The effect of environmental temperature on growth and muscle development in the European lobster *Homarus gammarus*

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Candidate's Declaration

I declare that the work recorded in this thesis is entirely my own, unless otherwise stated and that it is of my own composition. No part of this work has been submitted for any other degree.

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Summary

1. Although growth and moulting rates of larval and post-larval lobsters have often been monitored, very little is known about the effects of such regimes on the physiology of the animals, on their muscle properties, or on their performance and behaviour when released into the wild in re-stocking or ranching programmes. The extensive research on effects of environmental variables on fish development (Johnston and Temple, 2002), is unlikely to provide an accurate model for a crustacean such as the lobster, which have distinct larval and adult stages in different environments, and a development pattern with intermittent growth during the moult and metamorphosis. This project addresses these issues in the context of the practicalities of rearing the European lobster *Homarus gammarus* in a laboratory environment. It poses the question: do lobster rearing programmes produce animals that are suitably equipped to compete against their wild counterparts for food and space, once released? To address this question, groups of post-larval European lobsters, *Homarus gammarus*, were reared at 11°C, 15°C and 19°C from post-larval Stage 8 to 18 months old, and also from the egg through to 6 months of age at 15°C and 19°C at the larval rearing facilities of the Centre for Environmental, Fisheries and Aquaculture Science (CEFAS), Conwy, North Wales.

2. Rates of mortality were low at all rearing temperatures. Moulting frequencies were temperature-dependent, as shown by the complete moult records and also by the data based only on the median group. Long Inter-moult periods for the postlarval stages at 11°C indicate that this temperature was close to the null point for moulting. The standard measure of size, carapace length (CL) increased with age in a temperature-dependent manner from an initial value of 6 mm in the Stage 8 lobsters. Daily increases in CL were 18 μm, 28 μm and 64 μm for the 11°C, 15°C and 19°C groups respectively. A temperature-dependent difference also remained in the size-at-stage measures, suggesting that temperature is having an effect on growth *per se*, in addition to its effect on moulting frequency. These results imply that higher temperatures have a greater effect on the rate process for protein synthesis than for degradation, resulting in net production, i.e. growth. As a result,

rearing at elevated temperatures is a viable procedure for accelerating the growth rates of juvenile lobsters, so that they achieve later moult stages and larger sizes in a given time.

3. The growth, dimorphic switching, muscle development and contractile performance of the claws were monitored in lobsters from the 3 rearing temperatures, using morphometric measurements of claw dimensions, histochemical staining of their muscles to determine their fibre phenotypes, and non-invasive measurements of the isometric forces produced during claw closing. The dactyl length of the claws gave a reliable indication of ultimate claw type, being longer in the ultimate cutter claw after the point of dimorphic divergence at Stage 5. Also, the crusher claw heights were greater at all points during the rearing programme. The rate of dimorphic switching was temperature-dependent.

4. The time course of muscle fibre transformation in the claws of lobsters reared at the different temperatures was determined by mapping the distribution of fast and slow muscle fibre types in the claw muscles using myofibrillar ATPase enzyme histochemistry. From their initial bilaterally symmetrical distribution in the claw closer muscles, some of the initially slow fibres were transformed to the fast type during differentiation of the cutter claw, while some of the initially fast fibres were transformed to the slow type during differentiation of the crusher claw. The effect of rearing temperature was less influential on the rate of fibre transformation than on the rate of claw growth, since fully differentiated claw muscles were attained by animals at the lower rearing temperatures, despite the claws being of a smaller size. Dimorphic switching occurred most consistently in the cutter claw muscles, and the rate of fibre transformation showed a degree of temperature-dependence. Dimorphic switching was less complete in the crusher claw muscles (leading to 'false' crushers), possibly due to the lack of substrates to manipulate.

5. Measurements of the forces produced by voluntary contractions of the claw closing muscles were made using strain gauges, and were compared with the maximum elicitable forces induced by pharmacological stimulation. The peak closing forces for the crusher claws were 6.6 mN ± 1.32 for 11°C, 19.8 mN ±

6.8 for 15°C and 65.8 mN ± 3.7 for 19°C at 9 months of age increased to 41.2 mN ± 4.2, 53.5 mN ± 6.7, and 206.8 mN ± 31.7 respectively. The corresponding forces produced by the cutter claws were smaller in all cases. The rates of force development increased with age, and were greater at higher rearing temperatures, for both claw types. 'False' crusher claws often produced forces as great as true crushers, indicating that factors other than muscle fibre composition influence force production.

6. The tail flip escape response of lobsters was used to test the effect of rearing temperature on locomotory performance. The distance travelled per flip increased with age and with rearing temperature. The average swimming velocity also increased with age, and with temperature, except that at 19°C, which showed no change over the rearing period. Average swimming speed expressed as body lengths per second decreased with rearing temperature at each age point, and also with age at each temperature of rearing. Tail flip duration increased with temperature at each age point, and with age for each temperature group. Some of these relationships were not apparent in the immediate post-larval stages (1999 cohort). Compared at the same stage points, the performance of animals at 11°C was equal to that of animals reared at higher temperatures, suggesting that one or more forms of physiological compensation have occurred. Allometric changes in the relative size of the abdomen may contribute, as well as metabolic compensation and phenotypic plasticity.

7. The extent to which agonistic behaviour of *H. gammarus* is pre-determined, whether it is affected by the time of the first encounter, and whether these changes are size-, age- or moult-dependent was examined using animals in the 3 temperature groups. The frequency, composition and sequence of the behavioural displays associated with agonistic interactions were recorded and compared between the ultimate winners and losers. The eventual winning lobsters showed more instances of meral spreads and strike/rip behaviour but fewer retreats than the eventual losing lobsters. Two main loops of behaviour present: a low intensity display loop and a high intensity fighting loop, which is consistent with Game Theory. The total number of behaviours increased with

age, and this was accounted for predominantly by an increase in wrestling behaviour, indicating a higher intensity of fighting in older animals.

8. Temperature-dependent processes in the developing tissues at both the biochemical and the genetic levels may be causal to the effects reported in this thesis. These are discussed in relation to parallel molecular studies on *H. gammarus* muscle that provide evidence for temperature-sensitive phenotypic plasticity that is also stage-dependent. Also, temperature-dependent differences found in the structure of the ATP binding pocket of myosin (loop 1) may contribute to the physiological compensation of swimming performance at lower temperatures. Such molecular approaches offer the possibility of explaining phenotypic plasticity in relation to development at different temperatures in terms of the underlying genetic switching,

Contents

Chapter 1 - General Introduction

Although growth and moulting rates of larval and post-larval lobsters have often been monitored, very little is known about the effects of such regimes on the physiology of the animals, on their muscle properties, or on their performance and behaviour when released into the wild. If they are affected by temperature this will have important consequences for the ability of the juvenile lobsters to adapt to the marine environment when released, and must be taken into account by re-stocking programmes or ranching projects. A fundamental understanding of the developmental biology of the muscle in the edible lobster and of the influences of environmental factors such as temperature on this development are needed in order to address these issues. Although extensive research has been carried out into the effects of environmental variables on fish development (Johnston and Temple, 2002), it is unlikely that this can provide an accurate model for a crustacean such as the lobster, which have distinct larval and adult stages in different environments, and a development pattern with intermittent growth during the moult and metamorphosis.

This project addresses these issues in the context of the practicalities of rearing the European lobster *Homarus gammarus* in a laboratory environment. As a background, the life history of lobsters and their relation to commercial aspects of the fishery will be reviewed, and the underlying biological processes that are of most importance with regard to hatchery rearing will be considered.

1.1 The life cycle of a lobster

The life cycle of a clawed lobster such as the European lobster, *Homarus gammarus*, or the American lobster, *Homarus americanus*, comprises pre-larval, larval (Fig. 1.1), post-larval (early benthic) (to 20mm Cl), young juvenile (adolescent > 40mm Cl) (for example a stage 8 lobster would be classed as a juvenile) and adult phases (Fig. 1.2) (see Aiken and Waddy, 1980; Cobb and Wahle, 1994; Ennis, 1995 for reviews). After the eggs are spawned they are retained on the abdominal appendages of the female for up to one year, during which time the embryos pass through a series of pre-larval stages (naupilus, prezoea). The pre-larval hatchlings emerge from the egg envelopes and moult

into Stage 1 larvae, which are planktonic, migrating vertically in the water column in response to changes in light intensity, and feeding on the rich supplies of food within the plankton. They also undergo three moults during this planktonic phase, gradually increasing in size (from 8mm at Stage 1 to 11mm at Stage 3) and then transforming more extensively through a metamorphosis to Stage 4 post-larvae, which are morphologically very similar to the adult lobster. These post-larvae swim actively to the sea floor to begin a benthic existence. The duration of the larval and post-larval stage can vary from 60 days at 10°C to 11 days at 22°C, dependent upon food availability and particularly upon temperature (Aiken and Waddy, 1986; MacKenzie, 1988), and thus the development times are influenced by seasonal changes in the weather and the flow of oceanic currents. The seawater temperatures that lobsters can endure, ranges from 2-25°C in the wild, however below 5°C moult induction can be blocked and at the other extreme, a reduced increment per moult can be seen (Aiken, 1977). Seawater temperatures in the coastal habitats of lobsters around the Irish Sea range from 4-6°C in January to 16-18°C in August (Beal *et al.*, 2002). Furthermore, post-larvae can delay settlement for up to 5 weeks, until they find a suitable benthic environment for further development.

During the early benthic phase, the post-larvae are still extremely vulnerable, and in order to survive they either excavate burrows within the sediment or find small crevices within the rocky substrate, and feed by creating a current of water through the burrow, to provide a supply of small planktonic prey items (Whale and Steneck, 1991). Juvenile lobsters may spend between 3 and 4 years within their burrows, but as they grow and become less vulnerable they begin to undertake longer forays. Sexual maturity is attained between the age of 5 and 8 years, and is dependent on food availability and water temperature (Waddy *et al.*, 1995).

1.2 Lobster fisheries

The European lobster, *Homarus gammarus* (*L.*), is one of the most important shellfish species in the Great British food industry. It can be found from above the Arctic Circle (Norway) to Morocco and into the Mediterranean (Mercer *et al.*, 2001), but it is found mainly around the coast of the British Isles. There is a huge

demand for the consumption of this species in most of the Northern Hemisphere countries and this is where a problem arises: the demand exceeds the stock. In 1977, MAFF (UK) introduced a minimum catch size of 80mm carapace length (measured from the rear of the eye socket to the centre line of the body shell (along a parallel line)) and protection during breeding seasons, in response to fluctuating landings of 500 – 700 tonnes per year, between 1967 – 1976 (Shelton, 1978). In comparison with the European lobster, the American lobster (*H. americanus*) is caught in much larger quantities and in the period 1984 – 1988 catches increased from 48638 – 61936 tonnes. The principal landings of the European lobster now occur in the United Kingdom, Ireland, the Channel Islands and France (Browne, 1999b). Historically these landings have averaged 2500 tonnes per year and are of considerable socio-economic importance to coastal fishing communities throughout the range (Browne and Mercer, 1998; Browne, 1999a). Due to this increased demand, a number of rearing programmes have been initiated in an effort to restock the natural populations of lobsters both in Europe and America, alongside projects such as LEAR (Lobster Ecology and Recruitment) which aim to monitor the stocks of juvenile (early benthic phase - EBP) lobsters (Mercer, 2001).

Lobsters are usually caught and sold on the same day; otherwise they are stored in pots or '*cawfs*', until the fisherman has numbers high enough to make selling them worthwhile. Lobsters need to be treated very carefully at all times, due to their high value and the need to keep them in good condition for the live market. Lobsters have been kept successfully in tanks, which is a useful development for supplying the European market, where they sell for much higher prices than in Britain, and it is also a way of overcoming the seasonal fluctuations in numbers. However the need for rearing programmes is vital in order to maintain the culinary trade for lobsters.

In the UK, the economic importance of marine shellfisheries and research into the development of this resource has now been recognised (Mercer *et al.*, 2001). In the case of the fishery for the European lobster, *Homarus gammarus,* there is an urgent need for research into the underlying biology of its rearing, particularly for maximising yields in muscle mass and for understanding the effects of the artificial rearing environment. Due to the expense of lobster aquaculture

(involving 6-7 years of rearing prior to reaching marketable size), the majority of studies have centred on stock enhancement measures. Large scale rearing of young juveniles and release at key sites around the coasts of the United Kingdom (Bannister, 1998), Ireland, France and Norway (Mercer *et al.*, 2001), has been used to assess the feasibility of restocking the fisheries. This programme has been further supported by the observations that juvenile European lobsters remain very close to the sites of release. This implies that potential benefits can be gained by localised fisheries, and has also stimulated further research into the production of artificial reefs.

In order to reduce the times of rearing, elevated temperatures are often employed. The effect of temperature on the larval development rates of invertebrates has long been recognised (reviewed in Pechenik, 1987). Temperature has the most pervasive influence on lobster growth, from the egg to the adult, and seawater temperature cycles are a dominant regulator of embryonic development (Perkins, 1972; Branford, 1978). The temperature limit for normal successful development of the larvae is 10°C, when the time from hatching to Stage 4 is 60 days, but this can be reduced to 10 days at 22-24°C (Waddy *et al.*, 1995). Subsequently, the growth rate of juvenile lobsters is approximately proportional to temperature within the range 8-25°C.

1.3 Lobster aquaculture and ranching

The commercial importance of lobsters as a luxury food item has resulted in the implementation of many aquaculture programmes (Bannister and Howard, 1991; Aiken and Waddy, 1995). The length of time that is required for the lobsters to develop into mature adults (up to 8 years) and consequently the space and maintenance costs that this requires, is the main obstacle faced by these rearing programmes. The aquaculture of lobsters, following the life cycle, begins with collecting the hatchlings as they emerge from the gravid females, then holding the free-swimming larvae (Stages 1-3) in rapidly circulating seawater bins, to mimic the planktonic phase in the water column. This method reduces the incidences of cannibalism, and can sustain survival rates at 70-80%. The planktonic phase can be reduced to less than 30 days by elevating the water temperature to 18°C. On settlement, the post-larvae are distributed into individual

trays in order to prevent agonistic interactions between these highly aggressive animals. However, keeping lobsters under these conditions is very labour intensive and hence costly, and can fail if the rearing conditions are not strictly maintained.

A less expensive alternative to aquaculture is that of a lobster re-stocking programme, which is used to supplement the natural lobster populations after they have been depleted by over-fishing, or to seed artificial reefs. This involves rearing the planktonic larvae in bins, as described above, but then growing-on the post-larvae for only a short time. This method thus eliminates the most expensive and time-consuming phase of the rearing programme. The on-growing time can be as short as 1-2 months (to Stages 5-6) before release, as favoured by US operatives. The Massachusetts State Lobster Hatchery released half a million Stage 4 lobsters annually into coastal waters over a period of 40 years, but with no measurable effect on stock enhancement (Aiken and Waddy, 1995). Rearing for around 6 months (to approximately Stage 9) before release is more common in Europe (Bannister and Addison, 1998). An interesting recent variant of re-stocking is the attempt to establish a lobster ranch facility near the Island of Lismore, off the Scottish Argyll coast (Adam, 2001). The reef will consist of blocks of compressed quarry by-products, placed on an area of soft sediment to act as a nursery area for released post-larvae (Wilding and Sayer, 2002). When established, it is the intention that the lobsters on this reef will be fished by local fishermen.

Since lobsters released into the environment from rearing facilities will not only have to survive from predators, but will also interact with resident lobsters, and compete with them for food, refuges and mates, their ability to perform locomotory tasks such as rapid escape swimming, their ability to open shellfish by breaking their shells with their claws and their performance in agonistic encounters will all dictate their survival success. The present study addresses these issues by measuring the performance of laboratory-reared animals in these behaviours.

1.4 Background to the research

The consequences of raising water temperature during rearing on the growth, muscle physiology, performance and behaviour of these lobsters, once they are released into the marine environment, is not known. It is apparent, that modulation of the thermal environment during the early stages of development, when moulting frequency is naturally at its highest level, may have a significant impact on muscle growth, and may also affect plasticity. Additionally, there may be physiological compensations for the changes in rearing temperature, which may offset their impact on muscle performance.

1.4.1 Effects of temperature on metabolism, growth and development

Since the kinetic energy of molecules increases with temperature (the 'rate effect'), it affects the physico-chemical processes underlying metabolism, and thus also all the biological processes that depend on metabolism (cellular physiology, muscle activity, development and growth). Standard measures include Q_{10}, the ratio of rates over a 10°C interval, and the Arrhenius plot, which estimates the thermal increment (Cossins and Bowler, 1987). Metabolism involves enzyme-catalysed reactions, which follow Michaelis-Menton kinetics. These depend on the relative concentrations of the cellular substrates and of the enzyme, and on their binding kinetics. In addition they depend upon the rates at which reactants and products can diffuse through membranes to and from the sites of enzyme reactions, eg. the mitochondria. All of these chemical and physical factors may be influenced directly or indirectly by temperature. For both *in vitro* biochemical systems, and for whole-animal processes, such thermal dependency falls within narrow bounds, with Q_{10} values typically in the range 2-3 for chemical processes such as enzyme reactions, or close to 1 for purely physical processes such as diffusion.

Three levels of metabolism have been recognised by Fry (1957): **standard metabolism** (required for vital functions in the absence of spontaneous activity), **routine metabolism** (which also includes that for normal activity) and **active metabolism** (which includes that for forced levels of activity). In developing animals the processes of growth are sustained by increases in the level of routine metabolism, and the net production of tissue that is involved in growth results from a greater rate of anabolism than of catabolism (Von Bertalanffy, 1960). This

involves mainly protein synthesis and degradation, and it is both the relative rates of these processes, and also the absolute rate of protein turnover that have to be considered when interpreting the effect of temperature on growth (Hawkins and Day, 1996). Thus, for example, if the enzyme systems for protein synthesis and protein degradation have different temperature coefficients, then net production may change with temperature. High protein turnover rates will also have consequences, since they may increase the cellular substrate concentrations to levels that saturate the enzymes, so that reaction rates become increasingly temperature dependent (i.e. have elevated Q_{10} values) (Hochachka and Somero, 1984). These and other factors underlie the observed effects of temperature on the growth rates of animals, and will be considered in Chapters 2 and 4.

However animals also show adaptive responses in their physiology to temperature, whereby they compensate for direct temperature effects, and tend to maintain physiological rates in the face of temperature change (Precht, 1958). Such capacity adaptation, or physiological compensation gives a degree of functional independence from variations in temperature, although the degree of compensation can vary from complete (eg. microtubule assembly), to partial (eg. swimming performance in fish) to virtually none (eg. the speed of nerve conduction) (Clarke, 1998). Hazel and Prosser (1974) distinguished the physiological responses of **acclimatisation**, involving changes in response to environmental temperature, from those of **acclimation**, that are laboratory-induced adaptations. The cellular basis of compensatory responses ultimately depends on the activity levels of key regulatory enzymes, and Hochachka and Somero (1984) recognise three strategies: quantitative (changes in the cellular concentration of enzymes), qualitative (the expression of different enzyme isoforms) and modulatory (changes in the kinetics of pre-existing enzymes). These processes can occur not only in muscle tissue, but also in other organ systems such as the nervous and digestive systems. Additionally, changes in membrane structure, and mitochondrial density may be involved.

The majority of work investigating the effects of temperature on the physiological compensation of muscle to temperature change has been carried out on fish, most notably by the laboratories of I.A. Johnston (reviewed by Johnston and Temple, 2002) and of G. Goldspink (Goldspink, 1995), and recently involving

molecular techniques. Adaptation in muscular performance may result from changes in neural function (synaptic transmission), contractile protein composition and/or muscle metabolism. Early work by Johnston and Lucking (1978) on goldfish acclimated to environmental temperatures of 8, 18 and 31°C, for a period of three months, demonstrated a shift to more aerobic metabolism and changes in the size and relative distribution of different fibre types in the myotome. This work also suggests that there are different kinetic forms of myofibrillar ATPase at different environmental temperatures, which has led to many studies investigating both acclimation and adaptation in these enzymes, and the generation of myofibrils with different enzyme thermo-stabilities. More recently, Watabe *et al.* (1995) demonstrated that there are different myosin heavy chain (S1) isoforms in warm and cold acclimated carp. Gerlach *et al.* (1990) have shown that there are up to 28 different isoform genes in fish and suggest that some of these isoforms are temperature-sensitive, with subsequent variation in ATPase structure and activity. Ennion and Goldspink (1996) have further characterised 7 of the carp myosin isoforms, and have identified two potential isoforms, expressed in the slow muscle of warm-acclimated carp, as well as shifts in the temporal expression of developmental isoforms in response to increases in temperature.

In Crustacea and in particular lobsters, little is known about the number of myosin isoforms or the existence of temperature dependent isoforms. The effects of temperature on gene transcription and protein synthesis rates in a marine isopod and a freshwater crayfish have been investigated, and have demonstrated that rates of whole protein synthesis and mRNA transcription rates for actin are directly related to temperature, with Q_{10} values of around 2 (Whiteley and El Haj, 2001).

Aspects of the physiological compensation of lobsters to temperature changes are considered in Chapters 5-7, and the underlying molecular mechanisms are discussed in Chapter 9.

1.5 Muscle systems studied

Embryonic development of the muscle and nervous systems of lobsters during their planktonic stages is important to the subsequent differentiation of the muscle fibres in the juvenile. The pattern of muscle development follows that described in a number of invertebrate and vertebrate species, in particular the differentiation of mesodermal precursors to the myogenic lineage with the presence of skeletal muscle specific transcription factors, which are further characterised in vertebrate muscle as myoD, myogenin and a number of others, which regulate the expression of muscle specific genes. The characteristics of muscles, including sarcomere length, actin-myosin ratio, enzymatic content, myofibrillar protein profile, are established early in development. Growth involves fibre enlargement, and possibly also in some cases increasing fibre number. Vertebrate and invertebrate sarcomeric proteins have been demonstrated to have developmental isoforms, which are expressed temporally throughout embryonic and postnatal stages, but little is known about the corresponding isoforms in crustacean species.

1.5.1 The claw muscles

The claw muscles comprise both fast and slow (predominantly S1) phenotypes, according to the various myofibrillar protein isoforms that they express (Govind *et al.*, 1987; Mykles, 1997). The claw muscles undergo a considerable transformation during the post-larval stages (Govind and Lang, 1978; Ogonowski *et al.*, 1980). This change occurs in relation to morphological changes in the claws themselves, which are symmetrical in the early post-larval lobsters, but differentiate into the characteristic cutter and crusher claws of the adult lobster over the early benthic and juvenile periods (Govind, 1992). Post-larvally, the closer muscles of the symmetrical claws of the lobster have identical fibre arrangements, where fast fibres are located centrally and slow fibres are located peripherally (Govind and Lang, 1978). During Stages 5-13, changes in fibre composition take place, in the developing dimorphic claws. In the presumptive cutter claw, the fast-fibre region of the closer muscle expands in area (comprising at least 80% by completion), whilst in the developing crusher claw, the fast-fibre region contracts in area (almost entirely composed of slow fibres), this has been reported to be complete by the Stage 13, but in some cases is not until the lobster is 1-2 years old.

Fibre type transformation occurs through the switching of pre-existing, fully differentiated fibres from one type to the other, under the influence of a stimulus related to claw use. It is an interesting example of muscle plasticity, and is not a result of fibre degeneration and replacement of new fibres, generated *de novo* by hyperplasia. The muscles assume different contractile properties, through the expression of fibre-type specific isoforms, and changes in this expression result in the fibre type transformation. This process of fibre transformation occurs along a distinct boundary between the regions of fast and slow fibres, and takes place immediately post-moult, when protein synthesis is elevated and haemolymph ecdysteroid concentrations are low. It is only at this time that fibres at the boundary between the fast and slow regions have been found to display intermediate myofibrillar ATPase activities, or to co-express the messenger RNAs for fast and slow myosin heavy chains (Mykles, 1997). A key feature of this process is that the genetics of the dimorphic claws is not fixed, with the claws showing plasticity of development according to an 'environmental' influence, the pattern of claw usage. In the natural environment, the claw that is used more, differentiates into the crusher (Govind, 1992), but changes in substrate can affect this development. The commitment to development is thought to occur in the Stage 5, but this is partially as a result of the types of experiments, which have been conducted. There is very little known about the effects of temperature during the early larval rearing of European lobsters on the subsequent differentiation of the claw muscle and of its performance in later life, and for this reason, it has been addressed in the present study.

Many studies have targeted the claw muscles as they display an obvious external phenotypic difference, which develops through the postlarval stages of development. However, it has been suggested that this same plasticity, involving fibre type transformation during differentiation, may extend to other heterogenous muscles of the lobster, which do not show related changes in external morphology. It is known that lobster muscles differentiate at different rates. Muscles within the thoracic exopodites and the abdomen undergo myogenesis in embryonic development (Govind *et al.*, 1988) and are functional at the time of hatching, whereas those of the pleopods become larval and post-larval stages. This correlates with the different locomotory behaviours of the larval and post-

larval stages. The larvae swim strongly using the thoracic exopodites and tail flips (Ennis, 1986; Rooney and Cobb, 1991) and sustained swimming plays a crucial role in their dispersal. In contrast, the bottom-dwelling post-larvae tail flip only infrequently and also use pleopod beating to propel their swimming (Neil *et al.*, 1976). Any changes in the normal pattern of development of these muscles, or the expression of their myofibrillar proteins, will therefore have serious consequences for the survival of the larvae and their recruitment into the adult stock.

1.5.2 The abdominal muscles

The abdominal muscles form the major muscle system in the body. The organisation and segregation of the abdominal musculature is established early in development, and these muscles are functional when the larvae first hatch (Cole and Lang, 1980). The majority of the abdominal muscle mass is made up of deep fast flexor and extensor muscles, which control the powerful flexions and extensions of the abdomen, propelling the lobster through the water during the escape tail flip response (Wine and Krasne, 1982). The deep fast muscle is surrounded by thin sheets of slow superficial extensor and flexor muscles these control the posture of the abdomen (Pilgrim and Wiersma, 1963; Kennedy and Takeda, 1965). Unlike the deep muscles, which are homogenous populations of fast type fibres, the slow superficial muscles can be further sub-divided into two subtypes, S1 and S2, which play different roles in postural control (Fowler and Neil, 1992; Holmes *et al.*, 1999).

The fast, S1 and S2 fibre phenotypes, which are found within the skeletal muscles of decapod crustaceans, are well characterised and can be identified by their morphological (Govind *et al.*, 1987; Fowler and Neil, 1992), mechanical (Galler and Neil, 1994; Holmes *et al.*, 1999) biochemical (Mykles, 1988) and histological (Mykles, 1985a, b; Li and Mykles, 1990; Neil *et al.*, 1993) properties. As in other crustaceans, fibres persist throughout development, and attain their different contractile properties through the expression of fibre-type specific isoforms, which can be visualised using one-dimensional SDS PAGE gel electrophoresis. Thus at least seven isoforms are unique to the abdominal fast fibres: myosin heavy chain (fMHC), fast tropomyosin (fTM), unidentified band at

75kD (P75), Troponin I_1, I_3 and I_5; five isoforms are unique to the S1 slow fibres: slow myosin heavy chain (sMHC), slow tropomyosin 1 (sTM1), slow tropomyosin 2 (sTM2), Troponin T_3 and Troponin I_4 (Mykles, 1985a, b; Neil *et al.,* 1993). The development of the abdominal muscles will be studied with regard to the structural characteristics and the swimming performance attainable by the lobsters, reared at the different temperatures.

1.6 The moult cycle

The indeterminate growth of lobsters is coupled to the moult cycle (El Haj, 1996; El Haj and Whiteley, 1997; Whiteley and El Haj, 1997), unlike that of ectothermic marine vertebrates, such as fish, which grow continuously throughout the year. The moult cycle can be divided into a number of phases: premoult (animal is preparing to moult); moult or ecdysis (when old exoskeleton is shed); postmoult (when the new external cuticle is hardening) and intermoult. Before moulting, the lobster absorbs minerals from the old exoskeleton, which causes it to become soft, and by taking in water, the blood volume is increased, which swells the new exoskeleton and causes the old one to split. Longitudinal growth in crustacean muscle fibres, which occurs by either the addition of new sarcomeres or by sarcomere lengthening, has been shown to occur in the abdominal and leg muscles, over ecdysis and during the immediate post moult period (El Haj *et al.*, 1984; Houlihan and El Haj, 1985; El Haj and Houlihan, 1987). Similarly, myofibrillar splitting, which indicates an increased number of fibres per cross sectional area, has been suggested to occur over the moult (El Haj *et al.*, 1984).

In contrast, the claw muscle undergoes atrophy during the premoult to enable the muscles to pass through the narrow basi-ischum joints during ecdysis (Mykles and Skinner, 1982). Claw muscle atrophy is associated with elevated levels of protein synthesis during the pre-moult and post-moult periods (Skinner, 1965; Mykles and Skinner, 1985a, b; El Haj *et al.*, 1996), whereas synthesis rates are only slightly raised during late pre-moult in the muscles of the abdomen and legs (El Haj *et al.*, 1996). The complex patterns of cellular and physiological processes associated with the moult cycle (El Haj *et al.*, 1992; El Haj, 1996; El Haj and Whiteley, 1997) make it extremely difficult to establish how muscle growth and

phenotype are modified in response to other variables such as environmental temperature.

1.7 Lobster Behaviour

1.7.1 Escape response swimming

Like other malocostracan crustaceans with extended abdomens, *H. gammarus* uses the tail-flip escape swimming response as an evasive reaction to noxious (chemical) or threatening stimuli such as a predator or when in an agonistic encounter (Wine and Krasne, 1982; Newland *et al.*, 1988a; Neil and Ansell, 1995; Edwards, 1995; Arnott *et al.*, 1998, 1999). The ability for a lobster to maintain it's swimming (flexing and extending the abdomen) is vital for it avoiding being eaten by a predator or wounded by a conspecific, so that it can remove itself from the threat. This study has utilised the convenience of the tail flip escape response of lobsters to test the effect of rearing temperature on locomotory performance, and hence muscle development and contractile activity. It was performed in conjunction with biochemical studies of protein turnover, and with a molecular study of myosin gene expression (Holmes *et al.*, 2001), which has allowed changes in the structure of key parts of the myosin molecule involved in the contractile and metabolic properties, to be correlated with these changes in its performance.

1.7.2 Agonistic behaviour

The ability of the laboratory reared juvenile lobsters to compete with their wild counterparts, if released for re-stocking purposes, would be vital to their existence. If the lobsters from a rearing programme were found to be agonistically naïve, then their chances of finding shelter and eventually winning a mate would be greatly reduced. The ritualistic nature of the aggressive behaviour in juvenile *H. americanus* has been well documented by Kravitz and co-workers (Huber and Kravitz, 1995), and the present study has adopted a similar approach to the study of juvenile *H. gammarus,* in order to assess whether changes occur in agonistic behaviour during the course of postlarval development, and if so, whether rearing temperature modulates these changes (Chapter 8).

1.8 Rationale for the project

A multidisciplinary study of the effects temperature on lobster development represents a powerful approach, since investigations of the growth and development of the muscles in the tail and the claws can be related to molecular mechanisms of gene expression, to physiological measures of muscle contraction, and to performance indicators such as claw strength, swimming speed and use in agonistic encounters. The present project represents part of this strategy, and is focussed on measures of growth, phenotypic expression of muscle fibres and physiological measures of performance in post-larval European lobsters. A parallel study, which was integrated with this work through common grant funding (NERC DEMA Thematic Programme), has provided complementary information about the expression of relevant muscle genes. Such a combined approach, which has never previously been applied to lobster development, presents an excellent opportunity to investigate how environmental change can influence the ontogeny of lobsters and the resultant fitness of a sustainable marine resource.

This present study has posed the question: do lobster rearing programmes produce animals that are suitably equipped to compete against their wild counterparts for food and space, once released? To address this question, groups of post-larval European lobsters, *Homarus gammarus*, were reared at 11°C, 15°C and 19°C from post-larval Stage 8 to 18 months old, and also from the egg through to 6 months of age at 15°C and 19°C at the larval rearing facilities of the Centre for Environmental, Fisheries and Aquaculture Science (CEFAS), Conwy, North Wales.

As well as keeping full records of their moult history, regular morphometric measurements of the body and the claws, histochemical analysis of their muscle fibre phenotypes, physiological measurements of muscle performance and behavioural analyses of agonistic encounters were made throughout these periods of development. Particular attention was paid to the abdominal muscles, which are responsible for the swimming escape behaviour, and to the muscles of the cutter and crusher claws, which are important for feeding and for agonistic

behaviour, since both of these also represent the commercially important body tissues.

The results of this study identify certain changes in muscle growth and phenotype that may affect how these hatchery-reared lobsters perform and behave once released into the natural marine environment. When considered together with molecular studies of gene expression in the same animals, they also provide insights into the adaptive mechanisms operating at both the phenotypic and genotypic levels.

1.9 Figures

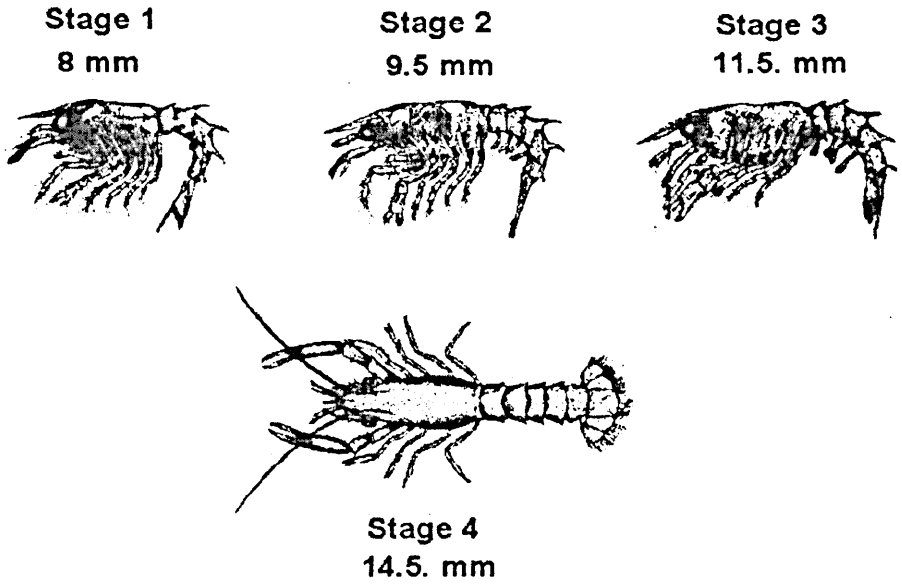


Figure 1.1: The larval stages of the lobster, *Homarus gammarus*.

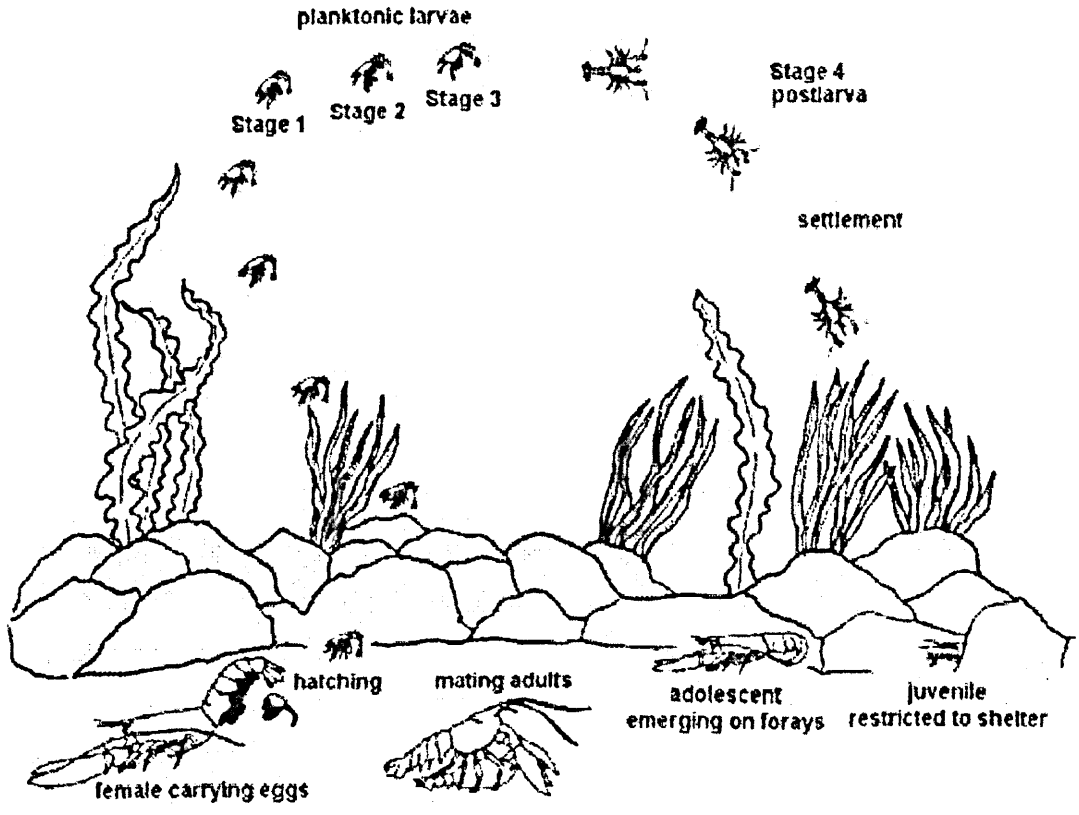


Figure 1.2: The life cycle of the European lobster, *Homarus gammarus* (taken from Factor, 1995).

Chapter 2 - The Growth of the European lobster, *Homarus gammarus*, at different temperatures of rearing

2.1 Introduction

Both European and American lobsters have been reared commercially, but the success in this has been variable. The husbandry has most commonly involved keeping lobsters in individual containers, to avoid fighting and cannibalism. However, the lack of space, the unnaturalness of the substrate and the lack of a varied diet, have all contributed to this lack of success. The need for daily inspection and the feeding of animals individually also makes it very labour intensive, and hence costly. Recently, communal rearing of lobsters is being attempted, and has produced good rates of survival and growth. It is an important progression, as it reduces the need for large holding areas and is less labour intensive.

As with all crustaceans, morphogenesis and differentiation of tissues can occur in lobsters throughout an instar, although they do not lead to any visible change in size. Lobsters are able to increase their body size only when they shed their exoskeleton at the moult (ecdysis). Lobsters spend much of their lives preparing for or recovering from ecdysis, although the actual process of shedding the old exoskeleton can take only a few minutes (Guyselman, 1953). Pre-moult induction and preparation for ecdysis are under the control of the endocrine system, which in turn responds to internal (*e.g.* nutritional state and health) and external (e.g. temperature and day length) cues. Pre-moult activities include limb regeneration, resorption and storage of cuticular components, deposition of new cuticle, histolysis of somatic muscle, selective water and ion absorption and shifts in biochemical pathways. These all culminate in ecdysis, after which the lobster assumes its new length and volume by actively absorbing water, the epidermis regresses, additional cuticle is secreted, mineralization occurs, water is replaced by new tissue and metabolic reserves are replaced (Waddy, 1995). After ecdysis the exoskeleton of the lobster is very soft, and can take 3-4 days to harden, during which time the lobster is very susceptible to predation. The lobster may then move directly into preparation for the next moult (di-ecdysis) or pause for an extended period (an-ecdysis) (Carlisle and Dohm, 1953).

Even though there is extensive knowledge about the mechanisms of the moulting cycle, it is still not possible to establish the age of the lobster, based on its size, as lobsters grow at different rates depending on both internal and external factors. Size is not a reliable indicator of age, as growth can be delayed in adverse conditions. Recently it has been reported that a 'waste' pigment called lipofuscin accumulates in the olfactory lobe of the brain as the animal gets older, and may serve as a measure for aging (O' Donavan and Tully, 1996). As lipofuscin accumulation is actually a function of 'metabolic age' (O' Donavan and Tully, 1996), factors such as temperature and population density, which affect growth rates in the wild, could potentially affect the metabolic rate and therefore lipofuscin accumulation. However, independence between growth and lipofuscin accumulation in laboratory animals indicates that this might not be a problem (O' Donavan and Tully, 1996). Use of lipofuscin is promoted primarily for the aging of wild-caught animals, although controlled laboratory rearing at different temperatures, as in the present study, could yield relevant material for validating the use of lipofuscin as an age marker.

When rearing lobsters, food type and ration are also very important, as insufficient amounts cause a lengthening of the larval life and reduced survival (Templeman, 1936b; Sandoz and Rogers, 1944). Fresh or freshly-frozen flesh of selected molluscs, crustaceans and polychates are some of the best food types for laboratory reared crustaceans (especially lobsters), as they mimic the natural diet, and support good growth and survival (Ali and Wickens, 1994). Diet quality can influence growth by affecting both inter-moult period and length increment. If moult frequency is determined by the rate of tissue growth following ecdysis (Adelung, 1971), then nutrition influences the size increase over time. This is reported to occur primarily through an effect on moult frequency, not size increment per moult (Aiken, 1977).

The environmental conditions also have to be strictly maintained. The most critical ones are salinity, diet, the level of illumination, photoperiod and temperature. The effects of light are specific, and can be both direct and indirect. The main effect is that of photoperiodism, which operates through the central nervous system, to control physiological processes. Light intensity has an indirect

effect, which can influence growth through a secondary mechanism, such as behaviour and activity levels. There is little evidence that photoperiodism directly controls either moulting or growth in juveniles or adults, but there are strong indications that light influences related processes. Bright light depresses lobster activity, and so less food is consumed, and as a result growth suffers. In contrast, darkness causes hyperactivity, which can reduce growth because of an increase in antagonistic behaviour, the level of stress and injury (Aiken, 1980). Small juvenile lobsters moult predominantly during the day (Tamm and Cobb, 1976) and their food consumption and growth is enhanced by a short photoperiod (Bordner and Conklin, 1981). Larger juvenile lobsters can increase their moult frequency in long photoperiods at 10°C but not at 15°C and 20°C, which suggests that response to day length may vary with life history, moult stage and temperature (Aiken and Waddy, 1976).

Lobster growth is also directly related to the size of space within which the animal is contained (Sastry *et al.*, 1975; Van Olst *et al.*, 1975; Aiken, 1977). Shlesser (1975) found that this relationship was not altered by the shape of the container. With limited space, Van Olst *et al.* (1975) found the inter-moult period to be the same, but that the size increase at moult was reduced. Based on this, it has been reported that for optimum growth and economy, the space needed for the lobsters is $(1.2 \times \text{Tail length})^2$ (Adiyodi, 1968; Van Olst *et al.*, 1975). In conjunction with the size needed, lobsters that find themselves downstream can have a reduction in size, potentially due to the chemicals and hormones released into the water by the other lobsters. It has also been demonstrated that moulting can be delayed if Stage 4 lobsters are held in pairs, which is not seen in those held individually (Cobb, 1970), this phenomenon can persist to Stage 11 (Cobb and Tamm, 1974). The dominant animal moults first, but this itself is delayed in comparison to the control (single lobster). This will be the case to some extent in communal rearing systems, which are known to produce a mixture of fast- and slow-growing lobsters, but it cannot explain how communally reared lobsters are often larger than individually reared ones (Aiken, 1977).

A lot of work has been carried out on the development of *H. americanus* at different temperatures of rearing, both at larval and post-larval stages, in order to improve husbandry procedures and also to understand growth processes in the

wild. Upper and lower temperature limits of 10°C and 22°C respectively are normally applied for rearing. Larval development cannot be completed at low temperatures, and in the wild hatching occurs well before the water temperature declines in the autumn. The temperature limit for larval development is ~10°C, below which survival is poor (Waddy *et al.*, 1995). The time taken to reach postlarval Stage 4 is considered to be 10 days at 22-24°C and 2 months at 10°C (Huntsman, 1923; Templeman, 1936a, c; Hughes and Matthiessen, 1962; MacKenzie, 1988). The most rapid development occurs in spring before the normal hatching season, and the slowest occurs in late summer/autumn (Waddy and Aiken, 1986). This also affects the survival rates, as the larvae that hatch early survive better than those that hatch later (Sastry and Vargo, 1977; Aiken and Waddy, 1986a). In relation to this it has been shown that lobsters of a given locality have a relatively consistent length increase at the moult from year to year (Ennis, 1971, 1972; Conan, 1978; Campbell, 1983), but can vary significantly between areas (Wilder, 1953; Ennis, 1972, 1986; Conan, 1978), being slightly greater in the warm waters of the southern Gulf of St. Lawrence than in the cooler waters of the Bay of Fundy. Temperature also has a great effect on the survival rates of Stage 3 and of postlarval lobsters. At 10°C the survival of Stage 3 and post-larvae is <26%, compared to >75% at higher temperatures (MacKenzie, 1988). Cumulative survival to Stage 5 is 4% at 10°C, 56% at 12°C, 64% at 15°C, 68% at 18°C and 47% at 22°C. Survival in nature depends very much on the time of hatching in relation to the summer time portion of the annual temperature cycle (Ennis, 1995).

It can therefore be seen that temperature is one of the most pervasive influences on lobster growth, and it was for this reason that it was chosen as the environmental variable for the present study. The aim was to examine the effect of three rearing temperatures, 11°C, 15°C and 19°C, on the growth and moulting of three large groups of postlarval lobsters. In later chapters, measures have been performed to identify whether increased growth rates could be achieved without detriment to the normal morphogenic processes (eg. dimorphic claw development; tail muscle development) or consequent physiological performance (claw force development; swimming speed).

2.2 Materials and Methods

The rearing of the larval and juvenile lobsters was carried out at the Centre for Environment, Fisheries and Aquaculture Science (CEFAS) laboratory in Conwy, North Wales, following procedures developed by the director John Wickens. Gravid female lobsters were held in the aquarium at a temperature of 15°C ± 1°C, and on hatching the larvae (typically in May/June) were transferred into circulating bulk containers, to mimic the planktonic phase of the life cycle. These re-circulated water containers were set up at the three temperatures of rearing, 11°C ± 1°C, 15°C ± 1°C and 19°C ± 1°C. The salinity of the seawater used was approximately 32‰.

The lobsters were held in this way until moulting to the Stage 4 occurred, when the lobsters take on a form more closely resembling the adult lobster. At this point, the animals were transferred to separate numbered chambers, where they were held individually. The dimensions of the containers into which they were transferred initially were: 5.2 x 5.4 x 7.5 cm and were filled to about 6 mm deep with seawater at the specific temperatures. The containers had a mesh floor, to allow the exchange of water and to control the water circulation system. The containers were cleaned on a daily basis, and the animals were fed daily on the mantle tissue of mussel (*Mytilus edulis*), mysid shrimps and occasionally on chopped *Artemia salina*.

When the lobsters reached approximately 10-15 mm carapace length, they were transferred to larger chambers (12.5 x 12.3 x 9 cm), so that growth was not limited by their available space (see Introduction). They remained in isolation in this way for up to 18 months. No external characteristics were visible in animals of this age range that allowed their sex to be reliably distinguished, and so the juveniles have been treated as a single set.

The postlarval lobsters were checked twice a day, and if a moult or death had occurred, then it was recorded, so building up a life history for each lobster. Since cast exuvia were consumed rapidly after moulting by the post-moult animal (being a rich source of protein and calcium) there was sometimes no evidence of exuvial fragments. For this reason, lobsters were also touched to ascertain

whether their exoskeleton was soft, and if so were recorded as having just moulted.

At regular intervals, the following measurements of growth were made on all lobsters: weight (g), body length (telson to tail fan), carapace length, (bottom of eye socket back parallel to the mid dorsal line of the carapace) and for each claw: claw length, dactyl length, claw height and claw width (all measured with callipers in mm) (See Chapter 4, Fig. 4.1). The total length measurement is unsatisfactory if measurements are made within a few days of moulting, so carapace length has been used as a more suitable standard of growth (Templeman, 1936).

The moulting records and growth measures for each lobster were combined in a single database, and from these data various growth statistics for individual lobsters were derived: the frequency of moulting, the duration of the successive intermoult periods, the body dimensions at given ages and in given moult stages. By considering the data for particular temperature groups, average measures and population statistics could be obtained. In some cases the data for animals in the median stage at a given time point (the median group) were calculated separately, to overcome the problem of averaging values from a population of animals in which there is a wide range of growth rates. The median stage was calculated by considering the stages occupied by the total number of lobsters at a given time point, and evaluating the stage reached by the animal at the mid-point of the distribution.

Following a pilot trial in 1997, a rearing programme was initiated in the summer of 1998, but due to high mortalities in all temperature groups, due to unforeseen circumstances, insufficient numbers of postlarval animals were available for on-growing. As a substitute, a stock of 1058 Stage 8 lobsters was obtained from a commercial rearing facility in Orkney in October 1998 (previously held at ambient temperatures of 14-16°C), and these were held as described above (11°C – 324 animals, 15°C – 374 animals and 19°C – 360 animals) for period up to December 1999 (17 months post-hatching).

In 1999 a second cohort of lobsters was reared from hatching in June at CEFAS Conwy, and again the larvae were held in bins at the three temperatures of 11°C,

15°C and 19°C. On this occasion 160 animals at 15°C and 240 animals at 19°C reached postlarval Stage 4, and were on-grown in individual chambers until December 1999 (6 months post-hatching). At 11°C, however, which is just above the lower limit of successful rearing of *H. americanus* in laboratory conditions (Templeman, 1936b), the larvae did not survive in large numbers, and on-growing trials were not attempted on the few post-larvae that were produced at this temperature.

As well as making regular morphometric measurements, as detailed above, small numbers of animals were sacrificed at certain times in order to obtain tissues for histological, histochemical, biochemical and molecular analysis. For the 1998 cohort of lobsters, the main sampling times were in March 1999 (9 months post-hatch), August/September 1999 (15 months post-hatch) and December 1999 (17 months post-hatch). For the 1999 cohort of lobsters, the main sampling times were in September 1999 (3 months post-hatch) and December 1999 (6 months post-hatch).

2.3 Results

2.3.1 1998 cohort

2.3.1.1 Rates of mortality

Rates of mortality calculated month-on-month as a percentage of the remaining stock (Figs. 2.1.a-c) indicate that animals survived in large numbers at all rearing temperatures, with the values at 11°C (mean of 12% mortality) being similar to those at 19°C (mean of 9% mortality). Values at 15°C were similar up to 9 months (April 1999) but high rates of mortality (60%) occurred thereafter, which were almost certainly due to an event, which interrupted the water flow and caused a drop in its salinity over a period of 24-48 hours. Only small numbers survived this event, to contribute to the data for the 15°C group thereafter.

2.3.1.2 Moulting frequency

From the moult records, the numbers of lobsters at each moult stage were calculated at monthly intervals. In *Figures 2.2.a-c* these values for the three temperature groups are expressed as a percentage of the total number of lobsters alive at the monthly time points, taking into account any mortality between sampling points. At each temperature there was a variation in the moulting frequencies of individuals, so that at any point in time animals at a number of different stages co-existed in a temperature group. The range of stages expressed increased with time, up to a value of approximately five stage increments by the end of the rearing period (i.e. Stages 10-15 at 11°C; Stages 12-17 at 15°C; Stages 14-19 at 19°C). For this reason, the 'median stage' for each group was also calculated at each time point, and comparative growth measures have been based on values for these median groups.

The change in moult stage over the rearing period was temperature-dependent, as shown by the complete moult records (Figs. 2.2.a-c) and also by the data based only on the median group (Fig. 2.3). Starting from the same initial stage, Stage 8, the median stages for the three temperature groups diverged, and by the end of the rearing period (17 months) were separated from each other by two stage increments: Stage 12 at 11°C, Stage 14 at 15°C and Stage 16 at 19°C (Fig. 2.3). The times at which a given median stage was reached by the different temperature groups are also informative. Thus Stage 12 was reached at 9 months for the 19°C group, 10 months for the 15°C group but not until 13 months for the 11°C group.

2.3.1.3 Inter-moult period

The Inter-moult period, i.e. the duration spent in a stage between successive moults gives a more precise indication of moulting frequency, and how this changes with stage. Values of the Inter-moult periods for all animals at the three rearing temperatures are shown in *Figure 2.4.* There were systematic differences between the groups, with Inter-moult period decreasing with increasing temperature at all stages throughout the rearing period. Another trend was for Inter-moult period to increase with moult number, which was clear for both the 15°C and 19°C groups. At 15°C this increase was from ~25 days at Stage 8 to ~50 days at Stage 16, and for 19°C it was from ~ 20 days at Stage 8 to ~55 days

at Stage 18. In contrast, the Inter-moult periods of the 11°C group were significantly longer (at 50-70 days) than those of the higher temperature groups at all stages from Stage 8, and showed no trend with stage number. These long Inter-moult periods for the early stages at 11°C indicate that this temperature was close to the null point for moulting. There are significant differences between all the mean values for the stage 8 animals (ANOVA F-statistic, $F_{2, 849} = 53.21$, $p = 0.000$). Tukey pairwise comparisons indicate significant differences between all three temperature groups. By stage 13, significant differences between the inter-moult periods are also seen (ANOVA F-statistic, $F_{2, 181} = 12.20$, $p = 0.000$). Tukey pairwise comparisons indicate significant differences between the 11 and 19°C and the 15 and 19°C groups but not between the 11 and 15°C groups.

2.3.1.4 Rates of Growth

The morphometric measurements made are plotted for the median groups of animals. The standard measure of size, carapace length, increased with age in a temperature-dependent manner from an initial value of 6 mm in the Stage 8 lobsters, which had been raised to that point at a common temperature. By the end of the rearing period the values for the 11°C, 15°C and 19°C groups had increased to 12 mm, 16 mm and 25 mm respectively (Fig. 2.5). The slopes for the three temperature groups are statistically significantly different from one another (Univariate ANOVA; comparison of regression slopes; $F_{2, 6} = 107.541$, $p = 0.000$). Plotting these growth data in relation to moult stage (Fig. 2.6), rather than age (see Fig. 2.5), shows that the lobsters raised at the different temperatures do not attain the same size when they reach a given stage, even though they pass through it at a greater age, but that a temperature-dependent difference remains (Univariate ANOVA; comparison of regression slope intercepts; $F_{2, 24} = 13.509$, $p = 0.000$). This suggests that temperature is having an effect on growth *per se*, in addition to its effect on moulting frequency.

The underlying relationship between moulting frequency and growth rate was sought by calculating the moult increment, i.e. the increase in size (represented as a % increase in carapace length) from one stage to the next. However the moult increment values for the 1998 cohort were variable (between 3% and

>30%) and showed no systematic trend with either moult stage or temperature (Fig. 2.7).

Plotting one body dimension, carapace length (a standard measure of growth in studies on Crustacea), against another, abdomen length, shows the proportionality of growth in the lobsters, i.e. the degree of allometry. These data are presented in *figure 2.8*. The fact that the regression lines fitted to the data for all animals (Fig. 2.8) are statistically significantly different (Univariate ANOVA; comparison of regression slopes; $F_{2, 204} = 9{,}810$, $p = 0.000$) indicates that there is a some deviation from isometry during growth at the different temperatures, with the 11°C lobsters having slightly larger abdomens in proportion to their carapace lengths than those from the other two temperature regimes.

2.3.2 1999 cohort

2.3.2.1 Rates of mortality

The monthly rates of mortality were higher in the 1999 cohort, than in the 1998 cohort over the equivalent rearing periods (months 3-6 post-hatch) (Figs. 2.9.a+b). In the first month with postlarval animals (July 1999) the rate of mortality was 25% for the 15°C group and 20% for the 19°C group. Subsequently mortalities increased at both temperatures to 65% at 15°C and 40% at 19°C at the end of the rearing period (6 months).

2.3.2.2 Moulting frequency

The frequencies of moulting (Figs. 2.10.a+b) are shown in the same way as for the 1998 cohort. Measurements began at Stage 4, and after 6 months of rearing the 15°C group contained Stages 4-8, with a median of Stage 7, and the 19°C group contained Stages 5-10, with a median also of Stage 7 (Fig. 2.11).

2.3.2.3 Inter-moult period

Considering the post-larval stages, in the 15°C group the Inter-moult periods were shorter than in the 19°C group for Stages 4 and 5, but were the same as the

19°C group by Stage 6-8, and were longer than the 19°C group thereafter (Fig. 2.12). A progressive reduction in the duration of the moult stages of the 19°C group over Stages 5-10 underlies this shift.

2.3.2.4 Rates of Growth

The morphometric measurements for the median groups show that carapace length increased with age in a temperature-dependent manner (Fig. 2.13). In this case the Stage 4 post-larvae differed in size in the two temperature groups: 5.5 mm CL at 15°C and 6.5 mm at 19°C, which was due to them being raised at these two temperatures throughout the larval stages, unlike the situation for the 1998 cohort, which was separated only at Stage 8. Considering the rate of growth up to 6 months, these size differences are maintained, as the growth curves have different elevations. (Univariate ANOVA not possible on this data set).

Plotting these growth data in relation to moult stage (Fig. 2.14), rather than age (see Fig. 2.13), illustrates that the lobsters raised at 15°C remain significantly smaller in size than those at 19°C when they reach a given stage, even though they have a greater age (Univariate ANOVA; comparison of regression slope intercepts; $F_{1,9} = 24.726$, $p = 0.001$).

Moult increments for the 1999 cohort were in the range of 10-30% (Fig. 2.15). They showed no definite trend with temperature of rearing, although there was some indication that the moult increment decreased in the 19°C group from the Stage 7/8 transition and beyond.

The relationship between carapace length and abdomen length (Fig. 2.16) is a ratio of ~2, with there being no difference in the slopes of the relationships for the two temperature groups (Univariate ANOVA; comparison of regression slopes and intercepts: no significant differences).

2.4 Discussion

One of the most consistent findings in the literature is that a higher temperature of rearing increases the rate of growth of the lobster populations. The data shown here confirm this correlation between rearing temperature and increase in body size for juvenile *H. gammarus* (Figs. 2.5 & 2.13).

The fact that growth has such a clear temperature dependence indicates that the thermal 'rate effect' dominates the relationship, and there is no apparent physiological compensation. This might be expected from the fact that net production of protein occurs through high turnover rates around the time of the moulting process (El Haj *et al.*, 1996), and, as discussed in Chapter 1, at such high turnover rates enzyme reaction kinetics are highly temperature dependent, with high Q_{10} values.

However, there are at least two distinct processes affected by rearing temperature. The first and main one is moulting frequency, which increases with temperature, so that more growth increments accumulate in a given time. The second process is the rate of underlying morphogenesis, which determines the amount of new tissue that is formed between moults. These will be considered in turn.

2.4.1 Moulting frequency

In *H. americanus*, moulting frequency in both the larval and post-larval stages increases with increasing temperature within the range of 8-25°C (Aiken, 1977). Thus there is an inverse relationship between temperature and the time required from hatching to attain the post-larval stage (Templeman, 1936; MacKenzie, 1988). However, moulting frequency is also reduced with increasing age, as the successive stages become progressively longer (Ennis, 1995). MacKenzie (1988) examined the relationship between these two factors and found the ratio of the duration of a particular stage to total larval development time to be constant along temperature treatments (i.e. equi-proportional development).

The present study has demonstrated similar effects, since inter moult periods both decreased with increasing temperature (comparing 15°C and 19°C) and increased with age (Figs. 2.4, 2.12), confirming the powerful influence of these

factors on moult frequency. An exception to this, however, was the moulting rate of the 11°C group of the 1998 cohort, which was significantly lower, with long Inter-moult periods that persisted throughout the rearing period (when tested at stages 8 and 13, however there was no difference between the stage 13 11 and 15°C groups). The most likely explanation of this is that 11°C was close to the null point (i.e. the lower temperature limit for development), which has been identified by a number of studies on *H. americanus* as being 10°C (Templeman, 1936; MacKenzie, 1988). The fact that the 1999 cohort failed to metamorphose successfully from the larval stages lobsters at 11°C probably may have a similar explanation. Post-larval Stage 4 *H. americanus* can be achieved at 10°C, but moulting to Stage 5 does not occur (Waddy *et al.*, 1995). If the water temperature drops to approximately 5°C (as is possible in the wild) then moult induction is blocked. The 11°C group should therefore be regarded as close to the low temperature null point, with a low survival rate, and minimal rates of growth.

It has been suggested that such null points for crustacean moulting are related to an inhibition of the hormonal systems that initiate this process (Cossins and Bowler, 1987) by regulating ecdysteroid release (El Haj *et al.*, 1996; Whiteley and El Haj, 1997). It would therefore seem appropriate to test this hypothesis directly by measuring ecdysteroid titres in the different temperature groups.

2.4.2 Evidence for differences in rates of morphogenesis

For a given increase in size at each moult (moult increment) increasing moult frequency will produce greater rates of growth. Moult increments for both juvenile and adult *H. americanus* are on average, 15% in length and 50 – 55% in weight (Wilder, 1953; Thomas, 1958; Kensler, 1970; Aiken, 1977). However, moult increments themselves may change, reflecting changes in underlying morphogenic processes, which can separately influence measured rates of development. It has in fact been shown that moult increment may be sex-dependent, being greater in adult males than in females (Hughes and Matthiessen, 1962; Wilder, 1963; Aiken, 1977), age-dependent, reducing with increasing age (Hughes and Matthiessen, 1962; Wilder, 1963), as well as being temperature-dependent. The nature of this temperature-dependence in different crustaceans is not clear-cut, however, as reports have been made of a positive

correlation (Hartnoll, 1977), a negative correlation (Carlberg and Van Olst, 1976), and no correlation (Knowlton, 1974; Serling and Ford, 1975).

The moult increments measured in the present study (3-30%) encompass the average value of 15% reported for both juveniles and adult *H. americanus* (Wilder, 1953; Thomas, 1958; Klenster, 1970; Aiken, 1977), but were variable, and give no indication either way as to whether growth differences involve temperature-dependent morphogenic processes, in addition to differences in moult frequency.

The plots of stage-dependent changes in size provide more convincing evidence for an effect of temperature on the morphogenesis, over and above its effect on moulting frequency. These show that animals reared at higher temperatures have a larger size as they pass through a given moult stage, compared with those reared at lower temperatures (Figs. 2.6 and 2.14). The only exception to this occurs in the final months of rearing of the 1998 cohort, when there is some indication of a compensatory recovery by the 15°C group, and its stage-dependent size approaches that of the 19°C group. For the 1999 cohort, however, the size difference inherited from the larval stages persists through the postlarval period.

These data can be compared with those of a survey of size-at-stage results from a wide range of ectotherms reported by Atkinson (1994). While the majority of species studied show a reduced size at a given stage with increasing temperature, others show an increased size-at-stage, as found here for lobsters. An explanation for these different possible relationships is that the two processes involved, development (reflected in moulting) and growth, can have different temperature co-efficients, and depending on which is the greater, size-at-stage can either increase or decrease with temperature.

2.4.3 Net production

As growth is dependent on overall tissue turnover, which is a function of tissue synthesis and degradation, the finding that lobsters have a larger size-at-stage with increased rearing temperatures implies that higher temperatures have a

greater effect on the rate process for synthesis, resulting in net production, i.e. growth. Previous work on intermoult crustaceans has demonstrated that the effect of temperature on protein synthesis rates is tissue- and species-dependent (Whiteley *et al.*, 1997). In general, the protein synthesis rate increases with temperature and is accompanied by an elevation in RNA activity at a constant RNA:protein ratio (Whiteley *et al.*, 1992; Whiteley and El Haj, 1997; Whiteley *et al.*, 1997). This occurs in crustaceans with different thermal tolerances, as demonstrated by the temperate eurythermal isopod *Idotea rescata* (2-24°C) (Whiteley *et al.*, 1996), the stenothermal Antarctic isopod, *Glypotonotus antarcticus* (0-5°C) (Whiteley *et al.*, 2001) and the tropical tiger prawn, *Penaeus esculentus* (Hewitt, 1992). Measures of whole body protein synthesis rates in the post-larval lobsters used in the present study have shown that they increase with rearing temperature. However, corresponding measures of tissue degradation rates have yet to be made and related to growth measurements, so the hypothesis that temperature has a greater effect on rates of synthesis than on rates of degradation remains to be tested.

2.4.4 Variations in growth rate

Within populations of *H. americanus* lobsters, growth rates are known to be variable; some lobsters are slow growers and others grow fast (Aiken and Waddy, 1988; Waddy *et al.*, 1988). The size (as expressed by carapace length) attained at various stages by individually reared larvae is quite variable, and larvae that are large at the time of hatching, usually remain larger than average during development (Ennis, 1995), indicating that their initial rate of growth persists through successive stages (MacKenzie, 1988).

Measures of the relative increases in various body and limb dimensions, made during post-larval development, also demonstrate that the growth of post-larval lobsters is isometric at all temperatures in the 1999 cohort, but not in the 1998 cohort, where a deviation from isometry occurs, mostly in the 11°C cohort, which is seen to have proportionally longer abdomens to their carapace lengths.

Effects of rearing temperature on growth and differentiation have been demonstrated in other aquatic ectotherms, notably fish. Thus in the development

of the axial muscles in Atlantic herring *Clupea harengus*, myofibril synthesis and acetylcholinesterase activity at the neuromuscular junctions occurs later with respect to embryonic stage at 5°C than at 8°C and 12°C (Johnston *et al.*, 2001). Another example consistent with such a differential effect of temperature is the Japanese flounder *Paralichthys olivaceus*, in which the final stage of metamorphosis is completed at significantly shorter body lengths at 19°C rather than 13°C (Seikai *et al.* 1986). These results indicate that there is an uncoupling between the effects of temperature on growth and tissue differentiation.

Evidence also exists that a thermal regime limited to a particular life stage can affect subsequent growth processes. In herring, the temperature experienced by the eggs is a significant source of phenotypic variation in the larvae, and when Johnston *et al.* (2001) incubated eggs at 5°C and 12°C until shortly after hatching, and then reared the larvae at ambient temperature, a number of growth and performance features were different. Thus flexion of the notochord and the development of the fin ray muscles occurred at shorter body lengths in the 12 than 5°C groups, the maximum escape velocity of larvae during fast-starts was 24% higher in larvae hatched from 12°C than 5°C eggs, and cruising swimming behaviour of the 12°C group displayed the more developmentally advanced sub-carangiform style of locomotion.

The results of the present study suggest that the same variations occur in post-larval *H. gammarus* reared under laboratory conditions, with both slow and fast growers in groups kept under the same conditions (Fig. 2.2). The median stage animals were chosen to be representative of the average rate of growth and frequency of moulting, excluding the effects of the slow-growing and fast-growing animals. By comparing the outliers with the median group, it was found that animals that were slow growers in the early stages remained behind, and the initial fast growers stayed ahead in relation to the median stage, throughout the rearing period, (data not shown). The range of moult stages was greater at 19°C than at 15°C for the 1999 cohort, even though by 6 months the median stage was the same.

2.4.4 Conclusion

In conclusion, the results indicate that rearing at elevated temperatures is a viable procedure for accelerating the growth rates of juvenile lobsters, so that they achieve later moult stages and larger sizes in a given time. If size alone was the criterion for successful survival on release, then elevated temperatures of rearing would be recommended for hatchery rearing to supply juvenile lobsters for stock-enhancement purposes. Shorter rearing times would translate into economic savings for an industrial operation. However, measures of size alone provide an incomplete picture of the fitness of artificially-reared juvenile lobsters, and more detailed information on tissue development, motor abilities and behaviour is necessary before the effectiveness of rearing at elevated temperature can be fully assessed. The following chapters address these issues.

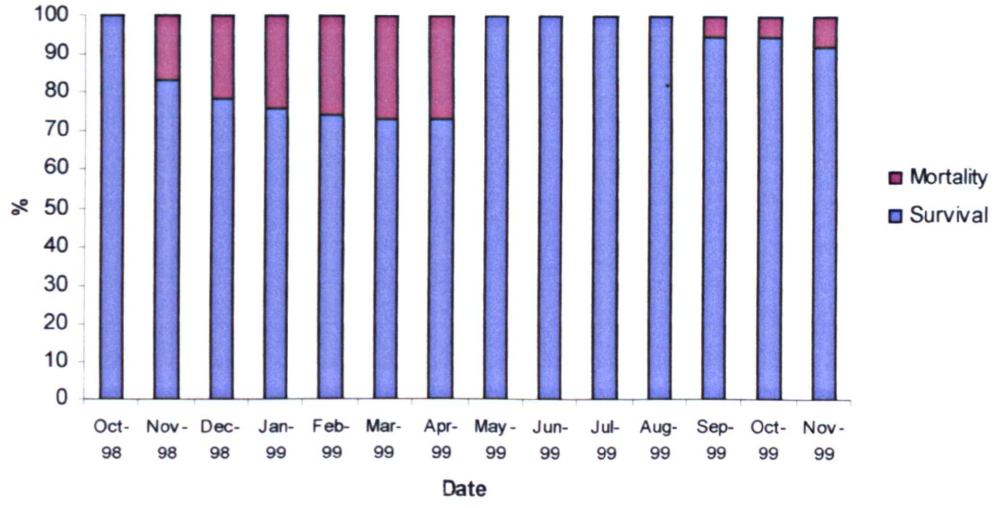


Figure 2.1.a: The rate of mortality of the 1998 cohort at 11°C, expressed as a percentage, for each month of the acclimation period.

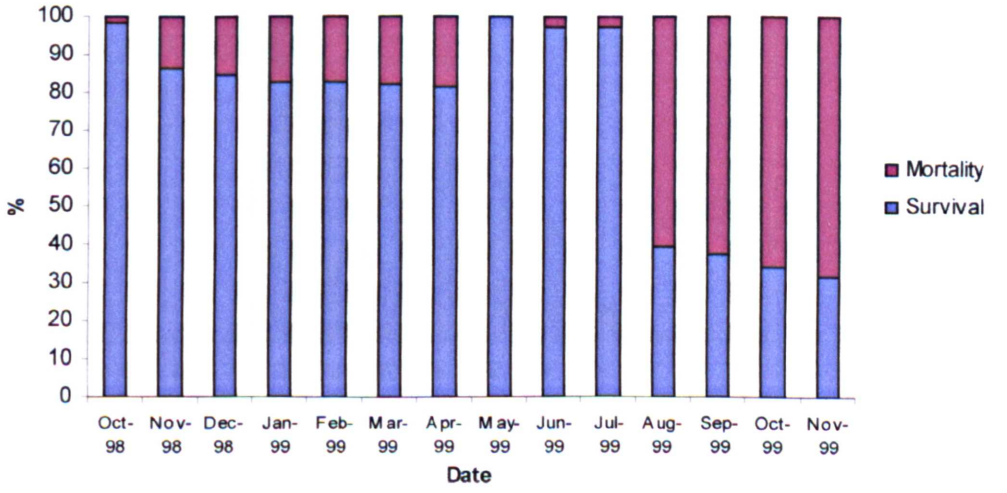


Figure 2.1.b: The rate of mortality of the 1998 cohort at 15°C, expressed as a percentage, for each month of the acclimation period.

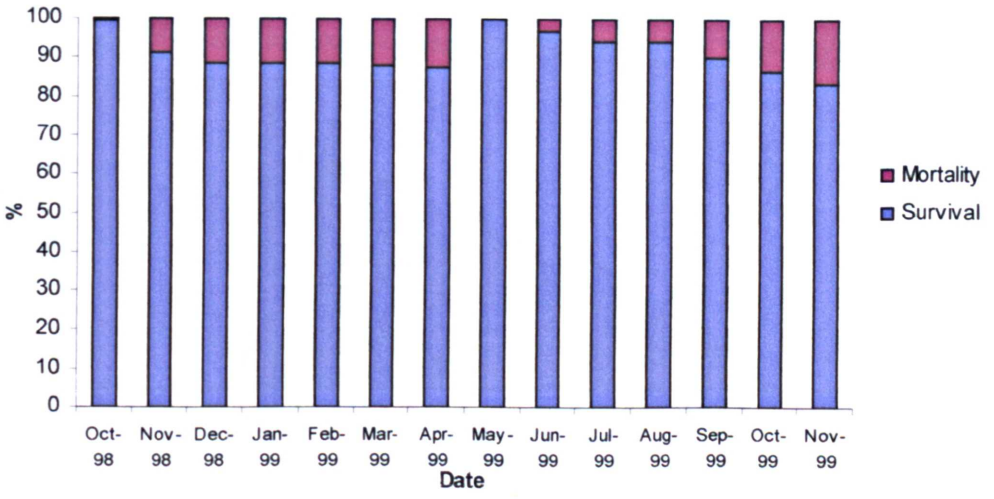


Figure 2.1.c: The rate of mortality of the 1998 cohort at 19°C, expressed as a percentage, for each month of the acclimation period.

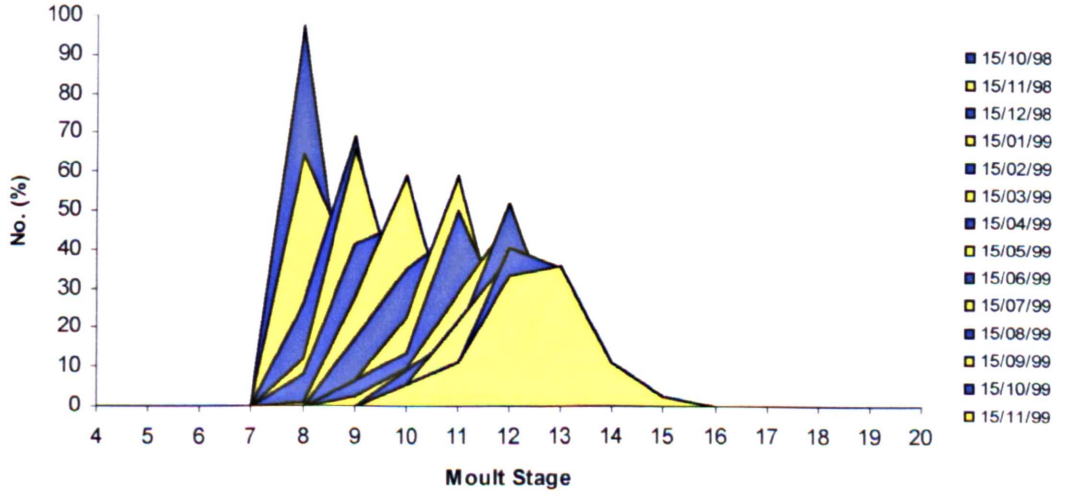


Figure 2.2.a: Percentage of surviving lobsters from the 1998 cohort 11°C group at each stage at monthly intervals (all starting from Stage 8).

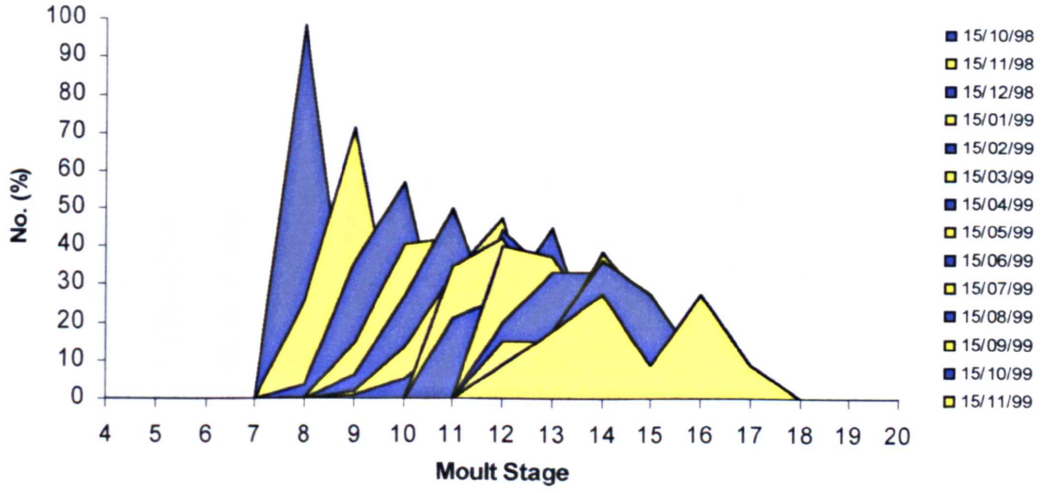


Figure 2.2.b: Percentage of surviving lobsters from the 1998 cohort 15°C group at each stage at monthly intervals (all starting from Stage 8).

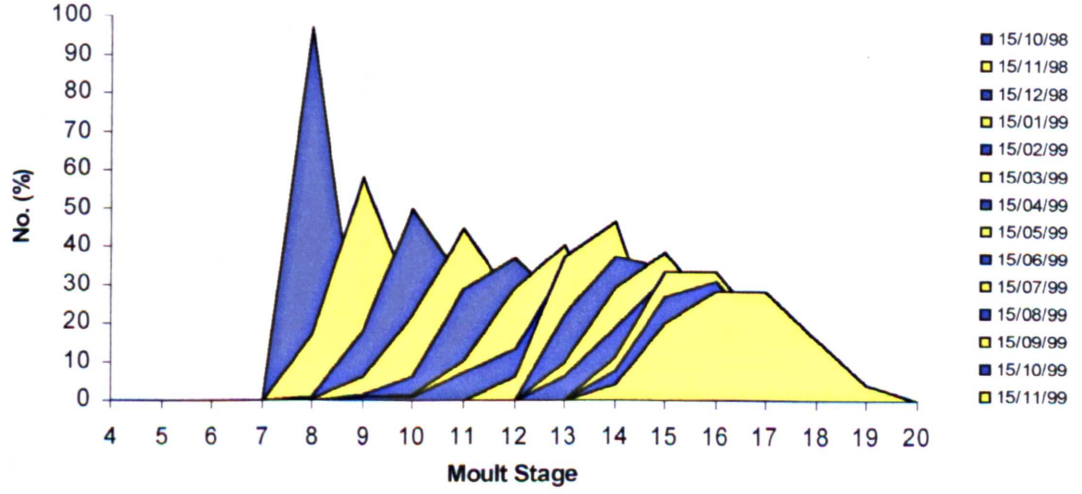


Figure 2.2.c: Percentage of surviving lobsters from the 1998 cohort 19°C group at each stage at monthly intervals (all starting from Stage 8).

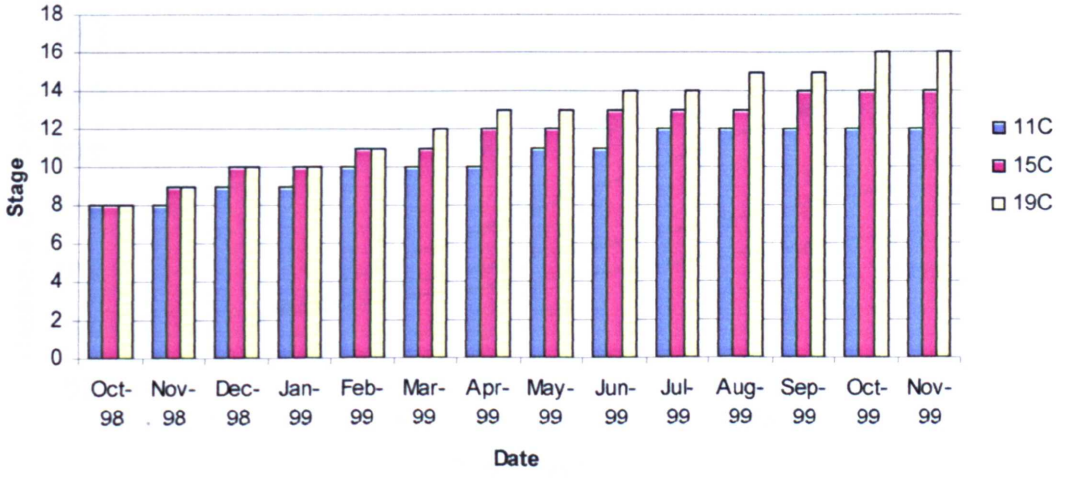


Figure 2.3: The median stages for the three temperature groups of the 1998 cohort, during the rearing period.

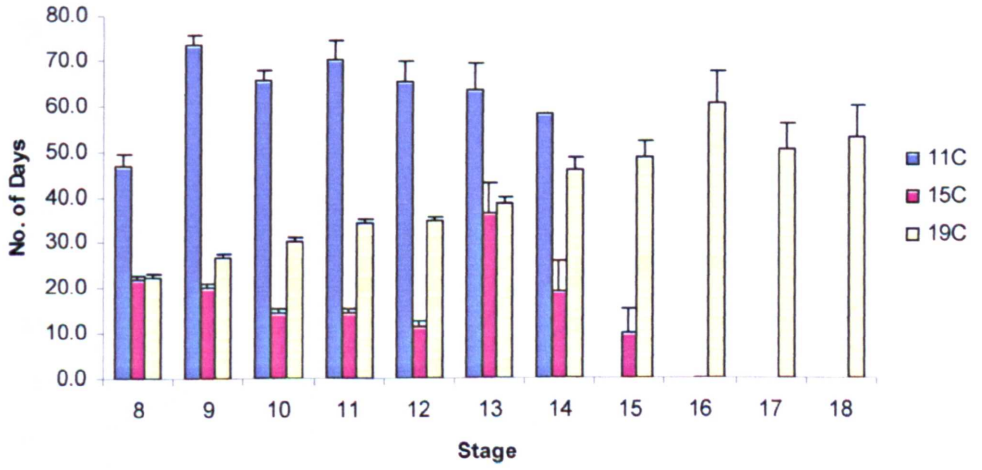


Figure 2.4: Inter-moult period (mean number of days spent in a moult stage) for the median group of the 1998 cohort at three temperatures over the rearing period of 17 months. Stage 8 animals (ANOVA F-statistic, $F_{2, 849} = 53.21$, $p = 0.000$). Tukey pairwise comparisons indicate significant differences between all three temperature groups. Stage 13 animals (ANOVA F-statistic, $F_{2, 181} = 12.20$, $p = 0.000$). Tukey pairwise comparisons indicate significant differences between the 11 and 19°C and the 15 and 19°C groups but not between the 11 and 15°C groups.

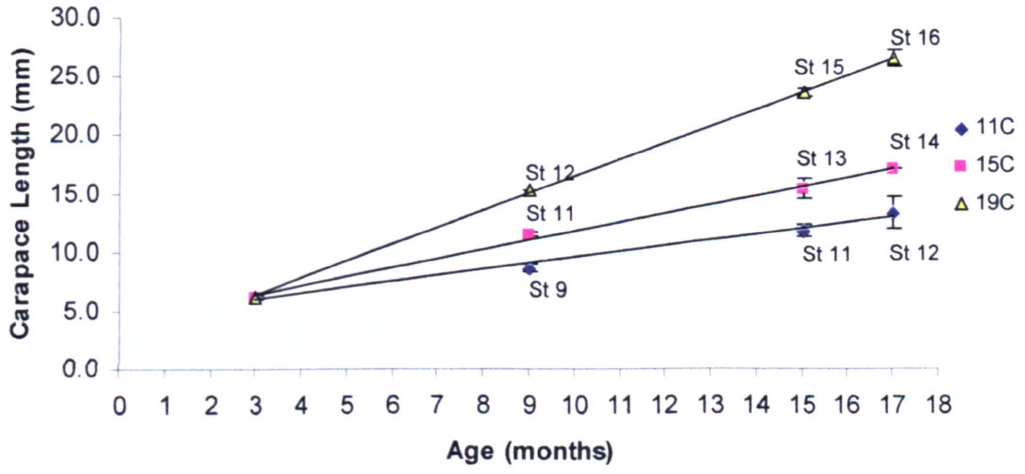


Figure 2.5: The median stages of the three temperature groups of the 1998 cohort at the main sampling points during the rearing period. Univariate ANOVA; comparison of regression slopes; $F_{2, 6} = 107.541$, $p = 0.000$; highlighting, that there is a significant difference between the growth rates at each temperature of rearing.

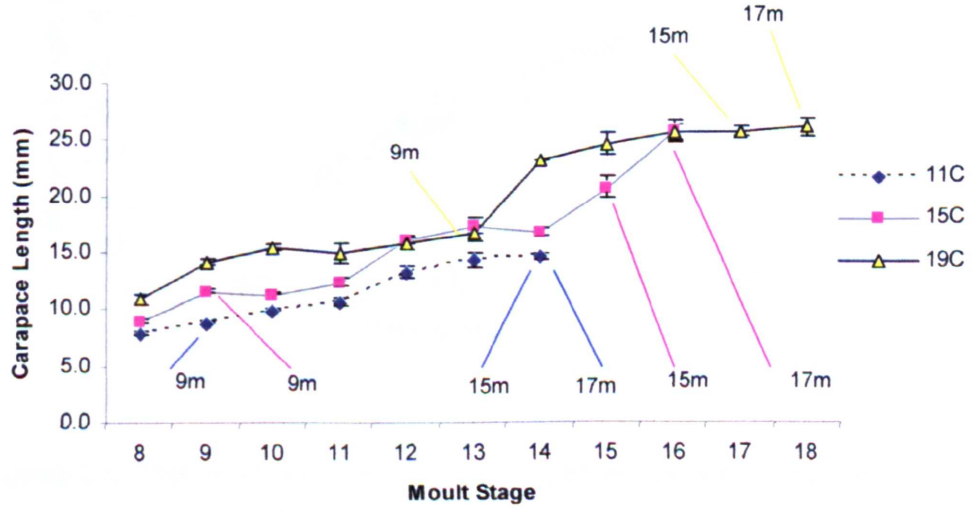


Figure 2.6: The relationship between carapace length (mm) and moult stage for the 1998 cohort of lobsters. The labels 9m (months) etc, mark the age of the lobsters at the particular moult stages. Univariate ANOVA; comparison of regression slope intercepts; $F_{2, 24} = 13.509$, $p = 0.000$.

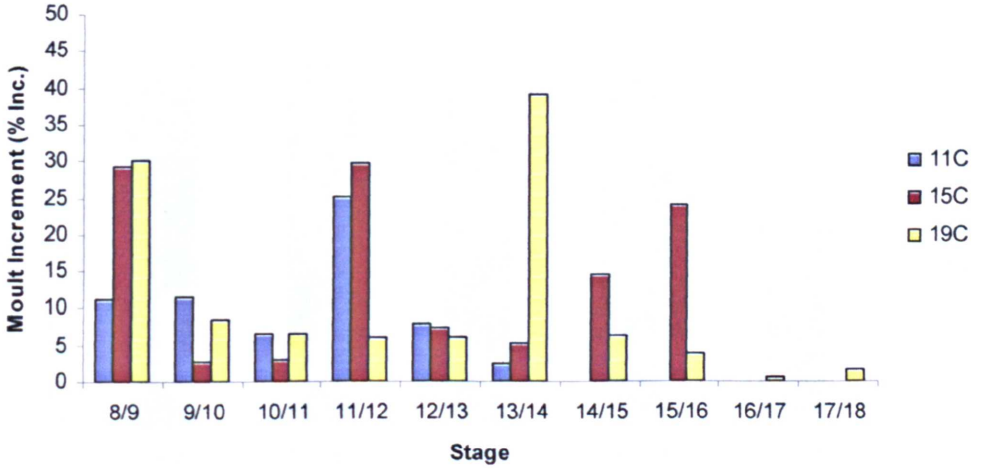


Figure 2.7: Moult increment of the of the 1998 cohort, expressed as a percentage increase from one stage to the next, for all temperatures of rearing.

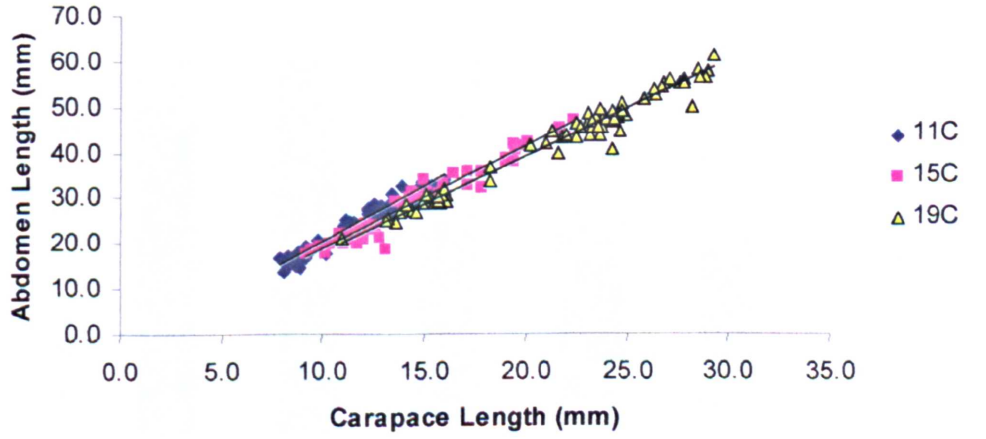


Figure 2.8: The relative increases in abdomen length and carapace length of all the measured lobsters from the 1998 cohort, from all sampling times. Univariate ANOVA; comparison of regression slopes; $F_{2, 204} = 9.810$, $p = 0.000$.

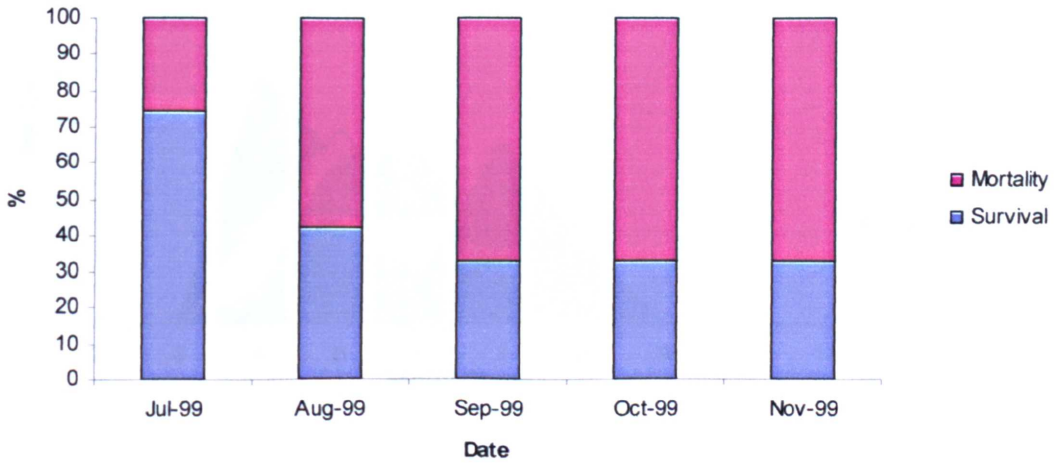


Figure 2.9.a: The rate of mortality of the 1999 cohort at 15ºC, expressed as a percentage, for each month of the acclimation period.

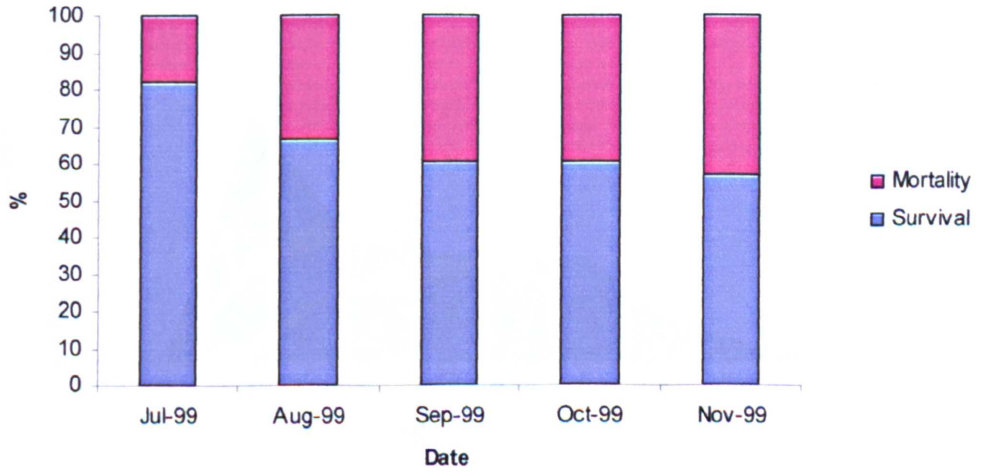


Figure 2.9.b: The rate of mortality of the 1999 cohort at 19ºC, expressed as a percentage, for each month of the acclimation period.

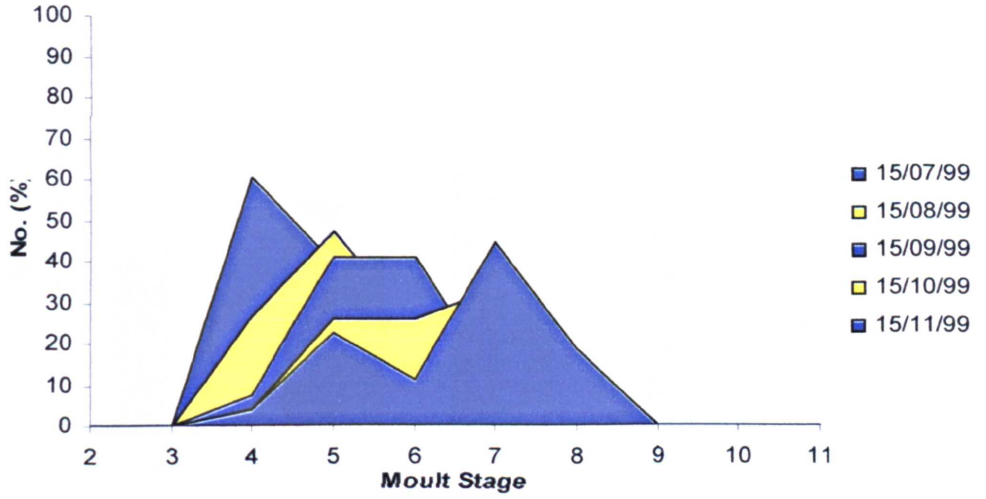


Figure 2.10.a: Percentage of surviving lobsters from the 1999 cohort 15ºC group at each stage at monthly intervals.

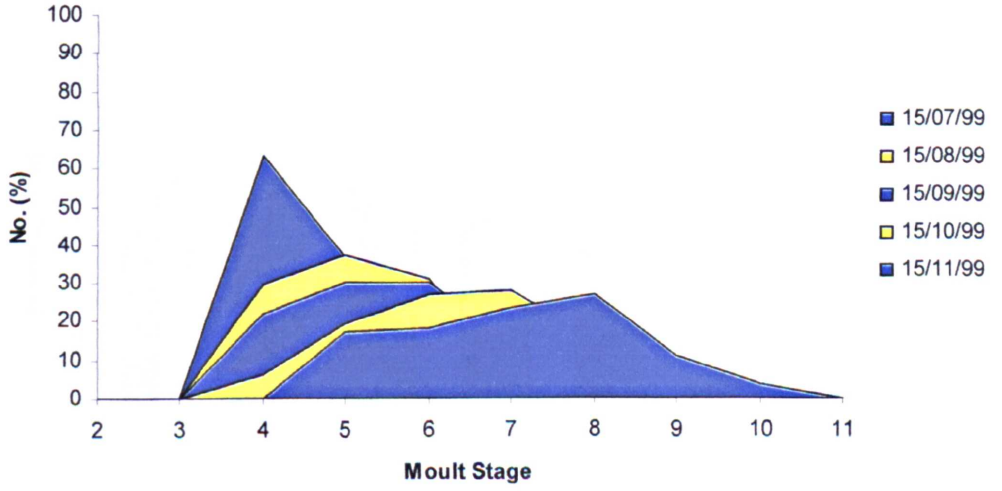


Figure 2.10.b: Percentage of surviving lobsters from the 1999 cohort 19ºC group at each stage at monthly intervals

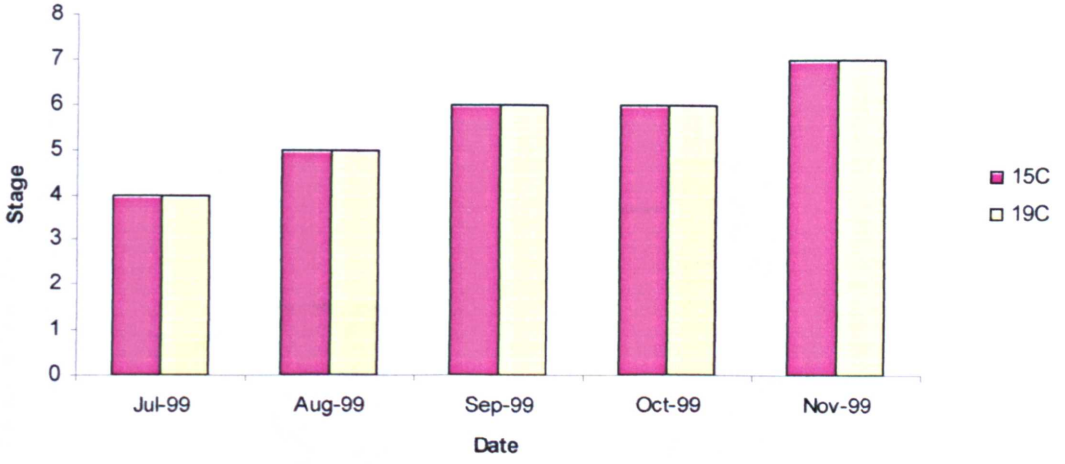


Figure 2.11: The median stages for the two temperature groups of the 1999 cohort, during the rearing period.

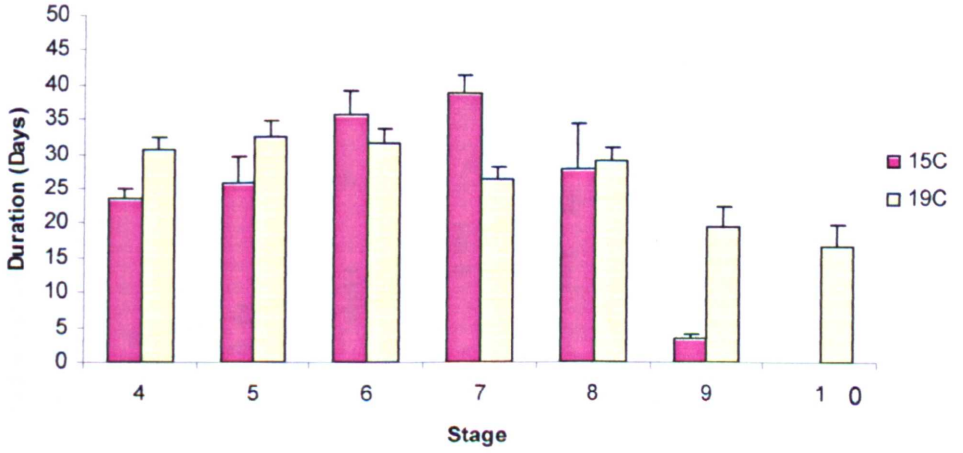


Figure 2.12: Inter-moult period (mean number of days spent in a moult stage) for the median group of the 1999 cohort at two temperatures over the rearing period of 6 months.

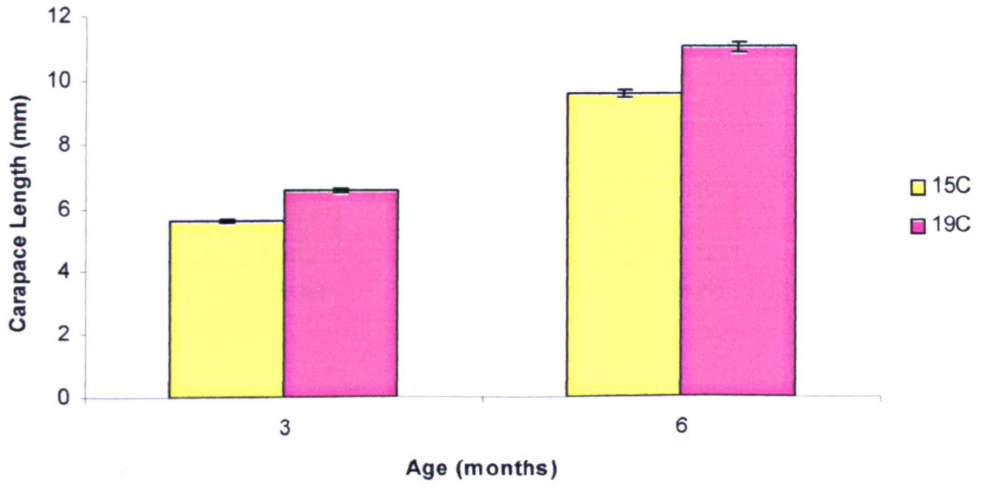


Figure 2.13: The median stages of the 1999 cohort at the two temperatures of rearing.

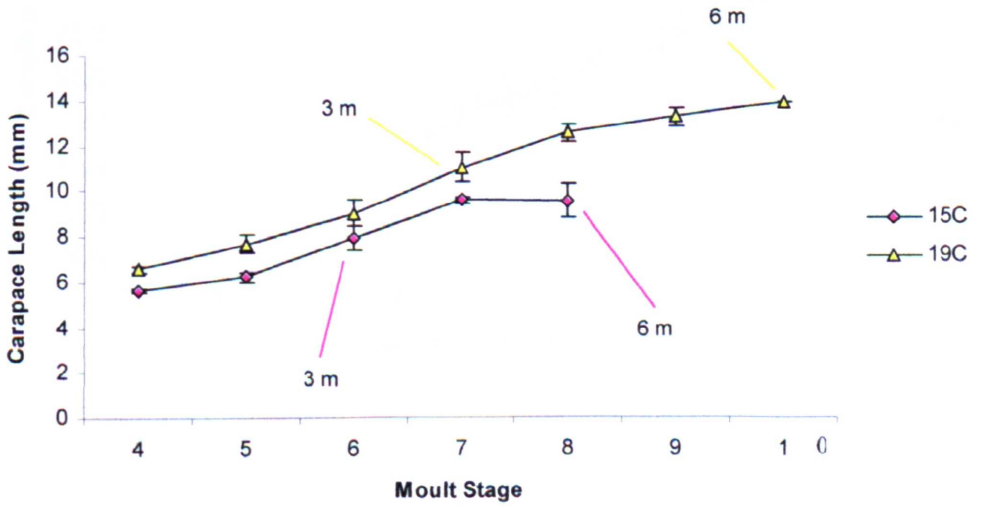


Figure 2.14: The relationship between carapace length (mm) and moult stage for the 1999 cohort. Univariate ANOVA; comparison of regression slope intercepts; $F_{1,9} = 24.726$, $p = 0.001$.

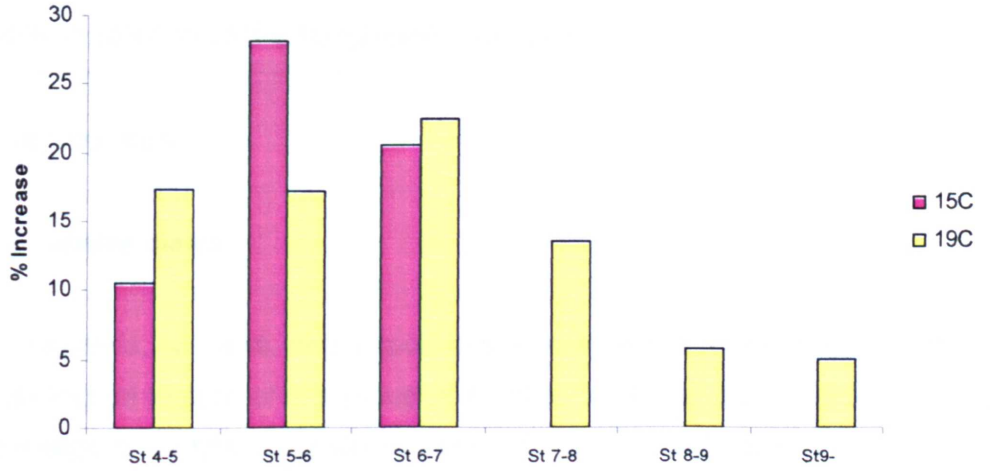


Figure 2.15: Moult increment of the of the 1999 cohort, expressed as a percentage increase from one stage to the next, for the two temperatures of rearing.

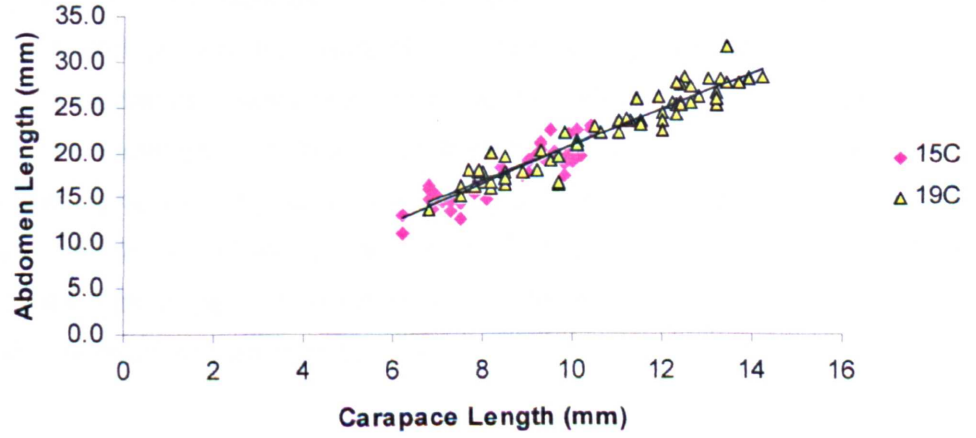


Figure 2.16: The relative increases in abdomen length and carapace length of all the measured lobsters from the 1999 cohort, from all sampling times. Univariate ANOVA: comparison of regression slopes and intercepts; no significant differences: $F_{1, 101} = 0.413$, $p = 0.522$.

Chapter 3 - The dimorphic development of the claws of the European lobster in relation to the temperature of rearing

3.1 Introduction

3.1.1 Lobster claws

The chelipeds, or claws, of decapod crustaceans are probably the most striking morphological feature of this group of animals, and they often represent a high percentage of the total body weight (Stein, 1976), or a high size ratio with respect to overall body dimensions (Vermeijj, 1977; Lee and Seed, 1992). For example, an American lobster, *Homarus americanus*, caught off Cape Cod (17.0 kg male), was found to have a crusher claw of 39.5cm in length and 48.3cm in girth (Wolff, 1978), highlighting the fact that lobsters possess the largest claws of any of the crustaceans (Elner and Campbell, 1981).

Decapod claws are prime examples of multi-functional organs, and are used in all aspects of the crustacean life, including foraging, agonistic interactions, and competition for, and handling of mates (Lee and Seed, 1991). On the assumption that form and function are interrelated, Brown *et al.* (1979) has argued that most, if not all, decapod claws are poly-functional, and that calling the claws crusher and cutter seriously underestimates their potential functional capabilities. While acknowledging that each claw can display variety and plasticity in its actions, the distinct differences in shape, muscle fibre composition and physiology between the claws makes their classification into cutter and crusher not only convenient, but also relevant to their division of labour.

The morphology and biomechanics of lobster claws are of great interest and very relevant in this study, due to their importance for the ecology and evolution of the species (Block and Rebach, 1998). In addition to this, the fact that the claws attain a dimorphic form in adult lobsters, as the cutter and crusher, from an initially symmetrical form in the larval stages provides a most suitable model system in which to study the factors that influence developmental change in postlarval lobsters.

3.1.2 The functions of the claws

In their fully developed form, the claws of the European lobster and other crustaceans are very important for a number of purposes: defence and aggression, shelter acquisition (which is linked to aggressive ability), the capture and handling of food items and sexual selection (Crane, 1975). As tools, the claws of crustaceans have many different functions: gripping, squeezing, cutting and tearing. In some particular situations they are also used for scraping, digging or the resistance of displacement by hydrodynamic forces (Blake, 1985; Hughes, 2000). The loss of one (or more) of the claws reduces the ability of the animal to forage for food and consume their prey effectively, reduces mating success and also increases their vulnerability to intra- and inter-specific attack (Juanes and Smith, 1995).

Autotomy of the claws is a reflexive response to injury or its threat that results in the casting off of an appendage at a predetermined breakage plane (Wood and Wood, 1932; Hopkins, 1993). There are benefits of autonomy, such as predator avoidance and the limitation of wounding (Bliss, 1960; McVean, 1982). However these can be offset by the potential energetic costs that are incurred, such as reduced growth. Damaged claws (broken dactylus or propodus) or worn chelae increase the handling time of prey, and reduce their ability to handle hard-shelled prey (Juanes and Smith, 1995). In the Blue crab (*Callinectes sapidus*), the absence of one cheliped does not affect the feeding rate or size selectivity on armoured bivalves, and the compensatory use of the walking legs in the absence of cheliped (s) has been observed (Smith and Hines, 1991a). However, in the shore crab (*Carcinus maenas*) if smaller claws than average are present (during the regeneration process), then these animals have a lower energy intake rate than intact crabs, due to them choosing smaller prey (Elner, 1980). It has also been observed that in injured crabs there is a weakening of the chelipeds, which reflects the fact that the degree of exercise influences claw size and crushing force (Smith and Palmer, 1994). Limb loss may also reduce the growth increment at the moult (Juanes and Smith, 1995).

With regard to foraging and feeding ability, the mechanical advantage (MA) of the crustacean claw has often been examined (Warner and Jones, 1976; Elner and

Campbell, 1981; Block and Rebach, 1998). Mechanical advantage decreases with distance along the claw/dactyl from the pivot point, and as a result the largest force is produced near to the pivot point (Elner and Campbell, 1981). At developmental stage 4 the mechanical advantage is the same in the two claws of American lobsters, but in the adult it has increased in the crusher claw to an average of 2.8 times that of the cutter claw (Costello and Lang, 1979). Elner and Campbell (1981) also found that large differences in mechanical advantage enable the more heavily built crusher claw to generate larger output forces than the cutter claw, and they related these differences to their different usages in dealing with prey.

3.1.3 Claws and aggressive behaviour

The role of the lobster claws in aggressive encounters is of vital importance, due to the signals that they can make to an opponent, as well as to the injuries that they can inflict. As with other animal weapons, such as the horns of ungulates, the spurs of birds and the claws and mandibles of other arthropods, lobster claws have been shown to be important in agonistic behaviour (Andersson, 1994). The antlers of the red deer stags are important in combat (the 'rut'), and are said to have a visual role in the behaviour of stags. They act both as a status symbol, influencing the outcome of encounters between unfamiliar animals (Beninde, 1938), and as a courtship display symbol involved in attracting hinds (Bubenik, 1968; Lincoln, 1972). Removing the antlers of male deer reduces their fighting ability and dominance status (Lincoln, 1972). This may also be true for the claws of decapod crustaceans.

The importance of weapon size in the agonistic behaviours of many animals has been well documented (e. g. the horned beetle, *Bolithotherus cornutus*, Brown and Bartalon, 1986; red deer, Clutton-Brock *et al.*, 1982; pseudoscorpians, *Dinocherius arizonensis*, Zeh, 1987a, b). Animals which possess large weapons may be in better condition, and claw size may therefore be a good predictor of phenotypic and genotypic quality (Kodric-Brown and Brown, 1984; Andersson, 1986; Sneddon *et al*, 1997). The claws of decapod crustaceans are of vital importance in aggressive behaviour, both functionally and visually. In the shore crab, *C. maenas*, claw size is said to be the most important factor influencing the

outcome of agonistic contests between males, and in aggressive encounters the winners perform significantly more claw displays than the losers (Sneddon *et al.*, 1997). Lee and Seed (1992) reported that larger-clawed *C. maenas* are more successful in obtaining a mate, and individuals with larger claws could prove more attractive to females if weapon size is a good indicator of fitness.

Explanations for these differences in display could be that if claw size is a decisive factor in the winning of aggressive encounters, then increased presentation of the claws, such as in the wide spread of the claws (meral spread), could help to establish dominance over an opponent (Sneddon, 1997; Hughes, 2000). This has been shown to be of great importance in crayfish, where large claws are beneficial in a number of ways, conferring reduced susceptibility to fish predation (Stein, 1976) and dominance over similar-sized crayfish with smaller chelae (Bovbjerg, 1956; Stein, 1976; Sneddon, 1990; Garvey, 1992). Also, males with larger claws are better able to secure and hold females during mating (Stein, 1976; Sneddon, 1990). Large crayfish use their superior chela size, via aggression, to displace small crayfish from shelter, exposing them to predation (Capelli and Munjal, 1982; Garvey and Stein, 1993). In response to this the evicted or beaten animal will employ escape swimming, but when in the water column, and unable to use their claws, they are easily attacked and consumed by fish predators (Stein, 1977; Mather, 1990; Garvey, 1992; Garvey and Stein, 1993). This factor will be relevant to juvenile lobsters, which prefer escape behaviour to defence.

3.1.4 The dimorphic and functional development of lobster claws

Adult lobsters possess dimorphic claws (Elner and Campbell, 1981). The larger, more heavily built crusher claw has blunt molar-like processes on the occluding surface, and has a closer muscle composed entirely of slow muscle fibres. The smaller and slimmer cutter claw has small needle-like teeth, and has a closer muscle composed of predominantly fast fibres (Govind and Pearce, 1988). Elner and Campbell (1981) found that great differences exist between the cutter and the crusher in the mechanical advantage of the closer muscle action about the PD joint. The greater MA values of the heavily-built crusher chela enable it to generate larger output forces than the cutter claw. These different properties

allow the two claws to perform different roles in the behaviour of the animal, such as in defence and prey capture (Lang *et al.*, 1977). Elner and Jamieson (1979) observed this in lobsters predating on sea scallops, *Placopecten magellanicus*, where the cutter claw holds the prey while the crusher claw cracks the shell.

Cutter and crusher claws occur with equal probability on the right and left sides of the lobster. Claw laterality is not genetically fixed, but is determined during a critical juvenile period of early postlarval development. During this period there is a switch from the bilateral symmetry of claws in the immediately postlarval stages (both being initially cutter-like) to the asymmetry that is very apparent in adults (Govind and Lang, 1974, 1978; Costello and Lang, 1979; Ogonowski *et al.*, 1980; Costello and Govind, 1984). This appears to be determined primarily by extrinsic factors related to the use of the claws in manipulation (Lang *et al.*, 1978). It is therefore influenced by exercise, which under natural conditions occurs during the manipulation of food items, and in the laboratory can be induced by providing the animals with oyster spat as food rations (Wickens, 1986).

The lateralisation of the claws is thought to involve mechanoreceptive (primarily proprioceptive) signals, which produce central effects in the thoracic ganglion, and subsequent reflex activity. The side of the ganglion with the greater sensory input becomes the 'crusher side' and induces the claw on that side to become a crusher, while the other becomes the 'cutter side' and induces the claw on that side to become a cutter (Govind and Pearce, 1986). The process can be likened to a teeter-totter effect, and helps to explain why almost all wild lobsters have bilaterally asymmetrical claws, with a 50:50 ratio of the crusher being on the left or right side. Mykles (1997) states that this decision is determined during the first 2-3 days of the fifth stage of postlarval development, and that before this period the claws are essentially identical in their morphology, their muscle fibre composition and their patterns of innervation. Once laterality is established, however, it remains fixed for the rest of the lobster's life.

Several variants on this pattern of development have been identified. In the first, which occurs in only a small number of instances of lobsters caught from the wild, there are two crusher claws. In the second, which occurs especially under captive conditions, an incomplete lateralisation is found to occur whereby the crusher

claw is only partly differentiated, forming a 'false crusher'. The condition with 'double crushers' has shown that one of the 'crusher' claws has the makeup of a cutter claw (Emmel, 1908; Govind and Lang, 1979; Govind, 1982), whereas the occurrence of a 'false crusher' is most probably related to inadequate manipulation during the critical period, leading to only a sub-threshold level of sensory input, which fails to lateralise the ganglion or claws (Govind, 1995). Without stimulation for the claws during the critical period of development, symmetrical claws can persist in laboratory reared-lobsters for up to a year or more into their postlarval lives (Lang *et al.*, 1978). Since it occurs much more commonly under captive conditions, the 'false crusher' state (which can, sometimes be misinterpreted as the 'double cutter' state) represents a major difference between cultivated lobsters and their wild counterparts (Agnalt *et al.*, 1999). It is unlikely to see a lobster with two crusher like claws, and if so it will be probable that one will behave like a cutter claw, with a rapid closing behaviour and ~40% fast fibres (Govind and Lang, 1979).

It was observed by Lang *et al.* (1977) that in the larval and early juvenile stages of the American lobster's life, escape is the favoured response to conspecifics or predators. In adult lobsters the defensive behaviour is preferred to escape, unless the claws have been lost. In larval and early post-larval animals, the claw weight and length are a small fraction of total weight and carapace length, respectively. During growth, claw length increases disproportionately faster than carapace length until the animal reaches about 5 cm. As growth continues the abdomen length becomes proportionally smaller in relation to the total length, and the conduction time of the medial giant nerve axons increases. Both these factors may facilitate a change towards the predominance of defensive behaviour over escape.

3.1.5 Crustacean muscle structure and function

Crustacean muscles, including those of lobsters, are located within and attached to the exoskeleton. The primary function of these muscles is to bring about the movement of the exoskeleton. To enable this, the muscles are organised into functional groups within the cephalothorax, abdomen and various appendages, and they usually operate as antagonistic pairs, e.g. extensor/flexor,

levator/depressor and promoter/remoter (Atwood, 1967; Chapple, 1982). Crustacean muscle has the characteristic features of skeletal muscle. The two main contractile proteins, myosin and actin occur as filaments, and are grouped into bundles which are termed myofibrils. Within these myofibrils, filaments occur in a highly organised linear manner that repeats itself, forming a series of sarcomeres delimited by adjacent Z-lines. Thin actin filaments project from the Z-line towards the middle of the sarcomere and surround the centrally located myosin filaments. This appears in light microscopy as a central A-band (the overlapping actin and myosin filaments) and flanking I-bands (actin filaments alone) (Govind, 1995).

Based on a number of criteria, the muscle fibres of crustaceans can be divided into two categories, fast (phasic) and slow (tonic) (predominantly S1) phenotypes, according to the various myofibrillar protein isoforms that they express (Atwood, 1973; Govind and Atwood, 1982; Govind *et al.*, 1987; Mykles, 1997). These two fibre types can also be distinguished by their contractile properties: in response to a standard burst of electrical stimulation, fast fibres contract rapidly, and maintain their tension for the duration of the stimulus, while slow fibres show a continual increase in tension for the duration of the stimulus (Jahromi and Atwood, 1971; Costello and Govind, 1983). Intermediate fibres display a combination of both types of contractile responses, i.e. an initial rapid rise followed by a slower rise (Govind, 1995).

The muscles of the crustaceans display a wide range in sarcomere length, from 2- 20μm, a diversity unparalleled in the Animal Kingdom. Other things being equal, a muscle fibre with more sarcomeres per unit of length, will contract more rapidly than one with fewer sarcomeres (Jahromi and Atwood, 1969; Josephson, 1975). Due to this, sarcomere length is a very reliable indicator of the contraction speed of a muscle fibre. The fast fibres of claw closer muscles have shorter sarcomere lengths than the slow fibres, typical lengths for each type being 2-6μm for fast and 6-15μm for slow (Lang *et al.*, 1977), although within each category there may be numerous subtypes (Govind *et al.*, 1981).

Each fibre type also has a characteristic assemblage of structural and regulatory proteins (Mykles, 1985a, Neil *et al.*, 1993). From analysis of myosin in the fast

and slow muscle fibres of the lobster, it appears that their light-chain isoforms are similar, if not identical (Mykles, 1997). However, there are two distinct forms of myosin heavy chain molecule present in lobster muscle, which regulate the differences in ATPase activity between the fast and slow fibres. Eleven peptides are unique to fast-muscle myosin and seven to slow-muscle myosin (Li & Mykles, 1990). Comparison of the regulatory proteins reveals that the fast fibres (F) possess two isoforms of paramyosin (P_1 & P_2) while slow fibres (S) have only one paramyosin isoform (P_2) (Mykles, 1985b, 1988). The slow fibres can be further subdivided into two subtypes: slow twitch (S1) and tonic (S2), on the basis of their complements of Troponin T and Troponin I isoforms (Neil *et al.*, 1993).

The fast and slow fibres can also be distinguished according to histochemical tests for enzymes such as myofibrillar ATPase, NADH-diaphorase or succinic dehydrogenase (SDH). Myofibrillar ATPase is correlated with the speed of muscle contraction. Fast muscle fibres stain much more intensely for myofibrillar ATPase than slow muscle fibres (Ogonowski and Lang, 1979). A correlation between high ATPase and low NADH-diaphorase and low oxidative capacity (SDH) for fast fibres and the reverse for slow fibres applies to most of the muscles in the lobster. Succinic dehydrogenase (SDH), a known mitochondrial enzyme, indicates the oxidative capacity of the fibres, which determines fatigue resistance. Slow muscle fibres have high levels of SDH reactivity, while fast fibres have low levels of SDH activity (Fowler and Neil, 1992). The subtypes of slow muscle fibre exhibit further differences in staining intensity for the two enzymes (Kent and Govind, 1981; Mykles, 1985b), and can also be distinguished according to the pH stability of their myofibrillar ATPases (Fowler, 1990; Neil *et al.*, 1993).

3.1.6 Lobster claw muscle morphology and development

The lobster claw closer muscles undergo a developmentally-regulated transformation during the differentiation of the claws into the heteromorphic cutter and crusher types, during the post-larval stages (Govind and Lang, 1978; Ogonowski *et al.*, 1980). The change in fibre phenotype is due to the changes in the expression of certain myofibrillar protein isoforms, rather than to the replacement of fibres of one phenotype by fibres of another, generated *de novo*

by hyperplasia. This process of fibre transformation occurs along a distinct boundary between the regions of fast and slow fibres, and takes place over a relatively short period of the moult cycle, i.e. immediately post moult, when protein synthesis is elevated and haemolymph ecdysteroid concentrations are low. These transformations occur in both directions simultaneously in the same individual. Thus in the presumptive cutter claw the fast-fibre region of the closer muscle expands in area, while in the presumptive crusher, this fast-fibre region contracts in area. It is only at this time that fibres at the boundary between the fast and slow regions have been found to display intermediate myofibrillar ATPase activities, or to co-express the messenger RNAs for fast and slow myosin heavy chains (Mykles, 1997).

Generally, in adult lobsters, the cutter claw has 60-70% short sarcomere fast fibres and 30-40% long sarcomere slow fibres, while the crusher has almost all long-sarcomere slow fibres; a small proportion (2-4%) of intermediate sarcomere fibres is occasionally found in both muscles (Lang *et al.*, 1977). In contrast, in the first and second larval stages, the closer muscles are composed of over 50% intermediate sarcomere fibres, 30-40% short sarcomere fibres and less than 10% long sarcomere fibres. By the late third stage the long sarcomere fibres have increased to a maximum of 40% with a corresponding decrease in number of intermediate fibres. So at the end of the last larval stage, the closer muscles are symmetrical with muscle fibres about equally distributed among short, intermediate and long sarcomere fibre types (Lang *et al.*, 1977). In post-larval animals (stages 4-6), a mixed population of fibres is present in the dorsal groups of the closer muscles of both claws (Costello and Lang, 1979). At Stage 5, one of the muscles differentiates into a cutter muscle with over 60% short sarcomere fibres. The other claw closer muscle slowly loses its short sarcomere muscle fibres and is transformed into a crusher claw, a process that is usually complete by Stages 13-15 (Govind and Lang, 1978). The ventral groups in both claws and the small claw opener muscle never contain fast fibres (Costello and Lang, 1979). Govind and Lang (1978) also reported that the change of the closer muscles from a symmetric to an asymmetric condition is correlated with the loss of the ability for the claws to undergo a 'reversal', i.e. to revert to the phenotypic pattern of the opposite claw.

3.1.7 The relationship between moulting, growth and muscle myogenesis

Unlike vertebrates, such as fish, which grow continuously, the indeterminate growth of a crustacean such as a lobster is coupled to the moult cycle (El Haj, 1996; El Haj and Whiteley, 1997; Whiteley and El Haj, 1997), and as lobsters lack a terminal moult, individuals of up to 15-20kg are not uncommon (Herrick, 1895). It has been estimated lobsters of this size are 50-70 years old, based on the knowledge that moulting frequency declines with age and that body weight at each moult is not doubled (Cooper and Uzmann, 1980). Lobsters show very few signs of conventional ageing, despite their long life spans, e.g. the motor reflexes of claw closing ability are as rapid in old animals as in young (Govind, 1995). Due to this, the development and growth of these muscles are of great interest. (See chapter 2 for the mechanisms of the crustacean moult cycle)

Longitudinal growth in crustacean muscle fibres, which occurs by either the addition of new sarcomeres, or by sarcomere lengthening, has been shown to occur in the abdominal and leg muscles both over the period of ecdysis and during the immediate postmoult period (El Haj *et al.*, 1984; Houlihan and El Haj, 1985; El Haj and Houlihan, 1987). Myofibrillar splitting (hyperplasia), which indicates an increased number of fibres per cross sectional area, may also occur over the period of moulting (El Haj *et al.*, 1984). In contrast, the claw muscle undergoes atrophy during the premoult to enable the muscle to pass through the narrow basi-ischum joints during ecdysis (Mykles and Skinner, 1982). Claw muscle atrophy is associated with elevated levels of protein synthesis during both the pre-moult and post-moult periods (Skinner, 1965; Mykles and Skinner, 1985a, b; El Haj *et al.*, 1986), whereas synthesis rates are only slightly raised during late pre-moult in the muscles of the abdomen and the legs (El Haj *et al.*, 1996).

The complex patterns of cellular and physiological processes, associated with the moult cycle (El Haj *et al.*, 1992; El Haj, 1996; El Haj and Whiteley, 1997), make it extremely difficult to establish how muscle growth and phenotype are modified in response to other variables, such as environmental temperature. For example, elevated protein synthesis rates may simply reflect the early onset of moult and not an effect of temperature. Moreover, regulation of different sets of muscles occurs independently.

Myogenesis (the initial appearance of the myofibrils and their subsequent divergence into fibre types) takes place at different times during the primary development of the lobster. This is also dependent on the time at which the muscle becomes functional during ontogeny. For example, the muscles in the abdomen undergo myogenesis early in their embryonic development and are functional at the time of hatching (Govind *et al.*, 1988), while those in abdominal swimmerets form late in the embryo and the larval forms (Kirk and Govind, 1992), and are functional only in late larval stages.

Structural observations of myogenesis reveal that it is similar in muscle with different developmental timetables. The presumptive myotubules (syncytia of muscle cells) are recognised by the appearance of a tissue mass, which contains patches of myofilaments and many prominent nuclei, with diffuse chromatin. Next, myofilaments are organised in longitudinal arrays and the characteristic latticework of thin filaments surrounds the thick-filament forms. No Z-lines or I-bands are visible at this point, which suggests that the arrangement of thick and thin filaments is the initial event in sarcomere formation. The appearance of neuromuscular terminals with clear synaptic vesicles and the occasional synaptic contacts with muscle sarcoplasm are the first signs of innervation. Myofilament patches become distinguishable as myofibrils, displaying characteristic sarcomeres, defined by Z-lines, A- and I-bands. At this point the sarcomeres are shorter than when fully developed, then they elongate to their final length. Slow sarcomeres elongate by a factor of 3-5 (from 3µm to 9-15µm) while fast sarcomeres elongate by only 2-3 fold (from 1.5µm to 3µm) (Govind, 1995).

During primary development, the muscles acquire their individual characteristics, of sarcomere length, actin-myosin ratio, enzymatic and protein profiles. As there is no terminal moult in lobsters, the muscles continue to enlarge in longitudinal and cross sectional area. Lobster muscle fibres are complex, multi-branched structures, with cytoplasmic links to each other and to adjacent fibres. This makes it difficult to assess whether fibre number increases during the increase in cross-sectional area of a muscle. In the limb accessory flexor muscle, the number of muscle fibres is predicted to stay the same (Govind *et al.*, 1977), but in the claw closer muscle, it is believed to increase (Jahromi and Atwood, 1971). El Haj

et al. (1984) found enlargement of existing fibres taking place over the inter-moult period by the addition of actin and myosin filaments to individual myofibrils, just prior to the moult these enlarged myofibrils split and in turn undergo hypertrophy during the inter-moult.

Growth in length occurs through the addition of sarcomeres in series, which takes place a few hours after the animal has moulted (Govind *et al.*, 1977; El Haj *et al.*, 1984). Immediately after the moult the integument increases in size due to water uptake, and this subsequently causes the muscles to be stretched, triggering the addition of sarcomeres of a fairly constant size. The presence of slightly shorter sarcomeres at the exoskeletal ends of the fibres suggests that addition takes place here. As fibres continue to grow in size with every moult, the serial addition of sarcomeres persists throughout life (Govind, 1995).

3.1.8 Aims of the study

As part of an overall aim to determine the fitness of laboratory-reared European lobsters for release into the wild, a study of the growth, dimorphic switching, muscle development and contractile performance of the claws has been carried out at different temperatures of rearing (11°C, 15°C and 19°C) in order to determine the direct and indirect effects of temperature on these processes.

Their development has been tracked using a number of methods: morphometry of the claws to determine their external size and shape, histochemical staining of their muscles to determine their fibre phenotypes, and non-invasive measurements of the isometric forces produced during claw closing. These different approaches are described in the three following chapters (Chapters 4, 5 and 6).

Chapter 4 – The growth and external development of the claws of juvenile *Homarus gammarus*

4.1 Introduction

The claws of lobsters are prime examples of multi-functional organs, used for food acquisition, mate selection, habitat choice and defence. Claw size is important for the survival of the lobsters not only because it reflects the bulk of the muscle within, and hence the potential of the claw to develop force, but also because the claws act as signalling devices, and convey important information to conspecifics and predators about the animal's size and potential power. Large weapons convey good overall condition, are indicative of phenotypic and genotypic quality (Kodric-Brown and Brown, 1984; Andersson, 1986), and hence confer more successful mating chances (Lee and Seed, 1992).

Post-larval (Stage 4) lobsters possess bilaterally-symmetrical claws, but following a period of determination at around Stage 5, one of the two claws develops into the bulky and powerful crusher claw, while the other becomes the slender cutter claw. Therefore both growth and differentiation are occurring simultaneously in the claws during the postlarval stages, providing an opportunity to study the effects of environmental conditions on these two distinct developmental processes. The temperature of rearing represents such a stimulus, since it produces a dramatic effect on the rate of growth of the animal (Chapter 2). The aim of this part of the study was therefore to determine the equivalent rates of growth of the claws, together with measures that reflect the rate of their dimorphic change to the crusher and crusher forms, to provide an insight into the relative effects of rearing temperature on growth and differentiation of the claws.

4.2 Materials and Methods

4.2.1 External Claw Measurements

The body dimensions of the lobsters (see Chapter 2 methods) were measured at intervals of no more than 4 weeks, and three main sampling periods (for the 1998

cohort) took place in March 1999, when the 1998 cohort was 9 months old, August/September 1999 (15 months old) and November 1999 (17 months old). Two main sampling periods occurred for the 1999 cohort, in September 1999 (3 months old) and in November 1999 (6 months old). Together with the moult records these measurements provide a complete growth history of the lobsters.

In addition to wet weight, the following body measurements were made using Vernier callipers (to the nearest mm): body length (telson to tail fan), carapace length (back of eye socket to end of carapace), and for each claw: claw length (CL) (tip of the propus to join at manus), dactyl length (DL), claw thickness (CT) (widest part), and claw height (CH) (highest part – mid point) (Fig. 4.1).

In addition, the cross sectional areas of the claws and the claw muscles were calculated from mid points of frozen sections of the specimens used for histochemical analysis (1998: 11°C n=19; 15°C n=14; 19°C n=17; 1999: 15°C n=11; 19°C n=17). These measurements were always made in the inter-moult period (at least seven days after the previous moult) to ensure that the lobsters had reached their full size after moulting.

4.2.2 Data analysis

The morphometric measurements of the lobster claws have been plotted against Carapace length to show the differences in size that result from rearing them at the three different temperatures. Any significant differences in the size and shape of the claws between temperature treatments and claw types have been determined by Univariate ANOVA of regression slopes.

4.3 Results

4.3.1 Nomenclature

Since animals were reared individually, and measured regularly for increases in claw dimensions (Fig. 4.1) (and for increases in claw closing forces - see Chapter 6), it was possible to designate them as cutter or crusher claws from their shape and their mechanical performance after dimorphic differentiation had become established. It has therefore been possible for their ultimate identities to be

applied retrospectively to times before differentiation had occurred. It is on this basis that, in the following account, the terms 'cutter' and 'crusher' can be applied to the claws of animals at all postlarval stages, although at the time of measuring their identities were not known (i.e. they were presumptive).

4.3.2 Claw Growth in the 1998 Cohort

Measurements of the four main dimensions of the developing claws of the post-larval lobsters: claw length, dactyl length, claw height and claw width have allowed the growth of the dimorphic cutter and crusher claws during development to be defined.

As for the whole body, the higher temperatures of rearing increased the rate of growth of both the cutter and crusher claws. The highest temperature (19°C) showed increased growth at all sampling points (Figs. 4.2-4.5) (Stage ranges represented are as follows: 11°C: 9-14, 15°C: 9-16 and 19°C: 9-19). Comparing the crusher claw lengths for all temperatures, it can be seen that there is a difference between the regression slopes (Univariate ANOVA; comparison of regression slopes; $F_{2, 199} = 6.366$, $p = 0.002$) (Fig. 4.2.a), indicating the growth of the claws is allometric to the body growth, between the temperature regimes, this can also be said to be the case for the respective cutter claw lengths (Univariate ANOVA; comparison of regression slopes; $F_{2, 223} = 9.736$, $p = 0.000$) (Fig. 4.2.b). The same trend of different growth at the different rearing temperatures is seen with the dactyl lengths for both the crusher (Univariate ANOVA; comparison of regression slope; $F_{2, 199} = 5.039$, $p = 0.007$) (Fig. 4.3.b) and cutter claws (Univariate ANOVA; comparison of regression slope; $F_{2, 223} = 8.294$, $p = 0.000$) (Fig. 4.3.b). There are also significant differences indicated between claw heights (Univariate ANOVA; comparison of regression slope; $F_{2, 199} = 7.191$, $p = 0.001$) (Fig. 4.4.a) and widths (Univariate ANOVA; comparison of regression slope; $F_{2, 199} = 13.638$, $p = 0.000$) (Fig. 4.5.a) in crusher claw and in the claw heights (Univariate ANOVA; comparison of regression slope; $F_{2, 223} = 19.420$, $p = 0.000$) (Fig. 4.4.b) and widths (Univariate ANOVA; comparison of regression slope; $F_{2, 223} = 12.817$, $p = 0.000$) (Fig. 4.5.b) in the cutter claws. The differences in both the crusher and cutter claw widths are interesting, because if the regression lines are compared it can be seen that the lower temperature lobsters have a greater

width at a given body size than the 15 and 19°C animals, which highlights that the lobsters at 19°C may have the largest claws, but are not as great as they might be if the lower temperatures had reached the same sizes. These results indicate that there are different rates of growth for both claws at the different temperature regimes, possibly indicating that the lower temperature lobsters have a better quality of claw shape/dimorphism for a given size, also indicating allometric growth of the claws to the body at all regimes.

Comparing the cutter and crusher claws, they initially have the same dimensions, but as growth takes place the cutter claw becomes greater in both total length (Figs. 4.6.a-c) and dactyl length (Figs. 4.7.a-c) relative to its other dimensions. This is more apparent at 15°C (Claw lengths: Univariate ANOVA; comparison of regression slopes; $F_{1, 105} = 6.452$, $p = 0.013$) and 19°C (Claw lengths and dactyl lengths: Univariate ANOVA; comparison of regression slopes; effect of claw; $F_{1, 168} = 4.505$, $p = 0.035$; $F_{1, 168} = 21.001$, $p = 0.000$) (Figs. 4.6.b, 4.6.c and 4.7.c), but is also present to a certain extent in the 11°C cohort (Fig. 4.7.a) (Dactyl lengths: Univariate ANOVA; comparison of regression slopes; $F_{1, 150} = 12.921$, $p = 0.000$). Thus the total length of the claws and their dactyl lengths are useful predictors of the laterality of the dimorphic change.

At all rearing temperatures, the crusher claws also had greater claw heights than the respective cutter claws (Figs. 4.8.a-c), particularly at 15°C (Univariate ANOVA; comparison of regression slopes; $F_{1, 105} = 48.301$, $p = 0.000$) and 19°C (Univariate ANOVA; comparison of regression slopes; $F_{1, 168} = 42.466$, $p = 0.000$), but also at 11°C (Univariate ANOVA; comparison of regression slopes; $F_{1, 150} = 21.726$, $p = 0.000$). This is a very valid indication that externally the dimorphic difference between the claws is apparent in these juvenile lobsters. However, the dimension of claw width varied little between cutter and crusher claws over the whole growth period at all temperatures (Figs. 4.9.a-c), except at 15°C (Univariate ANOVA; comparison of regression slopes; $F_{1, 105} = 15.949$, $p = 0.000$), and to a certain extent at 11°C (Univariate ANOVA; comparison of regression slope intercepts; $F_{1, 151} = 23.960$, $p = 0.000$).

In Figure 4.10 (a-d), the claw growth data for all temperatures are expressed relative to carapace length. This emphasises that the two claws grow at similar

The claw height of crusher claws in adult lobsters, above approximately 80-93mm carapace length, is known to be invariably greater than the height of the cutter claw (Aiken and Waddy, 1980; Elner and Campbell, 1981). It is also above this size that the differences between the sexes become apparent. The males have more enlarged crusher claws, and in general body size the females are slightly smaller, with wider abdomens for carrying the eggs (Aiken and Waddy, 1989). The lobsters in the present rearing programme never attained the above stated size (they were <30mm carapace length), so the differences between the developing cutter and crusher claws due to size and sexual maturation were not to be expected.

There were no great differences between the crusher and cutter claw lengths of the 1998 lobsters, but the dactyl length of the claws (Univariate ANOVA; comparison of regression slopes; $F_{1, 430} = 10.741$, $p = 0.001$) (Fig. 4.10.b) gave a reliable indication of ultimate claw type, the cutter claw having a longer dactyl than the respective crusher at all ages/sizes, after the point of dimorphic divergence which occurs at Stage 5.

In *H. gammarus* the main predictor of which claw will become the future crusher is claw height (Elner and Campbell, 1981). The data from the present study on juveniles are consistent with this in showing that the crusher claw heights are greater at all points during the rearing programme (11°C: Univariate ANOVA; comparison of regression slopes; $F_{1, 150} = 21.726$, $p = 0.000$; 15°C: Univariate ANOVA; comparison of regression slopes; $F_{1, 105} = 48.301$, $p = 0.000$; 19°C: Univariate ANOVA; comparison of regression slope intercepts; $F_{1, 168} = 42.466$, $p = 0.000$) (Figs. 4.8.a-c). This finding is significant as the animals studied were all below the carapace length at which this difference was thought to become apparent (Aiken and Waddy, 1980; Elner and Campbell, 1981).

The dimension of claw width is the only one of the four measures that does not show a great divergence between claw types (Univariate ANOVA; comparison of regression slope intercepts; $F_{1, 431} = 11.347$, $p = 0.000$), indicating that there is a difference in the intercepts of the two claw types (Fig. 4.10.d) (1998 cohort). This is somewhat surprising, as one would expect the bulkier crusher claws to have a far greater claw width, as it is reported that the closer apodeme (the point of

muscle attachment within the claw) is wider in the crusher claws than the cutters, leading to greater claw areas (Elner and Campbell, 1981). This may reflect the fact that the claws of the lobsters used in the present study were below the size threshold at which these differences occur.

The values for claw cross-sectional area can be related to those for the area of the claw apodeme of the crusher claw, which in mature lobsters is known to be larger and thicker than the respective cutter claw apodemes, and to act as an indicator of claw size or type (Elner and Campbell, 1981). Apodeme area reflects closer muscle size and ultimately chela strength. The results for claw area and claw closer muscle area (Figs. 4.12.a-c) show how the crusher claw values diverge from those of the cutter claw. It is of interest that this phenomenon has occurred within the time scale of 18 months of age, as a lot of studies have had difficulty in producing the dimorphic claws (especially that of the crusher) within a laboratory rearing programme (Meeren and Uksnøy, 2000).

For the 1998 cohort, the majority of animals at all temperatures possessed distinguishable crusher and cutter claws by the end of the rearing programme (17 months). It can therefore be concluded that the level of mechanosensory stimulation received by the animals in their growth chambers was adequate to initiate and sustain this dimorphic change in external morphology (Wickens, 1986), even if the proportions of these claws to the body sizes were different at the three temperatures indicating allometric growth, not isometric growth as one might expect.

The 1999 cohort allows the early part of claw development to be analysed, and since these lobsters were reared from the egg, their development conditions were uniform. The most significant finding in this cohort was that a divergence in the dactyl lengths was apparent in the claws of the 19°C group, even at 3 months of age, with the cutter claw dactyl lengths beginning to become greater than those of the respective crusher claw (Univariate ANOVA; comparison of regression slopes; $F_{1, 215} = 7.926$, $p = 0.005$) (Fig. 4.18.b). Since this difference was not apparent at 3 months in the 15°C group, this defines a size and or moult stage at which the dimorphic divergence can first be detected (approximately 6-8 mm carapace length and at stage 5-6).

By the end of the rearing period (6 months) both claw height (15°C: Univariate ANOVA; comparison of regression slopes; $F_{1, 130} = 15.768$, $p = 0.000$; 19°C: Univariate ANOVA; comparison of regression slopes; $F_{1, 214} = 44.913$, $p = 0.000$) (Figs. 4.19.a+b) and in this case also claw width (15°C: Univariate ANOVA; comparison of regression slopes; $F_{1, 130} = 4.969$, $p = 0.028$; 19°C: Univariate ANOVA; comparison of regression slopes; $F_{1, 214} = 19.252$, $p = 0.000$) (Figs. 4.20.a+b), were greater in the crusher claws at both temperatures, which is a significant finding since growth at 15°C was slower than that at 19°C. This confirms the reliability of these claw dimensions as predictors of the ultimate crusher claw, and as early indicators of the laterality of the dimorphic switch (stage 5). The greater claw area in the crusher compared to the cutter claw area (Figs. 4.23.a-c), is expected from the above.

The fact that claw width differs between crushers and cutters to such an extent in the 1999 cohort, but is not as obvious in the 1998 cohort, may be a reflection of the more uniform rearing conditions of the former group, or may be because these younger juveniles, with smaller claws, received more effective stimulation from their surroundings (the mesh bottom of their holding compartments), effectively increasing dimorphic development.

As is the case with *Homarus americanus* (Costello and Lang, 1979), *Homarus gammarus* begins life with symmetrical claws, which then change dramatically in morphology, performance and muscle composition as the lobster grows (Block and Rebach, 1998). Here the aim was to ascertain how the development of the two claws of juvenile lobsters progressed to dimorphism at different rearing temperatures, in relation to their rates of growth. What has been observed is that dimorphic divergence/switching of the claws occurs at an early age/stage (1999 cohort) and the higher temperatures of rearing increase the rate of this process as seen with the 19°C 1999 group of lobsters. This however is not necessarily the case with the 1998 lobsters as the growth rates of certain dimensions (height and width) indicate that the lower temperatures (11 and 15°C) have a greater trend towards dimorphic transformation at a given body size, especially given that claw height is seen as one of the main predictors of dimorphic divergence. Hence by manipulating rearing temperature external dimorphism can be achieved

in smaller lobsters. It is interesting that this phenomenon occurs in the 1999 cohort, but not in the 1998 cohort, indicating that high temperatures do not always accelerate the growth rate in predicted fashion. Hence from these results one could postulate that rearing from the egg at higher temperatures, could lead to increased claw sizes at a given body size, which would be very useful for animals if they were released into the wild as they would posses what could be classed as super claws for their size conferring a selection advantage, but this is not the case with the acclimated (from Stage 8) 1998 cohort.

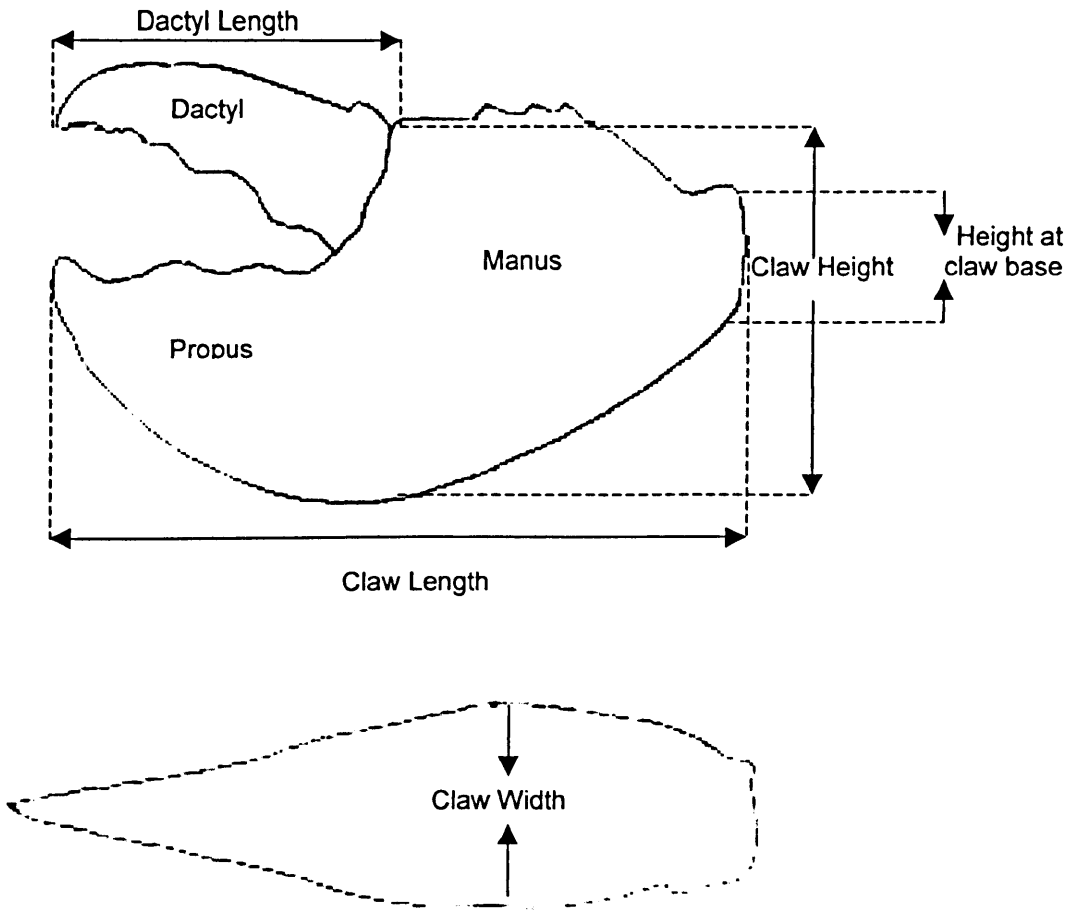


Figure 4.1: Diagram of where the measurements were made on the claws of the juvenile lobsters, using Vernier callipers to 0.1mm.

1998 Cohort

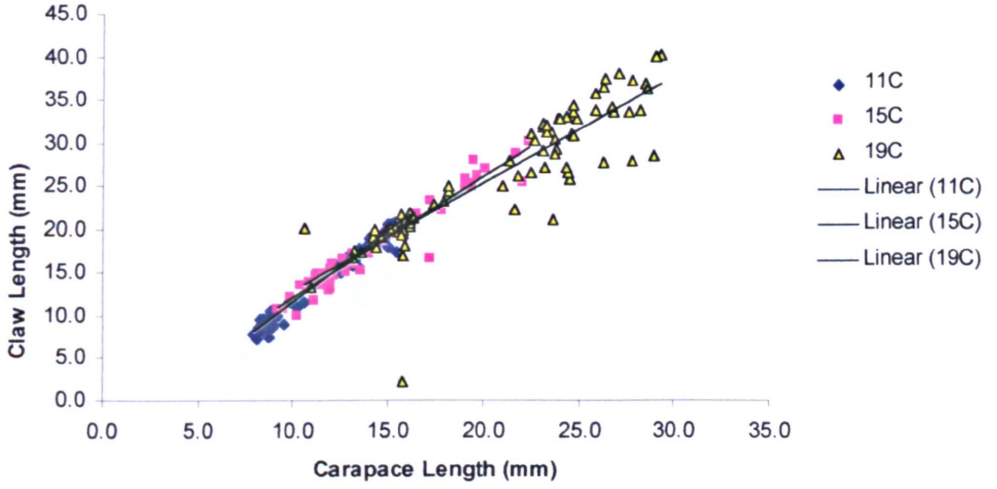


Figure 4.2.a: The Crusher claw lengths for all 1998 lobsters at all rearing temperatures. Univariate ANOVA; comparison of regression slopes; $F_{2,\ 199}$ = 6.366, p = 0.002.

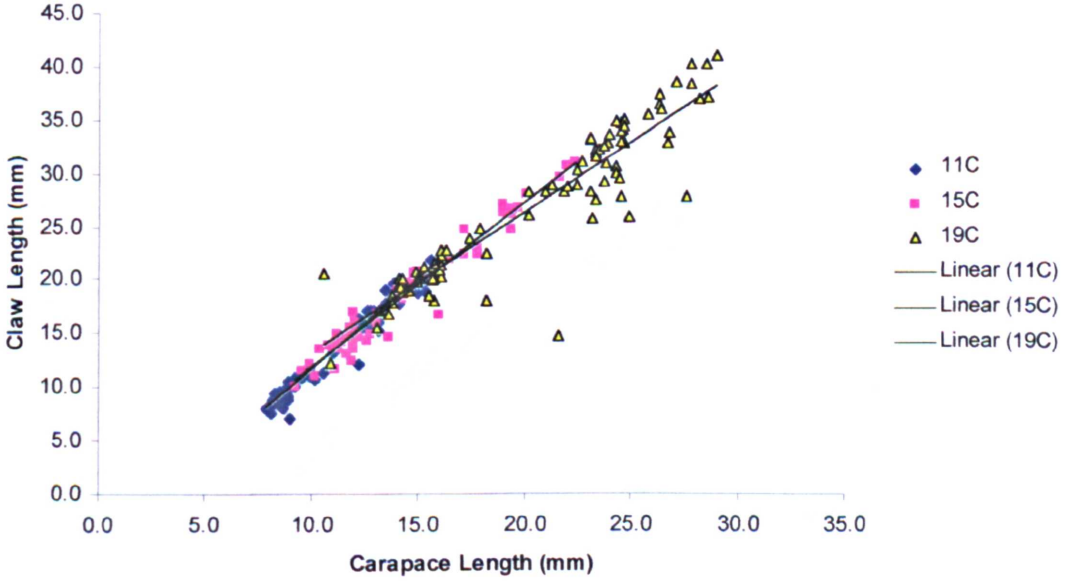


Figure 4.2.b: The Cutter claw lengths for all 1998 lobsters at all rearing temperatures. Univariate ANOVA; comparison of regression slopes; $F_{2,\ 223}$ = 9.736, p = 0.000.

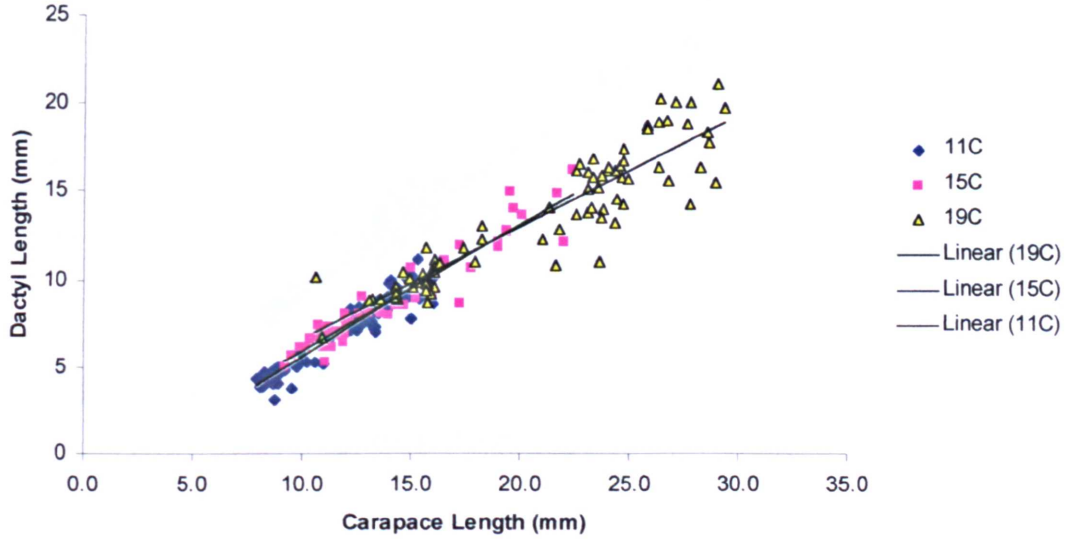


Figure 4.3.a: The crusher claw dactyl lengths for all 1998 lobsters at all temperatures of rearing. Univariate ANOVA; comparison of regression slopes; $F_{2, 199} = 5.039$, $p = 0.007$.

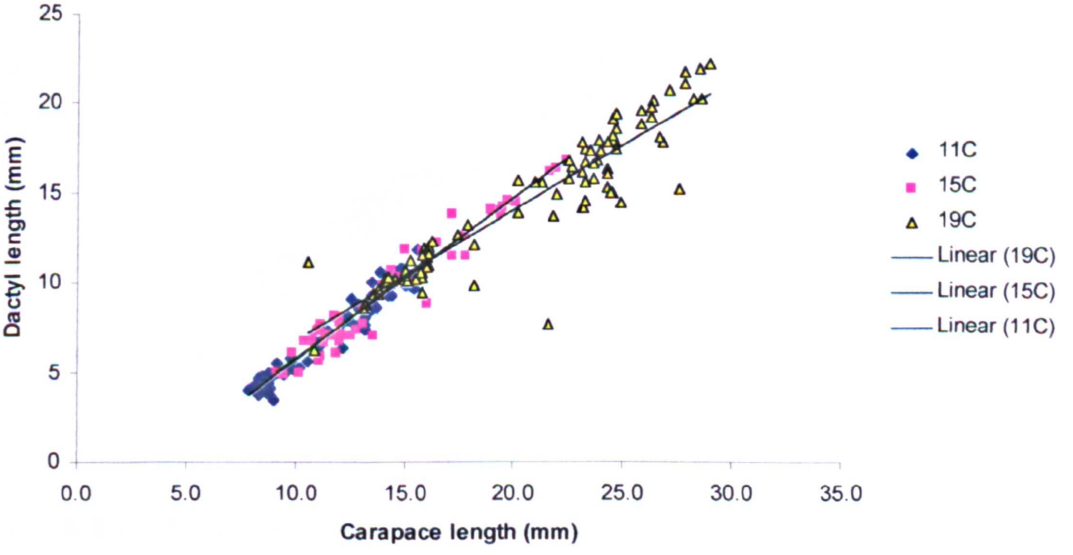


Figure 4.3.b: The cutter claw dactyl lengths for all 1998 lobsters at all temperatures of rearing. Univariate ANOVA; comparison of regression slopes; $F_{2, 223} = 8.294$, $p = 0.000$.

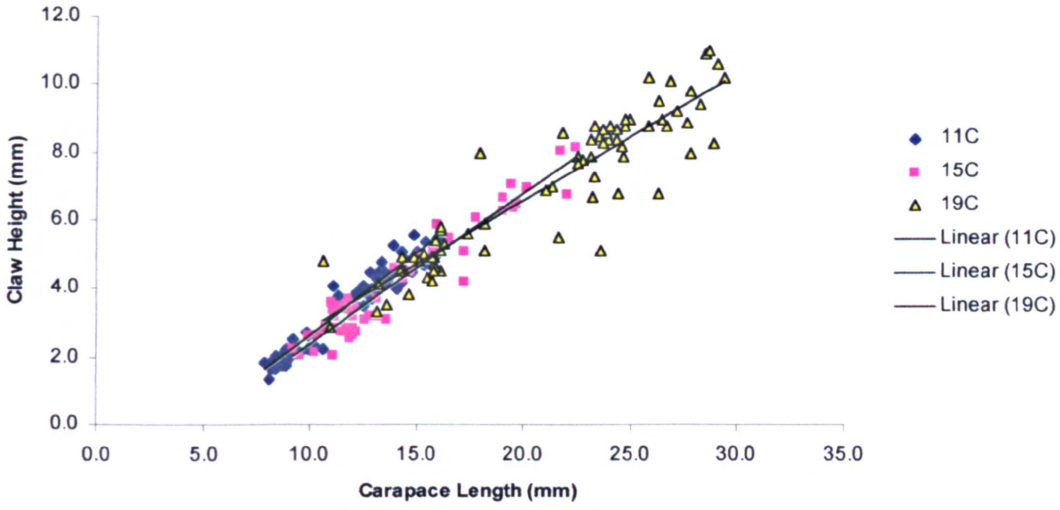


Figure 4.4.a: The crusher claw heights for all 1998 lobsters at all temperatures of rearing. Univariate ANOVA; comparison of regression slopes; $F_{2, 199} = 7.191$, $p = 0.001$.

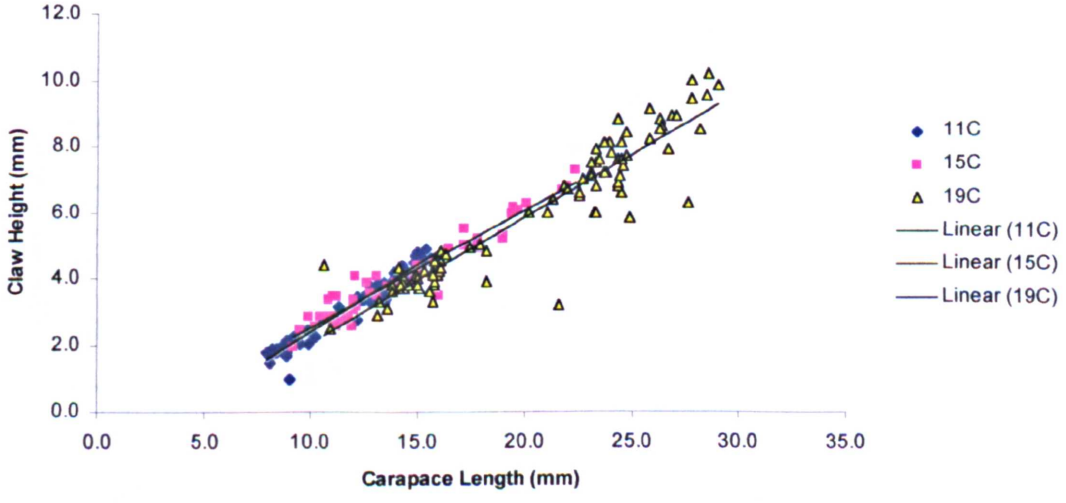


Figure 4.4.b: The cutter claw heights for all 1998 lobsters at all temperatures of rearing. Univariate ANOVA; comparison of regression slopes; $F_{2, 223} = 19.420$, $p = 0.000$.

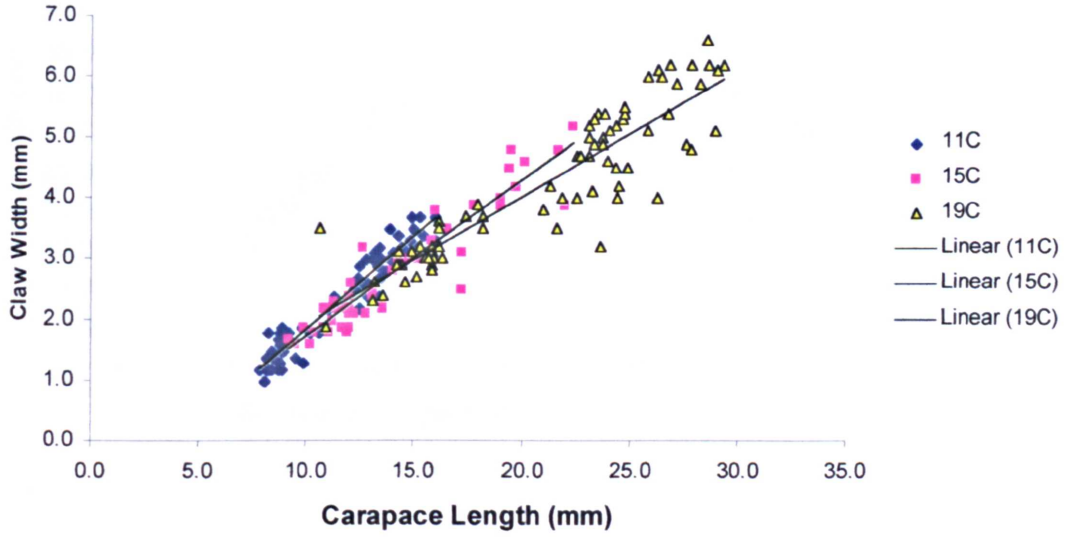


Figure 4.5.a: The crusher claw widths for all 1998 lobsters at all temperatures of rearing. Univariate ANOVA; comparison of regression slopes; $F_{2, 199} = 13.638$, $p = 0.000$.

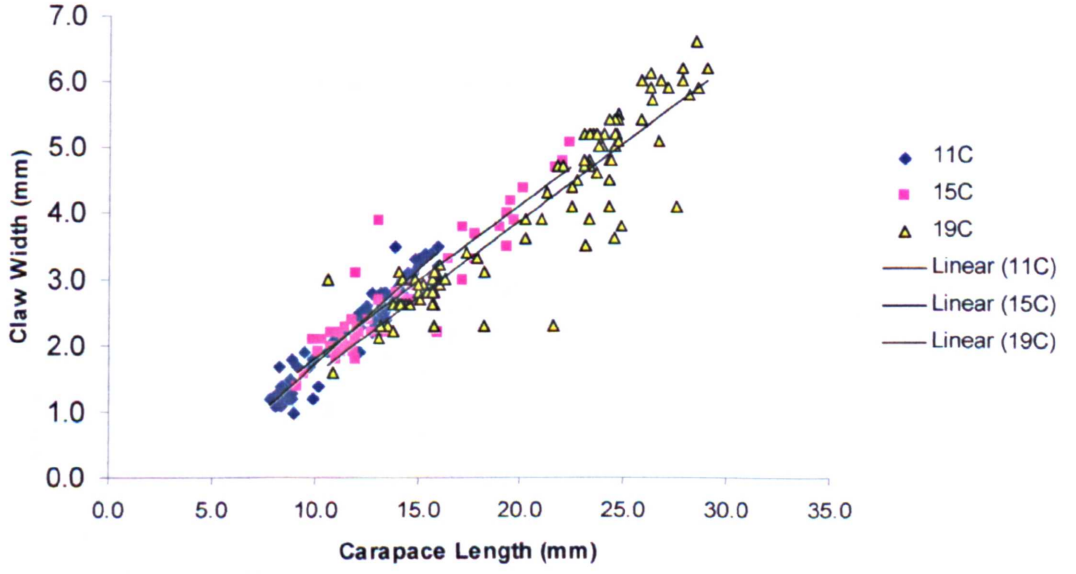


Figure 4.5.b: The cutter claw widths for all 1998 lobsters at all temperatures of rearing. Univariate ANOVA; comparison of regression slopes; $F_{2, 223} = 12.817$, $p = 0.000$.

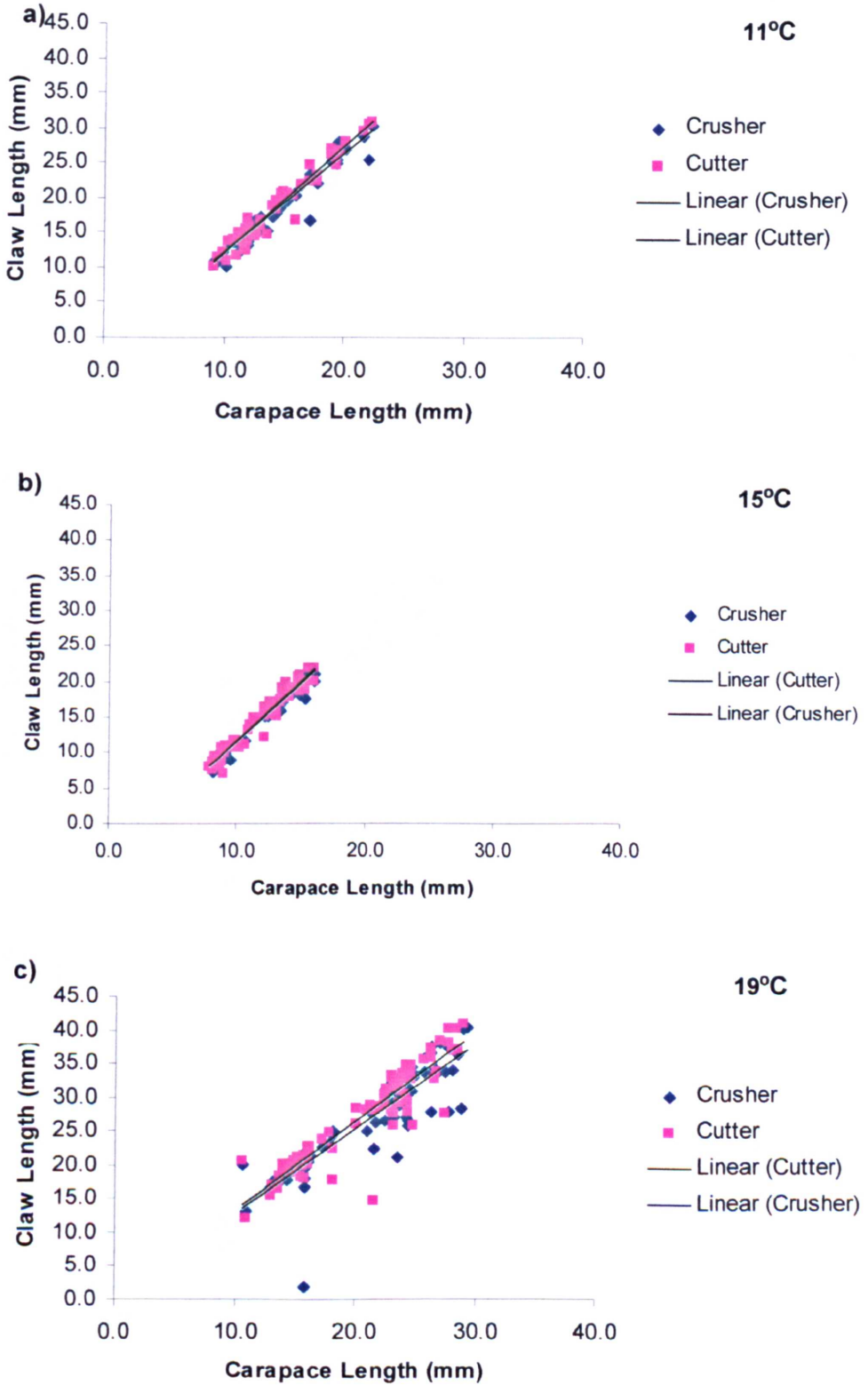


Figure 4.6.a-c: Comparison between the crusher and cutter claw lengths at each temperature of rearing (1998 cohort). Univariate ANOVA; 11°C: n.s.: $F_{1, 150} = 0.812$, $p = 0.369$; $F_{1, 151} = 0.223$, $p = 0.638$; slopes; 15°C: $F_{1, 105} = 6.452$, $p = 0.013$; intercepts; 19°C: $F_{1, 168} = 4.405$, $p = 0.035$.

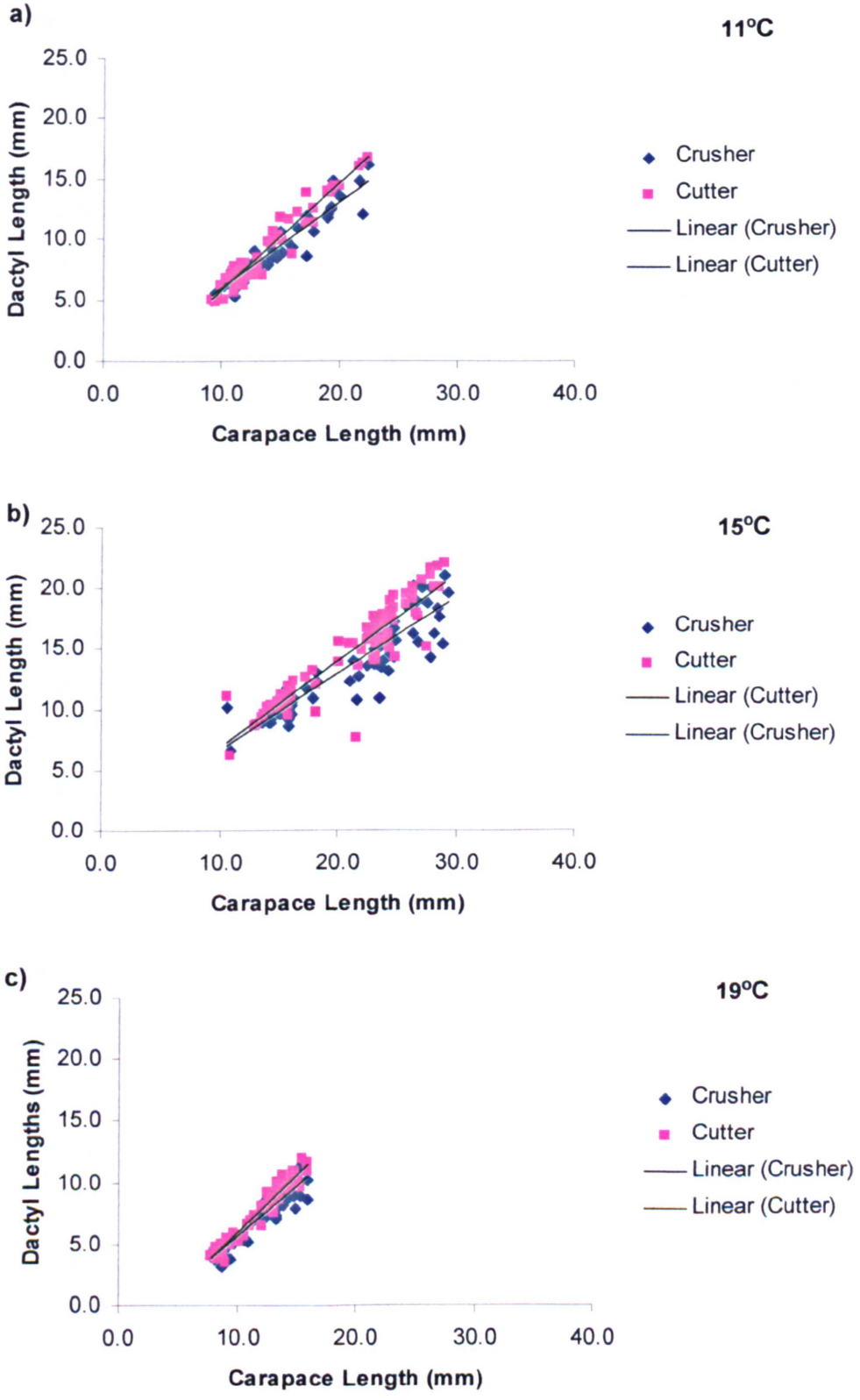


Figure 4.7.a-c: Comparison between the crusher and cutter claw dactyl lengths at each temperature of rearing (1998 cohort). Univariate ANOVA; comparison of regression slopes; 11°C: $F_{1, 150} = 12.921$, $p = 0.000$; 15°C: n.s.: $F_{1, 105} = 0.555$, $p = 0.458$; $F_{1, 106} = 2.675$, $p = 0.105$; intercepts; 19°C: $F_{1, 168} = 21.001$, $p = 0.000$.

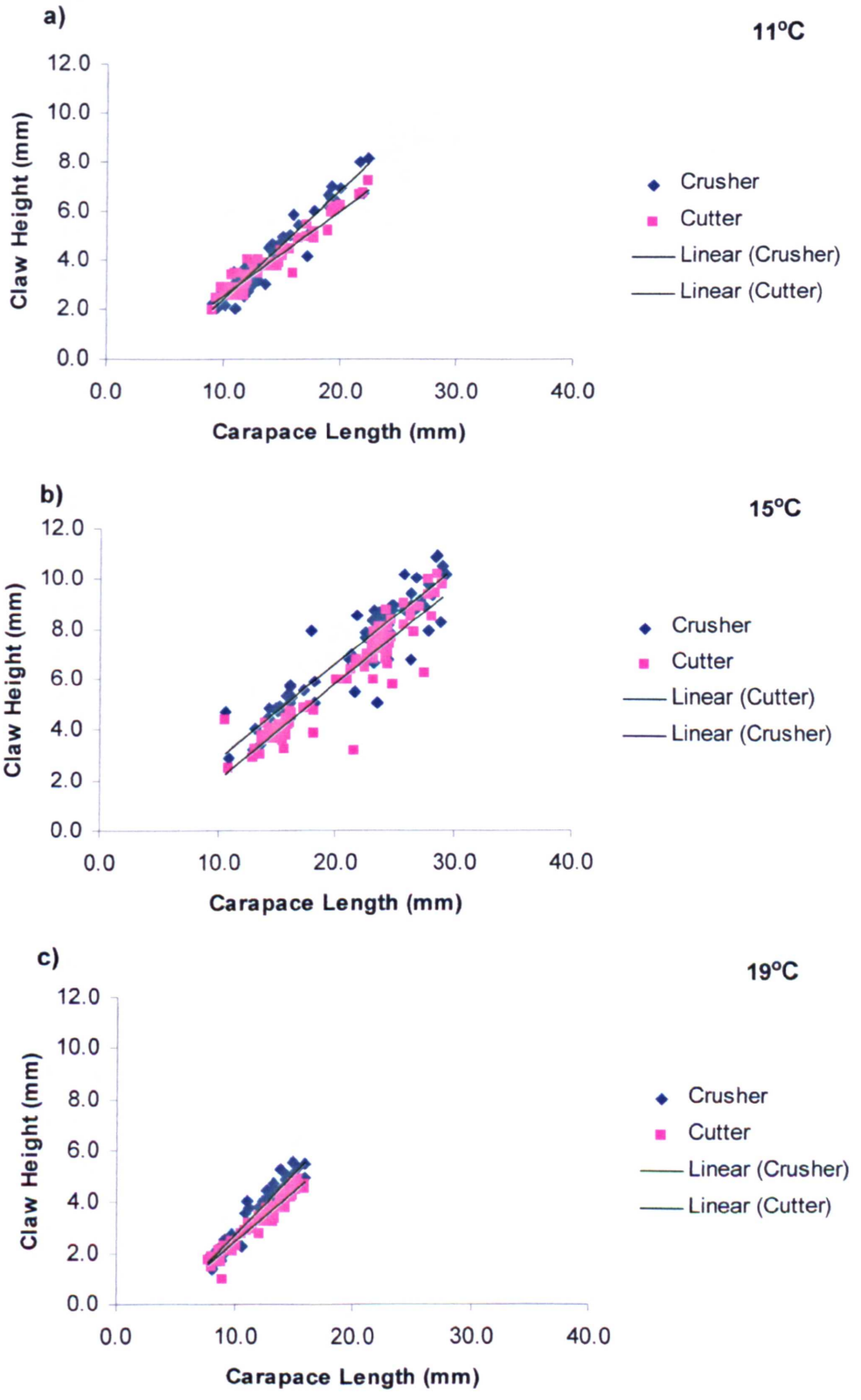


Figure 4.8.a-c: Comparison between the crusher and cutter claw heights at each temperature of rearing (1998 cohort). Univariate ANOVA; comparison of regression slopes; 11°C: $F_{1, 150} = 21.726$, $p = 0.000$; 15°C: $F_{1, 105} = 48.301$, $p = 0.000$; intercepts; 19°C: $F_{1, 168} = 42.466$, $p = 0.000$.

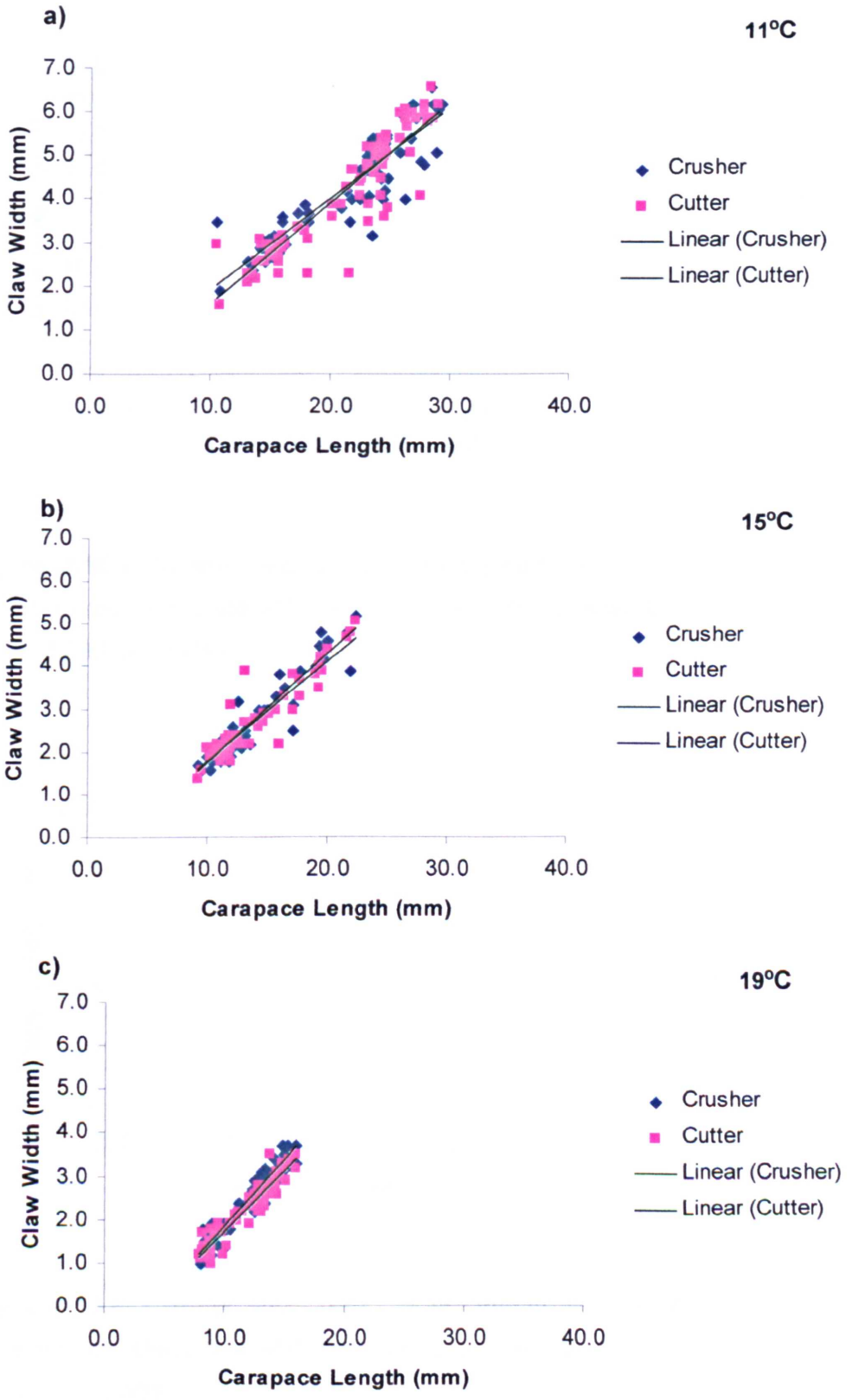


Figure 4.9.a-c: Comparison between the crusher and cutter claw widths at each temperature of rearing (1998). Univariate ANOVA; comparison of regression slope intercepts; 11°C: $F_{1, 151} = 23.960$, $p = 0.000$; slopes; 15°C: $F_{1, 105} = 15.949$, $p = 0.000$; 19°C: n.s.: $F_{1, 167} = 2.565$, $p = 0.111$; $F_{1, 168} = 1.822$, $p = 0.179$.

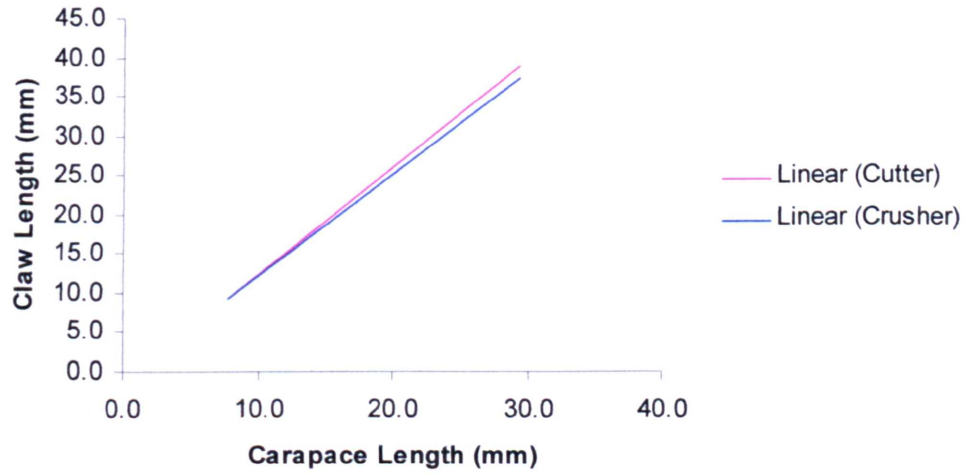


Figure 4.10.a: Crusher and Cutter Claw Lengths for the 1998 lobsters at all temperatures. Univariate ANOVA; comparison of regressions slope intercepts: $F_{1, 431} = 4.069$, $p = 0.044$.

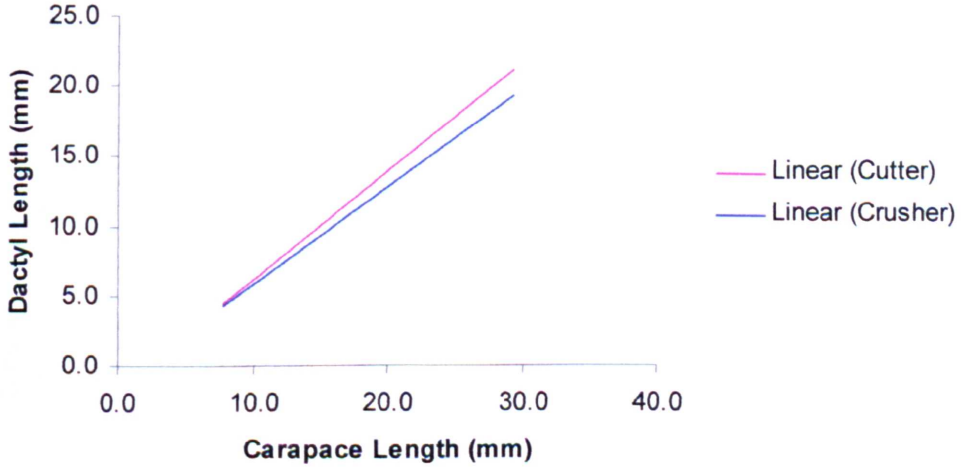


Figure 4.10.b: Crusher and Cutter Dactyl Lengths for the 1998 lobsters at all temperatures. Univariate ANOVA; comparison of regressions slopes: $F_{1, 430} = 10.741$, $p = 0.001$.

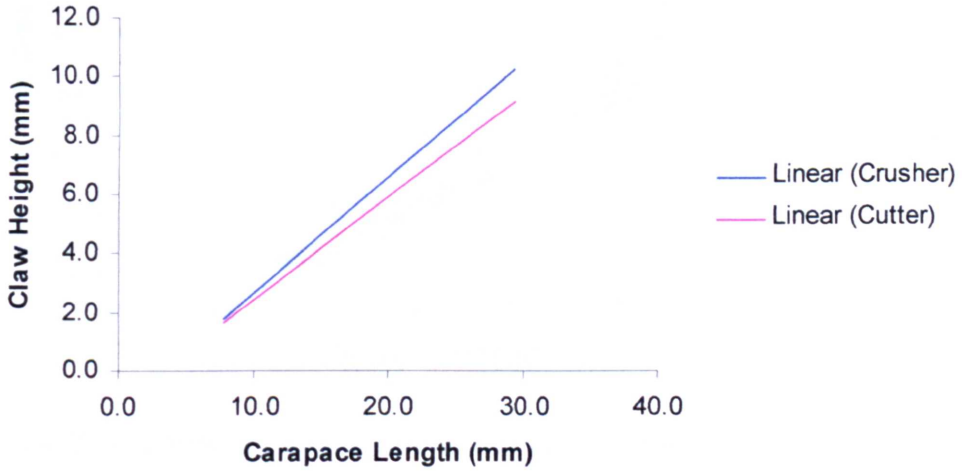


Figure 4.10.c: Crusher and cutter claw heights for the 1998 lobsters at all temperatures. Univariate ANOVA; comparison of regressions slopes: $F_{1, 430} = 25.524$, $p = 0.000$.

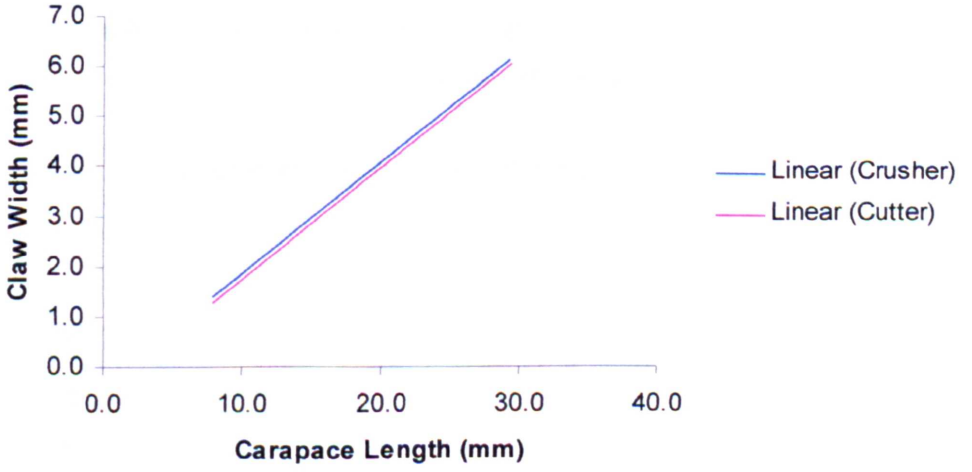


Figure 4.10.d: Crusher and cutter claw widths for the 1998 lobsters at all temperatures. Univariate ANOVA; comparison of regressions slope intercepts: $F_{1, 431} = 11.347$, $p = 0.000$.

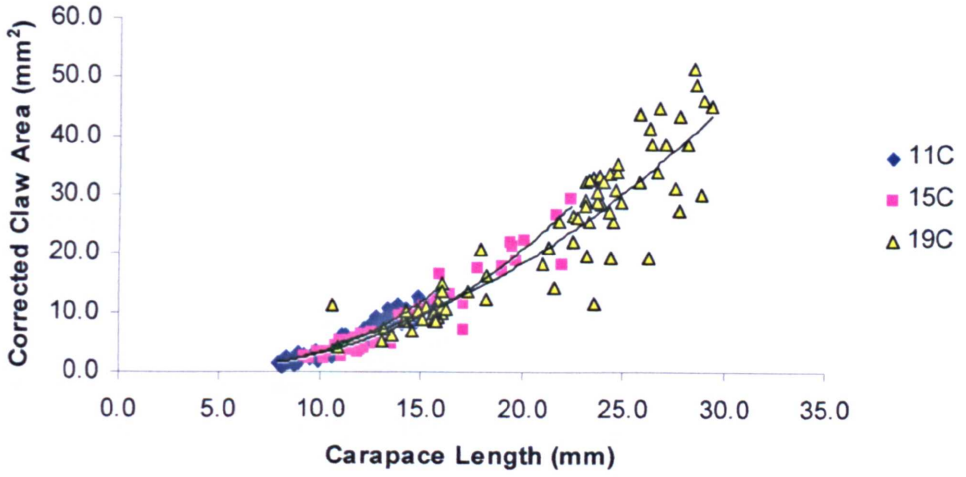


Figure 4.11.a: Corrected crusher claw areas for the 1998 lobsters.

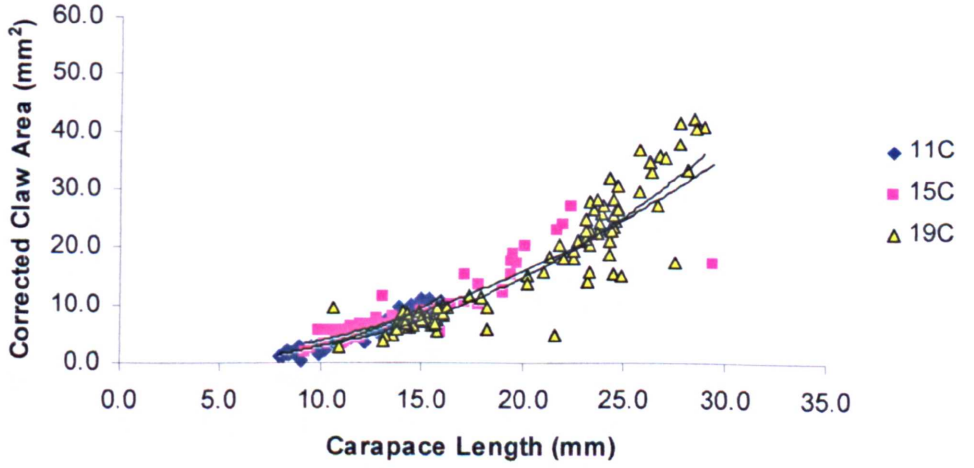


Figure 4.11.b: Corrected cutter claw areas for the 1998 lobsters.

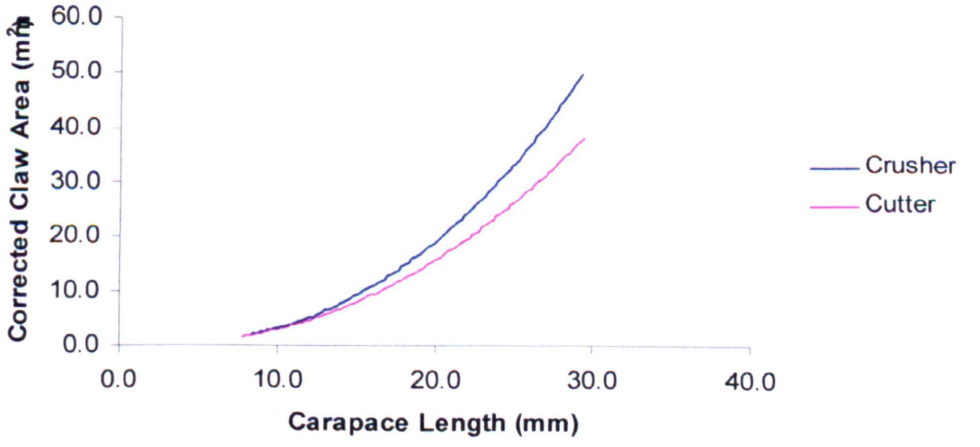


Figure 4.11.c: Corrected crusher and cutter claw areas for the 1998 lobsters at all temperatures.

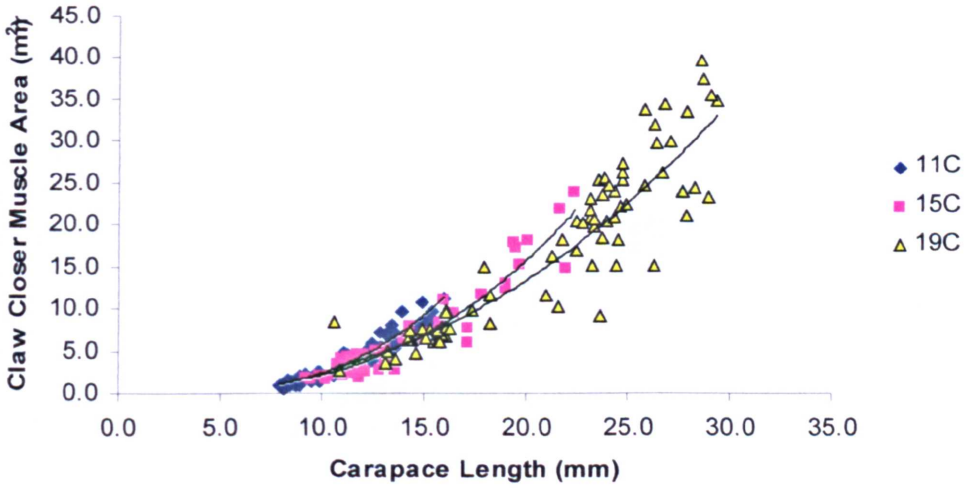


Figure 4.12.a: Crusher claw closer muscle areas for all 1998 lobsters.

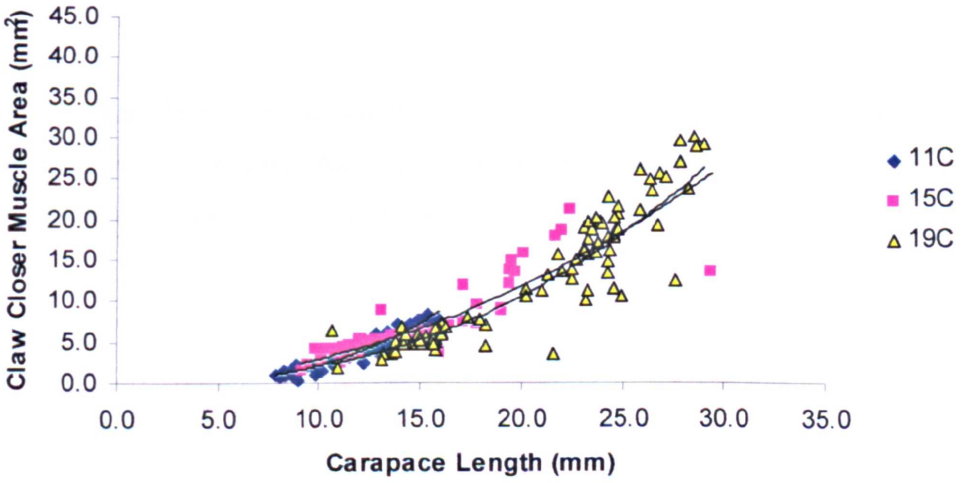


Figure 4.12.b: Cutter claw closer muscle areas for all 1998 lobsters.

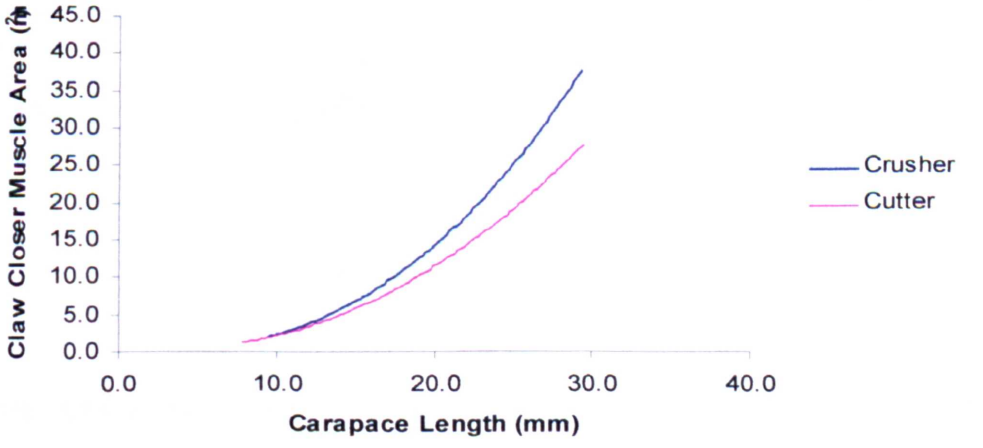


Figure 4.12.c: Crusher and cutter closer muscle areas for all 1998 lobsters at all temperatures.

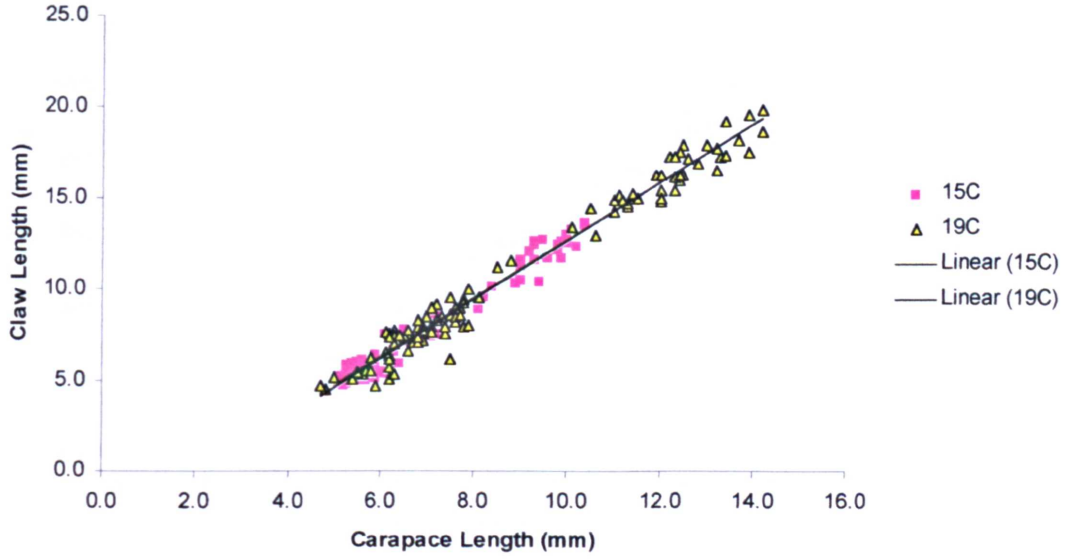


Figure 4.13.a: The Crusher claw lengths for all 1999 lobsters at all rearing temperatures. Univariate ANOVA; comparison of regression slopes and intercepts: n.s.: $F_{1, 171} = 0.000$, $p = 0.986$; $F_{1, 171} = 0.124$, $p = 0.726$.

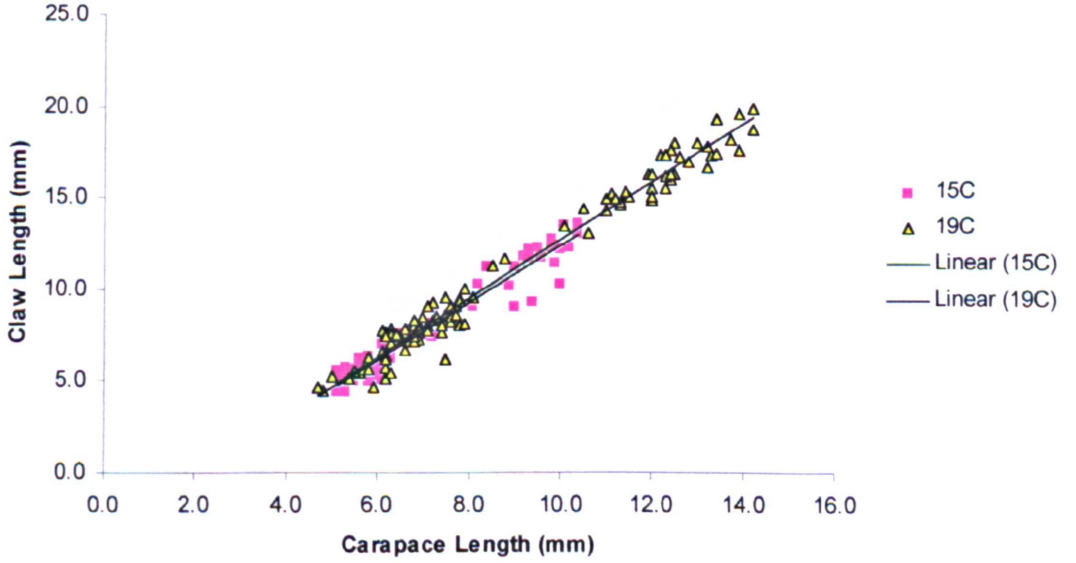


Figure 4.13.b: The cutter claw lengths for all 1999 lobsters at all rearing temperatures. Univariate ANOVA; comparison of regression slope intercepts; $F_{1, 175} = 9.235$, $p = 0.003$.

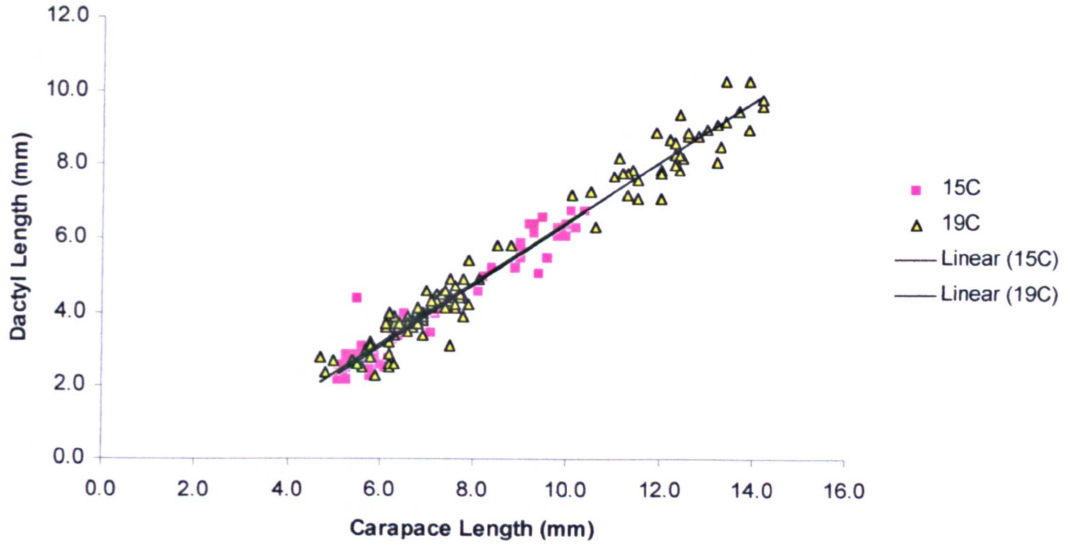


Figure 4.14.a: The Crusher claw dactyl lengths for all 1999 lobsters at all rearing temperatures. Univariate ANOVA; comparison of regression slopes and intercepts: n.s.: $F_{1, 170} = 0.124$, $p = 0.725$; $F_{1, 171} = 1.098$, $p = 0.296$.

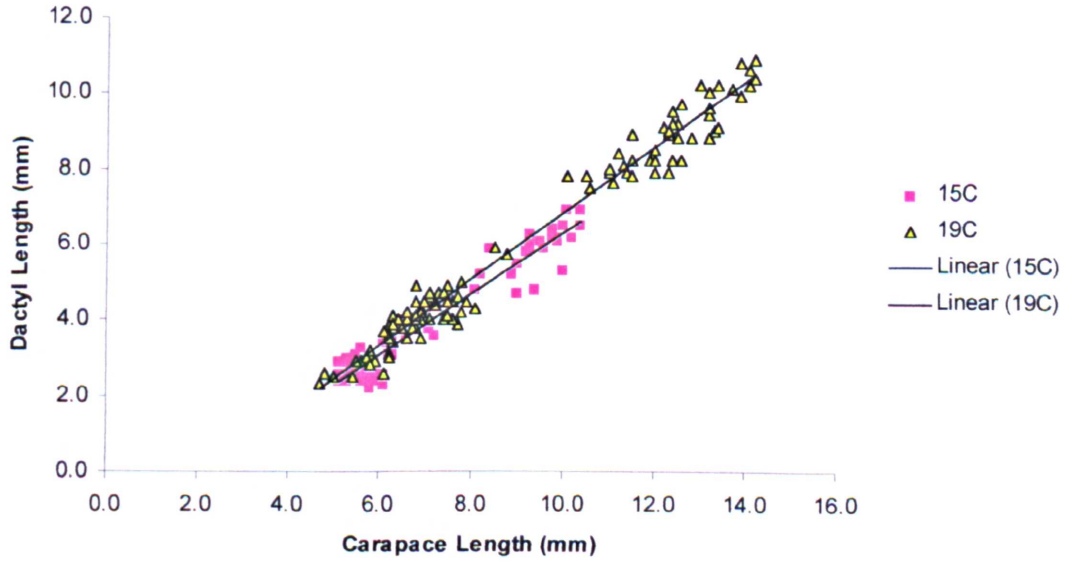


Figure 4.14.b: The Cutter claw dactyl lengths for all 1999 lobsters at all rearing temperatures. Univariate ANOVA; comparison of regression slopes; $F_{1, 174} = 5.767$, $p = 0.017$.

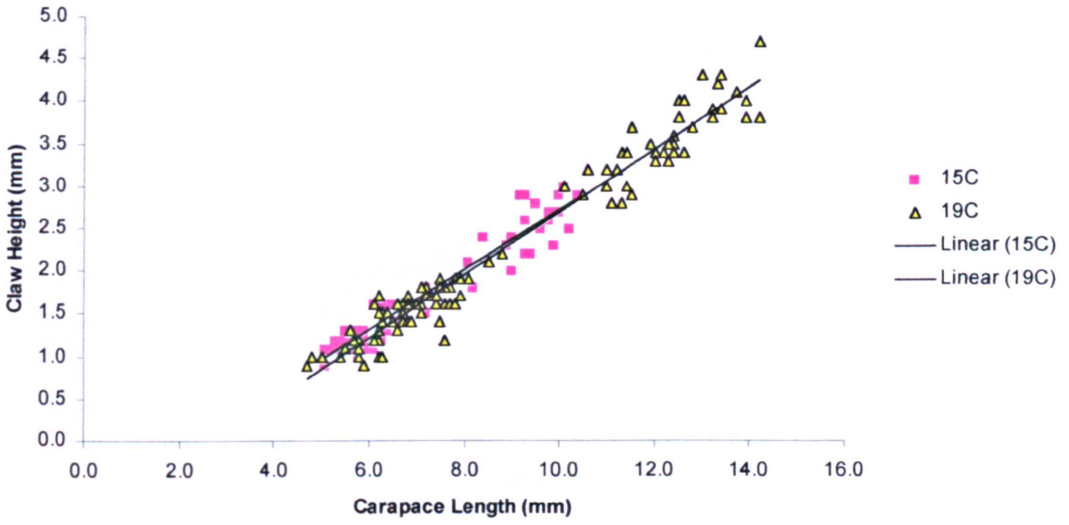


Figure 4.15.a: The Crusher claw heights for all 1999 lobsters at all rearing temperatures. Univariate ANOVA; comparison of regression slope intercepts; $F_{1, 171} = 4.261$, $p = 0.041$.

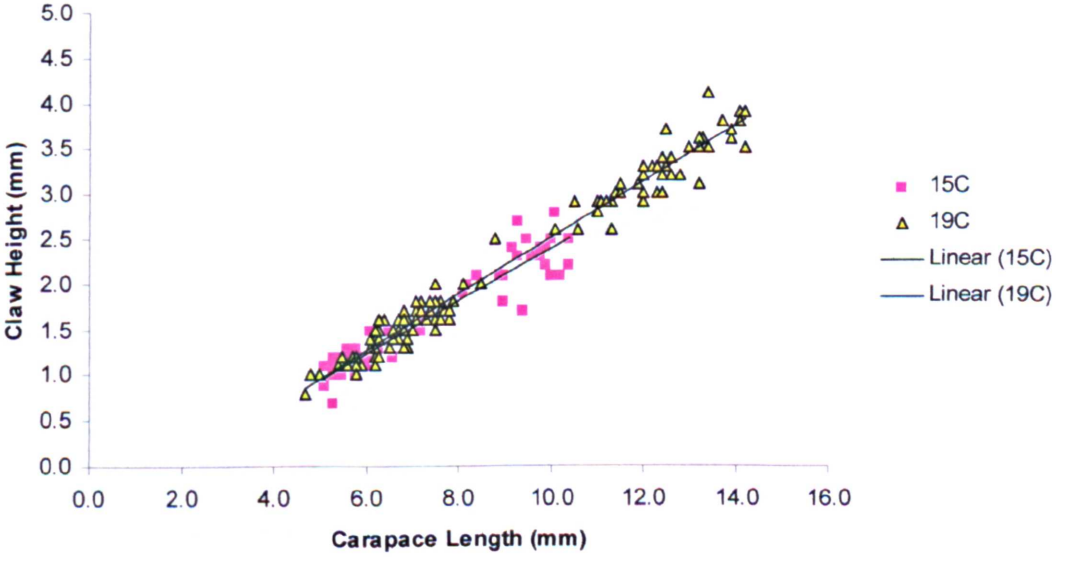


Figure 4.15.b: The Cutter claw heights for all 1999 lobsters at all rearing temperatures. Univariate ANOVA; comparison of regression slopes; $F_{1, 174} = 4.192$, $p = 0.042$.

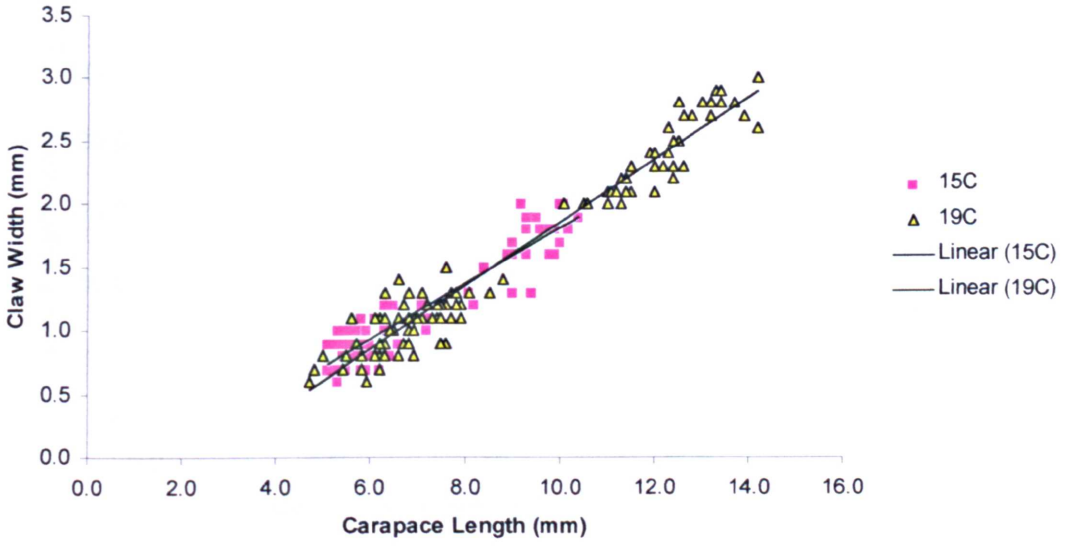


Figure 4.16.a: The Crusher claw widths for all 1999 lobsters at all rearing temperatures. Univariate ANOVA; comparison of regression slopes; $F_{1,\ 170} = 6.597$, $p = 0.011$.

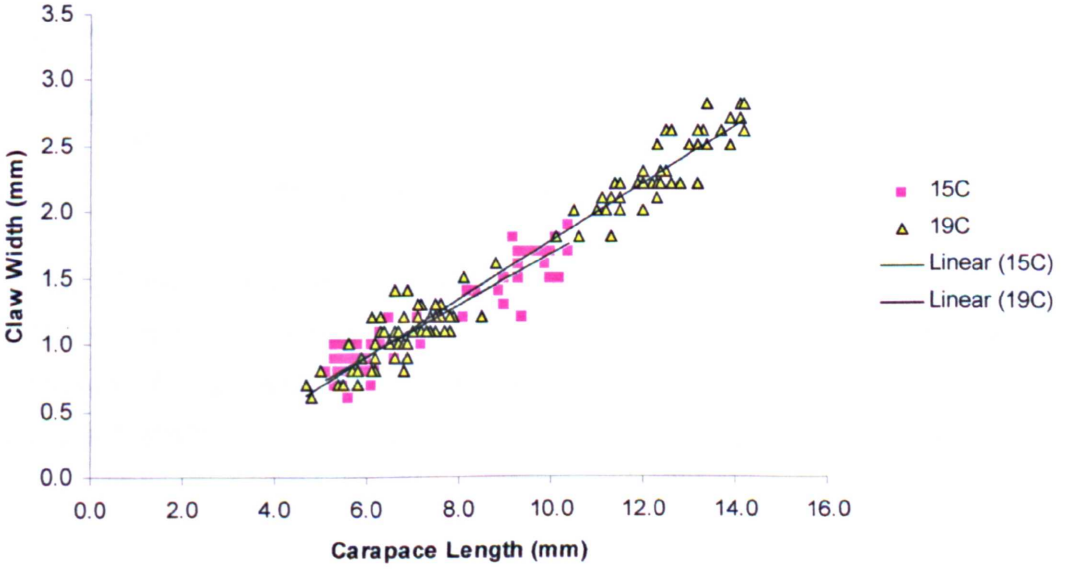


Figure 4.16.b: The Cutter claw widths for all 1999 lobsters at all rearing temperatures. Univariate ANOVA; comparison of regression slopes; $F_{1,\ 174} = 8.583$, $p = 0.004$.

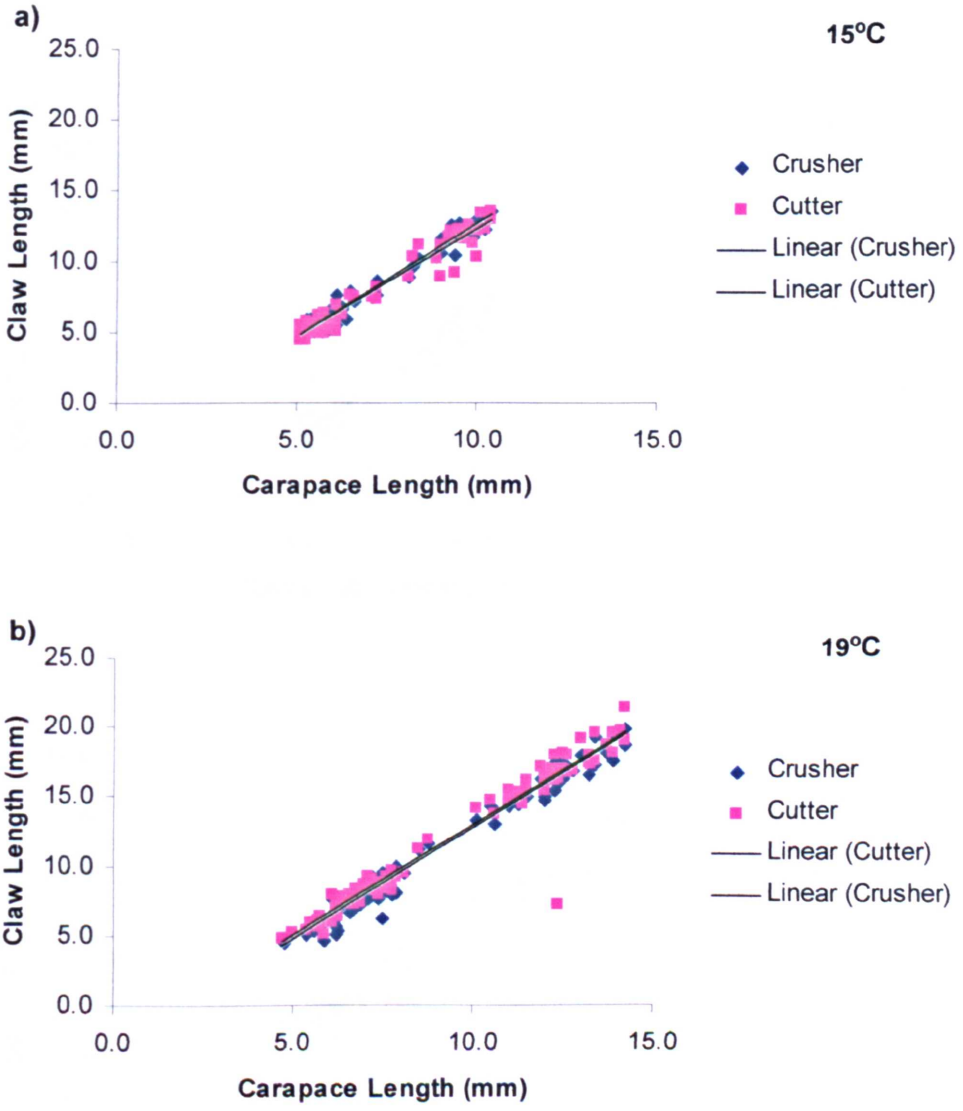


Figure 4.17.a+b: Comparison between the crusher and cutter claw lengths for the 1999 cohort at each temperature of rearing. 15°C: Univariate ANOVA; comparison of regression slope intercepts; $F_{1,\ 131} = 4.448$, $p = 0.037$; 19°C: Univariate ANOVA; comparison of regression slope intercepts; $F_{1,\ 215} = 4.125$, $p = 0.043$.

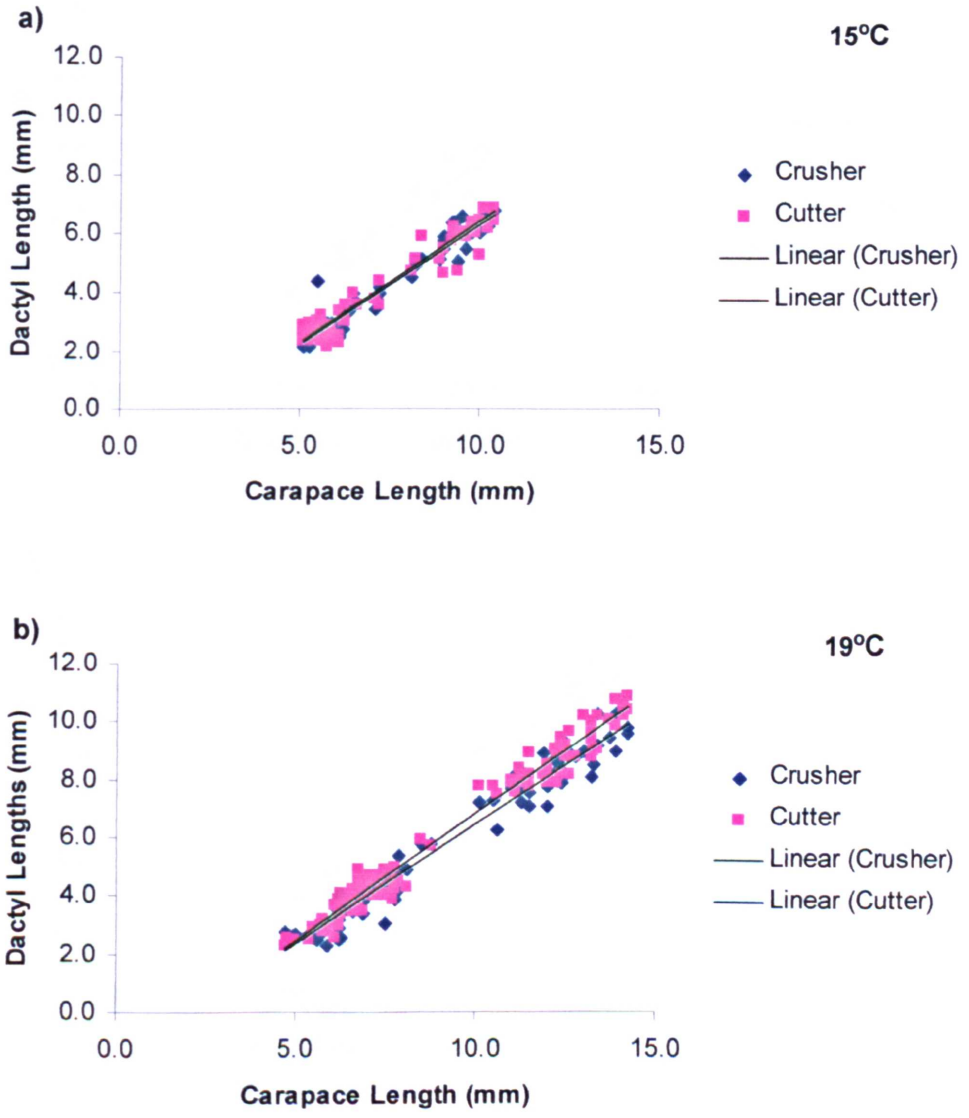


Figure 4.18.a+b: Comparison between the crusher and cutter claw dactyl lengths lengths for the 1999 cohort at each temperature of rearing. 15°C: Univariate ANOVA; comparison of regression slopes and intercepts: n.s.: $F_{1, 130} = 0.541$, $p = 0.463$; $F_{1, 131} = 0.989$, $p = 0.322$; 19°C: Univariate ANOVA; comparison of regression slopes; $F_{1, 215} = 7.926$, $p = 0.005$.

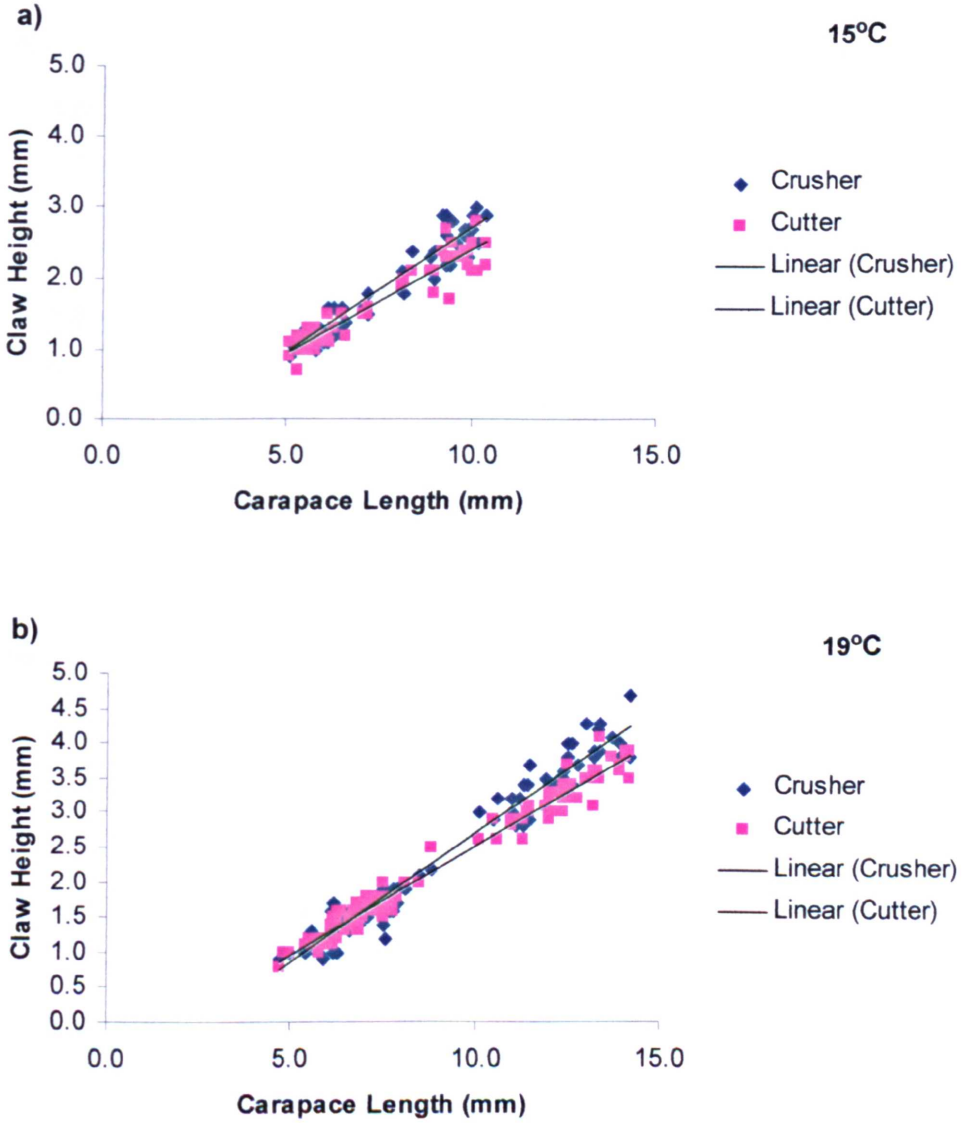


Figure 4.19.a+b: Comparison between the crusher and cutter claw heights lengths for the 1999 cohort at each temperature of rearing. 15°C: Univariate ANOVA; comparison of regression slopes; $F_{1, 130} = 15.768$, $p = 0.000$; 19°C: Univariate ANOVA; comparison of regression slopes; $F_{1, 214} = 44.913$, $p = 0.000$.

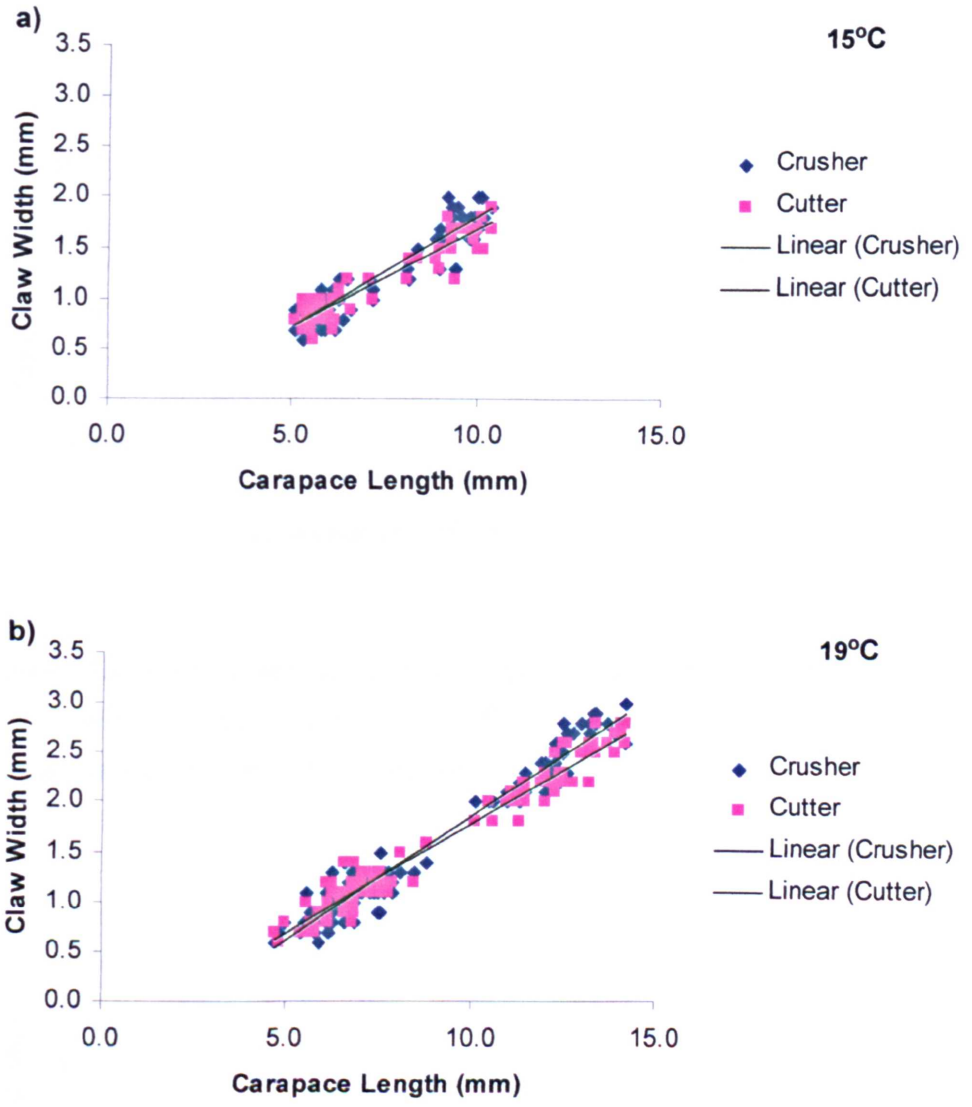


Figure 4.20.a+b: Comparison between the crusher and cutter claw widths lengths for the 1999 cohort at each temperature of rearing. 15°C: Univariate ANOVA; comparison of regression slopes; $F_{1,\ 130} = 4.969$, $p = 0.028$; 19°C: Univariate ANOVA; comparison of regression slopes; $F_{1,\ 214} = 19.252$, $p = 0.000$.

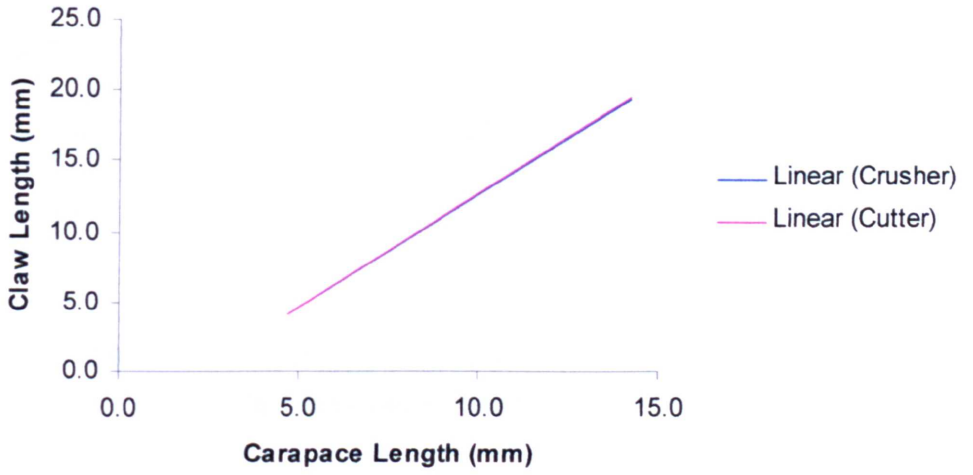


Figure 4.21.a: Crusher and cutter claw lengths for all lobsters lengths of the 1999 cohort at all temperatures. Univariate ANOVA; comparison of regression slopes and intercepts: no significant differences.

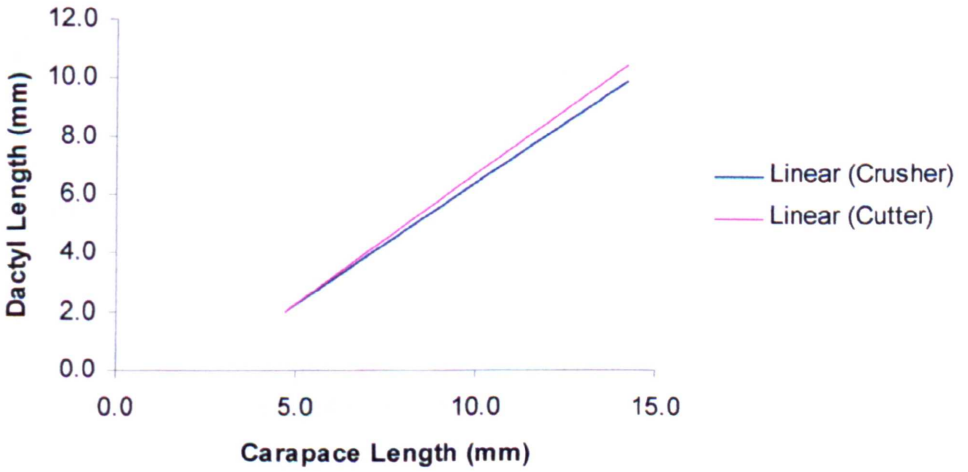


Figure 4.21.b: Crusher and cutter claw dactyl lengths for all lobsters of the 1999 cohort at all temperatures. Univariate ANOVA; comparison of regression slopes and intercepts: no significant differences.

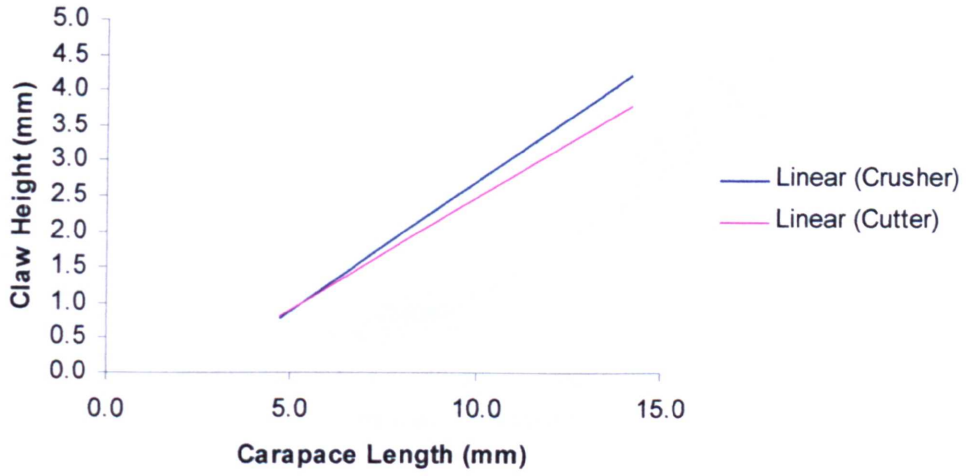


Figure 4.21.c: Crusher and cutter claw heights for all lobsters of the 1999 cohort at all temperatures. Univariate ANOVA; comparison of regression slope intercepts; $F_{1, 171} = 4.261$, $p = 0.041$.

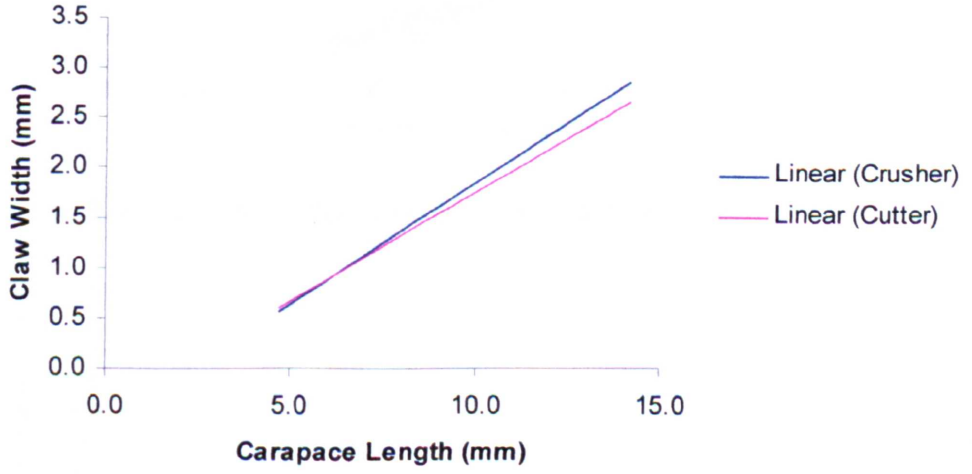


Figure 4.21.d: Crusher and cutter claw widths for all lobsters of the 1999 cohort at all temperatures. Univariate ANOVA; comparison of regression slopes; $F_{1, 170} = 6.597$, $p = 0.011$.

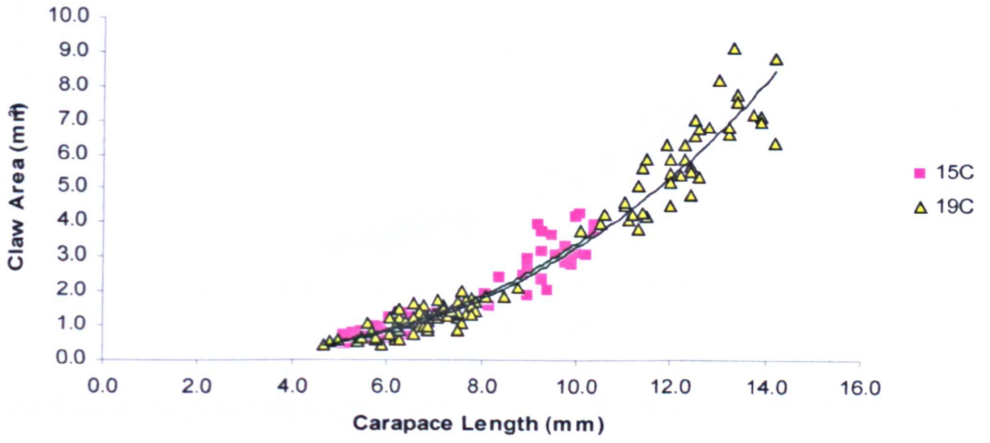


Figure 4.22.a: Corrected crusher claw areas for the 1999 lobsters.

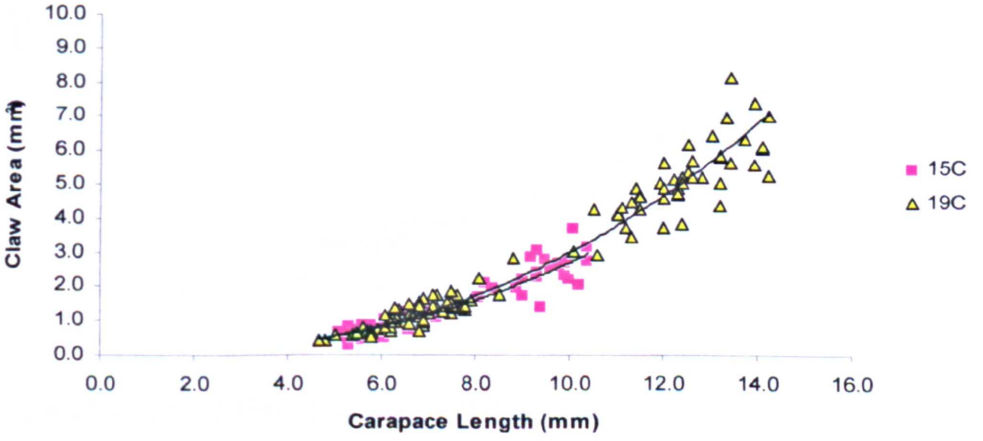


Figure 4.22.b: Corrected cutter claw areas for the 1999 lobsters.

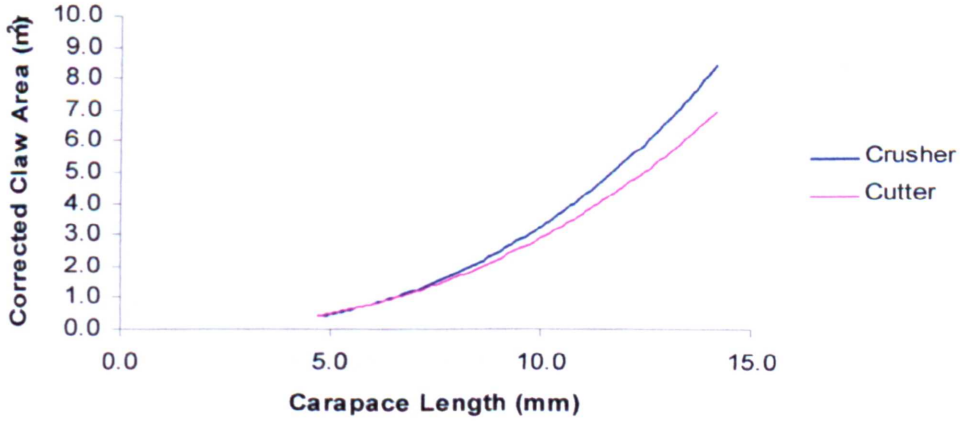


Figure 4.22.c: Corrected crusher and cutter claw areas for all 1999 lobsters.

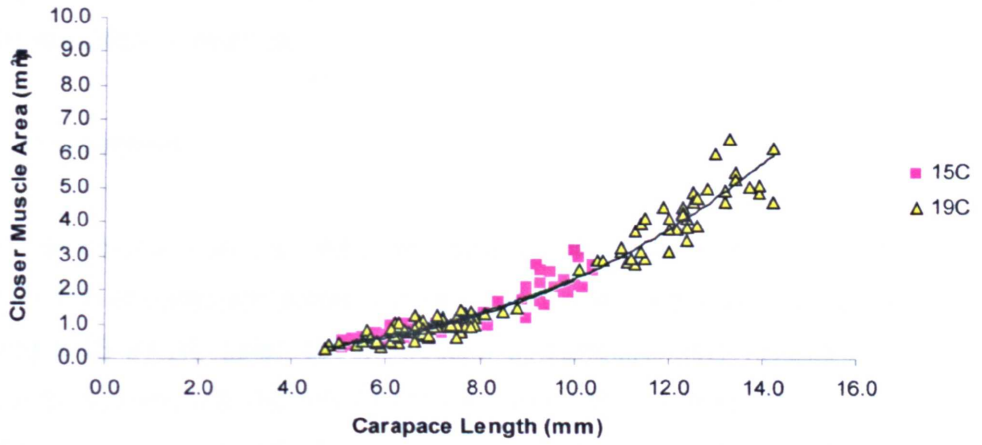


Figure 4.23.a: Crusher claw closer muscle areas for the 1999 lobsters.

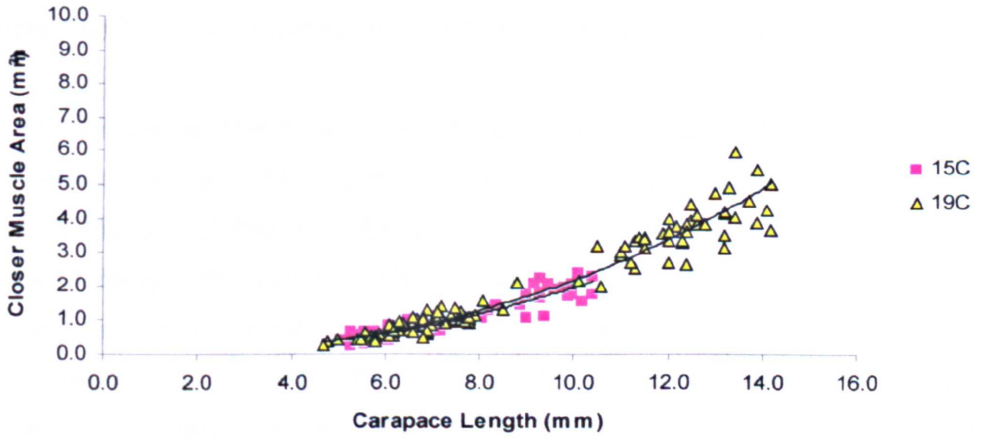


Figure 4.23.b: Cutter claw closer muscle areas for the 1999 lobsters.

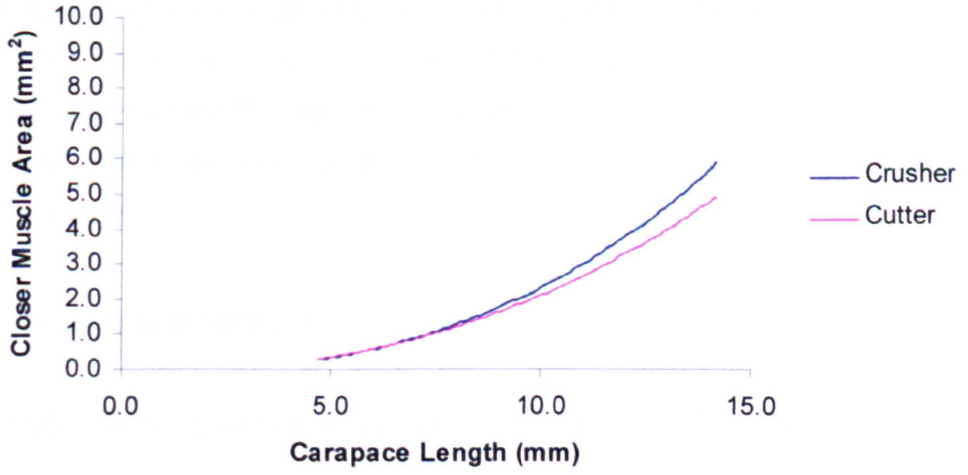


Figure 4.23.c: Crusher and cutter claw closer muscle areas for all 1999 lobsters.

Chapter 5 – The internal development of the claw muscles at different temperatures of rearing

5.1 Introduction

Various criteria can be used to identify the claw muscle fibre types: the expression of particular isoforms of regulatory and contractile myofibrillar proteins (using SDS PAGE gels), the production of corresponding messenger (using *in situ* hybridization with the mRNA for the particular isoforms), and the presence of certain enzymes (myofibrillar ATPase, oxidative enzymes such as succinic dehydrogenase). Other methods such as the measurement of fibre sarcomere lengths or the determination of their mitochondrial densities give corresponding results, but these are inconvenient methods for mapping whole muscle blocks.

It is known that lobster claw muscle fibres can be either of the fast (F) or slow (S1) phenotypes, according to the various myofibrillar protein isoforms that they express (Mykles, 1985a, b; 1988; Neil *et al.*, 1993). At least seven isoforms are unique to the fast fibres (fMHC, fTm, paramyosin$_1$, P75, Troponin I_1, I_3 and I_5) and five isoforms are unique to the S1 slow fibres (sMHC, sTm$_1$, sTm$_2$, troponin T_3, and Troponin I_4). The individual claw muscle fibres of lobsters in intermoult express one or other of these fibre types, but never intermediate forms, which simplifies their identification.

Corresponding to these different protein signatures, fast fibres have higher levels of myofibrillar ATPase and lower levels of SDH than S1 slow fibres, features that can be used to map the distribution of fast and slow fibres in whole muscles by using enzyme histochemistry, a task for which single fibre gel electrophoresis is inappropriate.

5.2 Materials and Methods

5.2.1 Myofibrillar proteins determined by gel electrophoresis

Discontinuous SDS-PAGE was performed according to the method of Laemmli (1970). Gels containing a 2.5% acrylamide stacking gel and a 10% or 5%

separating gel were prepared from a 30% (w/v) acrylamide and 0.8% (w/v) N, N'-methylene bisacrylamide stock solution.

Muscle fibres were prepared as described by Neil *et al.* (1993) by putting pieces of muscle (individual whole fibres; protein load not determined) into cold glyceration buffer containing 20mmol l^{-1} Tris-acetate (pH 7.5), 50% glycerol, 0.1 $moll^{-1}$ KCL, 1 $mmoll^{-1}$ EDTA and 0.1% Triton X-100 for 2-3h. Individual fibres were then separated and transferred to 100 µl of SDS sample buffer [62.5 $mmoll^{-1}$ Tris-HCl (pH 6.8), 12.5% glycerol, 1.25% SDS, 1.25% ß-mercaptoethanol]. Samples were immediately boiled for 3 min, and then stored at -20°C until required.

Samples and standards of known relative molecular mass (M_r) (Sigma Dalton Mark VII-L) were applied to the wells in the stacking gel. The gels were then mounted in a chamber containing a reservoir buffer of 0.2mol l^{-1} Tris-HCL and 0.1% SDS, and run with applied currents of up to 40mA per gel.

Gels were fixed and stained in 0.2% (w/v) Coomassic Blue in 45% (v/v) methanol and 10% (v/v) acetic acid for up to 8 h, and subsequently destained in a methanol/acetic acid mixture. In some cases, gels were further stained with silver. The relative amounts of protein in different bands were determined using scanning densitometry (Hoefer GS 300).

5.2.2 Sarcomere length measurements

Fibres for sarcomere measurements were taken from the claw muscles of animals in different temperature groups and at different ages. Fibre bundles were taken from the central or peripheral parts of the cutter and crusher claws (marked regions in Fig. 5.1), and, for reference, fibres from the deep abdominal flexor muscle and the superficial abdominal flexor muscle were also taken. In some cases the fibres were subsequently subjected to SDS PAGE, in order to define their myofibrillar proteins.

To prepare the muscle for sarcomere length measurements, the claw or abdomen was pinned out in a partly extended position so that the muscles were held at resting lengths. One of the two following procedures was then followed:

1. The muscles were fixed in alcoholic Bouin's solution for 24 hours. Individual fibres were dissected from the mid and ventral areas of the closer muscle, teased apart in 70% alcohol on a microscope slide, and viewed with phase-contrast optics. The lengths of 8 successive sarcomeres were measured using a calibrated ocular micrometer. Average values were calculated from 3 such measurements on a single fibre.

2. The muscles were fixed *in situ* for two hours in a solution containing 4% glutaraldehyde and 2% paraformaldehyde in Millonig's phosphate buffer. After dissection, the muscles were post-fixed in 1% osmium tetroxide in the same buffer, dehydrated and embedded in Araldite. Thick sections (1μm) were cut on an ultramicrotome (REICHERT), stained with aniline blue and examined by light microscopy. Thin sections (60-70 nm) were also cut, stained with uranyl acetate and examined on an electron microscope (ZEISS), at a magnification of x400.

The 2 methods gave similar results.

5.2.3 Histochemical analysis of the claw muscle

Histochemical staining of the lobster abdomen and claw muscles was performed to determine the ATPase activity which is 2-3 times higher in fast muscle than slow muscle (Ogonowski and Lang, 1979). The procedures followed were adapted from those of Fowler and Neil (1992).

At various different times during development (9 months, 15 months 17 months for 1998 cohort; 3 months, 6 months for 1999 cohort). Subgroups of lobsters from the three temperature groups were sacrificed for histochemical analysis. The abdomens and autotomised claws of lobsters were frozen in Isopentane, cooled by Liquid Nitrogen, then mounted in OCT, and sectioned at 20μm in a Cryostat at -25°C. The sections obtained were transferred to cover slips, air dried, and used for various histochemical procedures to test for myofibrillar ATPase activity. The

total myofibrillar ATPase activity of the muscle fibres was determined using a method derived from those used by Mabuchi and Sreter (1980) and Snow *et al.* (1982). From stock solutions of Buffer – N glycylglycine (0.05M) (pH 8.0), calcium chloride (1M), magnesium chloride (0.1M), and ATP (0.1M) (pH 7.0), an incubation medium was prepared. This uses 76ml glycylglycine, 0.72ml $CaCL_2$, 0.5ml $MgCl_2$, and 1.2ml ATP, pH at 8.0 with KOH. The sections were incubated for 30 minutes in a fridge (4°C), washed in distilled water for 1.5 minutes, immersed in 1% $CaCl_2$ for 3 minutes, washed, immersed in 2% $CoCl_2$, for 3 minutes, washed, immersed in $(NH_4)_2S$ for 30 seconds, washed, and then dehydrated through an alcohol series and mounted (Fowler and Neil, 1992).

In addition some sections were stained for Succinic dehydrogenase (SDH) activity, a known mitochondrial enzyme, as a measure of the oxidative capacity of the fibres, which is indicative of their fatigue resistance (Lojda *et al.*, 1976). The incubation medium contained 1M sodium succinate, 0.1M sodium phosphate, and 1.0 mg/ml nitro blue tetrazolium. The cryosections were placed in a petri dish on moist filter paper, and a drop of the incubation medium was applied to each cover slip. Incubation was for up to 2 hours at 40°C. The sections were then dehydrated in an alcohol series, cleared in Histoclear and mounted in Histomount.

The sections were viewed with a light microscope. Individual frames from a video camera attachment were captured by an image capture card (Pinnacle DV10+) and were stored on a PC as image files. Measurements were made using an image analysis software package system (Scion V. 4.2) to obtain values for the cross sectional areas of the claw (at its mid-point) and of the opener and closer muscle. By comparing the claw area measurements with calculations of 'rectangular' cross-sectional areas derived from height and width measures made on the same claws using callipers, a series of correction factors were produced that could be applied to the much larger number of claws that were measured only externally using callipers. The areas of the claw closer muscle area that stained in different ways, indicating different fibre types, were also determined, so that the relative areas of fast and slow muscle could be calculated.

5.3 Results

The objective was to map the time course of fibre transformation in the claws of lobsters reared at the different temperatures. The methodology chosen for mapping the distribution of fast and slow muscle fibre types in the claw muscles was myofibrillar ATPase enzyme histochemistry applied to claws of representative animals at regular intervals during development. For quantitative purposes, changes in the relative sizes of the fibre regions of the claw closer muscles with low ATPase activity (dorsal and ventral regions) and high ATPase activity (the central band) were used as convenient measures of dimorphic transformation.

In a small number of claws from these representative animals, muscle fibres taken from the dorsal and ventral regions and from the central band were also be characterised in terms of their myofibrillar proteins, their sarcomere lengths and certain other morphological features.

5.3.1 Sarcomere Lengths

Table 5.1 shows the mean sarcomere lengths (SL) taken from lobsters at the end the two rearing periods (18 and 6 months, respectively). The 1998 cohort is represented by an 11°C Stage 13 lobster and a 19°C Stage 15 lobster; these were chosen to represent the extremes of the temperature regimes.

For the 1998 cohort at 18 months, the central band of the cutter claw was sampled as it is composed of fast fibres at all ages. The values for fibres in this area (1.98 µm for the 11° C group and 2.95 µm for the 19° C group) fall within the typical range for fast fibres (2 - 3 µm). The ventral area of the crusher closer muscle was sampled as it is composed of slow fibres at all ages. The values for fibres in this area (10.24 µm for the 11° C group and 6.16 µm for the 19° C group) fall within the typical range for slow fibres (6 - 13 µm).

The results for the 1999 lobsters follow the same pattern as for the 1998 cohort, with short SL fibres in the central band of the cutter claw (1.83 µm for the 15°C

group and 1.81 μm for 19°C group) and long SL fibres in the ventral area of the crusher claw (8.62 μm for the 15°C group and 7.36 μm for 19°C group).

5.3.2 Myofibrillar proteins

Figure 5.1 shows the expression of myofibrillar protein isoforms for individual slow and fast fibres taken from the central band and ventral region of a *H. gammarus* crusher claw, and, for comparison, from the deep abdominal flexor muscle of the same lobster. Particular proteins (eg P75) and isoforms (eg of TnI) identify fibres with a fast (F) phenotype in the deep abdominal muscle and in the central region of the crusher claw closer muscle, while the ventral region of this claw muscle has fibres of the S1 phenotype, with no P75 band and a different combination of TnI isoforms. The sarcomere lengths of fibres of these two phenotypes also show a consistent difference: 2-3 μm for F fibres and 6-7μm for S1 fibres.

5.3.3 The Initial condition in postlarval lobsters

Cryosections taken from the midpoint of the claws of lobsters at different stages of development were subjected to myofibrillar ATPase enzyme histochemistry. The distribution of fibre types in the claws of post-larval lobsters (Stage 6) was taken to represent the initial bilaterally symmetrical state (Fig. 5.2). In each of the animals examined, the relative proportions of the central band of high ATPase fibres (dark staining) (~40% of the closer muscle cross-sectional area) and the dorsal and ventral regions of low ATPase fibres (light staining) (each ~30% of the muscle area) were the same in the two claws. All the fibres in the smaller opener muscle also displayed light staining.

For each claw, in addition to the set of sections used for the ATPase reaction, a number of sections were also stained for SDH activity, so that the relative enzyme properties of identifiable fibres could be compared in adjacent sections (Fig. 5.3). The SDH staining shows a similar distribution to that for ATPase, being high in fibres with high ATPase activity (the central band) and low in fibres with low ATPase activity (the dorsal and ventral regions). A distinct boundary was always present between the fibres with these different staining properties (Figs. 5.4 and 5.6), and no evidence for intermediate strengths of staining for either

ATPase or SDH were ever obtained from the claw muscle fibres of the animals that were used (all of which were in the intermoult stage).

5.3.4 Dimorphic claw development in the 1998 cohort

Figure 5.5 shows typical histochemical results from the 1998 cohort of lobsters reared at the different temperatures (11°C, 15°C and 19°C). These sections also serve to demonstrate the growth in size of the claws at different and ages and moult stages through development. The opener muscles of the two claws retained their slow fibre composition throughout development, but the closer muscles of the cutter and crusher claws showed systematic changes.

The 11°C group is represented by lobsters at Stage 10 (9 months), Stage 12 (15 months) and Stage 13 (17 months). It can be seen that the cutter claws (depicted on the right of each image pair) exhibit a progressive change with age in closer muscle fibre type distribution, involving an expansion of the central band of high ATPase (fast) fibres. On the other hand the crusher claws (depicted on the left of each image pair) show a contraction in the central band of fast fibres in the closer muscle.

The 15°C group is represented by lobsters at Stage 11 (9 months), Stage 13 (15 months) and Stage 15 (17 months). This group shows the same trends in the claw closer muscle fibre development as the 11°C, with the cutter closer muscle eventually being composed of almost all fast muscle fibres (with small fringes of slow fibres). The crusher claws do not reach the full dimorphic state of being composed entirely of slow muscle fibres, even in the Stage 15 lobster (17 months) but a central band of fast fibres persists.

The 19°C group is represented by lobsters at Stage 11 (9 months), Stage 15 (15 months) and Stage 17 (17 months). At this temperature there are clear examples of claws in the advanced dimorphic state. At Stages 15 and 17 there are predominantly fast fibres in the cutter claw closer muscles, with very small areas of slow muscle fibres around the margins. The crusher claw closer muscles are composed almost entirely low ATPase slow muscle fibres, except for a narrow central band of fast fibres (best seen in the examples at Stages 11 and 15).

In terms of growth rates, the measures of claw area from the stained cryosections confirm those of the external dimensions (Chapter 4) in showing that the lowest temperature of 11°C restricts the growth to a very low rate. Thus the 11°C Stage 13 lobsters at 17 months have claws with virtually the same areas as 19°C Stage 11 lobsters at 9 months (i.e. at approximately half the age). Comparing lobsters of the same age, 17 months (Fig. 5.10), the 11°C lobsters (at Stage 13) have smaller claws than the 19°C lobsters (at Stage 17), but the dimorphic differentiation of their claw closer muscles is no less advanced. This indicates that, even though a higher temperature of rearing increases the growth of the lobster and its claws, muscle fibre transformation does not proceed at a rate that is directly linked to the increased growth rate or moulting rate. Conversely, a rearing temperature of 11°C therefore seems to have a greater retarding effect on the rate of growth than on the rate of fibre type transformation.

Although the transformation of the cutter claw closer muscles from the initial postlarval condition (Fig. 5.2) to the final state with a predominance of fast fibres was ultimately achieved at all temperatures (11°C = 100%, 15°C = 91.6% and 19°C = 100%), the changes in the crusher claw were more variable (Figs. 5.4 and 5.5). In numerous cases (11°C = 67%, 15°C = 91.7% and 19°C = 78.6%), there was incomplete transformation of the crusher claw closer muscle fibres even after 17 months of development, an example of which is shown in figure 5.7. In this Stage 15 animal reared at 19°C (aged 15 months) the cutter claw closer muscle (left hand side), is fully transformed to predominantly fast muscle fibres, with some persisting slow muscle fibres peripherally. However the crusher claw (right hand side) still has a large proportion of fast muscle fibres centrally. This is an example of what has been termed a 'false' crusher claw, to distinguish it from a 'true' crusher in which the closer muscle comprises only slow muscle fibres (Jahromi and Atwood, 1971; Lang *et al.*, 1977a; Ogonowski *et al.*, 1980).

5.3.5 Rates of claw muscle fibre transformations

In order to quantify the rates of claw muscle fibre transformations at the different temperatures of rearing, the percentages of the muscle cross sectional area represented by the central band of fast fibres and the dorsal and ventral regions

of slow fibres were calculated. However, in obtaining these data the following facts had to be taken into account. By taking serial sections of one claw, it was established that changes in fibre type distribution occurred along the length of the claw closer muscles. *Figure 5.9* illustrates the results for a crusher claw. This shows that fast fibres are present in a central band in the middle of the muscle, but not at its proximal and distal ends, where slow fibres predominate. It was for this reason that the sections used for calculating relative areas of fast and slow fibre regions were taken only from the midpoint of the claw.

The change in the relative size of the dorsal region of slow fibres in the cutter closer muscle was used as the most reliable measure of dimorphic transformation (Fig. 5.11). Here the data obtained from animals at all three sampling points are plotted relative to stage (averages), for each rearing temperature (11°C n=18, 15°C n=12, 19°C n=16). The initial value for the dorsal slow fibre region is 10% at stage 6 (3 months), and at the endpoint it is <1% (when most fibres are fast). Following a statistical analysis on stage 10 data (ANOVA F-statistic, $F_{2,6} = 10.76$, $p = 0.01$) it can be seen through a Tukey's pairwise comparison that there is a significant difference between the 11 and 19°C groups but not between the other combinations. There are also differences between the stage 11 animals (ANOVA F-statistic, $F_{2,7} = 5.61$, $p = 0.035$), at the 11 and 19°C groups but not between the other combinations, as determined by a Tukey's pairwise comparison. In light of this it can be seen that at stages 10 and 11 the 11°C lobsters have a slower rate of dimorphic switching than the 19°C lobsters, but not at the other points. However the complete cutter condition was reached at all temperature regimes by stage 14.

The same measure of dimorphic transformation has been used for the crusher claw (Fig. 5.12). Again starting from its initial value of 10% at Stage 6, for this claw it should rise to values in some cases in excess of 60%, as would be expected for a transformation to the 'true' crusher pattern with mainly slow fibres. In many individuals, however, the values remain lower than this, which is a reflection of the 'false' crusher state. An arbitrary threshold for distinguishing between 'true' crushers and 'false' crushers has been taken to be a value of 20% for the relative area dorsal slow fibre region (marked on Fig. 5.8). From the spread of data points for individual animals, it can be seen that:

- 'true' crushers are produced at all moult stages from Stage 9 onwards
- 'true' crushers and 'false' crushers are produced at all temperatures
- There is no trend for 'true' crushers to be produced more at later stages or at higher temperatures.

5.3.6 Dimorphic claw development in the 1999 cohort

The claws from the 1999 cohort of lobsters (which were raised from the egg at Conwy) were first examined in animals of 3 months age. *Figure 5.13* shows examples from the group reared at 19°C. They show a greater individual variability in the claw muscle fibre composition than was apparent in the 1998 cohort (see Fig. 5.5), since a number already show a trend towards dimorphic development, with a reduction in the central band of fast muscle fibres in the presumptive crusher claw, and an expansion in the presumptive cutter the left claw is the cutter, while others dimorphic switching has not begun. Thus dimorphic development is relatively advanced in Stage 5 lobster #73, Stage 6 lobster #33 and Stage 7 lobster #47 (Fig. 5.13.b), but is barely apparent in Stage 6 lobster #13. This highlights the extent of individual variability that occurred within a population of lobsters reared in the same conditions, and at the same temperature.

At 6 months of age, the group of lobsters raised at 19°C has increased sizes of claws, but the degree of dimorphic transformation remains variable (Fig. 5.14.b). Thus Stage 10 lobster #69b has fully dimorphic claws, Stage 9 lobster #35 has partially dimorphic claws, and Stage 8 lobsters #1 and #69 have claw muscles in the untransformed condition.

These results for the 1999 cohort back up the results for the 1998 cohort, in so much as the higher temperature of rearing does not increase the incidence of dimorphic development in the claws through the increased growth and moulting rates.

As before, the change in the relative size of the dorsal region of slow fibres in the cutter closer muscle was used as a measure of dimorphic transformation (Fig.

5.16). Here the data obtained from both sampling points (3 and 6 months) of juvenile animals are plotted relative to stage, for each of the two rearing temperatures (15°C and 19°C). At Stage 4, starting from a value of less than 10%, the relative size of the dorsal slow fibre area initially increases, but then decreases with further development, at both rearing temperatures. The rate of transformation to the fully dimorphic cutter condition is faster than that for the 1998 cohort, as it is complete by 6 months of age.

In contrast to this consistent and relatively rapid transformation in the cutter claws, the transformation in the crusher claws was in many cases incomplete by 6 months of postlarval development, and there seemed to be no apparent trend with either temperature or increasing stage. Indeed in some cases the relative area of the dorsal slow fibre area was either unchanged, or even decreased relative to the earlier postlarval stages.

5.4 Discussion

The analysis of myofibrillar proteins in the claw closer muscles of juvenile *H. gammarus* by SDS PAGE demonstrates that their fibres divide into two distinct phenotypes, fast (F) and slow (S1) according to the presence of key isoforms. These data are consistent with the measures of sarcomere length, which also define two populations of fibres with short (<2μm) and long (>7μm) sarcomeres. Since the distribution of these fibre populations within the claw closer muscle correspond to each other, i.e. F phenotype and short SL from the central band, S1 phenotype and long SL from the dorsal and ventral regions, it is reasonable to conclude that the F phenotype fibres have short SL, and the S1 phenotype fibres have long SL. This relationship is found extensively in crustacean skeletal muscles (Neil *et al.*, 1993), and is taken to indicate that these fibres have distinct contractile properties.

The histochemical staining of the claw closer muscles of juvenile *H. gammarus* has revealed the distribution of fibres with high and low levels of myofibrillar ATPase activity (indicative of contraction speed) within them. In some, but not all cases, it was also possible to demonstrate a relationship between the level of

ATPase activity and the level of the SDH staining, although the latter was not consistently revealed. The fact that this was not the expected reciprocal relationship, was possibly due to the incubation conditions, which were originally developed for use on Norway lobsters (Fowler and Neil, 1992), being sub-optimal for the succinate reaction in *H. gammarus*.

These distributions determined by histochemistry are similar to those found previously in the claw muscles of *H. americanus* (Govind, 1995) and when considered in conjunction with the spot measures of myofibrillar proteins and SL from individual muscle fibres of juvenile *H. gammarus*, they indicate that areas with fibres of the F phenotype have high ATPase activity, while those with fibres of the S1 phenotype have low ATPase activity. This relationship between the myofibrillar protein composition and metabolic properties of muscle fibres is well established in other crustacean skeletal muscles (Neil *et al.*, 1993), and has recently been shown to correlate with the pattern of expression of genes specific for fast and slow fibre types, as indicated by *in situ* hybridisation using anti-sense probes for mRNA's of fast and slow myosin heavy chains (Mykles, 1997). This therefore justifies the use of the histochemical method to map the distribution of muscle fibre phenotypes within the claw muscles during the development period.

From their initial bilaterally symmetrical distribution in the claw closer muscles, some of the initially slow fibres were transformed to the fast type during differentiation of the cutter claw, while some of the initially fast fibres were transformed to the slow type during differentiation of the crusher claw. This corresponds to the changes described by Govind and Kent (1982) for *H. americanus*. In the juvenile lobsters used for sectioning no intermediate fibres were detectable at this boundary, and fibres in the process of transformation were encountered only very rarely. This is to be expected since specimens for sectioning were taken at the intermoult stage, as judged from moult records, in order to avoid post-moult softness of the cuticle, whereas fibre transformation is known to occur immediately postmoult, and to be completed within a few hours (Costello and Lang, 1979; Mykles, 1997).

Dimorphic development of the juvenile *H. gammarus* claw muscles occurred at all rearing temperatures, but to different extents and at different rates. The most

consistent trends were shown by the cutter claws, in which the closer muscles became composed almost entirely of fast muscle fibres. In contrast, the development in the crushers was more variable, as also found in *H. americanus* (Goudey and Lang, 1974).

5.4.1 Cutter Claw

The developmental switch in the properties of the cutter claw closer muscle fibres of *H. americanus* is reported to be determined by Stage 5, and is expressed over the subsequent moult stages when the fast fibre area begins to expand. The rate of this change increases at each moult until the fully dimorphic state is reached at around Stage 12 (Lang *et al.*, 1978; Ogonowski *et al.*, 1980). These changes occur irrespective of other factors, and thus cutter claw development has been designated as 'pre-programmed', implying a predominantly genetic control.

In the present study, the cutter claw muscles of *H. gammarus* changed progressively to their final state at all temperatures of rearing. However, evidence was obtained that this rate of change was to some extent temperature-dependent, since differences were seen between the 11 and 19°C groups at stages 10 and 11 (Fig. 5.11). This finding is important, as it suggests that the timing of a set of developmental changes in the expression of particular muscle genes, i.e. those defining the phenotypic characteristics of F and S1 fibre types, can be influenced to a degree by an identified environmental factor, i.e. temperature. This brings into doubt the validity of the term 'pre-programmed' in its strictest sense, since variations were induced in the rate of transformation, if not its final outcome, as all temperatures exhibited the complete cutter claw condition by stage 14.

A number of other, less-well-defined factors also influenced the stage at which the final dimorphic state of the cutter claw was achieved. Thus uniform rearing conditions from the larval through to the post-larval stages (1999 cohort) speeded up the rate of transformation, when compared with conditions that were subject to change (1998 cohort) (compare Figs. 5.11 and 5.15). However, the nature of these effects is difficult to specify. In contrast, the particular effects of rearing temperature on the timing of a switch in the expression of particular muscle

genes represent exploitable features of lobster claw muscle development for relating environmental changes to molecular mechanisms. This rationale has been used in a parallel study, in which molecular genetic methods have been applied to seek more direct evidence for gene switching in the claw muscles at different times in the three temperature groups (Holmes *et al.*, 2001) (see Final Discussion).

When these results of muscle fibre transformations in the cutter claw are taken together with those for claw growth (Chapter 4), it becomes possible to compare the effect of temperature on these two distinct, but related processes. The effect of rearing temperature appears to be less influential on the rate of fibre transformation than on the rate of claw growth, since fully differentiated claw muscles were attained by animals at the lower rearing temperatures, despite the claws being of a smaller size. From this finding it can be said that irrespective of rearing temperature, fully dimorphic cutter claws are achieved in all temperature regimes by stages 13-14, which is indicative of a pre-programmed process timed by moult stage.

5.4.2 Crusher Claw

In a series of elegant experiments on *H. americanus*, Govind and colleagues were able to show that the dimorphic transformation of the crusher claw closer muscle only occurred if the animal was allowed to manipulate hard objects, such as shell fragments or plastic chips, at a critical stage of post-larval growth (Stage 5) (Govind and Pearce, 1989a). Once determined, this laterality is fixed for life (Lang *et al.*, 1978), but before this point, it is dictated by which claw receives the greater mechanosensory input and produces more reflex activity. This claw becomes the crusher, while the other becomes the cutter (Govind and Pearce, 1986). The mechanism underlying this effect is thought to involve the claw ganglion, and to relate to the levels of neuronal activity on the two sides.

Govind and Lang (1982) found that 91% of lobsters (*H. americanus*) given chips to manipulate became dimorphic, while 97% of those without chips were non-dimorphic (double cutter claws). Wickens (1986) showed that the proportion of Stage 10-11 *H. gammarus* bearing fully functional, well-differentiated crusher

claws increased from approximately 30% to over 80%, following the provision of a few oyster spat to the rearing chambers. If this external stimulation was denied or lacking, then the transformation of the crusher claw closer muscle was most often incomplete or arrested, resulting in a 'false crusher'. Double cutters were found in some of the animals that were sectioned, but most were seen to have some trend towards crusher claw development, and it must be taken into consideration, that once sectioned the lobsters can develop no further, and so any double cutter claws found, could be attributed to slow development and not simply that they would not develop into a 'true' or 'false' crusher.

Under the rearing programmes of the current study, lobsters were kept in chambers with a mesh floor, which gave some opportunity for their claws to grasp, and they were also provided with soft food items which they would manipulate with their claws. However they were not offered any hard items to manipulate. Without this additional stimulation to ensure normal crusher development, 'false' crushers or double cutter claws were to be expected, and indeed the former were found to occur frequently. There was a great deal of individual variation in this within both the 1998 and 1999 cohorts, with both true and false crushers being produced at all temperatures of rearing. The fact that the variability was just as evident in the 1999 cohort, which was raised under more uniform holding conditions, is consistent with a lack of manipulation being the cause of false crushers. However, the true crushers that developed in lobsters of the 1999 cohort did so at a more rapid rate than in the 1998 cohort, suggesting that uniform rearing conditions did promote crusher claw transformation once it had been initiated.

Another feature of the false crushers was the variable degrees of transformation in their closer muscles from fast to slow fibres, which was still evident in the 1998 cohort even after 17 months. This was despite the fact that at this time the animals reared at all three temperatures had reached at least moult stage 13, which is reported to be the stage at which dimorphic switching is complete in *H. americanus* (Govind, 1992). This suggests that the false crusher state may be a persistent one, and that some animals may never progress to the fully dimorphic condition.

In contrast to the extensive occurrence of false crushers, the juvenile *H. gammarus* in this study rarely displayed the 'double cutter' claw condition (the occurrence being higher in the 1999 cohort (~40%) than the 1998 cohort (~36%)). Measures of claw dimensions (Chapter 4) and claw forces (Chapter 6) corroborate this finding. This differs from the results of other studies done in the absence of a manipulative substrate, in which high percentages of double cutters have been reported, both in *H. americanus* (Govind and Lang, 1982) and *H. gammarus* (Wickens, 1986). Taken together with the relatively high frequency of false crushers, the most likely explanation for this absence of 'double cutters' in the present study is that the holding conditions (mesh floor, food items) offered sufficient opportunity for exercise of the claws to initiate dimorphic switching, but insufficient manipulation to ensure that the transformation of the crusher claw muscle fibres from fast to slow was complete.

While the frequent occurrence of false crushers confounds any systematic attempt to compare the effects of different rearing temperature on crusher claw muscle development, this circumstance is nevertheless informative, since it provides an opportunity to compare the functional properties of 'true' and 'false' crushers in relation to their size, and to the age and moult stage of the lobsters possessing them. These are described in Chapter 6.

Mean Sarcomere Lengths:				Muscle Fibres From:		Cutter	Crusher
Temperature	Cohort	Age at Sampling	Stage	FF (Fast Flexor)	MSF (Medial Super Flexor)	M (Medial (claw))	V (Ventral (claw))
11	98	18 months	13	1.99 (0.20)	7.46 (0.18)	2.95 (0.39)	10.24 (0.05)
19	98	18 months	15	3.40 (0.03)	6.16 (0.13)	1.98 (0.01)	6.16 (0.05)
15	99	6 months	9	2.12 (0.01)	7.91 (0.24)	1.83 (0.02)	8.62 (0.11)
19	99	6 months	11	2.87 (0.06)	8.45 (0.42)	1.81 (0.02)	7.36 (0.10)

Table 5.1: Mean values for the sarcomere lengths (μm), derived from measures of at least 50 sarcomeres in each of 4-8 fibres, from the same area of muscle tissue for each lobster. The values for cutter closer medial band have been taken to ensure that fast type fibres are sampled, and from the crusher closer ventral region to ensure that slow type fibres are sampled. Values in brackets are +/- Standard Error.

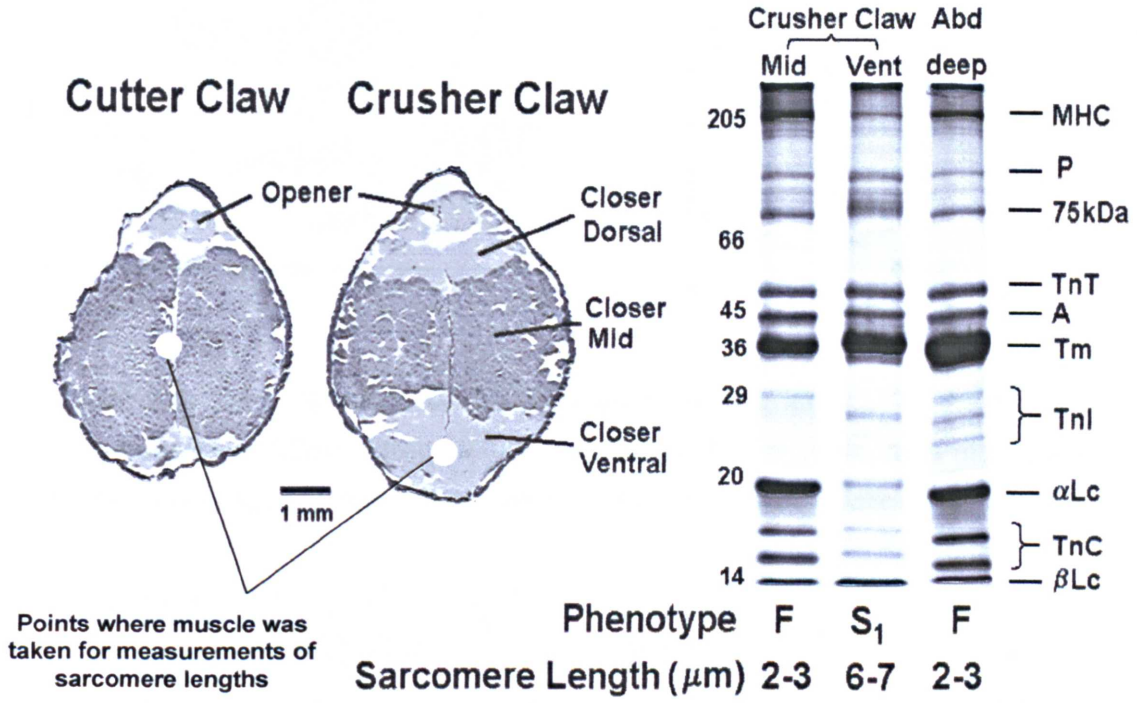


Figure 5.1: Criteria for identifying claw muscle fibre types: the expression of *particular isoforms or regulatory and contractile (gels or in situ hybridization)*, lobster claw muscle fibres can be either of the fast (F) or slow (S_1) phenotypes, according to the various myofibrillar protein isoforms that they express.

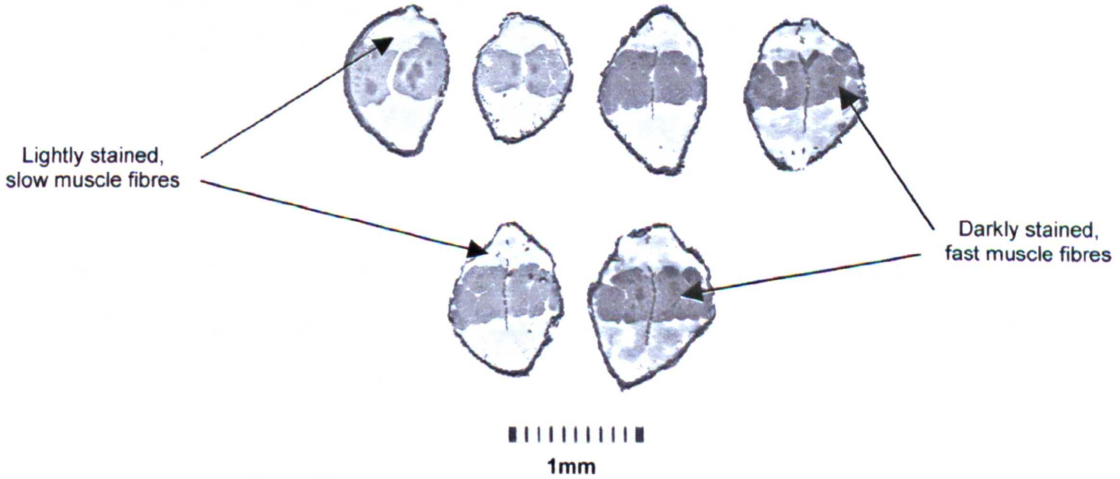


Figure 5.2: The claw closer muscles of animals imported from Orkney, which we *have used as a reference point for immediately post-larval animals (Stage 8).*

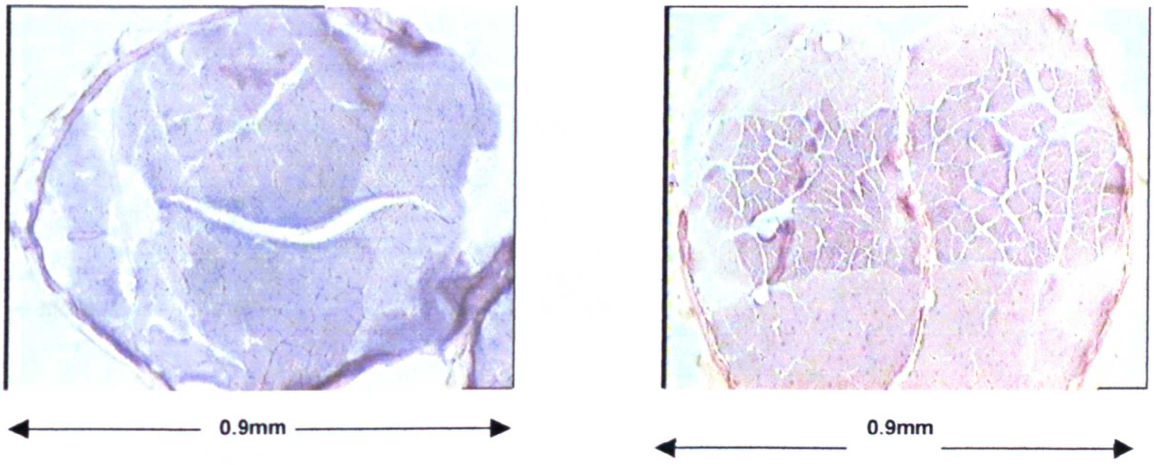


Figure 5.3: These sections of the claw were stained, looking for the Succinic Dehydrogenase (SDH) activity, a known mitochondrial enzyme, as a measure of the oxidative capacity of the fibres, which is indicative of their fatigue resistance.

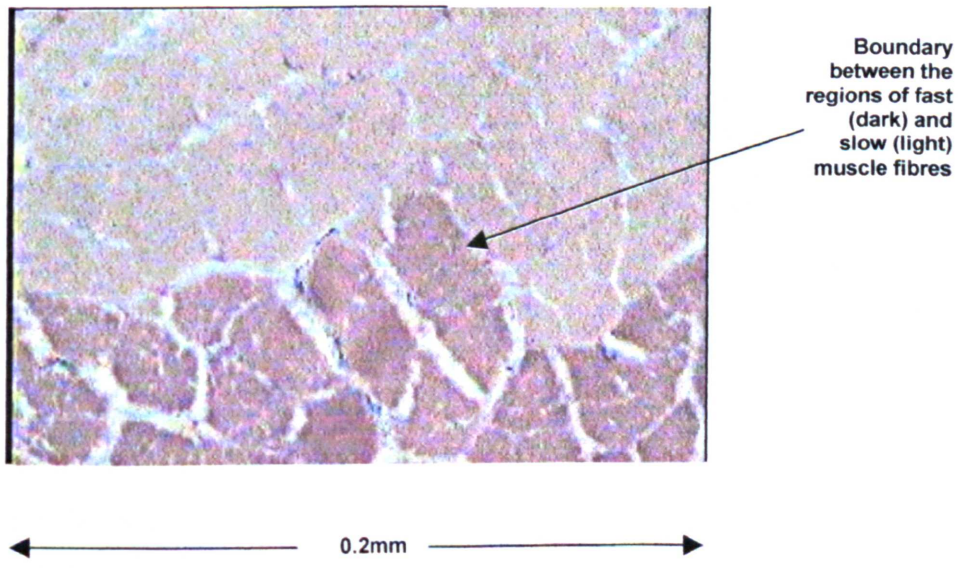


Figure 5.4: Using the stain for SDH again, this section shows the distinct boundary between the fast muscle fibres (darkly stained) and the highly oxidative slow muscle fibres (lightly stained).

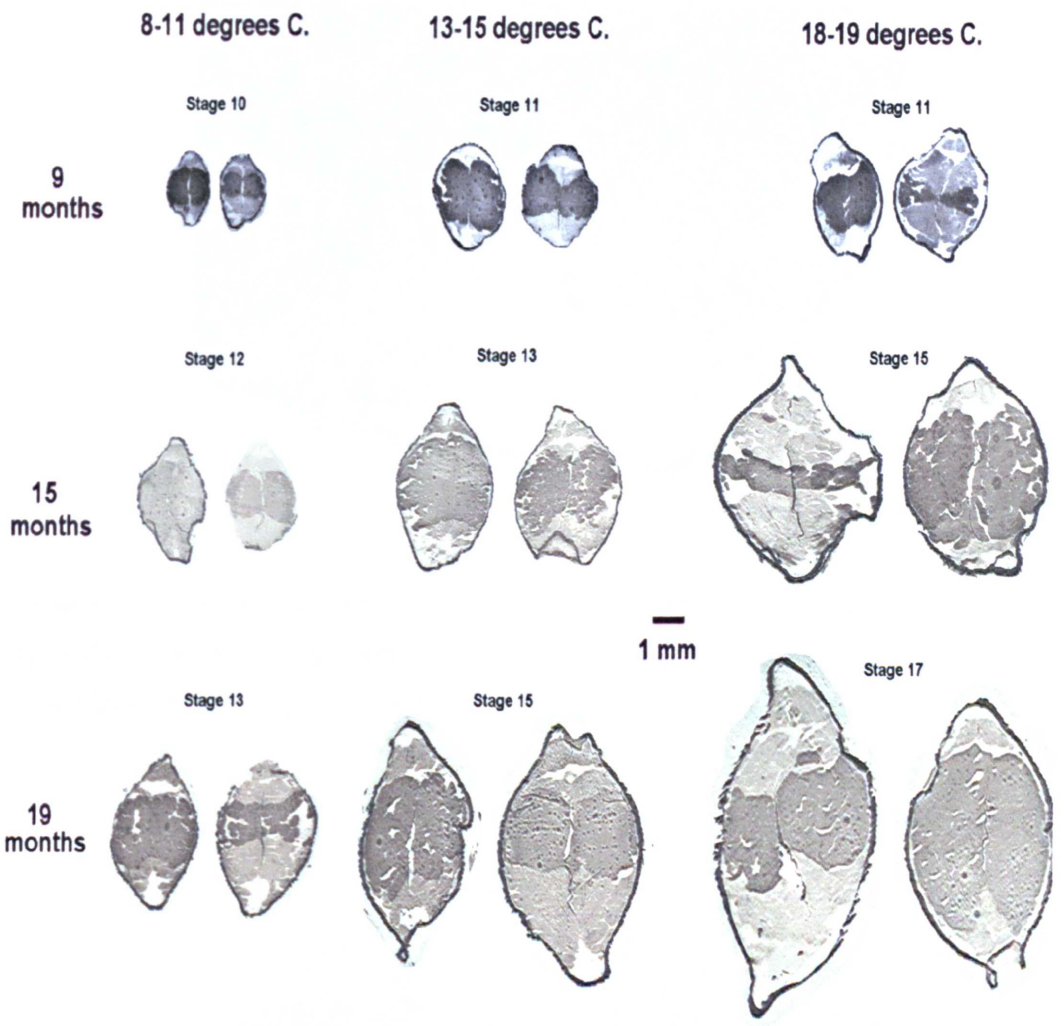


Figure 5.5: Summary of the histochemical results from the lobsters of the 1998 cohort reared at the different temperatures (11, 15 and 19°C) (NB: 19°C stages 15 and 17, Crusher claws are on the left and cutter claws are on the right, for all *others cutter claws are on the left and crusher claws on the right*).

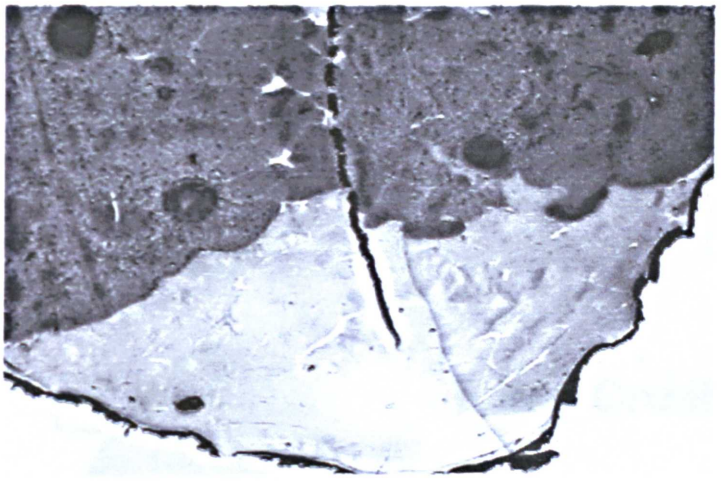


Figure 5.6: A section from a 15°C stage 11 lobster. It shows the clear cut boundary between the fast and slow muscle, highlighted by the different characteristic staining of fast and slow muscle myofibrillar ATPase.

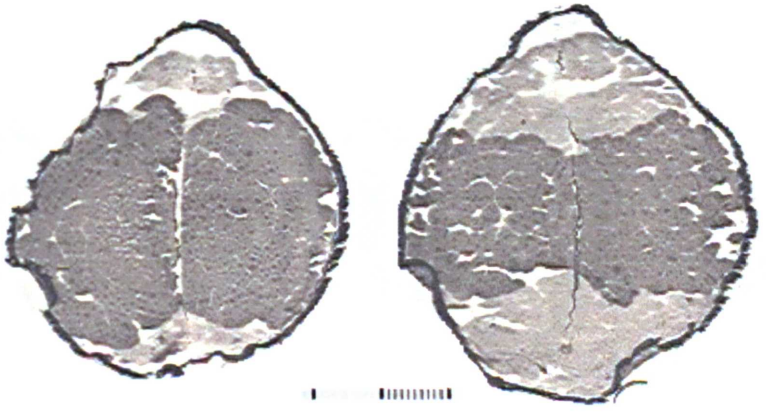


Figure 5.7: This pair of claws is from a stage 15 animal reared at 19°C (aged 15 months). Scale bar – 2mm.

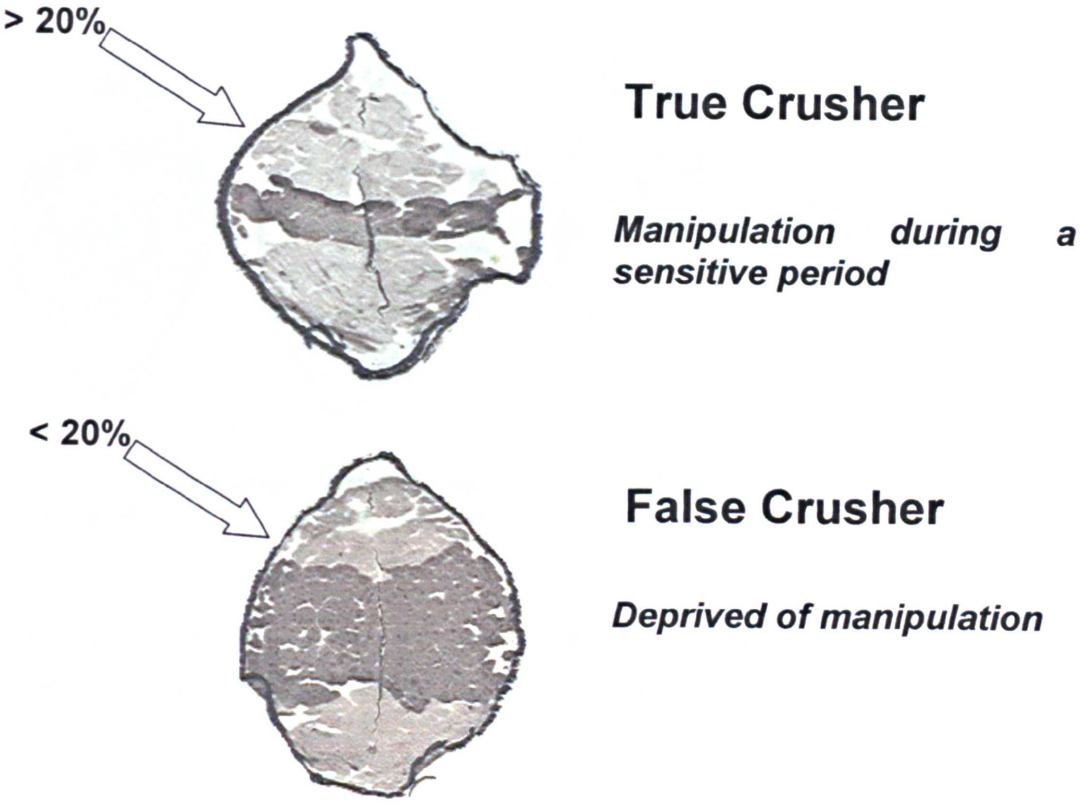


Figure 5.8: Criteria for the characterisation of 'true' and 'false' crusher claws

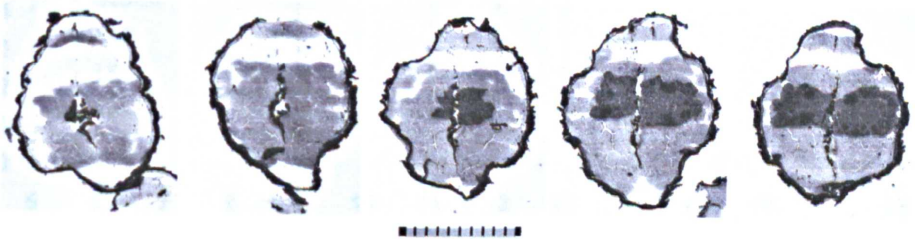


Figure 5.9: Serial sections of one claw to show how the muscle fibres change along the length of the claw. Scale bar – 1mm.

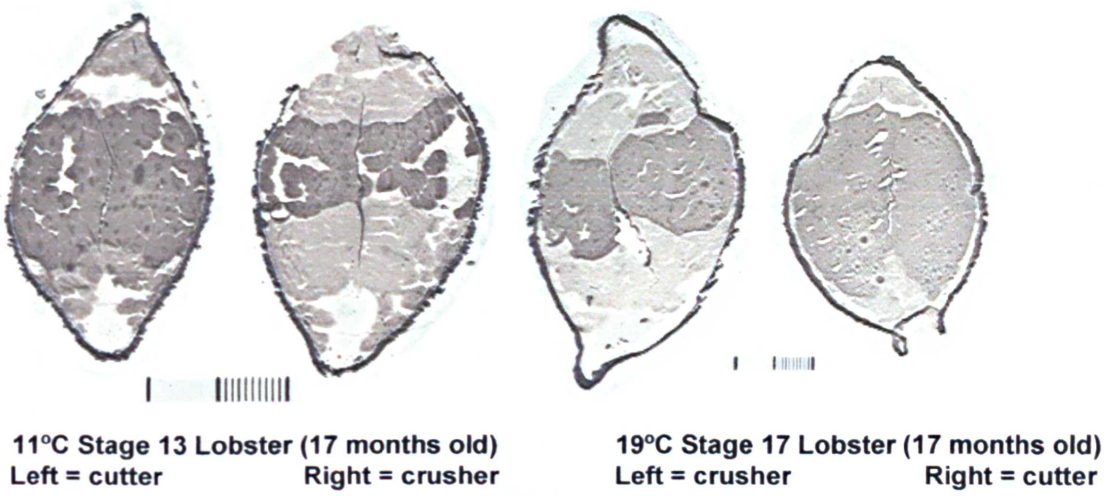


Figure 5.10: The claws of two lobsters of the 1998 cohort from the extremes of rearing temperature, at the end of the 17 month acclimation period. Scale bar – 1mm.

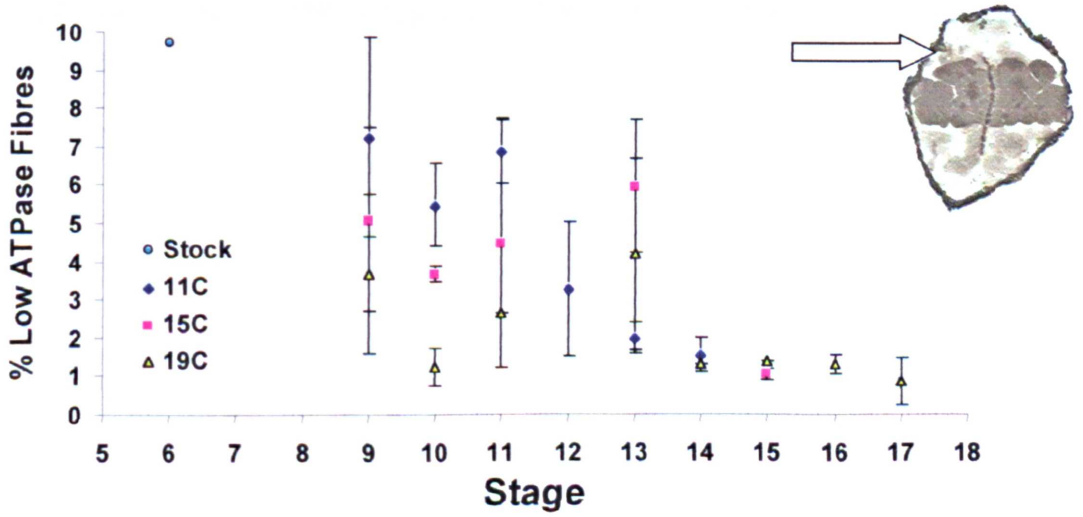


Figure 5.11: The change in the relative size of the dorsal low ATPase area of the cutter closer muscle plotted relative to stage, for each temperature group of the 1998 cohort. Stock: the initial value for the dorsal low ATPase region at stage 6 (3 months). Mean values from: n = 19 at 11°C, n = 13 at 15°C, n = 15 at 19°C.

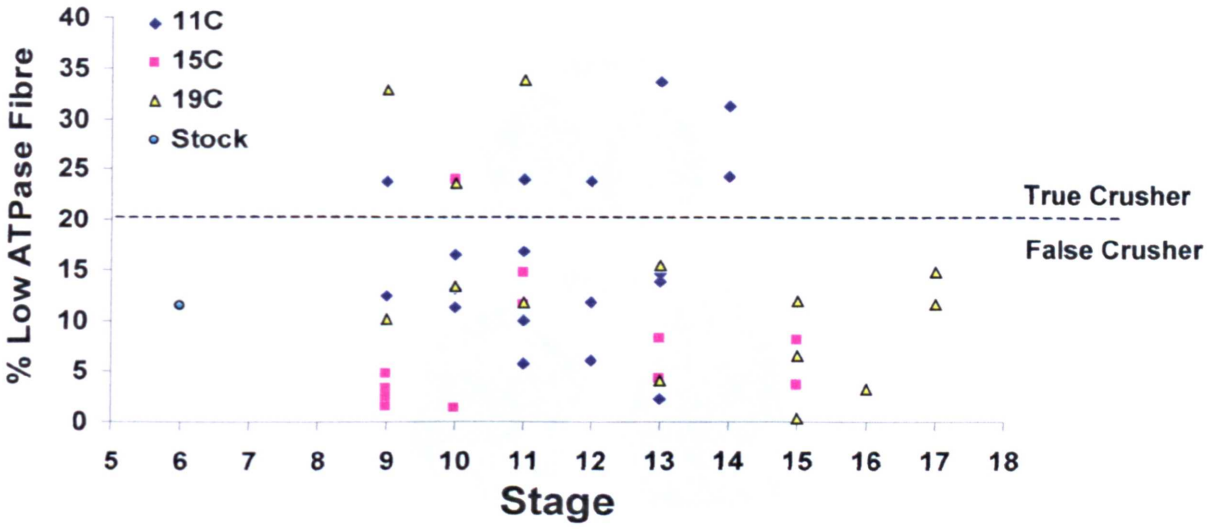


Figure 5.12: The change in the relative size of the dorsal low ATPase area of the crusher closer muscle plotted relative to stage, for each temperature group of the 1998 cohort. Stock: the initial value for the dorsal low ATPase region at stage 6 (3 months). Dashed line: the arbitrary threshold for distinguishing between True and False Crushers (20% dorsal low ATPase region) Individual claw measures are plotted.

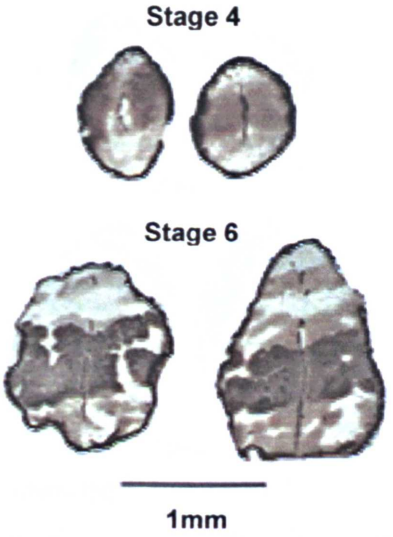


Figure 5.13.a: Examples of claws from the 1999 15°C cohort of lobsters, at the age of 3 months. The pairs of claws are undifferentiated at this time. Scale bar – 1mm.

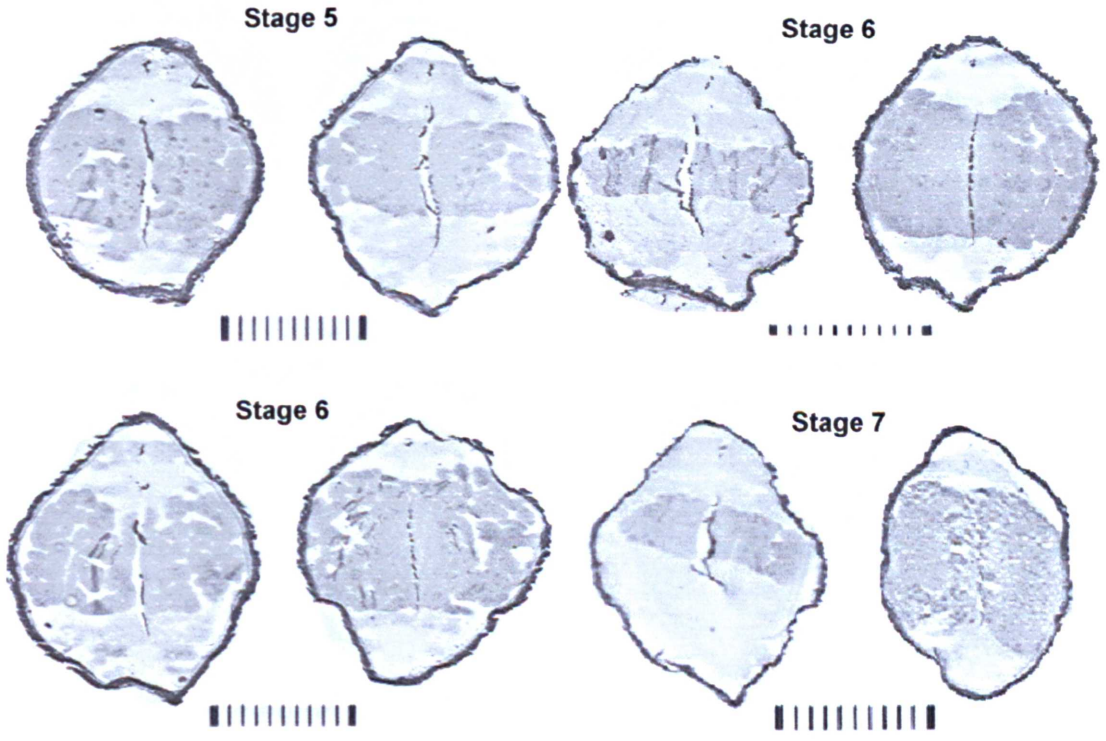


Figure 5.13.b: Examples of claws from the 1999 19°C cohort of lobsters, at the age of 3 months. There is individual variability in the claw muscle fibre compositions. Scale bars - 1 mm.

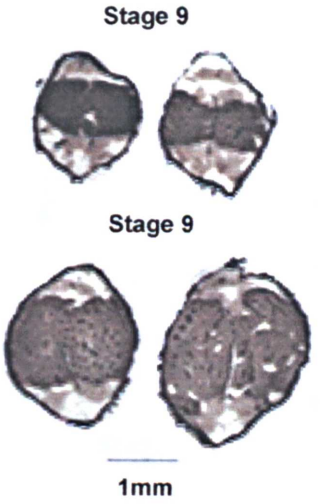


Figure 5.14.b: Examples of claws from the 1999 15°C cohort of lobsters, at the age of 6 months. The development of the dimorphic claws of the 15°C raised animals has not progressed, with both pairs of claws being identical. Scale bar - 1 mm.

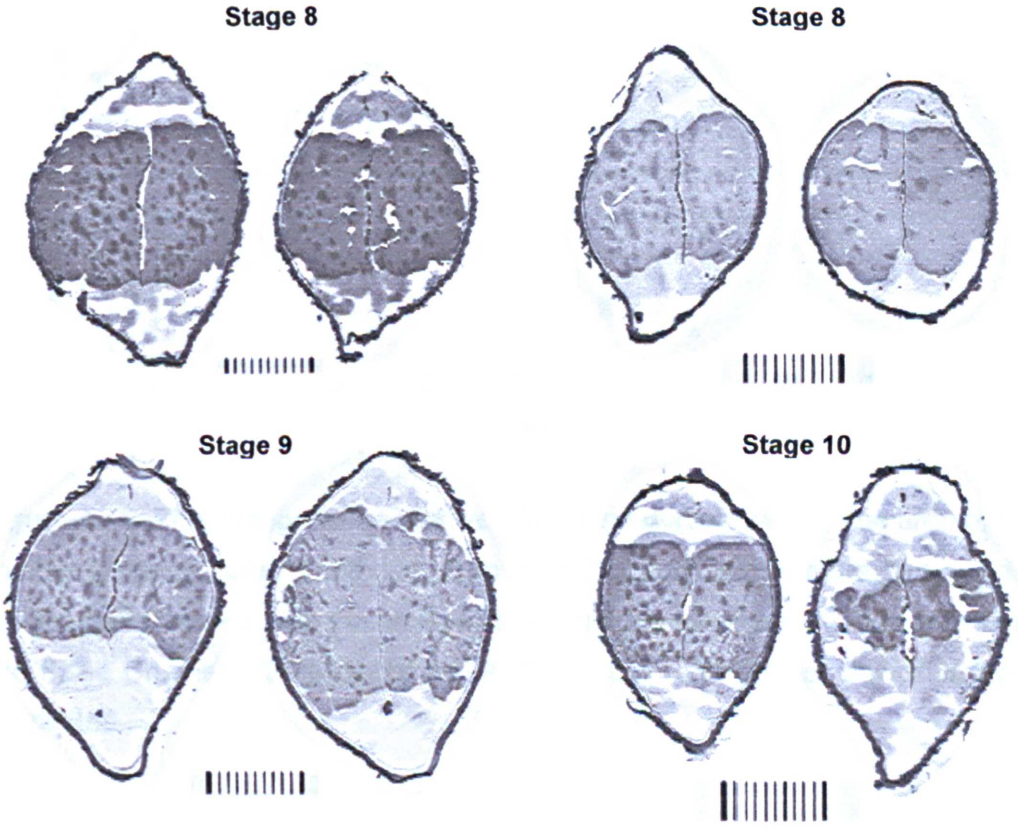


Figure 5.14: Examples of claws from the 1999 19°C cohort of lobsters, at the age of 6months. The development of the dimorphic claws has progressed in these animals. Scale bars - 1 mm.

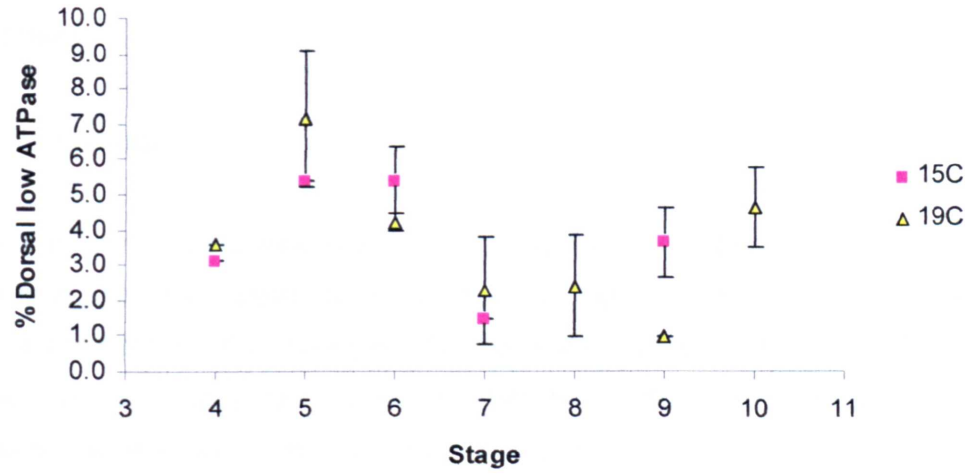


Figure 5.15: The change in the relative size of the dorsal low ATPase area of the cutter closer muscle plotted relative to stage, for each temperature group of the 1999 cohort. Mean values from: n = 11 at 15°C, n = 17 at 19°C.

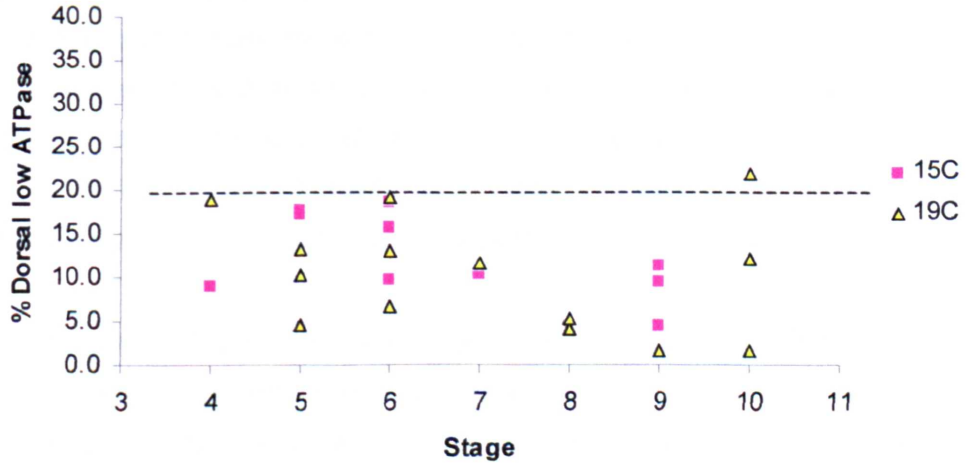


Figure 5.16: The change in the relative size of the dorsal low ATPase area of the crusher closer muscle plotted relative to stage, for each temperature group of the 1999 cohort. Dashed line: the arbitrary threshold for distinguishing between True and False Crushers (20% dorsal low ATPase region) Individual claw measures are plotted.

Chapter 6 – The voluntary claw contractions of the juvenile *Homarus gammarus*

6.1 Introduction

In conjunction with the measurements of growth and muscle differentiation in the developing juvenile lobster claws, measures of the forces produced by voluntary contractions of the claw closing muscles were also made, in order to assess how these changed with growth at the different temperatures of rearing, and also between the two claw types: the crusher and cutter.

By their nature, voluntary claw contractions are subject to variability, especially when produced in response to an artificial stimulus such as the presence of a strain gauge. However, preliminary trials indicated that the response was consistently elicited by this artificial stimulus, and occurred repeatedly in response to successive presentations. Since the test could be applied quickly and non-invasively, with minimum disturbance to the animal, it provided a useful screening procedure that could be applied repeatedly to individuals at different times during their period of growth. In this way, the need to sacrifice animals for the purpose of determining their physiological responses was avoided.

In order to determine how the forces generated in these voluntary contractions compared with the maximum elicitable claw closing force, a series of calibration tests was also performed on a subset of animals using the technique developed by Jahromi and Atwood (1969) of injecting a high potassium / caffeine solution into the claw in order to fully depolarise and activate all the fibres of the claw closer muscles.

6.2 Materials and Methods

6.2.1 Measurement of isometric force of claw closing

To test claw closing force, the lobsters were taken from their holding compartments and transferred to a 'dry' room in chambers of water at the

temperature of rearing immediately prior to testing the lobsters were removed from the chambers, placed on a damp towel, held stationary and voluntarily grasped a calibrated force meter (Figs. 6.1.a+b). Three gauges with different ranges of force sensitivity were used according to the size of the claws. The largest (see Fig 6.1) was of the design described by Preston *et al.* (1996), the other two were based on the Grass FT03 gauge, one with and one without the springs inserted so that they operated in different working ranges. Generally, the animals performed this test readily over numerous replicate trials; however on some occasions the lobsters were completely unresponsive.

The output signal of the force transducer was fed to an A/D converter (MACLAB) and displayed on a Macintosh computer using custom software (CHART). From the recorded traces, the initial squeeze force and the sustained grip force of both the cutter and crusher claws of lobsters were measured. These results were then transferred to a spreadsheet (Microsoft EXCEL), for calculations, analysis and plotting to be carried out. Prior to claw force testing, the strain gauges were calibrated using weights (g), to allow conversion of the voltage readings to values in milli-Newtons (mN) as a standard measure of force. The peak force values attained by the cutter and crusher claws were also used in conjunction with measures of claw dimensions (claw cross-sectional areas derived as reported in Chapter 4), to derive values of force per unit area (mN mm^{-2}). The rate of isometric force development to the peak force (mN s^{-1}) was also measured (Fig. 6.2.a).

As well as *in vivo* force measurements, *in vitro* tests were performed, based on those carried out by Warner and Jones (1976) on *Cancer paguras* and *Macropipus depurator*, in order to ascertain the maximal closing force that could be produced by the claws. This involved detaching the cutter and crusher claws and cutting the opener muscle apodeme at the point of insertion onto the dactyl, to prevent any interference from the opener muscle. The propus was clamped into a fixed position, and the dactyl was attached to a calibrated balance via a wire loop. The chela were induced to close by injecting a high concentration of K+ with added caffeine (400mM K+/40mM caffeine in lobster saline), (Jahromi and Atwood, 1969), into the middle of the closer muscle, thus producing the maximum attainable force of the chela (Elner and Campbell, 1981). This was a

more convenient alternative to exposing the nerve of the chela and electrically stimulating it.

In some cases this procedure was modified, so that instead of removing the claws the lobsters were held in a position with the claws open over the strain gauge before injection of the high K+/caffeine solution (approximately 5ml, but depending on the size of the animal). The resultant force was recorded in the same way as for the voluntary contractions. This method on intact animals was equally effective to the use of autotomised claws, but often itself induced autotomy.

There were three main sampling points for the 1998 cohort, the first of which was in March 1999, when the lobsters were 9 months old and generally ranged from post-larval stages 9-11 (13 at 19°C). The second was in September 1999, when the lobsters were 15 months old and the last occurred in November 1999, when the lobsters were 17 months old. The 1999 cohort was tested twice, at 3 and 6 months of age, in August/September 1999 and November 1999, respectively.

6.3 Results

6.3.1 Force profiles

Standard responses elicited by the two claws of adult (3 year) lobsters when presented with the strain gauge were obtained as a reference. Typically, the crusher claw produced a large initial peak of closing force, followed by a series of less powerful closures producing a plateau of force. This could either rise or fall in magnitude until the grip was released. Similar patterns were produced by the juvenile lobsters, and the characteristic differences in the force profiles of the presumptive crusher and cutter claws was discernible from as early as Stage 4 (the youngest animals tested) (Fig. 6.2.a).

6.3.2 Maximum elicitable responses

Measurements of the maximum elicitable closing forces were performed on autotomised claws of a small number of lobsters at different ages. Following the measurement of its voluntary response (left panels in Fig. 6.2.b) each claw was autotomised by applying pressure close to the basi-ischiopodite joint. This, itself induced claw closure during the autotomy fracture, and this force was also monitored (middle panels in Fig. 6.2.b). When this force had subsided, the claw was injected with the high K+/caffeine solution (see Methods) to induce maximal contraction, which produced a persistent plateau of force (left panels in Fig. 6.2.b). The cutter and crusher claws of the sampled individuals were tested in this way in turn.

Comparing the peak voluntary responses with the induced maximal responses of individual claws, it can be seen that in all the tested animals the former were within ~10% of the latter.

6.3.3 Peak isometric forces

6.3.3.1 1998 Cohort

For the 1998 cohort at 9 months of age (March 1999), the peak closing forces for the crusher claws, as represented by the mean values for each of the stages represented in the figure legends, at each sampling point for each of the temperature regimes, were as follows: 6.6 mN ± 1.32 for 11°C, 19.8 mN ± 6.8 for 15°C and 65.8 mN ± 3.7 for 19°C. These peak closing forces were therefore greater in the crusher claws (65.8 mN ± 3.7) than in the respective cutter claws (36.1 mN ± 17.1) of the 19°C (Figs. 6.3.a+b) (ANOVA F-statistic, $F_{1, 68} = 11.40$, $p = 0.001$) and 11°C groups (ANOVA F-statistic, $F_{1, 54} = 5.96$, $p = 0.018$) but there was virtually no difference between the claws in the 15°C (ANOVA F-statistic, $F_{1, 56} = 1.48$, $p = 0.229$) (Figs. 6.3.a+b). Between the crusher and cutter claw areas significant differences occur at 15°C (ANOVA F-statistic, $F_{1, 56} = 6.94$, $p = 0.011$) and 19°C (ANOVA F-statistic, $F_{1, 68} = 14.70$, $p = 0.000$), but not at 11°C (ANOVA F-statistic, $F_{1, 54} = 0.82$, $p = 0.370$).

Analysing the data that has given rise to these means (means of claw area and peak force for the each of the stages in the represented range in the figure legend), shows that for both crusher and cutter claw areas, there are significant differences between all the temperature groups at 9 months (Crusher: ANOVA F-statistic, $F_{2, 86} = 123.59$, $p = 0.000$; Cutter: ANOVA F-statistic, $F_{2, 92} = 96.8$, $p = 0.000$). For the crusher claw peak forces, differences between the 11 and 19°C and the 15 and 19°C group are apparent (ANOVA F-statistic, $F_{2, 86} = 23.89$, $p = 0.000$), showing the higher temperature group to be developing at a greater rate. When the cutter claw peak forces are analysed, the differences are between the 11 and 15°C and the 11 and 19°C group (ANOVA F-statistic, $F_{2, 92} = 22.54$, $p = 0.000$) showing how the 11°C group is reduced in it's development compared to the others.

At 15 months the peak forces produced by the crusher claws had increased to 206.8 mN ± 31.7 for the 19°C group and 53.5 mN ± 6.7 and 41.2 mN ± 4.2 for the 15°C and 11°C groups. Also, by this time, the difference between the crusher and cutter claw peak closing forces was apparent between the 11 and 19°C temperature groups (Figs. 6.4.a+b) (11°C: ANOVA F-statistic, $F_{1, 44} = 34.31$, $p = 0.000$; 15°C: ANOVA F-statistic, $F_{1, 26} = 1.94$, $p = 0.176$; 19°C: ANOVA F-statistic, $F_{1, 50} = 32.45$, $p = 0.000$). For both crusher and cutter claw areas at 15 months there are significant differences between all the temperature groups (Crusher: ANOVA F-statistic, $F_{2, 54} = 111.45$, $p = 0.000$; Cutter: ANOVA F-statistic, $F_{2, 67} = 137.39$, $p = 0.000$). There are significant differences in the crusher and cutter claw areas at each temperature group at 15 months of age (11°C: ANOVA F-statistic, $F_{1, 44} = 13.08$, $p = 0.001$; 15°C: ANOVA F-statistic, $F_{1, 26} = 10.19$, $p = 0.004$; 19°C: ANOVA F-statistic, $F_{1, 50} = 18.81$, $p = 0.000$).

Plotting peak closing force against claw area, it can be seen that the force per unit area of crusher closer muscle has values of: 7.6 ± 0.9, 4.1 ± 0.5 & 5.9 ± 0.9 ($mN\ mm^{-2}$) for 19°C, 15°C and 11°C respectively. The values for the 15°C and 11°C groups are similar to each other, indicating that the smaller claws of the 11°C lobsters are capable of producing similar forces to the larger claws of the 15°C group, with significant differences between the 11 and 19°C and the 15 and 19°C groups (ANOVA F-statistic, $F_{2, 54} = 24.29$, $p = 0.000$), indicating the advancement of the 19°C claw growth. The values for the cutter claws (Fig. 6.4.b)

show that at 19°C the claw area has increased by an amount similar to the crusher (three fold), but the force produced has increased only two fold (compared with three fold for the crusher). The 11°C cohort has increased in force production by approximately 6x, and in claw area by around three fold, whereas the 15°C cohort has increased the least amount, with a force increase of just over half the previous value (March 1999), and the claw area has doubled in the 6 months since previously being measured. For the cutter claw it can be seen that there are significant differences between the 11 and 19°C and the 15 and 19°C groups (ANOVA F-statistic, $F_{2, 67} = 26.46$, $p = 0.000$), indicating how the 19°C claws are stronger than the lower two temperature regimes.

At 17 months, compared with the values at 15 months, force production by the 19°C group remained very similar, even though claw closer muscle area increased by a factor of 1.3 (Fig. 6.5.a). In the 15°C group the crusher claws increased their force production 3 fold, while the claw area increased by a factor of 1.5 times. At all temperatures there were significantly higher forces produced by the crusher claws to the respective cutter claws (11°C: ANOVA F-statistic, $F_{1, 38} = 41.50$, $p = 0.000$; 15°C: ANOVA F-statistic, $F_{1, 17} = 19.73$, $p = 0.000$; 19°C: ANOVA F-statistic, $F_{1, 38} = 9.98$, $p = 0.003$). Between all the temperatures, there are again differences between the claw area values at 17 months (ANOVA F-statistic, $F_{2, 45} = 53.2$, $p = 0.000$). In the 11°C group force production was doubled, even though the increase in claw area was very small, but was the only one of the temperatures to have significantly different cutter and crusher claw areas at 17 months (11°C: ANOVA F-statistic, $F_{1, 38} = 5.46$, $p = 0.025$; 15°C: ANOVA F-statistic, $F_{1, 17} = 0.46$, $p = 0.506$; 19°C: ANOVA F-statistic, $F_{1, 38} = 0.71$, $p = 0.406$).

Therefore despite a general trend for closing forces to increase with increasing claw area, most clearly seen in the 19°C group, exceptions occurred such that claws with smaller areas (eg. of 15°C group compared to the 19°C group at 17 months) produced similar forces as can be seen from the statistical analysis, where there are significant differences between the 11 and 15°C and the 11 and 19°C groups but not between the 15 and 19°C groups (ANOVA F-statistic, $F_{2, 45} = 10.32$, $p = 0.000$).

This effect can also be seen in the results for the 1998 cutter claws at 17 months (Fig. 6.5.b). The cutter claws of the 19°C group have doubled their forces, whereas their claw closer muscles have increased in area by only 1.5 times. The 15°C group has doubled both the forces produced and the claw closer area, bringing them to the values similar to those of the 19°C group after 15 months. In the 11°C group, however, there is no increase in attainable peak force or claw area, compared with the equivalent values at 15 months. The temperature regimes, all show a significant difference in the claw areas at 17 months (ANOVA F-statistic, $F_{2, 48} = 102.08$, $p = 0.000$). When the cutter claw forces are examined at 17 months, it can be seen that there are differences between the 11 and 19°C and 15 and 19°C groups (ANOVA F-statistic, $F_{2, 48} = 13.93$, $p = 0.000$), showing the continued greater differences exhibited by the 19°C groups.

When the crusher claw force values for individual animals at all temperatures of rearing and for all sampling points are plotted against their claw closer muscle areas, it can be seen that there is a large variation in the peak forces at the different claw areas *(*Fig. 6.6.a*)*. Moreover, individuals with a 'false' crusher (i.e. incomplete transformation to claw closer muscles to slow fibres - see Chapter 5) ('F' on Fig. 6.6.a) appear able to produce forces as large as those with a 'true' crusher (unmarked on Fig. 6.6.a).

When the cutter claw force values for individual animals at all temperatures of rearing and for all sampling points are plotted relative to their claw closer muscle areas, there are some differences to the crusher claws, which are apparent (Fig. 6.6.b*)*. The crusher claws show greater variation in both attainable peak forces and claw areas, compared to the cutter claws, which show a more uniform trend. The main differences are that by 17 months (November 1999), the crusher claws for all temperatures show an elevated rate of change compared to the previous sampling sessions, whereas the cutter claws at all temperatures continue to follow the earlier trend. These trends are most obvious in the 11 and 15°C groups, whereas the 19°C group shows more of an increase at the end of the rearing period in the cutter claw values, deviating away from the almost flat trends exhibited by the lower temperature treatments. The respective claw areas of the two types of claws combined with the force results highlight the differences

in claw properties, as the differences in size are minor, but the forces attained are increased.

6.3.3.2 1999 Cohort

The 1999 cohort of lobsters was examined in the same way as the 1998 cohort. At Stage 5 (3 months) the 19°C lobsters exhibited greater maximal closing forces than the 15°C group (Fig. 6.7.a). Also, at this stage there were differences between the presumptive crusher and cutter claws in each temperature group (Fig. 6.7.b). For both the 15°C and 19°C groups the cutter claw peak forces were around the same value, between 5-10 mN, whereas the crusher claw values were 10-20 mN for the 15°C group and 10-32 mN for the 19°C group. Claw area differences between crusher and cutter exist for the 19°C group, but not for the 15°C group, and so cannot be the sole basis for these differences in performance.

At 6 months of age, when body and claw dimensions have increased (Body Length – from 21.81 mm ± 0.46 to 26.8 mm ± 2.55 for the 15°C group and from 26.97 mm ± 0.89 to 36.6 mm ± 0.79 for the 19°C group), but there is still only a small difference in the claw areas between crusher and cutter (Figs. 6.8.a+b) (15°C mean crusher claw area = 2.92 ± 0.27, cutter = 2.30 ± 0.20, 19°C mean crusher claw area = 5.89 ± 0.17, cutter = 4.87 ± 0.15 (mm^2)), the peak forces produced by crusher closing are similar in the 15°C and 19°C groups (40 mN). A greater temperature-dependent difference is seen in the cutter peak closing forces (Fig. 6.8.b), although this is not as large as might be expected from the differences in claw area. Dimorphic differences are apparent between crusher and cutter for both temperature groups.

The peak closing force values for the crusher claws of individual animals (Fig. 6.9.a) show that the attainable forces reach the same level in both the 15°C and 19°C groups, even though the claws grow to a larger size at 19°C (see above). This suggests that a larger claw area does not necessarily increase the force production of the juvenile claws.

The peak closing force values for the cutter claws of individual animals (Fig. 6.9.b) show the more expected relationship between force and area for the two temperature groups. Moreover, the development of the 15°C lobsters seems to be more uniform than the cutter claws of the 19°C group, which show a great deal of variation.

6.3.4 The rate of isometric force development

6.3.4.1 1998 cohort

In the 1998 cohort at 9 months, the rates of isometric force development increased with the temperature of rearing for both the cutter and crusher claws, and were greater for the crusher claws compared with their respective cutter claws (Figs. 6.10.a-c).

At 15 months the rates of force development were greater (eg. for the 19°C cohort by ~4x in the crusher and ~2x in the cutter) but the relationships between claws and temperature groups were similar (Figs. 6.11.a-c).

At 17 months (Figs. 6.12.a-c), the 11°C group shows very little difference from the rates shown at 15 months. The 15°C cohort crusher claw contraction rates have increased the most, as the values at Stages 11 and 15 are comparable to the 19°C rates at 15 and 17 months. The 15°C cutter claw rates have remained at approximately the same range of 20 mN s^{-1}, between the two sampling points. The 19°C cohort contraction speeds are very similar to the rates expressed at 15 months, with the crusher claw values being ~100 mN s^{-1} and those of the cutter at ~30 mN s^{-1}. When the data for the crusher claws at the three temperatures is compared over the acclimation period (Fig. 6.13.a), it can clearly be seen how the 19°C crusher claws show the greatest increase in contraction rate over the time period, with the 11°C cohort showing very little change over the same period, and could be compared to the cutter claw rates for the 15 and 19°C groups. The same effect of increasing contraction rates with size/age and temperature is seen within the cutter claws of the lobster populations but at a far reduced level than for the respective crusher claws, especially at 11°C (Fig. 6.13.b).

From these results it can be deduced that at a higher rearing temperature the rates of force development are greater than those at the lower temperatures, for both claw types. These rates also increase with age/size within each temperature group.

6.3.4.2 1999 Cohort

When the 1999 cohort is analysed in the same way, the predominant difference between the temperature groups (15 and 19°C) is for the crusher claws to develop force at a greater rate than their respective cutter claws, both at 3 months (Figs. 6.14.a+b) and at 6 months (Figs. 6.15.a+b). The 19°C group shows the greatest increase in rates of contraction for the cutter claws from 3 and 6 months, but it is apparent that by the end of the rearing period the rates of contraction exhibited by the crusher claws at the two temperatures are almost identical.

6.4 Discussion

The functionality of the developing claws in the juvenile *H. gammarus* is highly relevant to this study, as the claws are vital to the survival of this and other clawed species of crustaceans. The measures of the claw forces of the juvenile lobsters both define the power of the two claw types, and also provide useful predictors of claw dimorphic development.

6.4.1 Voluntary claw contractions

The choice of voluntary contraction tests to measure claw force was enforced by the need to make repeated measures at different stages of development, and for the stock of animals to be maintained through to the end of the growth period for other purposes. However this approach was made feasible by the fact that the animals showed a great readiness to grasp the strain gauge, a finding that is consistent with the general observation that animals kept in captivity are known to have higher aggression levels (Huntingford and Turner, 1987). The tests of force production by isolated claws made *in vitro* by flushing them with high K^+/caffeine

solution (Fig. 6.2.b), as used by Jahromi and Atwood (1969) and Elner and Campbell (1981), indicate that the voluntary forces were in fact comparable to those produced by the maximal pharmacological activation. This finding justifies the use of the voluntary protocol for comparing the force production by different claws at different stages.

The patterns of contraction shown by the crusher claws of juvenile lobsters are in accordance with those described by Elner and Campbell (1981) for adult lobsters, in that the peak tension was sustained only briefly. On the basis of muscle fibre properties, Costello and Lang (1979) postulated that the crusher claw is highly specialised for maintaining a large amount of tension for prolonged periods. In the present study a plateau of force was observed after the initial peak that was often sustained at quite high levels for long periods of time, with a degree of oscillation, indicating repeated contractions around the same point. This may represent an effective way to open a shell, since repeated applications of stress will induce and extend fractures (Boulding and LaBarbera, 1986; Block and Rebach, 1998). Similar behaviour is shown by the crusher claw of the stone crab, *Menippe mercenaria* (Blundon, 1988); when presented with a force transducer, it immediately applied maximum force to the transducer for less than a second, followed by a sustained contraction for several seconds that was 50 to 70% of the maximum force. Blundon (1988) speculates that the initial maximum peak in tension is generated by a modulated pattern of neuronal firing.

The action of the cutter claw was somewhat different to that of the crusher. It showed an initial rise to a peak force (which was substantially lower than that produced by the respective crusher claw), then stabilised at a plateau value that was only slightly lower than that of its peak. Therefore the analyses performed were based upon the sizes of the initial peaks of force for both claws.

6.4.2 Factors influencing force production

Three factors can contribute to force production by a claw: muscle bulk, mechanical advantage at the joint and muscle fibre composition:

- Muscle bulk is a function of claw size, as reflected in measures of its height and width (and hence claw area), and of the closer muscle

apodeme area. Elner and Campbell (1981) found that the maximum forces produced by the two claws of adult lobsters increased with claw height, and differences in muscle bulk between crusher and cutter claws must account for some of the difference in their force production (Block and Rebach, 1998).

- Mechanical advantage is another influential factor, which differs between the more heavily built crusher claws and the more slender cutter claw (Elner and Campbell, 1981), and it has been argued by Warner and Jones (1976) that the different architecture of crusher claw contributes to its greater strength. This difference between the two claws will increase as they grow.

- Differences in muscle fibre composition underlie differences in both contraction rate and contraction forces (Huxley and Nierdergerke, 1954), and represent a third variable that differs between the claws, as documented in Chapter 5. Huxley and Nierdergerke (1954) argued that, other things being equal, a muscle composed of short sarcomeres will contract faster than one composed of long sarcomeres, and also that longer sarcomeres are capable of producing a greater tension. It is likely that this is related to the ratio of actin to myosin filaments (which is higher in slow muscle fibres) and to the physiology of the stimulatory system and sarcoplasmic reticulum (Atwood, 1973).

The combined measures of morphometry, histochemistry and force production on juvenile lobsters reared under different temperature regimes provide a basis for disentangling the effects of these variables.

A more intractable factor is that of temperature itself. Over and above its effects on the rate of growth and moulting, which are directly measurable; temperature also acts in a dual capacity as an acclimating stimulus during the rearing period and as a physiological variable during the contraction testing itself. The former effect can be expected to lead to metabolic acclimation, which can in turn partially or completely offset the latter effect (i.e. the Q_{10} relationship). Tests on acclimated animals performed, as here, at their temperature of rearing will

therefore be influenced by both factors, but the balance of these influences cannot be known. Using a common testing temperature would eliminate the effect of the Q_{10} relationship, but this was rejected as a testing paradigm, as it would have broken the uniformity of the rearing regime if the temperature shifts had been made gradually, or would have introduced stress responses if temperature shifts had been made rapidly. The extent to which these two temperature effects influenced the measured claw forces was therefore not directly determined. Only in cases where expectations based on the Q_{10} relationship are not met can it be certain that other effects are being influential (one of which is discussed below). These considerations do not, of course, apply to comparisons between measures within individual temperature groups, both on different claws at the same time and on the same claw at different times.

The most reliable indication of the relationship between claw dimensions and closing force is provided by the data for the cutter claws (in which muscle fibre transformation is unaffected by the level of manipulation) from animals that were reared from the egg under uniform temperature conditions (the 1999 cohort). At both temperatures there was a positive correlation between claw area and contraction force (Figs. 6.9.a+b), and since any fibre transformation would have been expected to increase the proportion of the less powerful muscle fibres (fast phenotype), this can be taken as evidence that the relationship between claw size and closing force is causal. What cannot be determined from these measures is whether the increasing claw dimensions increase the mechanical advantage of the dactyl joint, as well as the bulk of the closer muscle, as the former was not measured separately.

Comparisons between the cutter and crusher claws of the 1999 cohort provide further information about the factors underlying force production. Within both temperature groups, the cutter and crusher claws are different in their mechanical strength even at the first sampling point (3 months). This indicates that a physiological differentiation has already begun, but since the presumptive cutter and crusher claws still have very similar dimensions, (Fig. 6.7.a+b) this difference most probably reflects the dimorphic transformation of their closer muscles, which is quite advanced by this time in the 1999 cohort (Chapter 5). The general remark may also be made that the differences in the pattern and amount of force

production by voluntary claw contractions provide both reliable and early indications of dimorphic switching, before external morphological differences between the cutter and crusher claws are discernible. This is an exploitable finding for further studies on the early stages of dimorphic development.

The crusher claws provide further evidence for an influence of muscle fibre composition on contraction force. Thus at 6 months of age, the forces produced by the 15°C and 19°C groups are almost identical, yet the 19°C crushers are considerably larger (Figs. 6.8.a+b). Despite a smaller muscle bulk, and a lower temperature of testing, the 15°C group perform equally to the 19°C group, which implicates muscle physiology in the performance difference. The observed greater rate of transformation of muscle fibres to the slow type in the 15°C crusher claws (Fig. 6.8.a) suggests that muscle fibre composition underlies their relatively better performance. It has also been noted in *Menippe mercenaria* (Stone crab) (Blundon, 1988) and *Homarus americanus* (American lobster) (Elner and Campbell, 1981), that a decline in muscle stress with increasing claw size occurs, which may represent an adaptation to an environment that changes as decapods grow, conferring a fitness advantage on large-clawed individuals, that are actually relatively weaker (Taylor, 2001). Other selection pressures, such as sexual selection or defence, may act on decapod claws to enhance attributes other than crushing strength, such as increased size without an increase in strength, or increased speed rather than strength, e.g. mature males may have large claws for display, and this size may be inflated relative to actual closing force capabilities (Elner and Campbell, 1981; Taylor, 2001).

The trends in the 1998 cohort are less clear, but are not inconsistent with the above interpretations in identifying differences in closer muscle fibre composition as a major contributor to differences in attainable force (see Fig. 6.6.a). However of particular interest in this cohort is the occurrence of three different variants of the claw opposite to the cutter claw: a true crusher, a false cutter, or a second cutter. Reasons for the occurrence of these different forms are discussed elsewhere (Chapter 5), but in the context of force development, they represent claws with different sizes, shapes and muscle fibre compositions that can be related to the forces produced. Of particular interest is the comparison between

true crushers and false crushers, since these two forms differ only in their internal muscle fibre composition, but not in their external size or shape.

The longer rearing period of the 1998 cohort allowed time not only for considerable increase in the sizes of the claws, but also for the complete transformation of one claw to the true crusher state in some animals. Others from the same temperature group, however, attained only the false crusher status. Tests on these true and false crushers (as identified subsequently by histochemical analysis) indicated that the latter could produce closing forces (per unit area) as large as the former. This suggests that a factor (or factors) relating to size is the primary determinant of force production in these larger crusher claws of older animals, while the closer muscle fibre composition has less influence. This does not of course mean that muscle fibre composition is of no consequence to the action of the crusher claw, for its primary importance may be related to aspects of claw muscle action other than maximal contractile force. For example, the rate of force development, the ability to sustain force for long periods, or the capacity to make repeated contractions may be conferred by the slow muscle fibres of the crusher closer, and it would be relevant to devise methods to test these properties directly. Only the first of these was measured in this study.

For both the 1998 and 1999 cohorts the rate of force development (mN s^{-1}) in the crusher claws was greater than in the respective cutter claws, and also increased with size in both claws (Figs. 6.13.a+b and 6.16.a+b). Since the measures were made under isometric conditions, this difference between the claws is to be expected, since it relates to properties that are enhanced in the slow fibres of the crusher closer muscle: more fibres in parallel within the larger cross-sectional area of the crusher compared to the cutter; slow muscle fibres that have myofibrils with a higher actin:myosin ratio than fast muscle myofibrils. The increase in the rate of force development with size in both claws is also expected, since this involves an increase in the cross sectional areas of the closer muscles in each case. Elongation of the muscle fibres as the claws enlarge may also contribute to this effect. However, if stages 15-17 are disregarded on figures 6.13.a+b, then the relationships would be different in relation to the effect of increasing speed of contraction with stage and temperature. This would highlight

that at all the common stages to the three temperature regimes, there is little change with developmental stage, of the claw contraction speeds for both the crusher and cutter claws.

6.4.3 Functional consequences

Functionality of the claws is vital to survival, and Block and Rebach (1998) have detailed several factors that drive the selection of large and powerful claws: claw strength directly affects crushing ability (Boulding and LaBarbera, 1986), and so the prey type and or the size of prey that is eaten. Greater claw strength theoretically expands the critical size of prey items and potentially increases the breadth of the crab or lobster's diet. So, increased crushing forces allow for potential prey variation. Certainly Wickens (1986) found that only lobsters with claws above a certain size could open the oyster spat that were offered as food items, and thus acquire nutrition that was unavailable to smaller lobsters. In the context of a laboratory-rearing, early claw development would be an advantage by providing increased feeding opportunities for the newly released lobsters in stock enhancement programmes.

Claw strength in relation to claw size will also be important for the ability of laboratory-reared animals to compete with wild conspecifics in agonistic encounters. Large claws are combined display and combat structures, but if the displays escalate to aggressive actions, then claw strength relative to size becomes an important factor (Levinton *et al.*, 1995). This relationship has been investigated in the present study through staged encounters between juvenile lobsters at different times during the rearing programme (Chapter 8).

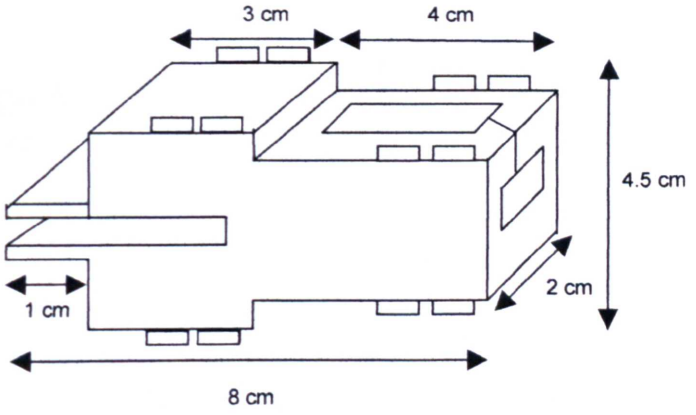


Figure 6.1.a: Diagram of largest strain gauge used for measuring the voluntary force contractions of the juvenile lobsters. Two other smaller and more sensitive strain gauges were used, as appropriate (see Materials and Methods).

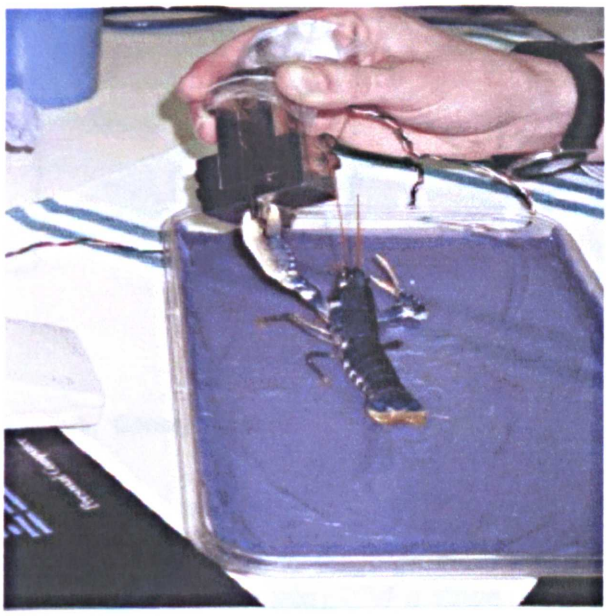


Figure 6.1.b: Picture of a lobster voluntarily gripping the strain gauge (diagram above), at Conwy, North Wales.

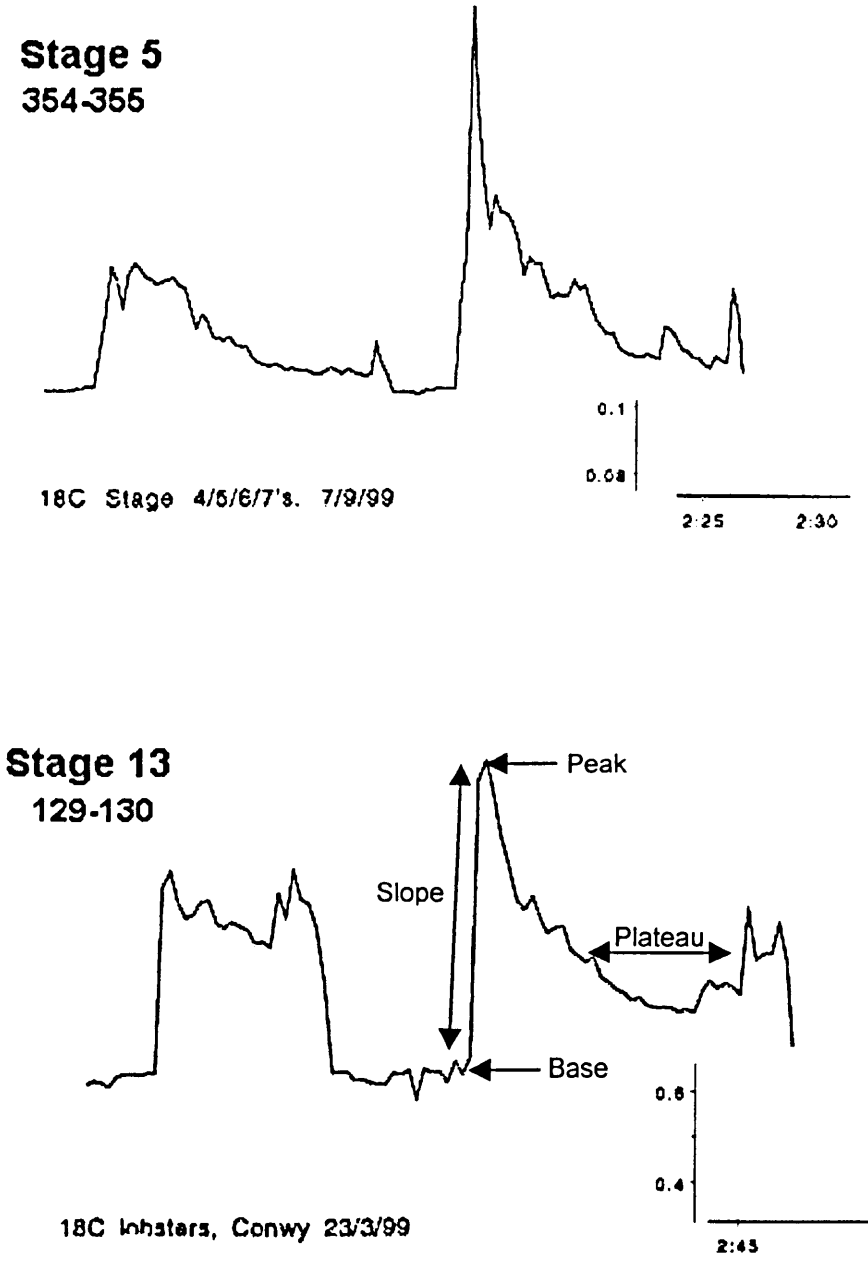


Figure 6.2.a: Examples of the force recordings produced by a stage 5, 3 month old lobster from the 1999 cohort (top trace), and a stage 13, 9 month old lobster from the 1998 cohort (bottom trace), both from the 19°C temperature regime. The crusher claw (second peak in each trace) has an initial peak of force, followed by an undulating plateau, consisting of repeated sub-threshold contractions. The cutter claw (first peak in each trace) has an indistinct initial peak of force, which is lower than that of the crusher claw, and a variable plateau. Scale bars: horizontal, s; vertical, voltage of strain gauge amplifier (traces not to same scale).

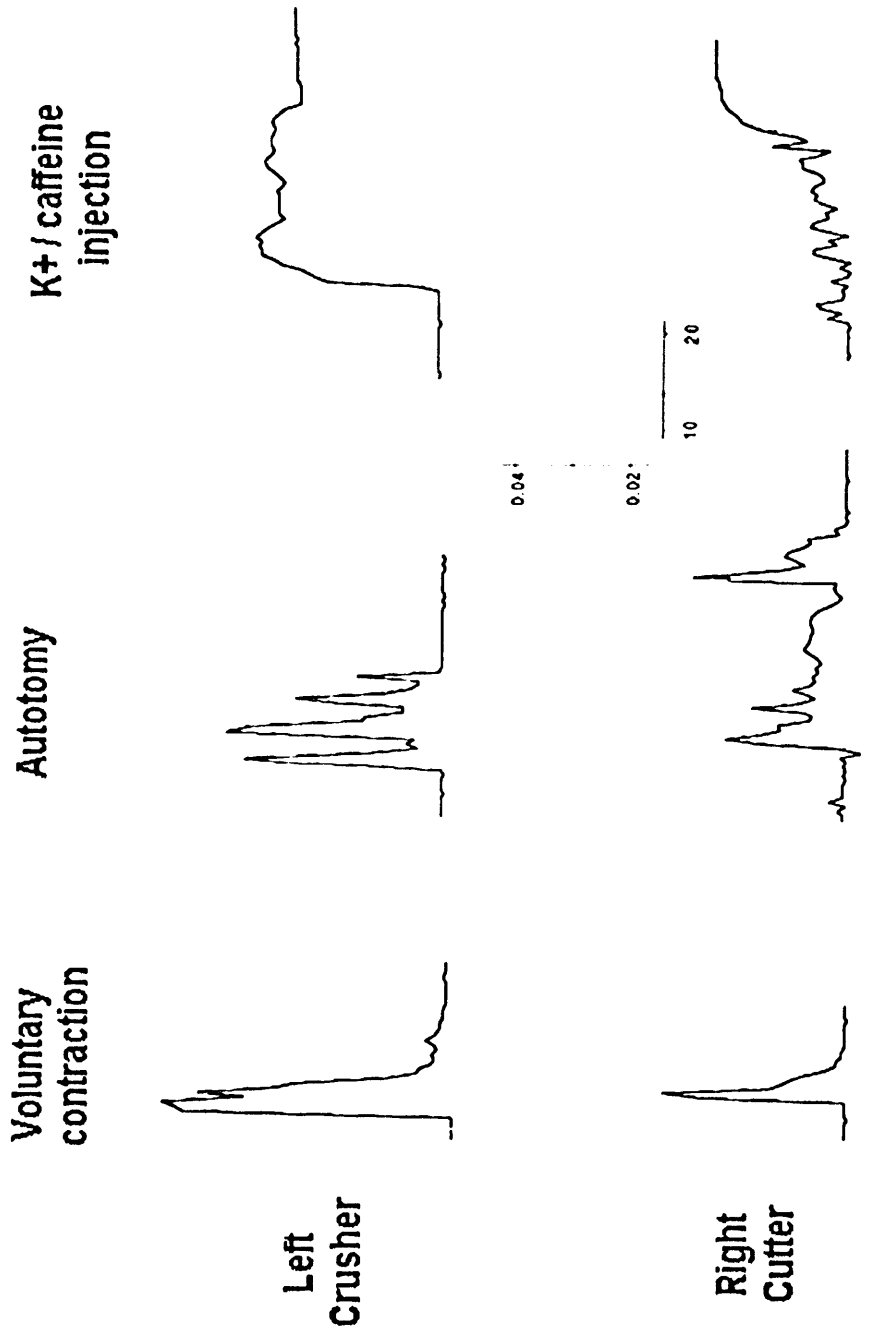


Figure 6.2.b: Examples of contractions of the crusher claw (top trace) and the cutter claw (bottom trace) from a lobster of the 1998 cohort at Stage 15. First traces: voluntary contractions; second traces force measurement during autotomy of the claw; third traces: maximum elicitable forces produced by the injection of the K+/Caffeine solution. Scale bars: horizontal, s; vertical, voltage of strain gauge amplifier (0.02 - 0.04 = 100 mN).

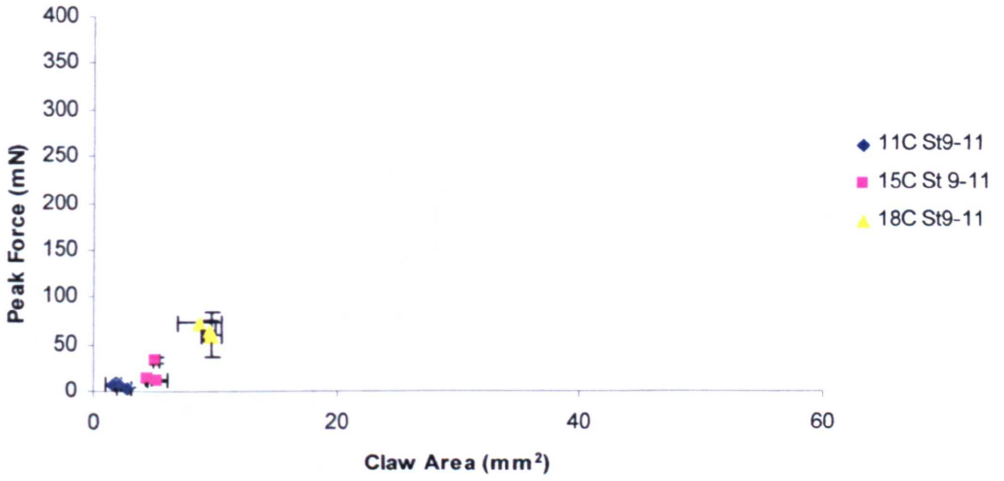


Figure 6.3.a: The crusher claw peak forces of the 1998 lobsters at 9 months old plotted against claw area. Each graph represents the mean values for claw area and peak force production for each of the stages represented in the figure legend, for each temperature regime (Figs. 6.3-6.5).

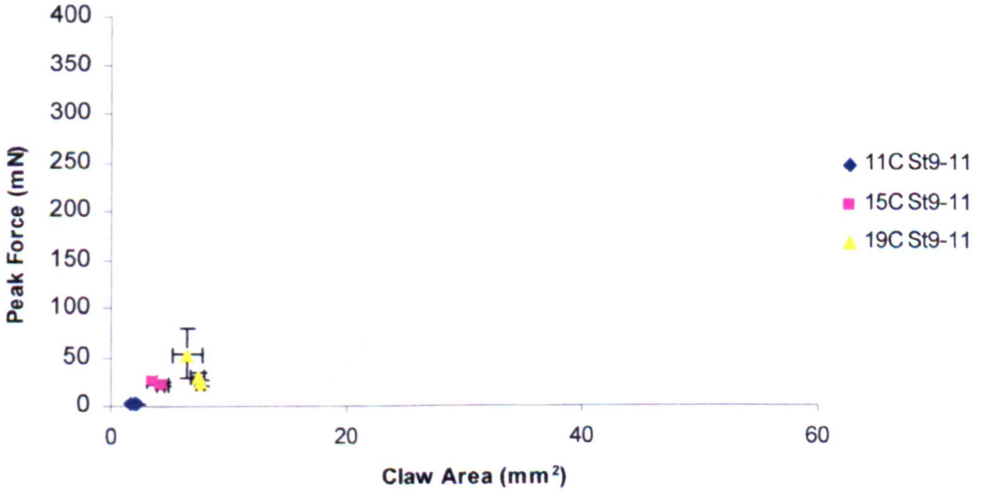


Figure 6.3.b: The cutter claw peak forces of the 1998 lobsters at 9 months old plotted against claw area.

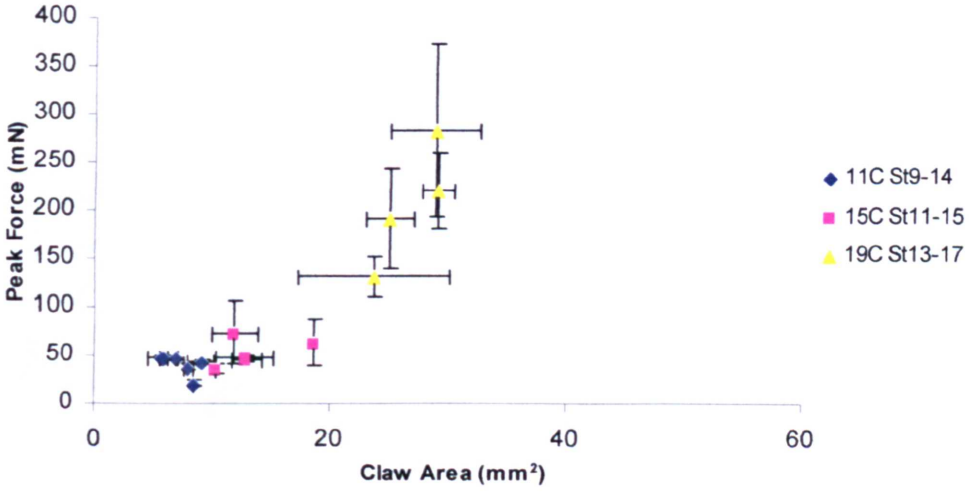


Figure 6.4.a: The crusher claw peak forces of the 1998 lobsters at 15 months old plotted against claw area.

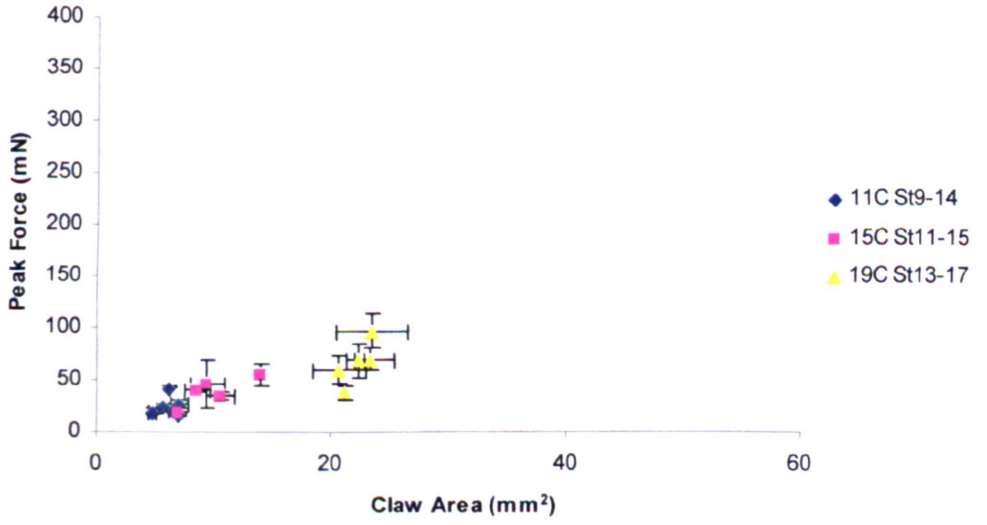


Figure 6.4.b: The cutter claw peak forces of the 1998 lobsters at 15 months old plotted against claw area.

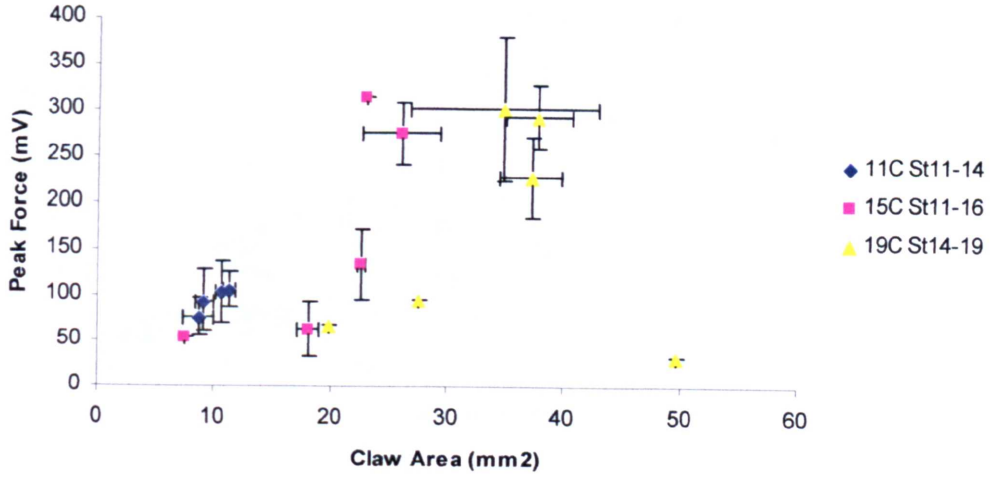


Figure 6.5.a: The crusher claw peak forces of the 1998 lobsters at 17 months old plotted against claw area.

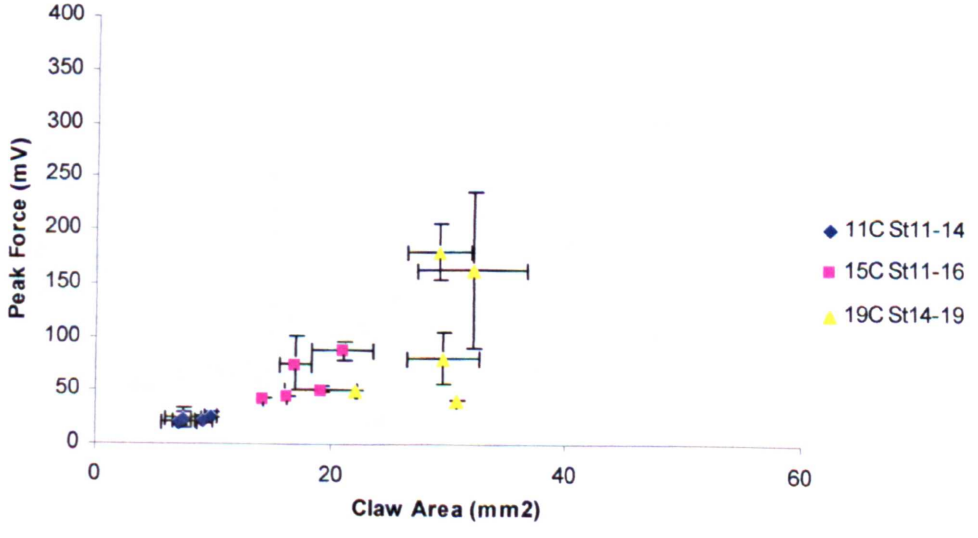


Figure 6.5.b: The cutter claw peak forces of the 1998 lobsters at 17 months old plotted against claw area.

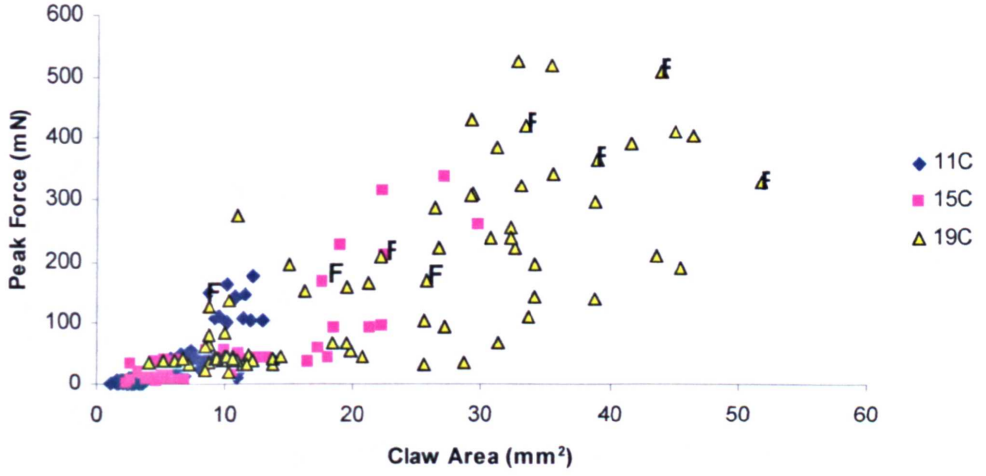


Figure 6.6.a: The crusher claw forces for individual lobsters of the 1998 cohort at all temperatures, at all 3 sampling points plotted against claw area. The 'F's indicate the presence of false crusher claws (see text).

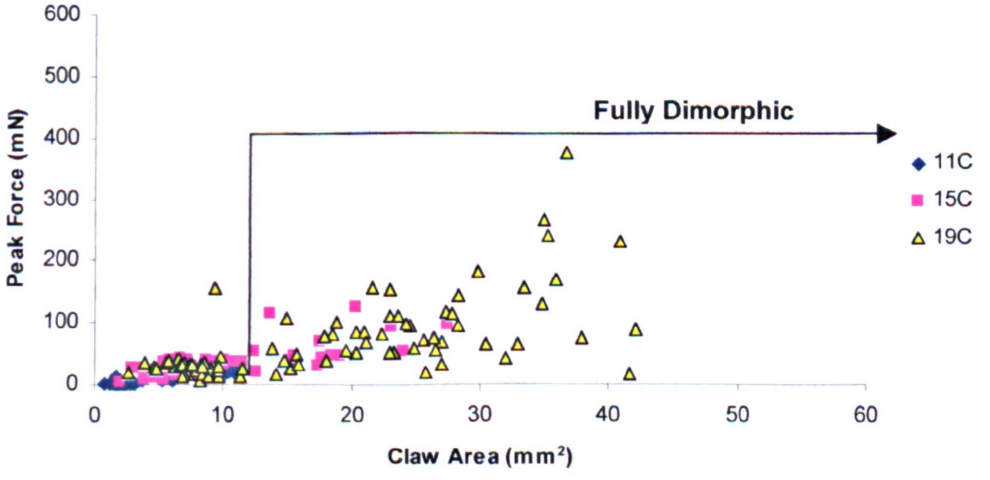


Figure 6.6.b: The cutter claw forces for individual lobsters of the 1998 cohort at all temperatures, at all 3 sampling points plotted against claw area. The claw area at which dimorphic switching is complete is indicated.

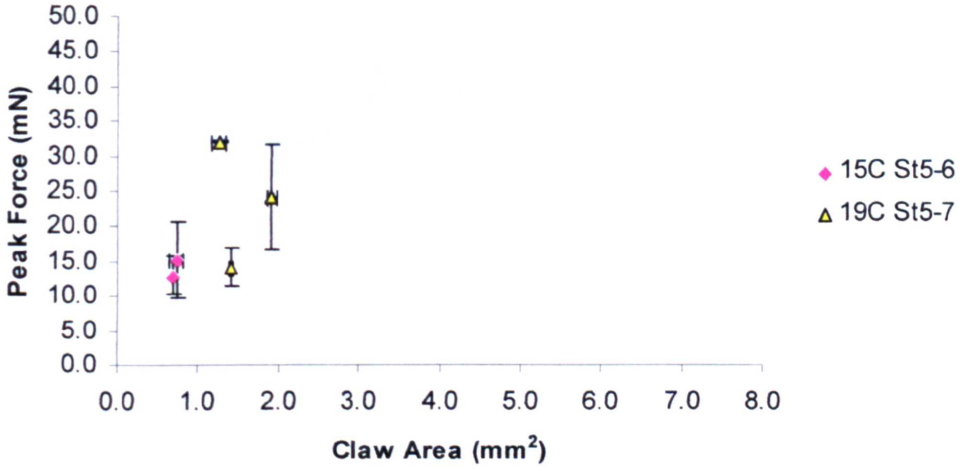


Figure 6.7.a: The crusher claw peak forces of the 1999 lobsters at 3 months old plotted against claw area.

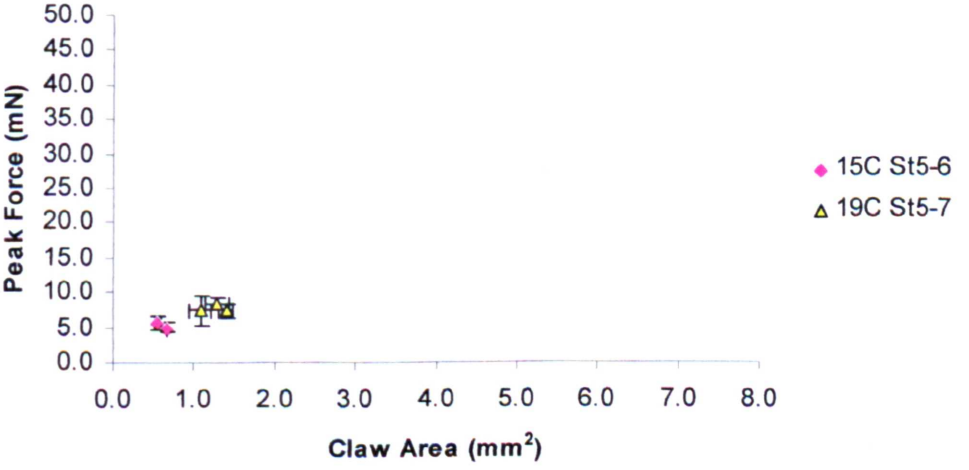


Figure 6.7.b: The cutter claw peak forces of the 1999 lobsters at 3 months old plotted against claw area.

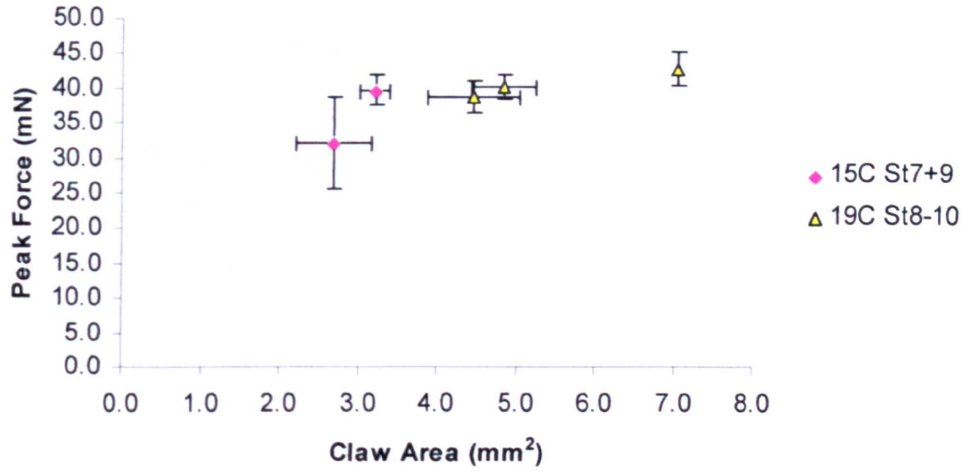


Figure 6.8.a: The crusher claw peak forces of the 1999 lobsters at 6 months old plotted against claw area.

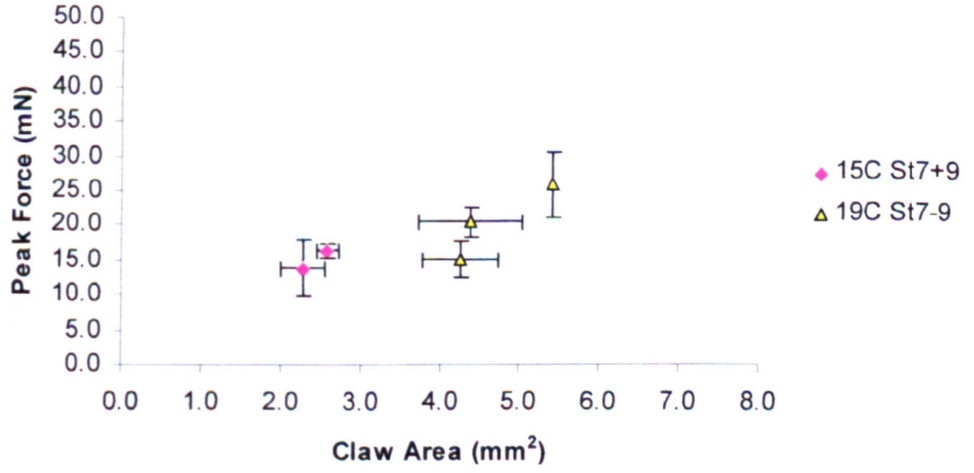


Figure 6.8.b: The cutter claw peak forces of the 1999 lobsters at 6 months old plotted against claw area.

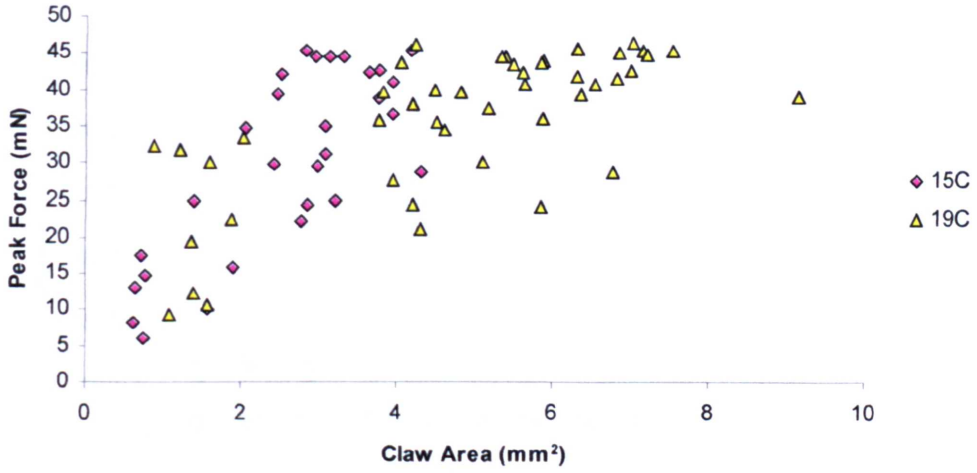


Figure 6.9.a: The crusher claw forces for individual lobsters of the 1999 cohort at all temperatures, at both sampling points plotted against claw area.

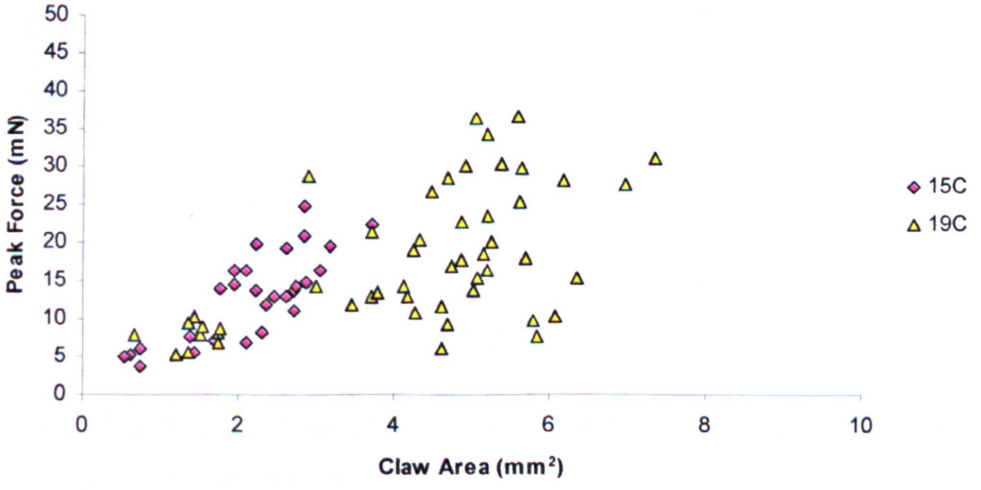


Figure 6.9.b: The cutter claw forces for individual lobsters of the 1999 cohort at all temperatures, at both sampling points plotted against claw area.

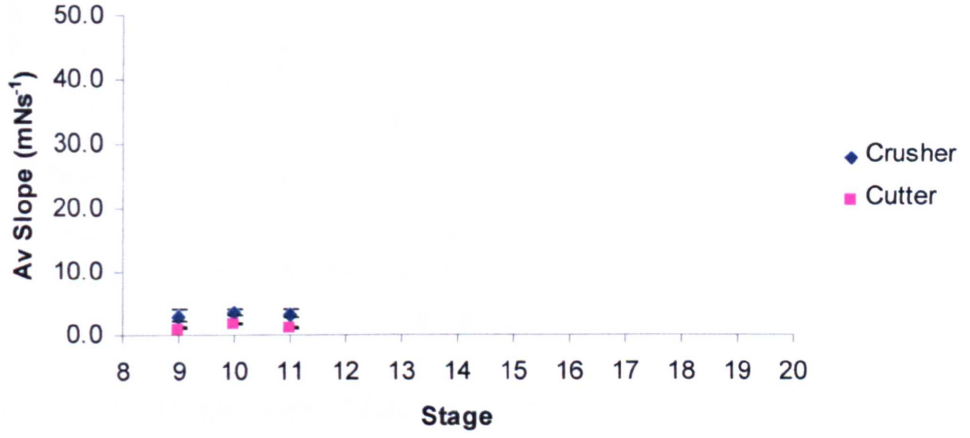


Figure 6.10.a: Average rates of force production for crusher and cutter claws of the 11°C group of the 1998 cohort at 9 months old.

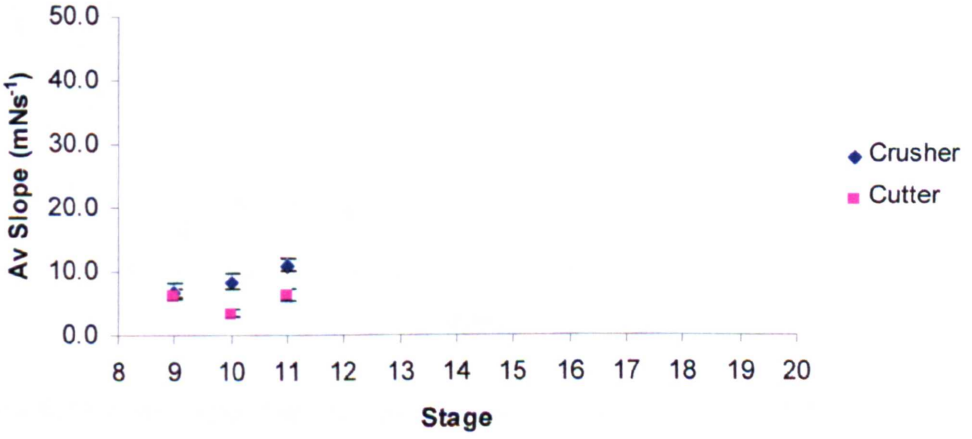


Figure 6.10.b: Average rates of force production for crusher and cutter claws of the 15°C group of the 1998 cohort at 9 months old.

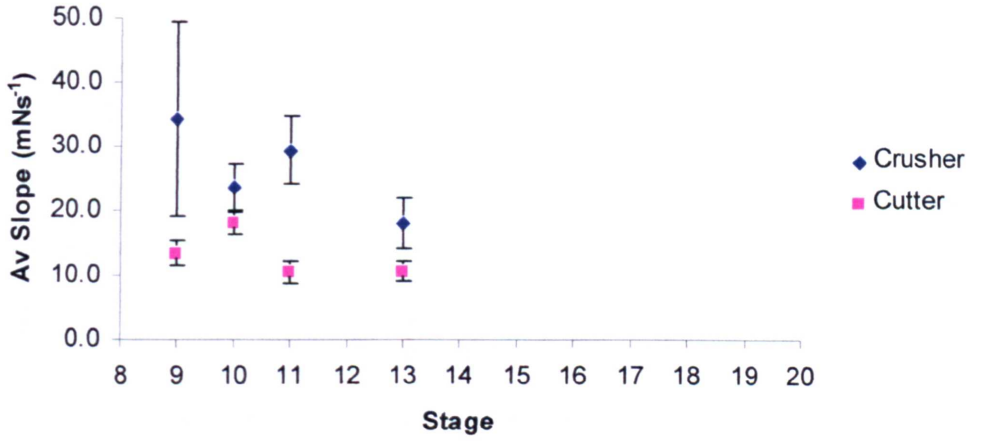


Figure 6.10.c Average rates of force production for crusher and cutter claws of the 19°C group of the 1998 cohort at 9 months old.

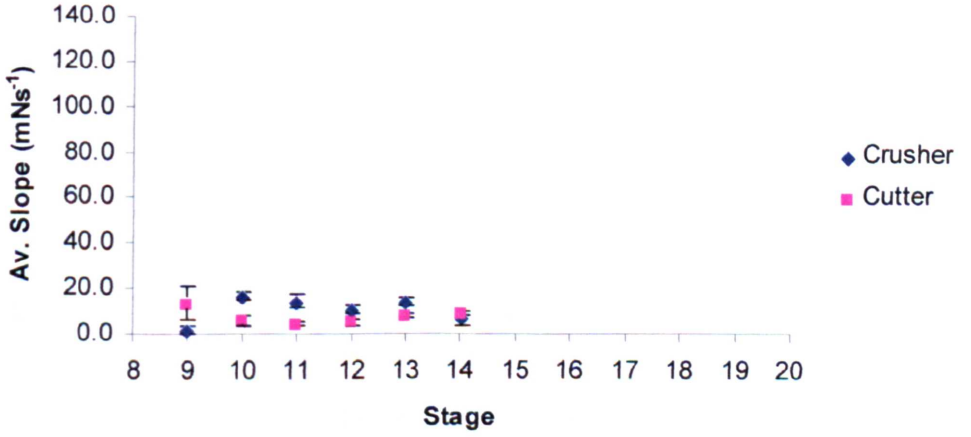


Figure 6.11.a Average rates of force production for crusher and cutter claws of the 11°C group of the 1998 cohort at 15 months old.

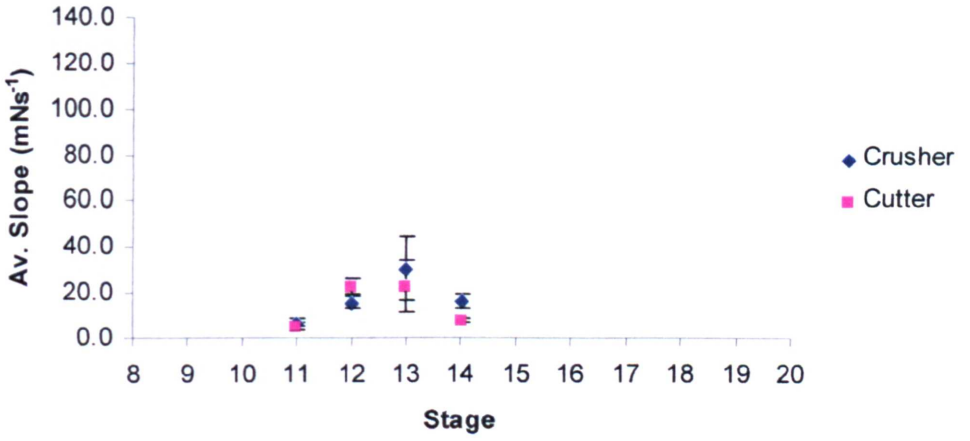


Figure 6.11.b Average rates of force production for crusher and cutter claws of the 15°C group of the 1998 cohort at 15 months old.

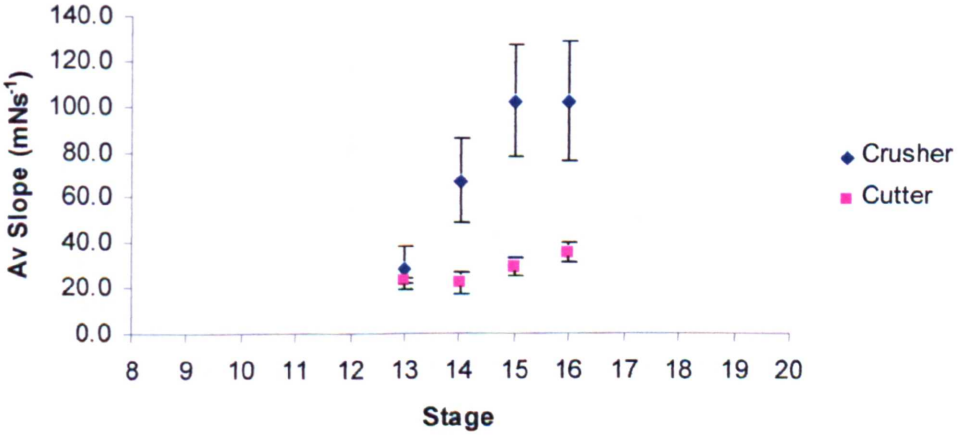


Figure 6.11.c: Average rates of force production for crusher and cutter claws of the 19°C group of the 1998 cohort at 15 months old.

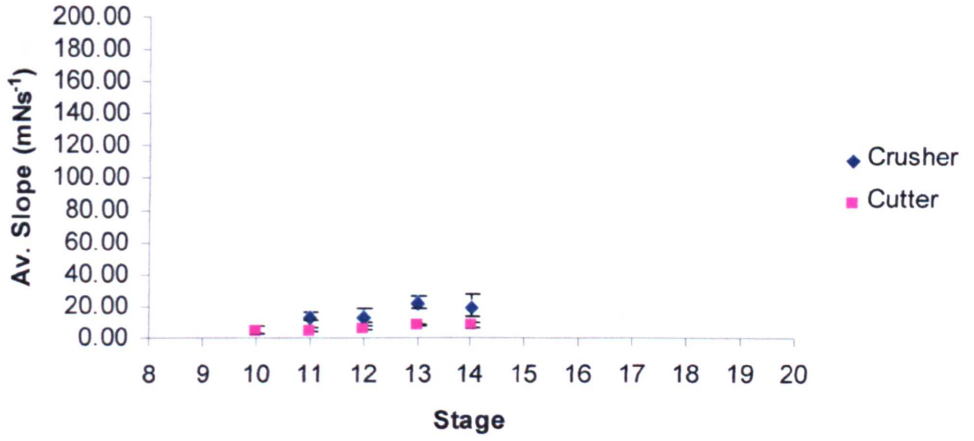


Figure 6.12.a Average rates of force production for crusher and cutter claws of the 11°C group of the 1998 cohort at 17 months old.

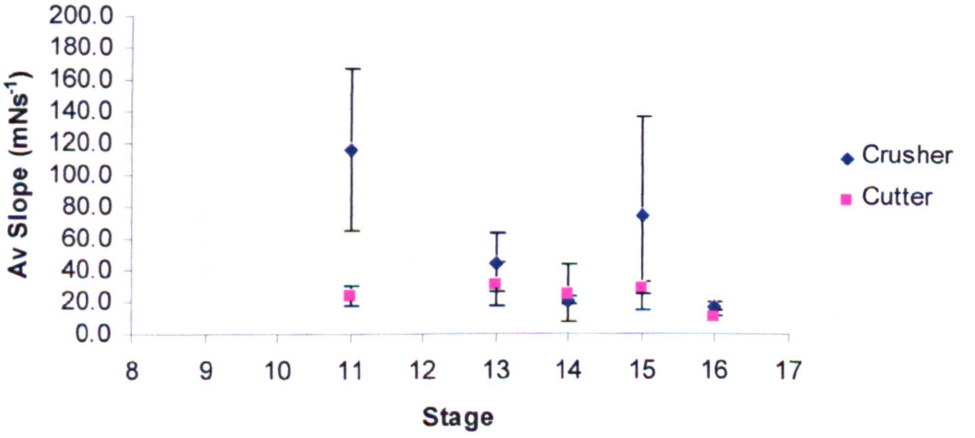


Figure 6.12.b: Average rates of force production for crusher and cutter claws of the 15°C group of the 1998 cohort at 17 months old.

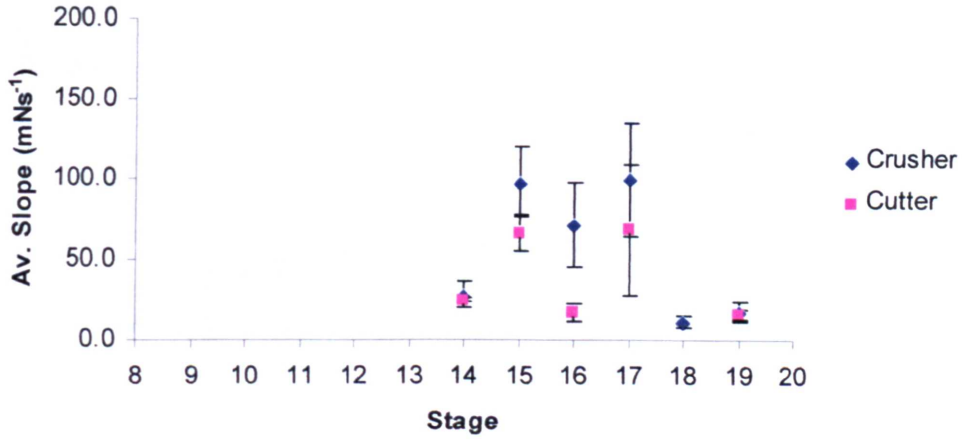


Figure 6.12.c Average rates of force production for crusher and cutter claws of the 19°C group of the 1998 cohort at 17 months old.

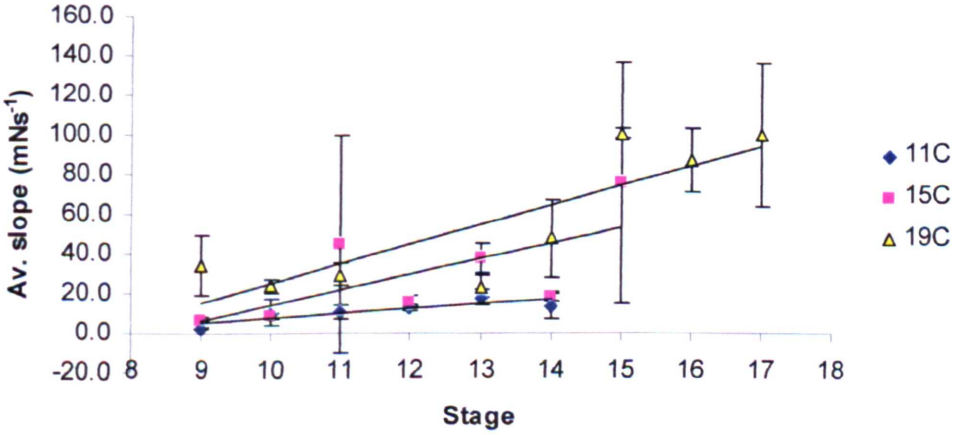


Figure 6.13.a: Average rates of force production for crusher claws of the 1998 cohort. The regression equations for the three temperature groups are: 11°C: y = 2.3257x - 15.498, R2 = 0.7069; 15°C: y = 7.8265x - 64.217, R2 = 0.4683; 19°C: y = 9.9263x - 74.684, R2 = 0.7101.

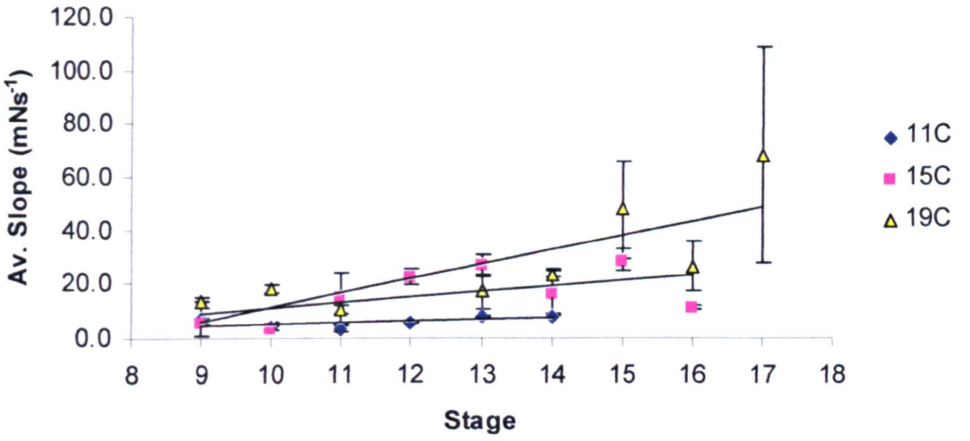


Figure 6.13.b: Average rates of force production for cutter claws of the 1998 cohort. The regression equations for the three temperature groups are: 11ºC: y = 0.5803x - 0.4024, R2 = 0.2769; 15ºC: y = 2.0345x - 9.1042, R2 = 0.2922; 19ºC: y = 5.343x - 42.028, R2 = 0.6042.

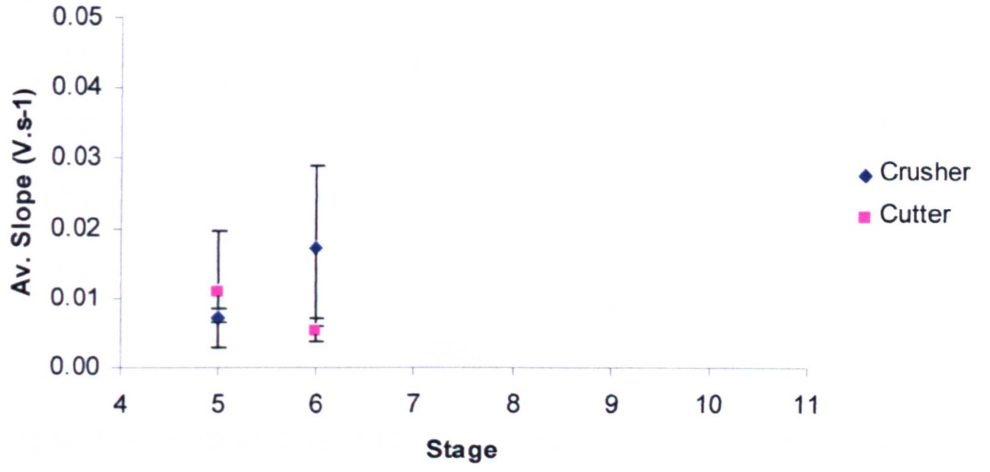


Figure 6.14.a Average rates of force production for crusher and cutter claws of the 15°C group of the 1999 cohort at 3 months old.

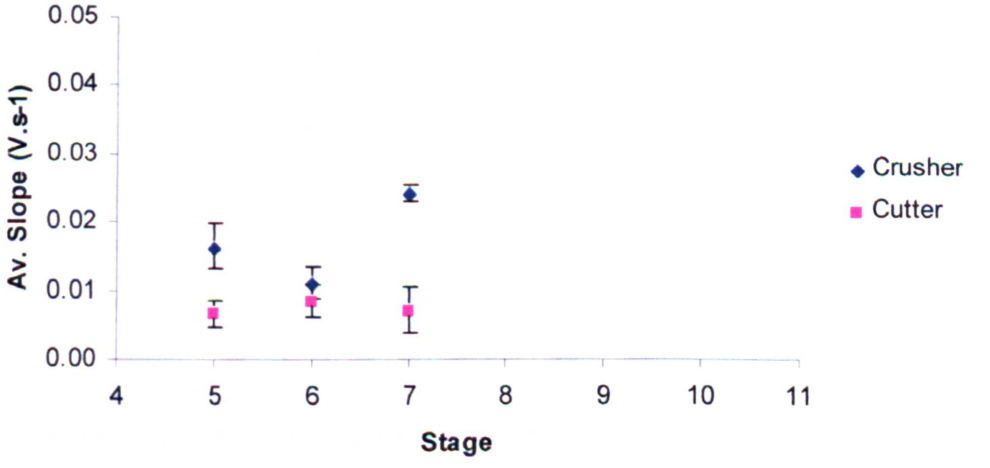


Figure 6.14.b: Average rates of force production for crusher and cutter claws of the 19°C group of the 1999 cohort at 3 months old.

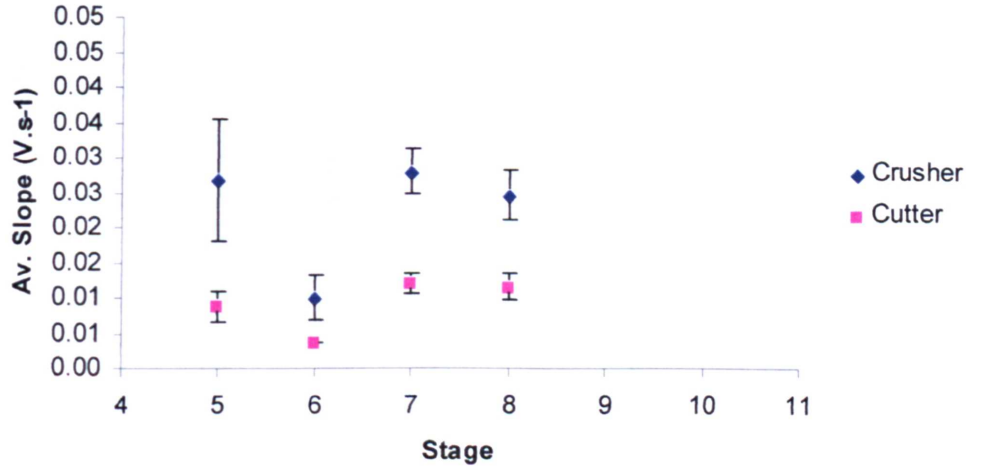


Figure 6.15.a Average rates of force production for crusher and cutter claws of the 15°C group of the 1999 cohort at 6 months old.

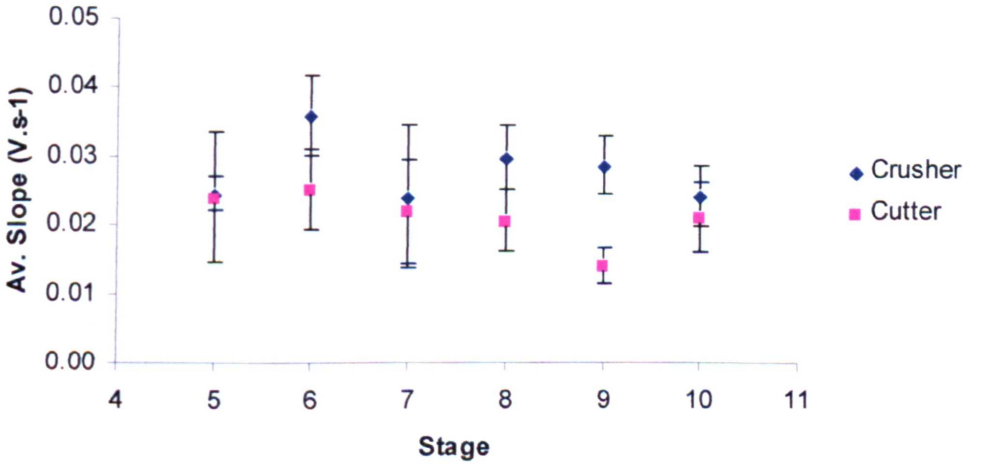


Figure 6.15.b: Average rates of force production for crusher and cutter claws of the 19°C group of the 1999 cohort at 6 months old.

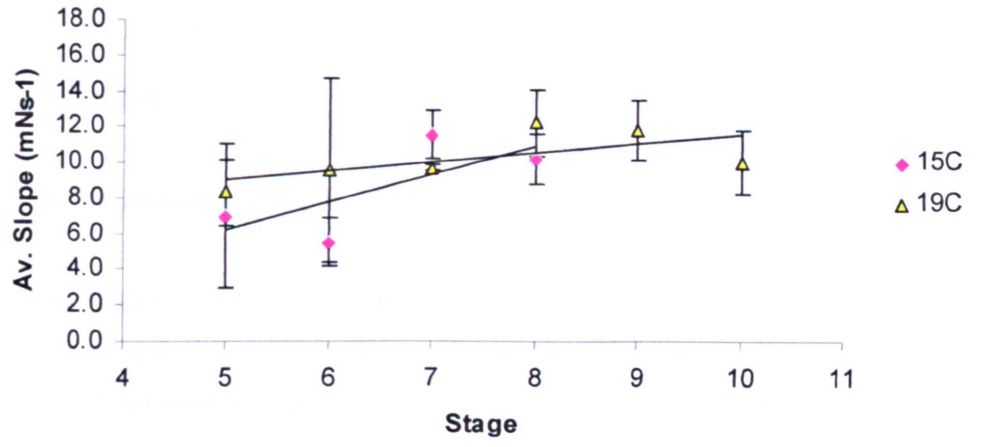


Figure 6.16.a: Average rates of force production for crusher claws of the 1999 cohort. The regression equations for the three temperature groups are: 15°C: y = 1.5629x - 1.5754, R2 = 0.5165; 19°C: y = 0.5087x + 6.4487, R2 = 0.4113.

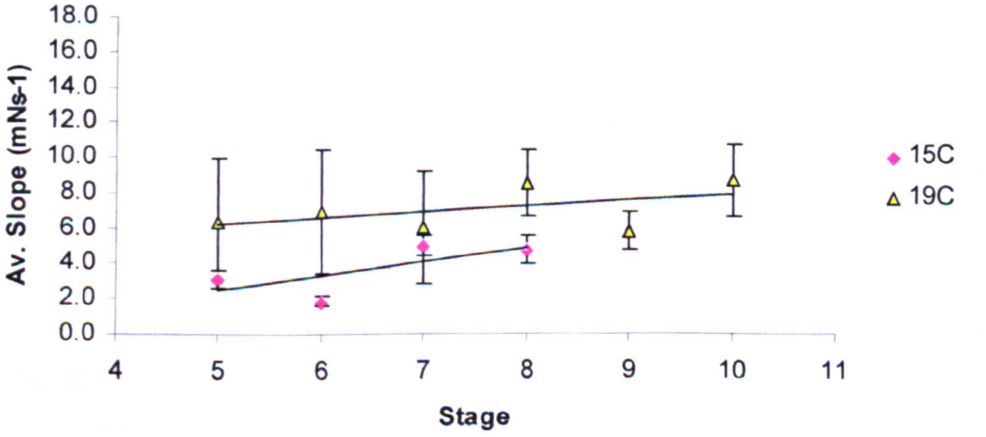


Figure 6.16.b: Average rates of force production for cutter claws of the 1999 cohort. The regression equations for the three temperature groups are: 15°C: y = 0.8154x - 1.6409, R2 = 0.5041, R2 = 0.5165; 19°C: y = 0.3329x + 4.4943, R2 = 0.2328.

Chapter 7 – The effect of different rearing temperatures on the swimming behaviour of the European lobster, *Homarus gammarus*

7.1 Introduction

7.1.1 The crustacean tail flip

The European lobster, *Homarus gammarus*, like other malocostracan crustaceans with extended abdomens, uses the tail-flip escape swimming response as an evasive reaction to noxious (chemical) or threatening stimuli such as a predator or when in an agonistic encounter (Wine and Krasne, 1982; Newland *et al.*, 1988a; Neil and Ansell, 1995; Edwards, 1995; Arnott *et al.*, 1998, 1999). The rapid alternate flexions and extensions of the abdomen which constitute the tail flip propel the animal backwards (Newland *et al.*, 1992; Arnott *et al.*, 1998). The tail-flip response is a startle reaction (Bullock, 1984), *i.e.* it has a high threshold for activation, involves a rapid propulsive movement and propels the animal at a high velocity, and is most effectively triggered by abrupt mechanosensory or visual stimuli (Wine, 1984).

The success of the tail-flip as an avoidance reaction depends on its timing in response to the threat (too early and the predator may adjust its attack, too late and the prey may be caught), on its velocity and on its orientation (Neil and Ansell, 1995). The tail-flip is regarded as one of the best known examples of a neuronal motor act (Wine, 1984; Krasne and Wine, 1988), and has been well documented in lobsters and crayfish. Tail flipping also occurs in smaller decapods, such as cariddean shrimps and those of other malostracan orders, such as mysids and euphausids. This shows the diversity of use, but also the effectiveness of the behaviour favoured by many similar species.

7.1.2 Mechanics of the tail flip

The tail-flip swimming of crustaceans is an intermittent form of locomotion, in which, bio-mechanically, the flexion of the abdomen represents rowing with a single appendage (Daniel *et al.*, 1992). The rapid flexion of the extended malostracan 'tail' is thought to have evolved as a mechanism to assist backward

escape jumping (Heitler and Fraser, 1989) and this tail-flip reaction is mediated in all groups by giant nerve fibres (Paul, 1990; Neil and Ansell, 1995). The malocostracan abdomen is anatomically specialised for tail-flipping, as it is segmented, and contains a large spiral musculature (Hessler, 1983) and an expandable tail fan which represents the main thrust generating surface (Webb, 1979).

The propulsion arises from a combination of a reactive force (the added mass) and a resistive force (drag). The first of these is dominant in producing the instantaneous thrust, with a major contribution being made by the expanded tail fan (Webb, 1979). At the end of the tail-flip movement a hydrodynamic squeeze force is produced, which occurs as the abdomen is pressed against the cephalothorax. This squeeze force is similar to the fling mechanism in hovering insects (Ellington, 1984) and the jetting action of the squid, medusae and salps (O'Dor, 1988; Daniel *et al.*, 1992). During the single oar rowing of the tail-flip motion the 'lateral' forces do not cancel out, and significant rotational movements can arise. The instantaneous force and movements that are generated about the animal's centre of mass can be predicted and used to estimate the translational movements of the whole animal. Daniel and Meyhöfer (1989) conducted a theoretical analysis of the escape swimming in the penaeid shrimp, *Pandalus danae*, and this showed that there is a unique relative abdomen size that maximises the performance. Small abdomens (<50% body length) yield very small forces that barely move the body, but as abdomen size increases, both the thrust and movement arm increase. The latter may become so large that it dominates the body kinematics, however, so producing mainly rotational rather than translational forces.

7.1.3 Neuronal control of the tail flip

The neuronal control of the crustacean tail-flip, most extensively studied in the crayfish, *Procambarus clarkii* (Wine, 1984; Krasne and Wine, 1988), involves two pairs of giant fibres in the ventral nerve cord, which are known as the lateral giants (LGs) and the medial giants (MGs). These initiate the initial short-latency flexion movement of the abdomen, in response to an adequate stimulus to the telson, rostrum or abdomen (Newland *et al.*, 1992). The subsequent tail-flips in

the swimming sequence, termed non-giant or swimming tail-flips, are generated by a neuronal network that does not involve the giant fibres (Wine and Krasne, 1982). Through the activation of the giant fibre pathways (LGs and MGs) two forms of escape can be triggered. The lateral giant axons (LGs) are activated by sudden caudal stimuli (abdomen, tail fan) and this elicits a bending in only the more rostral abdominal segments to produce an upward, forward-pitching trajectory, which takes the animal away from the signal. The medial pair of giant axons (MGs) are activated by stimuli located rostrally (cephalothorax, legs, vision) and cause bending in all abdominal segments, which thrusts the animal directly backwards away from the stimulus (Wiersma, 1947; Wine and Krasne, 1972, 1982).

The primary circuitry associated with these neurones has been elucidated (Wine and Krasne, 1982). The LGs receive convergent input from the primary afferents and sensory interneurons of the abdomen, and these make powerful excitatory synapses with giant interneurons (MoGs), which in turn innervate phasic flexor muscles that will bend the rostral segments. Correspondingly, the MGs receive input from the head and thorax and excite the MoGs of all abdominal segments, eliciting a uniform abdominal flexion. When the activity in either sensory field is sufficient, the corresponding giant neurone fires (usually only once) causing the appropriate type of tail flip (Wine and Krasne, 1972; Mittenthal and Wine, 1973). The giant fibres are regarded as classical examples of 'command neurones', which elicit fixed motor acts (Kupfermann and Weis, 1978). Now there is certain evidence to suggest that their activity may be 'necessary' for tail-flip production, but is not alone 'sufficient' to produce a flip with completely normal characteristics (Wine, 1984). The 'normal' flips will only occur if the parallel sensory pathways are activated along with the giant fibres themselves (Edwards and Mulloney, 1987; Krasne and Wine, 1988), providing the neuronal basis for a steering component in giant-fibre tail-flip.

Sudden stimuli received by the lobster tend to fire the giant fibres. The decision to fire is itself rapid, the central delay is often only a few milliseconds, and immediate effector consequences are assured by a system of large axons and minimal synaptic delays. The cost of such speed and reliability is great, however;

the responses are very stereotyped and essential flexibility requires the two sets of giant fibres, MGs and LGs.

Less sudden stimuli, presumably associated with less immediate threats, do not activate the giant fibres, but instead are dealt with by a slower acting but more flexible non-giant system(s). In contrast to the giant-fibre reactions, which can be classed as reflexive, the non-giant reactions seem to be essentially voluntary in nature. Their latencies can be an order of magnitude greater than those of the giant-fibre reactions, and the eliciting stimuli are more variable, e.g. visual stimuli and mechanosensory somatic stimuli such as pinching or even cutting (Wine and Krasne, 1972). The subsequent swimming sequence after an initial giant fibre flip is also mediated by the non-giant neuronal system.

7.1.4 The tail flip as a predator avoidance reaction

Tail-flip escape responses in lobsters and other crustaceans possessing an elongated abdomen are extremely effective in allowing the animal to avoid capture by a predator or a con-specific. The interactions between predatory fish and their crustacean prey are some of the most common examples of this. The predator-prey interactions of the 15-spined stickleback, *Spinachia spinachia*, which is an ambush predator, and the mysid *Neomysis integer*, have been studied (Kislalioglu and Gibson, 1975; Kaiser *et al.*, 1992; Neil and Ansell, 1995). Here, the maximum attack velocity of the stickleback is 846m s^{-1}, which is slightly higher than the maximum escape velocity of the mysid (796m s^{-1}), but the capture success is only 25% (Rademacher and Kils, unpublished observations, in: Neil and Ansell, 1995). One possible reason for this is that up to 75% of the mysid tail-flips induced by the fish incorporate a lateral shift (Kaiser *et al.*, 1992), and this effectively removes the mysid from the 'zone of interception' of the fish (Hart and Hamrin, 1990). The success of this strategy is increasingly strengthened by the mysid delaying its escape until the last moment, beginning flexion when the fish is only about 6mm away. Similar strategies are employed by the brown shrimp *Crangon crangon* (Arnott *et al.*, 1998). This is an example of 'Avoidance Case I' behaviour (Weiks and Webb, 1984), since the crustacean moves out of the fish's field of view and so reduces the probability of further detection, enhancing it's future chances of escape.

7.1.5 Factors influencing the tail flip

7.1.5.1 Development stage

The capacity to tail-flip first develops in lobsters during the pelagic larval stages, although these larvae produce only individual weak twitches (Laverack *et al.*, 1976). During these pelagic stages the larvae are found in the upper metre of the water column (Fogarty, 1983; Harding *et al.*, 1987; Rooney and Cobb, 1991) and perform rapid directional swimming (Smith, 1873; Herrick, 1909; Ennis, 1986a; Cobb *et al.*, 1989) propelled by the thoracic exopodites, and later the abdominal swimmerets (Macmillan *et al.*, 1976; Neil *et al.*, 1976). It is believed that this ability for distance swimming contributes to the distribution of the species, before the lobsters settle, metamorphose to Stage 4 post larvae and gain the capacity for tail-flipping escape behaviour (Laverack *et al.*, 1976; Rooney and Cobb, 1991).

Juvenile lobsters of *H. americanus* generally respond to a threat with tail-flip escape behaviour, but in adults the probability of eliciting such a response appears to be a function of moult stage: the more hard-shelled intermoult (stage C) and premoult (stage D) animals tail-flip more than soft-shelled post-moult animals (stages A+B) (Cromarty *et al.*, 1991). Thus, as well as the type of stimulus received by the animal, many other factors (including also their weight, allometry and physiology) are involved in determining whether the escape response of a lobster will occur (Cromarty *et al.*, 1991).

7.1.5.2 Moult state

It was first noted by Huxley (1880) that immediately after moulting, lobsters appear to be 'timid', whereas later they display aggressive behaviour (Scrivener, 1971). Similar changes in behaviour have been documented in relation to the moult cycle of stomatopods (Steger and Caldwell, 1983). Lobsters are most vulnerable to predation immediately post-moult, when they are soft shelled, and if threatened they are not likely to respond aggressively, but will try to escape (Atema and Cobb, 1980). Experiments by Lang *et al.* (1977) showed that the

escape response occurs more often in juveniles and small adults, than in larger animals or adults. When confronted by a threat, large lobsters are more likely to show aggressive behavioural postures such as the meral spread. According to Lang *et al.* (1977) as the animal grows, the escape behaviour is gradually replaced with defensive or aggressive behaviour. The tail-flip behaviour becomes less effective as an escape response for two reasons. Firstly, the conduction time of the medial giant impulses that travel from the brain to the sixth abdominal ganglion is increased as the animal grows, resulting in an increased latency for the escape response. Secondly, the ratio of abdomen length to carapace length decreases from around 2.0 to 1.4, from the first larval stage to the adult, so that the abdominal flexor muscles have to propel an increasing body mass (represented to a large extent by the claws) through the water during an escape sequence (Cromarty *et al.*, 1991).

A lot of work has been carried out on the effect of moult state on the behaviour of *H. americanus* (Herrick, 1909; Cobb and Tamm, 1975; Tamm and Cobb, 1978; Atema *et al.*, 1979; Cromarty *et al.*, 1991). The effect of moulting on the behaviour of lobsters is important, as it is not a short-lasting or isolated event; it is a prolonged process involving the entire physiology of the animal (Cromarty *et al.*, 1991). On average, 70% of the time between moults is spent in preparation for, and then recovery from, ecdysis (Drach, 1939). Directly after the moult the exoskeleton is very soft, this makes the lobster very vulnerable to predation, injury or cannibalism (Reaka, 1975, 1976; Tamm and Cobb, 1976; Sastry and Ehinger, 1980; Cromarty *et al.*, 1991). Due to these effects, certain defensive behaviours are common, and are similar in many crustacean species. It has been reported that *Panulirus argus* is submissive immediately before and directly after the moult, but at other times of the moult cycle, regularly tail-flips when approached by conspecifics (Lipcius and Herrnkind, 1982).

Soft-shelled, post-moult lobsters (stage B) travel further, produce more tail-flips and swim longer, at a sustained velocity, than do pre-moult lobsters, and this is also the case for the animals in the earliest moult stage (stage A) (Cromarty *et al.*, 1991). Due to the soft exoskeleton, the first flip is slower and the subsequent flips produce reduced forces and acceleration, but this could be compensated for by the animals having an increased propensity to swim (Cromarty *et al.*, 1991).

Cromarty *et al.* (1991) found no significant differences between the distances travelled in the initial tail flip by the lobsters in different moult stages. In contrast, Tamm and Cobb (1978) found that pre-moult animals, which are hard-shelled, responded to threats with a meral spread rather than escape swimming, but when they did swim they produced a rapid initial tail flip followed by swimming with a decreasing velocity. This highlights how different behaviours are exhibited by lobsters in different moult states, and that each behaviour, be it escape swimming or meral spread, is the most appropriate given the state of the individual.

7.1.6 Temperature effects on locomotion

Temperature has an important effect on the performance of the muscle, and especially on its mechanical power output, although ectothermal animals appear to locomote in a kinematically similar fashion at different temperatures (Rome, 1982, 1990). Temperature affects physiological processes through effects on both reaction rates and the equilibria determining the non-covalent interactions that stabilise macromolecules and membranes. Organisms can adjust their responses to both short- and long-term thermal fluctuations through the exact biochemical composition or physiological rates and metabolic processes within their muscle fibres. Mitochondria are the centres of oxidative phosphorylation in the muscles of animals and are the sites for ATP provision in aerobic tissues. The primary role of mitochondria in the oxidative muscle fibres is that of the provision of ATP for muscle contraction. Both burst and sustained locomotor capacity are likely to be modified during adaptation to changed environmental temperatures. It has been found that during cold acclimation, fish increase their mitochondrial volumes and density in the muscles. This suggests that aerobic capacity of muscle is limiting at low temperatures. These are important considerations when looking at the swimming performance of the juvenile lobsters at the different temperatures of rearing.

In crustaceans, several biochemical processes are affected by elevated rearing temperature, such as the rate of protein turnover (synthesis and degradation). Thermal experience has immediate effects on metabolic rate (Hazel and Prosser, 1974), locomotory activity, protein turnover rates and enzyme kinetics. For example, in the muscles of isopod crustaceans, elevated water temperature has

direct effects on protein synthesis and transcription rates of actin and MyHc (Whiteley *et al.*, 1997). Similarly, protein synthesis rates in the leg and abdominal muscles of freshwater crayfish showed an exponential decrease as water temperature dropped in the winter from 12-1°C and was linked to a corresponding decrease in ribosomal activity (Whiteley *et al.*, 1997).

7.1.7 Aims of present study

The present study has utilised the convenience of the tail flip escape response of lobsters to test the effect of rearing temperature on locomotory performance, and hence muscle development and contractile activity. It was performed in conjunction with biochemical studies of protein turnover, and with a molecular study of myosin gene expression (Holmes *et al.*, 2001), which has allowed changes in the structure of key parts of the myosin molecule involved in the contractile and metabolic properties of muscle to be correlated with these changes in its performance.

7.2 Materials and Methods

7.2.1 Husbandry

All the juvenile lobsters, *H. gammarus*, used in this experiment, were held in isolated compartments in a rearing facility at CEFAS, Conwy. The lobsters were held at different water temperatures (11°C, 15°C and 19°C – 1998 cohort) (15°C and 19°C – 1999 cohort), in water of 32‰ salinity. All animals were tested at the CEFAS Laboratory at the temperatures of rearing. By consulting the records of moulting times maintained for every individual throughout the rearing programme, it was possible to ensure that all animals tested were in the intermoult state as defined by Aiken (1980).

The juvenile lobsters were tested at three different sampling sessions, March, September and November 1999 (1998 cohort) and August/September and November 1999 (1999 cohort). During these times, lobsters from moult stages 4-17 were analysed for their swimming performance.

7.2.2 Experimental Set-up

All experiments were carried out in a tank 55cm length x 15cm width x 25cm depth (Fig. 7.1) containing water at the temperature of rearing. Within this tank, a distance marker (5cm intervals) was placed on the back wall to facilitate the analysis of distance travelled and speed of swimming. The swimming sequences of all lobsters were recorded using a digital video camera (Panasonic AG EZ1E). The camera was positioned to view the whole length of the testing tank. Once recorded the swimming sequences of each lobster were transferred to standard VHS tapes, for later analysis.

7.2.3 Experimental Protocol

For recording the swimming sequences, the juvenile lobsters were transferred from their individual holding compartments, and allowed to settle in the testing tank for 10 minutes prior to stimulation. After settlement, the animal was subjected to mechanical stimulation (rostrum taps) using a plastic rod, which evoked the MG tail-flip response (as evidenced by the bending of all the abdominal segments, and a tail flip with a flat rearwards trajectory) followed by a series of a variable number of swimming flips. MG-mediated tail flips were activated in order to simply measure the swimming performance; LG mediated flips involve extensive twisting of the body.

After the initial swimming sequence was completed, and the animal had settled back on the substrate, the stimulus was repeated in order to elicit another swimming sequence. In some cases a third or fourth sequence was elicited, in order to provide sufficient data for analysis. At the end of this period, the animal was removed from the tank, then measured (mm) for carapace length (back of the eye socket to end of the carapace), body length (telson to tail-fan), wet weight (g) and abdomen length (derived by subtracting the carapace length from the body length) and returned to its individually numbered compartment in the rearing facility. This procedure was carried out for each of the animals, for both annual cohorts.

7.2.4 Video Analysis

The recordings of juvenile lobster swimming were analysed at the Division of Environmental and Evolutionary Biology at the University of Glasgow. The VHS tapes were replayed through a Panasonic AG-6730 VCR, which was capable of single frame freeze and advance, onto a 28" colour monitor.

For each sequence, an acetate overlay was placed on the monitor screen, the scale was marked out and a reference point (the centre of the eye of the lobster) was marked for every frame (20 ms intervals). In addition the times of endpoints of abdominal flexion (f) and full extension (e) were marked (see Arnott *et al.*, 1998). In this way, the durations of individual tail flips (defined according to Newland *et al.* (1988) as single cycles of flexion and extension of the abdominal segments) were also obtained. These swimming tracks were scanned and saved to computer as image files, where the *x*, *y* co-ordinates of each reference point were generated automatically using the software package Scion Image V. 3.0b (Scion Corporation).

7.2.5 Data Analysis

From the digitised tracks, measurements of mean distance per flip (mm), mean velocity of flip (ms^{-1}), mean duration of flip (ms) and total duration of the swimming sequence (ms), were calculated and plotted. From the automatically generated x, y co-ordinates, the distance travelled for each of the 20ms periods was calculated using the following equation: $\sqrt{}$ (((x2-x1)*(x2-x1)) + ((y2-y1)*(y2-y1))) (where x and y = co-ordinates). For the mean distance per flip, the beginning and endpoints of the flips were used as markers allowing the distance travelled in the flip to be summed from the values generated for each 20ms period. Values 'per flip' were averages based on the measurement of the three swimming flips following the initial MG-mediated flips. This first flip itself was not analysed, as it took place from rest, and also because the values obtained from the 4-6 frames that encompassed this flip (at the frame rate used, of $50s^{-1}$) would have been subject to an unacceptable error, as discussed by Arnott *et al.* (1998). The values generated for the distances travelled were used to attain the velocities reached during the flip, using the following equation: ((distance

travelled for each set of co-ordinates *50)/1000, these were then averaged for each flip). The velocity expressed as body lengths per second (BIs^{-1}) was derived as follows: (1000/body length)*velocity (Vs-1).

From the results of these analyses, comparisons of the kinematic performance of the juvenile lobsters reared at the different temperatures were tested statistically using Univariate analysis of variance (ANOVA). All tests were considered significant if $p<0.05$.

7.3 Results

Figure 7.2 shows three frames taken from a sequence of escape swimming by a juvenile lobster in response to a rostral stimulus. During the flexion phase, all the abdominal segments are bent to thrust the animal backwards. From such sequences, the trajectory of the escape swimming path and the durations of the individual flips could be re-constructed. A typical example for a Stage 11 lobster (9 months old) at 11°C is shown in *Figure 7.3.a*. Its trajectory is initially directly away from the point of stimulation, but later in the sequence it rises in the water before ceasing to swim. The points of flexion and extension have been marked on this plot, to indicate the individual tail-flips.

From such data, plots were derived of the distance travelled between successive frames (20 ms intervals) (Fig. 7.3.b) and corresponding inter-frame velocities (Fig. 7.3.c). For both the distance travelled and the velocity, the highest values occurred during flexion of the abdomen, and the lowest values during the extension of the abdomen. This pattern stayed relatively constant throughout the swimming sequence, which make the choice of flips 2-4 for deriving average values per tail-flip a valid one (Fig. 7.3.b).

7.3.1 1998 Cohort

Figures 7.4.a & 7.4.b, show the average distances travelled per tail-flip in swimming sequences performed by juvenile lobsters reared at the different temperature regimes. *Figure 7.4.a* shows the mean values for each temperature

group at the three main sampling ages of 9, 15 and 17 months. At each sampling age the distance travelled per flip increased with temperature, and the differences in this parameter between the three temperature groups can be clearly seen. Through a comparison of the means, significant differences at both 9 (ANOVA F-statistic, $F_{2, 23} = 18.38$, $p = 0.000$) and 15 months (ANOVA F-statistic, $F_{2, 45} = 18.44$, $p = 0.000$) can be shown to be between the 19°C group and both the 11 and 15°C groups, through Tukey's pairwise comparisons. At 17 months there are significant differences between all the temperature groups for distance travelled per flip (ANOVA F-statistic, $F_{2, 41} = 25.04$, $p = 0.000$). Significant differences between the age points within the temperature regimes as highlighted by Tukey's pairwise comparisons of the mean values, show the differences at 11°C to be between the 9 – 15 and 9 – 17 month periods (ANOVA F-statistic, $F_{2, 38} = 6.19$, $p = 0.005$); 15°C: 15 – 17 and 9 – 17 months (ANOVA F-statistic, $F_{2, 26} = 14.49$, $p = 0.000$); 19°C: 9 and 17 months (ANOVA F-statistic, $F_{2, 44} = 3.95$, $p = 0.026$). Hence all temperatures show a difference between the beginning and the end of the rearing period.

Figure 7.4.b shows the measures of distance travelled per flip for individual animals plotted relative to CL (used as a measure of body size). This allows comparisons to be made between animals as they reach a given size (achieved at different ages in the different temperature groups). This parameter correlates positively with size for all rearing temperatures, and although there appears to be a difference between the temperature groups, there is no difference between the regression slopes or their intercepts.

Figures 7.5.a & 7.5.b show the average velocity per flip of swimming sequences performed by juvenile lobsters reared at the different temperatures. The mean values for each temperature group at the three main sampling ages (Fig. 7.5.a) show that the average swimming velocity increased with temperature at 9 and 15 months, while at 17 months the 19°C group had a lower swimming velocity than the 15°C group. Also, average swimming velocity per flip increased with age from 9 to 15 months at both 11 (ANOVA F-statistic, $F_{2, 38} = 6.19$, $p = 0.005$) and 15°C (ANOVA F-statistic, $F_{2, 26} = 5.44$, $p = 0.011$), but not from 15 to 17 months, both temperature groups show a significant increase in average swimming velocity between 9 and 17 months of age (Tukey's pairwise comparison). The 19°C

lobsters show no increase in swimming velocity over the rearing period (ANOVA F-statistic, $F_{2, 44} = 0.52$, $p = 0.600$). When a comparison is done between temperature treatments at the three age points, it can be seen that there are significant differences between both the 11 and 19°C groups at 9 and 15 months (ANOVA F-statistic, $F_{2, 23} = 6.11$, $p = 0.007$; ANOVA F-statistic, $F_{2, 45} = 3.89$, $p = 0.028$). At 17 months there is only a difference between the 11 and 15°C groups (ANOVA F-statistic, $F_{2, 41} = 3.70$, $p = 0.033$). Data plotted relative to CL (Fig. 7.5.b) show that these trends also occur in relation to size and that the calculated regressions for the 11°C and 15°C temperature groups are very similar to each other (Univariate ANOVA; comparison of regression slopes and intercepts: no significant differences), but that the regression line for the 19°C group is flat ($R^2 = 0.0006$), indicating almost no change in average swimming velocity with size (Univariate ANOVA; comparison of regression slopes; $F_{2, 111} = 4.403$, $p = 0.014$).

When the average swimming speed is expressed as body lengths per second (BL s^{-1}) (Fig. 7.6.a), the values decrease with rearing temperature at each age point, and also decrease with age at each temperature of rearing. It can be seen that at 9 months there are no significant differences between the temperature regimes (ANOVA F-statistic, $F_{2, 23} = 6.11$, $p = 0.007$). At both 15 (ANOVA F-statistic, $F_{2, 45} = 19.88$, $p = 0.000$) and 17 months (ANOVA F-statistic, $F_{2, 41} = 14.51$, $p = 0.000$), the 11 and 19°C and 15 and 19°C lobsters have significantly different swimming speeds (Bl s^{-1}), but is not seen between the 11 and 15°C groups, highlighting a greater reduction at the higher temperature. When the differences are compared within the temperature groups, all show a significant reduction in swimming speed (Bl s^{-1}), between the beginning (9m) and the end (17m) of the rearing period (11°C: ANOVA F-statistic, $F_{2, 38} = 5.21$, $p = 0.010$; 15°C: ANOVA F-statistic, $F_{2, 26} = 3.82$, $p = 0.035$; 19°C: ANOVA F-statistic, $F_{2, 44} = 19.60$, $p = 0.000$ (also significantly different between 9 and 15m)).

Plotting these data relative to CL (Fig. 7.6.b) shows that these trends also occur in relation to size, although the regression line for the 11°C group is steeper than for the two higher rearing temperatures (Univariate ANOVA; comparison of regression slope intercepts; effect of temperature, 11°C vs. 15°C; $F_{1, 67} = 4.009$, $p = 0.049$).

For animals reared at 11°C an apparent discrepancy exists between the size-dependent values of average swimming velocity expressed as m s^{-1} (Fig 7.5.a) and those expressed as Bl s^{-1} (Fig 7.6.a), since the regression line for the former is similar to that from the 15°C temperature group, while the one for the latter has a steeper negative slope. The basis for this discrepancy was sought in the relative body dimensions of the abdomen and the cephalothorax (i.e. the ratio between the abdomen length (AL) and the carapace length (CL)) of lobsters tested for swimming performance (Fig. 7.7.a-c). The results show that the 11°C lobsters have longer abdomens relative to their carapace length, compared to the two higher temperature groups, at the 15 (ANOVA F-statistic, $F_{2, 45} = 12.71$, $p = 0.000$) and 17 month (ANOVA F-statistic, $F_{2, 41} = 5.81$, $p = 0.006$) age points and at 9 months are significantly longer than the 15°C group (ANOVA F-statistic, $F_{2, 23} = 3.93$, $p = 0.034$) (Fig. 7.7.a). All temperature treatments show an increase in the abdomen to carapace length ration within the rearing period, at 11°C (ANOVA F-statistic, $F_{2, 38} = 7.88$, $p = 0.001$) and 19°C (ANOVA F-statistic, $F_{2, 44} = 5.08$, $p = 0.010$), increases can be seen between 9 – 15 and 9 – 17 months. However at 15°C the only significant increase in Al:Cl ratio occurs between 9 – 15 months (ANOVA F-statistic, $F_{2, 26} = 3.49$, $p = 0.046$).

When compared against carapace length (Fig. 7.7.b), there is a significant difference between the regression lines of the three temperature treatments (Univariate ANOVA; comparison of regression slopes; $F_{2, 214} = 4.854$, $p = 0.010$). Therefore, a fixed proportionality between carapace length and either abdomen length or overall body length cannot be assumed, as these relationships can vary. *Figure 7.7.c* shows the ratio between the abdomen length and the carapace length, and again a significant difference between the regression slopes is seen (Univariate ANOVA; comparison of regression slopes; $F_{2, 112} = 5.266$, $p = 0.007$).

Figures 7.8.a &7.8.b show the average durations of the tail-flips in swimming sequences performed by juvenile lobsters reared at the different temperatures. The mean values for each temperature group at the three main sampling ages show trends for increasing duration with temperature at each age point, and with age for each temperature group (Fig. 7.8.a). These trends are seen significantly in the 19°C group (ANOVA F-statistic, $F_{2, 44} = 4.23$, $p = 0.021$) between 9 and 17 months, compared to the other two temperatures which showed no differences in

duration over the rearing period (11°C: ANOVA F-statistic, $F_{2, 38} = 0.24$, $p = 0.787$; 15°C: ANOVA F-statistic, $F_{2, 26} = 3.17$, $p = 0.058$). Between temperatures at the 3 sampling periods, there are no differences at 9 months (ANOVA F-statistic, $F_{2, 23} = 1.74$, $p = 0.199$). At 15 (ANOVA F-statistic, $F_{2, 45} = 8.85$, $p = 0.001$) and 17 months (ANOVA F-statistic, $F_{2, 41} = 15.64$, $p = 0.000$) the 19°C lobsters have significantly longer tail-flip duration's than the 11 and 15°C groups. Data plotted relative to CL (Fig. 7.8.b) show that the 11°C and 15°C groups show no change with increasing size ($R^2 = 0.0001$ & 0.0923 respectively), but that in the 19°C group there is a positive correlation between size and tail-flip duration ($R^2 = 0.235$), there is however, no significant difference between the slope or the intercepts of the regression lines (Univariate ANOVA; comparison of regression slopes and intercepts: no significant differences).

7.3.2 1999 Cohort

The swimming performance of the 1999 cohort of lobsters, reared from the egg at either 15°C or 19°C, was recorded and analysed in the same way as for the 1998 cohort.

Figures 7.9.a & 7.9.b show the average distances travelled per tail-flip in swimming sequences performed by juvenile lobsters reared at 15°C or 19°C. *Figure 7.9.a* shows the mean values for each temperature group at the two sampling ages of 3 months and 6 months. For each temperature group, the distance travelled per flip increased with age, but there is very little difference between the groups. When the values for individual animals are plotted relative to CL (Fig. 7.9.b), it can be seen that the distance travelled is positively correlated with size at both temperatures, although this relationship is steeper for the 15°C group than the 19°C group (Univariate ANOVA; comparison of regression slopes; $F_{1, 75} = 7.910$, $p = 0.006$). Extrapolating these trends, it would be expected that when the animals raised at 15°C attain the sizes of those raised at 19°C for 6 months, they would be travelling greater distances per tail-flip.

Figures 7.10.a & 7.10.b show the average velocity per flip of swimming sequences performed by juvenile lobsters reared at 15°C or 19°C. The mean values for each temperature group at the two sampling ages (Fig. 7.10.a) show

that for each temperature group the average swimming velocity increased with age, but there is no systematic difference between the temperature groups. When the values for individual animals are plotted relative to CL (Fig. 7.10.b) a clearer picture emerges: mean swimming velocity is positively correlated with size for the 15°C group, but has only a weak correlation with size for the 19°C group, these trends are also highly significantly different from each other (Univariate ANOVA; comparison of regression slopes; $F_{1, 75} = 10.663$, $p = 0.002$). In this respect the results are similar to those obtained from the 1998 cohort (*Fig. 7.5.b*).

The 19°C lobsters display a negative correlation between size and velocity, expressed as Bl s^{-1} (*Figs. 7.11.a & 7.11.b*): as CL doubles from 6.7mm to 13.4mm, relative velocity drops from c.a.17 Bl s^{-1} to c.a. 5.4 Bls^{-1}. Over the same growth period animals in the 15°C group grow from CL 5.0mm to 10.0mm, but maintain a relative velocity of c.a.13 Bl s^{-1} (R^2=0.0006) throughout. These regression slopes are significantly different from each other (Univariate ANOVA; comparison of regression slopes; $F_{1, 75} = 7.679$, $p = 0.007$). Since the ratio of abdomen length to carapace length for the 1999 cohort is very similar (at around 2.0) for both temperature groups at both age points (Figs. 7.12.a-c), it is unlikely that allometric changes underlie these observed differences in relative swimming velocity. Rather, it is the larger size of the 19°C lobsters, *per se*, compared to those at 15°C at 6 months that may best explain the reduction in velocity (Bl s^{-1}) shown in the results.

Figures 7.13.a & 7.13.b show the average durations of the tail-flips in swimming sequences performed by postlarval lobsters reared at 15°C and 19°C. The mean values for the 15°C group at the two sampling ages of 3 and 6 months (around 85ms) are lower than for the values at 9 months for the 1998 cohort (around 120ms) (*Fig. 8b*). However, the data for the 19°C group (Figs. 7.13.a and 7.13.b respectively) (Univariate ANOVA; comparison of regression slopes and intercepts: no significant difference) follow more clearly the pattern shown by the 1998 cohort at that temperature, with an increasing tail-flip duration with both age (Fig. 7.7a) and with size (Fig 7.7b).

7.4 Discussion

The analysis of the swimming performance of juvenile *Homarus gammarus* was carried out to investigate how different rearing temperatures affect this intrinsic escape behaviour. The results that have arisen are interesting because they do not necessarily conform to other similar investigations into the effect of temperature on swimming in fish and other animals.

The duration of tail flips can be correlated with the distance travelled per tail-flip. The 19°C group travels the furthest distance during the tail-flips, and so one would expect this group of lobsters to have tail-flip with the longest duration. This can especially be expected, as the velocities attained by the 19°C lobsters are not that much greater than the lower two temperature treatments. Overall the distance travelled (and hence the duration time) in a tail-flip appears to be positively correlated to the increasing size of the juvenile lobsters, as can be seen for both cohorts (1998 and 1999). So, the reduced distances travelled per flip at 11°C, could be attributed to the limited sizes that they reach during the acclimation period, when compared to the other two treatments.

The 1998 cohort showed that tail-flip duration increases with increasing size, but this is not the case for the 1999 lobsters. An explanation for the fact that tail flip durations do not increase with age from 3 to 6 months could be that the size range of the 15°C lobsters is below the level that increasing tail-flip duration occurs, and that the 19°C lobsters could be just reaching the sizes where this relationship emerges. It is also worth noting, that the durations of the 15°C tail flips might be expected to increase, in relation to the increase in distance travelled per flip exhibited. However, this correlates with the greater increase in velocity shown by the 15°C cohort, in comparison to the 19°C cohort, which shows very little increase in velocity over the rearing period.

7.4.1 Temperature compensation

The 11°C animals grow slowly, and it has been argued they are close to the null point for growth (Chapter 2). However, in terms of swimming speed, their performance is similar to the higher temperature animals, suggesting that some form of physiological compensation has taken place. For a given size, the 15°C lobsters are also capable of swimming faster than the 19°C lobsters. This may also represent compensation, or may reflect the fact that the 19°C animals are performing sub-optimally – their rapid growth rate may be responsible for this. Certainly, the 19°C lobsters show no real increase in velocity with increased size, in both of the cohorts tested.

Comparisons at the same age points indicate that animals reared and tested at the higher temperatures swim at a higher velocity than those reared at the lower temperatures, especially that of 11°C. Meaningful comparisons are difficult to make between these results, however, since the groups differ in size, allometry, rearing temperature and testing temperature. However, when animals from these groups are compared at the same body size, it would still be expected, on the basis of temperature rate effects alone, that those reared at higher temperatures would swim with a higher velocity than those reared and tested at lower temperatures. The fact that this relationship does not hold indicates that compensatory factors must be operating to reverse these expectations. Several possible compensatory responses may be involved.

7.4.2 Metabolic compensation

The most likely form of metabolic compensation is an acclimation response of the animals to the temperature of rearing, involving changes in enzyme activities so that they operate most effectively at the animal's rearing temperature. Much research on such thermal acclimation responses has focused on fish, and in many species it has been found that sustained swimming performance increases with temperature to an optimum, but then decreases (Fry and Hart, 1948; Brett, 1967). Several biochemical processes are affected by elevated rearing temperature, such as increased rates of protein turnover (synthesis and degradation), and in some cases these have been linked with swimming speed and the functional properties of muscles (Gerlach *et al.,* 1990). Carp, for example swim faster at low temperatures following acclimation to 10°C than fish

acclimated to 30°C, and vice versa (Johnston, 1985; Watabe *et al.,* 1998). More importantly, a minimum acclimation period of 2 weeks is required for this response, to allow time for protein turnover.

However, the optimal temperature for sustained swimming will vary with the thermal habitat of the animal and with acclimation status. Antarctic fish swim at an optimal temperature of 0°C, a temperature that would render most temperate fish inactive or dead, whereas in temperate fish, the temperature range for maximal aerobic performance is between 15-25°C. A common response by fish to short-term cold acclimation is to increase the mitochondrial volumes and densities in the red and white muscle, or to increase in the activity of the mitochondrial enzymes, all of which increase the aerobic capacity of the swimming musculature. The proportions of aerobic fibres in the swimming musculature may also rise in cold acclimation (Johnston and Lucking, 1978; Sidell, 1980).

An increase in mitochondrial volume and density during cold acclimation suggests that mitochondrial function becomes limiting during activity at low temperatures. These functional limitations could reflect the maximum oxidative capacities of mitochondria, limitations in diffusive transfer between the myofibrillar and mitochondrial compartments (Eggington and Sidell, 1989) or a loss of regulatory sensitivity (Dudley *et al.*, 1987) with a decrease in temperature (Guderley and St. Pierre, 1995). The muscle mitochondria of temperate zone species seem capable of more complete compensation of maximal capacities than those from Antarctic species. In response to the constraints of functioning at continuously low temperatures, Antarctic species have enhanced the levels of mitochondrial enzymes in the red swimming muscle (Crockett and Sidell, 1990). Johnston *et al.* (1988) also found that the Antarctic species of fish have mitochondrial muscle volume densities far greater than those of temperate species, reaching up to 60% in some cases.

In addition to increasing the mitochondrial volume densities, the more active Antarctic fish species have decreased the mitochondrial clustering and increased the intracellular lipid contents, which may minimise diffusive limitations and maximise rates of mitochondrial ATP generation. There are also potential modifications in the mitochondria themselves, such as membrane properties,

cristae density and variants of mitochondrial enzymes and membrane proteins, which could be implicated in the evolutionary adaptation of mitochondrial properties to distinct thermal habitats (Guderley and St. Pierre, 1995).

No studies on the mitochondrial densities of crustacean abdominal muscle have yet been performed in acclimated animals, so it not known whether similar metabolic compensations occur, as described for fish. In the present project ultra-thin sections of the abdominal muscle fibres from animals raised at the three temperatures have been prepared, which will allow measurements of mitochondrial densities to be made.

7.4.3 Phenotypic plasticity

Changes may also have occurred at the molecular level, as documented in parallel studies on these stocks of lobsters (Holmes *et al.*, 2001). Corresponding to the greater growth rates found at elevated temperatures, evidence has been obtained for up-regulation of fast myosin, actin and tropomyosin with rearing temperature. This suggests that elevated rates of protein synthesis and degradation were occurring with temperature, and this has been confirmed by measures of protein synthesis rates in Stage 10 larvae acclimated to 10, 14 and 19°C, using flooding doses of 3H phenylalanine (Holmes, Whiteley and El Haj, unpublished observations).

Corresponding to possible temperature acclimation, changes in the molecular structure of loop 1 on the myosin head have been identified in the different temperature groups (Holmes *et al.*, 2001). Since loop 1 acts as the ATP binding site it determines the rate of myofibrillar ATPase activity, and influences both the stability of the enzyme and the speed of contraction. Changes in its amino acid sequence will alter these properties, and hence would be expected to affect contractile performance in relation to temperature. Further discussion of the molecular correlates of temperature effects on muscle performance, is provided in chapter 9.

7.4.1.2. Allometry effects

It is known from other studies that the length of the abdomen is ~2x that of the carapace in juvenile lobsters, but that this value drops to around 1.4 as an adult Cromarty *et al.* (1991). This is discussed by these authors in the context of a shift in behaviour from escape to defence as ageing occurs. In the present project, the finding that animals reared at lower temperatures have relatively larger abdomens could represent a form of compensation, leading to increased propulsion relative to body mass, as a result of their larger surface areas and increased muscle volumes. These two findings also indicate that it is inappropriate to use CL as an estimator of body length, as the relationship between these two dimensions is temperature dependent, and may also be age dependent.

The analysis of the effects of allometry on the swimming propulsion of long-bodied crustaceans performed by Daniel and Meyerhoff (1989) (see Introduction to this chapter) indicates that thrust increases with relative abdomen size up to a point where rotational forces come to dominate over translational forces, when there is a consequent reduction in linear swimming velocities. Results obtained by Arnott *et al.*, (1998) on the tail flip swimming velocities of the brown shrimp *Crangon crangon* over a large size range conform to the predictions of this model, and it might be expected that similar relationships exist in *H. gammarus* post-larvae. It is interesting that in the 1999 cohort, the lobsters have not reached the stage in development where the abdomen starts to reduce in length, relatively, and so this allometry effect cannot contribute to changes in their swimming performance.

As seen with the 1998 data and other data published on the swimming performance of crustaceans, the velocity (BIs^{-1}) should decrease with increasing size. The lack of reduction of the BIs^{-1} value for the 15°C 1999 cohort could be attributed to the fact that within the 3 months between sampling, the 15°C lobsters have not grown in size enough for it to have an effect on the velocity rates (BIs^{-1}). The reduction in velocity of the 19°C lobsters expressed as $BI\ s^{-1}$ may be a function of two factors acting in combination: the rapid rate of growth of the animals at this temperature over the rearing period, and the small change in absolute swimming velocity that occurs with this increasing size. These could reflect the common finding across a range of species, including tail- flipping

crustaceans that relative swimming velocity decreases with size (Newland *et al.*, 1988; Daniel and Meyhöfer, 1989; Cromarty *et al.*, 1991; Smith, 1993; Neil and Ansell, 1995). This could, in turn, be due to a number of factors such as a greater increase in drag than in thrust (change in body shape) and/or a greater increase in body mass relative to that of the tail muscle that acts as the propulsor (allometric growth, especially of claws).

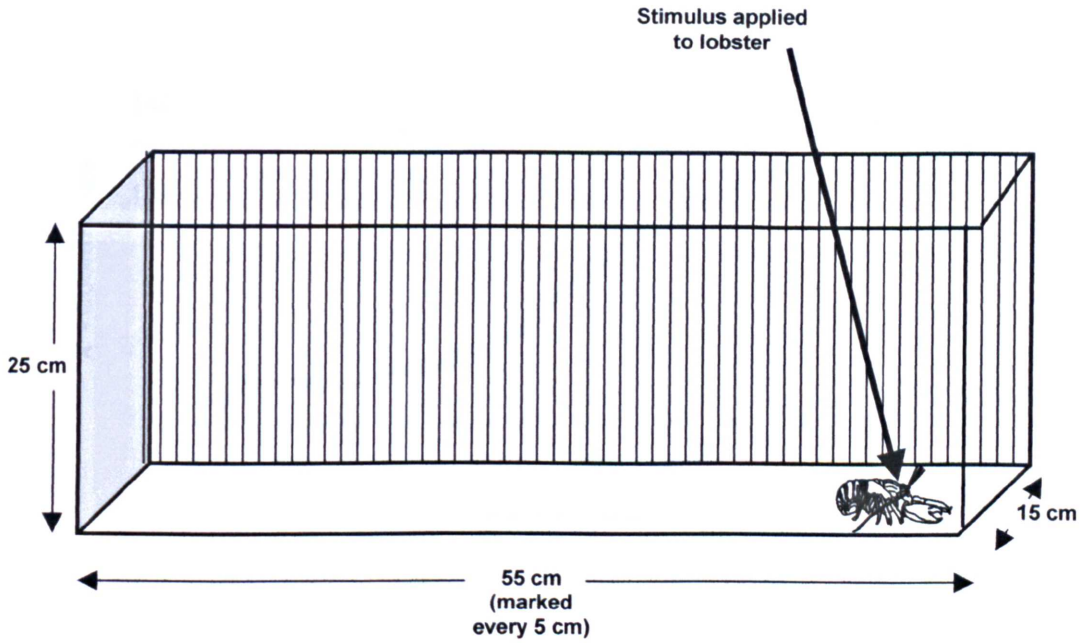


Figure 7.1: Diagram of the tank used to video the swimming performance of the juvenile *Homarus gammarus* (Dimensions: 55cm (length) x 15cm (width) x 25cm (depth). A scale (5 cm intervals) was marked on the back of the tank.

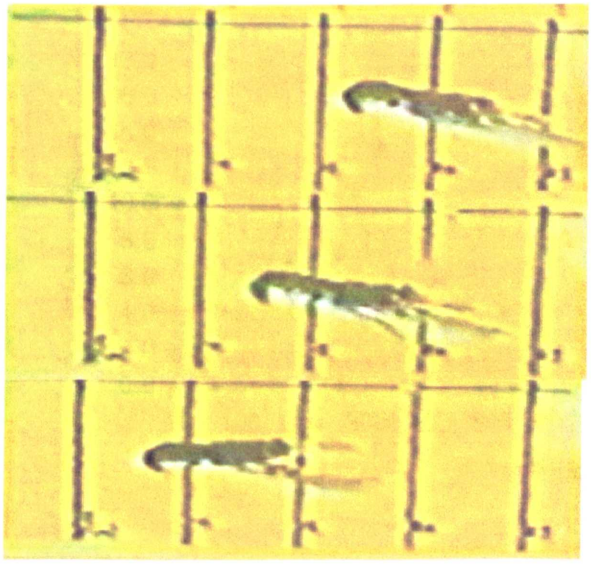


Figure 7.2: Still pictures taken from a swimming sequence of a juvenile lobster, *H. gammarus*, to highlight the flexion and re-extension of the abdomen during this escape behaviour.

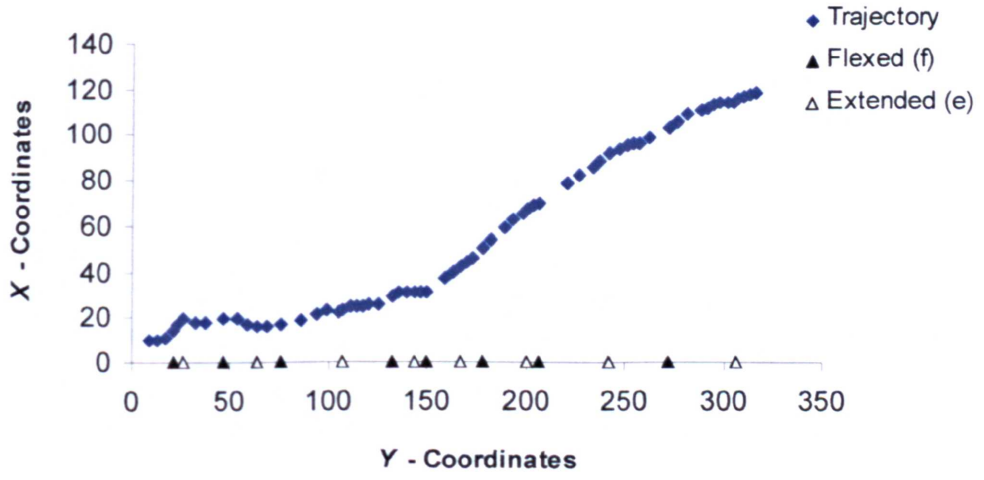


Figure 7.3.a: A reconstruction of the swimming trajectory of an 11°C stage 11 j lobster, with the flexion and re-extension points of the abdomen during the escape reaction marked on the horizontal axis.

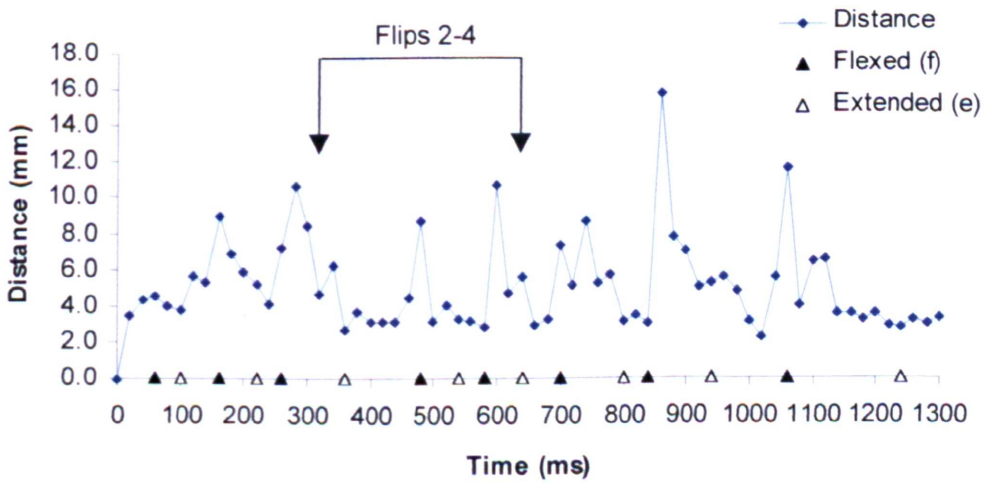


Figure 7.3.b: A plot of the distance travelled by a Stage 11 lobster reared at 11°C, to show the changes between the power stroke and return stroke phases of the tail-flip swimming sequence.

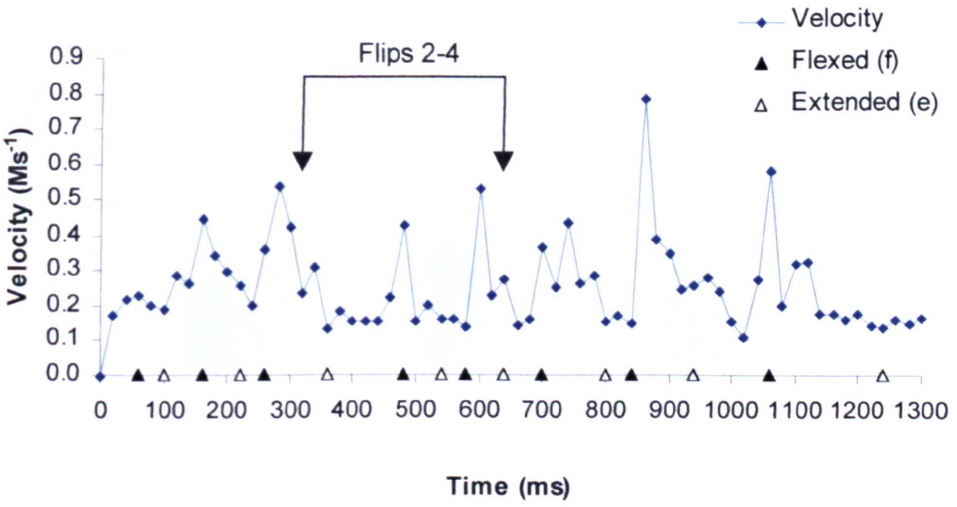


Figure 7.3.c: The velocity attained for each tail-flip in a swimming sequence from an 11°C stage 11 lobster, showing how the velocity attained is greatest at the point of abdominal flexion (each peak).

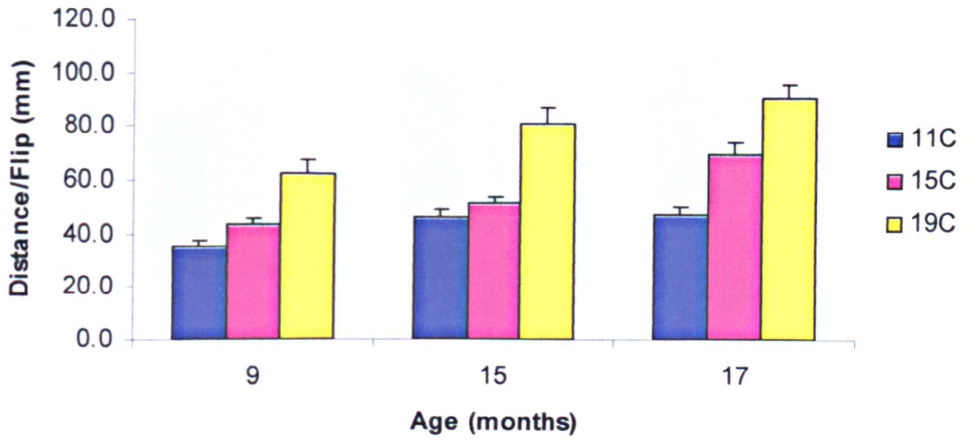


Figure 7.4.a: The mean distances travelled per tail-flip for the lobsters of the 1998 cohort at the different temperatures at the three main sampling points, of 9, 15 and 17 months of age.

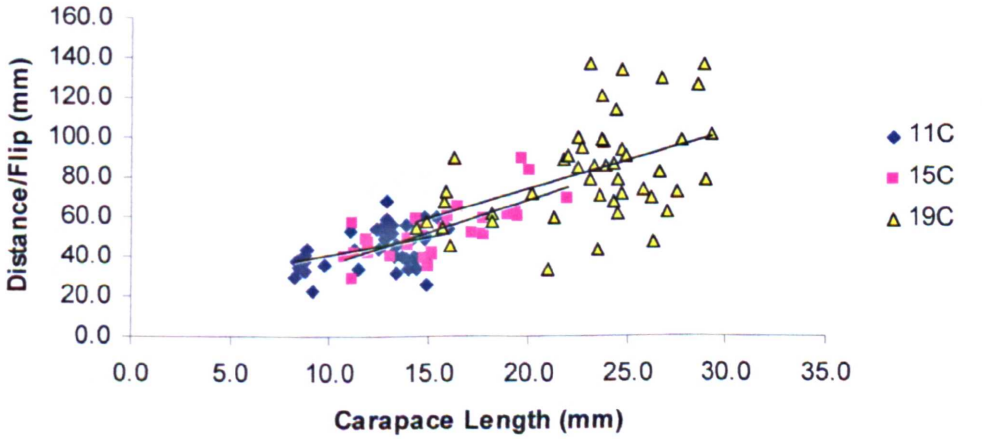


Figure 7.4.b: The average distance travelled per tail-flip, for each of the individual lobsters of the 1998 cohort tested at the three temperatures of rearing, over the rearing period. Regression equations: 11°C: y = 1.8634x + 21.243, R2 = 0.1641; 15°C: y = 3.1656x + 3.9799, R2 = 0.5297; 19°C: y = 2.8599x + 16.153, R2 = 0.2061. Univariate ANOVA: comparison of regression slopes and intercepts; n.s.: $F_{2, 111}$ = 0.411, p = 0.664; $F_{2, 113}$ = 1.322, p = 0.271.

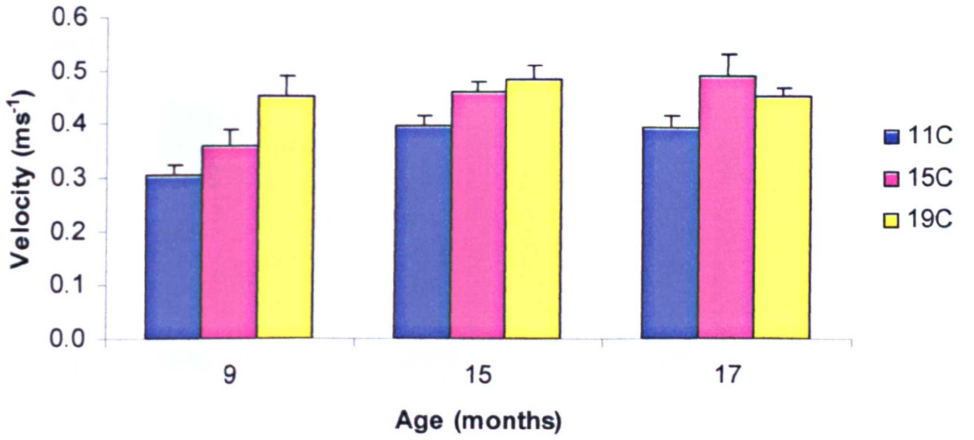


Figure 7.5.a: The mean velocities attained during the tail-flips for the lobsters of the 1998 cohort reared at the different temperatures at the three main sampling points of 9, 15 and 17 months of age.

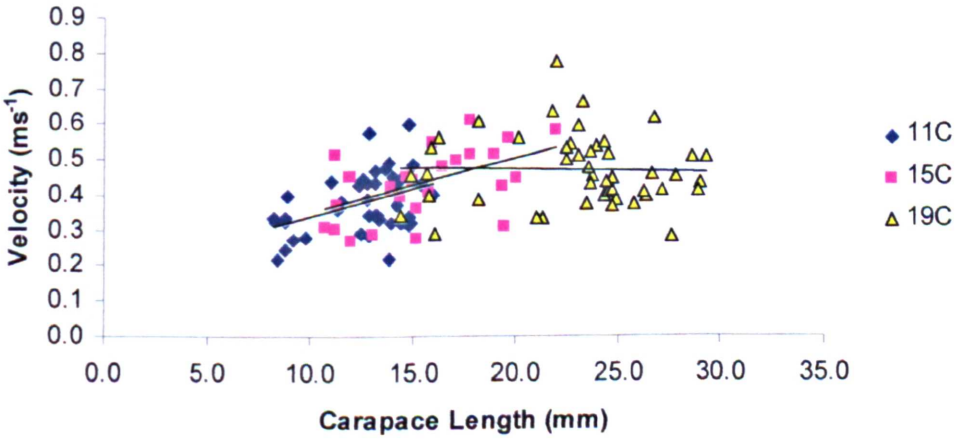


Figure 7.5.b: The mean velocities (ms^{-1}) attained by the individual lobsters of the 1998 cohort reared at the three temperatures, over the acclimation period. Regression equations: 11°C: y = 0.0152x + 0.1849, R2 = 0.1617; 15°C: y = 0.0149x + 0.1998, R2 = 0.2481; 19°C: y = -0.0006x + 0.4792, R2 = 0.0006. Univariate ANOVA; comparison of regression slopes; $F_{2, 111} = 4.403$, p = 0.014.

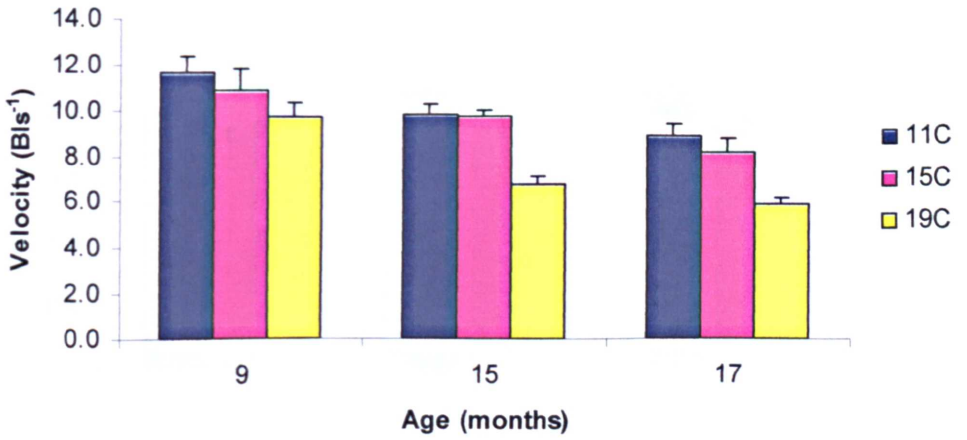


Figure 7.6.a: The mean velocities (Bls^{-1}) attained during the tail-flips for the lobsters of the 1998 cohort at the different temperatures at the three main sampling points, of 9, 15 and 17 months of age.

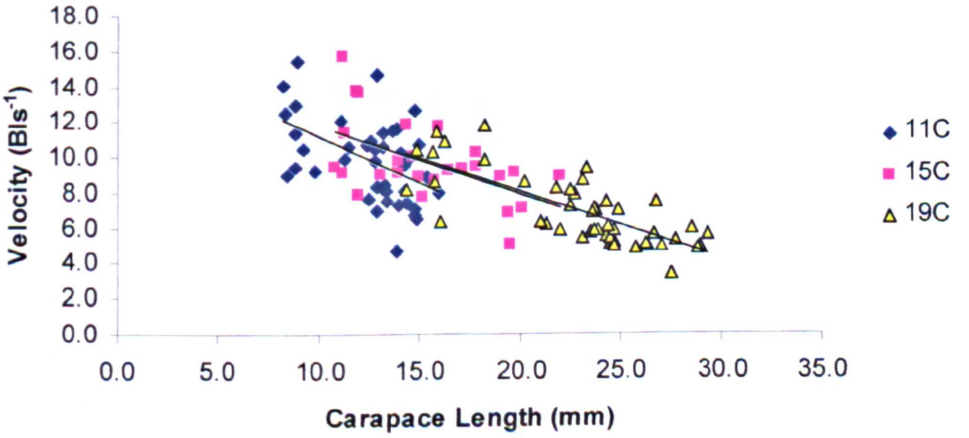


Figure 7.6.b: The mean velocities (Bls^{-1}) attained by the individual lobsters of the 1998 cohort reared at the three temperatures, over the acclimation period. Regression equations: 11°C y = -0.5159x + 16.274, R2 = 0.2507; 15°C y = -0.381x + 15.5, R2 = 0.299; 19°C y = -0.3727x + 15.453, R2 = 0.5818. Univariate ANOVA; comparison of regression slope intercepts; effect of temperature, 11°C vs. 15°C; $F_{1, 67} = 4.009$, $p = 0.049$.

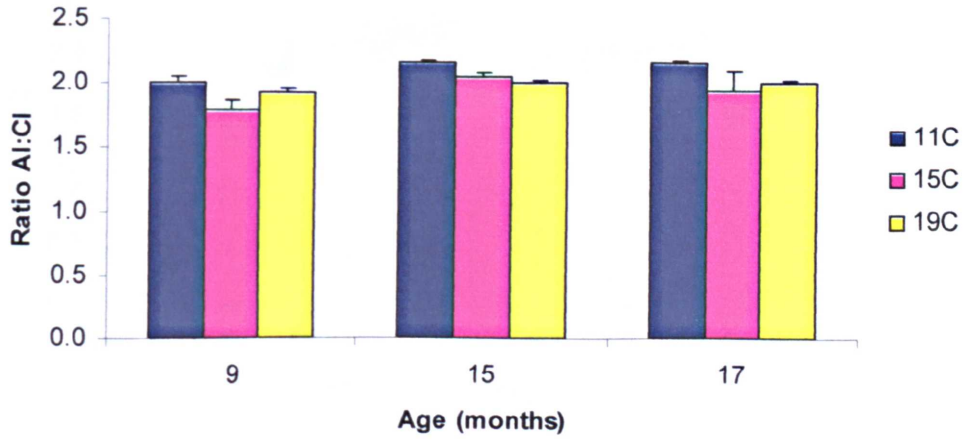


Figure 7.7.a: The mean ratios of abdomen length to carapace length, for the lobsters of the 1998 cohort at the different temperatures at the three main sampling points, of 9, 15 and 17 months of age.

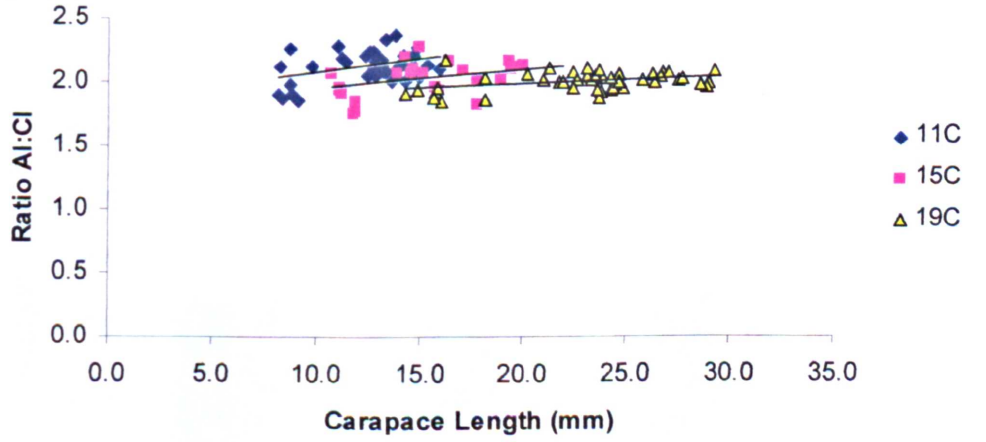


Figure 7.7.b: The ratio of abdomen length to carapace length for the individual lobsters of the 1998 cohort at the three temperatures, over the acclimation period. Regression equations: 11°C y = 0.0216x + 1.8482, R2 = 0.1722; 15°C y = 0.0148x + 1.7867, R2 = 0.1374; 19°C y = 0.0061x + 1.8465, R2 = 0.1142. Univariate ANOVA; comparison of regression slopes; $F_{2, 214} = 4.854$, $p = 0.010$.

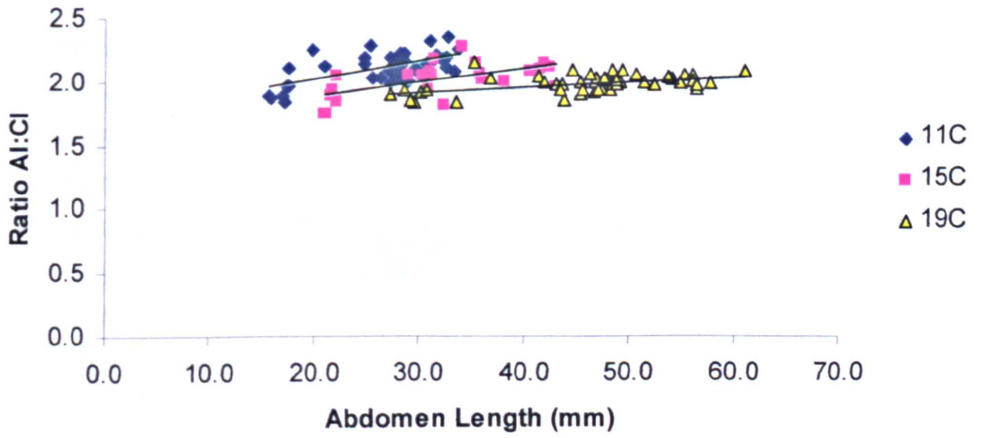


Figure 7.7.c: The ratio of abdomen length to carapace length for the individual lobsters of the 1998 cohort at the three temperatures, over the acclimation period, expressed against the abdomen lengths of the lobsters. Regression equations: 11°C y = 0.0133x + 1.7639, R2 = 0.3731; 15°C y = 0.0101x + 1.6974, R2 = 0.3369; 19°C y = 0.0041x + 1.7977, R2 = 0.2381. Univariate ANOVA; comparison of regression slopes; $F_{2, 112} = 5.266$, $p = 0.007$.

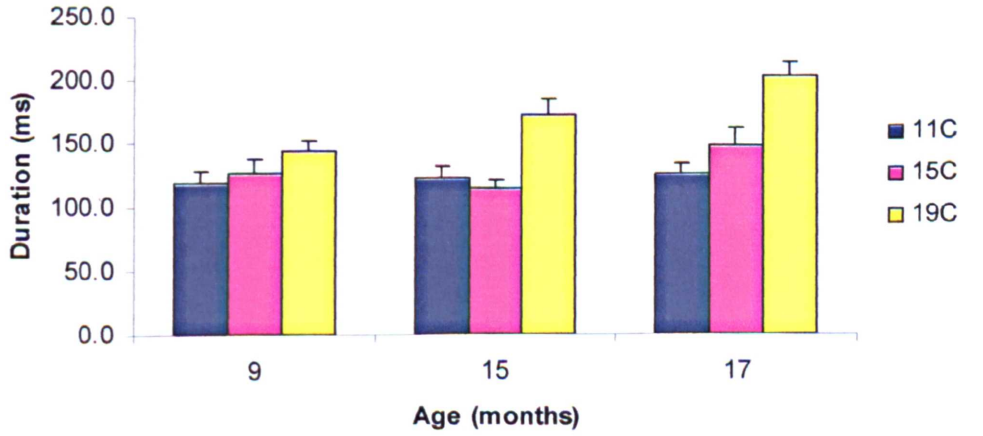


Figure 7.8.a: The mean durations (ms) of the tail-flips, for the lobsters of the 1998 cohort at the different temperatures at the three main sampling points, of 9, 15 and 17 months of age.

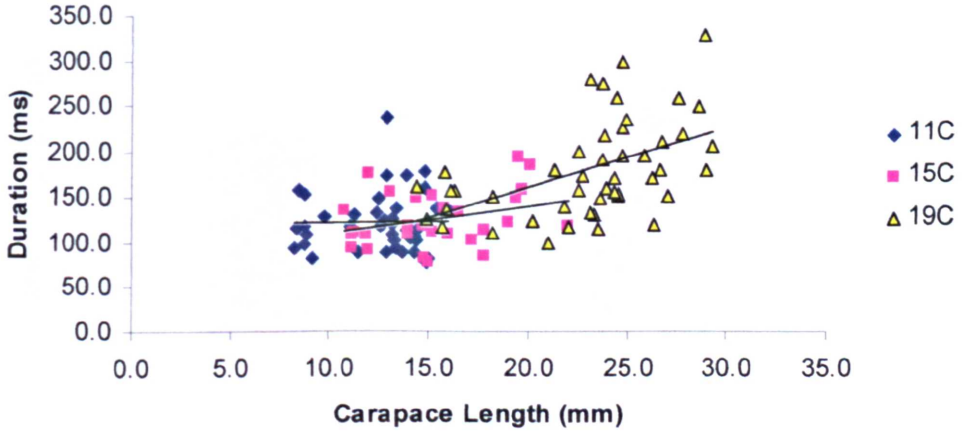


Figure 7.8.b: The mean durations (ms) of the tail-flips, attained by the individual lobsters of the 1998 cohort reared at the three temperatures, over the acclimation period. Regression equations: 11°C y = 0.1668x + 120.74, R2 = 0.0001; 15°C y = 2.9325x + 81.523, R2 = 0.0923; 19°C y = 6.5032x + 29.989, R2 = 0.235. Univariate ANOVA: comparison of regression slopes and intercepts: n.s.: $F_{2, 111} = 2.482$, p = 0.088; $F_{2, 113} = 1.177$, p = 0.312.

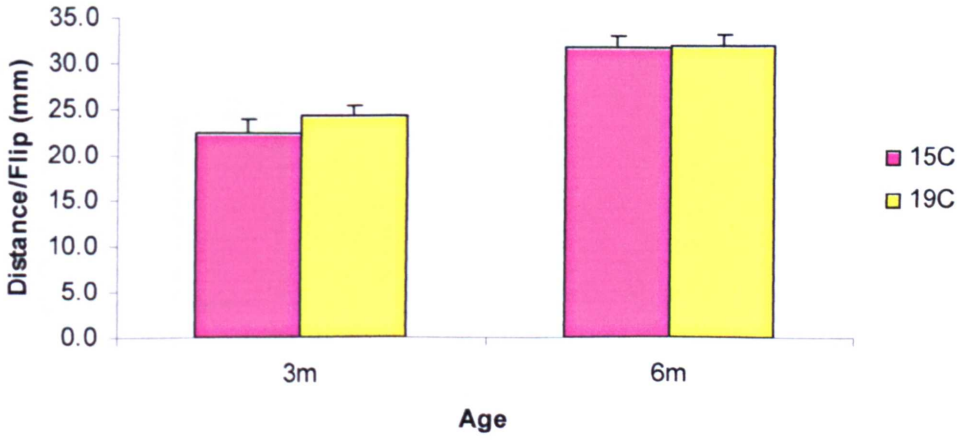


Figure 7.9.a: The mean distances travelled per tail-flip for the lobsters of the 1999 cohort at the different temperatures at the two main sampling points, of 3 and 6 months of age.

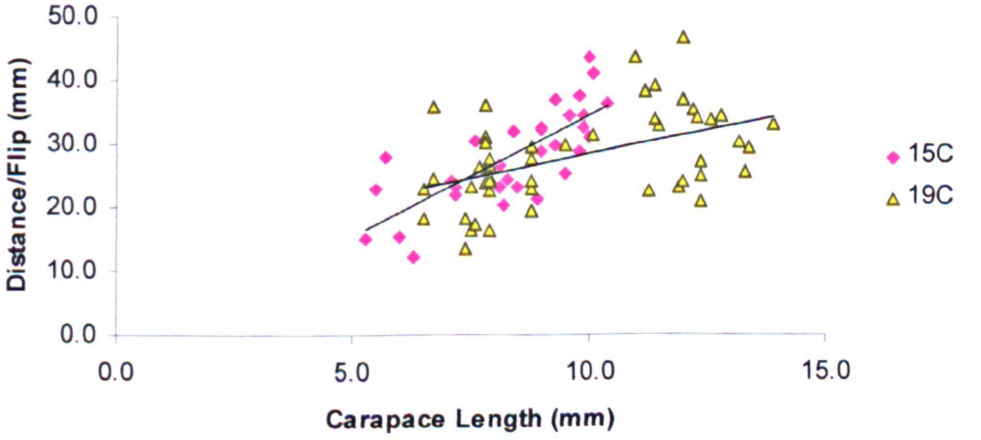


Figure 7.9.b: The average distance travelled per tail-flip, for each of the individual lobsters of the 1999 cohort tested at the two temperatures of rearing, over the growth period. Regression equations: 15°C y = 3.7925x - 3.9248, R2 = 0.5765; 19°C y = 1.5042x + 13.008, R2 = 0.2283. Univariate ANOVA; comparison of regression slopes; $F_{1, 75} = 7.910$, $p = 0.006$.

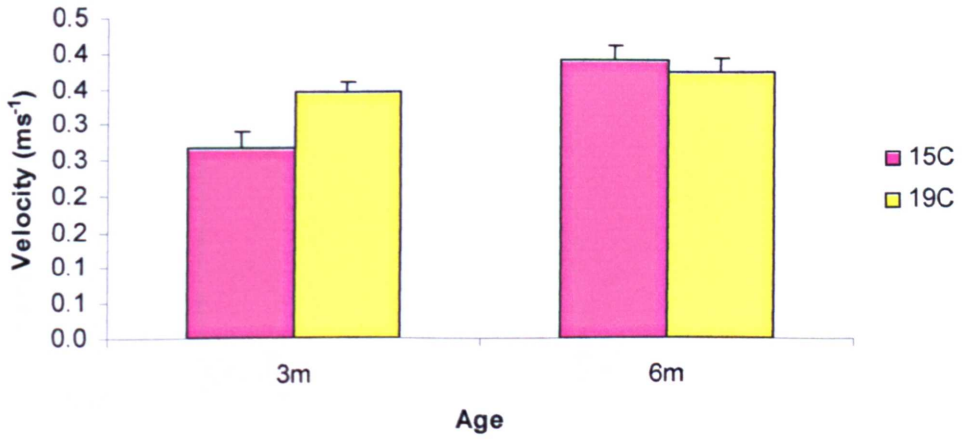


Figure 7.10.a: The mean velocities (Vs^{-1}) attained during the tail-flips for the lobsters of the 1999 cohort at the different temperatures at the two main sampling points, of 3 and 6 months of age.

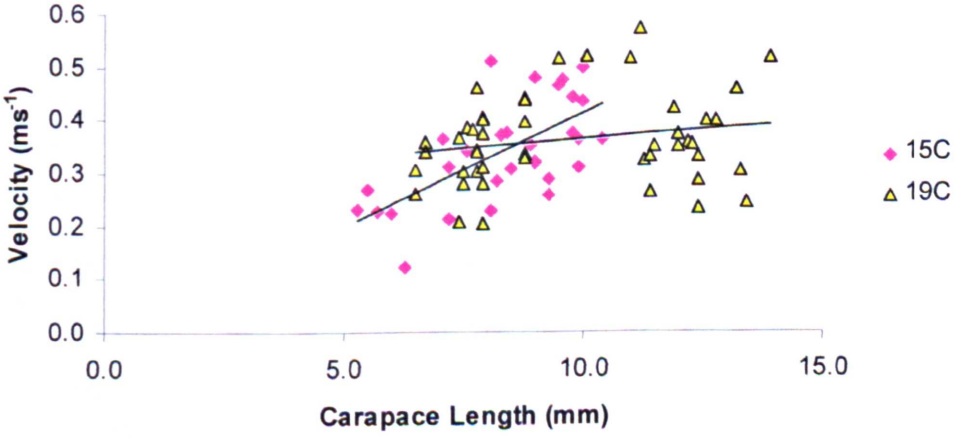


Figure 7.10.b: The mean velocities (ms^{-1}) attained by the individual lobsters of the 1999 cohort reared at the two temperatures, over the growth period. Regression equations: 15°C y = 0.0432x - 0.0198, R2 = 0.4221; 19°C y = 0.0071x + 0.2902, R2 = 0.0382. Univariate ANOVA; comparison of regression slopes; $F_{1, 75} = 10.663$, p = 0.002.

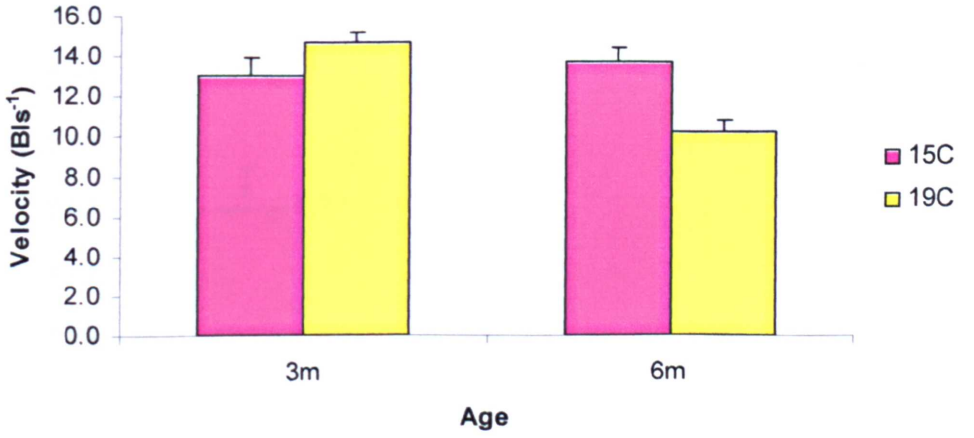


Figure 7.11.a: The mean velocities (Bls^{-1}) attained during the tail-flips for the lobsters of the 1999 cohort at the different temperatures at the two main sampling points, of 3 and 6 months of age.

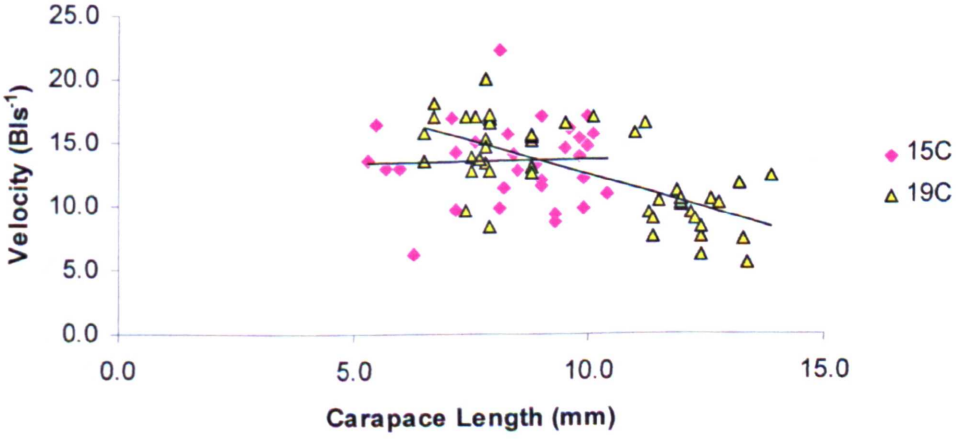


Figure 7.11.b: The mean velocities (Bls^{-1}) attained by the individual lobsters reared at the two temperatures, over the growth period. Regression equations: 15°C y = 0.0515x + 12.991, R2 = 0.0006; 19°C y = -1.0416x + 22.824, R2 = 0.4611. Univariate ANOVA; comparison of regression slopes; $F_{1, 75} = 7.679$, $p = 0.007$.

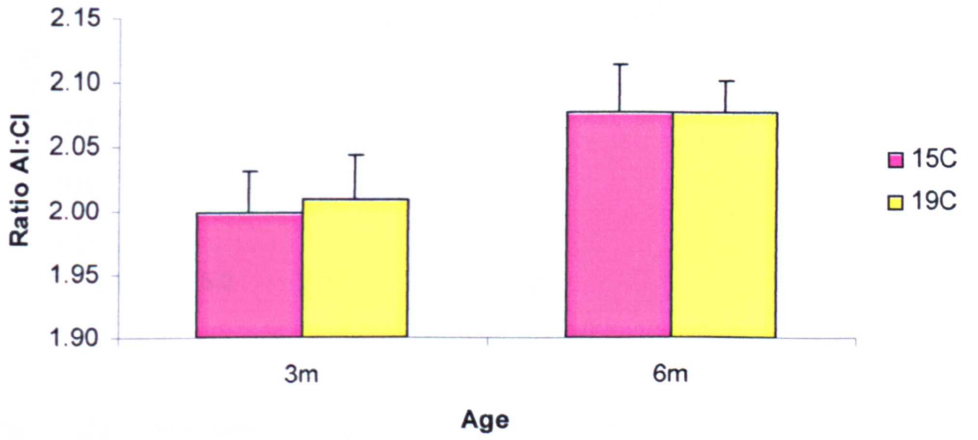


Figure 7.12.a: The mean ratios of abdomen length to carapace length, for the lobsters of the 1999 cohort at the two temperatures at the main sampling points, of 3 and 6 months of age.

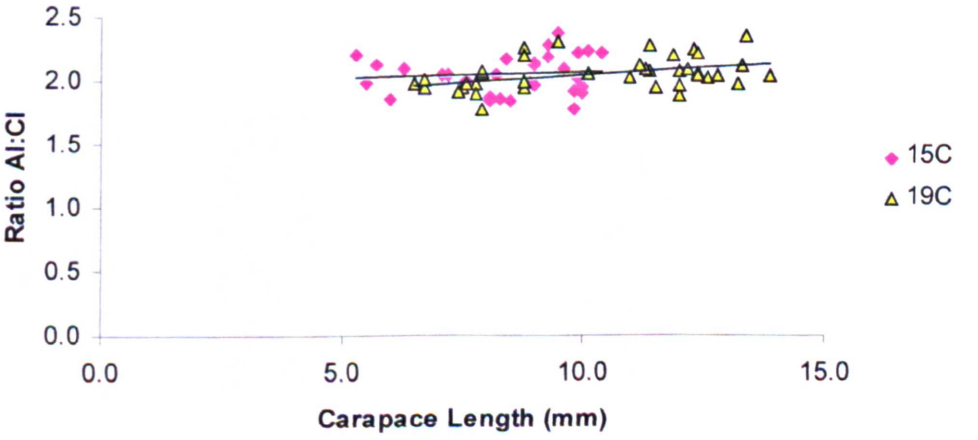


Figure 7.12.b: The ratio of abdomen length to carapace length for the individual lobsters of the 1999 cohort at the two temperatures, over the growth period. Regression equations: 15°C: y = 0.0106x + 1.957, R2 = 0.0115; 19C: y = 0.0241x + 1.7909, R2 = 0.1939. Univariate ANOVA; comparison of regression slopes and intercepts: n.s.: $F_{1, 75} = 0.156$, $p = 0.694$; $F_{1, 76} = 0.688$, $p = 0.409$.

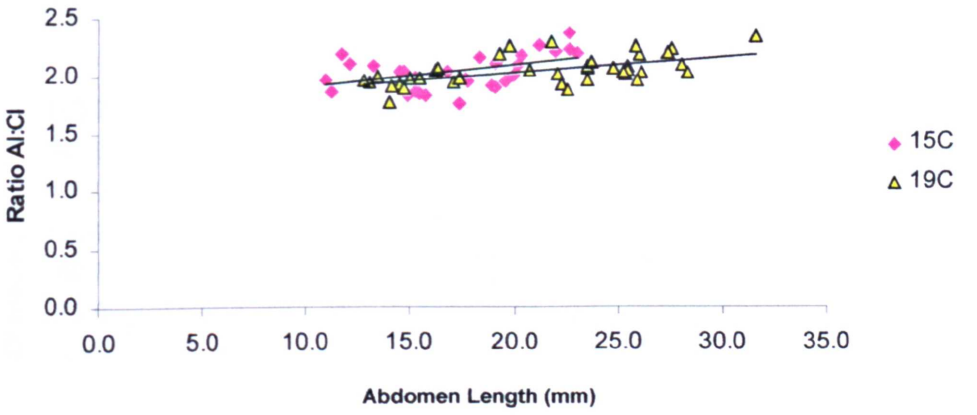


Figure 7.12.c: The ratio of abdomen length to carapace length for the individual lobsters of the 1999 cohort at the two temperatures, over the growth period, against abdomen length. Regression equations: 15°C: y = 0.02x + 1.7008, R2 = 0.2222; 19°C: y = 0.0147x + 1.7331, R2 = 0.3878. Univariate ANOVA; comparison of regression slopes and intercepts: n.s.: $F_{1, 75} = 0.459$, p = 0.500; $F_{1, 76} = 2.633$, p = 0.109.

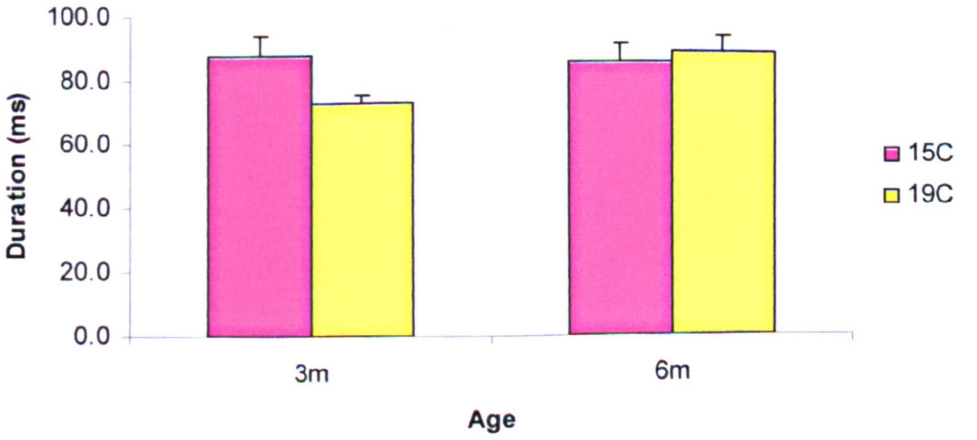


Figure 7.13.a: The mean durations (ms) of the tail-flips, for the lobsters of the 1999 cohort at the different temperatures at the two main sampling points, of 3 and 6 months of age.

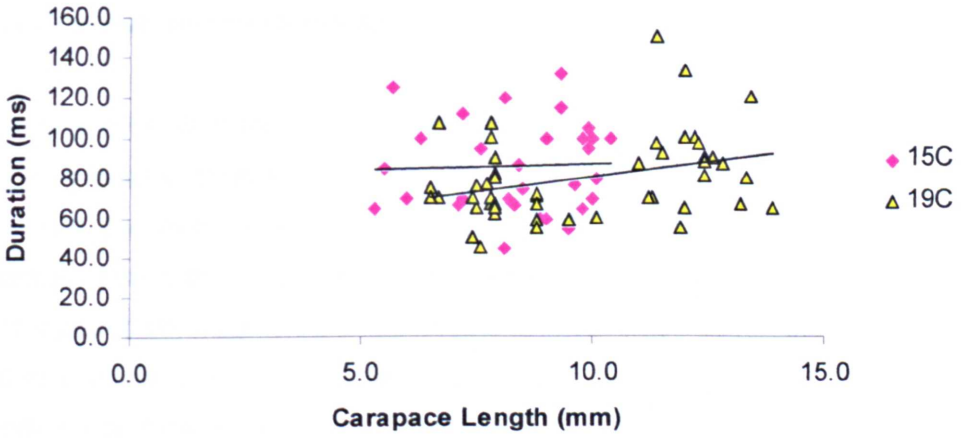


Figure 7.13.b: The mean durations (ms) of the tail-flips, attained by the individual lobsters of the 1999 cohort reared at the two temperatures, over the growth period. Regression equations: 15°C y = 0.5928x + 81.084, R2 = 0.0016; 19°C y = 3.0117x + 50.138, R2 = 0.1112. Univariate ANOVA; comparison of regression slopes and intercepts: n.s.: $F_{1, 75} = 0.701$, $p = 0.405$; $F_{1, 76} = 3.757$, $p = 0.056$.

Chapter 8 - Agonistic encounters in juvenile, naïve lobsters: does rearing temperature or age affect the behaviour?

8.1 Introduction

8.1.1 Aggression and Dominance

Protective rock shelters or burrows are used by lobsters to avoid predation, and to gain access to food and mates (Atema and Voigt, 1995). They are particularly important for early juveniles, because attacks from benthic predators are common during the first year of life (Larvelli and Barshaw, 1986). In natural lobster populations agonistic encounters are used for the acquisition and defence of these shelters, and a dominance hierarchy is established which functions to determine preferential access to them (Hyatt, 1983; Atema, 1986). If two juvenile lobsters are introduced into a common space, fighting usually results, which varies in its intensity and duration. The eventual outcome, however, is that one individual becomes dominant over the other (Huber and Kravitz, 1995). It is this ability to dominate con-specifics that makes juvenile lobsters such good subjects for the study of agonistic behaviour.

There are some predictors of success in agonistic encounters such as size, sex, moult state, physical condition and prior experience (Atema and Cobb, 1980). Generally the outcome of contests between two individuals with large size asymmetries is easy to predict by an observer (Vye *et al.*, 1997). These contests are usually very short, with minimal physical contact, and the outcome is determined very quickly. As often found in a number of species, the initial approach of a bigger individual towards a smaller one can cause the latter to move away (Jachowski, 1974; Helfman, 1977). If these factors are closely matched, then the confrontation is more likely to escalate. In almost symmetrical contests the prediction of a winner is more difficult (each individual has close to a 50% chance of winning) and the contest comprises different behavioural components compared to an asymmetrical contest (Vye *et al.*, 1997).

It is the winning or losing of these agonistic encounters that determines dominance and it is these dominance relationships that allow the lobsters to

establish their place within the social hierarchy of the population. Physical fights and behavioural displays are used to establish these dominance relationships, but they are maintained almost solely by displays, and only rarely involve combat. Dominance relationships require each participant to memorise the encounter. Agonistic encounters tend to occur less in the wild due to the large amount of space, resulting in a lower likelihood of two individuals being forced together. Confrontations in small experimental arenas are therefore atypical in this respect.

8.1.2 Game Theory

As detailed in Immelman and Beer (1989), extensive and conspicuous threat displays or ritualised combat often take place between two conspecifics before physical combat occurs. Such routines are restricted to harmless manoeuvres and avoid the use of damaging weapons; they allow individuals to gather information on their opponent's fighting ability and associated aspects such as strength (Enquist and Leimar, 1983). This can be linked to Game Theory (Parker, 1974), which states that agonistic behaviour may provide an assessment of an opponent's fighting ability, while effectively reducing the risk of injury to both combatants, especially in asymmetric contests. It is also apparent that while attempting to assess an opponent's fighting ability, an animal should give no indication as to how long or hard it, itself, is willing to fight for (Maynard-Smith, 1982). In the presence of a prominent asymmetry, for example in size, encounters among juvenile lobsters are quickly resolved. Also according to Game Theory (Maynard-Smith and Price, 1973; Caryl, 1979), in a symmetrical contest or if the asymmetries are not obvious, the eventual winner and loser should display a similar agonistic behaviour, through various levels of intensity, until the point of decision is reached, often after an escalated fight (Parker, 1974; Parker and Rubenstein, 1981; Maynard-Smith, 1982).

Another major factor in animal populations is that of an 'Evolutionary Stable Strategy' (ESS), which can be defined as a strategy that, if all members of a population adopt it, no 'mutant' strategy can do better, a rule that specifies which action to take for each combination of causal factors (Enquist and Leimar, 1983). Most real contests are asymmetric, to some degree, and so the ESS in asymmetric contests will usually be to permit the asymmetry to settle the contest

without escalation. However, if information to the contestants about the asymmetry is imperfect, escalated contests will occur (Maynard-Smith and Parker, 1976). It has been thought for some time that these inhibiting mechanisms and threat displays are beneficial for the long term survival of a species (Lumsden and Holldobler, 1983), and so will be strongly selected for. Fighting within juvenile lobsters, and other animals, is potentially very costly, due to the high chance of injury, so it makes sense for the fights to be resolved as early as possible.

8.1.3 The Nature of Lobster Aggression

The following account is based on studies of *H. americanus* (Huber and Kravitz, 1995). Initially when lobsters approach each other, both engage in a variety of threat postures, generally without physical contact. The most common display is ***'meral spread'***, in which the lobster raises its claws high and wide in front of it and its conspecific (Dingle, 1969). A highly ritualised aspect of lobster fighting is where the animals touch both claw tips in meral spread and push each other back and forth (***'do-si-do'***) (Huber and Kravitz, 1995). Then a period of restrained combat generally occurs, in which the animals touch each other with their claws (usually kept in the closed position). 'Reaching' occurs when the claws are repeatedly stretched far apart, as if measuring each other's span. A continuation of the fight develops whereby the lobsters lock claws and attempt to displace one another by pulling or pushing i.e. ***'wrestling'***. Also attempts are made to lift the opponent off the substrate and turn it on its back. In some cases the encounter does not proceed further, due to the highly structural behavioural system, which avoids physical injury (Huber and Kravitz, 1995).

Such sequences conform to Game Theory for asymmetric fights (Parker, 1974). However if no decision is reached the increase in intensity of the fight is dramatic. This last phase of combat is usually short in duration. Here both animals make unrestrained and extensive use of their claws ***'strike/rip'***. Antennae are grasped, as are the chelipeds and legs, and attempts are made to rip these appendages from the opponent. The juvenile lobsters amplify this ripping motion by using short upward directed tail flips, keeping the animals in close proximity. After some time, one animal will generally ***'retreat'*** using a tail flip, and often remains in the

corner of the tank. This animal is classed as the sub-dominant and maintains a body position close to the ground. The dominant of the pair initiates (or attempts to initiate) further bouts until the sub-dominant consistently retreats (Huber and Kravitz, 1995).

It is also believed that during the various stages of interaction, animals may be assessing the other's fighting potential. Ploys are used to deceive the opponent, such as meral spread, and raising the body high on its walking legs to try and make itself look bigger to the opponent, as also found in crabs (Glass and Huntingford, 1988). In the periods of restrained combat, the animals obtain information about the other's vigour and stamina, which cannot be falsified (Parker, 1974).

Vye *et al.*, (1997) have shown in *H. americanus* that the outcome of seemingly symmetric contests can be predicted if subtle cues such as claw dimensions, exoskeleton calcium content or plasma protein level. These are essential for the prediction of winners in these contests by an observer and possibly also for competing lobsters when assessing the relative resource holding potential (RHP) (Parker, 1974) of the other contestant. Knowledge of the relative values of the above key variables is vital in a combat situation (Vye *et al.*, 1997). It has been shown that measurement of the serum protein concentration is most important predictor of winning contestants (Stewart *et al.*, 1967) as a measure of muscle weight. The relationship of the claw dimensions is the second most important predictor of a contest, especially the width of the crusher and cutter claws, or their relative volumes. These variables have been shown to correlate positively and so probably relate to muscle biomass and hence contraction power (Vye *et al.*, 1997). It is possible that when lobsters are in an encounter they could assess, indirectly, the relative plasma protein level, calcium exoskeleton concentration, and the claw dimensions of their rivals. This would be done through assessing the effect on claw contraction forces, resistance of exoskeleton to pressure and general fighting vigour. Mechanoreceptors distributed over certain areas of the exoskeleton would provide such information (Solon and Cobb, 1980a).

8.1.4 Chemical Signalling and Urine

The role of urine signalling is important in the establishment and maintenance of dominance relationships. Studies in this area form a large part of current research into aggressive behaviour (Karavanich and Atema, 1998a, b; Breithaupt *et al.*, 1999; Douglas, 2000; Breithaupt & Atema, 2000). While it has been known for some time that lobster displays play an important role in dominance relationships, it has mostly been the case that the displays examined were visual or acoustic. However, in terms of conveying information, it is equally likely that chemical signals play an important role (Atema and Voigt, 1995). Lobster urine, excreted from the base of the 2nd antennae, carries a vast amount of information about the producer. It can provide the identity, sex, aggressive motivation as well as other attributes (Breithaupt and Atema, 2000). The amount of urine release is increased during aggressive encounters (Breithaupt, 1999) and this increase is also linked to increases in the intensity of fighting and would, therefore seem to be used for signalling during fights. Eventual winners use urine signals more often in the early stages of an encounter than do the losers (Breithaupt and Atema, 2000). This may prevent the escalation of the fight and limit the risk of potential injury. Being aware of the urine of other individuals is essential to maintain the dominance hierarchy within lobster groups, but is an ability rarely found in invertebrates (Douglas, 2000). This recognition of the smell of another individual coupled with aggressive encounters may establish and reinforce the dominance relationships (Karavanich and Atema, 1998). For example, the losing animal stops urinating, continually retreats and tail flips to escape the advances of the winner. The memory of losing persists for many days, and alters the willingness of the animal to engage others in combat. In addition to its role in shelter competition, dominance also confers greater female attractiveness through the odour produced by the dominant male, compared to the subordinate (Bushmann and Atema, 2000).

8.1.5 Development and Aggression

Aggressive behaviour in lobsters is known to be expressed in juveniles before the attainment of sexual maturity. This is because, unlike cases where aggression is related to sexual behaviour alone (Atema and Voigt, 1995), lobsters also interact

agonistically with conspecifics in order to secure access to shelters or burrows. The willingness of juvenile lobsters to display aggression is therefore appropriate from the start of postlarval life (Stage 4), when they first seek burrows in soft sediments (Atema and Voigt, 1995). The main modulating influences on this behaviour during further development are related to the developmental processes themselves, and include both growth and differentiation of the body and limbs. Changes might therefore be expected to occur progressively with age, but also recurrently with the cycles of moulting, since immediately after moulting lobsters are known to be reclusive and less willing to interact agonistically (Tamm and Cobb, 1978). Since moulting occurs around ten times in the first year (reducing progressively to once per year from year 3) (Phillips *et al.*, 1980), postlarval lobsters will spend much time in this state. These considerations have led to the following rationale for an experimental study of agonistic interactions in developing postlarval lobsters.

8.1.6 Rationale for the Experimental Study

- An aggressive attitude affects the lobster's survival chances at all ages, so an ability to interact, aggressively, from the time that they reach the first postlarval stage is expected. The rituals of agonistic encounters may be pre-wired in the nervous system and so not dependent on learning.

- However, the nature of agonistic behaviours might be expected to change with age and experience, to deal with changing requirements. The patterns used by the juveniles may develop into more sophisticated displays in the adult (Huber and Kravitz, 1995).

- The fact that animals were reared as isolates up to the time of testing allows the effects of experiencing social interactions to be excluded.

- The rearing programme at different temperatures has altered the rate of moulting relative to age, and so allows the effects of age and moult stage to be determined separately.

Therefore the opportunity was taken to determine the extent to which agonistic behaviour of the European lobster, *H. gammarus,* is pre-determined, whether it is affected by the time of the first encounter, and, by examining animals growing and moulting at different rates, whether these changes are age- or moult-dependent. This has been done by recording the frequency, composition and sequence of the behavioural displays associated with agonistic interactions, and comparing these between the ultimate winners and losers.

From such results, it may be possible to predict the success of laboratory-reared animals in competing with wild populations (which would probably have had more fighting experience).

8.2 Materials and Methods

8.2.1 Experimental Setup

The agonistic encounters between the juvenile *H. gammarus* were arranged under standard conditions. In each case the two protagonists were naïve animals of the same stage from the same temperature group, and they were also closely matched for size. The lobsters used were all at least 7 days post moulting, to avoid the reclusive period and weakness due to a soft exoskeleton. The two animals were placed in a tank (140 x 70 x 50 mm) containing seawater at the temperature of rearing, and with a layer of sand at the bottom. They were initially separated by a partition for a period of 30 minutes, and after that time the partition was raised and the encounter was recorded for a period of one hour on a digital video camera (Panasonic AG EZ1E).

8.2.2 Video analysis

The fights were analysed by replaying the video tapes through a recorder with a single frame advance facility (Panasonic AG-6730). The beginning of each fight was determined as the first approach or point of contact between the two lobsters, and the subsequent bout was separated into eight 30 second periods (a total of 240 seconds), which encompassed the duration of the whole encounter. The behaviours occurring within these periods were noted in sequence, for each

of the lobsters involved. Splitting the record in this way and resetting the counts at the beginning of each 30 seconds was a convenient way to keep the numbers small.

8.2.3 Behaviours analysed

The behaviours that were identified and recorded in these encounters were:

A) Meral Spread (Body Up, Antenna Up, Claws Up, Claws Touch)
B) Wrestling (Antenna Up, Claws Grasp, Push/Pull)
C) Do-Si-Do (Antenna Whip, Claws Down, Approach)
D) Retreat (Approach, Retreat)
E) Strike/Rip

These behaviours were noted down in the order that they occurred in the encounter for each of the 30 second periods, allowing a sequence of behaviour to be built up. Table 1 shows a hypothetical example of a 30 second period of such an analysis.

Time (seconds)	Sequence of events	Behaviour of Lobster No. X	Behaviour of Lobster No. Y
	1	A (Meral)	
	2		A (Meral)
	3	B (Wrestling)	
	4		B (Wrestling)
30	5	D (Retreat)	

Table 8.1: A hypothetical example of a recording an encounter.

It would be interpreted as – Lobster X approaches with meral spread, this meral spread is returned by Lobster Y, which then initiates a wrestle by both protagonists, and finally lobster X retreats. In a real fight this 30-second fragment would be repeated eight times to complete the four-minute (240 second) encounter. The winner of each contest was determined retrospectively and was

designated as the animal that retreats the least number of times during the encounter (Vye and Cobb, 1997).

A total of 38 paired encounters between lobsters of the three temperature groups at various moult stages were analysed in this way. Encounters between pairs of lobsters raised at the three temperatures (11°C, 15°C and 19°C) were arranged from the postlarval stages (Stages 9) through to 18 months post-hatching (i.e. up to Stage 17). The numbers of bouts at each stage for each temperature are shown in *Table 8.2.*

Temp	St. 9	St. 10	St. 11	St. 12	St. 13	St. 14	St. 15	St. 16
11°C	4	3	0	2	3	1	0	0
15°C	4	1	2	1	2	0	1	0
19°C	3	1	2	0	1	1	2	2

Table 8.2: The number of fights per moult stage (St.) for analysis at the three rearing temperatures

Only the 1998 cohort was used in this assessment of agonistic behaviour, so it is not possible to comment on the behaviour immediately post-larvally, but as all the lobsters used were naïve, the behaviours shown should be indicative of pre-wired nature of these contests. This provided a large data set for analysis, allowing the following parameters to be measured:

- The total number of agonistic behaviours performed in an encounter by both winner and loser
- The frequency of each behaviour exhibited in an encounter by both winner and loser
- The sequence of behaviours displayed by an individual winner or loser
- The sequence of behaviours displayed between protagonists, measured both as winner action / loser reaction and loser action / winner reaction.

8.3 Results

In almost all cases the fights were resolved within the 240 s of the recording.

8.3.1. Differences between winners and losers

8.3.1.1 Total Number of Behaviours Exhibited

The 11°C group values for total behaviours exhibited begin at the same point for winners and losers. This does separate by the end of the acclimation period, but not by a great margin (Univariate ANOVA; comparison of regression slopes: no significant differences) (Fig. 8.3.a). The 15°C lobsters generally have a higher level of behaviours exhibited by the winning lobsters in bouts than by those that lost. There is a slight increase in the number of behaviours with an increase in moult stage/development, however there is no difference between the regression lines of their intercepts (Univariate ANOVA; comparison of regression slopes: no significant differences) (Fig. 8.3.b). The 19°C lobsters show the same pattern as the 15°C lobsters, with the winners exhibiting more behaviours than the losers (Fig. 8.3.c). This group also shows a rise in number of behaviours shown with increasing stage, causing there to be a difference in the intercepts of the slopes, indicating that at a given stage the winners exhibit more behaviours than the losers (Univariate ANOVA; comparison of regression slope intercepts; $F_{1, 21}$ = 4.423, p = 0.048). These lobsters reach a higher number of behaviours shown than the 11°C and 15°C groups. When the losing and winning lobsters are plotted at the three different temperatures (Figs. 8.8.a+b), there appears to be very little difference between the temperature treatments in relation to the number of behaviours exhibited by the lobsters in the encounters (Univariate ANOVA; comparison of regression slopes and intercepts: for both winners and losers: no significant differences).

8.3.1.2 Number of Retreats

There are significantly more retreats made by losers than by winners, at all stage points and at all rearing temperatures. This has an increasing trend for the 11°C group (Univariate ANOVA; comparison of regression slopes; $F_{1, 22}$ = 4.666, p =

0.042) (Fig. 8.2.a), but at 15°C (Fig. 8.2.b) (Univariate ANOVA; comparison of regression slope intercepts; $F_{1, 19} = 22.533$, $p = 0.000$) and 19°C the initial difference is maintained with stage (Fig 8.2.c) (Univariate ANOVA; comparison of regression slope intercepts; $F_{1, 21} = 9.904$, $p = 0.005$).

8.3.1.3 Number of Meral Spreads

Winning lobsters show more incidences of meral spread behaviour than the losers, a difference that is significant at 15°C (Fig. 8.4.b) (Univariate ANOVA; comparison of regression slope intercepts; $F_{1, 19} = 8.692$, $p = 0.008$) and 19°C (Fig. 8.4.c) (Univariate ANOVA; comparison of regression slope intercepts; $F_{1, 21} = 7.607$, $p = 0.012$) but not at 11°C (Fig. 8.4.a) (Univariate ANOVA; comparison of regression slopes and intercepts: no significant differences). However these differences do not seem to increase with stage, as the trend lines are flat.

8.3.1.4 Wrestling Behaviour

The amount of wrestling behaviour is the same between winners and losers 11°C (Fig. 8.5.a) and 19°C (Fig. 8.5.c) (Univariate ANOVA; comparison of regression slopes and intercepts: no significant differences), but is significantly greater in winners than losers at 15°C (Fig. 8.5.b) where there is also an increasing trend with stage (Univariate ANOVA; comparison of regression slope intercepts; $F_{1, 19} = 4.462$, $p = 0.048$),

8.3.1.5 Do-Si-Do Behaviour

At all temperatures there is no difference in the do-si-do behaviour exhibited between the winning and losing lobsters, and there are no trends with moult stage (Fig. 8.6) (Univariate ANOVA; comparison of regression slopes and intercepts: no significant differences).

8.3.1.6 Strike/Rip Behaviour:

Strike/rip behaviour was generally higher in winners than losers (Fig. 8.7), although this was statistically significant only in the 15°C group (Fig. 8.7.b). There was no trend for these relationships to change with stage.

8.3.2 Changes with Age and Moult Stage

8.3.2.1 Individual behavioural acts

To further assess whether any differences with the behaviours exhibited by the juvenile lobsters occur with increasing age/stage, the data have been split in to three stage ranges (9-11, 12-14 and 15-16) and the averages taken for the individual behaviours. The five behaviours (retreats, meral spreads, wrestling, do-si-do and strike/rip) are shown for each temperature, at each of the stage ranges (Figs. 8.9-8.11).

8.3.2.2 Stages 9-11

All temperatures show the winning and losing lobsters to have a high level of wrestling behaviour (Figs. 8.9.a-c). The fact that the levels are high in both the winners and the losers is due to the nature of the wrestling behaviour where both participants are required. At all temperatures, the winning lobsters showed a slightly greater level of the strike/rip behaviour than the losers, whereas the levels of do-si-do were almost identical in both the winning and losing lobsters at all temperatures. This behaviour often involved the mirroring of posture or movement between the two individuals.

At 11°C, it can be seen that there is very little difference between the number of meral spreads and retreats shown by the winning and losing lobsters, although it might be expected that the losing lobster would retreat more than the winner (Fig. 8.9.a). At 15 and 19°C, the winners show higher levels of the meral spread behaviour compared to low incidences of retreating. In contrast the losing lobsters show the opposite relationship, with significantly higher levels of retreating in the losers compared to the winners (15C: ANOVA F-statistic, $F_{1, 12} = 9.81$, $p = 0.009$; 19C: ANOVA F-statistic, $F_{1, 10} = 6.86$, $p = 0.026$), and low levels of the meral spread behaviour (Figs. 8.9.b+c). This is an expected relationship,

behaviourally, as a retreat will be shown in response to a meral spread, by a lobster if it is losing against a conspecific that has already asserted its dominance through a simple size asymmetry or through an escalation of the fight through the various levels of aggression, this relationship will be explored later in this chapter.

8.3.2.3 Stages 12-14

The values for all the behaviours displayed by the winning and losing lobsters appear to be similar to those seen at stages 9-11, at all temperatures (Figs. 8.10.a-c), the only exception is that of the values for the wrestling behaviour, which has increased considerably at all temperatures, and as detailed for both winners and losers. However, the proportions of high meral spread to low retreats in the winners and vice versa in the losers, is more apparent than at stages 9-11 for both the 11°C and 15°C. This could indicate that once the encounters have been through the rigorous wrestling, with a significantly higher incidence of wrestling occurring between the winners and losers at 15°C (ANOVA F-statistic, $F_{1,4} = 19.17$, $p = 0.012$) (as seen by the high numbers) the winners are able to force a retreat simply by using a meral spread and not attacking. The values for the 19°C lobsters is around the same level as seen at stages 9-11, as is the case for the strike/rip behaviour. However at 11°C it can be seen that the winners show far more incidences of the meral spread behaviour than the losers (ANOVA F-statistic, $F_{1,10} = 12.60$, $p = 0.005$) and the losers show a higher level of retreats than the winners (ANOVA F-statistic, $F_{1,10} = 12.34$, $p = 0.006$). The latter is also shown at 15°C (ANOVA F-statistic, $F_{1,4} = 108.00$, $p = 0.000$).

8.3.2.4 Stages 15-16

At stage 15-16, only the 15°C and 19°C lobsters have representatives, as the 11°C cohort has not reached this developmental stage due to it low moulting rate (see chapter 2). As before, both cohorts show high levels of wrestling, indicating a close match between combatants. It can be seen in the 15°C lobsters (Fig. 8.11.a) (n=1 bout) that the winner does not retreat at all and dominates the meral spread behaviour over its opponent. The strike/rip behaviour is also, unexpectedly, dominated by the eventual winner of the 15°C winner. The winning lobsters of the 19°C show a high level of meral spreads to the high levels of

retreats by the losers (Fig. 8.11.b). The winning lobsters also dominate the strike/rip behaviour. In both cases the levels of do-si-do are approximately equal between the winning and losing lobsters. However, as the 15°C cohort only had one pair of lobsters at this stage range a statistical comparison could not be made. A statistical comparison on the stage 15-16 19°C lobsters showed no differences between winners and losers in the behaviours analysed.

8.3.3 Total number of behaviours

8.3.3.1 Differences between the stage ranges within the temperature groups

At 11°C the total number of behaviours exhibited increases from stages 9-11 to 12-14 in the winning lobsters, but not in the losing lobsters (Fig. 8.12.a). Neither of these increases was statistically different. In the 15°C cohort there is a general trend of increasing number of behaviours shown by the winning lobsters with increasing age (Fig. 8.12.b). However this trend is affected by a considerable increase in the total number of behaviours shown by the losing lobsters at stages 9-11 to 12-14, which is statistically different (ANOVA F-statistic, $F_{2,8} = 9.86$, $p = 0.007$). The values for the losing lobsters at stages 9-11 and 15-16 are very similar. Both the winning and losing lobsters at 19°C show an increase in the total number of behaviours from stage 9-11 to stages 12-14 (Fig. 8.12.c). The values at Stages 12-14 and 15-16, for both winning and losing lobsters are very similar. However a significant difference between the total number of behaviours exhibited by the winning lobsters can be seen between stages 9-11 and 15-16 (ANOVA F-statistic, $F_{2,9} = 6.62$, $p = 0.017$). The lobsters at this temperature exhibit the most behaviours in the analysed time period, possibly indicating that the level of aggression is higher at this temperature, or just simply that the lobsters are so closely matched for size that there is a great tendency for the encounters to escalate aggressively, until the eventual winner is decided.

8.3.3.1 Differences between the temperature groups within the stage ranges

When the values for total behaviours are compared between the temperature treatments, it can be seen that there is very little difference between them at the

Stage range of 9-11 (Figs. 8.13.a) with the 19°C lobsters displaying the most behaviours, but this is not statistically different (Winners: ANOVA F-statistic, $F_{2, 17}$ = 0.83, p = 0.451; Losers: ANOVA F-statistic, $F_{2, 17}$ = 0.98, p = 0.387). At Stages 12-14, it can be seen that the 11°C lobsters show fewer behavioural acts than the 15°C and 19°C groups of lobsters (Fig. 8.13.b), for both winning and losing lobsters there are no statistical differences between the temperature regimes (Winners: ANOVA F-statistic, $F_{2, 8}$ = 1.07, p = 0.387; Losers: ANOVA F-statistic, $F_{2, 8}$ = 1.19, p = 0.352). At stages 15-16, as already mentioned, the 11°C cohort is not present. Here it can be seen that the 19°C lobsters show far more behaviours than the 15°C cohort (NB: n=1 bout). However, in this stage range the 19°C lobsters appear to be showing the greatest number of total behaviours, but there are no differences for winners or losers between the two temperatures (Winners: ANOVA F-statistic, $F_{1, 3}$ = 1.90, p = 0.262; Losers: ANOVA F-statistic, $F_{1, 3}$ = 0.68, p = 0.469).

8.3.4 Sequences of behaviour

In order to test for the significant patterns of behaviour that arise within the encounters, Chi-squared tests were performed to ascertain which of the behavioural sequences occurred more or less than expected. From this analysis the main patterns of behaviour could be identified for the following interactions: winner action to loser reaction and loser action to winner reaction, allowing coverage of both aspects of the encounters. This analysis was split into three main components: total behaviour over the whole population and age range, up to 9 months of age and 15 to 17 months of age, for all three temperatures. The results from the Chi-squared tables have been converted into behavioural sequence diagrams, which show whether or not each behavioural pairing elicits a response more or less than expected. The arrows indicate the direction of movement and the colour indicates whether the sequences are displayed more (red) or less (blue) times than expected, with respect to the observed results. In addition to this, the dashed lines represent the behavioural interactions that contribute most to the overall interaction (derived from the individual chi-squared values assigned to each of the interactions, which make up the overall Chi-squared total). For all of the Chi-squared tests, the values were significant (for a 0.01 level of confidence, for 16 degrees of freedom, a value above 32.0 is

required), thus indicating that somewhere within the observed data there are behavioural patterns occurring more often than would be expected by chance.

The sequences that did occur more often than expected in the encounters account for a high proportion of the behaviours in the interactions, and were often reciprocated between the winner and loser. These were as follows: meral to meral, do-si-do to do-si-do and wrestle to wrestle. This indicates the great extent to which lobsters exhibit 'mirroring' tactics involving 'low intensity' behaviours without resorting to 'high intensity' behaviours such strike/rip. When this does occur, however, it is also in most cases mirrored, showing that both the winners and losers are willing to attack the other, and to reciprocate the action of the other. This indicates that the situation is often finely balanced up to a point of resolution. After resolution, the winning lobster re-iterates its dominance over the losing lobster by further meral spreads, asserting its presence and size.

A large proportion of the encounters are made up of the reciprocal acts of meral spread to meral spread, do-si-do to do-si-do and wrestling to wrestling. These acts can be classed as part of the low intensity loops of behaviour, except for that of wrestling, as that is indicative of an escalation in aggression, to a high intensity loop of aggression. Meral spread and do-si-do both allow the opponents to assess each other for size and power, and are part of the decision making process, that could lead a fight on to a higher level of aggression or to stop as one of the animals would see the other as too much of a threat, as game theory states. Strike/rip to strike/rip is also found to be of a high proportion of the encounters in both winning and losing lobsters, actions and reactions. This is part of a high level intensity loop of behaviour within the encounters, and indicates how both participants are willing to risk injury to become the dominant of the pair.

8.4 Discussion

A number of factors are known to influence the outcome of agonistic interactions between two combatant lobsters: body size, which is the most important factor in dominance (Scrivener, 1971); moult stage (Tamm and Cobb, 1980); sex (Scrivener, 1971); previous social experience (Atema and Cobb, 1980; Bakker *et*

al, 1989; Chase *et al.*, 1994). In the present study the first of these factors was eliminated by pairing animals of the same size, and the last factor was not involved as animals were held in isolation up to the time of the encounter. The discussion below will deal with the other factors.

8.4.1 The effect of stage

Increased numbers of behaviours were expressed with stage both for winning and losing lobsters, which suggests that the agonistic encounters become more complex (i.e. include a greater number of interactions) with increasing development. Game theory states that fights are based on the gradual acquisition of information regarding the opponents fighting ability (Enquist and Leimar, 1983), and since all the lobsters were naïve learning must take place in the fight. It may be that the older lobsters either require increased levels of assessment, or that they are so closely matched that the fights have to escalate, increasing the interactions and levels of aggression.

Comparing animals at different moult stages, they all showed the same repertoires of behaviour in the same sequences. A lot of mirroring behaviour took place involving low intensity behaviours (meral spread, do-si-do) but these almost always escalated to the high intensity fighting, in order to acquire more information about the opponent and then to reach a decision through the attack of one of the combatants, forcing the other to retreat. Both winners and losers exhibited the full range of behaviours, but there was a difference between the frequency of these. Winners performed more meral spreads than losers, which can be related to the dual purpose of the meral spreads (Figs. 8.4.a-c), as a display tactic used initially by both to impose their size and potential fighting ability and by the winner after dominance has been achieved, to reinforce the result in the sub-dominant, often after it has retreated. Wrestling interactions allow the assessment of each other. The losing lobsters invariably performed more retreats than the winning lobsters (Figs. 8.2.a-c), which is not unexpected as the higher number of retreats dictates which of the two is the loser. As the animals get older, there is a tendency for the interactions to become more complex, allowing a greater assessment of the opponent's ability, and thus to make more informed decisions, expressed through the increased levels of

wrestling behaviour. However, when comparing the total number of behaviours of the winning and losing lobsters, between the stage ranges at the different temperatures (Figs. 8.9-8.11) very few differences can be seen. Differences with increasing stage range for the three temperatures (Figs. 8.12.a-c) are also very limited when the means are compared, as is also seen when the mean values for the total number of behaviours exhibited by all temperature cohorts are compared (Figs. 8.13.a-c).

Meral spread is one of the most common behaviours in the agonistic interactions of crustaceans (Dingle, 1969; Huber and Kravitz, 1995), and often initiates an agonistic encounter (Glass and Huntingford, 1988). It is the most visual of the behaviours exhibited by the lobsters, and it is thought to convey an increase in the perceived size of the individual. The body is raised up high, on the walking legs and its weaponry (claws) is displayed. Game theory states that during behavioural displays, selection should favour a behaviour that gives the most accurate indication of Resource Holding Power (RHP), which is an indicator of the fighting ability of an individual (Parker, 1974). Both lobsters in an encounter were found to use this behaviour initially, but after the outcome of a fight was decided only the winner would use it to assert and reinforce the victory and make the sub-dominant retreat. These expressions of meral spread by the winner and retreat by the loser after the outcome was determined contribute significantly to their high total numbers in the repertoires of the two combatants.

A significant finding of the study is that higher intensity behaviours such as wrestling and strike/rip are expressed in all the encounters. The lobsters in this study were all highly aggressive, which fits with the findings of Huntingford and Turner, 1987) who found that the aggression levels of animals held in captivity are very high, and they show a reduced ability to use and recognise social signals (Huntingford and Turner, 1987). This could explain why the lobsters in this study did not resolve their encounters through the low intensity display behaviours alone, as predicted by Game Theory. Close matching of sizes may also have contributed to this progression to high intensity behaviours before resolution.

The fact that strike/rip behaviour was greater in the winning lobsters (Fig. 8.7.a-c) suggests that once the eventual winner perceives its dominance in the encounter it is willing to follow the costly option of striking its opponent (i.e. an action that could result in injury to itself). This may then represent the point in the encounter when the outcome is determined, and be the deciding factor in causing the loser to retreat (Huber and Kravitz, 1995).

8.4.2 Assessment cues

The outcome of contests between *H. americanus* can be predicted on the basis of subtle cues, such as plasma protein levels, claw dimensions and exoskeleton calcium concentration (Vye *et al.*, 1997). A knowledge of the relative value of the above key variables is essential for the prediction of winners by an observer, and is probably also of great value for competing lobsters, when assessing the relative resource holding potential (Vye *et al.*, 1997). Stewart *et al.*, (1967), found the most important predictor of winning contests, was the serum protein concentration, which is a reliable indicator of the physiological condition of the lobsters. The second factor for predictions of the winner is related to the claw dimensions: as cutter width occasionally interchanged as a predictor with cutter volume and crusher width, which would reflect muscle biomass, which is likely to be manifested in differential contraction power (Vye *et al.*, 1997). When considering the contraction power of the lobsters, it could be postulated that if one of the lobsters possessed a 'false' crusher claw (see Chapters 5 and 6) and the other a developed crusher claw, if there were no size differences, the assessment during the wrestling interactions could lead the lobster with fully developed claws to realise that the other was inferior, through the different ability, and this could lead to a decision being made as to the eventual victor.

Through the highly ritualised fighting behaviour of juvenile lobsters, there is a possibility that they can indirectly assess relative plasma protein level, calcium exoskeleton concentration and claw dimensions of their rivals, through the effect of the above variables on claw contraction forces, resistance of exoskeleton to pressure and general fighting vigour, through mechano-receptors distributed over different areas of the exoskeleton, especially that of the claws (Solon and Cobb, 1980a). It can only be speculated as to the actual cues used by lobsters in

symmetrical contests to assess RHP, however one can be sure that during the wrestling behaviour the lobsters are assessing the strength of each other and assessing whether one is to become the eventual aggressor and the eventual subordinate, which then leads one of the pair to strike/rip and hence become the winner.

8.4.3 Sequences of behaviour

Within the agonistic encounters of juvenile lobsters there are two main loops of behaviour present: a low intensity display loop involving the meral spread and do-si-do behaviours and a high intensity fighting loop involving wrestling and strike/rip behaviours. The low intensity display loop allows the lobsters to resolve a fight while avoiding any potential injury that could arise if a large size asymmetry is present. However this is not the case with the lobsters studied here. They displayed large numbers of low intensity behaviours, such as meral to meral, do-si-do to do-si-do and retreats to a certain extent, but nevertheless all the fights did escalate to the high intensity fight loop, particularly involving wrestling, which allows further mutual assessment, and strike/rip. Once in the high intensity fighting loop there are only two possible outcomes: to continue wrestling (i.e. continuing assessment) or proceed to the strike/rip behaviour. The point of resolution probably occurred during such wrestling, and return to the low intensity display loop was rare, if not impossible.

With regard to the behaviour of laboratory reared lobsters if released into the wild, it is reasonable to assume that the former would be at a disadvantage, due to a lack of experience through solitary confinement. A difference between wild and cultivated lobsters has been observed, in the way that lobsters use their claws when performing behavioural patterns during agonistic encounters (Meeren and Uksnøy, 2000). In meral spreads and pushing behaviour, cultivated lobsters keep both claws closed, whereas wild lobsters keep the crusher claw open and in front of their heads. Also, it was observed that wild lobster's only lock claws with the crusher, whereas the cultivated lobsters seem to have no priority between the major and minor claws (Meeren, 2000). It would thus be informative to analyse staged encounters between lobsters with prior experience to see if the

frequencies and patterns of agonistic behaviour are modulated with increased experience of social interactions.

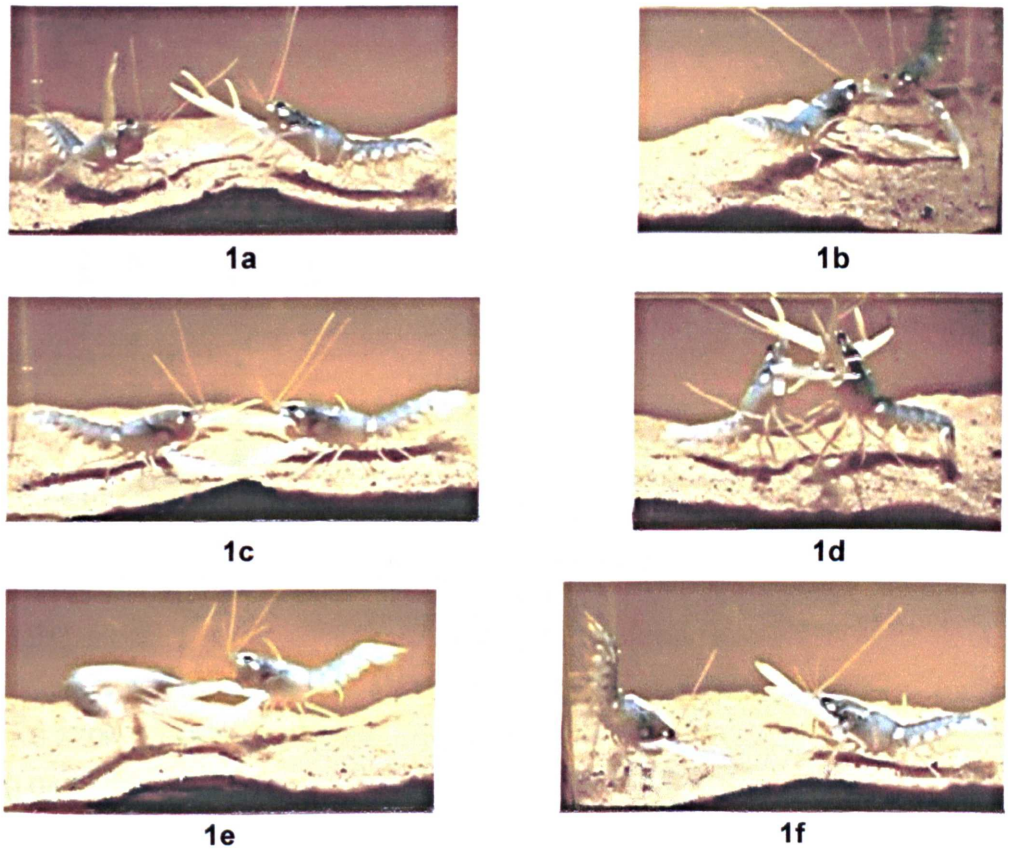


Figure 8.1.a-f: The six main behavioural components of agonistic interactions between juvenile *Homarus gammarus:* a) meral spread; b) reach; c) do-si-so; d) wrestling; e) retreat; f) sub-dominant/dominant postures (All still frames taken from an encounter between two 19°C stage 11 lobsters (9 months)).

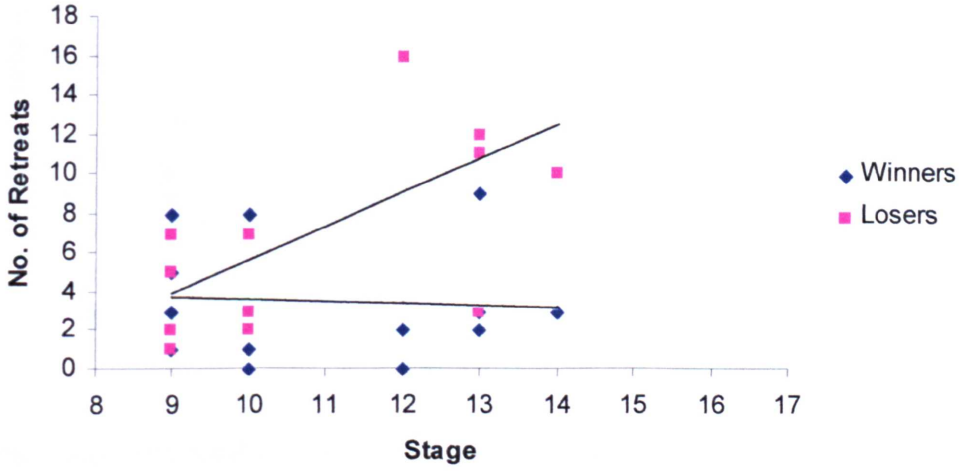


Figure 8.2.a: The total number of retreats exhibited by the winning and losing 11°C lobsters, over the growth period (Stages 9-14). Regression equations: Winners: y = -0.0952x + 4.5092, R2 = 0.0033; Losers: y = 1.7143x – 11.549, R2 = 0.3709. Univariate ANOVA; comparison of regression slopes; $F_{1, 22} = 4.666$, p = 0.042.

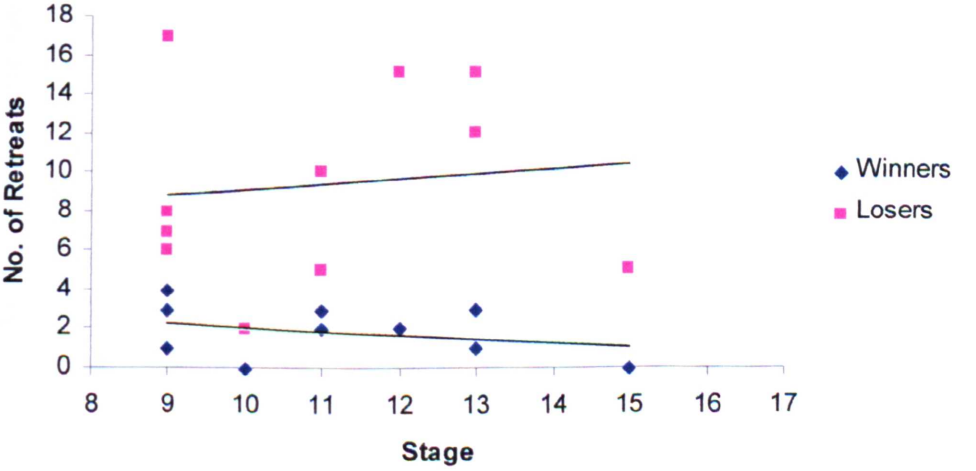


Figure 8.2.b: The total number of retreats exhibited by the winning and losing 15°C lobsters, over the growth period (Stages 9-15). Regression equations: Winners: y = -0.1905x + 3.9134, R2 = 0.0864 Losers: y = 0.2619x + 6.3918, R2 = 0.012. Univariate ANOVA; comparison of regression slope intercepts; $F_{1, 19} = 22.533$, p = 0.000.

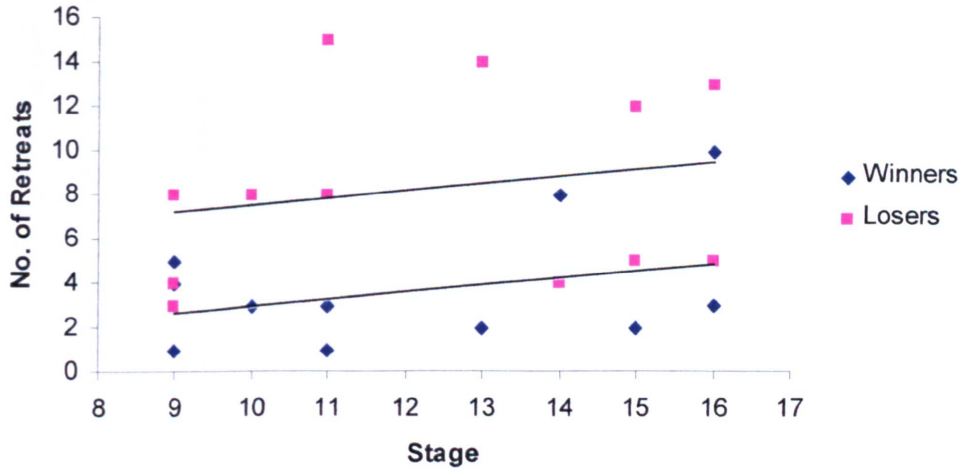


Figure 8.2.c: The total number of retreats exhibited by the winning and losing 19°C lobsters, over the growth period (Stages 9-16). Regression equations: Winners: y = 0.3154x - 0.2231, R2 = 0.1018; Losers: y = 0.3231x + 4.2654, R2 = 0.0452. Univariate ANOVA; comparison of regression slope intercepts; $F_{1, 21}$ = 9.904, p = 0.005.

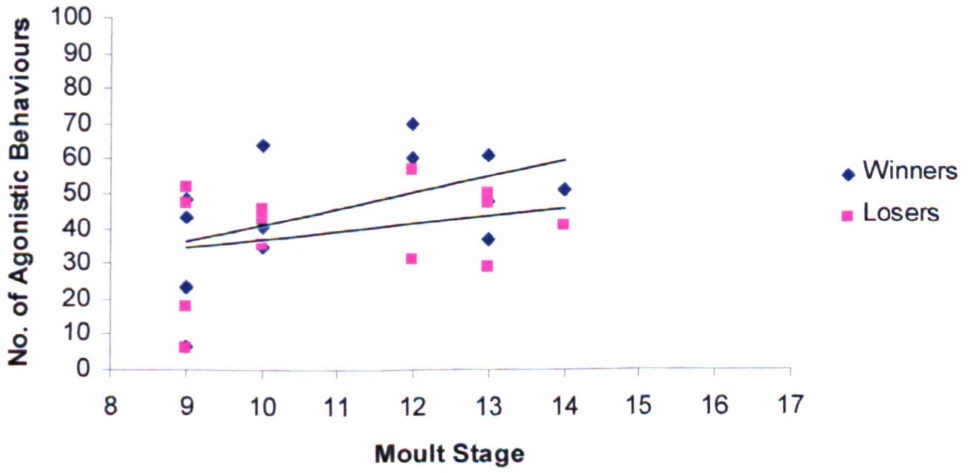


Figure 8.3.a: The total number of agonistic behaviours exhibited (see *figs. 1a-f*) by the winning and losing 11°C lobsters, over the growth period (Stages 9-14). Regression equations: Winners: y = 4.4524x – 3.5147, R2 = 0.2318; Losers: y = 2.1429x + 15.198, R2 = 0.0762. Univariate ANOVA; comparison of regression slopes and intercepts: n.s.: $F_{1, 22}$ = 0.484, p = 0.494; $F_{1, 23}$ = 1.286, p = 0.269.

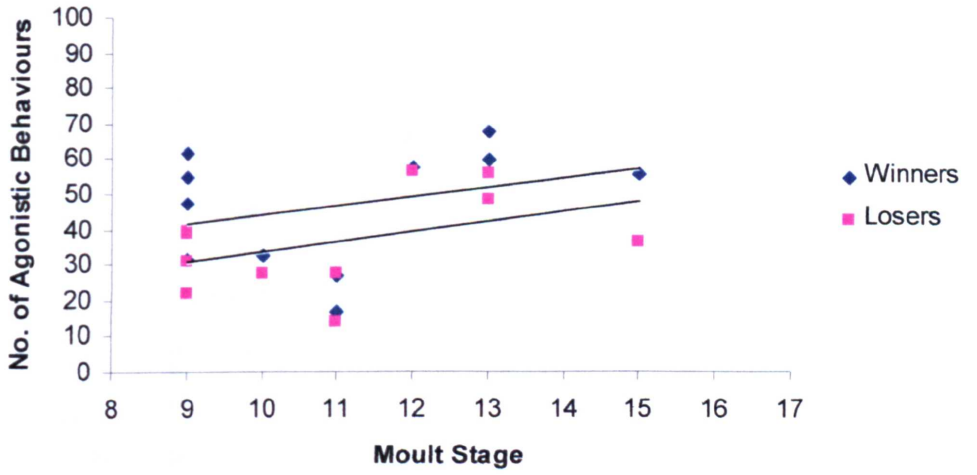


Figure 8.3.b: The total number of agonistic behaviours (see *figs. 1a-f*) exhibited by the winning and losing 15°C lobsters, over the growth period (Stages 9-15). Regression equations: Winners: y = 2.6429x + 17.838, R2 = 0.1039; Losers: y = 2.9286x + 4.2403, R2 = 0.193. Univariate ANOVA; comparison of regression slopes and intercepts: n.s.: $F_{1, 22} = 0.008$, p = 0.931; $F_{1, 19} = 2.829$, p = 0.109.

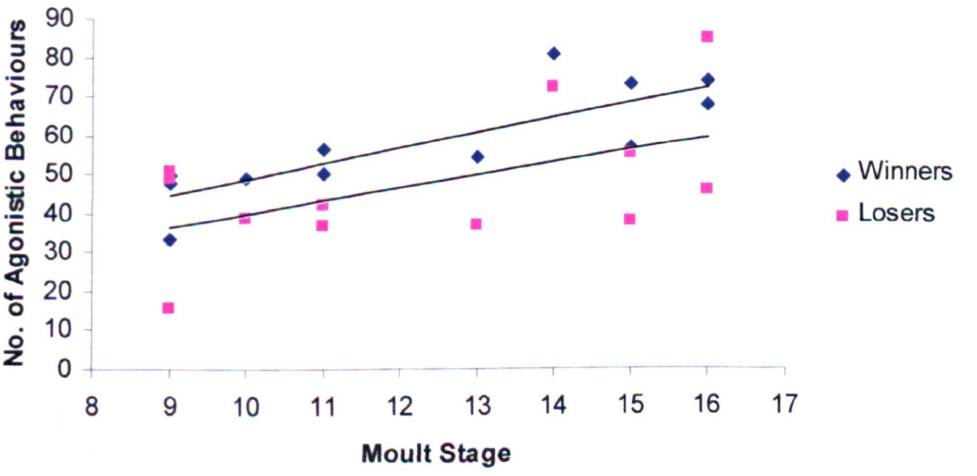


Figure 8.3.c: The total number of agonistic behaviours (see *figs. 1a-f*) exhibited by the winning and losing 19°C lobsters, over the growth period (Stages 9-16). Regression equations: Winners: y = 3.9538x + 9.0692, R2 = 0.6722; Losers: y = 3.3462x + 5.9808, R2 = 0.2785. Univariate ANOVA; comparison of regression slope intercepts; $F_{1, 21} = 4.423$, p = 0.048.

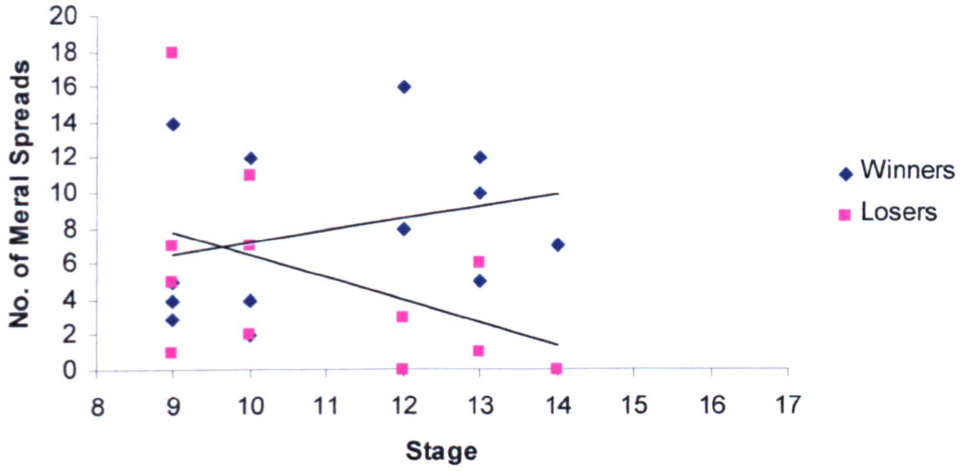


Figure 8.4.a: The total number of meral spreads exhibited by the winning and losing 11°C lobsters, over the growth period (Stages 9-14). Regression equations: Winners: y = 0.6905x – 0.2509, R2 = 0.0808; Losers: y = -1.2619x + 19.035, R2 = 0.216. Univariate ANOVA; comparison of regression slopes and intercepts: n.s.: $F_{1, 22} = 3.743$, p = 0.066; $F_{1, 23} = 1.968$, p = 0.174.

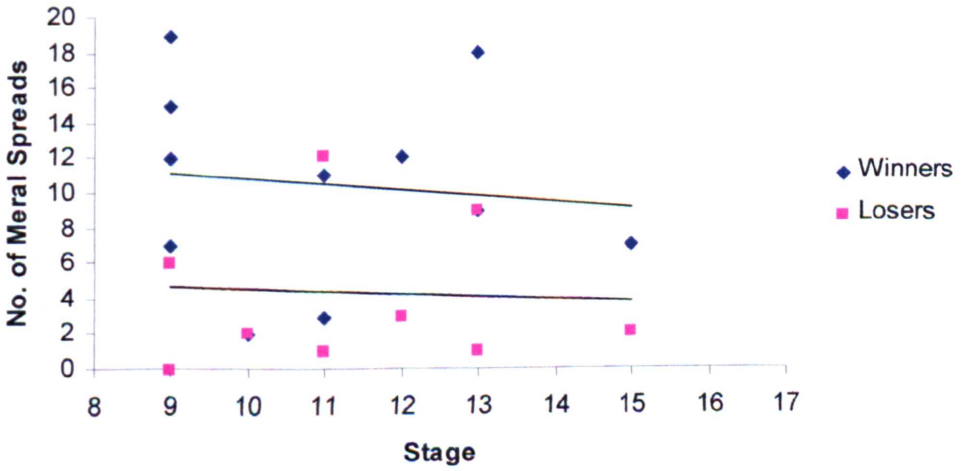


Figure 8.4.b: The total number of meral spreads exhibited by the winning and losing 15°C lobsters, over the growth period (Stages 9-15). Regression equations: Winners: y = -0.3333x + 14.121, R2 = 0.0151; Losers: y = -0.1667x + 6.197, R2 = 0.0082. Univariate ANOVA; comparison of regression slope intercepts; $F_{1, 19} = 8.692$, p = 0.008.

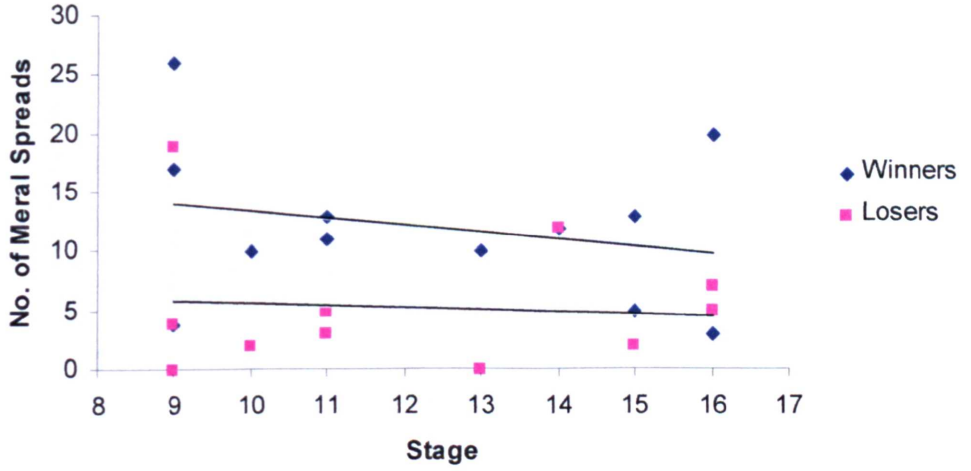


Figure 8.4.c: The total number of meral spreads exhibited by the winning and losing 19°C lobsters, over the growth period (Stages 9-16). Regression equations: Winners: y = -0.6115x + 19.542, R2 = 0.0661; Losers: y = -0.2x + 7.55, R2 = 0.0105. Univariate ANOVA; comparison of regression slope intercepts; $F_{1, 21} = 7.607$, p = 0.012.

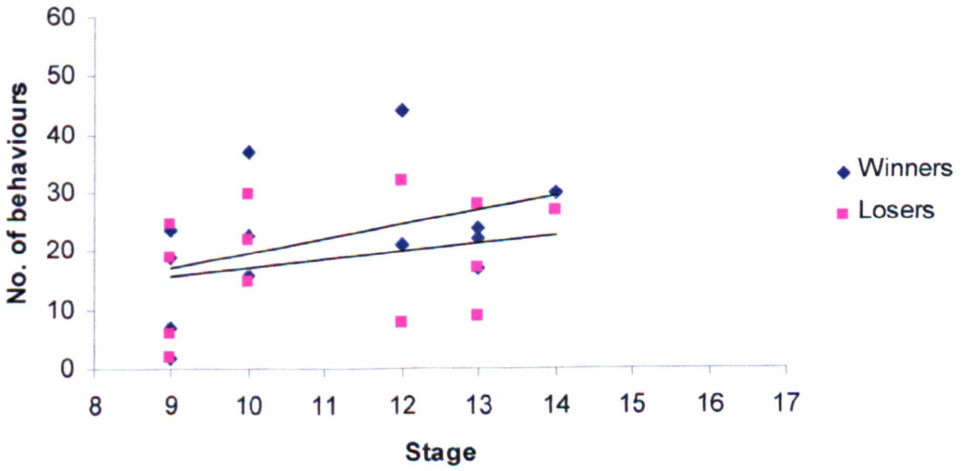


Figure 8.5.a: The total number of wrestling behaviours exhibited by the winning and losing 11°C lobsters, over the growth period (Stages 9-14). Regression equations: Winners: y = 2.4048x - 4.4524, R2 = 0.1643; Losers: y = 1.381x + 3.2711, R2 = 0.0682. Univariate ANOVA; comparison of regression slopes and intercepts: n.s.: $F_{1, 22} = 0.208$, p = 0.653; $F_{1, 23} = 0.796$, p = 0.382.

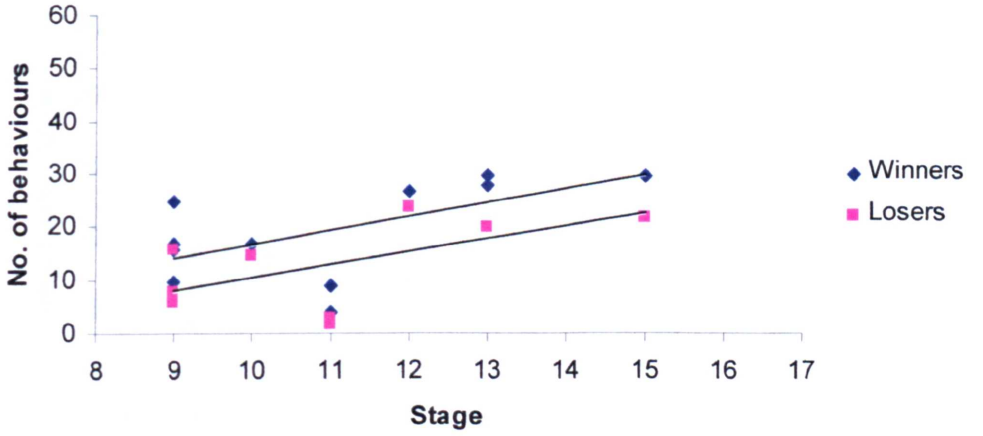


Figure 8.5.b: The total number of wrestling behaviours exhibited by the winning and losing 15°C lobsters, over the growth period (Stages 9-15). Regression equations: Winners: y = 2.619x - 9.4459, R2 = 0.3411; Losers: y = 2.4524x - 13.976, R2 = 0.3922. Univariate ANOVA; comparison of regression slope intercepts; $F_{1, 19} = 4.462$, p = 0.048.

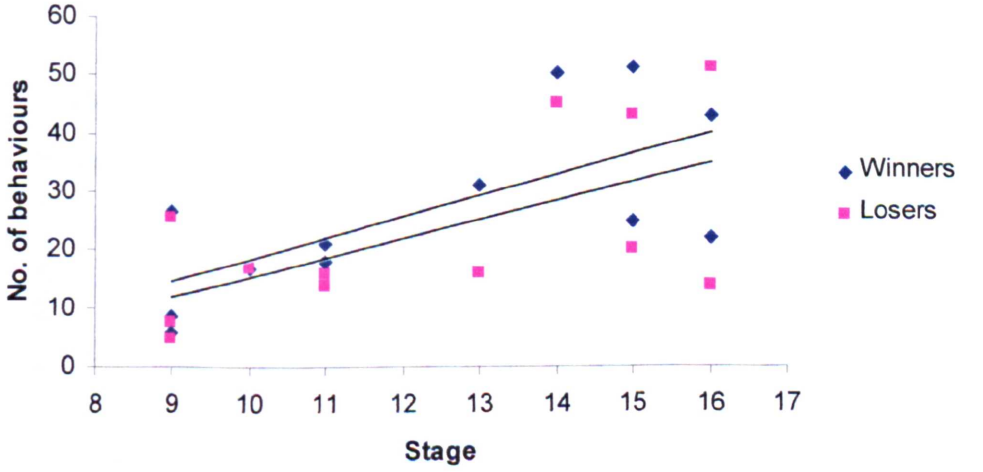


Figure 8.5.c: The total number of wrestling behaviours exhibited by the winning and losing 19°C lobsters, over the growth period (Stages 9-16). Regression equations: Winners: y = 3.6154x - 17.923, R2 = 0.4746; Losers: y = 3.2577x - 17.262, R2 = 0.3634. Univariate ANOVA; comparison of regression slopes and intercepts: n.s.: $F_{1, 20} = 0.039$, p = 0.846; $F_{1, 21} = 0.617$, p = 0.441.

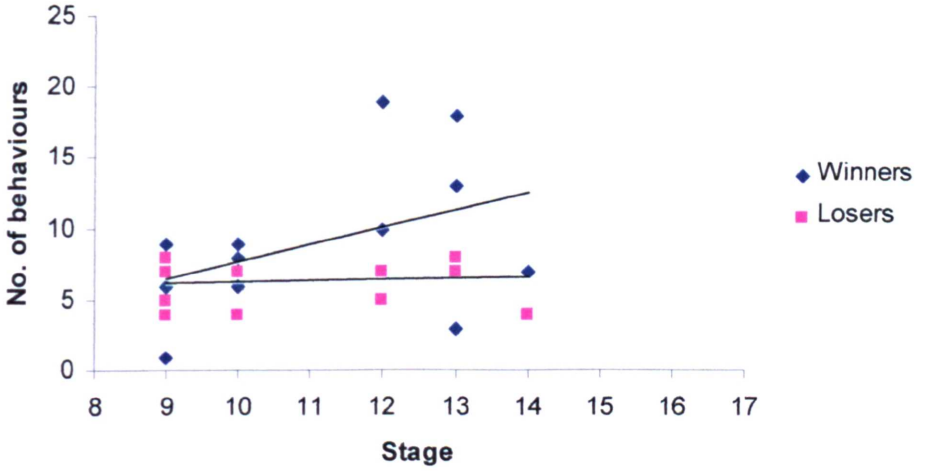


Figure 8.6.a: The total number of do-si-do behaviours exhibited by the winning and losing 11°C lobsters, over the growth period (Stages 9-14). Regression equations: Winners: y = 1.2143x - 4.511, R2 = 0.1878; Losers: y = 0.0952x + 5.1832, R2 = 0.0126. Univariate ANOVA; comparison of regression slopes and intercepts: n.s.: $F_{1, 22} = 1.943$, p = 0.177; $F_{1, 23} = 3.156$, p = 0.089.

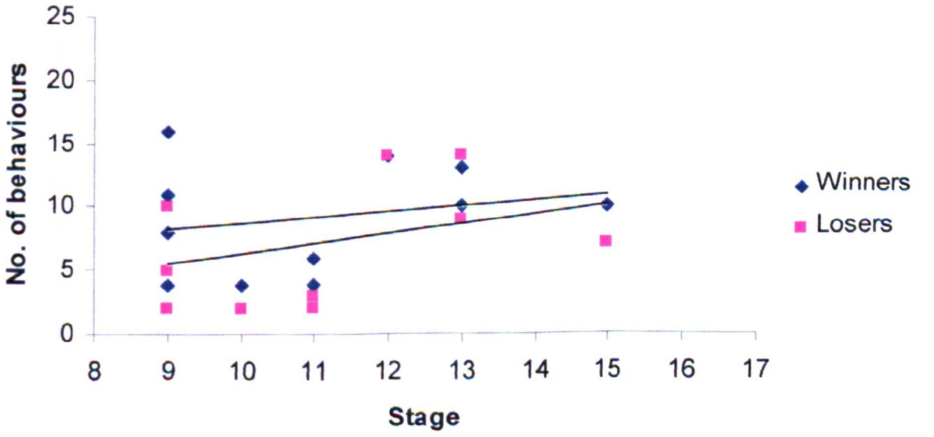


Figure 8.6.b: The total number of do-si-do behaviours exhibited by the winning and losing 15°C lobsters, over the growth period (Stages 9-15). Regression equations: Winners: y = 0.4286x + 4.3766, R2 = 0.0426; Losers: y = 0.7619x - 1.29, R2 = 0.1134. Univariate ANOVA; comparison of regression slopes and intercepts: n.s.: $F_{1, 22} = 0.115$, p = 0.738; $F_{1, 19} = 1.142$, p = 0.299.

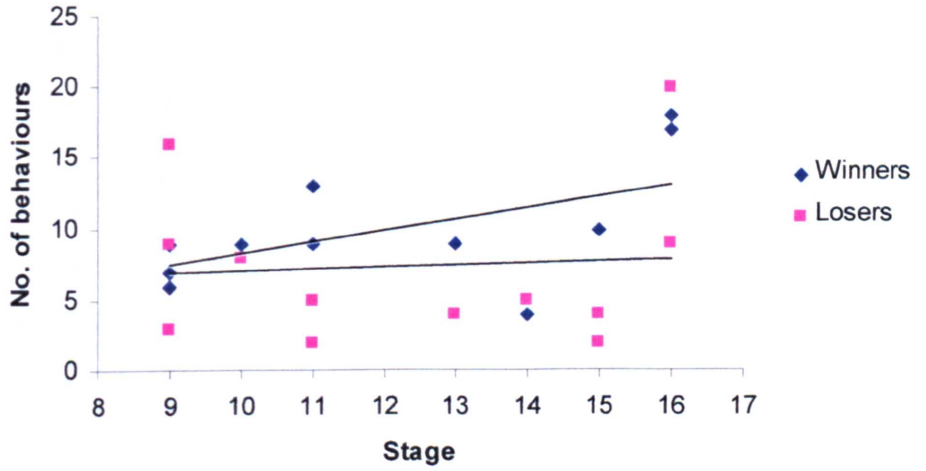


Figure 8.6.c: The total number of do-si-do behaviours exhibited by the winning and losing 19°C lobsters, over the growth period (Stages 9-16). Regression equations: Winners: y = 0.8154x + 0.0269, R2 = 0.3083; Losers: y = 0.1385x + 5.5423, R2 = 0.0047. Univariate ANOVA; comparison of regression slopes and intercepts: n.s.: $F_{1, 20} = 0.831$, p = 0.373; $F_{1, 21} = 2.032$, p = 0.169.

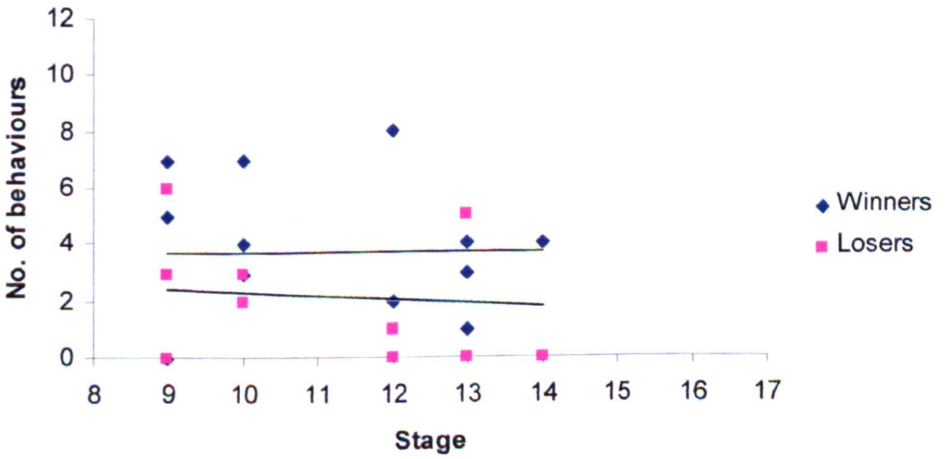


Figure 8.7.a: The total number of strike/rip behaviours exhibited by the winning and losing 11°C lobsters, over the growth period (Stages 9-14). Regression equations: Winners: y = 3.6923, R2 = 0; Losers: y = -0.119x + 3.4634, R2 = 0.0103. Univariate ANOVA; comparison of regression slopes and intercepts: n.s.: $F_{1, 22} = 0.047$, p = 0.829; $F_{1, 21} = 2.561$, p = 0.123.

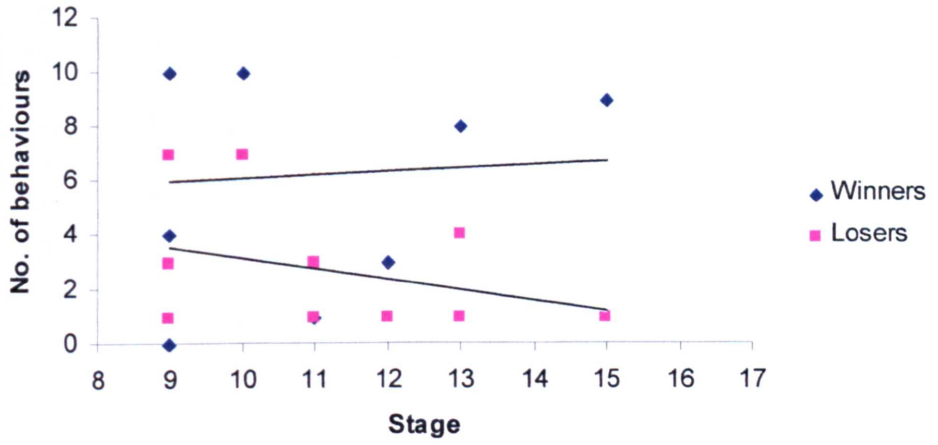


Figure 8.7.b: The total number of strike/rip behaviours exhibited by the winning and losing 15°C lobsters, over the growth period (Stages 9-15). Regression equations: Winners: y = 0.119x + 4.8723, R2 = 0.0028; Losers: y = -0.381x + 6.9177, R2 = 0.1085. Univariate ANOVA; comparison of regression slope intercepts; $F_{1, 19} = 4.682$, p = 0.043.

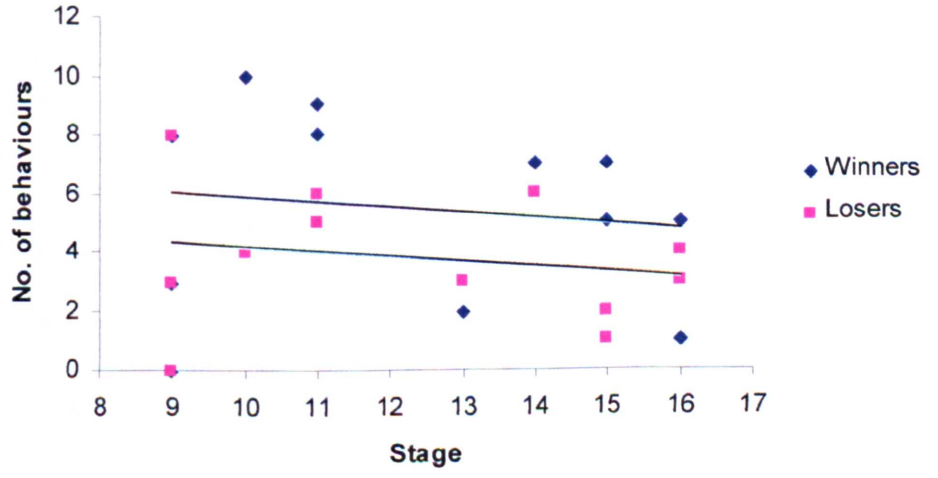


Figure 8.7.c: The total number of strike/rip behaviours exhibited by the winning and losing 19°C lobsters, over the growth period (Stages 9-16). Regression equations: Winners: y = -0.1808x + 7.6462, R2 = 0.0238; Losers: y = -0.1731x + 5.8846, R2 = 0.0462. Univariate ANOVA; comparison of regression slopes and intercepts: n.s.: $F_{1, 20} = 0.000$, p = 0.986; $F_{1, 21} = 2.062$, p = 0.166.

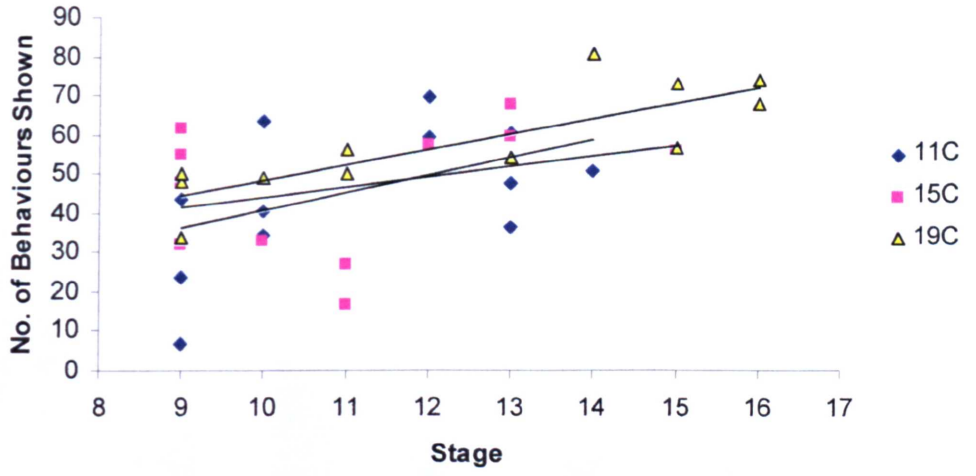


Figure 8.8.a: Total number of behaviours exhibited by the winning lobsters at the three temperatures of rearing. Regression equations: 11ºC: y = 4.4524x - 3.5147, R2 = 0.2318; 15ºC: y = 2.6429x + 17.838, R2 = 0.1039; 19ºC: y = 3.9538x + 9.0692, R2 = 0.6722. Univariate ANOVA; comparison of regression slopes and intercepts: n.s.: $F_{2, 30} = 0.191$, p = 0.827; $F_{2, 32} = 0.914$, p = 0.411.

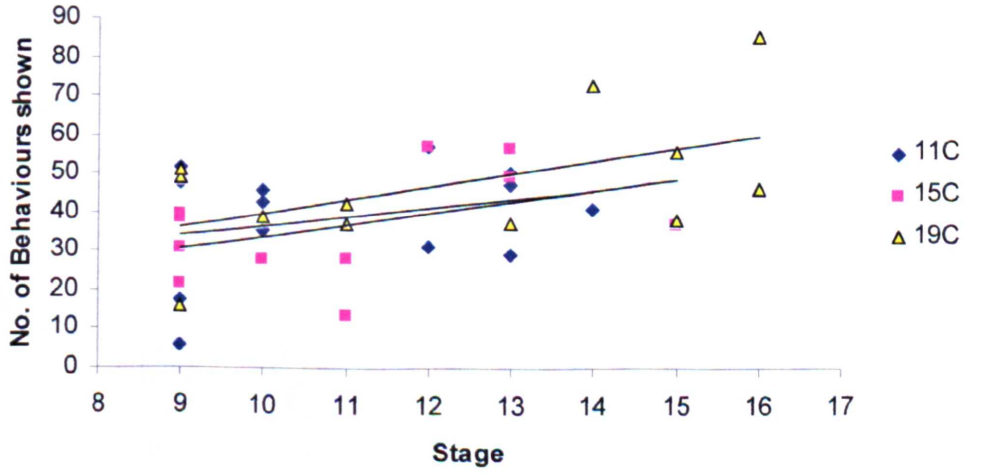


Figure 8.8.b: Total number of behaviours exhibited by the losing lobsters at the three temperatures of rearing. Regression equations: 11ºC: y = 2.1429x + 15.198, R2 = 0.0762; 15ºC: y = 2.9286x + 4.2403, R2 = 0.193; 19ºC: y = 3.3462x + 5.9808, R2 = 0.2785. Univariate ANOVA; comparison of regression slopes and intercepts: n.s.: $F_{2, 30} = 0.191$, p = 0.908; $F_{2, 32} = 0.661$, p = 0.523.

Figure 8.9.a: The mean values for the 5 main behaviours exhibited by the 11°C lobsters (Stages 9-11).

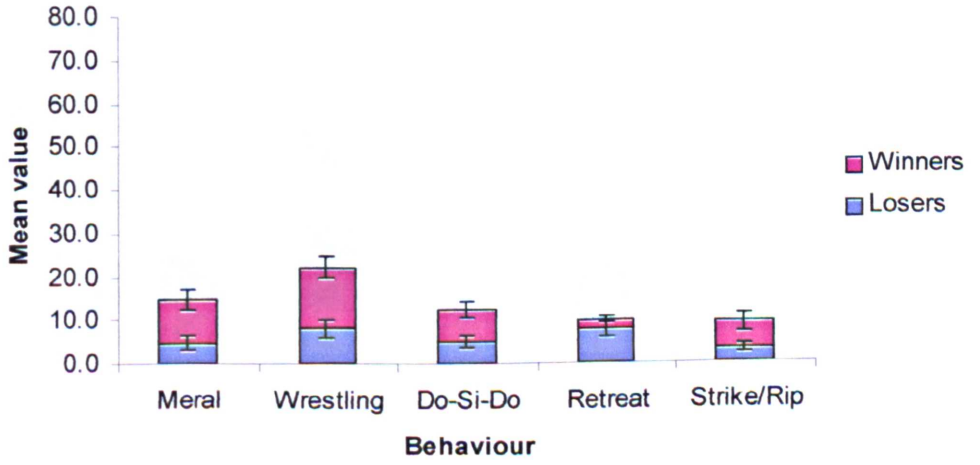


Figure 8.9.b: The mean values for the 5 main behaviours exhibited by the 15°C lobsters (Stages 9-11).

Figure 8.9.c: The mean values for the 5 main behaviours exhibited by the 19°C lobsters (Stages 9-11).

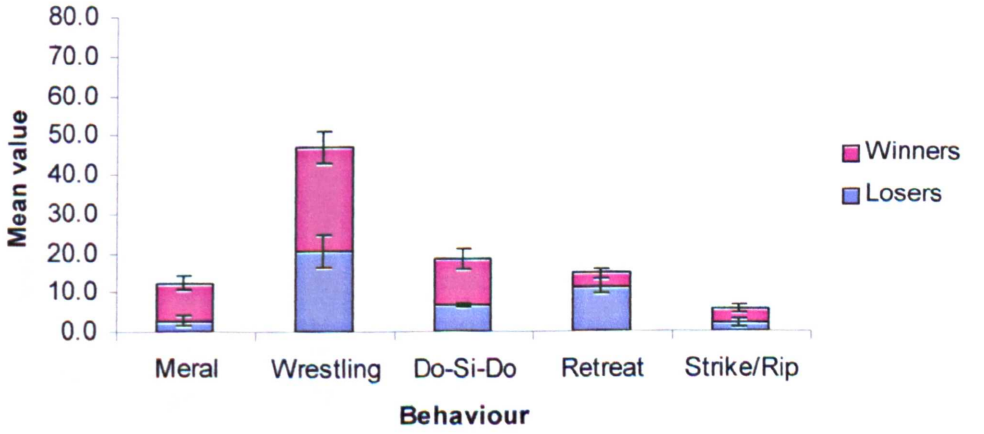


Figure 8.10.a: The mean values for the 5 main behaviours exhibited by the 11°C lobsters (Stages 12-14).

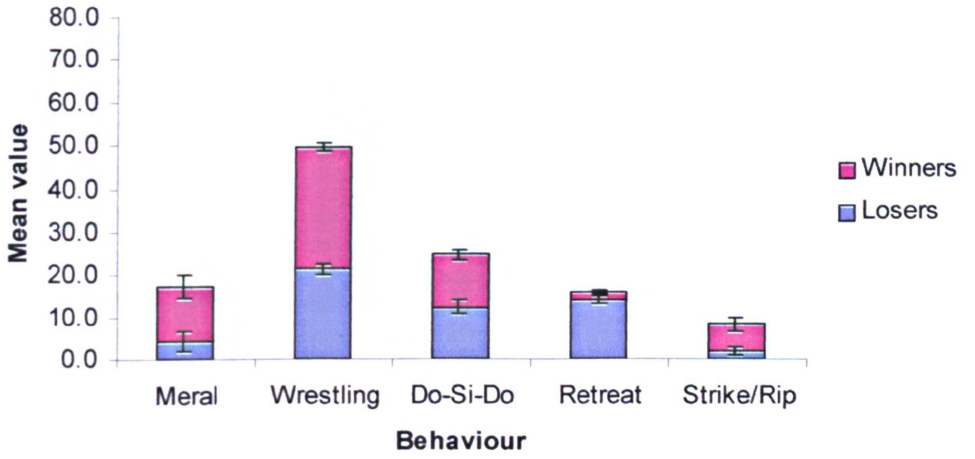


Figure 8.10.b: The mean values for the 5 main behaviours exhibited by the 15°C lobsters (Stages 12-14).

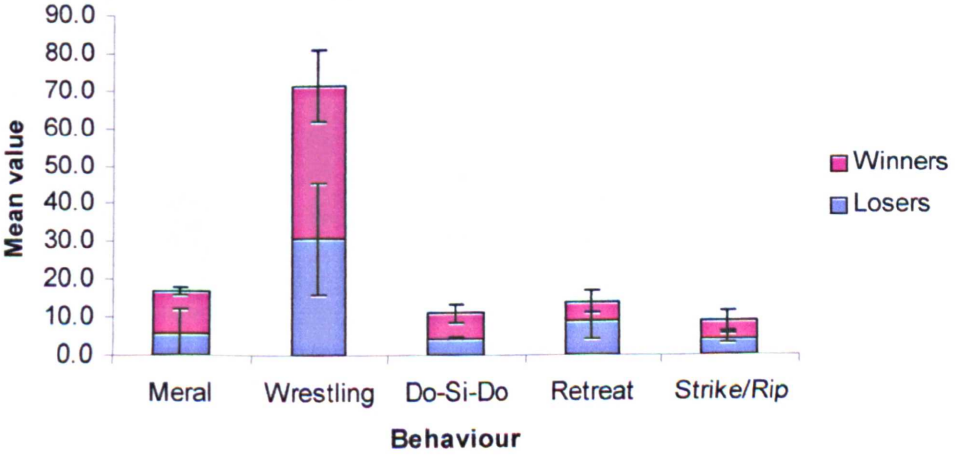


Figure 8.10.c: The mean values for the 5 main behaviours exhibited by the 19°C lobsters (Stages 12-14).

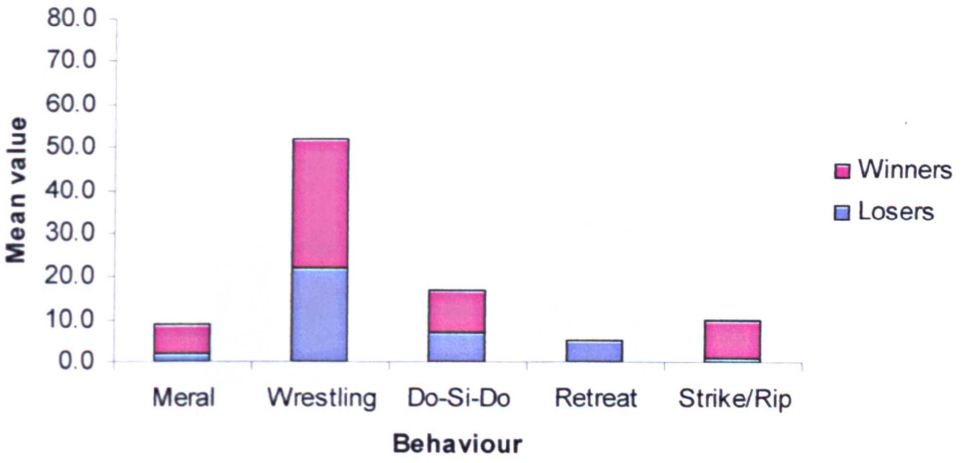


Figure 8.11.a: The mean values for the 5 main behaviours exhibited by the 15°C lobsters (Stages 14-16).

Figure 8.11.b: The mean values for the 5 main behaviours exhibited by the 19°C lobsters (Stages 14-16).

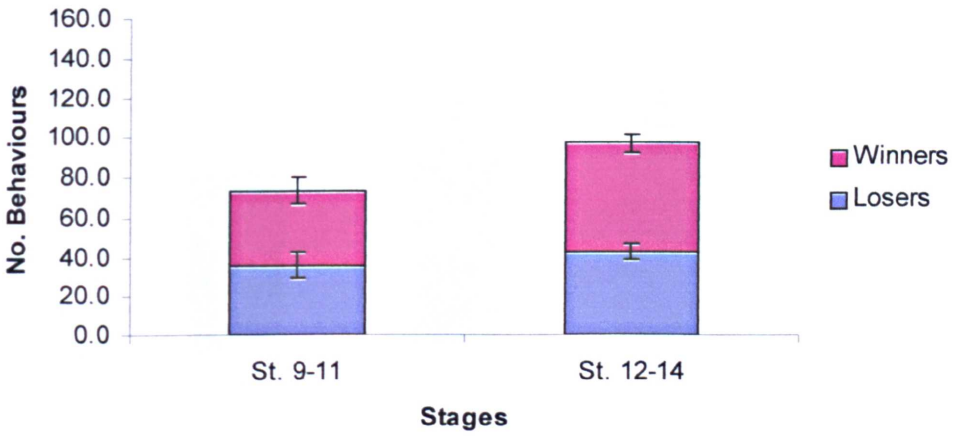


Figure 8.12.a: The mean values for the total number of behaviours exhibited by the 11°C lobsters.

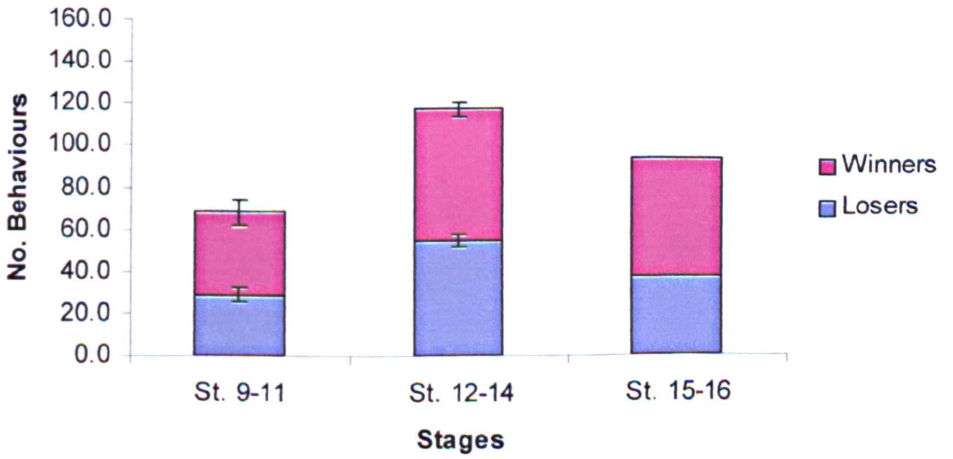


Figure 8.12.b: The mean values for the total number of behaviours exhibited by the 15°C lobsters.

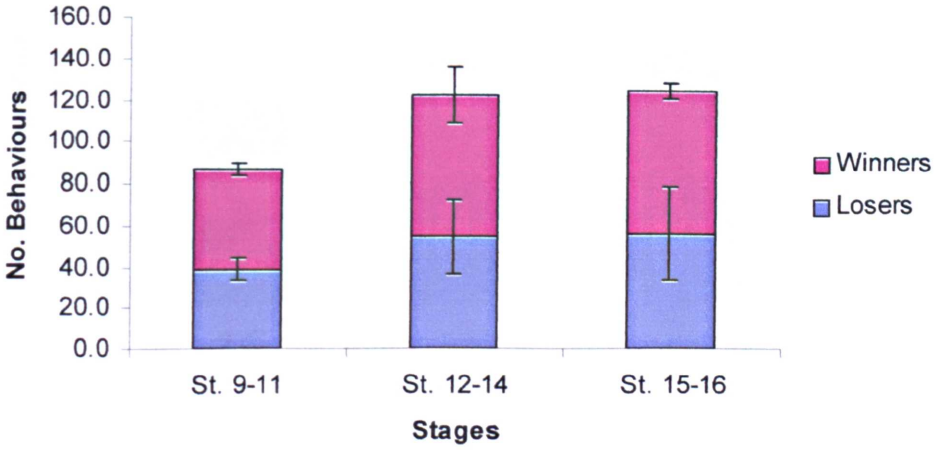


Figure 8.12.c: The mean values for the total number of behaviours exhibited by the 19°C lobsters.

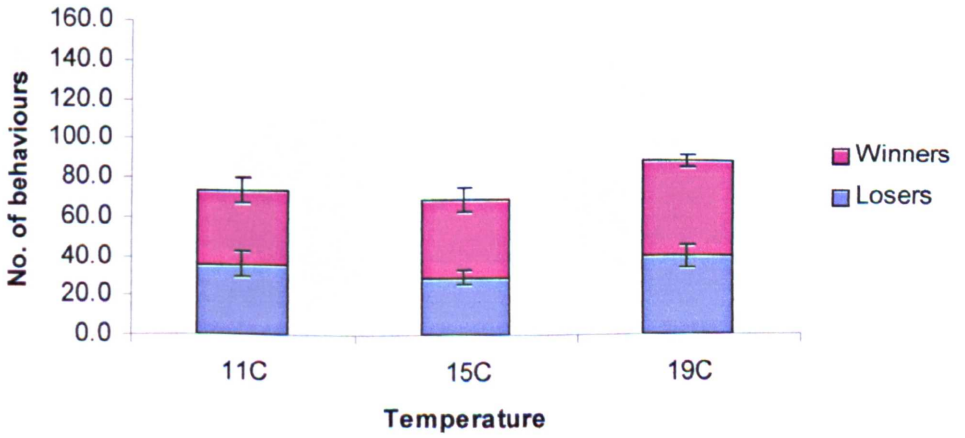


Figure 8.13.a: A comparison of the mean values for the total number of behaviours exhibited by all temperature cohorts (stages 9-11).

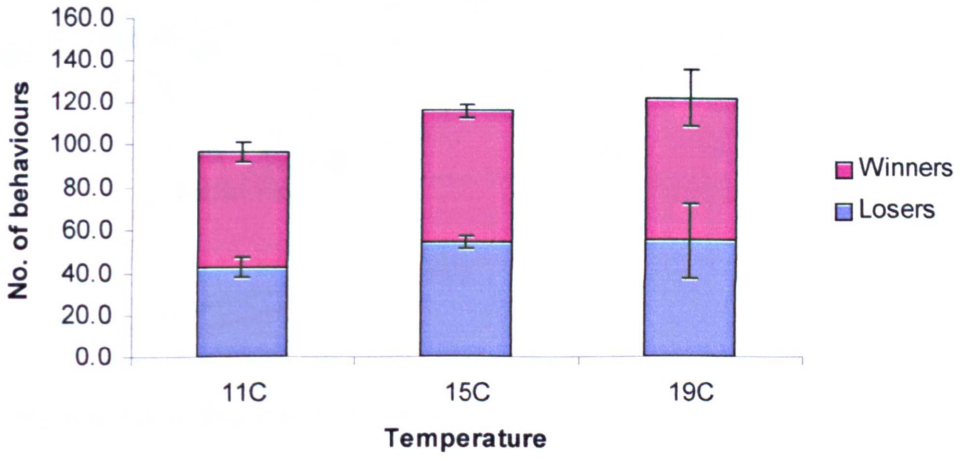


Figure 8.13.b: A comparison of the mean values for the total number of behaviours exhibited by all temperature cohorts (stages 12-14).

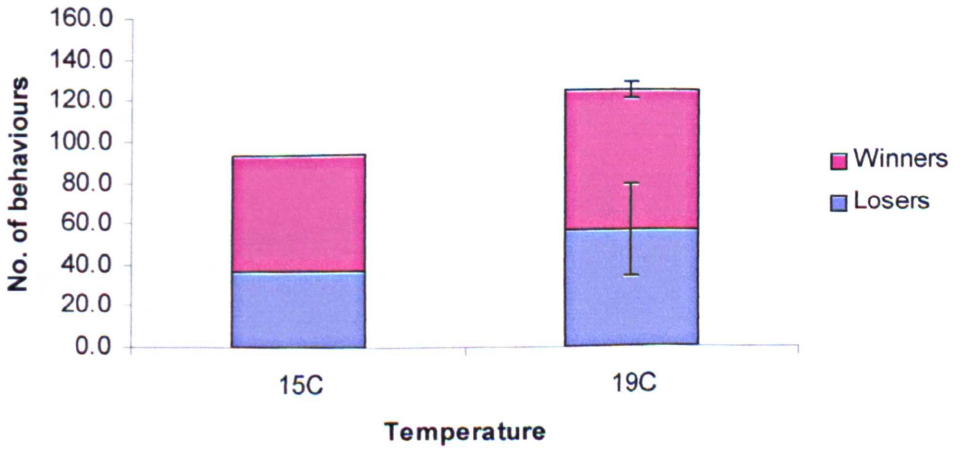
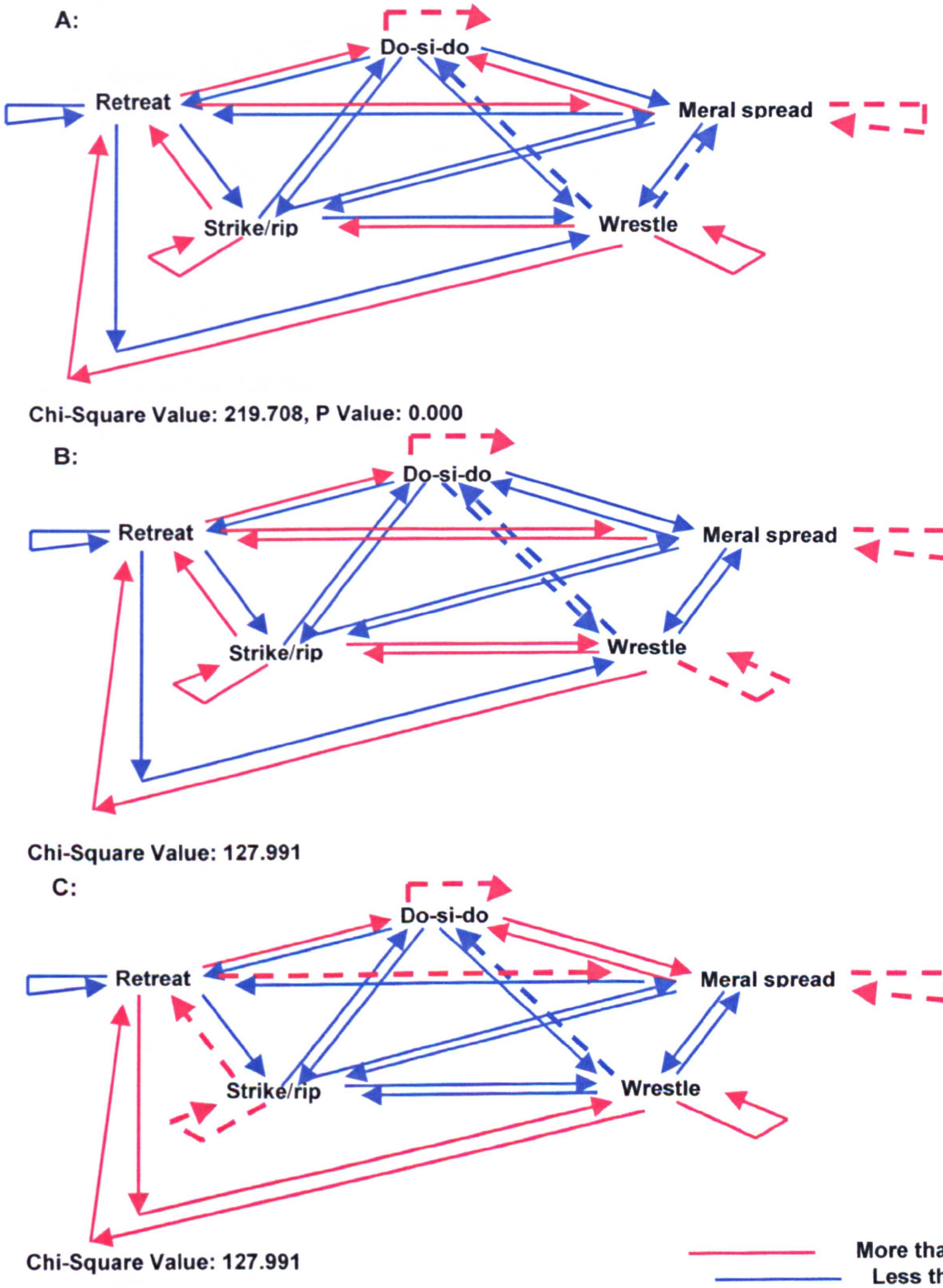
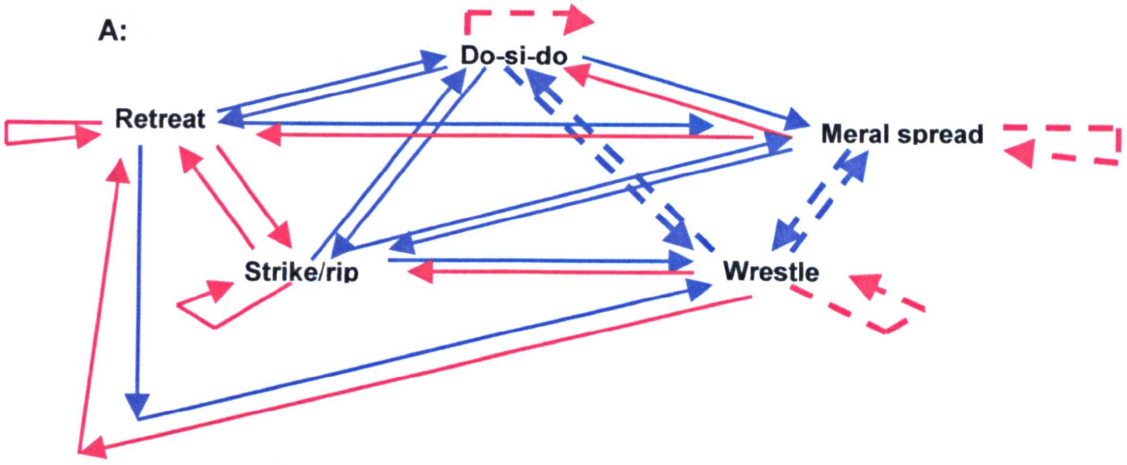


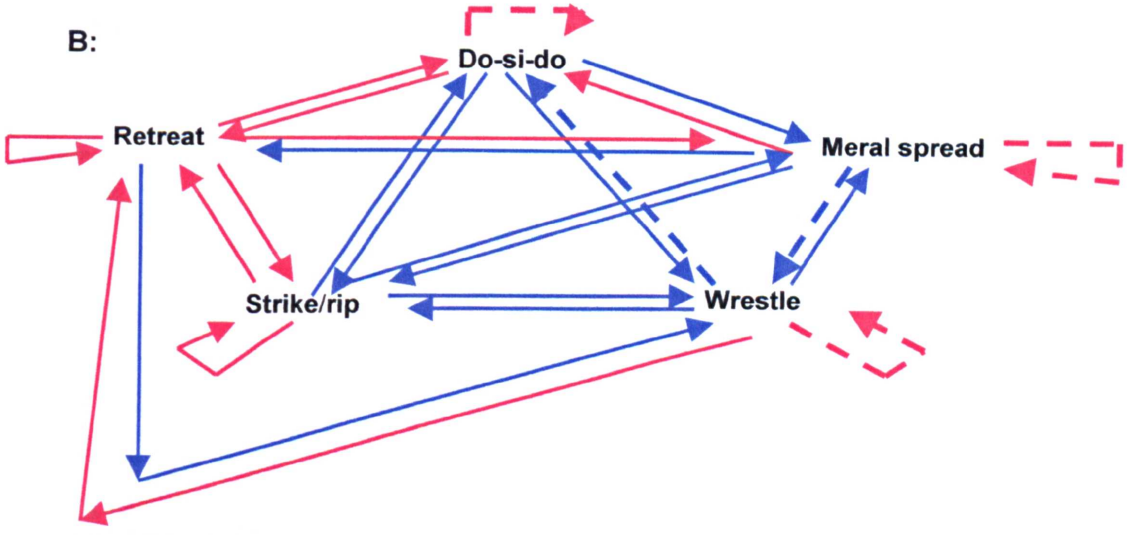
Figure 8.13.c: A comparison of the mean values for the total number of behaviours exhibited by the 15 and 19°C temperature cohorts *(stages 15-16)*.



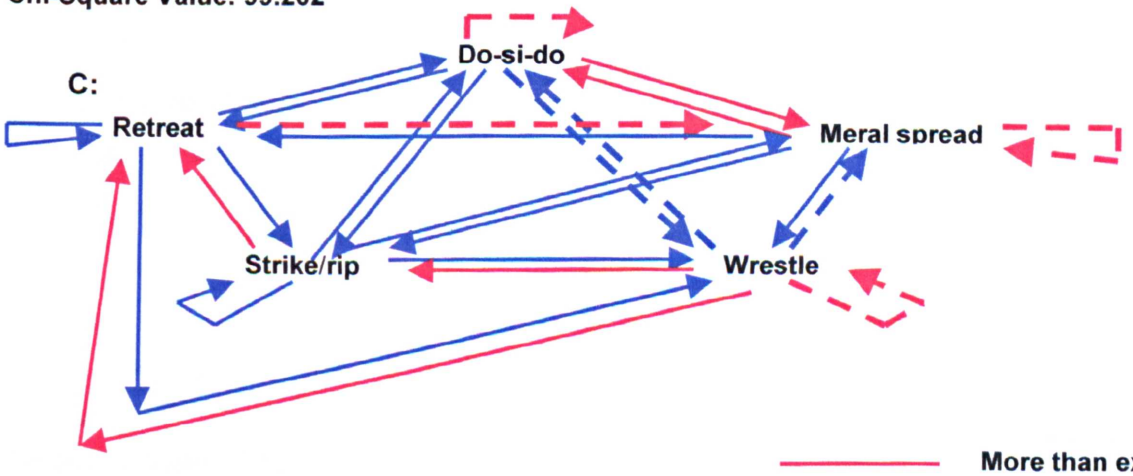
Figures 8.14.a-c: 11°C winner action to loser reaction based on chi-squared analysis: **a**: totals for whole population; **b**: totals for lobsters up to 9 months; **c**: totals for lobsters from 15-17 months. (**Dashed lines indicate the strongest relationships).**



Chi-Square Value: 222.198



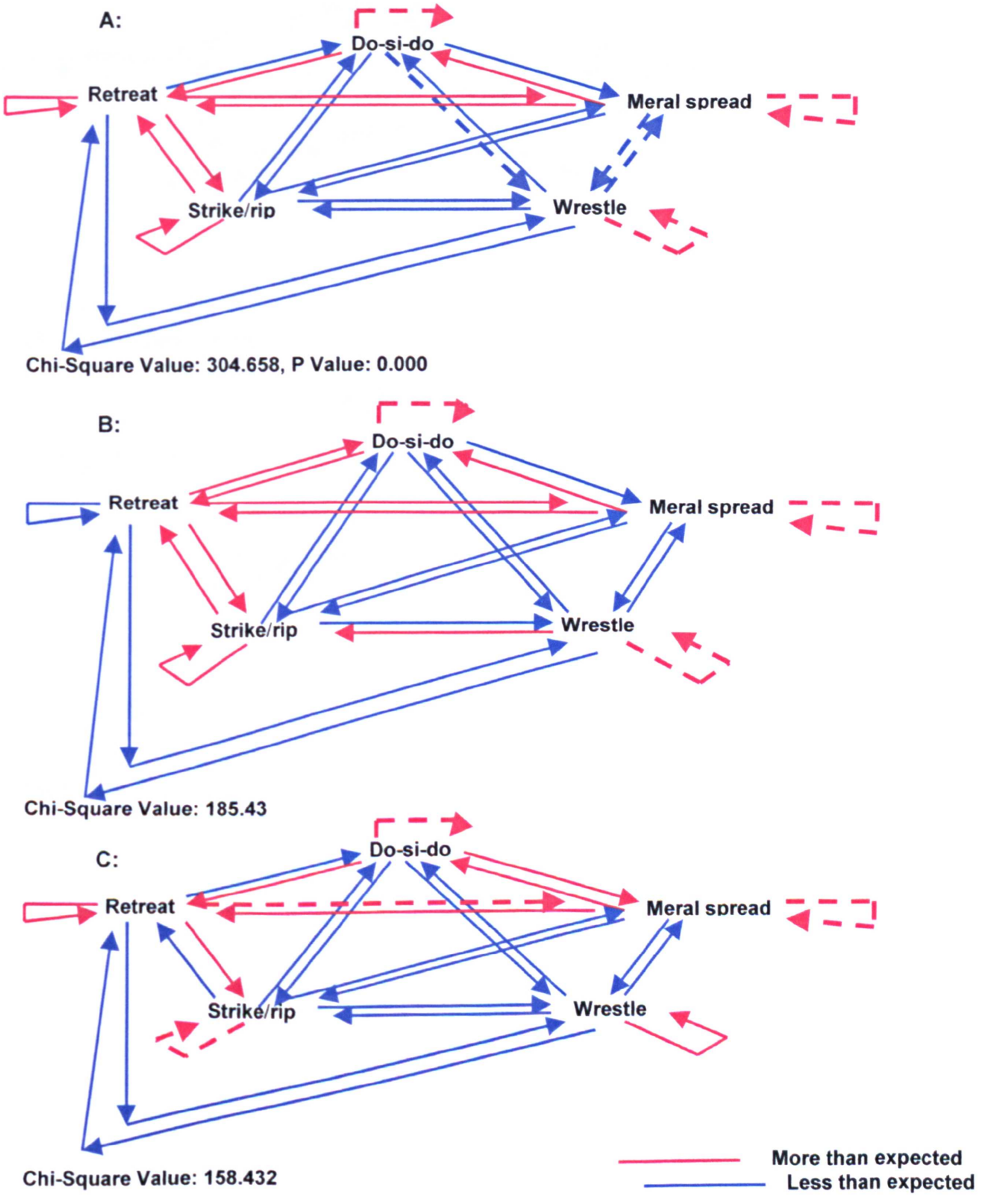
Chi-Square Value: 99.262



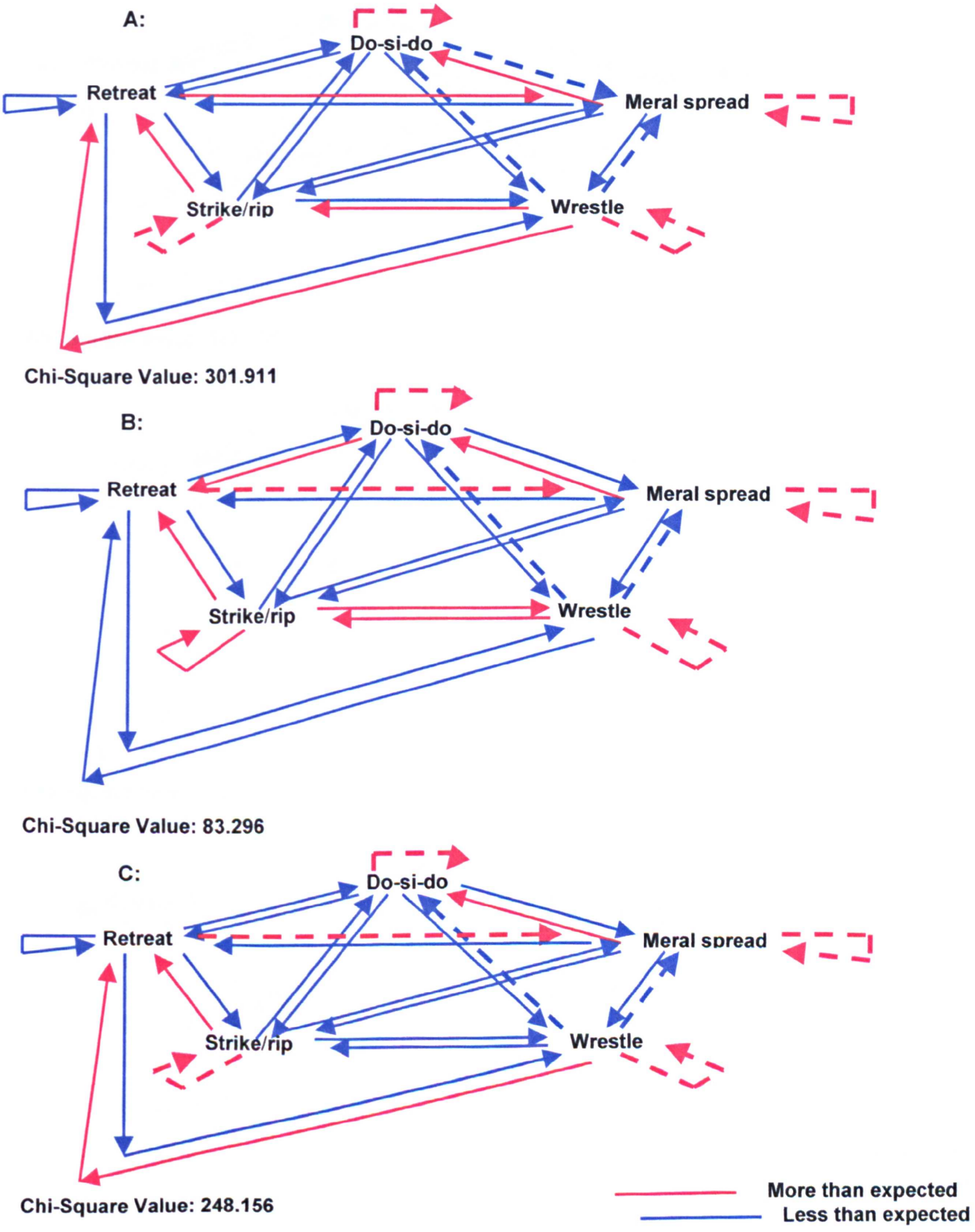
Chi-Square Value: 138.39

More than expected
Less than expected

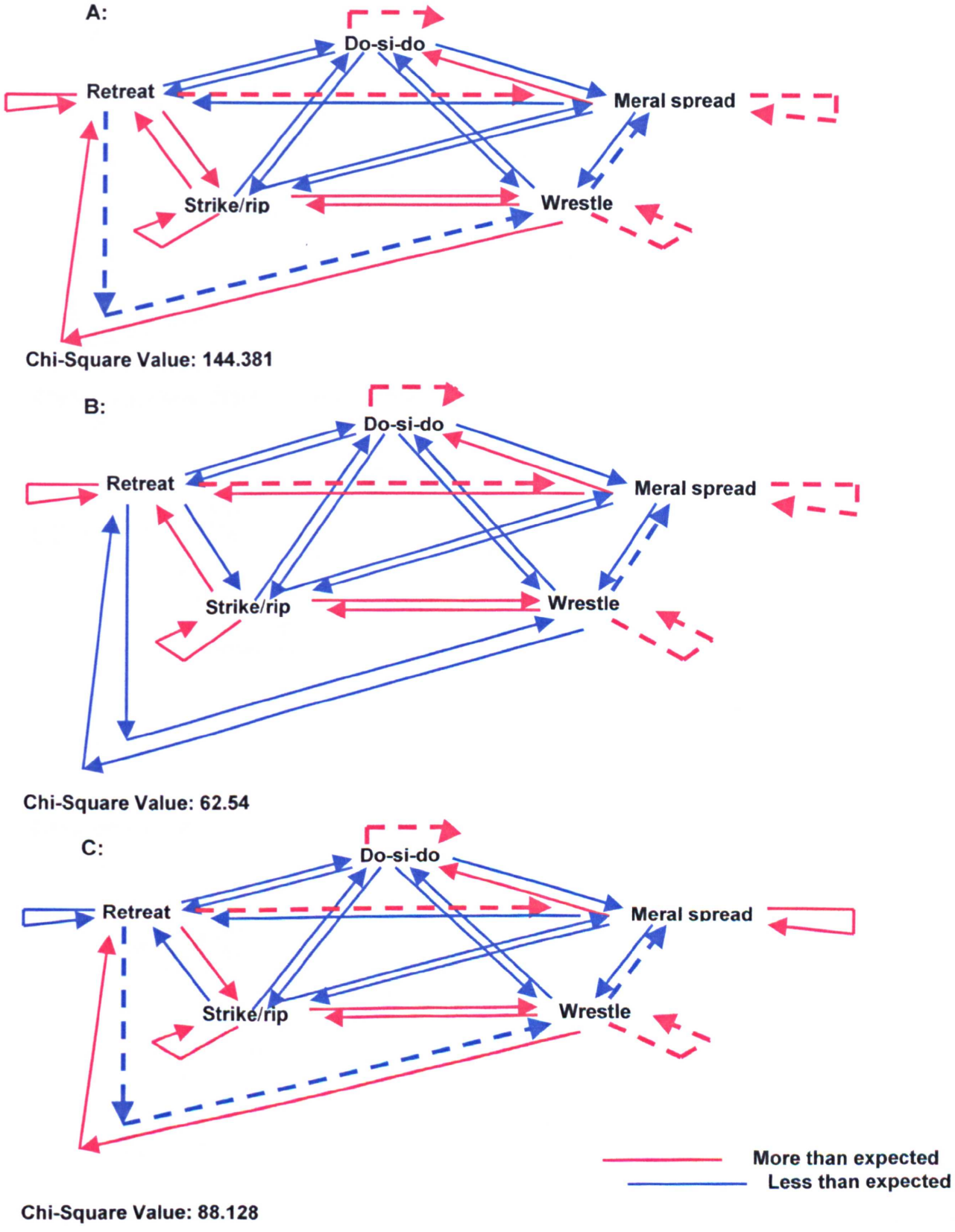
Figures 8.15.a-c: 15°C winner action to loser reaction based on chi-squared analysis: **a**: totals for whole population; **b**: totals for lobsters up to 9 months; **c**: totals for lobsters from 15-17 months.



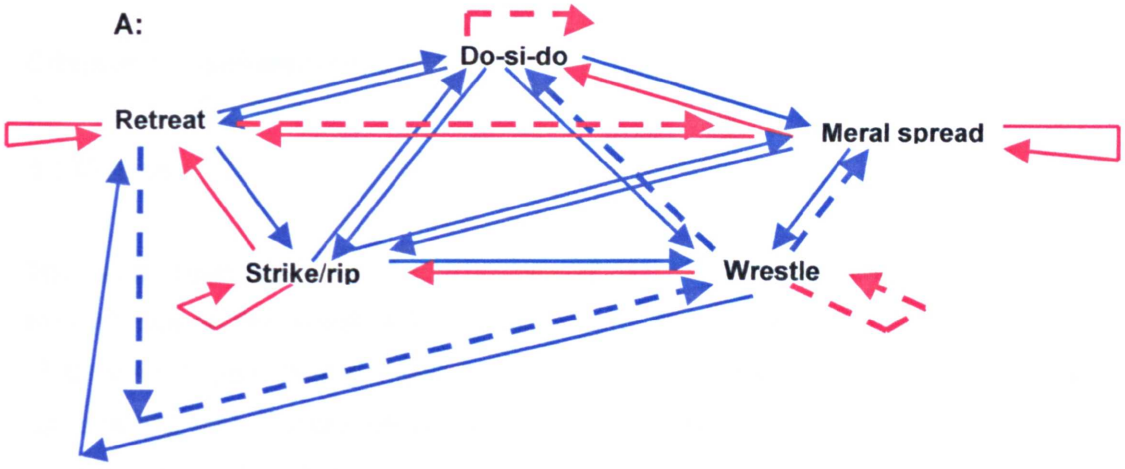
Figures 8.16.a-c: 19°C winner action to loser reaction based on chi-squared analysis: **a**: totals for whole population; **b**: totals for lobsters up to 9 months; **c**: totals for lobsters from 15-17 months.



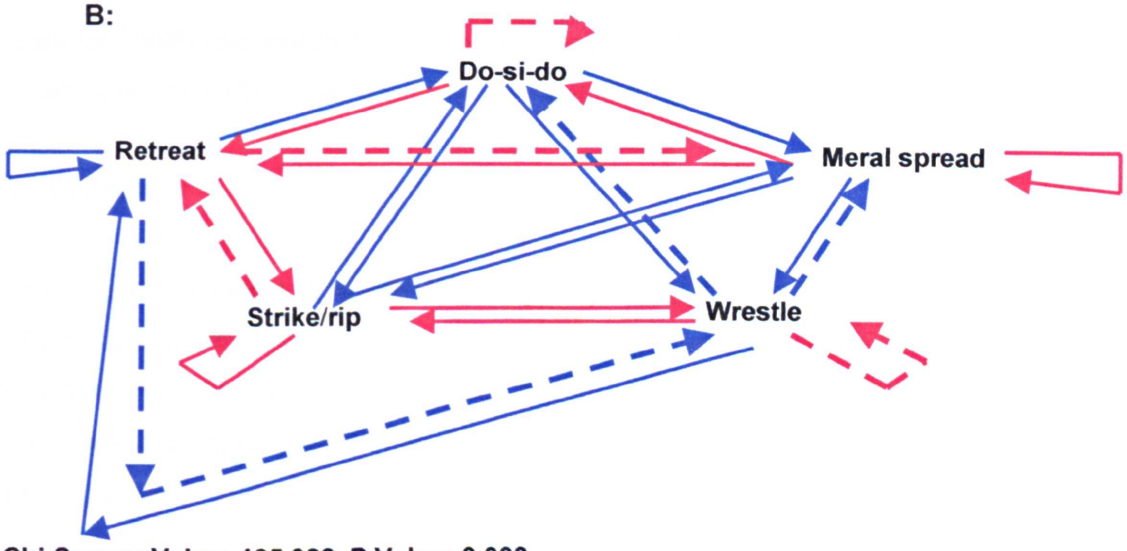
Figures 8.17.a-c: 11°C loser action to winner reaction based on chi-squared analysis: **a**: totals for whole population; **b**: totals for lobsters up to 9 months; **c**: totals for lobsters from 15-17 months.



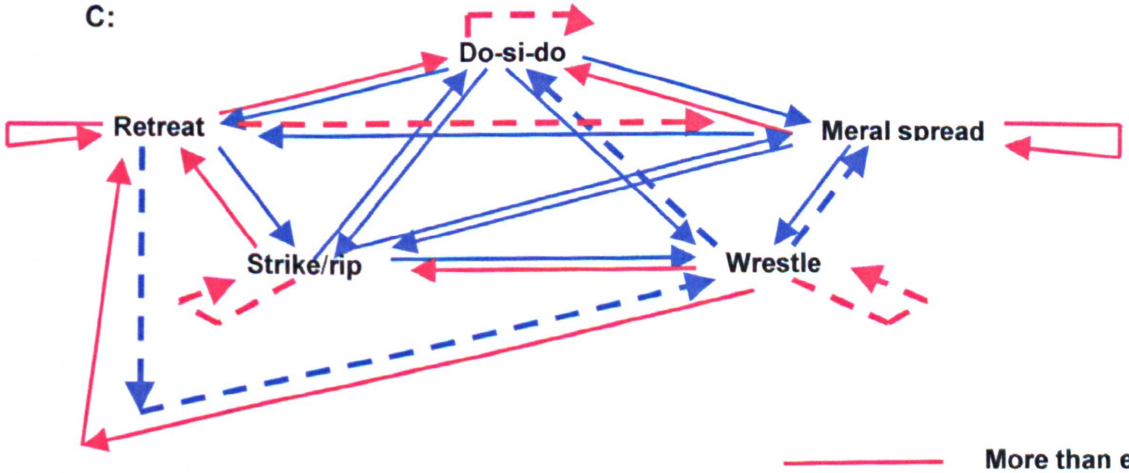
Figures 8.18.a-c: 15°C loser action to winner reaction based on chi-squared analysis: **a**: totals for whole population; **b**: totals for lobsters up to 9 months; **c**: totals for lobsters from 15-17 months.



Chi-Square Value: 205.671, P Value: 0.000



Chi-Square Value: 105.922, P Value: 0.000



Chi-Square Value: 113.643

More than expected
Less than expected

Figures 8.19.a-c: 19°C loser action to winner reaction based on chi-squared analysis: **a**: totals for whole population; **b**: totals for lobsters up to 9 months; **c**: totals for lobsters from 15-17 months.

Chapter 9 - General Discussion

9.1 Outline

The work reported in this thesis has identified numerous effects of rearing temperature on the growth and moulting rates of the European lobster, on the rate of dimorphic claw development relative to moult stage and related claw force parameters, and on locomotory performance. These effects may reflect a number of underlying temperature-dependent processes in the developing tissues at both the biochemical and the genetic levels, affecting growth, morphogenesis and the rates of metabolic reactions. The discussion below considers some of these underlying factors that may be causal to the observed effects.

9.2 Growth

The growth of lobsters is strongly temperature-dependent (Chapters 2 and 4), and it has been demonstrated that as well as an effect on moulting rate, there is also an underlying effect on growth *per se*, indicated by the values for size at stage. At the lowest temperature (11°C) the long inter-moult periods for the juvenile lobsters show that it was close to the null point for growth. When the growth (CI) of the lobsters was compared between the temperature regimes, it can be seen that carapace length increased in a temperature dependant manner from an initial value of 6 mm in the stage 8 lobsters. Hence, it can be said that growing lobsters at higher temperatures is a viable procedure for accelerating the growth rates of juvenile lobsters. When the growth of the dimorphic claws are viewed it has been found that at a given size the lower temperature animals have a greater claw size than the higher temperature lobsters, potentially indicating that the growth at 11°C may be slower, but is more productive. On this note, from personal observations in the laboratory, it was apparent that the 19°C lobsters showed the greatest incidences of deformities, potentially demonstrating the consequences of accelerated growth, which has also been found in studies on fish reared at elevated temperatures. In all temperatures the crusher claws were greater in cross-sectional area than the cutter claws. The dactyl length was found to a good predictor of the potential cutter claws and that of claw height and width for the crushers.

The differences between the lobsters at the three temperatures can be related to the underlying factors of growth, since corresponding whole body protein synthesis rates measured on lobsters used in the present study increased with rearing temperature (Intanai and Taylor, pers. com.) this suggests that the temperature affects synthesis and degradation rates to different extents, resulting in a net increase in protein production. Hence in addition to the developmental processes being accelerated, the growth at any stage point is also increased between rearing temperatures.

In other crustacean studies the effect of temperature on protein synthesis rates has been shown to be tissue- and species-dependent according to the normal thermal regime (Whiteley *et al.*, 1997). In general, the protein synthesis rate increases with temperature and this is accompanied by an elevation in RNA activity at a constant RNA:protein ratio (Whiteley *et al.*, 1992; Whiteley and El Haj, 1997; Whiteley *et al.*, 1997). This occurs in crustaceans with different thermal tolerances, as demonstrated by the temperate eurythermal isopod *Idotea rescata* (2-24°C) (Whiteley *et al.*, 1996), the stenothermal Antarctic isopod, *Glypotonotus antarcticus* (0-5°C) (Whiteley *et al.*, 2001) and the tropical tiger prawn, *Penaeus esculentus* (Hewitt, 1992). Little is known, however, of the effects of rearing temperature on tissue degradation rates. If such measures could be applied to the tissues of lobsters reared at 11°C, the issue of why these animals grow so slowly might be resolved.

9.3 Larval muscle differentiation - the abdominal muscles

In lobsters the abdominal muscle systems differentiate early in the larval stages, when the deep flexor and extensor muscles develop fast fibres, and the superficial muscle sheets develop slow fibres (of two phenotypes). In contrast, the claw muscles transform to their dimorphic state during the postlarval phase of growth (Chapter 5). The differentiation of these two muscle systems would therefore be expected to be sensitive to environmental conditions such as temperature at different periods in the developmental process.

The abdominal muscles of lobsters undergo myogenesis early in embryonic

development (Govind *et al.,* 1988) and are fully functional prior to hatching (Cole and Lang, 1980). The fast and slow phenotypes within the abdomen of the embryo become distinct when myofibrils first organise into sarcomeres (Govind *et al.,* 1974), indicating that they are established at the time of assembly, and are genetically determined. Behavioural studies, which have recorded embryos tail-flipping within the egg, confirm the requirement for functional protein isoforms prior to hatching (Cole and Lang, 1980). Such a mechanical conditioning of the abdominal muscles may be essential in order to meet the immediate functional demands for larval motility placed on the abdominal muscle system, on release into the water column.

Based on our knowledge of the life cycle of the lobster (Aiken and Waddy 1980; Ennis, 1995), it is likely that the myosin isoforms expressed in pelagic larvae are switched off around the time of settlement, and replaced by adult isoforms, reflecting changes in muscle function associated with the behaviour and growth of the young juvenile lobsters. Such developmental-stage specific isoforms of myosin have been described in fish. For example, two developmentally regulated MHC gene transcripts have been identified in the fast myotomal muscles of common carp that are expressed between 22h post-fertilisation and 2 weeks post-hatch (Ennion *et al.*, 1999). Also, embryonic/larval protein isoforms of MHC have been identified in common carp (Wakeling *et al.*, 2000), herring (Johnston *et al.*, 1997; 1998), plaice (Johnston and Horne, 1994) Arctic charr (Martinez and Christiansen, 1994) and sea bream (Mascarello et al, 1995).

The influence of environmental cues on the expression of developmentally programmed isoforms, and of the rate of switching between isoforms at a given life history stage may not be constant, however, there is accumulating evidence from other species that they vary with the stage of development. Certainly in fish, there appears to be critical stages during development when the animals are more responsive to varying temperature regimes (Johnston *et al.*, 1997). For example, acclimation responses of carp to low temperatures leading to the appearance of characteristic low-temperature MHC isoforms for myofibrillar ATPase activity are achieved in 10 weeks by the larvae (Wakeling *et al.*, 2000), but in only 2-3 weeks by the adults (Heap *et al.*, 1985). This suggests that some developmental competency is required to adjust myosin isoform composition to

decreasing water temperatures. The ability to modify MHC expression with temperature acclimation thus seems to be gradually acquired during development, and becomes evident at some specific stage (recently determined to be 37mm total length in the case of carp: Cole and Johnston, 2001).

In order to investigate whether equivalent effects of development and temperature can be detected in the expression of muscle genes for key sarcomeric proteins of lobsters, an extensive molecular study has been carried out on muscle tissue samples taken from lobsters of the three temperature groups at different developmental stages (reviewed by Holmes *et al,* 2001). These molecular studies, which were performed in parallel to the work reported in this thesis, provide preliminary evidence for differences in the responsiveness of myosin isoform switching in lobster muscles to temperature with the stage of development, which has important consequences for interpreting the results reported in earlier chapters.

Using the adult fast and slow MHC cDNA clones, it has been demonstrated that the molecular transformation of the abdominal muscles during development switches on prior to hatching, and up-regulates throughout the larval period with both development and temperature (Holmes *et al.*, 2001). Actin and tropomyosin are both expressed in the eggs and are up-regulated with age and temperature. The slow MHC isoform switches on prior to hatching and up-regulates throughout larval development. The fast myosin switches on later, around Stages 1-2, and elevated rearing temperature appears to accelerate the developmental cues for its expression. In settled lobsters, this pattern of expression reverses and the slow myosin signal down-regulates at a rate that is also temperature-dependent. One possible interpretation of these results is that the adult slow myosin clone recognises certain larval myosin isoforms and it is these isoforms that are being down-regulated at postlarval stages. If this is so, then these data provide evidence for a development sequence in the expression of myosin isoforms, which is responsive to rearing temperature.

This question has been addressed further by comparing sequence data for key-regulatory regions associated with the nucleotide and actin binding pockets of the MHC gene (loop 1 and loop 2 respectively) from larval and postlarval lobsters.

Loop 2 is highly conserved and does not appear to be a key site for acclimation during development at varying temperatures, but loop 1 does show sensitivity to both developmental stage and environmental temperature. Due to its position, loop 1 modulates ATPase activity and sliding velocity (Spudich, 1994). Larger and more positively charged loops cause changes in the tertiary structure of the nucleotide binding pocket, producing faster rates of ADP release and shortening, while smaller loops show increased rigidity (Sweeney *et al.,* 1998). Sequence variation measured within the loops of myosin from lobster larvae has identified developmental shifts involving its amino acid sequence and charge which suggest that myofibrils will show increased rates of *in vitro* motility and ADP release between Stages 1 and 2, as charge increases from +1 to +2. Significantly, these shifts are not advanced by elevated rearing temperature in the larvae, but they do become temperature sensitive in the post larvae. This therefore represents an example in crustaceans of a temperature-sensitive molecular expression that is limited to particular developmental stages.

The temperature-dependent differences found in loop 1 in the post larvae, and their consequent effect on the binding the ATP molecule may ultimately produce differences in the rate of ATPase activity, and thus have an important effect on the rate and efficiency of the contraction process. This could be a significant contributory factor to the swimming performance measured in the lower temperature groups, in addition to other biochemical compensatory mechanisms, and to any effects of allometric growth of the abdomen, the main propulsor, as previously discussed (Chapter 7).

9.4 Postlarval muscle differentiation - the claw muscles

The claw muscles of lobsters, like the abdominal muscles, comprise fibres of either the fast or slow (predominantly S1) phenotypes, but unlike the abdominal muscles, they undergo considerable transformation during the postlarval stages (Govind and Lang, 1978; Ogonowski *et al.*, 1980) in conjunction with differentiation into the characteristic cutter and crusher claws of the adult lobster (Govind, 1992). During the dimorphic change, transformations of the closer muscle fibre types take place in both directions. Thus, in the presumptive cutter claw the fast-fibre region of the closer muscle expands in area, while in the

presumptive crusher-claw this fast-fibre region contracts in area. Fibre type transformation is due to the switching of pre-existing, fully-differentiated fibres from one type to the other (slow to fast in the presumptive cutter and fast to slow in the presumptive crusher). This change in fibre phenotype is due to the changes in the expression of certain myofibrillar protein isoforms, rather than to the replacement of fibres of one phenotype by fibres of another, generated *de novo* by hyperplasia (Mykles, 1997).

In the American lobster, *H. americanus*, this process of transformation to the dimorphic adult pattern can be complete as early as stage 13, but in some cases is not attained until the lobster is 1-2 years old (Govind, 1992). In the present study (Chapter 5) it has been found that the rate of fibre transformation in the cutter claw closer muscle (from predominantly the slow phenotype to completely the fast phenotype) displayed a degree of moult stage dependence, with the final state being reached by all temperature by stage 14, indicating a transformation that is not greatly influenced by rearing temperature (Fig. 5.11). It was found that the development of the crusher claws was not in as uniform a way as for the cutter claws. This development is influenced by exercise, however we have demonstrated that partial transformation to the crusher claw type takes place within these juvenile lobsters (False crushers). It can be seen that even though the muscle is not fully dimorphically developed, internally it can still behave in the manner of a 'true' crusher. Between the age points within the temperature groups the peak forces of the crusher claws were seen to be consistently greater than those of the cutter claws. This data also shows how the higher temperature of rearing has had a greater effect on the size and subsequent force production of the claws in the 19°C group is generally significantly higher in force production than the 11 and 15°C groups.

The transformation in fibre phenotype during dimorphic claw development in the lobster represents a useful model system in which to correlate changes at the phenotypic and genotypic levels. Thus, as shown by *in situ* hybridisation using the mRNAs for fast and slow myosin heavy chains, fibre transformation involves the expression of fast isoforms of myosin in the high ATPase fibres, and of slow isoforms of myosin in low ATPase fibres (Mykles, 1997). These results correspond to those derived from histochemical mapping of fibre properties

(Chapter 5), and suggest that within claw muscle fibres there is a high correlation between the expression of myosin chain isoforms and the level of activity of the myofibrillar ATPase. Since a number of other contractile and regulatory proteins are also expressed as different isoforms in the two fibre phenotypes (Fig. 5.1), switching involving a suite of different genes is implied.

A study of the expression of fast and slow mRNAs in claws of the postlarval *H. gammarus* lobsters used in the present project has also shown that changes in fibre type correlate with the expression of the genes for fast and slow myosin (Holmes *et al.*, 2001). Thus there is an up-regulation of the mRNA for fast myosin in the cutter claw and an up-regulation of the mRNA for slow myosin in the crusher claw. Moreover, these changes in expression are affected by temperature in a way that corresponds closely to the changes measured using the histochemical mapping of fibre phenotypes (Fig. 5.11). Both levels of analysis identify transitory differences in the relative timing of claw muscle differentiation with temperature that could be of behavioural or ecological importance.

Sequence analysis has also been used to identify transitions in a key region of functional importance associated with the nucleotide binding on the myosin heavy chain gene of claw muscles. Amino acid residues within loop 1 showed temperature-dependent changes: with increasing temperature the total charge decreased in the cutter claw, and increased in the crusher claw (El Haj and Holmes, pers. com.).

9.5 Behaviour

The swimming behaviour of the juvenile lobsters is interesting as it could help to determine the survival if released into the wild after growing in a lab. Here as detailed previously with relation to the molecular mechanisms, some forms of compensations tend to take place within the lower temperature animals such as allometric changes in the relative size of the abdomen, alongside forms of metabolic compensation and phenotypic plasticity. Alongside this the agonistic behaviour was also analysed to try and see if temperature affects the intrinsic nature of the fighting rituals in juvenile lobsters. The main differences that occurred between winning and losing lobsters at the different temperature

regimes, was that of a ratio of high numbers of meral spreads in the winning lobsters to low in the losing lobsters and high levels of retreat behaviour in the losing lobsters to low in the winners, which seems to be almost reciprocal between the factors. Two main loops of behaviour were present: a low level display loop and a high intensity-fighting loop, which is consistent with Game Theory. As the lobsters increased in age, the total numbers of behaviour increased, which was predominantly accounted for by wrestling behaviour, indicating that older animals (juveniles) were more likely to let the fights escalate to the high intensity level of fighting. Behavioural repertoire was not altered with age or with rearing temperature.

9.5 Conclusions/Prospects

The finding that molecular methods provide results that are consistent with phenotypic mapping opens up the opportunity to study the gene regulation processes underlying the changes in muscle fibre phenotype, and the mechanism whereby they are influenced by internal factors (nerve stimulation, hormone titre) and external factors (environmental conditions, muscle exercise). This offers the possibility of explaining phenotypic plasticity in relation to development, use, or environmental influences in terms of the underlying genetic switching, which is an obvious direction for future research.

Through this project various aspects of the development of the juvenile lobsters (*Homarus gammarus*) have been dealt with. It has been demonstrated that the growth is greater at higher temperatures but this also shows it's constraints, through factors such as deformities and greater claw size at a given body size for the lower temperature animals. However it can be seen that the performance of the claws of the higher temperature lobsters far exceed those of the lower two groups, but here again, problems arose due to the lack of temperature dependant development of the crusher and cutter claws, giving rise to the phenomenon of false crusher claws. With the compensation effects of the lower two temperature relating to the swimming behaviour and the lack of difference between temperature groups when agonistic encounters are compared, the question of what the most suitable temperature of rearing lobsters in a laboratory environment would be and whether these animals would be able to fare against a natural

population if released into the sea? From the findings of this project, it would be recommended that 19°C is too high a rearing temperature, as too many anomalies seem to occur, even though the greatest sizes are seen in this regime. A temperature of ~14-15°C would be recommended for the on-growing of post-larvae/juvenile lobsters because this temperature regime increases the sizes of the lobsters in a uniform manner and shows all the attribute of the higher temperature lobsters with fewer anomalies. It can be said that ideally the quality of the 11°C lobsters could be classed as the best, but the development at this temperature is just too slow. As to whether the laboratory-reared lobsters would be able to compete in the wild is hard to say. However through the analysis of the agonistic behaviour the lobsters should be able to compete on a par or at a slightly reduced level, as all the behavioural components are present. The main problem that might occur is that of social naïveté and whether they could adapt in time to avoid being out competed by the natural population, which will have had more life experience than those reared in a laboratory.

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