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Fish Skeletal Muscle:Adenoisine Triphosphate and Adenine Nucleotide Metabolites in Relation to the Texture and Quality of Fish

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FISH SKELETAL MUSCLE: ADENOSINE TRIPHOSPHATE AND ADENINE NUCLEOTIDE METABOLITES IN RELATION TO THE TEXTURE AND QUALITY OF FISH

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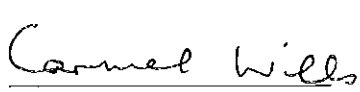

Carmel Wills

DEDICATION

To my husband Tom and my son John

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FISH SKELETAL MUSCLE: ADENOSINE TRIPHOSPHATE AND ADENINE NUCLEOTIDE METABOLITES IN RELATION TO THE TEXTURE AND QUALITY OF FISH

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Summary

The changes which occurred in the concentrations of ATP, ADP, AMP, inosine monophosphate, inosine and hypoxanthine in skeletal muscle of rainbow trout, salmon and goldfish during the onset and resolution of *rigor mortis* were investigated. The effects of *ante mortem* handling and methods of slaughter on the concentrations of these nucleotides in muscle immediately after death and during storage of fish at 3°C and at –30°C were examined. Very careful handling of fish and killing by a method which did not cause contraction of muscle were essential if concentrations of ATP were to be at levels indicative of resting muscle (4 to 6μmol/g tissue). Fish which were anaesthetised *ante mortem* and killed by cervical fracture or by clubbing had a high concentration of ATP and a relatively slow onset of *rigor*. Fish slaughtered by CO_2 stunning or asphyxiation struggled violently at death and the muscle had very low concentrations of ATP, very high concentrations of inosine monophosphate and a rapid onset of *rigor*. The ratio of the sum of the concentrations of inosine and hypoxanthine to the total concentration of the six nucleotides was used as a criterion of freshness.

Quality and freshness were also evaluated by physical and sensory procedures, texture by measurement of muscle shear force and freshness by measurement of the dielectric constant of the whole fish and by a standard method of visual appraisal of the fish. The results demonstrated that there is a relationship between biochemical data, texture, dielectric constant and visual evaluation for both fresh (chilled) and frozen fish. During *rigor*, the resolution of *rigor* and in the *post rigor* state freshness and quality declined on the basis of an increase in K value and a decrease in shear force, dielectric constant and visual assessment.

There were significant differences in all four assessments of quality between fish which died tranquilly (clubbing) and those which struggled during asphyxiation. The slaughter procedure therefore appears to have an influence on several aspects of quality in fish during storage at 3°C and –30°C.

Abbreviations used in this Thesis

ADP	adenosine diphosphate
AMP	adenosine monophosphate
AQUI-S	2-methoxy-4(propenyl)-phenol
ATP	adenosine triphosphate
CP	creatine phosphate
G-6-P	glucose-6-phosphate
HPLC	high performance liquid chromatography
Hx	hypoxanthine
IMP	inosine monophosphate
INO	inosine
MS-222	ethyl m-amino benzoate methane sulphonate
~ P	high energy phosphate
QIM	quality index method
RPS	rapid paper strips
SR	sarcoplasmic reticulum
s.e.m.	standard error of the mean
*	significant at the 5% level ($p < 0.05$)

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1. INTRODUCTION

1. INTRODUCTION

1.1 Foreword

Fishes inhabit the largest ecosystem on Earth - the water. Life is thought to have originated in water, and many species of plants and animals living today have the sea or freshwater as their habitat. The term fish is applied to a variety of cold-blooded aquatic vertebrates of several evolutionary lines. Fishes belong to the phylum Chordata, which also include the amphibians, reptiles, birds and mammals. Fishes are among the most numerous vertebrate animals. There are known to be in excess of 40,000 different kinds, an abundance and diversity unequalled among all other vertebrates. The most primitive class, *Agnatha*, comprises species of lampreys and hagfish – soft bodied vertebrates that lack jaws, and those vertebrates that possess jaws, the *Gnathostomata* which include the cartilaginous Elasmobranchs and the bony Teleosts. The Teleosts are the dominant fish of the world today. In terms of diversification, they are the most successful of all vertebrate groups, with more species than all other vertebrates combined. They are widely distributed in fresh and saltwater from Antarctic regions below freezing to hot springs of more than 40°C.

The characteristic adaptations of fishes are related to propulsion through water and extraction of oxygen from water. Locomotion in vertebrate fishes is dependent on body and tail muscles as well as fins. The body is streamlined to minimise energy spent on swimming, the tail is well developed, to provide power and aid steering and the fins work in various ways as hydrofoils. Fishes take in water through the mouth and expel it to the exterior across the gills. The gills are internal organs rich in blood that extract oxygen from the water, which is required for respiration and the release of energy from food. The most obvious sense organs of fish are the eyes, with a

reflective layer (the tapetum) inside the eye. This allows fishes to make maximum use of light filtering through water. Light triggers and directs migrations and movements, influences rate and pattern of growth and has a timing role in reproduction.

Fish may be classified scientifically according to taxanomic norms. However, because of the diversity in fishes in practice many systems of classification are used informally. Fish may, for example, be categorised under headings referring to physiology, habitat, recreational pursuits, as foods, and in terms of economic importance. The ESRI (1980) classified fishes found in Irish waters into four groups which encompasses features such as habitat, lifestyle and structure as follows:

(i) demersal fish, which are found on or near the sea floor. These include round fish such as cod, haddock, whiting and pollock, and flatfish such as plaice, sole, brill and turbot.

(ii) pelagic fish which live in surface or middle depths of the sea. Species include herring, mackerel, pilchards and sprats.

(iii) anadromous fish which live in salt water but spawn in fresh water and include salmon and sea trout.

(iv) invertebrate shellfish.

The world's annual catch of fish and marine invertebrates is currently at about 90-100 million metric tons (Sikorski and Pan, 1994). Nonetheless, only 20% of the total catch, at a yield of 30% is used for direct human food consumption (Shahidi, 1994). A depletion of certain species has resulted from over-fishing and thus conservation strategies have been implemented. In recent years aquaculture developments have led to the production of increasing amounts of fish and shellfish.

Within the Irish Fishing Industry both freshwater and marine fish farming constitute an increasingly important activity. The total aquaculture production in Ireland in 1996 was 35,000 tonnes valued at £55 million, of which, finfish accounted for approximately 16,000 tonnes, and shellfish realised 19,000 tonnes (BIM, 1997). Whiting and plaice are important species within the Irish domestic market. Records show that whiting landings from January 1996 to December 1996 realised 9,778 tonnes valued at £5.8 million while landings of plaice for the same period were 1,525 tonnes with a value of £1.996 million (BIM, 1997). Although consumption of seafood in Ireland is low compared with other European countries, it has been growing steadily to its present level of 8.5kg per person per annum. This increase has been due in part to better marketing of fish. The greater availability of fresh fish daily, as well as the health qualities of fish and the expanding range of consumer-ready products has also been responsible for the increase in fish consumption.

1.2 Functional Aspects of Fish Muscle

All animals need to move at some stage in their lives – to find food, to invade new habitats, to reproduce. Most animals move by using muscles that can contract and, when anchored to a rigid skeleton, are able to move the parts of the body that are attached to them. Since muscles are capable only of contraction, not extension they are generally arranged in antagonistic pairs, where the contraction of one muscle extends another. Muscle generates motion by converting chemical energy in the form of ATP which is derived from the metabolism of food, into mechanical energy. The primary source of ATP in living cells is aerobic respiration and the Lohmann reaction. At the death of an animal the concentrations of ATP fall and leads to the sliding together of the actin and myosin filaments and the onset of *rigor mortis* (Erdos, 1943; Bendall, 1973).

The onset of *rigor* in fish varies from species to species and is dependent on factors such as the physiological status of the animal *ante mortem*, the method of harvesting and the temperature at time of death. Changes in muscle biochemistry *post mortem* have important physiological consequences for the fish in terms of the texture of the muscle. Fish muscle from newly captured animals is a highly desirable food due to its flaky, soft, moist texture, its pleasant mild flavour, and high nutritional value. Fresh fish however, is susceptible to rapid bacterial deterioration. Loss of quality in stored fish is characterised by the development of unpleasant odours and flavours, excessively soft texture, loss of liquid-holding capacity and the development of a dry, tough texture when cooked. Contrary to mammalian meat, where ageing brings about a desirable flavour and texture in the product, fish and seafoods tend to deteriorate rapidly *post mortem* (Shahidi, 1994). This phenomenon is one of the major challenges facing the fishing industry today. Researchers worldwide are actively seeking strategies to

extend the quality of fresh fish. The Concerted Action "Evaluation of Fish Freshness" (AIR3 CT94 2283) project funded by The European Union (1995-1997) focussed on harmonising research activities on the evaluation of fish freshness. The overall aim of the concerted action project was to validate methods for the assessment of fish freshness and to discuss the freshness criteria for fish within the European Union. The research participants worked in subgroups on ATP metabolites and physical measurements; lipids, proteins, microbial methods and predictive modelling: methods to monitor changes in volatile compounds and sensory analysis. Representatives from the fish industry expressed the need for rapid and reliable methods to evaluate fish freshness. A number of rapid techniques such as instruments that can monitor changes in the electrical properties of the muscle and electronic noses to detect volatile degradation compounds as indicators of freshness are available. Although there are no rapid methods available to detect changes in lipids and protein, rapid methods to measure ATP metabolites have been developed, however, these methods have not been implemented in industry. The Concerted Action concluded that sensory evaluation was the most important method used to evaluate fish freshness and proposed the Quality Index Method (QIM). While there is no real definition of the word freshness the Concerted Action took the view that freshness referred to fish which had been recently harvested and which exhibited those properties attributed to the fish in the living state.

Structural links between the muscle cells and the connective tissue of fish provide the necessary integrity for the flesh to withstand the effects of post-harvest handling, processing and storage. In order to understand the changes which take place in fish skeletal muscle *post mortem* a fundamental knowledge of the components of the delicate and complex structure of fish flesh is essential (Bremner, 1992). The

progressive decline in quality of fish muscle *post mortem* is due to two major factors: (i) chemical and physical changes due to endogenous enzyme activity, and (ii) changes due to microbial growth. While a great deal of attention has been focussed on the role of microbial activity in the deterioration of fish quality, the contribution of autolysis had been mainly overlooked (Simpson, 1997).

The analysis of ATP and nucleotides provides the food technologist with a useful tool for assessment of the quality of fish muscle using a ratio of the concentrations of inosine (INO) and hypoxanthine (Hx) to the total amount of ATP derived compounds called the K value (Saito *et al* 1959).

1.3 Morphology of Fish Muscle

Muscle has been the subject of scientific investigation for several centuries and it is not surprising that the early scientists Hooke (1665) and van Leeuwenhoek (1712) first turned their attention to this tissue. Fishes, like all other vertebrates, have three major types of muscle. These are smooth muscle and cardiac and skeletal muscle (both striated muscle). Skeletal muscle is the most widely distributed tissue in mammals and fish and is responsible for generating body motion. The anatomy of fish skeletal muscle is different from that of terrestrial mammals, in that fish lack the tendinous system connecting muscle bundles to the skeleton. Instead, fish have muscle cells running in parallel and connected to sheaths of connective tissue, which are anchored to the skeleton and the skin (Love, 1970). Because of the support provided by the water surrounding a fish, the requirements for fish muscle to support the skeletal framework is much reduced and as a result a very different type of muscle organisation has evolved. The skeletal muscle of fish represents from 45 to 65% of the total body mass and from 50 to 70% of the eviscerated weight depending on the species (Lefèvre,

1997). The muscles that form the fish fillet are the large lateral muscles which run on both sides of the body from head to tail. These muscles have a characteristic metameric structure quite uncommon in mammalian tissues. A horizontal septum called the lateral line separates the upper and lower parts of the fillet. The upper part is referred to as the dorsal or epaxial muscle and the lower part the ventral or hypaxial muscle. The body muscle of vertebrate fish is divided into segments, or myotomes, corresponding in numbers to those of the vertebrae (Dunajski, 1979). The myotomes are separated by sheets of connective tissue, or myocommata (Fig.1). In transverse section, the sheets of the connective tissue between the myotomes curve in a complex manner. The exact shape of the myotomes is characteristic for the species of fish and depends also on the location of the myotome in the muscle (Dunajski, 1979).

When seen at the surface of the body after the skin has been removed, the myocommata have the shape of a horizontal 'W' set across the long axis of the body with their central points directed towards the head. This simple shape is only superficial; in three dimensions a myotome can be visualised as consisting of two hollow cones, one on each side of a plane bisecting the fillet at the level of the backbone, with the apices of the cones pointing towards the tail (Fig.1).

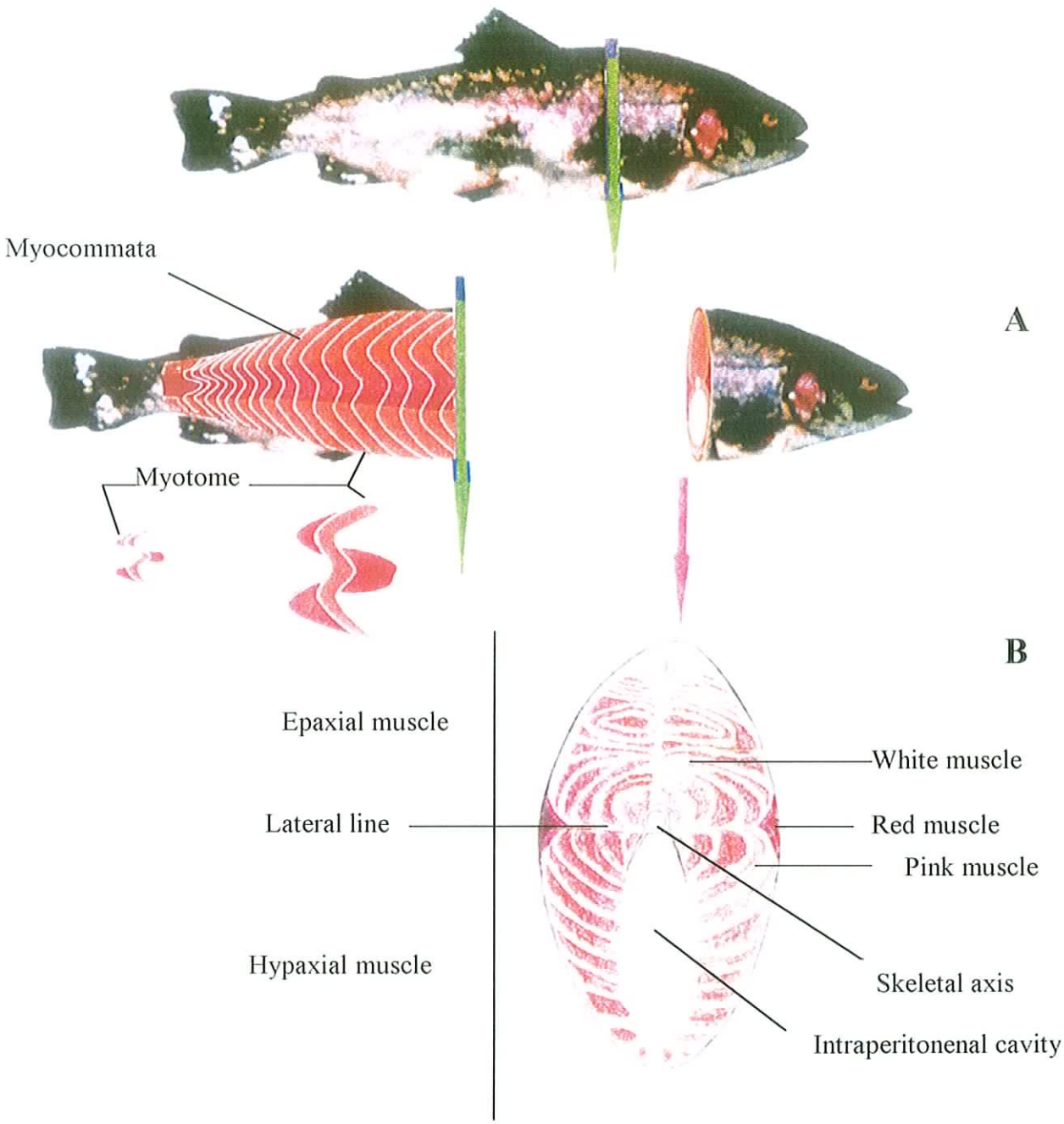

Fig. 1. Description of myotomal muscle of teleost fish. External arrangement of the myotomes (A), and transverse section showing the different types of muscle (B). (Gauvry, 1995).

The cones of one myotome nest into those of the next (Le Danois, 1952). This arrangement derives from the very complicated swimming mechanism of the fish (Nursall, 1956; Bainbridge, 1963; Ganguly and Nag, 1964).

Fish musculature is heterogenous in that it contains varying proportions of white (fast) muscle, dark (red or slow) muscle and connective tissue. The lengths of the muscle cells vary from the head (anterior) to the tail (posterior). Measurements of the width of myotomes show that the longest muscle cells in cod are found at about the 11^{th} to 14^{th} myotome from the point of severance of the head and the diameters of the individual cells follow a similar pattern (Love, 1970; Dunajski, 1979). This finding is of great importance in sampling. It is important to decide on one particular region of the fish, and use none other for all samples which are to be compared (Love, 1970). Johnston *et al* (1995) reported that the largest myotomes in short-horned sculpin (*Myoxocephalus scorpius*) are at the centre of the fish.

Historically, fish muscles have been classified according to their colour. Although classifications based on muscle colour have lost favour with scientists for other vertebrate groups, they are widely applied to fish muscle (Johnston, 1981). In many fish, the myotomal muscle is largely composed of two muscle fibre types, namely red fibres and white fibres which are arranged in anatomically distinct regions (Arloing and Lavocat, 1875). A third intermediate type of muscle fibre termed pink also exists (Bone, 1978a).

1.3.1 Red muscle

Red muscle forms a superficial layer of tissue lying just below the skin at the lateral line. The muscle runs from behind the head to the caudal fin (Johnston *et al* 1975) and

the content increases towards the tail (Greer-Walker and Pull, 1975; Johnston, 1982; Lampila, 1990). When the trunk of a typical teleost is viewed in cross-section the red muscle appears in the form of a triangle (Fig.1). The cells of red muscle are narrower and more uniform than those of white muscle (Stirling, 1886). These are the slow twitch fibres (Bone, 1975) and are associated with slower or sustained swimming speeds (Bone, 1966). In addition to the high myoglobin content giving the muscle its characteristic red colour, the fibres are characterized by an extensive blood supply, low myofibrillar ATPase activity, greater numbers of mitochondria, and high concentrations of lipids and glycogen (Venugopal and Shahidi, 1996). Red muscle has a predominantly oxidative type of metabolism with high activities of enzymes of the citric acid cycle utilising both carbohydrates and lipids for fuel (Johnston *et al* 1977).

1.3.2 White muscle

White muscle represents more than 90% of the myotomal mass and contains fibres ranging from 10 to 200μm in diameter (Lefèvre, 1997; Table 1). It is generally agreed that the fibres of white muscle are better equipped than the red for the anaerobic provision of short bursts of energy. The less extensive vascularisation of these fibres imparts both the characteristic white colour and culinary importance. The ATPase activity is higher in white than in red muscle (Ogata, 1960; Sreter *et al* 1966) and the fibres have a higher activity of various glycolytic enzymes, for example, phosphorylase (Engel, 1962), phosphofructokinase and fructose-1-6 diphosphatase (Opie and Newsholme, 1967), glyceraldehyde phosphate dehydrogenase (Pette, 1966) and lactate dehydrogenase (Dawson and Romanul, 1964). Ogata (1960) and Tarrant *et al* (1972) reported higher concentrations of CP, ATP and glycogen in resting white myofibres of mammalian muscle compared to red myofibres. In some species of fish, for example

salmon and trout, small diameter fibres occur throughout the myotome intermingled with the larger fibres, giving the muscle a characteristic mosaic appearance (Boddeke *et al* 1959). White muscle increases in size with the age of the animal (Kiessling *et al* 1991).

1.3.3 Pink muscle

Pink muscle is intermediate between white and red muscles and is so-called because of its colouring (Johnston *et al* 1977). The presence of intermediate muscle fibre types in fish myotomes was first noted by Ogata (1958). The limited information on intermediate fibres shows that they are less well equipped with glycolytic enzymes than are fast fibres, but that they are between slow and fast fibres in aerobic capacity and ATPase activity (Johnston *et al* 1977; Johnston, 1982). The fibres of pink muscle can be distinguished from other fibre types histochemically by their alkaline stable (pH 10.40) and myofibrillar ATPase activity (Johnston *et al* 1974; Johnston *et al* 1975). The number of pink fibres varies from one species to another (Zhang *et al* 1996). In mirror carp (*Cyprinus carpio*) these fibres comprise around 10% of the trunk musculature, making them slightly more numerous than red fibres (Johnston *et al* 1974). The precise functional role of the pink fibres is uncertain but is thought to be associated with the ability to sustain swimming for long periods (Bokdawala, 1967). Coughlin *et al* (1996a) reported that in certain species pink muscle was associated with normal swimming in complement with white muscle.

Fish species	Length or age/weight	Type of muscle	Average diameter (µm)	Range of variation (µm)	Reference
Rainbow trout (*Oncorhynchus mykiss*)	16-18 months	white red	- -	10-200 20-30	Fauconneau *et al* 1993a
Rainbow trout	10-15cm	white	-	10-95	Johnston, 1982b
Rainbow trout	1.9-2.6kg	white pink red	- - -	19-304 41-187 45-66	Kiessling *et al* 1995
Cod (*Gadus morhua*)	95cm	White	-	150-300	Love, 1958 cited by Dunajski, 1979
Tuna/Skipjack (*Euthynnus pelamis*)	-	White	50	-	Lampila, 1990
Horse mackerel (*Trachurus trachurus*)	-	White	90	-	Lampila, 1990
Plaice (*Pleuronectes platessa*)	-	White	130	-	Lampila, 1990
Wolf fish (*Anarhichas lupus*)	-	White	257	-	Lampila, 1990
Horse mackerel (*Trachurus murphyi*)	35-45cm	White Red	- -	30-80 10-30	Rutman *et al* 1988
Scup (*Stenotomus chorysops*)	~20cm	White Pink Red	64 56.3 34	27-120 22-73 16-54	Zhang *et al* 1996
Carpe (*Cyprinus carpio*)	19-24cm	White red	44.6 32.4	30-58 21-43	Spierts *et al* 1996
Carpe (*Cyprinus carpio*)	Adult	White Pink Red	~100 ~60 ~36	- - -	Fauconneau *et al* 1995
Beef	-	-	-	10-100	Lampila, 1990

Table 1. Diameter of muscle fibre in white, red and pink muscle taken from a number of fish species (Lefèvre, 1997).

1.3.4 Myocommata

The myocommata are situated between the myotomes and are essentially made up of connective tissue which forms a supporting network throughout the whole muscle. Compared with mammalian muscle the connective tissue content in fish muscle is lower since the water environment lends support to the body (Hultin, 1984). Connective tissue is more uniformly distributed in fish muscle than in the muscles of warm-blooded animals, although its content varies in different parts of the musculature because of the size distribution of the myotomes (Dunajski, 1979). There have been reports that the proportion of connective tissue increases towards the tail of the fish (Love, 1988; Lampila, 1990). In hake, for example, the proportion of connective tissue varies from 0.2% at the head region to 3.3% at the caudal region and in trout from 2.6 to 4.6% (Montero and Borderias, 1989). The thickness of the myocommata depends on the species, anatomical location, age, season and the nutritional state of the fish. Hultin (1984) indicated that fish collagens appear to turn over annually rather than being more highly cross-linked and tougher with age.

1.3.5 Structure of Muscle

Each myotome is composed of muscle cells or fibres running parallel to the long axis of the fish. Compared with mammalian muscle the fibres of fish muscle are short and are generally between 10 and 30mm long. Their diameter depends mainly on the species, the age of the fish and the function of the muscle. In large adult fish for example, cod (95cm long) the fibre diameter varies from 150 to 300μm depending on the location in the fillet (Love, 1958). In mammals, the muscle fibres can run the whole length of the muscle and may be as long as 300mm (Lockhart, 1972). Each fibre contains between 1000 to 2000 elongated, thread-like structures or myofibrils,

each up to 5μm in diameter. The myofibrils are the contractile elements and occupy most of the volume of the cell and give the characteristic striated pattern of alternate light and dark bands when viewed with the light microscope. The myofibrils are segmented into sarcomeres and are composed of thick and thin filaments, showing alternate arrangements of anisotropic A-bands and isotropic I-bands. At the centre of each I-band is a dark line called the Z-line or Z-disc. At the centre of each A-band is a light area which is called the H-zone. In the centre of the H-zone there is a darker M-line. The distance between the two Z-lines is termed the "sarcomere" and is the basic contractile unit of the fibril (Huxley, 1963). The sarcolemma or cell membrane surrounds the muscle fibre within which lie the SR and the transverse (T) tubules. The length of the sarcomere depends on the species and on the degree of contraction or relaxation of the muscle. In resting mammalian muscle the sarcomere length is 2.3 to 2.8μm, whereas in the muscle of crab sarcomere lengths ranging from 4 to 7μm have been recorded (Gillis, 1969; Fahrenback, 1967; Franzini-Armstrong, 1970). The thick filaments of myosin molecules, and the thin filaments of double helical strings of actin, overlap and slide over one another during muscle contraction. The thick filaments of a vertebrate contain 200-400 molecules of myosin. Tropomyosin and troponin are regulatory proteins involved in the contractile mechanism and are present in the actin filaments (Suzuki, 1981; Skaara and Regenstein, 1990). The electron microscopy of muscle fibres has been described by Huxley (1963) and reviewed by Johnston (1981).

1.3.6 Proteins of Fish Muscle

1.3.6.1 Sarcoplasmic proteins

The sarcoplasmic proteins are water-soluble and account for 20-35% of the total protein content of muscle (Connell, 1968; Matsumoto, 1980). They are present within the muscle cell and consist mostly of enzymes responsible for the metabolism of the cell (Connell, 1968; Matsumoto, 1980; Mackie, 1993). This group of proteins includes myoglobin which is responsible for the storage and transport of oxygen within the cell and is present in higher concentrations in red muscle than in white muscle. Although the sarcoplasmic proteins are heat sensitive, they do not appear to have much involvement in the textural characteristics of fish flesh. However, they are believed to contribute to oxidative changes within the muscle during storage *post mortem*. The concentration of sarcoplasmic proteins is higher in the muscle of pelagic fish than in demersal fish (Sikorski, 1990).

1.3.6.2 Myofibrillar proteins

The myofibrillar or contractile proteins account for 65-75% of the total protein content of fish muscle, compared with 52-56% in mammals (Mackie, 1993). The myofibrillar proteins are responsible for the water-holding capacity and texture of muscle. Hydrolysis of muscle proteins in fish *post mortem* can lead to changes in texture (Haard, 1992). The three main myofibrillar proteins are the myosin, actin and tropomyosin (Asghar *et al* 1985; Bechtel, 1986).

Myosin

Myosin, accounts for 40-60% of the total myofibrillar protein and is located in the thick filaments (Asghar, 1985; Bechtel, 1986; Hamm, 1986; Ohtsuki *et al* 1986;

Mackie, 1993). It has a molecular weight of approximately 500,000 Daltons. Myosin contains two identical heavy polypeptide chains (HC) each with a molecular weight of 200,000 Daltons and four light chains (LC) with a molecular weight of ~20,000 Daltons. The two heavy chains are wound around each other in a supercoiled helical conformation. The myosin molecule is characterised by two globular head sections at one end of the molecule which are responsible for ATPase activity and binding to actin.

Actin

Actin represents 15-30% of the total protein (Connell, 1968; Matsumoto, 1980; Asghar, 1985; Bechtel, 1986; Ohtsuki *et al* 1986). Monomeric actin is a globular protein with a molecular weight of 43,000 Daltons. It exists in muscle as a polymeric form of F-actin, which has a double helical structure. Each actin monomer in F-actin binds to one globular head of myosin. The F-actin filaments on opposite sides of the Z-discs point in opposite directions, allowing the thick filaments in adjacent sarcomeres to move toward the Z-disc. Actin from fish muscle is more stable during frozen storage than myosin.

Tropomyosin and Troponins

These myofibrillar proteins together account for 10% of the contractile proteins (Asghar, 1985; Bechtel, 1986; Ohtsuki *et al* 1986). Tropomyosin contains two strands coiled. The molecular weight of tropomyosin is 66,000 Daltons and its subunit is 33,000 Daltons. Troponin is made up of three subunits designated troponin-C (18,000 Daltons), troponin-I (21,000 Daltons) and troponin-T (31,000 Daltons) respectively.

Troponin-T is the site for binding troponin to tropomyosin; troponin-C binds calcium and troponin-I inhibits the enzymatic activity of actomyosin.

Connective Tissue Proteins

The connective tissue proteins are mainly collagens and account for between 3 to 10% of the total protein in fish muscle. These proteins represent between 10 and 15% of the total protein in mammalian muscle. Fish collagens contain less proline and hydroxyproline than mammalian collagen, and are thermally less stable and more soluble than those of mammals. Fifteen genetically distinct types of collagen have been identified (Bailey, 1985; Burgeson, 1988) of which five are associated with skeletal muscle (Types 1, 11, 111, 1V, V). However, in the intramuscular tissue of teleost fish collagen types1 and V only have been demonstrated to be present (Sato *et al* 1988). Connective tissue imparts structural support for the muscular system and contributes to the texture of the tissue. The different structural arrangement of fish muscle is derived from the lower content of connective tissue proteins. Also, fish do not need the extensive support as land animals. The content of collagen in the muscle depends upon the species as well as on the state of maturation and feeding of the fish (Sikorski *et al* 1990).

1.4 Changes in Skeletal Muscle *Post Mortem*

The circulation of blood stops with cardiac arrest and death and the tissues are deprived of oxygen. The most apparent physical change which occurs after death is the development of the stiff and rigid state of the muscle. This phenomenon is referred to as *rigor mortis*. Biochemical changes within the dying muscle determine the rate of onset, duration and extent of *rigor*. The rate at which biochemical changes occur is

very important in that it affects the subsequent keeping quality of the muscle as food with respect to colour, texture, water-holding capacity and potential for bacterial growth.

Current knowledge of the biochemistry of *rigor* was initiated by the discovery of the ATPase activity of myosin (Engelhardt and Ljubimova, 1939) and the observation by Erdos (1943) that the loss of ATP from muscle after death was associated with the onset of *rigor*. Bate-Smith and Bendall (1947a) confirmed this observation which was further extended by Bendall (1951) and Lawrie (1953) and Marsh (1954). Partmann (1965) showed that the onset of *rigor* in fish skeletal muscle was associated with a fall in the concentration of ATP.

The biochemistry of *rigor* was long misunderstood. Kuhne (1864) and Schipiloff (1882) proposed the lactic acid theory. It was thought that *rigor* onset was due to precipitation of muscle proteins by lactic acid. However, Claude Bernard (1877) described alkaline *rigor* in animals which had died from starvation and in which no lactic acid was formed in muscle *post mortem*. Hoet and Marks (1926) observed a rapid onset of alkaline *rigor* in rabbits injected with convulsant doses of insulin, which demonstrated that lactic acid was not necessarily involved.

1.4.1 Theory of *rigor*

The main biochemical processes associated with *rigor* are the initial disappearance of glycogen and CP and finally the disappearance of ATP. These reactions are accompanied by the production of lactic acid by anaerobic glycolysis and a fall in pH. It has not yet been established which ATPases are responsible for the hydrolysis of

ATP. Greaser *et al* (1969) and Heffron and Mc Loughlin (1971) investigated the role of myofibrillar and SR ATPases in determining the rate of glycolysis *post mortem*.

The concentration of ATP remains constant for a variable time in the muscle after death as a result of the degradation of CP and glycolysis. The period where the concentration of ATP remains steady is known as the delay phase of *rigor*. ATP is regenerated anaerobically *post mortem* by the transfer of high energy phosphate (~P) from CP to ADP *via* the Lohmann reaction and by the Embden Myerhof pathway of glycolysis. ATP concentrations fall when concentrations of CP fall below a certain critical level. This is the rapid phase of *rigor*. Studies on relaxed muscle in rabbits *m. psoas* showed that CP levels were high immediately *post mortem* but rapidly declined to one-third of the original level before any detectable loss in ATP occurred (Bendall, 1951). The rate of loss of ATP from muscle, is determined by the initial concentrations of ATP, CP and glycogen. Bate-Smith and Bendall (1956) considered that ATP dephosphorylation was the principal reaction determining the rate of other chemical changes.

1.4.2 Time course of *rigor*

Bate-Smith and Bendall (1947b, 1949) described three main types of *rigor* in mammalian muscle. They are described as acid *rigor*, alkaline *rigor* and an intermediate type of *rigor*. These three types of *rigor* differ in the rate of onset and degree of shortening of the muscle. The initial concentrations of ATP, CP and glycogen in the muscle at any particular temperature determine the type of *rigor* present (Marsh, 1954). The magnitude of the glycogen reserve of the muscle determines the pH at onset of *rigor*. Factors such as exhaustion whether induced by insulin injection (Bate-Smith and Bendall, 1956) or starvation or hunting, deplete the

concentrations of glycogen and CP so that the resynthesis of ATP from ADP is limited and alkaline *rigor* develops rapidly with formation of small amounts of lactate.

A wide range of factors influence the rate of onset of *rigor* but the primary determinants are the concentrations of ATP, CP and glycogen in the muscle at the time of death. Stress *ante mortem* and a violent death reaction reduce the concentrations of ATP, CP and glycogen in the muscle. McLoughlin (1964) reported that muscle contraction associated with struggling at time of death accelerated glycolysis in pig muscle. Bate-Smith and Bendall (1947b, 1949; Bendall, 1973) studied the effect of the muscle relaxant myanesin injected in rabbits. These authors reported that the initial concentrations of glycogen, CP and ATP in the *m. psoas* were high and the time course of *rigor* was extended. Bate-Smith and Bendall (1956) compared two groups of well-fed rabbits, one group of which was killed under anaesthesia and the other which was stunned by a blow to the head. The concentration of glycogen decreased by more than 50% during killing in the "violent death" group compared with the group which was killed under anaesthesia. Bendall (1973) noted that there was considerable variation in the time course of *rigor* and in the rate and extent of the accompanying biochemical changes in mammalian and amphibian muscle. These differences are due to the nutritional and physiological status of the animal at the time of death and to differences between and within species.

1.4.3 *Rigor mortis* in fish muscle

The concentrations of ATP in mammalian skeletal muscle may, under certain circumstances remain constant for a number of hours *post mortem* (Bate-Smith and Bendall, 1947; Marsh, 1952; Lawrie, 1953; Marsh, 1954; Briskey and Wismer-Pedersen, 1961). Tomlinson and Geiger (1962) reported a rapid decline of ATP in fish

skeletal muscle *post mortem*. However, some species of fish retained a constant level of ATP in the muscle *post mortem* when they were in an unexercised condition before being killed. Degradation of fish glycogen *post mortem* involves two pathways, the Embden-Myerhoff phosphorylytic pathway (from glycogen to pyruvic acid) and the direct amylolytic hydrolysis of glycogen via maltose and glucose. The *post mortem* conversion of glucose-6-phosphate to glucose by glucose-6-phosphatase only occurs to a slight degree in fish muscle. Consequently the hydrolytic pathway appears to be the main one operating in fish (Ghaneker *et al* 1956; Eskin *et al* 1971). Tarr (1965) and Burt (1966) confirmed this as the main degradative pathway of glycogen to glucose for most fish. However, it is the phosphorylytic pathway that is responsible for glycogen degradation in mammalian muscle. Eskin *et al* (1971) remarked that irrespective of which pathway is involved in the early stages of glycogen breakdown, the final pathway involving glycolysis is the same for both mammalian and fish muscle.

Rigor in fish is generally of shorter duration than in mammals (Amlacher, 1961). The rate of onset of *rigor* varies from species to species and is dependent on such factors as the physiological condition of the animal at time of death, environmental factors and temperature. In some species of pig onset of *rigor* in muscle (*l. dorsi*) has been recorded in less than 1 hour at 37°C (Ludvigsen, 1954; Briskey and Wismer-Pedersen, 1961; Bendall and Lawrie, 1964). There is a striking contrast between animals such as amphibians which show a long drawn out *rigor* process lasting more than 24 hours at 20°C and certain birds (pigeon) in which the pectoral muscle goes into *rigor* in less than 4 hours at this temperature (Bendall, 1970). Eskin *et al* (1971) and Govindan (1979) noted that the appearance of *rigor* in fish commenced 1 to 7 hours *post mortem* (Table 2). In slaughtered fish kept on ice it peaked between 5 and 22 hours *post*

mortem and the complete duration of *rigor* covered 30 to 120 hours. The time to onset of *rigor* of 1 to 7 hours was quoted by Eskin *et al* (1971) and by Govindan (1979).

Amlacher (1961) described the onset of *rigor* in fish as follows: Stiffening starts in the lower jaw and around the gill cover which in many fish, e.g. cod and whiting, is visible as a slight opening of the mouth and the gill covers. Further *rigor* spreads from the head *via* the chief body muscles to the caudal areas. At this stage the fin muscles may also stiffen. It has been observed in some cases that the caudal muscles go into *rigor* first. Weiss (1914), Pohl (1953) and Messtorff (1954) attributed this to the fact that these muscles are particularly active during the death struggle. Resolution of *rigor* follows the same pattern starting from the head region and spreading to the tail area. Mori (1954) confirmed this sequence for mackerel anchovy, garfish, gray mullet, umbrine, sea bream and sole, except in all of these fish it was observed that the onset of *rigor* takes place simultaneously over the entire body.

The onset, extent and duration of *rigor* in fish are influenced by the activity of the fish before slaughter. The more tranquil a fish is before death the longer the time to onset of *rigor* (Amano *et al* 1953; Fujikama and Kogo, 1953; Noguchi and Yamamoto, 1955) and the longer the duration of *rigor*. Conversely, the metabolic processes in the muscle tissue are accelerated due to intense muscular activity of the fish before death causing a decrease in content of glycogen reserves. In such fish, time to onset of *rigor* is shortened and the duration of *rigor* is reduced (Amlacher, 1961). Amlacher (1961) also noted that muscle from salt-water fish, handled in a careful way during capture, resulted in a better quality raw product for the processing industry than muscle from fish which had been exhausted prior to slaughter. Similar observations were made for freshwater fish (Pohl, 1953). The effects of rough handling and electrical stimulation

may hasten the onset and course of *rigor* in fish muscle (Partmann, 1961). Amlacher (1961) noted that environmental temperatures and manner of death of fish influenced the duration of *rigor*. *Rigor* is prolonged when fish are slaughtered immediately after capture, although not to the same extent as through lowering the temperature (Messtorff, 1954a,b; Gianelli, 1954; Pavlov, 1956). Gianelli (1954) reported a reverse reaction in the initiation of *rigor* in sole, *viz*; 30 minutes at 2°C to 3°C and 3 to 8 hours at 23°C to 24°C. Flatfish show a more extensive *rigor* than roundfish; *rigor* develops more slowly in flatfish, but becomes more intense (Gianelli, 1954). Amlacher (1961) studied the effect of temperature on *rigor* and concluded that temperature has less influence on the rate of onset of *rigor* than on its duration. Amlacher (1961) summarised the relationship between intensity and duration of *rigor* in fish in relation to other factors as follows:

(1) the intensity of *rigor* in various fish species becomes greater the longer the *rigor* lasts *ceteris paribus* (*c.p.*) (Messtorff, 1954b);

(2) in fish of the same species which die in the same way, the duration and intensity of *rigor* increase with a lowering of the body temperature *c.p.* (Cacacciolo, 1954; Gianelli, 1954; Messtorff, 1954b; Pavlov, 1956).

(3) slaughtered fish show a later onset of *rigor* than do asphyxiated fish *c.p.* *Rigor* also became more intense and lasted longer;

(4) for fish of the same species and presumably of the same biotypes and same size, the duration of *rigor* is equally long *c.p.*;

(5) fish of varying size and age show differences with regard to onset and duration of *rigor*. In small sized fish, *rigor* may last longer than in large sized specimens *c.p.*

Pohl (1953) studied the nature of *rigor* in freshwater fish killed by different methods. Fish were killed by asphyxiation, by hitting on the head, by beheading and by electrical shock. In the fish which were killed by asphyxiation and beheading the duration of *rigor* was 21 to 25 hours at 16°C, whereas in fish that were killed by hitting on the head the duration of *rigor* was 30 to 32 hours at 16°C. The duration of *rigor* in fish killed by electrical shock was considerably shortened. The differences in duration of *rigor* were less influenced by the method of kill at temperatures above 20°C and below 5°C. Pohl (1953) remarked that the size and age of fish have a considerable influence on the onset and duration of *rigor*. The influence of temperature on the development of *rigor* in freshwater fish was described by Pohl (1953). At 0°C *rigor* started about 24 hours after death in roach, redeye and perch and lasted for 72 hours to 80 hours. In the same species at 35°C it began 20 to 25 minutes *post mortem* and had an average duration of 3 hours. Studies on the onset, duration and resolution of *rigor* in the tropical fish tilapia at 5°C and 22°C showed that stiffening occurred within minutes of the fish being iced; maximum stiffening was reached after 8 hours at 0°C and lasted for 72 hours. The immediate stiffening of tilapia was described as a cold shock reaction and was not accompanied by contraction of the muscle fibres. The onset of *rigor* occurred at 7 hours *post mortem* when tilapia were maintained at 22°C after death. Tilapia which developed the cold shock reaction were shown to give low filleting yields with high drip loss (Curran, 1986).

Proctor *et al* (1992) studied the concentrations of ATP, CP, glycogen, glucose-6-phosphate and lactate present in skeletal muscle of a number of marine and freshwater

species anaesthetised with the inhalant anaesthetic ethyl m-amino benzoate methane sulphonate (MS-222). These authors reported concentrations of ATP in the muscle immediately *post mortem* similar to those found in mammalian skeletal muscle. The concentrations of lactate and the high pH (≥ 7) indicated that the fish had not been stressed *ante mortem*, nor had significant contraction of muscle occurred at death. They also reported that both electrocution and carbon dioxide (CO_2) stunning significantly reduced the initial concentrations of ATP and CP in muscle of farmed salmon (*Salmo salar*) compared with fish which had been anaesthetised before being killed by a sharp blow to the head.

Proctor and McLoughlin (1992) studied the effects of MS-222 and electrical stunning on chemical changes in the myotomal muscle of wild salmon (*Salmo salar*) *post mortem*. Electrical stunning accelerated the rate of disappearance of ATP and CP from the muscle, thus accelerating the onset of *rigor*.

The rate of hydrolysis of ATP in spiked plaice *post mortem* was reported to be higher at 0°C than at 5 to 15°C (Iwamoto *et al* 1985; 1987; Iwamoto and Yamanaka, 1986). McLoughlin and Proctor (1993) studied biochemical changes in myotomal muscle of anaesthetised (MS-222) rainbow trout (*Oncorhynchus mykiss* Walbaum; until recently called *Salmo gairdneri*, Bone *et al* 1995) and plaice (*Pleuronectes platessa*) during the onset of *rigor* at different temperatures. The rate of hydrolysis of ATP in the muscle of both species decreased as the temperature was reduced from 37°C to 1°C. An increase in the concentration of CP occurred at and below 20°C. The results did not indicate that a temperature of 1°C accelerated the rate of onset of *rigor* in these species, as had been reported previously by other workers.

Azam *et al* (1989) studied three different methods of killing rainbow trout (*Salmo gairdneri*). The fish were killed by electrocution, by exposure to elevated concentrations of CO_2 and by a blow to the head. The fish which were killed by electrocution and by CO_2 narcotization had a higher initial production of lactic acid and a slightly reduced pH, compared with the fish which had been killed by a blow to the head. Proctor (1992) reported frenzied activity in salmon (*Salmo salar*) stunned with CO_2 which was accompanied by depleted concentrations of CP and ATP immediately *post mortem*. Similar observations were made by Robb and Warriss (1997) who commented on the violent reaction of salmon stunned with CO_2 gas and reported thrashing of the fish at the surface of the container for about two minutes compared with fish which had been anaesthetised with the anaesthetic Aqui-S. They also reported that fish which were stunned with CO_2 had a lower pH immediately *post mortem* and onset of *rigor* was faster compared with the anaesthetised fish. The use of traumatic methods such as CO_2 stunning to slaughter fish in bulk is prevalent in the harvesting of farmed salmon and has been the subject of recent discussion (Paterson *et al* 1997). Kestin *et al* (1991) reported that use of an electroencephalography in an evoked response study of trout (*Oncorhynchus mykiss*) indicated that CO_2 stunning is not instantaneous and that the fish reacted adversely during the induction phase of anaesthesia. Sebastio *et al* (1996) studied the onset of *rigor* in rainbow trout (*Oncorhynchus gairdneri*) killed by four different methods. Fish were killed by percussive stunning (blow to the head), asphyxiation, electrocution and CO_2 narcotization and electrocution. In fish killed by electrocution and CO_2 onset of *rigor* was observed at 1-2 hours and 7 hours and the duration of *rigor* was 65 and 47 hours respectively at 2-3°C. In fish which were killed by percussive stunning onset of *rigor* was observed at 12 hours *post mortem* and the duration of *rigor* was 84 hours at 2-3°C.

Fish which were asphyxiated exhibited the most rapid onset of *rigor* (15-20 minutes) and the shortest duration (46 hours) at the same temperature. Marx *et al* (1997) compared three methods of stunning on freshwater fish. Trout (*Oncorhynchus mykiss*), carp (*Cyprinus carpio*) and eels (*Anguilla anguilla*) were killed by methods which included a manual technique delivered by a blow to the head, stunning by electrocution and stunning using CO_2. The fish which were stunned using CO_2 had a significantly lower pH immediately *post mortem* compared with fish which were electrically stunned or manually stunned, and onset of *rigor* was faster particularly in carp anaesthetised with CO_2.

Hattula *et al* (1995) reported that the onset of *rigor* was faster and the concentrations of IMP were lower in fish caught by gill-net compared to fish which had been harvested by trawling or pound-netting. It has been proposed that rapid killing of fish should lead to enhanced shelf life although this may be so only when the fish are not stressed at the time of killing (Proctor, 1987). The use of the inhalant anaesthetic MS-222 is considered to be generally unsuitable for use on fish for human consumption. Jerrett *et al* (1998) studied the effect of the food-grade anaesthetic Aqui-S on Atlantic salmon. These animals exhibited a delay in the onset of *rigor* of up to 20 hours *post mortem* and maintained significantly higher muscle pH (7.2) values for up to 40 hours *post mortem* compared with fish which had been subjected to CO_2. Paterson *et al* (1997) suggested that AQUI-S may be a suitable anaesthetic for fish in terms of good colour and appearance, reduced gaping and better quality. While AQUI-S has approval in New Zealand and Australia it is currently undergoing review by the US FDA and it is presently not available for sale in the USA.

Species	Condition	Temperature (°C)	Time *post mortem* to onset of rigor (hours)	Time *post mortem* to end of *rigor* (hours)	Reference
Cod (*Gadus morhua*)	Stressed	0	2-8	20-65	Stroud (1969)
	Stressed	10-12	1	20-30	
	Stressed	30	0.5	1-2	
	Unstressed	0	14-15	72-96	
Blue Tilapia (*Areochromis aureus*)	Stressed	0	1		Korhonen *et al* (1990)
	Unstressed	0	6		
Tilapia (*Tilapia mossambica*) small 60g	Unstressed	0-2	2-9	26.5	Pawar and Magar (1965)
Grouper (*Epinephalus malabaricus*)	Unstressed	2	2	18	Nazir and Magar (1963)
Anchovy (*Engraulis anchoita*)	Stressed	0	20-30	35-55	Trucco *et al* (1982)
Plaice (*Pleuronectes platessa*)	Stressed	0	7-11	18	Iwamoto *et al* (1987)
Socckeye salmon (*Oncorhynchus nerka*)	Unstressed	0	4	17	Tomlinson *et al* (1961)
	Unstressed	20-24	1	6	
	Stressed	0	<1	15	
	Stressed	20-24	1	3	
Rainbow trout (*Oncorhynchus mykiss*)	Stressed	0	1		Tomlinson *et al* (1961)
	Stressed	17-19	1	14.5	
	Unstressed	0	14.5	8.5	
	Unstressed	17-19	3.5	79.5	
				34.5	
Carp (*Cyprinus carpio*)		0	8		Hwang *et al* (1991)
		10	60		
		20	16		
		0	1		
		0	6		

Table 2. Onset and duration of *rigor mortis* in a number of fish species. Adapted from Huss (1995).

1.5 Methods of Harvest and Animal Welfare

The humane killing of mammals and poultry is regulated by legislation which requires the animal to be slaughtered by a method which will render it instantaneously insensible to pain until death occurs. There are, however, exceptions to this in the instance of religious slaughter and for the slaughter of poultry, where methods of decapitation and neck dislocation are used (Kestin *et al* 1991). Legislation requires a two-stage slaughter process. The animal must be stunned, followed by a killing procedure. However, where slaughter of fish is concerned there are no such specific regulations and methods of killing have evolved to suit local needs. It is not known whether fish are capable of feeling pain or distress in the same way as mammals. In the absence of such knowledge it is fitting to afford fish similar protection to that given to higher vertebrates. There is a need to assess the principal slaughter methods of fish on humanitarian grounds.

The most frequently used method of harvest is to remove fish from water and allow them to asphyxiate in air. In many instances fish are harvested in big numbers into large containers of ice slurry. The water is then drained off and the fish die from anoxia while packed in ice. Reducing the temperature of the tissues could increase the time to cerebral anoxia and thus prolong the time to loss of sensibility. Since fish are poikilotherms, this slaughter method could therefore prolong the time during which the fish are conscious. Kestin *et al* (1991) investigated the time required for fish to lose sensibility after removal from water by using visual evoked responses (EEG) as an index of brain function. They concluded that fish killed by removal from water can take from 144 to 866 seconds to loose sensibility. Such a time span falls short of rendering the fish insensible instantaneously.

There have been fewer studies on specific killing methods for fish (Boggess *et al* 1973; Azam *et al* 1989; 1990). Stress *ante mortem* can be reduced by careful handling of the fish before and during harvesting. The effects of neural stimulation of muscle can be minimised by destroying the brain, or by severing its connection with the spinal cord, a procedure known as 'spiking'or *ike jime* (Boyd *et al* 1984; Iwamoto *et al* 1987; Oka *et al* 1990; Ando *et al* 1991; Lowe *et al* 1993; Mochizuki *et al* 1994; Jerrett *et al* 1996). Sebastio *et al* (1996) found that effective percussive stunning of fish resulted in higher concentrations of glycogen and glucose-6-phosphate in the muscle immediately *post mortem* and appeared to cause the least stress during slaughter. Although arguably more humane, these practices are labour intensive and their commercial use is not acceptable for the time being. In recent years consumer attention has focussed on animal welfare in relation to methods of killing. Many consumers want to be assured that the food from the animal has been produced in an ethical way and that the animal has been killed humanely (Warriss and Robb, 1997).

1.6 Concept of Quality

In any discussion of quality or quality attributes of a food material, it is important that the terms used are understood. Fish is one of the most highly perishable commodities and the public has always required continuous reassurance about its quality. It is clear from the earliest hieroglyphic records that fish were an important source of nutrition and quality issues were both recognised and taken seriously (Lupien *et al* 1996). In earlier times fish were eaten close to where they were caught and for the most part very shortly after harvesting. However, today fish are traded as a commodity and are normally transported large distances under some form of refrigeration. In any such trade high quality standards are important. Thus, food safety control systems such as

Hazard Analysis Critical Control Point (HACCP) and ISO 9000 series may improve the global marketability of seafoods (Notermans and Jouve, 1995) Furthermore, predictive microbiology may serve as a very important tool in fish handling and processing operations (Valin, 1995; Whiting, 1995; Buchanan and Whiting, 1996). Recently a Code of Practice for Quality Farmed Freshwater Trout (BIM, 1999) was introduced to help fish farmers implement an Irish Aquaculture Quality Assurance Scheme (I-AQUAS) for the production of fresh and prepared rainbow trout (*Oncorhynchus mykiss*) and other species of salmonids, such as brown trout (*Salmo trutta*) and Arctic char (*Salvenius*). This document was intended to compliment the current European Legislation on the production, processing and marketing of fishery products.

The literature cites many definitions of food quality (Kramer, 1959; Jacobson, 1972; Wodica, 1976; Hall, 1981; Fishken, 1990). Kramer (1959) defines food quality as "the composite of those characteristics that differentiate individual units of a product and have significance in determining the degree of acceptability of that unit by the user". A more recent definition from the consumer point of view defines quality as: "sensory quality is that complex set of sensory characteristics, including appearance, aroma, taste and texture, that is maximally acceptable to a specific audience of consumers, those who are regular users of the product category, or those who, by some definition, comprise the target market" (Fishken, 1990). While these terms are concise and appear appropriate to a discussion of fish as an edible product, it is difficult to talk in terms of fish quality without taking into consideration the concept of freshness.

Botta (1994) refers to the freshness of a particular seafood and describes it as the "degree of excellence" of that seafood and must refer to appearance, texture, odour and colour.

The degree of excellence depends upon a number of factors for example, the location of harvest (Love, 1975, 1988; Huss, 1988), the time of year during which the fish is caught (Love, 1975b), the method of catching (Botta *et al* 1987; Huss, 1988), and the manner in which the seafood is handled (Reay and Shewan, 1949; Reay, 1951; Botta *et al* 1986, 1989; Botta and Bonnell, 1988; Huss, 1988). These factors seriously affect the degree of excellence with regard to the sensory, physical and chemical attributes of the fish.

1.6.1 Factors that Affect Quality

Location of harvest

Fish living in the wild are subjected to a number of physical factors such as salinity, temperature, pressure and oxygen concentration. The variation within these conditions may change the metabolism of the fish in such a way that it affects the quality of the fish *post mortem* (Borresen, 1992). Variation within a species caused by age, sexual maturity and spawning, and the availability of food may also have a profound effect on the quality of fish as food by influencing characteristics such as chemical composition, texture, flavour and colour (Love, 1980). Thompson and van Cleve (1936) cited by Dunlop (1955) reported differences in the body proportions of Pacific halibut from different locations. Lundbeck (1953) observed marked differences in cod depending on the geographical location and reported the mean weight of 4 to 7 year old fish ranged from 4.9kg in the North Sea to 1.4kg in the "North East area". Thus in conventional fisheries there are a number of difficulties in controlling the properties of

the product. However, aquaculture opens interesting possibilities where the fish farmer has additional ways to control a certain amount of variation by selection of a particular species of fish raised under specified conditions. In this way, control of the physiological factors, such as age and growth rate; management of environmental factors, such as water temperature and control of dietary factors can help to produce fish of a consistent quality.

Time of year

The time of year during which the seafood is caught has a profound effect upon the quality of fish (Love, 1975). Sometimes fish are bright-eyed and lively, while at other times they just lie down and die (Love, 1980). Their flesh may feel firm and springy or soft and flabby, their shape and colour may vary, and sometimes they make a more appetising food for man than they did even a few weeks previously. The diet of cod for example differs qualitatively at different times of the year and the flesh is watery and contains less protein. Fish of this kind is said to be in poor 'condition' or 'out of season'.

Fish are in poor condition after spawning. During spawning and for some time afterwards most fish do not feed and the fish become depleted of protein, carbohydrate and fat and are described as being 'run down'. However, this depletion suffered by cod at time of spawning has also been observed to a small extent in sexually immature fish during the same period. Rae (1967) suggested that fish appear to be affected by some factor other than spawning. Hickling (1934) found seasonal changes in the size of the ovaries of immature hake (*Merluccius merluccius*) which corresponded with those of mature fish. He concluded that the loss of condition in summer time of immature and adolescent fish cannot be attributed to nutrition. To some extent, it is

thought that the key to understanding certain seasonal variations lies in the concept of a biological 'clock', an inner rhythm which persists in the absence of outside stimulus (Love, 1980).

The attributes which govern the quality of fish are flavour, odour, texture, colour and appearance, all of which are said to vary according to the season (Love, 1979). Gaping, or the sectional separation which occurs in the fillets of fish is related to weakening of the strength of the collagen allowing the flesh to fall apart. This phenomenon appears to vary seasonally and is associated with variations in pH (Love, 1979). Factors which weaken the connective tissue in fish and result in gaping have been summarised as follows by Love *et al* (1982): (a) freezing the whole fish as distinct from the fillets (Love and Robertson, 1968), (b) freezing in *rigor* as distinct from pre *rigor* (Love et al 1969), (c) freezing fish which have been allowed to age after *rigor* (Love *et al* 1969), (d) allowing fish to enter *rigor* at higher temperatures rather than at lower temperatures (Love and Haq, 1970), and (e) reduction in pH (Love *et al* 1972).

Seasonal variation in the gaping of cod muscle was studied during the period of 1969-1970, when the average pH was low in the summer months and again in December-January when a secondary fall in pH was observed (Love *et al* 1972). The highest incidence of gaping was recorded between June-July, with a smaller increase in November- December. The phenomenon of gaping poses a serious problem for the fishing industry in terms of processing and ultimately for the consumer, particularly when fish have been frozen.

Method of catching

Diverse opinions have been expressed concerning the effects of methods of catching on fish quality (Botta *et al* 1987; Huss, 1988). Most capture techniques used for wild fish can involve long delays between time of catch and landing on the fishing vessel. As a result some fish may become stressed due to overcrowding, prolonged struggling and asphyxiation (Wagner, 1978; Davis, 1995). Studies have shown that fish which struggle during capture can have a core temperature exceeding 27°C on landing (Zhao *et al* 1998). Fish allowed to struggle prior to death have a higher rate of glycolysis, depletion of ATP and a corresponding shorter time for onset of *rigor* (Izquierdo-Pulido *et al* (1992). Sigholt *et al* (1997) showed that handling stress due to confinement for ten minutes before stunning with CO_2 in farmed Atlantic salmon (*Salmo salar*) resulted in lower concentrations of CP and ATP and a shorter *pre rigor* period compared with the fish which were not confined before stunning.

There have been suggestions that *rigor* commences earlier and lasts longer in trawled fish than in hand-line caught fish of the same species (Sikorski and Pan 1994). The quality of Antarctic krill is very significantly affected by the time of trawling and the size of the hauls. In large hauls of up to 15 tons some krill are still alive when hauled on board. However, in longer trawls consisting of many hours even though the haul size is very small, the fish are crushed and broken to a large extent (Kolakowski and Gajowiecki, 1991). Where the fish are killed as part of the catching operation, care should be taken to ensure that the fish die as tranquilly as possible.

Handling of seafood

The quality of fish from the time of capture until it reaches the consumer is subject to changes arising from post-harvest handling, standard of hygiene during handling and environmental factors (Sikorski and Pan, 1994). Large fish brought on board, if not stunned immediately, can bruise during struggling on deck thereby affecting the overall appearance of the fish. Some post harvest treatments including scaling, washing, gutting and heading and bleeding indicated that poor post-harvest handling practices may enhance the rate of deterioration of fish (Ashie *et al* 1996). Love (1980) discussed the importance of handling and attributed gaping in cod muscle to rough handling when the fish was in *rigor*. Rough handling also results in a faster rate of spoilage due to the physical damage to the fish resulting in easy access for endogenous enzymes and spoilage bacteria.

The types and rate of deterioration in quality during post-harvest handling of fish are often found to be species specific. Scott *et al* (1984) found that careful handling of orange roughy which had been headed and gutted only slightly increased the shelf-life. It is a common experience that the quality and storage life of many fish decrease if they have not been gutted immediately after stunning. In most North European countries, gutting of lean species is compulsory. Gutting removes the source of digestive enzymes responsible for early autolytic changes and prevents the spreading of bacteria from the gut. In cod (*Gadus morhua*) for example, it has been shown that there is a reduction in the storage life of five or six days if the fish have not been gutted Huss (1995).

Experiments carried out with a number of cod-like species showed a different picture. In haddock (*Melanogrammus aeglefinus*), whiting, (*Merlangius* merlangu), saithe

(*Pollachius virens*), and blue whiting (*Micromesistius poutassou*), ungutted fish stored at 0°C showed a loss in quality compared with gutted fish, but not to the same degree as that observed for cod. Although some off-odours and off-flavours were detected in the haddock, whiting and saithe, the fish were still considered to be acceptable after storage on ice for one week (Huss and Asengo, 1976). However, in South American (*Merluccius gayi*) hake there was no difference between gutted and ungutted fish (Huss and Asengo, 1977).

Icing

The single most important factor in reducing the deterioration of fish is temperature (Connell, 1975). Historical evidence shows that the Ancient Chinese used natural ice to preserve fish more than three thousand years ago. In developed countries, particularly in the USA and some European countries, the tradition of chilling fish with ice dates back to more than a century. It has been suggested that rapid chilling of fish to about 0°C (FAO/WHO 1977; FAO 1982) reduces the growth of spoilage and pathogenic microorganisms thereby reducing the rate of deterioration of the fish and reducing or eliminating certain safety risks (Huss, 1995). In some tropical species, ageing of the fish for a few hours before chilling can prevent cold-shock reactions and a loss in yield of the fillets (Curran *et al* 1986). Tropical fish held on ice are thought to have an extended shelf-life. A reason for this is that tropical fish have a mesophilic bacterial flora compared with the psychotrophic flora of cold-water fish. To ensure the most efficient chilling rate it is necessary to surround each fish completely with ice. Although this is possible in laboratory experiments, the conditions on a fishing vessel may not always allow for such individual treatment of all fish.

Superchilling

Storage of fish at temperatures just below the freezing point, at –3°C, where 70% of the water in the muscle is in the ice state is referred to as 'superchilling' or partial freezing. Chilling at this temperature can retard biochemical reactions and effectively extend the shelf-life (Ehira and Fujii, 1980; Lee and Park, 1985; Simpson and Hard; 1987). Superchilling gained much attention in the 1960's, but does not appear to have been very successful. However, recently, it has received renewed interest (Keizer, 1995). It has been shown that storage life could be doubled by partial freezing, for example from 14 days on ice to 28 days at partially frozen conditions, although there have also been reports of a loss of sensory quality in fish after about five days. In respect of the K value not exceeding 20%, Ehira and Uchiyama (1986) reported excellent quality was maintained for a number of fish species held at –3°C compared with ice storage. They also stressed the critical aspect of minimal temperature variation (<0.5°C) for partial freezing temperatures to be effective in maintaining high quality.

Freezing

Freezing food means reducing the temperature below the freezing point so that most of the water contained in the material turns to ice. The freezing point depends upon the concentration of different solutes in the tissue fluids (Sikorski and Kolakowska, 1990). The freezing point of fish muscle ranges from – 0.6 to – 2.0°C (Kato, 1985). Over the past few decades, freezing has developed into a widely used method for the preservation, distribution and marketing of fish and fishery products (Subasinghe, 1992). According to Lavety (1991) a stable temperature of –30°C is required to avoid serious deterioration of most fish. This temperature is much lower than the lowest temperature (-12°C) at which the activity of most microorganisms is arrested (Sikorski

and Pan, 1992). For species of lean fish for example, cod and plaice, a temperature of --20°C is permitted and for fatty fish such as herring and mackerel the temperature should be lower (-30°C). In Japan it is recommended that fish destined for the sashimi market should be stored at a temperature as low as –50°C (Lavety, 1991) and in some instances –60°C (Sikorski and Pan, 1992). Good quality lean fish which has been properly frozen can normally be held at –20 to –30°C for more than one year without appreciable loss in consumer acceptability (Dyer, 1968; Mackie *et al* 1986). However, measurements of organoleptic, chemical and physical change have clearly demonstrated that the quality of fish deteriorates during frozen storage. The palatibility of frozen fish stored for extended times is limited by losses in flavour or texture which in turn are influenced by other biochemical reactions for example, lipid hydrolysis, proteolysis and nucleotide catabolism (Haard, 1992).

Frozen storage may lead to extensive alterations in the proteins of fish muscle known as freeze denaturation. Freeze denaturation of fish proteins has been reviewed by several authors (Connell, 1968; Sikorski *et al* 1976; Matsumoto, 1979; Shenouda, 1980). It is believed that the main contractile proteins myosin and actin are largely responsible for the functional properties of flesh foods and that during frozen storage myosin in particular undergoes aggregation reactions which lead to toughening of muscle and a loss of water-holding capacity (Mackie, 1993).

1.7 Methods to Determine the Freshness of Fish

For almost 100 years, scientists have been developing physical and/or chemical methods of determining the freshness of fish. In 1891, Eber developed a chemical test, based on volatile amines, for the putrefaction of fish. It was thought that chemical and physical methods were objective and that all evaluations using human senses were

subjective (Botta, 1994). However, the development of sensory evaluation commenced in the early 1930s and by the late 1960s the procedures for conducting sensory evaluations were clearly defined (IFT, 1964; Amerine *et al* 1965; Larmond, 1967; ASTM, 1968a, 1968b).

Sensory evaluation may be defined as the scientific discipline used to evoke, measure, analyse and interpret human reactions to characteristics of food perceived through the senses of sight, smell, taste, touch and hearing. Sensory methods must be performed scientifically under carefully controlled conditions so that the effects of test environment and personal bias, may be reduced (Nielsen and Jessen, 1995).

It is now accepted that it is incorrect to assume there is no objectivity in sensory evaluation and no subjectivity in chemical procedures or the operation of instruments (Trant *et al* 1981; Pangborn, 1989). The literature dealing with the different sensory methods which can be used as objective tests has been reviewed by Larmond (1987). Connell and Shewan (1980) reviewed the sensory and non sensory methods used in the evaluation of fish freshness and pointed out that where chemical or physical tests were employed they must correlate with the sensory judgements for which they are to substitute (Trant *et al* 1981).

1.7.1 Sensory methods

The most frequently used sensory method for assessing the freshness of fish is structured scaling, usually a point scale. In Europe today, the most commonly used method for quality grading is the EU scheme (currently under review No. 103/76 of January 1976 and updated by decision No. 2406/96). The EU scheme requires that fish must be sorted into lots which are homogenous with regard to freshness and size.

There are three categories included in the regulations, namely, E (Extra), A and B where E is the highest quality and below B is the level where fish is no longer fit for human consumption and is discarded. The validity of such a scheme is questionable since it is subjective, requires trained personnel and does not take into account differences between species. More recently the West European Fish Technologists' Association (WEFTA) has compiled a Multilingual Guide to EC Freshness Grades for Fishery Products (Howgate *et al* 1992) and has a glossary of odours and flavours which can be very useful in searching for descriptive words for sensory evaluation of fish freshness. Special schemes for whitefish, dogfish, herring and mackerel have been developed.

Sensory evaluation is the most important method today for freshness evaluation in the fish sector. A new method, the Quality Index Method (QIM) which was originally developed by the Tasmanian Food Research Unit (Bremner *et al* 1985) is now used widely in Europe and is being implemented in the fish industry. The method involves inspection of sensory parameters of the fish (eyes, skin, gills, odour) and a specific demerit point is recorded. Scores for each attribute are then combined to give an overall score called the quality index. A score system from 0 to 3 or 4 demerit points is employed to grade the fish. Fish which are very fresh get a score of zero. As the fish deteriorate the total score increases. The individual scores never exceed 4, so no parameter can excessively unbalance the score. There is a linear correlation between the sensory quality expressed the demerit score and storage life on ice. It is necessary to develop a separate scheme for each species. The QIM method has been developed for fresh herring, saithe, cod and turbot to name but a few. The method is currently being developed for rainbow trout. Compared with the EU scheme the main

advantages of the QIM method is that it is specific for each species and minor differences in judgements of any one attribute cannot unduly influence the total score.

The non sensory methods for assessment of fish quality include the use of biochemical, microbiological and physical procedures.

1.7.2. Biochemical methods

The number and diversity of fish species commonly exploited for food is much greater than the number of mammalian species. It is generally agreed that the biochemical changes which accompany the onset of *rigor* and bring about the conversion of muscle to meat are also responsible for the conversion of fish muscle into edible fish. The exact relationship between the rate and extent of the biochemical events which take place in skeletal muscle during harvesting and catching have not been clearly established in fish (Mestorff, 1954; Amlacher, 1961; Partmann, 1961; Proctor *et al* 1992). Also the influences of environmental factors on these changes have not been clearly elucidated.

A variety of methods has been used for assessing freshness in fish. However, it appears that there is no single biochemical marker compound which may be used universally to indicate freshness or to predict future shelf life. In recent years much attention has been focussed on the potential use of nucleotide degradation, particularly that of ATP as a determinant of freshness. In a number of fish the sequence of nucleotide degradation follows a well-defined process. ATP undergoes enzymatic dephosphorylation to form ADP and then AMP. Deamination of AMP by the tissue enzyme AMP deaminase produces IMP. These steps are fast and give rise to an accumulation of IMP. Saito and Arai (1959) found that these reactions took place

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during slow freezing of carp. Jones and Murray (1962) reported that trawled cod had little ATP and a substantial amount of IMP at death compared with rested well-fed fish. Similar results have been observed in salmon caught by gill-net (Kramer and Kennish, 1987). Most of the ATP in trawl-caught fish is lost by the struggling of the fish during capture and the remainder is converted to IMP within a day or two if the fish is chill-stored (Kassemsarn *et al* 1963). IMP is further dephosphorylated to inosine (INO) which is hydrolysed to hypoxanthine (Hx). The rate of breakdown of IMP varies considerably with species but is slow in comparison with the formation of IMP in both chill-stored and frozen fish (Tarr *et al* 1969). Ehira and Uchiyama (1986) reported that concentrations of IMP in muscle of Pacific cod and Alaska pollock decreased to less than 1μmol/g in only two days, whereas in sea bream the concentration of IMP was 5μmol/g after ten days. The breakdown of INO and Hx in fish muscle during storage is the basis of one of the most important objective criteria for determining early loss of fish freshness (Kobayashi 1966; Gould and Peters, 1971; Hiltz *et al* 1971; Spinelli 1971; Burt 1977; Jacober and Rand, 1982). The rate of formation of INO and Hx varies widely with species and with storage conditions. In some species such as haddock, lemon sole, plaice, cod, INO is broken down rapidly and Hx accumulates, hence Hx levels would be reliable indicator of time of storage on ice in these species (Kassemsarn *et al* 1963).

In certain other species namely halibut (Spinelli, 1967) and a number of tropical species (Bremner *et al* 1988), INO accumulates and is converted slowly to Hx. Ehira and Uchiyama (1973) investigated 98 species and established three categories of fish: those that form INO, those that form Hx and those that are intermediate. This diversity in pattern of nucleotide degradation supports the contention that it is not possible to use any individual compound as an indicator of freshness.

Saito *et al* (1959) first proposed the term "K value" as an indicator of fish freshness. K value is defined as the ratio of INO and Hx to the total concentrations of ATP derived compounds expressed as a percentage. In practice, ATP is quickly converted to IMP within 24 hours after death for a great many species (Tarr, 1966). Karube *et al* (1984) simplified the definition of the K value by excluding the adenine metabolites (ATP, ADP and AMP) from the equation and referred to the K_1 value. Karube *et al* (1984) also showed a strong correlation between the K_1 value and the K value proposed by Saito *et al* (1959) for certain species of fish. The purpose of applying the K value formula is to obtain a specific number which will quantitatively reflect the state of freshness, thus providing a guideline (Malle and Pezennec, 1992). Many studies have confirmed the relationship between freshness and the K value (Ehira *et al* 1970; Ehira and Uchiyama 1974). In Japan a K value of 20% or less is considered safe for fish which may be eaten raw as sashimi. Where the K value exceeds this value the fish must be cooked first. In fresh fish in which the concentrations of HxR and Hx are low, the K value will be low. As storage time increases and further ATP degradation occurs, the concentrations of INO and Hx accumulate and the K value increases.

It is well known that the deterioration of freshness in fish is species specific (Ehira and Uchiyama 1986). Huynh *et al* (1992) reported a rapid increase in K value for Pacific cod during early storage. The K value reached 80% after only two days in ice compared with Pacific herring and sockeye salmon which had K values of 45% and 40% respectively at the same time. Hattula and Kiesvaara (1992) showed that for Sea Trout the K value increased linearly up to 17 days *post mortem* compared with White Bream where the K value was only linear for 6 days. The White Sea Bream reached a K value of 85% after 6 days of iced storage while the same K value was reached by Sea Trout after 17 days storage and was thought to be due to the different rates of

nucleotide catabolism in the two species. It is clear that the use of K value as a freshness indicator depends on the species under consideration. It is not possible to set a single K value below which all fish may be considered fresh.

The methods available for estimating K value may be broadly divided into two categories, namely, chromatographic methods and methods based on enzyme technology. The high performance liquid chromatography (HPLC) method gives accurate and reliable results and is often used as a benchmark with which other methods are compared. The system uses reverse-phase separation with a commercially available column, which provides rapid, quantitative analyses of ATP and its breakdown products from which K values may be determined. Huynh *et al* (1992) employed the method of Burns and Ke (1985) with some modification. This method is reported to be reliable and was the choice of method used in this thesis. While HPLC methods are effective and accurate, they are expensive, difficult to use and require trained personnel.

Considerable effort has been made to develop a rapid and automated system for monitoring fish freshness (Uchiyama and Kakuda, 1984; Karube *et al* 1984; Watanabe and Karube, 1986). The development of the KV-101 Freshness Meter from Oriental Electric, Japan, the BIOFRESH system from New Japan Radio Company, Japan and the Microfresh system from Pegasus Instruments, Canada has led to faster methods of measuring fish freshness (Gill, 1992). These instruments use an oxygen electrode for enzymatic measurement of ATP-related compounds and the K value is then determined. While these instruments are reliable and accurate, they are expensive and electrodes have to be replaced frequently.

The use of rapid paper strips for estimating the K_1 value has received some attention in recent years (Hattula and Wallin, 1996). The method uses the immobilization of enzymes to paper strips. The action of the enzymes on the particular metabolite is coupled to the colour change of an indicator dye. Ehira *et al* (1986) and Negishi and Karube (1989) independently reported test strips capable of measuring several of the nucleotide catabolites. The method of Ehira *et al* (1986) involved the immobilization of xanthine oxidase (XO) and nucleoside phosphorylase (NP) to a paper strip. In this reaction, the consumption of dissolved oxygen to form hydrogen peroxide was used to change the colour of an indicator dye. The method of Negishi and Karube (1989) involved the use of IMP dehydrogenase, diaphorase and a tetrazolium salt for the quantitative determination of IMP. A similar paper strip method has been commercialised by Transia-Diffchamb, France. This method involves soaking the paper strip in a buffer extract of the fish muscle. The resulting colour change is compared to a colour chart.

1.7.3 Biochemical changes during frozen storage

During frozen storage, deterioration in fish quality due to microorganisms and some biochemical processes is delayed. Jones (1965) reported that nucleotide compounds were stable in cod muscle at temperatures –26°C to –30°C. Similar findings have been reported for Ocean perch (Spinelli *et al* 1964) and swordfish (Dyer *et al* 1966). However, at higher temperatures (-10°C) at 4 months in both bonito (Sameshima, 1965) and swordfish (-4°C) (Dyer *et al* 1966) dephosphorylation of IMP was observed. Studies carried out on swordfish showed that IMP phosphorylase was inactive at –26°C up to a year, but at –18°C and –8°C, the rates of loss of IMP were 0.03 and 0.24μmol/g/week respectively (Dyer and Hiltz, 1969).

Licciardello *et al* (1982) observed excellent storage life at very low temperatures for species of fish like red hake which are particularly sensitive to frozen storage. Some studies showed that the deterioration in quality was greater when the fish were held on ice before freezing. This pattern was observed in species of milkfish (Joseph and Perigreen, 1980), Baltic herring (Kolakowska, 1981), chub mackerel (Fukuda, 1982) and gemfish (Thrower *et al* 1982). It has been reported that ADP, AMP and IMP which are known to occur in fresh fish can retard the denaturation of actomyosin during storage at -20°C, whereas the nucleotides which accumulate in aged fish-inosine (INO) and hypoxanthine (Hx) accelerate this reaction.

Degradation of nucleotides was studied in the muscle of iced barramundi (*Lates calcarifer*) which had been blast frozen and held at –20°C for two months, and in Nile perch (*Lates niloticus*) which were also blast frozen and stored at < -15°C for five months (Williams *et al* 1993). These authors reported that despite the differences in frozen storage between these two closely related species, the nucleotide profiles were remarkably similar during storage. Also, the K values of barramundi and Nile perch were similar. If a cut-off K value of 30% is used, then the storage life of both barramundi and Nile perch could be estimated at three days on ice on thawing after frozen storage.

1.7.4 Physical methods

Physical methods which have been used in the assessment of fish spoilage include measurements of viscosity of muscle homogenate, measurements of opacity and refractive index of eye lens and measurements of capacitance and resistance of the flesh. Changes in muscle during storage on ice are associated with changes in appearance, colour, texture and flavour which are normally used to judge freshness

(Jason and Lees, 1971). These changes are reflected by changes in the electrical resistance and capacitance of the tissue. The Torrymeter - a small hand-held unit, can give readings on individual fish or, compute the average of 16 successive measurements across a batch of fish. The Torrymeter which was developed by Jason and Lees (1971) is independent of the geometry of the fish muscle or precise location of the region of measurement. The main advantage of electrical testers is their immediate response and they can be operated by non-trained personnel.

1.8 Texture

Texture is a difficult term to define since it means different things to various people. The dictionary defines texture as "the disposition or manner of union of the particles of a body or structure". This definition does not describe texture in terms of a food property. In fact, the term texture was first used in the textile industry in connection with the art of weaving as "disposition or connection of threads as in a fabric". It is only with the development of textured foods that this original meaning of texture could be applied directly to the evaluation of food quality. A wider definition such as "the disposition or manner of union of particles of a body or substance", could also apply to all natural and processed solid foods, where the "disposition or manner of union" of different types of cells and tissues in the food material could be considered as the "texture" of the food. This, therefore, is a major attribute of food quality which can be included under a definition of texture and which would be directly related to the internal structure of natural or fabricated foods (Sherman, 1972). The problem of defining texture as a major component of sensory food quality arose during the 1920's when there developed a gradual awareness that sensory quality of foods does not consist of a single well-defined attribute, but a composite of any number of attributes

which are perceived by the human senses individually and are then integrated by the brain into a total, or overall, impression of quality.

For many years scientists have endeavoured to produce their own definition of texture. Although there is no entirely satisfactory definition, Bourne (1982) stated that texture of food has the following characteristics:

1. It is a group of physical properties that are derived from the structure of the food.
2. It belongs under the mechanical or rheological subheading of physical properties.
3. It consists of a group of properties not a single property.
4. It is not related to the chemical senses of taste or odour.
5. It is sensed by a feeling of touch, usually in the mouth, but also by the hands.

Since texture consists of a number of different physical sensations, it is preferable to talk about "textural properties" rather than "texture "which infers a single parameter. It is important to realize that texture is a multifaceted group of properties of foods. Most of the instrumental methods used to measure texture are based on mechanical tests which measure the resistance of the food to applied forces greater than gravity. Hankoczy (1905) designed an apparatus for measuring the strength of gluten (Brabender, 1965) and Lehmann (1907) described the first objective method for meat tenderness evaluation.

Much attention and discussion has been devoted to what actually is measured by these devices. It is important to remember that texture is not "one thing" but a spectrum of parameters. Each of the instruments detects only a portion of that spectrum, some a larger portion than others. It is only the human being who can perceive, analyze, integrate and interpret the entire spectrum of texture and other characteristics in one

evaluation. Moreover, it is generally agreed that the concept of texture is meaningful only when viewed as an "interaction of the human with the mechanical properties of the material" (Corey, 1970).

Instrumental methods for measuring texture may be classified into several groups. Scott Blair (1958) recognised three categories based on the general nature of the test, namely, fundamental, empirical and imitative. Fundamental tests measure well-defined properties and generally assume the material is continuous, isotropic (exhibiting the same physical properties in every direction) and the test piece is of uniform shape. Most tests made on foods fail to comply with these assumptions. Fundamental tests are slow to perform, do not correlate well with sensory evaluation as do empirical tests and use expensive equipment.

Empirical tests measure characteristics related to texture and are the most widely used class of tests in the food industry. The tests are usually easy to perform, rapid and frequently use inexpensive equipment. The problems associated with this type of test are the poor definition of what is being measured, the arbitrariness of the test, that there is usually no absolute standard available and that the tests are usually only effective with a limited number of commodities. Instruments used to perform such tests include: (a) penetrometers which register the force required to penetrate the material, or the depth of penetration following impact; (b) compressors, which determine the hardness or firmness of foodstuffs by measuring resistance to a compression force; (c) consistometers, which measure the consistency of liquids and semisolids by testing their resistance to flow and (d) shearing devices, which record the force needed to shear the test material.

The shearing devices used to test solid foods employ either a single blade or a multi-blade probe. Their most popular use is in studies on meat texture where the force required to shear the specimen is taken as a quantitative measure of tenderness. An example of a single blade shear apparatus is the well-known Warner-Bratzler Shear. Much work has been done with this device regarding its performance and correlation with other objective tests. However, there have been suggestions that small variations in sample diameter have a very large influence on force readings (Poole and Klose, 1969; Davey and Gilber, 1969). An example of a multi-blade shearing devices is the Kramer Shear Press. Dunajski (1979) found that tenderness of fish muscle could best be measured using a Kramer shear/compression cell. Szczesniak *et al* (1970) have demonstrated that measurement with the Kramer Shear Press involves shear, compression, and extrusion, and that different combinations of these forces may act on a given food depending on its rheological nature. They also suggested the possibility of a similar situation with respect to other shearing devices.

Imitative tests are performed under conditions simulating those to which the material is subjected in practice. Devices used are those which measure the properties of the material during handling, and those which measure the properties of the food during consumption. Examples of this type of test are the Farinograph and other dough-testing apparatuses that imitate the handling and working of bread dough, and the general Foods Texturometer which imitates the chewing action of the teeth.

All texture measuring devices have five essential elements: driving mechanism; probe element in contact with the food; force-direction, type and rate of application; sensing element; and read-out system. The driving mechanism may vary from a simple weight and pully arrangement to a more sophisticated variable drive electric motor hydraulic

system. The probe in contact with the food sample may be a plunger, a pair of shearing jaws, a tooth-shaped attachment, a piercing rod, a cutting blade or a set of cutting wires. The force may be applied in a vertical, horizontal, rotational or levered manner and may be of the cutting, piercing, puncturing, compressing grinding, shearing or pulling type. The sensing element for detecting the resistance of the foodstuff to the applied force may be a simple spring or a more sophisticated hydraulic strain gauge transducer. The read-out system may be a maximum force dial, an oscilloscope, or a recorder tracing the force-distance relationship.

1.8.1 Texture of fish muscle

The role of texture in the overall sensory acceptability of fresh fish muscle is not as important as it is in beef. This is due to the fact that fish muscle has a significantly lower content of highly crosslinked connective tissue proteins and a different morphological structure (Dunajski, 1979). Factors such as chemical composition, *ante mortem* factors, treatment after catch and the biochemical state of the fish have an important impact on the texture and overall acceptability of the fish meat. There have been suggestions that palatability of fish is influenced by texture (Hatae *et al* 1984).

Similar to many other foods, fish meat is structurally and rheologically a complex material. It consists of a mixture of solid and fluid components. The characteristics, both those which can be seen and those which determine the manner in which the muscle tissue handles and feels in the mouth, are derived from properties and concentrations of the structural elements of the tissue and of their complex arrangement in the muscle (Dunajski, 1979). The texture of whole fish muscle is difficult to measure because of the lack of a uniform structure (Love, 1973). Texture of fish muscle is also related to the diameter of the muscle fibres. Muscle fibres in the

caudal area of a fish are smaller but more numerous, thereby increasing the strength of the muscle (Hatae *et al* 1990). This lack of uniformity in the muscle has made it difficult to prepare muscle samples of standard size, and has led to a variety of sample preparation procedures and to some variation in results. Many terms have been used to describe the texture of fish muscle. (Szczesniak, 1963) commented on the apparent lack of definitions relating to the texture of fish. Fennema *et al* (1973) used the term "tenderness", Bremner (1977) referred to "hardness" and Sikorski (1980) described "toughness".

1.8.2 Factors that affect texture

The texture of fish muscle is influenced by a number of parameters arising from such conditions as the physiological state of the fish *ante mortem*, whether the fish was fed, exhaustion due to hunting, transport or manner of death. The initial biological condition of the fish is important in terms of the overall quality, as a fish in poor condition will not improve during storage. Ostenfeld *et al* (1995) evaluated the effect of live haulage on concentrations of metabolites and on the texture of farmed rainbow trout (*Oncorhynchus mykiss*) muscle using a two-cycle compression test. They found that haulage had limited effects upon the mechanical parameters tested (Hardness, Elasticity and Breakpoint), and did not appear to effect the quality of the fish muscle.

The size of the muscle segments (myotomes) appears to depend largely on the type of fish and its size, and may vary with the location along the fish (Johnston *et al* 1980a,b). It has also been suggested that connective tissue is more concentrated in the caudal area of the fish (Love, 1988; Lampila, 1990). Montero and Borderias (1989) used a Warner-Bratzler cell to study shear resistance of both the muscle and connective tissue of hake (*Merluccius merluccius* L.) and trout (*Salmo irideus* Gibb) stored at cold

temperatures. They observed two peaks in the force deformation curves obtained for both hake and trout. The first peak was considered to correspond to the muscle fibres whereas the second peak which was sharper and more pronounced was taken to be that of connective tissue. They also observed an increase in the concentration of collagen towards the tail of the fish in both species. The trend was more apparent in the muscle of hake than of trout. Their observations were in agreement with those reported by Love (1970) for cod muscle. Lefèvre, (1997) studied the texture of muscle taken from different locations in trout (*Salmo trutta*) using the Kramer Shear press and reported significantly higher shear resistance in muscle samples taken from the caudal region. Histological studies also showed that the concentration of connective tissue was higher in the caudal region and that the myotomes were thinner in this region. This author also reported a variation in the orientation of the muscle using the Kramer Shear press. Muscle which was sheared in perpendicular configuration had a significantly higher shear force value compared with muscle which was sheared in axial configuration.

The literature referring to texture measurements at different locations on the fish fillet is limited, although some indication of the importance of sampling technique has been published (Borresen, 1986; Botta, 1991). The main emphasis has been that the muscle sample must be representative of the whole fillet. Azam *et al* (1989) measured the texture at three locations along the fillet. Botta (1991) recommended the use of three locations on cod fillets for the purpose of sensory analyses. Recent studies have shown that salmon (4kg in weight) fillets varied in thickness from the head (3cm) to the tail (2cm) region (Sigurisladottir *et al* 1999). These authors measured the texture at seven locations along the fillet and reported differences in shear force measurements which increased (locations 6 and 7) towards the tail of the fish, but not between locations 2, 3 and 4 which were under the dorsal fin.

Conrad *et al* (1994) developed a 1-blade cell similar to the Warner-Bratzler cell to study the influence of four different types of feeding on the texture of muscle of catfish (*Silurus glanis*). They concluded that diet did not appear to influence the texture of the fish muscle. This report appears to be contrary to that reported by other workers

The texture of fish muscle in *rigor* may be tough (Love 1962, 1988). The importance of *rigor* in relation to the texture of muscle has not received as much attention in fish as in land animals (Iwamoto *et al* 1987; Ando *et al* 1991). This is probably due to the fact the duration of *rigor* in most fish is shorter than in muscle tissues derived from mammals and birds.

1.8. 3 Changes in texture during cold storage

The mechanical properties of raw fish can be attributed to the strength of the connective tissues and to that of the muscle bundles (Dunajski, 1979). In fresh fish, the flesh is firm and the pattern of underlying blocks of muscle is easily seen (Davis, 1995). However, during storage on ice over several days the firmness of fish flesh decreases rapidly with few exceptions (Hatae *et al* 1985; Toyohara and Shimizu, 1988; Ando *et al* 1991a-c, 1992, 1993, 1995; Sato *et al* 1991). These changes in firmness of fish flesh are reflected in the texture of the muscle.

Changes in texture associated with chilled storage have been measured mechanically using numerous different methods. One of the simplest and most frequently used tests is the puncture test. Parameters such as penetration force, depth of penetration or breaking strength decrease with time of storage. Kairiyama *et al* (1990) reported a decrease in penetration force values for hake muscle (*Merluccius hubbisi*) after 18 days storage at 3°C. Gelman *et al* (1990) used a cone to measure the depth of penetration in

the muscle of common carp (*Cyprinus carpio*) during storage at different temperatures. They showed that fish which were stored at 5 to 6°C had a sharper decrease in firmness than those fish which were stored at 0 to 2°C. Tenderization of fish muscle may be caused by disintegration of collagen fibres in the pericellular connective tissue. Ando *et al* (1991a, b, c) reported early softening (0-72h) in the muscle of rainbow trout fillets (*Oncorhynchus mykiss*) using a cylindrical plunger to measure the maximum force or breaking strength of the muscle. They also reported early softening in eight different fishes stored at 4-5°C for 72 hours.

Azam *et al* (1989) measured the elasticity of raw rainbow trout fillets by double compression, using a Stevens Compression Response Analyser. The literature relating to the texture of fish muscle suggests that feeding strategy, method of slaughter and storage *post mortem* may have an affect on the overall texture of the fish. Faergemand *et al* (1995) used a one-cycle compression test to measure the texture of farmed rainbow trout (*Oncorhynchus mykiss*) muscle held on ice for 9 days. From the different factors studied (feeding strategy, method of killing and storage time *post mortem*) storage on ice for 9 days was judged to be the single most important factor affecting the texture of the muscle.

There have been reports that the cooked meat derived from farmed sturgeon (*Acipenser transmontanusi*) has an undesirably tough texture. Izquierdo-Pulido *et al* (1992) used the force of compression and depth of penetration using a needle to measure the texture of cooked fish flesh from sturgeon which had struggled at time of death and from anaesthetised sturgeon. Compression tests showed that the texture of cooked meat was firmer when the muscle was in *rigor* and also showed that meat from fish which had struggled at time of death was softer than that obtained from anaesthetised fish.

A number of authors have reported that no significant changes in texture occurred during chilled storage. Barassi *et al* (1981) studied the texture of silver blue whiting (*Micromesistius australis*) using resistance to penetration force. They found no significant changes in texture during 15 days storage at 0°C. Pastoriza *et al* (1994) also used force of penetration to measure the texture of ray wing muscle (*Raja clavatai*) stored at 2°C for 15 days and recorded no changes during time of storage. Conrad *et al* (1994) used a modified 4-blade Kramer shear-compression cell to measure the texture of raw fillets of catfish (*Silurus glanis*) which had been stored at 0-2°C for 30 days. Again, they found no significant changes in texture during storage; this was attributed to the existence of some firm fibres in the muscle which remained unaltered during storage. However, the authors pointed out that the measurement of maximum force may not have been a suitable method for the purpose of their experiment. Texture measurements using depth of penetration in carp muscle (*Cyprinus carpio*) showed no significant changes during storage at 10-15°C for 26 days (Icekson *et al* 1996).

1.8.4 Changes in texture during frozen storage

Most fish species undergo deteriorative changes during frozen storage due to denaturation and aggregation of the myofibrillar proteins. Such changes result in alteration of the functional properties of the muscle proteins, loss of water-holding capacity and changes in texture (Shenouda, 1980). The rates of these deteriorative changes vary markedly from one species to another, for example it is known that gadoid species develop toughness and a decrease in water-binding capacity more rapidly than flatfish, and that fatty species produce rancid flavours more quickly than white fish species (Shenouda, 1980). The stability of a given species during frozen storage may also vary with the season of harvest, nutritional status or sexual maturity

(Love, 1988). There have been reports that some species of fish for example, spot (*Leiostomus xanthurus*) harvested in the spring, rather than at other times of the year, have a greater tendency to develop rancidity during frozen storage (Waters, 1982), while weakfish (*Cynoscion regalis*) harvested at different times of the year show little variation (Waters, 1983).

The texture of fish muscle stored for long periods at about –18°C is often described as tough, chewy, rubbery or fibrous (Sikorski, 1990). Recent research indicates that the rate of freezing may cause deterioration of quality during subsequent storage (Haard, 1994). Some authors have reported that species of fish like yellowtail rockfish (Kramer and Peters, 1981), red hake (Kelleher *et al* 1982), Atlantic cod (Reece, 1985; Ragnarsson and Regenstein, 1989) and ocean perch (Ragnarsson and Regenstein,1989) show a slower rate of deterioration in texture when they are aged on ice for several days prior to frozen storage. This finding may be related to depletion of trimethylamine oxide (TMAO) in aged fish (Lundstrum *et al* 1981; Kelleher *et al* 1982; Reece 1985; Ragnarsson and Regenstein 1989) and the formation of trimethylamine (TMA); (Parkin and Hultin 1982). Lee (1982) observed that freezing the fish in liquid nitrogen resulted in less muscle toughening and less drip than conventional freezing after storage at –20°C for 2 months.

The shear resistance increases during frozen storage in a number of fish species (Racicot *et al* 1984; Careche and Tejada 1991; Montero and Borderias 1992). Kelleher *et al* (1981) used a Warner-Bratzler cell to study the shear resistance of blocks of red hake (*Urophycis chuss*) muscle which had been subjected to pre-processing modifications prior to frozen storage for 6 months. They observed that the sharpest increase in shear values occurred after 1 month of storage. They also commented on

the consistently low shear values for fish held at –90°C indicating the effectiveness of extremely low temperatures in preserving the red hake in a condition most closely resembling the fresh state.

It has also been suggested that the temperature of frozen storage has an effect on the texture of the fish muscle. Moral *et al* (1986) studied the minced muscle of trout (*Salmo irideus* Gibb) stored at –12, -18 and –24°C for 1 year and showed that the higher the temperature of frozen storage, the higher the shear force value. More recently Dias *et al* (1994) studied the effect of frozen storage on the physical properties of black scabbardfish (*Aphanopus carbo*) and silver scabbardfish (*Lepidopus caudatus*). They reported an increase in muscle toughness after four weeks at –18°C for the silver scabbardfish and after five weeks at the same temperature for the black scabbardfish.

1.9 The Work Described in this Thesis

The work described in this thesis is a development of the research on biochemical changes *post mortem* in fish skeletal muscle undertaken by McLoughlin and Proctor. Their research was concerned with the effects of tranquillisation and killing methods on concentrations of ATP, CP, glycogen, glucose-6-phosphate and lactate and pH in the muscle of marine and freshwater fish *post mortem* during onset of *rigor* (Proctor *et al* 1992; Proctor and McLoughlin, 1992; McLoughlin and Proctor, 1993). The objective of the work described here was to further explore the theme of onset and resolution of *rigor* in fish skeletal muscle (a) by studying the effects of handling and killing procedures on the biochemical changes which take place in skeletal muscle during the harvesting of farmed fish with particular reference to the high energy

phosphate compound ATP, (b) by observing the pattern of the degradation of ATP *post mortem* and (c) by investigateing changes in texture during storage.

A study of the biochemical changes and changes in texture of fish skeletal muscle *post mortem* was made. The pattern of the degradation of ATP was observed in the early *post mortem* hours (6), at daily intervals (20) and during frozen storage (24 weeks). As part of the study the anatomical location of muscle was examined.

2. MATERIALS AND METHODS

2. MATERIALS AND METHODS

2.1 Fish

The fish studied were rainbow trout (*Oncorhynchus mykiss* Walbaum), salmon (*Salmo salar*) and goldfish (*Carassius auratus*).

Rainbow trout

Rainbow trout were obtained from Annamoe Fish Farm, Co. Wicklow and from Idas Fish Farm, Woodenbridge, Co. Wicklow. There were two major differences in handling of fish at this point which related to the experiments in which the fish were used. These differences were that (1) certain fish were killed at the fish farm and (2) other fish were transported live to the laboratory.

(1) The experiments which were carried out on the fish which were killed at the fish farm involved a number of different harvesting procedures:

(a) Fish were used in experiments to determine differences which may exist between individual muscle sites. Seven groups of fish were used. Each group was subjected to a different method of harvest. The fish were two years old, approximately 480g in weight and measured 34cm in length. They had been not been fed for three hours prior to killing with the exception of the fish in group 1 which had been starved for five days. The appearance of the fish was noted and muscle samples taken from four specific anatomical sites in each fish, a procedure called anatomical site of sampling. The muscle samples were put into pre-labelled freezer bags, frozen in liquid nitrogen and stored at –30°C for biochemical analysis.

(b) Fish were killed at the fish farm. Zero hour muscle samples were taken and pH and Torrymeter measurements were made. The fish carcasses were transported in ice in insulated boxes to the laboratory at the Dublin Institute of Technology and were stored at 3°C.

(c) Fish were killed and zero hour muscle samples taken as described in (b) and the fish carcasses were transported in ice to the laboratory and stored in a freezer at –30°C. In preparation for other experiments, groups of fish were killed at the fish farm as in (b) and muscle samples taken. The muscle samples were put into pre-labelled freezer bags, transported to the laboratory, and stored in a freezer at –30°C.

(2) The fish which were transported live to the laboratory were used in experiments to study nucleotide catabolism and texture of fish muscle immediately *post mortem* and at time intervals during storage. Individual specimens were taken from the pond by means of a net and transferred to the water-storage tank which had been previously filled to a capacity of approximately 100 litres with water from the fish farm. The tank was fitted with a lid of clear perspex and plywood. Two air-vents (diameter 45mm) were inserted in the lid which was secured by means of screws to prevent loss of water and fish during transit. A small capacity pump was used to aerate the water. Aeration was achieved by drawing the water from the tank and discharging it back over the water level. A polyethylene water-storage tank (800mm x 450mm x 450mm) was used to transport the fish. The fish were transported to the laboratory within two hours of capture. They were then quickly removed from the water-storage tank, placed in plastic buckets containing water and transferred to a holding tank in the laboratory. They were kept in the holding tank at a temperature of 10°C for 24 hours before experiments. The fish were not fed during this time. The holding tank was obtained

from Mailbox International. It was a rectangular tank measuring (864mm x 559mm x 559mm) which was filled with tap water to a capacity of approximately 200 litres and was connected to a Water cooling system (Maxi-cool 14) by means of an Eheim (model 1018) circulating pump. The water was allowed to circulate for 48 hours to drive off chlorine. This procedure was carried out 48 hours before experiments.

The handling procedures for fish which were transported live to the laboratory were as follows:

(a) Fish were taken from the tank using a fishnet and killed. The appearance of the fish was noted and muscle samples were taken immediately after death, put into pre-labelled freezer bags and frozen in liquid nitrogen before being stored at –30°C for biochemical analysis. The fish carcasses were then put into polystyrene boxes, layered with ice and stored at 3°C.

(b) Fish were killed and zero hour muscle samples were taken as described in (a). A number of muscle samples were excised from the epaxial muscle mass, put into pre-labelled freezer bags and held on ice for two hours *post mortem* during transit before being stored in a freezer at –30°C.

Salmon

Harvested salmon were had been stunned with CO_2 were supplied by Kilkerrin Hatcheries, Connemara, Co. Galway. They were approximately two years old and ranged between 3kg and 5kg in weight and 60cm to 70cm in length. The fish had been starved for five days before stunning. When anaesthesia was complete the gills were cut and the fish allowed to bleed. Four grades of salmon were used namely, Superior Grade, Superior-S Grade, Ordinary Grade and Production Grade. The fish were graded

using criteria devised by the Irish Salmon Growers Association and An Bord Iascaigh Mhara (1991). These gradings were based mainly on visual observations relating to apparent freshness and absence of physical blemishes. Three round and three gutted specimens from each grade were packed into polythene-lined polystyrene cartons, layered with ice and transported to the laboratory. The fish reached the laboratory at about 30 hours *post mortem* and were stored at 3°C. Sampling of muscle was carried out at 30 hours *post mortem* and at 7 days.

Goldfish

Goldfish were obtained from a local retail outlet and were used in experiments using the anaesthetic MS-222. They were transported live in water to the laboratory. Individual fish were placed in separate bowls containing water to allow recovery from the stress of catching and transport. The fish were held under ambient conditions in the laboratory, two for 23 hours and three for 42 hours before the experiments. The fish were not fed during this time.

2.2 Stunning and Killing

2.2.1 Carbon dioxide stunning

The stunning procedure employed was based on that used by the Irish Salmon Growers Association and An Bord Iascaigh Mhara (1991). When stunning was complete the gills were cut and the fish were allowed to bleed.

2.2.2 Clubbing

Fish were struck sharply on the head with a wooden stick. This procedure is subsequently referred to in this thesis as clubbing. Clubbing resulted in the death of the fish.

2.2.3 Asphyxiation

Fish were taken from the pond by means of a fish net and placed in a large plastic bin. They struggled for 15 to 20 minutes after removal from the water and were clubbed.

2.2.4 MS-222

Goldfish were anaesthetised using a solution of ethyl-m-aminobenzoate methane sulphonate (MS-222). The solution (0.002M) was prepared by adding 3.6g of MS 222 to 6 litres of water in the fishbowl containing the fish. Within two minutes the fish exhibited reduced reflexes although there was still some movement. Anaesthesia was complete within three minutes at which time the fish lost balance and fin and gill movement ceased. The anaesthetised fish were removed from the water and killed by cervical fracture.

2.3 Harvest Methods

Rainbow trout were used in these experiments.

Group 1. The fish (4) were handled and harvested under conditions aimed at reducing stress that inevitably occurs under industry based conditions. Individual specimens were taken from the pond by means of a net and clubbed at ambient temperature (section 2.2.2). Immediately after death muscle samples were taken as described

(section 2.4). One zero hour muscle sample was taken from each of four separate anatomical locations in each of the fish.

Group 2. The fish (4) were handled and harvested as for the fish in group 1. Immediately after death individual specimens were put in a plastic container and held at ambient temperature for 30 minutes *post mortem* before being sampled. Muscle samples were taken as described above.

Group 3. The fish (5) were handled as for the fish in group 1. Immediately after death they were put in a plastic container in a cold room, layered with ice and chilled for 30 minutes *post mortem* before sampling.

Group 4. The fish (5) were killed as for the fish in group 1. Immediately after death they were put into a large plastic container in a cold room, layered with ice and chilled for 90 minutes *post mortem* before being sampled.

Group 5. The fish (5) were taken from the pond as previously described. Individual fish were put into separate containers in ice slurry. On introduction to the ice slurry the fish became agitated for a few minutes, then became still. The temperature of the ice slurry was 0.3°C. The fish were allowed to chill in the ice slurry for fifteen minutes *ante mortem*. They were then removed, clubbed and sampled immediately *post mortem*. Muscle samples were taken as described.

Group 6. The fish (5) were treated as for the fish in group 5. These fish were chilled as a group in ice slurry for 15 minutes *ante mortem*, clubbed and sampled immediately *post mortem*.

Group 7. The fish (5) were taken from the pond using a net. Each fish was put in a separate box in a cold room at 3°C and asphyxiated. Immediately after death muscle samples were taken as described.

2.4 Muscle Specimens

Specimens free of skin, adipose tissue and blood were taken immediately *post mortem*, frozen in liquid nitrogen and stored at –30°C for biochemical analysis. Muscle specimens were taken from the epaxial muscle mass starting at a point posterior to the pectoral fin. Further sampling was carried out posteriorally along the length of the fish with a space between the site of adjacent samples. Frozen whole fish were placed firmly in a press and the muscle samples were excised using a hammer and chisel. In rainbow trout muscle samples for biochemical analysis and for texture measurements were taken from the same fish. This was to allow for a closer comparison between the biochemistry of the muscle and the textural properties of the muscle. The muscle samples for biochemical analysis were taken from the left side of the fish and the muscle samples for texture measurements were taken from the right side.

2.4.1 Anatomical Site of Sampling

Four muscle samples (section 2.4) were taken from specific locations in each fish. The muscle samples were put into liquid nitrogen and stored at –30°C for biochemical analysis. The key to the anatomical muscle site is shown in Fig. 2.

2.5 Extraction of Muscle

Frozen muscle samples (stored for not more than three months at –30°C with the exception of the samples used in the frozen storage experiments) were broken into

small pieces using a hammer. One gram (approximately) of tissue was weighed in a pre-weighed centrifuge tube containing perchloric acid (8.0ml, 0.6M) and the volume of perchloric acid was adjusted to give a 1 in 10 dilution of the sample. The sample was homogenised by administering 4 x 15 second bursts (24000 rpm) from a Polytron (model PT-MR-3000) homogeniser. The centrifuge tube containing the muscle sample was held on ice during the procedure. The homogenised sample was immediately filtered under vacuum using a Millipore sampling manifold (model 1225), fitted with a Millipore membrane filter (diameter 25mm; pore size 0.45μm). The filtrate was mixed well. A one ml aliquot of the filtrate was neutralised with an equal volume of a solution of KH_2PO_4 (0.2M) containing KOH (0.6M). This gave a 1:1 dilution of the filtrate. The diluted filtrate (pH 6.5-7.0) was filtered under vacuum using a Millipore sampling manifold (model 1225) to give the final muscle extract (Murray *et al* 1984).

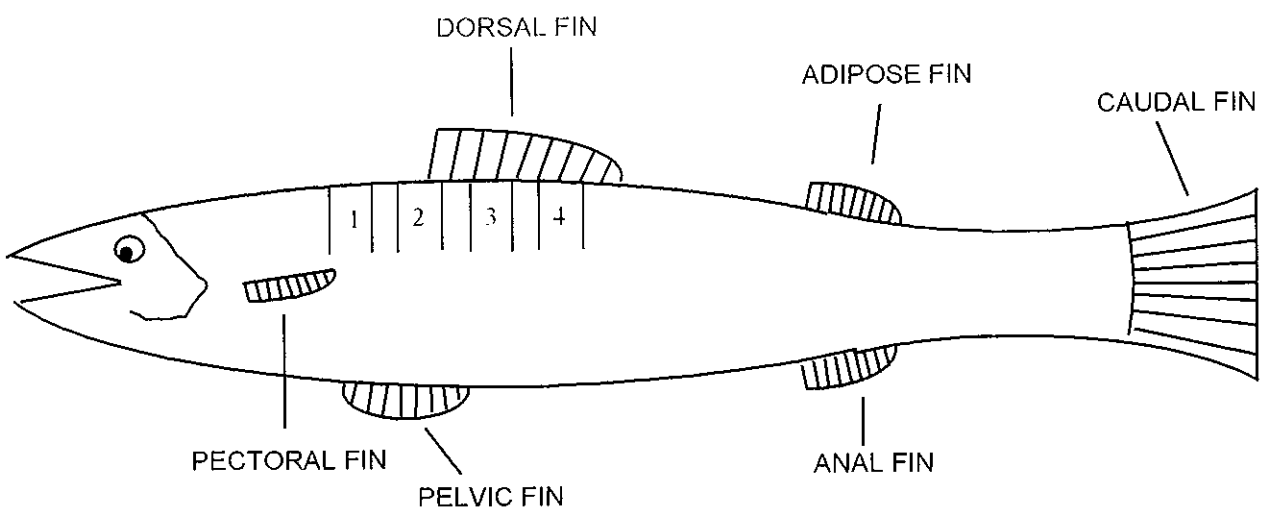


Fig. 2. A schematic representation of the anatomical location of muscle taken from rainbow trout.

2.6 Biochemical Analysis

The concentrations of ATP, ADP, AMP, IMP, INO and Hx were measured in muscle extracts using a method based on that of Burns and Ke (1985). The method employs isocratic separation of nucleotides by ion-pair reversed-phase high pressure liquid chromatography (HPLC) and entails their determination by UV detection. The apparatus comprised a pump (Waters, model 501) equipped with a variable detector (Waters, model 486) and was operated at 254nm. The column was a Waters Nova-Pak C_{18} steel analytical column (length 150mm, internal diameter 3.9mm; particle size 4μm; pore size 0.006μm). The mobile phase used for the separation of ATP and the nucleotide derivative compounds consisted of potassium dihydrogen phosphate (0.01M) adjusted to pH 4.5 with KOH or H_3PO_4 as necessary.

2.6.1 Reagents

Adenosine 5'-triphosphate (ATP; crystalline disodium salt), adenosine 5'-diphosphate (ADP; disodium salt) and adenosine 5'-monophosphate (AMP; crystalline disodium salt) [Boehringer, Mannheim, Germany]; inosine 5'-monophosphate (IMP; disodium salt), inosine (INO) and hypoxanthine (Hx) [Sigma, St. Louis] were used. ATP, AMP and IMP were kept under desiccated conditions at 4°C, and ADP under desiccated conditions at –30°C. INO and Hx were stored in the laboratory at ambient temperature. Analytical reagent grade potassium dihydrogen phosphate, potassium hydroxide and orthophosphoric acid (Fisher Scientific UK) and methanol HPLC grade (Fisher Scientific) were used. Milli-U10 grade water (Millipore Corporation, USA) was used for the preparation of the mobile phase and the standard solutions.

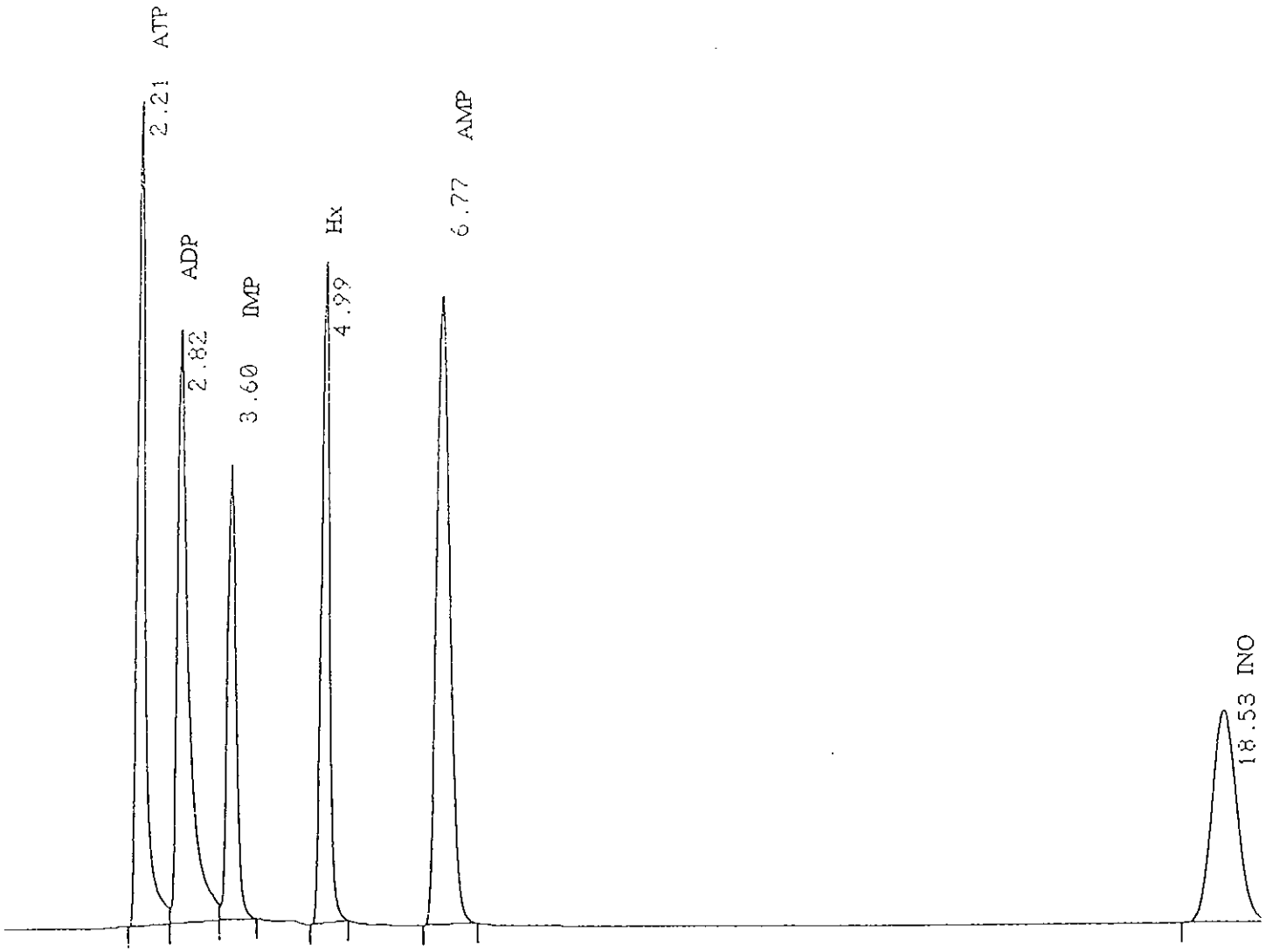
2.6.2 Standard Solutions of Nucleotides

A mixed standards solution containing ATP, ADP, AMP, IMP, INO and Hx was made up as follows:

ATP (0.01816g), ADP (0.01404g), AMP (0.01498g), IMP (0.01177g), INO (0.00805g), and Hx (0.00408g) were weighed into a 100ml volumetric flask, dissolved and made up to volume with water (Millu-U10 grade). This gave a concentration of 300μmoles/l of each compound. Dilutions of the stock solution were made to give concentrations of 250, 200, 150 and 100μmoles/l. HPLC chromatograms were obtained for each compound. These chromatograms were used to prepare standard curves for each nucleotide by plotting concentrations (μmoles/l) against the peak area responses on the chromatogram. These standard curves were used to calculate the concentrations of ATP, ADP, AMP, IMP, INO and Hx present in the fish samples. An example of a typical HPLC chromatogram of these compounds is given in Fig. 3.

External standards were injected twice each day to identify the peaks and quantify the compounds. Separation of the nucleotides was completed in 20 minutes.

A Hamilton microlitre syringe (SZR-605-0706) was used to inject the neutralised filtrate for HPLC analysis. Duplicate 5μl (fixed loop) injections were carried out on all samples. The samples were diluted in water (Milli-U10 grade) when necessary.



PEAK#	AREA%	RT	AREA BC
1	18.269	2.21	2876156 02
2	19.11	2.82	3008428 02
3	10.51	3.6	1654624 03
4	14.689	4.99	2312492 01
5	23.061	6.77	3630578 01
6	14.361	18.53	2260805 01
TOTAL	100.		15743083

Fig. 3. HPLC chromatogram for ATP, ADP, AMP, IMP, INO and Hx. Standard solution containing 200µmol/l of each compound.

2.7 K Value

K value (Saito *et al* 1959) is defined as the ratio of the concentration (μmol/l) of INO and Hx to the total concentration (μmol/g) of ATP and other ATP metabolites and is calculated by the following formula:

$$\text{K value \%} = \frac{[\text{INO}]+[\text{Hx}]}{[\text{ATP}]+[\text{ADP}]+[\text{AMP}]+[\text{IMP}]+[\text{INO}]+[\text{Hx}]} \times 100$$

The magnitude of the K value is inversely proportional to freshness i.e., the fresher the fish the lower the K value.

Karube *et al* (1984) simplified the definition of K value by excluding the adenosine phosphates and proposed the K_1 index as follows:

$$K_1 \text{ value \%} = \frac{[\text{INO}]+[\text{Hx}]}{[\text{IMP}]+[\text{INO}]+[\text{Hx}]} \times 100$$

2.7.1 Rapid Paper Strip Method for measuring K value

A simple paper strip method for measuring K value was developed by Transia-Difchamb, France. The strip consists of two discrete zones each impregnated with enzymes for the estimation of ATP and its metabolites. Zone A contains immobilised nucleoside phosphorylase and xanthine oxidase. The reaction of these enzymes with the ATP metabolites INO and Hx leads to the production of a resazurin dye to produce a colour. The intensity of the colour produced is proportional to the total concentration of INO and Hx present in the sample. Zone B of the paper strip contains an enzyme system IMP dehydrogenase to estimate the IMP concentration. A muscle sample (0.5g) was weighed in a pre-weighed centrifuge tube. Fresh tester-buffer solution

(10ml, Transia) were added to the centrifuge tube and the muscle sample homogenised for one minute using a Polytron (model PT-MR-300) homogeniser. The paper was immersed in the homogenised sample for 10 seconds. It was then removed, put into a freezer bag and stored in the dark in the laboratory for 10 minutes to allow the colour to develop. An estimate of the K value was obtained by comparing the colours developed on the paper strip to those colours supplied using a reference colour table. The reference colour table for estimation of the K value is shown in Fig. 4.

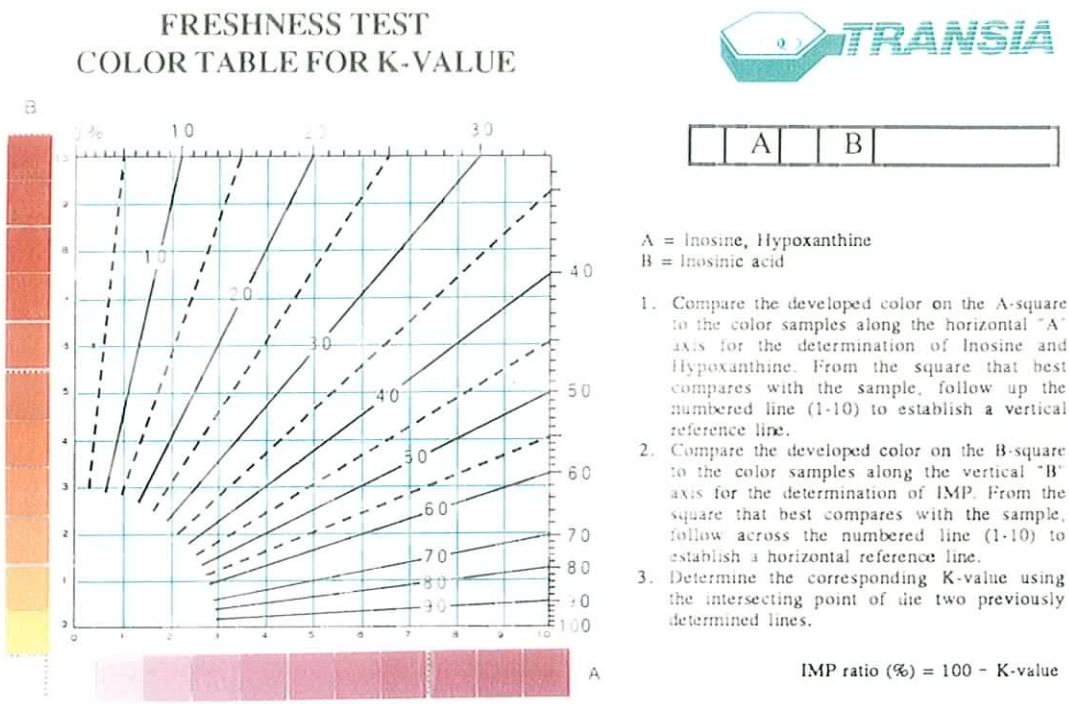


Fig. 4. Reference Colour Table for estimation of the K value.

2.8 pH

The pH value was determined on the intact muscle using a pH meter. The meter (pH Stick, Fisons PHK-121-800) with a spear electrode equipped with an inbuilt temperature compensation probe (Fisons PHK-124-510A) was inserted into the muscle mass above the lateral line and within the space between the pectoral fin and the gill cover. The meter was standardised using phosphate buffers Orion type 910107, (pH 7.0) and Orion type 910104, (pH 4.01). The spear electrode was rinsed with water (Milli-U10 grade) and dried with a disposable paper towel between pH measurements.

2.9 Temperature

A portable probe thermometer (Ebro, model TTX 181) was used to record the temperature of fish. The thermometer was equipped with a silicon sensor and was protected by a stainless steel probe. The probe was inserted into the muscle mass above the lateral line and posterior to the pectoral fin. The thermometer was held in position for 10 seconds until a steady reading was obtained. The stainless steel probe was rinsed with water (Milli-U10 grade) and dried with a disposable paper towel between measurements.

2.10 Torrymeter Readings

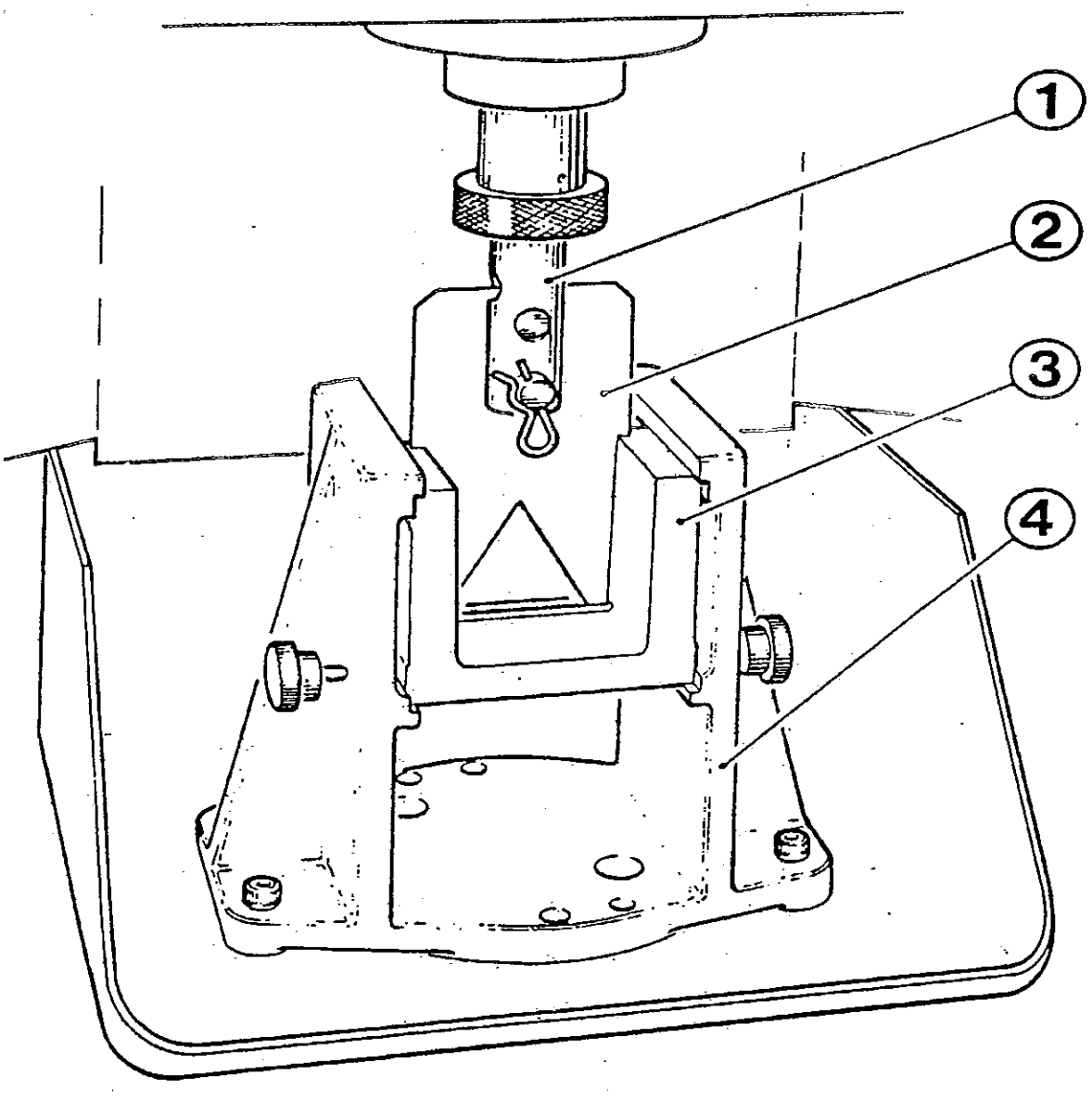
The gr Torrymeter (G R International Electronics Lt., Perth, Scotland) was used to measure the dielectric properties of fish muscle after death. The base of the instrument, which was applied to the fish, had two pairs of concentrically arranged electrodes. An alternating current of approximately 1 milliamp passed through the fish between the outer pairs and the inner pair sensed the resulting voltage. The phase angle between the current and the voltage was measured and converted electronically for digital display on a scale of 0 to 16. The phase angle and hence the meter reading

decreased with storage. Since the phase angle depended on the temperature as well as the freshness the instrument was equipped with a thermister which measured the fish temperature and corrected the reading to the value it should have shown at a reference temperature of 0°C. The mode switch on the Torrymeter had three positions, namely, off, 1 and 16. When the switch is in the 1 position a reading is obtained after each measurement. This mode was used to obtain readings for individual fish immediately *post mortem* and during storage at 3°C. Torrymeter measurements were made on rainbow trout and salmon.

2.11 Texture

Texture was measured using an Instron Universal Testing Machine, model 4464 (Instron UK Ltd., High Wycombe, Bucks, HP 12 3SY) which was interfaced with a personal computer (PC Siemens Nixdorff, type 5H PC1). The sampling rate of the computer was 10 pts/sec. Instron Series 1X Automated Materials Testing System Software for Windows (Instron Corporation, High Wycombe, Bucks, HP123 SY) was used. A 5 kg Newton load cell (type UK 149) with full scale range 50-500N was mounted on a moving crosshead. The load cell was a self identifying and electrically calibrated load cell. Shear force measurements were made using a Warner-Bratzler shear cell (type 2830-013). The Warner-Bratzler shear cell (Fig. 5), developed by Warner (1928) and by Bratzler (1949) consisted of a moving stainless steel inverted "V"-Shaped Blade which was secured to the blade holder by means of a hitch and clevis pins, and a stationary thick shear blade which contained a centrally located slot. The upper attachment of the Warner-Bratzler Shear cell was fixed in the load cell while the stationary blade was fitted to the sample support frame of the Instron. The crosshead speed was set at 80mm/min. This deformation rate chosen was based on force deformation curves obtained at different crosshead speeds. Tornberg *et al* (1985)

claimed that the average deformation rates imposed on foods during mastication were in the range of 100-150cm/min. Rectangular muscle samples measuring (15mm in width x 10mm in thickness x 20 mm in length) were cut from the epaxial muscle mass using a scalpel. Each sample contained at least three collagen bands that traversed the entire cross-sectional area of the muscle sample. Shear force was applied to the muscle samples at an angle perpendicular to the alignment of the myotomes. The blade travelled at a distance of 20mm before it was stopped. During operation the Warner-Bratzler shear blade was lowered into the shear fixture blade through the slot shearing the muscle sample. A force versus displacement graph was recognised by the computer. Peak height was taken as the maximum force required to cut through the muscle sample. Force was expressed as Newtons (N).



① Upper Blade Holder (with hitch and clevis pins)

② Upper Blade

③ Sample Shear Fixture and thick blade

④ Sample Support Frame

Fig. 5. Warner-Bratzler shear cell. Drawing adapted from Instron catalogue.

2. 12 Quality Index Method (QIM)

A visual examination of the fish was carried out immediately *post mortem* and during storage to assess the general appearance of the fish. Fish exhibiting bright eyes, pink gills, firm body texture, absence of loose scales and fresh fish odour were considered to be of good appearance. The extent of any deterioration associated with softening of the body musculature, wateriness, loose scales, dull eyes and unpleasant smell was noted. A sensory grading system (Table 3) based on the Quality Index Method (QIM) was used to evaluate the freshness of the fish (Bremner *et al* 1985).

The Quality Index Method assigned demerit points to the general appearance of the fish and to the state of *rigor*, condition of the eyes, gills, surface and belly. A score system from 0 to 3 demerit points was employed to grade the fish. Individual fish were inspected and the specific number of demerit points was recorded for each parameter. Fish which were inspected immediately after death and had exhibited firm body texture and bright eyes got a score of zero for the parameters studied. As the fish deteriorated with time the number of demerit points for each characteristic increased. The scores for all the characteristics were then added to give an overall sensory score, the so-called quality index. Factors of less importance were given lower total scores. The individual scores never exceeded 3, so no factor excessively unbalanced the sum score.

FRESHNESS QUALITY GRADING SYSTEM FOR RAINBOW TROUT

PARAMETER	CHARACTERISTIC	DEMERIT POINTS
General Appearance	Lustrous, translucent slime	0
	Loss of lustre, cloudy slime	1
	Pale appearance, cloudy slime	2
	Pale appearance, thick slime	3
Rigor	*Pre rigor*	0
	Rigor	1
	Post rigor	2
Scales	Absence of loose scales	0
	Initial loss of scales	1
	Loss of scales	2
Eyes		
• clarity	Clear, translucent	0
	Slightly dull	1
	Cloudy	2
• pupil	Black, shiny	0
	Slightly opaque	1
	Opaque	2
	Opaque and sunken	3
Gills		
• colour	Red, uniform in colour	0
	Slightly brown	1
	Dark brown	2
• odour	Fresh Oceanic	0
	Odourless	1
	Fishy	2
	Putrefactive	3
Flesh		
• texture	Very firm	0
	Less firm	1
	Flaccid	2
• colour	Pink, fresh bloom	0
	Slightly pale	1
	Very pale, almost white	2
Total demerit points (0-21)		

Table 3. Freshness quality grading system based on the Quality Index Method (Bremner *et al* 1985).

2.13 Statistical Analysis

Statistical analyses were carried out using GLM General Factorial Analysis procedure (SPSS, version 8.0). Statistical significance was determined by least squared difference (LSD) and Scheffe's tests.

Student t-tests (unpaired) were performed to test the differences between mean values.

3. RESULTS

3. RESULTS

3.1 Biochemistry of Fish Muscle

A. Rainbow Trout

3.1.1 Anatomical Site of Sampling and Concentrations of Adenine Nucleotides

Rainbow trout obtained from Annamoe Fish Farms Co. Wicklow were used in experiments carried out to study the effect on the anatomical site of sampling on the concentrations of adenine nucleotides. The concentrations of ATP, ADP, AMP, IMP, INO and Hx were used to calculate the K value (section 2.7). Four muscle samples were taken from specific locations in each fish (section 2.4.1). Seven groups of fish were used. Each group was subjected to a different method of harvest (section 2.3).

3.1.1.1 ATP and Metabolites

The mean concentration of ATP in muscle taken from the four anatomical sites from fish which were clubbed and sampled immediately *post mortem* (harvest method 1) ranged from 2.84 ± 0.49μmol/g to 5.20 ± 1.35μmol/g. The results are given in Table 4. The highest concentration of ATP (5.20 ± 0.48μmol/g) was found in the third muscle site which was taken under the dorsal fin and the lowest value (2.84 ± 0.49μmol/g) was found in the first muscle site which was taken posterior to the pectoral fin. The difference between these values was not significant ($p = 0.05$). The concentrations of ADP ranged from 0.8 1 ± 0.12μmol/g to 1.47 ± 0.36μmol/g. Concentrations of AMP were very low (< 0.2μmol/g) for the four muscle samples. The concentrations of IMP ranged from 2.03 ± 1.18μmol/g which was found in the third muscle location to 3.14 ± 0.61μmol/g in the first muscle location. The difference between these values was not significant ($p = 0.05$). Concentrations of INO and Hx were negligible for all muscle

samples. The mean concentrations of ATP and its metabolites for anatomical muscle samples taken from fish which were subjected to the different methods of harvest are given in Tables 4 to 10.

Muscle Sample Location	ATP (μmol/g)	ADP (μmol/g)	AMP (μmol/g)	IMP (μmol/g)	INO (μmol/g)	Hx (μmol/g)
1	2.84 ± 0.49	1.17 ± 0.13	0.11 ± 0.04	3.14 ± 0.61	0.18 ± 0.19	0.14 ± 0.02
2	5.00 ± 0.48	1.47 ± 0.36	0.14 ± 0.06	2.69 ± 1.35	0.01 ± 0.02	0.30 ± 0.05
3	5.20 ± 1.35	0.81 ± 0.12	0.04 ± 0.03	2.03 ± 1.18	0.01 ± 0.01	0.17 ± 0.06
4	3.74 ± 0.65	1.19 ± 0.28	0.06 ± 0.02	2.54 ± 1.38	< 0.01	0.33 ± 0.12

Table 4. Rainbow trout: mean concentrations of ATP and metabolites in muscle taken from four specific locations in clubbed fish immediately *post mortem*. Mean values ± s.e.m. for four fish.

Muscle Sample Location	ATP (μmol/g)	ADP (μmol/g)	AMP (μmol/g)	IMP (μmol/g)	INO (μmol/g)	Hx (μmol/g)
1	3.12 ± 1.30	1.23 ± 0.39	0.12 ± 0.01	6.22 ± 0.91	0.32 ± 0.18	0.10 ± 0.03
2	3.30 ± 1.15	1.10 ± 0.16	0.10 ± 0.01	4.54 ± 0.88	0.28 ± 0.15	0.09 ± 0.03
3	4.08 ± 1.32	0.79 ± 0.31	0.32 ± 0.27	4.16 ± 1.17	0.54 ± 0.10	0.05 ± 0.02
4	5.80 ± 1.48	1.01 ± 0.20	0.09 ± 0.02	3.30 ± 0.60	0.46 ± 0.09	0.06 ± 0.03

Table 5. Rainbow trout: mean concentrations of ATP and metabolites in muscle taken from four specific locations in fish which were clubbed and held at ambient temperature for 30 minutes *post mortem* before sampling. Mean values ± s.e.m. for four fish.

Muscle Sample Location	ATP (μmol/g)	ADP (μmol/g)	AMP (μmol/g)	IMP (μmol/g)	INO (μmol/g)	Hx (μmol/g)
1	3.07 ± 0.95	1.25 ± 0.13	0.20 ± 0.02	5.25 ± 0.43	0.23 ± 0.11	0.20 ± 0.08
2	4.54 ± 1.01	1.06 ± 0.16	0.15 ± 0.05	4.79 ± 0.97	0.08 ± 0.06	0.15 ± 0.03
3	4.94 ± 1.18	1.47 ± 0.17	0.15 ± 0.04	4.81 ± 1.01	0.07 ± 0.07	0.17 ± 0.10
4	3.83 ± 0.90	1.58 ± 0.31	0.33 ± 0.24	5.01 ± 1.27	0.24 ± 0.14	0.14 ± 0.08

Table 6. Rainbow trout: mean concentrations of ATP and metabolites in muscle taken from four specific locations in fish which were clubbed and held at 3°C for 30 minutes *post mortem* before sampling.
Mean values ± s.e.m. for five fish.

Muscle Sample Location	ATP (μmol/g)	ADP (μmol/g)	AMP (μmol/g)	IMP (μmol/g)	INO (μmol/g)	Hx (μmol/g)
1	3.85 ± 0.73	1.19 ± 0.07	0.14 ± 0.04	5.04 ± 0.83	0.44 ± 0.17	0.09 ±0.03
2	3.05 ± 1.28	1.50 ± 0.27	0.16 ± 0.03	5.90 ± 1.04	0.50 ± 0.15	0.03 ± 0.01
3	4.20 ± 1.29	1.39 ± 0.42	0.11 ± 0.03	4.93 ± 1.10	0.52 ± 0.23	0.13 ± 0.10
4	5.31 ± 0.68	1.35 ± 0.11	0.08 ± 0.01	3.39 ± 0.55	0.70 ± 0.08	0.15 ± 0.08

Table 8. Rainbow trout: mean concentrations of ATP and metabolites in muscle taken from four specific locations in fish which were individually chilled in ice slurry for 15 minutes *ante mortem* and clubbed before sampling. Mean values ± s.e.m. for five fish.

Muscle Sample Location	ATP (μmol/g)	ADP (μmol/g)	AMP (μmol/g)	IMP (μmol/g)	INO (μmol/g)	Hx (μmol/g)
1	3.21 ± 0.58	1.33 ± 0.23	0.13 ± 0.01	4.34 ± 0.77	0.39 ± 0.12	0.14 ± 0.04
2	3.86 ± 0.88	1.40 ± 0.29	0.10 ± 0.03	4.32 ± 1.09	0.14 ± 0.15	0.16 ± 0.04
3	4.89 ± 0.57	1.25 ± 0.13	0.09 ± 0.01	4.99 ± 1.29	0.19 ± 0.14	0.18 ± 0.04
4	3.25 ± 0.70	1.27 ± 0.19	0.12 ± 0.01	5.14 ± 1.08	<0.01	0.18 ± 0.07

Table 7. Rainbow trout: mean concentrations of ATP and metabolites in muscle taken from four specific locations in fish which were clubbed and held at 3°C for 90 minutes *post mortem* before sampling.
Mean values ± s.e.m. for five fish.

Muscle Sample Location	ATP (μmol/g)	ADP (μmol/g)	AMP (μmol/g)	IMP (μmol/g)	INO (μmol/g)	Hx (μmol/g)
1	3.37 ± 2.10	1.12 ± 0.21	0.13 ± 0.04	6.06 ± 1.91	0.21 ± 0.12	0.12 ± 0.05
2	2.72 ± 1.04	1.13 ± 0.12	0.11 ± 0.03	4.53 ± 1.02	0.02 ± 0.02	0.32 ± 0.07
3	3.34 ± 1.37	1.12 ± 0.26	0.11 ± 0.02	3.90 ± 1.20	0.01 ± 0.01	0.21 0.09
4	4.14 ± 1.58	1.03 ± 0.15	0.11 ± 0.03	2.94 ± 1.00	0.16 ± 0.17	0.15 ± 0.03

Table 9. Rainbow trout: mean concentrations of ATP and metabolites in muscle taken from four specific locations in fish which were chilled as a group in ice slurry for 15 minutes *ante mortem* and clubbed before sampling. Mean values ± s.e.m. for five fish.

Muscle Location Number	ATP (µmol/g)	ADP (µmol/g)	AMP (µmol/g)	IMP (µmol/g)	INO (µmol/g)	Hx (µmol/g)1
1	1.26 ± 0.80	0.98 ± 0.12	0.20 ± 0.04	7.15 ± 0.97	0.61 ± 0.19	< 0.01
2	1.23 ± 0.76	1.02 ± 0.16	0.19 ± 0.03	7.31 ± 0.79	0.75 ± 0.09	0.12 ± 0.13
3	1.09 ± 0.90	1.14 ± 0.33	0.19 ± 0.03	7.54 ± 0.74	0.70 ± 0.15	0.01 ± 0.01
4	1.32 ± 1.24	1.28 ± 0.56	0.20 ± 0.08	6.79 ± 1.73	0.71 ± 0.33	0.26 ± 0.31

Table 10. Rainbow trout: mean concentrations of ATP and metabolites in muscle taken from four specific locations in fish which asphyxiated at 3°C before sampling.
Mean values ± s.e.m. for five fish.

3.1.1.2 Harvest Methods and Nucleotide Degradation

The concentrations of ATP and its metabolites are the overall mean of the muscle samples taken from the four sites and looked at as a function of the harvesting method. The mean zero hour concentration of ATP for the fish harvested by seven different methods ranged from 1.21 ± 0.36μmol/g to 4.19 ± 0.41μmol/g. The results are shown in Table 11. The highest concentration of ATP (4.19 ± 0.41μmol/g) was found in the fish which were clubbed and sampled immediately *post mortem* (harvest method 1) and the lowest value (1.21 ± 0.36μmol/g) in the asphyxiated fish (harvest method 7). The difference between these values was significant ($p < 0.001$). There was no significant difference in the concentration of ATP between fish which were clubbed and sampled immediately *post mortem* (4.19 ± 0.41μmol/g; harvest method 1), and fish which were clubbed and held at 3°C for 90 minutes *post mortem* (3.39 ± 0.66μmol/g; harvest method 4) before sampling. The zero hour concentrations of ADP were between 1.02 ± 0.12μmol/g and 1.36 ± 0.13μmol/g for all the fish. The mean initial concentrations of the metabolites AMP, INO and Hx were very low (all < 0.7μmol/g). The mean concentrations of IMP were in the range of 2.60 ± 0.46μmol/g to 7.25 ± 0.40μmol/g. The highest concentration of IMP (7.25 ± 0.40μmol/g) was found in the asphyxiated fish (harvest method 7) and the lowest (2.60 ± 0.46μmol/g) in the fish which were clubbed and sampled immediately *post mortem* (harvest method 1). The difference between these values was significant ($p < 0.001$).

3.1.1.3 K value

The mean K value for the seven groups of fish ranged from 2.96 ± 0.48% to 7.15 ± 0.24%. The results are shown in Fig. 8. The highest K value (7.15 ± 0.24%) was found in the asphyxiated fish (harvest method 7) and the lowest (2.96 ± 0.48%) in the

fish which were chilled as a group for 15 minutes *ante mortem* and then clubbed. The difference between these values was significant ($p < 0.001$). There was no significant difference in the K values between fish which were clubbed and sampled immediately *post mortem* and fish which were clubbed and held on ice for 30 minutes *post mortem*.

Harvest Method	ATP (μmol/g)	ADP (μmol/g)	AMP (μmol/g)	IMP (μmol/g)	INO (μmol/g)	Hx (μmol/g)
1. Clubbed and sampled immediately *post mortem*	4.19 ± 0.41	1.16 ± 0.12	0.09 ± 0.02	2.60 ± 0.46	0.05 ± 0.04	0.24 ± 0.04
2. Clubbed and held at ambient temperature for 30 minutes *post mortem* before sampling.	4.07 ± 0.60	1.02 ± 0.12	0.16 ± 0.06	4.56 ± 0.46	0.40 ± 0.06	0.07 ± 0.01
3. Clubbed and held on ice for 30 minutes *post mortem* before sampling.	4.09 ± 0.45	1.34 ± 0.11	0.21 ± 0.06	4.96 ± 0.40	0.18 ± 0.06	0.17 ± 0.04
4. Clubbed and held on ice for 90 minutes *post mortem* before sampling.	3.80 ± 0.33	1.32 ± 0.11	0.11 ± 0.01	4.70 ± 0.45	0.18 ± 0.07	0.16 ± 0.02
5. Chilled individually for 15 minutes *ante mortem*. clubbed and sampled.	4.10 ± 0.47	1.36 ± 0.13	0.12 ± 0.11	4.82 ± 0.43	0.54 ± 0.08	0.10 ± 0.04
6. Chilled as a group for 15 minutes *ante mortem*. clubbed and sampled.	3.39 ± 0.66	1.10 ± 0.08	0.11 ± 0.01	4.36 ± 0.61	0.10 ± 0.05	0.20 ± 0.03
7. Asphyxiated at 3°C and sampled immediately *post mortem*.	1.21 ± 0.36	1.09 ± 0.11	0.19 ± 0.02	7.25 ± 0.40	0.69 ± 0.07	0.08 ± 0.05

Table 11. Rainbow trout: mean concentrations of ATP and metabolites in muscle samples taken from four sites and looked at as a function of the harvesting method.
Mean values ± s.e.m. for groups of four (harvest methods 1and 2) and five (harvest methods 3 to 7) fish.

3.1.2 Statistical Analysis of Anatomical Muscle Site and Nucleotides

A statistical analysis involving GLM General Factorial Analysis procedure (SPSS, version 8.0) was conducted to determine the effects of anatomical muscle location on concentrations of ATP and IMP and on the K value. The analysis was carried out using the seven different harvest methods (section 2.3) and four specific anatomical muscle locations. The key to the anatomical muscle locations is shown in Fig.2 (Materials and Methods). No significant differences were found in the concentrations of ATP, IMP or K value between muscle samples taken from the different anatomical sites. The results for the analysis are shown in Fig. 6, 7 and 8.

A statistical analysis was conducted to determine the differences between the harvest methods on the concentrations of ATP and IMP and on the K value. The results showed a significant effect ($p < 0.05$) with harvest method 7. This method was different to the others as determined by post-hoc analysis using the least squared differences (LSD) and Scheffe's tests. The results for these analyses are shown in Fig. 9, 10 and 11. No significant differences were found in concentrations of ATP between any of the other harvest method methods (1 to 6).

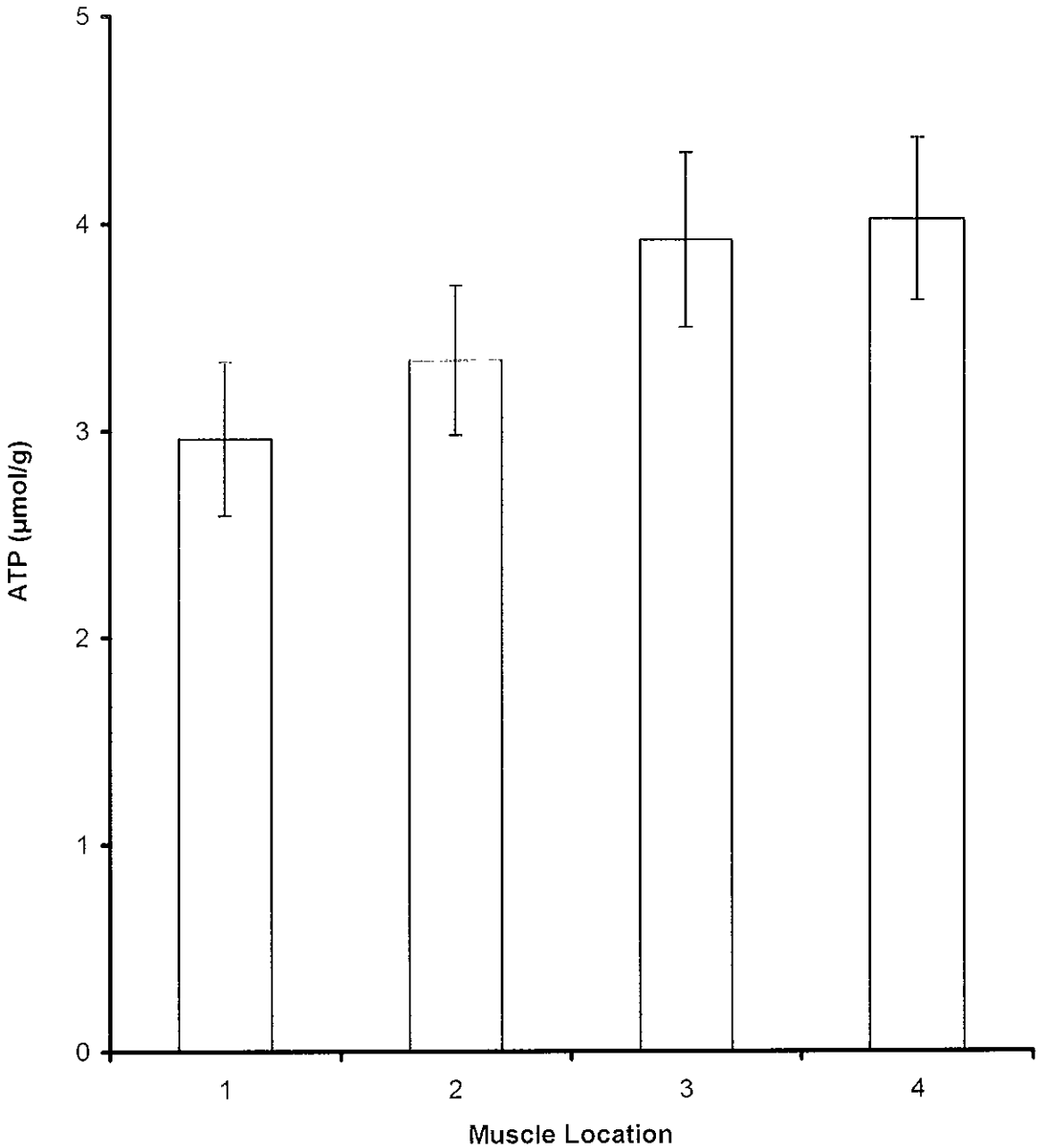


Fig. 6. Rainbow trout: concentrations of ATP for the mean of four muscle locations
Mean values ± s.e.m. for 33 fish.

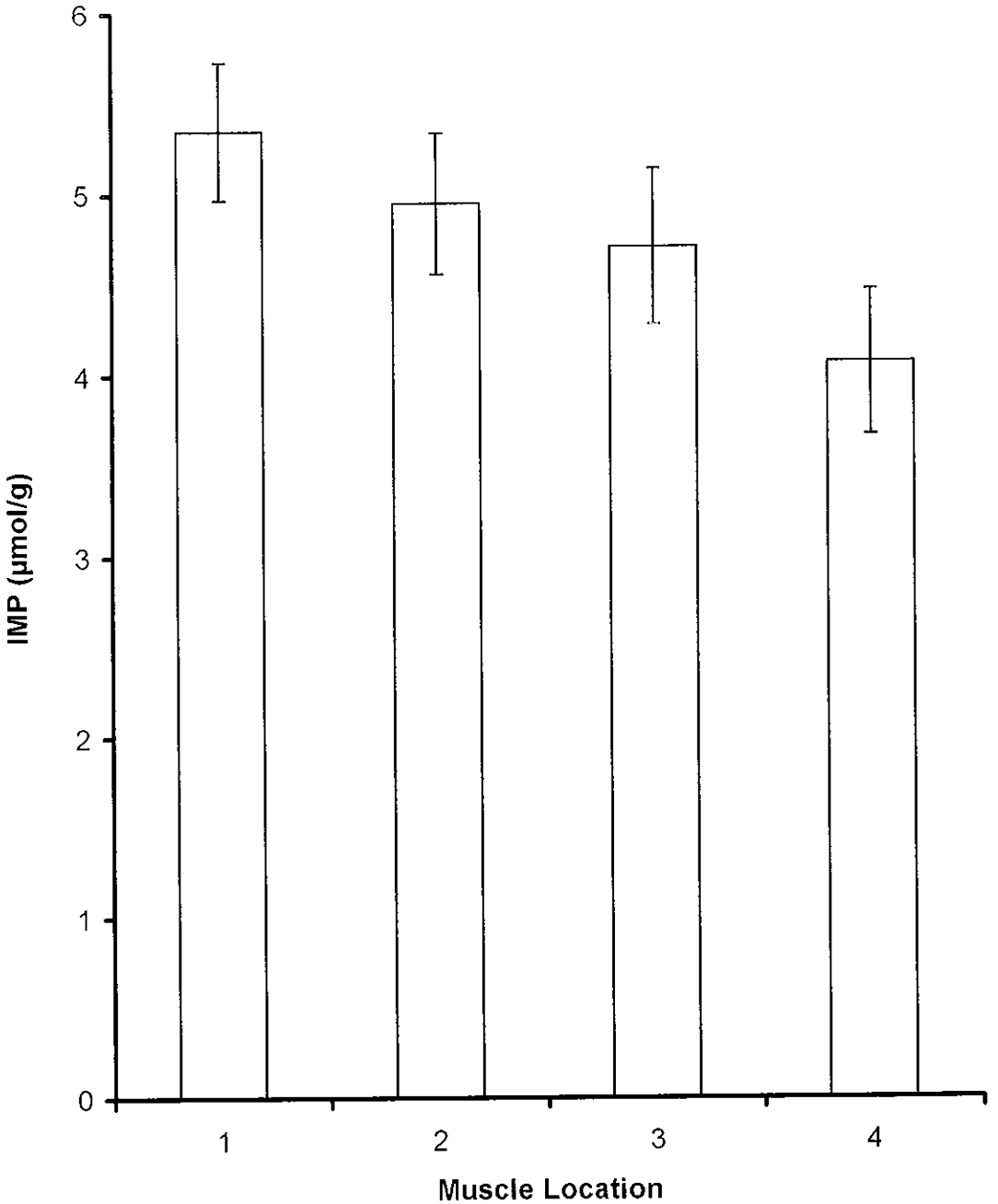


Fig. 7. Rainbow trout: concentrations of IMP for the mean of four muscle locations. Mean values ± s.e.m for 33 fish.

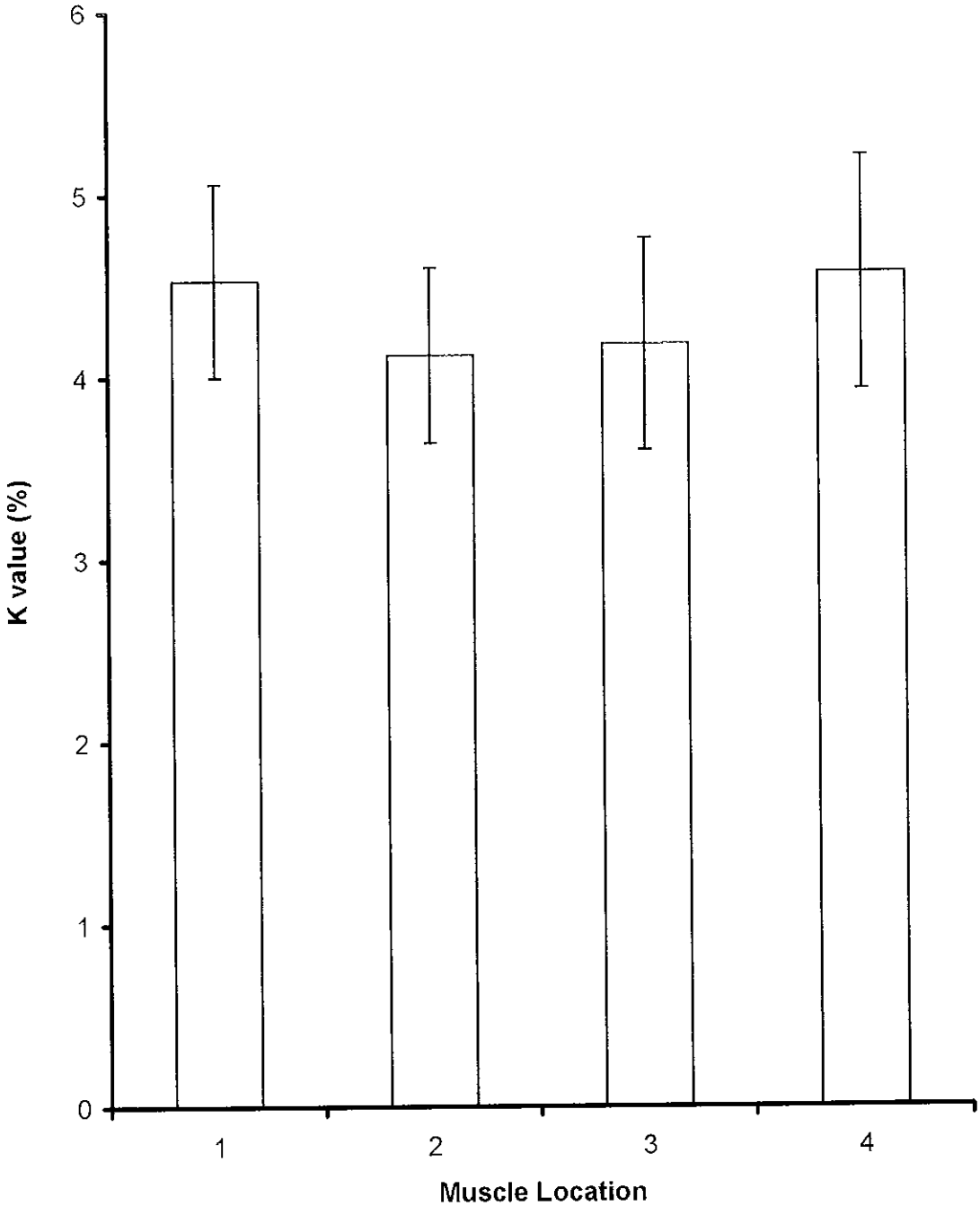


Fig. 8. Rainbow trout: K value for the mean of four muscle locations.
Mean values ± s.e.m. for 33 fish.

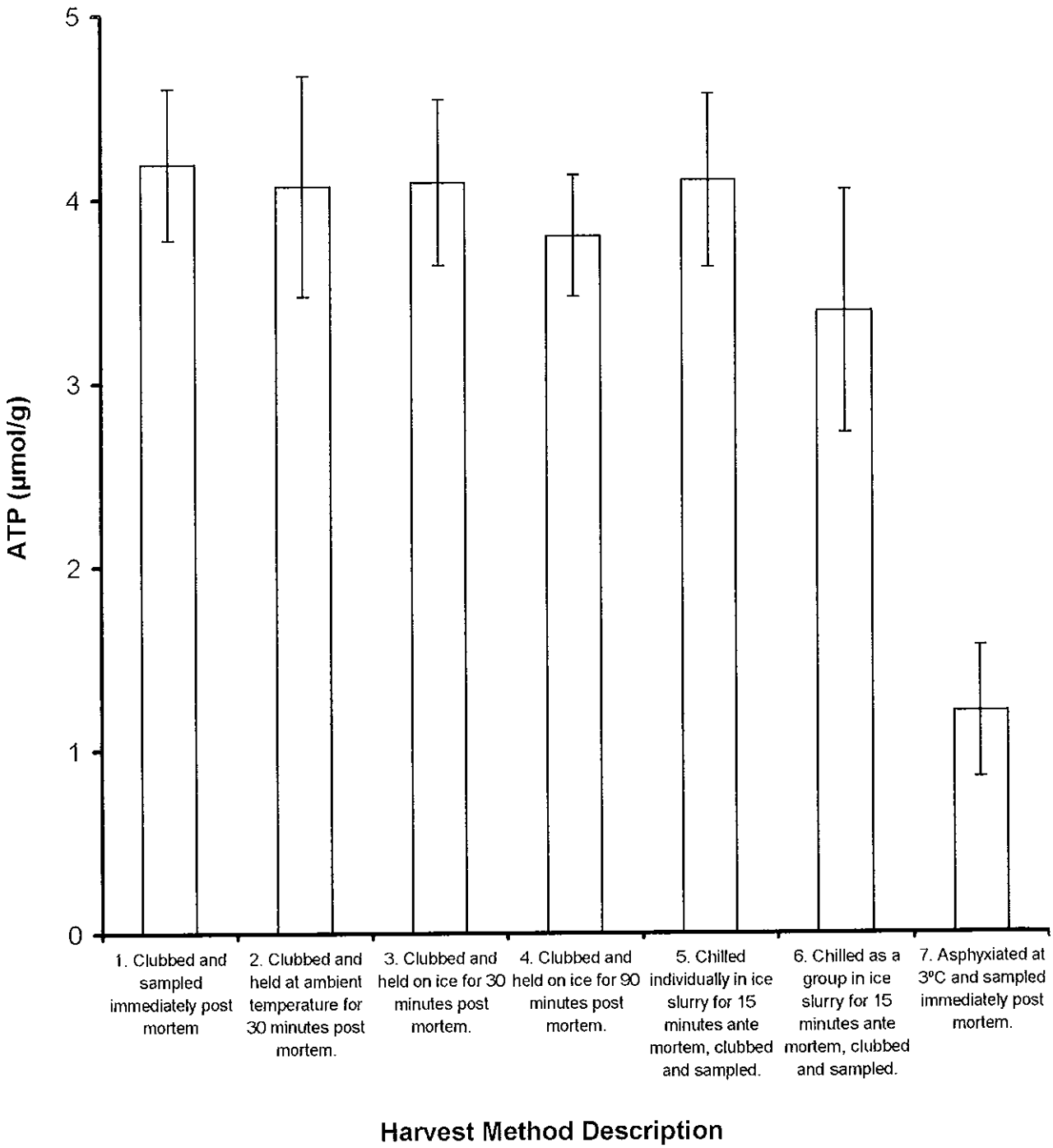


Fig. 9. Rainbow trout: mean concentrations of ATP for four muscle samples taken from fish harvested at the method indicated.
Mean values ± s.e.m. for groups of four (harvest methods 1and 2) and five (harvest methods 3 to 7) fish.

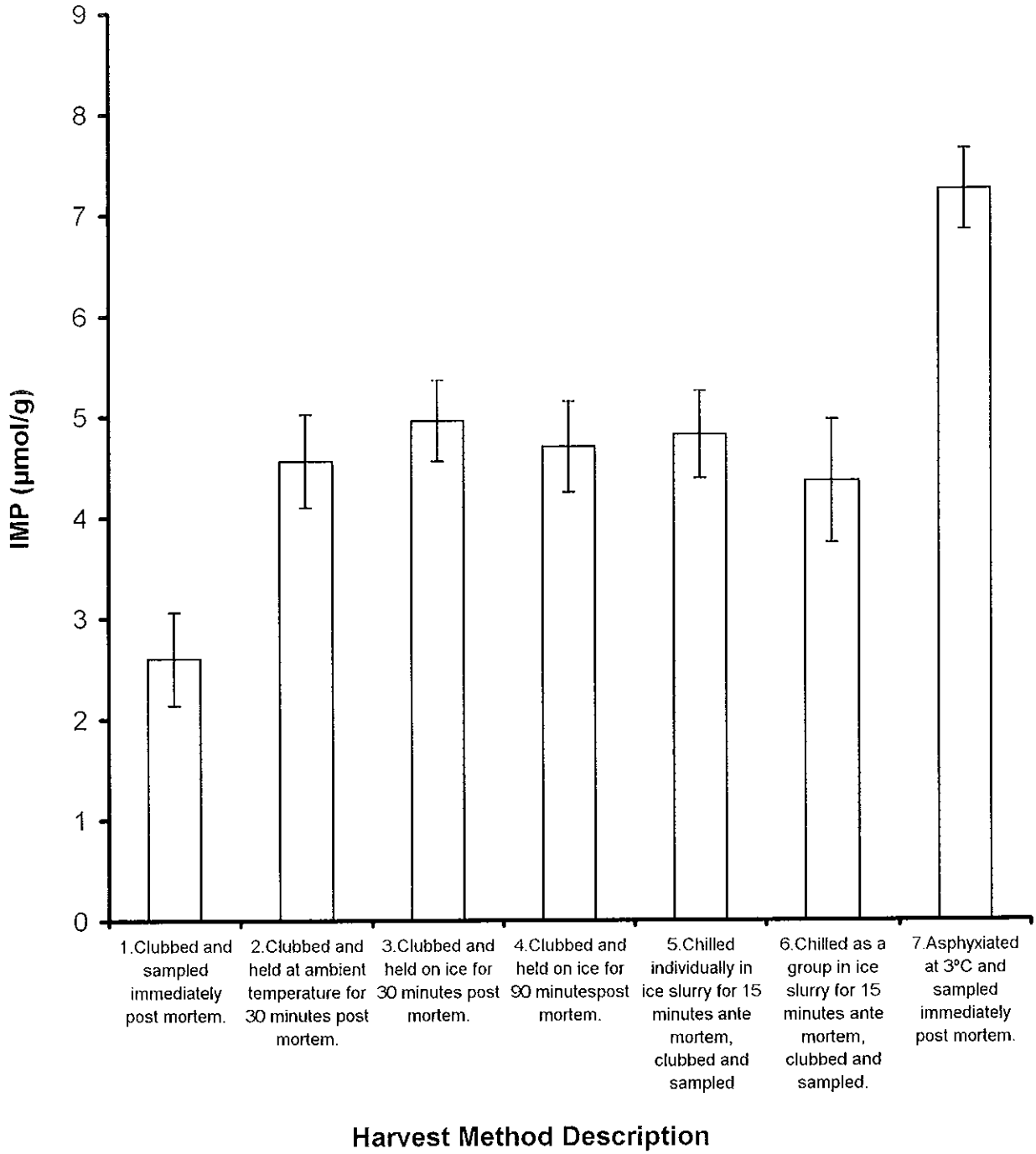


Fig. 10. Rainbow trout: mean concentrations of IMP for four muscle samples taken from fish harvested at the method indicated.
Mean values ± s.e.m. for groups of four (harvest method 1and 2) and five (harvest methods 3 to 7) fish.

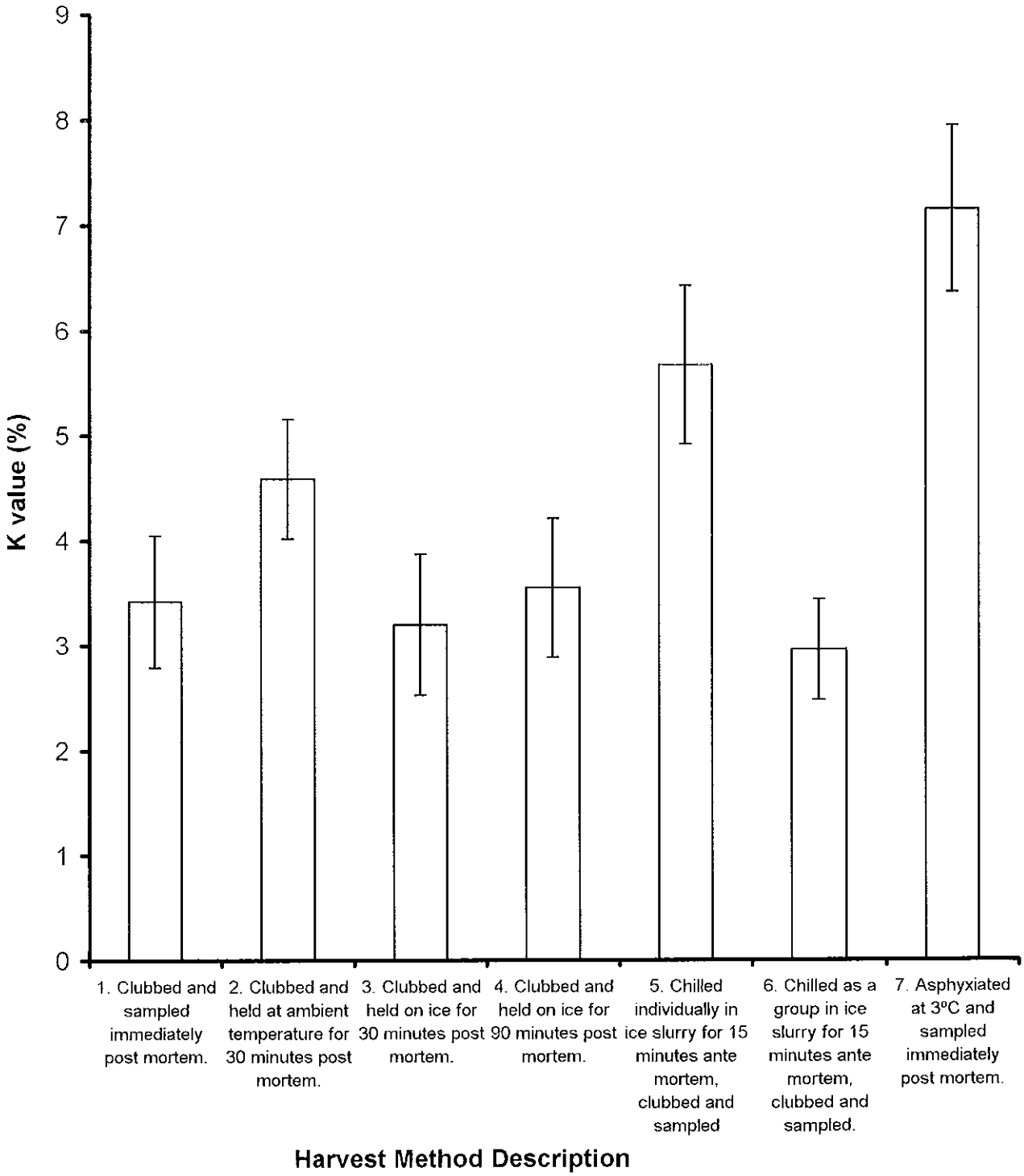


Fig. 11. Rainbow trout: K value for the mean of four muscle samples taken from fish harvest at the method indicated.
Mean values ± s.e.m. for groups of four (harvest methods 1and 2) and five (harvest methods 3 to 7) fish.

3.1.3 Adenine Nucleotides and pH in Rainbow Trout at 3°C

In these experiments, specimens of muscle were taken immediately after death, at 1h, 2h, 3h and at 30h. In the first experiment, the changes were studied in fish killed by clubbing. In the second series of experiments fish killed by clubbing and asphyxiation were compared.

3.1.3.1 Clubbed Rainbow Trout

Fish (530g in weight and 37cm in length) were obtained from Annamoe Fish Farms, Co. Wicklow and transported live to the laboratory (section 2.1). They had been starved for two days prior to killing. Fifteen fish were used and were handled in three groups of five fish. The fish were clubbed as described in Materials and Methods (section 2.2.2). Muscle samples were taken as described (section 2.4). The concentrations of ATP, ADP, AMP, IMP, INO and Hx were determined (section 2.6) and were used to calculate the K value. Measurements of pH were made on the intact muscle sample (section 2.8.). Sampling was carried out at zero hour and at one, two and three hours and at 30 hours *post mortem*. The fish were held on ice at 3°C during the experiments.

3.1.3.1.1 ATP and Metabolites

The results for ATP and metabolites are the mean for fifteen fish. The changes in the concentration of ATP are shown in Fig.12. The mean zero hour concentration of ATP was 4.15 ± 0.48μmol/g. At one hour *post mortem* the concentration of ATP was 4.26 ±0.68μmol/g and at two hours *post mortem* the concentration of ATP was 4.59 ± 0.70μmol/g. The increase in the concentration of ATP from 4.15 ± 0.48 to 4.26 ± 0.68μmol/g and 4.59 ± 0.70μmol/g at one and two hours *post mortem* respectfully was not significant ($p > 0.5$). At 30 hours *post mortem* the concentration of ATP was

almost negligible (0.05 ± 0.02μmol/g). The mean initial concentration of ADP was 1.12 ± 0.14μmol/g. There was a slight increase in the concentration of ADP at one hour *post mortem* after which time the concentrations of ADP decreased slightly. At 30 hours *post mortem* the concentration of ADP was very low (0.50 ± 0.07μmol/g). The results are shown in Fig. 13. The mean zero hour concentration of AMP was 0.11 ± 0.02μmol/g (Fig. 14). At one hour *post mortem* the concentration of AMP was 1.15 ± 0.05μmol/g. The difference between these values was not significant ($p > 0.05$). At two hours *post mortem* the concentration of AMP was 0.09 ±0.02μmol/ and remained constant for up to 30 hours. Changes in the concentrations of IMP are shown in Fig. 15. The mean zero hour concentration of IMP was 4.10 ± 0.56μmol/g. At one hour *post mortem* there was a slight increase in the concentration of IMP and at three hours there was a further increase. At 30 hours *post mortem* the concentration of IMP was 8.23 ± 0.82 μmol/g. The increase in the initial concentration of IMP from 4.10 ± 0.56μmol/g to 8.23 ± 0.82μmol/g at 30 hours *post mortem* was significant ($p < 0.01$). The mean zero hour concentration of INO was very low (0.08 ± 0.04μmol/g; Fig. 16). There was a slight increase in the concentration of INO during the first three hours. At 30 hours *post mortem* the concentration of INO was 1.37 ± 0.17μmol/g. The increase in the initial concentration of INO from 0.08 ± 0.04μmol/g to 1.37 ± 0.17μmol/g at 30 hours *post mortem* was significant ($p < 0.01$). Changes in the concentration of Hx are shown in Fig. 17. The mean zero hour concentration of Hx was 0.15 ± 0.02μmol/g. The concentrations of Hx decreased during the first three hours of storage after which time there was an increase. At 30 hours *post mortem* the concentration of Hx was 0.10 ± 0.01μmol/g and was not significantly ($p > 0.05$) lower than the initial concentration (0.15 ± 0.02μmol/g).

3.1.3.2 K value

Changes in the K value during storage are shown in Fig. 18. The zero hour K value was 2.22 ± 0.60%. At one hour *post mortem* the K value was 2.60 ± 0.81%. The K value increased slightly up to three hours *post mortem*. At 30 hours *post mortem* the K value was 15.31 ± 2.07%.

3.1.4 pH

The pH measurements were taken on the intact muscle as described in Materials and Methods, section 2.8. Changes in pH values during storage are shown in Fig.19. The zero hour pH value was 7.09 ± 0.03. This value would indicate that the fish were in a pre *rigor* state at time of sampling.

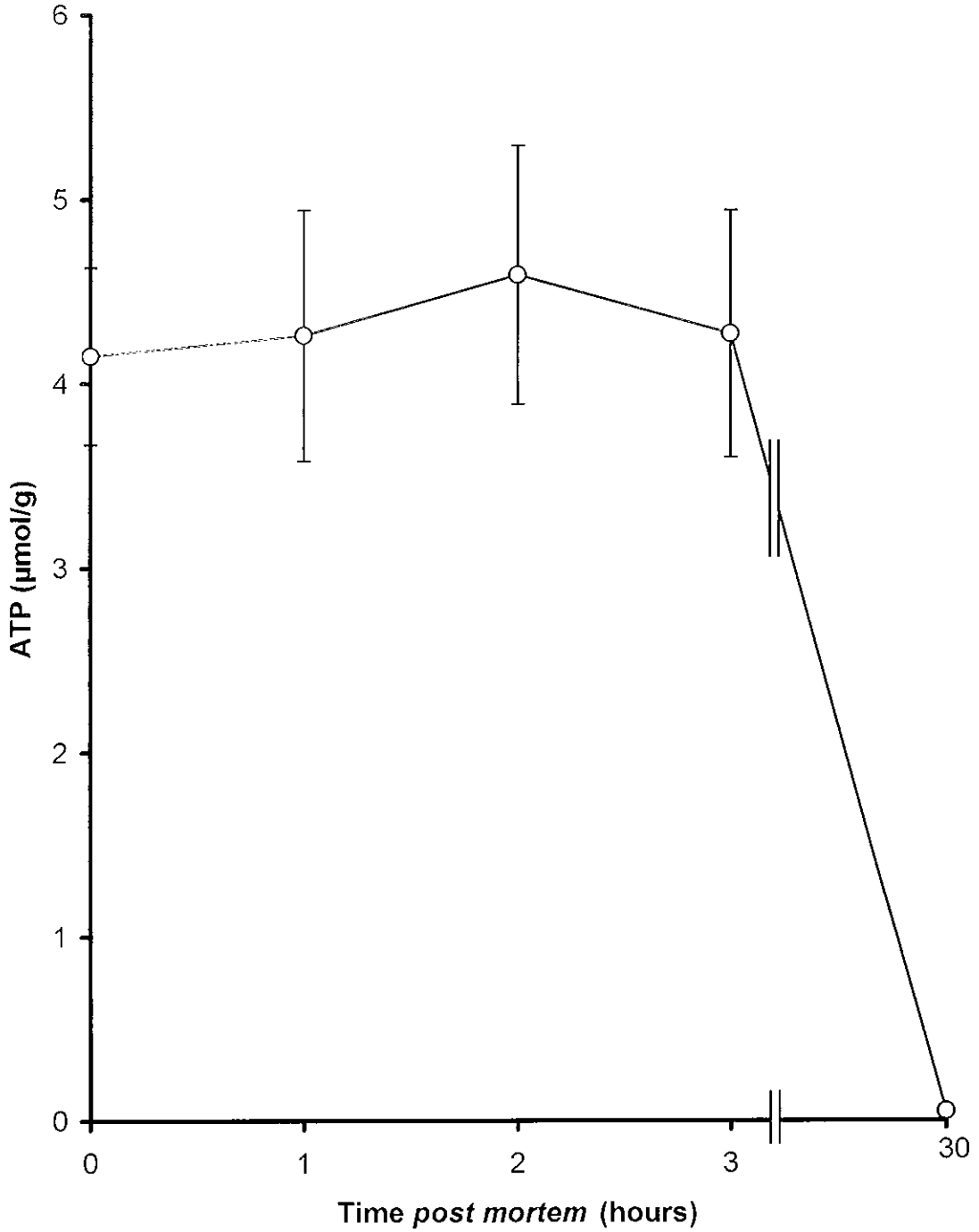


Fig. 12. Myotomal muscle of clubbed rainbow trout: changes in the concentration of ATP at 3°C.
Mean values ± s.e.m. for fifteen fish.

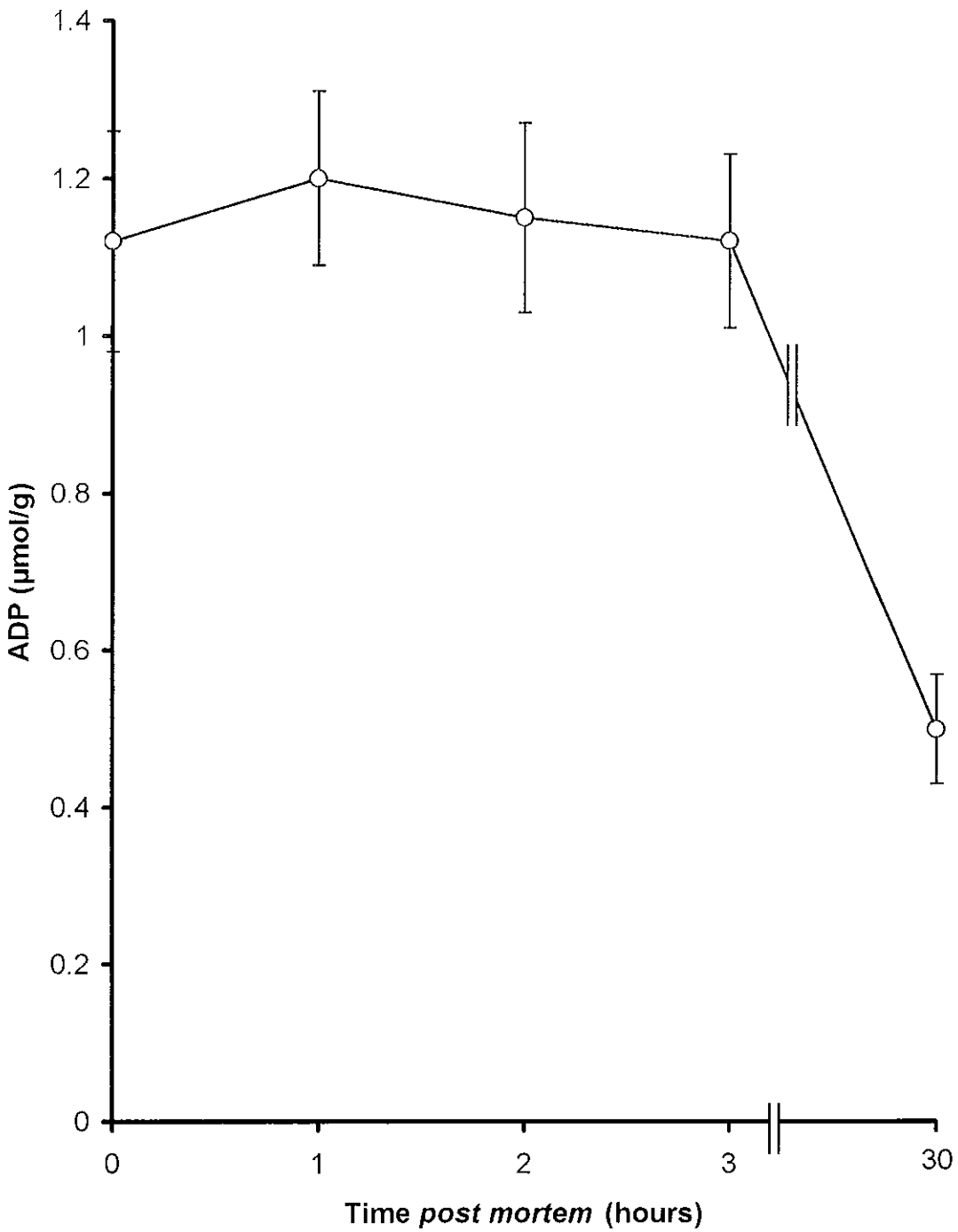


Fig. 13. Myotomal muscle of clubbed rainbow trout: changes in the concentration of ADP at 3°C.
Mean values ± s.e.m. for fifteen fish.

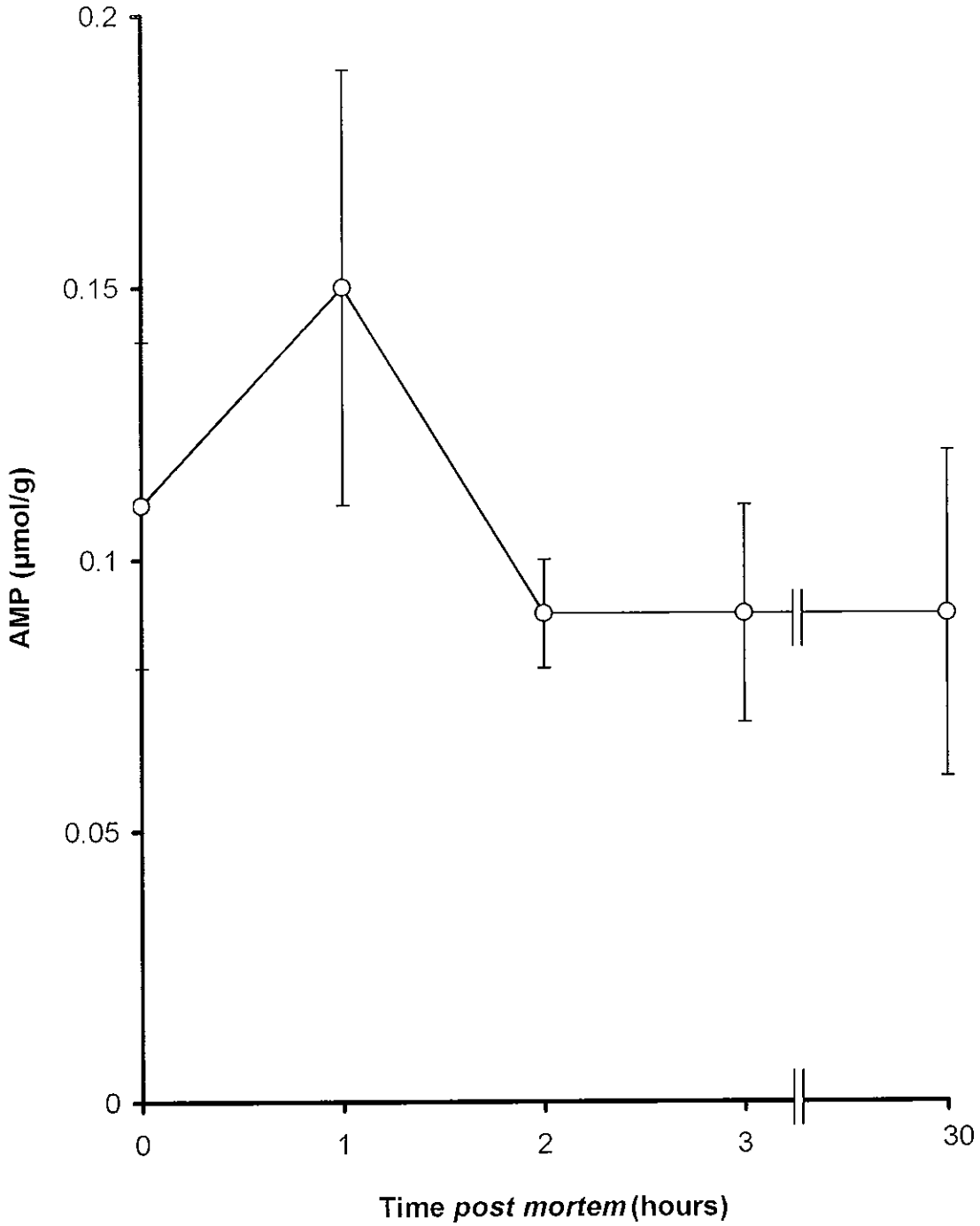


Fig. 14. Myotomal muscle of clubbed rainbow trout: changes in the concentration of AMP at 3°C.
Mean values ± s.e.m. fifteen fish.

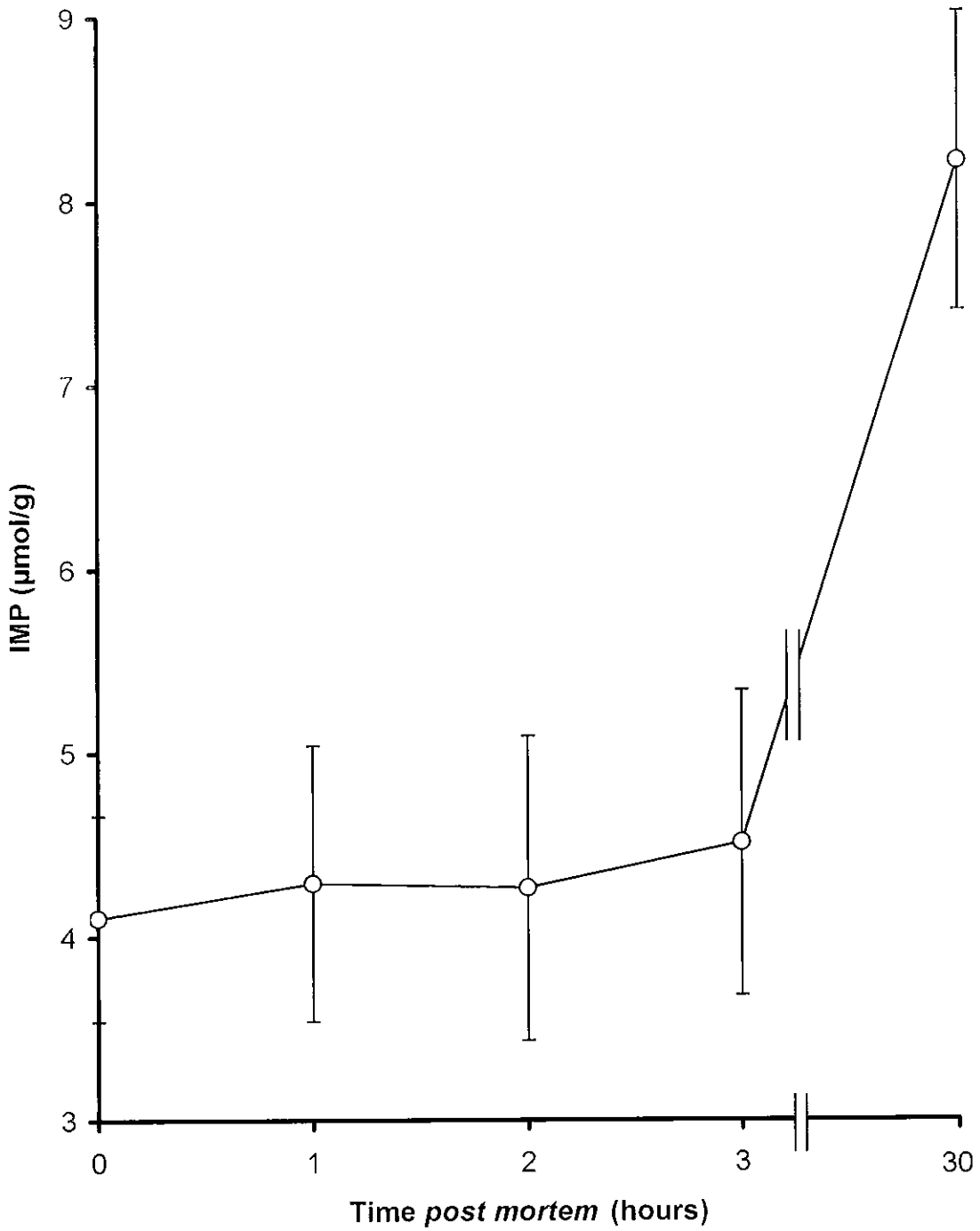


Fig. 15. Myotomal muscle of clubbed rainbow trout: changes in the concentration of IMP at 3°C.
Mean values ± s.e.m. for fifteen fish.

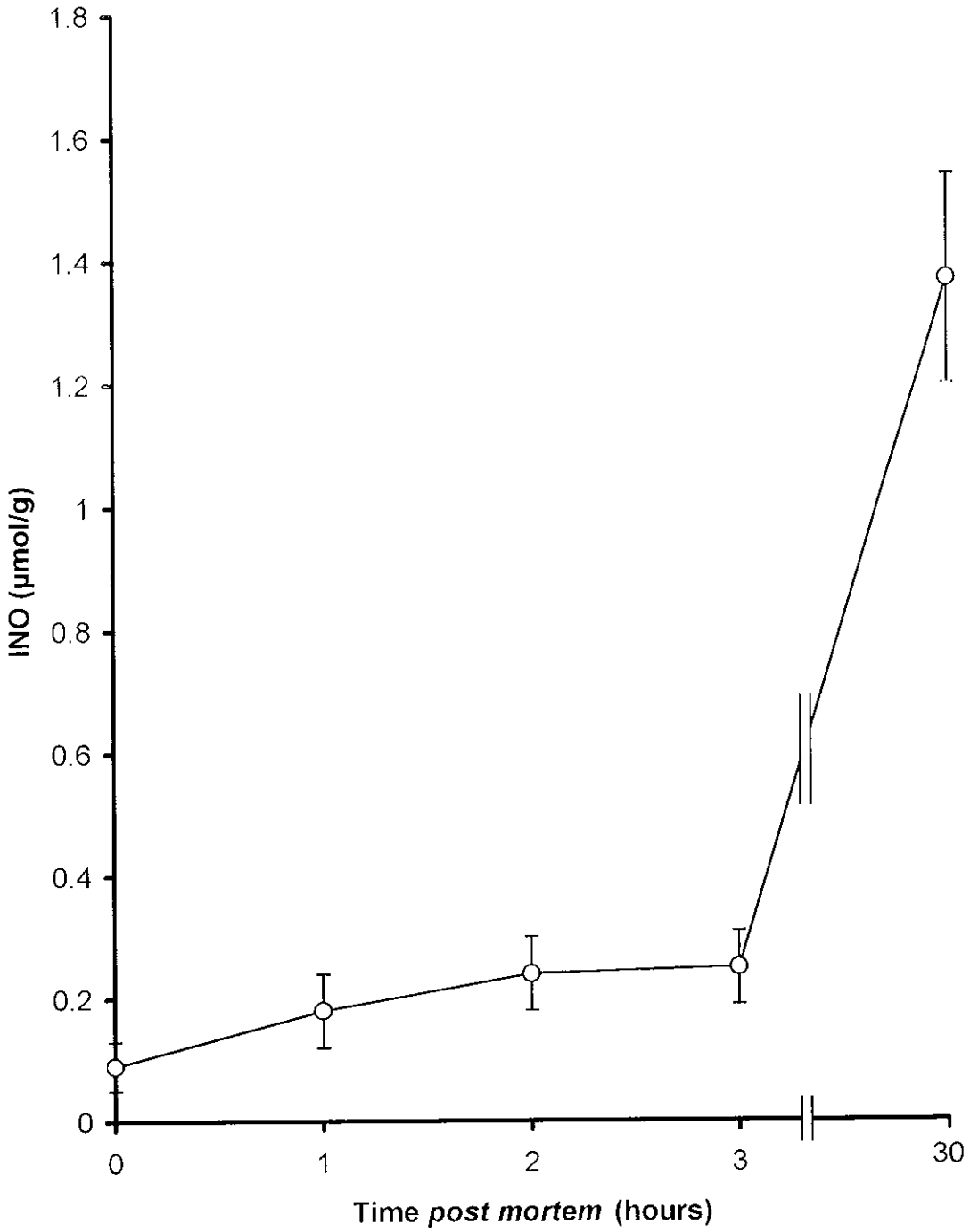


Fig. 16. Myotomal muscle of clubbed rainbow trout: changes in the concentration of INO at 3°C.
Mean values ± s.e.m. for fifteen fish.

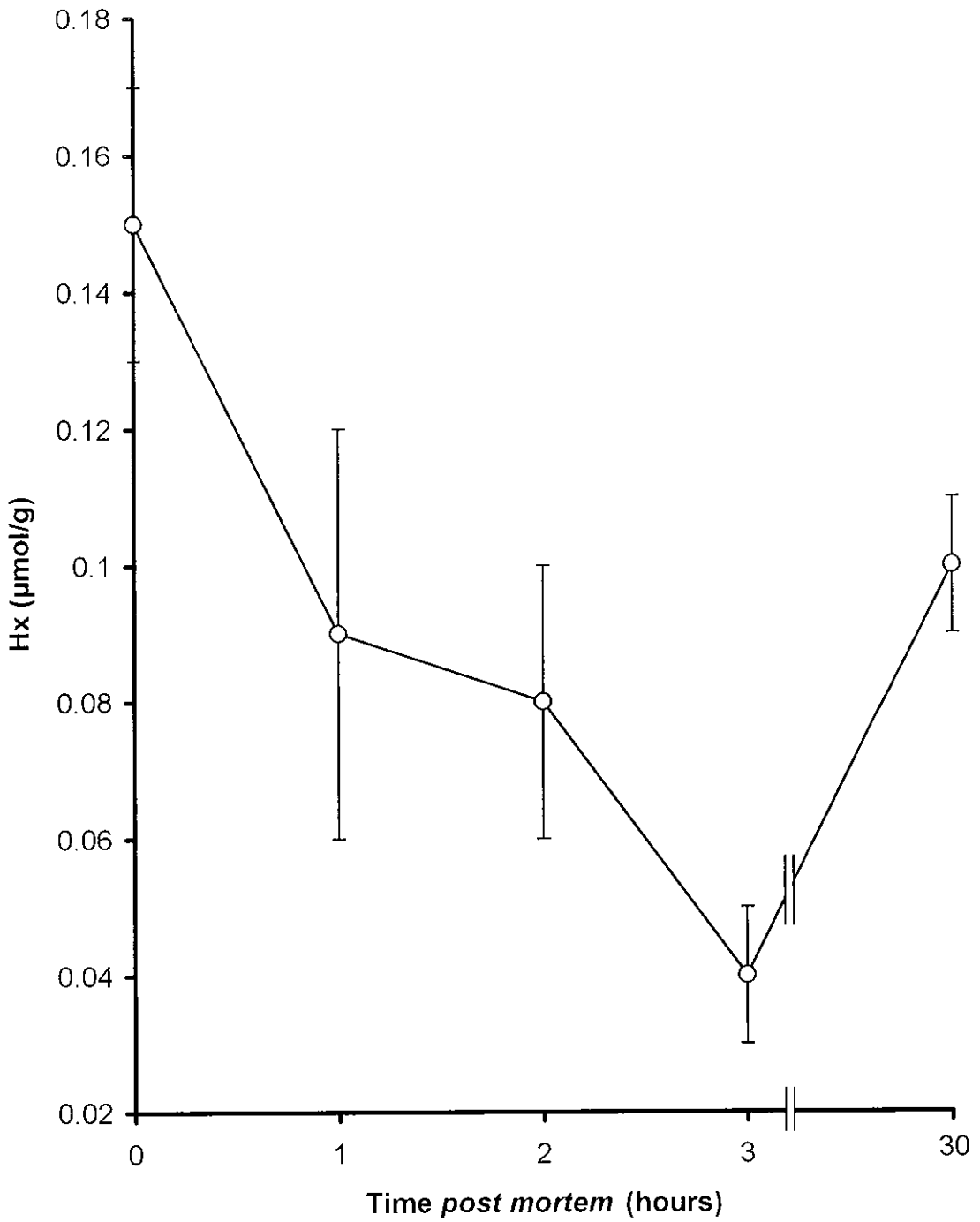


Fig. 17. Myotomal muscle of clubbed rainbow trout: changes in the concentration of Hx at 3°C.
Mean values ± s.e.m. for fifteen fish.

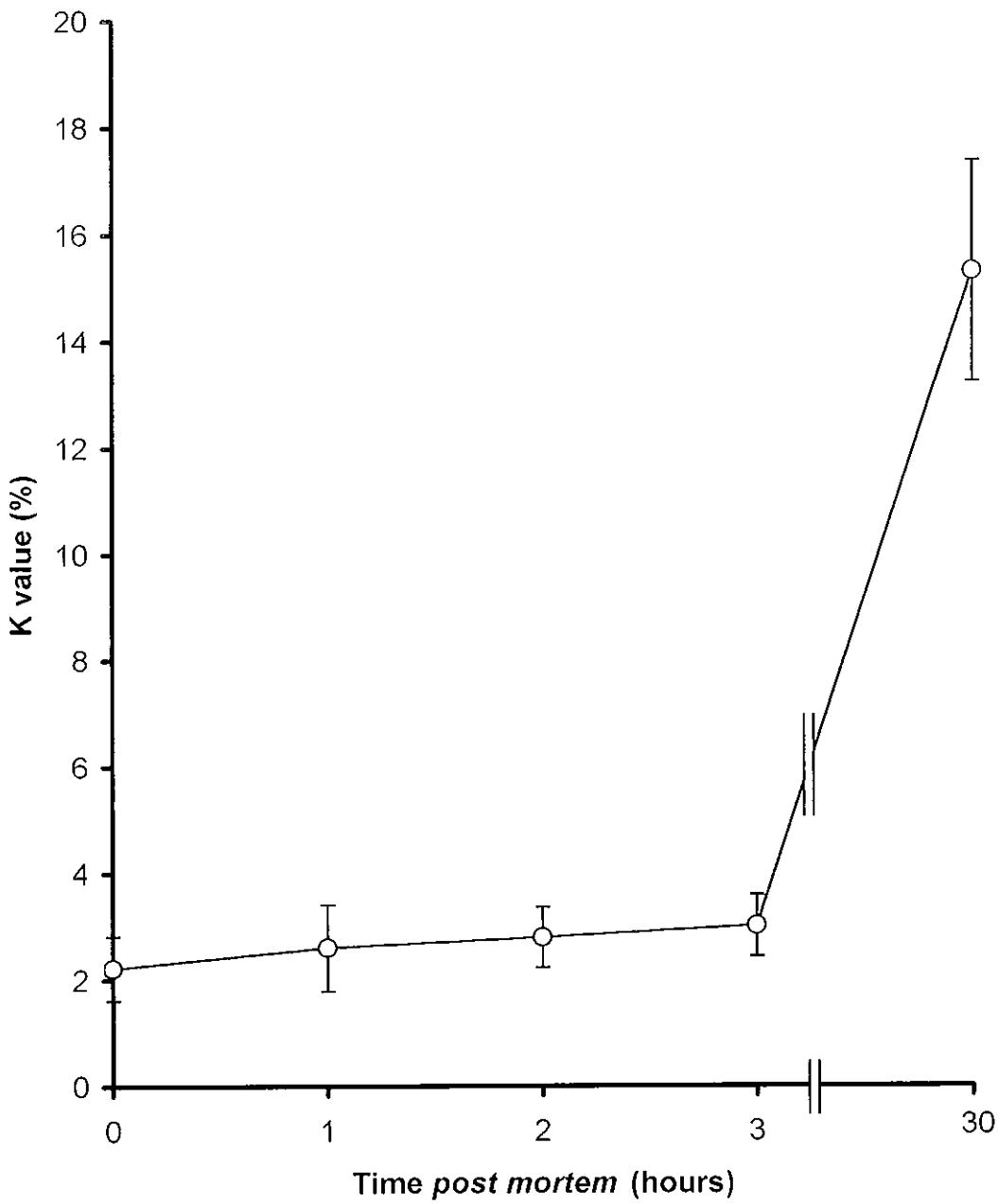


Fig. 18. Myotomal muscle of clubbed rainbow trout: changes in K value at 3°C.
Mean values ± s.e.m. for fifteen fish.

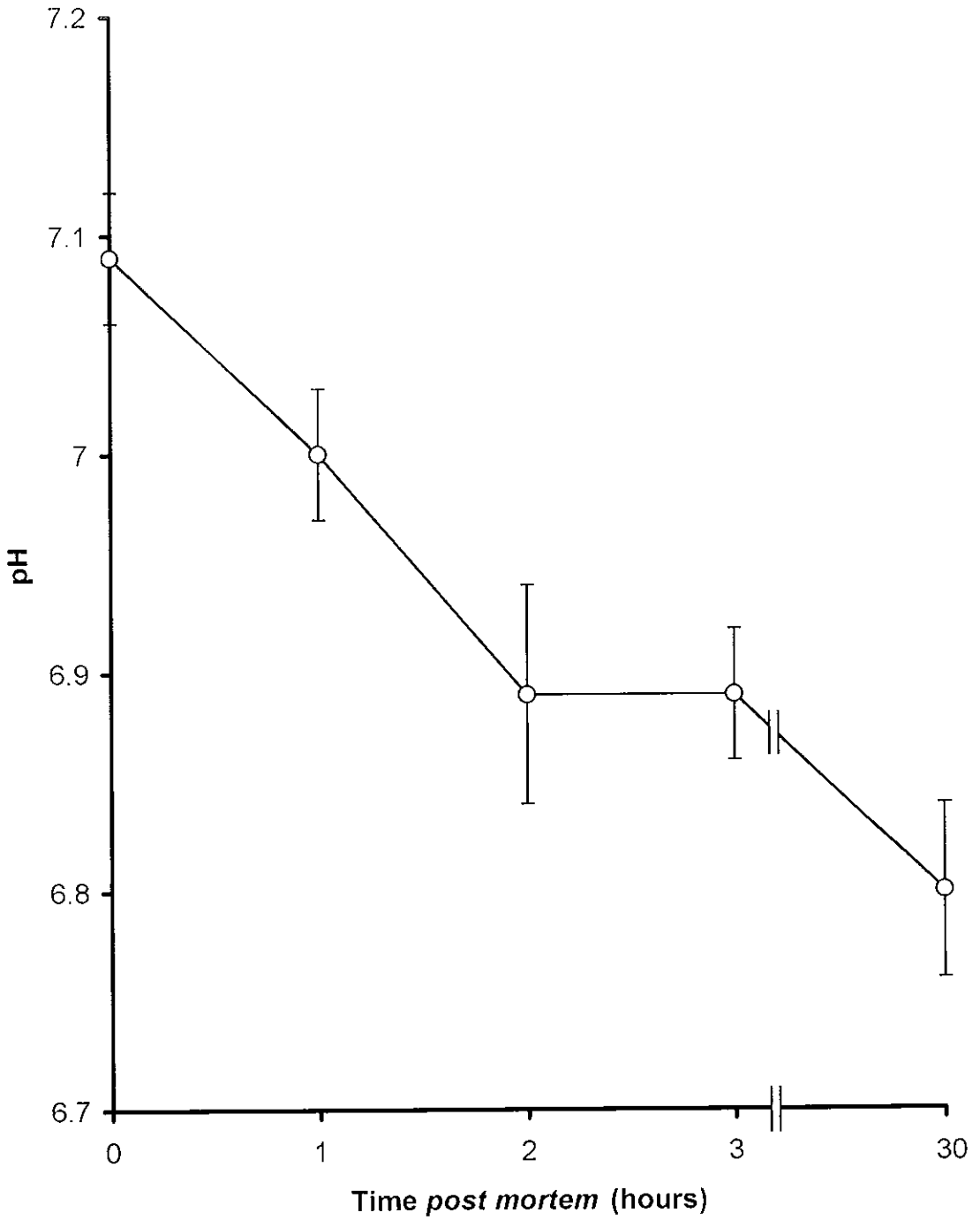


Fig. 19. Myotomal muscle of clubbed rainbow trout: changes in pH at 3°C. Mean values ± s.e.m. for fifteen fish.

Comment

The mean initial concentration of ATP in muscle samples taken from fifteen fish indicates that the fish were in a *pre rigor* state at time of sampling. There was no significant decrease in the concentration of ATP after storage on ice for three hours. The very low K values for each of the three groups of fish immediately *post mortem* and the pH values indicate that the fish did not struggle significantly during killing and probably were not subjected to any serious stressors before death. The K values did not increase significantly during the first three hours. However, by 30 hours *post mortem* the concentration of ATP had fallen to very low levels while the K value increased for each group.

3.1.5 A Comparison of Clubbed and Asphyxiated Fish

Rainbow trout (460g in weight and 33cm in length) were obtained from Annamoe Fish Farms, Co. Wicklow. Two groups were used in these experiments. The fish (5) in group 1 were clubbed and those (5) in group 2 were asphyxiated as described in Materials and Methods (section 2.2.2 and 2.2.3). Zero hour muscle samples were taken at the fish farm (section 2.4). The fish carcasses were packed into polystyrene boxes, layered with ice and transported to the laboratory as previously described. The fish were stored at 3°C. Sampling was carried at daily intervals up to 20 days *post mortem*.

3.1.5.1 ATP and Metabolites

The mean initial concentration of ATP was 0.58 ± 0.28μmol/g for the clubbed fish and 0.14 ± 0.07μmol/g for the asphyxiated. Changes in the concentration of ATP are shown in Fig. 20. The initial values for ATP in the asphyxiated fish were very low. These fish exhibited a strong death reaction (vigorous muscular activity at time of death) and a rapid onset of *rigor*. The initial concentration of ATP in the clubbed fish was considerably lower than anticipated (0.58 ± 0.28μmol/g) an observation which suggested that the fish may have been stressed *ante mortem* or had very low glycogen. The zero hour concentrations of ADP were 0.71 ± 0.06μmol/g for the clubbed fish and 0.57 ± 0.09μmol/g for the asphyxiated. The results are shown in Fig. 21. Concentrations of AMP for the clubbed and asphyxiated fish were very low (<0.04μmol/g. Fig. 22.). Changes in the concentration of IMP are shown in Fig. 23. The mean initial concentration of IMP was 10.00 ± 0.60μmol/g for the clubbed fish and 9.99 ± 0.50μmol/g for the asphyxiated. At day 1 the concentration of IMP was 8.55 ± 0.49μmol/g for the clubbed fish and 6.37 ± 0.77μmol/g for the asphyxiated.

The difference between these values was significant ($p < 0.01$). IMP continued to decrease with time of storage for the two groups of fish. At day 20 the concentrations of IMP were very low (< 0.05μmol/g) for the clubbed and asphyxiated fish. The mean zero hour concentrations of INO and Hx were very low (< 0.3μmol/g) for the two groups of fish (Fig. 24 and 26). The concentrations of INO and Hx increased with storage up to 13 days for the clubbed and asphyxiated fish. At 20 days there was a significant ($P < 0.01$) decrease in the concentration of INO for the two groups of fish. The concentrations of INO (1.61 ± 0.06μmol/g) and Hx (1.64 ± 0.19μmol/g) for the asphyxiated fish were almost the same at day 20 *post mortem*.

3.1.5.2 K value

The K values at zero time and during storage are shown in Fig. 26. The value for the asphyxiated (2.30 ± 0.70%) fish was significantly higher ($p< 0.01$) than that for the clubbed (0.50 ± 0.00%). Changes in K value during storage are shown in Fig. 25. At day 1 there was a significant ($p> 0.001$) difference in K value between clubbed (7.00 ± 1.90%) fish and asphyxiated (21.90 ± 1.90%). The K values increased up to day 13 for the two groups of fish but did not increase further by 20 days.

3.1.6 pH

The mean zero hour pH was 6.98 ± 0.06 for the clubbed fish and 6.46 ± 0.05 for the asphyxiated. The difference between these values was significant ($p< 0.001$). The pH time curves for the two groups of fish are shown in Fig. 27.

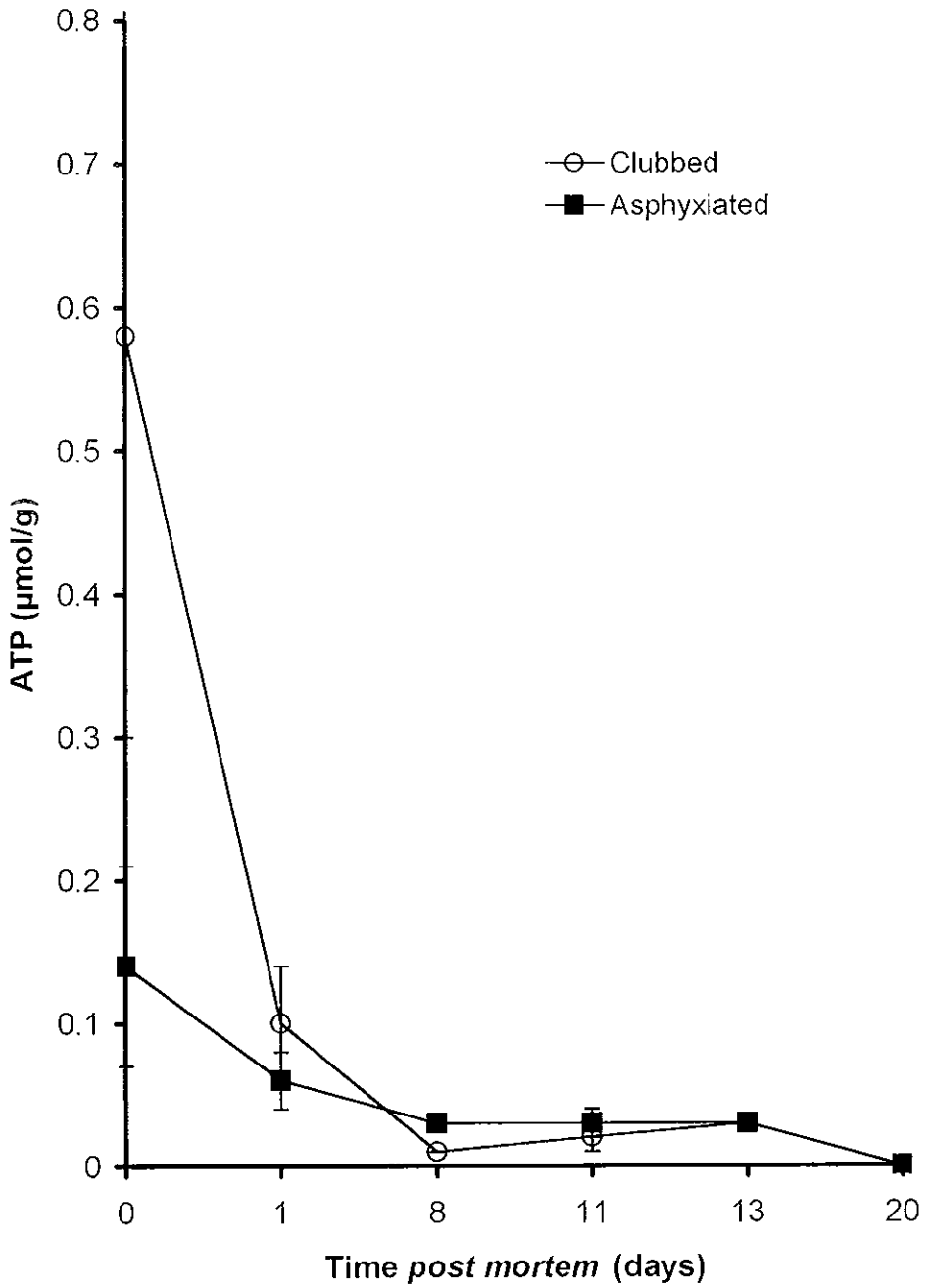


Fig. 20. Myotomal muscle of clubbed and asphyxiated rainbow trout: changes in the concentration of ATP at 3°C.
Mean values ± s.e.m. for groups of five fish.

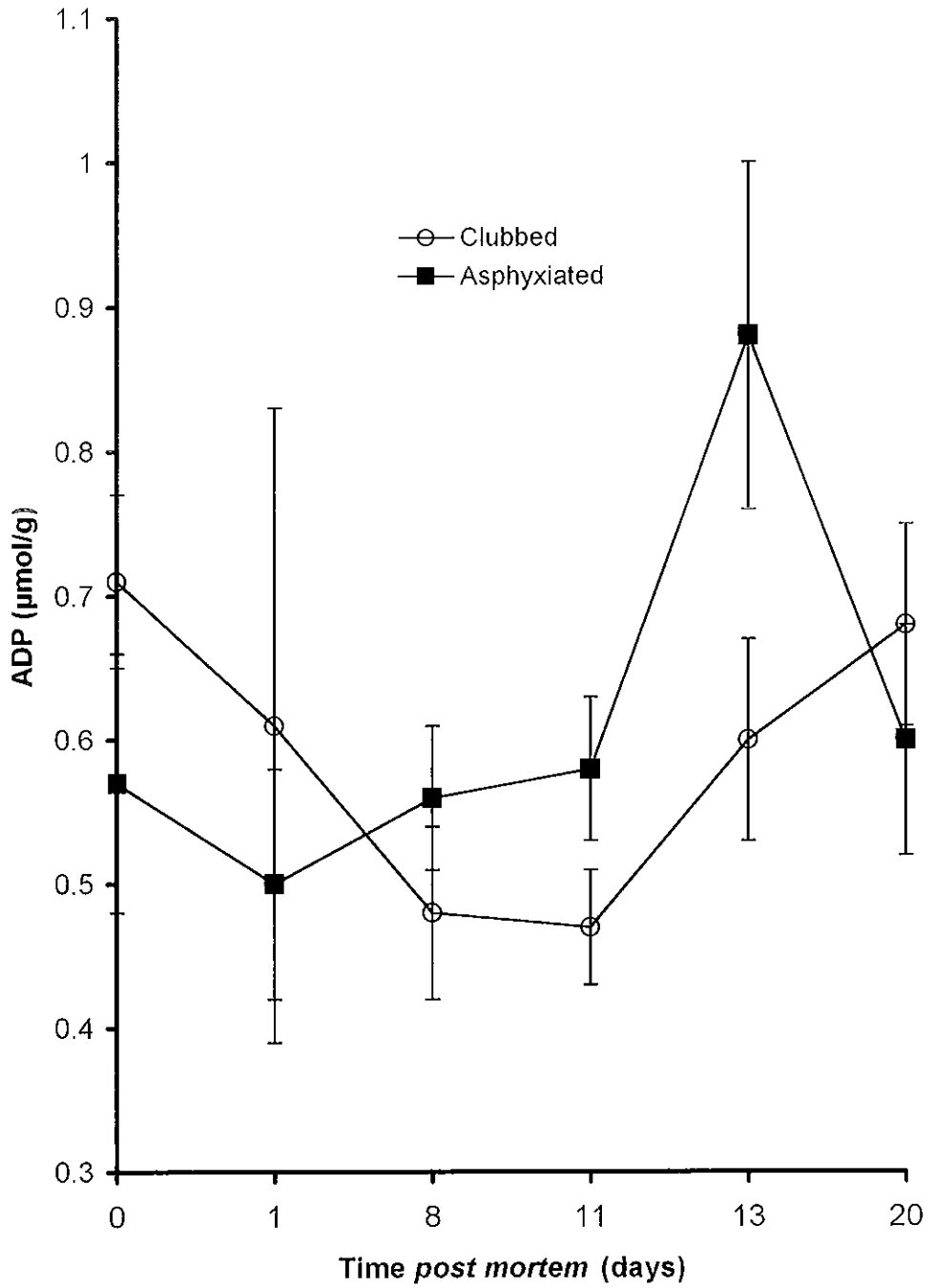


Fig. 21. Myotomal muscle of clubbed and asphyxiated rainbow trout: changes in the concentration of ADP at 3°C.
Mean values ± s.e.m. for groups of five fish.

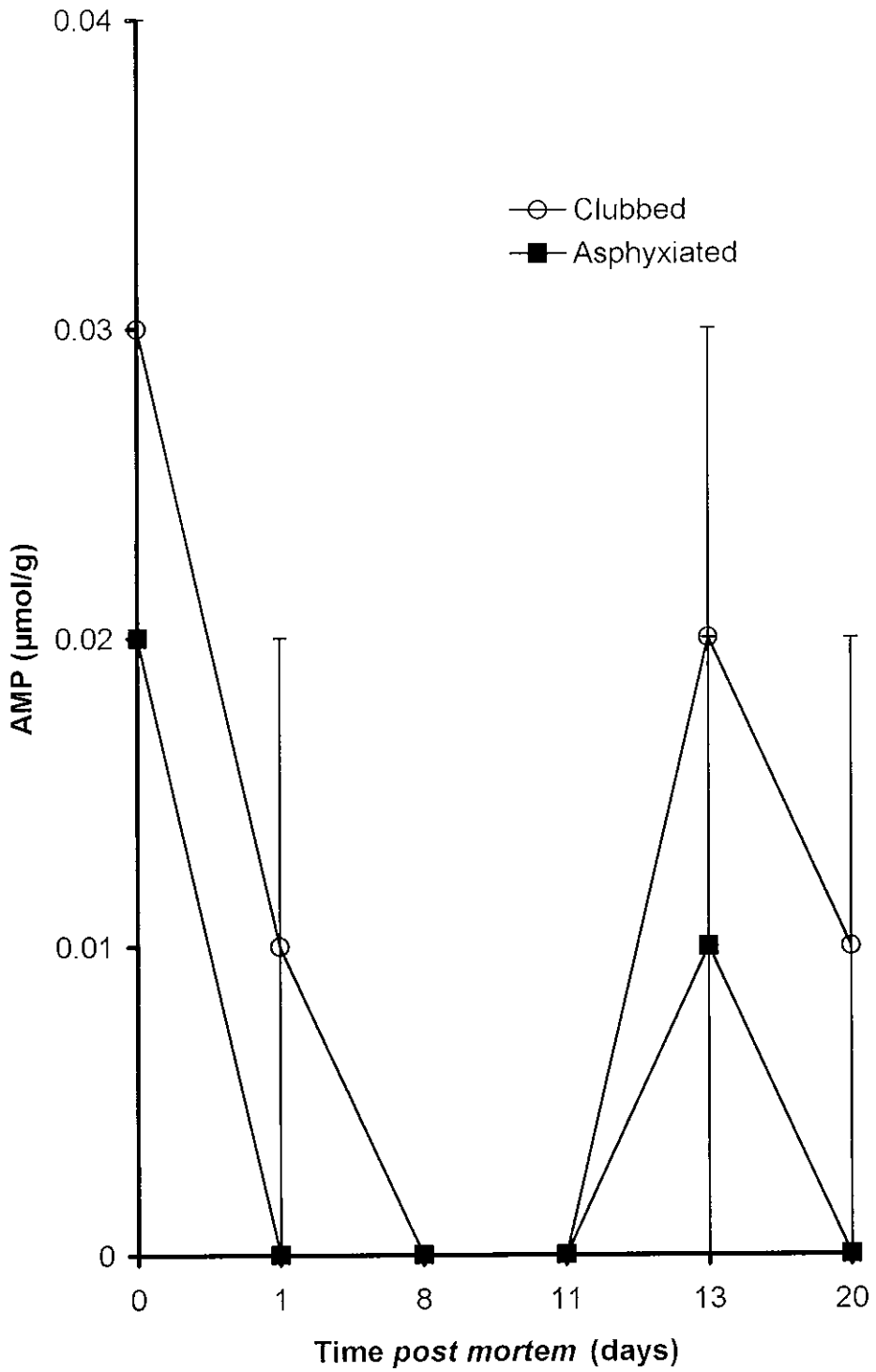


Fig. 22. Myotomal muscle of clubbed and asphyxiated rainbow trout: changes in the concentration of AMP at 3°C.
Mean values ± s.e.m. for groups of five fish.

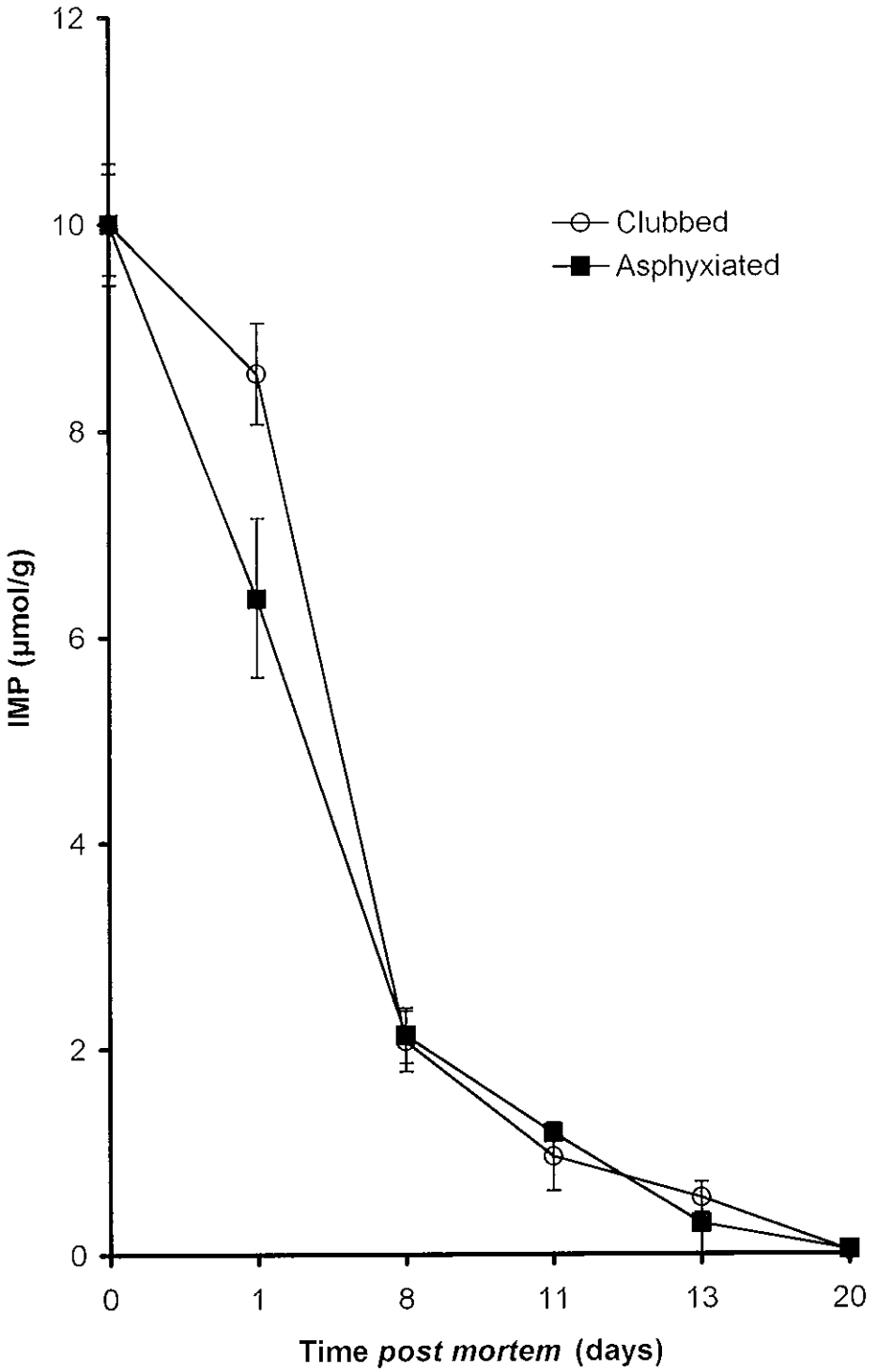


Fig. 23. Myotomal muscle of clubbed and asphyxiated rainbow trout: changes in the concentration of IMP at 3°C.
Mean values ± s.e.m. for groups of five fish.

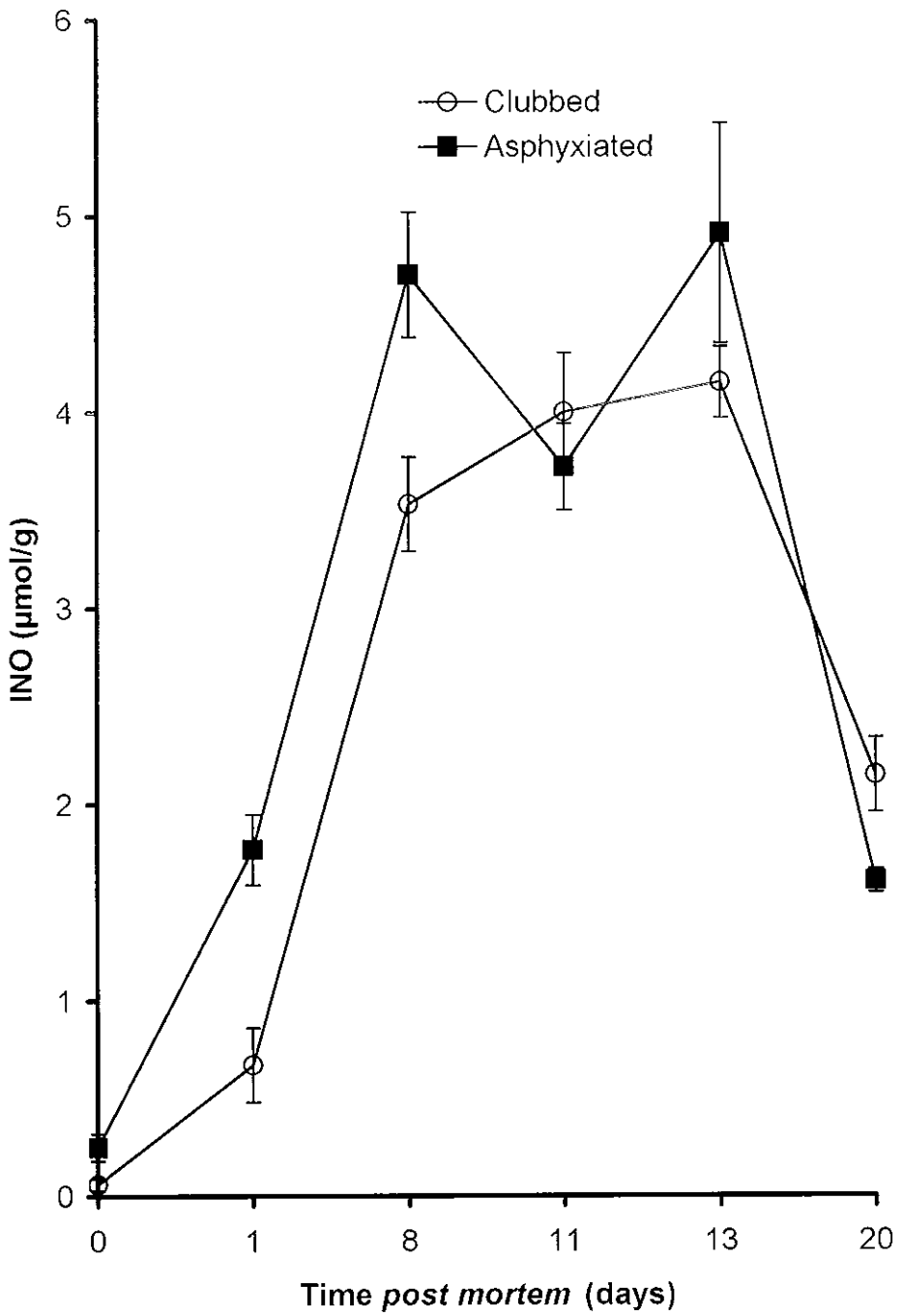


Fig. 24. Myotomal muscle of clubbed and asphyxiated rainbow trout: changes in the concentration of INO at 3°C.
Mean values ± s.e.m. for groups of five fish.

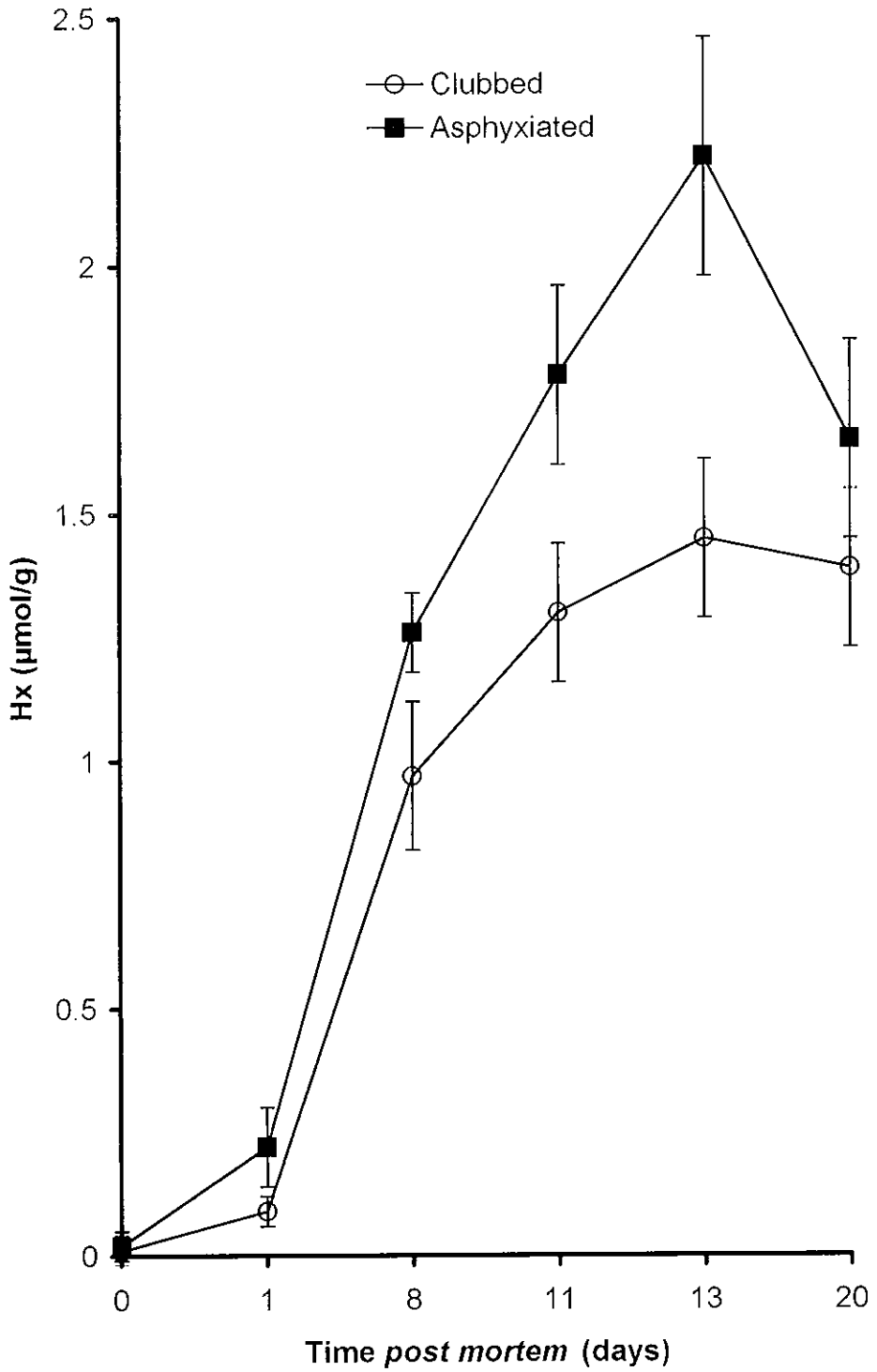


Fig. 25. Myotomal muscle of clubbed and asphyxiated rainbow trout: changes in the concentration of Hx at 3°C.
Mean values ± s.e.m. for groups of five fish.

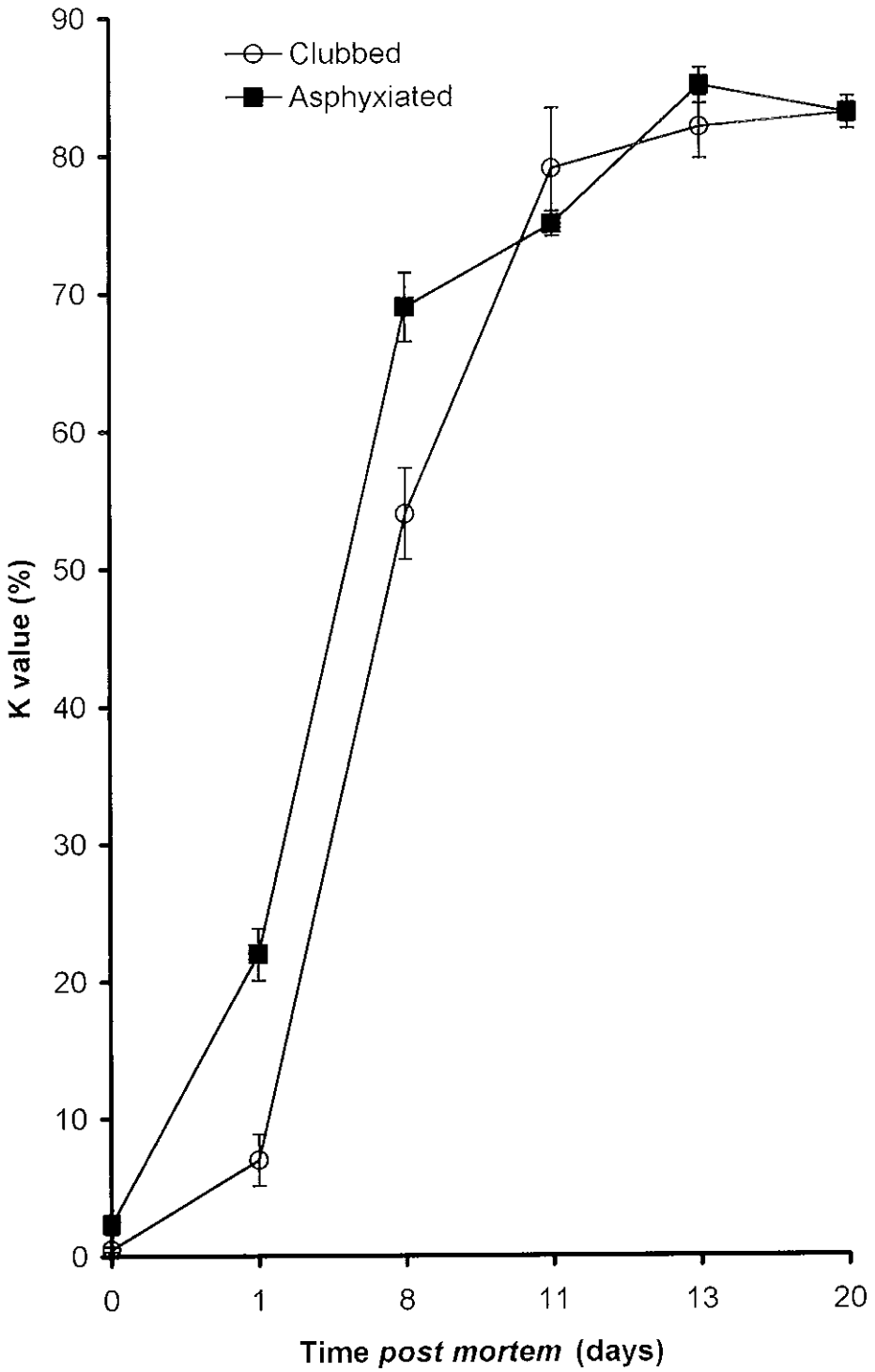


Fig. 26. Myotomal muscle of clubbed and asphyxiated rainbow trout: changes in the K value at 3°C.
Mean values ± s.e.m. for groups of five fish.

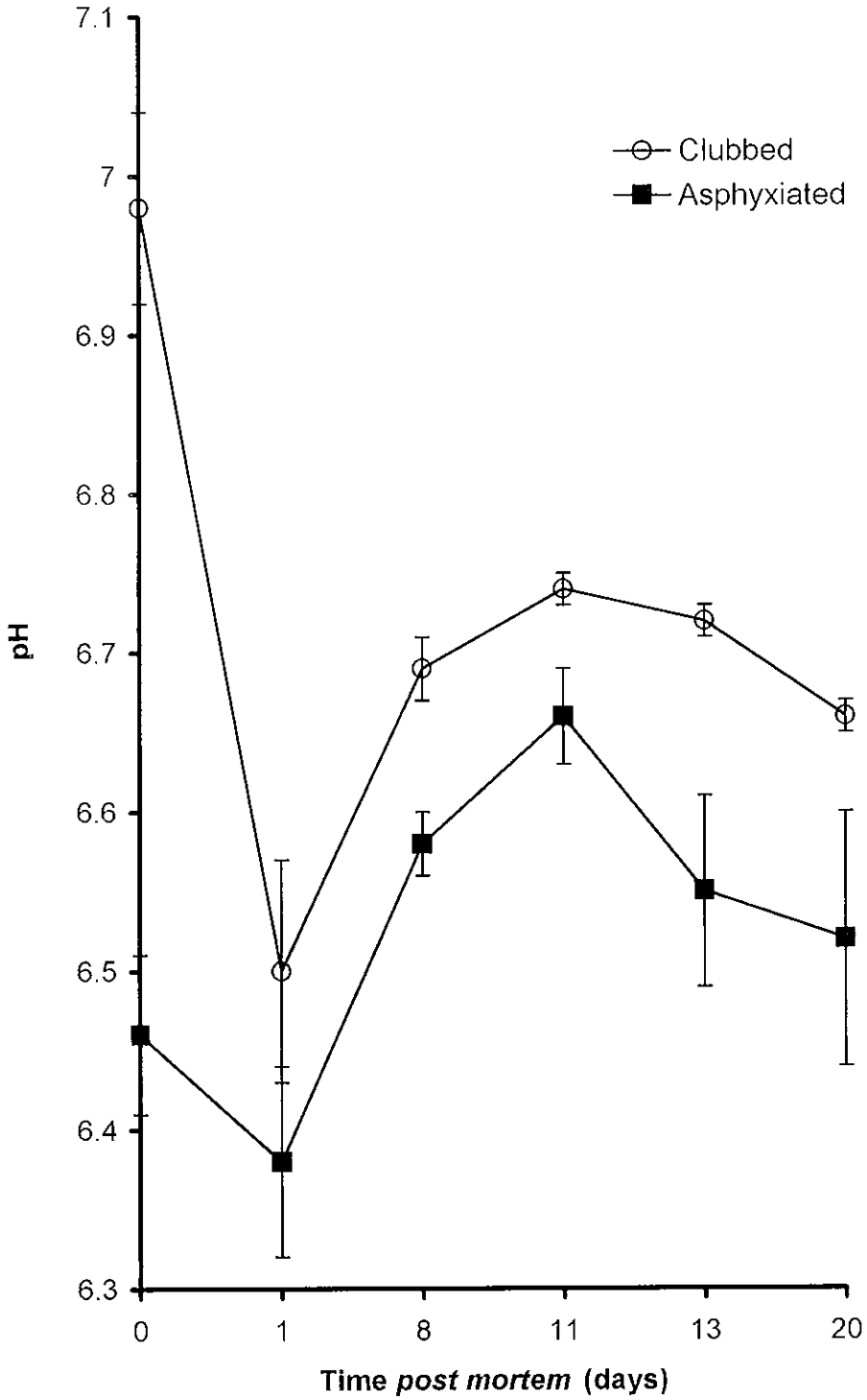


Fig. 27. Myotomal muscle of clubbed and asphyxiated rainbow trout: changes in pH at 3°C.
Mean values ± s.e.m. for groups of five fish.

Comment

The results of these experiments indicate that asphyxiation accelerated the onset of *rigor* compared with the effects of killing by clubbing. There was evidence of *rigor* in the asphyxiated fish immediately *post mortem*. The initial concentration of ATP for the clubbed fish was very low (0.58 ± 0.28μmol/g) and would suggest that the fish were going into *rigor*, although there was no evidence of *rigor* present in these fish immediately *post mortem*. At 1 hour *post mortem* there was slight *rigor* and the tissue was dry which would indicate that some degree of change in texture had taken place. The low initial concentration of ATP (0.58 ± 0.28μmol/g) would suggest that the fish may have had muscle contraction during killing. K values were significantly higher for the asphyxiated fish at the start of the experiment and during storage up to day 8. The K value continued to increase with time of storage for the two groups of fish up to day 13, although significant differences were not found between the clubbed and the asphyxiated fish. There was no further increase in K value for clubbed or asphyxiated fish at day 20.

3.1.7 Clubbed and Asphyxiated Fish Maintained at –30°C

Fish (490g in weight and 37cm in length) were obtained from Annamoe Fish Farms, Co. Wicklow. Four groups of rainbow trout were used. The fish (5) in groups 1 and 2 were clubbed and the fish (5) in groups 3 and 4 were asphyxiated as described in Materials and Methods (sections 2.2.2 and 2.2.3 respectively). The fish in groups 1 and 3 were sampled immediately after death at the fish farm (section 2.4) and the fish carcasses were packed into polystyrene boxes, layered with ice and transported to the laboratory where they were stored at -30°C. Sampling was carried out at intervals of 6, 12, 18 and 24 weeks. Zero hour muscle samples were taken from the fish in groups 2 and 4 and were put into liquid nitrogen. Further muscle samples were taken from the fish carcasses, put into pre-labelled freezer bags in ice and transported to the laboratory. They were stored at -30°C and sampled at intervals.

3.1.7.1 ATP and Metabolites

The concentrations of ATP are shown in Fig. 28. The mean zero hour concentration of ATP for the two groups of clubbed fish were 4.32 ± 0.83μmol/g and 3.31 ± 0.77μmol/g. The mean concentrations for the two groups of asphyxiated fish were 0.32 ± 0.31μmol/g and 0.14 ± 0.09μmol/g. The asphyxiated fish in group 3 exhibited a strong death reaction compared with the fish in group 4. These fish struggled for about 5 minutes following removal from the water, then became quite still. The concentrations of ATP in the four groups were close to zero at 6 weeks. The zero hour concentrations of ADP were 0.83 ± 0.04μmol/g and 1.00 ± 0.04μmol/g for the two groups of clubbed fish and 0.46 ± 0.16μmol/g and 0.70 ± 0.08μmol/g for the two groups of asphyxiated fish (Fig. 29). Concentrations of AMP for the two groups of clubbed fish were 0.10 ± 0.03μmol/g and 1.00 ± 0.01μmol/g. The difference between

these values was significant ($p > 0.001$). The zero hour concentrations of AMP for the two groups of asphyxiated fish were 0.14 ± 0.02μmol/g and 0.06 ± 0.03μmol/g (Fig. 30). The mean initial concentrations of IMP were 4.08 ± 1.15μmol/g and 5.25 ± 0.69μmol/g for the two groups of clubbed fish, and 8.37 ± 0.80μmol/g and 9.05 ± 0.56μmol/g for the asphyxiated. Changes in the concentration of IMP during storage are shown in Fig. 31. At 6 weeks there was a significant (p 0.001) increase in the concentration of IMP for the two groups of clubbed fish to 10.46 ± 0.53μmol/g and 9.91 ± 0.42μmol/g respectively. At 18 weeks there was a significant ($p<0.01$) decrease in IMP levels with the exception of the excised muscle taken from the asphyxiated fish which increased then marginally declined. At 24 weeks the concentration of IMP for the two groups of clubbed fish increased significantly ($p < 0.05$). The zero hour concentrations of INO and Hx were very low (<0.6μmol/g) for the four groups. Changes in the concentrations of INO and Hx are shown in Fig. 32 and 33. At 24 weeks the concentrations of INO were 0.54 ± 0.09μmol/g and 0.67 ± 0.08μmol/g for the two groups of clubbed fish and 1.80 ± 0.14μmol/g and 0.59 ± 0.09μmol/g for the asphyxiated fish.

3.1.7.2 K value

Changes in K value during storage are shown in Fig. 34. The mean initial K values were 1.91 ± 1.16% and 1.93 ± 1.06% for the two groups of clubbed fish and 3.74 ± 0.34% and 5.01 ± 1.08% for the asphyxiated. At 6 weeks there was a significant ($p<0.001$) increase in K value for the excised muscle taken from the asphyxiated fish. Muscle samples taken from these fish were significantly The K value increased up to 12 weeks storage then marginally declined.

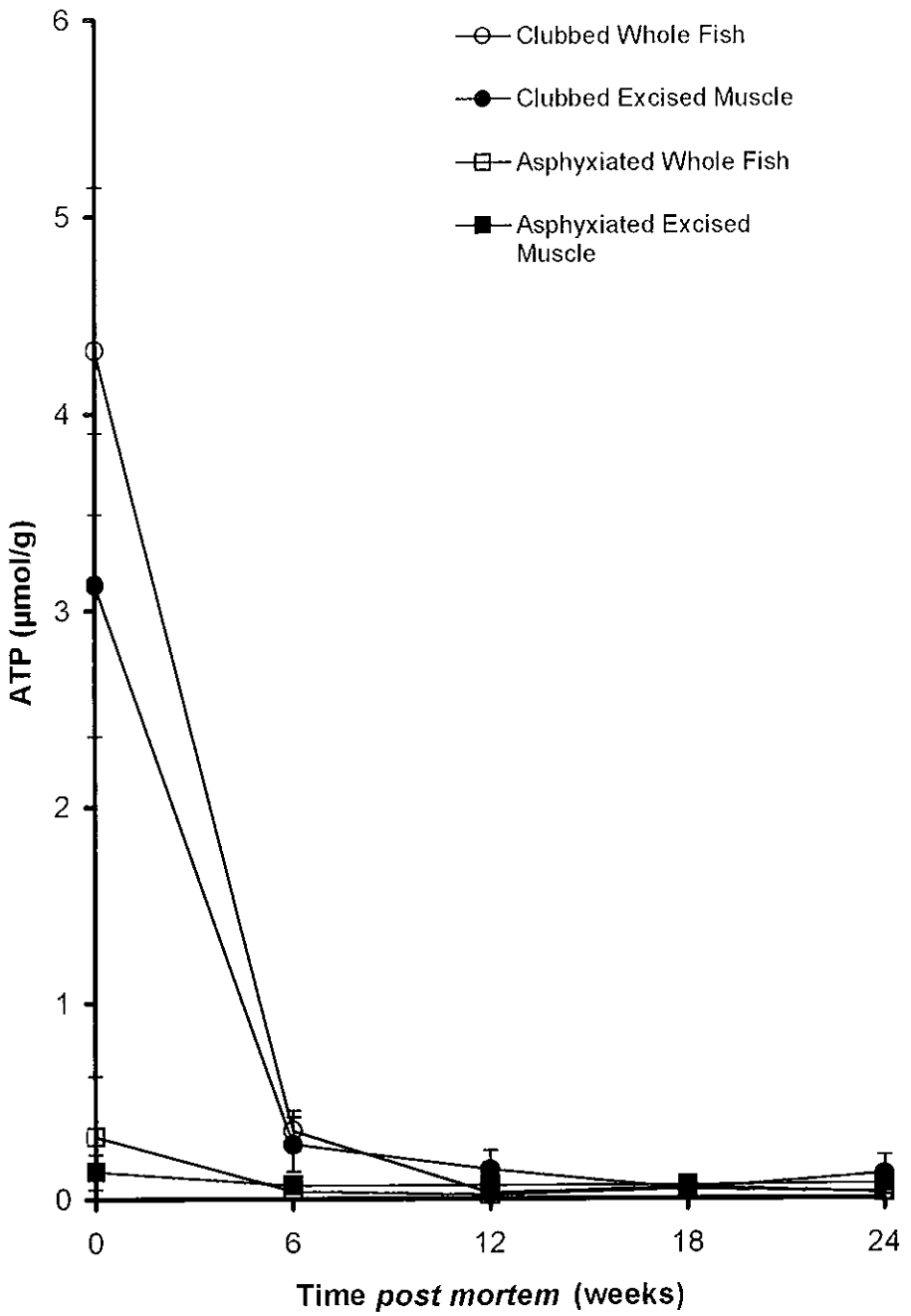


Fig. 28. Myotomal muscle of clubbed and asphyxiated rainbow trout: changes in the concentration of ATP at –30°C. Mean values ± s.e.m. for groups of five fish.

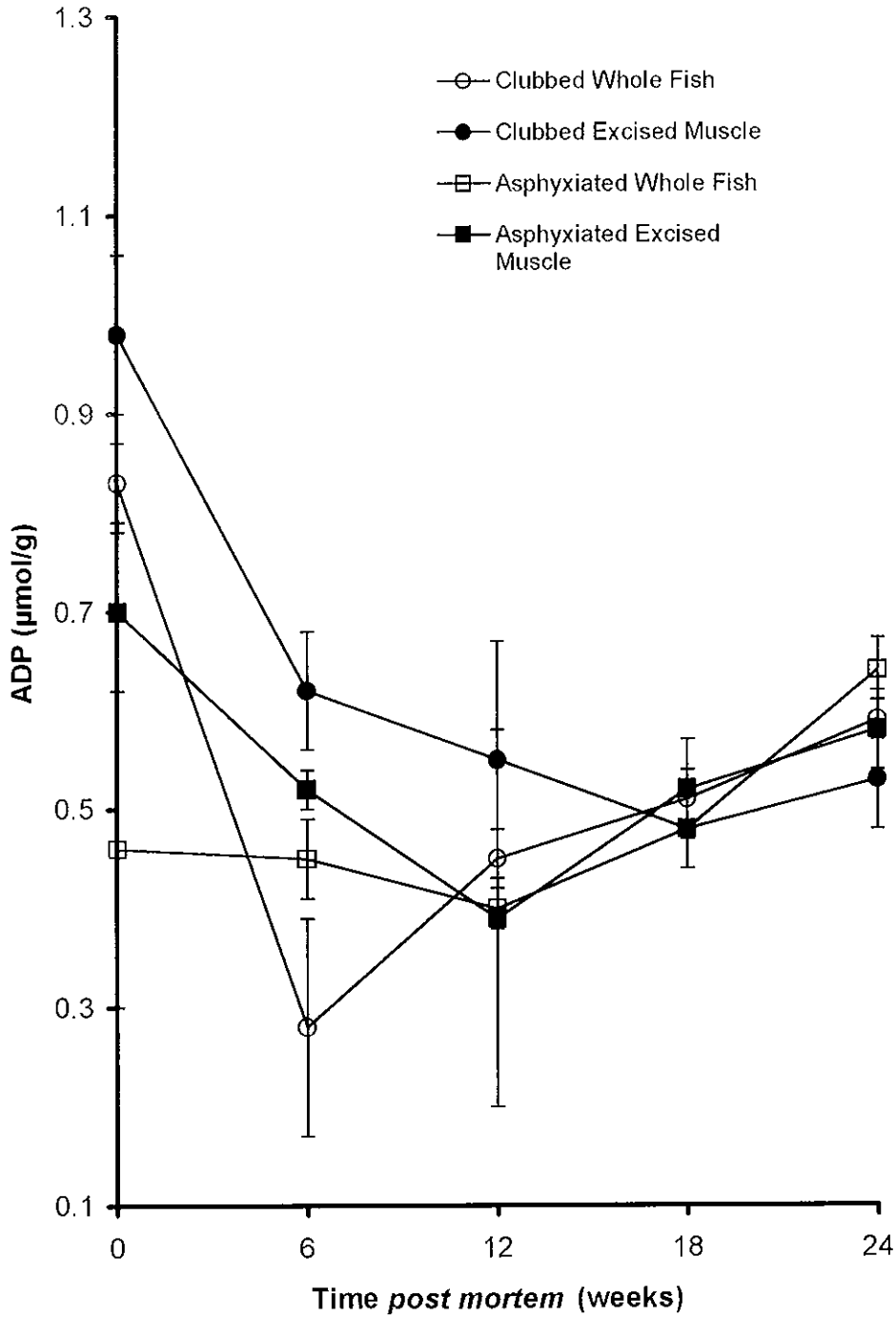


Fig. 29. Myotomal muscle of clubbed and asphyxiated rainbow trout: changes in the concentration of ADP at –30°C.
Mean values ± s.e.m. for groups of five fish.

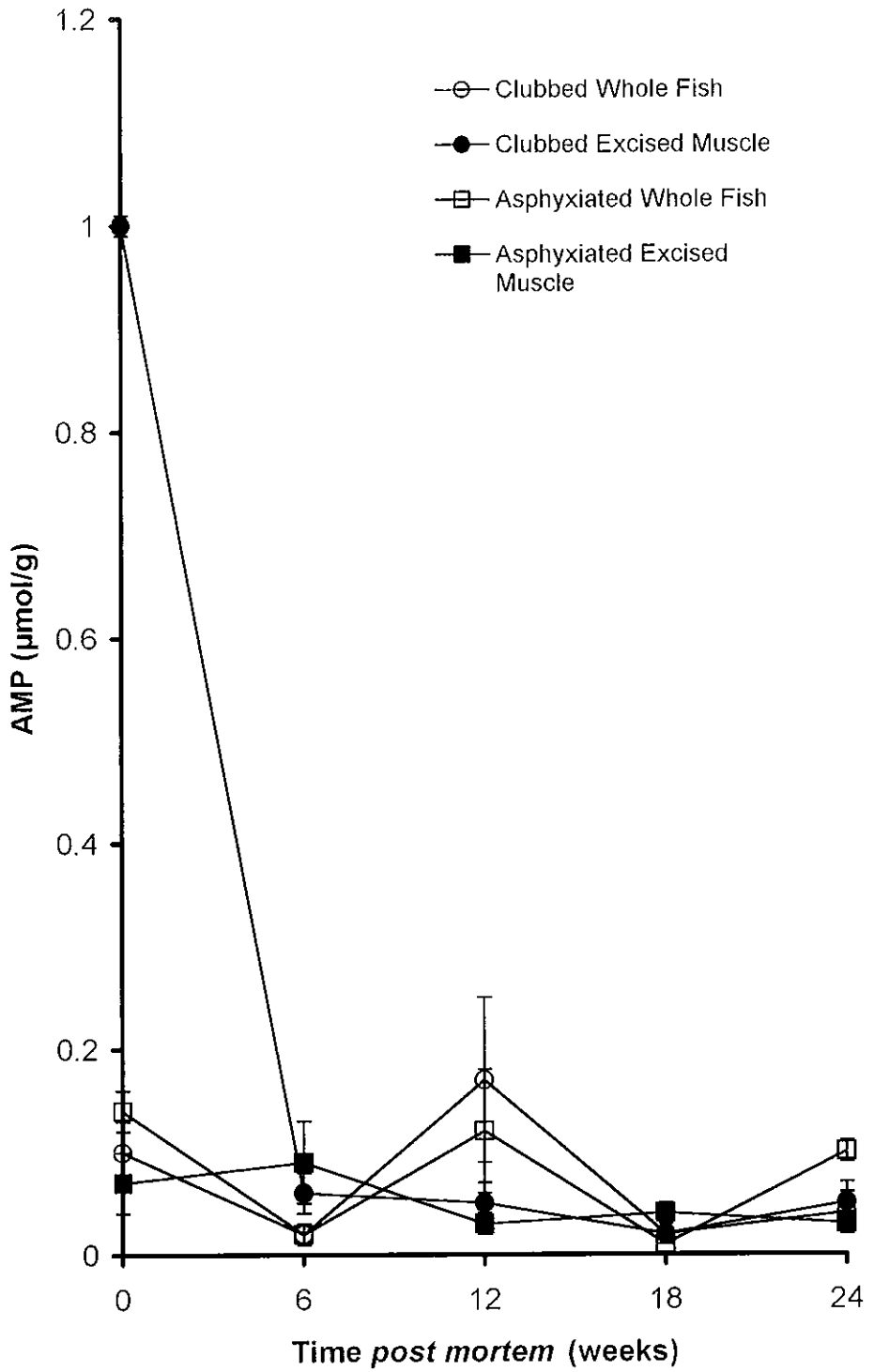


Fig. 30. Myotomal muscle of clubbed and asphyxiated rainbow trout: changes in the concentration of AMP at -30°C. Mean values ± s.e.m. for groups of five fish.

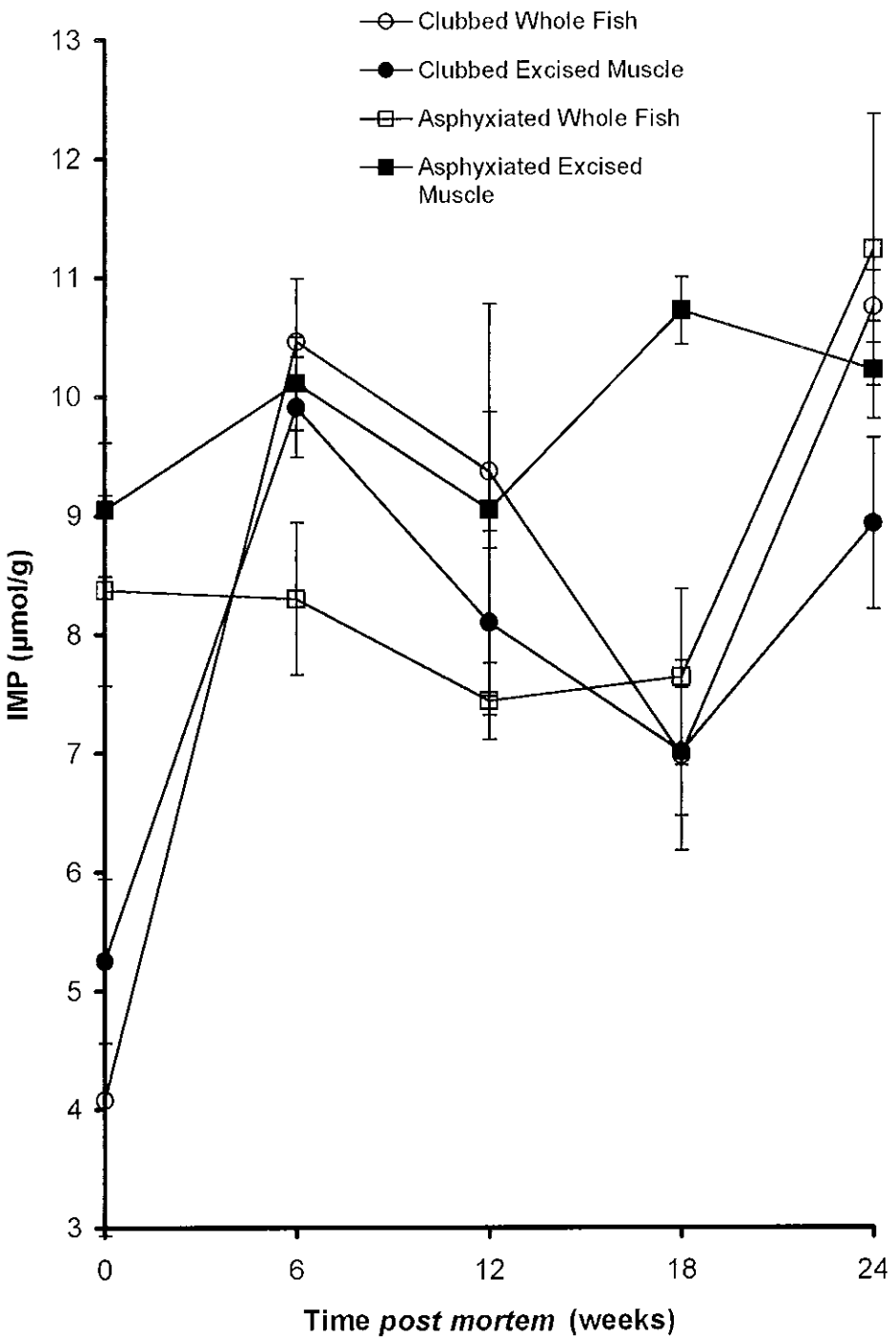


Fig. 31. Myotomal muscle of clubbed and asphyxiated rainbow trout: changes in the concentration of IMP at –30°C.
Mean values ± s.e.m. for groups of five fish.

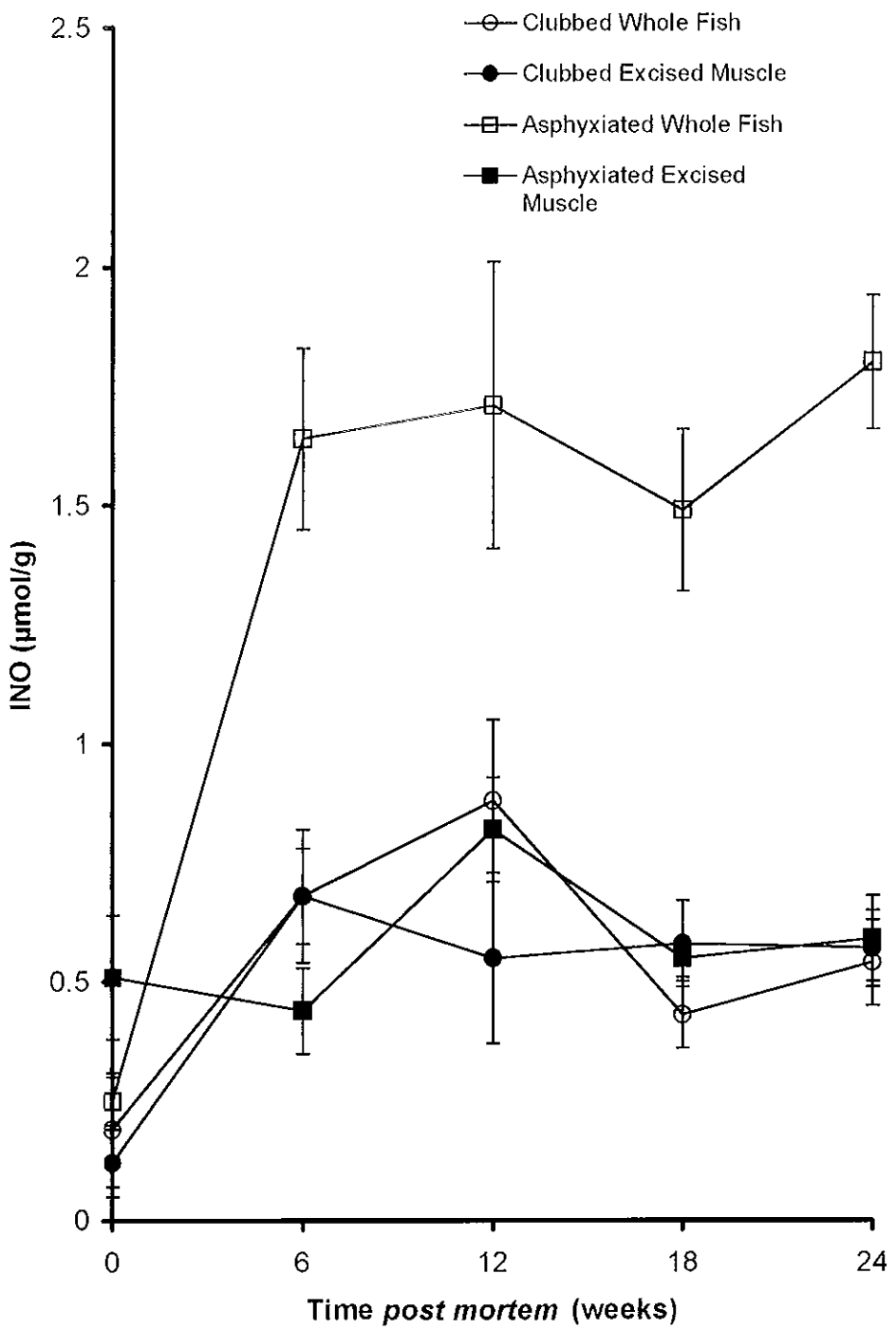


Fig. 32. Myotomal muscle of clubbed and asphyxiated rainbow trout: changes in the concentration of INO at –30°C. Mean values ± s.e.m. for groups of five fish.

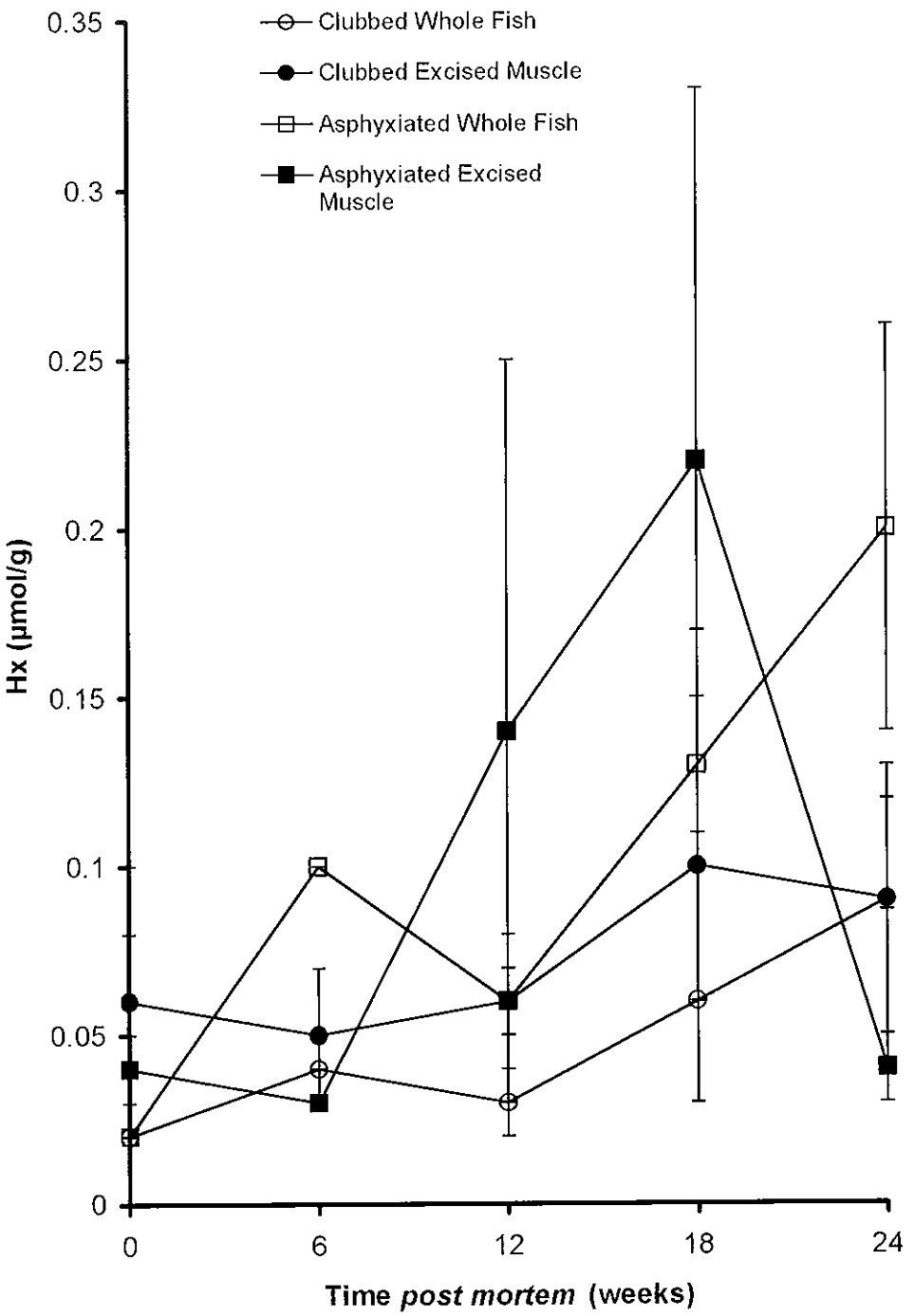


Fig. 33. Myotomal muscle of clubbed and asphyxiated rainbow trout: changes in the concentration of Hx at –30°C.
Mean values ± s.e.m. for groups of five fish.

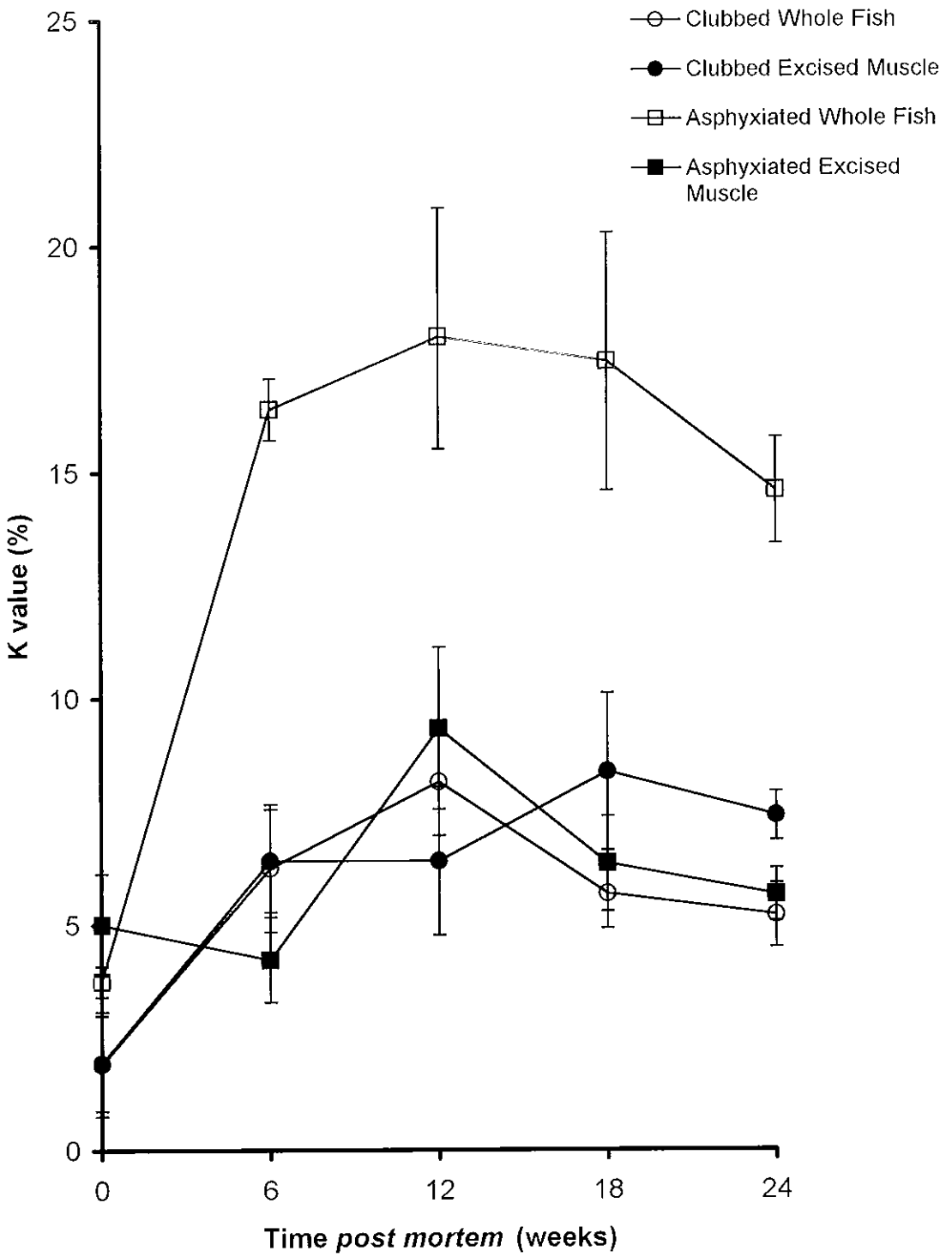


Fig. 34. Myotomal muscle of clubbed and asphyxiated rainbow trout: changes in the K value at –30°C.
Mean values ± s.e.m. for groups of five fish.

Comment

The results of these experiments showed that concentrations of IMP for the four groups of fish stored at -30°C for 24 weeks were at values comparable with those reported for muscle which had been stored on ice for 24 hours *post mortem* (Fig. 23). The results showed that the K value for the asphyxiated fish was significantly lower when muscle had been removed from the bone than when the procedure had not been carried out.

B. SALMON

3.1.8 Adenine Nucleotides and pH in Salmon at 3°C

Salmon (3kg to 5kg in weight and 60cm to70cm in length) were supplied by Kilkerrin Hatcheries, Connemara, Co. Galway. The fish had been starved for five days before stunning using CO_2 gas (section 2.2.1). Four grades of salmon were used namely, Superior Grade, Superior-S Grade, Ordinary Grade and Production Grade (section 2.1). The fish reached the laboratory at about 30 hours *post mortem* and were stored at 3°C. Sampling was carried out at 30 hours *post mortem* and at seven days storage.

3.1.8.1 ATP and Metabolites

The results are given in Table 12. The mean values for the concentrations of ATP at 30 hours *post mortem* were in the range of 0.04 ± 0.00μmol/g to 0.40 ± 0.22μmol/g. The highest concentration of ATP was found in the Superior-S round fish (0.40 ± 0.22μmol/g) and the lowest value was found in the Production grade fish (0.04 ± 0.00μmol/g). At day 7 the concentrations of ATP were < 0.3μmol/g for all the fish. The individual concentrations of ATP and its metabolites are given in Table 13. The mean 30 hour values for the concentrations of ADP ranged from 0.41 ± 0.02μmol/g (Production grade gutted fish) to 1.05 ± 0.15μmol/g (Superior grade gutted fish). At 7 days storage the highest concentration of ADP (0.91 ± 0.06μmol/g) was found in the Superior grade round fish and the lowest concentration (0.40 ± 0.04μmol/g) in the Production grade gutted fish. AMP was not detected in any fish at 30 hours *post mortem.* At day 7 the concentration of AMP was 0.05 ± 0.03μmol/g for the Superior round fish only. The mean concentrations of IMP at 30 hours *post mortem* were in the range of 1.12 ± 0.12μmol/g to 5.21 ± 0.64μmol/g. The highest concentration (5.21 ± 0.64μmol/g) was found in the Superior-S round fish and the lowest (1.12 ±

0.12μmol/g) in the Production grade gutted fish. The difference between these values was significant ($p > 0.001$). At 7 days storage the concentration of IMP for Superior-S round fish was 3.01 ± 0.91μmol/g while the concentration of IMP for the Production grade gutted fish was 0.17 ± 0.05μmol/g. The difference between these values was significant ($p< 0.01$). The highest concentration of INO (2.53 ± 0.26μmol/g) was found in the Superior round fish at 30 hours *post mortem* and the lowest 1.46 ± 0.25μmol/g) in the Ordinary round fish. At 7 days there was a significant ($p> 0.05$) increase in the concentration of INO (3.66 ± 0.24μmol/g) for the Superior round fish. There was also a significant ($p < 0.01$) increase in the concentration of INO (2.92 ± 0.54μmol/g) for the Ordinary round fish. The concentrations of Hx were in the range of 0.00μmol/g to 1.80 ± 1.01μmol/g at 30 hours *post mortem.* The highest concentration was found in the Ordinary grade gutted fish and the lowest in the Ordinary grade round fish. At 7 days the highest concentrations of Hx (1.94 ± 0.64μmol/g) were found in the Superior round and gutted fish and the lowest (0.76 ± 0.28μmol/g) in the Production grade gutted fish.

3.1.8.2 K value

The mean K values at 30 hours *post mortem* ranged between 25.36 ± 6.27% and 54.08 ± 2.80 %. The individual K values at 30 hours *post mortem* and at 7 days storage are given in Table 14. The highest K value was found in the Production Grade gutted fish and the lowest in the Ordinary Grade round fish. The K values increased significantly ($p < 0.05$) at 7 days for all grades of fish.

3.1.9 pH

The pH values for all fish at 30 hours *post mortem* and at 7 days storage at 3°C are given in Table 15.

Grade	ATP (μmol/g)	ADP (μmol/g)	AMP (μmol/g)	IMP (μmol/g)	INO (μmol/g)	Hx (μmol/g)
Superior						
Round	0.09	0.77	< 0.01.	4.20	2.53	1.06
	± 0.04	± 0.13		± 0.35	± 0.26	± 0.33
Gutted	0.06	1.05	< 0.01	5.00	2.46	1.10
	± 0.02	± 0.15		± 0.66	± 0.17	± 0.43
Superior						
S-Round	0.40	0.90	< 0.01	5.21	2.50	1.40
	± 0.22	± 0.06		± 0.64	± 0.42	± 0.40
S-Gutted	0.12	0.64	< 0.01	4.52	1.46	1.14
	± 0.04	± 0.47		± 0.80	± 0.26	± 0.91
Ordinary						
Round	0.10	0.52	< 0.01	3.87	1.46	<0.01
	± 0.01	± 0.02		± 0.89	± 0.25	
Gutted	0.14	0.54	< 0.01	3.47	1.72	1.89
	± 0.02	± 0.11		± 0.25	± 0.01	± 1.01
Production						
Round	0.09	0.62	< 0.01	2.40	1.62	0.30
	± 0.00	± 0.09		± 0.78	± 0.36	± 0.10
Gutted	0.04	0.41	< 0.01	1.12	1.57	0.27
	± 0.00	± 0.02		± 0.12	± 0.04	± 0.00

Table 12. Concentrations of ATP and metabolites (at 30 hours *post mortem*) in myotomal muscle of salmon (stunned with CO_2 before bleeding). Mean values ± s.e.m. for groups of three fish.

Grade	ATP (μmol/g)	ADP (μmol/g)	AMP (μmol/g)	IMP (μmol/g)	INO (μmol/g)	Hx (μmol/g)
Superior						
Round	0.29	0.91	0.05	2.31	3.66	1.94
	± 0.06	± 0.06	± 0.03	± 043	± 0.24	± 0.64
Gutted	0.14	0.73	< 0.01	2.39	3.22	1.94
	± 0.04	± 0.67		± 0.19	± 0.07	± 0.44
Superior						
S-Round	0.23	0.60	< 0.01	3.01	2.52	1.63
	± 0.07	± 0.09		± 0.91	± 0.15	± 0.38
S-Gutted	0.18	0.47	< 0.01	1.96	2.34	1.09
	± 0.02	± 0.06		± 0.24	± 0.33	± 0.59
Ordinary						
Round	0.20	0.46	< 0.01	1.88	2.92	1.11
	± 0.05	± 0.05		± 0.48	± 0.54	± 0.24
Gutted	0.13	0.57	< 0.01	1.71	2.74	0.79
	± 0.00	± 0.00		± 0.23	± 0.13	± 1.12
Production						
Round	0.02	0.45	< 0.01	0.22	2.43	0.87
	± 0.01	± 0.03		± 0.04	± 0.28	± 0.03
Gutted	0.05	0.40	< 0.01	0.17	2.35	0.76
	± 0.02	± 0.04		± 0.05	± 0.02	± 0.28

Table 13. Concentrations of ATP and metabolites (at 7 days *post mortem*) in myotomal muscle of salmon (stunned with CO_2 before bleeding) Mean values ± s.e.m. for groups of three fish.

Grade		K value (%) 30 hours *post mortem*	K value (%) 7 days *post mortem*
Superior	Round	40 50 ± 3.96	55.41 ± 4.66
	Gutted	36.50 ± 2.47	54.53 ± 2.57
Superior-S	Round	37.20 ± 1.72	46.02 ± 4.74
	Gutted	31.60 ± 4.38	50.79 ± 6.19
Ordinary	Round	25.36 ± 6.27	61 31 ± 5.58
	Gutted	37.10 ± 8.58	59.53 ± 3.82
Production	Round	39.63 ± 9.83	82.14 ± 2.46
	Gutted	54 09 ± 2.81	84.00 ± 2.14

Table 14. K values (30 hours and 7 days *post mortem*) for myotomal muscle of salmon (stunned with CO_2 before bleeding).
Mean values ± for groups of three fish.

Grade		pH 30 hours *post mortem*	pH 7 days *post mortem*
Superior	Round	6.40 ± 0.02	6.41 ± 0.64
	Gutted	6.42 ± 0.02	6.41 ± 0.02
Superior-S	Round	6.45 ± 0.01	6.38 ± 0.03
	S-Gutted	6.48 ± 0.03	6.44 ± 0.02
Ordinary	Round	6.46 ± 0.04	6.47 ± 0.0
	Gutted	6.47 ± 0.03	6.47 ± 0.01
Production	Round	6.48 ± 0.07	6.46 ± 0.03
	Gutted	6.47 ± 0.06	6.46 ± 0.03

Table 15. Values for pH (30 hours and 7 days *post mortem*) for myotomal muscle of salmon (stunned with CO_2 before bleeding) Mean values ± s.e.m. for groups of three fish.

Comment

The concentration of ATP (< 0.5µmol/g) at 30 hours *post mortem* was very low for all grades of fish. More than 50% of the fish were considered to be in partial *rigor* on arrival at the laboratory at 30 hours *post mortem*. The K values at 30 hours *post mortem* ranged from 25.36 ± 6.27% for the Ordinary Grade round fish to 54.08 ± 2.80% for the Production Grade gutted fish. At 7 days storage the K value was 46.02 ± 4.74% for the Superior-S round fish and 84.00 ± 2.14% for both round and gutted specimens of the Production Grade fish.

C. *GOLDFISH*

3.1.10 Concentrations of Adenine Nucleotides and Metabolites

Five goldfish were anaesthetised with MS-222, killed and sampled as described in Materials and Methods (sections 2.2.4 and 2.4) and the concentrations of ATP, ADP, AMP, IMP, INO and Hx determined. Two zero hour muscle samples were taken immediately *post mortem* from three fish. The muscle samples were difficult to dissect due to the tough nature of the skin. The first sample was extracted immediately on removal (section 2.4), the second sample was put into liquid nitrogen, stored at -30°C for five days and then extracted.

1.1.10.1. ATP and Metabolites

The results are given in Tables 16 and 17. The mean initial concentration of ATP was 5.40 ± 1.17μmol/g for the samples which had been extracted immediately *post mortem* and 8.09 ± 2.13μmol/g for the samples taken at zero hour, put into liquid nitrogen and stored at –30°C for 5 days. The difference between these values was not significant ($p > 0.05$). The zero hour concentration of ADP was 2.94 ± 0.36μmol/g and that of AMP very low (0.13 ± 0.05μmol/g). The mean initial concentration of IMP was 6.81 ± 1.38μmol/g while the concentrations of INO and Hx were 0.34 ± 0.09 and 1.11 ± 0.60μmol/g respectively.

3.1.10.2 K value

The zero hour K value for the fish which were extracted immediately *post mortem* was 8.10 ± 3.37% and 4.50 ± 2.50% for those fish which were put into liquid nitrogen and stored at –30°C for 5 days (Table 18). There was no significant difference between these values.

ATP (μmol/g)	ADP (μmol/g)	AMP (μmol/g)	IMP (μmol/g)	INO (μmol/g)	Hx (μmol/g)
5.40 ±1.17	2.94 ±0.36	0.13 ±0.05	6.81 ±1.38	0.34 ±0.09	1.11 ±0.60

Table 16. Zero hour concentrations of ATP and metabolites in skeletal muscle of goldfish anaesthetised with MS-222. The muscle samples were extracted immediately on removal from the fish.
Mean values ± s.e.m. for five fish.

ATP (μmol/g)	ADP (μmol/g)	AMP (μmol/g)	IMP (μmol/g)	INO (μmol/g)	Hx (μmol/g)
8.09 ± 2.13	2.02 ± 0.50	0.24 ± 0.05	2.98 ± 0.41	0.37 ± 0.25	0.26 ± 0.13

Table 17: Zero hour concentrations of ATP and metabolites in skeletal muscle of goldfish anaesthetised with MS-222. The muscle samples were put into liquid nitrogen and stored at -30°C for five days prior to extraction. Mean values ± s.e.m. for three fish.

Method of Sampling	K value (%)
Muscle extracted immediately *post mortem* on removal from the fish	4.50 ± 2.50
Muscle put into liquid nitrogen and stored at –30°C for 5 days prior to extraction.	8.10 ± 3.37

Table 18. Goldfish: Zero hour K values for fish anaesthetised with MS-222. Mean values ± s.e.m. for three and five fish.

Comment

The results reported in these experiments indicate that the muscle samples were in a pre *rigor* state biochemically on removal from the goldfish. All of the fish were in a tranquil state at time of death. This was reflected in the concentrations of ATP obtained from all the muscle samples. The mean initial concentration of ATP (5.40 ± 1.17μmol/g) was obtained from the muscle samples which were extracted immediately on removal from the fish. The individual concentrations of ATP in these muscle samples (5) ranged from 2.80 to 8.0μmol/g. The mean initial concentration of ATP (8.09 ± 2.13μmol/g) was obtained from the muscle samples which were put into liquid nitrogen on removal from the fish and stored at –30°C for five days prior to extraction. The individual concentrations of ATP in these samples (3) ranged from 5.00μmol/g to 13.80μmol/g. There was no significant difference between the mean initial concentration of ATP (5.40 ± 1.17μmol/g) and muscle samples which were put into liquid nitrogen and stored at –30°C for 5 days prior to extraction. This was due to the diverse range in the concentrations of ATP between individual muscle samples.

There was no significant difference in K value between muscle samples which were extracted immediately on removal from the goldfish (4.50 ± 2.50%) and muscle samples which were put into liquid nitrogen and stored for five days prior to extraction (8.10 ± 3.37%).

3.2 Texture of Fish Muscle

Texture of fish muscle was measured using an Instron Universal Testing Machine (model 4464) which was equipped for use with a Warner-Bratzler shear cell. Shear force was applied to the muscle samples at an angle perpendicular to the myotomes at a speed of 80mm/min (Section 2.10).

3.2.1 Anatomical Site of Sampling and Texture Measurements

The fish were used in experiments carried out to study differences that may exist between the texture of the left and right sides of the epaxial muscle mass. Rainbow trout (4) obtained from Idas Trout Farms, Woodenbridge, Co. Wicklow were transported live to the laboratory as described. The fish were clubbed as described in Materials and Methods (section 2.2.2). Immediately after death, four muscle samples were taken from specific locations from the left and right sides of each fish (section 2.4).

3.2.1.1 Shear Force

The results are shown in Table 19. The shear force required to cut the muscle samples ranged from 9.75 ± 0.99N to 12.75 ± 0.73N and the difference between these values was significant at $p < 0.05$. The highest value (12.75 ± 0.73N) was found in muscle taken from the left side of the third fish and the lowest (9.75 ± 0.98N) in muscle taken from the right side of the fourth fish. There were no significant ($p > 0.05$) differences in shear force measurements between muscle samples taken from the left side of the fish and the right within individual fish. The results are shown in Fig. 35. Dunajski (1979) reported that in round-bodied fish no difference exists between the composition of the left and right fillets, but such differences can occur in flat-fish.

Fish Number	Shear Force (N) Left Side	Shear Force (N) Right Side
1	12.00 ± 0.67	12.25 ± 0.55
2	10 50 ± 0.58	10.75 ± 0.55
3	12.75 ± 0.73	11.50 ± 1.53
4	10.00 ± 0.47	9.75 ± 0.99

Table 19. Rainbow trout: Shear force measurements for four muscle samples taken from the left and right sides of the fish immediately *post mortem*. Mean values ± s.e.m. for four muscle samples.

3.2.2 Nucleotides and Texture of Rainbow Trout at 3°C

Two groups of fish were obtained from Idas Trout Farms, Woodenbridge, Co. Wicklow. The fish were transported live to the laboratory as previously described. They ranged from 310g to 811g in weight and 33cm to 43cm in length and had been starved for two days prior to killing. The fish (5) in group 1 were clubbed as described in Materials and Methods, (section 2.2.2) and the fish (6) in group 2 were asphyxiated (section 2.2.3). Muscle samples were taken (section 2.4). To ensure the muscle samples for biochemical analysis and texture measurements were comparable they were taken from the same fish. The muscle samples for biochemical analysis were taken from the left side and the muscle samples for texture were taken from the right side.

3.2.2.1 ATP and Metabolites

The concentrations of ATP for the clubbed and asphyxiated fish are shown in Fig. 36. The initial concentration were 4.41 ± 0.86μmol/g for the clubbed fish and 2.00 ± 0.69μmol/g for the asphyxiated and the difference was significant at $p < 0.01$. At one hour *post mortem* there was a significant ($p < 0.001$) difference in the concentration of ATP between asphyxiated (0.67 ± 0.22μmol/g) and clubbed (4.59 ± 0 ± 0.62μmol/g) fish. The concentration of ATP for the clubbed fish remained constant for five hours *post mortem* then marginally declined at six hours. The mean initial concentration of ADP was 1.11 ± 0.13μmol/g for the asphyxiated fish and 1.08 ± 0.13μmol/g for the clubbed (Fig. 37). Zero hour concentrations of AMP were very low in both groups of fish (< 0.5μmol/g). The mean zero concentration of IMP was 6.99 ± 0.78μmol/g in the asphyxiated fish and 3.53 ± 1.22μmol/g in the clubbed fish. The difference between these values was significant ($p < 0.01$). Changes in the concentration of IMP are

shown in Fig. 39. At 5 hours *post mortem* the concentrations of IMP were 3.97 ± 1.22μmol/g for the clubbed fish and 8.65 ± 0.50μmol/g for the asphyxiated. Concentrations of INO and Hx were very low for both groups of fish at the start of the experiment. At five hours the concentration of INO was 0.61 ± 0.18μmol/g for the asphyxiated fish and 0.38 ± 0.17μmol/g for the clubbed fish (Fig. 40 and 41).

3.2.2.2 K value

The mean initial K value (2.47 ± 1.05% for the clubbed fish and 2.53 ± 0.58% for the asphyxiated) and changes in K value are shown in Fig. 42. At five hours *post mortem* the K value for the clubbed fish (4.59 ± 2.19%) was significantly ($p < 0.05$) different to that (6.86 ± 1.73%) for the asphyxiated.

3.2.3 pH

Changes in pH during storage are shown in Fig. 43. The mean zero hour pH was 7.19 ± 0.08 for the clubbed fish and 6.75 ± 0.04 for the asphyxiated.

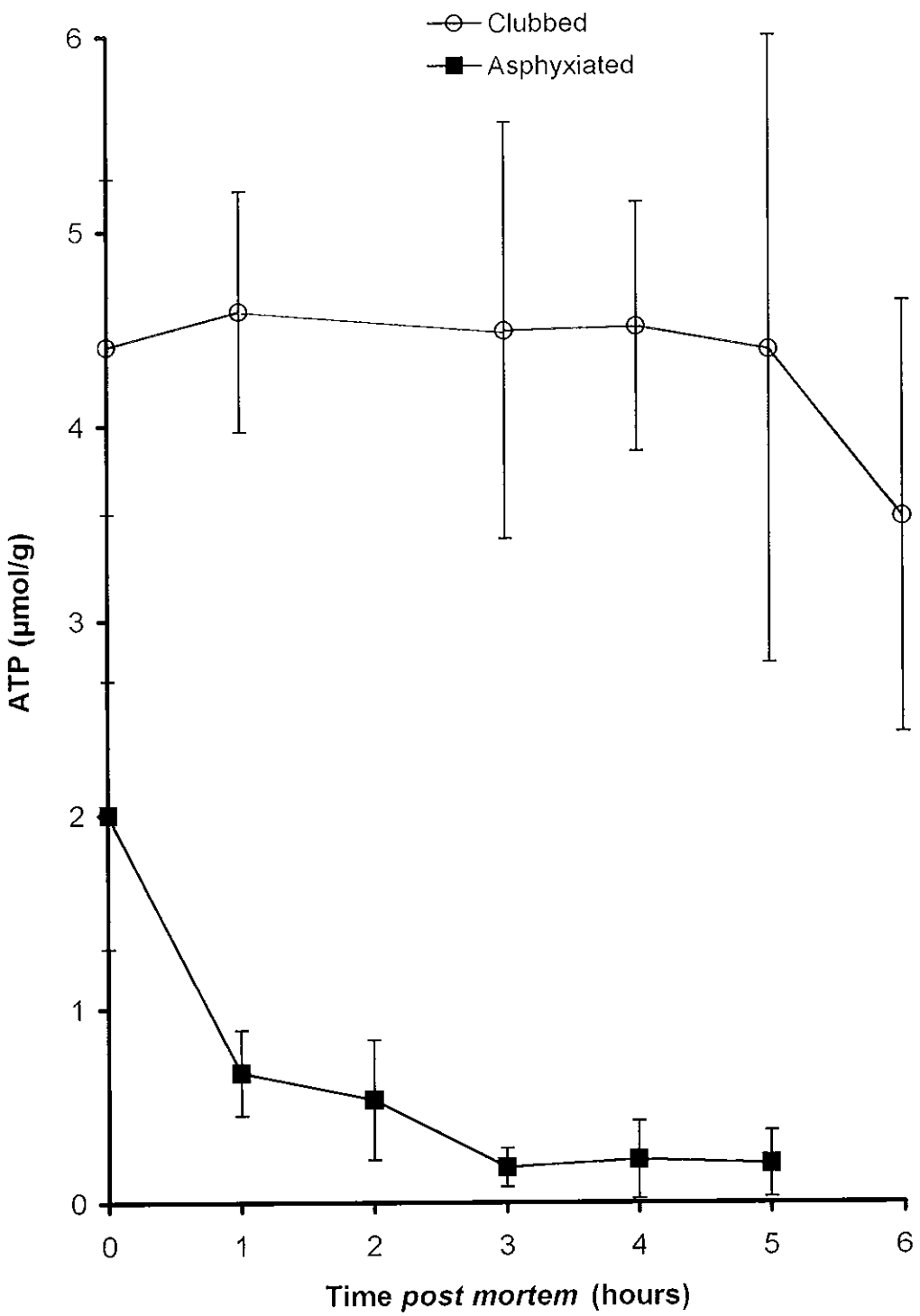


Fig. 36. Myotomal muscle of clubbed and asphyxiated rainbow trout: changes in the concentration of ATP at 3°C.
Mean values ± s.e.m. for groups of five (clubbed) and six (asphyxiated) fish.

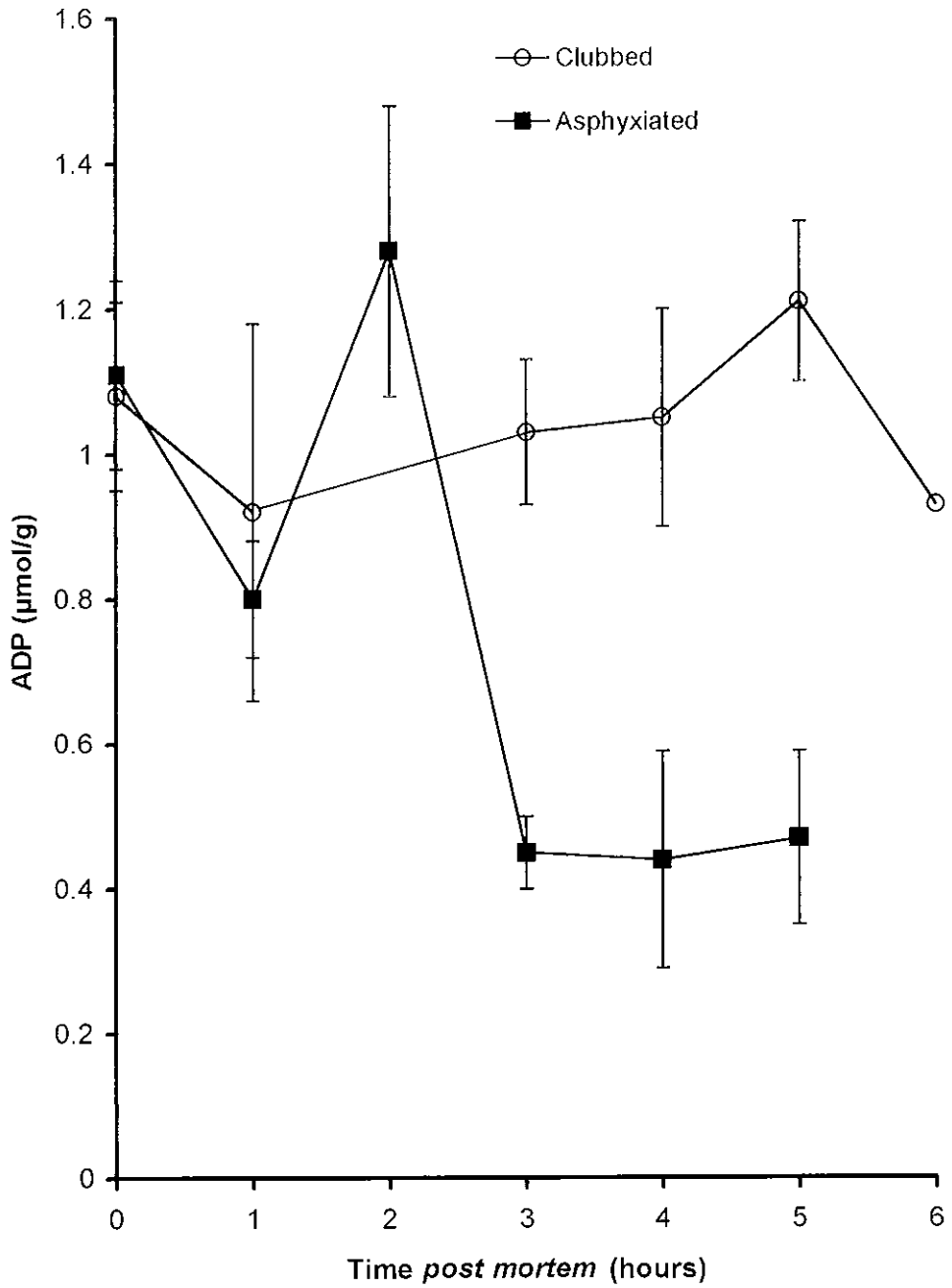


Fig. 37. Myotomal muscle of clubbed and asphyxiated rainbow trout: changes in the concentration of ADP at 3°C.
Mean values ± s.e.m. for groups of five (clubbed) and six (asphyxiated) fish.

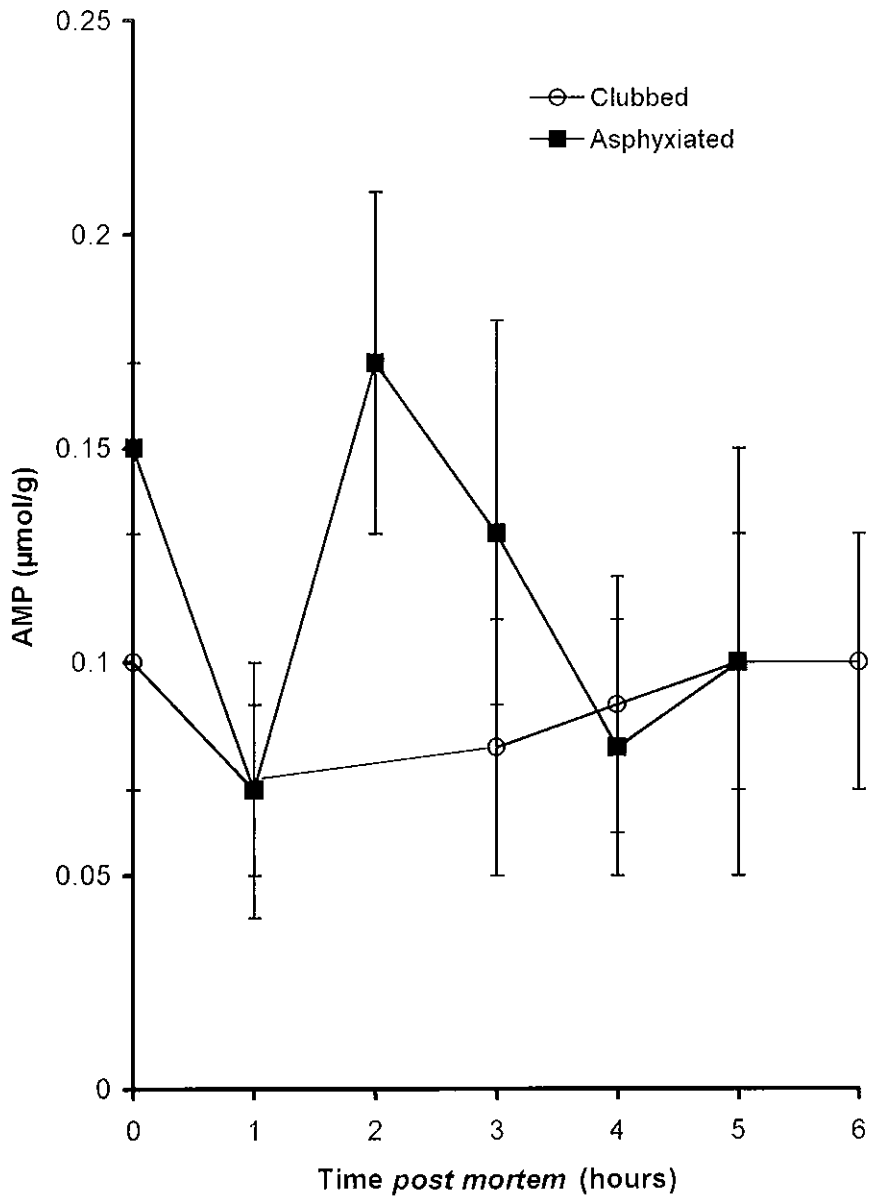


Fig. 38. Myotomal muscle of clubbed and asphyxiated rainbow trout: changes in the concentration of AMP at 3°C.
Mean values ± s.e.m for groups of five (clubbed) and six (asphyxiated) fish.

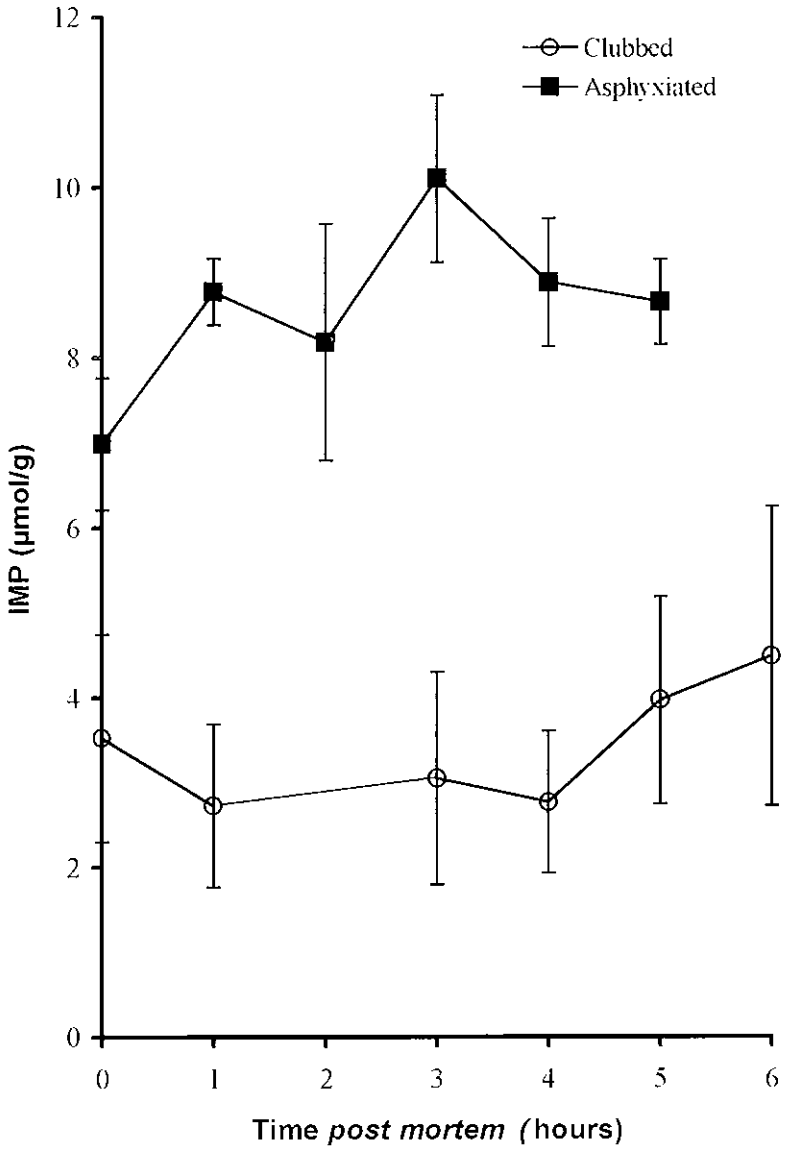


Fig. 39. Myotomal muscle of clubbed and asphyxiated rainbow trout: changes in the concentration of IMP at 3°C.
Mean values ± s.e.m. for groups of five (clubbed) and six (asphyxiated) fish.

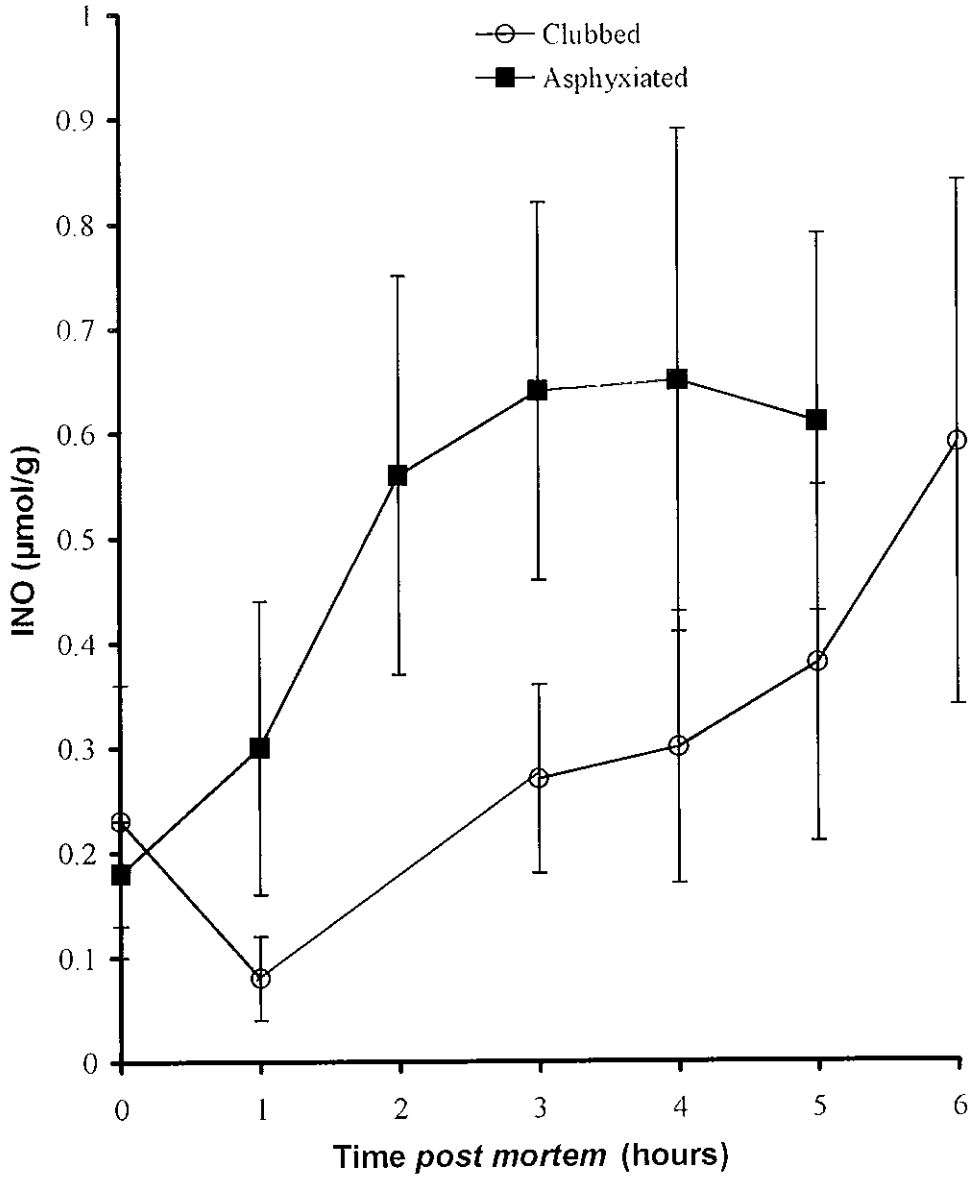


Fig. 40. Myotomal muscle of clubbed and asphyxiated rainbow trout: changes in the concentration of INO at 3°C.
Mean values ± s.e.m. for groups of five (clubbed) and six (asphyxiated) fish.

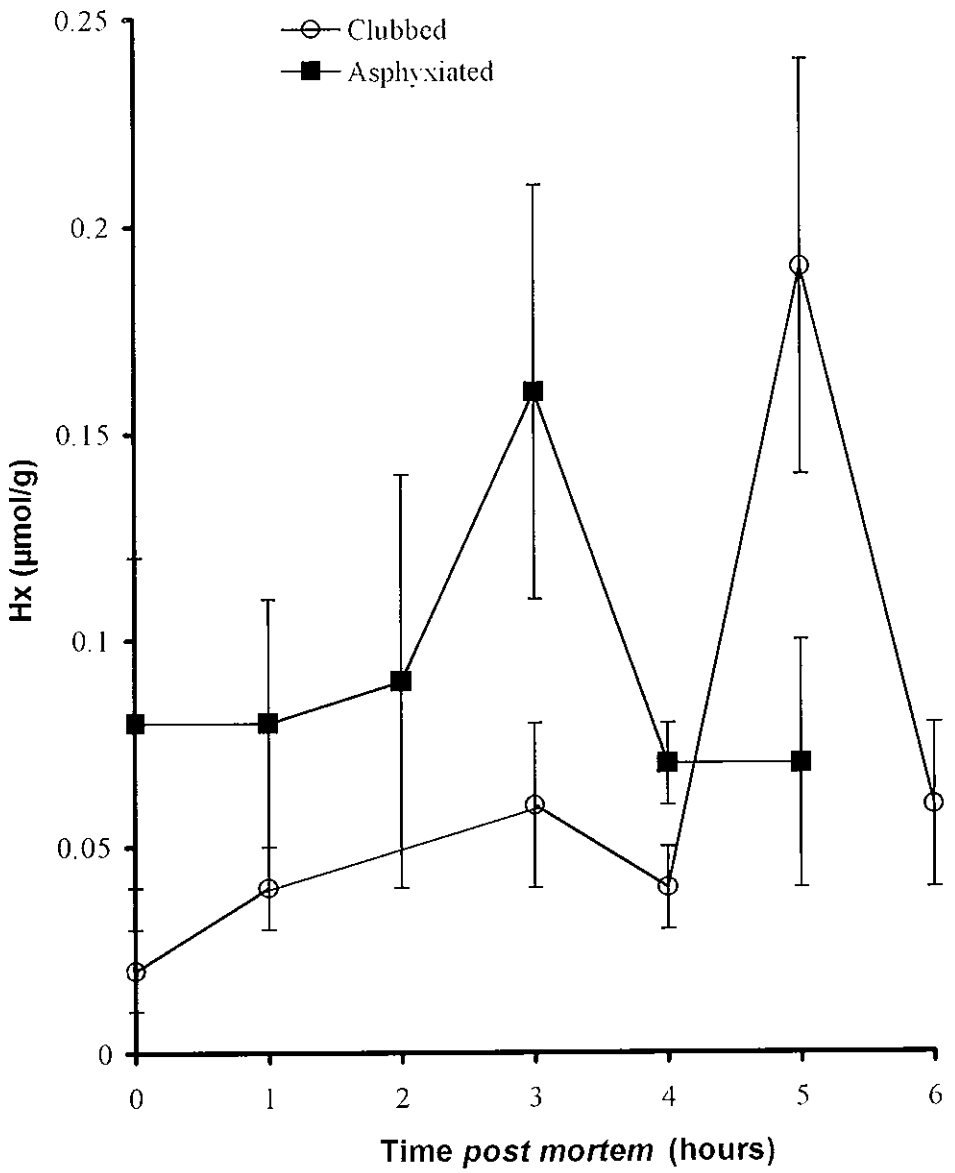


Fig. 41. Myotomal muscle of clubbed and asphyxiated rainbow trout: changes in the concentration of Hx at 3°C.
Mean values ± s.e.m. for groups of five (clubbed) and six (asphyxiated) fish.

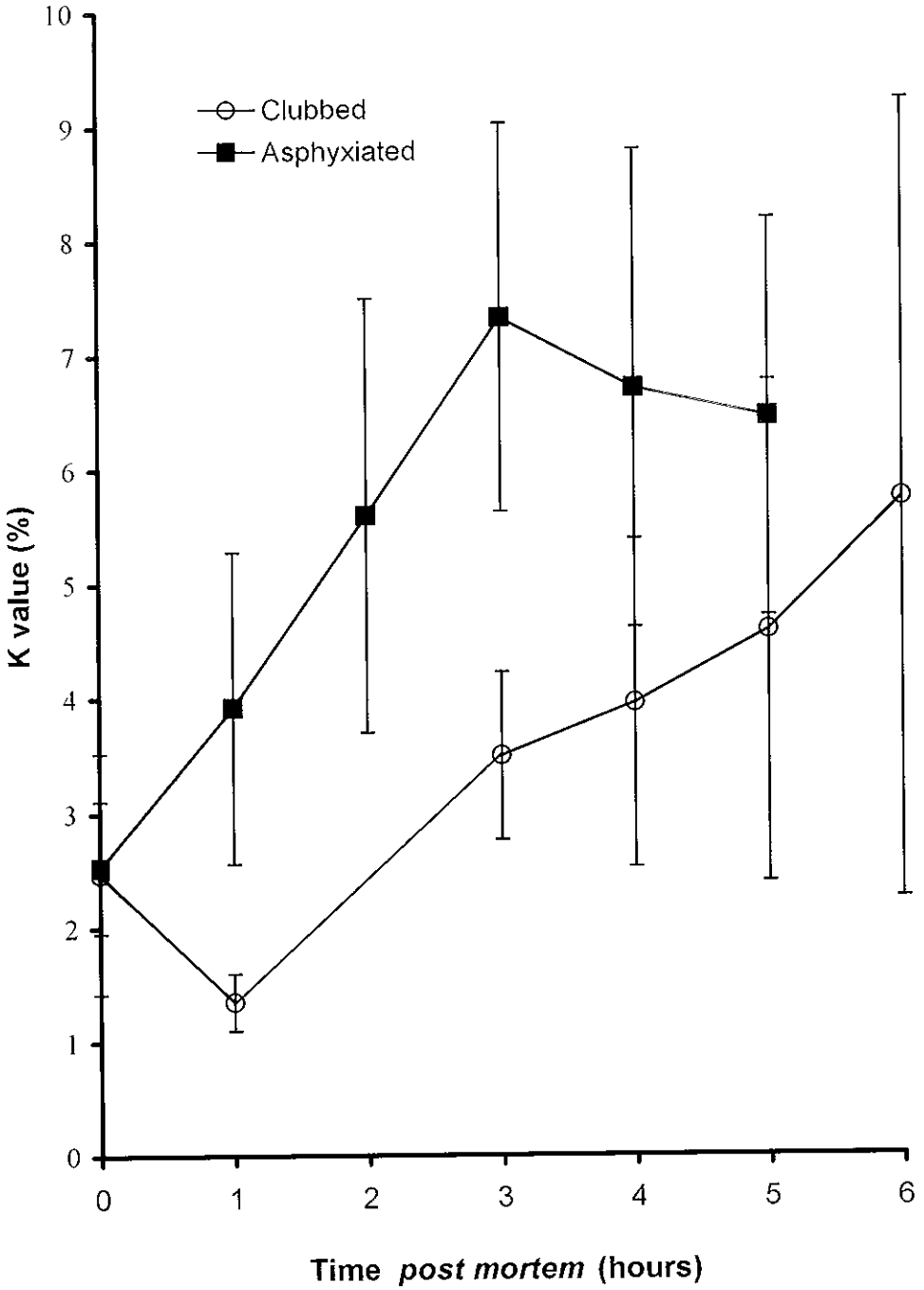


Fig. 42. Myotomal muscle of clubbed and asphyxiated rainbow trout: changes in K value at 3°C.
Mean values ± s.e.m. for groups of five (clubbed) and six (asphyxiated) fish.

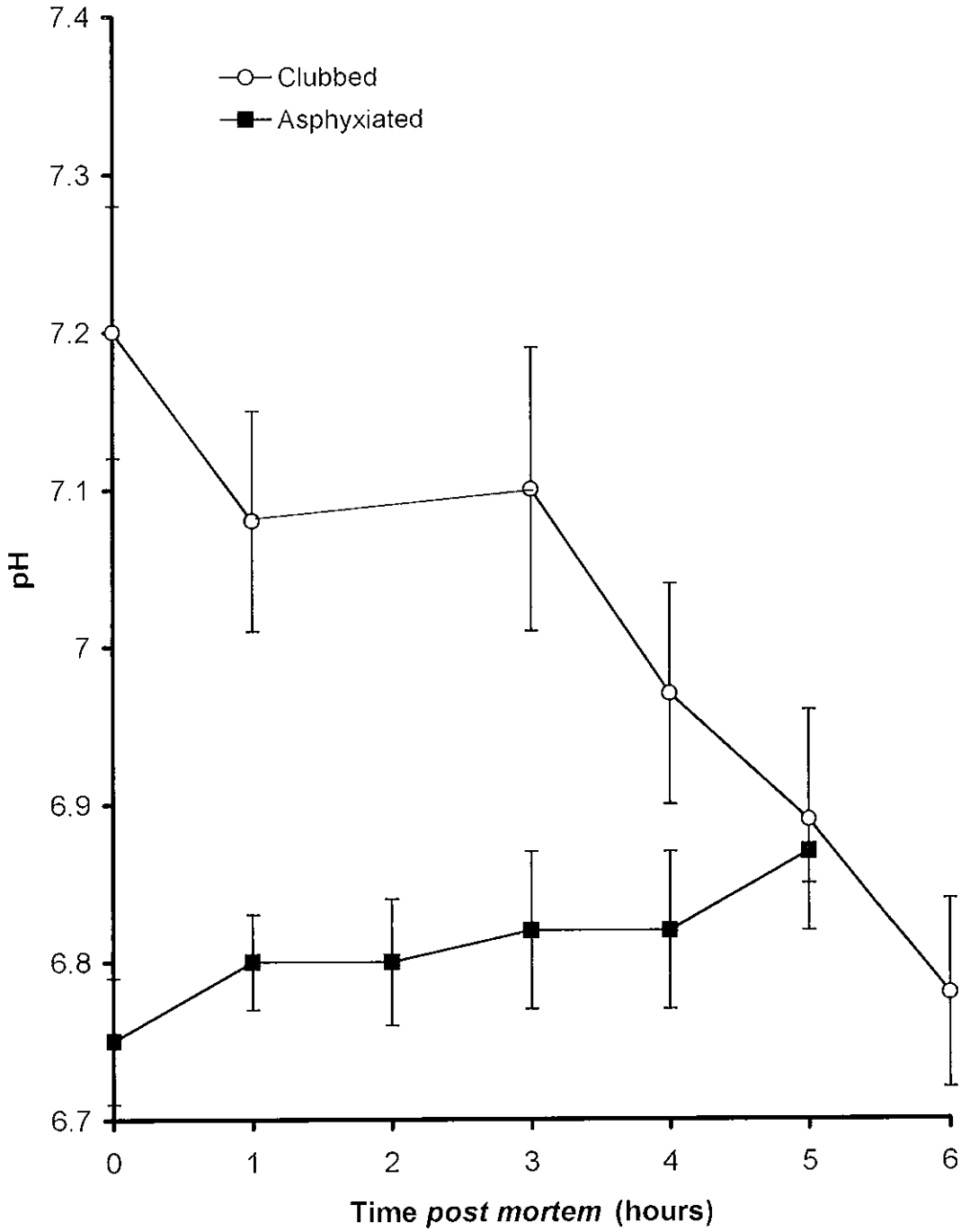


Fig. 43. Myotomal muscle of clubbed and asphyxiated rainbow trout: changes in pH at 3°C.
Mean values ± s.e.m. for groups of five (clubbed) and six (asphyxiated) fish.

3.2.4 Shear Force

Changes in shear force for clubbed and asphyxiated fish are shown in Fig. 44. The shear force required to cut the muscle of clubbed fish immediately *post mortem* was 8.33 ± 61N compared with 6.85 ± 0.98N for the asphyxiated fish. The difference between these values was not significant ($p > 0.01$). At 1 hour *post mortem* there was a marginal increase in shear force for the clubbed fish from 8.33 ± 0.61N to 8.83 ± 0.91N. This phenomenon was not observed in the asphyxiated fish. There was a significant ($p < 0.05$) difference in shear force between the clubbed (8.83 ± 0.91N) and asphyxiated (6.42 ± 0.47N) fish at 1 hour *post mortem*. Shear force decreased for clubbed and asphyxiated fish up to 3 hours after which time the values remained constant. A similar pattern was observed for the biochemical analysis of clubbed fish where the concentrations of ATP remained constant for up to 5 hours *post mortem*. Changes in the concentration of ATP and changes in shear force are shown in Fig. 45.

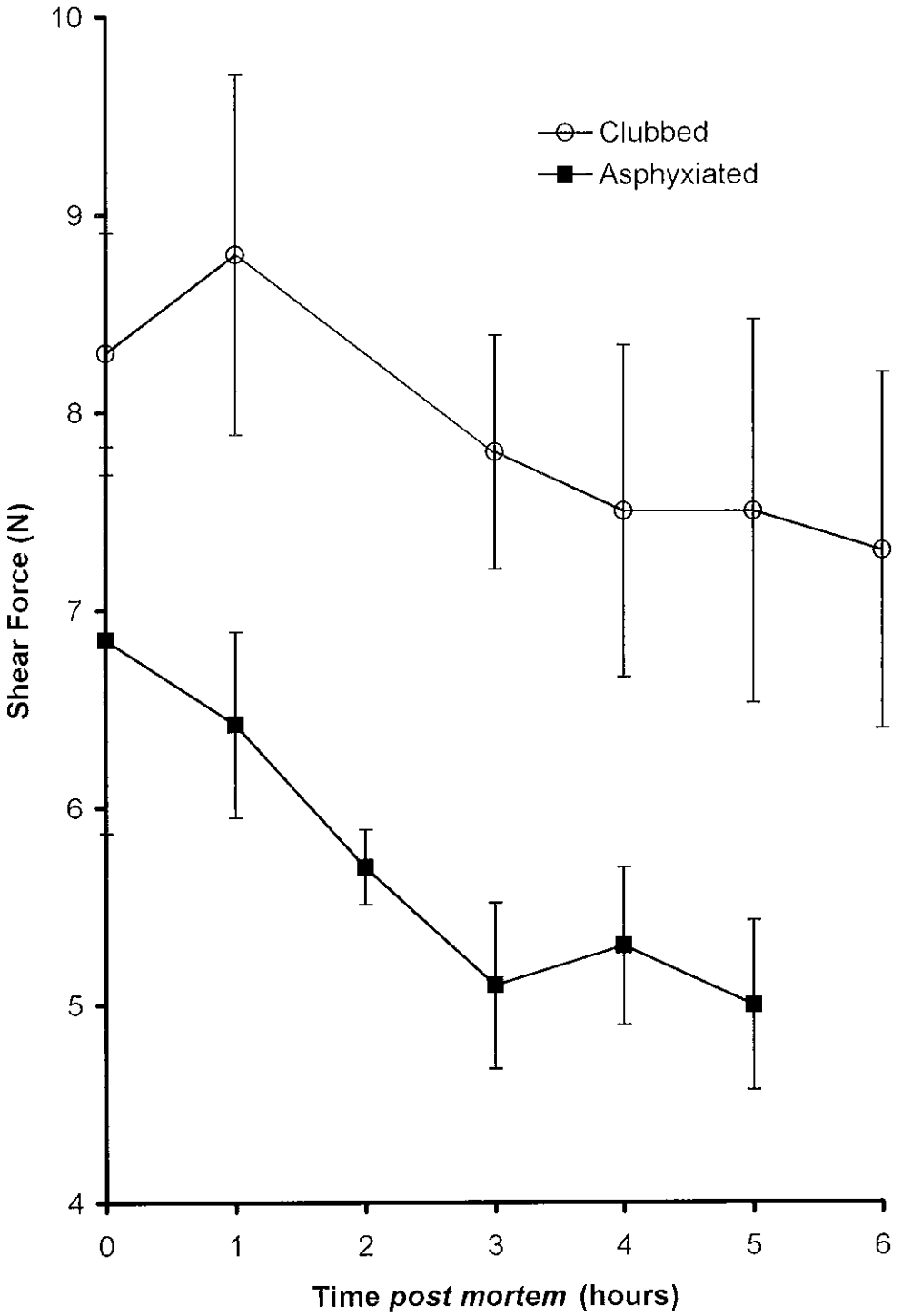


Fig. 44. Myotomal muscle of clubbed and asphyxiated rainbow trout: changes in shear force at 3°C.
Mean values ± s.e.m. for groups of five (clubbed) and six (asphyxiated) fish.

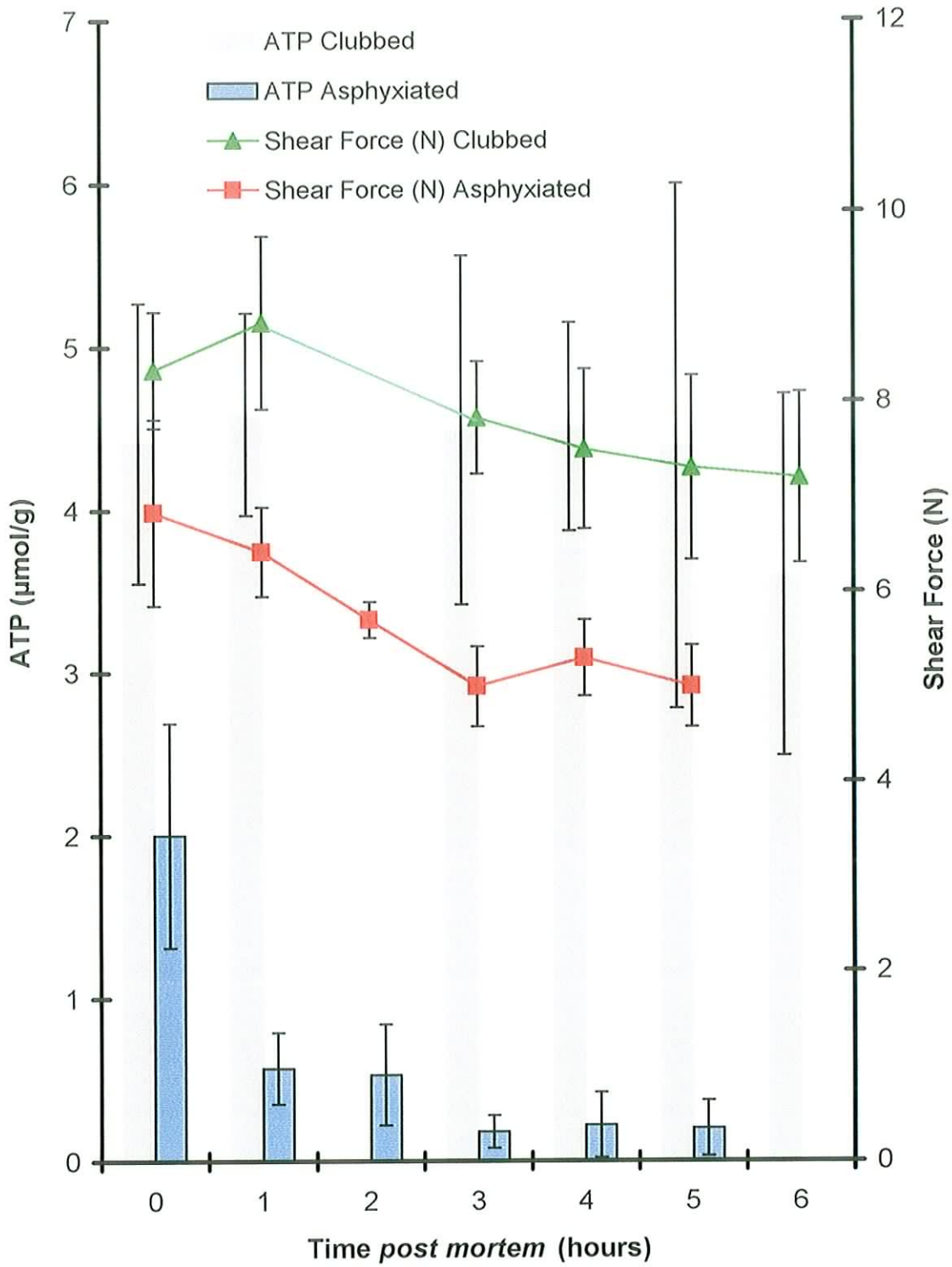


Fig. 45. Myotomal muscle of clubbed and asphyxiated rainbow trout: changes in the concentration of ATP and changes in shear force at 3°C.
Mean values ± s.e.m. for groups of five (clubbed) and six (asphyxiated) fish.

Comment

The results of these experiments showed that the myotomal muscle of clubbed fish had a higher concentration of ATP immediately after death than did that of asphyxiated fish. The concentration of ATP in the clubbed fish remained constant for 5 hours *post mortem*. These results are in agreement with those reported in Section 1.2 where the concentration of ATP remained constant for up to 3 hours *post mortem* in three groups of clubbed fish. A similar pattern was observed for shear force measurements of clubbed fish. The shear force marginally declined in the clubbed and asphyxiated fish during the first 3 hours *post mortem* after which time the values remained constant. The muscle samples taken from the clubbed fish were soft and pliable at time of sampling compared with those from the asphyxiated fish. The K values were similar for both groups of fish at zero hour and increased with time for the two groups. The asphyxiated fish had significantly higher K values at each time of sampling compared with the clubbed.

3.2.5 A Comparison of Clubbed and Asphyxiated Fish held at 3°C

Rainbow trout obtained from Idas Fish Farms, Woodenbridge, Co. Wicklow were transported live to the laboratory. Two groups of fish were used in the experiments. The fish (6) in group 1 were clubbed and the fish (6) in group 2 were asphyxiated as described in materials and Methods (section 2.2.2 and 2.2.3). Muscle samples were taken as (section 2.4). The fish carcasses were then packed into polystyrene boxes, layered with ice and stored at 3°C. Sampling was carried out immediately after death and at time intervals up to ten days *post mortem*.

3.2.5.1 ATP and Metabolites

Changes in the concentration of ATP are shown in Fig. 46. The mean zero hour concentration of ATP was 0.77 ± 0.75μmol/g in the asphyxiated fish and 6.67 ± 1.51μmol/g in the clubbed. The difference between these values was significant ($p <$ 0.001). The asphyxiated fish exhibited a strong death reaction and a rapid onset of *rigor*. The concentration of ATP in the fish which were clubbed fell from an initial concentration of 6.67 ± 1.51μmol/g to a value that was very low (0.03 ± 0.02μmol/g) at 24 hours *post mortem*. The mean initial concentration of ADP (0.98 ± 0.19μmol/g) in the muscle of the asphyxiated fish was lower than that in the clubbed (1.93 ± 0.16μmol/g). The results are shown in Fig. 47. Zero hour concentrations of AMP (< 0.2μmol/g) were very low for both groups of fish (Fig. 48). The mean zero hour concentration of IMP in the clubbed fish was 4.00 ± 1.30μmol/g compared with 8.90 ± 0.89μmol<0.001). Changes in the concentration of IMP are shown in Fig.49. The IMP values at 24 hours storage were 9.80 ± 0.61μmol/g for the clubbed fish and 9.49 ± 0.72μmol/g for the asphyxiated. Concentrations of IMP decreased in both groups of fish although in the clubbed fish there was an increase in the concentration from 5.40 ±

0.27μmol/g at day 3 to 6.69 ± 0.20μmol/g at day 6 which was significant ($p < 0.01$). At day 10, the values for IMP were 2.61 ± 0.26μmol/g for the clubbed fish and 1.73 ± 0.45μmol/g for the asphyxiated. Concentrations of INO and Hx were very low (<0.5μmol/g) for the two groups of fish at the start of the experiment (Fig. 50 and 51). At day 10 the concentrations of INO were 5.12 ± 0.46μmol/g and 4.22 ± 0.13μmol/g for the asphyxiated and the clubbed fish respectively, while the concentrations of Hx were 1.05 ± 0.15μmol/g for the clubbed and 0.84 ± 0.10μmol/g for the asphyxiated fish.

3.2.5.2 K value

Changes in K value during storage are shown in Fig. 52. The mean initial K value for the clubbed fish was 1.84 ± 0.64μmol/g and 3.72 ± 0.78μmol/g for the asphyxiated. The K value increased during storage for both groups. The K values were marginally higher for the asphyxiated fish at each time of sampling. There was a significant ($p < 0.01$) difference between the K values for the clubbed (44.23 ± 2.29%) and asphyxiated (61.09 ± 6.58%) fish at day 6. At day 10, the difference in the K value between clubbed and asphyxiated fish was not significant.

3.2.6 pH

The pH/time curves for both groups of fish are shown in Fig. 53. The value for the clubbed fish (7.00 ± 0.14) was significantly ($p < 0.001$) higher than that for the asphyxiated (6.77 ± 0.06).

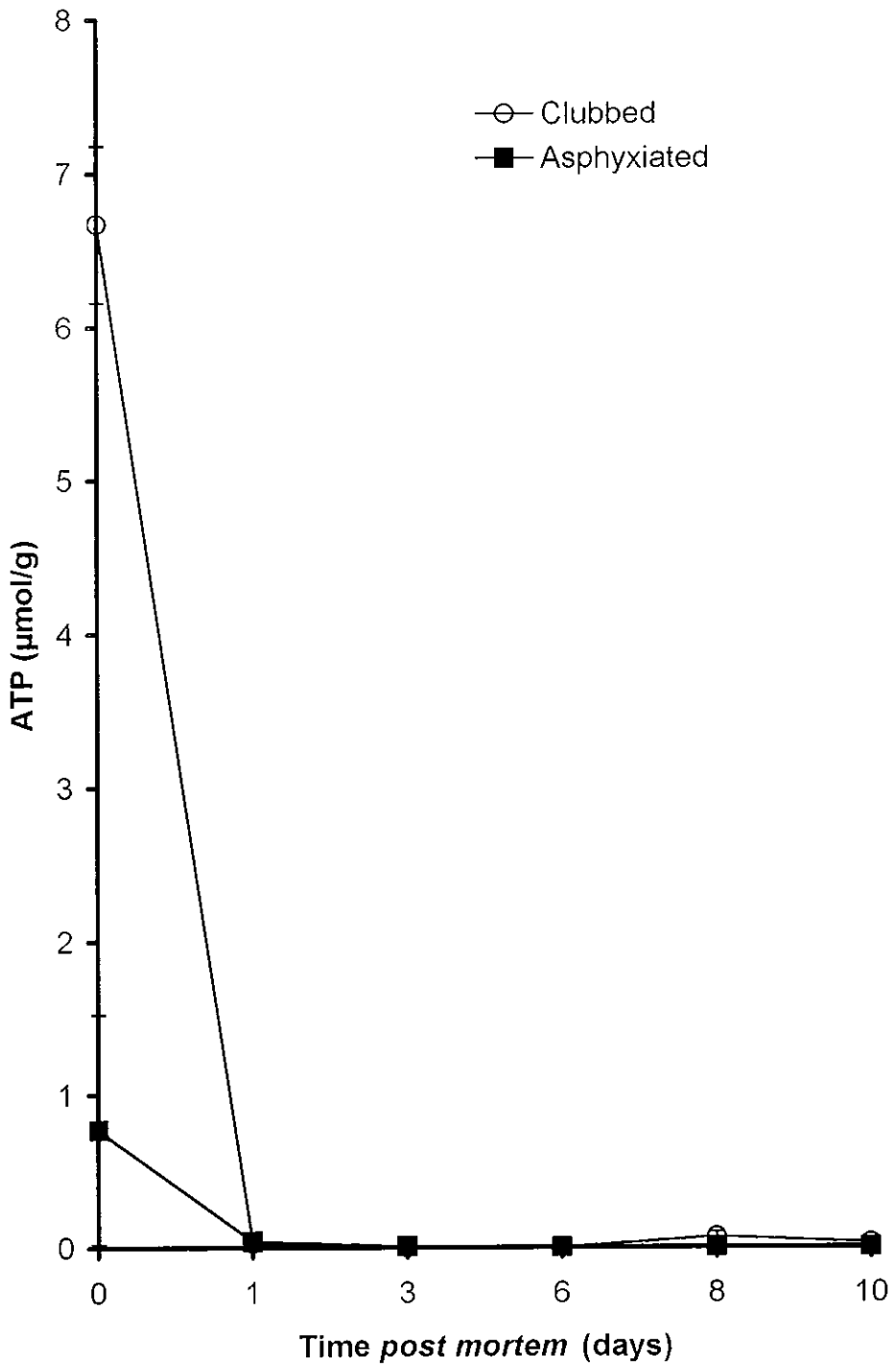


Fig. 46. Myotomal muscle of clubbed and asphyxiated rainbow trout: changes in the concentration of ATP at 3°C.
Mean values ± s.e.m. for groups of six fish.

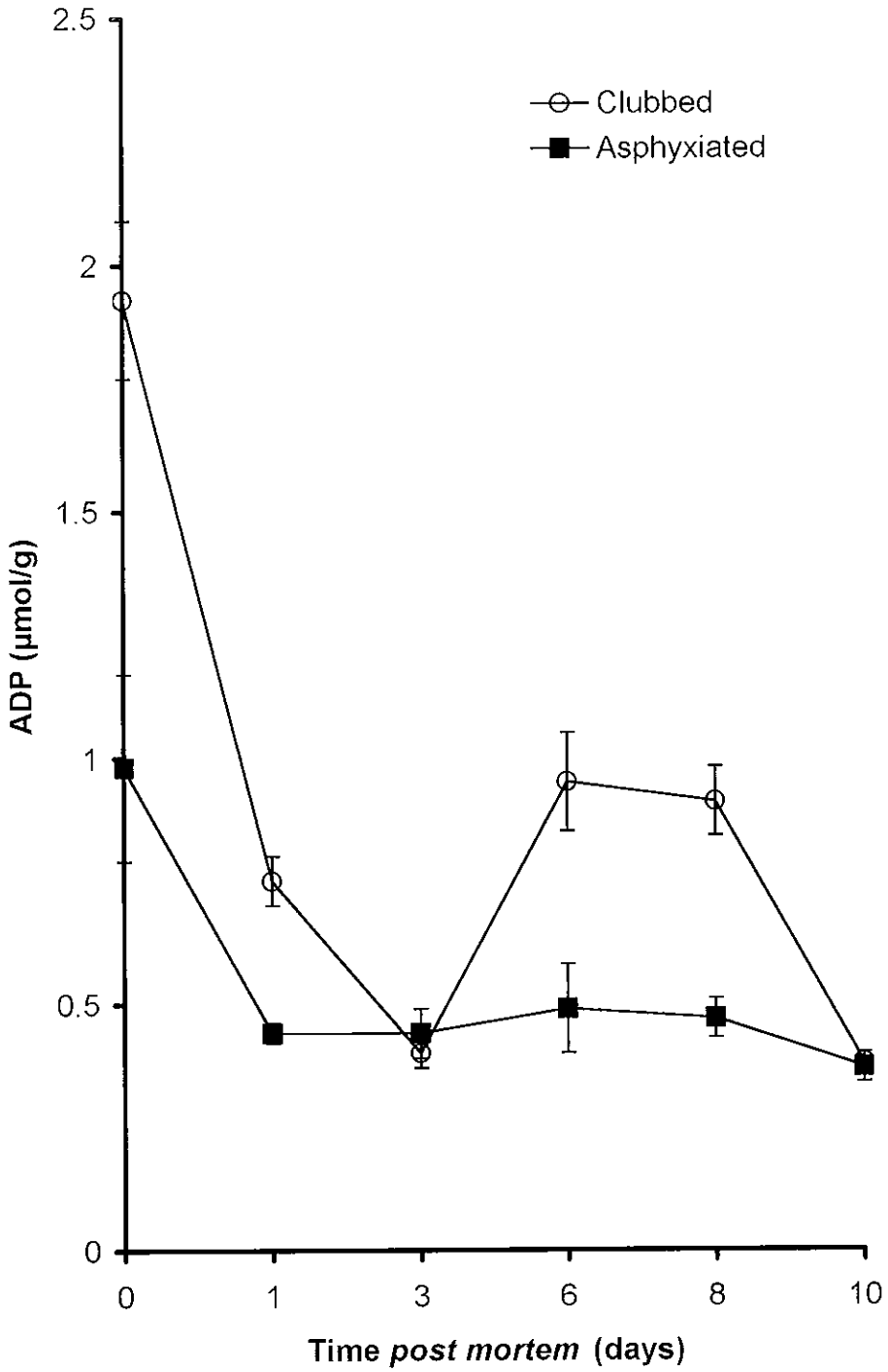


Fig. 47. Myotomal muscle of clubbed and asphyxiated rainbow trout: changes in the concentration of ADP at 3°C.
Mean values ± s.e.m. for groups of six fish.

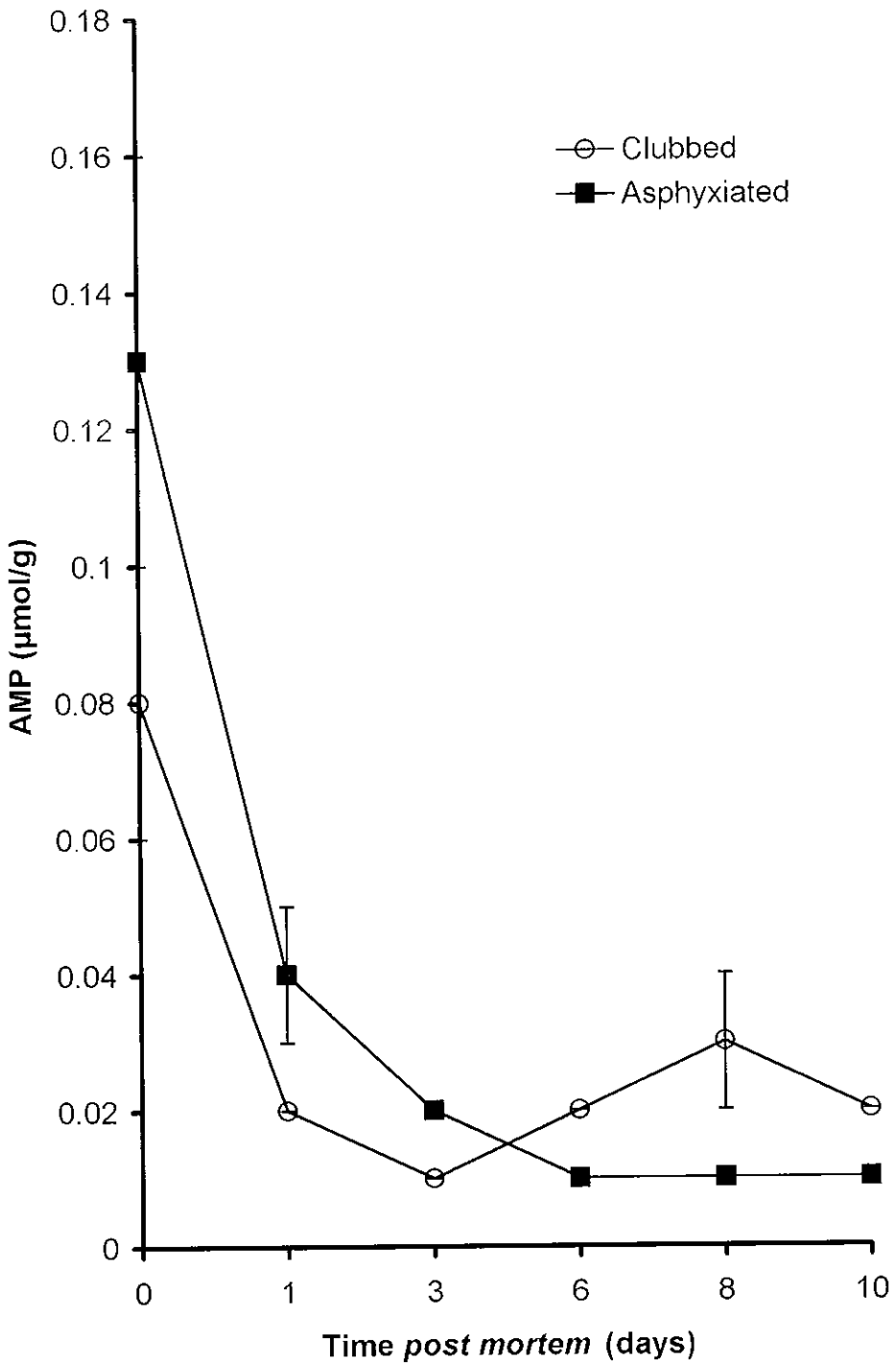


Fig. 48. Myotomal muscle of clubbed and asphyxiated rainbow trout: changes in the concentration of AMP at 3°C.
Mean values ± s.e.m. for groups of six fish.

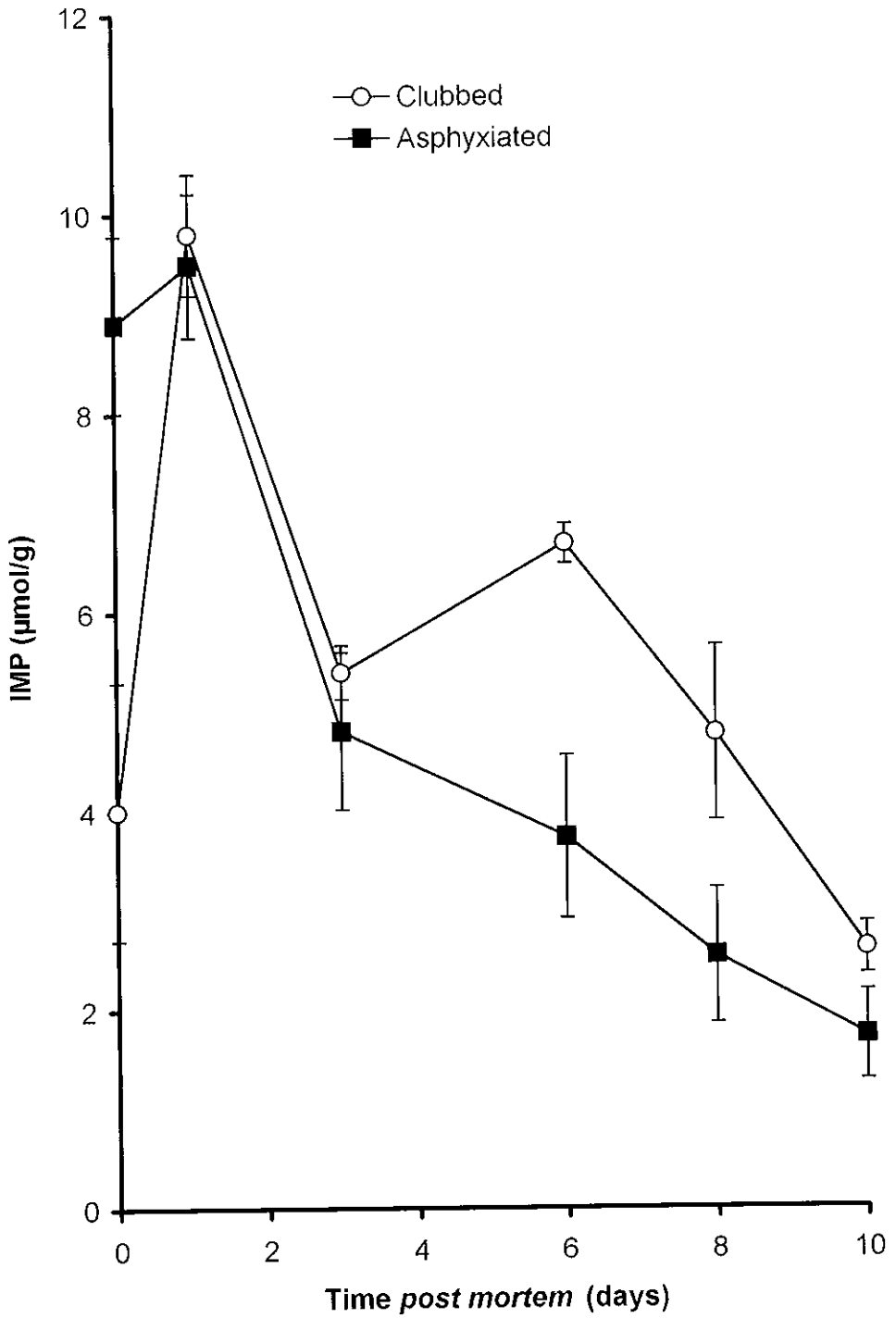


Fig. 49. Myotomal muscle of clubbed and asphyxiated rainbow trout: changes in the concentration of IMP at 3°C.
Mean values ± s.e.m. for groups of six fish.

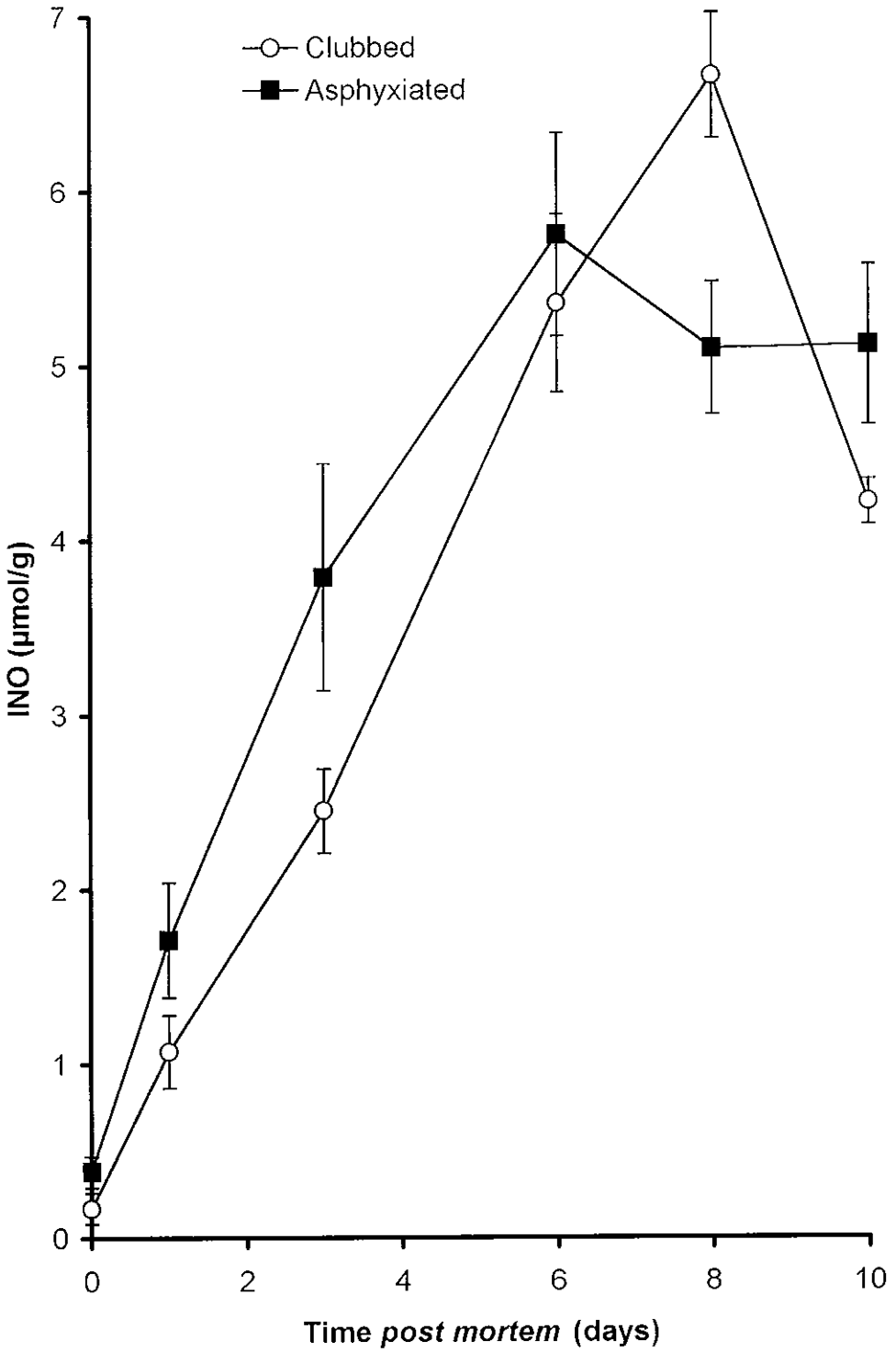


Fig. 50. Myotomal muscle of clubbed and asphyxiated rainbow trout: changes in the concentration of INO at 3°C.
Mean values ± s.e.m. for groups of six fish.

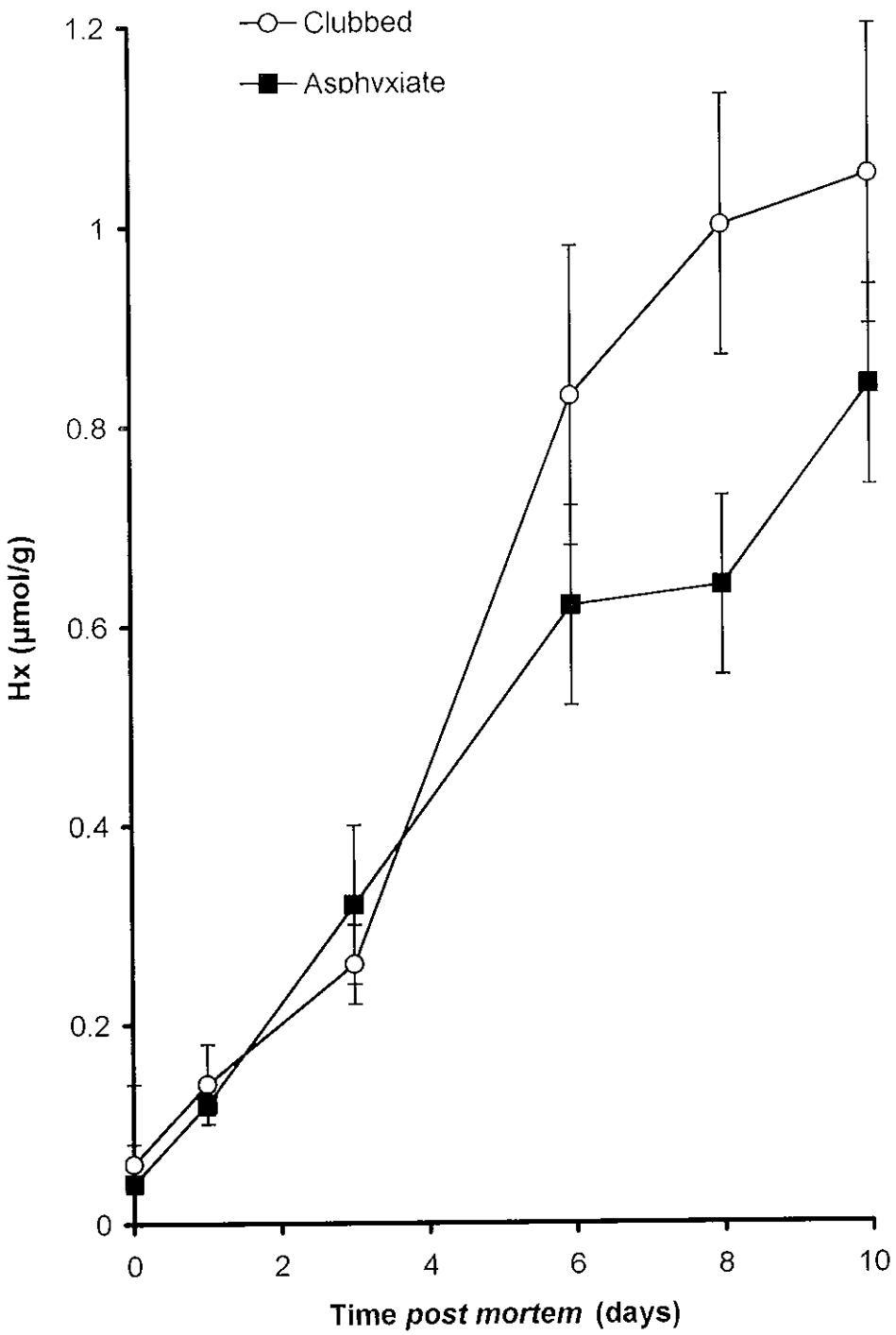


Fig. 51. Myotomal muscle of clubbed and asphyxiated rainbow trout: changes in the concentration of Hx at 3°C.
Mean values ± s.e.m. for groups of six fish.

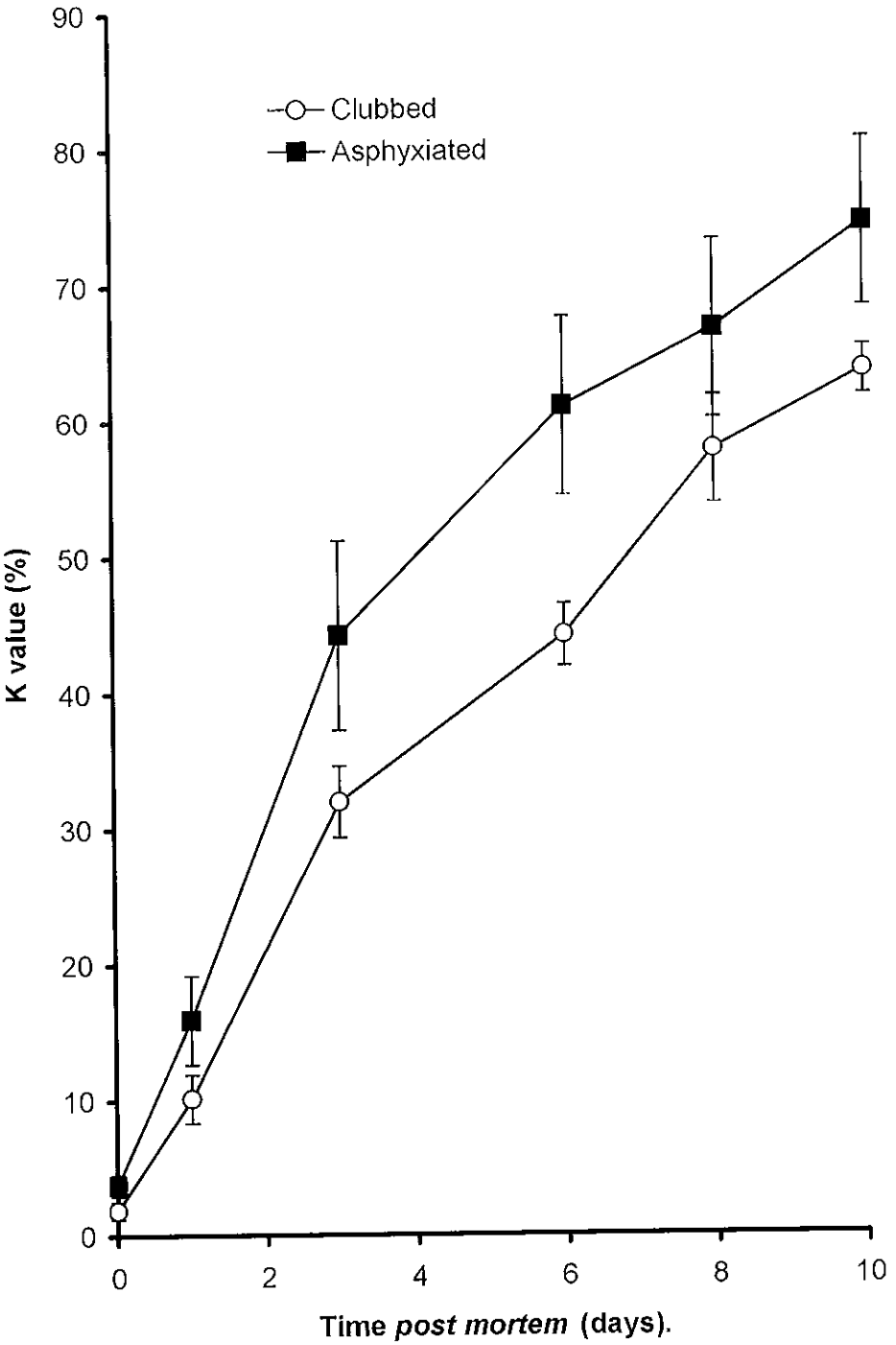


Fig. 52. Myotomal muscle of clubbed and asphyxiated rainbow trout: changes in K value at 3°C.
Mean values ± s.e.m. for groups of six fish.

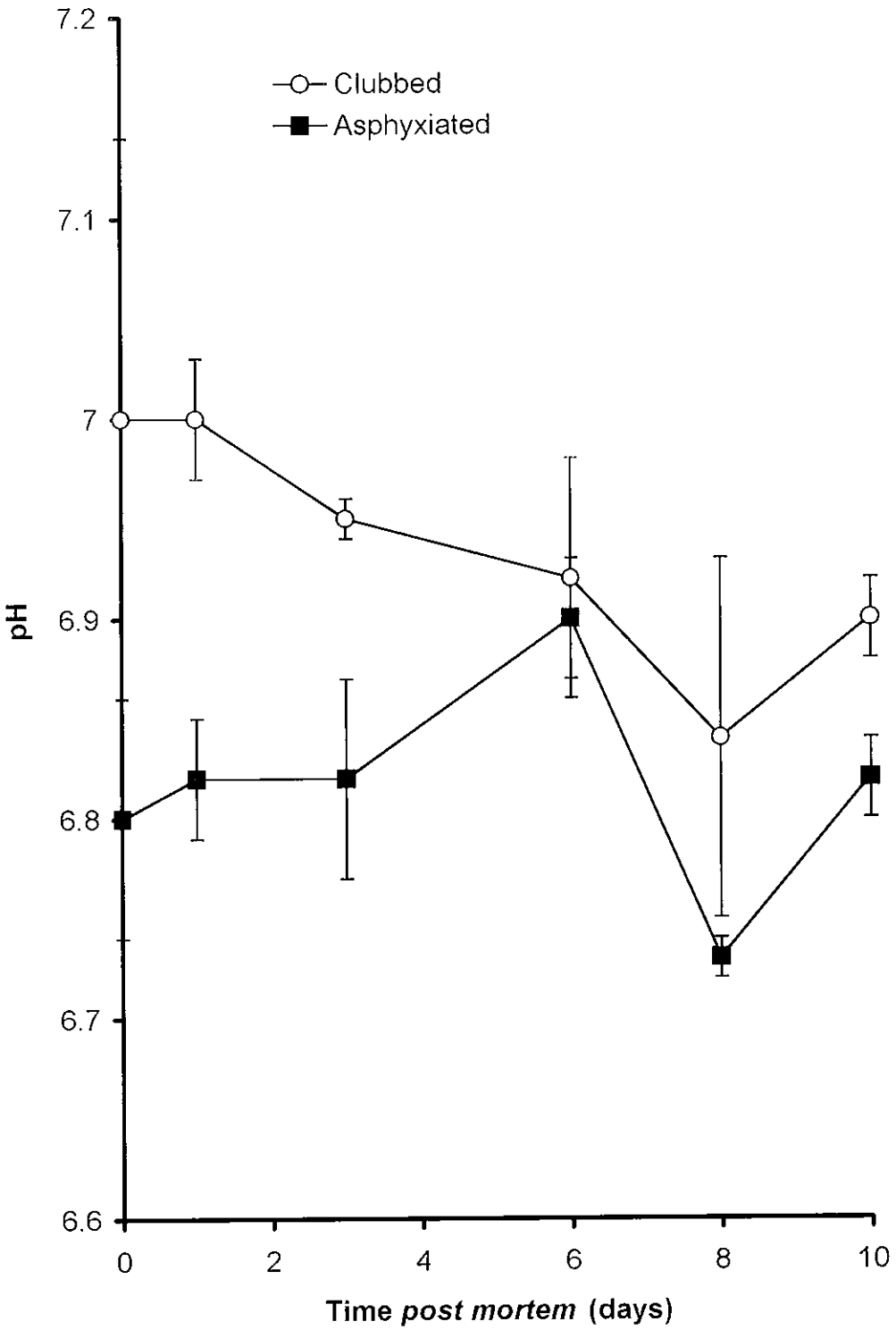


Fig. 53. Myotomal muscle of clubbed and asphyxiated rainbow trout: changes in pH at 3°C.
Mean values ± s.e.m. for groups of six fish.

3.2.7 Shear Force

The shear force required to cut the muscle of fish immediately after death was 8.40 ± 0.82N for the clubbed fish and 10.72 ± 0.55N for the asphyxiated. The difference between these values was significant at $p < 0.01$ and may be explained by the fact that the asphyxiated fish were in *rigor* at time of sampling. The force/displacement curves for the clubbed and asphyxiated fish are shown in Fig. 54 and 55. The curve for the asphyxiated fish showed two distinct peaks in contrast to that for the clubbed. Changes in shear force during storage for clubbed and asphyxiated fish are shown in Fig. 56. At day 1 there was a significant ($p < 0.05$) difference in shear force values for clubbed (5.86 ± 0.41N) and asphyxiated (6.92 ± 0.70N) fish. Shear force decreased relatively rapidly over the first day (from 8.40 ± 0.82N to 5.89 ± 0.42N) for the clubbed fish and (from 10.72 ± 0.55N to 6.90 ± 0.70N) for the asphyxiated and then more slowly over the following 9 days. At day 10, the force values were 3.40 ± 0.33N for the clubbed fish and 3.64 ± 0.21N for the asphyxiated. Differences in shear force between the two groups of fish during storage were not significant.

3.2.8 K value and Shear force

Changes in the K value and in shear force are shown in Fig 57a. The mean initial K values were low for the clubbed (1.84 ± 0.64%) and asphyxiated (3. 72 ± 0.78%) fish. The mean initial shear force values were high in the clubbed (8.40 ± 0.82N) and the asphyxiated (10.72 ± 0.55N) fish. At day 1 there was a rapid increase in the K value from 1.84 ± 0.64% to 10.10 ± 3.98% for the clubbed fish and from 3.72 ± 0.78% to 15.91 ± 3.98% for the asphyxiated. Shear force decreased relatively rapidly at day 1 from 8.40 ± 0.82N to 5.89 ± 0.42N for the clubbed fish and from 10.72 ± 0.55N to 6.90 ± 0.70N for the asphyxiated. The difference between the K values for the clubbed

(44.23 ± 2.29%) and asphyxiated (61. 09 ± 6.58%) fish at day 6 was significant ($p < 0.01$). However, there were no significant differences between shear force measurements for clubbed (5.2 ± 0.4N) and asphyxiated (4.8 ± 0.44N) fish at day 6. There was a gradual increase in the K values for the two groups of fish over the following two days while the shear force values slowly decreased in both clubbed and asphyxiated fish during this time. The K values were marginally higher and the shear force values slightly lower for asphyxiated fish.

There was an inverse relationship between the K value and the shear force during storage (Fig. 57b). As the K value increased with time, there was a decrease in the force required to cut the muscle sample.

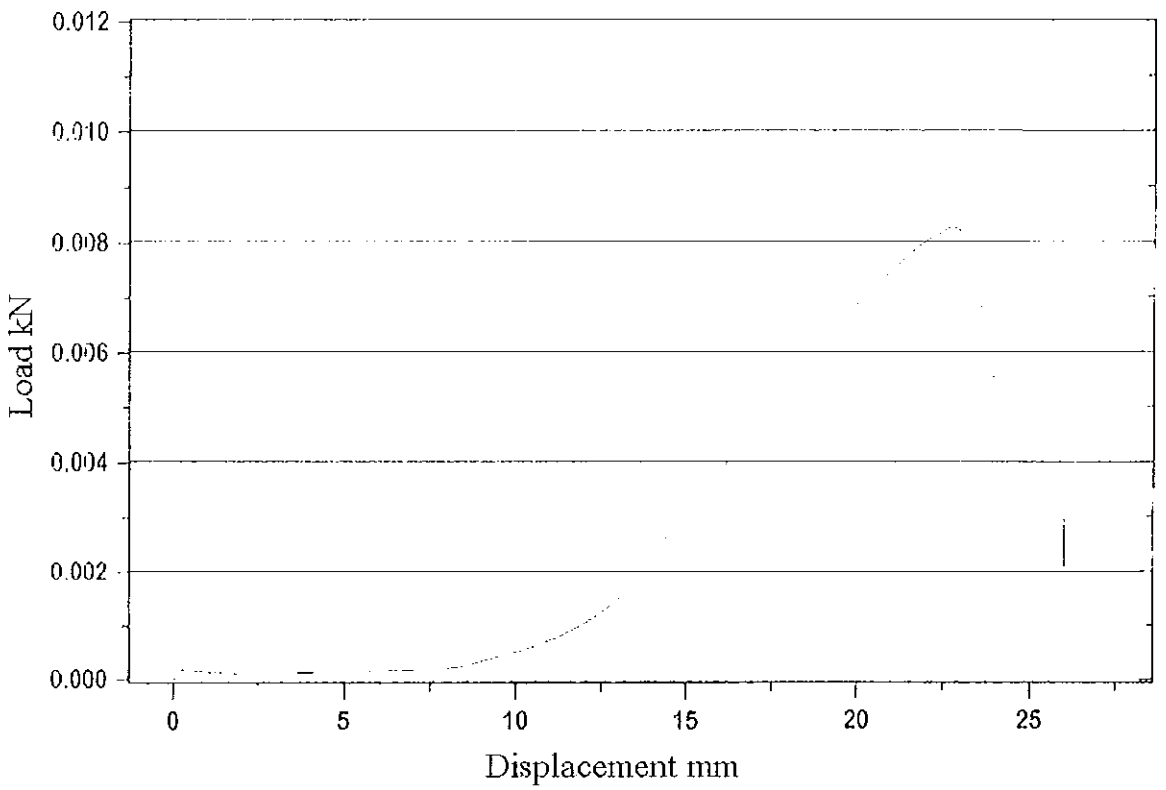


Fig. 54. Myotomal muscle of clubbed rainbow trout: shear force/displacement curve for muscle taken immediately *post mortem*.
Mean values ± s.e.m. for six fish.

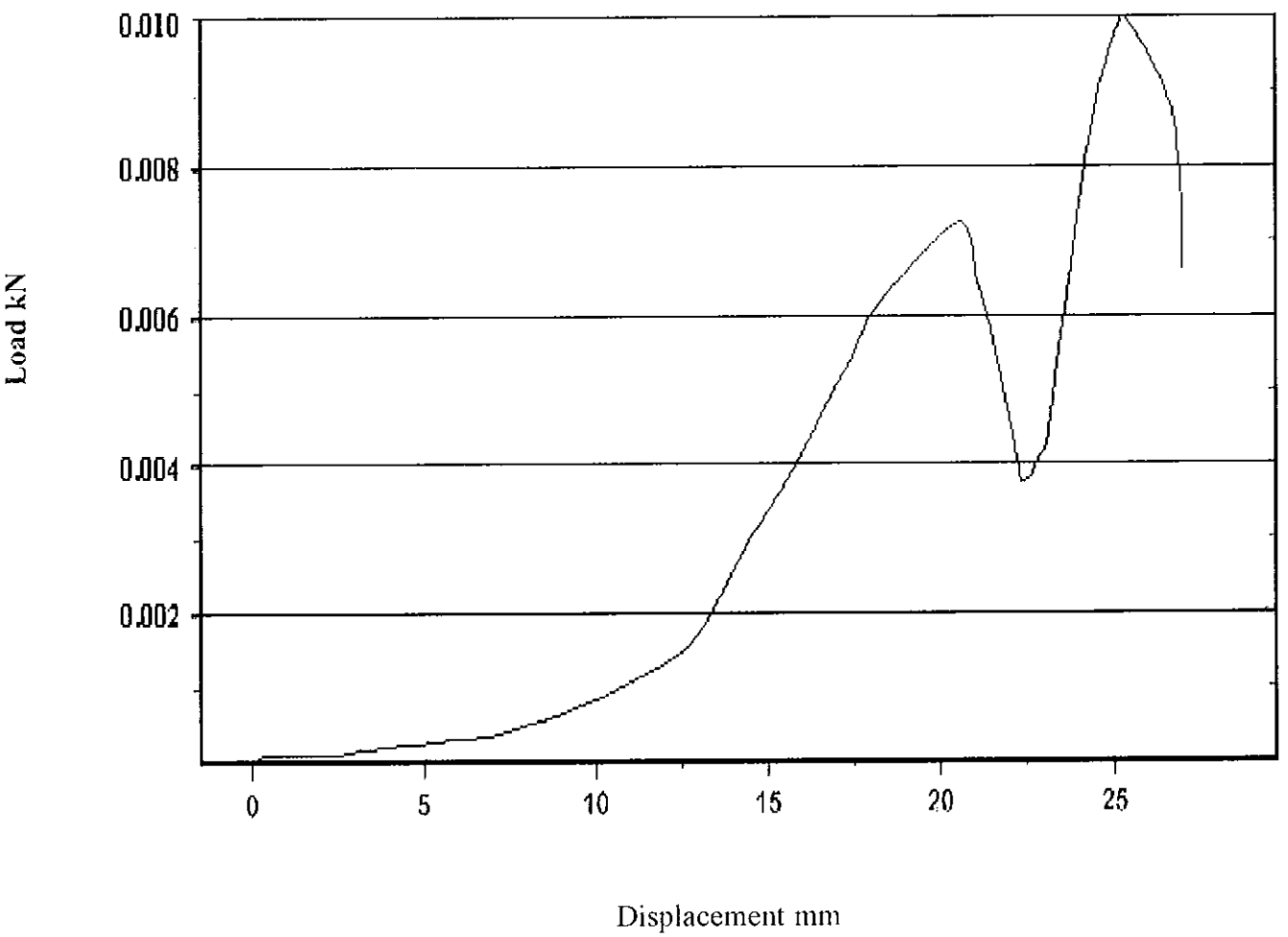


Fig. 55. Myotomal muscle of asphyxiated rainbow trout: shear force/displacement curve for muscle taken immediately *post mortem*.
Mean values ± s.e.m. for six fish

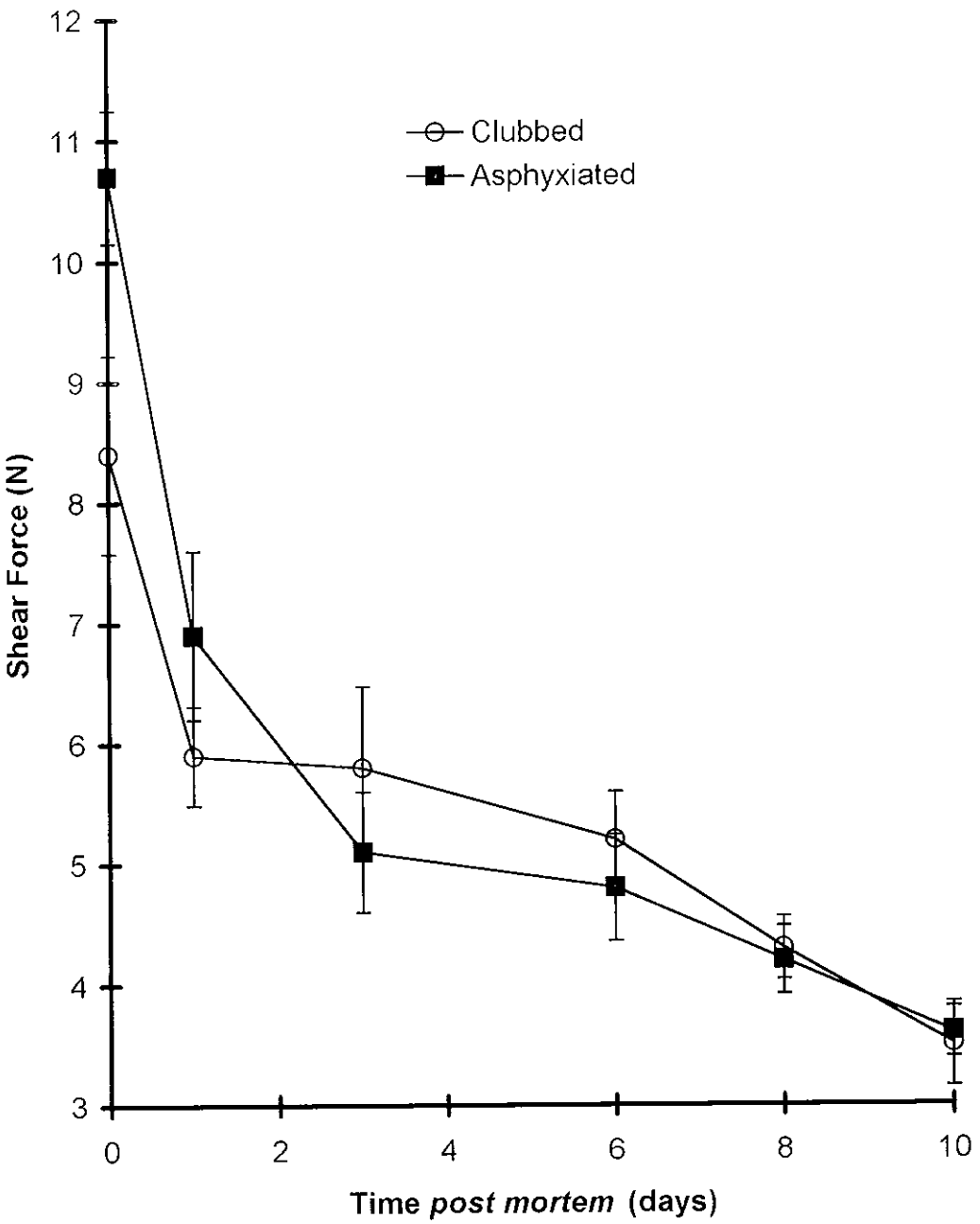


Fig. 56. Myotomal muscle of clubbed and asphyxiated rainbow trout: changes in shear force at 3°C.
Mean values ± s.e.m. for groups of six fish.

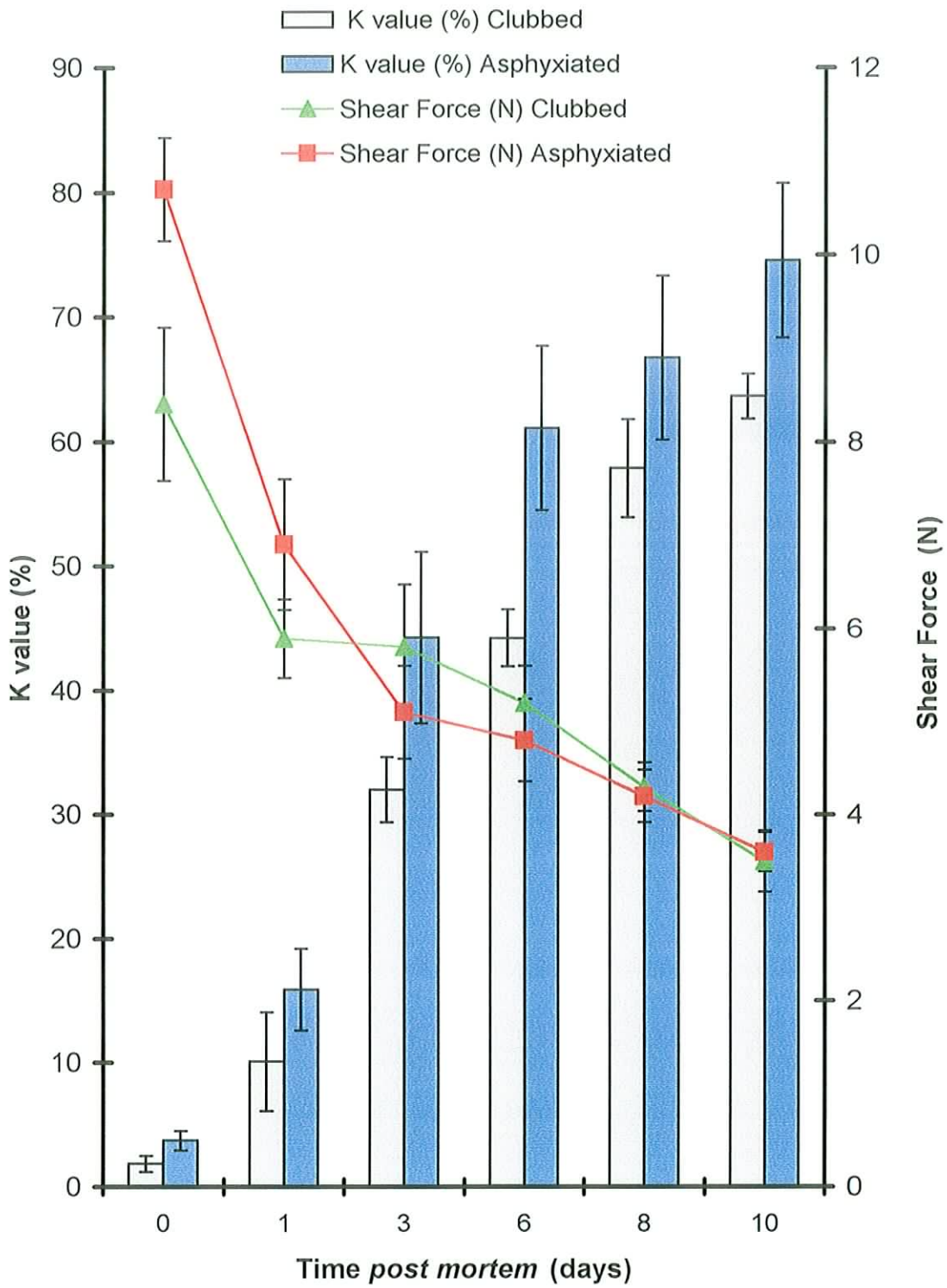


Fig. 57a. Myotomal muscle of clubbed and asphyxiated rainbow trout: changes in K value and changes in shear force at 3°C.
Mean values ± s.e.m. for groups of six fish.

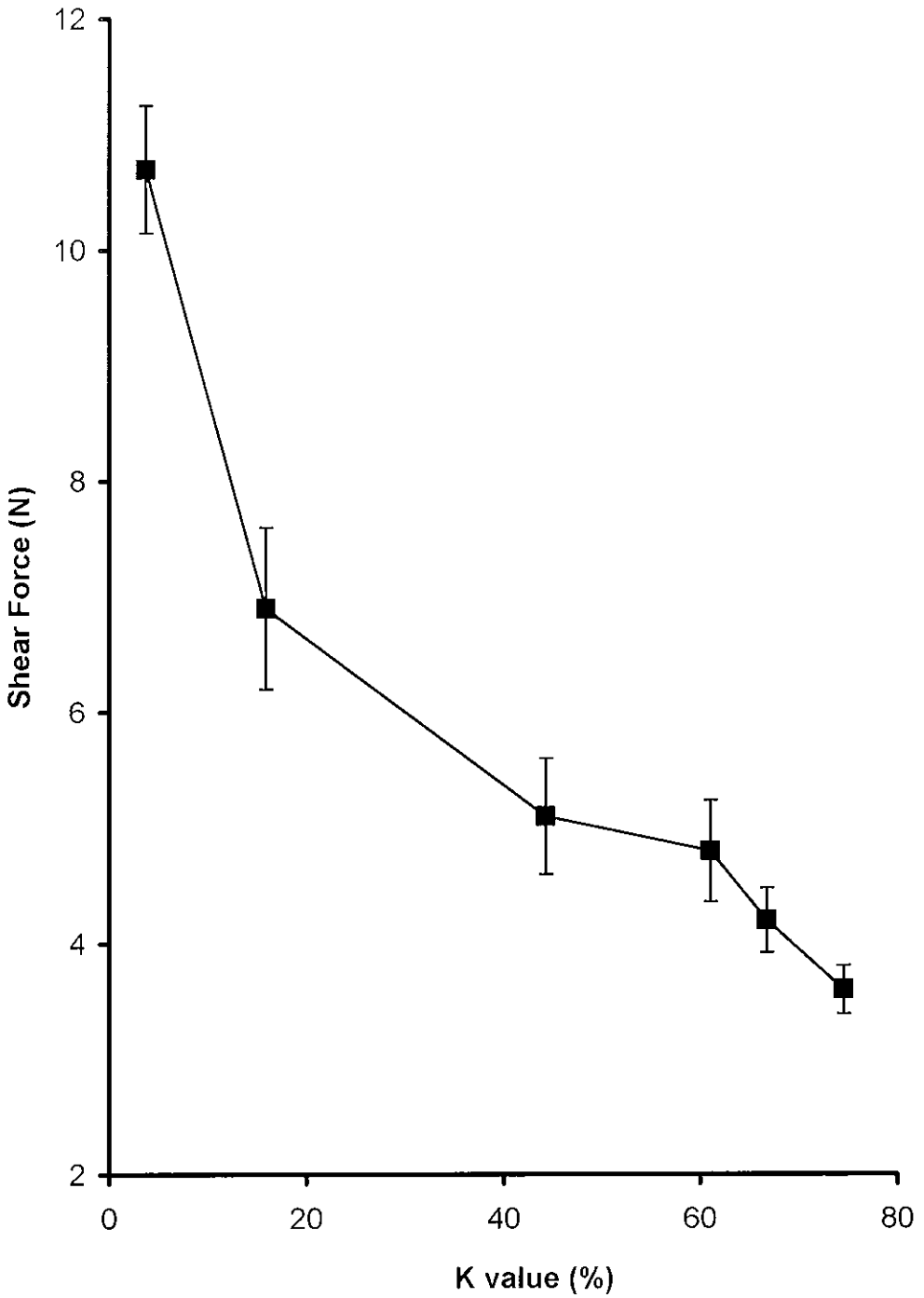


Fig. 57b. Shear force and K values in myotomal muscle of rainbow trout at 3°C.

3.2.8 Muscle held at –30°C

In these experiments the muscle samples rather than the fish carcasses were held at –30°C. This procedure was carried out because of the difficulty encountered in trying to excise muscle from the frozen fish carcass.

Fish (approximately 500g in weight and 37cm in length) were obtained from Idas Fish Farms and were transported live to the laboratory as previously described. Two groups were used. The fish (6) in group 1 were clubbed and the fish (6) in group 2 were asphyxiated (sections, 2.2.2 and 2.2.3). Zero hour muscle samples were taken (section 2.4). Further muscle samples were excised from the right-hand side and left-hand side of the epaxial muscle mass, put into pre-labelled freezer bags and held on ice for two hours prior to frozen storage. The muscle samples taken from the left side were used for biochemical analysis and those from the right side were used for texture. Biochemical analysis and texture measurements were carried out at 6,12, 18 and 24 weeks.

3.2.8.1 ATP and Metabolites

Changes in the concentration of ATP during frozen storage are shown in Fig. 58. The mean zero hour concentration of ATP was 6.86 ± 0.51μmol/g for the clubbed fish and 3.36 ± 0.92μmol/g for the asphyxiated fish. The difference between these values was significant ($p < 0.001$). At 6 weeks storage the concentration of ATP for the clubbed and asphyxiated fish were very low (< 0.1μmol/g). The concentrations of ADP were 1.00 ± 04μmol/g for the clubbed fish and 0.85 ± 0.19μmol/g for the asphyxiated (Fig. 59). Zero hour concentrations of AMP were very low (< 0.1μmol/g) for both groups of fish (Fig. 60). The mean initial concentrations of IMP were 1.49 ± 0.33μmol/g for the clubbed fish and 4.02 ± 1.15μmol/g for the asphyxiated (Fig. 61). At 24 weeks the

concentrations of IMP were 8.67 ± 0.25μmol/g for the clubbed fish and 9.09 ± 0.88μmol/g for the asphyxiated. The zero hour concentrations of INO and Hx were very low at the start of the experiment but increased in both groups of fish (Fig. 62 and 63). At 24 weeks the concentrations of INO were 1.14 ± 0.11μmol/g for the clubbed fish and 1.27 ±0.43μmol/g for the asphyxiated fish while the concentrations of Hx were < 0.2μmol/g for both groups of fish.

3.2.8.2 K value

Changes in the K value are given in Fig. 64. The zero hour K value for the clubbed fish (1.99 ± 1.06%) and asphyxiated fish (2.53 ± 1.09%) were not significantly ($p > 0.05$) different. The K value increased during storage for the two groups of fish. At 6 weeks the K value for the clubbed fish had increased significantly ($p < 0.001$) from an initial value of 1.99 ± 1.06% to 10.43 ± 2.06% while that for the asphyxiated increased from 2.53 ± 1.09% to 13.49 ± 4.69%. At 24 weeks the K values for the clubbed fish (12.21 ± 0.80%) and the asphyxiated (13.49 ± 5.08%) were not significantly different.

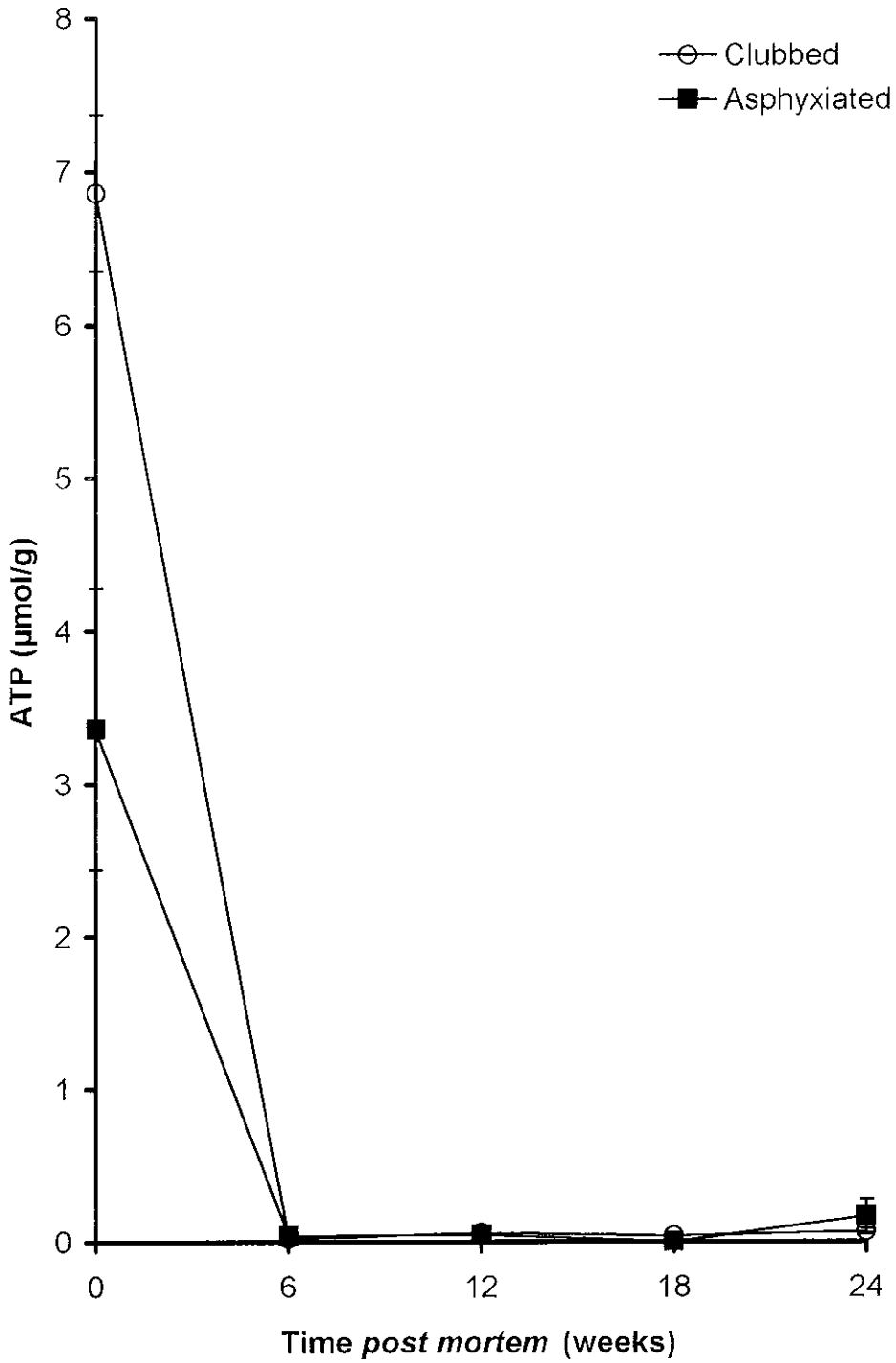


Fig. 58. Myotomal muscle of clubbed and asphyxiated rainbow trout: changes in the concentration of ATP at –30°C.
Mean values ± s.e.m. for groups of six fish.

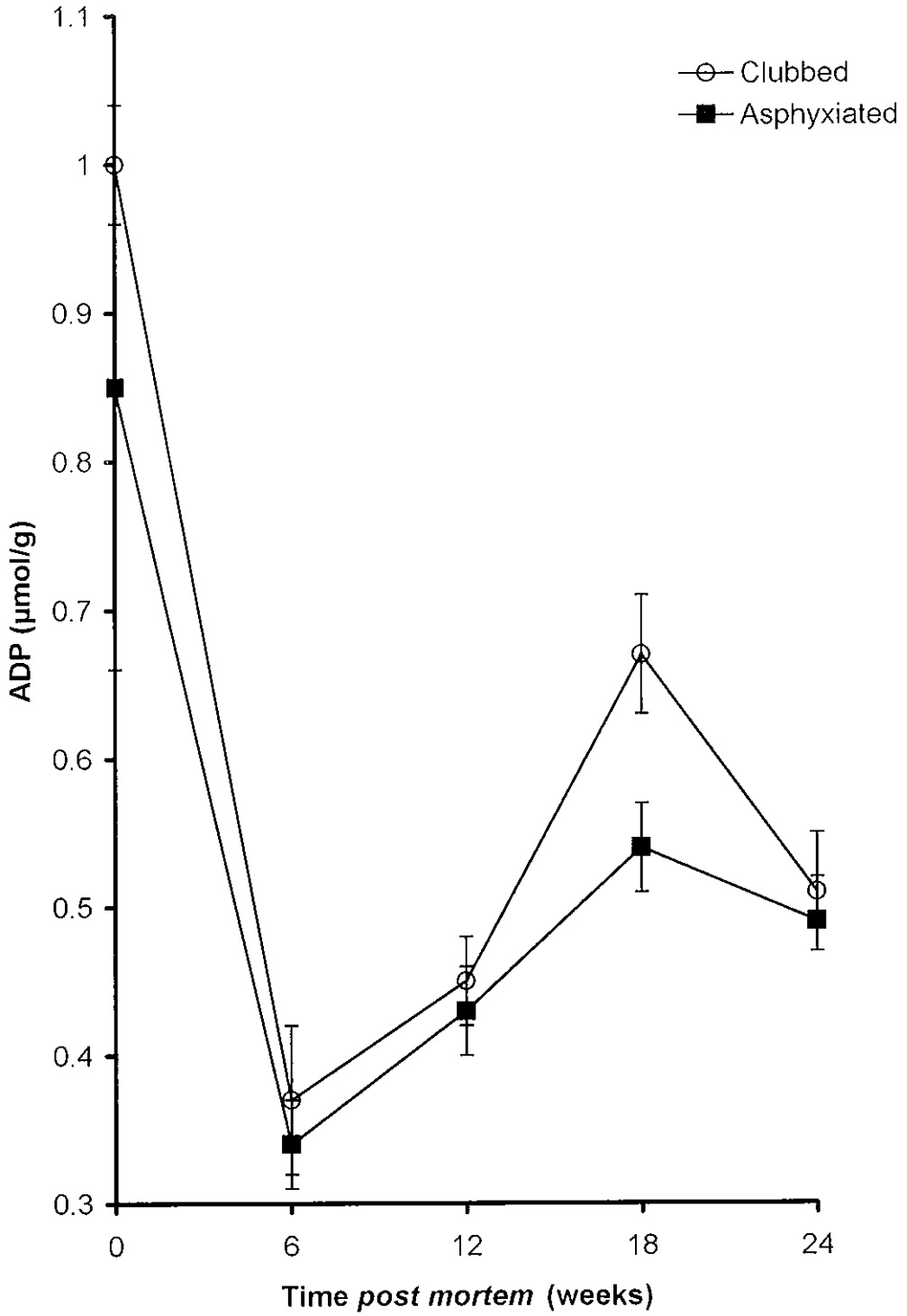


Fig. 59. Myotomal muscle of clubbed and asphyxiated rainbow trout: changes in the concentration of ADP at –30°C.
Mean values ± s.e.m. for groups of six fish.

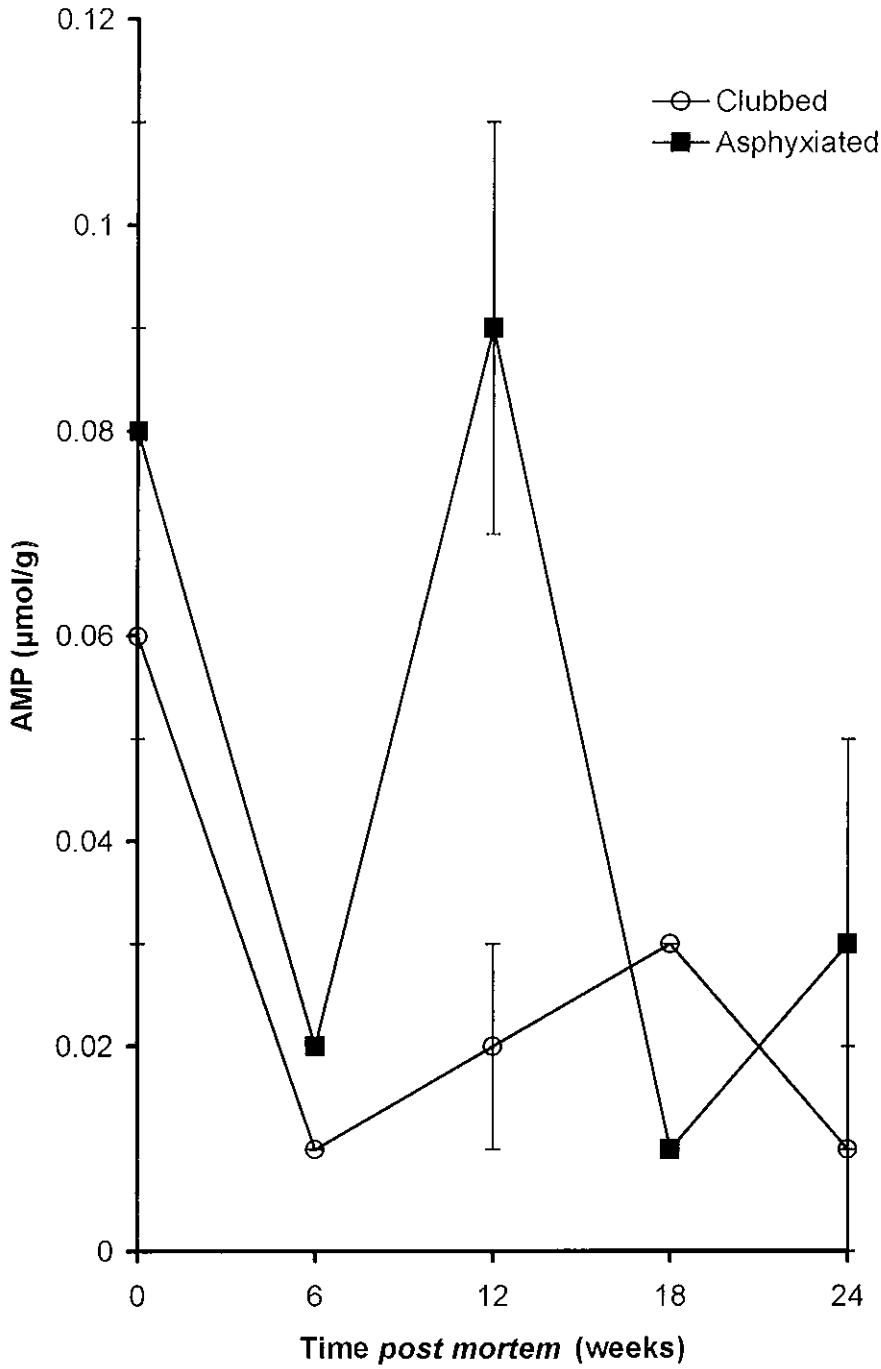


Fig. 60. Myotomal muscle of clubbed and asphyxiated rainbow trout: changes in the concentration of AMP at –30°C.
Mean values ± s.e.m. for groups of six fish.

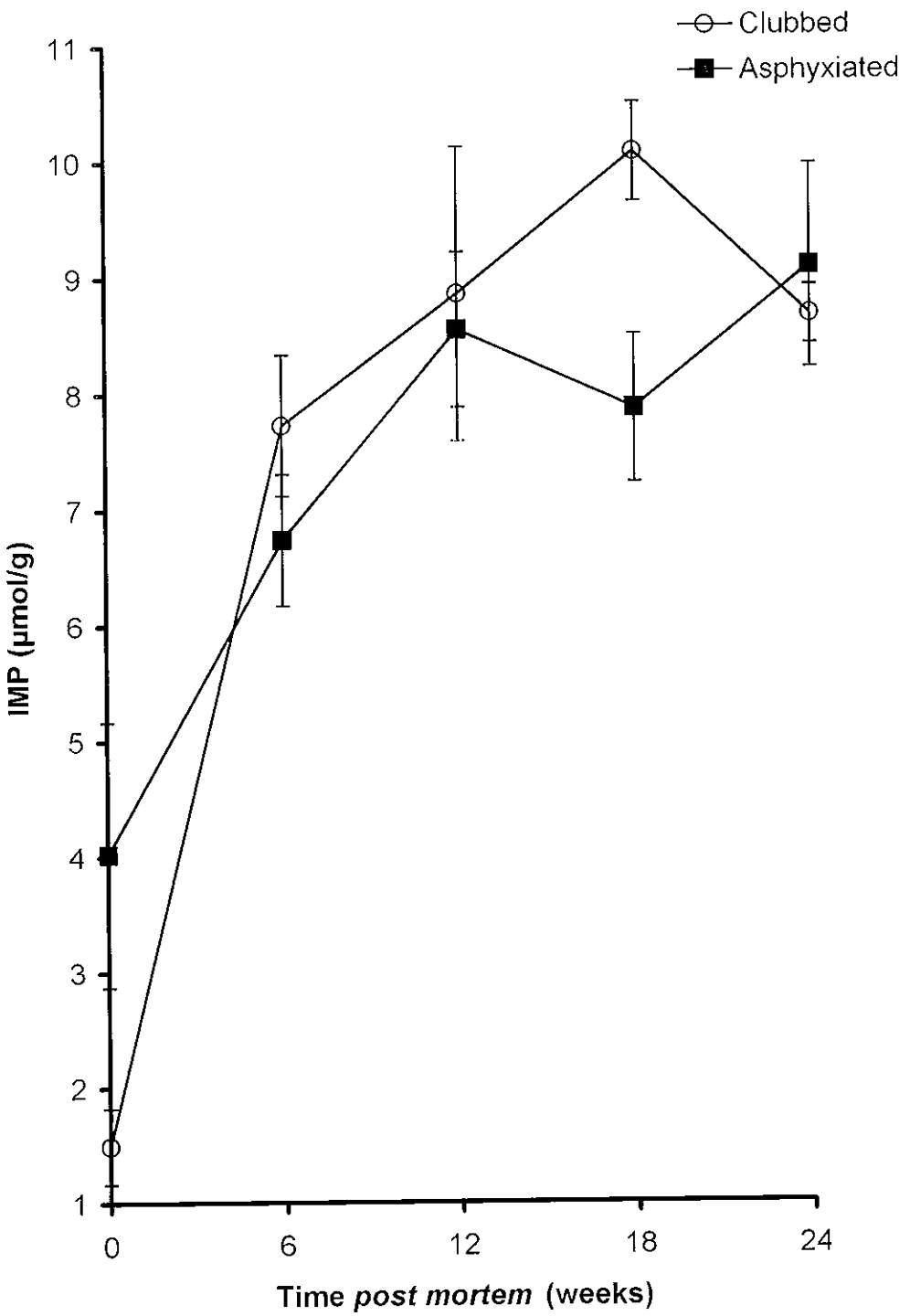


Fig. 61. Myotomal muscle of clubbed and asphyxiated rainbow trout: changes in the concentration of IMP at –30°C.
Mean values ± s.e.m. for groups six fish.

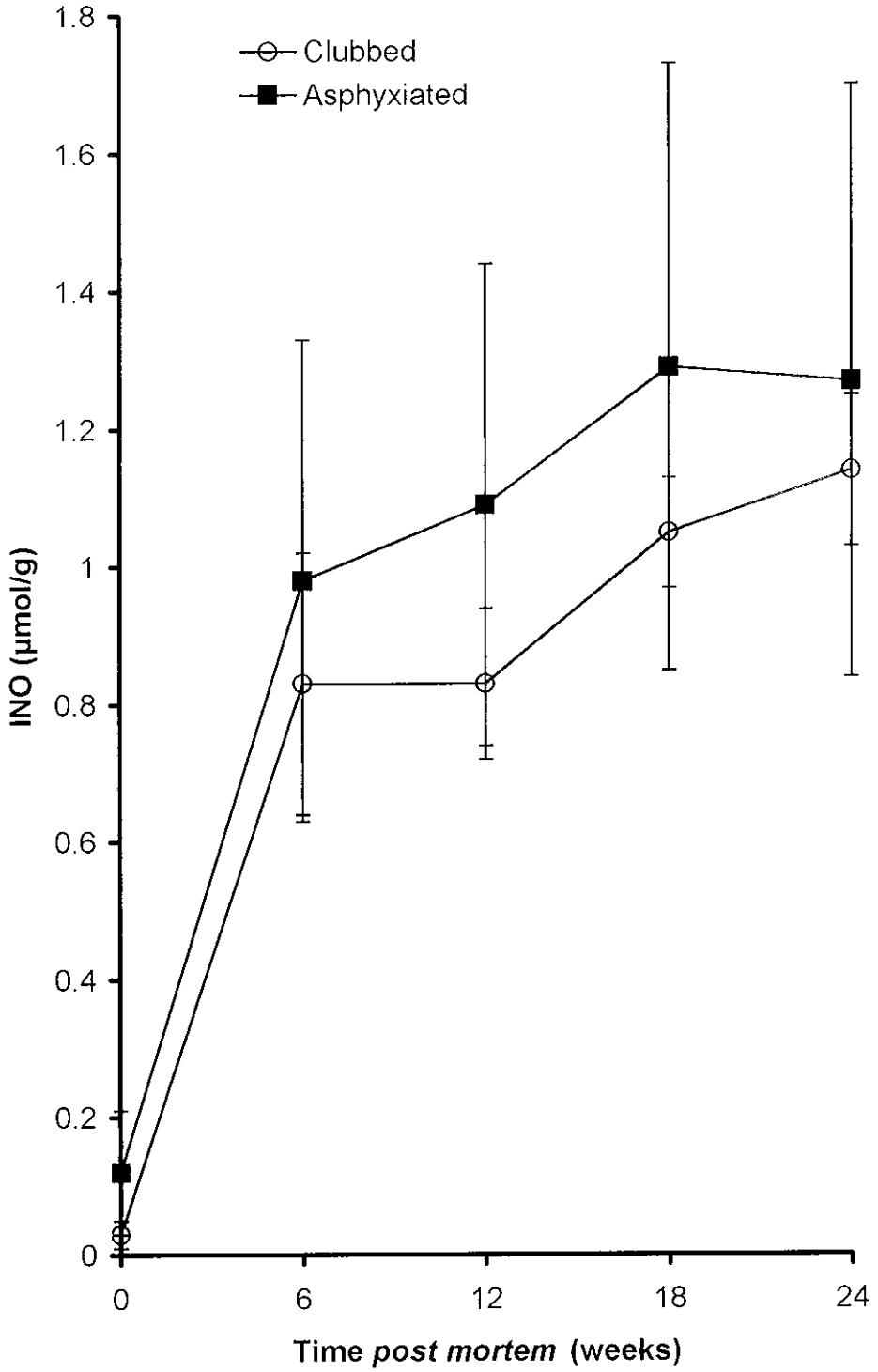


Fig. 62. Myotomal muscle of clubbed and asphyxiated rainbow trout: changes in the concentration of INO at –30°C.
Mean values ± s.e.m. for groups of six fish.

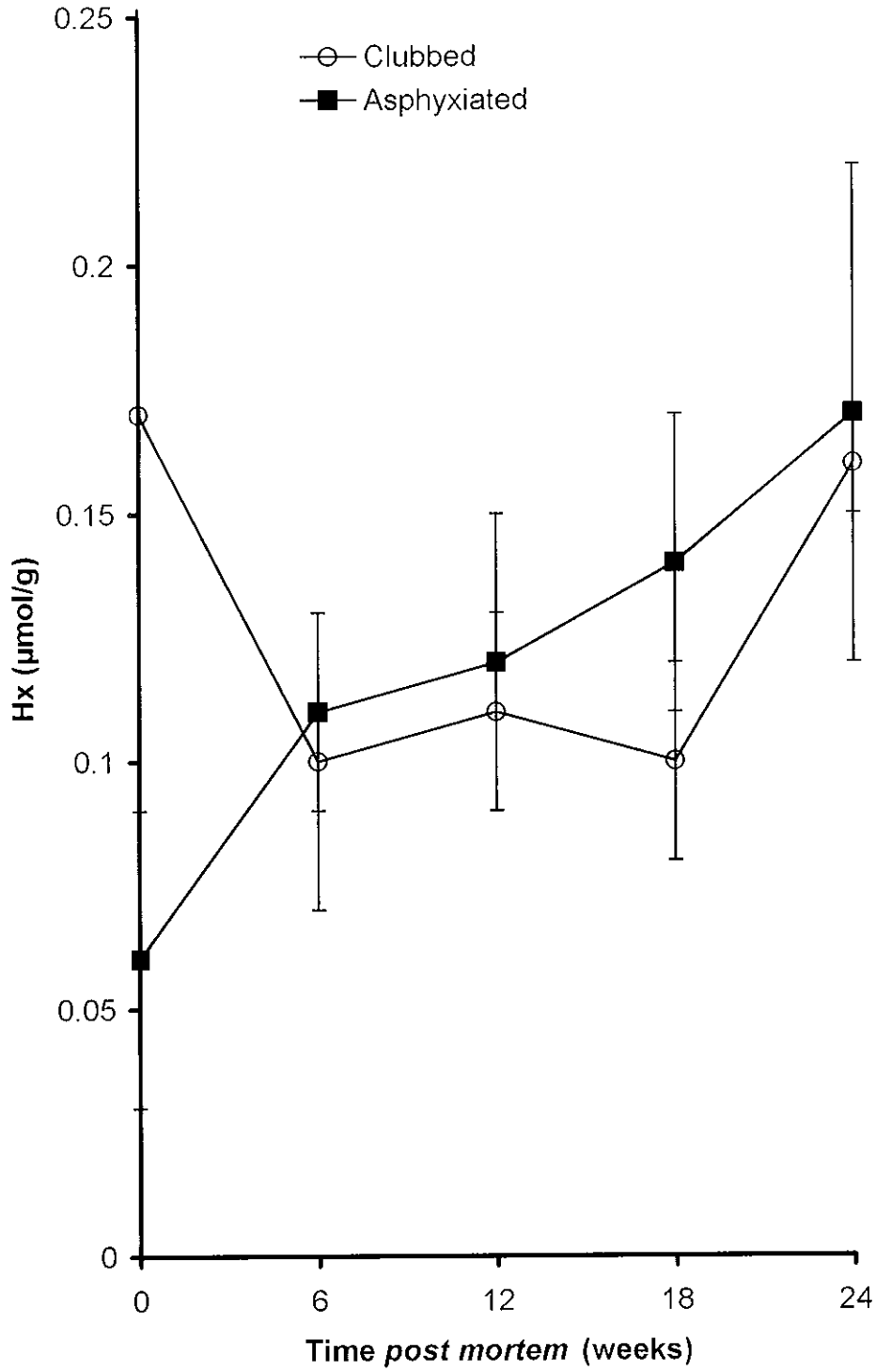


Fig. 63. Myotomal muscle of clubbed and asphyxiated rainbow trout: changes in the concentration of Hx at –30°C.
Mean values s.e.m. for groups of six fish.

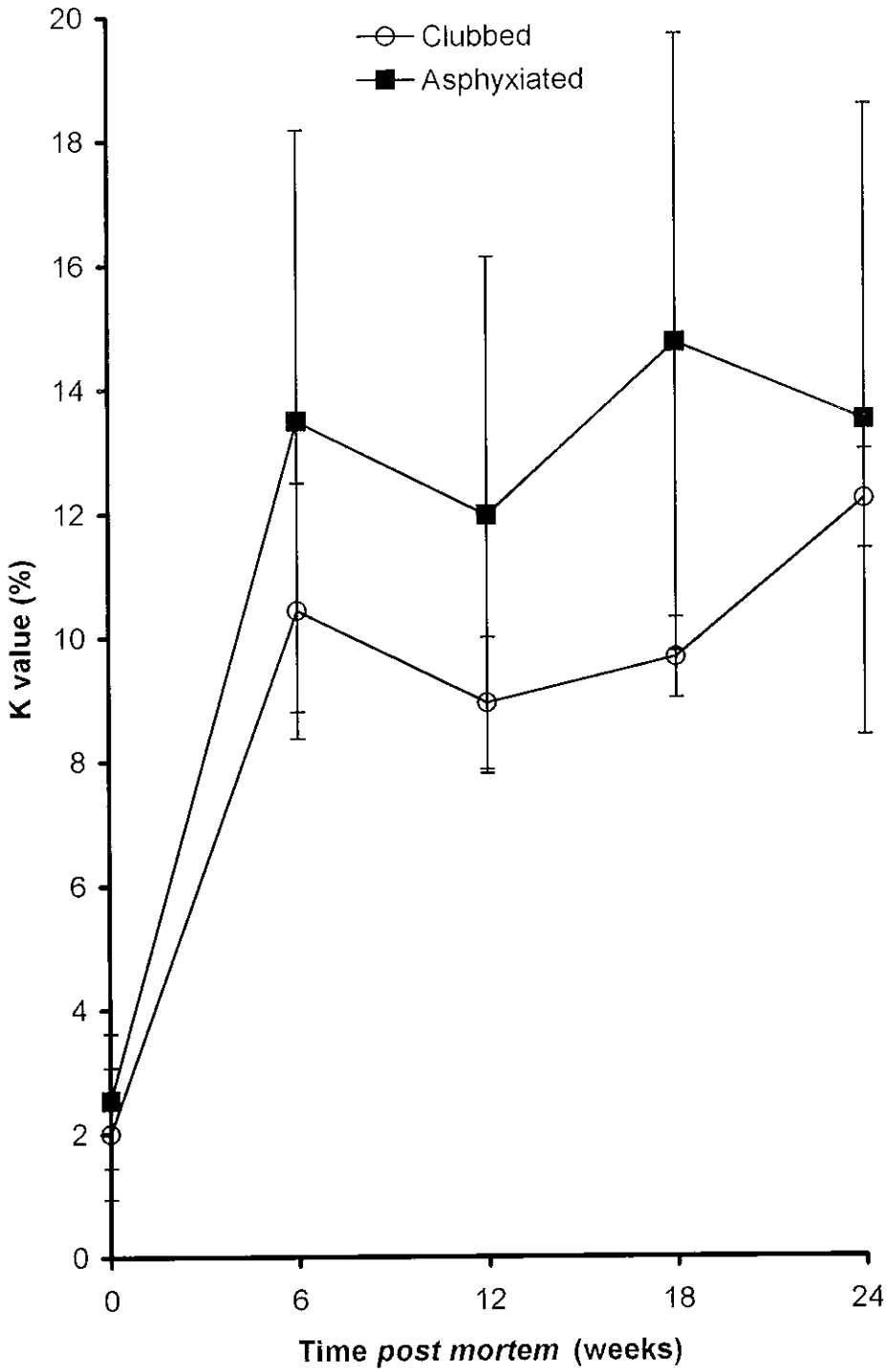


Fig. 64. Myotomal muscle of clubbed and asphyxiated rainbow trout: changes in K value at –30°C.
Mean values ± s.e.m. for groups of six fish.

3.2.9 Shear Force

Changes in shear force for clubbed and asphyxiated fish during storage are shown in Fig. 65. The shear force required to cut the muscle sample of clubbed fish immediately *post mortem* was 10.00 ± 0.40N compared with 8.50 ± 0.47N for the asphyxiated fish and the difference was significant ($p < 0.05$). At 6 weeks there was a significant (p 0.001) decrease in the shear force values for clubbed (4.00 ± 0.28N) and asphyxiated (4.00 ± 0.48N) fish. The muscle samples were very soft for the clubbed and asphyxiated fish at this time and a loss of liquid was observed as the Warner-Bratzler blade sheared through the muscle sample. Shear force values remained constant for the two groups of fish up to 18 weeks. At 24 weeks there was a slight decrease in shear force for the clubbed fish.

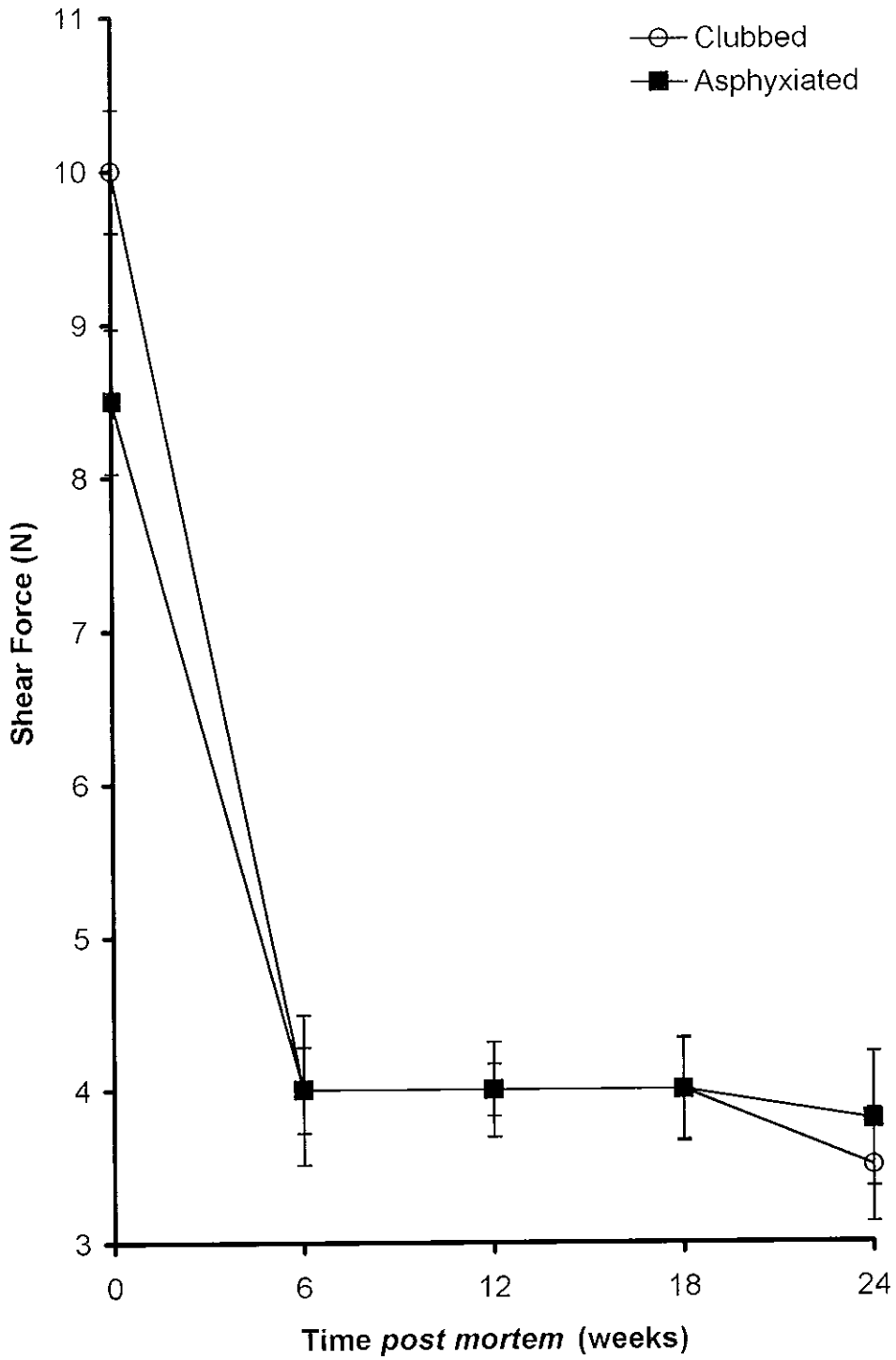


Fig. 65. Myotomal muscle of clubbed and asphyxiated rainbow trout: changes in Shear force at –30°C.
Mean values ± s.e.m. for groups of six fish.

Overall Comment

In these experiments the depletion of ATP and other biochemical changes were related to texture measurements of clubbed and asphyxiated fish. The results showed that concentrations of ATP in the clubbed fish remained constant for up to 5 hours *post mortem* compared with the asphyxiated fish (Fig. 36). A similar pattern was observed for texture measurements where the shear force required to cut the muscle of clubbed fish remained constant after 3 hours *post mortem* (Fig. 44). Muscle samples maintained at -30°C showed no significant increase in K values after 6 weeks (Fig. 64) A similar pattern was observed with the texture of the fish muscle, where shear force values did not decrease after 6 weeks storage (Fig. 65). There appeared to be a relationship between K value and shear force. As the K value increased with time indicating a loss of freshness, there was a decrease in shear force required to cut the muscle.

3.3 Evaluation of Fish Freshness

Three rapid methods were used to assess the freshness of rainbow trout. The methods were (a) biochemical using a Rapid Paper Strip Method (RPS) for measuring the K value, (b) physical using the Torrymeter and (c) sensory using the Quality Index Method (QIM). Since the Rapid Paper Strip Method is semiquantitative the results obtained using the Rapid Paper Strip method were compared with the K_1 value and the K value, both of which were derived from the HPLC method.

3.3.1 Rainbow Trout

The fish used in these experiments were obtained from Idas Fish Farms, Woodenbridge, Co Wicklow and were the same fish that were used in the experiments reported in Results, Section 3.2.5. The results for the biochemical analysis and texture measurements for these fish are described in Sections, 3.2.5.1 and 3.2.7 respectively.

3.3.1.1 K value (RPS) Method to Assess Fish Freshness

Changes in K value in rainbow trout maintained at 3°C are shown in Fig. 66. The zero hour K value was 5.83 ± 0.91% for the clubbed fish and 6.67 ± 1.15% for the asphyxiated fish. At day 1 the K value for the clubbed fish was 10.83 ± 0.91% while that for asphyxiated fish was 15.00 ± 2.00%. The difference between these values was significant ($p < 0.01$). K values increased with time for the two groups of fish. Significantly ($p < 0.01$) higher K values were obtained for the asphyxiated fish during storage, with the exception of day 8 when there was no significant difference between clubbed and asphyxiated fish.

3.3.1.1.1 K value, K_1 value and K (RPS) value

The results are given in Table 20. The zero hour K value was 1.84 ± 0.64% for the clubbed and 3.72 ± 0.78% for the asphyxiated fish. The difference between these values was significant ($p < 0.01$). The K_1 values for the clubbed and asphyxiated fish were 5.55 ± 1.12% and 4.32 ± 0.68% respectively while the RPS values were 5.83 ± 0.91% for the clubbed and 6.67 ± 1.15% for the asphyxiated fish. There was a significant ($p < 0.01$) difference between the K value (1.84 ± 0.64%) and the K_1 value (5.54 ± 1.12%) for the clubbed fish immediately *post mortem*. There was also a significant ($p < 0.01$) difference between the K value (1.84 ± 0.64%) and the RPS method (5.83 ± 0.91%) for the clubbed fish immediately after death, but not between the K_1 (5.55 ± 1.12%) and the RPS (5.83 ± 0.91%) for these fish immediately *post mortem*. The mean initial K value for the asphyxiated was 3.72 ± 0.78% compared with 6.67 ± 1.15% using the RPS. At day 1 the K value was 10.08 ± 3.39% for the clubbed fish and 15.91 ± 3.29% for the asphyxiated. The difference between these values was significant ($p < 0.01$). Significant ($p < 0.05$) differences were also found between clubbed and asphyxiated fish using the K_1 value and the RPS method. However, differences between the three methods used to estimate the K value were not significant ($p > 0.05$). The K values increased during storage for both groups of fish. At day 6, there was a significant ($p < 0.01$) difference between the K_1 value (47.59 ± 2.61%) and the RPS method (39.17 ± 2.19%) for the clubbed fish but not for the asphyxiated fish. At day 10, the K value was 63.66 ± 1.79% for the clubbed and 74.54 ± 6.19% for the asphyxiated fish, but the difference was not significant ($p > 0.05$). The K_1 values for the clubbed and asphyxiated fish were 67.13 ± 2.00% and 78.34 ± 6.64% respectively, while the RPS values were 60.83 ± 2.61% for the clubbed and 73.33 ± 6.11% for the asphyxiated fish. These differences were not significant ($p > 0.05$).

There were no significant differences between the K value, the K_1 value and the Rapid Paper Strip method for the two groups of fish during storage at 3°C. Hattula (1996) found that results with the RPS method did not agree with the K_1 value obtained by HPLC but correlated well with the sensory quality of fish.

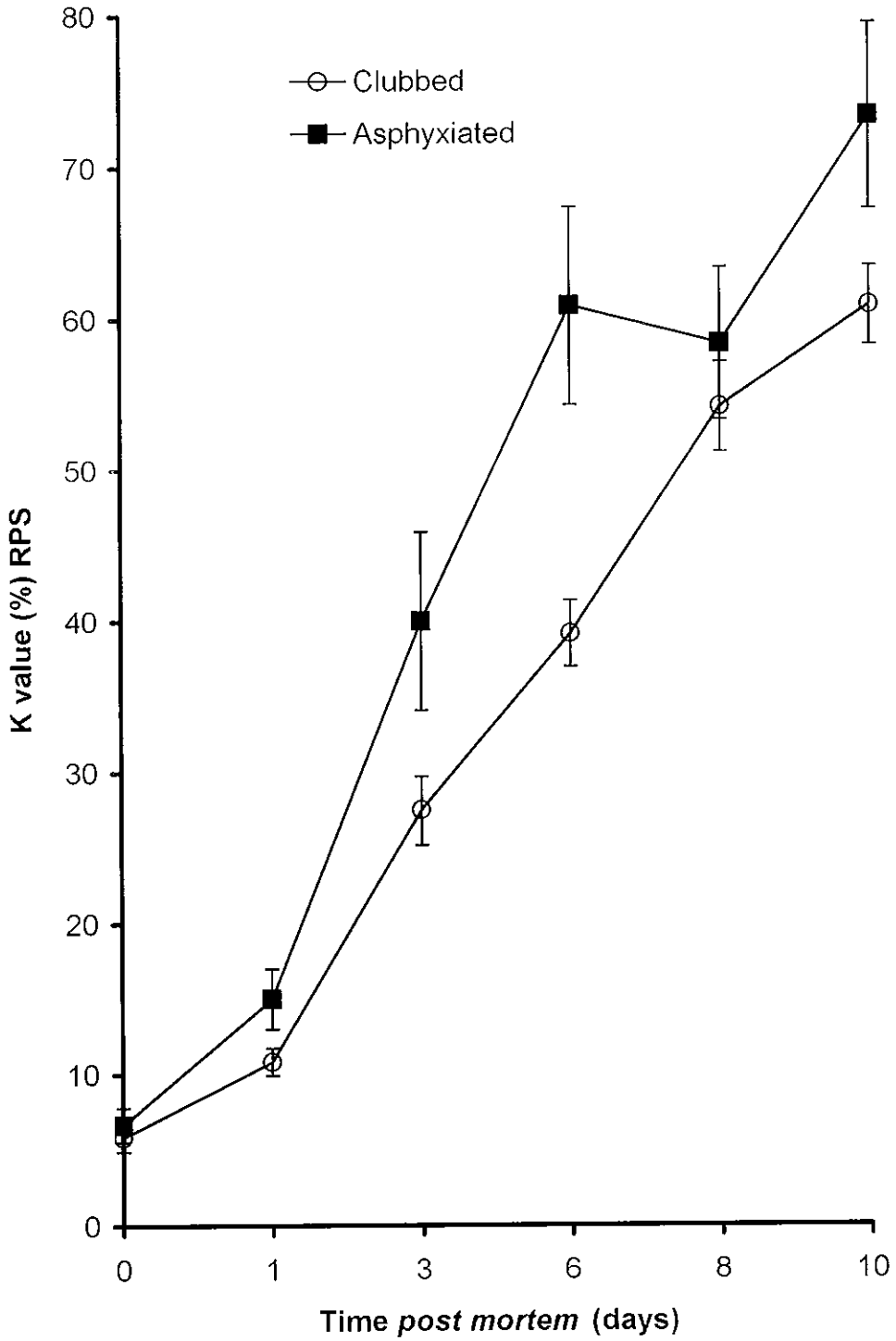


Fig. 66. Myotomal muscle of clubbed and asphyxiated rainbow trout: K value (RPS) at 3°C.
Mean values ± s.e.m. for groups of six fish.

Time *post mortem*	Method of Killing	K value (%)	K_1 value (%)	K value (%) (RPS)
0 hour	Clubbed	1.84 ± 0.64	5.55 ± 1.12	5.83 ± 0.91
	Asphyxiated	3.72 ± 0.78	4.32 ± 0.68	6.67 ±1.15
Day 1	Clubbed	10.08 ± 3.98	10.81 ± 1.93	10.83 ± 0.91
	Asphyxiated	15.91 ± 3.29	16.68 ± 3.45	15.00 ± 2.00
Day 3	Clubbed	32.00 ± 2.63	33.29 ± 2.60	27.50 ± 2.35
	Asphyxiated	44.26 ± 6.90	46.50 ± 7.28	40.00 ± 5.90
Day 6	Clubbed	44.23 ± 2.29	47.59 ± 2.61	39.17 ± 2.19
	Asphyxiated	61.09 ± 6.58	64.08 ± 6.91	60.83 ± 6.54
Day 8	Clubbed	57.87 ± 3.93	62.49 ± 4.15	54.16 ± 2.97
	Asphyxiated	66.74 ± 6.56	70.56 ± 6.64	58.33 ± 5.03
Day 10	Clubbed	63.66 ± 1.79	67.13 ± 2.00	60.83 ± 2.61
	Asphyxiated	74.54 ± 6.19	78.34 ± 6.64	73.33 ± 6.11

Table 20. Myotomal muscle of clubbed and asphyxiated rainbow trout: changes in the K value, the K_1 value and K value (RPS) at 3°C.
Mean values ± s.e.m. for groups of six fish.

3.3.2 Excised Muscle Maintained at –30°C

The fish were obtained from Idas Fish Farms and were the same fish which were used in the experiments reported in Results, Section 3.2.8. Two groups of fish were used and were killed by clubbing and asphyxiation (sections, 2.2.2 and 2.2.3). The results of the biochemical analysis and texture measurements for these fish are described in Results, Sections, 3.2.8.1 and 3.2.9 respectively.

3.3.2.1 A Comparison of the Rapid Paper Strip and the K_1 value

The zero hour K_1 values for the clubbed and asphyxiated fish were 8.19 ± 2.16% and 5.10 ± 1.24% respectively and were significantly ($p < 0.05$) different (Fig. 67). The K values by the Rapid Paper Strip were 5.83 ± 0.91% for the clubbed and 6.00 ± 1.12% for the asphyxiated fish. At 6 weeks there was a significant ($p < 0.01$) increase in K values for clubbed and asphyxiated fish using the Rapid Paper Strip, and for the K_1 value for the asphyxiated fish, but not for the clubbed fish. At 24 weeks the K_1 values for the clubbed and asphyxiated fish were 12.96 ± 0.90% and 14.40 ± 5.36% while the K values by the Rapid Paper Strip were 15.83 ± 0.91% for the clubbed and 17.00 ± 4.60% for the asphyxiated fish. These values were not significantly ($p > 0.05$) different to those values obtained after 6 weeks storage.

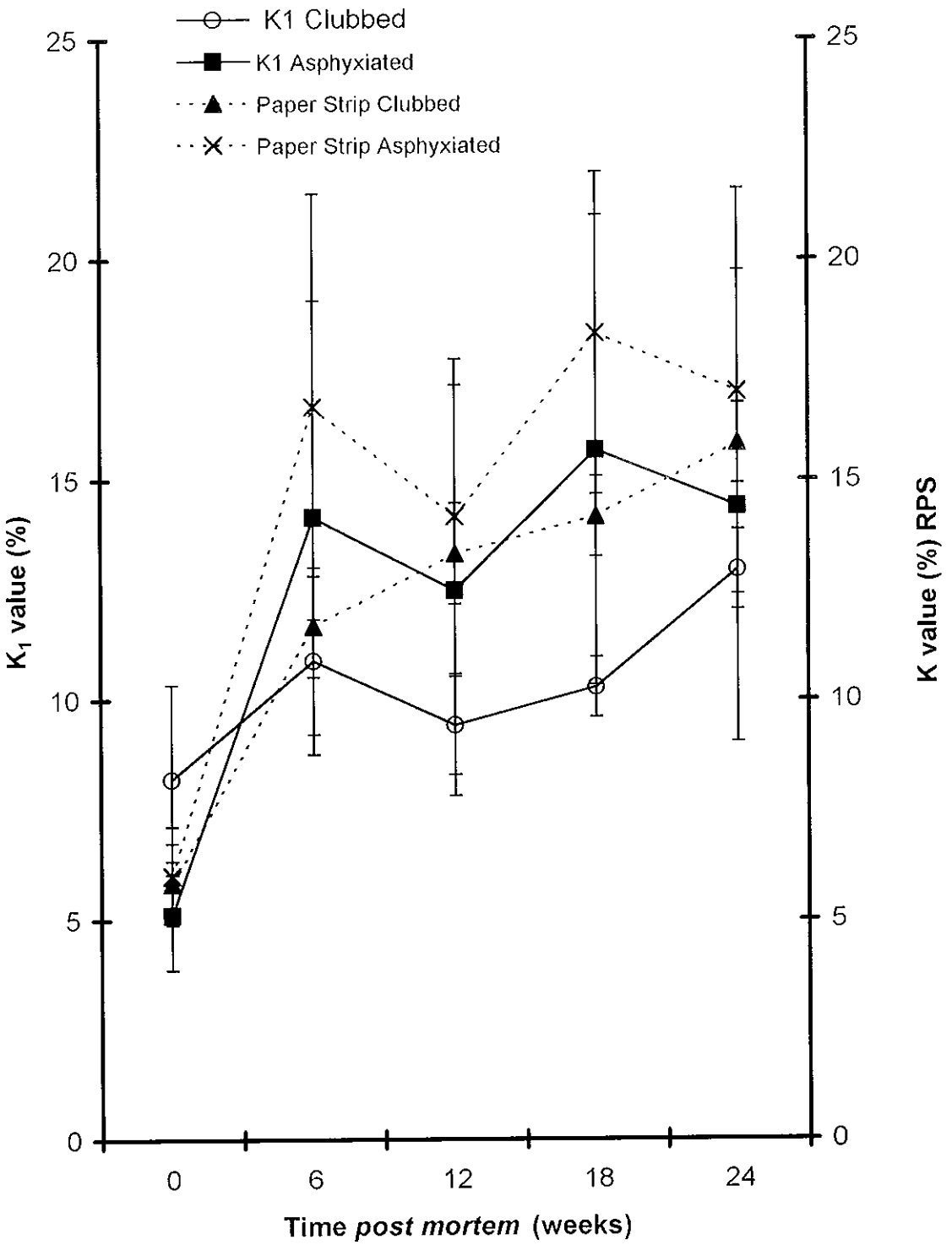


Fig. 67. Myotomal muscle of clubbed and asphyxiated rainbow trout: K_1 value and K value (RPS) at –30°C.
Mean values ± s.e.m. for groups of six fish.

Comment

There was good agreement between the K value and the K_1 value obtained by HPLC and the K value by the Rapid Paper Strip method. The zero hour K value for the clubbed (1.84 ± 0.64%) fish was significantly (p <0.01) lower than the K_1 value (5.55 ± 1.12%). This can be explained by the fact that the adenine nucleotides, namely, ATP ADP and AMP were omitted when using the K_1 method. The K values for excised fish muscle maintained at -30°C for 24 weeks were comparable to those reported for fish maintained at 3°C for one day (Fig. 66).

3.3.3 Assessment of Freshness of Fish using the Torrymeter

In these experiments the dielectric constant of fish muscle in rainbow trout was used as an index of fish freshness. Changes in the dielectric constant were measured using the Torrymeter as described in Materials and Methods (section 2.9). Torrymeter measurements were made on individual fish immediately *post mortem*, at hourly intervals up to 6 h and at 30 h. In the first experiment, the changes were studied in fish killed by clubbing. In the second series of experiments, fish killed by clubbing and asphyxiation were compared.

3.3.3.1 Clubbed Rainbow Trout

Three groups of fish were transported live to the laboratory, killed by clubbing (section 2.2.2) and held on ice during the experiments. Torrymeter readings were taken at zero, one, two and three hours and at 30 hours *post mortem*. The mean zero hour Torrymeter measurement for the fifteen clubbed fish was 10.80 ± 0.21 (Fig. 68). At 1 hour the value was 12.00 ± 0.18. The increase in the Torrymeter measurement from 10. 80 ± 0.21 to 12.00 ± 0.18 at 1 hour *post mortem* was significant ($p < 0.01$). Torrymeter measurements did not increase up to 3 hours *post mortem*. However, at 30 hours the value had increased to 12.6 ± 0.42 and was not significant (p 0.05). The biochemical analysis for these fish are described in Results, Section 3.1.3.

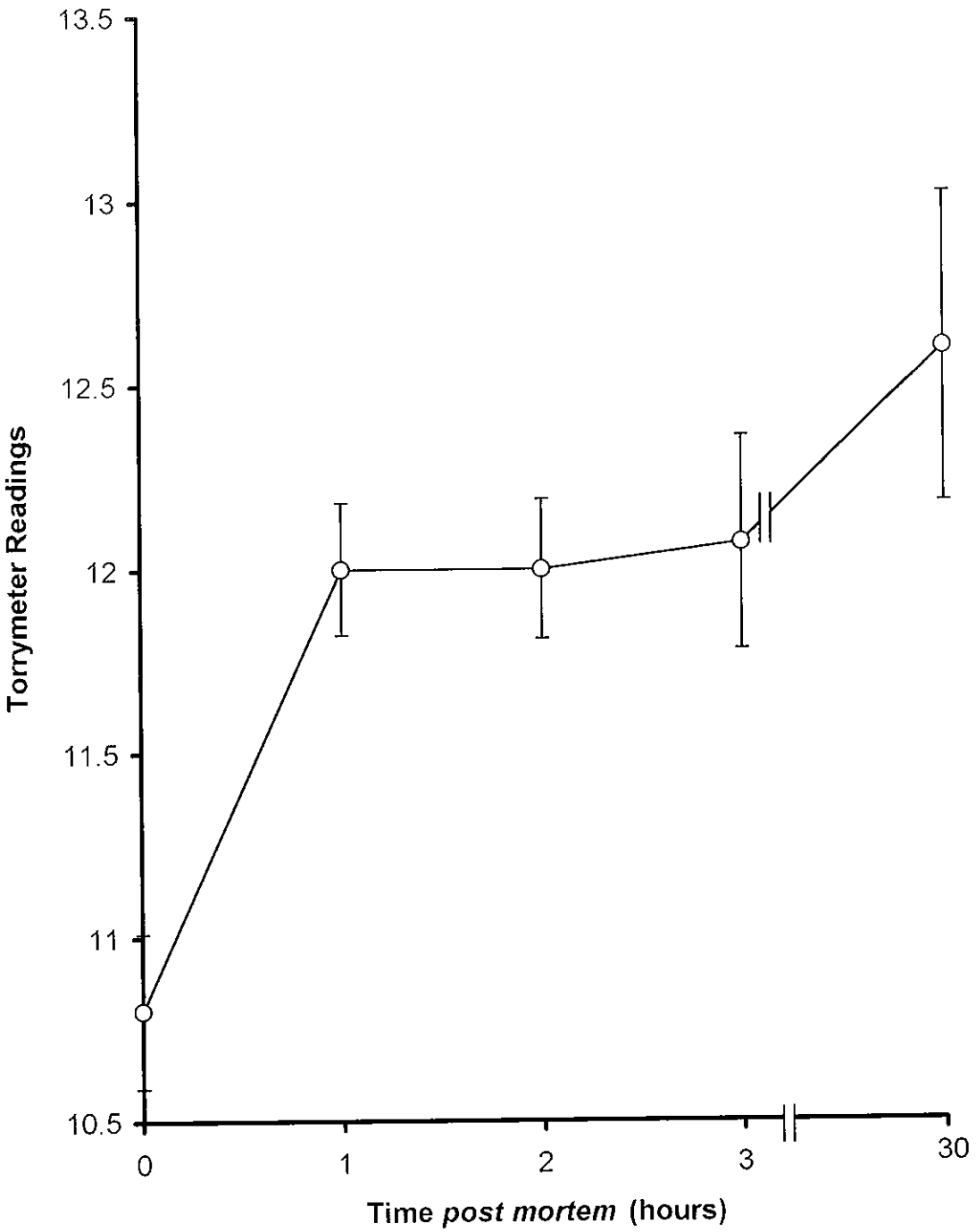


Fig. 68. Myotomal muscle of clubbed rainbow trout: Torrymeter readings at 3°C. Mean values ± s.e.m. for fifteen fish.

3.3.3.2 A Comparison of Clubbed and Asphyxiated Fish

In this series of experiments Torrymeter measurements were made on clubbed and asphyxiated fish. The fish were transported live to the laboratory and were the same fish that were used in the experiments reported in Results, Section 3.2.2. They were killed by clubbing and asphyxiation as described in Materials and Methods, sections 2.2.2 and 2.2.3 respectively. Torrymeter readings were made (a) immediately after death and at hourly intervals up to six hours *post mortem*, (b) immediately after death and daily intervals up to 10 days *post mortem* and (c) immediately after death and at daily intervals up to 20 days *post mortem*. The fish were held at 3°C during the experiments.

(a) Changes in Torrymeter readings during storage are shown in Fig. 69. The mean zero hour Torrymeter measurements for the clubbed and asphyxiated fish were 9.80 ± 0.22 and 11.30 ± 0.23. At 1 hour *post mortem* there was a slight increase in readings for the clubbed fish to 10.20 ± 0.22. Torrymeter readings for the asphyxiated fish increased slightly at 2 hours, then decreased and remained constant for up to 5 hours *post mortem*. The biochemical changes *post mortem* for these fish are described in Results, Section 3.2.2.1.

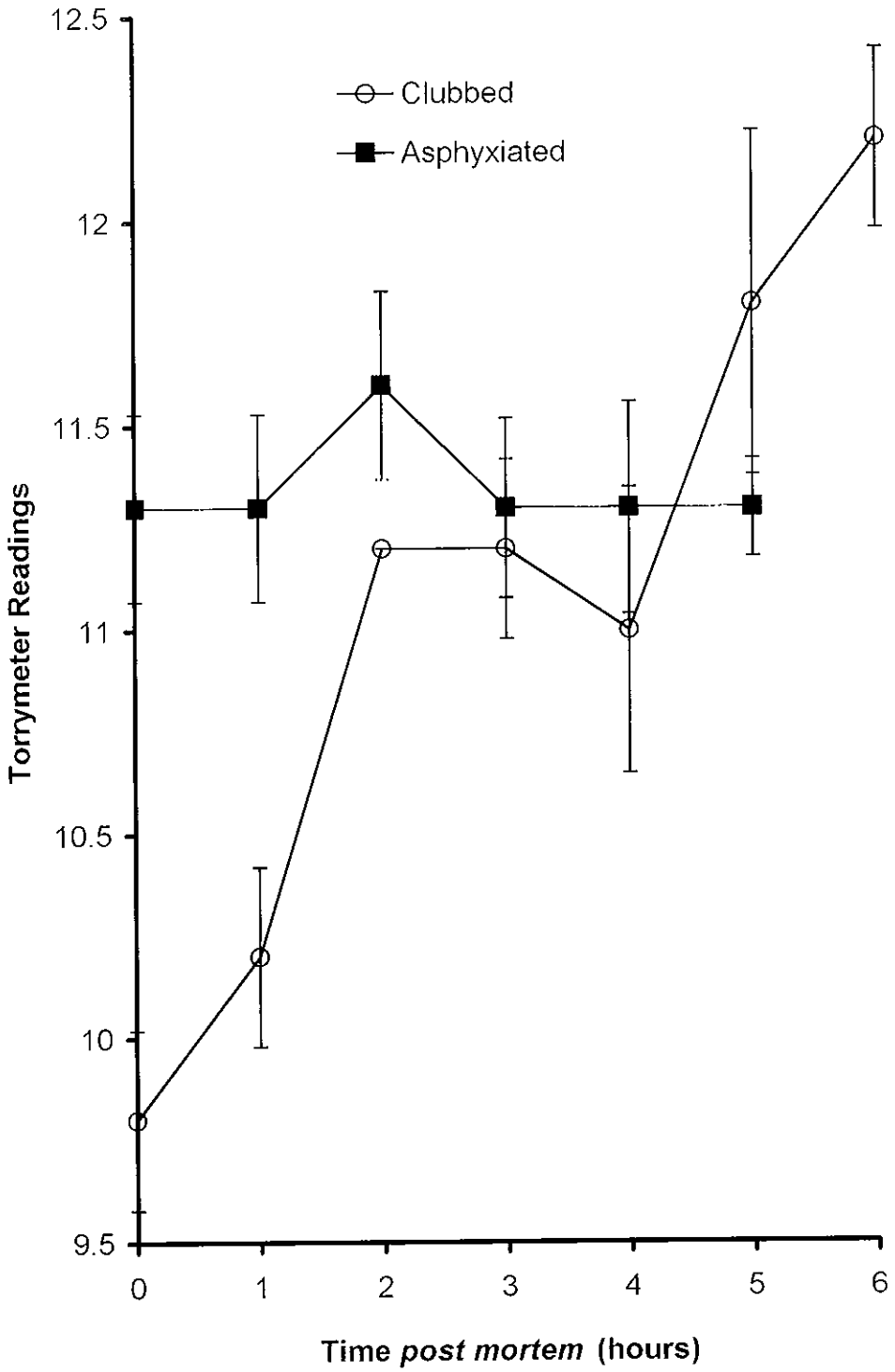


Fig. 69. Myotomal muscle of clubbed and asphyxiated rainbow trout: Torrymeter readings at 3°C.
Mean values ± s.e.m. for groups of five (clubbed) and six (asphyxiated) fish.

(b) The mean zero hour Torrymeter readings for the clubbed and asphyxiated fish were 10.17 ± 0. and 10.10.16 ± 0.18 respectively. Changes in Torrymeter readings are shown in Fig. 70. At day 1 there was a significant ($p< 0.001$) increase in the readings for the clubbed and asphyxiated fish. There was a steady decline in readings for the two groups of fish during storage. At day 6 the respective readings for the clubbed and asphyxiated fish were 10.00 ± 0.28 and 8.67 ± 0.23. The difference between these values was significant at $p < 0.001$. At day 10 *post mortem* the Torrymeter readings for the clubbed fish were 6.91 ± 0.02 and 6.82 ± 0.02 for the asphyxiated fish. The biochemical analyses and texture are described in Results, Section 3.2.5.1, and 3.2.7 respectively.

(c) The mean zero hour Torrymeter measurements for the clubbed and asphyxiated fish was fish were 10.05 ± 0.24 and 11.00 ± 0.35 respectively (Fig.71). By day 1 *post mortem* there was an increase to 11.83 ± 0.18 for the clubbed fish and 11.67 ± 0.23 for the asphyxiated. Torrymeter readings decreased subsequently for both groups of fish. A similar trend was observed for the fish in the previous experiment (section 3.4). By day 8 there was a significant ($p < 0.001$) difference in Torrymeter readings between clubbed (8.67 ± 0.23) and asphyxiated (7.00 ± 0.00) fish. At day 11 the values continued to fall to 7.17 ± 0.18 and 6.33 ± 0.23 for the clubbed and asphyxiated fish respectively. However, by day 13 there was a significant ($p< 0.001$) increase to 8.50 ± 0.37 for both groups of fish. By day 20 the Torrymeter readings for the clubbed and asphyxiated fish were 8.50 ± 0.68 and 6.33 ± 0.37 respectively. The difference between these values was significant ($p < 0.001$). The biochemical analyses for these fish are described in Results, Section 3.1.5.1.

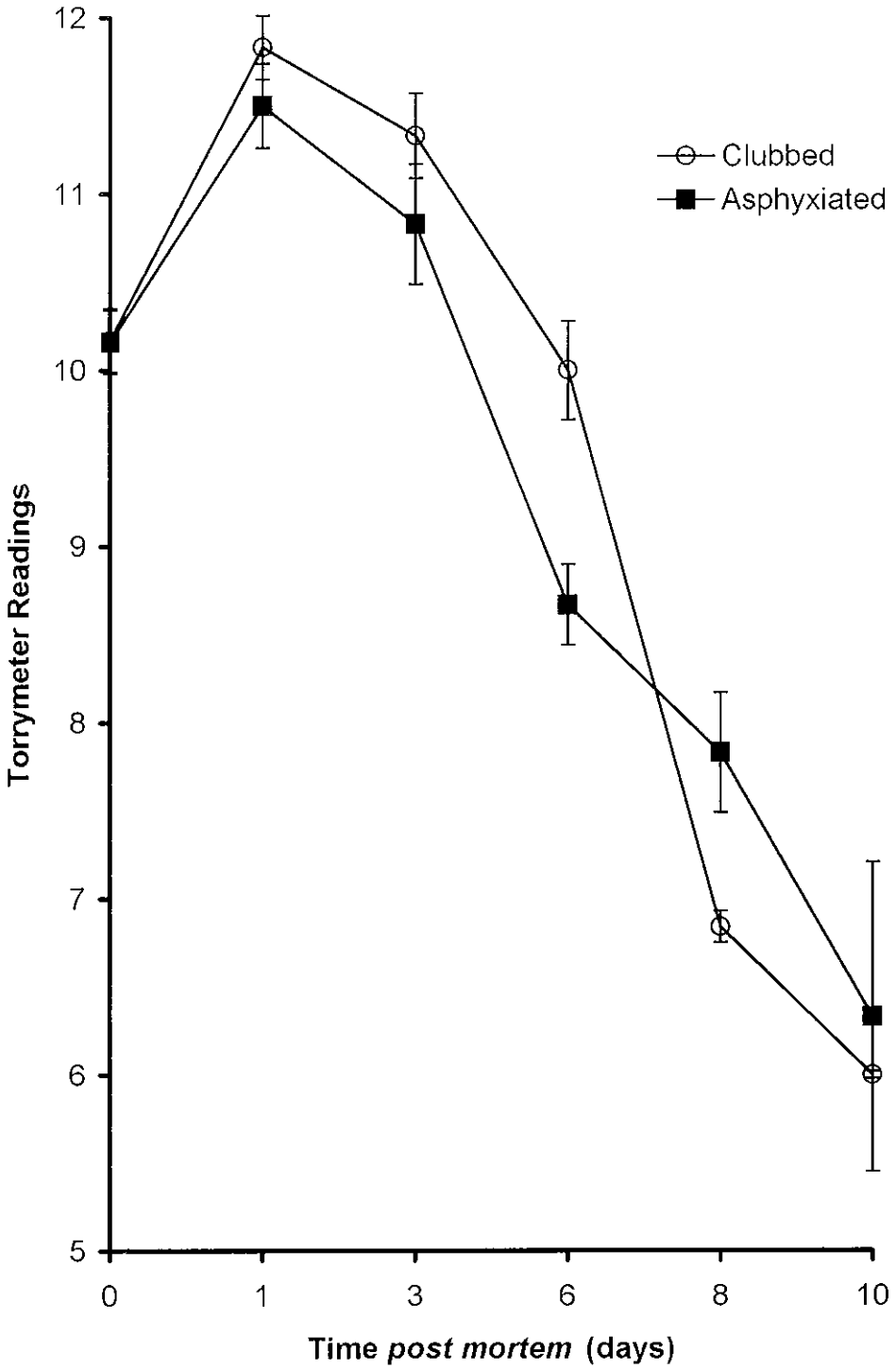


Fig. 70. Myotomal muscle of clubbed and asphyxiated rainbow trout: Torrymeter readings at 3°C.
Mean values ± s.e.m. for groups of six fish.

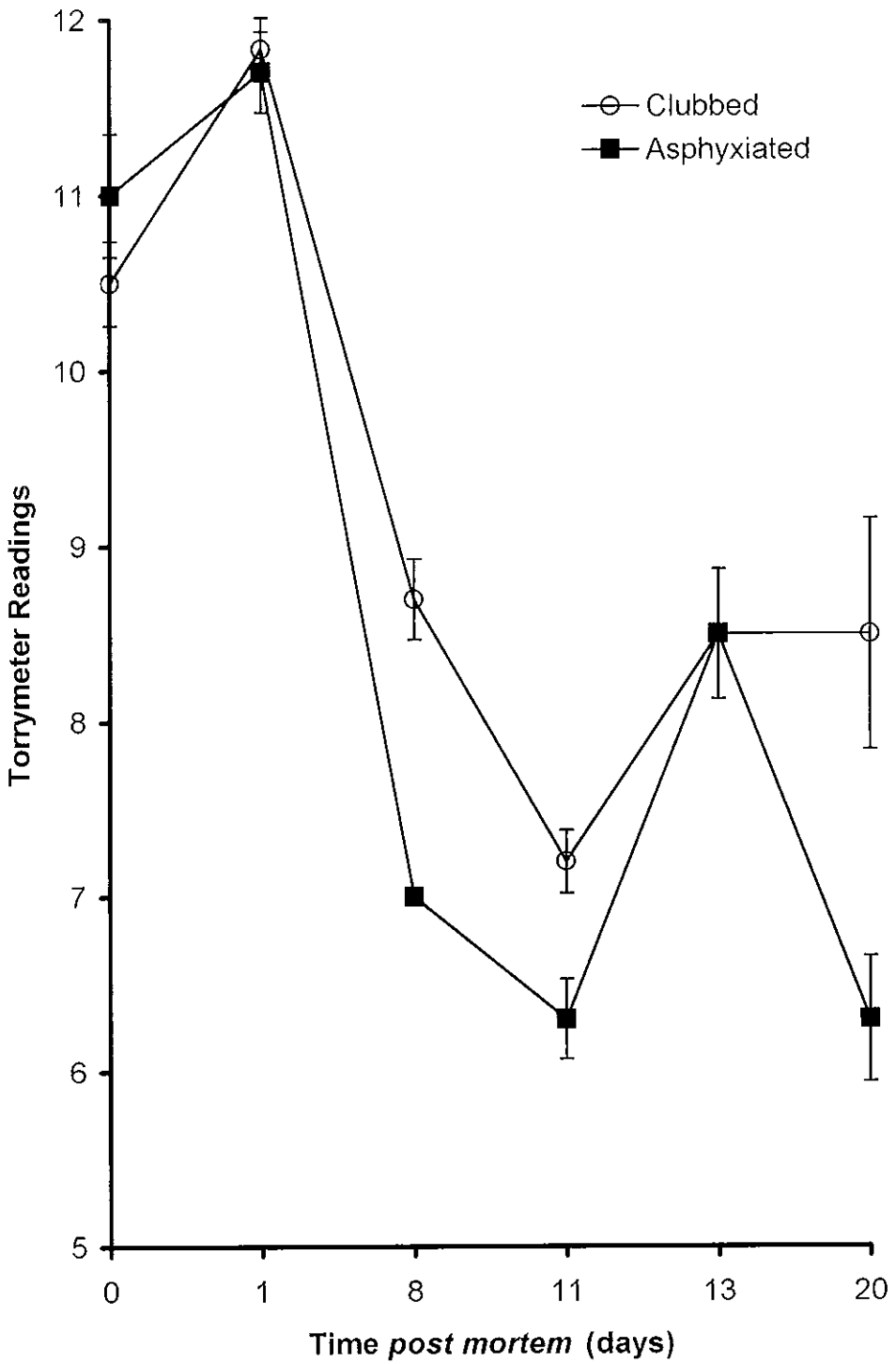


Fig. 71. Myotomal muscle of clubbed and asphyxiated rainbow trout: Torrymeter readings at 3°C.
Mean values ± s.e.m. for groups of six fish.

Comment

Torrymeter readings increased at 1 hour *post mortem*. At 2 hours there was a marginal decrease in the readings followed by an increase at 30 hours *post mortem* for the three groups of clubbed fish. Torrymeter readings increased at day 1 *post mortem* for clubbed and asphyxiated fish maintained at 3°C. This phenomenon has been reported in the literature. Proctor (1987) suggested that the observed increase may have been associated with the particular time course of the biochemical events associated with the onset and resolution of *rigor* in fish skeletal muscle. Torrymeter readings declined steadily during storage for both groups of fish. For fish maintained at 3°C for 20 days, the Torrymeter readings decreased up to 11 days, then rose at 13 days. The readings for the clubbed fish remained the same at day 20, while the readings for the asphyxiated fish fell at this time.

3.3.4 Quality Index Method (QIM) to Assess Fish Freshness

In these experiments the Quality Index Method (QIM) was used to evaluate the freshness of clubbed and asphyxiated fish. The fish were obtained from Idas Fish Farms (section 3.2.5). The fish were killed by clubbing and asphyxiation (sections 2.2.2 and 2.2.3). A visual examination of all fish immediately *post mortem* and during storage was made as described in Materials and Methods, Section 2.11. The fish were held at 3°C during the experiments.

3.3.4.1 Demerit Points

Changes in demerit points are shown in Fig. 72. The mean demerit score was 2.00 ± 0.48 for the clubbed fish and 4.00 ± 0.56 for the asphyxiated. The demerit points increased with time of storage with higher values for the asphyxiated fish at each time of sampling. At day 6 the demerit score for the clubbed fish was 11.16 ± 0. 49 while that for the asphyxiated fish was 16.00 ± 0.59. The difference between these values was significant at $p < 0.01$. At day 20 there was no significant difference between clubbed or asphyxiated fish.

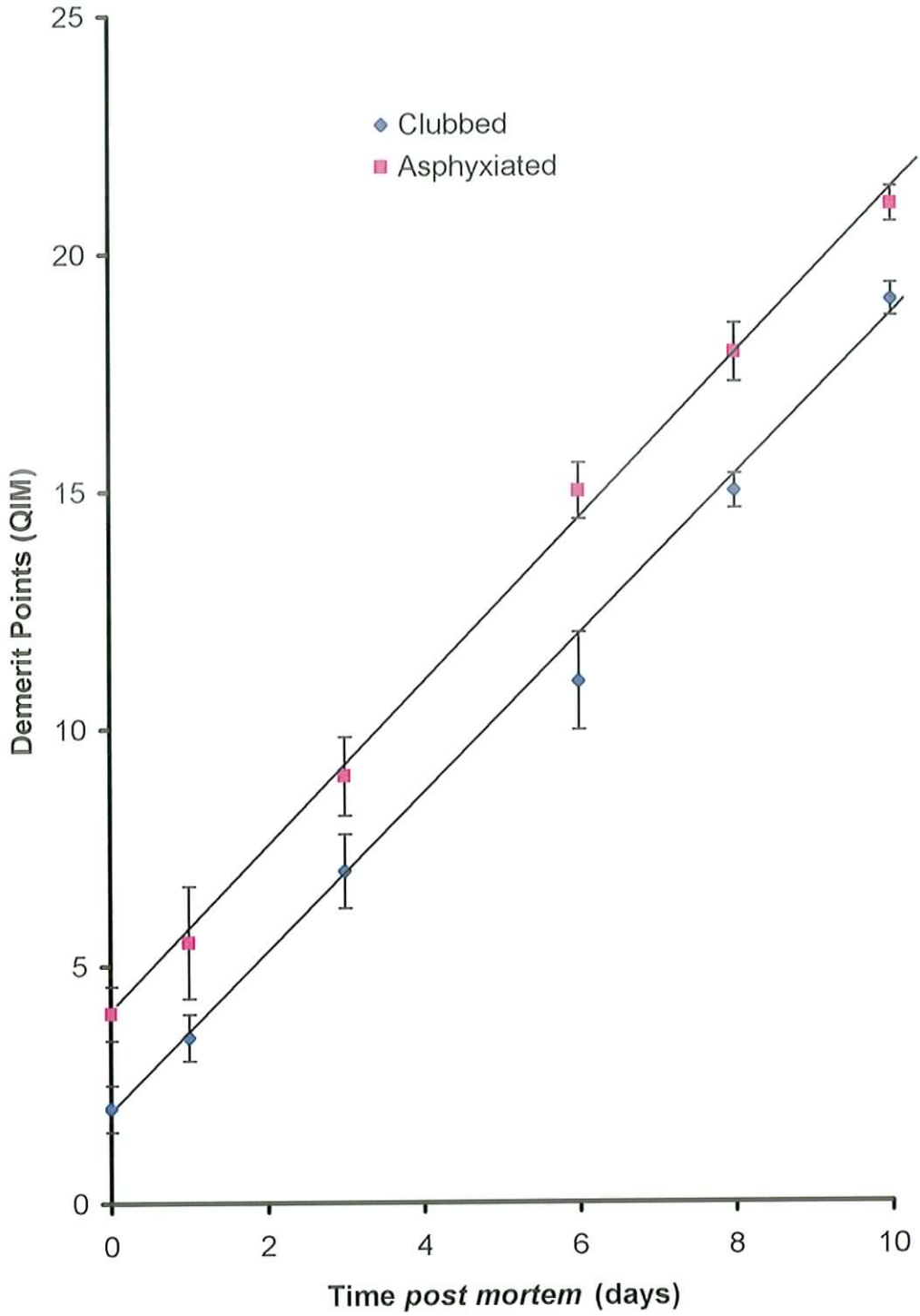


Fig. 72. Sensory curves for clubbed and asphyxiated rainbow trout at 3°C.

Overall Comment

The results of the three methods used to evaluate the freshness of fish showed that the increase in demerit points (QIM) with time of storage was accompanied by an increase in the K value. Torrymeter readings decreased with time for both groups of fish indicating a decline in the freshness of the fish. Significant differences between clubbed and asphyxiated fish were observed at day 6 for the K value, Torrymeter readings and the QIM method.

4. DISCUSSION

4. DISCUSSION

The work in this thesis may be divided into three overlapping sections. The objective of the first section was to study the biochemical changes which take place in skeletal muscle during the harvesting of farmed fish with particular reference to the high energy phosphate compound ATP. It is known that the onset of *rigor* in mammals and fish is accompanied by the depletion of ATP in the muscle after death (Erdos, 1943; Bate-Smith and Bendall 1949; Lawrie. 1953; Partmann, 1965). Factors before death such as exhaustion, starvation, hunting and manner of death influence the time course and the extent of the biochemical changes which accompany the onset of *rigor*. The exact relationship between these changes and environmental factors has not been clearly established for fish. In addition it is difficult to determine the exact time of onset and resolution of *rigor* in fish. There are several reports which suggest that onset of *rigor* in fish is accelerated by exhaustion before death (Amano *et al* 1953; Buttkus, 1963; Jones *et al* 1965; Korhonen *et al* 1990). Wagner (1978) referred to stress in fish as trawl time and catch size increased. Hattula *et al* (1995) reported that onset of *rigor* in Baltic herring was faster when the fish had been caught by gillnet than when methods of trawling or pound-netting had been used. Sebastio *et al* (1996) reported that effective percussive stunning of fish appeared to cause the least activity in the fish at time of death. There is considerable industrial interest in optimising the conditions of harvesting of farmed fish. Diverse opinions have been expressed concerning the effects of the method of harvest on the quality of fish. It has been suggested that rapid killing might lead to enhanced shelf life but an overview of the research on this topic indicates that this may be valid only when the fish are not stressed *ante mortem*.

Farmed rainbow trout, salmon and goldfish were chosen as experimental subjects as they are readily available and are not subjected to the variation imposed by nature on the wild harvest. Observations of the physical aspects of *rigor* were also carried out where possible.

Fish were subjected to various methods of harvest and environmental conditions. The harvest methods used in this study were clubbing and asphyxiation. Some of the clubbed fish had been chilled *ante mortem* and others had been chilled after death. Differences were not detected in the concentrations of ATP for these fish regardless of whether they were chilled before or after death. Fish which were held at 3°C for 90 minutes *post mortem* had concentrations of ATP which were comparable to those values in fish which had been clubbed and sampled immediately *post mortem*. This finding has implications for the fishing industry particularly when large catches are to be handled, or if for other reasons catch handling cannot start immediately. Fish which had been transported live to the laboratory and held in the laboratory for 24 hours prior to experiments appeared to be in good condition. The observation was consistent with the biochemistry of the muscle as reflected in the initial concentrations of ATP and in the pH immediately *post mortem*, and in the physical (texture and dielectric properties) and sensory (QIM) parameters evaluated. This finding was contrary to that reported by Pickering *et al* (1982) who referred to stress in brown trout which had been subjected to handling and suggested that a period of at least two weeks was required to allow recovery. The initial concentrations of ATP were low in the asphyxiated fish, which exhibited most activity at time of death. Proctor (1987) showed that onset of *rigor* took place more rapidly in asphyxiated fish than in fish which had been killed by cervical fracture or anaesthetised prior to death. Hattula (1993) referred to the importance of the physiological state of the fish and the stress caused by the method of

harvest which can accelerate the degradation of ATP in muscle. A similar observation was made by Thomas *et al* (1999) who described a faster onset of *rigor* and a lower pH in muscle of fish which had been stressed (a combination of lowering of the water volume and chasing) at time of death compared with fish which had not been stressed.

A comparison of the concentrations of ATP in the muscle of clubbed and asphyxiated rainbow trout showed that the concentrations were higher in fish which had been clubbed than in fish which had been asphyxiated and had exhibited a vigorous death reaction. The concentrations of ATP in clubbed fish remained constant for up to five hours *post mortem* at 3°C. This finding was contrary to that reported by Tomlinson and Geiger (1962) who recorded a rapid depletion of ATP in fish muscle *post mortem.* The delay in the onset of *rigor* was associated with high pH values (7.00) in the muscle. In individual fish, however, there were fluctuations in the concentrations of ATP between one and two hours *post mortem*. A similar observation was reported by Proctor (1987) who recorded fluctuations in the concentrations of the high energy phosphate potential in rainbow trout during onset of *rigor*. In one batch of clubbed fish however, the concentrations of ATP immediately *post mortem* were very low (<1µmol/g). This may be explained by the fact that there may have been some activity in these fish *ante mortem* or that they had very little glycogen.

Since the musculature of fish contains different types and sizes of cells, variations may exist within an individual specimen according to the anatomical locations (Dunajski 1979). In order to determine whether this might be so specimens were taken from four locations within the epaxial muscle mass and the concentrations of ATP and IMP measured. While there was some variation in the concentrations of ATP between the locations sampled the results did not show that there were significant differences. The

results agreed with those reported by Hattula (1993) who did not find differences in the concentrations of ATP and metabolites at different locations in muscle of whitefish. Muscle taken from the right and left sides of rainbow trout showed no differences in shear force measurements. Dunajski (1979) reported no differences between the composition of the right and left fillets in round-bodied fish but that such differences may occur in flat-fish. Lefèvre (1997) found no differences in texture measurements between muscle samples taken from the epaxial muscle and the hypaxial muscle of rainbow trout but reported differences in samples taken from the caudal area. Sigurisladottir *et al* (1999) reported differences in shear force measurements of muscle taken from seven locations between the head and the tail of raw Atlantic salmon but reported no differences between three locations taken under the dorsal fin.

Fish which had struggled at death had initial concentrations of ATP which were very low and the onset of *rigor* occurred within thirty minutes *post mortem*. These fish had higher concentrations of IMP immediately after death which indicated a rapid conversion of ATP to IMP. The results were consistent with accounts in the literature that trawled cod had little ATP but had a substantial amount of IMP in muscle at time of death compared with rested well-fed fish (Jones and Murray, 1962). Most of the ATP in trawl-caught fish is lost by struggling during capture and the remainder is converted to IMP within a day or two (Kassemsarn *et al* 1963). Similar results have been recorded for salmon caught by gill-net (Kramer and Kennish, 1987). At the later stages of storage inosine and hypoxanthine accumulated. The use of a single adenine nucleotide metabolite as a freshness indicator has not been successful since the rate of formation of inosine and hypoxanthine varies widely with species and with storage conditions. In some species of fish inosine accumulates whereas in other species there is an accumulation of hypoxanthine (Dingle and Hines, 1971). Ehira and Uchiyama

(1973) investigated 98 species of fish and proposed that those species of fish that develop an inosine:hypoxanthine ratio of 5:1 or greater be classified as inosine-forming species: fish that develop a hypoxanthine:inosine ratio of 5:1 or greater be classified as hypoxanthine-forming species whereas those fish with a lower ratio be classified as an intermediate type-species. There are suggestions in the literature that salmonids are an intermediate type species. The results reported in the study in this thesis suggest that rainbow trout are inosine-forming species. Izquierdo-Pulido *et al* (1992) suggested that sturgeon which struggled prior to death were inosine and hypoxanthine producers, whereas anaesthetised sturgeon were inosine producers. Nucleotide degradation of iced barramundi and Nile perch showed that these closely related species were both hypoxanthine formers (Williams *et al* 1993).

In the contemporary fishing industry freezing is considered to be one of the best methods of preserving fish and has been employed increasingly both on shore and on board fishing vessels (Jiang *et al* 1987). The degradation of ATP in the muscle of clubbed and asphyxiated rainbow trout stored at –30°C for up to 24 weeks showed that while the concentrations of ATP were depleted after six weeks storage, the concentrations of IMP at this time were at those levels reported for fish which had been stored at 3°C for one day and had not decreased during storage. It is thought that loss of IMP during frozen storage may result in flavour loss in fish. However, studies on cod muscle (Jones, 1965) showed that IMP phosphohydrolase to be inactive when stored at –30°C.

Salmon of four different grades, which indicated different physiological conditions of the fish, were stunned with CO_2. The results showed that the concentrations of ATP were depleted and the pH values were low (6.40-6.48) in all the fish at 30 hours *post*

mortem. The concentrations of IMP for all the salmon were lower than those values normally found in asphyxiated rainbow trout at 24 hours *post mortem*. The degradation of IMP to inosine and hypoxanthine occurred in all the fish during storage. The ratio of inosine:hypoxanthine in these fish would indicate they are intermediate-type species and is consistent with that reported by Ehira and Uchiyama (1973). The use of CO_2 as a stunning agent is widely employed in commercial practice at salmon hatcheries and is considered to produce a more acceptable fish and an enhanced shelf life. However, Proctor (1987) reported low levels of ATP and CP in rainbow trout and salmon immediately *post mortem* using CO_2 and some of these fish had exhibited extreme agitation *ante mortem*. Further research has shown that fish reacted violently during induction of CO_2 (Kestin *et al* 1991) and Marx *et al* (1997) noted motor activity in eel, carp, and trout during anaesthesia induced by CO_2. Methods of stunning that act quickly and minimize violent activity in the fish at time of death are considered acceptable, not only from a humanitarian standpoint but also in terms of the quality of the fish (Marx *et al* 1997).

Goldfish were anaesthetised to determine zero hour values for ATP in rested muscle. The results showed that the concentrations of ATP were similar to those values reported for mammalian muscle. A similar observation was made by Proctor *et al* (1992) who studied the concentrations of ATP in skeletal muscle of marine and freshwater species anaesthetised with MS-222. Fish have been anaesthetised for many reasons, for example, handling of dangerous species, sorting and grading of farmed fish, tagging, transport, biological and physiological research, surgery, and photography and the literature refers to the use of various chemicals including ethanol, urethane, ether, narcotics, chlorbutol and MS-222 for this purpose. MS-222 was originally developed during a search for a cocaine substitute and is now employed as

the preferred anaesthetic for teleosts. Aquatic animals readily absorb it across the gill tissue, the degree of sedation or anaesthesia is easily varied for a wide variety of applications and animals recover rapidly after exposure and resume normal physiological and behavioural functions. In the United States MS-222 is the only anaesthetic permitted for the use of fish intended for human consumption provided that at least 21 days have elapsed since the fish were last exposed to the substance (US FDA, 1995). In Ireland, the recommended withdrawal period of MS-222 in fish sold for human consumption is 14 days (NDAB, 1991). More recently AQUI-S was developed as an aquatic anaesthetic which would allow fish to be harvested in a rested state (Jerret *et al* 1998). However, AQUI-S is currently undergoing review by the US FDA and is not available for sale in the USA.

Conditions arising during harvesting and handling of fish which induce stress lower the high energy phosphate potential and also the pH. Both the rate and extent of the fall in pH are important variables in terms of the quality of the muscle as a food. The abnormally rapid decline in muscle pH *post mortem* has a detrimental effect on the water-holding capacity of pork meat (Bendall and Wismer-Pedersen 1962). In a review of the literature on the quality of pig meat Naude (1983) reported that stress immediately before slaughter results in meat which has a lower pH, lighter colour and reduced water binding capacity. The ultimate pH of muscle is dependent both on species and handling conditions. Beef generally has an ultimate pH in the range of 5.5-5.7, chicken is around 6.2 and fish may be as high as 6.6-6.7 (Hultin, 1984). The pH was lower in asphyxiated fish than in clubbed fish. This difference was due to the fact that the asphyxiated fish struggled at death thus increasing the rate of anaerobic breakdown of glycogen. On the other hand the ultimate pH, i.e., the final pH attained

in the muscle was the same in both groups, an observation which indicated that the mean concentration of glycogen at death had been similar in the muscle of both groups. This result was in agreement with that of Korhonen *et al* (1990) who observed that when fish were stressed the pH was lower in the *pre-rigor* state and during early onset of *rigor* compared with non-stressed fish, but that the ultimate pH obtained in the fish muscle was similar in both stressed and non-stressed fish.

In the second section of the thesis changes in the concentration of adenine nucleotides and texture were studied in clubbed and asphyxiated rainbow trout at 3°C. The results of the study showed that the results of the biochemical analysis and measurement of texture were complementary. The fluctuations in the concentrations of ATP in the clubbed fish were accompanied by similar fluctuations in the shear force required to cut the muscle. When the concentrations of ATP remained constant for a period of time *post mortem*, the shear values also remained constant; and as the biochemical composition of the fish muscle changed with time, the shear force values decreased. The shear force values were lower for the asphyxiated fish up to 8 days after which time there were no differences between the clubbed and asphyxiated fish. Ando *et al* (1992) suggested that *post mortem* tenderization of fish muscle during chilled storage was due to the disintegration of collagen fibre in the pericellular connective tissue rather than the weakening of the Z-disc in the muscle fibres. The force/displacement curves for the asphyxiated fish showed two distinct peaks. These peaks did not appear in the curves for the clubbed fish. Borderias *et al* (1983) recorded two distinct peaks in texture measurements of muscle of rainbow trout taken between the dorsal fin and the caudal fin. The first peak was taken as that of the muscle fibres and the second peak which was sharper and more pronounced was taken to be that of the connective tissue.

Lefèvre (1997) showed that muscle from the caudal region had more connective tissue and that the myotomes were thinner than the muscle from the anterior part of the fish. However, for some of the asphyxiated fish described in this thesis, only one peak was observed during shearing. These fish were in *rigor* and perhaps there was no clear distinction detected in the shear force measurements between the muscle fibres and the connective tissue at this time. It has been suggested that when the myofibres are rigid, as in cooked meat, there is a simultaneous shearing of muscle and connective tissue fibres (Salé, 1971). When meat is in *rigor*, there is a simultaneous shearing of the muscle fibres and the connective tissue.

Muscle of fish stored at –30°C for up to 24 weeks showed that while the concentrations of ATP were depleted at six weeks there were no further changes in the texture of the muscle after this time. There were no differences in texture between fish that struggled at time of death and fish which did not. There are reports in the literature which suggest that during the freezing process and during frozen storage, fish muscle can undergo a number of changes, such as denaturation and aggregation of the myofibrillar proteins. This may result in muscle which is tough and fibrous giving rise to sensory attributes described as dryness, rubbery texture, loss of juiciness and loss of water holding properties (Haard, 1992). While the texture of the fish reported in the study in this thesis did not appear to be tough, a certain decrease in the water holding capacity was observed for the clubbed and asphyxiated fish.

The results of this part of the study showed that there is a relationship between biochemical and shear force changes in fish muscle *post mortem*. This relationship has not been reported in the literature previously. The observations were made during a

large number of experiments but of course the relationship remains to be confirmed or refuted by other workers. The relationship if confirmed, has an obvious practical significance.

In the third section of this thesis three rapid methods were used to assess the freshness of fish. These were (a) a Rapid Paper Strip to measure the K value, (b) measurement of the dielectric constant (Torrymeter) and (c) the Quality Index Method (QIM). The experiments were carried out on groups of rainbow trout which had been maintained *ante mortem* and killed (clubbed) in such a way as to reduce stress *ante mortem* and motor activity and on other groups which had been allowed to asphyxiate, a method which caused strong muscular activity at the time of death.

(a) The results showed that the K value obtained from the Rapid Paper Strip (RPS) was a reliable method for estimating the freshness of fish during storage. A comparison of the Rapid Paper Strip and the K_1 value and K value obtained by the HPLC method showed that there was good agreement between the three methods. The results were contrary to those reported by Hattula *et al* (1993) who found a poor correlation between the Rapid Paper Strip Method and the K_1 value. He did, however, report that there was good agreement between the Rapid Paper Strip Method and sensory analysis. The development of the K value during storage as an indicator of freshness in salmonids has been reported by a number of authors (Uchiyama *et al* 1978; Uchiyama, 1988; Boyle *et al* 1991; Luong *et al* 1991; Hattula and Kiesvaara, 1992). However, the studies provided limited information regarding the history of the fish prior to the experiments. In the case of clubbed fish immediately *post mortem* which were used in the study reported in this thesis the K values obtained were

considerably lower than those reported for the Rapid Paper Strip or the K_1 value. This was due to the fact that, ATP, ADP and AMP had not been included in the calculation when using the K_1 value or the Rapid Paper Strip. The K values for the clubbed and the asphyxiated fish increased during storage. The K values for the asphyxiated fish were higher than for the clubbed fish at 6 days storage after which time the differences appeared to have been offset by the effects of storage time. The results were consistent with those reported by Izquierdo-Pulido *et al* (1992) who showed that K values in anaesthetised sturgeon were lower than when the fish struggled at time of death during the first 5 days of storage. Lowe *et al* (1993) reported lower K values in muscle of rested snapper up to 3 days *post mortem*. Erikson *et al* (1997) studied the effect of the stress of handling on K values for salmon and reported lower values for unstressed fish than for stressed fish. The K values for rainbow trout which had been stored at –30°C were higher for the asphyxiated fish at 6 weeks storage after which time there was no further significant increase for either clubbed or asphyxiated fish.

The significance of the K value was studied as a function of both the duration of storage and the freshness index. Texture, evaluated by shear force measurements, was the basis of comparison of the fish quality. There appeared to be a relationship between the K value and the shear force. As the K values increased during storage the force required to cut the muscle decreased. The usefulness of the K value is that it changes from the beginning of storage. The K value reflects autolytic phenomena prior to bacterial growth in the muscle, which explains its reliability as an index of fish freshness. Ehira and Uchiyama (1986) reported that the K value was influenced by the rate of IMP decomposition. The low initial K value ($3.72 \pm 0.78\%$) for the asphyxiated fish immediately *post mortem* showed that there was no breakdown of IMP in the

muscle of these fish. The mean initial shear force (10.72 ± 0.55N) value was high, indicating the firmness of the muscle. Hatae *et al* (1986) found a significant correlation between the collagen content and the firmness of the muscle and noted that species of fish with firmer texture contained higher collagen content than those species with softer texture. Bremner and Hallet (1986) found that collagenous fibrils, rather than the myofibrillar proteins, were broken down in spotted travella (*Seriolella punctata*) during 14 days storage on ice. Ando *et al* (1992) studied tenderization of rainbow trout *post mortem* and found that the myofibrillar proteins showed no disintegration during storage for 72 hours. However, they reported a decrease in the breaking strength of the collagen fibres in the pericellular connective tissue and concluded that the tenderization *post mortem* was caused by disintegration of collagen.

(b) Measurements of changes in the dielectric constant of muscle were used as an indicator of freshness. Attention was focussed on fish held at 3°C rather than on the frozen product. An increase was observed in the dielectric constant at 24 hours *post mortem* for clubbed and asphyxiated rainbow trout after which time there was a steady decline for all fish during further storage. This observation was similar to that reported by Proctor (1987) for rainbow trout. Jason and Lees (1971) referred to the initial increase in Torrymeter readings over the first 1 or 2 days associated with onset and resolution of *rigor*. Fish which had exhibited muscular activity at time of death had lower Torrymeter readings than fish in which this activity was not present. Such a finding suggests that the retention of quality was poorer in the asphyxiated fish than in the clubbed fish. A direct relationship was observed between the decline in the dielectric constant of the fish muscle and a decline in freshness measured by the K value. As the dielectric constant of the fish muscle decreased during storage, there was

an increase in K values and this was particularly noticeable in asphyxiated fish at day 6.

(c) The Quality Index Method (QIM) was used to grade the fish by attributing demerit points for the different parameters during time course studies. There was an increase in QIM score as the fish deteriorated on ice. Fish which had not struggled at time of death received a lower demerit score than those fish which exhibited a violent death reaction i.e., the former were of better quality on the basis of appearance. At day 6 there was a significant difference between the QIM score for the asphyxiated fish and that score obtained for the clubbed fish. There appeared to be direct relationship between the QIM method, the K value and the dielectric constant (Torrymeter readings). As the fish muscle deteriorated an increase in the QIM score was accompanied by an increase in the K value and a decline in the dielectric constant. Such a relationship, between freshness expressed biochemically, freshness expressed by measurement of dielectrical constant and appearance and texture of muscle have not been reported in the literature previously.

5. CONCLUSIONS

5. CONCLUSIONS

1. The method of killing influenced the rate of onset of *rigor* in rainbow trout, salmon and goldfish. The rate of depletion of ATP and the accumulation of IMP were higher in fish which were killed by asphyxiation, a method which caused severe struggling and, presumably, anoxia *ante mortem*, than in fish which were killed by a sharp blow to the head and in which struggling did not occur. Muscle contraction at death in the former group of fish was a major factor influencing the rate of onset of *rigor*. The use of CO_2 stunning gave rise to low concentrations of ATP (<1.0μmol/g) and low pH values (6.40-6.48) in the fish muscle. Fish which were anaesthetised with MS-222 were in a tranquil state at time of death and the concentrations of ATP were high.

2. There were no differences in nucleotide metabolites nor in texture in this study between samples taken from different locations within the muscle nor between samples taken from muscles on the right and left sides of the fish.

3. Clubbed fish could be kept at 3°C for up to 90 minutes *post mortem* without there being any significant difference in the concentration of ATP at the various time intervals.

4. Fish which did not struggle at death retained their freshness on the basis of their biochemical K value for a longer time than fish which did struggle.

5. A rapid paper strip method for measuring K_1 value was satisfactory when compared with results obtained by the HPLC method.

6. Fish stored at –30°C did not undergo further changes in freshness (K value) nor in texture (shear force) after six weeks.

7. A relationship was demonstrated between nucleotide metabolism (K value) of muscle, texture of muscle (shear force), dielectric constant and sensory (visual) evaluation of fish. During the onset and resolution of *rigor* and during the *post rigor* state K values increased, shear force values (texture) decreased, dielectric constant decreased and visual sensory quality decreased.

8. As far as the author is aware there have been no published studies which have demonstrated a relationship between the nucleotide metabolism of muscle and the physical and sensory methods used to evaluate quality.

6. REFERENCES

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