990; Maltby 1992; Roast et al. 1999a; Roast et al. 1999b).

The energy balance of lagoon organisms is likely to be affected by the extremes of environmental variation evident in the habitat, however scope for growth is also known to be affected by various intrinsic factors (Bayne et al. 1985). All components of the energy equation increase with body mass in *M. edulis* while the weight-specific rates of the components declines. Respiration (R) is 33% higher when the mantle tissue contains mature gametes (Widdows 1978b; Widdows 1978a). Respiration rate is significantly higher in male *Neomysis integer* (Crustacea: Peracarida) than females (Roast et al. 1999 a). The higher respiration rates recorded in male *N. integer* has been attributed to reproductive condition or weight specific rate because of morphological differences (Roast et al. 1999 a).

Many extrinsic factors are known to affect the energy balance and therefore SFG of organisms and it is these that are of significance in the consideration of the interactions between lagoonal organisms and their environment. For aquatic species temperature is perhaps the dominant influence. However, acclimatization results in the maintenance of many physiological rates independent of temperature (Bayne et al. 1985) so SFG is not necessarily affected by changes within the normal experience range of an animal. Between 5˚C and 20˚C *M. edulis* had Q_{10} values of 1 to 1.6 after acclimation so the effect of temperature on SFG was reduced. Above 20˚C however, R increased and clearance rate (C) decreased, thus SFG was reduced. Above 25˚C SFG was negative indicating stress on the animals at the highest temperature. The results presented by Bayne et al. (1985) correlate with the maximum experienced temperature in the ecological range of *M. edulis.* Relatively constant feeding rates despite changes in water temperature (Thompson and Newell 1985) and positive scope for growth at temperatures reaching -1˚C (Loo 1992) demonstrate the importance of acclimation ability to the SFG of animals.

Respiration rate was shown to increase significantly in *N. integer* in response to increasing temperature (Roast et al. 1999 a, b). The study also showed that increasing salinity caused a decrease in R. This was concluded to be an adaptation to the estuarine environment where the tidal cycle subjects the species to conflicting changes in salinity and temperature so that energy lost through respiration remains relatively constant during the cycle.

For estuarine and coastal species salinity is often variable and may be one of the more important physico-chemical challenges to organisms from these habitats. “The oxygen consumption of euryhaline organisms increases in sub-normal and/or decreases in supra-normal salinities” (Kinne 1971). This is supported by the study of *N. integer* (Roast et al. 1999a) and *M. edulis* (Stickle and Sabourin 1979). Acclimation is again of importance with respect to salinity in *M. edulis*. Within the normal salinity experience range (20 to 35), all components of the energy equation were independent of salinity but below 20 R, C and SFG were reduced (Shumway and Youngson 1979; Stickle and Sabourin 1979). There was also variation in the ability to acclimate between different populations (Sickle & Sabourin, 1979).

While extrinsic, physico-chemical factors are important to the energy balance of any organism, the energy gained from the food is perhaps of greater significance. This can be affected by a variety of factors including food availability and food quality. Despite the importance of food quantity, there is a plateau in SFG increase as food concentration increases because maximal SFG is limited by the rate of energy absorption (A) (Griffiths and King 1979; Bayne et al. 1985). Food quality was also shown to be of importance to SFG (Widdows et al. 1979); when there are high levels of suspended particles in the planktonic food source *M. edulis* showed a reduction in absorption efficiency.

The quality of food was shown to affect growth rate in the stichaeid fish *Cebidichthys violaceus* (Horn et al. 1995). Fish fed on a natural diet were compared with those fed on higher protein diets. The fish assimilated equivalent amounts of energy on medium and high protein diets which were both of significantly greater protein content than the natural diet. Growth rate was highest in fish fed on a medium protein diet (30%), while food consumption reduced with increased dietary protein. It was postulated that the medium diet was of highest quality, the natural diet lacked sufficient protein for rapid growth while the high protein diet lacked other vital energy sources so protein was being metabolised. If the SFG assay had been carried out on the fish it may have been possible to detect changing respiration rates to determine whether metabolism was in fact affected.

One of the most important considerations in energy balance is the energy available from the food consumed. *Gammarus insensibilis* feeds on the filamentous green alga *Chaetomorpha linum* but this varies in abundance during the course of year suggesting differential growth rates and therefore biochemical composition, leading to potential variable energy content. The energy content of marine macroalgae has been shown to vary spatially and temporally. Lamare and Wing (2001) demonstrated higher calorific content for *Macrocystis pyrifera* (Phaeophyta) in the summer but the opposite trend for *Ulva lactuca* (Chlorophyta). The energy content of *Pilayella littoralis* (Phaeophyta) was greater in the summer than spring and autumn (Steele and Whittick 1991). In contrast there was no significant seasonal variation in calorific content of a number of macroalgae from the Chlorophyta, Phaeophyta and Rhodophyta (Paine and Vadas 1969).

Although there is evidence to suggest that growth rate may be increased when the food source is of greater calorific value (Larson et al. 1980) there is evidence to suggest that there is no selection of foods with greater calorific value by herbivores and food selection behaviour has evolved in response to availability (Paine and Vadas 1969)

There is an increase in metabolism (termed Specific Dynamic Action), which can be measured as increased respiration rate, that is the direct result of feeding. SDA reflects the energy requirements of the behavioural, physiological and biochemical processes involved in feeding including ingestion, digestion, absorption, assimilation and the synthesis of proteins and lipids (Robertson et al. 2001). Various factors affect SDA including the composition of the diet. The high energetic cost of the breakdown of cellulose caused the giant freshwater prawn *Macrobrachium rosenbergii* to demonstrate an increase in metabolism of 326% four hours after feeding on a high cellulose diet (15%) compared to a 61 % increase when fed on a diet with no cellulose (Gonzalez-Pena and Moreira 2003). In the same study the metabolism of *M. rosenbergii* was approaching pre-feeding rates 24 hours after the feeding episode.

1.7. Parasites associated with lagoon species

All organisms host a variety of parasites. The enclosed nature of saline lagoons, high abundance of certain species and the importance as feeding grounds for sea birds and waders suggest that lagoons are habitats conducive to the transmission of parasites. The importance of parasites to the understanding of the biology of individuals, populations and ecosystems is increasingly recognized but until recently, was to a large extent ignored (Sousa 1991). To gain a full understanding of the functioning of an ecosystem such as the saline lagoon, for its conservation, it is essential to investigate all the major interactions within it, including those between host and parasite. *Gammarus insensibilis* can be infected by the metacercarial stage of a number of species of microphallid trematodes. *Microphallus papillorobustus, Microphallus hoffmanni*, *Maritrema subdolum* and *Levinseniella propinqua* were all recorded in Black Sea *G. insensibilis* (Kostadinova and Mavrodieva 2005b). These infections can have important implications to the ecology of the lagoon, influencing *G. insensibilis* intraspecifically through effects of reproductive behaviour (Thomas et al. 1995; Thomas et al. 1996a) and interspecifically via predator-prey interactions (Thomas et al. 1997; Thomas et al. 1999; Thomas et al. 2005a).

The Trematoda is a class of the phylum Platyhelminthes (the flatworms) with complex life cycles, characterized by a variety of different larval stages inhabiting a series of host species (Figure 1.2). They are generally endoparasitic and in the majority of species the adult stages occur in vertebrate definitive hosts. The larval stages may occur in a succession of several invertebrate species, frequently including a molluscan intermediate host. The adults feed on intestinal debris, blood or mucus depending on the particular host environment. There are two orders of Trematoda; the Aspidogastra (endoparasites with large adhesive organs), and the Digenea (endoparasites with simple adhesive organs and indirect life cycles) (Smythe 1994).

One of the features of the life cycles of parasites that infect amphipods that has received attention in the scientific literature is the ability of some species to manipulate the behaviour of their host to increase the likelihood of transmission to the next host (Poulin 1994; Thomas et al. 2005a).

Parasite manipulation of host behaviour is common (Poulin 1994) and has been shown to occur in a number of different species. Evidence suggests that the aberrant escape behaviour exhibited by *G. insensibilis*, also seen in several other species, is related to levels of the neurotransmitter serotonin (Overli et al. 1982; Helluy and Holmes 1990; Maynard et al. 1996). In *G.* insensibilis serotonin levels are altered in the specific areas of the brain infected with the cerebral metacercaria of *Microphallus papillorobustus* (Rankin), resulting in deformed optic tracts degraded projections of the tritocerebral giant neurons (TGN) (Helluy and Thomas 2003).

A result of infection with the behaviour modifying *Microphallus papillorobustus* is altered reproductive behaviour. The phenomenon of assortative pairing (parasitized males entering precopula with infected females) occurs as a result of vertical segregation in the water column (infected individuals are more likely to be located and therefore form pairs in the surface waters), because of behavioural manipulation rather the influence of mate choice by parasitic prevalence (Thomas et al. 1996a) as has been theorized for vertebrate hosts (Hamilton and Zuk 1982; Möller 1994)

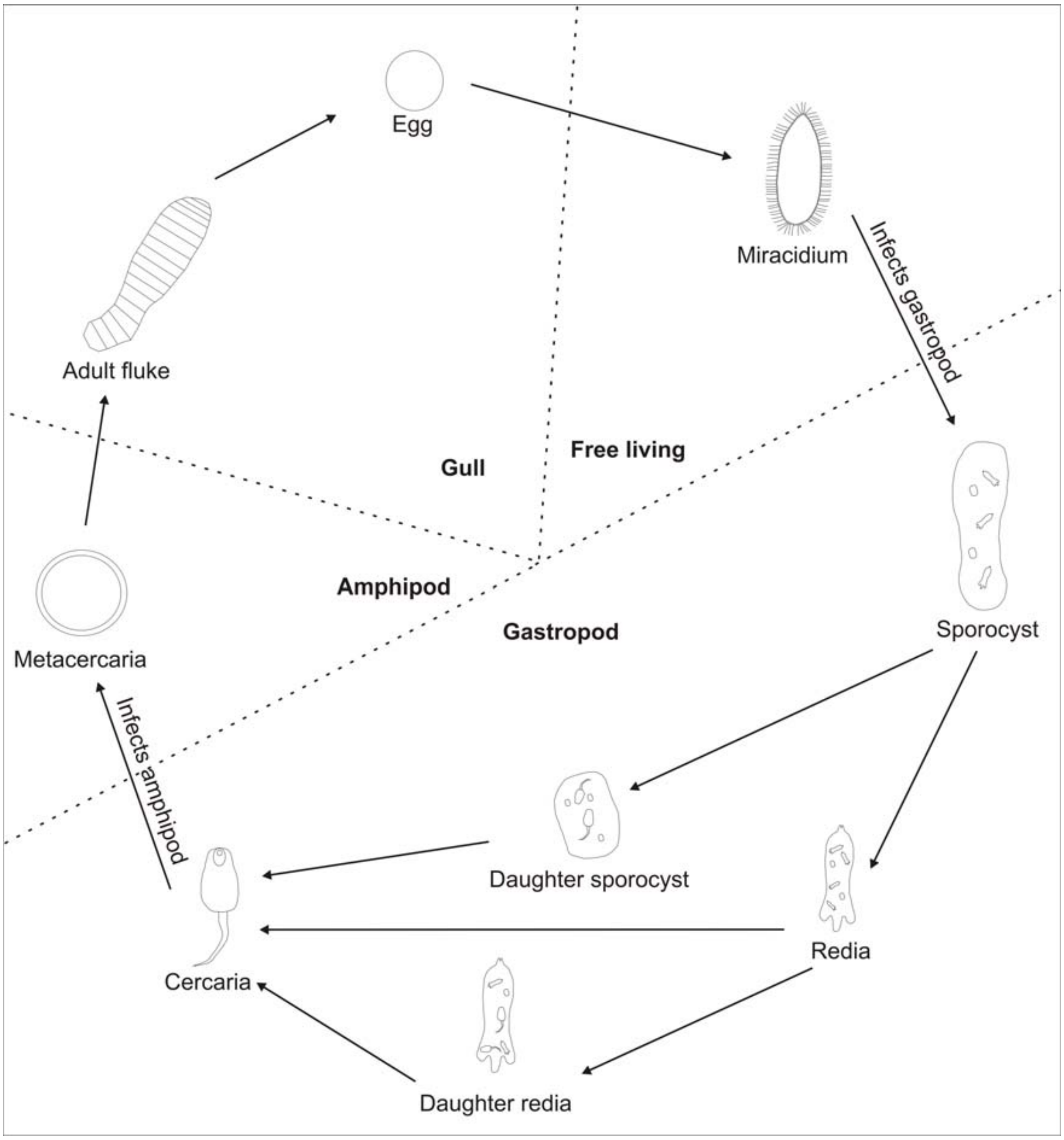

Figure 1.2: The complex life cycle of microphallid trematodes infecting amphipods

1.8. Aims and objectives of the project

This study monitors the population dynamics, reproductive investment, bioenergetics and parasitism of *Gammarus insensibilis* and the population dynamics and reproductive investment of *Idotea chelipes* from lagoons on the south coast of England in order to improve the understanding of the ecology of lagoon specialists. The particular aims of the investigation were as follows

- The primary aim of this work is to provide the detailed information needed to understand the ecology of *Gammarus insensibilis* in order to ensure lagoon management is directed to preserve the species in its rare habitat.
- Secondarily the lagoon habitat and its specialist fauna provide an unusual system for the study of adaptation to environmental stress. This study aims to determine the reproductive and metabolic adaptations of organisms to the dynamic and stressful nature of the lagoon.
- Finally the study aims to highlight the importance of parasites to ecosystem function. All organisms are subject to parasitism and the effects of parasites can have important consequences on their hosts. Prior to this study the lagoon habitat and the specialist biota had been identified as of conservation importance but the high rate of infection of *G. insensibilis* with trematode parasites had not been identified, so it is important to an understanding of how these interactions affect the lagoon system.

Chapter 2

Site descriptions

The saline lagoon habitat is generally variable in terms of the physicochemical environment. Physicochemical parameters are affected by the nature of the barrier with the sea, the size of the opening to the sea, the depth of the lagoon, the macrophyte abundance and the level of tidal influence resulting diversity between lagoon habitats. This chapter describes the physicochemical environment of the lagoons used in this study. Initially, previous work is discussed and then the temperature and salinity data collected during the project are presented. The data describe the temperature and salinity conditions to which animals were subjected in the lagoon at the time of collection, and were used as the basis for the bioenergetics studies (Chapter 5).

2.1 Study sites

Animals from three lagoon sites were used in this investigation. The majority of the work was carried out on *Gammarus insensibilis* from Gilkicker lagoon (Hampshire) but for the parasitology studies, samples were also collected from the Lymington-Keyhaven lagoon system (Hampshire) and on a single occasion from the Moulton Marsh lagoons in Lincolnshire. The locations of Gilkicker and the Lymington-Keyhaven lagoons are shown in Figure 2.1.

2.1.1 Gilkicker

The Gilkicker system lies on the shores of The Eastern Solent, surrounded by a golf course, near Gosport, Hampshire on the south coast of England. Its 36750 m^2 area consists of two connected lagoons and is separated from the sea by a shingle bank and sea wall. The larger (seaward lagoon) is 210 m long and 145 m wide and connected to the sea by a channel of 85 m in length and 10 m wide ending in a metal pipe (0.8 m diameter) that penetrates the barrier. The landward lagoon is 130 m long and 80 m wide and is connected to the seaward lagoon by two pairs of metal pipes of 0.5 m diameter (Al-Suwailem 1991).

The lagoons do not exceed 0.75 m in depth with a low tide mean of 0.46 m. There is a substratum comprised predominantly of mud in the middle and sand on the western and eastern sides with a 1 m belt of shingle around the shores. In much of the lagoon the surface sediments lie above a layer of shingle or silt (Al-Suwailem 1991).

Tidal influence on the lagoon is reduced because of its elevation above sea level (approximately 3.2 m above chart datum) and restricted opening to the sea; at high water neap tides, inflow of water may only occur for about 15 minutes. A 0.12 m tidal range was recorded during a tidal cycle (between springs and neaps) and it was estimated that the range would be 20 – 25 cm during spring tides (Al-Suwailem 1991).

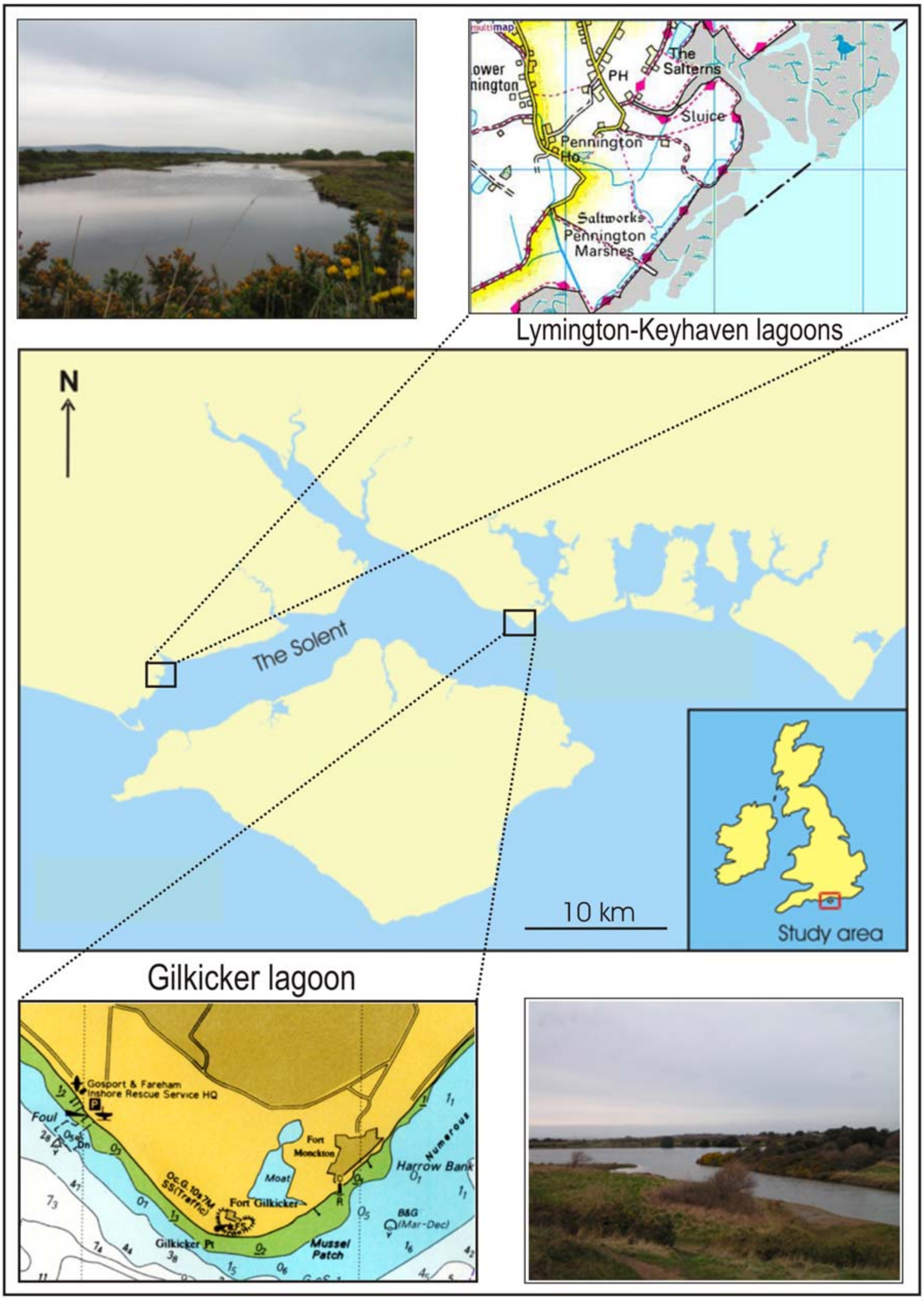

Figure 2.1: The location of Gilkicker lagoon and the Lymington-Keyhaven lagoon system on the shores of the Solent on the south coast of England. Photographs were taken from the barrier with the sea.

There is already a good understanding of the long term (seasonal) and short term (daily) trends in the most important environmental factors affecting Gilkicker lagoon (Al-Suwailem 1991). These are summarised below:

2.1.1.1 Salinity

The mean annual salinity of Gilkicker lagoon was 34. It ranged between 36 in the summer of 1989 and 29 in February 1990. In the lagoons there was a highly seasonal cycle of salinity in comparison to the sea. The data for the seaward lagoon is summarised in Table 2.1, it was suggested that the salinity of Gilkicker can be characterized by three distinct salinity periods (Al-Suwailem 1991):

Table 2.1: The periods of extreme low and extreme high salinity in Gilkicker lagoon (Al-Suwailem 1991)

Period	**Time of Year**	**Salinity**
Low salinity	January – February	20-25
High salinity	July – August	38-41
Moderate salinity	Rest of year	25-37 (mean = 30)

The landward lagoon experienced greater extremes of salinity (9-46) and greater variation during the rest of the year (20-38). These long term salinity trends are related directly to the balance between evaporation and precipitation (Al-Suwailem 1991).

Gilkicker lagoon demonstrates remarkably high spatial salinity variation for a small lagoon system. Al-Suwailem (1991) described salinity ranging from 18-27 in different locations in the seaward lagoon and 12-18 in the landward lagoon on an occasion in April 1987. The spatial salinity variation was attributed to the effects of macrophyte growth on water circulation. In areas of dense macropohyte growth salinity was higher than in areas with little plant growth, when evaporation was dominant, while high precipitation resulted in lower than average salinity where macrophytes were dense.

Tidal effects are also important to the salinity of the lagoon system and are highlighted by the differences in the salinity regimes of the landward and seaward lagoons. The salinity of the seaward lagoon, with its direct connection to the sea, is far more stable than the landward lagoon. Salinity stratification did not occur in the lagoon, because of its shallow nature.

2.1.1.2 Temperature

There were similar seasonal trends in the temperature of the sea and the lagoon but the lagoon showed greater extremes (Table 2.2).

Table 2.2: The ranges of temperature in the Solent and Gilkicker lagoon in summer and winter (Al-Suwailem 1991)

	Summer (°C)	**Winter (°C)**
Sea	16 – 19	6 – 8
Lagoon	17 - >24	2.5 - 8.5

Wind-induced mixing of the water column resulted in spatial temperature variation (the windward side was usually 0.5 °C cooler). Macrophytes induced some spatial variation; the water was 1 – 3 °C warmer over *Ruppia* beds than in open water. Thermal stratification only occurred in areas of dense macrophyte growth on still days (Al-Suwailem 1991).

2.1.1.3 Dissolved Oxygen

There was diurnal variation in oxygen concentration ranging from 6.4 mg l^{-1} just before dawn to 16.4 mg l^{-1} in mid afternoon resulting from respiration and photosynthetic activity (Al-Suwailem 1991). Like salinity and temperature, seasonal oxygen concentration variation showed similar trends in the sea and the lagoon, although the lagoon variation was greater. Oxygen concentration was highest in winter and spring and reached low levels in the summer (5.5 mg l^{-1}) although the validity of the seasonal dissolved oxygen conditions have been doubted because of the variable times of analysis and the significance of the diurnal cycle. There was some spatial variation directly related to macrophyte presence.

Vertical variation was also observed in areas of dense macrophyte growth, for example under a mat of *Chaetomorpha linum* the oxygen concentration was 3 mg l^{-1} while above the mat it was 10.2 mg l^{-1}.

2.1.1.4 pH

Resulting from the photosynthetic activity of the lagoon macrophytes, the pH showed a similar diurnal cycle to dissolved oxygen concentration, ranging from 8.2 at 0900 to 9.45 at 1600. Seasonal trends were also apparent; pH was high (9.6) in the spring and summer months but fell to 7.9 in the autumn. Areas of high macrophyte growth had slightly higher pH and sometimes induced stratification. With depth, through a mat of *C. linum*, as light intensity and photosynthesis reduced, the pH dropped from 8.9 – 8.06.

2.1.1.5 Nutrients

Nutrient levels in the lagoon were generally lower than the sea. As there was high input of nutrients (freshwater input, sediment disturbance and decay of both aquatic and terrestrial vegetation) it was suggested that the low levels were related to the utilization by the abundance of macrophytes and other primary producers.

The lagoon phosphate concentration was always lower than the sea but similar trends were observed. The concentration was highest in the winter (>1.0 µg l^{-1} in January and February) and lowest in the summer (0.6 µg l^{-1} in May). The highest phosphate levels may be related to import from the sea, reduced phosphate usage by fewer and inactive macrophytes in the winter months or build up from the death and decay of macrophytes.

Nitrate levels in the sea were high in the winter, low in the summer and followed a clear seasonal cycle. The lagoon showed no seasonal cycle in nitrate concentration and never reached more than the lowest levels recorded in the sea (40 µg l^{-1}). These peaks of nitrate concentration were brief and rare, occurring only twice in 20 months of sampling and levels returned to normal (5-15 µg l^{-1}). The lack of a trend in the lagoon nitrate concentration was related to fertilizer runoff from the golf course that surrounds the lagoon.

2.1.1.6 Sediment total organic matter (TOM)

The sediment TOM originates from many sources including macrophytes, microalgae, phytoplankton, terrestrial organisms, imported marine sources, faecal material and dead macrofauna. TOM was variable throughout the year and different patterns were recorded in different areas of the lagoon. The TOM was at its highest levels in areas where the sediment was of high percentage silt/clay while it was lowest in the sandy areas. Some trends in TOM were also related to areas of dense macrophyte coverage. In such an area, the TOM was highest in the autumn and winter, related to the high levels of detrital material present at the end of the growing season. The areas of greatest TOM were concluded to be under macrophyte growth (source of material) sheltered from water movement (so the particles remain where they were deposited).

2.1.2 Keyhaven-Lymington Lagoon system

The Keyhaven-Lymington lagoon system is located approximately 20 km to the west of Gilkicker (Figure 1). There are a series of lagoons stretching from Normandy Farm lagoon (near Lymington) towards Keyhaven at the mouth of the Lymington river estuary. The lagoons are artificial and were created from relict salterns when the sea wall was repaired in the 1980s in order to maintain the populations of lagoon specialists present in the salterns (Bamber et al. 2000). The lagoons to the west of the system are prone to low salinity resulting from excess freshwater input after high precipitation so specialist lagoon biota is low. In the western-most lagoon, Keyhaven pond, the salinity is typically 6. The lagoons used in this study were to the east of the system where salinity is usually around 25-35.

2.1.3 Moulton Marsh lagoons

Samples were taken from the Moulton Marsh lagoon system in Lincolnshire on a single occasion. At this site there are four lagoons and a series of ‘scrapes’ (small lagoon-like habitats). Specialist lagoon species have previously been recorded in lagoons 1 and 2 so samples were taken at these sites. The salinity was 38 in lagoons 1 and 2 on a previous survey on 30th October 2003 and temperature was 8 – 10°C (Bamber et al. 2004).

2.2 Temperature and salinity records for the present study

2.2.1 Gilkicker

Temperature and salinity showed typical seasonal patterns, both within the Gilkicker lagoon and in the adjacent Solent. There were clear seasonal temperature cycles in both environments, directly related to the air temperature. Salinity was stable throughout the year in The Solent but showed seasonal cycling in the lagoon. *C. linum* was present throughout the year although its abundance reduced in the winter months. In the warmest months (notably August 2003) there was evidence of high stress on the population of *G. insensibilis* with many dead animals evident in the margins of the lagoon. In the coldest winter months there was occasionally ice formation around the margins.

2.2.1.1 Temperature

During the sampling period from February 2003 to May 2005 the temperature of Gilkicker lagoon varied between extremes of 2.1 and 27.7 °C. The temperature was more variable than the adjacent sea (Figure 2.2), reaching higher maximum values in the summer (e.g. 27.7 °C in August 2003 – 6 °C warmer than the sea) (Figure 2.3, a). In the winter the lagoon was between 2-4 °C cooler than the sea.

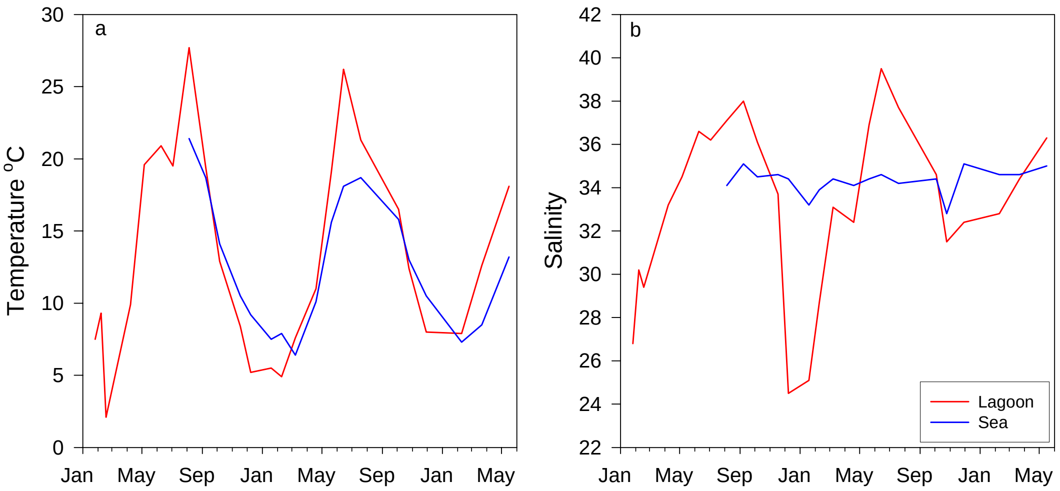

Figure 2.2: The temperature (a) and salinity (b) of Gilkicker lagoon and the adjacent Solent on sampling occasions from January 2003 until May 2005

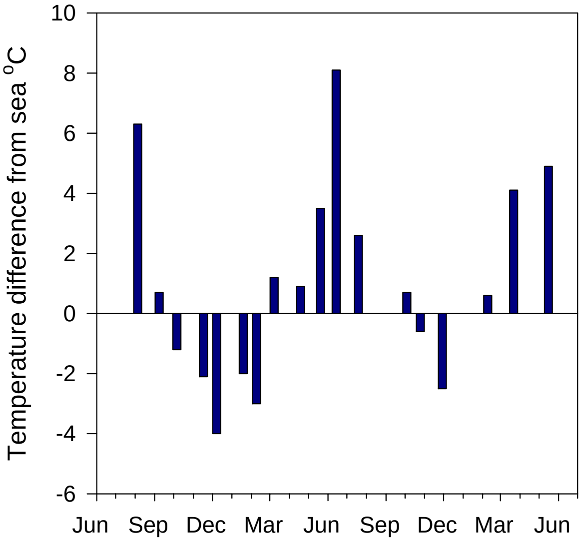

Figure 2.3: The difference between Gilkicker lagoon and the Solent sea temperature on 18 sampling visits between August 2003 and June 2005

2.2.1.2 Salinity

There was great variability in the salinity of the lagoon (Figure 2.2 b). The clear salinity cycling evident in the lagoon was not present in The Solent although there were some drops in salinity presumably related to local rainfall levels because of the coastal nature of where the readings were taken. In the peak summer, lagoon salinity was up to 15 % (around 4 psu) higher than the sea. In winter it was up to 30% lower than the sea (Figure 2.4).

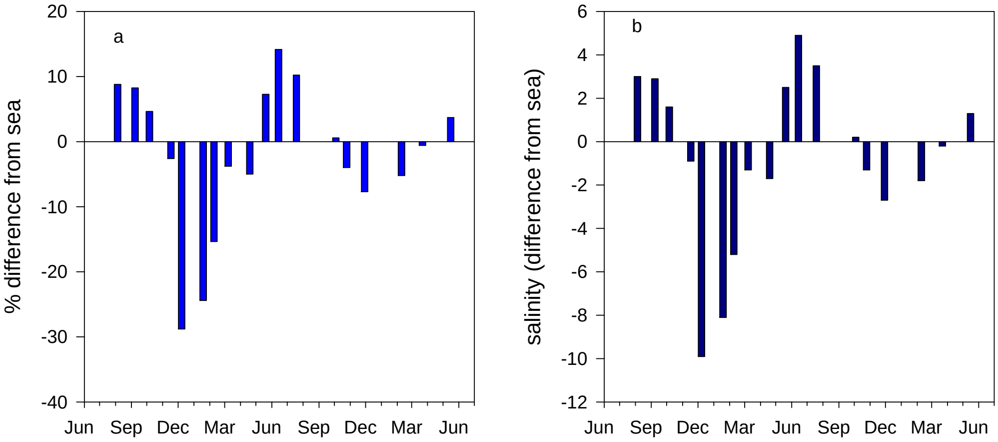

Figure 2.4: The percentage difference (a) and the actual difference (b) between the lagoon and the sea salinity throughout the year

2.2.2 Lymington – Keyhaven lagoon system

Temperature and salinity measurements were taken on sampling visits to "The Salterns" and "8-Acre Pond"; two of the known habitats for *Gammarus insensibilis* within the Lymington – Keyhaven lagoons. Temperature in these lagoons showed the same seasonal trend as Gilkicker (Figure 2.5 a). The pattern was almost identical in 8 Acre Pond to The Salterns. Like Gilkicker the salinity was variable but was generally lower at the Lymington lagoons than at Gilkicker. Salinity was lower in The Salterns when compared to 8 Acre Pond (Figure 2.5 b).

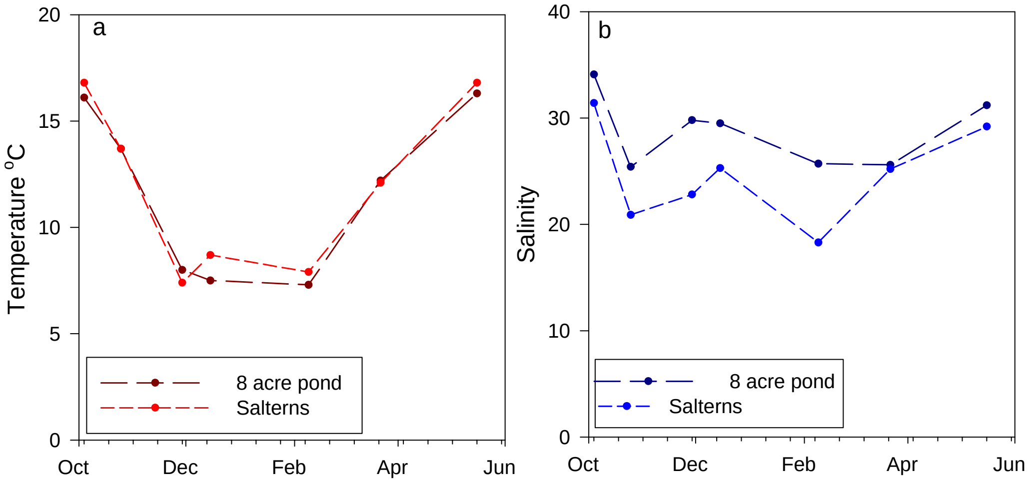

Figure 2.5; The temperature (a) and salinity (b) of 8 Acre Pond and Salterns lagoons from the Lymington-Keyhaven lagoon system between October 2004 and May 2005

2.2.3 Moulton Marsh lagoons

Measurements were taken on one occasion only, the 12th October 2004, at the Moulton Marsh lagoons. Temperature was 10.7 and 11.4°C in lagoons 1 and 2 respectively and salinity was 26.6 and 29.1.

2.3 Discussion

The shallow and enclosed nature of coastal lagoons means they characteristically demonstrate variable temperature and salinity (Barnes 1980; Bamber et al. 2000). Gilkicker lagoon and the Lymington-Keyhaven lagoons follow this pattern with greater extremes of both parameters, than in The Solent, evident seasonally. The trends at Gilkicker are similar to those shown in previous studies (Al-Suwailem 1991) suggesting that management has remained constant over the 15 years since. The extremes of temperature can be up to 8 ºC higher or lower than The Solent. The high summer temperatures can be greater than would normally be experienced by marine organisms at this latitude and may last for extended periods of time. Lagoon organisms must be able to acclimatize to such temperatures but also have the ability to survive in the extremely low temperatures experienced in the winter in this habitat. Salinity at Gilkicker is subject to comparable variation, showing differences of up to 30% from the sea.

The temperature and salinity at the Lymington-Keyhaven lagoons show similar variation to Gilkicker but salinity is generally lower. The lagoons are located at the mouth of the estuary of the Lymington River so may be subject to lower salinity water entering the lagoon depending on the state of the tide. There is also a salinity gradient with reduced salinity with distance west from the first site (Normandy Farm lagoon) at which salinity readings in the sites furthest west being as low as 6 (Bamber et al. 2000), although it has since been shown to be fully freshwater (Bamber pers.comm.). The slightly lower salinity in the Salterns lagoon can be explained by the presence of a freshwater input to the lagoon. 8-Acre Pond has no such input. There is very little data on the physicochemical parameters of the Moulton Marsh lagoons but the individual readings for this study were lower than those of Bamber *et al* (2004).

Variable temperature and salinity are not unique in aquatic environments; estuaries and rock pools experience cycles of both parameters related to the tides, and fresh water habitats may reach either extreme of temperature depending on the season. Lakes are subject to temperature variation and although this is extremely demanding on an animal’s metabolism it does not coincide with variable salinity. The importance of the lagoon environment is that the animals are subject to variation in both parameters for extended periods. In estuaries or rock pools, the tidal cycle ensures that organisms are never exposed to unfavourable salinity or temperature for extended time periods and behavioural strategies such as burrowing (annelids, bivalves), closing (gastropods, bivalves, anemones) or movement away (decapods, fish) can counteract the effects with limited physiological adjustment. In the lagoon the temperature can be high all summer in combination with high salinities. For an organism to be successful in this situation it must be able to adjust physiologically and employ reproductive strategies to ensure its offspring can survive in a similar situation.

All the sites studied provide habitats for several specialist lagoon species. These data are used in the remainder of this thesis as guides to how changing environmental factors can affect the population dynamics, reproductive investment and physiology of lagoon species and to highlight, for conservation purposes, the times of year when these protected species are at most risk of environmental stress.

Chapter 3

Materials and Methods

Peracarid crustaceans, particularly amphipods and isopods are extremely useful organisms for the analysis of reproductive patterns and physiological rates. There are a large number of parameters that can be measured when studying these organisms, because of the direct, marsupial development of the offspring. This chapter describes the methods used during the course of the study and presents data from preliminary studies and some initial analysis of survey data, particularly on the size-to-weight ratios and the effects of body size on reproductive factors, in order to explain how it was used in later chapters.

3.1 Sample collection

Working under the under license of Section 16 (3) (a) of the Wildlife & Countryside Act 1981 (amended by the Environmental Protection Act 1990) (Licenses held by Dr. R. Bamber, numbers: 2003-2004 no. 20031279; 2004-2005 no. 20041787) samples of *Gammarus insensibilis* and *Idotea chelipes* were collected monthly from Gilkicker lagoon, Gosport, UK (OS ref. SZ608978) (Figure 3.1). Animals were collected with a hand net from within the mats of *Chaetomorpha linum* (Figure 3.2) near the southern shore of the lagoon, where they were abundant. This varied, especially during the winter months, depending on the location of the mats of *C. linum,* which was affected by the particular wind and wave conditions.

The samples were collected using a non-quantitative method, because of the nature of the habitat. Methods have been trialled where a known amount of *C. linum* was collected and the animals within this amount quantified (Bamber *pers. com.*). However, a large amount of *C. linum* would have been required to ensure sufficient animals for the intended purposes, and the drifting nature of the alga, and the fact that it dies back to a certain extent in the winter (Al-Suwailem 1991) means that simply, alga from a greater area would be needed to provide the a useful number of organisms. The non-quantitative collection provided the large number of animals required to ensure a good representation of population was obtained. As the method was non-quantitative, the population structure data was presented as percentages of the population, under the assumption the collection procedure ensured that all sizes and life history stages were correctly represented in the samples.

Figure 3.1: Gilkicker lagoon viewed from the barrier between it and The Solent

Samples were collected for two purposes; firstly, representations of the populations of both species were needed to examine the population dynamics, so samples of the alga were collected to ensure that all size groups were considered. Secondly, brooding females of *Gammarus insensibilis* were required for use in bioenergetics studies and to analyse egg sizes so these were actively sought, after population samples had been taken to minimize animal numbers removed from the lagoon.

Animals for physiological study and studies of the size of embryos were returned to the laboratory aquarium within 1 hour of collection and maintained, with other lagoon fauna, at ambient (lagoon) temperature and salinity, and provided, in excess, with a natural diet of *C. linum* and other lagoon macrophytes. Animals for population dynamics studies were immediately fixed in 10 % formalin.

Salinity and temperature of the lagoon and adjacent sea were recorded on sampling visits (presented in Chapter 2). In the lagoon, the measurements were taken from approximately the midpoint in the water column, although there is little stratification in the lagoon because of the shallow nature and mixing effects of the wind (Al-Suwailem 1991). The measurements from the sea were taken from the shore, at a substantial distance from the lagoon outlet (Figure 3.3) in order to prevent effects of water exchange between The Solent and the lagoon influencing the results.

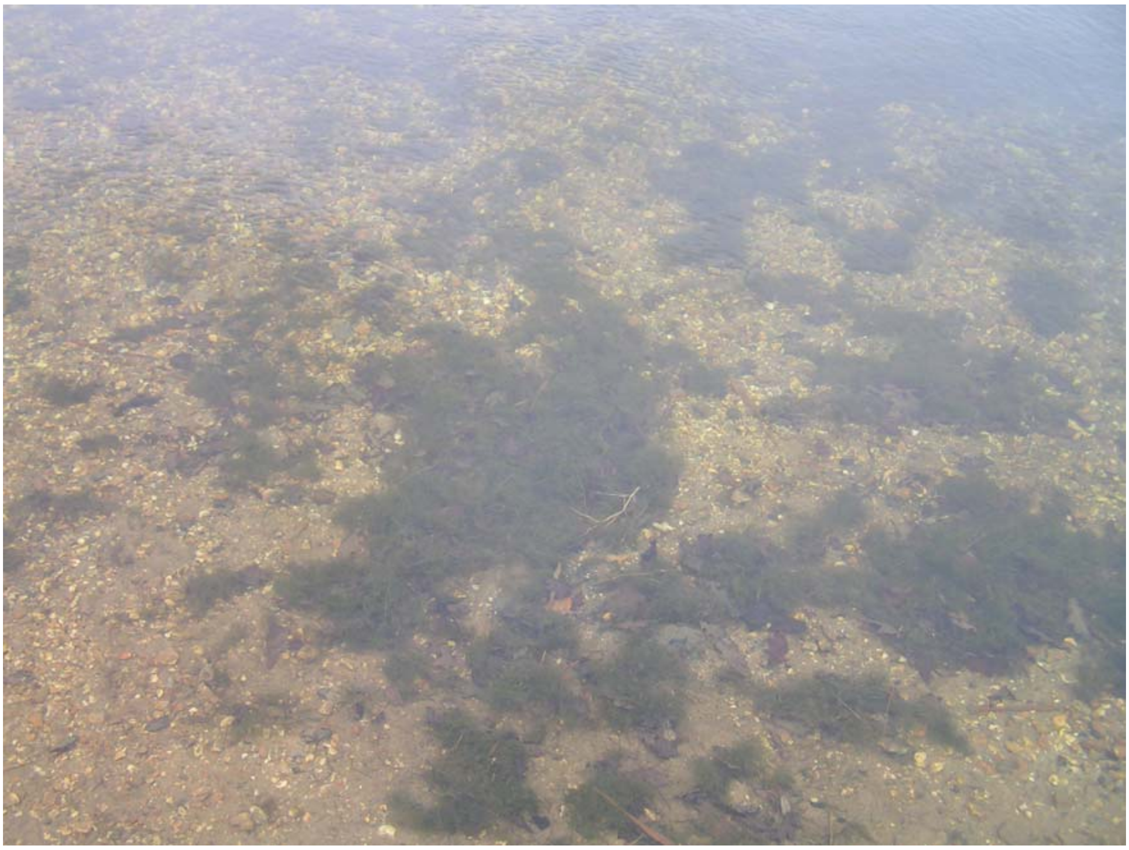

Figure 3.2: Chaetomorpha linum in a shallow area of Gilkicker lagoon, a typical habitat for *Gammarus insensibilis* and *Idotea chelipes*

Figure 3.3: The water outlet from Gilkicker lagoon at low tide

3.2. *Population dynamics and reproductive investment*

The majority of work in this study focused on *Gammarus insensibilis*. This section briefly describes some of the important aspects of the biology and anatomy of *G. insensibilis* as well as the general methods that were used regularly in the following chapters. The basic data that were obtained and analysed to determine how various parameters were presented are also explained. Any additional, specific or alternative methodological issues are addressed in the chapters for which they are necessary.

3.2.1. Population structure

Samples of between 100 and 200 animals were taken monthly. Each individual animal was measured for its head, first pereon segment and body length (anterior end of head to distal end of telson) as indicated in Figure 3.4. They were measured using an eyepiece graticule, calibrated with a stage micrometer. Each animal was dried and weighed, and the ash-free dry weight (AFDW):Dry weight ratio was calculated. The animals were divided into the following categories of life history stage, as described in Table 3.1; males (m), mature brooding females (Fb), mature (empty marsupia) females, immature females (non-setose oöstegites) and juveniles (no sexual differentiation). The various stages of oöstegite development are shown in Figure 3.5, the oöstegites of immature females are represented by stages a – c, while those of mature females have fully developed oöstegites as represented by stage d. Use of the term mature females in this study refers to animals with setose oöstegites.

Data collected from these observations were used to monitor sex ratio, size at maturity and the reproductively active proportion of the population. As it is possible to determine the sex of males at a smaller size than females (earlier development of secondary sexual characteristics) the sex ratio was monitored for animals over 7 mm in length (the size above which all animals were of determinable sex). Following recent publications, animals were also examined for intersex, an apparently common phenomenon in amphipods under stressful environmental conditions, often associated with pollution events (Ford et al. 2003b).

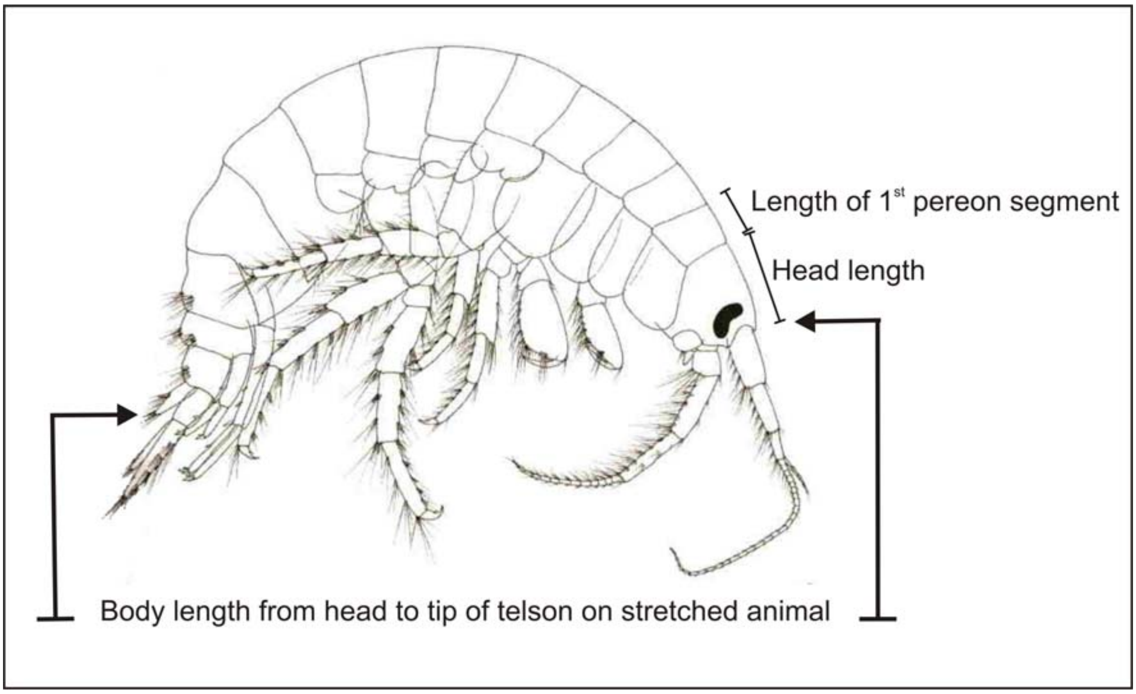

Figure 3.4: The length measurements taken on the *G. insensibilis* specimens (after Lincoln, 1979)

Table 3.1: The characteristics used to divide the populations of *Gammarus insensibilis* and *Idotea chelipes* into recognizable life stages

Life stage	**Abbreviation**	**Description**
Mature male	M	Animal with genital papillae and large gnathopods
Mature brooding female	Fb	Female carrying embryos in oostegites
Mature empty female	Fe	Mature female with no embryos in setose oöstegites
Immature female	Fi	Female with non-setose oöstegites
Juvenile	J	Animals with no visible sexual differentiation

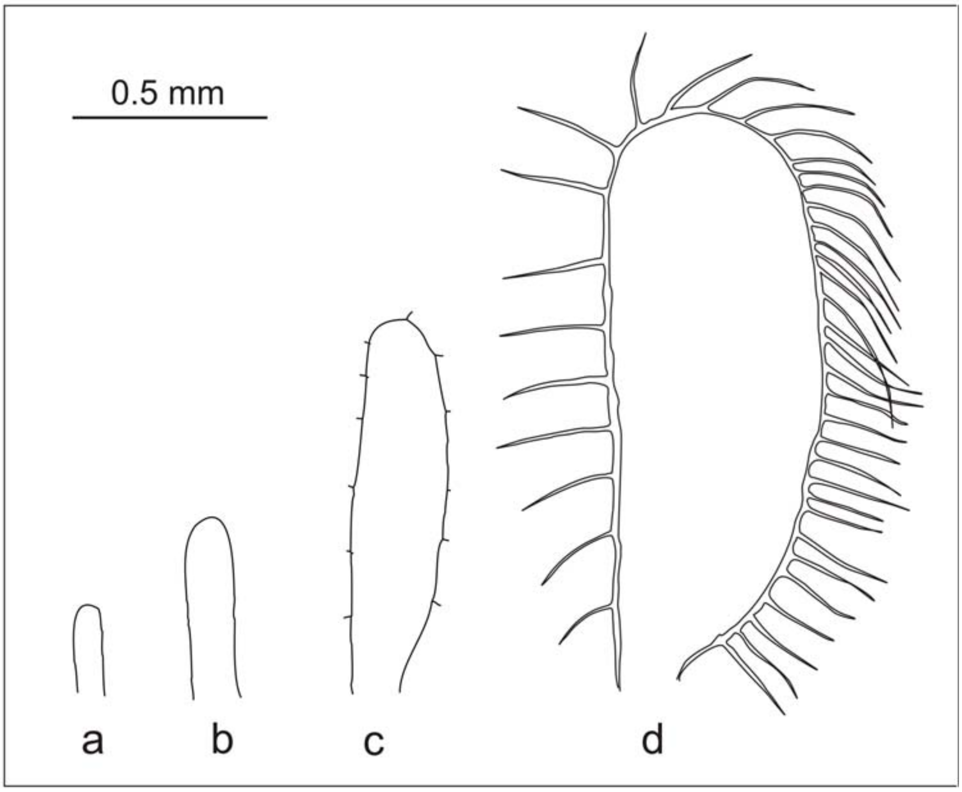

Figure 3.5: Oöstegite development in female *Gammarus insensibilis*, a – c are immature (Fi). d is from a brooding female (Fb) of 8mm from a sample taken in August 2003. Empty females (Fe) also have type d oöstegites.

The relationship between animal size and dry weight was typical for gammarid amphipods (Figure 3.6, Table 3.2).

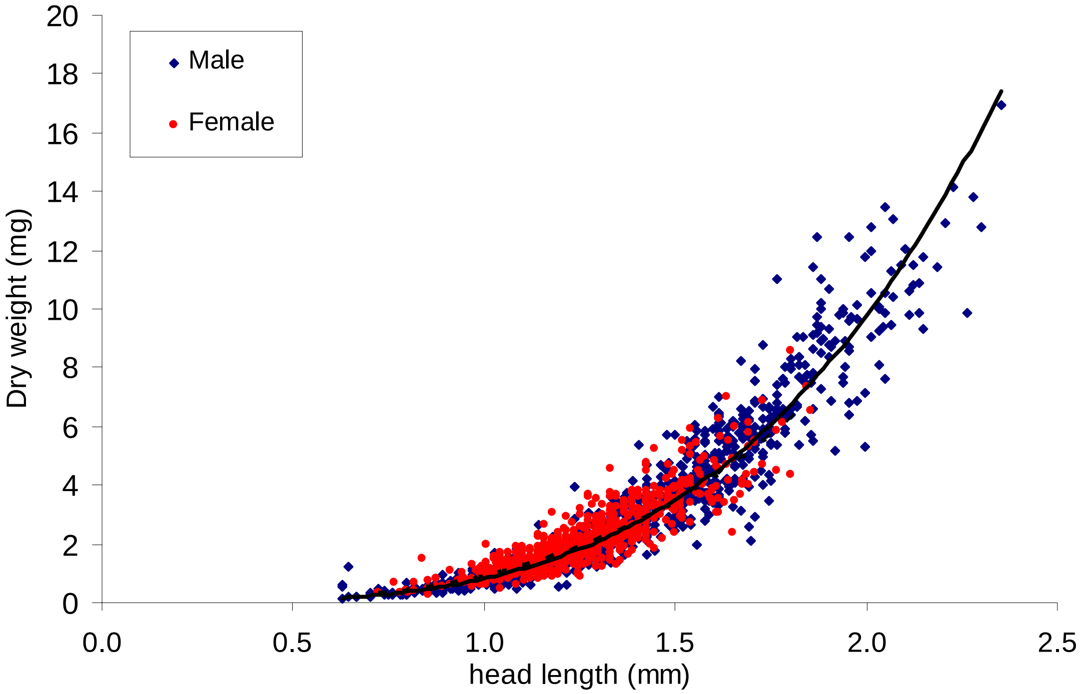

Figure 3.6: The relationship between head length and dry weight of male and female *Gammarus insensibilis*. Trendlines included; dashed line represents females while filled line represents males.

Table 3.2 The equations describing the relationship between the dry weight (DW) and head length (HL) of male, female and the whole population (including juveniles) *G. insensibilis*

Gender	Equation	r^2
Male	$DW = 0.9459\ HL^{3.1638}$	0.9459
Female	$DW = 0.9265\ HL^{3.2526}$	0.8179
All animals (inc. juv.)	$DW = 0.85277\ HL^{3.49177}$	0.9389

3.2.2. Size of individual embryos

Embryos were removed from the marsupium of live females within 24 hours of sample collection. They were counted and staged according to the method proposed by (Sheader and Chia 1970) based on 6 (I, II, III, IV, V, J) developmental stages in which the early cleavage stages are represented by stage I and newly hatched juveniles are represented by stage J. Measurements of the volume of up to 8 embryos (8 if the brood contained more than 8 eggs) from each brood were taken using the three dimensions (length, width and depth) of the embryo and the formula for an ellipsoid – Equation 3.1 (Sheader 1996; Costa and Costa 1999).

$$\text{Volume (mm}^3\text{)} = 4/3\pi abc$$

Equation 3.1: the volume of an ellipsoid (a = embryo length, b = embryo width and c = embryo depth)

The length measurements of the female were also taken (Figure 3.4). Live embryos were measured because when preserved they show variation in volume (Sheader 1996) and the egg membranes may become separated (personal observations). There was no effect of the size of the animal on the size of embryos it was incubating so it was not necessary to standardise embryo volume to female size (Figure 3.7). There was therefore no significant variation in embryo size from individual months (P > 0.05) so all eggs measured from a sample of a particular stage could be pooled to ensure maximum data.

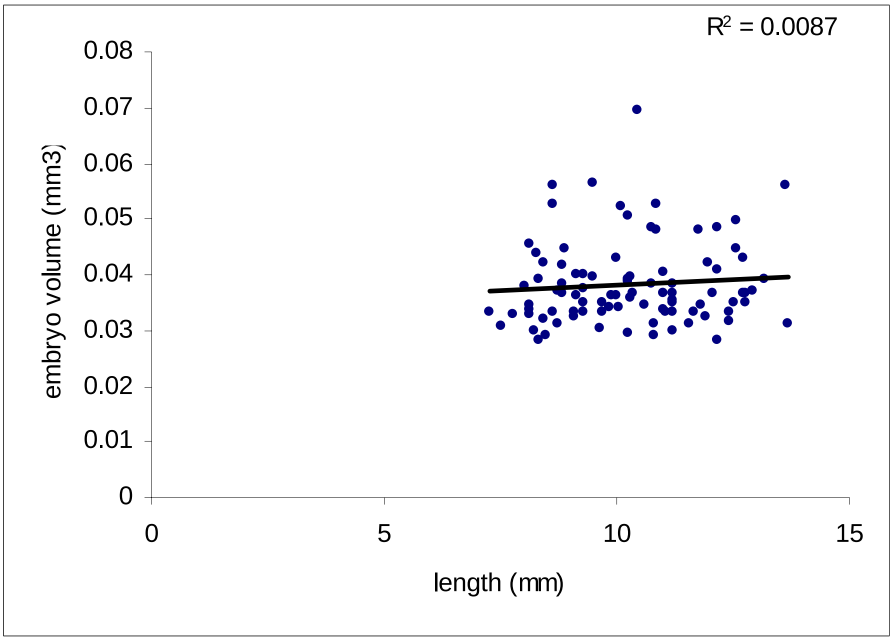

Figure 3.7: The effect of the size of female *Gammarus insensibilis* on embryo size (including linear regression trend line and r^2 value)

In agreement with Sheader (1996) and as would be expected during development there were significant increases in the volume of *G. insensibilis* embryos between the 6 developmental stages (Kruskal-Wallis, H = 2707.750, 4 df P = <0.001), (Figure 3.8).

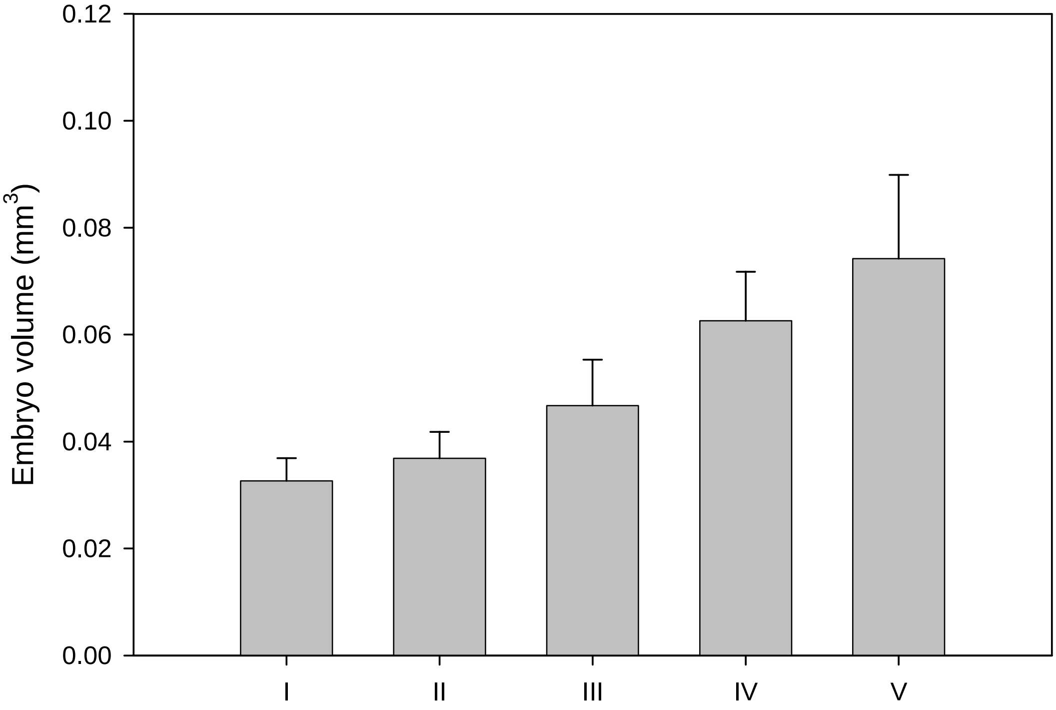

Figure 3.8: The change in embryo volume with developmental stage (± 1 sd) includes all data from all 4 seasons.

3.2.3. Brood size

Individual fitness can be increased in female amphipods by increasing their body size, because larger females are able to carry more embryos in their marsupium (Watt and Adams 1993). Figure 3.9 shows that the findings in the present study were in agreement with this. There was a significant increase in embryo number with larger body size, so for comparable analysis of reproductive investment, brood sizes were standardized to female dry body weight (mg).

Similar relationships between female body size and brood size have been described for many amphipods including estuarine *Echinogammarus marinus* (Maranhao et al. 2001), freshwater amphipods *Echinogammarus longisetosus* (Guerao 2003) and *Gammarus lacustris* (Wilhelm and Schindler 2000; Ford et al. 2003a), intertidal *Marinogammarus obtusatus* (Sheader and Chia 1970) and lagoonal *Corophium insidiosum* (Kevrekidis 2004). The studies referenced above also showed that linear regression fitted brood size to weight data best while non-linear regression with a power trend line best fitted the length measurements to brood size regression analysis.

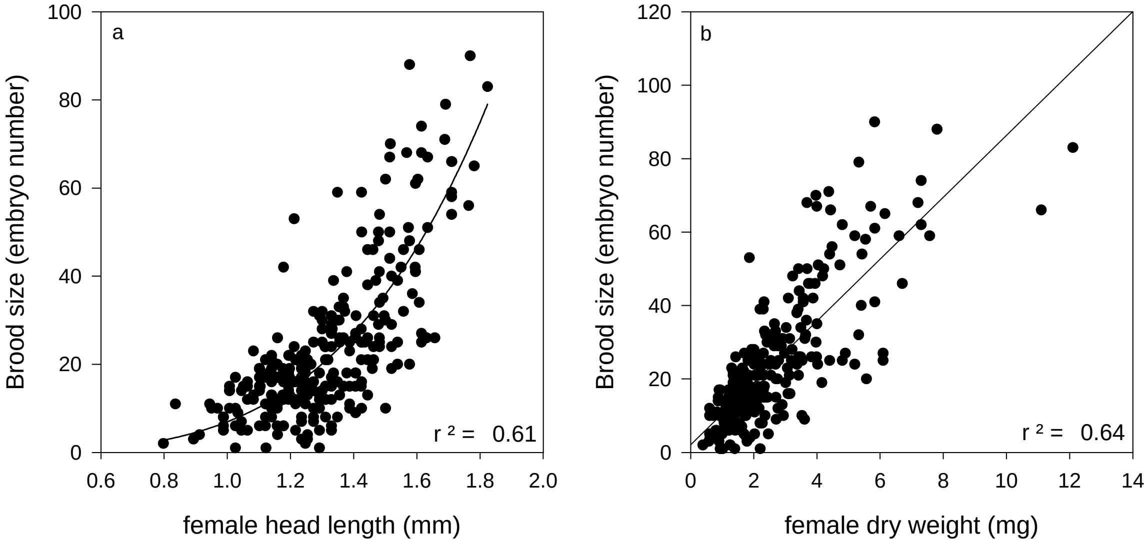

Figure 3.9: The relationship between a) female body length (head length, mm) and b) dry body weight (mg) on brood size in *G. insensibilis*; r^2 value from regression analysis is shown along with regression trend line, (n = 280).

There was no clear effect of body mass on weight-specific brood size in *G. insensibilis* (Figure 3.10). In many circumstances it is known that there are important size specific effects of body size. For example, the general trend is for larger animals to demonstrate lower size specific metabolic rates than their smaller counterparts. As there was an expected, significant effect of body size on brood size, but no size specific effect, in later chapters brood size is expressed as weight-specific brood size (embryos mg dry wt.$^{-1}$).

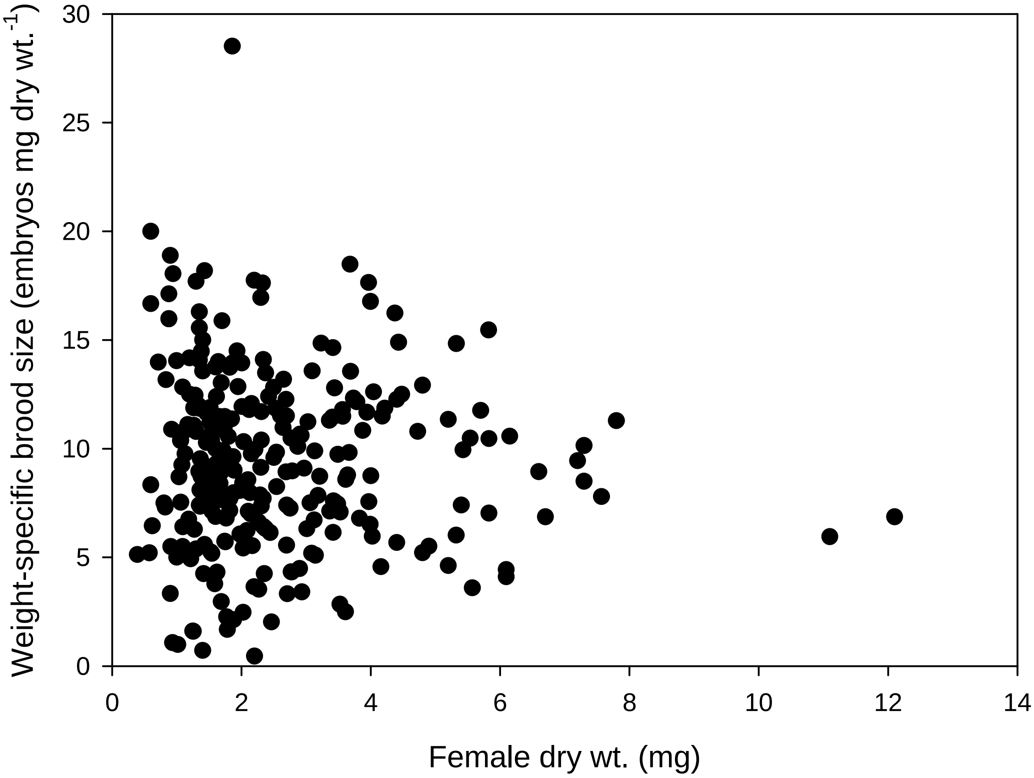

Figure 3.10: The effect of female *G. insensibilis* dry weight (mg) on weight specific brood size (n = 280).

The number of embryos and their stage of development were determined for gravid females. As previously mentioned, embryos were staged according to Sheader and Chia (1970). Brood mortality is high in some gammarid amphipods so it is not possible to use all stages in brood size analysis. In order to maximise the data available to analyse brood sizes a study was carried out to determine embryo loss during development from Stage I to J (Figure 3.11 and 3.12). Combining all data from all months (Figure 3.11) showed significant differences in the number of embryos in each stage class (Kruskal-Wallis One Way Analysis of Variance on Ranks H = 162.173 5 degrees of freedom, P = <0.001). Pairwise multiple comparison procedures (Dunn's Method) showed each of stages I-III to differ from IV-J but to be no significant difference between stages I-III. The same trends were present when the data were analysed by season (Figure 3.12). For this reason all analysis of brood sizes combined data into early (Stage I-III) and late (IV-V) stage embryos. Juveniles were generally not included as they regularly left the marsupium during the fixation process or after disturbance when working with live specimens. A similar simplified system has been used to analyse brood sizes of *Echinogammarus marinus* (Ford et al. 2003a).

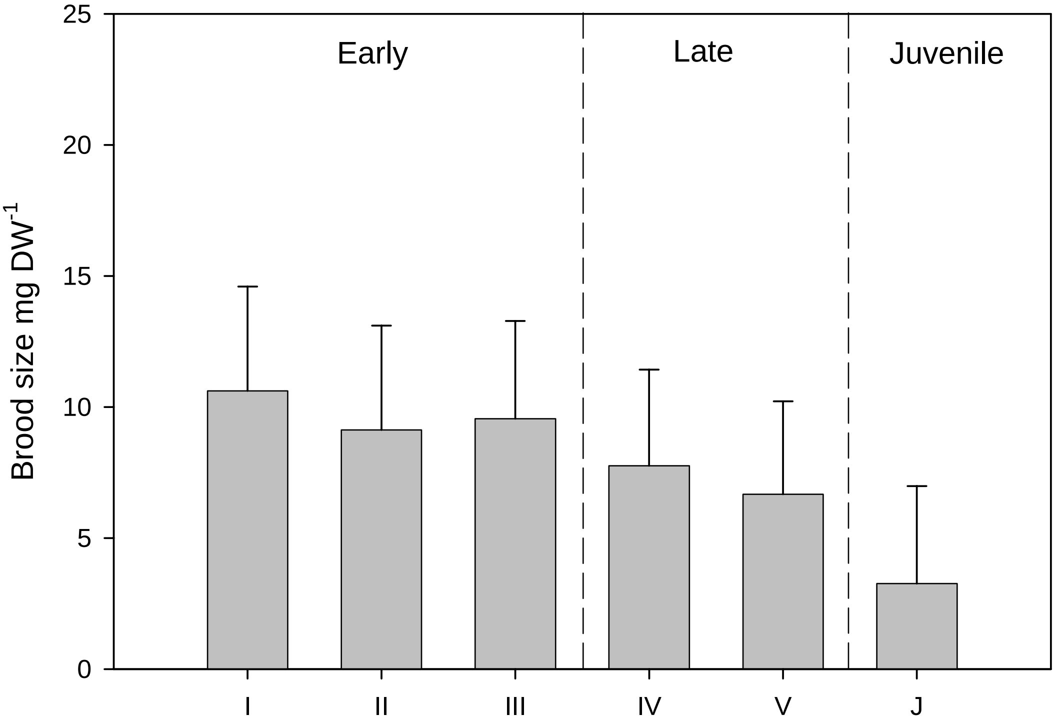

Figure 3.11: Mean weight-specific brood sizes (± 1 sd) with development of all females measured.

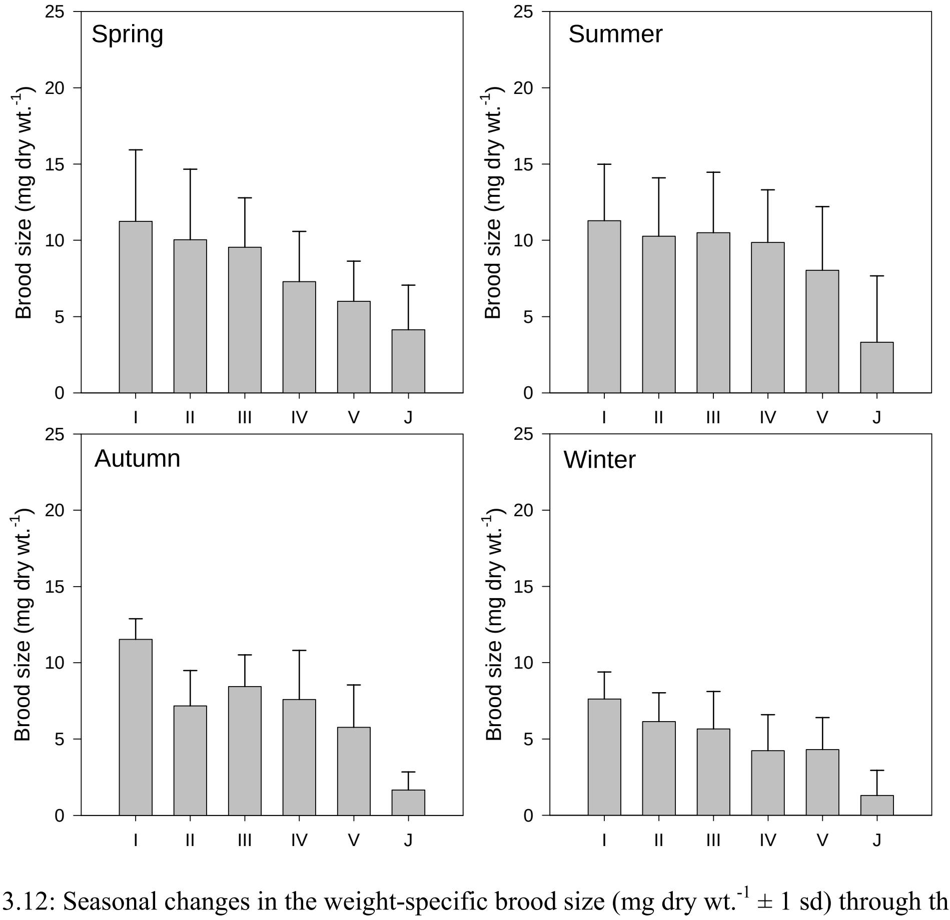

Figure 3.12: Seasonal changes in the weight-specific brood size (mg dry wt.$^{-1}$ ± 1 sd) through the embryonic developmental stages of *Gammarus insensibilis*

3.2.4. Brood mass

A separate investigation was carried out in order to determine the validity of the use of weight-specific brood size in the long term population dynamics survey. The weight of broods and individual embryos were determined using preserved animals from Gilkicker. Broods were weighed in tin foil capsules and dried for 24 hours at 50 °C.

The patterns shown by the brood mass investigation were similar to the weight-specific brood size (embryo number) trends. There was a reduction in both brood mass and embryo number with development (Figures 3.13 and 3.14). However, there was also a decrease in the weight of individual embryos with development owing to the conversion of high energy storage material of the egg into the structural material of the embryo (Figure 3.15). For the relative simplicity of measuring the volume of embryos as well as for continuity with previous work by Sheader (1996), it was decided to use this method to study the effects on investment into individual offspring rather than the use of brood mass.

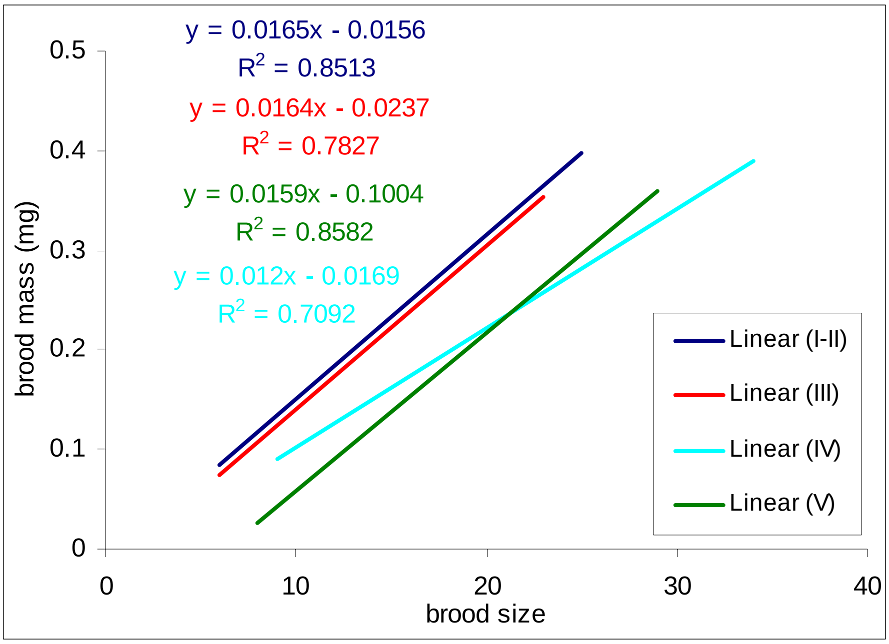

Figure 3.13: The regression lines showing the relationship between brood size (embryo number) and brood mass, line equations and r^2 values are also shown

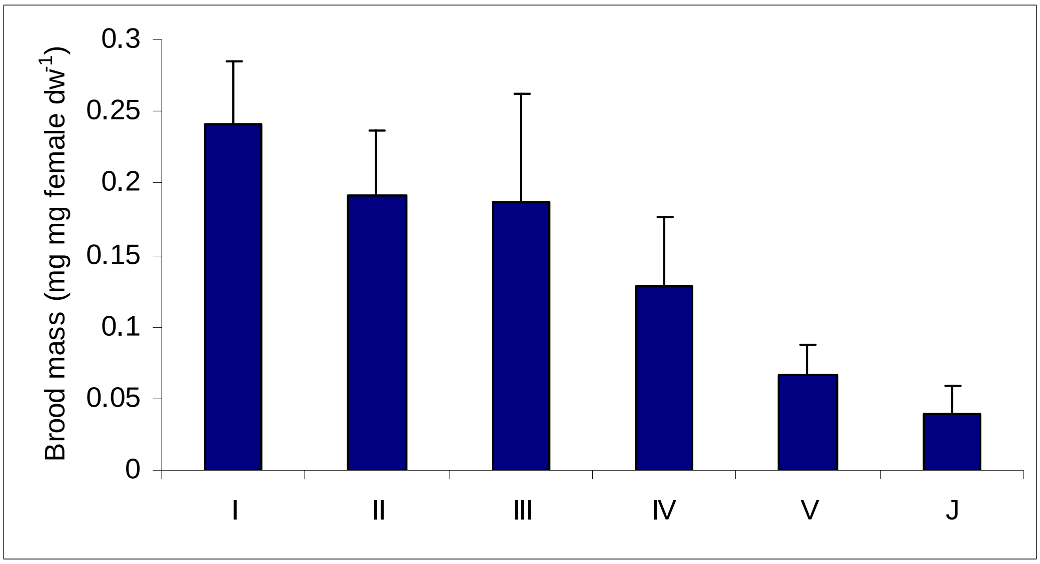

Figure 3.14: The relationship between developmental stage and mean (± 1 sd) weight-specific brood mass of female *G. insensibilis*

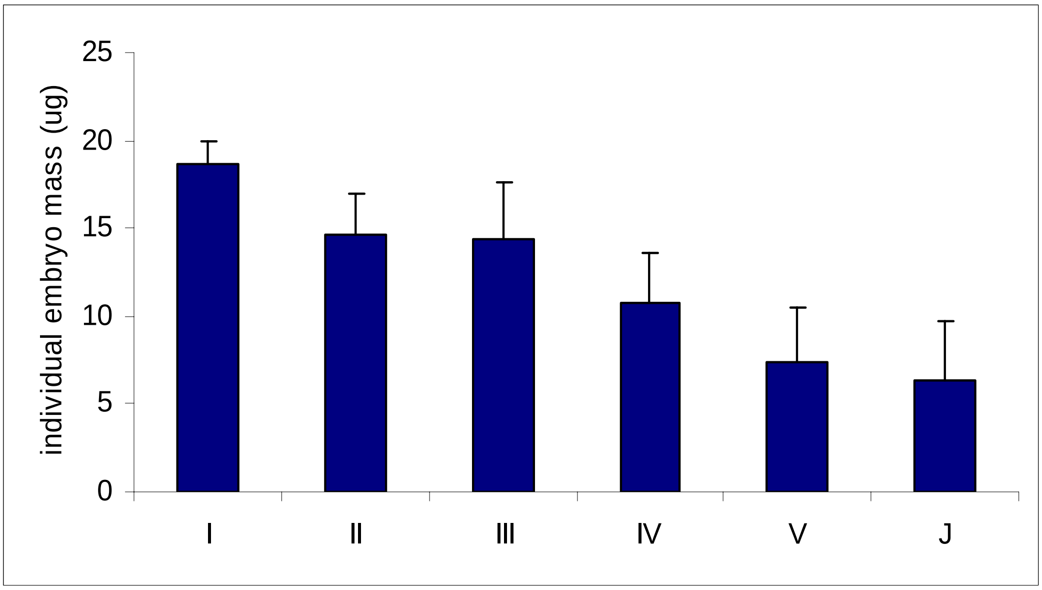

Figure 3.15: The relationship between developmental stage and mean (± 1 sd) mass of individual *G. insensibilis* embryos.

3.2.5. Clutch volume

To gain an understanding of the overall investment in reproduction, and the survival of the embryos through the brooding period, the clutch size was also determined. This represents the overall investment in reproduction, i.e. the number of embryos in the brood multiplied by the mean volume of embryos. Data from the embryo size investigation were used for this analysis.

As with brood size there was an effect of body size on clutch volume (larger females carried larger clutches – Figure 3.16). As there were significant influences of female body size on the clutch volume it was necessary in later applications of this parameter to express it as a weight specific clutch volume.

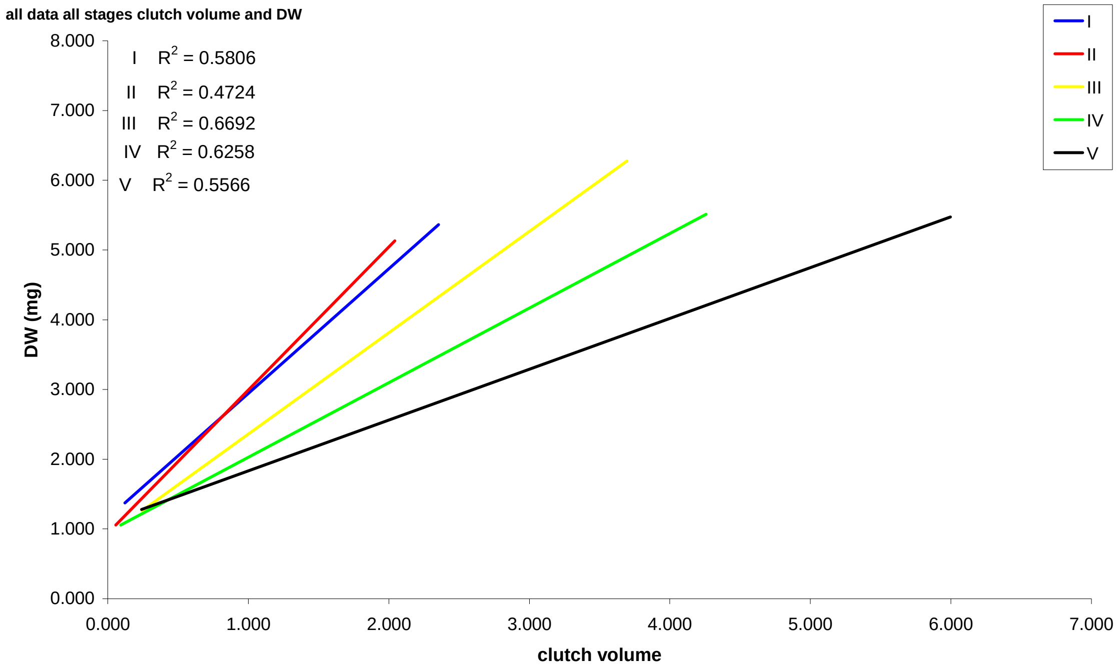

Figure 3.16: The linear regression lines showing effect of female dry weight (mg) on the clutch volume of embryos in the 5 developmental stages, r^2 values are shown for each trend line.

As expected, clutch volume also increased as the embryos developed. Combining all data from all animals measured (Figure 3.17) showed that there were significant differences between all stages apart from I and II (Kruskal-Wallis One Way Analysis of Variance on Ranks, H = 157.705 with 4 degrees of freedom. P = <0.001. Pairwise Multiple Comparison Procedures: Dunn's Method). As there was no significant difference between stages I and II these were combined for analysis of seasonal trends in clutch volume.

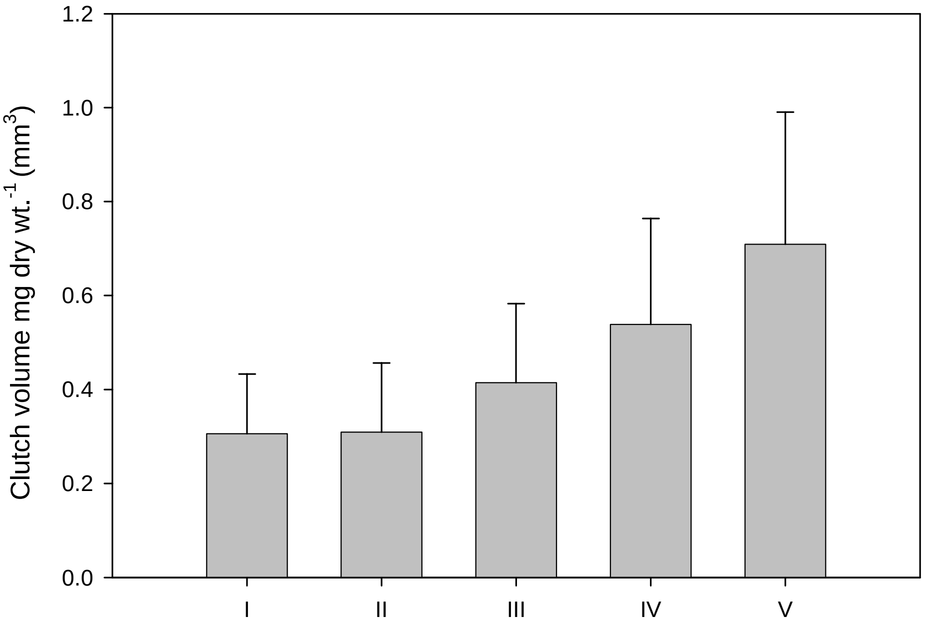

Figure 3.17: The change in weight-specific clutch volume for all specimens of *G. insensibilis* examined from all the months studied

3.3. *Bioenergetics*

The Scope for Growth assay (SFG), used by various workers (Bayne et al. 1985; Maltby et al. 1990; Lesser et al. 1994; Roast et al. 1999), was developed for the study of the energetics of *G. insensibilis*. This involved the determination of the physiological rates that make up the energy equation (Equation 1.2).

Monthly SFG assays used gravid (egg stage I-III) females with eggs removed, to standardize the stage in the life cycle of the animals. Preliminary studies showed variation in physiological rates with size and between sexes. Similarly, published evidence suggests variation in physiological rates at different stages in the moult cycle of crustaceans, for example muscle protein synthesis rates (El Haj and Houlihan 1987). The assays began within 48 hours of collection from the lagoon and were run in filtered sea water at conditions (salinity and temperature) resembling the ambient lagoon environment. Temperature was maintained (± 0.5 °C) in controlled temperature rooms in the NOC,S aquarium, salinity measured with a t/s probe and experiments were run under a 12 h light, 12 h dark regime. Assays were run for 24 hours and results were standardized to body mass. Before the assays began the animals were acclimatized to the filtered aquarium water and starved for 24 hours to ensure there were no effects of specific dynamic action.

3.3.1. Energy Loss (Respiration and Ammonia Excretion)

Air-tight control chambers containing filtered seawater and experimental chambers containing an individual amphipod were incubated for 24 hours at the temperature and salinity recorded in the lagoon when the animals were collected. After 24 hours water samples were extracted from the chambers for analysis of oxygen and ammonia concentrations.

3.3.1.1 Respiration rate

Respiration rate was determined as the difference between oxygen concentrations before (control) and after (experiment) the amphipods had been incubated in the chambers, using Winkler titrations as outlined by Parsons et al. (1984).

3.3.1.2. Ammonia Excretion rate

Ammonia excretion rate was determined as the difference between ammonia concentrations in the control chambers and the chambers containing an amphipod. The concentrations were determined using a colorimetric method (Solorzano 1969) with a CECIL Series 2 CE292 digital ultraviolet spectrophotometer. Respiration and excretion rates were converted to energy equivalents according to Bayne et al. (1985).

3.3.2. Energy Uptake (Feeding and Assimilation)

3.3.2.1. Feeding rate (C)

Feeding rate was measured using a standardised method developed to show rate of consumption of *C. linum*, the normal food under natural conditions (Sheader and Sheader 1985; Bamber et al. 2000). Animals were incubated individually in feeding chambers, with mesh bottoms to prevent the re-ingestion of faecal pellets, containing a known initial wet weight of *C. linum,* for 24 hours. Wet weights were determined using a standardised procedure to remove excess water in which the alga was placed in tissue paper for 40 seconds prior to weighing. On completion of the feeding assay the animals were removed, the final wet weight of the alga recorded using the same method and all the faeces removed. The mass of the faeces produced was also recorded. Determination of the energy content of the food source is described in detail in Chapter 5.

3.3.2.2. Assimilation efficiency

Faecal pellets were collected from the feeding chambers, rinsed in deionised water and dried to constant weight on pre-burned, pre-wieghed Whatmann GF/F filters at 60 °C. The ash free dry weight (AFDW) was calculated by heating the filters and faeces to 550 °C. The same procedure was carried out on food samples. The AFDW:DW ratio of both the food and faeces samples were then compared in order to determine the differences in organic content between the two and therefore the efficiency of digestion, presented as a percentage of the organic content of the food consumed (Conover 1966). The faeces collected from each individual were combined to for the determination of assimilation efficiency as such small quantities were produced (Roast et al. 1999a).

3.3.2.3. Assimilation Rate

The actual energy consumed (Assimilation rate - A) was determined from the rate of feeding (C) and the assimilation efficiency (e). Assimilation rate can be expressed by the following equation: $A = C \times e$.

3.4 Statistical analysis

Statistical analyses were carried out using Sigma Stat 3.0 and Sigma Plot 8.0 statistics and graphical programmes. To compare differences in means Equality of Variance and Normality tests were carried out, if the data passed these tests One-Way ANOVA was used with pairwise comparisons using the Holm-Sidak Test. If either or both tests were failed the non-parametric equivalent Kruskal-Wallis One-Way ANOVA on ranks test was used with pairwise comparisons testing using Dunn's Method. To compare the effects of both temperature and salinity, two factor ANOVA was used.

To determine relationships between parameters regression analysis was used while in order to compare the effects of both temperature and salinity on the metabolic rates multiple linear regression analyses were used.

Chapter 4

The effects of environmental factors on the reproductive investment and population dynamics of the lagoon amphipod *Gammarus insensibilis*

4.1 Introduction

The success of the Amphipoda can be attributed to some extent, to the plasticity of their reproductive strategies. The strategies employed are variable between families and species but may also vary intraspecifically and can frequently be related to the environmental conditions of their habitat (Steele and Steele 1975; Nelson 1980; Saintemarie 1991; Johnson et al. 2001). To understand the biology of a species in order to propose effective conservation measures, it is essential have an awareness of how populations of that species function over time. Although there is some published information on the population dynamics of *Gammarus insensibilis* (Karakiri and Nicolaidou 1987; Sheader 1996), it is limited and, in part, obtained from different geographical regions to that of The Solent populations. The intraspecific plasticity of reproductive strategy demonstrated by amphipods suggests that there may be few similarities in reproductive traits between the latitudinally separate *G. insensibilis* from The Solent lagoons and populations studied by Karakiri and Nicolaidou (1987) from the Mediterranean (Highsmith and Coyle 1991).

The environmental factors considered in this study were temperature and salinity. Individually or in combination, these factors have been described as “ecological master factors” for aquatic organisms (Brett 1971; McKenney 1996). Temperature effects that are important to organism function include membrane fluidity and rates of protein synthesis (Whiteley et al. 1997). Salinity is also of great consequence to aquatic organisms and frequently their osmoregulatory ability determines their distribution (Bolt 1983; Lignot et al. 2000). Both factors may have important energetic consequences for organisms, related to the costs of maintaining homeostasis. These energetic costs can be translated through levels of biological organisation leading to reduced energy available for growth and reproduction; which could ultimately, have effects at the population level.

Total reproductive investment is a combination of various components. The number of eggs in the brood, the size of the eggs, the capacity of the brood chamber, the incubation period and the number of broods produced, are all factors that combine to determine how different organisms are adapted to produce maximum surviving offspring. Over the course of a year, depending on the different stressors encountered, these factors may vary.

Perhaps the most important reproductive factor is brood size (potential fecundity). The number of embryos produced per brood ultimately determines potential offspring survival. Intraspecifically, amphipod brood size is directly related to body size (marsupium volume limits the number of embryos that can be carried) so increased body size increases potential reproductive output (Steele and Steele 1975; Nelson 1980; Van Dolah and Bird 1980; Johnson et al. 2001).

Trade-offs between investment in individual offspring and in numbers of offspring have been discussed at length in the literature (Steele and Steele 1975; Nelson 1980; Van Dolah and Bird 1980; Kolding and Fenchel 1981; Stearns 1992; Sheader 1996; Glazier 2000; Johnson et al. 2001). When the young are likely to encounter stressful environmental conditions it would appear beneficial to invest more resources into fewer individuals to increase their chance of survival, rather than produce many animals poorly prepared for the conditions (Williams 1994). It would be expected that total reproductive output (clutch volume or brood mass) would remain constant despite variations in either individual egg size or fecundity, if there was a fixed amount of energy available for reproduction (egg size x egg number = constant). The evolutionary stable investment would be the situation where effective fecundity (most surviving offspring) is maximised (Kolding and Fenchel 1981). This may vary over the course of the year depending on how environmental conditions affect juvenile survival (Figure 4.1), for example if there is lower survival in winter, selection would favour a strategy where the chance of juvenile survival is increased e.g. larger eggs (Skadsheim 1984). The winter and summer divide in the model in Figure 4.1 represents any situation where there are times of differing stress levels that have an effect on survival of the offspring.

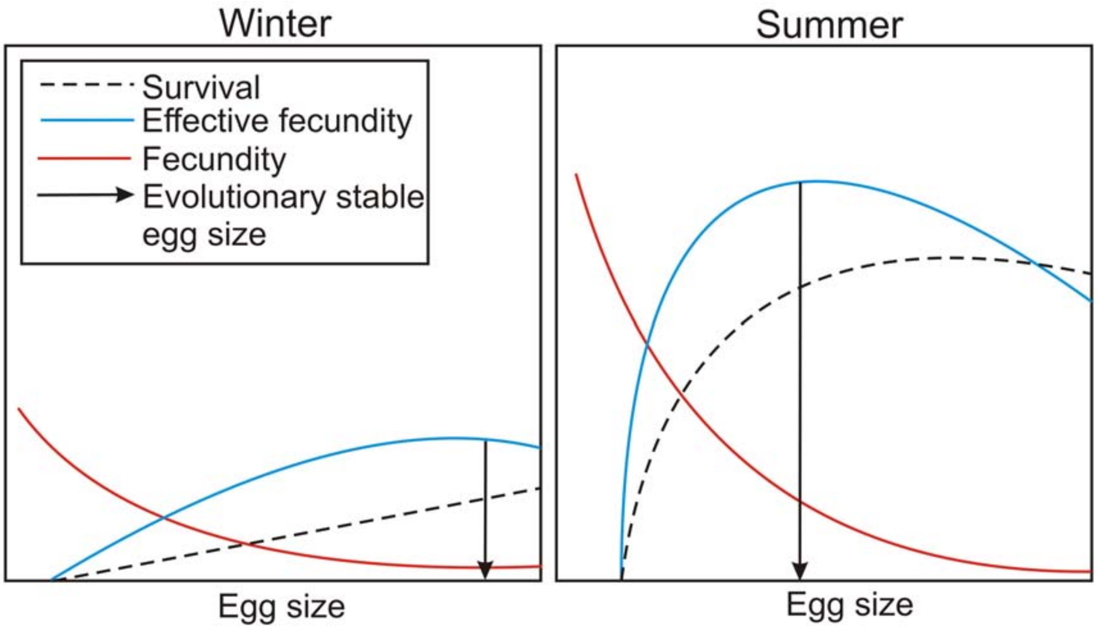

Figure 4.1: A model of the trade off between investment into individuals and investment into numbers. After Kolding & Fenchel (1981) and Skadsheim (1984)

There is frequently loss of amphipod embryos from the marsupium as they increase in size during development; this may be the result of maximum capacity of the brood chamber being reached or it may be because of stress induced consumption of the embryos by the mother. Whatever the reason for the loss, there are important bioenergetic considerations; there are values of up to 38% loss quoted in the literature for *Gammarus duebeni* (Sheader 1983). If 30 % of the energy allocated for the reproduction component of the energy balance equation (Equation 1.1) is wasted, animals under severe energetic stress are likely to be compromised.

The analysis of the population structure over the course of a year is important to understand how the environmental effects on reproductive investment are translated up to the higher levels of biological organisation. The age at maturity, sex ratio, proportion of the population that were reproductively active and percentage that were juvenile give insights into the times of the year when the population maybe more prone to suffer from the effects of stressors (Naylor et al. 1990).

This study monitors the reproductive investment made by female *G. insensibilis* in relation to the environmental temperature and salinity of Gilkicker lagoon. There is also some consideration of how the structure of the population varies over the year and whether the environmental effects on reproduction drive any changes in population structure.

4.2. Methods

Samples of *Gammarus insensibilis* were collected on a monthly basis from amongst the *Chaetomorpha linum* in Gilkicker lagoon. The studies of brood size and population structure were carried out on preserved specimens. All animals in a sample were measured, sexed and weighed. The reproductive condition of the female specimens was determined based on the categories described in Chapter 3 (brooding – Fb, immature – Fi and those with fully developed oöstegites but no eggs – Fe). Embryos in the marsupium were counted and staged and the brood size described standardized to female dry weight.

Embryo volumes were measured in live animals to ensure the dimensions were not affected by preservation techniques. The clutch volume was also determined using live animals, by multiplying the mean size of the individual embryos from each animal by the number of embryos in the brood. Population structure data was presented as percentage representations of the population rather than actual numbers because of the non-quantitative sampling method.

While division of the year into calendar months and seasons is purely arbitrary from the perspective of amphipod life cycles and reproductive biology (complex interaction of inherent genetic and external environmental stimuli determine such cycles) it is important for conservation management of natural systems to assess the general temporal trends. For this reason data were presented on both a monthly and seasonal basis. Monthly data represent the sample collection regime while for seasonal analyses data were divided into Spring (March, April and May), Summer (June, July and August), Autumn (September, October and November) and Winter (December January and February).

On each sampling visit temperature and salinity measurements were taken in the lagoon and from the beach of the adjacent Solent (presented in Chapter 2). Observations were also made of the distribution and abundance of the *C. linum*.

4.3 Results

4.3.1. Summary of reproduction over all months studied

Gammarus insensibilis reproduced throughout the year. The largest brood size measured was 90 embryos. The mean brood size across all stages for the year was 20.72 embryos, ranging from 26.78 at stage I to 16.54 at stage V. Sixty three percent of all females were brooding over all the data recorded. The mean size of females with developed oöstegites was 9.52 mm with the smallest reproductive female measuring 5.26 mm. The overall sex ratio (male:female) of 1.01 suggests no sex bias over time.

A summary of seasonal reproductive traits can be seen for *Gammarus insensibilis* in Table 4.1, additionally, similar data from the literature for some other gammarid amphipods. Table 4.1 gives an indication of the inter- and intraspecific variation in a number of reproductive parameters, both geographically and seasonally, that occurs within the framework of the marsupial brooding method of offspring care characteristic of gammarid amphipods and other peracarid crustaceans. The *G. insensibilis* data collected in this study were comparable with previous studies of the same organism (Karakiri and Nicolaidou 1987; Sheader 1996). Most of the other gammarids listed show similar trends to those seen in *G. insensibilis*. Reproductive females tend to be larger at lower temperatures e.g. *G. duebeni* (Steele and Steele 1969) with smaller embryos and larger broods in summer (or high laboratory temperature) and larger embryos and small broods in winter (or low temperature), notably *Echinogammarus marinus* (Maranhao et al. 2001).

Table 4.1 Reproductive investment by several species of gammarid amphipods. Values from the literature are as published, values from this study are means (± sd, where known)

Species	**Temperature °C**	**Salinity**	**Female length (mm)**	**Gravid %**	**Brood size (egg number)**	**Egg volume (mm^3)**	**Location**	**Reference**
Gammarus insensibilis	7.6-19.1	33.1-6.2	10.78 (±2.0)	64	23.49 (±14.2)	0.034 (±0.003)	Gilkicker Lagoon	this study
Gammarus insensibilis	19.5-27.7	36.2-9.5	9.19 (±1.6)	64.51	20.62 (±14.9)	0.033 (±0.004)		
Gammarus insensibilis	8.4-16.5	31.5-6.1	8.80 (± 0.95)	60.71	14.14 (±8.3)	0.037 (± 0.004)		
Gammarus insensibilis	2.1-9.3	24.5-2.8	10.27 (±0.97)	45.99	17.38 (±8.0)	0.040 (± 0.004)		
Gammarus insensibilis			4-14				Black Sea	(Greze 1972)
Gammarus insensibilis	winter	Brackish			e.g. 84	0.054 (max st1)	Gilkicker Lagoon	Sheader 1996
Gammarus insensibilis	summer					0.034 (min st1)		
Gammarus insensibilis		14-37			30-60		Mazoma Lagoon, Greece	Karakiri & Nicolaidou 1987
Gammarus locusta					max 143		England	(Sexton 1924)
Gammarus locusta			7-16		max 156		Sado Esturary, Portugal	(Costa and Costa 1999)
Gammarus locusta		Brackish	9.4		40.2		Poland	(Jazdzewski 1973)
Gammarus locusta		32	10.04 (±1.0)	47.3 ±12	21.4 (±12.7)	0.063 (±0.01)	Sado Estuary, Portugal	(Costa et al. 2005)
Gammarus locusta		32	9.96 (±0.7)	27.6 ±20	17.7 (±6.4)	0.066 (±0.01)		
Gammarus duebeni	summer	Brackish	8.7		10-60	0.061 (min st2)	River Test, UK	(Sheader 1983)
Gammarus duebeni	winter		9.5			0.076 (max st2)		
Gammarus duebeni	14	2-32	10.5				Conception Bay, Canada	(Steele and Steele 1969)
Gammarus duebeni	2	2-32	11.5		29			
Gammarus lawrencianus		2.5	7		6-18		Avalon Peninisula, Canada	(Steele and Steele 1991)
Gammarus lawrencianus		25	7		9.5-27			
Echinogammarus marinus	10	4-31			30		Mondego Estuary, Portugal	(Maranhao and Marques 2003)
Echinogammarus marinus	15	4-31			20			
Echinogammarus marinus	20	4-31			10			
Echinogammarus marinus	7.5-20	0.5-20			10-31		Mondego Estuary, Portugal	(Maranhao et al. 2001)
Echinogammarus marinus	10-24	15-30			14-38			
Echinogammarus marinus	20-30	20-30			6-27			
Echinogammarus marinus	24-13	0-26						

4.3.2. Monthly brood sizes

There was an annual cycle of early stage (I-III) weight-specific brood size (mg dry wt.$^{-1}$) that generally followed the temperature and salinity curves with the smallest weight specific brood sizes occurring in the winter months (Figure 4.2). The smallest broods were produced in the winter while those produced in the summer were larger. The difference in brood size through the year was significant (Kruskall Wallis One Way ANOVA on ranks H = 98.092 with 15 degrees of freedom P = <0.001). Pairwise analysis (Dunn's method) showed June, May, April and July 2004 to differ significantly from December 2003, January and February 2004. The largest weight-specific brood sizes (mg dry wt^{-1}) were recorded in the spring and early summer (April, May and June). This trend was verified when these data were combined and analysed seasonally (Figure 4.3 b). Brood sizes varied significantly on a seasonal basis (One Way ANOVA $F_{3, 113}$ = 3.801, P = 0.012) with All Pairwise Multiple Comparison Procedures (Holm-Sidak method) showing spring, summer and autumn to all differ significantly from winter.

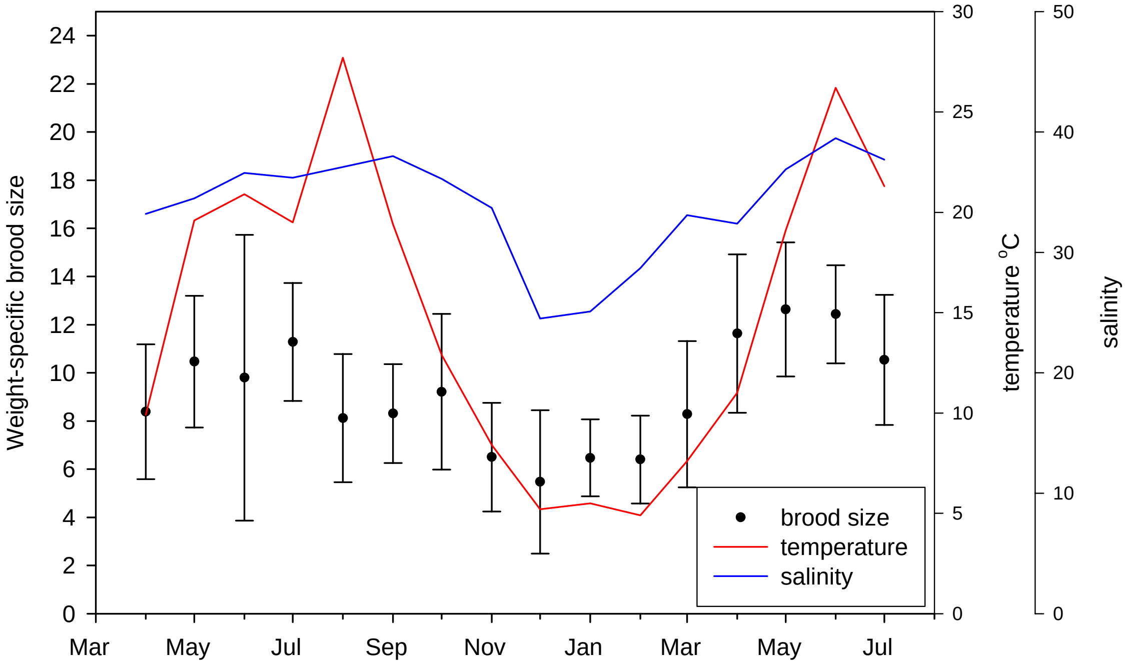

Figure 4.2: The seasonal effects of salinity and temperature on the weight-specific size (embryos mg dry wt.$^{-1}$) of stage I-III broods (± 1 sd) produced by of *Gammarus insensibilis* from April '03 to July '04.

Late stage (IV and V) brood size showed a similar trend to that of the early stages. The late stage broods were smaller than the early stage broods and the peaks were in June of both 2003 and 2004 rather than May (Figure 4.3 a). The differences in the mean values for the 16 months in the study were significant (One Way ANOVA $F_{15, 232}$ = 8.007, P = <0.001). Pairwise comparison (Holm-Sidak) suggested significant seasonal differences in late stage brood size because the females in June in 2003 and 2004 had significantly larger late stage broods than those in August, November, December, January, February and April. The females in April, May, July and September also had larger broods than those in November and the three winter months. When analysed seasonally the same trend was highlighted (Figure 4.3 b). The late stage brood size followed the pattern of the early stages, but the broods were smaller. The peak brood size was in the summer and there were significant differences between seasons (Kruskall-Wallis ANOVA on ranks H = 30.430 with 3 degrees of freedom, P = <0.001). Multiple pairwise analysis showed the late stage winter broods to be significantly smaller than those in the rest of the year. Figure 4.12 (egg loss) shows the seasonal differences between early (I-III) and late stage (V) brood size in order to investigate the rate of embryo loss from the marsupium. It demonstrates that the percentage loss of embryos from the marsupium is greater in the spring when broods are largest, suggesting that late stage brood sizes are not as variable seasonally as the early stages.

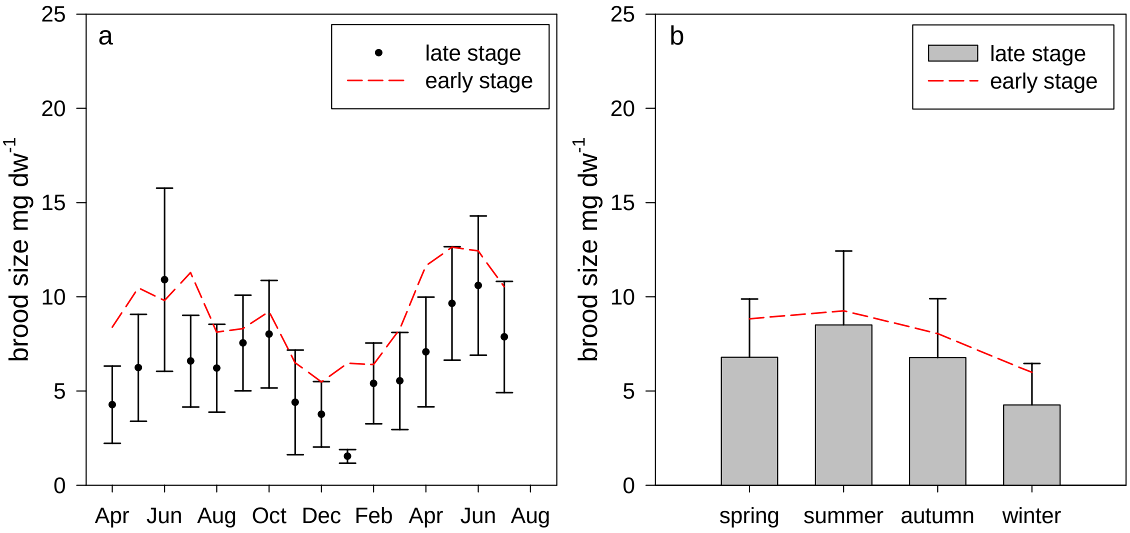

Figure 4.3: (a) monthly variation in the size of late stage brood size mg dry wt. $^{-1}$ (± 1 sd) produced by *G. insenbilis*. The equivalent early stage brood sizes are also shown (dashed line); (b) seasonal late stage weight specific brood sizes (± 1 sd). The equivalent early stage brood size is also shown.

Maximum brood size is shown in Figure 4.4 but it is not standardized to animal weight. Like the mean weight specific brood size (mg dry wt.$^{-1}$) the largest maximum brood sizes were recorded in the spring and early summer (April, May and June), after which they dropped by approximately 50 % as the year progressed through the summer and into the winter. The maximum brood sizes in the winter were only half that of those in the early summer. The largest brood sizes were produced (spring and early summer) when the largest females were present in the population (Figures 4.15 & 4.17).

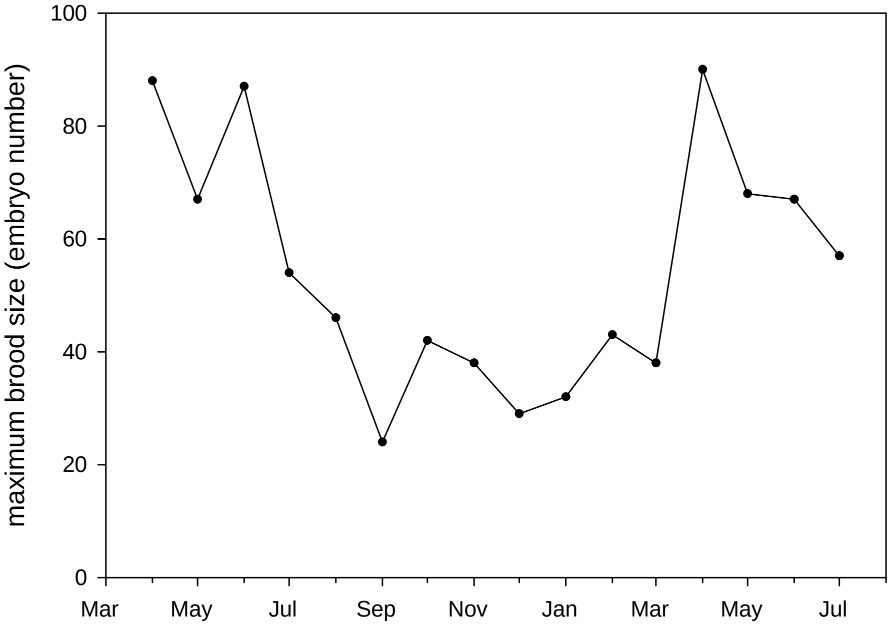

Figure 4.4: The maximum brood size carried by female *G. insensibilis* over a 16 month period, includes all developmental stages.

4.3.3. Monthly embryo sizes

The monthly trend in embryo volume contrasted to that of brood size (Figures 4.6 & 4.9). Figure 4.5 shows that data for stage I-II embryos suggested greater investment into individual embryos during the winter months while the embryos produced in the summer were relatively small; mean winter embryo volume was 0.039 mm^3 in comparison to 0.031 mm^3 in the summer while the mean brood size was approximately 35 % smaller in the winter. The variation was significant (Kruskall Wallis One Way ANOVA on ranks H = 68.755 with 13 degrees of freedom, P = <0.001). Multiple pairwise comparisons (Dunn’s method) showed the individual summer months to differ significantly from the winter months. The trend evident contrasted with the temperature and salinity curves, with small embryo sizes coinciding with high temperature and salinity.

The trend over the course of the year was verified when these data were combined and analysed seasonally (Figure 4.6 a), when the egg volumes varied significantly seasonally (Kruskall-Wallis One Way ANOVA on ranks H = 296.238 with 3 degrees of freedom P = <0.001), All Pairwise Multiple Comparison Procedures (Dunn's Method) showed each season to differ significantly from the others. The seasonal analysis of the brood and embryo size data (Figure 4.6) and monthly analysis (Figure 4.9) clearly show that embryo size decreased with increase in the brood size.

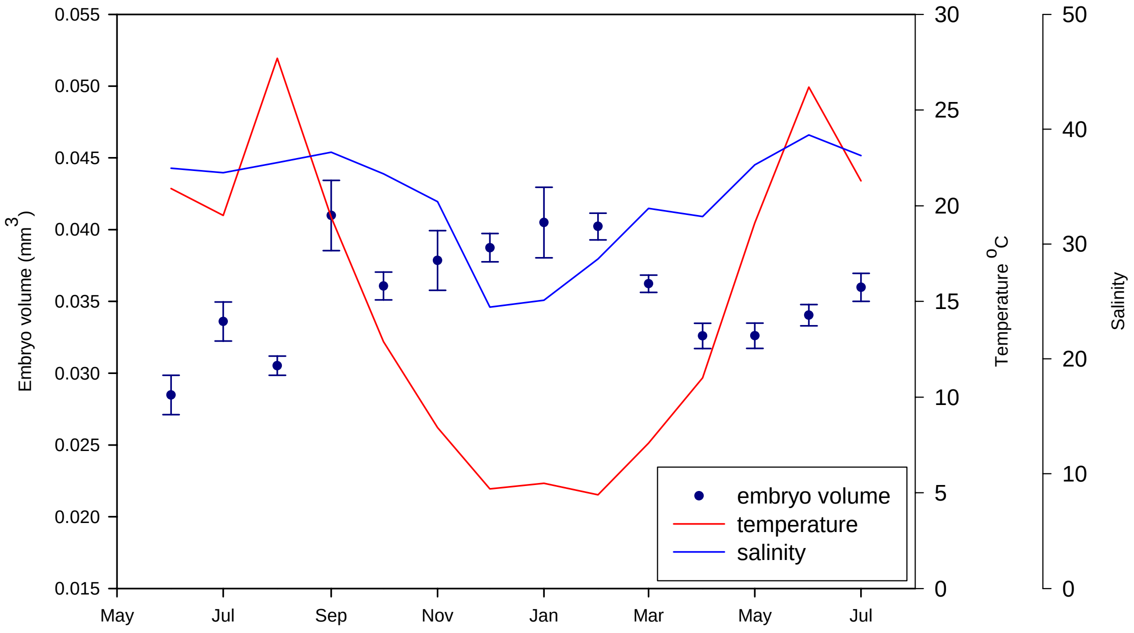

Figure 4.5: The seasonal effects of salinity and temperature on the size (± 1 standard error) of stage I-II embryos produced by *Gammarus insensibilis*.

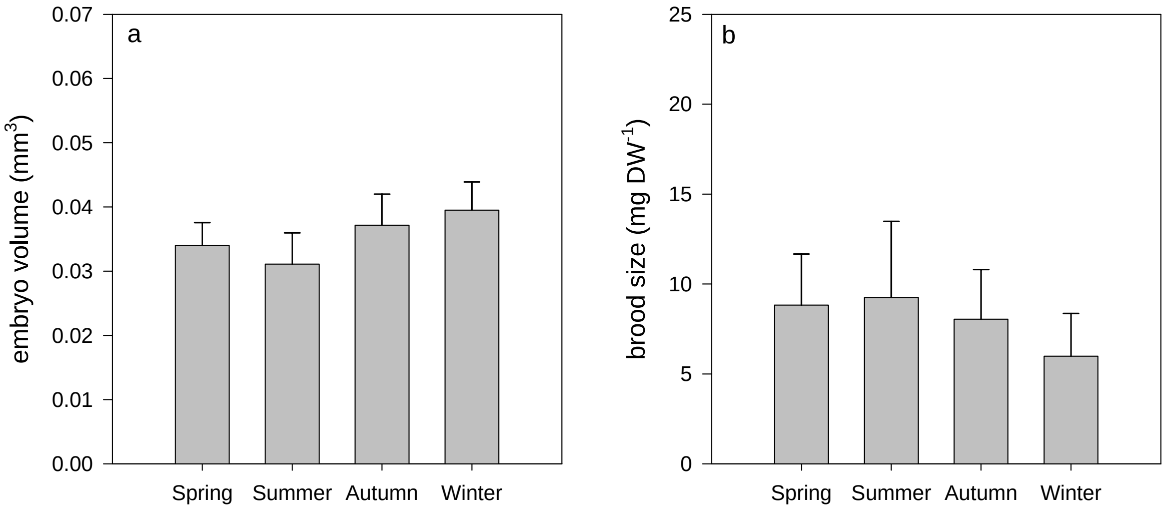

Figure 4.6: The seasonal variation in (a) mean embryo volume (mm^3) (± 1 se) and (b) mean weight specific brood size mg dry wt.$^{-1}$ (± 1 sd) for early stage embryos

4.3.4. Clutch volumes and brood sizes

Clutch volume showed less variability through the year than embryo size or brood size (Figure 4.7), there was a significant difference in clutch volume (Kruskal-Wallis One Way Analysis of Variance on Ranks H = 40.448 with 13 degrees of freedom, P = <0.001) but pairwise analysis showed that the only significant difference occurred when the peak clutch volume in May 2004 differed from December and November 2003.

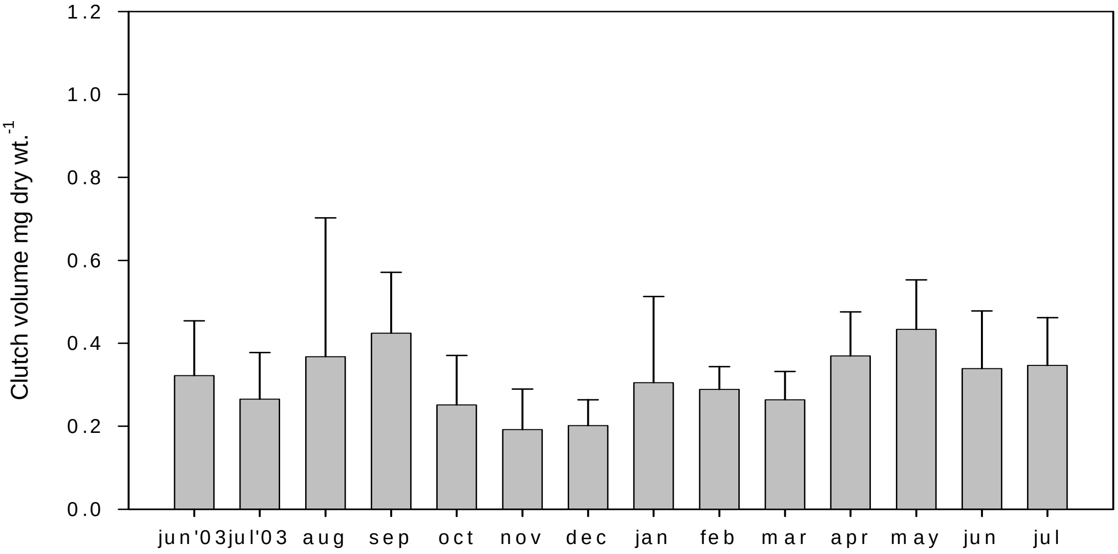

Figure 4.7: The weight-specific clutch volume (mg dry wt.$^{-1}$) of *G. insensibilis* for combined stage I & II embryos over 14 months from June 2003 to July 2004 (± 1 sd)

When the data for stages I and II were combined into seasons (Figure 4.8) there was a significant difference between (Kruskal-Wallis One Way Analysis of Variance on Ranks H = 11.121 with 3 degrees of freedom, P = 0.011). Pairwise comparisons showed spring to differ from winter; there were no other significant differences.

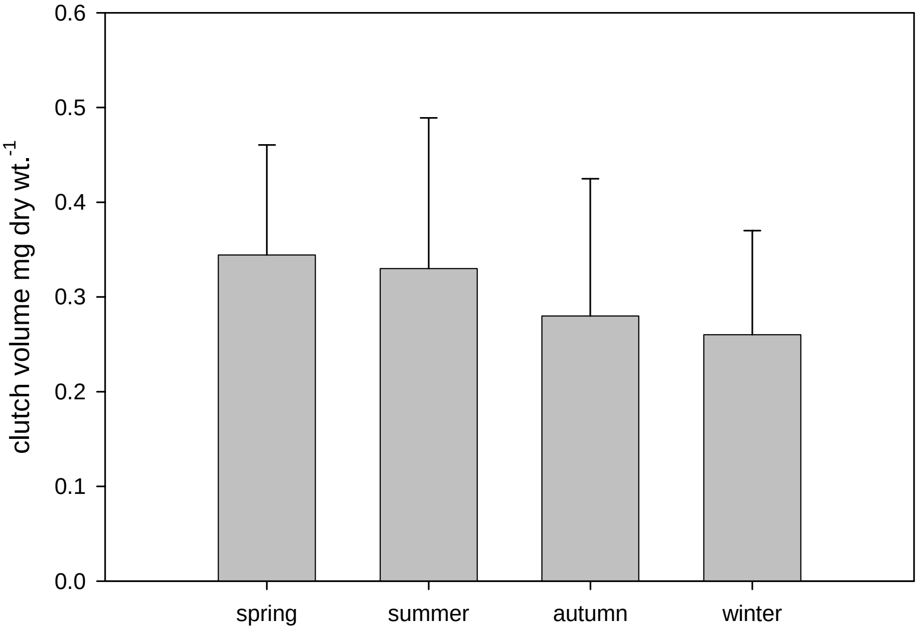

Figure 4.8: Clutch volume mg dry wt.$^{-1}$ seasonally for stages I & II combined (± 1 sd) for the 14 month period from June 2003 to July 2004

Although there was variation in clutch volume it was not as great as the variation in brood and embryo sizes, suggesting that there was a finite allocation to overall reproductive investment, within which each component varied.

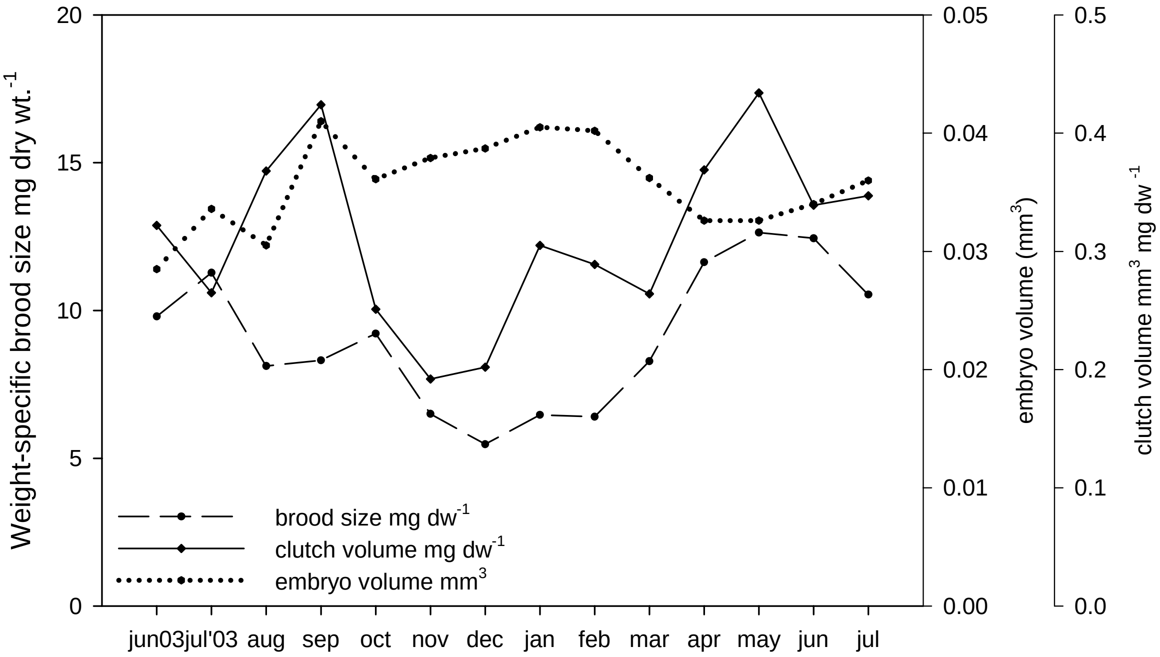

Figure 4.9: The comparative effects of time of year mean brood size, clutch volume and embryo volume in *G. insensibilis*

4.3.5. Juvenile size

It is clearly not possible, under field conditions, to estimate size of the offspring of a particular female. Despite the common behaviour of juveniles to leave the marsupium during the fixing procedure, estimates of the size of juveniles were made from those remaining in the marsupium after fixation. There was no effect of female size on the size of the juveniles yet to leave the marsupium (Figure 4.10), $r^2 = 0.0396$. All juveniles had left the marsupium before they reach approximately 2 mm in length.

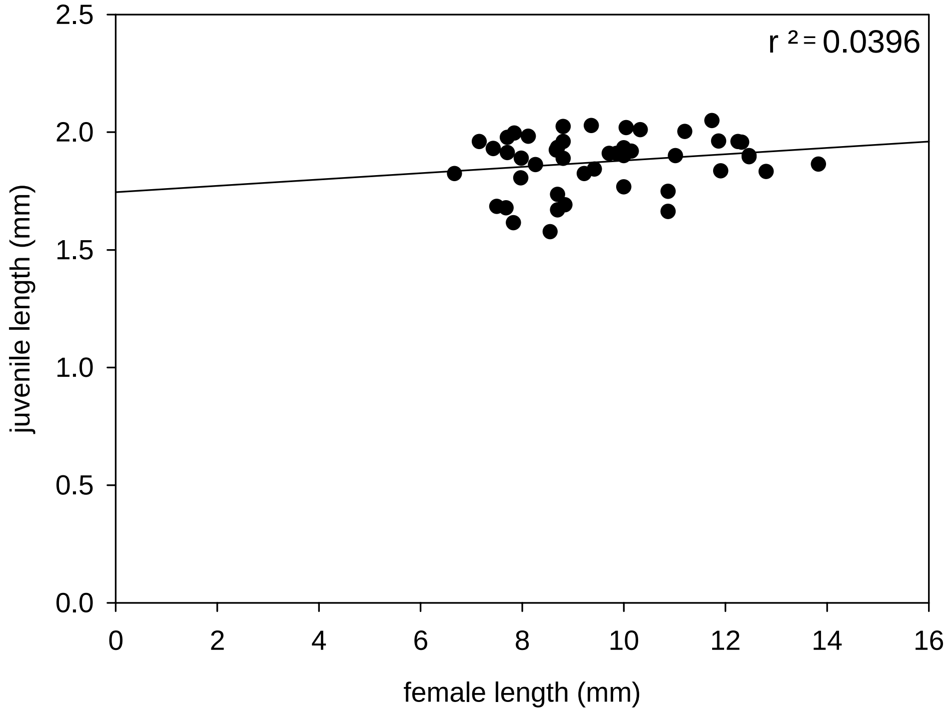

Figure 4.10: The relationship between female size and the size of their offspring in *G. insensibilis*. Trend line is linear regression line and the r^2 value for the regression analysis is shown.

There were differences in the size of juveniles in the brood chamber over the course of a year, both when these data were examined monthly and seasonally. Figure 4.11 shows the mean size of juveniles in the marsupium fluctuated around 2mm in length. On a monthly basis (Figure 4.11 a) Kruskall-Wallis One Way ANOVA on ranks showed there was a significant difference between months (H = 47.803 with 11 degrees of freedom, P = <0.001). Pairwise comparison procedures (Dunn's method) showed that there were no significant differences between most months but the juveniles were significantly larger in February, April, June and July than they were in October. In April and July the juveniles were also larger than in September. Figure 4.11 b shows the same data on a seasonal basis. Kruskall-Wallis showed a significant difference between seasons (H = 25.054 with 3 degrees of freedom, P = <0.001) with pairwise comparisons (Dunn's method) showing that the juveniles in the marsupium in autumn were significantly smaller than the other seasons. However there does not appear to be a seasonal trend in the size of juveniles in the brood chamber and the differences may be the result of small sample sizes resulting from the escape responses of the juveniles during the preservation process.

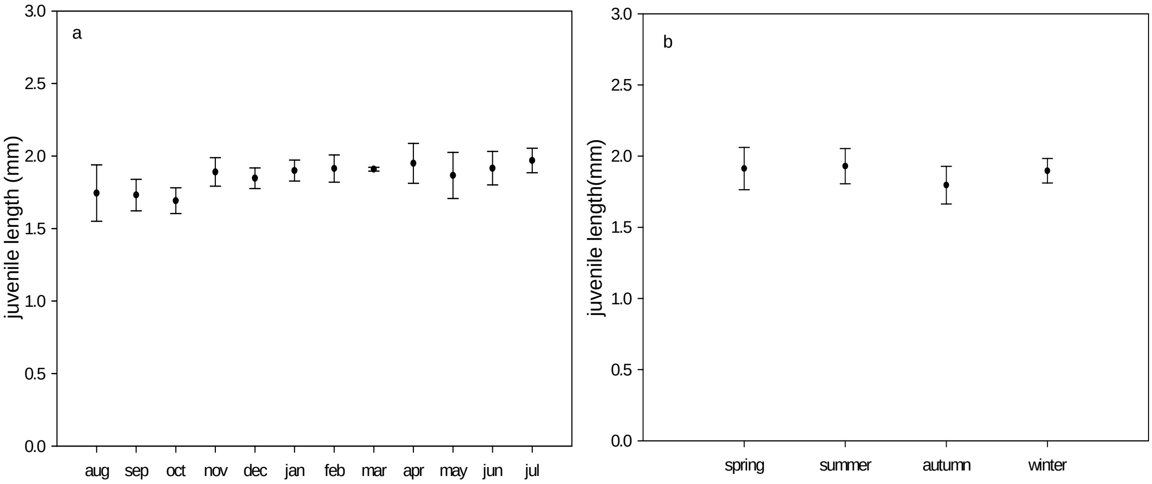

Figure 4.11: The mean size (± 1 sd) of juveniles in the brood chamber of *G. insensibilis* on both a monthly (a) and seasonal (b) basis.

4.3.6. *Loss of embryos during development and brood parasitism*

Embryos were frequently lost from the marsupium during development. When all data for stage I-III embryos were compared with stage V (inset Figure 4.12) there was a significant reduction in mean weight-specific brood size (ANOVA $F_{1\text{-}382}$ = 44.67, P<0.01). This equates to a loss of 30% of embryos from the brood chamber during development throughout the year. Embryo loss varied seasonally with the greatest percentage (40%) loss in the spring (Figure 4.12). The amount of loss from the brood means there is less variation in late stage brood size than early stage. This was verified statistically; there were significant differences in early stage brood size (Kruskall-Wallis One Way ANOVA on Ranks H = 41.274 with 3 degrees of freedom, P = <0.001) and late stage brood size (Kruskall-Wallis One Way ANOVA on Ranks, H = 11.760 with 3 degrees of freedom, P = 0.008) but pairwise analysis showed in the case of early stage embryos the spring and summer broods were significantly larger than those in the winter while summer broods were also larger than autumn broods. Late stage brood sizes did not show as much variation, significant differences were only evident between summer and winter.

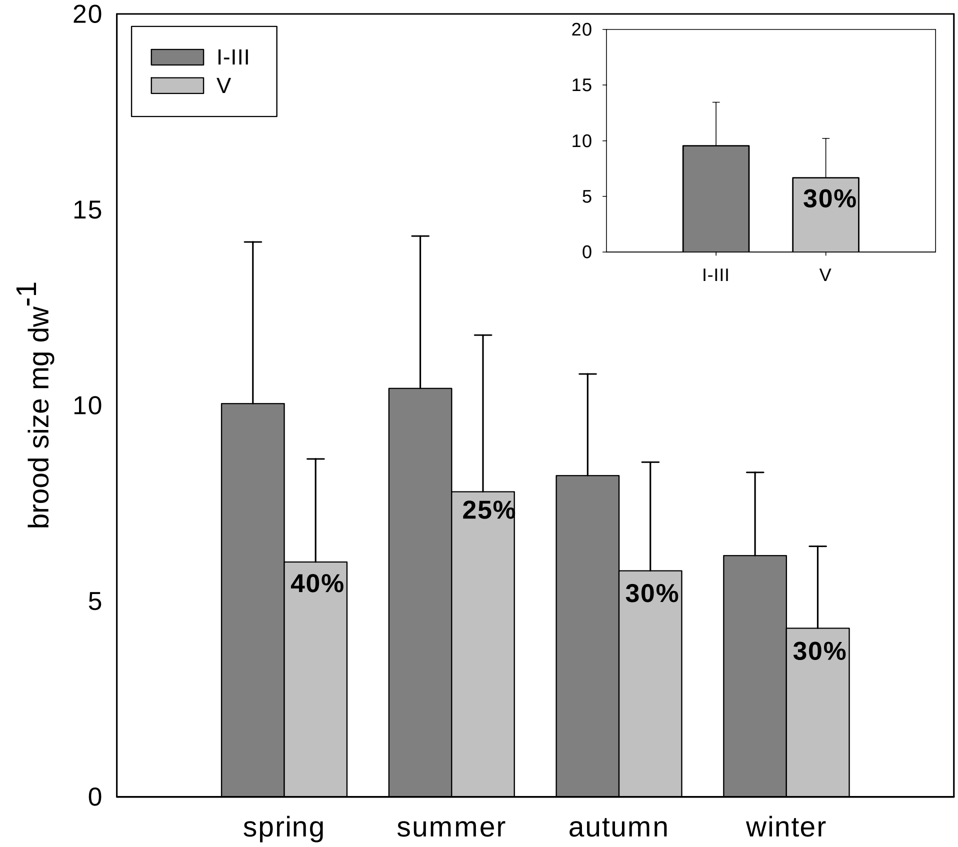

Figure 4.12: Seasonal embryo loss in *G. insensibilis*. Dark bars represent mean early stage brood size mg dw^{-1} (± 1 sd) and light bars represent late stage brood size (± 1 sd). Values on the dark bars are the percentage loss during development. The inset is the combined data for all females over a year; y axis is equivalent to main plot.

Between December and April there were a number of cases of infection of the brood by an unidentified fungus (Figure 4.13). This never exceeded 11% of the brooding female population (January) but was recorded in embryos from stage I-IV.

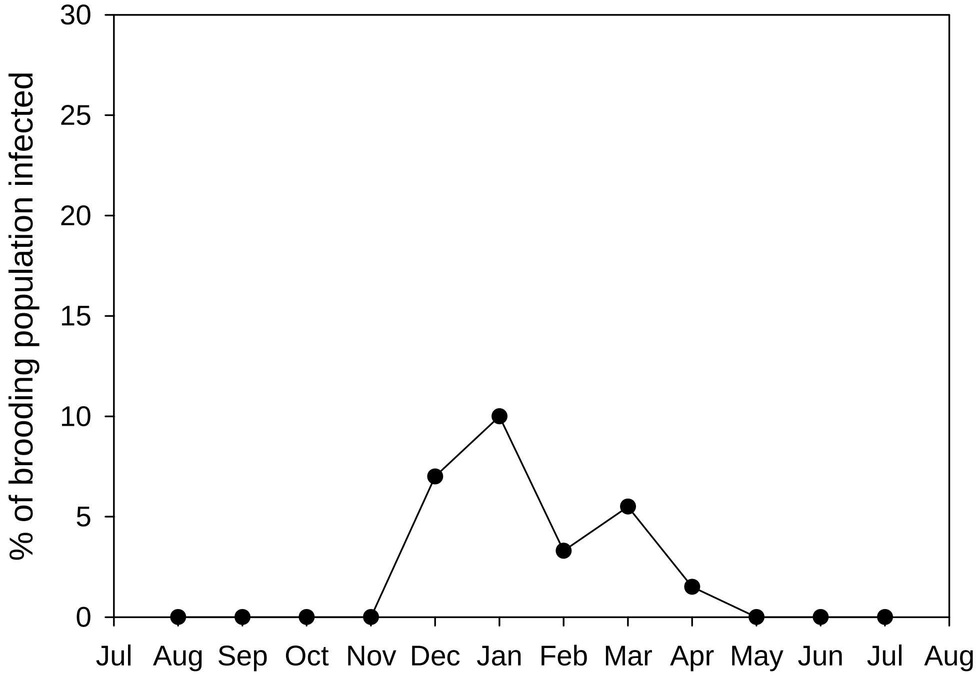

Figure 4.13: The percentage of the population of brooding female *G. insensibilis* infected by an unidentified fungal brood parasite

4.3.7. Sex ratio

The sex ratio was measured as the number of females per male, for animals over 7 mm. Animals over 7 mm in length were used because males are distinguishable at a smaller size than females. The sex ratio was variable, but over the year there was no bias towards one sex. Dominance fluctuated but overall the sex ratio was 1.01 (Figure 4.14). The absence of a seasonal trend in sex ratio suggests that temperature and photoperiod, the main environmental factors known to cause environmental sex determination (ESD), did not influence this aspect of the reproductive biology of *G. insensibilis*.

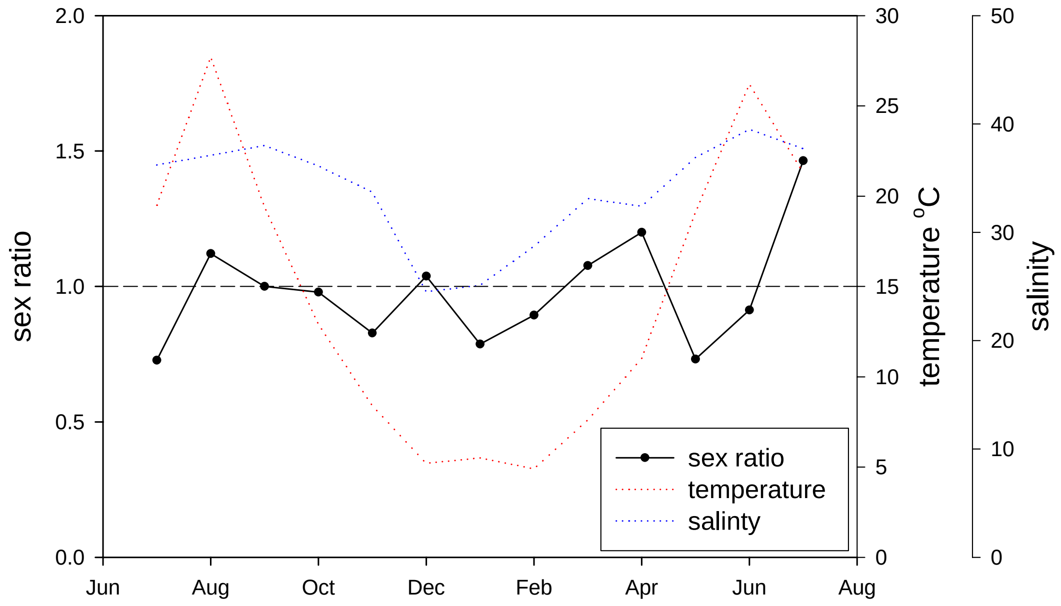

Figure 4.14: The sex ratio of *Gammarus insensibilis* (number of females per male over 7 mm)

4.3.8. Population structure

Figure 4.15 shows the mean size of reproductively active females was variable during the course of the year. Kruskal-Wallis One Way Analysis of Variance on Ranks (H = 127.307 with 13 degrees of freedom; P = <0.001) shows that the differences between months were significant, with Pairwise Multiple Comparison Procedures (Dunn's Method) showing that brooding females were larger in January and February than in the summer and autumn months, while March and April also showed larger females than some of the summer months. The dotted line on Figure 4.15 represents the smallest reproductive female recorded. The size of the smallest female neatly follows the trend for mean reproductive female size, which varied between 5.4 mm in June 2003 and 9.2 mm in January 2004.

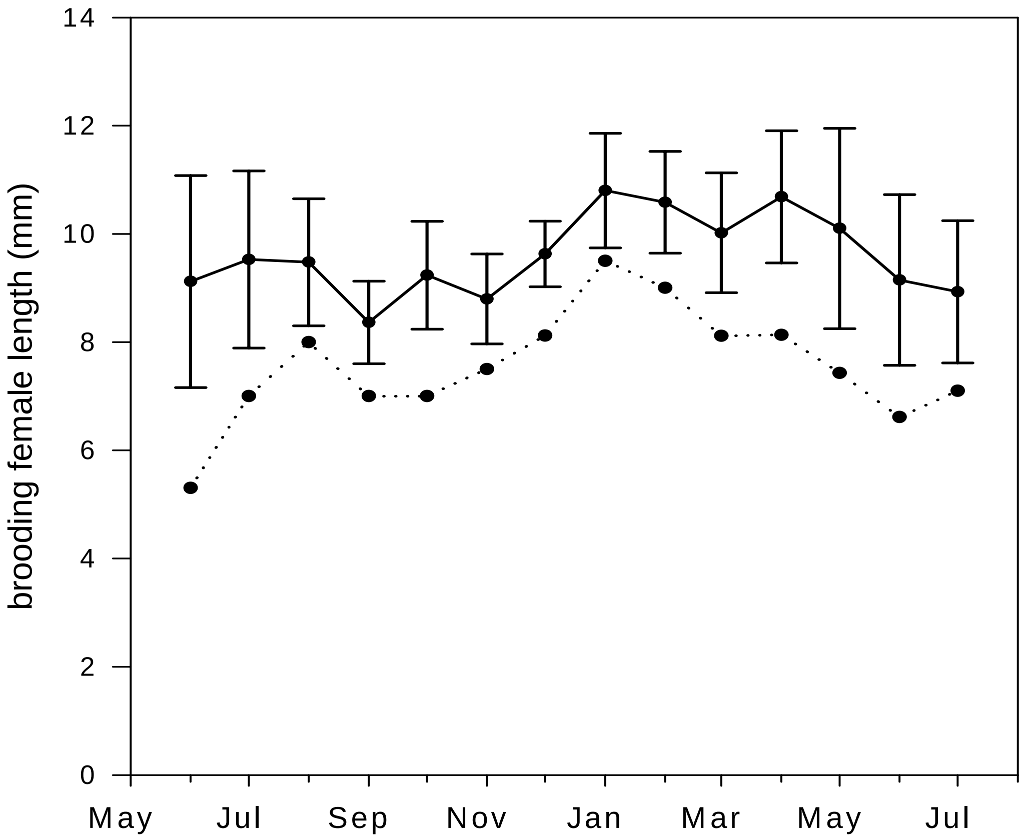

Figure 4.15: Monthly variation in the size of brooding female *G. insensibilis*. Solid line - the mean length of brooding females (± 1 sd) over a 16 month period; dotted line – the smallest brooding female recorded

The percentage of mature females dropped to 50% in the winter (January) and reached peaks of 93% (April, May and October) in the spring and autumn (Figure 4.16 a). The percentage of females brooding was highly variable but there were also peaks in autumn (October – 76%) and spring (April – 78%), while the proportion of mature winter females was low (January – 25%) (Figure 4.16 b). The percentage of mature females declined from the spring to the late summer/early autumn (April-September).

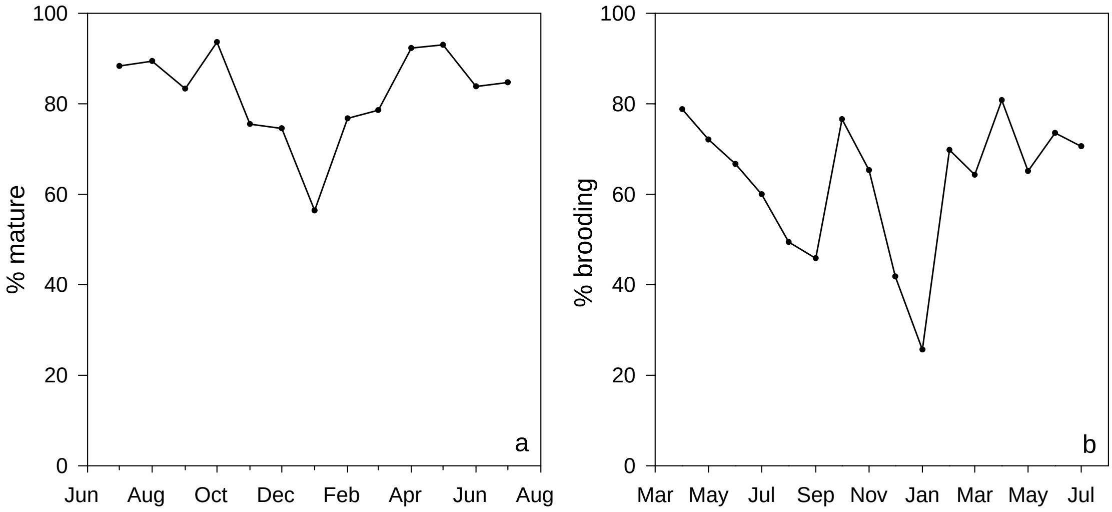

Figure 4.16: The structure of the female population of *G. insensibilis* – percentage of the female population that was a) mature and b) carrying embryos in the marsupium

Adult male, female (Fe, Fb, Fi) and juvenile *Gammarus insensibilis* were present in all samples in the 12 month sampling programme (Figure 4.17). The size range of males (5 - 19 mm) was more extensive than that of females (5 – 15 mm). The highest proportion of females fell in two or three size categories which varied during the year. Between July and November the majority of females were 8 – 10 mm in length, but from December to April there were more females in the 9 – 11 mm range. Females over 12 mm were only present in the first half of the year, coinciding with the largest brood sizes. In May and June there was a much more even distribution of females across the size ranges. The male population was much more evenly distributed through the year and spanned a greater range of sizes although peaks of a greater proportion of smaller males occurred in September and November. The proportion of juveniles was highest between September and January (population graphs, Figure 4.17) and the majority of juveniles fell in the 5mm and 7 mm size categories. The proportion of both immature females and juveniles in the larger (7 mm and over) size categories increased in the winter months between November and March.

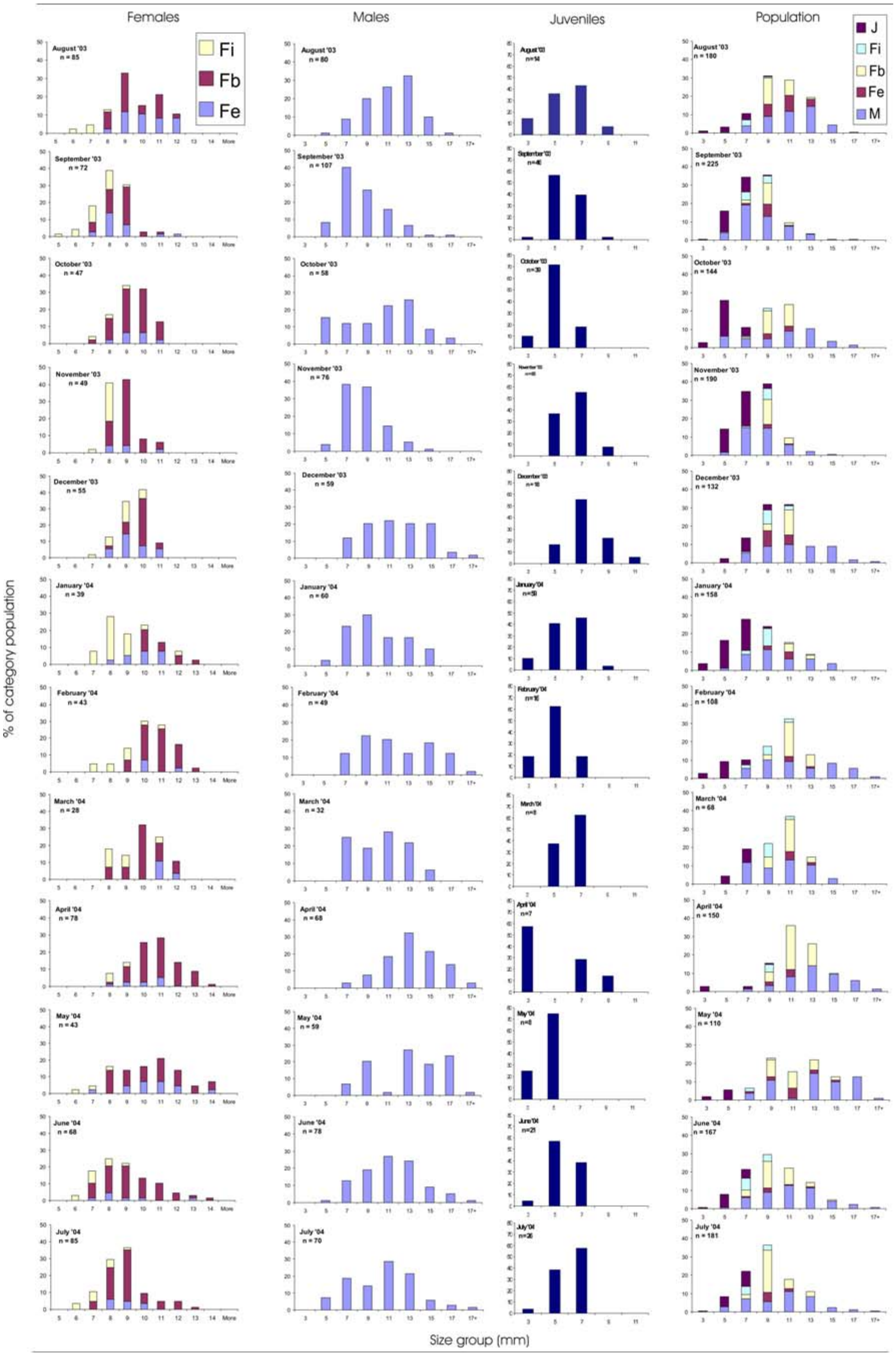

Figure 4.17: The population structure of female (divided into brooding, empty and immature animals), male, juvenile *G. insensibilis* (as percentage of each particular category) and the overall population structure from Gilkicker lagoon over the course of the 12 month sampling programme.

4.4. Discussion

4.4.1. Reproductive biology of gammarid amphipods

Gammarus insensibilis in this study showed similar reproductive traits to those found in previous studies of the same organism. The data on brood and embryo size were comparable to the values published by Sheader (1996) and Karakiri and Nicolaidou (1987). Significantly, the previous studies were from different latitudes (Gilkicker lagoon, England and Mazoma lagoon, Greece) so it might be expected that reproductive strategies could vary (Highsmith and Coyle 1991) but both authors and the current study showed that *G. insensibilis* reproduced continuously through the year implying that, despite being near the northern limit of its range, similar reproductive strategies are employed on the south coast of England to the Mediterranean. However, there are only limited data on the actual reproductive output in the Mazoma lagoon population; according to the models of Kolding and Fenchel (1981) and Skadsheim (1984) (Figure 4.1) it would be expected that fecundity would be higher in the Mediterranean population.

Gammarus insensibilis shows certain reproductive similarities to other gammarid amphipods. The females of the six species in Table 1 are of similar size when reproductively active and produce similar sized broods. Marsupium size limits the clutch volume that can be produced and successfully brooded to juvenile stage by a female amphipod and explains why the six species in Table 1 all have similar sized broods. Variation in reproductive parameters within the limits imposed by the anatomy of the marsupium may result from differential investment in individual embryos or in the number produced.

4.4.2. Reproductive investment

Gammarus insensibilis reproduces throughout the year; however there are periods of peak reproduction. Brood sizes are largest in the spring and early summer and drop away later in the summer and into the winter. This could be the result of optimal temperature or salinity for *G. insensibilis* in these more intermediate seasons. In the winter the amphipods are likely to be suffering from stress induced by low temperature and salinity whilst the high temperature and high salinity experienced in the warmest summer months could also stress the organism.

There is evidence for the trade-off between egg size and number discussed by Kolding and Fenchel (1981) and Skadsheim (1984). Brood sizes were larger in the summer while the embryos were small, and in the winter there was apparent increased investment into individual offspring.

However there have been other explanations proposed for diversity of egg size. The production of large eggs in the Antarctic has been attributed to food limitations rather than cold adaptation as it is expected that physiological cold adaptation will have previously occurred so there should be no energetic stress induced by the low temperature (Clarke 1982). It has been proposed that small embryos may result from situations where rapid development time is required because of low rates of female survival (Van Dolah and Bird 1980). This implies that it would be advantageous for older females to produce smaller eggs as increased age reduces the probability of survival until completion of brooding.

Reduced egg size as a strategy to minimize development time can not be dismissed for animals such as *G. insensibilis*. Amphipods are abundant, small animals that provide a food source to numerous predators including fish and birds, so survival rates are may be low. For example in mid-July, migrating Sandpiper in the Bay of Fundy (NW Atlantic) may consume up to 27 % of the standing biomass of *Corophium* sp. at certain mudflat sites (Hilton et al 2002). *G. insensibilis* (like many other gammarids) is subject to infection by various parasites including some that alter behaviour to increase the chance of transmission to the definitive host by predation (Thomas et al. 1996a), suggesting minimal brood incubation times would be beneficial, particularly at later stages of life, when infection probability is higher.

Despite these alternative explanations of variations in brood and embryo size, it appears that in the case of *G. insensibilis* there is a trade off in energy allocation. There is clear cycling of early stage embryo size over the course of the year, following the temperature and salinity curves, with gradual changes between months leading to the significant seasonal differences. There are females of all sizes in all samples suggesting that the larger females do not produce broods of smaller eggs (if this was the case, the winter eggs would be smaller because the smallest reproductive females were larger in the colder months). The numbers of *G. insensibilis* decline in the winter in Gilkicker lagoon (Al-Suwailem 1991) so survival rate is reduced; under Van Dolah and Bird’s hypothesis there would be smaller eggs in winter (because the female survival rate is low) contradicting the evidence of these data.

The trematodes that *G. insensibilis* host are known to alter the behaviour of the amphipod in order increase its probability of predation by the definitive host (discussed at length in Chapter 8). However, the rate of infection with *Microphallus* sp. is similar through the year and at Gilkicker the infection rate is high (Chapter 8) so most animals are at a similar ‘increased’ risk of predation by birds, whilst some are subjected to higher levels of infection but these cases are rare. The parasites were shown to incur some reproductive costs on *G. insensibilis* but this was low (Chapter 8). Any trend in parasite infection seemed to indicate greater infection rates in the summer, which reduced brood sizes. The data presented here show increased brood sizes in the summer, so it would appear that parasitism has little bearing on the trends in seasonal reproductive investment, it may however serve as an explanation for some of the variation in brood size within each month’s sample. Also, when parasitism was more prevalent, the egg numbers were reduced but the sizes were unaffected so this would appear to have minimal bearing on the discussion of seasonal trends in reproductive investment.

Food supply is known to be an important factor affecting the reproductive biology of amphipods. For example, the hydrothermal vent amphipod *Bouvierella curtirama* was shown to be more successful in a number of reproductive traits, including size and maturity and brood size, at one “Lucky Strike” hydrothermal vent site only 400 metres from another (Sheader et al. 2004). This was proposed to be the result of increased primary resources and quality of food. Significantly, the food availability in Gilkicker lagoon appeared to be constant over the year. Despite the fact that dense mats of summer *Chaetomorpha linum* are known to die back to a certain extent in the winter (Al-Suwailem 1991), a large quantity of the alga remained in large mats throughout the lagoon over the winter (the photograph of the mat of *C. linum* in Figure 3.1, Chapter 3, was taken in winter) and it still provided a habitat and abundant food source to the amphipods. There was however, some evidence for some variability in food quality (discussed in Chapter 5) but this was not related to reproductive factors, and the energy content of the food source was greater in the winter months. It seems that embryo size and number are determined by distribution of energy allocation to ensure maximum effective fecundity under the particular level of environmental stress the organism is subjected to, as predicted by the models of Kolding & Fenchel (1981) and Skadsheim (1984) and summarised in Figure 4.1.

Examining clutch volume or brood mass supports the trade-off hypothesis because it becomes clear that the investment was shifting from one parameter to the other if clutch volume remains relatively constant, despite fluctuation in brood size and embryo volume. Clutch volume is a useful measure because it shows the overall capacity of the marsupium, and the data were available as a consequence of measuring individual embryo sizes and brood size. In this case, the clutch volume of early stage embryos showed a peak in May which was significantly larger than November and December but was otherwise relatively constant (Figure 4.7).

Although the trade off is evident, it can not be confirmed how it functions. Consistent clutch volume suggests that energy allocation to reproduction remains relatively constant despite environmental stress. If this is the case then the partitioning of resources is not driven by the quantity of energy available. Variable SFG (Chapter 5) implies reproduction is the most important recipient of energy allocation in *G. insensibilis* with brooding occurring throughout the year, despite periods of severe energy shortage (negative SFG) so the same amount of energy is partitioned despite the animals being under energetic stress. The partitioning of energy must be driven by the environmental stress itself. It could feasibly be the result of incubation time. Low temperatures slow metabolic rates so embryonic development time increases. Perhaps when ovaries or oocytes are developing slowly more resources are provided to the eggs. The time of hatching of females could in some circumstances drive the resource-partitioning, but the multiple broods per year, continuous reproduction and lack of obvious cohorts would appear to discount this method of control. Some kind of environmental cue, temperature or photoperiod may be involved in driving the partitioning of the resources.

Variable brood size has been used as a measure of stress in amphipods in ecotoxicology studies (Ford et al. 2003). It is important to distinguish between reduced brood sizes as a response to an environmental stress and separate reproductive strategies being employed seasonally. Ford et al. (2003) fails to consider the size of the embryos or clutch volume when suggesting that pollution affects reproductive investment. It is feasible that in the polluted environment embryos are larger to increase survival rates so effective fecundity may not be as adversely affected as Ford et al. (2003) suggests. Brood size investigations usually use early stage embryos as indicators of investment or stress (Sheader 1996; Ford et al. 2003). This is an appropriate measure of the initial investment and is useful in investigations of energy balance. Perhaps a more relevant index is late stage brood size, as this provides greater insight into actual offspring recruitment.

When the late stage (V) brood sizes were analysed on a seasonal basis they were less variable than early stage (I-III) brood sizes, this equates with the rate of loss of embryos from the marsupium, suggesting that when early stage brood sizes are large the clutch develops to become too large for the marsupium. This seems an unlikely strategy as energy is clearly wasted investing in embryos that will not become part of the recruitment. Embryo loss during development is common in gammarid amphipods (Sheader and Chia 1970) and rates of loss can be high; it seems unlikely that in other organisms employing brood care strategies that rates of loss would be as high as 40% before they hatch. It is possible that this does occur and the energy is regained by the ingestion of embryos failing to develop correctly. However, it seems doubtful that female amphipods could select from their marsupium which embryos to re-ingest and which to continue to brood. Brood cannibalism by the female does occur under nutritional stress in gammarids (Sheader 1983) and under disturbance stress (pers. obs.) but it is doubtful whether such explanations could account for the generally high levels of brood mortality found in certain peracarids (Johnson et al. 2001).

Overproduction of offspring is a recognized phenomenon suggested to provide the opportunity to capitalize when resources are unusually rich; act as food for other brood members; or act as a stock of replacements for offspring that fail (Mock and Forbes 1995; Forbes and Mock 1998). Of these hypotheses overproduction of offspring cannot be to provide food sources for other brood members because in gammarids the brood develops at the same rate so there are no early stage embryos in the marsupium when the first juveniles emerge and embryo loss occurs before any juveniles have emerged. Increased egg output when conditions are favourable seems a viable strategy in a variable habitat such as the lagoon but the limits determined by the marsupium anatomy suggests that this is not possible. Replacements for offspring that fail would imply that failing embryos would be found in the broods; this is not the case. It is possible however, that females could determine failing embryos from the differences in size evident across the developmental stages (Figure 3.8) and reingest these to recover investment of resources. The re-absorption of oocytes prior to the release into the marsupium has been recorded for the terrestrial isopod *Porcellio ficulneus* in response to laboratory initiated temperature stress (Hornung and Warburg 1994).

In other instances brood mortality has been attributed to brood parasites including fungus and copepods. 4% of female *Idotea granulosa* (Isopoda) from the Irish Sea were infested by the parasitic isopod *Clypeoniscus* sp. This parasite destroys the brood but because of its limited distribution and ability to destroy the whole brood it clearly does not account for the 20% loss of embryos between early and late stage (Salemaa 1986). The unidentified fungus infecting winter broods in this study reached up to 10% infection rate of females in the population, but is also likely to destroy the entire brood. Although recorded in stage I-IV broods it is assumed that the later stage broods were recently infected or they would not have survived. Female amphipods carrying unidentifiable material in their marsupia were recorded on some occasions, presumably previously destroyed broods.

Brood mortality could be a phenomenon associated with the anatomy of the marsupium. There is diversity of oöstegite morphology across the Peracarida. Gammarid amphipods possess slender oöstegites with long setae along the margins, which interlock to contain the brood while in most other peracarid groups they are broad flat structures which completely enclose the brood below the ventral body wall (Johnson et al. 2001). The open mesh created by the interlocking setae in gammarids enables freer gas exchange possibly allowing brooding of larger eggs (Steele 1991) than the large brood plates of other peracarids but may be less secure leading to greater rates of embryo loss. For example, rates of embryo loss are lower in the lagoon isopod *Idotea chelipes* which have a more secure marsupium (Chapter 9). The most plausible explanation for the high levels of brood mortality during development seems to be overproduction, to act as replacements for those that fail.

There appears to be no advantage to juveniles remaining in the marsupium above a certain size as larger females do not brood their offspring until they reach a larger size. This may result from a number of factors including no feeding opportunities in the marsupium and competition for space with siblings as it is known that larger females produce larger broods. There is also no seasonal trend in the size of juveniles in the brood chamber, it would be expected that the large winter eggs would produce larger juveniles than the small summer eggs. However, it should be noted that juvenile amphipods, particularly the larger individuals often left the marsupium during fixation. Hence, the juveniles measured were probably slightly smaller in mean length than in the natural environment, because the fully developed juveniles left the marsupium so were not included in the study.

There are some differences in juvenile size evident statistically but they may arise from low sample sizes resulting from the escape response exhibited by the juveniles when the animals are being preserved. There were no data available on the dry weight of juveniles in the marsupium. Winter juveniles, having received more parental investment may have a greater body mass for their length than summer individual. They may also have utilized the extra resources in attaining the maximum size before leaving the marsupium so there appears that there is no difference in size of summer and winter brooded juveniles.

4.4.3. Population structure

Sex ratio is environmentally determined in some amphipod species (Watt and Adams 1993; McCabe and Dunn 1994; Watt 1994) but it does not seem to be the case in *G. insensibilis* as the sex ratio was relatively stable over the year. Any variation that was apparent (Figure 4.14), is not related to photoperiod, the main environmental factor that controls sex ratio in *G. duebeni* (Watt and Adams 1994).

The greater size range of males was partly because males can be distinguished from a smaller size than females and also because they reach a larger maximum size. Sexual size dimorphism is common in gammarid amphipods and is adaptive (Watt and Adams 1993). Large size increases female fitness as brood size increases with female size (Figure 3.9). It is of greater significance to male fitness however because larger males are able to guard large females in precopula. Sheader and Chia (1970) discuss a maximum fecundity at a certain size in some gammarid females so larger animals suffer reduced fecundity. In this case further increases in size would decrease fitness. This does not occur in *G. insensibilis* so larger animals have greater reproductive potential.

The juvenile proportion of the population increased between September and January. This is unlikely to be the result of increased recruitment over the winter but probably because animals are maturing later in the winter. Reaching sexual maturity at a larger size in the winter may be a strategy to compensate for constant investment in reproduction. *Gammarus insensibilis* reproduces despite low energy availability (SFG – Chapter 5), so larger reproductive size may allow for the utilization of a greater proportion of stored resources. The declining percentage of females brooding from October to January may reflect the fact that it is only the larger females that have the stored resources to brood their eggs to full development in the winter. Peaks in the proportion of females carrying embryos in the spring and autumn probably highlight the times of the year when temperature and salinity stresses are minimal so a greater proportion of the female population is able to invest in reproduction.

There was a steady decline in the percentage of brooding females (Figure 4.16, Table 1) from the spring (April 2003 – 78%) to the autumn (September 2003 – 46%) but subsequently it was highly variable. This may be the result of the high temperature and salinity stresses associated with summer in the lagoon. There were peaks in autumn and spring suggesting that these months, with their more intermediate temperature and salinity, may prove optimal for reproduction. The peaks in the percentage of mature females fall in the same months as those for the percentage brooding.

The reproductive investment studies show that juveniles were only found in the marsupium until approximately 2mm in length however animals smaller than 5mm were rare in the population structure analysis. These small individuals may exhibit different habitat preferences than the adult animals and consequently be excluded from the samples, for example there may be a greater detrital component to the diet, so they maybe found on the sediment surface to a greater extent. Loss of these small individuals as a result of a sampling error seems unlikely as many *Corophium* sp. and *Idotea chelipes* specimens of small size were recorded in the samples.

The reproductive data highlights important areas of consideration for the conservation of *G. insensibilis* and the overall lagoon ecosystem. *G. insensibilis* is well adapted to the lagoon environment and the plasticity of reproductive strategy shown here, enables it to maintain a healthy reproductive rate throughout the year. Despite this, lagoon management is still important. *G. insensibilis* is an important component of the lagoon ecosystem and its interactions with *C. linum* are perhaps central to the functioning of the system. Mismanagement could lead to extremes of temperature or salinity that are too great for energy partitioning adaptations to compensate for, leading to reductions in amphipod abundance and consequential changes in the functioning of the lagoon system through disruption to the interaction between *G. insensibilis* and *C. linum* (although *Idotea chelipes* may be of equal importance) or perhaps more importantly from a conservation perspective, for birds that utilize the feeding opportunities lagoons offer. With too little variation in environmental parameters *G. insensibilis* may be out-competed by marine or freshwater congeners leading to a break down of the lagoon ecosystem and formation of a more typical marine or freshwater habitat.

Chapter 5

Seasonal variation in physiological rates and energy balance of *Gammarus insensibilis*

5.1. Introduction

Environmental stress can cause detrimental changes to populations and communities of any living organism. This becomes of particular interest when the organisms concerned are of importance to mankind, such as marine organisms of conservation or commercial interest. Monitoring populations and biodiversity is expensive and labour intensive, so various assays have been developed to indicate possible changes at higher levels of biological organisation, by observing the effects of stress at lower levels (Widdows 1993). Examples of the use of peracarids for such tests include the Scope For Growth assay at the individual amphipod level (Maltby and Naylor 1990), cellular energy allocation in mysids (Verslycke et al. 2004) and heat shock protein expression at the molecular level in amphipods (Schill et al. 2003).

The analysis of the effects of stress at the lower levels of organisation can highlight which specific stressors, and at what levels, could potentially have population level effects, giving important information for the implementation of relevant environmental management or conservation strategies. However, despite the importance of the assumption that environmental effects on the individual are translated up through levels of biological organisation, there have been few studies linking the two (Crowe et al. 2004).

Environmental changes can affect the physiology of organisms in various ways. Temperature affects the rates of metabolic reactions and membrane fluidity, while salinity affects the osmotic balance of cells (Randall et al. 1997). To maintain homeostasis in these functions, energy is required, which may be obtained by increasing energy uptake through feeding, or reducing energetically costly functions such as reproduction or locomotion (Schneider 2004).

The laws of energy balance dictate that for an organism to survive for any length of time energy intake must at least equal energy loss. Based on the energy balance equation (Winberg 1960) and previously outlined in Chapter 3, the Scope For Growth (SFG) assay is a sublethal test that can detect how organisms are affected by extrinsic factors. It describes the amount of energy available for somatic growth and reproduction, and depends on the remaining energy after that gained from assimilated food has been distributed between general maintenance costs. Equation 1 describes the SFG equation[1].

The SFG assay was initially developed through work on the bioenergetics of fish (Warren and Davis 1967). It was later modified and used extensively on intertidal bivalves (Widdows and Bayne 1971; Bayne et al. 1985), and has since been developed for use on peracarids in toxicity testing (Naylor et al. 1989; Maltby and Naylor 1990; Maltby et al. 1990; Roast et al. 1999b). It has predominantly been used as an indicator of the effects of anthropogenic pollution stress (Widdows et al. 1995; Widdows et al. 2002) but should perhaps also be considered to a greater extent as an important tool in the understanding of whole organism ecology (Smaal et al. 1997; Chaparro and Thompson 1998; Crowe et al. 2004).

$$SFG = A - (R + U)$$
$$\text{where}$$
$$A = C \times e$$

Equation 5.1: The SFG equation. SFG = Scope For Growth; A = assimilation rate; R = respiration rate; U = excretion rate; C = feeding rate; e = assimilation efficiency (%).

SFG tests can be run relatively cheaply, quickly and gives greater insight into the actual effects of a stressor than tests such as the LC_{50}. All organisms are able to adjust to the variability of their environment to some extent in order to maintain homeostasis. However, the maintenance of homeostasis is energetically costly and a stressor that has no apparent effect in an LC_{50} assay may negatively affect SFG. The SFG assay consequently indicates stress which may not immediately affect the survival of individuals but may have consequences at higher levels of biological organisation at a later time. If there is a change in one of the parameters of the energy equation it can be compensated for by adjusting another so homeostasis can be maintained. However, consequences at the organism level may arise because energy used in maintaining homeostasis reduces that available for reproductive investment. Resulting from this reduction in allocation, reproduction is either reduced (decreasing lifetime fitness) or supported by the utilization of stored resources (reduced survival prospects). Ultimately, if the stress affects all the animals in the population, it may lead to population level effects owing to reduced recruitment or lower quality juveniles.

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[1] The abbreviations in Equation 1 will be used throughout the description of the results in this chapter.

5.1.1. Energy loss

The use of energy can be detected from the quantity of oxygen used (respiration rate) and the rate of production of nitrogenous waste material (excretion rate). The usage of oxygen results from the oxidation of metabolic products and is closely related to actual metabolic rate[2]. It describes the energy used in the full range of activities carried out by organisms.

Respiration rate, like each component of the SFG equation, is affected by intrinsic factors including gender, age, size, Specific Dynamic Action (SDA) and reproductive condition. Extrinsic factors including temperature, salinity, and anthropogenic pollution can also have important effects on respiration rate and it is these that the SFG assay was designed to monitor.

Excretion rate describes the rate of energy loss through the degradation of ammonia products but has been shown to represent minimal energetic loss in peracarids, to the extent that it has been ignored in previous uses of peracarids for SFG assays e.g. *G. pulex* (Naylor et al. 1989; Maltby and Naylor 1990; Maltby et al. 1990) and *Neomysis integer* (Roast et al. 1999b).

5.1.2. Energy uptake

Assimilation rate describes the energy intake of an organism. It describes the rate of intake of the component of the consumed food from which energy is gained by the organism and is dependent on the rate of consumption of food material (feeding rate) and the efficiency of digestion (assimilation efficiency). Assimilation efficiency is usually relatively stable therefore feeding rate is often regarded as the most important factor in the SFG assay. The quality of the food source is also an important factor; for example, growth or reproductive rates of grazing organisms may vary depending on their food source (Larson et al. 1980; Steele and Whittick 1991; Cruz-Rivera and Hay 2001).

The main recognized food source of *Gammarus insensibilis* is the filamentous green macroalga *Chaetomorpha linum* (Sheader and Sheader 1985; Bamber et al. 2000). This is present in Gilkicker lagoon (and other lagoons that support populations of *G. insensibilis*) throughout the year, however it is not so abundant in the winter months (Al-Suwailem 1991). The energy content of marine macroalgae can vary spatially and temporally. For example the calorific content of *Macrocystis pyrifera* and *Ulva lactuca* varied seasonally (Lamare and Wing 2001)

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[2] Metabolic rate (MR) refers to all the reactions involved in organism function. The oxidation of metabolic products means MR is closely correlated with respiration rate, which is frequently used as a surrogate for MR. Actual MR can only be measured through direct calorimetry.

while the energy content of the ephemeral brown alga *Pilayella littoralis* was greater in the summer than spring and autumn (Steele and Whittick 1991). The energy content varied in different anatomical regions of the algae (Paine and Vadas 1969) and reproductive anatomy was of greater calorific value. In each case the calorific content was inversely related to the inorganic (ash) content of the alga.

The aim of this survey was to monitor, over the course of a year (on the same sampling visits as the population dynamics survey), the basic rates of metabolic loss and gain of *Gammarus insensibilis* under the temperature and salinity conditions of the lagoon, in order to determine how the dynamic lagoonal environment affects the amphipods at the individual organism level. Most studies of SFG are carried out under a number of different treatments to simulate the different environmental conditions that the test organism experiences. This study ran the assay on a regular basis throughout a year so the seasonal variation in the test organisms and the food source, usually ignored, is considered.

5.2 Methods

The materials and methods used in the 12 month bioenergetics survey are outlined in Chapter 3. The rates of energy loss and gain were recorded under lagoon temperature and salinity conditions for reproductive female *Gammarus insensibilis* carrying early stage embryos (which were removed for the assays). After collection the animals were taken to the aquarium facilities. They were maintained in controlled temperature rooms under a 12 hour photoperiod system in filtered sea water diluted to correct salinity and starved for 24 hours prior to the start of the assays. The salinity and temperature conditions used were those recorded on sampling visits.

In feeding experiments *Chaetomorpha linum* (Chlorophyta) was used as a food source. The energy content of *C. linum* was determined using the relationship between ash content and calorific content described by Lamare and Wing (2001) and the data on green macroalgae (Chlorophyta) presented by Paine and Vadas (1969). To verify the estimates of the energy content of *C. linum* the total lipid content (determined gravimetrically in this study), total ash content (from this study), total protein content (Cruz-Rivera and Hay 2001) and the total carbohydrate content (estimated as the remaining component) were determined. The energy content of the component parts of *C. linum* were obtained from the literature and are summarised in Table 5.1 (Omori and Ikeda 1992; Harris et al. 2000):

Table 5.1: The energy content of the main biochemical constituents of *Chaetomorpha linum*

Biochemical component	**Energy content (kcal mg^{-1})**	**Energy content (KJ g^{-1})**
Protein	4.2	23.7
Carbohydrate	4.2	17.2
Lipid	9.5	39.4

Similarly to the reproductive data in Chapter 4, bioenergetics data were presented on both a monthly and seasonal basis, using the same division of the year into Spring, Summer, Autumn and Winter.

5.3. Results

5.3.1. Feeding and assimilation rate

The rate that *Gammarus insensibilis* consumed the green alga *Chaetomorpha linum* was greatest in the summer months when temperature and salinity were at their highest (Figure 5.1). Feeding rate varied significantly over the 12 month sampling period (Kruskall Wallis One Way ANOVA on ranks H = 91.575 with 11 degrees of freedom P = <0.001). Pairwise comparisons (Dunn's method) showed feeding rates in March and April were significantly lower than May, June, July, August and September; the rate in February was significantly lower than in May, July and August; December was lower than July and August while November also differed significantly from August.

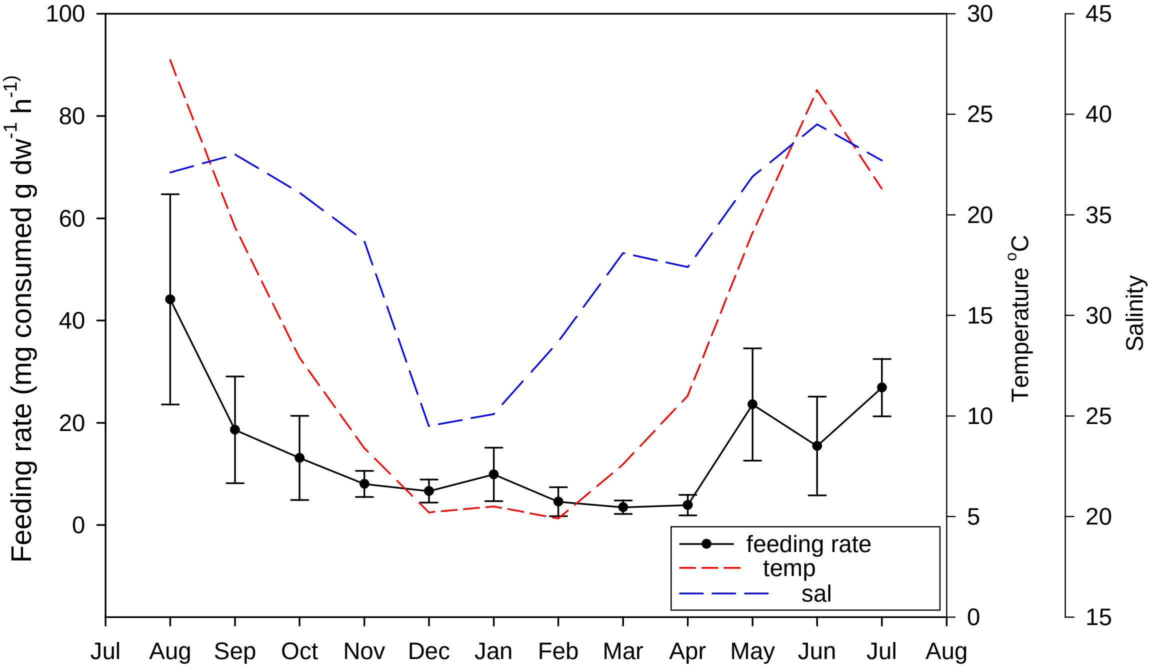

Figure 5.1: Mean feeding rate (mg of *C. linum* consumed g dw^{-1} h^{-1} ± 1 sd) by *G. insensibilis* over the 12 month bioenergetics survey (n = 7 – 12). The temperature and salinity conditions of the experiment are also shown.

Figure 5.2 shows that this variation in feeding rate was also evident when these data were analysed on a seasonal basis (Kruskall Wallis One Way ANOVA on ranks H = 50.237 P<0.001). The feeding rates measured in summer were significantly higher than the other seasons (pairwise comparisons, Dunn's method).

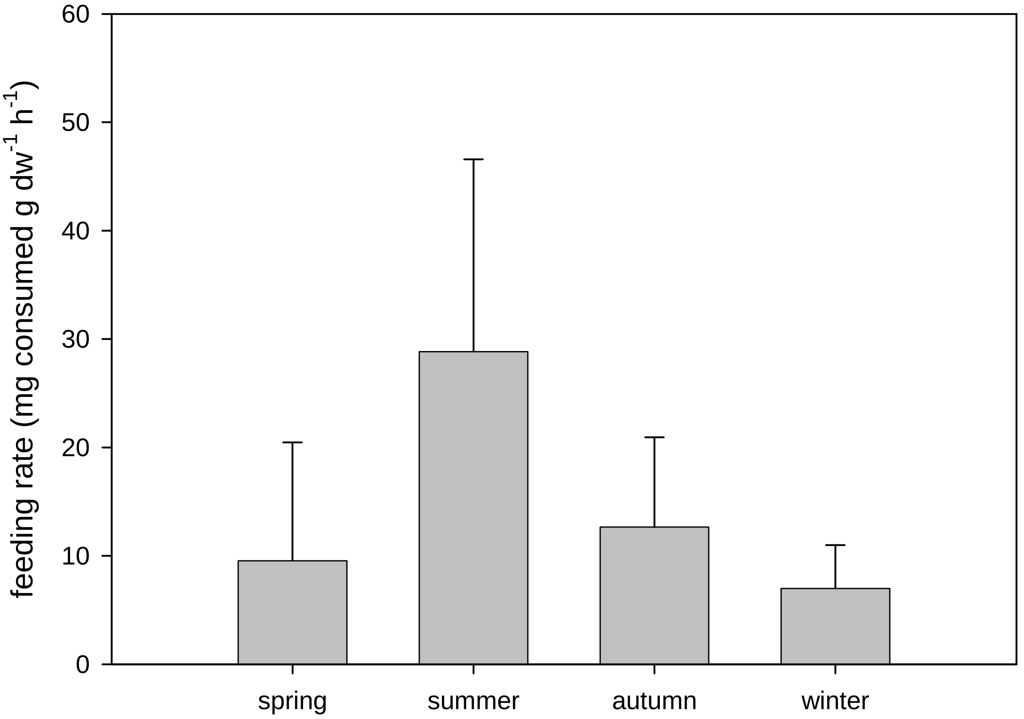

Figure 5.2: Seasonal feeding rate (mg consumed g dw^{-1} h^{-1}) of *Gammarus insensibilis* over the 12 month bioenergetics survey (±1 sd, n = 26-36)

The occurrence of the highest feeding rates in the summer months suggests that there was a relationship with temperature, salinity or their combined effects. Figure 5.3a describes the results of linear regression analysis and shows a positive relationship between temperature and feeding rate ($r^2 = 0.69$). Feeding rate was lowest at times of intermediate salinity (32-34) and highest when salinity was approaching 40. The highest salinity was recorded when temperature was at its highest in Gilkicker lagoon (Chapter 2) so the high feeding rates at this time can probably be explained by a combination of the two environmental factors. The lower feeding rates at intermediate salinity may explain some of the variation from the line in Figure 5.3a.

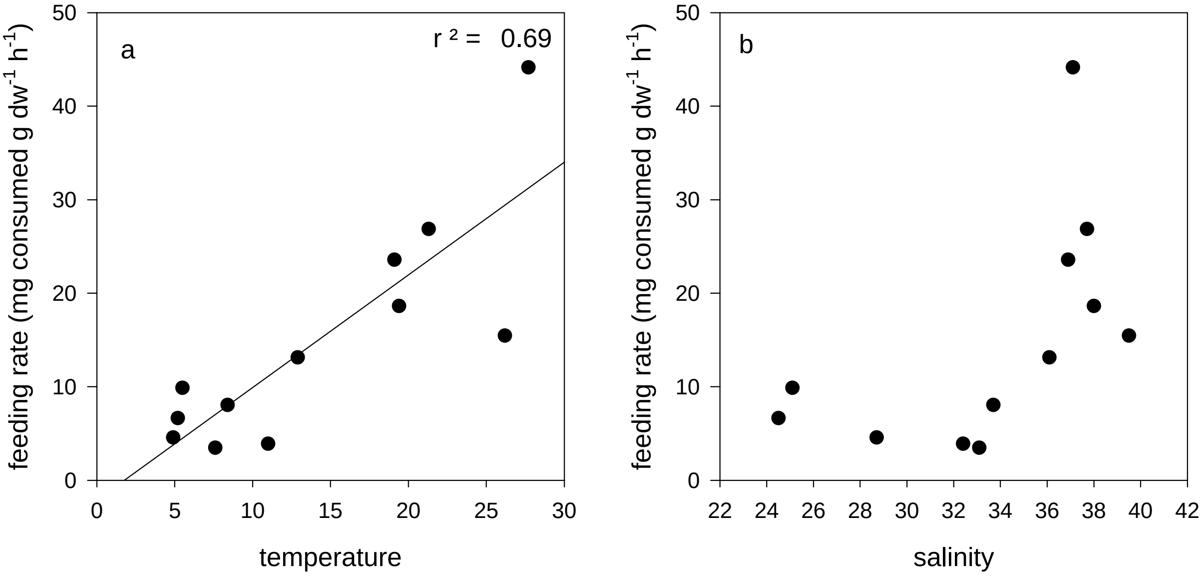

Figure 5.3: The effects of (a) temperature and (b) salinity on mean feeding rate of *G. insensibilis* feeding on *C. linum* during the 12 month bioenergetics survey (regression trend line and r^2 value are shown)

The feeding rate was variable during the course of the year but there was inherent variation in the energy available from feeding on *C. linum*. The energy content of marine macroalgae is directly related to the proportion of inorganic matter (% ash content of dry weight). Figure 5.4, based on a study of 9 species of green algae (Paine and Vadas, 1969) shows a clear negative relationship between the ash content (%) of Chlorophyta and their calorific content (r^2 = 0.97). The equation of the line (Equation 5.2) for this relationship was used to determine the calorific content of *C. linum*.

Equation 5.2: $\text{kcal g dry wt}^{-1} = -0.0513 (\% \text{ ash}) + 4.9403$

These data were used to determine the energetic equivalents in KJ for feeding rate using the 1 kcal = 4.184 KJ conversion factor (Lamare and Wing, 2001). When the feeding rate was presented as the amount of energy consumed rather that the actual quantity of food eaten the pattern was slightly different. For example, the absolute rate of feeding (mg consumed) was higher in May and July when compared to June (Figure 5.1). When the greater energy availability from the food source in June was considered, the rate of energy consumption was almost identical between the three months (Figure 5.10). The feeding rate (mg consumed) in August was double that in September but when energy content was considered, the August rate of consumption was just 1.5 times that of September.

The effect of the energy content of the alga on feeding and energy consumption is highlighted by the comparison of the lines for the graphs for feeding rate (Figure 5.3 – $r^2 = 0.69$) and energy consumption rate (Figure 5.7 – $r^2 = 0.73$) plotted against temperature. Some of the variation around the line is taken away by the consideration of the actual energy content of the food source.

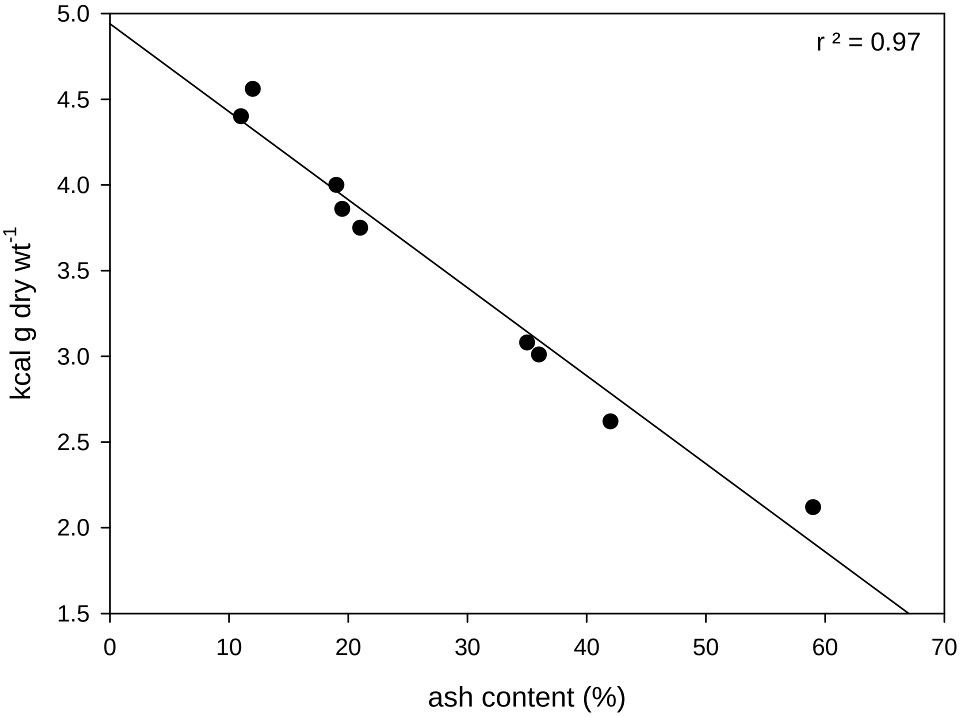

Figure 5.4: The relationship between the ash content (%) of 9 species of Chlorophyta and their calorific content based on the data of Paine and Vadas (1969). Regression line and r^2 value from regression analysis are also shown.

As the ash content of *C. linum* was variable over the course of the year (Figure 5.5 a) the energy content was determined on a monthly basis. There was significant variation between months (Kruskal Wallis ANOVA on ranks H = 82.43 with 11 degrees of freedom, P <0.001) with the inorganic content generally lower in the winter, although there were “unseasonably” low ash contents in September and June. When the data were combined into seasons there were also significant differences ($F_{3\text{-}63} = 6.483$, $P<0.001$). Pairwise multiple comparisons (Holm-Sidak) showed that each season differed significantly from the others except between spring vs. autumn and summer vs. autumn.

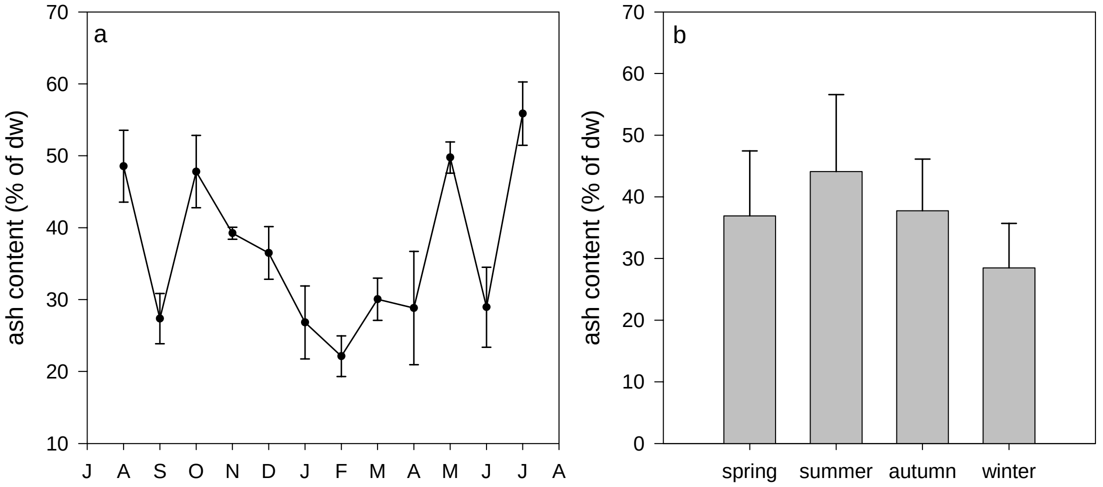

Figure 5.5: a) The monthly variation in ash content (±1 sd) of *C. linum*, n = minimum of 5. b) The seasonal ash content as a percentage of dry weight (±1 sd) of *C. linum*, n = minimum of 15.

Derived from Equation 5.2, Figure 5.6 describes the energy content on a monthly basis during the year long survey. It is simply a reflection of the ash content data (Figure 5.5) and indicates that there was variable energy content with an apparent seasonal trend. Higher energy content was recorded in the winter and lower values in the summer, although the September and June data appear to be unseasonably high.

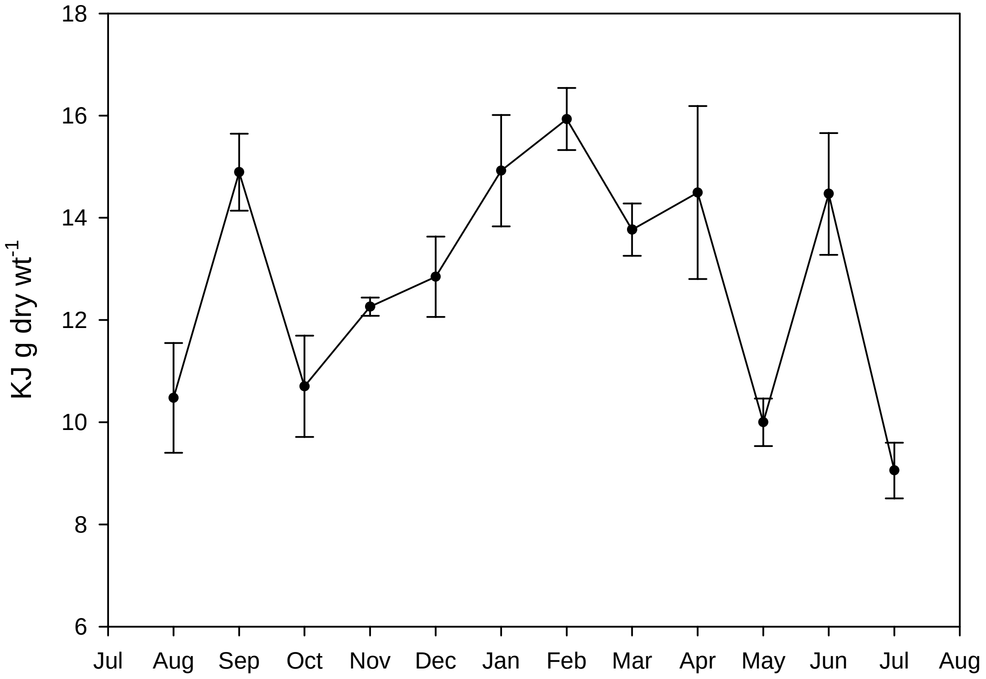

Figure 5.6: The energy content (± 1 sd) of *C. linum* during the course of the 12 month bioenergetics survey (n = minmum of 5)

To verify the energy content of the *C. linum* another estimate was made of its value using data from the literature and the total lipid and ash content of the alga determined during this investigation. The estimated percentage composition is summarised in Table 5. 2. At 12.04 KJ g dry wt^{-1} the estimated value was similar to the annual mean energy content (12.80 KJ g dry wt^{-1}) calculated using the relationship described in Figure 5.4. Both these values for energy content are lower than the mean value obtained for Chlorophyta of 14.6 KJ g dry wt^{-1} by Paine and Vadas (1969).

Table 5.2: The % biochemical composition of *C. linum* (± SD where experimentally derived) and energetic equivalent (KJ g DW^{-1}) derived from the energetic equivalents for each component

	%	KJ g dry wt.$^{-1}$ of *C. linum*
Protein	1.5	0.36
Lipid	4.82 (± 2.8)	1.9
Carbohydrate	56.88	9.78
Ash	36.8 (± 11.5)	0
Total	100	12.04

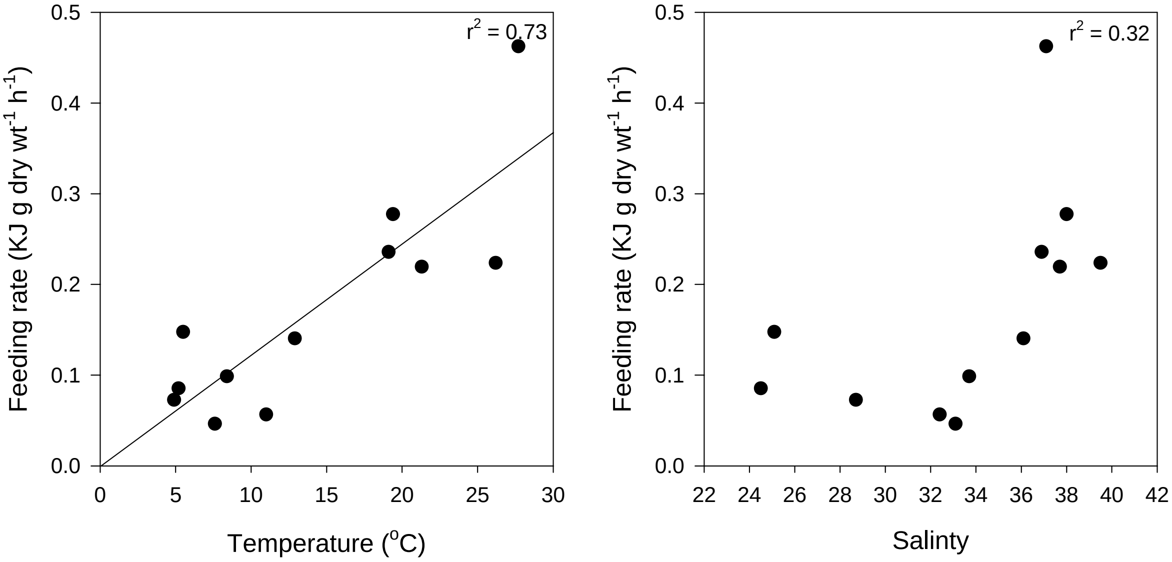

Figure 5.7: The effects of (a) temperature and (b) salinity on the energy consumption of *Gammarus insensibilis* feeding on *C. linum* during the course of the 12 month bioenergetic survey. Regression lines and r^2 values are also shown.

Assimilation efficiency (e) (percentage of the food that was digested) was variable during the year, although the general trend was for greater efficiency in the winter months (Figure 5.8). There are no error bars in Figure 5.8 because the faeces samples were pooled to ensure enough material to measure the dry and ash weights in order to determine assimilation efficiency. Regression analysis of the assimilation efficiency in relation to environmental factors showed a negative relationship between assimilation efficiency and temperature (Figure 5.9 a) during the 12 month survey (r^2 = 0.47). There was a similar, although weaker, relationship with salinity (Figure 5.9 b, r^2 = 0.24).

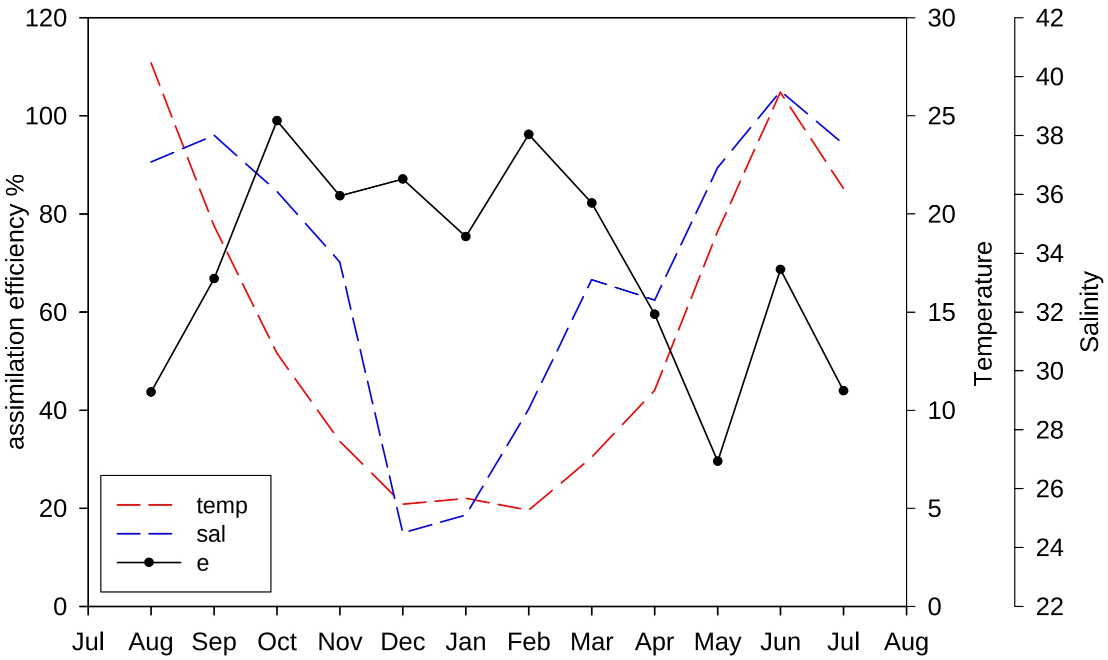

Figure 5.8: The assimilation efficiency (%) of *G. insensibilis* over the 12 month bioenergetic survey. Lagoon temperature and salinity are also shown.

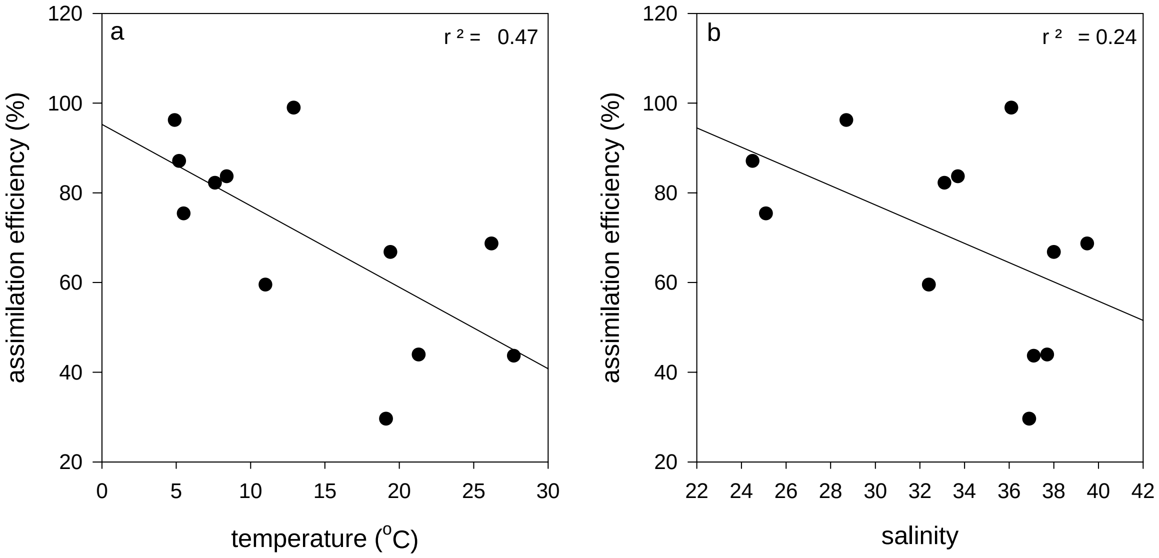

Figure 5.9: The effects of temperature (a) and salinity (b) on the assimilation efficiency of *G. insensibilis* feeding on *C. linum* during the 12 month bioenergetics survey (linear regression line and r^2 value are shown)

Multiple linear regression analysis suggested that in combination, there were no significant effects of either temperature or salinity on the assimilation efficiency of *G. insensibilis*. The negative coefficient for temperature confirms that assimilation efficiency decreased with increasing temperature but this was not significant (multiple linear regression, t = - 2.136 degrees of freedom = 2, p = 0.061). There was no significant salinity effect (multiple linear regression, t = 0.690, degrees of freedom = 2, p = 0.508).

The relatively lower assimilation efficiency (e) in the summer (Figure 5.10) coincided with increased feeding rates (C) resulting in less variation in assimilation rate (A) than feeding rate. There was however significant variation in A (Kruskall-Wallis one way ANOVA on ranks H = 65.062 with 11 degrees of freedom, P = <0.001). The pairwise comparisons (Dunn's method) showed that August, September, October and June differed significantly from April and March, although there were fewer significant differences than in the feeding rate assay (Figure 5.1). There was significant variation in A on a seasonal basis (Kruskall-Wallis one way ANOVA on ranks H = 19.581 with 3 degrees of freedom, P = <0.001). Dunn's pairwise comparisons showed that assimilation rates in summer and autumn were significantly greater than in spring. Winter rates were also significantly lower than those in the summer (Figure 5.11).

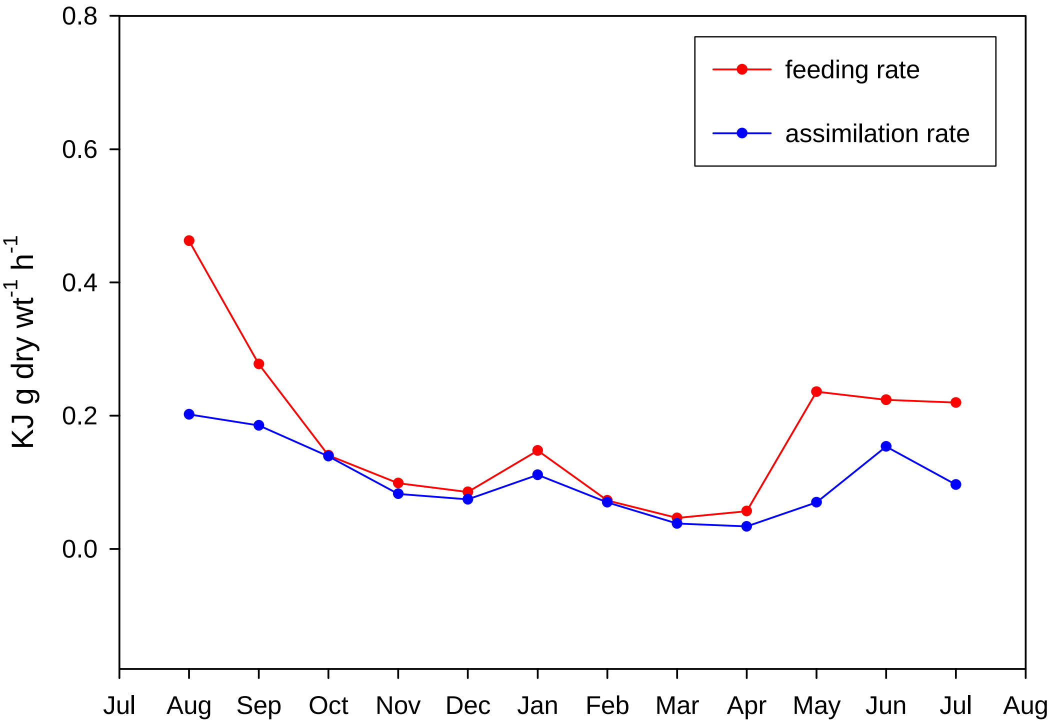

Figure 5.10: The feeding rate and assimilation rate of *G. insensibilis* over the 12 month bioenergetics survey

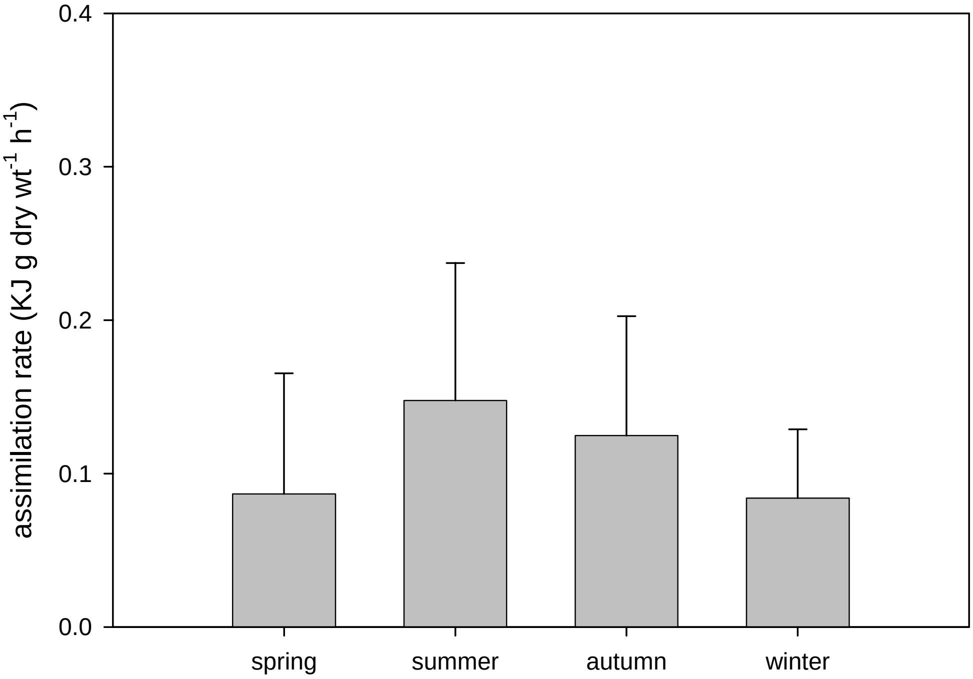

Figure 5.11: Seasonal assimilation rate (± SD) of *G. insensibilis* during the 12 month bioenergetic survey (n = 34, 36, 26 & 27).

The lower variability in the assimilation rate data can be seen from the regression analysis of A with temperature. There is a stronger relationship between feeding rate and temperature (Figure 5.7a, r^2 = 0.73) when compared with assimilation rate and temperature (Figure 5.12a, r^2 = 0.46). The relationship between assimilation rate and salinity (Figure 5.12b) shows a similar pattern to that shown between C and salinity with lowest assimilation rates evident at intermediate salinity.

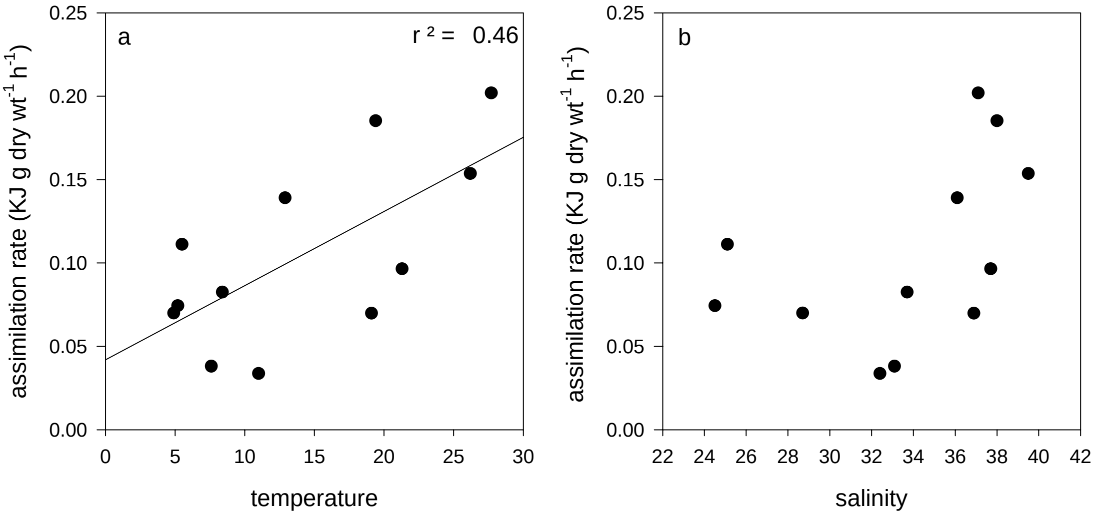

Figure 5.12: The effects of temperature (a) and salinity (b) on the assimilation rate of *G. insensibilis* feeding on *C. linum* over the course of the 12 month bioenergetics survey (regression line and r^2 value are shown for temperature)

In the feeding assays, when feeding rate of *G. insensibilis* on *C. linum* was high, assimilation efficiency was low (Figure 5.13a, $r^2 = 0.48$). There was however, no effect of ash content (inversely related to energy content) of the alga on amphipod feeding rate (Figure 5.13b, $r^2 = 0.002$).

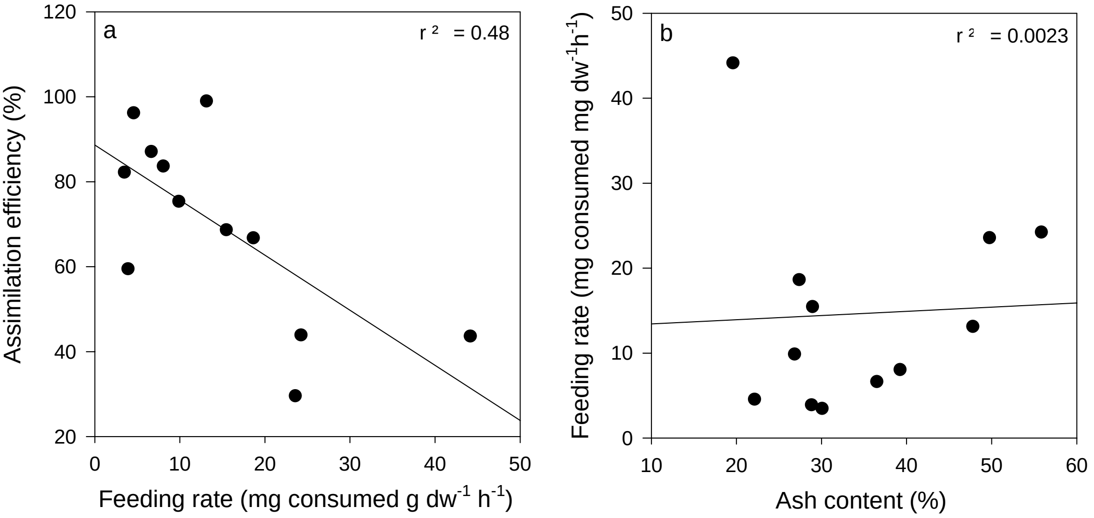

Figure 5.13: The effects of a) mean feeding rate on mean assimilation efficiency in the feeding assays in the 12 month bioenergetics survey and b) the effects of mean ash content of the algal food source on feeding rate (trend lines are linear regression, r^2 values are also shown)

5.3.2. Respiration rate

Respiration rate dropped steadily from a maximum in August into the winter and then began to increase again as the water warmed and salinity increased (Figure 5.14). There appeared to be variations from this seasonal trend when respiration rate was high in November and low in May. The variation over the 12 month bioenergetic survey was significant (Kruskall Wallis One ANOVA on ranks H = 42.488 with 11 degrees of freedom P = <0.001), all pairwise comparisons (Dunn's method) showed that respiration rate was significantly higher in August than December, January and May; as well as September differing from January. Apart from the differences highlighted by the pairwise comparisons, there was limited difference in the respiration rate of *Gammarus insensibilis* over the course of the year. However, there were significant differences in respiration rate between seasons (Figure 5.15) (Kruskall Wallis One ANOVA on ranks H = 24.82 with 3 degrees of freedom P = <0.001), when the rates were significantly higher in the summer and autumn than in winter (pairwise comparisons, Dunn's method).

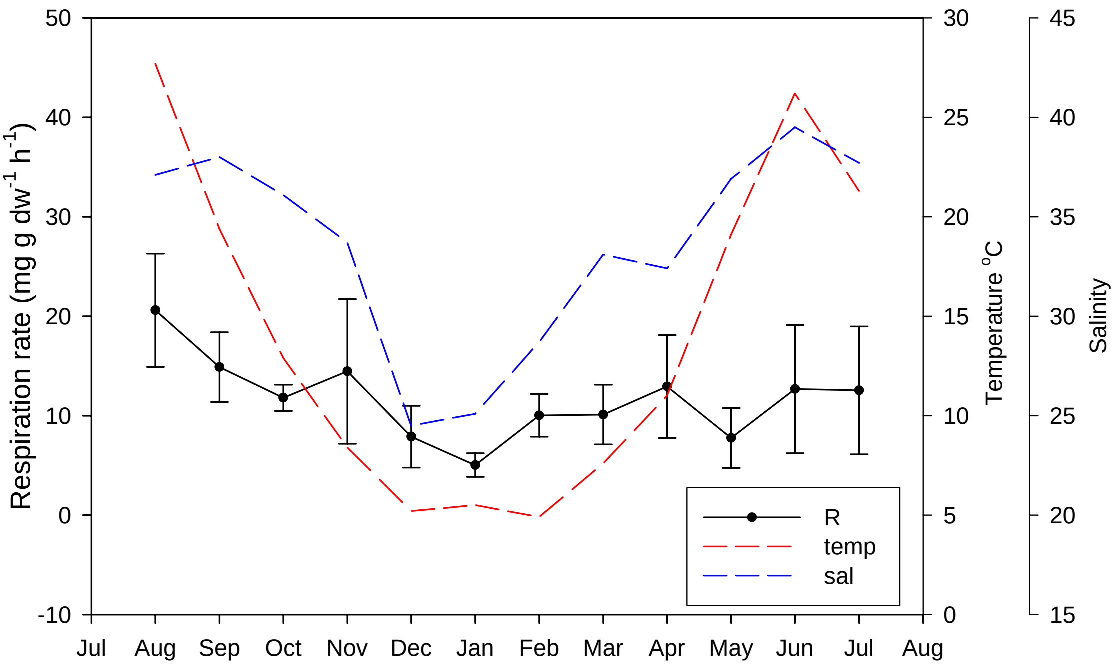

Figure 5.14: The mean respiration rate (mg g dw^{-1} h^{-1} ± 1 sd) of *G. insensibilis* over the 12 month bioenergetic survey (n=5-9). Temperature and salinity are also shown.

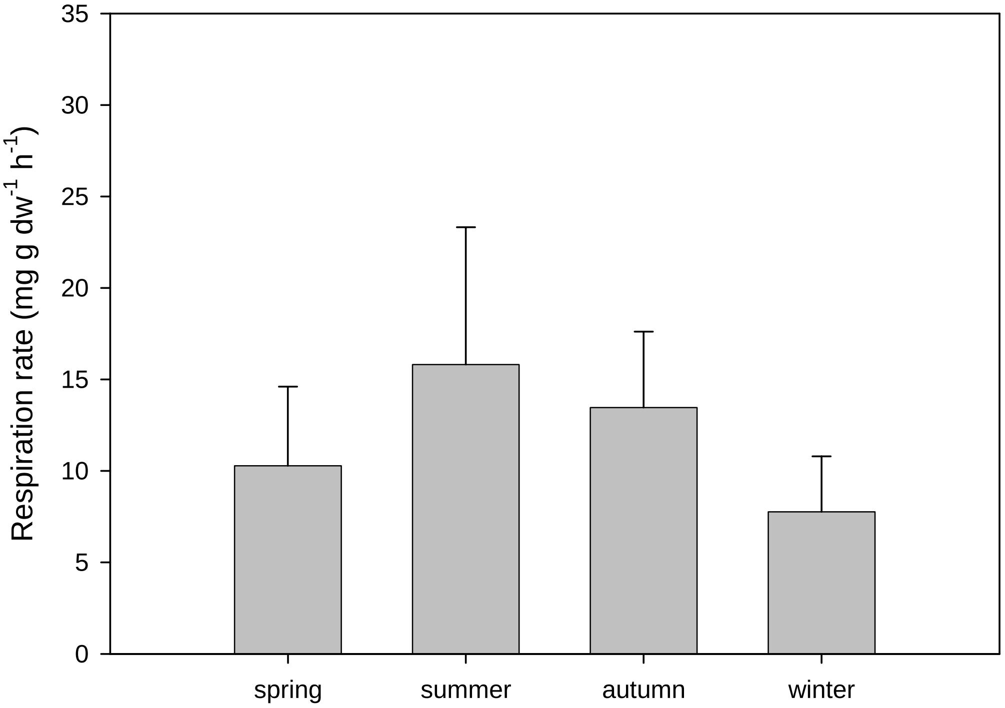

Figure 5.15: Seasonal respiration rate (mg g dw^{-1} h^{-1}) of *Gammarus insensibilis* over the 12 month bioenergetic survey (± 1 sd, n = 21 – 23)

The greater respiration rates in the summer months were, in part, evident from the regression analysis of R with the environmental parameters but these relationships were weak; temperature (Figure 5.16a) $r^2 = 0.41$ and salinity (Figure 5.16b) $r^2 = 0.37$. Multiple linear regression analysis showed no significant effect of temperature or salinity on the respiration rate of *G. insensibilis*. The positive coefficients for temperature and salinity suggested that respiration rate did increase with both parameters although this was not significant (multiple linear regression, temperature; t = 0.757 with 2 degrees of freedom; p = 0.469, salinity; t = 0.806; with 2 degrees of freedom, p = 0.510).

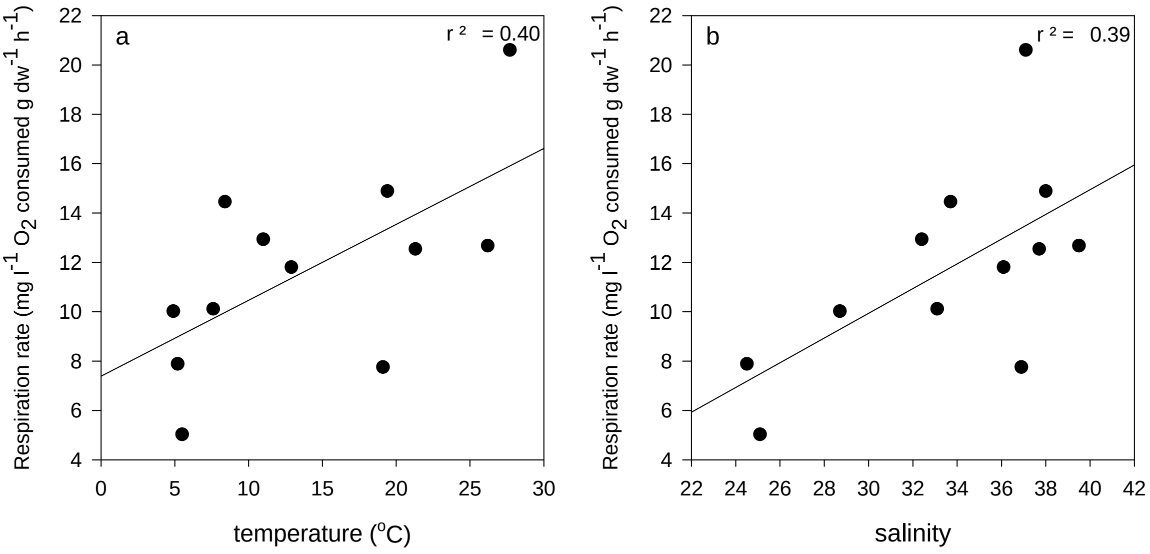

Figure 5.16: The effects of temperature (a) and salinity (b) on the respiration rate of *G. insensibilis* over the 12 month bioenergetics survey (regression line and r^2 values are shown)

5.3.3. Excretion rate

Ammonia excretion rate (U) was variable over the year, with the highest rate in August and lowest rates in June and March (Figure 5.17). With the exception of the high mean excretion rate in August there was no obvious seasonal trend. However, there were significant differences between months (Kruskall Wallis one way ANOVA on ranks, H = 62.657 with 11 degrees of freedom, P = <0.001). Pairwise analysis showed that the excretion rates in August and September were significantly higher than March and June while in August the excretion rate was also higher than in October and February. The excretion rate was high again in November and again differed significantly from March and June, as was the case in July. The excretion rate in January was significantly higher than in March. When combined into seasons there were also significant differences in excretion rate (Figure 5.18, Kruskall Wallis ANOVA on ranks, H = 13.794 with 3 degrees of freedom, P = 0.003) Pairwise comparisons showed that the only significant difference was between autumn and spring, probably the result of the extremely high excretion rates evident in August and high standard deviation. For this reason and for continuity with previous uses of the SFG assay on peracarids the excretion rate data was excluded from further calculations of SFG.

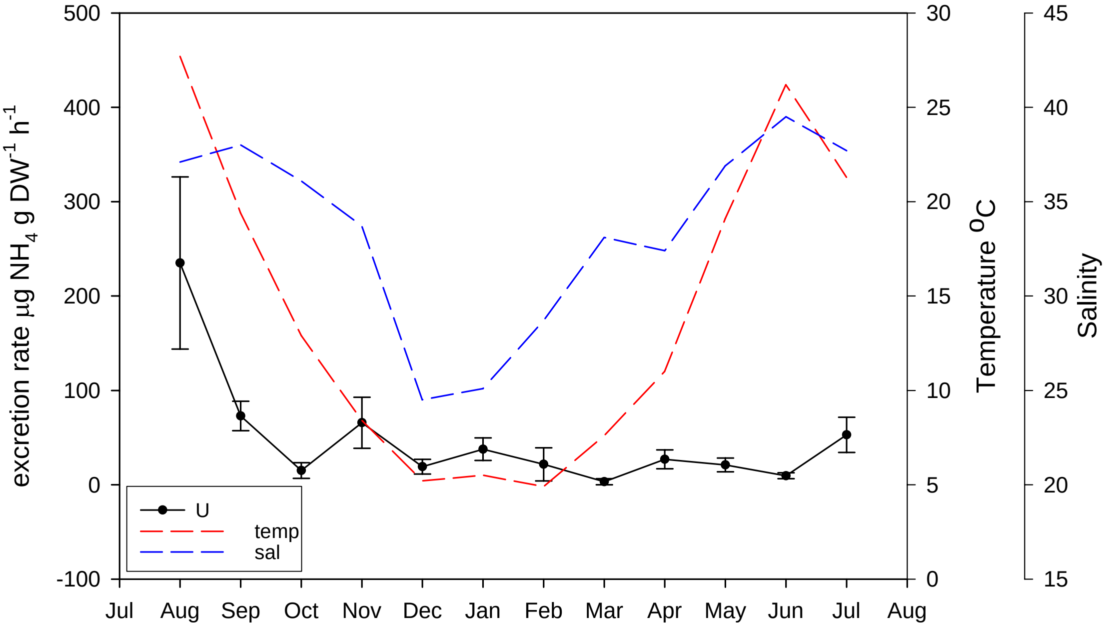

Figure 5.17: The mean excretion rate (U) µg NH_4 g dw^{-1} h^{-1} (± 1 sd) of *G. insensibilis* over the 12 month bioenergetic survey (n=5-9). Lagoon temperature and salinity are also shown.

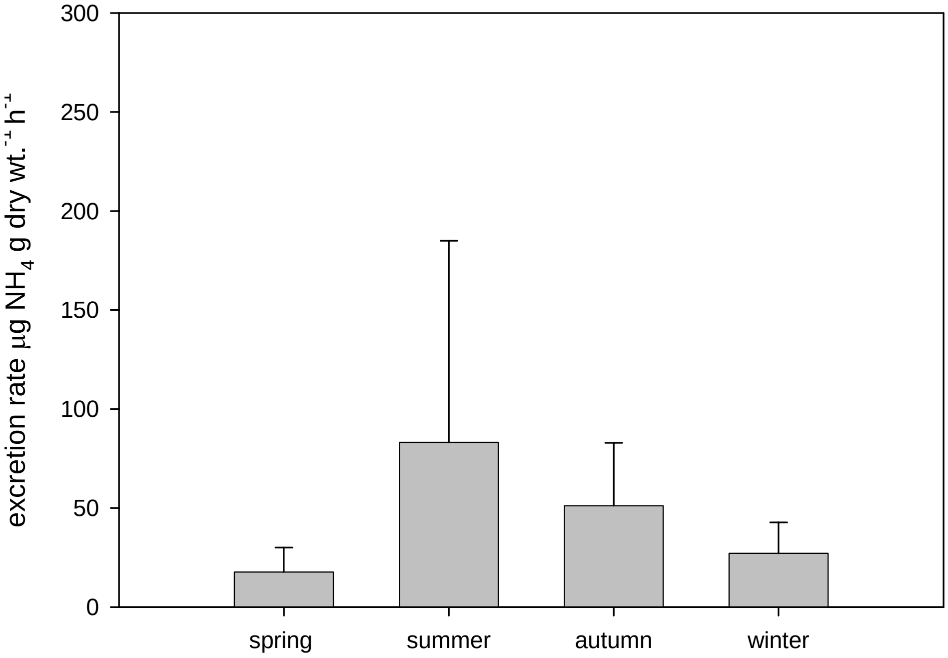

Figure 5.18: The seasonal excretion rate (± 1 sd) of *G. insensibilis* during the 12 month bioenergetics survey (n = 18 - 23)

The physiological rates (excluding excretion rate as has become standard in peracarid SFG assays) were converted to energetic equivalents from which monthly and seasonal energy budgets were constructed (Figure 5.19 – 5.21). As previously displayed, energy consumption rate was different to the absolute feeding rate after the energy content of the food source had been considered. Respiration rate was converted to energy 'loss' but this was a straight conversion so the patterns obtained were the same as the rates expressed above. Scope For Growth represents the difference between A and R, and for much of the year it was close to zero or negative. SFG closely reflected respiration rate, with increased respiration rate causing a negative effect on SFG. SFG was most negative in November, March, April and February. There were no trends between SFG and salinity or temperature.

When SFG was grouped seasonally, high respiration rates accounted for negative SFG in all seasons (Figure 5,20). Energy, represented by the SFG, was most imbalanced in the spring when assimilation efficiency was low leading to the rate of energy consumption failing to cover the costs of energy loss. Winter SFG was least negative, reflecting the lower rates of all components of the energy balance equation.

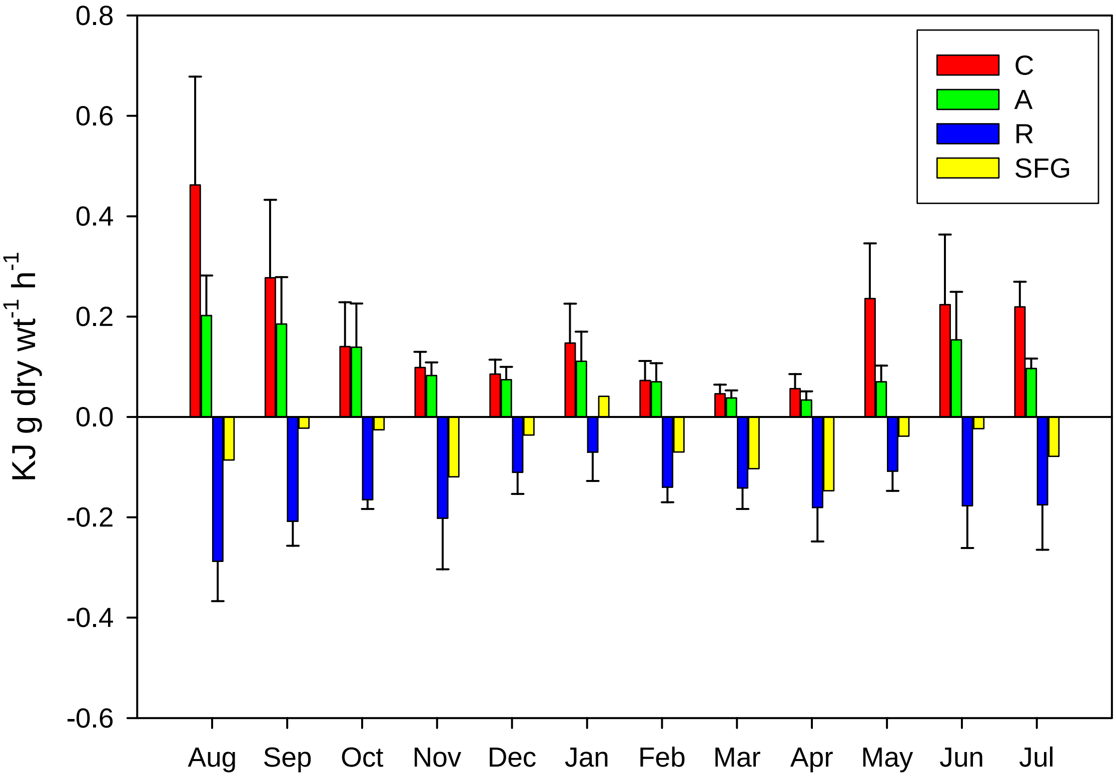

Figure 5.19: Energy balance of *Gammarus insensibilis* over the 12 month bioenergtics survey. C = feeding rate; A = assimilation rate; R = energy loss through respiration; SFG = scope for growth (error bars shown are 1 standard deviation)

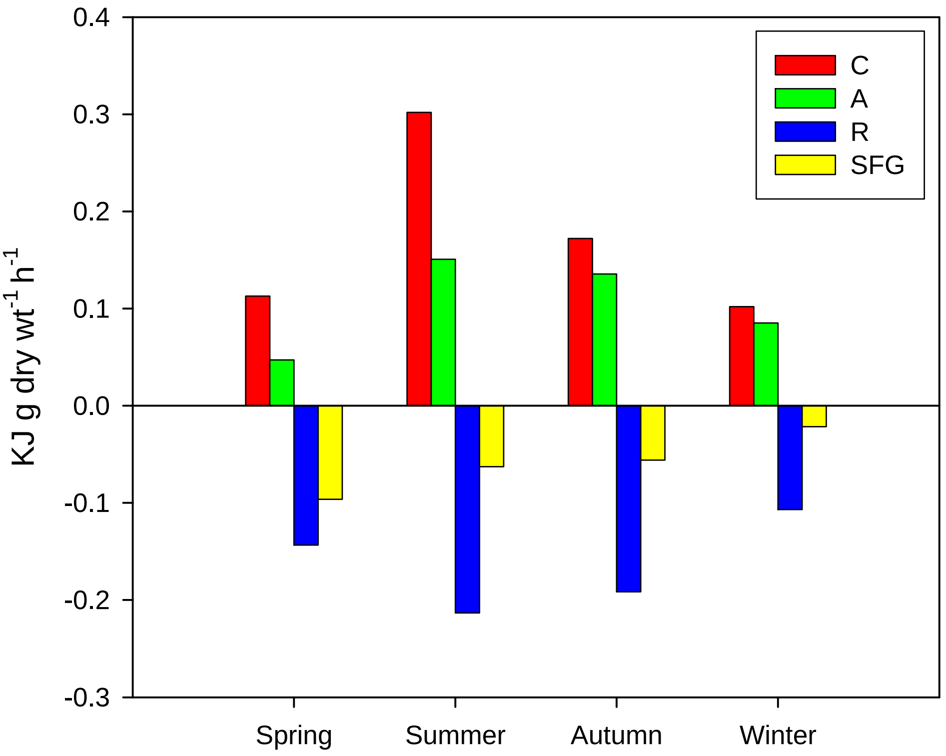

Figure 5.20: Seasonal SFG during the course of the 12 month bioenergetics survey; C = energy consumption rate, A = energy assimilation rate, R = rate of energy loss through respiration, SFG = scope for growth

When feeding rate was expressed using a single mean value for the energy content of the Chlorophyta (14.6 KJ g dry wt^{-1}), some of the variation in energy gained from food consumption resulting from variable food source was removed but overall similar patterns still emerged. August showed SFG of almost 0 KJ g dry wt^{-1} h^{-1} rather than the -1 KJ g dry wt^{-1} h^{-1} value obtained with the variable food source values. There was still highly negative SFG in the spring months but over all the values for SFG were closer to zero. Using this value for the food source showed that the warm half of the year (May - October) SFG was around zero, and energy was much more balanced than in the winter half of the year.

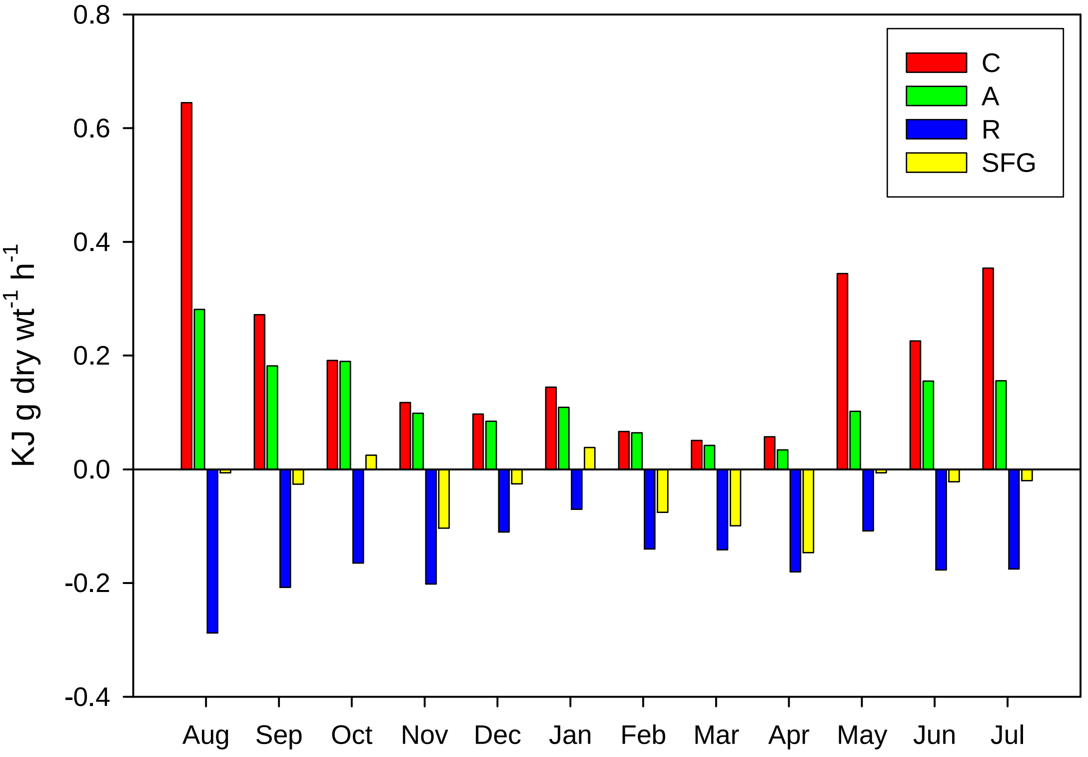

Figure 5.21: The energy budget for *Gammarus insensibilis* over the course of the 12 month bioenergetics survey using a constant energy content for Chlorophyta to determine feeding rate.

5.4. Discussion

In Gilkicker lagoon temperature and salinity were closely related, with the high temperatures in the summer leading to increased evaporation and therefore high salinity, while in the winter higher rainfall resulted in lower salinity – see Chapter 2. As the temperature and salinity used in the assays were the same as the conditions in the lagoon and they were so closely linked to each other, it was difficult to determine which factor had the greater effect. This consideration is therefore re-visited and the factors experimentally manipulated in Chapter 7.

This survey was designed to observe *Gammarus insensibilis* acclimated to the natural, seasonal conditions over the course of a year, in order to gain an overview of how the individual organisms were affected in this highly dynamic, stressful environment. The majority of studies focusing on environmental influences on the metabolic rates and energy balance of marine organisms have been carried out under laboratory conditions with animals exposed to various different treatments to determine tolerances and acclimation capabilities for inter- and intraspecific variation (Bulnheim 1974; Chapelle and Peck 1995; Hahn 2005; Lapucki et al. 2005).

Many factors affect the physiology and therefore the energetic status of marine invertebrates. In the lagoon situation temperature and salinity are of great significance and are considered in this chapter, based on the physicochemical data from Chapter 3. Food source is another important consideration and many adaptations in many organisms are the result of increased ability to obtain and gain energy from food. *Chaetomorpha linum* is the main food source for *Gammarus insensibilis* so some consideration into its nutritional information was necessary before analysis of the seasonal energy balance of *G. insensibilis*.

Both the calculation from literature values and percentage composition methods of determination of the mean energy content of the alga resulted in similar values (12.04 and 12.80 KJ g dry wt^{-1}) for a mean energy content over the course of a year. The method based on the work of Lamare and Wing (2001) was used for further analysis of feeding rates as it gave insight into how the food source varied seasonally. Energetically *C. linum* is comparable to other species of marine macroalgae. The mean energy content over the course of the year in this study was 12.8 KJ g dry wt^{-1} (or 12.04 KJ g dry wt^{-1} from biochemical composition) while Paine and Vadas (1969) reported a mean value of the Chlorophyta as being 14.6 KJ g dry wt^{-1} and Lamare and Wing (2001) suggested a mean value of 11.1 KJ g dry wt^{-1}. Work on the biochemical composition of *C. linum* showed that it had lower protein content (approximately 1% of algal dry weight) than other macroalgae, but it did have a high Nitrogen content (Cruz-

Rivera and Hay 2001) suggesting increased calorific value (Lamare and Wing 2001). The ash content of over 50% reported by Cruz-Rivera and Hay (2001) was higher than generally obtained in this study (annual mean = 36%) and may have been related to when the samples were taken (although this was not reported) as ash content was around 50% in some months during this study.

There was a seasonal trend in the energy content of *C. linum* in this study, with greatest energy content in the winter months. Seasonal variation in the food quality of marine macroalgae is well documented. Steele and Whittick (1991) showed that energy content of the brown alga *Pilayella littoralis* was highest in July and August, when the proportion of reproductive cells was greatest. The energy content of *Macrocystis pyrifera* (Phaeophyta) was also higher in the summer months when the nitrogen content was highest. *Ulva lactuca* (Chlorophyta) showed a similar trend to the *C. linum* used in this study with the greatest energy content occurring in the spring and autumn months. This was potentially the result of increased photosynthetic activity at these times (Lamare and Wing 2001). Paine and Vadas (1969) reported higher calorific content in ephemeral algae such as *Ectocarpus* (Phaeophyta) or *Monostroma* (Chlorophyta) than perennial genera such as *Laminaria* (Phaeophyta) or *Codium* (Chlorophyta). Perennial species are always available as a food source for herbivores so it has been suggested that grazing is a selective pressure resulting in reduced energy content or secondary metabolites to inhibit grazers. As availability of the short-lived algae is not predictable they have not been subject to the same selection pressures, possibly resulting in the higher energy content. *Chaetomorpha linum* is present throughout the year but dies back in the winter months. It has definite growth periods in the spring and summer (Al-Suwailem 1991), but its presence cannot be considered unpredictable in the sense suggested by Paine and Vadas (1969), as *G. insensibilis* and *I. chelipes* are found feeding on it throughout the year.

Feeding rate was considered as both the absolute amount of *Chaetomorpha linum* consumed and as the amount of energy consumed because the seasonal variation in energy content of the alga affected the energy available to *G. insensibilis*. Similar seasonal trends were evident in both the expressions of the rates of feeding, but energy content did have an effect on the rate of energy consumption. This was most notable when the actual feeding rate dropped in June but when presented as energy consumption rate, however, it was almost identical to May and July. This variation in energy content highlights the importance of seasonal variation in food source to invertebrate grazers and has important implications for the construction of energy budgets for organisms under natural conditions.

The positive correlation between feeding rate and temperature (both absolute feeding rate and energy consumption rate) was comparable to previous information on the effects of environmental factors on feeding rate. For example, Roast et al. (2000) showed the egestion rate (directly representative of feeding rate in the case of the mysids studied) to increase significantly with temperature. Roast et al. (2000) also showed a positive relationship between feeding rate and salinity but suggested that there was also a more complex relationship involving both temperature and salinity. The complex relationship between temperature, salinity and metabolic rates is also apparent in this study. Multiple linear regression analysis showed a significant effect of temperature on the rate of energy consumption but there was no significant effect of salinity. There was however an increase in feeding rate associated with salinity but only above a certain level. Above 32 any increase in salinity caused an increase in feeding rate. However below 32, feeding rate increased as salinity decreased (Figure 5.3). The same pattern occurred when assimilation rate was compared with salinity (Figure 5.12). This may suggest an optimum salinity where energetic needs can be met by lower energy intake or that at these times *G. insensibilis* was feeding less because of other factors such as reduced need for energy intake because of lower reproductive costs.

The preferred diet of invertebrates feeding on a variety of algae is known to improve certain aspects of physiological performance. Both somatic and gonad growth in the sea urchin *Strongylocentrotus drobachiensis* were highest when it fed on its preferred diet of *Laminaria longicruris* (Larson et al. 1980). The amphipod *Amphithoe longimana* showed differential survival, grew at different rates and displayed variable reproductive traits when it fed on a range of algal species (Cruz-Rivera and Hay 2001). Despite these observations there is little evidence for macroalgal grazers choosing higher quality food. The gastropods *Littorina striata* and *Osilinus atratus* showed no correlation between choice of food and calorific, ash, nitrogen, carbon, soluble carbohydrate or soluble protein content (Granado and Caballero 2001). Cruz-Rivera and Hay (2001) showed that in choice experiments amphipod grazers (*Amphithoe longimana*) did not preferentially feed on the higher quality food sources but when feeding on lower quality algal or artificial material compensatory feeding occurred. Compensatory feeding was also evident when the mysid *Neomysis integer* fed on low quality diets (Roast et al. 2000).

It should be noted that organisms evolve the appropriate feeding apparatus and mechanisms for their preferred food source. The diversity of macroalgal morphology and chemical properties will therefore affect the choice of food by grazers. The lack of correlations between choice of food and algal quality may reflect an organism’s inability to manipulate and utilize the energy from a particular alga, irrespective of its nutritional quality. Analysis of a range of algae which organisms routinely feed on may show preferences for those of greatest energetic value.

However the co-evolution that occurs between grazers and their food plants would probably result in the choices having been previously made and the grazers would be adapted to make most efficient use of that particular alga.

In this study increased ash content of *C. linum* (lower energy content) had no effect on the absolute feeding rate (mg *C. linum* consumed) suggesting no compensatory feeding mechanism. This may suggest that ash content is not the main determinant of the food quality of the alga to *G. insensibilis*. Alternatively, under natural conditions the amphipods may select the area of the plant to feed on so as to avoid lower quality algae. Under laboratory conditions, where they were restricted to the food presented to them, they would have fed, at the temperature dependant rate, on the food source available to them rather than selecting higher quality food. The algae used in the feeding assays were chosen based on consistency of colour and thickness of the filaments (there was colour variation depending on the position within the mass of alga). This method of selection probably did not reflect the algal quality.

There was however a relationship between assimilation efficiency and feeding rate (Figure 5.13); high feeding rate resulted in low assimilation efficiency. This was probably the result of increased food intake causing decreased gut residence time, rather than a temperature associated decrease in digestion capabilities (the opposite effect on digestion would be expected owing to improved enzyme reaction rates). Reduced feeding rate has been associated with increased gut residence times and resultant increased absorption (assimilation) efficiency in bivalves (Hawkins and Bayne 1984). The absorption efficiency of the urchin *Strongylocentrotus intermedius* feeding on *Laminaria japonica* increased as the food ration decreased because the food spent more time in the bolus (Lucas 1996). Although this was not directly the result of feeding rate, the higher food ration would increase the amount of material in the gut possibly decreasing passage time, if the gut is of fixed volume. In *Mysis relicta* gut residence time was negatively correlated with temperature but at high temperatures feeding rate became disassociated from gut residence time (Chipps 1998).

Respiration rate was significantly higher in August and September than in the winter months (Figure 5.14). However, for the majority of the year the differences were not statistically significant, suggesting that *Gammarus insensibilis* is adapted to a variable environmental regime and has the ability to acclimate to the temperature and salinity conditions it is subjected to rapidly. This is also suggested by the multiple linear regression analysis where there was no significant effect of temperature or salinity when analysed in combination. Relatively lower Q_{10} values and comparatively temperature independent respiration rates for intertidal and shallow water amphipods in comparison to deeper water species, indicate highly developed adaptation to the temperature regimes of the environment to which organisms are adapted (Bulnheim 1979).

When analysed on a seasonal basis however the summer respiration rates were higher than the spring and winter suggesting despite well developed adaptation to variable environmental conditions, there was some seasonal effect on respiration rate owing to extended periods of higher temperature and salinity. It is unsurprising that this is the case in the lagoon environment because under laboratory circumstances it is widely known that increased temperature can be positively correlated with respiration rate, even when organisms are well acclimated e.g. amphipods (Mathias 1971; Einarson 1993; Yamada and Ikeda 2003); isopods (Bulnheim 1974; Salomon and Buchholz 2000) and mysids (Roast et al. 1999a).

The significantly higher respiration rates evident in August were almost certainly related to the extremely high temperature. On the August 2003 sample date the lagoon temperature was 27.7 °C and similar temperatures were experienced for an extended period in the summer of 2003. The increased respiration rates at higher temperatures are in part the result of increased rates of protein synthesis which are closely correlated with temperature (Robertson et al. 2001; Whiteley et al. 2001). Whole body protein synthesis accounts for a minimum of 18-26% of the heat (energy) loss of marine organisms (Hawkins 1991). For example 22% of the energy loss through respiration rate of the isopod *Idotea rescata* was the direct result of the rate of whole body protein synthesis (Whiteley et al. 1997).

Excretion rate showed a similar pattern to respiration rate with a high rate in August. This can again be attributed to the extremely high temperature and salinity experienced in that summer. Excretion rate has been ignored from the SFG assay in other peracarids because it is labour intensive and it tends to be low in these animals so the resulting effects on the energy budget are negligible. The assay was carried out in this survey because of the importance of salinity variation to the lagoon habitat. Variation in salinity causes osmotic stress in organisms so the maintenance of water balance can be compromised and excretion rates may be higher in order to osmoregulate. However, as described in the results section the high standard deviation in these assays may reduce the validity of these results so it was ignored in the construction of the final energy budget.

There have been few long term studies of energy budgets i.e. several months to a year. Most studies have focused on analysis of various laboratory experiments on certain influences on the energy budget. Longer term studies that have been carried out have been on bivalves, in which energy budgets have been followed for an extended period of time so that they reflect the natural conditions of the organism. In an evaluation of the SFG over the course of a year *Mytilus edulis* showed no seasonal pattern in any of the components of SFG whereas *Cerastoderma edule* were sensitive to reduced temperature. This was credited to the more northerly distribution of *M. edulis* and the reduced temperature acclimation ability of *C. edule* (Smaal et al. 1997). Seasonal patterns in SFG were also apparent for the bivalve *Spisula subtruncata* with much lower values for SFG in the autumn and winter months (Rueda and Smaal 2004). It would be expected therefore that *G. insensibilis*, adapted to variable conditions of coastal lagoons would demonstrate relative stability in its energy balance over the course of a year, or be able to maintain reproductive output despite periods of stress.

In the present study, when SFG was calculated for *G. insensibilis* using the monthly derived calorific content of the food source there was no apparent seasonal trend, despite the various components showing some seasonality. The same is true with *C. edule* (Smaal et al. 1997) but in the cases of *M. edulis* and *S. subtruncata* reduced or negative SFG were related to times of peak reproduction. A similar relationship account for the most negative SFG values recorded for *G. insensibilis* in the spring (March and April). The relationship between the energy budget and reproductive factors is considered in more detail in the Chapter 6. Alternatively, using a fixed value for energy content of *C. linum* of 14.6 KJ/g indicates that SFG was far more balanced in the summer half of the year with values predominantly around zero. This may be more accurately reflect the actual lagoon conditions because, as previously discussed the methods of selection of algae for the assays may not reflect the selection made by the amphipods under natural circumstances.

While SFG was frequently negative in this survey and there are several reasons which may help to explain this phenomenon (discussed below), it must be noted that low or negative SFG does not necessarily represent a severe problem for an organism. Roast et al. (1999 b) explained that reduced SFG simply represents less energy available from that that was ingested. This could, as the definition of the term states, be utilized in growth or reproductive costs of the organism. SFG is only an indicator of stress and low values must be shown to be in response to a particular challenge to be interpreted as harmful to an organism. This highlights the need to use animals for which there is sufficient information available on the life history strategies in order to reduce intrinsic effects that may indicate low SFG.

Scope For Growth was low and often negative in this survey indicating that *G. insensibilis* was under severe stress for a large part of the year. The animals clearly can not be so energetically compromised for such a large proportion of the time or they would not be so abundant in the lagoon. There maybe a number of reasons for the negative SFG. The organisms used were

gravid females carrying early stage embryos (the embryos were removed for the assays). Although this ensured that there was consistency of life stage and reproductive state of specimens for the experiments, it did mean that the organisms may have been experiencing or until recently experiencing increased metabolic demands associated with reproduction.
In a monthly study on the SFG of the economically important bivalve *Spisula subtruncata*, there was an increase in respiration rate of up to twice the non-reproductive rate during the period of peak reproduction (Rueda and Smaal 2004). The SFG and body mass of the Chilean oyster *Ostrea chilensis* was much reduced in brooding organisms, resulting from a decrease in the feeding rate component of SFG (Chaparro and Thompson 1998). For *G. insensibilis* this can be supported by the fact that it is a continuously reproducing species (Chapter 4) and by the results of comparative respiration rates in Chapter 7 which show that under most temperature/salinity combinations reproductive females demonstrate higher respiration rates than males.

The food source used for the feeding assays was *C. linum*, on which the *Gammarus insensibilis* routinely feeds upon in the lagoon. Gammarid amphipods are frequently omnivorous (Lincoln 1979) so this probably does not solely represent its diet. They were observed feeding on a number of other materials under laboratory conditions including injured members of their own species and their own embryos and those of other females if they were removed or spilled. They would also readily consume artificial fish foods presented to them. They were also commonly found amongst detritus of both aquatic and terrestrial origin in the margins of the lagoon so presumably the diet can be supplemented under natural conditions. Opportunistic feeding is common among benthic peracarids, a number of species of *Idotea*, which had previously been fed on a normal algal food source, would switch to feed on polychaete, mollusc or fish material when it was presented to them (Naylor 1955). Furthermore, a potential source of experimental stress occurred because in the respiration rate assays there was no substratum in the respiratory chambers. While preliminary observations suggested that this was not a problem, because the animals rested in the corners of the chambers, it has been suggested that a lack of substrate may cause additional stress to experimental animals (Normant et al. 2004).

The rate of feeding was measured as the rate of consumption of *C. linum* and there was a mesh bottom to the feeding chamber to prevent the amphipods from re-ingesting the faeces. Observations during this investigation have shown that *G. insensibilis* does re-ingest faeces under laboratory conditions so prevention of this in the assays may also reduce the energy available from feeding.

Paine and Vadas (1969) and Steele and Whittick (1991) urged caution when using calorific content as indicators of the potential value of macroalgae as food sources to grazers as they represent the total amount of stored energy which is “at best a quantitative estimate” of the energy available to herbivores. Carbohydrate, lipid and protein and non-energetic dietary components such as vitamins vary inter- and intraspecifically both spatially and temporally, thus affecting the quality of the food source for invertebrate grazers (Larson et al. 1980; Bowen et al. 1995; Haroon et al. 2000; Cruz-Rivera and Hay 2001).

The indigestible structural polysaccharides of marine algae may represent a large part of the calorific content (Granado and Caballero 2001). The relative proportions of these components more accurately describe the value of a food source and how this changes. However, the nutritional requirements of marine invertebrates with the exception of certain species of commercial importance are poorly known (Granado and Caballero 2001), so the benefit of calorific content is that it gives a simple estimate of the energy that can be gained from a particular food source. This can then be used as a value to estimate SFG. For a more accurate energy budget the complete biochemical composition of the food source would be required along with a far greater understanding of the abilities of the test organisms to assimilate these components. In this kind of survey, where the aim was to determine the trends to gain a greater understanding of the ecology of an important animal seasonal calorific content is sufficient.

In summary, the metabolic rates of *Gammarus insensibilis* in Gilkicker lagoon showed the usual trends of increased rates when the temperatures were high in the summer but also demonstrated strong ability to adjust to the variable environment. The amphipod appears to be able to survive and reproduce to maintain a viable population despite being at the limit of its energy balance. The added metabolic cost of increased reproductive effort in the spring indicates severe stress in the SFG assay.

Chapter 6

Scope For Growth, reproduction and links between levels of biological organisation

The Scope For Growth assay is a useful tool for monitoring when organisms are under stress, and is frequently used to determine how they are affected by anthropogenic pollution stress. However it is not just extrinsic factors that can induce negative effects on SFG. By definition SFG, as previously discussed, may be reduced at times of peak reproduction or somatic growth in any organism (Smaal et al. 1997). When SFG is lowered, for any reason, including high reproductive investment, organisms have reduced energetic capacity for the initiation of the stress response. This may lead to increased risk of disease or reduced ability to respond to variable environmental conditions. For example, Pacific oysters (*Crassostrea gigas*) were more susceptible to bacterial infection after a stress response had been initiated by a mechanical stressor (Lacoste et al. 2001). Similar reduced immune capabilities or diversion of energetic resources away from reproduction can obviously have important implications for populations of conservation importance that are subjected to severe environmental variability.

The data from chapters 4 and 5 represent an opportunity for the comparison of a relatively long term survey of an organism’s energy budget with the actual situation that was apparent in the population at the time of the energy balance assays. This chapter compares these data in order to investigate the interactions between reproductive investment and energetic status of *G. insensibilis* under the lagoon temperature and salinity regime. It must be noted that these data do not represent individual organisms but are mean values from the month’s population dynamics samples and the mean values from the SFG assays.

The trends in reproductive parameters were more seasonal than the trends in SFG. For example reproductive trends included larger summer brood sizes (Figure 4.2) or larger winter embryos (Figure 4.5) while there were no seasonal trends in SFG (Figure 5.19), suggesting that the number and size of eggs produced was not directly related to energy availability at the time (Figure 6.1). There were also no monthly similarities between the SFG pattern and the other reproductive parameters measured in Chapter 4 (mean female size, smallest female size, late stage brood size, the rate of embryo loss or mean juvenile size).

The lack of a trend between SFG and brood size or egg volume (Figure 6.1) would be expected because neither represents total reproductive investment (Chapter 4). Clutch volume was a more reliable determinant of overall reproductive output, because it represented the trade-off between investment into number of offspring and investment into the individual, and it showed a closer relationship with SFG (Figure 6.2). This was not a linear relationship but for the first seven months of the survey the two parameters followed the same pattern as one another i.e. when SFG increased clutch volume increased and when SFG decreased so did clutch volume. The peaks and troughs in clutch volume occurred at the same time as those in SFG (peaks; September and January, troughs; November) implying that total reproductive investment occurs at a rate determined by the energy availability.

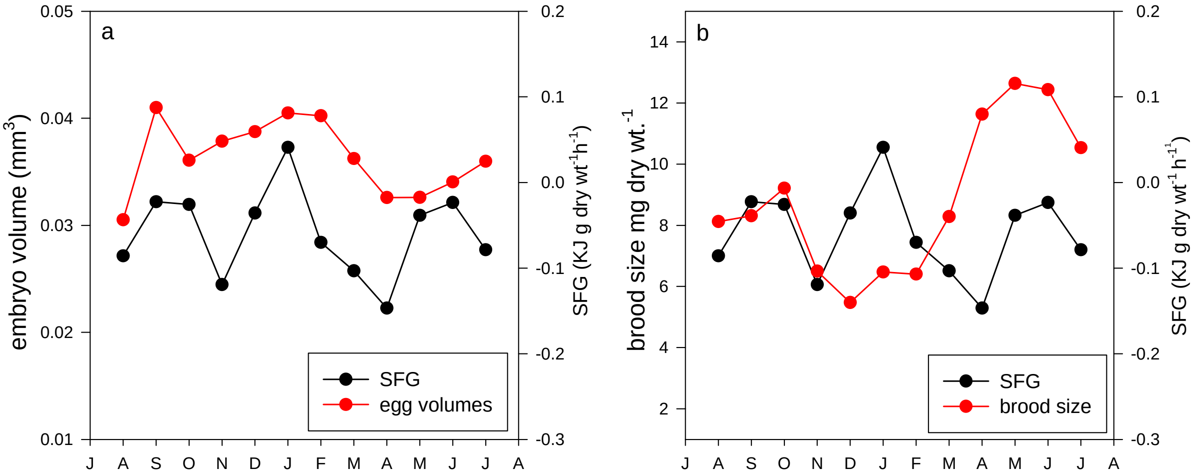

Figure 6.1: Comparisons of monthly early-stage reproductive investment parameters with Scope For Growth in *G. insensibilis* a) Embryo volume and b) Brood size.

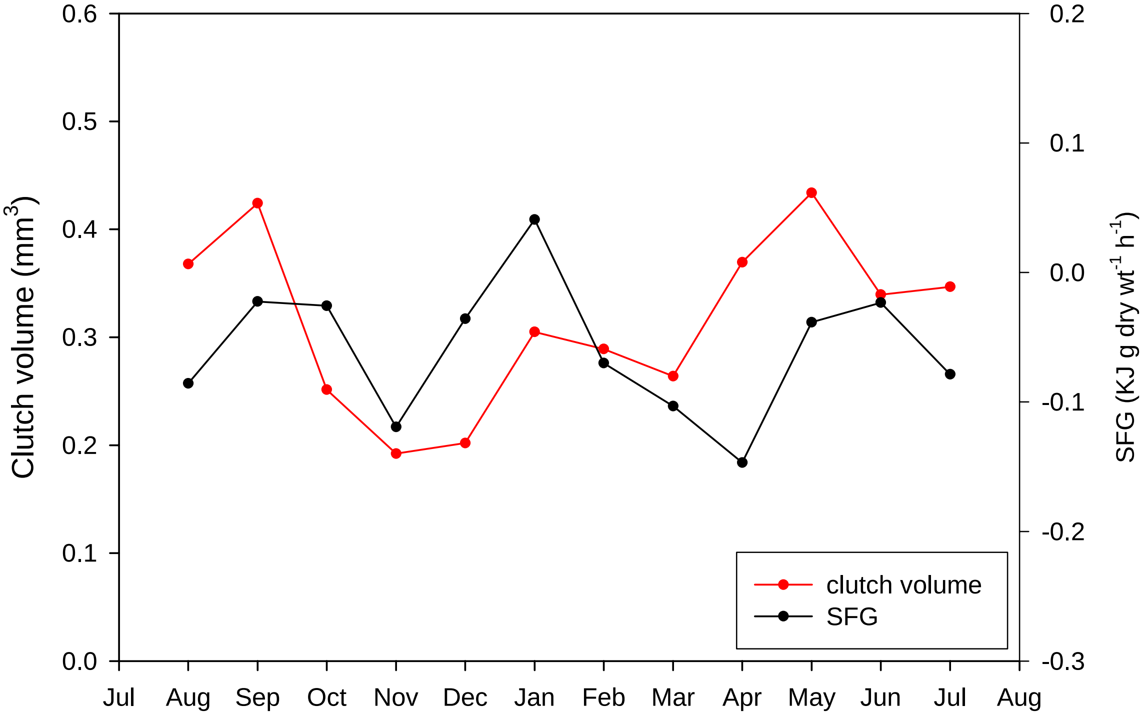

Figure 6.2: Monthly comparison between the early stage clutch volume and SFG of *G. insensibilis*.

The manner in which the clutch volume and SFG oscillated similarly suggests that they were in some way linked. The amphipods appear to reproduce to the greatest extent that the energy balance will allow, for the majority of the year, i.e. there was a fixed proportion of the energy budget allocated to reproduction. In the spring however, when environmental conditions may be more conducive to high juvenile survival, the clutch volume became disassociated from SFG. In April the SFG continued a negative trend while clutch volume began the rise to its highest level, suggesting increased energy allocation to produce the larger clutches associated with the spring. Presumably fitness was maximised under a strategy in which the amphipods invested heavily in reproduction, potentially at the expense of personal survival to ensure maximum offspring production during the most satisfactory environmental conditions for juvenile growth and survival.

When analysed on a seasonal basis there were significant differences in the reproductive parameters (Chapter 4). There were also more obvious seasonal trends in SFG. Consequently there were trends between SFG and the reproductive parameters; these are shown in Figure 6.3. Early stage clutch volume, late stage brood size and early stage brood size were all highest when SFG was low demonstrating the energetic cost of reproduction for *G. insensibilis*. Mean embryo size was greatest when SFG was highest. Despite the larger embryos in the winter, clutch volume was still low. This suggests that when energy investment into reproduction was low (i.e. when environmental conditions may not be conducive to high juvenile survival) the allocation to reproduction was directed preferentially towards individual egg quality rather than to numbers in order to ensure that those juveniles had a greater chance of survival.

The monthly SFG and clutch volumes, to a great extent, followed the same pattern. This may have been because reproduction was allocated based on the condition of the organism. Therefore in this case SFG did not represent the actual cost of reproduction, but highlighted the time when reproduction was likely to be highest. Over an increased period of time, i.e. seasonally, the energetic status of the organism was negatively affected by increased reproduction.

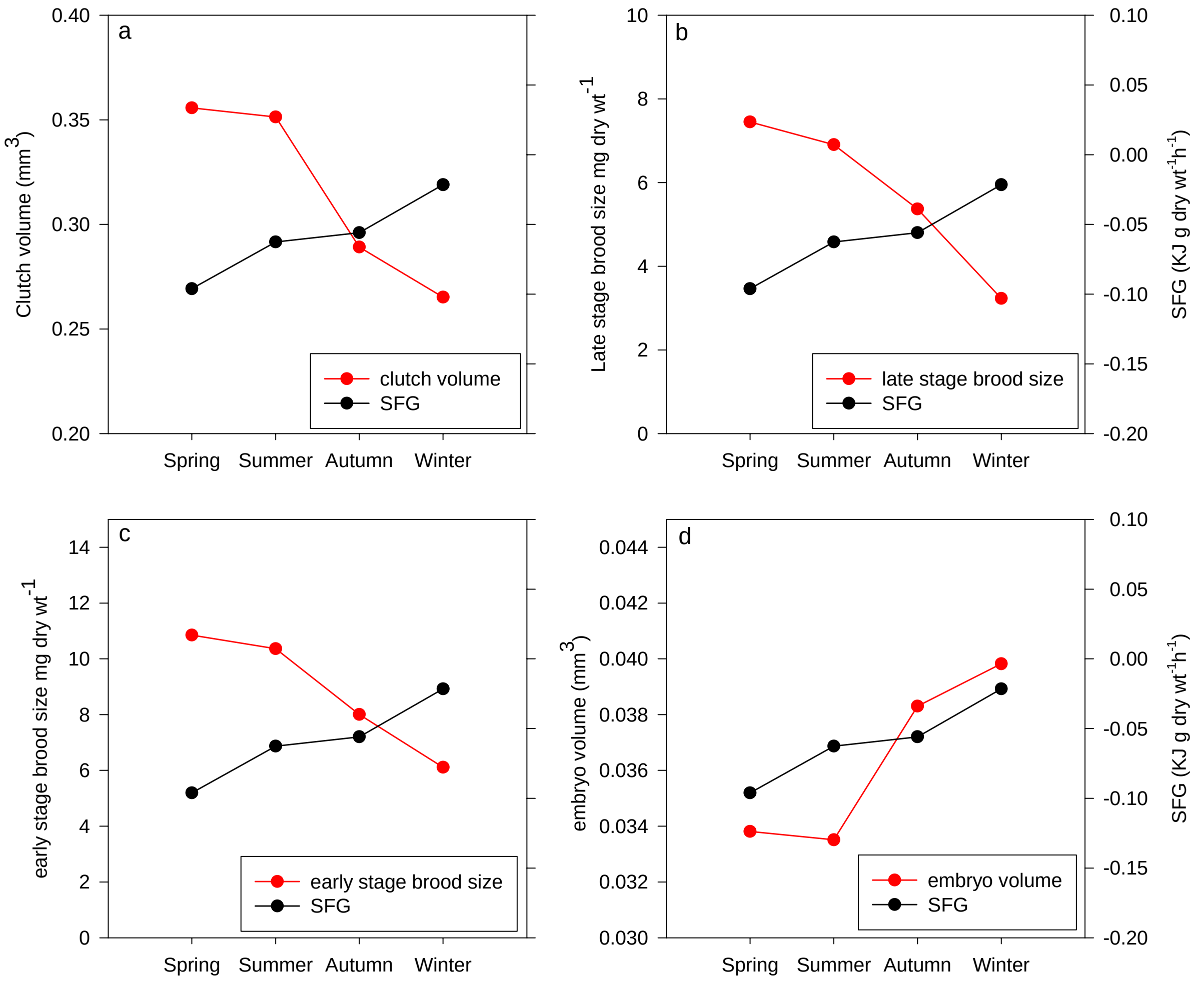

Figure 6.3: Comparisons between seasonal reproductive parameters and SFG; a) clutch volume, b) late stage brood size, c) early stage brood size and d) embryo volume (unlabeled y axes represent SFG).

During the course of the population dynamics survey, the ash-free dry-weight: dry-weight ratio of the *G. insensibilis* specimens was determined. The ash-free dry-weight to dry-weight ratio (AFDW:DW) describes the ratio of organic material to inorganic material in the body of an organism. It can be used as a measure of the condition of animals, as the higher the proportion of organic material, the more stored products such as lipid are present in the organism. Figure 6.4, however, shows that there was no trend between the mean AFDW:DW of the population (all animals, male female and juvenile from the population dynamics survey) and SFG. This may be expected as the SFG assay detects immediate stress but the level of stored materials indicates the condition of the organism over a more extended period of time. There was however significant variation in AFDW:DW over the year long population dynamics survey (Kruskall-Wallis: H = 260.456, 11 df, P = <0.001). In general, when the AFDW:DW was highest (October and July) it was significantly higher than the rest of the year, and when at its lowest (February) it was significantly lower than the other months (Pairwise comparisons – Dunn’s method).

The monthly patterns for AFDW:DW of male, brooding female, non-brooding female and juvenile *G. insensibilis* were very similar over the course of the year. The whole population analysis in Figure 6.4 can therefore be used to represent any group (there were slight differences between groups, e.g. brooding and non-brooding females as seen in Figure 6.6 but the general patterns over the year were the same). On a seasonal basis (Figure 6.5 a) the AFDW:DW of females carrying early stage embryos differed significantly (ANOVA $F_{3,122}$ = 3.175, P = 0.027). The winter AFDW:DW was significantly lower than the other seasons (Holm-Sidak). Again however, there was little or no trend apparent with SFG (Figure 6.5 b).

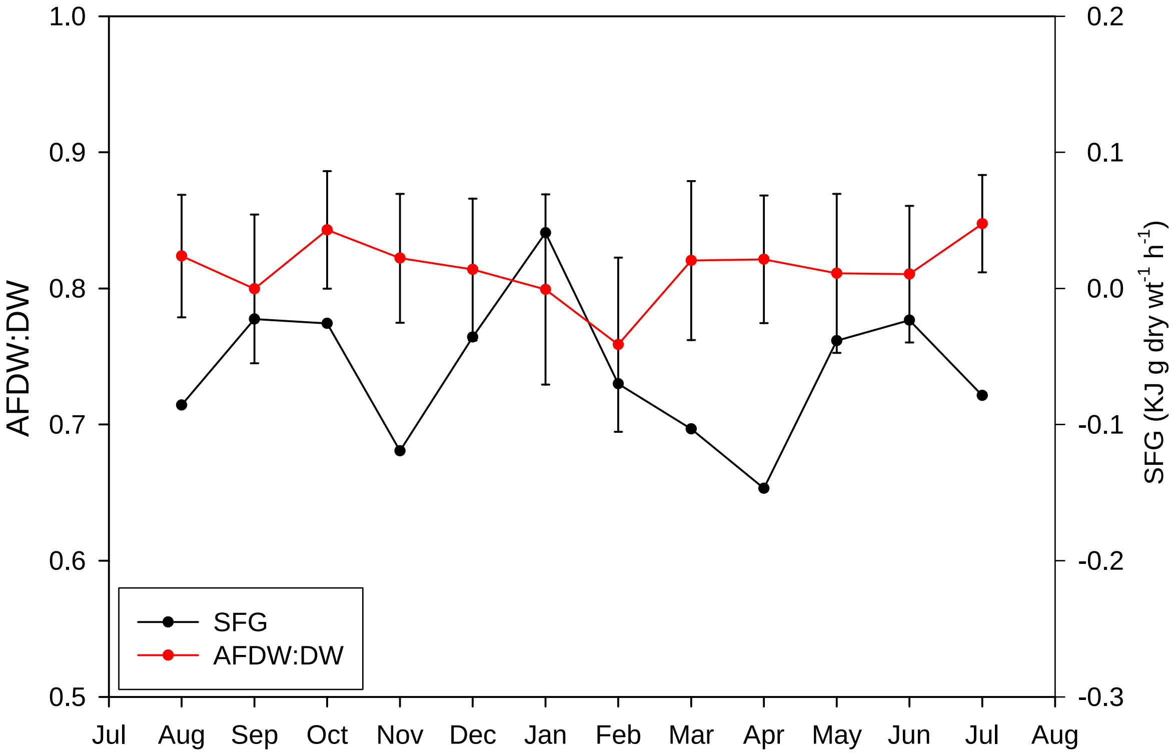

Figure 6.4: The AFDW:DW of all *Gammarus insensibilis* combined over the course of the 12 month population dynamics survey. SFG is also shown for the same 12 month period in the bioenergetics survey

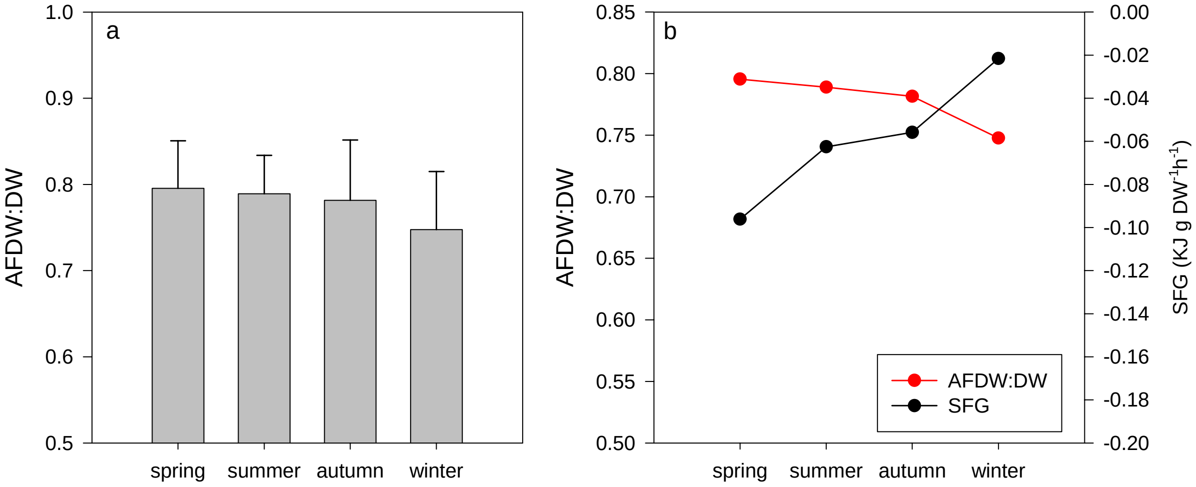

Figure 6.5: a) AFDW:DW of female *G. insensibilis* carrying early stage embryos (±sd). b) The same data, plotted alongside SFG

Egg-carrying female *G. insensibilis* demonstrated lower AFDW:DW than non-brooding females (Figure 6.6) indicating a degree of usage of stored materials related to the brooding of the embryos. There was a significant difference between the combined data for all the non-brooding and early stage brooding females from the population dynamics survey (Kruskall-Wallis: H = 65.985, P = <0.001: Figure 6.6 inset). There was however no difference between non-brooding females with developed oöstegites and those with undeveloped oöstegites suggesting that the animals can rapidly return to pre-reproduction condition after the juveniles were released. This is also suggested by the increase in the AFDW:DW ratio for females carrying embryos of later development stages shown in Figure 6.7.

The lower AFDW:DW in brooding females further indicates that the reproductive females used for the SFG assays in the 12 month bioenergetics survey were suffering from reproductive stress. The mean organic proportion of the females brooding early stage embryos was approximately 6% lower than non-brooding equivalents. They had clearly experienced a significant excess use of stored resources, which may, in part explain the generally negative values demonstrated for SFG in the 12 month bioenergetics survey and supports the evidence for the increased reproductive stress that the amphipods experienced during the increased reproductive investment of the spring months.

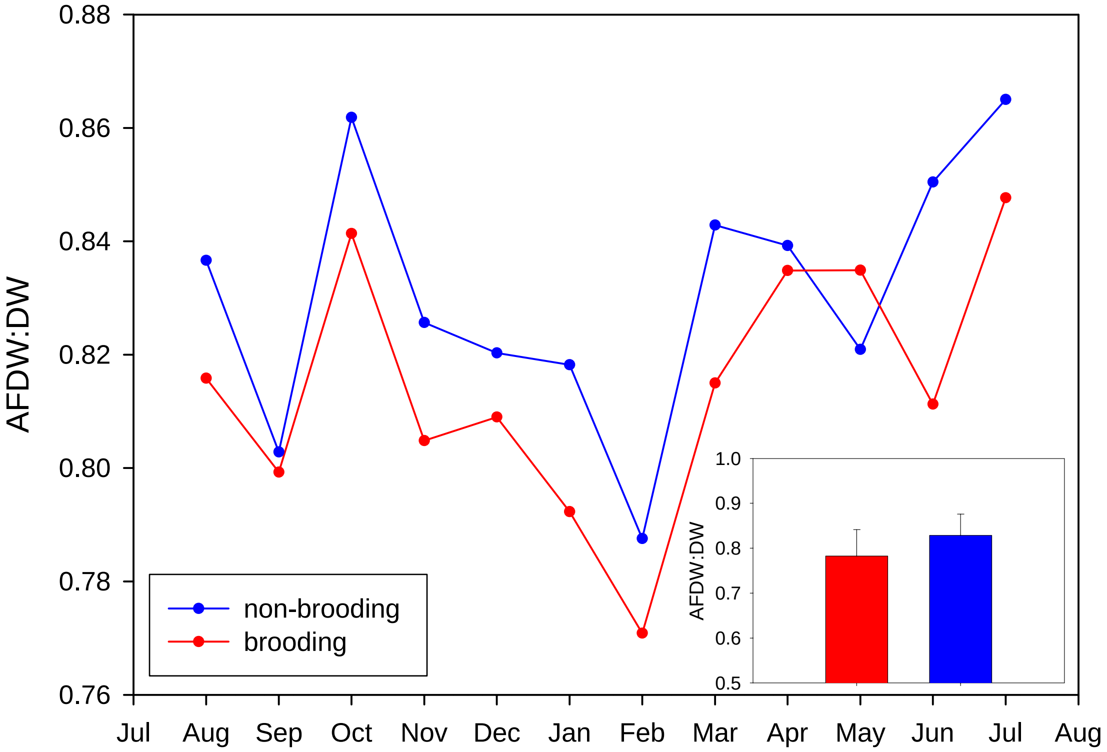

Figure 6.6: The monthly AFDW:DW of brooding and non-brooding female *Gammarus insensibilis* over the course of the 12 month population dynamics survey. Inset – the mean AFDW:DW of all females brooding early stage embryos (red) and all those with empty marsupia (blue).

Figure 6.7 shows the combined annual data for the AFDW:DW of female *G. insensibilis*, brooding embryos of different developmental stages. AFDW:DW was generally greater in females carrying later stage embryos. These differences were significant (Kruskall-Wallis, H = 113.783 with 5 degrees of freedom, P = <0.001) and Pairwise comparisons showed that females carrying stage I and II embryos differed significantly from those carrying embryos of later stages. This suggests that there was an initial reproductive stress related to the production of the early stage embryos. The initial stress is probably the result of the actual production of the eggs for the next reproductive event because the cycle of oogenesis in amphipods, and therefore utilization of energetic resources, occurs concurrently with the development of the embryos (Sheader and Chia 1970; Johnson et al. 2001). In the later stages of development the AFDW:DW rose to higher than the mean for non brooding females suggesting resources were being stored for the beginning of the next reproductive output after the next moult.

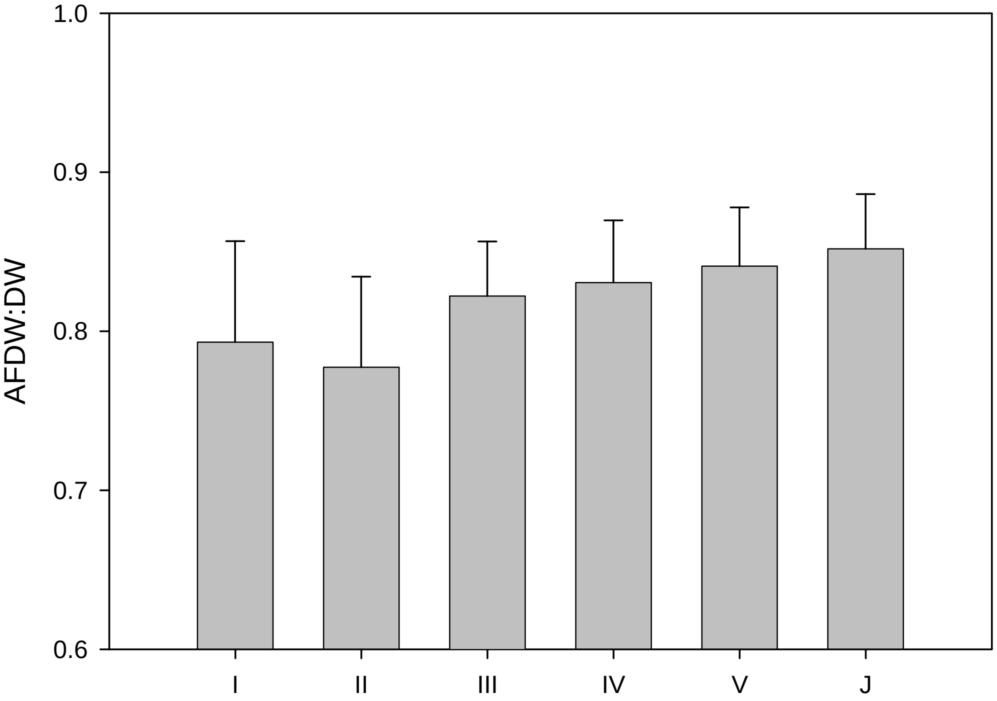

Figure 6.7: The relationship between of egg stage on the AFDW:DW of brooding female *Gammarus insensibilis* (± 1 sd)

In conclusion it appears that the energetic status of *Gammarus insensibilis* is more closely related to the level of investment into reproduction than the environmental conditions it is subjected to. However, this leads to the suggestion that at the times when *G. insensibilis* invests most heavily into reproductive output it is more likely to be negatively affected by stressful environmental conditions. Under the normal environmental regime of the saline lagoon, however, the animals appear well adapted, and have an energetic strategy where reproduction can be maintained constantly through the year with the capacity for an increased allocation in spring, when environmental conditions are favourable for the subsequent months to ensure juvenile survival and to maximise lifetime fitness.

The management of saline lagoons must therefore ensure that excess environmental stress is avoided at these times. Factors such as poor management, pollution events and in the longer term, climate change could all result in increased environmental stress on lagoon organisms. All three are genuine risks to the long term survival of these uncommon habitats and their specialist biota. Poor management is a potential risk because of financial constraints limiting funding for schemes to monitor such habitats. Pollution events are common in near shore ecosystems affected by run off from surrounding urban areas. Climate change poses various risks to the lagoon; physically, rising sea levels may threaten their existence, while temperature change has obvious effects on the physiology of poikilotherms and variable precipitation has important salinity consequences. These aspects are discussed further in Chapter 10.

Chapter 7
The physiological tolerances of *Gammarus insensibilis*

7.1. Introduction

The monthly surveys of the bioenergetics, population dynamics and reproductive investment of *Gammarus insensibilis* provide useful data for its conservation. They described when the population was at greatest risk, because of the effects of environmental stressors or because reproductive output was high. However, the results in these surveys were characteristic of the animals under natural circumstances, i.e. physiologically acclimatized to the environmental conditions used in the assays. It is still essential to understand how the animals respond to wider ranges of temperature and salinity, of the kind normally uncommon, but which may occur with extreme weather conditions in combination with neglected management of lagoons.

Over an extended time period all organisms can adjust, through the processes of acclimatization, to variation in their ambient environment. It is widely accepted that part of the evolutionary adaptation to variable habitats, for example coastal waters or estuaries, is increased acclimatization ability. For example, the amphipod *Gammarus locusta*, which is preferentially distributed in a subtidal environment, demonstrated significantly increased respiration rates for over 30 hours after transfer to lower salinity. In comparison, the more euryhaline *G. duebeni* (commonly found in supralittoral habitats) reached a steady level of metabolism within 3 hours, following the same treatment (Bulnheim 1974). Both *G. duebeni* and *G. locusta* are found in relatively variable habitats in contrast to organisms from the characteristically stable habitat of the deep sea or the polar sublittoral. Organisms from stable environments tend to demonstrate far lower acclimatory ability (Aarset and Aunaas 1990). It would be expected therefore, from the high abundance of *G. insensibilis* in saline lagoons, that like *G. duebeni*, it has the ability to adjust physiologically to the demands imposed upon it by the environmental variability of its habitat. This has already been proposed as a consequence of the relatively minor variation in the respiration rates in response to the long term change in the bioenergetic survey (Figure 5.14).

In addition, the population dynamics survey showed that at some times of the year the population was biased toward a certain demographic e.g. a higher proportion of reproductive females in the summer, while at the same time these animals were smaller in size. Various life history characteristics impose important physiological considerations for the study of all organisms. Age and size (Spanopoulos-Hernandez et al. 2005) and gender and reproductive condition (Smaal et al. 1997; Rueda and Smaal 2004) are known to have effects on metabolic rates. The bioenergetic survey of Chapter 5, used animals of similar size and the same reproductive condition; in order to increase the understanding of the ecophysiology of *G. insensibilis* it is important to consider a range of animals, of varying size, gender and reproductive condition.

Respiration rate is widely used as an indicator of animal condition, particularly in the aquaculture industry (Spanopoulos-Hernandez et al. 2005). The bioenergetic survey in Chapter 5 of this study showed that although the rate of energy intake was important, the majority of effects on SFG resulted from respiration rate. Therefore, it can be considered that respiration rate can be used as a good indicator of the general condition of *G. insensibilis*.

Data collected over a range of temperatures allows the calculation of the temperature coefficient (Q_{10}). This describes the sensitivity of an organism to temperature change. A Q_{10} value of approximately 2 is commonly accepted to represent the normal physiological adjustments made by organisms with increases in temperature, related to enzymatic reaction rates. Values differing from 2, in either direction, suggest that the organism is not effectively compensating for the effects of the stressor (Randall et al. 1997). Temperature coefficient values greater than 2 show that the respiration rate has increased at a rate greater than the expected temperature related increase in enzymatic reaction rates. This suggests that the animal has increased energy usage in order to survive the effects of the stressor. Q_{10} values below 2 suggest that there has been no increase in enzymatic reaction rates, possibly because of their inactivity, because of low temperature dependence of the reactions (Garnacho et al. 2001).

The bioenergetic survey also indicated seasonal variation in feeding rates. Like respiration rate, various factors are known to affect the rate of energy acquisition through feeding in crustaceans, including extrinsic parameters such as temperature, salinity (Roast et al. 2000) and anthropogenic pollution (Maltby et al. 1990, Roast et al. 1999b, Verslycke et al. 2004) as well as intrinsic factors including size (Lozano et al. 2003), gender (Moss and Moss 2006, Schuwerack et al. 2006) or life history stage (Randall et al. 1997).

Various methods have been used to determine the rate of energy acquisition by marine invertebrates. The wide ranging sizes, life histories, feeding mechanisms and food preferences of the commonly studied aquatic species means that it is difficult of propose simple, standardized methods to determine these rates. The use of wet weight to dry weight ratios of *C. linum* in the bioenergetics survey, while adequate for the measurement of larger scale trends in metabolism, resulted in the inherent error associated with the measurement of wet weight of aquatic organisms.

This study further investigates the metabolic responses of *G. insensibilis* under a range of temperature and salinity with the aim of supplementing the information provided by the bioenergetics survey of Chapter 5. In response to the evidence that reproductive female *G. insensibilis* from Gilkicker lagoon appear to be living on the limit of their energy balance, various intrinsic factors are considered in an attempt to explain this apparent stress. The secondary aim was to trial a new method to infer the rate of grazing by gammarid amphipods, which eliminated the need for the potentially clumsy use of wet weight to dry weight ratios, as was necessary in Chapter 5. This method, based on the measurement of the rate of egestion of faeces, allows the use of naturally occurring food sources rather than artificial feeds, while reducing the error associated with the use of the wet weight of algal material.

7.2 Methods

The experiments were carried out in the spring of 2005. Prior to both feeding and respiration rate assays the animals were maintained in water collected from Gilkicker lagoon in covered outdoor tanks containing a variety of lagoon fauna and excess *C. linum* as a food source. As a consequence of the time of year the temperature of the holding tanks was approximately 10 ºC although this clearly varied on a diurnal cycle, while salinity was close to 30. The animals were transferred to filtered aquarium seawater previously diluted and prepared to the required the experimental salinity and temperature conditions 48 hours prior to the start of the feeding and respiration assays. They were maintained under a 12 hour light, 12 hour dark regime in controlled temperature rooms or water baths (±1ºC).

The method used for the measurement of respiration rate was the same as that used in the bioenergetic survey. However, in order to reduce handling stress there was no examination of animals prior to the assays. They were selected at random from the aquaria in which they were held. They were then starved for 24 hours prior to the start of the assay. On completion of the respiration assays, the animals were examined for gender, body length, dry weight and the presence of cerebral metacercaria of the microphallid trematode parasite that they harbour. The results of the parasite data are considered in Chapter 8.

Using the means from all the animals at each treatment the temperature coefficient for respiration rate (Q_{10}) was calculated, in order to investigate the range of tolerance of temperature and salinity for *G. insensibilis* using equation 7.1:

$$Q_{10} = (K_2/K_1)^{10/t1\text{-}t2}$$

Equation 7.1: Where Q_{10} = the thermal coefficient and K_1 and K_2 are the respiration rates at temperatures t_1 and t_2 respectively.

The general maintenance of the animals used in the feeding assays was the same as the respiration rate assays. The feeding rate experiments again used feeding chambers with a mesh bottom, to prevent the amphipods from re-ingesting faeces, and *Chaetomorpha linum* was again used as a food source. Like the respiration rate assays the animals were examined after the experiments. However, there was a slight difference in the methodology for the determination of the amount of the alga consumed in this study in an attempt to improve the method to negate the need to use wet weight to dry weight ratios of the alga. Some studies on the feeding rate of peracarids have used egestion rate as a surrogate for feeding rate e.g. mysids (Roast et al. 2000). Preliminary investigations in this study showed that the rate of egestion of faecal pellets by *Gammarus insensibilis* closely followed the pattern of feeding rate. However, this was obviously lower than feeding rate, owing to the effects of digestion.

Measurement of the quantity of alga ingested was replaced with the rate of egestion of faeces. After the animals had been incubated with the food source for the duration of the assay the food was removed and the animals left until there was no material visible in the gut. The faecal pellets were then collected using a Pasteur pipette under a dissecting microscope. These were gently rinsed with milli-Q water. They were then transferred to pre-ashed, pre-weighed GF/F filters, dried and weighed to determine dry weight. They were then burned in a muffle furnace to determine ash-free dry-weight. From this information the rate of production of faeces and the assimilation efficiency were determined. From these data the actual rate of feeding was determined.

Where the bioenergetics survey used a mean value for assimilation efficiency for all the animals in the assay this study used the egestion rate and assimilation efficiency of each individual amphipod to ensure greater accuracy in order to enable the comparison of the different life history stages. For the latter months of the bioenergetics survey this method was employed alongside the ww:dw ratio method for testing purposes. The results of this comparison are presented in Figure 7.1 and show that the two methods for the determination of assimilation rate produce similar data. The February results are excluded because a problem occurred with the collection of faeces, and it was necessary to re-run the assimilation efficiency assay. As a result the rate of faeces egestion was not related to the rate of consumption of *C. linum* in the assay. The discrepancy in the results presented in Figure 7.1 (January, June and July) can be explained by the inadequate time period allowed for the egestion of all faecal pellets, thus indicating lower assimilation rates. The problem was addressed, and in this study sufficient time was allowed to ensure all the faeces produced from feeding in the period of the assay were egested.

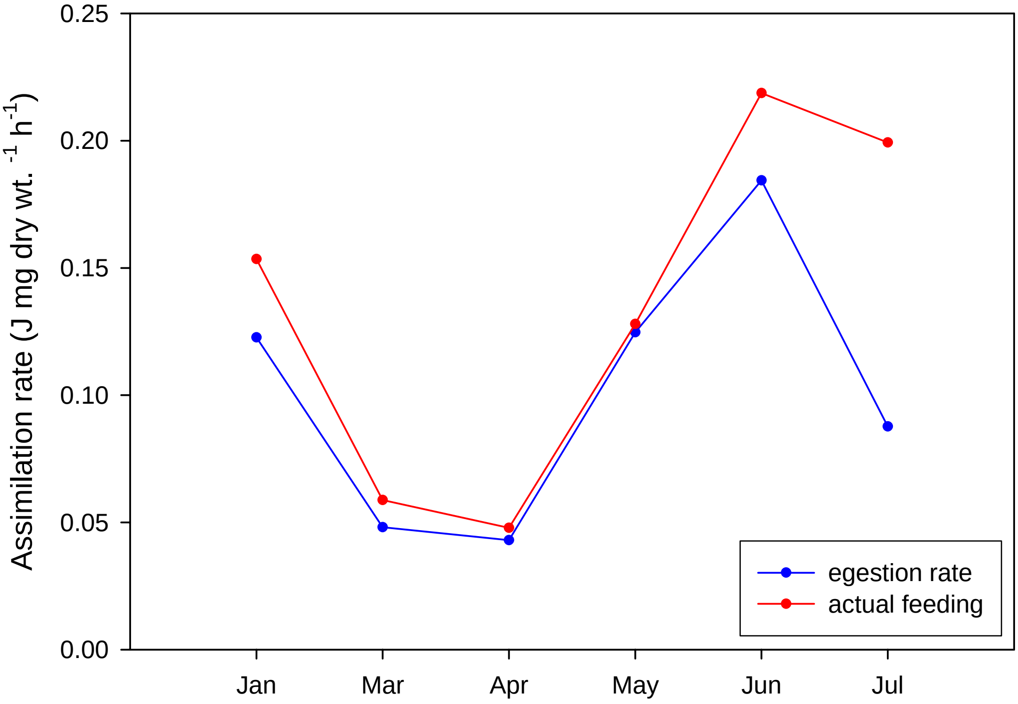

Figure 7.1 A comparison of the methods for the determination of assimilation rate over 6 months of the bioenergetic survey. February was excluded from the analysis because of problems in the assay, these are explained in the text.

7.3 Results

Temperature had the greatest influence on respiration rate of *Gammarus insensibilis* with much increased rates over the range, particularly between 15 and 20 ºC (Figure 7.2). Two factor ANOVA showed the temperature related differences to be significant (Table 7.1, $p < 0.01$) but the salinity effects were not ($p > 0.05$). However, there was a significant interaction between the two factors ($p = 0.014$).

When each salinity treatment was analysed individually there were clear significant differences between the temperature treatments (20 salinity: $H = 13.47$, $P = 0.001$; 25 salinity: $F_{2,23} = 15.694$, $P < 0.001$; 30 salinity: $F_{2,26} = 3.897$, $P = 0.034$; salinity 35 $F_{2,22} = 6.351$, $P = 0.007$). However, there was the unusual occurrence of no rise in respiration rate with the temperature increase between 5 and 15 ºC at salinities of 20 and 35. Pairwise comparisons (Holm-Sidak) highlighted that the respiration rate at 20 ºC was significantly higher than at 5 and 15 ºC at each salinity treatment while there was generally no significant difference between the 5 and 15 ºC treatments.

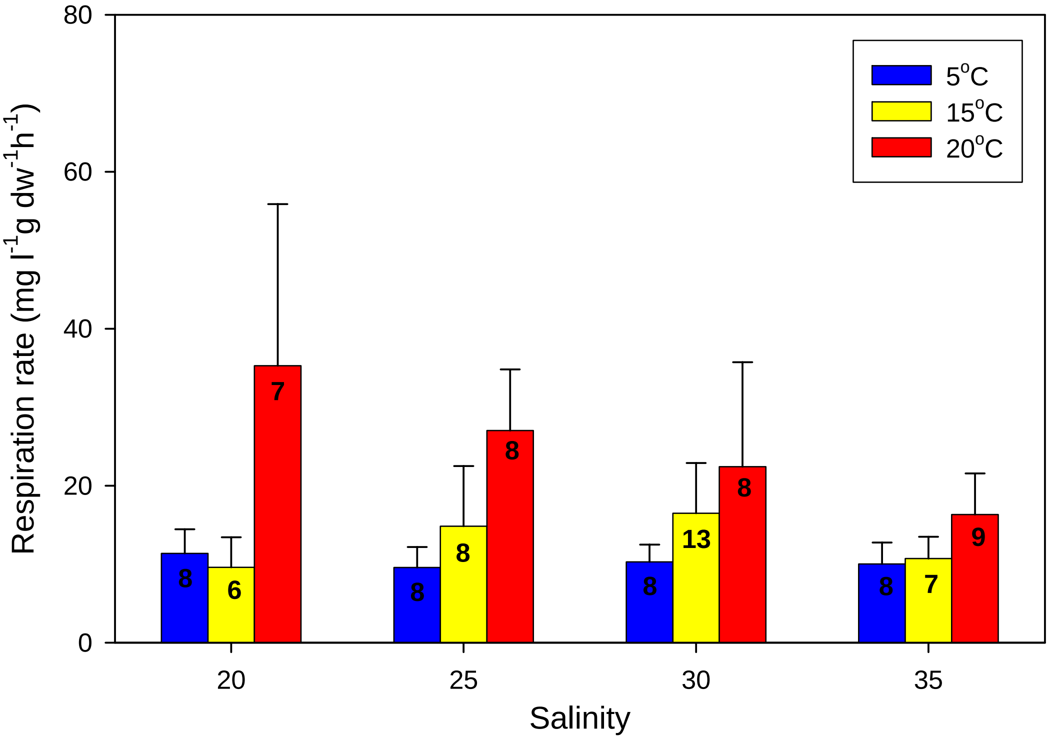

Figure 7.2: mean weight specific respiration rate (mg g dry wt.$^{-1}$ h^{-1}) at a range of salinity and temperature conditions (± 1 sd). The values on the bars represent sample size (n).

Table 7.1: Analysis of respiratory responses to temperature and salinity by *Gammarus insensibilis*

Two factor ANOVA of salinity, temperature and their interactions

Source	Degrees of freedom	Sum of squares	MS	F	P
Temperature	2	3819.20	1909.60	29.39	<0.001
Salinity	3	492.93	164.311	2.53	0.063
Interaction	6	1109.22	184.869	2.85	0.014
Total	94	10716.89	114.01		

The calculation of the thermal coefficient showed that at the lower temperatures mid range salinity produced Q_{10} values closest to 2 while at higher temperatures Q_{10} was closest to 2 at the higher salinities. Table 7.2 demonstrates this; Q_{10} was 1.85 and 2.32 in the 15 – 20 °C temperature range at the higher salinities (30 and 35). However, in the higher temperature range at low salinity Q_{10} was high, resulting from the low respiration rate at 15 °C in the 20 salinity range. In lower temperature range, intermediate salinity resulted in Q_{10} values closest to 2. At the extremes of salinity Q_{10} was closer to 1. The Q_{10} values for the entire range of temperature change at all salinities were close to 2.

Table 7.2: The thermal coefficient (Q_{10}) for *Gammarus insensibilis* at 2 temperature ranges and four salinities.

Salinity	Temperature intervals	
	5-15 °C	15-20 °C
20	0.84	3.68
25	1.55	3.32
30	1.60	1.85
35	1.07	2.32

The respiration rate data from Figure 7.3 were further analysed to determine the effect of gender. As there were no significant salinity effects the data for the 4 salinity treatments at each temperature were combined to increase the number of animals for which data were available. The respiration rate of female *G. insensibilis* was significantly higher than males at both 5 and 20 °C ($p < 0.05$). There was however no significant difference between the sexes at 15 °C.

The females were divided into those brooding embryos and those with empty marsupia. There was no significant difference between the groups at each of the temperature treatments. There was also no significant difference between females with empty marsupia and the state of development of their oöstegites had no significant effect. There were general trends however including the trend towards females with undeveloped oöstegites to show increased respiration rates but limited data resulted in no significant difference. These data were used, however, in the construction of the energy budgets. It should be noted that there were also effects on respiration related to the presence of the microphallid trematode parasite, commonly found infecting *G. insensibilis* from Gilkicker lagoon. These effects on amphipod respiration rate are discussed in the next chapter.

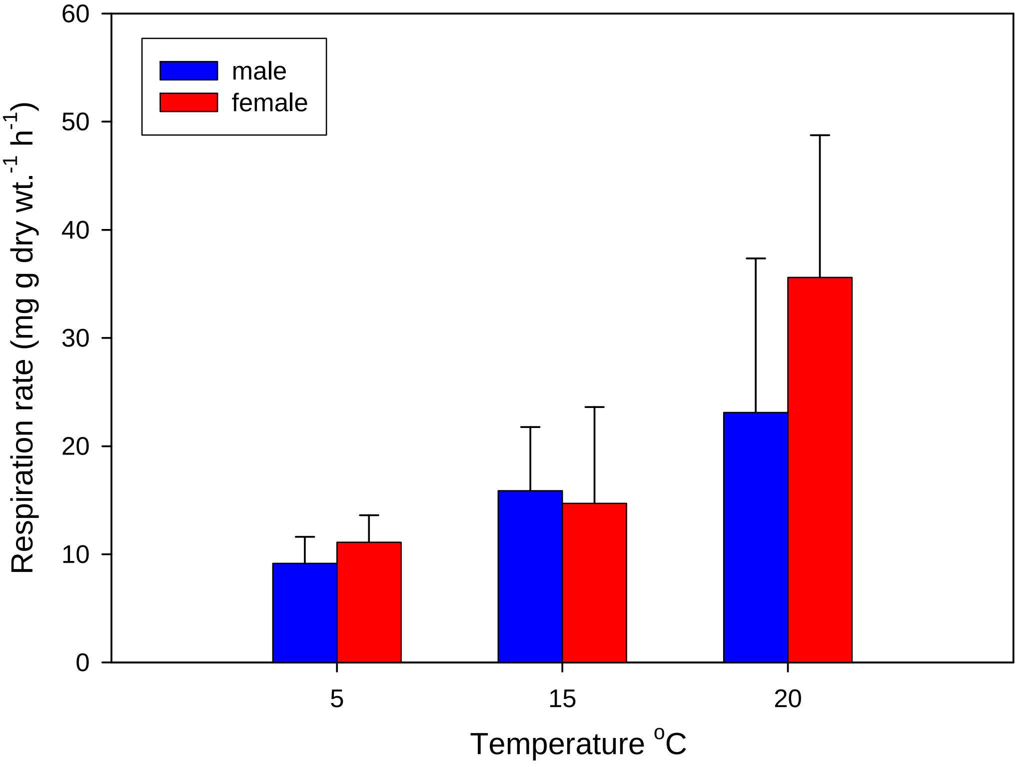

Figure 7.3: The effect of temperature on the respiration rate (mg g dry wt.$^{-1}$ h^{-1}) of *Gammarus insensibilis* (± 1 sd) of different gender.

The effect of animal size on the weight specific respiration rate was analysed. Although negative trends were generally evident there was only one treatment at which there was a significant negative relationship between size specific respiration rate and body mass (males at 5°C, Table 7.3). Some of the respiration rates to size relationships were best as described power relationships.

Table 7.3: Regression equations and r^2 values of weight specific respiration rate in male and female *G. insensibilis* at different temperatures. (dw = dry weight)

Temperature	Gender	n	Slope	r^2
5	♀	19	y = -0.7733dw + 13.685	0.16
	♂	13	y = -0.5846dw + 12.86	0.59
	total	32	y = -0.6297dw + 13.18	0.41
15	♀	9	y = -0.36878dw + 4.7362	0.22
	♂	12	y = -0.6879dw + 18.849	0.05
	total	21	y = 0.2588dw + 12.44	0.04
20	♀	9	y = -10.31dw + 51.866	0.38
	♂	12	$y = 22.605dw^{-0.1115}$	0.05
	total	21	$y = 28.346dw^{-0.3207}$	0.24

Figure 7.4 showed that, between 5 and 15 ºC, feeding rate increased with increased temperature, but high standard deviation resulted in insignificant differences between the treatments. There were no significant effects of salinity so data were grouped.

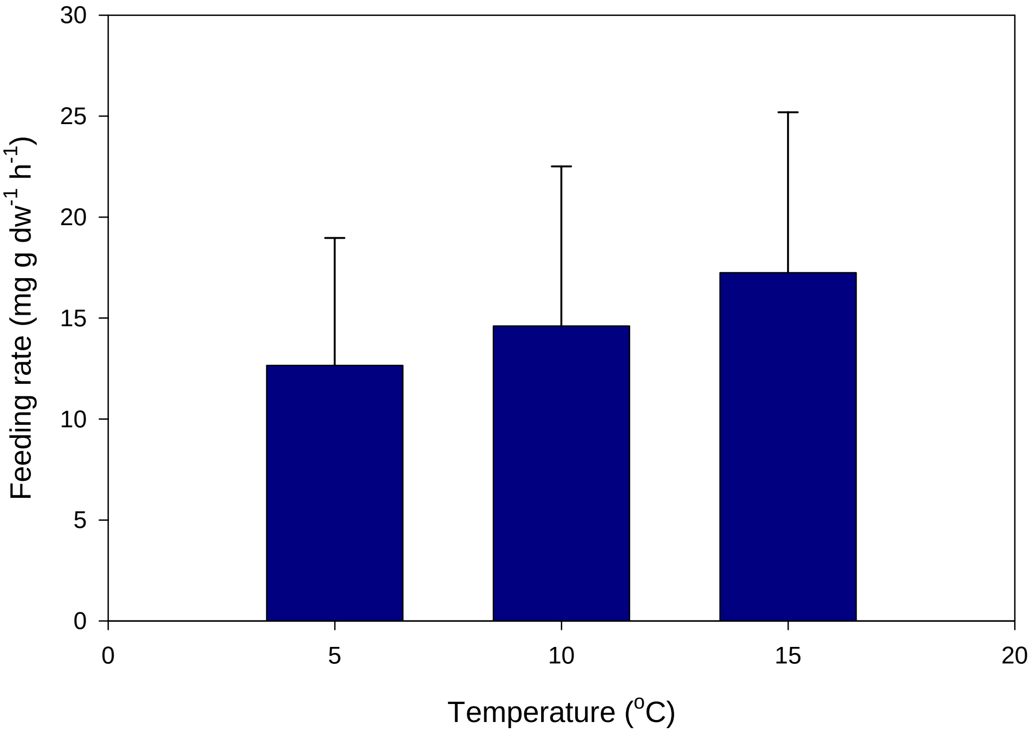

Figure 7.4: the effect of temperature on the feeding rate (± 1 sd) of *G. insensibilis*

Owing to the lack of significance between feeding rates at different temperatures, analysis was performed on combined data as well as on the individual temperature treatments in order to increase the number of animals available to test the effects of size, gender and, within the females, brooding of embryos on feeding rate (Figure 7.5). The combined data suggested no significant difference between the groups (ANOVA, $P = 0.255$) but females with empty marsupia demonstrated slightly increased feeding rates. At 5 ºC this pattern was repeated (Figure 7.5, inset) but at this temperature females with empty marsupia fed at a significantly greater rate than those carrying embryos (ANOVA, $F_{1,11} = 10.286$, $P = 0.009$). There was no significant difference between males and either gravid or non-gravid females. At 10 and 15 ºC there was no significant difference between the feeding rates of the animals of different life history stage.

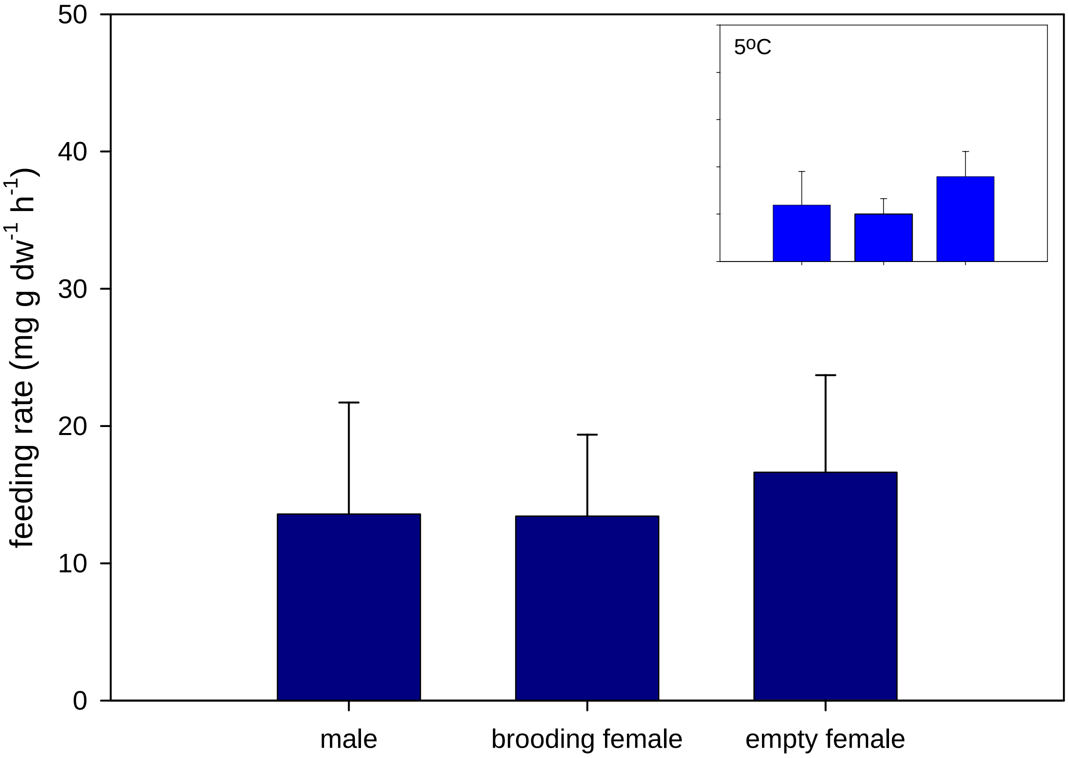

Figure 7.5: The combined data at 5, 10 and 15 ºC, for feeding rate of male *G. insensibilis*, brooding females and females with empty marsupia (± 1 sd). Inset; the same data at 5 ºC (scale is the same as the main graph).

When the mean values for energy losses and gains for all the animals were considered, Scope For Growth was positive at both 5 and 15ºC (Figure 7.6). There were significant differences between assimilation rates at 5 and 15ºC (ANOVA, $F_{1, 29} = 4.289$, $P < 0.05$) and respiration rates ($F_{1, 64} = 6.375$, $P = 0.01$).

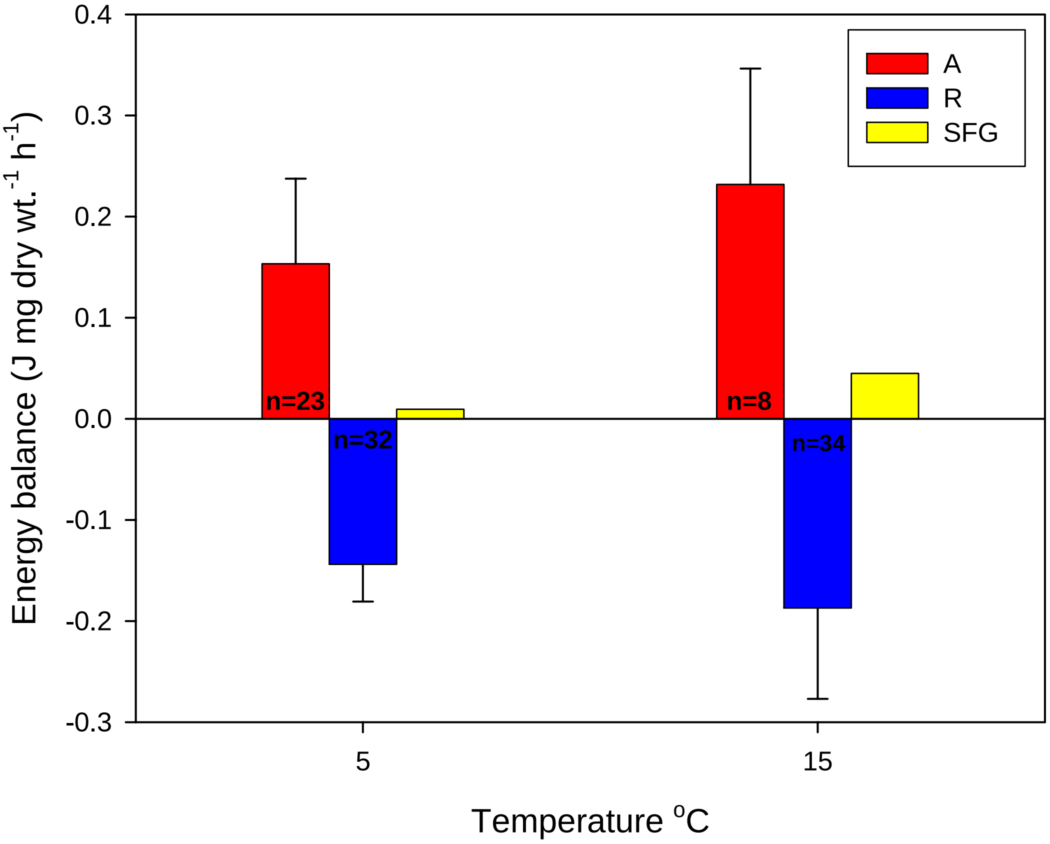

Figure 7.6: The energy balance of the population. Assimilation rate (A), Respiration rate (R) and Scope For Growth (SFG) for *G. insensibilis* at 5 and 15 ºC. Error bars = 1 sd, n is shown on the bars.

At 5 ºC males and females with empty marsupia demonstrated positive SFG. .However, owing to lower feeding rate, SFG was negative for gravid females. Male animals displayed positive SFG at 15 ºC, while gravid females were on the balance between energy intake and energy loss with a slightly positive SFG value of just over 0. The discrepancies between the SFG values obtained in Figure 7.6 resulted from variation in the number of animals used in the assays; animals were included in Figure 7.6 for which there were no data available on their gender. Therefore, the results from these animals could clearly not be included in Figure 7.7.

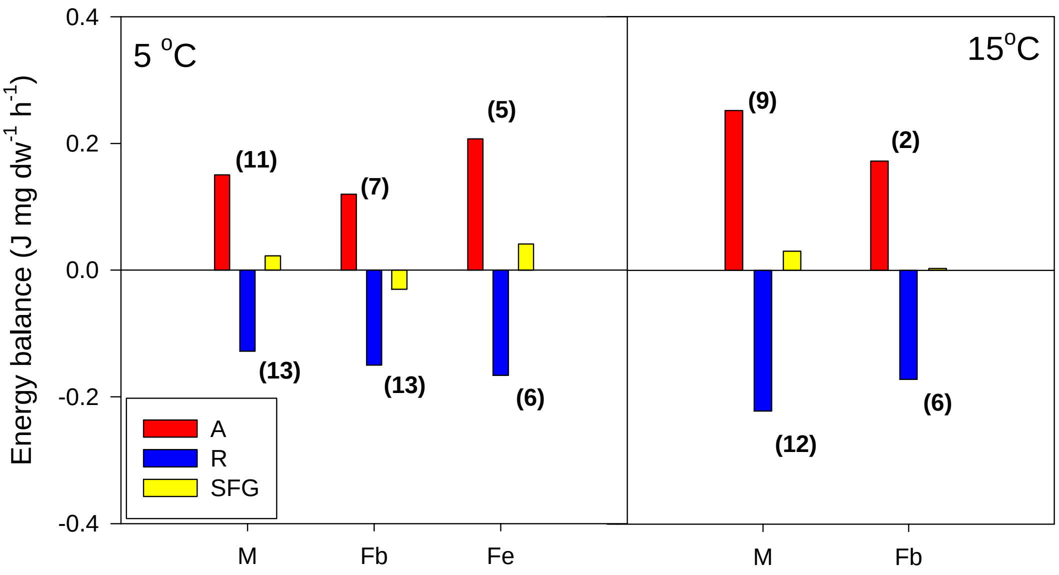

Figure 7.7: The energy balance of *G. insensibilis* of different life history stages. Assimilation rate (A), Respiration rate (R) and Scope For Growth (SFG) for male (M), brooding female (Fb) and females with empty marsupia (Fe) at 5 and 15 ºC. Values in parentheses represent *n*.

7.4 Discussion

The monthly bioenergetic survey showed that despite the environmental variation of the lagoon, the *Gammarus insensibilis* population was successfully able to adjust physiologically in order to survive and invest energy in reproduction. The habitat variability of the lagoon occurs over an extended time period with changes resulting from the ambient weather conditions, so acclimation can occur over time. Under certain circumstances organisms are subjected to more rapid changes in their environment, organisms from the marine intertidal zone readily adjust to the short term, tidally induced habitat variability that they are subjected to. However in the short term, because lagoons are essentially subtidal habitats, *G. insensibilis* experiences less variability in environmental temperature and salinity than the rock-pool example (Al-Suwailem 1991). This study showed the responses of *G. insensibilis* to changes in habitat temperature and salinity over a shorter time scale than the variation studied in the bioenergetic survey, where the metabolic rates were determined under ambient conditions.

Respiration rate shows the amount of oxygen consumed for the oxidation of metabolic substrates and describes the physiological energy usage by organisms in all aspects of their life histories (Svetlichny and Hubareva 2005) and closely reflects actual metabolic rate. Peracarid respiration rates have been determined using various methods by a number of authors and as such, there is much variation in data presented in the literature. They have also been presented in a number of different formats and units. As a result of this variation and the range of conditions under which oxygen consumption has been measured, meaningful comparisons between organisms can be hard to come by (Garnacho et al. 2001). Weight specific respiration rate has been determined for many peracarid crustaceans and values in the literature are highly variable. When presented in Joules, for use in Scope For Growth applications, the freshwater amphipod, *Gammarus pulex*, showed respiration rates varying between 0.2 – 0.3 J mg dw^{-1} day^{-1} (or approximately 0.01 J mg dw^{-1} h^{-1}) (Maltby and Naylor 1990; Maltby et al. 1990; Maltby 1992) while the coastal marine amphipod, *Melita longidactyla* lost energy through respiration at a rate of 0.4 J mg dw^{-1} h^{-1} (Wu and Or 2005). When presented as the amount of oxygen consumed (ml O_2 g dry $wt.^{-1}$ h^{-1}) there is equivalent variation in the literature. The *Gammarus* species from a salinity gradient demonstrated oxygen consumption rates between 0.7 – 2.8 ml O_2 g dry $wt.^{-1}$ h^{-1} (Dorgelo 1977a) while *G. pulex* rates were determined to be between approximately 5 – 30 ml O_2 g dry $wt.^{-1}$ h^{-1} (Rumpus and Kennedy 1974). In this study the respiration rates were within this range and were determined to be approximately 0.1 J mg dw^{-1} h^{-1} or 3.5 to 9 ml O_2 g dry $wt.^{-1}$ h^{-1}. These values are based on February and July from the bioenergetics survey.

The general trend for increased respiration rate at higher temperature (Figure 7.1), at each of the salinity treatments, was expected and reflects the usual situation for poikilotherms. Peracarid examples include the amphipod *Gammarus pulex*, which demonstrated higher respiration rates at 20 ºC than at 10 ºC (Rumpus and Kennedy 1974), the mysid *Praunus flexuosus* which consumed oxygen at a rate of 2 to 3 times higher in the summer than the winter (Garnacho et al. 2001) and the gammarids *G. tigrinus* and *G. fossarum* for which respiration rates were significantly influenced temperature raises between 5 and 15 ºC (Dorgelo 1977a).

An increased respiration rate at higher temperature also reflects the situation shown by *G. insensibilis* from in the lagoon, as represented by the bioenergetic survey (Chapter 5). The effects of temperature in the lagoon however, were frequently not significant leading to the suggestion that *G. insensibilis* can maintain homeostasis without the need for large scale changes in routine metabolism. The temperature dependence of respiration rate has also been questioned elsewhere. Low Q_{10} values for the mysid, *Praunus flexuosus* imply that the respiration rate of these estuarine peracarids is not as temperature dependent as other animals (Garnacho et al. 2001). Dorgelo (1977a) showed that a series of gammarids from freshwater and fully marine habitats were significantly affected by temperature and temperature-salinity combinations, but the intertidal *Chaetogammarus marinus* was not. It was concluded that this was an adaptation to the fluctuating environmental regime of the intertidal zone. This is also likely to be the case with *G. insensibilis* in the lagoon situation where variable environmental salinity and temperature characterize the habitat.

The effects of salinity on amphipod respiration rates have also been discussed at length in the literature. The respiration rates of *G. duebeni* and *G. oceanicus* were lowest at ambient salinity; they increased with lower salinity and decreased as salinity rose (Tedengren et al. 1988). In Dorgelo's (1977a) study of the gammarids from a range of environmental salinity *Chaetogammarus marinus* (marine), *G. tigrinus* (brackish) and *G. fossarum* (freshwater) also demonstrated decreased oxygen consumption rates with increased salinity (Dorgelo 1977b) but there was no evidence of lower oxygen uptake at ambient salinity. In this study, salinity appeared to present few energetic problems to *G. insensibilis,* and at the 5 °C temperature treatment there was no trend in respiration rate, related to salinity. At 15 °C the respiration rate was lower at salinities of 20 and 35, which contradicts the work of Tedengren *et al*. (1988), as these are the extremes that were measured, and are unlikely to be experienced at the times of the year when the lagoon is around 15 ºC in temperature, but this difference was not significant. This suggests that at these ranges this amphipod can maintain osmotic balance with limited energetic expense. This seems similar to the ability of the brackish water amphipod, *G. duebeni,* to acclimate to lower salinity within 3 hours of transfer to the new medium (Bulnheim 1979).

At higher temperature, salinity had more influence. Although the respiration rates across the range of salinity treatments did not differ significantly, there was an apparent trend towards higher respiration rates at lowest salinity which appears important to lagoon management and suggests that further comparative studies are required. This apparent trend may be explained by the unnatural situation of high temperature and low salinity, which the amphipods would rarely experience under natural lagoon conditions. In most lagoons, salinity is normally high in the summer, when temperature is high, because of greater evaporation in the summer months. This was also reflected in the Q_{10} analysis in which a high Q_{10} value between 15 and 20 ºC at a salinity of 5 suggests that low salinity poses a problem to the ability of the amphipod to adjust enzymatically when it is acclimated to high temperature. There was a general trend for increased Q_{10} with decreasing salinity at the high temperature, indicating lower salinity poses energetic problems for summer acclimated lagoon amphipods, and suggesting that during the summer months, to ensure the continued survival of *G. insensibilis* in the lagoon, salinity should be maintained at the more marine end of the scale.

A similar trend was noted at the lower temperature, where Q_{10} was closest to 2 at the intermediate salinities, indicating that winter animals are acclimated to the intermediate salinities of the lagoon, evident during the colder months at Gilkicker. When the salinity was either high (35) or low (20), conditions naturally rare at the site when the temperature is low, the Q_{10} values fell away from 2. This data is summarised by the model below in Figure 7.8.

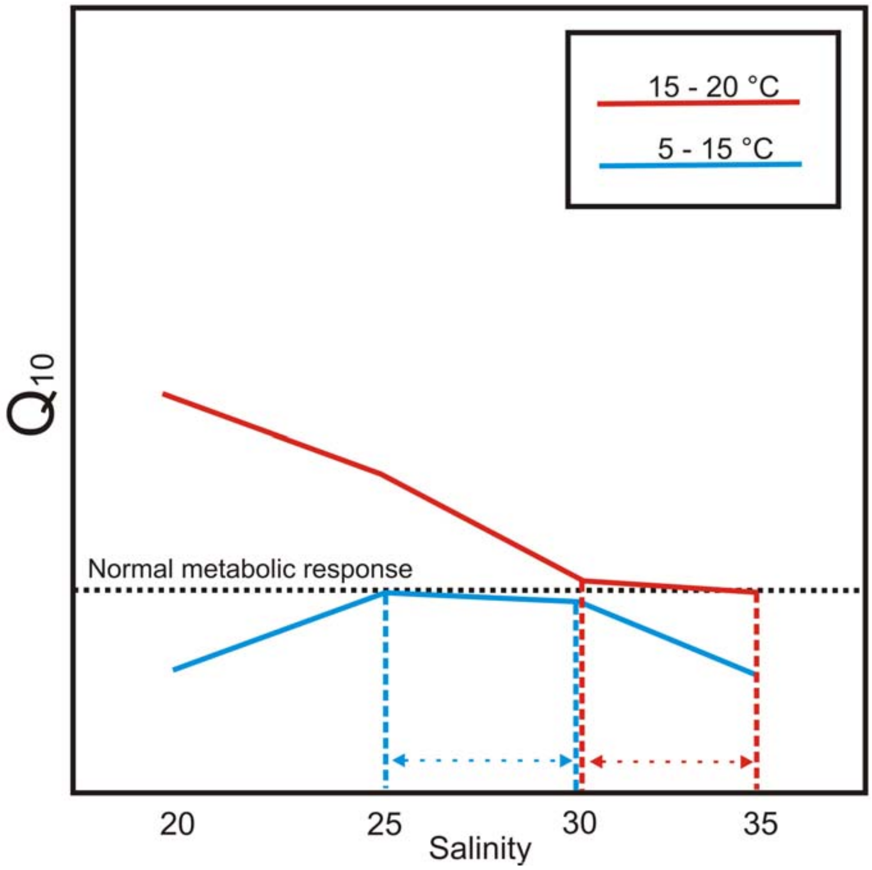

Figure 7.8: A model describing the effects of salinity on the thermal coefficient of *G. insensibilis* at a range of salinity conditions. The dotted lines and arrows represent the optimum salinity conditions at the given temperature as a guide for the management of lagoons

Over the entire range of temperature Q_{10} was approximately 2, which suggests that *G. insensibilis* is well adapted to the variable habitat of the saline lagoon. Q_{10} values of close to 2 reflect the usual adjustments an organism makes with increased temperature resulting from the temperature dependent properties of enzyme controlled reactions, implying that over this range of temperatures *G. insensibilis* suffered little or no stress.

The effect of temperature on respiration rate was significant over the range of treatments but between 5 and 15 ºC there was only a rise in rate at the mid range salinities and this was not significant. This may reflect the fact that prior to the assays the animals were maintained in ambient conditions which were approximately 10 ºC. To an animal well-adapted to variable habitat conditions the 5 ºC change in temperature may represent minimal stress and cause barely-detectable increases in respiration rate. The increase from 10 to 20 ºC, however, is a situation which is unlikely to occur under the normal circumstances in lagoons in the UK and this may explain the high respiration rates recorded in this assay, these rates are substantially higher than the respiration rates recorded at similar temperatures in the bioenergetic survey, further indicating that the more rapid change in temperature had important metabolic consequences.

The range of different life history stages within the population of any organism may result in certain members being more susceptible to the effects of environmental stress or disease. In this study female *G. insensibilis* demonstrated higher respiration rates than their male counterparts. This may reflect a greater need to allocate resources towards reproduction rather than the ability to survive at the extremes of temperature but may also be the result of the effects of size on the size specific metabolic rate. There are few published data on the metabolic costs of reproduction in peracarids. Across the Crustacea in general there is also a lack of information describing the costs. For example, there was no detectable effect of the brooding of eggs or embryos on the respiration rate of adult females of the cladoceran *Daphnia magna* (Glazier 1991) and Garancho (2001) was also unable to find a metabolic cost of reproduction.

The size of an organism has important implications to its metabolism. The general trend, inter-specifically, across the animal kingdom is for lower weight-specific-metabolism with increased body mass (Randall et al. 1997). This trend is also apparent intra-specifically, as demonstrated with respiration rates in the Crustacea, for the shrimp *Litopenaeus stylirostris* (Spanopoulos-Hernandez et al. 2005). In this study there were negative relationships between weight-specific-respiration-rate and body-weight for *G. insensibilis* but the trends were weak. This may be the result of the restricted size available when working on amphipods; in this survey the animals were up to 11 mg in weight, whereas in the study on *L. stylirostris* the shrimps ranged from 5 – 15 g.

There has been limited study of the effects of life stage or gender on the metabolic rates of marine organisms (Roast et al. 1999; Lapucki et al. 2005). Studies that have been carried out on peracarids suggest there are minimal effects. There were no significant differences in size-specific oxygen consumption rate between males, females or brooding females in the mysid *Praunus flexuosus*. Juvenile animals did however demonstrate significantly higher respiration rates than the adults, although this was almost certainly a size-related effect (Garnacho et al. 2001). Differences in weight specific metabolic rates between genders of *G. oceanicus* have been reported. The males and females were of similar length but the males were generally of greater body mass so these differences were actually attributed to mass rather than gender (Normant et al. 2004). In the methods chapter of this study however, it was shown males and females of similar length demonstrated similar body weights (Figure 3.6, p. 46) so gender related differences may occur, probably a result of reproductive investment by the female.

Feeding rate is difficult to measure as there is such variety of feeding mechanisms and food preferences demonstrated within the Crustacea. The method used in the bioenergetic survey was adequate for its purpose of monitoring the general trends in energy intake over the year. However the inherent error associated with the use of dry weight to wet weight ratios meant that it was necessary to use a different method in order to determine the relatively small differences that may occur between separate life history stages of small animals, such as gammarid amphipods.

As a method for the determination of feeding rate the measurement of egestion rate was successful. The values were similar in range to those of the wet weight measurement method. The aim of reducing the need for the use of wet weight to dry weight ratios was accomplished but there was still relatively high standard deviation from the mean. This level of error meant that it was not possible to gain the desired data on the variability in feeding rate between the different life history stages, for which the method was designed. There was further error with the method as the need for both assimilation efficiency values and feeding rate values meant that there was double the chance for experimental error, which occurred with both methods of determination of feeding rate but were abundant in this survey leading to the discard of many samples.

The feeding rates of peracarids have been determined using a variety of methods but similar animals appear to feed at similar rates despite the variation in the method of recording it. The rate at which *Gammarus pulex* consumed fungus-inoculated leaf discs was approximately 15 mg consumed g dw^{-1} h^{-1} (Maltby 1992) while in this study the rate of consumption of *C. linum* was between 12 and 17 mg consumed g dw^{-1} h^{-1}. Egestion rate of the mysid *N. integer* was between 10 and 50 mg faeces g ww^{-1} h^{-1} (Roast et al. 2000).

Feeding rates were also comparable with those exhibited in the bioenergetic survey although the range was lower. While feeding rates ranged from 3.5 mg g dw^{-1} h^{-1} in the winter to 44.1 mg g dw^{-1} h^{-1} in the summer in the bioenergetic survey (with an annual mean of 15 mg g dw^{-1} h^{-1}), they were between 12 mg g dw^{-1} h^{-1} at 5 ºC and 17 mg g dw^{-1} h^{-1} at 15 ºC (with a mean across all three temperatures of 14.1). The feeding rate of 44 in the bioenergetic survey was recorded at 28 ºC, this survey did not record feeding rate at such high temperature. Pairwise comparisons in the bioenergetics survey showed that generally the significant differences were evident between the highest summer rates and the lowest winter rates. As in the bioenergetic survey, feeding rate appeared to rise with temperature. However, this was not significant. There was also no apparent effect of salinity. There was an apparent increase in the feeding rate of females with empty marsupia indicating increased need to intake energy before the onset of a reproductive event, although this may be the result of size related effects. However, there was relatively high standard deviation in the feeding rates. Perhaps as a result of this variation, it was not possible to detect a relationship between size specific feeding rate and body mass.

In Chapter 5 there was in-depth discussion about the negative values that were recorded for Scope For Growth. In this study, mean SFG for the population (males, brooding females and empty females) was positive at both 5 and 15 ºC, as would be expected for a healthy population such as that *G. insensibilis* from Gilkicker lagoon. This suggests that in the bioenergetics survey there was an additional cost being represented in the negative SFG values. At both 5 and 15 ºC there was a cost of reproduction evident. Figure 7.7 shows that at 5 ºC the SFG of brooding females was negative, while at 15 ºC it was very low (approximately zero). This provides further evidence to explain the generally negative results for SFG in the bioenergetics survey (Chapter 5). It suggests that the SFG values may have been influenced by the fact that the gravid females used, in order to ensure consistency of life history stage, were affected by a reproductive stress.

In summary, this study provides further information on the effects of dominant environmental factors in the lagoon on the physiology of *G. insensibilis*. The main conclusion is that under normal temperature regime salinity stress on *G. insensibilis* is minimal but if one of the parameters is unseasonal, *G. insensibilis* can become compromised as a result of increased respiration rates. There is important evidence for energetic stress on reproductive female *G. insensibilis* and as reproductive females are abundant throughout the year it is important for lagoon management strategies to ensure that unseasonably extreme salinity does not occur as this could have important consequences to the most vulnerable members of the population.

Chapter 8

The effects of the cerebral metacercariae of microphallid trematode parasites on the biology of *Gammarus insensibilis*

8.1. Introduction

Many gammarid amphipods are involved, as intermediate hosts, in the complex life cycle of certain trematode parasite species. One of the more notable features of these associations is that some trematodes have evolved the ability to alter the behaviour of their host, in order to increase the probability of transmission to their definitive host. During the course of the investigations in this project it became apparent that *Gammarus insensibilis* from Gilkicker lagoon was frequently infected with trematode metacercariae. *Gammarus insensibilis* is known to host microphallid species in various localities in continental Europe, including *Microphallus papillorobustus, M. hoffmanni* and *M. subdolum* from Pomorie lagoon in the Black Sea (Kostadinova and Mavrodieva 2005) and *Microphallus papillorobustus* and *Maritrema subdolum* from the Thau lagoon in France (Thomas et al. 1998b). Trematode life cycles are complex, involving a variety of stages infecting a number of different host species. In the case of the *G. insensibilis* and *M. papillorobustus* interaction, the definitive host is avian but before infecting the amphipod, the life cycle begins within a hydrobid snail. The amphipod is infected by the cercarial stage of the trematode which encysts as a metacercarium in the amphipod. The life cycle the trematode is described in full on page 26, Chapter 1, Figure 1.1.

Given the conservation importance of this amphipod, and the desire to maintain lagoon habitats, in part because of their importance to wading seabirds, some consideration of the relationship between *G. insensibilis* and its trematode parasite is necessary. This is not necessarily because of potential detrimental effects on the amphipods or birds, but because parasites can have direct (mortality) and indirect (behavioural modification) effects on populations and communities (Thomas et al. 1998a; Mouritsen and Poulin 2002), to the point where they appear to play a key role in lagoon ecosystems (Thomas and Renaud 2001). The range of effects that some parasites have on the ecology of their hosts has lead to the suggestion that parasites should be considered in the conservation of coastal and wetland habitats (Thomas et al. 1997).

In addition to the interest from conservation perspective, this provides a good opportunity to investigate the effects of parasite infection in conjunction with the more general ecology of an organism. The importance of parasites to the function of ecosystems has until recently has been largely ignored, it is however increasing in recognition and is now accepted as an important aspect when considering ecological systems (Sousa 1991). For example, despite numerous studies on the ecology and ecophysiology of *Corophium volutator* between 1967 and 1993, none of them considered the fact that the animals are continuously infected with microphallid trematodes (Meissner and Schaarschmidt 2000).

Differential susceptibility to infection by a particular parasite, or variation in the symptoms of infection, may be as important a factor in determining success and distribution of a species as many other more traditionally studied factors such as resource availability, physicochemical parameters, and intra/interspecific interactions (Fredensborg et al. 2004). Some parasites cause modifications in their hosts, through host responses to the infection or through parasite mediated modification which can have important implications for the host ecology (Thomas et al. 2005).

As described in Chapter 1, when infected with *M. papillorobustus*, *G. insensibilis* undergoes behavioural modification. When infected by cerebral metacercaria of *M. papillorobustus* it demonstrates aberrant escape behaviour, responding to disturbance with negative geotaxis and positive phototaxis, an adaptation demonstrated by several parasites, to increase the probability of host predation and subsequent transmission to the definitive host (Thomas et al. 1996). This has been postulated to lead to a divide in the population, with infected individuals frequently located near the surface of the water column and uninfected individuals found at the bottom. The resultant mate choice is therefore assortative, with infected individuals pairing with similarly infected animals and vice versa (Thomas et al. 1995; Thomas et al. 1996). The mechanism that leads to this behavioural modification is not fully understood but it is known that in the case of the *G. insensibilis* the behavioural modification only occurs when infected by cerebral metacercariae, when the metacercariae are located in the abdomen the modification does not occur. It has been proposed, because of variations in neurological architecture related to levels of the neurotransmitter serotonin (5 hydroxytryptamine, 5-HT) and induction of the behaviour after injection with serotonin, that the actual mechanism of modification is related to levels of the neurotransmitter in the amphipod (Helluy and Holmes 1990; Maynard et al. 1996; Helluy and Thomas 2003). There has been discussion as to whether this is secreted by the metacercaria itself or whether it is a fortunate (for the parasite) result of a host response to the infection (Thomas et al. 2005).

The manipulation of hosts by their parasites in order to increase likelihood of predation by their definitive host, clearly affects the predator-prey interactions in such a community, affects reproductive factors, and may cause a divide in the population; therefore, the implication is that

the ecology of *G. insensibilis* can not be fully understood without considering the implications of infection with *Microphallus papillorobustus* (Ponton et al. 2005).
Various facts are known about the effects of trematode infection on crustacean hosts. However, there is little published information on the immune responses of amphipods to their parasites. It is known that there is a melanization response, common in crustaceans in response to infection. Kostadinova and Mavrodieva (2005) found that microphallid parasites induce a cellular defence consisting of encapsulation of the metacercaria and then melanization, which prevented the behavioural alteration. They compared their findings to the those of (Thomas et al. 2000) who had studied the same microphallid system in *G. aequicauda* and found only *M. papillorobustus* to induce the defence. Kostadinova and Mavrodieva (2005) suggested that this may be the result of the higher infection density in the Black Sea population than the Thau lagoon in France requiring a more effective defence from the hosts thereby attacking all the species of parasite. However, in these two situations the hosts studied were different.

It would be expected that, as with any form of immune response, the melanization response discussed above would require the use of resources. This kind of energetically costly response to infection would direct resources away from growth and reproduction, a potentially dangerous strategy in a highly variable habitat. It has been shown that infection with the acathocephalan, *Pomphorhynchus laevis*, can increase the sensitivity of *Gammarus pulex* to pollutants suggesting that fitness is reduced, possibly resulting from the energy strain of hosting the parasite (Brown and Pascoe 1989; McCahon and Poulton 1991). *Gammarus pulex* displayed reduced feeding rate under the stress of pollution when infected by the parasite.

The principle of Scope For Growth indicates that such stresses may lead to reduced energy available for growth and reproduction. Parasite infection is known to have an impact on reproductive output. Ovigerous females were underrepresented in samples of isopods (*Cyathura carinata*) infected with microphallids, it was proposed that the infections interfere with ecdysis and gametogenesis, leading to suggestions that microphallid infections could have significant impacts on population development (Jensen et al. 2004).

The evidence for parasite-induced variation in the levels of neurotransmitters has implications beyond behaviour. The neuroendocrine system is complex, with many molecules, often associated with one another, involved in many functions in the physiology of invertebrates (Ottaviani and Franceschi 1996; Weiger 1997). Within the Crustacea serotonin has been shown to induce ovarian maturation in aquaculture shrimps, *Litopenaeus stylirostris* and *L. vannamei* (Alfaro et al. 2004), modulate aggressive behaviour in lobsters (Livingstone et al. 1980), increase the heart rate of decapods (Florey and Rathmayer 1978) and it is known to affect the digestive tract (Weiger 1997). It has also been demonstrated to be present in several members of the Peracarida although functions are not so well known (Moreau et al. 2002). Although the functions are not clear it seems probable that parasite-induced variation in levels of serotonin will not just affect the behavioural response for which these interactions have been studied to date.

This study used a variety of methods to assess how infection with *Microphallus papillorobustus* may have an impact on the ecology of *G. insensibilis*. After an investigation into the abundance of the parasite, data were collected on its effects on the metabolic rates of the amphipods. In addition, the effects of the intensity of infection on reproductive output were considered, because this has important considerations to higher levels of biological organisation. The suggestion that behavioural modification is achieved through the alteration of serotonin levels within the amphipod is considered using a method to determine whole body catecholamine levels.

In a recent review of the state of knowledge of parasitic manipulation Thomas et al (2005) discussed the fact that modified organisms are complex, the parasites have important effects on ecosystems and there is still much research needed to fully understand these systems. Researchers were urged to look beyond the obviously manipulated traits and understand the wider scale consequences of manipulation. This study views parasite manipulation from the point of view of the host and considers the effect of the parasite on the lagoon ecosystem.

8.2. Materials and Methods

The presence of cerebral metacercariae in the amphipods became apparent during the course of the Population Dynamics survey of Gilkicker. During the latter half of this survey the animals were carefully examined for parasite load. This information provided a large amount of the data on parasite prevalence from Gilkicker and the effects on reproduction. An additional sample of approximately 100 reproductive females was analysed from the August immediately following the end of the population dynamics survey to provide further information at higher parasite infection intensities.

Additional samples were collected from a number of lagoons around the south and east coasts of England to assess the extent of the microphallid trematodes beyond Gilkicker; 3 lagoons from the Lymington – Keyhaven system (Eight-Acre Pond, The Salterns and Normandy Farm) and the Moulton Marsh lagoons in Lincolnshire were sampled. Parasite prevalence was determined from each of these sites.

As infection with non-self material generally induces immune responses, a study was carried out on the effects of parasites on metabolic rate (indicated by respiration rate) of the hosts. Initially a study was conducted using obviously parasitized individuals and those that showed no obvious signs of infection. Amphipods were determined as infected by observation of their behaviour; infected animals were those that demonstrated the aberrant escape behaviour described by Poulin (1994) i.e. the amphipods that were found actively swimming towards the surface of the water when disturbed. Upon examination of these animals it was possible to verify infection as the metacercariae were visible through the cuticle of the head. Uninfected individuals were those that were found to swim away from the surface and into the *C. linum* in their aquaria and with no obvious metacercariae present when examined (without dissection).

Further investigations were carried out to determine the effect of the actual numbers of metacercariae present. Animals for physiology studies were collected from Gilkicker and The Salterns (from the Keyhaven-Lymington lagoon system). Following preliminary investigations it was shown that there was no significant difference in feeding or respiration rate between Gilkicker and Salterns animals. It was therefore decided that as uninfected animals were very rare at Gilkicker, Salterns animals would be used to provide uninfected animals for comparison.

Metabolic rate experiments were carried out with animals from Gilkicker and Salterns acclimated to the same temperature and salinity and using the same batch of filtered sea water. As determining whether an animal was parasitized could cause it stress, metabolic rate experiments were conducted using randomly selected animals. After the test had been completed the animals were dissected and the metacercariae removed and counted.

Part of the stress response includes the production of catecholamines including noradrenalin. The aberrant escape behaviour observed in gammarids infected with certain trematode parasites has been proposed to be related to changes in serotonin levels in the protocerebrum (Helluy and Holmes 1990; Helluy and Thomas 2003). Noradrenaline and serotonin are both amine neurotransmitters and can therefore be used as a means to crudely estimate the levels of serotonin and associated molecules in the body haemolymph. The whole body level of noradrenaline was therefore measured to determine whether the parasites induce whole body increases in catecholamine levels. If so, this production would indicate a stress on the organism, requiring energy otherwise available for somatic growth and reproduction, i.e. SFG would be reduced. Catecholamine concentration was determined for the whole body haemolymph, colorimetrically reacting the noradrenalin with sodium bismuth using noradrenalin as a standard following the procedure of (Sorouraddin et al. 1998). This method had previously been successfully attempted using *G. duebeni* infected with *Polymorphus minutus* from a saline lagoon near Gilkicker (McHenry 2005).

8.3. Results

The presence of metacercariae of microphallid trematodes became apparent during the examination of animals for the population dynamics survey. They were noted in the head of the amphipod, sometimes in large numbers, to the point where, on occasions, the shape of the head was distorted. Occasionally they were also present in the abdomen but this study concentrated on the cerebral metacercariae, which have the potential for behavioural modification. A sample of *Gammarus insensibilis* taken from Gilkicker in 1982 was examined and it was established that these metacercariae have been present in the population for at least 24 years.

Analysis of the parasite prevalence of animals from the latter months of the population dynamics survey (March – July) showed that most *G. insensibilis* specimens were infected with cerebral metacercariae (84 %). In nearly all cases the metacercariae were located in the cephalic region of the amphipods. The presence in the head suggested that the metacercariae were of the species *Microphallus papillorobustus*, as this species is the only one that actively seeks the brain in which to encyst and cause the behavioural manipulation (Helluy 1983) and the size and shape of the metacercariae were similar to the 270 x 350 µm ovoid cysts described by Rebecq (1964). On occasions there were smaller metacercariae present in the abdomen, these may represent a different species of trematode or may be from new infections.

In the Keyhaven-Lymington lagoon system prevalence was lower. Amphipods from The Salterns were rarely infected, while animals from Eight-Acre Pond showed levels of infection of approximately 60 %. In the only sample from Moulton Marsh lagoon *G. insensibilis* was not abundant, but the limited data suggested low prevalence, of the few animals found 8 % were infected (Table 8.1). Additionally, at each site, it should be noted that the trematode metacercariae were not solely restricted to *G. insensibilis*. They were also present, although at low prevalence and intensity, in *Idotea chelipes*. However, they were not recorded in the cephalic region of the isopods, only in the abdomen.

Table 8.1: The prevalence of cerebral metacercariae of microphallid trematodes in *G. insensibilis* from several sites on the UK coast.

Site	Sampling date	N	% prevalence
Gilkicker	March – July 2004	677	84.3
Salterns	October 2004	51	0
Salterns	January 2005	69	3.9
8-Acre Pond	January 2005	24	58.3
Moulton Marsh	October 2004	12	8.0

The Gilkicker data set shown in Table 8.1 was analysed in order to determine the intensity of infection with cerebral metacercariae. This showed that most amphipods from Gilkicker were infected by 1-2 parasites (Figure 8.1). From the maximum infection intensity (45 % of the animals were infected by 1 metacercaria) there was a steady decrease in the number of amphipods harbouring more trematodes. Approximately 15% of the animals were uninfected and the maximum number of cerebral metacercariae recorded was 20. Amphipods infected with more than 5 metacercariae were only occasionally recorded.

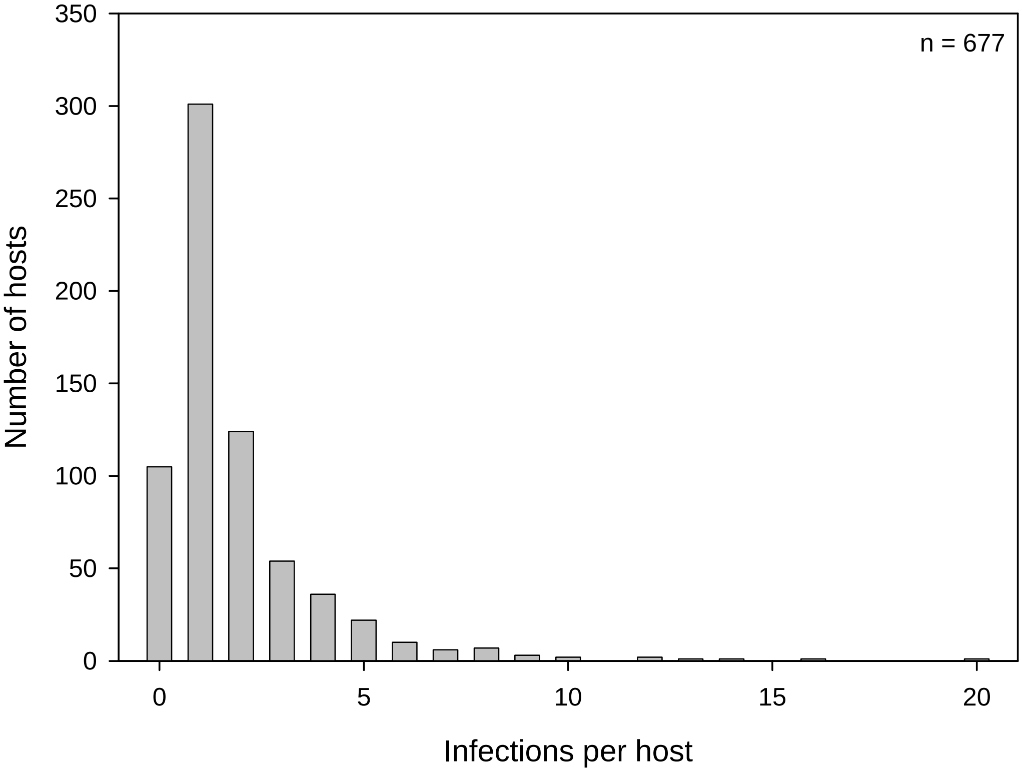

Figure 8.1: The frequency of cerebral metacercaria infection in the population of *G. insensibilis* from Gilkicker between March and July 2004

Figure 8.2 suggests that there was no relationship between mature amphipod size and the number of cerebral metacercariae recorded, with no significant differences evident between the groups. As previously shown in the population dynamics survey (Chapter 4), males were generally larger than females. Juveniles were obviously smaller than the males and females and it should be noted that infected juveniles were generally larger than uninfected individuals (Figure 8.2).

This apparent trend in the size of infected juveniles was tested. Owing to the low numbers of juveniles infected by larger numbers of metacercariae the data were combined into three categories (0, 1 and 2+ cerebral infections) in order to perform statistical analysis; these data are shown, before combination into groups, in Figure 8.2. There was a significant difference in mean juvenile length between the infection groups (ANOVA; $F_{2, 69} = 21.512$, $P < 0.001$). Holm-Sidak multiple pairwise comparisons showed that mean juvenile length was significantly lower in uninfected animals than either those infected with one, or two or more trematode metacercariae. There was however no difference in juvenile length between either infected group.

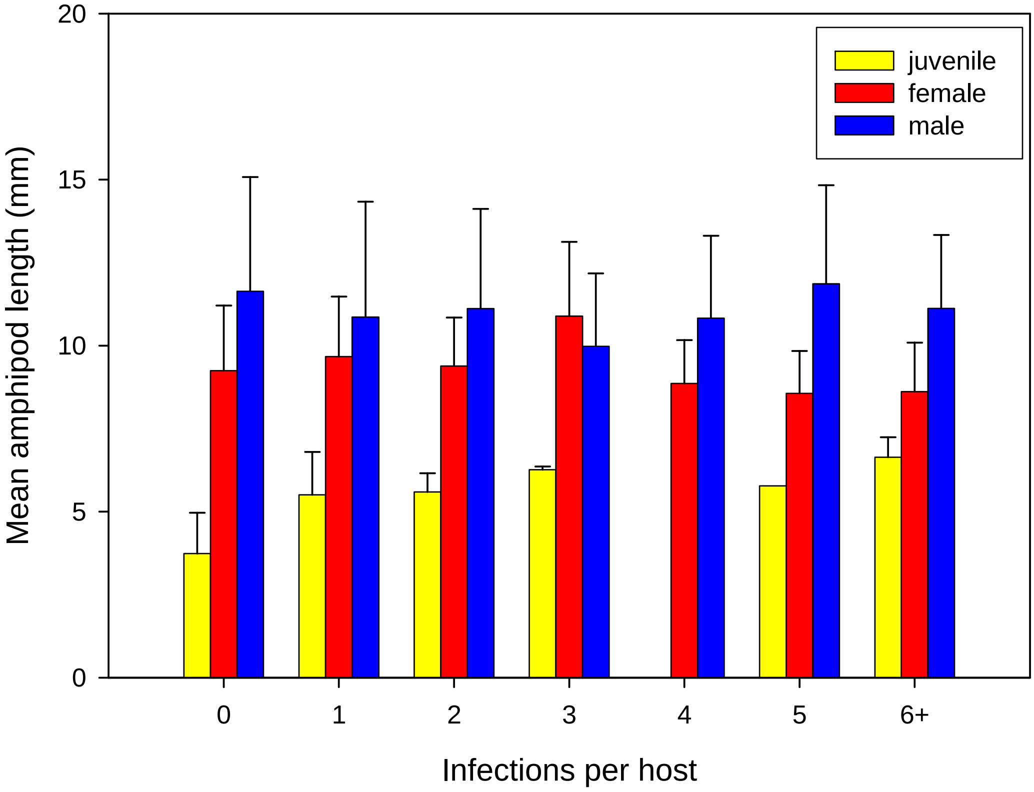

Figure 8.2: The size of amphipods (mm ± 1 sd) from different life history categories with differential infection intensities

The life history stage composition of the population is shown for animals with varying degrees of infection (Figure 8.3). Generally there was a slight male bias to the sex ratio probably related to the size at which it was possible to differentiate the genders (see Chapter 4). Within the uninfected proportion of the population juvenile animals comprised 20 %. Notably, coinciding with increased parasite load, there was a decrease in the juvenile percentage. This continued until the infection rate reached four cerebral metacercariae per host, when it began to increase again. It should be noted however, that it was rare to find juveniles infected by more than two metacercariae.

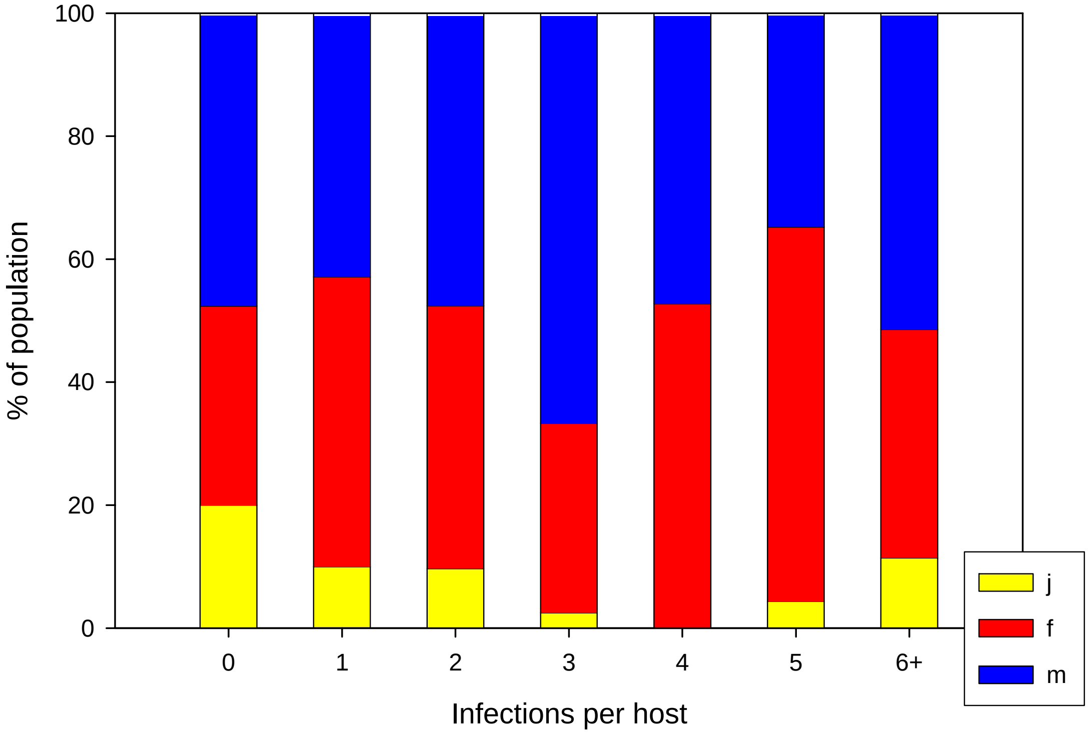

Figure 8.3: The composition of the male, female and juvenile populations of amphipods with differential infection intensity.

8.3.1 Respiration rate

Initially, a respiration rate assay was run to monitor the effects of trematode infection on the respiration rate of *G. insensibilis*. The animals, all from Gilkicker lagoon were crudely divided into “normal” healthy individuals and those that were deemed to be parasitized from visual observation and the consideration of their behaviour (strongly positively phototactic and negatively geotactic when disturbed). Figure 8.4 shows that at 5 °C and salinity of 35, the animals clearly infected by trematodes displayed significantly reduced mean respiration rates in comparison to the uninfected individuals (ANOVA; $F_{1\text{-}17} = 6.533$, $P = 0.021$).

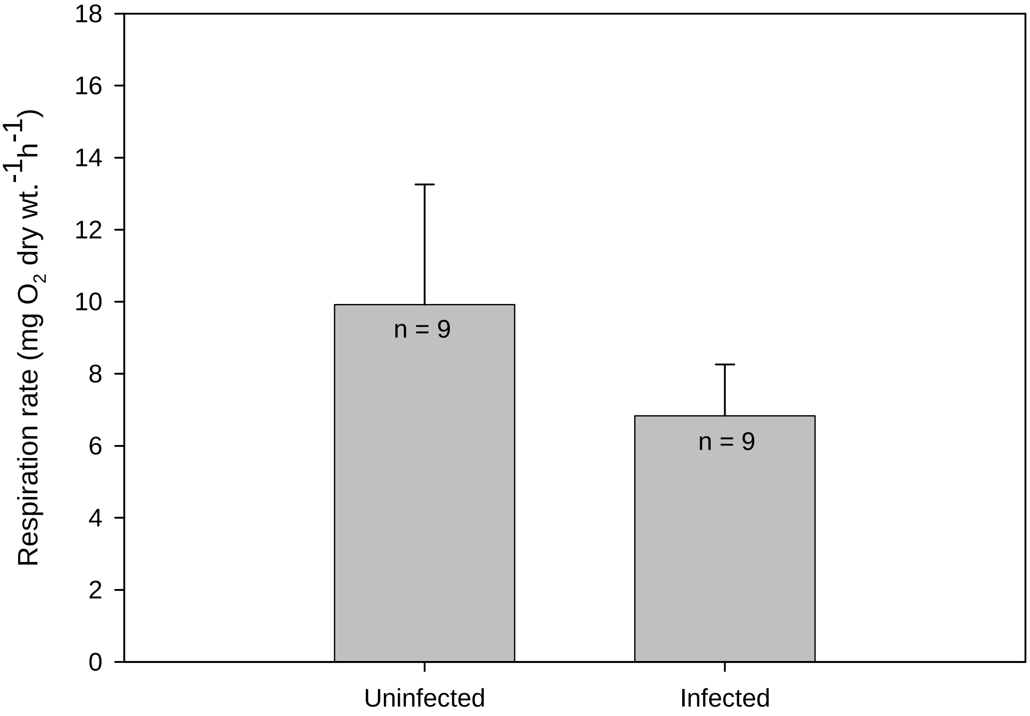

Figure 8.4: The mean respiration rates (± 1 sd) at 5ºC and 35 psu of uninfected *G. insensibilis* and those infected by cerebral microphallid trematodes, in each case n = 9.

More detailed respiration rate experiments were conducted which compared animals from The Salterns (uninfected) and Gilkicker (infected). For each test the animals were randomly selected and analysed after the assay to determine metacercaria abundance. At 5 °C (over a range of salinity, for which there was no significant effect on oxygen consumption), the mean respiration rate of the infected amphipods was slightly lower than that of the uninfected individuals but this difference was not significant (Figure 8.5 a). At 15°C (35 salinity) there was a significant difference in the mean respiration rate of infected and uninfected amphipods (Figure 8.5 b, ANOVA: $F_{1,13}$ = 8.463, P=0.013).

It must be noted however that there were other influences besides parasite infection acting on the respiration rate. As shown in Chapter 7, at certain temperatures there were significant effects of gender on the respiration rate of *G. insensibilis*. At 5 ºC, there was no significant effect of parasite infection on respiration rate, but the respiration rate of females was greater than that of males, although there was no significant difference. At 15 ºC, there was a significant parasite related effect on the respiration rate, but opposing previous findings male respiration rate was higher than that of females. The respiration rates of the individual animals, male and female, are plotted in Figure 8.6 for each of the temperatures analysed.

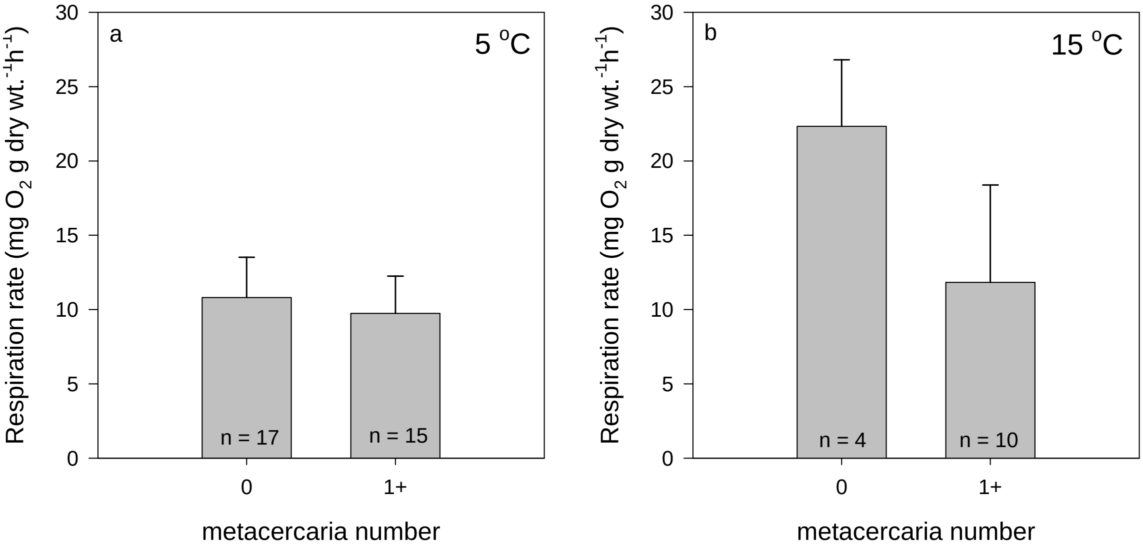

Figure 8.5: The mean respiration rates (± 1 sd) at 5 and 15 ºC of infected (Gilkicker lagoon) and uninfected (Salterns lagoon) *G. insensibilis* (n is also shown)

A further assay was conducted on *G. insensibilis* respiration rates; this was only carried out on animals from Gilkicker at a temperature of 20 ºC (Figure 8.6). Infection rate in this assay was 100 % so there were no data available for uninfected animals. In this assay there were several animals with very high infection intensities. Males demonstrated generally lower respiration rates but also had higher rates of infection. All the specimens with 6 or more cerebral metacercariae showed dramatically reduced oxygen consumption rates (Figure 8.6, 20 ºC).

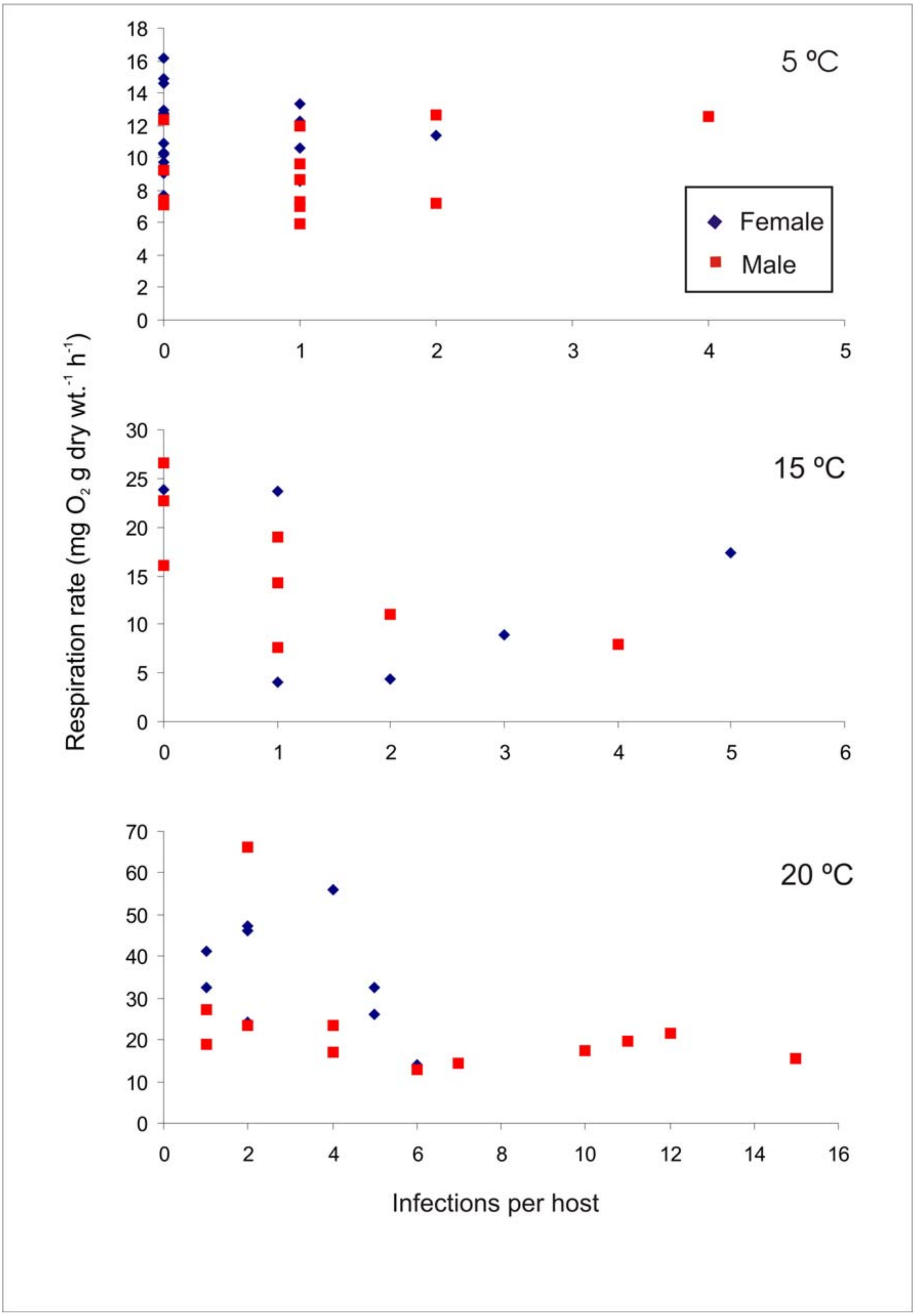

Figure 8.6: The respiration rate in relation to number of infections per host for male and female *G. insensibilis* over a range of temperatures

8.3.2 Feeding rate

Assays were carried out to determine the effects of infection with trematode metacercariae on the rates of energy acquisition. Despite a higher mean feeding rate for infected animals, comparisons between animals from Gilkicker lagoon with uninfected specimens from Salterns lagoon, showed no significant variation at 5 ºC, Figure 8.7 (ANOVA; P = 0.37). When the specimens were examined after the assay it was noted that many of the apparently infected animals from Gilkicker, in fact, had no cerebral metacercariae. Feeding assays were also run at 10 and 15 ºC (Figure 8.8). At 15 ºC, feeding rates were high when infected by 1 metacercaria. They were low at 2 infections but increased steadily with increasing parasite infection number. The results were inconclusive, in part because at the higher temperature, all the animals were infected and at the lower temperature there were few infected animals. There was however a suggestion of increased feeding rates with increased parasite load. There were no parasite related effects on assimilation efficiency.

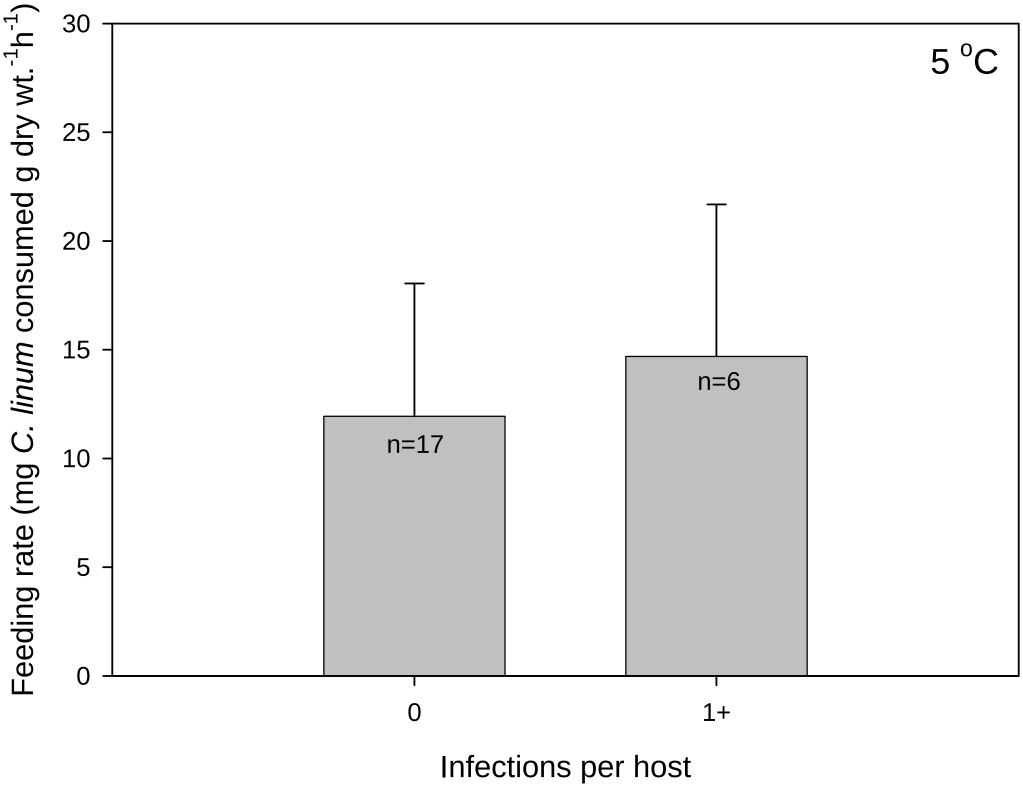

Figure 8.7: The feeding rate of uninfected and infected *G. insensibilis* (± 1 sd) at 5 ºC, number of animals are also shown.

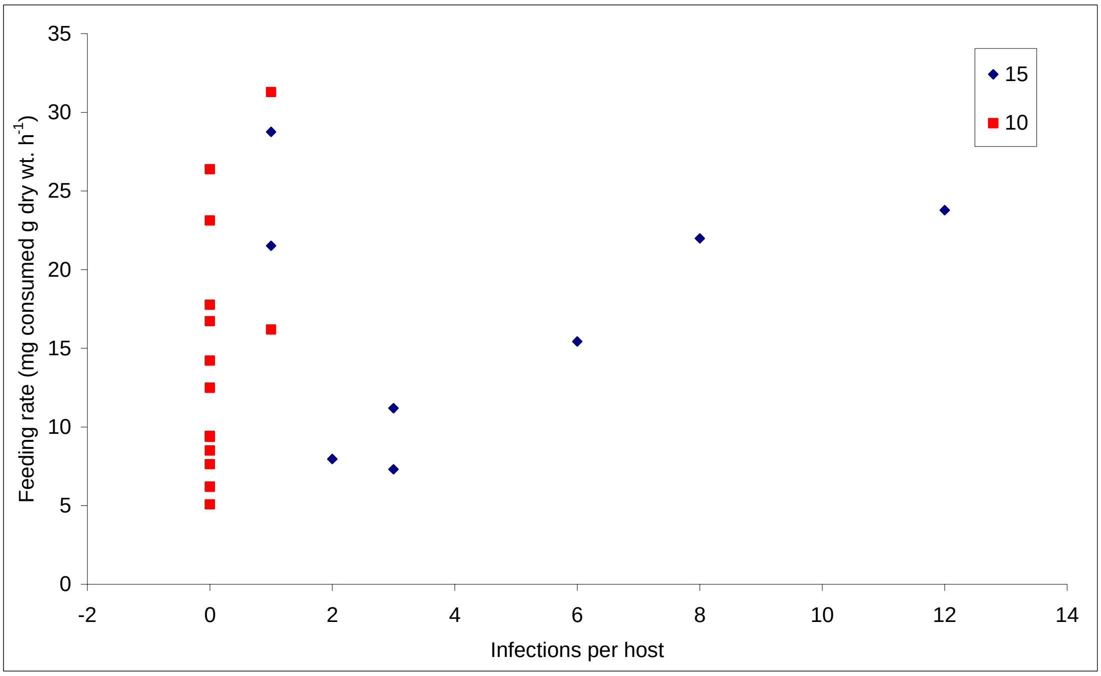

Figure 8.8: The feeding rates (mg *C. linum* consumed g dry wt. h^{-1}) of individual *G. insensibilis* with varying intensities of infection with cerebral metacercariae at 10 and 15 ºC.

8.3.3. *Reproductive effects*

Figure 8.9 shows that there was a significant difference in the mean weight-specific brood size of gammarids infected by the metacercariae of *M. papillorobustus*. (Kruskall-Wallis ANOVA on ranks H = 19.808, 2 df; $P < 0.001$). This was a reduction of 36.6% between uninfected and heavily infected (3+) individuals. Pairwise multiple comparisons (Dunn’s method) showed females infected with more than 3 cysts to have significantly lower reproductive output than both uninfected animals, and those infected with 1 or 2 metacercariae. There was no difference between uninfected and those with 1 or 2 infections. The inset in Figure 8.9 shows that with increased infection intensity, there was a steady reduction in the weight specific brood size. However, the sample size was low in some of the increased infection categories so data were combined, as shown in the main figure. Subsequent additional studies were carried out on the effects of trematode infection on reproductive investment using stored samples from August 2004 and a newly collected sample from March 2005; in each of these investigations similar trends were found to that shown in Figure 8.9 but the differences were not significant.

While there was a parasite-induced decrease in weight specific brood size (embryos mg dw^{-1}), the size of the embryos of *G. insensibilis* was unaffected. Both embryo volume (mm^3) and the individual mass (μg) of the embryos within the brood were unaffected by the intensity of infection of the female by trematodes indicating that only the changes in clutch size were directly related to the number of embryos.

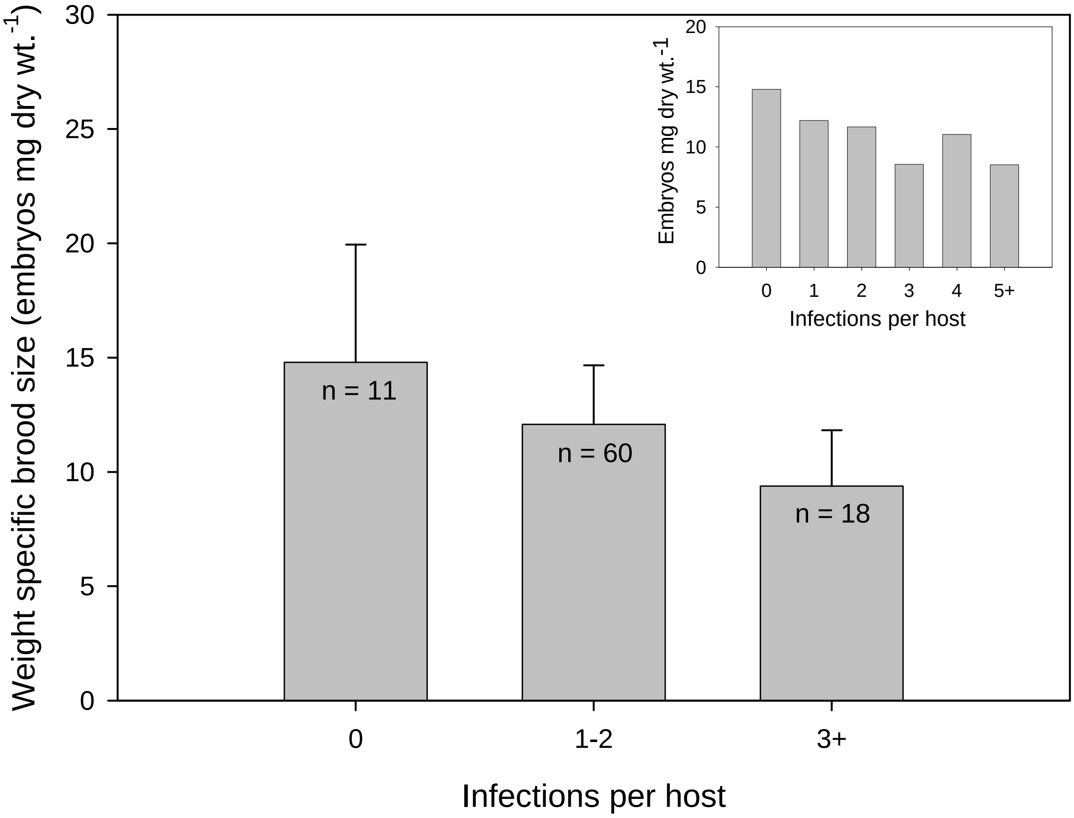

Figure 8.9: The effect of intensity of cerebral metacercaria infection on the early stage brood size (embryos mg dry wt.$^{-1}$) of *G. insensibilis*. The data represent April-July in the population dynamics survey. Inset shows the same data for each individual infection number.

The reduced early stage brood size indicated by Figure 8.9 implied that final offspring output may be reduced in relation to parasite load. As explained in Chapter 4, measurement of final offspring output is best estimated by the Stage V brood size, rather than counting juveniles, because juveniles leave the marsupium during the fixing procedure. Figure 8.10 shows the effect of parasite load on the late stage brood sizes. Mean late-stage brood size was higher in uninfected individuals but, resulting from low numbers of uninfected females there was no significant difference in embryo number in either stage IV or V broods (IV – ANOVA, P = 0.808; V – ANOVA = 0.065).

Gammarid amphipods demonstrate high rates of loss of embryos from the marsupium during development (see Chapter 4). The effects of the presence of parasites were estimated from Figures 8.9 and 8.10. Uninfected females showed a rate of loss of embryos of 27.8 % while females infected with three or more metacercariae lost 28.9 % of their embryos during development from early stage (I-III) to late stage (V). Females with intermediate parasite load demonstrated higher rates of embryo loss during development of 44.5 %.

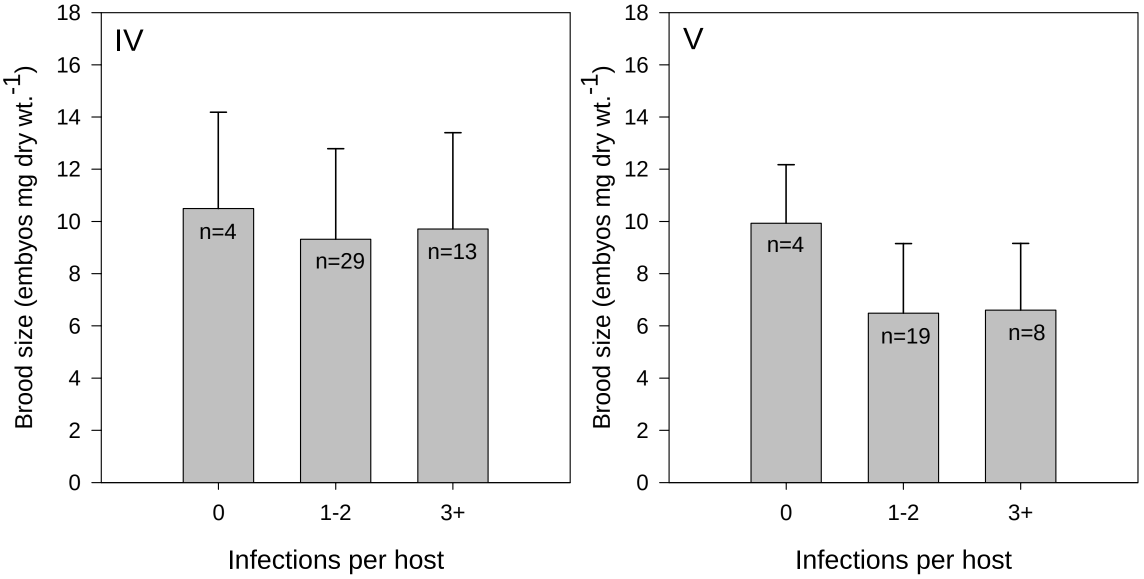

Figure 8.10: The effect of intensity of parasite infection on the late stage brood sizes (embryos mg dry wt.$^{-1}$ ± 1 sd) of *G. insensibilis* (IV and V shown separately) between April and July 2004. Number of animals is also shown

In addition to the use of the data from the population dynamics survey, to investigate further the effects of microphallids on reproduction, another sample was analysed. This sample was taken from a stored sample of female *G. insensibilis* collected from Gilkicker in August 2004. In this instance there were no uninfected organisms found, all animals had at least 1 cerebral metacercaria. The data, presented in Figure 8.11 were grouped into early stage (I-III) embryos, Stage IV and Stage V embryos. In each case there was no significant difference in brood size between animals infected with a low intensity (1-2 metacercariae) or higher intensity (3+). Brood mortality between the early stages and Stage V was 26 % in both the intermediate and high infection rates.

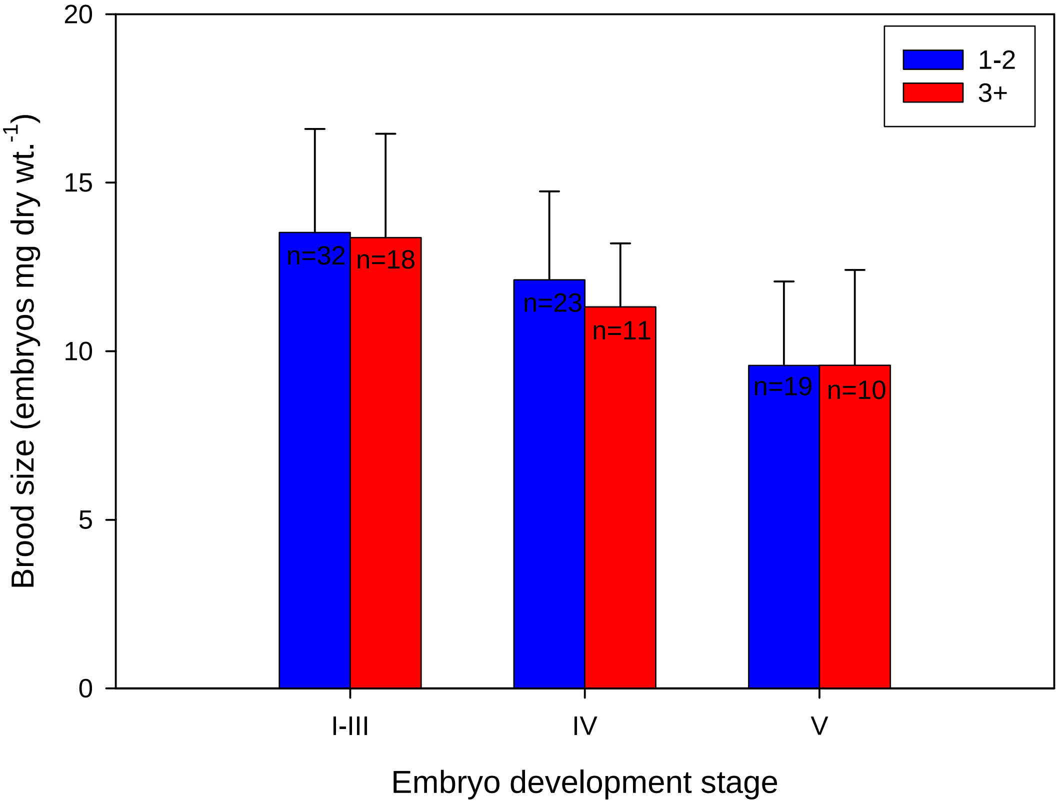

Figure 8.11: The effect of intensity of parasite infection on brood sizes at different development stages (embryos mg dry wt.$^{-1}$ ± 1 sd) of *G. insensibilis* in August 2004. Blue bars = low infection intensity (1-2 metacercariae), Red bars = high intensity (3+ metacercariae). The number of animals is also shown.

Figure 8.12 shows the effects of parasite load on the whole body noradrenaline levels of *G. insensibilis*. There was high variability in the data resulting in inconclusive statistical analysis. However, it was only animals from the "infected" treatment groups that displayed the remarkably high levels of catecholamines (CA). When the animals were grouped into infected and uninfected categories there was no significant difference within the males, but the infected females demonstrated significantly increased CA levels in comparison to uninfected females (Kruskall-Wallis: H = 6.776, P = 0.009), indicating that there was at least some effect of parasite infection intensity on the whole body catecholamine level.

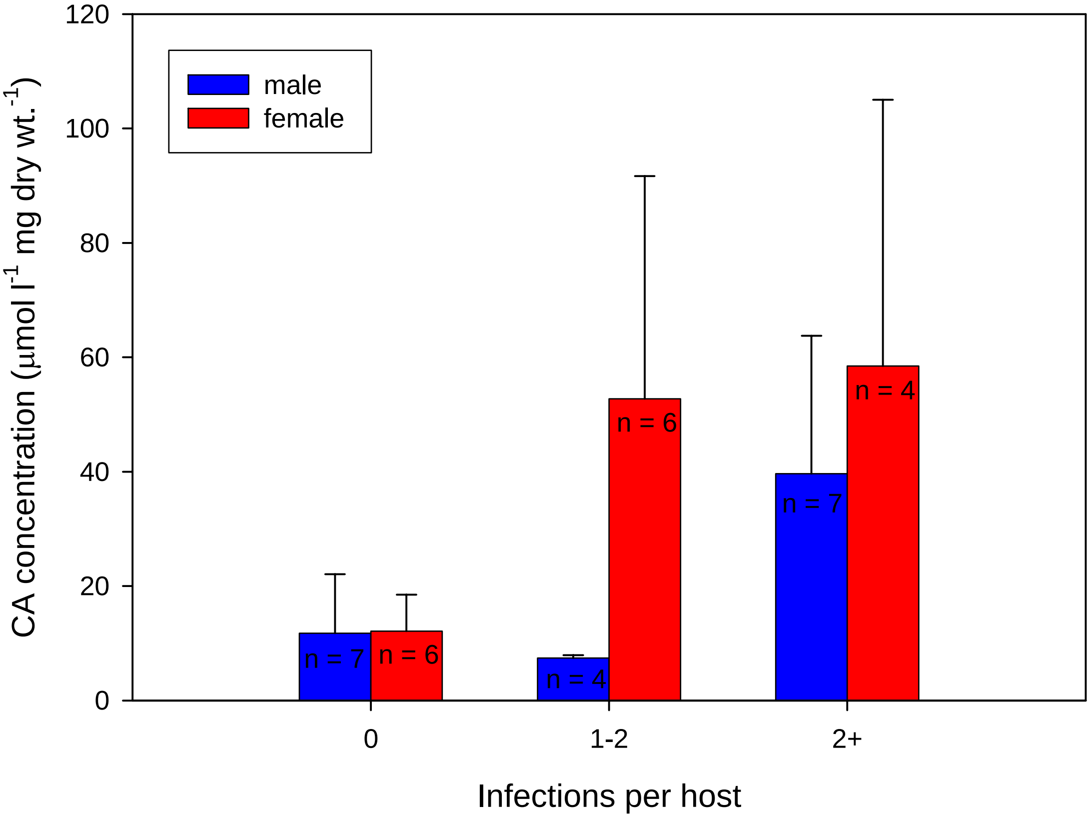

Figure 8.12: the whole body catecholamine (CA) concentration (± 1 sd) in male and female *G. insensibilis* from Salterns (uninfected) and Gilkicker (infected), the number replicates in each treatment are also shown.

8.4 Discussion

The presence of *Microphallus papillorobustus* in populations of *Gammarus insensibilis* from UK lagoons has implications for the understanding of the ecology of the species. This study showed that the parasite is abundant in UK populations, particularly at Gilkicker, and has effects on the reproductive output and metabolic rates of its host. However, the presence of the metacercariae had not previously been recorded in UK populations of *G. insensibilis*, despite studies on the amphipod from sites where the parasite is common (Sheader 1996; Pearson 2003). Schwartz and Cameron (1993) state a similar situation in the cladoceran *Daphnia obtusa* and suggest that when researchers do not have a “search image” for the parasite they can be easily missed.

The prevalence of the cerebral metacercariae of *Microphallus papillorobustus* was shown to be variable in *Gammarus insensibilis* from different sites around the coast of England (Table 8.1). The fragmented nature of lagoon habitats could plausibly be regarded as a potential reason for this variable distribution. However, in this case it does not appear to explain the situation. Parasite prevalence was high at Gilkicker and 8-Acre Pond; these lagoons are separated by approximately 30km. However, prevalence at The Salterns lagoon was low. The Salterns is adjacent to 8-Acre Pond in the Lymington-Keyhaven system and separated by less than 50 metres.

Increased environmental stress is known to increase probability of infection with bacterial pathogens in the freshwater giant prawn, because stress-induced dopamine depresses the immune response (Li et al. 2005). Increased environmental stress from the physicochemical parameters for which data are available does not appear to explain the increased infection rates in this case; temperature and salinity profiles of the Gilkicker, The Salterns and 8-Acre Pond were similar (see Chapter 2). Various other factors have been discussed in the literature to explain variable distribution of parasites. Both abiotic and biotic factors such as reduced tidal submergence time and variable abundance of the first intermediate host were discounted as reasons to explain differences in the prevalence of echinostomatid metacercariae infecting the New Zealand cockle horizontally along a shore. Other explanations suggested in the same study were, the abundance of predators and larger scale hydrodynamic processes (Poulin and Mouritsen 2004). Amphipods are more mobile organisms than the bivalve molluscs studied by Poulin and Mouritsen (2004) so the horizontal gradients are unlikely to be evident in their populations unless there is some form of isolating barrier, such as that between lagoons in the Keyhaven-Lymington system.

One of the notable similarities between Gilkicker lagoon and 8-Acre Pond is that they are both wide, shallow lagoons with a gravel sediment in the margins (from where the samples were collected). In contrast, the Salterns and the Moulton Marsh lagoons are narrower, deeper bodies of water. The differential infection rates between the two lagoon types could be explained by a similar phenomenon to that which explains the assortative pairing by infected and healthy *G. insensibilis* from different depths in the water column (Thomas et al. 1995; Thomas et al. 1996). This phenomenon has lead to the suggestion that *M. papillorobustus* splits its host population into two discrete subunits; one infected, living at the surface of the water, and the other, uninfected, living near the bottom (Ponton et al. 2005). Within Gilkicker and 8-Acre Pond, the water is very shallow, so these discrete populations are less feasible. It can also be argued that the shallower lagoon environments may be more suitable as feeding grounds for wading birds, thus increasing the potential for successful completion of the trematode life cycle, and increasing parasite prevalence at these sites.

There was also some seasonal variation in the prevalence of *M. papillorobustus*. Within the Gilkicker samples from the population dynamics survey infection rates were nearly 100 % in July. The same was the case in the additional reproductive-female study carried out in August. It must be noted that the August study was selective for reproductive females so the smaller, commonly uninfected animals may have been ignored. In the March-June population dynamics survey the infection rates were variable, but lower then 100 %. The release activity of microphallid cercariae from the initial molluscan host is known to increase in relation to warmer environmental temperature (Mouritsen and Jensen 1997). This may explain the high infection rates in July and August. Similar temperatures were evident in June but cerebral infections were not as common as they were in the later months. This may be attributed to a time lag between the release of cercariae from the hydrobid, infection of the amphipod and passage to the protocerebrum of the amphipod. However, this has been shown to take days rather than weeks (Fredensborg et al. 2004).

The number of cerebral metacercariae that were recorded infecting animals in the Gilkicker population ranged between zero and 20 (Figure 8.1). Those animals with high rates of infection were rare because, presumably, increased infection rate caused an increase in the rate of predation by the definitive host so these animals were removed from the population (Lafferty 1996). The other possibility is that high infection rates lead to death through other means. The amphipod *Paracalliope novizealandiae* demonstrated significantly increased mortality at both “intermediate” and “high” levels of *Maritrema novaezelandensis* (Microphallidae) infection (Fredensborg et al. 2004). The penetration of the amphipod cuticle by the cercariae caused wounding and loss of haemolymph in all cases of infection. High infections rates would increase this wounding and potentially be fatal to the amphipods (Fredensborg et al. 2004). A further possibility of stress caused by the wounding from penetration by the cercariae is reduced osmoregulatory ability associated with damage to the cuticle. There has also been discussion of a maximum volume of infection, related to both size and number of metacercariae of microphallids infecting *Cyathura carinata* (Jensen et al. 2004). At low infection intensities a greater proportion of the host animals were juveniles. Again, this was probably because they were young and therefore the probability of infection was reduced.

There was little relationship between animal size and the number of infections. The only evident trend involved juvenile amphipods. The uninfected juveniles were significantly smaller than those infected by trematodes (Figure 8.2). This is likely to be because the smaller animals were younger and had therefore had less time to become infected, rather than any delayed maturity effect of parasite infection, or, that those that had become infected had died because they were too small to tolerate the parasite. A similar trend was noted in *Daphnia obtusa* infected by trematode parasites (Schwartz and Cameron 1993). An additional possibility could be related to the suggestion that there is a maximum volume of metacercariae that an amphipod can sustain, in smaller animals this volume is clearly reduced so higher infection intensity could cause death. Also, the rate of haemolymph loss incurred by the penetration by metacercariae could be too great for juveniles to tolerate. Interestingly, at high parasite infection level the juveniles were much larger than usual. This may represent a parasite effect on the size at maturity, but examples of juveniles surviving with high infection rates were rare, so sample sizes were small.

Rumpus and Kennedy (1974) discussed early literature on parasite effects on metabolic rates and showed, across a range of parasite and host species, that patterns were conflicting. However, they showed that respiration rate in *G. pulex* infected by *Pomphorhynchus laevis* was significantly reduced in comparison to uninfected individuals. They also noted that the difference in respiration rate was not significant at lower temperatures. They suggested that it probably occurred, but as respiration rate was low at 10 ºC they were unable to detect it. Aside from parasite effects on the physiology of respiration, a plausible explanation for reduced respiration rates in animals parasitized with behaviour modifying parasites is reduced activity. Although the escape behaviour is described as aberrant, this is reported to occur in response to disturbance (Ottaviani and Franceschi 1996). In a laboratory assay where disturbance is minimal, activity may be reduced in parasitized animals.

In this study, the initial respiration assay was carried out at a lower temperature (5 ºC) than the 10 ºC used by Rumpus and Kennedy (1972). As with the Rumpus and Kennedy (1972) study there was a significant difference between infected and uninfected individuals with the infected organisms demonstrating lower respiration rates (Figure 8.4). When the assay was re-run, again at 5 ºC, the difference was not significant (Figure 8.5a). A potential reason for this observation is the method of selection of animals. For the second assay, the animals came from separate sites (infected from Gilkicker and uninfected from The Salterns). The animals were randomly selected from their aquaria prior to the assay and examined for parasite infection after the assay. For the initial experiment, clearly parasitized animals (displaying the aberrant escape behaviour described by Thomas et al 1995) were actively selected, so animals showing clear responses to infection were used.

In other investigations on the effects of parasites on metabolism the intensity of microphallid trematode infection was shown to only have minor effects (measured by direct micro-calorimetry) on *C. volutator* (Meissner and Schaarschmidt 2000). In this case, however the microphallids were *Maritrema subdolum* and *Levinseniella brachysoma*, neither of these species are known to be manipulative, so the effects would be expected to be different from the cerebral infections demonstrated by *G. insensibilis* in this investigation.

It has been noted, that when ambient temperature was high, there was “substantial” transmission of microphallid cercariae between *Hydrobia* and *Corophium* (Mouritsen and Jensen 1997). This fact was utilized to increase infection rates in a study of host survival (Jensen et al. 1998). A similar phenomenon may be apparent in the *G. insensibilis* population from Gilkicker lagoon. In the July and August reproductive investment samples infection rates were high in comparison to other months. Mouritsen and Jensen (1997) suggested that the increased infection intensities at higher temperature could be related to the increase in respiration rate that occurs with temperature increases. Invertebrates increase the ventilation of their gills to aid in oxygen uptake at higher temperature (Kristensen 1983), it was suggested that this increases contact between the amphipods and the trematode cercariae.

Fredensborg et al (2004) expanded on the issue of temperature dependent release of cercariae from the molluscan host. They stated that the temperature on hot summer days, in the shallow tide pools in which *Corophium* were collected sometimes exceeded 25 °C. This had the possibility to induce severe host (amphipod) mortality as a result of the increased infection intensity that occurred at these times. This could help to explain the large numbers of dead amphipods noted in the margins of Gilkicker lagoon, in the summer months of this study. The highly increased chance of injury associated with cercarial penetration, in conjunction with the stressors that characterize lagoonal habitats during the warmest summer months (extremes of temperature and salinity, along with temperature related reduced oxygen levels) are probably too great a physiological challenge for the amphipods to tolerate.

The stress induced by the combined effects of parasites and environmental parameters is supported by previous work on feeding rates. Feeding rate was been shown to be depressed by the presence of parasites in addition to anthropogenic pollution stressors (Brown and Pascoe 1989; McCahon et al. 1991; McCahon and Poulton 1991). In this study, however, there was no significant difference in the feeding rate between infected and uninfected individuals although extra environmental stressors were not applied (Figure 8.7). Any differences in feeding rate appeared to be an increase in energy intake.

It has been noted, in the case of *Corophium volutator* infected by *Maritrema subdolum*, that a possible effect of microphallid infection is the absorption of nutrients from the amphipod haemolymph by the parasites as they develop from the cercariae stage that penetrates the amphipod through to the metacercaria stage that encysts in the body of the amphipod (Galaktionov et al. 1996). The increase shown in feeding rate in this investigation was not significant but it suggests that further work is necessary in order to determine whether organisms parasitized by trematodes increase their energy consumption rate in order to compensate for nutrients lost to the parasites.

The diversity of parasite and host species, along with the variety of life history stages of trematodes, means that more research is needed into the actual effects on individual animals. This should focus on timing of infection; in this investigation the only factors considered were the presence or absence of metacercariae in the amphipods. The animals used, despite being parasitized, were generally healthy. It is probably the case that the main costs associated with parasite infection are related to the high levels of cercarial infection that occur in the hot summer days in lagoon-like habitats.

The effects of parasites on individual animals are important considerations, but it is the question as to whether these effects are translated to higher levels of biological organisation that concerns the study of populations and ecosystems. Such effects may occur through high levels of mortality resulting from, for example, high rates of cercaria release at high temperature or through the long-term reduction of reproductive output, either through direct effects on reproductive capability (parasitic castration), alteration of the structure of the population (sex ratio distortion) or through diversion of resources away from reproduction.

While there is no obvious selective advantage for parasites to have notable, detrimental influences on population sizes of their hosts, it is evident that increasing the likelihood of predation and the induction of immune responses and wound-healing costs may, reduce the energy available for growth and reproduction, which may consequently have such an influence. Data on the percentage of animals preyed on by birds, and the proportion of these that are reproductive females infected by trematodes are clearly difficult to determine, so the costs to the population of such losses will remain unknown. In terms of impact on the amphipod population as a whole, the predation rates by birds must be low because assortative pairing is well documented. If the aberrant behaviour dramatically increased predation rates then the parasitized animals would not form distinct population subunits divided by the depth of the lagoon as described by Ponton et al. (2005).

Individual reproductive costs relating to infection are more straightforward to study. In this study it was evident that there was a reduction in the weight-specific early-stage brood size with increased parasite load (Figure 8.9). The same trend was evident at the later stages of embryo development (Figure 8.10). Uninfected and heavily infected (3+) animals showed similar rates of brood mortality, both approximately 29 %, suggesting that there is no parasite-related effect on brood mortality. The similar rates of brood mortality suggest that smaller broods in infected animals was not related to the re-ingestion of the developing embryos at a later stage of development in order to compensate for energetic imbalances because of responses to parasite infections. This implies that the effects of trematode infection on reproduction were initiated prior to the release of eggs into the marsupium, probably because of reduced energy to initially allocate to reproduction.

In the second analysis of the effects of trematode infection on reproduction there were no uninfected females present. As previously explained, there might have been a temperature-dependent increase in the activity of cercariae, as these samples were from the warm water in August. The lack of uninfected females prevented the possibility of comparison of uninfected individuals with those hosting cerebral metacercariae. Like the data from the population dynamics survey there was no significant difference between the different intensities of infection. However, the mean brood size of females infected with three or more trematodes was lower than those with a lower parasite load in the population dynamics survey. In the second study the mean brood sizes were very similar in the intermediate and high infection rate groups. Brood mortality in the second study was 26 % between the early stages and Stage V.

Trematode effects on host reproduction have been reported in the literature. For the microphallid-gammarid situation reports have mostly related to the phenomenon of assortative pairing (Thomas et al. 1996). There is, however, little mention as to how this actually has an impact on potential recruitment. It has been shown that the fitness of infected males can be reduced because they pair with smaller, infected females (Ponton et al. 2005). As the fitness of female gammarids is increased by being of larger body size, and that of males is increased by pairing with larger females (Watt and Adams 1993), overall lifetime fitness of infected males would be lower.

More direct effects on individual reproductive output have also been reported; the number of juveniles per female was lower in infected animals (Ponton et al. 2005) but this was not significant when corrected to size, and, as it was shown in the same study that infected females were smaller than uninfected individuals, this difference in juvenile number could result from size-specific factors. Reproductive costs have also been inferred for isopods infected by microphallids (Jensen et al. 2004). There was an under-representation of ovigerous females of *Cyathura carinata* infected with microphallids, suggested to be the result of interference with ecdysis and gametogenesis. It has also been reported that infected specimens of the same isopod species were unable to produce embryos and showed increased mortality than uninfected individuals (Ferreira et al. 2005). More generally in the Crustacea, the fecundity of the cladoceran *Daphnia obtusa* was reduced by infection with more than two trematodes (Schwartz and Cameron 1993). This general lack of information on actual costs of the parasites suggests the evidence for reduced reproductive output shown in this study is valuable in increasing the understanding of the effects of trematode-amphipod interactions.

There are many factors involved in the control of reproduction within amphipods, and numerous ways in which reproduction could be affected. The evidence from the embryo-loss data suggested that parasite-related reduction in reproductive output occurred prior to the release of eggs into the marsupium. A conceivable explanation for reduced embryo number, given the evidence of previous chapters, which imply that reproductive effort in *G. insensibilis* is related to energy levels, is that parasite infection induces an energetic cost through immune response. It is known that melanization and encapsulation responses are induced following infection with *Microphallius* spp (Kostadinova and Mavrodieva 2005), which theoretically must be energetically costly. In addition to the initial immune response it is also known that the penetration of the abdomen by the cercariae wounds the amphipods and leads to a loss of haemolymph (Fredensborg et al. 2004), so replacement of body fluids and repair of wounds must also be energetically costly. The reduced respiration rates associated with infection shown in this study, however, would not be associated with high rates of protein synthsis associated with wound healing or stress response. A further, indirect, potential energetic cost involves the impact from utilization of amphipod nutrients (Galaktionov et al. 1996). This may not be as relevant a cost when considering cerebral metacercariae, but at some stage these animals penetrated the abdomen. It must also be noted that in certain specimens there were some abdominal infections, but this study was focused on the behaviour-altering cerebral metacercariae.

Other potential reasons for lower reproductive output involve the levels of catecholamines. Although variation was great there was an apparent increase in noradrenaline levels in parasitized females in this study (Figure 8.12). It is known that in certain crustaceans, ovarian maturation can be induced by varying the levels of dopamine and serotonin (Alfaro et al. 2004). In the Alfaro (2004) study, increased serotonin/spiperone induced spawning and had no effect on the quality of the offspring. In this investigation increased levels of catecholamines occurred in conjunction with decreased reproductive success.

Increased catecholamine levels, notably serotonin, have been reported as an explanation for the behavioural modification that many parasitized amphipods undergo. Injection of serotonin into the haemolymph of *Gammarus lacustris* reproduced the effect that *Polymorphus paradoxus* had on the amphipod (Helluy and Holmes 1990). Serotonergic imbalances were also shown for *G. insensibilis* infected with *M. papillorobustus* (Helluy and Thomas 2003). There has been debate as to whether these changes in serotonin levels result from secretion by the parasite or form part of a response by the amphipod to infection (Thomas et al. 2005).

Trematode parasites are not just confined to crustacean intermediate hosts. The New Zealand cockle, *Austrovenus stutchburyi,* from the intertidal zone, hosts echinostomatid trematodes which, when infection rates are high, prevent the normal behaviour of burrowing into the sediment, which increases probability of transmission to the definitive host, oystercatchers (Poulin and Mouritsen 2004). Although there are no data available on parasite infections, such a system may add to the explanation as to why the lagoon cockle (*Cerastoderma glaucum*) is frequently found on the surface sediments in lagoons rather than buried like its closely related congener, *C. edule*. This behaviour has previously been attributed to a mechanism to escape the low oxygen levels that sometimes occur just above the benthic layer in lagoons (Bamber et al. 2000).

It is clear that the amphipod-trematode interaction has persisted at Gilkicker for over 20 years and is likely to continue, because while there are mortality effects associated with parasite infection, not all amphipods are consumed by birds before reproduction. If inherited, there is a selective advantage to those amphipods which are able to escape predation when infected. The animals which are more susceptible to infection produce fewer (Figure 8.9), potentially lower-quality offspring and have an increased probability of being consumed by the parasite's definitive avian host. Natural selection could therefore remove these organisms from the population and the animals which can withstand parasite infection and the variable lagoon habitat, while still maintaining healthy rates of reproduction would survive leading to a population that is well adapted to the lagoon environment and parasite infection. While natural selection would drive increased amphipod resistance to the manipulative effects of the parasite, manipulation merely increases the probability of consumption by a bird. Lagoons are productive ecosystems and the high density of peracarids present at these sites means that they are important feeding grounds for certain bird species so they will continue to favour parasite adaptation to increase transmission probability.

Microphallid trematodes are frequently associated with lagoons or habitats where temperature can reach high levels, such as the intertidal zone on the seashore. The success of organisms such as *G. insensibilis* in lagoon habitats may be the result of an ability to maintain healthy reproductive rates and population sizes despite stressful environmental conditions, continual parasite infection and occasional parasite-induced reductions in population size.

In summary, there are interesting conclusions to be drawn about the role of parasites in ecosystems. The abundance of trematodes in Gilkicker and their effects on the reproductive investment of *G. insensibilis* show that the parasite must be considered an important part of the environment of the lagoon. More generally it is important that care should be taken by researchers when studying ecological systems to ensure that their study organisms are not the only organisms affecting the outcome of the results. The situation shown by the study of *Corophium* sp. described in this chapter highlights the need to consider parasites when studying any organism.

In addition to the necessity to consider the parasite in the study of lagoon habitats it must be noted that this microphallid-gammarid interaction has persisted, within the variable habitat of Gilkicker lagoon for over 20 years. This suggests that, despite near 100% infection rates at times, the detrimental effects of the trematode on the amphipods do not have severe implications to the population, despite the fact that the most intense bouts of parasite infection seem to coincide with the more extreme temperatures. This, and the fact that manipulated hosts demonstrate some normal attributes and some parasite-induced novel attributes, have led to the suggestion that situations such as the *G. insensibilis* and *M. papillorobustus* association should perhaps be considered as a discrete entity (Curtis 1990). However, to gain a more comprehensive understanding of the interactions between the amphipod and trematode, more research is necessary on the seasonal variation in the abundance of the parasite as well as the predation-related mortality rates caused by the trematodes. Microphallid trematodes appear to have important effects on the functioning of lagoon ecosystems and should perhaps be considered part of the specialist biota.

Chapter 9

The monthly reproductive biology of *Idotea chelipes*

9.1. Introduction

Within saline lagoons, one of the most important interactions is that between the filamentous green alga, *Chaetomorpha linum* and the peracarid crustaceans which use it as a habitat and a food source (Al-Suwailem 1991; Bamber et al. 2000). While *Gammarus insensibilis* is clearly a major contributor in this important interaction the isopod, *Idotea chelipes* is similarly abundant within the matrix of filaments of the alga and is a similarly voracious consumer of it. These interactions imply that the alga is a very productive habitat as two similar organisms apparently utilize it to such a great extent and therefore occupy similar ecological niches. Lagoons are complex systems with many interacting factors, both biotic and abiotic, and although the *C. linum*/peracarid interaction persists in many lagoon sites, their management should not be tailored to just one species.

As shown and discussed in Chapter 4, peracarids have evolved remarkable variability in life history and reproductive traits, frequently in response to environmental selective pressures. In saline lagoon habitats however, *I. chelipes* and *G. insensibilis* utilize similar habitats and food sources (Bamber et al. 2000). As a result of the equivalent selection pressures, it would be expected that they would demonstrate similar reproductive strategies. The importance of the specialist lagoon peracarids offers an ideal opportunity to study the adaptation of two similar organisms to the same variable habitat conditions.

On the Atlantic coast *I. chelipes* is found from France to Morroco and it is also known from the Baltic and the Mediterranean (Charfi-cheikhrouha 1996). From the UK it is reported from coastal saline lagoons and is described as a lagoon specialist, and is considered nationally scarce. It is found at a number of sites on the south and east coast of England (Bamber et al. 2000). Variation in the reproductive strategy of *I. chelipes* is documented. French *I. chelipes* were reported to breed all year round and produce up to four broods per year (Labourgh 1971) but, in contrast, a population from Gilkicker lagoon has been described that has a distinct breeding season between April and November with two broods per year (Al-Suwailem 1991) and has a maximum lifespan of 10-11 months (Kroer 1989). It is known to have important controlling effects on macroalgal densities in coastal ecosystems (Lotze and Worm 2000).

This chapter describes the population dynamics and reproductive investment of *I. chelipes* in order to gain a greater understanding of the ecology of another important lagoon specialist and to compare the strategies of similar organisms inhabiting the same environment.

9.2. Methods

Samples of *Idotea chelipes* were collected, with a hand net, from among the mats of *C. linum* in Gilkicker lagoon on the same occasions as the *Gammarus insensibilis* used in the population dynamics survey (Chapter 4). They were taken to the laboratory, fixed in 10% formalin and stored until analysis.

The samples were examined from each month under a dissecting microscope and the population was divided into the same classes as the amphipod (Fe, Fb, Fi, M & J). Population structure data were presented as percentages of the population because of the non-quantitative sampling method. Body length was measured from the head to the tip of the telson and additionally, head length was measured (Figure 9.1), dry weight was also determined by drying in an oven at 60 °C for 24 hours. As with *G. insensibilis* brood size was also counted and the embryos were staged using a similar method to the amphipods; the classification system used was based on that of Naylor (1972) in which there are 4 stages (A, B, C, D), in which A represents a newly fertilized embryo, and D describes the newly hatched juveniles. Like *G. insensibilis*, brood size was also determined and standardised to female body weight.

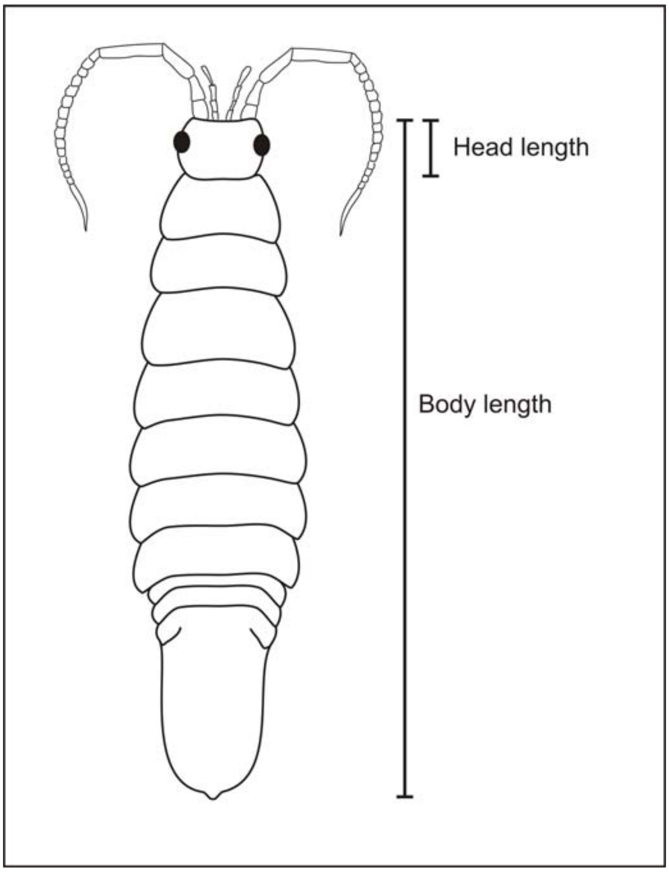

Figure 9.1: The measurements taken on *Idotea chelipes* after Naylor (1972)

9.3. Results

Similar to *Gammarus insensibilis*, there was a clear relationship (Figure 9.2) between dry weight and body length ($r^2 = 0.93$). The animals measured ranged between 2 and 15 mm in length and 0.024 and 6 mg in dry weight. Males covered a larger size range, being evident in the population at a smaller size than females, and also reached far larger sizes than females which were never more than 10 mm long. The separate relationships for the plots in Figure 9.2 are described by the equations in Table 9.1.

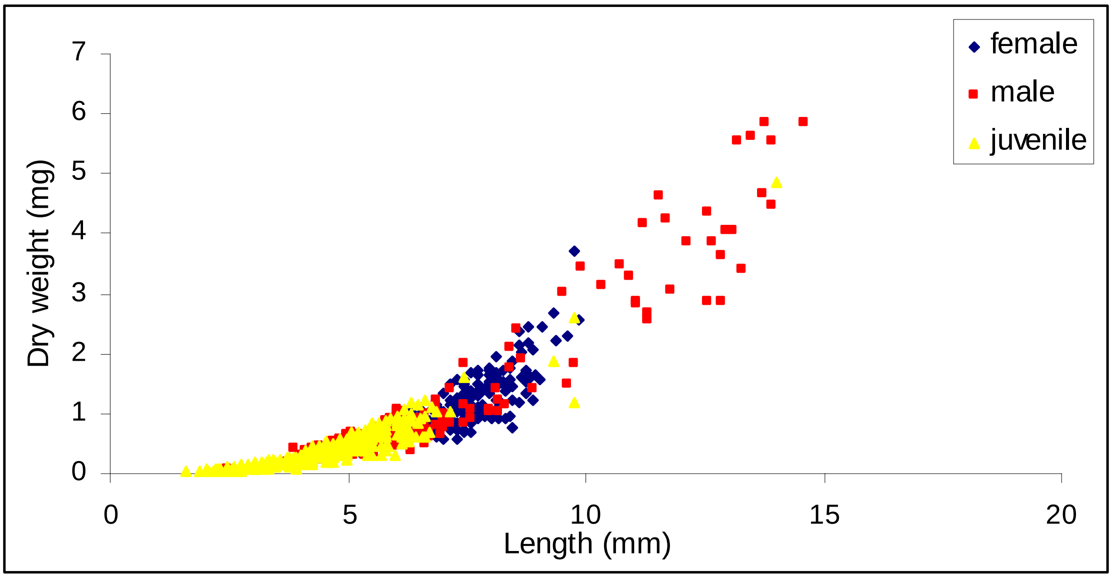

Figure 9.2: The relationship between body length and dry weight for the population of *Idotea chelipes* divided into male, female, juvenile categories (regression equations and r^2 values are shown in Table 9.1).

Table 9.1: The equations and regression lines for the plots shown in Figure 9.2

Life history stage	Equation	r^2
Female	$y = 0.005x^{2.692}$	0.60
Male	$y = 0.0104x^{2.3412}$	0.91
Juvenile	$y = 0.0064x^{2.6324}$	0.90
Population	$y = 0{,}0078x^{2.4892}$	0.93

Figure 9.3 shows that across the first two stages of embryo development, for all the months studied, there was an increase in brood size with increased female body weight (linear regression $r^2 = 0.58$), the later stages of development (Stages C and D) were excluded from the analysis because of the effects of brood mortality (Figure 9.4).

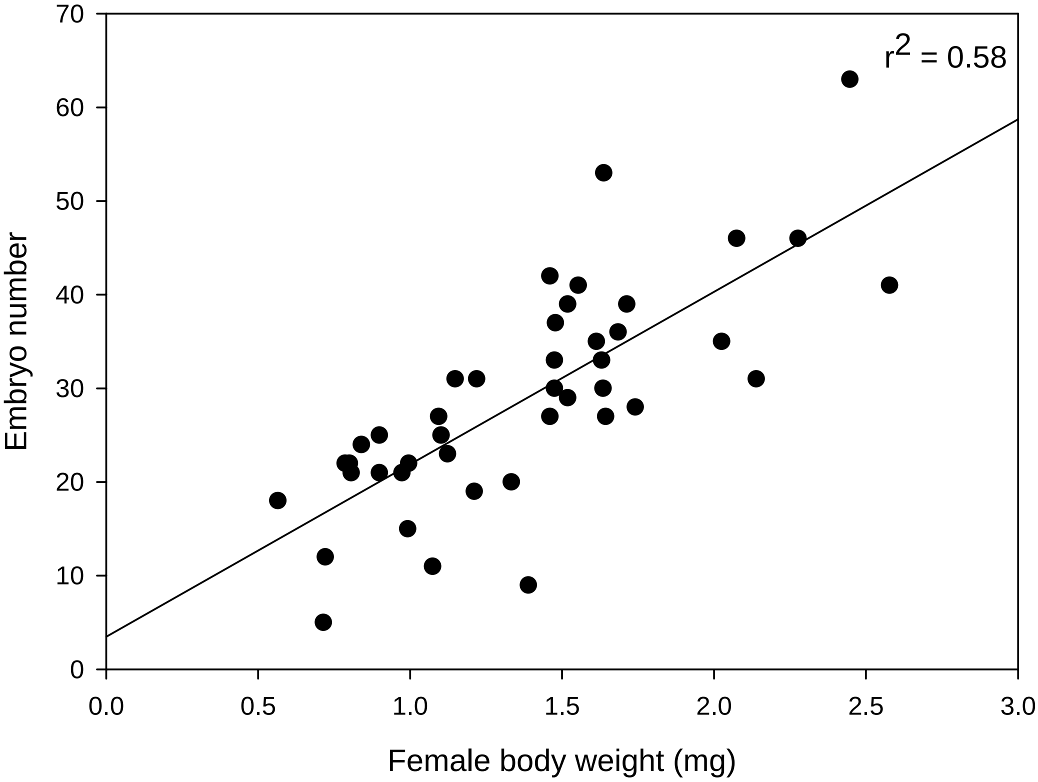

Figure 9.3: The relationship between female body weight (mg) and the number of embryos (Stages A & B) in the marsupium for *Idotea chelipes.*

The weight specific brood size of *I. chelipes* declined during the course of embryonic development (Figure 9.4). There was a significant difference in weight-specific brood size between the stages of development (ANOVA, $F_{3, 76}$ = 3.349, P = 0.024). Pairwise comparisons (Holm –Sidak) showed that mean Stage A brood size was significantly larger than Stages C and D. The rate of embryo loss was 25% between Stage A and Stage D (equivalent to between Stage I and Stage J in the amphipod system). This was a lower rate of mortality than demonstrated by *G. insensibilis* which lost 30% of its brood during development from Stage I – V (V in the amphipod classification is an earlier stage of development that D in the isopod scheme). Between stage A and C the isopod lost 19% of its brood (D is equivalent to stages IV and V in the amphipod classification).

During the survey, some of the mature female isopods were observed to have degraded organic material in their marsupia, occasionally identifiable as embryos. In each of these occasions the material was accompanied by a number of nematodes. There was no seasonal trend in this occurrence.

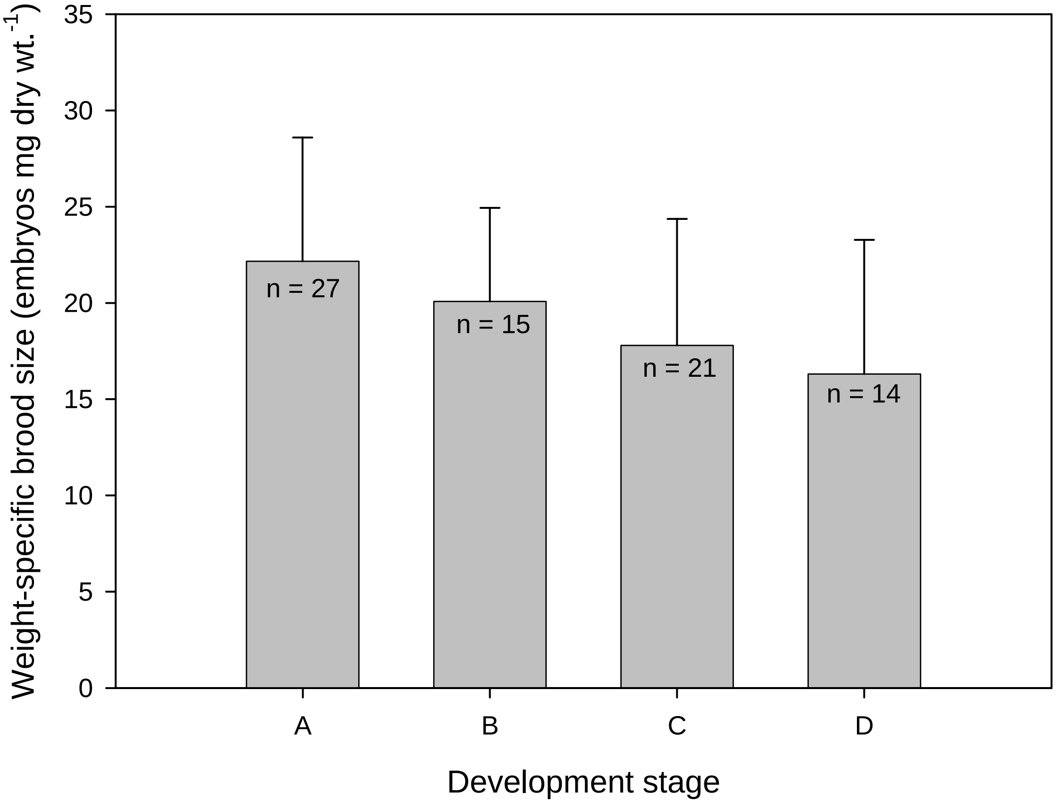

Figure 9.4: The mean weight-specific brood size (± 1 sd) of female *I. chelipes* carrying embryos of different stages of development (n is also shown), from all the months studied.

The percentage of the reproductive female component of the population often seemed low because of the effect of large numbers of juvenile animals; therefore the proportion carrying embryos was presented as a percentage of mature females (Figure 9.5). Reproductive females were found in all samples, however they were rare in the winter months, with less than 20 % carrying broods in their marsupia.

A large proportion of the population in each sample comprised of juvenile animals. The smallest animals for which it was possible to determine gender were approximately 3.8 – 3.9 mm in length and these animals were male. As demonstrated by Figure 9.6 the highest proportion of animals for which it was possible to differentiate gender (animals over 4 mm) occurred in the later summer months (July and August), and in the autumn (October) while the lowest percentage of mature animals occurred in February. These trends are also evident from the greater number of larger juveniles in winter months in the population structure profiles (Figure 9.9).

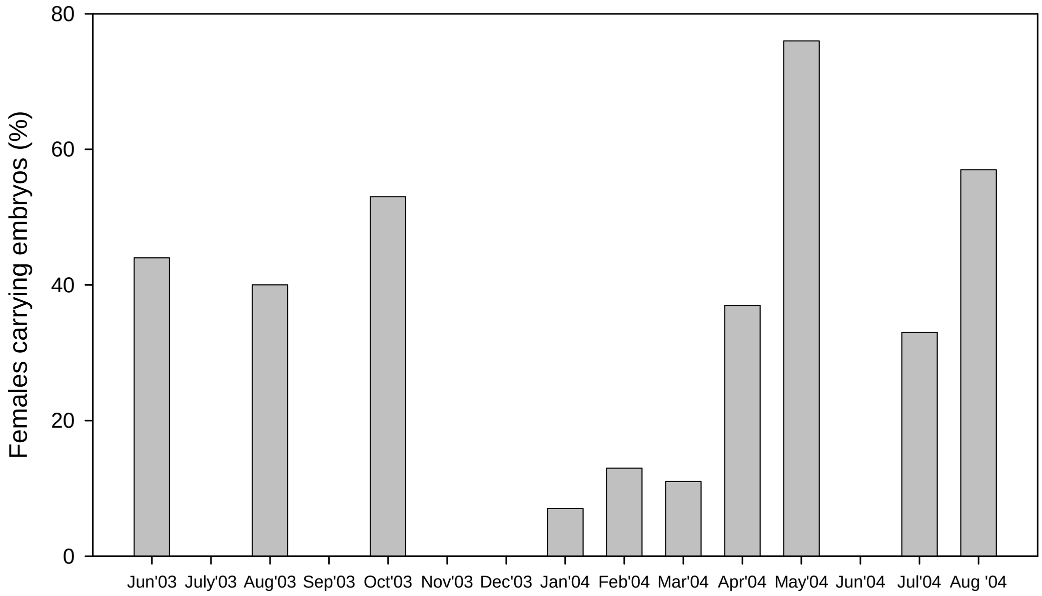

Figure 9.5: The percentage of the female population of *I. chelipes* from Gilkicker carrying embryos between June 2003 and August 2004. (There were no collections in July'03 September '03, November '03, December '03 and June '04)

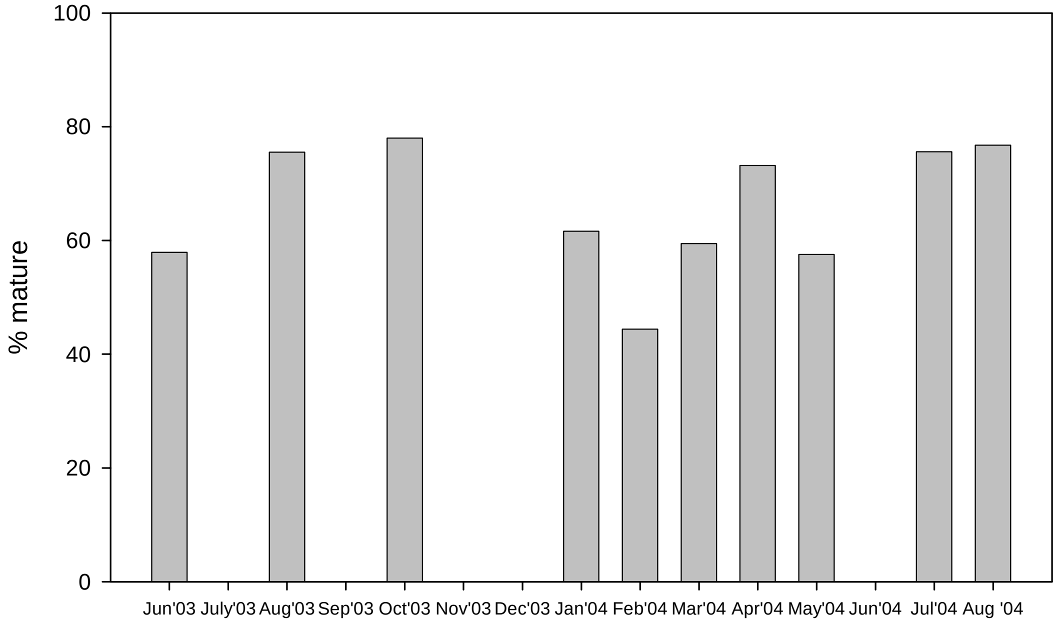

Figure 9.6: The percentage of the Gilkicker population of *Idotea chelipes* for which it was possible to determine gender, larger than 4 mm in length between July 2003 and August 2004.

There was variability in the mean weight specific brood size of *Idotea chelipes* during the course of the year (Figure 9.7). As a result of low numbers of reproductive females recorded in the samples the categorisation into broods of different stages reduced the data available for comparison. Consequently early stage broods showed no significant variation between months (ANOVA, P = 0.345). When all stages of development were included in the analysis the variation in brood size was significant (ANOVA, $F_{7,75}$ = 3.799, P = 0.002). Holm-Sidak pairwise analysis showed that the weight specific brood sizes in the winter months were significantly smaller than the summer broods.

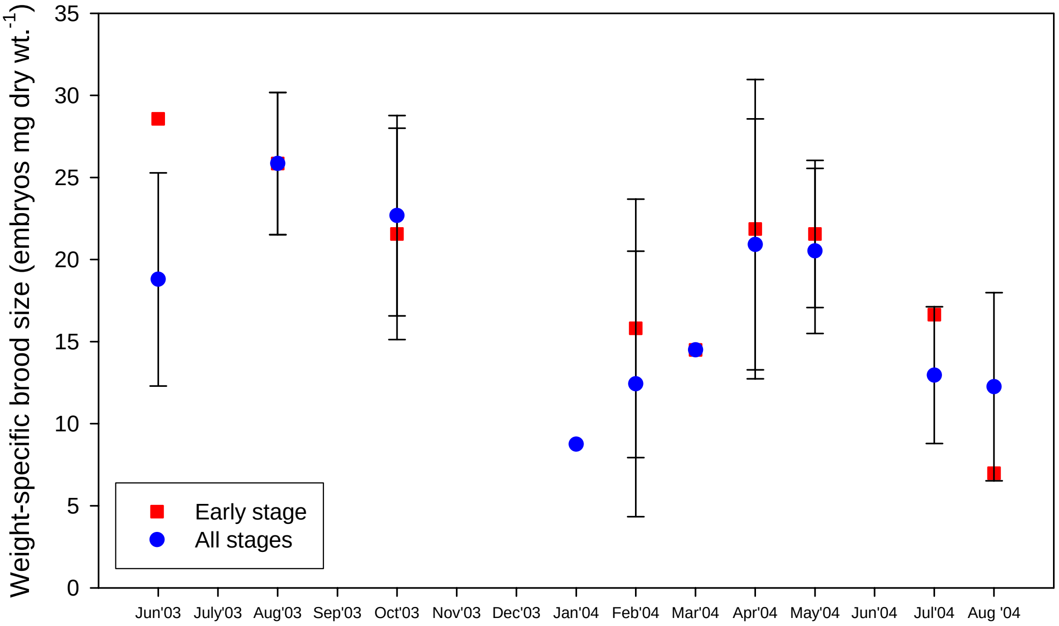

Figure 9.7: The monthly variation in the mean (± 1 sd) weight-specific early-stage (A-B) and all-stage (A-D) brood sizes of *Idotea chelipes*

On a seasonal basis there was also variation in weight specific brood size (Figure 9.8). Mean winter early stage brood size was smaller than the rest of the year (Figure 9.8a), however this difference was not significant (ANOVA, P = 0.61). When all stages were included in the analysis there was a significant difference in mean weight specific brood size (ANOVA $F_{3\text{-}77}$ = 4.08, P = 0.01), mean autumn brood size was significantly larger than the other seasons (Holm-Sidak pairwise analysis).

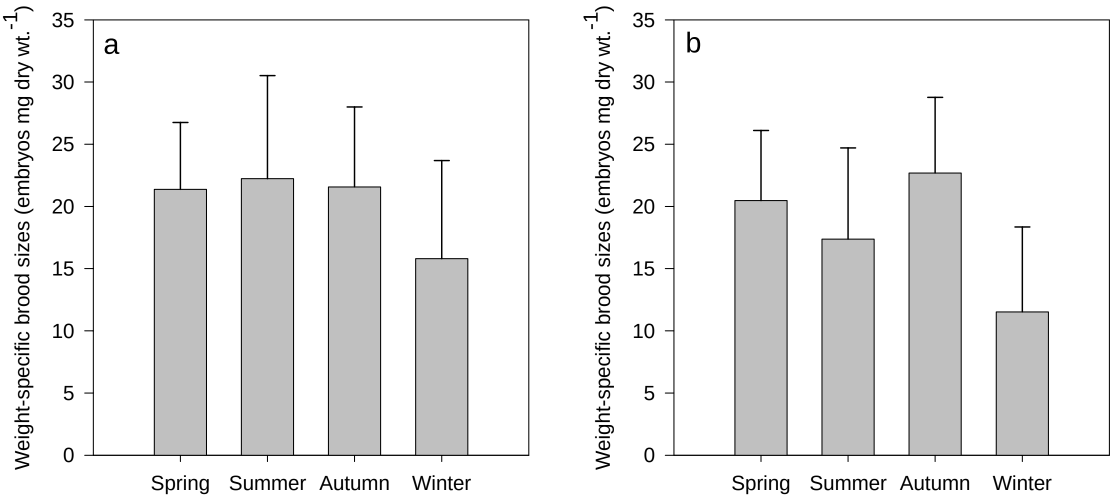

Figure 9.8: Mean seasonal weight-specific brood size of *I. chelipes* (± 1 sd), a) early stage embryos (A-B), b) all stages (A-D)

The size structure of the population was clearly variable during the course of the sampling programme (Figure 9.9). There were periods when small juveniles (less than 3 mm in length) were dominant, this was evident in both the ‘juvenile’ and ‘overall population’ population structure graphs in Figure 9.9 This was most notable in June 2003 and May 2004 although there were high proportions of juveniles in all summer months. Between January and April 2004 there were a greater proportion of larger juvenile animals.

The largest individuals recorded were males, reaching a maximum of 15.3 mm in the samples. During the course of the various sampling visits to lagoons much larger specimens of *I. chelipes* were observed around rocks of submerged wood but these large animals were not recorded in any samples taken from among the *C. linum*.

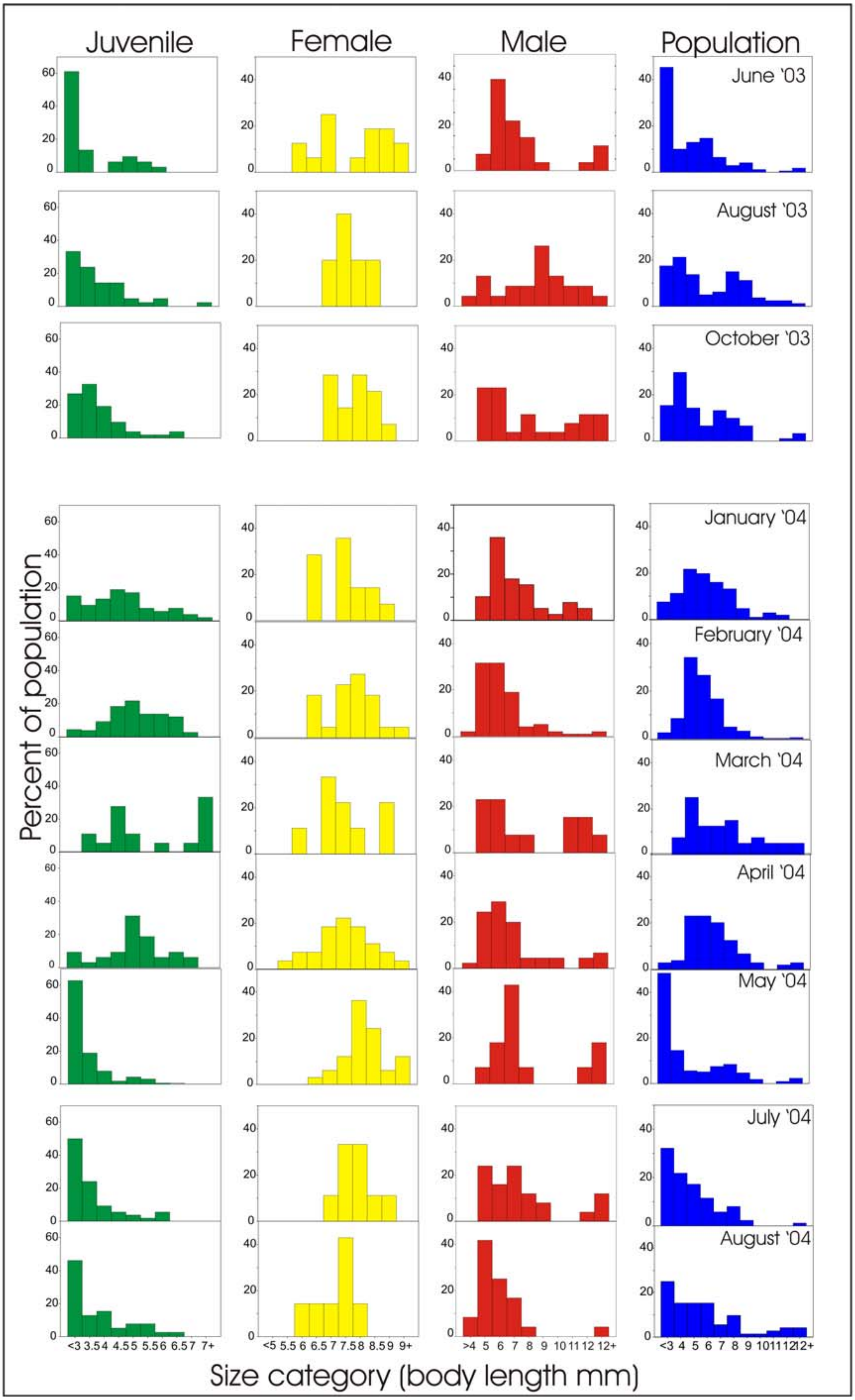

Figure 9.9: The population structure of juvenile, female, male and the total population of *Idotea chelipes* (as percentage of each particular category) and the overall population structure from Gilkicker lagoon between June 2003 and August 2004.

9.4. Discussion

This study of *Idotea chelipes* was carried out in order to provide comparative material for the work on *Gammarus insensibilis*. The initial intention was to demonstrate concurrent variation in the reproductive investment of both species in relation to the environmentally induced patterns in Scope For Growth. However, the relative stability in the SFG of *G. insensibilis* in relation to the environmental influences prevented this possibility. However, the data remain valuable in relation to the management and understanding of the ecology of lagoon systems.

A common feature of peracarid crustaceans, as demonstrated and discussed for *Gammarus insensibilis* in Chapter 4, is plasticity of reproductive strategy as an adaptation to variable environmental parameters. Intraspecifically this may involve variation in investment into the components of total brood size (embryo number and size). While *G. insensibilis* reproduced continuously all year round, this is not necessarily the case for all species of peracarid, and interspecifically there is a greater variety of life history strategies evident in the group involving timing of reproduction, number of broods per year, size of broods or size of eggs often in order to ensure optimum conditions for juveniles when they emerge from the marsupium. The giant isopod, *Bathynomus giganteus*, for example, breeds in the winter months, ensuring juveniles emerge when food is more abundant (Barradas-Ortiz et al. 2003).

The results of this investigation showed that, like *G. insensibilis*, lagoonal *Idotea chelipes* demonstrated typical peracarid patterns of body mass change with increased body length and increased brood size with increased body size. Thus, like gammarid amphipods, there is an adaptive value in increased body size of both female and male *Idotea*. Females because they can brood more embryos and males because they have the ability to guard larger females (Adams and Greenwood 1983). However, for comparison of results between seasons, when the size of the animals varied, the brood sizes were presented as weight specific brood sizes throughout the results.

Amphipod brood mortality was discussed in Chapter 4. This also occurred in the isopod, although the rate of loss was generally lower. The stages of development of isopods are not as clear as in the amphipods so there was the complication of a different embryo staging methodology for the isopod broods. However, between stages A and D (equivalent to I – J in the amphipod model) the rate of loss of embryos from *I. chelipes* was 25% (Figure 9.4). This was lower than the 30% loss that occurred between stages I-V in the amphipods. In the isopod this loss includes the more active juvenile animals, which, in the amphipod example readily leave the marsupium when the female is disturbed. This behaviour clearly does not occur to the same extent in the isopod.

A potential reason for the reduced loss of embryos may result from the differential oöstegite morphology between the two species. The general trend for isopod oöstegites, and evident in this study, is for them to be larger and completely cover the brood, while the gammarid amphipod oöstegites are more slender with setae along the margins, which form a mesh holding the embryos in place. This may prevent the juveniles from leaving the marsupium when the female is disturbed either physically or by the addition of fixative. However, the lower brood mortality between the first and final stage of the isopod and the first and final non-active stage of the amphipod clearly suggests that brood mortality is a less important factor in *I. chelipes* than *G. insensibilis.*

There were a number of occasions when there was a mass of organic material held in the marsupium by the oöstegites, this material appeared to be degraded embryos and was normally associated with nematodes in the marsupium. It would appear therefore that in the case of the isopod, brood mortality does occur but it is generally less likely to be in the form of loss of individual embryos from the brood, but more likely to be the loss of an entire brood. It is not clear if the nematodes found in the marsupium were the cause of the mortality or whether they colonized the marsupium after mortality to consume the dead brood.

There was some evidence for continuous breeding throughout the year; in each month studied there were females carrying broods (Figure 9.5). However there were obvious seasonal trends in reproductive parameters. The percentage of reproductive females reduced in the colder months (winter and early spring) with less than 15% of the female population carrying embryos in January, February and March. This percentage increased dramatically through the spring and reached a maximum of almost 80% in May, coinciding with a high proportion of small juveniles in the population (Figure 9.9). These data suggest that the reproductive strategy of the isopod is more seasonal than that of the amphipod. Female *G. insensibilis* were present in the population throughout the year but there was some variation. However, it was only in January that the percentage carrying embryos fell below 40% and generally, even in the colder months it was above 50% (Chapter 4, Figure 4.16). *I. chelipes* seems to have a seasonal reproductive strategy, with greatest investment in the late spring and early summer. This is supported by the findings in a previous study in which *I. chelipes* from Gilkicker (Al-Suwailem, 1991) was reported to breed between April and November. *Idotea chelipes* from Essex, England also demonstrated a seasonal strategy in which juveniles were released between May and August and in December (Howes 1939). Other species of isopod are also known to have discrete breeding seasons, for example *I. pelagica* from north-east England released juveniles between June and September (Sheader 1977).

Al- Suwailem's (1991) findings suggest a wider breeding period for *I. chelipes* from Gilkicker than Howes (1939) found in Essex. In this study however, there were a small number of individuals that reproduced throughout the year and the presence of early stage embryos in the winter months (Figure 9.7) suggest that this was not just a case of later summer embryos developing slowly as a result of the cooler winter temperatures. This is feasibly a reproductive adaptation to the variable environmental habitat conditions of the lagoon. High summer temperature and salinity, unseasonably low salinity resulting from heavy summer rainfall, high winter salinity from poor lagoon management or parasite-induced mass-mortality events are all potential threats to lagoonal populations. If there is some degree of continuous recruitment there is more likelihood of a population surviving a mass-mortality event.

While this strategy may resemble a group-selection hypothesis, it is not. It is clear that there may be a selective advantage to the individual in seasonal reproduction because of the more optimal conditions for growth and survival that occur in the summer. However, it is known that lagoons are subject to severe environmental stress related to their shallow nature and proximity to land, particularly in relation to extremely high summer temperatures, so a minority of animals that reproduce on a more continuous basis are likely to be those which produce the offspring which begin the recovery process after a population bottleneck, and therefore benefit from a significant selective advantage at these times. However, further work is required on the history of bottleneck events in coastal lagoons. An initial study using genetic analysis of *I. chelipes* from a number of lagoons in the Solent area suggested that there was no evidence of recent bottleneck events (Jolly et al. 2003).

In terms of size at maturity, Figure 9.6 showed that above 4 mm in length a large proportion of the population were mature (females with fully developed oöstegites). In several months this was approximately 80% while in February the mature proportion of the population fell to under 50%. This suggests that at the cooler winter temperatures reproductive maturity may be delayed in order to achieve more whole body reserves to ensure the animal is able to tolerate the intrinsic stress related to reproduction.

Gammarus insensibilis demonstrated considerable environmentally-induced variation in the weight-specific brood size (Figure 4.2). Figures 9.7 and 9.8 showed that *I. chelipes* also demonstrated some similar variation. The mean winter weight specific brood size was smaller than those from the rest of the year, suggesting reduced investment into numbers of offspring.

However, the seasonal variation in embryo size, shown in *G. insensibilis*, was not recorded because live animals were not studied. There was therefore no information available for investment into individual embryos. Embryo volume measurements were attempted but no variation could be found. Embryo volume can vary as a result of fixation (Chapter 3) so conclusions can not be made about the investment into the individual embryos of *I. chelipes*.

Despite the lower mean early-stage weight-specific brood size the difference was not significant (Figure 9.8a). The low proportion of the females that were brooding in the winter months, together with the fact that in late spring and early summer a large proportion of the population comprised juvenile animals further supports the argument that *I. chelipes* had a more seasonal reproductive strategy than *G. insensibilis*.

It has already been suggested that *G. insensibilis* and *I. chelipes* share a very similar habitat and to a large extent feed upon a similar food source (Al-Suwailem 1991; Bamber et al. 2000). However, basic ecological theory states that species can not persist together if they share the same niche. There are a number of factors that appear to separate these two species ecologically. Firstly, as discussed in this chapter, they demonstrate slight differences in reproductive strategy so that, in general, there will not be large numbers of juveniles of each species competing for the same resources. Secondly, in the samples of *I. chelipes*, there were large numbers of small juvenile individuals (<3mm), while in the amphipod samples the equivalent small individuals were not so abundant. The same sample collection methods were used for both the isopod and amphipod, suggesting that the small amphipods were not missed through sampling error. This may represent a slightly different life history strategy for the amphipod, in which the juveniles inhabit and feed in a different part of the lagoon, for instance the sediment. It is clear that the juvenile isopods live within, and feed upon the *C. linum* like the adults. It may be that the amphipods only move to the alga when they reach a larger size.

An additional observation made in the course of this investigation, during the general maintenance of lagoonal organisms in aquaria, suggested that there may also be some differences in the food source of the isopods and the amphipods. Although both fed on *C. linum* when maintained in the same aquarium tanks, when presented with other food sources *I. chelipes* would more readily consume animal material and artificial fish foods than *G. insensibilis*.

In summary, the isopod *I. chelipes* plays a similarly prominent role in coastal lagoon systems as *G. insensibilis*. It shows some variation in reproductive strategy but seems to demonstrate a more seasonal reproductive strategy under which the majority of recruitment occurs during the spring months which may ensure more optimal conditions for the growth of the juveniles during the summer. There is also an element of continuous reproduction, suggested to be an adaptation to the characteristic variable environmental conditions of saline lagoons. The slight differences in the reproductive strategies of the specialist lagoon peracarid crustaceans may help to explain how species utilizing a similar ecological niche can coexist. It is important for the management of coastal lagoons to understand the life history strategies of a number of the ecologically important species, in order to ensure that conservation strategies aimed at one species are not detrimental to the other contributing members of the overall lagoon ecosystem.

Chapter 10

Summary, general discussion and further work

Biological systems are complex, with many interacting past, present, biotic and abiotic factors contributing to the phenotypes displayed. Coastal lagoons are useful habitats to study because they present a rare opportunity to examine populations, of conservation importance, from a relatively closed ecosystem in order to determine the various selection agents that act upon them to produce the life history characteristics demonstrated.

The lagoons around the coast of the UK have attracted scientific interest because of their rarity, specialist biota, unusual habitat conditions and their wider importance as feeding grounds to animals of popular human interest, such as wading birds. In coastal lagoons it seems that there is an important balance which, when met, ensures all these features of lagoons are able to persist. Without the protection afforded to these rare habitats, lagoon specialists could potentially be lost, in a similar manner to *Edswardia ivelli*, the sea anemone thought to be extinct from Widewater lagoon in Sussex, England – its only known site worldwide (Bamber et al. 2000), or replaced by a more common estuarine, marine or freshwater fauna. The loss of the rare animals would reduce the importance of these habitats as conservation concerns, and the loss of either the fauna or the sites would clearly have an impact on the value as feeding sites for birds. While lagoons have been designated as EU priority habitats, and some of the specialist species scheduled, there has been relatively little scientific investigation of the function of these systems in order to determine how they can best be conserved. This investigation attempted to provide some of the vital information on the biology of lagoon specialists in order to facilitate the conservation of the habitats.

Primarily, the aim of the investigation was to provide detailed information on the ecology of *Gammarus insensibilis* in order to provide data for the management of the conservation of the species. This was attempted through the monitoring of various aspects of the population dynamics, reproductive biology and energy balance of *G. insensibilis* in combination with the examination of the dominant physico-chemical parameters (temperature and salinity). However, lagoons are dynamic systems, with each site demonstrating differing physico-chemical regimes as a result of their shape, location and marine and terrestrial influences. It is therefore not possible to propose one strategy to conserve *G. insensibilis* in all lagoons. Instead, the information gained by this study is representative of the sites used and can be utilized as a guideline for use in combination with specific data on the lagoon concerned.

One of the main areas of study was the survey of the reproductive investment made by *G. insensibilis* across an extended time period. This was because the ultimate expression of the energy status of an organism is shown by its reproductive potential. Therefore, the ultimate consequence of high environmental stress, as expected in lagoons, would be reduced reproductive output. Various components of reproductive output were therefore studied in order to determine how the extreme environment of the lagoon affects its inhabitants. Although most environmental parameters vary in lagoons (Barnes 1980; Al-Suwailem 1991; Bamber et al. 1992; Bamber et al. 2000) temperature and salinity are accepted to be ecological "master-factors" so were used as the basis for the studies in this investigation.

The temperature and salinity of the lagoons studied in this investigation were characteristically variable, with similar seasonal patterns in the extremes of both temperature and salinity. This variation had clear consequences for *G. insensibilis*, which demonstrated differential allocation of total reproductive resources; while clutch volume remained relatively constant through the year, the number of offspring and the size of the individual embryos varied. This was suggested to be a reproductive adaptation to increase total fitness in the prevailing conditions, under the assumption that fewer larger embryos should increase survival under a harsher environmental regime. In terms of lagoon management this is an important consideration. Many species reproduce seasonally, experiencing increased intrinsic energetic stress at times of peak reproduction (Lucas 1996; Smaal et al. 1997). While *G. insensibilis* demonstrated its highest reproductive output in the spring months it is essential that conservation management strategies ensure that there is consideration of the fact that *G. insensibilis* reproduces throughout the year.

To enable these reproductive adaptations to the lagoon environment, there was also physiological adaptation to the variable habitat conditions. Respiration rate was variable during the year, with higher rates in the summer, but overall there was no large scale variation, suggesting that under the gradual temperature and salinity changes that occur over a year, the animals were physiologically well adapted to the environmental variation. Scope For Growth was variable but the trends were not seasonal. Instead, the greatest "stress" on the animals was intrinsic, i.e. reproductive stress. Over the year there was a pattern in which overall reproductive investment (clutch volume) reflected that of SFG, suggesting that in order to prosper under a stressful habitat regime *G. insensibilis* maximized reproductive effort when the conditions were optimal and energy was available. For the management of coastal lagoons it is essential that this wide range of tolerance is used as a tool to ensure that the variable environmental conditions of lagoon environments are maintained. The variable yet sub-littoral habitat created by lagoons is unusual. *Gammarus insensibilis*, and the other species with which it co-exists, are well adapted to the habitat variation they experience, and this is one of the selection pressures that prevents the colonization of lagoons by marine, estuarine fauna, it is therefore important to ensure that these conditions persist in lagoons.

Despite the successful adaptation to the variable habitat conditions of the coastal lagoon it must be noted that there are limits within which lagoons should be maintained. While *G. insensibilis* was able to acclimate successfully to demonstrate relatively stable respiration rates over the course of the year, unseasonal variation between salinity and temperature caused greater stress to *G. insensibilis*. Low salinity in combination with high temperature or high salinity with low temperature are situations unlikely to arise under natural circumstances in the lagoon sites used in this study. Therefore, lagoon management strategies should involve continued monitoring of temperature and salinity of lagoons. The control of temperature is purely the result of the prevailing environmental conditions but use of sluices should be encouraged to ensure that salinity maintained at higher levels in the summer and at no times allowed to become excessively high or reach freshwater levels. Problems of this nature have occurred at the Lymington-Keyhaven lagoon system where salinity was allowed to reduce to zero for an extended period, resulting in the loss of specialist species from the affected lagoons (Bamber et al. 2000).

An important consideration for the conservation of any habitat is protection from anthropogenic influences. The enclosed nature of lagoons and their many terrestrial influences places them at a higher risk from anthropogenic activities than most other marine habitats. For example the Mar Menor lagoon in Spain is subjected to metal pollution after heavy rainfall as a result of run-off from nearby mining facilities (Marin-Guirao et al. 2005). Gilkicker lagoon is surrounded on its landward margins by a golf course, so it is subject to fertilisers, pesticides, herbicides and freshwater run-off from the course. Lagoon specialist species have a wide ranging tolerance to the typical stressors so, as a result of the generalized nature of the stress response it would be expected that they have a certain degree of tolerance to anthropogenic stressors. While lagoon specialists are successful and abundant in Gilkicker despite the variable habitat and potential anthropogenic influences (from the activities of the golf course), a particular problem with lagoons in relation to pollution events can be attributed to their enclosed nature. There is reduced possibility of dispersal of polluting agents in lagoons, in comparison to estuaries or the open sea, so the organisms in the vicinity are subjected to higher concentrations of pollutants for longer periods of time than their marine counterparts, in similar circumstances. Such events can cause devastating effects on lagoon communities. The Little Anglesey lagoon on the south coast of England suffered such an event in 2004 when effluent from a nearby sewage works was released into the lagoon leading to dramatically reduced numbers of lagoon species (Sheader *pers. com.*). It is therefore essential that the management of lagoons controls terrestrial inputs to the systems in order to reduce the risk of such events.

Beside the physico-chemical properties of the lagoons, a further influence on the ecology of *G. insensibilis* involves its interaction with *Microphallus papillorobustus*. Although only recently increasing in ecological recognition, parasites are important members of ecological systems, to the point where, in some situations, they have been described as ecosystem engineers (Thomas et al. 1998). The effects of trematode infection on the *Gammarus insensibilis* population from Gilkicker lagoon were clear; there was a decrease in fecundity as a result of infection. Although the actual effects on the population dynamics are unclear, it is evident that high infection intensities in conjunction with severe environmental stress may pose problems to the conservation of lagoons. However, from the lagoon management perspective, trematode infection should purely be considered as one of the environmental variables which affect lagoon populations. One, perhaps alarming, consideration of the high rates of trematode infection is that the definitive hosts of the trematode parasites are birds. Despite this, lagoons should still be protected as feeding grounds for birds, as the success of the avocet shows. It is thought that the adult trematodes have no pathological effects on the birds that host them, and they simply mature and produce eggs in the digestive system of the bird (Thomas et al. 2005).

The importance of the interaction between the amphipod and *M. papillorobustus* could extend to explaining the dominance *G. insensibilis* in coastal lagoons. Microphallid trematodes are clearly abundant in the hydrobiid populations in the lagoons. The ability of *G. insensibilis* to maintain successful reproductive output rates despite the variable environmental conditions and the high parasite infection rates may explain why it is the dominant gammarid species lagoons. Gammarids are abundant in most marine habitats and it would be expected that species such as *G. locusta*, a closely related marine species, may also prosper in lagoons. The resistance to the parasite may be a defining feature in explaining the low numbers of *G. locusta* found in lagoons. It is therefore essential that parasites are considered as important components of the understanding of the functioning of ecosystems.

A further point to conclude from this investigation is related to the concept of Scope For Growth. While SFG is a useful tool to monitor the condition of organisms, and its value has frequently been demonstrated in the literature to be closely related to many stressors on various organisms, it must be noted that it does not necessarily indicate that organisms are under severe stress. When an artificial stressor is applied under controlled laboratory conditions and SFG is shown to reduce it can be concluded that there is a cause and effect relationship. However, when animals are monitored over time and SFG varies, as demonstrated in this study, the reduced SFG cannot immediately be concluded as a detrimental effect on the organism. Scope For Growth, by definition, describes the energy available to be utilized in growth and reproduction. If SFG is low in a long-term survey of a particular species, unless this is directly related to an obvious environmental stimulus, it must be considered that the production (P) component of the energy equation is actually being utilized in production; either somatic growth or reproduction as appeared to be the case in this study, where SFG was frequently low, while at all times the animals were investing in reproduction. This highlights the importance of the long-term monitoring of populations in order to understand both the intrinsic and the extrinsic stressors acting upon species of conservation importance seasonally.

While *G. insensibilis* employs a continuous reproductive strategy, *I. chelipes* demonstrated that it was equally successful with a more seasonal strategy. However, there was an element of continuous reproduction as some brooding females were found in the winter months. This probably represents a situation where, in a small proportion of the population, an element of continuous reproduction persists because there is a selective advantage to those individuals under the conditions in which population bottlenecks or founder events may occur, both likely scenarios in the environmentally variable habitat of coastal lagoons.

It is unclear how specialist lagoon species colonize the lagoons in which they are found. There have been suggestions of rafting on mats of floating algae (characteristic of lagoon biota) or transport following the algae or individual animals becoming caught on the legs of wading birds while feeding (Barnes 1988). Whichever method, discussed or unknown, by which specialist lagoon species colonize new habitats, continuous rather than seasonal reproduction is an adaptation which would enable them to establish within the new site. The importance of colonization of new sites is highlighted by the transient nature of lagoon habitats. If the process involves transport on floating mats of algae or results from the feeding activities of wading birds it is important that lagoon management strategies allow for these mechanisms. For these processes to occur it is important to encourage the use of lagoons by birds and to ensure that in sluiced lagoons or those with a natural output into the sea that there is some exchange of water during which the algal mats may be transported from the lagoon.

In addition to the management of existing coastal lagoons, the creation of new sites is a method of conserving these habitats (Bamber et al. 1992; Bamber et al. 2000). This has been carried out by the RSPB at Freiston Shore in Lincolnshire, UK (RSPB 2004) but as yet lagoonal specialists have not been recorded at the site (*personal observation*). In order to enhance the development of the lagoon fauna it would appear beneficial to allow the development of the lagoon macrophytes such as *Chaetomorpha linum*. Whether this would be through the introduction of the algae, or management of the lagoon to encourage colonisation is an issue that would require discussion. The proximity of established lagoon sites would probably determine the likelihood of natural colonization by lagoon specialist biota, although further research is required to establish the mechanisms of dispersal of lagoon specialist biota. An important part of this research would involve the determination of how specific lagoon specialists are to lagoonal habitats. While it is clear that they are successful in lagoons it is likely that they are also able to survive in more typical marine conditions or other brackish habitats. Such, non-lagoonal populations may provide links between lagoon sites.

While the conservation of lagoons demands scientific study in its own right, there are also wider implications to their study. Monitoring habitats such as coastal lagoons can provide useful information for the global issue of climate change. Related issues such as rising sea levels will result in the destruction of current lagoon habitats, but also the possible creation of others, highlighting the importance of encouraging the establishment of new lagoon populations. Also, the study of the physiological effects of variable environments provides information on the kinds of organisms that may prosper under the more extreme habitats that may occur with the increased global temperatures. A study such as this one, using habitats such as saline lagoons in which organisms are subjected to extremes of temperature and salinity yet still prosper, can provide useful insights into the potential marine situation when shallow coastal seas are exposed to greater climate variability.

The effects of climate change on the biology of invertebrates are increasing in recognition as an important consequence of global changes. For example, the knock-on effects of climate change could have impacts on fecundity and recruitment, causing problems for over-wintering birds reliant on invertebrate populations as food sources (Lawrence and Soame 2004). The abundance of animals such as *G. insensibilis* and *I. chelipes* in lagoon habitats imply that the vast diversity of the Crustacea, not to mention the invertebrates as a whole, will certainly have the ability to adapt to changing future environments. It is unlikely that food sources for important migratory birds will be dramatically depleted. The likely result of climate change, in terms of invertebrate species, is that geographic distribution and ranges will change. Animals known from a more southerly distribution may extend further north as temperatures, ocean currents and prevailing conditions change.

An additional threat of climate change (in areas which would be subjected to warming of coastal waters) could be related to the temperature dependence of cercaria release by the initial molluscan hosts of trematode parasites. High temperatures are known to increase the rates of release of cercariae, resulting in increased infection rates, and potentially the mortality of various species that host the metacercarial stages of the trematodes. As a result of the increased mortality that occurs when amphipods are subjected to the physical damage associated the penetration of higher numbers of infections, it has been suggested that this could be a threat to intertidal amphipod populations as a direct result of global warming (Mouritsen et al. 2005; Poulin 2006). However, this is likely to be only associated with intertidal or lagoonal populations as few other systems would be likely to reach the temperatures necessary for these increased rates of release of cercariae.

The importance of parasites to the functioning of ecosystems has been recognized as an essential area of study. Sousa (1980) suggested that the study of soft sediment communities cannot be complete without consideration of parasites. The situation in the lagoon environment supports this argument because the interaction between *Gammarus insensibilis* and *Microphallus papillorobustus* is so important that the ecology of the amphipod cannot possibly be fully understood without serious consideration of the trematode.

This suggests that further research is vital in increase the understanding of the interaction. This extends to the predator-prey interactions within lagoon ecosystems. However, despite the previous discussion focussing on the detrimental effects of parasite infection, it may not necessarily be a totally negative influence on the amphipod population. It has been noted that predation by birds may have a controlling effect on the population of *Corophium volutator,* because when feeding, the birds select the larger amphipods. This was suggested to reduce the competition for the smaller individuals which were able to increase in density. When the predation factor was removed amphipod population densities actually reduced (Wilson and Parker 1996). As behavioural manipulation supposedly increases predations rates, and larger amphipods are more prone to infection by metacercariae, this could also be a controlling factor within the lagoon habitat, assisting in ensuring that both the dominant peracarids in the *C. linum* habitat are able to coexist.

Predation may also affect life history traits of amphipods. It has been shown for *C. volutator* populations that predictable, periodic predation events may alter reproductive factors such as size at maturity, the timing of reproductive events and size of broods because of a strong selection pressure against reproductive activity during the predation events (Hilton et al. 2002). Therefore, parasite-induced changes in predator-prey interactions are likely to have important effects on the reproductive biology of a species. An additional aspect of further interest in light of the work produced in this thesis would be to monitor the predation rates by wading birds to determine whether any of the variation in reproductive strategy, described and related to physico-chemical parameters in this thesis, is in fact the result of parasite effects leading to increased predation at certain times of the year.

Despite the advances in the understanding of the ecology of lagoon species made during this investigation, it can not be assumed that these rules apply for all lagoon specialists. While it is likely that the management schemes that favour *G. insensibilis* would be beneficial to all the fauna associated with *C. linum*, there are other species that utilize different ecological niches within lagoons. Similar studies, based on those organisms more commonly found within the sediment in lagoons, such as *Corophium insidiosum*, *Nematostella vectensis*, and *Armandia cirrhosa* are required. These species are likely to have alternative adaptations to the variable lagoon environment as a result of their different life styles.

In order to determine how lagoon specialists differ from their freshwater, estuarine or marine congeners, comparative studies are necessary. While there was some comparison with literature values for the various parameters measured in this project, it was not possible to maintain consistency of spatial or temporal factors. Detailed comparisons of individuals from a range of similar species (preferably congeners) from within lagoons, just outside the lagoon and further away may provide insights into the adaptations that allow an organism specialize in the lagoon habitat.

The examination of large samples of amphipods in this investigation indicated that there is some morphological variation within the species, particularly at the Lymington-Keyhaven lagoons. One of the diagnostic traits that distinguish *G. insensibilis* from *G. locusta* is the number of setae on the third epimeral plate. In *G. insensibilis* there is one seta while *G. locusta* has many. During the course of this study it became apparent that there were frequently examples of *G. insensibilis* with more than one seta on this epimeral plate. Although there has been a brief study of the taxonomic differences between closely related gammarids (Costa et al. 2004), the animals used were not from the UK. Lagoons are fragmented habitats and therefore genetic drift may be an important factor in the speciation processes that occur. It would be of value to carry out genetic studies, like the one of Costa et al. (2004) on the populations of *G. insensibilis* to determine whether the geographical isolation and differences in selection pressures between sites are driving speciation events. This may also infer how the populations in the various lagoons establish and provide information on the processes by which lagoon specialists colonize new habitats.

Finally it would be of interest to determine how the adaptations to the lagoon environment described in this thesis function. In terms of temperature and salinity stress it would be beneficial to examine the mechanisms that allow the survival of lagoons specialists in their habitat. The expression of heat shock proteins (hsp) is presumably of significance in the general cell maintenance. The question as to whether hsp expression and other stress response mechanisms are greater in lagoon systems than marine habitats seems vital to the understanding of how lagoon specialists respond to stress. This area of research is increasing in popularity and bioassays to monitor pollution effects based on hsp production have been developed (Werner et al. 1998). This may also provide insights into whether investment in stress resistance reduces the resource allocation to the control of pathogens, potentially explaining why *G. insensibilis* is so heavily infected by parasites.

The coastal lagoons of the UK are important habitats with an interesting ecology. They are of conservation value because, as a result of their rarity, the species are uncommon. In addition to the “applied” aspect of this study, lagoons also provide academic scientific interest for a number of reasons. Future work on lagoon systems should continue in building an understanding of the physiological adaptation to stressful habitats, but should also continue work on the functioning of fragmented habitats and their value as sites where the interactions between parasites and their hosts can be studied. Their continued protection is important, not just because of their value in terms of biodiversity but also because of their scientific importance.

Chapter 11

References

Aarset AV, Aunaas T (1990) Metabolic responses of the sympagic amphipods *Gammarus wilkitzkii* and *Onisimus glacialis* to acute temperature variations. Marine Biology 107: 433-438

Adams J, Greenwood PJ (1983) Why are males bigger than females in pre-copula pairs of *Gammarus pulex*. Behavioral Ecology and Sociobiology 13: 239-241

Alfaro J, Zuniga G, Komen J (2004) Induction of ovarian maturation and spawning by combined treatment of serotonin and a dopamine antagonist, spiperone in *Litopenaeus stylirostris* and *Litopenaeus vannamei*. Aquaculture 236: 511-522

Al-Suwailem AM (1991) The ecology of a saline lagoon in southern England. PhD. School of Ocean and Earth Sciences, Southampton

Bamber RN (2004) Temporal variation and monitoring of important lagoonal communities and species in Wales. Natural History Museum, 12, London

Bamber RN, Batten SD, Sheader M, Bridgewater ND (1992) On the ecology of brackish water lagoons in Great Britain. Aquatic Conservation: Marine and Freshwater Systems 2: 65-94

Bamber RN, Evans NJ, Chimonides PJ (2004) Saline lagoon survey, Moulton Marsh nature reserve. English Nature, ECM 635L/04

Bamber RN, Gilliland PM, Shardlow EA (2000) Saline Lagoons: A guide to their management and creation (interim version). Saline Lagoon Working Group

Barnes RSK (1980) Coastal Lagoons. The natural history of a neglected habitat. Cambridge University Press, Cambridge

Barnes RSK (1988) The faunas of land-locked lagoons: Chance differences and the problems of dispersal. Estuarine, Coastal and Shelf Science 26: 309-318

Barnes RSK (1991) Dilemmas in the theory and practice of biological conservation as exemplified by british coastal lagoons. Biological Conservation 55: 315-328

Barnes RSK (1994) Investment in eggs in lagoonal *Hydrobia ventrosa* and life-history strategies in north-west European *Hydrobia* species. Journal of the Marine Biological Association of the United Kingdom 74: 637-650

Barradas-Ortiz C, Briones-Fourzan P, Lozano-Alvarez E (2003) Seasonal reproduction and feeding ecology of giant isopods *Bathynomus giganteus* from the continental slope of the Yucatan peninsula. Deep-Sea Research Part I-Oceanographic Research Papers 50: 495-513

Bayne BL, Brown DA, Burns K, Dixon DR, Ivanovici A, Livingstone DR, Lowe DM, Moore MN, Stebbing ARD, Widdows J (1985) The effects of stress and pollution on marine animals. Praeger Special Studies, New York

Bird ECF (1994) Physical setting and geomorphology of coastal lagoons. In: Kjerfve B (ed) Coastal Lagoon Processes. Elsevier Science, Amsterdam, pp 9-39

Bolt SRL (1983) Haemolymph concentration and apparent permeability in varying salinity conditions of *Gammarus duebeni, Chaetogammarus marinus* and *Gammarus locusta*. Journal of Experimental Biology 107: 129-140

Bonga SEW (1997) The stress response in fish. Physiological Reviews 77: 591-625

Borowsky B (1980) Reproductive patterns of three intertidal salt-marsh gammaridean amphipods. Marine Biology 55: 327-334

Bowen SH, Lutz EV, Ahlgren MO (1995) Dietry protein and energy as determinants of food quality: trophic strategies compared. Ecology 76: 899-907

Brett JR (1958) Implications and assessments of environmental stress. In: Larkin PA (ed) Investigations of fish-power problems. University of British Columbia Press, Vancouver, pp 69-83

Brett JR (1971) Energetic Responses of Salmon to Temperature - Study of Some Thermal Relations in Physiology and Freshwater Ecology of Sockeye Salmon (*Oncorhynchus nerka*). American Zoologist 11: 99-&

Brown AF, Pascoe D (1989) Parasitism and host sensitivity to cadmium - an acanthocephalan infection of the freshwater amphipod *Gammarus pulex*. Journal of Applied Ecology 26: 473-487

Brown SP, De Lorgeril J, Joly C, Thomas F (2003) Field evidence for density-dependent effects in the trematode *Microphallus papillorobustus* in its manipulated host, *Gammarus insensibilis*. Journal of Parasitology 89: 668-672

Bulnheim H-P (1974) Respiratory metabolism of *Idotea balthica* (Crustacea, Isopoda) in relation to environmental variables, acclimation processes and moulting. Helgolander Meeresuntersuchungen 26: 464-480

Bulnheim H-P (1979) Comparative studies on the physiological ecology of five euryhaline *Gammarus* species. Oecologia 44: 80-86

Castanares AA, Phleger FB (1969) Coastal Lagoons: a symposium. Universidad Nacional Autonoma de Mexico

Chaparro OR, Thompson RJ (1998) Physiological energetics of brooding in Chilean oyster *Ostrea chilensis*. Marine Ecology-Progress Series 171: 151-163

Chapelle G, Peck LS (1995) The influence of acclimation and substratum on the metabolism of the Antarctic amphipods *Waldeckia obesa* (Chevreux 1905) and *Bovallia gigantea* (Pfeffer 1888). Polar Biology 15: 225-232

Charfi-cheikhrouha F (1996) *Idotea chelipes* (Pallas, 1966), espèce polytypique: description de ses sous-espèces (Isopodes, Valviferes). Crustaceana 69: 187-203

Chipps SR (1998) Temperature-dependent consumption and gut-residence time in the opossum shrimp *Mysis relicta*. Journal of Plankton Research 20: 2401-2411

Cimmaruta R, Scialanca F, Luccioli F, Nascetti G (2003) Genetic diversity and environmental stress in Italian populations of the cyprinodont fish *Aphanius fasciatus*. Oceanologica Acta 26: 101-110

Clarke A (1982) Temperature and embryonic development in polar marine invertebrates. International Journal of Invertebrate Reproduction 5: 71-82

Council of the European Communities (1992) Council directive 92/43/EEC of 21 May 1992 on conservation of natural habitats and of wild fauna and flora

Conover RJ (1966) Assimilation of organic material by zooplankton. Limnology and Oceanography 11: 338-345

Costa FO, Costa MH (1999) Life history of the amphipod *Gammarus locusta* in the Sado estuary (Portugal). Acta Oecologica 20: 305-314

Costa FO, Cunha MR, Neuparth T, Theodorakis CW, Costa MH, Shugart LR (2004) Application of RAPD DNA fingerprinting in taxonomic identification of amphipods: a case-study with *Gammarus* species (Crustacea: Amphipoda). Journal of the Marine Biological Association of the United Kingdom 84: 171-178

Costa FO, Neuparth T, Correia AD, Costa MH (2005) Multi-level assessment of chronic toxicity of estuarine sediments with the amphipod *Gammarus locusta*: II. Organism and population-level endpoints. Marine Environmental Research 60: 93-110

Crowe TP, Smith EL, Donkin P, Barnaby DL, Rowland SJ (2004) Measurements of sublethal effects on individual organisms indicate community-level impacts of pollution. Journal of Applied Ecology 41: 114-123

Cruz-Rivera E, Hay ME (2001) Macroalgal traits and the feeding and fitness of an herbivorous amphipod: the roles of selectivity, mixing, and compensation. Marine Ecology-Progress Series 218: 249-266

Curtis LA (1990) Parasitism and the movements of intertidal gastropod individuals. Biological Bulletin 179: 105-112

Dorey AE, Little C, Barnes RSK (1973) An ecological study of the Swanpool, Falmouth 2. Hydrography and its relation to animal distributions. Estuarine and Coastal Marine Science 1: 153-176

Dorgelo J (1977a) Comparative ecophysiology of gammarids (Crustacea: Amphipoda) from marine, brackish and fresh-water habitats exposed to the influence of salinity-temperature combinations. III. Oxygen uptake. Netherlands Journal of Sea Research 7: 253-266

Dorgelo J (1977b) Comparative Ecophysiology of Gammarids (Crustacea-Amphipoda) from Marine, Brackish-Water and Freshwater Habitats Exposed to Influence of Salinity Temperature Combinations .4. Blood Sodium Regulation. Netherlands Journal of Sea Research 11: 184-199

Dunn AM, Adams J, Smith JE (1993) Transovarial transmission and sex-ratio distortion by a microsporidian parasite in a shrimp. Journal of Invertebrate Pathology 61: 248-252

Einarson S (1993) Effects of temperature, seawater osmolality and season on oxygen consumption and osmoregulation of the amphipod *Gammarus oceanicus*. Marine Biology 117: 599-606

El Haj AJ, Houlihan DF (1987) *In vitro* and *in vivo* protein synthesis rates in a crustacean muscle during the moult cycle. Journal of Experimental Biology 1217: 413-426

Feder JH, Rossi JM, Solomon J, Solomon N, Lindquist S (1992) The Consequences of Expressing Hsp70 in Drosophila Cells at Normal Temperatures. Genes & Development 6: 1402-1413
Feder ME, Hofmann GE (1999) Heat-shock proteins, molecular chaperones, and the stress response: Evolutionary and ecological physiology. Annual Review of Physiology 61: 243-282

Ferreira SM, Jensen KT, Martins P, Sousa SF, Marques JC, Pardal MA (2005) Impact of microphallid trematodes on the survivorship, growth, and reproduction of an isopod (*Cyathura carinata*). Journal of Experimental Marine Biology and Ecology 318: 191-199

Florey E, Rathmayer M (1978) The effects of octopamine and other amines on the heart and neuromuscular transmission in decapod crustaceans: further evidence for a role as a neurohormone. Comparative Biochemistry and Physiology 61C

Forbes LS, Mock DW (1998) Parental optimism and progeny choice: When is screening for offspring quality affordable? Journal of Theoretical Biology 192: 3-14

Ford AT, Fernandes TF, Rider SA, Read PA, Robinson CD, Davies IM (2003a) Measuring sublethal imapacts of pollution on reproductive output of marine Crustacea. Marine Ecology - Progress Series 265: 303-309

Ford AT, Fernandes TF, Rider SA, Read PA, Robinson CD, Davies IM (2003b) Reproduction in the amphipod, *Echinogammarus marinus*: a comparison between normal and intersex specimens. Journal of the Marine Biological Association of the United Kingdom 83: 937-940

Fredensborg BL, Mouritsen KN, Poulin R (2004) Intensity-dependent mortality of *Paracalliope novizealandiae* (Amphipoda : Crustacea) infected by a trematode: experimental infections and field observations. Journal of Experimental Marine Biology and Ecology 311: 253-265

Galaktionov KV, Malkova II, Irwin SWB, Saville DH, Maguire JG (1996) Developmental changes in the tegument of four microphallid metacercariae in their second (crustacean) intermediate hosts. Journal of Helminthology 70: 201-210

Garnacho E, Peck LS, Tyler PA (2001) Effects of copper exposure on the metabolism of the mysid *Praunus flexuosus*. Journal of Experimental Marine Biology and Ecology 265: 181-201

Gilliland PM, Sanderson WG (2000) Re-evaluation of marine benthic species of nature conservation importance: a new perspective on certain 'lagoonal specialists' with particular emphasis on *Alkmaria romijni* Horst (Polychaeta : Ampharetidae). Aquatic Conservation-Marine and Freshwater Ecosystems 10: 1-12

Glazier DS (1991) Separating the respiration rates of embryos and brooding females of *Daphnia magna*: Implications for the cost of brooding and the allometry of metabolic rate. Limnology and Oceanography 36: 354-362

Glazier DS (2000) Smaller amphipod mothers show stronger trade-offs between offspring size and number. Ecology Letters 3: 142-149

Gonzalez-Pena MD, Moreira M (2003) Effect of dietary cellulose level on specific dynamic action and ammonia excretion of the prawn *Macrobrachium rosenbergii* (De Man 1879). Aquaculture Research 34: 821-827

Granado I, Caballero P (2001) Feeding rates of *Littorina striata* and *Osilinus atratus* in relation to nutritional quality and chemical defenses of seaweeds. Marine Biology 138: 1213-1224

Greze II (1972) Sur quelques rythmes du biologique des especes d'amphipodes de la Mer Noire presentant des populations nombreuses. Marine Biology 16: 75-80

Griffiths CL, King JA (1979) Some relationships between size, food availability and energy-balance in the ribbed mussel *Aulacomya ater*. Marine Biology 51: 141-149

Grime JP (1989) The stress debate: symptom of impending synthesis? Biological Journal of the Linnean Society 37: 3-17

Guerao G (2003) Some observations on the life history of the freshwater amphipod *Echinogammarus longisetosus* Pinkster, 1973 (Gammaridae) from Catalonia (Spain, N Iberian pensinsula). Animal Biodiversity and Conservation 26: 31-39

Hahn T (2005) Respiration rates in *Bithynia tentaculata* (L.) (Gastropoda : Bithyniidae) in response to acclimation temperature and acute temperature change. Journal of Molluscan Studies 71: 127-131

Hamilton WD, Zuk M (1982) Heritable true fitness and bright birds: a role for parasites. Science 218: 384-387

Haroon AM, Szaniawska A, Normant M, Janas U (2000) The biological composition of *Enteromorpha* spp. from the Gulf of Gdansk coast on the southern Baltic Sea. Oceanologica 42: 19-28

Harris R, Wiebe P, Lenz J, Skjoldal HR, Huntley M (2000) ICES zooplankton methodology manual. Elsevier Academic Press, San Diego, CA

Hatcher MJ, Dunn AM, Tofts C (2000) Co-existence of hosts and sex ratio distorters in structured populations. Evolutionary Ecology Research 2: 185-205

Hawkins AJS (1991) Protein-Turnover - a Functional Appraisal. Functional Ecology 5: 222-233

Hawkins AJS, Bayne BL (1984) Seasonal variation in the balance between physiological-mechanisms of feeding and digestion in *Mytilus edulis* (Bivalvia, Mollusca). Marine Biology 82: 233-240

Helluy S (1983) Relations hôtes-parasites du trématode *Microphallus papillorobustus* (Rankin 1940). II. Modifications du comportement des *Gammarus* hôtes intermediaires et localisation des métacercaires. Annales de Parasitologie Humaine et Comparee 58:1–17

Helluy S (1984) Relations hotes-parasites du trematode *Microphallus papillorobustus* (Rankin, 1940). III Facteurs impliques dans les modifications du comporttement des *Gammarus* hotes intermediaires et tests de predation. Annales de Parasitologie Humaine et Comparee 59: 41-56

Helluy S, Holmes JC (1990) Serotonin, octopamine and the clinging behaviour induced by the parasite *Polymorphus paradoxus* (Acanthocephala) in *Gammarus lacustris* (Crustacea). Canadian Journal of Zoology 68: 1214-1220

Helluy S, Thomas F (2003) Effects of *Microphallus papillorobustus* (Platyhelminthes : Trematoda) on serotonergic immunoreactivity and neuronal architecture in the brain of *Gammarus insensibilis* (Crustacea : Amphipoda). Proceedings of the Royal Society of London Series B-Biological Sciences 270: 563-568

Highsmith RC, Coyle KO (1991) Amphipod life histories - community structure, impact of temperature on decoupled growth and maturation rates, productivity, and P-B ratios. American Zoologist 31: 861-873

Hill D, Rushton SP, Clark N, Green P, Prysjones R (1993) Shorebird Communities on British Estuaries - Factors Affecting Community Composition. Journal of Applied Ecology 30: 220-234

Hilton C, Walde SJ, Leonard ML (2002) Intense episodic predation by shorebirds may influence life history strategy of an intertidal amphipod. Oikos 99: 368-376

Horlyck V (1973) The osmoregulartory ability in three species of the genus *Idotea* (Isopoda, Crustacea). Ophelia 12: 129-140

Horn MH, Mailhiot KF, Fris MB, McClanahan LL (1995) Growth, consumption, assimilation and excretion in the marine herbivorous fish *Cebidichthys violaceus* (Girard) fed natural and high protein diets. Journal of Experimental Marine Biology and Ecology 190: 97-108

Hornung E, Warburg MR (1994) Oosorption and oocyte loss in a terrestrial isopod under stressful conditions. Tissue & Cell 26: 277-284

Howes NH (1939) Observations on the biology and post-embryonic development of *Idotea viridis* (Slabber) (Isopoda, Valvifera) from New England Creek, South -East Essex. Journal of the Marine Biological Association of the United Kingdom 23: 279-310

Hynes HBN (1954) The ecology of *Gammarus duebeni* Lilljeborg and its occurrence in freshwater in western Britain. Journal of Animal Ecology 23: 38-84

Jazdzewski K (1973) Ecology of gammarids in the Bay of Puck. Oikos Supplement 15: 121-216

Jensen KT, Ferreira SM, Pardal MA (2004) Trematodes in a *Cyathura carinata* population from a temperate intertidal estuary: infection patterns and impact on host. Journal of the Marine Biological Association of the United Kingdom 84: 1151-1158

Jensen T, Jensen KT, Mouritsen KN (1998) The influence of the trematode *Microphallus claviformis* on two congeneric intermediate host species (*Corophium*): infection characteristics and host survival. Journal of Experimental Marine Biology and Ecology 227: 35-48

Johnson WS, Stevens M, Watling L (2001) Reproduction and development of marine peracaridans. In: Southward AJ, Tyler PA, Young CM, Fuiman LA (eds) Advances in Marine Biology. Academic Press, London

Jolly MT, Rogers AD, Sheader M (2003) Microgeographic genetic variation of populations of *Idotea chelipes* (Crustacea : Isopoda) in lagoons of the southern English coast. Cahiers De Biologie Marine 44: 319-327

Karakiri M, Nicolaidou A (1987) Population studies on the Amphipoda of Mazoma Lagoon (Greece). Helgolander Meeresuntersuchungen 41: 453-464

Kelly A, Dunn AM, Hatcher MJ (2001) Population dynamics of a vertically transmitted, parasitic sex ratio distorter and its amphipod host. Oikos 94: 392-402

Kevrekidis T (2004) Population dynamics, growth and reproduction of *Corophium insidiosum* (Crustacea : Amphipoda) at low salinities in Monolimni lagoon (Evros Delta, North Aegean Sea). Hydrobiologia 522: 117-132

Kinne O (1971) Marine ecology: a comprehensive, integrated treatise on life in oceans and coastal waters. Volume I. Environmental factors. Part 2. Wiley-Interscience, London

Kjerfve B (1994) Coastal lagoons. In: Kjerfve B (ed) Coastal Lagoon Processes. Elsevier Science, Amsterdam, pp 1-8

Koehn RK, Bayne BL (1989) Towards a physiological and genetic understanding of the energetics of the stress response. Biological Journal of the Linnean Society 37: 157-171

Kolding S, Fenchel TM (1981) Patterns of reproduction in different populations of five species of the amphipod genus *Gammarus*. Oikos 37: 167-172

Kostadinova A, Mavrodieva RS (2005a) Microphallids in *Gammarus insensibilis* Stock, 1966 from a Black Sea lagoon: manipulation hypothesis going East? Parasitology 131: 337-346

Kostadinova A, Mavrodieva RS (2005b) Microphallids in *Gammarus insensibilis* Stock, 1966 from a Black Sea lagoon: host response to infection. Parasitology 131: 347-354

Krebs RA, Feder ME (1997) Natural variation in the expression of the heat-shock protein Hsp70 in a population of *Drosophila melanogaster* and its correlation with tolerance of ecologically relevant thermal stress. Evolution 51: 173-179

Krebs RA, Loeschcke V (1994) Costs and Benefits of Activation of the Heat-Shock Response in *Drosophila melanogaster*. Functional Ecology 8: 730-737

Kristensen E (1983) Ventilation and oxygen uptake by three species of *Nereis* (Annelida: Polychaeta). II. Effects of temperature and salinity changes. Marine Ecology - Progress Series 12: 299-306

Kristensen TN, Dahlgaard J, Loeschcke V (2002) Inbreeding affects Hsp70 expression in two species of *Drosophila* even at benign temperatures. Evolutionary Ecology Research 4: 1209-1216

Kroer N (1989) Life cycle characteristics and reproductive patterns of *Idotea* spp. (Isopoda) in the Limfjord, Denmark. Ophelia 30: 63-74

Labourgh P-J (1971) Cycle reproducteur d'un Isopode des etange saumatres de la region d'Archachon: *Idotea viridis* (Slabber). Vie Millieu, suppl. 22: 155-166

Lacoste A, Jalabert F, Malham SK, Cueff A, Poulet SA (2001a) Stress and stress-induced neuroendocrine changes increase the susceptibility of juvenile oysters (*Crassostrea gigas*) to *Vibrio splendidus*. Applied and Environmental Microbiology 67: 2304-2309

Lacoste A, Malham SK, Cueff A, Poulet SA (2001b) Stress-induced catecholamine changes in the hemolymph of the oyster *Crassostrea gigas*. General and Comparative Endocrinology 122: 181-188

Lacoste A, Malham SK, Gelebart F, Cueff A, Poulet SA (2002) Stress-induced immune changes in the oyster *Crassostrea gigas*. Developmental and Comparative Immunology 26: 1-9

Lafferty KD (1996) Altered behaviour of parasitized killifish increased susceptibility to predation by bird final hosts. Ecology 77: 1390-1397

Lamare MD, Wing SR (2001) Calorific content of New Zealand marine macrophytes. New Zealand Journal of Marine and Freshwater Research 35: 335-341

Lapucki T, Normant M, Feike M, Graf G, Szaniawska A (2005) Comparative studies on the metabolic rate of the isopod *Idotea chelipes* (Pallas) inhabiting different regions of the Baltic Sea. Thermochimica Acta 435: 6-10

Larson BR, Vadas RL, Keser M (1980) Feeding and nutritional ecology of the sea urchin *Strongylocentrotus drobachiensis* in Maine, USA. Marine Biology 59: 49-62

Lawrence AJ, Soame JM (2004) The effects of climate change on the reproduction of coastal invertebrates. Ibis 146: 29-39

Lesser MP, Witman JD, Sebens KP (1994) Effects of flow and seston availability on scope for growth of benthic suspension-feeding invertebrates from the Gulf of Maine. Biological Bulletin 187: 319-335

Li JT, Lee PP, Chen OC, Cheng W, Kuo CM (2005) Dopamine depresses the immune ability and increases susceptibility to *Lactococcus garvieae* in the freshwater giant prawn, *Macrobrachium rosenbergii*. Fish & Shellfish Immunology 19: 269-280

Lignot JH, Spanings-Pierrot C, Charmantier G (2000) Osmoregulatory capacity as a tool in monitoring the physiological condition and the effect of stress in crustaceans. Aquaculture 191: 209-245

Lincoln RJ (1979) British marine Amphipoda: Gammaridea. British Museum (Natural History), London

Livingstone MS, Harris-Warrick RM, Kravitz EA (1980) Serotonin and octopamine produce opposite postures in lobsters. Science 208: 76-79

Loo LO (1992) Filtration, assimilation, respiration and growth of *Mytilus edulis* (L) at low temperatures. Ophelia 35: 123-131

Lotze HK, Worm B (2000) Variable and complementary effects of herbivores on different life stages of bloom-forming macroalgae. Marine Ecology-Progress Series 200: 167-175

Lozano, SJ, Gedeon, ML, Landrum, PF (2003) The effects of temperature and organism size on the feeding rate and modeled chemical accumulation in *Diporeia* spp. for Lake Michigan sediments. Journal of Great Lakes Research 29: 79-88

Lucas A (1996) Bioenergetics of Aquatic Animals. Taylor & Francis, London

Maltby L (1992) The use of the physiological energentics of *Gammarus pulex* to assess toxicity: a study using artificial streams. Environmental Toxicology and Chemistry 11: 79-85

Maltby L (1999) Studying stress: The importance of organism-level responses. Ecological Applications 9: 431-440

Maltby L, Naylor C (1990) Preliminary observations on the ecological relevance of the *Gammarus* 'scope for growth' assay: effect of zinc on reproduction. Functional Ecology 4: 393-397

Maltby L, Naylor C, Calow P (1990) Effect of stress on a freshwater benthic detritivore: Scope for growth in *Gammarus pulex*. Ecotoxicology and environmental safety 19: 285-291

Maranhao P, Bengala N, Pardal M, Marques JC (2001) The influence of environmental factors on the population dynamics, reproductive biology and productivity of *Echinogammarus marinus* Leach (Amphipoda, Gammaridae) in the Mondego estuary (Portugal). Acta Oecologica-International Journal of Ecology 22: 139-152

Maranhao P, Marques JC (2003) The influence of temperature and salinity on the duration of embryonic development, fecundity and growth of the amphipod *Echinogammarus marinus* Leach (Gammaridae). Acta Oecologica-International Journal of Ecology 24: 5-13

Marin-Guirao L, Cesar A, Marin A, Lloret J, Vita R (2005) Establishing the ecological quality status of soft-bottom mining-impacted coastal water bodies in the scope of the Water Framework Directive. Marine Pollution Bulletin 50: 374-387

Mathias JA (1971) Energy flow and secondary production of the amphipods *Hyalella azteca* and *Crangonyx richmondensis occidentalis* in Marion Lake, British Columbia. Journal of the Fisheries Research Board of Canada 28: 711726

Maynard BJ, DeMartini L, Wright WG (1996) *Gammarus lacustris* harboring *Polymorphus paradoxus* show altered patterns of serotonin-like immunoreativity. Journal of Parasitology 82: 663-666

McCabe J, Dunn AM (1994) Is resource partitioning among offspring a response to brood sex-ratio in an amphipod with environmental sex determination. Oikos 69: 203-206

McCahon CP, Maund SJ, Poulton MJ (1991) The effect of the acanthocephalan parasite (*Pomphorhynchus laevis*) on the drift of its intermediate host (*Gammarus pulex*). Freshwater Biology 25: 507-513

McCahon CP, Pascoe D (1988) Culture techniques for 3 freshwater macroinvertebrate species and their use in toxicity tests. Chemosphere 17: 2471-2480

McCahon CP, Poulton MJ (1991) Lethal and sublethal effects of acid, aluminum and lime on *Gammarus pulex* during repeated simulated episodes in a welsh stream. Freshwater Biology 25: 169-178

McHenry SC (2005) The impact of an acanthocephalan parasite *Polymorphus minutus* on aspects of the physiological ecology of the lagoonal amphipod *Gammarus duebeni* (Lilljeborg, 1881). MSc. School of Ocean and Earth Science, Southampton

McKenney CL (1996) The combined effects of salinity and temperature on various aspects of the reproductive biology of the estuarine mysid, *Mysidopsis bahia*. Invertebrate Reproduction & Development 29: 9-18

Meiβner K, Schaarschmidt T (2000) Ecophysiological studies of Corophium volutator (Amphipoda) infested by microphallid trematodes. Marine Ecology-Progress Series 202: 143-151

Mock DW, Forbes LS (1995) The Evolution of Parental Optimism. Trends in Ecology & Evolution 10: 130-134

Möller AP (1994) Sexual selection in the barn swallow. Oxford University Press, Oxford

Moreau X, Benzid D, De Jong-Brink L (2002) Evidence for the presence of serotonin in Mysidacea (Crustacea, Peracarida) as revealed by fluorescence immunohistochemistry. Cell Tissue Research 310: 359-371

Moss DR, Moss SM (2006) Effects of gender and size on feed acquisition in the Pacific white shrimp *Litopenaeus vannamei*. Journal of the World Aquaculture Society 37: 161-167

Mouritsen KN, Jensen KT (1997) Parasite transmission between soft-bottom invertebrates: Temperature mediated infection rates and mortality in *Corophium volutator*. Marine Ecology-Progress Series 151: 123-134

Mouritsen KN, Poulin R (2002) Parasitism, community structure and biodiversity in intertidal ecosystems. Parasitology 124: S101-S117

Mouritsen KN, Tompkins DM, Poulin R (2005) Climate warming may cause a parasite-induced collapse in coastal amphipod populations. Oecologia 146: 476-483

Naylor C, Maltby L, Calow P (1989) Scope for growth in *Gammarus pulex*, a freshwater benthic detritivore. Hydrobiologia 188/189: 517-523

Naylor C, Pindar L, Calow P (1990) Inter- and intraspecific variation in sensitivity to toxins: the effects of acidity and zinc on the freshwater crustaceans *Asellus aquaticus* (L.) and *Gammarus pulex* (L.). Water Research 24: 757-762

Naylor E (1955) The diet and feeding mechanism of *Idotea*. Journal of the Marine Biological Association of the United Kingdom 34: 347-355

Naylor E (1972) British marine isopods. Academic Press, London and New York

Nelson WG (1980) Reproductive patterns of gammaridean amphipods. Sarsia 65: 61-71

Normant M, Lapucki T, Schmolz E, Lamprecht I (2004) Direct calorimetric studies on the metabolic rate of *Gammarus oceanicus* from the brackish waters of the Baltic Sea. Thermochimica Acta 422: 49-54

Omori M, Ikeda T (1992) Methods in marine zooplankton ecology. Krieger Publishing Company, Malabar

Ottaviani E, Franceschi C (1996) The neuroimmunology of stress from invertebrates to man. Progress in Neurobiology 48: 421-440

Overli O, Pall M, Borg B, Jobling M, Winberg S (1982) Effects of *Shistocephalus solidus* infection on the brain monoaminergic activity in female three-spined sticklebacks *Gasterosteus aculeatus*. Proceedings of the Royal Society of London Series B - Biological Sciences 268: 1411-1415

Paine RT, Vadas RL (1969) Calorific values of benthic marine algae and their postulated relation to invertebrate food preference. Marine Biology 4: 79-86

Parsons TR, Maita Y, Lalli CM (1984) A manual of chemical and biological methods for seawater analysis. Pergamon Press, Oxford

Pearson C (2003) The genetic population structure of the lagoonal specialists *Nematostella vectensis, Cerastoderma glaucum* and *Gammarus insensibilis* from populations along the southern and eastern coasts of the United Kingdom. School of Ocean and Earth Sciences, Southampton

Pearson CVM, Rogers AD, Sheader M (2002) The genetic structure of the rare lagoonal sea anemone, *Nematostella vectensis* Stephenson (Cnidaria; Anthozoa) in the United Kingdom based on RAPD analysis. Molecular Ecology 11: 2285-2293

Peck LS, Chapelle G (2003) Reduced oxygen at high altitude limits maximum size. Proceedings of the Royal Society of London Series B-Biological Sciences 270: S166-S167

Ponton F, Biron DG, Joly C, Helluy S, Duneau D, Thomas F (2005) Ecology of parasitically modified populations: a case study from a gammarid-trematode system. Marine Ecology - Progress Series 299: 205-215

Porter JS, Dyrynda PEJ, Ryland JS, Carvalho GR (2001) Morphological and genetic adaptation to a lagoon environment: a case study in the bryozoan genus *Alcyonidium*. Marine Biology 139: 575-585

Poulin R (1994) The evolution of parasite manipulation of host behaviour - a theoretical analysis. Parasitology 109: S109-S118

Poulin R (2006) Global warming and temperature-mediated increases in cercarial emergence in trematode parasites. Parasitology 132: 143-151

Poulin R, Mouritsen KN (2004) Small-scale spatial variation in rates of metacercarial accumulation by a bivalve second intermediate host. Journal of the Marine Biological Association of the United Kingdom 84: 1209-1212

Randall D, Burggren W, French K (1997) Eckert Animal Physiology: mechanisms and adaptations. W. H. Freeman and company, New York

Rebecq J (1964) Recherches systématiques, biologiques et écologiques sur les formes larvaires de quelques Trémato des de Camargue. PhD thesis Faculté de Sciences de Marseille

Roast SD, Widdows J, Jones MB (1999a) Respiratory responses of the estuarine mysid *Neomysis integer* (Peracarida: Mysidacea) in relation to a variable environment. Marine Biology 133: 643-649

Roast SD, Widdows J, Jones MB (1999b) Scope for growth of the estuarine mysid *Neomysis integer* (Peracarida: Mysidacea): effects of the organophosphate pesticide chlorpyrifos. Marine Ecology - Progress Series 191: 233-241

Roast SD, Widdows J, Jones MB (2000) Egestion rates of the estuarine mysid *Neomysis integer* (Peracarida: Mysidacea) in relation to a variable environment. Journal of Experimental Marine Biology and Ecology 245: 69-81

Robertson RF, El-Haj AJ, Clarke A, Taylor EW (2001) Effects of temperature on specific dynamic action and protein synthesis rates in the Baltic isopod crustacean, *Saduria entomon*. Journal of Experimental Marine Biology and Ecology 262: 113-129

RSPB (2004) Freiston Shore, The Wash, Lincolnshire. Draft case study, prepared on behalf of the RSPB

Rueda JL, Smaal AC (2004) Variation of the physiological energetics of the bivalve *Spisula subtruncata* (da Costa, 1778) within an annual cycle. Journal of Experimental Marine Biology and Ecology 301: 141-157

Rumpus AE, Kennedy CR (1974) Effect of acanthocephalan *Pomphorhynchus laevis* upon respiration of its intermediate host, *Gammarus pulex*. Parasitology 68: 271-284

Saintemarie B (1991) A review of the reproductive bionomics of aquatic gammaridean amphipods - variation of life-history traits with latitude, depth, salinity and Superfamily. Hydrobiologia 223: 189-227

Salemaa H (1986) Breeding biology and microhabitat utilization of the intertidal isopod *Idotea granulosa* Rathke, in the Irish Sea. Estuarine, Coastal and Shelf Science 22: 335-355

Salomon M, Buchholz F (2000) Effects of temperature on the respiration rates and the kinetics of citrate synthasein two species of *Idotea* (Isopoda, Crustacea). Comparitive Biochemistry and Physiology Part B. 125: 71-81

Schill RO, Gorlitz H, Kohler HR (2003) Laboratory simulation of a mining accident: acute toxicity, hsc/hsp70 response, and recovery from stress in *Gammarus fossarum* (Crustacea, Amphipoda) exposed to a pulse of cadmium. Biometals 16: 391-401

Schlekat CE, Scott KJ, Swartz RC, Albrecht B, Antrim L, Doe K, Douglas S, Ferretti JA, Hansen DJ, Moore DW, Mueller C, Tang A (1995) Interlaboratory comparison of a 10-day sediment toxicity test method using *Ampelisca abdita*, *Eohaustorius estuarius* and *Leptocheirus plumulosus*. Environmental Toxicology and Chemistry 14: 2163-2174

Schneider JE (2004) Energy balance and reproduction. Physiology & Behavior 81: 289-317

Schwartz SS, Cameron GN (1993) How do parasites cost their hosts? Preliminary answers from trematodes and *Daphnia obtusa*. Limnology and Oceanography 38: 602-612

Schuwerack, PMM, Barnes, RSK, Underwood, GJC, Jones, PW (2006) Gender and species differences in sentinal crabs (*Macrophthalmus*) feeding on an Indonesian mudflat. Journal of Crustacean Biology 26: 119-123

Sexton EW (1924) The moulting and growth-stages of *Gammarus*, with descriptions of the normals and intersexes of *G. chevreuxi*. Journal of the Marine Biological Association of the United Kingdom 13: 340-401

Sheader M (1977) The breeding biology of *Idotea pelagica* (Isopoda: Valvifera) with notes on the occurrence and biology of its parasite *Clypeoniscus hanseni* (Isopoda: Epicaridea). Journal of the Marine Biological Association of the United Kingdom 57: 659-674

Sheader M (1983) The reproductive biology and ecology of *Gammarus duebeni* (Crustacea: Amphipoda) in southern England. Journal of the Marine Biological Association of the United Kingdom 63: 517-540

Sheader M (1996) Factors influencing egg size in the gammarid amphipod *Gammarus insensibilis*. Marine Biology 124: 519-536

Sheader M, Chia F-S (1970) Development, fecundity and brooding behaviour of the amphipod, *Marinogammarus obtusatus*. Journal of the Marine Biological Association of the United Kingdom 50: 1079-1099

Sheader M, Sheader AL (1985) New distribution records for *Gammarus insensibilis* Stock, 1966, in Britain. Crustaceana 49: 101-105

Sheader M, Sheader AL (1987) The distribution of the lagoonal amphipod *Gammarus insensibilis* Stock in England. Porcupine Newsletter 3: 220-223

Shumway SE, Youngson A (1979) Effects of fluctuating salinity on the physiology of *Modiolus demissus* (Dillwyn). Journal of Experimental Marine Biology and Ecology 40: 167-181

Sibly RM, Calow P (1989) A life-cycle theory of responses to stress. Biological Journal of the Linnean Society 37: 101-116

Silbermann R, Tatar M (2000) Reproductive costs of heat shock protein in transgenic *Drosophila melanogaster*. Evolution 54: 2038-2045

Skadsheim A (1984) Coexistance and reproductive adaptations of amphipods: the role of environmental heterogeneity. Oikos 43: 94-103

Smaal AC, Vonck A, Bakker M (1997) Seasonal variation in physiological energetics of *Mytilus edulis* and *Cerastoderma edule* of different size classes. Journal of the Marine Biological Association of the United Kingdom 77: 817-838

Smythe JD (1994) Introduction to animal parasitology. Cambridge University Press, Cambridge

Solorzano L (1969) Determination of ammonia in natural waters by the phenolhypochlorite method. Limnology and Oceanography 14: 799-801

Sorensen JG, Kristensen TN, Loeschcke V (2003) The evolutionary and ecological role of heat shock proteins. Ecology Letters 6: 1025-1037

Sorouraddin MH, Manzoori JL, Kargarzadeh E, Shabani AMH (1998) Spectrophotometric determination of some catecholamine drugs using sodium bismuthate. Journal of Pharmaceutical and Biomedical Analysis 18: 877-881

Sousa WP (1991) Can models of soft-sediment community structure be complete without parasites. American Zoologist 31: 821-830

Spanopoulos-Hernandez M, Martinez-Palacios CA, Vanegas-Perez RC, Rosas C, Ross LG (2005) The combined effects of salinity and temperature on the oxygen consumption of juvenile shrimps *Litopenaeus stylirostris* (Stimpson, 1874). Aquaculture 244: 341-348

Spicer JI, Gaston KJ, (1999) Physiological diversity and its ecological implications. Blackwell, Oxford

Stearns SC (1992) The Evolution of Life History Strategies. Oxford University Press, Oxford

Stebbing ARD (1981) Stress Health and Homeostasis. Marine Pollution Bulletin 12: 326-329

Steele DH (1991) Is the oöstegite structure of amphipods determined by their phylogeny or is it an adaptation to their environment. Hydrobiologia 223: 27-34

Steele DH, Steele VJ (1969) The biology of Gammarus (Crustacea, Amphipoda) in the northwest Atlantic. I. *Gammarus duebeni* Lillj. Canadian Journal of Zoology 47: 235-244

Steele DH, Steele VJ (1975) The biology of *Gammarus* (Crustacea, Amphipoda) in the northwestern Atlantic. XI. Comparison and discussion. Canadian Journal of Zoology 53: 1116-1126

Steele DH, Steele VJ (1991) Effects of salinity on the survival, growth rate, and reproduction output of *Gammarus lawrencianus* (Crustacea, Amphipoda). Marine Ecology - Progress Series 78: 49-56

Steele DH, Whittick A (1991) Seasonal variation in *Pilayella littoralis* (Phaeophyceae) and it consequences as a food source for the amphipod, *Gammarus lawrencianus*, in the intertidal of Newfoundland. Journal of the Marine Biological Association of the United Kingdom 71: 883-889

Stickle WB, Sabourin TD (1979) Effects of salinity on the respiration and heart-rate of the common mussel, *Mytilus edulis* (L), and the black chiton, *Katherina tunicata* (Wood). Journal of Experimental Marine Biology and Ecology 41: 257-268

Svetlichny LS, Hubareva ES (2005) The energetics of *Calanus euxinus*: locomotion, filtration of food and specific dynamic action. Journal of Plankton Research 27: 671-682

Tait RV, Dipper FA (1998) Elements of marine ecology. Butterworth-Heinemann, Oxford

Tedengren M, Arner M, Kautsky N (1988) Ecophysiology and stress response of marine and brackish water *Gammarus* species (Crustacea, Amphipoda) to changes in salinity and exposure to cadmium and diesel-oil. Marine Ecology - Progress Series 47: 107-116

Thomas F, Adamo S, Moore J (2005a) Parasitic manipulation: where are we and where should we go? Behavioural Processes 68: 185-199

Thomas F, Crivelli A, Cezilly F, Renaud F, deMeeus T (1997) Parasitism and ecology of wetlands: A review. Estuaries 20: 646-654

Thomas F, Guldner E, Renaud F (2000) Differential parasite (Trematoda) encapsulation in *Gammarus aequicauda* (Amphipoda). Journal of Parasitology 86: 650-654

Thomas F, Poulin R, de Meeus T (1999) Parasites and ecosystem engineering: what roles could they play? Oikos 84: 167-171

Thomas F, Renaud F (2001) *Microphallus papillorobustus* (Trematoda): A review of its effects in lagoon ecosystems. Revue D Ecologie-La Terre Et La Vie 56: 147-156

Thomas F, Renaud F, Cezilly F (1996a) Assortative pairing by parasitic prevalence in *Gammarus insensibilis* (Amphipoda): Patterns and processes. Animal Behaviour 52: 683-690

Thomas F, Renaud F, de Meeus T, Poulin R (1998a) Manipulation of host behaviour by parasites: ecosystem engineering in the intertidal zone? Proceedings of the Royal Society of London Series B-Biological Sciences 265: 1091-1096

Thomas F, Renaud F, Derothe JM, Lambert A, Meeüs T, Cézilly F (1995) Assortative pairing in *Gammarus insensibilis* (Amphipoda) infected by a trematode parasite. Oecologia 104: 259-264

Thomas F, Renaud F, Guégan J-F (2005b) Parasitism and Ecosystems. Oxford University Press

Thomas F, Verneau O, Santalla F, Cezilly F, Renaud F (1996b) The influence of intensity of infection by a trematode parasite on the reproductive biology of *Gammarus insensibilis* (Amphipoda). International Journal for Parasitology 26: 1205-1209

Thomas F, Villa M, Montoliu I, Santalla F, Cezilly F, Renaud F (1998b) Analyses of a debilitating parasite (*Microphallus papillorobustus*, Trematoda) and its "hitchhiker" parasite (*Maritrema subdolum*, Trematoda) on survival of their intermediate host (*Gammarus insensibilis*, Amphipoda). Journal of the Helminthological Society of Washington 65: 1-5

Thompson RJ, Newell RIE (1985) Physiological responses to temperature in two latitudinally separated populations of the musssel, *Mytilus edulis* 19th European Marine Biology Symposium, pp 481-495

Van Dolah RF, Bird E (1980) A comparison of reproductive patterns in epifaunal and infaunal gammaridean amphipods. Estuarine and Coastal Marine Science 11: 593-604

Verslycke T, Roast SD, Widdows J, Jones MB, Janssen CR (2004) Cellular energy allocation and scope for growth in the estuarine mysid *Neomysis integer* (Crustacea : Mysidacea) following chlorpyrifos exposure: a method comparison. Journal of Experimental Marine Biology and Ecology 306: 1-16

Vlasblom AG (1969) A study of a population of *Marinogammarus marinus* (Leach) in the Oosterschelde. Netherlands Journal of Sea Research 4: 317-338

Warren GE, Davis GE (1967) Laboratory studies on the feeding, bioenergetics and growth of fish. In: Gerking SD (ed) The biological basis of freshwater fish production. Blackwell Scientific Publications, Oxford, pp 175-214

Watt PJ (1994a) Parental control of sex ratio in *Gammarus duebeni*, an organism with environmental sex determination. Journal of Evolutionary Biology 7: 177-187

Watt PJ, Adams J (1993a) Adaptive sex determination and population dynamics in a brackish water amphipod. Estuarine Coastal and Shelf Science 37: 237-250

Watt PJ, Adams J (1994) Adaptive variation in sex determination in a crustacean, *Gammarus duebeni*. Journal of Zoology 232: 109-116

Weiger WA (1997) Serotonergic modulation of behaviour: A phylogenetic overview. Biological Reviews 72: 61-96

Werner I, Kline KF, Hollibaugh JT (1998) Stress protein expression in *Ampelisca abdita* (Amphipoda) exposed to sediments from San Francisco Bay. Marine Environmental Research 45: 417-430

Wheeler JC (1999) Sequence requirements for upregulated expression of *Drosophila hsp70* transgenes during aging. Neurobiology of Aging 20: 545-553

Whiteley NM, Robertson RF, Meagor J, El Haj AJ, Taylor EW (2001) Protein synthesis and specific dynamic action in crustaceans: effects of temperature. Comparative Biochemistry and Physiology a-Molecular & Integrative Physiology 128: 595-606

Whiteley NM, Taylor EW, El Haj AJ (1997) Seasonal and latitudinal adaptation to temperature in crustaceans. Journal of Thermal Biology 22: 419-427

Widdows J (1978a) Combined effects of body size, food concentration, and season on the physiology of *Mytilus edulis*. Journal of the Marine Biological Association of the United Kingdom 58: 109-124

Widdows J (1978b) Physiological indices of stress in *Mytilus edulis*. Journal of the Marine Biological Association of the United Kingdom 58: 125-142

Widdows J (1993) Marine and Estuarine Invertebrate Toxicity Tests. In: Calow P (ed) Handbook of ecotoxicology. Blackwell Scientific, Oxford, pp 145-166

Widdows J, Bayne BL (1971) Temperature acclimation of *Mytilus edulis* with reference to its energy budget. Journal of the Marine Biological Association of the United Kingdom 51: 827-843

Widdows J, Donkin P, Brinsley MD, Evans SV, Salked PN, Franklin A, Law RJ, Waldock MJ (1995) Scope for growth and contaminant levels in North Sea mussels *Mytilus edulis*. Marine Ecology - Progress Series 127: 131-148

Widdows J, Donkin P, Staff FJ, Matthiessen P, Law RJ, Allen YT, Thain JE, Allchin CR, Jones BR (2002) Measurement of stress effects (scope for growth) and contaminant levels in mussels (*Mytilus edulis*) colllected from the Irish Sea. Marine Environmental Research 53: 327-356

Widdows J, Fieth P, Worrall CM (1979) Relationships between seston, available food and feeding activity in the common mussel *Mytilus edulis*. Marine Biology 50

Wilhelm FM, Schindler DW (2000) Reproductive strategies of *Gammarus lacustris* (Crustacea: Amphipoda) along an elevation gradient. Functional Ecology 14: 413-422

Williams TD (1994) Intraspecific variation in egg size and egg composition in birds - effects on offspring fitness. Biological Reviews of the Cambridge Philosophical Society 69: 35-59

Wilson JG, Elkaim B (1997) Seasonal and geographical differences in oxygen consumption with temperature of *Cerastoderma glaucum* (Poiret) and a comparison with *C. edule* (L.). Estuarine Coastal and Shelf Science 45: 571-577

Wilson WH, Parker K (1996) The life history of the amphipod, *Corophium volutator*: The effects of temperature and shorebird predation. Journal of Experimental Marine Biology and Ecology 196: 239-250

Winberg GG (1960) Rate of metabolism and food requirements of fishes. Fisheries Research Board of Canada Translated Series 194: 1-253

Wu RSS, Or YY (2005) Bioenergetics, growth and reproduction of amphipods are affected by moderately low oxygen regimes. Marine Ecology-Progress Series 297: 215-223

Yamada Y, Ikeda T (2003) Metabolism and chemical composition of four pelagic amphipods in the Oyashio region, western subarctic Pacific Ocean. Marine Ecology-Progress Series 253: 233-241

Zohar S, Holmes JC (1998) Pairing success of male *Gammarus lacustris* infected by two acanthocephalans: A comparative study. Behavioral Ecology 9: 206-211