

**THE THERMAL DEPENDENCE OF SWIMMING AND MUSCLE  
PHYSIOLOGY IN TEMPERATE AND ANTARCTIC SCALLOPS**

**David Mark Bailey**

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**The thermal dependence of swimming and  
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scallops**

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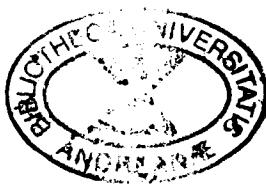
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**July, 2001**



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## **SUMMARY**

### **Chapter 1**

Swimming is important to the ecology of many species of scallop but the effects of temperature upon swimming are not clear. The ecology and natural history of scallops is introduced followed by a description of the state of current knowledge of scallop swimming, muscle physiology and energetics. The effects of temperature and the mechanisms used by ectotherms to compensate for such changes over acute, seasonal and evolutionary timescales are discussed.

### **Chapter 2**

Scallops are active molluscs, able to escape from predators using jet propelled swimming. Queen scallops (*Aequipecten opercularis*) were acclimated to 5, 10 and 15°C in the laboratory and collected in Autumn ( $13\pm 3^\circ\text{C}$ ) and Winter ( $8\pm 2^\circ\text{C}$ ) in order to investigate seasonal acclimatisation. The first jetting cycle of escape responses in these animals was recorded using high-speed video (200-250fps). Whole-animal velocity and acceleration were determined while measurements of valve movement and jet area allowed the calculation of muscle shortening velocity, force and power output.

Peak swimming speed was significantly higher at 15°C ( $0.37\text{m}\cdot\text{s}^{-1}$ ) than at 5°C ( $0.28\text{m}\cdot\text{s}^{-1}$ ). Peak acceleration was 77% higher at 15°C ( $7.88\text{m}\cdot\text{s}^{-2}$ ) than at 5°C ( $4.44\text{m}\cdot\text{s}^{-2}$ ). Mean cyclic power output was also higher at 15°C ( $31.3\text{W}\cdot\text{kg}^{-1}$ ) than at 5°C ( $23.3\text{W}\cdot\text{kg}^{-1}$ ). Seasonal comparison of swimming in freshly caught animals revealed significantly greater acceleration (x2 at 11°C) and velocity during jetting in Winter than in Autumn animals (ANCOVA). These were associated with significant increases in peak power output (x8 at 11°C), force production and muscle shortening velocity. Actomyosin ATPase activity was

significantly higher (31% at 15°C) in winter animals with peptide mapping of the Myosin heavy chain showing no differences between groups.

Increases in muscle power output were associated with reductions in the length of the jetting phase as a proportion of the overall cycle. As a result large changes in muscle performance resulted in large short-term whole body performance enhancement but little difference to velocity over the cycle.

### Chapter 3

Measurements of the swimming performance of the Antarctic scallop were made from videos of escape responses. Animals were acclimated to +2 and -1°C in the laboratory and compared to animals maintained at natural water temperature ( $0\pm 0.5^\circ\text{C}$ ) at the time of experimentation.

*Adamussium* was very sensitive to temperature change with the proportion of swimming responses being less common at higher temperatures and where an individual was exposed to temperatures above its maintenance temperature. Analysis of the first jetting cycle of swimming was carried out as described in Chapter 2. These analyses revealed that over the small temperature range that the animals can tolerate swimming performance is strongly temperature dependent.  $Q_{10}$ s above 2 included those for thrust (3.74), mean cyclic swimming speed (2.46), mean cyclic power output (5.71) and mean muscle fibre shortening velocity (2.16).

*Adamussium* did not demonstrate strong phenotypic plasticity with no significant differences in swimming of muscle performance between animals acclimated to different temperatures. Comparison of the relationship between swimming

velocity and temperature in *Adamussium* and other species showed little evidence for evolutionary compensation for temperature with all data fitting to a single relationship with a  $Q_{10}$  of 1.96 (0-20°C). Similar results were obtained for power output and the performance of *in vitro* muscle preparations. These results are discussed in the light of field studies revealing the low predator pressure and escape performance of wild *Adamussium*.

#### Chapter 4

*In vivo*  $^{31}\text{P}$ -Nuclear Magnetic Resonance Spectrometry (MRS) was used to measure the levels of ATP, Phospho-L-arginine (PLA) and inorganic phosphorous (PI) in the adductor muscle of the Antarctic scallop, *Adamussium colbecki*, and two temperate species, *Aequipecten opercularis* and *Pecten maximus*. Graded exercise regimes from light (1-2 contractions) to exhausting (failing to respond to further stimulation) were imposed upon animals of each species. MRS allowed non-invasive measurement of metabolite levels and intracellular pH at high time resolution (30-120s intervals) during exercise and throughout the prolonged recovery period. Significant differences were shown between the magnitude and form of the metabolic response with increasing levels of exercise. Short-term (first 15 minutes) muscle alkalosis was followed by acidosis of up to 0.2 pH units during the recovery process.

*Aequipecten* had significantly higher resting muscle PLA levels than either *Pecten* or *Adamussium*, used a five-fold greater proportion of this store per contraction and was able to perform only half as many claps (maximum of 24) as the other species before exhaustion. All species regenerated their PLA store at a similar rate despite widely different environmental temperatures.

## **Chapter 5**

The major results and their impact on our knowledge of biomechanics and its temperature dependence are discussed. Suggestions for future research based upon the experimental findings and techniques developed are presented.

## **ACKNOWLEDGEMENTS**

I want to thank my supervisors Ian Johnston and Lloyd Peck for all their help and guidance, Rob James for introducing me to the world of muscle physiology and Nick Cole for all his teaching and help with the horrors of gels and enzyme assays. I want to pick out James Wakeling for particular thanks, the programming techniques he taught and his input during the write-up were absolutely invaluable. Christian Bock, Rolf Wittig and Hans-Otto Portner at the Alfred Wegener Institute were also great to work with, thanks for your amazing NMR, looking after me, and making me feel so welcome. I can't thank Alan Ansell for his help unfortunately. Alan did more to point me in the right direction in the first months of the study than anyone else.

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## CONTENTS

|  | Page |
|--|------|
| <b>Declarations</b>  | i    |
| <b>Summary</b>   | ii   |
| <b>Acknowledgements</b>  | vi   |
| <b>Contents</b>  | vii  |
| <b>Chapter 1 General Introduction</b>  | 1    |
| <b>1.1 Scallop natural history</b>   | 1    |
| <b>1.2 Scallop swimming</b>  | 2    |
| <i>1.2.1 Jet propulsion</i>  | 2    |
| <i>1.2.2 Scallops as model animals</i>   | 4    |
| <i>1.2.3 Structure and regulation of scallop muscle</i>  | 7    |
| <i>1.2.4 Energetics and cost of swimming</i>   | 11   |
| <b>1.3 Adaptive trade-offs in performance and energetics</b>   | 12   |
| <b>1.4 Temperature</b>   | 14   |
| <i>1.4.1 Acute temperature change</i>  | 15   |
| <i>1.4.2 Temperature acclimation and acclimatisation</i>   | 16   |
| <i>1.4.3 Seasons are not just temperature changes</i>  | 19   |
| <i>1.4.4 Temperature adaptation</i>  | 20   |
| <b>Chapter 2 Modelling muscle performance during swimming in the European Queen Scallop: effects of acute and seasonal temperature change.</b> | 23   |
| <b>2.1 Introduction</b>  | 23   |

|            |   |    |
|------------|---|----|
| <b>2.2</b> | <b>Materials and methods</b>  | 26 |
|            | <i>2.2.1 Animals</i>  | 26 |
|            | <i>2.2.2 Analysis of swimming behaviour</i>   | 27 |
|            | <i>2.2.3 Morphological measurements</i>   | 29 |
|            | <i>2.2.4 Hinge ligament properties</i>  | 29 |
|            | <i>2.2.5 Analysis of filming</i>  | 30 |
|            | <i>2.2.6 Determination of Actomyosin ATPase activity</i>                              | 33 |
|            | <i>2.2.7 SDS-PAGE electrophoresis</i>   | 36 |
| <b>2.3</b> | <b>Results</b>  | 39 |
|            | <i>2.3.1 Ligament stress-strain properties</i>  | 39 |
|            | <i>2.3.2 Behaviour</i>  | 39 |
|            | <i>2.3.3 Biomechanics of swimming in acclimated animals at different temperatures</i> | 39 |
|            | <i>2.3.4 Effects of seasonal acclimatisation on swimming</i>                          | 42 |
|            | <i>2.3.5 Interaction of variables</i>   | 44 |
|            | <i>2.3.6 Scaling</i>  | 45 |
|            | <i>2.3.7 Actomyosin ATPase activity</i>   | 45 |
|            | <i>2.3.8 SDS-PAGE electrophoresis</i>   | 45 |
| <b>2.4</b> | <b>Discussion</b>   | 47 |
|            | <i>2.4.1 Effects of temperature on swimming</i>                                       | 47 |
|            | <i>2.4.2 Effects of field acclimatisation on swimming</i>                             | 50 |
|            | <i>2.4.3 Conclusions</i>  | 53 |
|            | <b>Appendix A</b>   | 54 |
|            | Mathematical model  | 54 |
|            | Anatomical parameters and glossary  | 62 |

|                  |  |           |
|------------------|--|-----------|
| <b>Chapter 3</b> | <b>Effects of temperature and acclimation on the swimming performance and in vitro muscle characteristics of the Antarctic Scallop, <i>Adamussium colbecki</i></b> | <b>64</b> |
| <b>3.1</b>       | <b>Introduction</b>  | <b>64</b> |
| <b>3.2</b>       | <b>Materials and methods</b>   | <b>68</b> |
|                  | <i>3.2.1 Swimming activity in wild animals</i>   | 68        |
|                  | <i>3.2.2 Experimental animals</i>  | 69        |
|                  | <i>3.2.3 Filming</i>   | 71        |
|                  | <i>3.2.4 In vitro muscle twitch characteristics</i>  | 74        |
|                  | <i>3.2.5 Statistics</i>  | 76        |
| <b>3.3</b>       | <b>Results</b>   | <b>77</b> |
|                  | <i>3.3.1 Field studies of activity in Adamussium</i>   | 77        |
|                  | <i>3.3.2 Behaviour</i>   | 77        |
|                  | <i>3.3.3 Swimming performance and effects of temperature</i>   | 78        |
|                  | <i>3.3.4 Comparative whole-body and muscle performance</i>   | 79        |
|                  | <i>3.3.5 Comparative in vitro muscle characteristics</i>   | 80        |
| <b>3.4</b>       | <b>Discussion</b>  | <b>81</b> |
|                  | <i>3.4.1 Effects of temperature change and thermal history</i>   | 81        |
|                  | <i>3.4.2 Evidence of evolutionary adaptation</i>   | 82        |
| <b>Chapter 4</b> | <b>High-energy phosphate metabolism during exercise and recovery in temperate and Antarctic scallops - an <i>In vivo</i> <sup>31</sup>P-NMR study.</b>             | <b>86</b> |
| <b>4.1</b>       | <b>Introduction</b>  | <b>86</b> |
| <b>4.2</b>       | <b>Materials and methods</b>   | <b>86</b> |
|                  | <i>4.2.1 <sup>31</sup>P-NMR Spectroscopy</i>   | 91        |
|                  | <i>4.2.2 Graded exercise</i>   | 92        |

|                  |   |     |
|------------------|---|-----|
| 4.2.3            | <i>Analyses</i>   | 93  |
| 4.2.4            | <i>Technical constraints</i>  | 94  |
| <b>4.3</b>       | <b>Results</b>  | 95  |
| 4.3.1            | <i>Resting metabolite levels</i>                                      | 95  |
| 4.3.2            | <i>Muscle metabolism during exercise</i>                              | 96  |
| 4.3.3            | <i>Recovery of PLA levels following exercise</i>                      | 97  |
| 4.3.4            | <i>Interspecific differences in muscle metabolism during exercise</i> | 97  |
| 4.3.5            | <i>Interspecific differences in recovery rates</i>                    | 98  |
| 4.3.6            | <i>Muscle pH during exercise and recovery</i>                         | 99  |
| <b>4.4</b>       | <b>Discussion</b>   | 100 |
| 4.4.1            | <i>Observations of metabolism during exercise and recovery</i>        | 100 |
| 4.4.2            | <i>Causes of fatigue</i>  | 103 |
| 4.4.3            | <i>Interspecific differences in high-energy phosphate metabolism</i>  | 104 |
| 4.4.4            | <i>Conclusions</i>  | 107 |
| <b>Chapter 5</b> | <b>General discussion</b>   | 108 |
| <b>5.1</b>       | <b>Findings of the present study</b>                                  | 108 |
| 5.1.1            | <i>Biomechanics of escape responses</i>                               | 108 |
| 5.1.2            | <i>Significance of escape responses and their thermal dependence</i>  | 111 |
| 5.1.3            | <i>Trade-offs in performance and energetic costs</i>                  | 112 |
| <b>5.2</b>       | <b>Future studies</b>   | 114 |
| 5.2.1            | <i>Scallops as model animals</i>                                      | 114 |
| 5.2.2            | <i>Closing remarks</i>  | 115 |
|                  | <b>References</b>   | 116 |

## **Chapter 1**

### **GENERAL INTRODUCTION**

#### **1.1 Scallop natural history**

The scallops are members of the Pectinids, a family of around 400 extant species found throughout the oceans of the world from the poles (Arsenault and Himmelman, 1998a; Berkman, 1988) to the tropics (Boadas et al., 1997; Velez et al., 1995) and to depths of over 1000m (Dell, 1972). A large body of literature exists on the natural history of scallops due to their importance as fisheries species, particularly focussed on growth and reproductive biology. Seasonal cycles are important to the ecology of scallops with interrelated changes in both reproductive and food availability (Allison, 1994; Barber et al., 1987; MacDonald and Thompson, 1986; Navarro and Thompson, 1995).

As lamellibranch molluscs scallops feed by filtering seawater through hypertrophied gills (Bayne and Hawkins, 1997; Jorgensen, 1996) and are thus directly affected by changes in seasonal productivity. Seasonal cycles of phytoplankton productivity are linked to the scallop's reproductive cycles (Arsenault and Himmelman, 1998b) with spawning in the study species occurring before or during the summer period of highest productivity (Allison, 1994), (D.Powell, unpublished data). In ripe individuals reproductive events may be triggered by changes in water temperature (Bonardelli et al., 1996). Reproductive cycles result in changes in energy resource allocation within the scallop's body resulting in reduced muscle mass during gametogenesis (Allison, 1994) and reductions in the activity of metabolic enzymes immediately following spawning (Boadas et al., 1997; Brokordt et al., 2000).

Scallops are close to the base of benthic food webs, fixing primary productivity and recovering precipitated organic material (Chiantore et al., 1998). Scallops are predated on by starfish (Barbeau and Scheibling, 1994a; Barbeau and Scheibling, 1994c; Thomas and Gruffydd, 1971), crabs (Cliche et al., 1994; Freitas et al., 2000), fish (Digiacommo and Perier, 1996), gastropods (Freitas et al., 2000), and octopus (Wolf and White, 1997). The interactions of scallops with their predators is substantially modified in many species by the scallop's active escape behaviour.

## **1.2 Scallop swimming**

### *1.2.1 Jet propulsion*

In contrast to most swimming animals scallops do not locomote by the action of external structures against the surrounding water. Swimming pectinids, cephalopods, salps and jellyfish (amongst others) "have chosen to separate a body of water from its surroundings and manipulate it so as to produce locomotion by jet propulsion" (Weihs, 1977). While this may seem a fine distinction it has important consequences for the energetics of swimming. The specific nature of scallop swimming is discussed in depth below a short introduction to this mode of locomotion is of value here. All forms of jet propulsion described have certain common elements, namely a "power stroke", during which water is ejected and the animal propelled in the opposite direction. This is followed by the recovery phase as water is drawn into the animal, often under the partial or complete action of an elastic element (DeMont, 1988; Macgillivray et al., 1999).

As a large proportion of animals (including ourselves) have the ability to eject water under pressure it is perhaps surprising that jet propulsion is not more

common amongst animals. Newtonian physics provides part of the explanation. Unlike a fish, which has the entire ocean with which to interact, a jet propelled animal has only that which can be accommodated within its body. Secondly, as the elastic mechanisms responsible for the recovery stroke must be tensioned during the power stroke even a perfectly elastic structure reduces the power available to jetting. Although this can be minimised by changing the area of the inhalent and exhalent openings in general the faster the recovery required, the more power is lost to locomotion itself. Elastic elements in the skin and other connective tissues of vertebrates may increase the efficiency of their swimming though they remain relatively poorly understood compared to those of cephalopods and molluscs (Pabst, 1996).

The net result of the anatomical restrictions described above, is that the jet propelled animal ejects a relatively small amount of water over a relatively small proportion of the time it spends swimming. In order to gain sufficient thrust (the product of mass and acceleration) this small mass must be ejected at high speed. Unfortunately the work required to do this increases with the square of velocity ( $0.5 \times \text{mass} \times \text{velocity of the jet}^2$ ) so small amounts of water ejected at high speed require higher power inputs for the same thrust gains as larger, slower moving bodies of water. The ratio of the work done on the animal (resulting thrust x animal mass) to the work done by the animal (jet power requirement) is the Froude efficiency. This is lower in jet propelled animals than for fish of the same size due to the larger mass of water that a fish tail can push rearwards (Trueman, 1980).

Fortunately things are not quite so bleak as they appear and for some animals with high pulse rates jets may be more efficient. As the jets enter the

surrounding water their vorticity causes them to roll up into toroids. These entrain additional water and drive each other along by Biot-Savard Induction. Injection of pulsed jets into a fluid of similar density results in a jet of higher momentum than could be expected from the initial impetus of each pulse due to the induced velocity of the toroids produced (Weihs, 1977). By manipulation then of the jet cavity pressure, jet area and pulse frequency, toroids may be stacked upon each other in order to increase the thrust derived from the jet.

### 1.2.2 *Scallops as model animals*

What makes scallops relatively unusual among molluscs, and an excellent model group in which to investigate biomechanical questions, is their ability to swim. In many species swimming is used to escape predators (Barbeau and Scheibling, 1994a; Thomas and Gruffydd, 1971) or hostile environmental conditions (Berkman, 1988), move to a better habitat (Hamilton and Koch, 1996) or possibly even migrate (Anderson et al., 1997). Swimming appears to be important to scallops with observations of encounters between Sea Scallops (*Placopecten magellanicus*) and its predators confirming its important role in reducing predation by starfish (Barbeau and Scheibling, 1994a; Barbeau and Scheibling, 1994c). Predation on scallops in the wild is high (Barbeau et al., 1994; Cliche et al., 1994; Hatcher et al., 1996; Stokesbury and Himmelman, 1995); being able to move to suitable habitats both reduces predation rate and improves feeding success (Arsenault et al., 1997; Arsenault and Himmelman, 1996a; Bologna and Heck, 1999).

The jet mechanism by which scallops swim has been well described previously in a range of temperate (Baird, 1957; Dadswell and Weihs, 1990; Moore and Trueman, 1971; Stephens and Boyle, 1978), tropical (Morton, 1980), and polar

(Ansell et al., 1998) species. Briefly, while the shell is open the soft mantle encloses the body of water between the valves. In response to a predator, a rapid contraction of the fast, striated, adductor muscle closes the shell. The enclosed water is forced out of openings formed by the mantle as one or two jets propelling the scallop in the opposite direction. When attacked by a starfish a single jet may be formed anywhere in the mantle (Thomas and Gruffydd, 1971), while for swimming two jets are formed, one on either side of the hinge (Cheng and DeMont, 1996; Moore and Trueman, 1971; Vogel, 1997). Following adduction, the hinge ligament forces the shell open again ready for the next contraction. The elastic properties of this ligament have been well studied (Alexander, 1965; Bowie et al., 1993; DeMont, 1988; Trueman, 1953). Stretching of the adductor by the ligament initiates consecutive contractions (Mellon, 1968) with the animal swimming gape first.

As the anatomy and swimming behaviour of scallops is relatively simple, they have been widely used as model animals in which the work done by the entire locomotory system can be accurately determined. Two major experimental series have so far investigated the muscle performances involved in scallop locomotion. Marsh et al, (1992) calculated *in vivo* power output in the Bay Scallop *Argopecten irradians* using intramantle pressure recordings and flow measurements estimated from sonomicrometry. The results were then compared to those derived from experiments on dissected muscle preparations (Marsh and Olson, 1994) using the workloop technique (Josephson, 1993).

In Marsh and Olson's (1994) study the *in vivo* muscle length and activation patterns during swimming were recorded by sonomicrometry and electromyography. Natural and sinusoidal length changes were then imposed

on the muscle preparation by a lever arm attached to a servo-controlled ergometer with electrical stimulation given at the appropriate point in the cycle. At any point in the cycle the muscle length and force produced was measured by the ergometer and recorded digitally. As length changes and force production were known the cyclic power output could be calculated. The *in vivo* and *in vitro* techniques gave similar peak power outputs of around 175 and 150 W.Kg<sup>-1</sup> respectively at 10°C (Marsh and Olson, 1994; Marsh et al., 1992).

In an alternative approach, a detailed hydrodynamic model of scallop swimming was developed from high-speed cinematography and studies of ligament properties in *Placopecten magellanicus*. The model estimates the lift and drag forces and thrust forces involved for various sizes of scallop (Cheng and DeMont, 1996) and the power output and force production of the adductor muscle (Cheng et al., 1996b; Vogel, 1997). A modelled workloop was produced (Cheng and Demont, 1997) which was compared to Marsh and Olson's (1993) *in vitro* recordings.

The estimates of power output derived from the model were greater (185 W.Kg<sup>-1</sup>) than those previously measured (Cheng et al., 1996b) though the peak predicted force was similar (100 KN.m<sup>-2</sup>) to that measured *in vitro* (Cheng and Demont, 1997; Marsh and Olson, 1994). Interestingly the form of the modelled workloop (stress vs. strain) differed fundamentally from the experimentally developed one. Marsh and Olson (1994) measured two peaks in force during the muscle shortening while Cheng and Demont (1997) predicted only one. While both methods agree in that very low forces are involved during muscle lengthening their predictions of the timing of muscle stress during shortening are very different. Cheng and Demont (1997) predict the peak stress much earlier in

the cycle, when the strain rate is higher, and therefore predicts a higher power requirement. Because of this discrepancy Cheng and Demont (1997) concluded that in vitro experiments could only describe the general form of muscle performance characteristics. In fact the modelled workloop (Cheng and Demont, 1997) looks much like the Marsh and Olson's (1993) experimental workloop using a sinusoidal muscle length change. This is possibly due to the assumptions of the model, in particular that closure rate is constant (Cheng and Demont, 1996b).

These power outputs are high and approach those found in workloop experiments on vertebrate striated muscles such as lizard leg muscle (Swoap et al., 1993), mouse extensor digitorum longus (Askew and Marsh, 1997), and fish white muscle (James and Johnston, 1998). No study has combined muscle and whole-body performance measures in scallops or determined the performances necessary to escape particular predators.

### *1.2.3 Structure and regulation of scallop muscle*

While scallop muscle delivers power outputs and produces forces similar to those of vertebrates its structure, regulation and physiology differs in a variety of ways. Control of muscle activity may be by actin or myosin filaments, varying between species and even between muscles in an individual (Lehman and Szentgyorgi, 1975). In molluscs control is achieved during direct Calcium-Myosin interaction (Kendrick-Jones et al., 1970). Scallop muscle is much like vertebrate striated muscle in gross appearance and contractile performance though quite different at a cellular level (Olson and Marsh, 1993) and without thin filament control. Regulatory light chains inhibit the action of calcium in the

muscle fulfilling the role played by troponin in other species (Simmons and Szent-Gyorgyi, 1978).

Each Myosin molecule in the fast adductor muscle of *Placopecten magellanicus* is associated with two regulatory light chains. This complex may bind to calcium ions; one ion per light chain (Chantler and Szent-Gyorgyi, 1980). Removal of regulatory light chains (using EDTA) results in loss of control of ATPase activity (Chantler and Szent-Gyorgyi, 1980; Szent-Gyorgyi and Szentkiralyi, 1973), replacement of these light chains, even with material from another species results in recovery of control.

In the absence of calcium the presence of light chains reduces ATPase activity with full control only when two chains per thick filament are present. Calcium ion reduces ATPase activity when no light chains are associated with myosin but this level of activity is increased four-fold as light chain content increases to two per thick filament (Simmons and Szent-Gyorgyi, 1978). Over the same range of light chain content the force developed by the muscle doubles. The discrepancy between the change in ATPase activity and the change in tension may be due to an increase in the rate of cross bridge formation without a similar change in the number exerting force. This would indicate an increase in the rate of detachment during crossbridging with increased light chain content (Simmons and Szent-Gyorgyi, 1984).

Contractile force developed changes with the calcium content of the solution in which the muscle strip is bathed as defined by the Hill equation where  $\alpha H = 5.5$  (Simmons and Szent-Gyorgyi, 1984). EDTA treated muscle develops tension in the absence of calcium, muscle in this state is described as "desensitised" to the

action of calcium (Simmons and Szent-Gyorgi, 1984) and control is not aided by increasing the magnesium ion concentration. Control of contraction is regained after recombination with regulatory light chains though the form of the force:light chain content relationship is not the same as that for ATPase activity.

No differences in muscle microstructure were observed between *Pecten maximus* and *Placopecten magellanicus* (Millman and Bennett, 1976). In the fast adductor muscle, fibres were elongated in transverse section ( $1 \times 10\mu\text{m}$ ) (Nunzi and Franzini-Armstrong, 1981) with sarcomere and filament lengths similar to those observed in vertebrate striated muscle. Slow muscle contains fibres which are more rounded with cross sections of around  $3 \times 5\mu\text{m}$ . The ratio of thin to thick filaments was around 5.5:1 with an average of 11 thin fibres around each thick one. These myosin fibres form a hexagonal array. The sarcoplasmic reticulum is thought to act as a calcium store in scallop muscle with surface couplings receiving signals from the surface membrane and allowing calcium release (Nunzi and Franzini-Armstrong, 1981). The increased development of the system in fast muscle is presumably to facilitate the more rapid release of calcium into the muscle and allow the entire muscle to contract simultaneously (Marsh *et al*, 1992).

Differences between scallop muscle and vertebrate striated muscle include (From Olson and Marsh, 1993) the absence of a transverse (T-) tubule system in the sarcoplasmic reticulum, uninucleate cells, long sarcomeres ( $3.1\mu\text{m}$  at  $L_0$ ), fibres of only 3% muscle length and electrical coupling between cells via gap junctions.

The effect of having longer sarcomeres, higher actin:myosin ratio and myosin head density could be to increase force generation per myofibril and may compensate for the seasonal reduction in fibril density due to the use of the muscle as a glycogen store (Morrison and Odense, 1968). Force is maximised and shortening velocity reduced by minimising the number of sarcomeres between the load and static point of muscle attachment. The small size and ribbon shape of the muscle cells may compensate for the lack of T-tubules by increasing the cells' surface area to volume ratio. A single myofibril occupies the centre of the cell though glycogen granules and, in some cases, sarcoplasmic reticulum may be inserted between groups of filaments dividing them into submyofibrils (Nunzi and Franzini Armstrong, 1981). These glycogen granules may constitute a large proportion of the cross-sectional area in some parts of the fibre (Nunzi and Franzini Armstrong, 1981). Total dry mass of muscle as a function of shell size is highly variable and relates to the amount of glycogen stored in the adductor. This is in turn related to reproductive state (Cattaneo-Vietti *et al*, unpublished).

The junctions at which muscle cells are connected to each other are similar to the fascia adherentes of myocardial intercalated disks (Nunzi and Franzini Armstrong, 1981; Rall, 1981; Rall, 1982). Nunzi and Franzini-Armstrong (1981) suggested that the increased reliance on connective tissue between and around muscle fibres rather than direct cell to cell junctions could be associated with high levels of force generation. The finding that slow muscle, which has longer myosin filaments, has a more developed system of hemifascia adherentes supports this.

#### 1.2.4 Energetics and costs of swimming

The use of swimming incurs an expense by the scallop that must be met from the energy available to the animal both in terms of the direct costs of muscle use (see below) and in increased investment in muscle growth. When forced to swim to exhaustion once per day for six weeks scallops invested more heavily in their adductor muscle and reduced gonad growth. This effect was greatest at higher water temperature, even with unlimited food (Kleinman et al., 1996).

Scallops minimise costs by detecting predators prior to attack and can discriminate between the predatory and non-predatory starfish found in their ecosystem (Ordzie and Garofalo, 1980; Thomas and Gruffydd, 1971). Following an escape response, or where swimming is less “urgent” e.g. moving into a better habitat, efficiency may be of greater importance than speed. Scallops have various mechanisms to improve the efficiency of their swimming. These include the extremely high energy return of the hinge ligament (Cheng et al., 1996b; DeMont, 1988), passive re-opening of the shell by water flow (Vogel, 1985) and drag reducing shell formations such as riblets (Anderson et al., 1997). Despite these, scallop swimming remains energetically expensive compared to other swimming molluscs such as *Limaria fragilis* and the cephalopods (Baldwin and Opie, 1977; Donovan and Baldwin, 1999; O'Dor and Webber, 1991).

Several studies have examined the metabolism of scallops following exhausting exercise. In scallop muscle, contraction requires the hydrolysis of ATP by myosin ATPase. This is then regenerated by transphosphorylation, in this case by Phospho-l-arginine (equivalent to phosphocreatine in vertebrates). In most animals phosphagen levels are typically only sufficient to meet the needs of initial work (Stryer, 1995), after which ATP must be replenished by other means.

In scallops the energy storage substance used is probably glycogen, it is abundant in scallop muscle and hexokinase activity is low compared to phosphorylase (so glucose use is probably relatively low) (deZwaan et al., 1980).

During anaerobic exercise in scallops the pyruvate released during glycolysis is combined with the arginine from PLA breakdown to produce octopine (Baldwin and Opie, 1977; Grieshaber and Gäde, 1977) and provide the NAD required for further glycolysis. The breakdown of Phospho-l-arginine (PLA) and the formation of octopine is progressive with increasing numbers of muscle contractions. Recovery following exhaustion takes several hours and returns PLA levels to resting levels (Gäde et al., 1978). This recovery could be prevented by denying the animal the ability to respire aerobically (Grieshaber, 1978). The mitochondria of scallop phasic muscle are specifically adapted for maximum enzyme activity under the low pH conditions existing following exercise. (Guderley et al., 1995).

### **1.3 Adaptive trade-offs in performance and energetics**

Studies of the biomechanics and physiology of exercise are extremely numerous. However surprisingly few studies explicitly demonstrate the adaptive benefits of muscle performance. In Garter snakes, higher burst speed and stamina correlate with increased survival a year after testing (Jayne and Bennett, 1990). Similarly, tadpoles that had high burst swimming speed were more likely to survive encounters with garter snakes in experimental mesocosms (Watkins, 1995). The survival of individuals does not necessarily correlate closely with fitness. The optimum strategy for investment in locomotory capacity might result in a higher proportion of the animals sharing that strategy dying.

The strategy could still succeed if, as a result of increased reproductive investment, the survivors were able to be more fecund. This possible where resource partitioning between locomotory and reproductive systems compete. This leads to one reason why absolute performance and fitness are not directly correlated. Increased performance may incur increased physiological costs which might reduce survival in other ways and/or lessen fecundity. It is therefore important to consider both performance and energetics in biomechanical studies.

Throughout the study reference will be made to selective pressures on ecological and physiological trade-offs made by animals. That scallops swim at all represents the first and perhaps most important of these. The scallop invests more heavily in its adductor muscle than other comparable bivalves, both in terms of muscle mass and proportion of fast adductor tissue (Morrison and Odense, 1973). Swimming scallops partition resources to the adductor development and maintenance, using resources that could be used for gonad growth. Trade-off strategies are only maintained through successful reproduction so why does a strategy of reduced gonadal investment persist?

The conclusion would then be that at the optimum position of trade-off the scallops adopting that strategy are more successful as their investment in adductor growth enables increased resource acquisition (by moving to better habitat) and reduced predation. If these advantages outweigh the marginal costs of adductor investment then animals end up with larger gonads and/or reproduce more times than would be possible if they remained stationary and partitioned a greater proportion of resources directly to the gonad. This also

implies that the animal will be most successful if it minimises the costs of swimming while maximising its improved survival chances.

#### **1.4 Temperature**

The effectiveness of scallop swimming in response to predators and the performances and energetic consequences involved may be strongly affected by environmental and seasonal changes of which the best described is temperature. Due to their wide distribution scallops are found in regions of widely differing thermal regimes, both in terms of absolute temperature and in its variation over a range of temporal scales.

As aquatic ectotherms their physiological rates are strongly affected by the temperature of the surrounding water as well as the indirect effects of temperature on its viscosity (Johnson et al., 1998; Podolsky and Emlet, 1993), density and oxygen content (Regoli et al., 1997; Viarengo et al., 1995).

The effect of a given temperature on a biological system may differ according to the timescale of the change leading to that temperature. Acute temperature changes and their effects occur quickly (from immediate changes to hours or days). Slower, seasonal changes may allow the animal to acclimatise to the change, modifying its behaviour or physiology and these changes may be associated with a variety of other cues such as day length. Where the animal is allowed to adjust to a new temperature in the laboratory this is termed acclimation. Longer-term changes may result in sufficient changes in selective pressure for the population to adapt by evolution to improve its fitness at the new temperature. Each of these cases may apply to scallops according to their location, latitude, and depth of occurrence.

#### 1.4.1 Acute temperature change

The effects of temperature on activity capacity have been documented in a range of terrestrial, freshwater and marine animal groups including frogs (Navas et al., 1999; Peplowski and Marsh, 1997), fish (Rome et al., 1984; Swank and Rome, 2000), and lizards (Hertz et al., 1982; Marsh, 1990). Lowered temperature typically causes reductions in jumping, running or swimming performance. At their most extreme temperature changes can kill (Somero and DeVries, 1967; van Dijk et al., 1999) or sufficiently disable an animal to make it vulnerable to predators (Yocom and Edsall, 1974).

Few studies of the effects of temperature on scallop swimming exist. Marsh (1990) reported changes in the form of the clap cycle in *Argopecten irradians* at 10 and 20°C. Temperature strongly affected the duration of the clap cycle due to changes in relaxation rate rather than changes to the shortening or lengthening phases themselves (Marsh, 1990). In contrast, studies of free swimming *Placopecten magellanicus* showed changes in the duration of shortening, as well as in clap period and swimming velocity over the temperature range 3.4-11.2°C (Dadswell and Weihs, 1990).

Olson and Marsh's (1993) study provides the only existing measurements of the direct effects of temperature on scallop muscle. This *in vitro* study showed that, in common with most muscle preparations, intrinsic shortening velocity was affected by temperature though isometric force production was not. These properties appear to conflict with the behaviour observed in this species *in vivo* which showed no change in shortening velocity with temperature.

Increases in intrinsic shortening velocity without changes to the form of the force-velocity relationship allow either the same force to be exerted at a higher speed or higher force at the same speed (Marsh, 1990). Both scenarios result in increased power output (force.distance.time<sup>-1</sup>). The conditions under which force velocity relationships are determined are unrealistic with the workloop technique giving better information on the likely *in vivo* performance of the muscle. Such experiments with muscle dissected from fish (Altringham and Block, 1997), frog (Navas et al., 1999) and lizards (Swoap et al., 1993) have shown power output increases with increases in temperature

The significance of short-term temperature changes to the survival of the Sea Scallop *Placopecten magellanicus* has been demonstrated. Acute temperature changes are known to modify interactions between scallops and their predators with differential changes in the predation rates of crabs and seastars (Barbeau and Scheibling, 1994c).

#### *1.4.2 Temperature acclimation and acclimatisation*

The effects of temperature on metabolism and activity capacity are not fixed and may vary seasonally or following a period of acclimation in the laboratory. Where capacity acclimatisation returns physiological rates or performance measures to values recorded before the temperature change the animal is said to have perfectly compensated for the change (Precht et al., 1973).

Numerous studies of the responses to periods of temperature change exist in groups as diverse as fish (Beddow et al., 1995; Heap and Goldspink, 1986; Johnston et al., 1995), crabs (Pearson et al., 1999), and bacteria (Hoffman, 1995). Acclimation may result in changes in locomotory performance and

associated muscle, enzyme, and neurological systems (Heap and Goldspink, 1986; Johnson and Johnston, 1991; Johnston et al., 1990; Pearson et al., 1999). While these studies have shown changes following acclimation the selective advantages of the responses obtained have not always been clear.

Acclimatisation responses are most likely where they are cheap (energetically), the environment experienced by the animal is variable and the selective pressure on the trait of interest is equally strong in all (equally common) environmental conditions. An environmental cue, such as a change in photoperiod, must be present which correlates to the selective advantage of the acclimatisation response (Scheiner, 1993).

For these reasons the ability to acclimatise to temperature is less common in stenothermic animals (Heap et al., 1985; van Dijk et al., 1999), which lack the variability of environment or many terrestrial vertebrates (Else and Bennett, 1987) which may lack cues which reliably correlate to future conditions due to the greater variability of air temperature. A similar situation exists in certain shallow-water marine fish that experience large tidal variations in water temperature (Johnson and Bennett, 1995a).

Where the criteria of a strongly signalled, highly variable environment with constant selective pressure do not exist a generalist strategy may be favoured (Scheiner, 1993). An effective reproductive strategy for an unpredictable but variable environment may be "shotgun generalism". Large numbers of offspring with varying activity capacities (Jayne and Bennett, 1990) presumably also show concurrent differences in their investment in muscle mass.

Even where changes in performance as a result of acclimation are shown demonstrating improved fitness as a result of acclimation is difficult (Bennett, 1990). Improved swimming performance may result in increased prey-capture success in laboratory trials (Beddow et al., 1995). Whether the extra food gained outweighs the metabolic costs of activity and acclimation combined is not demonstrated. Even more difficult to justify would be to determine fitness based on escape performance in fish, without knowledge of the speeds or accelerations required to escape (unknown) predators. If the low-performance escape response is adequate to survive 90% of encounters then the additional performance gained by acclimation may be of negligible benefit. Escape performance in fish may be a correlate to fitness but more ecological information is required before its importance can be determined (Temple and Johnston, 1998).

Experiments in model populations of *Escherichia coli* (Bennett and Lenski, 1997; Leroi et al., 1994) and *Drosophila* (Gibbs et al., 1998) have failed to demonstrate competitive advantages through increased fitness at their acclimation temperature. Such species have the advantage of short generation times, animals with differing thermal histories can be easily labelled, and actual fitness can be determined rather than any chosen correlate. One of the reasons why acclimation responses do not always confer the expected advantages may be that the costs associated either with the response or survival at the acclimation temperature reduce the animal's competitiveness (Hoffman, 1995).

The costs of acclimation are poorly understood and difficult to measure directly. Carp are normally extremely good at acclimating to temperature with reversible changes in myofibrillar ATPase activity (Heap et al., 1985) associated with

changes in the locomotory ability of the fish (Heap and Goldspink, 1986). These changes do not occur if the fish is starved during acclimation and are reversed if a fish that was fed during acclimation is then starved (Heap et al., 1986). This implies that initial acclimation and its maintenance in carp has an energetic cost which must be met from the animal's food budget.

The potential costs depend on the mechanism used to modify enzyme activity. These typically include changes to enzyme systems by the synthesis of replacement ATPase isoforms and changes in cellular environment. As there appears to be a strong negative relationship between the thermal stability and metabolic activity of the enzymes involved with exercise and recovery the net effect of acclimatory changes is to maintain the enzyme "population" at the lowest reasonable stability for the expected range of environmental temperatures. In order to maintain the closest possible match between enzyme properties and environmental temperature, ATPase activity changes in fish are associated with the turnover of regulatory chains, rather than the larger and more slowly replaced myosin fibres, which are the source of variability in evolutionary differences in myofibrillar ATPase activity. The direct costs of acclimation, in terms of any up-regulation of protein turn-over are not known and difficult to discriminate from the direct effects of temperature on metabolism.

#### *1.4.3 Seasons are not just temperature changes*

Where temperature changes are caused by seasonal differences in solar irradiance the situation is considerably more complex than in the laboratory acclimation. The physiology of an animal may undergo variations throughout the year which, although they may be affected by temperature change (MacDonald and Thompson, 1986; Navarro and Thompson, 1995) are not

driven by it. In temperate latitudes the changes in solar radiation input that affect water temperature also result in dramatic alterations in primary production (Lampitt et al., 2000). These changes may affect the timing of gonad maturity and the optimum timing of gamete release. This is in turn governed by the type of any larval phase used (i.e. whether the larvae need to feed themselves or will be endowed with an adequate energy store by the parent). In polar waters and areas of upwelling temperature and primary productivity may be weakly related or completely decoupled. This may lead to misleading interpretations of the effect of any seasonal temperature change on physiological processes.

In scallops the interacting effects of reproductive cycles and water temperature can affect the properties of muscle mitochondria resulting in changes in oxidative capacity and recovery rate from exercise (Boadas et al., 1997), though not in apparent swimming performance itself. This is perhaps surprising as muscle mass may vary seasonally as energy is transferred from the adductor to the gonad (Allison, 1994).

#### *1.4.4 Temperature adaptation*

The presence of animals throughout the world's oceans has required groups to evolve tolerance to the challenges of different thermal environments. Challenges of temperature may involve life at the edges of the envelope but, as significantly, the ability to survive changes in temperature.

The "extreme" environments of the polar seas, tropics and deep ocean are regions of relative thermal stability. For the majority of Antarctic fauna the extreme cold is not harmful in itself because marine invertebrates are iso- or hyperosmotic with seawater and therefore do not freeze unless the water around

them does (Clarke, 1983). The major impact of very low temperatures on these animals may be as much physical (iceberg impact and anchor ice) as biochemical. Fish however have evolved antifreezes (De Vries, 1982), an example of “Resistance Adaptation”.

Much interest has been taken in the ability of animals to compensate for their environmental temperature. “Capacity Adaptations” have been investigated in fish muscle characteristics (Johnston and Altringham, 1985), locomotion (Johnston et al., 1991b; Wakeling and Johnston, 1998), muscle oxidative capacity (Johnston et al., 1998) and recovery from exercise (Hardewig et al., 1998). Despite the apparent adaptive advantages Antarctic fish do not appear to show compensation for muscle performance or glycolytic capacity. Chapters 3 and 4 of this study investigate the muscular performances and muscle physiology of the Antarctic Scallop, *Adamussium colbecki*. Studies are less common in invertebrates but have included swimming in the Antarctic scallop (Ansell et al., 1998), and oxygen consumption by isopods (Whiteley et al., 1996).

It has been difficult to conclusively demonstrate temperature adaptation due to the inherent differences between species which might have nothing to do with differences in their normal environmental temperature. Finding independent “replicate” species in each temperature group whose phylogeny is known is a requirement (Bennett, 1990; Garland and Adolph, 1994), which may be difficult to achieve. In scallops the divergence of individual populations across environmental gradients may result in measurable changes in gene frequencies (Krause et al., 1994) and morphology (Wilbur and Gaffney, 1997) though even here the selective pressures leading to any changes are not clear.

Given the difficulty in comparing species across latitudinal clines, and the limited number of species available during this study's "within-species" experiments have been undertaken wherever possible and differences in the responses of individual species compared on this basis.

## **Chapter 2.**

# **MODELLING MUSCLE PERFORMANCE DURING SWIMMING IN THE EUROPEAN QUEEN SCALLOP: EFFECTS OF ACUTE AND SEASONAL TEMPERATURE CHANGE.**

### ***2.1 Introduction***

Scallops swim using a form of jet propulsion, the simplicity of which has attracted researchers seeking to study a complete locomotory system. Swimming is powered by a single adductor muscle, acting in opposition to an elastic hinge ligament. When the muscle is activated the shell is pulled closed and water is ejected through jets situated close to the hinge. The animal accelerates forwards (gape first) taking successive "bites" out of the water. The general form of swimming is well described for a number of temperate (Manuel and Dadswell, 1990; Moore and Trueman, 1971), tropical (Joll, 1989; Morton, 1980) and polar (Ansell et al., 1998) species.

Several mechanical aspects of this behaviour have been previously examined including the hydrodynamics of the shells (Anderson et al., 1997; Milward and Whyte, 1991; Thorburn and Gruffydd, 1979b; Vogel, 1985), the properties of the hinge ligament (Alexander, 1965; DeMont, 1988) and the activation of contraction (Stephens and Boyle, 1978). The muscle performances involved in scallop swimming have been investigated using *in vitro* muscle preparations (Marsh and Olson, 1994; Olson and Marsh, 1993), intramantle pressure recordings (Marsh et al., 1992), and mathematical modelling (Cheng et al., 1996a; Cheng and Demont, 1996a; Cheng and Demont, 1996b; Cheng and Demont, 1997).

Temperature may directly affect the muscle performance of the adductor muscle resulting in changes in fibre shortening velocity *in vitro* (Olson and Marsh, 1993), swimming speed (Manuel and Dadswell, 1990) and in the form of the clap cycle (Marsh, 1990). However the effects of temperature on muscle performance *in vivo* have not been studied and the effect of thermal history on swimming performance is unknown.

Environmental temperature has a major effect on locomotory performance and behaviour in many ectothermic animals including lizards (Hertz et al., 1982), frogs (Peplowski and Marsh, 1997) and fish (Beddow et al., 1995; Johnson et al., 1998; Rome et al., 1990). Using an integrated approach at different levels of organisation the origins of these changes can sometimes be traced to the effects of temperature on muscle (Navas et al., 1999) and enzyme systems (Johnson and Bennett, 1995b). The relationship between temperature, muscle characteristics and whole-body performance is, however, not simple (Bennett et al., 1989; Navas et al., 1999; Peplowski and Marsh, 1997).

Furthermore, the effects of temperature on activity are not fixed, even within a species or individual, and may differ following a period of temperature acclimation. Changes in whole-body performance or locomotory physiology following acclimation have been shown in numerous species including temperate fish (Heap and Goldspink, 1986; Johnson and Johnston, 1991; Johnston et al., 1990), and crabs (Pearson et al., 1999). Acclimation responses are a labile form of phenotypic plasticity. Such traits are most likely if the changes required are energetically cheap, the environment is variable, and selection favours plasticity equally strongly amongst equally common conditions (Scheiner, 1993). A cue must be present that correlates to the strength of the

selective advantage of plasticity. If these conditions do not apply then a generalist strategy is favoured (Scheiner, 1993). Examples of species which show little or no change with acclimation include Antarctic fish (Hardewig et al., 1999), which are subject to very small temperature fluctuations, and salamanders, which lack a reliable cue (Else and Bennett, 1987).

The European Queen Scallop, *Aequipecten opercularis* may meet the criteria for the evolution of an acclimatory response. The environment inhabited by the species is variable temporally with the seasonal changes being both predictable and associated with a strong photoperiod cue. In *Placopecten magellanicus*, a scallop with similar predators and temperature range to the study species, escape response performance appears to be important in predator-prey interactions with the effectiveness of escapes depending on environmental temperature (Barbeau and Scheibling, 1994b; Barbeau and Scheibling, 1994c). It seems likely then that both escape performance and its relationship to water temperature may be subject to selective pressure. While high performance during escape responses may be adaptive there is little evidence for the benefits of high performance during the steady swimming that has been the focus of previous studies (Cheng and DeMont, 1996; Marsh et al., 1992).

In the present study swimming behaviour has been analysed during the critical period immediately following first contact between the scallop and its predator. The effects of acute temperature change and acclimatisation state on behaviour were determined. A simple biomechanical model was used to estimate the muscle performances required to support the observed behaviour.

## 2.2 Methods

### 2.2.1 Animals

24 *Aequipecten opercularis* (mean shell height 63.6mm, sd=3.0mm) were obtained from the University of the Highland and Islands aquaculture facility Ardtoe, Scotland in November 1997. The scallops were allocated to one of 3 groups of the same average body size and acclimated to  $5\pm 0.4^{\circ}\text{C}$ ,  $10\pm 0.6^{\circ}\text{C}$  and  $15\pm 0.6^{\circ}\text{C}$  (Mean and range) in temperature controlled recirculating aquaria (Grants Ltd., Cambridge) for 6 weeks. During acclimation and experimentation the animals were fed *ad libitum* on cultured algae (*Isochrysis galbana*, (Lu and Blake, 1996)) and a 12h light:dark regime was maintained. The acclimation group to be tested was rotated daily and experiments were completed within 3 weeks of the end of acclimation.

A further 300 *Aequipecten opercularis* were obtained from Loch Fyne Seafarms in November 1999 and February 2000. Water temperatures at the Seafarms site for the month prior to collection were determined as  $13\pm 3^{\circ}\text{C}$  and  $8\pm 2^{\circ}\text{C}$  (mean $\pm$ range) respectively using temperature loggers deployed at the same depth (TinyTalk loggers, RS Electronics, UK). Scallops were maintained at their mean monthly temperature ( $\pm 0.3^{\circ}\text{C}$ ) in a purpose built temperature controlled tank (3m x 1m x 0.3m, length x width x height) under a 12h light:dark regime for 48h before beginning experiments. Filming was completed within 2 weeks of the arrival of the animals in the laboratory.

## 2.2.2 Analysis of swimming behaviour

### Filming

Scallop escape responses were recorded on video at 200 or 250 frames per second (NAC 400 and NAC HSV500c<sup>3</sup> respectively (NAC Inc., Japan)). The camera was mounted on a tripod at least 3m from the swim tank in order to minimise perspective error. The tank was illuminated by pair of 100W spotlights. Swimming was filmed in a purpose built tank (80x40x40cm, length x width x height) with a mirror mounted above the bottom at 45° to the line of sight of the camera, z' (fig. 1). The mirror allowed simultaneous top and side views of the tank. Tank temperature was controlled ( $\pm 0.2^{\circ}\text{C}$ ) by a heat exchanger (Grants, Cambridge). A raised glass stage with an attached reference length bar allowed clear views of the scallop during stimulation. The stage was placed so that the animal was within 10cm of the tank wall nearest the camera in order to minimise "back-scatter" due to particles in the water and maximise the amount of light reaching the animal from the spotlights. A submerged pump and the action of the aeration system ensured that no temperature microclimates existed within the tank. Where present, the movement of suspended sediment/detritus particles past the stage was used to determine water flow. These values did not exceed  $0.05\text{m}\cdot\text{s}^{-1}$ .

### Experimental protocol

24h before filming 6 points on each scallop (fig. 1) were marked using foil patches in order to increase the consistency with which those sites were located during digitising. Animals were transferred into the swim tank and allowed to rest for a minimum of 1h before the first escape response was stimulated using a dissected starfish (*Asterias rubens*) limb attached to a glass rod. Contact was

made at the rear (hinge edge) of the scallop between the tube feet of the starfish and the sensory tentacles of the scallop mantle. This starfish species is a known predator of *Aequipecten* (Thomas and Gruffydd, 1971) and therefore provides an ecologically valid stimulus for eliciting escape responses. There was minimal disturbance of the water around the animal and no force was exerted on the body of the scallop itself. Animals were filmed in rotation with a minimum of 30min between stimulations.

For each stimulation the animal ID number, tank temperature ( $\pm 0.1^{\circ}\text{C}$ ), stimulus, and type of response was recorded. A counter was inscribed onto each frame of the tape during recording. In addition to the stimuli described above responses resulting from handling or occurring spontaneously were recorded. Responses were categorised as "Claps" - animal rapidly closes shell with little or no lateral movement, "Jumps" - animal displaced laterally due to ejection of a jet of water, or "Swims" - animal displaced laterally and vertically making 1 or more claps while above the bottom. This classification was similar to that used by Thomas (1971). Only "swims" were analysed.

Animals acclimated in the laboratory were swum only within  $0.5^{\circ}\text{C}$  of their acclimation temperature. Field acclimated animals were swum anywhere within the temperature range recorded at the sample site for the month prior to sampling (Autumn,  $8.7\text{-}16^{\circ}\text{C}$ , mean  $12.5^{\circ}\text{C}$ , Winter  $6\text{-}12.6^{\circ}\text{C}$ , mean  $9.7^{\circ}\text{C}$ ). Swim tank temperatures were altered in  $1^{\circ}\text{C}$  increments with stimulation to swim only given following a minimum of 1h at the new temperature.

### *2.2.3 Morphological measurements*

Following filming, the scallops were dissected and a range of morphological measurements was taken, including: whole body and shell wet masses, shell height, length and depth (left and right valve), muscle mass and cross sectional area (shell open and closed), ligament depth and width. Mantle depth and hinge width was required for the calculations of water intake/jetting. The distances from the muscle scars to the anterior and left margins of the upper valve were measured in the x and z directions respectively. This enabled the anterior-posterior and lateral obliqueness of the adductor to be calculated. Measured and static calculated parameters are shown in Appendix A, fig. A1. All variables used are also described in the glossary (Table A1). The relationship between each variable and shell height was determined. These were significant ( $p < 0.05$ ,  $RSq > 0.6$ ,  $df = 32$ ) in all cases.

### *2.2.4 Hinge ligament properties*

Stress-strain curves were obtained for the hinge ligament. The lower valves of 3 live scallops were glued to round plastic plates. Once the glue had set the animals were rapidly eviscerated in water so that all soft tissues were removed. The shell was entirely submerged in a shallow dish of cooled seawater placed on a balance (Mettler Instruments, High Wycombe). The lower valve was kept stationary while the upper valve was pushed downwards in 2mm increments by the wooden end of a seeker attached to a micromanipulator. The shell was then allowed to open in 2mm increments. The mass measured by the balance was read off for each step ( $\pm 0.1g$ ).

Experiments were carried out between 5 and 25°C, temperature rose by up to 0.3°C during each experiment. Force (N) was calculated from the mass

recorded on the balance and plotted against distance moved by the valve (m). The area of the hysteresis loops formed equals the energy lost during the closing and opening cycle (J).

### *2.2.5 Analysis of filming*

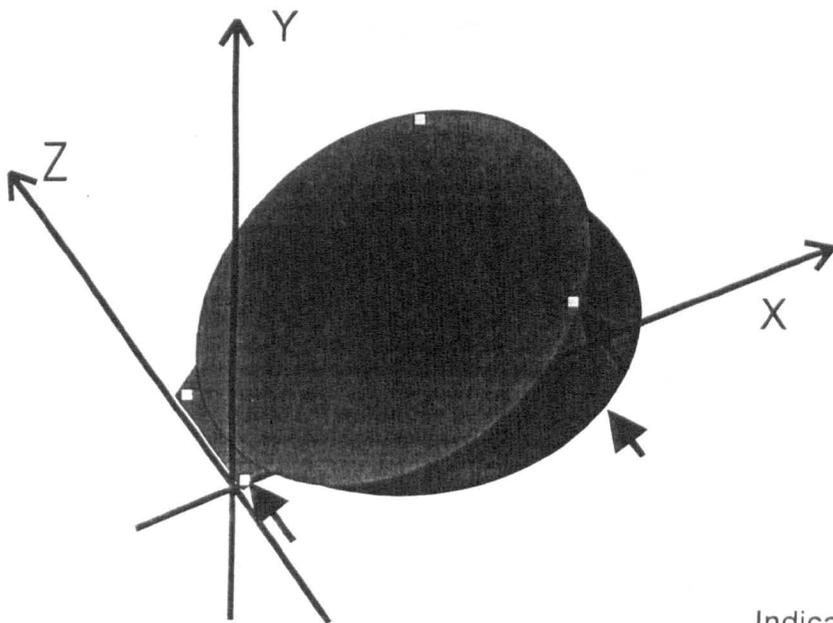
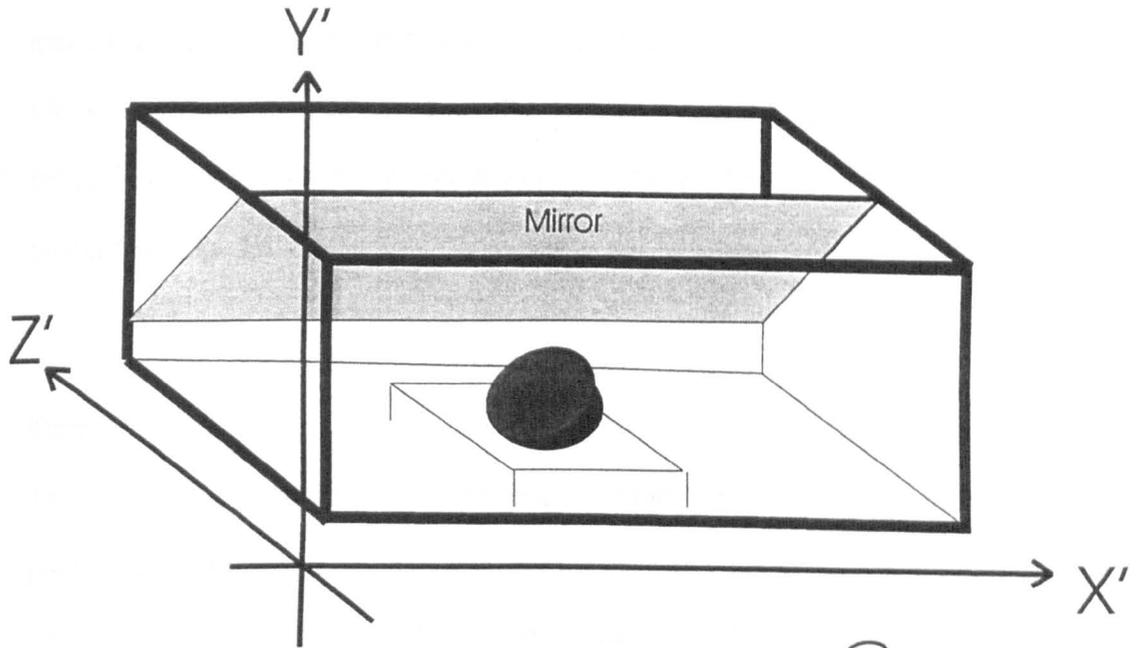
Video recordings were played frame-by-frame to a PC (Gateway 2000 G6-266) with a video capture board (Hauppauge Win/TV). The co-ordinates of the marked points on both valves were selected by hand and recorded by a purpose-designed package obtained from Dunstaffnage Marine Laboratory (Visual Basic 4, Microsoft). The spatial resolution of the data was limited by the image analysis program to 5 pixels.mm<sup>-1</sup> (average of 300 pixels.body length<sup>-1</sup>). On-screen analysis did not allow mis-position of digitising cursor due to parrallax as the cursor was on the same plane as the point being digitised. Jet areas (*ajet*) during swimming were measured from close-up videos (x4 on-screen magnification) with the animal rotated such that the jet opening was aligned across the film plane (*x',y'*).

### Data processing

The co-ordinate data for each swim sequence were broken down into individual clap cycles for analysis in Mathematica (Wolfram Research). Only the first clap cycle following stimulation was analysed. A clap cycle started from rest and consisted of the animal closing and then re-opening its shell. For each cycle the animal's shell height, the water temperature and salinity (for calculation of water density) were manually entered into the program. At the start of each sequence a point at the extreme front and rear of the animal and the position of the marked points were digitised with the animal perpendicular to the line of sight of

Fig. 1, Filming and co-ordinate system. a) A mirror allowed top and side views of the animals. b) A fixed co-ordinate system ( $x',y',z'$ ) describes positions within the tank while the instantaneous co-ordinate system ( $x,y,z$ ) describes scallop orientation. The line of sight of the camera is the  $z'$  direction while the animal swum in the  $x'$  direction. The  $x,z$  plane is the commissural plane of the animal. The marked points on the scallop shell were used to improve digitising accuracy.

Fig.1 Filming tank setup and co-ordinate systems



Indicates position of digitising points. These were repeated on the opposite side

the camera. The ends of a measured ( $\pm 0.1\text{mm}$ ) reference bar within the tank were also digitised. Animal orientation was described with reference to an instantaneous co-ordinate system ( $x,y,z$ ) attached to the animal with the  $x,z$  plane the commissural plane of the animal. A fixed system within the tank ( $x',y'z'$ ) described animal position with the film plane of the camera in the  $x',y'$  plane (See fig. 1).

### Initial smoothing

The  $x$  and  $y$  co-ordinates of each marker point were smoothed using moving, piecewise cubic regression. The centre of each piece was recorded as the smoothed  $x$  or  $y$  position. Whole animal velocity and acceleration in the  $x$  and  $y$  directions was determined by differentiation of  $x$  and  $y$  co-ordinates of the hinge with respect to time. Resultant velocity ( $Ub$ ) and acceleration ( $Xb_{total}$ ) were then calculated. Tangential acceleration ( $Xb_{tang}$ ) was calculated from the differentiation of resultant velocity with respect to time.

The smooth width ( $n$ ) chosen greatly affected the results obtained especially after differentiation to estimate rate values. Digitising inaccuracies produce apparent rapid changes in gape values; low values of  $n$  cause a drop in the estimates of work. As  $n$  increased a plateau was achieved after the removal of digitising errors before  $n$  became too great and began to cut out "real" data. Additional points were added at the start and end of each cycle by copying the co-ordinates of the first and last frame 33 times. The number of extra points included in the analysis was  $(n-1)/2$  before and after the sequence. This prevented the start and end of the real sequence data from being chopped off by the smoothing process. The standard error of the real data around the smoothed positions were calculated for each processed sequence. At the

correct  $n$  this error was similar to that obtained from repeatedly digitising stationary positions on the screen (approximately 0.007 body lengths).

### Errors of filming

Errors may appear during filming and kinematic analysis due to digitising inaccuracy, perspective error, yaw and roll. Yaw and roll were defined as deviation of  $x$  from  $x'$  or  $z$  from  $z'$  respectively (See fig 1). Yaw was detected by measuring the projected distance between the hinge and gape marker points in each frame. Depending on the direction of yaw velocity and acceleration may be over or underestimated. Velocity or acceleration of the hinge due to rotation of the animal were subtracted from raw values. Velocity and acceleration data from sequences in which rotational velocity exceeded 5% of the total measured were not used.

Only a small amount of roll ( $18^\circ$ ) was required to significantly change ( $>5\%$ ) the apparent gape recorded. Good images showing top and side views simultaneously were rare so sequences showing roll were used only for the determination of clap period.

These problems were minimised by placement of the camera a minimum of 3m from the swim tank. At 3m a range difference of 150mm between the scale bar and the object being measured is necessary for a significant (5%) change in measured size. Initial placement of the animal was within 40mm of the scale bar. Similarly, the object must move 150mm towards or away from the camera during filming for a 5% difference in measured size, gape etc. As the entire field of view of the camera was limited to 200mm, this would require the animal to swim at an angle of at least  $36.8^\circ$  from  $x'$  (fig 1).

Lens height above the floor was adjusted to match the height of the centre of the field of view. Remaining errors were corrected for by digitising known lengths in the  $x'$  and  $y'$  directions in the camera's field of view. The resulting aspect ratios were used within the modelling process. There was a mean aspect ratio of 0.87. There was no parallax error during digitising as the cursor was on the same plane as the points being digitised.

Walker (1998) criticised the use of double-differentiated moving regressions as a method for determining velocity and acceleration in kinematic studies. At similar frame rates and magnifications to those used in the present study this technique produced good estimates of both velocity and acceleration in Walker's (1998) study. The double cubic regression used in here gives a closer fit to the data than the quadratic regressions used by Walker (1998). In addition, the smooth width was objectively optimised to the data in the present study rather than being chosen arbitrarily.

### Whole animal performance

Animal mass ( $M_b$ ) was estimated from its relationship with shell height ( $L_{sh}$ ), added mass of water and the instantaneous enclosed mass of water were calculated from the jet cavity data and the volume of the shell (See appendix, Fig A1 and table A1).

The total net force exerted on the animal by thrust and drag ( $T$ ) was calculated from the product of the animal's mass (+associated water) and its tangential acceleration (Daniel, 1984). Internal water mass was calculated from the volume of the shells and the instantaneous volume of the jet cavity (see

Appendix A). The external added mass of water of was calculated according to the formula for flat discs (Cheng, 1996). Total acceleration was greater than tangential acceleration by a factor of up to 37% but there was no significant difference between experimental groups in the ratio of total to tangential acceleration.

### Modelling

A simple model of swimming was developed to calculate muscle shortening velocity, hydrodynamic power output and force production from the kinematic data. A full explanation of the model's mathematics and assumptions can be found in Appendix A). Jet flow and jet area measured from video recordings of escape responses (n=7, representing the entire size range studied) were used to calculate the velocity of the jet and its power output (Appendix A, Table A1). The force production of the adductor was calculated from its power output and shortening velocity.

Not all animals produced a analysable response at every temperature and in several cases sequences were not suitable for all analyses. Where more than one suitable sequence was obtained for an individual at a single test temperature all were processed and the sequence with the highest whole body thrust ( $T$ ) was used in subsequent analyses.

#### *2.2.6 Determination of Actomyosin ATPase activity*

##### Actomyosin preparation.

12 Animals of similar mean shell height from each acclimatisation group were sampled 48h after arrival in the laboratory. The entire fast adductor muscle was

rapidly removed and separated from the associated soft tissues and slow adductor muscle. The fast muscle was split into 4 pieces and placed in ice-cold glycerol buffer (50% glycerol, 20mM NaCl, 2.5mM Phosphate, 0.5mM MgCl<sub>2</sub>, 0.05mM EDTA, 1.5mM NaN<sub>3</sub> and 0.5mM PMSF. pH 7 at 20°C). After 30min at 0°C the glycerinated muscle samples were placed in a freezer at -20°C. Storage in glycerol buffer at low temperature has been shown to preserve enzyme activity in scallop muscle for months or years (Szent-Gyorgyi, 1975).

Muscle tissue was macerated on ice using scissors and homogenised on ice in 10 vol of 100mM KCl, 20mM Imidazole, 0.01% Triton X and 0.001% Antifoam A (pH 7.2 at 20°C) for 3 x 20s using a Polyton blender (Kinetica GMBH, Switzerland). The homogenate was centrifuged 3 times for 5 min at 5000g. Following the first two centrifugations the pellet was resuspended in 4 vol 100mM KCl and 20mM Imidazole (pH 7.2). After the 3rd centrifugation the pellet was resuspended in approximately 3 volumes of 67.5mM KCl, 7.5mM MgCl<sub>2</sub> and 1.5mM DTT (pH 6.4 at 20°C) and mixed by shaking on ice for 10 minutes. The resulting mixture was centrifuged for 10min at 10,000g and the resulting supernatant was added to 12 volumes of ice cold deionised water and left on ice for 30min. A further centrifugation for 10min at 10,000g pelleted out the precipitated actomyosin. This pellet was resuspended in 40mM Imidazole, 25mM KCl, 7mM MgCl<sub>2</sub> and 5mM CaCl<sub>2</sub>. pH 7.4 at 20°C (assay medium). The protein concentration was determined using Microprotein-PR (Sigma Diagnostics) and adjusted to 0.1mg.ml<sup>-1</sup> using assay medium.

#### ATPase activity assay procedure

ATPase activity was determined by incubation of actomyosin at 15, 10 and 5°C. pH was allowed to vary freely with the pK of imidazole ( $\Delta\text{pH}/\Delta\text{T} = -0.0229$  per °C).

The reaction was started by the addition of 50ul ATP (50mM) and stopped by the addition of 100ul TCA (20% w/v) after 10, 20 or 30 minutes respectively. For each assay triplicate incubations and controls without ATP and with denatured protein were performed. After stopping the tubes were placed on ice to prevent further ATP breakdown. Protein was precipitated out by centrifugation at 5000g for 20min and the Inorganic phosphorous content determined using the Fiske and Subarrow (1925) method (Sigma Diagnostics).

### *2.2.7 SDS-PAGE electrophoresis*

Actomyosin was dissolved in 60 mM Tris-HCl pH 6.8, 2% (w/v) SDS, 10% (v/v) glycerol, 1 mM DTT and 0.001% (w/v) Bromophenol Blue to give a final protein concentration of 2 mg/ml. The samples were denatured by heating to 100°C for 3 minutes. Any solid particles were removed by centrifugation at 5000g for 10 min. Mini-slab (70x80x0.75mm) SDS-Page gels were prepared in BioRad Mini-Protean II electrophoresis cells (Bio-Rad Laboratories, UK). All equipment was thoroughly cleaned in Decon 90 (Sigma, UK), rinsed in filtered, distilled water and air dried prior to use. In addition gel plates were wiped clean with 70% ethanol immediately before assembling the cells.

Stacking gel: 0.125 M Tris-HCl pH 6.8, 4% total acrylamide of which 2.67% was BIS crosslinker and 0.1% SDS. Polymerising agents were 1 ml of TEMED and 0.5 mg ammonium persulphate per 1 ml of gel. Resolving gel: 0.375M Tris-HCl pH 8.8, and a varying amount of total acrylamide depending on requirements. This ranged from 10% to 15% of which 2.67% was BIS crosslinker, and 0.1% SDS. Again polymerising agents were 1ml of TEMED and 0.5mg ammonium persulphate per ml of gel. Gel solutions were degassed using a vacuum pump immediately prior to the addition of TEMED, Ammonium Persulphate and SDS.

Resolving gels were overlaid with 40µl of Butan-1-ol to give a level surface to the gel by preventing meniscus formation. A well-forming comb was inserted into stacking gels immediately following loading. Gels that set in less than 10min or had not set after 60min were discarded. Wells were filled with electrode buffer (25 mM Tris, 0.192 M glycine, 0.1 % SDS pH 8.3 at 20 °C). Running Conditions: the gels were run at 50 volts until the samples had entered the stacking gel. The voltage was then increased to 200 volts until the bromophenol blue dye front reached the end of the gel. Gels were then fixed in 12% TCA and 3% sulphosalicylic acid for 1h, rinsed twice in Mili-Q water and once with Mili-Q and 3% Phosphoric Acid. The gels were stained in Coomassie Blue G250 colloidal stain (2% Phosphoric Acid, 15% (w/v) Ammonium Sulphate, 0,01% (w/v) Coomassie Blue G-250 and 20% Methanol) for 12h.

### Peptide mapping

Unfixed gels were rapidly stained in Coomassie Blue R-250. The myosin heavy chain (MHC) bands were cut out and inserted into the wells of 1mm thick SDS-PAGE gels. These gels were identical to the 1st dimension gels other than in their greater thickness and the increased depth (20mm) of the stacking gel. The width of the wells in the stacking gel was slightly greater in order to allow easy positioning of the myosin band in the bottom of the well.

The proteolytic enzymes used were Chymotrypsin (bovine pancreas), Papain (Papaya latex), Endoprotease Glu-c (*Staphylococcus aureus* V8) and Clostripain (*Clostripain histolyticum*). The enzyme required for the digest was dissolved in 60 mM Tris-HCl pH 6.8, 5% glycerol, 1 mM DTT and 0.001% BPB. 5µl of overlay buffer and 5-15µl of proteolytic enzyme was injected into each well. The amount of enzyme varied according to the enzyme used. The gels were initially

run at 50V with a current limit of 50mA for 30 min, by which time the bromophenol blue front had entered the gel. The power was then switched off for 12 h to allow the digestion of the MHCs. The power was restored until the dye front had entered the resolving gel. The voltage was then increased to 200v until the dye front ran off the bottom of the gel. The resulting peptride maps were silver stained using a Plus One Silver Stain Kit (Pharmacia Biotech Ltd.). All gels were analysed for differences in band density and position using Phoretix 1D Advanced software (NonLinear Dynamics Ltd, UK).

## 2.3 Results

### 2.3.1 Ligament stress-strain properties

The relationship between ligament force production and gape was determined for opening and closing of the shell. The area of the hysteresis loops formed is proportional to the loss of energy to heat within the hinge during one clap cycle. There was no significant relationship between temperature and the peak ligament stress (mean  $366\text{kN}\cdot\text{m}^{-2}$ ) or the energy loss per cycle (mean  $0.005\text{J}$ ).

### 2.3.2 Behaviour

Above  $5^{\circ}\text{C}$  the proportion of swimming responses to total responses to stimulation was 0.95 ( $n=311$ ) and did not vary with temperature. Below  $5^{\circ}\text{C}$  the proportion of swimming responses fell to 0.57 ( $n=26$ ). There was no difference in response type between animals swimming above or below their acclimation temperature.

### 2.3.3 Biomechanics of swimming in acclimated animals to different temperatures

Clap cycles consisted of a short period of rapid shell closing (adduction), reducing in rate until reaching zero velocity, after which re-opening was rapid and returned the gape to the length prior to the beginning of the cycle. Temperature strongly affected the first clap cycle during simulated escape responses in animals swimming at their acclimation temperature. At higher temperatures shell closure was completed earlier without an apparent change in the rate of reopening. This resulted in a change in both the form and duration ( $0.53\text{s}$  at  $5^{\circ}\text{C}$  to  $0.30\text{s}$  at  $15^{\circ}\text{C}$ ,  $\text{RSq}=0.95$ ,  $p<0.001$ ,  $\text{df}=9$ ) of the clap cycle (fig. 2A).

Measurements of the animal's acceleration ( $Xb_{tang}$ ) the instantaneous mass of the animal,  $Mb_{inst}$ , (including associated water) allowed the net thrust ( $T$ ) experienced by the animal to be calculated (fig. 2B). A short period of high net thrust was observed ending at the end of shell closure. Thrust remained close to zero until the beginning of reopening when rapid deceleration resulted from the increased drag of the valves.  $T_{max}$  was significantly higher at 15°C (1.05N) than at 5°C (0.56N) and was attained earlier in the cycle (fig. 2B, table 1).

Velocity ( $Ub$ ) increased rapidly until reaching a maximum at the same time as closure of the valves was completed (fig. 2C).  $Ub$  reduced slowly as jetting ended and then fell rapidly on valve opening. Increased thrust ( $T$ ) at higher temperatures resulted in increased mean swimming velocity during jetting,  $\bar{U}b_{jet}$ , (table 3) with peak velocity,  $Ub_{max}$ , attained earlier in the cycle than at lower temperatures (fig. 2C). The  $Q_{10}$  of  $Ub_{jet}$  was higher than that for  $\bar{U}b$  or  $Ub_{max}$  (table 3) although  $Ub_{max}$  did significantly increase from 0.28ms<sup>-1</sup> at 5°C to 0.37ms<sup>-1</sup> at 15°C (table 1 and 3).

The modelling of muscle performance during the escape responses described above revealed the underlying changes in muscle properties involved. Instantaneous muscle length ( $Lm$ ) was calculated from the gape of the shell (fig. 2A) and detailed anatomical measurements (Appendix A). Fibre velocity ( $\hat{U}m$ ) rose rapidly then fell throughout closure (fig. 3). Lengthening was more rapid and reached a peak after around 0.07s of reopening (at all test temperatures).  $\hat{U}m$  then fell until the end of the cycle. Mean muscle shortening velocity during jetting increased with increasing temperature while muscle lengthening velocity did not (table 1 and 3).

Fig. 2A. Gape against time during escape responses, representative clap cycles at 5, 10 and 15°C. The cycle at 5°C is indicated by the solid line (—), at 10°C by the dotted line (.....) and by the dashed line (---) at 15°C. The form of the clap cycle changed with increasing temperature. The closing phase was completed more quickly with increasing temperature while the period of muscle lengthening remaining relatively unchanged. Traces were normalised - there were no significant differences in strain between "raw" cycle data.

B) Net force experienced by the animal due to the thrust and drag forces during escape responses at 5, 10 and 15°C. The cycle at 5°C is indicated by the solid line (—), at 10°C by the dotted line (.....) and by the dashed line (---) at 15°C. Peak thrust was higher at increased temperatures, with the point where drag exceeds thrust occurring earlier.

C) The effects of temperature on swimming velocity ( $U_b$ ) during escape responses. Velocity against time plots are for representative animals at 5, 10 and 15°C. The cycle at 5°C is indicated by the solid line (—), at 10°C by the dotted line (.....) and by the dashed line (---) at 15°C. Increases in temperature resulted in higher maximum speed ( $U_{bmax}$ ) and higher residual speed at the end of the cycle.  $U_{bmax}$  was also attained earlier in the cycle as temperature increased.

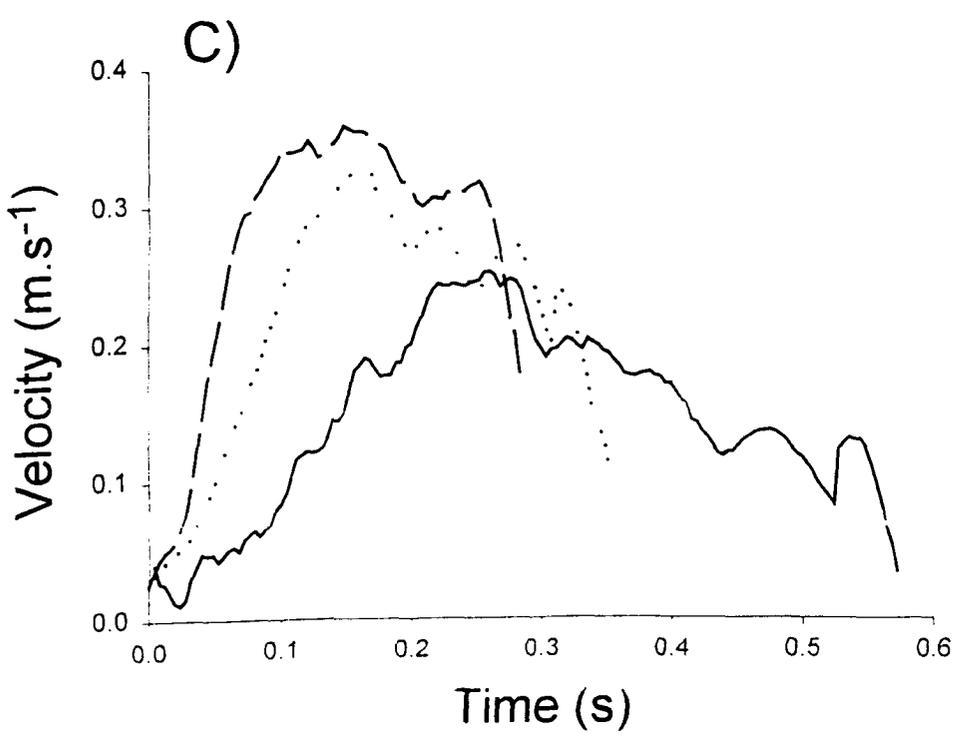
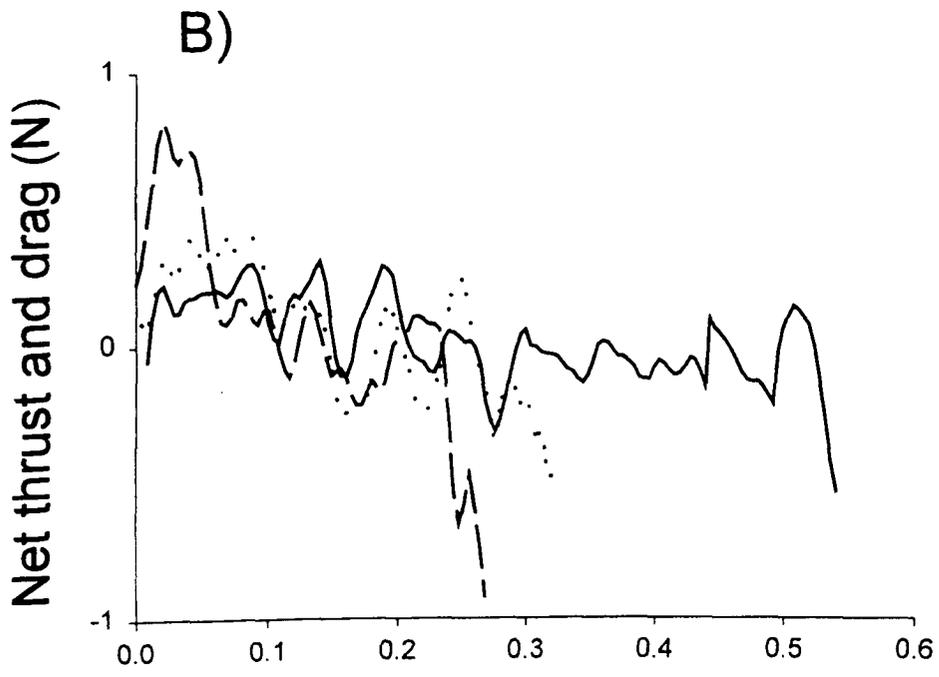
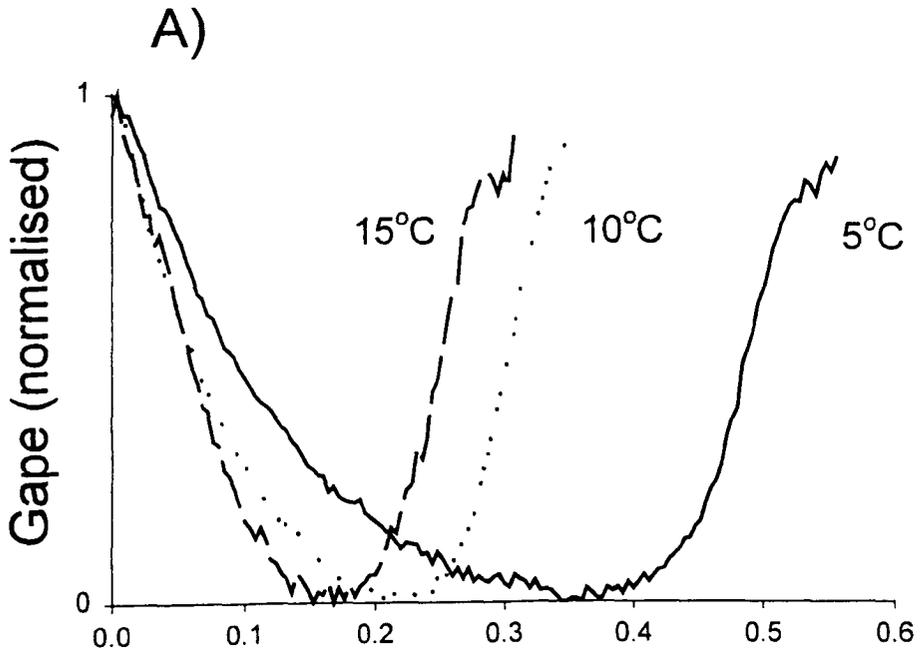


Fig. 3. Calculated adductor muscle fibre shortening and lengthening velocities during escape responses. Representative cycles at 5°C (—), 10°C (.....) and 15°C (— —). While peak shortening (negative values) and lengthening (positive) velocities were similar at all temperatures shortening was completed earlier (Fibre velocity=0 Fl.s<sup>-1</sup>) with increasing temperature.

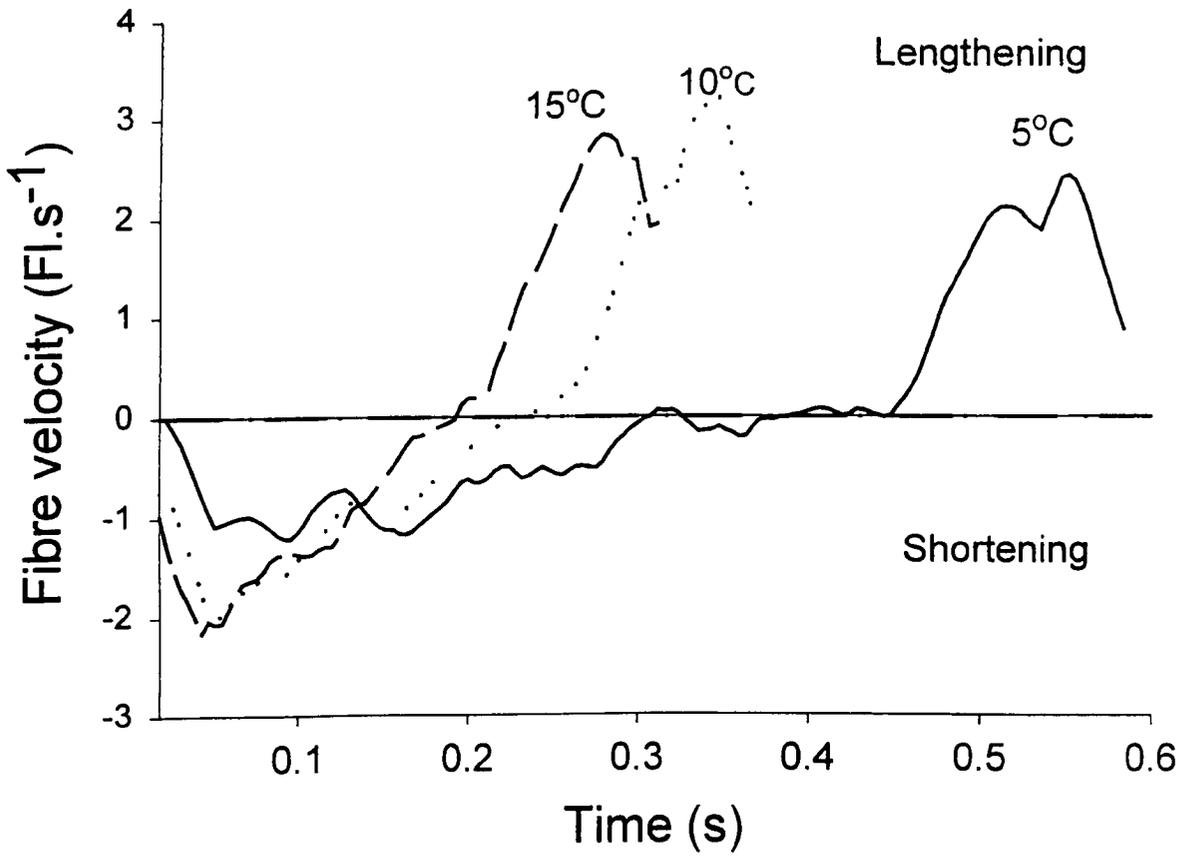


Table 1

| Performance type              | Variable   |       |       |
|-------------------------------|--|-------|-------|
|                               |  | RSq   | p     |
| <b>Whole body performance</b> | Maximum velocity (m.s <sup>-1</sup> )              | 0.673 | 0.002 |
|                               | Mean velocity (m.s <sup>-1</sup> )                 | 0.839 | 0.001 |
|                               | Mean velocity during jetting (m.s <sup>-1</sup> )  | 0.762 | 0.001 |
|                               | Maximum acceleration (m.s <sup>-2</sup> )          | 0.758 | 0.001 |
|                               | Maximum thrust (N)                                 | 0.741 | 0.001 |
|                               | Maximum drag (N)                                   | 0.546 | 0.009 |
| <b>Muscle performance</b>     | Maximum power output (W.kg <sup>-1</sup> )         |       | N/S   |
|                               | Mean power output (W.kg <sup>-1</sup> )            | 0.471 | 0.02  |
|                               | Maximum tension (kN.m <sup>-2</sup> )              |       | N/S   |
|                               | Mean tension (kN.m <sup>-2</sup> )                 | 0.512 | 0.013 |
|                               | Maximum shortening velocity (Fl.s <sup>-1</sup> )  |       | N/S   |
|                               | Mean shortening velocity (Fl.s <sup>-1</sup> )     | 0.38  | 0.003 |
|                               | Maximum lengthening velocity (Fl.s <sup>-1</sup> ) |       | N/S   |
|                               | Maximum flow (kg.s <sup>-1</sup> )                 |       | N/S   |
|                               | Maximum pressure (kPa)                             |       | N/S   |
|                               | Maximum jet velocity (m.s <sup>-1</sup> )          |       | N/S   |
|                               | Maximum jet thrust (N)                             |       | N/S   |

Table 1. Summary of regression results for whole-body and muscle performance measures against temperature. Shaded fields denote regressions which were not significant at  $p=0.05$ . Degrees of freedom(df)=9 in all cases.

Table 2

|                             |                      | RSq   | p     |
|-----------------------------|----------------------|-------|-------|
| <b>Maximum power output</b> | Maximum velocity     |       | N/S   |
|                             | Mean velocity        |       | N/S   |
|                             | Maximum acceleration |       | N/S   |
|                             | Maximum thrust       |       | N/S   |
|                             | Mean power output    | 0.609 | 0.005 |
| <b>Mean power output</b>    | Maximum velocity     | 0.369 | 0.047 |
|                             | Mean velocity        | 0.479 | 0.018 |
|                             | Maximum acceleration |       | N/S   |
|                             | Maximum thrust       |       | N/S   |
| <b>Maximum velocity</b>     | Mean velocity        | 0.844 | 0.001 |

Table 2. Interaction of whole-body and muscle performance measures. Shaded fields denote regressions which were not significant at  $p=0.05$ . Degrees of freedom are as for Table 1, relationships with temperature.  $df=9$  in all cases.

Power output ( $P$ ) and force production ( $F$ ) were calculated from the shortening velocity of the muscle, the jet area ( $ajet$ ) and flow rate ( $f$ ) of water through the jets (see Appendix A). Tension ( $F_{spec}$ ), was calculated from the resting cross-sectional area of the adductor ( $am$ ). Predicted workloops ( $F_{spec}$ , against muscle strain,  $S$ ) and tension-velocity ( $F_{spec}$  vs.  $\hat{U}m$ ) trajectories were plotted from the model output (fig. 4A and B). Workloops showed a rapid increase in  $F_{spec}$  with decreasing muscle length reaching a maximum tension at approximately 90% of starting length.  $F_{spec}$  fell sharply then continued to fall more slowly to zero with a shortening of around 20%. Lengthening was associated with a small negative force requirement to open the valves. This force fell during lengthening.  $F_{max}$  occurred at slightly greater fibre length at 5°C than at the other two temperatures.

During shortening force-velocity trajectories fitted to a quadratic function (RSq=0.998,  $p < 0.001$ ,  $df=34$ ,  $y = 29.872x^2 + 7.0347x + 2.3295$ ) with  $F_{max}$  occurring simultaneously with peak shortening velocity,  $\hat{U}m_{max}$ , (fig. 3). As force development and velocity are directly interdependent in this locomotory system the force during increases and decreases in velocity are overlaid. During lengthening (positive values)  $F_{spec}$  is low due to the large area through which the flow into the mantle occurs. This negative force requirement is reduced as the shell opens due to the increasing area of gape. The shape of the predicted force-velocity trajectories did not differ with temperature although the length of the trajectory depended on  $\hat{U}m_{max}$ .

Power output ( $P$ ) increased to a maximum ( $P_{max}$ ) almost immediately upon the commencement of adduction with a rapid fall in power output as shortening

Fig 4 A). Workloops ( $S$  vs.  $F_{spec}$ ) during escape responses. Representative cycles at 5°C (—), 10°C (⋯) and 15°C (— —). Zero strain indicates that the adductor muscle is at its maximum length prior to the initiation of contraction. The area of the loop is proportional to the total work done during the cycle.

B) Force-velocity trajectories ( $Um$  vs.  $F_{spec}$ ) during escape responses, representative cycles at 5°C (—), 10°C (⋯) and 15°C (— —). Trajectories were similar at all temperatures though the length of the shortening phase (negative values for muscle velocity) differed with temperature.

C) Muscle mass specific hydrodynamic power output ( $P_{spec}$ ) during escape responses at 5°C (—), 10°C (⋯) and 15°C (— —). Peak values of  $P_{spec}$  ( $P_{max}$ ) were greater with increasing temperature but the "active" phase of positive power output was completed earlier. The negative power required to re-fill the mantle was small and similar at all test temperatures.

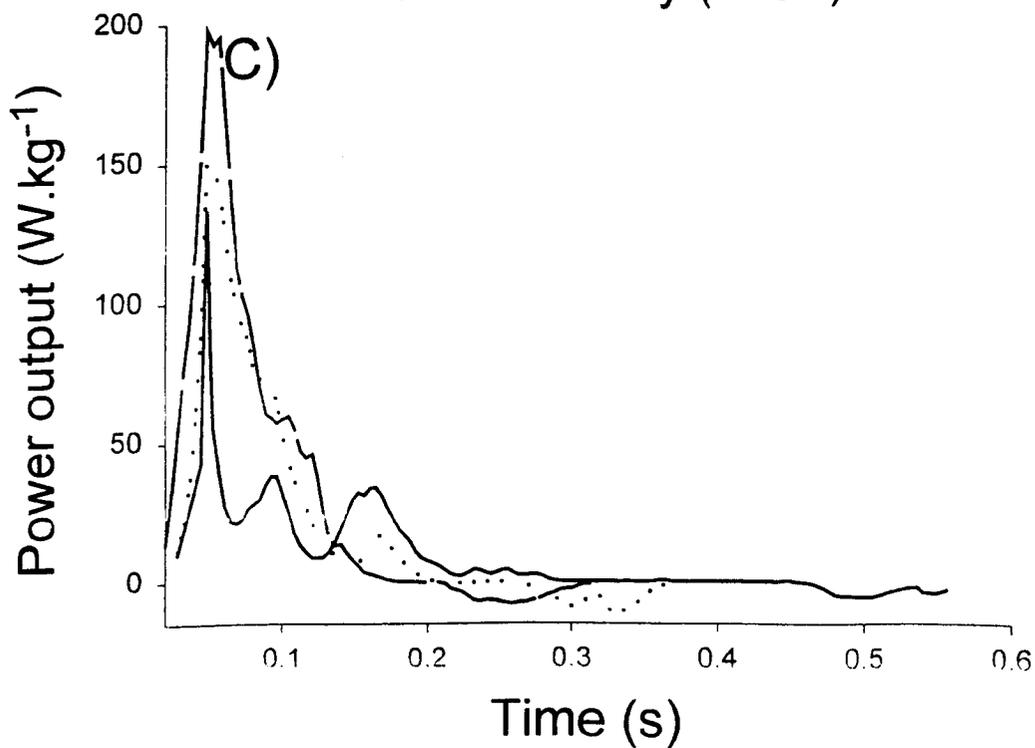
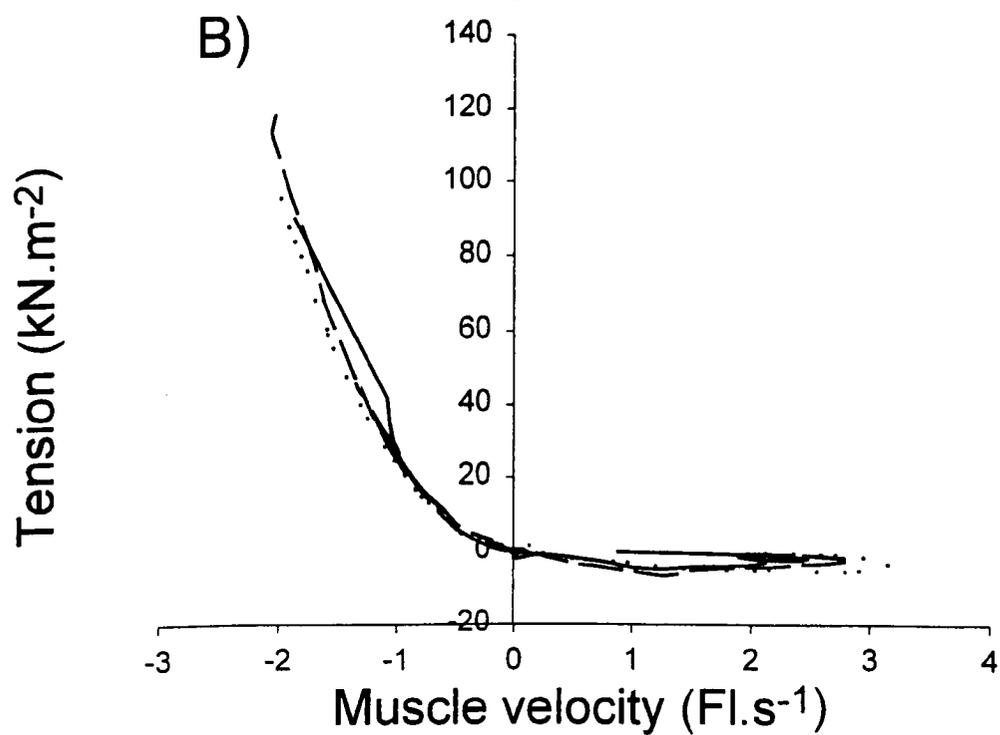
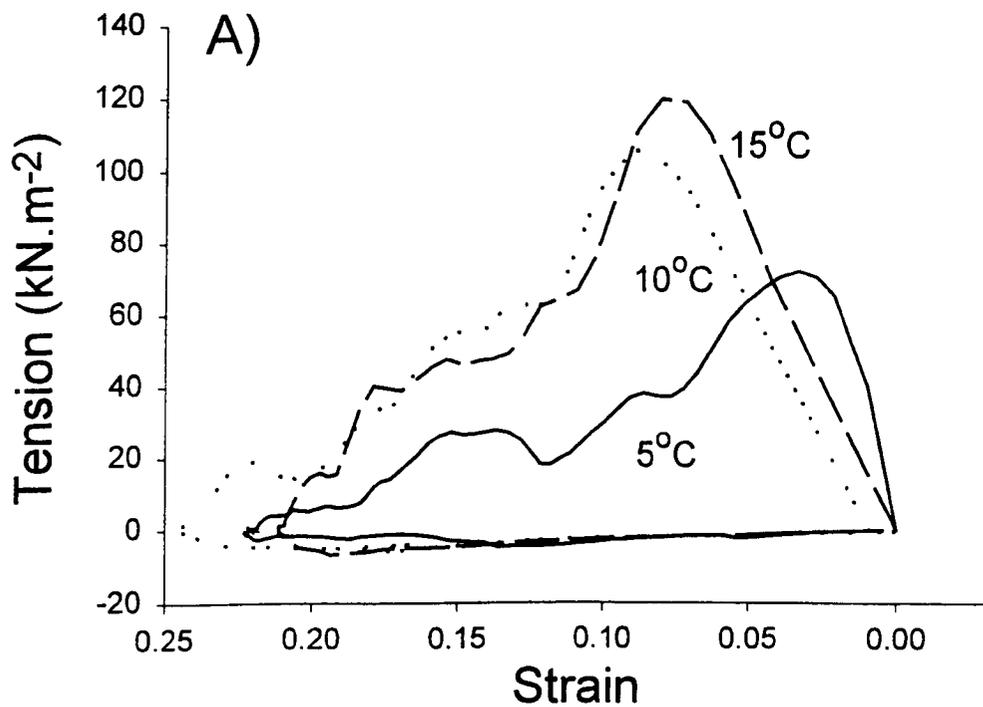


Table 3. Key whole-body and muscle performance parameters for animal acclimatised to 5, 10 and 15°C. Values given are means and standard errors for parameter for each group and the overall Q10 5-15°C for each parameter.

|   | 5       |       | 10      |       | 15      |       | Q10 (5-15°C) |
|---|---------|-------|---------|-------|---------|-------|--------------|
|   | Mean    | SE    | Mean    | SE    | Mean    | SE    |              |
| Peak swimming velocity, $U_{bmax}$ (m.s <sup>-1</sup> )               | 0.28    | 0.01  | 0.33    | 0.03  | 0.37    | 0.01  | 1.34         |
| Mean cyclic swimming velocity, $\bar{U}_b$ (m.s <sup>-1</sup> )       | 0.16    | 0.02  | 0.22    | 0.01  | 0.27    | 0.01  | 1.66         |
| Mean swimming velocity during jetting, $U_{jet}$ (m.s <sup>-1</sup> ) | 0.08    | 0.01  | 0.11    | 0.05  | 0.16    | 0.02  | 2.01         |
| Peak fibre shortening velocity, $U_{max}$ (F.l.s <sup>-1</sup> )      | -2.16   | 0.05  | -1.78   | 0.26  | -2.18   | 0.07  | 1.01         |
| Peak fibre lengthening velocity, (F.l.s <sup>-1</sup> )               | 3.45    | 0.30  | 2.88    | 0.25  | 2.93    | 0.20  | 0.85         |
| Mean fibre velocity during shortening (F.l.s <sup>-1</sup> )          | -0.51   | 0.04  | -0.92   | 0.07  | -1.05   | 0.08  | 2.04         |
| Mean fibre velocity during opening (F.l.s <sup>-1</sup> )             | 4.44    | 0.35  | 3.32    | 0.48  | 7.88    | 0.64  | 1.78         |
| Maximum thrust, $T_{max}$ , (N)                                       | 0.56    | 0.05  | 0.45    | 0.07  | 1.05    | 0.08  | 1.87         |
| Peak power output, $P_{max}$ , (W.kg <sup>-1</sup> )                  | -139.16 | 40.02 | -135.16 | 50.72 | -221.64 | 33.65 | 1.59         |
| Mean cyclic power output, $\bar{P}_{spec}$ , (W.kg <sup>-1</sup> )    | -23.30  | 4.67  | -11.66  | 3.78  | -31.10  | 5.32  | 1.33         |

velocity fell. The active phase of positive power production was completed earlier at higher temperature. A small negative power requirement was observed beginning at the start of valve opening. Only mean cyclic power output,  $\bar{P}_{spec}$ , and mean tension,  $\bar{F}_{spec}$ , were significantly related to temperature due to the high variance of  $P_{max}$  and  $F_{max}$  (table 1).  $\bar{P}_{spec}$  increased from 11.7 to 31.1W.kg<sup>-1</sup> and  $\bar{F}_{spec}$  from 12.5 to 28.8kN.m<sup>-2</sup> as temperature increased from 5 to 15°C. In general muscle performance was higher and the active phase of jetting completed sooner at higher temperatures.

#### *2.3.4 Effects of seasonal acclimatisation on swimming*

The swimming performance of scallops acclimatised to different temperatures in the field were compared over a range of temperatures. Representative cycles at a common temperature (11°C) are presented in figs 5A-C. In this case the winter animal was swimming above its mean acclimatisation temperature and the autumn animal below its mean acclimatisation temperature.

The general form of the clap cycle was as described for acclimated animals with a declining rate of shortening followed by a rapid opening phase. The form of the clap cycle was modified by temperature change and differed according to the season of collection (fig. 5A). Time to close the shell was negatively related to temperature in both groups (table 4A) with winter animals completing the closing phase of the clap cycle significantly sooner (ANCOVA,  $p < 0.012$ ,  $df = 16$ ) than autumn animals and having lower clap durations (fig. 10).

A period of high thrust ( $T$ ) was observed, ending close to the end of shell closure (fig. 5B).  $T$  remained low until reopening when negative net thrust increased.  $T_{max}$  increased significantly with increasing temperature in both groups (fig. 6B,

Fig 5A) Gape ( $G$ ) plotted against time in representative autumn (solid line, —) and winter (dotted line, ..... ) acclimatised animals at  $10^{\circ}\text{C}$ . Although both cycles took place at the same temperature the clap cycle was of reduced duration in the winter animal as a result of the reduced time taken to complete shortening.

B) Representative net thrust and drag ( $T$ ) traces in autumn (solid line, —) and winter (dotted line, ..... ) acclimatised animals at  $10^{\circ}\text{C}$ . Peak thrust ( $T_{max}$ ) was greater in the winter acclimatised animal as is net drag ( $T_{min}$ ) at the end of the cycle.

C) Swimming velocity ( $U_b$ ) plotted against time in representative autumn ( $13^{\circ}\text{C}$ ) and winter ( $8^{\circ}\text{C}$ ) acclimatised animals swimming at  $10^{\circ}\text{C}$ . The winter acclimatised animal (dotted line, ..... ) was swimming above it's normal temperature and accelerated earlier reaching peak velocity ( $U_{bmax}$ ) earlier than the autumn acclimatised animal (solid line, —). In this case  $U_{bmax}$  was also higher.

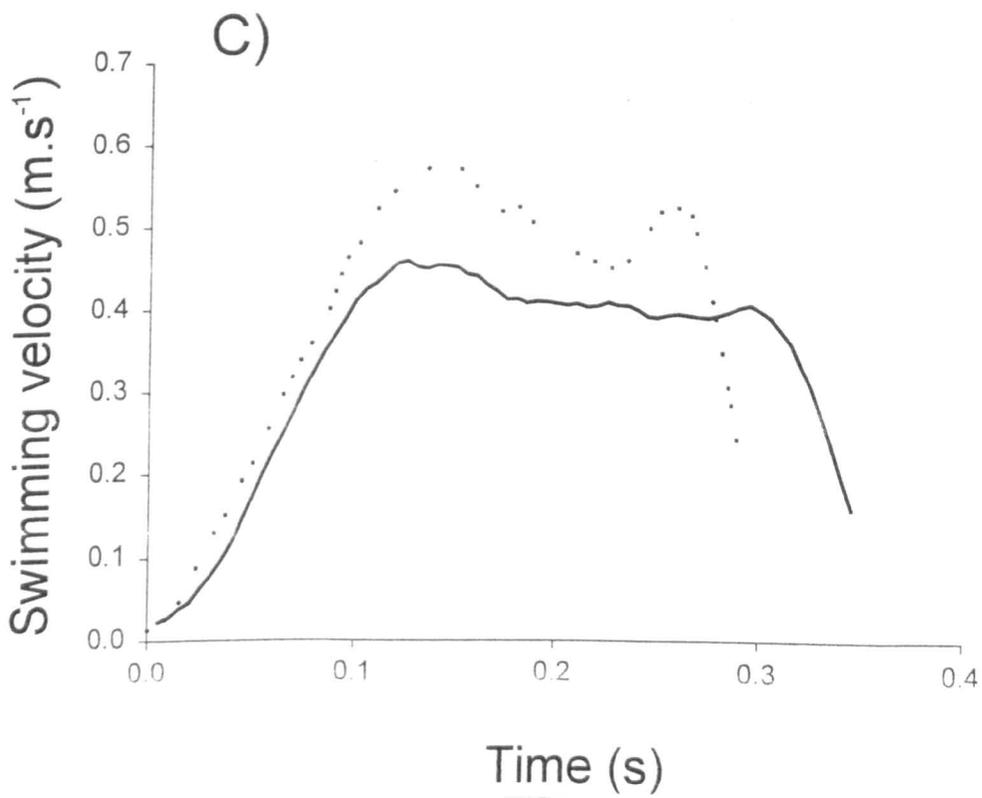
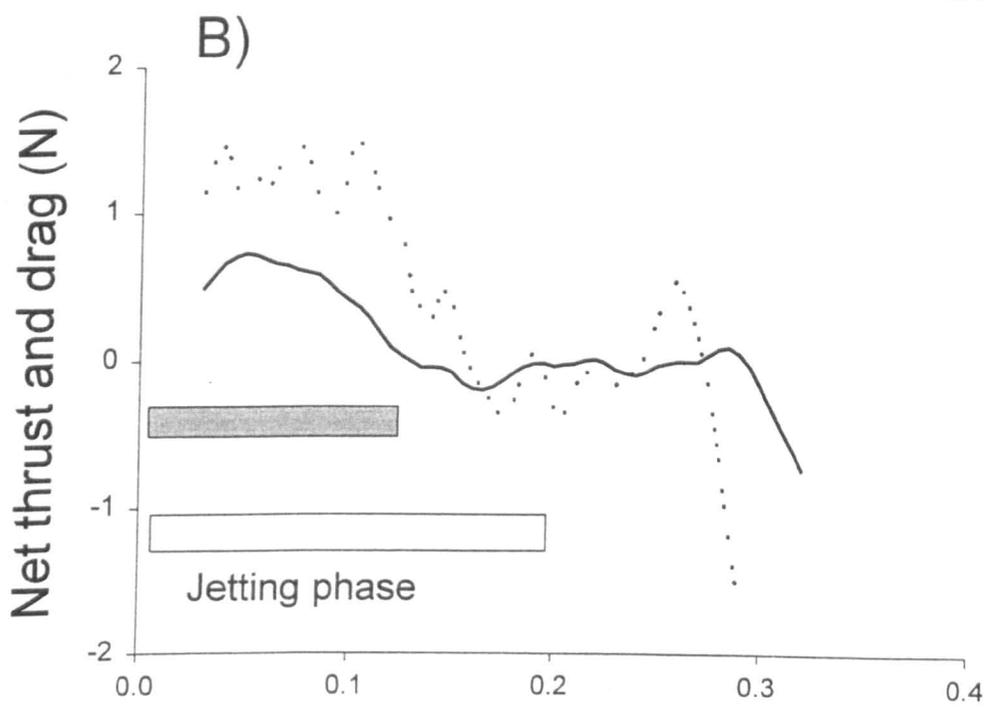
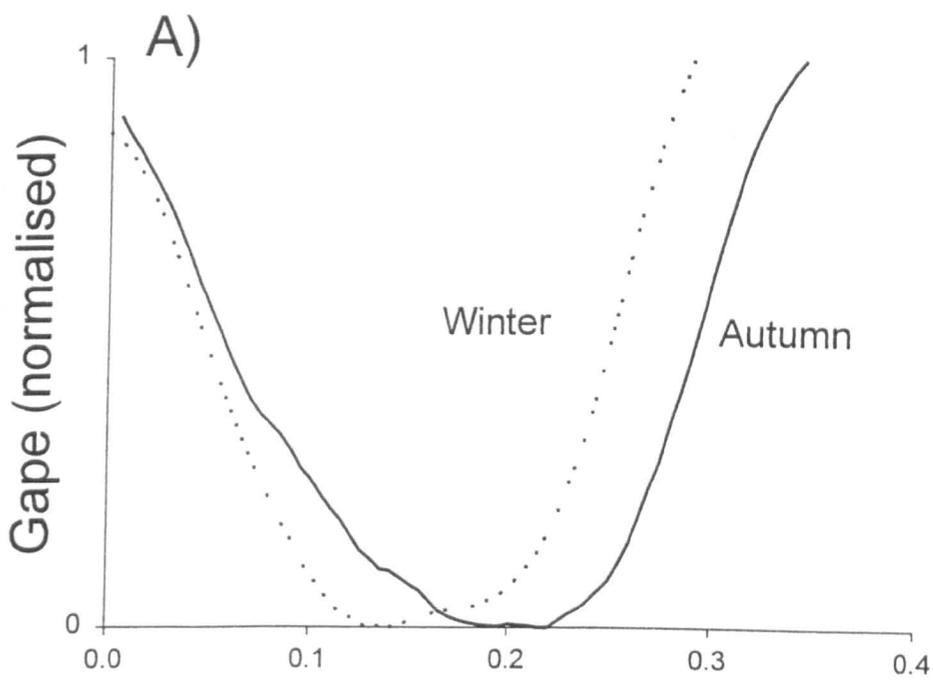


Fig 6A) Peak acceleration ( $Xb_{max}$ ) against temperature in autumn (closed circles, ●) and winter animals (open circles, ○).  $Xb_{max}$  increased significantly with increasing temperature in both groups. Dashed lines are 95% Confidence Intervals. Winter,  $y = 1.79x - 6.46$ ,  $RSq=0.56$ ,  $p=0.006$ ,  $df=9$ . Autumn,  $y = 0.950x - 3.58$ ,  $RSq=0.48$ ,  $p=0.008$ ,  $df=12$ . The lines were significantly different (ANCOVA,  $p=0.002$ ,  $df=16$ ). See also Table 3.

B) Peak thrust ( $T_{max}$ ) against temperature in autumn (closed circles, ●) and winter animals (open circles, ○).  $T_{max}$  increased significantly with increasing temperature in both groups. Dashed lines are 95% Confidence Intervals. Winter,  $y = 0.24x - 0.89$ ,  $RSq=0.53$ ,  $p=0.009$ ,  $df=9$ . Autumn,  $y = 0.12x - 0.52$ ,  $RSq=0.33$ ,  $p=0.032$ ,  $df=12$ . The lines were significantly different (ANCOVA,  $p=0.002$ ,  $df=16$ ). See also Table 3.

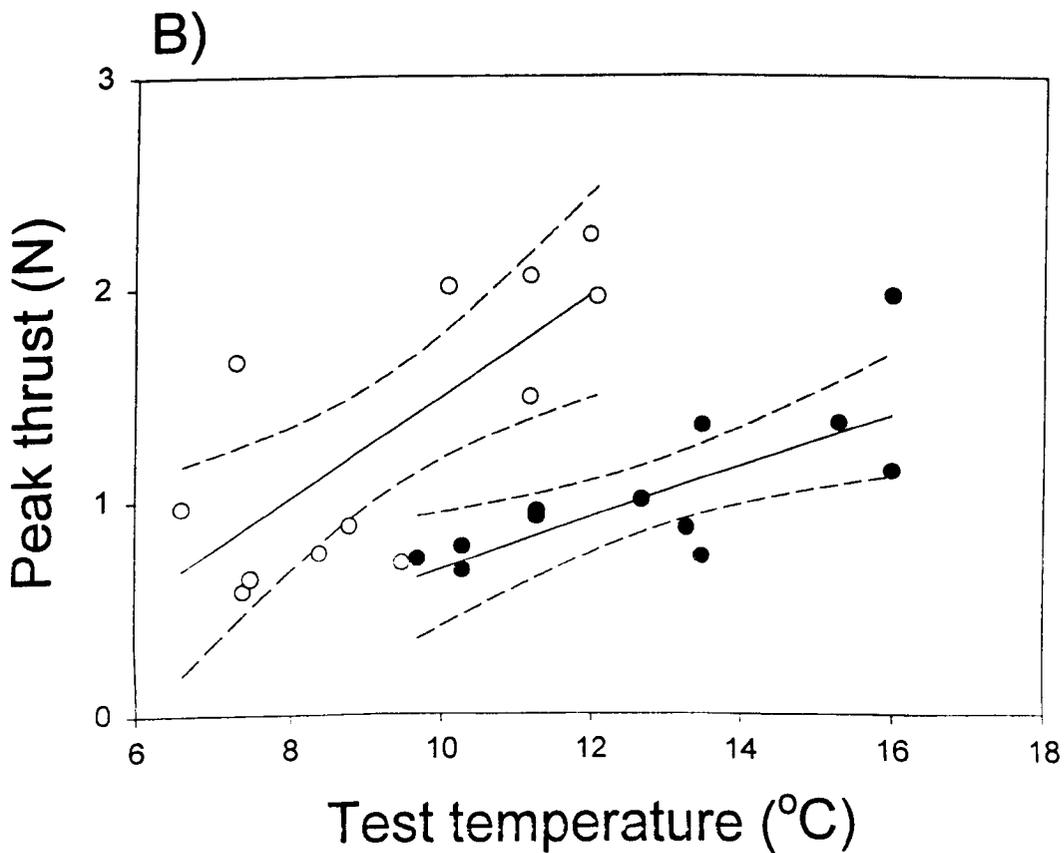
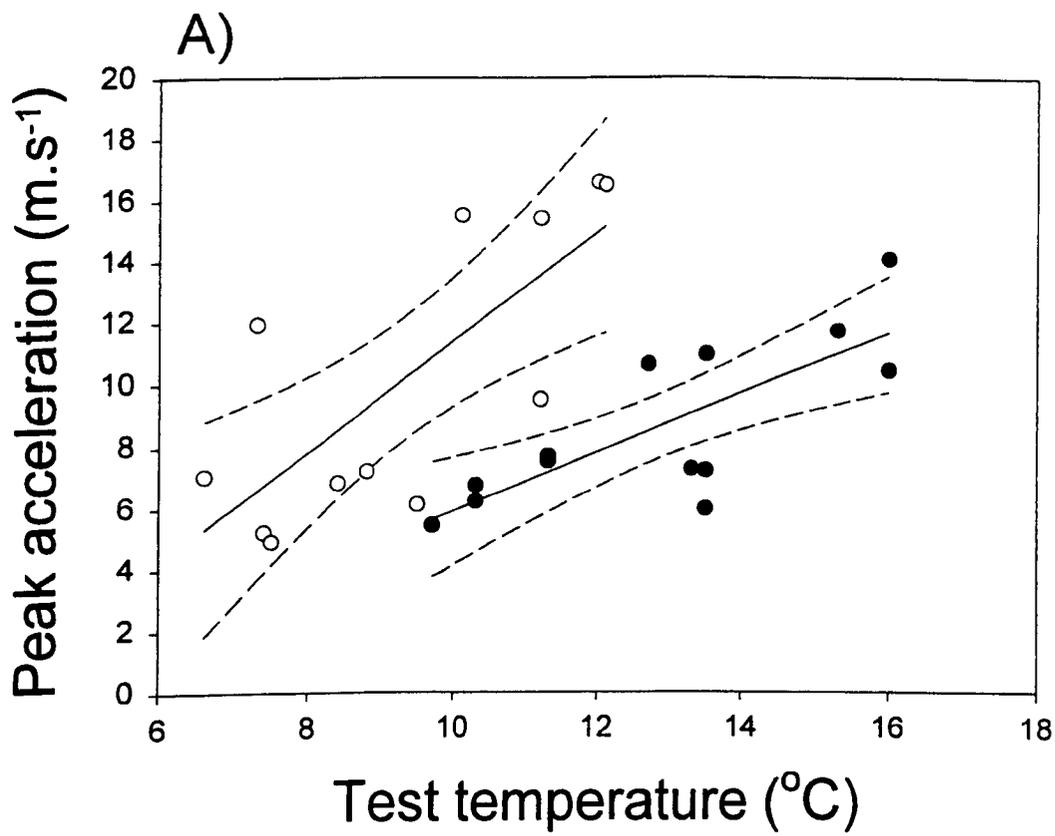


Fig 7A) Mean swimming velocity during jetting,  $\bar{U}_{b_{jet}}$ , in winter (open circles, o) and autumn (solid circles, ●) acclimated animals. The period of jetting was defined as the period from the beginning of the first movement of the valves to jet velocity returning to zero. Peak swimming velocity was typically attained at the end of this period.  $\bar{U}_{b_{jet}}$  increased with increasing temperature in both groups though this was not significant in winter animals. Dashed lines are 95% Confidence Intervals. Autumn animals,  $y=0.015x+0.0345$ ,  $RSq=0.46$ ,  $p=0.02$ ,  $df=10$ . Winter animals,  $y=0.012x+0.188$ .  $RSq=0.23$ ,  $p=0.19$ ,  $df=8$ . The groups were significantly different (ANOVA),  $p=0.007$ ,  $df=19$ ,  $F=9.18$ .

B) Mean cyclic swimming velocity,  $\bar{U}_b$ , in winter (open circles, o) and autumn (solid circles, ●) acclimated animals.  $\bar{U}_b$  did not increase significantly with increasing water temperature in either group. There was no significant difference between groups.  $\bar{U}_b$  may be higher than  $\bar{U}_{b_{jet}}$ , especially in Autumn animals where the glide period (where velocity was relatively high) was prolonged.  $\bar{U}_b$  depended on both performance during jetting (which increases with temperature) and the length of time before opening the shell for the next cycle (which decreased with temperature).

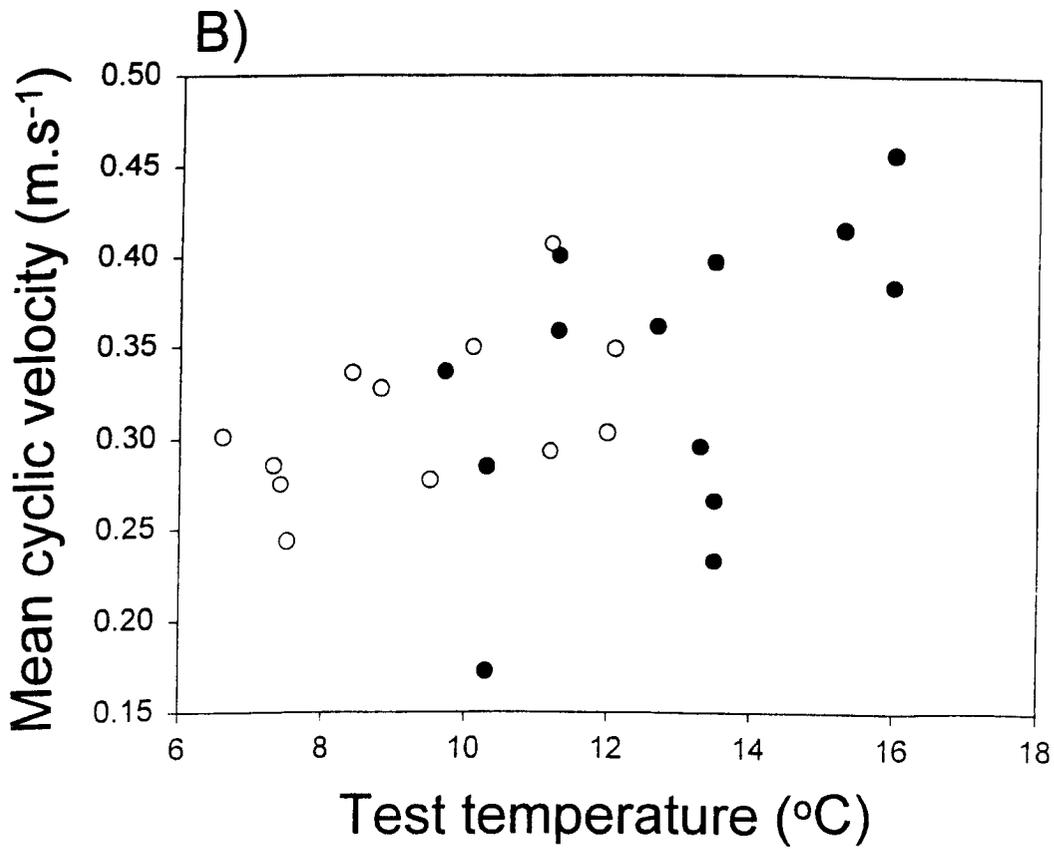
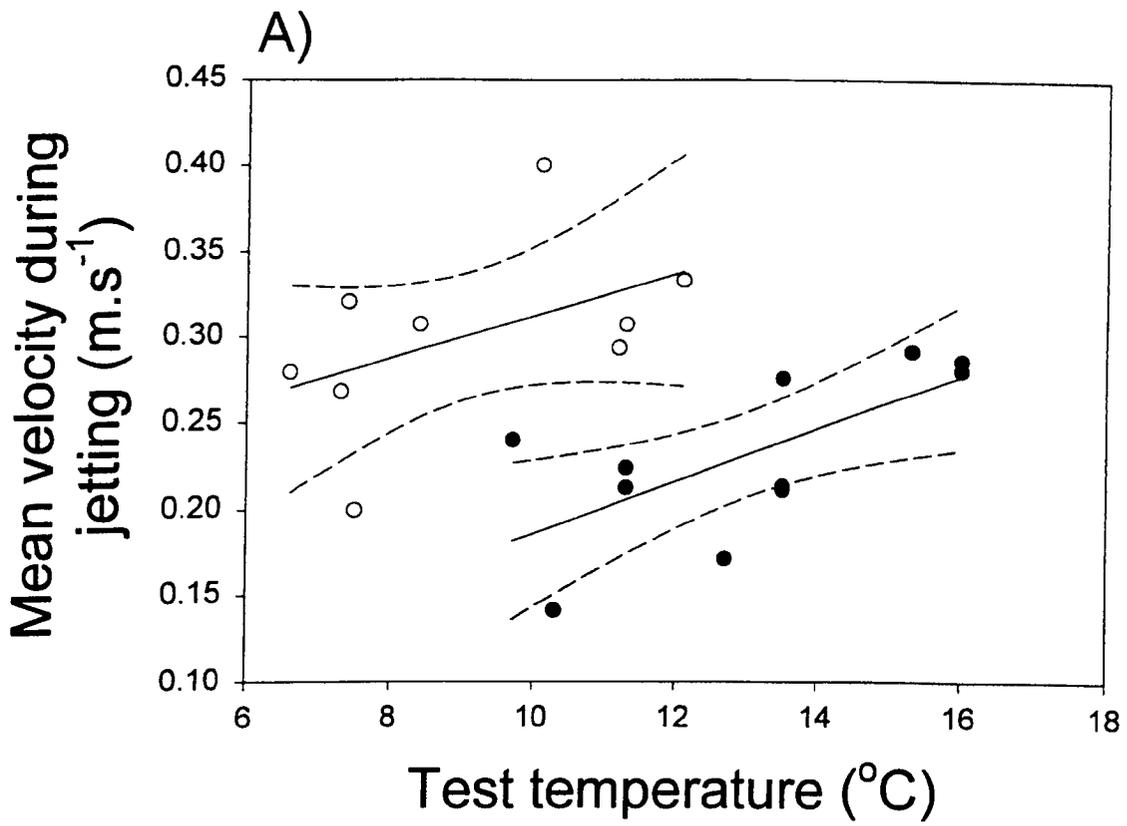


Fig. 8A) Fibre shortening and lengthening velocity during escape responses in representative autumn (solid line, —) and winter (dotted line, ..... ) acclimatised animals at 10°C. Peak shortening velocity occurred later and shortening takes longer in Autumn than in Winter animals. Peak shortening velocity ( $U_{max}$ ) at this temperature was approximately half the maximum value in the autumn compared to the winter acclimatised animal.

B) Muscle mass specific power output ( $P_{spec}$ ) during escape responses in representative autumn (solid line, —) and winter (dotted line, ..... ) acclimatised animals at 10°C.  $P_{spec}$  is higher in the winter animal and the power stroke is completed earlier. Negative work (to re-fill jet cavity) is similar in both animals and small due to the large area through which the influx of water may occur.

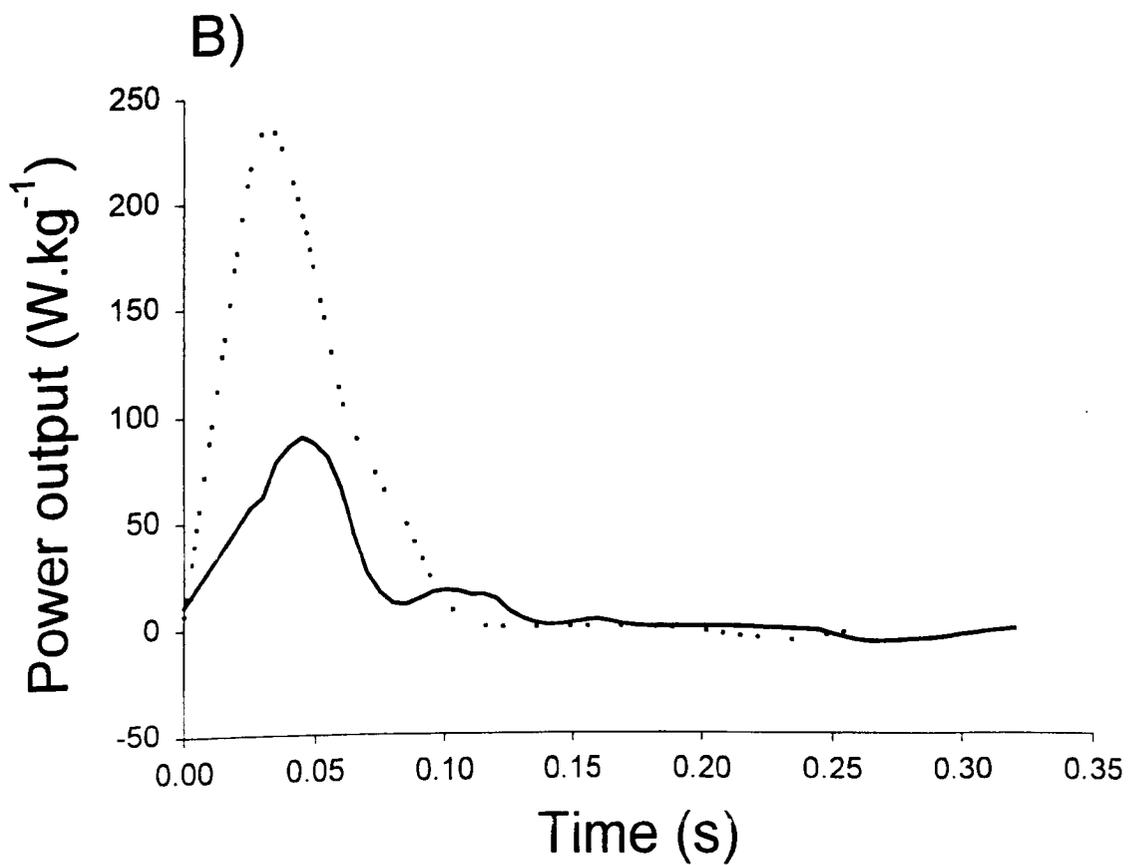
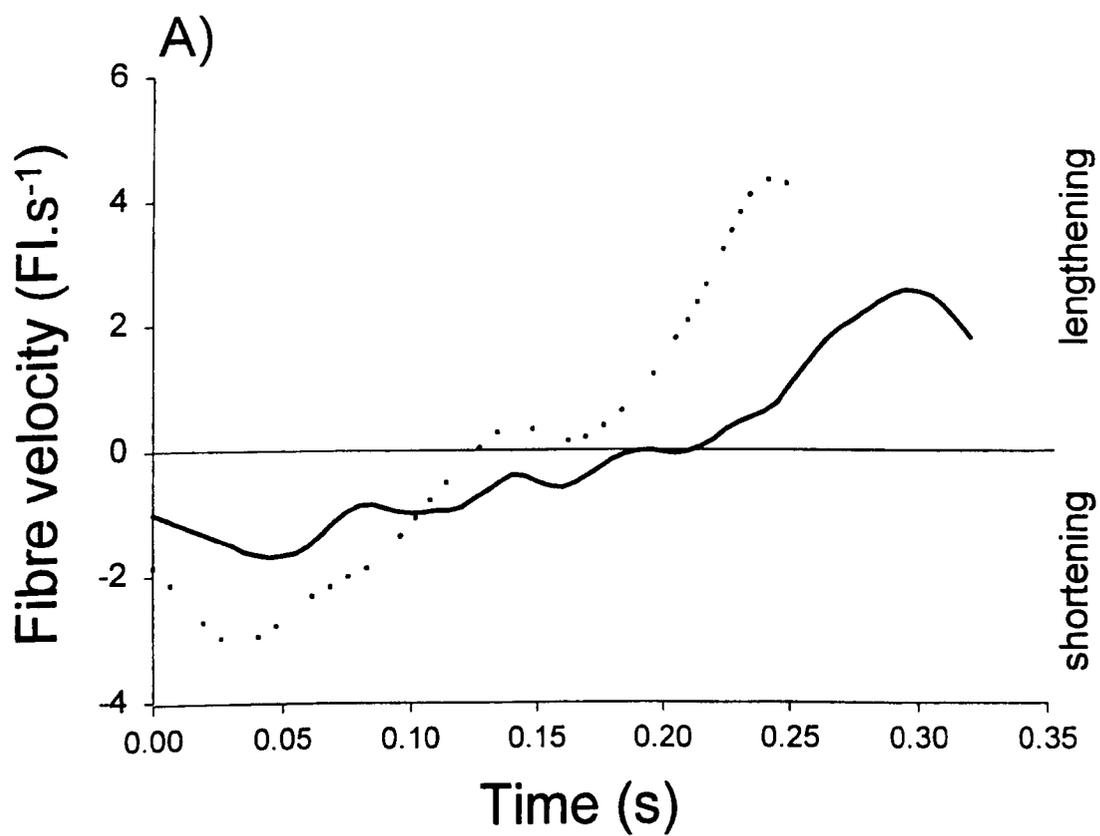


Table 4A

| Performance type       | Variable                     | Group  |       |        |       | Between seasons |
|------------------------|------------------------------|--------|-------|--------|-------|-----------------|
|                        |                              | Autumn |       | Winter |       |                 |
|                        |                              | RSq    | p     | RSq    | p     | p               |
| Whole body performance | Maximum velocity             | N/S    |       | N/S    |       | N/S             |
|                        | Mean velocity                | N/S    |       | N/S    |       | N/S             |
|                        | Mean velocity during jetting | 0.460  | 0.020 | N/S    |       | 0.007           |
|                        | Maximum acceleration         | 0.477  | 0.006 | 0.561  | 0.008 | 0.002           |
|                        | Maximum thrust               | 0.330  | 0.032 | 0.532  | 0.009 | 0.002           |
| Muscle performance     | Maximum drag                 | N/S    |       | 0.466  | 0.022 | 0.010           |
|                        | Maximum power output         | 0.452  | 0.024 | 0.802  | 0.003 | <0.001          |
|                        | Mean power output            | 0.572  | 0.007 | 0.759  | 0.006 | <0.001          |
|                        | Maximum force production     | N/S    |       | 0.748  | 0.006 | <0.001          |
|                        | Mean force production        | 0.397  | 0.038 | 0.709  | 0.009 | <0.001          |
|                        | Maximum shortening velocity  | 0.681  | 0.002 | N/S    |       | 0.003           |
|                        | Maximum lengthening velocity | N/S    |       | N/S    |       | N/S             |
|                        | Maximum flow                 | N/S    |       | 0.563  | 0.032 | <0.001          |
|                        | Maximum pressure             | N/S    |       | 0.568  | 0.031 | <0.001          |
|                        | Maximum jet velocity         | N/S    |       | 0.563  | 0.032 | <0.001          |
|                        | Maximum jet thrust           | N/S    |       | 0.568  | 0.031 | <0.001          |

Table 4B

| Performance type       | Variable                     | Group  |       |        |       |
|------------------------|------------------------------|--------|-------|--------|-------|
|                        |                              | Autumn |       | Winter |       |
|                        |                              | RSq    | p     | RSq    | p     |
| Whole body performance | Maximum velocity             | N/S    |       | N/S    |       |
|                        | Mean velocity                | N/S    |       | N/S    |       |
|                        | Maximum acceleration         | N/S    |       | N/S    |       |
|                        | Maximum thrust               | N/S    |       | 0.462  | 0.021 |
|                        | Maximum drag                 | N/S    |       | 0.436  | 0.027 |
| Muscle performance     | Maximum power output         | N/S    |       | N/S    |       |
|                        | Mean power output            | N/S    |       | N/S    |       |
|                        | Maximum force production     | N/S    |       | N/S    |       |
|                        | Mean force production        | N/S    |       | N/S    |       |
|                        | Maximum shortening velocity  | N/S    |       | 0.533  | 0.04  |
|                        | Maximum lengthening velocity | N/S    |       | N/S    |       |
|                        | Maximum flow                 | 0.471  | 0.02  | 0.527  | 0.041 |
|                        | Maximum pressure             | N/S    |       | 0.513  | 0.046 |
|                        | Maximum jet velocity         | 0.471  | 0.02  | 0.527  | 0.041 |
|                        | Maximum jet thrust           | 0.378  | 0.044 | 0.513  | 0.046 |

Table 4. Summary of regression results for whole-body and muscle performance measures against A) temperature and B) shell height. Shaded fields denote regressions which were not significant at  $p=0.05$ . Error degrees of freedom were 9 (winter animals) and 12 (autumn animals) for whole body performance, and 9 and 6 respectively for muscle performance. The difference between the number of sequences analysed results from sequences in which the digitising points were not sufficiently visible to allow accurate determination of gape angle. Differences between seasonal groups were compared using GLM Univariate ANOVA with temperature or shell height as a covariate. Error degrees of freedom for temperature was 16 in each case. No scaling differences were detected between seasonal groups.

table 4A) and was significantly greater in winter acclimatised than in autumn acclimatised animals with values ranging from 0.58-2.25N and 0.67-1.96N respectively (see table 4A). Swimming velocity,  $U_b$ , increased rapidly as closing began to a peak value after 0.1-0.15s. The animal then decelerated slowly after the end of valve closure before decelerating rapidly after valve reopening (fig. 5C).  $\hat{U}b_{jet}$  was significantly higher in winter than in autumn animals while  $U_{bmax}$  and  $\hat{U}b$  did not differ (table 4A).  $\hat{U}b_{jet}$  was significantly related to temperature in both groups (fig. 6B) Gains in performance at higher temperatures occurred early in the cycle and were derived from the shortening in the duration of jetting.

Muscle fibre velocity,  $\bar{U}m$ , during adduction fell to zero before lengthening rapidly. Mean shortening velocity was significantly greater in winter than in autumn animals (table 4A) while lengthening velocity did not differ.

Predicted workloops (fig. 9A) were similar to those described previously and consisted of a single large peak in predicted tension ( $F_{spec}$ ), reached at highest muscle length, followed by a slow reduction in  $F_{spec}$  during subsequent shortening. While the shape and duration of the workloops in autumn and winter animals were similar  $F_{max}$  was lower in the autumn animals. Force-velocity trajectories (fig. 9B) were similar to those described in acclimated animals with no difference in shape between seasons. In all cases the quadratic function presented earlier fully describes the relationship between  $\hat{U}m$  and  $F_{spec}$ . Differences in force production between groups was therefore related to the length of the trajectory rather than its shape.

Power output ( $P_{spec}$ ) peaked early in the cycle, falling rapidly as shortening velocity ( $\hat{U}m$ ) decreased with jetting being completed earlier in winter than in

Fig. 9A) Workloops ( $S$  vs.  $F_{spec}$ ) during escape responses in representative autumn (solid line, —) and winter (dotted line, ·····) acclimatised animals at 10°C. Maximum tension ( $F_{max}$ ) is greater in the winter acclimatised animal, negative force requirements to refill the shell are small and similar in both animals.

B) Force-velocity trajectories ( $U_m$  vs.  $F_{spec}$ ) during escape responses in representative autumn (solid line, —) and winter (dotted line, ·····) acclimatised animals at 10°C. The shape of the trajectory is similar in both animals with the difference in fibre velocity increasing the length of the winter curve.

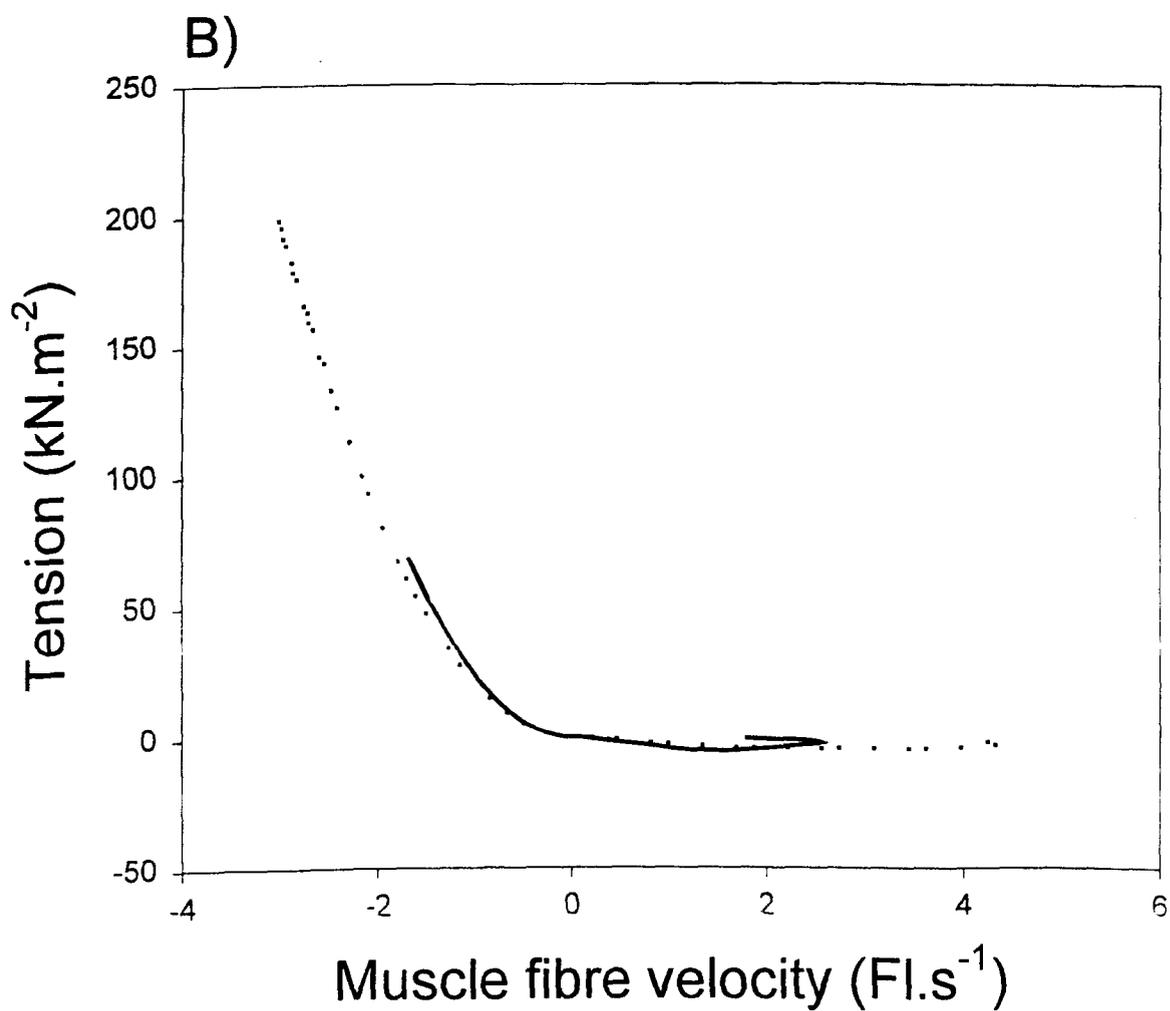
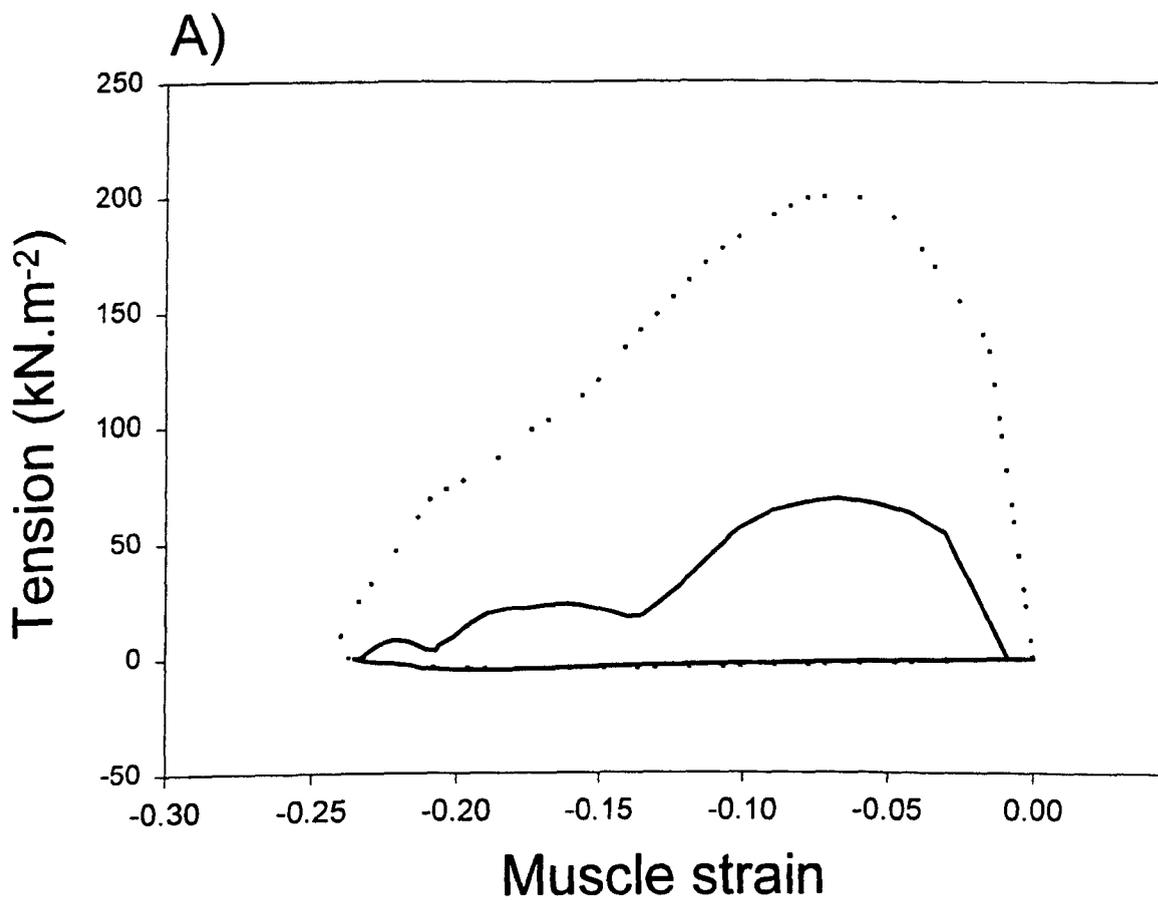


Fig. 10. Clap frequency against temperature in autumn (closed circles, ●) and winter (open circles, ○) acclimatised animals. Clap frequency increases with increasing temperature in both autumn and winter animals. Dashed lines are 95% Confidence Intervals. Autumn,  $y=0.1849x+0.85$ ,  $RSq=0.80$ ,  $p<0.001$ ,  $df=12$ . Winter,  $y=0.22x+ 0.71$ ,  $RSq=0.90$ ,  $p<0.001$ ,  $df=9$ . The lines were significantly different (ANCOVA,  $p=0.002$ ,  $df=16$ ).

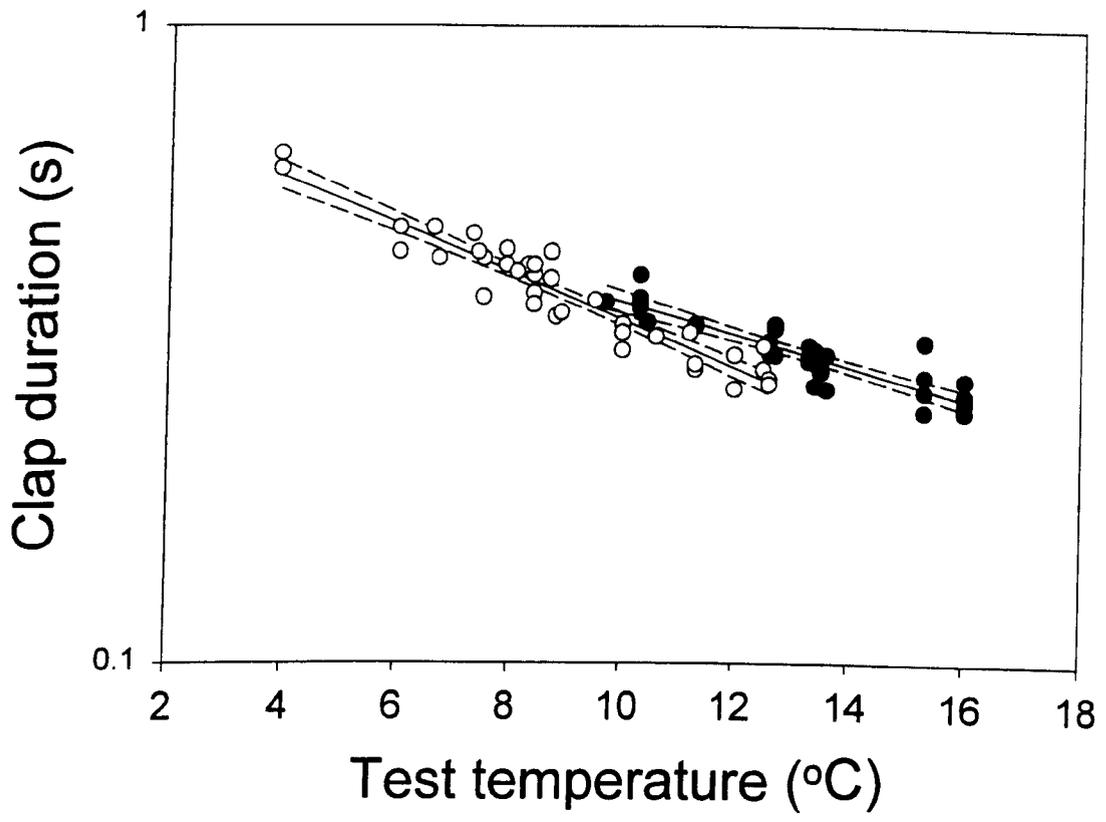


Fig. 11A) The relationship between peak muscle mass specific power output ( $P_{max}$ ) and temperature in autumn (closed circles, ●) and winter (open circles, ○) animals.  $P_{max}$  increased significantly with increasing temperature in both groups. Winter,  $y=34.69x - 149.99$ ,  $RSq=0.80$ ,  $p=0.003$ ,  $df=6$ . Autumn  $y=16.553x - 140.08$ ,  $RSq=0.452$ ,  $p=0.024$ ,  $df=9$ . The lines were significantly different (ANCOVA,  $p<0.001$ ,  $df=16$ ). See also Table 4.

B) The relationship between mean cyclic muscle mass specific power output,  $\bar{P}_{spec}$ , and temperature autumn (closed circles, ●) and winter (open circles, ○) animals.  $\bar{P}_{spec}$  increased significantly with increasing temperature in both groups. Winter,  $y=6.80x - 31.3$ ,  $RSq=0.74$ ,  $p=0.006$ ,  $df=6$ . Autumn,  $y=2.90x - 18.8$ ,  $RSq=0.572$ ,  $p=0.007$ ,  $df=9$ . The lines were significantly different (ANCOVA,  $p<0.001$ ,  $df=16$ ). See also Table 4.

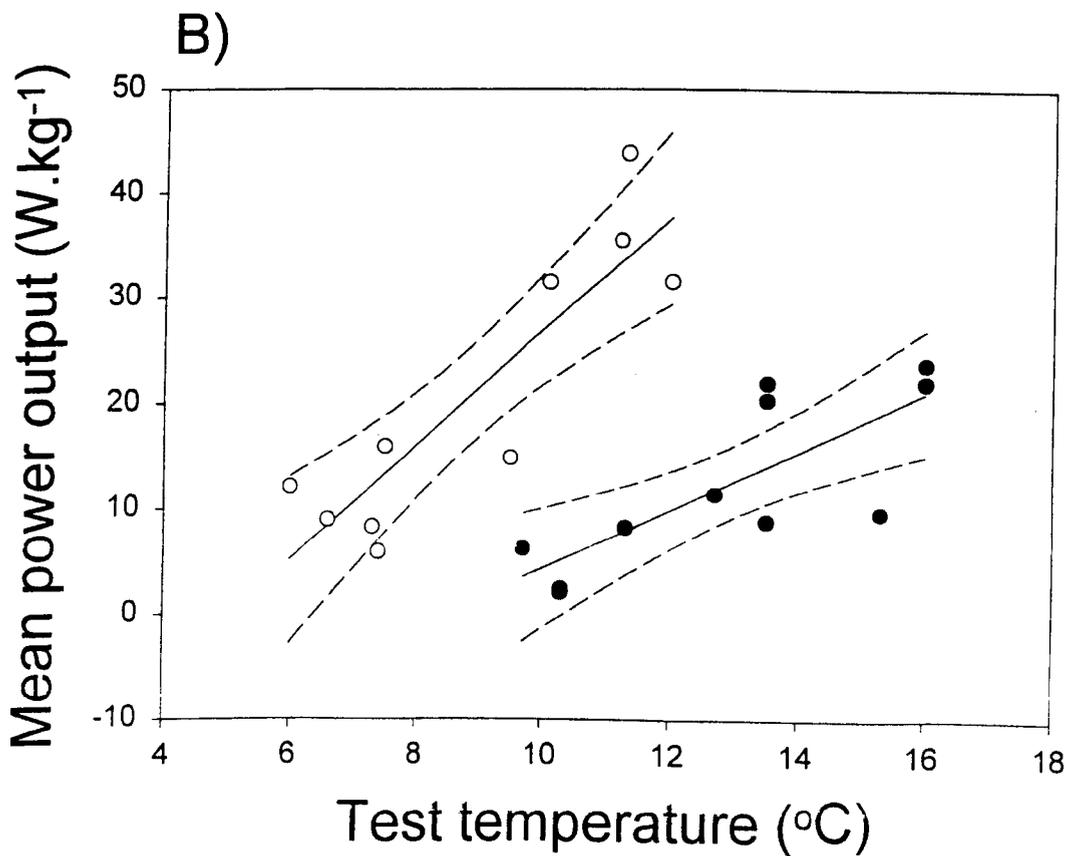
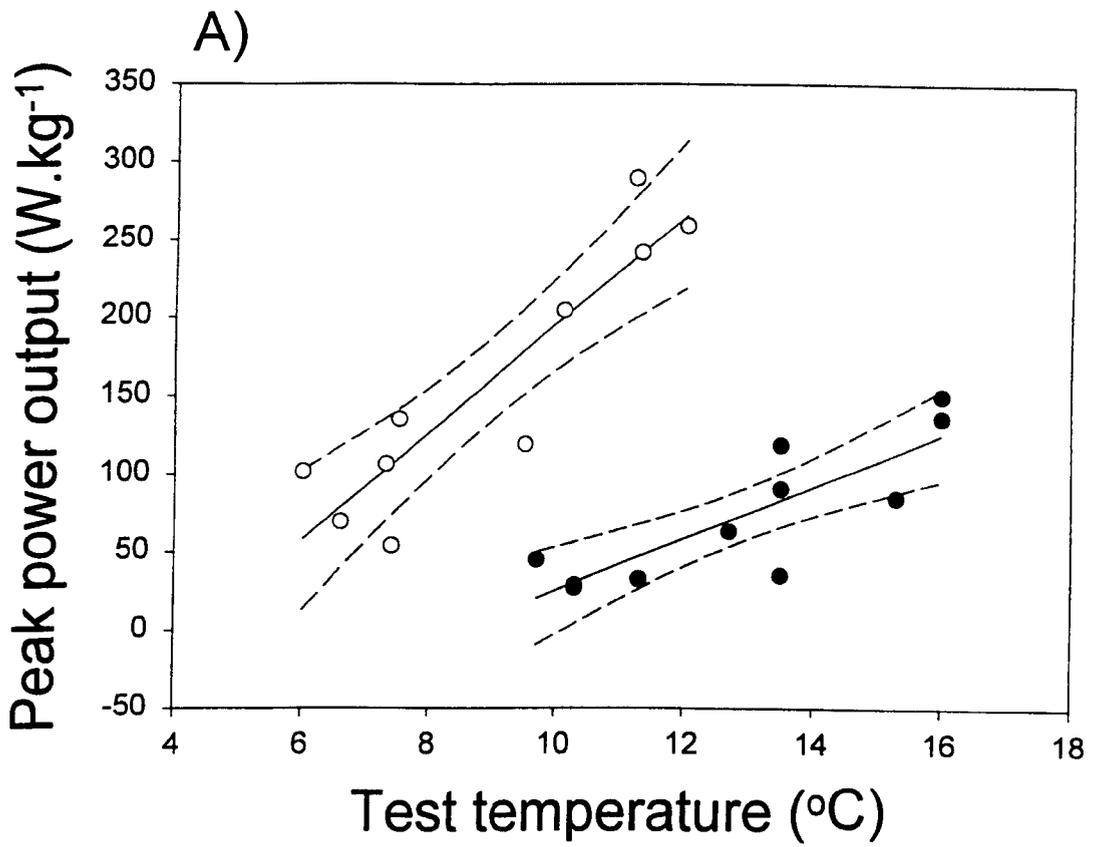
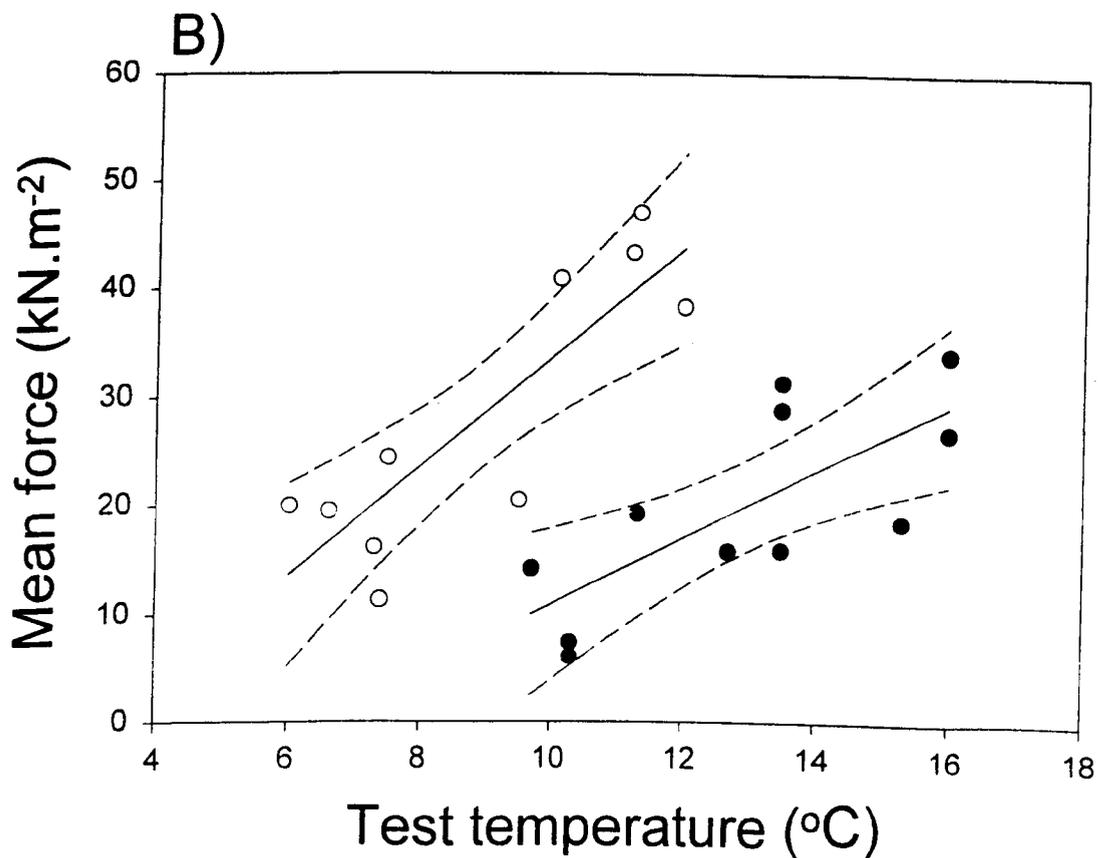
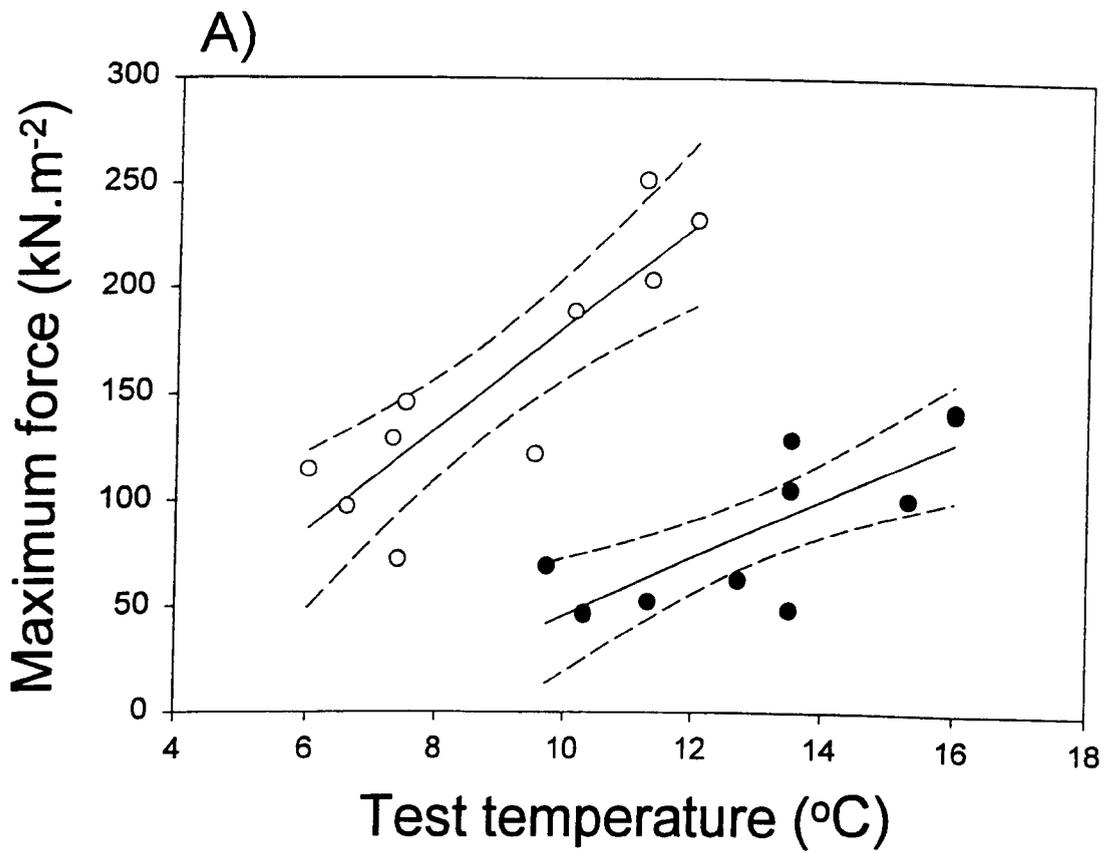


Fig. 12A) The relationship between peak muscle cross-sectional-area specific force production ( $F_{max}$ ) and temperature in autumn (closed circles, ●) and winter (open circles, ○) animals.  $F_{max}$  increased significantly with increasing temperature in winter but not in autumn animals. Winter,  $y=24.00x -57.41$ ,  $RSq=0.75$ ,  $p=0.006$ ,  $df=6$ . The groups were significantly different (ANCOVA,  $p<0.001$ ,  $df=16$ ). See also Table 4.

B) The relationship between mean muscle cross-sectional-area force production,  $\bar{F}_{spec}$ , and temperature in autumn (closed circles, ●) and winter (open circles, ○) animals.  $\bar{F}_{spec}$  increased significantly with increasing temperature in both groups. Winter,  $y=5.02x-16.40$ ,  $RSq=0.71$ ,  $p=0.009$ ,  $df=6$ . Autumn,  $y=4.60x-29.64$ ,  $RSq=0.40$ ,  $p=0.038$ ,  $df=9$ . The groups were significantly different (ANCOVA,  $p<0.001$ ,  $df=16$ ). See also Table 4.



autumn animals (fig. 8B). Increased temperature resulted in significant increases in  $P_{spec}$  and  $F_{spec}$  (peak and mean) in both acclimatisation groups (figs 11A and B). All muscle performance parameters differed between acclimatisation groups except muscle lengthening velocity (table 4). Lengthening velocity was temperature independent in both groups. Maximum muscle length (as a proportion of length with the shell closed) did not differ between groups or with temperature change and averaged 1.28. The power required to accelerate the valves and their associated added mass of water remained negligible (<1%) compared to the power requirements of jetting.

### 2.3.5 Interaction of variables

The relationship between peak and mean muscle mass specific power output,  $P_{max}$ , and  $\bar{P}_{spec}$ , was significantly different between the autumn and winter groups (table 5) with acclimated animals producing higher  $P_{max}$  for a given  $\bar{P}_{spec}$  (t-test,  $p < 0.05$ ,  $df = 16$ ). Winter animals swam in a much more jerky manner with  $U_{bmax}$  significantly higher for the same  $\hat{U}_b$  in autumn acclimated animals (t-test,  $p < 0.001$ ,  $df = 16$ ).

Whole body and muscle performance measures were plotted against each other to determine the strength of these relationships.  $P_{max}$  and  $\bar{P}_{spec}$  were both significantly related to maximum acceleration,  $X_{bmax}$  (table 5) but not to  $U_{bmax}$  or  $\hat{U}_b$ . In each group the relationship between power output and thrust (T) based on the animal's acceleration, body mass and associated water had higher  $R^2$  values than acceleration itself. There was no significant difference in any of these relationships between acclimatisation groups (table 5).

Table 5

|                             |                      | Group  |       |        |       | Between seasons |            |
|-----------------------------|----------------------|--------|-------|--------|-------|-----------------|------------|
|                             |                      | Autumn |       | Winter |       | Slope           | Elevation  |
|                             |                      | RSq    | p     | RSq    | p     | p               | p          |
| <b>Maximum power output</b> | Maximum velocity     | N/S    |       | N/S    |       | N/S             | N/S        |
|                             | Mean velocity        | N/S    |       | N/S    |       | N/S             | N/S        |
|                             | Maximum acceleration | 0.389  | 0.04  | N/S    |       | N/S             | N/S        |
|                             | Maximum thrust       | 0.41   | 0.034 | N/S    |       | N/S             | N/S        |
|                             | Mean power output    | 0.88   | 0.001 | 0.913  | 0.003 | 0.05            | Not tested |
| <b>Mean power output</b>    | Maximum velocity     | N/S    |       | N/S    |       | N/S             | N/S        |
|                             | Mean velocity        | 0.397  | 0.038 | N/S    |       | N/S             | N/S        |
|                             | Maximum acceleration | 0.447  | 0.022 | N/S    |       | N/S             | N/S        |
|                             | Maximum thrust       | 0.459  | 0.025 | N/S    |       | N/S             | N/S        |
| <b>Maximum velocity</b>     | Mean velocity        | 0.688  | 0.004 | 0.558  | 0.05  | 0.001           | Not tested |

Table 5. Interaction of whole-body and muscle performance measures. Differences in interactions were investigated using t-test on the slopes and elevations of the regression lines. Shaded fields denote regressions and t-tests which were not significant at  $p=0.05$ . Where the slopes of the lines were significantly different differences in elevations were not tested for.  $df=16$  in each case.

### 2.3.6 Scaling

Although the body size range of the animals used in the current study was small, (49-68mm) all whole-body and muscle performance variables were plotted against shell height (*Lsh*) in order to investigate the effects of scale. Increasing *Lsh* significantly increased peak flow and jet velocity in both acclimatisation groups. This did not translate into an increase in muscle mass or area specific performance due to concurrent increases in muscle size (table 4B). The relationship between muscle mass and shell height differed between seasons with winter animals having a slight but significant reduction in shell-height specific muscle mass.  $\dot{U}b$  was significantly higher in the freshly caught scallops (Autumn and Winter animals combined) than in the laboratory acclimated animals (ANCOVA,  $p < 0.001$ ,  $df = 33$ ).

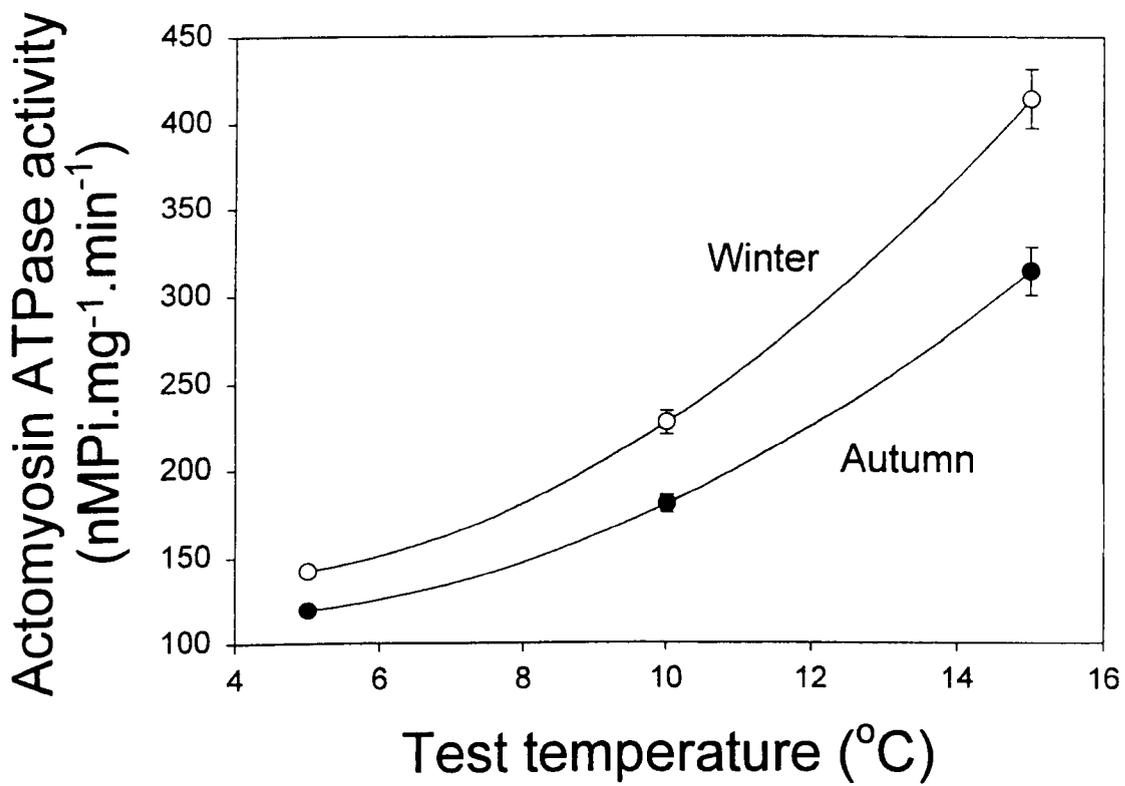
### 2.3.7 Actomyosin ATPase activity

Actomyosin ATPase activity was significantly higher in the Winter group than in the Autumn group (fig. 1.31). At 15°C mean ATPase activity in the winter group was 414 nMPi.mg protein.min<sup>-1</sup> while in the autumn group it was 314 nMPi.mg protein.min<sup>-1</sup>. This represents an average difference in ATPase activity of 31% between the two seasonal groups when assayed at the same temperature. Significant differences in ATPase activity were observed between acclimatisation groups at all temperatures (ANOVA,  $p < 0.003$ ,  $df = 71$  at each temperature).

### 2.3.8 SDS-PAGE electrophoresis

No differences in apparent molecular mass or density could be detected in the 1st Dimension SDS-PAGE gels or the myosin heavy chain peptide maps. 1D

Fig. 13 Actomyosin ATPase activity in Autumn (closed circles, ●) and Winter (open circles, ○) animal fast adductor muscle at 5, 10 and 15°C. Activity was significantly higher in the winter animals at all test temperatures. ANOVA,  $p < 0.001$ ,  $df = 71$ . Points presented are pooled data for all 3 experiments,  $\text{mean} \pm 1\text{SE}$ .



Gels allowed the apparent discrimination of myosin heavy chain (approx. 200,000 daltons), paramyosin (100,000), actin, (40,000), tropomyosin (35,000) and light chain (20,000). Protease digestion resulted in multiple bands. The identities of individual bands were not investigated further as no difference in pattern was detectable.

## 2.4 Discussion

### 2.4.1 Effects of temperature on swimming

Whole-body performance was strongly affected by temperature in animals swimming at their acclimation temperature. While all animals attempted to swim at all temperatures, the proportion which succeeded in leaving the bottom was greatly reduced below 5°C. Animals acclimated to, and swimming at, higher temperatures swam faster, and “took-off” sooner with higher accelerations than animals at lower temperatures. These changes in whole-body performance were associated with a reduction in the time spent shortening the adductor and a resultant decrease in clap period. The difference between the 5°C animals and the other groups is particularly apparent. This group was the only one whose members failed to swim on more than 15% of occasions, failing to take off almost as often as succeeding. This may be associated with a fall in mean swim velocity below that required for the lift developed by the shell to support the weight of the animal (Thorburn and Gruffydd, 1979a). It is therefore unlikely that the 6 week acclimation used in the present study caused a compensatory reaction and may even have caused dormancy.

Previous studies on the swimming of *Aequipecten opercularis* recorded peak swimming speeds of only 25cm.s<sup>-1</sup> (Moore and Trueman, 1971). This is slower than was found in the present study and below the velocity at which the animals ought to have been able to sustain swimming according to the above criteria. Unfortunately many details of the previous study Moore and Trueman’s (1971) study (e.g. water temperature) are not recorded and the frame rate of the camera used was low (16 frames.s<sup>-1</sup>) so it is difficult to make further comparisons. Few studies of scallop swimming at different temperatures exist. The only study using high-speed recordings at different temperatures was

reported by Marsh (1990) in *Argopecten irradians* at 10 and 20°C. Marsh (1990) determined that clap frequency was controlled by the time from the end of shortening to the beginning of lengthening, with little modification to either the opening or closing phases with temperature. Again, few details of the study were provided and the representative data presented could be interpreted differently. Video recordings of *P. magellanicus* indicated a shortening of the power stroke with increasing temperature with shortening velocity increasing by a factor of 1.5 as temperature increased from 3.4 to 11.2°C (Dadswell and Weihs, 1990).

The results of the present study also show changes to the length and velocity of contraction during the shortening phase of the clap cycle with temperature change. Increased shortening velocity resulted in increased power output and force production. As these changes are confined to the closing phase relatively large changes in muscle performance cause only small changes in clap duration. Changes to whole-body performance early in the cycle show that modification by temperature is occurring throughout closure. Temperature dependence of the lengthening phase was not expected given that ligament properties were unchanged by the temperatures used in the study. A change in stress-strain relationship with temperature was shown by Alexander (1965) though this required a much higher temperature range (up to 67°C). Further investigation of the dynamic properties of the ligament at different temperatures is certainly needed to fully quantify its effect on work during the swimming cycle.

In scallops, muscle lengthening velocity is controlled by the elastic hinge ligament (whose properties change little with temperature) and the flow of water around the shell (Vogel, 1985). During opening swimming velocity was similar

at all temperatures so flow-assisted reopening is also unlikely to vary significantly with temperature. As opening is rapid compared to closing the force available from flow assisted reopening would have to be large in order to increase this velocity by a significant proportion. Vogel (1985) calculated that 18% of the total force during reopening was due to flow at a flow velocity of  $0.5\text{m}\cdot\text{s}^{-1}$ .

If muscle relaxation rate in *Argopecten* does modulate clap frequency (Marsh, 1990) this may be a mechanism to increase efficiency by reducing the energy expenditure of rapid calcium transport. Possibly *Argopecten* uses swimming more routinely than *Aequipecten*, the species is known to use swimming to move into seagrass beds in order to improve feeding efficiency and reduce predation (Hamilton and Koch, 1996; Wolf and White, 1997).

*In vitro* experiments with *Argopecten irradians* muscle show elevated intrinsic shortening velocity with increasing temperature and higher force production for a given shortening velocity (Olson and Marsh, 1993). Little change in the form of the workloops and force-velocity trajectories were observed in the experiments reported here. These results are in good agreement with those of Cheng and Demont (1997) and therefore differ from the experimentally derived workloops obtained by Olson and Marsh (1993). Temperature acts upon the shortening velocity of the muscle, changing the position on the force-velocity curve but not the form of the relationship. This results in peak tension at greater muscle length in cold acclimated ( $5^{\circ}\text{C}$ ). The form of the force-velocity trajectory is fixed by the relationship between shortening velocity and flow for an individual animal. This relationship is controlled by the plane area of the mantle cavity and the areas through which water enters and leaves the shell.

The transient force and power peak observed in workloop (Marsh and Olson, 1994) and pressure-flow experiments (Marsh et al., 1992) were not observed here. This is possibly a result of our incomplete understanding of mantle behaviour during early jetting. This is a significant limitation of the method used in the present study. The entire scallop fast adductor muscle is thought to contract simultaneously during swimming (Marsh et al., 1992) with the initiation of further contractions depending on muscle stretch (Mellon, 1968). Once escape swimming has begun the locomotory system is a cyclical pump, the performance of which is not controlled by fast muscle innervation. The modulation of shortening velocity and force production by the adductor then depends on the cross-sectional area of the jet. This is an unusual case where muscle and swimming performance is under the neurological control of a muscle system which itself does little or none of the actual work. The mechanism of interaction between the muscle and mantle systems remains uncharacterised at this time.

#### *2.4.2 Effects of field acclimatisation on swimming*

Whole-body and muscle performance characteristics differed between animals acclimatised to winter and autumn conditions in the field when swum at similar temperatures. Cold acclimatised animals accelerated faster and attained higher mean speeds during jetting. These changes were reflected in higher muscle mass specific power outputs and muscle tension in winter animals than in warmer (autumn) acclimatised animals from the same population.

Mean swimming velocity during jetting was significantly increased by temperature but mean cyclic swimming velocity was not significantly related to

temperature. This is probably because at higher temperature the length of the high-speed glide phase is reduced. The animal spends a greater proportion of the time during the first clap accelerating and decelerating and less at peak velocity as temperature increases. In most cases temperature dependency and differences between groups were strongest within the period of jetting. Reductions in the duration of this active phase of the cycle with increasing temperature reduce the effects of the increased performance obtained. The force-velocity trajectories and workloops obtained were similar in form between acclimatisation groups. Again the differences in performance observed result from changes in the range of the force-velocity trajectory rather than changes in the relationship.

The small differences in ATPase activity between groups present a possible mechanism for the occurrence of the differences in muscle performance observed. Changes in muscle myofibrillar ATPase with temperature acclimation have been observed in several fish species including goldfish (Johnston, 1979), carp (Heap et al., 1986). Integrated studies in have demonstrated that the observed differences in whole-animal and muscle performance following acclimation may be associated with these changes in myofibrillar ATPase activity (Johnson and Bennett, 1995a). In Johnson and Bennett's (1995) study the proportional increase in ATPase activity was an order of magnitude greater than that observed here and was marked by measurable changes in myosin heavy chain (as molecular weight of digested fragments). In the present study it was not possible to identify any changes in molecular weight of contractile proteins or the protease digested fragments of myosin. Various other techniques are available for the detection of differences in the molecular mass of contractile and regulatory proteins between groups of animals. In the absence

of differences in ATPase activity and in peptide maps to explain differences in muscle shortening velocity between groups of acclimated fish, Ball and Johnston (1996) utilised SDS-capillary electrophoresis revealing changes in the ratio of myosin light chain isoforms. Changes in light chain ratios with temperature acclimation has now also been documented in carp (Hirayama et al., 1997). This remains an interesting avenue for future study especially in the light of the differences the mechanisms of regulation of scallop muscle from other striated muscle and the differing effects of temperature on these mechanisms (Shiraishi et al., 1999).

The adaptive significance, if any, of the changes in performance described is not clear. Absolute swimming speed is unlikely to be of selective importance as the predators from which the scallop can normally escape are slow moving invertebrates such as seastars (Barbeau and Scheibling, 1994a). Acceleration and thrust provide the impetus to break free of a predator and escape and are therefore most likely to be important, with the requirement to gain sufficient speed (and therefore lift) to sustain swimming following. However, without more information on the physics of predator-prey interactions it is not possible to evaluate this further. Similarly the relationship between distance travelled and chance of re-location by the predator is not known. It would be of particular interest to know whether current flow is utilised by scallop to attain a downstream position relative to olfactory predators. In order for the increase in thrust observed in cold acclimated animals to be of adaptive significance it would have to demonstrably increase survival in actual encounters between scallops and their predators.

The only known difference between the groups was the date of sampling and therefore their immediate thermal history. Preparation for the summer reproductive season may reduce muscle mass in this species (Allison, 1994). A small reduction in mean muscle mass did occur between samplings though this is not enough to account for the difference in muscle specific performance observed.

### 2.4.3 Conclusions

Temperature dependent changes in swimming behaviour and performance have been demonstrated in the eurythermal scallop *Aequipecten opercularis*. In acclimated animals both whole-body and muscle performance measures were strongly modified by temperature with animals at 5°C often failing to swim at all. Force-velocity trajectories of muscle fibres do not differ in form with temperature because, in the absence of jet aperture changes, they are fundamentally constrained by the morphology of the animal.

The swimming behaviour of freshly caught animals differed according to their season of collection. Winter collected scallops demonstrated higher swimming velocities during jetting, muscle mass specific power output and muscle tensions than scallops caught in November. These changes were associated with changes in scallop muscle actomyosin ATPase activity.

In all groups temperature dependency was greatest during the period of jetting with reductions in the length of the active phase of the cycle preventing these changes from translating into proportional increases in overall swimming velocity.

## **Appendix A,**

### *A1 Modelling scallop swimming*

#### A1 Location of shell centre of mass.

In order to investigate the kinetics of valve movement the shell mass (left and right valves), centre of mass and 2<sup>nd</sup> moment of mass (moment of inertia) determined. The centre of mass of each valve was found by trigonometry from digital photographs (QV Link software) of dry, eviscerated shells. 25 pairs of valves covering the entire size range were used. 2mm diameter holes were drilled through the valves; one close to the anterior margin and one close to the margin halfway between the anterior margin and the hinge. The valve was suspended by a pin through each hole and a digital photograph taken. The distance between the holes (Lholes) and the angles (A and B) between vertical and the line joining the holes in the two photos was used to estimate the distance of the centre of mass from the holes (Lcom).

As the hinge hung directly beneath the hole on the anterior margin shell height minus the distance between the centre of mass and the anterior hole was used as an estimate of the distance from the hinge to the centre of mass (*Lhcom*).

#### A2 Measurement of projected shell areas

Shell areas (as) in the commissural plane were determined from digital photographs by digitising 50 points around the outline of eviscerated shells (QV link and WinTV software with a Visual Basic co-ordinate taking program). The areas of the non-right-angled triangles formed by the digitised points and the first digitised point according to the formula below where a, b and c are the

lengths of the lines joining the digitised points. The sum of the areas enclosed in the non-equilateral triangles gave the total projected area of the shell.

$$as = \sqrt{s(s-a)(s-b)(s-c)}$$

where :

$$s = \frac{a+b+c}{2}$$

Plane area ( $as$ ) approximates well (Table A1, allometric relationships) to an ellipse where:

$$as = \pi \left( \frac{Lsh \cdot Lsl}{4} \right)^2$$

The volume of each valve ( $V_s$ ) approximates ( $R^2=0.71$ ,  $p<0.001$ ,  $df=9$ ) to the volume of a paraboloid with an elliptical flat surface according to the formula (comparison with weight of valves filled with water). It is necessary to use this approximation to ensure consistency with the preceding equation when calculating instantaneous animal mass.

$$V_s = \frac{\pi}{8} \cdot Lsh \cdot Lsl \cdot (Lsdh + Lsdb)$$

### A3 The mathematical model

A simple model of scallop swimming was constructed which used the detailed morphological measurements and high-speed video sequences of take-off behaviour to estimate muscle performance. Briefly, the change in volume of water within the shell during clapping (flow,  $f$ ) and the jet cross-sectional area ( $a_{jet}$ ) were used to calculate jet mass and velocity. From this jet power output was determined, and with knowledge of muscle shortening velocity ( $Um$ ) force production ( $F$ ) could also be calculated.

## Assumptions

The mathematical model assumed that the valves are rigid, and pivoted only at the hinge. Digitised silhouettes of scallops during swimming showed no change in lateral section area not accounted for by the volume of the mantle cavity. It was not possible to determine whether or not the shell was deformed laterally (i.e. gets wider or narrower). Shell deformation could affect swimming significantly if it allowed further water to be jetted after shell closure by reducing internal volume. This does not appear to be possible in this case.

Water was assumed to be inviscid and incompressible at the pressures involved in this study. Viscous forces might act at the whole body level and in the formation of the jets. Scallops are relatively large ( $Lsh \geq 57\text{mm}$  in all experiments) and their whole-body swimming velocity ( $Ub$ ) reasonably fast so Reynolds numbers ( $Re$ ) as derived from the formula below exceed 10,000 at the experimental temperatures.

While jet areas are necessarily much smaller the water velocities during jetting

$$Re = \frac{(Lsh \times Ub)}{\nu}$$

are relatively large ( $>2\text{m}\cdot\text{s}^{-1}$ ). Experiments in fish have demonstrated that the effects of temperature on viscosity are insignificant compared to its effects on physiology for anything bigger than larvae and early juvenile fish (Johnson et al., 1998; Podolsky and Emlet, 1993; Weihs, 1980)

The scallop makes use of extremely efficient energy return by the hinge ligament (DeMont, 1988) assisted by water flow (Vogel, 1985) to effect the non-power producing phase of the cycle. Indeed over 99% of the work done by the adductor muscle goes to power the jets (Cheng et al., 1996a), the power output

of which was determined in a similar way in the present study. The error caused by not modelling the complete hydrodynamics of the scallop is therefore small.

### Calculation of muscle length

The gape angle ( $A_g$ ) was calculated from the distance between the digitised points ( $L_{g_{points}}$ ) on the valve edges and their distance from the hinge ( $L_{sh_{points}}$ ), fig. A1). The distance from the points to the hinge was measured in the first shot of the sequence and remains the same throughout. The angles between the centreline and the muscle scars were calculated from the depth of the valves ( $L_{sd_t}$  and  $L_{sd_b}$ ) and the distances from the scars to the hinge parallel to the centreline. These measurements gave the internal top and bottom angles,  $A_{i_t}$  and  $A_{i_b}$ , and remain constant throughout. The sum of these angles was then the angle over which the muscle was extended ( $A_{mext}$ ).

$$A_{mext} = A_g + A_{i_t} + A_{i_b}$$

$$= 2 \text{ArcSin} \left( \frac{L_{g_{points}}}{2L_{sh_{points}}} \right) + \text{ArcTan} \left( \frac{L_{sd_t}}{L_{sh} - L_{mf_t}} \right) + \text{ArcTan} \left( \frac{L_{sd_b}}{L_{sh} - L_{mf_b}} \right)$$

Muscle length was calculated by considering the two valves separately in order to keep the geometry relatively simple. Muscle length above the centreline was calculated from angle  $A_{i_t}$  the angle from the commissural plane to the valve edge (equal to half the gape angle  $A_g$ ), and the direct distance between muscle scar and hinge ( $L_{mh}$ ). The length of the lower portion of the muscle was calculated in the same way and the two summed to give the total muscle length,  $L_{m_v}$ .

$$Lm_{ht} = \sqrt{Ls_{dt}^2 + (Lsh - Lm_{fi})^2}$$

$$Lm_{hb} = \sqrt{Ls_{db}^2 + (Lsh - Lm_{fb})^2}$$

$$Lm_v = \sin\left(\left(\frac{Ag}{2}\right) + Ai_u\right) \cdot Lm_{ht} + \sin\left(\left(\frac{Ag}{2}\right) + Ai_b\right) \cdot Lm_{hb}$$

The adductor muscle may be obliquely positioned within the shell, both laterally and front to rear. The lateral displacement ( $Lmm$ ) was measured upon dissection and used to calculate a corrected muscle length where the length taking into account lateral displacement was the hypotenuse, and the uncorrected length and lateral displacement the known two sides of a triangle (fig. A1).

$$Lm_{lat} = \sqrt{M_v^2 + Lmm^2}$$

The front to rear displacement ( $Lm_{anti}$ ) was variable due to the rotation of the shells during movement. This displacement was maximal at Gape ( $Lg$ )=zero and equal to the horizontal difference between the two muscle scars. This displacement was reduced as gape increases until at  $Lg=180$  degrees the displacement would be zero as the scars would be above each other. Lateral displacement would remain at the same value throughout.

The front to rear displacement parallel to the commissural (x,z) plane is needed to calculate muscle length where the actual muscle length ( $Lm$ ) was the hypotenuse and the front to rear displacement ( $Lm_{anti}$ ) and previously calculated length ( $Lm_{lat}$ ) were the known sides (fig. A1).

$$Lm_{anti} = \cos\left(\left(\frac{Ag}{2}\right) + Ai_u\right) \cdot Lm_{ht} - \cos\left(\left(\frac{Ag}{2}\right) + Ai_b\right) \cdot Lm_{hb}$$

$$Lm = \sqrt{Lm_{lat}^2 + Lm_{anti}^2}$$

### Jet characteristics

Jet area ( $a_{jet}$ ) (see fig. A1) was calculated from gape ( $Lg$ ) based on measurements of gape distance and jet area using high-speed (250 fps) video (table A1). A scaling formula was generated which relates shell height to peak jet area (see table A1); this may slightly under estimate jet performance late in the cycle but gives realistic values for the peak muscle performance. Cheng and Demont (1996) used mean jet area in their calculations and possibly overestimate peak performance as a result. The circumference ( $Lc$ ) of the projected shell area ( $as_{proj}$ ), the length of the arc blocked by the hinge ( $Lh_{arc}$ ) and gape were used to calculate the gape area through which water enters the shell on re-opening,  $ag$ .

$$ag = (Lc - Lh_{arc}) \cdot \left( \frac{Lg}{2} \right)$$

Jet cavity volume ( $vc$ ) was calculated from the projection of the elliptical plane area of the valves onto the commissural plane,  $as_{proj}$ , (based on shell length,  $Lsl$ , and projected shell height,  $Lsh_{proj}$ ) and gape distance,  $Lg$  (fig. A1).

$$Lsh_{proj} = \sqrt{Lsh^2 + \left( \frac{Lg}{2} \right)^2}$$
$$as_{proj} = \pi \left( \frac{Lsh_{proj} \cdot Lsl}{2} \right)$$
$$vc = \left( \frac{as_{proj} \times Lg}{2} \right)$$

Flow ( $f$ ) was calculated from the cavity volume data by differentiating a moving cubic regression of cavity volume against time. Jet velocity ( $U_{jet}$ ) was calculated by dividing flow by vent area ( $a_{jet}$ ). Intake velocity ( $U_{in}$ ) was calculated by dividing flow by gape area ( $ag$ ). Changes in the sign of the flow term were used to trigger the change from output through the vent to intake through the gape.

$$U_{in} = \frac{f}{ag}$$

$$U_{jet} = \frac{f}{a_{jet}}$$

Power output ( $P$ ) was calculated from flow ( $f$ ), jet or intake water velocity ( $U_{jet}$  or  $U_{in}$  respectively) and seawater density at the experimental temperature ( $\rho$ ).  $U_{jet}$  was used in the formula below, where  $U_{in}$  was used to calculate power required during opening. Dividing by adductor mass ( $M_m$ ) gave specific power output per kg adductor wet weight ( $P_{spec}$ ).

$$P_{spec} = \frac{\left( \frac{1}{2} f \cdot \rho \cdot U_{jet}^2 \right)}{M_m}$$

#### Muscle area specific force development

Muscle shortening velocity ( $U_m$ ) was calculated from the muscle length data by differentiating the moving cubic regression. Absolute power output in W ( $\text{Nm} \cdot \text{s}^{-1}$ ) was divided by shortening velocity,  $U_m$ , ( $\text{m} \cdot \text{s}^{-1}$ ) and adductor cross sectional area,  $ma$ , ( $\text{m}^2$ ) to give instantaneous force production per  $\text{m}^2$  of adductor tissue,  $F_{spec}$ . This was corrected for the rotational movement of the valves which results in the force developed by the muscle acting in a different direction to the direction of movement of the muscle scar and the oblique position of the muscle within the shell.

$$F_{spec} = \frac{P}{U_m \cdot ma} \cdot \left( \frac{1}{\cos\left(\frac{Ag}{2}\right)} \right) \cdot \left( \frac{1}{\left( \frac{L_m}{L_{mv}} \right)} \right)$$

#### Work done to accelerate valves

The angular velocity of the valves was calculated. From this the instantaneous velocity of the shell at the centre of mass ( $L_{hcom}$ ) was calculated. The kinetic energy of the shells (from the mass of the upper and lower valves,  $M_{st}$  and  $M_{sb}$ ) plus the added mass of water (Cheng et al., 1996a) could then be estimated and

its rate of change used to estimate power output required to accelerate the valves.

| Table A1  | Scaling coefficients |         | Abbreviation      |
|---|----------------------|---------|-------------------|
| Parameter   | b0                   | b1      |                   |
| <b>Angles (degrees)</b>   |                      |         |                   |
| Gape angle  |                      |         | $A_g$             |
| Internal angle from commissural plane to line joining hinge to muscle scar ( $L_{mh}$ ) |                      |         | $A_i$             |
| Muscle extension angle (in x,y plane)   |                      |         | $A_{m_{ext}}$     |
| Muscle pull angle (from y direction)  |                      |         | $A_p$             |
| <b>Areas (m<sup>2</sup>)</b>  |                      |         |                   |
| Gape area   |                      |         | $a_g$             |
| Jet area  | 0.187                | 4       | $a_{jet}$         |
| Adductor cross sectional area (resting)   |                      |         | $a_m$             |
| Instantaneous Adductor cross sectional area   |                      |         | $a_{m_{inst}}$    |
| Shell plane area  |                      |         | $a_s$             |
| Projected shell area  |                      |         | $a_{s_{proj}}$    |
| <b>Forces (kN or kN.m<sup>-2</sup>)</b>   |                      |         |                   |
| Force production of adductor muscle (kN)  |                      |         | $F$               |
| Peak tension (kN.m <sup>-2</sup> )  |                      |         | $F_{max}$         |
| Tension (muscle cross area specific force, kN.m <sup>-2</sup> )                         |                      |         | $F_{spec}$        |
| Mean cyclic tension (kN.m <sup>-2</sup> )   |                      |         | $\bar{F}_{spec}$  |
| <b>Lengths (m)</b>  |                      |         |                   |
| Shell circumference   |                      |         | $L_c$             |
| Gape  |                      |         | $L_g$             |
| Gape at digitising points   |                      |         | $L_{g_{points}}$  |
| Hinge length  | -0.0226              | 0.8822  | $L_h$             |
| Hinge arc   |                      |         | $L_{h_{arc}}$     |
| Hinge to centre of mass of valve  |                      | 0.491   | $L_{h_{com}}$     |
| Adductor length (taking into account lateral and anterior-posterior obliqueness)        |                      |         | $L_m$             |
| Anterior-posterior displacement of muscle scars   | 0                    | 0.00601 | $L_{m_{anti}}$    |
| Muscle scar to front lip of shell   |                      |         | $L_{mf}$          |
| Muscle scar to hinge  |                      |         | $L_{mh}$          |
| Adductor length (corrected for lateral offset)  |                      |         | $L_{m_{lat}}$     |
| Lateral displacement of muscle scars in x direction                                     | 0.0061               | -0.0975 | $L_{mm}$          |
| Maximum adductor length   |                      |         | $L_{m_{max}}$     |
| Adductor length (assuming vertical)   |                      |         | $L_{m_v}$         |
| Shell depth   | -0.0041              | 0.4333  | $L_{sd}$          |
| Shell height  |                      |         | $L_{sh}$          |
| Hinge to digitising points  |                      |         | $L_{sh_{points}}$ |
| Projected shell height  |                      |         | $L_{sh_{proj}}$   |
| Shell length  | -0.0041              | 1.11146 | $L_{sl}$          |

|   |         |        |                       |
|---|---------|--------|-----------------------|
| <b>Masses (kg)</b>  |         |        |                       |
| Body mass   | -0.0574 | 1.5889 | $M_b$                 |
| Instantaneous body mass                                       |         |        | $M_{b_{inst}}$        |
| Adductor mass*  | -0.0237 | 0.5258 | $M_m$                 |
| Mass upper valve  | -0.0075 | 0.2336 | $M_s$                 |
| <b>Power outputs (W or W.kg<sup>-1</sup>)</b>                 |         |        |                       |
| Power (W)   |         |        | $P$                   |
| Mass specific power (W.kg <sup>-1</sup> )                     |         |        | $P_{spec}$            |
| Peak mass specific power output (W.kg <sup>-1</sup> )         |         |        | $P_{max}$             |
| Mean cyclic mass specific power output (W.kg <sup>-1</sup> )  |         |        | $\bar{P}_{spec}$      |
| <b>Thrusts (N)</b>  |         |        |                       |
| Thrust, as calculated from acceleration                       |         |        | $T$                   |
| Maximum thrust  |         |        | $T_{max}$             |
| Minimum thrust (effective) – net drag                         |         |        | $T_{min}$             |
| <b>Velocities (m.s<sup>-1</sup>)</b>                          |         |        |                       |
| Swimming velocity   |         |        | $U_b$                 |
| Peak swimming velocity  |         |        | $U_{b_{max}}$         |
| Mean cyclic swimming velocity                                 |         |        | $\bar{U}_b$           |
| Mean swimming velocity during jetting                         |         |        | $\bar{U}_{b_{jet}}$   |
| Intake velocity   |         |        | $U_{in}$              |
| Jet velocity  |         |        | $U_{jet}$             |
| Adductor shortening velocity (m.s <sup>-1</sup> )             |         |        | $U_m$                 |
| Adductor shortening velocity (Fibre lengths.s <sup>-1</sup> ) |         |        | $\hat{U}_m$           |
| Peak shortening velocity (Fibre length.s <sup>-1</sup> )      |         |        | $\hat{U}_{m_{max}}$   |
| <b>Volumes (m<sup>3</sup>)</b>                                |         |        |                       |
| Jet cavity volume   |         |        | $V_C$                 |
| Shell volume  |         |        | $V_S$                 |
| <b>Accelerations (m.s<sup>-2</sup>)</b>                       |         |        |                       |
| Peak whole body acceleration (tangential)                     |         |        | $X_{b_{max}}$         |
| Whole body acceleration (tangential)                          |         |        | $X_{b_{tang}}$        |
| Whole body acceleration (total)                               |         |        | $X_{b_{total}}$       |
| Peak whole body acceleration (total)                          |         |        | $X_{b_{total_{max}}}$ |
| <b>Miscellaneous</b>  |         |        |                       |
| Flow (m <sup>3</sup> )  |         |        | $f$                   |
| Strain ( $L_m/L_{m_{max}}$ )                                  |         |        | $S$                   |
| Kinematic viscosity (m <sup>2</sup> .s)                       |         |        | $u$                   |
| Density of seawater (kg.m <sup>-3</sup> )                     |         |        | $\rho$                |

The subscripts <sub>t</sub> and <sub>b</sub> added to the above variables are used where appropriate to denote the top and bottom valves respectively

Fig A1, Side view, anatomical dimensions used in the calculations

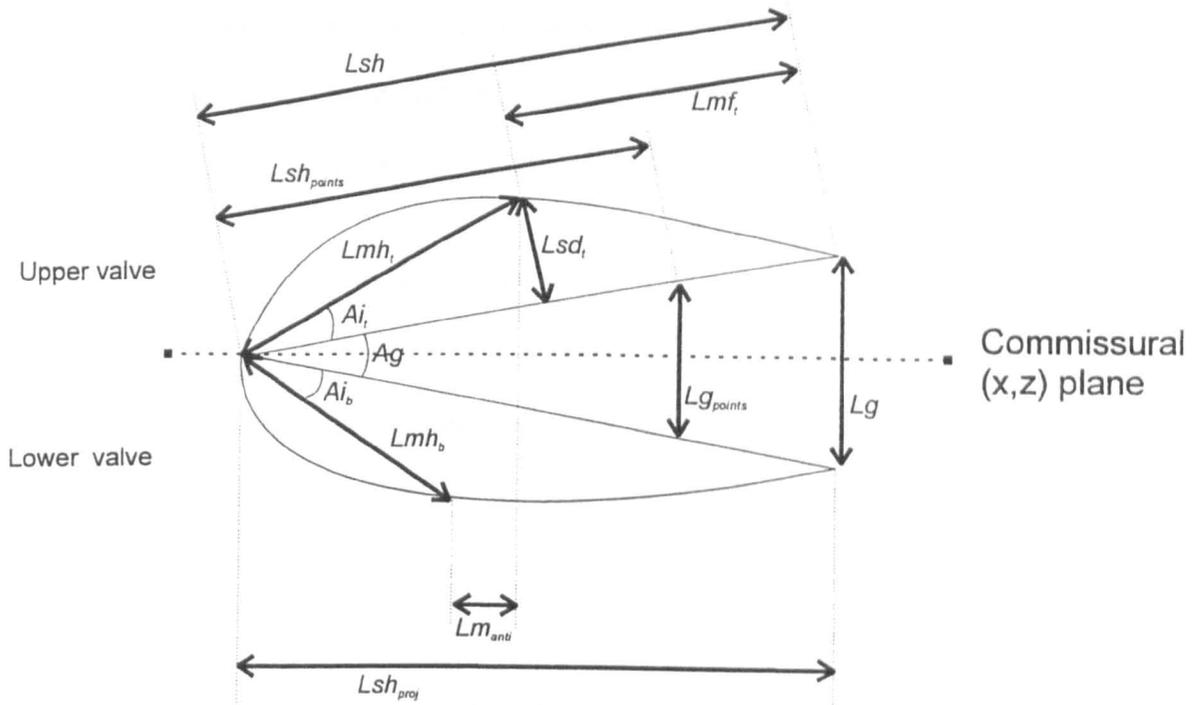
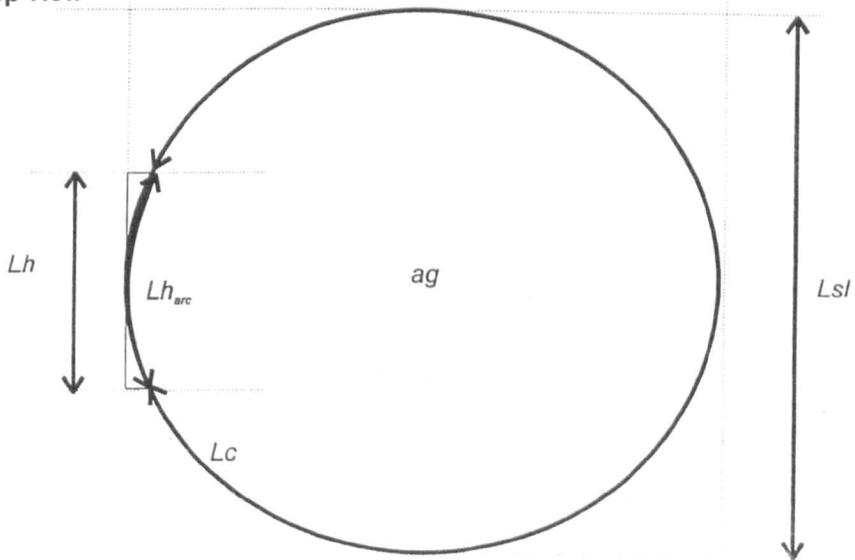


Fig A2, Top view



### **Chapter 3**

## **EFFECTS OF TEMPERATURE AND ACCLIMATION ON OF THE SWIMMING PERFORMANCE AND *IN VITRO* MUSCLE CHARACTERISTICS OF THE ANTARCTIC SCALLOP, *ADAMUSSIUM COLBECKI*.**

### **3.1 Introduction**

The scallop *Adamussium colbecki* is a common Antarctic invertebrate, with a circum-polar distribution. In the waters close to the Antarctic continent the physical environment is typified by a cold but stable temperature, highly seasonal changes in day length and light intensity, and ice impact in exposed and/or shallow areas (Arntz et al., 1994). These cold conditions are believed to have developed gradually since the opening of Drake's Passage and the formation of the Circum-Antarctic Current around 20 million years ago (Clarke, 1983). The resulting fauna show a high degree of endemism (Clarke, 1996; Dayton, 1990) and a range of adaptations to the physical challenges of the polar marine environment.

*AdamussiumA. colbecki*s reaches abundances exceeding 60 individuals per square metre (Berkman, 1988; Chiantore et al., 1998). Swimming in this species has been described by Ansell et al (1998) although the muscular performances required were not estimated. The ecological importance of swimming in this animal has also not been assessed. Predation rates are thought to be low in the wild and dispersal distances reduced compared to temperate scallops (Berkman, 1990; Cliche et al., 1994). The only reported natural stimulus for the initiation of swimming was the presence of a hyposaline lens below sea ice (Berkman, 1988). Other suggested functions of scallop swimming in other species have included seasonal migration (Anderson et al.,

1997) and movement to better habitats (Hamilton and Koch, 1996; Wolf and White, 1997). In *A. colbecki* this might include exploiting habitats made available by iceberg scour and anchor ice.

Antarctica is bounded by the polar frontal zone, which hydrographically separates the Southern Ocean from the South Atlantic. *A. colbecki* is found only within the Polar-frontal zone and therefore experiences extremely low water temperatures throughout the year. Environmental temperature has strong effects on the physiology of ectothermic animals. For example in scallops swimming velocity and clap frequency increase as temperature rises with associated changes in the form of the clap cycle (Dadswell and Weihs, 1990; Marsh, 1990). These changes are associated with direct effects of temperature on the intrinsic properties of scallop muscle (Olson and Marsh, 1993). Similar effects have been shown in a range of terrestrial, freshwater and marine species. (See Bennett (1990) for a review).

Much interest has been shown in the mechanisms used by polar animals to survive at low temperatures and the extent to which adaptation has allowed them to compensate for their environmental temperature in terms of physiological rates. While resistance adaptations such as antifreezes (Duman and DeVries, 1975), cold-resistant cellular microtubules (William et al., 1985) and antioxidant defences (Viarengo et al., 1995) are well documented, detecting capacity adaptation has proved more difficult.

Skeletal muscle myofibrillar ATPase activity (Johnston et al., 1975) and mitochondrial density (Johnston et al., 1998) are both elevated in Antarctic fish, however, when fish larvae or adult fish of similar morphology from different

latitudes were compared there was no evidence of compensation in swimming performance (Johnston et al., 1991b; Wakeling and Johnston, 1998). Similarly the oxygen consumption of individual mitochondria shows no compensation for low temperature (Johnston et al., 1998). Indeed, the need to increase mitochondrial density in order to maintain aerobic capacity in the absence of cold-adapted aerobic enzymes may reduce the potential performance of Antarctic fish muscle (Johnston et al., 1991a). The differences in temperature tolerance between species make representative comparisons more difficult although *in vitro* experiments are possible. Experiments with dissected muscle preparations have shown that Antarctic fish white muscle produces similar forces as temperate and tropical fish muscle but contracts more slowly (Altringham and Johnston, 1986; Johnston and Altringham, 1985) resulting in the limitation of swimming performance observed (Johnston et al., 1991b; Wakeling and Johnston, 1998).

In the present study measurements of swimming were made from video recordings of the Antarctic scallop *Adamussium colbecki*. A simple model based on measurements of animal position, jet area and valve motion was used to calculate whole-body velocity and acceleration and to predict the power output and force production of the adductor muscle. The potential of laboratory acclimation temperature and acute temperature change to modify swimming performance was investigated. Animals were intolerant of temperature change and no modification of performance by acclimation was detected. It appears that the phenotypic plasticity of this species is limited with respect to temperature change. The relationships between water temperature and power output or swimming velocity in *Adamussium* were compared to published data for a range of scallop species over a 20°C temperature range. In both cases a

single function described the relationship in all species. *Adamussium* does not demonstrate temperature adaptation in terms of its swimming performance.

## 3.2 Materials and methods

### 3.2.1 Swimming activity in wild animals.

#### Study site

The scallop population studied was confined to one area approximately 30m across at 20-28m depth and a smaller group at 12msw in North Cove, Rothera Point, Adelaide Island (67°34' S, 68°08' W). The bottom is an artificial boulder slope forming the base of the station airstrip, which extends to a maximum depth of 34msw where it meets the natural sand/silt bottom. The bottom shelves upward to beaches to the South of the scallop sites so that at the location of the smaller group of animals the sediment bottom is at 15m.

All sites in the area are heavily ice-impacted, soft bottom areas are dominated by the infaunal bivalve *Laternula elliptica* with high numbers of the predators such as the nemertean *Paborlasia*, the starfish *Odontaster validus* and the gastropod *Neobuccinum eatoni* present (personal observations).

#### Escape behaviour

The dominant mode of life for *A. colbecki* at Rothera Point is byssally attached with very few free-living animals (personal observations). Animals were stimulated to attempt escape swimming in order to determine whether the attached animals could break free of the bottom and make active escapes.

Two seastars (*Odontaster validus*) were homogenised in 1 litre of seawater and the resulting homogenate transferred into two 500ml (deionised water) bottles. A further 500ml of ice-cold freshwater was placed in a third bottle. Homogenised seastar and freshwater reliably produce an escape response in

*Adamussium colbecki* in the laboratory (Personal observations). Freshwater simulates the effects of hyposaline lens encroachment, an observed trigger to swim in wild populations (Berkman, 1988). Scallops (n=7) were approached *in situ* on the seabed and the seastar homogenate or freshwater squirted over the animal. The type of stimulus, response and whether the animal broke free of the rocks were recorded on a slate.

### Movement and mortality

Due to the low numbers of scallops on the site (approximately 30 at the time of study) and their sparse distribution, it was not feasible to reliably survey the existing population to determine survivorship. Fifteen scallops were collected from the general population and brought into the laboratory. They were then marked by gluing a numbered foil patch on each valve and aggregated in one site where part of the population was located.

The scallops were placed on a level, rock surface (50cm x 120cm) at 8.5m depth which was marked with a pellet buoy. The site was chosen to give a degree of protection from the North (direction of iceberg approach) but was otherwise open. This site was visited 24hr after placement of the animals and then weekly for 2 months. Whether or not the animals were attached and the distance moved by each animal was recorded.

### 3.2.2 *Experimental animals*

#### *Adamussium colbecki*

78 animals were obtained using SCUBA diving in 3 batches. The first batch of 27 animals was collected 29/11/98 and split into 3 groups of the same mean

shell height and acclimated to  $-1^{\circ}\text{C}$  ( $\pm 0.2$ ) and  $+2^{\circ}\text{C}$  ( $\pm 0.3$ ) (mean  $\pm$  range) in the controlled temperature room of the Bonner Laboratory, Rothera for 6 weeks prior to experimentation. Attempts to acclimate *A. colbecki* to  $5^{\circ}\text{C}$  failed, as did a following attempt at  $4^{\circ}\text{C}$ , due to the rapid loss of condition and consequent mortality in the groups before the end of acclimation (50% mortality in 14 and 19 days respectively).

Animals were maintained in 30l temperature controlled recirculating seawater aquaria (Grants, Cambridge). Independent electric pumps aerated the tanks and approximately one third of the water in each tank was changed each day after the replacement water had been brought to the appropriate temperature. A constant low-light regime was maintained in order to mimic the Antarctic summer conditions. Limitations on space and equipment prevented the use of replicate tanks.

The other collections were 12/12/98 ( $n=36$ ) and 07/01/99 ( $n=15$ ). These animals were maintained at ambient light and temperature levels ( $0^{\circ}\pm 0.5^{\circ}\text{C}$ ) in 1.75m diameter tanks of through-flow seawater in the main aquarium area. The swimming behaviour of animals of these animals were compared to those of animals acclimated to temperatures above and below the natural summer temperature. In such experiments animals were referred to as "natural" if they were maintained at local sea temperature or acclimated if their maintenance temperature was manipulated.

Experimentation began on "natural" animals after 24hr of arriving in the laboratory and continued for 4 weeks. Experimentation began on the acclimated animals on 30/01/99 and continued for 3 weeks.

A further 20 animals were collected immediately prior to the end of the field season (04/03/99) for experiments in the UK. These animals were maintained at in 400l aerated recirculating seawater tanks while being transported by ship and subsequently in the aquarium in St. Andrews, UK. These animals were maintained under constant very low light conditions to mimic, as far as practicable, Antarctic marine winter conditions.

#### *Zygochlamys patagonica*

24 animals were obtained from the Falkland Islands Government Fisheries Service in November 1997. Animals were maintained at the Gatty Marine Laboratory, St. Andrews in temperature controlled recirculating seawater at 5°C prior to use, 12hr light:dark. All scallops were fed, *ad libitum*, on the green alga *Isochrysis galbana*.

#### *Aequipecten opercularis*

50 *Aequipecten opercularis* were obtained in March 1998 from the University of Highlands and Islands aquaculture centre at Ardtoe, Scotland. These animals were maintained at 10°C, other details of husbandry were as for *Zygochlamys patagonica*.

### 3.2.3 Filming

Scallop escape responses were recorded on video at 25 frames (50 fields) per second (Panasonic WVP-F10E camera with WV-LZ14/8AFE 8x Auto Focus Power Zoom Lens). The camera was mounted on a tripod at least 3m from the swim tank to minimise perspective error. Twin 100W Spotlights were mounted on a second tripod 1m from the tank providing illumination at approximately 45°

to the line of sight of the camera. This level of light is much higher than experienced by the animals during holding but was required for a sufficiently high-quality image to be obtained. Tank setup was as described previously (Chapter 2)

### Experimental protocol

24hr before experimentation animals were marked with foil digitising points as described previously (this study, Chapter 2). Animals were moved into the swim tank and allowed to rest for a minimum of 6h before the first escape response was stimulated. Handling was minimised and animals were transported between tanks in water. The water in the original tank was slowly mixed with the water in the new tank in order to minimise any differences in temperature or salinity. 6h was the maximum time to 90% recovery recorded in this species for exhaustive exercise (This study, Chapter 4). The amount of swimming activity by each animal was well below this level even during swimming experiments.

Escape responses were then stimulated using freshwater at (or as close as possible to) tank temperature. Water was introduced to the rear of the animal, directly beneath the hinge, using a tube attached to a 20cm<sup>3</sup> syringe. Typically 10cm<sup>3</sup> was injected at approximately 2cm<sup>3</sup>.s<sup>-1</sup>. Freshwater is known to stimulate swimming in wild *A. colbecki* (Berkman, 1988) and therefore represents an ecologically valid stimulus with which to initiate escape responses. There was minimal disturbance of the water around the animal and no force was exerted on the body of the scallop itself. The area of reduced salinity visible around the animal typically dispersed before adduction. Animals were used in rotation with a minimum of 2hr between stimulations. For each stimulation the animal ID number, tank temperature ( $\pm 0.1^{\circ}\text{C}$ ), stimulus, and type of response was

recorded. In addition to the stimuli described above responses resulting from handling or occurring spontaneously were recorded.

Animals were swum initially at their maintenance temperature (acclimation temperature or the local water temperature at the time of the experiment, as appropriate). In order to investigate acute responses to temperature animals were placed in the swim tank, at their maintenance temperature, and the water temperature of the swim tank was increased or decreased by up to 1°C.

### Morphological measurements

Measurements of muscle mass and position and shell dimensions were taken in order to allow the calculation of muscle strain and mantle cavity volume during jetting. Relationships between shell height and each parameter of interest were determined. The full procedure was as described previously (Chapter 2, this study).

### Analysis of filming

Videos were analysed frame by frame using a PC (Gateway 2000 G6-266) with a video capture board (Hauppauge Win/TV). The x,y "on-screen" co-ordinates of the marked points on both valves were selected by hand and recorded by a purpose-designed package obtained from Dunstaffnage Marine Laboratory (Visual Basic 4, Microsoft) and output to an Excel spreadsheet. The spatial resolution of the data was limited by the image analysis program to 5 pixels.mm<sup>-1</sup> at a magnification of x2. On-screen analysis did not allow parallax error during digitising as the cursor was on the same plane as the point being digitised.

Initial processing was at 25 frames.s<sup>-1</sup>, on return to the UK the sequences which fulfilled the criteria for analysis were re-recorded onto Umatic video tapes and re-analysed field by field (50 fields.s<sup>-1</sup>). The criteria for analysis were as described previously (Chapter 2) with only the first clap of sequences being analysed. Close-up videos (x4 on-screen magnification) were also analysed to measure the behaviour of the mantle and jets during swimming. These determined the relationship between jet area and gape and the point at which the mantle seals during initial closure.

#### Data processing

Whole-body and muscle performances were calculated as described previously (Chapter 2, this study).

#### 3.2.4 *In vitro* muscle twitch characteristics

Studies of the contractile properties of isolated muscle fibres from *A. colbecki*, *Aequipecten* and *Zygochlamys* were carried out at the Gatty Marine Laboratory, St. Andrews. Experiments on *A. colbecki* were undertaken in June 1999, approximately 1 month after the arrival of the scallops in the UK. Experiments on *Zygochlamys* and *Aequipecten* took place from the 3-15 March 1998.

The ventral portion of the shell was cut back using a diamond edged circular saw blade on a variable speed electric drill (RS electronics, UK) exposing the adductor still attached to both valves. The majority of the adductor and other soft tissues were then dissected away to leave a small (<5mm width) bundle of fast muscle fibres attached to the valves. The muscle preparation was then removed from the valves with pieces of shell still attached to the ends. Foil

hooks were attached to the ends of the muscle preparation for connection to force and length transducers. The diameter of the preparation was then reduced by additional fibre dissected in ice-cold ringer (composition in mM; NaCl, 440; KCl, 10; MgSO<sub>4</sub>, 14; MgCl<sub>2</sub>, 30; CaCl<sub>2</sub>, 10; Imadazole, 20, pH 7.9 at 20°C, (Olson and Marsh, 1993)) and attached to the transducers in the muscle chamber. Once placed within the apparatus each preparation was allowed to rest for 1 hour in oxygenated, re-circulating ringer before experimentation.

Temperature control within the apparatus was maintained ( $\pm 0.2^\circ\text{C}$ ) by a pair of thermocirculators (Grants, Cambridge). One, containing a water/alcohol mix, cooled the ringer reservoir while the second contained a 50:50 mix of ethylene glycol antifreeze and water and cooled the jacket surrounding the rig. Oxygen was continuously bubbled through the ringer in the reservoir. The experimental chamber and cooling jacket was wrapped in foam insulation and the tubing from the two thermocirculators ran as a counter-current flow system within an insulated wrapping. This allowed the maintenance of low temperatures (down to 0°C in the chamber of the apparatus) without going below the freezing point of the ringer.

Stimuli were delivered by a pair of platinum wire electrodes and the force developed was detected by a silicon beam force transducer (AME 801, Sensor-Nor, Norway). A LabView (National Instruments) program on a desktop PC (Elonex PC-433) with a LabPC Plus data input/output board (National Instruments) controlled the stimulator and captured the resulting force data.

Muscle preparation length was adjusted and single stimuli delivered in order to find the optimum length ( $L_{opt}$ ) for twitch force production. Stimulus amplitude

and pulse width were then optimised in turn. Twitch experiments were then conducted and the temperature of the ringer was adjusted over the range 0-5°C. Chamber temperature was returned to zero and the initial twitch repeated hourly in order to check for deterioration of the muscle preparation. No change in twitch properties (time to max twitch, force produced) was observed even overnight once  $L_{opt}$  had been determined. Muscle cross-sectional areas were estimated by weighing the preparation after use and calculating the area from its length and an assumed density of 1060Kg.m<sup>-3</sup> (James et al., 1998).

### *3.2.5 Statistics*

Relationships between whole body and muscle performance measures and water temperature and shell height were investigated using linear regression and curve fitting (SPSS, Jandel Scientific). The slopes and elevations of the lines for each experimental group were compared using t-tests (Zar, 1996). Relationships between whole-body and muscle performances were investigated and compared between groups. Proportional data were normalised by Arc-Sine transformation prior to regression analysis.

### 3.3 Results

#### 3.3.1 Field studies of activity in *A. colbecki*

##### Escape responses *in situ*

All seven animals attempted to escape by swimming when stimulated by the presence of homogenised seastar or freshwater. All were unable to detach themselves from the rocks. Attempts to escape were characterised by several rapid adductions and rotation about the attached side of the hinge. The experiment was terminated after 7 animals had been observed due to deteriorating diving conditions.

##### Movement and survival in the wild

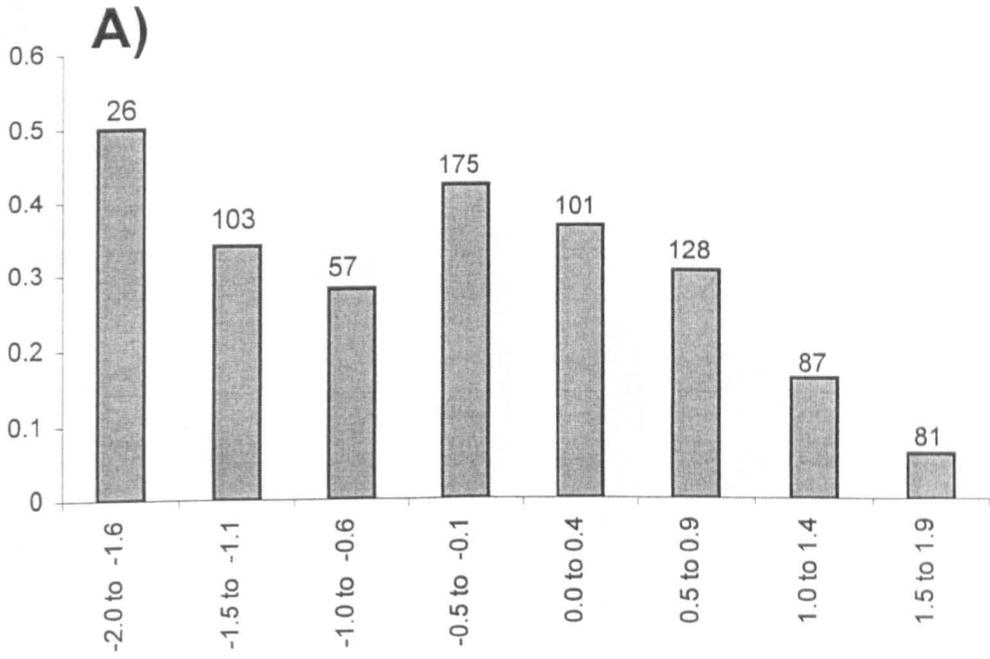
All scallops were attached to the site 24hr after placement. During the 2 months of observation a boulder disturbed by ice impact landed on the site and killed 3 animals. No movement or mortality was observed amongst the remaining 12 animals.

#### 3.3.2 Behaviour

The responses of all experimental animals to stimulation were recorded and categorised as swims, jumps, claps or no response (see previous chapter). The effect of ambient water temperature on the proportion of swims to other responses was determined. The escape behaviour of *A.colbecki* was strongly affected by temperature. Below  $-1.5^{\circ}\text{C}$ , 50% of scallops stimulated by freshwater responded by swimming (n=26). With increasing temperature this proportion fell until above  $1.4^{\circ}\text{C}$  only 6% of animals swum (n=81). The

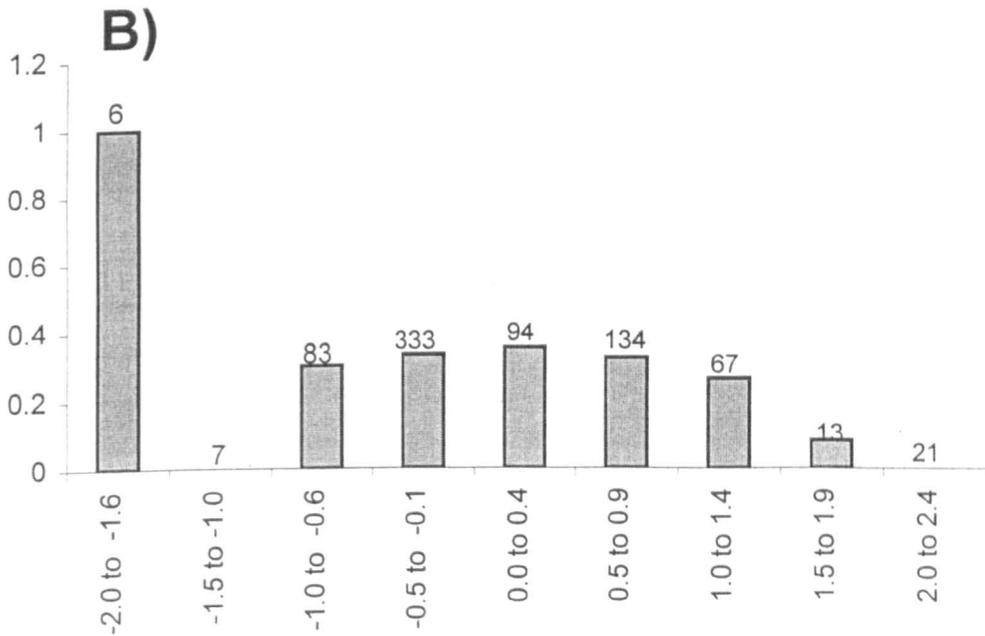
Figure 1. The effects of temperature and body size on the probability of swimming responses following stimulation by freshwater. Proportion of swims to total responses: A, at different temperatures, B at temperatures above and below acclimation/maintenance temperature and C, for different body sizes. Swimming responses were significantly less likely at higher temperatures. Swimming was less common where the animal's temperature was higher than its acclimation temperature and larger animals were less likely to swim than smaller ones. These relationships were not significant, see text for details.

Proportion of swims  
to total stimulations



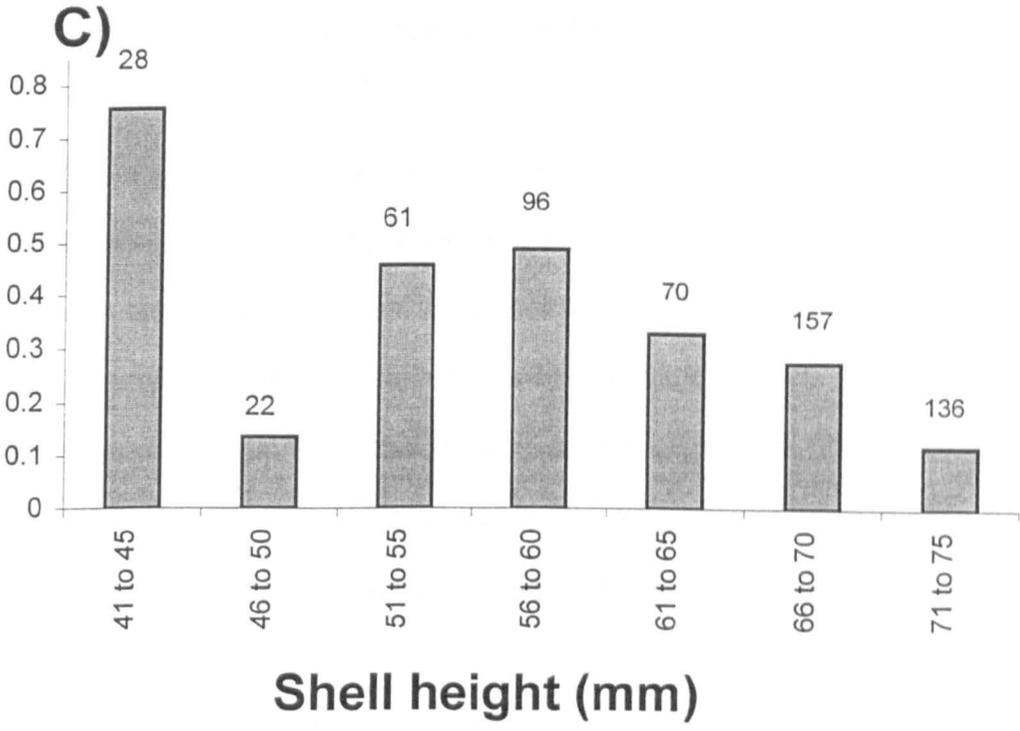
Test temperature (°C)

Proportion of swims  
to total stimulations



Test temperature (°C)

**Proportion of swims  
to total stimulations**



relationship between water temperature and the proportion of swims to total stimulations was significant ( $RSq=0.67$ ,  $p=0.013$ ,  $df=6$ ) (fig. 1 A).

The importance of thermal history in determining the scallop's behaviour was investigated by the relationship between the proportion of swims and the difference between ambient and acclimation temperature. The proportion of swims to total stimulations fell as the animals were warmed above their acclimation temperature though this relationship was not significant ( $RSq=0.28$ ,  $p=0.14$ ,  $df=7$ ) (fig. 1B). The proportion of swims also fell with increasing shell height but this relationship was not significant ( $RSq=0.36$ ,  $p=0.16$ ,  $df=5$ ) (fig. 1C).

### 3.3.3 *Swimming performance and effects of temperature*

No measures of whole-body or muscle specific performance differed significantly between groups when temperature was taken into account (Table 1A). As thermal history did not appear to strongly modify swimming performance in this species the remaining analyses were performed on pooled data for the groups combined (Table 2A).

The clap cycle in *A. colbecki* is similar to that described previously in *Aequipecten opercularis* (this study chapter 2) beginning with a period of rapid closing which declines in velocity as it continues. A period of low gape velocity is followed by rapid re-opening (fig. 1A). It is this period of low velocity that appears to modify the form and duration (fig. 2) of the clap cycle as temperature changes.

Swimming velocity rose rapidly during valve closure with peak velocity being attained close to the end of adduction. Velocity reduced slowly as reopening

Table 1A. Summary of regression analysis. Whole-body and muscle performance measures were regressed against water temperature.  $df=30$  for natural and 11 for acclimated animals. Shaded fields denote non-significant regressions. Differences between groups were investigated using ANCOVA with temperature as the covariate. No significant differences were detected between groups. No differences in relationships with temperature were detected between groups.

B. Summary of regression results for relationships between whole-body and muscle performance measures. Shaded fields denote non-significant regressions. Where significant relationships in both groups existed the slopes and elevations of the lines were compared using t-tests. No significant differences in relationships between variables were detected between groups in any case

Table 1A

| Performance type              | Variable                     | Group   |        |             |       |
|-------------------------------|------------------------------|---------|--------|-------------|-------|
|                               |                              | Natural |        | Acclimation |       |
|                               |                              | RSq     | p      | RSq         | p     |
| <b>Whole body performance</b> | Maximum velocity             | 0.369   | <0.001 | 0.125       | 0.236 |
|                               | Mean velocity                | 0.221   | 0.007  | 0.267       | 0.071 |
|                               | Maximum acceleration         | 0.280   | 0.002  | 0.072       | 0.375 |
|                               | Maximum thrust               | 0.381   | <0.001 | 0.161       | 0.174 |
|                               | Maximum drag                 | 0.367   | <0.001 | 0.055       | 0.442 |
| <b>Muscle performance</b>     | Maximum power output         | 0.255   | 0.003  | 0.257       | 0.077 |
|                               | Mean power output            | 0.138   | 0.036  | 0.553       | 0.004 |
|                               | Maximum force production     | 0.292   | 0.001  | 0.359       | 0.031 |
|                               | Mean force production        | 0.187   | 0.013  | 0.608       | 0.002 |
|                               | Maximum shortening velocity  | 0.007   | 0.653  | 0           | 0.977 |
|                               | Maximum lengthening velocity | 0.006   | 0.673  | 0.084       | 0.336 |
|                               | Maximum flow                 | 0.276   | 0.002  | 0.376       | 0.026 |
|                               | Maximum pressure             | 0.292   | 0.001  | 0.355       | 0.032 |
|                               | Maximum jet velocity         | 0.280   | 0.002  | 0.376       | 0.026 |
|                               | Maximum jet thrust           | 0.291   | 0.001  | 0.355       | 0.032 |

Table 1B

|                             |                      | Group   |       |             |       |
|-----------------------------|----------------------|---------|-------|-------------|-------|
|                             |                      | Natural |       | Acclimation |       |
|                             |                      | RSq     | p     | RSq         | p     |
| <b>Maximum power output</b> | Maximum velocity     | 0.274   | 0.002 | 0.072       | 0.376 |
|                             | Mean velocity        | 0.193   | 0.012 | 0.088       | 0.325 |
|                             | Maximum acceleration | 0.101   | 0.076 | 0.003       | 0.868 |
|                             | Maximum thrust       | 0.220   | 0.007 | 0.017       | 0.667 |
|                             | Mean power output    | 0.708   | 0.001 | 0.719       | 0.001 |
| <b>Mean power output</b>    | Maximum velocity     | 0.142   | 0.034 | 0.015       | 0.694 |
|                             | Mean velocity        | 0.197   | 0.011 | 0.009       | 0.754 |
|                             | Maximum acceleration | 0.062   | 0.189 | 0.001       | 0.913 |
|                             | Maximum thrust       | 0.141   | 0.034 | 0.052       | 0.452 |
| <b>Maximum velocity</b>     | Mean velocity        | 0.690   | 0.001 | 0.615       | 0.002 |

Table 2. Summary of regression results for all animals pooled. A) relationships with temperature and B) relationships with shell height. Significances, degrees of freedom and regression coefficients are presented. For relationships with temperature the  $Q_{10}$  (5-10°C) is also given, a  $Q_{10}$  of 2 denotes a doubling in rate over a 10°C temperature range. Shaded fields denote non-significant regressions.

Table 2A

| Performance type       | Variable  | Parameter |      |       |        |          |         |                 |
|------------------------|---|-----------|------|-------|--------|----------|---------|-----------------|
|                        |   | Rsq       | d.f. | F     | p      | b0       | b1      | Q <sub>10</sub> |
| Whole body performance | Maximum velocity (m.s <sup>-1</sup> )                 | 0.146     | 43   | 7.32  | 0.01   | 0.2347   | 0.0314  | 2.34            |
|                        | Mean velocity (m.s <sup>-1</sup> )                    | 0.147     | 43   | 7.39  | 0.009  | 0.1104   | 0.0162  | 2.47            |
|                        | Maximum acceleration (m.s <sup>-2</sup> )             | 0.106     | 43   | 5.11  | 0.029  | 1.4028   | 0.2521  | 2.80            |
|                        | Maximum thrust (N)                                    | 0.165     | 43   | 8.49  | 0.006  | 0.2003   | 0.0549  | 3.74            |
|                        | Maximum drag (N)                                      | 0.131     | 43   | 6.47  | 0.015  | -0.1521  | -0.042  | 3.76            |
| Muscle performance     | Maximum power output (W.kg <sup>-1</sup> )            | 0.241     | 43   | 13.64 | 0.001  | 64.62    | 24.72   | 4.83            |
|                        | Mean power output (W.kg <sup>-1</sup> )               | 0.299     | 43   | 18.36 | <0.001 | 15.58    | 7.345   | 5.71            |
|                        | Maximum tension (kN.m <sup>-2</sup> )                 | 0.276     | 43   | 16.41 | <0.001 | 81.1511  | 28.7144 | 4.54            |
|                        | Mean tension (kN.m <sup>-2</sup> )                    | 0.331     | 43   | 21.27 | <0.001 | 23.3241  | 10.3362 | 5.43            |
|                        | Maximum shortening velocity (Fl.s <sup>-1</sup> )     | 0.001     | 43   | 0.03  | 0.857  | -1.0729  | -0.005  | 1.05            |
|                        | Maximum lengthening velocity (Fl.s <sup>-1</sup> )    | 0         | 43   | 0.006 | 0.935  | 0.9088   | -0.0044 | 0.95            |
|                        | Mean velocity during shortening (Fl.s <sup>-1</sup> ) | 0.146     | 43   | 7.33  | 0.01   | -0.65127 | -0.0773 | 2.19            |
|                        | Maximum flow (kg.s <sup>-1</sup> )                    | 0.252     | 43   | 14.52 | <0.001 | -0.0001  | 0.0000  | 3.00            |
|                        | Maximum pressure (kPa)                                | 0.275     | 43   | 16.29 | <0.001 | 2.2776   | 0.8063  | 4.54            |
|                        | Maximum jet velocity (m.s <sup>-1</sup> )             | 0.254     | 43   | 14.63 | <0.001 | -1.9931  | -0.3706 | 2.86            |
|                        | Maximum jet thrust (N)                                | 0.275     | 43   | 16.29 | <0.001 | 0.2733   | 0.0968  | 4.54            |

Table 2B

| Performance type       | Variable   | Parameter |      |         |        |          |        |
|------------------------|--|-----------|------|---------|--------|----------|--------|
|                        |  | Rsq       | d.f. | F       | p      | b0       | b1     |
| Whole body performance | Maximum velocity (m.s <sup>-1</sup> )              | 0.126     | 43   | 6.180   | 0.017  | 0.010    | 0.003  |
|                        | Mean velocity (m.s <sup>-1</sup> )                 | 0.079     | 43   | 3.680   | 0.062  | 0.019    | 0.001  |
|                        | Maximum acceleration (m.s <sup>-2</sup> )          | 0.110     | 43   | 5.330   | 0.026  | -0.584   | 0.030  |
|                        | Maximum thrust (N)                                 | 0.286     | 43   | 17.200  | 0.000  | -0.360   | 0.008  |
|                        | Maximum drag (N)                                   | 0.218     | 43   | 11.970  | 0.001  | 0.268    | -0.006 |
| Muscle performance     | Maximum power output (W.kg <sup>-1</sup> )         | 0.367     | 43   | 24.970  | <0.001 | 172.284  | -3.557 |
|                        | Mean power output (W.kg <sup>-1</sup> )            | 0.313     | 43   | 19.580  | <0.001 | 42.617   | -0.876 |
|                        | Maximum tension (kN.m <sup>-2</sup> )              | 0.590     | 43   | 61.810  | <0.001 | -244.670 | 4.884  |
|                        | Mean tension (kN.m <sup>-2</sup> )                 | 0.469     | 43   | 37.940  | <0.001 | -71.999  | 1.432  |
|                        | Maximum shortening velocity (Fl.s <sup>-1</sup> )  | 0.226     | 43   | 12.570  | 0.001  | -1.747   | 0.010  |
|                        | Maximum lengthening velocity (Fl.s <sup>-1</sup> ) | 0.023     | 43   | 1.020   | 0.318  | 1.328    | -0.006 |
|                        | Maximum flow (kg.s <sup>-1</sup> )                 | 0.721     | 43   | 111.260 | 0.000  | 0.000    | 0.000  |
|                        | Maximum pressure (kPa)                             | 0.589     | 43   | 61.720  | 0.000  | -6.893   | 0.138  |
|                        | Maximum jet velocity (m.s <sup>-1</sup> )          | 0.725     | 43   | 113.470 | 0.000  | 2.877    | -0.073 |
|                        | Maximum jet thrust (N)                             | 0.589     | 43   | 61.720  | 0.000  | -0.827   | 0.017  |

Figure 1A. Shell gape plotted against time during the first clap of escape responses in *Adamussium colbecki* at -1.4 (solid line, —) and +1.7°C (dotted line, .....). Closure (adduction) is rapid initially and declines in rate until closure upon which rapid opening occurs, powered by the elastic hinge ligament. Adduction is completed earlier at 1.7 than at -1.4°C although maximum rates of opening and closing are similar.

Figure 1B. Swimming velocity plotted against time for escape responses at -1.4 (solid line, —) and +1.7°C (dotted line, .....). After a short delay animals accelerated rapidly reaching peak velocity just before the end of adduction. Velocity declined slowly as the valves opened until falling close to zero at the end of the cycle. A higher peak velocity was reached at 1.7 than at -1.4°C and this peak was reached sooner. Deceleration was also more rapid with zero velocity occurring earlier.

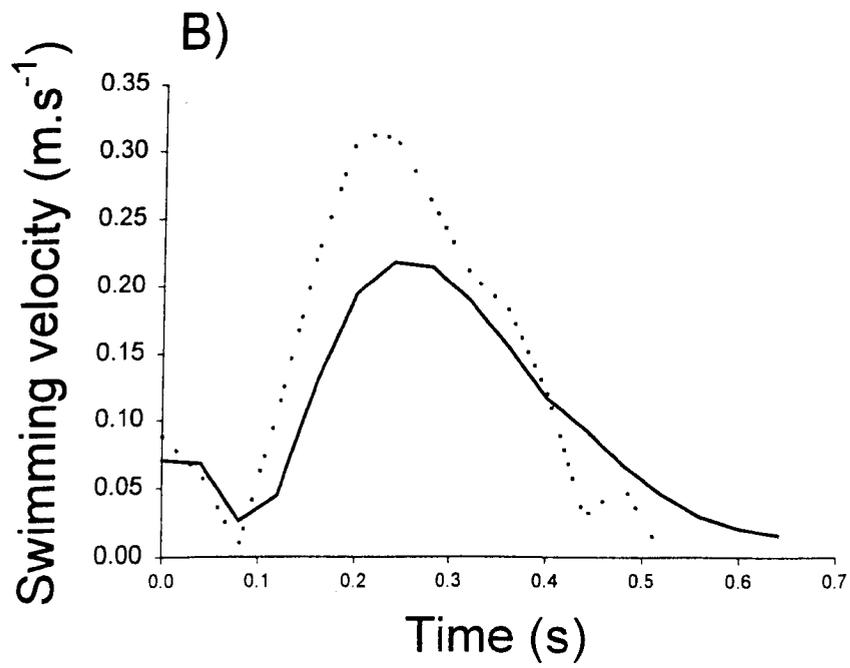
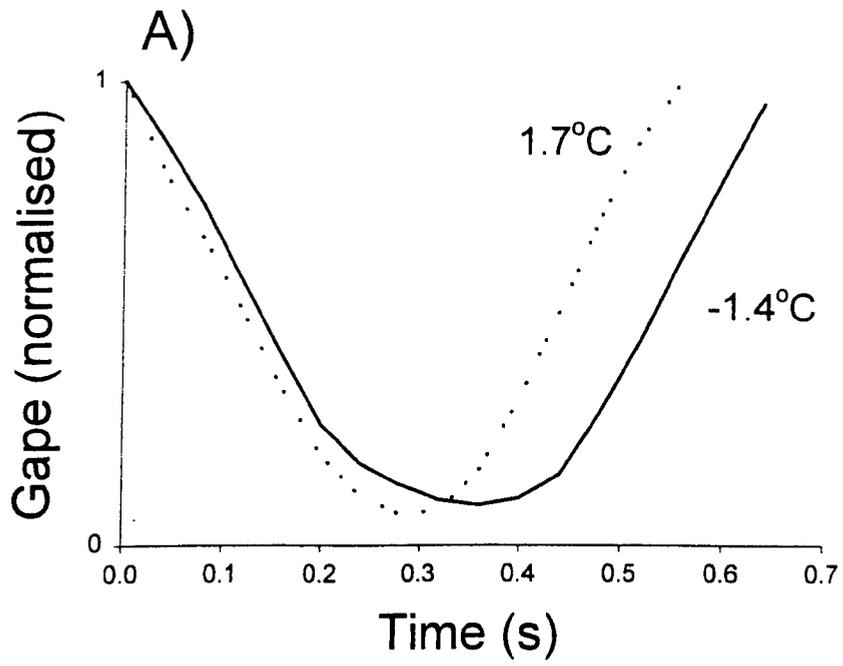
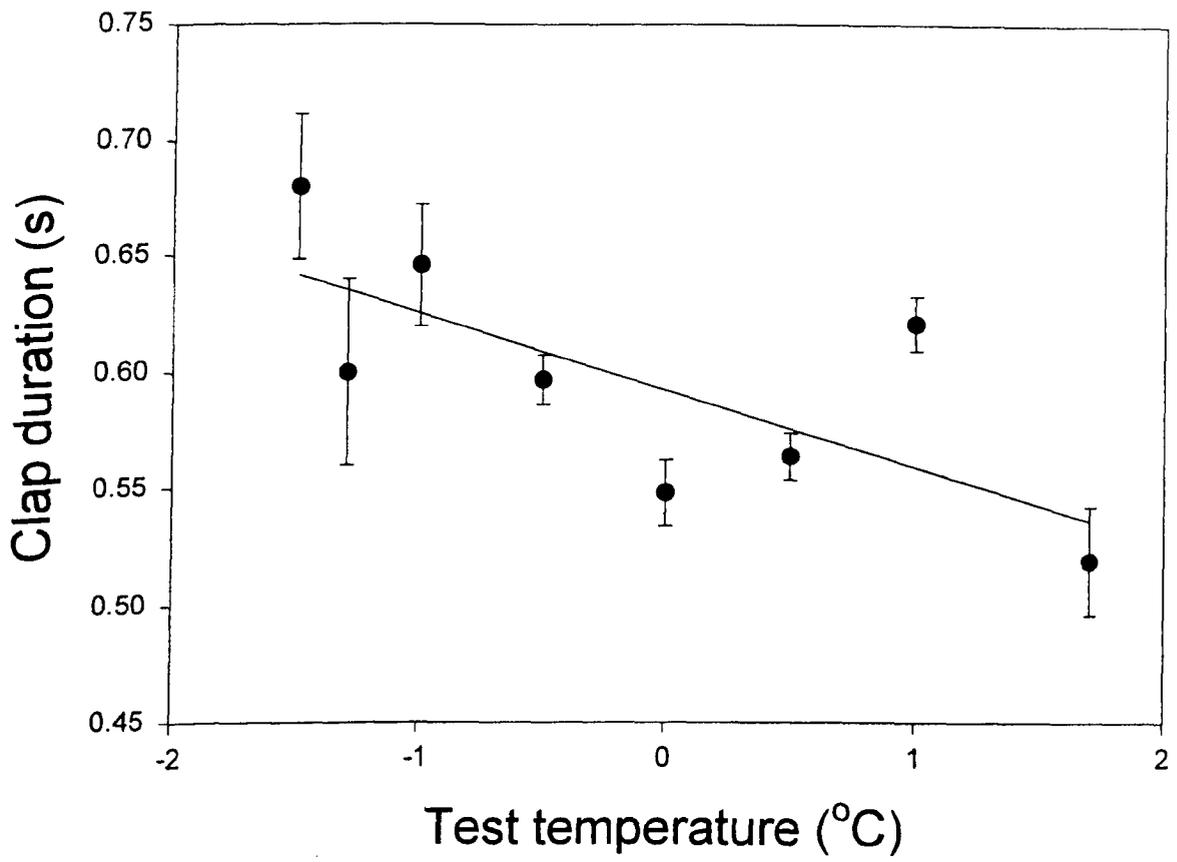


Figure 2. Clap duration against temperature in pooled animals. Data presented are means $\pm$ SE. Clap duration was significantly negatively related to temperature. Regression for the full data set, RSq=0.45,  $p < 0.001$ ,  $df=42$ ,  $y = -0.048x + 0.578$ .



began falling close to zero by the end of the cycle. At higher temperature the peak velocity was significantly (Table 2A) greater and was reached earlier (fig. 2B). Mean cyclic swimming velocity, acceleration and thrust were all significantly related to water temperature (Table 2A).

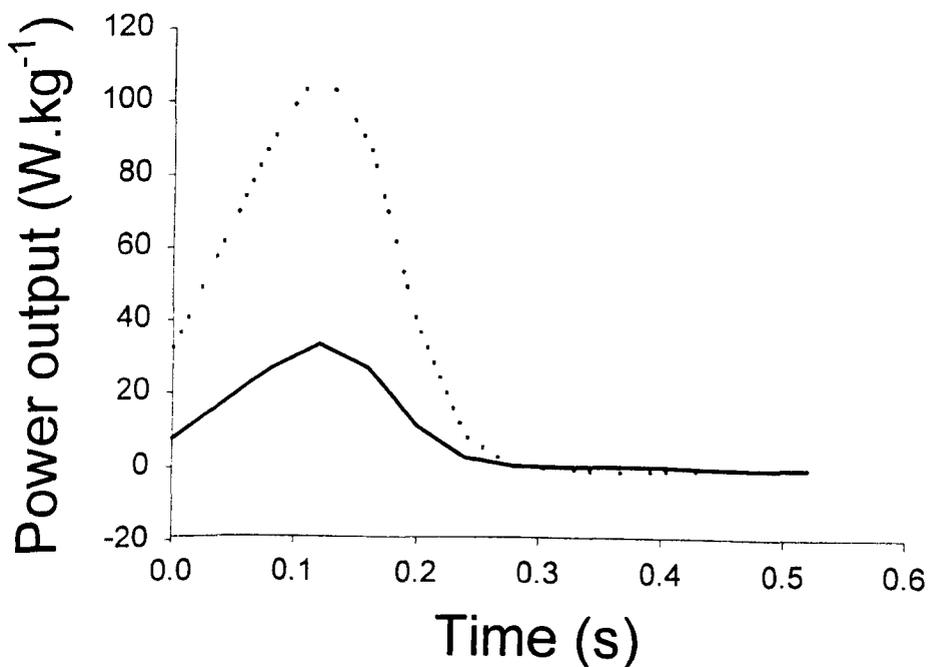
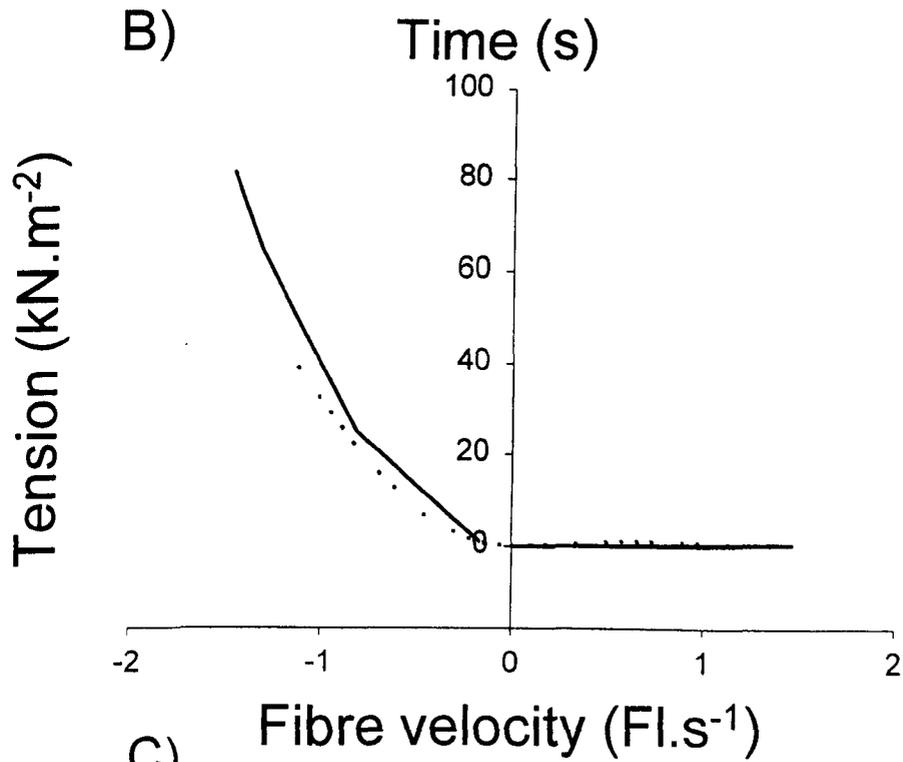
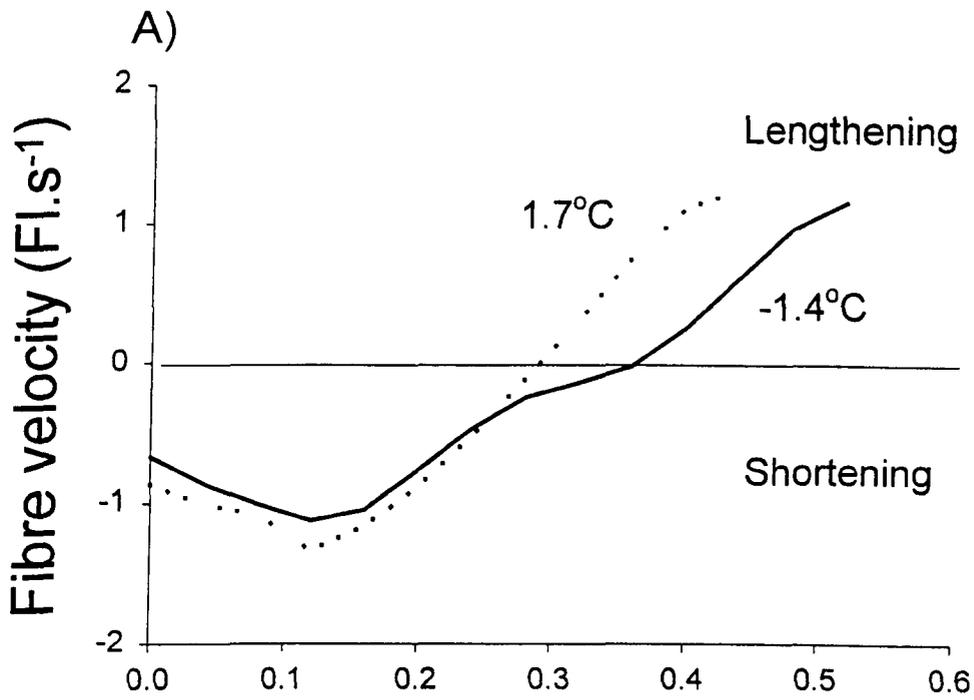
Muscle shortening velocity during swimming fell from a maximum close to the beginning of adduction, with the timing of the onset of lengthening depending on temperature. While peak shortening velocity did not change significantly with temperature (fig. 3A) mean shortening velocity increased due to the shorter time spent at low shortening velocity. As peak shortening velocity occurs almost instantaneously and very close to the beginning of adduction and because the frame rate of the camera was low, variance in the data was extremely high. This was an unfortunate limitation of the equipment available in the field.

Force-velocity trajectories of the adductor muscle were plotted from the model output as described previously (Chapter 2). The form of the trajectories at the two different temperatures did not differ and consisted of a high tension shortening phase followed by lengthening, during which negative force was low (fig. 3B). Power output peaked quickly (within the range of the measurements and smoothing necessary) falling rapidly to zero as shortening was completed (fig. 3C). Peak muscle mass specific power output varied from a mean of  $40\text{W}\cdot\text{kg}^{-1}$  to over  $100\text{W}\cdot\text{kg}^{-1}$  as temperature rose from  $-1.5$  to  $1.7^\circ\text{C}$ . The power requirement during reopening was small (equivalent to  $<5\text{W}\cdot\text{kg}^{-1}$ ) and similar at both temperatures. Again this measure suffered from the lack of time resolution available and the loss of data during smoothing. The presented peak values may be a slight underestimate as a result. The limited number of high-speed

Figure 3. A) Muscle fibre shortening and lengthening velocity during escape responses at  $-1.4^{\circ}\text{C}$  (solid line, —) and  $+1.7^{\circ}\text{C}$  (dotted line, .....). Shortening velocity fell during shortening (negative values) before lengthening as the shell reopened (positive values). While peak shortening and lengthening velocities were similar shortening was completed earlier at the higher temperature with higher mean shortening velocity.

B) Predicted muscle fibre force-velocity trajectories during escape responses at  $-1.4^{\circ}\text{C}$  (solid line, —) and  $+1.7^{\circ}\text{C}$  (dotted line, .....). A quadratic function described the relationship between force and velocity during shortening.  $RSq=1$ ,  $p<0.001$ ,  $y=30.211x^2 - 1.266x - 0.108$ . This was fixed by the constraints of the animal's morphology and did not differ between temperatures. The low force region during reopening indicates the low force required to reopen the shell. The force does not include the cost of stretching the relaxing adductor muscle though *in vitro* studies suggest that this requirement is also low (Marsh and Olson, 1994).

C) Predicted muscle power output during escape responses at  $-1.4^{\circ}\text{C}$  (solid line, —) and  $+1.7^{\circ}\text{C}$  (dotted line, .....). Power output was high initially, falling rapidly to zero at the end of shortening. Peak power output was greater at the higher temperature with the active jetting phase ending sooner. The negative power requirement for reopening was small and similar at both temperatures.



video sequences available match well to the sequences used here and suggest that peak value is not significantly higher than reported here.

Shell height was an important determinant of performance with all whole-body variables except mean swimming velocity scaling significantly. Amongst the muscle specific measures only muscle lengthening velocity was not significantly related to shell height (Table 2B)

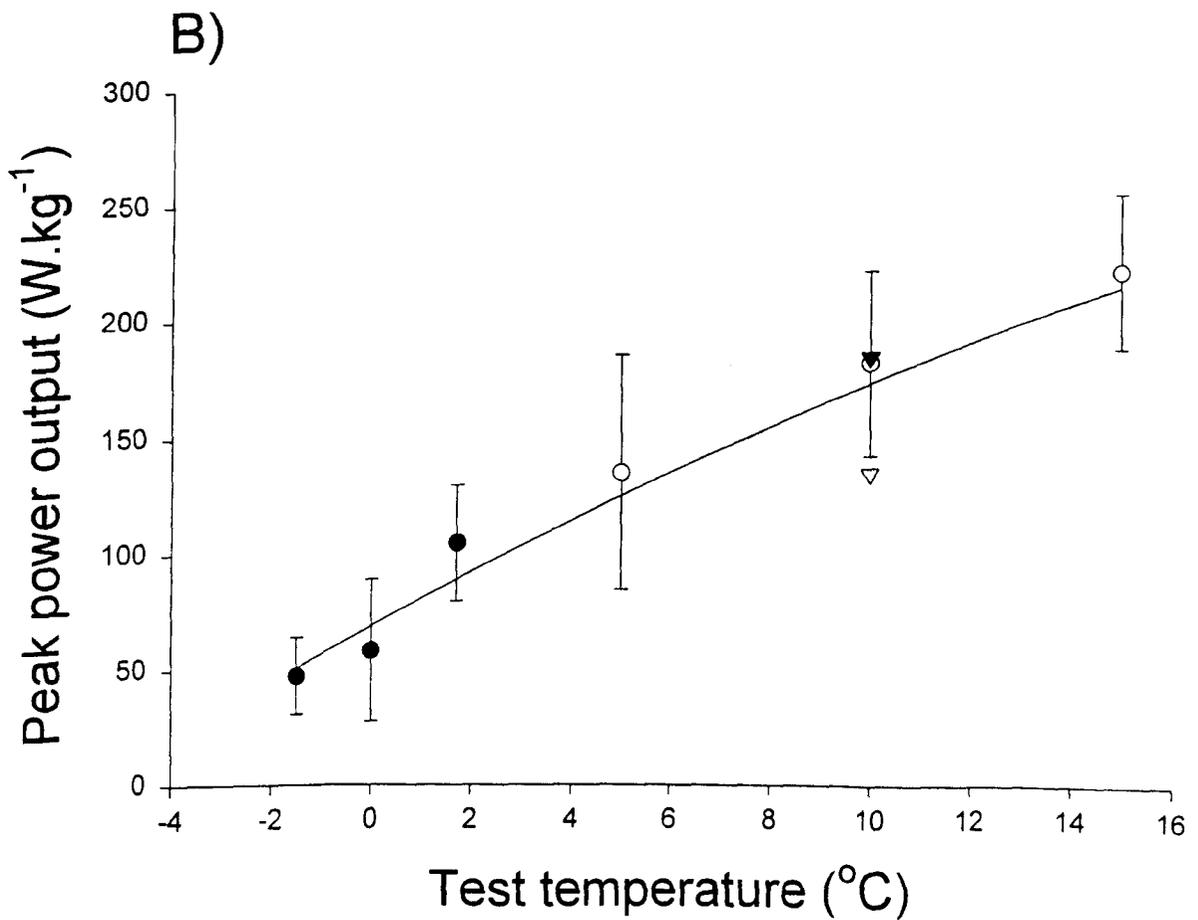
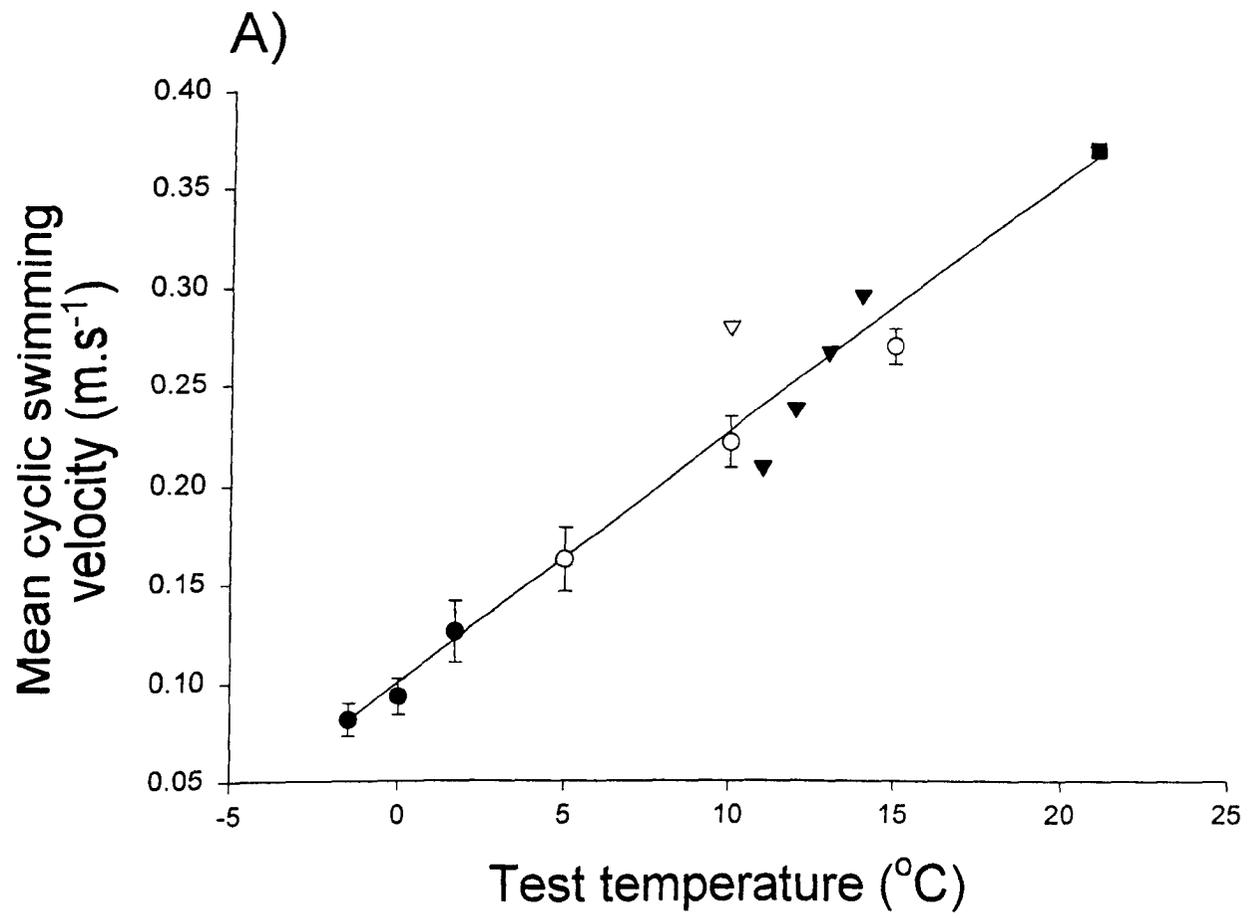
#### 3.3.4 Comparative whole body and muscle performance

Only limited comparisons of temperature acclimated animals of both *A. opercularis* and *A. colbecki* were possible due to the lack of a significant relationship between temperature and whole body performance in acclimated *A. colbecki* (Table 1A). It is also not possible to swim the animals at the same temperature due to the differences in temperature tolerance between species. Simple comparisons have been made of *A. colbecki* with the data collected for *Aequipecten opercularis* and *Zygochlamys patagonica* and published data in *Placopecten magellanicus*, (Cheng and DeMont, 1996; Manuel and Dadswell, 1990) *Argopecten irradians* (Marsh et al., 1992) and *Amusium pleuronectes* (Morton, 1980)

A single function describes the effects of mean swimming velocity on temperature in the species reviewed here (fig. 4A), over a wide thermal range (-1.5 to 20°C). The  $Q_{10}$  for the entire relationship is 1.96. Similarly peak muscle power output increased (fig., 4B) with increasing temperature and again there was little evidence of capacity adaptation in the muscle performance of *A. colbecki*.

Figure 4A. Comparison of the whole-body performance of *Adamussium* (solid circles) to that of other species. Mean velocity is plotted against temperature with data for *Aequipecten opercularis* (open circles, this study Chapter 1), *Placopecten magellanicus* #1, (Manuel and Dadswell, 1990) and #2, (Cheng and DeMont, 1996) (closed and solid triangles respectively) and *Amussium pleuronectes* (Morton, 1980) (solid squares). Data for all species were fitted to a single linear function (RSq=0.94, p<0.001, df=9,  $y=0.013x+0.101$ ).

Figure 4B. Muscle mean cyclic power outputs in *Adamussium* and other species. Data are presented for *Adamussium* (solid circle), *Aequipecten* (open circles, this study, Chapter 1), *Argopecten irradians* (open triangles) (Marsh et al, 1992) and *Placopecten magellanicus* (solid circles) (Cheng and Demont, 1997). All the available data fit a single quadratic function, RSq=0.92, p<0.001, df=6,  $y=12.00x^2 - 0.15x + 69.03$ .



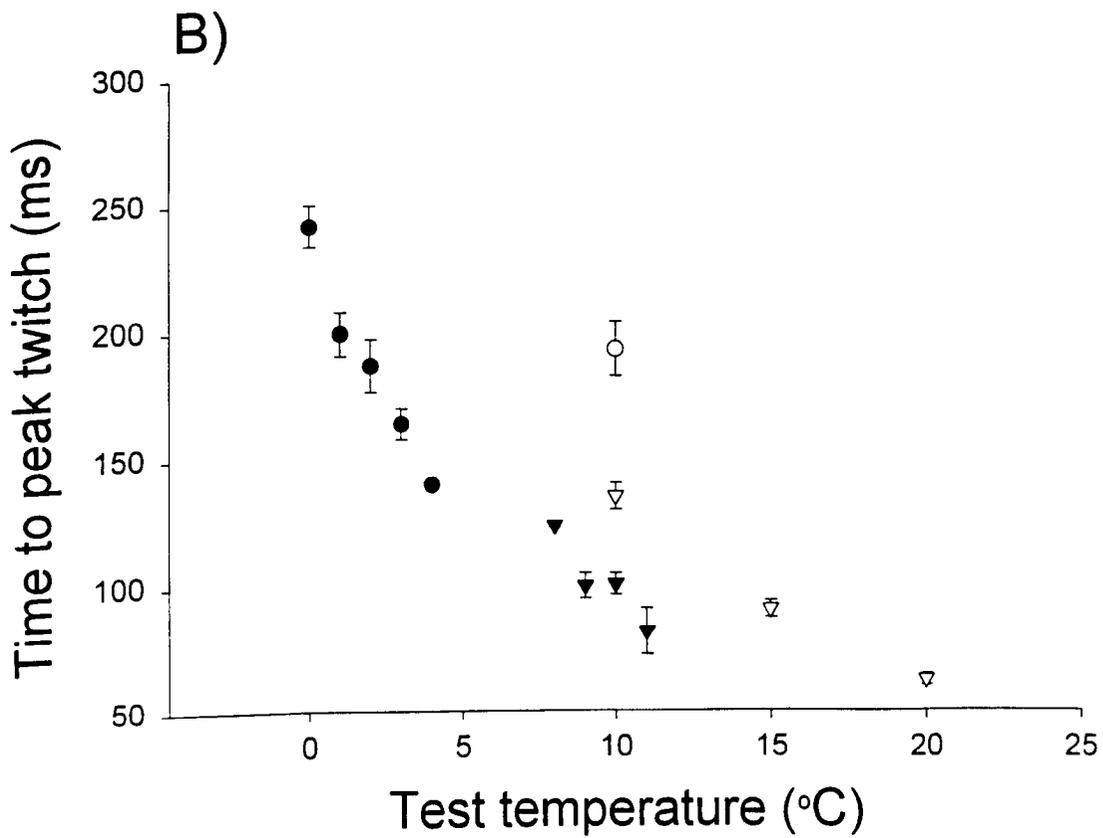
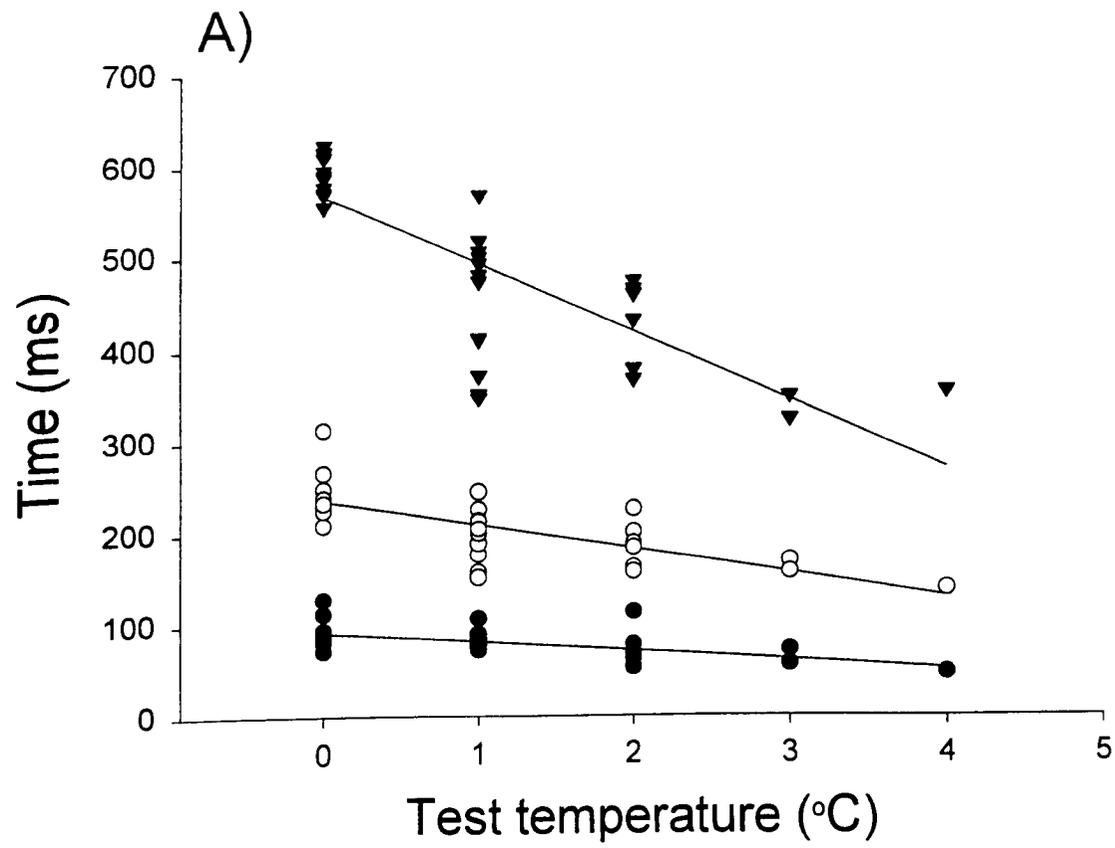
The swimming styles of the two species studies in detail (*A. colbecki* and *A. opercularis*) differed with the ratio of peak to mean swim velocity much higher in *A. colbecki* than in *A. opercularis* (t-test,  $t=8.00$ ,  $p<0.001$ ,  $df=41$ ). The power stroke in the Antarctic species was much shorter compared to the length of the overall cycle. Shell mass makes up a smaller proportion and the adductor muscle a higher proportion of total mass in *A. colbecki* than in *A. opercularis* (chapter 2) (GLM with total animal mass as covariate,  $p<0.001$ ,  $df=32$ ). In *Aequipecten opercularis* the shell is approximately twice the mass of the adductor muscle (paired t-test,  $t=12.3$ ,  $df=14$ ,  $p<0.001$ ) whereas in *A. colbecki* there was no significant difference between shell and adductor mass (paired t-test,  $t=1.78$ ,  $df=33$ ,  $p=0.08$ ).

### 3.3.5 Comparative In vitro muscle characteristics

Time to peak muscle force development, time to 50% force and time to 50% relaxation were recorded for 5 animals over the temperature range 0-5°C (fig. 5A). All contraction and relaxation durations decreased significantly with increasing temperature (See fig. 5A) with the slopes of the lines all being significantly different from each other. Muscle cross-sectional area specific force production was estimated from two muscle preparations. The peak value obtained was 103KN.m<sup>-2</sup>. The slopes of the regression lines relating temperature to time to peak twitch tension differed significantly between *A. colbecki* and the Southern temperate species *Zygochlamys patagonica* (fig. 5B). There was insufficient data to compare *Argopecten irradians* statistically (Olson and Marsh, 1993) but the points for this species fall outside the 95% Confidence Intervals for the other species. While these data indicate differences in the intrinsic muscle properties between species more work in a much larger range of species will be required in order to evaluate this further.

Figure 5A. Time to 50% peak force, peak force and 50% relaxation in *Adamussium* in relation to temperature. Relaxation time was prolonged at low temperature contributing to the "gliding" swimming style at low temperature. Presented are time to 50% peak force (solid circles), peak force (open circles) and 50% relaxation (solid triangles).

Figure 5B. Time to peak twitch force during isometric contractions. Data are presented for *Adamussium colbecki* (solid circles), *Aequipecten opercularis* (open circles), *Zygochlamys patagonica* (solid triangles), this study, and *Argopecten irradians* (open triangles) (Olson and Marsh, 1993). Time to peak twitch falls with increasing temperature in all species. The slopes of the relationship between temperature and time to peak twitch differed between *Adamussium* and *Zygochlamys* (t-test,  $p=0.05$ ,  $df=16$ ).



### 3.4 Discussion

The Rothera *A. colbecki* population apparently exists without the need for active swimming. The animals were unable to escape when stimulated and despite this appear to suffer minimal predation. The ability to swim was retained by the animals and once detached from the rocks and stimulated in the laboratory they attained swimming speeds and clap frequencies similar to those reported in free-swimming, wild *A. colbecki* at  $-1.7^{\circ}\text{C}$  (Ansell et al., 1998). Mean cyclic swimming speed was recorded as  $15.7\text{cm}\cdot\text{s}^{-1}$  by Ansell et al (1998) and  $11\text{cm}\cdot\text{s}^{-1}$  in the present study. Peak swimming speed were  $28\text{cm}\cdot\text{s}^{-1}$  and  $23\text{cm}\cdot\text{s}^{-1}$  respectively.

#### 3.4.1 Effects of temperature change and thermal history

The scallops behaviour was highly temperature dependent with the proportion of swims to total stimulations falling with increased temperature and as temperature rose above their acclimation temperature. No differences were detected between the effects of temperature on acclimated animals and those maintained at natural temperatures for that time of year.

Temperature affected all measured aspects of whole-body and muscle performance except peak muscle shortening and lengthening velocity. As in *A. opercularis* temperature significantly increased mean fibre shortening velocity. Clap duration is modulated by changes in adduction with the effects of temperature being greatest late in the cycle immediately prior to reopening. This is similar to the effect documented by Marsh (1990) in *Argopecten irradians*. The “clap and glide” style of swimming (with a long period between closing and reopening) reported in this species (Ansell et al., 1998) is caused by the effects of low temperature on muscle relaxation rate and is lost at higher temperature.

No convincing evidence for a compensatory acclimation response could be found in *A. colbecki*. The low numbers of animals available for study, their general lack of responsiveness and the poor time resolution of the recording equipment available may have obscured a small but genuine acclimation.

#### 3.4.2 Evidence of evolutionary adaptation.

Comparison of whole body, and *in vivo* and *in vitro* muscle characteristics has also been used to test for evolutionary adaptation to temperature (Altringham and Johnston, 1986; Johnston and Altringham, 1985; Johnston et al., 1991b; Wakeling and Johnston, 1998). This procedure is best carried out using a range of species across a temperature gradient (Garland and Adolph, 1994) amongst which the strengths of their evolutionary relationships can be quantified. Few detailed studies of the effects of temperature on scallop swimming and the properties of scallop muscle exist, limiting the possibilities for comparison between the data presented here for *A. colbecki* with other species. Simple comparisons of mean swim speeds and muscle twitch times were made between this and previous studies as were comparisons of peak muscle mass specific power output. Contrary to the predictions of metabolic cold adaptation (Clarke, 1983) there is no evidence of mean swimming speeds or power outputs above those that might be reasonably expected for a temperate or tropical scallop at the same temperature. While the relationships between temperature and twitch tension development differ between species this probably reflects differential temperature sensitivity rather than capacity adaptation. It appears then, that muscle performance is limited by temperature in *A. colbecki* in the same way as it is for fish.

*A. colbecki* swims at temperatures below those at which *A. opercularis* fails to leave the bottom but its muscle power outputs, swimming speeds and accelerations are extremely low. *A. colbecki* resists the effects of cold on its ability to swim without apparently increasing its capacity for muscular work. This appears to be a paradox but *A. colbecki* achieves this by reducing the work required for swimming. The much reduced shell mass must allow the animal to swim at velocities which would not be sufficient to support the mass of more heavily shelled species such as *Aequipecten opercularis* (Thorburn and Gruffydd, 1979a). Reduced skin mass has been suggested as an adaptation to high accelerations in some fish (Webb, 1979), this would be more likely in scallops due to the relative densities of shell and soft tissue.

Predation pressure appears to be low at the study site, the animals observed seldom moved position and suffered no predation despite this. Indeed attached animals were incapable of escaping from simulated threats. Low likelihood of predator attacks reduces the need for high accelerations and the need for a strong, heavy shell. The absence of decapod predators is likely to be key to this difference as these animals exert heavy predation pressure on many scallop species (Arsenault and Himmelman, 1996b; Barbeau et al., 1998; Freites et al., 2000; Nadeau and Cliche, 1998; Stokesbury and Himmelman, 1995) and often feed by crushing the shells of their prey (Barbeau and Scheibling, 1994a).

Although crabs are not found in Antarctica other benthic invertebrates which prey on scallops such as seastars and gastropods are present at Rothera. *A. colbecki*'s relatively rapid growth rate compared to other Antarctic benthos (Berkman, 1990; Brey and Clarke, 1993) may give it a degree of size protection from small predatory seastars such as *Odontaster validus*. Without the weight

of the shell the requirement for rapid swimming is lost. This “clap and glide” swimming style does not result in increased distance travelled per clap but may increase efficiency through drag reduction. Slow rates of muscle relaxation may also reduce the costs of calcium transport. The energetics of swimming in *A. colbecki* and temperate species will be examined in Chapter 4.

It may be surprising that *A. colbecki* does not show capacity adaptation or the ability to acclimate to temperature change in the way shown for temperate species. The importance of swimming has been documented in the laboratory and field (Barbeau and Scheibling, 1994b; Barbeau and Scheibling, 1994c) in other scallop species and the effects of temperature change in weakening its effectiveness demonstrated (Barbeau and Scheibling, 1994c). Inability to acclimate to temperature change has been shown in other stenothermal animals (Coughlin and Rome, 1999; Hardewig et al., 1999) (Hofmann et al., 2000). Despite the apparent advantages of temperature-compensated swimming ability *A. colbecki*'s performances were no higher than could be expected of a temperate animal cooled to similar temperatures. That *A. colbecki* is such a common species throughout Antarctica demonstrates that maintaining a relatively low level of expenditure on its locomotory system is a successful strategy.

Success for an animal depends on correctly trading-off reproductive investment with the costs of maintaining a body which can support the reproductive system to maturity. While basal metabolism may be reduced by low temperatures (Brey and Clarke, 1993) constructing and maintaining the machinery required for an active lifestyle appears to be more expensive than would be necessary in warmer waters (e.g. increased mitochondria production and more expensive protein synthesis and calcium uptake). With a lower absolute amount of energy

to utilise each marginal improvement in muscle performance reduces reproductive investment by a greater proportional amount than would be lost in an individual capable of garnering more resources.

Each animal in a population will trade-off escape performance with reproductive investment at a slightly different point with numbers in subsequent populations determining the resulting mean position of trade-off. In the *Rothera Adamussium colbecki* population at least, we can speculate that the potential benefits of a greater proportion of escapes from predators do not outweigh the costs of greater locomotory performance, and the additional costs of acclimation responses.

Table 3. Scaling of anatomical parameters used in the calculations. All relationships are with shell height and were significant at  $p < 0.05$   $RSq > 0.6$ ,  $df = 24$ . Linear and cubic regression coefficients are provided as appropriate.

| Parameter  | Scaling coefficients |         |        | Abbreviation           |
|--|----------------------|---------|--------|------------------------|
|  | b0                   | b1      | b2     |                        |
| Maximum jet area   | 0.00005              | -0.0013 | 0.0118 | <i>ajet</i>            |
| Hinge length   | 0.0036               | 0.0024  |        | <i>Lh</i>              |
| Distance from hinge to<br>centre of mass of valves       |                      | 0.491   |        | <i>Lhcom</i>           |
| Distance from the lip of the<br>shell to adductor muscle |                      |         |        |                        |
| Top  | -0.0068              | 0.5252  |        | <i>Lmf<sub>t</sub></i> |
| Bottom   | -0.0018              | 0.5646  |        | <i>Lmf<sub>b</sub></i> |
| Shell depth  | -0.094               | 0.4633  |        | <i>Lsd</i>             |
| Shell length   | -0.087               | 1.1524  |        | <i>Lsl</i>             |
| Adductor width, shell closed<br>(m)                      | -0.0013              | 0.3451  |        |                        |
| Total wet mass (kg)                                      | -0.0574              | 1.5889  |        | <i>Mb</i>              |
| Adductor wet mass (kg)                                   | 0.0036               | -0.238  | 4.2238 | <i>Mm</i>              |
| Valve mass (kg)  |                      |         |        |                        |
| Top  | -0.0075              | 0.2336  |        | <i>Ms<sub>t</sub></i>  |
| Bottom   | -0.0167              | 0.4136  |        | <i>Ms<sub>b</sub></i>  |

## **Chapter 4**

# **HIGH-ENERGY PHOSPHATE METABOLISM DURING EXERCISE AND RECOVERY IN TEMPERATE AND ANTARCTIC SCALLOPS**

## **- AN *IN VIVO* <sup>31</sup>P-NMR STUDY.**

### **4.1 Introduction**

The ability to swim is of great short-term importance to the survival and success of individual scallops (Barbeau and Scheibling, 1994b) (Barbeau and Scheibling, 1994a). In the longer-term repeated swimming affects the partitioning of resources resulting in greater muscle growth at the expense of the gonad (Kleinman et al., 1996). Avoiding unnecessary escape responses by predator recognition (Thomas and Gruffydd, 1971), minimising the cost of swimming in terms of energy use per effective escape and recovering as quickly and efficiently as possible may be selectable traits.

Several descriptions of the metabolic processes used by scallop fast muscle to power contractions and the processes involved in recovery exist. In scallops the ATP used during muscular activity is initially regenerated by the breakdown of Phospho-L-arginine (PLA) followed by anaerobically and aerobically supported glycolytic ATP production (Grieshaber, 1978; Livingstone et al., 1981). In anaerobic metabolism octopine is formed from pyruvate, arginine and NADH, releasing NAD. This replaces the pyruvate to lactate pathway of vertebrates (Gäde et al., 1978)

A range of tools has been used in the assessment of energy consumption and metabolic change during exercise in scallops. *In vivo* blood sampling, pulse rate and oxygen consumption measurements were taken by Thompson and co-

workers (Thompson et al., 1980) from scallops during work and recovery. Blood samples showed that during the period immediately after swimming blood oxygen fell and carbon dioxide rose while other metabolites showed little change. Oxygen uptake from the water was zero for at least one hour after exercise. This leaves a substantial "black box" of muscle based metabolism which, until now, has only been accessible by sampling muscle for the biochemical analyses, on which the existing literature is based (Baldwin and Opie, 1977; de Zwaan et al., 1979; Gäde et al., 1978; Grieshaber, 1978; Livingstone et al., 1981). This constraint reduced the number of experimental conditions possible and the time resolution at which recovery was followed.

<sup>31</sup>P-NMR Spectroscopy provides a sensitive and non-invasive tool for the measurement of changes in high-energy phosphate levels and tissue pH before, during and after exercise. It allows several experiments to be carried out on each animal (Hitzig et al., 1987), in the present study the observation of graded exercise from light (1-3 muscle contractions) to exhausting. Substances bound to proteins and therefore not in the free metabolite pool will give widened and shifted peaks, if substances are in separate pools their different pHs may be detected (Bagshaw, 1993).

Numerous studies using NMR to observe changes in metabolite levels during and after muscular activity exist, mostly focussing on a single muscle *in vivo* or dissected muscle preparations (See (Argov et al., 1987; Curtin et al., 1997) for similar work on dogfish and rat respectively). NMR experiments on whole locomotory systems in live animals are rare, an example being Thebault's (1991; 1987; 1997; 1994) work on the tail musculature of prawns. In the scallop the muscle is very prominent and provides the dominant signal within the scanning

area of the MRS. As existing studies have shown that metabolite transport to and from the fast adductor is limited (de Zwaan et al., 1979) focussing the NMR on the fast adductor allows the fate of all relevant phosphagens to be observed. Few NMR studies exist into scallop physiology. MRS has been successfully used on scallops in order to assess seasonal changes in metabolic state (Jackson et al., 1994) and the kinetics of scallop arginine phosphokinase have been examined *in vitro* (Graham et al., 1986). No NMR studies of scallop metabolism during work and recovery exist.

The ability of cold-water ectotherms to perform work during locomotion has been studied in a variety of Antarctic fish species (Franklin, 1998; Franklin and Johnston, 1997; Wakeling and Johnston, 1998) and in the Antarctic scallop *Adamussium colbecki* (Ansell et al., 1998). Studies in Antarctic fish have shown compensation for force production without a corresponding amount of ATP use, making isometric force maintenance more cost-effective at low temperature due to changes in the force development per cross-bridge cycle (Altringham and Johnston, 1986). These effects are associated with changes to both the myosin heavy and light chains which increase ATPase activity at low temperature (Johnston and Walesby, 1978; Johnston et al., 1975). However, when compared to a range of tropical and temperate fish species with similar morphologies Antarctic fish show little compensation in terms of whole-body acceleration or muscle power output (Wakeling and Johnston, 1998)

Although muscle performance in Antarctic marine animals is relatively well described, their ability to withstand fatigue (Altringham and Johnston, 1985; Lowe and Wells, 1997) and recover from exercise (Hardewig et al., 1998) is less well known. The increased mitochondrial density observed in Antarctic fish red

muscle has been proposed as an adaptation to facilitate aerobic metabolism by compensating for reduced diffusion rates at low temperatures (Tyler and Sidell, 1984). While this explanation is appealing no difference could be found in the diffusion of high-energy phosphates with temperature in goldfish (Hubley et al., 1997). Mitochondrial density may increase diffusion rates of other substances, e.g. carbon compounds and maximise aerobic capacity as the mass-specific aerobic capacity of mitochondria falls with temperature (Johnston et al., 1998).

Antarctic eelpout recover faster than temperate relatives at the same temperature (Hardewig et al., 1998). While aerobic processes may be maintained at high levels in Antarctic fish, the Notothenioids show reduced glycolytic capacity (Dunn, 1988; Dunn and Johnston, 1986). The current study on scallops represents a preliminary investigation of metabolism during exercise and recovery in an Antarctic invertebrate with similar, temperate, species for comparison.

## 4.2 Materials and methods

*Adamussium colbecki* were collected using SCUBA at Rothera Research Station in March 1999 and maintained at 0°C in through-flow seawater at Rothera, and aerated recirculating seawater while shipped to Bremerhaven via Cambridge. Two temperate species, *Aequipecten opercularis* and *Pecten maximus* were obtained by from the marine biological station, Roscoff, Brittany and maintained at 12°C at the Alfred Wegener Institute for Polar and Marine Research, Bremerhaven, Germany (AWI). Experiments on *Pecten maximus* were undertaken in Feb 1999, and on *A. opercularis* and *A. colbecki* in June 1999. Temperate animals were used within 2 weeks of collection, *A. colbecki* specimens had been in captivity for a longer period but were highly responsive to light and handling, indicating reasonable physical condition.

24h before experimentation a 2cm<sup>2</sup> piece of Velcro was glued to the lower valve of each animal. Each scallop was also intubated (via the rear jet aperture) with 2 pieces of narrow-bore plastic tubing. These tubes were held in place by dental wax, one allowed the injection of saturated saline into the mantle cavity to act as a stimulus to swim. Saline was used instead of freshwater (see Chapter 3) to reduce osmotic shock to the animals due to the introduction of the stimulating solution directly into the mantle cavity. Use of liquidised starfish (Chapter 2) was avoided as a possible contaminant of the NMR data. Monitoring of swimming activity was achieved with a pressure transducer fitted to the end of the other tube.

Experimental animals were fixed to the bottom of a 1.5 litre insulated plastic chamber filled with 1.2 l of seawater, by Velcro glued to the bottom of the

chamber. Cooled, aerated seawater was continuously pumped through the chamber.

Two thermocirculators were utilised to maintain the low temperatures in the chamber required for experiments in *A. colbecki* without freezing the seawater. The first controlled the temperature of the seawater to be pumped to the chamber and returned it to the reservoir where it was aerated. The second pumped cooled antifreeze through a coil around the water tubes. Temperature was measured inside the chamber using a fluoroptic thermometer (Luxtron, Polytec). Temperatures remained within 2°C of the animal's maintenance temperature throughout and were within 1°C during experimentation. Temperature and mantle pressure readings were collected continuously throughout experimentation and captured by a Macintosh computer running MacLab software.

#### 4.2.1 <sup>31</sup>P-NMR Spectroscopy

Phosphorous NMR of the live scallops was undertaken using a Bruker 47/40 Biospec DBX system. The chamber containing the experimental animal was placed within the magnet of the NMR so that the scallop's adductor muscle was centred over a triple tunable surface coil (5cm diameter). Spectra were obtained following tuning and matching of <sup>31</sup>P and <sup>1</sup>H channels of the surface coil and shimming to ensure a linear field using the proton signal of the water within the chamber. Free induction decays were Fourier transformed into spectra (XWIN NMR, Bruker).

Scan times were kept to the minimum necessary to reliably distinguish the 3 ATP signals in order to maximise temporal resolution. The time required varied

between animals (from 90seconds to 12 minutes) according to the signal-to-noise ratio of the sample, which in turn depended on adductor volume. Sequential spectra of the chosen duration were recorded using a purpose-designed program in "C". During processing, each spectrum was scaled relative to the first file processed.

#### *4.2.2 Graded exercise*

Each animal was allowed to rest for a minimum of 1h after placement within the chamber before beginning any experimentation. Manipulation of the animal was minimised during transfer to the chamber; however, initial spectra typically showed elevated inorganic Phosphorus (Pi) levels because of muscular activity during handling. No stimulation was given until an appropriate control spectrum (no detectable Pi) was obtained. Measurements were begun before exercise and continued at the chosen time interval until full recovery in most cases.

Saturated saline was prepared using natural seawater and Instant Ocean salts. This saline was introduced into the mantle cavity until a response was measured on the pressure transducer, indicating activity by the animal. An injection typically contained 2-5cm<sup>3</sup> saline, injected at 1cm<sup>3</sup>.s<sup>-1</sup>. Further injections were made until the desired number of "claps" (1-20) had been achieved. In addition to these, exhaustive exercise was imposed. This was defined as the animal failing to respond to further stimulation. Following experimentation all animals were returned to the holding aquarium and were still alive and responding normally 3 months later.

### Workloads in each exercise regime

It was not possible to measure the power output of the adductor from the pressure trace without a synchronised measure of flow through the jets of the animal (Marsh et al., 1992). This was not possible in the current equipment set-up but will be incorporated into future designs. Muscle cross-sectional area specific peak force production ( $F$ ,  $\text{KNm}^{-2}$ ) was estimated from the peak pressure recorded within the mantle ( $P$ , Pa), the shell area ( $Sa$ ,  $\text{m}^2$ ) and the muscle cross sectional area ( $Ma$ ,  $\text{m}^2$ ) determined from coronal MRI images obtained for each individual.

$$F = ((P \times Sa) / Ma) \times 1000$$

### 4.2.3 Analyses

A purpose-designed program identified the peaks of interest from the spectra and calculated their exact resonance frequency, height, width and area (XWIN NMR, Bruker). Peak area correlates with the amount of the substance within the detection area of the coil. During Phosphorus MR the peaks chosen were for Inorganic Phosphorous, Phospho-L-Arginine and ATP. Muscle pH was calculated from the chemical shift of the Inorganic Phosphorus signal relative to Phospho-L-Arginine using a calibration curve provided by Pörtner et al., 2000. The ratio of inorganic phosphorus to Phospho-L-Arginine was calculated. This is a widely used measure of muscle energetic status appropriate to the metabolism of scallops (Jackson et al., 1994). Time codes in the temperature and pressure (activity) data collected by the MacLab program allowed these data to be related to the Phosphorus NMR data.

Pre-exercise "resting" levels of PLA and total ATP were measured where possible and compared between species using GLM Univariate ANOVA with the Tukey Post-Hoc test. Estimates of changes in absolute concentrations of

metabolites have been made based on published data for *A. opercularis* from the same area (Grieshaber, 1978). Resting pH cannot be measured directly, as the calculation requires a Pi peak to be present. Resting pH was estimated by back-calculation of the claps vs. pH relationship to zero claps. Relationships between exercise (number of claps) and pH, Pi/PLA etc. were determined using Least Squares Regression (SPSS, Jandel Scientific). Slopes and elevations of regression lines were compared using Student's t-test (Zar, 1996).

#### 4.2.4 Technical constraints

During MRS the sample must be stationary, this is a constraint when investigating locomotion in animals. The hydrodynamics of "swimming" while in a fixed position are very different from those experienced while moving. A moving animal "levers" its way past the water rather than forcing water backward while remaining stationary itself. An example of this would be a rower attempting to row while the boat was held stationary. The force required to maintain strokes of the same frequency would be higher than if the boat was moving. The power requirement (the product of force and velocity) would therefore be higher than for a moving rower.

This effect is minimised by using scallops as a model animal. The biomechanics of swimming in *A. colbecki* ((Ansell et al., 1998), Chapter 3 of this study, and *P. maximus* (pers. obs) show that the animals come close to a standstill during each clap cycle as the valves open to refill the mantle cavity. During swimming 99% of the work done is used to form the propulsive jets (Cheng et al., 1996a) with only a small amount required to accelerate the shells themselves and the associated external mass of water. The work of jet formation and therefore the proportion of the animal's energy store used will therefore be similar in a fixed, stationary scallop to one that is swimming.

## 4.3 Results

### 4.3.1 Resting metabolite levels

A control reading, without detectable inorganic phosphorus, was obtained from each animal prior to experimentation. Since more than 90 % of cellular ATP and PLA pools are unbound the peak areas for these metabolites were converted to absolute values using the measured ATP level in *A. opercularis* ( $5.18 \mu\text{mol.g}^{-1}$  wet weight) obtained by Grieshaber (1978). The animals used by Grieshaber (1978) were of the same size and caught in the same location as the animals used in the current study. ATP level changes less during handling than the other metabolites so is the most reliable metabolite with which to scale the others.

Before first stimulation ATP level per gram of fast muscle tissue was significantly higher in *A. opercularis* than in either *A. colbecki* or *P. maximus* (GLM Univariate ANOVA with Tukey post-hoc,  $p=0.029$  and  $p=0.019$  respectively,  $df=9$ ). There was no significant difference between ATP level in these species. There was also no significant difference in PLA content of the adductors of the different species (fig. 1).

The time resolution of the measurements varied between experiments due to differences in the adductor volume of the animals and thus the signal to noise ratios of the spectra obtained. Noisy spectra were combined, reducing random noise but also reducing time resolution. The greater adductor volume of the King Scallop (*Pecten maximus*) allowed substantially higher temporal resolution (90 or 91 seconds) than was possible with the Antarctic Scallop (*Adamussium colbecki*) or Queen Scallop (*Aequipecten opercularis*) (7 to 12 minutes). The data for *P. maximus* allowed the observation of extremely short-term changes in

metabolite levels and therefore permitted the study of all grades of exercise. The majority of the data presented is for this species.

At medium to high workloads the observed effects of work on muscle metabolite levels were similar in all three species. Although of reduced temporal quality the data from the smaller bodied *A. colbecki* and *A. opercularis* did allow comparison between species for similar numbers of muscle contractions. Further improvements to the system will allow comparison of lower levels of work. Even at its poorest performance and resolution the data presented from this study are still significantly more detailed than any comparable published study.

#### 4.3.2 Muscle metabolism during exercise

A range of exercise regimes were imposed on the animal from 1-2 claps to exhausting. Exhaustion was defined as the animal becoming unresponsive to further stimulation. The number of claps required ranged from a mean of 13 in *A. opercularis* to 36.8 in *P. maximus*. The greatest number of claps performed was 57 by a *P. maximus* (Table 1).

Exercise in all species was associated with a drop in phospho-L-arginine (PLA) and rise in inorganic phosphate (Pi) levels. These changes were progressive with increasing workload (See fig. 4, fig. 2A and B). Changes in ATP level were small and within the bounds of the noise in the data (fig. 3C).

For each spectrum the ratio of Pi to PLA was calculated. At rest, with little free Pi, Pi/PLA was close to zero, increasing with increasing numbers of contractions (fig. 3) as Pi was produced from ATP and PLA was broken down to regenerate

Table 1. Interspecific differences in the number of claps (muscle contractions) performed before exhaustion. For the purposes of the experiment exhaustion was defined as the animal becoming unresponsive to further stimulation. *Aequipecten opercularis* performed the fewest claps before exhaustion and *Pecten maximus* the most. There was no significant difference between species (ANOVA,  $p > 0.05$ ,  $df = 10$ ) in any case, mainly due to the high variability of the data.

Table 1

| Species                        | Highest number of claps to exhaustion | Mean number of claps to exhaustion | Differences between species    |                       |
|--------------------------------|---------------------------------------|------------------------------------|--------------------------------|-----------------------|
|                                |                                       |                                    | <i>Aequipecten opercularis</i> | <i>Pecten maximus</i> |
| <i>Aequipecten opercularis</i> | 24                                    | 13                                 |                                | 0.052                 |
| <i>Pecten maximus</i>          | 57                                    | 36.8                               | 0.775                          |                       |
| <i>Adamussium colbecki</i>     | 48                                    | 30.8                               | 0.183                          | 0.775                 |

Figure 2 A-C. Typical traces of Pi, PLA and ATP levels in *Pecten maximus* with increasing exercise. The arrows in Fig. 2A indicate the timing of the onset of exercise and number of claps imposed (2, 7, 17 and 35). Units are scaled relative to the Pi trace of the first experiment. Pi level rises following exercise (A) while PLA level falls (B). Note the double peak in the PLA trace typical at high workloads in all species. No consistent pattern of ATP change could be detected as any transient changes (C, time=7500s) were within the range of the noise in the measurements.

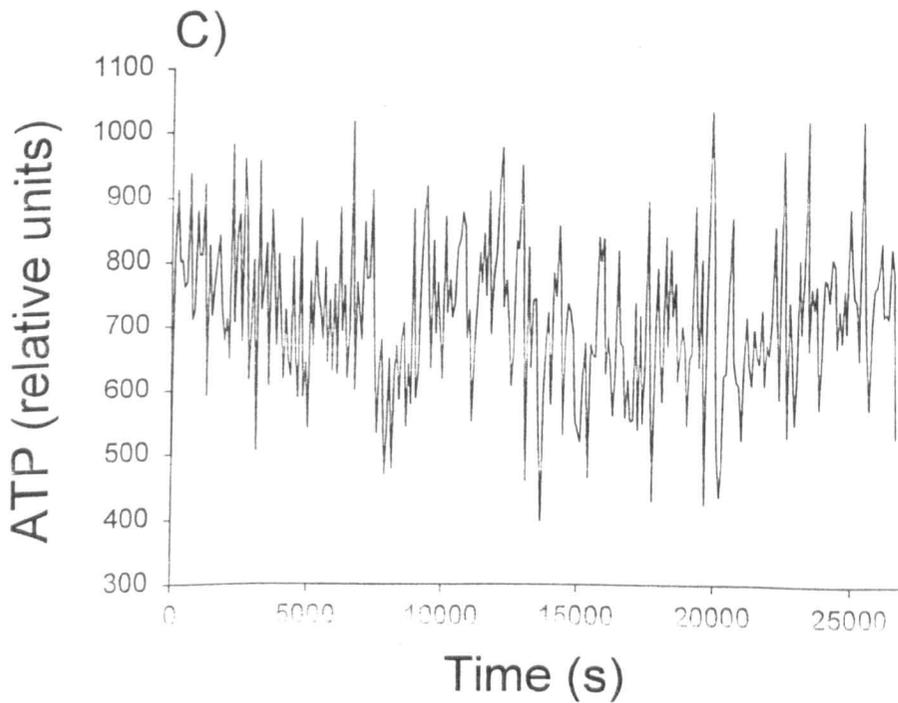
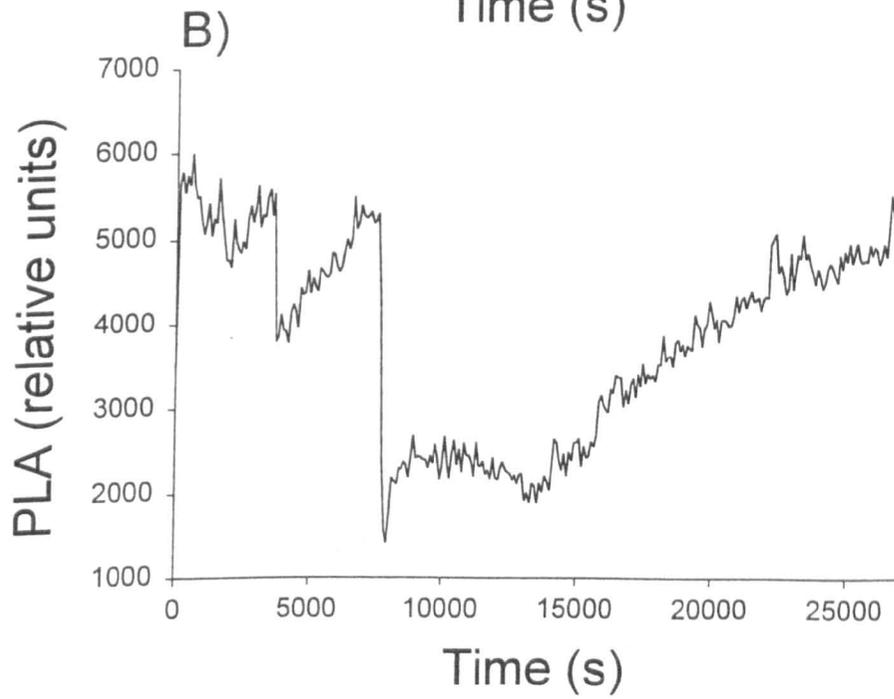
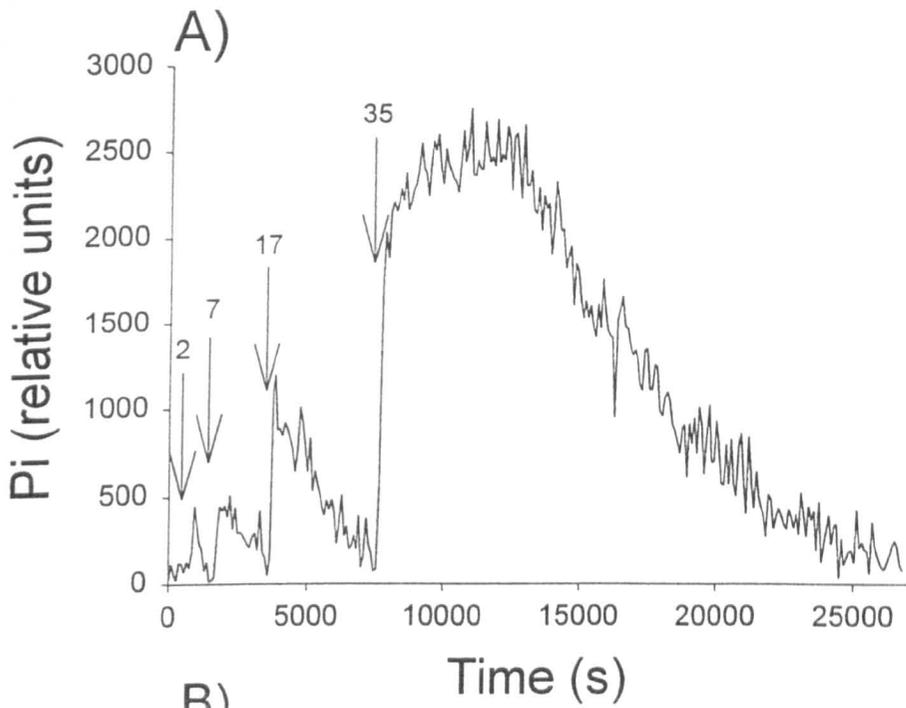


Figure. 3. Pi/PLA and muscle pH during graded exercise in *Pecten maximus*. This is the same sequence as Figure 4. As before arrows indicate the timing of onset of exercise and number of claps. The adductor muscle experienced a period of alkalosis (raised pH) followed by acidosis. pH was calculated from the chemical shifts of the Pi and PLA peaks (Portner et al, 2000), the noise at the beginning and end of the experiment was caused by the disappearance of the Pi peak. At high workloads Pi level exceeded PLA level

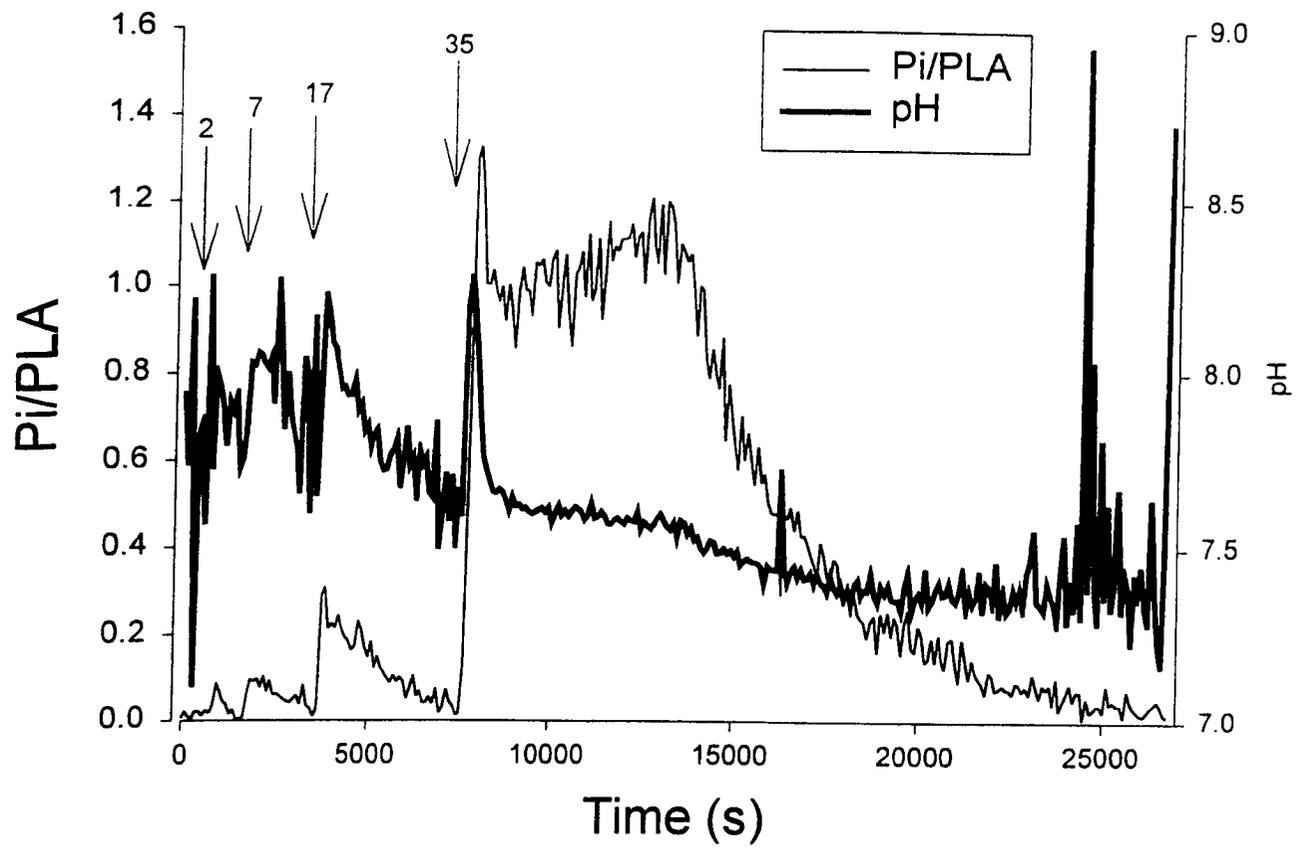
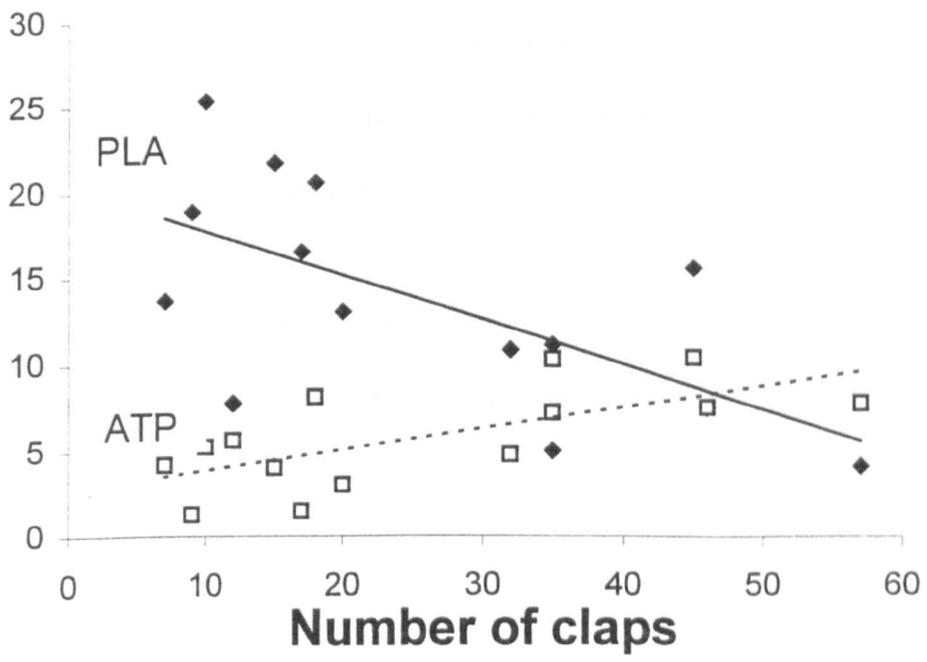


Figure 4. Absolute Pi and PLA level immediately after exercise against the number of claps in *Pecten maximus*. Symbols are maximum Pi (open squares) and minimum PLA (solid diamonds) recorded after the completion of the experiment. Concentrations were estimated on the basis of the Pi and PLA concentrations measured in resting animals by Grieshaber (1978), see text for details. Both PLA and Pi level immediately following exercise were significantly related to the number of claps performed by the animal. PLA,  $y=-0.261x+20.356$ ,  $RSq=0.41$ ,  $p=0.01$ ,  $df=13$ . Pi,  $y=0.119x+2.737$ ,  $RSq=0.44$ ,  $p=0.01$ ,  $df=13$ .

Pi and PLA levels  
( $\mu\text{mol}\cdot\text{g}^{-1}$ )



ATP. A transient increase in muscle pH was followed by a fall during the recovery phase (fig. 3), leading to muscle acidosis in most cases.

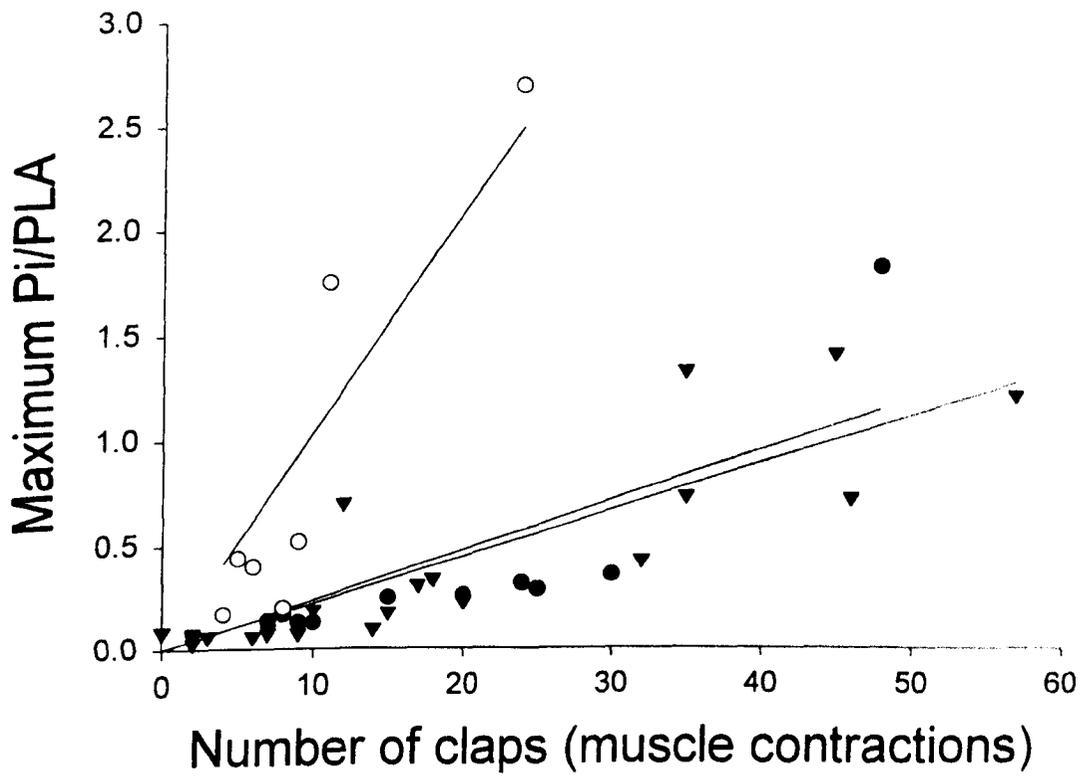
#### 4.3.3 Recovery of PLA levels following exercise

For the purposes of this study recovery was defined as the return of PLA levels to those prior to exercise and where no free Pi could be detected. The elevation of PLA level was “mirrored” by a reduction in free Pi during recovery from most grades of exercise (fig. 2, t=0 to t=7500s) and is well described by changes in Pi/PLA (fig. 3). At higher workloads Pi levels increase after the end of exercise (fig. 3, t>7500s). MR images during this period showed that this increase was associated with the animal remaining closed. Pi levels began to fall after the animal opened its shell and began to ventilate its gills. Water flows within the mantle were clearly visible in MR images. Typically at higher workloads a double peak was observed in the PLA plot (fig. 2). PLA level fell during exercise then increased rapidly for approximately for 5-7 minutes. Following this period, and throughout the period of valve closure, PLA level fell. This fall in PLA level and subsequent PLA recovery mirrored the Pi levels. Other than the period immediately following heavy exercise Pi+PLA remained relatively constant.

#### 4.3.4 Interspecific differences in muscle metabolism during exercise

Workload was defined as the number of claps (muscle contractions) as no direct measure of work was available during experimentation. Peak force production was estimated for *P. maximus* based on peak intramantle pressures during 20 claps from 2 animals. Peak force production averaged  $98\text{kNm}^{-2}$  ( $\pm 12$ ). The pressure traces for the other two species were not of sufficiently high resolution to allow the intramantle pressures to be measured accurately. Estimates of force production exist for both *A. opercularis* and *A. colbecki* from kinematic

Figure 5. Maximum Pi/PLA following exercise with increasing number of claps. Increases in the number of muscle contractions resulted in higher levels of Pi release and PLA breakdown, indicated by changes in the Pi/PLA ratio. Data points are the maximum Pi/PLA recorded following exercise in *Adamussium colbecki* (solid circles), *Aequipecten opercularis* (open circles) and *Pecten maximus* (solid triangles). Regression lines were driven through the origin, the known resting Pi/PLA value. *Adamussium*,  $y=0.024x$ ,  $RSq=0.787$ ,  $p<0.001$ ,  $df=9$ . *Aequipecten*,  $y=0.104x$ ,  $RSq=0.901$ ,  $p<0.001$ ,  $df=7$ . *Pecten*,  $y=0.0222x$ ,  $RSq=0.863$ ,  $p<0.001$ ,  $df=25$ . The slope of the relationship between number of claps and peak Pi/PLA was significantly steeper in *Aequipecten* than in *Adamussium* (t-test  $p<0.001$ ,  $df=7$ ) or *Pecten* ( $p<0.001$ ,  $df=25$ ).



studies of scallops acclimated to, and swum at, the same temperatures as the NMR experiments ( $109\text{kN}\cdot\text{m}^{-2}$  at  $10^{\circ}\text{C}$  and  $100\text{kN}\cdot\text{m}^{-2}$  at  $0^{\circ}\text{C}$  respectively) (Chapters 2 and 3 of this study).

The relationship between the number of muscle contractions and metabolic change was investigated. The slope of the relationship between number of claps and Pi/PLA (linear regression) differed between species with *A. opercularis* showing a greater change in Pi/PLA than either of the other two species (fig. 5). There was no significant difference in the proportion of the PLA store utilised in *A. colbecki* and *P. maximus*. There was no detectable relationship between number of claps and the time to peak Pi/PLA at different exercise levels. This is probably because the Pi increase was very rapid compared to the time resolution of the measurements.

#### 4.3.5 Interspecific differences in recovery rates

Time to 50% recovery, i.e. the mid-point between maximum Pi/PLA perturbation and full recovery, was used to compare between species. This parameter was used as it was seldom possible to accurately measure the time to a return to pre-exercise levels due to spontaneous activity by the animals in the later stages of recovery. There were significant relationships between maximum Pi/PLA and the time required for 50% recovery in Pi/PLA ratios in both *A. colbecki* and *P. maximus* (fig. 6). This relationship was weaker in *A. colbecki* than in *P. maximus* (Linear regression,  $\text{RSq}=0.63$ ,  $p<0.05$ ,  $\text{df}=4$  and  $\text{RSq}=0.56$ ,  $p<0.001$ ,  $\text{df}=18$  respectively). There was no significant difference between the slopes or intercepts of the lines. The effects of workload on Pi/PLA and time to recover are summarised in fig. 7.

Figure 6. Time to 50% recovery from increasing levels of Pi/PLA perturbation. Full recovery was defined at the return of Pi/PLA to resting levels. Data points are the time at which Pi/PLA reached 50% of the maximum Pi/PLA recorded following exercise. Symbols are for *Adamussium colbecki* (solid circles), *Aequipecten opercularis* (open circles) and *Pecten maximus* (solid triangles). The time to 50% recovery increased with increasing Pi/PLA, see text for statistics. *Adamussium*,  $y=19443x$ ,  $RSq=0.63$ ,  $p<0.05$ ,  $df=4$  and *Pecten*,  $y=8455.7$ ,  $RSq=0.56$ ,  $p<0.001$   $df=18$  respectively).

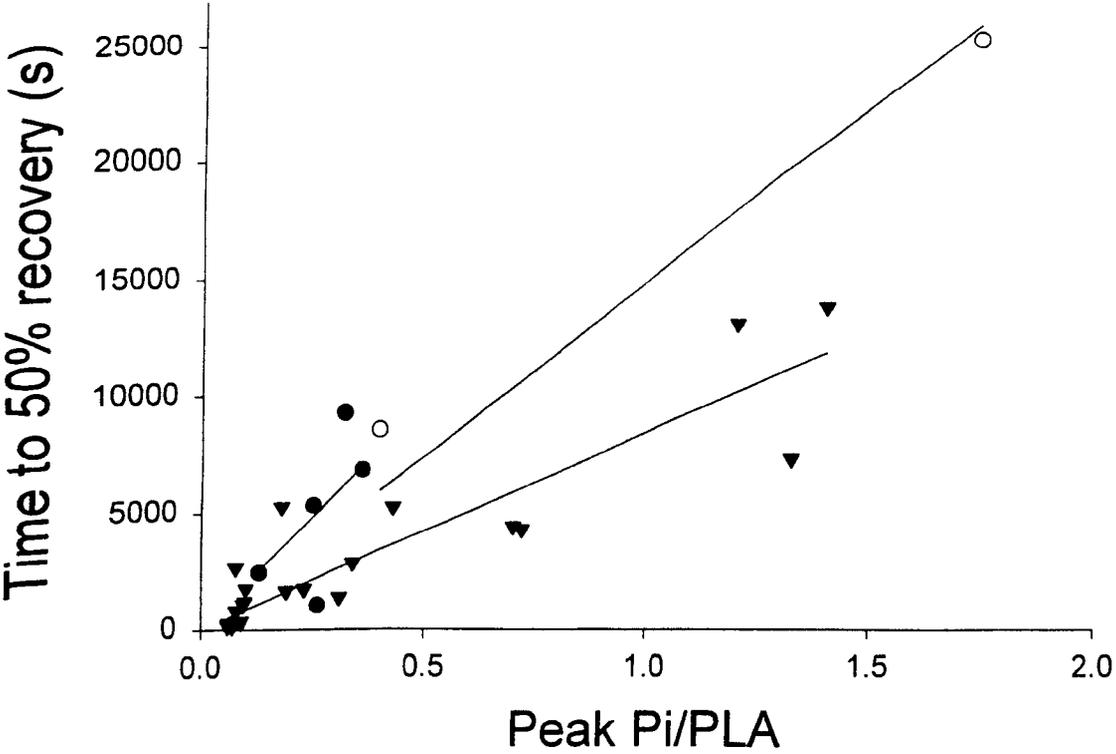
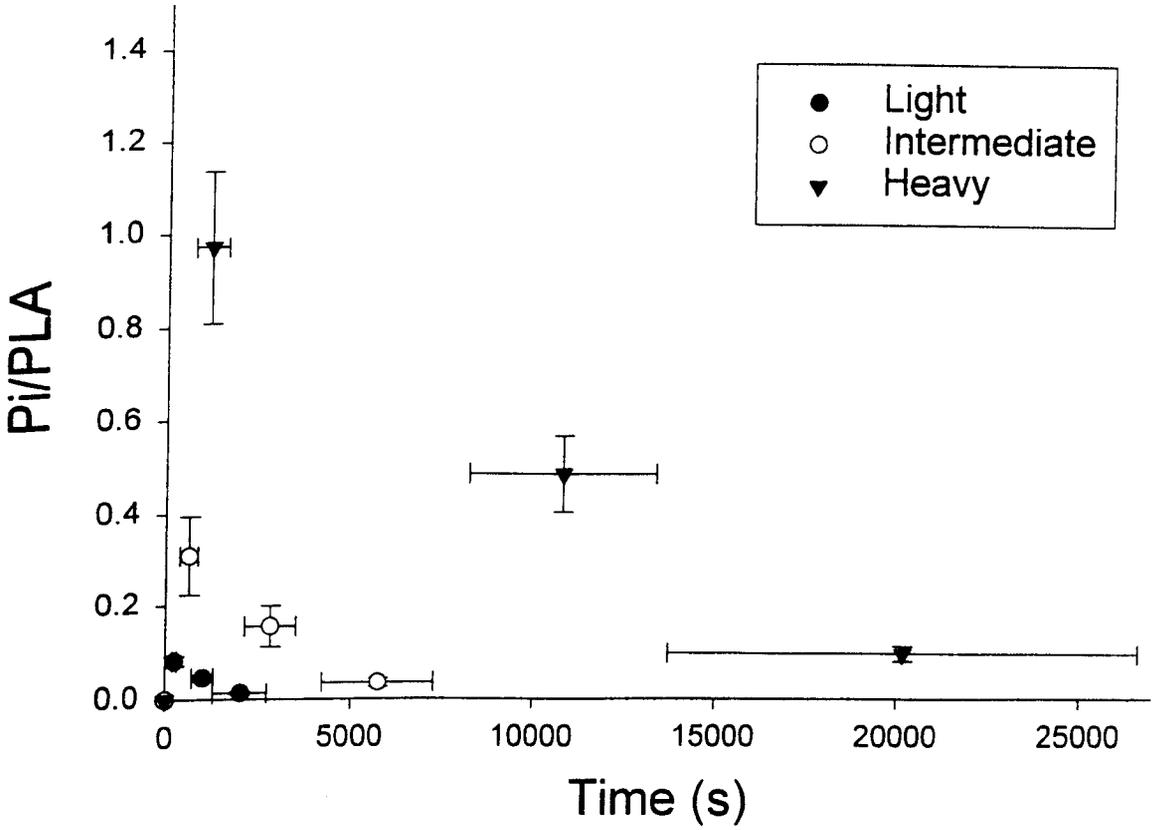


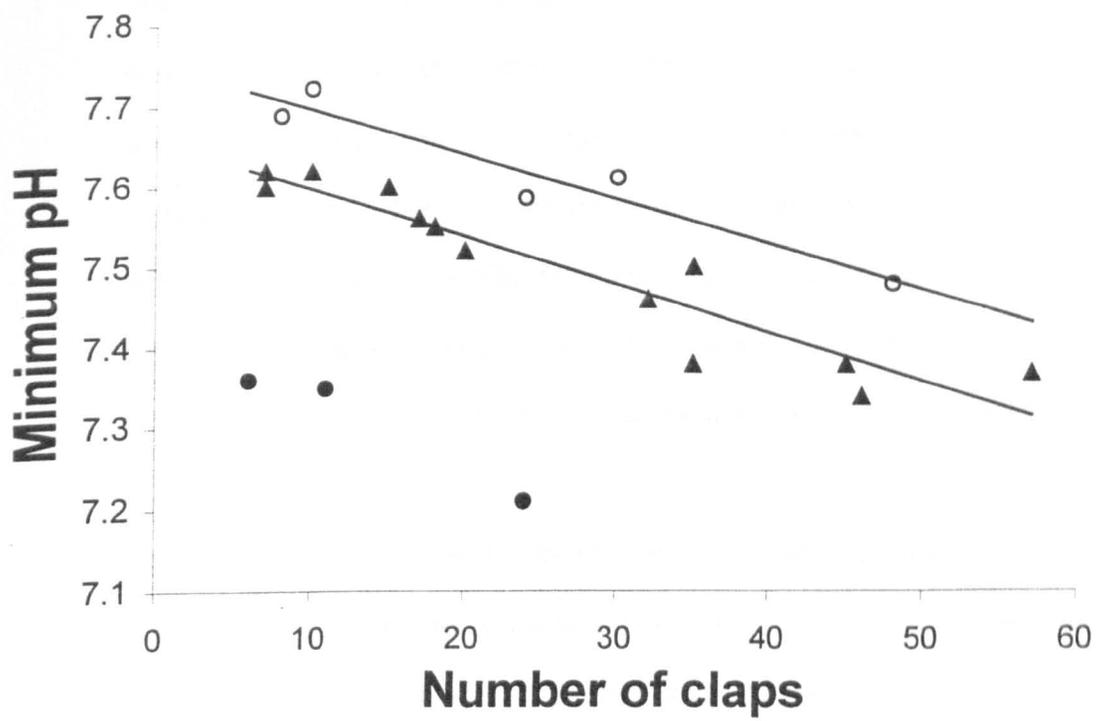
Figure 7. Summary of the effects of workload on the peak Pi/PLA ratio observed and the time taken to return Pi/PLA to resting levels in *Pecten maximus*. The three workloads presented are light (solid circles, <11 claps), intermediate (open circles, 11-21 claps) and heavy (solid triangles, >21 claps). Error bars are 1 S.E. At higher workloads higher Pi/PLA changes occur and recovery takes longer.



#### 4.3.6 Muscle pH during exercise and recovery

Exercise was followed by a short period of muscle alkalosis (rise of up to 0.5pH units), followed by more prolonged acidosis (Figures 3 and 8). The low number of data points obtained for *A. opercularis* prevented it from being included in most of these analyses. The alkalosis (maximum pH) reached was not significantly related to the number of muscle contractions in any species. The acidosis (minimum pH reached) following exercise was negatively related to the number of muscle contractions in *A. colbecki* and *P. maximus* (See fig. 8 for details). The minimum pH reached was significantly more alkaline in *A. colbecki* than in *P. maximus* (GLM Univariate ANOVA with clap number as a covariate). The intercepts of the pH vs. clap number lines at zero claps gave resting pHs of 7.75 and 7.65 for *A. colbecki* and *P. maximus* respectively. The net muscle acidification (maximum-minimum pH) increased significantly with increasing clap number in *A. colbecki* ( $RSq=0.869$ ,  $p=0.021$ ,  $df=3$ ) but not in *P. maximus*. There was insufficient data to compare *A. opercularis*. The relationships between peak  $P_i/PLA$  and maximum pH shift were significant in both *A. colbecki* and *P. maximus* (Linear regressions,  $RSq=0.805$ ,  $p=0.03$  and  $RSq=0.767$ ,  $p<0.001$  respectively).

Figure 8. The effect of workload (number of claps) on minimum muscle pH in the study species. Data points represent the minimum value recorded after the completion of the experiment. The drop in muscle pH following exercise increased significantly with increasing workload in both *Pecten* and *Adamussium*. *Pecten*,  $y = -0.006x + 7.658$ ,  $RSq = 0.889$ ,  $p < 0.001$ ,  $df = 11$ . *Adamussium*,  $y = -0.006x + 7.754$ ,  $RSq = 0.923$ ,  $p = 0.009$ ,  $df = 3$ . Minimum pH was significantly lower in *Aequipecten* than in *Pecten* (ANCOVA,  $p < 0.001$ ,  $df = 13$ ) and significantly higher in *Adamussium* than *Pecten* (ANCOVA,  $p < 0.001$ ,  $df = 5$ ).



## 4.4 Discussion

### 4.4.1 Observations of metabolism during exercise and recovery

The good time resolution of the data obtained show the metabolic changes during exercise and the recovery process in more detail than has previously been possible. As expected PLA level fell on initial work as the ATP used during exercise was regenerated from this phosphogen. As ATP level remained stable and arginine level rose, PLA began to reform. At lower workloads recovery set in early indicating that aerobic metabolism began shortly following work and ADP and Pi were removed by oxidative phosphorylation, ATP was produced and allowed the regeneration of PLA. The PLA store fell during exercise and rose during recovery. The contribution of anaerobic metabolism was minimal.

Considering the findings in earlier literature (see Introduction) the following scenario arises for higher workloads (fig. 2, t=7500s to end of experiment): Here anaerobic metabolism was prolonged, and was often extended further by the animal remaining closed. As before PLA level fell and Pi level rose following exercise with the magnitude of the changes reflecting the increased workload. Immediately following exercise PLA was rapidly reformed resulting in a rapid increase in level (fig. 2B). There was no concurrent fall in Pi level at this stage with Pi (fig. 2A) and PLA levels decoupling briefly. At this point there was an apparent drop in ATP level though this is within the range of the noise in the data (fig. 2C). This may suggest that the increase in PLA was derived from the breakdown of ATP by arginine phosphokinase at the very low PLA levels following heavy exercise. PLA reformation is probably arrested by the use of arginine by the octopine dehydrogenase pathway. The use of Octopine in anaerobic metabolism may increase the ATP available from Phospho-l-arginine. This anaerobic pathway regenerates NAD, allowing glycolysis to produce further

ATP. Supported by the developing acidosis PLA level started to fall again producing ATP.

The theory that use of Octopine in anaerobic metabolism may increase the ATP available from Phospho-l-arginine due to reduced arginine availability to the arginine phosphokinase reaction has been rejected by other authors. This position was on the basis that PLA use and octopine formation occurred over different timescales (Gäde et al., 1978) and that arginine removal was due to PLA regeneration (Livingstone et al., 1981). The increased resolution of the current study demonstrates that PLA utilisation may occur throughout the first half of the recovery process when octopine is probably being formed (fig. 3.03b). The transient recovery and subsequent fall in PLA levels indicates the activation of octopine dehydrogenase by pyruvate production. Another suggestion was that octopine use allows the animal to survive a further attack by closing its shell (Grieshaber, 1978). In predator-prey interaction experiments animals would almost certainly be consumed upon capture, closing its shell will not help (Barbeau and Scheibling, 1994a). In accordance with observations in squid (Pörtner et al., 1996) it appears that octopine metabolism enables the maximum use to be made of the PLA store and produces additional ATP by glycolysis, recovering the adenylate energy charge.

Natural selection presumably acts on the activities and substrate affinities of the various enzymes to maintain the balance between competing aerobic and anaerobic uses for substrates. Citrate synthase is four times as active in the file shell *Limaria fragilis* (which can swim for much longer than any described scallop) as in *Pecten alba* causing earlier initiation of the aerobic pathway. *Limaria's* octopine dehydrogenase activity was less than 1% of *P. maximus's*

(Baldwin and Opie, 1977), its reduced power output and inability to close its shell (Donovan and Baldwin, 1999) making the anaerobic pathway much less important.

So why don't scallops swim more slowly and respire aerobically during swimming? High-speed swimming is not a high priority for scallops as the predators against which swimming escapes are most effective are slow moving starfish and gastropods. Scallops probably cannot swim slowly. Observations in this study (chapter 2) show that at low power outputs (due to reduced water temperatures) *A. opercularis* often fails to leave the bottom. The requirement for relatively high power outputs in order to sustain swimming may be a constraint to this type of locomotion. Higher muscle mass specific power output is available from anaerobic muscle due to the reduced proportion of the muscle cross-section which is taken by mitochondria.

The results of the present study are in reasonably good agreement with PLA depletions during exercise in *Pecten jacobaeus* (Grieshaber and Gäde, 1977) and *Placopecten magellanicus* (Livingstone et al., 1981). Recovery took substantially longer in *Placopecten* with complete return of PLA levels not occurring for up to 24hr compared to a maximum of 17hr in the *P. maximus* in this study. The razor shell (*Ensis directus*) recovers from total exhaustion within 3hr probably since its PLA depletion was lower than was recorded in the present study and previously in scallops (Schiedek and Zebe, 1987); de Zwaan, 1979 #2016; Grieshaber, 1978].

Thompson and co-workers (Thompson et al., 1980) showed that the metabolism of *Placopecten magellanicus* recovering from exercise was very different from

one which was merely closing its shell and keeping it closed. Whilst using the catch muscle *Placopecten magellanicus* was able to maintain a relatively stable blood oxygen and pH level. An exhausted scallop was unable to take up sufficient oxygen even upon opening its valves to return blood oxygen and pH to baseline values for over 8 hours.

Acidosis was most rapid after the resumption of aerobic metabolism. Blood acidosis to the same pH as found in muscle in this study has also been recorded following exercise in *Placopecten magellanicus* (Thompson et al., 1980). They concluded that respiratory acidosis was more important than metabolic acidosis due to the low acidity of octopine and its low levels in the blood. However, it became clear later that octopine formation during anaerobic glycolysis causes the same extent of proton production as lactic acid formation (Hochachka and Mommsen, 1983; Pörtner, 1987; Pörtner et al., 1984) evidenced by a severe acidosis found during octopine formation in squid muscle (Pörtner et al., 1996). Buffering capacity may be reduced as increases in plasma calcium were not utilised by this species (Thompson et al., 1980). Mitochondrial metabolism was elevated at pH 6.4 compared to pH7.0 in *Euvola ziczac* at 22-28°C and indicated adaptation for aerobic recovery following acidosis (Boadas et al., 1997). The maximum pH shift recorded in the present study (around 0.4 units) is similar to that recorded by Curtin et al, (1997) for exercise in Dogfish muscle and relates in a similar way to the proportion of phosphagen utilised.

#### 4.4.2 Causes of fatigue

Exhaustion in the animals was defined as failure to respond to further stimulation. The causes of this remain unclear. As pH is elevated following exercise owing to the early consumption of phospho-L-arginine (Pörtner, 1987),

proton levels are not likely to be the cause. More likely is a relaxation effect by the accumulation of inorganic phosphate at high pH (cf. Pörtner et al., 1996). The relationships between number of claps, Pi/PLA, Pi or PLA do not change as the animal approaches exhaustion with no obvious threshold being crossed. There is a great deal of variability in the number of claps achieved (and therefore the metabolic changes) within species. As no consistent ATP depletion was detected during exercise or recovery, this is not implicated as a cause of fatigue.

#### 4.4.3 Interspecific differences in high-energy phosphate metabolism

Individual *Aequipecten opercularis* were able to perform around half as many claps as either of the other two species despite having the highest resting ATP levels. *P. maximus* clapped at a very low rate (typically 0.6-0.7Hz), as was found previously in response to stimulation by starfish homogenate (Gäde et al., 1978). Swimming responses were rare in both this and the present study. Higher numbers of claps have been recorded in *A. opercularis* when stimulated with a starfish leg (Grieshaber, 1978) and pers. obs). *A. opercularis* demonstrates clap frequencies of 2.4-3.2 Hz at the test temperatures (Chapter 2, this study). Ansell *et al*, (1997) recorded a maximum number of 18 claps from a single stimulation in *A. colbecki* in the wild. This species has a clap frequency of 1.7-2.1Hz at the test temperature.

The principle measure of metabolic change during exercise and recovery in this study was the ratio of Pi to PLA. ATP changes were small and within the range of the noise in the ATP signal. The use of the Pi/PLA ratio brings out trends not

apparent from either trace independently, reduces random noise and is easily comparable between animals.

The number of claps (muscle contractions) was significantly related to the PI/PLA ratios of muscles following exercise in all species, though the slopes of the relationships differed. The Pi/PLA attained by *A. opercularis* was significantly higher (2-3 times) than either of the other two species for the same number of claps. The relationship between the number of clap cycles and the maximum PI/PLA achieved was strong in all three species and the relationship was significantly different in each species from the others.

As there was no significant difference between the resting PLA stores of the three different species so *A. opercularis* used significantly more PLA than the other two species for the same number of muscle contractions. Peak muscle power output was significantly higher in *A. opercularis* (mean  $169 \text{ WKg}^{-1}$ ) than in *A. colbecki* ( $100 \text{ WKg}^{-1}$ ) at the temperatures used in the current study (Chapters 2 and 3). No comparable power output data exist for *P. maximus*. Muscle cross-sectional area specific force production (as calculated from the pressure trace) in *P. maximus* was similar to that produced by both the other species. Power output cannot be calculated directly from force production as this calculation requires data on the muscle strain rate not available in the present study (power=force x distance/time). Future studies will integrate direct measures of shell gape during clapping in order that muscle strain can then be calculated as described previously (this study, Chapter 2).

The increased PLA use per muscle contraction in *A. opercularis* may be related to the relatively high power output of this species. It should be noted that the

muscle performance estimates from the previous studies were not intended for interspecific comparison, only for the investigation of changes as a result of temperature and season within species so must be used with care.

Recovery rates were similar in *A. colbecki* and *P. maximus* with the available data for *A. opercularis* falling in between. Following heavy exercise (>22 claps) 50% recovery in *P. maximus* took an average of 3hr, with 90% recovery taking an average of 5.6hr. The increased PLA depletion observed in *A. opercularis* indicates a greater use of ATP and would initiate anaerobic glycolysis earlier. This species is the smallest and is an active swimmer in the wild and the laboratory, apparently at high energetic cost. The high swimming performance of *Aequipecten opercularis* is demonstrated by the much reduced mortality this species suffers from trawls compared with less motile species such as *Pecten jacobaeus* (Hall-Spencer et al., 1999). Although *Aequipecten* uses its energy stores the most quickly its rate of PLA regeneration is not appear to be higher than those of the other two species.

Recovery as defined in this experiment is not necessarily ecologically relevant, ability to perform further escape responses being the aim of the recovery, not simply returning metabolites to resting levels. The link between metabolic recovery and the return of full recovery is not demonstrated here. Possibly *A. opercularis* can initiate further claps at an earlier stage of recovery. Differences in reproductive state between species might also confound these results (Brokordt et al., 2000). Spawning is possible in *A. opercularis* at the time of the experiment (Allison, 1994) though none was observed. Spawning was less likely in the other species as they were both during their respective winters.

#### 4.4.4 Conclusions

There was more difference between the two temperate species than there was between *P. maximus* and *A. colbecki*. While this study (Chapter 3) found no evidence of cold adaptation in terms of the muscle performance of *A. colbecki* these new NMR data appear to demonstrate that this Antarctic species recovers from exercise at a similar rate at 0°C to *Pecten* at 10°C. The differences in mode of life between the two temperate species were more significant in terms of modifying its muscle physiology during exercise and recovery than environmental temperature.

## Chapter 5

### GENERAL DISCUSSION

#### 5.1 The findings of the present study

The performances available during an escape response and the total energetic cost of the performance determine in part whether the behaviour impacts positively upon fitness. The escape behaviour of two scallop species has been examined in great detail and the metabolic processes undergone observed at the highest resolution possible with existing technology.

At this point it is useful to reflect back on the findings of the study and discuss how they impact on our current knowledge. This discussion will cover each of the fundamental areas in turn by answering the following questions. Do we know anything new about the biomechanics and temperature dependence of escape responses? Are escape responses sufficiently important for scallops to attempt to maintain performance in the face of environmental change? What light might the findings of the study shed on our understanding of the trade-offs between performance and energetics?

##### *5.1.1 The biomechanics of escape responses*

The swimming of temperate and Antarctic scallops was studied using video of escape behaviour. The animals' whole-body performances were measured and mathematical predictions were made of the muscular performance required. As scallop swimming has been widely studied and the predictions of performance made here are in good agreement with those recorded previously (Cheng and Demont, 1997; Marsh and Olson, 1994; Marsh et al., 1992) this is not a major advance in itself. The large number of animals studied in this case has revealed

how variable scallop locomotion is even between animals of the same size and ambient temperature. A wide range of locomotory abilities appears to exist within the populations studied as has been reported for Garter snakes inhabiting highly variable thermal environments (Jayne and Bennett, 1990). This may be a mechanism by which highly fecund animals hedge their bets in terms of locomotory investment. Jayne and Bennett's (1990) study is one of few to correlate increased survival (let alone fitness) with animal performance, in this case the speed and stamina of garter snakes. Laboratory mesocosm studies have demonstrated that increased speed in tadpoles significantly improves their chances of surviving attacks by garter snakes (Watkins, 1995). Linking performance to actual fitness is not a trivial task and one which will be discussed later. Even correlating performance with survival suffers from a lack of detailed understanding of exactly which attributes a behaviour must have to improve fitness and the marginal costs of incremental improvements in these attributes.

On a technical level the results of this study match most closely to those of Cheng and Demont (1997) and share many of the same advantages and limitations. More detailed study of the behaviour of the mantle during jetting will enable a more detailed model to be constructed than either this study or those undertaken previously.

Unlike the other detailed studies of the biomechanics of scallop swimming (Cheng et al., 1996a; Cheng and Demont, 1996a; Cheng and Demont, 1996b; Cheng and Demont, 1997; Marsh and Olson, 1994; Marsh et al., 1992) and other studies of scallop locomotion (Ansell et al., 1998; Carsen et al., 1996a; Carsen et al., 1996b; Dadswell and Weihs, 1990; Joll, 1989; Manuel and Dadswell, 1990; Morton, 1980) the present study focussed upon the mechanics

of the first jetting cycle of escape swimming. As escape swimming is most effective against slow-moving non-visual predators such as seastars (Barbeau and Scheibling, 1994a; Barbeau and Scheibling, 1994c) it is this initial movement and the thrust of the jets against the predator which is most likely to determine survival.

This allowed the cycle in both *A. colbecki* and *A. opercularis* to be studied in detail and the effects of temperature to be determined. The thermal dependence of all stages in the swimming cycle were not equal with the  $Q_{10}$ s of peak and mean power output, and mean shortening velocity exceeding those of swimming velocity. Similarly animals acclimatised in the field to cold winter conditions had significantly greater power outputs than warm acclimatised animals collected in the autumn though their peak and mean swimming speeds did not differ.

Weak relationships between muscle and whole-body performance have been observed in other animals including frogs (Navas et al., 1999; Peplowski and Marsh, 1997) and salamander (Bennett et al., 1989) though in the present study it was possible to discern the cause. As temperature increases the shell is closed more rapidly due to the increased shortening velocity of the fast adductor muscle. As peak muscle strain did not differ with temperature or between groups adduction was completed sooner. Over the range of temperatures investigated ligament properties remained similar, resulting in a similar rate of reopening of the shell following adduction. The net result was a shortening of the active jetting phase relative to the cycle as a whole. Thrust during jetting was greater but this did not translate into increased swimming speed as the period of reopening, when drag is high and no thrust is delivered, was relatively

longer. This appears to be an unfortunate consequence for the animal, expending more energy for the same net result. If this is the case then why was muscle power output also elevated in cold acclimated animals?

#### 5.1.2 Significance of escape responses and their temperature dependence

Mean swimming speed is an important parameter in foraging for food but in most studied situations is mainly about escape (Barbeau and Scheibling, 1994a; Thomas and Gruffydd, 1971). Acceleration is likely to be more important than absolute speed and this was conserved by seasonal acclimatisation in *A. opercularis*. The adaptive significance of this difference in performance cannot be quantified as the physics of predator-prey interaction in scallops are not known. Data on the force required to break free of a Starfish would be useful in determining the marginal benefits of increased acceleration and thrust on survival. Acclimation has resulted in improved success in simulated predator-prey interactions though in this case it was the acclimated predator (the fish *Myoxocephalus scorpius*) which captured more prey (Beddow et al., 1995). The role of acclimation in fitness is not proven with laboratory studies in thermally acclimated bacteria and *Drosophila* demonstrating reduced fitness at that temperature in many cases (Gibbs et al., 1998; Leroi et al., 1994).

Scallops are negatively buoyant with a large proportion of their clap frequency dictated by a ligament whose properties are temperature independent. Depending on the angle of attack a minimum speed must be attained before lift supports the body of the animal (Thorburn and Gruffydd, 1979b); at low temperature (5°C) *A. opercularis* swam below this velocity and failed to swim at all following half of the experimental stimulations. Unlike file shells (Donovan and Baldwin, 1999) scallops probably must swim relatively quickly or not swim at

all. Seasonal acclimatisation may maintain this ability in the face of reduced water temperature.

The eurythermal scallop appeared to modify its muscle physiology in order to maintain muscle performance at low temperature. When the Antarctic scallop was investigated it showed no such evidence of ability to acclimate to temperature change. When compared to a four other scallop species across a 20°C temperature range its muscle power output and mean swimming velocity did not appear to be compensated for temperature change. These findings are in common with Antarctic fish (Franklin, 1998; Hardewig et al., 1999; Wakeling and Johnston, 1998). In this case a possible explanation was presented on the basis of the field observations and laboratory performance. *A. colbecki* exists in an ecosystem that is nutritionally poor (Albertelli et al., 1998; Berkman, 1990), but with no decapods and low starfish predator pressure (Berkman, 1988; Berkman, 1990). At Rothera the *A. colbecki* population was attached to the rocks by byssal threads, was unable to escape when stimulated, and suffered low natural mortality despite this. *A. colbecki* probably does not have to swim as quickly as *A. opercularis* due to the reduced weight of its shell, with a combination of low food availability and low selective pressure for escape performance resulting in a low-performance animal.

### *5.1.3 Trade-offs between performance and energetic costs*

In order to compare the energetic costs of exercise between scallop species animals were forced to simulate swimming movements while the high-energy phosphate content of their adductor muscle was measured using Magnetic Resonance Spectroscopy. This study provided the most detailed description yet

available on scallop muscle physiology following exercise and demonstrated the importance of the switch from anaerobic to aerobic respiration.

Two European species, *Aequipecten opercularis* and *Pecten maximus* were compared to *Adamussium colbecki*, the Antarctic species. These measurements demonstrated that *A. opercularis* had larger resting ATP stores and used a greater proportion of the energy available from phospho-l-arginine per muscle contraction and was able to produce fewer muscle contractions than either of the other two species. *P. maximus* and *A. colbecki* did not differ in any measured respect. Ecological differences amongst the species are probably more important than environmental temperature in determining the costs of swimming in scallops.

*A. opercularis* demonstrates higher muscle performance, maintains potentially important aspects of this performance by seasonal acclimatisation and has higher energetic costs than *A. colbecki*. However these remain the only species analysed using both NMR and high-speed video techniques. Non-invasive measures of both the performance and energy use during swimming have been developed. Application of these techniques to wild animals in order to answer the bigger question of how swimming performance relates to fitness in scallops was beyond the scope of this study. The design of a possible experimental design will, however, now be presented.

## 5.2 Future studies

### 5.2.1 *Scallops as model animals*

Does differential locomotory performance, acclimatory ability and efficiency within a population impact upon the fitness of individuals? Scallops are ideally suited to this type of experiment being highly variable, locally common and relatively sessile. Using the techniques developed here detailed measurements of swimming performance and muscle metabolism can be made in collected scallops. Minimally all that is required is to determine whether a relationship between performance and energetic cost exists in a representative subset of the filmed animals. Measurements of basal metabolism might also be useful in order to determine whether maintenance costs differ between animals.

The survival of these animals in the wild could then be monitored and their potential fecundity assessed prior to spawning. A further refinement would be the collection of individuals on a seasonal basis in order to compare individual differences in acclimatory ability and compare these to seasonal changes in mortality. Another interesting study would be to compare animals along an environmental and morphological gradient as exists in the bay scallop, *Argopecten irradians*, on the East coast of the United States (Wilbur and Gaffney, 1997). These animals exhibit differential investment in valve construction with genetically based differences in shell mass and morphology. In this case differences in predator-mediated selection were suggested as a possible mechanism for this divergence (Wilbur and Gaffney, 1997). Numerous examples of local scale population differentiation exist (Cruz et al., 1998; Krause et al., 1994) setting up a natural experiment into the effects of measurable animal performances on natural mortality. The effect of raising juveniles of each population under different levels of predator presence (e.g. kept in baskets in the

same tank or used to stimulate escapes at intervals) to investigate the developmental plasticity of morphology would also be interesting.

### *5.2.2 Closing remarks*

The adaptive importance of performance is a much under-studied area with advances in technology making its study easier all the time. Animals are observed and tagged world-wide, animals whose ecology, family history and fecundity is known in very great detail in some cases. To make biomechanical measurements from high-speed video of hunting in terrestrial animals is much more practicable now due to the low weight and power requirements of high-speed systems. A logging GPS tag with post-hoc differential corrections and an accelerometer could already give detailed enough measurements of position and movement to allow routine and peak performance to be accurately determined in individuals. It would be fascinating to measure locomotion and environmental temperature in ectotherms with natural, successful and unsuccessful, predator-prey encounters.

Integration of biomechanical and physiological studies with detailed knowledge of the ecology of the animals to be studied will be essential in furthering our understanding of the importance of animal performance. We need to know more about the behaviours of interest and the environmental conditions under which they occur, the life history of the animals under study and the periods in their life cycle which impact most strongly on differential survival. Integration should not end at the door of the biology department, making use of the advances in electronic and optical engineering available to us will enable the evolutionary importance of biomechanics to be studied where it belongs, in the field.

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