Effects of Water Quality Parameters on Prolonged Swimming Ability of Freshwater Fishes

A thesis
submitted in partial fulfilment of
the requirements for the degree
of
Doctor of Philosophy in Biological Sciences
at
The University of Waikato
by

Henry James Bannon

The University of Waikato

ABSTRACT

The critical swimming speed (U_{crit}) of rainbow trout parr (*Oncorhynchus mykiss*) and three life stages of *Galaxias maculatus*, larval (whitebait), postlarval inanga and adult inanga, were tested at temperatures from 5°C to 25°C. All fish were swum at their acclimation temperature under normoxic conditions to determine the optimal aerobic exercise temperature. To determine whether acclimation affected swimming ability, trout parr acclimated to either 10°C or 20°C were swum at 20°C and 10°C, respectively.

The potential effect of mild hypoxia (75% saturation) on trout parr and whitebait was also examined at 10°C, 15°C and 20°C, and also tested separately and in combination were the effects of mild hypoxia and severe anaemia on the prolonged swimming ability of trout smolts at temperatures from 10°C to 20°C. For all trout experiments, blood samples were taken from non-exercised and exercised fish by acute caudal venepuncture to determine haematological responses to both acclimation and exercise.

Under normoxic conditions, $U_{crit\ max}$ for trout parr (7.0 ± 0.5 cm fork length) was calculated to be 5.8 body lengths per second ($BL\ s^{-1}$) at 15.1°C, but declined at lower and higher temperatures. This result implies that swimming performance was limited by temperature below 15°C, whereas performance at higher temperatures was limited by oxygen availability. In support of this hypothesis, mild hypoxia (75% saturation) had no effect at 10°C or 15°C but caused a significant reduction in U_{crit} at 20°C. However, fish acclimated at 20°C showed an adaptive elevation in oxygen carrying capacity due to an increase in mean erythrocyte volume and haemoglobin content. Furthermore, acclimation to 20°C improved warm water swimming performance. Trout parr acclimated to 10°C performed significantly worse than fish acclimated to 20°C when swum at 20°C. However, trout parr acclimated to 20°C performed as well as fish acclimated to 10°C when swum at 10°C. Following exercise, haematocrit was elevated under both normoxic and hypoxic conditions. However, the primary cause of this apparent increase in oxygen carrying capacity was splenic release of erythrocytes under normoxic conditions, whereas stress-induced erythrocytic swelling

contributed to the observed increase in hypoxia. This contrasting response was most pronounced at 10°C.

Larval whitebait (4.7 – 5.0 cm total length (TL)) also showed a temperature dependence of prolonged swimming ability with $U_{crit\ max}$ calculated to be 5.1 *BL* s^{-1} at 17.7°C. Hypoxia significantly reduced U_{crit} at 15°C and 20°C, lowering the optimal aerobic temperature to 13.9°C and reducing U_{crit} to 4.2 *BL* s^{-1}. Mild hypoxia therefore had a more pronounced impact on inanga whitebait than trout.

Postlarval inanga (3.9 – 4.0 cm TL) performed poorly at higher temperatures with $U_{crit\ max}$ of 5.6 *BL* s^{-1} at 9.4°C indicating an ontogenetic change in swimming ability, possibly resulting from a developmental shift in red muscle kinetics or a greater dependence on anaerobic muscle.

Adult inanga (5.5 – 6.8 cm TL) prolonged swimming ability showed similar temperature dependence to that of inanga whitebait but lower relative swimming speeds due to their larger size. The dramatic decline in performance exhibited by juveniles at warmer temperatures was not apparent in adults. $U_{crit\ max}$ for adults was 4.0 *BL* s^{-1} at 18.3°C.

The critical swimming speed of trout smolts, subjected to mild hypoxia (6.8 mg O_2 L^{-1}, being 75% of the saturation value at 20°C) and severe anaemia (50%), separately and in combination, showed a significant reduction only in anaemic fish under hypoxic conditions at 20°C. However, although not statistically significant, 50% anaemia or hypoxia (6.8 mg O_2 L^{-1}) independently resulted in slight reductions in U_{crit} at both 15°C and 20°C. However, at 20°C a small reduction in ambient oxygen had as great an effect on U_{crit} as a much larger decrease in oxygen carrying capacity, indicating that environmental oxygen availability may be a more significant determinant of aerobic scope in these fish than physiological oxygen transport.

ACKNOWLEDGEMENTS

Firstly, I would like to thank my supervisor Dr Nicholas Ling for his help and support throughout this project. I would also like to acknowledge the assistance of Chris Sistern, Bruce Rhodes, Stephen Hardy, and Peter Jarman for equipment construction and technical support, and my daughter Kim for her assistance with statistical analysis. Thanks also to Michael Landman, Lee Laboyrie, and Charles Mitchell, for supplying fish.

I am very grateful to the following for their generous travel grants: University of Waikato Biological Sciences Special Purposes Fund for assistance to present work from this thesis at the Sixth International Congress of Comparative Physiology and Biochemistry. Mt. Buller, Victoria, Australia; Commonwealth Science Council for financial assistance to present work from this thesis at The Seventh International Symposium on Fish Physiology, Toxicology and Water Quality. Tallinn, Estonia;
The Student Travel Award Committee of the Physiology Section of the American Fisheries Society for assistance to present work from this thesis at the VI International Congress on the Biology of Fish. Manaus, Amazonas, Brazil.

Barrie Todd, thanks for exposure to trout in the wild, and your enthusiasm and interest in this project.

Finally, I am thankful to my wife, Judy Margaret for her support, encouragement and patience.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

Effects of Water Quality Parameters on Prolonged Swimming Ability of Freshwater Fishes

A thesis
submitted in partial fulfilment of
the requirements for the degree
of
Doctor of Philosophy in Biological Sciences
at
The University of Waikato
by

Henry James Bannon

The University of Waikato

ABSTRACT

The critical swimming speed (U_{crit}) of rainbow trout parr (*Oncorhynchus mykiss*) and three life stages of *Galaxias maculatus*, larval (whitebait), postlarval inanga and adult inanga, were tested at temperatures from 5°C to 25°C. All fish were swum at their acclimation temperature under normoxic conditions to determine the optimal aerobic exercise temperature. To determine whether acclimation affected swimming ability, trout parr acclimated to either 10°C or 20°C were swum at 20°C and 10°C, respectively.

The potential effect of mild hypoxia (75% saturation) on trout parr and whitebait was also examined at 10°C, 15°C and 20°C, and also tested separately and in combination were the effects of mild hypoxia and severe anaemia on the prolonged swimming ability of trout smolts at temperatures from 10°C to 20°C. For all trout experiments, blood samples were taken from non-exercised and exercised fish by acute caudal venepuncture to determine haematological responses to both acclimation and exercise.

Under normoxic conditions, $U_{crit\ max}$ for trout parr (7.0 ± 0.5 cm fork length) was calculated to be 5.8 body lengths per second ($BL\ s^{-1}$) at 15.1°C, but declined at lower and higher temperatures. This result implies that swimming performance was limited by temperature below 15°C, whereas performance at higher temperatures was limited by oxygen availability. In support of this hypothesis, mild hypoxia (75% saturation) had no effect at 10°C or 15°C but caused a significant reduction in U_{crit} at 20°C. However, fish acclimated at 20°C showed an adaptive elevation in oxygen carrying capacity due to an increase in mean erythrocyte volume and haemoglobin content. Furthermore, acclimation to 20°C improved warm water swimming performance. Trout parr acclimated to 10°C performed significantly worse than fish acclimated to 20°C when swum at 20°C. However, trout parr acclimated to 20°C performed as well as fish acclimated to 10°C when swum at 10°C. Following exercise, haematocrit was elevated under both normoxic and hypoxic conditions. However, the primary cause of this apparent increase in oxygen carrying capacity was splenic release of erythrocytes under normoxic conditions, whereas stress-induced erythrocytic swelling

contributed to the observed increase in hypoxia. This contrasting response was most pronounced at 10°C.

Larval whitebait (4.7 – 5.0 cm total length (TL)) also showed a temperature dependence of prolonged swimming ability with $U_{crit\ max}$ calculated to be 5.1 *BL* s^{-1} at 17.7°C. Hypoxia significantly reduced U_{crit} at 15°C and 20°C, lowering the optimal aerobic temperature to 13.9°C and reducing U_{crit} to 4.2 *BL* s^{-1}. Mild hypoxia therefore had a more pronounced impact on inanga whitebait than trout.

Postlarval inanga (3.9 – 4.0 cm TL) performed poorly at higher temperatures with $U_{crit\ max}$ of 5.6 *BL* s^{-1} at 9.4°C indicating an ontogenetic change in swimming ability, possibly resulting from a developmental shift in red muscle kinetics or a greater dependence on anaerobic muscle.

Adult inanga (5.5 – 6.8 cm TL) prolonged swimming ability showed similar temperature dependence to that of inanga whitebait but lower relative swimming speeds due to their larger size. The dramatic decline in performance exhibited by juveniles at warmer temperatures was not apparent in adults. $U_{crit\ max}$ for adults was 4.0 *BL* s^{-1} at 18.3°C.

The critical swimming speed of trout smolts, subjected to mild hypoxia (6.8 mg O_2 L^{-1}, being 75% of the saturation value at 20°C) and severe anaemia (50%), separately and in combination, showed a significant reduction only in anaemic fish under hypoxic conditions at 20°C. However, although not statistically significant, 50% anaemia or hypoxia (6.8 mg O_2 L^{-1}) independently resulted in slight reductions in U_{crit} at both 15°C and 20°C. However, at 20°C a small reduction in ambient oxygen had as great an effect on U_{crit} as a much larger decrease in oxygen carrying capacity, indicating that environmental oxygen availability may be a more significant determinant of aerobic scope in these fish than physiological oxygen transport.

ACKNOWLEDGEMENTS

Firstly, I would like to thank my supervisor Dr Nicholas Ling for his help and support throughout this project. I would also like to acknowledge the assistance of Chris Sistern, Bruce Rhodes, Stephen Hardy, and Peter Jarman for equipment construction and technical support, and my daughter Kim for her assistance with statistical analysis. Thanks also to Michael Landman, Lee Laboyrie, and Charles Mitchell, for supplying fish.

I am very grateful to the following for their generous travel grants: University of Waikato Biological Sciences Special Purposes Fund for assistance to present work from this thesis at the Sixth International Congress of Comparative Physiology and Biochemistry. Mt. Buller, Victoria, Australia; Commonwealth Science Council for financial assistance to present work from this thesis at The Seventh International Symposium on Fish Physiology, Toxicology and Water Quality. Tallinn, Estonia;
The Student Travel Award Committee of the Physiology Section of the American Fisheries Society for assistance to present work from this thesis at the VI International Congress on the Biology of Fish. Manaus, Amazonas, Brazil.

Barrie Todd, thanks for exposure to trout in the wild, and your enthusiasm and interest in this project.

Finally, I am thankful to my wife, Judy Margaret for her support, encouragement and patience.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

CHAPTER ONE

General introduction

Introduction

The sustained swimming performance of fishes depends on many extrinsic and intrinsic variables (Hammer, 1995), especially those that affect oxygen uptake and delivery to aerobic musculature (Jones, 1971; Gallaugher et al., 1995). To evaluate the effects of different environmental conditions or pollutants on fish fitness or survival in an ecological system, a reliable ecologically relevant measurement of swimming capability is required (Plaut, 2001). Previously, observations were made on either cruising speed; maximum sustained speed (U_{crit}), or burst speed, resulting in a variety of records, which are not readily compared (Brett, 1967). This chapter reviews the scientific literature with regard to fish locomotion, U_{crit} evaluation methodology, and identifies environmental and physiological factors from the scientific literature that affect U_{crit} values.

Fish locomotion

Fish locomotion can be classified within three categories: sustained, prolonged and burst swimming (Beamish, 1978). Each category reflects the constraints imposed by time and by the biochemical processes which supply fuels for its application (Farlinger & Beamish, 1977). Burst swimming may be sprint or acceleration (Webb, 1975) undertaken during predation-prey interactions, and when required to pass both natural and anthropogenic barriers e.g. falls and culverts. It is generally accepted that burst swimming is performed anaerobically, powered almost exclusively by white glycolytic muscle (Wood, 1991; Moyes & West, 1995; Milligan, 1996). Consequently, burst swimming can be only maintained for brief periods (< 20 s Beamish, 1978) and results in fatigue (Beamish, 1978; Wood, 1991). Fatigue is reported in the scientific literature to result from accumulation of waste products

(Bainbridge, 1960, 1962; Brett, 1964), in particular large quantities of lactate and metabolic protons (Milligan & Wood, 1986a, 1986b; Wood, 1991; Kieffer et al., 1994), which results in intracellular acidosis. However, the association of acidosis with fatigue in fish may require revision. During high-intensity exercise in mammalian muscles, Westerblad et al. (2002) postulated that an increase in organic phosphate (P_i), resulting from the breakdown of phosphocreatine (PCr) to creatine (Cr) and P_i, may be the primary cause of fatigue. Currently it is proposed by Allen & Westerblad (2001) that, "P_i may enter the sarcoplasmic reticulum (SR), combine with Ca^{2+} and form an insoluble precipitate of calcium phosphate (CaP_i), leading to a reduced SR Ca^{2+} release and consequent decline in muscle performance".

Throughout sustained swimming, in contrast to burst swimming, metabolic demand matches supply, while waste production is balanced by disposal (Jones, 1982), thus can be maintained for long periods [> 240 min (Beamish, 1966), >200 min (Brett, 1967)] without resulting in muscle fatigue (Farlinger & Beamish, 1977). Sustained swimming is primarily fuelled by fatty acid oxidation (Hochachka & Somero, 1984) and is supported by red, slow-twitch, lateral muscle fibres and a steady, undulatory locomotory gait (Coughlin & Rome, 1996). However, Johnston & Moon (1980) have demonstrated that sustained swimming is also supported by the aerobic capacity of the white muscle mass, with the aerobic capacity appearing to be around 25 - 35% that of red muscle.

Prolonged swimming is an intermediate mode utilizing both oxidative muscle fibres and white glycolytic muscle, lasting between 20 s - 200 min (Beamish, 1978), and, depending on swimming speed, is terminated by exhaustion of white muscles.

U_{crit} methodology

Prolonged swimming is frequently used to evaluate the maximum aerobic capacity of fish, and is assessed using the U_{crit} protocol first described and employed by Brett (1964) to evaluate the relative ability of salmonid fishes to ascend lotic waters to natal streams.

U_{crit} is determined as follows:

$U_{crit} = U_i + U_{ii}\ T_i\ /\ T_{ii}$

Where U_i is the highest velocity maintained for a complete time interval, U_{ii} is the velocity increment, T_i is the interval time elapsed at fatigue velocity and T_{ii} is the interval time.

This measurement is directly analogous to the treadmill tests performed on elite athletes to assess aerobic capacity. For fish, swimming performance was suggested by Brett (1967) as a sub-lethal criterion for measuring effects of toxicants, and Hammer (1995) concluded that critical swimming speed should provide a sensitive measure for environmental or physiological stress factors.

For U_{crit} measurement, a fish is placed in a water tunnel or flume, and exposed to a low water velocity for a period to aid recovery from handling and transfer to the flume. Following the recovery period, the fish is forced to swim against an increasing current velocity, which is increased in equal increments, with each increment being maintained for a set time period. This methodology is continued until the fish is fatigued.

Although prolonged swimming employs both aerobic and anaerobic muscle masses, Webb (1971) reported that swimming speeds greater than 80% of U_{crit} were required to detect the deviation of the overall efficiency from aerobic efficiency. Webb's (1971) findings have been supported more recently by electromyographic studies. Beddow & McKinley (1999) reported that white muscle recruitment starts at about 86% of U_{crit}, and Reidy et al. (2000) recorded glycolytic fibre recruitment only towards the end of U_{crit} tests. Further evidence of the threshold of anaerobic white muscle recruitment is provided by Driedzic & Kiceniuk (1976), who reported no accumulation of lactate in the blood of rainbow trout (*Salmo gairdneri*) until 93% of U_{crit} was reached, and Kicenuik & Jones (1977) found blood pH remained fairly constant

at speeds around 91% of U_{crit}, indicating exercise at this level was principally aerobic.

Maximum uptake of oxygen was assumed by Farrell & Steffensen (1987) to occur at $U_{crit\ max}$. However, Soofiani & Priede (1985) reported that oxygen consumption at maximum sustained swimming speed was below oxygen consumption during recovery from exhaustive exercise. Nevertheless, Brauner et al. (1994) considered U_{crit} provides a rough estimate of aerobic swimming velocity, and a reduction in U_{crit} values is generally accepted as being the result of interference of oxygen uptake in the gills or reduction of blood oxygen transport (Jones, 1971; Gallaugher et al., 1995).

Temperature / Hypoxia

Temperature increase is a particularly important factor that presents compounding problems for sustained swimming, due to an increase in metabolic oxygen demand while simultaneously decreasing oxygen availability. In eurythermal fishes, U_{crit} increases with increasing temperature to a maximum several degrees below the upper thermal limit, performance then declines markedly as the upper thermal limit is approached (Brett, 1971; Griffiths & Alderdice, 1972; Beamish, 1978; Myrick & Cech, 2000; Koumoundouros et al., 2002).

Hypoxia over a wide range of temperatures has been demonstrated to reduce U_{crit}. Graham (1949) found that at 8^{o}C, the cruising speed of brook trout (*Salvelinus fontinalis*) was appreciably reduced only when the oxygen concentration was lowered to about 50% of air-saturation. At temperatures between 10^{o}C and 20^{o}C, Davis et al. (1963) found that even a slight reduction of the dissolved oxygen concentration, from the air-saturation level, usually resulted in some reduction of the maximum sustained swimming speed of chinook salmon (*Oncorhynchus tshawytscha*) and coho salmon (*Oncorhynchus kisutch*). At 20^{o}C and carbon dioxide concentrations near 2 mg L^{-1}, Dahlenberg et al. (1968) reported that any considerable reduction of the oxygen concentration from about 9 mg L^{-1} (air saturation level) resulted

in some reduction of the final swimming speed of coho salmon. Similarly, at 25^{o}C and low carbon dioxide concentrations, the swimming speed of largemouth bass (*Micropterus salmoides*) decreased with oxygen concentrations below 5 or 6 mg L^{-1} (Dahlenberg et al., 1968).

Temperature has also been demonstrated to influence cardiac output, increasing linearly with increasing temperature while circulation time decreases (Barron et al., 1987). Farrell (1997) reported that at temperatures above the preferred temperature, cardiac performance begins to plateau, and suggested that the primary limitations to both maximum metabolic performance and swimming performance was the result of the decline in cardiac performance. Farrell's (1997) suggestion was validated by Taylor et al. (1997), who provided evidence, by way of measured swimming performance, that the capacity of the cardiovascular system for convective transport of oxygen, and the capacity of the microcirculation system for diffusional supply of oxygen to respiring cells, is maximised in the mid-temperature range. Taylor et al. (1997), however, proposed that the limits to swimming capacity were not exclusively determined by cardiac capacity: a contributory factor limiting endurance swimming at high temperatures could be the structural extent of capillary supply to muscle.

pH and metal ions

A consequence of low pH values in natural water is reported by Beaumont et al. (1995a) to be the mobilisation of metal ions such as aluminium, zinc and copper. Acute exposure of fish to dissolved metals can result in the production and coagulation of excess mucus, which may result in a lower diffusion rate across the gill (Leland & Kuwabara, 1985), which ultimately may limit the aerobic performance of the fish. The exposure of rainbow trout (*Oncorhynchus mykiss*) to pH 6.5 (Wilson et al., 1994) and pH 6 (Waiwood & Beamish, 1978) had no effect on critical speed. However, Ye & Randall (1991) and Wilson et al. (1994) reported that exposure of rainbow trout to a low pH (<pH 6.0)

reduced swimming performance. Furthermore, Graham & Wood (1981) and Butler & Day (1993) reported a significant reduction in U_{crit} of fish exposed to pH 4.6 and pH 4.5 respectively.

Copper exposure has been reported by Waiwood & Beamish (1978) to depress the critical speed of rainbow trout. Furthermore, Waiwood & Beamish (1978) reported that copper exposure, in general, had a more pronounced effect on U_{crit} at low pH than at high pH, and the effects of copper varied greatly with pH, hardness and exposure time. Taylor et al. (2004), to evaluate chronic exposure of yellow perch (*Perca flavescens*), collected fish from a reference lake (pH 7.5, 5 µg Cu L^{-1}), a metal-contaminated lake (pH 6.5, 18 µg Cu L^{-1}) and from a local hatchery (pH 8.0, 3 µg Cu L^{-1}) and subjected them to U_{crit} evaluation. U_{crit} swimming values were not significantly different between the groups. However, the hatchery fish had the highest U_{crit} and the metal-contaminated the lowest.

Beaumont et al. (1995b) exposed brown trout (*Salmo trutta*) to sub-lethal copper levels at pH 5.0, and reported swimming performance was reduced. In these fish, Beaumont et al. (1995a) reported that U_{crit} reduction was correlated with ammonia levels in the plasma, and systemic hypoxia was absent; indicating that oxygen uptake by diffusion was not limiting performance despite apparent ultra-structural damage to the gill. It was suggested that the reduced swimming performance may be attributed to haematological disturbances disrupting the transport of oxygen to the working muscle. Previously, Waiwood & Beamish (1978) had proposed that responses that may limit swimming performance of fish exposed to copper may be a result of impairment of transport or exchange of respiratory gases, alterations in energy transformations, or inhibition of nervous or muscular activity. Ionoregulatory disturbances (Lauren & McDonald, 1985; Wilson & Taylor, 1993) and gill damage (Wilson & Taylor, 1993; Beaumont et al., 1995a) resulting from copper exposure have been suggested by Shingles et al. (2001) to contribute to the accumulation of ammonia by inhibiting its efflux over the gills. Shingles et al. (2001) investigated

whether increased plasma ammonia levels alone reduce swimming performance. Exposing fish to sub-lethal total ammonia (288 μmol L^{-1}, pH 8.4) for 24 hours resulted in a marked effect on aerobic metabolism, and was associated with a 28% reduction in U_{crit} compared to controls.

Exposure of fish to aluminium has also been demonstrated to reduce U_{crit} values. Wilson et al. (1994) reported a decline of 17% in U_{crit} of juvenile rainbow trout acclimated for 10 days to soft water at pH 5.2 with 38 μg Al L^{-1}. Wilson et al. (1994) also reported that no evidence of recovery was observed, U_{crit} stabilizing at 15 – 20% below that of controls. Ytrestøyl et al. (2001) also reported a significant reduction in U_{crit} for Atlantic salmon (*Salmo salar*) exposed to aluminium. After one week's exposure to water from a moderately acidified brook (pH 5.0 – 5.2) mean U_{crit} was reduced by 19% in females but not in males (control, pH 6.5 – pH 6.7). Exposure for 24 hours to pH 5.0 – pH 5.2 with extra aluminium added led to a further reduction in U_{crit}, 34% for females and 23% for males. The difference was attributed to higher plasma cortisol and blood glucose levels in females, indicating more severe stress responses, a possible consequence being a greater reduction in aerobic scope compared to males (Ytrestøyl et al., 2001). Further contributory factors that may reduce aerobic scope of fish exposed to aluminium are structural and biochemical changes that make the gill epithelium more permeable to ions (Exley et al., 1991), increased blood water diffusion distance and reduced gill surface area (Wilson et al., 1994), and colloidal aluminium precipitation on fish gills causing excessive mucus secretion that clogs interlamellar spaces (Poléo, 1995).

Nitrite, found in both natural aquatic systems and aquacultural facilities (Brauner, 1993), may accumulate in fish to levels far in excess of that dissolved in the environmental water (Eddy et al., 1983). Taken up across the gills into the circulatory system, nitrite crosses the membrane of the erythrocyte, where it oxidizes the iron in haemoglobin from ferrous to its ferric state, creating methaemoglobin which does not bind oxygen, thus limiting oxygen transport. Limited oxygen transport

has been demonstrated by Jones (1971) to reduce U_{crit}. Phenylhydrazine hydrochloride injected by Jones (1971) into rainbow trout reduced haematocrit by 50 - 66% and resulted in a 34 - 40% reduction in U_{crit}. Brauner et al. (1993) injected 30, 60, or 90 mg kg^{-1} of sodium nitrite into groups of chinook salmon. However, only the 90 mg kg^{-1} treatment showed a significant reduction in U_{crit}.

Body size

Body size also places limitations on sustained swimming. Bainbridge (1958) and Brett (1965) demonstrated that absolute swimming speed of fish increased with size, however, as size increased, relative speed decreased. Glova & McInerney (1977) demonstrated that the performance of coho salmon was inversely related to size, varying from a peak of 7.3 *BL* s^{-1} in fry to 5.5 *BL* s^{-1} in smolts. A positive correlation between the critical performance and length of rainbow trout was calculated by Waiwood & Beamish (1978); a 1 cm increase in fork length (FL) would result in an approximate increase of 3.5 cm s^{-1} in critical speed FL s^{-1}. The effects of temperature in combination with size on the active metabolic rates of sockeye salmon (*Oncorhynchus nerka*) was investigated by Brett & Glass (1973), who concluded from isopleth graphs that doubling the length of the fish has a greater effect on U_{crit} than doubling the temperature.

Training

Aerobic training of fish has resulted in; lower oxygen consumption rates in resting and swimming rainbow trout when compared to untrained fish (Woodward & Smith, 1985); increased Hct and oxygen capacity of the blood of rainbow trout when compared to controls (Thorarensen et al., 1993), and higher arterial blood oxygen content at the highest swimming speed (Holk & Lykkeboe, 1998). However, Davie et al. (1986) reported no increase in Hct, but suggested their results indicated that exercise increased the aerobic capacity of red and particularly of

white muscle. However, in terms of skeletal muscle, the reported effects in the scientific literature of training are variable (Farrell, et al., 1990), and may be attributed to differences in training regimes. Training periods have varied from a few days to a year or longer (Davison, 1989). However, experiments that extend across seasons are not only a major time commitment requiring constant monitoring of water quality, but require interpretation of seasonal changes in the physiology of the fish (Davison, 1989).

Many other studies have demonstrated improvements in performance following aerobic training. Brett et al. (1958) reported that coho salmon reared in a water velocity of 24 cm s^{-1} exhibited a higher cruising speed and less susceptibility to fatigue when compared to salmon reared in lower current velocity. Largemouth bass fingerlings exposed to a training regime increased their maximum swimming speed from 18 to 30 cm s^{-1} (MacLeod, 1967). Cultured and wild striped bass (*Morone saxatilis*) subjected to a water velocity of 1.2 – 2.4 *BL* s^{-1} for 60 days significantly improved U_{crit} performance of both groups (Young & Cech, 1993). Furthermore, cultured striped bass exposed for 50 and 60 days to four different water velocities: < 0.02 (control), 0.5 – 1.2 (slow), 1.5 – 2.4 (moderate), and 2.4 – 3.6 *BL* s^{-1} (fast), resulted in U_{crit} values showing a linear trend with increasing exercise-conditioning velocity. After 50 days of conditioning, the fast-velocity fish U_{crit} values were significantly higher than those of fish exposed to other treatments (Young & Cech, 1994).

Other studies contradict the effects of exercise training. Rainbow trout, exposed to a water velocity of 30 cm s^{-1} for 28 - 52 days, had significantly higher U_{crit} values (12%) than the control group, which had been exposed to a water velocity of 1 cm s^{-1} (Farrell et al., 1990). In contrast, Farrell et al. (1991) recorded no statistically significant increase in U_{crit} of rainbow trout subjected to exercise-training for 18 hours per day over 28 days at a water velocity up to 60% of their measured U_{crit}. However, they reported that the caudal fin of two trained fish were abraded, resulting from contact with the rear grid of the

swimming tube. These two fish had the lowest U_{crit} values of all the fish tested, thus possibly skewing statistical results. Thorarensen et al. (1993) also reported non-significant increase in U_{crit} of rainbow trout following 8 months exposure to 1.5 *BL* s^{-1} when compared to control fish swum at 0.5 *BL* s^{-1}. Nevertheless, similar increases to those reported by Young & Cech (1994) and Farrell et al. (1990) have been reported in the scientific literature for large mouth bass (Farlinger & Beamish, 1978), coho salmon (Besner & Smith, 1983), and rainbow trout (Nahhas et al., 1982; Houlihan & Laurent, 1987; Holk & Lykkeboe, 1998).

Hct

Jones (1971) has demonstrated, by way of induced haemolytic anaemia in rainbow trout by intra peritoneal injections of phenylhydrazine hydrochloride, that a reduction in haematocrit to one-half or one-third normal resulted in a 34% reduction in maximum swimming speed at low temperature (8 ^{o}C - 10^{o}C) and a 40% reduction at high temperature (21 ^{o}C - 23^{o}C) compared with control animals (blank injected). However, no strict relationship between U_{crit} and Hct could be determined from these data (Jones, 1971). Subsequently it has been demonstrated that following exhaustive exercise, the spleen contracts (Wells & Weber, 1990, 1991; Pearson & Stevens, 1991) releasing red blood cells, and Yamamoto et al. (1980) have provided evidence of graded release of red blood cells from the spleen during aerobic swimming in yellowtail kingfish (*Seriola quinqueradiata*). Suspending such releases has been demonstrated to depress U_{crit}. Pearson & Stevens (1991) reported that splenectomized rainbow trout returned a 21% lower U_{crit} and 16% lower Hct value than sham-operated fish. But Gallaugher et al. (1992) were unable to demonstrate such a relationship, reporting no significant difference between the U_{crit} values obtained for splenectomized and sham-operated fish, and no significant correlation between U_{crit} and Hct for either group. However, comparison between the experiments of Pearson & Stevens (1991) and Gallaugher et al. (1992) show methodology discrepancies that veto scientific

comparisons. Pearson & Steven's (1991) experiment was carried out at 8.2°C, 6 months after spleen removal, whereas that of Gallaugher et al. (1992) was carried out at between 18°C and 19°C, approximately 48 hours after acute splenectomy. Furthermore, Pearson & Stevens (1991) sampled blood from the caudal peduncle, whereas Gallaugher et al. (1992) used cannulated fish.

Gallaugher et al. (1995) undertook manipulation of Hct of two acclimated groups of rainbow trout, (seawater at 12°C - 14°C and freshwater at 4°C - 6°C), at both above and below normocythaemia, and subsequently exposed the fish to U_{cirt} assessment. They reported that U_{crit} was positively correlated with Hct over the range of 8% to 55%, with the positive effect of Hct on swimming performance being more profound in anaemic (Hct < 22%) than in either normocythemic or polycythemic fish (Hct 23% to 55%). Furthermore, Gallaugher et al. (1995) found that a clear break point in the relationship between Hct and U_{crit} coincided with the lowest Hct value (about 22%) for the normocythemic range, indicating that exercise performance in anaemic fish is dependent on perfusion-limited O_2 transport, and diffusion limited O_2 transport in polycythemic fish (Gallaugher et al., 1995).

Nutrient

Beamish et al. (1989) reported critical swimming speed of lake trout (*Salvelinus namaycush*) increased with dietary and carcass protein content, indicating a possible association between muscle mass and exercise performance. Furthermore, McKenzie et al. (1998) found an association between dietary fatty acid composition and swimming performance of Atlantic salmon fed a diet of menhaden oil had a significantly lower U_{crit} than those fed a diet in which the supplemented lipid was an equal blend of menhaden oil and canola oil rich in 18-carbon unsaturated fatty acids. McKenzie et al. (1998) suggest that metabolism of these 18-carbon unsaturates accounted for the effects of diet on exercise performance.

Alsop & Wood (1997) investigated the impact of feeding on the rate of O_2 consumption, aerobic swimming performance, nitrogenous waste excretion and protein utilization as an aerobic fuel. Experimental design required rainbow trout to be assigned to one of three treatment groups on the basis of feeding quality: group 1 fasting, group 2 fed 1% of body mass per day, and group 3 satiation feeding per day. Fish were kept on their respective diets for at least 4 days prior to swimming tests. Swimming tests showed fasted fish had the highest U_{crit}, whereas U_{crit} for fish fed to satiation was significantly lower (15%). U_{crit} values for the 1% ration fish were intermediate but not different from values for either of the two treatments (Alsop & Wood, 1997). Collectively, the results demonstrated that once nutrients from the food have reached the specific dynamic tissue, the metabolic process cannot be turned off, and the absolute stimulation of MO_2 by the specific dynamic action effect of feeding is maintained virtually constant as swimming speed increases. Subsequently, fed fish were forced to stop aerobic swimming at a lower speed because of this specific dynamic action effect load (Alsop & Wood, 1997). From the collective data, Alsop & Wood (1997) concluded that protein does not become more important as a fuel during exercise, even when abundantly available in the diet.

The impact of starvation on sprint and U_{crit} performance of Atlantic cod (*Gadus morhua*) was investigated by Martínez et al. (2004). Two groups of cod were fed or starved for 12 weeks, resulting in a two-fold difference in Fulton's condition (0.5 for starved and 1.0 for fed cod), then evaluated for sprint and U_{crit} performance. Both sprint and U_{crit} performance were decreased, 30% and 38% respectively by starvation. Interestingly, starved cod had 81% less white muscle but only 56% less red muscle mass than fed cod, but sprint and U_{crit} values differed by virtually the same amount (Martínez et al., 2004).

Freshwater / Sea water

Teleost fishes are osmoregulators, maintaining their body fluids' osmotic concentration within a relatively narrow range, from ~260 to

400 mOsmol (Jobling, 1995). Exposure to salinities substantially outside their normal range may exceed their osmoregulatory capabilities and reduce their performance (Beamish, 1978; Webb, 1994).

Glova & McInerney (1977) tested and compared the swimming abilities of coho salmon from early fry to smolt stages over a range of salinity-temperature combinations representative of freshwater, estuarine, and inshore marine environments. Glova & McInerney (1977) concluded from the results that underyearling coho should be able to perform important locomotor-dependent behaviours in salinities up to 20‰. However, the anomalous situation in which large numbers of underyearling coho migrate prematurely to sea but few if any survive remains unexplained (Glova & McInerney, 1977).

Brauner et al. (1994) conducted a study to determine if hypo-osmoregulatory ability affects swimming performance and haematology in wild and hatchery-reared coho salmon at the time of smoltification. Hatchery-reared and wild juvenile coho salmon were held in freshwater for periods of not less than 1 week prior to commencement of the experiment. Fish were exposed for 24 hours, separately, to both freshwater and seawater. Fish were then introduced to a Brett-type respirometer, filled with water at the salinity to which the fish had previously been exposed, and subjected to U_{crit} assessment, followed 2 hours later by a second U_{crit} assessment. Brauner et al. (1994) reported that the effects of seawater exposure and repeated swimming in both seawater and fresh-water differed markedly between the groups of hatchery and wild fish. In wild fish, the only significant reduction in U_{crit} was observed in fish forced to swim a second trial in seawater ($U_{crit\,2}$). In contrast, Brauner et al. (1994) reported that hatchery fish exhibited a significant reduction in $U_{crit\,1}$ upon transfer to seawater, and in both fresh water and seawater $U_{crit\,2}$ was significantly reduced relative to $U_{crit\,1}$ in the respective water types. Previously, Maxime et al. (1991) had recorded, 24 hours after seawater transfer, a 38% reduction in arterial oxygen uptake at the gill of resting rainbow trout. Such a reduction Brauner et al. (1994) allied with the differential impairment in

swimming performance between hatchery and wild coho in seawater, concluding that "impairment in swimming performance was largely a result of the difference in hypo-osmoregulatory ability between the groups".

Increases in the metabolic costs of osmoregulation was implicated by Kolok & Sharkey (1997) in attempting to explain why gulf killifish (*Fundulus grandis*) in freshwater had significantly lower U_{crit} values than fish in brackish water. Kolok & Sharkey (1997) acclimated gulf killifish for at least 10 days at 23^{o}C to either freshwater or brackish water (10‰ salinity) before commencing U_{crit} assessment at acclimation temperature and salinity. The mean U_{crit} of the freshwater fish was significantly less than that of the fish from brackish water. Kolok & Sharkey (1997) concluded that, "if this additional cost of osmoregulation for euryhaline fish is met at the expense of oxygen available for aerobic swimming, then fish in freshwater are likely to fatigue more rapidly". However, Plaut (2000) suggested the reduction in sustained swimming recorded by Kolok & Sharkey (1997) may also be a result of reduced gill membrane and integument permeability, because of the increasing difference in osmotic pressure between body fluids and the environment.

Plaut (2000) collected euryhaline cyprinodontid fish, *Aphanius dispar*, and acclimated groups to salinities of <1 (freshwater), 35 (seawater), 70, 105, and 140‰ for 4 weeks before measurement of oxygen consumption, critical swimming speed, and routine activity level. Oxygen consumption was reported by Plaut (2000) to be similar in <1, 35, and 70‰, but decreased in 105 and 140 ‰. Critical swimming speed and routine activity levels showed the same trend. Plaut (2000) proposed that these results "suggest a general decrease in physiological function of *A. dispar at* extreme salinities".

Sea lice

The sea louse, *Lepeophtheirus salmonis*, a parasitic copepod, infects salmonids and is increasing along salmonid migratory routes in the

northern hemisphere. Sea lice create open lesions on the surface of the fish that compromise the osmotic balance of fishes, which may result in death (Grimnes & Jakobsen, 1996). The level of sea lice required to compromise osmoregulatory capacity, cardiac output, and swimming ability, was investigated by Wagner et al. (2003). Two groups of fish were infected with sea lice; infection levels prior to U_{crit} assessment were 0.013 and 0.020 sea lice g^{-1}.Wagner et al. (2003) reported that sub-lethal infection by sea lice compromised the overall fitness of the fish and significantly lowered U_{crit} (19%) of the higher sea lice infected fish compared to controls. However, lower-infected fish swam as well as the controls.

Wagner et al. (2003) concluded that the reduction in swimming performance of sub-lethal infected fish resulted from detrimental physiological changes, in particular, a compromise between osmoregulatory ability and cardiac performance.

Mycobacterium

Lescenko et al. (2003) reported that in areas of the temperate zone and in the tropics, mycobacterial infection is among the most common chronic disease of freshwater and sea fish. In the last few years, mycobacteroisis has been described in many species of freshwater and sea fish in the wild, and kept in captivity, in which it causes major economic losses (Lescenko et al., 2003). Swanson et al. (2002) evaluated the effects of *Mycobacterium* by comparison of critical swimming velocities of non-symptomatic infected delta smelt (*Hypomesus transpacificus*) with fish displaying no detectable infection. Swanson et al. (2002) reported that infection with *Mycobacterium* spp. impaired the capacity of delta smelt for sustained, high intensity activity. The critical swimming velocities were significantly higher in uninfected fish than in infected fish, which had a reduced U_{crit} on average by nearly 20%. However, Swanson et al. (2002) reported for infected fish, infection levels were variable (range 14.3-47.8 μg bacteria g^{-1}) and not significantly related to U_{crit}.

Pregnancy

Plaut (2002) reported that both body mass and cross-section area at the widest part along the body of *Gambusia affinis* increased continuously during pregnancy, resulting in a total increase of 55% and 52% respectively. Both these increases were accompanied by a decrease in U_{crit} to about 80% of that of non-pregnant females.

Plaut (2002) theorised that, if increased body cross-section area was the reason for decreased U_{crit}, one would expect the pregnant fish to increase its tail beat frequency and /or amplitude at any given swimming speed to overcome the extra drag compared with non-pregnant fish. As neither tail beat frequency nor tail beat amplitude differed between pregnant and non-pregnant fish at any given speed, Plaut (2002) hypothesized that "the reduction in U_{crit} is mainly because part of the oxygen absorbed in the gills is used by the embryos in pregnant females, rather than to power swimming activity". However, Plaut (2002) concluded that this should be a subject for further study.

Photoperiod

Kolok (1991) undertook research to determine whether the critical swimming speed of largemouth bass was influenced by prolonged exposure to seasonally inconsistent photoperiods. He suggested that exposure to different photoperiods alters the U_{crit} of juvenile largemouth bass but that this effect was not constant across water temperatures. In cold water (5^{o}C or 10^{o}C) performance was affected by photoperiod, but the effect depended upon the season in which the experiment was conducted (i.e., fish held under long photoperiods in the early winter had significantly reduced performance relative to field-acclimatized fish, whereas in the early summer, fish held under short photoperiods had reduced performances). In warm water (15 ^{o}C, 17 ^{o}C, or 19^{o}C), performance seemed to be insensitive to change in photoperiod and he concluded that "further research with centrarchids in water approaching

10°C should mention the season in which the experiment was conducted and should use a photoperiod consistent with the natural one".

Thesis Objectives

Successful colonisation of suitable adult habitats by migratory juveniles of New Zealand's diadromous fishes requires sustained swimming over many kilometres in lowland rivers and streams. Furthermore, fishes resident in such habitats must maintain station by constant swimming to counteract the flow. These lowland environments are often impacted on by elevated temperatures, high turbidity, low dissolved oxygen, and chemical and nutrient pollution.

Some aspects of the swimming abilities of some of New Zealand's migratory species have been investigated (Mitchell, 1989). However, these studies were invariably limited to short-term trials of burst swimming designed to assess the ability of species to pass limited migratory barriers, such as culverts or fish passes, or studied the avoidance responses of fish to factors such as elevated turbidity (Boubée et al., 1997; Rowe et al., 2000; Richardson et al., 2001). Although this research gives us some insights into the preferences of fish and whether they might avoid river tributaries that display elevated turbidity or temperatures, it tells us nothing about the chronic effects of reduced water quality in lowland rivers and streams and the potential impact of poor water quality on fish migration.

The aim of this thesis was to test the compounding effects of temperature and hypoxia on the prolonged swimming abilities of different life stages of *Galaxias maculatus* (inanga whitebait larvae, postlarval inanga and adults) and *Oncorhynchus mykiss* (rainbow trout parr and smolt) over a wide thermal range (5°C –25°C). The design of these experiments required the establishment of optimum aerobic temperature and associated U_{crit} performance curves for these fish species, measured in normoxic clean water. The establishment of these

U_{crit} performance curves providing a reference for evaluating the compounding effects of temperature and hypoxia on prolonged swimming.

Changes in haematocrit (Hct) and haemoglobin (Hb) have been identified as an efficient stress response mechanism, to compensate for a reduction in oxygen availability (Bernier et al., 1996). Thus, experiment design was also directed towards investigating these haematological responses as well as cortisol, glucose and lactate. In addition to responses to exercise, the effect of acclimation was also studied in trout parr to determine whether temperature acclimation affected prolonged swimming ability.

A further aim of this thesis was to identify the most significant exercise-limiting factor, oxygen uptake or oxygen delivery, because both physiological and environmental factors limit the aerobic performance of fish. To test this theory a study was designed to separately and in combination investigate the effects of mild hypoxia and severe anaemia on juvenile trout in prolonged swimming trials at 10^{o}C, 15^{o}C and 20^{o}C.

References

Allen, D. G.; Westerblad, H**.** 2001: Role of phosphate and calcium stores in muscle fatigue. *J. Physiol. 536.3*: 657-665.

Alsop, D. H.; Wood, C. M**.** 1997: The interactive effects of feeding and exercise on oxygen consumption, swimming performance and protein usage in juvenile rainbow trout (*Oncorhynchus mykiss*). *J. Exp. Biol. 200*: 2337-2346.

Bainbridge, R**.** 1958: The speed of swimming of fish as related to size and to the frequency and amplitude of the tail beat. *J. Exp. Biol. 35*: 109-133.

Bainbridge, R**.** 1960: Speed and stamina in three fish. *J. Exp. Biol. 37*: 129-153.

Bainbridge, R**.** 1962: Training, speed and stamina in trout. *J. Exp. Biol. 39*: 537-555.

Barron, M. G.; Tarr, B. D.; Hayton, W. L**.** 1987: Temperature-dependence of cardiac output and regional blood flow in rainbow trout, *Salmo gairdneri* Richardson. *J. Fish Biol. 31*: 735-744.

Beamish, F. W. H**.** 1966: Swimming endurance of some northwest Atlantic fishes. *J. Fish. Res. Bd. Can. 23*: 341-347.

Beamish, F. W. H**.** 1978: Swimming capacity. In: Hoar, W. H.; Randall, D. J. ed. Fish Physiology Vol. VII. New York, Academic Press. Pp 101-187.

Beamish, F. W. H.; Howlett, J. C.; Medland, T. E. 1989: Impact of diet on metabolism and swimming performance in juvenile lake trout, *Salvelinus namaycush*. *Can. J. Fish. Aquat. Sci. 46***:** 384-388.

Beaumont, M. W.; Butler, P. J.; Taylor, E. W. 1995a: Plasma ammonia concentration in brown trout in soft acidic water and its relationship to decreased swimming performance. *J. Exp. Biol. 198*: 2213-2220.

Beaumont, M. W.; Butler, P. J.; Taylor, E. W**.** 1995b: Exposure of brow trout, *Salmo trutta*, to sub-lethal copper concentrations in soft acidic water and its effects upon sustained swimming performance. *Aquat. Toxicol*. *33*: 45-63.

Beddow, T. A.; McKinley, R. S**.** 1999: Importance of electrode positioning in biotelemetry studies estimating muscle activity in fish. *J. Fish Biol. 54*: 819-831.

Bernier, N.; Fuentes, J.; Randall, D. 1996: Adenosine receptor blockade and hypoxia-tolerance in rainbow trout and Pacific hagfish. II. Effects on plasma catecholamines and erythrocytes. *J. Exp. Biol. 199*: 497-507.

Besner, M.; Smith, L. S**.** 1983: Modification of swimming mode and stamina in two stocks of coho salmon (*Oncorhynchus kisutch*) by differing levels of long-term continuous exercise. *Can. J. Fish. Aquat. Sci. 40*: 933-939.

Boubée, J. A. T.; Dean, T. L.; West, D. W.; Barrier, R. F. G**.** 1997: Avoidance of suspended sediment by the juvenile migrtory stage of six New Zealand native fish species. *N. Z. J. Mar. Freshwat. Res. 31*: 61-69.

Brauner, C. J.; Val, A. L.; Randall, D. J**.** 1993: The effect of graded methaemoglobin levels on the swimming performance of chinook salmon (*Oncorhynchus tshawytscha*). *J. Exp. Biol. 185*: 121-135.

Brauner, C. J.; Iwama, G. K.; Randall, D. J. 1994: The effects of short-duration seawater exposure on the swimming performance of wild and hatchery-reared juvenile coho salmon (*Oncorhynchus kisutch*) during smoltification. *Can. J. Fish. Aquat. Sci. 51*: 2188-2194.

Brett, J. R. 1964: The respiratory metabolism and swimming performance of young sockeye salmon. *J. Fish. Res. Bd. Can. 21*: 1183-1226.

Brett, J. R. 1965: The relation of size to rate of oxygen consumption and sustained swimming speed of sockeye salmon (*Oncorhynchus nerka*). *J. Fish. Res. Bd. Can. 22*: 1491-1501.

Brett, J. R**.** 1967: Swimming performance of sockeye salmon (*Oncorhynchus nerka*) in relation to fatigue time and temperature. *J. Fish. Res. Bd. Can. 24*: 1731-1741.

Brett, J. R**.** 1971: Energetic responses of salmon to temperature. A study of some thermal relations in the physiology and freshwater ecology of sockeye salmon (*Oncorhynchus nerka*). *Am. Zool. 11*: 99-113.

Brett, J. R.; Hollands, M.; Alderdice, D. F. 1958: The effect of temperature on the cruising speed of young sockeye and coho salmon. *J. Fish. Res. Bd. Can. 15*: 587-605.

Brett, J. R.; Glass, N. R. 1973: Metabolic rates and critical swimming speeds of sockeye salmon (*Oncorhynchus nerka*) in relation to size and temperature. *J. Fish. Res. Bd. Can. 30*: 379-387.

Butler, P. J.; Day, N. 1993: The relationship between intracellular pH and swimming performance of brown trout exposed to neutral and sublethal pH. *J. Exp. Biol. 176*: 271-284.

Coughlin, D. J.; Rome. L. C. 1996: The roles of pink and red muscle in powering steady swimming in scup, *Stenotomus Chrysops*. *Am. Zool. 36*: 666-677.

Dahlberg, M. L.; Shumway, D. L.; Doudoroff, P. 1968: Influence of dissolved oxygen and carbon dioxide on swimming performance of largemouth bass and coho salmon. *J. Fish. Res. Bd. Can. 25*: 49-70.

Davie, P. S.; Wells, R. M. G.; Tetens, V. 1986: Effects of sustained swimming on rainbow trout muscle structure, blood oxygen transport, and lactate dehydrogenase isoxymes: Evidence for increased aerobic capacity of white muscle. *J. Exp. Zool. 237*: 159-171.

Davis, G. E.; Foster, J.; Warren, C. E.; Doudoroff, P. 1963: The influence of oxygen concentration on the swimming performance of juvenile Pacific salmon at various temperatures. *Trans. Am. Fish. Soc. 92*: 111-124.

Davison, W. 1989: Training and its effects on teleost fish. *Comp. Biochem. Physiol. 94A*: 1-10.

Driedzic, W. R.; Kiceniuk, J. W. 1976: Blood lactate levels in free-swimming rainbow trout (*Salmo gairdneri*) before and after strenuous exercise resulting in fatigue. *J. Fish. Res. Bd. Can. 33*: 173-176.

Eddy, F. B.; Kunzlik, P. A.; Bath, R. N. 1983: Uptake and loss of nitrite from the blood of raibow trout, *Salmo gairdneri* Richardson, and Atlantic salmon, *Salmo salar* L. in fresh water and in dilute sea water. *J. Fish Biol. 23*: 105-116.

Exley, C.; Chappel, J. S.; Birchall, J. D. 1991: A mechanism for acute aluminium toxicity in fish. *J. Theor. Biol. 151*: 417-428.

Farlinger, S.; Beamish, F. W. H. 1977: Effects of time and velocity increments on the critical swimming speed of largemouth bass (*Micropterus salmoides*). *Trans. Am. Fish. Soc. 106*: 436-439.

Farlinger, S.; Beamish, F. W. H. 1978: Changes in blood chemistry and critical swimming speed of largemouth bass, *Micropterus salmoides*, with physical conditioning. *Trans. Am. Fish. Soc. 107*: 523-527.

Farrell, A. P. 1997: Effects of temperature on cardiovascular performance. In: Wood, C. M.; McDonald, D. G. ed. Global Warming Implications for Freshwater and Marine Fish. Cambridge, Cambridge University Press. Pp 135-158.

Farrell, A .P.; Steffensen, J. F. 1987: An analysis of the energetic costs of the branchial and cardiac pumps during sustained swimming in trout. *Fish Physiol. Biochem 4*: 73-79.

Farrell, A. P.; Johansen, J. A.; Steffensen, J. F.; Moyes, C. D.; West, T. G.; Suarez, R. K. 1990: Effects of exercise training and coronary ablation on swimming performance, heart size, and cardiac enzymes in rainbow trout, *Oncorhynchus mykiss*. *Can. J. Zool. 68*: 1174-1179.

Farrell, A. P.; Johansen, J. A.; Suarez, R. K. 1991: Effects of exercise-training on cardiac performance and muscle enzymes in rainbow trout, *Oncorhynchus mykiss*. *Fish Physiol. Biochem*. *9*: 303-312.

Gallaugher, P.; Axelsson, M.; Farrell, A. P. 1992: Swimming performance and haematological variables in splenectomized rainbow trout, *Oncorhynchus mykiss. J. Exp. Biol. 171*: 301-314.

Gallaugher, P.; Thorarensen, H.; Farrell, A. P. 1995: Hematocrit in oxygen transport and swimming in rainbow trout (*Oncorhynchus mykiss*). *Respir. Physiol. 102*: 279-292.

Glova, G. J.; McInerney, J. E. 1977: Critical swimming speeds of coho salmon (*Oncorhynchus kisutch*) fry to smolt stages in relation to salinity and temperature. *J. Fish. Res. Bd. Can. 34*: 151-154.

Graham, J. M. 1949: Some effects of temperature and oxygen pressure on the metabolism and activity of the speckled trout, *Salvelinus fontinalis*. *Can. J. Res. D27*: 270-288.

Graham, M. S.; Wood, C. M. 1981: Toxicity of environmental acid to the rainbow trout: interactions of water hardness, acid type, and exercise. *Can. J. Zool. 59*: 1518-1526.

Griffiths, J. S.; Alderdice, D. F. 1972: Effects of acclimation and acute temperature experience on the swimming speed of juvenile coho salmon. *J. Fish. Res. Bd. Can. 29*: 251-264.

Grimnes, A.; Jakobsen, P. J. 1996: The physiological effects of salmon lice infection on post-smolt of Atlantic salmon. *J. Fish Biol*. *48*: 1179-1194.

Hammer, C. 1995: Fatigue and exercise tests with fish. *Comp. Biochem. Physiol. 112A*: 1-20.

Hochachka, P. W.; Somero, G. N. 1984: Biochemical Adaptation. Princeton, Princeton University Press. p 355.

Holk, K.; Lykkeboe, G. 1998: The impact of endurance training on arterial plasma K^+ levels and swimming performance in rainbow trout. *J. Exp. Biol*. *201*: 1373-1380.

Houlihan, D. F.; Laurent, P. 1987: Effects of exercise training on the performance, growth, and protein turnover of rainbow trout (*Salmo gairdneri*). *Can. J. Fish. Aquat. Sci. 44*: 1614-1621.

Jobling, M. 1995: Environmental Biology of Fishes. London, Chapman and Hall. p 455.

Johnston, I. A.; Moon, T. W. 1980: Exercise training in skeletal muscle of brook trout (*Salvelinus fontinalis*). *J. Exp. Biol. 87*: 177-194.

Jones, D. R. 1971: The effect of hypoxia and anaemia on the swimming performance of rainbow trout (*Salmo gairdneri*). *J. Exp. Biol. 55*: 541-551.

Jones, D. R. 1982: Anaerobic exercise in teleost fish. *Can. J. Zool. 60*: 1131-1134.

Kicenuik, J. W.; Jones, D. R. 1977: The oxygen transport system in trout (*Salmo gairdneri*) during sustained exercise. *J. Exp. Biol. 69*: 247-260.

Kieffer, J. D.; Currie, S.; Tufts, B. L. 1994: Effects of environmental temperature on the metabolic and acid-base responses of rainbow trout to exhaustive exercise. *J. Exp. Biol. 194*: 299-317.

Kolok, A. S**.** 1991: Photoperiod alters the critical swimming speed of juvenile largemouth bass, *Micropterus salmoides*, acclimated to cold water. *Copeia 4*: 1085-1090.

Kolok, A. S.; Sharkey, D**.** 1997: Effect of freshwater acclimation on the swimming performance and plasma osmolarity of the euryhaline gulf killifish. *Trans. Am. Fish. Soc. 126*: 866-870.

Koumoundouros, G.; Sfakianakis, D. G.; Divanach, P.; Kentouri, M**.** 2002: Effect of temperature on swimming performance of sea bass juveniles. *J. Fish Biol. 60*: 923-932.

Lauren, D. J.; McDonald, D. G. 1985: Effects of copper on branchial ionoregulation in the rainbow trout, *Salmo gairdneri* Richardson. *J. Comp. Physiol. 155B*: 635-644.

Leland, H.V.; Kuwabara, J.S. 1985: Trace metals. In: Rand, G. M.; Petrocelli, S. R. ed. Fundamentals of Aquatic Toxicology: Methods and Applications. New York, Hemisphere Publishing Corporation, New York, NY. Pp 374-415.

Lescenko, P.; Matlova, L.; Dvorska, L.; Bartos, M.; Vavra, O.; Navratil, L.; Pavlik, I. 2003: Mycobacterial infection in aquarium fish. *Vet. Med.-Czech, 48*: 71-78.

MacLeod, J. C**.** 1967: A new apparatus for measuring maximum swimming speeds of small fish. *J. Fish. Res. Bd. Can. 24*: 1241-1252.

Martínez, M.; Bédard, M.; Dutil J.-D.; Guderley, H. 2004: Does condition of Atlantic cod (*Gradus morhua*) have a greater impact upon swimming performance at U_{crit} or sprint speeds? *J. Exp. Biol. 207*: 2979-29990.

Maxime, V.; Pennec, J.-P.; Peyraud, C. 1991: Effects of direct transfer from freshwater to seawater on respiratory and circulatory variables and acid-base status in rainbow trout. *J. Comp. Physiol. 161B*: 557-568.

McKenzie, D. J.; Higgs, D. A.; Dosanjh, B. S.; Deacon, G.; Randall, D. J. 1998: Dietary fatty acid composition influences swimming performance in Atlantic salmon (*Salmo salar*) in sea water. *Fish Physiol. Biochem. 19*: 111-122.

Milligan, C. L**.** 1996: Metabolic recovery from exhaustive exercise in rainbow trout. *Comp. Biochem. Physiol. 113A*: 51-60.

Milligan, C. L.; Wood, C. M**.** 1986a: Intracellular and extracellular acid-base status and H^+ exchange with the environment after exhaustive exercise in the rainbow trout: *J. Exp. Biol*. *123*: 93-121.

Milligan, C. L.; Wood, C. M**.** 1986b: Tissue intracellular acid-base status and the fate of lactate after exhaustive exercise in the rainbow trout. *J. Exp. Biol. 123*: 123-144.

Mitchell, C. P**.** 1989: Swimming performance of some native freshwater fishes. *N. Z. J. Mar. Freshwat. Res. 23*: 181-187.

Moyes, C. D.; West, T. G**.** 1995: Exercise metabolism of fish. In: Hochachka, P. W.; Mommsen, T. P. ed. Biochemistry and Molecular Biology of Fishes, Vol.4. Amsterdam, Elsevier. Pp 367-392.

Myrick, C. A.; Cech, J. J. Jr**.** 2000: Swimming performances of four California stream fishes: temperature effects. *Env. Biol. Fish. 58*: 289-295.

Nahhas, R.; Jones. N. V.; Goldspink, G. 1982: Some aspects of sustained training of rainbow trout, *Salmo gairdneri* Richardson. *J. Fish Biol. 20*: 351-358.

Pearson, M. P.; Stevens, E. D**.** 1991: Splenectomy impairs aerobic swim performance in trout. *Can. J. Zool. 69*: 2089-2092.

Plaut, I. 2000: Resting metabolic rate, critical swimming speed, and routine activity of the euryhaline cyprinodontid, *Aphanius dispar*, acclimated to a wide range of salinities. *Physiol. Biochem. Zool. 73*: 590-596.

Plaut, I. 2001: Critical swimming speed: its ecological relevance. *Comp. Biochem. Physiol. 131A*: 41-50.

Plaut, I**.** 2002: Does pregnancy affect swimming performance of female Mosquitofish, *Gambusia affinis*?. *Funct. Ecol. 16*: 290-296.

Poléo, A. B. S**.** 1995: Aluminium polymerization – a mechanism of acute toxicity of aqueous aluminium to fish. *Aquat. Toxicol. 31*: 347-356.

Reidy, S. P.; Kerr, S. R.; Nelson, J. A. 2000: Aerobic and anaerobic swimming performance of individual Atlantic cod. *J. Exp. Biol. 203*: 347-357.

Richardson, J.; Rowe, D. K.; Smith, J. P. 2001: Effects of turbidity on the migration of juvenile banded kokopu (*Galaxias faciatus*) in a natural stream. *N. Z. J. Mar. Freshwat. Res., 35*: 191-196.

Rowe, D.; Hicks, M.; Richardson J. 2000: Reduced abundance of banded kokopu (*Galaxias fasciatus*) and other native fish in turbid rivers of the North Island of New Zealand. *N. Z. J. Mar. Freshwat. Res. 34*: 547-558.

Shingles, A.; McKenzie, D. J.; Taylor, E. W.; Moretti, A.; Butler, P. J.; Ceradini, S. 2001: Effects of sublethal ammonia exposure on swimming performance in rainbow trout (*Oncorhynchus mykiss*). *J. Exp. Biol. 204*: 2691-2698.

Soofiani, N. M.; Priede, I. G. 1985: Aerobic metabolic scope and swimming performance in juvenile cod, *Gadus morhua* L. *J. Fish Biol*. *26*. 127-138.

Swanson, C.; Baxa, D. V.; Young, P. S.; Cech, J. J. Jr.; Hedrick, R. P. 2002: Reduced swimming performance in delta smelt infected with *Mycobacterium* spp. *J. Fish Biol*. *61*: 1012-1020.

Taylor, E. W.; Egginton, S.; Taylor, S. E.; Butler, P. J. 1997: Factors which may limit performance at different temperatures. In: Wood, M. C.; McDonald, D. G. ed. Global Warming Implications for Freshwater and Marine Fish. Cambridge, Cambridge University Press. Pp. 105-133.

Taylor, L. N.; McFarlane, W. J.; Pyle, G. G.; Couture, P.; McDonald, D. G. 2004: Use of performance indicators in evaluating chronic metal exposure in wild yellow perch (*Perca flavescens*). *Aquat. Toxicol. 67*: 371-385.

Thorarensen, H.; Gallaugher , P. E.; Kiessling, A. K.; Farrell, A. P. 1993: Intestinal blood flow in swimming chinook salmon *Oncorhynchus tshawytscha* and the effects of haematocrit on blood flow distribution. *J. Exp. Biol. 179*: 115-129.

Wagner, G. N.; McKinley, R. S..; Bjørn, P. A.; Finstad, B. 2003: Physiological impact of sea lice on swimming performance of Atlantic salmon. *J. Fish Biol. 62*: 1000-1009.

Waiwood, K. G.; Beamish, F. W. H. 1978: Effects of copper, pH and hardness on the critical swimming performance of rainbow trout (*Salmo gairdneri* Richardson). W*ater Res. 12*: 611-619.

Webb, P. W. 1971: The swimming energetics of trout. II. Oxygen consumption and swimming efficiency. *J. Exp. Biol. 55*: 521-540.

Webb, P. W.1975: Hydrodynamics and energetics of fish propulsion. *Bull. Fish. Res. Bd. Can. 190*: p 159.

Webb, P. W. 1994: The biology of fish swimming. In: Maddock, L.; Bone, Q.; Rayner, J. M. V. ed. Mechanics and Physiology of Animal Swimming . Cambridge, Cambridge University Press. Pp. 45-62.

Wells, R. M. G.; Weber, R. E. 1990: The spleen in hypoxic and exercised rainbow trout. *J. Exp. Biol. 150*: 461-466.

Wells, R. M. G.; Weber, R. E. 1991: Is there an optimal haematocrit for rainbow trout, *Oncorhynchus mykiss* (Walbaum)? An interpretation of recent data based on blood viscosity measurements. *J. Fish Biol. 38*: 53-65.

Westerblad, H.; Allen, D. G.; Lännergren, J. 2002: Muscle fatigue: lactic acid or inorganic phosphate the major cause? *News Physiol. Sci. 17*: 17-21.

Wilson, R. W.; Taylor, E. W. 1993: The physiological responses of freshwater rainbow trout, *Oncorhynchus mykiss*, during acutely lethal copper exposure. *J. Comp. Physiol. 163B*: 38-47.

Wilson, R. W.; Bergman, H. L.; Wood, C. M. 1994: Metabolic costs and physiological consequences of acclimation to aluminium in juvenile rainbow trout (*Oncorhynchus mykiss*). 2: Gill morphology, swimming performance, and aerobic scope. *Can. J. Fish. Aquat. Sc. 51*: 536-544.

Wood, C. M. 1991: Acid-base and ion balance, metabolism, and their interactions, after exhaustive exercise in fish. *J. Exp. Biol. 160*: 285-308.

Woodward, J. J.; Smith, L. S. 1985: Exercise training and the stress response in rainbow trout, *Salmo gairdneri* Richardson. *J. Fish Biol. 26*: 435-447.

Yamamoto, K.; Itazawa, Y.; Kobayashi, H. 1980: Supply of erthrocytes into the circulating blood from the spleen of exercised fish. *Comp. Biochem. Physiol. 65A*: 5-11.

Ye, X.; Randall. D. J. 1991: The effect of water pH on the swimming performance in rainbow trout (*Salmo gairdneri*, Richardson). *Fish Physiol. Biochem. 9:* 15-21.

Young, P. S.; Cech, J. J**.** Jr. 1993: Improved growth, swimming performance, and muscular development in exercise-conditioned young -of-the-year striped bass (*Morone saxatilis*). *Can. J. Fish. Aquat. Sci. 50*: 703-707.

Young, P. S.; Cech, J. J**.** Jr. 1994: Optimum exercise conditioning velocity for growth, muscular development, and swimming performance in young-of-the-year striped bass (*Morone saxatilis*). *Can. J. Fish. Aquat. Sci. 51*: 1519-1527.

Ytrestøyl, T.; Finstad, B.; McKinley, R. S**.** 2001: Swimming performance and blood chemistry in Atlantic salmon spawners exposed to acid river water with elevated aluminium concentrations. *J. Fish Biol. 58*: 1025-1038.

CHAPTER TWO

Methodological development and ancillary studies

Introduction

An initial U_{crit} experiment was designed to determine if the maximum turbidity level (100 NTU) recorded in the lower Waikato River during 2000 would limit the aerobic swimming ability of rainbow trout fry (*Oncorhynchus mykiss*) and sub-adult rainbow trout (24 ± 0.5 cm fork length, FL and 5 cm ± 0.2 FL respectively). The experiment was conducted in a rectangular acrylic channel that was 7.23 m long, 0.5 m wide, and 0.5 m deep. A 0.4 m diameter return pipe ran beneath the flume (Figure 2.1). An impeller in the descending arm of the return pipe regulated flow speed via a variable-speed AC motor, which produced velocities up to 0.65 m s^{-1}.

.

Figure 2.1. The 7.23 m long, 0.5 m wide, and 0.5 m deep acrylic flume.

Trout proved to be difficult to observe in the rectangular channel at 100 NTU, so to improve viewing, a multi-channel insert was designed and built (Figure 2.2). The insert comprised three channels, each measuring 0.16 x 0.16 m, and a wing extending upwards at 45 degrees that directed and forced the flow into the three channels, increasing water velocity from 0.65 m s^{-1} to ~ 1 m s^{-1}. Grills were installed to straighten the flow and contain the fish in each experimental area that measured 0.16 m x 0.16 m x 0.5 m.

Containment restricted burst swimming, and associated anaerobiosis, which will distort U_{crit} values. The insert ceiling removed the free surface effects, which can produce turbulence behind the grills (standing wave) that fish can utilise to reduce swimming effort.

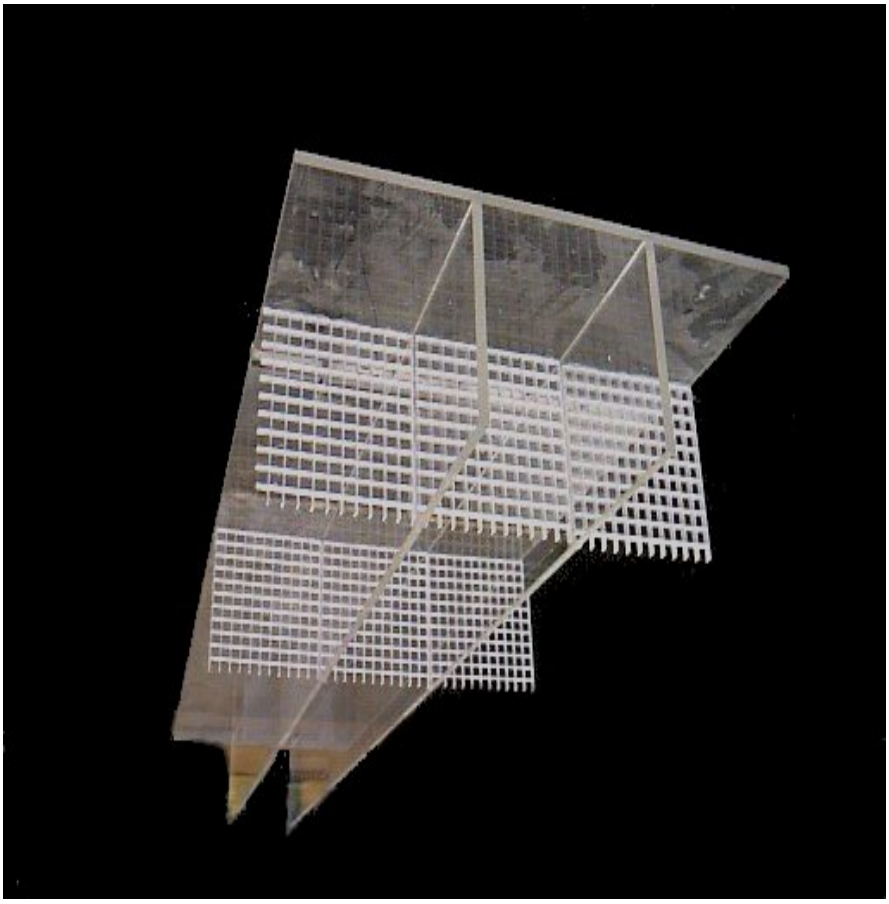

Figure 2.2. Multi-channel insert. White grills straightened the flow and contained the fish in the experimental area.

The flow profile of the insert channel (Figure 2.3) was determined by increasing the speed of the AC flume motor in increments of 5 Hertz and at each increment measuring channel velocity at a number of depths using an acoustic Doppler velocity probe (Sontek ADV, ProbeS/NA104). Flow profile results of the insert channels show that, at the lower velocities, the flow profiles were near linear throughout the water column, but at the higher velocity range they distorted close to the channel sides. This distortion has little or no effect on U_{crit} values, since small fish that would occupy the

distorted area, become fatigued before such velocities are reached, and larger fish are excluded from these boundary layers by their size.

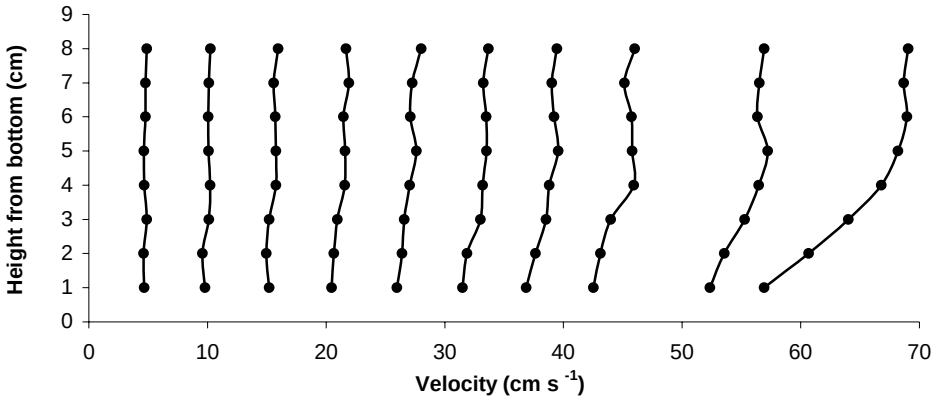

Figure 2.3. Flow profile of the insert channel obtained using an acoustic Doppler flow probe.

Preliminary experiment: turbidity

Introduction

A small preliminary experiment was designed to determine the capability of the 7.23 x 0.5 x 0.5 m flume with insert to assess U_{crit} values. The design of the experiment was to determine if turbidity levels, recorded in the Waikato River, would limit aerobic swimming ability of trout fry and sub-adults. The effects were determined by comparison of clear vs turbid water U_{crit} values.

In the Waikato River, mean and maximum turbidity levels, recorded at permanent monitoring sites by Environment Waikato during 2000, showed NTU values at upland river sites were low, and showed small variation between mean and maximum readings; differences ranging from 0.2 to 0.8 NTU. However at the lowland sites, variation between mean and maximum NTU levels ranged between 80 to 100 NTU.

Materials and methods

Experimental animals

Rainbow trout fry *Oncorhynchus mykiss* (Walbaum), (5 cm ± 0.2 FL) and sub-adults (24 cm ± 0.5 FL), were obtained from the Ngongataha Trout Hatchery, Rotorua, New Zealand. Both age groups were held indoors in aerated fibreglass aquaria, having a continuous supply of circulating dechlorinated Hamilton tap water. Temperature was maintained at 17^{o}C and fish were fed daily with commercial trout pellets to satiation. A photoperiod of 12:12 L:D was maintained.

Flume turbidity levels and water temperature

To undertake turbidity testing of trout, Waipa River silt was collected (Lat. 37^{o} 41$^{/}$ 04$^{//}$ S; Long. 175^{o} 09$^{/}$ 01$^{//}$ E) and, in the laboratory, the silt was mixed with water to a stock concentration of 784 NTU. Concentrate was then added to the flume to achieve 100 NTU. Water temperature of the flume was maintained at 17^{o}C for the duration of the experiment.

Experimental protocol and U_{crit} measurements

Thirty minutes after feeding, three fish were randomly selected, length estimated and then placed in the flume, one per experimental channel. The fish were acclimated in the test chamber (17^{o}C) for 2 hours and velocity was maintained at 0.5 *BL* s^{-1} (*BL* is fork body length), to aid recovery from handling and transfer stress. The two hour exposure of fish to a water velocity of 0.5 *BL* s^{-1} was based on the findings of Milligan et al. (2000) who reported that sustained swimming at 0.9 *BL* s^{-1} for two hours, following a bout of exhaustive exercise, enhanced recovery of metabolic and acid-base status in rainbow trout compared with fish held in still water.

Following this recovery period, U_{crit} for individual fish was determined by increasing velocity in 0.5 *BL* s^{-1} increments every 15 minutes, until the fish became exhausted. Exhaustion was determined when fish were forced onto and, with light prodding, remained on the rear grill for 20 s. Immediately following exhaustion, all fish were weighed to ± 0.01 g, and fork length measured to ± 0.1 cm. Swimming velocities in cm s^{-1} were

converted to body lengths per second (*BL* s^{-1}), for in all studies of fish swimming ability it is essential that velocity is recorded relative to fish length, and that results are standardised to appropriate size classes.

U_{crit} was determined using the equation of Brett (1964)

$U_{crit} = U_i + U_{ii}\ T_i\ /\ T_{ii}$

where U_i is the highest velocity maintained for a complete time interval, U_{ii} is the velocity increment (0.5 *BL* s^{-1}), T_i is the interval time elapsed at fatigue velocity, and T_{ii} is the interval time (15 min).

A velocity increment of 0.5 *BL* s^{-1} was chosen as a speed that was sufficiently large enough to complete the experiment in a reasonable time, but not large enough to affect U_{crit} values. Consequently, velocity increments must be of a magnitude that the initial increments exclude spontaneous movements, which are defined by Weatherley et al. (1982) as being dominated by turns, fin movements, sudden velocity changes, breaks, etc, and are possibly fuelled by anaerobic processes (Webb, 1971a). Moreover, velocity increments must not be of a magnitude that extends the period of restlessness that Webb (1971a,b, 1975) reports fish display after the current speed is increased, due to the possible recruitment of anaerobic processes, which could distort U_{crit} values. Swimming velocities were not corrected for the solid blocking effect of the fish because the cross-sectional area of the fish was not greater than 10% of the cross-sectional area of the flume (Brett, 1964).

An interval time of 15 minutes was selected as a minimum time to provide consistent determinations of critical swimming speed. Peterson (1974) reported that intervals less than 15 minutes resulted in increased critical swimming speeds, whereas intervals longer than 15 minutes resulted in a reasonably constant critical velocity.

Statistics

Data were analysed by Student's *t*-test using Statistica version 6.

Results

Trout fry showed no significant difference in U_{crit} values between treatments (Table 2.1). However, results for sub–adults were inconclusive due to velocity constraints, but preliminary results indicate turbidity has little if any effect on this age group with U_{crit} for both treatments being > 4.5 *BL* s^{-1}.

Table 2.1. Effects of turbidity on U_{crit} of two age groups of trout. Values are means with S.E.M. in parentheses.

	Treatment	***Number***	***Length (cm)***	***Turbidity (NTU)***	**U_{crit} (BL s^{-1})**
Trout fry	***Clear***	***7***	***5.1 (±1.1)***	***~0***	***7.09 (±0.25)***
	Turbid	***6***	***5.5 (±0.2)***	***92***	***7.06 (±0.47)***
Trout sub-adults	***Clear***	***4***	***24.0 (±0.3)***	***~0***	***>4.5***
	Turbid	***4***	***24.8 (±1.4)***	***115***	***>4.5***

Flume design

Velocity constraints of the 7.23 x 0.5 x 0.5 m flume with insert resulted in the design, construction, and calibration of an additional flume. Design and construction resulted in an enclosed variable velocity recirculating flume, 2 m in length and having a 230 litre capacity (Figure 2.4). Water was circulated by means of a propeller positioned in the descending arm of the return pipe, and four flow straighteners maintained a steady even flow and eliminated the vortices created by the propeller. The experimental area comprised a single channel measuring 0.16 m x 0.16 m x 0.5 m. Two of the four flow straighteners not only straightened the flow, but also contained the fish in the experimental area. Containment restricted burst swimming and associated anaerobiosis which can distort U_{crit} values.

Figure 2.4. 2 metre, 230 litre recirculating flume.

A flume of this size, 230 litres, allowed for precise temperature and oxygen control. Water temperature was maintained by circulating flume water through two flow-through water baths (Julabo, 20B). Hypoxic conditions were achieved by design and construction of a dissolved oxygen reduction system (bubble tower; Figure 2.5), which was installed into the water bath reticulation system.

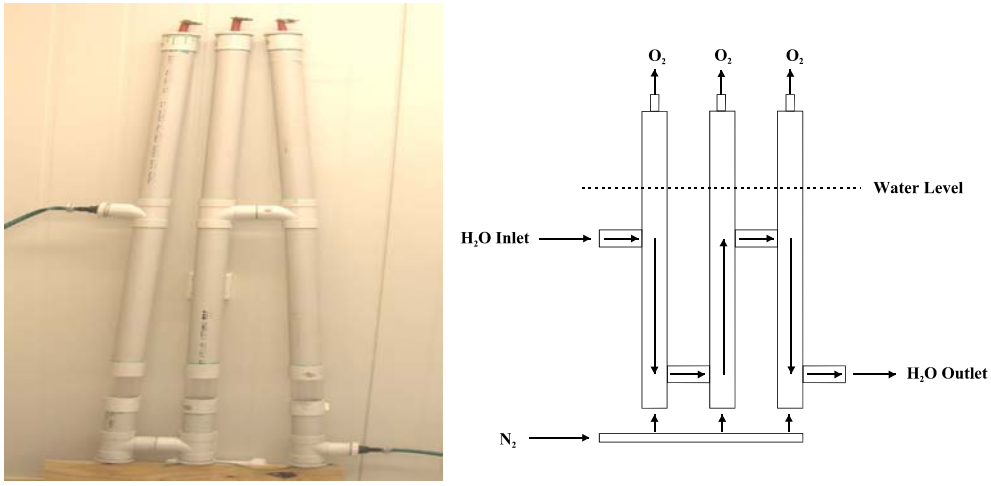

Figure 2.5. Oxygen reduction system and diagram showing oxygen stripping process.

Hypoxia (75% sat.) was produced by nitrogen-stripping of oxygen from water in the bubble tower. Oxygen levels and temperature were measured at each speed during the U_{crit} trials using oxygen (YSI) and temperature (thermo-couple) probes installed in the roof of the flume, both being connected to a chart recorder. The rear grill of the containment area was electrified, via a signal generator (Topward Electric Instrument Co. Ltd., TFG-462), to restrict fish from resting on the rear grill during U_{crit} assessment. The signal generator output was set to 8 Hertz and the initial applied voltage was 2 volts. If required, voltage could be increased to 8 volts to inhibit fish resting on the grill.

Flow calibration

The flow velocity within the experimental channel was determined by increasing the speed of the AC flume motor, in increments of 5 Hertz. At each increment, velocity within the experimental channel was measured, using a Marsh-McBirney model 2000 Flow Mate. Intermediate velocities were determined for experiments by interpolation of the flow calibration (Figure 2.6).

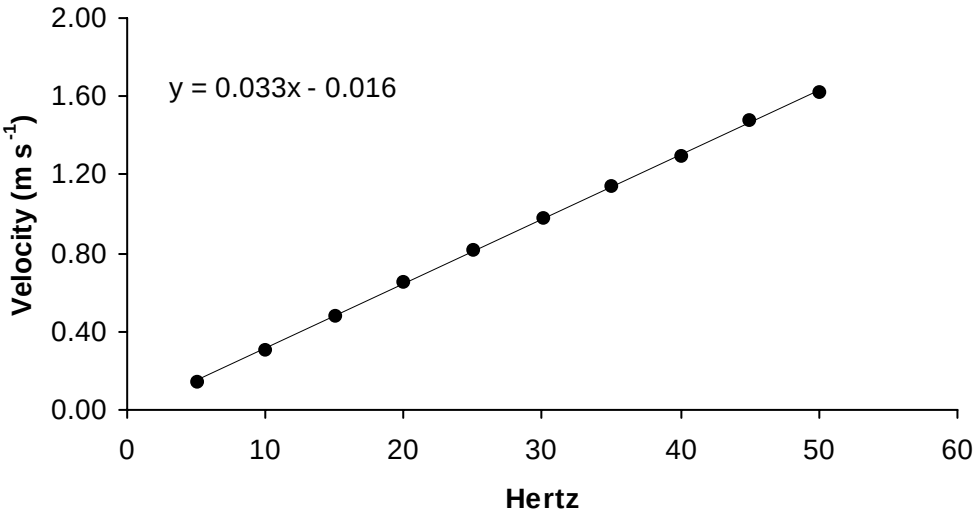

Figure 2.6. Motor speed (applied alternating current frequency in Hertz) vs flow velocity for 2 m recirculating flume.

Preliminary experiment:

Recovery from handling and stress

Introduction

Milligan et al. (2000) reported that sustained swimming at 0.9 *BL* s^{-1} for two hours, following a bout of exhaustive exercise, enhanced recovery of metabolite and acid-base status in rainbow trout. This enhanced recovery was suggested by Milligan et al. (2000) to be allied to a total absence of the elevation in cortisol concentration.

It was assumed from the findings of Milligan et al. (2000) that, acclimating fish in the test chamber for 2 hours at a velocity of 0.5 *BL* s^{-1}, may aid recovery from handling and transfer stress. Thus, this ancillary study investigated if rainbow trout exposed to a water velocity of 0.5 *BL* s^{-1} for 2 hours, results in an absence of the elevation of cortisol concentration when compared to recovering fish at rest.

Materials and methods

Experimental animals

Ngongataha Trout Hatchery-reared rainbow trout, *Oncorhynchus mykiss* (Walbaum), 14 ± 0.5 cm fork length, were randomly allocated to treatments of 10°C, 15°C and 20°C (± 0.5°C) (n = 5 fish per treatment group). Increasing or decreasing water temperature from rearing temperature (16.0°C) by 1°C per day achieved acclimation temperatures. Fish were maintained in aerated dechlorinated tap water for at least 21 days prior to U_{crit} determinations, and were fed daily with commercial trout pellets to satiation. A photoperiod of 12:12 L:D was maintained.

Experimental protocol

A fish was randomly selected from one of the treatment groups, length estimated, then placed in the flume for 2 hours, with velocity maintained at 0.5 *BL* s^{-1}.

Blood sampling

Blood samples were taken either at rest or immediately following 2 hours exposure to a velocity of 0.5 *BL* s^{-1}. All fish were stunned and bled (~200 µL sample volume) by acute caudal venepuncture using pre-heparinized syringes, weighed (± 0.01 g) and measured (± 1 mm). For blood sampling, fish were netted and held between two wet towels to facilitate rapid (30 s) sampling (Figure 2.7). Plasma cortisol was determined by radioimmunoassay at Gribbles Veterinary Pathology, Hamilton, New Zealand.

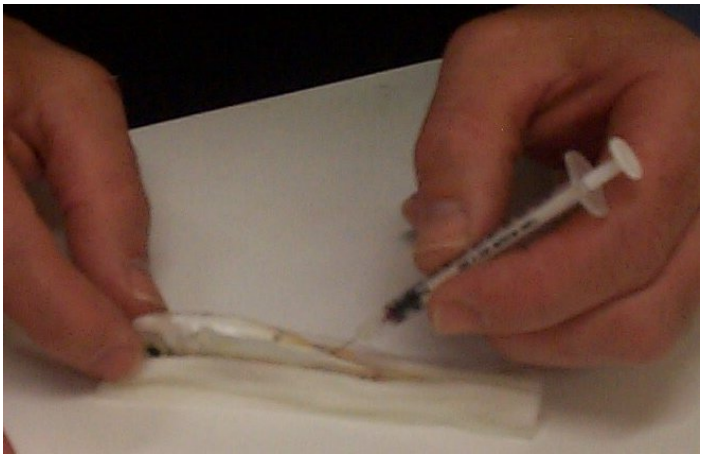

Figure 2.7. For blood sampling, fish were held between two wet towels to facilitate rapid (30 s) acute caudal venepuncture using pre-heparinized syringes.

Results

When exposed to a velocity of 0.5 *BL* s^{-1} for two hours, fish were observed swimming to maintain station for the duration of the experiment. Following 2 hours exposure to 0.5 *BL* s^{-1} cortisol concentrations were elevated at all temperatures (Table 2.2) and significantly so at 15^{o}C and 20^{o}C (P<0.05).

Table 2.2. Plasma cortisol values (nmol L^{-1}) of fish acclimated to 10 ^{o}C, 15 ^{o}C and 20^{o}C, and sampled at rest or following swimming at 0.5 *BL* s^{-1} for 2 hours at their acclimated temperatures. Values are means with S.E.M. in parentheses. N = 5 fish per group.

	Treatment	
Temperature (^{o}C)	Control Resting	0.5 *BL* s^{-1} 2 Hrs
10	40.8 (26.3)	182 (76)
15	15.0 (6.7)	510 (74) $P<0.001$
20	42.4 (12)	236 (66) $P=0.02$

Discussion

There was no apparent recovery of cortisol concentrations to baseline values in fish that had been transferred to the flume and swum at 0.5 *BL* s^{-1} for 2 hours. Landman (2001) reported that rainbow trout, exposed to a velocity of 0.5 *BL* s^{-1} for 24 hours, exhibited a cortisol concentration of 91 nmoles L^{-1}. Comparison of this value with fish at rest in this experiment, and reported resting values of trout, 24.3 nmoles L^{-1} (Landman 2001), 27.6 nmoles L^{-1} and 5.5 nmoles L^{-1} for wild and hatchery fish, respectively (Woodward & Strange 1987), show cortisol concentration had not returned to the baseline, thus supporting the recorded elevation in cortisol of this experiment. The continued elevation of cortisol following 2 hours at 0.5 *BL* s^{-1} is presumably due to both the effects of handling necessary to transfer the fish to the flume and the stress of transferring the fish to a novel environment.

The elevation in cortisol concentration, recorded in this experiment, clearly differ from the findings of Milligan et al. (2000). Consequently, the assumption that recovery from handling and stress transfer would be mitigated by exposure to a velocity of 0.5 *BL* s^{-1} for two hours was not validated.

Preliminary experiment:

Haematological responses of blood cells to captivity in common smelt

Introduction

Neilson (1996) reported that common smelt (*Retropinna retropinna*) showed a significant reduction in the number of circulating red blood cells following a 5-day captive period in still water. This was evident in decreases in haematocrit (PCV), haemoglobin concentration ([Hb]) and red blood cell count (RBCC). This response was postulated by Neilson (1996) to be mainly due to differences in activity, and thus metabolic rate, between wild and captive populations, rather than chronic stress responses to confinement.

This ancillary study investigated if captive smelt, exposed to water velocities of 0 *BL* s^{-1}, 1 *BL* s^{-1} and 2 *BL* s^{-1} for 5 days, resulted in a significant reduction in circulating red blood cells when compared to wild smelt.

Materials and methods

Experimental animals

Common smelt were netted at Wellington Street Beach, Waikato River, Hamilton, New Zealand, using a beach seine net (mesh size, 5 mm). Within 1 – 2 minutes of capture, a blood sample was taken from 10 fish by caudal venepuncture using heparinized syringes, fish were then weighed (± 0.01 g) and measured (± 1 mm). For blood sampling, fish were stunned and held between two wet towels to facilitate rapid (30 s) sampling (Figure 2.7). Following bleeding, each syringe was immediately placed onto ice and transported to the laboratory, where haematological analyses were undertaken within 2 hours of sampling.

Forty five fish were transported back to the laboratory, where they were immediately introduced to the flume and divided equally between three water velocities (0, 1 and 2 *BL* s^{-1}).

Experimental protocol

The 7.2 x 0.5 x 0.5 m flume with insert was used for this experiment. Flow of the three channels were manipulated to produce three different velocities, 0 *BL* s^{-1}, 1 *BL* s^{-1} and 2 *BL* s^{-1}. 0 *BL* s^{-1} was achieved by blocking water flow, into one of the channels, with the placement of a solid piece of acrylic across the channel entrance, 1 and 2 *BL* s^{-1} was attained by the addition of wire mesh across two of the channel entrances, restricting flow, until the required flow was reached in each channel. Into each of these three velocities were placed 15 randomly selected fish. After 5 days exposure five fish were removed and bled as described previously. A further five fish from each treatment were swum to determine U_{crit}. U_{crit} was determined using the equation of Brett (1964)

$U_{crit} = U_i + U_{ii} T_i / T_{ii}$

where U_i is the highest velocity maintained for a complete time interval, U_{ii} is the velocity increment (0.5 *BL* s^{-1}), T_i is the interval time elapsed at fatigue velocity, and T_{ii} is the interval time (15 min).

Haematological analyses

Blood was analysed for haematocrit (PCV), whole blood haemoglobin ([Hb]), mean cell haemoglobin concentration (MCHC), mean cell haemoglobin (MCH), mean cell volume (MCV) and red blood cell count (RBCC) according to standard methods (Dacie& Lewis, 1991).

Statistics

Data were analysed by one-way ANOVA using Statistica version 6.

Results

Statistical analysis of wild fish haematological parameters with fish at rest (0 *BL* s^{-1}), for 5 days, showed the latter having significant decreases in PCV, [Hb] and MCH ($P < 0.05$). However, in fish exposed to 2 *BL* s^{-1} for 5 days, only PCV was significantly decreased from that of wild fish (Table 2.3).The prolonged swimming velocity of common smelt was maintained for each of the treatment groups; 0 *BL* s^{-1}, 1 *BL* s^{-1} and 2 *BL* s^{-1}.

Table 2.3. Haematological parameters of wild common smelt (*Retropinna retropinna*) and of three groups of common smelt exposed to 0 *BL* s^{-1}, 1 *BL* s^{-1} or 2 *BL* s^{-1} water velocities for 5 days. N = 10 for wild and n =5 for all other treatment groups. Values with * are significantly different to wild fish (Student's *t*-test, $P<0.05$)

	Treatment	Length (mm)	Weight (g)	PCV (%)	[Hb} (g/L)	MCHC (g/L)	RBCC 10^{12}cells/L	MCH (pg)	MCV (fl)	U_{crit} *BL* s^{-1}
Mean	Wild	80.1	4.40	30.0	54.7	186	1.10	51.0	274	N/A
S.E.M		1.6	0.30	1.7	2.2	10	0.07	2.7	8	N/A
Mean	0.*BL* s^{-1} 5 days	77.4	2.96*	21.2*	29.4*	142	0.85	37.0*	261	6.0
S.E.M		2.2	0.15	3.2	3.2	10	0.14	6.8	41	0.2
Mean	1 *BL* s^{-1} 5 days	70.4 *	2.03*	23.3*	37.4*	170	1.07	35.2*	214	6.2
S.E.M		1.8	0.23	4.1	4.1	21	0.12	3.2	25	0.2
Mean	2 *BL* s^{-1} 5 days	70.0*	2.02*	22.9*	45.3	199	0.98	49.1	261	5.9
S.E.M		3.4	0.33	1.2	8.92	42	0.21	8.9	48	0.5

Discussion

This study has shown that captivity results in a significant decline in PCV. This reduction may be attributed to a decline in activity resulting from the absence of a current. However, causes of changes in PCV have also been attributed to sex of fish, age, and nutritional state (Larsson et al., 1976; Clark et al., 1979). Consequently, further research is required to determine

the haematological changes that fish undergo when subjected to captivity in the absence of water flow.

References

Brett, J. R. 1964: The respiratory metabolism and swimming performance of young sockeye salmon.* J. Fish. Res. Bd. Can. 21*: 1183-1226.

Clark, S.; Whitmore, D. H. Jr.; McMahon, R. F.1979: Considerarions of blood parameters of largemouth bass, *Micropterus salmoides. J. Fish Biol. 14*: 147-158.

Dacie, J. V.; Lewis, S. M. 1991: Practical Haematology. 7th edition. London, Churchill. p 557.

Landman, M. J. 2001: Physical and psychological stress in adult and juvenile rainbow trout (*Oncorhynchus mykiss*). Unpublished MSc thesis, University of Waikato , Hamilton, New Zealand.

Larsson, A.; Johansson-Sjobeck, M-L.; Fänge, R. 1976: Comparative study of some haematological and biochemical blood parameters in fishes from the Skagerrak. *J. Fish Biol. 9*: 425-440.

Milligan, C. L.; Hooke, G. B.; Johnson, C. 2000: Sustained swimming at low velocity following a bout of exhaustive exercise enhances metabolic recovery in rainbow trout. *J. Exp. Biol. 203*: 921-926.

Neilson, K. A. 1996: Haematological responses to capture and confinement in selected New Zealand native freshwater fish species. Unpublished MSc thesis, University of Waikato, Hamilton, New Zealand.

Petersen, R. H. 1974: Influence of Fenitrothion on swimming velocities of brook trout (*Salvelinus fontinalis*). *J. Fish. Res. Bd. Can. 31*: 1175-1762.

Weatherley, A. H.; Rogers, S. C.; Pincock, D. G.; Patch, J. R. 1982: Oxygen consumption of active rainbow trout, *Salmo gairdneri* Richardson, derived from electromyograms obtained by radiotelemetry. *J. Fish Biol. 20*: 479-489.

Webb, P. W. 1971a: The swimming energetics of trout. II. Oxygen consumption and swimming efficiency. *J. Exp. Biol. 55*: 521-540.

Webb, P. W. 1971b: The swimming energetics of trout. I. Thrust and power output at cruising speeds. *J. Exp. Biol. 55*: 489-520.

Webb, P. W. 1975**:** Hydrodynamics and energetics of fish propulsion**.** *Bull. Fish. Res Bd. Can. 190*: p 159.

Wood, C. M. 1991: Acid-base and ion balance, metabolism, and their interactions, after exhaustive exercise in fish. *J. Exp. Biol. 160*: 285-308.

Woodward, C. C.; Strange, R. J**.** 1987: Physiological stress responses in wild and hatchery-reared rainbow trout. *Trans. Am. Fish. Soc. 116*: 574-579.

CHAPTER THREE

Compounding effects of temperature, hypoxia and exercise for trout parr (*Oncorhynchus mykiss*)

Abstract

Prolonged swimming ability (U_{crit}) of rainbow trout parr (*Oncorhynchus mykiss*), was tested at temperatures from 5°C to 25°C. Acclimated fish were swum to exhaustion under normoxic conditions to determine optimal aerobic exercise temperature. The potential effect of mild environmental hypoxia (75% sat.) was examined in trout parr at 10°C, 15°C and 20°C. Haematological responses of trout were examined by acute caudal venepuncture.

Under normoxic conditions, $U_{crit\ max}$ for trout parr was 5.8 body lengths per second ($BL\ s^{-1}$) at 15.1°C. Hypoxia caused a significant reduction in U_{crit} at 20°C, but had no effect on trout at 10°C or 15°C. $U_{crit\ max}$ in hypoxia was 6 $BL\ s^{-1}$ at 14.1°C.

Temperature acclimation significantly improved trout parr swimming ability. Fish acclimated to 20°C for two weeks performed better at 20°C than fish acclimated to 10°C. Trout parr acclimated at 20°C showed an adaptive elevation in oxygen carrying capacity due to an increase in mean erythrocyte volume and haemoglobin content. Following exercise, haematocrit was elevated under both normoxic and hypoxic conditions. However, the primary cause of this apparent increase in oxygen carrying capacity was splenic release of erythrocytes under normoxic conditions, whereas stress-induced erythrocytic swelling contributed to the observed increase in hypoxia. This contrasting response was most pronounced at 10°C.

These results demonstrate that even mild hypoxia can significantly reduce the swimming abilities of migratory fishes in warm water, although acclimation can assist performance. The implications for the management of lowland rivers are clear: elevated water temperatures and hypoxia could significantly limit fish migration.

Introduction

The combined effects of elevated temperatures and hypoxia create compounding bioenergetic problems for sustained swimming in fish. Elevated temperatures increase metabolic rate, resulting in increased oxygen demand, whilst decreasing oxygen solubility and eutrophic hypoxia reduce environmental oxygen availability. However, these negative impacts could be offset by reduced water and blood viscosity, increased diffusion rates, and a temperature dependent increase in muscle performance so that the effect on swimming ability is difficult to predict. Taylor et al. (1997) concluded that a small acute temperature increase is unlikely to be a problem for sustained swimming unless the ambient temperature is close to the upper thermal limit of the species. However, sustained swimming ability is temperature dependent. In eurythermal fishes, the critical swimming velocity (U_{crit}; Brett, 1964, 1967) increases with temperature to a maximum several degrees below the upper thermal limit; performance then declines markedly as the upper thermal limit is approached (Brett, 1971).

The compounding effects of increased temperature and decreased oxygen availability on sustained swimming have not been investigated. Most fishes function as oxygen regulators, increasing gill ventilation as ambient oxygen decreases until a critical oxygen saturation (S_{crit}) is reached (Randall, 1982). Although S_{crit} increases with temperature in resting fish (Schurmann & Steffensen, 1997), there is little information available on the combined effects of decreased oxygen and increased temperature on active swimming. Brett (1964) concluded that any reduction in saturation is likely to reduce activity above the optimum temperature. However, it is widely assumed that the metabolic performance of fish decreases only at ambient oxygen concentrations below 70% saturation (Hammer, 1995). Although Bushnell et al. (1984) observed a significant decline in U_{crit} of rainbow trout at an ambient oxygen concentration of 27.5% saturation and an optimal performance temperature of 15^{o}C, the metabolic responses of fish to hypoxia in the upper thermal range have not been studied.

The aim of this study was to investigate possible anthropogenic impacts on fish by examining the effect of mild hypoxia (75% saturation) on the prolonged swimming abilities of diadromous fish over a wide thermal range. Rainbow trout parr (*Oncorhynchus mykiss*) were used to assess the compounding effects of temperature, hypoxia, and exercise. Rainbow trout (*Oncorhynchus mykiss*), native to North America, have diadromous populations only in the northern, colder regions of their natural range. In New Zealand, rainbow trout are potamodromous, with lake and lowland river fish migrating upstream to spawn in colder upland tributaries.

Materials and methods

Experimental animals

Rainbow trout parr, *Oncorhynchus mykiss* (Walbaum), (7.0 ± 0.5 cm fork length) were obtained from the Ngongataha Trout Hatchery and Forest Research, Rotorua, New Zealand. Fish were held at the University of Waikato in fibreglass aquaria supplied with dechlorinated tap water (17^{o}C).

Acclimation of fish

Trout parr were randomly allocated to treatment groups and acclimated to 5^{o}C, 10^{o}C, 15^{o}C, 20^{o}C or 25^{o}C (± 0.5^{o}C) for at least 2 weeks prior to swimming tests. Acclimation temperatures were achieved by increasing or decreasing water temperature by 1^{o}C per day. Fish were fed every day to satiation.

Effects of temperature and hypoxia on U_{crit}

Critical swimming speeds (U_{crit}) were measured in an enclosed, 2 metre, 230 litre, variable velocity recirculating flume. The flume allowed for precise temperature and oxygen control. The flume channel was 16 cm x 16 cm in cross-section. Hypoxia (75% sat.) was produced by nitrogen stripping in a bubble tower, and oxygen saturation and temperature were continuously recorded during U_{crit} trials.

For all experiments and at all temperatures, trout parr were swum individually. Fish were selected at random from the acclimated groups and their length was estimated visually to the nearest 0.5 cm. Handling was kept to a minimum to reduce stress; immediately after transfer to the flume, fish were swum for 2 hours at a low speed of 0.5 *BL* s^{-1} to aid recovery from handling and transfer stress (Milligan et al., 2000). During experiments, fish were swum to exhaustion (U_{crit}) at their acclimation temperatures under conditions of normoxia (>96% sat. at 5^{o}C, 10^{o}C, 15^{o}C, 20^{o}C or 25^{o}C) or mild hypoxia (75% sat. at 10^{o}C, 15^{o}C or 20^{o}C) by increasing water velocity in 0.5 *BL* s^{-1} increments every 15 minutes. Exhaustion was determined when the fish was forced on to, and remained on, an electrified rear grill. After

exhaustion, all fish were weighed to the nearest 0.01 g, and length was measured to the nearest 0.1 cm.

Data analysis

U_{crit} was determined for each fish using the equation of Brett (1964):

$U_{crit} = U_i + U_{ii} T_i / T_{ii}$

where U_i is the highest velocity maintained for a complete time interval, U_{ii} is the velocity increment (0.5 *BL* s^{-1}), T_i is interval time elapsed at fatigue velocity, and T_{ii} is the interval time (15 min). U_{crit} values were corrected for the measured lengths of individual fish. Swimming velocities were not corrected for the solid blocking effect of the fish because the cross-sectional area of the fish was not greater than 10% of the cross-sectional area of the flume (Brett, 1964).

Polynomial lines of best fit were fitted to the data in Microsoft Excel 2002 and maximal U_{crit} values calculated by interpolation.

Haematological acclimation in rainbow trout

To determine the effects of thermal acclimation on rainbow trout, five individuals from normoxic and hypoxic U_{crit} trials, as well as non-swum, rested fish removed from the acclimation aquaria at 10°C, 15°C and 20°C, were sampled by acute caudal venepuncture. Blood samples (~50 µl) were drawn into pre-heparinized syringes and analysed for haematocrit (Hct), whole blood haemoglobin (Hb), red blood cell count (RBCC), mean cell haemoglobin concentration (MCHC), mean cell haemoglobin (MCH), and mean cell volume (MCV) according to standard methods (Dacie & Lewis, 1991). A deproteinated extract was made by adding 25 µl of whole blood to 50 µl of perchloric acid (8% solution). Whole blood lactate, glucose and triglyceride concentrations were measured from the supernatant of the deproteinated extract (following centrifugation) using a micro-method adapted from Sigma methods 826-UV, 18-UV and 334-UV, respectively.

To determine whether acclimation affected swimming ability in trout, 5 fish acclimated to either 10°C or 20°C were swum at 20°C and 10°C,

respectively. Their performances were then compared with fish swum at the respective acclimation temperatures.

Statistical analyses

Data were analysed by Student's *t*-test using Statistica version 6.

Results

Swimming performance

The critical swimming speeds for trout parr in normoxia are shown in Figure.3.1. $U_{crit\ max}$ for trout parr was 5.8 *BL* s^{-1} at 15.1°C, but decreased at lower and higher temperatures. This result implied that swimming performance was limited by temperature below 15°C, whereas performance at higher temperatures was limited by oxygen availability. In support of this hypothesis, mild hypoxia caused a significant reduction in U_{crit} at 20°C (P=0.013), but had no effect at 10°C or 15°C (Figure 3.1). $U_{crit\ max}$ in hypoxia was 6 *BL* s^{-1} at 14.1°C.

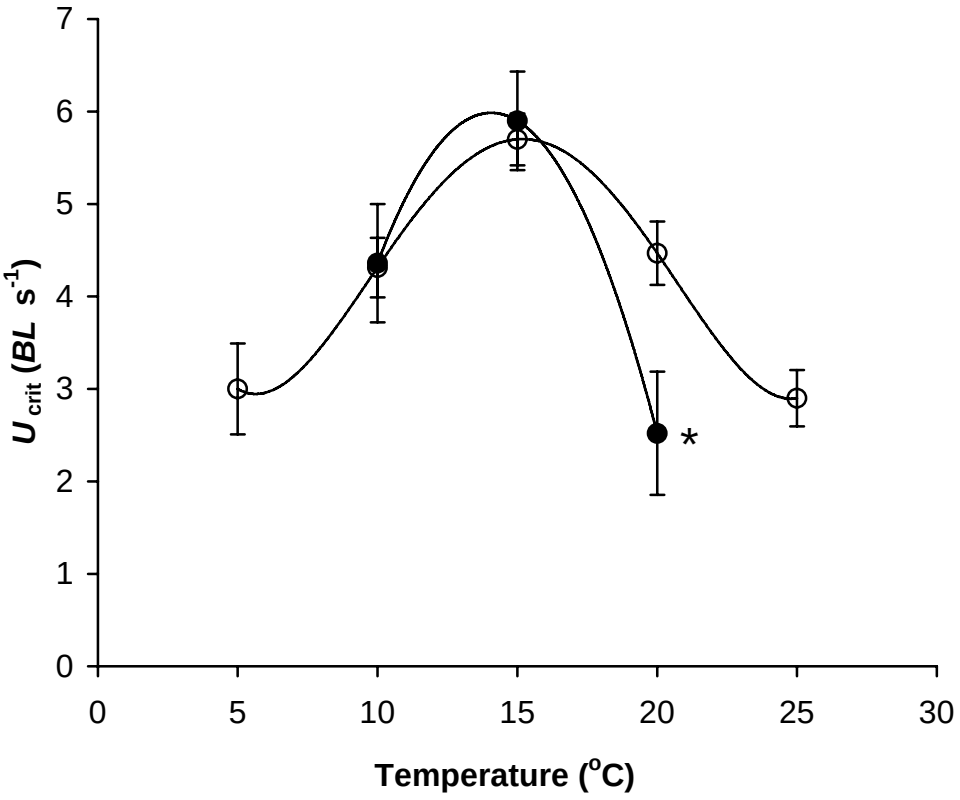

Figure 3.1. Sustained swimming speeds (U_{crit}) of rainbow trout parr at different temperatures in normoxia (>96% sat.; open circles, n=15) and mild hypoxia (75% sat.; closed circles, n=15). Means ± S.E.M. * = significantly different from other treatments at 20°C.

Acclimation to 20°C improved warm water swimming performance. Parr acclimated to 10°C performed significantly worse (P=0.022) than fish

acclimated to 20°C when swum at 20°C (Figure 3.2). However, fish acclimated to 20°C performed as well as fish acclimated to 10°C when swum at 10°C (P=0.83).

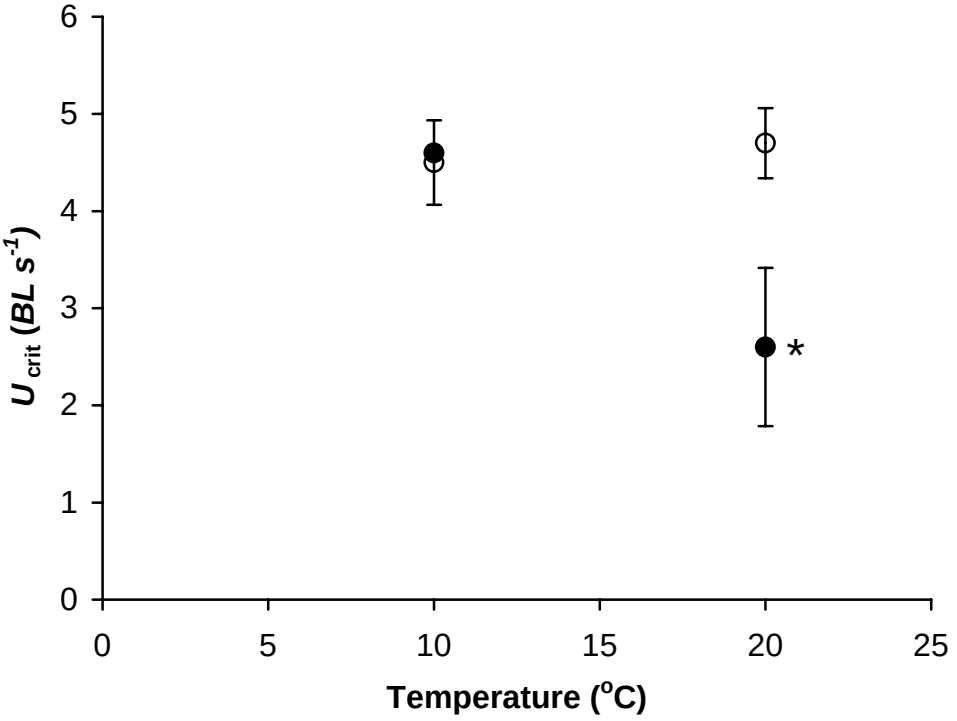

Figure 3.2. Sustained swimming speeds (U_{crit}) of rainbow trout parr acclimated for two weeks at 10°C (closed circles) or 20°C (open circles) and swum at 10°C and 20°C (n = 5 for each group). Means ± S.E.M.
* = significantly different from other treatments at 20°C.

Haematological responses of rainbow trout

Trout acclimated at 20°C showed an adaptive elevation in oxygen carrying capacity due to a significant increase in blood cell volume (MCV) (P=0.022) and an increase, although not significant (P=0.065), in cell haemoglobin content (MCH), compared with fish acclimated to 10°C (Table 3.1). This accounted for the observed increase in packed cell volume and whole blood haemoglobin concentration in these fish. Packed cell volume was further elevated in all acclimated groups following exhaustive exercise under both normoxic and hypoxic conditions. However, the primary cause

of this increase in oxygen carrying capacity appeared to be the splenic release of stored erythrocytes under normoxic conditions, indicated by unchanged MCHC and increased red cell numbers. Conversely, stress-induced adrenergic erythrocytic swelling accounted for much of the observed increase in hypoxia as evidenced by the significant decline in mean cell haemoglobin concentration at all temperatures. This contrasting response was most pronounced at 10^{o}C, whereas hypoxic fish at 20^{o}C employed both strategies. Whole blood lactate and glucose were elevated in all exhausted fish indicating anaerobiosis and stress, respectively. Although triglycerides were slightly elevated in some exercised fish, differences between treatments were not significant.

Table 3.1. Haematological values of trout parr acclimated to 10°C, 15°C and 20°C and sampled at rest or following exhaustive exercise (U_{crit}) under normoxic (>96% sat.) or hypoxic (75% sat.) conditions. Values are means with S.E.M. in parenthesis. Values with the same superscript are significantly different ($P < 0.05$). Superscripts a-i denote comparisons between treatments at each temperature. Superscripts r-z denote comparisons between acclimated groups within the same treatment. n = 5 for all groups.

Temperature (°C)	Variable	Control Resting	Normoxic Exercise	Hypoxic Exercise
10	PCV	25.0 (2.0)^{a,b}	32.8 (1.5)^{a}	30.6 (0.4)^{b}
15	(%)	24.0 (1.8)^{e,t}	26.0 (2.7)^{w}	32.4 (2.0)^{e}
20		29.6 (0.9)^{g,t}	38.0 (1.9)^{g,w}	34.8 (2.4)
10	[Hb]	63.7 (4.2)^{a,s}	81.8 (6.3)^{a}	68.0 (2.3)
15	(g L^{-1})	60.6 (4.3)^{t}	64.2 (6.3)^{w}	75.1 (5.8)
20		75.2 (2.2)^{s,t}	101 (13)^{w}	75.9 (3.9)
10	MCHC	257 (6)^{b}	250 (13)	222 (6)^{b}
15	(g L^{-1})	252 (4)^{e}	247 (10)	230 (6)^{e}
20		254 (7)^{h}	261 (26)	218 (4)^{h}
10	MCH	59.9 (3.5)	62.9 (3.9)	65.0 (1.9)
15	(pg)	70.5 (5.5)	66.3 (3.6)	73.4 (5.0)
20		74.3 (5.8)	86.1 (12.4)	62.5 (4.0)
10	MCV	233 (11)^{b,s}	251 (4)^{c,v}	294 (14)^{b,c}
15	(fL)	280 (20)	269 (9)^{f,w}	319 (17)^{f}
20		292 (17)^{s}	325 (23)^{v,w}	286 (17)
10	RBCC	1.08 (0.09)	1.30 (0.05)^{c}	1.05 (0.06)^{c}
15	(x 10^{12} cells L^{-1})	0.90 (0.14)	0.99 (0.21)	1.04 (0.09)
20		1.03 (0.06)	1.19 (0.07)	1.23 (0.10)
10	Lactate	1.79 (0.31)^{a,b}	4.79 (1.14)^{a}	4.82 (0.42)^{b}
15	(mM)	1.21 (0.26)^{e}	4.47 (1.54)	4.62 (0.82)^{e}
20		1.74 (0.25)^{g,h}	5.46 (1.50)^{g}	7.20 (1.53)^{h}
10	Glucose	3.51 (0.06)^{a,b}	5.28 (0.56)^{a}	4.39 (0.15)^{b,x,y}
15	(mM)	4.01 (0.64)	4.81 (0.35)	5.57 (0.35)^{x}
20		3.56 (0.22)^{h}	4.44 (0.33)	5.08 (0.18)^{h,y}
10	Triglycerides	222 (9.1)	230 (8.4)	252 (11.2)
15	(mg dL^{-1})	214 (5.4)	212 (10.7)	210 (10.5)
20		240 (10.2)	258 (19.4)	228 (4.9)

Discussion

The critical swimming speed of fish is dependent on many factors including size, temperature and ambient gas concentrations (Hammer, 1995). Temperature dependence of U_{crit} is well established; the typical response is an increase to an optimum as temperature rises, followed by a subsequent decline at temperatures greater than optimal (Beamish, 1978). Optimal temperature varies with species and often coincides with the preferred temperature of the fish (Brett, 1971; Reynolds & Casterlin, 1980; Gunderly & Blier, 1988). However, significant and fundamental differences in the temperature dependence of critical swimming speed may occur between species that are anatomically or ecologically similar (Beamish, 1980; Duthie, 1982).

The establishment of optimum aerobic temperature and associated U_{crit} performance curves for fish species, measured in normoxic clean water, provides a reference for evaluating the effects of water quality parameters on sustained swimming of fish. Hammer (1995) concluded that critical swimming speed should provide a sensitive measure for environmental or physiological stress factors.

U_{crit} performance curves for rainbow trout parr exhibited the characteristic temperature dependence. However, the effects of hypoxia on sustained swimming over a wide range of temperatures are unpredictable, given that most studies have exposed fish to hypoxia at temperatures at or below the optimum. The declining solubility of oxygen and the concomitant increase in metabolic oxygen requirements would be expected to constrain aerobic metabolism at higher temperatures. Graham (1949) reported that the cruising speed of brook trout was noticeably reduced when oxygen concentrations were decreased to about 50% of the air-saturated level at 8^{o}C; however, this is below the optimum temperature for this species. Davis et al. (1963) reported that juvenile coho (*Oncorhynchus kisutch*) and chinook salmon (*O. tshawytscha*) usually showed some reduction in sustained swimming speeds when exposed to even a slight reduction in dissolved oxygen concentration from the air-saturated level. At the optimum

temperature for rainbow trout (15°C), Bushnell et al. (1984) observed a significant reduction in U_{crit} with severe hypoxia (27.5% sat.). Brett (1964) reported that for yearling sockeye salmon in air-saturated freshwater, oxygen could become a limiting factor for active metabolism above the optimum temperature. Our results show that even a small reduction in ambient oxygen (75% sat.) above the optimum temperature can result in a significant reduction in U_{crit}. These results confirm that the decline in swimming performance at temperatures above the optimum is probably due to limited oxygen delivery to active muscles. Further evidence for the dependence of swimming performance on oxygen transport at higher temperatures is the enhanced performance of warm-acclimated trout. Such adaptive acclimation may be a factor responsible for the observed dependence of swimming performance on season (Brett, 1964). Trout parr acclimated to 20°C for two weeks performed nearly twice as well at that temperature when compared with fish acclimated at 10°C, due to an adaptive elevation in oxygen carrying capacity in the former. Moreover, mild hypoxia imparts some degree of physiological impact, even at colder temperatures; rainbow trout swum at 10°C showed a significant decrease in MCHC, indicating adrenergic swelling of erythrocytes. This response increases haemoglobin oxygen affinity by a reversal of the Bohr and Root effects (Nikinmaa, 1990) and would enhance oxygen uptake at the gills. Surprisingly, this effect did not occur in normoxic fish swum at 20°C, where normoxic oxygen saturation is almost equivalent to the 75% saturated value at 10°C.

The consequences of this study for lowland river water quality are clear. Hypoxia resulting from eutrophication is unlikely to markedly affect swimming abilities of fish at temperatures below optimum unless the reduction in ambient oxygen is very large. However, at temperatures above the species' optimum, even minor reductions in oxygen will severely impede swimming ability and fish migration. Anthropogenic influences that collectively increase water temperatures and decrease oxygen availability will have the greatest impact on fish populations.

Acknowledgements

Special thanks to Chris Sistern, Bruce Rhodes, Stephen Hardy, and Peter Jarman for equipment construction and technical support. Thanks also to Michael Landman, Lee Laboyrie and Gavin Reynolds for supplying fish and aquarium maintenance.

References

Beamish, F. W. H. 1978: Swimming capacity. In: Hoar, W. H.; Randall, D. J. ed. Fish Physiology Vol. VII. New York, Academic Press. Pp 101-187.

Beamish, F. W. H. 1980: Swimming performance and oxygen consumption of the Charrs. In: Balon, E. K. ed. Charrs. Salmonid Fishes of the Genus *Salvelinus*. The Hague, Dr W. Junk Publishing. Pp 739-748.

Brett, J. R. 1964: The respiratory metabolism and swimming performance of young sockeye salmon. *J. Fish. Res. Bd. Can. 21***:** 1183-1226.

Brett, J. R. 1967: Swimming performance of sockeye salmon (*Oncorhynchus nerka*) in relation to fatigue time and temperature. *J. Fish. Res. Bd. Can. 24***:**1731-1741.

Brett, J. R. 1971: Energetic responses of salmon to temperature. A study of some thermal relations in the physiology and freshwater ecology of sockeye salmon (*Oncorhynchus nerka*). *Am. Zool. 11***:** 99-113.

Bushnell, P. G.; Steffensen, J. F.; Johansen, K. 1984: Oxygen consumption and swimming performance in hypoxia-acclimated rainbow trout *Salmo gairdneri*. *J. Exp. Biol. 113***:** 225-235.

Davis, G. E.; Foster, J.; Warren, C. E.; Doudoroff, P. 1963: The influence of oxygen concentration on the swimming performance of juvenile Pacific salmon at various temperatures. *Trans. Am. Fish. Soc. 92***:** 111-124.

Duthie, G. G. 1982: The respiratory metabolism of temperature-adapted flatfish at rest and during swimming activity and the use of anaerobic metabolism at moderate swimming speeds. *J. Exp. Biol. 97***:** 359-373.

Graham, J. M. 1949: Some effects of temperature and oxygen pressure on the metabolism and activity of the speckled trout, *Salvelinus fontinalis. Can. J. Res. D27*: 270-289.

Gunderly, H.; Blier, P. 1988: Thermal acclimation in fish: conservative and labile properties of swimming muscle. *Can. J. Zool. 66***:** 1105-1115.

Hammer, C. 1995: Fatigue and exercise tests with fish. *Comp. Biochem. Physiol. 112A***:** 1-20.

Milligan, C. L.; Hooke, G. B.; Johnson, C. 2000: Sustained swimming at low velocity following a bout of exhaustive exercise enhances metabolic recovery in rainbow trout. *J. Exp. Biol. 203*: 921-926.

Nikinmaa, M. 1990: Vertebrate Red Blood Cells: Adaptations of Function to Respiratory Requirements. Berlin, Springer. p 262.

Randall, D. J. 1982: The control of respiration and circulation in fish during exercise and hypoxia. *J. Exp. Biol. 100***:** 275-288.

Reynolds, W. W.; Casterlin, M. E. 1980: The role of temperature in the environmental physiology of fishes. In: Ali, M. A. ed. Environmental Physiology of Fishes. New York, Plenum Press. Pp. 497-518.

Schurmann, H.; Steffensen, J. F. 1997: Effects of temperature, hypoxia and activity on the metabolism of juvenile Atlantic cod. *J. Fish Biol. 50***:** 1166-1180.

Taylor, E. W.; Egginton, S.; Taylor, S. E.; Butler, P. J**.** 1997: Factors which may limit performance at different temperatures. In Wood, M. C.; McDonald, D. G. ed. Global Warming Implications for Freshwater and Marine Fish. Cambridge, Cambridge University Press. Pp. 105-133.

CHAPTER FOUR

Effects of temperature, hypoxia, exercise and metamorphosis on prolonged swimming ability of inanga (*Galaxias maculatus*)

Abstract

The prolonged swimming abilities (U_{crit}) of larval and post-larval inanga (*Galaxias maculatus*) were tested at temperatures from 5°C to 25°C, and adults from 5°C to 20°C. Acclimated fish were swum to exhaustion under normoxic conditions to determine the optimal aerobic exercise temperatures for these groups. $U_{crit\ max}$ for larvae was 5.1 body lengths per second ($BL\ s^{-1}$) at 17.7°C, and decreased at lower and higher temperatures. Post-larval inanga performed poorly at higher temperatures with a $U_{crit\ max}$ of 5.6 $BL\ s^{-1}$ at 9.4°C, indicating an ontogenetic change in swimming ability. Adult inanga showed similar temperature dependence to that of larvae but lower relative swimming speeds due to their larger size. $U_{crit\ max}$ for adults was 4.0 $BL\ s^{-1}$ at 18.3°C.

The potential effect of mild environmental hypoxia (75% sat.) was examined in larval inanga at 10°C, 15°C and 20°C. Hypoxia significantly reduced U_{crit} at 15°C and 20°C in larval inanga, lowering the optimal aerobic temperature to 13.9°C and reducing $U_{crit\ max}$ to 4.2 $BL\ s^{-1}$.

These results demonstrate that a significant thermal sensitivity is shown by inanga as they undergo metamorphosis from larvae to juveniles. Furthermore, mild hypoxia can significantly reduce the swimming abilities of larval inanga. The implications for the management of lowland rivers are clear: elevated water temperatures could significantly limit fish migration.

Introduction

Diadromous fishes use lowland riverine systems as migratory pathways when moving between freshwater and marine habitats. Successful migration and colonization of suitable adult habitats by diadromous fishes requires sustained swimming over many kilometres in these lowland riverine systems. Globally, these migratory pathways are becoming increasingly degraded by anthropogenic impacts including chemical, thermal and nutrient pollution, canalisation, abstraction, loss of riparian shading, construction of physical barriers, and global climate change. Proximate consequences include eutrophication and hypoxia, loss of habitat complexity, alterations to water flow, and elevated temperatures. Migratory fishes, therefore, run a gauntlet of diverse stressors to reach adult or reproductive habitats. While some impacts, such as physical barriers and chemical pollution, represent obvious threats to migration, hypoxia and elevated temperatures represent an invisible challenge.

Inanga (*Galaxias maculatus* Jenyns 1842) is one of the world's most widespread freshwater species (McDowall, 1990), distributed throughout much of the southern hemisphere south of latitude 30^{o} S. Inanga are diadromous, having a four stage, catadromous life cycle: terrestrial spawning, marine growth (c. 6 months), saltwater-freshwater migration, and stream residence (c. 7 months).

Inanga larvae, on entering freshwater, appear to have no food in their stomachs and are almost transparent in appearance, and constitute a major recreational and commercial fishery as whitebait. However, within a few days of entering freshwater they become dark in colour (McDowall & Eldon, 1980), possibly associated with feeding, which begins 10-12 km or more upriver, and the development of the spleen, evident by a bright red spot in the anterior abdomen (McDowall, 1990). In addition to changing colour, juvenile inanga also shrink several millimetres after entering freshwater (McDowall, 1990). Inanga juveniles on returning to freshwater show a positive rheotactic response, and migrate upstream seeking suitable habitat, possibly aided by odours of adult galaxiids, which inanga juveniles

can discriminate and are attracted to during their migratory stage (Baker & Hicks, 2003).

Inanga are confined to low velocity, low altitude habitat, reflecting their poor climbing ability; inanga juveniles being restricted by falls of 10 cm or higher (Baker, 2003; Jowett & Richardson, 2003). However, the habitat of inanga is diverse, ranging from clear to tannin-stained waters, high to low pH, open pasture to forest cover and cold to warm waters, but fish are most abundant above the influence of sea water (McDowall, 1990).

Temperature has been demonstrated as an important determinant in the distribution, abundance and physiology of fish species (Alabaster & Lloyd, 1982; Coutant, 1987). Field studies undertaken on migratory inanga in the Waikato River have shown that migration ceases when thermal discharge from the Huntly Power Station exceeds 27^{o}C (Stancliff et al., 1989). At such temperatures the schooling behaviour of inanga is lost (Simmons, 1986). Preferred temperatures of inanga juveniles and adults are 18.7^{o}C and 18.1^{o}C, respectively, when acclimated to 15^{o}C (Richardson et al., 1994). Comparisons by Richardson et al. (1994) of these preferred temperatures with field records from a database having national coverage (McDowall & Richardson, 1983) show inhabited sites recorded mean temperatures within about 1^{o}C of their preferred temperatures. Boubée et al. (1991) reported that the final preferred temperature of adult inanga acclimated to 20^{o}C is about 20^{o}C. However, fish acclimated to 15^{o}C and 17^{o}C preferred warmer waters of up to 23^{o}C and 26^{o}C respectively, with total avoidance occurring at 29.5^{o}C, 31^{o}C and 31.5^{o}C, for fish acclimated to 15^{o}C, 17^{o}C and 20^{o}C, respectively.

Biological factors such as swimming ability and bio-energetic requirements are suggested by Jowett (2002) to dictate suitable habitat for inanga. Field observations by Jowett (2002) have shown that inanga maintain station in current patterns and water velocities that concentrate food, with optimum feeding velocity for 55 mm length inanga of 0.5-1.3 *BL* s^{-1}. In contrast, velocity of 1 m s^{-1} can only be maintained by 50 mm inanga for between 1 and 10 s (Boubée et al., 1999). Nikora et al. (2003) suggested that inanga, without rest and depending on size, can maintain

burst swimming for a distance of 10-100 m and prolonged swimming for 1-10 km.

The importance of understanding possible consequences of anthropogenic impacts, elevated temperature and hypoxia, on prolonged swimming is that successful colonisation of suitable adult habitat by juvenile migratory inanga requires migration over many kilometres in lowland riverine systems. The establishment of U_{crit} performance curves for a fish species measured in clean normoxic water not only identifies the optimum aerobic temperature, which often coincides with the preferred temperature (Brett, 1971; Reynolds & Casterlin, 1980; Guderley & Blier, 1988), but also provides a benchmark for assessing the effects of degraded water quality on prolonged swimming. Hammer (1995) concluded that critical swimming speed should provide a sensitive measure for the effects of environmental or physiological stressors.

The aim of this study was to investigate the prolonged swimming abilities of larval, post-larval and adult inanga over a wide thermal range (5^{o}C - 25^{o}C). A further aim was to investigate possible anthropogenic impacts on larval migration in lowland rivers by examining the effects of mild hypoxia (75% saturation) on their prolonged swimming ability.

Materials and methods

Experimental animals

Inanga whitebait larvae, *Galaxias maculatus* (Jenyns), 4.7 – 5.0 cm total length (TL), netted in the Waikato River near Port Waikato, North Island, New Zealand, were purchased from commercial whitebait fishermen. Inanga were kept in glass aquaria in dechlorinated tap water (17^{o}C) containing 0.35% NaCl. Inanga larvae were fed to satiation daily on live *Daphnia carinata*. To obtain post-larval inanga, larvae were grown in the laboratory until pigmented and flushed with red blood. Because inanga shrink during metamorphois, post-larval fish were 3.9 – 4.0 cm TL. Fish were fed live *Daphnia*, and after three days in the laboratory were also fed frozen blood worms (chironomid larvae) once a day to satiation. Post-larval individuals showed a well-developed spleen and a marked pink colouration due to the presence of red blood cells in circulation. The body was typically more opaque than the clear glass-like appearance of the larvae. Post-metamorphic adult inanga (5.5 – 6.8 cm TL) were kindly supplied by, C. Mitchell, Raglan, North Island, New Zealand.

Temperature conditioning of fish

Migrating larvae metamorphosed into the post-larval form within two to three weeks of capture. Larvae were therefore randomly allocated to treatment groups as soon as they were obtained and were conditioned to the experimental temperatures of 5^{o}C, 10^{o}C, 15^{o}C, 20^{o}C and 25^{o}C (± 0.5^{o}C) over the course of three days. Temperatures were adjusted in three even increments over the three day conditioning period.

Post-larval and adult inanga were randomly allocated to treatment groups and conditioned to 5^{o}C, 10^{o}C, 15^{o}C, 20^{o}C (± 0.5^{o}C) for at least two weeks prior to swimming tests. Conditioning temperatures were achieved by increasing or decreasing water temperature by 1^{o}C per day. A further group of post-larval inanga were conditioned to 25^{o}C but experienced high mortality and a general failure in swimming trials (88% failure), and this temperature was therefore not tested in adults. Larvae, post-larval and adult inanga were maintained on a 12:12 photoperiod during conditioning.

Effects of temperature and hypoxia on U_{crit}

Critical swimming speeds (U_{crit}) were measured in an enclosed 2 metre, 230 litre, variable velocity, recirculating flume. The flume allowed for precise temperature and oxygen control. The flume channel was 16 cm x 16 cm in cross-section. Hypoxia (75% sat.) was produced by nitrogen stripping in a bubble tower, and oxygen saturation and temperature were continuously recorded during U_{crit} trials.

For all experiments and at all temperatures, larvae and post-larval inanga were swum in groups of five, whereas adults were swum individually. Fish were swum in a circular cross-sectional insert within the flume to prevent them from seeking low-flow boundary regions at the corners of the flume channel. Prior to commencement of U_{crit} trials, fish were randomly selected from the acclimated groups and a visual estimate of their length was made to the nearest 0.5 cm. Fish were then transferred to the flume and swum for 2 hours at 0.5 *BL* s^{-1} to aid recovery from handling and transfer stress (Milligan et al., 2000). Following this recovery period, fish were swum to exhaustion by increasing water velocity in 0.5 *BL* s^{-1} increments every 15 minutes. Exhaustion was determined when the fish were forced on to, and remained on, an electrified rear grill. Immediately following exhaustion, all fish were weighed to the nearest 0.01 g and length measured to ± 0.1 cm. Measuring lengths of individual fish allowed correction of U_{crit} values. Swimming velocities were not corrected for the solid blocking effect of the fish because the cross-sectional area of the fish was not greater than 10% of the cross-sectional area of the flume (Brett, 1964).

Experiments were approved by the University of Waikato Animal Ethics Committee.

Data analyses

U_{crit} was determined using the equation of Brett (1964):

$U_{crit} = U_i + U_{ii}\ T_i\ /\ T_{ii}$

where U_i is the highest velocity maintained for a complete time interval, U_{ii} is the velocity increment (0.5 *BL* s^{-1}), T_i is the interval time elapsed at fatigue velocity and T_{ii} is the interval time (15 min).

Polynomial lines of best fit were fitted to the data in Microsoft Excel 2002 and maximal U_{crit} values calculated by interpolation.

Statistical analysis

Data were analysed by Student's *t*-test using Statistica version 6.

Results

Swimming performance

The critical swimming speeds for inanga larvae, post-larvae and adults are shown in (Figure 4 1).

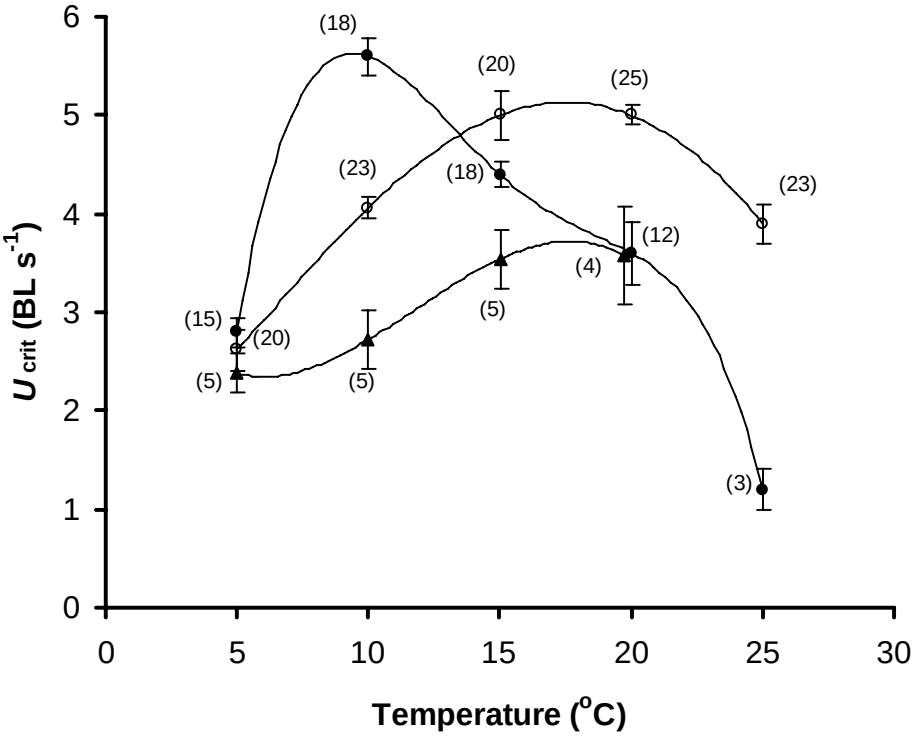

Figure 4.1**.** Prolonged swimming speeds (U_{crit}) of inanga larvae (open circles) post-larval (closed circles) and adult inanga (closed triangles) at different temperatures under normoxic conditions (>96% sat.). Means ± S.E.M., numbers of fish in each group in parentheses.

$U_{crit\ max}$ for inanga larvae was 5.1 *BL* s^{-1} at 17.7°C, but decreased at lower and higher temperatures. Post-larval inanga performed poorly at higher temperatures compared to larvae; $U_{crit\ max}$ was 5.6 *BL* s^{-1} at 9.4°C. This indicated an ontogenetic shift in swimming ability, possibly resulting from a developmental change in red muscle kinetics or a greater reliance on anaerobic muscle. Not only did post-larval inanga swim poorly at warmer temperatures, but few individuals at 25°C survived the two hour acclimation period in the flume prior to the start of the U_{crit} experiment (0.5 *BL* s^{-1}).

Adult inanga prolonged swimming showed similar temperature dependence to that of larvae but lower relative swimming speeds due to

their larger size. The dramatic decline in performance exhibited by juveniles at warmer temperatures was not apparent in adults. $U_{crit\ max}$ for adults was 4.0 *BL* s^{-1} at 18.3°C.

Mild hypoxia caused a significant reduction in U_{crit} of inanga larvae at 15°C (P=0.021) and 20°C (P=0.004), lowering the optimal aerobic temperature to 13.9°C and reducing $U_{crit\ max}$ to 4.2 *BL* s^{-1}. However mild hypoxia had no effect at 10°C (Figure 4.2).

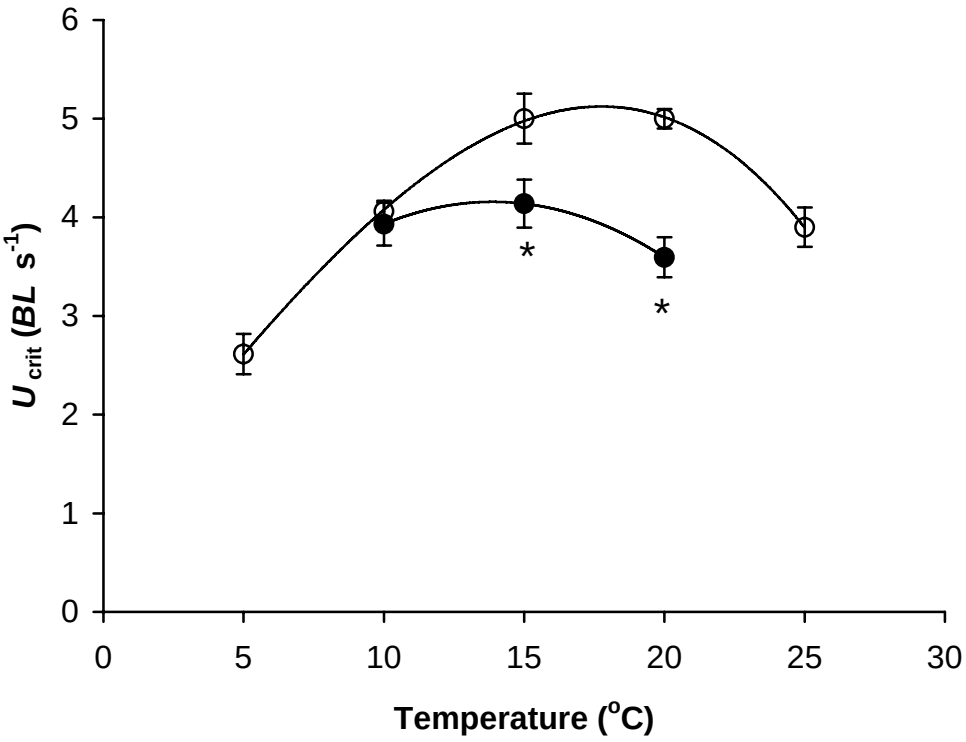

Figure 4.2. Sustained swimming speeds (U_{crit}) of inanga larvae at different temperatures in normoxia (>96% sat.; open circles, n = 20 to 25 in each group) and hypoxia (75% sat.; closed circles, n = 16 to 18 in each group). Mean ± S.E.M. * = significantly different from other treatments at 20°C.

Discussion

U_{crit} performance curves for inanga larvae, juveniles and adult inanga exhibited the characteristic temperature dependence seen in other studies of prolonged swimming (Beamish, 1978), and optimum performance temperatures for larvae (17.7^{o}C) and adults (18.3^{o}C) closely match those determined by Richardson et al. (1994) of 18.7 and 18.1^{o}C, respectively. The reduced performance of juvenile inanga at warmer temperatures was somewhat surprising compared to the swimming abilities of larvae and adults. The developmental transition of inanga from whitebait to the juvenile stage coincides with development and enlargement of the spleen; unlike the clear, glass-like whitebait, juvenile fish become noticeably suffused with red blood. It was therefore expected that the increased oxygen carrying capacity of juvenile inanga would improve aerobic performance. Although relative swimming performance is dependent on size and declines as fish increase in length (Bainbridge, 1960, 1962), juvenile inanga are actually shorter than larvae, so the decline in relative swimming speed of juveniles at warmer temperatures is not related to growth and the U_{crit} max value is indeed slightly greater than that of larvae. Richardson et al. (1994) also tested the preferred temperature of juvenile inanga and found that it corresponded closely with that of larvae and adults, however, fish used in their experiments were fully pigmented, post-metamorphic individuals (D. West, pers. comm.).

Developmental shifts in red muscle kinetics and swimming kinematics have been demonstrated in juvenile rainbow trout (Coughlin et al., 2001), therefore the observed ontogenetic shift in swimming performance may have resulted from developmental changes in swimming musculature. However, the restoration of warm temperature performance in adult fish implies that the observed performance in post-larval inanga is simply a temporary loss of function during metamorphosis. Baker (2003) found that adult inanga of the same total length as larvae were better able to negotiate increasing fall heights over an experimental weir and although this enhanced sprint performance was primarily attributed to a difference in

experimental temperatures, it is possible that greater anaerobic muscle performance of adult fish is also a factor. Ecologically, a developmental shift in locomotory performance is compelling as aerobically migrating larvae metamorphose into adults in low velocity lowland habitats to anaerobically-dominant individuals capable of fast-start sprints to catch prey and avoid predators. It is clear from this study that the temperature dependence of inanga larvae and adults is unchanged but that a significant thermal sensitivity is shown by inanga as they undergo metamorphosis from larvae to juveniles.

Although untested, the significant effect of hypoxia on larvae is likely to further limit the aerobic performance of inanga juveniles in warmer water. Hypoxia is therefore expected to limit inanga migration and survival during the sensitive migratory and metamorphic stages. Assessment of the potential impacts of environmental factors on migratory fish species must therefore take into account the possibility of ontogenetic shifts in swimming ability and the likelihood of particularly sensitive life stages.

Acknowledgements

We thank Chris Sistern, Bruce Rhodes, Stephen Hardy, and Peter Jarman for equipment construction and technical support. We also thank Lee Laboyrie for collecting inanga whitebait and C. Mitchell for supplying inanga adults.

References

Alabaster, J. S.; Lloyd, R. 1982: Water Quality Criteria For Freshwater Fish. London, Butterworth. p 361.

Bainbridge, R. 1960: Speed and stamina in three fish. *J. Exp. Biol. 37*: 129-153.

Bainbridge, R. 1962: Training, speed and stamina in trout. *J. Exp. Biol*. *39*: 537-555.

Baker, C. 2003: Effects of fall height and notch shape on the passage of inanga (*Galaxias maculatus*) and common bullies (*Gobiomorphus cotidianus*) over an experimental weir. *N. Z. J. Mar. Freshwat. Res. 37*: 283-290.

Baker, C. F.; Hicks, B. J. 2003: Attraction of migratory inanga (*Galaxias maculatus*) and koaro (*Galaxias brevipinnis*) juveniles to adult galaxiid odours. *N. Z. J. Mar. Freshwat. Res. 37*: 291-299.

Beamish, F. W. H**.** 1978: Swimming capacity. In: Hoar, W. H.; Randall, D. J. ed. Fish Physiology Vol. VII. New York, Academic Press. Pp 101-187.

Boubée, J. A.; Schicker, K. P.; Stancliff, A. G. 1991: Thermal avoidance in inanga, *Galaxias maculatus* (Jenyns), from the Waikato River, New Zealand. *N. Z. J. Mar. Freshwat. Res. 25*: 177-180.

Boubée, J. A. T.; Jowett, I. G.; Nichols, S.; Williams, E. K. 1999: Fish passage at culverts: a review with possible solutions for New Zealand indigenous species. Wellington, Department of Conservation. p 63.

Brett, J. R. 1964: The respiratory metabolism and swimming performance of young sockeye salmon. *J. Fish. Res. Bd. Can. 21*: 1183-1226.

Brett, J. R. 1971: Energetic responses of salmon to temperature. A study of some thermal relations in the physiology and freshwater ecology of sockeye salmon (*Oncorhynchus nerka*). *Am. Zool. 11*: 99-113.

Coughlin, D. J.; Burdick, J.; Stauffer, K. A.; Weaver, F. E. 2001: Rainbow trout display a developmental shift in red muscle kinetics, swimming kinematics and myosin heavy chain isoform. *J. Fish Biol. 58*: 701-715.

Coutant, C. C. 1987: Thermal preference: when does an asset become a liability? *Env. Biol. Fish. 18*: 161-172.

Guderley, H.; Blier, P. 1988: Thermal acclimation in fish: conservative and labile properties of swimming muscle. *Can. J. Zool. 66*: 1105-1115.

Hammer, C. 1995: Fatigue and exercise tests with fish. *Comp. Biochem. Physiol. 112A*: 1-20.

Jowett, I. G. 2002: In-stream habitat suitability criteria for feeding inanga (*Galaxias maculatus*). *N. Z. J. Mar. Freshwat. Res. 36*: 399-407.

Jowett, I. G.; Richardson, J. 2003: Fish communities in New Zealand riversand their relationship to environmental variables. *N. Z .J. Mar. Freshwat. Res. 37*: 347-366.

McDowall, R. M. 1990: New Zealand Freshwater Fishes. A Natural History and Guide. Auckland, Heinemann Reed. p 553.

McDowall, R. M.; Eldon, G. A. 1980: The ecology of whitebait migrations (Galaxiidae, *Galaxias* spp.). New Zealand Ministry of Agriculture and Fisheries. *Fish. Res. Bull. 20*. p 171.

McDowall, R. M.; Richardson, J. 1983: New Zealand freshwater fish survey: a guide to input and output. Fisheries Research Division. *Information Leaflet 12*: p 15.

Milligan, C. L.; Hooke, G. B.; Johnson, C. 2000: Sustained swimming at low velocity following a bout of exhaustive exercise enhances metabolic recovery in rainbow trout. *J. Exp. Biol. 203*: 921-926.

Nikora, V. I.; Aberle, J.; Biggs, B. J. F.; Jowett, I. G.; Sykes, J. R. E. 2003: Effects of fish size, time-to-fatigue and turbulence on swimming performance: a case study of *Galaxias maculatus*. *J. Fish Biol. 63*: 1365-1382.

Reynolds, W. W.; Casterlin, M. E .1980: The role of temperature in the environmental physiology of fishes. In: Ali, M. A. ed. Environmental Physiology of Fishes. New York, Plenum Press. Pp. 497-518.

Richardson, J.; Boubée, J. A. T.; West, D. W. 1994: Thermal tolerance and preference of some native New Zealand freshwater fish. *N. Z. J. Mar. Freshwat. Res. 28*: 399-407.

Simons, M. J. 1986: Effects of elevated temperature on three migratory fish from the Waikato River. Waikato Valley Authority Technical Report 40. p 62.

Stancliff, A. G.; Boubée, J. A. T.; Mitchell, C. P. 1989: Cross-channel movement of small migratory fish and shrimps in the Waikato River near Huntly thermal power station. New Zealand Freshwater Fisheries Report 30 p 107.

CHAPTER FIVE

Does environment or physiology limit prolonged swimming performance in trout: effects of temperature, hypoxia and anaemia

Abstract

Both physiological and environmental factors can limit the aerobic performance of fish, thus raising the question, what is the most significant limiting factor, O_2 uptake or O_2 delivery? To test this theory we separately and in combination investigated the effects of mild hypoxia (75% saturation or 6.8 mg O_2 L^{-1}) and anaemia (75% and 50% of normocythaemia) on juvenile trout in prolonged swimming trials (U_{crit}) at temperatures from 10°C to 20°C. Anaemia (50%) or hypoxia (6.8 mg O_2 L^{-1}) independently resulted in slight but comparable reductions in U_{crit} at 15°C and 20°C. However, at 20°C, the combination of hypoxia (6.8 mg O_2 L^{-1}) and anaemia (50%) caused a significant reduction in U_{crit}. The availability of oxygen at the gill surface may be a more significant limiting factor on aerobic exercise than carrying capacity of the circulation at elevated temperatures.
Key words: rainbow trout, environmental oxygen uptake, physiological oxygen transport, normocythaemia, anaemia, normoxia, hypoxia, swimming performance.

Introduction

Active predatory fish like salmonids typically utilise three distinct modes of swimming, termed burst, prolonged and sustained. Burst swimming, consisting of fast starts and sprints typically lasting less than 20 s (Beamish, 1978) is powered almost exclusively by white glycolytic muscle and is terminated by exhaustion of intracellular energy supplies (Jones, 1982) or accumulation of waste products (Brett, 1964). By contrast, sustained swimming used for migration or maintaining station in currents principally relies on red oxidative muscle fibres and can be maintained for long periods (> 240 min; Beamish, 1978) as metabolic demand matches supply, and waste production is balanced by disposal (Jones, 1982).

Prolonged swimming is intermediate between these two extremes and is frequently used to evaluate the swimming performance of fish using the U_{crit} protocol, whereby fish are subjected to incremental increases in swimming speeds until fatigued.

Although prolonged swimming employs both aerobic and anaerobic muscle masses, electromyographic studies of salmonids indicate that white muscle is only recruited at speeds above around 70% of U_{crit}. Moreover, salmonid white muscle is relatively well vascularised and Johnston & Moon (1980) demonstrated that sustained swimming is supported by the aerobic capacity of the white muscle mass, therefore U_{crit} is expected to be highly dependent on aerobic muscle function and is generally used as a surrogate for the aerobic performance capabilities of fish. Many environmental and physiological factors can limit the swimming performance of fish, although aerobic performance is ultimately limited by mitochondrial ATP production and usage. Vertebrate skeletal muscle mitochondria are generally assumed to be O_2-limited during maximal aerobic exercise and dependent on convective and diffusive steps involved in O_2 transport from the environment. Factors limiting mitochondrial oxygen supply include capillary perfusion, blood oxygen carrying capacity, cardiac output, and respiratory exchange, therefore changes in any of these parameters would be expected to alter prolonged swimming performance in fish.

Overriding all of these considerations is the master ecological variable, temperature, which strongly influences swimming performance. Fish show characteristic species-specific temperature optima for many important physiological functions including maximum metabolic performance and swimming performance, and Brett (1971) concluded that oxygen availability was the rate limiting condition for prolonged locomotory performance at high temperatures. Subsequent research by Barron et al. (1987), Farrell (1997), and Pörtner et al. (2004) found that cardiac performance was limited by temperature, suggesting that cardiac performance is likely to be a primary limitation to swimming at high temperatures.

While oxygen delivery influences swimming capacity, it is not exclusively determined by cardiac capacity. The structural extent of capillary supply to musculature may be inadequate to maintain endurance swimming (Taylor et al., 1997), as aerobic swimming requires well-perfused red muscle (Beamish, 1978). Sustained swimming also requires a haematocrit (Hct) that is adequate for maintaining oxygen transport from the gills to the muscle, as a reduction in Hct, thus a reduction in oxygen transport results in a reduced U_{crit} value (Jones, 1971; Gallaugher et al., 1995) while U_{crit} values for polycythaemic fish increase (Gallaugher et al., 1995). Gallaugher et al. (1995) suggest these results indicate a transition from perfusion-limited O_2 transport in anaemic fish to diffusion-limited O_2 transport in polycythaemic fish.

Gill oxygen transfer, under normal conditions, is influenced predominantly by rates of perfusion and or ventilation, whereas carbon dioxide transfer is believed to be diffusion limited (Perry, 1998). Lamellar chloride cell proliferation, as a response to maintaining ionic homeostasis, results in a thickening of the lamellar diffusion barrier, which impedes respiratory gas transfer (Thomas et al., 1988). Compensatory physiological responses to a thickened diffusion barrier include increased affinity of haemoglobin-oxygen binding, earlier onset of catecholamine release during acute hypoxia, and hyperventilation (Perry, 1998). While hyperventilation is the most important response it brings with it a negative impact, an elevation in energetic cost. Consequently this may limit the energy available for

normal activity, and normal physiological adjustment during exercise and when exposed to environmental hypoxia (Perry, 1998). Furthermore, Duthie and Hughes (1987) reported that rainbow trout with a cautery-induced 30% reduction in functional gill area showed significant proportional reductions in maximum oxygen consumption and U_{crit}. Under conditions of maximum aerobic demand, all available gill area would appear to be fully perfused but diffusion limited because conditions of hyperoxia (~ 200% sat.) did not reverse this impairment.

Temperature is a particularly important factor that presents compounding problems for sustained swimming due to an increase in metabolic oxygen demand while simultaneously decreasing oxygen availability. Consequently, U_{crit} increases to an optimum as temperature rises and then decreases again (Beamish, 1978, 1980). Jones (1971) showed that hypoxia or anaemia could decrease U_{crit} in juvenile trout at both high (21-23^{o}C) and low (8-10^{o}C) temperatures. However, Bannon & Ling (2004) found that mild hypoxia (75% sat.) only affected U_{crit} at temperatures above the optimum value of 15^{o}C. Moreover, fish acclimated for 2 weeks at 20^{o}C showed compensatory increases in resting haematocrit that improved aerobic exercise performance at that temperature, implying that oxygen carrying capacity was crucial to performance at higher temperatures. Bannon & Ling (2004) proposed that this mechanism may contribute to the adaptive response observed between swimming performance and season reported by Brett (1964). The dependence of swimming performance on temperature acclimation is further confirmed by the findings of Jain & Farrell (2003), who reported that U_{crit} values of warm acclimated fish (14.9 ± 1.0^{o}C) were significantly greater than those of cold acclimated fish (8.4 ± 0.9^{o}C).

The importance of haematocrit to U_{crit} is further supported by the study of Pearson & Stevens (1991) who observed a decrease in aerobic performance in splenectomized trout, because splenic release of erythrocytes contributes around 25% of the increased circulating haemoglobin mass in exercised trout (Pearson & Stevens, 1991). However,

Gallaugher et al. (1992) obtained contrary results from splenectomized trout and found no relationship between haematocrit and U_{crit}.

Performance at temperatures below the optimum is possibly limited by red muscle shortening velocity (V_{max}), and subsequent earlier recruitment of white muscle to maintain swimming velocity. Rome et al. (1984) reported that white muscle recruitment commenced at 26 cm s^{-1} at 10°C, but not until 46 cm s^{-1} at 20°C. Although Johnston & Moon (1980) demonstrated that prolonged swimming is supported by the aerobic capacity of the white muscle, earlier recruitment of white muscle at temperatures below the optimum probably accelerates anaerobic processes and associated accumulation of lactic acid, contributing to fatigue.

Swimming ability of trout has been indicated to increase with increase in migration distance (Tsuyuki & Williscroft, 1977). Experimental evidence supporting this assumption has been documented. Brett et al. (1958) reported that underyearling *Oncorhynchus kisutch* reared at a water velocity of 24 cm s^{-1} were not only less susceptible to fatigue, but exhibited a higher cruising speed when compared to underyearling *O. kisutch* reared at a lower water velocity. Swimming ability also increases when older fish are subjected to training. Besner & Smith (1983) reported that subjecting coho salmon to 40 days training resulted in an increase in U_{crit} from 3.4 – 4.3 body lengths (*BL*) s^{-1} (untrained) to 5.3 - 6.0 *BL* s^{-1} (trained). Training regimes have also been demonstrated to elevate Hct. Thorarensen et al. (1993) exposed fish for 8 months to one of two current velocities, 0.5 *BL* s^{-1} or 1.5 *BL* s^{-1}; the Hct of the latter being significantly higher. However, U_{crit} values for the two groups were not significantly different.

Body size also places limitations on sustained swimming. Bainbridge (1958) and Brett (1965) demonstrated that absolute swimming speed of fish increased with size, however, as size increased, relative speed decreased. Tail beat frequencies during sustained swimming in adult trout are lower than in juveniles, corresponding to a developmental shift in red muscle kinetics (Coughlin et al., 2001). Furthermore, size affects the ratio of white muscle energy stores and extent of lactate production. Ferguson et al. (1993) found that white muscle of larger rainbow trout at rest had higher

concentrations of ATP and glycogen when compared to smaller fish, whereas smaller fish had higher levels of phosphocreatine (PCr). However, Kieffer et al. (1996) found elevated resting values of both ATP and PCr in large brook trout compared to smaller individuals. Following exhaustive exercise, rainbow trout (Goolish, 1989; Ferguson et al., 1993) and brook trout (Kieffer et al., 1996) demonstrated a size-dependent increase in the production of lactate in the white muscle. These studies indicate a greater capacity for anaerobic swimming in larger fish.

Both physiological and environmental factors limit the aerobic performance of fish, thus raising the question, what is the most significant limiting factor, O_2 uptake or O_2 delivery? To test this theory we separately and in combination investigated the effects of mild hypoxia and severe anaemia on juvenile trout in prolonged swimming trials at temperatures from 10^{o}C to 20^{o}C.

Materials and methods

Experimental animals

Hatchery-reared rainbow trout, *Oncorhynchus mykiss* (Walbaum), 14 ± 0.5 cm fork length, were randomly allocated to normocythaemic treatments at 10°C, 15°C and 20°C (± 0.5°C), and anaemic treatments (75% and 50% of normocythaemia) at 15°C and 20°C (± 0.5°C). Increasing or decreasing water temperature by 1°C per day achieved acclimation temperatures. Fish were maintained in aerated dechlorinated tap water at acclimation temperatures for at least 21 days prior to commencement of treatments, and were fed daily with commercial trout pellets to satiation and subjected to a photoperiod of 12:12 L:D. Normocythaemic and anaemic fish were sampled at rest or swum to exhaustion under normoxic (>96% sat.) or hypoxic conditions (either 75% saturation or 6.8 mg O_2 L^{-1} at each temperature. n = 5 fish per treatment group).

Anaemia (75% and 50% of normocythaemia) was induced in fish acclimated for 14 days at 15°C or 20°C by caudal venepuncture. Fish were anaesthetized, weighed, and total blood volume was calculated (4% of total weight. Nikinmaa et al., 1981). 25% anaemia (75% of normocythaemia) was induced by a single withdrawal of 25% of total blood volume. 50% anaemia was induced by a single withdrawal of 25% of total blood volume, followed by a further 25% of total blood volume 24 hours later. Both anaemic fish groups (75% and 50%) were allowed to recover for 7 days prior to U_{crit} determinations.

Exercise testing

Critical swimming speeds (U_{crit}) of individual fish were measured in a 2 metre, 230 litre, variable velocity, recirculating flume. The flume channel measured 16 cm x 16 cm in cross-section, and flume design allowed for precise temperature and oxygen control, both of which were recorded continuously during U_{crit} trials. Hypoxia was achieved by nitrogen stripping water in a bubble tower. Prior to commencement of U_{crit} trials, fish were randomly selected from the acclimated groups and a visual estimate of their

length was made to the nearest 0.5 cm. Fish were then transferred to the flume and swum individually for 2 hours at 0.5 body lengths (*BL* s^{-1}) to aid recovery from handling and transfer stress (Milligan et al., 2000). Following recovery, fish were swum to exhaustion by increasing the water velocity in 0.5 *BL* s^{-1} increments every 15 minutes. Exhaustion was determined when the fish was forced on to, and remained on, an electrified rear grill.

U_{crit} was determined using the equation of Brett (1964)

$$U_{crit} = U_i + U_{ii} T_i / T_{ii}$$

where U_i is the highest velocity maintained for a complete time interval, U_{ii} is the velocity increment (0.5 *BL* s^{-1}), T_i is the interval time elapsed at fatigue velocity and T_{ii} is the interval time (15 min).

Haematological analyses

Immediately following exhaustion, all fish were bled (~200 μL sample volume) by acute caudal venepuncture using pre-heparinized syringes, weighed (± 0.01 g) and measured (± 1 mm). Blood was then analyzed for haematocrit (PCV), whole blood haemoglobin ([Hb]), mean cell haemoglobin concentration (MCHC), mean cell haemoglobin (MCH), mean cell volume (MCV) and red blood cell count (RBCC) according to standard methods (Dacie and Lewis, 1991); glucose and lactate by enzymatic colorimetry, and cortisol by radioimmunoassay. U_{crit} values were corrected for the measured fish lengths.

Experiments were approved by the University of Waikato Animal Ethics Committee.

Statistical analyses

Data were analysed by factorial ANOVA with temperature, exercise, hypoxia and anaemia as factors using Statistica version 6. Differences between individual treatments were tested using Student's *t*-test.

Results

Haematological responses to anaemia

Bleeding of rainbow trout acclimated to 15^{o}C and 20^{o}C resulted in reductions in red blood cell mass of around 25% and 50% (as measured by PCV, [Hb], and RBCC) 7 days after bleeding (Table 5.1).

Haematological responses to hypoxia

Exercise of normocythaemic fish under hypoxic conditions (75% sat. or 6.8 mg L^{-1}**)** resulted in significant increases in PCV (P <0.001), [Hb] (P <0.001), RBCC (P =0.022), cortisol (P <0.001) and lactate (P <0.001), and a significant decrease in MCHC (P <0.001) indicating erythrocytic swelling, splenic erythrocyte release, stress and anaerobiosis. There was also a significant interaction between exercise and temperature on MCH, MCV and glucose. Exercise of 50% anaemic fish under hypoxic conditions (6.8 mg L^{-1}**)** resulted in significant temperature dependent decreases in MCH (P=0.024) and MCHC (P=0.038), and exercise increased MCV (P<0.013), cortisol (P<0.001), glucose (P<0.001) and lactate (P<0.001). In 50% anaemic fish there was a significant interaction between dissolved oxygen and temperature on MCHC (P<0.005).

Haematological responses to exercise

Exercise of normocythaemic fish under normoxic conditions resulted in significant increases in PCV (P<0.001), [Hb] (P=0.004), RBCC (P=0.030), MCV (P=0.039), cortisol (P<0.001), glucose (P=0.0019) and lactate (P<0.001), and a significant decrease in MCHC (P<0.001), indicating that both splenic release and erythrocytic swelling also contributed to the increased PCV in these fish. Stress and anaerobiosis resulted in elevated glucose, cortisol and lactate. Exercise of 50% anaemic fish under normoxic conditions resulted in a significant increase in MCV (P=0.003), cortisol (P<0.001), glucose (P<0.001) and lactate (P<0.001) and a significant decrease in MCHC (P=0.016), again implicating eryhthrocytic swelling, stress and anaerobiosis. However, unlike normocythaemic fish, 50%

anaemic fish displayed no significant contribution from splenic erythrocyte release, indicating that in 50% anaemic fish the spleen is spent of stored erythrocytes.

In a comparison of exercised fish groups there was a significant interaction between hypoxia (6.8 mg L^{-1}**)** and temperature on MCHC (P=0.017), hypoxia (6.8 mg L^{-1}**)** and bleeding (50%) on MCHC (P=0.038), and bleeding (50%) and temperature on lactate (P=0.020). Exercise and hypoxia (6.8 mg L^{-1}**)** resulted in a significant increase in glucose (P<0.001).

Critical swimming speed

The critical swimming speed was significantly reduced only in 50% anaemic fish under hypoxic conditions (6.8 mg L^{-1}**)** at 20°C (P=0.003). However, although not statistically significant, 50% anaemia or hypoxia (6.8 mg L^{-1}**)** independently resulted in slight reductions in U_{crit} at both 15°C and 20°C (Figure 5.1).

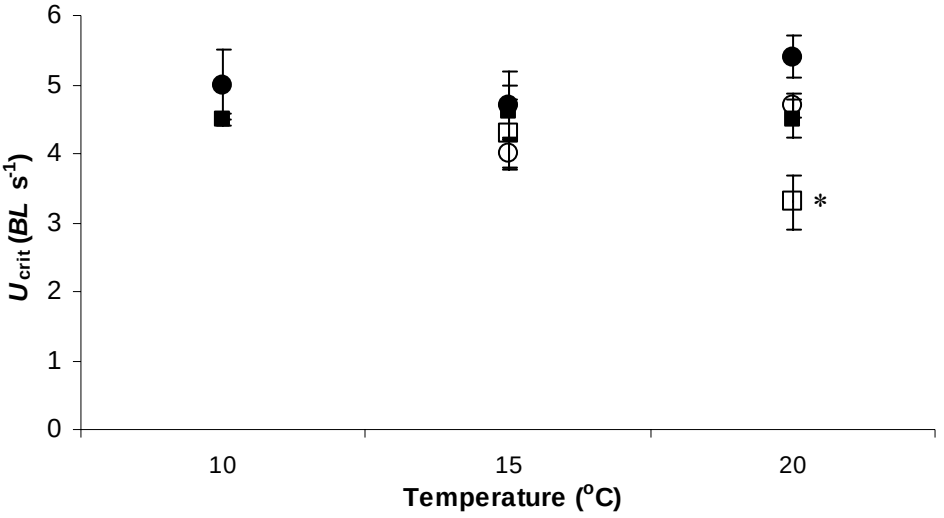

Figure 5.1. Temperature dependence of prolonged swimming speed (U_{crit}) of normocythaemic (closed symbols) and 50% anaemic (open symbols) juvenile trout in normoxia (>96%.sat.; circles) or mild hypoxia (6.8 mg L^{-1}; squares). Values are means ± S.E.M. * = significantly different from other treatments at 20°C (P<0.05).

Table 5.1 Haematological values of normocythaemic and anaemic (50% or 75% of the normocythaemic value) juvenile trout acclimated to 10°C, 15°C and 20°C and sampled at rest or following exhaustive exercise (U_{crit}) under normoxia (>96% sat.) or hypoxia (75% sat. or 6.8 mg L^{-1}). Values are means with S.E.M. in parentheses, N=5 for all treatments, ND = not done, N/A = not applicable, PCV = packed cell volume, [Hb] = whole blood haemoglobin, MCHC = mean cell haemoglobin concentration, MCH = mean cell haemoglobin, MCV = mean cell volume, RBCC = red blood cell count. Statistical comparisons have been omitted from the table for clarity but are fully discussed in the text.

Temp ^{0}C	Variable	Control (>96% sat) Resting Normocyth	Normoxic (>96% sat) Exercise Normocyth	Hypoxia (6.8 mg L^{-1}) Exercise Normocyth	Hypoxic (75% sat) Exercise Normocyth	Control (>96% sat) Resting Anaemic (50%)	Normoxic (>96% sat) Exercise Anaemic (50%)	Hypoxic (6.8 mg L^{-1}) Exercise Anaemic (50%)	Control (>96% sat) Resting Anaemic (75%)	Normoxic (>96% sat) Exercise Anaemic (75%)	Hypoxic (6.8 mg L^{-1}) Exercise Anaemic (75%)
10	U_{crit}	N/A	5.1 (0.5)	4.5 (0.1)	4.9 (0.2)	N/A	ND	ND	N/A	ND	ND
15	(BL s^{-1})	N/A	4.7 (0.5)	4.6 (0.4)	4.9 (0.4)	N/A	4.0 (0.2)	4.3 (0.5)	N/A	4.5 (0.4)	4.8 (0.2)
20		N/A	5.4 (0.3)	4.5 (0.2)	N/A	N/A	4.7 (0.2)	3.3 (0.4)	N/A	4.5 (0.1)	4.4 (0.6)
10	PCV	28.0 (3.0)	36.4 (2.2)	38.0 (2.1)	41.2 (1.4)	ND	ND	ND	ND	ND	ND
15	(%)	28.8 (1.0)	44.0 (3.0)	41.6 (0.6)	43.8 (1.5)	15.6 (2.0)	16.8 (1.2)	18.2 (1.7)	23.6 (0.7)	29.2 (1.1)	28.0 (2.5)
20		30.4 (1.3)	40.8 (2.5)	45.0 (3.0)	N/A	14.8 (1.7)	16.6 (1.1)	19.6 (3.4)	20.2 (1.7)	25.6 (2.2)	26.2 (2.5)
10	[Hb]	76.0 (8.5)	83.3 (5.8)	81.5 (4.9)	89.6 (3.8)	ND	ND	ND	ND	ND	ND
15	(g L^{-1})	71.3 (4.0)	91.3 (5.8)	91.5 (1.3)	93.7 (5.1)	34.3 (3.3)	34.8 (2.6)	36.8 (3.4)	57.3 (1.6)	61.2 (2.6)	63.8 (4.5)
20		77.1 (4.0)	93.8 (4.8)	102 (6.3)	N/A	32.6 (3.2)	36.8 (3.0)	35.3 (6.5)	45.7 (8.1)	56.7 (5.4)	58.4 (5.4)
10	RBCC	0.99 (0.15)	1.18 (0.05)	1.36 (0.05	1.31 (0.09)	ND	ND	ND	ND	ND	ND
15	(x 10^{12}	1.03 (0.13)	1.31 (0.10)	1.30 (0.10)	1.27 (0.07)	0.53 (0.06)	0.48 (0.05)	0.57 (0.08)	0.76 (0.05)	1.00 (0.05)	0.81 (0.04)
20	cells L^{-1})	1.15 (0.09)	1.30 (0.12)	1.19 (0.14)	N/A	0.68 (0.10)	0.51 (0.04)	0.60 (0.06)	0.74 (0.07)	0.85 (0.05)	0.70 (0.11)
10	MCHC	272 (8.0)	229 (7)	215 (4)	217 (6)	ND	ND	ND	ND	ND	ND
15	(g L^{-1})	245 (8.0)	211 (14)	220 (5)	215 (14)	223 (8)	205 (3)	203 (6)	245 (9)	209 (5)	229 (11)
20		255 (11)	232 (9)	226 (4)	N/A	220 (10)	219 (6)	179 (8)	255 (19)	219 (9)	221 (5)
10	MCH	80.3 (6.9)	70.2 (2.2)	56.9 (2.3)	68.5 (2.0)	ND	ND	ND	ND	ND	ND
15	(pg)	71.0 (5.1)	70.9 (6.1)	72.3 (6.3)	74.9 (6.8)	66.2 (4.3)	73.0 (5.0)	71.6 (3)	76.8 (5)	61.9 (1.8)	79.1 (4.3)
20		68.3 (4.6)	74.7 (8.9)	87.4 (6.4)	N/A	59 (4.5)	73.5 (7.0)	56.9 (5)	68.8 (3)	65.9 (3.1)	71.5 (6.8)
10	MCV	294 (22)	307 (13)	279 (8)	317 (15)	ND	ND	Nd	ND	ND	ND
15	(fL)	291 (22)	336 (14)	328 (26)	346 (14)	297 (18)	358 (24)	353 (19)	313 (19)	296 (13)	345 (22)
20		269 (16)	324 (33)	393 (32)	N/A	267 (12)	334 (27)	318 (26)	270 (5)	301 (14)	323 (31)
10	Cortisol	40.8 (26.3)	717 (84)	808 (85)	771 (100)	ND	ND	ND	ND	ND	ND
15	(nmol L^{-1})	15 (6.7)	931 (193)	861 (122)	960 (147)	13 (5.6)	547 (75)	742 (171)	137 (107)	796 (302)	753 (85)
20		42.4 (12.0)	1073 (242)	1030 (150)	N/A	4.6 (2.9)	749 (78)	1024 (97)	21 (8.7)	928 (86)	792 (133)
10	Glucose	4.3 (0.4)	8.9 (1.9)	6.9 (0.7)	7.0 (0.8)	ND	ND	ND	ND	ND	ND
15	(mM)	6.1 (0.9)	6.2 (1.0)	5.8 (0.3)	6.7 (0.8)	6.0 (0.7)	10.7 (0.7)	9.8 (1.2)	5.6 (1.2)	8.0 (1.2)	7.4 (1.5)
20		3.4 (0.5)	7.9 (1.1)	7.6 (0.6)	N/A	4.3 (0.3)	10.1(0.7)	9.3 (1.1)	3.8 (0.1)	7.8 (0.8)	8.5 (1.1)
10	Lactate	1.38 (0.20)	8.12 (1.03)	8.74 (0.99)	10.9 (2.66)	ND	ND	ND	Nd	ND	ND
15	(mM)	2.28 (0.60	7.88 (1.62)	9.9 (0.99)	11.94 (2.2)	1.42 (0.3)	16.73 (2.07)	12.66 (1.78)	1.90 (0.35)	12.56 (1.50)	12.25(1.74)
20		1.64 (0.43))	13.24 (2.80)	15.2 (3.26)	N/A	1.14 (0.07)	11.25 (3.83)	11.84 (1.73)	1.58 (0.46)	15.04 (2.00)	13.62 (1.33)

Discussion

Exercise of normocythaemic fish under normoxic and hypoxic conditions (6.8 mg L^{-1}**)** resulted in significant increases in PCV, [Hb] and RBCC and a significant decrease in MCHC. Significant changes in MCH and MCV occurred only in normocythaemic fish subjected to exercise in hypoxic conditions (6.8 mg L^{-1}**)**. However, the pattern of responses indicates that erythrocytic swelling occurs in all exercised fish but most significantly under hypoxic conditions (6.8 mg L^{-1}**)** at 20°C. Responses of PCV, [Hb] and RBCC indicate that splenic release of erythrocytes also contributed to increased carrying capacity in exercised fish (Table 5.1). The significant increase in MCH in normocythaemic hypoxic (6.8 mg L^{-1}**)** fish at 20°C and a slight decrease in MCH in 50% anaemic fish under the same conditions is possible evidence of splenic release of a cohort of atypical erythrocytes, haemoglobin-rich in normocythaemic fish and haemoglobin-poor (immature) in anaemic fish. Corresponding decreases in MCV under these conditions corroborate this hypothesis. Respective changes in MCV and MCHC may not be fully attributable to adrenergic erythrocytic swelling indicating that normocythaemic and 50% anaemic fish may release larger or smaller erythrocytes, respectively.

Exercise of 50% anaemic fish at 15°C and 20°C in normoxic and hypoxic conditions (6.8 mg L^{-1}**)** resulted in non-significant increases in PCV, [Hb] or RBCC, but MCHC significantly decreased and MCV significantly increased again indicating that erythrocytic swelling occurs under these conditions. Stress-induced increases in cortisol and glucose, and lactate, generally occurred in all exercised fish.

The prolonged swimming velocity of juvenile trout was maintained from 10°C to 20°C, and hypoxia (6.8 mg L^{-1}) or 50% anaemia, either singly or in combination, did not result in significant reductions in U_{crit} except at 20°C (Figure 5.1). The maintenance of U_{crit} values in normocythaemic fish swum in normoxic and hypoxic conditions (6.8 mg L^{-1}) at 10°C and 15°C, demonstrate that environmental oxygen availability is not a limiting factor at these temperatures. Furthermore, at 15°C physiological oxygen transport

is also demonstrated as a non-limiting factor, as 50% anaemic fish showed no significant difference in $U_{crit,}$ when compared with normocythaemic fish, having corresponding treatments. Therefore, these results do imply that at 10^{o}C and 15^{o}C prolonged swimming is limited solely by muscle contractile dynamics.

Brett (1964) speculated that when temperatures exceeded 15^{o}C, oxygen could become a limiting factor. Subsequently, Jones (1971) reported a decline in U_{crit} for juvenile trout when subjected to either severe hypoxia (50% sat.) or anaemia (haematocrit 10.9%) at 22.4^{o}C and 21.6 ^{o}C, respectively. At 20^{o}C these results show both mild hypoxia (6.8 mg L^{-1}, being 75% sat at 20^{o}C).) and 50% anaemia affected U_{crit} by a comparable amount, and the combination of hypoxia (6.8 mg L^{-1}) and 50% anaemia caused a more significant reduction in U_{crit} (Figure 5.1). Rather surprisingly, a 50% reduction in red cell volume had only the same degree of effect as a 25% reduction in environmental oxygen concentration, indicating that the availability of oxygen at the gill surface may be a more significant limiting factor on aerobic exercise than carrying capacity of the circulation. The circulation may contain a large surplus capacity for oxygen transport during exercise in fish, as has previously been demonstrated in mammals (Weibel, et al., 1991), but the gill clearly does not (Duthie and Hughes, 1987). Although exercised normocythaemic fish do respond by compensatory changes in haematology, including splenic release of erythrocytes and erythrocytic swelling (Table 5.1), it would appear that these may play a relatively minor role in improving aerobic exercise performance.

It is possible that decreased blood viscosity and increased cardiac output in anaemic fish, may contribute to sustaining oxygen delivery to exercising aerobic musculature in anaemic fish, although such speculation requires further experimental validation. The improved performance of warm acclimated fish may involve more than a compensatory increase in oxygen carrying capacity as implied by Bannon & Ling (2004).

The results of this study are significant since as they indicate that environmental oxygen availability, and not oxygen transport, is the limiting

factor for prolonged swimming at temperatures exceeding 15^{o}C. These results have clear implications for freshwater managers of migratory pathways of trout that are impacted on by anthropogenic activities that limit oxygen availability when water temperatures are high. Although at 20^{o}C the reduction in U_{crit} of trout subjected to mild hypoxia (6.8 mg L^{-1}) was not significant, any further decline in environmental oxygen will impede the migration of trout.

Acknowledgements

We thank Chris Sistern, Bruce Rhodes, Stephen Hardy, and Peter Jarman for equipment construction and technical support.

References

Bainbridge, R. 1958: The speed of swimming of fish as related to size and to the frequency and amplitude of the tail beat. *J. Exp. Biol. 35*, 109-133.

Bannon, H. J.; Ling, N. 2004: Running the unseen, lowland gauntlet: compounding effects of temperature, hypoxia and exercise for diadromous fishes. In: Rupp, G. L.; White, M. D. ed. Proceedings of the 7th International Symposium on Fish Physiology, Toxicology, and Water Quality, Tallinn, Estonia, May 12-15, 2003. Athens, Georgia, USA. U. S. Environmental Protection Agency, Ecosystems Research Division. EPA 600/R-04/049. Pp. 207-218.

Barron, M. G.; Tarr, B. D.; Hayton, W. L. 1987: Temperature-dependence of cardiac output and regional blood flow in rainbow trout, *Salmo gairdneri* Richardson. *J. Fish Biol*. *31*: 735-744.

Beamish, F. W. H. 1978: Swimming capacity. In: Hoar, W. H.; Randall, D. J. ed. Fish physiology Vol. VII. New York, Academic Press. Pp 101-187.

Beamish, F. W. H. 1980: Swimming performance and oxygen consumption of the Charrs. In: Balon, E. K. ed. Charrs. Salmonid Fishes of the Genus *Salvelinus*. The Hague, Dr W. Junk Publishing. Pp 739-748.

Besner, M.; Smith, L. S. 1983: Modification of swimming mode and stamina in two stocks of coho salmon (*Oncorhynchus mykiss*) by different levels of long-term continuous exercise. *Can. J. Fish. Aquat. Sci. 40*: 933-939.

Brett, J. R. 1964: The respiratory metabolism and swimming performance of young sockeye salmon. *J. Fish. Res. Bd. Can. 21*: 1183-1226.

Brett, J. R. 1965: The relation of size to rate of oxygen consumption and sustained swimming speed of sockeye salmom (*Oncorhynchus nerka*). *J. Fish. Res. Bd. Can*. *22*: 1491-1501.

Brett, J. R. 1971: Energetic responses of salmon to temperature. A study of some thermal relations in the physiology and freshwater ecology of sockeye salmon (*Oncorhynchus nerka*). *Am. Zool. 11*: 99-113.

Brett, J. R.; Hollands, M.; Alderdice, D. R. 1958: The effect of temperature on the cruising speed of young sockeye and coho salmon. *J. Fish. Res. Bd. Can. 15*: 587-605.

Coughlin, D. J.; Burdick, J.; Stauffer, K. A.; Weaver, F. E. 2001: Rainbow trout display a developmental shift in red muscle kinetics, swimming kinematics and myosin heavy chain isoform. *J. Fish Biol. 58*: 701-715.

Dacie, J. V.; Lewis, S. M. 1991: Practical Haematology.7th edition. London, Churchill. p 557.

Duthie, G. G., Hughes, G. M. 1987: The effects of reduced gill area and hyperoxia on the oxygen consumption and swimming speed of rainbow trout. *J. Exp. Biol. 127-354.*

Farrell, A. P. 1997: Effects of temperature on cardiovascular performance. In: Wood, C. M.; McDonald, D. G. ed. Global Warming Implications for Freshwater and Marine Fish. Cambridge, Cambridge University Press. Pp. 135-158.

Ferguson, R. A.; Kieffer, J. D.; Tufts, B. L. 1993: The effects of body size on the acid-base and metabolite status in the white muscle of rainbow trout before and following exhaustive exercise. *J. Exp. Biol. 180*: 195-207.

Gallaugher, P.; Thorarensen, H.; Farrell, A. P. 1992: Swimming performance and haematological variables in splenectomized rainbow trout, *Oncorhynchus mykiss*. *J. Exp. Biol. 171*: 301-314.

Gallaugher, P.; Thorarensen, H.; Farrell, A. P. 1995: Hematocrit in oxygen transport and swimming in rainbow trout (*Oncorhynchus mykiss*). *Respir. Physiol. 102*: 279-292.

Goolish, E. M. 1989: The scaling of aerobic and anaerobic muscle power in rainbow trout (*Salmo gairdneri*). *J. Exp. Biol*. *147*: 493-505.

Jain, K. E.; Farrell, P. 2003: Influence of seasonal temperature on the repeated swimming performance of rainbow trout *Oncorhynchus mykiss*. *J. Exp. Biol. 206*: 3569-3579.

Johnston, I. A.; Moon, T. W. 1980: Exercise training in skeletal muscle of brook trout (*Salvelinus fontinalis*). *J. Exp. Biol*. *87*: 177-194.

Jones, D. R. 1971: The effect of hypoxia and anaemia on the swimming performance of rainbow trout (*Salmo gairdneri*). *J. Exp. Biol*. *55*: 541-551.

Jones, D. R. 1982: Anaerobic exercise in teleost fish. *Can. J. Zool. 60*: 1131-1134.

Kieffer, J. D.; Ferguson, R. A.; Tompa, J. E.; Tufts, B. L. 1996: Relationship between body size and anaerobic metabolism in brook trout and large mouth bass. *Trans. Am. Fish. Soc. 125*: 760-767.

Milligan, C. L.; Hooke, G. B.; Johnson, C. 2000: Sustained swimming at low velocity following a bout of exhaustive exercise enhances metabolic recovery in rainbow trout. *J. Exp. Biol. 203*: 921-926.

Nikinmaa, M.; Soivio, A.; Railo, E. 1981: Blood volume of *Salmo gairdneri*: Influence of ambient temperature. *Comp. Biochem. Physiol*. *69A*: 767-769.

Pearson, M. P.; Stevens, E. D. 1991: Splenectomy impairs aerobic swim performance in trout. *Can. J. Zool. 69*: 2089-2092.

Perry, S. F. 1998: Relationships between branchial chloride cells and gas transfer in freshwater fish. *Comp. Biochem. Physiol*. *119A*: 9-16.

Pörtner, H. O.; Mark, F. C.; Bock, C. 2004: Oxygen limited thermal tolerance in fish? Answers obtained by nuclear magnetic resonance techniques. *Respir. Physiol. Neurobiol*. *141*: 243-260.

Rome, L. C.; Loughna, P. T.; Goldspink, G. 1984: Muscle fiber activity in carp as a function of swimming speed and muscle temperature. *Am. J. Physiol*. *247*: 272-279.

Taylor, E. W.; Egginton, S.; Taylor, S. E.; Butler, P. J**.** 1997: Factors which may limit performance at different temperatures. In: Wood, M. C.; McDonald, D. G. ed. Global Warming Implications for Freshwater and Marine Fish. Cambridge, Cambridge University Press. Pp. 105-133.

Thomas, S.; Fievet, B.; Motais, R. (1988): Adaptive respiratory response of trout to acute hypoxia. 1. Effects of water ionic composition on blood acid-base response and gill morphology. *Respir. Physiol*. *74*: 77-90.

Thorarensen, H.; Gallaugher, P. E.; Kiessling, A. K. 1993: Intestinal blood flow in swimming chinook salmon *Oncorhynus tshawytscha* and the effects of haematocrit on blood flow distribution. *J. Exp. Biol*. *179*: 115-129.

Tsuyuki, H.; Williscroft, S. N. (1977): Swimming stamina differences between genotypically distinct forms of rainbow trout (*Salmo gairdneri*) and steelhead trout. *J Fish. Res. Bd. Can*. 34: 996-1003.

Weibel, E. R.; Taylor, C. R.; Hoppeler, H. 1991: The concept of symmorphosis: A testable hypothesis of structure-function relationship. *Proc. Natl. Acad. Sci*. *88*: 10357-10361.

CHAPTER SIX

General discussion

The preliminary step for assessing the effects of water quality on prolonged swimming ability is the establishment of U_{crit} performance curves of fish in clean normoxic water. Their establishment not only identifies the optimum aerobic temperature, which often coincides with the preferred temperature (Brett, 1971; Reynolds & Casterlin, 1980; Guderley & Blier, 1988), but also provides a benchmark for assessing the effects of degraded water quality on prolonged swimming under defined physicochemical conditions (Hammer, 1995).

This thesis established, over a wide thermal range (5°C – 25°C) in normoxic conditions (>96% sat.), U_{crit} performance curves for *Galaxias maculatus* larval (inanga whitebait), post-larval and adults, and *Oncorhynchus mykiss* parr and smolt. U_{crit} performance curves for these fish exhibited the characteristic temperature dependence reported in the scientific literature for eurythermal fishes, U_{crit} increasing with temperature to a maximum several degrees below the upper thermal limit, performance then declining markedly as the upper thermal limit is approached (Brett, 1971; Griffiths & Alderdice, 1972; Beamish, 1978; Koumoundouros et al., 2002; Myrick & Cech, 2000).

The decline in U_{crit} as the thermal limit is approached may in part be attributed to an increase in metabolic oxygen demand while simultaneously decreasing oxygen availability as temperature increases above the optimum. This phenomenon resulted in Brett (1964) concluding that at temperatures above the optimum any reduction in saturation is likely to reduce U_{crit}, thus this study was extended to investigate the response of trout parr and inanga whitebait prolonged swimming ability to mild hypoxia (75% sat.) at 10°C, 15°C and 20°C.

U_{crit} values recorded for trout parr validate the conclusion reached by Brett (1964), mild hypoxia (75% sat.) at 20°C (5°C above the optimum) resulting in a significant reduction in U_{crit} (Figure 3.1) but had no effect at or below the optimum temperature (15°C). However, inanga whitebait, when subjected to exercise in hypoxic conditions, showed a significant decline in U_{crit} at and below the optimum temperature of 17°C (Figure

4.1), indicating that inanga whitebait are more sensitive to mild hypoxia than trout.

Aerobic activity in all vertebrates is dependent on circulatory oxygen supply. In order to prevent anaerobic fatigue, even during moderate exercise, the oxygen carrying capacity of the blood must exceed the aerobic demands of locomotion. As a consequence, haematological responses of acclimated rainbow trout parr in response to sustained aerobic stress (U_{crit}) were investigated.

Fish acclimated at 20^{o}C showed an adaptive elevation in oxygen carrying capacity due to an increase in red blood cell volume and haemoglobin content. Following exhaustive exercise, haematocrit was elevated under both normoxic and hypoxic conditions. However, the primary cause of this apparent increase in oxygen carrying capacity was splenic release of stored erythrocytes under normoxic conditions, whereas stress-induced erythrocytic swelling accounted for the increase in hypoxia. This contrasting response was most pronounced at 10^{o}C.

Exactly why splenic release of stored erythrocytes is inhibited in hypoxia at 10^{o}C is something of a mystery. It may be that an adaptive shift in oxygen affinity as a result of adrenergic erythrocyte swelling is more effective at maintaining oxygen delivery at this temperature. The subtleties of the circulatory responses of trout to increase oxygen demand under differing environmental conditions require further investigation.

The results of Chapter Four, effects of temperature and metamorphosis on prolonged swimming ability of inanga, clearly highlight the issue that prolonged swimming differs between life stages, particularly at warmer temperatures. Prolonged swimming ability in inanga larvae (whitebait) and adults showed similar temperature dependence but the poor performance of post-larval inanga at higher temperatures was surprising. This ontogenetic change in aerobic performance may be due to developmental changes in swimming muscles or the development of the spleen and production of red blood cells that accompanies metamorphosis.

Rombough (1998) reported that the skin of larval fish is an important site for respiratory gas exchange, with some species obtaining approximately 40% of their oxygen via their skin at the end of the larval stage. Inanga undergo a transition from a greater reliance on cutaneous respiration in the whitebait stage to fully functional gill respiration, which is accompanied by the development and enlargement of the spleen and noticeable suffusion with red blood cells. These post-larval fish may lack adaptive oxygen carrying response mechanisms that are fully developed in older fish.

Hypoxia has been shown to affect swimming ability, but acclimation to temperature also affects U_{crit}, and that acclimation effect seems to be mediated by adaptive changes in oxygen carrying capacity. Thus the question investigated in the final experiment was to address which of these is the most significant factor, oxygen carrying capacity or gill diffusion.

The maintenance of U_{crit} values in normoxic and hypoxic conditions at 10^{o}C and 15^{o}C demonstrated that environmental oxygen availability is not a limiting factor at these temperatures. Furthermore, at 15^{o}C, physiological oxygen transport is also non-limiting, as anaemic fish showed no significant difference in U_{crit} when compared with normocythaemic fish (Figure 5.1). However, at 20^{o}C these treatments resulted in a significant decline in U_{crit}. Moreover at 20^{o}C, a 50% reduction in red cell volume had only the same degree of effect as a 25% reduction in environmental oxygen concentration, indicating that the availability of oxygen at the gill surface may be a more significant limiting factor on aerobic exercise than carrying capacity.

The thermal sensitivity shown by inanga as they undergo metamorphosis from larvae to juveniles requires further scientific investigation. Future research should be directed towards identifying the dynamics of oxygen transport, details of haematopoietic development and changes in cutaneous respiration during metamorphosis. The effects of hypoxia on this life stage should also be investigated, as it is likely to further limit the aerobic performance in warmer water.

Conclusion

I have established that increased temperature and mild hypoxia limit the prolonged swimming performance of larval whitebait (*Galaxias maculatus*) and rainbow trout parr (*Oncorhynchus mykiss*), although physiological acclimation can redress impairment to some degree. Extending this study using older rainbow trout (smolt stage) and examining the effects of anaemia on swimming performance indicated that, although these older fish demonstrate greater capacity to perform under extreme conditions, it is environmental oxygen availability, rather than physiological oxygen transport, that is the major factor limiting performance at elevated temperatures.

Inanga U_{crit} performance curves for different life stages and trout parr U_{crit} performance curves, demonstrate that the potential impact of environmental stressors cannot be generalised between species or life stages, and that even mild hypoxia can significantly reduce the swimming abilities of migratory fishes in warm water.

The implications for the management of lowland rivers and streams are clear, elevated temperatures will impede migration of diadromous fishes, as warm, lowland, eutrophic migratory pathways will present serious exercise challenges. When setting the benchmark for water quality for lowland rivers and streams, consideration will need to be given to the species and life stage most vulnerable to environmental degradation.

References

Beamish, F. W. H. 1978: Swimming capacity. In: Hoar, W. H.; Randall, D. J. ed. Fish Physiology Vol. VII. New York, Academic Press. Pp. 101-187.

Brett, J. R. 1964: The respiratory metabolism and swimming performance of young sockeye salmon. *J. Fish. Res. Bd. Can. 21*: 1183-1226.

Brett, J. R. 1971: Energetic responses of salmon to temperature. A study of some thermal relations in the physiology and freshwater ecology of sockeye salmon (*Oncorhynchus nerka*). *Am. Zool. 11*: 99-113.

Guderly, H.; Blier, P. 1988: Thermal acclimation in fish: conservative and labile properties of swimming muscle. *Can. J. Zool. 66*: 1105-1115.

Griffiths, J. S.; Alderdice, D. F. 1972: Effects of acclimation and acute temperature experience on the swimming speed of juvenile coho salmon. *J. Fish. Res. Bd. Can. 29*: 251-264.

Hammer, C. 1995: Fatigue and exercise tests with fish. *Com. Biochem. Physiol. 112 A*: 1-20.

Koumoundouros, G.; Sfakianakis, D. G.; Divanach, P.; Kentouri, M. 2002: Effect of temperature on swimming performance of sea bass juveniles. *J. Fish Biol. 60*: 923-932.

Myrick, C. A.; Cech, J. J. Jr**.** 2000: Swimming performances of four California stream fishes: temperature effects. *Env. Biol. Fish. 58*: 289-295.

Reynolds, W. W.; Casterlin, M. E. 1980: The role of temperature in the environmental physiology of fishes. In: Ali, M. A. ed. Environmental Physiology of Fishes. New York, Plenum Press. Pp. 497-518.

Rombough, P. J. (1998). Partitioning of oxygen uptake between the gills and skin in fish larvae: a novel method for estimating cutaneous oxygen uptake. *J. Exp.Biol. 201*: 1763-1769.