# Nutritional limitation in populations of the Norway lobster, *Nephrops norvegicus* (L.) in the Firth of Clyde, Scotland.

by

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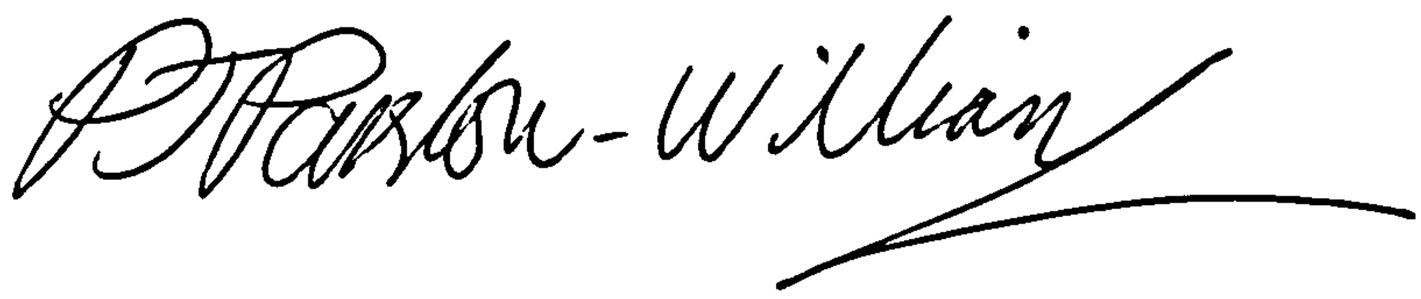
This thesis is dedicated to my parents and brothers.

# DECLARATION

I declare that the work reported in this thesis had been carried out by myself unless otherwise acknowledged. It is entirely of my own composition and has not, in whole or part, been submitted for any other degree.



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## ABSTRACT

Fishery-based research on the Norway lobster, *Nephrops norvegicus* (L.) has shown the existence of geographical variability in the size of the individual animals that comprise populations, and such differences in size composition can occur over relatively small geographical distances. This 'stocklet phenomenon' has been noted in *N. norvegicus* grounds in the Clyde Sea area, the Irish Sea, the Mediterranean Sea and elsewhere. The size disparity is usually associated with differences in density, since stocklets of *N. norvegicus* characterised by a small average size of individuals often live at high densities, whereas those which occur at lower densities have a larger mean animal size. There are also marked differences in other biological parameters, such as the size at which animals reach maturity. This thesis investigated methods of assessing these stocklet differences using both ecological and physiological methods, with the aim to provide insight into some of the underlying causes. Two stocklets were compared throughout the course of the study. The first is located south of Lesser Cumbrae, in the northern Clyde Sea area, where *N. norvegicus* are exposed to favourable conditions for growth, since they are sparsely populated on organically rich sediments. The other stocklet is found in the southern Clyde Sea area, south of Ailsa Craig, where *N. norvegicus* occur in high densities on a comparatively impoverished sediment.

Initially, the thesis investigated the ecological energetics of *N. norvegicus* in order to construct an energy budget which could be used to estimate scope for growth. The diet of the Norway lobster *Nephrops norvegicus* in the Firth of Clyde was found to

consist of crustaceans, molluscs, and to a lesser extent polychaetes and echinoderms. The proportion of bivalves was higher in the diet of *N. norvegicus* from south of Little Cumbrae than in the diet of *N. norvegicus* from south of Ailsa Craig which contained larger proportions of crustaceans and echinoderms. This appears to reflect the faunal composition of the areas. Despite a number of measures taken to preserve stomach contents, a large proportion of the diet was found to be macerated beyond recognition, particularly in the stomachs of animals from the south of Ailsa Craig stocklet. There were marked interspecific differences in the energy contents and organic contents of prey organisms. The calorific values of the prey items were found to vary according to the proportion of organic matter in the tissue. Organisms with low organic contents such as brittle stars had lower calorific values than organisms with a large organic proportion such as soft-bodied polychaetes.

Amounts of food consumption were calculated in 4h intervals over a 24h period. Results suggested that foraging is highest during the night at 2200-0200. A smaller increase in food consumption was also noticed during the morning at 0600-1000 and 1000-1400. The lowest amount of food was consumed between 1400-1800. Two different models were used to estimate the daily amounts of food consumption. The values obtained ranged from 476.5mg $day^{-1}$ (wet weight) at Ailsa Craig in November to 1206.2 mg $day^{-1}$ (wet weight) at Little Cumbrae in May.

The rates of weight specific oxygen consumption ($MO_2$) were determined for quiescent male and female *N. norvegicus*. No significant differences were detected between the sexes. In addition, the rates of active $MO_2$ were measured and found to

be significantly higher than standard $MO_2$. The scope for activity was found to be approximately four times basal metabolism. The recent feeding history was observed to have a marked effect on metabolic rate. The $MO_2$ significantly decreased following 8 and 12 weeks of starvation, and rapidly increased again after re-feeding. No significant differences in $MO_2$ were detected between *N. norvegicus* from the Little Cumbrae and Ailsa Craig sites.

An energy budget was constructed for Clyde Sea *N. norvegicus*. The scope for growth was significantly greater for animals in May than November. No significant differences in scope for growth were found between animals from the Little Cumbrae and Ailsa Craig sites. An additional energy budget was assembled to model *N. norvegicus* suspension-feeding. The model predicted that considerable energy is conserved due mainly to savings from operating at a lower level of activity.

As a result of the difficulties experienced with the application of theoretical energetics to animals in the field, it was decided to base the remainder of the thesis on biochemical indicators of nutritional condition. Rates of protein synthesis of the abdominal muscle tissue were calculated for *N. norvegicus* from the Little Cumbrae and Ailsa Craig stocklets. No significant differences in the rate of protein synthesis were found between animals from the different sites. Accurate methods were developed for the laboratory determination of RNA, DNA and protein concentration as indicators of nutritional condition. The effects of starvation on the nucleic acid content of three tissues were examined (abdominal muscle, gill and hepatopancreas). Significant reductions were observed in the RNA concentration, RNA:Protein ratio

and RNA:DNA ratio of abdominal muscle tissue of starved *N. norvegicus*. No significant changes were found in the other tissues examined, with the exception of a decrease in hepatopancreas DNA content. It was thus concluded that abdominal muscle tissue was the most suitable for assessment of nutritional condition.

Samples of abdominal muscle tissue were taken from lobsters from two sites in the Firth of Clyde, south of Little Cumbrae and south of Ailsa Craig. Evidence of nutritional limitation was provided by nucleic acid analysis which showed that the RNA concentration, RNA:Protein ratio and RNA:DNA ratio of lobsters from south of Ailsa Craig were significantly lower than lobsters of equivalent size from south of Little Cumbrae. Significant negative relationships were found with nucleic acid indices and animal weight. The correlation was strongest for RNA concentration expressed per unit dry weight and weakest for the RNA:DNA ratio. It was demonstrated that the differences in nucleic acid concentrations between the sites could be reversed by changing their feeding conditions. When starved, the RNA concentrations of lobsters from south of Little Cumbrae decreased to levels similar to those normally expected for lobsters south of Ailsa Craig. Furthermore, when lobsters from south of Ailsa Craig were fed to capacity, the abdominal muscle RNA concentrations reached levels found in lobsters from south of Little Cumbrae. The length of time taken for the Ailsa Craig lobsters to increase their nucleic acid contents to a level similar to that in Little Cumbrae lobsters may reflect the magnitude of the nutritional differences between these stocklets. The differences are clearly substantial since it required 4 weeks of continuous feeding on a high quality diet before they obtained a nutritional condition similar to Little Cumbrae animals.

Significant seasonal influences were noted on the biochemical condition of *N. norvegicus*. The hepatosomatic index decreased during the winter, presumably in response to a lack of feeding, whereas the RNA concentration increased during this time. The results of laboratory experiments on the thermal responses of RNA concentration suggested that this could be attributed to thermocompensation to the lower winter temperatures. Lobsters exposed to 5°C maintained significantly higher RNA concentrations than those exposed to 10°C and 15°C.

The *Hematodinium* sp. infection was found to have a considerable effect on the metabolism of *N. norvegicus*. The resting oxygen consumption rates ($MO_2$) of infected animals were significantly higher than uninfected animals. The $MO_2$ of quiescent lobsters with the heaviest parasite burdens (stages III/IV) was not significantly different from the $MO_2$ of uninfected lobsters which had been induced to become active. Further evidence of the physiological burden of infections was provided by measurements of abdominal muscle RNA concentration. The RNA concentrations of heavily infected lobsters (stages II-IV) were significantly higher than uninfected and moderately infected (stage I) lobsters. The dinoflagellate infection has no observable effect on *N. norvegicus* feeding in the field. Comparisons of stomach content weights between infected and uninfected lobsters failed to find any significant differences at any of the sampling times.

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# CHAPTER 1

# General Introduction

## 1.1. The *Nephrops norvegicus* (L.) fishery

The Norway lobster, *Nephrops norvegicus* (Linnaeus, 1758) is a decapod crustacean that is found in east Atlantic waters from Iceland to the Canary Islands, and in the Mediterranean (Chapman, 1980, Barquín *et al.*, 1998). It belongs to the family of clawed lobsters, Nephropidae, which also includes the American lobster, *Homarus americanus* and the European lobster, *H. gammarus*. In this thesis, the species is sometimes referred to by genus alone, particularly in the context of the fishery, following normal practice. *Nephrops norvegicus* is known by many other names throughout its range although these terms are often more gastronomic than taxonomic and are frequently confused with other Crustacea. An expression in common use around the British Isles, is Dublin-Bay prawn, usually shortened to 'prawn', which originates from the heavily exploited Irish-Sea stock west of the Isle of Man (Briggs, 1997). Additional names for *N. norvegicus* include langoustine (France, Morocco), langostino (Spain), lagostim (Portugal), sjøkreps (Norway), havskräfta (Sweden), Kaisergranat (Germany), Noorse kreeft (Netherlands), ksampu (Malta), scampi (UK), scampo (Italy), skamp (Yugoslavia), and astakos (Cyprus) (Farmer, 1973; Williams, 1988). The generic name of *Nephrops*, is derived from the characteristic 'kidney-shaped' eyes (see Plate 1).

**Plate 1. The Norway lobster, *Nephrops norvegicus* (L.) emerging from burrow in Loch Sween (photograph courtesy of C. Lumb, English Nature).**

The occurrence of *N. norvegicus* has been reported from depths of 5-800m (Atkinson, 1987; Briggs, 1997), although the fishery is primarily between depths of 14-500m (Chapman, 1980). In the Clyde Sea area the species occurs at depths of between 14 and 230m (Allen, 1967; Bailey *et al.*, 1986). Their distribution is limited to muddy sediments which are suitable for the construction of burrows. These range from muddy sands to soft clay-dominated muds. The patchy distribution of *N. norvegicus* reflects the distribution of sediment and this association has been found useful when designing stock assessment surveys by trawling and underwater television (Bailey *et al.*, 1993). Grounds which vary in their physical characteristics may support discrete sub-populations or 'stocklets' of *N. norvegicus*, which have differing size compositions and density that may vary dramatically (Chapman & Bailey, 1987). More is said on this topic later. *N. norvegicus* may make local migrations [e.g. into deeper water in the winter in some shallow sea loch locations (Marrs *et al.*, 1996)], but results from tagging experiments have shown that adult *N. norvegicus* do not migrate long distances (Chapman, 1980, Chapman & Bailey, 1987). Thus, once they have settled from the plankton and established burrows, they are unlikely to move far from that location, resulting in little exchange of adults between stocklets (Chapman & Bailey, 1987).

*N. norvegicus* has been exploited as a sea food in some parts of Europe for over 200 years (Olivi, 1792 cited in IMBC, UMBSM & IRPEM, 1994). In the Northern Adriatic, *N. norvegicus* were traditionally caught using a bottom trawl (coccia) which was towed by a pair of sail-boats (bragozzi). These vessels operated on grounds in the Gulf of Fiume (Croatian Rijeka) and their annual landing in the local fish-markets

could be as much as 30 metric tonnes (Faber, 1883 cited in IMBC, UMBSM & IRPEM, 1994). In contrast, up until comparatively recently, fisherman working in the Irish Sea regarded *N. norvegicus* as a nuisance and discarded large numbers of them from their trawls, complaining that they blocked their nets (Briggs, 1997). In Scottish waters the situation was similar, Bagenal (1952) commented that, "although there is not a definite fishery for prawns in the Clyde, man is a potential enemy. At present *N. norvegicus* are caught incidentally by fishing boats and in all probability are eaten on board". Nowadays, it is a different story, since there has been a marked increase in their commercial exploitation (see Figure 1.1). A total of 32,000 tonnes of *N. norvegicus* were landed by the UK fleet in 1995 (MAFF, 1995), from grounds in the West of Scotland, North Sea and Irish Sea, thus making *N. norvegicus* the most commercially valuable shellfish landed in the UK by a significant margin. Landings in the UK in 1995 were valued at £63.1 million, compared with £27.9 million for crabs and £24.3 million for scallops. Approximately three-quarters of the *N. norvegicus* caught around the UK coastline are landed in Scottish ports. In 1995 the commercial value of Scottish landings alone was worth £47.7 million (MAFF, 1995). *Nephrops* fisheries are currently the second most valuable in Scotland (see Figure 1.2) and the UK as whole, and the most valuable in Northern Ireland. Only cod (England and Wales) and haddock marginally exceed *Nephrops* in total value (MAFF, 1995).

Despite the commercial value of *N. norvegicus*, few fisheries are truly monospecific. In order to qualify as a single-species fishery, over 90% of the catch should consist of the target species (Briggs, 1997). Although the trawling gear is generally customised to their capture, some of the by-catch species can still greatly increase the variety and

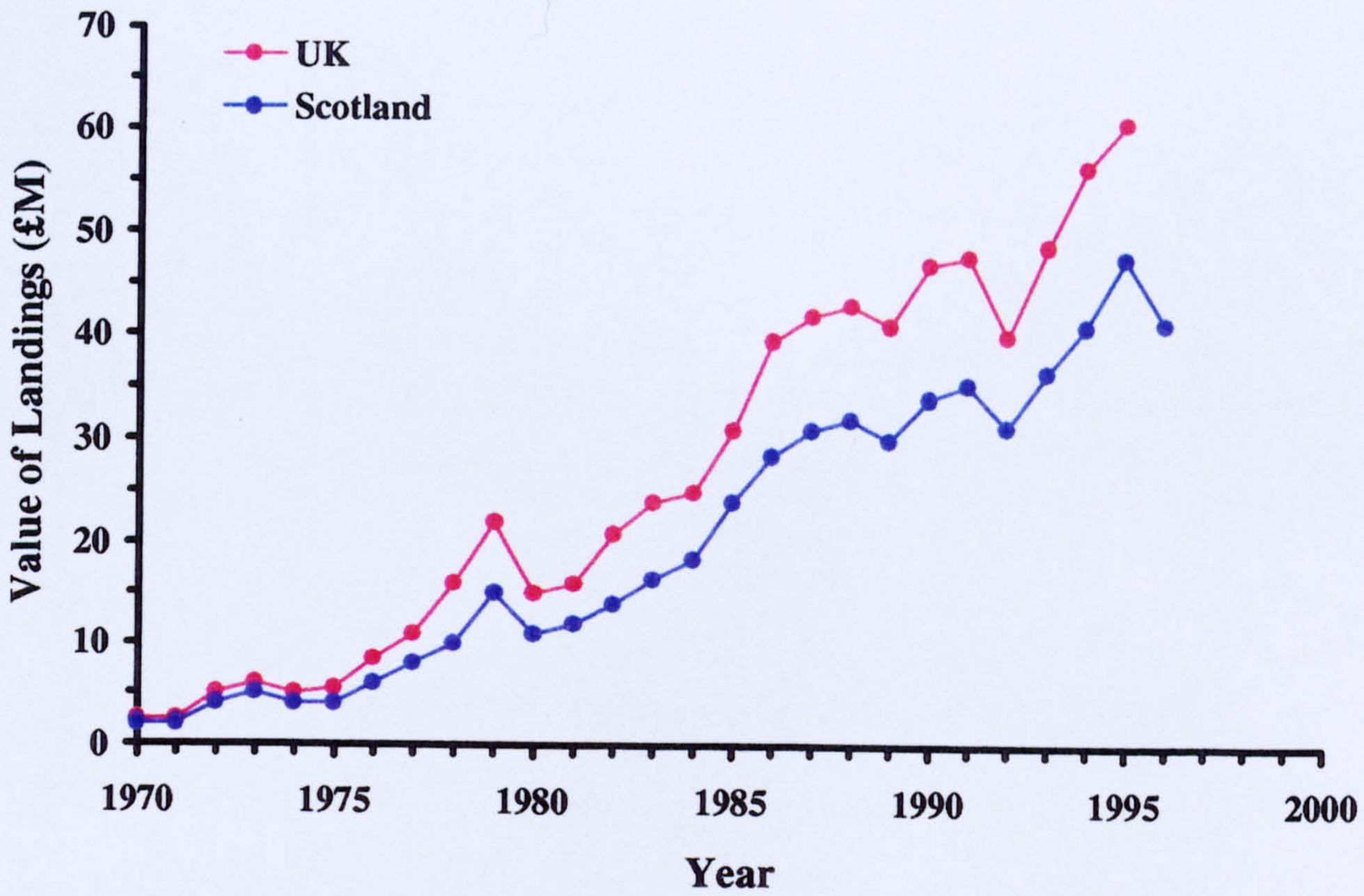


**Figure 1.1. Value of UK and Scottish *Nephrops norvegicus* landings, 1970-1996 (MAFF, 1995; 1998, I.D. Tuck, pers. comm.).**

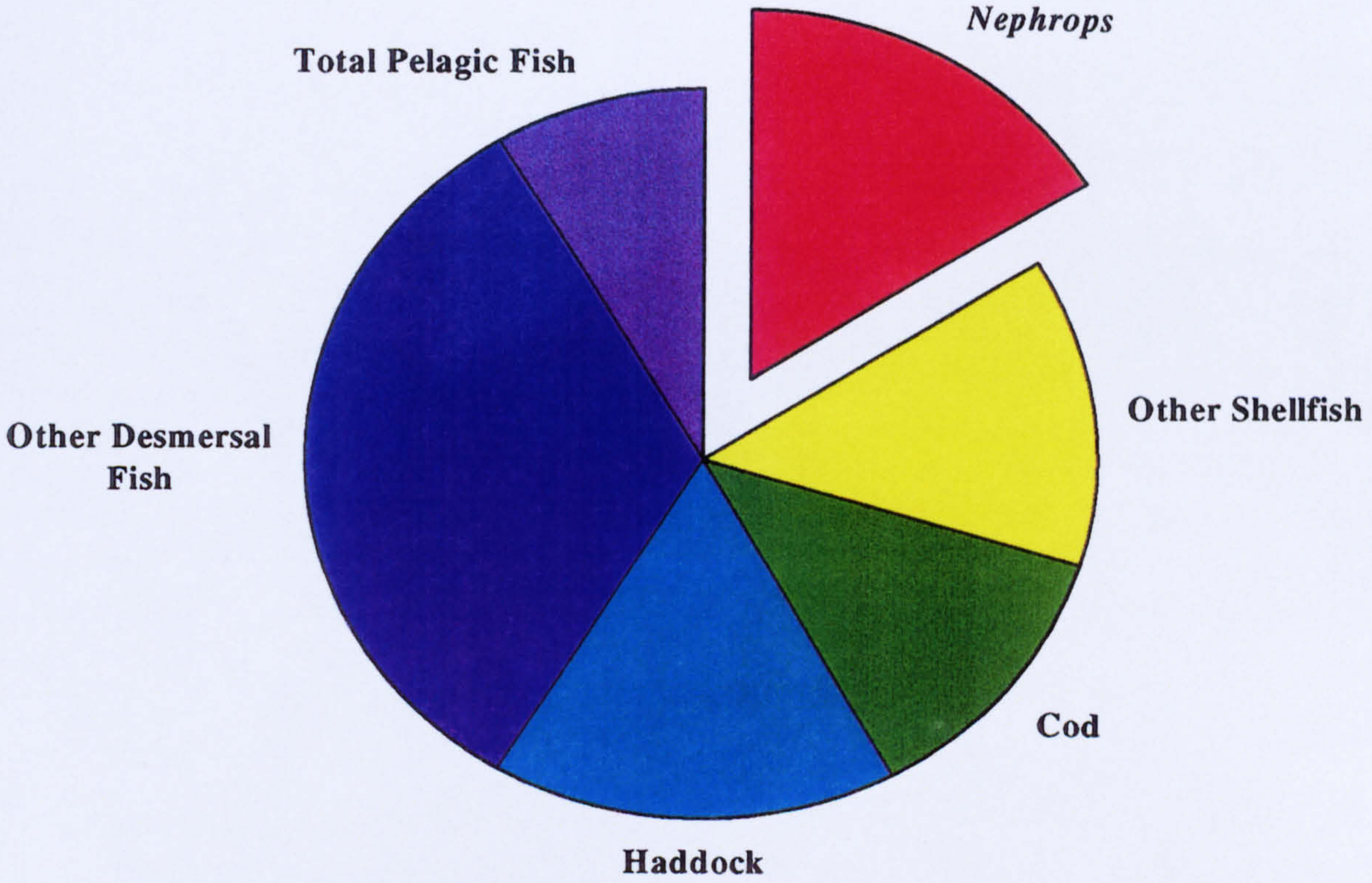


**Figure 1.2. Contibution of species groups to total Scottish Landings in 1995 (MAFF, 1995).**

quality of the catch, and consequently, the commercial value (Sarda *et al.*, 1993). Moreover, in winter months on some Adriatic grounds, *N. norvegicus* may only make up a minor proportion of the catch, so that it is effectively a by-catch species. In some parts of the Mediterranean, however, the concept of by-catch is of doubtful relevance, since the fisheries are multispecies ones and little is discarded. In the western Mediterranean, hake, monk fish, and shrimps are important by-catch species of *Nephrops* fishing (Sarda *et al.*, 1993), while further north in the Celtic and Irish Seas, whiting, cod, hake and more recently haddock are the major contributors to the by-catch (Briggs, 1997). However, the detrimental consequences of a varied trawl composition must also be considered, since not all the by-catch can be utilised. Although the discards which tend to consist of undersized *N. norvegicus* and white-fish as well as non-edible species (see Plate 2), may be promptly returned to the sea, few animals survive the combination of trawling, handling and predation from gulls (Edwards & Bennett, 1980) (see Plate 3). There have been a number of studies on discard survival. A study by carried out by in deck tanks indicated 50% survival of discarded *N. norvegicus* (Edwards & Bennett, 1980). However, most other studies indicated that mortality was higher (e.g. Simpson & Symonds, 1971; Charuau *et al.*, 1982). It was noted that Edwards & Bennett did not take into account sub-lethal effects such as the damage caused to *N. norvegicus* eyes as a result of the sudden exposure to bright light after capture (Loew, 1974; Shelton *et al.*, 1986), tissue damage, and the fact that animals may be discarded several kilometres away from the site of capture. The ICES *Nephrops* Assessment working group assumes 75% discard mortality, this figure being a compromise derived from studies carried out in the 1970s and 1980s (see

**Plate 2. Photograph showing the varied catch composition of a *Nephrops norvegicus* trawl in the Clyde Sea area (photograph courtesy of Melanie Bergmann).**

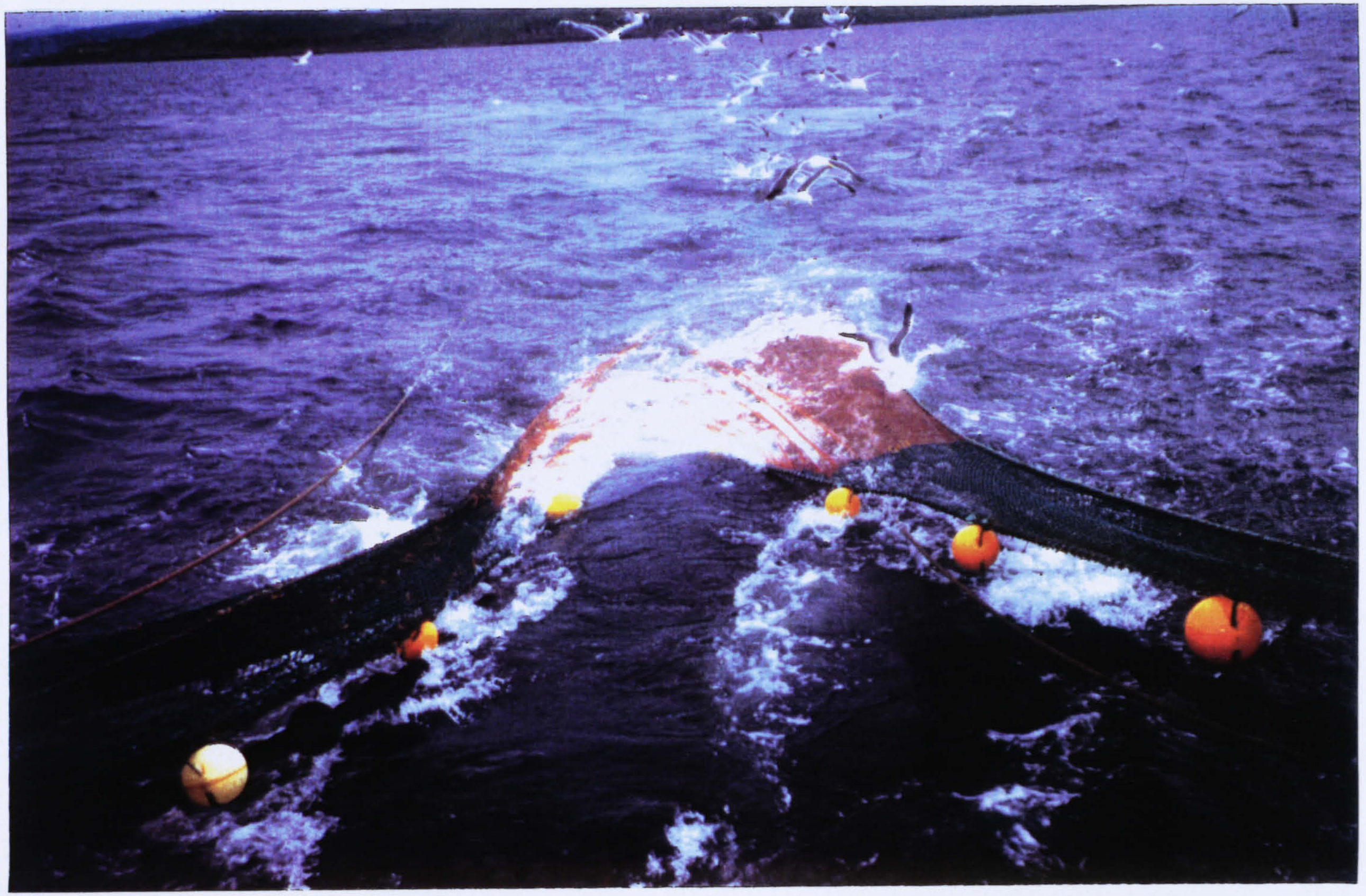

**Plate 3. Photograph showing gulls scavenging from a *Nephrops norvegicus* trawl in the Clyde Sea area (photograph courtesy of Melanie Bergmann).**

Ulmestrand, *et al.*, 1998). Recent work (Ulmestrand *et al.*, 1998) suggests around 69% discard mortality and 19% mortality in animals that have escaped from the gear.

## 1.2. Stocklet variability

Despite the variability in fishing effort and discard practices, there is evidence that the size of animals that comprise stocklets and the densities at which they occur are determined by factors other than fishing pressure, although the latter is not without an effect (Chapman & Bailey, 1987).

One possible effect of fishing is modification of the sex ratio of exploited stocklets. Reviews of sexual reproduction in *N. norvegicus* include those of Farmer (1974), Bailey (1984) and Sarda (1995), and can be summarised in a local context as follows, following Bailey *et al.* (1986). In Scottish waters, *N. norvegicus* spawn in early autumn. As with other decapods, the eggs are carried beneath the abdomen attached to the pleopods. Ovigerous females remain within their burrows throughout the winter and are therefore absent from trawl catches (some may enter creels if these are adjacent to a burrow- 1998, R.J.A. Atkinson, pers. comm.). In late spring, the eggs hatch and the females moult. Mating occurs shortly after a female has moulted. The reappearance of the females results in catches with large numbers of females during the late spring and summer. During the remainder of the year the catch mainly consists of males. In the Clyde Sea, Thomas & Figueiredo (1965) showed that the proportion of females in the catch varied from 11% in March to 44% in July.

There is therefore the potential for fishing to influence the sex ratio and to have differing effects on the size distribution of males and females. By remaining in their burrows for a significant proportion of the year, the females are effectively avoiding both predation and capture by trawls, resulting in a comparatively longer life-span than males. When only the larger size groups are considered (>50mm), females make up the majority of the catch throughout the year, and can contribute up to 92%. (Bailey, 1984). Little appears to be known about the reproductive biology of males and the effects of their heavy exploitation on the reproductive potential of stocklets.

The female reproductive cycle is also influenced by latitudinal effects. In stocks in the colder northerly waters, such as those found off Iceland, the duration of egg incubation can be over 10 months, and spawning is usually limited to biennially. In contrast, in the warmer southerly waters, such as the Adriatic Sea, eggs are incubated for about 6 months and spawning is commonly annual. However, in some populations in the Mediterranean, a significant proportion of the females may skip spawning in a particular year, which is thought to be a result of size or age, of asynchronies in the mating processes, and/or other unknown factors (Sarda, 1995). Bailey (1984) observed that a proportion of Clyde *Nephrops* had ovaries with resorbing oocytes and suggested that ovary maturation became progressively later each year until it was necessary to miss a year in order to get back into phase.

The *Nephrops* fisheries are regulated by a number of technical and other measures including regulation of mesh size, days at sea, size of vessel and gear, a minimum landing size, the incorporation of square mesh panels to aid roundfish escapes, and the

imposition of a landings quota. The majority of the landings into UK and Irish ports (80%) are 'tailed prawns' which consist of *N. norvegicus* abdomens (Briggs, 1997). The head region (cephalothorax) is often removed whilst the trawlers are still at sea. Once ashore, the white abdominal muscle tissue is removed from the shell and either frozen, or pre-coated with batter and bread crumbs and marketed as 'scampi', this form being the most familiar to consumers in the UK. A large proportion of marketed scampi is made up of reformed tissue. This usually consists of the minced abdominal muscle tissue of *N. norvegicus* moulded into tail shapes. However, whilst this process may make efficient use of smaller animals, it provides an opportunity for the product to be 'watered down' with bulking agents such as cheap white fish and squid. A survey of 25 different brands of scampi products in the UK carried out the Ministry of Agriculture, Food and Fisheries (MAFF, 1993) found that 8 contained organisms other than *N. norvegicus*. Both fishing and discard practices therefore have the potential to influence the size composition of animals within stocklets.

It is common for animals that are landed whole in UK ports to be transported to France and Spain, and to a lesser extent, the more salubrious restaurants in the UK. Consequently larger *N. norvegicus* tend to sell for a higher price than smaller ones (which will usually be tailed), making the size distribution of *N. norvegicus* catches a factor of economic importance. Much research on *N. norvegicus* has therefore concentrated on fisheries statistics such as stock abundance and size composition. Fishery-related physiological studies have included studies of tail-flick escape swimming and the stress of capture (Newland *et al.*, 1988; Spicer *et al.*, 1990). Since live landings can lead to animals spending a considerable amount of time emersed,

animals are exposed to stress, and often have to resort to anaerobic respiration. Spicer *et al.* (1990) suggested that emersion stress outweighed capture stress. Although, it might be expected that the method of capture would have a marked effect on lactate accumulation, Spicer *et al.* (1990) found that there were no differences in the tissue lactate concentrations between creel-caught and trawled *N. norvegicus*. However, it was noted that keeping emersed animals on ice greatly enhanced their chances of survival. Certainly, the longer undersized animals remain on deck before they are discarded, the greater the stress and risk of mortality. Nevertheless their robust physiology appears to aid their survival.

Whereas the size of *Nephrops* within stocklets is assessed by measuring animals from trawl catches, stock size estimates in Scotland increasingly rely on fishery independent methods, particularly the use of underwater television (UWTV) (Bailey *et al.*, 1993; Tuck *et al.*, 1997b). *N. norvegicus* stocks in European Atlantic waters are monitored by 'The Working Group on *Nephrops* Stocks' (Anon, 1997). The data on *N. norvegicus* gathered by this working group are analysed using techniques such as virtual-population analysis (VPA), and length-cohort analysis (LCA). The VPA model requires estimates of animal age, which is usually calculated using a growth curve to convert length to age (1998, C.J. Chapman pers. comm.). Unfortunately, it not possible to estimate age independently of size for *N. norvegicus*, because unlike the otolith in fish, there are no comparable hard structures which persist through the moulting process. However, this may be possible eventually, since there are new techniques of ageing *N. norvegicus* currently being developed based on the concentration of age-related pigment in the brain (Belcheier *et al.*, 1994). In addition,

a recent problem adding to the inaccuracy of the fishery-based methods, is that landings and effort data are commonly under-reported in certain European countries, in attempts to avoid quota restrictions.

Fisheries-independent methods appear to offer a more accurate assessment of *N. norvegicus* numbers, these include techniques such as spawning-stock estimation based on larval production estimates and the use of UWTV to estimate the density of *N. norvegicus* burrows, with allowances made for the level of burrow occupancy. These values are then computed to reflect the area of the fishing ground and when combined with data on mean animal size (converted to weight), stock biomass can be calculated. The distribution and production of *N. norvegicus* larvae have been used to estimate spawning stock sizes off the NE coast of England (Milligan & Nichols, 1988), in the Irish Sea (Nichols *et al.*, 1987), and in the Clyde (Tuck *et al.*, 1997b), but this method is more complicated than the UWTV method. Aside from this, the use of UWTV has confirmed the stocklet differences in animal size and population density evident from the trawl catches.

Another factor which may influence the size composition and density of *Nephrops* stocklets is parasitism and disease. Common parasites of *N. norvegicus* include *Histriobdella homari,* a polychaete found in the branchial chamber, on egg masses and pleopod setae (Uzmann, 1967; Briggs *et al.*, 1997). Its effect on *Nephrops* has never been quantified, but it can have a deleterious effect in lobster hatcheries (Briggs *et al.*, 1997). Potentially far more serious is the parasitic syndinean dinoflagellate, *Hematodinium* sp. which infects *Nephrops* in Scottish waters and elsewhere in

northern Europe (Field *et al.*, 1992; Appleton & Vickerman, 1998). Patent (parasite easily visible in the haemolymph) infection is fatal to the host and in the early 1990s over 50% of the *Nephrops* trawled in the Clyde Sea were infected. Prevalence was higher in females than males, and in animals of intermediate size. Levels are still around 20% (1998, R.J.A. Atkinson, pers. comm.) and this prevalence level is also seen in the Irish Sea (Briggs *et al.*, 1998). The effects of this on the stocks are still poorly known, the situation made more complicated by the fact infection makes the animals more susceptible to capture so that fishing mortality masks disease mortality (Appleton *et al.*, 1997).

The wealth of fishery-based research on *N. norvegicus* has shown the existence of geographical variability in the size of the individual *N. norvegicus* that comprise populations, and such differences in size composition can occur over relatively small geographical distances. This 'stocklet phenomenon' has been noted in *N. norvegicus* grounds in the Clyde Sea area, the Mediterranean and elsewhere (Chapman & Howard, 1988; IMBC, UMBSM & IRPEM, 1994; Tuck *et al.*, 1997a). The size disparity is usually associated with differences in density, since stocklets of *N. norvegicus* characterised by a small average size of individuals often live at high densities, whereas those which occur at lower densities have a larger mean animal size. There are also marked differences in other biological parameters, such as the size at which animals reach maturity. In the Clyde Sea, the size (carapace length in mm) at which 50% of the females are sexually mature is 23mm at Ailsa Craig compared with 33.5mm at a site south of Little Cumbrae (IMBC, UMBSM & IRPEM, 1994). Similarly, the sizes for Adriatic *N. norvegicus* were 26mm at the Pomo Pit and

31.5mm off NW Ancona (IMBC, UMBSM & IRPEM, 1994 ). Stocklet variation was first observed in British and Irish waters in the 1960s (Cole, 1962; O' Riordan, 1964; Thomas, 1965). The concurrent rise in the commercial exploitation of *N. norvegicus* at this time led to the size differences being attributed to variation in the intensity of fishing (Thomas, 1965). It was accepted that some other factors may also be involved, since variation in the size of individual *N. norvegicus* between some areas was observed before the fisheries had developed. With the information discussed thus far, it can been seen that there are a number of potential causes of this variation. Recently, attention has focused on environmental factors.

The sedimentary environment has been proposed as a possible cause of the variability. In the Clyde Sea area, stocklets of *N. norvegicus* living on coarse mud sediments (10-50% silt & clay) tend to consist of lobsters with a small average size, compared with the stocklets found on finer muds (>90% silt & clay), which have a larger mean size of individual. It has been speculated that the sediment may influence recruitment at the stage of larval settlement (Chapman & Bailey, 1987). Larval settlement is a crucial phase in the life history of lobsters; therefore factors affecting survival at this critical stage make marked differences to population size (reviewed by Cobb & Wahle, 1994). Similar instances have been observed with other lobsters. Pollock (1990) noted that in certain areas of the habitat range of the spiny lobster *Jasus tristani*, the increase in shelter available for newly-settled larvae greatly reduced mortality from predation, resulting in a higher lobster density than on the more exposed grounds, where shelter was limited. An alternative explanation was suggested by Chapman & Howard (1988). They proposed that sediments with high

silt/clay content are required for the construction of burrows sufficient in size for large *N. norvegicus*. However, it is not clear whether the relationship between sediment type, and animal size and stocklet density is causative, or simply an association resulting from the local hydrographic and topographic conditions. Furthermore, in the Adriatic Sea, the association between animal size and density, and the type of sediment differs from that found in the Clyde. Adriatic *N. norvegicus* living at low densities and with a larger mean animal size tend to be found on coarser sediments in the shallower waters off Ancona, whereas the smaller animals living at high densities are found on the fine sediment in the deep waters of the Pomo Pit (IMBC, UMBSM & IRPEM, 1994).

It has been proposed that in the Clyde Sea area, oceanographic features have a significant influence on *N. norvegicus* distribution. Tuck (1993) noted a southerly drift of *N. norvegicus* larvae associated with the surface water outflow from the Clyde Sea. When the larvae reach the southern limits of the Clyde Sea, an oceanographic front is thought to slow their passage, causing them to accumulate and settle onto a densely-populated area south of Ailsa Craig. In addition, Tuck *et al.* (1997b) noted that the production of larvae from the higher density *N. norvegicus* populations in the southern Clyde Sea was greater than in the northern reaches suggesting that high density populations may be maintained by higher levels of larval production. It is also possible that, as a result of the influences of these hydrographic factors, a proportion of the larvae produced by the large animals in the sparsely populated north-Clyde areas may settle in the more densely populated areas in the south. This potential for inter-stocklet reproduction greatly reduces the chances of genetic isolation. Indeed, a

study examining allozyme variation of *N. norvegicus* in the Clyde Sea and Adriatic Sea found no genetic differences between any of the stocklets in Clyde Sea area, or even between Scottish and Mediterranean *N. norvegicus* (Passamonti *et al.*, 1997).

The hydrography of the Clyde Sea area has been studied for over 100 years. Mill (1892) estimated that the total surface area covered approximately 1160 square miles (equivalent to 3,000km$^2$ Tuck, 1993). More recently, investigations have been carried out by Edwards *et al.* (1986). The glacial origins and fjordic origins of the Clyde Sea are expressed in the topography of the area which consists of deep basins separated by narrow sills. These characteristic formations provide a variety of marine environments ranging from shallow sea lochs to deep channels, and also create ideal conditions for oceanographic fronts. Similar hydrographic features have been in observed on other Norway lobster grounds. In the western Irish Sea for instance, a cyclonic near-surface gyre has been found to influence *N. norvegicus* populations (Hill *et al.*, 1994, 1996). It is a seasonal phenomenon, being limited to spring and summer. However, this is sufficient to have a marked effect on the life history of *N. norvegicus* since it is coincidental with the larval dispersal phase. The larvae are hatched into the water column each spring where they remain for approximately 50 days, usually being confined to the upper 40m (Hillis, 1974; Nichols *et al.*,1987). The gyre is thought to retain the larvae close to where they were spawned, thus isolating the *N. norvegicus* contained within. This could have important implications for the management of the *N. norvegicus* fishery. Hill *et al.* (1996) predicted that disasters such as large-scale pollution events and over-fishing, in particular, could be exacerbated by the properties of the gyre. In addition, the low genetic diversity resulting from the gyre's effects

could also increase the susceptibility of the population to disease, although Brown *et al.* (1995) suggested that the gyre could provide some protection from the spread of disease from other populations.

In addition to hydrographic and bathymetric influences, the heterogeneity of the sedimentary environment in the Clyde Sea area results largely from differences in terrestrial input. Since the northern reaches of the Clyde Sea are characterised by narrow sea-lochs and islands, these areas are thus liable to receive a greater amount of organic enrichment from rivers and land run-off, than the more open expanses of the southern Clyde. Values of organic carbon content range from 5 to 10mg.g$^{-1}$ in the sandy sediments in the southern Clyde Sea around Ailsa Craig, to 15 to 25mg.g$^{-1}$ in the northern Clyde (IMBC, UMBSM & IRPEM, 1994). However, there is also a possibility that the sewage dumping site NE of Arran (1.5 million tonnes.y$^{-1}$ wwt sewage) may influence these figures (IMBC, UMBSM & IRPEM, 1994). The faunal composition of *N. norvegicus* grounds in the Clyde Sea also varies considerably. Most of the biomass of the area around Ailsa Craig is composed of crustaceans and polychaetes, whereas bivalve molluscs dominate the fauna at sites around the Cumbraes (IMBC, UMBSM & IRPEM, 1994).

The coupling of hydrographic factors which are thought to concentrate the larvae in certain areas and the patchiness of the sedimentary environment creates the potential for a wide range of stocklet conditions. It has been suggested that animals living in the densely populated stocklets would face more intense competition for resources such as food and space, than animals in the sparser stocklets. In addition, differences

in the levels of competition are likely to be exacerbated by the inequality in prey availability. It is interesting to note that in the Clyde Sea, examples of stocklets approaching almost the 'best and worst case scenarios' can be observed. In the northern Clyde, *N. norvegicus* are exposed to favourable conditions, since they are sparsely populated on organically rich sediments associated with a rich infauna. In contrast, *N. norvegicus* in the southern Clyde occur in high densities on a comparatively impoverished sediment. The disparity in the size of individuals between stocklets has been clearly shown by Chapman & Howard (1988) who indicated a ten fold difference in number of animals per unit of weight when the north Clyde was compared with the sound of Jura. The differences between the north and south Clyde stocklets are similar, though the size difference is not so extreme (Tuck *et al.* 1997a; 1998, R.J.A.Atkinson, pers. comm.).

The differences in the intensity of competition are thought to be reflected in individual growth rates which are in turn, responsible for creating the size differences. Workers using length-frequency techniques have estimated lower rates of growth for animals on densely populated grounds than animals on sparsely populated grounds (Bailey & Chapman, 1983; Tuck *et al.*, 1997a). However, in common with some of the stock-assessment methods discussed earlier, there are a number of limitations associated with these methods, largely resulting from the absence of reliable age estimation. Furthermore, whilst these studies are useful in describing the growth variation, they provide little information on the factors responsible. Indeed, Sarda (1995) ended his review of the life history of *N. norvegicus* by stressing the importance of advancing knowledge on the actual causes of *N. norvegicus* size variation. To this end, the aim

of this thesis is to investigate methods of assessing the stocklet differences using both ecological and physiological methods, and gain insight into some of the underlying causes.

## 1.3. Outline of thesis

Thus, building on the background information provided in this first Chapter, Chapter 2 presents an investigation of the feeding energetics of Clyde Sea *N. norvegicus*. The types of prey eaten and their energy contents are examined. Comparisons are made between north Clyde and south Clyde stocklets. A 24h sampling programme was carried out in order to calculate daily food consumption. When used in conjunction with the information on prey composition and energy content, this enabled quantitative estimates of energy intake to be made.

The ecological energetics of Clyde Sea *Nephrops* are investigated in Chapter 3. Energy expenditure was determined using a combination of laboratory experiments on the energy cost of various levels of activity (using oxygen consumption) and previously-published work on animal behaviour in the field. Furthermore, these data, combined with those of Chapter 2 enabled an energy budget to be constructed. Chapter 4, presents an investigation of 'instantaneous' methods of predicting growth. Methods tried included estimation of protein synthesis using radio-labelled phenylalanine and measurement of nucleic acid concentrations. Following this laboratory calibration (Chapter 4), methods which successfully predicted nutritional condition were applied to field populations in order to detect any nutritional

differences between the *Nephrops* stocklets in the north and south Clyde Sea (Chapter 5).

Populations of *N. norvegicus* in the Clyde Sea are vulnerable to infection with a parasitic dinoflagelate (*Hematodinium* sp.). The methods used to investigate stocklet variability were also applied to infected animals in order to assess some of the physiological consequences of the disease (Chapter 6). Finally, the work reported in the earlier chapters is summarised in Chapter 7.

# CHAPTER 2

# Feeding energetics of the Norway lobster, *Nephrops norvegicus*

## 2.1. INTRODUCTION

The commercial value and the aquaculture of many decapod crustacean species has encouraged the publication of a number of works on their feeding and digestion e.g., Dall (1975) for *Panulirus longipes*, Logan & Epifanio (1978) for *Homarus americanus*, Pollock (1979) for *Jasus lalandi*, Williams (1981) for portunid crabs, Joll (1982) for *Panulirus cygnus*, Kurmaly *et al.* (1989) for *Penaeus monodon*, and Loya-Javellana *et al.* (1995) for the tropical freshwater crayfish, *Cherax quadricarinatus*. Due to the difficulties involved in observing their feeding behaviour in the field, most of these studies have been based on animals in the laboratory or under culture conditions. Information on the diet and feeding behaviour of marine organisms in the field often has to be obtained indirectly from stomach contents analysis. Whilst this process is relatively straightforward for species of fish such as the northern pike *Esox lucius*, which gulp down large chunks of prey (Diana, 1979), Hoglund (1924) commented that it was very difficult to identify prey items from the cardiac foregut of lobsters, since much of the food is ground into fine particles by the gastric mill prior to digestion. However, studies by Thomas & Davidson (1962), Oakley (1981), Bailey *et al.* (1986) and Goodheir (1995) all found that a large proportion of the dietary constituents of *N. norvegicus* could still be identified, provided that appropriate methods are taken to preserve the foregut contents. After

trying different preservation techniques, Goodheir (1995) noted that the highest proportion of identifiable prey was found in foreguts which had been fixed by the injection of a formaldehyde solution immediately after capture. Sarda & Valladres (1990) carried out a detailed investigation into gastric evacuation in *N. norvegicus*. The rates of digestion determined were observed to be higher than those found for fish; *N. norvegicus* was able to empty most of its digestive tract within 12h after feeding. In addition, Sarda & Valladres (1990) also noted that harder prey items such as crustaceans took considerably longer to digest than softer bodied prey such as polychaetes. Similarly, Hill (1976) observed that hard body parts tended to remain longer in the digestive tract of the crab *Scylla serrata*. As a result of the potential bias towards harder prey fragments which could accumulate in the stomach due to their greater resistance to digestive processes, Sarda & Valladres (1990) advised that the variation in the digestion rates of different prey items should be taken into account when examining stomach contents.

Observations on the diet of *N. norvegicus* (Thomas & Davidson, 1962; Oakley (1981); Bailey *et al.*, 1986; and Goodheir, 1995) suggest that it is an opportunistic predator; representatives of most invertebrate phyla have been found in their foreguts. Any dietary differences appear to be due more to changes in prey abundance than to prey preference. Thomas & Davidson (1962) also investigated the size range of prey eaten. They determined that the minimum food particle size which *N. norvegicus* could ingest was 1mm, and the maximum for hard particles such as shell pieces was 5mm. It was also noted that large soft-bodied organisms, such as polychaetes up to 6cm long, could be eaten if they were ingested lengthways. The size range of prey

thought to be available to *N. norvegicus* was extended by Loo *et al.* (1993). They proposed that *N. norvegicus* may have the capacity to suspension feed. Laboratory experiments revealed that lobsters kept in water enriched with *Artemia salina* were in a better nutritional state than lobsters kept in filtered seawater. Furthermore, fluorescently-marked *A. salina* were found in the gills, stomach and intestine. However, it is not clear how they were ingested. Although feeding on suspended food, this method of feeding may be 'micro-raptorial' rather than strictly filter-feeding (1998, R.J.A. Atkinson, pers. comm.). Despite the lack of direct evidence for this type of feeding behaviour to the field, Baden *et al.* (1990) did note that *N. norvegicus* were able to maintain a normal nutritional status (assessed by blood condition), during an extensive period of phytoplankton blooms.

This chapter aims to build on previous studies on the feeding ecology of the Norway lobster *Nephrops norvegicus* by providing quantification of their foraging behaviour. Information will also be gained about the energy content of prey items. In addition, when combined with estimates of digestion rates, stomach contents data will be used to investigate temporal patterns of feeding and also to calculate the daily rate of consumption.

## 2.2. MATERIALS AND METHODS

### 2.2.1. Analysis of cardiac-foregut contents

Samples of *Nephrops norvegicus* were collected from the north Clyde Sea area, south of Little Cumbrae (55°41'N, 04°57'W) (depth= 70m) , and from the south Clyde Sea area, south of Ailsa Craig (55°10'N, 05°13'W) (depth= 50m) during the months of May and November 1994 (see Figure 2.1). This work was carried out using the research vessel *Aora* (see Plate 4). A total of 91 male lobsters were examined ranging from 19 to 53g fresh weight. Immediately after capture, whilst still onboard the research vessel, lobsters were killed by swiftly inserting a needle into the brain. To prevent further digestion from occurring, the stomach contents of each lobster were fixed by injecting a 5% solution of formaldehyde in seawater, into the stomach through the mouth. The lobsters were then transported to the laboratory at the University of Glasgow for analysis. Each animal was weighed, and the fixed cardiac-foregut contents (hereafter referred to as stomach contents) were removed and identified as far as possible. In addition, the relative proportions that each taxa contributed to the *N. norvegicus* diet were estimated. This was carried out by developing work reported in Goodheir (1995). This study builds on previous investigations into the *N. norvegicus* diet by incorporating the energy contents of prey items. Furthermore, the total freeze-dried weights of stomach contents were also determined. Since many of the prey items identified in the stomachs were too small to weigh individually, quantification depended on an estimate of the percentage volume of each food item. The weight of each component of the diet was calculated by

multiplying the whole stomach contents weight by the estimated proportion of the stomach contents occupied by each taxa.

2.2.2. Calorific content of prey

Benthic invertebrates identified as prey were captured from *N. norvegicus* grounds south of Little Cumbrae using van Veen grabs. Energy contents of the most abundant prey items were measured by the combustion of freeze-dried, weighed samples in a micro-bomb calorimeter (Phillipson, 1964), calibrated against the thermochemical standard benzoic acid. Samples that were difficult to ignite were mixed with a known proportion of benzoic acid, and the appropriate corrections were made. The percentage organic matter was calculated by weighing the ash residue after each sample had been bombed. Energy contents were expressed as $kJ.g^{-1}$ dry weight and $kJ.g^{-1}$ ash-free dry weight. Minor corrections were made for the endothermic effect of calcium carbonate (Paine, 1966). The inorganic parts of some organisms, such as bivalve shells and polychaete cases were removed before combustion since prior observations on laboratory-held *N. norvegicus* had shown that they were not consumed. The prey organisms were collected throughout a year of sampling. The mean energy content of each species was calculated using between 5 and 20 samples of each animal species, depending on their availability.

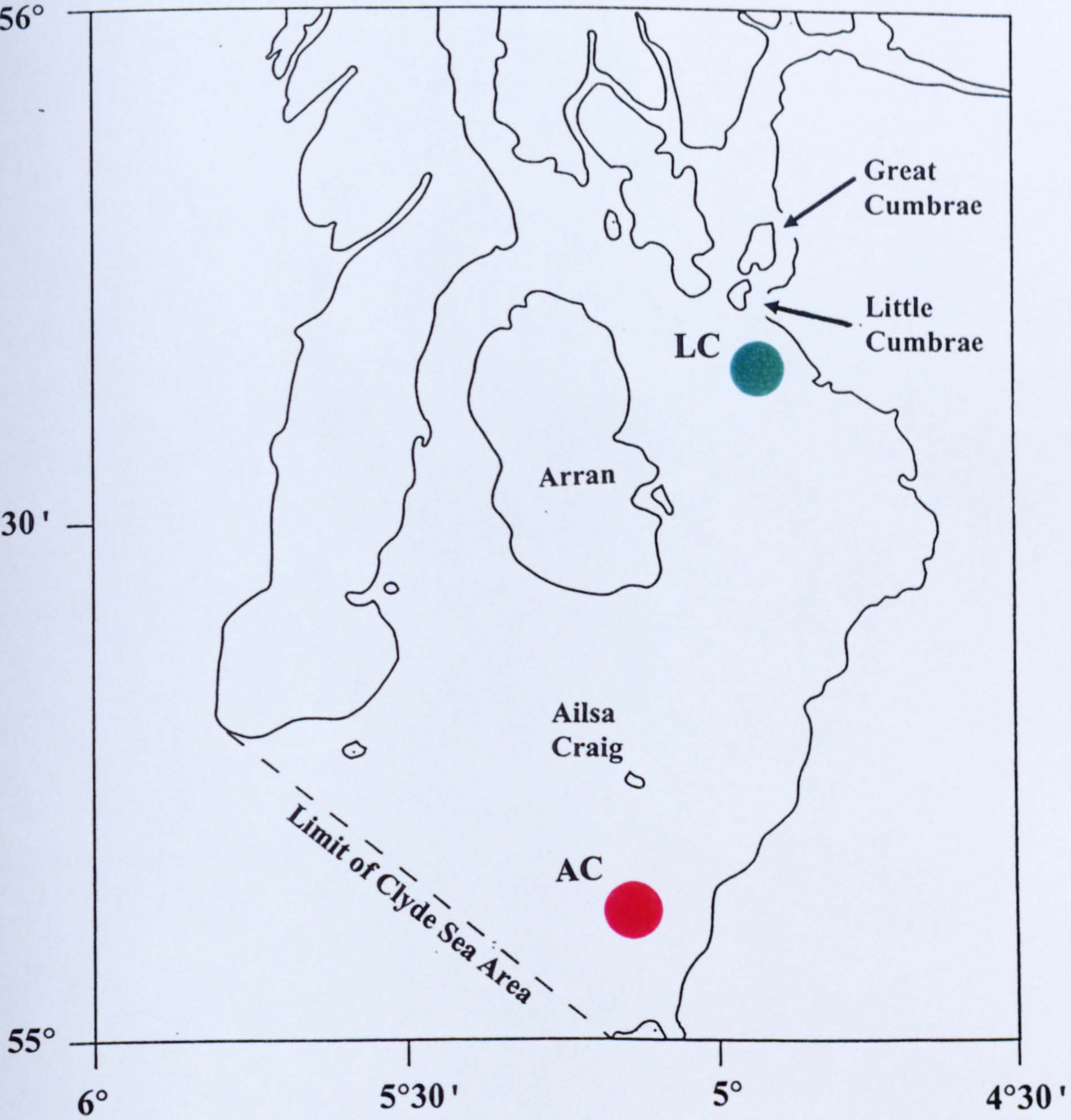


**Figure 2.1. Map of the Clyde Sea area showing *Nephrops norvegicus* sampling sites. LC=south of Little Cumbrae and AC= south of Ailsa Craig.**


**Plate 4. Photograph of *RV Aora* (photograph courtesy of Melanie Bergmann).**

## 2.2.3. Food consumption

Samples of male *N. norvegicus* were taken in March 1996, from south of Little Cumbrae at depths of between 69 and 79m. Samples were taken at 4h intervals over a 24h period; at the following times (GMT) 1000h, 1400h, 1800h, 2200h, 0200h and 0600h. Immediately after capture, lobsters were killed by swiftly inserting a needle into the brain, and a 5% formaldehyde in seawater solution was injected into the stomach through the mouth. The lobsters were then taken to the laboratory at the University of Glasgow for analysis. Each animal was weighed, and the fixed stomach contents were removed, freeze-dried and then weighed. The fresh weights of the majority of the animals (>80%) were between 15 and 45g. The mean animal weight was 26.2g. The amount of food consumed over each 4h time period was calculated by adding the difference in the amount of food in stomachs between the two sample points to the estimated amount of food evacuated from the stomach (digested) over that period (see below).

In order to estimate food consumption $C_t$ over a time period ($t$), three parameters were required:-

1) $S_0$ The weight of the stomach contents at the start of the sampling period.

2) $S_t$ The weight of the stomach contents after a given time-$t$ (in minutes).

3) $k$ The rate of gastric evacuation.

Two different methods were used to determine total daily food consumption, recommended by Thorpe (1977) and Elliott & Persson (1978). The method of Thorpe (1977) estimates the amount of food consumed in a period of time ($C_t$) by adding the difference between the stomach contents at the start and end of a time period ($t$), to an estimate of the amount food evacuated from the stomach during this time (A). The equation can thus be expressed as:-

$$C_t = S_t - S_0 + A$$

Where A is calculated by applying the digestion rate ($k$) to the average stomach contents over the sample period:-

$$A = \frac{(S_t + S_0)(1 - e^{kt})}{2}$$

The method of Elliott & Persson (1978) is more complex, and assumes that the rate of gastric evacuation is dependent on the amount of food in the stomach. Their equation for the estimate of food consumption can be expressed as:-

$$C_t = \frac{(S_t - S_0 e^{-kt})kt}{1 - e^{-kt}}$$

The rate of gastric evacuation ($k$) for *N. norvegicus* has previously been calculated for both hard and soft prey items by Sarda & Valladres (1990). The values of $k$ obtained were -0.0027 (using the crustacean *Pontocaris* sp.) and -0.0056 (using the polychaete *Hediste* sp.) respectively.

In order to account for the differences in the digestion rates of hard and soft prey (as recommended by Sarda & Valladres, 1990), the equations used to estimate food consumption ($C_t$) were modified. To achieve this, the amounts of food evacuated in each time period were determined by dividing each amount of food into relative proportions of hard and soft prey. The appropriate rate of digestion was then applied. The proportions of hard and soft food, in terms of volume, were calculated from stomach contents analysis in the study by Goodheir (1995). Crustaceans, molluscs and echinoderms were considered hard, whereas polycheates and cnidarians were considered as soft. The values can be compared with those of Sarda & Valladres (1990) and Thomas & Davidson (1961) (see Table 2.1).

**Table 2.1. Proportions of hard and soft items in the diet of *Nephrops norvegicus* (expressed as percentages). [a]=Goodheir (1995); [b]=Thomas & Davidson (1961); [c]=Sarda & Valladres (1990).**

| | Study Sites | | | |
|---|---|---|---|---|
| | Firth of Clyde | Firth of Clyde | Firth of Forth | Mediterranean Sea |
| **Hard** | 77.7[a] | 89.6[b] | 65.2[b] | 90.03[c] |
| **Soft** | 22.3[a] | 10.4[b] | 34.8[b] | 9.97[c] |

The modified equations can thus represented as:-

**Thorpe (1977):-**

$$C_t = S_t - S_0 + \left[0.777\left(\frac{S_0+S_t}{2}\right)\left(1-e^{-0.0027t}\right)\right] + \left[0.223\left(\frac{S_0+S_t}{2}\right)\left(1-e^{-0.0056t}\right)\right]$$

**Elliott & Persson (1978):-**

$$C_t = \left[0.777\left(\frac{S_t - S_0 e^{-0.0027t}}{1-e^{-0.0027t}}\right)(0.0027t)\right] + \left[0.223\left(\frac{S_t - S_0 e^{-0.0056t}}{1-e^{-0.0056t}}\right)(0.0056t)\right]$$

The total daily amounts of food consumed were then estimated by adding together the values calculated for each 4h period.

2.2.4. Geographical variation and the effects of sampling month on total daily food consumption and energy intake

Although a complete 24h sampling regime could be carried out on only one occasion at the South of Little Cumbrae site, stomach contents were examined during May and November at both the Little Cumbrae and Ailsa Craig sites to give some idea of seasonal and geographical variation. By comparing these single samples with the sample taken at the equivalent time (1000h) during the 24h sampling regime, and applying the same ratio to the total daily food consumption, an estimate of food intake ($mg.day^{-1}$) at these times was obtained. These values were also expressed per unit wet weight, by using a value of 76.2% water (the mean water content of all stomach contents) to allow comparison with other published data.

### 2.2.5. Statistical analysis

Parametric data were analysed using analysis of variance (ANOVA). Tukey's multiple comparison test was used to compare the organic and energy contents of the different phyla. Since the stomach contents data were proportional, they were arcsine transformed in order to achieve normality and homogeneity of variance. The effects of sample site and month on the transformed data were determined using two-way ANOVA. The temporal effects on food consumption were investigated using ANOVA coupled with Tukey's multiple comparison. The influences of sample site and month on the estimated daily food consumption of *N. norvegicus* were tested using two-way ANOVA.

## 2.3. RESULTS

### 2.3.1. General observations on stomach contents

Organisms found in the stomachs of *N. norvegicus* were classified as far as possible, and are listed in Table 2.2. There were several key characteristics which enabled some prey species to be easily identified. Glycerid polychaetes could be recognised by their shiny black jaws. Similarly, the presence of the polychaetes *Lagis koreni* and *Aphrodita aculeata* were indicated by fragments of bristles and scales respectively. Softer-bodied polychaetes such as *Nephtys* sp. were more difficult to distinguish due to their lack of conspicuous hard body parts, although it was much easier in cases where the head segments were eaten. Mud-shrimps were simple to identify by their chelipeds and stalked eyes, which tended to be ingested intact. Molluscs could be identified by their shell fragments and the velar folds in the mantle tissue. Ingested

brittle stars were characterised by their calcareous and spiny texture. Although it was possible to classify most of the stomach contents to a high taxonomic level, quantification was considerably more difficult. In terms of the proportions of prey species eaten, it was found to be more efficient if the prey were grouped into the following categories: polychaetes, crustaceans, molluscs, echinoderms, unidentified (prey which had been macerated beyond recognition), and inert objects such as stones and synthetic fibres.

### 2.3.2. Energy content of prey organisms

Analysis of variance coupled with Tukey's multiple comparison tests demonstrated that there were significant interspecific differences in organic content (ANOVA, $F_{8,88}=69.61$, $P<0.001$), energy content expressed as kJ per g dry weight (ANOVA, $F_{8,88}=59$, $P<0.001$), and energy content as kJ per g ash-free dry weight (ANOVA, $F_{8,88}=26.88$, $P<0.001$). Organisms with the highest organic contents and energy contents were soft-bodied polychaetes, and the organisms with the lowest organic contents and energy contents were echinoderms (see Table 2.3). The interspecific differences are summarised in Table 2.4.

The energy content (kJ.g$^{-1}$ dry weight) of the prey items was found to be significantly influenced by the relative proportion of organic/inorganic matter (Fig. 2.2). The relationship between percent organic content and calorific value (kJ.g$^{-1}$ dry weight) is explained by the following regression equation:-

**Calorific value=0.272(%Organic)-4.09 ($r^2$=0.95)**

Analysis of variance showed this relationship to be significant (ANOVA, $F_{87,1}$=1773.31, P<0.001).

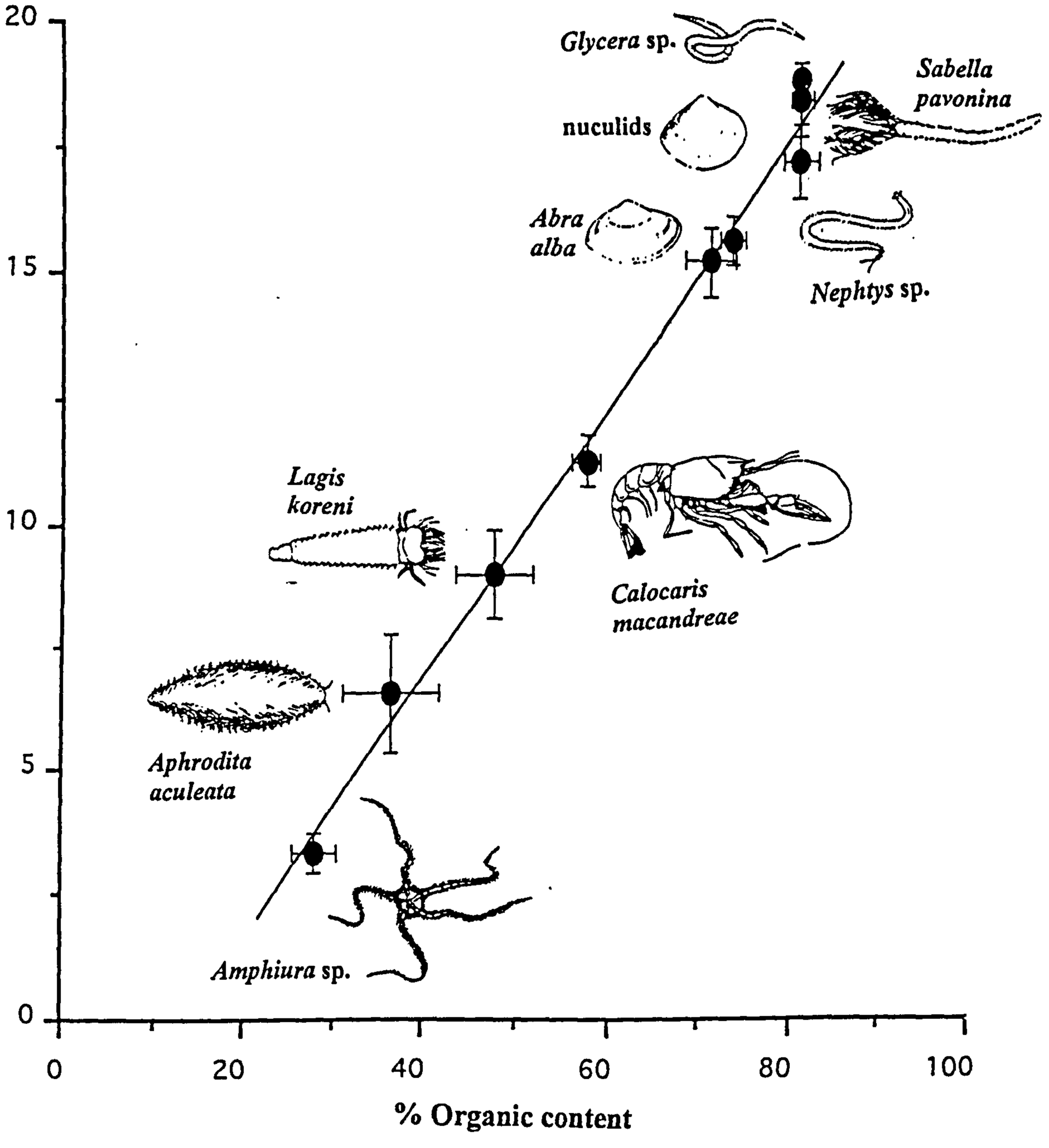


**Figure 2.2. Graph showing the relationship between organic content (%) and energy content (kJ.g$^{-1}$ dry weight) in the prey items of the Norway lobster, *Nephrops norvegicus*. Bars represent standard error (n=5 to 20).**

**Table 2.2. Benthic invertebrates identified in stomach contents of *N. norvegicus* sampled from Little Cumbrae and Ailsa Craig. Organisms marked with * have been analysed for energy content and organic content.**

| **Mollusca** | **Crustacea** |
|---|---|
| *Abra alba** | *Calocaris macandreae** |
| *Nucula nitidosa** | *Callianassa subterranea* |
| *Nucula sulcata** | *Jaxea nocturna* |
| *Nuculoma tenuis** | *Liocarcinus* sp. |
| *Phaxas pellucidus* | Carridean sp. |
| *Scaphander lignarius* | Cumacean sp. |
| *Spisula elliptica* | |
| **Polychaeta** | **Echinodermata** |
| *Glycera capitata* | *Amphiura chiajei** |
| *Glycera* sp.* | *Amphiura filiformis** |
| *Nephtys hombergii** | *Ophiura ophiura* |
| *Nephtys hystrix** | *Ophiura albida* |
| *Goniada maculata* | |
| *Lagis koreni** | |
| Maldanid sp | |
| *Aphrodita aculeata** | |
| *Sabella pavonina** | |
| **Plant material** | **Inert objects** |
| Macrophytes | Polypropylene wire |
| Eel grass (unidentifiable species) | Stones and mineral grains |

**Table 2.3. Energy content and organic content of representatives of prey items identified in stomach contents of *N. norvegicus*. DW=Energy content calculated per g dry weight. AFDW=Energy content calculated per g ash-free dry weight. *Values do not include shells or cases.**

| Name | n | Organic Content % | SE | Energy Content ($kJ.g^{-1}$) DW | SE | AFDW | SE |
|---|---|---|---|---|---|---|---|
| *Glycera* sp. | 20 | 81.18 | 0.76 | 18.68 | 0.28 | 23.00 | 0.21 |
| *Nephtys* sp. | 7 | 73.77 | 1.43 | 15.60 | 0.51 | 21.13 | 0.45 |
| *Sabella pavonina** | 9 | 81.27 | 1.84 | 17.10 | 0.70 | 20.98 | 0.51 |
| *Lagis koreni** | 5 | 47.50 | 3.73 | 8.50 | 0.82 | 19.25 | 0.44 |
| *Aphrodita aculeata* | 5 | 36.36 | 5.1 | 6.48 | 1.19 | 17.51 | 0.85 |
| *Abra* sp* | 10 | 71.38 | 2.92 | 15.14 | 0.70 | 21.21 | 0.35 |
| nuculids* | 8 | 81.24 | 1.22 | 18.29 | 0.71 | 22.47 | 0.61 |
| *Calocaris macandreae* | 20 | 57.56 | 1.59 | 11.22 | 0.55 | 19.34 | 0.52 |
| *Amphiura chiajei* | 5 | 29.54 | 2.61 | 3.75 | 0.48 | 12.38 | 0.67 |

**Table 2.4. Summary of Tukey multiple-comparison tests investigating interspecific variation in the energy content and organic content of *N. norvegicus* prey organisms. DW=Energy content calculated per g dry weight. AFDW=Energy content calculated per g ash-free dry weight. The energy contents of organisms sharing the same letter are not significantly different.**

| Name | n | % Organic | kJ. $g^{-1}$ DW | kJ.$g^{-1}$ AFDW |
|---|---|---|---|---|
| *Glycera* sp. | 20 | a | a | a |
| *Nephtys* sp. | 7 | a | b | ab |
| *Sabella pavonina* | 9 | a | ab | ab |
| *Lagis koreni* | 5 | c | cd | b |
| *Aphrodita aculeata* | 5 | cd | de | b |
| *Abra sp* | 10 | a | b | ab |
| nuculids | 8 | a | ab | a |
| *Calocaris macandreae* | 20 | b | c | b |
| *Amphiura* sp | 5 | d | e | c |

2.3.3. Effects of sampling month and site on the proportion of prey items in stomach contents

The mean proportions of each prey category in the *N. norvegicus* diet are illustrated in Figures 2.3-2.6. Statistical differences are illustrated in Table 2.5. The proportions of crustaceans, molluscs and echinoderms in stomachs were all significantly influenced by site. The proportions of molluscs were higher in the stomachs of *N. norvegicus* from Little Cumbrae, whereas the proportions of echinoderms were higher in the stomachs of *N. norvegicus* from Ailsa Craig. The weight of unidentifiable prey was significantly influenced by both sampling month and site, being highest in stomachs from Ailsa Craig sampled in May. There was also a significant effect of sampling month on the proportion of polychaetes in stomachs. At the Little Cumbrae site, the

proportion of polychaetes in stomachs was ten times greater in November than in May.

**Table 2.5. Summary of two-way ANOVA tests investigating the influences of geographical and temporal variation on the proportions of prey in the stomach contents of *N. norvegicus*. Shaded boxes denote significant effects.**

| Prey Category | Site | | Month | | Interaction | |
|---|---|---|---|---|---|---|
| | $F_{87,1}$ | P | $F_{87,1}$ | P | $F_{87,1}$ | P |
| **Polychaeta** | 0.40 | 0.529 | 4.23 | 0.043 | 1.07 | 0.304 |
| **Crustacea** | 4.09 | 0.046 | 0.05 | 0.821 | 3.50 | 0.065 |
| **Mollusca** | 10.06 | 0.002 | 0.83 | 0.366 | 2.86 | 0.095 |
| **Echinodermata** | 4.09 | 0.046 | 2.40 | 0.125 | 1.46 | 2.31 |
| **Unidentified** | 10.48 | 0.002 | 10.16 | 0.002 | 2.56 | 0.113 |
| **Inert Objects** | 0.01 | 0.936 | 3.07 | 0.083 | 1.79 | 0.184 |

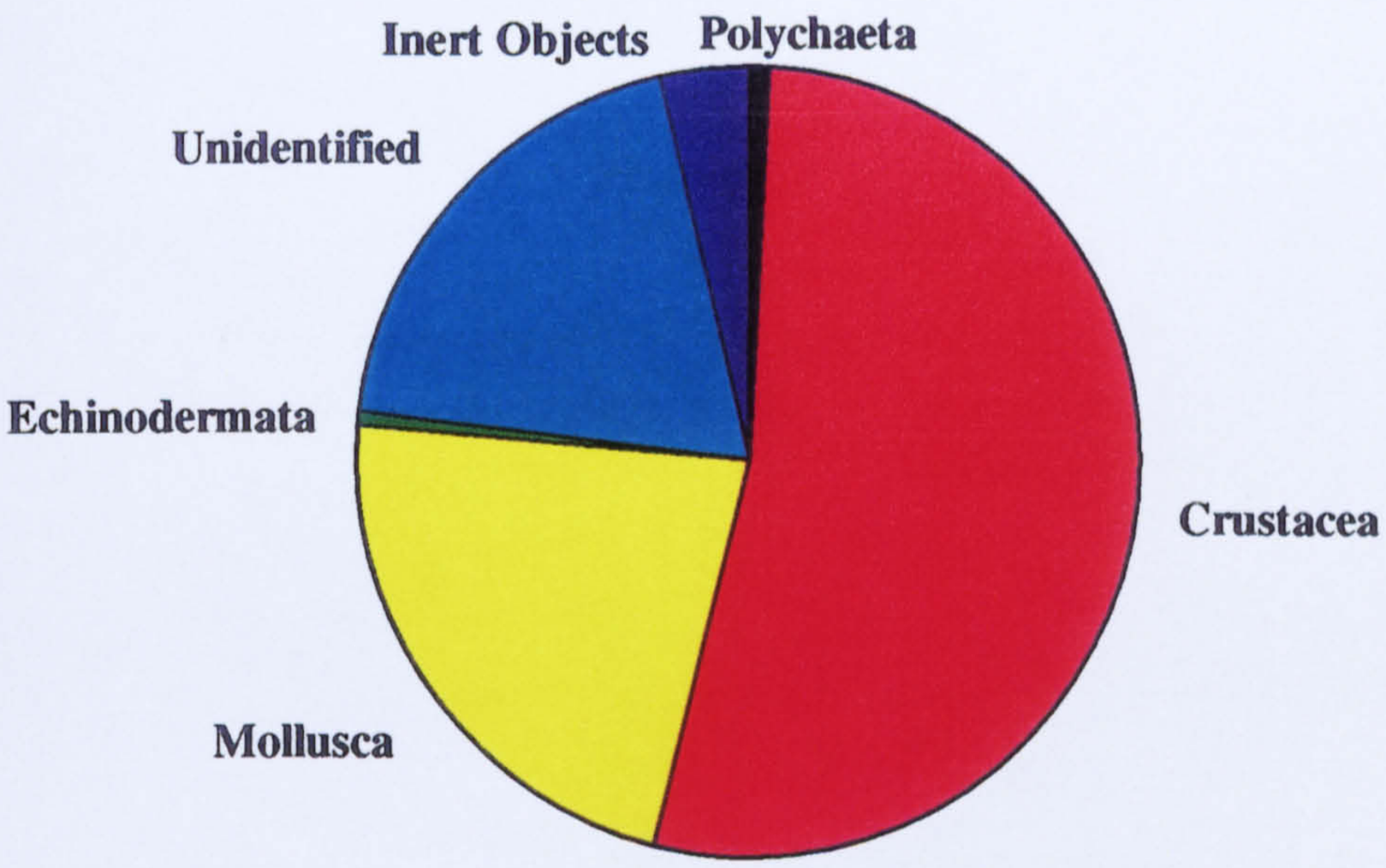


**Figure 2.3. Proportions of prey groups in the stomach contents of *Nephrops norvegicus* sampled from Little Cumbrae in May 1994. n=17.**

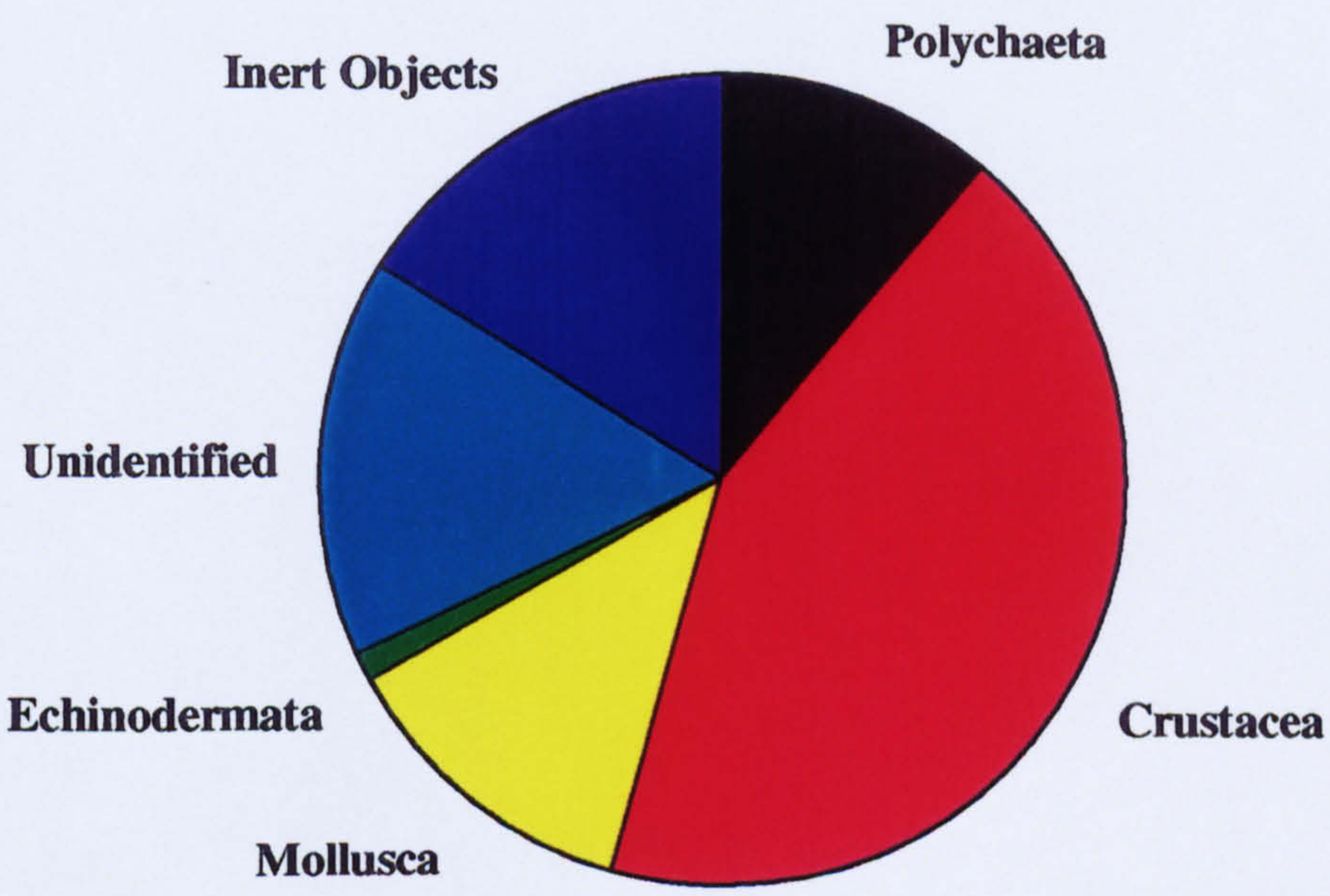


**Figure 2.4. Proportions of prey groups in the stomach contents of *Nephrops norvegicus* sampled from Little Cumbrae in November 1994. n=25.**

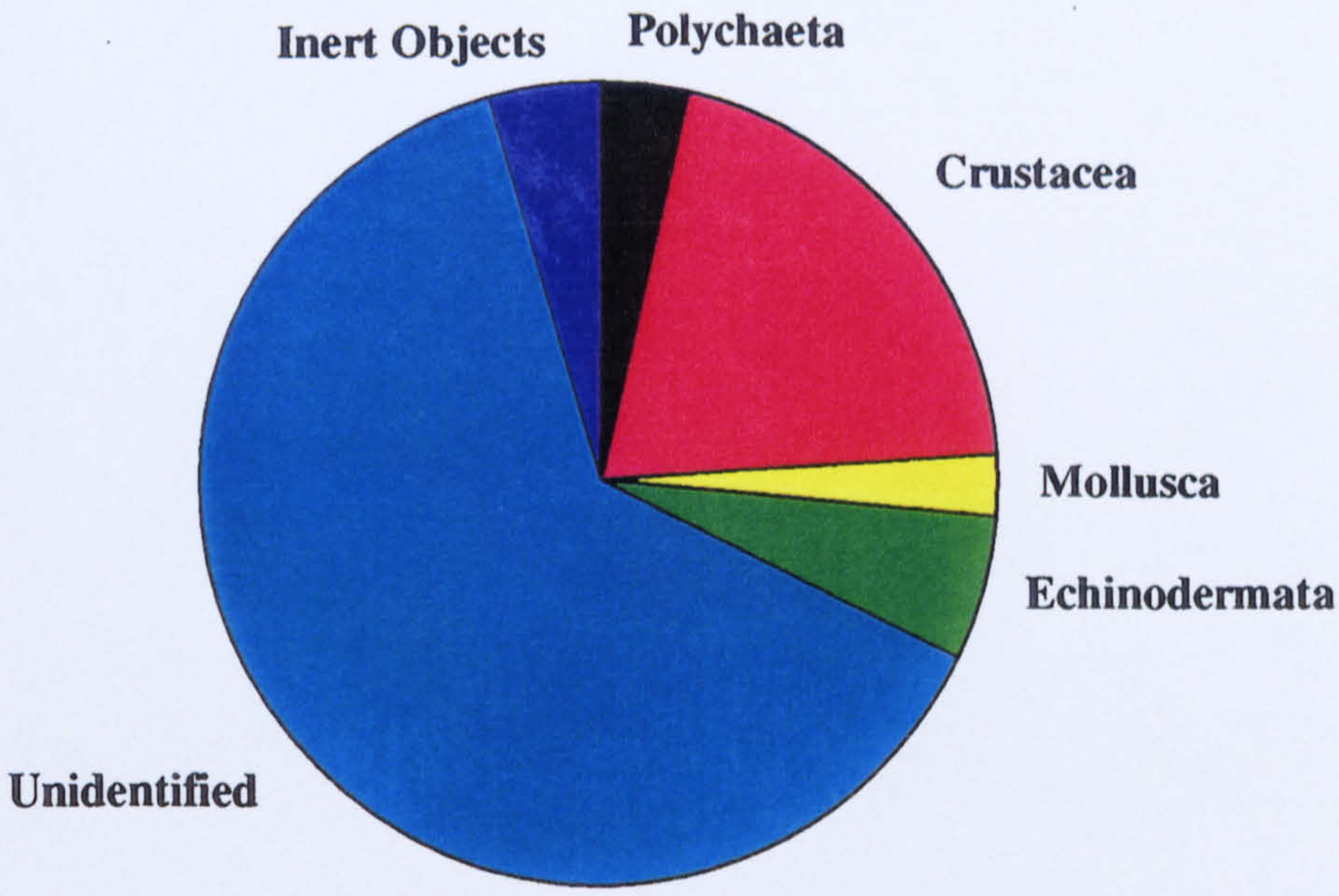


**Figure 2.5. Proportions of prey groups in the stomach contents of *Nephrops norvegicus* sampled from Ailsa Craig in May 1994. n=24.**

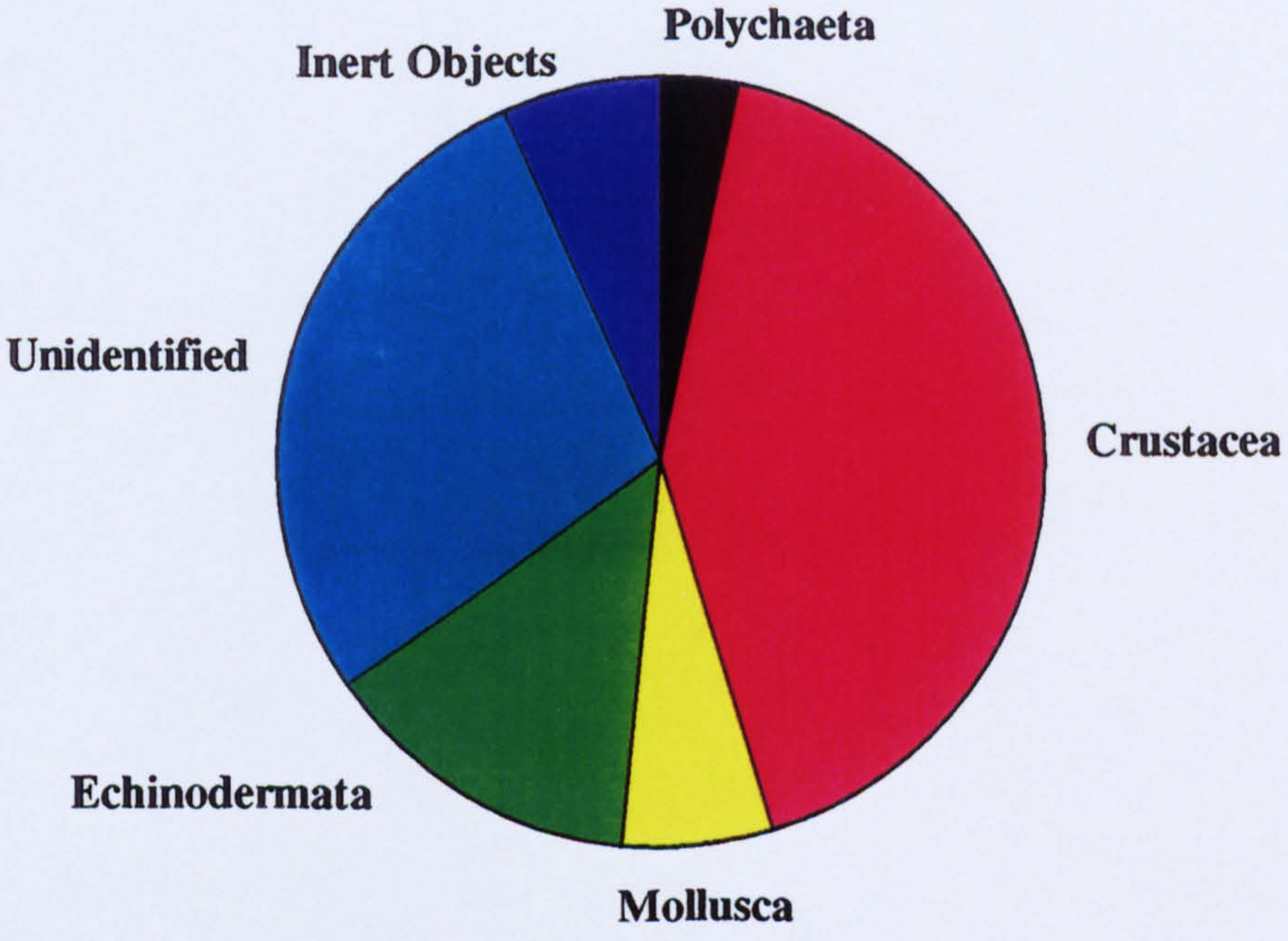


**Figure 2.6. Proportions of prey groups in the stomach contents of *Nephrops norvegicus* sampled from Ailsa Craig in November 1994. n=25.**

2.3.4. Investigation of temporal variation in food consumption and calculation of a daily total

The weights of dried-stomach contents at the various sample intervals throughout the 24h period are illustrated in Figure 2.7. The amounts of food consumed in each of these periods were estimated and are illustrated in Figures 2.8 and 2.9. There was a significant temporal influence on the amount of food consumed. This was determined using the estimation methods of Thorpe (1977) (ANOVA, $F_{263,5}=3.05$, $P<0.05$) (Figure 2.8) and Elliott & Persson (1978) (ANOVA, $F_{263,5}=2.64$, $P<0.05$) (Figure 2.9). The greatest amount of food consumption occurred after dark, between 2200h and 0200h, and at dawn, between 0600h and 1000h. The lowest amount of food consumption occurred at about mid-afternoon, between 1400h and 1800h, which was shown to be significantly different (Tukey's multiple comparison test, $P<0.05$) from the amount of food consumed during the period of 2200h-0200h. The daily total amount of food consumed was estimated by adding together all of the values of food consumed that were obtained for each 4h feeding period over the whole 24h period. The totals were estimated as 175.85mg per day (dry weight) using the method of Thorpe (1977) (Table 2.6) and 263.4mg per day (dry weight) using the method of Elliott & Persson (1978) (Table 2.7).

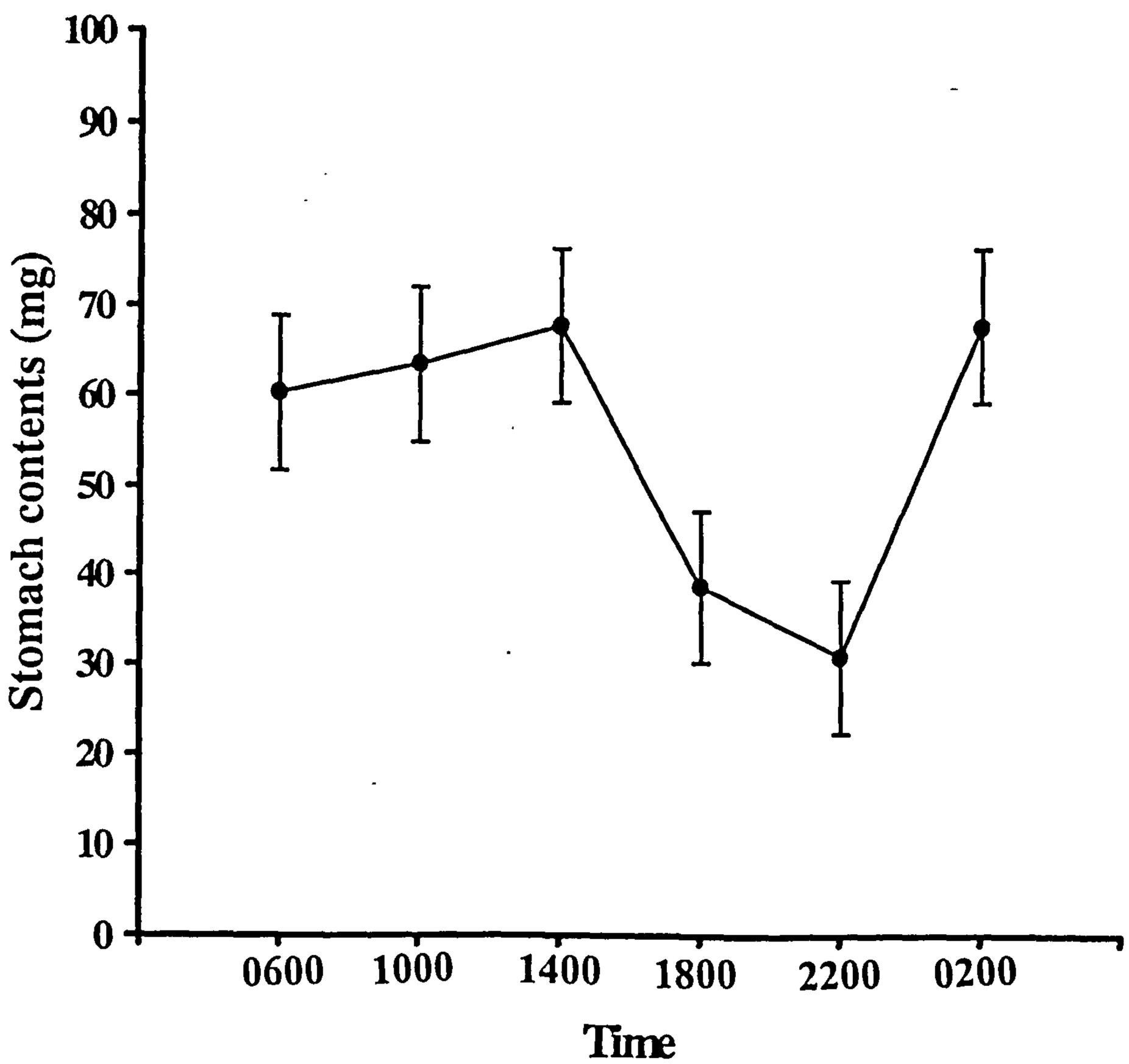


**Figure 2.7. Diel variation in the dry weight of stomach contents of *N. norvegicus*. Bars represent standard error. Number of animals sampled for each time period ranged from 20 to 56.**

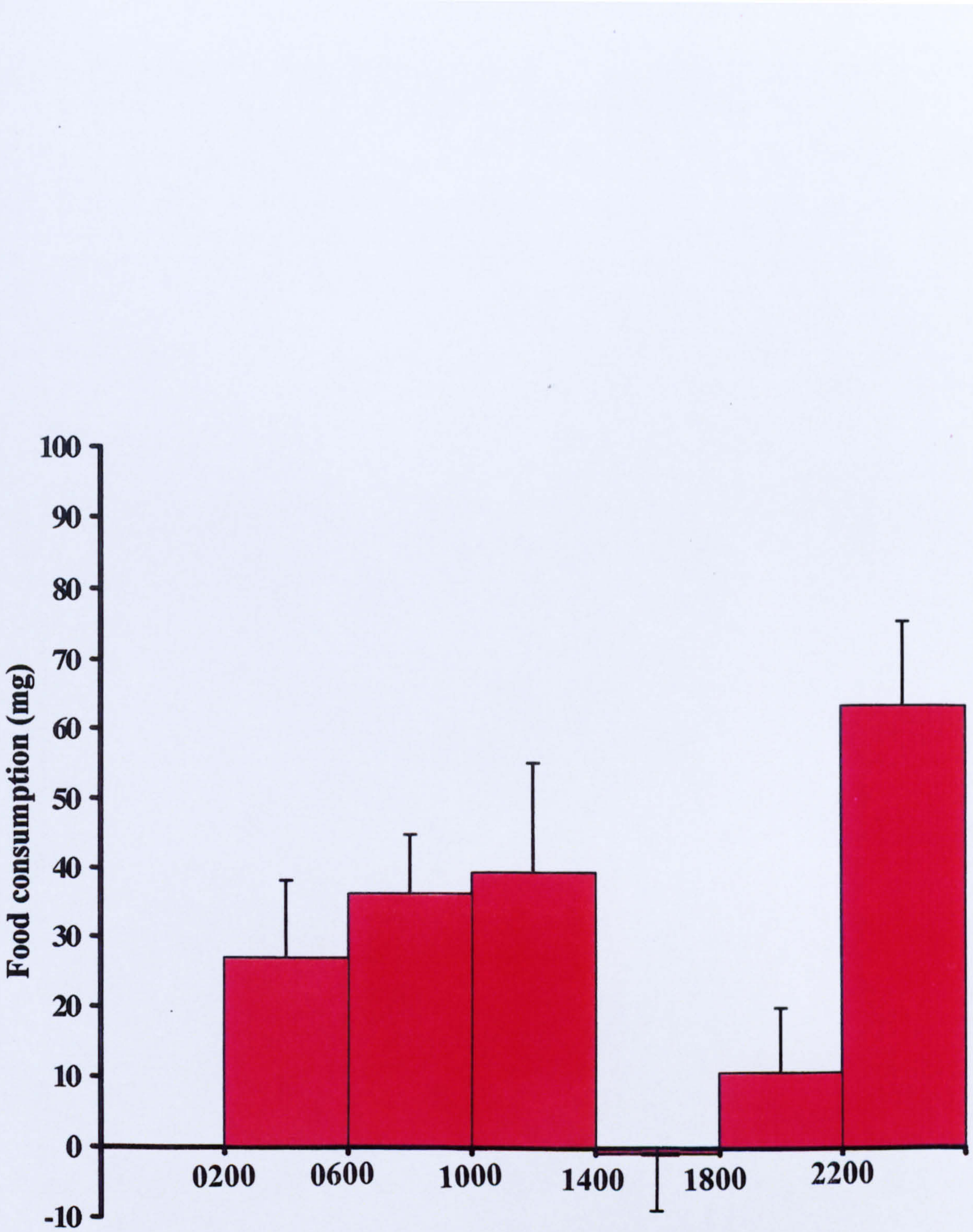


**Figure 2.8. Diel variation in the amount of food consumed (mg dry weight) by *Nephrops norvegicus* estimated using the method of Thorpe (1977). Bars represent standard error. Number of animals sampled for each time period ranged from 20 to 56.**

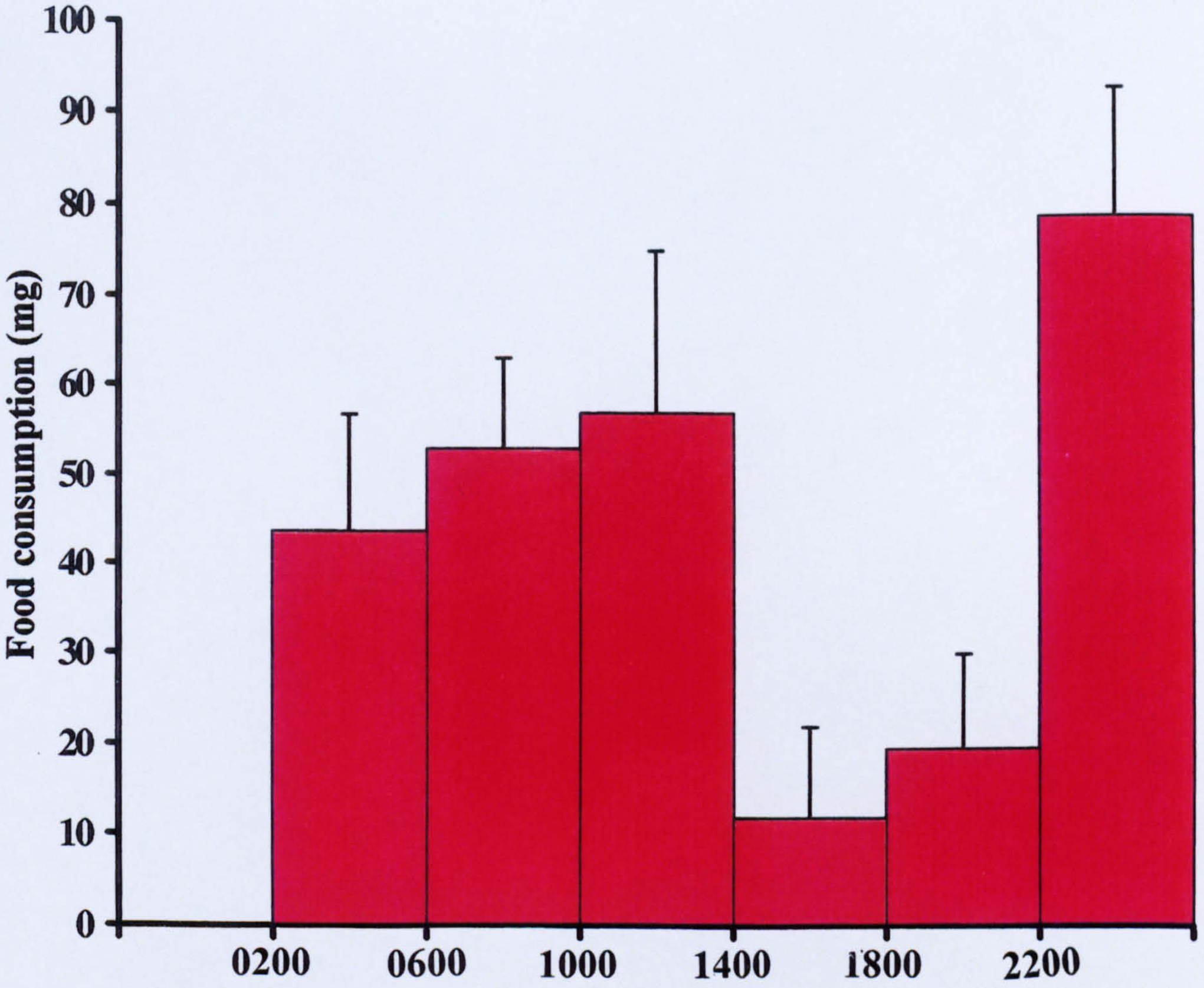


**Figure 2.9. Diel variation in the amount of food consumed (mg dry weight) by *Nephrops norvegicus* estimated using the method of Elliott & Persson (1977). Bars represent standard error. Number of animals sampled for each time period ranged from 20 to 56.**

2.3.5. Geographical variation and the effects of sampling month on total daily food consumption

Estimates of the amount of food consumed by a standard 26.2g *N. norvegicus* (average weight used in the 24h estimation) calculated using the method of Thorpe (1977) are presented in Table 2.6, those calculated using the method of Elliott & Persson (1978) are presented in Table 2.7. Two-way ANOVA showed that there was a significant influence of sampling month on food consumption. Although the estimates of food consumption for *N. norvegicus* from Little Cumbrae were higher than those from Ailsa Craig in both May and November, there was no significant effect of sample site (ANOVA, $F_{87,1}$=0.27, P=0.602). There were no significant interactions between site and month (ANOVA, $F_{87,1}$=0.04, P=0.851).

**Table 2.6. Calculation of the total daily food intake of *N. norvegicus* (mean weight 26.2g) from two sites in the Firth of Clyde using method of Thorpe (1977).**

| | Complete 24h estimation | Little Cumbrae | | Ailsa Craig | |
|---|---|---|---|---|---|
| | | May | Nov | May | Nov |
| **Mean weight of stomach contents in 1000h sample (mg dry weight)** | 63.48 | 66.5 | 43.9 | 64.4 | 39.5 |
| **Mean percentage of 1000h sample compared to complete 24h experiment** | 100 | 104.8 | 69.2 | 101.5 | 62.2 |
| **Predicted daily intake (mg dry weight) (standard error)** | 175.9 | 192.1 (19.5) | 126.9 (15.4) | 186.1 (20.9) | 114.0 (15.5) |
| **Predicted daily intake (mg wet weight) (standard error)** | 738.9 | 803.1 (81.5) | 530.4 (64.4) | 777.7 (87.3) | 476.5 (64.9) |

**Table 2.7. Calculation of total daily food intake of *N. norvegicus* (mean weight 26.2g) from two sites in the Firth of Clyde using method of Elliott & Persson (1978).**

| | Complete 24h estimation | Little Cumbrae | | Ailsa Craig | |
|---|---|---|---|---|---|
| | | May | Nov | May | Nov |
| **Mean weight of stomach contents in 1000h sample (mg dry weight)** | 63.48 | 66.51 | 43.92 | 64.41 | 39.48 |
| **Mean percentage of 1000h sample compared to complete 24h experiment** | 100 | 104.77 | 69.19 | 101.47 | 62.19 |
| **Predicted daily intake (mg dry weight) (standard error)** | 263.4 | 287.8 (29.2) | 190.1 (23.1) | 27.87 (31.3) | 170.8 (23.2) |
| **Predicted daily intake (mg wet weight) (standard error)** | 1089.9 | 1202.9 (122.1) | 794.5 (96.5) | 1164.9 (130.8) | 713.8 (97.14) |

## 2.4. DISCUSSION

There were marked interspecific differences in the energy contents of *N. norvegicus* prey organisms. A Norway lobster would receive over double the amount of energy from a glycerid polychaete than from a brittle star of equivalent mass. It was also demonstrated that the energy content of marine invertebrate prey can be accurately predicted by their proportion of organic matter. Taxa with low proportions of organic matter such as echinoderms, which contain large numbers of calcareous granules in their tissue, tended to have lower energy contents. Similarly, the lower energy content of the polychaetes *Lagis koreni* and *Aphrodita aculeata* can be explained by their body composition. *L. koreni* is characterised by a comb of stout cephalic spines and a chitinous plug which it uses to block the end of its tube, and *A. aculeata* is covered dorsally with scales overlaid with dense setae, thus it is likely that the low energy content of these body parts would reduce the average energy content for the whole animal. Similar trends were found by Wacasey & Atkinson (1987) and Thayer *et al.* (1973), measuring the energy contents and organic contents of a number of North Atlantic invertebrates. They stated that since the relationship was so strong, it may not even be necessary to go through the time-consuming procedures associated with bomb calorimetry if the organic contents are known. It is likely that the energy content of many of the prey organisms changes during the course of the year, as a result of factors such as cycles in reproductive status and changes in diet. Unfortunately, the difficulties involved in obtaining some of the prey organisms in this study, prevented an investigation of seasonal influences on their condition. In some

cases, it took a number of sampling trips over several months to acquire sufficient samples for analysis.

The greatest proportion of the diet of *N. norvegicus* was contributed by crustaceans. Other studies have also found that they form a significant part of the diet of other Crustacea. Wolcott (1978) observed that the mole crab *Emertia talpoidea* represented 62% of energy intake of the ghost crab *Ocypode quadrata.* Similarly, Hill (1979) reported that the crab *Ceratophthalmus algoense* contributed a large proportion to the diet of the crab *Scylla serrata.* The Crustacea preyed upon by *N. norvegicus* included mostly other decapods such as the thalassinidean mud-shrimps *Jaxea nocturna, Callianassa subterranea* and *Calocaris macandreae.* Although feeding on Crustacea does not give the highest energetic return, the consumption of closely related organisms such as mud-shrimps would provide an excellent source of minerals such as calcium which are important for the synthesis of the exoskeleton. A similar suggestion was made by Ennis (1993) from his study of the American lobster, *Homarus americanus.* He noted that there were seasonal changes in the proportion of calcium-rich organisms in the diet of the lobster, which were thought to correlate with moulting.

The molluscs found in the stomachs of Scottish *N. norvegicus* were predominantly bivalves of the genera *Abra* and *Nuculoma.* They were identified in stomachs by their shell fragments. Personal observations of feeding on aquarium held animals have shown that *N. norvegicus* crack open bivalve shells using the crusher claw and consume the tissue within. Furthermore, it was noted that the shell was not

deliberately eaten, small fragments tended to be ingested as they were attached to the adductor muscle. The proportion of bivalves was higher in the diet of *N. norvegicus* from south of Little Cumbrae than in the diet of *N. norvegicus* from south of Ailsa Craig which contained larger proportions of echinoderms. This appears to reflect the faunal composition of the areas, since the benthos south of Little Cumbrae has been found to be dominated by bivalve molluscs, whereas other prey such as crustaceans, echinoderms and polychaetes form a greater part of the faunal composition of grounds around Ailsa Craig (IMBC, UMBSM & IRPEM, 1994). This supports studies discussed earlier in this Chapter (Thomas & Davidson, 1962; Oakley (1981); Bailey *et al.*, 1986; and Goodheir, 1995) which suggested that *N. norvegicus* is an opportunistic predator,

The predator-prey relationship between decapods and molluscs (see reviews by Lau, 1987; Juanes, 1992) has received considerably more attention than that of decapods and any other prey taxa. This is largely due to the fact that they were used in investigations into optimal foraging behaviour. Early observations of decapod Crustacea feeding on molluscs appeared to suggest that they select an intermediate size of bivalve in order to maximise their energy intake (Elner & Hughes, 1978; Hughes & Seed, 1981). More recent studies have suggested however, that size-selectivity is more complex than originally thought. Juanes & Hartwick (1990) proposed that potential damage to claws as a result of cracking open mollusc shells could also be a limiting factor in prey-size selection. After reviewing a number of studies examining crab and lobster predation on molluscs, Juanes (1992) came to the conclusion that predatory decapods prefer small-size molluscan prey. Since

molluscan shell strength increases exponentially with shell thickness, mass, length and shape, it could be deduced that crabs and lobsters are selecting smaller shells for their ease of opening, or to limit claw damage.

The proportions of polychaetes in the stomachs of *N. norvegicus* were lower than those of crustaceans and molluscs. However, since polychaetes are soft-bodied and rapidly assimilated by *N. norvegicus* (Sarda & Valladres, 1990), these values may considerably underestimate their share of diet. They could form a large proportion of the material which remained unidentified, particularly in *N. norvegicus* from Ailsa Craig, where polychaetes form a large part of the local infauna (IMBC, UMBSM & IRPEM, 1994).

Although there were no significant size differences between the samples of *N. norvegicus* examined in this study, Goodheir (1995) noted that animal size can have an influence on the range of prey eaten. Smaller *N. norvegicus* were found to have consumed a greater proportion of polychaetes than larger conspecifics. It is possible that this may be due in part to mechanical reasons, since although molluscs and crustaceans could be readily abundant, the energetic cost and risk of claw damage may deter the smaller *N. norvegicus* from preying on them. Soft-bodied polychaetes are relatively less damaging to eat and would also give a higher energy return than molluscs.

The model of *N. norvegicus* feeding constructed in this chapter suggests that foraging occurs throughout most of the 24h, with the greatest amount of food being consumed

during the night. A smaller increase in food consumption was also observed in the morning, after dawn. This information is consistent with a number of other studies which have suggested that there may be an endogenous rhythm to the foraging activity of *N. norvegicus* (Aréchiga & Atkinson, 1975; Atkinson & Naylor, 1976; Chapman & Howard, 1979). This rhythm is thought to be largely associated with co-ordinating activity to an optimal range of light intensity, which on most commercial grounds is dawn and dusk. In shallower waters, foraging becomes nocturnal and in deeper waters with limited illumination, the optimal light conditions can be limited to the middle of the day (Chapman, 1980).

Work on *H. americanus* has shown that their feeding behaviour is carefully orchestrated in order to minimise exposure to predators (Lawton, 1987). Using time-lapse photography, lobsters were observed to collect individual mussels (*Mytilus edulis*), and immediately carry them back to shelter where they were consumed. Although the information on the foraging strategies of *N. norvegicus* is limited, there is some evidence to suggest that *N. norvegicus* may employ a similar predator-avoidance strategy. Core samples of the sediment taken around the openings to *N. norvegicus* burrows have been found to contain a higher biomass than the surrounding mud (McIntyre, 1973 possibly implying that prey may be captured and cached for later consumption. It may also be possible their foraging patterns are timed to coincide with the activity of certain prey items. Organisms such as other decapods and errant polychaetes may only be found above the sediment at certain times, such as during darkness. In contrast, less active prey such as bivalve molluscs and echinoderms could potentially be preyed upon anytime.

In a review of different methods of estimating food consumption from field samples, Hall *et al.* (1995) noted that some models predict negative food consumption. A negative rate of food consumption for the time period 1400h-1800h (GMT) was noted in this study using the method of Thorpe (1977). This occurred since the negative difference in stomach contents between two successive samples was greater than could be accounted for by the rate of gastric evacuation. Hall *et al.* (1995) explained that this did not necessarily mean that the animals were actually regurgitating food, and that it was more likely to be due to inadequacies in the predictive model, such as underestimating the rate of gastric evacuation, or an insufficient sampling frequency. Furthermore, other workers (Jobling, 1981; Elliott & Persson, 1978) commented that the Thorpe (1977) method was prone to underestimating food consumption. Elliott & Persson (1978) also noted that the duration of the sampling interval was crucial in determining the accuracy of food consumption models. Results from an experiment in which the actual amounts of food ingested were carefully measured, showed that estimated values for sampling periods of 2-3h were very close to the observed experimental values. However, when the sampling interval was increased to 6h the agreement was much poorer. In this study, the 4h sampling interval was about as short as possible, given the practical difficulties of animal collection.

The total daily amounts of food estimated to be consumed by *N. norvegicus* (fresh weight=26.2g) ranged from 476.5mg (wet weight).day$^{-1}$ at Ailsa Craig in November (using the method of Thorpe, 1977) to 1202.9mg (wet weight).day$^{-1}$ at Little

Cumbrae in May (using the method of Elliott and Persson, 1979). Although the food consumption of the Little Cumbrae stocklet was estimated to be greater than that of Ailsa Craig in both May and November, the difference was not significant. This could be a result of the large individual variation in stomach contents, which would have made it difficult to detect any differences statistically. Moreover, the data in this study only gave a rough estimate of temporal and geographical differences in diet. Ideally, full 24h sampling programmes would have been used to investigate the relationship between feeding and both seasonal variation and geographical location, in more detail. However, despite the individual variation, the values obtained are reasonably similar to those measured on laboratory *N. norvegicus* by Sarda & Valladres (1990) of approximately 650$mg^{-1}$.day (wet weight) for a 26.2g wet weight animal, which gives some credence to the accuracy of the model used in this study.

## 2.5. SUMMARY

- The diet of the Norway lobster *Nephrops norvegicus* in the Firth of Clyde consists of crustaceans, molluscs, and to a lesser extent polychaetes and echinoderms. However, a large proportion of the food was still found to be macerated beyond recognition.

- The proportion of bivalves was higher in the diet of *N. norvegicus* from south of Little Cumbrae than in the diet of *N. norvegicus* from south of Ailsa Craig which contained larger proportions of echinoderms. This appears to reflect the fauna composition of the areas

- There were marked interspecific differences in the energy contents and organic contents of prey organisms. The calorific values of the prey items were found to vary according to the proportion of organic matter in the tissue. Organisms with low organic contents such as brittle stars had lower calorific values than organisms with a large organic proportion such as soft-bodied polychaetes.

- Amounts of food consumed were calculated in 4h intervals over a 24h period. Results suggested that foraging is highest during the night at 2200h-0200h. A smaller increase in food consumption was also noticed during the morning at 0600-1000h and 1000h-1400h. The lowest amount of food was consumed between 1400h-1800h.

- Two different models were used to estimate the daily amounts of food consumption. The values obtained ranged from 476.5mg $day^{-1}$ (wet weight) at Ailsa Craig in November to 803.1 mg $day^{-1}$ (wet weight) at Little Cumbrae in May using the Thorpe (1977) method. Although these values were closest to the laboratory estimate of Sarda & Valladres (1990), this model predicted negative consumption for the time period of 1400h-1800h. The values obtained using the method of Elliott & Persson were higher, ranging from 713.8mg $day^{-1}$(wet weight) at Ailsa Craig in November, to 1202.9mg $day^{-1}$ (wet weight) at Little Cumbrae in May. There were no negative estimates of food consumption using this method.

# CHAPTER 3

# Metabolic rate and ecological energetics of the Norway lobster

# *Nephrops norvegicus*

## 3.1. INTRODUCTION

### 3.1.1. Metabolic rate

Measurement of the metabolic rate of an organism can provide a considerable amount of biological information. Changes or even the lack of changes in metabolic rate, under controlled experimental conditions, can enable the evaluation of the physiological adaptations of an organism. This has been demonstrated by many authors working on Crustacea, who have found that a variety of environmental and biological factors, such as temperature (Dalla Via, 1985), salinity (Jury *et al.*, 1994; Taylor, 1977), pH (Ultsch, 1980), pollution (Smith & Hargreaves, 1984), partial pressure of oxygen (Bridges & Brand, 1980), tidal cycles (Wallace, 1972), starvation (Marsden *et al.*, 1973), parasitic infection (see Chapter 6 and Taylor *et al.*, 1996), diet (Hewitt & Irving, 1990), sex (Jury *et al.*, 1994) and moult stage (Alcaraz & Sarda, 1981) influence the metabolic rate. Metabolic rate may also play an important part in social hierarchies. Although studies that have linked metabolic rate to social rank in crustaceans are lacking, work by Cutts (1997) on juvenile salmon *Salmo salar*, has shown that individuals with higher relative standard metabolic rates are more likely to acquire dominance. Metabolic rates can also be used in energetic studies. Oxygen consumption can be translated into energy utilisation using a conversion factor known

historically as the 'oxycalorific coefficient' (Bayne *et al.*, 1985). The value (in joules) can then be used to estimate the metabolic expenditure in energy budget equations.

Metabolic rate can easily be determined by means of heat evolution, oxygen depletion or carbon dioxide liberation (Bayne *et al.*, 1985). The most common method for aquatic invertebrates and fish is to measure the oxygen consumption ($MO_2$) of an organism contained in a respirometer. The design of such apparatus can vary considerably, both closed and flow-through designs are used and possess their own distinct advantages and disadvantages (Steffensen, 1989). The problem with closed systems is that during the course of the experiment, the test organism depletes environmental oxygen, which can itself markedly influence the metabolic rate (e.g. Bridges & Brand, 1980). In contrast, the influx of aerated water in flow-through systems enables the environmental partial pressure of oxygen to be maintained at a constant level. However, a potential problem associated with these systems is that they take time to stabilise, making it difficult to detect changes in metabolic rate over short time scales (Steffensen, 1989).

The total metabolic rate is made up of a number of components: a resting or standard metabolism, a post-feeding metabolism, and a metabolism associated with a high degree of activity. The standard metabolic rate is the metabolic rate of minimal maintenance, at which the animal is resting and not feeding. It can be determined by directly measuring the oxygen consumption of fasted, quiescent individuals, or by extrapolating measurements of $MO_2$ at varying levels of activity back to zero. Following a meal an increase in the metabolic rate occurs, resulting from requirements

for digestion, nutrient absorption and storage, deamination of amino acids and synthesis of excretory products, and for the turnover and deposition of tissue components (Houlihan, 1991). This characteristic post-prandial rise in metabolic rate was originally referred to as 'Specifische-Dynamische Wirkung' (Rübner, 1902 cited in Nelson *et al.*, 1985), which has subsequently been translated to specific dynamic action (SDA). SDA has been reported to last from several hours to 4-5 days (Crisp, 1979), although this largely depends on the quantity and quality of the diet (Jobling & Davies, 1981; Ross *et al.*, 1992). In general, SDA usually accounts for approximately 10% of the ingested energy, for example, Du Preez *et al.* (1992) working on the shrimp *Penaeus monodon* found that the calorigenic effect of food was between 2.0-19.5%.

The maximum metabolic rate shown by an animal is referred to as the active metabolic rate. There have been numerous studies that have measured this level of activity, and many have been based on fish (see review by Priede, 1985) and aquatic invertebrates, including the mollusc *Littorina littorea* (Newell & Pye, 1971), and the decapod crustaceans *Pacifastacus leniusculus* (Rutledge & Pritchard, 1981), *Panulirus interruptus* (1960) and *Palaemonetes vulgaris* (McFarland & Pickens, 1965). It is thought that both the standard and active metabolic rates of an organism are fixed (Priede, 1985). The resulting difference between these two levels of activity is termed the 'scope for activity' (Fry, 1947 cited in Rutledge and Pritchard, 1981). This metabolic scope represents the confines within which the organism must carry out aerobic activities such as digestion, locomotion and growth, which are additional to

standard metabolism. It can thus provide an indication of an organism's capacity for sustained aerobic activity.

### 3.1.2. Energy Budgets

Energy budget equations are based on the premise that there is a flux of energy and matter between organisms and their surroundings. Ingested resources (C) can be invested in the synthesis of tissues (P) or utilised as fuel for physiochemical activity (R). Alternatively, resources may be lost in the faeces (F), nitrogenous excretions such as ammonia and/or urea (U), and in the case of arthropods, a small proportion of energy may also be lost when the old exoskeleton is discarded during moulting. Thus the various pathways of energy flux may be simplified into the following equation:

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

Energy budgets are useful tools for biologists and are used extensively in agriculture. Their use in poultry and ruminant farming is particularly well advanced (MAFF, 1974, 1975). The primary aim of constructing energy budgets is often to predict the effects of changes in the animal's lifestyle on growth. These changes could range from simple alterations in the diet to chronic stresses resulting from handling or exposure to toxicants. Information on the effects of these factors is especially useful to aquaculturists because of the high densities and resulting stresses that their culture organisms are commonly held under (see review by Knights, 1985). Consequently, fish have been popular objects of study in the area of ecological energetics, and numerous energy budget equations have been constructed for them (e.g. Solomon and

Brafield, 1972; Hofer *et al.*, 1985; Cui & Jiankang, 1990 a,b,c,d). Energy budgets have also been compiled for invertebrates, although there are fewer studies than for fish. A number of these studies have been based on bivalve molluscs. Since it is difficult to determine growth directly for these organisms, measurement of energy available for growth, termed the 'scope for growth', has provided a convenient alternative. This technique has been successfully employed in investigating the biological effects of a number of pollutants (Bayne & Newell, 1983; Widdows, 1983; Widdows *et al.*, 1987; Widdows & Johnson, 1988). Other invertebrates for which energy budgets have been determined include the polychaete *Neanthes virens* (Kay & Brafield, 1973) and the barnacle *Balanus glandula* (Wu & Levings, 1978). Work on decapod crustacean energy budgets has mainly been concerned with optimising the rearing of commercially important species, such as *Penaeus monodon* (Kurmaly *et al.*, 1989) and *Homarus americanus* (Koshio *et al.*, 1992; Logan & Epifanio, 1978).

### 3.1.3. Chapter aims

The aim of this chapter is to estimate the oxygen consumption of *N. norvegicus* at standard and active levels. The effect of nutritional status on metabolism will also be investigated. The metabolic rates of animals from the Little Cumbrae and Ailsa Craig sites will also be compared. An energy budget for the Norway lobster *N. norvegicus* will be assembled using data on the food consumption rate (Chapter 2) and the metabolic expenditure. The others components will be determined using data from a published study on *Homarus americanus* (Logan & Epifanio, 1978). Although there are a number of limitations preventing the calculation of the complete equation, the budget should still give an indication of energy allocation in the Norway lobster *N.*

*norvegicus*. This will enable estimates of scope for growth to be made in relation to factors such as sampling site and season. In addition, since Loo *et al.* (1993) speculated that ovigerous females could supplement their nutritional requirements by suspension-feeding, a model of *N. norvegicus* utilising this feeding strategy will be constructed.

## 3.2. MATERIALS AND METHOD

### 3.2.1. Experimental animals

Adult *Nephrops norvegicus* were collected by trawling on grounds around the Isle of Cumbrae, Firth of Clyde, Scotland (Figure 2.1). Lobsters were returned to the University of Glasgow where they were maintained in aquaria containing circulating, aerated sea water (34‰, 10°C, 12:12h light:dark cycle) until required.

### 3.2.2. Standard oxygen consumption

The resting rates of oxygen consumption ($\mu mol.g^{-1}.h^{-1}$) of adult *Nephrops* of both sexes were determined using flow-through respirometry (Figure 3.1). All animals used were in the intermoult stage (Aiken, 1980), and starved for 48h before each experiment to eliminate potential complications resulting from moult stage effects or post-feeding metabolism. 49 animals were used, ranging in size from 5-105g (fresh body weight). The lobsters were introduced individually into clear 'Perspex' tubes of approximately 500ml capacity, through which fully aerated sea water (temperature=10°C; salinity=34‰) flowed at a low rate (<30 $ml.min^{-1}$), and left undisturbed overnight to settle.

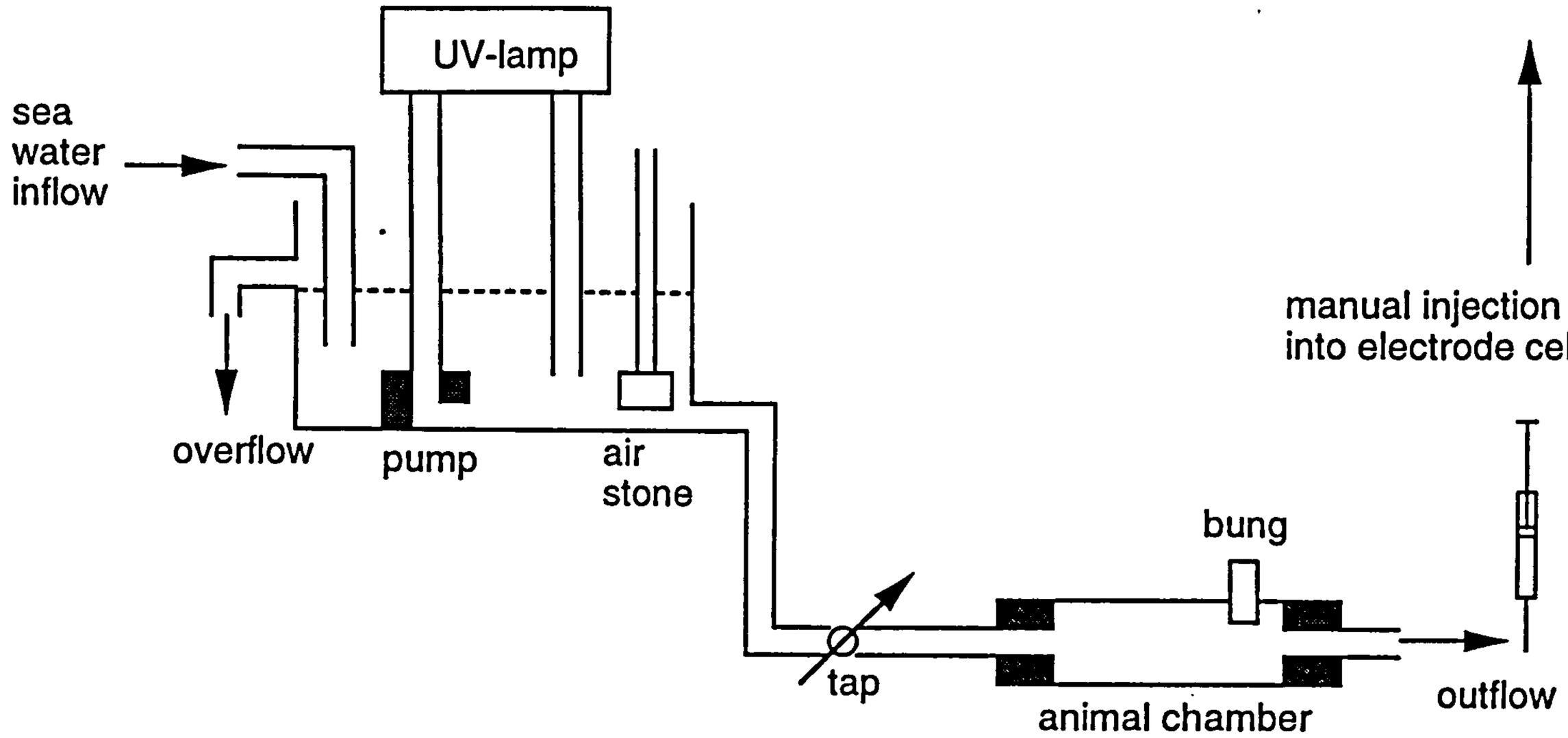


**Figure 3.1. Schematic diagram showing flow-through respirometry apparatus (not to scale).**

The sea water used in all the experiments was sterilised by exposure to UV light for approximately 1 h to eliminate microbial activity. The exact flow rate and the oxygen partial pressure ($PO_2$) of the sea water entering the respirometer and emerging from it were measured. Samples (approximately 0.5 ml) of the water leaving the respirometer were collected at regular intervals between 0800h and 1800h, allowing sufficient time between samples to ensure flush-out of tubes (Steffensen, 1989). Samples were taken by slowly drawing water into a 1ml syringe, using a needle inserted into respirometer outflows. The $PO_2$ of each water sample was determined by injecting samples into a glass cell (maintained at 10°C) containing a microcathode oxygen electrode which was in turn connected to an oxygen meter (Strathkelvin Instruments, Glasgow) and to a pen recorder. At the end of experiments, lobsters were removed from respirometry chambers, blotted dry and weighed. Throughout the course of the experiments, the respirometry chambers were regularly cleaned and sterilised in order to prevent bacterial growth. Furthermore, the electrodes were periodically serviced and calibrated against aerated sea water at the experimental temperature (10°C) and against a solution with a $PO_2$ of zero Torr (sodium sulphite in 0.01 M sodium tetraborate).

### 3.2.3. Active oxygen consumption

Active oxygen uptake of lobsters ($\mu mol.g^{-1}.h^{-1}$) was measured using a 'closed' respirometry system. Following a 2 minute period of activity, during which the animal was 'chased around a tank' in an attempt to induce the maximum rate of aerobic oxygen consumption (care was taken to ensure that no tail-flipping occurred), each lobster was placed in a 2l box-shaped Perspex respirometer containing seawater at a

temperature of 10°C and salinity of 34‰. An oxygen electrode (Strathkelvin Instruments, Glasgow) was inserted and the respirometer sealed. A magnetic stirrer ensured constant mixing of the water. The $PO_2$ of respirometer water was then measured over a 15min period. At no time during the experiment was the water allowed to become severely hypoxic; at all times, the oxygen was maintained at over 80% saturation. A measurement of the maximum rate of oxygen consumption was recorded three times for each animal. A blank run was performed at the end of each set of experiments to correct for microbial respiration. Changes in partial pressure of oxygen during this period of time were found to be negligible. Oxygen electrodes were calibrated prior to use against aerated sea water at the experimental temperature (10°C) and against a solution with a $PO_2$ of zero Torr (sodium sulphite in 0.01 M sodium tetraborate).

3.2.4. Effects of starvation on standard oxygen consumption

A sample of 30 *Nephrops*, all between 25-35mm carapace length, were placed inside plastic tubes (volume=2l), the ends of which were sealed with a cloth mesh (5mm). This was done to prevent cannibalism. The tubes containing lobsters were then placed in large plastic tanks. Measurements of standard oxygen consumption using flow-through respirometry as described above, were made on samples of 10 lobsters after 0, 8 and 12 weeks of starvation.

3.2.5. Effect of feeding on the $MO_2$ of previously starved animals

Measurements of standard oxygen consumption were made on the lobsters previously starved for 12 weeks. Following the final measurement, a 0.5g square of fresh squid

mantle tissue was fed to each lobster inside its tube. The lobsters were then left for 24h (to allow the lobsters to settle after being disturbed by the feeding), after which time the $MO_2$ was measured again.

3.2.6. Measurement of standard oxygen consumption in relation to sample site

Samples of *Nephrops norvegicus* were collected from the north Clyde Sea area, south of Little Cumbrae, and from the south Clyde Sea area, south of Ailsa Craig (see Figure 2.1). Lobsters were transported back to the aquarium at the University of Glasgow. The rate of standard oxygen consumption of 26 animals from Little Cumbrae and 25 animals from Ailsa Craig were measured using the flow-through respirometry method described earlier.

3.2.7. Energy Budget Equation for *N. norvegicus* in the Clyde Sea area

*Food consumption (C)*

The daily food consumption ($mg.day^{-1}$) was estimated in Chapter 2. Although a complete 24h sampling regime was only possible at the south of Little Cumbrae site on one occasion, stomach contents were examined during May and November at both the Little Cumbrae and Ailsa Craig sites to give some idea of seasonal and geographical variation. By comparing these single samples with the sample taken at the equivalent time (1000h) during the 24h sampling regime, and applying the same ratio to the total daily food consumption, an estimate of food intake at these times was obtained. These estimates were used in conjunction with the energy contents and proportion of each prey eaten to calculate the daily energy intake. The daily energy

intake ($kJ.day^{-1}$) for a 'standard' 26.2g *N. norvegicus* (from the average weight used in the previous chapter) was calculated by multiplying the estimated daily intake ($g.day^{-1}$) by the relative proportion of each dietary component and also by their respective energy content ($kJ.g^{-1}$). It should be noted that a small proportion of the stomach contents consisted of stones, spines, rope and various other inert objects. Since this material is largely indigestible, it was assumed to have an energy content of zero.

*Energy in metabolism (R)*

The total metabolic rate (R) is made up of a resting or standard metabolism ($R_S$), a post-feeding metabolism ($R_F$), and a metabolism associated with a high degree of activity ($R_A$) and can thus be represented as:

$$\mathbf{R = \alpha R_S + \beta R_A + R_F}$$

where the constants $\alpha$ and $\beta$ represent the proportion of the time that the animal spends at that level of metabolism. Since direct calorimetry or measurement of oxygen consumption could not be carried out in the field, approximations were made in this study by using a combination of laboratory experiments and published field observations. The standard and active levels of metabolism were measured in the laboratory, and the constants $\alpha$ and $\beta$ were estimated from field observations on *N. norvegicus* behaviour. There have been a number of studies of *N. norvegicus* behaviour, which concur in general, that animals are most active, i.e. they emerge from their burrows, during an optimal range of low-light intensity (Chapman & Rice,

1971; Aréchiga & Atkinson, 1975; Atkinson & Naylor, 1976; Chapman & Howard, 1979; Chapman, 1980). Since *N. norvegicus* on commercial fishing grounds tend to exhibit a crepuscular pattern of activity (Chapman, 1980), it was assumed in this study, that the animals were active for 12h of the 24h cycle, and quiescent for the remainder of this time. However, an additional model was constructed which incorporated a complete range of activity levels. The volume of oxygen consumed was translated into energetic equivalents by using the conversion factor of $4.83 \times 10^{-3}$ cal.$\mu lO_2^{-1}$ as used by Logan & Epifanio (1978) for *H. americanus*. These values were in turn converted into joules using the factor of 4.184 joules per calorie. The calorigenic effect of food ($R_F$) (also known as Specific Dynamic Action, SDA) is dependent on the quantity and quality of food intake (Jobling & Davies, 1981; Ross *et al.*, 1992). The value used in this study was 10% of ingested energy, taken from Logan & Epifanio (1978) working on the closely related *H. americanus*.

The total energy utilised in respiration was thus calculated using the following equation:-

$$\mathbf{R\ (kJ.day^{-1}) = 12R_S(kJ.h^{-1}) + 12R_A(kJ.h^{-1}) + 0.1C(kJ.day^{-1})}$$

*Energy lost in Faeces and Excreted Ammonia (F+U)*

Although ammonia excretion rates have been calculated for *N. norvegicus* (Hagerman & Uglow, 1985; Hagerman *et al.*, 1990), the amounts of food intake that these relate to are unknown. Kay & Brafield (1973) (working on *Neanthes virens*) found that excretion values were dependent on nutritional status, thus it was decided that it

would be more appropriate to calculate unassimilated energy using a previously calculated factor based on the food consumption. For consistency, as with the calorigenic effect of food, the energy lost in faeces and ammonia was also calculated using data on *H. americanus* from Logan & Epifanio (1978). The value used was 19% of consumed energy, C. This value is similar to the general model for carnivorous fish, of 20% of C, which was the value used by Miller *et al.* (1971) in an earlier version of a *H. americanus* energy budget.

*Energy utilised in growth (P)*

The energy invested in tissue synthesis was estimated by calculating the scope for growth. This was achieved by subtracting the values estimated for respiration, excretion and faeces from the amount of energy determined to be ingested in food.

3.2.8. Energy Budget Equation for *N. norvegicus* suspension-feeding on *Artemia*

Energy consumption was calculated by multiplying the clearance rates estimated by Loo *et al.* (1993) of 2,200-3,500 *Artemia*.$h^{-1}$, by the energy content of *Artemia* which was reported as 0.0495 J.individual$^{-1}$ (Kurmaly *et al.*, 1989). Since suspension-feeding *N. norvegicus* are thought to remain in their burrows, the energy expended as activity was assumed to be negligible, the calorigenic effect of food however, was calculated using the same method as above (10% of C).

## 3.3. RESULTS

### 3.3.1. Effect of sex

The relationship between wet weight and the mean resting weight specific oxygen consumption ($MO_2$) for male and female *Nephrops norvegicus* is shown in Figure 3.2. It was found that standard $MO_2$ decreased significantly with weight and regression lines could be fitted to the data using the expression $MO_2 = aw^{b-1}$, where the values of a and b-1 were the y intercept and slope respectively, derived from double-log regression. Analysis of covariance showed that there were no significant differences in the slope (ANCOVA $F_{1,46}$=1.26, NS) or elevation (ANCOVA $F_{1,47}$=0.01, NS) of the regression lines determined for the two sexes. Therefore, a combined regression was calculated:

**Std $MO_2$ ($\mu$mol $O_2$.g.$h^{-1}$)= 1.71 (wet weight)$^{-0.27}$ ($r^2$=43.3%, ANOVA, P<0.001)**

### 3.3.2. Effect of activity

The $MO_2$ of active lobsters also decreased with weight, although the relationship was weaker than with animals at a standard activity level (Fig. 3.3). The relationship could be described by the following equation:-

**Active $MO_2$ ($\mu$mol $O_2$.g.$h^{-1}$) = 6.60 (wet weight)$^{-0.25}$ ($r^2$=26.0%, ANOVA, P<0.05)**

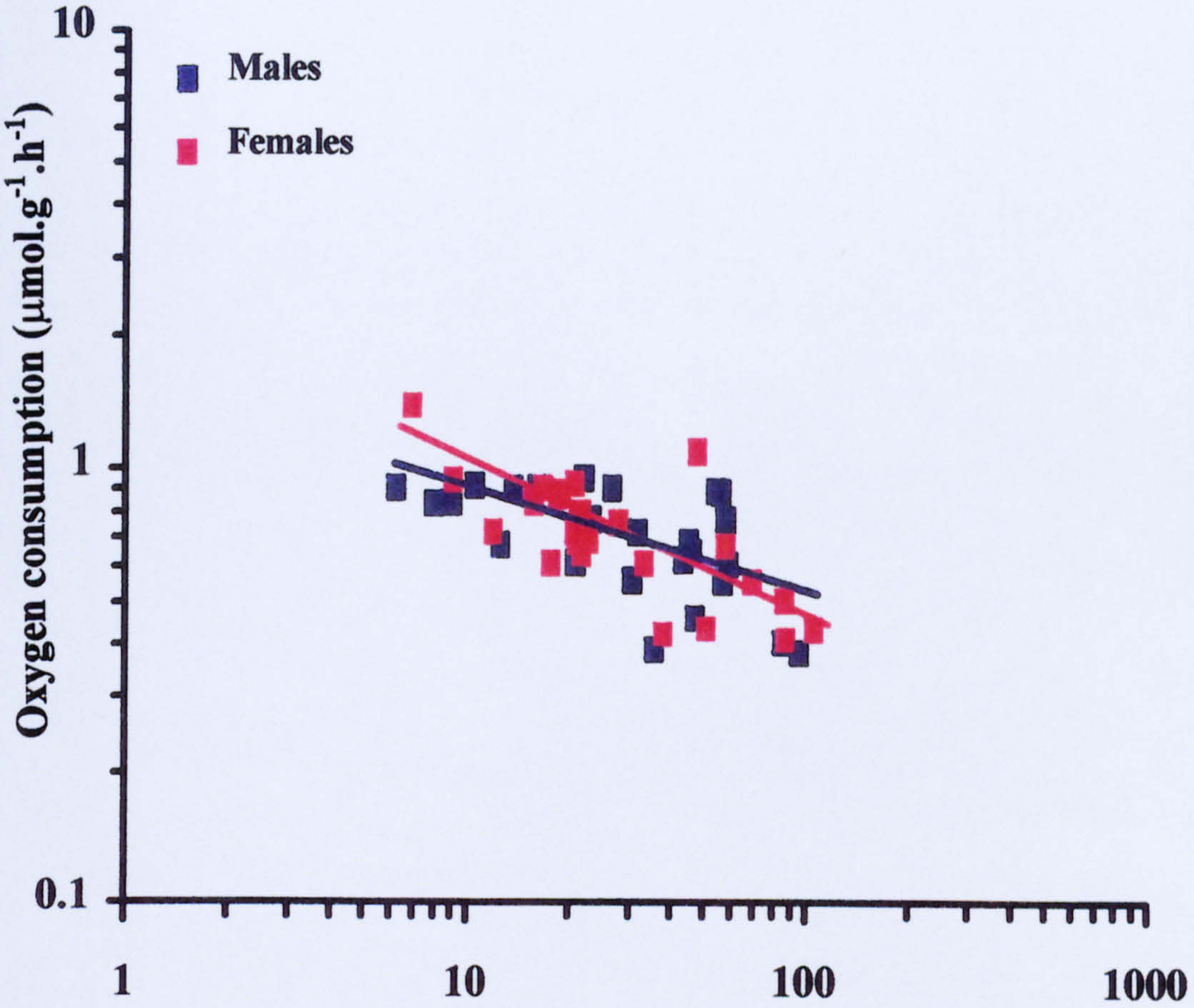


**Figure 3.2. A comparison of the relationship between fresh body weight (g) and weight-specific oxygen consumption ($\mu mol.g^{-1}.h^{-1}$) at 10°C, 34‰ salinity, in male and female *Nephrops norvegicus* from the Little Cumbrae site. Standard rates were calculated using flow-through respirometry. Lines represent the calculated regressions.**

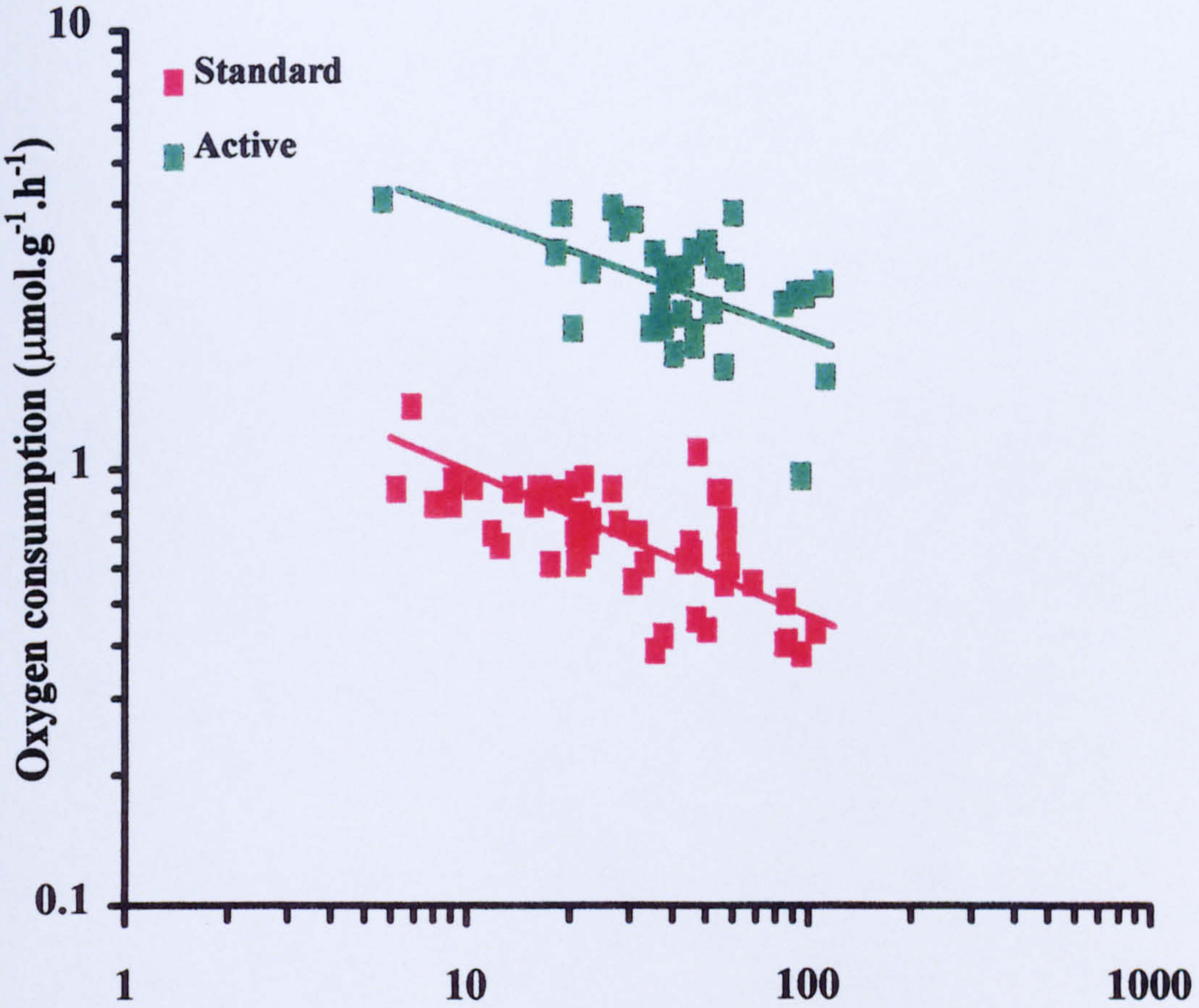


**Figure 3.3. A comparison of the relationship between fresh body weight (g) and weight-specific oxygen consumption ($\mu mol.g^{-1}.h^{-1}$) at 10°C, 34‰ salinity, in *Nephrops norvegicus* (from the Little Cumbrae site) at standard and active levels of metabolism. Standard rates were calculated using flow-through respirometry whereas active rates were calculated using closed-respirometry. Lines represent the calculated regressions.**

Analysis of covariance of resting and active animals revealed that while the two regressions did not differ in slope (ANCOVA $F_{1,78}$=0.84, NS), there was a significant difference in elevation (ANCOVA $F_{1,79}$=385.31, $p<0.001$). Active lobsters of a given weight had a significantly higher $MO_2$ than resting lobsters.

3.3.3. Effect of starvation and the effect of re-feeding on $MO_2$

The standard $MO_2$ of lobsters was significantly influenced by nutritional status (ANOVA, $F_{3,24}$=7.78, $P<0.005$) (Fig. 3.4). The mean $MO_2$ of lobsters measured after 8 weeks of starvation was significantly different from the initial mean $MO_2$ (Tukey pairwise comparison, $P<0.05$). However, there was no significant difference in the mean $MO_2$ between week 8 and week 12 (Tukey pairwise comparison, NS). 24h following feeding, the $MO_2$ in previously starved lobsters was found to almost double, and was no longer significantly lower than that measured at week 0, but was significantly higher than that measured after the animals had been starved for 8 and 12 weeks (Tukey pairwise comparison, $P<0.05$).

3.3.4. Results of geographical comparisons

Analysis of covariance showed that there were no significant differences in slope (ANCOVA $F_{1,47}$=0.14, NS) or elevation (ANCOVA $F_{1,48}$=0.32, NS) between the regression equations describing the relationship between $MO_2$ and weight of *N. norvegicus* from the Little Cumbrae and Ailsa Craig sites (Figure 3.5). Therefore, there are no significant differences between the $MO_2$ of *N. norvegicus* from Little Cumbrae and the $MO_2$ of animals of the same weight from the Ailsa Craig sites.

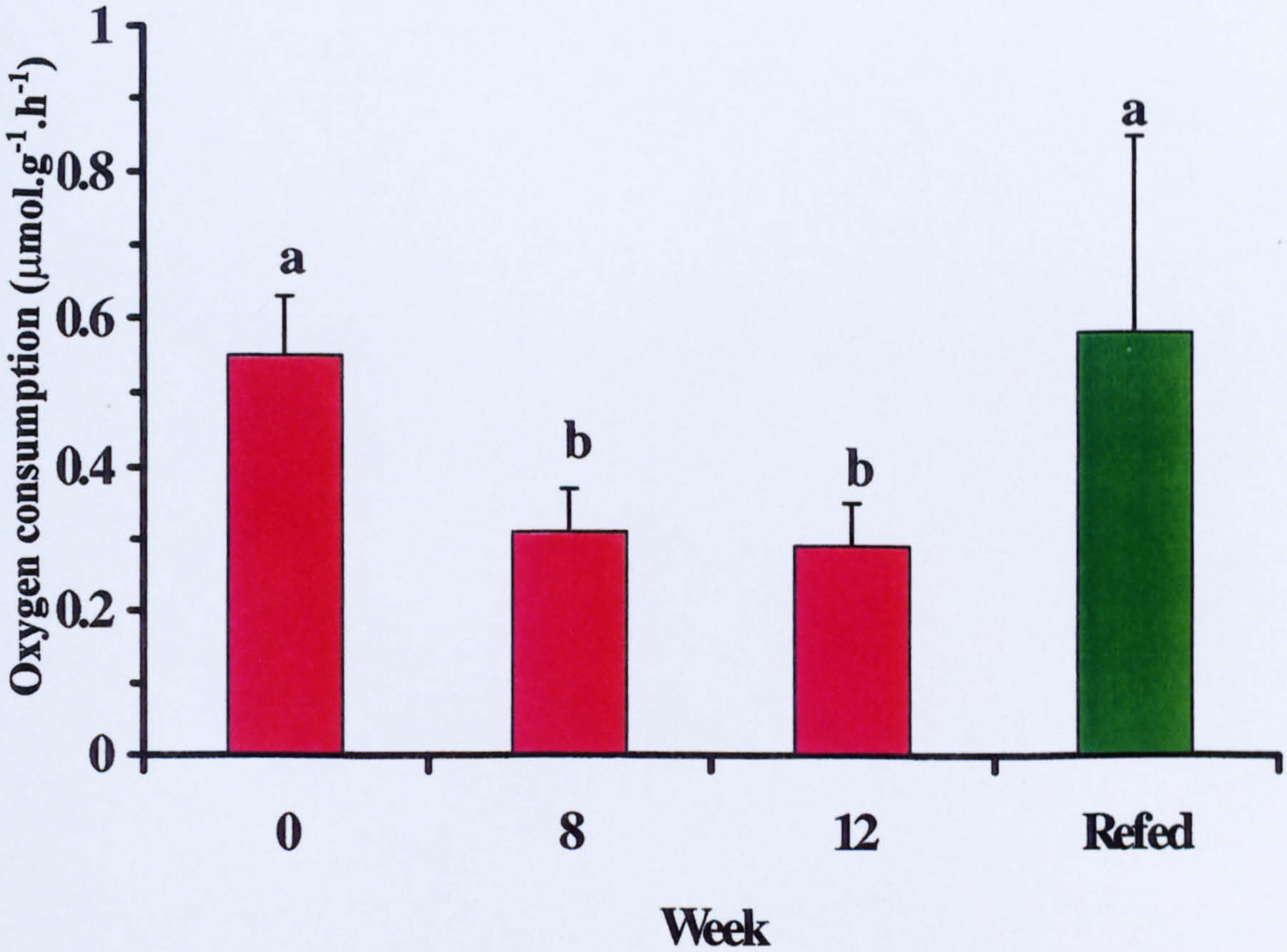


**Figure 3.4.** Graph illustrating the effects of starvation on the weight-specific oxygen consumption ($\mu mol.g^{-1}.h^{-1}$) at 10°C, 34‰ salinity, in *Nephrops norvegicus* from the Little Cumbrae site. Standard rates were calculated using flow-through respirometry. The effects of re-feeding on oxygen consumption were also measured (after 24h). Means +/- standard error are shown (n=10). Bars sharing the same letter are not significantly different.

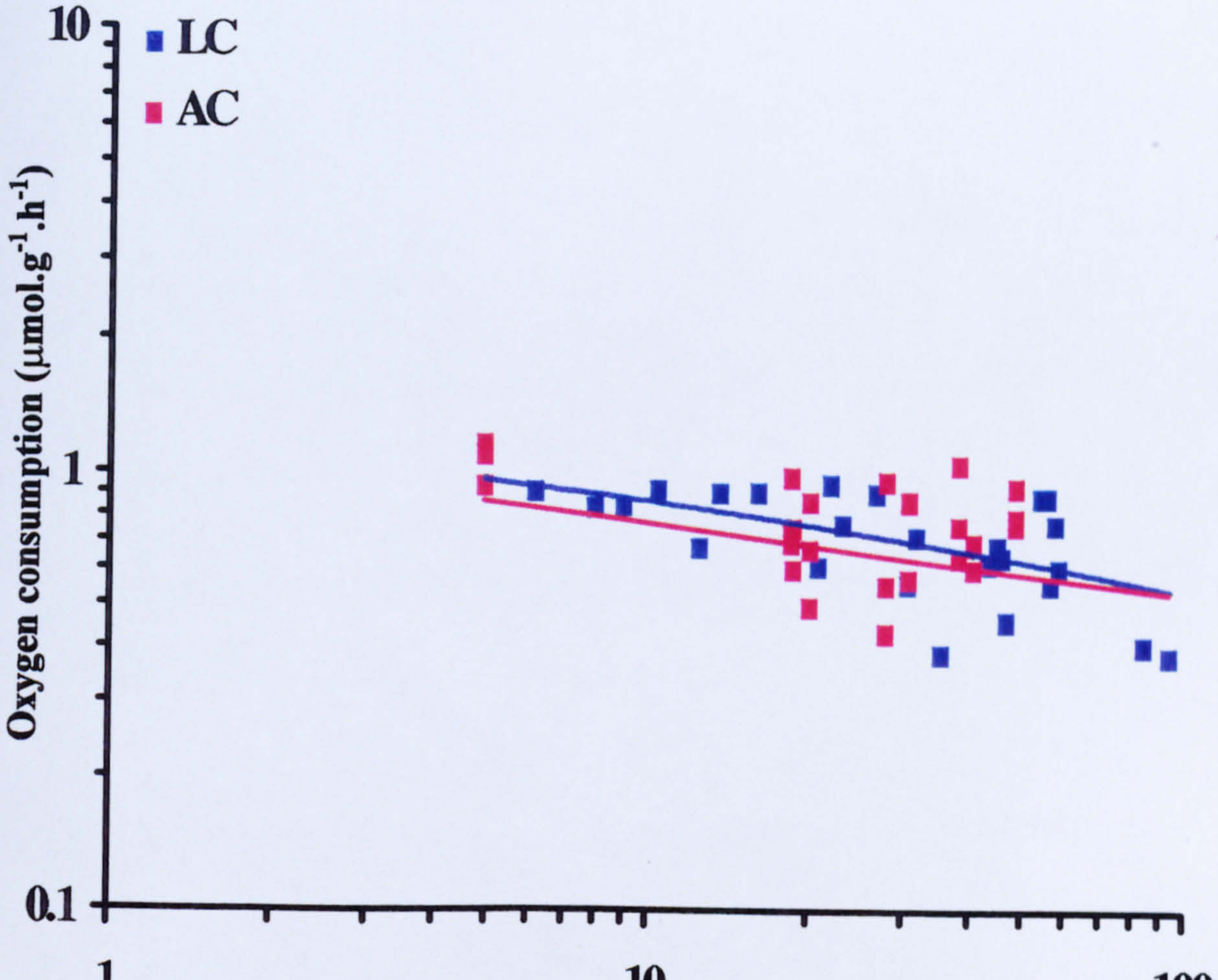


**Figure 3.5. A comparison of the relationship between fresh body weight (g) and weight-specific oxygen consumption ($\mu mol.g^{-1}.h^{-1}$) at 10°C, 34‰ salinity, in *Nephrops norvegicus* from the Little Cumbrae and Ailsa Craig sites. Standard rates were calculated using flow-through respirometry. Lines represent the calculated regressions.**

### 3.3.5. Energy budgets

Energy budgets complied for *N. norvegicus* from the two sites in the Clyde Sea area at two different times of the year are reported in Table 3.1, and illustrated in Figure 3.6. Two-way ANOVA investigating the influences of geographical and temporal variation indicated that scope for growth was significantly affected by sample month ($F_{87,1}$=19.39, P<0.001) but not by site ($F_{87,1}$=19.39, P=2.16). In addition, there were no significant interactions between site and month ($F_{87,1}$=0.11, P=0.744). The scope for growth estimated for suspension-feeding animals was found to be higher than the values predicted for animals in the field. The scopes for growth reported for animals in November were almost 50% of those suspension-feeding. Figure 3.7 illustrates that in addition to feeding, the level of activity also has a marked effect on the scope for growth. The minimum maintenance ration (scope for growth=0) required ranges from 0.27 $kJ.day^{-1}$ for quiescent animals, to 0.7 $kJ.day^{-1}$ for animals active for 50% of the time, and up to 1.1 $kJ.day^{-1}$ for animals which are continuously active.

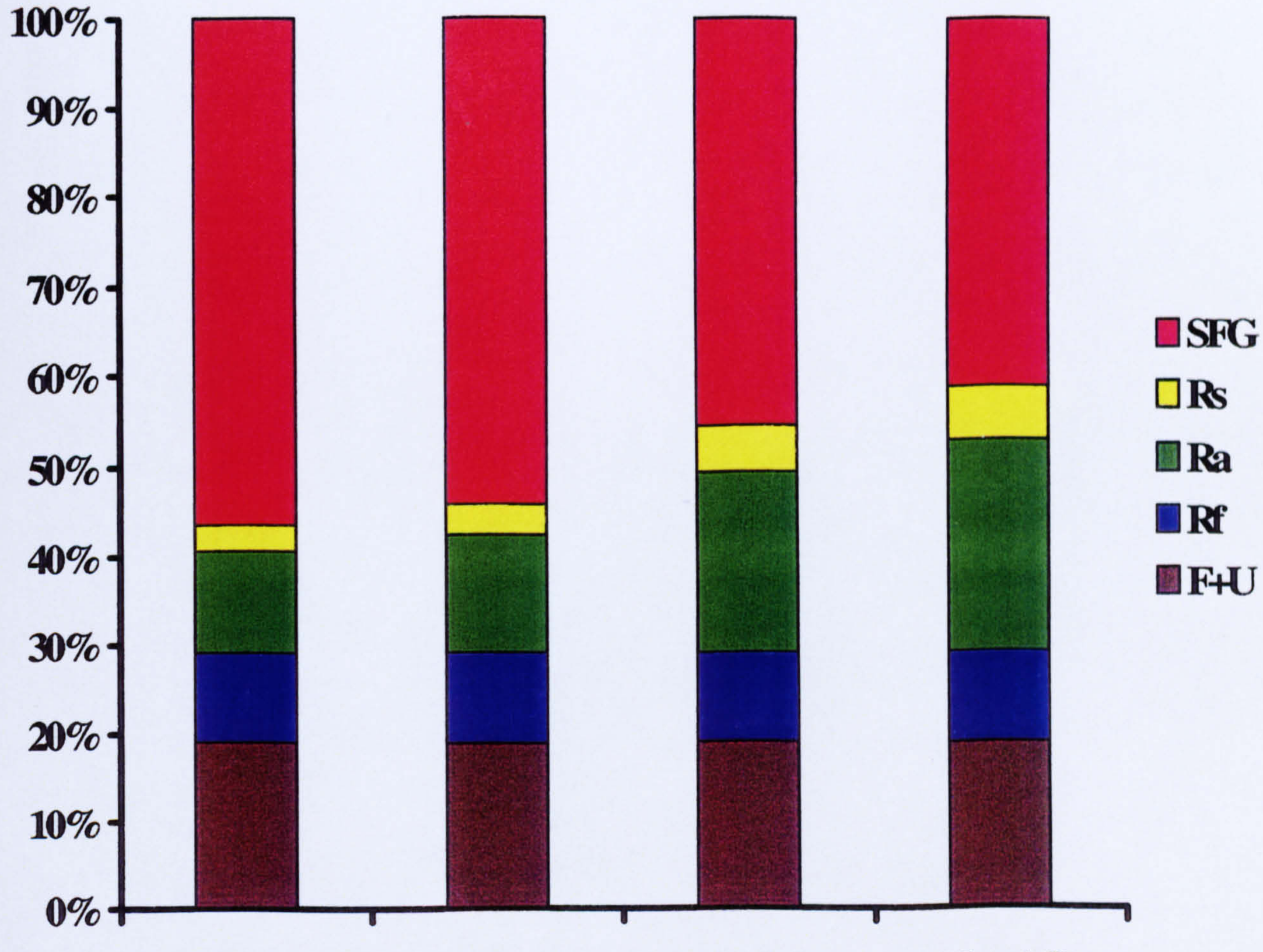


**Figure 3.6. Graph showing percentage energy allocation in *Nephrops norvegicus* from the Little Cumbrae (LC) and Ailsa Craig (AC) sites in May and November. SFG=scope for growth, Rs=standard metabolism, Ra=active metabolism, Rf=feeding metabolism and F+U=faecal and nitogenous excretions.**

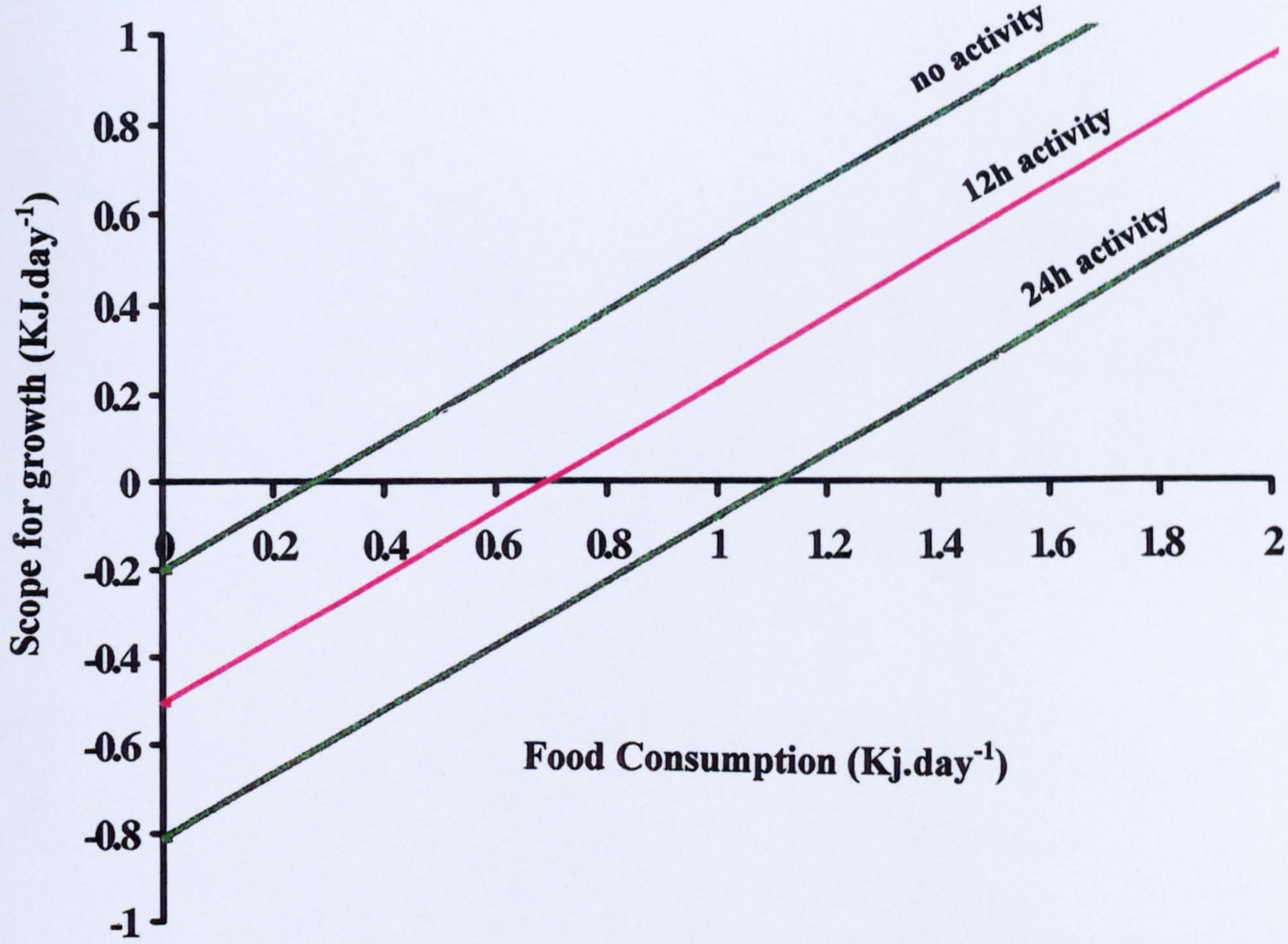


**Figure 3.7. Graph showing scope for growth (SFG) for *Nephrops norvegicus* (fresh weight=26.2g) at varying levels of activity and food consumption.**

**Table 3.1. Energy budgets of *Nephrops norvegicus* from the Clyde Sea area and a model of a standard 26.2g animal suspension-feeding on *Artemia*.**

| | | Energy ($kJ.day^{-1}$) | | | |
|---|---|---|---|---|---|
| | | ***C*** Estimated food intake | ***F+U*** Faeces & Excretion | ***R*** Total Respiration | ***P*** Scope for growth |
| **Little Cumbrae** | **May 94** | 3.47<br>100% | 0.66<br>19% | 0.85<br>24.6% | 1.96<br>56.5% |
| | **Nov 94** | 1.98<br>100% | 0.38<br>19% | 0.71<br>35.4% | 0.91<br>45.6% |
| **Ailsa Craig** | **May 94** | 2.99<br>100% | 0.57<br>19% | 0.81<br>26.9% | 1.62<br>54.1% |
| | **Nov 94** | 1.71<br>100% | 0.32<br>19% | 0.68<br>39.6% | 0.71<br>41.4% |
| **Filter feeding Loo *et al.* (1993)** | | 2.614-4.158<br>100% | 0.497-0.790<br>19% | 0.412-0.537<br>16-13% | 1.705-2.831<br>65-68% |

## 3.4. DISCUSSION

### 3.4.1. Metabolic rate

The decrease in weight-specific oxygen consumption of *Nephrops norvegicus* with body size shown in this study has also been found with a wide range of organisms (see Moore, 1995 for a review) including a number of other studies on lobsters, e.g. Thomas (1954) for *Homarus gammarus*, McLeese (1964) for *H. americanus*, Winget (1969) for *Panulirus interruptus*, and Bridges & Brand (1980) as well as Hagerman & Uglow (1985) for *N. norvegicus*. Although there was a markedly significant difference in the elevations of the regression equations describing the relationship

between quiescent and active metabolic rate with weight, the slopes of these equations were not significantly different, which suggests that the scope for activity does not change with size. This concurs with work on juvenile salmon (Cutts, 1997) and 3 species of cyprinids (Weiss & Forstner, 1986). However, this is contrary to a number of investigations which have shown that metabolic scope increases with weight in sockeye salmon (Brett & Weis, 1973), charr (Beamish, 1978), northern pike (Diana, 1982), and zebra fish (Lucus & Priede, 1992). Studies on fish have found that individuals with large metabolic scopes may also have a higher standard metabolic rate (Cutts, 1997). This has been attributed to the costs imposed by maintaining the additional metabolic apparatus. Proposed adaptive characteristics which could increase the metabolic scope, include a larger surface area of gills, and cells with an increased membrane permeability which facilitates the flux of metabolic substrates (Taigen, 1983). The potential detrimental side effect may be that these cells are leakier, which results in an increase in $Na^+$and $K^+$ transport that raises the standard rate of metabolism (Taigen, 1983).

When the metabolic scopes of different aquatic organisms are compared, they tend to reflect their respective lifestyles. Fish such as the skipjack tuna *Katsuwonus pelamis* which spend long periods of time swimming, tend to have large metabolic scopes (Gooding *et al.*, 1981), whereas those of less active fish including the plaice *Pleuronectes platessa,* have been found to be more limited (Priede & Holliday, 1980). Although salmon seldom show spontaneous activity, Brett & Glass (1973) suggested that their large metabolic capacity has evolved to meet the energy required during upstream migration, where approximately 70-75% of the maximum rate is sustained

for extensive periods of time. Similarly, active crustaceans also have high metabolic scopes. McFarland & Pickens (1965) found scopes of 22-26.8 μmol $O_2.g^{-1}h^{-1}$ for *Palaemonetes vulgaris*. In contrast, some crustaceans tend to exhibit relatively lower scopes, which may be indicative of their less-active behaviour. In this study, the metabolic scope for *N. norvegicus* (fresh weight of 26.2g) was found to be approximately 2.2 μmol $O_2.g.h^{-1}$ (approximately 4x standard rate), which is comparable to the findings of Ansell (1973) who found a scope of 2.8 μmol $O_2.g.h^{-1}$ at 11°C for *Cancer pagurus* and Spoeck (1974) who also found a scope of 2.8 μmol $O_2.g.h^{-1}$ at 15°C for *H. gammarus*. The lowest metabolic scopes appear to be found in Antarctic Crustacea. Peck (1989, 1996) found that the scope for activity of Antarctic isopod *Liothyrella uva* was only 1.64 times the basal metabolism.

As well as genuine ecophysiological differences, interspecific variability in metabolic rates could also result from inconsistencies in the procedures used for the measurements of 'standard' and 'active' rates of metabolism. Such disparity often arises from differences in the level of care taken by experimenters to ensure that organisms are truly quiescent (Steffenson, 1989), e.g. the duration that the organism has had to acclimatise to the experimental conditions, whether or not the animal has recently fed, or the degree of simulation of the natural surroundings of the animal. There is a noticeable variability in resting metabolic rates when studies on the same organism are compared. For instance, routine respiration rates reported for the deep-sea crabs *Chaceon fenneri* and *C. quinquedens* by Henry *et al.* (1990) were one third of those found by Erdman *et al.* (1991). Similarly, Hagerman *et al.* (1990) noted that *N. norvegicus* provided with a substratum and artificial burrow exhibited a lower rate

of lactate accumulation when exposed to hypoxia, than animals maintained in less natural conditions (Bridges & Brand, 1980).

The metabolic scope of *N. norvegicus* provides the capacity to increase the standard rate by a factor of 4. Although the magnitude of the scope may seem unnecessary for a temperate benthic crustacean such as *N. norvegicus* which is only moderately active, it could be argued that these animals do need a large buffer zone between a quiescent and an active rate when it is considered that the maximum rate that can be sustained over periods of several hours, will probably be lower than values that can be sustained over a much shorter period (Brett, 1964). The scope for activity represents the metabolic versatility of an organism, since a high scope can be a useful adaptation to fluctuating environmental conditions. For instance, studies have shown that decreasing salinity affects the metabolic rate of crustaceans. Taylor (1977) observed an increase in the oxygen consumption of *Carcinus maenas* as salinity was reduced, and suggested that this was due to the increased energy requirement for the active transport of ions. Similarly, Jury *et al.* (1994) noticed more than a two-fold increase in the oxygen consumption of *H. americanus* when they were moved from 20 to 10‰ salinity. They also found that energetic costs of osmoregulation were greater for females than for males, and suggested that this may partly influence the distribution of the sexes in estuarine habitats. However, it is unlikely that an increased metabolic scope would be required for the energetic costs of osmoregulation in most populations of *N. norvegicus*, since they tend to inhabit environments with a relatively stable salinity and temperature (IMBC, UMBSM & IRPEM, 1994). *N. norvegicus* are more likely to be subjected to periodic hypoxia in some locations (Hagerman &

Baden, 1988; Baden *et al.*, 1994). Exposure to these conditions results in a build up of the metabolic products of anaerobiosis such as lactate. With a large aerobic capability, *N. norvegicus* could minimise the accumulation of oxygen debts and speed repayment of the debts when they do occur. The metabolic scope would also need to incorporate elevated metabolism resulting from disease, injury and stress. This may be particularly important for the Clyde Sea area where infections of a *Hematodinium* sp. dinoflagellate parasite are common (Field *et al.*, 1992; see also Chapter 6). On a shorter time scale, the scope could enable short-term bursts of energy for agonistic behaviour (Smith & Taylor, 1993) or the tail-flipping escape response which *N. norvegicus* uses to evade predators (Newland *et al.*, 1992).

The decline in metabolic rate following starvation and the rapid increase when animals were refed, suggests that metabolic activities become adapted to changes in food availability. These data concur with work on other Crustacea, which also found that, metabolic rates declined during starvation and increased on feeding (Marsden *et al.*, 1973). This could form part of a strategy that enables *N. norvegicus* to survive long periods of food deprivation. The results of this study demonstrated that following a period of starvation, a small meal resulted in more than a doubling of the metabolic rate. Even this is likely to be an underestimate though, since animals were fed less than they would normally consume in their natural environment. It is therefore likely that a large proportion of the metabolic scope of *N. norvegicus* is occupied by the raised post-feeding metabolism. It is also worth noting that these animals were fed manually and that under natural conditions, additional foraging costs would also be incurred. Thus the nutritional status of *N. norvegicus* appears to be an important

factor in metabolic energy expenditure. However, the suspected nutritional differences between the *N. norvegicus* from south of Little Cumbrae and from Ailsa Craig, were not apparent when the metabolic rates of the two sites were compared.

### 3.4.2. Energy budgets

Although there are few complete investigations on the energetics of adult crustaceans for comparison with the budget constructed for *N. norvegicus*, published studies on fish shows that there are some differences in energy allocation. This is particularly evident with respect to the energy allocated to metabolism. The average energy utilised in metabolism for 14 species of predatory fish was found to be 51.5% (Cui & Jiankang, 1990d) which is greater than the range (25-40%) found for *N. norvegicus* in this study. This discrepancy is likely to be due to the greater amount of energy expended by fish whilst swimming. Although *N. norvegicus* may actively forage, swimming is generally limited to predator evasion (Newland *et al.*, 1988). Organisms feeding on herbivorous diets tend not to operate at such high levels of activity since prey capture usually requires less foraging effort as it is either sessile, or can be filtered from water currents. However, the types of food on which herbivorous organisms are feeding often have low energy contents or are difficult to digest resulting in a lower assimilation efficiency. For example, Hofer *et al.* (1985) noted that only 47.6% of consumed energy was assimilated for roach (*Rutilus rutilus*) feeding on grass, compared with 82.4% for roach feeding on mealworms.

Although the scope for growth incorporated qualitative differences in prey composition and the total energy intake between the samples, it appears that these

corrections may have been insufficient to compensate for the high individual variation. Significant differences in scope for growth between the months were detected, but not between sites. If indeed there are any differences in energy intake between the stocklets, in order to detect them, it may be necessary to correct for other potential geographical differences in the energy budget. For example, it is has been assumed that the assimilation efficiencies were the same for both sites. This is unlikely to be the case, since Chapter 2 demonstrated that there were some differences in diet composition between the sites. Furthermore, it was also assumed that the activity levels of the two stocklets were identical. Studies have shown that the environment of the south of Ailsa Craig stocklet is impoverished compared with that of Little Cumbrae (IMBC, UMBSM & IRPEM, 1994), thus it is probable that in order to consume the same amount of food as the Little Cumbrae animals, Ailsa Craig animals would have to spend more time foraging. A consequence of this would be that the animals are at a higher metabolic rate for longer periods which would result in an even lower scope for growth. The seasonal differences in scope for growth illustrate that at certain times of the year, *N. norvegicus* may be nutritional limited. This is supported by work (IMBC, UMBSM & IRPEM, 1994; see also Chapter 5) which has found that *N. norvegicus* are in poorer physical condition during the winter.

The simulated energy budget for *N. norvegicus* suspension-feeding on *Artemia* suggested that it is energetically efficient for animals to feed in this way, and at the food densities used by Loo *et al.* (1993) in their experiments, it is even more profitable than active foraging. However, this is unlikely to be true when applied to animals suspension-feeding in the field, since it is highly probable that environmental

concentrations of extractable food items in the water column are considerably lower than those simulated by Loo *et al.* (1993). In addition, the actual assimilation efficiency could also have been less than the value used. Work on other Crustacea such as *Penaeus monodon* (Kurmaly *et al.* (1989), has shown that when extractable cells are readily available, the assimilation efficiency is reduced, as it is more economical for food to be rapidly evacuated from the gut when it can be quickly replenished. Moreover, in an earlier study on *P. monodon,* Jones & Kurmaly, (1987) demonstrated that shrimps feeding in dense algal cultures were able to refill their guts 5-7 times per hour. Nevertheless, the model of *N. norvegicus* suspension feeding does suggest that this may be a compromising strategy for animals unwilling to leave their burrow environment, since *N. norvegicus* filter-feeding at rates of less than 15% of those used by Loo *et al.* (1993) will still have a positive scope for growth (approximately 1%) By remaining in the burrow *N. norvegicus* are able to conserve a large amount of energy which could otherwise have been spent on activity. It was estimated that actively foraging *N. norvegicus* spend 25-40% of energy on respiration whereas suspension-feeding animals spend just 13-16%. Similar energy conservation strategies have been observed in fish. Koch & Wieser (1983) found that reduction in locomotor activity compensated for the costs of synthesising gonadal tissue in the roach (*Rutilus rutilius*).

Although it is theoretically possible to determine all of the parameters of an energy budget experimentally, in practice, it is unlikely that any of the individual components could be measured with great precision (Soofiani & Hawkins, 1985). This is especially true when estimating energy budgets for organisms in the field. In this

investigation, estimates were made using a combination of information from field observations and laboratory-based energetics studies. The 'weakest link' in the energy budget equation was probably the measurement of the energy released in metabolism. The problems in measuring with precision arise since both the level of metabolism, and the duration of the periods at that level of metabolism at which organisms are operating, are difficult to predict accurately for animals in their natural environment. It is accepted that the method used to estimate total metabolism (R) in this study could be inaccurate since animals are unlikely to be at their maximum active rate during the whole dark period, and it is equally doubtful that they are operating exactly at the standard rate during the day. In addition, short bursts of energy involved in prey capture or predator evasion which could exceed the metabolic scope and force the animal to utilise anaerobic metabolism, may also be unaccounted for. However, despite these drawbacks, given the available apparatus and time, the method chosen seems to be the most suitable. This method also appears to be considerably more accurate when it is compared to some previous methods of estimating R. When calculating R for a population of *H. americanus*, Miller *et al.* (1971) completely ignored the effects of activity on metabolic rate, stating that "In extrapolating to field conditions, the values obtained in experimental bottles were taken to be fairly representative of the normal activity of the animals, all relatively sluggish in their habits". Other methods of estimating R have been limited to simply doubling the standard rate (Winberg, 1956 cited in Soofiani & Hawkins, 1985). Although, currently, the problems of actually obtaining accurate data on unrestrained animals in the field are considerable, there have been recent developments in remote sensing devices such as infra-red phototransducers that enable non-invasive

measurement of physiological functions such as heart rate (Depledge & Andersen, 1990). Since a number of studies have found heart rate to be a useful indicator of metabolic status (Deplege, 1985; Taylor, 1988), this system could be of potential use in recording the metabolic activity of *N. norvegicus* under field conditions.

Energy budgets can be extremely useful for aquaculturists where most of the environmental and biological variables are under strict control (Knights, 1985). However, the results from this study show that it is markedly more difficult to apply these theories to the field. The significant amount of individual variation exhibited can easily mask any subtle differences that could occur. It is also difficult to construct a budget without making a number of assumptions and estimates. It is likely that the compounding of these assumptions can contribute considerably to increasing the error in energy budget calculations. Unless a significant amount of information on the lifestyle of the organism of study is known, it might be more appropriate to use simpler and more reliable indicators of nutritional condition.

## 3.5. SUMMARY

- The rates of weight specific oxygen consumption ($MO_2$) were determined for quiescent male and female *N. norvegicus*. No significant differences were detected between the sexes.

- The rates of active $MO_2$ were measured and found to be significantly higher than standard $MO_2$. The scope for activity was found to be approximately four times basal metabolism.

- The recent feeding history was observed to have a marked effect on metabolic rate. The $MO_2$ significantly decreased following 8 and 12 weeks of starvation, and rapidly increased again after re-feeding.

- No significant differences in $MO_2$ were detected between *N. norvegicus* from the Little Cumbrae and Ailsa Craig sites.

- An energy budget was constructed for Clyde Sea *N. norvegicus*. The patterns of energy allocation were similar to those of predatory fish. The scope for growth was significantly greater for animals in May than November. No significant differences in scope for growth were found between animals from the Little Cumbrae and Ailsa Craig sites.

- An additional energy budget was assembled to model *N. norvegicus* suspension-feeding. The model predicted that considerable energy is conserved due mainly to savings from operating at a lower level of activity.

# CHAPTER 4

# Biochemical indicators of nutritional condition in the Norway lobster *Nephrops norvegicus*-a laboratory calibration

## 4.1. INTRODUCTION

### 4.1.1. Indicators of nutritional condition

A variety of methods for determining the physiological condition of aquatic organisms have been investigated. The most basic of these involve the use of morphometric measurements such as length:weight ratios. The advantages of these methods are that they are quick and cheap, enabling large numbers of samples to be processed, and they are relatively easy to carry out in the field. However, whilst these techniques are readily applicable to fish, they are not particularly suitable for crustaceans, since detectable changes in measurements such as length and weight occur in discrete intervals at the time of moulting (Aitken, 1980). Therefore, it is often necessary to use alternative procedures. One method that is growing in popularity is to measure nutritional condition and growth in Crustacea at the molecular level. Consequently, a number of methods have been developed that allows nutritional condition to be estimated from biochemical constituents of the blood or tissue. For instance, nutritional condition has been correlated with concentrations of the respiratory pigment haemocyanin in the haemolymph. Since haemocyanin constitutes as much as 90% of the circulating protein (Uglow, 1969), it has been suggested that it acts as an organic reserve during periods of starvation (Busselen, 1970). Changes in

haemocyanin content can be determined by measuring either the copper or the protein concentration of the haemolymph. A decline in haemocyanin content associated with reduced food intake has been found for a number of Crustacea, including isopods e.g. *Porcellio laevis* (Alikhan & Lysenko, 1973) and *Meinerta oestroides* (Romestand *et al.*, 1976) and decapods such as *Carcinus maenas* (Uglow, 1969), *Crangon crangon* (as *C. vulgaris*) (Djangmah, 1970; Hagerman, 1986), *Panulirus longipipes* (Dall, 1974), *Homarus americanus* (Stewert *et al.*, 1967) and *H. gammarus* (Hagerman, 1983). Hagerman & Baden (1988) also found low haemocyanin concentrations in *N. norvegicus* which had been exposed to prolonged periods of hypoxia. A decrease in haemocyanin content is usually associated with an increase in hepatopancreas copper. Djangmah (1970) noted that the copper content of starved *C. crangon* (as *C. vulgaris*) was 190 times that of fed animals. It is thought that as the haemocyanin protein is catabolised, the copper component is conserved through redistribution to the hepatopancreas.

The effects of starvation on the biochemical constituents of other crustacean tissues appear to be more variable. Studies suggest that there are marked differences in the way in which different crustaceans utilise their reserves. The effects of starvation in penaeid shrimps such as *Penaeus duorarum, P. japonicus* and *P. esculentus* are characterised by significant reductions in total lipid and protein (Schafer, 1968; Cuzon *et al.*, 1980; Barclay *et al.*, 1983). In contrast, Dall (1981) found no evidence of lipid depletion in the hepatopancreas of *N. norvegicus* during starvation. It is possible that for *N. norvegicus*, glycogen is the more important metabolic reserve. Baden *et al.* (1994) noted that 7 months of starvation resulted in more than a 90% reduction in

glycogen content of both muscle and hepatopancreas tissue. Of the different types of lipid, triacyglycerols (TAGs) are preferentially catabolised during periods of nutritional limitation (Heath & Barnes, 1970). Thus TAGs have been used as indicators of nutritional condition in a number of crustaceans including larval *H. americanus* (Sasaki *et al.*, 1984), sub-adult *P. esculentus* (Chandumpai *et al.*, 1991) and zoeae of the snow crab *Chionoecetes opilio* (Lovrich & Ouellet, 1994). Stuck *et al.* (1996) correlated TAG content with a variety of other nutritional indices in *P. vannamei*, and found that TAG was only useful as a short term nutritional indicator, since TAG content was almost totally depleted after less than a week of starvation. The study concluded that polyamine and nucleic acid contents are more accurate nutritional indicators.

### 4.1.2. Nucleic acids as indicators of nutritional condition

It has been estimated that ribosomal RNA accounts for approximately 80% of total cellular RNA. Therefore measurement of the concentration of RNA in a tissue will give an indication of ribosomal numbers. Since protein is synthesised by ribosomes, changes in the number of ribosomes in a cell will reflect changes in cellular activity. The monitoring of RNA content can thus provide information about the physiological status of an organism. The use of RNA concentration as a growth index has been applied to many animal groups, including microbes (Leick, 1968), echinoderms (Frantzis *et al.*, 1992), and mammals (Munro & Gray, 1969). RNA content can also be used to assess the condition of organisms in the field, which has led to its development for a number of species of commercially important fish (Miglavs & Jobling, 1988; Ferguson & Danzmann, 1989; Mustafa, 1991; Foster *et al.*, 1992;

Clemmesen, 1993, 1994, 1996) and shellfish including Crustacea such as penaeid shrimps (Moss, 1994a,b, Stuck *et al.*, 1996), lobsters (Juinio *et al.*, 1992, 1994), and blue crabs (Wang & Stickle, 1986), as well as bivalve molluscs such as scallops (Robbins *et al.* 1990; Kenchington, 1994; Lodeiros, 1996) and oysters (Wright & Hetzel, 1985). RNA content is commonly standardised by expressing the concentration as a ratio to the protein or DNA content of the tissue. As well as estimating RNA concentration, it is also possible to calculate RNA activity. Techniques have been developed from the free-pool method of Garlick *et al.* (1980) enabling measurement of the rate of protein synthesis in crustacean muscle tissue (El Haj & Houlihan, 1987; El Haj *et al.*, 1996). This can give an indication of instantaneous growth rates.

### 4.1.3. Chapter aims

Since previous work (e.g. Tuck *et al.*, 1997a) has suggested that there is a difference in the growth rate of *N. norvegicus* from the Little Cumbrae and Ailsa Craig stocklets, a small preliminary study was carried out in order to test this experimentally by measuring the rate of protein synthesis, an 'instantaneous' measure of growth. In addition, the calibration of nucleic acid indices as indicators of nutritional condition in *N. norvegicus* is also reported in this chapter. To this end, this chapter will examine the responses of RNA concentration, the RNA:Protein ratio and the RNA:DNA ratio to prolonged starvation in three different tissues; abdominal muscle, gill and hepatopancreas.

## 4.2. MATERIALS AND METHODS

### 4.2.1. Measurement of the rate of protein synthesis in the abdominal muscle tissue of *N. norvegicus* from the Little Cumbrae and Ailsa Craig sites.

*Experimental animals*

Samples of *Nephrops norvegicus* were collected from the north Clyde Sea area, south of Little Cumbrae, and from the south Clyde Sea area, south of Ailsa Craig (see Figure 2.1). Lobsters were then transported back to the aquarium at the University of Glasgow for analysis.

*Injection Procedure*

Protein synthesis was measured using the *in vivo* free pool technique as described by El Haj & Houlihan (1987) for *Carcinus maenas*. A single dose of L-[2,6-$^{3}$H] Phenylalanine (specific activity 40-60 $\mu Ci.ml^{-1}$) (Amersham, UK-TRK 552) at 50 $\mu Ciml^{-1}$ (2.85MBq.$ml^{-1}$) in *N. norvegicus* saline containing 150mmol.$l^{-1}$ phenylalanine at a dose of 1.0 ml $100g^{-1}$ body mass was injected into the sinus at the base of the 4th walking leg. After initial injection, animals were left undisturbed for 2h. Although 1h was used by for *Homarus americanus* (El Haj *et al.* 1996) and *C. maenas* (El Haj & Houlihan, 1987), a longer exposure time was chosen in this experiment to compensate for the lower temperatures at which the animals were maintained (10°C compared with 15-18°C), as well as the larger size of animals. 2h was also the time that Whiteley *et al.* (1996) used for measuring protein synthesis in isopods. All lobsters

were maintained in aerated sea water of 34‰ salinity at 10°C during this period. At the end of this time period, lobsters were rapidly frozen in liquid $N_2$ and stored at -70°C for subsequent biochemical analysis.

*Tissue preparation*

Approximately 100mg of abdominal muscle tissue was dissected out from each animal and homogenised in 1ml 0.5M chilled (<4°C) perchloric acid (PCA). A further 1ml of 0.5M PCA was added and the homogenate was centrifuged at 10,000g for 10min at 4°C. The supernatant was transferred to labelled stoppered centrifuge tubes for analysis of the specific activity of the free pool and the resulting pellet washed twice in chilled (<4°C) 0.5M PCA (centrifuging at 10,000g and 4°C, and discarding supernatant between washes). The pellet was resuspended by vortex mixing in 4.5ml distilled water. 0.5ml of 3M NaOH was added and mixed to give a final concentration of 0.3M NaOH. The pellet was disolved by incubating the tubes at 37°C for 1h. After cooling, 1.1ml of the solution was removed. 0.867ml 20% PCA was added to the remaining 3.9ml which was then centrifuged at 10,000g and 4°C in order to precipitate proteins and DNA. The remaining precipitate was washed twice with 0.5M PCA as before, discarding the supernatant each time. The washed precipitate was then transferred to hydrolysis tubes with 3x 2ml of 6M hydrochloric acid. The lids of the hydrolysis tubes were screwed on, and the tubes heated at 110°C in an oven for 18h. After cooling, the lids were removed, and the acid was removed by vacuum evaporation. The resulting hydrolysate was resuspended in 2ml 0.5M sodium citrate buffer (pH 6.3) and stored at -20°C prior to enzyme determination. To prepare the free pool samples for enzyme determination, 0.6ml of saturated tri-

potassium citrate was added, and the tubes were centrifuged at 10,000g at 4°C for 10 min.

*Preparation for Enzyme Determination and Scintillation Counting*

One ml of each of the hydrolysate and free pool samples were pipetted into stoppered test tubes. To test the efficiency of the enzymatic conversion of phenylalanine to phenethylamine, two standard curves of 30-150 nmole/ml phenylalanine solution were prepared. One set was treated as hydrolysate, the other as free pool. To each sample and standard, 0.5ml of a suspension of enzyme (L-tyrosine decarboxylase) suspended in 0.5M sodium citrate buffer (pH 6.3) containing 0.5mg/ml pyridoxal phosphate was added. The concentration of enzyme added was 1.4 units/ml for the hydrolysate samples and standards, and 0.7 units/ml for the free pool samples and standards. The tubes were stoppered, mixed and incubated overnight at 50°C in a water bath. After cooling, 1ml 3M NaOH was added to each tube, followed by 8ml n-heptane. Tubes were stoppered, shaken vigorously (15-20 secs) and frozen at -70°C for 2h or until the bottom layer had frozen. The top layer (n-heptane) of each tube was poured into labelled tubes which contained 3ml 0.01M sulphuric acid for the hydolysates and 2ml 0.01M sulphuric acid for the free pools. As before, the tubes were stoppered, shaken vigorously, and the bottom layer was frozen. The top layer of heptane was poured off as waste, and the remaining frozen sulphuric acid layer was defrosted and mixed prior to enzyme determination and scintillation counting.

*Enzyme Determination*

The radioactivity is expressed per unit L-phenylalanine. However, in order to determine the concentration of L-phenylalanine using standard laboratory techniques, it has to be converted into L-β-phenylethylamine prior to analysis. A standard curve of 4-40 nmole/ml L-β-phenylethylamine in 0.01M sulphuric acid was prepared. Aliquots of each sample and efficiency standard in sulphuric acid were transferred to test tubes. 1ml volumes were used for free pool samples and efficiency standards, and 0.1ml was the volume of hydrolysate pipetted, to which a further 0.9ml of 0.01M sulphuric acid was added to make up the volume to 1ml. To each tube, 0.5ml 2mM leucylalanine, 1.0ml 50mM ninhydrin and 2.5ml 1M $K_2HPO_4$ were added. The tubes were then covered to exclude light and incubated in a water bath at 1h for 60°C. After cooling under darkness for 30min, the fluorescence of each sample and standard were read at 495nm, with the excitation set at a wavelength of 388nm.

*Scintillation Counting*

The radioactivity in each sample was measured using a scintillation counter. 2ml of hydrolysate in 0.01M sulphuric acid samples were pipetted into plastic scintillation vials to which 10ml of scintillant (Ecoscint) was added. For the free pool samples, a smaller volume was used. 0.2ml 0.01M sulphuric acid was added to 2ml of scintillant.

The experimental results were calculated as $S_a$, the specific radioactivity of free L-phenylalanine (d.p.m.nmol$^{-1}$), and as $S_b$, the specific radioactivity of protein-bound phenylalanine (d.p.m.nmol$^{-1}$). The fractional rate of protein synthesis $k_s$ (% of protein mass synthesised per day), was determined as:

$$K_s = \frac{S_b}{S_a} \cdot \frac{100}{t}$$

where t is the duration in days (El Haj & Houlihan, 1987).

4.2.2. Method development for nucleic acid analysis

A large number of techniques have been developed for the quantitative estimation of nucleic acids. The most commonly used of these methods rely on colorimetric reactions, such as the orcinol test for RNA and the diphenylamine reaction for DNA. The orcinol method is based on the test for pentose sugars. When pentose sugars are heated with orcinol in the presence of HCl and the catalyst $FeCl_3$, a green colour develops. During the diphenylamine method of DNA estimation, a blue colour is produced when the diphenylamine reacts with 2-deoxyribose. This rate of blue colour development was later found to be increased when acetaldehyde was added to the reagents. Other common methods of DNA estimation include the indole reaction and the dual-wavelength method which calculates DNA content from its maximum absorbance (Munro & Fleck, 1966; Wilder & Stanley; 1983).

Developments in nucleic acid quantification have involved the use of fluorimetric methods (Clemmensen, 1992). These techniques are able to analyse much smaller tissue samples. A recent study using a fluorimetric method to determine the nucleic acid content of muscle biopsies from fish, demonstrated that it is not always necessary to kill the organism in order to sample its biochemical condition (Grant, 1996). However, it is unlikely that this method could ever be used in a truly non-invasive manner.

The orcinol method of RNA determination was found to be the most commonly used technique and so was used throughout the project. Since there were several different methods of calculating the DNA content of tissues, preliminary experiments were carried out to determine the most suitable assay, both in terms of accuracy and ease of use. The methods investigated were the diphenylamine reaction and the dual-wavelength method. To determine their repeatability, each assay was carried out on sub-samples of homogenised abdominal muscle tissue from a single *N. norvegicus*.

## 4.2.3. Biochemical procedures

### *RNA estimation-Orcinol method*

The procedure is based on the orcinol method, adapted from the modification by Moss (1994a). Lobsters were killed by immersion into liquid nitrogen. Samples (250mg) of fresh frozen tissue were dissected from each animal and placed into plastic centrifuge tubes, to which 4ml of cold (<4°C) 0.22N perchloric acid (PCA) was added. The tissue was broken up with an ultrasonic cell disrupter, and left to stand on

ice for 15 minutes. The samples were then centrifuged at 3000g for 15 min. The supernatant was discarded, and another 4ml of 0.22N PCA was added. The above step was repeated twice. Washing with cold acid removes most small acid-soluble molecules such as free pentoses, hexoses and lipids which may interfere with the assay. After the third wash, 4ml of 0.3N KOH was added to each precipitate. Samples were incubated in a water bath at 37°C for 2 hours or until the precipitate had dissolved, during which time standards were prepared using Torula yeast RNA (Sigma). Hot alkali hydrolyses RNA, rendering it acid soluble. Samples were cooled on ice for 15 min, and 1ml of chilled 2.3N PCA (<4°C) was added. The samples were left on ice for a further 15 min before being centrifuged at 3000g for 15 min. 1ml of supernatant from each sample and standard were transferred to test tubes. 2ml of orcinol reagent were added to each tube, which were then incubated in hot water bath (100°C) in a fume cupboard for 20 min. Samples and standards were cooled to room temperature and their absorbencies at 665nm were read using a Shimazdu UV-1201 spectrophotometer.

*DNA estimation*

*Sample preparation*

Samples (50 mg) of dry tissue were weighed into plastic centrifuge tubes. 5ml of 0.5N PCA were added to each tube which were then heated at 70°C for 30min. Samples were cooled on ice and centrifuged at 3000g for 20min, during which time standards were prepared using either calf thymus DNA (Sigma) or salmon sperm DNA (Sigma).

*DNA estimation-Dual wavelength method*

The absorbencies of samples from each supernatant at 232nm and 260nm were read using a Shimazdu UV-1201 spectrophotometer. DNA concentrations were determined using a formula based on that of Wilder & Stanley (1983), with modifications made for the absorbance spectrum of *N. norvegicus* tissue:-

$$\mathbf{DNA\ (\mu g.ml^{-1}) = 1.09.A_{260nm} - 0.19.A_{232nm}}$$

*Diphenylamine method*

1ml of each sample and standard were then pipetted into plastic screw-topped centrifuge tubes. 2ml of diphenylamine reagent containing acetaldehyde were added to each tube, the lids were replaced and the tubes were incubated for about 20h at 25-30°C. Samples and standards were cooled to room temperature and their absorbencies at 660nm were read using a Shimazdu UV-1201 spectrophotometer.

*Protein estimation*

Protein concentrations were determined using a commercially produced kit (Pierce), based on the method of Lowry *et al.* (1951). 10 mg samples of freeze-dried tissue were dissolved in 10ml of 0.1N NaOH, and left to stand overnight at room temperature. While the samples were left to stand, standards for the calibration curve were prepared using 0-10mg of bovine serum albumin (Sigma). Samples were centrifuged at 6000g for 20min. 0.5ml of supernatant were then pipetted from each sample into 1.5ml Eppendorf tubes. 1ml aliquots of 10% trichloroacetic acid were added, and the tubes were left to precipitate overnight. The samples were centrifuged

at 13000g for 20min. The supernatants were discarded, and 0.5ml of 0.1N Na0H was added to each tube. The tubes were mixed thoroughly to redissolve the precipitate. 0.05ml of each sample were then pipetted into 1.5ml Eppendorf tubes, to which 1ml of the Pierce protein reagent was added. Samples were incubated at 37°C for 30min, and their absorbencies at 562nm were read on a spectrophotometer.

*Laboratory calibration*

Adult *N. norvegicus* were collected by trawling on grounds around the Isle of Cumbrae, Firth of Clyde, Scotland. Lobsters were returned to the University of Glasgow where they were maintained in seawater aquaria at 10°C. The moult stage of animals was diagnosed by the method of Aiken (1980), and only intermoult animals were selected. A sample of 30 male lobsters was taken, from which a sub-sample of 10 animals was frozen immediately in liquid nitrogen and stored at -70°C. The remainder were placed individually into plastic tubes (30cm length x 10cm diameter), the ends of which were sealed with a cloth mesh (5mm). *N. norvegicus* were sealed in tubes in order to prevent cannibalism, the incidence of which is known to increase during starvation (Sarda & Valladres, 1990). The tubes containing the lobsters were then placed into large plastic tanks with circulating, aerated seawater (34‰, 10°C), where they were maintained without feeding, under a 12:12h light:dark cycle. Samples of 10 lobsters were taken after periods of 6 and 12 weeks of fasting and stored at -70°C. Samples of abdominal muscle, gill and hepatopancreas tissue were removed from each animal and analysed using the procedures detailed above.

4.2.4. A preliminary comparison of the abdominal muscle RNA concentration of male and female *N. norvegicus*.

Adult *N. norvegicus* of both sexes were collected by trawling on grounds around the Isle of Cumbrae, Firth of Clyde, Scotland. Lobsters were returned to the University of Glasgow where the abdominal muscle RNA concentrations (ug.mg$^{-1}$ dry weight) of samples (n=7) of similarly sized male (mean fresh body weight=18.9g) and female (mean fresh body weight=17.4g) *N. norvegicus* were determined using the procedures detailed above. The moult stage of animals was diagnosed by the method of Aiken (1980), and only intermoult animals were selected.

## 4.3. RESULTS

4.3.1. Protein synthesis

The results of the preliminary experiment (Figures 4.1-4.3) indicated that there were no significant differences in the rates of protein synthesis in abdominal muscle tissue between *N. norvegicus* from the Little Cumbrae and Ailsa Craig sites (t-test, t= 0.19, P=0.85).

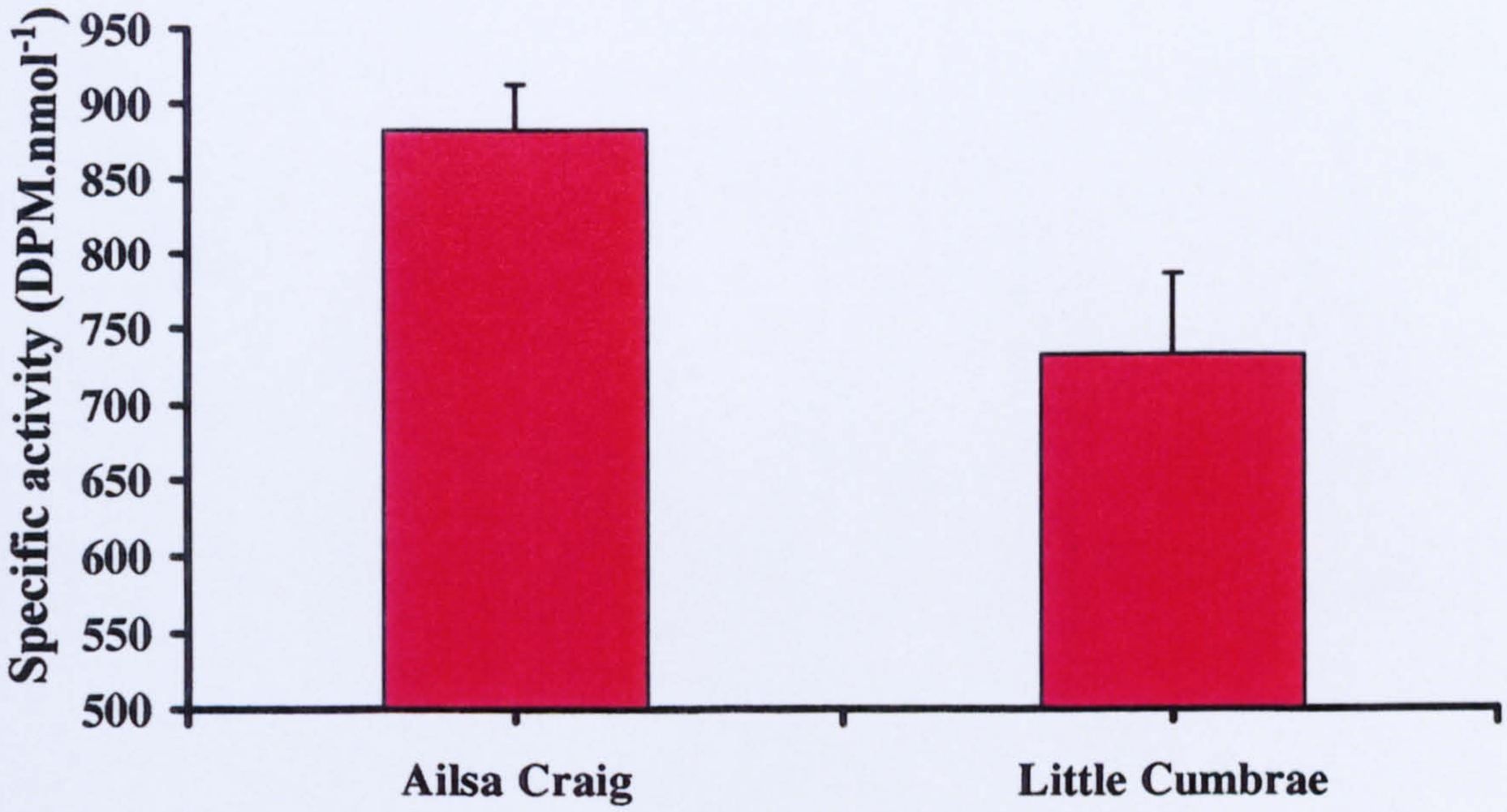


**Figure 4.1. Specific activity (DPM.nmol$^{-1}$) of free-pool phenylalanine extracted from the abdominal muscle tissue of *Nephrops norvegicus* from two sites in Clyde Sea area 2h after injection with L-[2,6-$^{3}$H] phenylalanine. Bars represent standard error (n=12).**

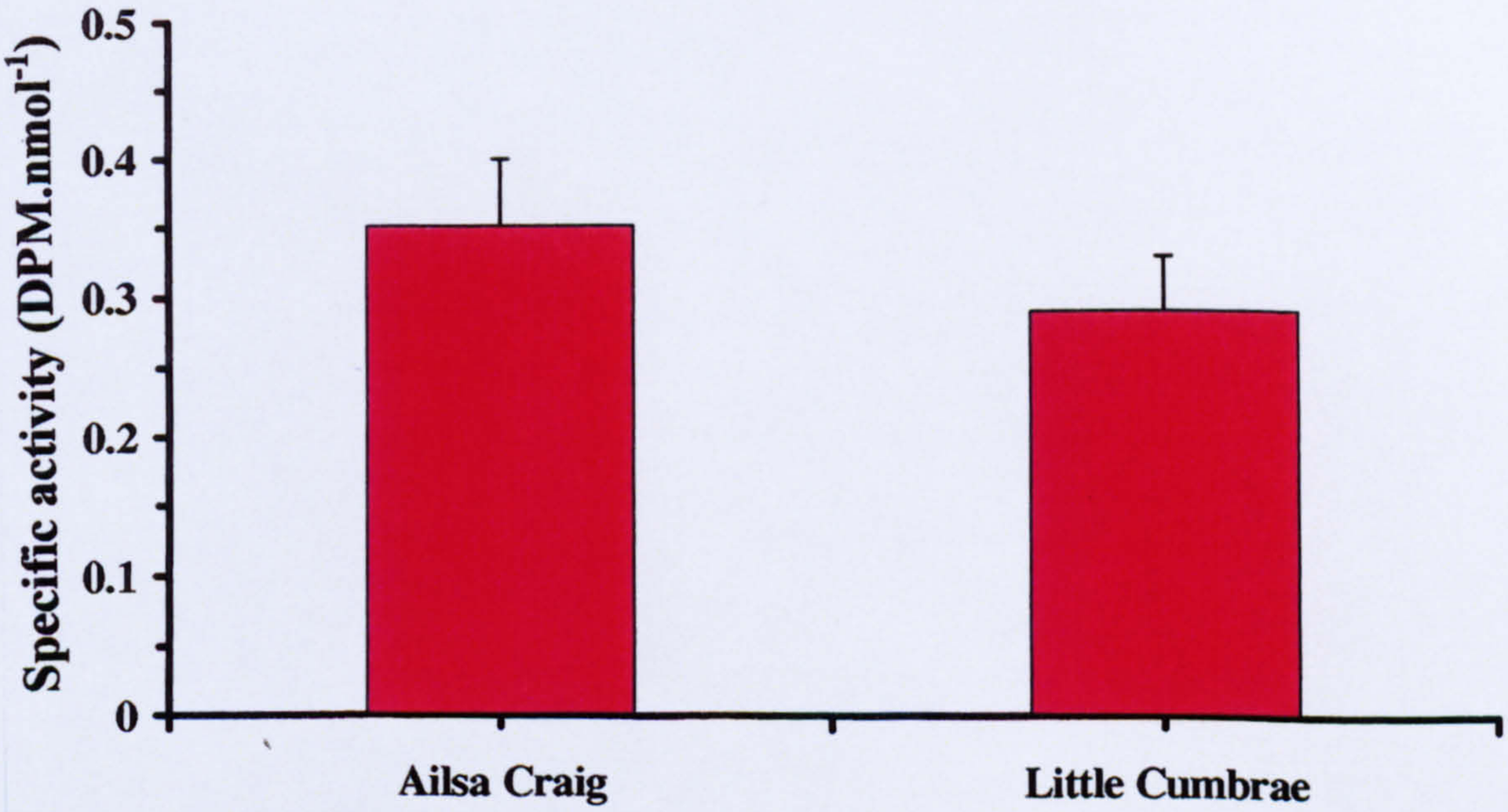


**Figure 4.2. Specific activity (DPM.nmol$^{-1}$) of protein-bound phenylalanine in the abdominal muscle tissue of *Nephrops norvegicus* from two sites in Clyde Sea area 2h after injection with L-[2,6-$^{3}$H] phenylalanine. Bars represent standard error (n=12).**

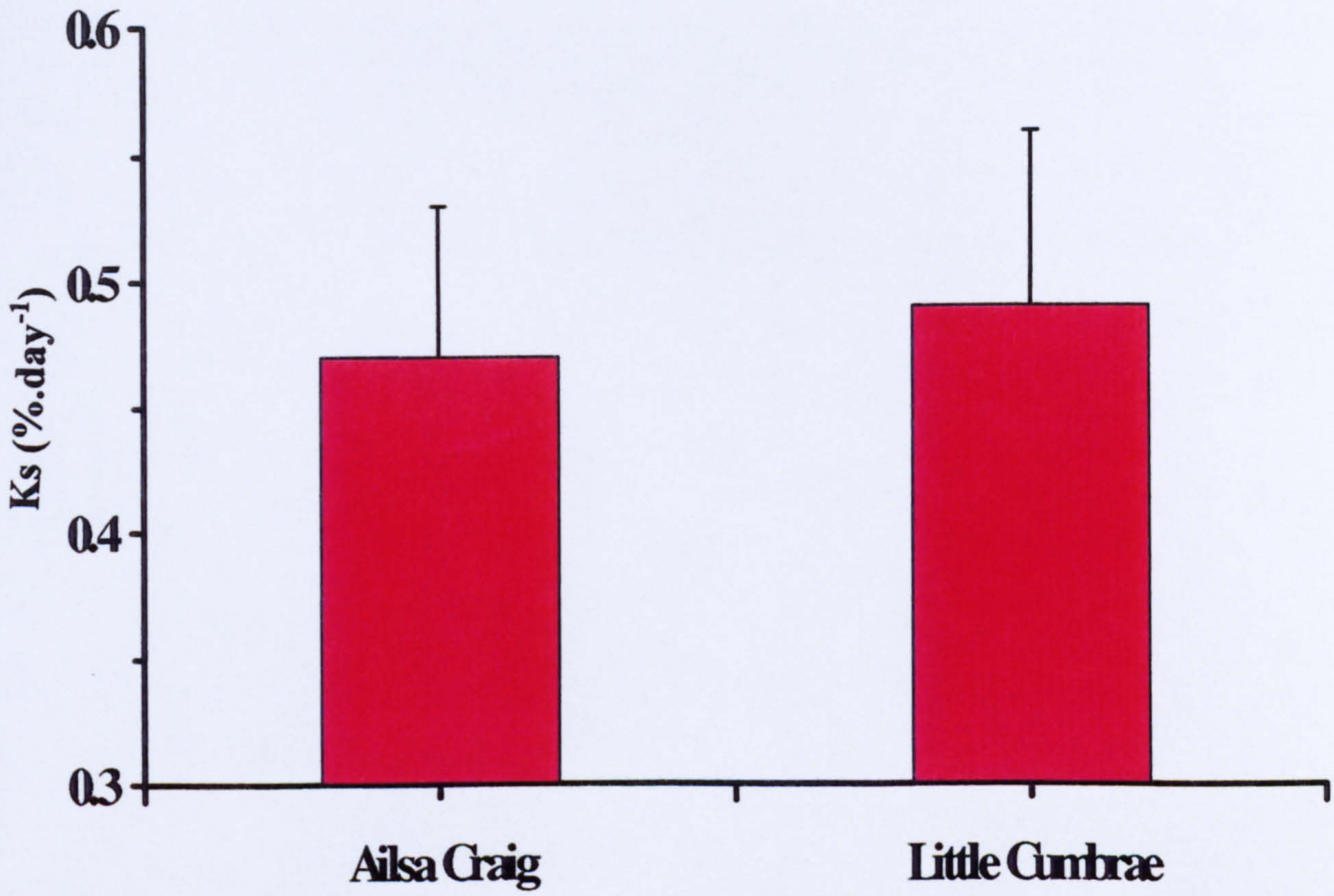


**Figure 4.3. Fractional rates of protein synthesis in the abdominal muscle tissue of *Nephrops norvegicus* from two sites in Clyde Sea area 2h after injection with L-[2,6-$^{3}$H] phenylalanine. Bars represent standard error (n=12).**

4.3.2. Method development for nucleic acid analysis

The results of the diphenlyamine reaction and dual wavelength methods of DNA estimation are shown in Table 4.1. No significant difference was found between the mean DNA concentrations obtained using the two different methods (t-test, t=0.29, p=0.8). However, while the dual-wavelength method was quicker and easier to carry out than the diphenylamine reaction, the greater accuracy afforded made the diphenylamine assay the preferred choice for further analysis. Moreover, it can be seen in Table 4.1 that the standard deviation of the diphenylamine method was almost an order of magnitude lower than for the dual-wavelength method. Further preliminary experiments also demonstrated (data not shown) that the RNA and DNA content of tissue did not change after being frozen at -70°C, and that the RNA and DNA concentrations remained the same if the analysis was performed on either fresh or freeze-dried tissue, following correction for water content.

**Table 4.1. Comparison of two methods of DNA estimation**

| | **Diphenyalmine method** | **Dual-wavelength method** |
|---|---|---|
| **Mean DNA content (ug.mg$^{-1}$)** | 0.205 | 0.223 |
| **Standard deviation (ug.mg$^{-1}$)** | 0.014 | 0.105 |
| **n** | 5 | 5 |

*Laboratory calibration*

One-way ANOVA applied to the nucleic acid contents of each tissue type indicated that for abdominal muscle tissue, the RNA concentration (Figure 4.4) and RNA:Protein ratio (Figure 4.5) decreased significantly ($P<0.05$) after 6 weeks of fasting and also showed a further significant decrease after 12 weeks of starvation ($P<0.01$). The RNA:DNA ratio (Figure 4.6) of abdominal muscle tissue was not significantly different from the initial ratio after 6 weeks but was significantly different after 12 weeks of starvation ($P<0.05$). There were no significant differences in RNA concentration, RNA:Protein or RNA:DNA in hepatopancreas or gill tissue after 6 or 12 weeks of starvation. The DNA concentration (Figure 4.7) of hepatopancreas tissue decreased significantly ($P<0.05$) after 12 weeks of starvation, but remained constant in gill and abdominal muscle tissue. The protein content (Figure 4.8) of any of the three tissues did not change significantly during the experiment. The water content (Figure 4.9) of abdominal muscle tissue increased significantly following 12 weeks of starvation ($P<0.05$), but remained unchanged in hepatopancreas and gill tissue.

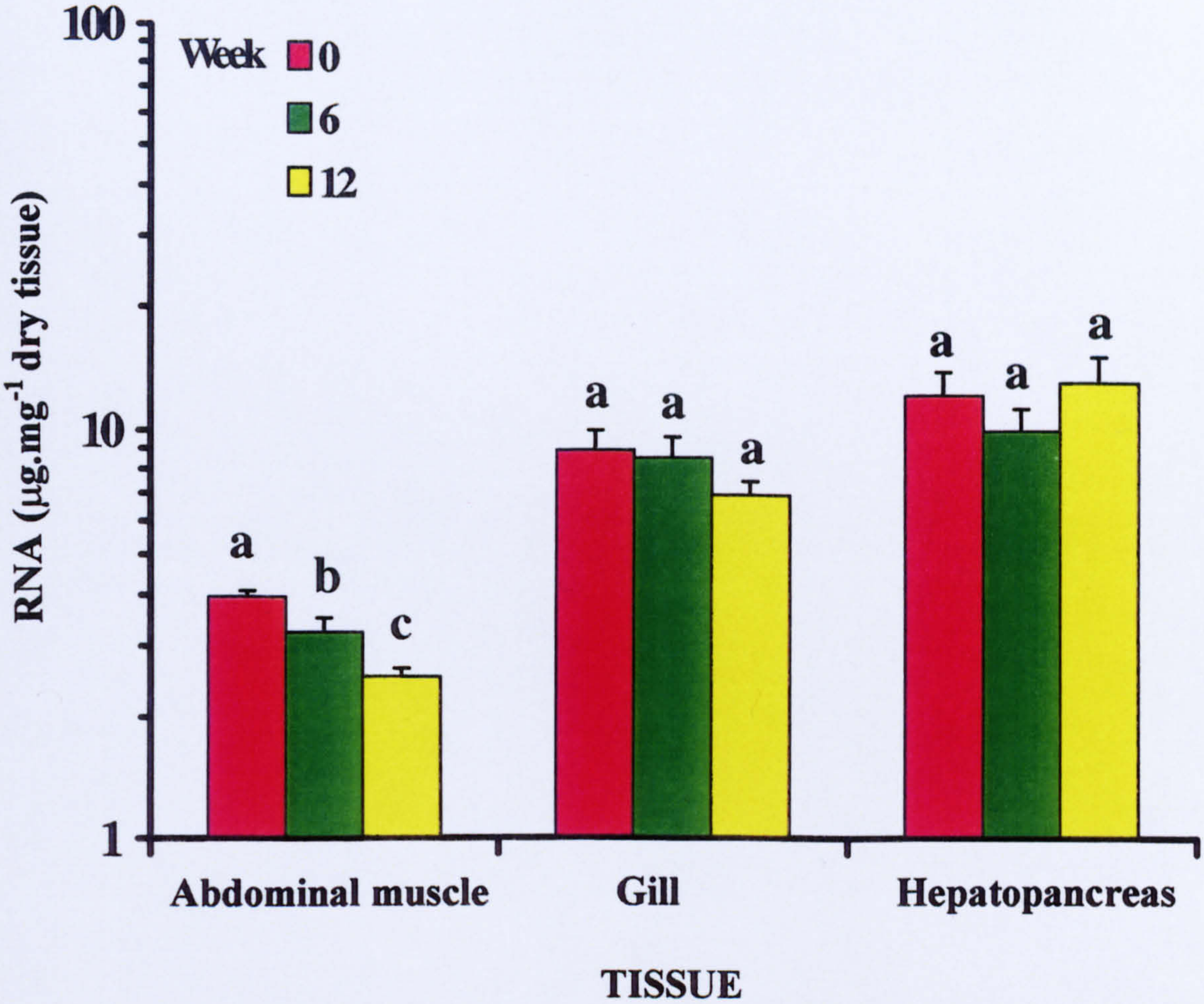


**Figure 4.4. Concentrations of RNA (µg.mg$^{-1}$ dry tissue) in the abdominal muscle, gill tissue and hepatopancreas tissue of *Nephrops norvegicus* (from the Little Cumbrae site) following starvation. Bars represent standard error (n=10). Bars sharing the same letter within each group are not significantly different.**

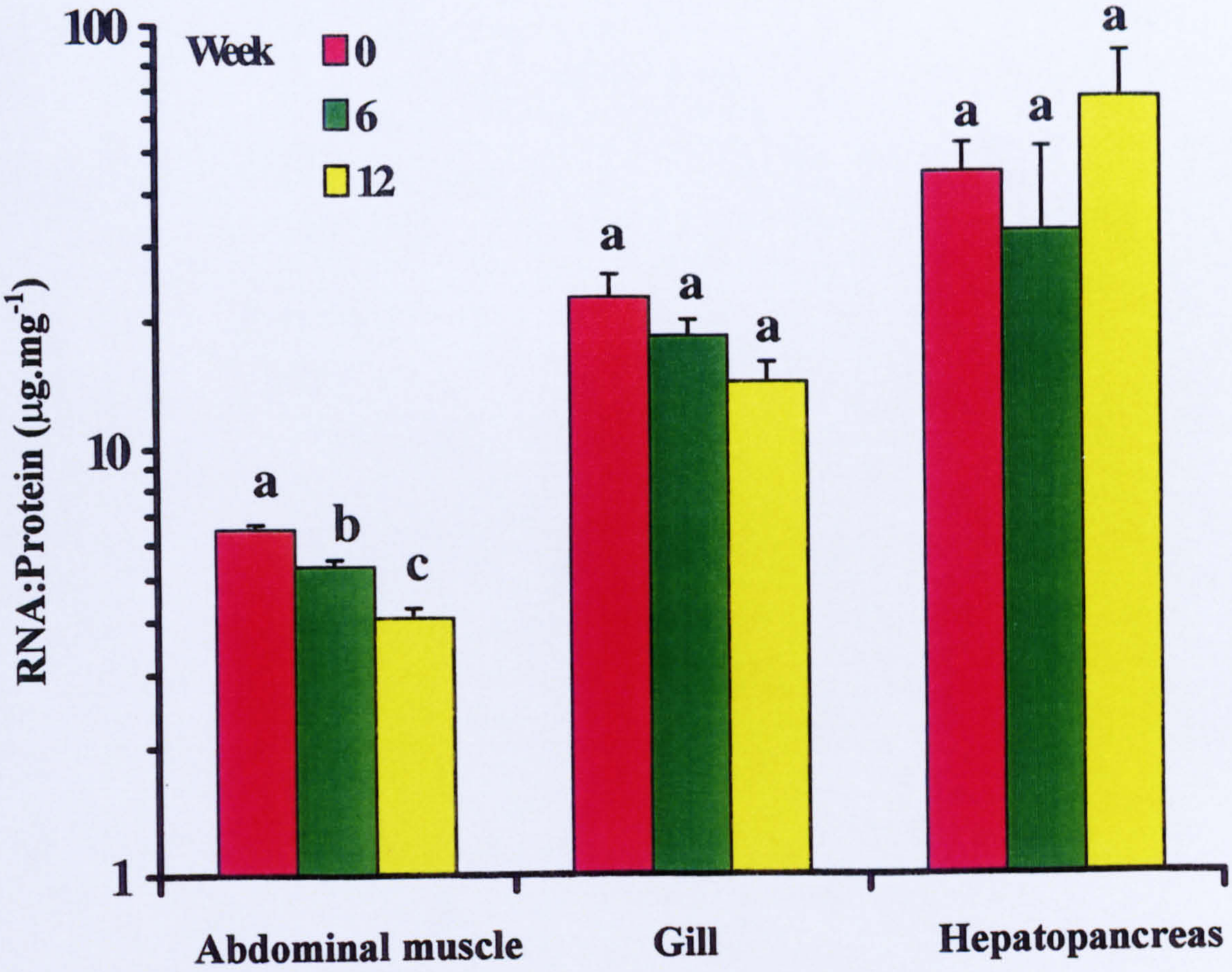


**Figure 4.5. Concentrations of RNA:Protein ($\mu g.mg^{-1}$) in the abdominal muscle, gill tissue and hepatopancreas tissue of *Nephrops norvegicus* (from the Little Cumbrae site) following starvation. Bars represent standard error (n=10). Bars sharing the same letter within each group are not significantly different.**

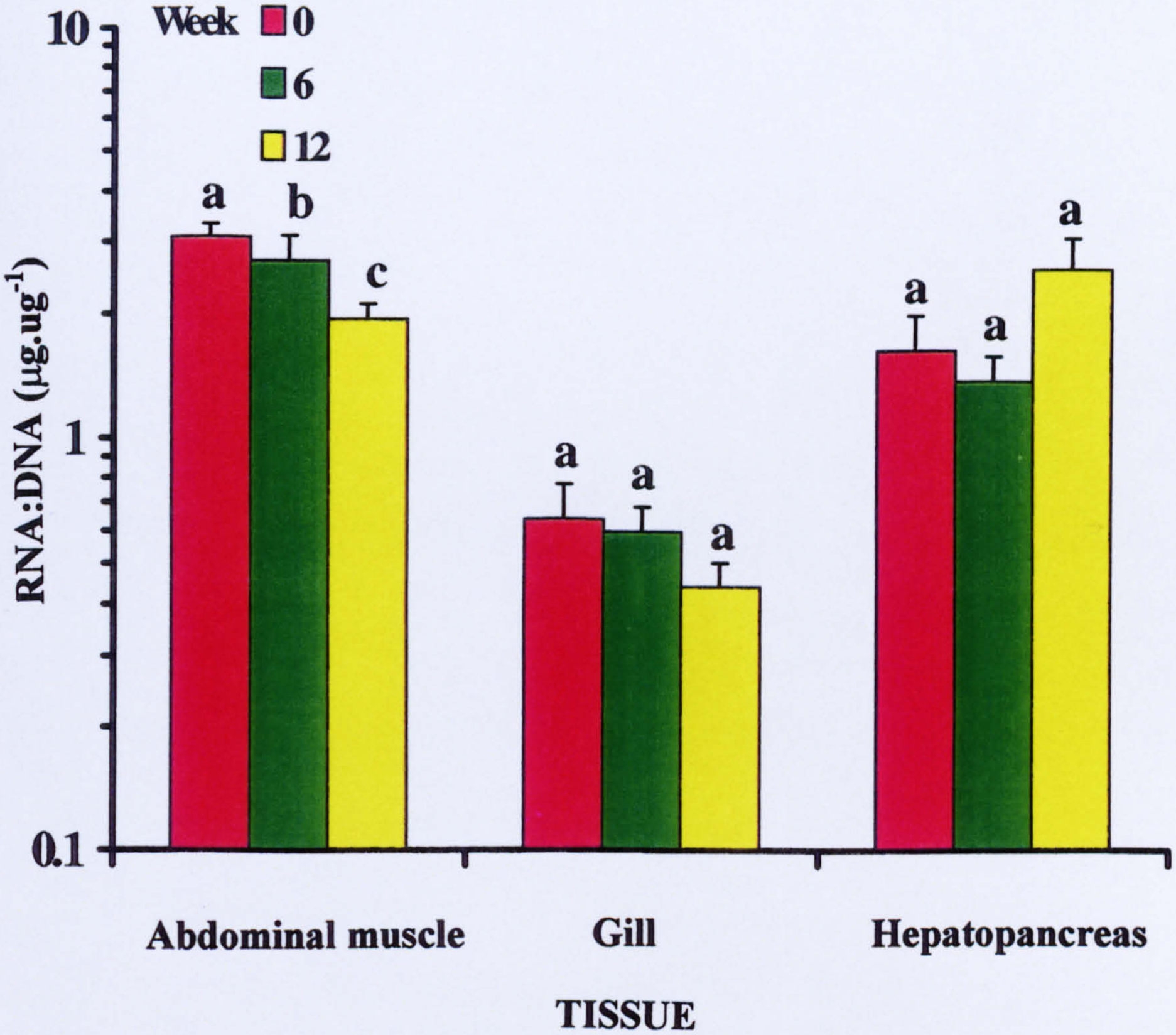


**Figure 4.6. Concentrations of RNA:DNA ($\mu g.\mu g^{-1}$) in the abdominal muscle, gill tissue and hepatopancreas tissue of *Nephrops norvegicus* (from the Little Cumbrae site) following starvation. Bars represent standard error (n=10). Bars sharing the same letter within each group are not significantly different.**

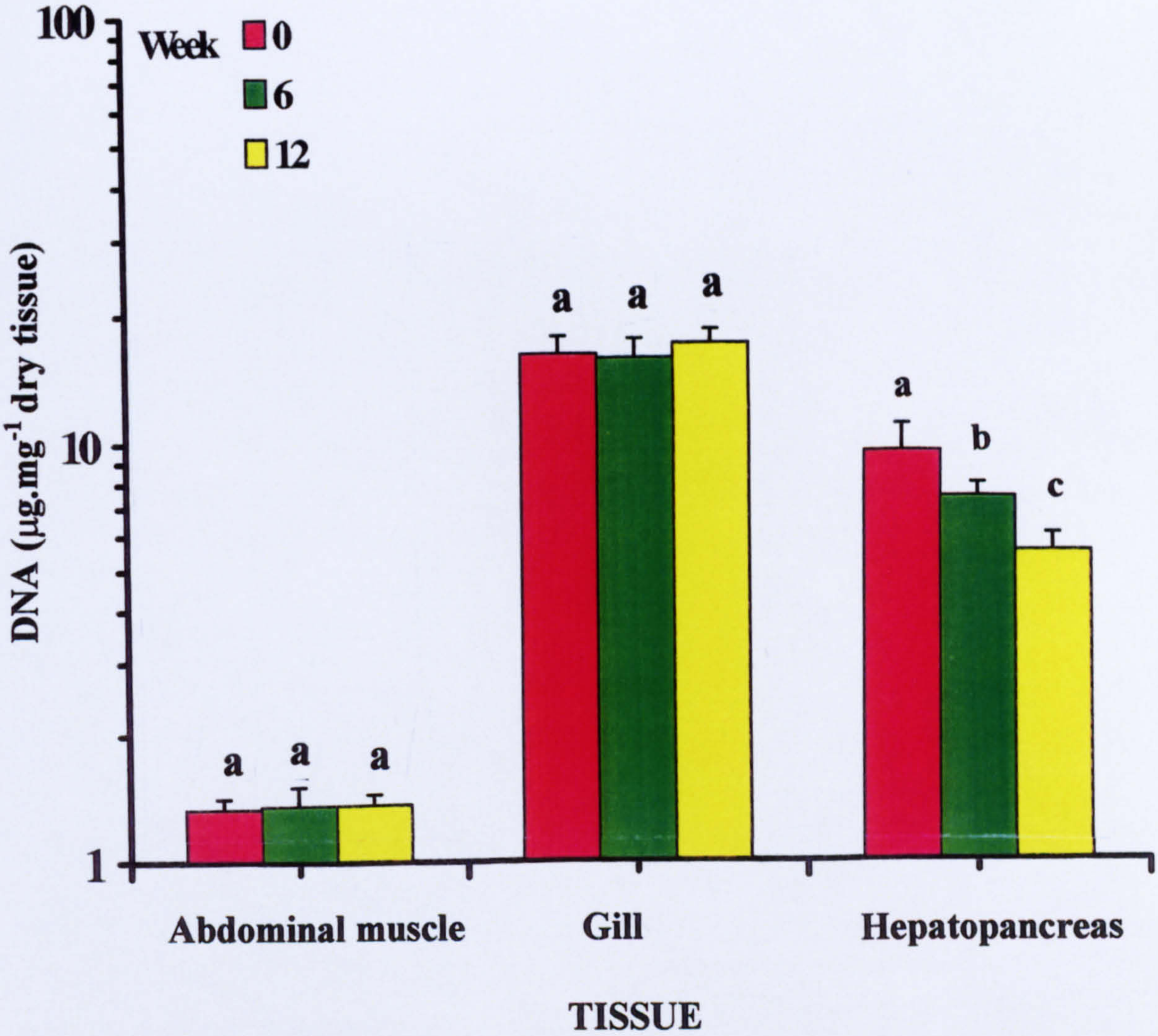


**Figure 4.7. Concentrations of DNA (μg.mg$^{-1}$ dry weight) in the abdominal muscle, gill tissue and hepatopancreas tissue of *Nephrops norvegicus* (from the Little Cumbrae site) following starvation. Bars represent standard error (n=10). Bars sharing the same letter within each group are not significantly different.**

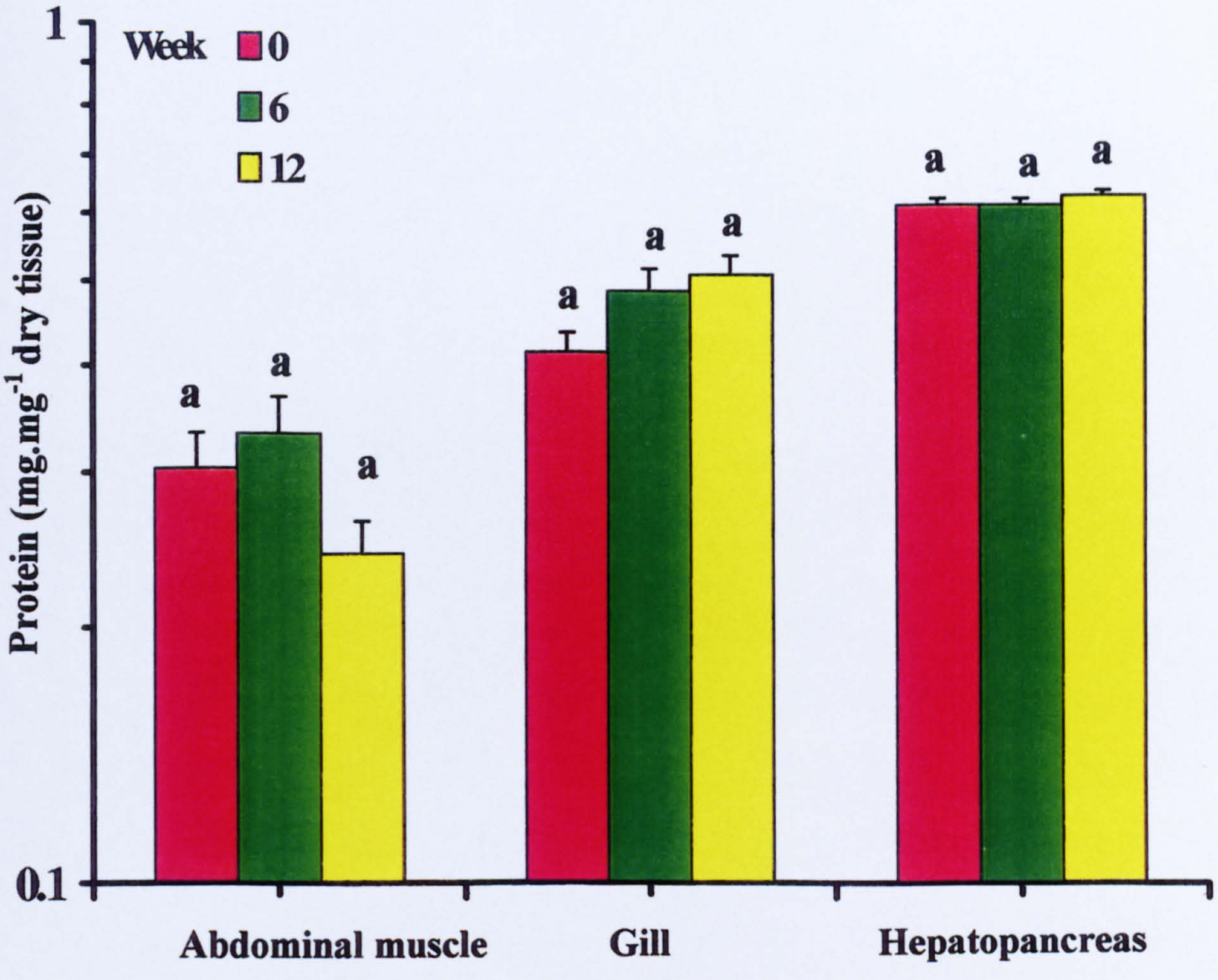


**Figure 4.8. Concentrations of Protein (mg.mg$^{-1}$) in the abdominal muscle, gill tissue and hepatopancreas tissue of *Nephrops norvegicus* (from the Little Cumbrae site) following starvation. Bars represent standard error (n=10). Bars sharing the same letter within each group are not significantly different.**

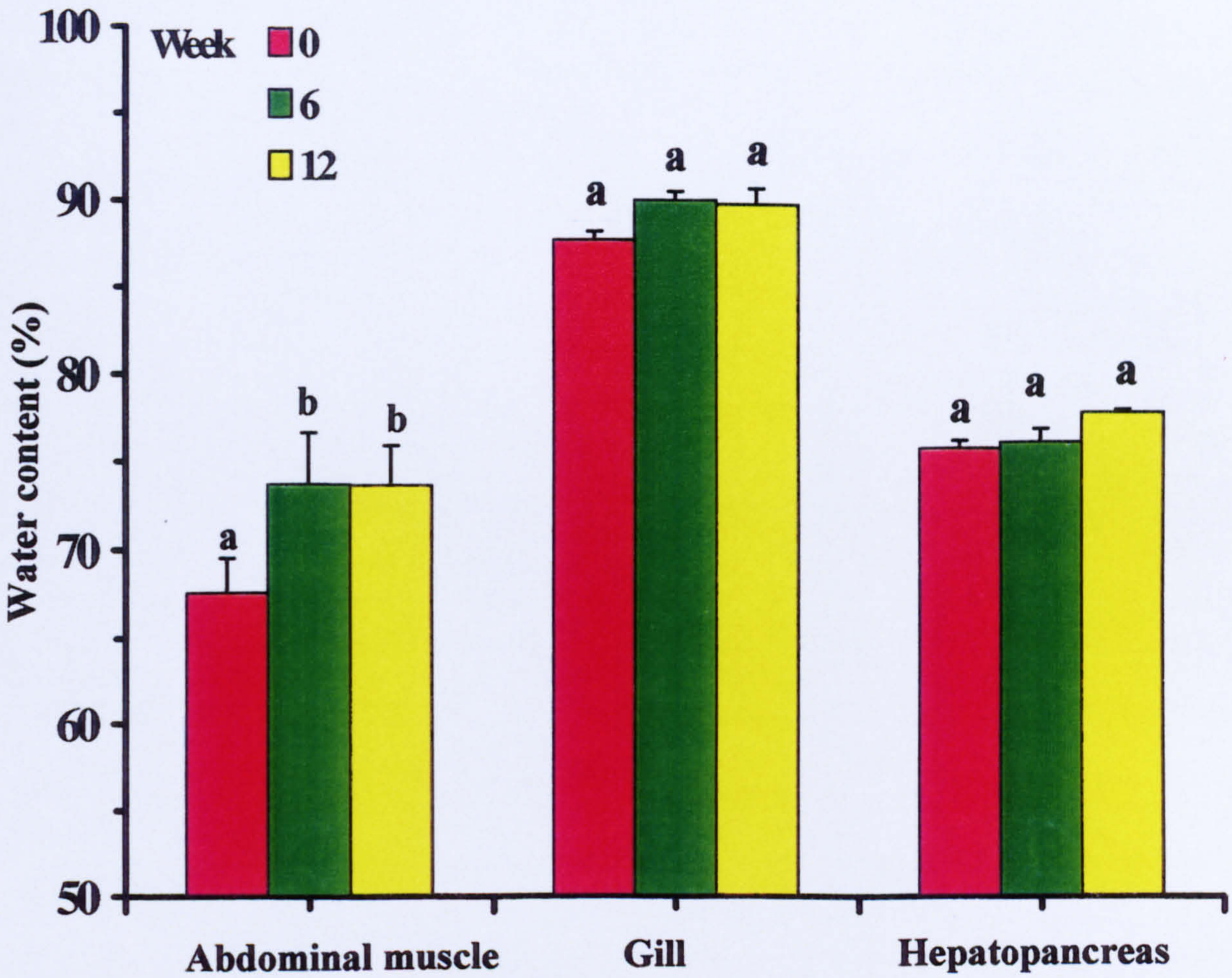


**Figure 4.9. Water content (%) in the abdominal muscle, gill tissue and hepatopancreas tissue of *Nephrops norvegicus* (from the Little Cumbrae site) following starvation. Bars represent standard error (n=10). Bars sharing the same letter within each group are not significantly different.**

4.3.3. A preliminary comparison of the abdominal muscle RNA concentration of male and female *N. norvegicus*.

It was found that the mean abdominal muscle RNA concentration of male *N. norvegicus* was significantly higher than the mean abdominal muscle RNA concentration of female animals (d.f.=11, t=3.23, P<0.01) (see Table 4.2). There were no significant differences observed between the mean fresh body weights of the male and female samples (d.f.=11, t=0.98, NS).

**Table 4.2. A comparison of the abdominal muscle RNA concentration (ug.mg$^{-1}$ dry weight) of male and female *N. norvegicus*. AM=abdominal muscle, DW=dry weight.**

| | | Fresh body weight (g) | | AM RNA (ug.mg$^{-1}$ DW) | |
|---|---|---|---|---|---|
| Sex | n | Mean | SE | Mean | SE |
| Female | 7 | 17.37 | 1.19 | 3.60 | 0.19 |
| Male | 7 | 18.91 | 1.03 | 4.46 | 0.18 |

## 4.4. DISCUSSION

The rates of protein synthesis obtained in this study, 0.4-0.5%.day$^{-1}$ are comparable with those found in work on other Crustacea; 0.5-1.0%.day$^{-1}$ for the leg muscle of the shore crab, *Carcinus maenas* (El Haj *et al.*, 1987), 0.1-0.3% for the isopod *Glyptonotus antarcticus* (Whitely *et al.*, 1996), 0.3-0.4% day$^{-1}$ for white muscle of *Homarus americanus* (El Haj *et al.*, 1996), and 0.5-1.5%.day$^{-1}$ for the brown tiger prawn *Penaeus esculentus* (Hewitt, 1992). In addition, fish also show similar values of white muscle protein synthesis, approximately 0.5%.day$^{-1}$ (Houlihan, 1991). Since

the processes of oxygen consumption and protein synthesis are linked (Houlihan, 1991), it could have been predicted from the lack of differences in $MO_2$ between the animals from the Little Cumbrae and Ailsa Craig stocklets (Chapter 2), that no differences would also be found in the rates of protein synthesis. From initial interpretation, these results could imply that there are no differences in the growth rates of the these stocklets, which is contrary to growth estimates of the animals from the stocklets reported in other studies (e.g. Tuck *et al.*, 1997a). However, these results may not represent a true indication of instantaneous growth, since the animals had not fed for several days prior to the experiments due to the delay in transportation. It is thus possible that during this time, the metabolism could have decreased. The protein synthesis measured in this experiment could therefore represent general tissue maintenance i.e. the 'ticking over' metabolism. In order to measure the 'growing' rate of metabolism, it would probably be necessary to measure protein synthesis shortly after feeding (preferably in their natural environment). The experimentation would therefore have to take place on board a research vessel. As a consequence of the logistic difficulties involved in carrying out such a project, it was decided to concentrate on developing alternative biochemical methods of estimating growth.

Further method development did indeed show, that RNA concentration is a good indicator of nutritional condition in *N. norvegicus*. Abdominal muscle tissue, showed a significant decline in RNA content, losing between 30-40% over the course of the experiment. There were, however, no significant changes in the nucleic acid content of gill tissue during starvation, implying that the metabolic status of this tissue is

preferentially maintained. Gill tissue is important for the respiratory requirements of the animal, whereas the primary function of the abdominal muscle possessed by macruran Crustacea is rapid backward swimming to escape from predators (Newland *et al.*, 1988). It is therefore possible that the infrequent use of this large mass of tissue could enable it to be utilised as an energy store. Loss of muscle condition during starvation has been reported for a number of Crustacea such as crayfish (Speck & Ulrich, 1969), crabs (Heath & Barnes, 1970) and lobsters (Dall, 1974). Even within different muscle types, there is an order in which different tissues were catabolised. Dall (1974) found that leg muscle protein was conserved in favour of that of the abdominal muscle, perhaps because the legs would need to be maintained in a suitable condition to enable foraging.

The hepatopancreas is a major organ in decapod Crustacea and carries out a variety of functions such as the secretion of digestive enzymes and storage of nutrients (Chang & O'Connor, 1983). Although this study did not find any significant differences in RNA content in hepatopancreas tissue following starvation, it was noted that the DNA content decreased by over 40%. Johnson (1980) observed cellular degeneration and nuclear lysis in the hepatopancreas of the crab *Callinectes sapidus*, and attributed this to nutritional stress, since the crabs were collected during the winter when little food was available. Nutrients are stored intracellularly in hepatopancreas tissue as reserve inclusions (Ris) (Chang & O'Connor, 1983). The progressive loss of DNA found in this study could be explained by the catabolism of Ris, since lysing of cells in order to access these intracellular nutrient stores could result a reduction in DNA content.

It is appropriate that the present study found abdominal muscle to be the most suitable tissue for nucleic acid analysis, as it is also the portion marketed for human consumption. As well as gaining ecological information, the biochemical analysis may also give some indication of the quality of the meat. Other studies have recommended white muscle tissue for this type of work, since RNA concentration in this tissue has been found to be proportional to whole body growth both for species of fish (Bulow, 1987) and Crustacea (Moss, 1994a).

Although this method has been applied to a number of species of fish and Crustacea, this study is the first application of the technique to the Norway lobster. The concentrations of nucleic acids for a number of species of aquatic organisms are displayed for comparison in Tables 4.3 & 4.4. As might be expected, the nucleic acid contents of *N. norvegicus* tissue are most similar to those of the closely related American lobster *Homarus americanus*, although comparable results are also found with many species of fish. Interestingly, it appears that the variation in nucleic acid concentration is greater between different tissue types than it is between taxa.

The RNA index was found to be most sensitive when concentrations were expressed per unit dry weight rather than as a ratio of protein or of DNA concentration. These findings agree with those of a number of workers who recorded increased variation when RNA was expressed as a ratio of protein or DNA, in both fish (Wilder & Stanley, 1983; Ferguson & Danzmann, 1989) and crustaceans (Moss, 1994 a,b).

| Organism | Tissue | RNA | RNA:protein | RNA:DNA | Source |
|---|---|---|---|---|---|
| **CRUSTACEA** | | | | | |
| *Nephrops norvegicus* | Abdominal muscle | 1.9-5 µg.mg dry wt$^{-1}$ | 3-8.5 µg.mg$^{-1}$ | 2-6 | This study |
| | Gill | 3-15.5 µg.mg dry wt$^{-1}$ | 7-40 µg.mg$^{-1}$ | 0.15-1.7 | |
| | Hepatopancreas | 2-26 ug.mg dry wt$^{-1}$ | 6-200 µg.mg$^{-1}$ | 0.5-5.5 | |
| *Homarus americanus* | Whole postlarvae | 0.03-0.18 mg.animal$^{-1}$ | | 1-4 | Juinio *et al.* (1992) |
| *Homarus americamus* | Abdominal muscle | | 2-10 µg.mg$^{-1}$ | | El Haj *et al.* (1996) |
| | Claw muscle | | 5-10 µg.mg$^{-1}$ | | |
| | Leg muscle | | 7-15 µg.mg$^{-1}$ | | |
| *Penaeus vannamei* | Head | 20-80 µg.mg dry wt$^{-1}$ | | 4-9 | Stuck *et al.* (1996) |
| *Penaeus vannamei* | Abdominal muscle | 2-5 µg.mg$^{-1}$ | | 2.5-7 | Moss (1994a,b) |
| *Crangon crangon* | Whole animal | | | 1-2 | Regnault & Luquet (1974) |
| *Callinectes sapidus* | Whole juvenile | 0.5-2.5 mg.g$^{-1}$ | | 1-3 | Wang & Stickle (1986) |
| *Hyas araneus* | Whole larvae | 2-14 µg.animal$^{-1}$ | | | Anger & Hirche (1990) |
| *Talorchestia martensii* | Whole animal | 9-15 µg.mg dry wt$^{-1}$ | | | Chandran & Michael (1968) |
| *Orchestia platensis* | Whole animal | 2-40 µg.mg dry wt$^{-1}$ | | | Sutcliffe (1965) |
| *Artemia salina* | Whole animal | 30-130 µg.mg dry wt$^{-1}$ | | | |
| *Artemia salina* | Whole animal | 5-80 µg.mg dry wt$^{-1}$ | 40-100 µg.mg$^{-1}$ | 2-4 | Dagg & Littlepage (1972) |
| *Euchaeta elongata* | Whole animal | 5-40 µg.mg dry wt$^{-1}$ | 10-60 µg.mg$^{-1}$ | | |
| *Paracalanus sp.* | Whole animal | | | 3-8 | Nakata *et al.* (1994) |
| *Calanus pacificus* | Whole animal | 10-70 µg.mg dry wt$^{-1}$ | | 0.1-4 | Ota & Landry (1984) |
| *Daphnia magna* | Whole animal | 40-120 µg.mg dry wt$^{-1}$ | 80-200 µg.mg$^{-1}$ | 30-75 | McKee & Knowles (1987) |
| **MOLLUSCA** | | | | | |
| *Pecten maximus* | Gonad | 2-14 mg.g$^{-1}$ | | 0.1-0.4 | Robbins *et al.* (1990) |
| *Placopecten magellanicus* | Adductor muscle | | | 0.07-0.26 | Kenchington (1994) |
| *Crassostrea virginica* | Mantle tissue | 1.5-3 g.mg$^{-1}$ | | 2-4 | Wright & Hetzel (1985) |
| *Euvolva ziczac* | | | | | |

**Table 4.3. Examples of tissue nucleic acid concentrations in aquatic invertebrates.**

| Organism | Tissue | RNA | RNA:protein | RNA:DNA | Source |
|---|---|---|---|---|---|
| **PISCES:** | | | | | |
| *Gadus morhua* | White muscle | 0.7-1.5 $\mu g.mg^{-1}$ | 5-11 $\mu g.mg^{-1}$ | 2.5-3.5 | Foster *et al.* (1992) |
| | Gill | 3.9-5 $\mu g.mg^{-1}$ | 60-65 $\mu g.mg^{-1}$ | 0.4-0.6 | |
| | Intestine | 2.4-4.2 $\mu g.mg^{-1}$ | 20-40 $\mu g.mg^{-1}$ | 0.5-1.5 | |
| | Stomach | 2.1-3.9 $\mu g.mg^{-1}$ | 35-65 $\mu g.mg^{-1}$ | 1-2 | |
| | Vent | 2.1-2.5 $\mu g.mg^{-1}$ | 15-30 $\mu g.mg^{-1}$ | 4-4.5 | |
| *Gadus morhua* | Whole larvae | | | 1-7 | Clemmesen (1996) |
| *Solea solea* | Whole larvae | 0.5-100 $\mu g.larva^{-1}$ | | 0.8-5 | Richard *et al.* (1991) |
| *Clupea harengus* | Whole larvae | 0.1-1.4 $\mu g.mm\ length^{-1}$ | | 0.5-5 | Clemmesen (1994) |
| *Dicentrarchus labrax* | White muscle | | | 1.2-3.2 | Mustafa *et al.* (1991) |
| *Salvelinus alpinus* | White muscle | | | 0.5-4.5 | Miglavs & Jobling (1989) |
| | Liver | | | 0.5-4.5 | |
| *Salvelinus alpinus* | Liver | 6-7 $\mu g.mg^{-1}$ | | 3-6 | Yang & Dick (1993) |
| *Salvelinus fontinalis* | White muscle | 1.2-1.6 $\mu g.mg^{-1}$ | | 3.3-5 | Wilder & Stanley (1983) |
| *Salvelinus fontinalis* | White muscle | 0.1-1.3 $\mu g.mg^{-1}$ | | 0.1-2.5 | McLaughlin *et al.* (1994) |
| *Oncorhynchus mykiss* | White muscle | 1-2.2 $\mu g.mg^{-1}$ | 50-120 $\mu g.mg^{-1}$ | 2-5 | Mugiya & Sugano (1991) |
| *Oncorhynchus mykiss* | Liver | 10-13 $\mu g.mg^{-1}$ | | 2-4 | Jurss *et al.* (1986) |
| *Salmo trutta* | White muscle biopsy | | | 2-4.5 | Grant (1996) |
| *Notegmigonus crysoleucus* | Whole fat-free fish | 1.5-4.2 $\mu g.mg\ dry\ wt^{-1}$ | | 1.3-5.7 | Bulow (1970) |
| *Lepomis macrochirus* | White muscle | 0.2-0.4 $\mu g.mg\ dry\ wt^{-1}$ | | 1-6 | Bulow *et al.* (1981) |
| | Liver | 4-12 $\mu g.mg\ dry\ wt^{-1}$ | | 1-7 | |
| *Cyprinus carpio* | White muscle | 6.7-10 $\mu g.mg^{-1}$ | | 1.5-6 | Goolish *et al.* (1983) |
| *Sciaenops ocellatus* | White muscle | 1-4 $\mu g\ .g^{-1}$ | | 1-30 | Chung *et al.*(1993) |
| | Red muscle | 0.2-5 $\mu g.g^{-1}$ | | 0.2-15 | |
| | Liver | 5-12 $\mu g.g^{-1}$ | | 7-30 | |

**Table 4.4. Examples of tissue nucleic acid concentrations in fish.**

Historically, the RNA:DNA ratio has been the preferred method of expressing RNA concentration since it is thought to correct for cell number. Some problems have been found, however, when using this ratio. For example, DNA concentration has been shown to exhibit a negative relationship with protein synthesis when different tissues are compared (Houlihan, 1991; Foster *et al.*, 1992).

Some workers have experienced problems when attempting to correlate nucleic contents with growth rate or condition in Crustacea (Dagg & Littlepage, 1972; Ota & Landry, 1984). These authors suggested that the complicating effects of the moult cycle limits the use of the assay. It is possible that since these studies were carried out on larval Crustacea, apart from the frequent moulting that occurs at this stage of life history, major developmental changes will be taking place, which could have a marked effect on tissue biochemistry. Whilst it is well known that the moult cycle does have a marked influence on the physiology of Crustacea (see review by Chang *et al.*, 1995), studies have shown that the moult cycle had no effect on the RNA content of abdominal muscle (Moss, 1994a). El Haj *et al.* (1996) demonstrated that it is the RNA activity that changes as opposed to RNA content. This change in activity is thought to be initiated by the moult hormone ecdysone. Some investigations such as those Regnault & Luquet (1974) have avoided complicating moult affects by only analysing animals of a particular moult stage, which was also the approach taken by the present study.

It was found that the mean abdominal muscle RNA concentration of male *N. norvegicus* was significantly higher than the mean abdominal muscle RNA

concentration of female animals. This is unlikely to be due to differences in the animal sizes, since no significant differences were observed between the mean fresh body weights of the male and female samples. The lower abdominal RNA concentrations found in the abdominal muscle of female *N. norvegicus* could suggest a lower rate of growth, which is in agreement with earlier work on Clyde *N. norvegicus*. Using length-frequency based techniques, these studies estimated that females appear to grow more slowly than males, presumably due to their reproductive expenditure (see reviews by Bailey & Chapman, 1987; Chapman & Howard, 1988; IMBC, UMBSM & IRPEM, 1994).

Nucleic acids have also been utilised as indicators of environmental stressors. Studies based both in the laboratory and in the field have demonstrated that a wide range of toxins affect concentrations of nucleic acids. Toxicant-induced changes in RNA content may even occur before a significant difference in body weight is detected. However, it may be difficult to decipher exactly how much of the decrease in RNA content can be attributed to a reallocation of resources away from protein synthesis towards xenobiotic metabolism, or to a reduction in feeding rate. Baron & Adelman (1984) found that following exposure to toxicants under laboratory conditions, the RNA and protein contents of whole fathead minnow larvae were significantly reduced. Similarly, Verma *et al.* (1984) noted that exposure to detergent caused a significant decrease in liver RNA concentration of the fish *Clarias batrachus.* Changes in RNA concentration due to toxin exposure have also been reported for Crustacea. Divavin (1975) found that exposure to petroleum and phenol resulted in a rapid fall in the RNA content of the shrimp, *Palaemon adsperus.* Kearns &

Atchinson (1979) observed the effects of the heavy metals cadmium and zinc on the yellow perch, *Perca flaviscens* in the field. They discovered a significant negative correlation between whole fish metal concentration and RNA:DNA ratio. However, it is important to take into account as many of the environmental factors as possible when interpreting field data. For example, Wilder & Stanley (1983) noted that brook trout *Salvelinus fontinalis* exposed to the pesticide carbonyl actually had significantly higher RNA:DNA ratios than laboratory fish. They suggested that the elevated RNA:DNA could be a result of the pesticide spraying. It was proposed that the pesticide may have killed large numbers of both terrestrial and benthic invertebrates, resulting in a higher density of insects in the stream drift and consequently an increased food supply.

Populations of *N. norvegicus* are subject to a variety of environmental stresses including high population densities (Tuck *et al.*, 1997), prolonged exposure to hypoxia (Hagerman & Baden 1988, Baden *et al.*, 1994) and parasitic infection (Field *et al.*, 1992, 1995, Field & Appleton 1995, Taylor *et al.*, 1996). Convenient methods of evaluating the condition of recently captured *N. norvegicus,* such as nucleic acid indices, therefore provide a valuable tool to aid in the management of the fishery.

## 4.5. SUMMARY

- Rates of protein synthesis of the abdominal muscle tissue were calculated for *N. norvegicus* from the Little Cumbrae and Ailsa Craig sites. The results obtained

were comparable to other published studies on crustacean muscle. No significant differences in $K_s$ were found between animals from the different sites.

- Accurate methods were developed for the laboratory determination of RNA, DNA and protein concentration as indicators of nutritional condition..

- A preliminary study showed a significant difference in abdominal muscle RNA concentrations between the sexes. Male *Nephrops* exhibited significantly higher abdominal muscle RNA concentrations than female *Nephrops* of the same fresh body weight.

- The effects of starvation on the nucleic acid content of three tissues were examined (abdominal muscle, gill and hepatopancreas). Significant reductions were observed in the RNA concentration, RNA:Protein ratio and RNA:DNA ratio of abdominal muscle tissue of starved *N. norvegicus*. No significant changes were found in the other tissues examined, with the exception of a decrease in hepatopancreas DNA content. It was thus concluded that abdominal muscle tissue was the most suitable for assessment of nutritional condition.

# CHAPTER 5

# Evidence of nutritional limitation in populations of the Norway lobster *Nephrops norvegicus* in the Firth of Clyde

## 5.1. INTRODUCTION

### 5.1.1. Geographical variation in nutritional condition

The existence of geographical variability in the abundance and size of *N. norvegicus* in Scottish waters has been well known since the 1960s (Cole, 1962; O' Riordan, 1964; Thomas, 1965). Because the fishery was rapidly expanding at this time, it was originally thought that the variation could be attributed to differences in the intensity of fishing (Thomas, 1965). However, it was also accepted that some other factors may be involved, since variation in the size of individual *N. norvegicus* between some areas was observed before commercial exploitation. More recent studies have suggested that the variability is associated with sediment type, and can occur over scales of a few km (Chapman & Bailey, 1987; Tuck *et al.*, 1997a). Stocklets of *N. norvegicus* living on coarse mud sediments (10-50% silt & clay) tend to consist of lobsters characterised by a small average size, living at high densities, whereas stocklets found on finer muds (>90% silt & clay) occur at lower densities and have a larger mean animal size. However, it is not clear whether this is a direct relationship, or simply a coincidence resulting from local hydrographic conditions, since the factors responsible for creating the sediment conditions could also be controlling larval distribution (Tuck, 1993). It has been suggested that in the Clyde Sea area, local

oceanographic features play a large role in *N. norvegicus* distribution. Tuck (1993) noted a southerly drift of *N. norvegicus* larvae associated with the surface water outflow from the Clyde Sea. When the larvae reach the southern limits of the Clyde Sea, an oceanographic front is thought to slow their passage, causing them to accumulate over a densely populated area south of Ailsa Craig. High densities of *N. norvegicus* larvae have also been found to be associated with frontal systems in the Irish Sea (Nichols *et al.*, 1987; White *et al.*, 1988).

An alternative explanation was provided by Chapman & Bailey (1987), who speculated that the recruitment could be controlled at the time of larval settlement. They proposed that larval numbers could be similar in different areas, but their mortality could be dictated by the type of sediment. Larval settlement is a crucial phase in the life history of lobsters; factors affecting survival at this stage have been found to make huge differences to animal density (reviewed by Cobb & Wahle, 1994). For example, Pollock (1991) observed that in certain areas of the habitat range of the spiny lobster *Jasus tristani*, the increase in available shelter afforded to recently settled larvae greatly reduced mortality from predation, resulting in a higher lobster density than on the more exposed grounds.

It has been observed that *N. norvegicus* from densely populated areas grow more slowly than conspecifics from less crowded areas (Bailey & Chapman, 1983; Tuck *et al.*, 1997). Other biological parameters associated with growth rate, such as size at maturity and fecundity have also been found to be related to density (Bailey & Chapman, 1983; Bailey, 1984; Tuck, 1993). Density-dependent growth variation has

also been observed in other Crustacea. A study by Jernakoff *et al.* (1994) found that the juvenile western rock lobster *Panulirus cygnus,* grew faster in areas of low population density. However, estimates of crustacean growth rate from field samples can be prone to error as the animals are intrinsically difficult to age independently of size. Current methods often involve the use of size-frequency distributions, and calculate growth by measuring the differences in carapace lengths between year classes (Jensen, 1965; Hillis, 1971, 1979; Farmer, 1973; Nicholson, 1979; Bailey & Chapman, 1983; Froglia & Gramitto, 1988; Tully *et al.,* 1989; Tuck *et al.,* 1997a). These methods are useful for estimating the growth rate of early year classes, but it is more difficult for older lobsters since, because growth rate decreases with age, the older year classes tend to merge together making them difficult to identify. There are new techniques of ageing *N. norvegicus* currently being developed based on the concentration of age-related pigment in the brain (Belcheier *et al.*, 1994), although there is no method available that is comparable in accuracy and ease of use as the use of otoliths to age fish. Estimates of growth rate in *N. norvegicus* have been improved by incorporating data from tagged animals (Jensen, 1965; Hillis, 1979; Bailey & Chapman, 1983), but the low rate of recapture (Chapman, 1980) ensures that the cost of this type of work is high. Consequently, biochemical indices that are able to estimate condition in field animals can be extremely useful.

5.1.2. Seasonal variation and the effects of temperature on nutritional condition

It is well known that temperature has a considerable influence on biological processes. An increase in temperature confers an increase in molecular kinetic energy leading to a greater probability of breakage of the chemical bonds necessary for the reaction to

occur. Therefore many physiological processes which are dependent on biochemical reactions such as enzymatic rates and membrane functions, are also markedly affected by changes in temperature. Most studies on the thermal responses of Crustacea have concentrated on their respiratory physiology (see review by Vernberg, 1983). The degree of temperature acclimation is often measured using the respiratory quotient-$Q_{10}$. The $Q_{10}$ of an organism describes the magnitude by which the respiration rate changes following a rise in temperature, which is commonly measured over a range of 10°C. High $Q_{10}$ values such as between 2.5-3.6, which Hagerman (1970) recorded for the shrimp *Crangon crangon* over the temperature range 0-20°C, suggests limited thermal compensation. In contrast, low values indicate thermal adaptation, such as 1.26 and 1.66 which were found for the freshwater copepods *Diaptomus dorsalis* and *Mesocyclops edax* respectively, over the temperature range 25-30°C (Smith *et al.*, 1978).

Temperature is also known to have a marked influence on crustacean feeding behaviour. Wyban *et al.* (1995) found that the feeding rate and food conversion ratio of the Pacific white shrimp *Penaeus vannamei* were directly related to temperature. In addition, it was also noted that the thermal effects were more pronounced in larger shrimps. A study by Ramirez *et al.* (1992) found that foraging can also be temperature dependent. The crayfish, *Procambarus clarkii* was observed to maintain a diel preference of foraging temperature, even when kept under constant darkness. The thermal responses of crustaceans can differ markedly both within and between species. Schneider (1967) (cited in Vernberg, 1983) studied the widely distributed xanthid crab *Rhithropanopeus harrisii* which can be found on the east coast of the

Americas, from Canada to Brazil. It was found that populations of crabs from Maine, North Carolina and Florida exhibited marked differences in their thermal responses. Since further studies on laboratory reared xanthid crabs from each site failed to find any significant interpopulation differences, Schneider (1967) (cited in Vernberg, 1983) concluded that the initial differences were not genetically based, but a consequence of the environmental conditions. Evidence of interspecific genetic differences in temperature adaptation has been provided by studies on metabolic enzymes. Dittrich (1992 a,b) investigated thermal adaptation by examining the kinetic properties of gastric enzymes in a number of different crustaceans. Cold adaptations of Antarctic benthic species (*Chorismus antarcticus*) were evident from the low activation energy of trypsin-like proteases as well as the comparatively high enzyme activities at 0°C. In contrast, the energies of activation of metabolic enzymes of tropical (*Ocypode ryderi*), temperate (*Cancer pagurus*) and subarctic (*Meganyctiphanes norvegica*) species were significantly higher. After a series of biochemical tests on these enzymes, species-specific reactions were observed, suggesting that differences are occurring at a molecular level. Metabolic temperature adaptations in the processes involved in protein synthesis have also been reported. Workers have recorded higher rates of both amino acid transport (Goolish & Adelman, 1983) and incorporation (Kent & Prosser, 1980) in cold-acclimated fish.

### 5.1.3. Chapter Aims

RNA concentration and the ratios of RNA concentration to DNA and protein have frequently been used as nutritional indicators (as discussed in Chapter 4). Their application as indicators of nutritional limitation for *N. norvegicus* under laboratory

conditions has been demonstrated in Chapter 4. The aim of this chapter was to determine if RNA concentration, RNA:protein ratio and RNA:DNA ratio could be used to investigate the nutritional condition of two contrasting *N. norvegicus* stocklets in the Clyde Sea area. Although it has already been shown that the recent feeding history has a marked effect on nucleic acid concentration, the extent to which other environmental variables such as temperature influence these indices also needs to be determined. The aim of this chapter was to examine the thermo-acclimatory response of abdominal muscle RNA concentration in the Norway lobster *Nephrops norvegicus*. Seasonal variation in RNA concentration was also investigated and compared with the hepatosomatic index, an established indicator of condition.

## 5.2. MATERIALS AND METHOD

### 5.2.1. Comparison of two stocklets

Adult *N. norvegicus* were collected from two sites, one from the north Clyde Sea area, south of Little Cumbrae and the other from the south Clyde Sea area, south of Ailsa Craig (see Figure 2.1). These two sites were chosen since they are the locations of two stocklets which are markedly different in population density. 28 male lobsters (15-35g fresh body weight) were removed from the samples collected at each site. The moult stage of lobsters was diagnosed by the method of Aiken (1980), and only intermoult males were selected. Samples of abdominal muscle tissue were removed from each animal and analysed for RNA, DNA and protein concentration using the procedures detailed in Chapter 4.

5.2.2. Diet manipulation experiment

Since it is thought that lobsters from Ailsa Craig have access to less food than those from the Little Cumbrae site, an experiment was designed in which lobsters which were captured from the food limited environment (Ailsa Craig) were well fed (maintained in the laboratory on a high-feeding regime) whereas those from the food rich site (Little Cumbrae) were starved. Further samples of adult *N. norvegicus* were collected from the south of Ailsa Craig and south of Little Cumbrae stocklets. 40 adult *N. norvegicus* (15-35g fresh body weight) from both sites were transferred to seawater aquaria at the University of Glasgow. Lobsters from south of Ailsa Craig were fed *ad lib.* on a diet of squid mantle, whereas those from south of Little Cumbrae were fasted. 8 lobsters from each site were sampled after 0, 6, 12, 24 and 36 days and rapidly frozen in liquid $N_2$ and stored at -70°C prior to nucleic acid analysis (see Chapter 4 for details of analytical procedures). Differences in biochemical parameters between lobsters from each site were tested for significance using Student's t-tests. Comparisons were made between the two sites at each sampling time.

5.2.3. Seasonal variation in abdominal muscle RNA concentration and hepatosomatic index

Bimonthly samples of *Nephrops norvegicus* were obtained from trawls in the Firth of Clyde, south of Little Cumbrae. Animals were sampled from 1995 to 1996 during the months of July, September, November, January, March and May. Due to the low representation of females in some catches, only male animals were selected. Animals

were killed by swiftly inserting a needle into the brain, and transported on ice to the University of Glasgow. Each animal was weighed whole, after which the hepatopancreas was removed and weighed separately. This enabled the calculation of the hepatosomatic index, ie. the percentage of the total body weight contributed by the hepatopancreas. Samples of abdominal muscle tissue were also removed and the RNA concentration was determined using the methods outlined in Chapter 4.

5.2.4. Effect of temperature on abdominal muscle RNA concentration

*Nephrops norvegicus* were obtained from trawls in the Firth of Clyde, south of Little Cumbrae and transported to aquaria at the University of Glasgow. Twenty animals (15-35g fresh body weight) were placed into tanks in three aquaria, in which the room temperatures were 5°C, 10°C and 15°C. Each tank was aerated and contained flowing seawater of 34‰ salintiy. Tubing and bottles were added to the tanks to provide shelter in order to reduce cannibalism. Samples of ten animals were frozen in liquid nitrogen and stored at -70° for future biochemical analysis. The remainder of the animals were fed cubes of squid mantle on alternate days throughout the six week experimental period. On feeding days, *N. norvegicus* were each fed 0.5g per animal. After 3 weeks, ten animals from each of the three temperature regimes were removed from their tanks, weighed and frozen immediately in liquid nitrogen. This procedure was repeated again at week 6. All the frozen samples were then analysed for abdominal muscle RNA concentration using the procedure described in Chapter 4. RNA concentration was expressed per unit wet weight.

## 5.3. RESULTS

### 5.3.1. Comparison of two stocklets

The relationships between nucleic acid concentrations (RNA, RNA:Protein and RNA:DNA) and body weight were compared between samples of lobsters from the south of Little Cumbrae and south of Ailsa Craig sites (see Figures 5.1-5.3 and Table 5.1 for details of regression equations). There was a significant correlation between RNA concentration and body weight for the Little Cumbrae ($r=0.5$, $P<0.005$) and Ailsa Craig ($r=0.39$, $P<0.05$) stocklets, and also between RNA:DNA ratio and body weight for the Little Cumbrae ($r=0.40$, $P<0.05$) lobsters. However, there were no significant relationships between body weight and RNA:Protein ratio for animals from both sites, and between fresh body weight and RNA:DNA ratio for animals from the Ailsa Craig site. Since there was an overall relationships between nucleic acid concentrations and body weight, the nucleic acid concentrations of animals from the different sites were compared relative to fresh body weight using analysis of covariance. No significant differences in the slopes of the regression lines were found for any of the indices. Analysis of covariance showed that lobsters from Little Cumbrae had significantly higher RNA concentrations (Figure 5.1) (ANCOVA $P<0.001$), RNA:Protein ratios (Figure 5.2) (ANCOVA $P<0.001$) and RNA:DNA ratios (Figure 5.3) (ANCOVA $P<0.001$) than lobsters of equivalent weight from the Ailsa Craig site. No significant relationships were observed between fresh body weight and Protein concentration, DNA concentration or water content. Comparisons between the two sites (see Table 5.2) showed that there were no significant

differences in abdominal muscle protein content or water content between sites . It was noted that the DNA concentration was significantly higher in the abdominal muscle tissue of animals from the Little Cumbrae site then in animals from the Ailsa Craig site.

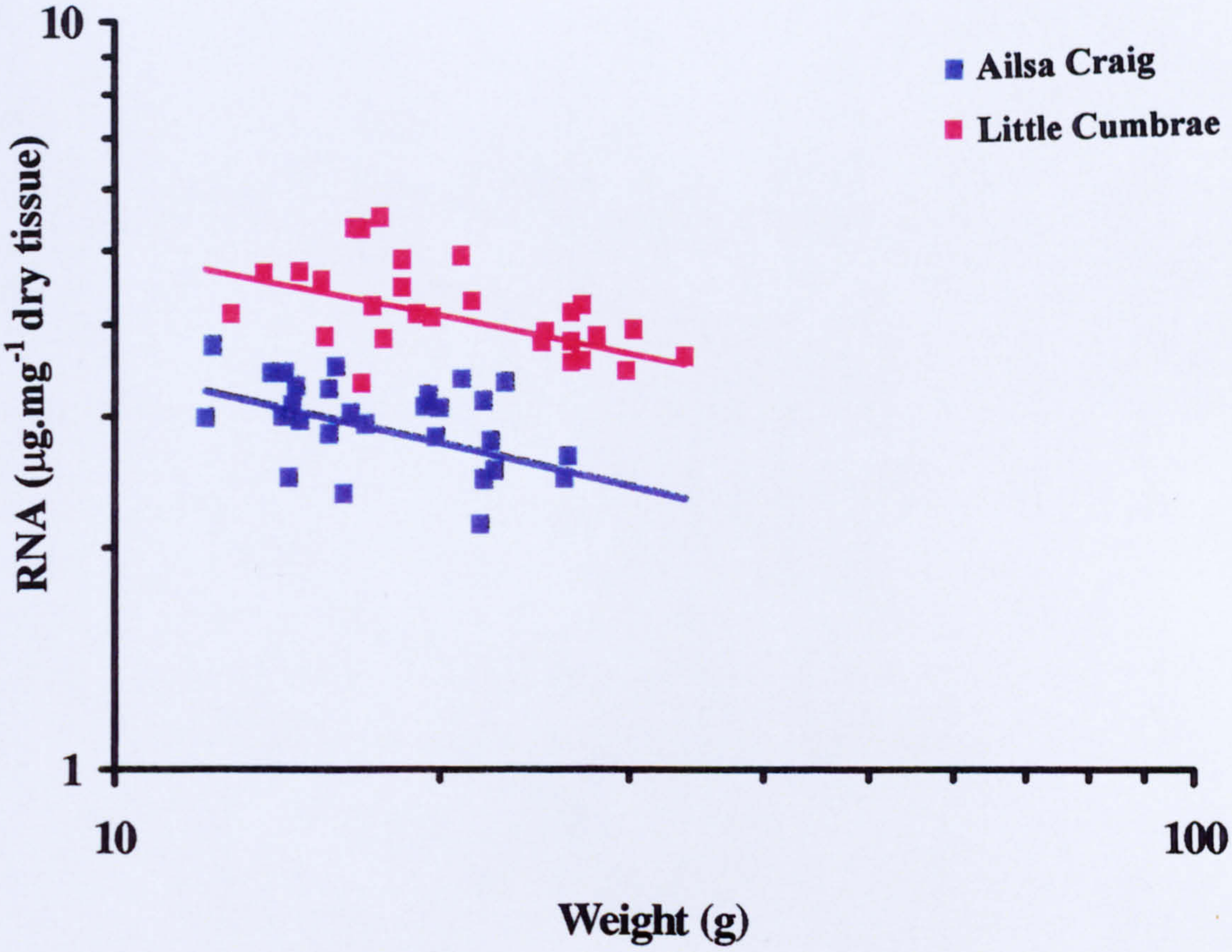


**Figure 5.1. The relationship between abdominal muscle RNA concentration (μg.mg$^{-1}$ dry weight) and fresh body weight in *Nephrops norvegicus* from two sites in the Clyde Sea area. Lines fitted are the calculated regression lines.**

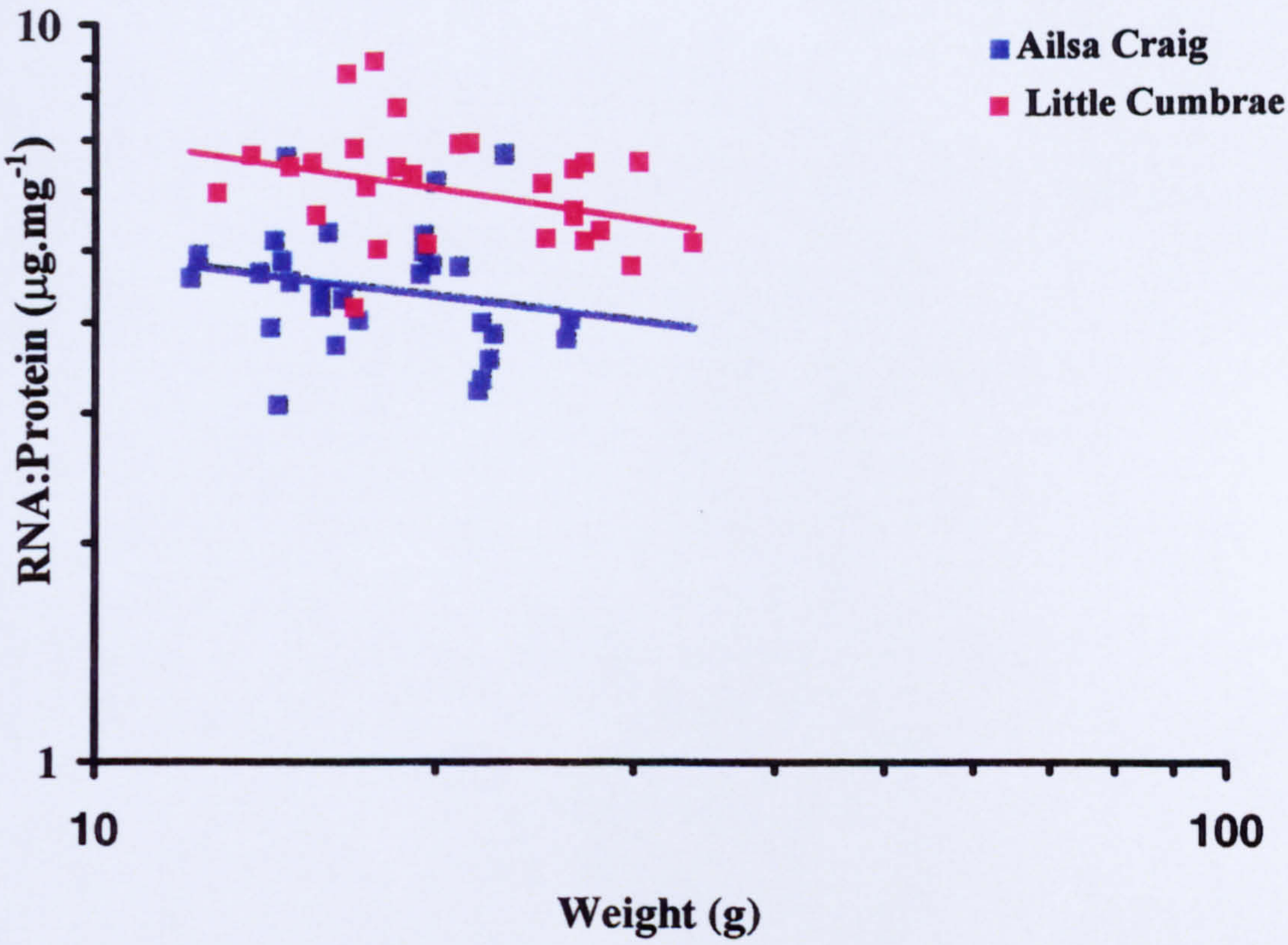


**Figure 5.2. The relationship between abdominal muscle RNA:protein ($\mu g.mg^{-1}$) and fresh body weight in *Nephrops norvegicus* from two sites in the Clyde Sea area. Lines fitted are the calculated regression lines.**

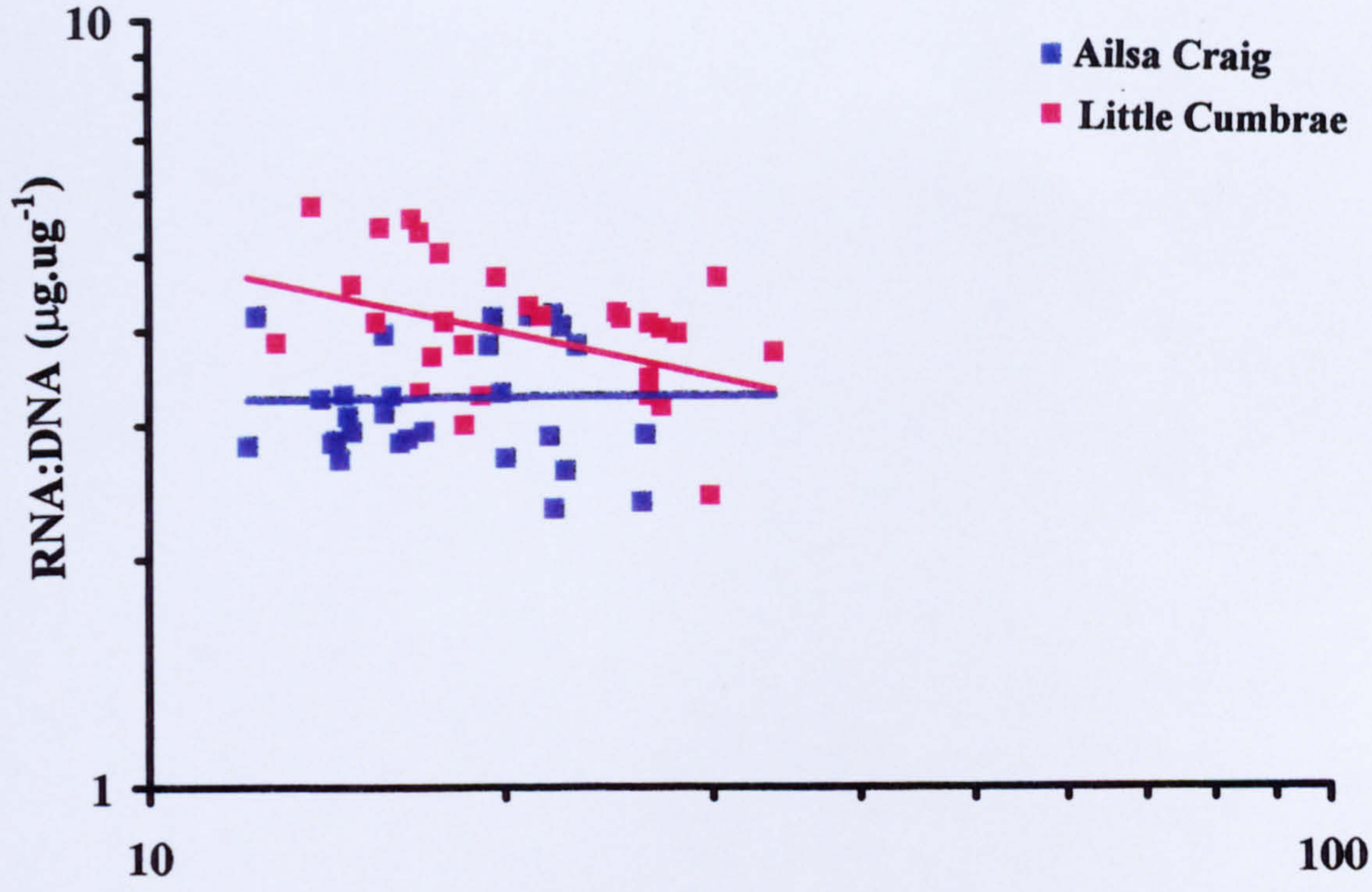


**Figure 5.3. The relationship between abdominal muscle RNA:DNA ($\mu g.\mu g^{-1}$) and fresh body weight in *Nephrops norvegicus* from two sites in the Clyde Sea area. Lines fitted are the calculated regression lines.**

**Table 5.1. Details of regression equations showing the relationship between abdominal muscle nucleic acid concentration and fresh weight (see Figs. 5.2, 5.3 & 5.4). LC=Little Cumbrae, AC=Ailsa Craig, *a* and *b* are parameters of the regression equation in the form of $y=ax^b$ and significance of regression (ANOVA).**

| Index | Site | *a* | *b* | r | P | ANCOVA |
|---|---|---|---|---|---|---|
| RNA | LC | 9.38 | -0.27 | 0.50 | P<0.005 | P<0.001 |
| $\mu g.mg^{-1}$ | AC | 6.35 | -0.27 | 0.39 | P<0.05 | |
| RNA:Protein | LC | 11.48 | -0.21 | 0.26 | NS | P<0.001 |
| $\mu g.mg^{-1}$ | AC | 7.21 | -0.17 | 0.19 | NS | |
| RNA:DNA | LC | 10.96 | -0.33 | 0.4 | P<0.05 | P<0.001 |
| $\mu g.\mu g^{-1}$ | AC | 3.21 | 0 | 0 | NS | |

**Table 5.2. Statistical comparison of protein, DNA and water content of abdominal muscle tissue using Student's t-test. LC=Little Cumbrae, AC=Ailsa Craig.**

| Index | Site | Mean | SD | t | P |
|---|---|---|---|---|---|
| Protein | LC | 0.69 | 0.06 | 1.02 | NS |
| % | AC | 0.67 | 0.08 | | |
| DNA | LC | 1.05 | 0.19 | 2.67 | <0.01 |
| $\mu g.mg^{-1}$ | AC | 0.93 | 0.13 | | |
| Water | LC | 78.6 | 1.42 | -1.87 | NS |
| % | AC | 79.3 | 1.47 | | |

5.3.2. Diet manipulation experiment

Differences in biochemical parameters between lobsters from each site were tested for significance using Student's t-tests. Comparisons were made at each sampling time. T-tests between sites showed that at the beginning of the experiment, RNA concentration (Figure 5.4) ($P<0.01$), RNA:Protein (Figure 5.5) ($P<0.05$) and RNA:DNA (Figure 5.6) ($P<0.05$) were all significantly higher for lobsters from the Little Cumbrae site. At day 6, RNA:Protein ratios ($P<0.05$) and RNA:DNA ratios($P<0.05$) were significantly higher for lobsters from the South Cumbrae site. However, at days 12 and 24, the coupling of a decrease in RNA concentration, RNA:Protein ratio or RNA:DNA ratio in the tissue of animals from the Little Cumbrae site with an increase in RNA concentration, RNA:Protein ratio or RNA:DNA ratio in the tissue of the animals from the Ailsa Craig site resulted in a lack of significant differences between the sites. Furthermore, by the end of the experiment, on day 36, the average RNA concentration, RNA:Protein ratio or RNA:DNA ratio of animals from each site had 'crossed-over' values observed in the opposing site and had changed to the extent that they were approaching similar concentrations exhibited by the other site at the beginning of the experiment. RNA concentration ($P<0.05$) and RNA:Protein ratio ($P<0.05$) were significantly higher for lobsters from Ailsa Craig site than lobsters from the Little Cumbrae site.

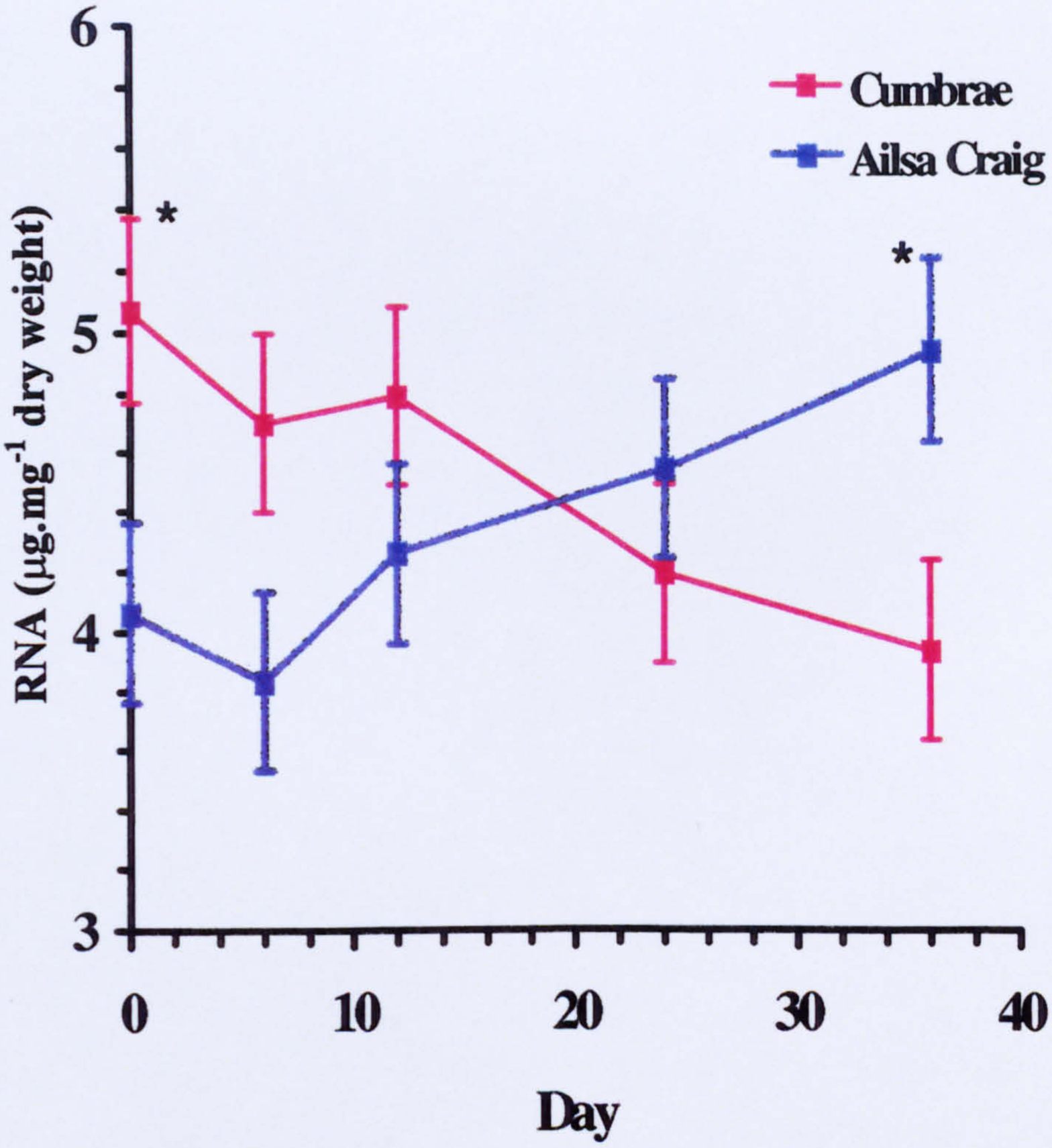


**Figure 5.4. Changes in the abdominal muscle RNA concentration (µg.mg$^{-1}$ dry weight) of *Nephrops norvegicus* after changes in diet. Animals from south of Little Cumbrae were starved, wheras animals from south of Ailsa Craig were fed to excess. Values are means +/- standard error. * denotes significant difference (P<0.05) between the two sites at that time.**

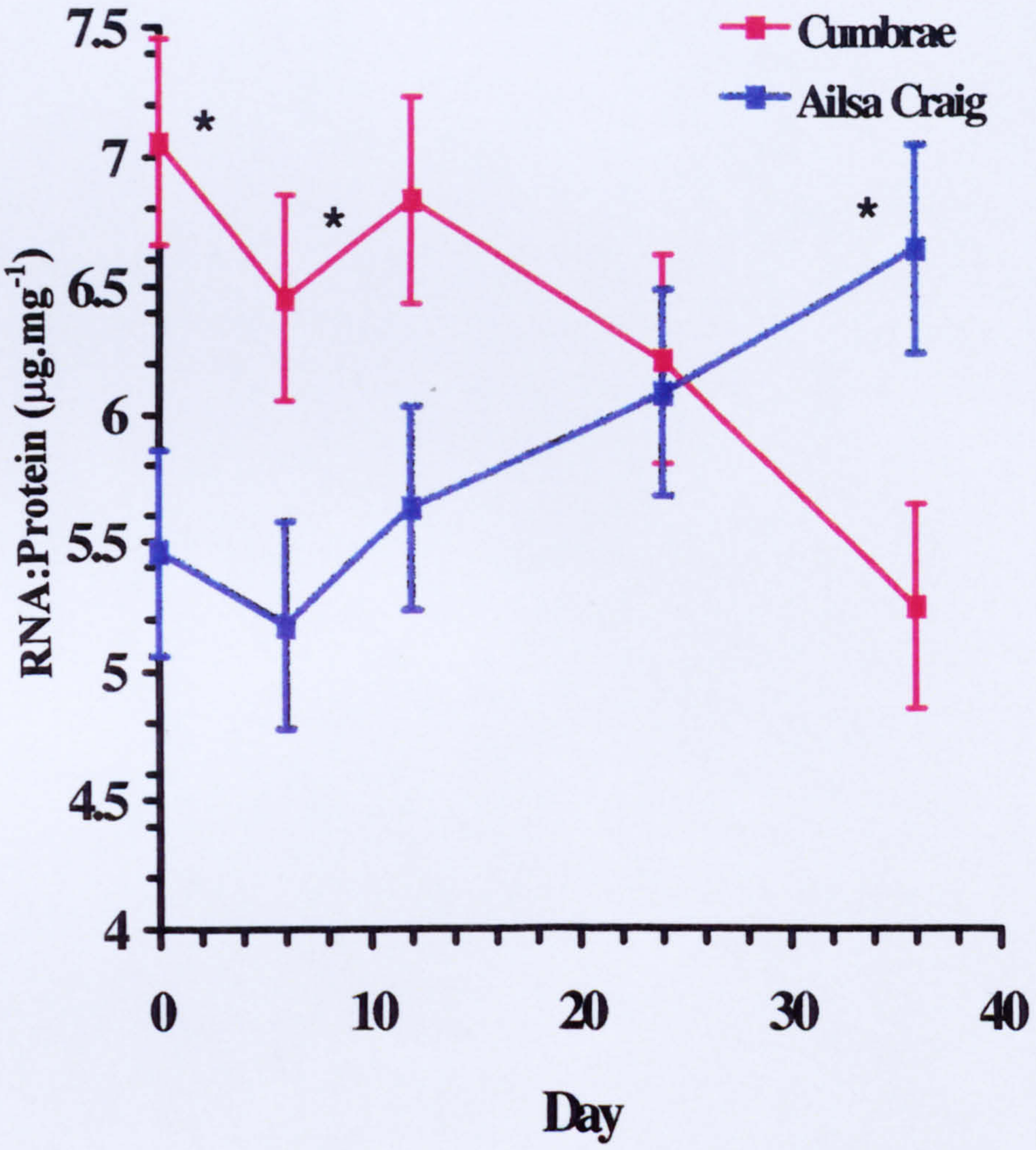


**Figure 5.5. Changes in the abdominal muscle RNA:protein (µg.mg$^{-1}$ dry weight) of *Nephrops norvegicus* after changes in diet. Animals from south of Little Cumbrae were starved, wheras animals from south of Ailsa Craig were fed to excess. Values are means +/- standard error. * denotes significant difference (P<0.05) between the two sites at that time.**

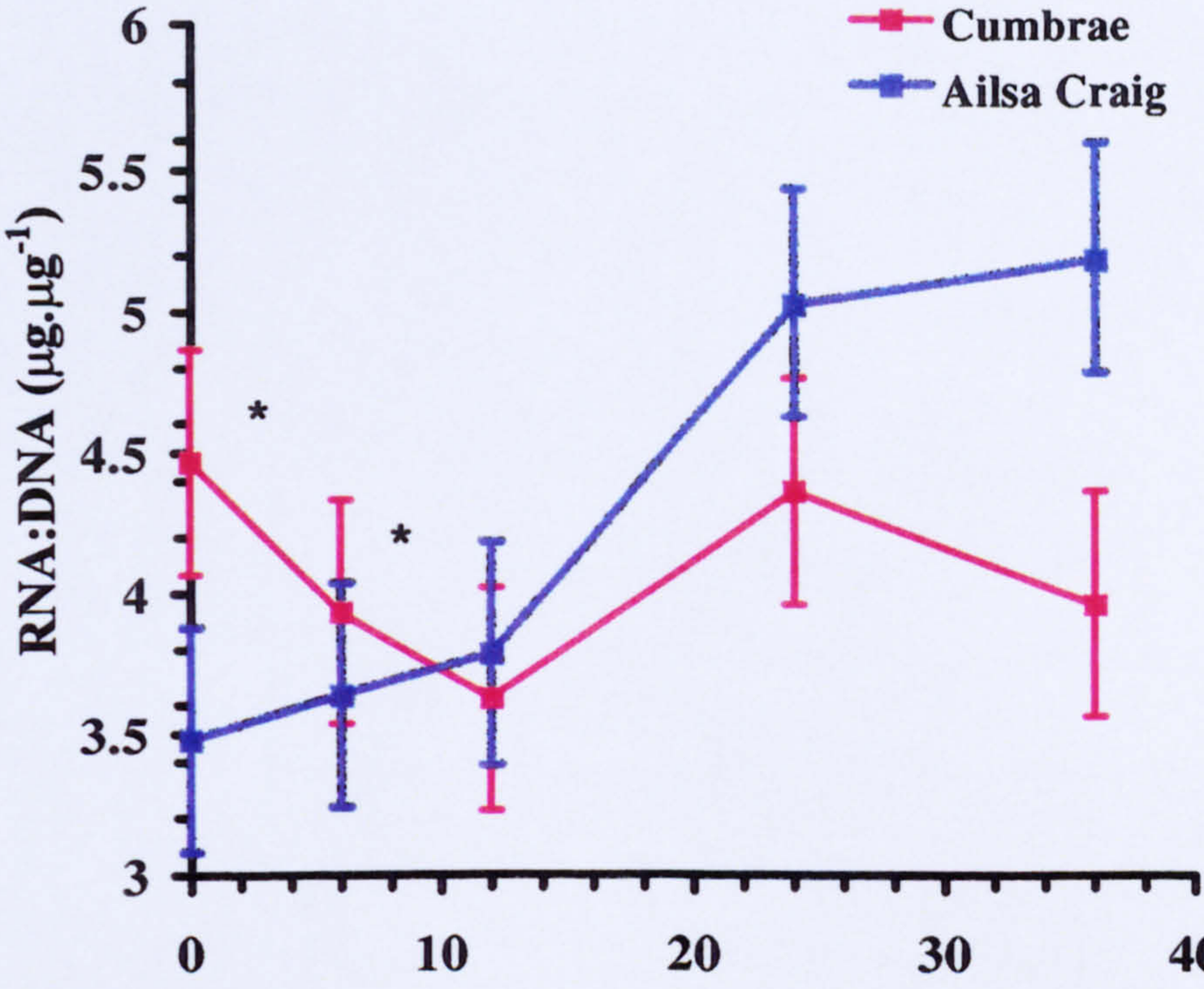


**Figure 5.6. Changes in the abdominal muscle RNA:DNA ($\mu g.\mu g^{-1}$ dry weight) of *Nephrops norvegicus* after changes in diet. Animals from south of Little Cumbrae were starved, wheras animals from south of Ailsa Craig were fed to excess. Values are means +/- standard error. * denotes significant difference ($P<0.05$) between the two sites at that time.**

5.3.4. Seasonality

There was a significant seasonal effect on the hepatosomatic index (ANOVA, $F_{54,5}$= 9.33, P<0.001) (Figure 5.7). Tukey's multiple comparison tests showed that the hepatosomatic indices were significantly lower (P<0.05) in the months of March and May than at any other time of the year. There were also significant temporal differences in abdominal RNA concentration (ANOVA, $F_{53,5}$= 5.01, P<0.001), the RNA concentrations were significantly higher (P<0.05) in November and January than in July (Figure 5.8).

5.3.5. Temperature

The abdominal muscle RNA concentrations were significantly influenced by both temperature (ANOVA $F_{50,2}$ =14.39, P<0.001) and the duration of exposure (ANOVA $F_{50,1}$ =7.59, P<0.01) (Figure 5.9). There was also a significant interaction between temperature and duration of exposure (ANOVA $F_{50,2}$ =5.19, P<0.01). Tukey's multiple comparison tests showed that at weeks 3 and 6, the abdominal RNA muscle concentration of animals exposed to 5°C were significantly higher (P<0.05) than those of animals exposed to 15°C.

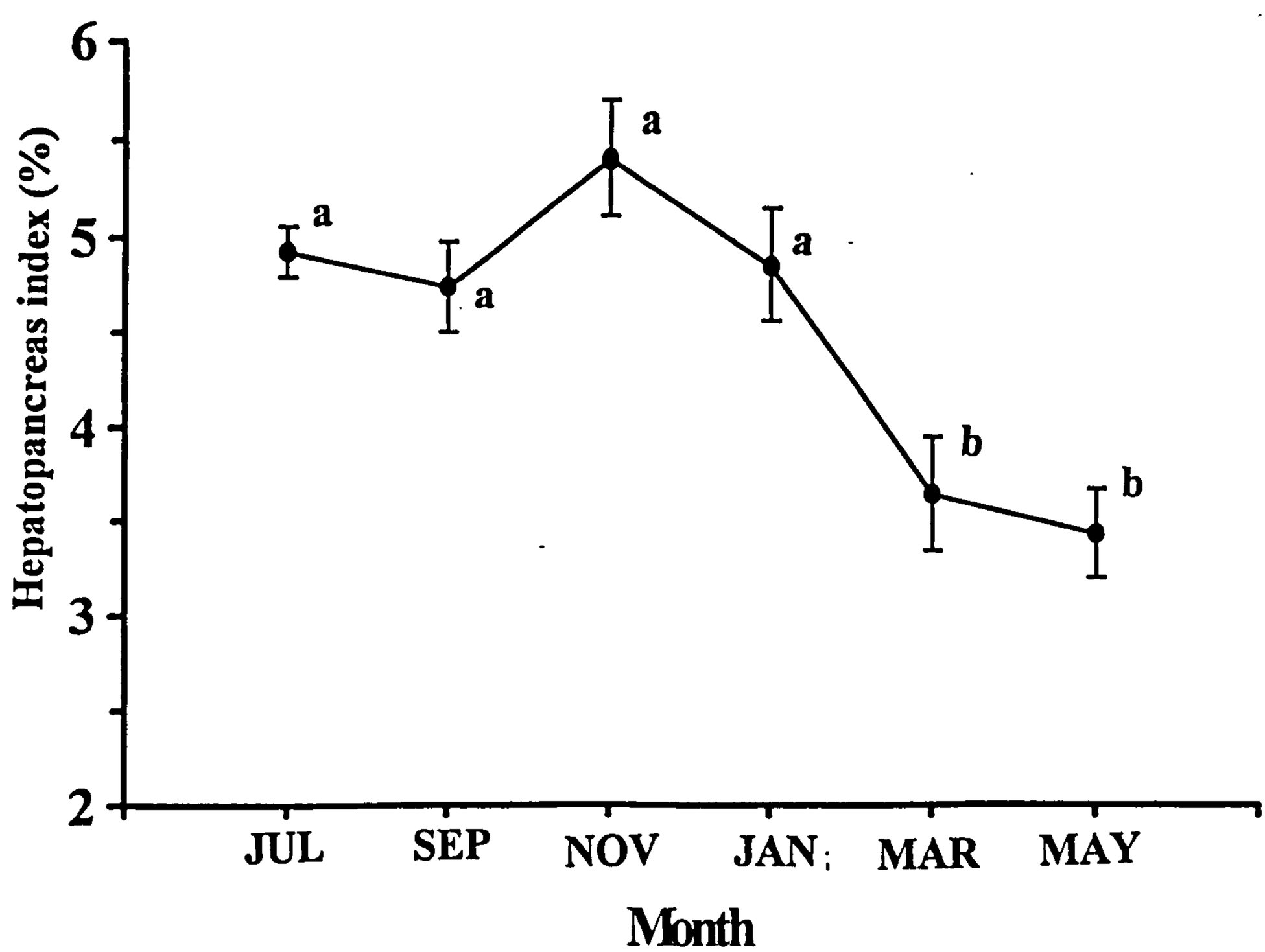


**Figure 5.7. Seasonal variation in the hepatosomatic index (%) of *Nephrops norvegicus* from the south of Little Cumbrae site. Values are means +/- standard error. Data sharing the same letter are not significantly different.**

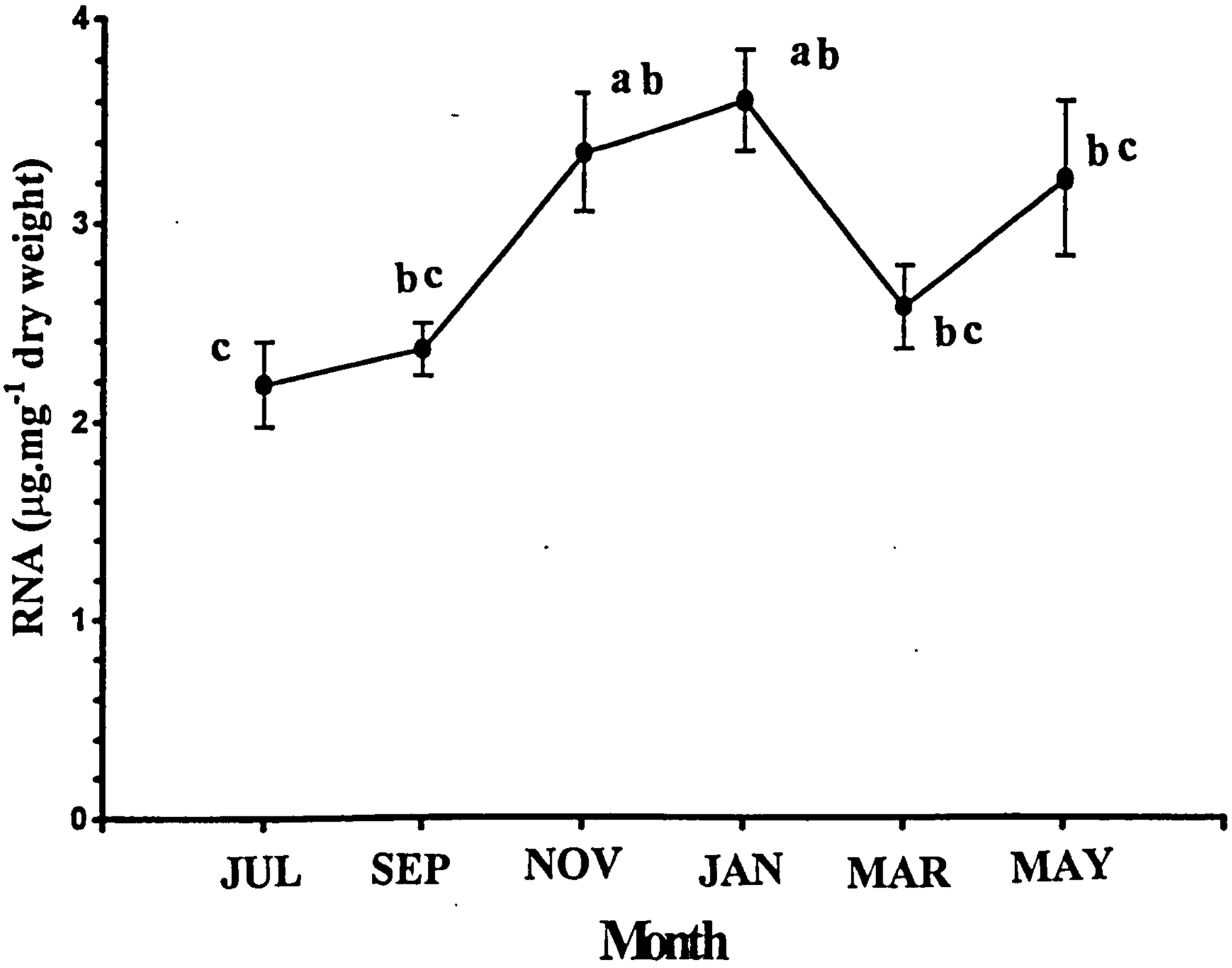


**Figure 5.8. Seasonal variation in the abdominal muscle RNA concentration ($\mu g.mg^{-1}$) of *Nephrops norvegicus* from the south of Little Cumbrae site. Values are means +/- standard error. Data sharing the same letter are not significantly different.**

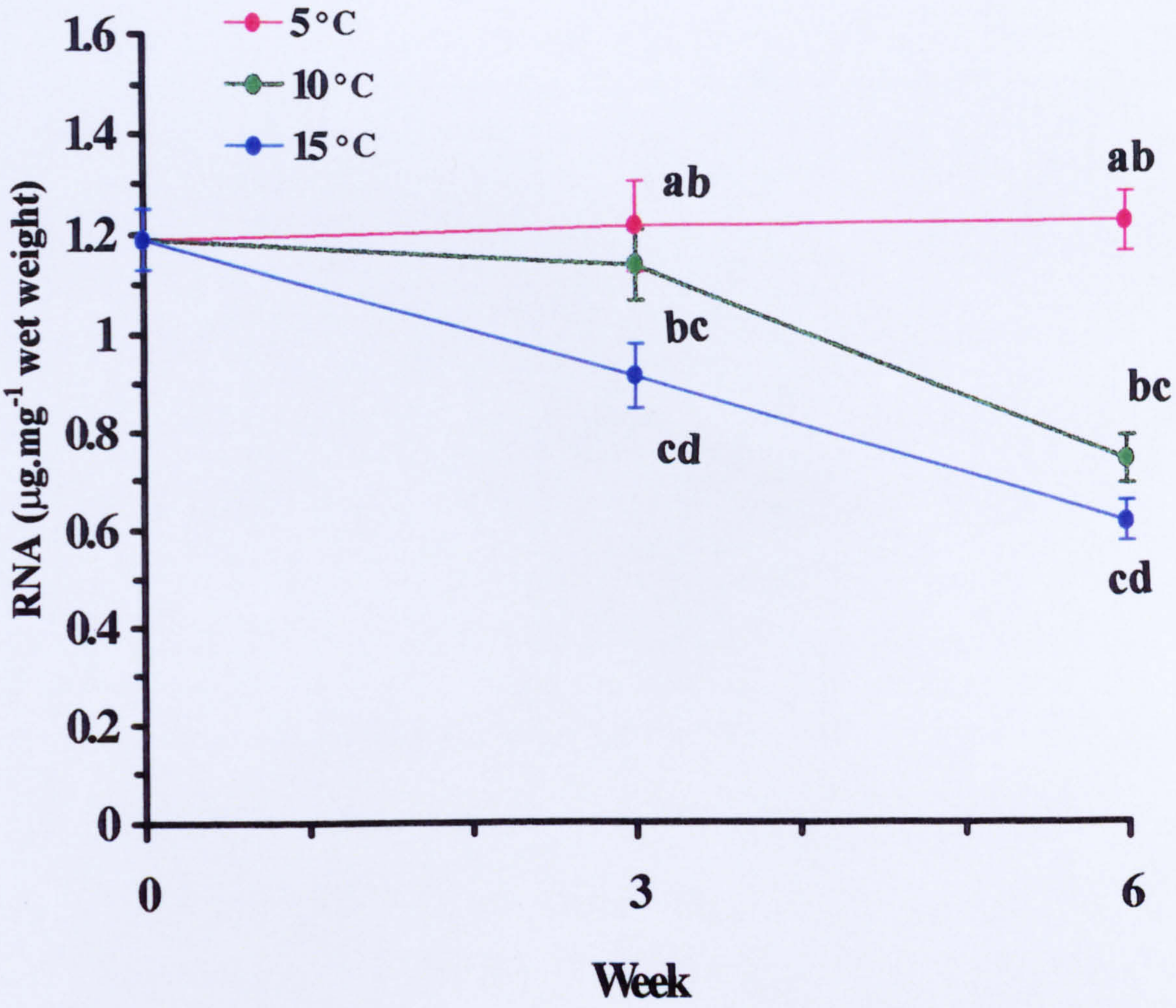


**Figure 5.9. Changes in the abdominal muscle RNA concentration (µg.mg$^{-1}$ wet weight) of *Nephrops norvegicus* from the south of Little Cumbrae site following exposure to different temperatures (5°C, 10°C and 15°C). Bars represent standard error. Data points sharing the same letter are not significantly different.**

## 5.4. DISCUSSION

### 5.4.1. Stocklet differences in abdominal muscle nucleic acid concentration

Since *Nephrops norvegicus* supports the most valuable invertebrate fishery in the UK, an understanding of the factors involved in stocklet heterogeneity is of considerable commercial interest. As discussed earlier, it is well documented that populations of *N. norvegicus* are distributed into patches of varying densities, and that lobsters from the densely populated stocklets appear to grow slower than those from less populated grounds. However, it is not fully understood why the lobsters differ in growth rate. Tuck (1993) speculated that the differences in growth rate between Clyde Seastocklets do not have a genetic basis since planktonic larvae produced by the different Clyde Sea stocklets are able to mix in the water column. Evidence to support this claim was provided by a study examining allozyme variation of *N. norvegicus* in the Clyde Sea and Adriatic Sea (Passamonti *et al.*, 1997). No genetic differences were found between any of the stocklets in the Clyde Sea area, or even between Scottish and Mediterranean *N. norvegicus*. The present study has shown significant differences in RNA concentration, RNA:protein ratio and RNA:DNA ratio of abdominal muscle tissue between similarly sized lobsters from the north and south Clyde stocklets. Since it was shown in Chapter 4 that nucleic acid concentrations reflect the recent feeding history, these results provide evidence of nutritional differences between the stocklets. The significantly lower nucleic acid concentrations in the abdominal muscle tissue of lobsters from south of Ailsa Craig suggests that these animals may have access to less food than lobsters from south of Little Cumbrae. Further evidence was provided by the diet manipulation experiments.

These demonstrated that the differences in nucleic acid concentration between the lobsters from each stocklet could be reversed by changing their feeding conditions. When fasted, the RNA concentrations of lobsters from south of Little Cumbrae decreased to levels similar to those normally expected for lobsters south of Ailsa Craig. In addition, when lobsters from south of Ailsa Craig were fed to capacity, the abdominal muscle RNA concentrations reached levels found in lobsters from south of Little Cumbrae. The length of time taken for the Ailsa Craig lobsters to increase their nucleic acid contents to a level similar to that in Little Cumbrae lobsters gives an indication of the magnitude of the nutritional differences between these stocklets. The differences are clearly substantial since it required over two weeks of continuous feeding on a high quality diet of squid mantle before the differences were no longer significant.

Although this is the first study of nutritional differences in *N. norvegicus* stocklets to be carried out using nucleic acid concentrations, an attempt has previously been made using a range of other biochemical techniques. It has been shown that *N. norvegicus* from slower-growing stocklets tended to have lower concentrations of protein, glycogen and lipid in their tissues (IMBC, UMBSM & IRPEM, 1994). It was also noted that the concentrations of some of the tissue constituents examined varied considerably between individuals. This may have prevented the detection of any significant differences in haemocyanin concentration, which have previously been shown to be correlated with nutritional status in lobsters (Hagerman, 1983). It has, however, been found that *N. norvegicus* from the Ailsa Craig stocklet contained significantly higher concentrations of metals such as Cu, Mn and Cd in their

hepatopancreas than animals of equivalent size from the Little Cumbrae stocklet (IMBC, UMBSM & IRPEM, 1994). Since no differences were found in the environmental concentrations of these metals, the differences in metal content between the stocklets could be due to differences in growth rates. Lobsters from slow-growing stocklets are likely to be older than similar sized lobsters from faster-growing stocklets and would therefore have had longer to bioaccumulate the metals. This is supported by previous studies which have demonstrated that the metal content of crustacean tissue is correlated with the duration of exposure (Rainbow, 1985; reviewed by Rainbow, 1988).

It was noted that a relationship exists between animal size and RNA concentration. Smaller lobsters tended to have higher RNA concentrations in their tissues than larger lobsters, which probably reflects the faster growth rates observed in the lower age classes (Tuck *et al.*, 1997a) . In addition, it was noted that the slope of this line was similar to that obtained with weight and oxygen consumption (see Chapter 3) providing some evidence of a link between the processes of protein synthesis and respiration. This relationship has been observed for a number of other organisms (Houlihan, 1991). The slope of the line was found to be the same for animals from both sites, only the elevation was significantly different. It is therefore important to recognise that when the RNA concentrations of individual organisms are being compared, the size of the organisms involved must be taken into account. The usual approaches are either to compare animals of similar sizes, use a scaling relationship to standardize to a given animal size or to compare the elevation of regression lines. It was also noted that the relationship between body weight and RNA was weaker when

RNA was expressed as a ratio to protein or DNA. It is unclear whether this is due to physiological reasons, or to technical reasons, as it might be expected that the variability would be increased when the error of two assays was incorporated into the ratio. The restricted size range of lobsters used is also likely to have contributed to the low correlation between weight and nucleic acid content.

The density of *N. norvegicus* burrows in the Clyde Sea area, measured using towed underwater television cameras, has been found to range from approximately 0.06$m^{-2}$ on north Clyde grounds to 0.47 $m^{-2}$ in the south Clyde (Tuck *et al.*, 1997b). The magnitude of these differences in density has led to the idea that intraspecific competition for food may be responsible for the nutritional differences between the stocklets (Bailey & Chapman, 1983; Bailey *et al.*, 1986; Tuck *et al.*, 1997a). In addition, competition is also likely to be increased further since the high densities tend to occur on sediments with a low organic content and a correspondingly low biomass of prey items (Chapman & Bailey, 1987; Chapman & Howard, 1988). Lobsters living at high densities could also be subject to frequent aggressive interaction with conspecifics, leading to animals spending longer at elevated metabolic levels (Lui & Brackesen, 1977). Experimental work on the American lobster, *Homarus americanus* (Cobb *et al.*, 1982) demonstrated a reduced moulting frequency in some individuals after the establishment of a dominance hierarchy, even when sufficient food was made available. Although similar work has not been carried out for *N. norvegicus*, agonistic behaviour has been observed in the field (Chapman & Rice, 1971).

5.4.2. Seasonal and thermal influences on nucleic acid concentration

The hepatosomatic index decreased throughout the winter months from November to March. Since one the functions of the hepatopancreas is nutrient storage (Chang & O'Connor, 1983), this supports previous work that suggested that *N. norvegicus* are nutritionally limited at this time of the year. For example, in Chapter 2 it was estimated that *N. norvegicus* were consuming considerably less food in November than in May. In addition, other work on Clyde Sea *N. norvegicus* (IMBC, UMBSM & IRPEM, 1994) found that muscle glycogen concentration, which Baden *et al.* (1994) associated with feeding history, were lowest during winter samples.

In contrast to the hepatosomatic index, the RNA contents were highest during the winter in January and March. Since feeding (or lack of feeding) has been found to have a marked effect on abdominal RNA concentration, the temporal changes in abdominal RNA concentration observed in this study appear to be more difficult to interpret. However, a possible explanation could be provided by the laboratory experiments on the effects of temperature. These demonstrated that the animals exposed to 5°C were able to maintain significantly higher RNA concentrations than those exposed to higher temperatures (10°C and 15°C), and that their abdominal muscle RNA concentrations even increased slightly from the initial value. Furthermore, these results probably underestimated the response of the animals exposed to 5°C, since the animals maintained their RNA concentration against an underlying decline, which was expressed by the animals exposed to 10°C and 15°C. This could suggest that the animals may not have been fed sufficiently and that the

decrease in RNA concentration occurred as a result of nutritional limitation (see Chapter 4). Similar effects of temperature on RNA concentration have also been observed in the lobster *Homarus americanus*. McCarthy *et al.* (1976) found that cold acclimation resulted in an increase in muscle and gill RNA concentrations, while the concentration of RNA in the hepatopancreas decreased. In a study on the seasonal acclimation of RNA concentration in juvenile cod, *Gadus morhua*, Foster *et al.* (1993) found that after 4 weeks of simulated winter acclimation, which comprised of exposure to lower temperatures and changes in photoperiod, white muscle RNA concentration was significantly higher than in fish held under simulated autumn conditions. These results supported earlier observations by Foster *et al.* (1992) who found that cod held at 5°C had significantly higher RNA concentrations than those held at 15°C. Goolish *et al.* (1984) also noted this effect in carp, and suggested that a 50% increase in RNA concentration in cold-acclimated tissue was a compensationary mechanism for reduced RNA activity at the lower temperature.

Field investigations have noted that temperature may also have indirect effects on nucleic acid concentrations. Bulow *et al.* (1981), working on the seasonal variation of nucleic acid content in the bluegill fish, *Lepomis macrochirus*, found that a negative relationship existed between nucleic acid concentration and temperature. Muscle and liver RNA concentrations appeared to be highest in the spring and autumn. Although, rather than as a direct consequence of the warmer temperature, the low nucleic acid concentrations in the summer were attributed to thermal stratification. It was deduced that the animals may have suffered a depression in growth due to the resulting low oxygen content of the water. This could be of

particular relevance to *N. norvegicus* since it is known to be under hypoxic stresses in some locations (Hagerman & Baden, 1988; Baden *et al.*, 1994).

For some species of Crustacea, including the freshwater crayfish *Orconectes virilis* (France, 1985) and the northern shrimp *Pandalus borealis* (Simard & Savard, 1990) spatial variation in growth rate is thought to be due to differences in water temperature. However, for the two stocklets examined in this study, water temperature is unlikely to be responsible, since there is very little difference between the two sites throughout the year (IMBC, UMBSM & IRPEM, 1994). Nevertheless, the thermal responses of RNA concentration expressed by *N. norvegicus* and reported in this Chapter have important considerations for the application of RNA as an indicator of nutritional status in the field. Difficulties may arise when investigating long-term changes in RNA concentration. The results from this and other studies have demonstrated that if RNA concentration is to be used as an nutritional index, temperature must also be taken in account. Furthermore, it not known to what extent other seasonal cues such as photoperiod influence nucleic acid concentrations. An investigation into one of these factors, could provide the basis of a future study. Nevertheless, in view of the difficulties involved in obtaining accurate growth data for *N. norvegicus* in the field the results obtained from this study give encouragement for the use of nucleic acids for future monitoring of the fishery.

## 5.5. SUMMARY

- Samples of abdominal muscle tissue were taken from lobsters from two sites in the Firth of Clyde, south of Ailsa Craig, where lobsters are thought to be nutritionally limited as a result of the high population densities that occur in this area, and south of Little Cumbrae, a less densely populated area. Evidence of nutritional limitation was provided by nucleic acid analysis which showed that the RNA concentration, RNA:Protein ratio and RNA:DNA ratio of lobsters from south of Ailsa Craig were significantly lower than lobsters of equivalent size from south of Little Cumbrae.

- Significant negative relationships were found with nucleic acid indices and animal weight. The correlation was strongest for RNA concentration expressed per unit dry weight and weakest for the RNA:DNA ratio.

- The results of this chapter demonstrate that the differences in nucleic acid concentrations between the sites are reversible. Lobsters from each site are capable of obtaining nucleic acid levels similar to the other, after a few weeks when exposed to different feeding regimes.

- Significant seasonal influences were noted on the biochemical condition of *N. norvegicus*. The hepatosomatic index decreased during the winter, presumably in response to a lack of feeding, whereas the RNA concentration increased during this time. The results of laboratory experiments on the thermal responses of RNA

concentration suggested that the winter increase in RNA could be attributed to thermocompensation to the lower winter temperatures. Lobsters exposed to 5°C maintained significantly higher RNA concentrations than those exposed to 15°C.

# CHAPTER 6

# The effects of a *Hematodinium*-type infection on the physiology of the Norway lobster *Nephrops norvegicus*.

## 6.1. INTRODUCTION

Stocks of the Norway lobster *Nephrops norvegicus*, off the west coast of Scotland have been found to be infected with a parasitic species of dinoflagellate, thought to belong to the genus *Hematodinium* (Field *et al.*, 1992). Dinoflagellate parasites are not limited to *N. norvegicus* or to Scottish waters; they are also responsible for diseases in other species of Crustacea world-wide. Infections have been reported in *Callinectes sapidus* (Newman & Johnson, 1975), *Ovalipes ocellatus*, *Cancer irroratus* and *C. borealis* (Maclean & Ruddell, 1978), *C. pagurus* (Latrouite *et al.*, 1988), *Necora puber* (Wilhelm & Boulo, 1988), *Chionoecetes bairdi* (Meyers *et al.*, 1987), *Portumus pelagicus* (Shields, 1992) and *Trapezia* sp. (Hudson *et al.*, 1992). In the Alaskan tanner crab *C. bairdi*, infections are known as Bitter Crab Disease (BCD) as a result of the effect that they have on the taste of crab meat (Meyer *et al.*, 1987). Commercial losses due to BCD in Alaska were estimated at $220,000 per annum in 1987 (Meyers *et al.* 1987). In view of the economic importance of dinoflagellate infections, there have been a number of investigations into the relationship with their crustacean hosts.

Studies have shown that there are several environmental and biological factors which appear to influence the susceptibility and progression of *Hematodinium* sp infections. For instance, investigations on crabs have speculated that water temperature and salinity are important in dinoflagellate transmission (Meyers *et al.* 1990; Eaton *et al.* 1991; Shields 1994). Crustacea are also thought to be particularly vulnerable to infection following moulting. Observations on blue crabs and tanner crabs have suggested that the infection could be acquired through spores or post spores entering the host via cuticular tears acquired during ecdysis (Eaton *et al.* 1991; Meyers *et al.* 1990). *Nephrops* are also thought to be susceptable to infection following ecdysis, since there is a strong correlation with disease infection and female moulting (Appleton *et al.*, 1997). However, a possible alternative explanation was proposed by Appleton & Vickerman (1998), who suggested that *Nephrops* could also be infected through the ingestion of parasite spores as a result of filter-feeding (Loo *et al.*, 1993).

Following initial infection, the disease is not thought to be expressed until the period after moulting in the following year (Appleton *et al.*, 1997). Disease patency in *N. norvegicus* begins in January or February showing a peak of patency between April and May. This seasonality coincides with the moult season of the Clyde stocklets. The onset of patency is thought to be triggered by the beginning of moulting and/or the low winter feeding rates at this time (Appleton *et al.*, 1997) (see Chapter 2).

The prevalence of *Hematodinium* sp. infections is higher in the Clyde Sea, compared with grounds in more open areas on the Scottish west coast and Irish Sea (Field *et al.*, 1995). It has been postulated that this may be a result of the local hydrography, since an oceanographic front has been observed to occur over the shallow sill between

Ayrshire and the Mull of Kintyre (other effects of this phenomena are discussed in Chapter 5 as well as in Tuck *et al.* 1997). The limited exchange of water between Clyde Sea and the Irish Sea (Tuck, 1993), may increase susceptibility to infection (Field *et al.*, 1995). A similar suggestion was made by Shields (1994), who proposed that the reduced water mixing may exaggerate *Hematodinium* sp. infections in tanner crabs and blue crabs from inshore areas of southern Alaska and Chesapeake Bay.

Since a large proportion of infected *N. norvegicus* are small females, it was initially thought that size and sex were important factors in governing susceptibility to infection (Field *et al.*, 1992). However, further work has suggested that it is age as opposed to size which is more significant (Field *et al.*, 1995). Comparisons of the north (Little Cumbrae site) and south (Ailsa Craig site) stocklets showed that the prevalence of infection is highest in larger animals at the Little Cumbrae site than in the Ailsa Craig site. The growth rates of animals at these sites are thought to be different as a result of the differences in animal density and subsequently nutritional condition (see Chapter 5 for details). Thus it is probable that the observed differences in the peak mean-size of infected *N. norvegicus* between the sites are due to differences in growth rate. Although the sizes of infected individials may differ, their ages are likely to be similar.

Infected *N. norvegicus* are easily distinguished by their abnormally bright yellow/orange carapace coloration. The lobsters also appear very sluggish. At advanced stages of infection, the haemolymph develops a milky-white appearance as it contains large numbers of vegetative parasite cells (Field *et al.* 1992). Infected blue

crabs are reported to appear normal externally, although infected animals are easily identified by internal observation, as the organs appear 'frosted' (Meyers *et al.*, 1987). Studies on the pathology of the disease in Crustacea have demonstrated that the majority of, if not all, infected animals will die from the infection. In an assessment of the impact of the disease on tanner crabs, Meyer *et al.* 1987 showed that over a 97 day period, 67% of the crabs died. Similarly, Field *et al.* (1992) noted that infected-trawl caught *N. norvegicus* had mortality rates of two to four times those of uninfected lobsters. In addition, the wide spread tissue damage indicated that meat quality and marketability were also affected.

The cause of death has yet to be clearly established, but it has been suggested that a contributing factor may be the presence of very large concentrations of parasite cells in the haemolymph, which in histological studies have been shown to occlude haemal spaces, especially in the gills (Meyers *et al.*, 1984; Field *et al.*, 1992). The disruption of circulation, together with the reduction in haemocyanin concentration which has been recorded previously (Field *et al.*, 1992) and the potentially high oxygen demand of the cells within the haemolymph may seriously compromise oxygen supply to the tissues of the host. It is also possible that infected animals may be unable to feed sufficiently. The present study has investigated these possibilities by comparing rates of oxygen consumption (see chapter 3), stomach contents (see chapter 2) and abdominal RNA concentration (see chapter 4) of infected and uninfected lobsters. Some of the results presented here, and further work on the consequences of a *Hematodinium* sp. infection on other aspects of oxygen transport by the haemolymph are reported in Taylor *et al.* (1996).

## 6.2. MATERIALS AND METHODS

### 6.2.1. Experimental animals

Adult *Nephrops norvegicus* (25-45mm carapace length) were collected by trawling on grounds around the Isle of Cumbrae, Firth of Clyde, Scotland between April and June when the prevalence of the disease among the population is at its highest (Field & Appleton, 1995). Immediately after capture, the lobsters were returned to the University of Glasgow where they were held in aquaria containing circulating aerated seawater (temperature 10°C, salinity 33‰, 12:12h light:dark cycle). The moult stage of all lobsters was estimated by the method of Aiken (1980), and only intermoult animals were selected. Dinoflagellate infection was diagnosed by the pleopod examination method reported by Field *et al.* (1992) and by Field & Appleton (1995), in which the degree of infection is classified into five qualitative stages based on the relative number of cells visible in the haemal spaces of the pleopod through the transparent cuticle (stage 0=uninfected, stages 1-4=increasing levels of infection) (see Plates 5 to 9). Diagnosis confirmation was by microscopical examination of fixed or fresh haemolymph (as detailed in Field & Appleton 1995).

**STAGE 0 (UNINFECTED)**

**Plate 5. Photograph of *Nephrops norvegicus* pleopod from which the degree of infection is classified into one of five stages ( 0=uninfected, stages 1-4=increasing levels of infection) based on the number of parasite cells visible in the haemal spaces through the transparent pleopod cuticle (photograph courtesy of Rob Field and Paul Appleton).**

## STAGE I INFECTION

**Plate 6. Photograph of *Nephrops norvegicus* pleopod from which the degree of infection is classified into one of five stages ( 0=uninfected, stages 1-4=increasing levels of infection) based on the number of parasite cells visible in the haemal spaces through the transparent pleopod cuticle(photograph courtesy of Rob Field and Paul Appleton).**

## STAGE II INFECTION

**Plate 7. Photograph of *Nephrops norvegicus* pleopod from which the degree of infection is classified into one of five stages ( 0=uninfected, stages 1-4=increasing levels of infection) based on the number of parasite cells visible in the haemal spaces through the transparent pleopod cuticle (photograph courtesy of Rob Field and Paul Appleton).**

## STAGE III INFECTION

**Plate 8. Photograph of *Nephrops norvegicus* pleopod from which the degree of infection is classified into one of five stages ( 0=uninfected, stages 1-4=increasing levels of infection) based on the number of parasite cells visible in the haemal spaces through the transparent pleopod cuticle (photograph courtesy of Rob Field and Paul Appleton).**

## STAGE IV INFECTION

**Plate 9. Photograph of *Nephrops norvegicus* pleopod from which the degree of infection is classified into one of five stages ( 0=uninfected, stages 1-4=increasing levels of infection) based on the number of parasite cells visible in the haemal spaces through the transparent pleopod cuticle (photograph courtesy of Rob Field and Paul Appleton).**

6.2.2. Oxygen consumption of whole animals

Resting oxygen consumption rates were determined for *N. norvegicus* diagnosed as being at infection stages I to IV using flow-through respirometry (temperature 10°C, salinity 33‰) (see Chapter 3 for details on method). These were compared with the resting and active oxygen consumption rates of a sample of apparently uninfected animals. The active rates of oxygen consumption were obtained using closed respirometry (see Chapter 3 for details on method). Since there were comparatively fewer highly infected animals, infection stages III and IV were combined for statistical analysis.

6.2.3. Abdominal RNA concentration of infected animals

Abdominal muscle nucleic acid concentrations (see Chapter 4 for details on method) were obtained for *N. norvegicus* diagnosed as being at infection stages I to IV, as well as a sample of apparently uninfected animals. Since only small samples of tissue were available, the amounts were only sufficient to determine RNA concentration. This did not present a major problem, since work in Chapters 4 & 5 demonstrated that RNA concentration is a better indicator of nutritional condition when expressed per unit dry weight compared with the ratio to protein or DNA.

6.2.4. Stomach contents of infected animals

Samples of *N. norvegicus* were collected from the vicinity of Little Cumbrae, every 4h over a 24h period; 1000h, 1400h, 1800h, 2200h, 0200h and 0600h GMT. Immediately after capture, lobsters were killed by swiftly inserting a needle into the brain, and a 5% solution of formaldehyde in seawater was injected into the stomach

through the mouth. The lobsters were then taken to the laboratory at the University of Glasgow where their levels of infection were diagnosed using the method detailed above. Each animal was weighed, and the fixed stomach contents were removed, freeze-dried and then weighed. To correct for differences in animal size, the weight of stomach contents were expressed as mg (dry weight) per g lobster(wet weight).

## 6.3. RESULTS

### 6.3.1. Oxygen consumption

The mean values for the $MO_2$ of quiescent lobsters, infected with *Hematodinium* sp. of all infection stages were significantly higher than those of 'apparently uninfected' *Nephrops* (individials exhibiting no visible signs of the disease) (ANOVA, $p<0.05$ Tukey pairwise comparison) (Figure 6.1). Tukey pairwise comparisons also indicated that mean $MO_2$ values of infected animals increased with infection stage; the $MO_2$ values of lobsters at stage III & IV were significantly higher than those of lobsters showing stage I infection ($p<0.05$). Furthermore, the mean $MO_2$ of active, uninfected *Nephrops* were significantly higher than those of stage I and II lobsters but not those at stages III & IV (ANOVA, Tukey pairwise comparison $p<0.05$).

### 6.3.2. RNA concentration

The degree of *Hematodinium* sp. infection was shown to have a significant effect on abdominal muscle RNA concentration (ANOVA, df=51, F=13.48, $p<0.001$) The concentrations of RNA in the abdominal muscle tissue of heavily infected lobsters (stages II-IV) were significantly higher than those of apparently uninfected lobsters

and stage I lobsters (Figure 6.2) There was no significant difference in RNA concentration between uninfected lobsters and lobsters at stage I of the infection. Furthermore, there were no significant differences in RNA concentration between stages of heavily infected lobsters (II-IV).

### 6.3.3. Stomach contents

The weight of dried stomach contents (mg per g fresh weight of lobster) were measured using the methods outlined in Chapter 2. The stomach contents of infected *N. norvegicus* were compared with a sample of apparently uninfected lobsters captured at the same sampling time (Figure 6.3). No significant differences were found between the weight of stomach contents of uninfected and infected lobsters captured at 0200h (df=4, T=1.84, p=0.14), 0600h (df=20, T=0.18, p=0.86), 1000h (df=17, T=-0.16, p=0.87) or 1400h (df=47, T=-0.57, p=0.57). No infected animals were captured in the 1800h and 2200h trawls.

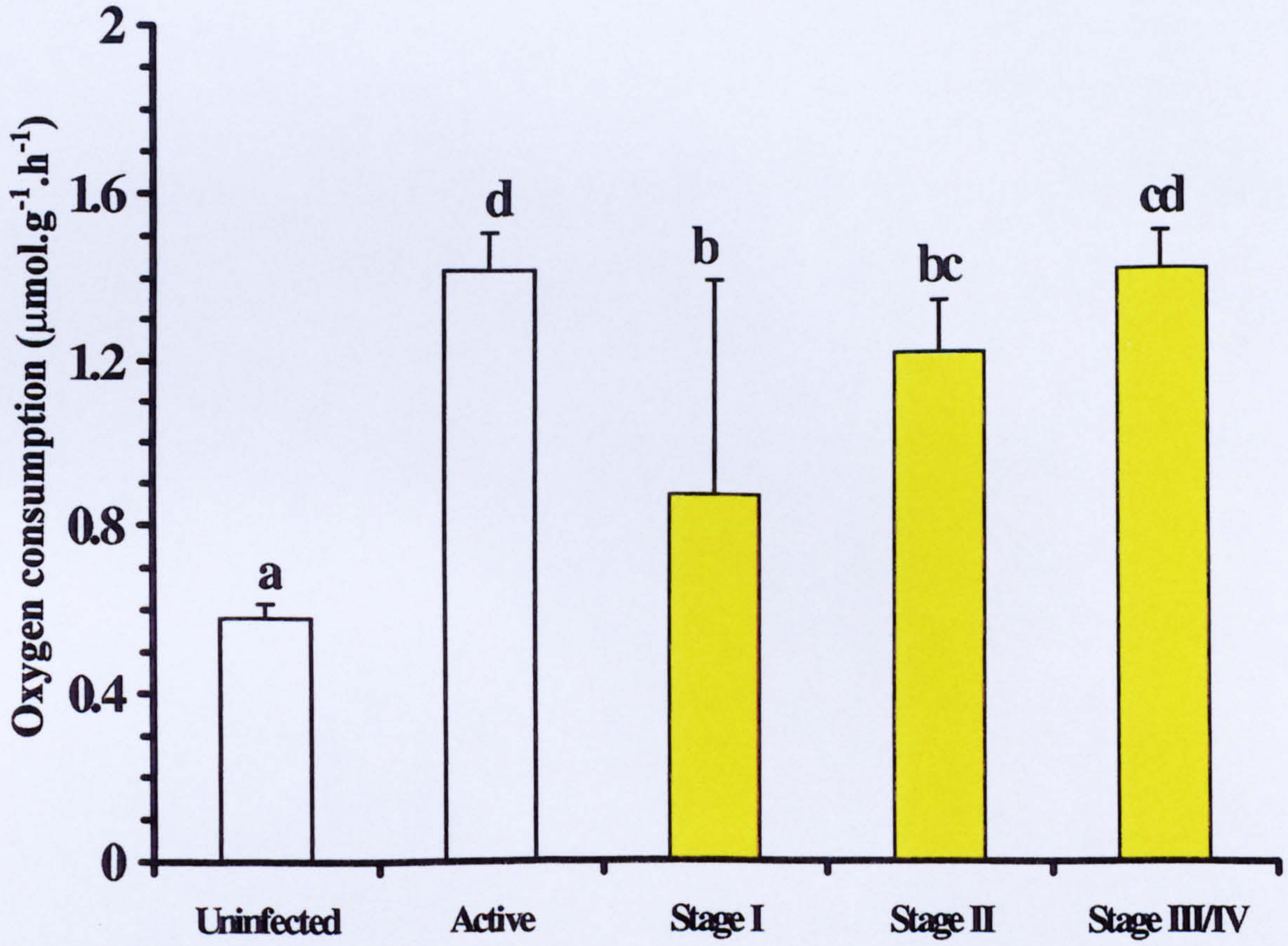


**Figure 6.1. Rates of oxygen consumption ($\mu mol.g^{-1}.h^{-1}$) of active and inactive, apparently uninfected *Nephrops norvegicus* and of lobsters showing different stages of infection with the *Hematodinium* sp. parasite (shaded bars). Error bars are standard error. Bars sharing the same letter are not significantly different.**

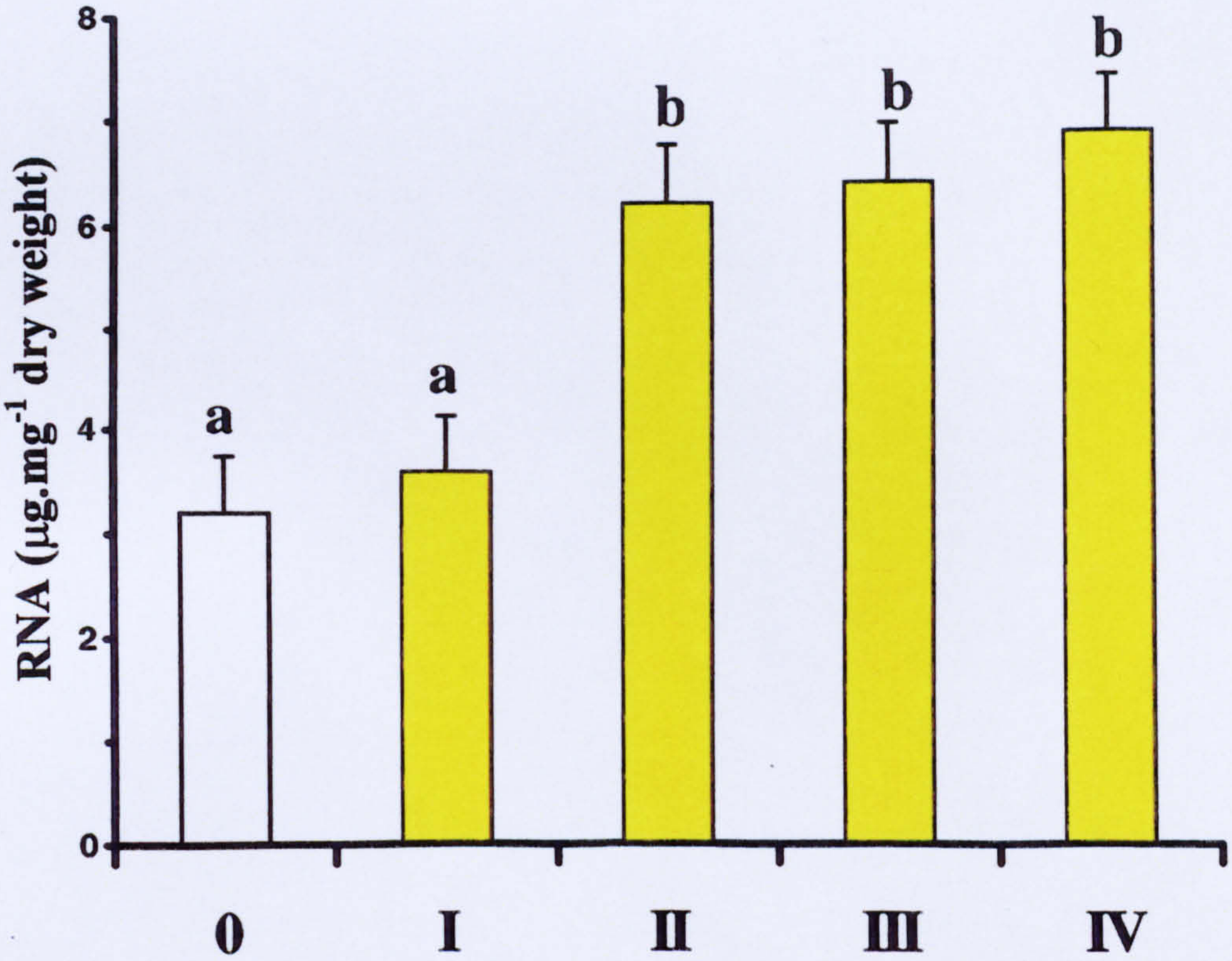


**Figure 6.2. Abdominal RNA concentration (µg.mg$^{-1}$ dry weight) of apparently uninfected *Nephrops norvegicus* and of lobsters showing different stages of infection with the Haematodinium sp. parasite (shaded bars). Error bars represent standard error. Bars sharing the same letter are not significantly different.**

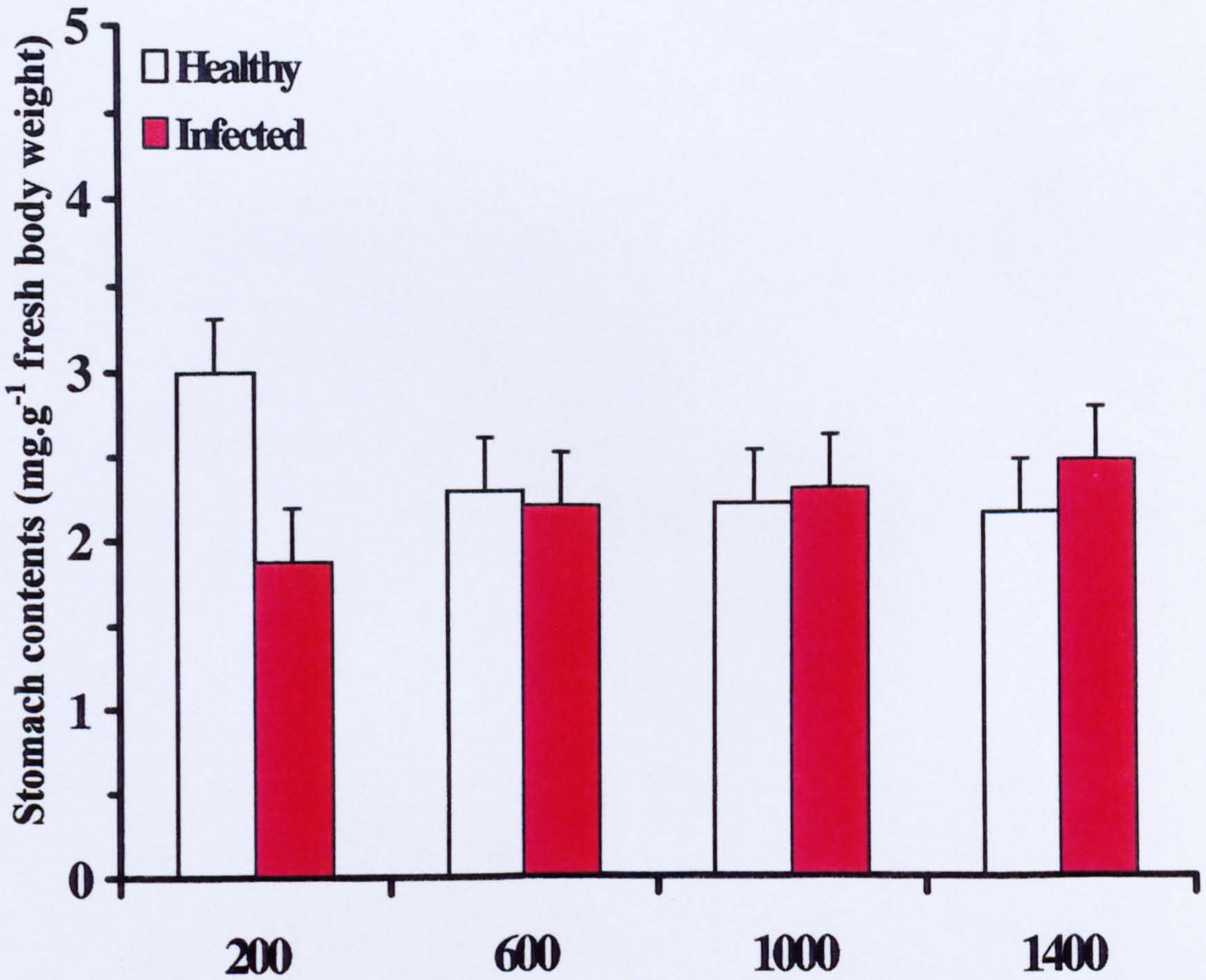


**Figure 6.3. Weight of dried stomach contents (mg.g$^{-1}$ fresh body weight) of infected (shaded) and apparently uninfected *Nephrops norvegicus* from the south of Little Cumbrae site. Error bars represent standard error.**

## 6.4. DISCUSSION

The present study has demonstrated that there is a significant increase in the rate of oxygen consumption of *Nephrops norvegicus* infected with *Hematodinium* sp., with the highest rates being shown by animals having the heaviest parasite burdens. These differences cannot be simply attributed to changes in the activity of infected animals, since care was taken to ensure that the comparisons were made only on quiescent lobsters. In fact, infected *N. norvegicus* are characteristically very lethargic when compared with uninfected individuals (Field *et al.* 1992).

The high $MO_2$ of infected lobsters may be due to an increase in the metabolic rate of the host in response to the infection or to the additional demand of high numbers of parasite cells in the haemolymph. The $MO_2$ values of heavily-infected, but quiescent lobsters (stages III and IV) were not significantly different from those of apparently uninfected animals that had been induced to become active. This suggests that the presence of *Hematodinium* sp. cells within infected hosts results in a significant additional oxygen demand.

The RNA concentration of abdominal muscle tissue was also significantly higher in infected animals. Since RNA concentration gives an indication of the capacity for protein synthesis, this provides additional evidence of the metabolic burden resulting from the infection. Unfortunately, however, it was not possible to determine what the relative contributions the host or the parasite made to the total RNA concentration. It is possible that the actual animal tissue is in a very poor condition, the majority of the

RNA expressed could belong to the parasites. Future studies should perhaps attempt to separate out the parasite cells from the tissue. The recent development of a fluorescent antibody diagnosis technique (Field & Appleton 1996) should aid this procedure.

Further work on the oxygen consumption of the haemolymph demonstrated that although $MO_2$ per cell was approximately the same for haemocytes and parasites, a significant relationship was observed between the number of parasites and haemocytes and the $MO_2$ of the haemolymph (Taylor *et al.*, 1996). Since total cell number increases with infection stage (Field *et al.*, 1992), the presence of the parasites is likely to impose an additional burden on the respiratory system. Evidence to support this suggestion was provided by the fact that values for the $PO_2$ of the pre- and post-branchial haemolymph of heavily infected *N. norvegicus* were significantly lower than in uninfected lobsters (Taylor *et al.* 1996).

The low values for the $PO_2$ of the prebranchial haemolymph were not unexpected given the huge numbers of parasite cells in the haemolymph. The fact that the $PO_2$ of the post-branchial haemolymph was also very much lower than in the uninfected animals suggests either that the haemolymph may be incapable of taking up sufficient oxygen during its passage through the gills (see below) or that the respiration of the high number of cells in the haemolymph has an immediate effect on the $PO_2$.

In an earlier study, Field *et al.* (1992) showed that the haemocyanain concentration (measured as the concentration of copper) and the oxygen carrying capacity of the

haemolymph of heavily-infected *N. norvegicus* were significantly lower than in uninfected lobsters. The study by Taylor *et al.* (1996) has confirmed this observation, and has shown that there was a progressive reduction in the carrying capacity of the haemolymph with increasing levels of infection. Although the reason for this reduction in the haemocyanin concentration of the haemolymph has yet to be clearly established, it may be due to direct effects of the parasite on the copper metabolism of the host, or to direct effects on their respiratory physiology. A similar reduction in haemocyanin concentration also occurs in *N. norvegicus* subjected to prolonged exposure to severe hypoxia (Hagerman & Baden,1988; Baden *et al.*, 1990). Internal hypoxia due to *Hematodinium* sp. infection may cause a similar response in this species. In a recent study, however, it was shown that, although the haemocyanin concentration of the haemolymph decreased under these conditions, the concentration of copper did not change significantly at this time (Baden *et al*, 1994).

An alternative explanation for the reduction in the haemocyanin concentration in infected lobsters could be that this results from a reduction in their feeding activity. Several previous studies have shown that starvation in decapods may lead to a significant reduction in the protein and/or haemocyanin concentration of the haemolymph (Uglow, 1969; Djangmah, 1970; Spoeck, 1974; Hagerman, 1983). Similarly, a recent study of the effects of starvation in uninfected *N. norvegicus* showed that haemoymph protein concentrations could be reduced by as much as 50% over an 8 week period of starvation (Coutts, Parslow-Williams and Taylor, unpubl. obs.). At present, starvation would not appear to be the likely cause of the reduction in haemocyanin content in infected lobsters since preliminary studies of feeding

behaviour under laboratory conditions have shown that these animals do not show any loss of appetite. In fact, given the high metabolic demands of the parasites, it might even be expected that infected *N. norvegicus* would consume more food. However, the results of this study have suggested that infected *N. norvegicus* are not feeding at lower or higher rates than uninfected lobsters. On all occasions when sufficient animals were captured, the weight of stomach contents of infected *N. norvegicus* were not significantly different from uninfected animals.

The results of a preliminary study on the behaviour of infected and uninfected *N. norvegicus* suggested that infected lobsters spend more time on the surface than uninfected animals, and showed little evidence of the characteristic diurnal emergence rhythm (Appleton *et al.*, 1997). This could be attributed to a reduction in the foraging ability of *N. norvegicus* by the infection. Although the results from this study imply that the amounts of food eaten by *N. norvegicus* are not influenced by the infection, the debilitating effect of the disease may mean the infected animals would have to spend a longer period of time on the surface foraging for food.

There is evidence that the presence of such large numbers of parasite cells in the haemolymph can result in the occlusion of haemal spaces, especially those in the gills (Field *et al.*, 1992 Field & Appleton, 1995). Clearly this is likely to impair gas exchange at the respiratory surfaces and the distribution of oxygen throughout the body. This, together with the reduction in the oxygen carrying capacity of the haemolymph of infected lobsters may compromise the ability of the haemolymph to supply sufficient oxygen to this host's tissues. Evidence that this does indeed occur is

provided by the increased L-lactate concentration and the associated reduction in the pH of the haemolymph of heavily-infected lobsters. The increased L-lactate concentration indicates that at least some of the host tissues have resorted to anaerobic metabolism in response to internal hypoxia. Vivares and Cuq (1981) observed increased concentrations of L-lactate in the haemolymph and muscle of *Carcinus mediterraneus* infected with *Thelohania maenadis*, which they attributed either to internal hypoxia induced by the parasite, or some indirect action of the parasite. Furthermore, another microsporidian, *Ameson michaelis*, was observed to cause hyperlactosis and a concomitant reduction in haemolymph pH in the blue crab, *Callinectes sapidus* (Findlay *et al.*, 1981). Elevated background L-lactate concentrations could also reduce the stamina of infected animals. Observations of the tail-flip responses of infected *N. norvegicus* have demonstrated that although the infection has no measurable effect on single tail flips, it significantly affects the ability of the animals to sustain prolonged swimming activity (Appleton *et al.*, 1997).

The presence of L-lactate in the haemolymph is now known to increase the oxygen affinity of the haemocyanin of many species of decapod and partly counteracts the effect of a reduction in pH and oxygen affinity *in vivo* (Truchot, 1980; Booth *et al.*, 1982; Morris, 1990). In the present study, however, this effect was unlikely to have offset the reduction in haemocyanin oxygen affinity caused by the large reduction in the pH of the haemolymph in heavily infected lobsters. The resulting reduction in haemocyanin oxygen affinity will also reduce the ability of these lobsters to take up oxygen at the gills which will further compromise their ability to maintain the supply of oxygen to the tissues. This may offer another explanation for why infected animals

tend to remain outside the hypoxic burrow environment (Appleton *et al.*, 1997). In some parts of the Clyde Sea, environmental hypoxia can perhaps present a problem when estimating prevalence of infection, since it is an additional factor bringing *N. norvegicus* out of their burrows, thereby increasing their susceptibility to capture (1998, R.J.A. Atkinson, pers comm.).

The cause of death of *N. norvegicus* infected with *Hematodinium* sp. requires further investigation but evidence suggests that it may be attributable to tissue hypoxia rather than nutritional limitation. This hypoxia occurs as a result of the massive systemic infection or perhaps to the acidosis resulting from the host switching to anaerobic metabolism in an attempt to combat this hypoxia.

## SUMMARY

- The *Hematodinium* sp. infection has a considerable effect on the metabolism of infected *N. norvegicus*. The resting oxygen consumption rates ($MO_2$) of infected animals were significantly higher than those of uninfected animals. The $MO_2$ of quiescent lobsters with the heaviest parasite burdens (stages III/IV) was not significantly different from the $MO_2$ of uninfected lobsters which had been induced to become active.

- Further evidence of the physiological burden of infections was provided by measurements of RNA concentration in abdominal muscle. The RNA

concentrations of heavily infected lobsters (stages II-IV) were significantly higher than uninfected and moderately infected (stage I) lobsters.

- The dinoflagellate infection has no observable effect on the feeding of *N. norvegicus* in the field. Comparisons of stomach content weights between infected and uninfected lobsters failed to find any significant differences at any of the sampling times.

# CHAPTER 7

# Final Discussion

## 7.1. Ecological energetics

The primary aim of this thesis was to investigate some of the consequences of stocklet variability in populations of *N. norvegicus* in the Clyde Sea area. This was addressed initially utilising methods based on the construction of energy budgets. This approach was selected since previous studies have demonstrated that they can be powerful tools for assessing the effects of lifestyle changes on growth. For example, they have been successfully employed in a series of studies investigating the impact of a number of environmental stresses on populations of bivalves (Bayne & Newell, 1983; Widdows, 1983; Widdows & Johnson, 1988). Growth was not measured directly using these techniques, but estimated as 'scope for growth', which was calculated by rearranging the energy budget equation. In order to determine 'scope for growth' for *N. norvegicus*, it was necessary to estimate both energy intake and expenditure. Energy intake was derived from the fixed-stomach contents of freshly-trawled animals. Analysis of these contents found that the diet of *Nephrops* consisted of crustaceans, molluscs, and to a lesser extent polychaetes and echinoderms. Despite a number of measures that were taken to preserve the stomach contents, a large proportion was still found to be macerated beyond recognition, particularly in stomachs of animals from south of Ailsa Craig. The dietary observations concurred with those of other workers (Thomas & Davidson, 1962; Bailey *et al.*, 1986; and Goodheir, 1995), who suggested that *N. norvegicus* is an opportunistic predator. The diet of *N. norvegicus*

from south of Little Cumbrae tended to contain a larger proportion of molluscs and lower proportions of crustaceans and echinoderms than animals from south of Little Cumbrae. However, any dietary differences were probably due more to changes in prey availability rather than prey selection, since prey organisms tended to appear in stomach contents in similar proportions to their abundance in the sediment infauna.

An alternative approach to using stomach contents in investigating suspected dietary differences between *N. norvegicus* stocklets, could involve the use of stable isotopes. These techniques have been found to be able to estimate the trophic status of organisms (see review by Gearing, 1991). The method works on the principle that heavier isotopes of elements such as carbon and nitrogen are selected against as they pass through metabolic processes. As a result of this, their ratio to lighter forms of the same element has been found to decrease down the food chain. Consequently, the tissue of primary producers contains a higher proportion of the heavier isotopes than primary consumers, which in turn have a higher proportion in their tissue than predators. In addition to comparing the trophic status of different *N. norvegicus* stocklets, it would interesting to apply these methods to the tissue of female lobsters, as it might be possible to determine how much of contribution suspension feeding makes to their diet at different times of the year. Animals which have been suspension feeding or deposit feeding could potentially be identified by their lower trophic status, as assessed by isotope ratios.

Marked interspecific differences were found between the energy contents and organic contents of prey organisms. The calorific values of the prey items were found to vary

relative to the proportion of organic matter in their tissue. Organisms with low organic contents such as brittle stars had lower calorific values than organisms with a large organic proportion such as soft-bodied polychaetes. The body composition of prey items can also influence digestibility. Since there is a potential bias in stomach contents towards the harder and less digestible prey fragments, Sarda & Valladres (1990) advised that variation in the assimilation rates of different prey items should be considered when studying stomach contents. Thus, when the amounts of food consumed were determined in Chapter 2, different rates of digestion were applied according to the relative proportions of hard and soft prey items. Amounts of food consumed were calculated in 4h intervals over a 24h period for the Little Cumbrae *N. norvegicus* stocklet. A model constructed using these data estimated that foraging is highest during the night and during the morning. The lowest amount of food was consumed in the afternoon. Previous observations both in the field and laboratory have suggested that there may be an endogenous rhythm to *N. norvegicus* foraging (Aréchiga & Atkinson, 1975; Atkinson & Naylor, 1976; Chapman & Howard, 1979). This rhythm is thought to be largely associated with the prevailing light regime. *N. norvegicus* have been observed to forage nocturnally, with peaks occurring around dawn and dusk. However, in areas with limited illumination such as deep waters, the optimal light conditions can be limited to the middle of the day, hence *N. norvegicus* in such habitats may exhibit a different pattern of activity. Similarly, in shallow waters, lobsters have been noted to forage in the middle of night. Although it is perhaps inappropriate to speculate too widely on their behaviour from just one small study, the results from Chapter 2 do seem to suggest a nocturnal-based feeding pattern, which would probably be expected for *N. norvegicus* in waters of

intermediate depth. Unfortunately, during the course of this investigation, the limited access to the south of Ailsa Craig site prevented an extensive 24h study on their foraging behaviour. This would have provided considerably more information for comparison than the stomach contents at one time of the day, as it could also enable temporal differences in foraging behaviour between the stocklets to be examined. For instance, it could help to answer questions such as whether the impoverished conditions at the south of Ailsa Craig site force animals to spend longer out of their burrows, or feed at different times of day. A potential consequence of Ailsa Craig animals having to spend more time foraging out their burrows would be that they are operating at higher metabolic rates for longer duration's, thereby increasing energy expenditure and resulting in an even lower scope for growth. Nevertheless, although the 24h feeding survey was limited to one site it demonstrated that it is possible to estimate feeding in *N. norvegicus* in the field from stomach content samples, since the amounts of food estimated compared favourably with estimates of feeding in laboratory-held animals (Sarda & Valladres, 1990).

Marked differences in 'scope for growth' between seasonal samples were detected, but not between sample sites, despite the fact that the site difference was between 20-30%. This could be attributed to the large amount of individual variation in stomach contents, from which the energy intake was determined, which may have masked any site-specific differences. In addition, in order to detect the suspected differences between the sites, it would probably have been necessary to correct for other potential stocklet-based differences, since a number of assumptions were made during the construction of the energy-budget equation. For example, it was assumed that the

assimilation efficiencies were the same for both sites. This is unlikely to be the case, as Chapter 2 demonstrated that there were some differences in diet composition between the sites. Furthermore, it was also assumed that the activity levels of the two stocklets were identical. As discussed earlier, this was necessary because the limited access to the south of Ailsa Craig site prevented detailed studies on their feeding behaviour and activity.

It was interesting to note from the energetic model of *N. norvegicus* filter-feeding on *Artemia,* that under certain circumstances, it could be profitable for animals to feed in this way, and at the food densities in the experiment of Loo *et al.* (1993), it may be even more efficient than their conventional method of feeding, opportunistic predation. It should be emphasised however, that this is due more to changes in lifestyle than diet. The higher scope for growth results from a lower level of activity, which compensates for lower-quality prey. This model, although limited, reinforces the idea that despite the fact that females may not actively forage for a significant part of the year, remaining in their burrows can be a feasible strategy in terms of maximising the protection of their reproductive investment. In the short term, they are less likely to lose eggs, both through physical loss, and to predators, and in the long term, the fact that the risks of being captured by a predator or trawl are dramatically reduced greatly enhances the chances of females reproducing in successive years.

Although energy budgets may be useful for aquaculturists, since they are able to maintain strict control of many of the environmental and biological variables (Knights,

1985), the results from Chapters 2 and 3 have demonstrated that it is considerably more difficult to apply these techniques to the field. In addition to the large amount of individual variation exhibited, it is likely that the compounding of assumptions and estimations contributed considerably to increasing the error in the energy-budget calculations. It was therefore decided that whilst useful ecological information was being gained from the energetics work, more information on the stocklet phenomena would probably be obtained from the development of physiological and biochemical measures.

## 7.2. Biochemical indicators of nutritional condition

Using established techniques for crustacean muscle tissue (El Haj & Houlihan, 1987; El Haj *et al.*, 1996), rates of protein synthesis were calculated for *N. norvegicus* from both the Little Cumbrae and Ailsa Craig stocklets. Although the rates obtained were comparable to other published studies on crustacean muscle, no significant differences in protein synthesis rates ($K_s$) were found between the two sites. However, a significant difference in RNA concentration between animals from each site was detected, which indicated the potential application of nucleic acids as nutritional indicators. Subsequently, accurate methods were developed for the laboratory determination of RNA, DNA and protein. To determine which *N. norvegicus* tissue would be most suitable for the assay, the effects of starvation of the nucleic acid content of abdominal muscle, gill and hepatopancreas were examined. The most noticeable changes were observed in the RNA concentration of abdominal muscle tissue of starved *N. norvegicus*. No significant changes were found in the other tissues examined, with the exception of a decline in hepatopancreas DNA content. It

was fortunate to find that abdominal muscle tissue was the most suitable tissue for assessment of nutritional condition as not only does it enable sampling of *N. norvegicus* landings which have been 'tailed' at sea, it is also the consumable part of the organism, thus monitoring of this tissue will also give an indication of the quality of the marketed product.

One of the major advantages in the use of the RNA indices was the rate at which it was found to respond to environmental change. Whilst Ks and $MO_2$ were noted to be capable of responding relatively quickly (over a scale of a few hours), to a changes in feeding, RNA concentration was shown to be somewhat slower, changing over periods of days and weeks. This level of sensitivity makes it ideal for use with field populations of *N. norvegicus* since the delays in transporting live animals resulting from long-boat journeys, make it difficult to use short-term methods. For instance, in Chapter 4 the lack of difference in the rates of protein synthesis between stocklets probably resulted from a decrease to baseline levels whilst the animals were being transported back to the laboratory. The maximum rates of protein synthesis are likely to be limited to periods when there are sufficient free amino-acids available, such as shortly after feeding.

The significant negative relationship found with RNA concentration and animal weight probably reflects the decrease in growth rate with age, which has been observed in other studies on *N. norvegicus* (Hillis, 1971, 1979; Farmer, 1973; Nicholson, 1979; Bailey & Chapman, 1983; Froglia & Gramitto, 1988; Tully *et al.*, 1989; Tuck *et al.*, 1997a). In these length-frequency based investigations, the

decrease in growth rate was indicated by the reduction in the distance between successive year-class modes as the animals aged. This illustrates a flaw in the application of these methods since, eventually, the distributions reach a particular point at which it becomes too difficult to distinguish the year classes, making estimates of growth rate for large animals difficult and inaccurate. In contrast, providing appropriate scaling relationships are applied, nucleic-acid indices can be used to estimate nutritional condition over a wide range of animal sizes.

Samples of abdominal muscle tissue were taken from lobsters from two sites in the Firth of Clyde, south of Ailsa Craig, where lobsters are thought to be nutritionally limited as a result of the high population densities and impoverished fauna that characterises this area, and south of Little Cumbrae, a less densely populated area. Evidence of nutritional limitation was provided by nucleic acid analysis which showed that the RNA-based indices in the abdominal muscle tissue of lobsters from south of Ailsa Craig were significantly lower than lobsters of equivalent size from south of Little Cumbrae. Furthermore, additional experiments demonstrated that the differences in nucleic acid concentrations between the sites were reversible. Lobsters from each site were able to obtain nucleic acid tissue concentrations similar to the other, after a few weeks when exposed to different feeding regimes. The abdominal muscle RNA concentration decreased in Little Cumbrae animals which had been starved, and increased in Ailsa Craig animals which had been fed. Interestingly, it was not possible to improve further the nutritional status of animals from the Little Cumbrae site. When these lobsters were maintained in the laboratory they tended to lose condition, even when fed regularly. This could imply that either the RNA

concentration has reached a physiological maximum in these animals, or perhaps more likely, that the conditions that the laboratory animals were held under, were not as good as those found at the Little Cumbrae site.

Whilst this evidence of nutritional limitation suggests that lobsters from south of Ailsa Craig have access to less food than lobsters from south of Little Cumbrae, is not clear whether this is due to density-dependent competition or the lower quality and quantity of prey available in the Ailsa Craig sediment, although it probably results from a compounding of both of these factors. Work has shown that the biomass of prey differs between the two sites (IMBC, UMBSM & IRPEM, 1994) and as mentioned earlier in this discussion, the effort required to capture the prey would probably be inversely proportional to prey density, leading to additional energy expenditure for animals living on poor grounds. Furthermore, the limited food and possibly even habitat space, may be contested over in areas of high density. It is unlikely however, that aggressive interactions occur as frequently in populations of *N. norvegicus* as in other lobsters such as *Homarus* spp., since the resource usually fought over by these animals are finite habitat spaces such as rock crevices. Although there may be some benefit in terms of energy investment from occupying a pre-constructed burrow (Reaka. & Manning, 1981), habitat availability is probably not as much of motivating factor for aggressive encounters between *N. norvegicus* as food, as they have the option of constructing their own burrows by digging into the sediment. Since studies on fish (Lui & Brackesen, 1979) and other species of lobster (Cobb *et al.*, 1982) have shown that agonistic behaviour may have a significant detrimental impact on growth, this area of *N. norvegicus* behaviour clearly warrants further investigation. Future

studies should aim to estimate the energy expenditure and time allocated to fighting at varying stocking densities, and determine how this effects individual food nutrition, possibly using RNA indices to estimate condition.

Significant seasonal influences on the biochemical condition of *N. norvegicus* were noted. The hepatosomatic index decreased during the winter, presumably due to a re-mobilisation of resources in response to a lack of feeding. In contrast, the abdominal muscle RNA concentration increased marginally during this time, suggesting the influence of some other factor. The results of laboratory experiments on the thermal responses of RNA concentration demonstrated that this could possibly be a result of thermocompensation to the lower winter temperatures. The synthesis of additional RNA in response to cold exposure enables protein synthesis and turnover to be maintained at relatively normal levels. Although the results from this part of the thesis illustrate that nucleic acid concentrations are significantly affected by temperature, thermal differences are not thought to be responsible for creating the disparity observed between the Little Cumbrae and Ailsa Craig stocklets, since the difference in water temperature between the sites is negligible (IMBC, UMBSM & IRPEM, 1994).

A preliminary comparison in Chapter 4 showed a significant difference in RNA concentration between the sexes. The lower abdominal RNA concentrations observed in the abdomens of female *N. norvegicus* implies a lower rate of growth, which concurs with earlier work on Clyde *N. norvegicus* using length-frequency based techniques showing that females appear to grow more slowly than males (Bailey & Chapman, 1987; Chapman & Howard, 1988; Tuck, 1993). This can probably be

attributed to the energy invested in egg production, which coupled with the reduction in foraging when animals are ovigerous, is likely to have a considerable effect on their somatic growth. Unfortunately, during the course of this thesis it was not possible to sample sufficient female *N. norvegicus* to investigate the effects of the ovarian development cycle in detail. Other studies in this area on *N. norvegicus* and other Crustacea, have noted that a certain proportion of females undergo ovary resorption prior to spawning. There have been several explanations proposed for this phenomenon, including lack of fertilisation, starvation and hormone deprivation (Aiken & Waddy, 1980; Sastry, 1983). Bailey (1984) suggested that females might utilise ovary resorption as a strategy for recycling nutrients, since late spawning, possibly predicted using photoperiod, would result in the release of larvae into the plankton at a time unsuitable for their development.

In order to survive a winter confined to their burrows, ovigerous female *N. norvegicus* would be required to build up sufficient metabolic reserves during ovarian development. This is supported by a recent study carried out on *N. norvegicus* by Tuck et al. (1997c), which noted that the metabolic reserves of both the ovary and hepatopancreas increased as the ovary matured. It would be interesting to determine if there is a physiological cue which governs whether or not resorption takes place. If resorption occurs as a result of nutritional limitation, it may even be possible to predict the occurrence of this event using RNA-indices.

Although the gonadal investment of males is likely to be less than that of females, males do, however, allocate energy to secondary sexual characteristics, such as

allometric claw growth. Subsequent work in this area should examine this type of growth further. Studies could involve comparisons of male and female tissues such as claw muscle and abdominal muscle using RNA concentration.

Chapter 6 demonstrated that the *Hematodinium* sp. infection has a marked effect on the metabolism of *N. norvegicus*. The resting oxygen consumption rates ($MO_2$) of infected animals were significantly higher than uninfected animals. This effect even reached the extent that the $MO_2$ of quiescent lobsters with the heaviest parasite burdens (stages III/IV) was not significantly different from the $MO_2$ of uninfected lobsters which had been induced to become active. Further evidence of the physiological burden of infection was provided by measurements of abdominal muscle RNA concentration. The RNA concentrations of heavily infected lobsters (stages II-IV) were significantly higher than uninfected and moderately infected (stage I) lobsters. The dinoflagellate infection had no observable effect on *N. norvegicus* feeding in the field. Comparisons of stomach content weights between infected and uninfected lobsters failed to find any significant differences at any of the sampling times. Further work reported in Taylor *et al.* (1996) suggests that the *Hematodinium* disease causes the host lobster to experience an 'internal hypoxia' since the parasite cells were found to be exerting a considerable oxygen demand on the host. Additional evidence of this metabolic burden was shown in Chapter 6 by the elevated tissue RNA concentration. However, since it was not possible to separate out the parasite cells from the muscle tissue, it could not be determined whether the RNA was crustacean or dinoflagellate in origin. It is possible that the abdominal muscle tissue could have

been in comparatively poor condition, which might seem quite likely, considering the physiological stress that the infected animals were experiencing.

## 7.3. Recommendations

Useful information may also be gained from the application of some of the physiological measures utilised in this thesis to crustacean populations experiencing other environmental stresses. Nucleic acids could for example, provide an insight into whether or not the apparent growth variability observed between islands in spiny lobster populations (Pollock, 1990), is also due to density-dependent nutritional limitation. In addition, it may also be possible to assess the effects of hypoxia on nutritional condition. This could be applied to Scandinavian populations of *N. norvegicus*, which are subjected to extended periods of hypoxia (Hagerman & Baden, 1988; Baden *et al.*, 1994). During these periods of hypoxia, *N. norvegicus* which are not killed outright are likely to have at least a period of depressed growth. Previous work carried out by Hagerman & Baden (1988) has found that *N. norvegicus* exposed to hypoxia experience nutritional limitation as assessed by a reduction in blood haemacyanin. This has the potential to create a physiological conflict of interest, since although the blood can be catabolised as an organic reserve in food shortages, it is also required for respiratory purposes, especially in hypoxic events. It would be interesting if future studies were to compare the ability of animals at different levels of nutritional status to respond to hypoxia. Lobsters from various laboratory-diet regimens or even different stocklets could be examined. After assessing their condition with a suitable index such as RNA concentration, their metabolic responses ($MO_2$) to declining $O_2$ tensions ($PO_2$) could be monitored, enabling the compensation

point, Pc (the oxygen concentration at which animals can no longer maintain a constant respiratory rate) of animals to be calculated. The existence of a negative relationship between RNA concentration and compensation point would suggest that stocklets under nutritional stress may have a compromised ability to survive hypoxic conditions.

Although it is not possible to influence the suspected causes of stocklet variability such as the prevailing hydrographic and sedimentary environments, strategic management of fishing could potentially increase the efficiency of *N. norvegicus* exploitation. If trawling efforts are directed towards the densely populated areas, the gradual 'thinning out' of these stocklets may reduce pressures from intraspecific competition, and encourage a greater rate of individual growth. Over the long term, this may lead to a higher-quality stocklet in terms of the size composition of catches. However, in order to implement such a strategy effectively, it would probably be necessary to relax a number of trawling restrictions such as the minimum animal landing size and net mesh sizes. In addition, while the fishing effort is concentrated on the densely-populated stocklets, the reduction in trawling mortality may provide the less-populated areas with an opportunity to increase their population size. Using other less-densely populated stocklets as a reference, the advantages of nucleic acid indices would enable their use in the detection of changes in nutrition as soon as they begin to occur and provide a novel method for the continued monitoring of the condition of *N. norvegicus* stocks.

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