THE INFLUENCE OF TEMPERATURE ON THE DEVELOPMENT AND SWIMMING PERFORMANCE OF FLATFISH

Sandra Gibson

A Thesis Submitted for the Degree of PhD
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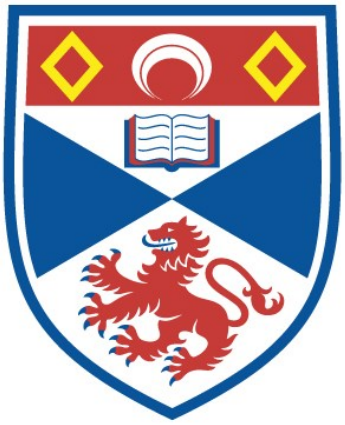

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The influence of temperature on the development and swimming performance of flatfish

A thesis submitted to the University of St Andrews for the degree of Doctor of Philosophy.

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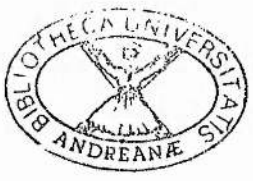

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This thesis is dedicated to the memory of Karen Fretwell

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Abstract

Growth and development were studied in turbot (*Scophthalmus maximus* L.) reared at 12°C and 16°C until 26 days after hatching. Muscle growth occurred by fibre hypertrophy and hyperplasia and was faster at 16°C. In larvae, the sequence of organogenesis was altered by temperature. The influence of temperature on the swimming performance of settled stages of turbot and plaice (*Pleuronectes platessa*) was studied. Maximum swimming speed (U_{max}), elicited following an escape response, scaled similarly between 13 and 23°C, for turbot, and could be fitted by the model:-

$$U_{max} = 28.4 + 10.9\left(\frac{Temp - 13}{5}\right) + 10.3TL$$

A comparison of U_{max} between wild caught and laboratory reared turbot showed that U_{max} for farmed turbot was lower than for wild fish filmed within 2 weeks of capture. 3 months after capture the average differences in escape performance were no longer significant, suggesting they were due to an acclimation. Standardised U_{max} for eighteen wild juvenile turbot was determined at 18°C and over a temperature change. Repeatability of ranking of the experimental U_{max} of individuals was maintained over a 6 week period, and through temperature change. For plaice U_{max} scaled in proportion to $TL^{0.65}$ between 5°C and 13°C. U_{max} did not increase at temperatures above 9°C. There was no difference in U_{max} or tail beat frequency (f) between laboratory reared and wild caught

plaice. U_{max} and f decreased after an acute temperature reduction from 9°C to 5°C and showed no compensation for the reduction temperature after a 29 days acclimation period. Stride length (X) was independent of temperature. After the 29 day period at 5°C, raising the temperature to 9°C resulted in an increase in U_{max} without a corresponding increase in f, although tail beat amplitude (A) was higher. The effects of temperature change during early development on locomotory performance and phenotype are discussed.

Summary

Chapter 1

A brief history of fish culture is given concentrating on the development of turbot and plaice production. A general introduction on constraints of animal size and hydrodynamics on swimming in fish is presented. Muscle morphology, development and action during swimming are discussed.

Chapter 2

Turbot (*Scophthalmus maximus L.*) were reared at 12°C and 16°C until 26 days (D26) after hatching. The time from fertilisation to 90% of the larvae hatching (development time, DT) was 144h at 12°C and 72h at 16°C. Expressed as a percentage of DT, somite formation occurred relatively earlier during embryogenesis at 12°C (45% DT) than at 16°C (55% DT). At both temperatures somites were initially formed every 75 min. At 12°C after the 32 somite stage somite formation decreased to one somite every 300 min. The larvae hatched with 40.0 ±4.0 somites at 12 °C and 36.2 ±3.3 somites at 16°C. Turbot hatch at a

primitive stage of development, with no functional eyes, gills, mouth or anus, and with a straight gut. Temperature was found to alter the relative timing of organogenesis in the larval stages. At 12°C, the swim bladder was formed at 360h post fertilisation time (PFT) whereas development of a loop in the gut coincided with complete yolk utilisation at 456h PFT, prior to the appearance of the caudal fin (552h PFT). In contrast, at 16 °C, complete yolk utilisation coincided with the formation of the swim bladder (216h PFT) and the caudal fin and the loop in the gut formed at the same time (312h PFT). Larvae were 3.19 ±0.19mm total length (TL) at 12°C (264h PFT) and 2.73 ±0.30mm at 16°C (168h PFT) when spines were formed on the operculum. Although spines formed in the auditory region of larvae at 16°C by 3.12 ±0.03mm TL (216h PFT), at 12 °C they still had not formed in larvae at 4.06 ±0.19 mm TL(552h PFT).

At hatching the myotomes contained around 200 inner muscle fibres with an average cross-sectional area of 60 - 80 μm^2, surrounded by a single layer of smaller area fibres, at both rearing temperatures. The initial growth of inner muscle was largely due to hypertrophy, but by D26 at 12°C and D11 at 16°C hyperplastic growth had become increasingly important, as evident by the increase in the number of small fibres (<10 μm^2) with an average cross-sectional area of 8.3 - 8.5 μm^2. By day 26 the number of inner muscle fibres had increased to 341 ±61 and 988 ±342 at 12°C and 16°C. New muscle fibres were added in distinct germinal zones at the dorsal and ventral apices of the myotomes.

Chapter 3

Maximum escape swimming speeds (U_{max}, cms^{-1}) were studied in settled turbot (*Scophthalmus maximus* L.) reared at 18°C. Metamorphosis was complete at 4.0 cm total length (TL). U_{max} scaled in proportion to $TL^{0.74}$ in fish 0.88 to 8.00 cm TL at 18°C. Stage of development had no effect on the relationship between TL and U_{max}. The scaling relationship for U_{max} was similar for temperature between 13 and 23°C and could be fitted by the model:-

$$U_{max} = 28.4 + 10.9\left(\frac{Temp - 13}{5}\right) + 10.3TL$$

U_{max} was temperature dependent with a Q10 of 1.77 over the temperature range studied.

Chapter 4

A comparison of the maximum swimming speed (U_{max}) between wild caught and laboratory reared turbot (*Scophthalmus maximus*) was carried out. Analysis of covariance showed that U_{max} for farmed turbot was 14% lower than for wild fish filmed within 2 weeks of capture. 3 months

after capture the average differences in escape performance were no longer significant, which suggests that the lower escape speeds of farmed fish are due to acclimation effects and not genetic stock differences. In order to assess the individual variability of U_{max}, eighteen wild juvenile turbot (TL = 6.2 ±0.4 cm week 1 to 7.5 ±0.5 cm week 17, Mean ±SD) were maintained in individual containers at 18°C. U_{max} was determined weekly for 6 weeks, standardised for fish length using the scaling relationship $U_{max} = 1.46TL^{0.74}$, and individuals ranked in order of performance. Temperature was reduced after 6 weeks to 13°C, resulting in a significant decline in U_{max} from 104.0 ±14.4 to 87.8 ±12.5 cm s^{-1} (Mean ±SD). After 3 weeks at 13°C U_{max} had increased to a level not significantly different from that at 18°C. Kendall's Coefficient of concordance showed that repeatability of ranking of the experimental U_{max} of individuals was maintained over a 6 week period, but not through temperature change. The results demonstrate that escape swimming speeds in juvenile turbot are repeatable, individually variable and can be modified in response to temperature acclimation.

Chapter 5

Maximum escape swimming speeds (U_{max}) were studied in wild caught settled plaice (*Pleuronectes platessa* L.). U_{max} scaled in proportion to $TL^{0.65}$ in fish ranging from 1.5 - 7.9 cm.

The scaling relationship for U_{max} was independent of temperature between 5 and 13°C. U_{max} increased between 5° and 9°C but did not increase further when temperature was raised to 13°C, (Q_{10} = 1.51 between 5° and 9°C). U_{max} and tail beat frequency (f) did not differ significantly between laboratory reared and wild caught plaice when compared at 5°, 9° and 13°C, giving a pooled Q_{10} = 1.61 and 1.68 for U_{max} and f respectively between 5° and 9 °C. U_{max} decreased by 22% and f by 36% after an acute temperature reduction from 9° to 5°C, but had shown no compensation for the reduced temperature after a 29 days acclimation period. Tail beat amplitude (A_{max}) and stride length (X) were independent of temperature. Temperature was acutely raised to 9°C after 29 days acclimation to 5°C, resulting in an increase in U_{max} from 17.75 ±5.1 to 25.27 ±5.4 cm s^{-1}. Tail beat frequency did not increase, but tail beat amplitude showed a marked increase which may account for the increase in U_{max}.

Chapter 6

The major findings of this thesis are discussed relating temperature to changes in development and burst swimming performance of turbot. Results for plaice are discussed comparatively with those of the turbot. Suggestions are made on expanding the work carried out in this study.

Chapter 1

General Introduction

General Introduction

A brief description of fish culture with particular reference to turbot and plaice

Fish culture arose independently in Asia, Africa and Europe about 2000 years ago, and has remained almost unchanged in many countries. Today, although important on a local scale, in general only a small percentage of fish consumed by humans comes from aquaculture (Weatherly and Gill 1987). In recent years world interest in aquaculture has increased as nations become increasingly anxious about the over exploitation of wild fish stocks. In the North Sea, herring catches have declined steadily since 1965 with the total adult biomass decreasing to a tenth of its original level (Hempel 1978). Fish culture practices range from intensive pond rearing to the management and harvesting of wild or semi-wild populations. Milkfish (*Chanos chanos*) culture, in Indonesia and the Philippines, is one of the oldest and simplest forms of fish culture. Wild caught larvae of the milk fish are placed in large shallow ponds, tambaks, carved out of mangroves. Fish feed on the rich algal mat that forms on the bottom of the pond. In the U.K, the most common method of fish culture is based on the rearing of salmonids in ponds, tanks or floating enclosures. Manipulation and control of factors that influence growth, such as density, nutrition, hormones and

genetics, have been important in determining the economical viability of fish culture.

Both plaice (*Pleuronectes platessa*) and turbot (*Scophthalmus maximus*) are commercially important flatfish species inhabiting the waters around the U.K. In spring, adult fish migrate into shallower water to spawn. Adult plaice make large migrations to the spawning grounds each year. Using radio telemetry Metcalfe *et al.* (1993), by tracking the movements of plaice in the North sea, have demonstrated that energy used during swimming can be reduced by remaining in the water column and exploiting tidal stream transport, whist making spawning migrations. Plaice begin spawning in March whereas turbot spawn later in the season beginning in late June. Eggs and larvae of both plaice and turbot are pelagic. Larvae of both species settle on to nursery areas, shallow sandy beaches, and are often found together. Turbot tend to occur in shallower water than plaice. Plaice have been artificially spawned and reared in captivity since 1901, but because of their low value are not commercially farmed in the U.K. Turbot carry a high commercial value, and are economically more viable to farm than plaice. The farming of turbot was begun by the Romans, who held wild caught fish in salt water ponds (Day 1880-1884). But it was not until the late nineteenth century that serious attempts were made to commercially cultivate turbot. In 1894, at the Fisheries Board of Scotland's laboratory at Dunbar, attempts were made to replenish natural turbot stocks with artificially spawned and reared fish (Anthony 1910). Many laboratory studies were undertaken to establish the viability of

mass turbot culture but it is only relatively recently that turbot have been successfully reared past metamorphosis. Jones (1972) succeeded in rearing turbot to metamorphosis at two temperatures. The failure to rear turbot past metamorphosis were attributed to deficiencies in nutrition (Kuhlmann *et al.* 1981). Turbot larvae show clear food preferences, and as the feeding and nutritional requirements of larvae were established, commercial turbot production became a viable proposition (Kuhlmann *et al.* 1981).

Flatfish development

Flatfish can be categorised into two subgroups, the dextral (right sided) forms such as plaice (*Pleuronectes platessa*) and halibut (*Hippoglossus hippoglossus*), and the sinistral (left sided) forms such as turbot (*Scophthalmus maximus*) and brill (*Scophthalmus rhombus*). Because flatfish larvae develop asymmetry whilst still planktonic their benthic existence is thought to be a secondary adaptation and not the reason for the asymmetry (Kyle 1921). Flatfish are a relatively ancient group, thought to have evolved on several occasions from different ancestors (Kyle 1921). Herdman (1901) concluded that during evolution flatfish became laterally compressed whilst still pelagic. Some modern species such as the megrim (*Lepidorhombus* sp.), turbot (*Scophthalmus maximus*) and halibut (*Hippoglossus hippoglossus*) are often pelagic, feeding

on fish and squid (Aflalo and Marston 1904, Whitehead *et al.* 1986).

Before the life histories of flatfish had been fully described, by Professor Tarquair in the 1860's, bizarre stories were invented to describe the development of flatfish. One such tale involves flounders hatching their young from pits in their backs, like the Surinam toad (Aflalo and Marston 1904). Another describes how shrimps were the intermediate stage between eggs and juveniles.

Depending on the species, flatfish lay pelagic eggs ranging from 0.6 mm to 4.5 mm in diameter. This spans the entire size range of marine fish eggs (Lasker 1981). The high water content (90-92%) rather than lipid content (10-15%) is thought to be the principal cause of buoyancy in marine eggs (Craik and Harvey 1987). Turbot's yolk sacs have a large visible oil droplet which constitutes 16% of the eggs dry weight and is composed principally of triglycerols and phospholipids. Flatfish hatch at a primitive stage of development, the mouth is closed, the eyes are not fully formed, the gut is a straight tube and there are no functional gills. The eye does not become fully pigmented, and functional, until a few days after hatching (Russell 1976). Escape response is triggered by the Mauthner cells in the hind brain (Blaxter 1986). Free neuromasts are present on the head and trunk of the body at hatching, as the fish develops the neuromasts are incorporated into the lateral line canal system, which becomes more concentrated on the ocular side of the body (Neave 1986, Harvey *et al.* 1992). Both the free neuromasts and

the lateral line canal are used in predator detection (Blaxter 1986, Harvey *et al*. 1992). There is usually one neuromast per myotome. Blaxter and Fuiman (1989) demonstrated that plaice larvae neuromast ablation decreased their response to a simulated predator. The development of a fully functioning eye coincides with first feeding in some flatfish such as the flounder (*Paralichthys olivaceus*) (Kawamura and Ishida 1985). The body surface and large fin fold act as a respiratory organ (Just *et al.* 1981, Liem 1981). At hatching, larvae are symmetrical, and at some point undergo dramatic morphological changes, developing asymmetry by the process of metamorphosis. Timing of metamorphosis is related to fish length, and environmental influences such as light and temperature (Hoar 1976, Thorpe and Morgan 1978, Youson 1988). The trigger for the onset of metamorphosis is not known. Thyroid hormone, thyroxine (T4), can induce metamorphosis in flounder larvae (Inui and Miwa 1985, Miwa and Inui 1987a, b). The pineal gland and day length are also thought to be involved in some species (Youson 1988). The most obvious morphological changes taking place during metamorphosis are lateral compression, eye migration, and a loss of pigmentation on the underside of the body. Asymmetry begins with alterations in the abdominal organs and jaws. In early studies, the process of eye migration was thought to be due to optic muscles pulling the eye around the top of the head once the fish was lying on its side and had become demersal (Cunningham 1893). During eye migration torsion occurs, with the frontal portion and otic region of the skull remaining approximately in the same plane whilst the orbital region twists through 90°. In plaice, although both eyes appear on the same side of the body they are still on either side of the cranium

(Herdman 1901). The telencephalic hemispheres and optic tectum in the brain of turbot develops asymmetry concurrent with metamorphosis. After metamorphosis the optic tectum recovers bilateral symmetry (Briñón *et al.* 1993). Just before larvae settle into their benthic habit there is a shift from single cones in the retina to twin cones and rods (Kawamura and Ishida 1985). After eye migration is complete the larvae are considered fully metamorphosed juveniles.

Metamorphosis has been associated with reductions in observed cruising speed in sole (*Solea solea*) and plaice (Blaxter and Staines 1971) and a reduction in oxygen consumption in the winter flounder (Laurence 1975). Sinistral species possess a swim bladder during early development which is thought to be responsible for buoyancy control during the larval pelagic phase (Kyle 1921, Jones 1972) and as a pneumatic cushion between the stomach and backbone (Al-Maghazachi and Gibson 1984). The swim bladder also shows asymmetry and Kyle (1921) suggests that it may be important in maintaining balance, allowing the fish to remain upright during metamorphosis.

Allometry

Comparisons between species, groups and individuals are often confounded by the effect size has upon the parameters being studied. Most physiological functions vary in level or intensity with the body size of the animals being studied (Packard

and Boardman 1987, Schmidt-Nielsen 1986). The term scaling is often used to describe variation due to body size and is defined by Schmidt-Nielsen (1977) as "the structural and functional consequences of a change in size or scale among similarly shaped animals". Scaling of function is usually described with the aid of a mathematical model, the allometric equation, which takes the general form $y = a \cdot x^b$, where b is the regression coefficient, and a is the proportionality. Allometry, the study of size and its consequences (Gould 1966), has become an important tool in comparative biology (Calder 1984). The first use of an allometric equation was by Snell (1891) to describe the mental capabilities of various mammals by mathematically describing the relationship between the mass of the brain and body mass (Schmidt-Nielsen 1986). The use of allometric equations is now widespread in biology and by knowing the body mass of an animal it is possible to predict a wide variety of its specifications including life span, heart and metabolic rate, each from an empirical allometric equation based on body size (Calder 1984). The majority of allometric equations are scaled to body mass, but functions such as fish swimming are better understood when scaled to a linear dimension such as length (Schmidt-Nielsen 1986). Fish can vary in size by between 2 and 3 orders of magnitude as they develop, having a dramatic effect on physiology. The hydrodynamical constraints on fish swimming are also related to fish size.

Hydrodynamics

Fish have nearly the same density as water, and the energy they use in locomotion goes into overcoming drag due to the resistance of the medium. The drag during swimming has two components, pressure drag (D_p) and friction drag (D_f). Pressure drag is difficult to evaluate and is due to the necessity to displace water during forward movement. Friction drag corresponds to the force used to overcome the viscosity of the fluid, and is dependent on swimming velocity, surface area, water density and the coefficient of drag (C_f). C_f in turn is related to the Reynolds number (Re), a hypothetical, dimension less, value which describes the hydrodynamic regime in the boundary layer of an object moving through a fluid. Re is defined as LV/μ, where L is fish length, V is swimming speed, and μ is the kinematic viscosity of the medium. Fish length is therefore important in determining the hydrodynamic constraints on swimming. In some species, as body length increases, the mode of swimming changes from bouts of continuous swimming to burst and coast swimming. The change in swimming mode has been shown to coincide with caudal fin development (Blaxter 1986). At Re <20 viscous forces are dominant, and the resistive model of propulsion concludes that bouts of continuous swimming are most appropriate (Webb and Weihs 1986). As fish length and swimming speed increases, at Re > 200, inertial forces are more important and burst and coast swimming becomes more efficient (Webb and Weihs 1986). Hunter (1972) described a shift in the swimming mode of northern anchovy larvae (*Engraulis mordax*) as they grew. Most larvae swim at 20 < Re

< 200. In small larvae, maximum burst swimming speeds can be sufficiently high to pass into the inertial dominated regime (Webb and Corolla 1981). Both larvae and adult stages of fish species swim in the same hydrodynamic regime for important behaviours such as escape swimming (Webb and Weihs 1986). It is at the low speeds (small Re) during the initial stages of acceleration, especially in small individuals, that factors such as water viscosity, density, and temperature have the greatest effect on swimming hydrodynamics. In small animals, at low Re values, changing temperature can alter locomotory ability by not only altering metabolic processes but also changing the viscosity of water and thereby the hydrodynamic regime of the animal. Podolsky and Emlet (1993) demonstrated in sand dollar larvae (*Dendraster excentricus*) that up to 40% of a reduction in swimming speed, with a 10°C reduction in temperature, was due to a change in water viscosity.

Swimming muscles

The lateral musculature of fish is segregated into myotomes delineated by sheets of collagen (myosepts) (Bone 1966, Alexander 1969, Videler 1993). In most teleosts, myotomes are cone-shaped and stacked in a metamerical arrangement on both sides of the median septum (Videler 1993). Muscle fibre types, red, white and intermediate, are arranged into anatomically discrete regions in the myotomes (Bone 1966,

Videler 1993). Red fibres have a simple arrangement forming a superficial layer running parallel to the body axis and can account for up to 29% of the locomotory musculature (Greer-Walker & Pull 1975). White fibres compose the majority of the cross sectional area of the fish, up to 95%. Deep fibres within the myotome can be arranged at up to an angle of 30° to the long axis of the body (Alexander 1969, Bone and Marshall 1982). In primitive fish, myotomes are V shaped and become more complex in higher teleosts, resembling a W. There is usually no septum separating the red and white fibres. The elaborate arrangement of the muscle fibres results in bending of the body with very little shortening of the sarcomeres (Alexander 1969). The proportion of red and white fibres is related to life style (Boddeke *et al.* 1959, McLaughlin and Kramer 1991, Akster *et al.* 1985). Active pelagic fish, such as mackerel (*Scomber scombrus*) (Bone 1978) and flying fish (Exocoetidae) (Davenport 1992), have a higher proportion of red fibres compared to demersal species, in which the lateral musculature is almost entirely composed of white fibres (Greer-Walker and Pull 1975) .

A variety of techniques, including histochemistry and electrophysiology, have been used to distinguish and characterise differences between muscle fibres. In fish, fibre colour and contraction speed have become the most widely accepted criteria for differentiating fibre types. The two main fibre types muscle identified are slow red fibres and fast white fibres. Myotomal red muscle fibres function aerobically and have high myoglobin and cytochrome concentrations, a high volume density of mitochondria and rich blood supply (Bone

1966, Bone 1978, Johnston 1981). Red fibres have slow contraction speeds and are used for sustained swimming. White fibres are designed for maximum power output and are characterised by a high myofibrillar density, low mitochondria content and poor blood supply (Johnston and Altringham 1991). White fibres function anaerobically converting stored glycogen to lactic acid. The immediate energy supply for contraction comes from the hydrolysis of phosphocreatine (Hochacha 1985).

Although fibre types can be divided into red and white, a whole spectrum of fibre types exists between the two, with varying properties (Johnston *et al.* 1974). Bone (1978), has identified five different fibre types in the dogfish (*Scyliorhinus canicula*). In some fish species, between the red muscle and white muscle fibres are pink muscle fibres with a range of characteristics somewhat intermediate between those of the red and white muscle fibres (Johnston *et al.* 1977). Having a diversity of fibre types, with different optimum rates of shortening, allows fish to swim at a wide range of speeds (Rome *et al.* 1988).

Swimming

The most prominent method of aquatic propulsion is the undulatory mode, in which a transverse wave, normally increasing in amplitude, passes backwards along the body from head to tail (Alexander 1967, Lighthill 1970). Breder (1926)

separated fish swimming into 4 basic forms on the basis of body and fin movement: 1. anguilliform, an undulatory mode in which the whole body participates, with a large amplitude wave passing down the length of the fish; 2. carangiform, where amplitude of lateral movements increase towards the tail such that the significant propulsive movements are concentrated at the posterior portion of the body and 3. ostraciform, where the body is rigid and propulsion is by means of oscillation of the caudal fin 4. balistiform, movement is the result of the simultaneous undulations of the dorsal and ventral fins. Some fish species have become specialised for each swimming type. Tuna have become highly streamlined with fins positioned far back on the body and possessing a semi lunate tail which is most efficient for their pelagic cruising life style. Tuna possess a large proportion of red muscle, and have developed a counter current exchange system to elevate body temperature above that of their external environment. In fish that specialise in fast starts, such as the flatfish, the body form has tended to increase in depth increasing the surface area available to provide the force needed to overcome inertia, and therefore produce high acceleration rates. Most fish are generalists with body shapes that give moderately good performance for accelerating, cruising and manoeuvring (Webb 1984). Although, the classification of swimming style according to extreme examples is useful, it should be noted that fish may use more than one mode of swimming. For example, in flatfish balistiform swimming is used to propel the fish slowly along the bottom, whereas at high speeds swimming is anguilliform. The swimming activity for many fish can be divided into two broad categories, low speed sustained cruising and high speed burst swimming (Blake 1983).

Based on experiments in flumes Brett (1967) further divided swimming activity. Sustained swimming was defined as any activity maintained for approximately 200 min or more, using purely aerobic metabolism, usually involving recruitment of the red muscle alone (Bone 1978, Davison *et al.* 1976). Many studies have measured the critical swimming speed of fish, U_{crit} (Brett 1964). U_{crit} is defined as the maximum swimming speed that can be maintained for a given time period before an oxygen debt is incurred. Burst swimming is defined as high speed movements of relatively short duration (<15s) that is powered anaerobically by recruitment of white muscle. Between sustained and burst swimming is prolonged swimming, in which cruising is interspersed with bouts of more vigorous activity. During prolonged swimming both aerobic and anaerobic metabolism are used.

Different fibre types power different levels of swimming. As speed increases there is a progressive recruitment, of first red, then intermediate and finally fast fibres being recruited (Johnston *et al.* 1977). Rome and colleagues have shown, in carp (*Cyprinus carpio*), that red fibres are used only at low swimming speeds, with the increasing numbers of white fibres recruited as speed increases (Rome *et al.* 1984, Rome *et al.* 1985). In flounder (*Pleuronectes flesus*) indirect evidence suggests that white fibres are recruited at moderate swimming speeds (Duthie 1982).

The flatfish are heavily camouflaged on the top (ocular) side with some capacity to alter colour to that of their

background (Harvey *et al.* 1992). Fast starts in flatfish are used for both prey capture and predator evasion. The majority of flatfish are ambush feeders, catching prey by a rapid, short duration lunge. Escape behaviour takes the form of a Mauthner cell-initiated startle response beginning with a C-start type contraction. The major advantage of the C-start for escaping predators is the high acceleration rate achieved within a short time (Firth and Blake 1990). The flatfish escape response was described in sole (*Solea solea*) by Kruuk (1963) as an omega jump, and involves the rapid lifting of the head followed by large amplitude tail flip which propels the fish off the bottom and into a position where rapid burst swimming is possible. Flatfish spend long periods of time inactive on the sea floor and their metabolic rates are low compared to more active species (Duthie 1982). Plaice make long migrations to their spawning grounds and it has been suggested that this may be done by swimming constantly at speeds above the critical level, then resting to repay the oxygen debt (Duthie 1982). Plaice also exploit selective tidal streams during these spawning migrations and therefore reduce the overall metabolic cost of transport (Metcalfe *et al.* 1990, Metcalfe *et al.* 1993).

Muscle structure and contraction

Fish lateral muscles fibres are multi-nucleated and surrounded by a plasma membrane, the sarcolemma. The contractile proteins are organised in myofibrils. In longitudinal sections, skeletal muscle has a striated appearance. Myofibrils

consist of a sequence of identical units, sarcomeres, interconnected by the Z-disc. Extending from the Z-disc in both directions are narrow thin filaments composed of actin molecules, about 5nm in diameter. In the centre of the sarcomere, arranged between the actin filaments, are the thick filaments. The thick filaments are made up of myosin and are about 10nm diameter. The basic structure of myosin consists of two heavy chain subunits (MHC) of 200kDa and four light chain subunits (LC) of about 20 kDa (Huriaux and Focant 1977, Focant, *et al.* 1981). During muscle contraction neither the actin or myosin filaments change length, but move relative to each other by the cycling of cross bridges projecting from the myosin filaments. The contraction of muscle is described by the sliding filament theory (Huxley and Niedergerke 1954, Huxley and Hanson 1954) in which the thick and thin filaments move in relation to each other due to conformational changes in the head of the myosin molecule (Finer *et al.* 1994). The maximum force produced during muscle contractions is directly proportional to the number of cross bridges in parallel (Goldspink 1980). The rate of shortening of a muscle depends on several factors including: the number of sarcomeres in series; the length of the filaments within the sarcomere; and the intrinsic rate of contraction (Goldspink 1980). Intrinsic rate of contraction is determined by the rate of force generation by the cross bridges and the duration of the cross bridge cycle.

During swimming, the myotomes undergo cycles of lengthening and shortening and are stimulated during each cycle (Altringham and Johnston 1990). Power output is a complex

function of strain, number and timing of stimuli, force generation and cycle frequency. Work done by the muscle is dependent on the timing of the stimulation in relation to the start of the length-change cycle and can be dramatically altered by the previous strain cycles (Johnston 1991). By imposing sinusoidal length changes on insect muscle and stimulus at selective phases in the strain cycle, Josephson (1985) was able to measure muscle power output under conditions more applicable to those that occur *in vivo*. Josephson's work loop technique was later modified by Altringham and Johnston (1990) to measure the power output of fish muscle under conditions of simulated swimming in the short-horned sculpin. Red fibres produced their maximum power output of 5-8 W kg^{-1} at 2Hz, whereas the fast fibres generated their maximum power output at 7-9 Hz (20 cm fish at 5°C) (Altringham and Johnston (1990).

Growth and scaling effects in fish muscle

Vertebrate skeletal muscle originates from the somatic mesoderm of the embryonic somites (Ordahl and Le Douarin 1992). In most vertebrates, myotubes are formed by the fusion of myoblasts withdrawn from the cell cycle, and which eventually differentiate and become mature muscle fibres (Nag and Nursall 1972). Soon after myotube formation, actin and myosin synthesis occurs (Johnston 1993). In fish, increases in muscle mass can occur by both increasing muscle fibre number (hyperplasia) and

increasing the size of existing muscle fibres (hypertrophy) (Stickland 1983). The relative proportions of larval muscle growth due to hyperplastic and hypertrophic differs between species. In herring (*Clupea harengus*), the number of myotomal muscle fibres remains relatively constant during early larval stages (Johnston 1993). During the early stages of development in other species, muscle fibres are formed in proliferation zones (Veggetti *et al.* 1990, Brooks and Johnston 1993). In the gilthead bream (*Sparus aurata*) (Rowlerson *et al.* 1994) addition of new fibres are confined to specific areas, especially at the apices of the myotomes. The relative contributions of hyperplasia and hypertrophy can also change throughout development. In the rainbow trout (*Salmo gairdneri*), the relative proportion of inner muscle fibre area due to hypertrophy increases with length (Stickland 1983). In fish which reach a large adult size, such as trout (Weatherley *et al.* 1980) and sea bass (Veggetti *et al.* 1990), post larval muscle growth is due to both hyperplasia and hypertrophy, whereas in fish that reach modest sizes relatively quickly, like the bluntnose minnow (Weatherly and Gill 1984) postlarval growth is exclusively hypertrophic. Hyperplasia can continue until fish reach 70% of their ultimate length (Weatherley and Gill 1985).

In the skeletal muscle of some fish species, there is a successive appearance and disappearance of different fibre types and myosin isozymes during development (van Raamsdonk *et al* 1978, Martinez *et al.* 1991). The existence of distinct larval and adult isoforms of myosin heavy chain (MHC), troponin T and troponin I have been demonstrated in the Atlantic herring

(*Clupea harengus*) (Crockford and Johnston 1993). Also in the barbel (*Barbus barbus*), up to the age of two months, larval muscle is characterised by specific myosin heavy chain isoforms distinct from those of adults (Focant *et al.* 1992).

The contractile properties of muscle are subject to scaling effects. During growth there are size-related changes in muscle mass and twitch kinetics. When calculated per unit volume of muscle, the work performed in one contraction is independent of body size (Hill 1950). The conclusion that maximum work is scale independent holds true for a variety of organisms ranging from higher vertebrates to some invertebrates (Schmidt-Nielsen 1977). It follows that power output per contraction will be directly related to speed of shortening (Power = work · time) and therefore contraction frequency. Maximum shortening velocity (V_{max}) is almost scale independent. For cod (*Gadus morhua*), isometric twitch contraction times scale in proportion to length (L) by the relationship $L^{0.29}$ (Archer *et al.* 1990). When measured under steady-state conditions, with imposed sinusoidal length changes, maximum power output scaled in proportion to standard length, $L_S^{-0.29}$ (Anderson and Johnston 1992). Contraction time of white muscle is related to tail beat frequency, which scales inversely with body size (Wardle 1975).

In order to provide the necessary fuel and oxygen for muscle power the respiratory and circulation systems must also scale with body size. In many marine species at hatching, the major site for gas exchange is the skin. As size increases the

demand for oxygen cannot be met by the skin alone, due to the reduction in the surface area to volume ratio and the skin becoming impermeable. Gills develop, increasing the area for gas exchange. In plaice (*Pleuronectes platessa*), gills are a scaled in proportion to body weight as $W^{1.59}$ up to metamorphosis when the relationship decreases to $W^{0.85}$ (De Silva 1974). Although a decrease in the mass exponent for gill area is observed during development in various fish species, the reason for this decrease has not yet been suitably explained (Hughes and Al-Kadhomiy 1988). In early larval life, active metabolism is mainly aerobic, with protein being the main source of energy. The capacity of anaerobic metabolic pathways increases during ontogeny (Wieser *et al.* 1987). Hinterleitner *et al.* (1987) demonstrated that the activity of glycolytic enzymes in three freshwater fish species increased during development, whereas the oxidative enzyme activity decreased.

Aims

The overall aim of this study was to investigate the influence of temperature on muscle development and swimming performance in turbot (*Scophthalmus maximus* L.). Some comparative experiments were also conducted on the swimming performance of plaice (*Pleuronectes platessa* L.). Scaling relationships between total length and maximum swimming speed were calculated for the two species. The swimming speeds

of wild caught and laboratory reared fish were compared (Chapters 4 and 5). It was noted during initial swimming experiments that there was some variation in maximum swimming speed between individuals. In order to investigate this further, a study was undertaken to determine the repeatability of burst swimming speed between individual turbot. The effects of temperature acclimation were also investigated.

Chapter 2

The effect of temperature on the development of turbot, *Scophthalmus maximus* L.

Introduction

Around the coast of Scotland, turbot (*Scophthalmus maximus* L.) produce small (diameter 0.91 - 1.20 mm) pelagic eggs from June to September (Russell 1976). Larvae remain pelagic until the onset of metamorphosis, at which time the body becomes laterally flattened and eye migration occurs, and they become demersal, settling onto shallow sandy beaches (Day 1880-1884). Turbot are a relatively eurythermal species with a geographic range from Norway (75° North) to the southern Mediterranean (30° North) (Wheeler 1978). Throughout this latitudinal range, sea temperature increases between the spawning season and the early juvenile phase, for example at Dunstaffnage Bay (Argyll, Scotland) mean sea temperature increases from 7°C to 14°C over this period (Morley personal communication). Eggs and larvae spawned early in the season may experience a different temperature regime from those spawned later.

Temperature influences the duration of the embryonic (Jones 1972) and larval (Lasker 1981) phases of the turbot, both of which are important factors in determining the dispersal of flatfish, by altering the time in the water column and hence movement due to waves and water current (Hovencamp 1991, Riley *et al.* 1981). In addition to effects on the rate of development, temperature has been reported to alter the relative timing of appearance of morphological characters. Fukuhara

(1990) found, in the Japanese flounder (*Paralichtys olivaceus*), that the sequence of appearance of features such as eye pigmentation and pectoral fin formation varied, relative to each other, between 15 and 21°C. Temperature has also been found to influence meristic characteristics such as vertebral and fin ray number in a number of fish species (Tåning 1952, Lindsey 1954, Fonds *et al.* 1974, Fahy 1981, Lindsey 1988). Somite formation and myogenesis proceed in a rostral to caudal sequence in fish embryos (Hannerman 1992). Johnston and co-workers have shown, in Atlantic herring (*Clupea harengus*) embryos that contractile protein synthesis and the differentiation of distinct muscle fibre types occurs relatively earlier with respect to somite formation at 12°C than 5°C (Johnston 1993, Johnston *et al.* 1995). Temperature has also been shown to alter the number and diameter of muscle fibres at hatching in salmon, herring (Johnston 1993) and plaice (*Pleuronectes platessa*) (Brooks and Johnston 1993). In herring, the volume density of mitochondria also varies with rearing temperature (Vieira and Johnston 1992). Heterochronies can also be observed at the molecular level, as well as the morphological level, for example, Crockford and Johnston (1993) found that the muscle of 1 day old herring reared at 5°C contained a mixture of embryonic and larval toponin T isoforms, whereas only larval isoforms were expressed at 10°C.

Al-Maghazachi and Gibson (1984) devised a comprehensive morphological staging procedure for larval turbot based on that already described for plaice (*Pleuronectes platessa*) by Shelbourne (1957) and modified by Ryland (1963, 1966). The existence of heterochronies means that length and/or

age cannot be used to compare satisfactorily fish from different groups or studies.

The aim of this study was to investigate the influence of temperature on development, in turbot, from fertilisation to the beginning of metamorphosis, with respect to the formation of the myotomal muscle.

Materials and Methods

Fish

Brood stock turbot (*Scophthalmus maximus* L.) were obtained from a commercial fish farm (Golden Sea Produce, Hunterston, Argyll, Scotland) and brought into season by varying the photo-period. Eggs, fertilised from a single female/male cross, were split into two batches and held in re-circulating sea water tanks at either 11.3 - 12.8°C or 15.2 - 16.8°C. Samples of approximately 10 eggs from each temperature were fixed in either neutral buffered formalin (NBF; 10% formalin, 0.03M sodium dihydrogen phosphate, 0.05M disodium hydrogen phosphate, distilled water) or Laverak's solution (50% glutaraldehyde, 100mM $CaCl_2$, 0.2M NaCl, 10% paraformaldehyde, 0.6M sodium cacodylate, 15% sucrose, pH 7). Samples were taken 3 times daily, from fertilisation, at 09:00, 12:00 and 17:00 hours, until hatching.

Embryonic development time (DT) was taken as the point at which 90% of live embryos had emerged from the eggs. Larvae were fed a diet of *Artemia* sp. nauplii enriched with a fatty acid diet supplement. The early pelagic larval stages were sampled daily until day 7 post hatching (D7). After D7 sampling frequency was reduced to every fourth day until day 30 days post hatching. Larvae were fixed in Bouin's solution (72% Picric acid, 24% formalin, 4% Glacial acetic acid) for 24h then transferred to 75% ethanol.

Somitogenesis

Embryos were dechorionated under a dissecting microscope and their yolk sacs removed. Embryos fixed in Laverak's solution were stained for acetyl cholinesterase activity, using the direct colouring method described by Karnovsky and Roots (1964), which enabled the somites to be counted more easily. Samples were incubated overnight, at 5°C in the dark, in 1.0 ml of incubation solution (5mM tri-sodium citrate, 3mM $CuSO_4.5H_2O$, 0.5mM potassium ferricyanide, 0.5mg/ml acetylthiocholine iodide). Somites were counted using a binocular microscope at a magnification of X64.

Growth, Morphology and Yolk Utilisation.

In order to make morphometric measurements embryos and larvae were traced with the aid of a *camera lucida*

microscope attachment. Total length (TL), standard length (SL) and yolk sac cross-sectional area were determined using an image analysis System (KONTRON Elektronic GmbH, Basel, Switzerland). The formation of internal structures was noted during development and staging was carried out using the criteria of Al-Maghazachi and Gibson (1984) (Table 1). Yolk sac volume was measured in two ways:

Embryonic yolk sacs shape was assumed to be spherical (Fig. 1a). The measured cross-sectional area of the yolk sac was used to estimate the average radius (r) of the yolk sac:

$$\text{average } r = \sqrt{\frac{\text{Area}}{\pi}}$$

Estimated average r was then used to calculate the volume of the yolk sac using the equation for the volume of a sphere:

$$\text{Volume} = \frac{4\pi r^3}{3}$$

At hatch the larvae straighten and the yolk sac becomes more ovoid in form (Fig 1b) and the formula for the volume of an ovoid was used : $\text{Volume} = \frac{4\pi l^2 h}{3}$ where l is the length, and h is the height of the yolk sac.

Post hatching muscle growth

Serial wax sections were cut transversely from larvae, at 1, 4, 11, and 26 days post hatching, from 12°C and 16°C, and

stained with haematoxylin-eosin. Morphometric measurements were made from a section through the trunk, midway down the gut (Fig 1b) and traced with the aid of a *camera lucida* microscope attachment. Fibre number and cross- sectional area were determined using an image analysis system (KONTRON Elektronic GmbH, Basel, Switzerland).

Statistical analysis

Rates of somite formation and yolk utilisation were compared between temperatures using the technique for comparison of regression coefficients described by Edwards (1962). All other results were compared using an analysis of variance (Minitab, data analysis software, Minitab Inc 1981). All results are expressed as mean ± standard deviation.

Results

Turbot development

Turbot lay small pelagic eggs (diameter 1.45 ± 0.04mm) with a single oil globule (diameter 0.36 ± 0.03mm) (n=10). Initially turbot eggs undergo meroblastic development. 50% epiboly occurred at approximately 30% development time (DT) at 12°C and 16°C. Somites were visible after 39h at 16°C and

63h at 12°C (Fig 2a). Expressed as a percentage of embryonic development time (DT) somite formation occurred relatively later during embryogenesis at 16°C (55% DT) than 12°C (45%) (Fig 2b). Somites were formed every 75 min at 16°C from the 10 somite stage to hatch. The rate of somite formation was initially similar at 12°C, but decreased to every 300 min after the formation of 32 somites ($t = 2.18$, $P < 0.05$). Embryos incubated at 12°C had more somites at any given DT than those at 16°C ($P < 0.05$). On day 1 post hatching (D1), larvae at 12°C had around 10% more somites than larvae at 16°C. As development proceeds the tail bud forms (88h PFT and 48h PFT at 12°C and 16°C respectively), the embryo elongates and the first somites segregate shortly after the formation of the neural plate and notochord (Fig. 3). Embryos had around 28 somites when the lens and the otic vesicle (64h PFT at 12°C and 39h PFT at 16°C) formed. Tail bud formation occurred relatively earlier at 12°C (61% DT, 88h PFT), with embryos having more somites (26.8 ±1.3 somites), than embryos at 16°C (67% DT, 45h PFT, 20.3 ±1.0 somites). Larvae on D1 were at a primitive stage in development, stage 1a : the mouth was closed, the gut was a straight tube, the rectum had not formed, eyes were unpigmented, and the gills and pectoral fins had not developed (Fig 4, Table 1)(Al-Magahazie and Gibson 1984). By D4 post hatching at 12°C and 16°C the eyes were fully pigmented and the mouth had open (Fig 4b). As larval development proceeded the body became longer and deeper, the gut more complex, spines formed on the operculum and flexion had occurred by day 26 at 16°C (Fig 4c).

The time from fertilisation to 90% of the larvae hatching halved over a 4°C increase in temperature and was 72h at 16°C and 144h at 12°C (Fig 5). The total length (TL) of the embryo increased more rapidly at 16°C than 12°C (Fig 6a.). Growth in length was linear at both temperatures and was 0.16 mm h^{-1} at 12°C and 0.25 mm h^{-1} at 16°C. When expressed in relation to number of somites, there is no difference in TL between the two temperatures up to the 19 somite stage (Fig 6b). After the 19 somite stage, initially embryos at 12°C are significantly shorter than those at 16°C for a given number of somites up to the 35 somite stage. At hatching (D1) 12°C larvae had a total length (TL) 10.8% greater than larvae reared at 16°C ($P < 0.05$). Larval growth, in length, was faster at high temperature than low, and was 0.07 mm d^{-1} at 12°C and 0.22 mm d^{-1} at 16°C ($t = 23.81$, $P = 0.05$) (Fig 7). By day 26 post hatching larvae at 16°C were 41% longer than larvae at 12°C.

Yolk utilisation was characterised by a rapid decrease in yolk volume, 0.08 mm^3 h^{-1}, to hatch at 16°C (Fig 8a). At 12°C the initial rapid phase of yolk utilisation was similar to that at 16°C, 0.08 mm^3 h^{-1}, then rate of yolk utilisation decreased to 0.0015 mm^3 h^{-1} ($t = 7.33$, $P < 0.05$). At hatching larvae reared at 16°C had 33% more yolk than those at 12°C ($P < 0.05$).

Larval muscle growth

At 1 day old larvae (D1), the appearance of the myotomes in transverse cross-section was characterised by a central mass of large diameter inner-muscle fibres, surrounded by a single layer of smaller diameter fibres, (presumptive red fibres) just under the skin (Fig. 3). D1 larvae at 16°C had a larger mean inner muscle cross-sectional area, 808 ±163 μm^2, than larvae at 12°C, 574 ±124 μm^2 ($P<0.05$, fig. 9). At both temperatures there was around 200 inner muscle fibres on D1 ($P=0.05$, Fig 10). Total inner muscle cross-sectional area was greater at 16°C than 12°C, due to the larger cross-sectional area of the inner muscle fibres (Fig. 11).

As turbot grow the body elongates in the dorso-ventral plane, and there is an increase in both inner muscle fibre number and the total cross-sectional area of inner muscle (Figs 9 and 10). The presumptive red fibre layer extends beyond the inner-muscle fibres to encircle the body cavity (Fig 3). Although inner muscle fibre cross-sectional area increases as the fibres grow, the mean cross sectional area remained relatively stable due to the constant addition of newly formed small fibres (Fig 11). The small newly formed fibres are concentrated in germinal zones at the dorso-ventral apices of the myotomes (Fig 3). Initial growth of inner muscle was due to hypertrophy, but by D26 at 12°C and D11 at 16°C hyperplastic growth became increasingly important, with the number of fibres <10 μm^2 beginning to increase (Fig 12c and 12h). On D26 at 16°C, small area fibres (<10 μm^2) made up

29% of the total fibre number (Fig 12d). On D1 at 12°C and 16°C, when plotted as a histogram, the percent of total muscle fibre number at each fibre cross-sectional area size class was normally distributed (Fig 12a and 12e). As the fish grew, the range of fibre areas became larger due to the hypertrophic muscle growth but the distribution of fibre areas became skewed to the left, due to addition of new fibres (Fig 12a-12h). By day 26 post hatching at 16°C, the red muscle layer had begun to thicken and was 2 cells thick (Fig 13). On day 26 post hatching at 16°C, small muscle fibres were also visible between the presumptive red layer and the inner muscle mass.

Relative timing of organogenesis

The relative timing of organogenesis was altered by temperature in larval turbot. The formation of a loop in the gut coincided with the onset of caudal fin formation, in larvae at 16°C. In contrast, at 12°C caudal fin formation occurred later than the formation of the loop in the gut (Fig 14). At 12°C the yolk sac persisted until a loop had formed in the gut, but at 16°C the yolk was fully utilised before the loop in the gut had developed. The formation of the swim bladder occurred before complete yolk utilisation at 12°C, but at 16°C yolk utilisation and swim bladder formation coincided (Fig 14).

Caudal fin formation began on D11, and flexion occurred by D26 at 16°C (576h post fertilisation time, PFT). At 12°C flexion had not occurred by D26 (768h PFT). Larvae were longer at 12°C at the formation of a given developmental feature, than larvae at 16°C ($P<0.05$). For example, larvae 3.44 ±0.28mm TL , at 12°C when the swim bladder is first observed, but at 16°C larvae had a TL of 3.12 ±0.03mm at the onset of swim bladder formation ($P=0.05$). Caudal fin formation occurred at approximately 4.1mm TL at both 12°C and 16°C.

Spines were first seen on the operculum on D6 post hatch at 12°C and on D5 post hatch at 16°C. At 16°C spines had developed in the auditory region by day 7 and a ridge above the eye by day 14, this is relatively later in the sequence of staging events than described by Al-Magahazie and Gibson (1984). At 12°C spines had not formed in the auditory region or above the eye by D26 post hatching, which suggests that they may be absent at low temperatures.

Discussion

Growth and muscle development

As for most fish species turbot develop faster at higher temperatures than lower ones within their naturally experienced range. Turbot embryonic development time doubled for a 4°C decrease in temperature. Turbot embryos reared at 12°C show a

decrease in the rate of somitogenesis after the 32 somite stage, which coincided with a decrease in the rate of yolk utilisation. In zebrafish (*Brachydanio rerio*), at 28.5°C somites were initially formed at 2 per hour until the 6 somite stage, then the rate of somitogenesis decreases to 3 somite per hour (Hannerman and Westerfield 1989). For plaice (*Pleuronectes platessa*) embryos a change in rate of somitogenesis during development has been shown to coincide with the spontaneous movement of the embryo (Brooks and Johnston 1993). Incubation of eggs at lower temperatures resulted in longer larvae (Ryland *et al.* 1975) with greater numbers of somites (Jordan 1892, Lindsey 1975). At hatching turbot larvae at 12°C were 10.8% longer with 10.1% more somites than larvae at 16°C. In the stickleback incubation at low temperature results in an increase in vertebrae number in larvae, the variation in vertebra phenotype has been shown to have a significant impact on burst swimming performance and predation (Swain 1992).

Muscle fibre development was more rapid at 16°C than at 12°C in larval turbot. At hatching, 16°C larvae had a greater cross-sectional area of inner muscle composed of larger cross-sectional area fibres, than larvae at 12°C. As fish grow, muscle mass can increases by both hypertrophy and hyperplasty (Weatherley *et al.* 1980). In 17 species of fish hyperplasia was found to continue until 70% of their ultimate length was reached (Weatherley and Gill 1985). In turbot larvae initial muscle fibre growth was predominantly by hypertrophy, with the relative proportion of muscle growth due to hyperplasty increasing as the larvae neared metamorphosis. At the onset of

metamorphosis the number of presumptive red fibres had also increased, and by day 26 at 16°C had formed a double layer of fibres surrounding the body. In plaice, no change in the distribution of red and white muscle fibres was associated with metamorphosis, with early juveniles having an inner mass of muscle surrounded by a single layer of presumptive red fibres (Brooks and Johnston 1993). Calvo and Johnston (1992) demonstrated that a 5°C change in temperature for juvenile turbot altered the relative proportions of red and white muscle fibres. As muscle growth is highly dependent on rearing temperature, the differences displayed in the distribution of red and white at metamorphosis, in turbot and plaice (Brooks and Johnston 1993) may be largely a function of differences in rearing temperature.

Temperature and development

Organogenesis proceeds throughout embryogenesis and the larval phase. Turbot hatch at a moderately primitive stage of development. Heterochronies were observed during the larval stage primarily in relation to the formation of the gut and fins. At 16 °C the caudal fin and a loop in the gut developed after all the yolk had been utilised. This could have important implications for the nutritional state of the larvae if the loop in the gut is associated with an increased digestion efficiency. Caudal fin development may be an important factor effecting swimming performance and therefore ability to catch prey.

Caudal fin development is associated with a change in swimming mode in the larvae of some species (Blaxter 1986).

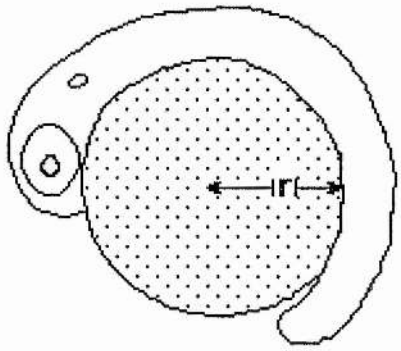
Turbot larvae developed a variety of spines in the head region during development. The cephalic armature of the turbot was first described by Holt (1892), who suggested it may be of protective or a remnant of a structure which was of ancestral significance . The exact function of the spines is not known. Blaxter (1988) suggests that the spines may be an anti-predator device. It has also been proposed that the spines may serve as temporary storage sites for bony material during metamorphosis as complete ossification may interfere with eye migration (Al-Maghazachi and Gibson 1984). The presence or absence of spines in the auditory region of turbot larvae varies from study to study. In this study, larvae developed spines in the auditory region at 16°C but spines still had not developed by day 26 at 12°C. It may be that the absence of spines in some studies are related to morphological differences between stocks or environmental factors such as temperature. The delay in formation, or absence of spines in larvae at 12°C may have important consequences for survival if these spines are important in deterring predators.

Table 1. *Scophthalmus maximus.* Table showing the morphological characteristics of the developmental stages of turbot larvae. Modified from Al-Maghazachi and Gibson (1984).

Stage	Substage	Morphological Characteristics
1		Larvae symmetrical, yolk sac present
	1a	Head bent round and attached to yolk sac; gut almost a straight tube.
	1b	Head pointed forward; anus not reaching primordial marginal fin.
	1c	Anus reaching the marginal fin; mouth open.
	1d	Intestine fully developed; mouth and anus open.
2		Larvae symmetrical, development of spines and air bladder
	2a	Yolk either completely absorbed or initially remaining as a small sac around oil globule; few spines apparent on the operculum.
	2b	Gut with a loop; swim bladder small; spines on operculum numerous; one or two spines in auditory region; a bony ridge developed above eyes.
	2c	Swim bladder larger; two rows of spines on operculum; spines around auditory region; spines growing out of bony ridge above eyes.
3		Appearance of fin rays, notochord straight
	3a	Swim bladder fully inflated; one or two fin rays barely visible.
	3b	Four to seven fin rays present; margin of fin slightly extended.
4		Asymmetry and eye migration, notochord slanted dorsally
	4a	Notochord caudally bent upwards but <45°; numerous fin rays present.
	4b	Notochord sloped upwards 45° or more but < 90°; eye migration commences.
	4c	Right eye position further upwards but not visible from left side; spines present on underside of each jaw.
	4d	Notochord bent straight upwards; caudal fin rays fully developed; fin rays visible in ventral fin ; upper edge of right eye can be seen from left side; spines on each jaw.
5		Completion of eye migration, spines and swim bladder reabsorbed
	5a	Half of right eye visible from left side; dentary spines completely reabsorbed; resorption of other spines commenced.
	5b	Right eye on top of head.
	5c	Right eye situated entirely on left side but still near upper edge; all remaining spines fully resorbed.
	5d	Upper eye placed away from upper edge; swim bladder disappeared; dorsal fin extends to above the front of the eyes: metamorphosis can be regarded as complete.

Figure 1a. Diagram of a turbot embryo showing parameters measured; shaded area: yolk sac cross-sectional area (mm^3); r: yolk sac radius (mm).

Figure 1b. Diagram of turbot larvae showing parameters measured; h: yolk sac height (mm); l: yolk sac length (mm); TL: total length (mm); SL: standard length (mm). Dashed line represents area where transverse cross-sections were cut for muscle fibre measurement.



a.

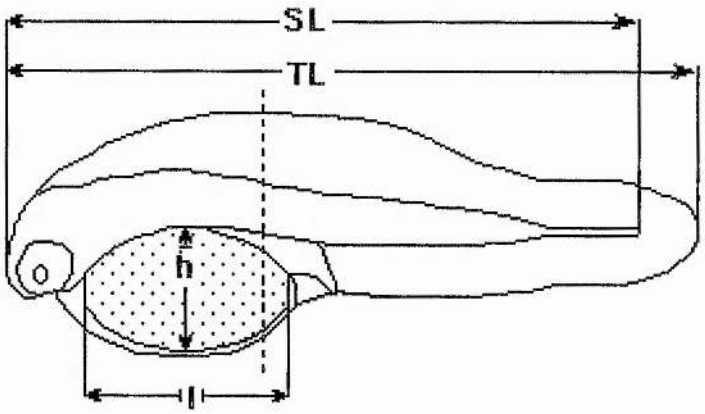


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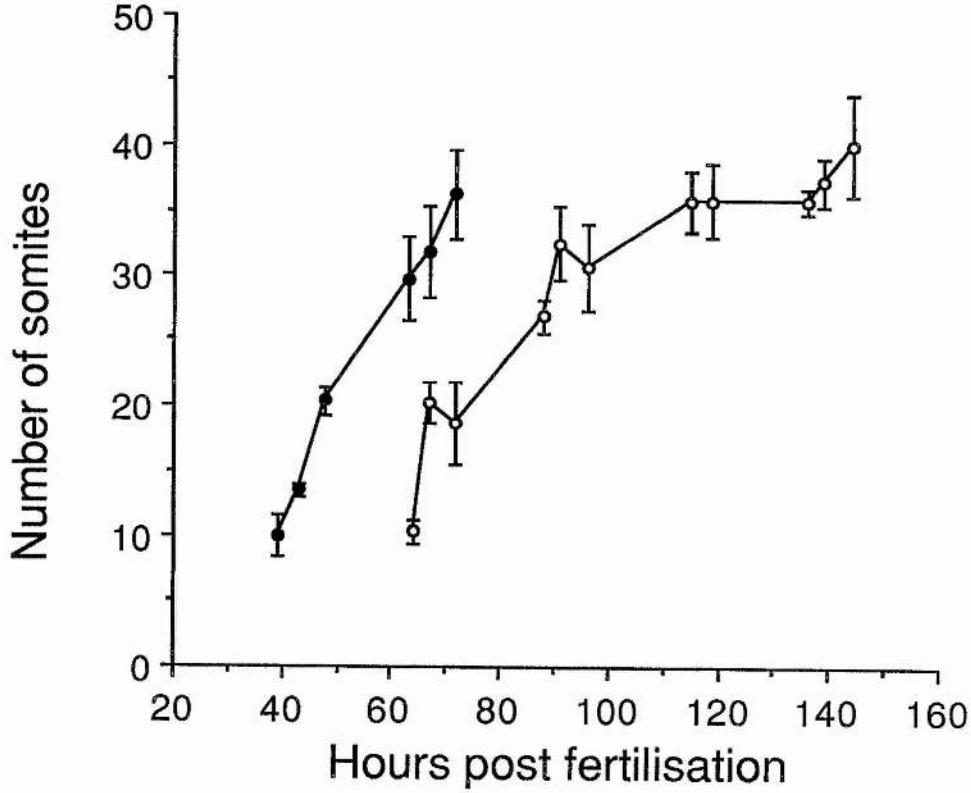
Figure 2a. *Scophthalmus maximus*. Increase in the number of somites from fertilisation to hatching, for turbot embryos maintained at 12°C (○) and 16°C (●). Values are mean ± SD.

Figure 2b. *Scophthalmus maximus*. Increase in the number of somites in turbot embryos maintained, at 12°C (○) and 16°C (●), expressed as a percentage of embryonic development time (DT). Values are mean ±SD.


Number of somites
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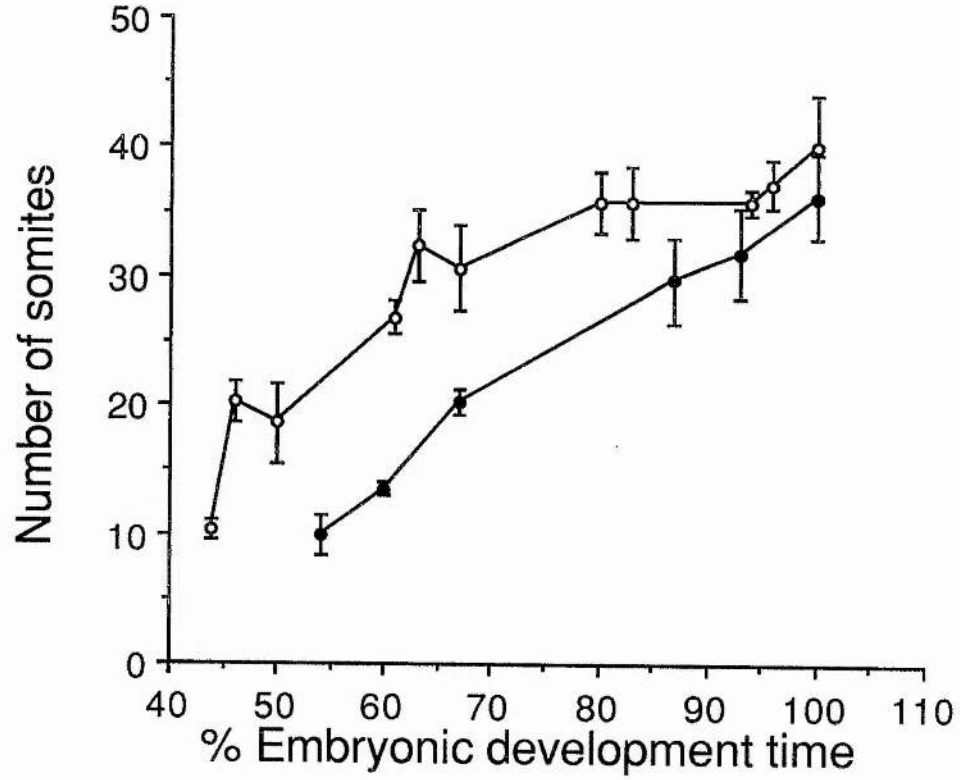
140
160
Hours post fertilisation


Number of somites
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110
% Embryonic development time

Figure 3. *Scophthalmus maximus.* Tracings of turbot embryos at 50% epiboly (a.), the beginning of somite and eye formation (b.) and just prior to hatch (c.). o: oil globule; y: yolk sac, e: eye; s: somites; l: lens; ov: otic vesicle; pfb: pectoral fin bud; n: notochord; pfm: primordial fin margin. Scale bar represents 1mm.

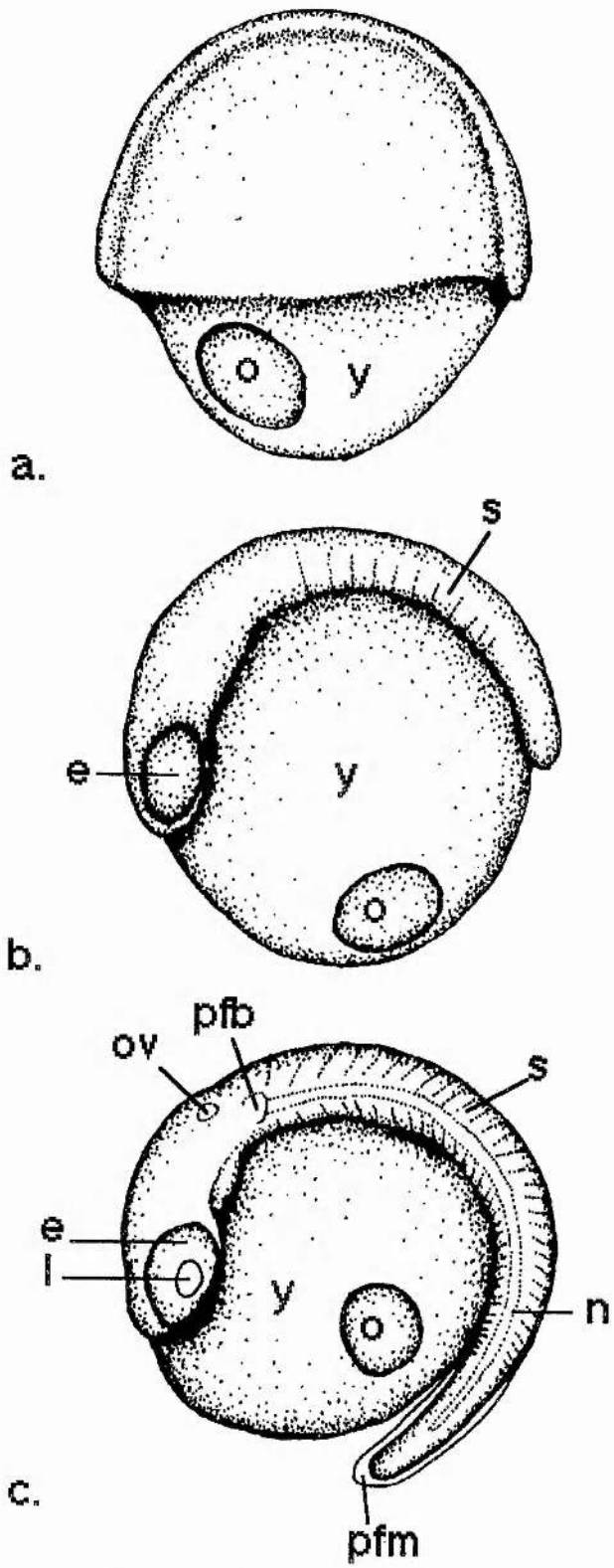
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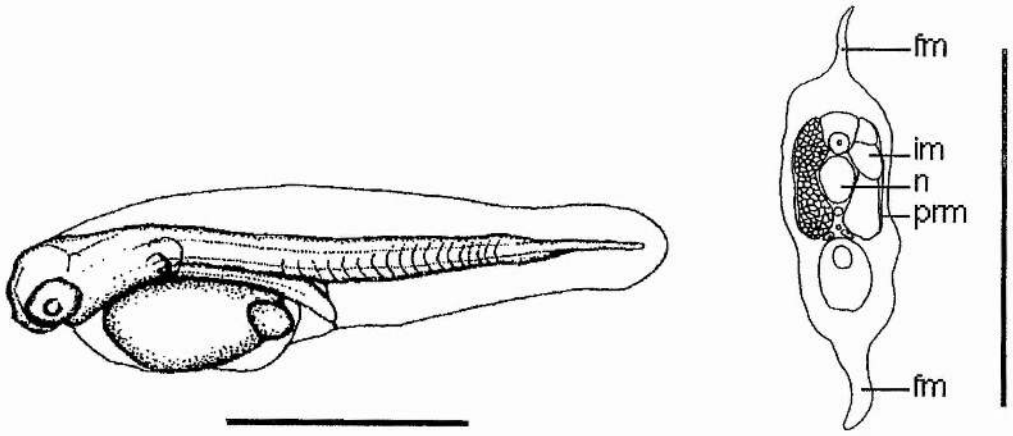
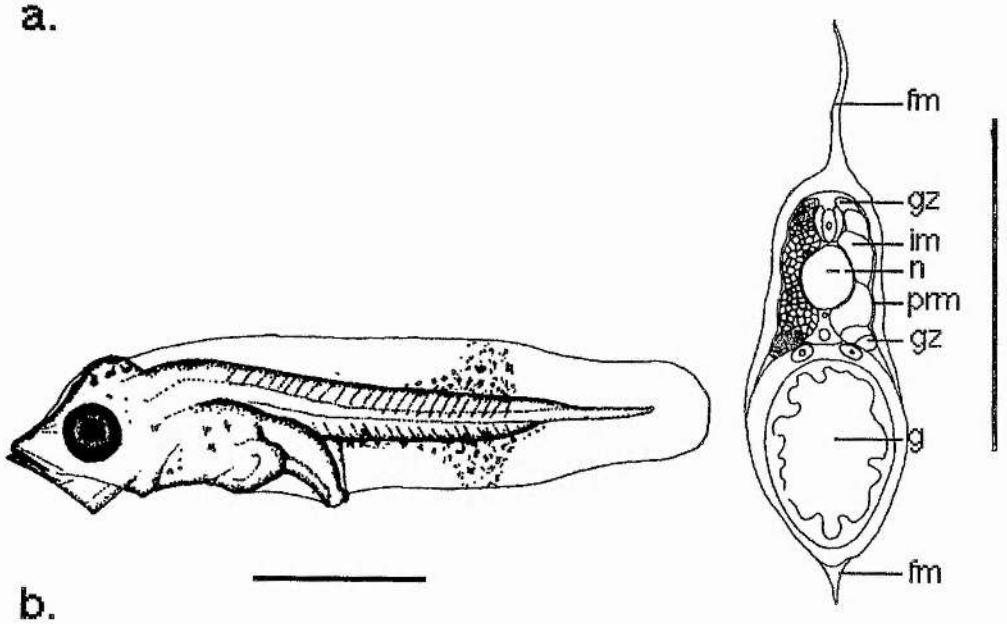
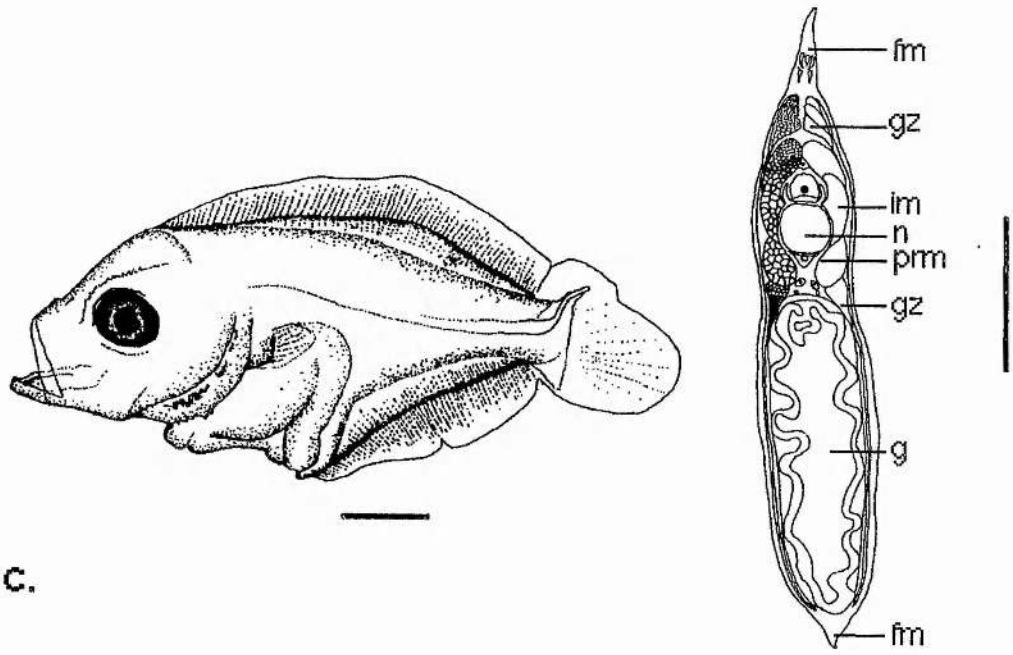
Figure 4. *Scophthalmus maximus*. Larval development at day 1 (a), day 11 (b) and day 26 (c) post hatching at 16°C. fm: fin margin; im: inner muscle; prm: presumptive red muscle; n: notochord; g: gut; gz: germinal zone. Scale bars represent 1mm.



a.



b.



c.

Figure 5. *Scophthalmus maximus.* Time from fertilisation to hatching for turbot embryos reared at various temperatures. ○: modified from Jones (1972), ●: this study.

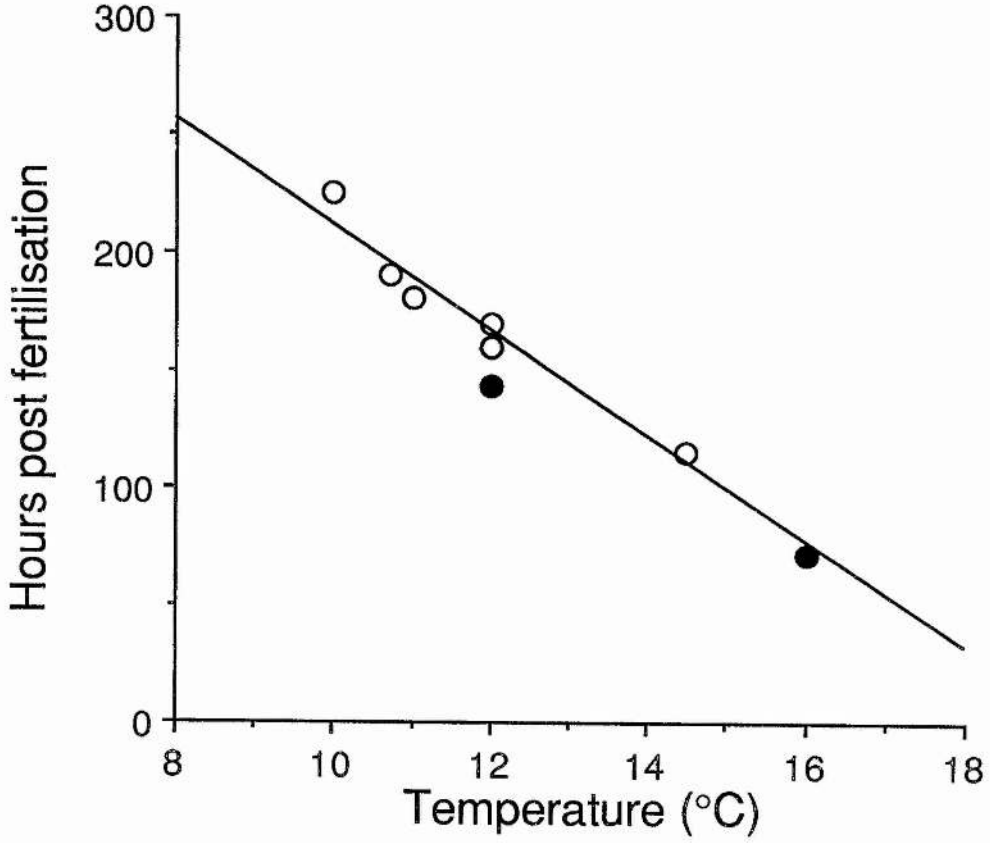
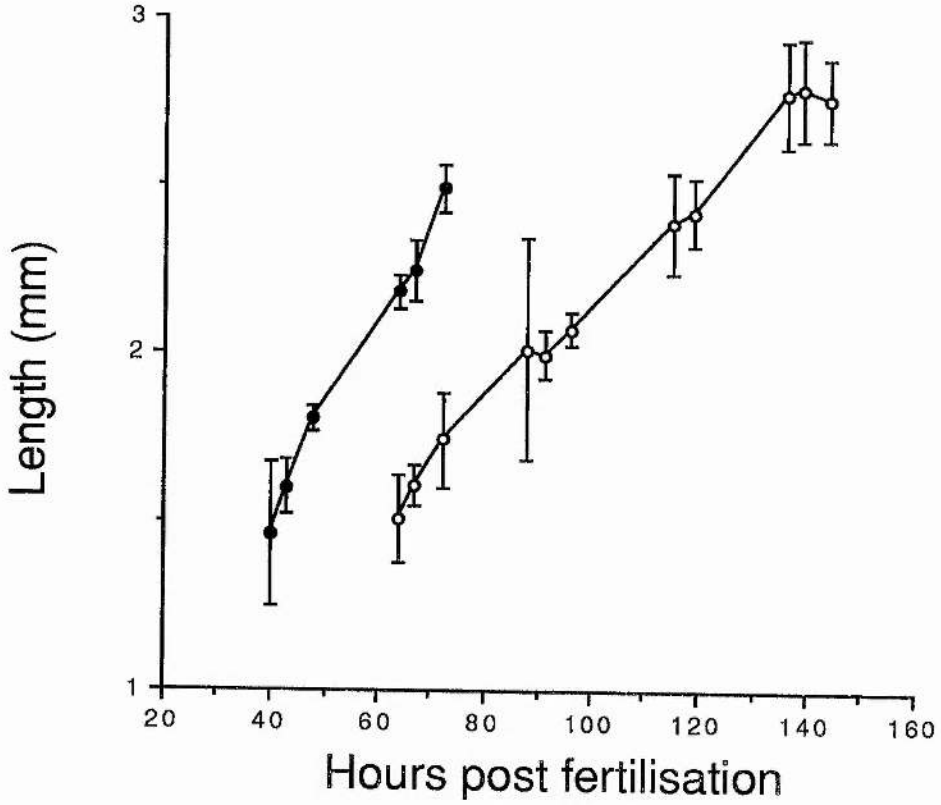

300
200
100
0
Hours post fertilisation
8
10
12
14
16
18
Temperature (°C)

Figure 6a. *Scophthalmus maximus*. Increase in length (mm) of turbot embryos, from fertilisation until hatching, reared at 12°C (o) and 16°C (●). Values are mean ±SD.

Figure 6b. *Scophthalmus maximus*. Increase in length (mm) for turbot embryos from fertilisation until hatching, reared at 12°C (o) and 16°C (●), expressed in relation to the number of somites. Values are mean ±SD.


Length (mm)
3
2
1
20
40
60
80
100
120
140
160
Hours post fertilisation

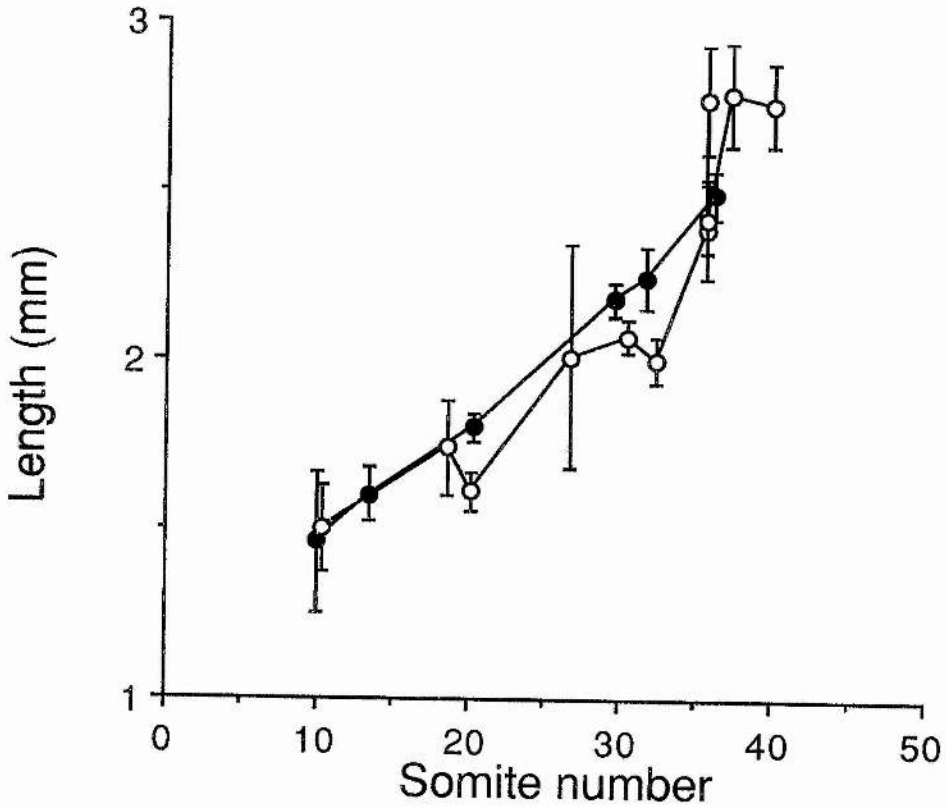

Length (mm)
3
2
1
0
10
20
30
40
50
Somite number

Figure 7. *Scophthalmus maximus.* Increase in total length (mm) of turbot larvae from hatching to day 26 post hatching, at 12°C (o) and 16°C (●).

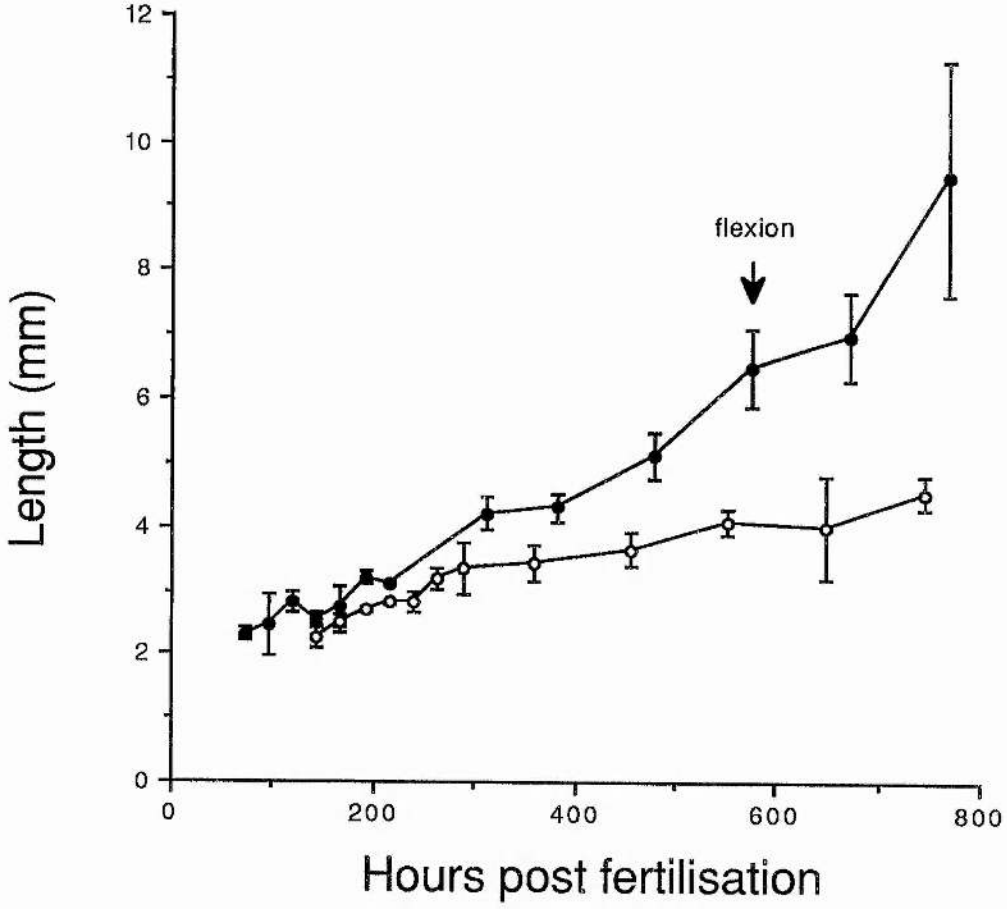
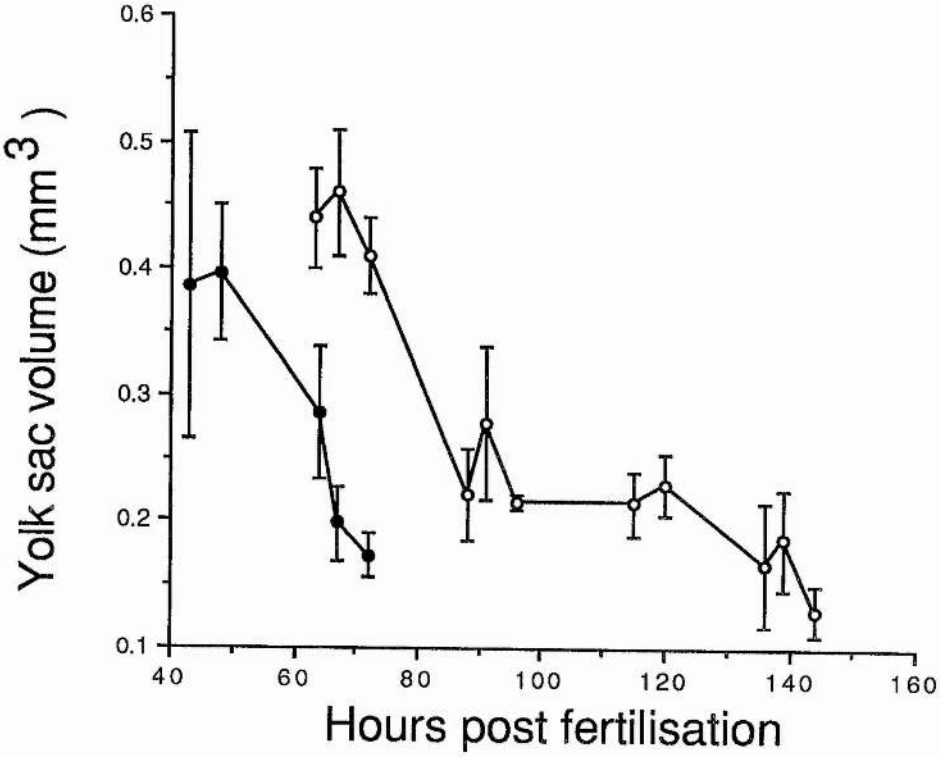

12
10
8
6
4
2
0
0
200
400
600
800
flexion
Length (mm)
Hours post fertilisation

Figure 8a. *Scophthalmus maximus.* Decrease in yolk sac volume (mm^3) in turbot embryos, from fertilisation until hatching, reared at 12°C (○) and 16°C (●). Values are mean ±SD.

Figure 8b. *Scophthalmus maximus.* Decrease in yolk sac volume (mm^3) for turbot embryos reared at 12°C (○) and 16°C (●) from fertilisation to hatching, expressed in relation to the number of somites. Values are mean ± SD.


Yolk sac volume (mm 3)
0.6
0.5
0.4
0.3
0.2
0.1
40
60
80
100
120
140
160
Hours post fertilisation

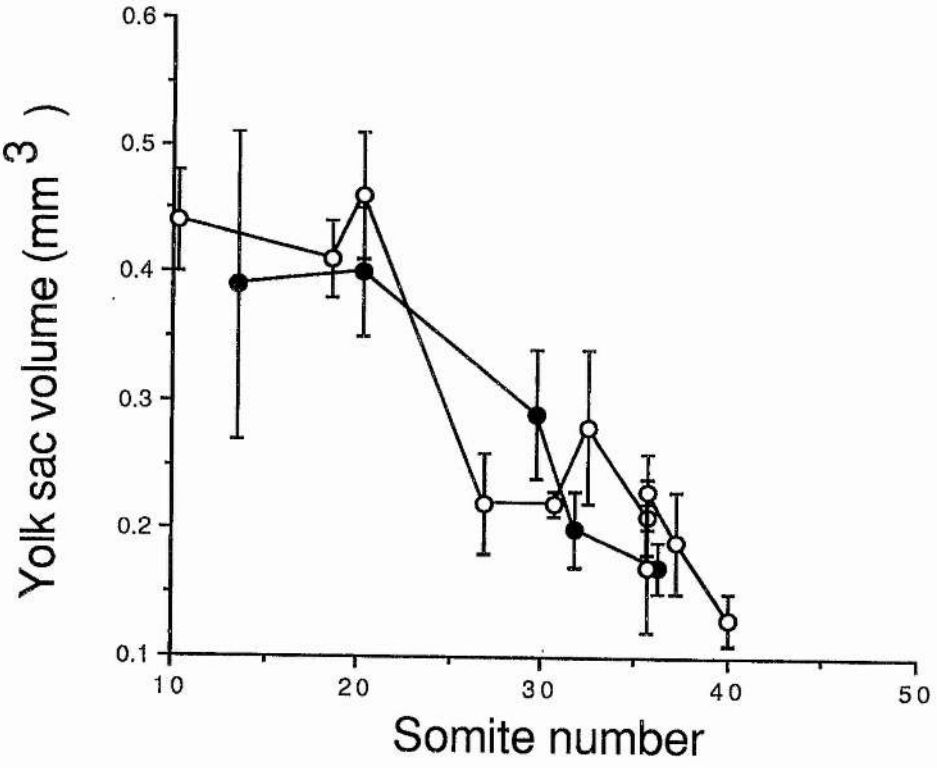

Yolk sac volume (mm 3)
0.6
0.5
0.4
0.3
0.2
0.1
10
20
30
40
50
Somite number

Figure 9. *Scophthalmus maximus.* Total cross-sectional area (mm^2) of the inner muscle of turbot larvae reared from hatching until day 26, at 12°C (○) and 16°C (●). Values are mean ± SD.

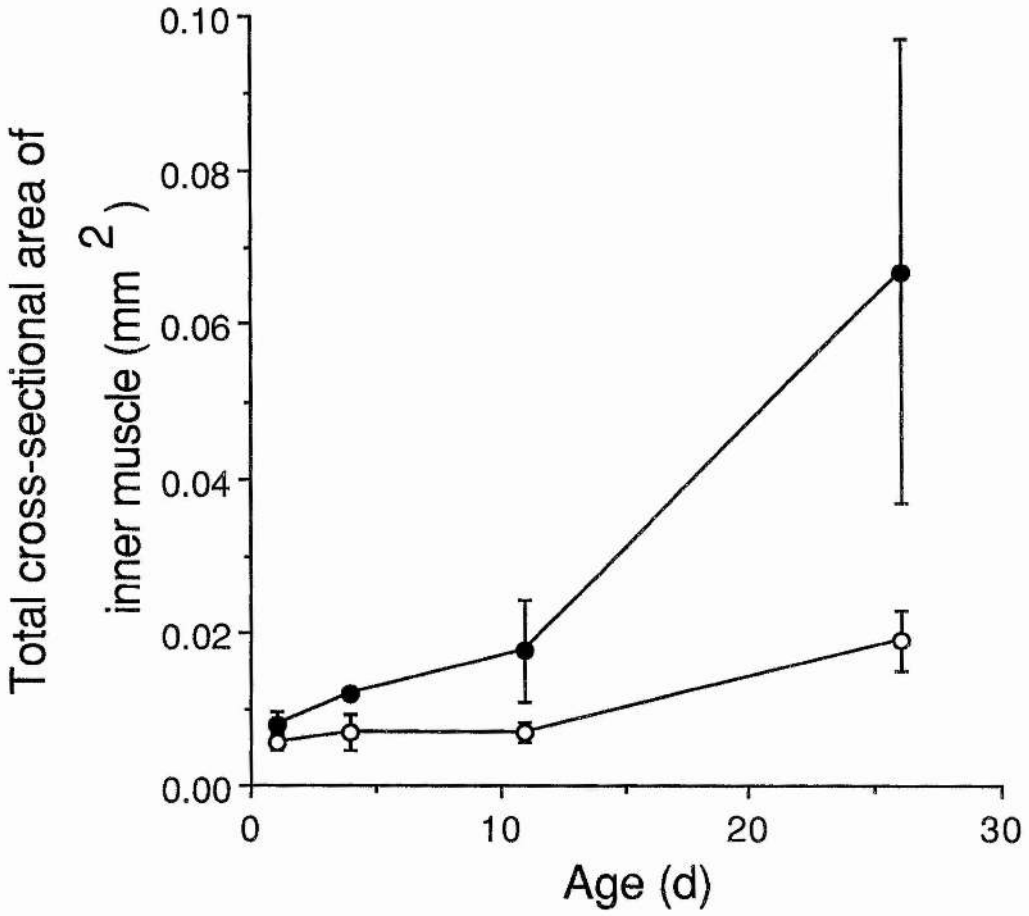

Total cross-sectional area of inner muscle (mm 2)
0.10
0.08
0.06
0.04
0.02
0.00
0
10
20
30
Age (d)

Figure 10. *Scophthalmus maximus.* Number of inner muscle fibres for turbot larvae reared from hatching until day 26 post hatching, at 12°C (○) and 16°C (●). Values are mean ± SD.

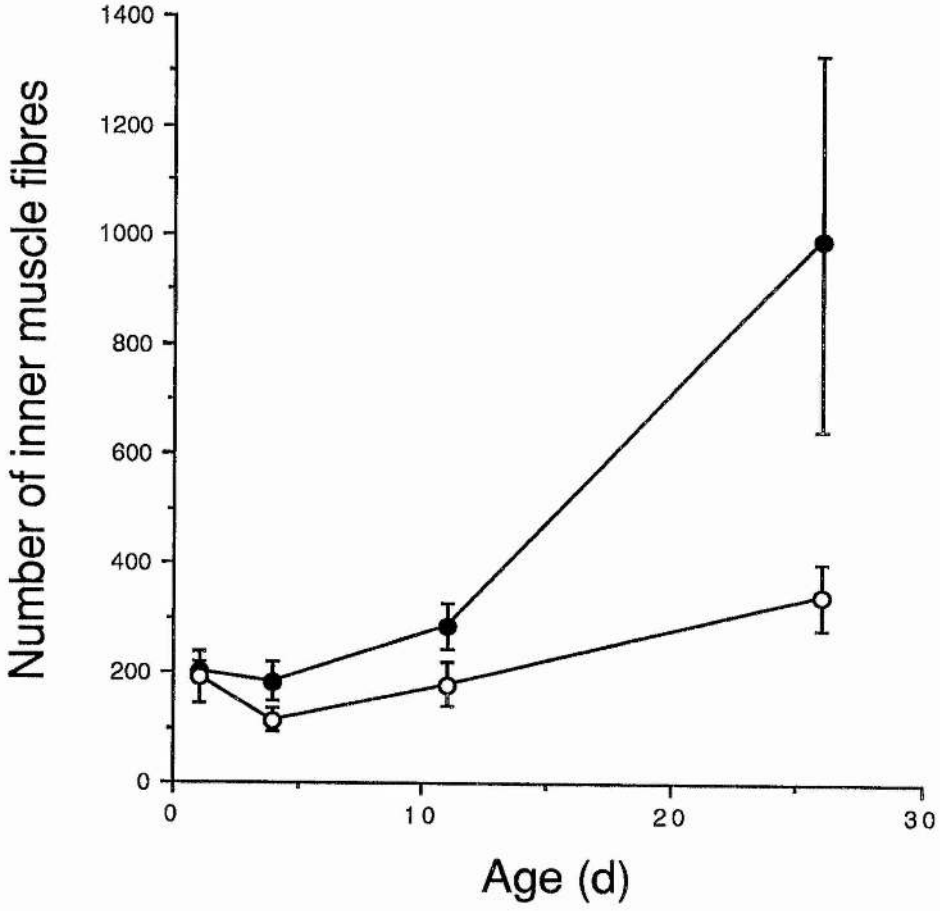

Number of inner muscle fibres
1400
1200
1000
800
600
400
200
0
0
10
20
30
Age (d)

Figure 11. *Scophthalmus maximus.* Average cross-sectional area (mm^2 $X10^{-5}$) of inner muscle fibres of turbot larvae reared from hatching to day 26 post hatching, at 12°C (○) and 16°C (●). Values are mean ± SD.

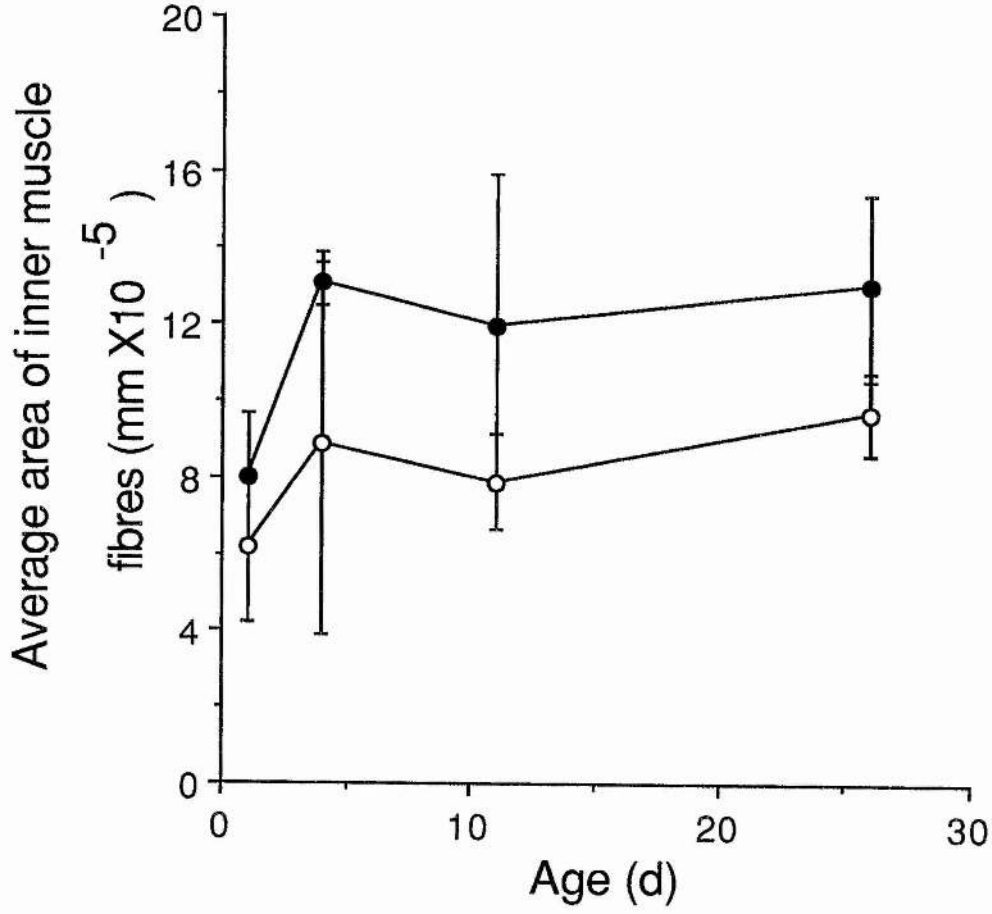
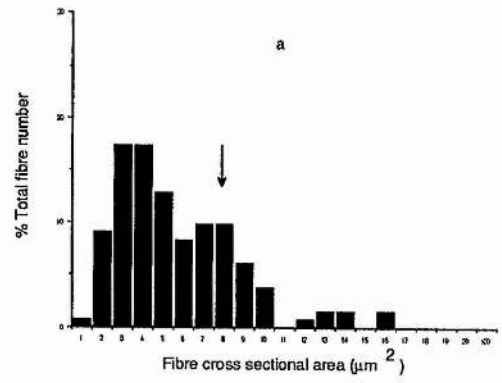
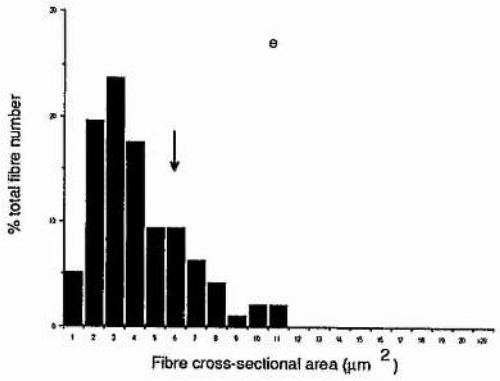
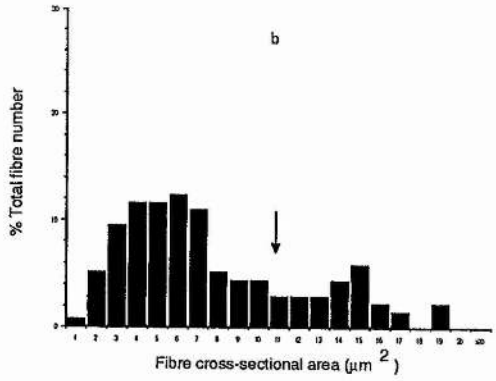
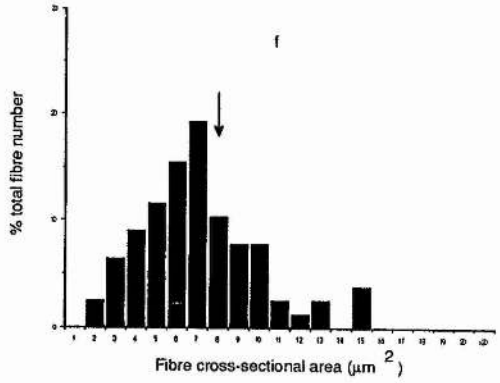

Average area of inner muscle fibres (mm X10 -5)
20
16
12
8
4
0
0
10
20
30
Age (d)

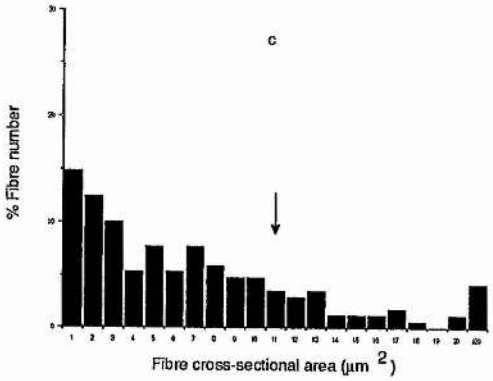
Figure 12. *Scophthalmus maximus.* Distribution of percentage total fibre number of inner muscle fibres in each fibre cross-sectional area size class ($1\mu m^2$), for turbot larvae at 12°C (e-h) and 16°C (a-d). a: D1 16°C; b: D4 16°C; c: D11 16°C; d: D26 16°C; e: D1 12°C; f: D4 12°C; g: D11 12°C; h: D26 12°C. Arrows represent mean fibre cross-sectional area (μm^2).

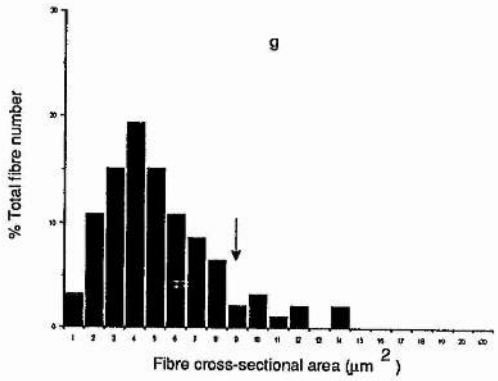

a
% Total fibre number
Fibre cross sectional area (µm 2)

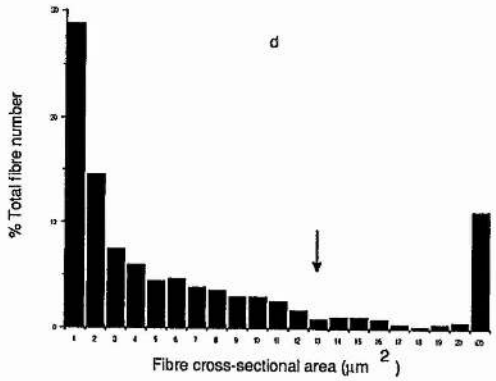

e
% total fibre number
Fibre cross-sectional area (µm 2)


b
% Total fibre number
Fibre cross-sectional area (µm 2)


f
% total fibre number
Fibre cross-sectional area (µm 2)


c
% Fibre number
Fibre cross-sectional area (µm 2)


g
% Total fibre number
Fibre cross-sectional area (µm 2)


d
% Total fibre number
Fibre cross-sectional area (µm 2)

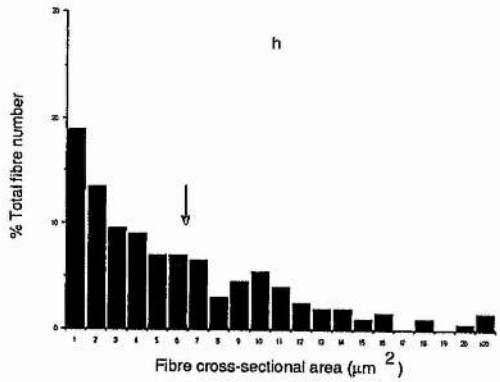

h
% Total fibre number
Fibre cross-sectional area (µm 2)

Figure 13. *Scophthalmus maximus.* Transverse section through the myotomes of larval turbot on day 26 after hatching at 16°C. if: inner muscle fibres; sf: superficial muscle fibres; sk: skin.

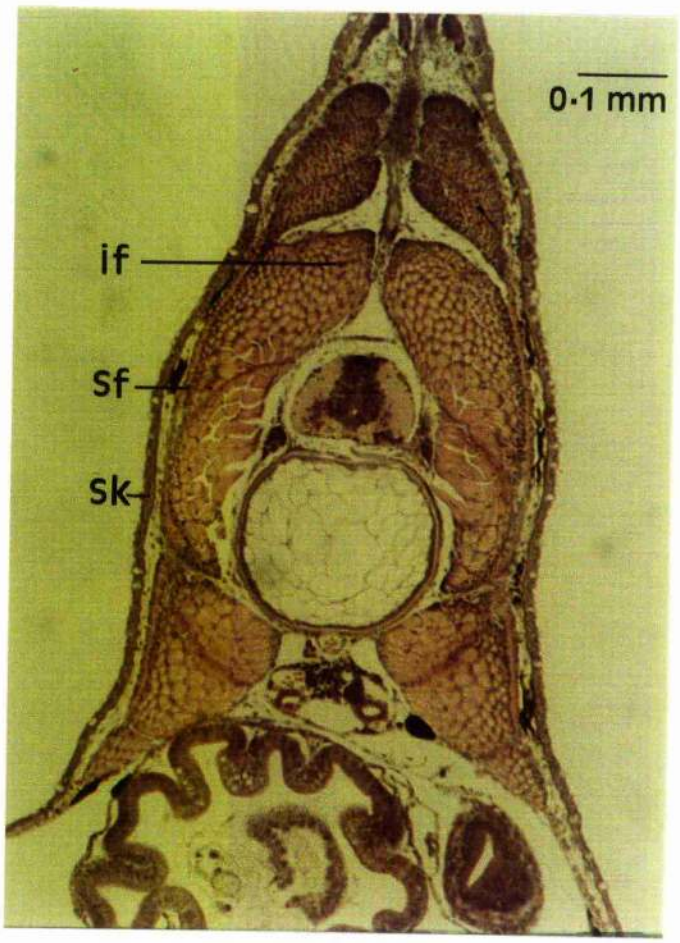
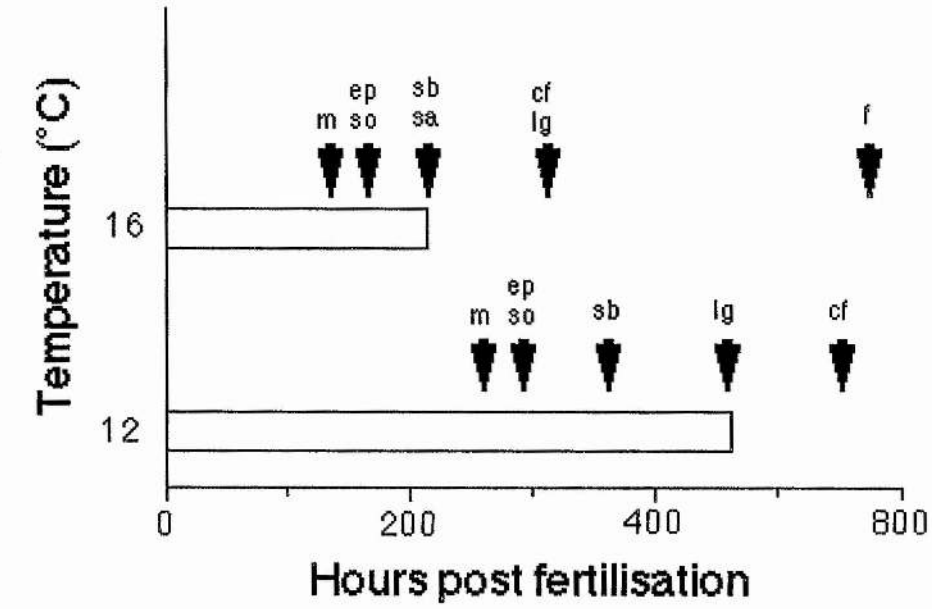
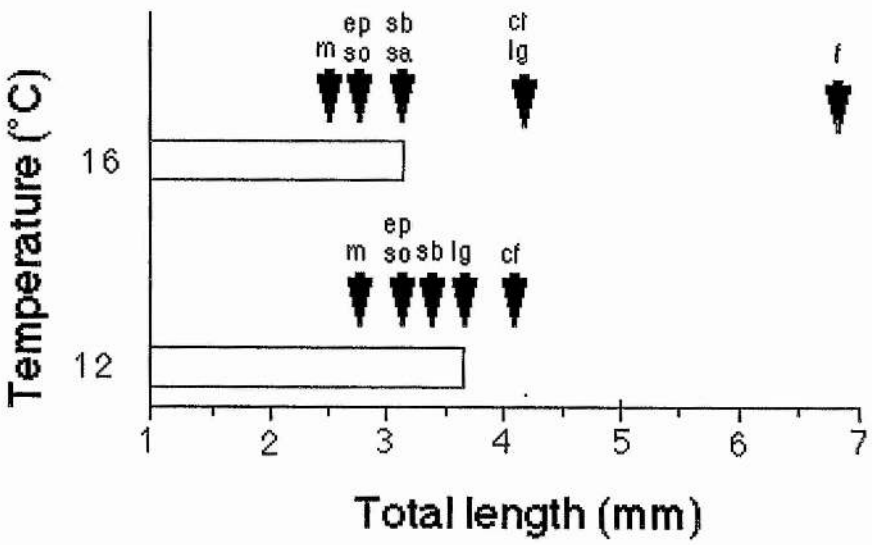

0·1 mm
if
Sf
Sk

Figure 14a. *Scophthalmus maximus.* Timing of the occurrence of various developmental features in turbot larvae at 12°C and 16°C, with respect to the presence of the yolk sac (horizontal bars). m: mouth opening; ep: eye pigmented; sb: swim bladder; cf: caudal fin; l: loop in gut; so: spines on the operculum; sa: spines in the auditory region; f: flexion. Values are mean ± SD.

Figure 14b. *Scophthalmus maximus.* Tining of the occurrence of developmental features in turbot larvae at 12°C and 16°C, with respect to larval length and the presence of the yolk sac (horizontal bars). Abbreviations explained in figure 14a.


Temperature (°C)
16
12
m
ep
so
sb
sa
cf
lg
f
m
ep
so
sb
lg
cf
0
200
400
800
Hours post fertilisation


Temperature (°C)
16
12
m
ep
so
sb
sa
cf
lg
f
m
ep
so
sb
lg
cf
1
2
3
4
5
6
7
Total length (mm)

Chapter 3

Scaling relationships and the influence of temperature on maximum swimming speed of turbot (*Scophthalmus maximus*)

Introduction

After a pelagic phase the larvae of the turbot (*Scophthalmus maximus*) undergo metamorphosis and settle in shallow water on sandy substrates (Day 1880-1884). Settled flatfish have a large variety of predators such as the shore crabs *Carcinus maenas*. The major factor affecting larval survival is predation and not starvation (May 1974). Predation may account for all the losses of small juvenile plaice (Van Der Veer and Bergman 1987). Bailey and Batty (1984) demonstrated that the escape success rate of larval fish from predators was related to burst swimming speed, which was correlated with fish length. Predator evasion behaviours, such as the burst escape swimming response, are therefore of major importance in determining the survival of early life stages and hence recruitment to the adult population (Batty and Blaxter 1992).

Settled turbot can experience a wide range of temperatures, varying on seasonal, diurnal and tidal cycles. For example, in Kiel Bay turbot encounter seasonal variations of ambient temperature between 8 and 24 °C (Waller 1992). Escape swimming behaviour often involves a C-start during which the head and the tail of the fish rotate in the same direction away from the centre of mass during the initial tail-beat (Webb 1976). Recent studies with larval stages of Atlantic herring (Batty *et al.* 1993, Batty and Blaxter 1992, Batty *et al.* 1991), plaice (Batty and Blaxter 1992), and the zebra danio (Fuiman 1986) have shown

that the maximum velocities attained during C-starts are highly temperature dependent, with Q_{10}'s in the range 1.4 to 1.9. In contrast, maximum velocity and acceleration during S-shaped fast-starts, which are used during prey capture, were found to be relatively independent of temperature in the short-horned sculpin (*Myoxocephalus scorpius*) over the range 5°C to 15°C (Beddow *et al.* 1994).

The aims of the present study were to investigate the influence of growth and temperature on escape swimming performance in early settled stages of the turbot.

Materials and Methods

Fish

Turbot larvae were obtained, at various stages of development, from a commercial supplier (Golden Sea Produce Ltd, Hunterston, Strathclyde, Scotland). Wild juvenile turbot (4.2 - 5.3 cm total length, TL) were caught using a hand-towed beam trawl at Tralee Bay, Argyll, Scotland. All fish were maintained at 17-19°C in filtered re-circulating sea water. Larval stages were initially fed *Artemia* sp. nauplii and gradually weaned onto a proprietary diet (Ecoline 17, Ecoline

Ltd, Middlesex, U. K.). Wild turbot were filmed within 2 weeks of capture, whereas the farmed turbot were maintained at 18°C in recirculating sea water aquarium at the Gatty Marine Laboratory for up to 6 months giving a size range of 8.8 - 80.0 mm TL.

Measurement of burst swimming performance

Swimming trials were carried out in one of two square perspex tanks (19 x 19 x 5 cm deep and 30 x 30 x 6 cm deep) suspended in a temperature-controlled water bath (±0.4°C). Sharp silhouettes of fish were obtained by using a reflective Scotchlite background beneath the tank and overhead illumination (2 x 60 W lamps). A transparent 0.5 or 1 cm square grid overlay was glued to the bottom of the tank to provide a scale. Fish were filmed at 50 Hz by recording the image from a mirror suspended, at an angle of 45°, above the tank using a Panasonic WVP-F10E video camera (Panasonic, Matsushita Industrial Trading Co., Ltd.., Osaka, Japan.). Some sequences were filmed from the side at 200 Hz, in a perspex tank (20 x 5 x 9 cm deep) using a NAC HSV-400 high-speed video system (NAC-Corp, Japan). Individual turbot were transferred to the filming arena at 18°C and the temperature was adjusted to the test temperature at a rate of either 0.1°C min^{-1} or 1.0°C min^{-1} (Table 1), this temperature range was chosen for the order of magnitude difference. Fish were left undisturbed for 60 min at

the test temperature prior to experiments. Burst swimming sequences were initiated by tactile stimulation of the fish with a fine probe (1.5 mm diameter). Three or four bursts of swimming were recorded from each individual during each trial. Video tapes of the fastest sequences were analysed frame-by-frame at 20 ms (50 Hz) or 5 ms (200 Hz) intervals. The maximum speed (U_{max}) was taken as the fastest values recorded over either a 20 or 5 ms interval. A simple measure of acceleration was taken as the change in speed from rest to U_{max} over the time taken.

Statistical analysis

Scaling relationships between TL and U_{max} of the form $U_{max} = aTL^b$ were calculated using least squares regression techniques and geometric mean regression. The length exponents (b) of larval (< 4.0 cm TL) and juvenile (> 4.0 cm TL) stages were compared using the technique described by Edwards (1962) for comparing regression coefficients. Since no significant differences were found in the b values for larvae and juveniles different temperature groups were subsequently compared using analysis of covariance (Sokal and Rohlf 1981). All results were expressed as Mean ± SD.

Results

Fish

All the turbot used in this study were either juveniles or settled larvae at various stages of metamorphosis. Larval staging was carried out using the following criteria (Al-Maghazachi and Gibson 1984); stage 3: larva has a swim bladder, but it is still symmetrical (<1.2 cm); stage 4: asymmetry beginning, eye migration to the point where the eye is just visible from the left side of the body (1.2 - 2.1 cm); stage 5: eye migration continues and re-absorption of swim bladder is complete (2 - 4 cm). In the present study, the length of larvae at the various stages was greater than previously reported (Al-Maghazachi and Gibson 1984). Metamorphosis was considered complete when the right eye had migrated away from the dorsal margin of the cranium, and occurred at approximately 4 cm TL.

The escape swimming response

The escape swimming response was characterised by a rapid acceleration to a maximum velocity (U_{max}) then a deceleration to rest. Trials where the edge of the swimming chamber were encountered were discounted. The beginning of

the response usually began with an "omega jump" (Fig. 1) in which the head was lifted slowly off the substrate then, in a rapid movement, the tail lifted and produced the propulsive stroke against the bottom of the filming chamber (Kruuk 1963). Fish length had a marked effect on the magnitude and duration of different components of the escape swimming response (Fig. 2). U_{max} occurred at approximately 0.04s, after stimulation, for all fish lengths (P=0.05). Acceleration from rest to U_{max} was positively correlated with length at 13°C ($R^2 = 0.34$, $P<0.01$) and 18°C ($R^2 = 0.28$, $P<0.01$), but not at 23°C. Acceleration was independent of temperature between 13° and 18°C, but increased from 10.0 ±6.6 m s^{-2} at 18° to 14.2 ±6.0 m s^{-2} at 23°C, with a Q_{10} of 1.43. As swimming sequences were often not followed to rest, deceleration was calculated as the change in speed over the time it took to reach $\frac{1}{2}U_{max}$. Deceleration times to $\frac{1}{2}U_{max}$ were calculated for juveniles and was found to increase with increasing fish length. The deceleration of larvae was not calculated as they often did not reach $\frac{1}{2}U_{max}$ in the time period measured, possibly due to the relatively slow framing (50 Hz) rate which may have slightly underestimated U_{max} in larvae.

Influence of rate of temperature change on swimming velocity

Two groups of larvae were subjected to varying rates of temperature change, differing by an order of magnitude, (0.1 or 1.0°C min^{-1}) from the acclimation temperature of 18°C, to either

13 or 23°C. The rate of temperature change had no significant effect on U_{max} at the trial temperatures and similar results were obtained with heating and cooling (Table 1.).

Developmental stage and swimming velocity

The maximum escape swimming speed (U_{max}) for both larvae and juveniles increased with fish length (TL) at each temperature. Relationships were fitted using least squares regressions of the form $\log U_{max} = \log a + b \log TL$ (Table 2). Length-specific swimming speed (U^*_{max}, TL s^{-1}) decreased with fish length (Table 2). Since regression analysis showed that there was no significant difference in slopes between larvae and juveniles, those groups were combined within each temperature treatment. The common slopes (b) calculated at each temperature are shown in Table 2.

Temperature and swimming velocity

Analysis of covariance showed that the scaling relationship between total length (TL) and U_{max} (cm s^{-1}) was not significantly different between temperatures (Table 2, $F = 31.13$, $P<0.01$). There was however a stepwise increase in the

intercepts with increasing temperature, indicating a significant increase in U_{max} with temperature (Fig. 3). Since both log and linear regressions were highly significant a model of the form $U_{max}=a+bTL$, incorporating the effect of test temperature was formulated (F= 0.0, 0.14, and 0.91 at 13, 18 and 23°C respectively):-

$$U_{max} = 28.4 + 10.9\left(\frac{Temp - 13}{5}\right) + 10.3TL$$

Predictions of U_{max} from the model did not differ significantly from measured U_{max} values at the test temperatures (P=0.05). The calculated Q10 for U_{max} was 1.77 ± 0.001 over the temperature range 13 to 23°C.

Discussion

Development and maximum swimming speed

Following settlement, the scaling relationship for maximum speed was unchanged throughout the latter stages of metamorphosis (Fig. 3). Length-specific escape swimming speed (U^*_{max}) in turbot was related to total length (TL cm) by a power equation of the form $U^*_{max} = a.\ TL^{-0.69}$(Table 2). U_{max} (cm s^{-1}) scaled in proportion to $TL^{0.74}$ at all temperatures (Table 2). There are limited data available on the scaling of U_{max} in other fish species. Archer and Johnston (1989) reported that U_{max}

scaled with $TL^{0.66}$ in the Antarctic fish, *Notothenia coriceps* at 1°C. Maximum tail beat frequency is related to maximum muscle contraction time (T) which in turn is related to U_{max} by the formula $U_{max} = A^*L/2T$, where A is stride length (Wardle 1975). Using hydrodynamic theory Webb (1977), postulated that maximum tail beat frequency (f) scaled in proportion to $L^{-0.44}$. Using Wardle's (1975) data, Webb (1977) calculated that f was proportional to $L^{-0.40}$ in 4 species of marine fish.

The myotomes of larval teleosts contain distinct muscle fibre types from those of juvenile stages (Batty 1984, El-Fiky *et al.* 1987, Scapolo *et al.* 1988, Crockford and Johnston 1993, Johnston and Horne 1994). In the larvae of Atlantic herring (Batty 1984), plaice (Brooks and Johnston 1993) and the turbot (Gibson and Johnston, unpublished results) there is a single superficial layer of small diameter muscle fibres staining intensely for succinic dehydrogenase (SDHase) activity, indicating strong oxidative capacity, which surrounds an inner mass of larger diameter fibres which stain less heavily for SDHase. The inner-muscle fibres of herring larvae contain a higher volume density of mitochondria (Vieira and Johnston 1992), and express different isoforms of myosin heavy chains, myosin Light Chain 2, tropomyosin, Troponin I and Troponin T than the white muscle fibres of juvenile stages (Crockford and Johnston 1993). In some species, such as the herring, larval muscle fibre types are replaced at metamorphosis, concomitant with the development of functional gill filaments (Batty 1984, Johnston and Horne 1994). In contrast, in flatfish, such as the plaice (*Pleuronectes platessa*), the muscle fibre types found in the

pelagic larvae are retained in the settled stages and are not replaced by adult types until several months after metamorphosis (Brooks and Johnston 1993). Metamorphosis in plaice was not correlated with changes in myosin heavy chain composition in the inner muscle fibres, although settled stages started to express myosin light chain 2 isoforms characteristic of the superficial fast muscle fibres in adult fish (Brooks and Johnson 1993). Williams and Brown (1992) found that in the winter flounder (*Pleuronectes americanus*) there was no change in relationship between U_{max} and TL between hatching and the end of metamorphosis. Thus in flatfish although metamorphosis is associated with a change in symmetry and major morphological adaptations, the myotomal muscles and swimming performance are relatively unchanged through the transition from pelagic to early demersal phases of the life history.

Influence of temperature on escape swimming behaviour

U_{max} was temperature dependent, the relationship between size and U_{max} was temperature independent. Over the temperature range 13°C to 23°C U_{max} could be described by the predictive model: $U_{max} = 28.4 + 10.9\left(\frac{\text{Temp} - 13}{5}\right) + 10.3\text{TL}$.

Turbot larvae begin settling in July in Tralee Bay, Argyll, and are found in shallow water until September. During this time fish are increasing in length and as the model predicts show an

increase in escape swimming speed. At the same time there is an increase in average temperature throughout the summer and early autumn, overlying temperature changes on daily and tidal cycles. At Tralee Bay, temperature varies seasonally between 7 and 15°C at a depth of 5m (Gibson *et al.* 1993). An acute change in temperature from that of acclimation temperature did not alter the scaling relationship between U_{max} and length (b values were not significantly different, Table 2), but did cause a stepwise increase in swimming speed between 13, 18 and 23°C (Q_{10} = 1.77) (a values were significantly different, Table 2). An increase in escape swimming velocity with increasing temperature has also been demonstrated in the pelagic larvae of herring (Batty *et al.* 1993, Batty and Blaxter 1992, Batty et al. 1991), plaice (Batty and Blaxter 1992) and the zebra danio (Fuiman 1986). Q_{10} values for U_{max} in all these species were in the range 1.4 to 1.9. In plaice U_{max} was a linear function of temperature below 13°C, but levelled off at higher temperatures (Batty *et al.* 1993, Batty and Blaxter 1992). Webb (1978) showed that in trout U_{max} has a Q_{10} of 1.7 between 5° and 15°C, but reached a plateau at higher temperatures. Fuiman (1986) suggested that maximum speed is linearly related to temperature within the normal thermal range experienced by each species. For turbot the relationship between U_{max} and TL was linear up to 23°C, which may be partly due to settled turbot occurring in shallower water than plaice and so experiencing higher temperatures.

Juvenile turbot acclimated to 18°C showed an increase in U_{max} around 3 weeks after an acute drop in temperature to 13°C, consistent with a partial capacity adaptation (Precht 1958).

Maximum sustained swimming speed in striped bass (*Morone saxatilis*) (Sisson and Sidell 1987) and common carp (*Cyprinus carpio*) (Johnston 1993) is also significantly increased following acclimation to low temperature, although the tail-beat frequency and amplitude required to swim at a given speed is unchanged (Johnston 1993). The mechanisms underlying adaptations of maximum sustained swimming performance with cold acclimation in cyprinids include an increase in the volume of red muscle fibres (Johnston and Lucking 1978, Sidell 1980), and an increase in the contraction speed and maximum force generated by the red muscle fibres (Johnston *et al.* 1985) which start to express myosin light isoforms normally associated with fast muscle (Langfeld *et al.* 1991). Less is known about the mechanisms underlying the plasticity of maximum swimming performance. In 5°C-acclimated short-horned sculpin the maximum velocity attained during S-shaped fast-starts at 15°C increases by 32% following 6-8 weeks acclimation to 15°C (Beddow *et al.* 1994). Improved swimming performance following warm acclimation was associated with an increase in maximum force generation and contraction velocity in fast muscle fibres (Beddow and Johnston 1994). For adult stages a temperature change of 10 to 15°C is usually required to elicit adaptations in swimming performance and in the metabolic and contractile properties of muscle fibres (Guderley and Blier 1988). Calvo and Johnston (1992) found that during metamorphosis in turbot a temperature change of only 5°C was sufficient to result in significant changes in the relative proportions of tonic and red muscle fibres in the myotomes. In the present study significant changes in U_{max} with acclimation also occurred with only a 5°C change in ambient temperature (Table 3). An interesting

possibility is that the responses of skeletal muscle to temperature acclimation are not fixed but vary during development and this deserves further study.

Table 1 *Scophthalmus maximus.* Maximum length-specific swimming speed (U^*_{max}) of larval turbot following an acute temperature change to the test temperature, and with two rates of heating and cooling. Values are means ±SD (n is number of fish).

Test temperature (°C)	Rate of temperature change (°C min^{-1})	(n)	TL (cm)	U^*_{max} (TL s^{-1})
13	0.1	(10)	1.97 ±0.76	25.35 ±2.99
	1.0	(7)	1.97 ±0.32	27.81 ±2.23
23	0.1	(7)	2.42 ±0.58	34.57 ±3.54
	1.0	(7)	2.00 ±0.86	37.09 ±4.65

Table 2 *Scophthalmus maximus.* Relationship between total length (TL) and maximum swimming speed (U_{max}) and maximum length-specific swimming speed (U^*_{max}), for settled turbot at three temperatures. Data were fitted by geometric mean regressions to power equation of form $U_{max} = a\ TL^b$. Values are means ±SD (n is number of fish). †: significantly different at 5% level.

Test temperature (°C)	(n)	TL (cm)	U_{max} (cm s^{-1})	$U_{max} = a\ TL^b$			U^*_{max} (TL s^{-1})	$U^*_{max} = a\ TL^b$		
				a	b	R^2		a	b	R^2
13	(33)	3.7 ±2.0	66.3 ±20.1	1.47	0.62	0.86	21.0±6.2	1.64	-0.57	0.82
18	(98)	3.7 ±1.9	76.3 ±24.4†	1.46	0.74	0.76	23.6±7.2†	1.75	-0.66	0.67
23	(38)	4.0 ±2.0	91.7 ±23.5†	1.60	0.60	0.77	27.0±9.4†	1.83	-0.66	0.82
Combined	(169)	3.8 ±2.0	77.8 ±24.8	1.46	0.74	0.73	23.8±7.8	1.77	-0.69	0.67

Fig. 1. *Scophthalmus maximus*. The height above the substrate of the head (●) and tail (○) of a turbot (5.3 cm TL) during the characteristic escape response behaviour, the 'omega jump'.

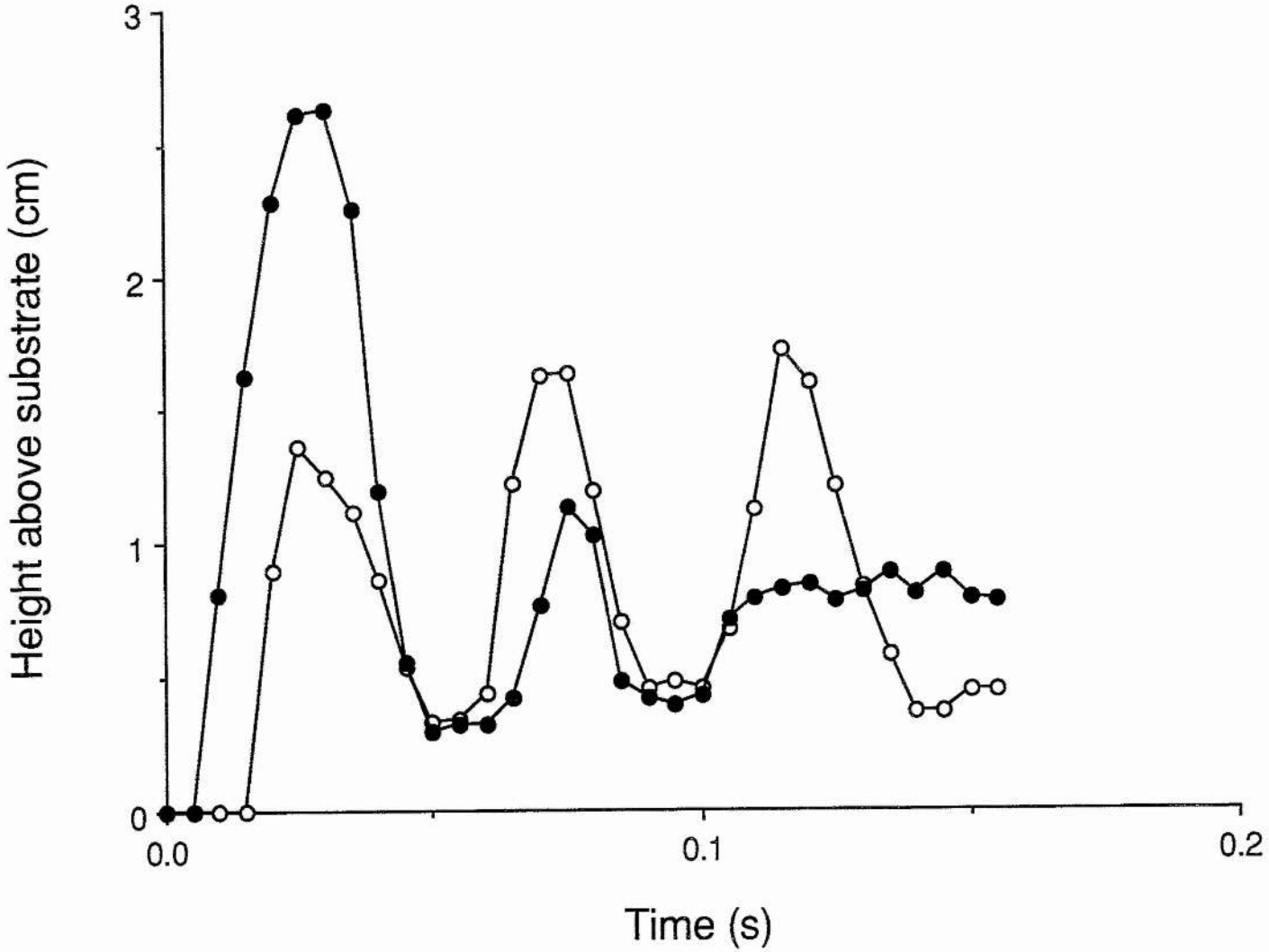

Height above substrate (cm)
3
2
1
0
0.0
0.1
0.2
Time (s)

Fig. 2. *Scophthalmus maximus*. Change in speed (U, cm s^{-1}) with time (s) at 13°C for three size classes of turbot, during the first 0.2 seconds of an escape swimming episode. Values are Mean ±SD. ●: TL = 2.0 ±0.4 cm, n=16. ○: TL = 4.7 ±0.4 cm, n=7. ▲: TL = 6.8 ±0.7 cm, n=7.

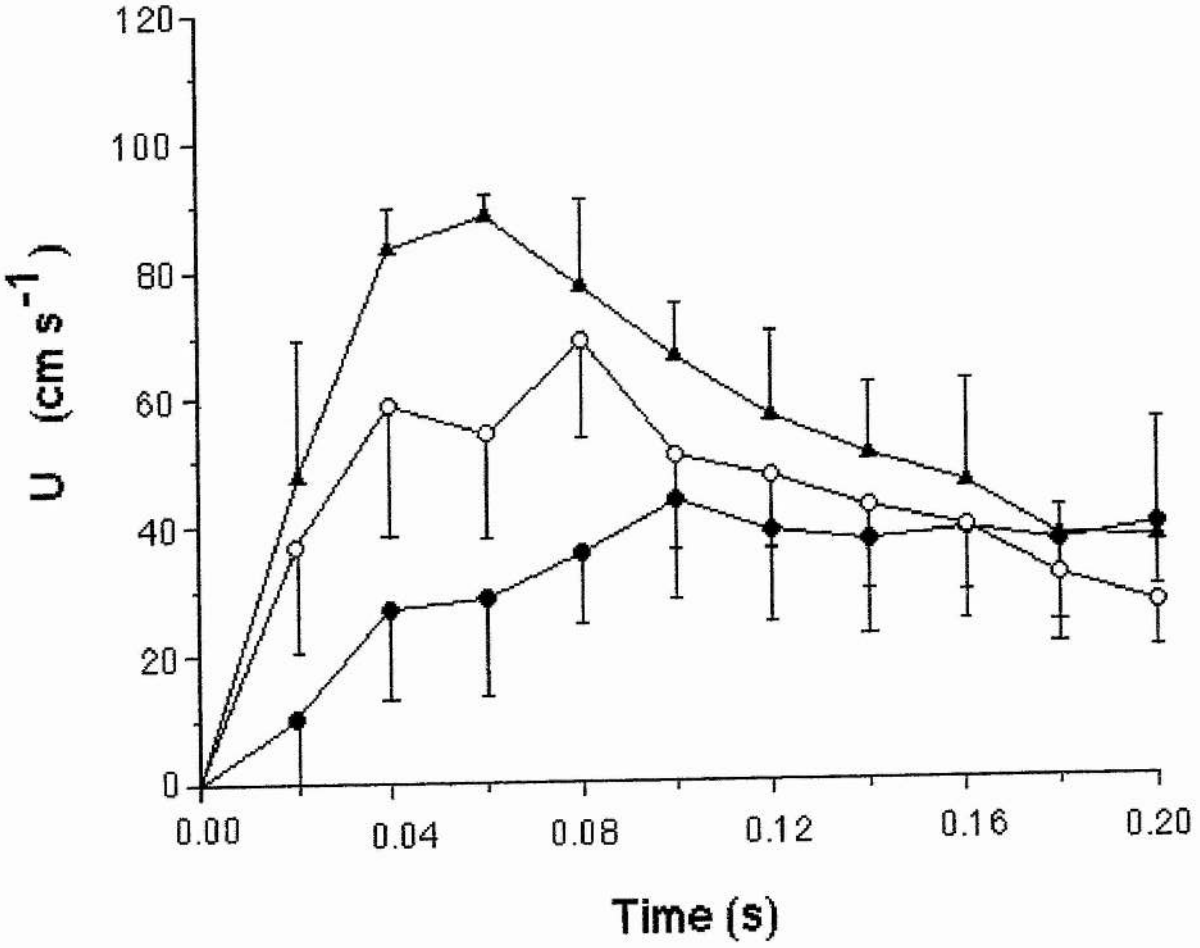
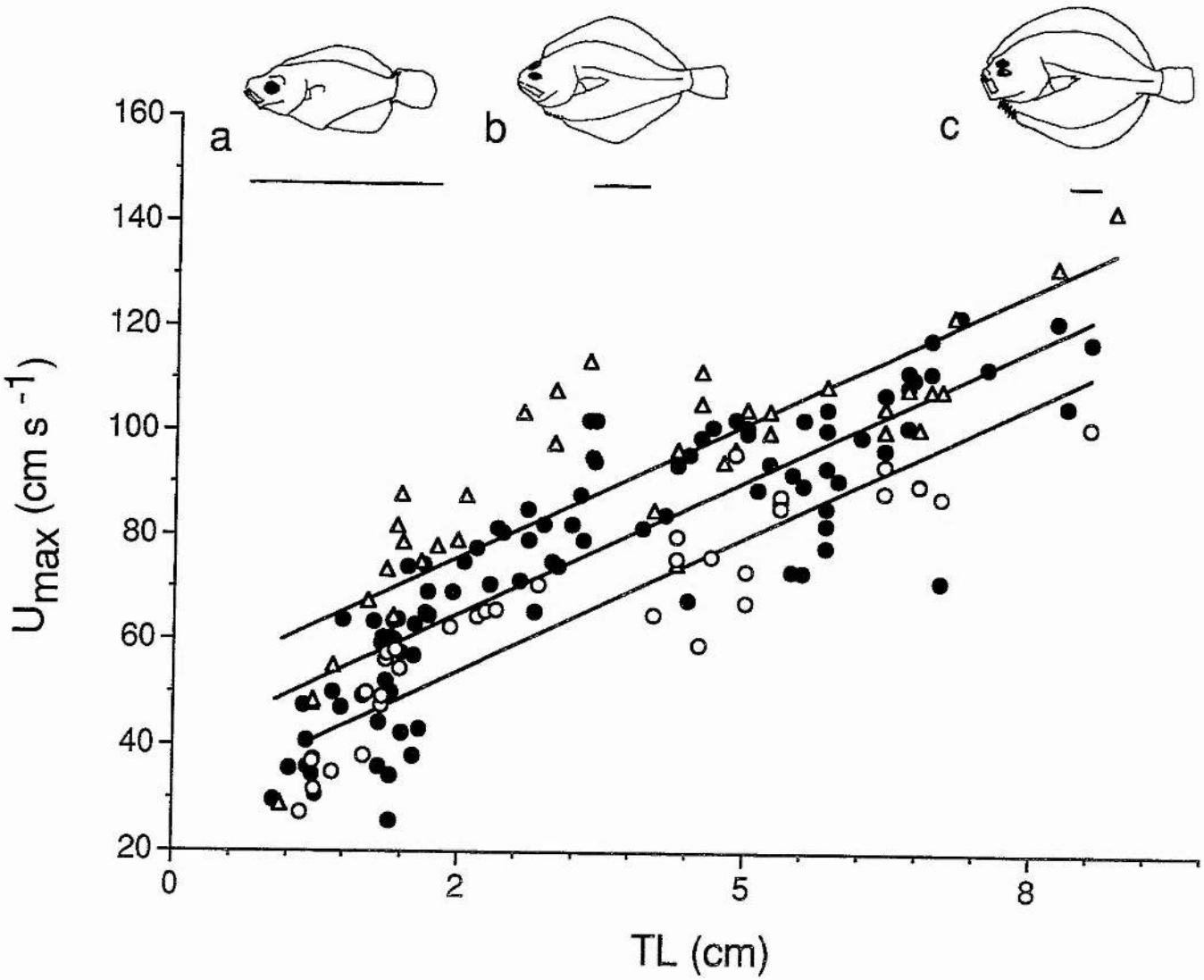

120
100
80
60
40
20
0
U (cm s-1)
0.00
0.04
0.08
0.12
0.16
0.20
Time (s)

Fig. 3. *Scophthalmus maximus*. Relationship between maximum burst swimming speed (U_{max}) and fish total length (TL) for turbot at three temperatures (○: 13°C; ●: 18°C; △: 23°C). (a) stage 3 $L_T < 1.2$cm; (b) stage 5 TL = 2-4cm; (c) juvenile TL> 4cm. Scale bars represent 1cm. Lines were fitted using least square regressions.


a
b
c
160
140
120
100
80
60
40
20
0
2
5
8
U_{max} (cm s^{-1})
TL (cm)

Chapter 4

Individual variation in the maximum swimming speed of turbot, *Scophthalmus maximus*, and a comparison between laboratory reared and wild caught fish.

Introduction

Studies of locomotory performance have, with a few exceptions used mean values to represent the behaviour of the population, treating the variation between individuals as statistical noise. Bennett (1987), studying the maximum running speed of lizards considered the variability between individuals, and demonstrated the consistency of an individual's rank performance over several trials. Kolok (1992 a) has also shown that maximum sustained swimming speed is repeatable for individual largemouth bass (*Micropterus salmoides*) and that ranks are maintained over a temperature change. Any variability in ecologically important behaviours such as escape swimming and maximum foraging speed may have important consequences for individual survival.

The aim of this study was to investigate whether maximum swimming performance in turbot was individually variable, and, if any ranking was established, whether it was maintained over a temperature change. A comparison was also carried out to test whether there was any difference in the swimming performance of laboratory reared and wild caught fish before and after acclimation to laboratory conditions.

Materials and methods

Fish

Wild juvenile turbot (42 - 53 mm total length, TL) were caught using a hand-towed beam trawl at Tralee Bay, Argyll, Scotland. All fish were maintained at 17-19°C in filtered a re-circulating sea water. Larval stages were initially fed *Artemia* sp. nauplii and gradually weaned onto a proprietary diet (Ecoline 17, Ecoline Ltd, Middlesex, U. K.). Wild turbot were filmed within 2 weeks of capture, whereas the farmed turbot were maintained at 18°C in recirculating sea water aquarium at the Gatty Marine Laboratory for up to 6 months giving a size range of 8.8 - 80.0 mm TL.

Comparison between laboratory reared and wild caught turbot

The maximum swimming speed of wild caught and laboratory turbot was measured at 18°C. Wild caught fish were filmed within two weeks of capture. U_{max} was then determined after 3 months acclimation to laboratory conditions. U_{max} was corrected for differences in length using the scaling equation calculated in Chapter 3 (Table 2).

Measurement of individual differences in performance

Eighteen wild turbot (62 ±4 mm week 1, to 75 ±5 mm week 17. Mean ±SD) were held individually in small chambers of netting suspended in the holding tank. Temperature was maintained by using a digistat controlling unit (ROCON Electronics, Gwynedd, Wales) and 3 x 300 W heaters. The maximum burst speed (U_{max}) for each individual was determined (see Chapter 3) weekly at 18°C for 6 weeks. The temperature was reduced to 13°C and the trials continued for a further 9 weeks. Temperature was raised again to 18°C for 1 further week. TL increased from 61.6 ±4.2 mm to 75.3 ±4.8 mm over the duration of the experiment. Speeds were corrected for changes due to growth by standardisation to mean TL using the calculated scaling relationship ($U_{max} = 1.46TL^{0.74}$) (Chapter 3, Table 2.). The rank performance of each individual in each trial was determined over the first 13 week period. The week 11 trial was omitted from the study due to a sudden drop in temperature (approximately 5°C) over 10 h which may have influenced the results.

Statistical analysis

All results were expressed as Mean ± SD. The ranking of U_{max} for individual fish was compared over the 13 week period using Kendall's coefficient of concordance (W), followed by a Chi^2 test for the significance of W. Horst's reliability coefficient

was used to determine the best estimate of mean ranks between the two temperature groups (Edwards 1962). Length corrected U_{max} values for wild and laboratory reared fish were compared using an analysis of variance (Minitab).

Results

Comparison of wild and laboratory reared fish

Analysis of covariance showed that U_{max} at 18°C was 14% lower in farmed turbot than in wild caught-fish of similar size, filmed within 2 weeks of capture ($F=14.21$, $P<0.001$). However when U_{max} was compared between the two groups 3 months later the differences were no longer statistically significant ($F = 0.5$, $P=0.48$, Table 1.). These results suggest that any original differences in U_{max} were due to an acclimation effect and not to genetic stock differences.

Individual variability in swimming performance

For wild turbot, at 18°C, the scaling relationship between TL and U_{max} was similar to that obtained for laboratory reared

fish (P<0.01). Therefore, the model derived for the laboratory reared turbot was used to correct U_{max} for size differences between individuals and for growth during the experiment. An individual's ranking for U_{max} was maintained over the 6 trials at 18°C ($\chi^2 = 40.94$; P<0.01, Fig. 1, Table 2). Ranking order was not maintained over a temperature change if only the trial prior to and after the change were compared (χ^2, 22.86. P=0.05). Over the 6 trials after the reduction in temperature to 13°C individual rankings of U_{max} were maintained $\chi^2 = 29.29$ (P<0.05). Horst's reliability coefficient ($r_{\bar{x}\bar{x}}$) was used to test whether the trials at 18°C and 13°C could be combined to yield a single best estimate of U_{max} ranking (Edwards 1962). Rankings at 18°C were more reliable, judged by Horst's reliability coefficient, than those at 13°C, but combining the two groups gave the best estimate of rank order $r_{\bar{x}\bar{x}} = 0.746$ (Fig. 1).

Over the initial 6 week period, at 18°C, mean U_{max} for the group (n = 18) remained relatively stable (105.3 ± 12.6 cm s^{-1}). Following a reduction in temperature to 13°C (0.2°C h^{-1}) there was a significant drop in average U_{max} (87.8 ± 12.5 cm s^{-1}). Over the next 3 weeks there was a gradual rise in average U_{max} to a level similar to that during the initial 18°C trials (P<0.05). U_{max} remained stable over the next 7 weeks. At the start of week 16 temperature was increased to 18°C but U_{max} did not increased further by the end of week 17 (Fig 2).

Discussion

Comparison of wild and laboratory reared fish

Although U_{max} in fish recently captured from the wild was higher than in laboratory reared fish, it declined after 2 months in captivity to a level similar to that of laboratory reared individuals. In rainbow trout sprint exercise training has been shown to improved endurance at burst speeds, minimising endogenous fuel depletion and enhancing rates of recovery (Pearson *et al.* 1990). Burst swimming performance has been shown to be correlated with heritable morphological differences such as body robustness in comparisons between wild and laboratory reared coho salmon (Taylor and McPhail 1985a), and in three spine stickleback morphs with two different life history strategies (Taylor and McPhail 1985b). In the present study the only obvious morphological difference between wild and laboratory reared turbot was in skin pigmentation. As wild caught plaice (Chapter 5) did not show any increased U_{max} just after capture, when compared to laboratory reared individuals, it was assumed that confinement stress in turbot would also be of minimum concern. The high U_{max} in wild-caught fish does not appear to be explained by genetic stock differences, but may be related to factors such as the temperatures experienced and training, due to predator avoidance and feeding competition, before capture.

Individual variability

The rank performances of U_{max} for individual fish were repeatable (Fig. 1). Burst speed performance has been shown to be individually variable in lizards and in snakes (Bennett 1987). Locomotory performance in garter snakes varies from litter to litter and has been correlated with survival in the wild (Jayne and Bennett 1990), therefore pointing towards a possibility that locomotory performance is heritable. Kolok (1992a.) demonstrated that in largemouth bass the rank order performance of critical swimming velocity (U_{crit}) (equivalent to the maximum sustainable speed for a 20 min period) was maintained before and after a reduction in temperature, and was correlated with fork length (Kolok 1992b). Speeds up to U_{crit} are powered by the recruitment of red muscle fibres, whereas U_{max} requires the recruitment of fast muscle fibres (Johnston *et al.* 1977). Individual differences in U_{max}, such as found in the present study, probably reflect genetic variation and/or the influence of environmental factors during early development. Factors which may influence the juvenile phenotype include maternal nutrition and hormone status and/or the salinity and temperature experienced by the eggs, and the exercise and feeding habits of the larval stages.

Acclimation

Turbot show partial compensation of U_{max} to a reduction in temperature after 3 weeks. There have been very few studies

on the effect of temperature acclimation on maximum swimming speeds in fish. The maximum swimming speeds of fish are powered by white muscle. In a study of the white muscle of plaice (*Pleuronectes platessa*) Wardle (1980) demonstrated that long term acclimation to higher temperature did not alter the contraction time of the muscle. In a more recent study using a different ambush predator, the short horned sculpin (*Myoxocephalus scorpius*) it was shown that the contractile properties of fast muscle fibres were not fixed but vary with acclimation temperature. (Beddow and Johnston 1994). For example, the maximum shortening speed of muscle fibres isolated from 15°C acclimated fish is 60% greater than in 5°C acclimated fish (Beddow and Johnston 1994). Beddow *et al.* 1994, demonstrated that an increase in U_{max} in warn acclimated short horned sculpin was associated with an increase in tail-beat frequency and tail beat amplitude, which reinforced the hypothesis that the muscle properties had been altered.

Table 1. *Scophthalmus maximus.* Comparison between the maximum swimming speed (U_{max}) of laboratory reared turbot and wild turbot just after capture and 3 months later following acclimation to laboratory conditions. Values represent Mean ±SD. †††: significantly different at 0.001% level.

Group	Time from capture to filming	n	Standardised U_{max} ±SD.
wild	2-3 weeks	10	122.7 ±8.7 †††
laboratory		10	105.6 ±11.4
wild	> 3 months	108	101.2 ±12.2
laboratory		98	102.6 ±16.7

Table 2. *Scophthalmus maximus*. Rank order of U_{max} for 18 wild caught turbot over 13 weeks and a temperature change.

	week 1	week 2	week 3	week 4	week 5	week 6	week 7	week 8	week 9	week 10	week 12	week 13
Individual	18°C						13°C					
a	1	7	4	4	3	6	6	1	3	12	1	13
b	4	2	2	14	12	4	5	3	1	3	4	8
c	2	1	5	16	8	3	9	2	5	7	8.5	2
d	5	15	1	17	4	5	13	18	16	9	3	12
e	3	6	12	11	7	1	3	6	9	8	5	6
f	7	3.5	16	5	1	7	10	7	15	14	2	11
g	6	5	10	2	15	8	4	9	8	11	7	7
h	12	3.5	3	10	6	14	2	8	6	17	12	14
i	17	8	9	8	10	13	14	11	12	4	14	1
j	10	12	7	15	2	12	11	15	13	2	8.5	17
k	15	9	18	1	11	11	17	10	10	10	10	4
l	14	14	8	9	9	2	7	5	7	13	6	10
m	13	13	6	13	13	18	15	12	2	15	15	15
n	8	17	13	6	16	17	18	16	18	1	13	18
o	9	10	15	3	17	16	1	13	11	6	16	3
p	18	18	11	7	18	15	12	4	4	18	11	16
q	11	16	17	18	5	10	8	17	14	16	18	5
r	16	11	14	12	14	9	16	14	17	5	17	9

Fig. 1. *Scophthalmus maximus*. Average ranking of the maximum burst swimming speed (U_{max}) for wild turbot over a 16 week period and following temperature change. Values are mean ±S.D.

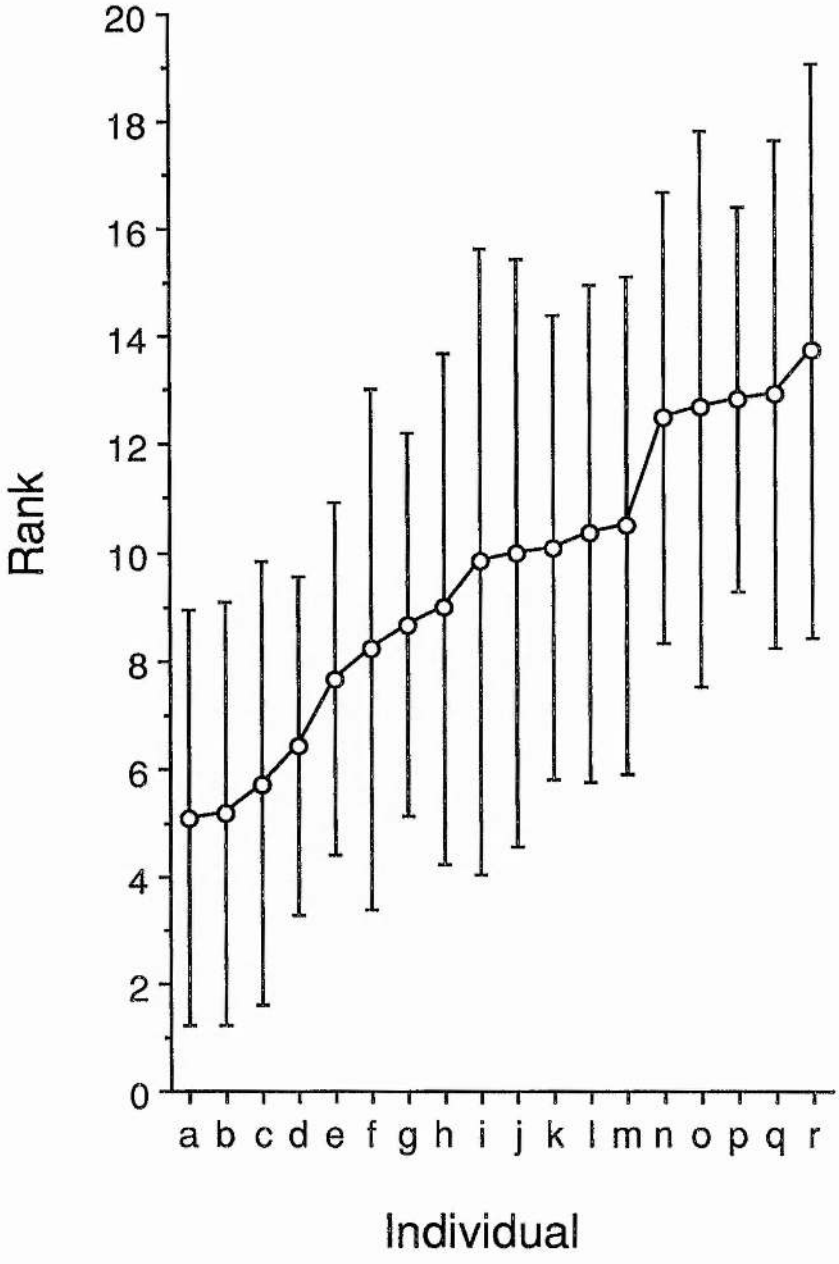
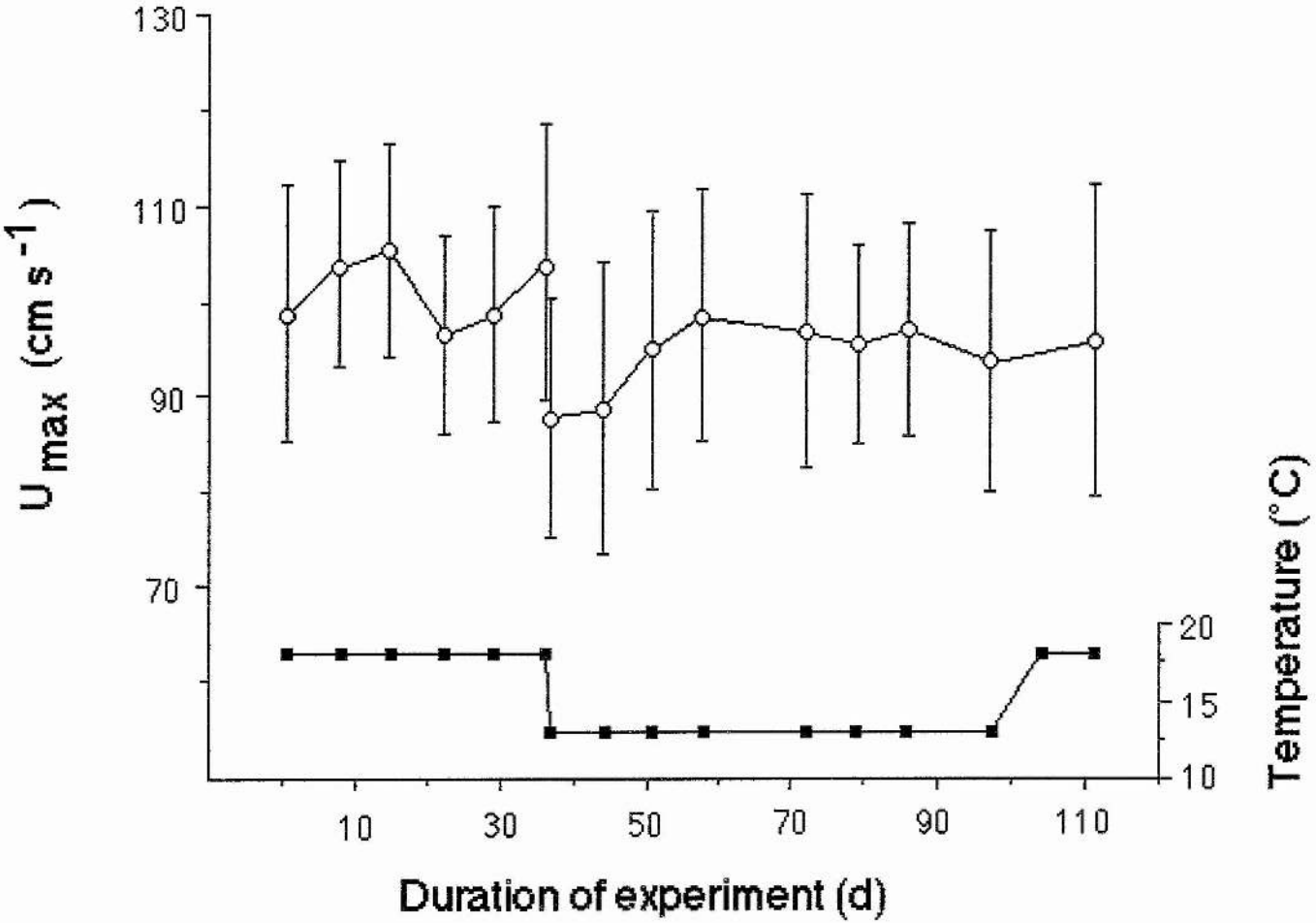

20
18
16
14
12
10
8
6
4
2
0
Rank
a b c d e f g h i j k l m n o p q r
Individual

Fig. 2. *Scophthalmus maximus.* Repeatability of maximum burst swimming speed U_{max} (o) in 18 juvenile wild turbot over a 16 week period. The temperature profile over the duration of the experiment is shown below (▪). Values are mean ±S.D.


130
110
90
70
U_{max} (cm s^{-1})
20
15
10
Temperature (°C)
10
30
50
70
90
110
Duration of experiment (d)

Chapter 5

The effect of temperature on the maximum swimming speed of wild and laboratory reared plaice, *Pleuronectes platessa* L.

Introduction

The pelagic eggs of plaice (*Pleuronectes platessa*) are spawned in early spring around the coast of Scotland. Hatched larvae remain in the plankton until near the end of metamorphosis when they settle onto shallow sandy beaches (Lasker 1981). After settling, juveniles still spend time in the water column. During development, eggs, larvae and juveniles are subject to a wide range of temperatures, fluctuating on a tidal, daily, and seasonal basis. In spring, water temperature can range between 9°C and 18°C in shallow water at Tralee Bay Scotland, with seasonal variations between 7 and 15°C at a depth of 5m (Gibson and Burrows personal communication., Gibson *et al.* 1993).

Fish larvae have high mortality rates, the majority of which is due to predation (Van der Veer and Bergman 1987). It is, therefore, important to study predator evasion behaviours such as burst swimming, and the effect that environmental factors such as temperature may have. Flatfish swim in an anguilliform mode, with large displacements of the head and tail (Webb 1975). Until recently, it was believed that the relatively high sprint speeds attained by fish were length and temperature independent (Webb, 1975). Batty and Blaxter (1992) have demonstrated that in plaice the C-start contraction time, tail beat frequency and swimming speed were all dependent on test temperature. There is little literature on the effect of long term

temperature change on swimming performance of fish larvae. In herring (*Clupea harengus*) rearing temperature had no effect on burst swimming performance except by affecting length at hatch (Batty *et al.* 1993). In plaice, Wardle (1980) has demonstrated that long term acclimation to an increase in temperature has no effect on the maximum contraction velocity of muscle. Beddow *et al.* (1994) have demonstrated that, in adult short horn sculpin (*Myoxocephalus scorpius*), S-shaped starts, which are associated with prey capture, shows some acclimatory response to temperature change. In fish acclimated to 15°C, fast start performance was improved by comparison with fish acutely transferred to the test temperature. The improved performance was associated with increased tail beat amplitude (Beddow *et al.* 1994).

The aim of this study was to look at the effect of acute and long term temperature change on the burst swimming behaviour of both newly settled wild caught plaice and laboratory reared fish.

Materials and methods

The fish

Mature plaice (*Pleuronectes platessa*) were trawled from the Firth of Clyde in February and March. Fish were maintained at ambient temperature, in the sea water aquarium at

Dunstaffnage Marine Laboratory, Oban. Eggs were stripped from adult females and fertilised with milt from mature males. Once fertilised, eggs were left to stand for an hour. Any unfertilised eggs were removed by increasing the salinity of the water to 40% by the addition of NaCl, which caused the unfertilised eggs to sink (Holliday and Pattie Jones 1967). Eggs were incubated in hopper tanks at ambient sea water temperatures and salinity. Larvae were transferred to 100 litre tanks and fed on rotifers (cultured on a diet of *Isochrisis, Pavlova and Rhodomonas*) and *Artemia* sp. (AF grade; Artemia systems, Baasrode, Belgium). Newly settled wild plaice were caught using a hand-towed beam trawl at Tralee Bay, Argyll, Scotland. Both laboratory reared and wild caught fish were maintained in filtered sea water in a flow through system at ambient temperature (8-10°C). Larvae and juveniles were initially fed *Artemia* sp. nauplii, later juveniles greater than 20 mm in length were also offered fresh mysids. Wild caught plaice were filmed within 2 weeks of capture.

Experimental protocol

Individual plaice were transferred to the filming arena at 9°C, the temperature was then adjusted to the test temperature at a rate of 1°C min^{-1}. Fish were left undisturbed for 30 minutes prior to the experiment. Burst swimming sequences were initiated by tactile stimulation with a fine probe (1.5 mm) Three or four bursts of swimming were recorded from each individual.

Measurement of swimming performance

Two filming set-ups were used to record swimming sequences. Swimming trials were carried out in a square perspex tank either 19 x19 x 5cm deep or 30 x 30 x 6cm deep depending on the size of the fish. Tanks were maintained at a constant temperature (±0.4°C) by being suspended in a temperature-controlled water bath. Some experiments were carried out in a controlled temperature room, negating the need for a water bath. Chambers were viewed from above. Sharp silhouettes of fish were obtained by using a retro-reflective Scotchlite background beneath the tank, light from a strobe was reflected onto the tank by a semi-silvered mirror suspended below the camera (Fig. 1a) (Batty 1984). A transparent 0.5 or 1 cm square grid overlay was glued to the bottom of the tank to provide a scale. Fish were filmed at 50 Hz using a Link video camera, fitted with an RCA ultricon tube, positioned approximately 75cm above the swimming chamber so that the filming chamber filled the entire field of view. Images were recorded on a U-format VCR (JVC (UK) Ltd, London).

Swimming trials recorded at high speed (400 Hz) were carried out in a perspex tank (20 x 5 x 9 cm deep) and viewed from the side. Light from a strobe was directed through a flexible light guide onto a fresnel lens which provided even illumination over the entire tank giving dark silhouettes of the fish (Fig. 1b). Sequences were recorded at 400 frames s^{-1} using a NAC HSV-400 high speed video system. Videotapes were

analysed frame-by-frame at 20 ms (50 Hz) or 2.5 ms (400 Hz), where the maximum speed was the fastest values recorded over the 20 ms interval or 5ms (200 Hz) intervals. Trials where the fish encountered the side of the filming arena early in the response were discounted. Frame-by-frame replay of recordings allowed swimming sequences to be digitised using MOVIAS software (NAC-Corp, Japan) on a personal computer linked to the high speed video system. Recordings were analysed at 200 Hz using alternate frames as movement between consecutive frames was very slight. Four parameters of the swimming that followed the response were measured, 1: Tail beat frequency (f), the reciprocal of the period for one complete tail-beat cycle (1/T), 2: Maximum swimming speed (U_{max}), the fastest recorded speed over one tail beat, 3: Tail beat amplitude (A), the maximum distance moved by the tip of the tail, normal to the swimming direction, during one tail beat , 4: Stride length (X), the distance covered by a larva in the direction of motion in one tail beat, expressed as a proportion of total fish length (Fig. 2).

Comparison between laboratory reared and wild juvenile plaice

During Spring 1992 wild juvenile plaice were captured and held in the laboratory at 9°C. After 3 days, maximum swimming speed and tail beat frequency were recorded at 400 Hz. Each fish was allowed to recover from its bout of swimming (approximately 1 hour) before the temperature was changed at 0.5°C min^{-1} to either 5 or 13°C. Swimming trials were then repeated at the new temperature.

Temperature acclimation of swimming response

Laboratory reared plaice were acclimated to 9°C. Escape swimming response was recorded at 400 Hz. This group of fish were then split into 3 groups and transferred to 3 re-circulating sea water tanks maintained at either 5°, 9° or 13°C. The video recording was the repeated at each temperature 24 h, 1 week, and 4 weeks after the initial temperature change. After 4 weeks' acclimation to 5°C half the fish were transferred acutely (<6h) to 9°C before swimming performance was tested. For the group at 9°C, half the fish were tested after an acute temperature change to 5°C.

Statistical analysis

Relationships between size, temperature and U_{max} were compared using analysis of covariance (Sokal and Rohlf 1981). Other comparisons were made using analysis of variance (Minitab). Results are expressed as mean ± standard deviation.

Results

Temperature, size and swimming speed

For wild caught juvenile plaice maximum swimming speed (U_{max}, $cm\ s^{-1}$) increased with fish total length (TL) at each temperature (regression coefficient b= 10.5, 14.5, and 12.9 at 5°,

9° and 13°C respectively). Length specific maximum swimming speed (U^*_{max}, TL s^{-1}) decreased with fish length. There was no significant difference in the scaling relationship of U_{max} and TL at each temperature (P=0.01). Therefore a common slope was calculated and new regression equations fitted giving the scaling relationship for $U_{max} = aTL^{0.65}$ which was independent of temperature (Table 1).

Speed was corrected for differences in fish length using the scaling relationship previously described, with U_{max} (cm s^{-1}) standardised to mean TL. At 5°C mean standardised U_{max} was significantly lower than at 9°C and 13°C (P<0.01), giving a Q_{10} = 1.51 between 5 and 9°C. Mean standardised U_{max} was not significantly different between 9° and 13°C (P=0.05) (Fig. 3). A reduction in temperature from that of acclimation caused a decrease in U_{max}, but an increase in temperature from that of acclimation caused no increase in U_{max}.

Comparison between laboratory reared and wild juvenile plaice

Both maximum swimming speed (U_{max}) and tail beat frequency (f) were related to fish total length. U_{max} (cm s^{-1}) scaled in proportion to $TL^{0.65}$, and f scaled in proportion to $TL^{-0.66}$. Before comparisons between laboratory reared and wild caught plaice were carried out, f and U_{max} were corrected for

differences in TL using the scaling relationships calculated from the data.

There were no significant differences in maximum swimming speed (U_{max}) and tail beat frequency (f, Hz) between laboratory reared and wild caught juvenile plaice at either 5°, 9°C or 13°C ($P = 0.05$). For both laboratory reared and wild caught plaice f was significantly lower at 5°C than at 9°C, or 13°C ($P < 0.05$). There was no significant difference in f between 9°C and 13°C ($P = 0.05$) (Fig. 4). For both groups the $Q_{10} = 1.68$ for tail beat frequency (f) between 5°C and 9°C. U_{max} for laboratory reared plaice showed a stepwise increase between 5°C, 9°C and 13°C ($P < 0.05$). For wild caught plaice U_{max} at 5°C was significantly lower than at either 9°C or 13°C ($P < 0.01$), U_{max} between 9°C and 13°C did not differ significantly ($P = 0.05$) (fig. 5). U_{max} had a $Q_{10} = 1.61$ between 5°C an 9°C for both groups.

Temperature acclimation of maximum swimming speed

A reduction in temperature on day 1 from 9°C to 5°C resulted in a decrease in U_{max} from 25.06 ±3.96 to 20.19 ±2.92 cm s^{-1}. When measured after 7 and 29 days acclimation to 5°C there was no change in U_{max}.($F = 0.37$, $P=0.05$, Fig. 6). U_{max} for fish acclimated to 9°C remained relatively stable for 29 days (F =

0.49, P = 0.05). Maximum tail beat frequency (f) followed a similar pattern to that of U_{max}, with a reduction in f from 25.5 ±2.8 to 19.0 ±1.9 Hz with an acute reduction in temperature from 9°C to 5°C. Tail beat frequency remained stable at each temperature during the 29 days acclimation period (F = 0.5 and F = 2.3, at 9°C and 5°C respectively, P=0.05, Fig. 7). After 29 days acclimation to 5°C temperature was increased to 9°C resulting in a significant increase in U_{max} from 17.75 ±5.1 to 25.27 ±5.4 cm s^{-1}. In the group acclimated to 9°C a reduction in temperature to 5°C resulted in a reduction in U_{max} from 26.0 ±3.1 to 18.8 ±2.7 cm s^{-1} (Fig. 8). For the 9°C acclimated group a reduction in temperature resulted in a decrease in f from 23.8 ±5.3 to 17.0 ±3.0 Hz. An increased in temperature for the 5°C acclimated group, to 9°C, did not affect f (Fig. 9). Tail beat amplitude (A) was related isometrically to fish total length by the power function $TL^{0.97}$. No significant differences in standardised A was found between laboratory reared and wild caught fish at any temperature (P=0.05). In the 5°C acclimated group an acute rise in temperature caused a noticeable increase in A although it was not statistically significant (Fig. 10). Stride length (X) was conserved throughout the duration of the experiment, giving an average value of 0.63 ±0.13. An acute drop in temperature from that of acclimation caused a reduction in U_{max} and f, but A and X did not change. After 29 days, acclimation to low temperature there was no change in any of these parameters. Once acclimated to low temperature an acute increase in temperature resulted in an increase in U_{max} but no increase in f, but there was an increase in A. Although not statistically significant the increase in A may be related to the increase in U_{max} but this needs further investigation.

Discussion

Size, temperature and maximum swimming

Maximum swimming speed (U_{max}) was related to total fish length (TL) by the power equation of the form $U_{max} = TL^{0.61}$, the relationship being parallel at each temperature. In a study of the Antarctic fish *Notothenia coriceps* U_{max} has been reported as scaling in proportion to $TL^{0.66}$ (Archer and Johnston, 1989). U_{max} was linearly related to temperature between 5° and 9°C ($Q_{10} = 1.15$) and did not increase further at temperatures above 9°C. Batty and Blaxter (1992) found for plaice a plateau in U_{max} at temperatures above 8°C. In rainbow trout, maximum velocity during fast starts is independent of temperature between 15°C and 25°C (Webb 1978). Plateauing of U_{max} at high temperatures has also been demonstrated in zebra danios (*Brachiodanio rerio*) and it has been suggested that the relationship between U_{max} and temperature is only linear in the temperature range normally encountered by the population (Fuiman, 1986). Videler and Wardle (1991) suggest that tail beat frequency is the major variable modulated to obtain different speeds. Tail beat frequency is related to both size and temperature. Maximum tail beat frequency is related to maximum muscle contraction time (T) which in turn is related to U_{max} by the formula $U_{max} = A*L/2T$, where A is stride length (Wardle 1975). Using hydrodynamic theory, Webb (1977) postulated that maximum tail beat frequency (f) scaled in proportion to $L^{-0.44}$ and, using Wardle's (1975) data, calculated

that f was proportional to $L^{-0.40}$ in 4 species of marine fish. In this study for plaice f scaled in proportion to $L^{-0.66}$, and had a Q_{10} of 1.68.

Comparison between laboratory reared and wild juvenile plaice

U_{max} and f did not vary between laboratory reared and wild caught plaice. Both U_{max} and f showed a plateau in the response at temperatures greater than 9°C. In a similar study on settled turbot (*Scophthalmus maximus*), wild caught fish outperformed laboratory reared fish when U_{max} was compared just after capture of wild individuals, but after acclimation to laboratory conditions no differences were found (Chapter 3). The reason for the different results in the two species is not clear, but may be related to the training condition and feeding of the laboratory reared fish. Laboratory reared plaice were fed on live food prior to and during the duration of the experimental period whereas laboratory reared turbot had been weaned onto a pellet diet. Wild caught turbot and plaice for both studies were caught and maintained under similar conditions. Pearson *et al.* (1990) in a study on rainbow trout demonstrated that sprint exercise training improved endurance at burst speed and enhanced rates of recovery.

Temperature acclimation

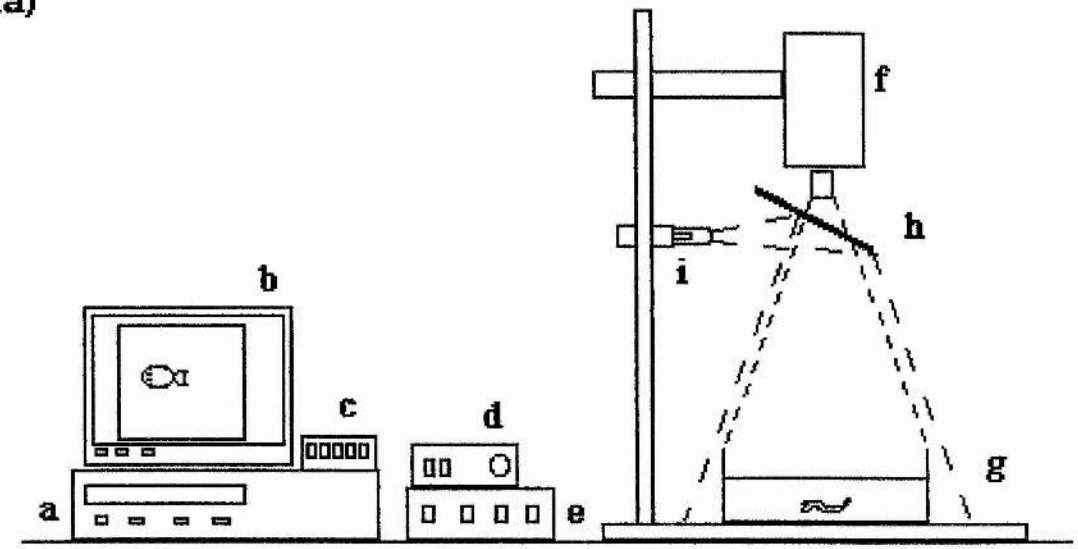
No response to cold acclimation was found in either U_{max} or f. Stride length was independent of temperature at 0.63 ±0.13 over the duration of the experiment. In a study by Wardle (1980) the maximum contraction time for white muscle in adult plaice did not show any compensation after a period of acclimation at a higher temperature. In short horned sculpin (*Myoxocephalus scorpius*) long term acclimation to a 10°C rise in temperature caused a 24% increase in tail-beat amplitude, as well as an increase in stride length during a S-shaped start (Beddow *et al.* 1994).

Table 1. *Pleuronectes platessa.* Relationship between total length (TL), maximum swimming speed (U_{max}) and maximum length-specific swimming speed (U^*_{max}), for settled plaice at three temperatures. Data were fitted by least square regressions to a power equation of the form $U_{max}=aL_T^b$. Values are mean ±SD (n is number of fish)

Test temperature (°C)	(n)	TL (cm)	U_{max} (cm s^{-1})	$U_{max}= aTL^b$			U^*max (TL s^{-1})	$U^*max=aTL^b$		
				a	b	R^2		a	b	R^2
5	(32)	3.61±1.59	57.52±18.80	1.40	0.61	0.79	16.48 ±4.41	1.40	-0.35	0.51
9	(44)	3.25±1.00	70.40±17.19	1.50	0.68	0.70	22.27 ±3.89	1.50	-0.32	0.35
13	(42)	3.56±1.46	75.45±20.17	1.52	0.65	0.75	22.34 ±4.31	1.52	-0.35	0.46
Combined	(118)	3.46 ±1.35	68.72 ±20.47	1.49	0.65	0.63	20.36 ±4.20	1.49	-0.35	0.33

Figure 1a. Diagram representing the apparatus used to film and record the burst swimming speed of flatfish at 50 Hz. **1b.** Diagram representing the arrangement used to film and record the swimming behaviour of flatfish at 400Hz. a: video cassette recorder, b: monitor, c: time code generator, d: strobe power pack, e: pulse generator, f: camera, g: swimming chamber, h: semi-silvered mirror, i: strobe, j: flexible light guide, k: freznil lens, l: computer.

(1a)



(1b)

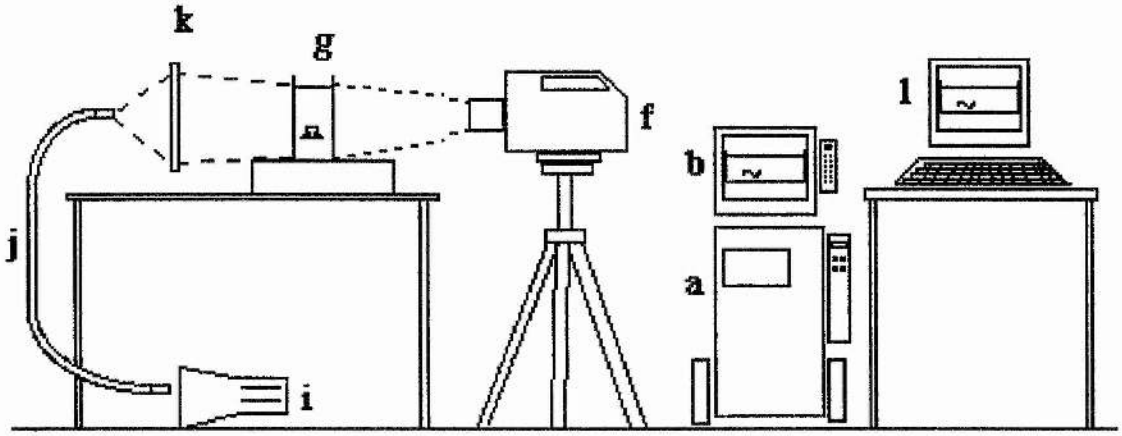


Figure 2. Diagram showing the measurements made when analysing flatfish swimming response. A: tail beat amplitude; X: stride length; T: time for complete tail beat cycle (tail beat frequency = 1/T).

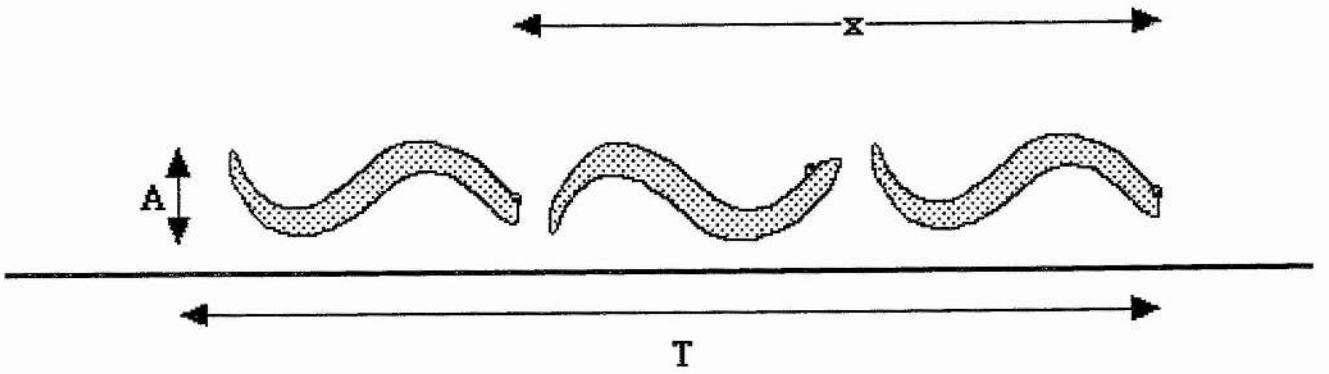

x
A
T

Figure 3. *Pleuronectes platessa.* Relationship between maximum burst swimming speed (U_{max}) and fish total length (TL) for juvenile plaice at three temperatures (○: 5°C; ●: 9°C; △: 13°C). Lines were fitted using least square regressions.

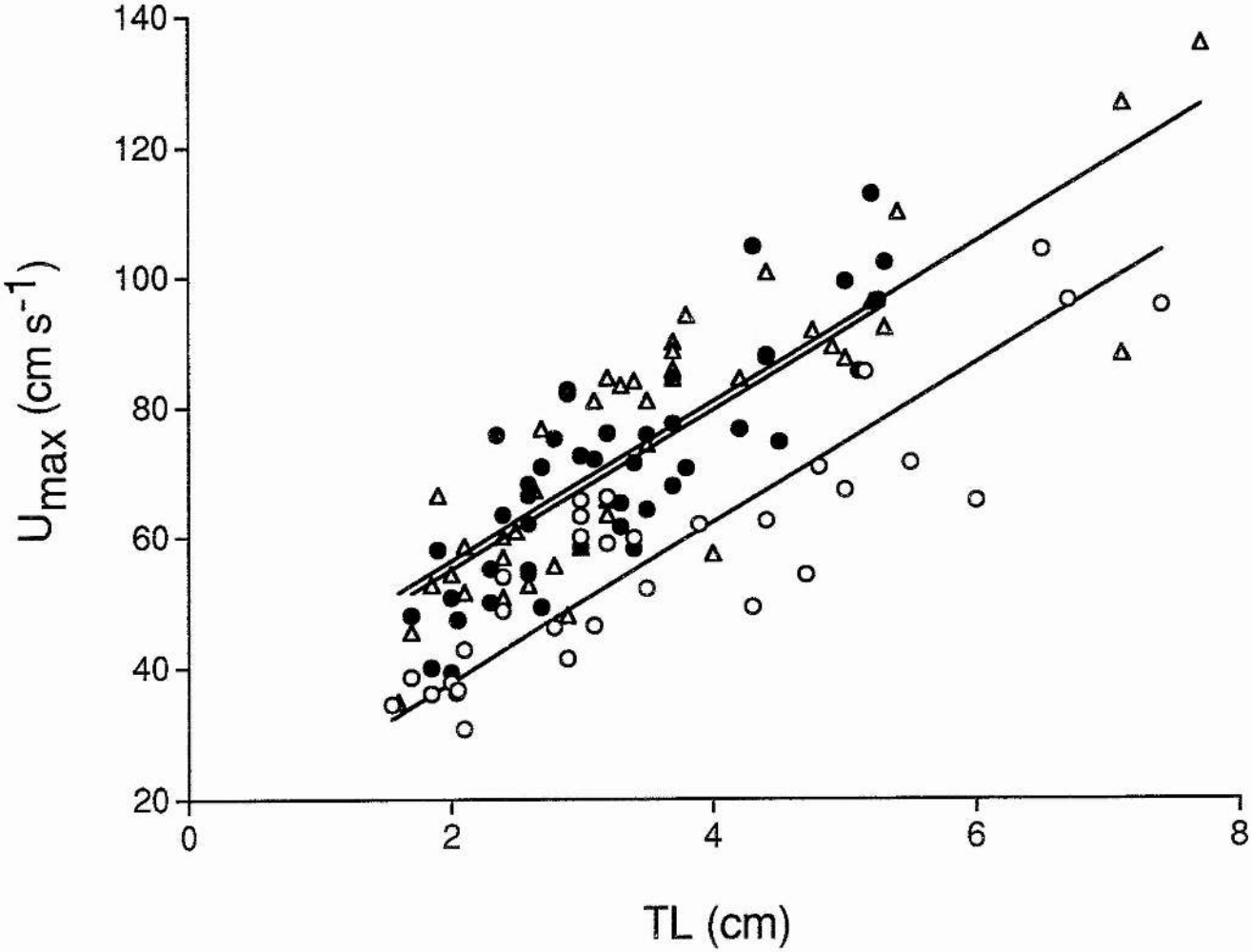
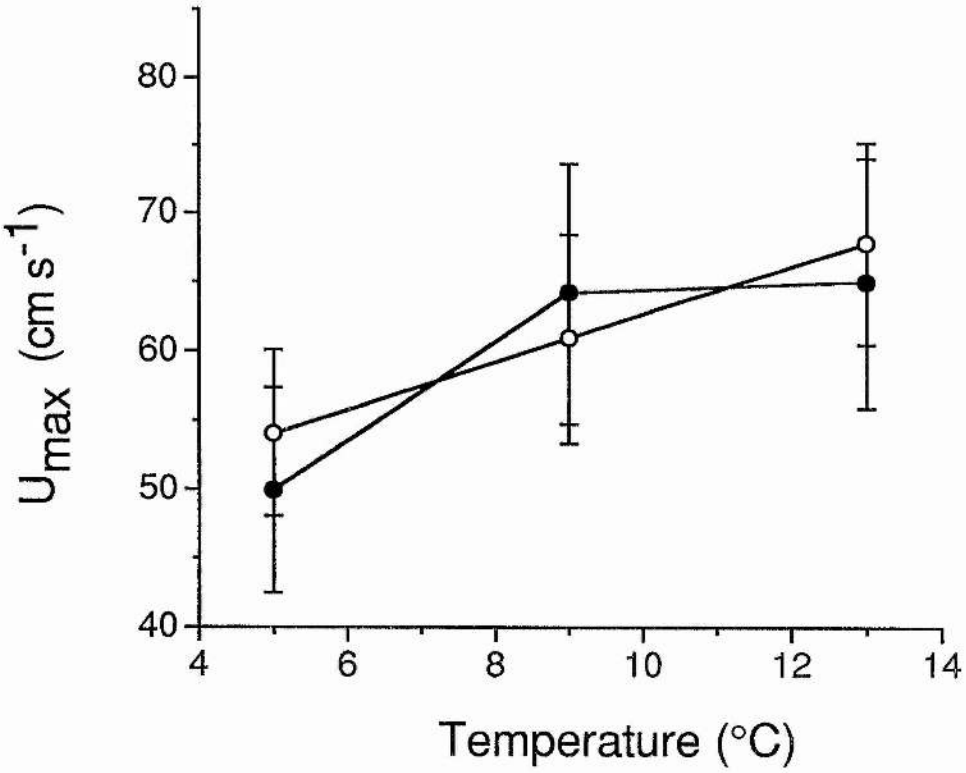

140
120
100
80
60
40
20
0
2
4
6
8
U_{max} (cm s^{-1})
TL (cm)

Figure 4. *Pleuronectes platessa.* Comparison of standardised maximum swimming speed (U_{max}) between laboratory reared (●) and wild caught (○) plaice, at three temperatures; 5°, 8°, and 13°C. Values are mean ±SD.

Figure 5. *Pleuronectes platessa.* Comparison of standardised maximum tail beat frequency (f) between laboratory reared (●) and wild caught (○) plaice, at three temperatures; 5°, 8°, and 13°C. Values are mean ±SD.


80
70
60
50
40
U_{max} (cm s^{-1})
4
6
8
10
12
14
Temperature (°C)

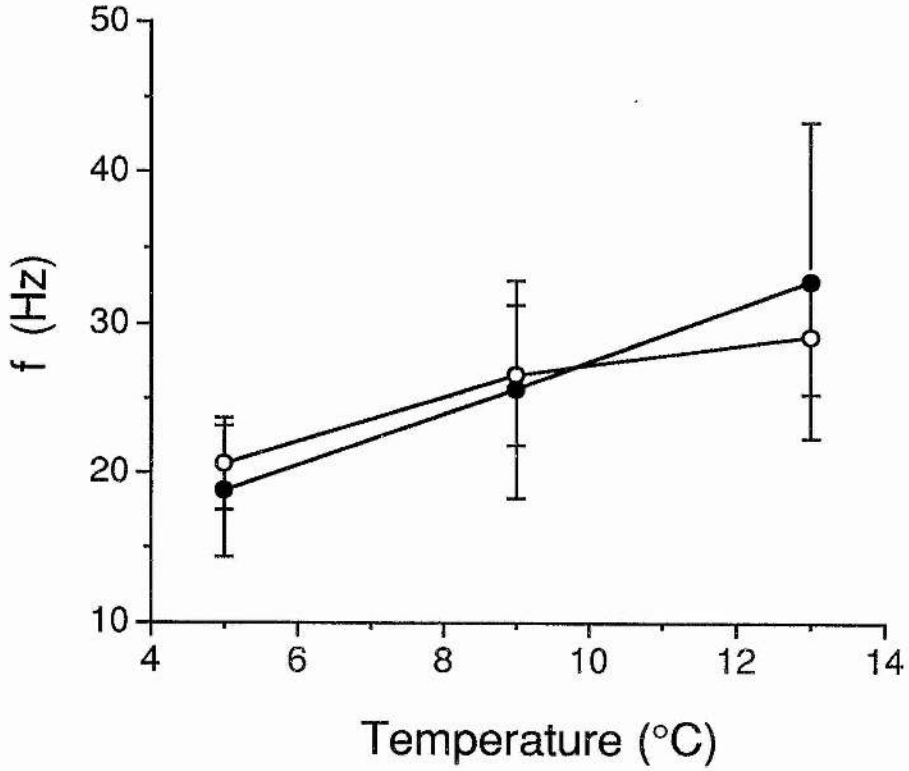
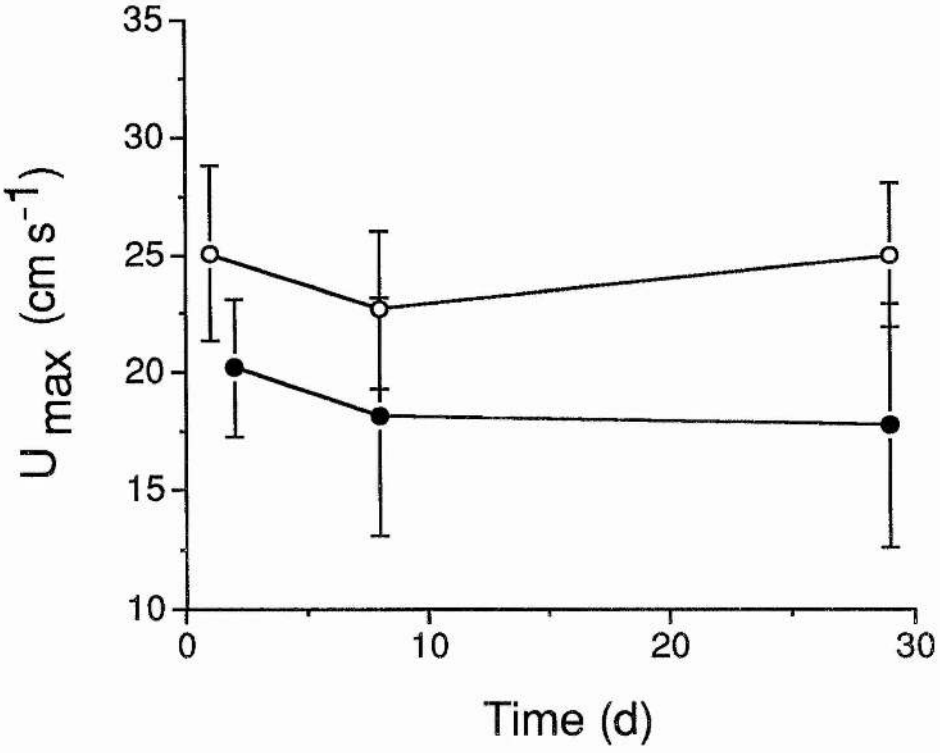

50
40
30
20
10
f (Hz)
4
6
8
10
12
14
Temperature (°C)

Figure 6. *Pleuronectes platessa.* Maximum swimming speed (U_{max}) for juvenile plaice at acclimation temperature of 9°C(○), after an acute temperature reduction to 5°C and period of acclimation at 5°C (●). Values are mean ±SD.

Figure 7. *Pleuronectes platessa.* Maximum tail beat frequency (f) at acclimation temperature of 9°C (○), after an acute temperature reduction to 5°C and a period of acclimation at 5°C (●). Values are mean ±SD.


35
30
25
20
15
10
0
10
20
30
U max (cm s-1)
Time (d)

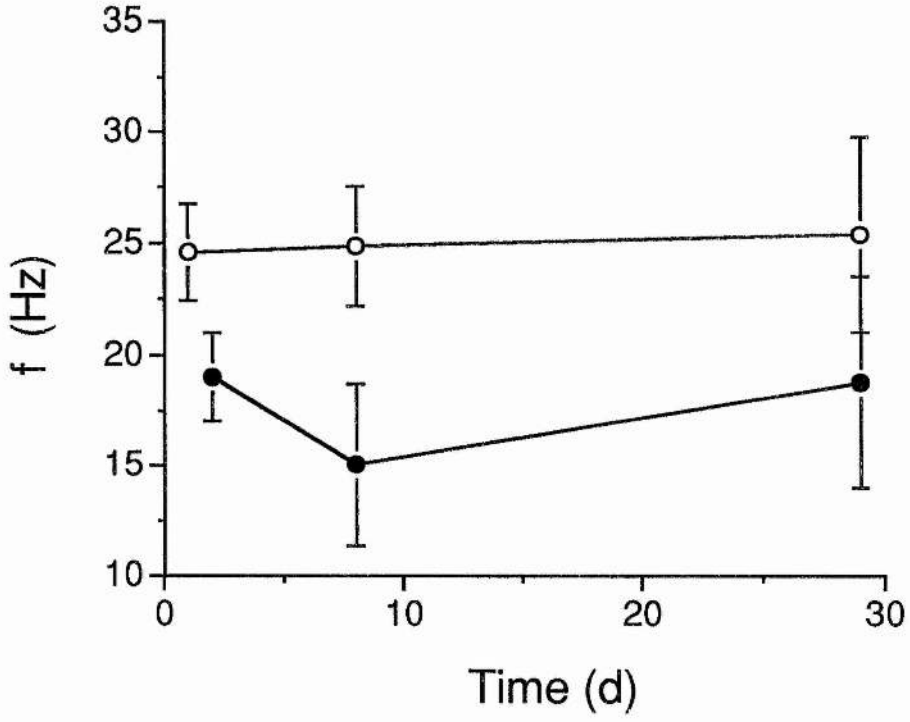
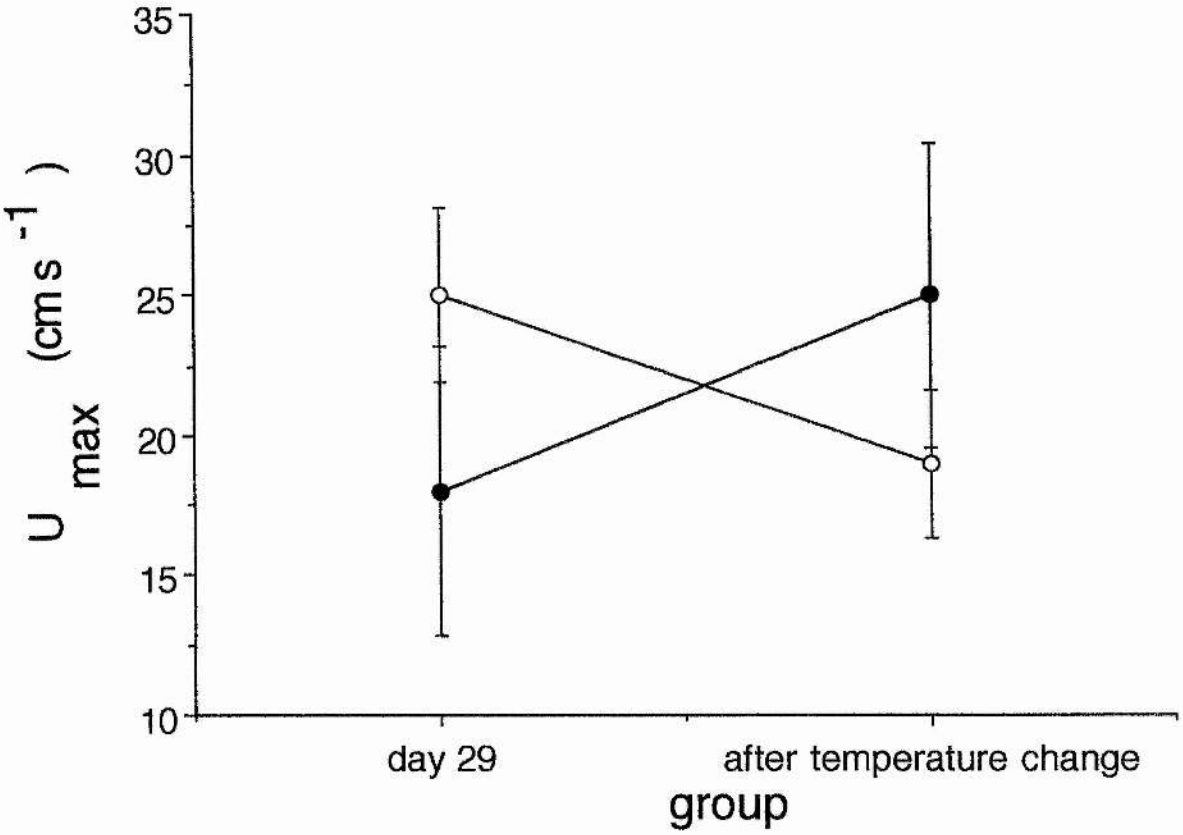

35
30
25
20
15
10
0
10
20
30
f (Hz)
Time (d)

Figure 8. *Pleuronectes platessa.* Maximum swimming speed (U_{max}) following an acute change in temperature for fish acclimated for 29 days at either 5° (●) and 9° (○) to the opposite test temperature (9°C or 5°C). Values are mean ±SD.

Figure 9. *Pleuronectes platessa.* Maximum tail beat frequency (f) following an acute change in temperature for fish acclimated for 29 days at either 5° (●) and 9° (○) to the opposite test temperature (9°C or 5°C). Values are mean ±SD.


35
30
25
20
15
10
U max (cm s -1)
day 29
after temperature change
group

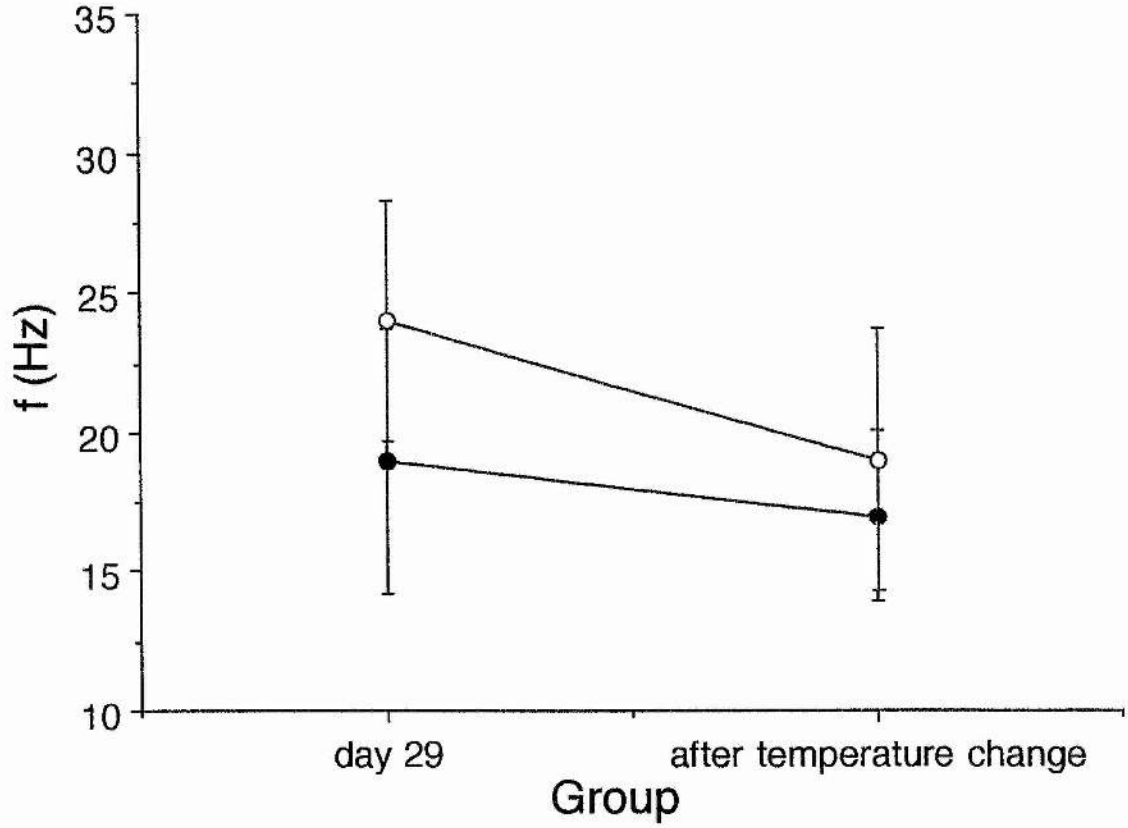
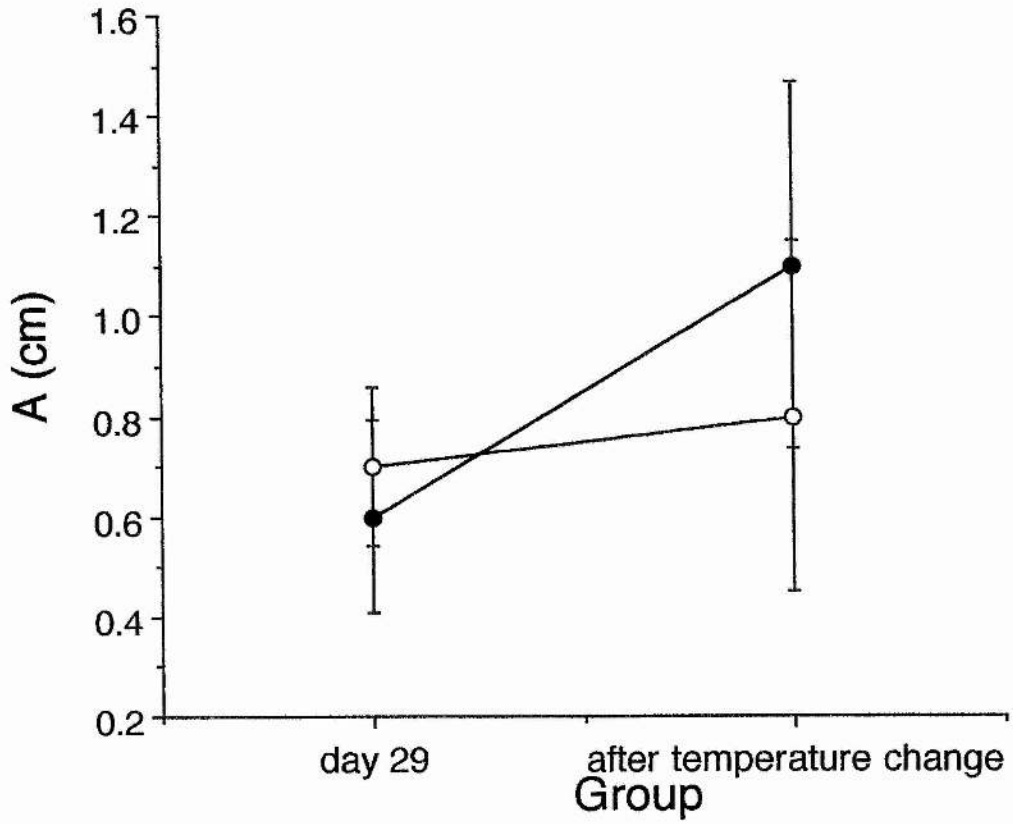

35
30
25
20
15
10
f (Hz)
day 29
after temperature change
Group

Figure 10. *Pleuronectes platessa.* Tail beat amplitude (A) following an acute change in temperature for fish acclimated for 29 days at either 5° (●) and 9° (○) to the opposite test temperature (9°C or 5°C). Values are mean ±SD.


1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
A (cm)
day 29
after temperature change
Group

Chapter 6

General Discussion

General Discussion

Scaling and maximum swimming speed

Size is an important factor in determining the maximum swimming speed of larval and juvenile fish. Maximum swimming speed (U_{max}) increased with fish length for both turbot (*Scophthalmus maximus*) and plaice (*Pleuronectes platessa*), scaling in proportion to $LT^{0.74}$ for turbot and $LT^{0.61}$ for plaice (Ch 3 and 5). Both species studied here display a similar escape behaviour, the omega jump (Kruuk 1963). Turbot and plaice settle onto the same beaches, and have similar prey, although turbot are usually found in shallower water than plaice (Ellis, personal communication). The different spatial distribution of the two species may be influenced by competition between the species. Newly caught wild turbot had a greater maximum swimming speed than laboratory reared turbot, whereas there was no difference in swimming performance between laboratory reared and wild caught plaice (Ch 4 and 5). Once acclimated to laboratory conditions there is no difference in U_{max} between wild caught and laboratory reared individuals (Ch 4). Wild fish are exposed to predation, therefore it would be expected that both turbot and plaice would have a training effect due to predator avoidance. Wild turbot exist in the first 2m depth of the sea, where the water is turbulent and more exposed to environmental conditions such as wind driven wave action, and temperature fluctuations, whereas, plaice are usually found

in deeper water with more stable conditions. It may be that enhanced swimming performance of wild turbot is due to training caused by water conditions.

Temperature and maximum swimming speed

For turbot an increase in temperature from 13°C to 23°C resulted in an increase in U_{max}, whereas in plaice U_{max} increase between 5°C and 9°C, but did not increase if the temperature was raised above 9°C (Ch 3 and 5). Turbot live in shallower water than plaice, and experience greater temperature variation. Fuiman (1986) suggests that the relationship between U_{max} and temperature is linear only over a temperature range normally experienced by the species.

Turbot demonstrated some ability to compensate for a reduction in U_{max} resulting from a decrease in temperature, with Umax increasing significantly after 3 weeks at the lower temperature (Ch 4). For plaice after 29 days acclimation to a reduced temperature there was no compensatory increase in U_{max} (Ch 5). After acclimation to a lower temperature turbot showed no increase in U_{max} when temperature was subsequently increased (Ch 4). In contrast, in plaice an increase in temperature after a period of acclimation to low temperature (5°C) resulted in an increase in U_{max} (Ch 4 and 5). The increase in U_{max} for plaice was not associated with an increase in tail beat frequency (Ch 5). The different responses of turbot and

plaice to temperature acclimation and subsequent temperature change are difficult to explain. It may be that the acclimatory responses are related to the environmental conditions experienced by the two species (discussed above). Both turbot and plaice used in the acclimation experiments were from wild stock. It could be possible that 29 days is not an adequate time for plaice to show any response to temperature acclimation, but this is doubtful as turbot demonstrated an acclimatory response after 3 weeks.

Temperature influences the swimming speed of larvae by changing the length of larvae at hatching. Larvae reared at lower temperatures tend to be longer and, therefore, can attain higher maximum swimming speeds (Ryland *et al.* 1975, Batty and Blaxter 1992). Changes in swimming speed and mode are also associated with the development of the caudal fin (Blaxter 1986). In this study temperature did not alter the larval length when the formation of the caudal fin occurred.

Temperature and development.

From the work done in this study (Ch 2)and in other studies (for example, Fukuhara 1990, Johnston 1993) it is clear that temperature plays an important role in the development of fish. By influencing the duration of egg development and the larval stage, dispersal is altered by changing the length of time the eggs and larvae are in the water column and subject to movement due

to waves and water current (Hovencamp 1991). As maximum swimming speed is related to fish length, altering the length at which metamorphosis occurs may influence the ability of fish to avoid predators. Heterochronies in the embryonic and larval development of have been observed in herring and the Japanese flounder (*Paralichtys olivaceus*) as a result of altered rearing temperature (Johnston 1993, Fukuhara 1990). In plaice (Brooks and Johnston 1993) and turbot (Ch 2) incubation at different temperatures did not alter the embryonic sequence of appearance of features, although it had a marked effect on muscle fibre development. Rearing at different temperatures did result in a change in the sequence of appearance of features in larval turbot (Ch 2). The altering of developmental sequence may have important consequences for survival in the wild. For example, the possible nutritional status of turbot larvae may be altered at higher temperatures by affecting the time of complete yolk utilisation with respect to the development of a more complex gut (Ch 2). There have been few relatively comprehensive studies carried out on the influence of temperature on development. There is a need for further knowledge of heterochronic changes during development, especially with respect to the existence of any behavioural or ecological consequences.

Individual variation

One of the most interesting findings of this work was the individual variability in the escape swimming speed of turbot

(Ch 4). For turbot, rank order of maximum swimming speeds amongst individuals was repeatable over time. After a disruption due to a temperature reduction, rank order of performance is re-established (Ch 4). Maximum swimming speed is important in predator avoidance (Bailey and Batty 1984), therefore fast swimming individuals have a survival advantage at any temperature. It would be very interesting to carry this research further, investigating whether there is any correlation of differences in body shape and structure amongst the individuals and rank order of their swimming performance. Individual variation in locomotory performance has been described in a number of species (Marker and Gatten 1993, Garland 1985, Garland 1988). However, perhaps the most important question raised by the existence of individual variation, is whether there is any genetic component to locomotory performance. Few studies have examined the theory that locomotory performance may be heritable. In both race horses (Langlois 1980) and humans (Bouchard and Malina 1983) running speed has been demonstrated to have significant heritabilites, and in the garter snake (*Thamnophis sirtalis fitchi*) locomotory performance is dependent on litter (Jayne and Bennett 1990). To my knowledge there have been no studies on the heritability of swimming performance in fish. Therefore, if a suitable fast breeding and easy to rear fish species could be found, a programme of selective breeding on the basis of swimming performance should be initiated to determine the existence, if any, of a genetic component to the swimming performance.

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